# SYNTHESIS OF NEW HETEROCYCLIC COMPOUNDS USING MULTICOMPONENT APPROACH AND THEIR BIOLOGICAL EVALUATION

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IN CHEMISTRY

By

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# CHAPTER-I

# INTRODUCTION

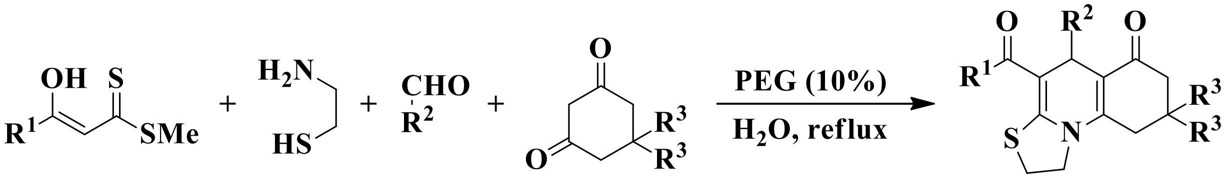
## Multi-component Reactions

MCRs are organic chemical transformations in which three or more reactants combine to generate a single product in a one-pot reaction and in a single operation under mild reaction conditions, in an efficient way with high atom economy. MCRs have several advantages over conventional linear step synthesis[1]. MCRs are widely used for the synthesis of natural products[2-4], organic materials, polymers[5-7], bioactive molecules[8,9], medicinal chemistry[10-12], agro chemistry[13-15], and combinatorial chemistry[16-20]. Multicomponent reactions (MCRs) are among the best synthetic methods to achieve the target molecule libraries and interest in MCRs has surged during recent years.

## Recent literature on multicomponent reactions

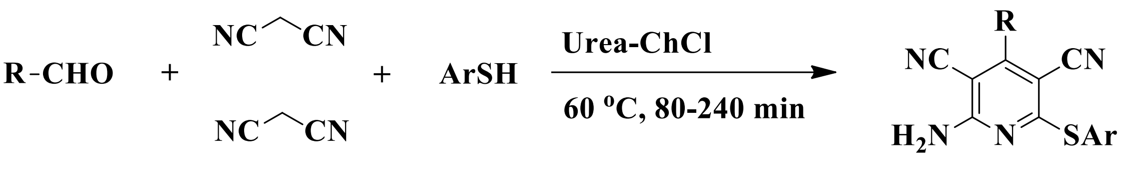
The following are a few interesting multicomponent reactions from recent literature.

Nagaraju *et al.*[21] synthesized highly diversified thiazoloquinoline derivatives (**1**) using α-enolic dithioesters, cysteamine, aldehydes, and cyclic 1,3-diketones in water–PEG-400. The advantage of the reaction is that all the starting materials are easily available, operational simplicity, and simple workup procedure.



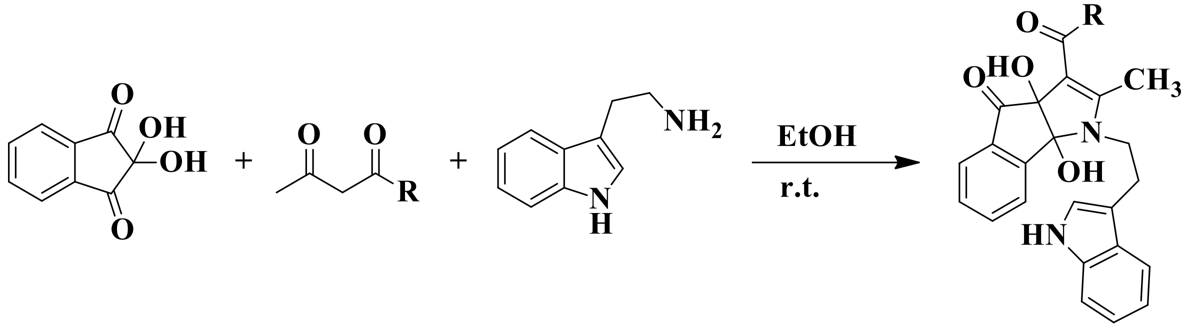
**1**

Azizi and Haghayegh[22] reported the synthesis of substituted pyridine derivatives (**2**) by the reaction of different aldehydes with various thiols and malononitrile using choline chloride-urea (1:2) as a green catalyst.



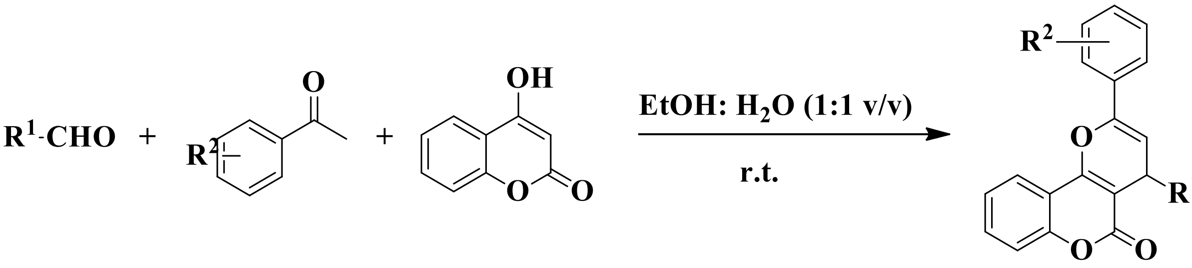
**2**

Tang *et al.*[23] described the synthesis of substituted indeno[1,2-b]pyrroles (**3**) by the domino threecomponent reaction of tryptamine, 1,3-dicarbonyl compounds and ninhydrin in ethanol at room temperature. The advantage of the reaction is mild reaction conditions, high yields, operational simplicity and it is being environmentally benign.



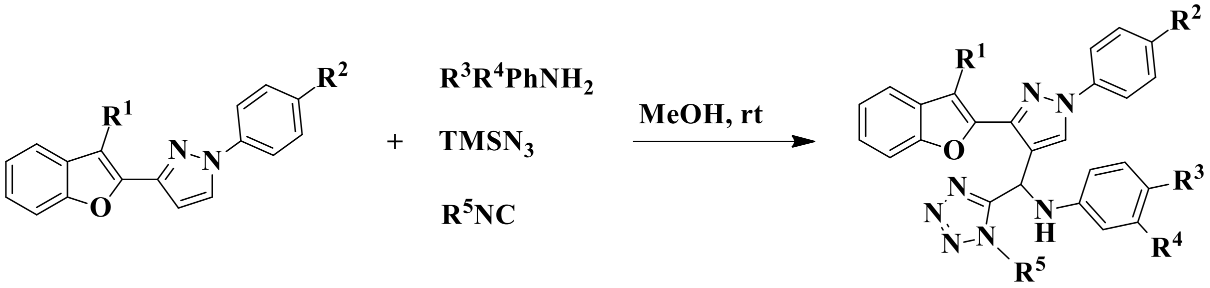
**3**

Brahmachari and Nurjamal[24] reported the synthesis of 2-aryl-4-alkyl/aryl-pyrano[3,2-*c*]chromen-5-(4*H*)-one derivatives (**4**). Condensation of alkyl/aryl aldehydes, acetophenones, and 4-hydroxycoumarin in aqueous ethanol gave title compounds.



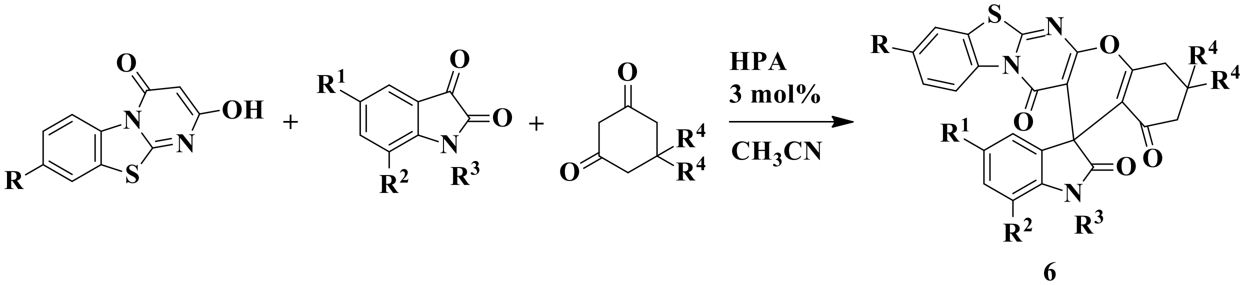
**4**

Kushwaha *et al.*[25] described the synthesis of novel benzofuran-tetrazole derivatives (**5**) by integrating multicomponent Ugi-azide reaction with the molecular hybridization approach. These compounds were screened for their anti-alzheimer activity.



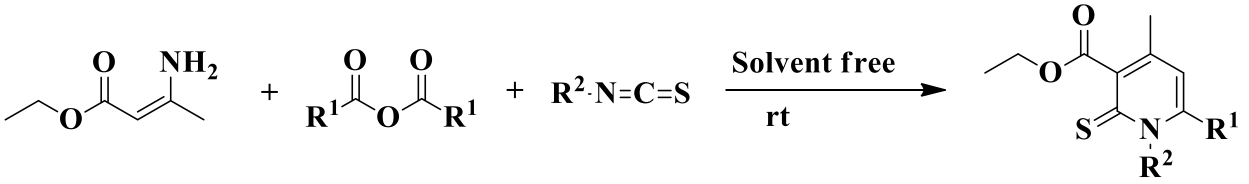
**5**

Jannati and Esmaeili[26] reported the synthesis of compound **6** via a three-component reaction of various substituted 2-hydroxy benzo[4,5]thiazolo[3,2-a]pyrimidin-4-ones with isatins, and cyclohexane-1,3-diones in presence of 12-tungstophosphoric acid catalyst. The reaction proceeds via Knoevenagel condensation, followed by Michael addition and intermolecular cyclization to give title compounds.



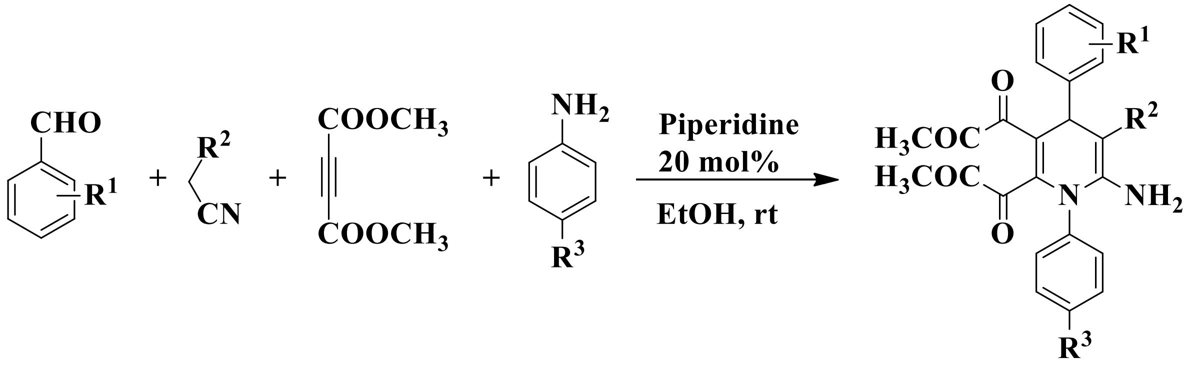
**6**

Trinh and McCluskey[27] described the synthesis of 1,3-thiazine-6-phenylimino-5-carboxylates (**7**) by the reaction of ethyl 3-aminocrotonate with phenylisothiocyanate and aceticanhydride under solvent free conditions.



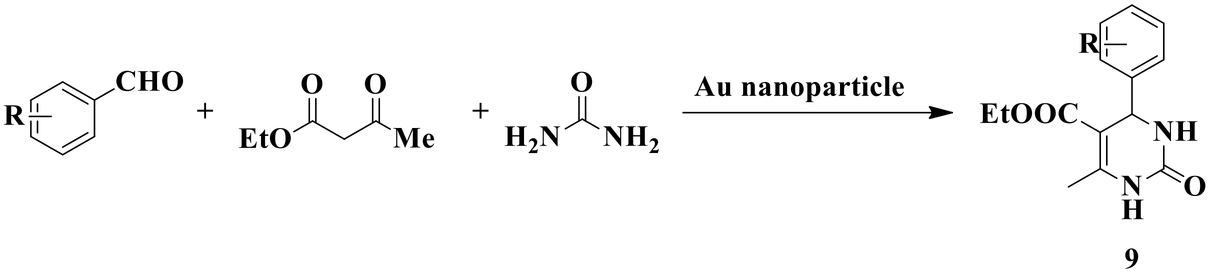
**7**

Polyfuctionalized 1,4-dihydropyridine derivatives (**8**) were synthesized by Ramesh *et al.*[28] via a four component reaction of aryl aldehydes with nitrile moiety, dimethyl acetylenedicarboxylate and aromatic/aliphatic amines using piperidine as base catalyst in ethanol at room temperature.



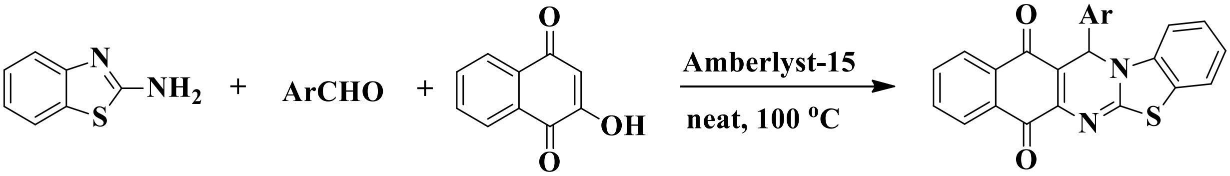
**8**

Nguyen *et al.*[29] synthesized 3,4-Dihydropyrimidin-2(1-*H*)-one derivatives (**9**) using Biginelli reaction. In this aryl aldehydes, ethyl acetoacetate, and urea were used along with Au nano rod as a catalyst.



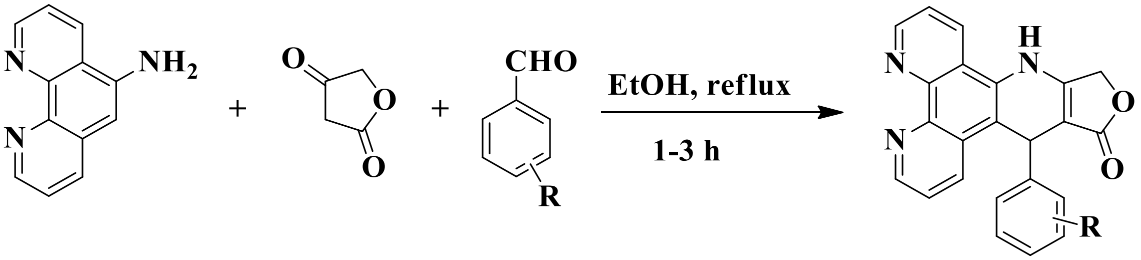
**9**

Liqiang Wu *et al.*[30] described the synthesis of novel 13-aryl-13*H*-benzo[*g*]benzothiazolo[2,3-*b*]quinazoline-5,14-dione derivatives (**10**). 2-Aminobenzothiazole, araldehydes and 2-hydroxy-1,4-naphthoquinone were condended in the presence of amberlyst-15. All the synthesized compounds were tested on two different human cancer cell lines (HepG2 and Hela).



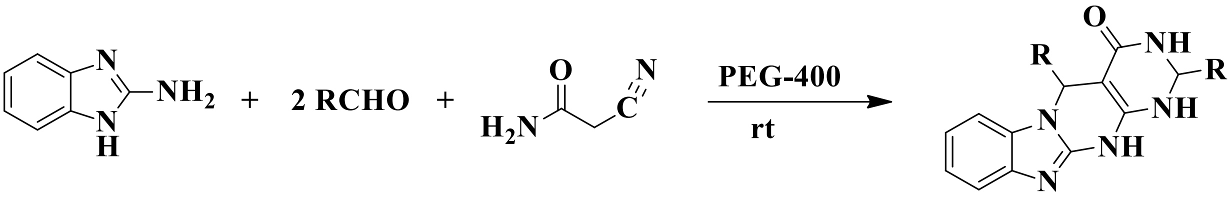
**10**

Kamal *et al.*[31] reported a series of novel 4-aza-2,3-dihydropyrido phenanthroline derivatives (**11**) from the reaction of a 1,10-phenanthroline amine, tetronic acid and various aromatic aldehydes via a multicomponent approach.



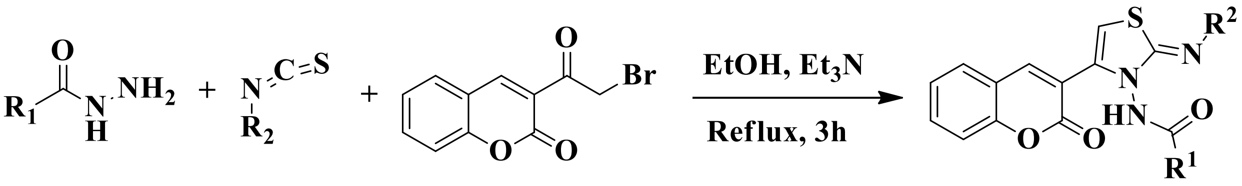
**11**

Reddy *et al.*[32] reported an efficient eco-friendly methodology for the synthesis of substituted benzo[4,5]imidazo[1,2-*a*]-pyrimido[4,5-*d*]pyrimidin-4(1*H*)-ones (12) via a one-pot multicomponent reaction in good yields by using inexpensive and non-toxic polyethylene glycol (PEG-400) medium.



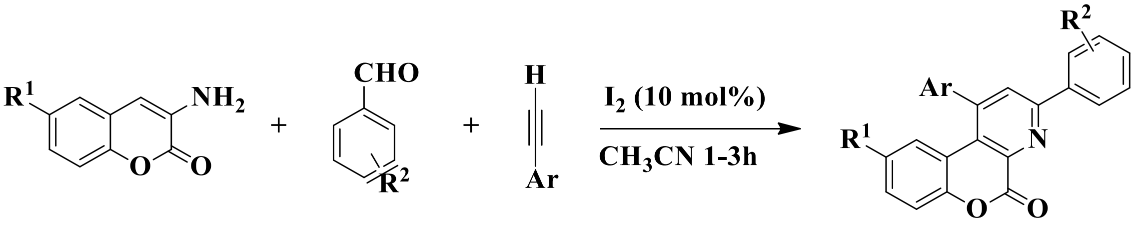
**12**

Salar *et al.*[33] described the synthesis of 3-thiazolyl coumarin derivatives (**13**) via a one-pot reaction of benzohydrazide, phenyl isothiocyanate and 3-(2-bromoacetyl)-2*H*-1-benzopyran-2-ones in ethanol.



**13**

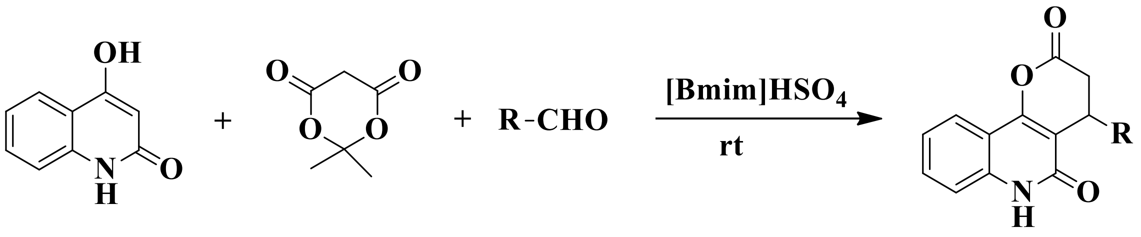
Khan *et al.*[34] described substituted pyrido[2,3-*c*]coumarins (**14**) synthesis by using 3-aminocoumarins, aromatic aldehydes and alkynes in presence of 10 mole% molecular iodine in acetonitrile under reflux conditions via a one-pot. The advantages of this method are that it gives a good yield, does not require the aqueous workup procedure, uses chromatographic separation and is environmentally benign.



**14**

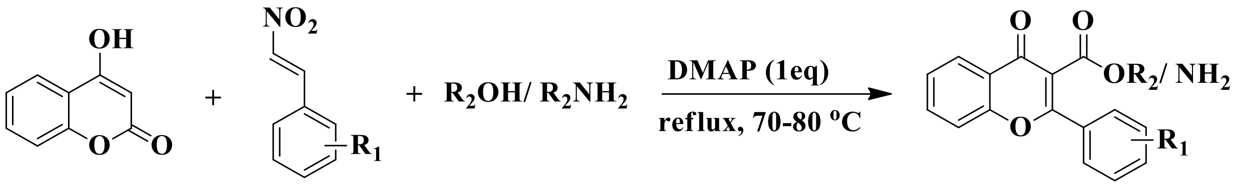
Rad-Moghadam *et al.*[35] described an efficient method to prepare a novel 4-substituted-3,4-dihydro-6*H*-pyrano[3,2-c]quinoline-2,5-dione derivatives (15) via a three component reaction of

4-hydroxy quinoline-2-one, meldrum's acid and an alkyl (or) aryl aldehydes in presence of [Bmim]$HSO_4$.



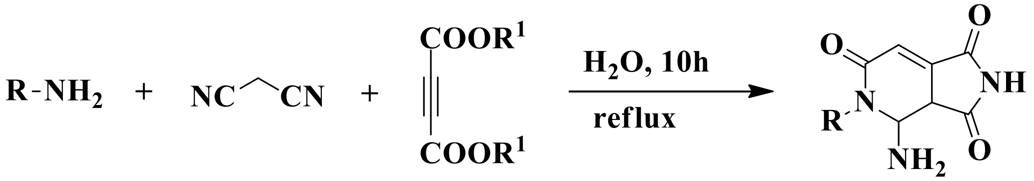
**15**

Synthesis of 3-substituted carboxylate/carboxamide flavone derivatives (**16**) was reported by Bhattacharjee and Khan[36]. The three-component reaction of 4-hydroxycoumarin with β-nitrostyrene and alcohol/amine in presence of *N,N*-dimethyl-4-aminopyridine (DMAP) to give title compounds.



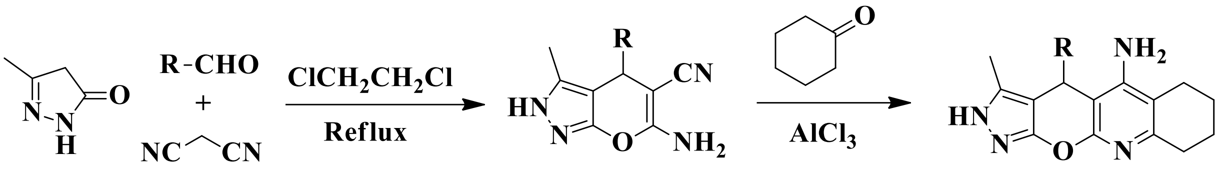
**16**

Maity *et al.*[37] described the catalyst-free synthesis of pyrrolo[3,4-c]pyridines (**17**) starting from primary amines, malononitrile and dialkylacetylene dicarboxylate in an aqueous medium. All the synthesized compounds are fluorescent in nature and their photophysical properties were also studied.



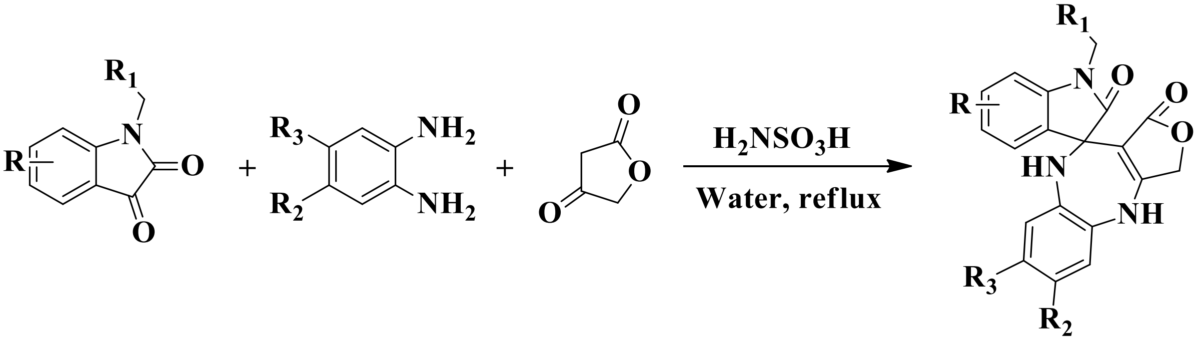
**17**

Derabli *et al.*[38] synthesized the tacrine-pyranopyrazole derivatives (**18**) by the reaction of 3-methyl-1*H*-pyrazol-5(4*H*)-one, aryl(or hetero)aldehydes, malononitrile, and cyclohexanone via Friedländer condensation.



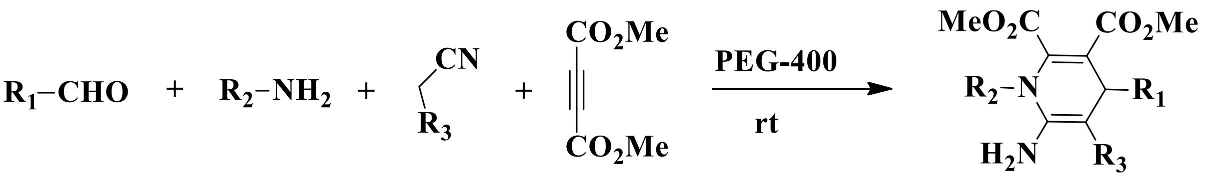
**18**

Synthesis of spiro-benzodiazepines (**19**) were reported by Nagaraju *et al.*[39] by the reaction of o-phenylenediamines, tetronic acid, and isatins by using mild and inexpensive catalyst like sulphamic acid in water.



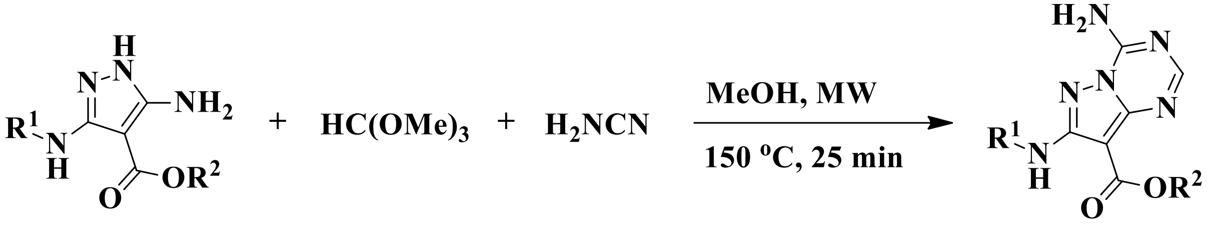
**19**

Pal*et al.*[40] described the synthesis of dihydropyridine derivatives (**20**) through a four component reaction of various aromatic aldehydes with anilines, malononitrile and dimethyl acetylenedicarboxylate in polyethylene glycol at room temperature.



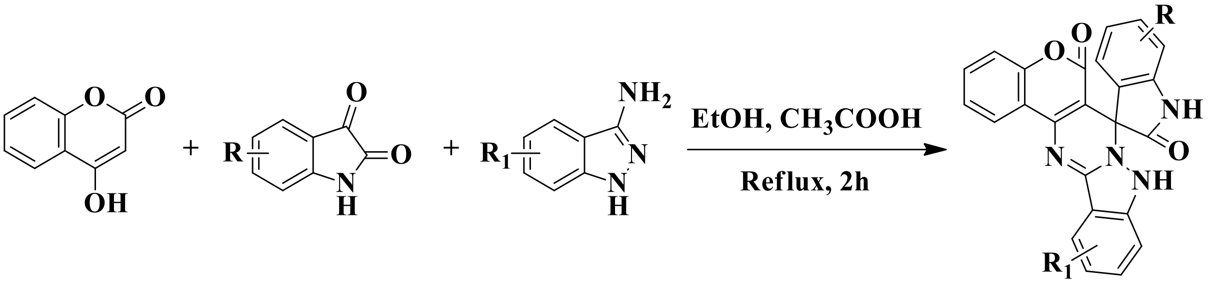
**20**

Lim *et al.*[41] synthesized the substituted 5-aza-9-deaza-adenine derivatives (**21**) using selective annulation of 1,3,5-triazine ring onto 5-aminopyrazole-4-carboxylates with cyanamide, and trimethyl orthoformate in methanol under microwave conditions.



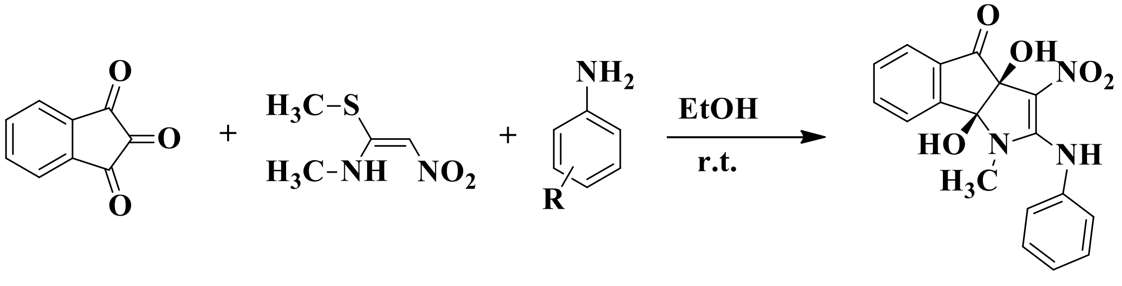
**21**

Jadhav *et al.*[42] discribed the synthesis of spiro[chromeno[4',3':4,5] pyrimido[1,2-b]indazole-7,30-indoline]-2',6(9 H)-dione derivatives (**22**) through a one-pot condensation of 4-hydroxy-2*H*-chromen-2-one, isatin, and 1H-indazole-3-amine in the presence of acetic acid and ethanol.



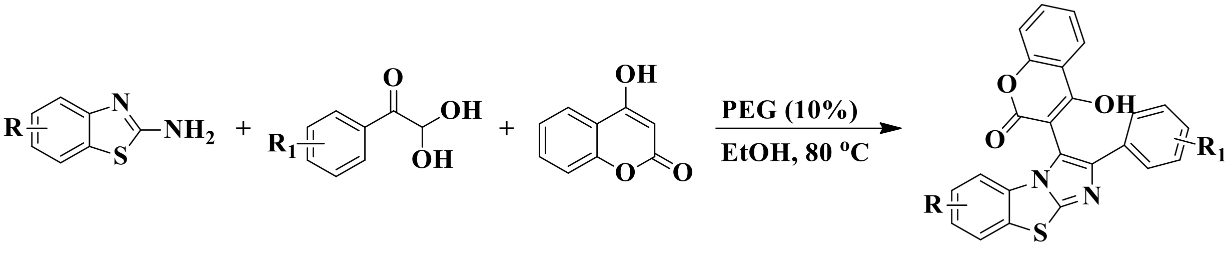
**22**

Rahimi *et al.*[43] reported the synthesis of dihydroxyoxoindeno[1,2-*b*]pyrrole derivatives (**23**) by the reaction of ninhydrin, *N*-methyl-1-(methylthio)-2-nitroethenamine and aromatic amines in ethanol at room temperature.



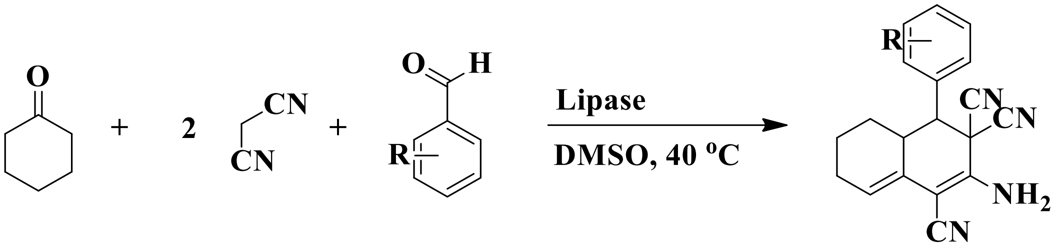
**23**

Benzo[d]imidazo[2,1-b]thiazol-3-yl-2*H*-chromen-2-ones (**24**) synthesis was described by Balwe *et al.*[44] The reaction consists of condensation of 2-amino benzothiazole, arylglyoxal monohydrate and 4-hydroxycoumarin in ethanol and PEG-600. The reaction is a catalyst-free, environmentally benign, and one-pot multicomponent protocol.



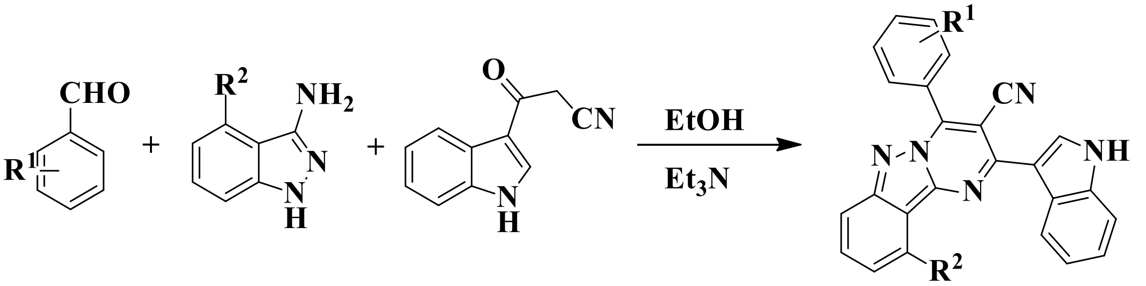
**24**

Dalal *et al.*[45] synthesized the aminocarbonitrile derivatives (**25**) through the three component reaction of aromatic aldehydes, cyclohexanone and two equivalents of malononitrile in DMSO solvent and lipase as enzyme.



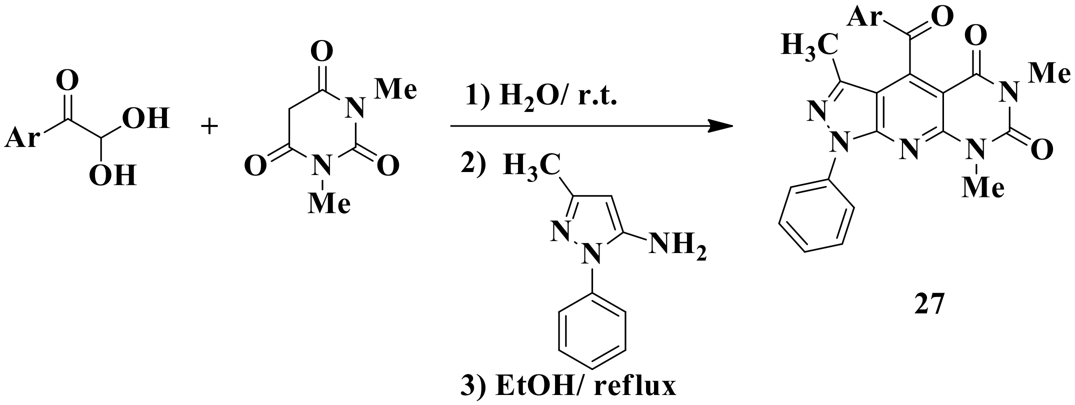
**25**

Li *et al.*[46] reported the metal-free synthesis of 4-aryl pyrimido[1,2-*b*]indazole-3-carbonitrile derivatives (**26**). The reaction starts from aromatic aldehydes, 1*H*-indazol-3-amine (4-chloro-1*H*-indazol-3-amine), and 3-(*1H*-indol-3-yl)-3-oxopropanenitrile in presence of ethanol and triethylamine.



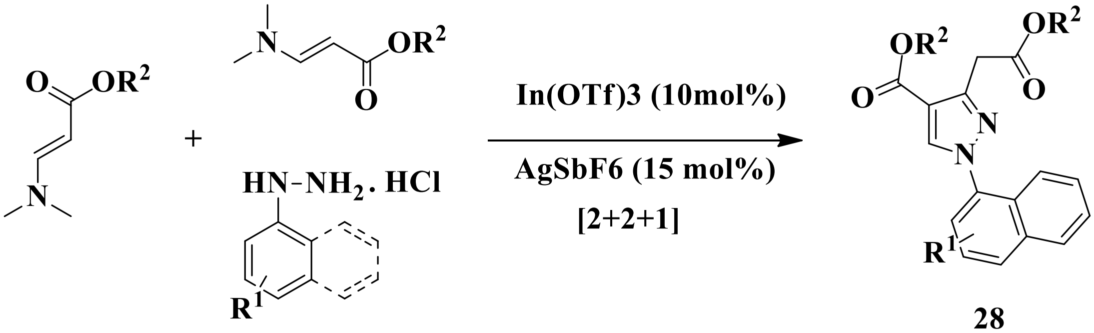
**26**

Ezzati *et al.*[47] described the synthesis of pyrazolo[4',3':5,6]pyrido[2,3-d]pyrimidin-5,7-dione derivatives (**27**). Cyclocondensation of 1,3-dimethylpyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione, 3-methyl-1-phenyl-1H-pyrazole-5-amine and arylglyoxals in $H_2O$/EtOH under reflux conditions to give title compounds. This reaction procedure involves catalyst-free, mild reaction conditions and with good yields.



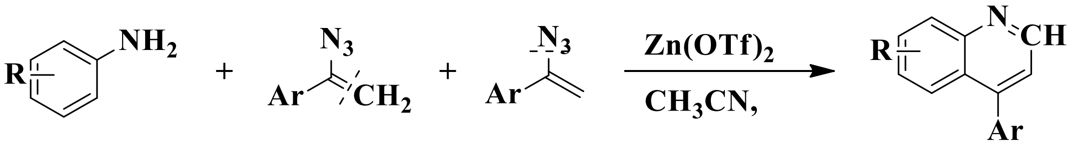
**27**

Thombal and Lee[48] synthesized the multisubstituted *N*-arylpyrazoles (**28**) via a multicomponent approach. Indium(III)/Silver(I)-catalyzed [2+2+1] oxidative annulation of arylhydrazine hydrochlorides with β-enamino esters gives the title compounds.



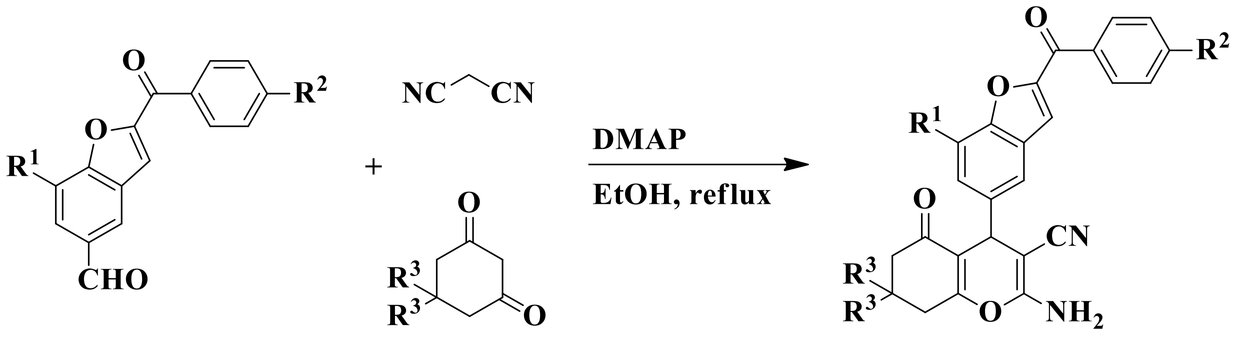
**28**

Cen *et al.*[49] synthesized the 4-substituted quinolines (**29**) via a multicomponent reaction of aryl amines with vinyl azides using Zn promoted catalyst in presence of acetonitrile solvent.



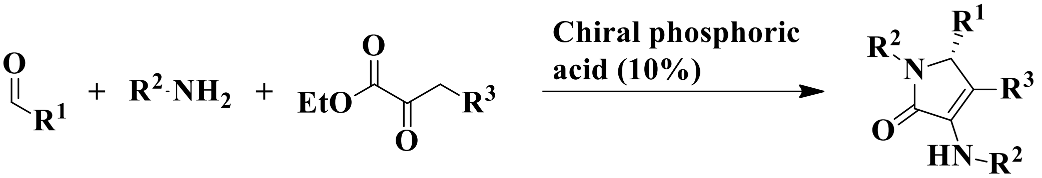
**29**

Benzofuran-pyran derivatives (**30**) were synthesized by Gupta *et al.*[50]. All the synthesized derivatives were evaluated for their osteogenic effects on primary osteoblast cells isolated from calvaria and the compounds were found to be potential bone anabolic agents.



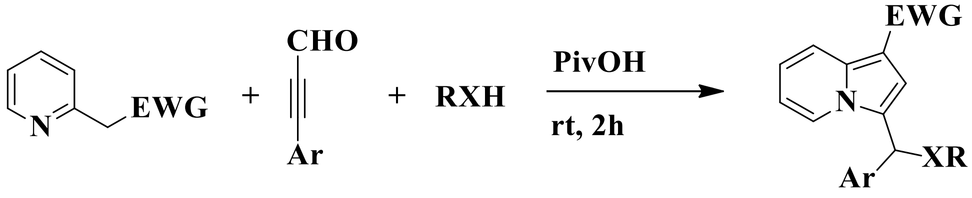
**30**

The highly functionalized γ-lactam derivatives (**31**) were synthesized by Corte *et al.*[51] from the reaction of substituted amines, aldehydes and pyruvates by using chiral phosphoric acid as a catalyst.



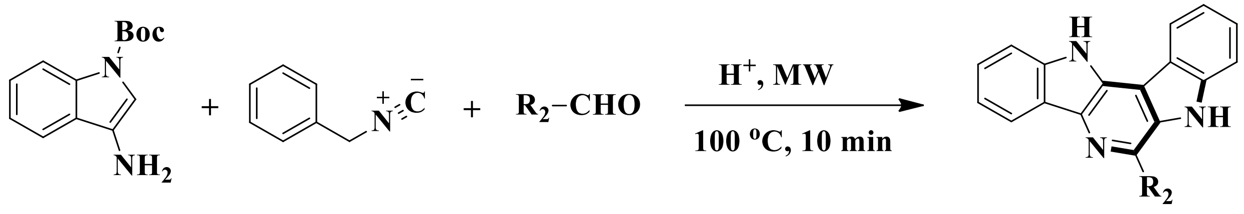
**31**

Yang *et al.*[52] described the regiospecific one-pot synthesis of indolizines (**32**) from 2-(pyridin-2-yl)acetates, ynals, and alcohols or thiols under the solvent-free and metal-free conditions.



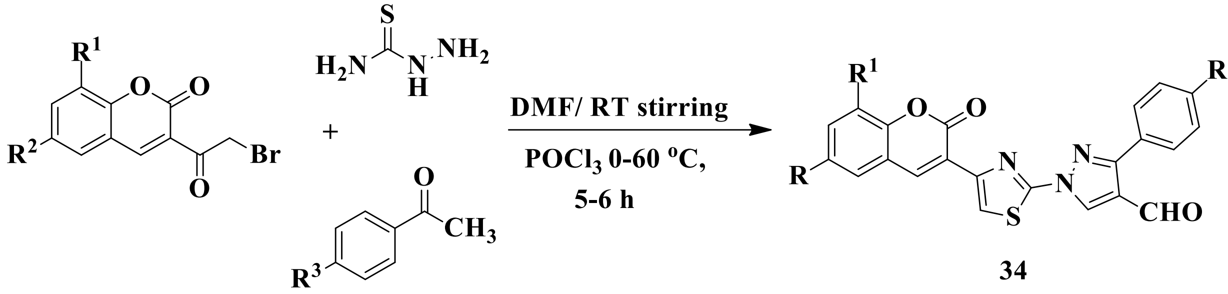
**32**

Chen *et al.*[53] reported the synthesis of pyridodiindole derivatives (**33**). A three component cascade condensation of Boc-protected 3-aminoindole, aldehyde and benzyl isocyanide with the assistance of microwave irradiation to give title compounds.



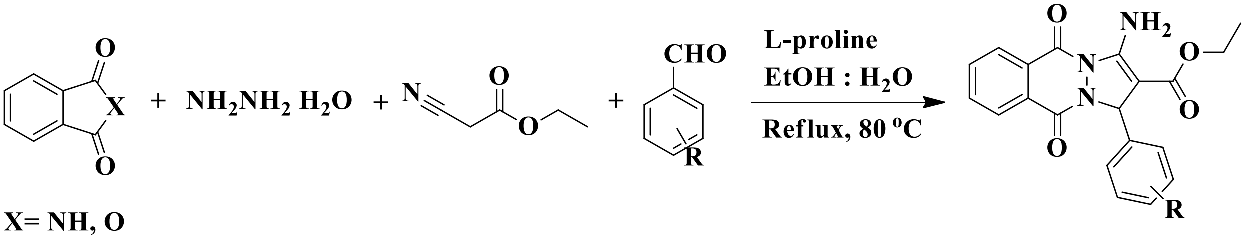
**33**

1-(4-(2-Oxo-2H-chromen-3-yl)thiazol-2-yl)-3-phenyl-1H-pyrazole-4-carbaldehydes (**34**) were synthesized by Krishnaiah *et al.*[54] via a multicomponent approach. 3-(2-Bromoacetyl)-2*H*-chromen-2-ones on reaction with thiosemicarbazide and substituted acetophenones using Vilsmeier-Haack reaction to give title compounds. All the synthesized compounds were tested for anticancer activity.



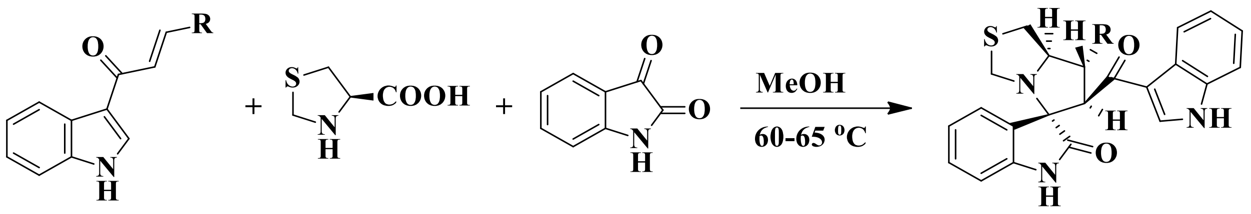
**34**

Roy *et al.*[55] described the synthesis of 1*H*-pyrazolo[1,2-*b*]phthalazine-5,10-diones (**35**) by the reaction of phthalimide or phthalic anhydride with aromatic aldehydes, and ethyl cyanoacetate in presence of L-proline.



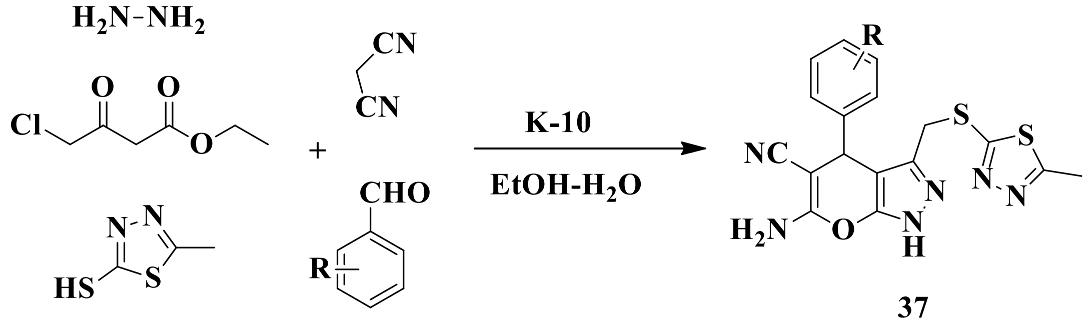
**35**

Thiazolo-pyrrolidine-(spirooxindole) derivatives (**36**) were synthesized by Islam *et al.*[56] via a multicomponent reaction of 3-acetyl indole chalcones with isatin, and l-4-thiazolidine carboxylic acid in presence of methanol.



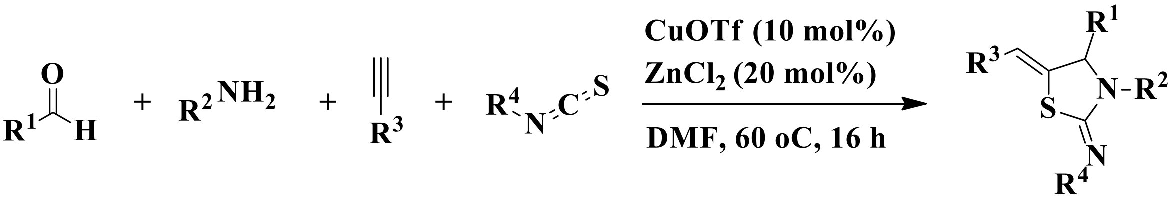
**36**

Reddy *et al.*[57] synthesized the thiadiazolyl-pyranopyrazoles (**37**) through a multicomponent reaction of 2-thiolyl-5-methyl-1,3,4-thiadiazole, ethyl 4-chloro-3-oxobutanoate, hydrazine, malononitrile, and benzaldehyde in presence of K-10 catalyst.



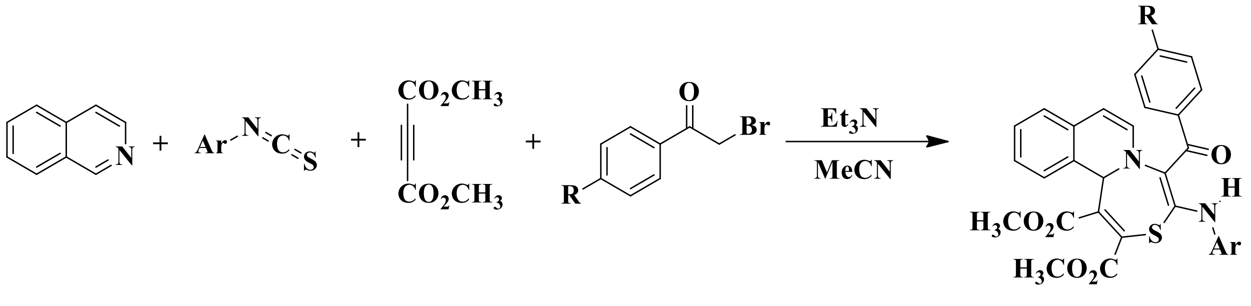
**37**

Thiazolidin-2-imines (**38**) were synthesized by Shehazadi *et al.*[58] through a multicomponent reaction of aldehydes, amines, alkynes and isothiocyanates by using Cu (I) / Zn (II) as catalysts and the synthesized compounds act as acetylcholinesterase inhibitors.



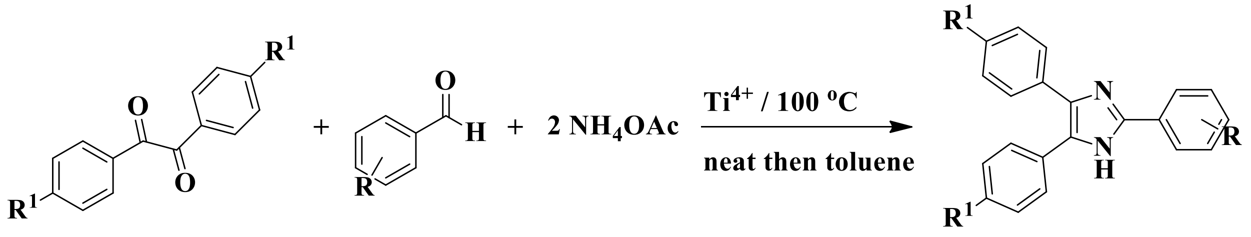
**38**

Alizadeh *et al.*[59] described the synthesis of [1,4]thiazepino[5,4-*a*]isoquinolines (**39**) by the reaction of isoquinoline with phenacyl bromide, aryl isothiocyanate, and dimethyl acetylenedicarboxylate in presence of methanol and triethylamine.



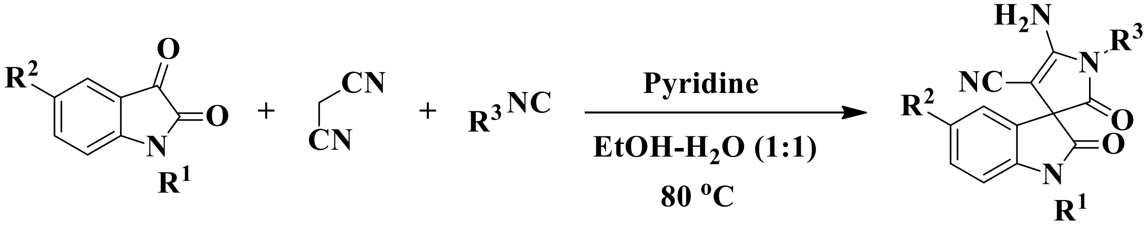
**39**

Magyar and Hell[60] synthesized the 2,4,5-trisubstituted-imidazoles (**40**) by the reaction of substituted benzil, benzaldehydes, and ammonium acetate in presence of molecular sieves supported titanium(IV) as heterogeneous catalyst.



**40**

Synthesis of 5′-Amino-2,2′-dioxospiro[indoline-3,3′-pyrrole]-4′-carbonitrile derivatives (**41**) was described by Adib *et al.*[61]. Reaction between isatin, malononitrile and isocyanides in presence of pyridine to yield title compounds.



**41**

## PRESENT WORK

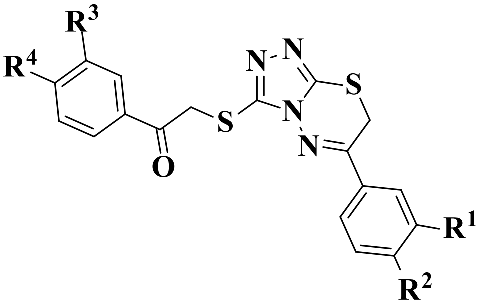
Previous section clearly explains the importance of MCR. MCRs have applications in high-throughput screening techniques that enabled rapid identification of potential new medicines among large collections of organic compounds. This required the development of new approaches to the synthesis of organic compounds. The methods that would give many compounds at a time.

## OBJECTIVES OF RESEARCH

1. To design the new heterocyclic compounds by simple methodologies
2. To explore the biological activities new entities.

Chapter-I gives an overview of MCRs and their resent literature.

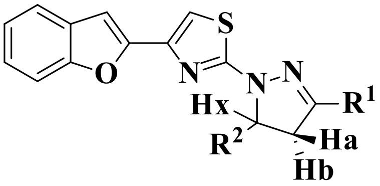
Chapter-II describes synthesis and anticancer activity of compound 42



**42**

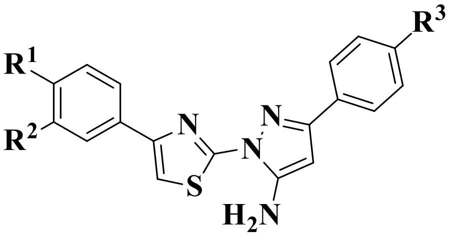
Chapter-III is divided in to two sections viz, section-A and section-B.

Section-A portrays the synthesis of 43



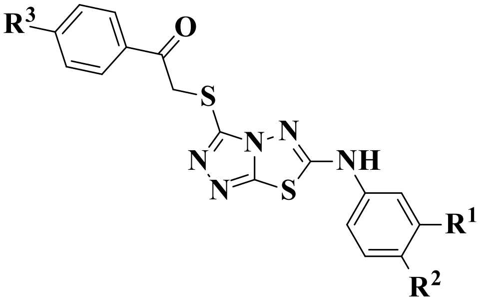
**43**

Section-B narrates the synthesis of 3-phenyl-1-(4-phenylthiazol-2-yl)-1*H*-pyrazole-5-amines and their biological evaluation: antiviral, antifungal and anticancer activities.



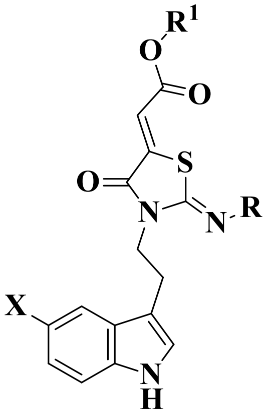
**44**

Chapter-IV explains about the multicomponent synthesis of substituted 1-phenyl-2-((6-(phenylamino)-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazol-3-yl)thio)ethanones and their anticancer activity**.**



**45**

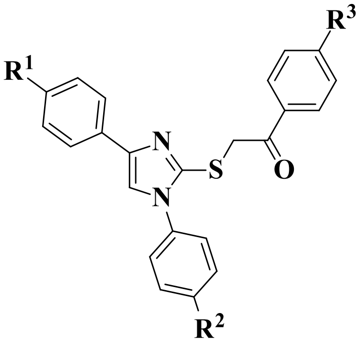
Chapter-V contains synthesis and anticancer activities of 46



**46**

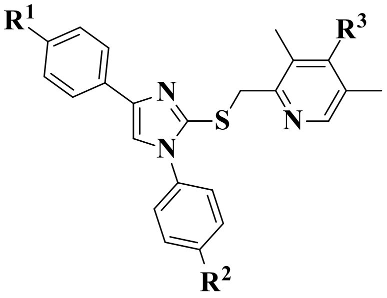
Chapter-VI is further divided in to two sections viz, section-A and section-B.

Section-A delineates synthesis of 2-((1,4-diphenyl-1*H*-imidazol-2-yl)thio)-1-phenylethanones and their biological evaluation: anticancer, antimicrobial and antiviral activity.



**47**

Section-B represent the synthesis and anticancer activity of 2-(((1,4-diphenyl-1H-imidazol-2-yl)thio)methyl)-3,5-dimethylpyridines.



**48**

The compounds 42, 43, 44, 45, 46, 47 and 48 structures were confirmed by spectral data.

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# CHAPTER-II

## SYNTHESIS AND ANTICANCER ACTIVITY OF 1-PHENYL-2-((6-PHENYL-7*H*-[1,2,4]TRIAZOLO[3,4-*b*][1,3,4]THIADIAZIN-3-YL)THIO)ETHANONES

## INTRODUCTION

Triazole is three nitrogen atoms containing five-membered heterocyclic system. It is having two isomeric forms and they are 1,2,3-triazole and 1,2,4-triazole (Fig.1).

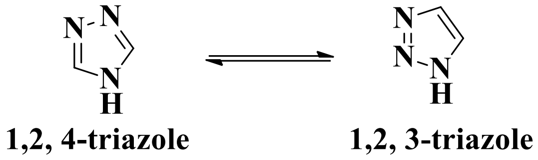
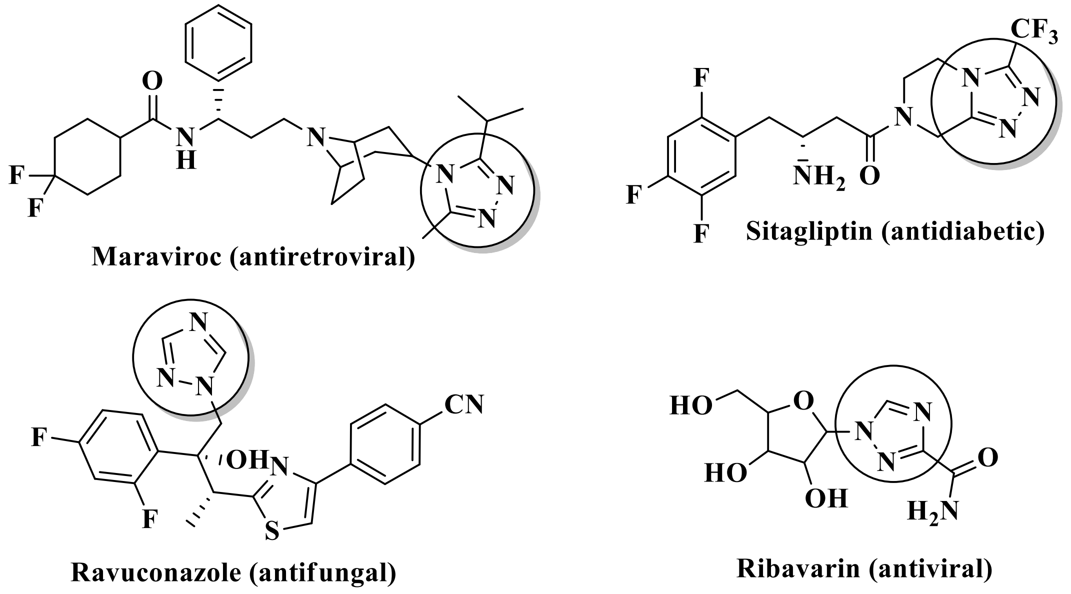


**Fig. 1** The two isomeric forms of triazole

1,2,4-triazole ring containing compounds have good therapeutic properties, which are widely available in the market such as triazolam, furacycline, etizolam, alprazolam, fluconazole, voriconazole, itraconazole. Triazoles exhibit antibacterial[1,2], antiviral[3,4], antifungal[5,6], anti-inflammatory[7,8], antimalarial[9], antitubercular[10], herbicides[11,12], insecticidal[13], anticonvulsant[14,15], and antidepressant[16] properties. Due to the versatile therapeutic applications, low toxicity, excellent selectivity, synthesis of these triazoles has gained much importance in the last few decades. Some of the important 1,2,4-triazole therapeutic molecules are shown in Fig. 2



**Maraviroc (antiretroviral)**

**Sitagliptin (antidiabetic)**

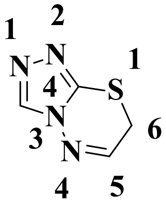
**Ravuconazole (antifungal)**

**Ribavarin (antiviral)**

Fig. 2 Some of the important 1,2,4-triazole therapeutic molecules

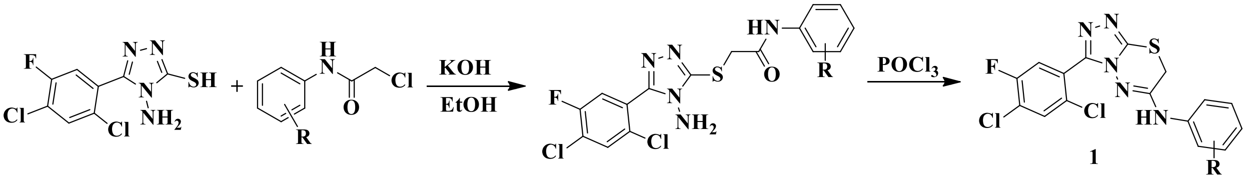
1,2,4-triazole ring fused with 1,3,4-thiadiazines are associated with various biological activities due to presence of NCS linkage[17,18].

Thiadiazines have various biological applications[19, 20]. Triazolothiadiazines are privileged heterocyclic compounds and are active for drug development due to their metabolic stability, high solubility and the ability of hetero atoms interaction with target proteins. These triazolothiadiazines were extensively studied for their vast therapeutic applications[21-27].

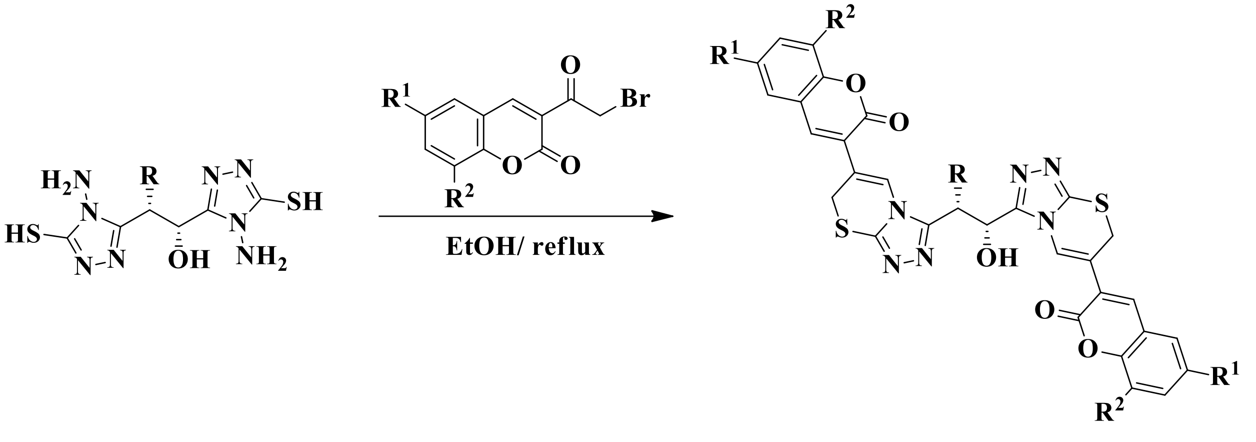


A concise review of the synthesis of 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazines was given below.

Karthikeyan[28] *et al.* described the synthesis of 6-arylamino-3-(2,4-dichloro5-fluorophenyl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazines (**1**). 4-Amino-5-(2,4-dichloro-5-fluorophenyl)-4*H*-1,2,4-triazole-3-thiol and 2-chloro-*N*-phenylacetamide in presence of ethanolic potassium hydroxide solution under reflux conditions to give the corresponding S-alkylated derivatives. These on further reaction with $POCl_3$ gave the cyclized final products.

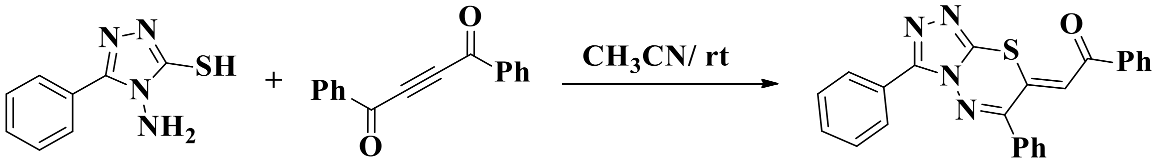


Pavurala[29] *et al.* synthesized the bis coumarinyl bis triazolothiadiazinyl ethane derivatives (**2**) through the reaction of 3,3'-(3,3'-(dihydroxy/hydroxyethane-1,2-diyl)bis(7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine-6,3-diyl))bis(2*H*-chromen-2-ones) and various 3-(2-bromoacetyl)coumarins in dry alcohol reflux conditions.



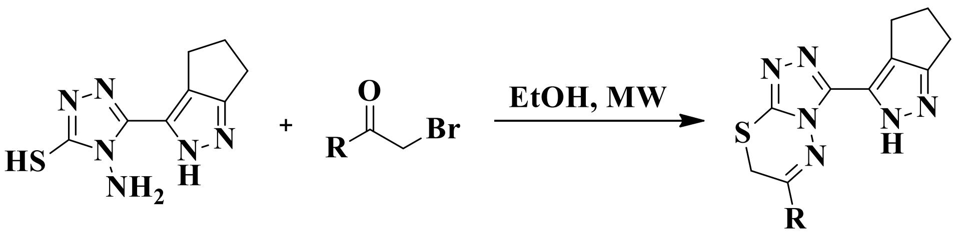
**2**

2-(3,6-Diphenyl-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-7-ylidene)-1-phenylethanone (**3**) synthesis was reported by Shah[30] *et al.* through the reaction of 4-amino-5-phenyl-4*H*-1,2,4-triazole-3-thiol and 1,4-diphenylbut-2-yne-1,4-dione in acetonitrile at room temperature.

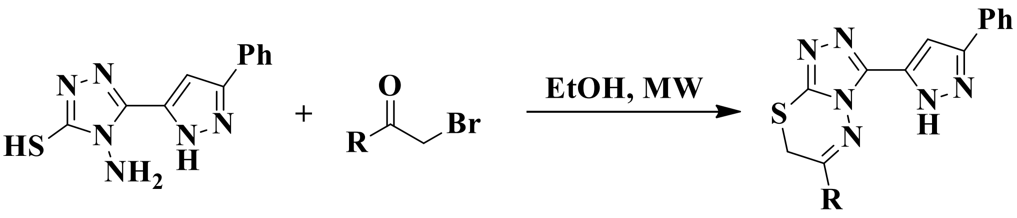


**3**

Laporte[31] *et al.* synthesized pyrazolo 1,2,4-triazolo-[3,4-*b*]thiadiazines (**4** & **5**).

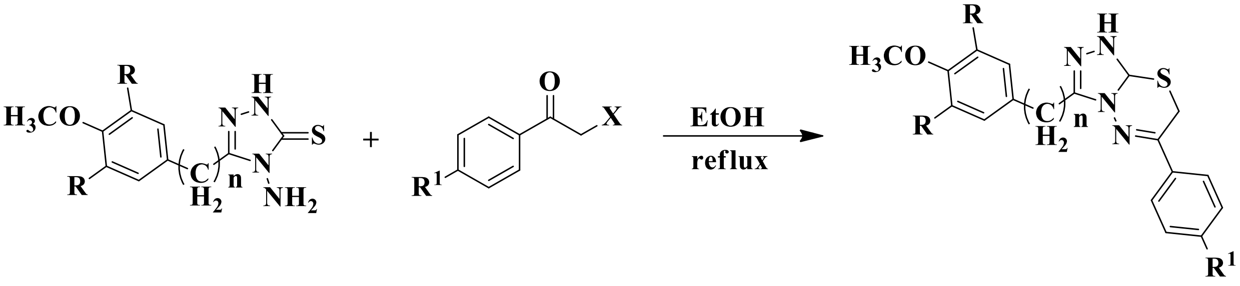


**4**



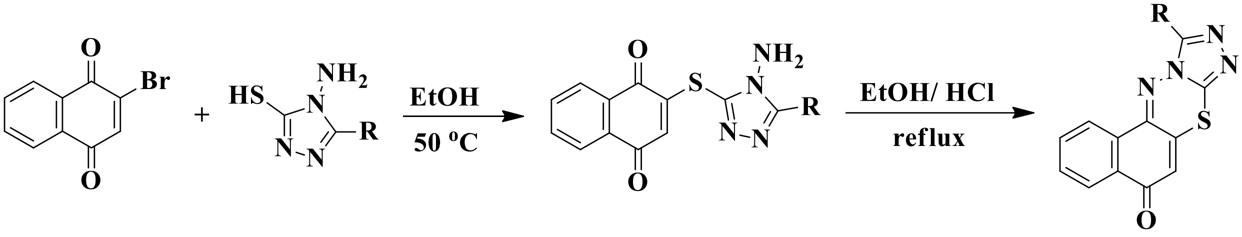
**5**

Aytac[32] *et al.* reported the compounds (**6**) by the condensation of 4-amino-3-substituted-1,2,4-triazole-5-thiones with various phenacyl bromides in alcohol.



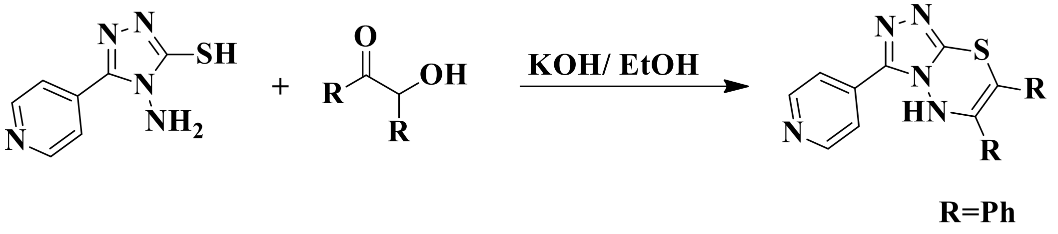
**6**

10-Substituted-5*H*-naphtho[1,2-e][1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-5-ones (**7**) were reported by Khalafy[33] *et al.* The condensation of 2-bromo-1,4-naphthoquinone with 4-amino-5-aryl-4*H*-1,2,4-triazole-3-thiols led to 2-[(4-amino-5-aryl-4*H*-1,2,4-triazol-3-yl)thio] naphthalene-1,4-diones which were further undergo intramolecular cyclization to give final compounds.



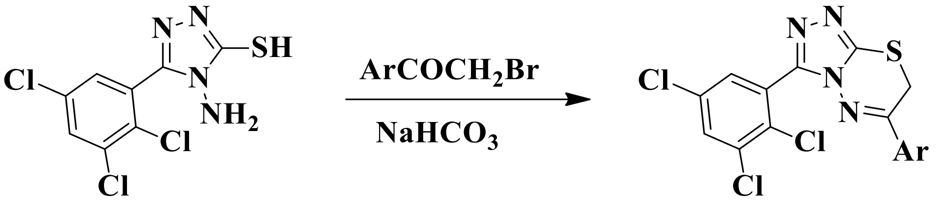
**7**

Deohate[34] reported the synthesis of 6,7-disubstituted phenyl-3-(pyridin-4-yl)-5*H*-[1,2,4]triazolo[3,4 *b*][1,3,4]thiadiazine derivatives (**8**). The reaction between 4-amino-5-(pyridin-4-yl)-4*H*-1,2,4-triazole-3-thiol and substituted benzoins in presence of ethanolic potassium hydroxide solution to give title compounds with good yields.



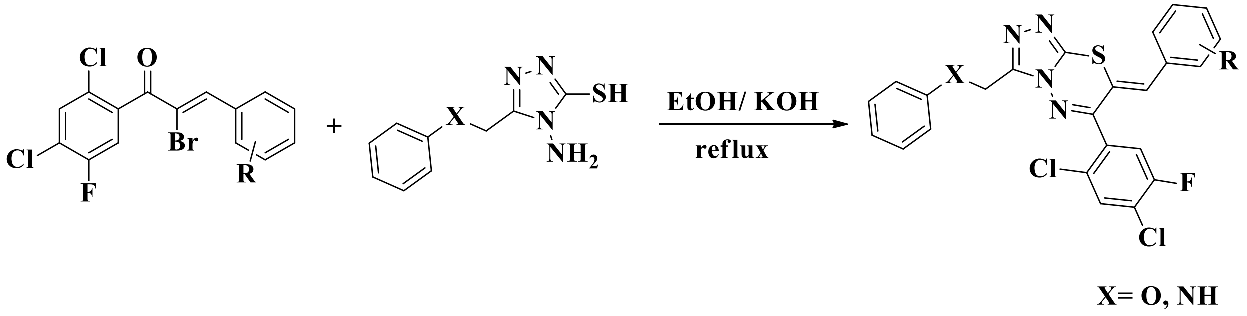
**8**

Karegoudar[35] *et al.* reported the synthesis of compounds (**9**). From the reaction of 3-(2,3,5-trichlorophenyl)-4-amino-1,2,4-triazole-5-thione with various phenacyl bromides in ethanol.



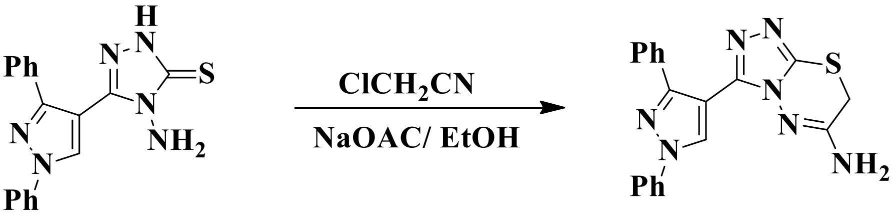
**9**

Holla[36] *et al.* reported the synthesis of 7-benzylidene-6-(2,4-dichloro-5-fluorophenyl)-3-(phenoxymethyl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine derivatives (**10**) by interacting halo substituted 3-phenylprop-2-en-1-one and 4-amino-5-(aryloxy/phenylamino methyl)-4*H*-1,2,4-triazole-3-thiol in ethanol potassium hydroxide solution under reflux conditions with good yields.



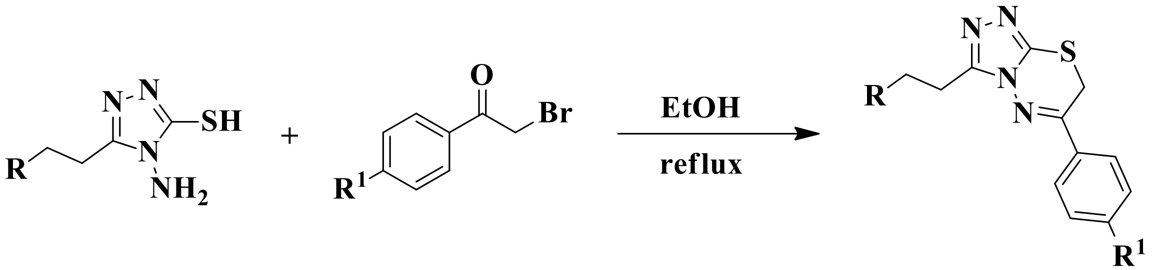
**10**

Farghaly[37] *et al.* synthesized compound (**11**) from the reaction of 4-amino-3-(1,3-diphenyl-1*H*-pyrazol-4-yl)-4,5-dihydro-[1,2,4]triazole(1*H*)-thione and chloroacetonitrile by using fused sodium acetate in ethanol.



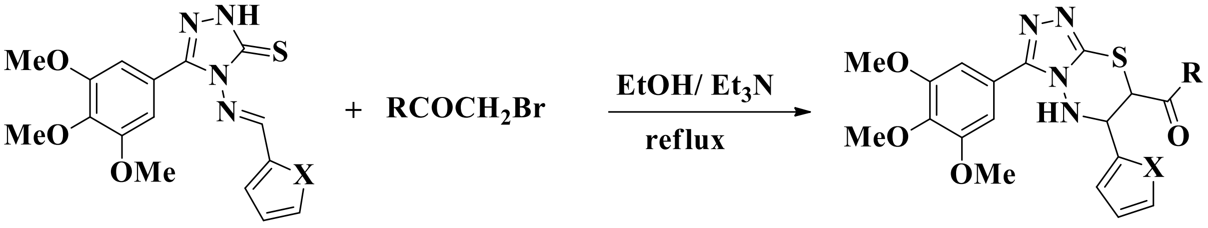
**11**

Altıntop[38] *et al.* synthesized triazolothiadiazine derivatives (**12**) by condensing 4-amino-5-substituted-2,4-dihydro-3*H*-1,2,4-triazol-3-thiones with 2-bromo-1-phenylethanones in ethanol. One of the compounds showed potent anticandidal activity.



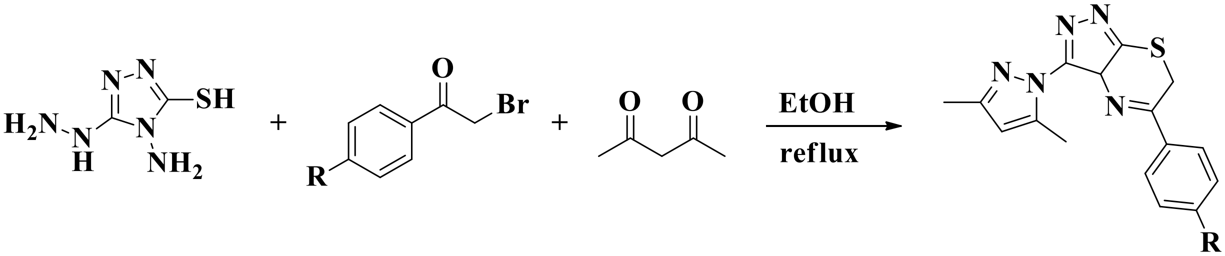
**12**

6-(Furan-2-yl)-3-(3,4,5-trimethoxyphenyl)-6,7-dihydro-5*H*-[1,2,4]triazolo[3,4-*b*][1,3,4] thiadiazin-7-yl)(phenyl)methanones[39] (**13**) were synthesized by taking 4-((furan / thiophene-2-ylmethylene)amino)-(3,4,5trimethoxyphenyl)-1*H*-1,2,4-triazole-5(4*H*)-thione and various α-bromoketones in ethanol and triethylamine. All the synthesized compounds were screened for their anticancer activity and few compounds shown good antiproliferative activity.



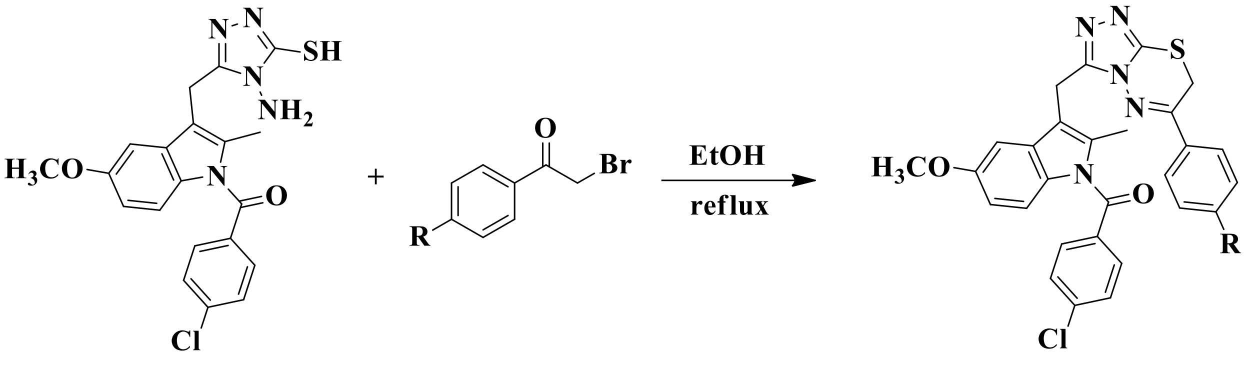
**13**

Tewodros and Rao[40] reported the synthesis of triazolothiadiazinyl pyrazolone derivatives (**14**) by the reaction of 4-amino-5-hydrazinyl-4*H*-1,2,4-triazole-3-thiol, various substituted phenacyl bromides and acetylacetone in dryalcohol.



**14**

Sever *et al.*[41] reported the synthesis and anticancer activity of (**15**). 4-amino-5-[(5-methoxy-2-methyl-1-(4-chlorobenzoyl)-1*H*-indol-3-yl)methyl]-2,4-dihydro-3*H*-1,2,4-triazol-3-thione reacts with various 2-bromo-1-phenylethanones in dry alcohol.

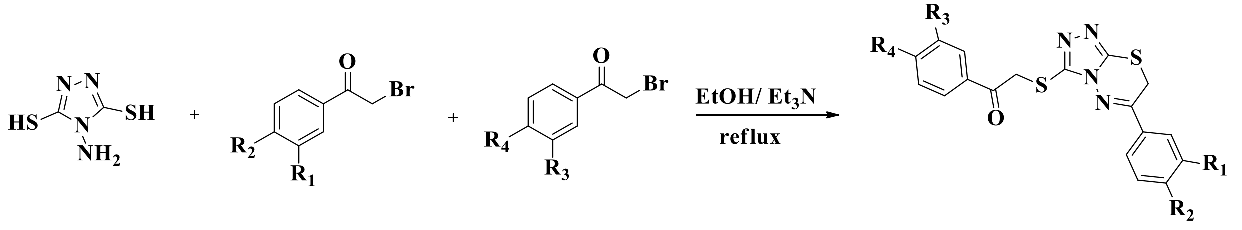


15

## PRESENT WORK

Based on the above mentioned synthetic and therapeutic importance, triazole scaffold would serve as a privileged structure due to their prevalence in anticancer agents and other biologically active compounds prompted by the latest developments in the field of [1,2,4]triazole and thiadiazine moieties as part of our continuous efforts to search for new biologically active compounds, we have planned to fuse two active moieties with an intention to afford better biological activity[42]. The synthetic route for the compounds 20 (a-l) was shown in the scheme-1.

The gist of the literature survey provided in the introduction clearly reveals the physiological and synthetic importance of 1,3,4-thiadiazines. Therefore, it was thought worth while to take up the synthesis of novel 1-Phenyl-2-((6-phenyl-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-3-yl)thio)ethanones and their anticancer activity. The title compounds 20 (a-l) have been synthesized by condensation of 4-amino-4*H*-1,2,4-triazole-3,5-dithiol (**17**) with various 2-bromo-1-phenylethanones (18 a-l & 19 a-l) under refluxing in ethanol in presence of $Et_3N$ as shown in scheme-1. The specialty of the reaction is that, there is simultaneous formation of two C-S and one C=N bond with high atom economy.

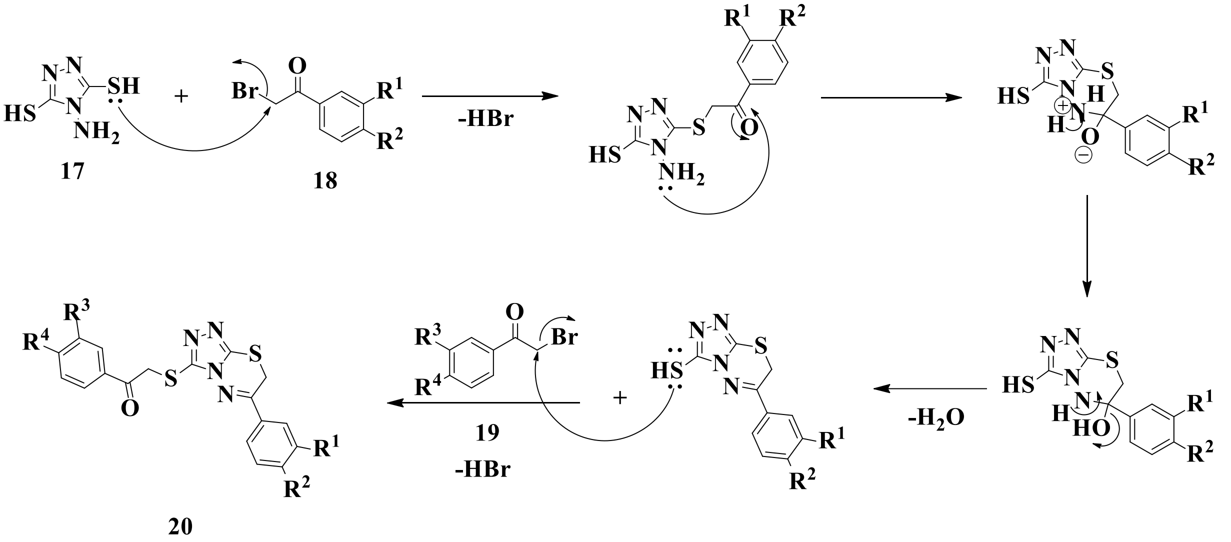


17 18 (a-l) 19 (a-l) 20 (a-l)

**Scheme-1.** Synthesis of **20 (a-l)**.

## Mechanism

The mechanisam of formation of compound 20 (a-l) has been established and is shown in the fallowing **scheme-2**.



**Scheme-2.** Plausible mechanism for formation of title compounds **20 (a-l)**.

From the above mechanism it is evident that the bromine atom of phenacyl bromide was substituted by thiol group of 17 to yield an open chain α- thioketone followed by cyclo condensation to give the 6-phenyl-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazine-3-thiol compound. This on further reaction with another equivalent of phenacyl bromide yielded the final compound 20.

Initially, we have optimized the reaction between 4-amino-4*H*-1,2,4-triazole-3,5-dithiol 17 (1 eq), 2-bromo-1-phenylethan-1-ones 18a (1 eq) and 19a (1 eq) by using different solvents and different catalysts. From the results, it was observed that the best reaction condition in terms of reaction yields and time to carry the reaction is by ethanol as solvent and triethylamine (0.1eq) as a base. The results are summarized in Table-1.

To check the generality of the reaction condition in ethanol and triethylamine (0.1 eq), a variety of substituted 2-bromo-1-phenylethan-1-ones such as methyl, methoxy, fluoro, chloro, bromo, nitro, dichloro, and biphenyl were utilized to react with 4-amino-4*H*-1,2,4-triazole-3,5-dithiol under similar optimized condition (Scheme-1). The reaction time for title compounds 20 (a-l) were shown in Table-2.

Table-1. Optimized reaction conditions for the synthesis of compound 20a.

| S. No. | Solvent | Catalyst | Time (h) | Yield (%) |
|---|---|---|---|---|
| 1 | methanol | - | 8 | 40 |
| 2 | ethanol | - | 8 | 46 |
| 3 | methanol | NaOH | 5 | 62 |
| 4 | ethanol | NaOH | 4 | 69 |
| 5 | methanol | Triethylamine | 5 | 76 |
| 6 | ethanol | Triethylamine | 4 | 80 |

Table-2. Reaction time of compounds 20 (a-l)

| **S. No.** | **Compound** | **$R^1$** | **$R^2$** | **$R^3$** | **$R^4$** | **Time (h)** |
|---|---|---|---|---|---|---|
| 1 | 20a | H | H | H | H | 4 |
| 2 | 20b | H | $CH_3$ | H | $CH_3$ | 3.5 |
| 3 | 20c | H | $OCH_3$ | H | $OCH_3$ | 3 |
| 4 | 20d | H | F | H | F | 6 |
| 5 | 20e | H | Cl | H | Cl | 5.5 |
| 6 | 20f | H | Br | H | Br | 5 |
| 7 | 20g | Cl | Cl | Cl | Cl | 9 |
| 8 | 20h | H | Ph | H | Ph | 5 |
| 9 | 20i | H | $NO_2$ | H | $NO_2$ | 9 |
| 10 | 20j | H | H | H | F | 5 |
| 11 | 20k | H | $OCH_3$ | H | F | 6 |
| 12 | 20l | H | Cl | H | F | 8 |

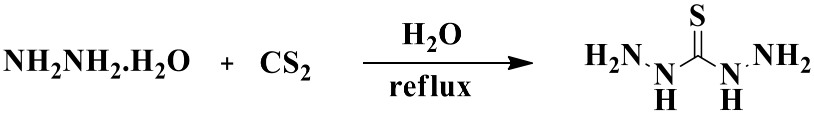
The $^1$H NMR spectrum of compound 20a exhibited characteristic peaks at $\delta$ 4.27 (s, 2H, thiadiazine methylene), 5.01 (s, 2H, -S-$CH_2$), the aromatic protons appeared in the range of $\delta$ 7.51-8.06 ppm. The $^{13}$C NMR spectrum of **20a** exhibited characteristic peaks at $\delta$ 23.88 ppm corresponds to thiadiazine –$C_6$ carbon, S-$CH_2$ carbon merging in DMSO-$d_6$ peaks and $\delta$ 192.82 ppm represents the carbonyl carbon. The mass spectrum of compound **20d** exhibited 367.15 as $[M+H]^{+\cdot}$ peak.

## EXPERIMENTAL SECTION

One of the starting materials 4-amino-4*H*-1,2,4-triazole 3,5-dithiol (**17**) was prepared by following procedure[43] and various substituted phenacyl bromides were procured from commercial source.

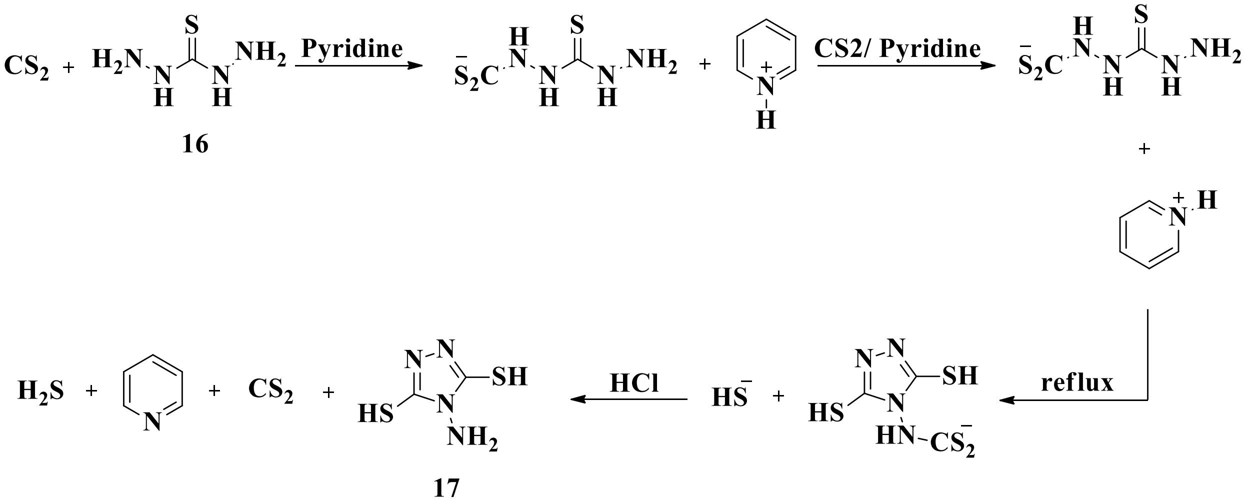
### Preparation of 4-amino-4*H*-1,2,4-triazole 3,5-dithiol (17)

It was prepared in two steps. In the first step, thiocarbohydrazide (**16**) was prepared[44] by reaction of hydrazine hydrate and carbon disulfide in water.



**16**

In second step thiocarbohydrazide (**16**) reacts with carbon disulfide in pyridine to give yellow pyridinium salt of 4-amino-4*H*-1,2,4-triazole 3,5-dithiol. This salt was neutralized by addition of 4N Conc. HCl which is dissolved in hot water to give the final product (**17**).



### General procedure for the synthesis of compounds 20 (a-i)

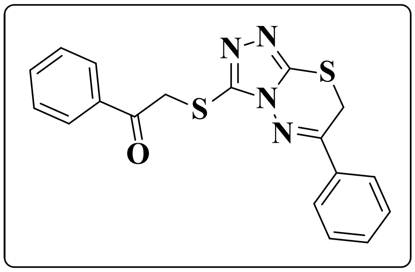
A mixture of 4-amino-4*H*-1,2,4-triazole-3,5-dithiol (**17** 1 mmol), appropriate phenacyl bromide (**18 a-i & 19 a-i,** 2 mmol), ethanol (5ml) and triethylamine (0.1 mmol)was taken in a round bottom flask. The reaction mixture was heated to 60°C and the progress of the reaction was checked by TLC. After completion of the reaction, the reaction mixture was cooled to room temperature and the solid separated was filtered, washed with ethanol, dried and recrystallized from ethanol.

### General procedure for the synthesis of compounds 20 (j-l)

A mixture of 4-amino-4*H*-1,2,4-triazole-3,5-dithiol (**17** 1mmol), appropriate phenacyl bromide (**18 j-l,** 1 mmol), ethanol (5ml) and triethylamine (0.1 mmol) were heated to 60°C and the progress of the reaction was checked by TLC. Then various substituted phenacyl bromides (**19 j-l,** 1 mmol) were added and the progress of the reaction was checked by TLC. After completion of the reaction, the reaction mixture was cooled to room temperature and the solid separated was filtered, washed with ethanol, dried and recrystallized from ethanol.

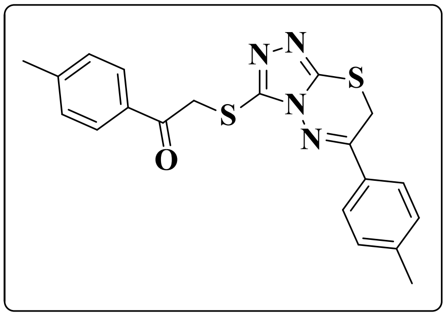
## SPECTRAL DATA

### 1-Phenyl-2-((6-phenyl-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-3-yl)thio)ethanone (20a)



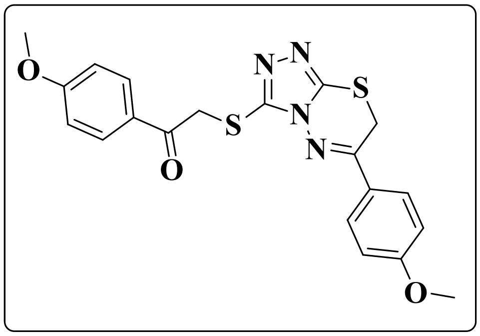
Color: yellow solid, mp**:** 256-258 °C, yield: (0.292g, 80%), FT-IR (KBr, $\nu_{max}$ cm$^{-1}$): 1672 (C=O), 1597 (C=N), **$^1$H NMR (400MHz, CDCl$_3$):** $\delta$ 4.27(s, 2H, thiadiazine methylene), 5.01(s, 2H, S-CH$_2$), 7.51-7.67(m, 6H, Ar-H), 7.98(d, *J* =7.6Hz, 2H, Ar-H), 8.06 (d, *J* = 8Hz, 2H, Ar-H) ppm, **$^{13}$C NMR (100MHz, CDCl$_3$ & DMSO-d$_6$):** $\delta$ 23.88, 127.58, 128.53, 128.92, 129.11, 129.63, 132.24, 133.17, 134.04, 135.19, 142.38, 154.61, 192.82 ppm; **MS (ESI) *m/z* (%):** 367.15 [M+H]$^+$; Anal. Calcd. For $C_{18}H_{14}N_4OS_2$; C, 59.00; H, 3.85; N, 15.29. Found: C, 58.96; H, 3.81; N, 15.34%.

### 1-(p-Tolyl)-2-((6-(p-tolyl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-3-yl)thio)ethanone (20b)



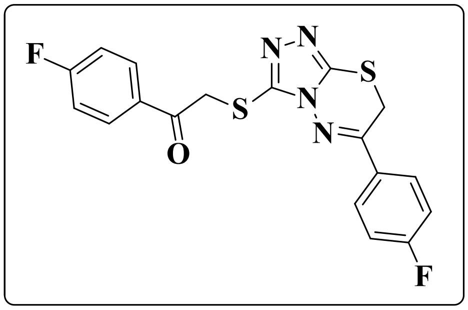
Color: yellow solid, mp**:** 237-239 °C, yield: (0.327g, 83%), FT-IR (KBr, $\nu_{max}$ cm$^{-1}$): 1677 (C=O), 1604 (C=N), **$^{1}$H NMR (400MHz, CDCl$_3$ & DMSO-*d*$_6$):** $\delta$ 2.45 (s, 6H, 2-methyl), 4.26 (s, 2H, thiadiazine methylene), 5.00 (s, 2H, S-CH$_2$), 7.31-7.34 (m, 4H, Ar-H), 7.88(d, $J$ = 8Hz, 2H, Ar-H), 7.96(d, $J$ = 8Hz, 2H, Ar-H) ppm, **$^{13}$C NMR (100MHz, CDCl$_3$ & DMSO-*d*$_6$):** $\delta$ 21.57, 21.75, 23.74, 127.57, 128.64, 129.28, 129.56, 129.78, 129.82, 130.07, 132.56, 142.64, 143.17, 145.07, 154.87, 192.19 ppm; **MS (ESI) *m/z* (%):** 395.20 [M+H]$^+$; Anal. Calcd. For $C_{20}H_{18}N_4OS_2$; C, 60.89; H, 4.60; N, 14.20. Found: C, 60.92; H, 4.56; N, 14.24%.

### 1-(4-Methoxyphenyl)-2-((6-(4-methoxyphenyl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-3-yl)thio)ethanone (20c)



Color: yellow solid, mp**:** 195-197 °C, yield: (0.341g, 80%), FT-IR (KBr, $\nu_{max}$ cm$^{-1}$): 1670 (C=O), 1600 (C=N), **$^{1}$H NMR (400MHz, CDCl$_3$ & DMSO-*d*$_6$):** $\delta$ 3.90 (s, 6H, -OCH$_3$), 4.22 (s, 2H, thiadiazine methylene), 4.97 (s, 2H, S-CH$_2$), 6.98-7.03 (m, 4H, Ar-H), 7.96 (d, $J$ = 8.4Hz, 2H, Ar-H), 8.04 (d, $J$ = 8.4Hz, 2H, Ar-H) ppm, **$^{13}$C NMR (100MHz, CDCl$_3$ & DMSO-*d*$_6$):** $\delta$ 23.57, 55.64, 114.13, 114.53, 125.20, 128.13, 129.58, 130.95, 132.01, 142.59, 154.53, 162.95, 164.17, 191.08 ppm; **MS (ESI) *m/z* (%):** 427.20 [M+H]$^+$; Anal. Calcd. For $C_{20}H_{18}N_4O_3S_2$; C, 56.32; H, 4.25; N, 13.14. Found: C, 56.38; H, 4.21; N, 13.19%.

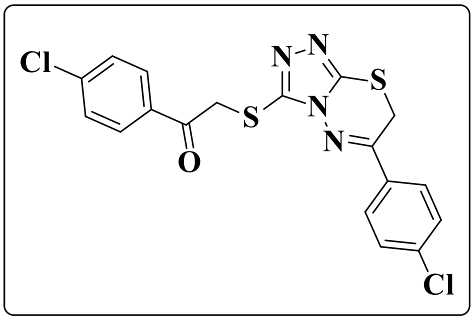
### 1-(4-Fluorophenyl)-2-((6-(4-fluorophenyl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-3-yl) thio)ethanone (20d)



Color: yellow solid, mp**:** 216-217 °C, yield: (0.346g, 86%), FT-IR (KBr, $\nu_{max}$ cm$^{-1}$): 1682 (C=O), 1596 (C=N), **$^{1}$H NMR (400MHz, CDCl$_3$ & DMSO-*d*$_6$):** $\delta$ 4.11 (s, 2H, thiadiazine methylene), 4.95 (s, 2H, -S-CH$_2$), 7.17-7.24 (m, 4H, Ar-H), 7.99 (d, $J$ = 7.6Hz, 2H, Ar-H), 8.12 (d, $J$ = 7.6Hz, 2H, Ar-H) ppm, **$^{13}$C NMR (100MHz, CDCl$_3$ &**

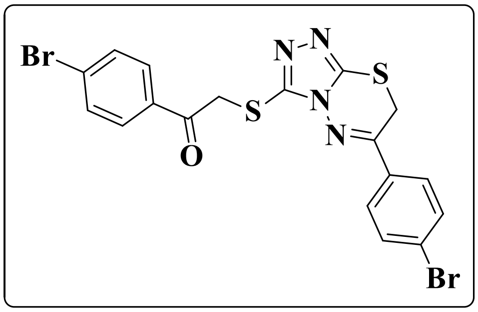
**DMSO-$d_6$):** $\delta$ 23.80, 39.63, 115.91, 116.39, 129.35, 129.78, 129.86, 131.30, 131.40, 131.64, 152.90, 163.73, 191.39 ppm; **MS (ESI) *m/z* (%):** 403.15 $[M+H]^+$; Anal. Calcd. For $C_{18}H_{12}F_2N_4OS_2$; C, 53.72; H, 3.01; N, 13.92. Found: C, 53.77; H, 2.95; N, 13.96%.

**1-(4-Chlorophenyl)-2-((6-(4-chlorophenyl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-3-yl)thio)ethanone (20e)**



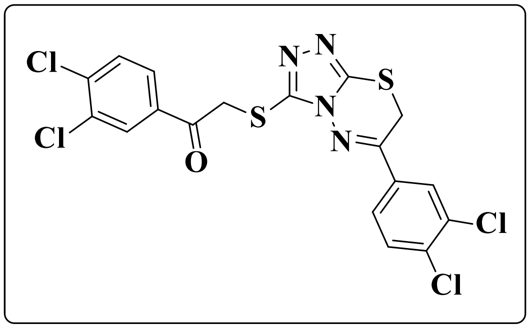
Color: yellow solid, mp**:** 191-193 °C, yield: (0.378g, 87%), FT-IR (KBr, $\nu_{max}$ cm$^{-1}$): 1678 (C=O), 1588 (C=N), **$^1$H NMR (400MHz, CDCl$_3$):** $\delta$ 3.97 (s, 2H, thiadiazine methylene), 4.93 (s, 2H, -S-CH$_2$), 7.47-7.50 (m, 4H, Ar-H), 7.85 (d, *J* = 8Hz, 2H, Ar-H), 8.01 (d, *J* = 8.4Hz, 2H, Ar-H) ppm, **$^{13}$C NMR (100MHz, CDCl$_3$):** $\delta$ 23.77, 39.81, 128.59, 129.24, 129.48, 130.01, 131.56, 133.48, 138.65, 140.64, 141.53, 151.00, 152.12, 191.90 ppm; Anal. Calcd. For $C_{18}H_{12}Cl_2N_4OS_2$; C, 49.66; H, 2.78; N, 12.87. Found: C, 49.62; H, 2.73; N, 12.84%.

**1-(4-Bromophenyl)-2-((6-(4-bromophenyl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-3-yl)thio)ethanone (20f)**



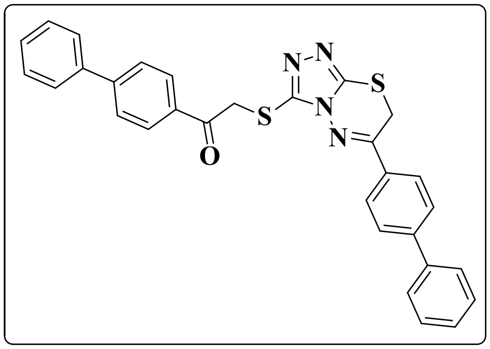
Color: brown solid, mp**:** 235-237 °C, yield: (0.466g, 89%), FT-IR (KBr, $\nu_{max}$ cm$^{-1}$): 1680 (C=O), 1583 (C=N), **$^1$H NMR (400MHz, CDCl$_3$ & DMSO-$d_6$):** $\delta$ 4.06 (s, 2H, thiadiazine methylene), 4.90 (s, 2H, -S-CH$_2$), 7.64-7.68 (m, 4H, Ar-H), 7.81 (d, *J* = 7.2Hz, 2H, Ar-H), 7.93 (d, *J* = 7.6Hz, 2H, Ar-H) ppm, **$^{13}$C NMR (100MHz, CDCl$_3$ & DMSO-$d_6$):** $\delta$ 23.63, 39.52, 126.91, 128.84, 129.20, 130.04, 132.13, 132.30, 133.90, 141.79, 150.62, 152.66, 192.05 ppm; **MS (ESI) *m/z* (%):** 525.05 $[M+H]^+$; Anal. Calcd. For $C_{18}H_{12}Br_2N_4OS_2$; C, 41.24; H, 2.31; N, 10.69. Found: C, 41.20; H, 2.35; N, 10.65%.

**1-(3,4-Dichlorophenyl)-2-((6-(3,4-dichlorophenyl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-3-yl)thio)ethanone (20g)**



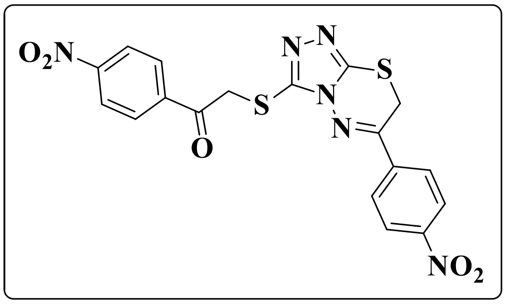
Color: brown solid, mp**:** 247-249 °C, yield: (0.463g, 91%), FT-IR (KBr, $\nu_{max}$ cm$^{-1}$): 1688 (C=O), 1582 (C=N), **$^1$H NMR (400MHz, CDCl$_3$):** $\delta$ 3.96 (s, 2H, thiadiazine methylene), 4.88 (s, 2H, -S-CH$_2$), 7.60 (d, *J* = 8.8 Hz, 2H, Ar-H), 7.74 (d, *J* = 8.8 Hz, 1H, Ar-H), 7.90 (d, *J* = 8 Hz, 1H, Ar-H), 7.99 (s, 1H, Ar-H), 8.14 (s, 1H, Ar-H) ppm, **$^{13}$C NMR (100MHz, CDCl$_3$):** $\delta$ 23.71, 39.55, 126.27, 127.60, 129.10, 130.51, 131.04, 131.18, 132.92, 133.73, 133.88, 134.67, 136.84, 138.80, 141.51, 150.85, 151.09, 190.95 ppm; **MS (ESI) *m/z* (%):** 505.05 [M+H]$^+$; Anal. Calcd. For $C_{18}H_{10}Cl_4N_4OS_2$; C, 42.88; H, 2.00; N, 11.11. Found: C, 42.84; H, 2.12; N, 11.15%.

### 1-([1,1'-Biphenyl]-4-yl)-2-((6-([1,1'-biphenyl]-4-yl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4] thiadiazin-3-yl)thio)ethanone (20h)



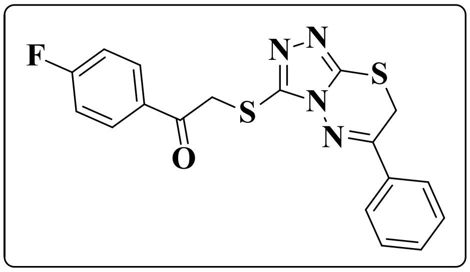
Color: red solid, mp**:** 211-213 °C, yield: (0.472g, 89%), FT-IR (KBr, $\nu_{max}$ cm$^{-1}$): 1678 (C=O), 1600 (C=N), **$^1$H NMR (400MHz, CDCl$_3$ & DMSO-*d*$_6$, ppm):** $\delta$ 4.08 (s, 2H, thiadiazine methylene), 4.90 (s, 2H, -S-CH$_2$), 7.35 (d, *J* = 7.2 Hz, 2H, Ar-H), 7.40 (d, *J* = 7.2 Hz, 2H, Ar-H), 7.55 - 7.57 (m, 5H, Ar-H), 7.64-7.67 (m, 5H, Ar-H), 7.94 (d, *J* = 8 Hz, 2H, Ar-H), 8.05 (d, *J* = 8 Hz, 2H, Ar-H), **$^{13}$C NMR (100MHz, CDCl$_3$ & DMSO-*d*$_6$):** $\delta$ 23.75, 127.07, 127.21, 127.34, 127.52, 127.97, 128.48, 129.03, 129.18, 129.53, 131.87, 133.83, 139.39, 142.05, 144.43, 146.43, 150.61, 153.50, 192.51 ppm; **MS (ESI) *m/z* (%):** 519.30 [M+H]$^+$; Anal. Calcd. For $C_{30}H_{22}N_4OS_2$; C, 69.47; H, 4.28; N, 10.80. Found: C, 69.43; H, 4.32; N, 10.75%.

### 1-(4-Nitrophenyl)-2-((6-(4-nitrophenyl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-3-yl) thio) ethanone (20i)



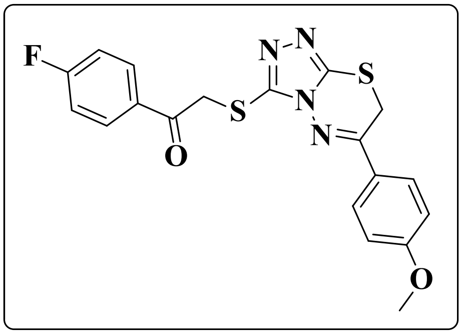
Color: brown solid, mp**:** 196-198 °C, yield: (0.406g, 81%), FT-IR (KBr, $\nu_{max}$ cm$^{-1}$): 1683 (C=O), 1601 (C=N), **$^1$H NMR (400MHz, $CDCl_3$ & DMSO-*$d_6$*):** $\delta$ 4.23 (s, 2H, thiadiazine methylene), 4.91 (s, 2H, -S-$CH_2$), 8.14 (d, *J* = 8.8 Hz, 2H, Ar-H), 8.20 (d, *J* = 8.4Hz, 2H, Ar-H) 8.26 - 8.29 (m, 4H, Ar-H) ppm; **MS (ESI -ve) *m/z* (%)**: 455.15 [M-H]; Anal. Calcd. For $C_{18}H_{12}N_6O_5S_2$; C, 47.36; H, 2.65; N, 18.41. Found: C, 47.40; H, 2.68; N, 18.45%.

### 1-(4-Fluorophenyl)-2-((6-phenyl-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-3-yl)thio) ethanone (20j)



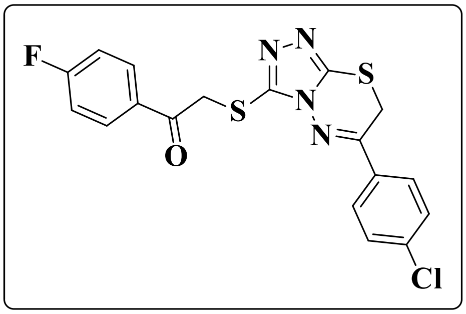
Color: yellow solid, Mp**:** 240-242 °C, yield: (0.311g, 83%), FT-IR (KBr, $\nu_{max}$ cm$^{-1}$): 1683 (C=O), 1601 (C=N), **$^1$H NMR (400MHz, $CDCl_3$ & DMSO-*$d_6$*):** $\delta$ 4.19 (s, 2H, thiadiazine methylene), 4.92 (s, 2H, -S-$CH_2$), 7.43-7.47 (m, 3H, Ar-H), 7.50-7.59 (m, 2H, Ar-H), 7.90 (d, *J* = 7.6Hz, 2H, Ar-H), 7.98 (d, *J* = 7.6Hz, 2H, Ar-H) ppm, Anal. Calcd. For $C_{18}H_{13}FN_4OS_2$; C, 56.24; H, 3.41; N, 14.57. Found: C, 56.28; H, 3.45; N, 14.54%.

### 1-(4-Fluorophenyl)-2-((6-(4-methoxyphenyl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-3-yl) thio)ethanone (20k)



Color: yellow solid, mp**:** 221-223 °C, yield: (0.344g, 90%), FT-IR (KBr, $\nu_{max}$ cm$^{-1}$): 1672 (C=O), 1601 (C=N), **$^1$H NMR (400MHz, $CDCl_3$):** $\delta$ 3.91 (s, 3H, -$OCH_3$), 3.97 (s, 2H, thiadiazine methylene), 4.98 (s, 2H, -S-$CH_2$), 6.97-7.03 (m, 4H, Ar-H), 7.90 (d, *J* = 8 Hz, 2H, Ar-H), 8.08 (d, *J* = 8 Hz, 2H, Ar-H) ppm, **$^{13}$C NMR (100MHz, $CDCl_3$):** $\delta$ 23.69, 40.02, 55.58, 114.03, 114.51, 125.40, 128.22, 129.17, 131.01, 141.59, 151.11, 152.70, 162.86, 164.22, 191.63 ppm; Anal. Calcd. For $C_{19}H_{15}FN_4O_2S_2$; C, 55.06; H, 3.65; N, 13.52. Found: C, 55.10; H, 3.69; N, 13.56%.

**2-((6-(4-Chlorophenyl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-3-yl)thio)-1-(4-fluoro phenyl)ethanone (20l)**



Color: pale yellow solid, mp**:** 232-234 °C, yield: (0.377g, %), FT-IR (KBr, $\nu_{max}$ cm$^{-1}$): 1679 (C=O), 1589 (C=N), **$^1$H NMR (400MHz, $CDCl_3$):** $\delta$ 3.99 (s, 2H, thiadiazine methylene), 4.93 (s, 2H, -S-$CH_2$), 7.47-7.51 (m, 4H, Ar-H), 7.86 (d, *J* = 8 Hz, 2H, Ar-H), 8.02 (d, *J* = 8 Hz, 2H, Ar-H) ppm, **$^{13}$C NMR (100MHz, $CDCl_3$):** $\delta$ 23.75, 39.79, 40.30, 128.60, 129.22, 130.00, 131.56, 133.48, 138.61, 140.60, 141.57, 150.94, 152.21, 191.87 ppm; Anal.Calcd. For $C_{18}H_{12}ClFN_4OS_2$; C, 51.61; H, 2.89; N, 13.38. Found: C, 51.57; H, 2.93; N, 13.34%.

## BIOLOGICAL ACTIVITY

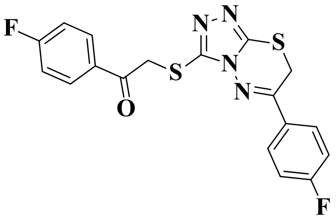
### *In vitro* anticancer assay

From all the synthesized compounds a few selected compounds were screened for their primary anticancer activity at 10 µM concentration in accordance with the National Cancer Institute (NCI), Bethesda, USA of Drug Evaluation Branch. The human tumor cell lines were grown in Roswell Park Memorial Institute (RPMI) 1640 medium having 5% fetal bovine serum and L- glutamine (2 mM). The tumor cells were inoculated in a 96 well plate. The microtiter plates were incubated at 37 °C, in 5% $CO_2$ environment for about 24 h. After incubation of the cells, the target compounds were added to the cell culture at single dose concentration (10 µM) and the cultures were incubated for 48 h. After incubation sulforhodamine B (SRB) protein binding dye was added and absorbance values were measured. The cell percentage growth was calculated for the test compounds with respect to untreated control cells.

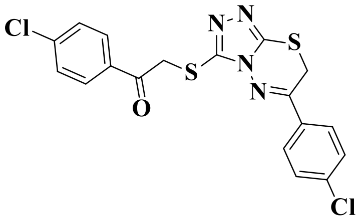
### *In vitro* anticancer activity results and discussion

All the synthesized compounds 20 (a-l) were evaluated for their *in vitro* anticancer activity against 60 human cancer cell lines comprising leukemia (6 sub-panels), non-small cell lung cancer (9 sub-panels), colon cancer (7 sub-panels), CNS cancer (6 sub-panels), melanoma (9 sub-panels), ovarian (7 sub-panels), renal (8 sub-panels), prostate cancer (2 sub-panels) and breast cancer (6 sub-panels) at $10^{-5}$ M concentration and results were given as growth percent as shown in table-3.

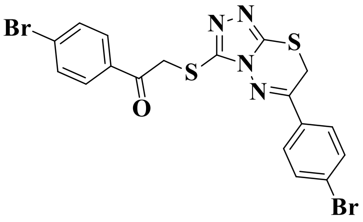
Among all the synthesized compounds, compound **20f** showed significant activity on renal cancer OU-31 (46.76% growth, 53.24% inhibition), moderate activity against non-small cell lung cancer A549/ATCC (58.58% growth, 41.42% inhibition), leukemia MOLT-4 (59.25% growth, 40.75% inhibition). Compound **20d** showed significant activity on renal cancer OU-31 (47.42% growth, 52.58% inhibition), moderate activity against melanoma UACC-257 (51.77% growth, 48.23% inhibition), non-small cell lung cancer HOP-92 (57.77% growth, 42.23% inhibition). Compound **20l** showed significant activity on renal cancer OU-31 (48.14% growth, 51.86% inhibition), leukemia MOLT-4 (49.82% growth, 50.18% inhibition), moderate activity against ovarian cancer IGROV1 (57.48% growth, 42.52% inhibition), breast cancer MCF7 (59.20% growth, 40.80% inhibition). Compound **20e** showed significant activity on ovarian cancer SK-OV-3 (50.40% growth, 49.60% inhibition), moderate activity against renal cancer UO-31 (54.64% growth, 45.36% inhibition), non-small cell lung cancer HOP-62 (55.46% growth, 44.54% inhibition).



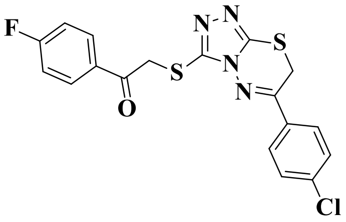
**(20d)**



**(20e)**



**(20f)**



**(20l)**

The compounds that have anticancer activity.

Table-3 *In vitro* anticancer activity of the title compounds.

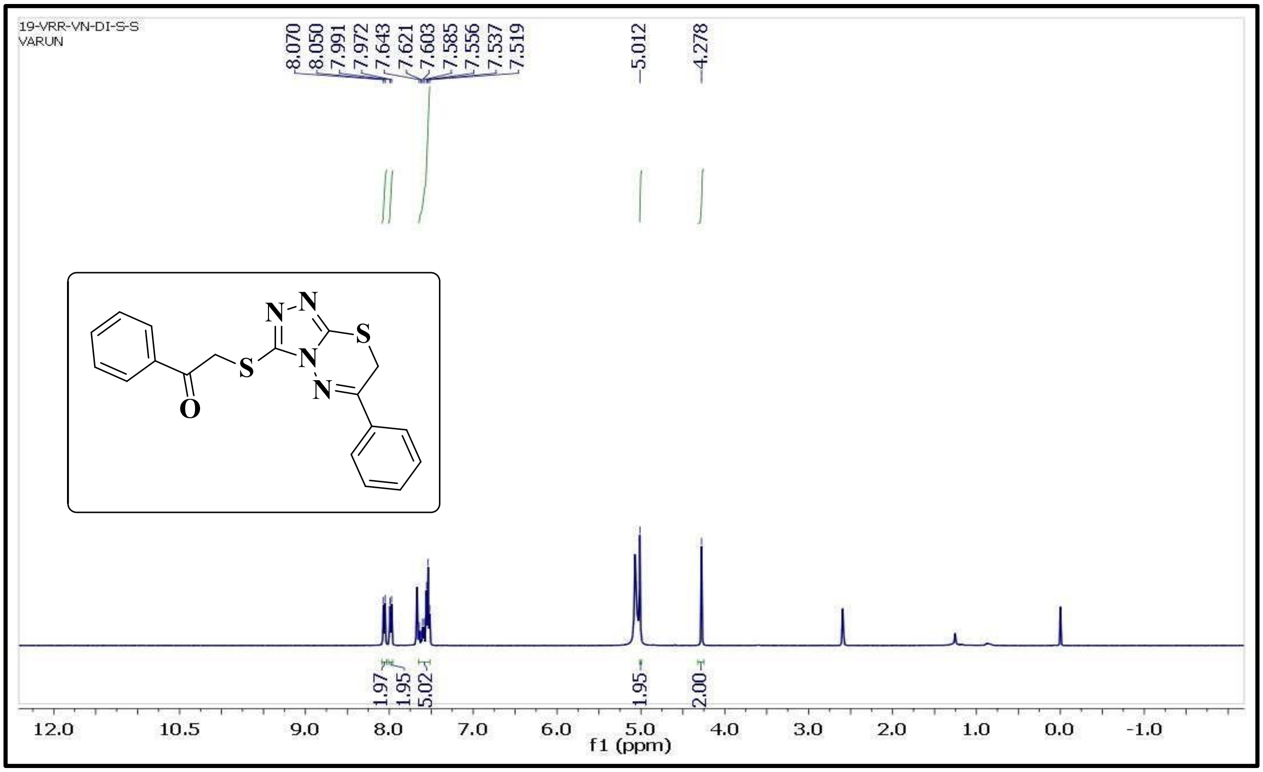
| | Growth percentage (%) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Cancer cell line | 20a | 20b | 20c | 20d | 20e | 20f | 20g | 20h | 20i | 20j | 20k | 20l |
| **Leukemia** | | | | | | | | | | | | |
| MOLT-4 | 75.51 | 73.60 | 85.91 | 61.48 | 61.40 | **59.25** | 84.01 | 83.98 | 105.12 | 75.68 | 73.01 | **49.82** |
| HL-60(TB) | 87.42 | 82.03 | 87.40 | 67.08 | 75.92 | 68.40 | 85.43 | 102.20 | 101.47 | 85.82 | 72.66 | 72.66 |
| **Non-small cell lung cancer** | | | | | | | | | | | | |
| A549/ATCC | 101.55 | 85.55 | 98.86 | 71.27 | 63.00 | **58.58** | 86.38 | 104.59 | 91.28 | 98.19 | 93.55 | 82.22 |
| HOP-62 | 92.44 | 102.24 | 93.40 | 65.16 | **55.46** | 70.69 | 70.55 | 102.83 | 96.39 | 94.16 | 78.40 | 88.73 |
| HOP-92 | 87.49 | 105.95 | 90.99 | **57.61** | 76.24 | 78.13 | 97.55 | 106.91 | 87.26 | 90.17 | 90.29 | 87.15 |
| **Melanoma** | | | | | | | | | | | | |
| UACC-257 | 96.32 | 96.43 | 104.28 | **51.77** | 74.66 | 68.66 | 95.52 | 105.58 | 98.28 | 98.47 | 93.30 | 88.31 |
| **Ovarian cancer** | | | | | | | | | | | | |
| IGROV1 | 88.77 | 78.26 | 82.54 | 66.39 | 64.95 | 62.20 | 92.75 | 90.33 | 105.16 | 81.69 | 83.12 | **57.48** |
| SK-OV-3 | 105.97 | 101.00 | 100.48 | 77.55 | **50.40** | 69.20 | 71.30 | 100.63 | 92.61 | 105.56 | 82.93 | 94.08 |
| **Renal cancer** | | | | | | | | | | | | |
| UO-31 | 83.56 | 69.86 | 82.88 | **47.42** | **54.64** | **46.76** | 68.04 | 70.99 | 69.62 | 73.67 | 76.36 | **48.14** |
| **Prostate cancer** | | | | | | | | | | | | |
| PC-3 | 97.67 | 100.62 | 101.89 | 68.22 | 68.51 | 62.64 | 82.98 | 96.80 | 88.75 | 101.96 | 94.66 | 83.60 |
| **Breast cancer** | | | | | | | | | | | | |
| MCF7 | 88.01 | 86.58 | 93.12 | 70.61 | 67.00 | 66.99 | 94.27 | 93.86 | 101.95 | 90.70 | 92.31 | **59.20** |

## Conclusion of anticancer activity

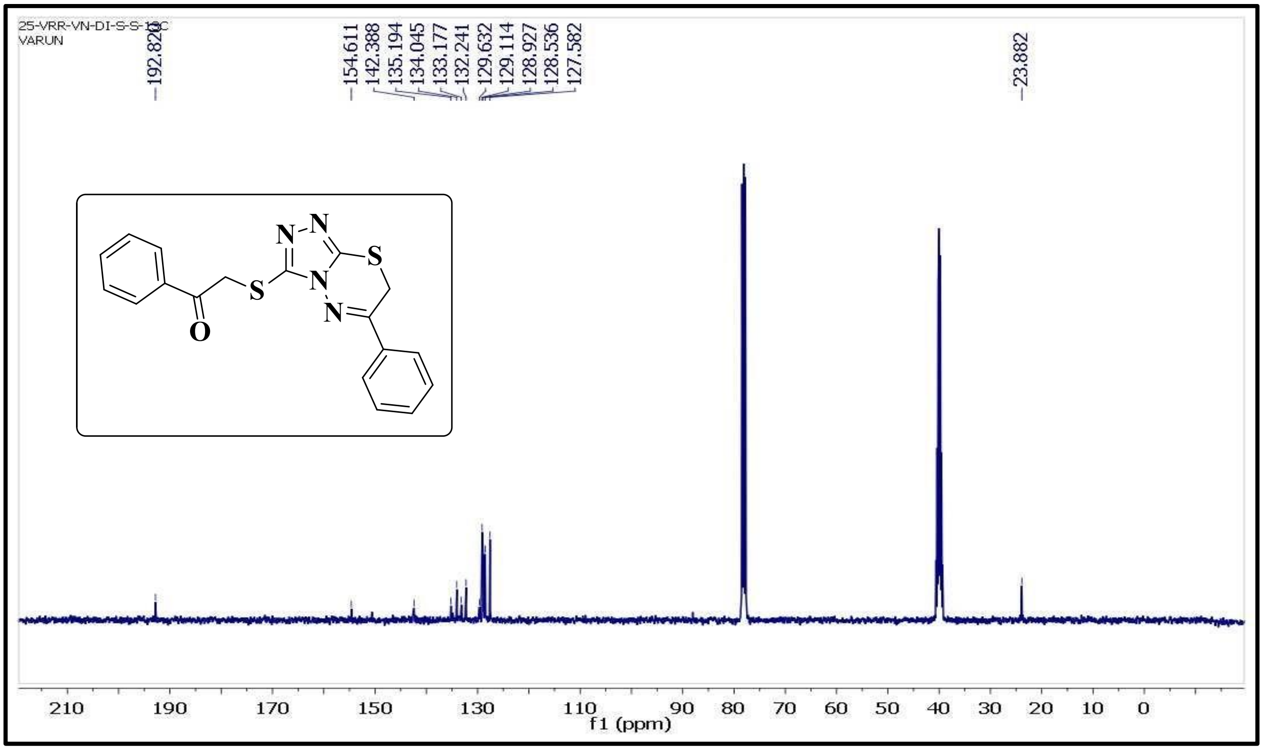
The *in vitro* anticancer activity data clearly reveals the presence of halogen substituents i.e., two fluorine atoms on compound 20d, two chlorine atoms on compound 20e, two bromine atoms on compound 20f, and one fluorine and one chlorine atoms on compound 20l shows significant activity.

## SUMMARY

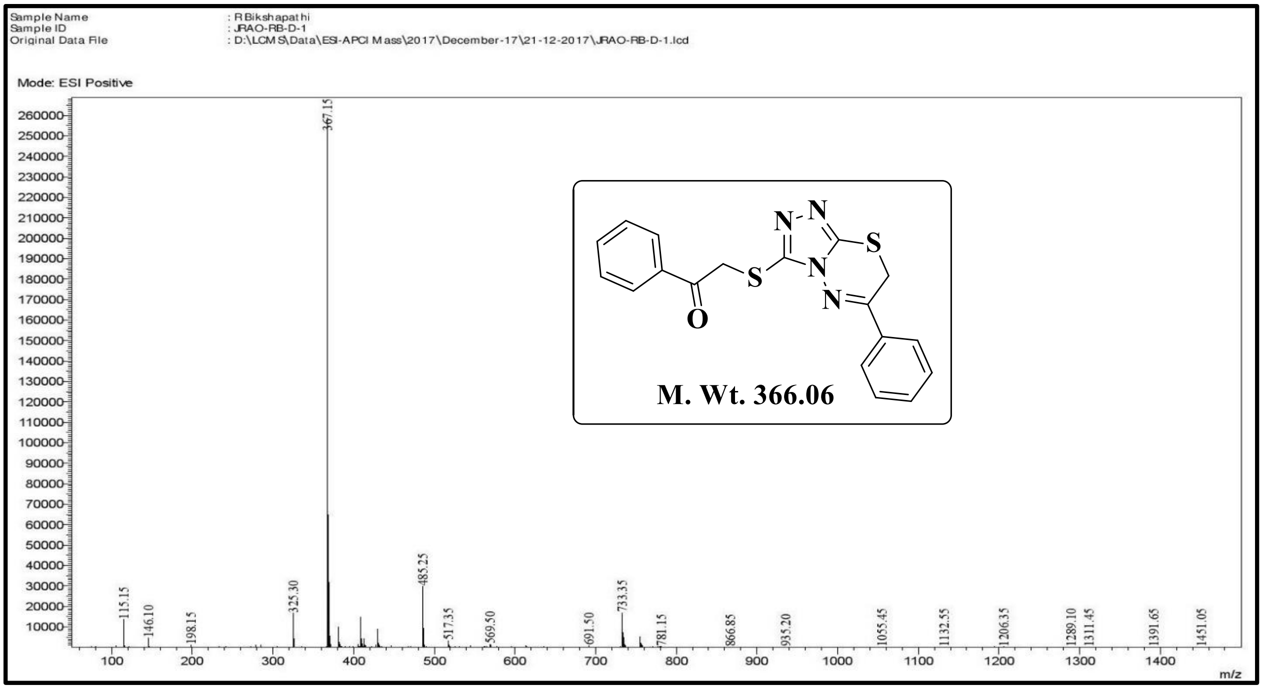
In summary, we have described an efficient synthesis of 1-phenyl-2-((6-phenyl-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazin-3-yl)thio)ethanones via a one-pot multicomponent approach. The method utilized for the synthesis has advantages such as shorter reaction time, easy workup, clean reaction profile and good yields. Compound 4d exhibited significant inhibitory activitie on renal cancer OU-31 and melanoma UACC-257 cell lines, compound 4e on ovarian cancer SKOV-3, compound 4f on renal cancer OU-31, and compound 4l on renal cancer OU-31 and leukemia MOLT-4.



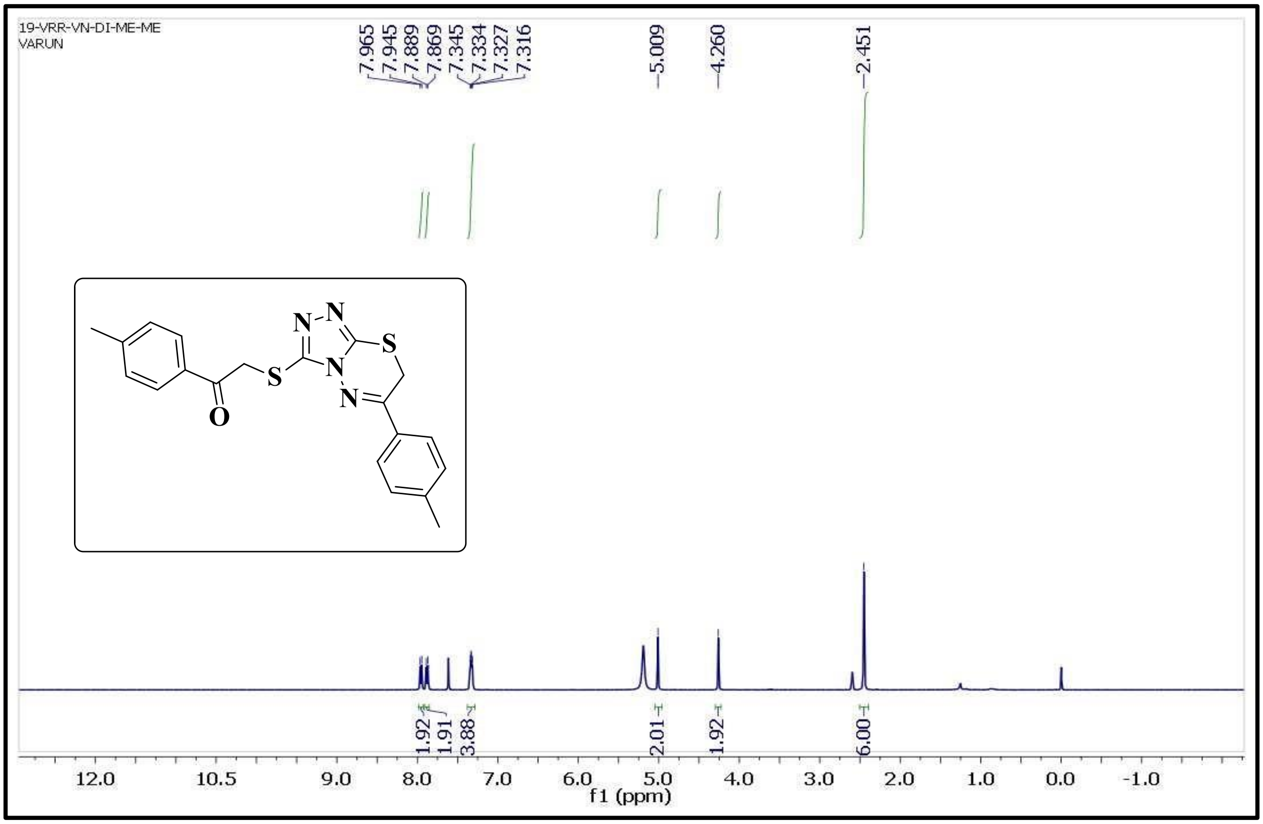
$^{1}$H NMR Spectrum of compound **20a**



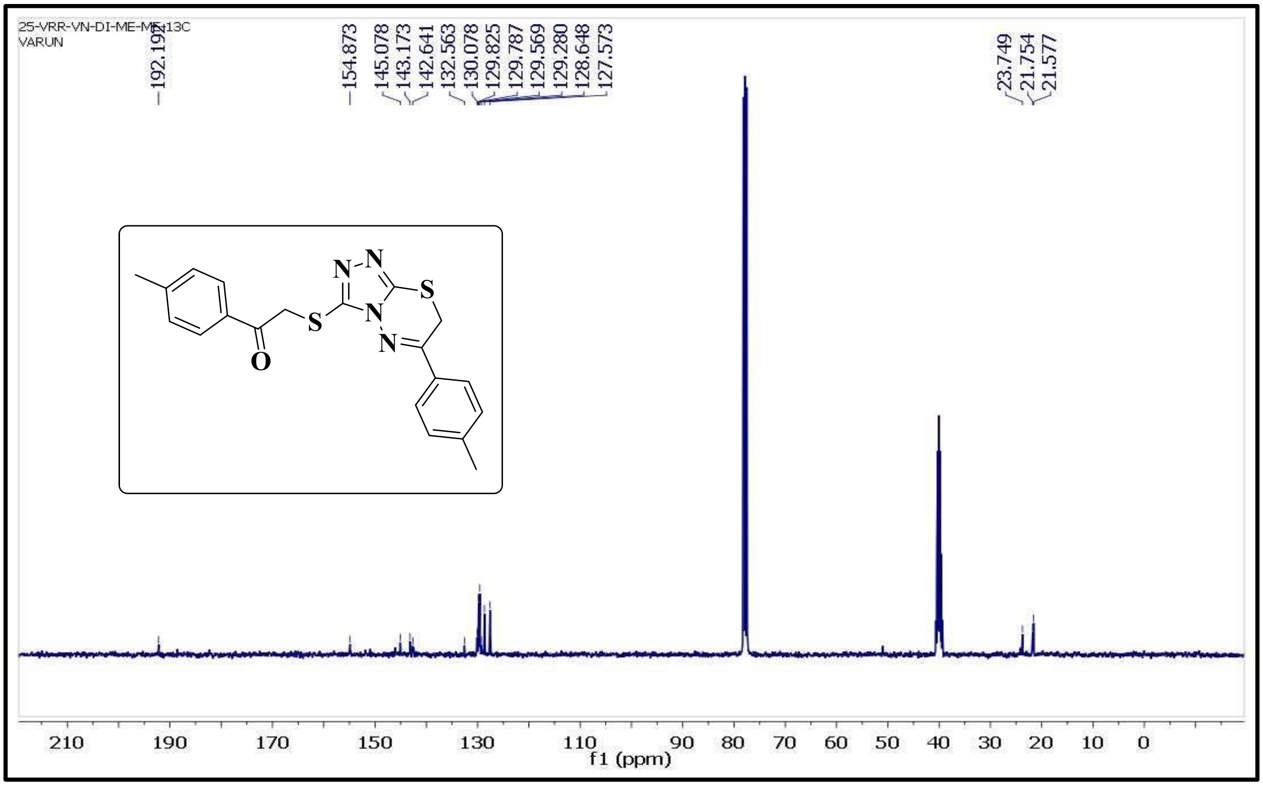
$^{13}$C NMR Spectrum of compound **20a**



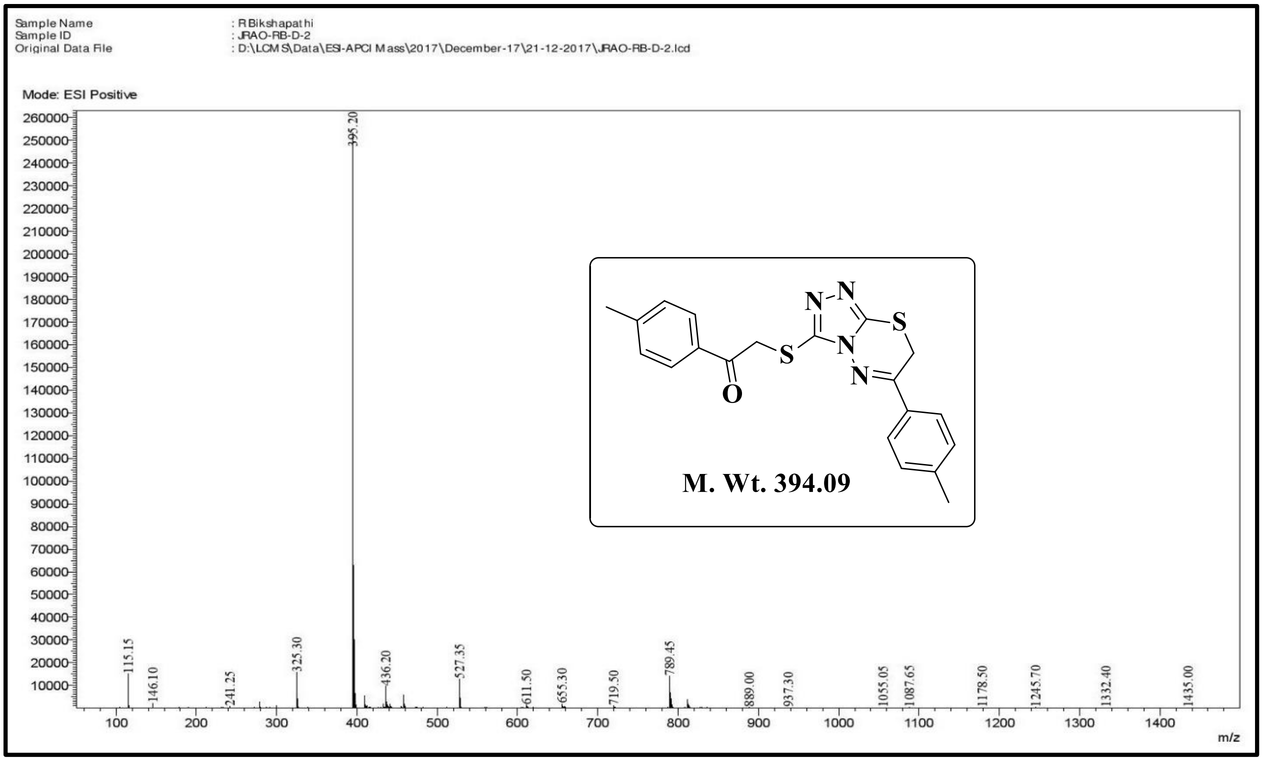
Mass Spectrum of compound **20a**



$^1$H NMR Spectrum of compound **20b**



$^{13}$C NMR Spectrum of compound **20b**



Mass Spectrum of compound **20b**

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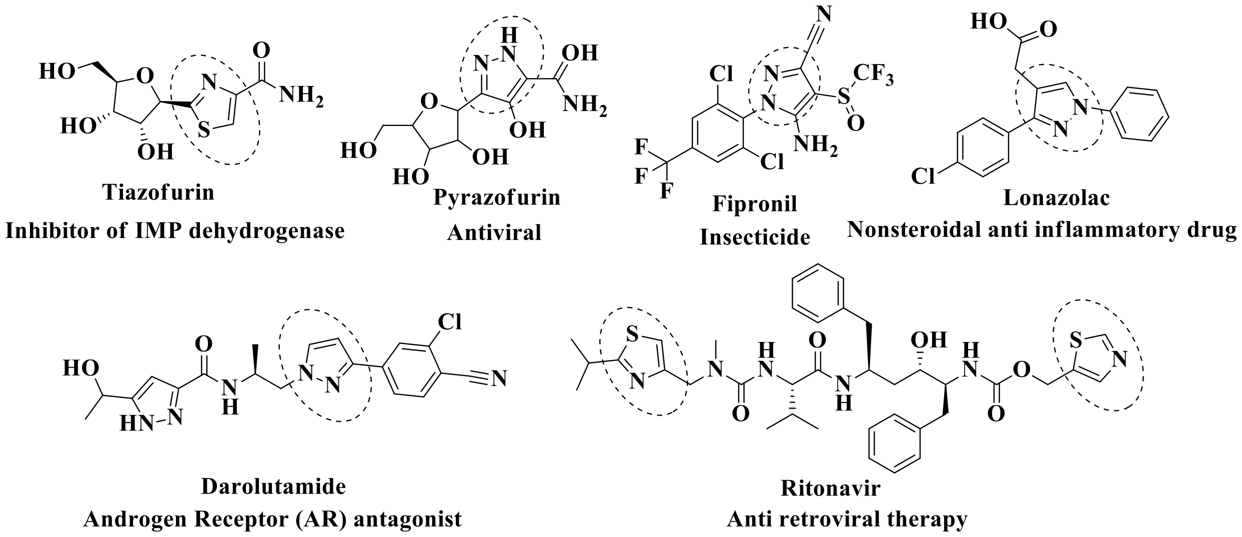
# CHAPTER-III

# ONE-POT SYNTHESIS OF SUBSTITUTED 4-(BENZOFURAN-2-YL)-2-(3-(ARYL/HETERYL)-5-(ARYL/HETERYL)-4,5-DIHYDRO-1*H*-PYRAZOL-1YL)THIAZOLES AND SYNTHESIS OF 3-PHENYL-1-(4-PHENYLTHIAZOL-2-YL)-1*H*-PYRAZOLE-5-AMINES AND THEIR BIOLOGICAL EVALUATION: ANTIVIRAL, ANTIMICROBIAL AND ANTICANCER ACTIVITIES

## INTRODUCTION

Thiazoles are important organic molecules containing sulfur and nitrogen heteroatoms in a five-membered heterocyclic system. Thiazoles are widely distributed in natural and synthetic medicines and have wide therapeutic applications [1-7] and that makes them one of the best-studied compounds. Some of the thiazoles are available as therapeutic drugs in the market these being antineoplastic (Dasatinib, Tiazofurin), anti-inflammatory (Fentiazac, Meloxicam), anti-ulcer (Nizatidine), antifungal (Ravuconazole), antiviral (Ritonavir) and these thiazoles are also widely used in agriculture (insecticides, fungicides and herbicides) and material applications.

Pyrazoles are also well studied heterocyclic compounds with huge therapeutic value [8-16]. Pyrazoles were found to possess antibacterial, antifungal, antiviral, anti-tubercular, antitumor and analgesic activities.  Some of the potential therapeutic agents are shown in figure. 1


**Tiazofurin**
**Inhibitor of IMP dehydrogenase**

**Pyrazofurin**
**Antiviral**

**Fipronil**
**Insecticide**

**Lonazolac**
**Nonsteroidal anti inflammatory drug**

**Darolutamide**
**Androgen Receptor (AR) antagonist**

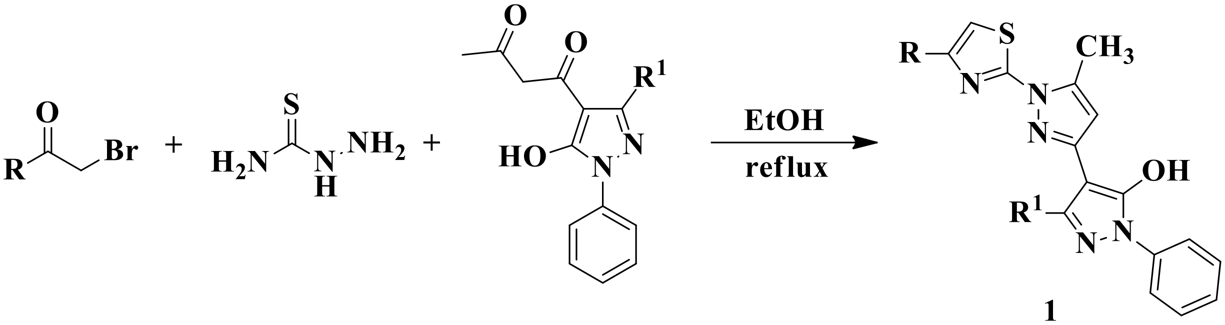
**Ritonavir**
**Anti retroviral therapy**

**Fig. 1**. Some of the pyrazole and thiazole ring containing drug molecules

In recent years, molecular hybridization technique has been used to generate library of compounds with good biological activities. With this intension we have added the pyrazole nucleus to thiazole at second position.

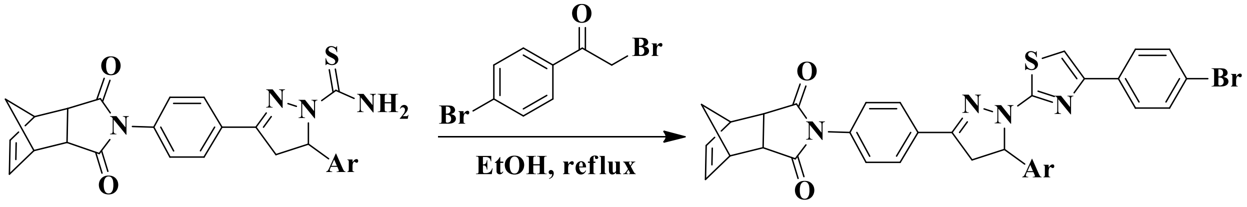
A concise review of the synthesis of pyrazolyl thiazoles and thiazolyl pyrazoles was given below.

Saidoun *et al.*[17] reported the synthesis of 5-methyl-1'-phenyl-1-(thiazol-2-yl)-1*H*,1'*H*-[3,4'-bipyrazol]-5'-ols (**1**) via a three component reaction of α-bromo acetylated pyran-2-ones, thiosemicarbazide, and polysubstituted-1-(1*H*pyrazol-4-yl)butane-1,3-dione in ethanol under reflux conditions.



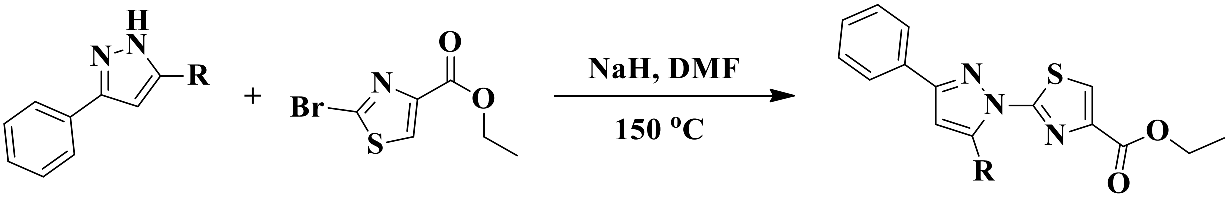
**1**

Budak *et al.*[18] synthesized (3*aR*,4*S*,7*R*,7*aS*)-2-(4-(1-(4-(4-bromophenyl)thiazol-2-yl)-5-phenyl-4,5-dihydro-1*H*-pyrazol-3-yl)phenyl)-3*a*,4,7,7*a*-tetrahydro-1*H*-4,7-methanoisoindole-1,3(2*H*)-dione derivatives (**2**) by the reaction of 2,4'-dibromoacetophenone and 3-(4-((3*aR*,4*S*,7*R*,7*aS*)-1,3-dioxo-3*a*,4,7,7*a*-tetrahydro-1*H*-4,7-methanoisoindol-2(3*H*)-yl)phenyl)-5-phenyl-4,5-dihydro-1*H*-pyrazole-1-carbothio amide.



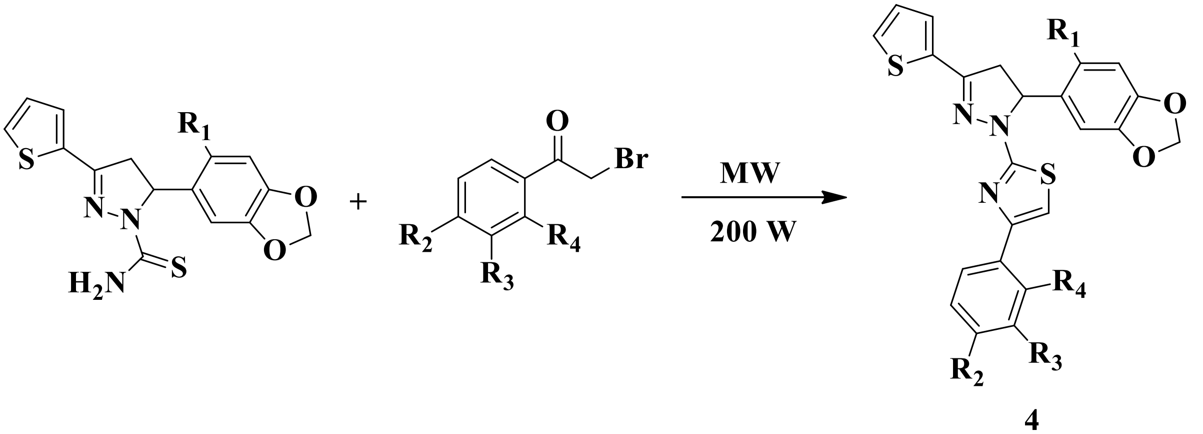
**2**

Atobe *et al.*[19] described the synthetic route to ethyl 2-(3-phenyl-1*H*-pyrazol-1-yl)thiazole-4-carboxylates (**3**) by the reaction of 3-phenyl-1*H*-pyrazoles and ethyl-2-bromo thiazolecarboxylate in the presence of sodium hydride and dimethylformamide.



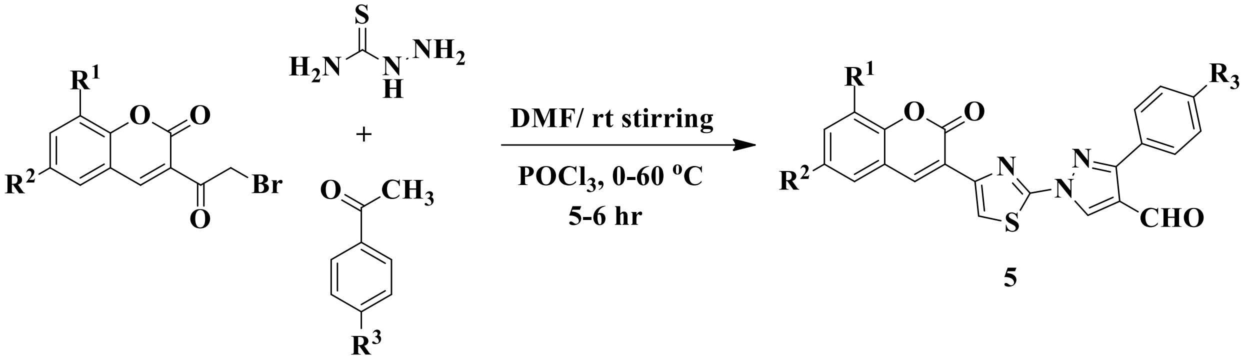
**3**

2-(5-(Benzo[*d*][1,3]dioxol-5-yl)-3-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)-4-phenyl thiazoles (**4**) were reported by Sulthana *et al.*[20]. The reaction of 5-(6-substituted benzo[*d*][1,3]dioxol-5-yl)-3-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazole-1-carbothioamide with various substituted phenacyl bromide in ethanol solvent under microwave conditions.



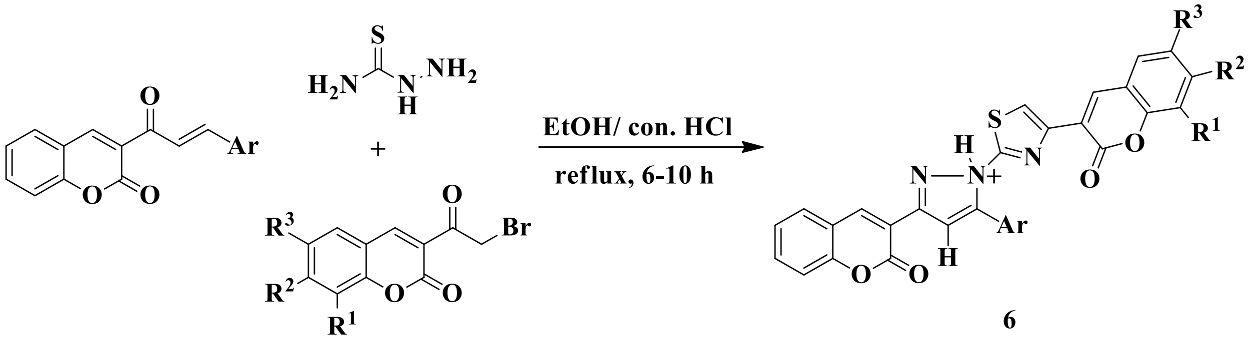
**4**

Vaarla *et al.*[21] described the synthesis of compounds (**5**). 3-(2-Bromoacetyl)-2*H*-1-benzopyran-2-ones on reaction with various substituted acetophenones and thiosemicarbazide under Vilsmeier-Haack reaction condition afforded title compounds.



**5**

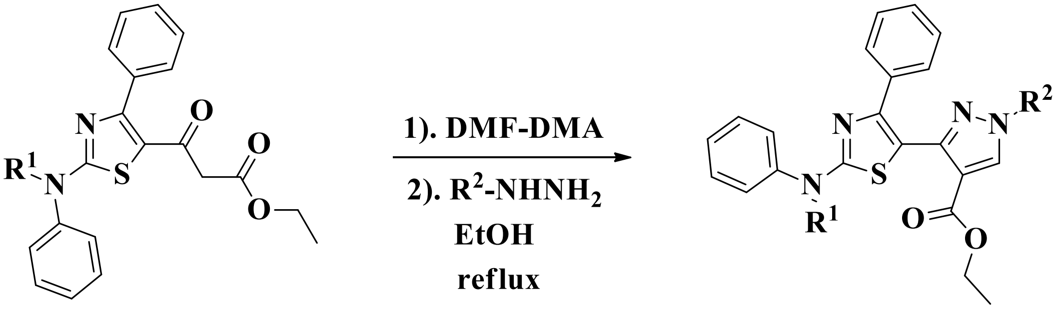
Mahmoodi and Ghodsi[22] reported the synthesis of (**6**) via a three-component cyclocondensation reaction. Various substituted coumarin chalcones react with thiosemicarbazide and substituted 3-(2-bromoacetyl) coumarins in ethanol and catalytic amount of con. HCl to give title compounds.



**6**

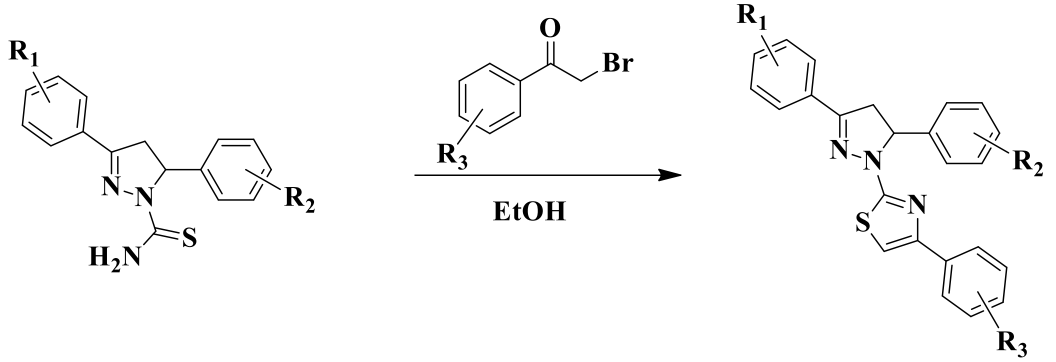
Yuvaraj *et al.*[23] reported the synthesis of 3-(substituted 1,3-thiazol-5-yl)-1-substituted 1*H*-pyrazole-4-carboxylates (**7**) in two steps. In the first step the reaction of *N*-substituted-1-[4-phenyl-

2-(phenylamino)-1,3-thiazol-5-yl] 3-oxo-propionic acid ethyl ester was heated with N, N-dimethylformamide dimethyl acetal (DMF-DMA) then fallowed by reaction with hydrazines in ethanol.



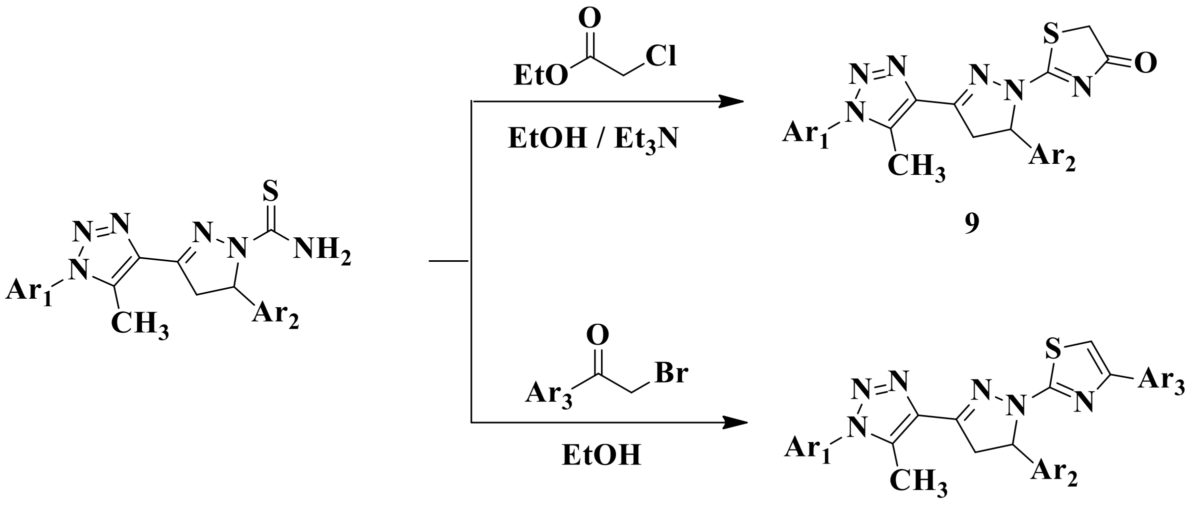
**7**

Lv *et al.*[24] reported the synthesis of 2-(3,5-diphenyl-4,5-dihydro-1*H*-pyrazol-1-yl)-4-phenylthiazoles (**8**) through the reaction of 3-(4-substituted phenyl)-5-(4-substituted phenyl)-4,5-dihydro-1*H*-pyrazole-1-carbothioamide and various phencyl bromides.



**8**

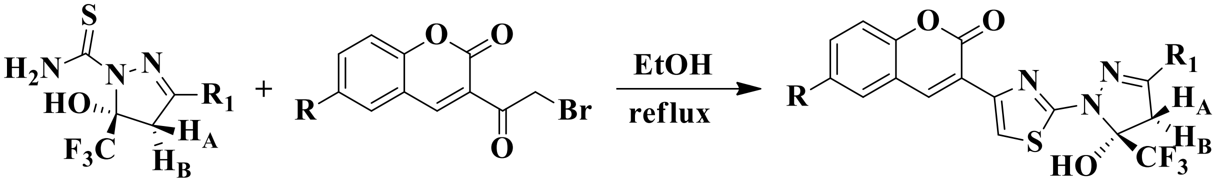
Compounds (**9**) and (**10**) synthesis was reported by Wahab *et al*[25] by the reaction of substituted pyrazole-1-carbothioamide, ethyl chloroacetate and 1-aryl 2-bromoethanones.



9

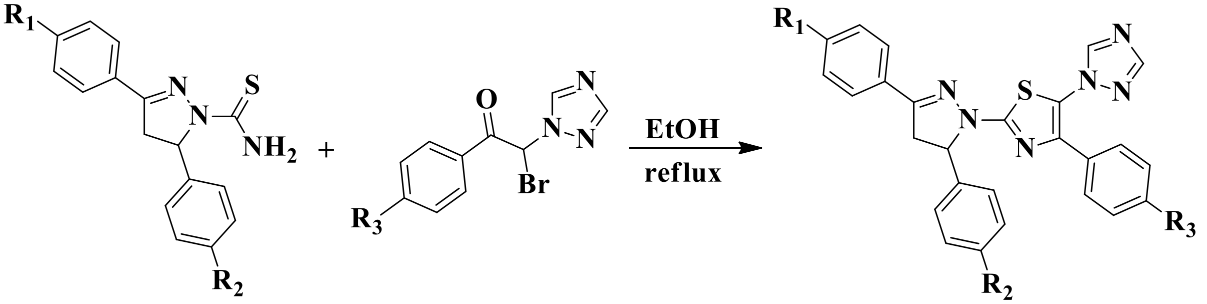
10

Aggarwal *et al.*[26] synthesized 2-(5-hydroxy-5-trifluoromethyl-4,5-dihydropyrazol-1-yl)-4-(coumarin-3-yl)thiazole derivatives (**11**) by employing 3-(2-bromoacetyl)-2*H*-1-benzopyran-2-ones and various 5-hydroxy-5-trifluoromethyl-4,5-dihydropyrazol-1-thiocarboxamides in ethanol.



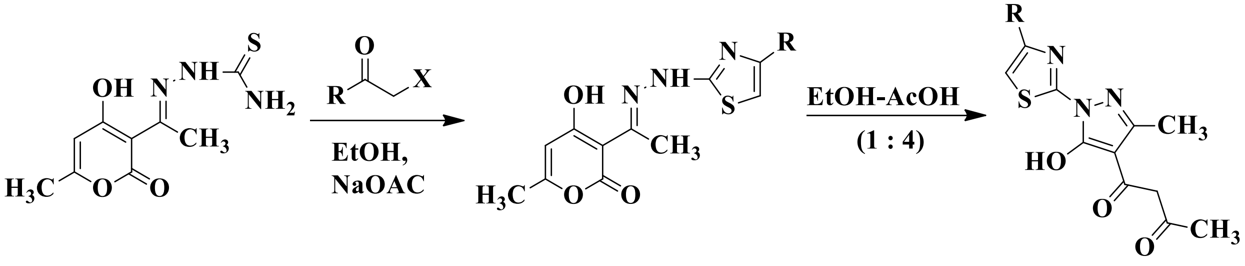
11

1-(4-Aryl-5-triazolyl-2-thiazolyl)-3,5-diaryl-2-pyrazolines (**12**) were synthesized by chen *et al*[27]. Condensation of 3,5-diaryl1-thiocarbamoyl-2-pyrazolines with 2-bromo-1-aryl-2-(1H-1,2,4-triazol-1-yl) ethanones in ethanol under reflux conditions.



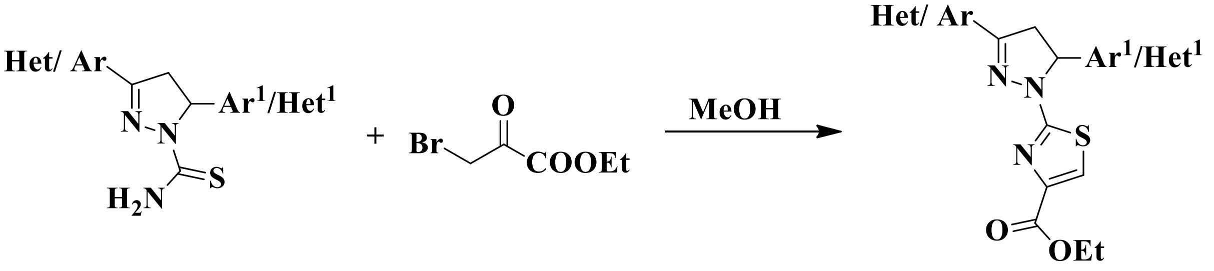
12

1-(5-Hydroxy-3-methyl-1-(4-substituted thiazol-2-yl)-1*H*-pyrazol-4-yl)butan-1,3-diones (**13**) were synthesized by Singh *et al.*[28]. Thiosemicarbazone of dehydroacetic acid was reacted with αhaloketones to give 2,4-disubstituted thaizoles. These on reaction with a mixture of ehanol and acetic acid gave the title compounds



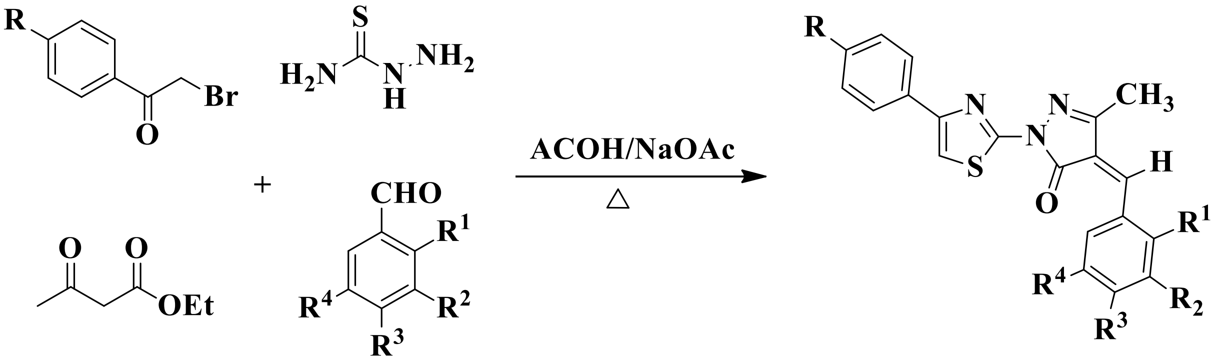
**13**

Carradori *et al.*[29] synthesized (**14**) by the reaction of 3,5-di(hetero)aryl-1-thiocarbamoyl-2-pyrazolines with ethyl ester of α-bromo-pyruvic acid.



**14**

Chunduru and Rao[30] reported the synthesis of compounds (**15**) via a multi-component approach. Condensation reaction of various 2-bromo-1-phenylethanones, with ethylacetoacetate, thiosemicarbazide, and aromaric aldehydes in presence of sodium acetate and acetic acid to give title compounds.



**15**

# CHAPTER-III (SECTION-A)

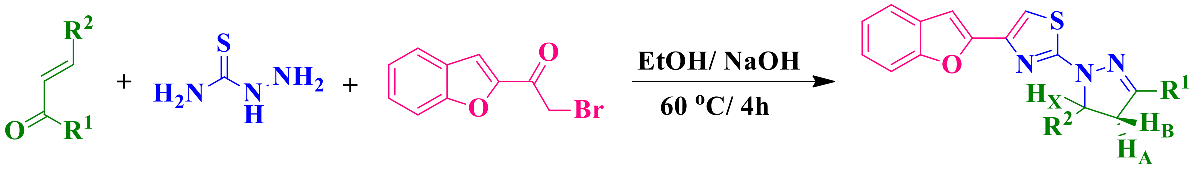
## ONE-POT SYNTHESIS OF SUBSTITUTED 4-(BENZOFURAN-2-YL)-2-(3-(ARYL/HETERYL)-5-(ARYL/HETERYL)-4,5-DIHYDRO-1*H*-PYRAZOL-1YL)THIAZOLES

# SECTION-A

## PRESENT WORK

Inspired by the biological importance of thiazoles and pyrazoles, we were particularly interested in the synthesis of 4-(benzofuran-2-yl)-2-(3-(aryl/ heteryl)-5-(aryl/ heteryl)-4,5-dihydro-1*H*-pyrazol-1yl)thiazoles 19 (a-k) via a one-pot multicomponent approach.

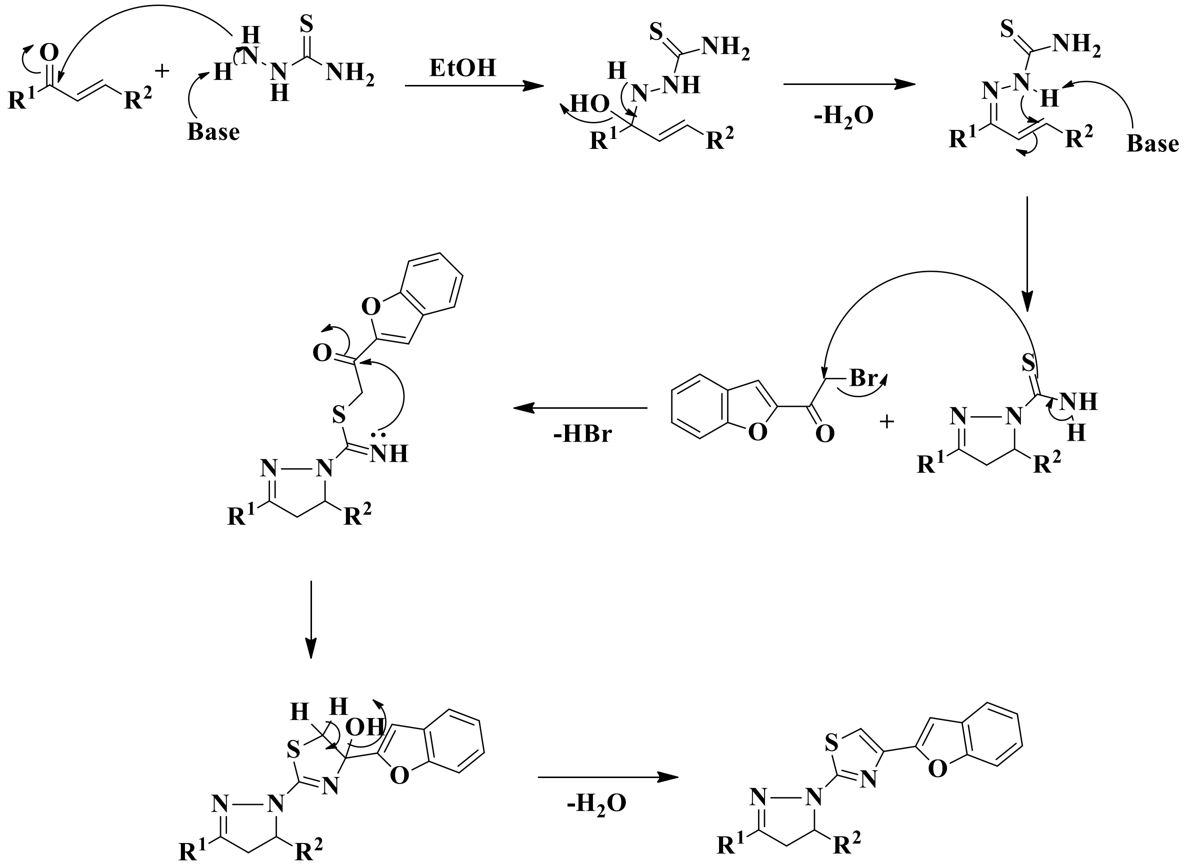
In the present work we have synthesized 4-(benzofuran-2-yl)-2-(3-(aryl/ heteryl)-5-(aryl/ heteryl)-4,5-dihydro-1*H*-pyrazol-1yl)thiazoles **19 (a-k)** by the reaction of aryl / hetryl chalcones **16 (a-k)**, thiosemicarbazide **(17)** and 1-(benzofuran-2-yl)-2-bromoethan-1-one **(18)** in presence of ethanol containing NaOH. In the scheme-1, we have found a new synthetic methodology involving simultaneous formation of two potential heterocyclic rings like thiazole and dihydropyrazole derivatives.



**16 (a-k)** **17** **18** **19 (a-k)**

**Scheme 1.** Synthesis of **19 (a-k).**

**Mechanism:** The mechanism for the formation of title compounds has been shown below.



**Scheme 2:** Mechanism of formation of .19 (a-k)

In the preliminary study, we performed the reaction of the desired product **19e** in presence of methanol/ethanol by quantifying the base equivalents as shown in Table 1. Initially, we tried the reaction at 60 °C with 0.5 equivalents of NaOH, but the reaction time was high and the yields of the products were poor. In the next trials, the concentration of NaOH was increased to 1.0, 1.5 and 2.0 equivalents and it was observed that the yields increased and the reaction time was minimized. Similarly, the reaction was performed with KOH and the findings are given in table 1.

**Table 1:** A model reaction for the synthesis of compound **19e**.

| Entry | Solvent | Base | % Yield | Reaction Time (h) |
|---|---|---|---|---|
| 1 | | NaOH (0.5 eq) | 30 | 12 |
| 2 | | KOH (0.5 eq) | 38 | 12 |
| 3 | | NaOH (1.0 eq) | 46 | 8 |
| 4 | Methanol | KOH (1.0 eq) | 45 | 8 |

| | | | | |
|---|---|---|---|---|
| 5 | | NaOH (1.5 eq) | 55 | 6 |
| 6 | | KOH (1.5 eq) | 54 | 6 |
| 7 | | NaOH (2.0 eq) | 55 | 6 |
| 8 | | KOH (2.0 eq) | 50 | 6 |
| 9 | Ethanol | NaOH (0.5 eq) | 44 | 12 |
| 10 | | KOH (0.5 eq) | 46 | 12 |
| 11 | | NaOH (1.0 eq) | 55 | 8 |
| 12 | | KOH (1.0 eq) | 48 | 8 |
| 13 | | NaOH (1.5 eq) | 93 | 4 |
| 14 | | KOH (1.5 eq) | 64 | 4 |
| 15 | | NaOH (2.0 eq) | 62 | 4 |
| 16 | | KOH (2.0 eq) | 57 | 4 |

It was observed that increasing the concentration of base beyond 2.0 equivalents did not increase the yield. After several trials of base concentration modifications, the base concentration (NaOH 1.5 equivalents) was optimized to synthesize the target compounds via a one-pot multi-component approach in short reaction time with good yields as shown in Table 2.

**Table 2:** Reaction time of compounds **19a-k**.

| **S. No.** | **compound** | **$R^1$** | **$R^2$** | **reaction time (h)** |
|---|---|---|---|---|
| 1 | 19a | Benzofuran-2-yl | 4-Bromophenyl | 4.5 |
| 2 | 19b | Benzofuran-2-yl | 4-Methoxyphenyl | 3.5 |
| 3 | 19c | 4-Fluorophenyl | Furan-2-yl | 6 |
| 4 | 19d | Thiophen-2-yl | 4-Chlorophenyl | 5 |
| 5 | 19e | Thiophen-2-yl | Phenyl | 4 |
| 6 | 19f | Thiophen-2-yl | 3,4,5-Trimethoxyphenyl | 3 |
| 7 | 19g | Thiophen-2-yl | 4-Bromophenyl | 4.5 |
| 8 | 19h | Thiophen-2-yl | 4-Fluorophenyl | 6 |
| 9 | 19i | 4-Methoxyphenyl | Furan-2-yl | 3.5 |
| 10 | 19j | Thiophen-2-yl | N,N-Dimethylaniline | 3 |
| 11 | 19k | Thiophen-2-yl | 4-Methoxyphenyl | 3.5 |

The synthesized derivatives were identified by their spectral data. The $^1$H NMR of the derivatives showed ABX type for the three protons present in the dihydropyrazole ring at the fourth and fifth position and displayed three double doublets (dd). The $^1$H NMR of **19a** showed characteristic peaks at δ 3.38 (dd, *J* =17.6Hz, 6.4Hz, 1H, $H_A$ ), 3.97 (dd, *J* =17.2Hz, 12Hz, 1H, $H_B$), 5.68 (dd, *J* = 12Hz, 6.8Hz, 1H, $H_X$) ppm of dihydropyrazole ring of $H_A$, $H_B$ and $H_X$ protons. Aromatic protons appeared at δ 6.85-7.62 ppm. In $^{13}$C NMR of compound **19a**, the methylene carbon and methine carbon atoms of dihydropyrazole appeared at δ 42.99 and 63.87 ppm respectively. The remaining $sp^2$ hybridized carbons appeared from 102.90-154.79 ppm. The most down fielded peak is due to the C-2 carbon of thiazole at 164.75 ppm. The mass spectrum for compound **19a** displayed $(M+H)^+$ at 540.25.

## EXPERIMENTAL SECTION

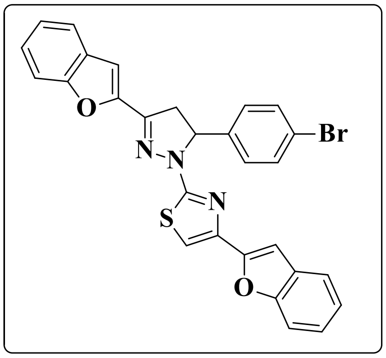
The starting materials aryl / hetryl chalcones **16 (a-k)** were prepared by Claisen -Schmidt condensation [31,32] of aryl / hetryl aldehyde with aryl / hetryl ketones by using 10% aq. NaOH in ethanol at room temperature. 1-(Benzofuran-2-yl)-2-bromoethan-1-one [33] **(18)** was prepared by following the procedure in literature using 2-acetyl benzofuran and bromine.

### General procedure for the synthesis of 19 (a-k)

In a reaction vessel chalcone (**16 a-k,** 1 mmol), thiosemicarbazide (**17,** 1mmol) and 1-(benzofuran-2-yl)-2-bromoethan-1-one (**18,** 1 mmol) were taken. To this 5 ml of ethanol was added and stirred at room temperature for about 10 minutes. Then the reaction mixture was treated with NaOH (1.5 eq) and heated at 60 °C for a specified time as shown in the table 2. The product separated was filtered, and recrystallized from ethanol.

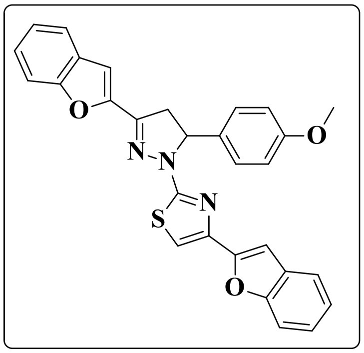
## SPECTRAL DATA

### 4-(Benzofuran-2-yl)-2-(3-(benzofuran-2-yl)-5-(4-bromophenyl)-4,5-dihydro-1*H* pyrazol-1-yl) thiazole (19a)



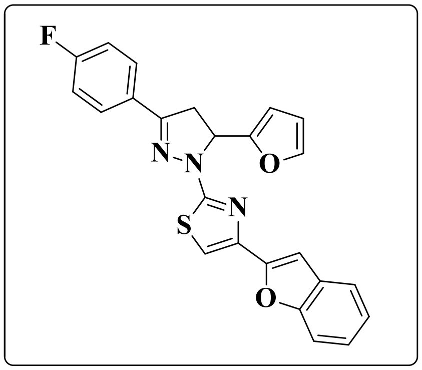
Color: yellow solid, mp: 256-258 $^o$C, yield: (0443g, 82%), FT-IR (KBr, $\nu_{max}$ cm$^{-1}$): 1614 (C=N), **$^1$H NMR (400 MHz, $CDCl_3$, ppm):** δ 3.38 (dd, *J* = 17.6Hz, 6.4Hz, 1H, Pyrazole-$H_A$ ), 3.97 (dd, *J* =17.2Hz, 12Hz, 1H, Pyrazole-$H_B$), 5.68 (dd, *J* =12Hz, 6.8Hz, 1H, Pyrazole-$H_X$), 6.85 (s, 1H, Ar-H), 7.07 (d, *J* = 9.6Hz, 2H, Ar-H), 7.18 - 7.23 (m, 2H, Ar-H), 7.36 - 7.45 (m, 5H, Ar-H), 7.54-7.62 (m, 5H, Ar-H), **$^{13}$C NMR (100 MHz, $CDCl_3$):** δ 42.99, 63.87, 102.90, 106.44, 108.32, 111.00, 111.76, 121.21, 121.63, 124.28, 125.18, 126.24, 130.05, 130.46, 143.10, 143.22, 143.49, 148.08, 152.22, 154.79, 155.55, 164.75 ppm; **MS (ESI *m/z* %):** 540.25 [M+H]$^+$; **Anal. Calcd**. for $C_{28}H_{18}BrN_3O_2S$: C, 62.23; H, 3.36; N, 7.78. Found: C, 62.29; H, 3.42; N, 7.73%.

### 4-(Benzofuran-2-yl)-2-(3-(benzofuran-2-yl)-5-(4-methoxyphenyl)-4,5-dihydro-1*H*-pyrazol-1-yl)thiazole (19b)



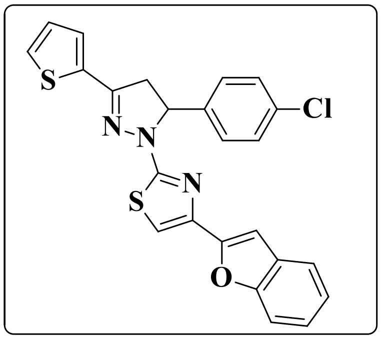
Color: yellow solid, mp: 237-239 $^o$C, yield: (0.373g, 76%), FT-IR (KBr, $\nu_{max}$ cm$^{-1}$): 1608 (C=N), **$^1$H NMR (400 MHz, $CDCl_3$, ppm):** δ 3.38 (dd, *J* = 17.2Hz, 6Hz, 1H, Pyrazole-$H_A$), 3.78 (s, 3H, -$OCH_3$), 3.94 (dd, *J* = 17.2Hz, 12Hz, 1H, Pyrazole-$H_B$), 5.70 (dd, *J* = 12Hz, 6.4Hz, 1H, Pyrazole-$H_X$), 6.87-6.90 (m, 3H, Ar-H), 7.05 (d, *J* = 8.4 Hz, 2H, Ar-H), 7.18-7.29 (m, 3H, Ar-H), 7.37 (d, *J* = 8.4Hz, 3H, Ar-H), 7.44 (d, *J* = 8Hz, 1H, Ar-H), 7.54-7.62 (m, 3H, Ar-H); **$^{13}$C NMR (100 MHz, $CDCl_3$):** δ 43.06, 55.28, 63.98, 102.77, 106.21, 108.09, 111.00, 111.75, 114.13, 121.14, 121.55, 122.87, 123.52, 124.21, 126.10, 127.97, 128.97, 133.05, 143.09, 143.53, 148.42, 152.37, 154.77, 155.53, 159.29, 164.89 ppm; **MS (ESI)*m/z*(%):** 492.34 [M+H]$^+$; **Anal. Calcd**. For $C_{29}H_{21}N_3O_3S$: C, 70.86; H, 4.31; N, 8.55. Found: C, 70.90; H, 4.38; N, 8.51%.

### 4-(Benzofuran-2-yl)-2-(3-(4-fluorophenyl)-5-(furan-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl) thiazole (19c)



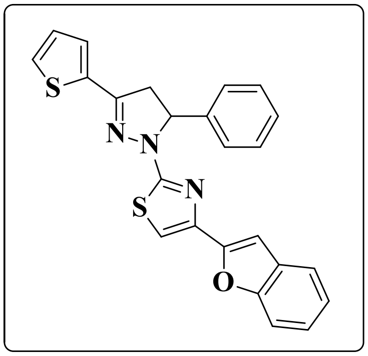
Color: white solid, mp: 195-197 °C, yield: (0.365g, 85%), FT-IR (KBr, ν$_{max}$ cm$^{-1}$): 1614 (C=N), **$^1$H NMR (400 MHz, CDCl$_3$, ppm):** δ 3.64 (dd, *J* = 17.2Hz, 6.8Hz, 1H, Pyrazole-H$_A$), 3.74 (dd, *J* = 17.2Hz, 11.6Hz, 1H, Pyrazole-H$_B$), 5.82 (dd, *J* = 10.8Hz, 6.4Hz, 1H, Pyrazole-H$_X$), 6.36 (s, 1H, Ar-H), 6.58 (s, 1H, Ar-H), 7.01 (s, 1H, Ar-H ), 7.07 (s, 1H, Ar-H), 7.13 (t, *J* = 8.8Hz, 2H, Ar-H), 7.19 - 7.28 (m, 2H, Ar-H), 7.36 (s, 1H, Ar-H), 7.47 (d, *J* = 7.6Hz, 1H, Ar-H), 7.58 (d, *J* =7.6Hz, 1H, Ar-H), 7.76 - 7.79 (m, 2H, Ar-H); **$^{13}$C NMR (100 MHz, CDCl$_3$):** δ 39.28, 58.03, 102.85, 106.10, 109.25, 110.65, 111.03, 115.80, 116.01, 121.19, 122.93, 124.30, 127.50, 128.40, 128.49, 128.99, 142.32, 142.80, 151.28, 151.52, 152.27, 154.81, 162.56, 165.52 ppm; **MS (ESI)*m/z*(%):** 430.34 [M+H]$^+$; **Anal. Calcd.** For C$_{24}$H$_{16}$FN$_3$O$_2$S: C, 67.12; H, 3.76; N, 9.78. Found: C, 67.18; H, 3.71; N, 9.72%.

### 4-(Benzofuran-2-yl)-2-(5-(4-chlorophenyl)-3-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl) thiazole (19d)



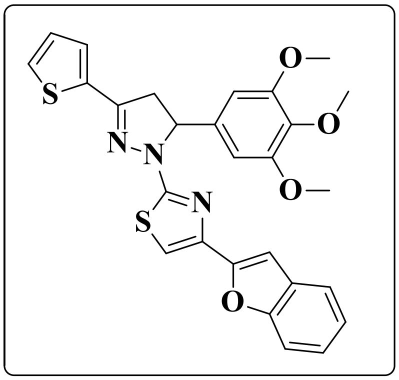
Color: yellow solid, mp: 216-217 °C, yield: (0.374g, 81%), FT-IR (KBr, ν$_{max}$ cm$^{-1}$): 1616 (C=N), **$^1$H NMR (400 MHz, CDCl$_3$, ppm):** δ 3.30 (dd, *J* = 17.2Hz, 6.8Hz, 1H, Pyrazole-H$_A$), 3.93 (dd, *J* = 17.2Hz, 11.6Hz, 1H, Pyrazole-H$_B$), 5.65 (dd, *J* = 12Hz, 6.8Hz, 1H, Pyrazole-H$_X$), 6.81 (s, 1H, thiazole-H), 7.05 (s, 1H, Ar-H), 7.07 - 7.09 (m, 1H, Ar-H), 7.17 - 7.24 (m, 3H, Ar-H), 7.33 - 7.38 (m, 4H, Ar-H), 7.43 (d, *J* = 6.0Hz, 2H, Ar-H), 7.53 - 7.755 (m, 1H, Ar-H); **$^{13}$C NMR (100 MHz, CDCl$_3$):** δ 44.27, 64.09, 102.85, 106.08, 110.97, 121.20, 122.89, 124.27, 127.66, 128.03, 128.16, 128.59, 128.92, 129.00, 133.69, 134.63, 139.73, 147.90, 152.13, 154.77, 165.21 ppm; **MS (ESI)*m/z*(%):** 462.24 [M+H]$^+$; **Anal. Calcd.** For C$_{24}$H$_{16}$ClN$_3$OS$_2$: C, 62.40; H, 3.49; N, 9.10. Found: C, 62.46; H, 3.42; N, 9.15%.

### 4-(Benzofuran-2-yl)-2-(5-phenyl-3-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)thiazole (19e)



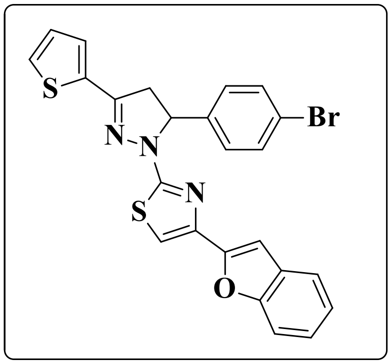
Color: white solid, mp: 191-193 ºC, yield: (0.397g, 93%), FT-IR (KBr, $\nu_{max}$ cm$^{-1}$): 1614 (C=N), **$^1$H NMR (400 MHz, CDCl$_3$, ppm):** δ 3.35 (dd, *J* = 17.2Hz, 6.8Hz, 1H, Pyrazole-H$_A$), 3.93 (dd, *J* = 17.6Hz, 12Hz, 1H, Pyrazole-H$_B$), 5.68 (dd, *J* = 12Hz, 6.8Hz, 1H, Pyrazole-H$_X$), 6.81 (s, 1H, thiazole-H), 7.03 (s, 1H, Ar-H), 7.06 – 7.08 (dd, *J* = 5.2Hz, 4Hz, 1H, Ar-H), 7.15 - 7.23 (m, 3H, Ar-H), 7.30 (d, *J* = 7.2, 1H, Ar-H), 7.37 (t, *J* = 7.6, 2H, Ar-H), 7.41 - 7.44 (m, 4H, Ar-H), 7.52 (d, *J* =7.2Hz, 1H, Ar-H); **$^{13}$C NMR (100 MHz, CDCl$_3$):** δ 44.30, 64.59, 102.67, 105.84, 110.88, 121.09, 122.84, 124.17, 126.51, 127.62, 127.86, 128.14, 128.39, 128.73, 128.84, 141.18, 165.18 ppm; **MS (ESI)*m/z*(%):** 428.36 [M+H]$^+$; **Anal. Calcd.** for $C_{24}H_{17}N_3OS_2$: C, 67.42; H, 4.01; N, 9.83. Found: C, 67.48; H, 4.10; N, 9.88%.

**4-(Benzofuran-2-yl)-2-(3-(thiophen-2-yl)-5-(3,4,5-trimethoxyphenyl)-4,5-dihydro-1*H*-pyrazol-1-yl)thiazole (19f)**



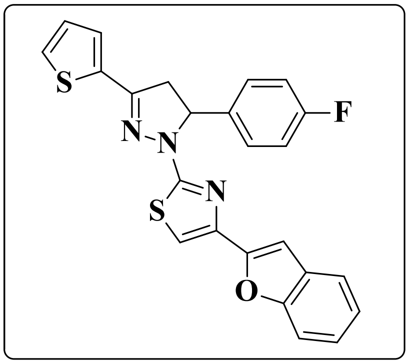
Color: light yellow solid, mp: 235-237 ºC, yield: (0.455g, 88%), FT-IR (KBr, $\nu_{max}$ cm$^{-1}$): 1614 (C=N), 1256 (-C-O); **$^1$H NMR (400 MHz, CDCl$_3$, ppm):** δ 3.35 (dd, *J* = 17.2Hz, 6.8Hz, 1H, Pyrazole-H$_A$), 3.83 (s, 3H,-OCH$_3$), 3.86 (s, 6H, -OCH$_3$), 3.94 (dd, *J* = 17.2Hz, 5.2Hz, 1H, Pyrazole-H$_B$), 5.59 (dd, *J* = 6.8Hz, 1H, Pyrazole-H$_X$), 6.64 (s, 2H, Ar-H), 6.84 (s, 1H, thiazole-H), 7.07 - 7.09 (m, 2H, Ar-H), 7.17 - 7.25 (m, 4H, Ar-H), 7.43 - 7.45 (m, 2H, Ar-H), 7.53 (d, *J* = 7.6Hz, 1H, Ar-H); **$^{13}$C NMR (100 MHz, CDCl$_3$):** δ 44.46, 56.23, 60.84, 65.07, 102.71, 103.46, 106.22, 110.98, 121.12, 122.90, 124.25, 127.65, 128.46, 128.90, 134.77, 136.97, 137.53, 143.06, 148.05, 152.36, 153.53, 154.75, 165.65 ppm; **MS (ESI)*m/z*(%):** 518.30 [M+H]$^+$; **Anal. Calcd.** for $C_{27}H_{23}N_3O_4S_2$: C, 62.65; H, 4.48; N, 8.12. Found: C, 62.60; H, 4.42; N, 8.17%.

**4-(Benzofuran-2-yl)-2-(5-(4-bromophenyl)-3-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl) thiazole (19g)**



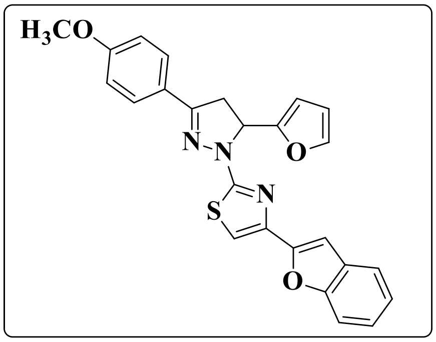
Color: yellow solid, mp: 247-249 ºC, yield: (0.415g, 82%), FT-IR (KBr, νmax $cm^{-1}$): 1608 (C=N), **$^1$H NMR (400 MHz, $CDCl_3$, ppm):** δ 3.33 (dd, *J* = 17.6Hz, 7.2Hz, 1H, Pyrazole-$H_A$), 3.93 (dd, *J* = 17.6Hz, 12Hz, 1H, Pyrazole-$H_B$), 5.62 (dd, *J* = 12Hz, 7.2Hz, 1H, Pyrazole-$H_X$), 6.83 (s, 1H, thiazole-H), 7.05 (s, 1H, Ar-H), 7.08 (dd, *J* = 4.8Hz, 1H, Ar-H), 7.16 - 7.22 (m, 4H, Ar-H), 7.36 (d, *J* = 8Hz, 1H, Ar-H), 7.41 - 7.44 (m, 3H, Ar-H), 7.54 (d, *J* = 8Hz, 1H, Ar-H), 7.60 - 7.61(m, 1H, Ar-H); **$^{13}$C NMR (100 MHz, $CDCl_3$):** δ 44.14, 64.5, 102.81, 106.17, 110.97, 121.17, 122.86, 124.22, 125.21, 127.64, 128.12, 128.54, 130.03, 130.40, 131.06, 143.52, 165.19 ppm; **MS (ESI)*m/z*(%):** 506.25 $[M+H]^+$; **Anal. Calcd.** for $C_{24}H_{16}BrN_3OS_2$: C, 56.92; H, 3.18; N, 8.30. Found: C, 56.97; H, 3.13; N, 8.35%.

**4-(Benzofuran-2-yl)-2-(5-(4-fluorophenyl)-3-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl) thiazole (19h)**



Color: yellow solid. mp: 211-213 ºC, yield: (0.334g, 75%), FT-IR (KBr, νmax $cm^{-1}$): 1613 (C=N), **$^1$H NMR (400 MHz, $CDCl_3$, ppm):** δ 3.32 (dd, *J* = 17.2Hz, 6.8Hz, 1H, Pyrazole-$H_A$), 3.93 (dd, *J* = 17.2Hz, 11.6Hz, 1H, Pyrazole-$H_B$), 5.66 (dd, *J* = 11.6Hz, 6.4Hz, 1H, Pyrazole-$H_X$), 6.80 (s, 1H, thiazole-H), 7.03 - 7.09 (m, 3H, Ar-H), 7.16 - 7.24 (m, 4H, Ar-H), 7.39 - 7.44 (m, 4H, Ar-H), 7.54 (d, *J* = 8 Hz, 1H, Ar-H); **MS (ESI)*m/z*(%):** 446.31 $[M+H]^+$; **Anal. Calcd**. for $C_{24}H_{16}FN_3OS_2$: C, 64.70; H, 3.62; N, 9.43. Found: C, 64.74; H, 3.67; N, 9.48%.

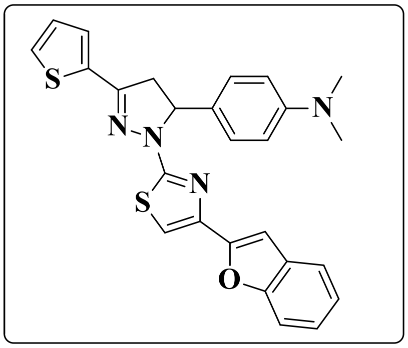
**4-(Benzofuran-2-yl)-2-(5-(furan-2-yl)-3-(4-methoxyphenyl)-4,5-dihydro-1*H*-pyrazol-1-yl) thiazole (19i)**



Color: yellow solid, mp: 196-198 ºC, yield: (0.401g, 91%), FT-IR (KBr, νmax $cm^{-1}$): 1609 (C=N), 1258 (-C-O); **$^1$H NMR (400 MHz, $CDCl_3$, ppm):** δ 3.63 (dd, *J* = 17.2Hz, 6.8Hz, 1H, Pyrazole-$H_A$), 3.73 (dd, *J* = 17.2Hz, 11.6Hz, 1H, Pyrazole-$H_B$), 3.86 (s, 3H, $-OCH_3$), 5.75 (dd, *J* = 11.2Hz, 6.8Hz, 1H, Pyrazole-$H_X$), 6.36 (dd, *J* = 3.2Hz, 1.6Hz, 1H, Ar-H ), 6.54

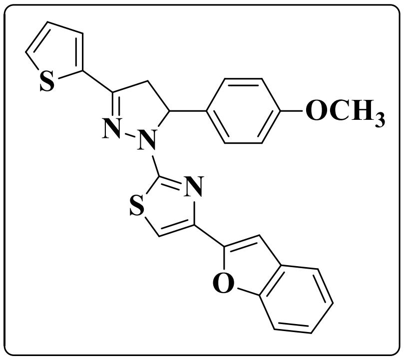
(d, J=3.2Hz, 1H, Ar-H), 6.94 - 6.99 (m, 3H, Ar-H), 7.05 (s, 1H, Ar-H), 7.18 - 7.24 (m, 2H, Ar-H), 7.35 - 7.36 (m, 1H, Ar-H), 7.45 - 7.47 (m, 1H, Ar-H), 7.56 - 7.59 (m, 1H, Ar-H ), 7.72 - 7.74 (m, 2H, Ar-H); **$^{13}$C NMR (100 MHz, CDCl$_3$):** δ 39.43, 55.41, 57.84, 102.71, 105.93, 108.97, 110.60, 111.01, 114.16, 121.15, 122.88, 123.92, 124.20, 128.10, 129.04, 142.20, 142.84, 151.65, 152.37, 154.80, 161.13, 165.79 ppm; **MS (ESI)*m/z*(%):** 442.32 $[M+H]^+$; **Anal. Calcd**. for $C_{25}H_{19}N_3O_3S$: C, 68.01; H, 4.34; N, 9.52. Found: C, 68.12; H, 4.38; N, 9.57%.

**4-(1-(4-(Benzofuran-2-yl)thiazol-2-yl)-3-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazol-5-yl)-N,N-dimethylaniline (19j)**



Color: red solid, mp: 240-242 °C, yield: (0.418g, 89%), FT-IR (KBr, $\nu_{max}$ cm$^{-1}$): 1612 (C=N), **$^1$H NMR (400 MHz, CDCl$_3$, ppm):** δ 2.93 (s, 6H, -CH$_3$), 3.35 (dd, *J* = 17.2Hz, 6.4Hz, 1H, Pyrazole-H$_A$), 3.88 (dd, *J* = 17.2Hz, 12Hz, 1H, Pyrazole-H$_B$), 5.60 (dd, *J* = 11.6Hz, 6Hz, 1H, Pyrazole-H$_X$), 6.71 (d, *J* = 9.2Hz, 2H, Ar-H), 6.90 (s, 1H, thiazole-H), 7.01 (s, 1H, Ar-H), 7.06 – 7.08 (dd, *J* = 3.6Hz, 1H, Ar-H), 7.16 -7.24 (m, 3H, Ar-H), 7.31 (d, *J* = 8.8Hz, 2H, Ar-H), 7.40 - 7.45 (m, 2H, Ar-H), 7.54 - 7.56 (m, 1H, Ar-H); **$^{13}$C NMR (100 MHz, CDCl$_3$):** δ 40.66, 44.07, 64.32, 102.66, 105.79, 110.97, 112.67, 121.09, 122.78, 124.07, 127.55, 127.76, 128.11, 129.07, 135.22, 143.08, 147.80, 150.00, 152.62, 154.75, 165.31 ppm; **MS (ESI)*m/z*(%):** 471.30 $[M+H]^+$; **Anal. Calcd**. for $C_{26}H_{22}N_4OS_2$: C, 66.36; H, 4.71; N, 11.91. Found: C, 66.31; H, 4.77; N, 11.97%.

**4-(Benzofuran-2-yl)-2-(5-(4-methoxyphenyl)-3-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)thiazole (19k)**

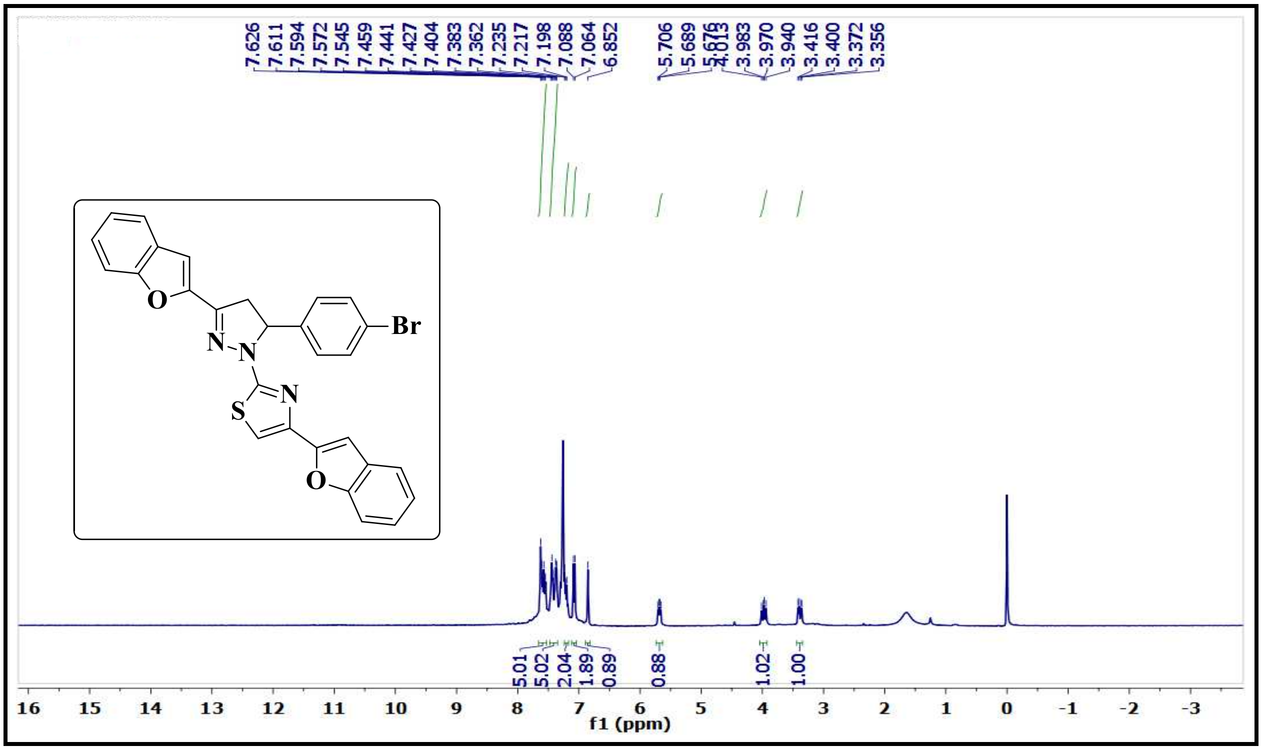


Color: yellow solid, mp: 221-223 °C, yield: (0.356g, 78%), FT-IR (KBr, $\nu_{max}$ cm$^{-1}$): 1614 (C=N), 1257 (-C-O); **$^1$H NMR (400 MHz, CDCl$_3$, ppm):** δ 3.33 (dd, *J* = 17.6Hz, 6.8Hz, 1H, Pyrazole-H$_A$), 3.78 (s, 3H, -OCH$_3$), 3.90 (dd, *J* = 17.6Hz, 12Hz, 1H, Pyrazole-H$_B$), 5.65 (dd, *J* = 12Hz, 6.4Hz, 1H, Pyrazole-H$_X$), 6.86 - 6.90 (m, 3H, 2Ar-H, thiazole), 7.02 (s, 1H, Ar-H), 7.06 - 7.08 (m, 1H, Ar-H), 7.16-7.23 (m, 3H, Ar-H), 7.36 (d, *J* = 8.8 Hz, 2H, Ar-H), 7.41 - 7.44 (m, 2H, Ar-H), 7.53 - 7.55 (m, 1H, Ar-H); **$^{13}$C NMR (100 MHz,**

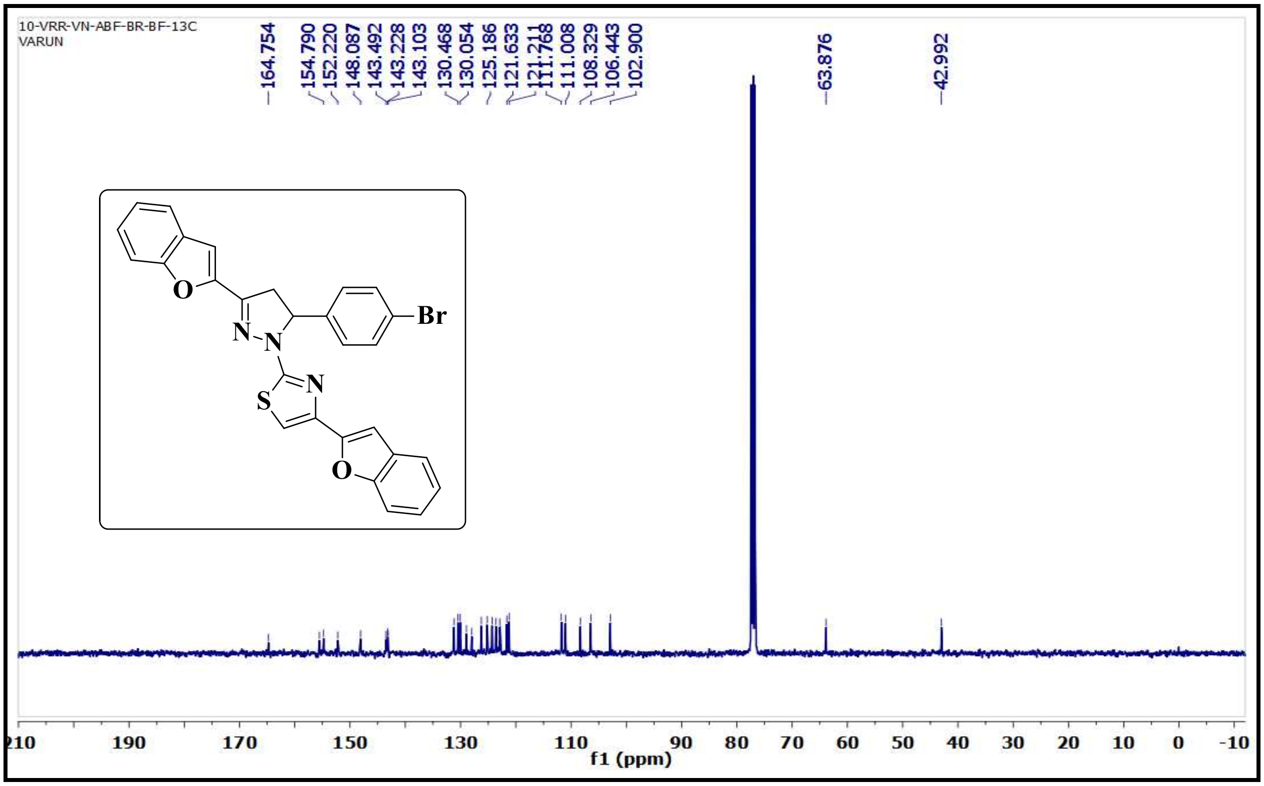
**CDCl$_3$):** δ 44.26, 55.27, 64.23, 102.76, 105.88, 110.97, 114.10, 121.13, 122.84, 124.17, 127.60, 127.95, 128.32, 129.00, 133.29, 134.98, 142.91, 147.90, 152.38, 154.77, 159.23, 165.27 ppm; **MS (ESI)*m/z*(%):** 458.30 $[M+H]^+$; **Anal. Calcd**. for $C_{25}H_{19}N_3O_2S_2$: C, 65.62; H, 4.19; N, 9.18. Found: C, 65.66; H, 4.23; N, 9.21%.

## SUMMARY

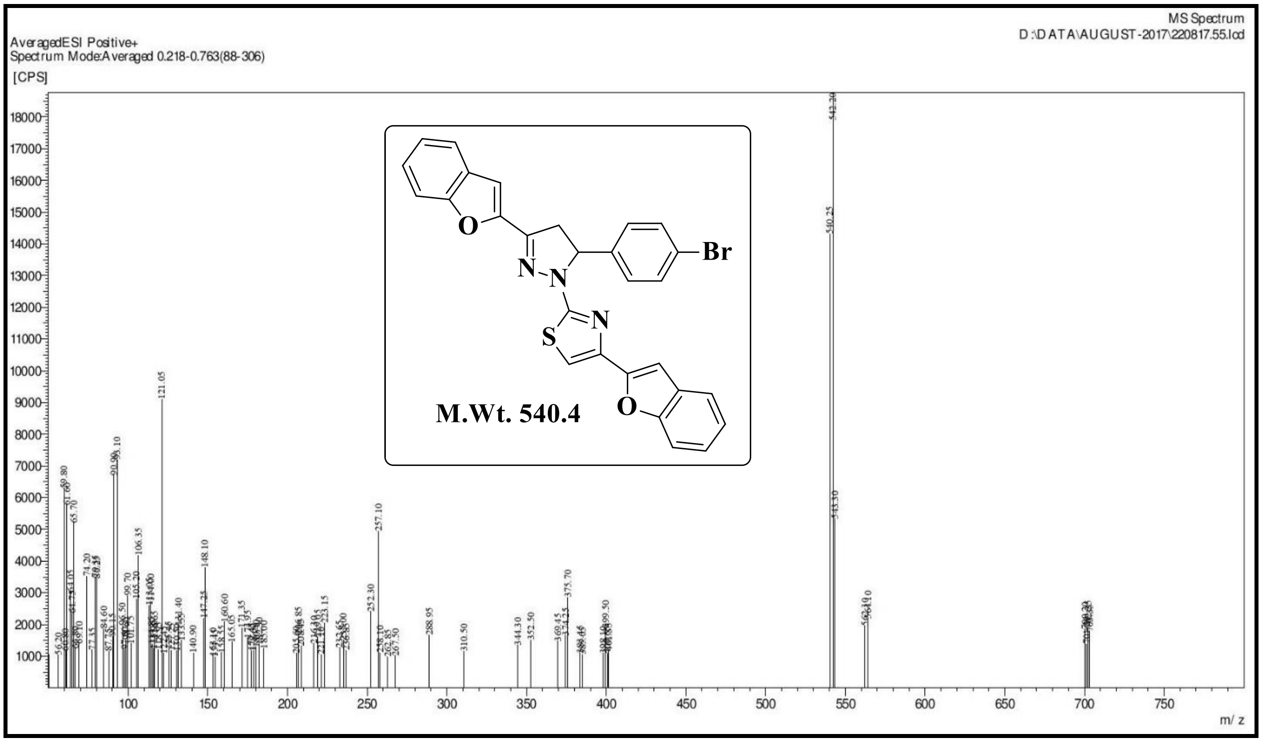
In summary, we have synthesized 4-(benzofuran-2-yl)-2-(3,5-diaryl/heteryl-4,5-dihydro-1*H*-pyrazol-1yl)thiazoles with high yields and short reaction times. This reaction is not only having operational simplicity but it also constructs two potential heterocyclic rings (thiazole and dihydropyrazole) in one step reaction.



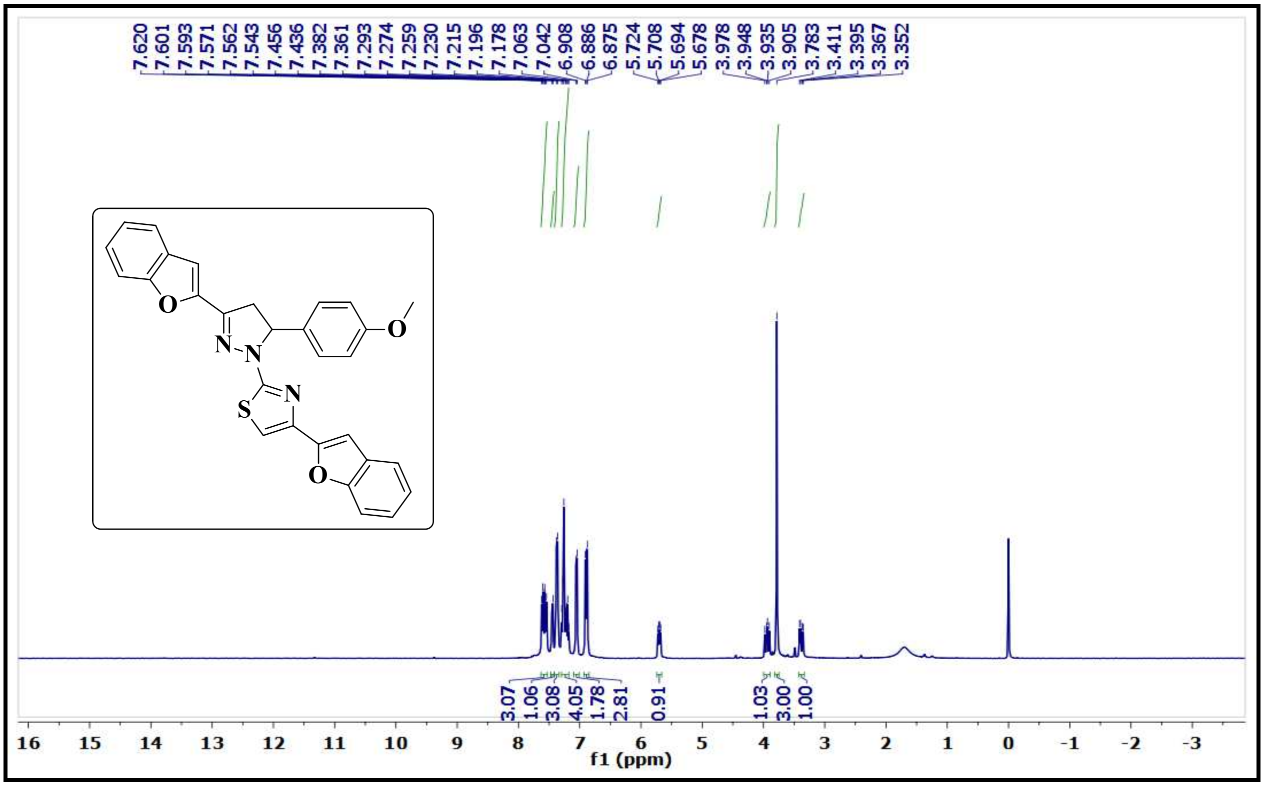
$^1$H NMR Spectrum of compound **19a**



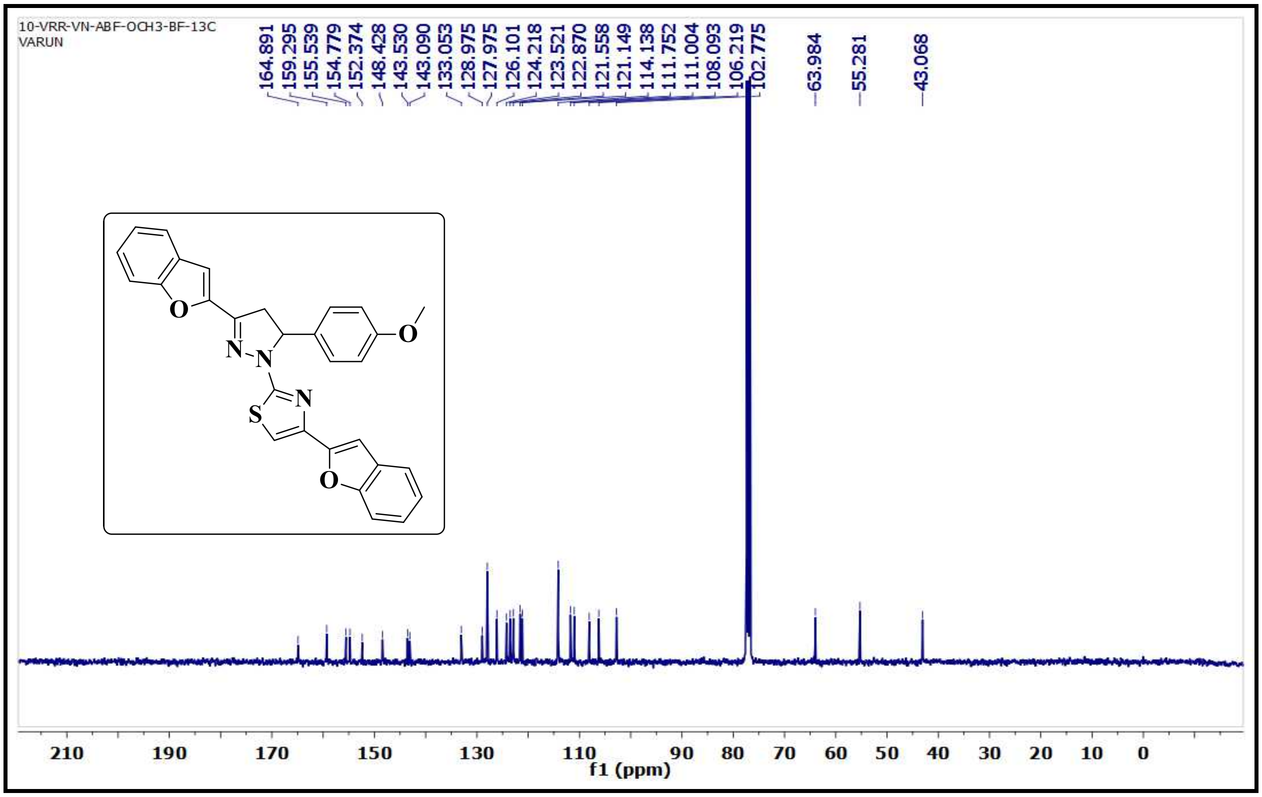
$^{13}$C NMR Spectrum of compound **19a**



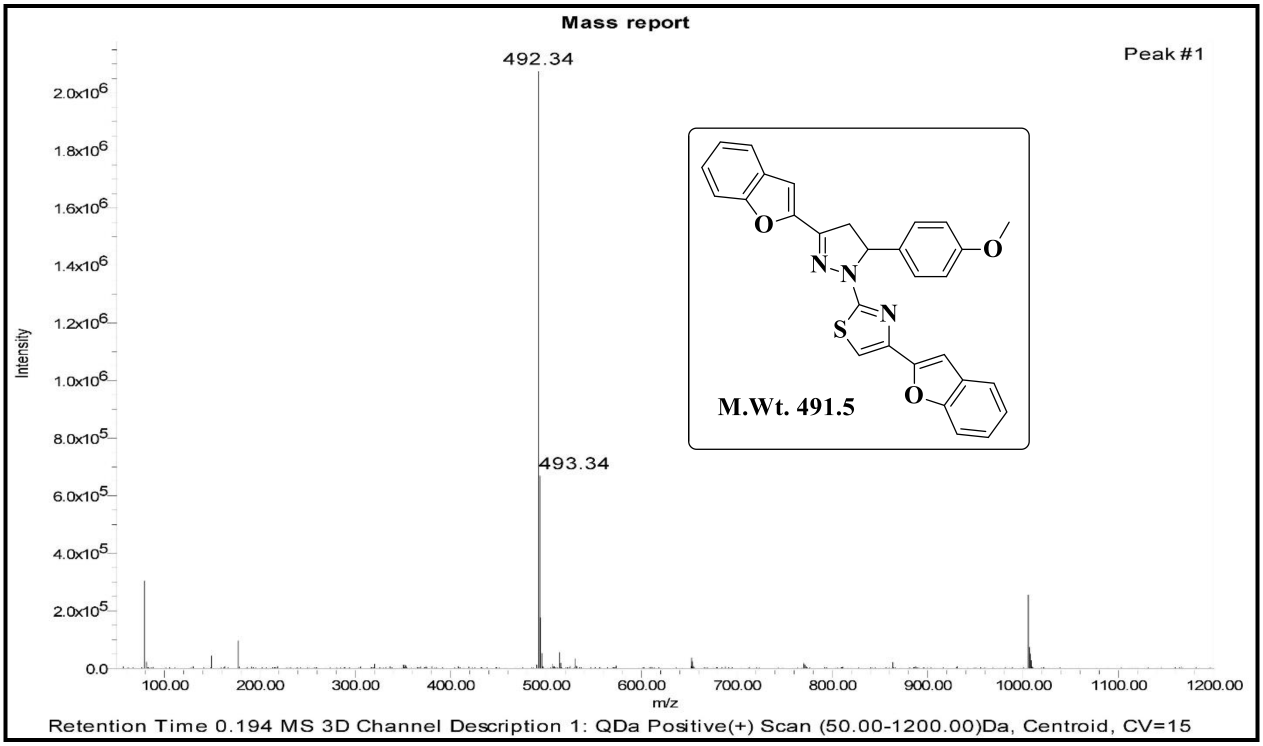
Mass Spectrum of compound **19a**



$^1$H NMR Spectrum of compound **19b**



$^{13}$C NMR Spectrum of compound **19b**



Mass Spectrum of compound **19b**

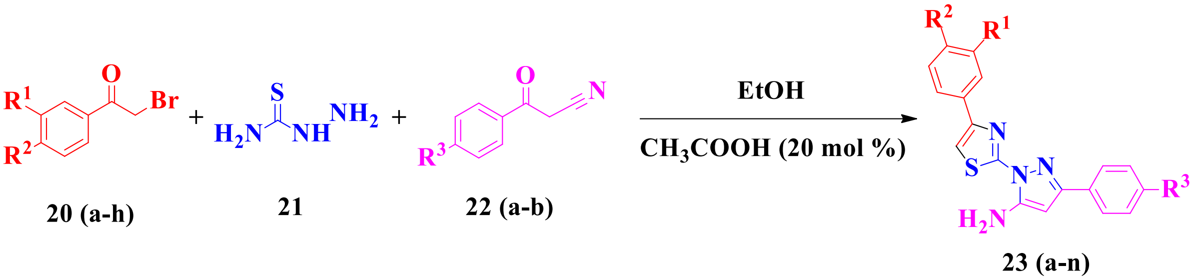
# CHAPTER-III (SECTION-B)

## SYNTHESIS OF 3-PHENYL-1-(4-PHENYLTHIAZOL-2-YL)-1*H*-PYRAZOLE-5-AMINES AND THEIR BIOLOGICAL EVALUATION: ANTIVIRAL, ANTIMICROBIAL AND ANTICANCER ACTIVITIES

# SECTION-B

## PRESENT WORK

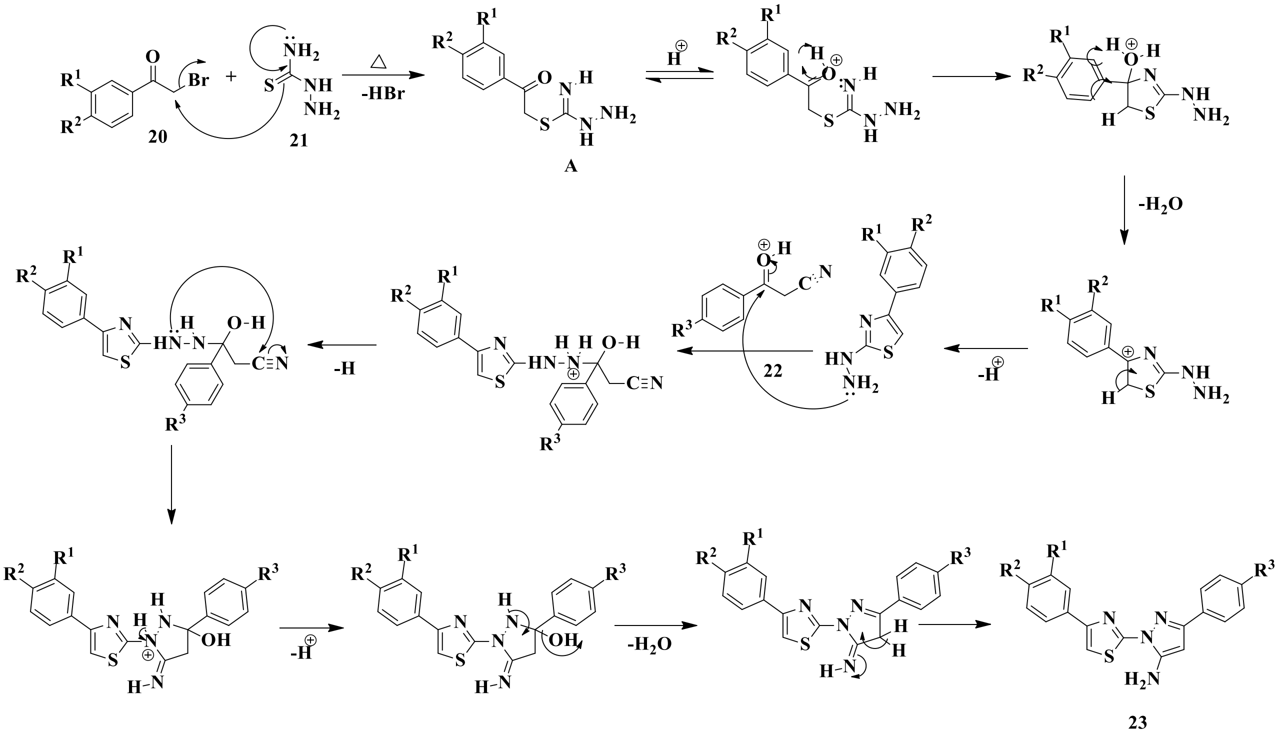
The investigation intends to synthesize new substituted 3-phenyl-1-(4-phenylthiazol-2-yl)-1*H*-pyrazole-5-amines **23 (a-n)** via a one-pot multicomponent approach (**scheme 3**). The title compounds were synthesized by the reaction of substituted phenacyl bromide **20 (a-h)**, thio-semicarbazide **21**, and phenacyl cyanides **22 (a-b)** in dry alcohol with small catalytic amount of $CH_3COOH$ (20 mol %) under reflux conditions. The derivatives were subjected to antiviral, antifungal and anticancer activity.



**Scheme 3:** One-pot synthesis of target compounds **23 (a-n).**

## Mechanism

From the mechanism it is evident that the substitution of α-bromine atom of the phenacyl bromide by the sulphur atom of thiosemicabazide occurs initially to yield an open chain thio ketone ‘A’, which under trans protonation gives 4-hydroxy-$\Delta^2$-thiazoline derivative. This on further loss of $H_2O$ gives intermediate 2-hydrazino-4-substituted thiazole. Finally the hydrazino group of thiazole reacts with protonated benzonitrile resulting in the formation of cyclo condensed product 23.



**Scheme 4** Mechanism for the formation of **23**

**Table 3:** Reaction time of compounds **23 (a-n)**.

| S. No. | Compound | $R^1$ | $R^2$ | $R^3$ | Time (h) |
|---|---|---|---|---|---|
| 1 | 23a | H | H | H | 5 |
| 2 | 23b | H | H | Cl | 5.5 |
| 3 | 23c | H | $CH_3$ | H | 4.5 |
| 4 | 23d | H | $CH_3$ | Cl | 5 |
| 5 | 23e | H | $OCH_3$ | H | 4 |
| 6 | 23f | H | $OCH_3$ | Cl | 4.5 |
| 7 | 23g | H | Cl | H | 5 |
| 8 | 23h | H | Cl | Cl | 6 |
| 9 | 23i | H | Br | H | 4.5 |
| 10 | 23j | H | Br | Cl | 5.5 |
| 11 | 23k | Cl | Cl | H | 8 |
| 12 | 23l | Cl | Cl | Cl | 8.5 |
| 13 | 23m | H | $NO_2$ | H | 7 |
| 14 | 23n | H | $NO_2$ | Cl | 7.5 |

The $^{1}$H NMR spectrum of compound 23a showed peaks at $\delta$ 5.95 (s, 1H, $C_4$-H of pyrazole), 6.93 (bs, 2H, -$NH_2$), the aromatic protons appeared in the range of $\delta$ 7.37-8.01 ppm. The $^{13}$C NMR spectrum of **23a** showed signals at at $\delta$ 85.66 ppm corresponds to pyrazole-$C_4$ carbon, $\delta$ 109.70

ppm corresponds to thiazole-$C_5$ carbon. The mass spectrum of compound **23a** exhibited 319.1001 $[M+H]^{+\cdot}$

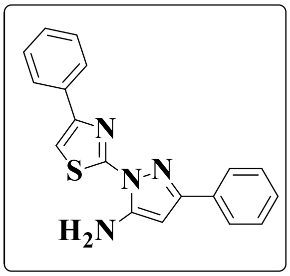
## EXPERIMENTAL

### Synthesis of compounds 23 (a-n)

A mixture of phenacyl bromide **20 (a-h)** (1 mmol), thio-semicarbazide **21** (1 mmol), and substituted 3-oxo-3-phenylpropanenitrile **22 (a-b)** (1 mmol) in ethanol was taken and catalytic amounts of acetic acid (20 mole %) was added under refluxing conditions to afforded corresponding substituted 3-phenyl-1-(4-phenylthiazol-2-yl)-1H-pyrazol-5-amine **23 (a-n)**. The progress of reaction was monitored with the help of TLC. After completion of reaction, cool the mixture and filter it, re-crystalized from ethanol.

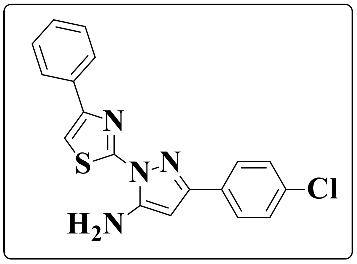
## SPECTRAL DATA

### 3-Phenyl-1-(4-phenylthiazol-2-yl)-1*H*-pyrazol-5-amine (23a)



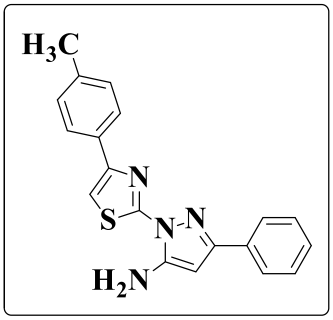
Color: light yellow solid, mp: 195-197 °C, yield (0.222g, 70%), FT-IR (KBr, $\nu_{max}$ $cm^{-1}$): 3417 ($NH_2$), 1614 (C=N), **$^1$H NMR (400MHz, DMSO-d$_6$, ppm):** $\delta$ 5.95 (s, 1H, $C_4$-H of pyrazole), 6.93 (bs, 2H, -$NH_2$), 7.37-7.42 (m, 2H, Ar-H), 7.47 (dd, *J*=16.4 Hz, *J*=8 Hz, 4H, Ar-H), 7.83-7.86 (m, 3H, Ar-H), 8.01 (d, J=7.6 Hz, 2H, Ar-H), ppm, **$^{13}$C NMR (100MHz, DMSO-d$_6$):** $\delta$ 85.66. 109.70, 126.14, 126.34, 128.70, 129.13, 129.29, 132.65, 134.00, 149.88, 151.54, 153.68, 162.50 ppm; **MS (ESI) *m/z* (%)**: 319.1001 $[M+H]^+$; Anal. Calcd. for $C_{18}H_{14}N_4S$; C, 67.90; H, 4.43; N, 17.60. Found: C, 67.84; H, 4.49; N, 17.65%

### 3-(4-Chlorophenyl)-1-(4-phenylthiazol-2-yl)-1*H*-pyrazol-5-amine (23b)



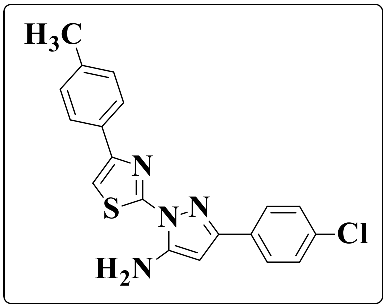
Color: light yellow solid, mp: 195-197 °C, yield (0.268g, 76%), FT-IR (KBr, $\nu_{max}$ $cm^{-1}$): 3417 ($NH_2$), 1614 (C=N), **$^1$H NMR (400MHz, DMSO-d$_6$, ppm):** $\delta$ 5.95 (s, 1H, $C_4$-H of pyrazole), 6.96 (bs, 2H, -$NH_2$), 7.38-7.49 (m, 5H, Ar-H), 7.84-8.00 (m, 5H, Ar-H) ppm; **$^{13}$C NMR (100MHz, DMSO-d$_6$):** $\delta$ 85.69, 109.86, 126.36, 127.86, 128.74, 129.20, 129.31, 131.61, 133.70, 133.97, 150.02, 151.57, 152.51 ppm; **MS (ESI) *m/z* (%):** $[M+H]^+$; Anal. Calcd. for $C_{18}H_{13}ClN_4S$; C, 61.27; H, 3.71; N, 15.88. Found: C, 61.23; H, 3.76; N, 15.83%

### 3-Phenyl-1-(4-(p-tolyl)thiazol-2-yl)-1*H*-pyrazol-5-amine (23c)



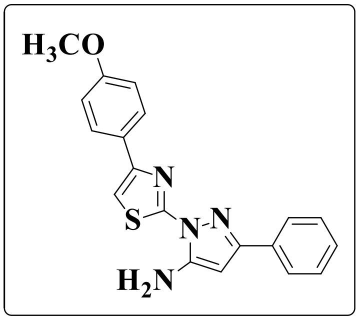
Color: white solid, mp: 195-197 °C, yield (0.332g, 81%), FT-IR (KBr, $\nu_{max}$ $cm^{-1}$): 3417 ($NH_2$), 1614 (C=N), **$^1$H NMR (400MHz, DMSO-$d_6$, ppm):** $\delta$ 2.36 (s, 3H, -$CH_3$), 5.94 (s, 1H, $C_4$-H of pyrazole), 6.92 (bs, 2H, -$NH_2$), 7.29 (d, *J*=7.6 Hz, 2H, Ar-H), 7.36-7.41 (m, 1H, Ar-H), 7.46 (t, *J*=7.6 Hz, 2H, Ar-H), 7.78 (s, 1H, Ar-H), 7.83 (d, *J*=7.2 Hz, 2H, Ar-H), 7.90 (d, *J*=8 Hz, 2H, Ar-H), ppm, **$^{13}$C NMR (100MHz, DMSO-$d_6$):** $\delta$ 21.47, 85.63, 108.83, 126.10, 126.26, 127.42, 129.13, 129.64, 129.84, 130.09, 130.61, 142.09, 149.83, 151.75, 164.53 ppm; **MS (ESI) *m/z* (%):** $[M+H]^+$; Anal. Calcd. for $C_{19}H_{16}N_4S$; C, 68.65; H, 4.85; N, 16.85. Found: C, 68.60; H, 4.80; N, 16.89%

### 3-(4-Chlorophenyl)-1-(4-(p-tolyl)thiazol-2-yl)-1*H*-pyrazol-5-amine (23d)



Color: white solid, mp: 195-197 °C, yield (0.311g, 85%), FT-IR (KBr, $\nu_{max}$ $cm^{-1}$): 3417 ($NH_2$), 1614 (C=N), **$^1$H NMR (400MHz, DMSO-$d_6$, ppm):** $\delta$ 2.35 (s, 3H, methyl), 5.95 (s, 1H, pyrazole), 6.96 (bs, 2H, -$NH_2$), 7.28 (d, *J*=7.6Hz, 2H, Ar-H), 7.51 (d, *J*=8.8Hz, 2H, Ar-H), 7.79 (s, 1H, Ar-H), 7.85 (d, *J*=8.4Hz, 2H, Ar-H), 7.89 (d, *J*=8.4Hz, 2H, Ar-H) ppm; **MS (ESI) *m/z* (%):** $[M+H]^+$; Anal. Calcd. for $C_{19}H_{15}ClN_4S$; C, 62.20; H, 4.12; N, 15.27. Found: C, 62.24; H, 4.17; N, 15.22%

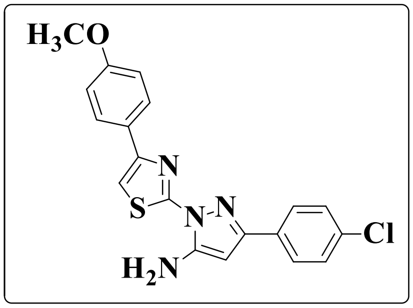
### 1-(4-(4-Methoxyphenyl)thiazol-2-yl)-3-phenyl-1*H*-pyrazol-5-amine (23e)



Color: yellow solid, mp: 195-197 °C, yield (0.275g, 79%), FT-IR (KBr, $\nu_{max}$ $cm^{-1}$): 3417 ($NH_2$), 1614 (C=N), **$^1$H NMR (400MHz, DMSO-$d_6$, ppm):** $\delta$ 3.82 (s, 3H, methoxy), 5.94 (s, 1H, pyrazole), 6.92 (bs, 2H, -$NH_2$), 7.03 (d, *J*=8.8Hz, 2H, Ar-H), 7.40 (t, *J*=7.6 Hz, 1H, Ar-H), 7.46 (t, *J*=7.2 Hz, 2H, Ar-H), 7.69 (s, 1H, Ar-H), 7.83 (d, *J*=7.6 Hz, 2H, Ar-H), 7.94 (d, *J*=8.8Hz, 2H, Ar-H) ppm, **$^{13}$C NMR (100MHz, DMSO-$d_6$):** $\delta$ 55.66, 85.60, 107.56, 114.63, 126.11, 126.86, 127.76, 129.13, 132.67, 149.84, 151.43, 153.57, 159.76, 162.35 ppm; **MS (ESI)**

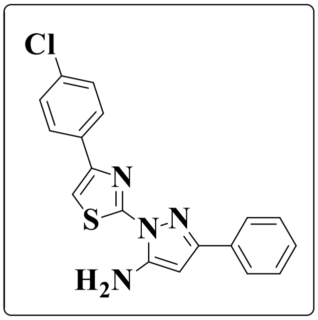
***m/z* (%):** [M+H]$^{+}$; Anal. Calcd. for $C_{19}H_{16}N_{4}OS$; C, 65.50; H, 4.63; N, 16.08. Found: C, 65.55; H, 4.67; N, 15.97%

### 3-(4-Chlorophenyl)-1-(4-(4-methoxyphenyl)thiazol-2-yl)-1*H*-pyrazol-5-amine (23f)



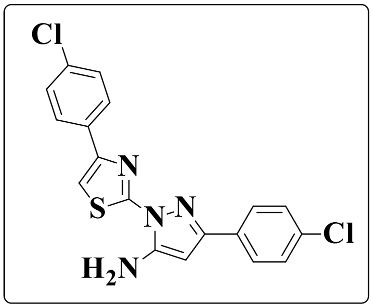
Color: light yellow solid, mp: 195-197 $^{o}$C, yield (0.317g, 83%), FT-IR (KBr, $\nu_{max}$ cm$^{-1}$): 3417 ($NH_2$), 1614 (C=N), **$^{1}$H NMR (400MHz, DMSO-$d_{63}$, ppm):** $\delta$ 3.81 (s, 3H, methoxy), 5.96 (s, 1H, pyrazole), 6.93 (bs, 2H, -$NH_2$), 7.03(d, *J*=8.8Hz, 2H, Ar-H), 7.51 (d, *J*=8.8Hz, 2H, Ar-H), 7.66 (s, 1H, Ar-H), 7.84 (d, *J*=8.8 Hz, 2H, Ar-H), 7.93 (d, *J*=7.6 Hz, 2H, Ar-H) ppm, **$^{13}$C NMR (100MHz, DMSO-$d_6$):** $\delta$ 55.15, 85.23, 107.56, 114.15, 126.26, 127.24, 127.31, 128.71, 130.97, 133.12, 149.43, 150.94, 151.96, 159.76, 161.73 ppm; **MS (ESI) *m/z* (%):** [M+H]$^{+}$; Anal. Calcd. for $C_{19}H_{15}ClN_{4}OS$; C, 59.60; H, 3.95; N, 14.63. Found: C, 59.65; H, 3.90; N, 14.68%

### 1-(4-(4-Chlorophenyl)thiazol-2-yl)-3-phenyl-1*H*-pyrazol-5-amine (23g)



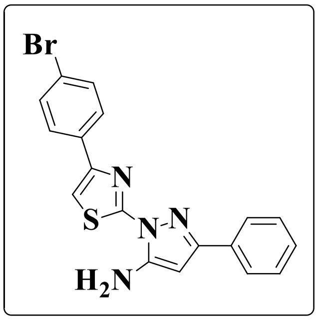
Color: white solid, mp: 195-197 $^{o}$C, yield (0.285g, 81%), FT-IR (KBr, $\nu_{max}$ cm$^{-1}$): 3417 ($NH_2$), 1614 (C=N), **$^{1}$H NMR (400MHz, DMSO-$d_6$, ppm):** $\delta$ 5.93 (s, 1H, pyrazole), 6.91 (bs, 2H, -$NH_2$), 7.30 (t, *J*=8.8 Hz, 2H, Ar-H), 7.39 (t, *J*=7.2Hz, 1H, Ar-H), 7.45 (t, *J*=7.6Hz, 2H, Ar-H), 7.83 (d, *J*=8 Hz, 3H, Ar-H), 8.07 (d, *J*=8.8 Hz, 5.6 Hz, 2H, Ar-H) ppm, **$^{13}$C NMR (100MHz, DMSO-$d_6$):** $\delta$ 85.14, 109.01, 115.52, 115.73, 125.62, 127.97, 128.06, 128.63, 130.97, 132.13, 149.37, 150.03, 153.20, 160.77, 162.05, 163.21 ppm; **MS (ESI) *m/z* (%):** 353.0616 [M+H]$^{+}$; Anal. Calcd. for $C_{18}H_{13}ClN_{4}S$; C, 61.27; H, 3.71; N, 15.88. Found: C, 61.23; H, 3.75; N, 15.84%

### 3-(4-Chlorophenyl)-1-(4-(4-chlorophenyl)thiazol-2-yl)-1*H*-pyrazol-5-amine (23h)



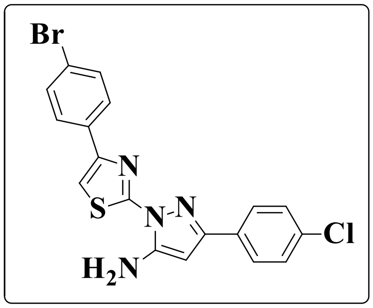
Color: white solid, mp: 195-197 $^{o}$C, yield (0.340g, 88%), FT-IR (KBr, $\nu_{max}$ cm$^{-1}$): 3401 ($NH_2$), 1609 (C=N), **$^{1}$H NMR (400MHz, DMSO-$d_6$, ppm):** $\delta$ 5.95 (s, 1H, pyrazole), 6.94 (bs, 2H, -$NH_2$), 7.30 (t, *J*=8.4Hz, 2H, Ar-H), 7.51 (d, *J*=8.8Hz, 2H, Ar-H), 7.84-7.86 (m, 3H, Ar-H), 8.07 (dd, *J*=8.8 Hz, 5.6 Hz, 2H, Ar-H) ppm, **$^{13}$C NMR (100MHz, DMSO-$d_6$):** $\delta$ 85.18, 109.13, 115.52, 115.73, 127.35, 127.99, 128.07, 128.69, 133.11, 149.51, 150.06, 152.03, 160.78, 161.94, 163.22 ppm; **MS (ESI) *m/z* (%):** [M+H]$^+$; Anal. Calcd. for $C_{18}H_{12}Cl_2N_4S$; C, 55.82; H, 3.12; N, 14.47. Found: C, 55.87; H, 3.16; N, 14.43%

**1-(4-(4-Bromophenyl)thiazol-2-yl)-3-phenyl-1*H*-pyrazol-5-amine (23i)**



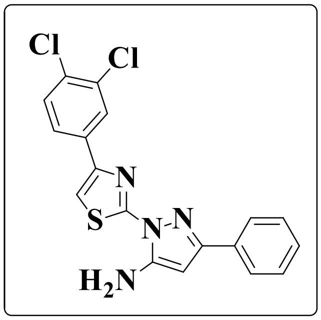
Color: Pale brown solid, mp: 195-197 $^o$C, yield (0.309g, 78%), FT-IR (KBr, $\nu_{max}$ cm$^{-1}$): 3417 ($NH_2$), 1614 (C=N), **$^1$H NMR (400MHz, DMSO-d$_6$, ppm):** $\delta$ 5.94 (s, 1H, pyrazole), 6.90 (bs, 2H, -$NH_2$), 7.40 (t, *J*=7.2 Hz, 1H, Ar-H), 7.46 (t, *J*=7.6 Hz, 2H, Ar-H), 7.66 (d, *J*=8.4 Hz, 2H, Ar-H), 7.83 (d, *J*=7.2 Hz, 2H, Ar-H), 7.93 (s, 1H, Ar-H), 7.98 (d, *J*=8.4 Hz, 2H, Ar-H) ppm, **$^{13}$C NMR (100MHz, DMSO-d$_6$):** $\delta$ 85.66, 110.60, 121.81, 126.14, 128.41, 129.14, 132.18, 132.60, 133.21, 149.87, 150.37, 153.76, 162.61 ppm; **MS (ESI) *m/z* (%):** [M+H]$^+$; Anal. Calcd. for $C_{18}H_{13}BrN_4S$; C, 54.42; H, 3.30; N, 14.10. Found: C, 54.47; H, 3.35; N, 14.15%

**1-(4-(4-Bromophenyl)thiazol-2-yl)-3-(4-chlorophenyl)-1*H*-pyrazol-5-amine (23j)**



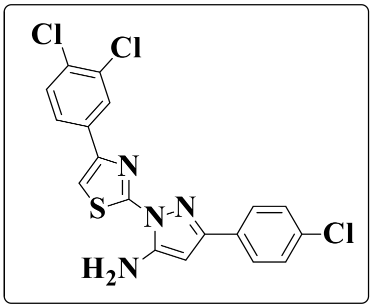
Color: pale brown solid, mp: 195-197 $^o$C, yield (0.362g, 84%), FT-IR (KBr, $\nu_{max}$ cm$^{-1}$): 3417 ($NH_2$), 1614 (C=N), **$^1$H NMR (400MHz, DMSO-d$_6$, ppm):** $\delta$ 5.95 (s, 1H, pyrazole), 6.94 (bs, 2H, -$NH_2$), 7.51 (d, *J*=8.4 Hz, 2H, Ar-H), 7.66 (d, *J*=8.4 Hz, 2H, Ar-H), 7.85 (d, *J*=8.8 Hz, 2H, Ar-H), 7.95 (s, 1H, Ar-H), 7.98 (d, *J*=8.4 Hz, 2H, Ar-H) ppm; **MS (ESI) *m/z* (%):** [M+H]$^+$; Anal. Calcd. for $C_{18}H_{12}BrClN_4S$; C, 50.08; H, 2.80; N, 12.98. Found: C, 49.95; H, 2.84; N, 12.95%

**1-(4-(3,4-Dichlorophenyl)thiazol-2-yl)-3-phenyl-1*H*-pyrazol-5-amine (23k)**



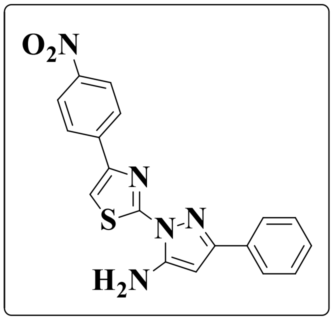
Color: pale brown solid, mp: 195-197 $^o$C, yield (0.333g, 86%), FT-IR (KBr, $\nu_{max}$ cm$^{-1}$): 3417 ($NH_2$), 1614 (C=N), **$^1$H NMR (400MHz, DMSO-d$_6$, ppm):** $\delta$ 5.93 (s, 1H, pyrazole), 6.88 (bs, 2H, -$NH_2$), 7.39 (d, *J*=7.2 Hz, 1H, Ar-H), 7.45 (d, *J*=7.6 Hz, 2H, Ar-H), 7.71 (d, *J*=8.4 Hz, 1H, Ar-H), 7.82 (d, *J*=7.2 Hz, 1H, Ar-H), 8.03 (dd, *J*=8.4 Hz, 2 Hz, 1H, Ar-H), 8.05(s, 1H, Ar-H), 8.30 (d, *J*=2 Hz, 1H, Ar-H) ppm, **$^{13}$C NMR (100MHz, DMSO-d$_6$):** $\delta$ 81.19, 111.44, 125.63, 126.07, 127.51, 128.63, 130.90, 131.68, 132.07, 134.07, 148.60, 149.39, 153.34, 162.17 ppm; **MS (ESI) *m/z* (%):** [M+H]$^+$; Anal. Calcd. for $C_{24}H_{18}N_4S$; C, 73.07; H, 4.60; N, 14.20. Found: C, 72.97; H, 4.65; N, 14.25%

### 3-(4-Chlorophenyl)-1-(4-(3,4-dichlorophenyl)thiazol-2-yl)-1*H*-pyrazol-5-amine (23l)



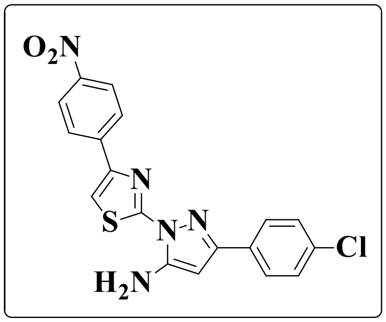
Color: pale brown solid, mp: 195-197 °C, yield (0.397g, 92%), FT-IR (KBr, $\nu_{max}$ cm$^{-1}$): 3417 (NH$_2$), 1614 (C=N), **$^1$H NMR (400MHz, DMSO-d$_6$, ppm):** *δ* 5.95 (s, 1H, pyrazole), 6.95 (bs, 2H, -NH$_2$), 7.52 (d, *J*=8.4 Hz, 2H, Ar-H), 7.71-7.75 (m, 2H, Ar-H), 7.85 (d, *J*=8.4 Hz, 2H, Ar-H), 8.07 (s, 1H, Ar-H), 8.30 (s, 1H, Ar-H) ppm; **MS (ESI) *m/z* (%):** [M+H]$^+$; Anal. Calcd. for $C_{24}H_{17}ClN_4S$; C, 67.20; H, 3.99; N, 13.06. Found: C, 67.25; H, 3.94; N, 12.95%

### 1-(4-(4-Nitrophenyl)thiazol-2-yl)-3-phenyl-1*H*-pyrazol-5-amine (23m)



Color: yellow solid, mp: 195-197 °C, yield (0.323g, 89%), FT-IR (KBr, $\nu_{max}$ cm$^{-1}$): 3417 (NH$_2$), 1614 (C=N), **$^1$H NMR (400MHz, DMSO-d$_6$, ppm):** *δ* 5.94 (s, 1H, pyrazole), 6.94 (bs, 2H, -NH$_2$), 7.40 (t, *J*=7.2 Hz, 1H, Ar-H), 7.46 (t, *J*=7.6 Hz, 2H, Ar-H), 7.83 (d, *J*=7.2 Hz, 2H, Ar-H), 8.15 (d, *J*=8.8Hz, 2H, Ar-H), 8.22 (s, 1H, Ar-H), 8.38 (d, *J*=8.8 Hz, 2H, Ar-H) ppm; **MS (ESI) *m/z* (%):** [M+H]$^+$; Anal. Calcd. for $C_{18}H_{13}N_5O_2S$; C, 59.49; H, 3.61; N, 19.27. Found: C, 59.44; H, 3.65; N, 19.24%

### 3-(4-Chlorophenyl)-(1-(4-(4-nitrophenyl)thiazol-2-yl)-3-phenyl-1*H*-pyrazol-5-amine (23n)



Color: yellow solid, mp: 195-197 °C, yield (0.367g, 91%), FT-IR (KBr, $\nu_{max}$ cm$^{-1}$): 3459 (NH$_2$), 1607 (C=N), **$^1$H NMR (400MHz, CDCl$_3$, ppm):** *δ* 5.95 (s, 1H, pyrazole), 6.86 (bs, 2H, -NH$_2$), 7.41 (t, *J*=8.4 Hz, 3H, Ar-H), 7.77 (d, *J*=8 Hz, 2H, Ar-H), 8.00 (d, *J*=8.4 Hz, 2H, Ar-H), 8.31 (d, *J*=8.4 Hz, 2H, Ar-H) ppm; **MS (ESI) *m/z* (%):** [M+H]$^+$; Anal. Calcd. for $C_{18}H_{12}ClN_5O_2S$; C, 54.34; H, 3.04; N, 17.60. Found: C, 54.30; H, 2.95; N, 17.56%

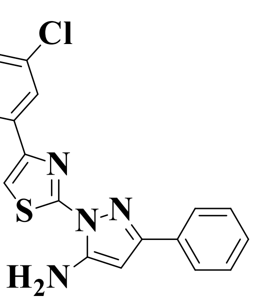
## BIOLOGICAL ACTIVITY

### *In vitro* anticancer assay

The *in vitro* anticancer activity procedure already mentioned in the chapter-II.

### *In vitro* anticancer activity results and discussion

Among all the synthesized compounds, 6 compounds were selected for anticancer activity screening against 60 human cancer cell lines at 10 µM. The results are shown in table 4. **23k** showed moderate activity on breast cancer MCF7 cell line (51.21% growth, 48.79% inhibition), T-47D cell line (58.18% growth, 41.82% inhibition).



**23k**

The compound **23k** shown moderate anticancer activity.

**Table 4:** ***In vitro*** **anticancer activity**

| | Growth percentage (%) | | | | | |
|---|---|---|---|---|---|---|
| **Cancer cell lines** | **23b** | **23g** | **23h** | **23j** | **23k** | **23l** |
| **Non-small cell lung cancer** | | | | | | |
| **A549/ATCC** | 91.08 | 81.18 | 84.38 | 88.05 | 78.98 | 79.71 |
| **EKVX** | 87.91 | 77.39 | 97.31 | 93.59 | 74.30 | 77.43 |
| **HOP-62** | 91.18 | 76.76 | 86.75 | 97.91 | 78.57 | 86.64 |
| **NCI-H322M** | 88.91 | 75.99 | 86.48 | 93.29 | 86.27 | 72.63 |
| **NCI-H522** | 97.37 | 75.16 | 76.60 | 84.18 | **69.09** | 83.21 |
| **Colon cancer** | | | | | | |
| **HT29** | 102.06 | 86.49 | 79.06 | 92.0 | 91.37 | 89.15 |
| **Melanoma** | | | | | | |
| **UACC-257** | 93.76 | 79.93 | 78.07 | 92.14 | 81.70 | 81.98 |
| **Ovarian cancer** | | | | | | |
| **IGROV1** | 100.37 | 79.55 | 93.78 | 99.90 | 84.89 | 80.02 |
| **SK-OV-3** | 87.63 | 82.67 | 100.34 | 92.36 | 78.29 | 94.18 |
| **Renal cancer** | | | | | | |
| **UO-31** | 77.90 | 84.09 | 100.04 | 93.41 | 79.73 | 85.22 |
| **Breast cancer** | | | | | | |
| **MCF7** | 82.78 | **66.95** | 86.75 | 89.83 | **51.21** | 81.31 |
| **T-47D** | 91.90 | **65.43** | 86.03 | 98.20 | **58.18** | 97.40 |

## *In vitro* antimicrobial activity

### Primary antimicrobial screening

Primary antimicrobial screening was done against 5 types of bacteria: *Escherichia coli, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa* and *Staphylococcus aureus,* and 2 yeast *Candida albicans* and *Cryptococcus neoformans* as the study by hole cell growth inhibition assays, using the provided samples at a single concentration - 32 μg/mL, n=2, where one or both replicates were deemed active the compound is deemed active. (I- Inactive- inhibition < 80% or Z-Score < 2.5), (A- Active- inhibition ≥ 80% and Z-Score ≥ 2.5)

### Hit-conformation Study

Hit confirmation of active compounds by whole cell growth inhibition assays was conducted as an 8-point dose response to determine the minimum inhibitory concentration (MIC), in duplicate (n=2). The inhibition of growth is measured against those microorganisms that showed susceptibility to the compounds tested in the primary screen.

Active compounds were also counter screened for cytotoxicity against a human embryonic kidney cell line, HEK293, by determining their $CC_{50}$ value.

### Experimental methods: Anti-microbial Hit-conformation

### Sample preparation

Samples were provided by the collaborator as dry material, and were mixed at the rate of 10mg/mL in DMSO or water and stored frozen at -20 °C. Samples were prepared in DMSO and water to a final testing concentration of 32 to 0.25 μg/mL and serially diluted 1:2 fold for 8 times. Each sample concentration was prepared in 384-well plates, non-binding surface plate (**NBS;** Corning 3640) for each bacterial/fungal strain or tissue-culture treated (**tc treated;** Corning 3712/3764) black for mammalian cell types, all in duplicate (n=2), and keeping the final DMSO concentration to a maximum of 0.5% DMSO. All the sample preparation was done using liquid handling robots.

### Antibacterial assay

All bacteria were cultured in Cation-adjusted Mueller Hinton broth (CAMHB) at 37 °C overnight. A sample of each culture was then diluted 40-fold in fresh broth and incubated at 37 °C for 1.5-3 h. The resultant mid-log phase cultures were diluted (CFU/mL measured by OD600), then added to each well of the compound containing plates, giving a cell density of 5×105 CFU/mL and a total volume of 50 µL. All the plates were covered and incubated at 37 °C for 18 h without shaking.

**Analysis**

Inhibition of bacterial growth was determined to measure the absorbance at 600 nm (OD600), using a Tecan M1000 Pro monochromator plate reader. The percentage of growth inhibition was calculated for each well, using the negative control (media only) and positive control (bacteria without inhibitors) on the same plate as references.

The percentage of growth inhibition was calculated for each well, using the negative control (media only) and positive control (bacteria without inhibitors) on the same plate. The MIC was determined as the lowest concentration at which the growth was fully inhibited, defined by an inhibition ≥ 80%. In addition, the maximal percentage of growth inhibition is reported as DMax, indicating any compounds with partial activity.

Hits were classified by MIC ≤ 16 µg/mL in either replicate (n=2 on different plates).

**Antifungal assay**

Fungi strains were cultured for 3 days on Yeast Extract-Peptone Dextrose (YPD) agar at 30 °C. A yeast suspension of 1 x $10^6$ to 5 x $10^6$ CFU/mL (as determined by OD530) was prepared from five colonies. The suspension was subsequently diluted and added to each well of the compound-containing plates giving a final cell density of fungi suspension of $2.5\times10^3$ CFU/mL and a total volume of 50 µL. All plates were covered and incubated at 35 °C for 36 h without shaking.

**Analysis**

Growth inhibition of *C. albicans* was determined to measure absorbance at 630 nm (OD630), while the growth inhibition of *C. neoformans* was determined to measure the difference in absorbance between 600 and 570 nm (OD600-570), after the addition of resazurin (0.001% final concentration) and incubation at 35 °C for about 2 hours. The absorbance was recorded using a Biotek Multiflo Synergy HTX plate reader.

In both cases, the percentage of growth inhibition was calculated for each well, using the negative control (media only) and positive control (fungi without inhibitors) on the same plate. The MIC was determined as the lowest concentration at which the growth was fully inhibited, defined by an inhibition ≥ 80% for *C. albicans* and an inhibition ≥ 70% for *C. neoformans*. Due to higher variance in growth and inhibition, a lower threshold was applied to the data for *C. neoformans*. In addition, the maximal percentage of growth inhibition is reported as $D_{Max}$, indicating any compounds with marginal activity.

Hits were classified by MIC ≤ 16 µg/mL in either replicate (n=2 on different plates).

### Cytotoxicity assay

HEK293 cells were counted manually in a Neubauer hemocytometer and then plated in the 384-well plates containing the compounds to give a density of 6000 cells/well in a final volume of 50 µL. DMEM supplemented with 10% FBS was used as growth media and the cells were incubated together with the compounds for 20 h at 37 $^{o}$ C in 5% $CO_2$.

### Analysis

Cytotoxicity (or cell viability) was measured by fluorescence, ex: 560/10 nm, em: 590/10 nm (F560/590), after addition of 5 µL of 25 µg/mL Resazurin (2.3 µg/mL final concentration) and after incubation for further 3 h at 37 °C in 5% $CO_2$. The fluorescence intensity was measured using a Tecan M1000 Pro monochromator plate reader, using automatic gain calculation.

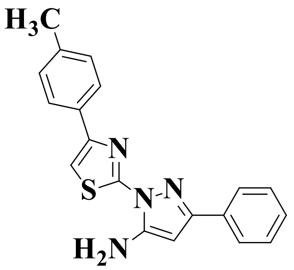
$CC_{50}$ (concentration at 50% cytotoxicity) were calculated by curve fitting the inhibition values vs. log(concentration) using sigmoidal dose-response function, with variable fitting values for the bottom, top, and slope. In addition, the maximal percentage of cytotoxicity is reported as $D_{Max}$, indicating compounds with partial cytotoxicity.

The curve fitting was implemented using Pipeline Pilot's dose-response component (giving similar results to similar tools such as GraphPad's Prism and IDBS's XlFit). Any value with > indicates sample with no activity (low $D_{Max}$ value) or samples with $CC_{50}$ values above the maximum tested concentration (higher $D_{Max}$ value).

Cytotoxic samples were classified by $CC_{50}$ < 32 µg/mL in either replicate (n=2 on different plates).

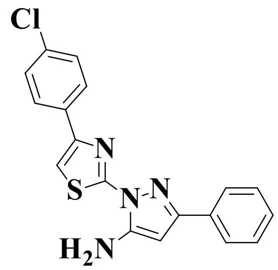
### Antibiotic and cytotoxic standards preparation and quality control

Colistin and Vancomycin were used as positive bacterial inhibitor standards for gram-negative and gram-positive bacteria, respectively. Fluconazole was used as a positive fungal inhibitor standard for *C. albicans and C. neoformans.* Tamoxifen was used as a positive cytoxicity standard. Each standard was provided in 4 concentrations, with 2 above and 2 below its MIC or CC50 value, and plated into the first 8 wells of column 23 of the 384-well NBS plates. Quality Control (QC) of the assays was determined by Z'-factor, calculated from the negative (media only) and positive controls (bacterial, fungal or cell culture without inhibitor), and the standards. Plates with a Z'-factor of ≥ 0.4 and standards active at the highest and inactive at the lowest concentration, were accepted for further data analysis.



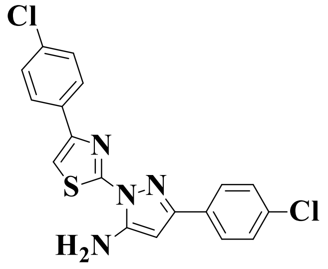
**23c**

***Candida albicans***
**(MIC- 8 µg/mL)**
***Cryptococcus neoformans***
**(MIC- 2 µg/mL)**



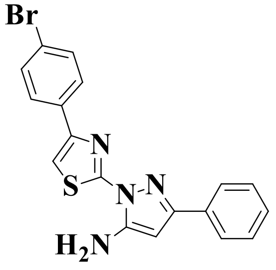
**23g**

***Candida albicans***
**(MIC- 4 µg/mL)**
***Cryptococcus neoformans***
**(MIC- 1 µg/mL)**



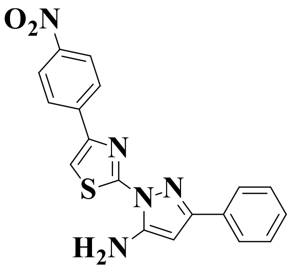
**23h**

***Cryptococcus neoformans***
**(MIC- 8 µg/mL)**



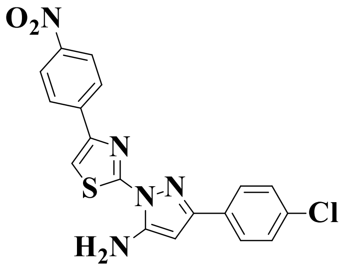
**23i**

***Cryptococcus neoformans***
**(MIC- 4 µg/mL)**



**23m**

***Cryptococcus neoformans***
**(MIC- 8 µg/mL)**



**23n**

***Cryptococcus neoformans***
**(MIC- 8 µg/mL)**

Potent antimicrobial activity containing compounds

**Table 5:** ***In vitro*** **antimicrobial primary screening.**

| **Compound** | **Antimicrobial activity at 32 μg/mL** | | | | | | |
|---|---|---|---|---|---|---|---|
| | ***Escherichia coli*** **ATCC 25922 FDA control** | ***Klebsiella pneumoniae*** **ATCC 700603 MDR** | ***Acinetobacter baumannii*** **ATCC 19606 Type strain** | ***Pseudomonas aeruginosa*** **ATCC 27853 Type strain** | ***Staphylococcus aureus*** **ATCC 43300 MRSA** | ***Candida albicans*** **ATCC 90028 CSLI Ref** | ***Cryptococcus neoformans*** **ATCC 20881 H99** |
| **23a** | I | I | I | I | I | I | I |
| **23b** | I | I | I | I | I | I | I |
| **23c** | I | I | I | I | I | **A** | I |
| **23d** | I | I | I | I | I | I | I |
| **23e** | I | I | I | I | I | I | I |
| **23f** | I | I | I | I | I | I | I |
| **23g** | I | I | I | I | I | **A** | I |
| **23h** | I | I | I | I | I | **A** | I |
| **23i** | I | I | I | I | I | **A** | **A** |
| **23j** | I | I | I | I | I | I | I |
| **23k** | I | I | I | I | I | I | I |
| **23l** | I | I | I | I | I | I | I |
| **23m** | I | I | I | I | I | **A** | **A** |
| **23n** | I | I | I | I | I | **A** | **A** |

(I- Inactive- inhibition < 80% or Z-Score < 2.5), (A- Active- inhibition ≥ 80% and Z-Score ≥ 2.5)

**Table 6: The *In vitro* antimicrobial activity (Hit- confirmation)**

| Compound | MIC [µg/mL] | | | | | | | CC50 [µg/mL] |
|---|---|---|---|---|---|---|---|---|
| | ***Escherichia coli*** **ATCC 25922 FDA control** | ***Klebsiella pneumoniae*** **ATCC 700603 MDR** | ***Acinetobacter baumannii*** **ATCC 19606 Type strain** | ***Pseudomonas aeruginosa*** **ATCC 27853 Type strain** | ***Staphylococcus aureus*** **ATCC 43300 MRSA** | ***Candida albicans*** **ATCC 90028 CSLI Ref** | ***Cryptococcus neoformans*** **ATCC 20881 H99** | **HEK293 ATCC CRL1573** |
| **23c** | >32 | >32 | >32 | >32 | >32 | **8** | **2** | >32 |
| **23g** | >32 | >32 | >32 | >32 | >32 | **4** | **1** | >32 |
| **23h** | >32 | >32 | >32 | >32 | >32 | >32 | **8** | >32 |
| **23i** | >32 | >32 | >32 | >32 | >32 | >32 | **4** | >32 |
| **23m** | >32 | >32 | >32 | >32 | >32 | >32 | **8** | >32 |
| **23n** | >32 | >32 | >32 | >32 | >32 | >32 | **8** | >32 |

**Anti-microbial activity conclusion**

The *in vitro* antimicrobial study identified **23c**, **23g**, **23h**, **23i**, **23m**, and **23n** compounds with inhibitory activity ≤ 16 µg/mL against the organisms including *C. albicans* and *C. neoformans*, with all six compounds displaying no cytotoxicity against mammalian HEK293 cells. In Hit-conformation, compounds **23c** and **23g** showed potent activity against *C. albicans* with MIC values of **8** and **4** µg/mL respectivly. Compounds **23c, 23g, 23i, 23m,** and **23n** 4 showed potent activity against *C. neoformans* with MIC values **2, 1, 8, 4, 8** and **8** µg/mL respectively.

**Antiviral activity assay**

The antiviral activity of the chemical compounds was assayed in various cell lines.

(i) HELA cells were seeded in 96-well plates and cultured for six days in CO2 (5%) incubator at 37°C. Old culture medium was replaced by serial dilutions of the test chemical compounds (100 µl per well). Different types of virus (*Herpes simplex virus type 1, Herpes simplex virus type 2, Herpes simplex virus type 1 TK- ACVr, Vaccinia virus, Adeno virus-2* or *Vesicular stomatitis virus*), diluted in culture medium and added to each well (to acquire a virus input of 100 CCID50). Mock-treated cell cultures receiving solely the test compounds were included, to determine the cytotoxicity. Four days later, microscopic analysis was employed to score the cytopathogenic effect (CPE) induced by virus.

(ii) Vero cells (derived from African green monkey) were seeded in 96-well plates and cultured for 24 hrs at 37°C in CO2 incubator. Culture medium was replaced by fresh medium with serial dilutions of the test chemical compounds. Various virus strains (*Coxsackie virus B4, Sindbis virus, Para influenza virus 3, Punta Toro virus* or *Reovirus*) were added to each well and diluted to obtain a virus input of 100 CCID50. To detect the cytotoxicity of the compound, mock treated cell cultures were treated with only compound (without any virus). Microscopic analysis was performed to detect the virus-induced pathogenicity (CPE) for Coxsackie virus B4 and Sindbis virus (three days post infection) or Parainfluenza virus 3, Punta Toro virus and Reovirus (six days of post infection).

(iii) To assay the antiviral activity HeLa cells (15,000/well) were seeded in 96-well plates and incubated for 24hrs at 37°C incubator until confluence was reached. Fresh medium was added with serial diluted the test chemical compounds (100 µl/well). One hundred microliters of the virus (*Coxsackie virus B4, Vesicular stomatitis virus* or *Respiratory syncytial virus*) were diluted in medium to obtain a virus input of 100 CCID50 and added to each well. Mock-treated cell cultures were added with only test compounds, to determine the compound induced cytotoxicity. Three days post infection (for Vesicular stomatitis virus and Coxsackie virus B4 and) or six days (for Respiratory syncytial virus) microscopic analysis was employed to score the virus-induced cytopathogenic effect (CPE).

(iv) Madin-Darby Canine Kidney (MDCK) cells were seeded in 96- well plates (7,500 cells/well) and incubated for 24hrs at 35°C. Existing culture medium was replaced with freshly prepared medium and serial diluted test compounds (100 µl/well). Different types of virus (*Influenza B*

*B/Ned/537/05 or Influenza A/H1N1 A/Ned/378/05, Influenza A/H3N2 A/HK/7/87*) were diluted in the culture medium (to acquire a virus input of 100 CCID50) and added to each well. Mock-treated cell cultures were added with only test compounds, to determine the compound induced cytotoxicity. Four days later, virus-induced cytopathogenic effect (CPE) was assayed by visual scoring, and by performing the formazan dye based MTS assay to determine cell viability.

(v) CRFK cells were cultured in 96-well plates (30,000 cells/well) and incubated in CO2 incubator at 37°C for 24 hrs. Serial dilutions of test compounds were prepared in fresh medium and added to each well (100 µl/well). *Feline herpes virus* or *Feline corona virus* were diluted in a medium (100 µl/well) to obtain a virus input of 100 CCID50. Mock-treated cell cultures were added with only test compounds, to determine the compound induced cytotoxicity. Four days later, virus-induced cytopathogenic effect (CPE) was assayed by visual scoring, and by performing the formazan dye based MTS assay to determine cell viability 65-67.

### ***In vitro* assays for antiviral activity**

Chemically synthesized various compounds were tested for the antiviral activity against different human viruses. The HELA cells were used to evaluate the compounds against *Herpes simplex* virus type I (KOS) [HSV-1 KOS], *Herpes simplex* virus type 2 (G) [HSV-2 G], *Herpes simplex virus-1* TK$^-$KOS ACV, thymidine kinase-deficient *Herpes simplex* virus type 1 (HSV-1 TK- KOS ACV), *Vaccinia* virus [VV] and *Adeno* virus type 2 (AV-2). HeLa cells were used for *Vesicular stomatitis*, *Coxsackie* virus B4, *Respiratory syncytial* virus. African green monkey derived Vero cells were used to estimate the chemical compounds against *Parainfluenza* type 3 virus (PI-3V), *Sindbis* virus (SV), *Coxsackie* virus B4 (CV B4), *Punto toro* virus (PTV) and *Yellow Fever virus* (YFV). The CRFK derived cell culture medium was used for *Feline Corona* Virus (FIPV) and *Feline Herpes* Virus. To infect *Influenza* A virus type H1N1 (strain A/Ned/378/05) and type H3N2 (strain A/HK/7/87), and *Influenza* B virus (strain B/Ned/537/05) MDCK cells were used. Based on the microscopica analysis of the virus-induced cytopathicity (CPE) the antiviral potency of the compounds was determined and compared to different reference antiviral drugs (brivudin, cidofovir, acyclovir, ganciclovir, zalcitabine, and alovudine). By using microscopic analysis virus-induced cytopathogenic effect (CPE) and antiviral potency of the chemical compounds were estimated and compared with various antiviral drugs (acyclovir, alovudine, brivudin, cidofovir, ganciclovir and zalcitabine). Results showed in **Table 7-11**.

**Table 7: Cytotoxicity and antiviral activity in HEL cell cultures**

| Compound | Con. unit | Minimum cytotoxic con.[a] | $EC_{50}$[b] | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Herpes simplex virus-1 (KOS) | Herpes simplex virus-2 (G) | Herpes simplex virus-1 $TK^{-}$KOS $ACV^{r}$ | Vaccinia virus | Adeno virus-2 | Human Corona virus (229E) |
| **23a** | µM | ≥20 | >100 | >100 | >100 | >100 | >100 | >100 |
| **23b** | µM | ≥20 | >100 | >100 | >100 | >100 | >100 | >100 |
| **23c** | µM | ≥100 | >100 | >100 | >100 | >100 | >100 | >100 |
| **23d** | µM | ≥100 | >100 | >100 | >100 | >100 | >100 | >100 |
| **23e** | µM | ≥20 | >100 | >100 | >100 | >100 | >100 | >100 |
| **23f** | µM | ≥20 | >100 | >100 | >100 | >100 | >100 | >100 |
| **23g** | µM | - | - | - | - | - | - | - |
| **23h** | µM | >100 | >100 | >100 | >100 | >100 | >100 | >100 |
| **23i** | µM | >100 | >100 | >100 | >100 | >100 | >100 | >100 |
| **23j** | µM | ≥20 | >100 | >100 | >100 | >100 | >100 | >100 |
| **23k** | µM | - | - | - | - | - | - | - |
| **23l** | µM | 4 | >100 | >100 | >100 | >100 | >100 | >100 |
| **23m** | µM | >100 | >100 | >100 | >100 | >100 | >100 | >100 |
| **23n** | µM | >100 | >100 | >100 | >100 | >100 | >100 | >100 |
| **Brivudin** | µM | >250 | 0.08 | 112 | 10 | 10 | - | - |
| **Cidofovir** | µM | >250 | 4.5 | 2 | 3.4 | 22 | 29 | - |
| **Acyclovir** | µM | >250 | 0.7 | 0.2 | 85 | >250 | - | - |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Ganciclovir** | µM | >100 | 0.16 | 0.06 | 4 | >100 | - | - |
| **Zalcitabine** | µM | >250 | - | - | - | - | 19 | - |
| **Alovudine** | µM | >250 | - | - | - | - | 50 | - |
| **UDA** | µg/ml | >100 | - | - | - | - | - | 1.8 |
| **Ribavirin** | µM | >250 | - | - | - | - | - | 112 |

[a] Required to cause a microscopically detectable alteration of normal cell morphology.

[b] Required to reduce virus-induced cytopathogenicity by 50%

Urtica dioica agglutinin (UDA)

**Table 8: Cytotoxicity and antiviral activity in HeLa cell cultures**

| | | | $EC_{50}$[b] | | |
|---|---|---|---|---|---|
| **Compound** | **Con. unit** | **Min cytotoxic con**[a] | **Vascular stomatitis virus** | **Coxsackie virus B4** | **Respiratory syncytial virus** |
| **23a** | µM | 100 | >100 | >100 | >100 |
| **23b** | µM | 100 | >100 | >100 | >100 |
| **23c** | µM | >100 | >100 | >100 | >100 |
| **23d** | µM | >100 | >100 | >100 | >100 |
| **23e** | µM | ≥100 | >100 | >100 | >100 |
| **23f** | µM | >100 | >100 | >100 | >100 |
| **23g** | µM | - | - | - | - |
| **23h** | µM | >100 | >100 | >100 | >100 |
| **23i** | µM | >100 | >100 | >100 | >100 |
| **23j** | µM | >100 | >100 | >100 | >100 |
| **23k** | µM | - | - | - | - |
| **23l** | µM | ≥4 | >100 | >100 | >100 |
| **23m** | µM | >100 | >100 | >100 | >100 |
| **23n** | µM | >100 | >100 | >100 | >100 |
| **DS-10.000** | µg/ml | >100 | 0.4 | 6.8 | 0.4 |
| **Ribavirin** | µM | >250 | 2.5 | 50 | 10 |

[a] Required to cause a microscopically detectable alteration of normal cell morphology.

[b] Required to reduce virus-induced cytopathogenicity by 50 %.

Table 9: Cytotoxicity and antiviral activity in Vero cell cultures

| Compound | Con. unit | Min. cytotoxic con.[a] | EC$_{50}$[b] Para-influenza-3 virus | Reovirus -1 | Sindbis virus | Coxsackie virus B4 | Punta Toro virus | Yellow Fever virus |
|---|---|---|---|---|---|---|---|---|
| **23a** | µM | 100 | >100 | >100 | >100 | >100 | >100 | >100 |
| **23b** | µM | 100 | >100 | >100 | >100 | >100 | >100 | >100 |
| **23c** | µM | ≥100 | >100 | >100 | >100 | >100 | >100 | >100 |
| **23d** | µM | ≥100 | >100 | >100 | >100 | >100 | >100 | >100 |
| **23e** | µM | 100 | >100 | >100 | >100 | >100 | >100 | >100 |
| **23f** | µM | 100 | >100 | >100 | >100 | >100 | >100 | >100 |
| **23g** | µM | - | - | - | - | - | - | - |
| **23h** | µM | 100 | >100 | >100 | >100 | >100 | >100 | >100 |
| **23i** | µM | 100 | >100 | >100 | >100 | >100 | >100 | >100 |
| **23j** | µM | 100 | >100 | >100 | >100 | >100 | >100 | >100 |
| **23k** | µM | - | - | - | - | - | - | - |
| **23l** | µM | ≥4 | >100 | >100 | >100 | >100 | >100 | >100 |
| **23m** | µM | ≥100 | >100 | >100 | >100 | >100 | >100 | >100 |
| **23n** | µM | 100 | >100 | >100 | >100 | >100 | >100 | >100 |
| **DS-10.000** | µg/ml | >100 | >100 | >100 | 100 | 45 | 2.3 | 0.8 |
| **Ribavirin** | µM | >250 | 146 | >250 | >250 | >250 | 125 | >250 |
| **Mycophenolic acid** | µM | ≥100 | 0.4 | 0.5 | 20 | 45 | 4.0 | 0.8 |

**Table 10: Cytotoxicity and antiviral activity in CRFK cell cultures**

| | | | $EC_{50}$[b] | |
|---|---|---|---|---|
| **Compound** | **Concentration unit** | **$CC_{50}$[a]** | **Feline Coronavirus (FIPV)** | **Feline Herpesvirus** |
| **23a** | µM | >100 | >100 | >100 |
| **23b** | µM | >100 | **4.3** | **10.5** |
| **23c** | µM | >100 | >100 | >100 |
| **23d** | µM | >100 | >100 | >100 |
| **23e** | µM | >100 | >100 | >100 |
| **23f** | µM | >100 | >100 | >100 |
| **23g** | µM | - | - | - |
| **23h** | µM | >100 | >100 | >100 |
| **23i** | µM | >100 | >100 | >100 |
| **23j** | µM | >100 | >100 | >100 |
| **23k** | µM | - | - | |
| **23l** | µM | >100 | >100 | >100 |
| **23m** | µM | >100 | >100 | >100 |
| **23n** | µM | >100 | >100 | >100 |
| **HHA** | µg/ml | 79.4 | 9.4 | 2.3 |
| **UDA** | µg/ml | >100 | 5.7 | 2.0 |
| **Ganciclovir** | µM | >100 | >100 | 3.4 |

[a] 50% Cytotoxic concentration, as determined by measuring the cell viability with colorimetric formazan-based MTS assay.

[b] 50% Effective concentration, or concentration producing 50% inhibition of virus-induced cytopathic effect, as determined by measuring the cell viability with colorimetric formazan-based MTS assay.

CRFK cells: Crandell-Rees Feline Kidney cells. (UDA: Urtica Dioica Agglutinin)

**Table 11: Cytotoxicity and antiviral activity in MDCK cell cultures**

| Compound | Con. unit | Cytotoxicity | | Antiviral $EC_{50}$[c] | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | $CC_{50}$[a] | Minimum cytotoxic con.[b] | Influenza A/H1N1 A/Ned/378/05 | | Influenza A/H3N2 A/HK/7/87 | | Influenza B B/Ned/537/05 | |
| | | | | Visual CPE score | MTS | Visual CPE score | MTS | Visual CPE score | MTS |
| **23a** | µM | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 |
| **23b** | µM | >100 | ≥100 | >100 | >100 | >100 | >100 | >100 | >100 |
| **23c** | µM | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 |
| **23d** | µM | >100 | ≥100 | >100 | >100 | >100 | >100 | >100 | >100 |
| **23e** | µM | 57.5 | 100 | >100 | >100 | >100 | >100 | >100 | >100 |
| **23f** | µM | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 |
| **23g** | µM | - | - | - | - | - | - | - | - |
| **23h** | µM | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 |
| **23i** | µM | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 |
| **23j** | µM | >100 | 100 | >100 | >100 | >100 | >100 | >100 | >100 |
| **23k** | µM | - | - | - | - | - | - | - | - |
| **23l** | µM | >100 | 100 | >100 | >100 | >100 | >100 | >100 | >100 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **23m** | µM | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 |
| **23n** | µM | >100 | 100 | >100 | >100 | >100 | >100 | >100 | >100 |
| **Zanamivir** | µM | >100 | >100 | 0.4 | 0.1 | 0.8 | 0.1 | 0.5 | 0.4 |
| **Rebavirin** | µM | >100 | 100 | 8.9 | 4.4 | 4.0 | 1.0 | 8.9 | 8.4 |
| **Amantadine** | µM | >100 | >100 | 100 | >100 | 0.2 | 0.1 | >100 | >100 |
| **Remantadine** | µM | >200 | >200 | >200 | >200 | 0.02 | 0.01 | >200 | >200 |

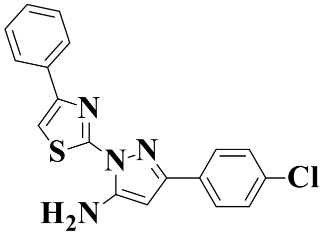
[a] 50% Cytotoxic concentration, as determined by measuring the cell viability with colometric formazan-based MTS assay.

[b] Minimum compound concentration that causes microscopically detectable alteration of normal cell morphology.

[c] 50% Effective concentration, or concentration producing 50% inhibition of virus-induced cytopathic effect, as determined by measuring the cell viability with colometric formazan-based MTS assay.

***In vitro* antiviral activity conclusion:**

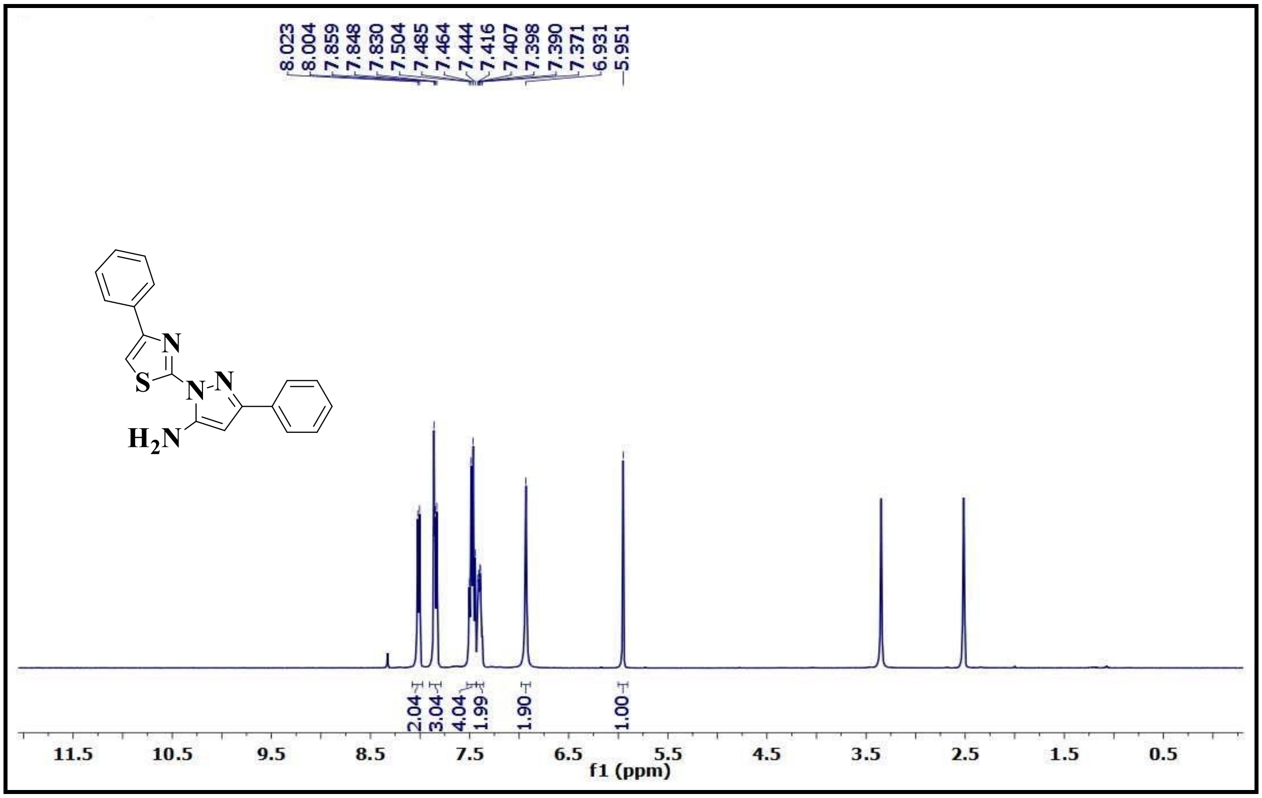
Among the tested compounds, compound **23b** showed potent activity against *feline corona virus* with $EC_{50}$ value **4.3** µM compared to standard drugs HHA and UDA, significant activity against *feline herpes virus* with $EC_{50}$ value **10.5** µM.



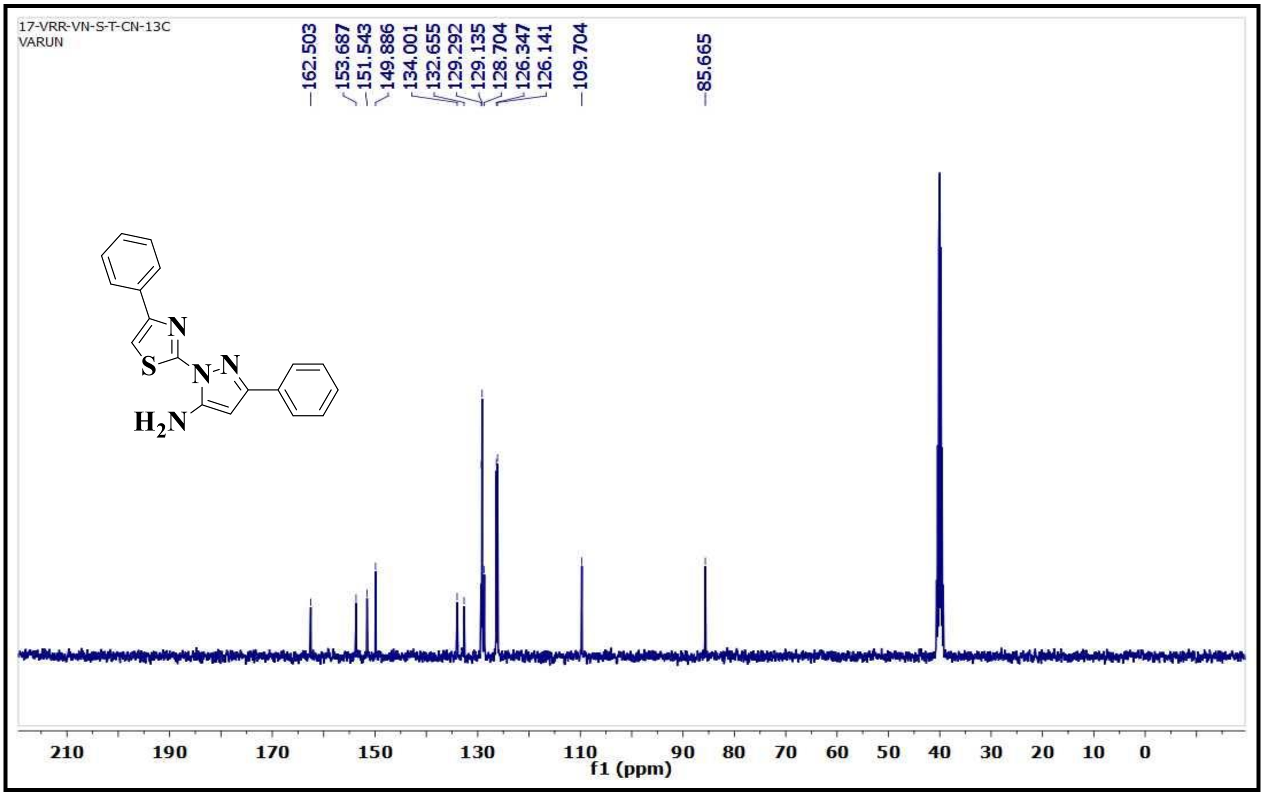
**23b**

## SUMMARY

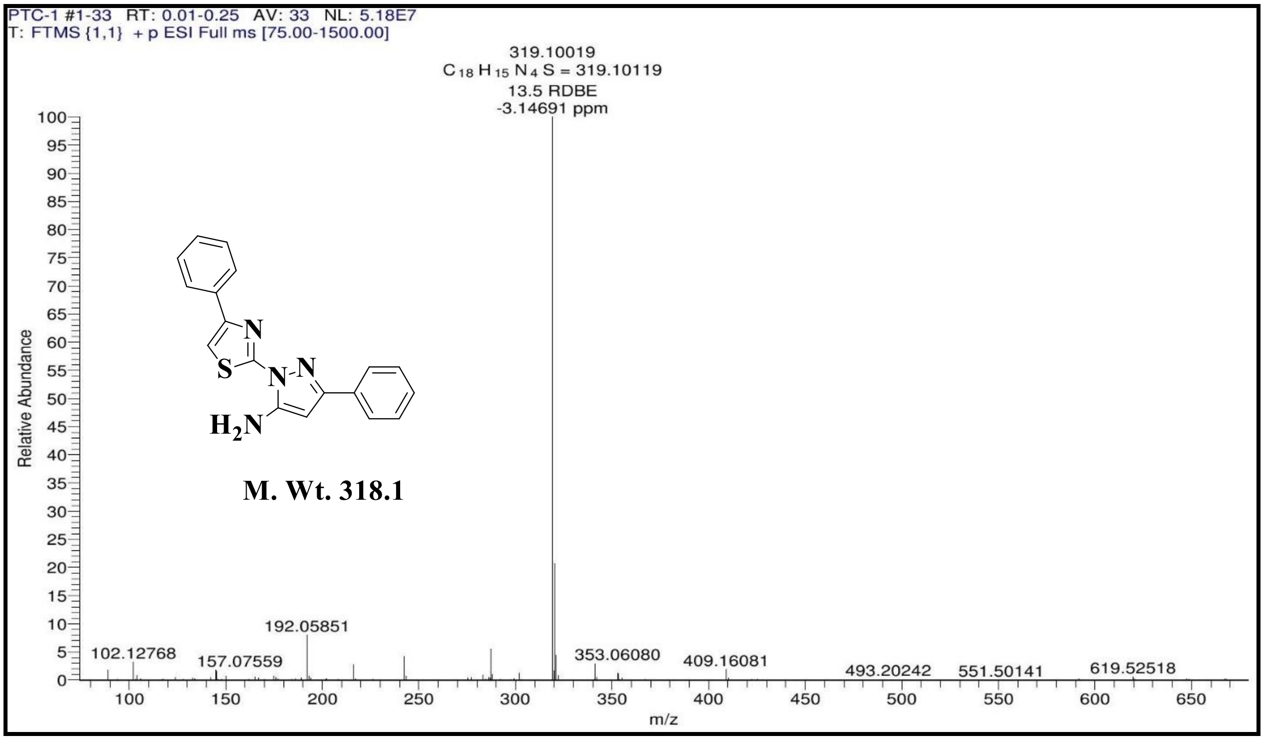
In summary, we have synthesized the above compounds through one-pot three component reaction. Among the synthesized compounds, compound **23b** showed potent activity against *feline corona virus*, significant activity against *feline herpes virus*. All synthesized compounds were tested for antimicrobial activity, Hit-conformation of active compounds by whole cell growth inhibition assays were conducted as an 8-point dose response to determine the minimum inhibitory concentration (MIC) values, compounds **23c, 23g, 23h, 23i, 23m** and **23n** showed potent antifungal activity. Compound **23k** showed moderate anticancer activity against breast cancer MCF7 cell line and T-47D cell line.



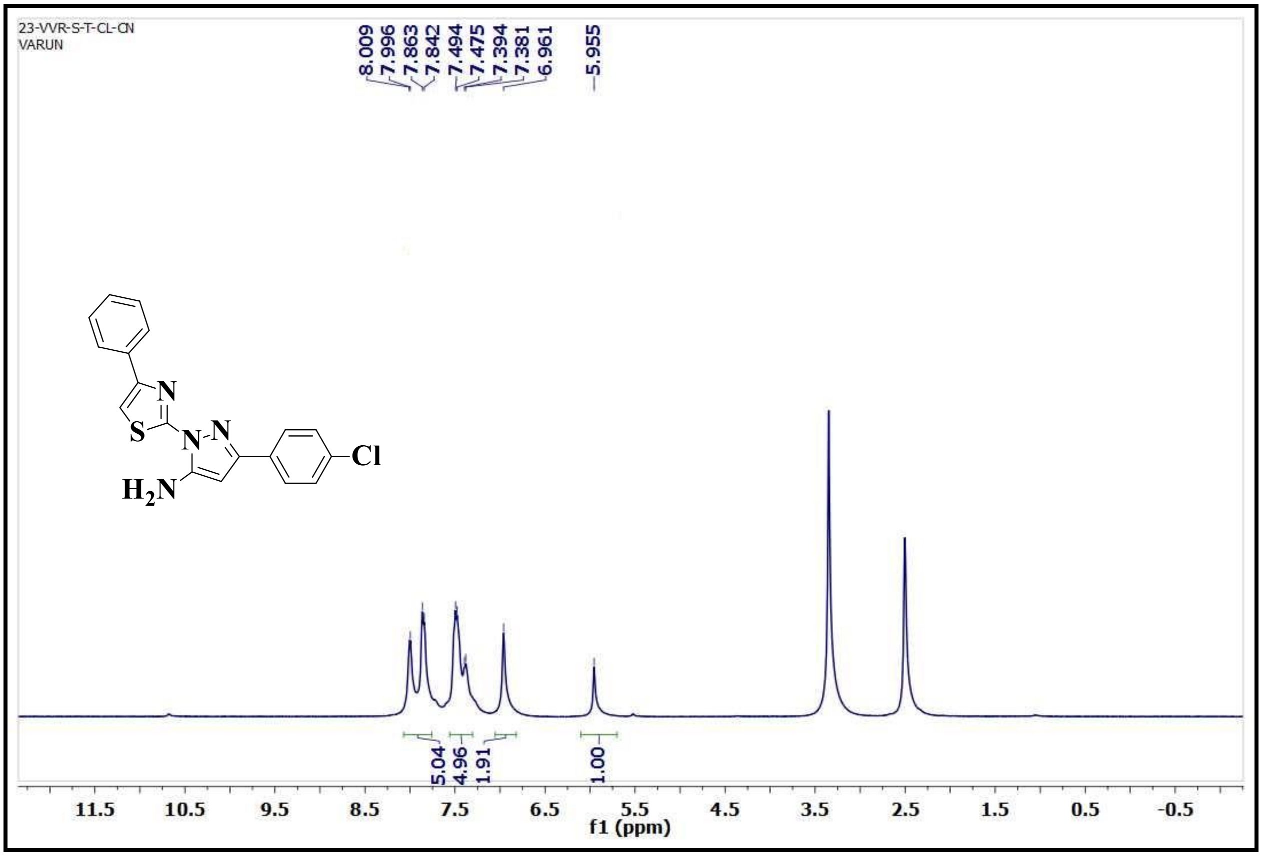
$^1$H NMR Spectrum of compound **23a**



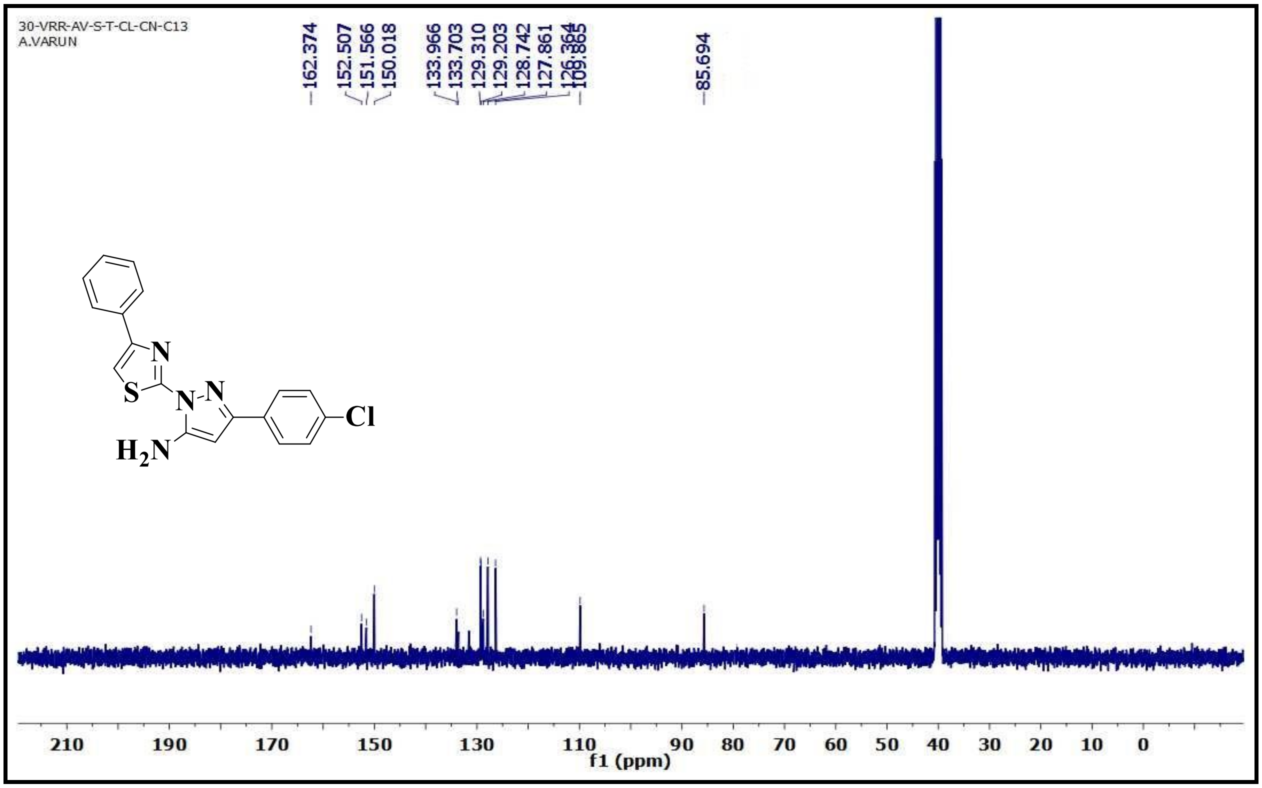
$^{13}$C NMR Spectrum of compound **23a**



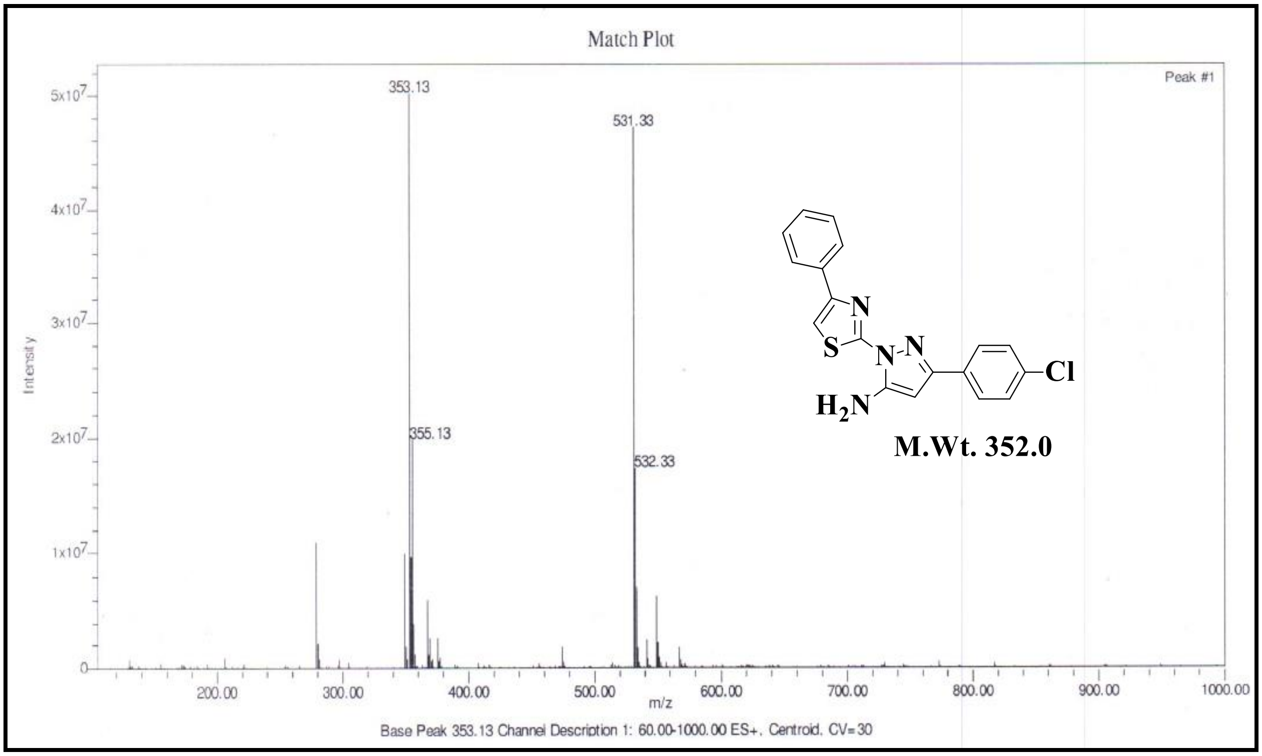
Mass Spectrum of compound **23a**



$^1$H NMR Spectrum of compound **23b**



$^{13}$C NMR Spectrum of compound **23b**



Mass Spectrum of compound **23b**

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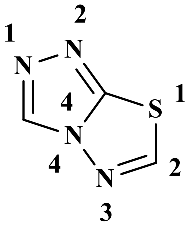
# CHAPTER-IV

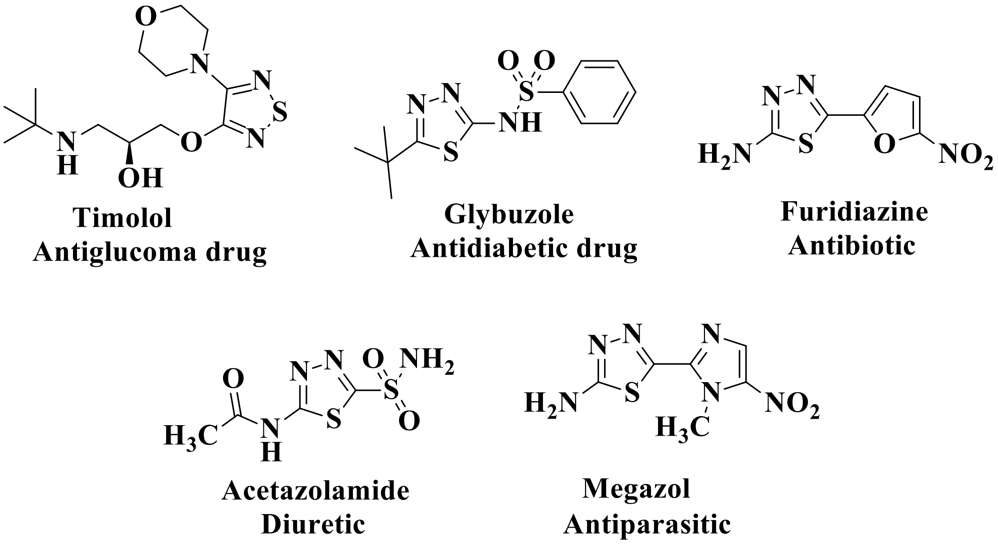
# MULTICOMPONENT SYNTHESIS OF SUBSTITUTED 1-PHENYL-2-((6-(PHENYLAMINO)-[1,2,4]TRIAZOLO[3,4-*b*][1,3,4]THIADIAZOL-3-YL)THIO)ETHANONES AND THEIR ANTICANCER ACTIVITY

## INTRODUCTION

In triazolo thiadiazole system, two five membered rings are fused, in which one ring of 1, 2, 4-triazole is fused with 1, 3, 4-thiadiazole ring. Nitrogen and sulphur containing five membered hetero cycles have significant pharmacological properties.



1,3,4-Thiadiazoles show various biological activities such as antibacterial[1-2], antifugal[3-5], anti-tuberculosis[6-8], anticancer[9,10], antioxidant properties[11],[12].


**Timolol**
**Antiglucoma drug**

**Glybuzole**
**Antidiabetic drug**

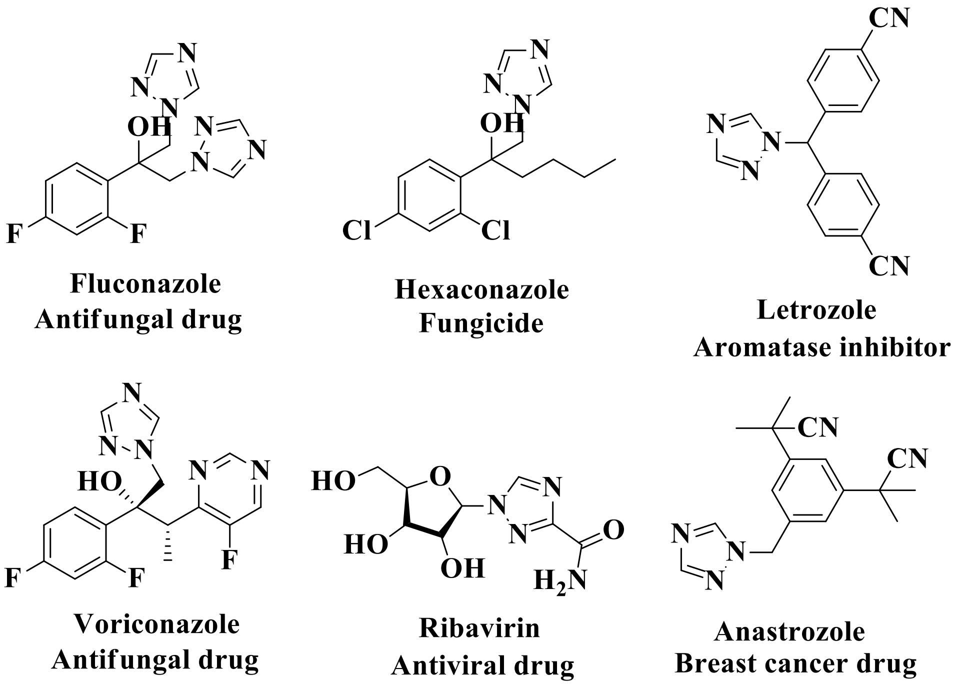
**Furidiazine**
**Antibiotic**

**Acetazolamide**
**Diuretic**

**Megazol**
**Antiparasitic**

**Fig. 1** Some of thiadiazole ring containing drug molecules.

Triazole is five-membered heterocyclic system containing three nitrogen atoms. It is having two isomeric forms like 1,2,3-triazole and 1,2,4-triazole. The 1,2,4-triazole ring containing compounds show antibacterial[13,14], antifungal[15,16], anti-inflammatory[17,18], antimalarial[19], antitubercular[20], antiviral[21,22], and antidepressants[23]. anticonvulsant[24,25], herbicides[26,27] and insecticidal[28].



**Fluconazole**
**Antifungal drug**

**Hexaconazole**
**Fungicide**

**Letrozole**
**Aromatase inhibitor**

**Voriconazole**
**Antifungal drug**

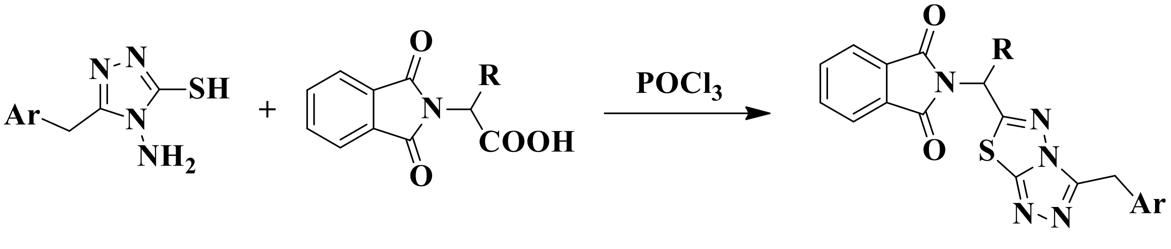
**Ribavirin**
**Antiviral drug**

**Anastrozole**
**Breast cancer drug**

**Fig. 2** Some of triazole ring containing drug molecules.

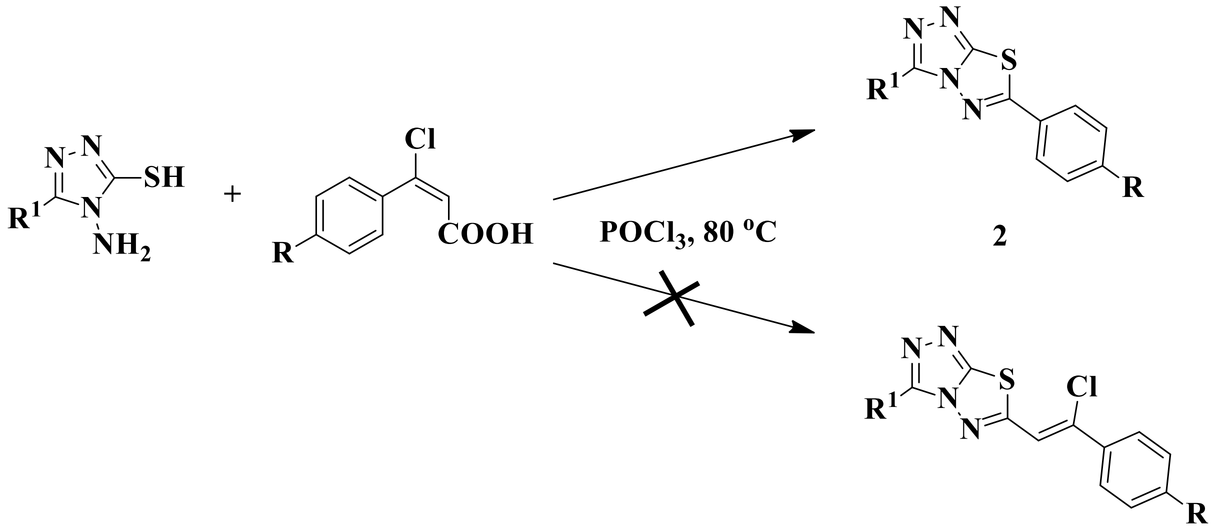
Fused 1,2,4-triazolo[3,4-*b*]1,3,4-thiadiazoles ring containing derivatives exhibit antitumor[29] and antiviral[30,31] activities.

Ebrahimi *et al.*[32] reported an efficient procedure for the synthesis of s-triazolothiadiazole derivatives (**1**) by the condensation of substituted amino triazoles and *N*-phethaloyl-L-amino acids in presence of $POCl_3$.

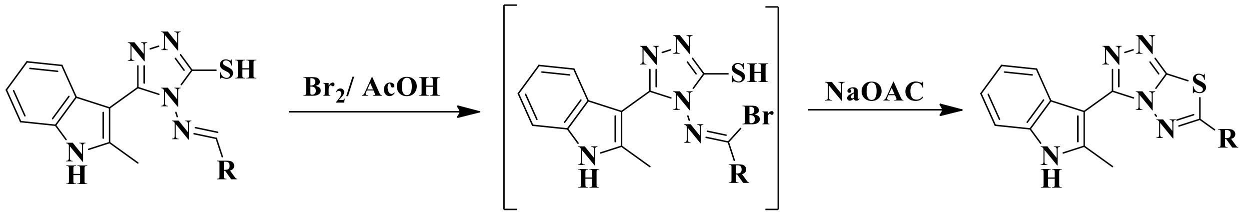


**1**

Sahi *et al.* [33] reported the synthesis of s-triazolo[3,4-*b*][1,3,4]thiadiazoles (**2**) by the reaction of 4-amino-5-aryl-3-mercapto-1,2,4-triazole and (Z)-3-chloro-3-arylacrylic acid in presence of $POCl_3$ at 80 °C. During the reaction, (*Z*)-3-chloro-(3-aryl) acrylic acid was converted to aryl carboxylic acid in the presence of phosphorous oxychloride.

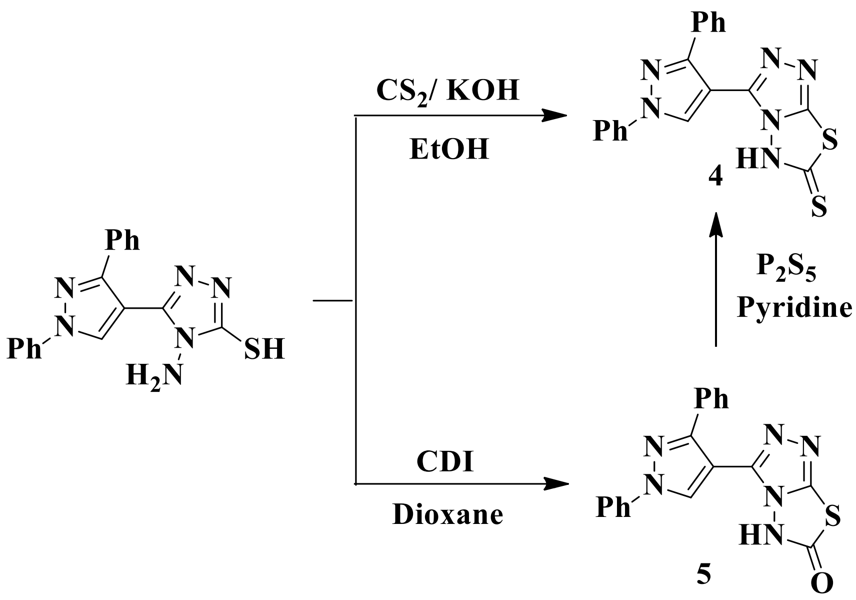


Gomha and Riyadh[34] reported the synthesis of [1,2,4-triazolo][3,4-*b*][1,3,4]thiadiazoles (**3**) through the bromination of 4-(arylideneamino)-5-mercapto-4*H*-[1,2,4] triazoles in acetic acid followed by elimination of HBr to give the title compound.

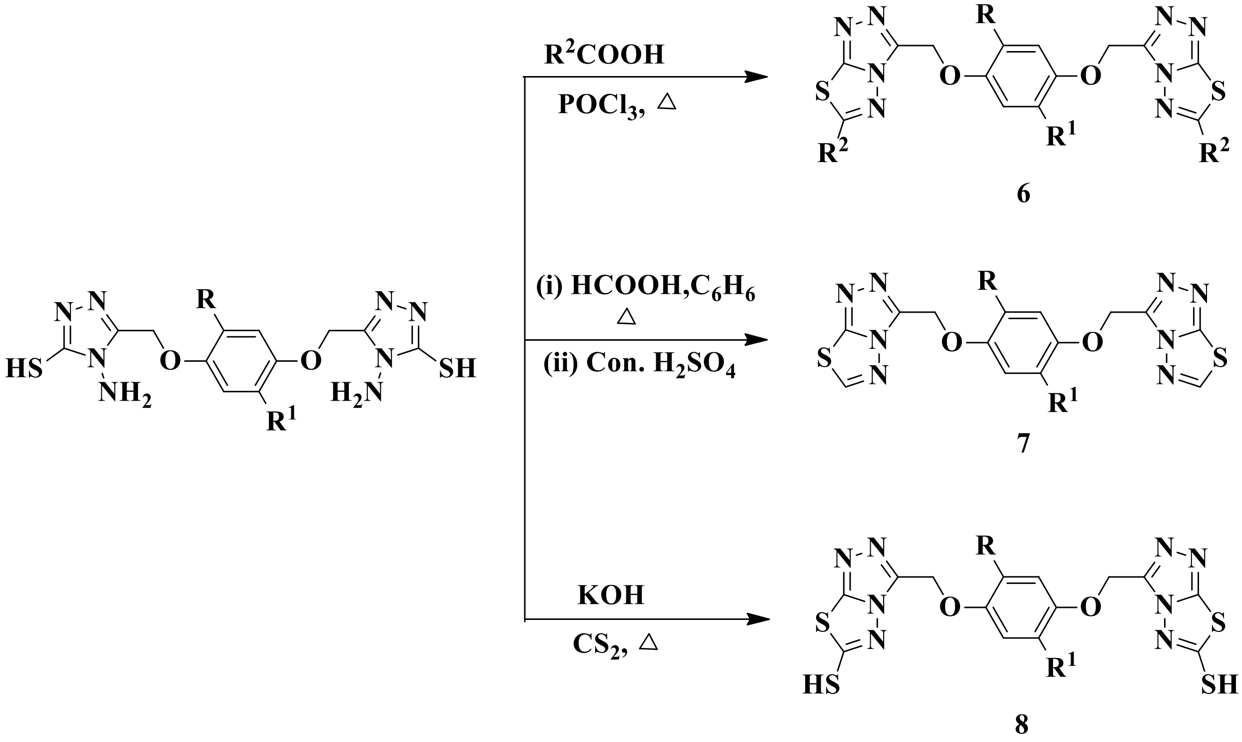


**3**

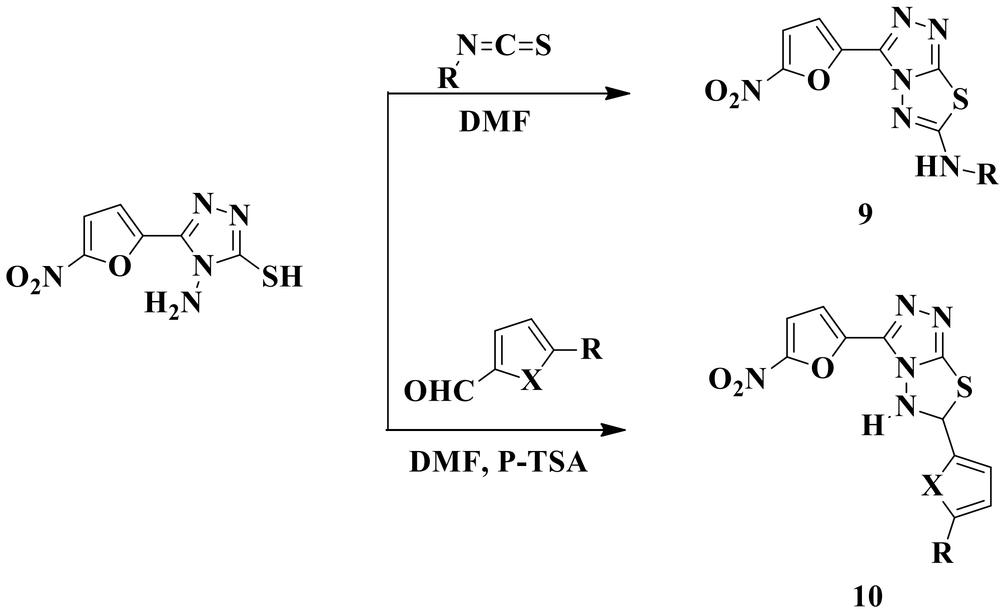
Farghaly *et al.*[35] reported the synthesis and antiviral activity of [1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazoles (**4 & 5**). The compounds **4** and **5** were obtained by condensation of 4-amino-3-mercapto-5-pyrazolyl derivative with $CS_2$ and CDI respectively.



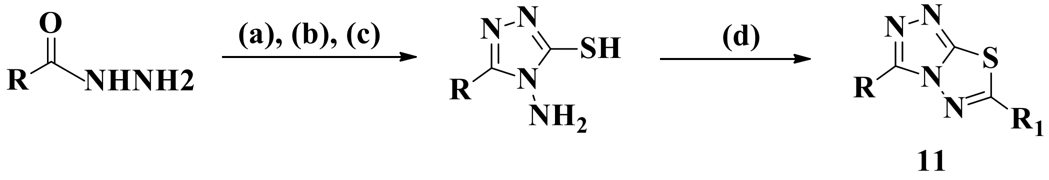
Holla *et al.*[12] synthesized bis-triazolothiadiazoles (**6-8**) as possible anticancer agents. The condensation of bis-(4-amino-5-mercapto-1,2,4-triazol-3-ylmethyleneoxy)phenylenes with various reagents to give title compounds. Among the tested compounds some of them were showed promising anticancer activity.



Badr and Barwa[36] synthesized compound 9 and 10 from 4-amino-5-(5-nitrofuran-2-yl)-4*H*-1,2,4-triazole-3-thiol with alkyl/aryl isothiocyanates, heteroaromatic aldehydes in presence of dimethylformamide to give the title compounds. Among the tested compounds, some compounds showed potent antibacterial activity against *Staphylococcus aureus.*



Lam *et al.*[37] synthesized biological active triazolothiadiazoles (**11**) in a multistep process starting from arylhydrazide. The title compounds showed mushroom tyrosinase inhibitor activity.



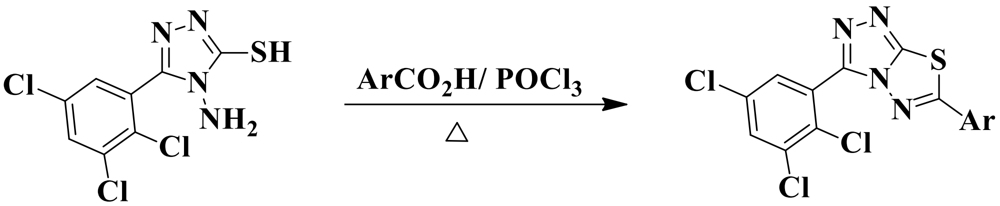
**(a). $CS_2$, KOH, EtOH, reflux**

**(b). $NH_2NH_2.H_2O$, reflux**

**(c). HCl, Cold distilled water**

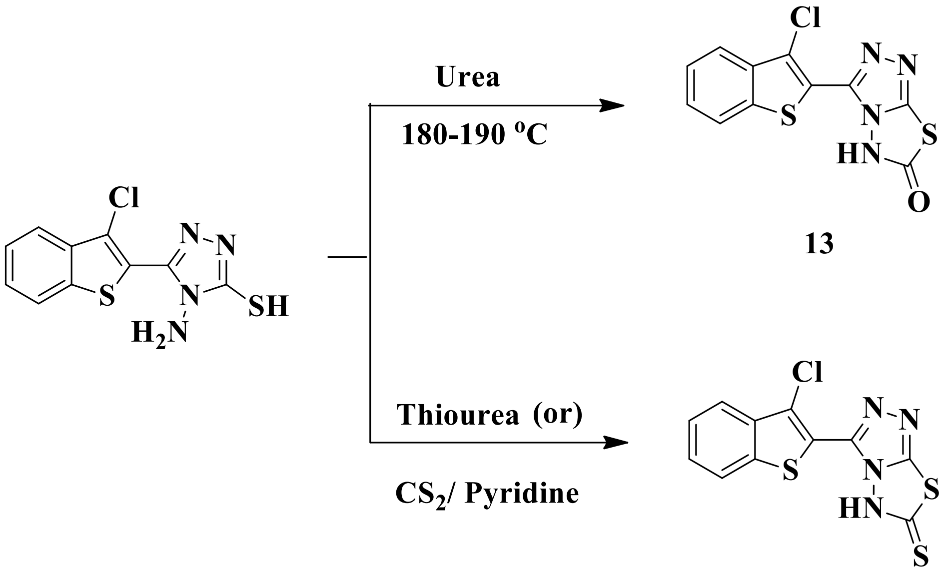
**(d). R-COOH, $POCl_3$, reflux**

Karegoudar *et al.*[38] synthesized compounds (**12**) through the condensation of 3-(2,3,5-trichlorophenyl)-4-amino-5-mercapto 1,2,4-triazole with various aromatic carboxylic acids phosphorous oxychloride. Among the tested compounds few compounds exhibited promising antimicrobial and anti-inflammatory activities.



**12**

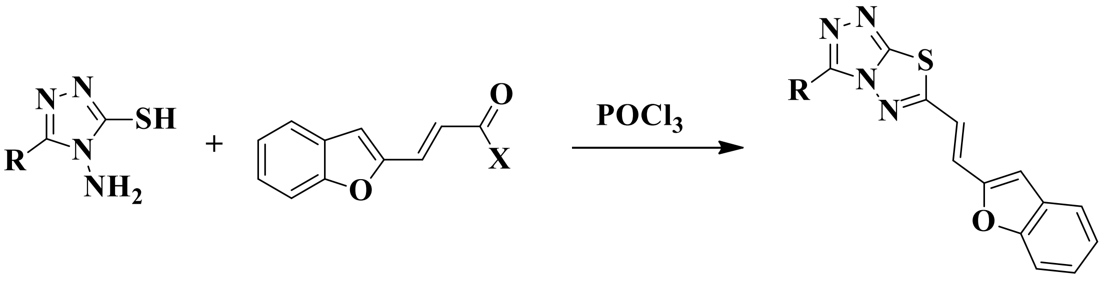
Ashry *et al.*[39] synthesized compound (**13**) and (**14**) by interaction of 4-amino-5-(3-chlorobenzo[*b*]thiophen-2-yl)-4*H*-1,2,4-triazole-3-thiol with urea and thiourea.



**13**

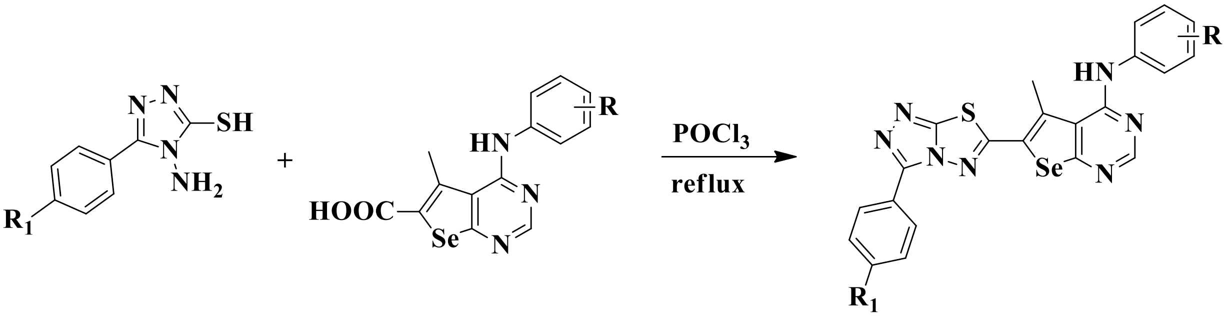
**14**

Obushak *et al.*[40] described the synthesis of (**15**) starting from 4-amino-5-methyl-4*H*-1,2,4-triazole-3-thiol with (*E*)-3-(benzofuran-2-yl) 2-propinoic acid or (*E*)-3-(benzofuran-2-yl) 2-propinoyl chloride in presence of $POCl_3$ gives title compounds.



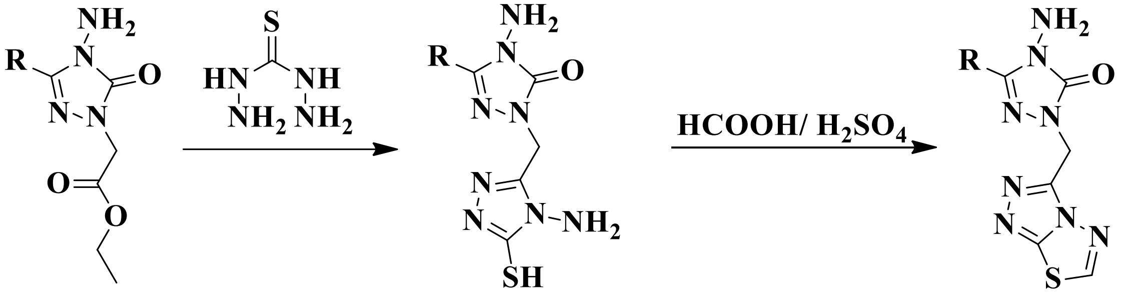
**15**

Kotaiah *et al.*[41] reported the synthesis, antioxidant activity of 1,2,4-(triazolo[3,4-*b*][1,3,4]thiadiazol-6-yl)selenopheno[2,3-*d*]pyrimidine derivatives (**16**). The condensation of various substituted 4-amino-5-aryl-4*H*-1,2,4-triazole-3-thiol and 5-methyl-4-(phenylamino) selenopheno[2,3-*d*]pyrimidine-6-carboxylic acids in $POCl_3$ yielded title compounds.



**16**

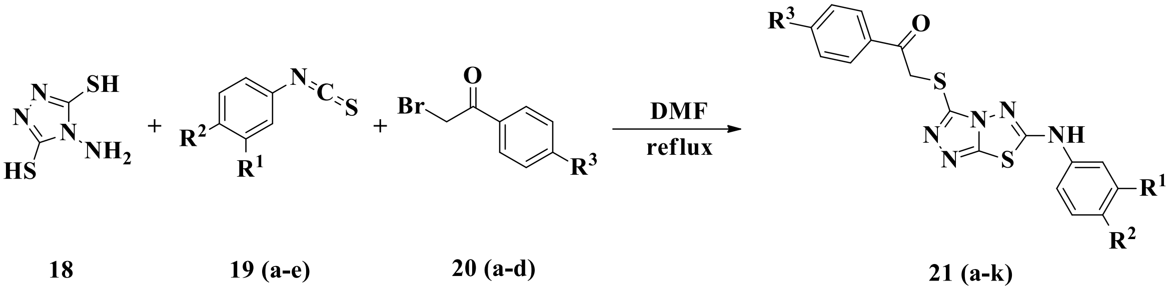
Demirbas *et al.*[42] synthesized 1-([1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazol-3-ylmethyl)-4-amino-3-methyl-1*H*-1,2,4-triazol-5(4*H*)-ones (**17**) by a two step process. The first step is condensation between ethyl 2-(4-amino-3-methyl-5-oxo-4,5-dihydro-1*H*-1,2,4-triazol-1-yl)acetate and thiocarbohydrazide and second step is reaction between formic acid and 4-amino-1-((4-amino-5-mercapto-4*H*-1,2,4-triazol-3-yl)methyl)-3-methyl-1*H*-1,2,4-triazol-5(4*H*)-one.



**17**

**PRESENT WORK**

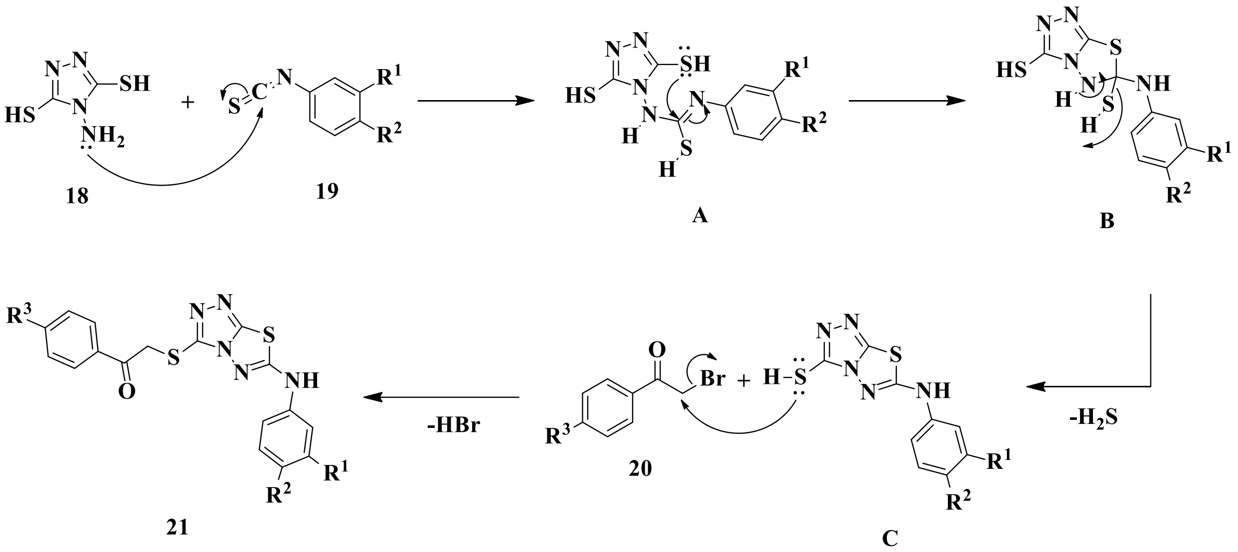
Inspired by these achievements, we surmised that 1-phenyl-2-((6-(phenylamino)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-3-yl)thio)ethanones **21 (a-k)** can be synthesized in one pot under mild and efficient conditions using starting from 4-amino-4*H*-1,2,4-triazole-3,5-dithiol **(18)**, phenyl isothiocyanate **19 (a-e)** and different subsituted 2-bromo-1-phenylethanone **20 (a-d)** in dimethylformamide under refulx conditons (as shown in scheme-1). The compounds 21 (a-k) were screened for anticancer activity.



**Scheme-1.** Synthesis of 21 (a-k).

## Mechanism

A Plausible mechanism for the formation of products has been proposed and was shown in scheme-2.



**Scheme-2**. Mechanism for formation of the title compounds

From the above mechanism it is evident that initially, the amino group of 18 reacts with carbon atom of –N=C=S group of phenyl isothiocyanate. This is a nulceophilic addition reaction. Then, the intermediate 'A' is formed. The –SH group of 'A' reacts intra-molecularly with carbon atom of –C=N– to form another intermediate 'B'. From 'B' $H_2S$ molecule is eliminated to yield 'C'. The thiol group of 'C' displaces the bromine atom of phenacyl bromide to give final product 21.

**Table 1:** Reaction time of the title compounds

| S. No. | Compound | $R^1$ | $R^2$ | $R^3$ | Reaction time (h) |
|---|---|---|---|---|---|
| 1 | 21a | H | H | $CH_3$ | 5 |
| 2 | 21b | H | H | $OCH_3$ | 4.5 |
| 3 | 21c | $OCH_3$ | H | H | 4.5 |
| 4 | 21d | $OCH_3$ | H | $CH_3$ | 4 |
| 5 | 21e | $OCH_3$ | H | $OCH_3$ | 4 |
| 6 | 21f | H | F | $CH_3$ | 6 |
| 7 | 21g | Cl | H | $CH_3$ | 5.5 |
| 8 | 21h | Cl | H | $OCH_3$ | 5 |
| 9 | 21i | H | CN | H | 6.5 |
| 10 | 21j | H | CN | $CH_3$ | 6 |
| 11 | 21k | H | H | Cl | 5 |

The $^1$H NMR spectrum of compound **21a** showed peaks at $\delta$ 2.34 (s, 3H, methyl), 4.75 (s, 2H, -S-$CH_2$), the Ar-H found in between $\delta$ 7.01-7.80 and NH proton appears at 10.24 ppm. The CMR of **21a** showed peaks at $\delta$ 21.74 ppm which corresponds to methyl carbon, $\delta$ 39.63 ppm is due to S-$CH_2$ carbon and $\delta$ 192.72 ppm indicates carbonyl carbon. The mass spectrum of **21a** exhibited 382.10 $[M+H]^+$.

## EXPERIMENTAL SECTION

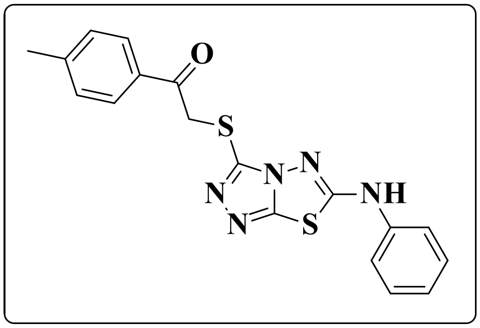
The compound **(18)** was prepared according to the procedure given in chapter-II.

### General procedure for the synthesis of 21 (a-k)

A mixture of (**18,** 1 mmol), phenyl isothiocyanate (**19,** 1 mmol) and 2-bromo-1-phenylethanones (**20,** 1 mmol) was placed in a round bottom flask. Then 5 ml of DMF was charged to it and heated at 80 °C. The progress of the reaction was monitored through thin layer chromatography using ethyl acetate and hexane (1:1) as mobile phase. After completion of the reaction, the reaction mixture was cooled to room temperature, poured in to ice water, so that the solid separated, was filtered, washed with water, dried and re-crystallized from ethanol.

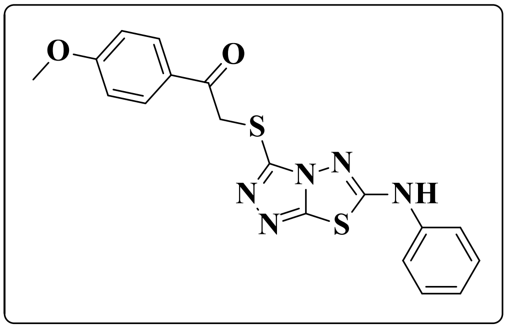
## SPECTRAL DATA

### 2-((6-(Phenylamino)-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazol-3-yl)thio)-1-(p-tolyl)ethanone (21a)



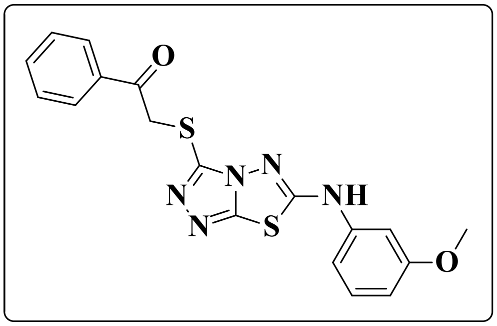
Color: white solid, mp: 191-193 °C, yield: (0.309g, 81%), FT-IR (KBr, $\nu_{max}$ $cm^{-1}$): 3202 (N-H), 1681 (C=O), 1604 (C=N), **$^1$H NMR (400MHz, $CDCl_3$ and DMSO-$d_6$, ppm):** $\delta$ 2.34(s, 3H, methyl), 4.75(s, 2H, methylene), 7.01(t, *J*=7.2Hz, 1H, Ar-H), 7.20(d, *J*=8Hz, 2H, Ar-H), 7.26(t, *J*=8Hz, 2H, Ar-H), 7.45(d, *J*=8Hz, 2H, Ar-H), 7.80(d, *J*=8Hz, 2H, Ar-H), 10.24(s, 1H, N-H) ppm, **$^{13}$C NMR (100MHz, $CDCl_3$ and DMSO-$d_6$):** $\delta$ 21.74, 118.64, 123.52, 128.69, 129.28, 129.55, 132.70, 139.25, 144.76, 160.72, 192.72; **MS (ESI *m/z* %):** 382.10 $[M+H]^+$; **Anal. Calcd.** For $C_{18}H_{15}N_5OS_2$: C, 56.67; H, 3.96; N, 18.36. Found: C, 56.62; H, 3.91; N, 18.41%

### 1-(4-Methoxyphenyl)-2-((6-(phenylamino)-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazol-3-yl)thio) ethanone (21b)



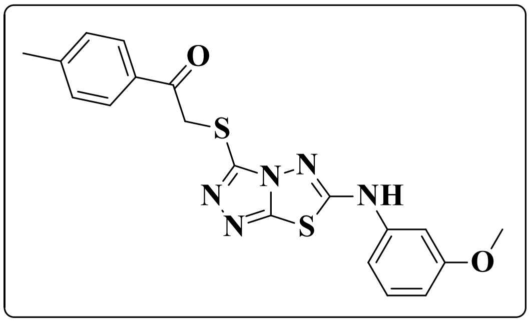
Color: pale yellow solid, mp: 205-207 °C, yield: (0.345g, 87%), FT-IR (KBr, $\nu_{max}$ $cm^{-1}$): 3201 (N-H), 1681 (C=O), 1599 (C=N), **$^1$H NMR (400MHz, DMSO-$d_6$, ppm):** $\delta$ 3.85(s, 3H, methoxy), 4.86(s, 2H, methylene), 7.05(d, *J*=8.4Hz, 2H, Ar-H), 7.10(t, *J*=7.6Hz, 1H, Ar-H), 7.37(t, *J*=7.6Hz, 2H, Ar-H), 7.51(d, *J*=8Hz, 2H, Ar-H), 7.98(d, *J*=8.4Hz, 2H, Ar-H), 10.74(s, 1H, N-H) ppm, **$^{13}$C NMR (100MHz, DMSO-$d_6$):** $\delta$ 56.09, 114.10, 114.49, 118.69, 123.78, 128.31, 129.33, 129.78, 129.99, 131.36, 139.43, 140.32, 150.77, 160.99, 164.05, 192.20; **MS (ESI *m/z* %):** 398.05 $[M+H]^+$; **Anal. Calcd.** For $C_{18}H_{15}N_5O_2S_2$: C, 54.39; H, 3.80; N, 17.62. Found: C, 54.34; H, 3.75; N, 17.67%

### 2-((6-((3-Methoxyphenyl)amino)-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazol-3-yl)thio)-1-phenyl ethanone (21c)



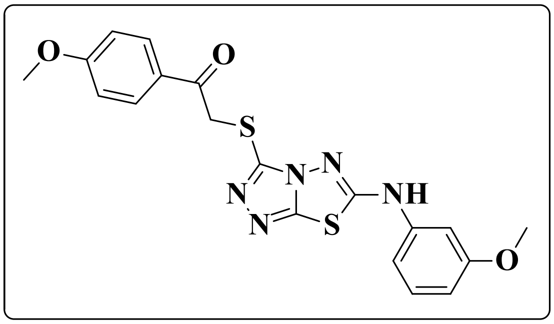
Color: pale yellow solid, mp: 211-213 °C, yield: (0.337g, 85%), FT-IR (KBr, $\nu_{max}$ cm$^{-1}$): 3211 (N-H), 1683 (C=O), 1607 (C=N), **$^{1}$H NMR (400MHz, DMSO-d$_6$, ppm):** *δ* 3.74(s, 3H, methoxy), 4.92(s, 2H, methylene), 6.69(d, *J*=8Hz, 1H, Ar-H), 7.04(d, *J*=7.6Hz, 1H, Ar-H), 7.20(s, 1H, Ar-H), 7.27(t, *J*=8Hz, 1H, Ar-H), 7.54(t, *J*=7.6Hz, 2H, Ar-H), 7.68(t, *J*=7.6Hz, 1H, Ar-H), 8.00(d, *J*=8Hz, 2H, Ar-H), 10.75(s, 1H, N-H) ppm, **$^{13}$C NMR (100MHz, DMSO-d$_6$):** *δ* 55.47, 104.72, 109.12, 111.06, 128.92, 129.26, 130.63, 134.27, 135.41, 140.21, 140.52,150.76, 160.35, 161.02, 193.81; **MS (ESI *m/z* %):** 398.05 [M+H]$^+$; **Anal. Calcd.** For $C_{18}H_{15}N_5O_2S_2$: C, 54.39; H, 3.80; N, 17.62. Found: C, 54.35; H, 3.85; N, 17.67%

### 2-((6-((3-Methoxyphenyl)amino)-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazol-3-yl)thio)-1-(p-tolyl) ethanone (21d)



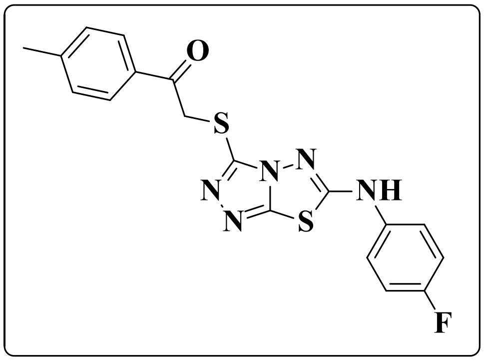
Color: pale yellow solid, mp: 223-225 °C, yield: (0.337g, 82%), FT-IR (KBr, $\nu_{max}$ cm$^{-1}$): 3212 (N-H), 1678 (C=O), 1605 (C=N), **$^{1}$H NMR (400MHz, DMSO-d$_6$, ppm):** *δ* 2.38(s, 3H, methyl), 3.74(s, 3H, methoxy), 4.88(s, 2H, methylene), 6.69(d, *J*=8.4Hz, 1H, Ar-H), 7.04(d, *J*=8Hz, 1H, Ar-H), 7.20(s, 1H, Ar-H), 7.28(t, *J*=8.4Hz, 1H, Ar-H), 7.33(d, *J*=7.6Hz, 2H, Ar-H), 7.89(d, *J*=7.6Hz, 2H, Ar-H), 10.75(s, 1H, N-H) ppm, **$^{13}$C NMR (100MHz, DMSO-d$_6$):** *δ* 21.66, 55.47, 104.71, 109.13, 111.06, 129.04, 129.79, 130.63, 132.92, 140.24, 140.51, 144.83, 150.75, 160.35, 160.98, 193.38; **MS (ESI *m/z* %):** 412.30 [M+H]$^+$; **Anal. Calcd.** For $C_{19}H_{17}N_5O_2S_2$: C, 55.46; H, 4.16; N, 17.02. Found: C, 55.50; H, 4.12; N, 17.16%

### 1-(4-Methoxyphenyl)-2-((6-((3-methoxyphenyl)amino)-[1,2,4]triazolo[3,4-*b*][1,3,4] thiadiazol-3-yl)thio)ethanone (21e)



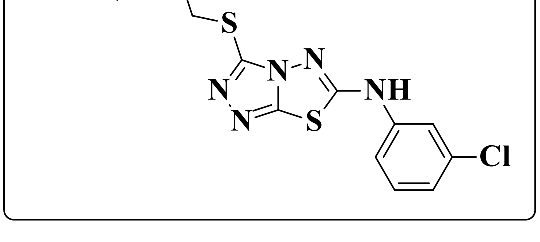
Color: yellow solid, mp: 227-229 $^{o}$C, yield: (0.389g, 91%), FT-IR (KBr, $\nu_{max}$ cm$^{-1}$): 3212 (N-H), 1675 (C=O), 1600 (C=N), **$^1$H NMR (400MHz, DMSO-d$_6$, ppm):** $\delta$ 3.74(s, 3H, methoxy), 3.85(s, 3H, methoxy), 4.85(s, 2H, methylene), 6.69(d, *J*=8.4Hz, 1H, Ar-H), 7.04(d, *J*=8Hz, 3H, Ar-H), 7.20(s, 1H, Ar-H), 7.28(d, *J*=8Hz, 1H, Ar-H), 7.97(d, *J*=8.8Hz, 2H, Ar-H), 10.75(s, 1H, N-H) ppm, **$^{13}$C NMR (100MHz, DMSO-d$_6$):** $\delta$ 55.46, 56.08, 104.70, 109.12, 114.47, 128.28, 130.63, 131.35, 140.30, 140.53, 150.73, 160.35, 160.96, 164.06, 192.20; **MS (ESI *m/z* %):** 428.10 [M+H]$^+$; **Anal. Calcd.** For $C_{19}H_{17}N_5O_3S_2$: C, 53.38; H, 4.01; N, 16.38. Found: C, 53.35; H, 4.15; N, 16.33%

### 2-((6-((4-Fluorophenyl)amino)-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazol-3-yl)thio)-1-(p-tolyl) ethanone (21f)



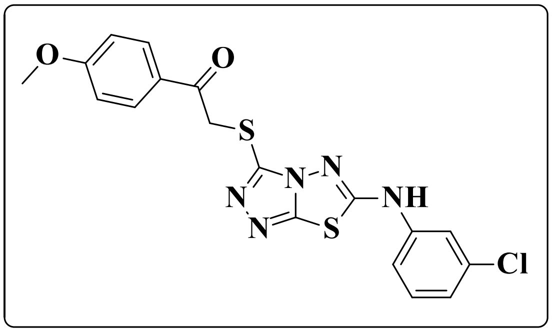
Color: white solid, mp: 215-217 $^{o}$C, yield: (0.343g, 86%), FT-IR (KBr, $\nu_{max}$ cm$^{-1}$): 3218 (N-H), 1684 (C=O), 1586 (C=N), **$^1$H NMR (400MHz, DMSO-d$_6$, ppm):** $\delta$ 2.38(s, 3H, methyl), 4.87(s, 2H, methylene), 7.20(t, *J*=8Hz, 2H, Ar-H), 7.33(d, *J*=7.6Hz, 2H, Ar-H), 7.51-754(m, 2H, Ar-H), 7.89(d, *J*=7.2Hz, 2H, Ar-H), 10.76(s, 1H, N-H) ppm, **$^{13}$C NMR (100MHz, DMSO-d$_6$):** $\delta$ 21.60, 116.23, 116.45, 120.61, 120.69, 129.01, 129.81, 132.77, 135.68, 140.34, 144.98, 150.84, 157.27, 159.66, 161.16, 193.43; **MS (ESI *m/z* %):** 400.21 [M+H]$^+$; **Anal. Calcd.** For $C_{18}H_{14}FN_5OS_2$: C, 54.12; H, 3.53; N, 17.53. Found: C, 54.16; H, 3.57; N, 17.58%

### 2-((6-((3-Chlorophenyl)amino)-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazol-3-yl)thio)-1-(p-tolyl) ethanone (21g)



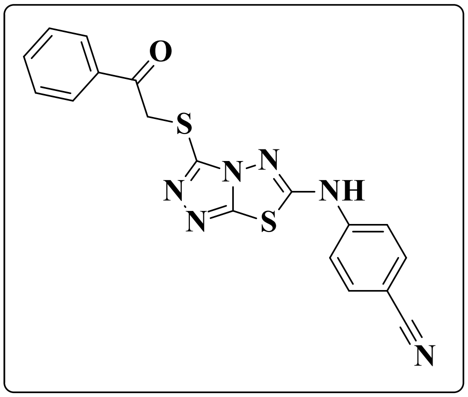
Color: white solid, mp: 235-237 $^{o}$C, yield: (0.370g, 89%), FT-IR (KBr, $\nu_{max}$ cm$^{-1}$): 3195 (N-H), 1682 (C=O), 1602 (C=N), **$^1$H NMR (400MHz, DMSO-d$_6$, ppm):** $\delta$ 2.37(s, 3H, methyl), 4.89(s, 2H, methylene), 7.16(d, *J*=3.6Hz, 1H, Ar-H), 7.32(d, *J*=8Hz, 2H, Ar-H), 7.40(d, *J*=4.4Hz, 2H, Ar-H), 7.66(s, 1H, Ar-H), 7.89(d, *J*=7.6Hz, 2H, Ar-H), 10.93(s, 1H, N-H) ppm; **MS (ESI *m/z* %):** 416.15 $[M+H]^+$; **Anal. Calcd.** For $C_{18}H_{14}ClN_5OS_2$: C, 51.98; H, 3.39; N, 16.84. Found: C, 51.93; H, 3.35; N, 16.80%

**2-((6-((3-Chlorophenyl)amino)-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazol-3-yl)thio)-1-(4-methoxyphenyl)ethanone (21h)**



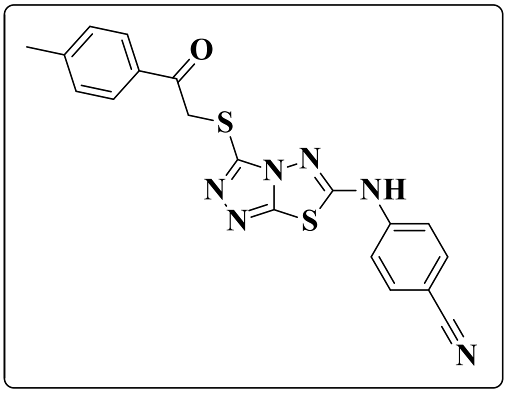
Color: pale yellow solid, mp: 243-245 °C, yield: (0.397g, 92%), FT-IR (KBr, $\nu_{max}$ $cm^{-1}$): 3192 (N-H), 1674 (C=O), 1582 (C=N), **$^1$H NMR (400MHz, DMSO-d$_6$, ppm):** $\delta$ 3.84(s, 3H, methoxy), 4.86(s, 2H, methylene), 7.03(d, *J*=8.4Hz, 2H, Ar-H), 7.16(d, *J*=3.6Hz, 1H, Ar-H), 7.40(d, *J*=4.4Hz, 2H, Ar-H), 7.66(s, 1H, Ar-H), 7.97(d, *J*=8.8Hz, 2H, Ar-H), 10.93(s, 1H, N-H) ppm, **$^{13}$C NMR (100MHz, DMSO-d$_6$):** $\delta$ 56.04, 114.45, 117.15, 118.10, 123.41, 128.21, 131.33, 131.40, 140.51, 140.66, 150.79, 160.79, 164.06, 192.19; **MS (ESI *m/z* %):** 432.17 $[M+H]^+$; **Anal. Calcd.** For $C_{18}H_{14}ClN_5O_2S_2$: C, 50.05; H, 3.27; N, 16.21. Found: C, 50.15; H, 3.23; N, 16.25%

**4-((3-((2-Oxo-2-phenylethyl)thio)-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazol-6-yl)amino) benzonitrile (21i)**



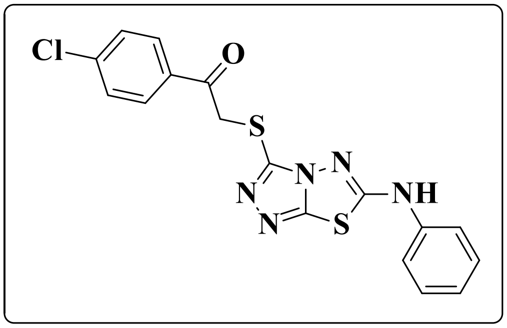
Color: white solid, mp: 233-235 °C, yield: (0.333g, 85%), FT-IR (KBr, $\nu_{max}$ $cm^{-1}$): 3187 (N-H), 2223 (CN), 1682 (C=O), 1595 (C=N), **$^1$H NMR (400MHz, DMSO-d$_6$, ppm):** $\delta$ 4.94(s, 2H, methylene), 7.54(t, *J*=7.6Hz, 2H, Ar-H), 7.66(t, *J*=8Hz, 3H, Ar-H), 7.82(d, *J*=8Hz, 2H, Ar-H), 8.01(d, *J*=7.6Hz, 2H, Ar-H), 11.24(s, 1H, N-H) ppm, **$^{13}$C NMR (100MHz, DMSO-d$_6$):** $\delta$ 105.13, 118.68, 119.42, 128.90, 129.31, 134.11, 134.36, 135.29, 140.65, 143.15, 150.92, 160.60, 193.84; **MS (ESI *m/z* %):** 393.16 $[M+H]^+$; **Anal. Calcd.** For $C_{18}H_{12}N_6OS_2$: C, 55.09; H, 3.08; N, 21.41. Found: C, 55.15; H, 3.19; N, 21.46%

**4-((3-((2-Oxo-2-(p-tolyl)ethyl)thio)-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazol-6-yl)amino) benzonitrile (21j)**



Color: white solid, mp: 245-247 °C, yield: (0.369g, 91%), FT-IR (KBr, $\nu_{max}$ cm$^{-1}$): 3202 (N-H), 2222 (CN), 1684 (C=O), 1605 (C=N), **$^1$H NMR (400MHz, DMSO-d$_6$, ppm):** $\delta$ 2.38(s, 3H, methyl), 4.89(s, 2H, methylene), 7.33(d, *J*=8Hz, 2H, Ar-H), 7.64(d, *J*=8.4Hz, 2H, Ar-H), 7.80(d, *J*=8.4Hz, 2H, Ar-H), 7.89(d, *J*=8Hz, 2H, Ar-H), 11.23(s, 1H, N-H) ppm; **MS (ESI *m/z* %):** 407.20 [M+H]$^+$; **Anal. Calcd.** For $C_{19}H_{14}N_6OS_2$: C, 56.14; H, 3.47; N, 20.67. Found: C, 56.19; H, 3.42; N, 20.63%

### 1-(4-Chlorophenyl)-2-((6-(phenylamino)-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazol-3-yl)thio) ethanone (21k)



Color: white solid, mp: 195-197 °C, yield: (0.349g, 87%), FT-IR (KBr, $\nu_{max}$ cm$^{-1}$): 3215 (N-H), 1685 (C=O), 1604 (C=N), **$^1$H NMR (400MHz, DMSO-d$_6$, ppm):** $\delta$ 4.88(s, 2H, methylene), 7.11(t, *J*=7.6Hz, 1H, Ar-H), 7.18(d, *J*=8.4Hz, 2H, Ar-H), 7.51(d, *J*=7.6Hz, 2H, Ar-H), 7.61(d, *J*=8.8Hz, 2H, Ar-H), 8.02(d, *J*=8.4Hz, 2H, Ar-H), 11.23(s, 1H, N-H) ppm; **MS (ESI *m/z* %):** 402.26 [M+H]$^+$; **Anal. Calcd.** For $C_{17}H_{12}ClN_5OS_2$: C, 50.81; H, 3.01; N, 17.43. Found: C, 50.89; H, 3.14; N, 17.48%

## BIOLOGICAL ACTIVITY

### *In vitro* anticancer assay

The in vitro anticancer activity procedure already mentioned in the chapter-II.

### *In vitro* anticancer activity results and discussion

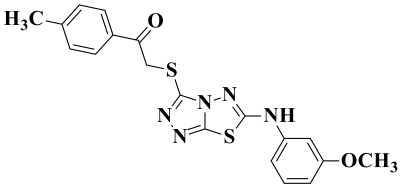
Among all the synthesized compounds nine compounds were selected for NCI cancer activity and given NSC codes, **21a** (NSC: D-803381 / 1), **21b** (NSC: D-803382 / 1), **21d** (NSC: D-804119 / 1), **21e** (NSC**:** D-804118 / 1), **21f** (NSC: D-804117 / 1), **21g** (NSC: D-804116 / 1), **21h** (NSC: D-804115 / 1), **21i** (NSC: D-804111 / 1), **21j** (NSC: D-804112 / 1), **21k** (NSC: D-804120 / 1). The summary of results were shown in table 2.

**Table 2.** *In vitro* anticancer activity of the title compounds

| | **Growth percentage (%)** | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Tumor cell line** | **21a** | **21b** | **21d** | **21e** | **21f** | **21g** | **21h** | **21i** | **21j** | **21k** |
| **Leukemia** | | | | | | | | | | |
| SR | 100.46 | 71.94 | 68.90 | 78.55 | 98.24 | 61.49 | 88.63 | 88.82 | 91.16 | 81.75 |
| **Non-small cell lung cancer** | | | | | | | | | | |
| NCI-H522 | 82.45 | 73.86 | 80.59 | 87.77 | 92.58 | 96.43 | 89.18 | 86.11 | 85.38 | 84.10 |
| **CNS cancer** | | | | | | | | | | |
| SNB-19 | 97.59 | 90.91 | 98.80 | 101.06 | 98.80 | 96.70 | 101.12 | 100.23 | 72.39 | 96.15 |
| **Melanoma** | | | | | | | | | | |
| UACC-62 | 79.19 | 70.41 | 83.73 | 90.74 | 87.90 | 86.89 | 88.27 | 90.21 | 92.37 | 90.43 |
| **Renal cancer** | | | | | | | | | | |
| CAKI-1 | 94.45 | 96.96 | 75.24 | 88.19 | 90.45 | 86.01 | 86.63 | 91.22 | 93.50 | 82.13 |
| UO-31 | 82.32 | 84.25 | **58.74** | 75.72 | 88.87 | 80.87 | 81.66 | 81.10 | 84.59 | 77.84 |
| **Breast cancer** | | | | | | | | | | |
| T-47D | 89.36 | 81.54 | 73.20 | 84.86 | 85.77 | 66.14 | 89.43 | 82.34 | 76.96 | 73.50 |

## Anticancer activity conclusion

Among all the tested compounds, compound **21d** showed moderate activity against renal cancer OU-31 cell line with 58.74% growth or 41.26% growth inhibition.



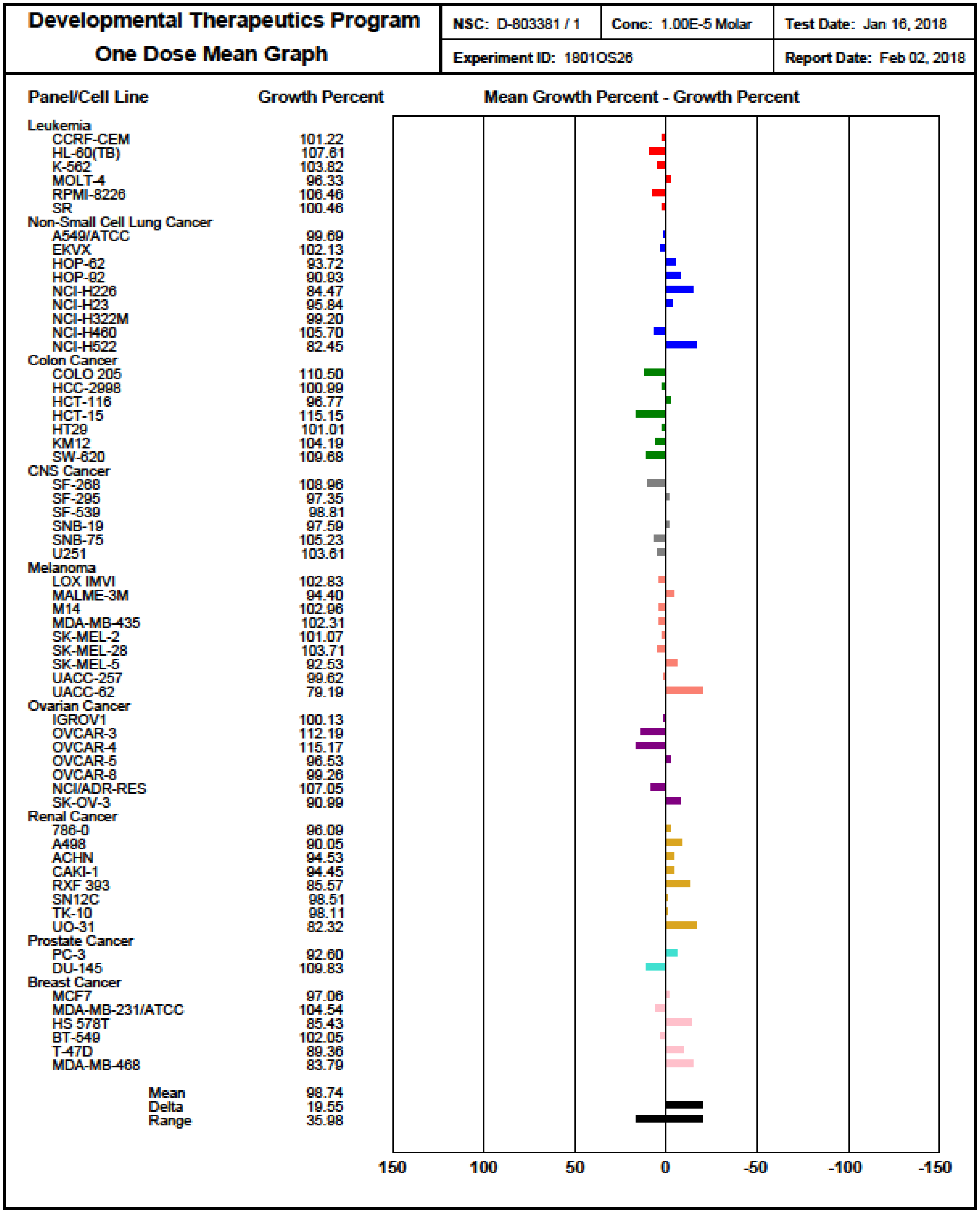
**21d**

The compound have moderate anticancer activity

In gist, we have synthesized compounds 21 by a three component condensation reaction. Compound 21a shows moderate activity against renal OU-31 cell line.

# Anticancer activity of compounds 21a to 21k.

## Table-3 Compound 21a



**Developmental Therapeutics Program**
**One Dose Mean Graph**

NSC: D-803381 / 1
Conc: 1.00E-5 Molar
Test Date: Jan 16, 2018
Experiment ID: 1801OS26
Report Date: Feb 02, 2018

| Panel/Cell Line | Growth Percent |
|---|---|
| Leukemia | |
| CCRF-CEM | 101.22 |
| HL-60(TB) | 107.61 |
| K-562 | 103.82 |
| MOLT-4 | 96.33 |
| RPMI-8226 | 106.46 |
| SR | 100.46 |
| Non-Small Cell Lung Cancer | |
| A549/ATCC | 99.69 |
| EKVX | 102.13 |
| HOP-62 | 93.72 |
| HOP-92 | 90.93 |
| NCI-H226 | 84.47 |
| NCI-H23 | 95.84 |
| NCI-H322M | 99.20 |
| NCI-H460 | 105.70 |
| NCI-H522 | 82.45 |
| Colon Cancer | |
| COLO 205 | 110.50 |
| HCC-2998 | 100.99 |
| HCT-116 | 96.77 |
| HCT-15 | 115.15 |
| HT29 | 101.01 |
| KM12 | 104.19 |
| SW-620 | 109.68 |
| CNS Cancer | |
| SF-268 | 108.96 |
| SF-295 | 97.35 |
| SF-539 | 98.81 |
| SNB-19 | 97.59 |
| SNB-75 | 105.23 |
| U251 | 103.61 |
| Melanoma | |
| LOX IMVI | 102.83 |
| MALME-3M | 94.40 |
| M14 | 102.96 |
| MDA-MB-435 | 102.31 |
| SK-MEL-2 | 101.07 |
| SK-MEL-28 | 103.71 |
| SK-MEL-5 | 92.53 |
| UACC-257 | 99.62 |
| UACC-62 | 79.19 |
| Ovarian Cancer | |
| IGROV1 | 100.13 |
| OVCAR-3 | 112.19 |
| OVCAR-4 | 115.17 |
| OVCAR-5 | 96.53 |
| OVCAR-8 | 99.26 |
| NCI/ADR-RES | 107.05 |
| SK-OV-3 | 90.99 |
| Renal Cancer | |
| 786-0 | 96.09 |
| A498 | 90.05 |
| ACHN | 94.53 |
| CAKI-1 | 94.45 |
| RXF 393 | 85.57 |
| SN12C | 98.51 |
| TK-10 | 98.11 |
| UO-31 | 82.32 |
| Prostate Cancer | |
| PC-3 | 92.60 |
| DU-145 | 109.83 |
| Breast Cancer | |
| MCF7 | 97.06 |
| MDA-MB-231/ATCC | 104.54 |
| HS 578T | 85.43 |
| BT-549 | 102.05 |
| T-47D | 89.36 |
| MDA-MB-468 | 83.79 |
| Mean | 98.74 |
| Delta | 19.55 |
| Range | 35.98 |

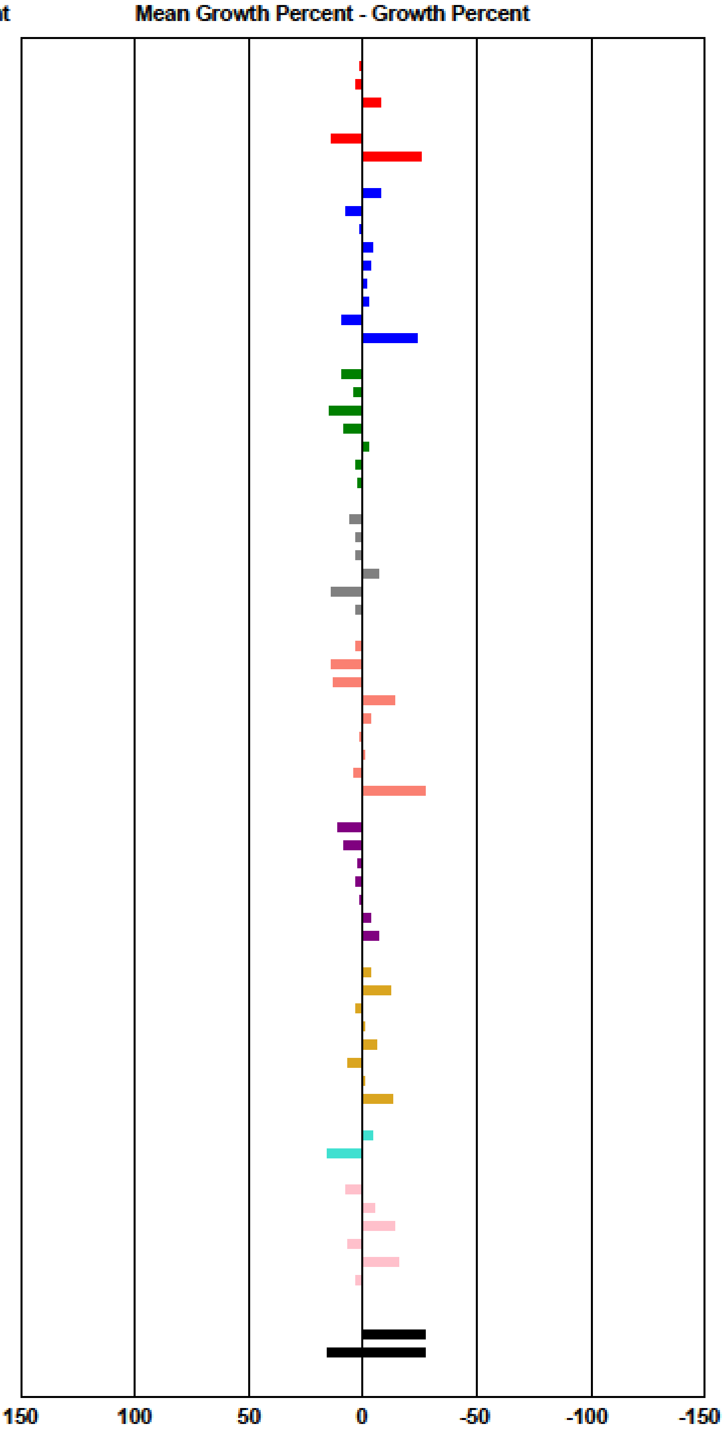
# Table-4 Compound 21b

**Developmental Therapeutics Program**

**One Dose Mean Graph**

NSC: D-803382 / 1
Conc: 1.00E-5 Molar
Test Date: Jan 16, 2018
Experiment ID: 1801OS26
Report Date: Feb 02, 2018

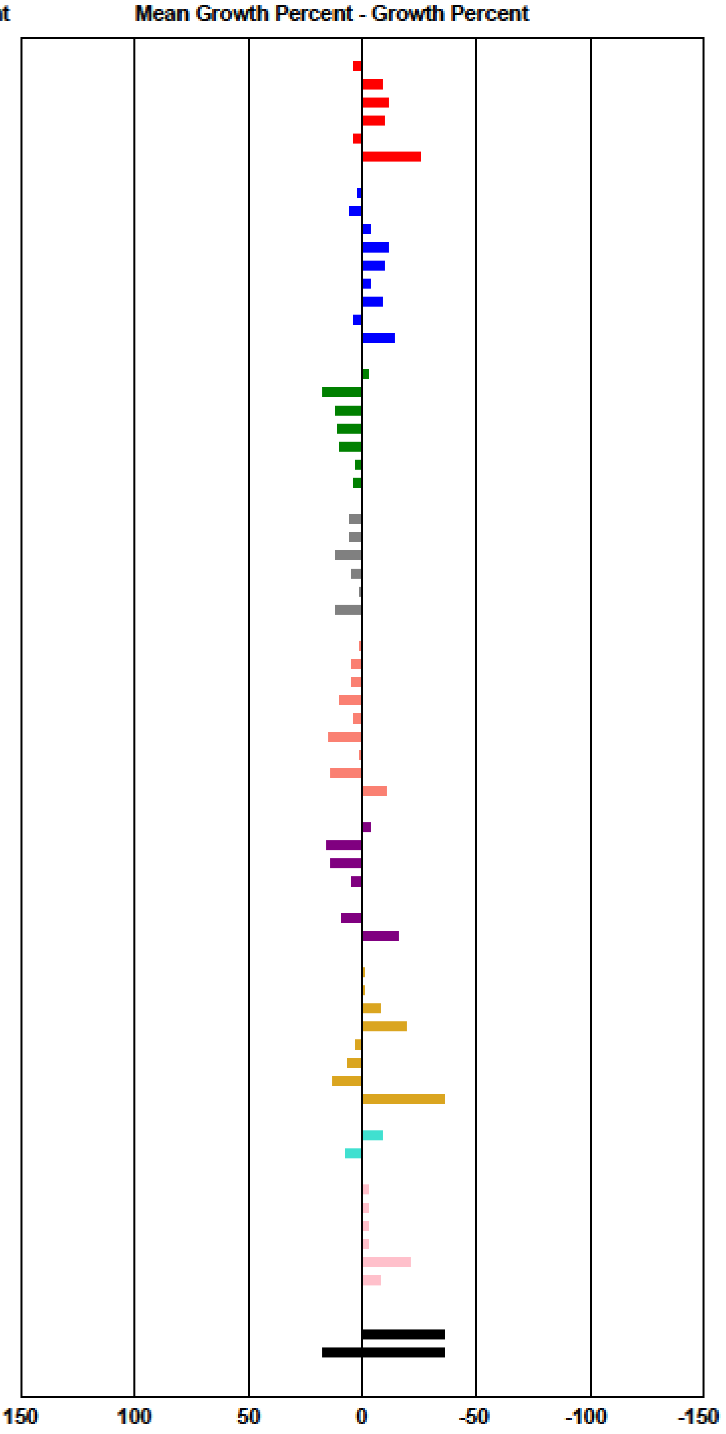
| Panel/Cell Line | Growth Percent |
|---|---|
| Leukemia | |
| CCRF-CEM | 98.51 |
| HL-60(TB) | 100.02 |
| K-562 | 89.93 |
| MOLT-4 | 98.05 |
| RPMI-8226 | 111.13 |
| SR | 71.94 |
| Non-Small Cell Lung Cancer | |
| A549/ATCC | 89.73 |
| EKVX | 104.71 |
| HOP-62 | 98.31 |
| HOP-92 | 93.28 |
| NCI-H226 | 93.67 |
| NCI-H23 | 95.48 |
| NCI-H322M | 94.83 |
| NCI-H460 | 106.51 |
| NCI-H522 | 73.86 |
| Colon Cancer | |
| COLO 205 | 106.66 |
| HCC-2998 | 101.57 |
| HCT-116 | 112.15 |
| HCT-15 | 105.15 |
| HT29 | 94.74 |
| KM12 | 100.68 |
| SW-620 | 99.61 |
| CNS Cancer | |
| SF-268 | 103.15 |
| SF-295 | 100.64 |
| SF-539 | 100.56 |
| SNB-19 | 90.91 |
| SNB-75 | 110.46 |
| U251 | 100.58 |
| Melanoma | |
| LOX IMVI | 100.35 |
| MALME-3M | 110.66 |
| M14 | 109.88 |
| MDA-MB-435 | 83.17 |
| SK-MEL-2 | 93.91 |
| SK-MEL-28 | 98.14 |
| SK-MEL-5 | 96.75 |
| UACC-257 | 100.78 |
| UACC-62 | 70.41 |
| Ovarian Cancer | |
| IGROV1 | 108.51 |
| OVCAR-3 | 105.71 |
| OVCAR-4 | 99.50 |
| OVCAR-5 | 100.11 |
| OVCAR-8 | 98.87 |
| NCI/ADR-RES | 94.14 |
| SK-OV-3 | 90.23 |
| Renal Cancer | |
| 786-0 | 94.23 |
| A498 | 85.21 |
| ACHN | 100.55 |
| CAKI-1 | 96.96 |
| RXF 393 | 91.75 |
| SN12C | 104.11 |
| TK-10 | 96.44 |
| UO-31 | 84.25 |
| Prostate Cancer | |
| PC-3 | 93.28 |
| DU-145 | 112.38 |
| Breast Cancer | |
| MCF7 | 104.58 |
| MDA-MB-231/ATCC | 92.71 |
| HS 578T | 83.76 |
| BT-549 | 104.17 |
| T-47D | 81.54 |
| MDA-MB-468 | 100.46 |
| Mean | 97.24 |
| Delta | 26.83 |
| Range | 41.97 |



# Table-5 Compound 21d

| | | | |
|---|---|---|---|
| **Developmental Therapeutics Program** | NSC: D-804119 / 1 | Conc: 1.00E-5 Molar | Test Date: Mar 05, 2018 |
| **One Dose Mean Graph** | Experiment ID: 1803OS47 | | Report Date: Mar 23, 2018 |

| Panel/Cell Line | Growth Percent |
|---|---|
| Leukemia | |
| CCRF-CEM | 98.10 |
| HL-60(TB) | 85.97 |
| K-562 | 83.47 |
| MOLT-4 | 85.36 |
| RPMI-8226 | 97.97 |
| SR | 68.90 |
| Non-Small Cell Lung Cancer | |
| A549/ATCC | 96.64 |
| EKVX | 99.89 |
| HOP-62 | 90.76 |
| HOP-92 | 83.36 |
| NCI-H226 | 84.85 |
| NCI-H23 | 91.08 |
| NCI-H322M | 86.19 |
| NCI-H460 | 97.89 |
| NCI-H522 | 80.59 |
| Colon Cancer | |
| COLO 205 | 91.80 |
| HCC-2998 | 111.11 |
| HCT-116 | 106.09 |
| HCT-15 | 105.04 |
| HT29 | 104.77 |
| KM12 | 97.26 |
| SW-620 | 98.09 |
| CNS Cancer | |
| SF-268 | 100.18 |
| SF-295 | 100.21 |
| SF-539 | 106.61 |
| SNB-19 | 98.80 |
| SNB-75 | 95.70 |
| U251 | 105.85 |
| Melanoma | |
| LOX IMVI | 95.92 |
| MALME-3M | 98.72 |
| M14 | 99.21 |
| MDA-MB-435 | 104.64 |
| SK-MEL-2 | 98.17 |
| SK-MEL-28 | 108.77 |
| SK-MEL-5 | 95.43 |
| UACC-257 | 107.57 |
| UACC-62 | 83.73 |
| Ovarian Cancer | |
| IGROV1 | 91.39 |
| OVCAR-3 | 109.59 |
| OVCAR-4 | 108.09 |
| OVCAR-5 | 99.32 |
| OVCAR-8 | 94.76 |
| NCI/ADR-RES | 103.88 |
| SK-OV-3 | 79.15 |
| Renal Cancer | |
| 786-0 | 93.53 |
| A498 | 93.67 |
| ACHN | 86.45 |
| CAKI-1 | 75.24 |
| RXF 393 | 97.10 |
| SN12C | 101.05 |
| TK-10 | 107.11 |
| UO-31 | 58.74 |
| Prostate Cancer | |
| PC-3 | 85.45 |
| DU-145 | 101.40 |
| Breast Cancer | |
| MCF7 | 91.83 |
| MDA-MB-231/ATCC | 91.74 |
| HS 578T | 92.19 |
| BT-549 | 92.19 |
| T-47D | 73.20 |
| MDA-MB-468 | 87.14 |
| Mean | 94.31 |
| Delta | 35.57 |
| Range | 52.37 |

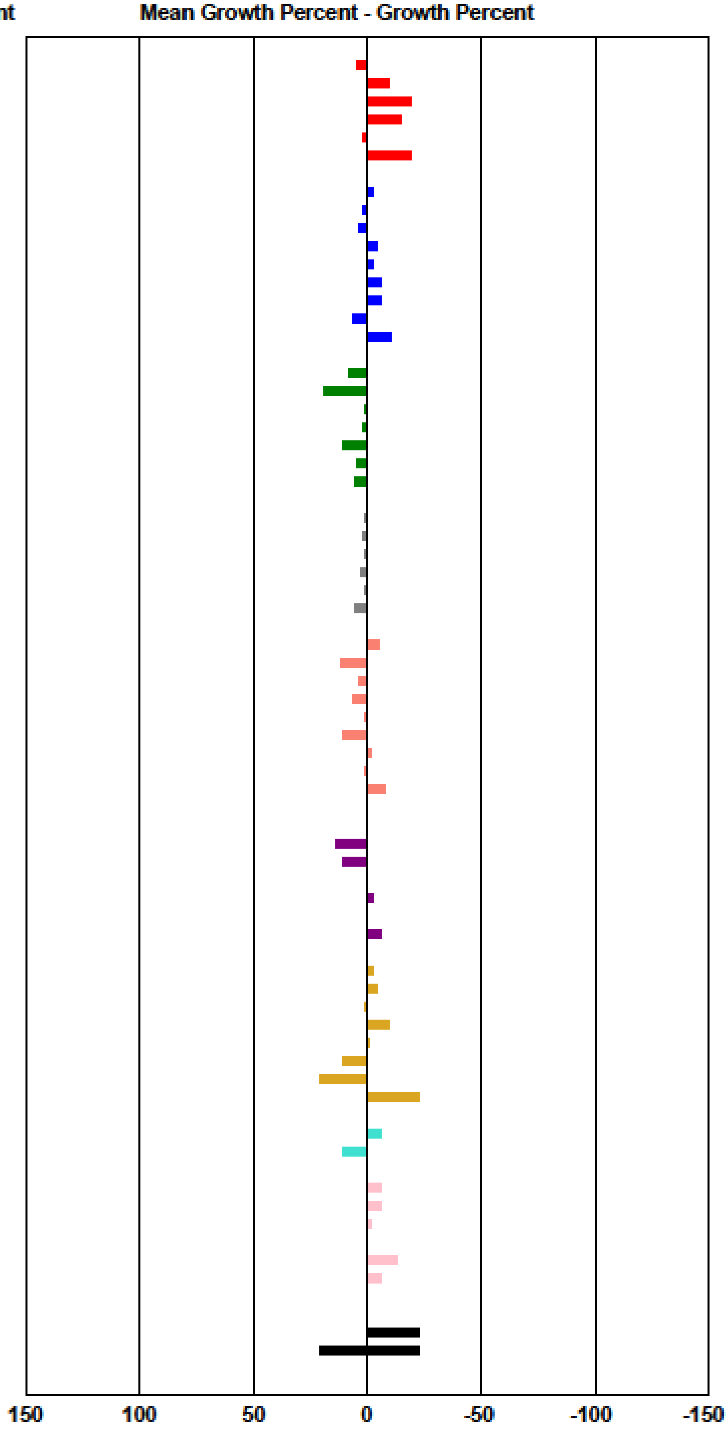


# Table-6 Compound 21e

## Developmental Therapeutics Program
## One Dose Mean Graph

| | | |
|---|---|---|
| **NSC:** D-804118 / 1 | **Conc:** 1.00E-5 Molar | **Test Date:** Mar 05, 2018 |
| **Experiment ID:** 1803OS47 | | **Report Date:** Mar 23, 2018 |

| Panel/Cell Line | Growth Percent |
|---|---|
| Leukemia | |
| CCRF-CEM | 102.57 |
| HL-60(TB) | 88.24 |
| K-562 | 79.06 |
| MOLT-4 | 83.25 |
| RPMI-8226 | 100.05 |
| SR | 78.55 |
| Non-Small Cell Lung Cancer | |
| A549/ATCC | 95.40 |
| EKVX | 100.50 |
| HOP-62 | 101.92 |
| HOP-92 | 94.07 |
| NCI-H226 | 95.52 |
| NCI-H23 | 92.49 |
| NCI-H322M | 91.73 |
| NCI-H460 | 104.33 |
| NCI-H522 | 87.77 |
| Colon Cancer | |
| COLO 205 | 106.31 |
| HCC-2998 | 116.49 |
| HCT-116 | 99.00 |
| HCT-15 | 100.31 |
| HT29 | 109.24 |
| KM12 | 102.95 |
| SW-620 | 103.38 |
| CNS Cancer | |
| SF-268 | 99.59 |
| SF-295 | 100.34 |
| SF-539 | 99.24 |
| SNB-19 | 101.06 |
| SNB-75 | 99.55 |
| U251 | 103.64 |
| Melanoma | |
| LOX IMVI | 93.28 |
| MALME-3M | 109.86 |
| M14 | 101.65 |
| MDA-MB-435 | 104.57 |
| SK-MEL-2 | 99.26 |
| SK-MEL-28 | 108.91 |
| SK-MEL-5 | 96.58 |
| UACC-257 | 99.23 |
| UACC-62 | 90.74 |
| Ovarian Cancer | |
| IGROV1 | 98.43 |
| OVCAR-3 | 111.90 |
| OVCAR-4 | 108.62 |
| OVCAR-5 | 98.02 |
| OVCAR-8 | 95.28 |
| NCI/ADR-RES | 98.19 |
| SK-OV-3 | 91.79 |
| Renal Cancer | |
| 786-0 | 95.43 |
| A498 | 93.83 |
| ACHN | 99.22 |
| CAKI-1 | 88.19 |
| RXF 393 | 97.17 |
| SN12C | 108.58 |
| TK-10 | 118.55 |
| UO-31 | 75.72 |
| Prostate Cancer | |
| PC-3 | 91.84 |
| DU-145 | 109.13 |
| Breast Cancer | |
| MCF7 | 92.51 |
| MDA-MB-231/ATCC | 92.26 |
| HS 578T | 96.46 |
| BT-549 | 98.01 |
| T-47D | 84.86 |
| MDA-MB-468 | 92.35 |
| Mean | 97.95 |
| Delta | 22.23 |
| Range | 42.83 |

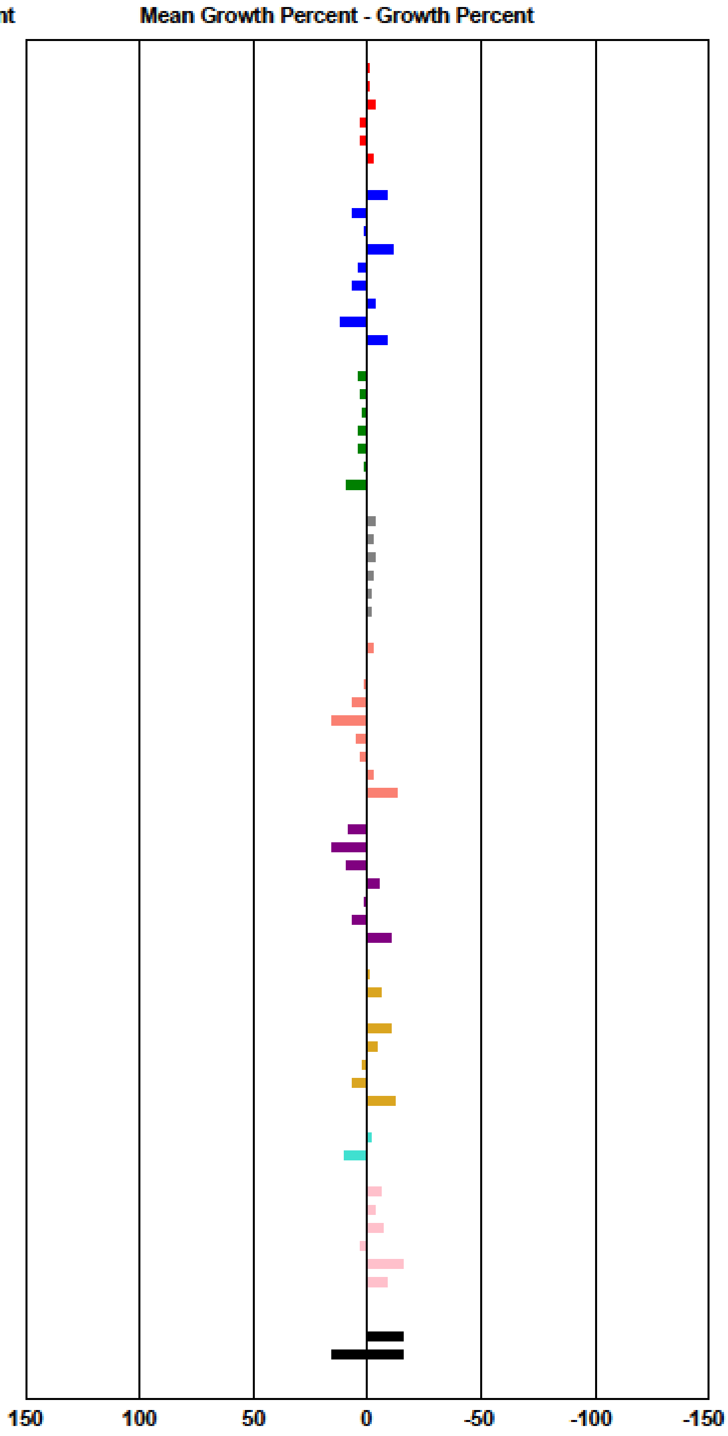


# Table-7 Compound 21f

## Developmental Therapeutics Program
## One Dose Mean Graph

**NSC:** D-804117 / 1
**Conc:** 1.00E-5 Molar
**Test Date:** Mar 05, 2018
**Experiment ID:** 1803OS47
**Report Date:** Mar 23, 2018

| Panel/Cell Line | Growth Percent |
|---|---|
| Leukemia | |
| CCRF-CEM | 100.55 |
| HL-60(TB) | 100.26 |
| K-562 | 97.72 |
| MOLT-4 | 103.66 |
| RPMI-8226 | 104.21 |
| SR | 98.24 |
| Non-Small Cell Lung Cancer | |
| A549/ATCC | 92.11 |
| EKVX | 107.01 |
| HOP-62 | 102.55 |
| HOP-92 | 89.60 |
| NCI-H226 | 104.58 |
| NCI-H23 | 107.22 |
| NCI-H322M | 97.83 |
| NCI-H460 | 113.16 |
| NCI-H522 | 92.58 |
| Colon Cancer | |
| COLO 205 | 105.15 |
| HCC-2998 | 103.51 |
| HCT-116 | 102.67 |
| HCT-15 | 105.08 |
| HT29 | 104.63 |
| KM12 | 101.97 |
| SW-620 | 109.89 |
| CNS Cancer | |
| SF-268 | 97.68 |
| SF-295 | 98.70 |
| SF-539 | 98.12 |
| SNB-19 | 98.80 |
| SNB-75 | 99.56 |
| U251 | 99.92 |
| Melanoma | |
| LOX IMVI | 98.18 |
| MALME-3M | 101.41 |
| M14 | 101.86 |
| MDA-MB-435 | 107.11 |
| SK-MEL-2 | 115.89 |
| SK-MEL-28 | 106.09 |
| SK-MEL-5 | 104.01 |
| UACC-257 | 98.50 |
| UACC-62 | 87.90 |
| Ovarian Cancer | |
| IGROV1 | 108.80 |
| OVCAR-3 | 115.89 |
| OVCAR-4 | 109.76 |
| OVCAR-5 | 95.86 |
| OVCAR-8 | 101.95 |
| NCI/ADR-RES | 107.18 |
| SK-OV-3 | 90.84 |
| Renal Cancer | |
| 786-0 | 100.28 |
| A498 | 95.36 |
| ACHN | 101.14 |
| CAKI-1 | 90.45 |
| RXF 393 | 96.89 |
| SN12C | 103.28 |
| TK-10 | 107.86 |
| UO-31 | 88.87 |
| Prostate Cancer | |
| PC-3 | 99.77 |
| DU-145 | 110.98 |
| Breast Cancer | |
| MCF7 | 94.90 |
| MDA-MB-231/ATCC | 97.52 |
| HS 578T | 94.35 |
| BT-549 | 104.28 |
| T-47D | 85.77 |
| MDA-MB-468 | 92.35 |
| Mean | 100.87 |
| Delta | 15.10 |
| Range | 30.12 |



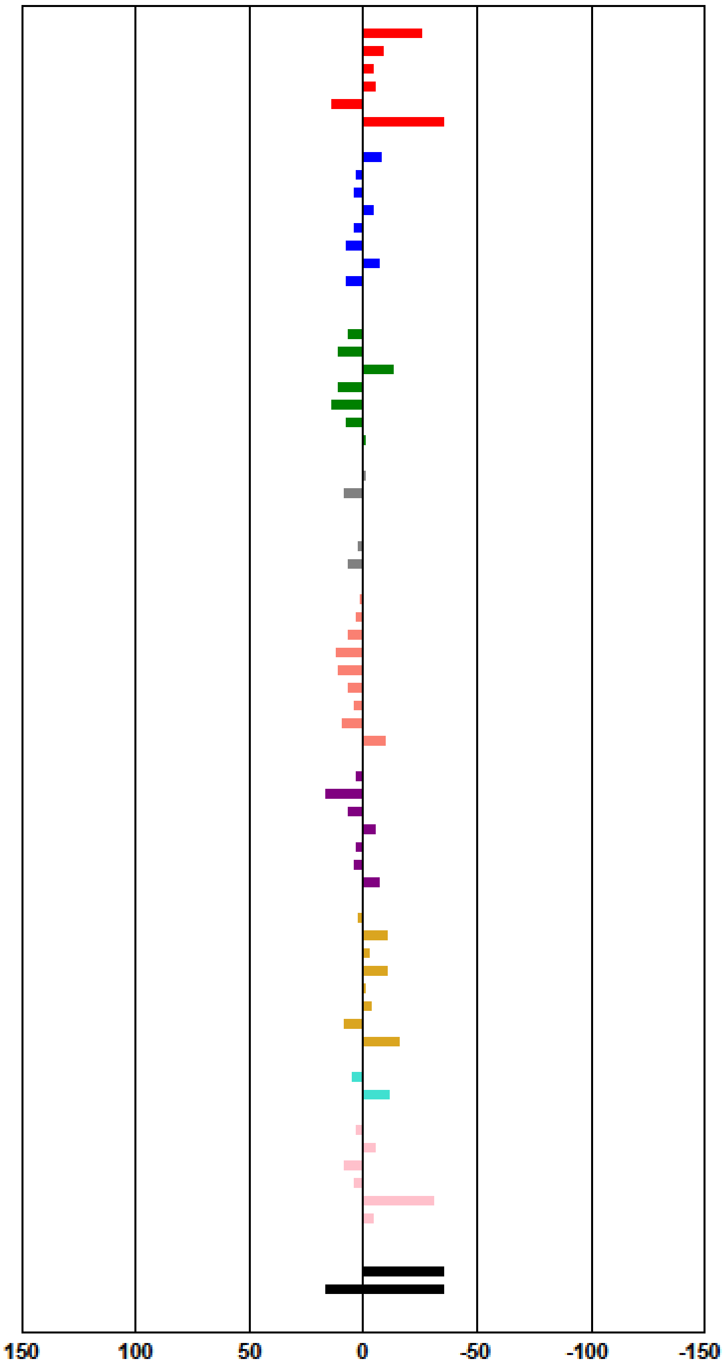
# Table-8 Compound 21g

## Developmental Therapeutics Program
## One Dose Mean Graph

| | | |
|---|---|---|
| **NSC:** D-804116 / 1 | **Conc:** 1.00E-5 Molar | **Test Date:** Mar 05, 2018 |
| **Experiment ID:** 1803OS47 | | **Report Date:** Mar 23, 2018 |

| Panel/Cell Line | Growth Percent |
|---|---|
| Leukemia | |
| CCRF-CEM | 71.12 |
| HL-60(TB) | 87.68 |
| K-562 | 92.21 |
| MOLT-4 | 91.20 |
| RPMI-8226 | 109.62 |
| SR | 61.49 |
| Non-Small Cell Lung Cancer | |
| A549/ATCC | 88.30 |
| EKVX | 99.50 |
| HOP-62 | 100.25 |
| HOP-92 | 92.59 |
| NCI-H226 | 99.86 |
| NCI-H23 | 103.32 |
| NCI-H322M | 89.58 |
| NCI-H460 | 103.80 |
| NCI-H522 | 96.43 |
| Colon Cancer | |
| COLO 205 | 102.84 |
| HCC-2998 | 106.99 |
| HCT-116 | 82.99 |
| HCT-15 | 106.95 |
| HT29 | 109.71 |
| KM12 | 103.84 |
| SW-620 | 95.84 |
| CNS Cancer | |
| SF-268 | 96.20 |
| SF-295 | 104.78 |
| SF-539 | 96.82 |
| SNB-19 | 96.70 |
| SNB-75 | 98.42 |
| U251 | 102.85 |
| Melanoma | |
| LOX IMVI | 97.68 |
| MALME-3M | 98.89 |
| M14 | 103.06 |
| MDA-MB-435 | 108.22 |
| SK-MEL-2 | 107.29 |
| SK-MEL-28 | 103.02 |
| SK-MEL-5 | 100.25 |
| UACC-257 | 105.55 |
| UACC-62 | 86.89 |
| Ovarian Cancer | |
| IGROV1 | 99.04 |
| OVCAR-3 | 112.56 |
| OVCAR-4 | 103.04 |
| OVCAR-5 | 91.74 |
| OVCAR-8 | 99.74 |
| NCI/ADR-RES | 100.45 |
| SK-OV-3 | 89.73 |
| Renal Cancer | |
| 786-0 | 98.24 |
| A498 | 85.83 |
| ACHN | 93.97 |
| CAKI-1 | 86.01 |
| RXF 393 | 96.22 |
| SN12C | 93.34 |
| TK-10 | 104.80 |
| UO-31 | 80.87 |
| Prostate Cancer | |
| PC-3 | 100.76 |
| DU-145 | 85.09 |
| Breast Cancer | |
| MCF7 | 98.93 |
| MDA-MB-231/ATCC | 91.00 |
| HS 578T | 104.69 |
| BT-549 | 100.34 |
| T-47D | 66.14 |
| MDA-MB-468 | 92.12 |
| Mean | 96.29 |
| Delta | 34.80 |
| Range | 51.07 |

Mean Growth Percent - Growth Percent



# Table-9 Compound 21h

**Developmental Therapeutics Program**

**One Dose Mean Graph**

**NSC:** D-804115 / 1

**Conc:** 1.00E-5 Molar

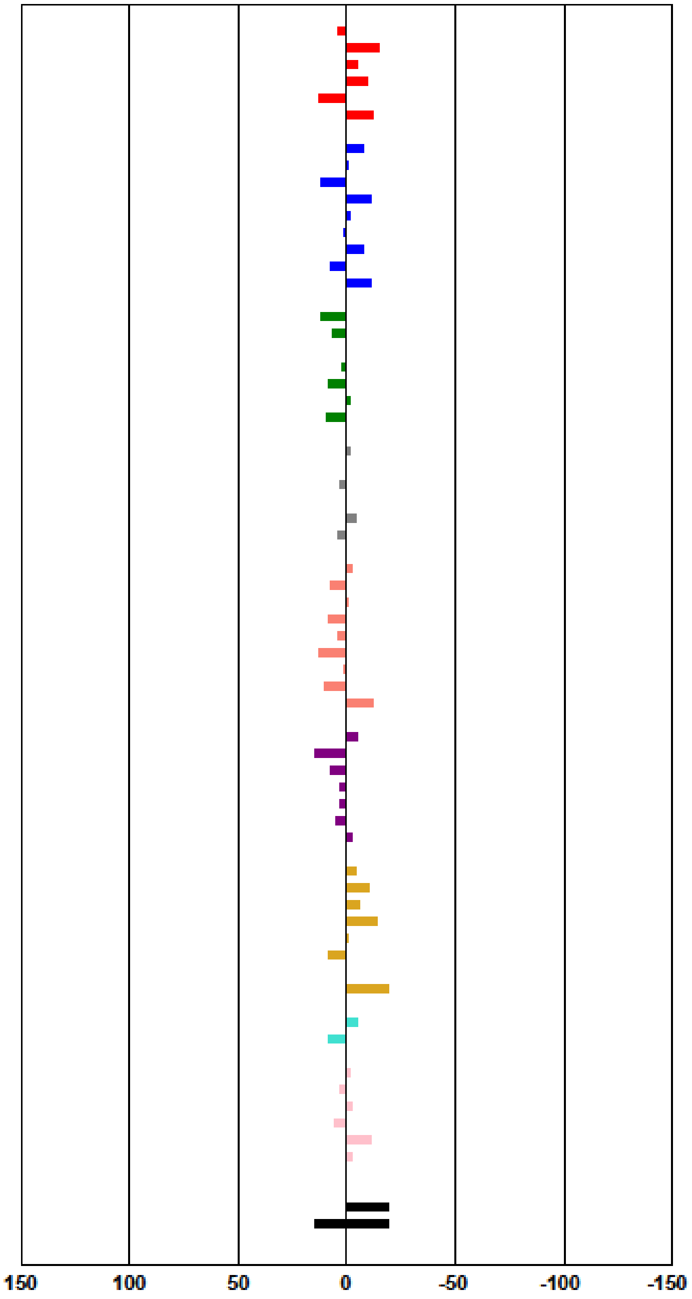
**Test Date:** Mar 05, 2018

**Experiment ID:** 1803OS47

**Report Date:** Mar 23, 2018

| Panel/Cell Line | Growth Percent |
|---|---|
| Leukemia | |
| CCRF-CEM | 104.31 |
| HL-60(TB) | 85.75 |
| K-562 | 95.19 |
| MOLT-4 | 91.32 |
| RPMI-8226 | 113.29 |
| SR | 88.63 |
| Non-Small Cell Lung Cancer | |
| A549/ATCC | 93.16 |
| EKVX | 100.25 |
| HOP-62 | 112.04 |
| HOP-92 | 89.59 |
| NCI-H226 | 99.08 |
| NCI-H23 | 101.78 |
| NCI-H322M | 92.56 |
| NCI-H460 | 107.70 |
| NCI-H522 | 89.18 |
| Colon Cancer | |
| COLO 205 | 112.17 |
| HCC-2998 | 107.31 |
| HCT-116 | 101.08 |
| HCT-15 | 102.66 |
| HT29 | 108.79 |
| KM12 | 99.20 |
| SW-620 | 109.34 |
| CNS Cancer | |
| SF-268 | 99.09 |
| SF-295 | 100.86 |
| SF-539 | 103.33 |
| SNB-19 | 101.12 |
| SNB-75 | 96.13 |
| U251 | 104.67 |
| Melanoma | |
| LOX IMVI | 98.56 |
| MALME-3M | 107.81 |
| M14 | 100.20 |
| MDA-MB-435 | 109.00 |
| SK-MEL-2 | 104.61 |
| SK-MEL-28 | 113.58 |
| SK-MEL-5 | 101.37 |
| UACC-257 | 110.73 |
| UACC-62 | 88.27 |
| Ovarian Cancer | |
| IGROV1 | 95.32 |
| OVCAR-3 | 114.74 |
| OVCAR-4 | 108.25 |
| OVCAR-5 | 103.21 |
| OVCAR-8 | 103.30 |
| NCI/ADR-RES | 104.92 |
| SK-OV-3 | 98.26 |
| Renal Cancer | |
| 786-0 | 96.68 |
| A498 | 90.70 |
| ACHN | 94.68 |
| CAKI-1 | 86.63 |
| RXF 393 | 99.82 |
| SN12C | 108.53 |
| TK-10 | 100.97 |
| UO-31 | 81.66 |
| Prostate Cancer | |
| PC-3 | 95.40 |
| DU-145 | 108.48 |
| Breast Cancer | |
| MCF7 | 98.95 |
| MDA-MB-231/ATCC | 103.68 |
| HS 578T | 98.46 |
| BT-549 | 105.95 |
| T-47D | 89.43 |
| MDA-MB-468 | 98.66 |
| Mean | 100.51 |
| Delta | 18.85 |
| Range | 33.08 |

Mean Growth Percent - Growth Percent



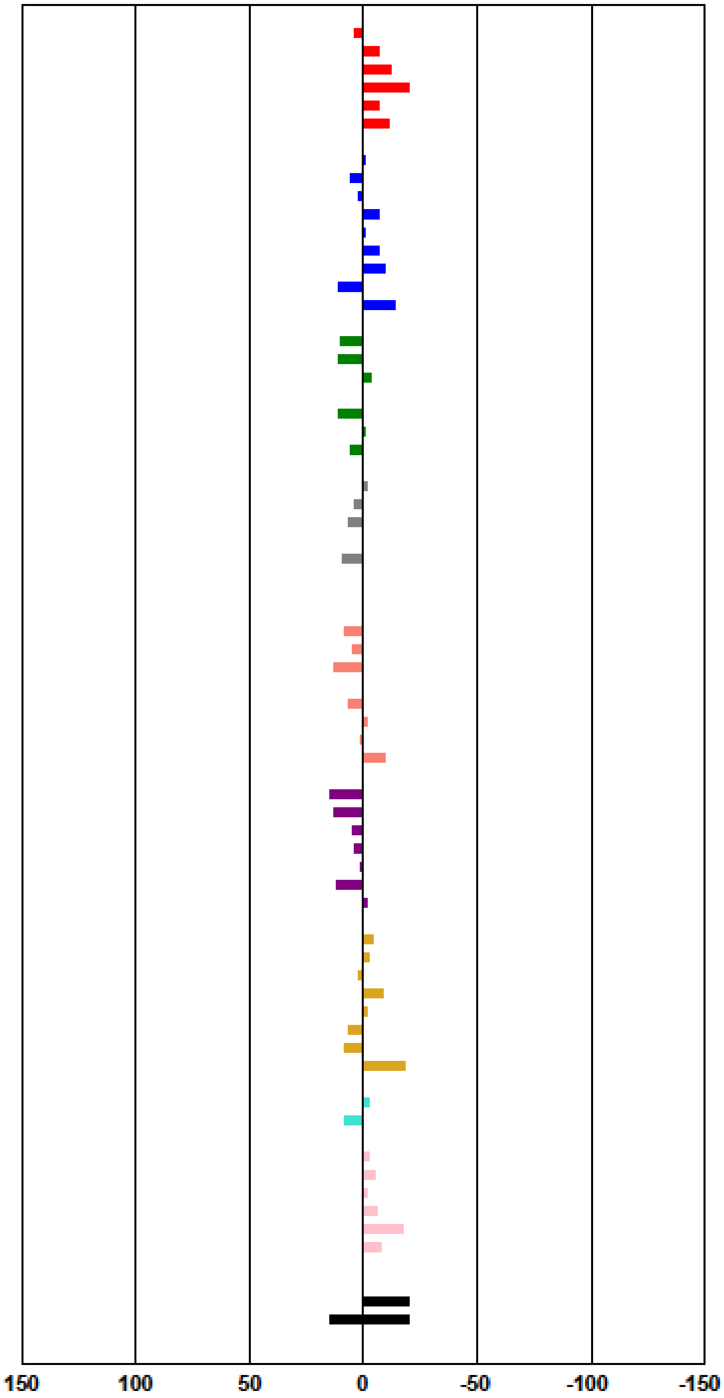
# Table-10 Compound 21j

**Developmental Therapeutics Program**
**One Dose Mean Graph**

**NSC:** D-804111 / 1
**Conc:** 1.00E-5 Molar
**Test Date:** Mar 05, 2018
**Experiment ID:** 1803OS47
**Report Date:** Mar 23, 2018

| Panel/Cell Line | Growth Percent |
|---|---|
| Leukemia | |
| CCRF-CEM | 103.32 |
| HL-60(TB) | 93.32 |
| K-562 | 87.55 |
| MOLT-4 | 79.83 |
| RPMI-8226 | 93.36 |
| SR | 88.82 |
| Non-Small Cell Lung Cancer | |
| A549/ATCC | 98.80 |
| EKVX | 105.66 |
| HOP-62 | 101.77 |
| HOP-92 | 92.60 |
| NCI-H226 | 98.87 |
| NCI-H23 | 92.53 |
| NCI-H322M | 89.87 |
| NCI-H460 | 110.35 |
| NCI-H522 | 86.11 |
| Colon Cancer | |
| COLO 205 | 109.37 |
| HCC-2998 | 110.95 |
| HCT-116 | 96.31 |
| HCT-15 | 100.43 |
| HT29 | 110.53 |
| KM12 | 98.86 |
| SW-620 | 104.88 |
| CNS Cancer | |
| SF-268 | 98.63 |
| SF-295 | 103.36 |
| SF-539 | 106.59 |
| SNB-19 | 100.23 |
| SNB-75 | 108.56 |
| U251 | 99.98 |
| Melanoma | |
| LOX IMVI | 99.68 |
| MALME-3M | 108.36 |
| M14 | 104.36 |
| MDA-MB-435 | 112.41 |
| SK-MEL-2 | 99.61 |
| SK-MEL-28 | 106.44 |
| SK-MEL-5 | 98.21 |
| UACC-257 | 100.80 |
| UACC-62 | 90.21 |
| Ovarian Cancer | |
| IGROV1 | 114.04 |
| OVCAR-3 | 112.19 |
| OVCAR-4 | 104.64 |
| OVCAR-5 | 103.23 |
| OVCAR-8 | 101.32 |
| NCI/ADR-RES | 111.88 |
| SK-OV-3 | 97.89 |
| Renal Cancer | |
| 786-0 | 95.54 |
| A498 | 97.25 |
| ACHN | 101.62 |
| CAKI-1 | 91.22 |
| RXF 393 | 98.45 |
| SN12C | 106.01 |
| TK-10 | 107.82 |
| UO-31 | 81.10 |
| Prostate Cancer | |
| PC-3 | 97.41 |
| DU-145 | 107.89 |
| Breast Cancer | |
| MCF7 | 97.08 |
| MDA-MB-231/ATCC | 94.30 |
| HS 578T | 97.84 |
| BT-549 | 93.52 |
| T-47D | 82.34 |
| MDA-MB-468 | 91.80 |
| Mean | 99.63 |
| Delta | 19.80 |
| Range | 34.21 |

**Mean Growth Percent - Growth Percent**

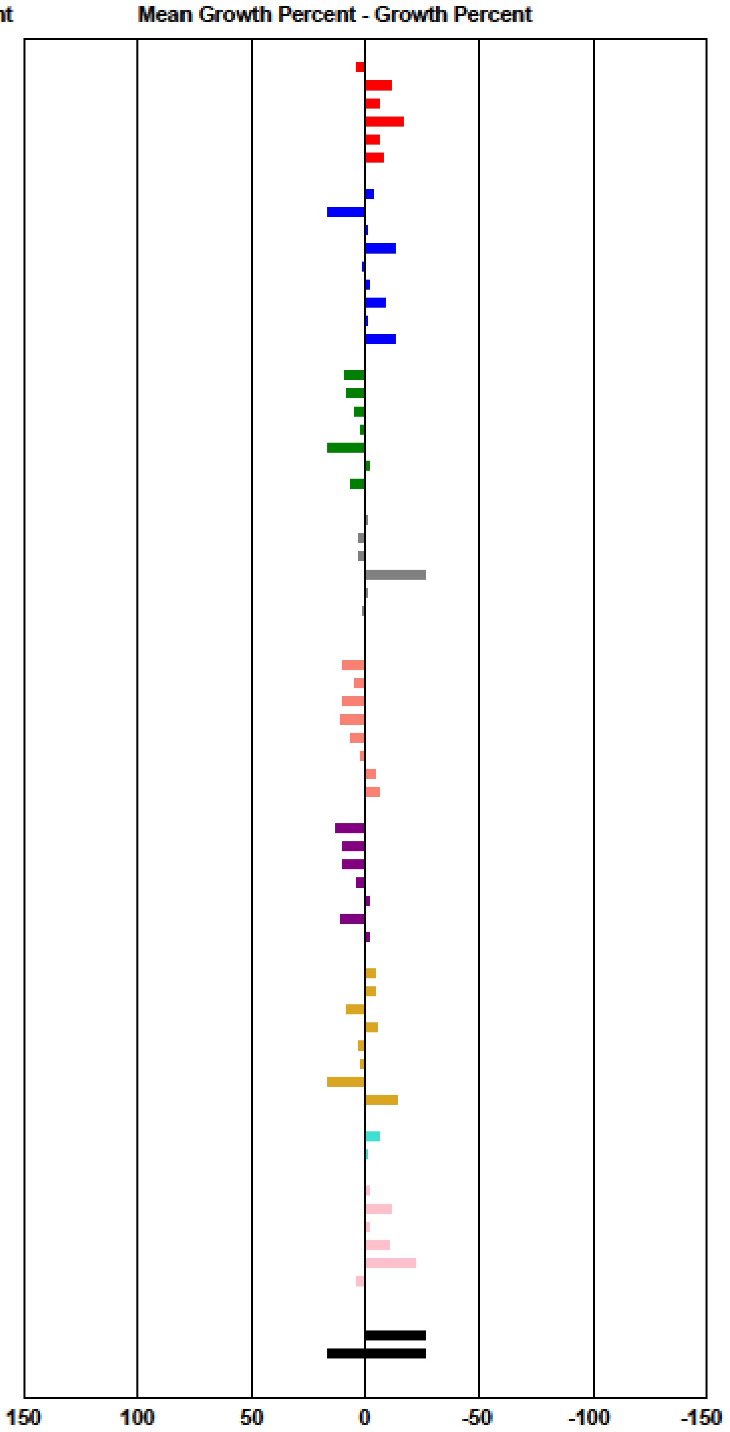


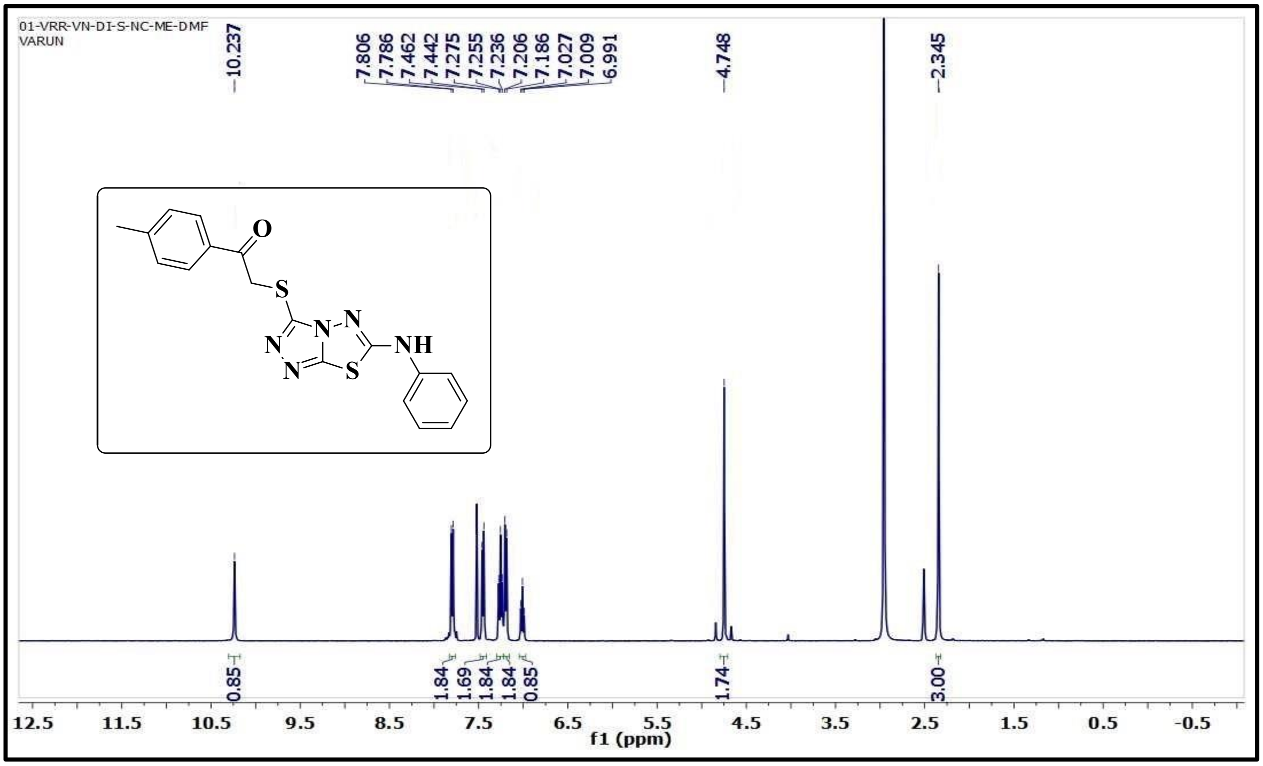
# Table-11 Compound 21k

**Developmental Therapeutics Program**
**One Dose Mean Graph**

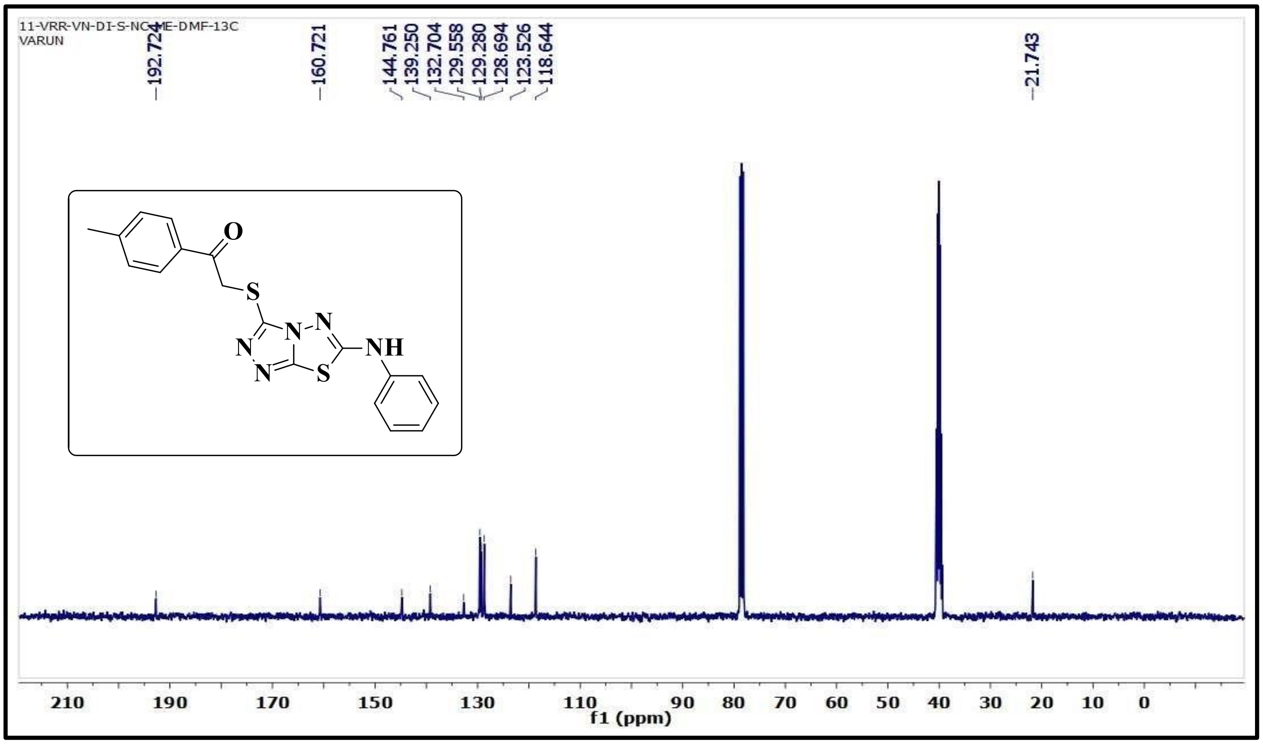
NSC: D-804112 / 1
Conc: 1.00E-5 Molar
Test Date: Mar 05, 2018
Experiment ID: 1803OS47
Report Date: Mar 23, 2018

| Panel/Cell Line | Growth Percent |
|---|---|
| Leukemia | |
| CCRF-CEM | 101.93 |
| HL-60(TB) | 87.07 |
| K-562 | 92.72 |
| MOLT-4 | 81.59 |
| RPMI-8226 | 92.83 |
| SR | 91.16 |
| Non-Small Cell Lung Cancer | |
| A549/ATCC | 95.63 |
| EKVX | 114.45 |
| HOP-62 | 97.93 |
| HOP-92 | 85.64 |
| NCI-H226 | 99.84 |
| NCI-H23 | 97.14 |
| NCI-H322M | 90.33 |
| NCI-H460 | 98.17 |
| NCI-H522 | 85.38 |
| Colon Cancer | |
| COLO 205 | 107.50 |
| HCC-2998 | 106.96 |
| HCT-116 | 103.35 |
| HCT-15 | 100.61 |
| HT29 | 114.60 |
| KM12 | 96.82 |
| SW-620 | 105.28 |
| CNS Cancer | |
| SF-268 | 97.81 |
| SF-295 | 101.10 |
| SF-539 | 101.00 |
| SNB-19 | 72.39 |
| SNB-75 | 97.95 |
| U251 | 99.80 |
| Melanoma | |
| LOX IMVI | 98.46 |
| MALME-3M | 108.55 |
| M14 | 103.11 |
| MDA-MB-435 | 108.55 |
| SK-MEL-2 | 109.74 |
| SK-MEL-28 | 105.22 |
| SK-MEL-5 | 100.67 |
| UACC-257 | 94.48 |
| UACC-62 | 92.37 |
| Ovarian Cancer | |
| IGROV1 | 111.26 |
| OVCAR-3 | 108.51 |
| OVCAR-4 | 108.64 |
| OVCAR-5 | 102.37 |
| OVCAR-8 | 97.13 |
| NCI/ADR-RES | 109.72 |
| SK-OV-3 | 97.37 |
| Renal Cancer | |
| 786-0 | 94.55 |
| A498 | 94.80 |
| ACHN | 106.51 |
| CAKI-1 | 93.50 |
| RXF 393 | 101.43 |
| SN12C | 100.49 |
| TK-10 | 115.12 |
| UO-31 | 84.59 |
| Prostate Cancer | |
| PC-3 | 93.00 |
| DU-145 | 98.02 |
| Breast Cancer | |
| MCF7 | 96.77 |
| MDA-MB-231/ATCC | 87.64 |
| HS 578T | 97.04 |
| BT-549 | 87.78 |
| T-47D | 76.96 |
| MDA-MB-468 | 101.98 |
| Mean | 98.39 |
| Delta | 26.00 |
| Range | 42.73 |

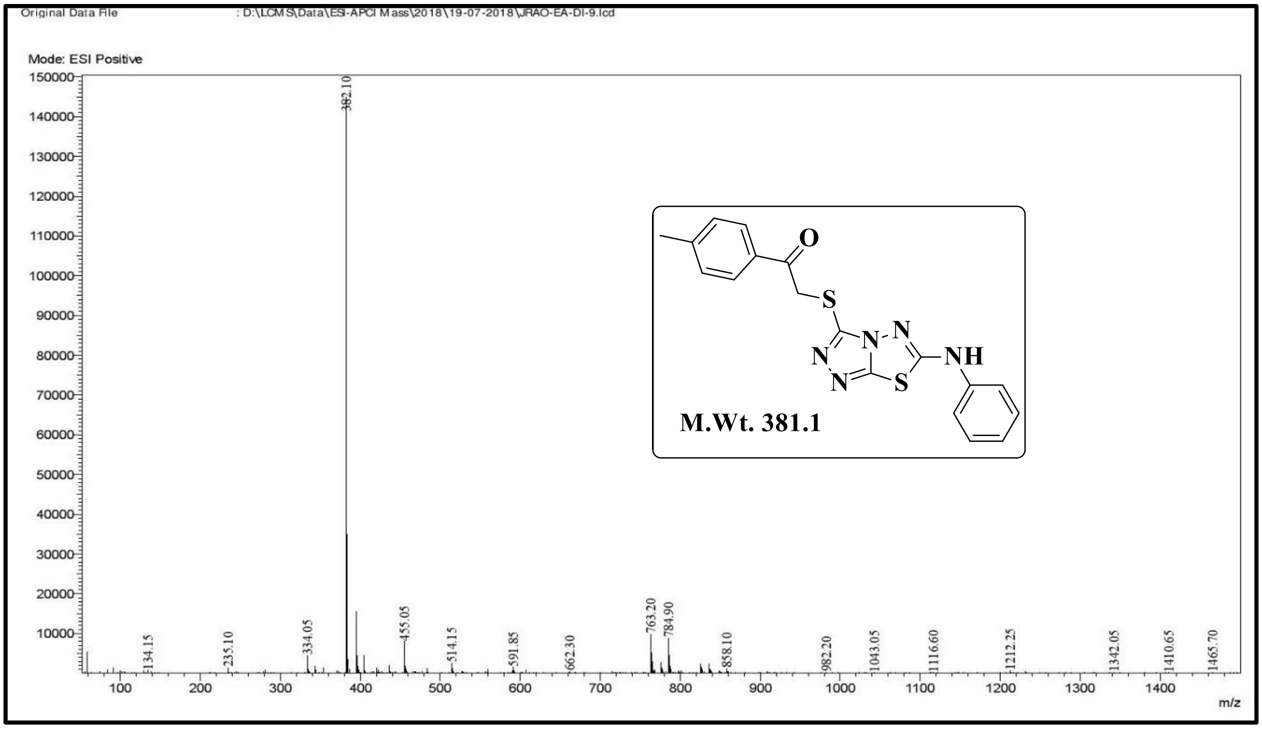




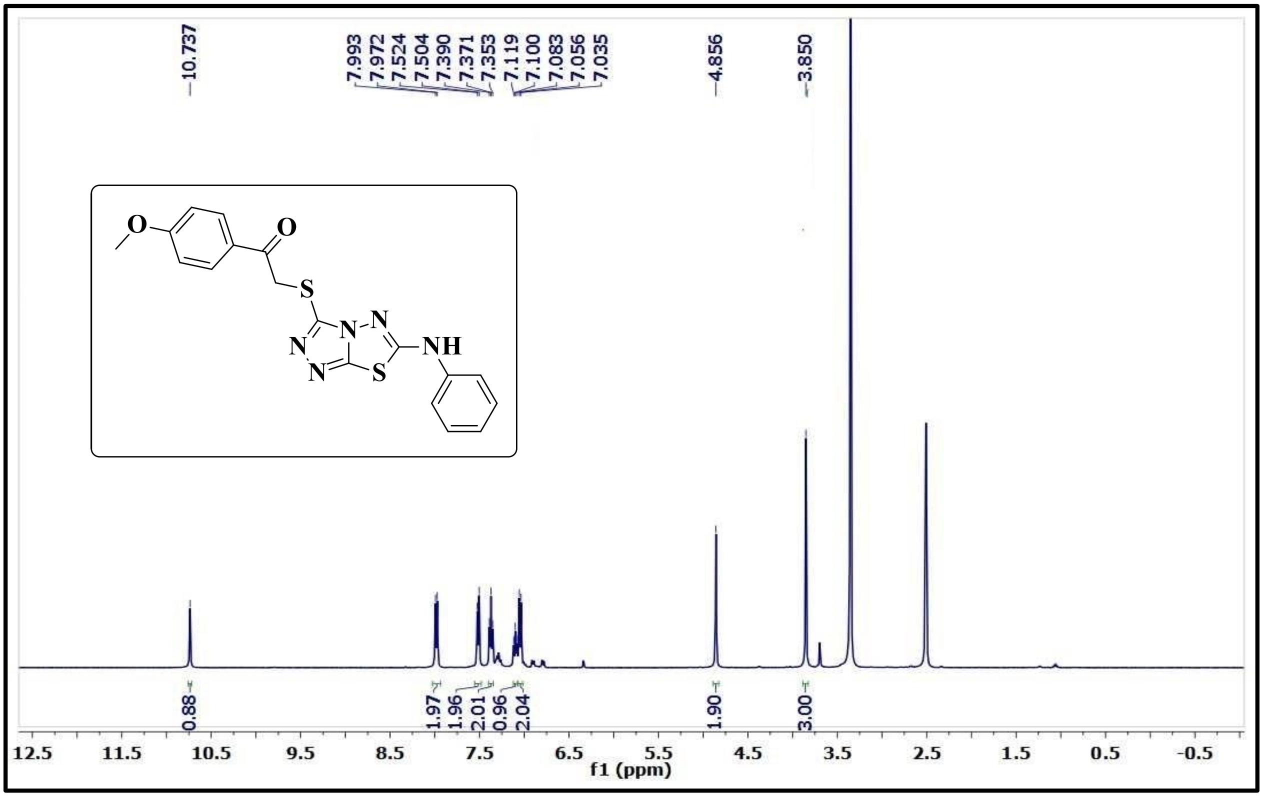
$^{1}$H NMR Spectrum of compound **21a**



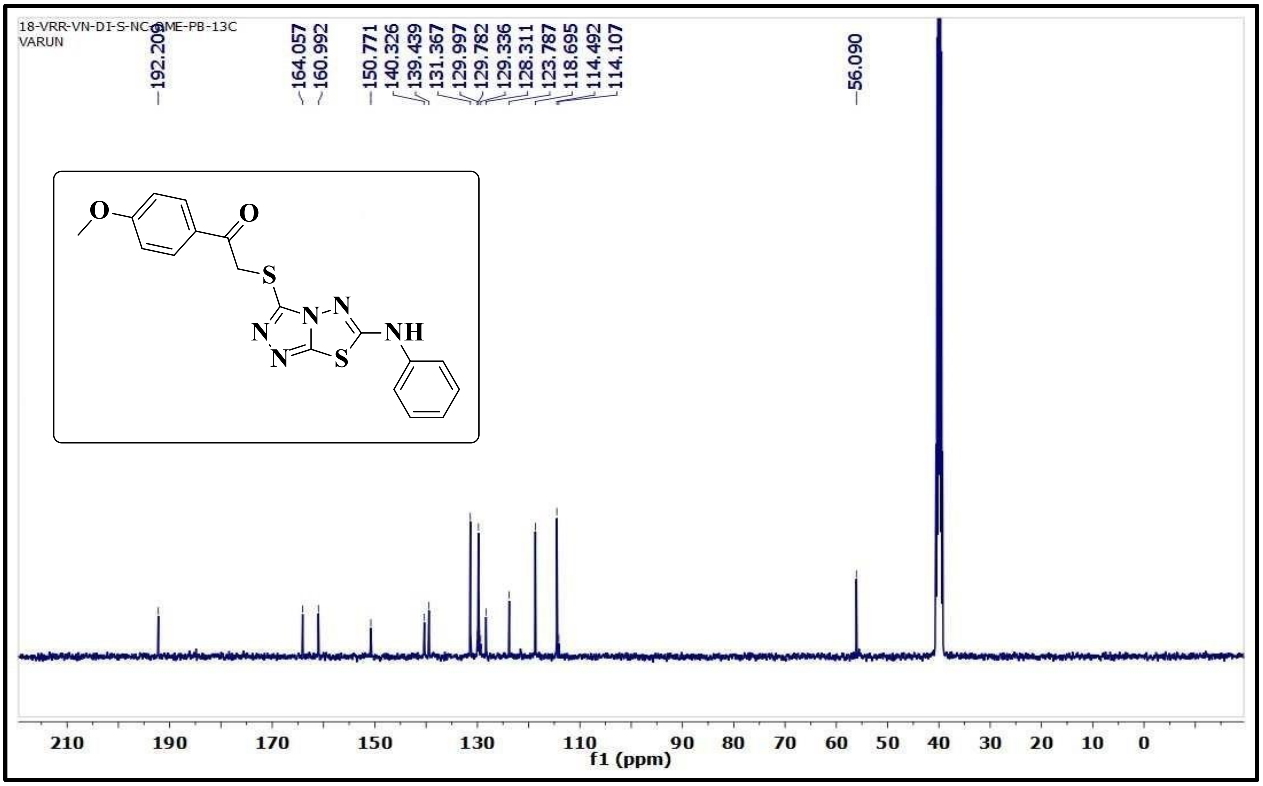
$^{13}$C NMR Spectrum of compound **21a**



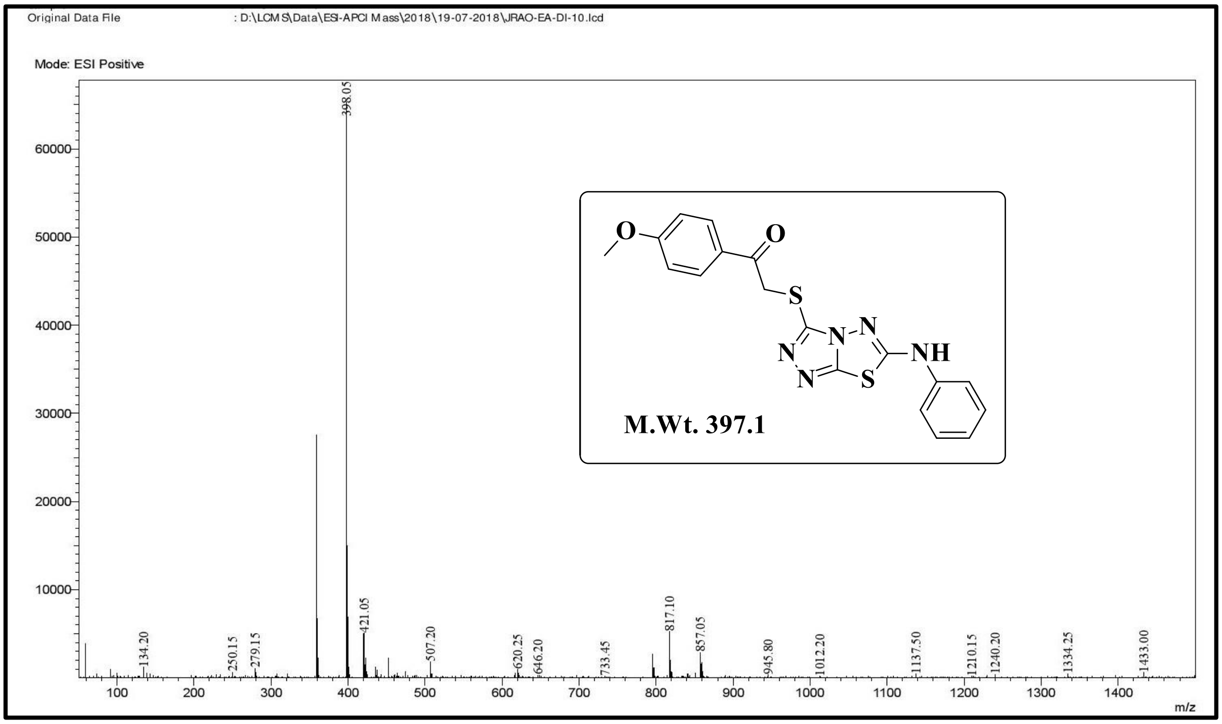
Mass Spectrum of compound **21a**



$^1$H NMR Spectrum of compound **21b**



$^{13}$C NMR Spectrum of compound **21b**



Mass Spectrum of compound **21b**

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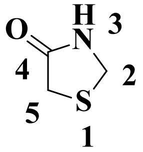
# CHAPTER-V

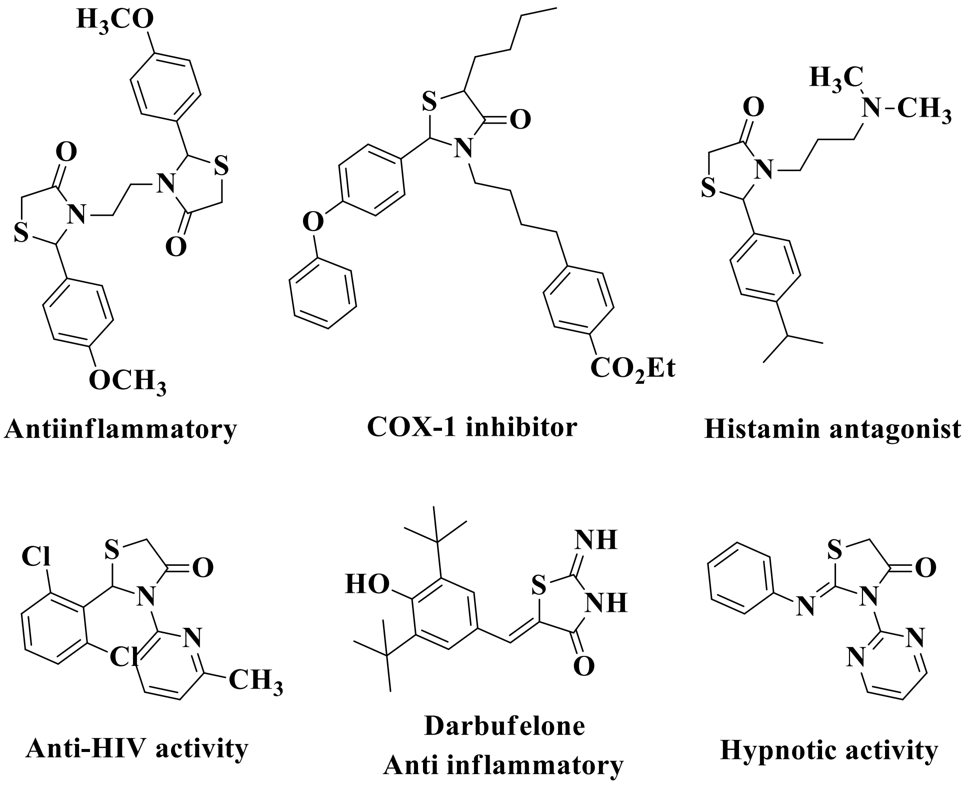
# ONE-POT MULTICOMPONENT SYNTHESIS OF (*Z*)-ALKYL 2-((*Z*)-3-(2-(1*H*-INDOL-3-YL)ETHYL)-4-OXO-2-(ARYLIMINO/BENZYLIMINO)THIAZOLIDIN-5-YLIDENE)ACETATES AND THEIR ANTICANCER ACTIVITY

# INTRODUCTION

4-Thiazolidinone is a five membered heterocyclic ring system, in which sulfur, nitrogen atoms are at 1st, 3rd positions and carbonyl functional group at 4th position respectively.



4-Thiazolidinone ring system exhibits a variety of biological applications such as antimicrobial[1,2] antifungal[3], antiviral[4,5], anticancer[6,7], anti-inflammatory[8,9], antioxidant[10], antitubercular[11], and antidiabetic[12].



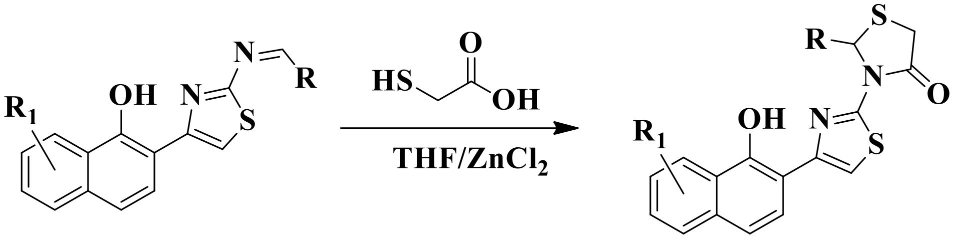
**Antiinflammatory** **COX-1 inhibitor** **Histamin antagonist**

**Anti-HIV activity** **Darbufelone Anti inflammatory** **Hypnotic activity**

**Fig.1** Some of the 4-thiazolidinone ring-containing drug molecules.

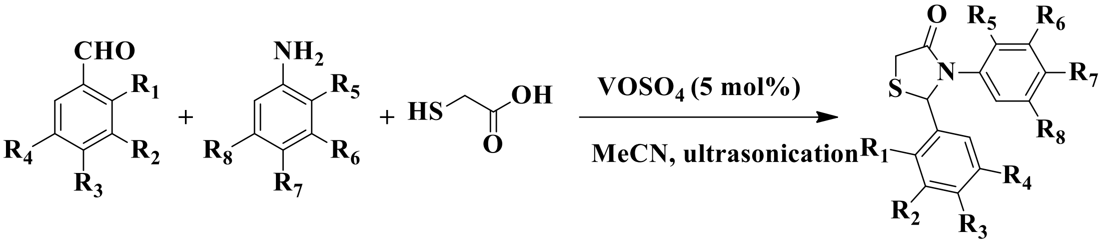
A concise review of the synthesis of thiazole is given below.

Gaikwad[13] *et al.* synthesized 3-(4-(1-hydroxynaphthalen-2-yl)thiazol-2-yl)-2-phenylthiazolidin-4-ones (1). Cyclocondensation reaction of 2-(2-(benzylideneamino)thiazol-4-yl)naphthalen-1-ol and 2-mercapto acetic acid in presence of THF and $ZnCl_2$ yields the title compounds.

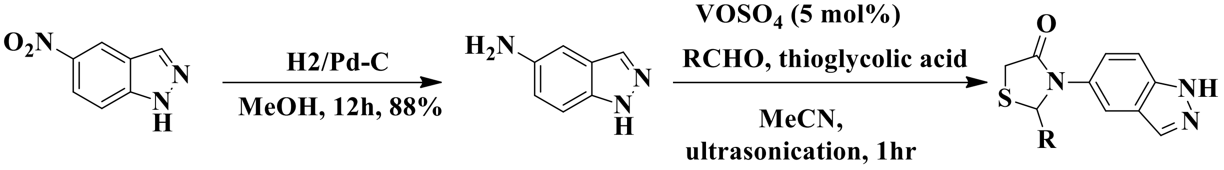


**1**

2,3-Diphenylthiazolidin-4-ones (**2**) and 4-(1*H*-indazol-5-yl)-5-phenyldihydrothiophen-3(2*H*)-ones (**3**) were synthesized by Angapelly[14] *et. al.*. A mixture of substituted benzaldehyde, aniline and thioglycolic acid react in presence of $VOSO_4$ as a catalyst in acetonitrile under ultrasonic irradiation to give compound (**2**). 5-nitro-1*H*-indazole on reduction with Pd-C to give 1*H*-indazol-5-amine, which further reacts with substituted benzaldehyde and thioglycolic acid using $VOSO_4$ as a catalyst in acetonitrile to give corresponding compound (**3**).

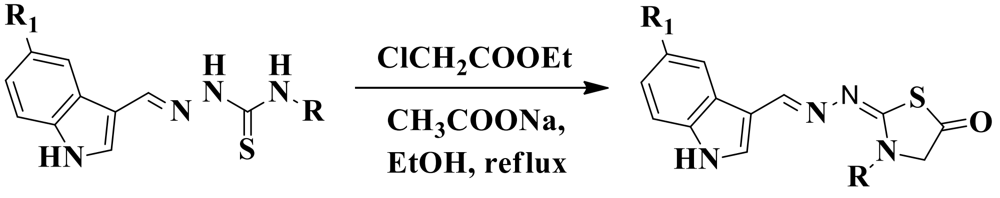


**2**



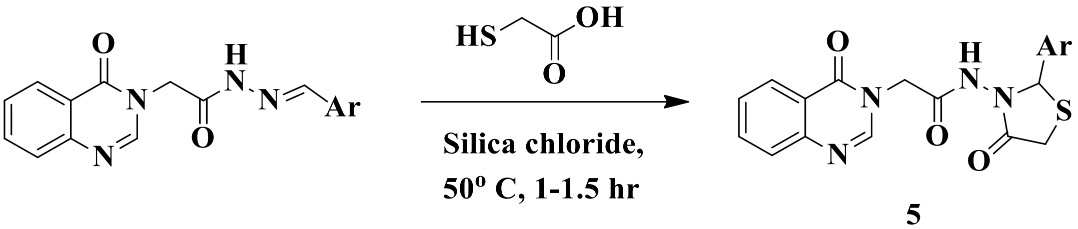
**3**

Oliveira[15] *et. al.* synthesized 2-(((1*H*-indol-3-yl)methylene)hydrazono)-3-arylthiazolidin-5-ones (**4**). Cyclocondensation reaction between substituted 2-((1*H*-indol-3-yl)methylene)-*N*-arylhydrazinecarbothioamide and $ClCH_2COOEt$ in ethanol to yield title compounds (**4**).



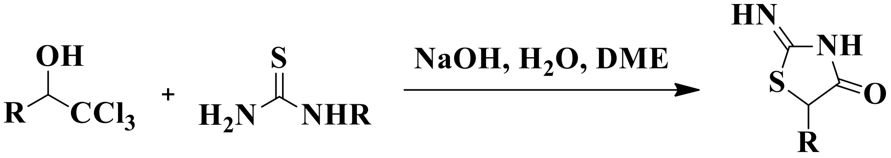
**4**

Mali[16] *et. al.* synthesized of *N*-(4-oxo-2-phenylthiazolidin-3-yl)-2-(4-oxoquinazolin-3(4*H*)-yl)acetamides (**5**). Cyclocondesation reaction of *N'*-benzylidene-2-(4-oxoquinazolin-3(4*H*)-yl)acetohydrazide and mercapto acetic acid resulted in formation of compound 5.



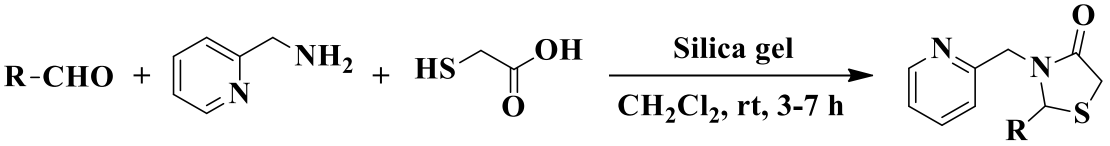
**5**

Blanchet and Zhu[17] reported the synthesis of 2-imino-5-alkyl/aryl thiazolidin-4-ones (**6**). Reaction between alkyl (aryl) trichloromethylcarbinols and thioureas under mild conditions to give title compounds.



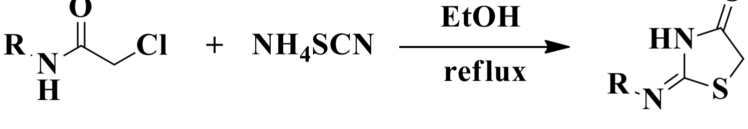
**6**

Thakare[18] *et al.* synthesized 2-alkyl/aryl/heteryl-3-(pyridin-2-ylmethyl)thiazolidin-4-ones (**7**). Three component reaction of alkyl/aryl/heteryl aldehyde, pyridin-2-ylmethanamine and thioglycolic acid in dichloromethane, silica gel as catalyst yielded the title compounds



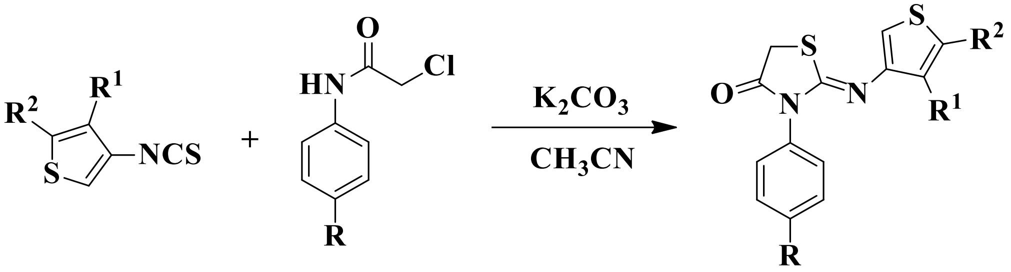
**7**

2-Heterylimino-4-thiazolidinones (**8**) were synthesized by Geronikaki[19] *et al.* by the reaction of 2-chloro-N-(thiazole/benzo[d]thiazole)acetamides and $NH_4SCN$



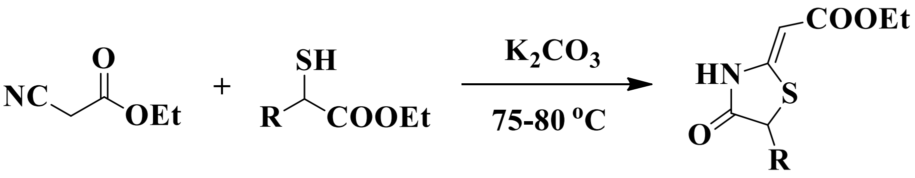
**8**

Revelant[20] *et al.* synthesized of 2-heteroarylimino-4-thiazolidinones (**9**) by the reaction of 2-chloro-*N*-arylacetamide with 3-isothiocyanatothiophene in presence of $K_2CO_3$ and acetonitrile as a solvent.



**9**

4-Oxo-thiazolidines (**10**) synthesis was given by Stojanovic[21] *et al.* under solvent free reaction condition by using ethyl cyanoacetate and α-mercaptoesters in presence of $K_2CO_3$.



**10**

## PRESENT WORK

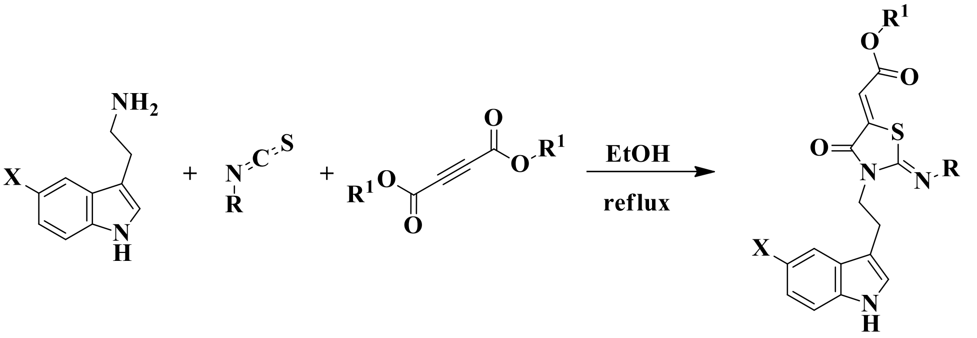
Review of the literature shows that thiazolidinones play an important role in the analogue synthesis of several medicinal chemistry programs. The concept of high through put screening has literally challenged the synthetic medicinal chemists to prepare structurally diverse and complex molecules of pharmacological interest. The growing demand for new chemical entities has fuelled our interest to try new approaches such as diversity oriented synthesis for the small molecular libraries.

In the literature, much importance has been given to 4-thiazolidinones due to their wide range of biological applications. In recent years, much attention has been given to explore the synthesis and biological applications of 4-thiazolidinones by many chemists. In view of importance of tryptamine, serotonin and thiazolidinones, an efficient multicomponent reaction was developed for the synthesis of substituted thiazolidinones by incorporating these heterocyclic moieties. It is hoped that by using tryptamine and serotonin scaffolds may be helpful for the enhancement of biological activity.

In the present chapter we describe the synthesis and anticancer activity of (*Z*)-alkyl 2-((*Z*)-3-(2-(1*H*-indol-3-yl)ethyl)-4-oxo-2-(arylimino/benzylimino)thiazolidin-5-ylidene)acetates **14 (a-za)**.

The reactants used for the synthesis of these heterocyclic compounds were procured from commercial sources and used further without any purification.

Tryptamine and its hydroxy derivative reacts with aryl/benzyl isothiocyanate and dialkylacetylene dicarboxylates in ethanol under reflux to yield corresponding cyclo products (*Z*)-alkyl2-((*Z*)-3-(2-(1*H*-indol-3-yl)ethyl)-4-oxo-2-(arylimino/benzylimino)thiazolidin-5-ylidene) acetates **14 (a-za)**. Among all synthesized compounds, some of the compounds were selected for their anticancer activity by NCI for screening against 60 human cancer cell lines.



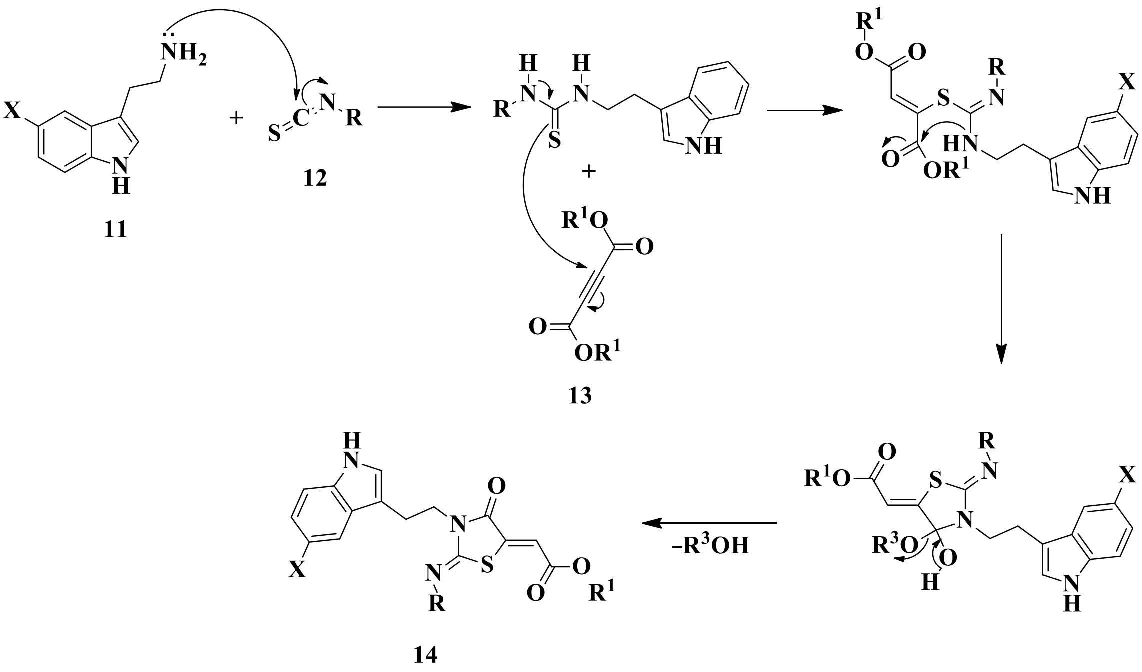
**X=H, 11 (a)** **12 (a-i)** **13 (a-b)** **X=H, 14 (a-r)**

**X=OH, 11 (b)** **X=OH, 14 (s-za)**

**Scheme-1:** One pot synthesis of **14 (a-za)**

## Mechanism

A plausible mechanism for the synthesis of (*Z*)-alkyl 2-((*Z*)-3-(2-(1*H*-indol-3-yl)ethyl)-4-oxo-2-(arylimino/benzylimino)thiazolidin-5-ylidene)acetates **14 (a-za)** is explained in scheme-2. The nucleophilic amino group of 16 readily reacts with electrophilic ‘C’ atom of isothiocyanate to give unsymmetrical thioureas i.e., 1-(2-(1*H*-indol-3-yl)ethyl)-3-phenylthiourea/3-benzylthiourea. The more nucleophilic sulphur atom of thiourea adds on to one of the alkyne carbon atom followed by loss of MeOH/EtOH results in the formation of final products 19.



**Scheme-2:** Plausible reaction mechanism for the formation of compounds **14 (a-za).**

**Table 1**. Various substituents on the product along with reaction time

| Product | X | R | $R^1$ | Reaction time (h) |
|---|---|---|---|---|
| 14a | H | phenyl | $OCH_2CH_3$ | 2.0 |
| 14b | H | phenyl | $OCH_3$ | 2.5 |
| 14c | H | 4-methyl phenyl | $OCH_2CH_3$ | 1.0 |
| 14d | H | 4-methyl phenyl | $OCH_3$ | 1.5 |
| 14e | H | 3-methoxy phenyl | $OCH_2CH_3$ | 0.5 |
| 14f | H | 3-methoxy phenyl | $OCH_3$ | 1.0 |
| 14g | H | 4-fluoro phenyl | $OCH_2CH_3$ | 4.0 |
| 14h | H | 4-fluoro phenyl | $OCH_3$ | 4.0 |
| 14i | H | 3-chloro phenyl | $OCH_2CH_3$ | 3.0 |
| 14j | H | 3-chloro phenyl | $OCH_3$ | 3.5 |
| 14k | H | 4-cyano phenyl | $OCH_2CH_3$ | 3.0 |
| 14l | H | 4-cyano phenyl | $OCH_3$ | 3.5 |
| 14m | H | 4-nitro phenyl | $OCH_2CH_3$ | 4.0 |
| 14n | H | 4-nitro phenyl | $OCH_3$ | 4.0 |
| 14o | H | 4-trifluoromethyl phenyl | $OCH_2CH_3$ | 4.5 |

|  |  |  |  |  |
|---|---|---|---|---|
| 14p | H | 4-trifluoromethyl phenyl | $OCH_3$ | 5 |
| 14q | OH | benzyl | $OCH_2CH_3$ | 1.0 |
| 14r | OH | benzyl | $OCH_3$ | 1.5 |
| 14s | OH | 3-methoxy phenyl | $OCH_2CH_3$ | 1.0 |
| 14t | OH | 3-methoxy phenyl | $OCH_3$ | 1.5 |
| 14u | OH | 3-chloro phenyl | $OCH_2CH_3$ | 3.5 |
| 14v | OH | 3-chloro phenyl | $OCH_3$ | 3.0 |
| 14w | OH | 4-fluoro phenyl | $OCH_3$ | 4.0 |
| 14x | OH | 4-trifluoromethyl phenyl | $OCH_3$ | 4.5 |
| 14y | OH | 4-methyl phenyl | $OCH_3$ | 1.5 |
| 14z | OH | 4-nitro phenyl | $OCH_2CH_3$ | 4.0 |
| 14za | OH | 4-nitro phenyl | $OCH_3$ | 4.5 |

All the synthesized compounds were characterized by physical and analytical spectral data. Compounds **14e** and **14i** were further confirmed by single crystal X-ray diffraction data

## Single crystal data

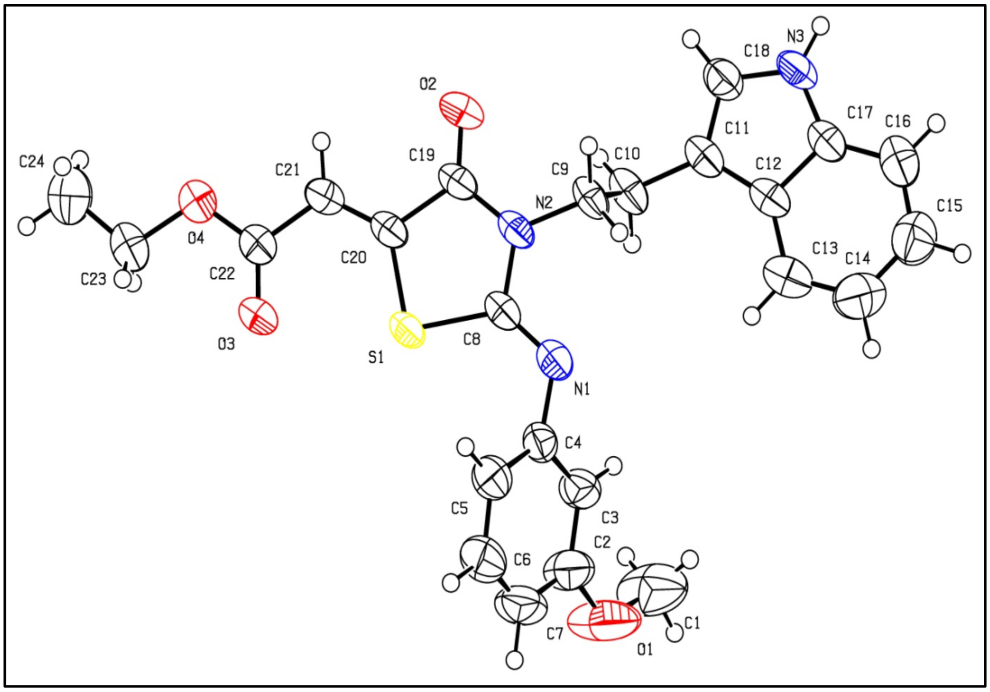


**Fig. 1** Platon diagram of compound **14e**

**Table 2**. Crystal data and structure refinement for the compound **14e**

| **Identification code** | **shelx** |
|---|---|
| Empirical formula | $C_{24} H_{23} N_3 O_4 S$ |
| Formula weight | 449.51 |
| Temperature | 296(2) K |
| Wavelength | 0.71073 A |
| Crystal system, space group | Monoclinic, P 21/c |
| Unit cell dimensions | a = 11.8095(10) (A°), b = 13.0904(8) (A°) |
| | c = 14.7933(12) (A°) |
| | $\alpha$ = 90 (°), $\beta$ = 97.811(3) (°), $\gamma$ = 90 (°) |
| Volume *V* (Å$^3$) | 2265.7(3) |
| Z, Calculated density | 4, 1.318 Mg/m 3 |
| Absorption coefficient | 0.179 mm$^{-1}$ |
| F(000) | 944 |
| Crystal size | 0.350 x 0.300 x 0.300 mm |
| Theta range for data collection | 1.741 to 28.424 (°) |
| Limiting indices | $-15 \leq h \leq 15$ |
| | $-17 \leq k \leq 11$ |
| | $-19 \leq l \leq 19$ |
| Reflections collected / unique | 18213 / 5682 [R(int) = 0.0366] |
| Completeness to theta = 25.242 | 100.0 % |
| Absorption correction | Semi-empirical from equivalents |
| Max. and min. transmission | 0.948 and 0.940 |
| Refinement method | Full-matrix least-squares on F 2 |
| Data / restraints / parameters | 5682 / 0 / 291 |
| Goodness-of-fit on F 2 | 0.986 |
| Final R indices [I>2sigma(I)] | R1 = 0.0502, wR2 = 0.1380 |
| R indices (all data) | R1 = 0.0936, wR2 = 0.1748 |
| Extinction coefficient | n/a |
| Largest diff. peak and hole | 0.441 and -0.333 e.A -3 |

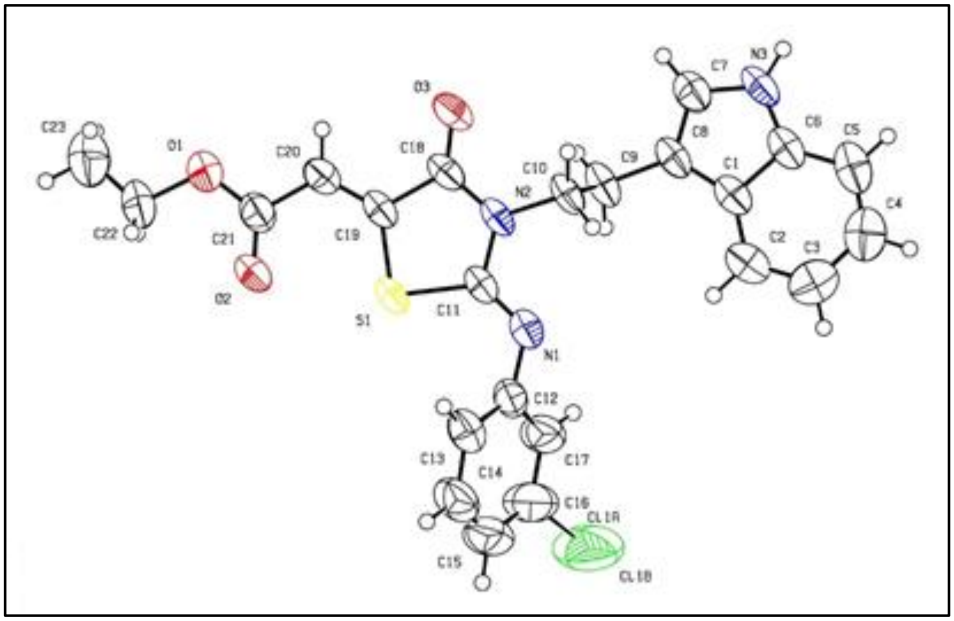


**Fig. 2** Platon diagram of compound **14i**

**Table 3**. Crystal data and structure refinement for the compound **14i**

| **Identification code** | **shelx** |
|---|---|
| Empirical formula | $C_{23} H_{20} Cl N_3 O_3 S$ |
| Formula weight | 453.93 |
| Temperature | 296(2) K |
| Wavelength | 0.71073 A |
| Crystal system, space group | Monoclinic, P 21/c |
| Unit cell dimensions | a = 11.7231(12) (A°), b = 13.1490(10) (A°), |
| | c = 14.6781(16) (A°) |
| | α = 90 (°), β = 99.523(6) (°), γ = 90 (°) |
| Volume *V* (Å$^3$) | 2231.4(4) |
| Z, Calculated density | 4, 1.351 Mg/m$^3$ |
| Absorption coefficient | 0.295 mm$^{-1}$ |
| F(000) | 944 |
| Crystal size | 0.350 x 0.350 x 0.300 mm |
| Theta range for data collection | 1.761 to 28.488 (°) |
| Limiting indices | $-15 \leq h \leq 15$, $-17 \leq k \leq 17$, $-19 \leq l \leq 19$ |
| Reflections collected / unique | 27219 / 5622 [R(int) = 0.0585] |

| | |
|---|---|
| Completeness to theta = 25.242 | 99.9 % |
| Absorption correction | Semi-empirical from equivalents |
| Max. and min. transmission | 0.917 and 0.904 |
| Refinement method | Full-matrix least-squares on F 2 |
| Data / restraints / parameters | 5622 / 0 / 292 |
| Goodness-of-fit on F 2 | 0.950 |
| Final R indices [I>2sigma(I)] | R1 = 0.0639, wR2 = 0.1597 |
| R indices (all data) | R1 = 0.1330, wR2 = 0.2117 |
| Extinction coefficient | 0.016(2) |
| Largest diff. peak and hole | 0.517 and -0.542 e.A -3 |

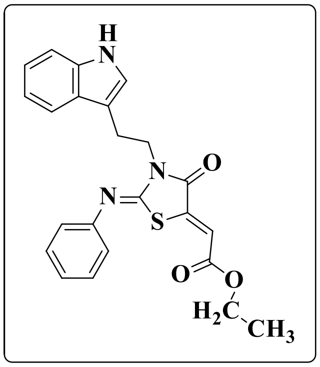
## EXPERIMENTAL SECTION

### General procedure for the synthesis of 14 (a-za)

Tryptamine/serotonin (1mmol), phenyl isothiocyanate (1mmol) in ethanol was stirred at room temperature for 20-30 minutes, then added dialkyl acetylenedicarboxylate (1mmol). The reaction mixture was refluxed, the progress of the reaction checked by TLC. After completion of the reaction, the reaction mixture was cooled, filtered and re-crystallized from ethanol.

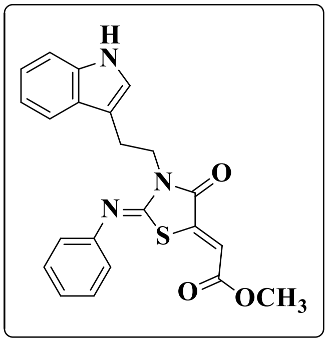
## SPECTRAL DATA

### (*Z*)-ethyl-2-((*Z*)-3-(2-(1*H*-indol-3-yl)ethyl)-4-oxo-2-(phenylimino)thiazolidin-5-ylidene) acetate (14a)



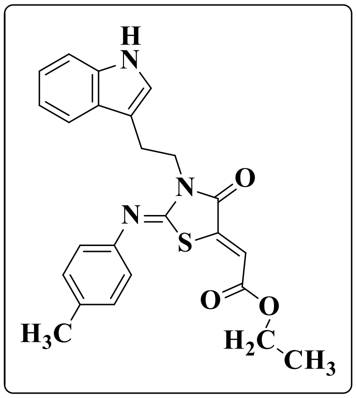
Color: yellow solid, mp**:** 216-217$^{o}$C, yield: (0.356g, 85%), FT-IR (KBr, $\nu_{max}$ cm$^{-1}$): 3356 (N-H), 1709 (Ester –C=O), 1688 (amide –C=O), 1612 (-C=N); **$^{1}$H NMR (400MHz, CDCl$_3$, ppm):** $\delta$ 1.30 (t, *J*=7.2Hz, 3H, methyl), 3.25 (t, *J*=7.6Hz, 2H, methylene), 4.25 (q, *J*=7.2Hz, 2H, methylene), 4.29 (t, *J*=7.6Hz, 2H, methylene), 6.87 (d, *J*=7.2Hz, 2H, Ar-H), 6.89 (s, 1H, Ar-H), 7.09 (s, 1H, Ar-H), 7.10-7.22 (m, 3H, Ar-H), 7.32-7.37 (m, 3H, Ar-H), 7.76 (d, *J*=8Hz, 1H, Ar-H), 8.01 (s, 1H, Ar-H) ppm, **$^{13}$C NMR (CDCl$_3$):** $\delta$ ppm; **MS (ESI) *m/z* (%):** 420.2 [M+H]$^{+}$; Anal. Calcd. for $C_{23}H_{21}N_3O_3S$; C, 65.85; H, 5.05; N, 10.02. Found: C, 65.81; H, 5.15; N, 10.12%

**(*Z*)-methyl-2-((*Z*)-3-(2-(1*H*-indol-3-yl)ethyl)-4-oxo-2-(phenylimino)thiazolidin-5-ylidene) acetate (14b)**



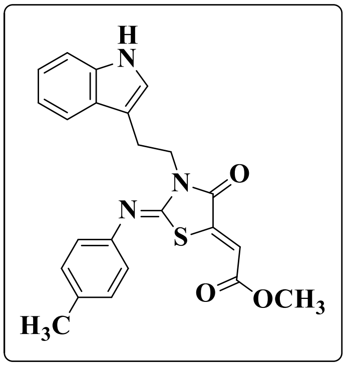
Color: yellow solid, mp**:** 216-217$^{o}$C, yield: (0.328g, 81%), FT-IR (KBr, $\nu_{max}$ cm$^{-1}$): 3356 (N-H), 1709 (Ester –C=O), 1688 (amide –C=O), 1612 (-C=N); **$^1$H NMR (400MHz, DMSO-d$_6$, ppm):** $\delta$ 3.14 (t, *J*=7.2Hz, 2H, methylene), 3.73 (s, 3H, methoxy), 4.15 (t, *J*=7.2Hz, 2H, methylene), 6.78 (s, 1H, Ar-H), 6.88 (d, *J*=8Hz, 2H, Ar-H), 6.98 (t, *J*=7.6Hz, 1H, Ar-H), 7.07 (t, *J*=7.2Hz, 1H, Ar-H), 7.19-7.22 (m, 2H, Ar-H), 7.35 (d, *J*=8Hz, 1H, Ar-H), 7.40 (t, *J*=7.2Hz, 2H, Ar-H), 7.59 (d, *J*=8Hz, 1H, Ar-H), 10.88 (s, 1H, Ar-H) ppm, **$^{13}$C NMR (DMSO-d$_6$):** $\delta$ 23.16, 43.77, 53.03, 110.79, 111.92, 115.60, 118.51, 118.89, 121.31, 121.49, 123.67, 125.52, 127.65, 129.12, 129.86, 131.97, 136.73, 141.66, 147.82, 151.21, 164.63, 166.22 ppm; **MS (ESI) *m/z* (%):** 406.1 [M+H]$^+$; Anal. Calcd. for $C_{22}H_{19}N_3O_3S$; C, 65.17; H, 4.72; N, 10.36. Found: C, 65.22; H, 4.78; N, 10.32%

**(*Z*)-ethyl-2-((*Z*)-3-(2-(1*H*-indol-3-yl)ethyl)-4-oxo-2-(p-tolylimino)thiazolidin-5-ylidene) acetate (14c)**



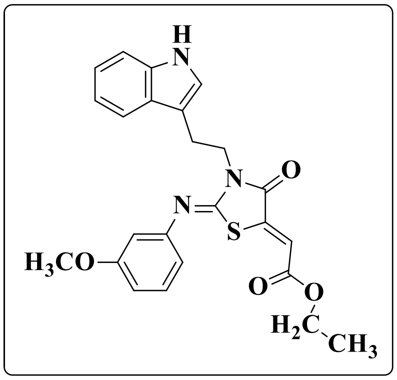
Color: yellow solid, mp**:** 216-217$^{o}$C, yield: (0.372g, 86%), FT-IR (KBr, $\nu_{max}$ cm$^{-1}$): 3356 (N-H), 1709 (Ester –C=O), 1688 (amide –C=O), 1612 (-C=N); **$^1$H NMR (400MHz, CDCl$_3$, ppm):** $\delta$ 1.30 (t, *J*=7.2Hz, 3H, methyl), 2.34 (s, 3H, methyl), 3.23 (t, *J*=7.6Hz, 2H, methylene), 4.21-4.28 (m, 4H, 2-methylene), 6.79 (d, *J*=8Hz, 2H, Ar-H), 6.88 (s, 1H, Ar-H), 7.05 (s, 1H, Ar-H), 7.09-7.15 (m, 3H, Ar-H), 7.19 (t, *J*=8Hz, 1H, Ar-H), 7.33 (d, *J*=8Hz, 1H, Ar-H), 7.76 (d, *J*=8Hz, 1H, Ar-H), 8.05 (s, 1H, Ar-H) ppm; **MS (ESI) *m/z* (%):** 434.3 [M+H]$^+$; Anal. Calcd. for $C_{24}H_{23}N_3O_3S$; C, 66.49; H, 5.35; N, 9.69. Found: C, 66.44; H, 5.39; N, 9.64%

**(*Z*)-methyl-2-((*Z*)-3-(2-(1*H*-indol-3-yl)ethyl)-4-oxo-2-(p-tolylimino)thiazolidin-5-ylidene) acetate (14d)**



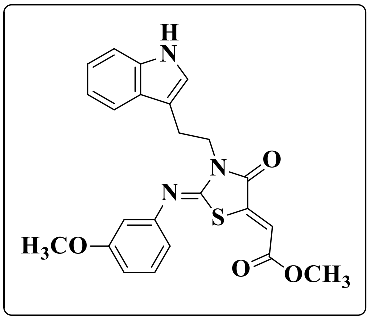
Color: yellow solid, mp**:** 216-217°C, yield: (0.348g, 83%), FT-IR (KBr, $\nu_{max}$ cm$^{-1}$): 3356 (N-H), 1709 (Ester –C=O), 1688 (amide –C=O), 1612 (-C=N); **$^1$H NMR (400MHz, CDCl$_3$, ppm):** $\delta$ 2.35 (t, *J*=8Hz, 3H, methyl), 3.24 (t, *J*=8Hz, 2H, methylene), 3.79 (s, 3H, methyl), 4.28 (t, *J*=2Hz, 2H, methylene), 6.78 (d, *J*=8.4Hz, 2H, Ar-H), 6.88 (s, 1H, Ar-H), 7.08-7.15 (m, 4H, Ar-H), 7.20 (t, *J*=8Hz, 1H, Ar-H), 7.36 (d, *J*=8Hz, 1H, Ar-H), 7.76 (d, *J*=8Hz, 1H, Ar-H), 8.00 (s, 1H, Ar-H) ) ppm; **MS (ESI) *m/z* (%):** 420.2 [M+H]$^+$; Anal. Calcd. for $C_{23}H_{21}N_3O_3S$; C, 65.85; H, 5.05; N, 10.02. Found: C, 65.81; H, 5.14; N, 10.07%

**(*Z*)-ethyl-2-((*Z*)-3-(2-(1*H*-indol-3-yl)ethyl)-2-((3-methoxyphenyl)imino)-4-oxothiazolidin-5-ylidene)acetate (14e)**



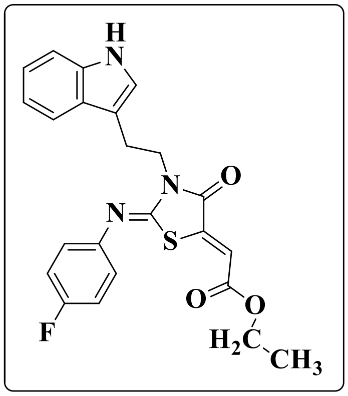
Color: yellow solid, mp**:** 216-217°C, yield: (0.400g, 89%), FT-IR (KBr, $\nu_{max}$ cm$^{-1}$): 3356 (N-H), 1709 (Ester –C=O), 1688 (amide –C=O), 1612 (-C=N); **$^1$H NMR (400MHz, CDCl$_3$, ppm):** $\delta$ 1.33 (t, *J*=7.2Hz, 3H, methyl), 3.27 (t, *J*=7.6Hz, 2H, methylene), 3.82 (s, 3H, methoxy), 4.25-4.34 (m, 4H, methylene), 6.42 (t, *J*=2Hz, 1H, Ar-H), 6.49 (dd, *J*=8Hz, 1H, Ar-H), 6.75 (dd, *J*=8Hz, 1H, Ar-H), 6.92 (s, 1H, Ar-H), 7.12-7.26 (m, 4H, Ar-H), 7.39 (d, *J*=8Hz, 1H, Ar-H), 7.78(d, *J*=7.6Hz, 1H, Ar-H), 8.04(s, 1H, Ar-H); **MS (ESI) *m/z* (%):** 450.2 [M+H]$^+$; Anal. Calcd. for $C_{24}H_{23}N_3O_4S$; C, 64.13; H, 5.16; N, 9.35. Found: C, 64.10; H, 5.12; N, 9.39%

**(*Z*)-methyl-2-((*Z*)-3-(2-(1*H*-indol-3-yl)ethyl)-2-((3-methoxyphenyl)imino)-4-oxothiazolidin-5-ylidene)acetate (14f)**



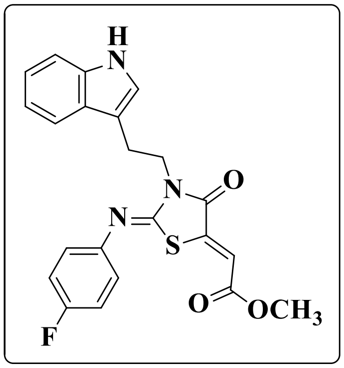
Color: yellow solid, mp**:** 216-217°C, yield: (0.365g, 84%), FT-IR (KBr, $\nu_{max}$ cm$^{-1}$): 3356 (N-H), 1709 (Ester –C=O), 1688 (amide –C=O), 1612 (-C=N); **$^1$H NMR (400MHz, CDCl$_3$, ppm):** $\delta$ 3.28 (t, *J*=7.6Hz, 2H, methylene), 3.82 (s, 3H, methoxy), 3.83 (s, 3H, methoxy), 4.24 (t, *J*=7.6Hz, 2H, methylene), 6.43 (t, *J*=2Hz, 1H, Ar-H), 6.50 (dd, *J*=8Hz, 1H, Ar-H), 6.76 (dd, *J*=8Hz, 1H, Ar-H), 6.93 (s, 1H, Ar-H), 7.10-7.29 (m, 4H, Ar-H), 7.38 (d, *J*=8Hz, 1H, Ar-H), 7.78 (d, *J*=8Hz, 1H, Ar-H), 8.08 (s, 1H, Ar-H) ppm; **MS (ESI) *m/z* (%):** 436.2 [M+H]$^+$; Anal. Calcd. for $C_{23}H_{21}N_3O_4S$; C, 63.43; H, 4.86; N, 9.65. Found: C, 63.49; H, 4.82; N, 9.69%

### (*Z*)-ethyl-2-((*Z*)-3-(2-(1*H*-indol-3-yl)ethyl)-2-((4-fluorophenyl)imino)-4-oxothiazolidin-5-ylidene)acetate (14g)



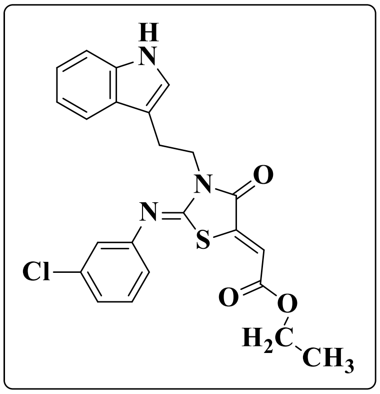
Color: yellow solid, mp**:** 216-217$^o$C, yield: (0.393g, 90%), FT-IR (KBr, ν$_{max}$ cm$^{-1}$): 3356 (N-H), 1709 (Ester –C=O), 1688 (amide –C=O), 1612 (-C=N); **$^1$H NMR (400MHz, CDCl$_3$, ppm):** *δ* 1.31 (t, *J*=7.2Hz, 3H, methyl), 3.23 (t, *J*=7.2Hz, 2H, methylene), 4.24 (t, *J*=7.2Hz, 2H, methylene), 4.28 (t, *J*=4Hz, 2H, methylene), 6.76-6.80 (m, 2H, Ar-H), 6.90 (s, 1H, Ar-H), 7.02 (t, *J*=8.4Hz, 2H, Ar-H), 7.07 (s, 1H, Ar-H), 7.13 (t, *J*=8Hz, 1H, Ar-H), 7.20 (t, *J*=8Hz, 1H, Ar-H), 7.36 (d, *J*=8Hz, 1H, Ar-H), 7.74 (d, *J*=7.6Hz, 1H, Ar-H), 8.03 (s, 1H, Ar-H) ppm; **MS (ESI) *m/z* (%):** 438.25 [M+H]$^+$; Anal. Calcd. for $C_{23}H_{20}FN_3O_3S$; C, 63.14; H, 4.61; N, 9.60. Found: C, 63.19; H, 4.65; N, 9.64%

### (*Z*)-methyl-2-((*Z*)-3-(2-(1*H*-indol-3-yl)ethyl)-2-((4-fluorophenyl)imino)-4-oxothiazolidin-5-ylidene)acetate (14h)



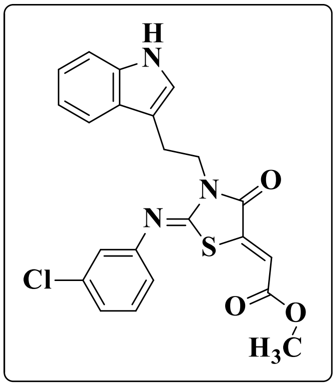
Color: yellow solid, mp**:** 216-217$^o$C, yield: (0.368g, 87%), FT-IR (KBr, ν$_{max}$ cm$^{-1}$): 3356 (N-H), 1709 (Ester –C=O), 1688 (amide –C=O), 1612 (-C=N); **$^1$H NMR (400MHz, DMSO-d$_6$, ppm):** *δ* 3.13 (t, *J*=7.6Hz, 2H, methylene), 3.74 (s, 3H, methoxy), 4.14 (t, *J*=7.2Hz, 2H, methylene), 6.80 (s, 1H, Ar-H), 6.88-6.91 (m, 2H, Ar-H), 6.98 (t, *J*=7.2Hz, 2H, Ar-H), 7.08 (t, *J*=8Hz, 1H, Ar-H), 7.21-7.26 (m, 3H, Ar-H), 7.35 (d, *J*=8Hz, 1H, Ar-H), 7.59 (d, *J*=8Hz, 1H, Ar-H), 10.88 (s, 1H, Ar-H) ppm; **$^{13}$C NMR (DMSO-d$_6$)** *δ* 23.15, 43.78, 53.05, 110.78, 111.92, 115.74, 116.47, 116.70, 118.52, 118.90, 121.49, 123.04, 123.13, 123.69, 127.65, 129.12, 131.96, 132.15, 136.73, 141.47, 144.19, 151.82, 158.83, 161.23, 164.62, 166.21, 167.39 ppm; **MS (ESI) *m/z* (%):** 424.25 [M+H]$^+$; Anal. Calcd. for $C_{22}H_{18}FN_3O_3S$; C, 62.40; H, 4.28; N, 9.92. Found: C, 62.44; H, 4.24; N, 9.97%

### (*Z*)-ethyl-2-((*Z*)-3-(2-(1*H*-indol-3-yl)ethyl)-2-((3-chlorophenyl)imino)-4-oxothiazolidin-5-ylidene)acetate (14i)



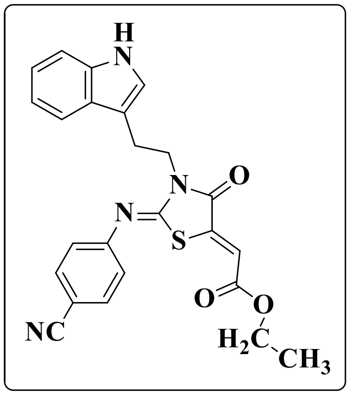
Color: yellow solid, mp**:** 216-217$^{o}$C, yield: (0.413g, 91%), FT-IR (KBr, ν$_{max}$ cm$^{-1}$): 3356 (N-H), 1709 (Ester –C=O), 1688 (amide –C=O), 1612 (-C=N); **$^{1}$H NMR (400MHz, CDCl$_3$, ppm):** $\delta$ 1.31 (t, *J*=7.2Hz, 3H, methyl), 3.23 (t, *J*=8Hz, 2H, methylene), 4.24 (q, *J*=7.2Hz, 2H, methylene), 4.28 (t, *J*=7.2Hz, 2H, methylene), 6.70 (d, *J*=8Hz, 1H, Ar-H), 6.72 (s, 1H, Ar-H), 6.91 (s, 1H, Ar-H), 7.05 (s, 1H, Ar-H), 7.11-7.14 (m, 2H, Ar-H), 7.18-7.23 (m, 2H, Ar-H), 7.36 (d, *J*=8Hz, 1H, Ar-H), 7.72 (d, *J*=7.6Hz, 1H, Ar-H), 8.03 (s, 1H, Ar-H) ) ppm,; **MS (ESI) *m/z* (%):** 454.25 [M+H]$^{+}$; Anal. Calcd. for $C_{23}H_{20}ClN_3O_3S$; C, 60.86; H, 4.44; N, 9.26. Found: C, 60.83; H, 4.40; N, 9.30%

**(*Z*)-methyl-2-((*Z*)-3-(2-(1*H*-indol-3-yl)ethyl)-2-((3-chlorophenyl)imino)-4-oxothiazolidin-5-ylidene)acetate (14j)**



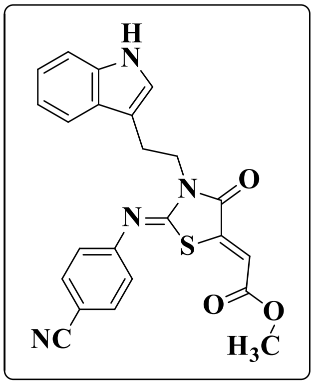
Color: yellow solid, mp**:** 216-217$^{o}$C, yield: (0.387g, 88%), FT-IR (KBr, ν$_{max}$ cm$^{-1}$): 3356 (N-H), 1709 (Ester –C=O), 1688 (amide –C=O), 1612 (-C=N); **$^{1}$H NMR (400MHz, CDCl$_3$, ppm):** $\delta$ 3.23 (t, *J*=8Hz, 2H, methylene), 3.81 (s, 3H, methyl), 4.27 (t, *J*=7.6Hz, 2H, methylene), 6.70 (d, *J*=8Hz, 1H, Ar-H), 6.72 (s, 1H, Ar-H), 6.92 (s, 1H, Ar-H), 7.06 (s, 1H, Ar-H), 7.10-7.14 (m, 2H, Ar-H), 7.20 (d, *J*=8.8Hz, 1H, Ar-H), 7.23 (d, *J*=7.6Hz, 1H, Ar-H), 7.37 (d, *J*=8Hz, 1H, Ar-H), 7.71 (d, *J*=8Hz, 1H, Ar-H), 8.00 (s, 1H, Ar-H) ppm; **MS (ESI) *m/z* (%):** 440.20 [M+H]$^{+}$; Anal. Calcd. for $C_{22}H_{18}ClN_3O_3S$; C, 60.07; H, 4.12; N, 9.55. Found: C, 60.18; H, 4.16; N, 9.51%

**(*Z*)-ethyl-2-((*Z*)-3-(2-(1*H*-indol-3-yl)ethyl)-2-((4-cyanophenyl)imino)-4-oxothiazolidin-5-ylidene)acetate (14k)**



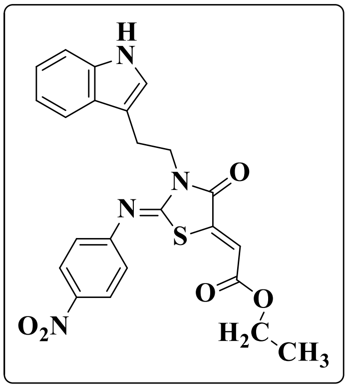
Color: yellow solid, mp**:** 216-217°C, yield: (0.395g, 89%), FT-IR (KBr, $\nu_{max}$ $cm^{-1}$): 3356 (N-H), 1709 (Ester –C=O), 1688 (amide –C=O), 1612 (-C=N); **$^1$H NMR (400MHz, $CDCl_3$, ppm):** $\delta$ 1.31 (t, *J*=7.2Hz, 3H, methyl), 3.23 (t, *J*=7.6Hz, 2H, methylene), 4.22 (q, *J*=7.6Hz, 2H, methylene), 4.28 (t, *J*=7.2Hz, 2H, methylene), 6.79 (d, *J*=7.6Hz, 2H, Ar-H), 6.93 (s, 1H, Ar-H), 7.04 (s, 1H, Ar-H), 7.11 (t, *J*=8Hz, 1H, Ar-H), 7.20 (t, *J*=8Hz, 1H, Ar-H), 7.36 (d, *J*=8Hz, 1H, Ar-H), 7.59 (d, *J*=8.4Hz, 2H, Ar-H), 7.69 (d, *J*=8Hz, 1H, Ar-H), 8.11(s, 1H, Ar-H) ppm; **MS (ESI) *m/z* (%):** 445.25 $[M+H]^+$; Anal. Calcd. for $C_{24}H_{20}N_4O_3S$; C, 64.85; H, 4.54; N, 12.60. Found: C, 64.80; H, 4.59; N, 12.64%

### (*Z*)-methyl-2-((*Z*)-3-(2-(1*H*-indol-3-yl)ethyl)-2-((4-cyanophenyl)imino)-4-oxothiazolidin-5-ylidene)acetate (14l)



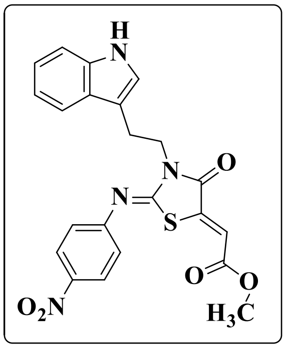
Color: yellow solid, mp**:** 216-217°C, yield: (0.353g, 82%), FT-IR (KBr, $\nu_{max}$ $cm^{-1}$): 3356 (N-H), 1709 (Ester –C=O), 1688 (amide –C=O), 1612 (-C=N); **$^1$H NMR (400MHz, $CDCl_3$, ppm):** $\delta$ 3.24 (t, *J*=7.6Hz, 2H, methylene), 3.81 (s, 3H, methoxy), 4.28 (q, *J*=7.2Hz, 2H, methylene), 4.28 (t, *J*=7.2Hz, 2H, methylene), 6.80 (d, *J*=8.4Hz, 2H, Ar-H), 6.94 (s, 1H, Ar-H), 7.06 (s, 1H, Ar-H), 7.11 (t, *J*=8Hz, 1H, Ar-H), 7.20 (t, *J*=8Hz, 1H, Ar-H), 7.37 (d, *J*=8.4Hz, 1H, Ar-H), 7.60 (d, *J*=8.4Hz, 2H, Ar-H), 7.68 (d, *J*=8Hz, 1H, Ar-H), 8.03 (s, 1H, Ar-H) ppm; **MS (ESI) *m/z* (%):** 431.25 $[M+H]^+$; Anal. Calcd. for $C_{23}H_{18}N_4O_3S$; C, 64.17; H, 4.21; N, 13.01. Found: C, 64.13; H, 4.25; N, 13.11%

### (*Z*)-ethyl-2-((*Z*)-3-(2-(1*H*-indol-3-yl)ethyl)-2-((4-nitrophenyl)imino)-4-oxothiazolidin-5-ylidene)acetate (14m)



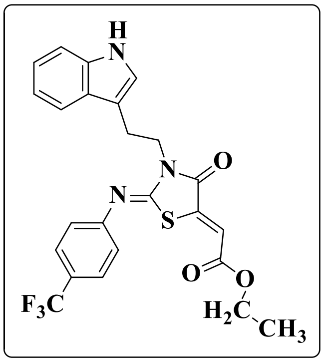
Color: yellow solid, mp: 216-217ºC, yield: (0.418g, 90%), FT-IR (KBr, νmax cm$^{-1}$): 3356 (N-H), 1709 (Ester –C=O), 1688 (amide –C=O), 1612 (-C=N); **$^1$H NMR (400MHz, CDCl$_3$, ppm):** $\delta$ 1.31 (t, *J*=7.2Hz, 3H, methyl), 3.24 (t, *J*=7.6Hz, 2H, methylene), 4.26 (q, *J*=7.2Hz, 2H, methylene), 4.30 (t, *J*=7.2Hz, 2H, methylene), 6.79 (d, *J*=8.8Hz, 2H, Ar-H), 6.95 (s, 1H, Ar-H), 7.06 (s, 1H, Ar-H), 7.12 (t, *J*=8Hz, 1H, Ar-H), 7.21 (t, *J*=8Hz, 1H, Ar-H), 7.38 (d, *J*=8.4Hz, 1H, Ar-H), 7.68 (d, *J*=8Hz, 1H, Ar-H), 8.02 (s, 1H, Ar-H), 8.18 (d, *J*=8.8Hz, 2H, Ar-H) ppm; **MS (ESI) *m/z* (%):** 465.25 [M+H]$^+$; Anal. Calcd. for $C_{23}H_{20}N_4O_5S$; C, 59.47; H, 4.34; N, 12.06. Found: C, 59.43; H, 4.30; N, 12.16%

**(*Z*)-methyl-2-((*Z*)-3-(2-(1*H*-indol-3-yl)ethyl)-2-((4-nitrophenyl)imino)-4-oxothiazolidin-5-ylidene)acetate (14n)**



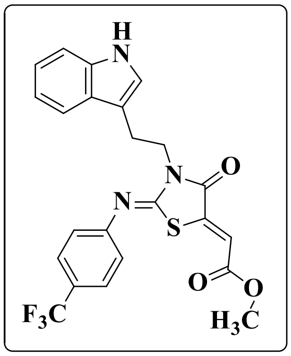
Color: yellow solid, mp: 216-217ºC, yield: (0.391g, 87%), FT-IR (KBr, νmax cm$^{-1}$): 3356 (N-H), 1709 (Ester –C=O), 1688 (amide –C=O), 1612 (-C=N); **$^1$H NMR (400MHz, CDCl$_3$, ppm):** $\delta$ 3.25 (t, *J*=7.6Hz, 2H, methylene), 3.81 (s, 3H, methoxy), 4.30 (t, *J*=7.2Hz, 2H, methylene), 6.79 (d, *J*=8.8Hz, 2H, Ar-H), 6.96 (s, 1H, Ar-H), 7.06 (s, 1H, Ar-H), 7.12 (t, *J*=8Hz, 1H, Ar-H), 7.21 (t, *J*=8Hz, 1H, Ar-H), 7.38 (d, J=8Hz, 1H, Ar-H), 7.68 (d, *J*=8Hz, 1H, Ar-H), 8.02 (s, 1H, Ar-H), 8.19 (d, *J*=8.8Hz, 2H, Ar-H) ppm; **MS (ESI) *m/z* (%):** 451.25 [M+H]$^+$; Anal. Calcd. for $C_{22}H_{18}N_4O_5S$; C, 58.66; H, 4.03; N, 12.44. Found: C, 58.62; H, 4.17; N, 12.40%

**(*Z*)-ethyl-2-((*Z*)-3-(2-(1*H*-indol-3-yl)ethyl)-4-oxo-2-((4-(trifluoromethyl)phenyl)imino) thiazolidin-5-ylidene)acetate (14o)**



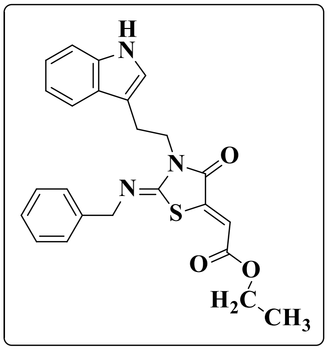
color: yellow solid, mp: 216-217ºC, yield: (0.453g, 93%), FT-IR (KBr, νmax cm$^{-1}$): 3356 (N-H), 1709 (Ester –C=O), 1688 (amide –C=O), 1612 (-C=N); **$^1$H NMR (400MHz, CDCl$_3$, ppm):** $\delta$ 1.31 (t, *J*=7.2Hz, 3H, methyl), 3.25 (t, *J*=8Hz, 2H, methylene), 4.25 (q, *J*=7.2Hz, 2H, methylene), 4.29 (t, *J*=7.6Hz, 2H, methylene), 6.85 (d, *J*=8.8Hz, 2H, Ar-H), 6.92 (s, 1H, Ar-H), 7.07 (s, 1H, Ar-H), 7.12 (t, *J*=8Hz, 1H, Ar-H), 7.21 (t, *J*=8Hz, 1H, Ar-H), 7.37 (d, *J*=8Hz, 1H, Ar-H), 7.57 (d, *J*=8.4Hz, 2H, Ar-H), 7.71 (d, *J*=7.6Hz, 1H, Ar-H), 8.01 (s, 1H, Ar-H) ppm; **MS (ESI) *m/z* (%):** 488.25 [M+H]$^+$; Anal. Calcd. for $C_{24}H_{20}F_3N_3O_3S$; C, 59.13; H, 4.14; N, 8.62. Found: C, 59.16; H, 4.10; N, 8.67%

### (*Z*)-methyl-2-((*Z*)-3-(2-(1*H*-indol-3-yl)ethyl)-4-oxo-2-((4-(trifluoromethyl)phenyl)imino) thiazolidin-5-ylidene)acetate (14p)



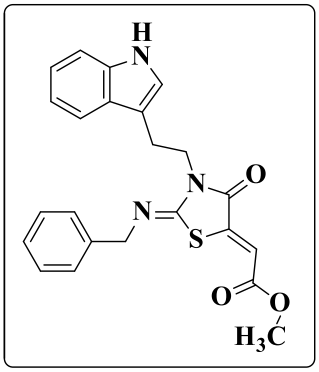
Color: yellow solid, mp**:** 216-217°C, yield: (0.421g, 89%), FT-IR (KBr, $\nu_{max}$ cm$^{-1}$): 3356 (N-H), 1709 (Ester –C=O), 1688 (amide –C=O), 1612 (-C=N); **$^1$H NMR (400MHz, $CDCl_3$, ppm):** $\delta$ 3.25 (t, *J*=8Hz, 2H, methylene), 3.81 (s, 3H, methoxy), 4.29 (t, *J*=7.2Hz, 2H, methylene), 6.85 (d, *J*=8Hz, 2H, Ar-H), 6.93 (s, 1H, Ar-H), 7.07 (s, 1H, Ar-H), 7.12 (t, *J*=8Hz, 1H, Ar-H), 7.21 (t, *J*=8Hz, 1H, Ar-H), 7.37 (d, *J*=8Hz, 1H, Ar-H), 7.58 (d, *J*=8Hz, 2H, Ar-H), 7.71 (d, *J*=7.6Hz, 1H, Ar-H), 8.01 (s, 1H, Ar-H); **MS (ESI) *m/z* (%):** 474.25 [M+H]$^+$; Anal. Calcd. for $C_{23}H_{18}F_3N_3O_3S$; C, 58.35; H, 3.83; N, 8.87. Found: C, 58.39; H, 3.80; N, 8.83%

### (*Z*)-ethyl-2-((*Z*)-3-(2-(1*H*-indol-3-yl)ethyl)-2-(benzylimino)-4-oxothiazolidin-5-ylidene) acetate (14q)



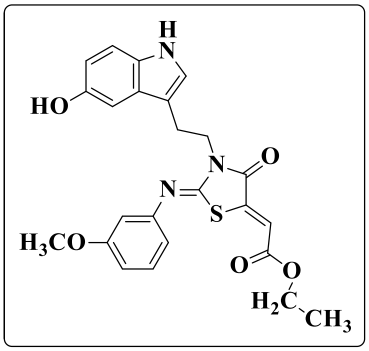
Color: yellow solid, mp**:** 216-217°C, yield: (0.368g, 85%), FT-IR (KBr, $\nu_{max}$ cm$^{-1}$): 3356 (N-H), 1709 (Ester –C=O), 1688 (amide –C=O), 1612 (-C=N); **$^1$H NMR (400MHz, $CDCl_3$, ppm):** $\delta$ 1.35 (t, *J*=7.2Hz, 3H, methyl), 3.15 (t, *J*=8Hz, 2H, methylene), 4.19 (t, *J*=8Hz, 2H, methylene), 4.31 (q, *J*=7.2Hz, 2H, methylene), 4.68 (s, 2H, methylene connected to phenyl), 6.90 (s, 1H, Ar-H), 7.04 (s, 1H, Ar-H), 7.08 (t, *J*=8Hz, 1H, Ar-H), 7.19 (t, *J*=8Hz, 1H, Ar-H), 7.30 (d, *J*=8.8Hz, 1H), 7.33-7.37 (m, 5H, Ar-H), 7.71 (d, *J*=7.6Hz, 1H, Ar-H), 7.96 (s, 1H, Ar-H) ppm; **MS (ESI) *m/z* (%):** 434.30 [M+H]$^+$; Anal. Calcd. for $C_{24}H_{23}N_3O_3S$; C, 66.49; H, 5.35; N, 9.69. Found: C, 66.45; H, 5.38; N, 9.73%

### (*Z*)-methyl-2-((*Z*)-3-(2-(1*H*-indol-3-yl)ethyl)-2-(benzylimino)-4-oxothiazolidin-5-ylidene) acetate (14r)



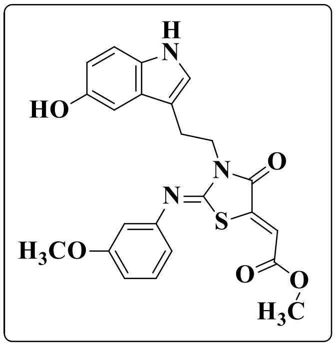
Color: yellow solid, mp**:** 216-217°C, yield: (0.339g, 81%), FT-IR (KBr, $\nu_{max}$ cm$^{-1}$): 3356 (N-H), 1709 (Ester –C=O), 1688 (amide –C=O), 1612 (-C=N); **$^{1}$H NMR (400MHz, CDCl$_3$, ppm):** $\delta$ 3.19 (t, *J*=7.6Hz, 2H, methylene), 3.88 (s, 3H, methoxy), 4.24 (t, *J*=7.6Hz, 2H, methylene), 4.71 (s, 2H, benzyl methylene), 4.68 (s, 2H, methylene connected to phenyl), 6.94 (s, 1H, Ar-H), 7.08-7.11 (m, 2H, Ar-H), 7.21 (t, *J*=7.6Hz, 1H, Ar-H), 7.32-7.40 (m, 6H, Ar-H), 7.72 (d, *J*=7.6Hz, 1H, Ar-H), 7.99 (s, 1H, Ar-H) ppm; **$^{13}$C NMR (CDCl$_3$):** $\delta$ 23.35, 43.49, 52.56, 56.05, 111.11, 112.23, 115.49, 119.03, 119.52, 122.13, 122.22, 127.23, 127.46, 127.68, 128.59, 136.20, 138.67, 141.36, 149.81, 164.71, 166.51; **MS (ESI) *m/z* (%):** 420.25 [M+H]$^+$; Anal. Calcd. for $C_{23}H_{21}N_3O_3S$; C, 65.85; H, 5.05; N, 10.02. Found: C, 65.89; H, 5.15; N, 10.15%

### (*Z*)-ethyl-2-((*Z*)-3-(2-(5-hydroxy-1*H*-indol-3-yl)ethyl)-2-((3-methoxyphenyl)imino)-4-oxothiazolidin-5-ylidene)acetate (14s)



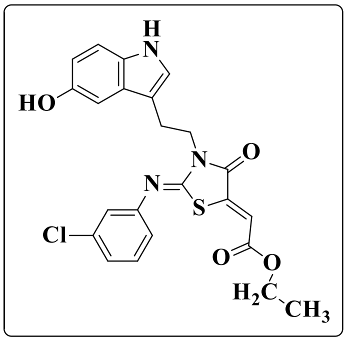
Color: yellow solid, mp**:** 216-217°C, yield: (0.400g, 85%), FT-IR (KBr, $\nu_{max}$ cm$^{-1}$): 3541 (O-H), 3356 (N-H), 1709 (Ester –C=O), 1688 (amide –C=O), 1612 (-C=N); **$^{1}$H NMR (400MHz, CDCl$_3$, ppm):** $\delta$ 1.23 (t, *J*=7.2Hz, 3H, methyl), 3.03 (t, *J*=7.2Hz, 2H, methylene), 3.76 (s, 3H, methoxy), 4.10 (t, *J*=7.2Hz, 2H, methylene), 4.20 (q, *J*=7.2Hz, 2H, methylene), 4.60 (s, 1H, OH), 6.47 (d, *J*=7.6Hz, 1H, Ar-H), 6.60 (d, *J*=8.4Hz, 1H, Ar-H), 6.77(d, *J*=6.8Hz, 2H, Ar-H), 6.88 (s, 1H, Ar-H), 7.09 (s, 1H, Ar-H), 7.14 (d, *J*=8.4Hz, 1H, Ar-H), 7.29 (t, *J*=7.6Hz, 1H, Ar-H), 8.62 (s, 1H, Ar-H), 10.57 (s, 1H, Ar-H) ppm; Anal. Calcd. for $C_{24}H_{23}N_3O_5S$; C, 61.92; H, 4.98; N, 9.03. Found: C, 61.96; H, 4.94; N, 9.15%

### (*Z*)-methyl-2-((*Z*)-3-(2-(1*H*-indol-3-yl)ethyl)-2-((3-methoxyphenyl)imino)-4-oxothiazolidin-5-ylidene)acetate (14t)



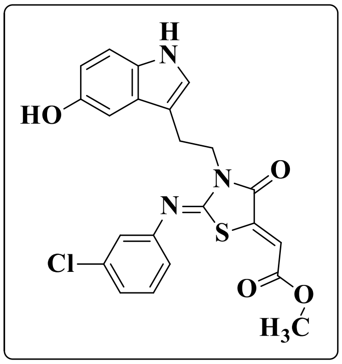
Color: yellow solid, mp**:** 216-217°C, yield: (0.365g, 81%), FT-IR (KBr, νmax cm$^{-1}$): 3541 (O-H), 3356 (N-H), 1709 (Ester –C=O), 1688 (amide –C=O), 1612 (-C=N); **$^1$H NMR (400MHz, CDCl$_3$, ppm):** *δ* 3.17 (t, *J*=8Hz, 2H, methylene), 3.80 (s, 6H, methoxy), 4.24 (t, *J*=7.6Hz, 2H, methylene), 4.68 (s, 1H, OH), 6.43 (s, 1H, Ar-H), 6.50 (d, *J*=7.6Hz, 1H, Ar-H), 6.73 (d, J=8.4Hz, 1H, Ar-H), 6.78 (d, *J*=8.4Hz, 1H, Ar-H), 6.90 (s, 1H, Ar-H), 7.06 (s, 1H, Ar-H), 7.15 (s, 1H, Ar-H), 7.22 (d, *J*=8.8Hz, 2H, Ar-H), 7.88 (s, 1H, Ar-H) ppm; Anal. Calcd. for $C_{23}H_{21}N_3O_5S$; C, 61.18; H, 4.69; N, 9.31. Found: C, 61.23; H, 4.64; N, 9.35%

**(*Z*)-ethyl-2-((*Z*)-2-((3-chlorophenyl)imino)-3-(2-(5-hydroxy-1*H*-indol-3-yl)ethyl)-4-oxothiazolidin-5-ylidene)acetate (14u)**



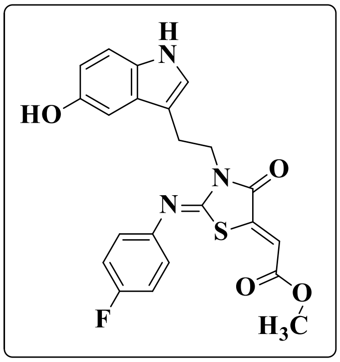
Color: yellow solid, mp**:** 216-217°C, yield: (0.427g, 91%), FT-IR (KBr, νmax cm$^{-1}$): 3541 (O-H), 3356 (N-H), 1709 (Ester –C=O), 1688 (amide –C=O), 1612 (-C=N); **$^1$H NMR (400MHz, DMSO-d$_6$, ppm):** *δ* 1.23 (t, *J*=7.2Hz, 3H, methyl), 3.03 (t, *J*=6.8Hz, 2H, methylene), 4.10 (t, *J*=6.8Hz, 2H, methylene), 4.20 (q, *J*=7.2Hz, 2H, methylene), 6.61 (d, *J*=8.4Hz, 1H, Ar-H), 6.80-6.86 (m, 4H, Ar-H), 7.08 (s, 1H, Ar-H), 7.14 (d, *J*=8.8Hz, 1H, Ar-H), 7.26 (d, *J*=8Hz, 1H, Ar-H), 7.41 (t, *J*=8Hz, 1H, Ar-H), 8.62 (s, 1H, Ar-H), 10.56 (s, 1H, Ar-H) ppm; **$^{13}$C NMR (DMSO-d$_6$):** *δ* ppm; **MS (ESI) *m/z* (%):** 470 [M+H]$^+$; Anal. Calcd. for $C_{22}H_{18}ClN_3O_4S$; C, 57.96; H, 3.98; N, 9.22. Found: C, 57.91; H, 3.94; N, 9.26%

**(*Z*)-methyl-2-((*Z*)-2-((3-chlorophenyl)imino)-3-(2-(5-hydroxy-1*H*-indol-3-yl)ethyl)-4-oxothiazolidin-5-ylidene)acetate (14v)**



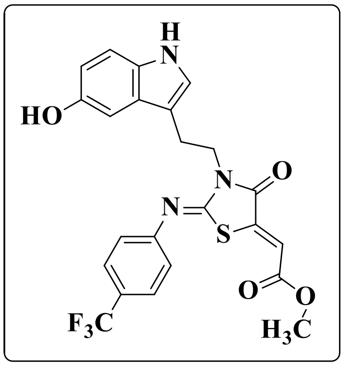
Color: yellow solid, mp**:** 216-217°C, yield: (0.396g, 87%), FT-IR (KBr, νmax cm$^{-1}$): 3541 (O-H), 3356 (N-H), 1709 (Ester –C=O), 1688 (amide –C=O), 1612 (-C=N); **$^1$H NMR (400MHz, DMSO-d$_6$, ppm):** *δ* 3.03 (t, *J*=7.6Hz, 2H, methylene), 3.75 (s, 3H, methoxy), 4.10 (t, *J*=7.2Hz, 2H, methylene), 6.61 (d, *J*=8.4Hz, 1H, Ar-H), 6.83-6.86 (m, 4H, Ar-H), 7.08 (s, 1H, Ar-H), 7.14 (d, *J*=8.4Hz, 1H, Ar-H), 7.26 (d, *J*=8Hz, 1H, Ar-H), 7.41 (t, *J*=7.2Hz, 1H, Ar-H), 8.62 (s, 1H, Ar-H), 10.57 (s, 1H, Ar-H) ppm; **$^{13}$C NMR (DMSO-$d_6$):** $\delta$ 23.20, 43.73, 45.98, 53.10, 102.58, 109.83, 111.87, 112.17, 115.98, 120.11, 121.31, 124.15, 125.27, 128.38, 131.26, 131.49, 134.07, 141.25, 149.33, 150.79, 152.42, 164.65, 166.24 ppm; **MS (ESI) *m/z* (%):** 456 $[M]^+$; Anal. Calcd. for $C_{22}H_{18}ClN_3O_4S$; C, 57.96; H, 3.98; N, 9.22. Found: C, 58.01; H, 3.94; N, 9.25%

### (*Z*)-methyl-2-((*Z*)-2-((4-fluorophenyl)imino)-3-(2-(5-hydroxy-1*H*-indol-3-yl)ethyl)-4-oxothiazolidin-5-ylidene)acetate (14w)



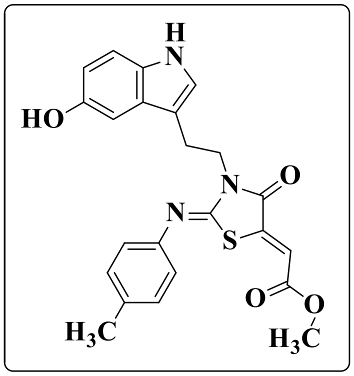
Color: yellow solid, mp**:** 216-217°C, yield: (0.391g, 87%), FT-IR (KBr, $\nu_{max}$ cm$^{-1}$): 3541 (O-H), 3356 (N-H), 1709 (Ester –C=O), 1688 (amide –C=O), 1612 (-C=N); **$^1$H NMR (400MHz, DMSO-$d_6$, ppm):** $\delta$ 3.03 (t, *J*=7.6Hz, 2H, methylene), 3.74 (s, 3H, methoxy), 4.10 (t, *J*=7.2Hz, 2H, methylene), 6.60 (d, *J*=8.4Hz, 1H, Ar-H), 6.80 (s, 1H, Ar-H), 6.88-6.93 (m, 3H, Ar-H), 7.09 (s, 1H, Ar-H), 7.13 (d, *J*=8.4Hz, 1H, Ar-H), 7.24 (t, *J*=8.4Hz, 2H, Ar-H), 8.61 (s, 1H, Ar-H), 10.56 (s, 1H, Ar-H) ppm; **$^{13}$C NMR (DMSO-$d_6$):** $\delta$ 23.2, 43.71, 53.05, 102.60, 109.83, 111.87, 112.18, 115.71, 116.44, 116.67, 123.10, 123.18, 124.09, 128.36, 131.26, 141.49, 144.26, 150.78, 151.87, 158.82, 161.22, 164.66, 166.23, ppm; **MS (ESI) *m/z* (%):** 440 $[M+H]^+$; Anal. Calcd. for $C_{22}H_{18}FN_3O_4S$; C, 60.13; H, 4.13; N, 9.56. Found: C, 60.16; H, 4.10; N, 9.60%

### (*Z*)-methyl-2-((*Z*)-3-(2-(5-hydroxy-1*H*-indol-3-yl)ethyl)-4-oxo-2-((4-(trifluoromethyl) phenyl)imino)thiazolidin-5-ylidene)acetate (14x)



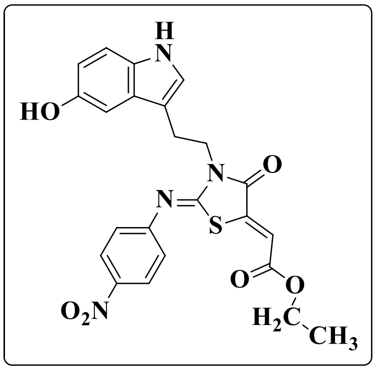
Color: yellow solid, mp**:** 216-217°C, yield: (0.450g, 92%), FT-IR (KBr, $\nu_{max}$ cm$^{-1}$): 3541 (O-H), 3356 (N-H), 1709 (Ester –C=O), 1688 (amide –C=O), 1612 (-C=N); **$^1$H NMR (400MHz, DMSO-$d_6$, ppm):** $\delta$ 3.05 (t, *J*=7.2Hz, 2H, methylene), 3.74 (s, 3H, methoxy), 4.11 (t, *J*=7.6Hz, 2H, methylene), 6.60 (d, *J*=8.4Hz, 1H, Ar-H), 6.84 (s, 1H, Ar-H), 6.87 (s, 1H, Ar-H), 7.05-7.15 (m, 4H, Ar-H), 7.76 (d, *J*=8Hz, 2H, Ar-H), 8.61 (s, 1H, Ar-H), 10.56 (s, 1H, Ar-H) ppm; **$^{13}$C NMR (DMSO-$d_6$):** $\delta$ 23.22, 43.76, 53.11, 102.57, 109.80, 111.88, 112.20, 116.16, 122.17, 124.14, 125.66, 125.97, 126.13, 127.05, 128.36, 131.27, 141.13, 150.81, 151.42, 152.48, 164.60, 166.22 ppm; **MS (ESI) *m/z* (%):** 490 $[M+H]^+$; Anal. Calcd. for $C_{23}H_{18}F_3N_3O_4S$; C, 56.44; H, 3.71; N, 8.58. Found: C, 56.49; H, 3.75; N, 8.60%

**(*Z*)-methyl-2-((*Z*)-3-(2-(5-hydroxy-1*H*-indol-3-yl)ethyl)-4-oxo-2-(p-tolylimino)thiazolidin-5-ylidene)acetate (14y)**



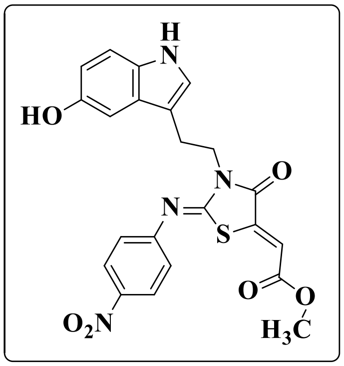
Color: yellow solid, mp**:** 216-217ºC, yield: (0.352g, 81%), FT-IR (KBr, $\nu_{max}$ cm$^{-1}$): 3541 (O-H), 3356 (N-H), 1709 (Ester –C=O), 1688 (amide –C=O), 1612 (-C=N); **$^1$H NMR (400MHz, CDCl$_3$, ppm):** $\delta$ 2.35 (s, 3H, methyl), 3.16 (t, *J*=7.6Hz, 2H, methylene), 3.80 (s, 3H, methoxy), 4.25 (t, *J*=7.6Hz, 2H, methylene), 4.68 (s, 1H, OH), 6.43 (s, 1H, Ar-H), 6.50 (d, *J*=7.6Hz, 1H, Ar-H), 6.73 (d, *J*=8.4Hz, 1H, Ar-H), 6.78 (d, *J*=8.4Hz, 1H, Ar-H), 6.90 (s, 1H, Ar-H), 7.06 (s, 1H, Ar-H), 7.15 (s, 1H, Ar-H), 7.22 (d, *J*=8.4Hz, 2H, Ar-H), 7.88 (s, 1H, Ar-H) ppm; Anal. Calcd. for $C_{23}H_{21}N_3O_4S$; C, 63.43; H, 4.86; N, 9.65. Found: C, 63.47; H, 4.90; N, 9.61%

**(*Z*)-ethyl-2-((*Z*)-3-(2-(5-hydroxy-1*H*-indol-3-yl)ethyl)-2-((4-nitrophenyl)imino)-4-oxothiazolidin-5-ylidene)acetate (14z)**



Color: yellow solid, mp**:** 216-217ºC, yield: (0.437g, 91%), FT-IR (KBr, $\nu_{max}$ cm$^{-1}$): 3541 (O-H), 3356 (N-H), 1709 (Ester –C=O), 1688 (amide –C=O), 1612 (-C=N); **$^1$H NMR (400MHz, DMSO-d$_6$, ppm):** $\delta$ 1.23 (t, *J*=7.2Hz, 3H, methyl), 3.04 (t, *J*=7.2Hz, 2H, methylene), 4.11 (t, *J*=7.2Hz, 2H, methylene), 4.20 (q, *J*=6.8Hz, 2H, methylene), 6.61 (d, *J*=8.4Hz, 1H, Ar-H), 6.83 (s, 1H, Ar-H), 6.87(s, 1H, Ar-H), 7.06-7.15 (m, 4H, Ar-H), 8.26 (d, *J*=8.4Hz, 2H, Ar-H), 8.62 (s, 1H, Ar-H), 10.57 (s, 1H, Ar-H) ppm; **MS (ESI) *m/z* (%):** 481 [M+H]$^+$; Anal. Calcd. for $C_{23}H_{20}N_4O_6S$; C, 57.49; H, 4.20; N, 11.66. Found: C, 57.45; H, 4.25; N, 11.70%

**(*Z*)-methyl-2-((*Z*)-3-(2-(5-hydroxy-1*H*-indol-3-yl)ethyl)-2-((4-nitrophenyl)imino)-4-oxothiazolidin-5-ylidene)acetate (14za)**



Color: yellow solid, mp**:** 216-217ºC, yield: (0.405g, 87%), FT-IR (KBr, $\nu_{max}$ cm$^{-1}$): 3541 (O-H), 3356 (N-H), 1709 (Ester –C=O), 1688 (amide –C=O), 1612 (-C=N);**$^1$H NMR (400MHz, DMSO-$d_6$, ppm):** $\delta$ 3.04 (t, *J*=7.2Hz, 2H, methylene), 3.74 (s, 3H, methoxy), 4.11 (t, *J*=7.2Hz, 2H, methylene), 6.61 (d, *J*=8.4Hz, 1H, Ar-H), 6.86 (s, 2H, Ar-H), 7.06-7.15 (m, 4H, Ar-H), 8.27 (d, *J*=8.4Hz, 2H, Ar-H), 8.62 (s, 1H, Ar-H), 10.56 (s, 1H, Ar-H) ppm; **MS (ESI) *m/z* (%):** 467 [M+H]$^+$; Anal. Calcd. for $C_{22}H_{18}N_4O_6S$; C, 56.65; H, 3.89; N, 12.01. Found: C, 56.69; H, 3.85; N, 12.10%

## BIOLOGICAL ACTIVITY

### *In vitro* anticancer activity

### *In vitro* anticancer activity assay

All the synthesized compounds were submitted for their primary anticancer activity evaluation. The selected compounds were screened for their primary anticancer activity at $10^{-5}$ M concentration at the National Cancer Institute (NCI), Bethesda, USA of drug evaluation branch[47-49]. The compounds were added to the cell culture at single dose concentration (10 μM) and the cultures were incubated for 48 h. After incubation sulforhodamine B (SRB) protein binding dye was added and absorbance values were measured. The cell percentage growth was calculated for the test compounds with respect to the untreated control cells.

The compounds exhibiting excellent cell percentage growth inhibition were tested for five dose concentrations ($10^{-4}$ to $10^{-8}$ μM) and cytotoxicity was measured and growth inhibitory effects in vitro against 60 human cancer cell lines after 48 h incubation and the absorbance values were measured using sulforhodamine B dye.

Using the seven absorbance values (time zero (Tz), the percentage growth was calculated using the test growth in presence of the compound (T*i*) and control growth in absence of the compound (C).

$[(Ti - Tz) / (C - Tz)] \times 100$ for concentrations for which $Ti \geq Tz$,

$[(Ti - Tz) / Tz] \times 100$ for concentrations for which $Ti < Tz$.

From the five dose concentration experiment, dose response parameters $GI_{50}$, TGI, $LC_{50}$ were calculated. Percentage growth inhibition ($GI_{50}$) of the compound was calculated using the formula $[(Ti - Tz)/(C - Tz)] \times 100 = 50$. The test compound concentration required for total growth inhibition (TGI) was calculated from $Ti = Tz$. The $LC_{50}$ (net loss of cells by treatment of the test compound) was calculated from $[(Ti - Tz)/ Tz] \times 100 = -50$. The compounds having $GI_{50}<100$ μM were considered as anticancer active compounds.

### ***In vitro*** **anticancer activity results and discussion**

The one dose screening results of selected fifteen compounds **14b, 14d, 14f, 14h, 14j, 14l, 14n, 14p, 14r, 14t, 14v, 14w, 14x, 14y** and **14za** are shown in table **4-18**. In one dose screening growth percent values were given. Among fifteen compounds four compounds **14p, 14v, 14w** and **14x** showed remarkable anticancer activity against all the tested cell lines, these compounds were further selected for five dose screening and the results are shown in table **19-34**.

In five dose screening $GI_{50}$, TGI, $LC_{50}$ values were given. $GI_{50}$ value is the concentration of the compound inhibiting 50% growth in net cell growth, TGI value is the concentration of the compound resulting in net total cell growth inhibition and $LC_{50}$ value is the concentration of compound causing net 50% cell loss of initial cells at 48 h incubation period. Compounds **14p, 14v, 14w** and **14x** exhibited remarkable antitumor activity ($GI_{50}$ and TGI <100μM) against most of the tested cancer cell lines.

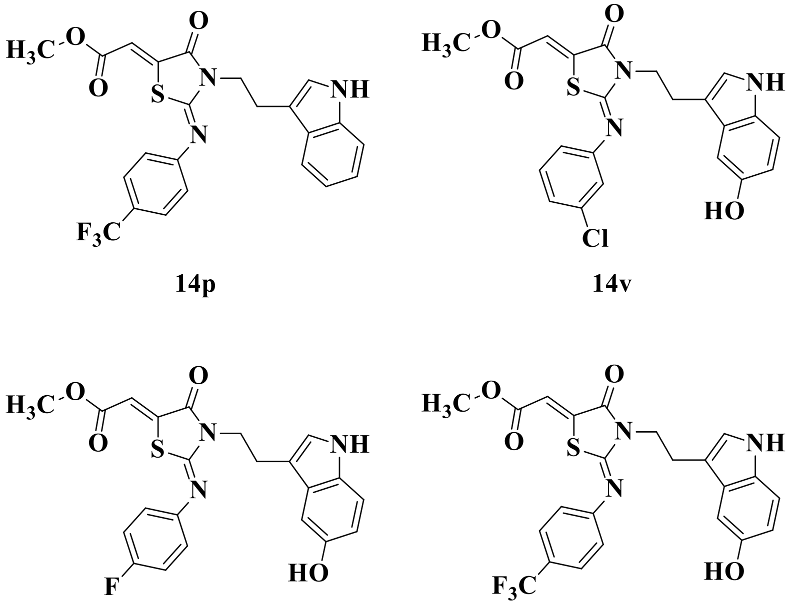
Compound **14p** exhibited a broad spectrum of anticancer activity against leukemia HL-60 cell line (TB) (**$GI_{50}$ 1.23 μM**), MOLT-4 (**$GI_{50}$ 1.22 μM**), RPMI-8226 (**$GI_{50}$ 1.13 μM**), SR (**$GI_{50}$ 0.613 μM**), non-small cell lung cancer NCI-H226 (**$GI_{50}$ 1.87 μM**), colon cancer HCT-116 (**$GI_{50}$ 1.21 μM**), HCT-15 (**$GI_{50}$ 1.38 μM**), CNS cancer SF-295 (**$GI_{50}$ 1.57 μM**), SF-539 (**$GI_{50}$ 1.62 μM**), SNB-75 (**$GI_{50}$ 1.21 μM**), U251 (**$GI_{50}$ 1.63 μM**), melanoma LOX IMVI (**$GI_{50}$ 1.49 μM**), M14 (**$GI_{50}$ 1.46 μM**), MDA-MB-435 (**$GI_{50}$ 1.56 μM**), UACC-62 (**$GI_{50}$ 1.62 μM**), ovarian cancer OVCAR-3 (**$GI_{50}$ 1.50 μM**), OVCAR-4 (**$GI_{50}$ 1.61 μM**), renal cancer 786-0 (**$GI_{50}$ 1.56 μM**), A498 (**$GI_{50}$ 1.50 μM**), ACHN (**$GI_{50}$ 1.56 μM**), RXF 393 (**$GI_{50}$ 1.43 μM**), UO-31 (**$GI_{50}$ 1.30 μM**), prostate cancer PC-3 (**$GI_{50}$ 1.62 μM**), breast cancer MDA-MB-231/ATCC (**$GI_{50}$ 1.50 μM**), BT-549 (**$GI_{50}$ 1.53 μM**), T-47D (**$GI_{50}$ 1.19 μM**).

Compound **14v** exhibited a conspicuously broad spectrum of anticancer activity against leukemia CCRF-CEM cell line (**GI$_{50}$ 0.931 µM**), MOLT-4 (**GI$_{50}$ 1.00 µM**), RPMI-8226 (**GI$_{50}$ 1.24 µM**), SR (**GI$_{50}$ 0.522 µM**), non-small cell lung cancer NCI-H226 (**GI$_{50}$ 1.65 µM**), NCI-H522 (**GI$_{50}$ 1.63 µM**), colon cancer HCT-116 (**GI$_{50}$ 1.18 µM**), HCT-15 (**GI$_{50}$ 1.50 µM**), KM12 (**GI$_{50}$ 1.53 µM**), CNS cancer SF-539 (**GI$_{50}$ 1.63 µM**), melanoma LOX IMVI (**GI$_{50}$ 1.52 µM**), M14 (**GI$_{50}$ 1.62 µM**), MDA-MB-435 (**GI$_{50}$ 1.68 µM**), SK-MEL-2 (**GI$_{50}$ 1.53 µM**), SK-MEL-28 (**GI$_{50}$ 1.69 µM**), UACC-62 (**GI$_{50}$ 1.68 µM**), ovarian cancer OVCAR-3 (**GI$_{50}$ 1.54 µM**), OVCAR-4 (**GI$_{50}$ 1.40 µM**), renal cancer 786-0 (**GI$_{50}$ 1.67 µM**), RXF 393 (**GI$_{50}$ 1.51 µM**), UO-31 (**GI$_{50}$ 1.43 µM**), prostate cancer PC-3 (**GI$_{50}$ 1.74 µM**), breast cancer MCF7 (**GI$_{50}$ 1.69 µM**), MDA-MB-231/ATCC (**GI$_{50}$ 1.74 µM**), BT-549 (**GI$_{50}$ 1.24 µM**).

Compound **14w** exhibited a broad spectrum of anticancer activity against leukemia RPMI-8226 cell line (**GI$_{50}$ 3.01 µM**), SR (**GI$_{50}$ 2.08 µM**), non-small cell lung cancer HOP-62 (**GI$_{50}$ 3.83 µM**), HOP-92 (**GI$_{50}$ 6.21 µM**), NCI-H226 (**GI$_{50}$ 4.17 µM**), NCI-H23 (**GI$_{50}$ 7.49 µM**), NCI-H460 (**GI$_{50}$ 4.71 µM**), NCI-H522 (**GI$_{50}$ 3.85 µM**), colon cancer HCT-116 (**GI$_{50}$ 4.89 µM**), KM12 (**GI$_{50}$ 5.56 µM**), CNS cancer SF-268 (**GI$_{50}$ 5.96 µM**), SF-539 (**GI$_{50}$ 6.42 µM**), SNB-75 (**GI$_{50}$ 4.01 µM**), U251 (**GI$_{50}$ 4.32 µM**), melanoma LOX IMVI (**GI$_{50}$ 2.58 µM**), MDA-MB-435 (**GI$_{50}$ 3.79 µM**), SK-MEL-2 (**GI$_{50}$ 2.76 µM**), SK-MEL-28 (**GI$_{50}$ 7.34 µM**), UACC-62 (**GI$_{50}$ 4.75 µM**), ovarian cancer IGROV1 (**GI$_{50}$ 5.79 µM**), OVCAR-3 (**GI$_{50}$ 4.41 µM**), OVCAR-4 (**GI$_{50}$ 3.37 µM**), OVCAR-8 (**GI$_{50}$ 4.44 µM**), NCI/ADR-RES (**GI$_{50}$ 4.69 µM**), renal cancer 786-0 (**GI$_{50}$ 6.85 µM**), ACHN (**GI$_{50}$ 7.29 µM**), RXF 393 (**GI$_{50}$ 2.23 µM**), UO-31 (**GI$_{50}$ 7.07 µM**), prostate cancer PC-3 (**GI$_{50}$ 7.05 µM**), DU-145 (**GI$_{50}$ 5.92 µM**), breast cancer **MCF7** (**GI$_{50}$ 1.73 µM**), HS 578T (**GI$_{50}$ 4.28 µM**), BT-549 (**GI$_{50}$ 3.75 µM**), T-47D (**GI$_{50}$ 4.40 µM**), MDA-MB-468 (**GI$_{50}$ 3.03 µM**).

Compound **14x** exhibited a broad spectrum of anticancer activity against leukemia CCRF-CEM cell line (**GI$_{50}$ 0.569 µM**), HL-60(TB) (**GI$_{50}$ 1.57 µM**), K-562 (**GI$_{50}$ 1.55 µM**), MOLT-4 (**GI$_{50}$ 0.349 µM**), RPMI-8226 (**GI$_{50}$ 0.722 µM**), SR (**GI$_{50}$ 0.416 µM**), non-small cell lung cancer NCI-H226 (**GI$_{50}$ 1.48 µM**), NCI-H522 (**GI$_{50}$ 1.50 µM**), colon cancer HCT-116 (**GI$_{50}$ 0.913 µM**), HCT-15 (**GI$_{50}$ 1.43 µM**), CNS cancer U251 (**GI$_{50}$ 1.46 µM**), melanoma LOX IMVI (**GI$_{50}$ 1.25 µM**), M14 (**GI$_{50}$ 1.45 µM**), MDA-MB-435 (**GI$_{50}$ 1.32 µM**), SK-MEL-2 (**GI$_{50}$ 1.52 µM**), SK-MEL-28 (**GI$_{50}$ 1.54 µM**), UACC-62 (**GI$_{50}$ 1.45 µM**), ovarian cancer IGROV1 (**GI$_{50}$ 1.62 µM**), OVCAR-3 (**GI$_{50}$ 1.23 µM**), OVCAR-4 (**GI$_{50}$ 1.54 µM**), OVCAR-8 (**GI$_{50}$ 1.62 µM**), renal cancer

786-0 (**GI$_{50}$ 1.56 µM**), ACHN (**GI$_{50}$ 1.55 µM**), RXF 393 (**GI$_{50}$ 1.16 µM**), SN12C (**GI$_{50}$ 1.29 µM**), UO-31 (**GI$_{50}$ 1.10 µM**), prostate cancer PC-3 (**GI$_{50}$ 1.45 µM**), breast cancer BT-549 (**GI$_{50}$ 1.29 µM**).



**14p** **14v**

**14w** **14x**

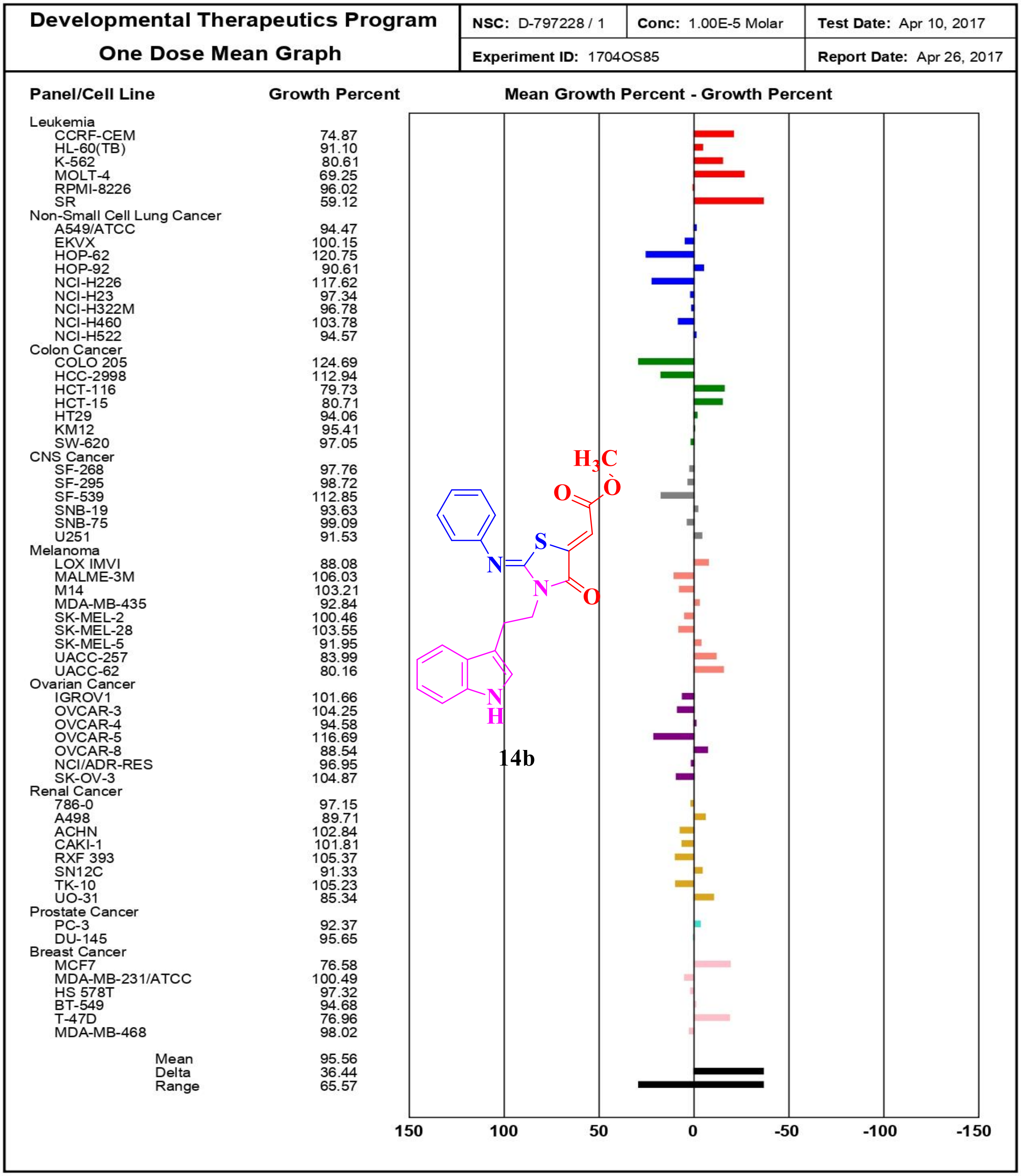
Compounds have potent anticancer activity.

## SUMMARY

In summary, we have developed an efficient synthetic protocol for (*Z*)-alkyl 2-((*Z*)-3-(2-(1*H*-indol-3-yl)ethyl)-4-oxo-2-(arylimino)thiazolidin-5-ylidene)acetates. The procedure applied for the synthesis of the title compounds were simple, with easy work up, short reaction time and high yields. Among the all synthesized compounds, compound **14p**, **14v**, **14w**, and **14x** exhibited potent activity against cancer cell lines.

## Anticancer activity one dose results

**Table-4** One dose results of 14b



**Developmental Therapeutics Program**

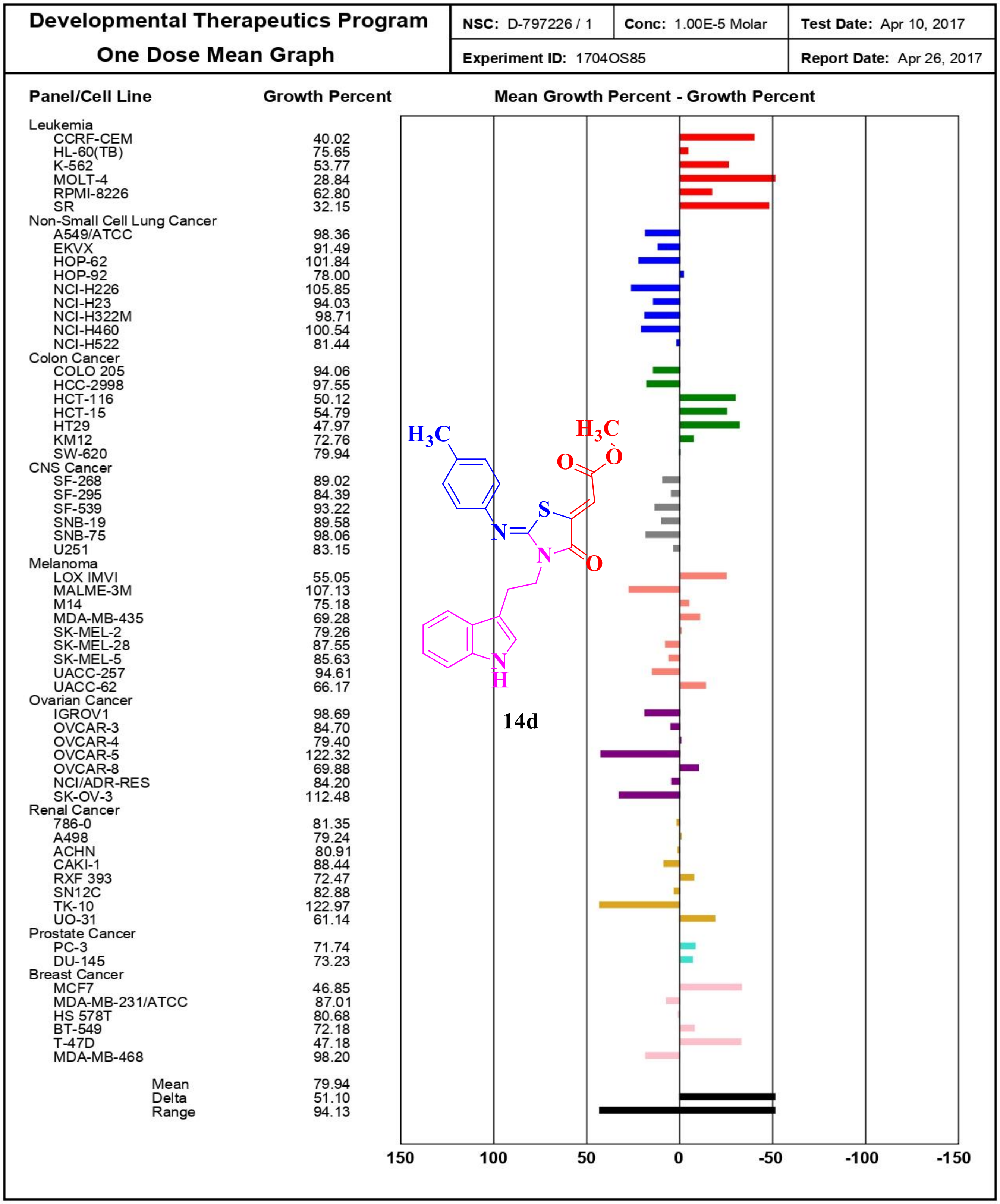
**One Dose Mean Graph**

| | |
|---|---|
| **NSC:** D-797228 / 1 | **Conc:** 1.00E-5 Molar |
| **Test Date:** Apr 10, 2017 | **Experiment ID:** 1704OS85 |
| **Report Date:** Apr 26, 2017 | |

| Panel/Cell Line | Growth Percent |
|---|---|
| Leukemia | |
| CCRF-CEM | 74.87 |
| HL-60(TB) | 91.10 |
| K-562 | 80.61 |
| MOLT-4 | 69.25 |
| RPMI-8226 | 96.02 |
| SR | 59.12 |
| Non-Small Cell Lung Cancer | |
| A549/ATCC | 94.47 |
| EKVX | 100.15 |
| HOP-62 | 120.75 |
| HOP-92 | 90.61 |
| NCI-H226 | 117.62 |
| NCI-H23 | 97.34 |
| NCI-H322M | 96.78 |
| NCI-H460 | 103.78 |
| NCI-H522 | 94.57 |
| Colon Cancer | |
| COLO 205 | 124.69 |
| HCC-2998 | 112.94 |
| HCT-116 | 79.73 |
| HCT-15 | 80.71 |
| HT29 | 94.06 |
| KM12 | 95.41 |
| SW-620 | 97.05 |
| CNS Cancer | |
| SF-268 | 97.76 |
| SF-295 | 98.72 |
| SF-539 | 112.85 |
| SNB-19 | 93.63 |
| SNB-75 | 99.09 |
| U251 | 91.53 |
| Melanoma | |
| LOX IMVI | 88.08 |
| MALME-3M | 106.03 |
| M14 | 103.21 |
| MDA-MB-435 | 92.84 |
| SK-MEL-2 | 100.46 |
| SK-MEL-28 | 103.55 |
| SK-MEL-5 | 91.95 |
| UACC-257 | 83.99 |
| UACC-62 | 80.16 |
| Ovarian Cancer | |
| IGROV1 | 101.66 |
| OVCAR-3 | 104.25 |
| OVCAR-4 | 94.58 |
| OVCAR-5 | 116.69 |
| OVCAR-8 | 88.54 |
| NCI/ADR-RES | 96.95 |
| SK-OV-3 | 104.87 |
| Renal Cancer | |
| 786-0 | 97.15 |
| A498 | 89.71 |
| ACHN | 102.84 |
| CAKI-1 | 101.81 |
| RXF 393 | 105.37 |
| SN12C | 91.33 |
| TK-10 | 105.23 |
| UO-31 | 85.34 |
| Prostate Cancer | |
| PC-3 | 92.37 |
| DU-145 | 95.65 |
| Breast Cancer | |
| MCF7 | 76.58 |
| MDA-MB-231/ATCC | 100.49 |
| HS 578T | 97.32 |
| BT-549 | 94.68 |
| T-47D | 76.96 |
| MDA-MB-468 | 98.02 |
| Mean | 95.56 |
| Delta | 36.44 |
| Range | 65.57 |

**14b**

**Table-5** One dose results of 14d



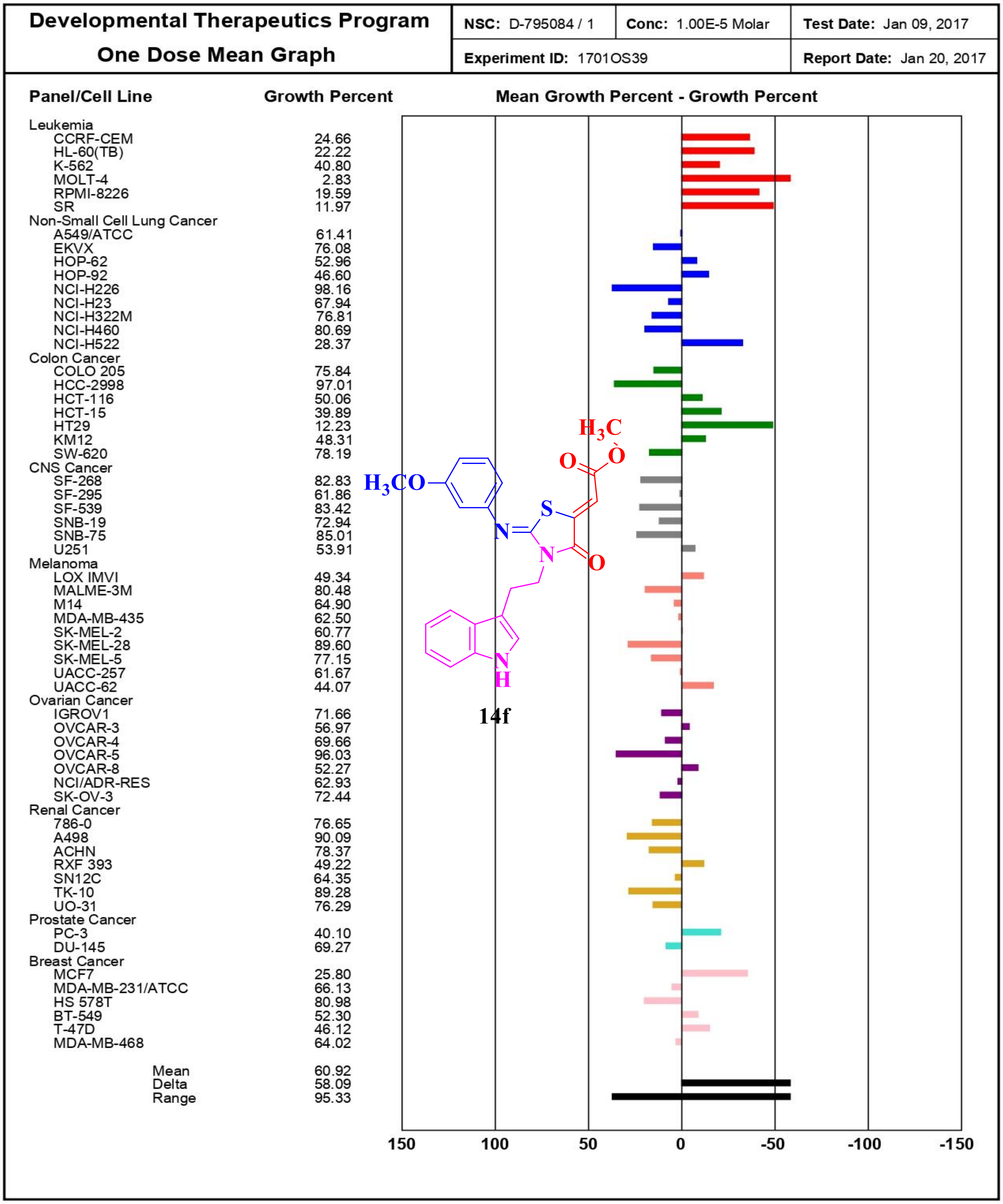
**Developmental Therapeutics Program**
**One Dose Mean Graph**

**NSC:** D-797226 / 1
**Conc:** 1.00E-5 Molar
**Test Date:** Apr 10, 2017
**Experiment ID:** 1704OS85
**Report Date:** Apr 26, 2017

**Mean Growth Percent - Growth Percent**

| **Panel/Cell Line** | **Growth Percent** |
|---|---|
| Leukemia | |
| CCRF-CEM | 40.02 |
| HL-60(TB) | 75.65 |
| K-562 | 53.77 |
| MOLT-4 | 28.84 |
| RPMI-8226 | 62.80 |
| SR | 32.15 |
| Non-Small Cell Lung Cancer | |
| A549/ATCC | 98.36 |
| EKVX | 91.49 |
| HOP-62 | 101.84 |
| HOP-92 | 78.00 |
| NCI-H226 | 105.85 |
| NCI-H23 | 94.03 |
| NCI-H322M | 98.71 |
| NCI-H460 | 100.54 |
| NCI-H522 | 81.44 |
| Colon Cancer | |
| COLO 205 | 94.06 |
| HCC-2998 | 97.55 |
| HCT-116 | 50.12 |
| HCT-15 | 54.79 |
| HT29 | 47.97 |
| KM12 | 72.76 |
| SW-620 | 79.94 |
| CNS Cancer | |
| SF-268 | 89.02 |
| SF-295 | 84.39 |
| SF-539 | 93.22 |
| SNB-19 | 89.58 |
| SNB-75 | 98.06 |
| U251 | 83.15 |
| Melanoma | |
| LOX IMVI | 55.05 |
| MALME-3M | 107.13 |
| M14 | 75.18 |
| MDA-MB-435 | 69.28 |
| SK-MEL-2 | 79.26 |
| SK-MEL-28 | 87.55 |
| SK-MEL-5 | 85.63 |
| UACC-257 | 94.61 |
| UACC-62 | 66.17 |
| Ovarian Cancer | |
| IGROV1 | 98.69 |
| OVCAR-3 | 84.70 |
| OVCAR-4 | 79.40 |
| OVCAR-5 | 122.32 |
| OVCAR-8 | 69.88 |
| NCI/ADR-RES | 84.20 |
| SK-OV-3 | 112.48 |
| Renal Cancer | |
| 786-0 | 81.35 |
| A498 | 79.24 |
| ACHN | 80.91 |
| CAKI-1 | 88.44 |
| RXF 393 | 72.47 |
| SN12C | 82.88 |
| TK-10 | 122.97 |
| UO-31 | 61.14 |
| Prostate Cancer | |
| PC-3 | 71.74 |
| DU-145 | 73.23 |
| Breast Cancer | |
| MCF7 | 46.85 |
| MDA-MB-231/ATCC | 87.01 |
| HS 578T | 80.68 |
| BT-549 | 72.18 |
| T-47D | 47.18 |
| MDA-MB-468 | 98.20 |
| Mean | 79.94 |
| Delta | 51.10 |
| Range | 94.13 |

**Table-6** One dose results of 14f



**Developmental Therapeutics Program**

**One Dose Mean Graph**

**NSC:** D-795084 / 1

**Conc:** 1.00E-5 Molar

**Test Date:** Jan 09, 2017

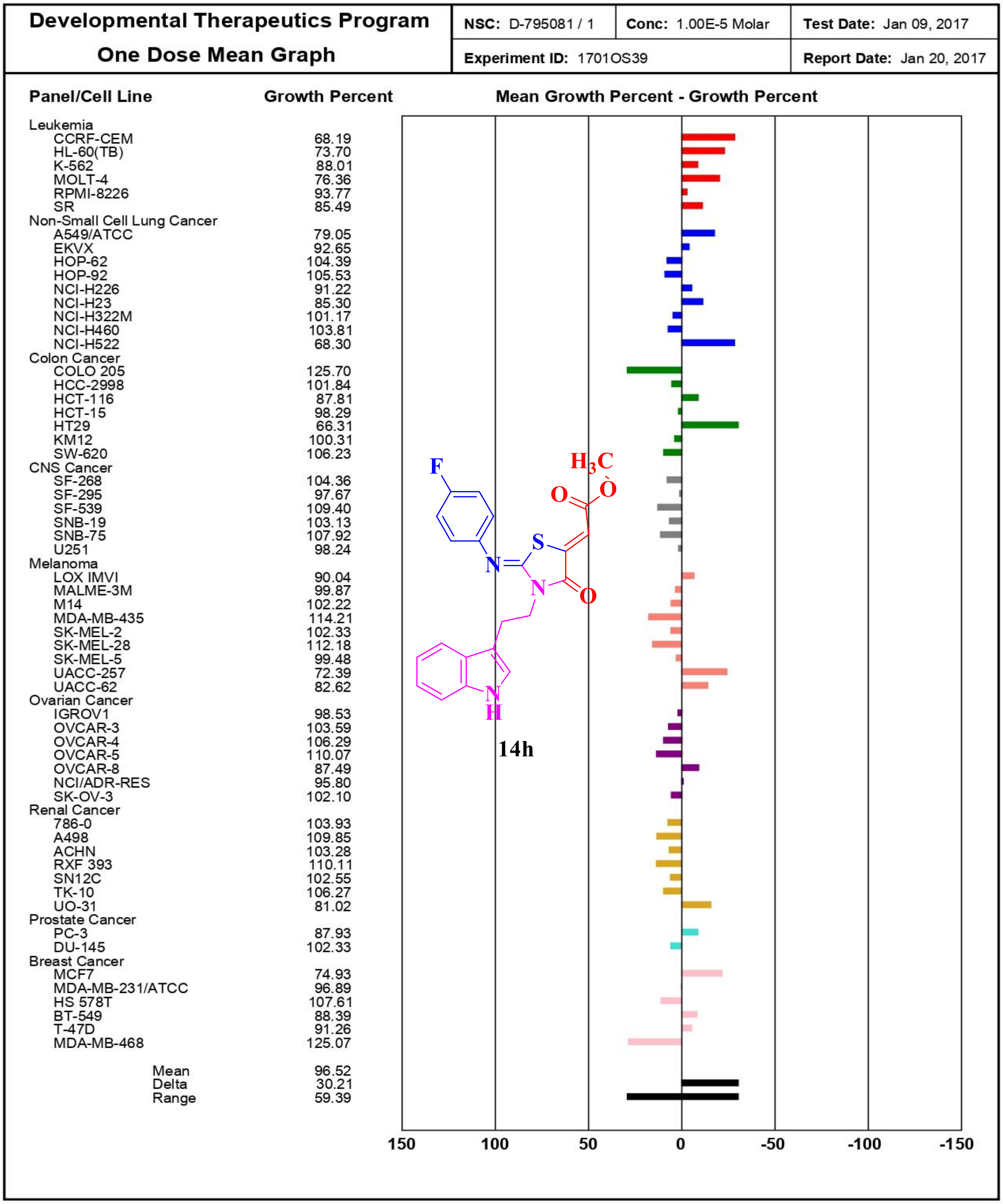
**Experiment ID:** 1701OS39

**Report Date:** Jan 20, 2017

| Panel/Cell Line | Growth Percent |
|---|---|
| Leukemia | |
| CCRF-CEM | 24.66 |
| HL-60(TB) | 22.22 |
| K-562 | 40.80 |
| MOLT-4 | 2.83 |
| RPMI-8226 | 19.59 |
| SR | 11.97 |
| Non-Small Cell Lung Cancer | |
| A549/ATCC | 61.41 |
| EKVX | 76.08 |
| HOP-62 | 52.96 |
| HOP-92 | 46.60 |
| NCI-H226 | 98.16 |
| NCI-H23 | 67.94 |
| NCI-H322M | 76.81 |
| NCI-H460 | 80.69 |
| NCI-H522 | 28.37 |
| Colon Cancer | |
| COLO 205 | 75.84 |
| HCC-2998 | 97.01 |
| HCT-116 | 50.06 |
| HCT-15 | 39.89 |
| HT29 | 12.23 |
| KM12 | 48.31 |
| SW-620 | 78.19 |
| CNS Cancer | |
| SF-268 | 82.83 |
| SF-295 | 61.86 |
| SF-539 | 83.42 |
| SNB-19 | 72.94 |
| SNB-75 | 85.01 |
| U251 | 53.91 |
| Melanoma | |
| LOX IMVI | 49.34 |
| MALME-3M | 80.48 |
| M14 | 64.90 |
| MDA-MB-435 | 62.50 |
| SK-MEL-2 | 60.77 |
| SK-MEL-28 | 89.60 |
| SK-MEL-5 | 77.15 |
| UACC-257 | 61.67 |
| UACC-62 | 44.07 |
| Ovarian Cancer | |
| IGROV1 | 71.66 |
| OVCAR-3 | 56.97 |
| OVCAR-4 | 69.66 |
| OVCAR-5 | 96.03 |
| OVCAR-8 | 52.27 |
| NCI/ADR-RES | 62.93 |
| SK-OV-3 | 72.44 |
| Renal Cancer | |
| 786-0 | 76.65 |
| A498 | 90.09 |
| ACHN | 78.37 |
| RXF 393 | 49.22 |
| SN12C | 64.35 |
| TK-10 | 89.28 |
| UO-31 | 76.29 |
| Prostate Cancer | |
| PC-3 | 40.10 |
| DU-145 | 69.27 |
| Breast Cancer | |
| MCF7 | 25.80 |
| MDA-MB-231/ATCC | 66.13 |
| HS 578T | 80.98 |
| BT-549 | 52.30 |
| T-47D | 46.12 |
| MDA-MB-468 | 64.02 |
| Mean | 60.92 |
| Delta | 58.09 |
| Range | 95.33 |

14f

**Table-7** One dose results of 14h



**Developmental Therapeutics Program**

**One Dose Mean Graph**

**NSC:** D-795081 / 1

**Conc:** 1.00E-5 Molar

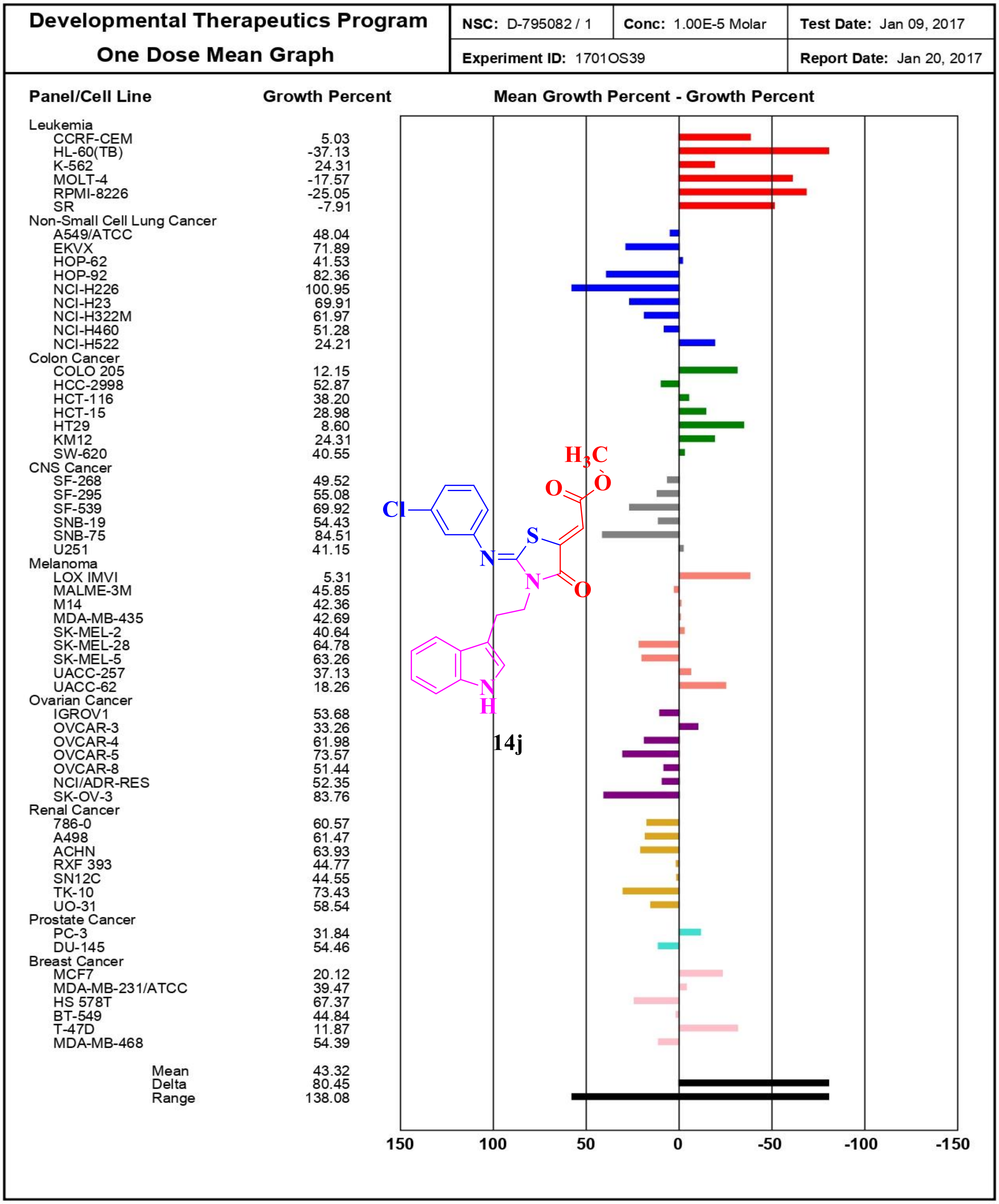
**Test Date:** Jan 09, 2017

**Experiment ID:** 1701OS39

**Report Date:** Jan 20, 2017

| Panel/Cell Line | Growth Percent |
|---|---|
| Leukemia | |
| CCRF-CEM | 68.19 |
| HL-60(TB) | 73.70 |
| K-562 | 88.01 |
| MOLT-4 | 76.36 |
| RPMI-8226 | 93.77 |
| SR | 85.49 |
| Non-Small Cell Lung Cancer | |
| A549/ATCC | 79.05 |
| EKVX | 92.65 |
| HOP-62 | 104.39 |
| HOP-92 | 105.53 |
| NCI-H226 | 91.22 |
| NCI-H23 | 85.30 |
| NCI-H322M | 101.17 |
| NCI-H460 | 103.81 |
| NCI-H522 | 68.30 |
| Colon Cancer | |
| COLO 205 | 125.70 |
| HCC-2998 | 101.84 |
| HCT-116 | 87.81 |
| HCT-15 | 98.29 |
| HT29 | 66.31 |
| KM12 | 100.31 |
| SW-620 | 106.23 |
| CNS Cancer | |
| SF-268 | 104.36 |
| SF-295 | 97.67 |
| SF-539 | 109.40 |
| SNB-19 | 103.13 |
| SNB-75 | 107.92 |
| U251 | 98.24 |
| Melanoma | |
| LOX IMVI | 90.04 |
| MALME-3M | 99.87 |
| M14 | 102.22 |
| MDA-MB-435 | 114.21 |
| SK-MEL-2 | 102.33 |
| SK-MEL-28 | 112.18 |
| SK-MEL-5 | 99.48 |
| UACC-257 | 72.39 |
| UACC-62 | 82.62 |
| Ovarian Cancer | |
| IGROV1 | 98.53 |
| OVCAR-3 | 103.59 |
| OVCAR-4 | 106.29 |
| OVCAR-5 | 110.07 |
| OVCAR-8 | 87.49 |
| NCI/ADR-RES | 95.80 |
| SK-OV-3 | 102.10 |
| Renal Cancer | |
| 786-0 | 103.93 |
| A498 | 109.85 |
| ACHN | 103.28 |
| RXF 393 | 110.11 |
| SN12C | 102.55 |
| TK-10 | 106.27 |
| UO-31 | 81.02 |
| Prostate Cancer | |
| PC-3 | 87.93 |
| DU-145 | 102.33 |
| Breast Cancer | |
| MCF7 | 74.93 |
| MDA-MB-231/ATCC | 96.89 |
| HS 578T | 107.61 |
| BT-549 | 88.39 |
| T-47D | 91.26 |
| MDA-MB-468 | 125.07 |
| Mean | 96.52 |
| Delta | 30.21 |
| Range | 59.39 |

**Table-8** One dose results of 14j



**Developmental Therapeutics Program**

**One Dose Mean Graph**

**NSC:** D-795082 / 1

**Conc:** 1.00E-5 Molar

**Test Date:** Jan 09, 2017

**Experiment ID:** 1701OS39

**Report Date:** Jan 20, 2017

| Panel/Cell Line | Growth Percent |
|---|---|
| Leukemia | |
| CCRF-CEM | 5.03 |
| HL-60(TB) | -37.13 |
| K-562 | 24.31 |
| MOLT-4 | -17.57 |
| RPMI-8226 | -25.05 |
| SR | -7.91 |
| Non-Small Cell Lung Cancer | |
| A549/ATCC | 48.04 |
| EKVX | 71.89 |
| HOP-62 | 41.53 |
| HOP-92 | 82.36 |
| NCI-H226 | 100.95 |
| NCI-H23 | 69.91 |
| NCI-H322M | 61.97 |
| NCI-H460 | 51.28 |
| NCI-H522 | 24.21 |
| Colon Cancer | |
| COLO 205 | 12.15 |
| HCC-2998 | 52.87 |
| HCT-116 | 38.20 |
| HCT-15 | 28.98 |
| HT29 | 8.60 |
| KM12 | 24.31 |
| SW-620 | 40.55 |
| CNS Cancer | |
| SF-268 | 49.52 |
| SF-295 | 55.08 |
| SF-539 | 69.92 |
| SNB-19 | 54.43 |
| SNB-75 | 84.51 |
| U251 | 41.15 |
| Melanoma | |
| LOX IMVI | 5.31 |
| MALME-3M | 45.85 |
| M14 | 42.36 |
| MDA-MB-435 | 42.69 |
| SK-MEL-2 | 40.64 |
| SK-MEL-28 | 64.78 |
| SK-MEL-5 | 63.26 |
| UACC-257 | 37.13 |
| UACC-62 | 18.26 |
| Ovarian Cancer | |
| IGROV1 | 53.68 |
| OVCAR-3 | 33.26 |
| OVCAR-4 | 61.98 |
| OVCAR-5 | 73.57 |
| OVCAR-8 | 51.44 |
| NCI/ADR-RES | 52.35 |
| SK-OV-3 | 83.76 |
| Renal Cancer | |
| 786-0 | 60.57 |
| A498 | 61.47 |
| ACHN | 63.93 |
| RXF 393 | 44.77 |
| SN12C | 44.55 |
| TK-10 | 73.43 |
| UO-31 | 58.54 |
| Prostate Cancer | |
| PC-3 | 31.84 |
| DU-145 | 54.46 |
| Breast Cancer | |
| MCF7 | 20.12 |
| MDA-MB-231/ATCC | 39.47 |
| HS 578T | 67.37 |
| BT-549 | 44.84 |
| T-47D | 11.87 |
| MDA-MB-468 | 54.39 |
| Mean | 43.32 |
| Delta | 80.45 |
| Range | 138.08 |

**Mean Growth Percent - Growth Percent**

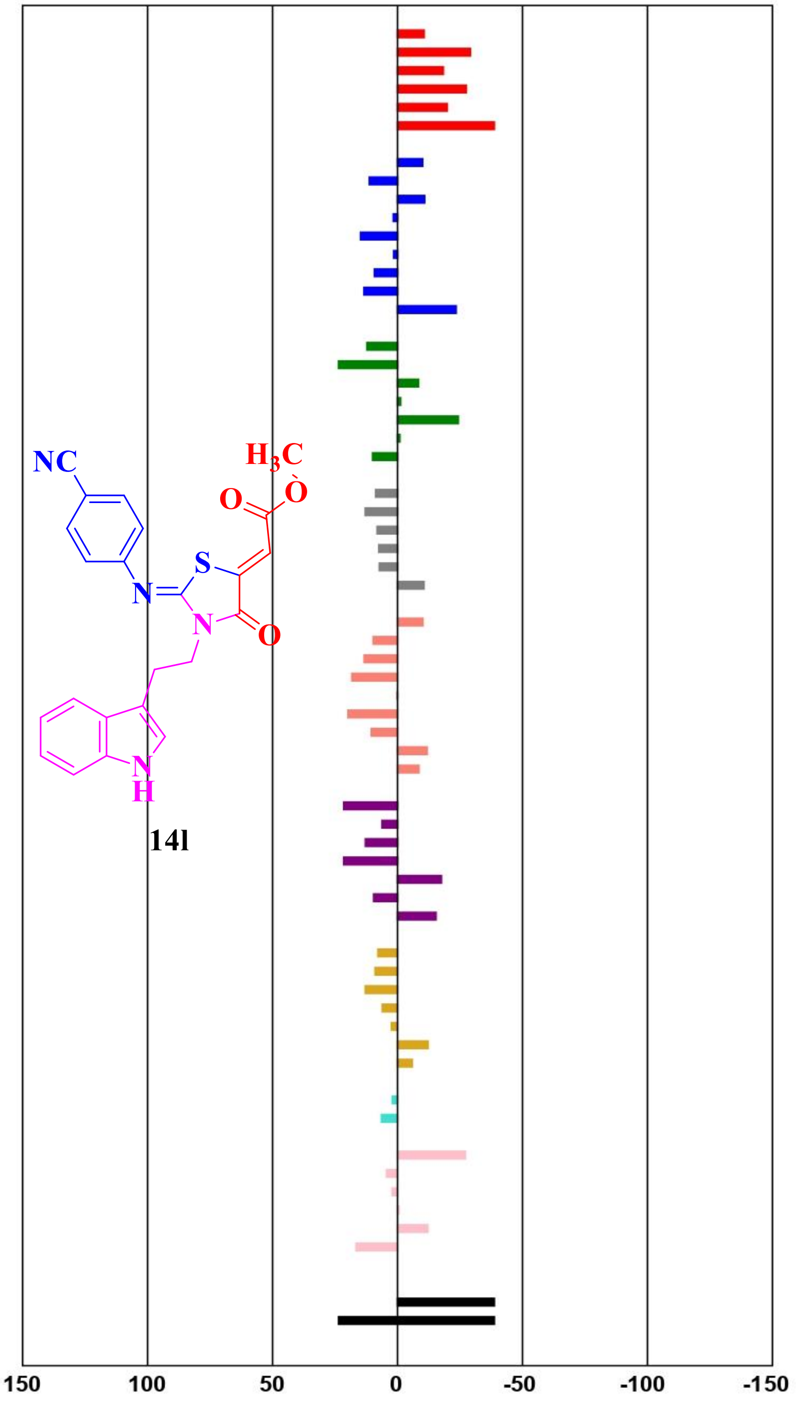
14j

**Table-9** One dose results of 14l

| Developmental Therapeutics Program One Dose Mean Graph | **NSC:** D-795085 / 1 | **Conc:** 1.00E-5 Molar | **Test Date:** Jan 09, 2017 |
|---|---|---|---|
| | **Experiment ID:** 1701OS39 | | **Report Date:** Jan 20, 2017 |

| Panel/Cell Line | Growth Percent |
|---|---|
| Leukemia | |
| CCRF-CEM | 78.52 |
| HL-60(TB) | 60.03 |
| K-562 | 70.85 |
| MOLT-4 | 61.67 |
| RPMI-8226 | 69.29 |
| SR | 50.45 |
| Non-Small Cell Lung Cancer | |
| A549/ATCC | 79.11 |
| EKVX | 100.47 |
| HOP-62 | 78.32 |
| HOP-92 | 90.89 |
| NCI-H226 | 103.98 |
| NCI-H23 | 90.72 |
| NCI-H322M | 98.43 |
| NCI-H460 | 102.61 |
| NCI-H522 | 65.73 |
| Colon Cancer | |
| COLO 205 | 101.44 |
| HCC-2998 | 112.76 |
| HCT-116 | 80.77 |
| HCT-15 | 87.91 |
| HT29 | 64.87 |
| KM12 | 88.24 |
| SW-620 | 99.15 |
| CNS Cancer | |
| SF-268 | 97.93 |
| SF-295 | 102.12 |
| SF-539 | 97.36 |
| SNB-19 | 96.66 |
| SNB-75 | 96.47 |
| U251 | 78.58 |
| Melanoma | |
| LOX IMVI | 79.01 |
| MALME-3M | 98.92 |
| M14 | 102.52 |
| MDA-MB-435 | 107.47 |
| SK-MEL-2 | 89.26 |
| SK-MEL-28 | 108.98 |
| SK-MEL-5 | 99.68 |
| UACC-257 | 77.31 |
| UACC-62 | 80.61 |
| Ovarian Cancer | |
| IGROV1 | 110.69 |
| OVCAR-3 | 95.38 |
| OVCAR-4 | 102.04 |
| OVCAR-5 | 110.72 |
| OVCAR-8 | 71.62 |
| NCI/ADR-RES | 98.73 |
| SK-OV-3 | 73.78 |
| Renal Cancer | |
| 786-0 | 97.00 |
| A498 | 98.16 |
| ACHN | 102.07 |
| RXF 393 | 95.30 |
| SN12C | 91.61 |
| TK-10 | 76.94 |
| UO-31 | 83.26 |
| Prostate Cancer | |
| PC-3 | 91.26 |
| DU-145 | 95.62 |
| Breast Cancer | |
| MCF7 | 62.03 |
| MDA-MB-231/ATCC | 93.56 |
| HS 578T | 91.35 |
| BT-549 | 88.64 |
| T-47D | 77.08 |
| MDA-MB-468 | 105.78 |
| Mean | 89.18 |
| Delta | 38.73 |
| Range | 62.31 |

Mean Growth Percent - Growth Percent



**14l**

**Table-10** One dose results of 14n

**Developmental Therapeutics Program**

**One Dose Mean Graph**

**NSC:** D-797229 / 1

**Conc:** 1.00E-5 Molar

**Test Date:** Apr 10, 2017

**Experiment ID:** 1704OS85

**Report Date:** Apr 26, 2017

| Panel/Cell Line | Growth Percent |
|---|---|
| Leukemia | |
| CCRF-CEM | 8.51 |
| HL-60(TB) | 69.77 |
| K-562 | 25.82 |
| MOLT-4 | -2.10 |
| RPMI-8226 | 2.18 |
| SR | 1.73 |
| Non-Small Cell Lung Cancer | |
| A549/ATCC | 90.84 |
| EKVX | 87.23 |
| HOP-62 | 96.72 |
| HOP-92 | 73.16 |
| NCI-H226 | 98.68 |
| NCI-H23 | 72.71 |
| NCI-H322M | 98.41 |
| NCI-H460 | 92.22 |
| NCI-H522 | 53.61 |
| Colon Cancer | |
| COLO 205 | 84.56 |
| HCC-2998 | 78.31 |
| HCT-116 | 21.83 |
| HCT-15 | 29.88 |
| HT29 | 51.75 |
| KM12 | 66.36 |
| SW-620 | 47.25 |
| CNS Cancer | |
| SF-268 | 75.69 |
| SF-295 | 84.16 |
| SF-539 | 79.56 |
| SNB-19 | 69.24 |
| SNB-75 | 84.28 |
| U251 | 46.88 |
| Melanoma | |
| LOX IMVI | -9.30 |
| MALME-3M | 86.54 |
| M14 | 51.96 |
| MDA-MB-435 | 55.49 |
| SK-MEL-2 | 68.11 |
| SK-MEL-28 | 76.79 |
| SK-MEL-5 | 84.88 |
| UACC-257 | 71.58 |
| UACC-62 | 50.97 |
| Ovarian Cancer | |
| IGROV1 | 62.03 |
| OVCAR-3 | 43.06 |
| OVCAR-4 | 66.21 |
| OVCAR-5 | 111.70 |
| OVCAR-8 | 42.03 |
| NCI/ADR-RES | 66.85 |
| SK-OV-3 | 104.66 |
| Renal Cancer | |
| 786-0 | 68.01 |
| A498 | 78.22 |
| ACHN | 64.64 |
| CAKI-1 | 73.13 |
| RXF 393 | 70.44 |
| SN12C | 58.82 |
| TK-10 | 102.71 |
| UO-31 | 48.84 |
| Prostate Cancer | |
| PC-3 | 63.43 |
| DU-145 | 66.84 |
| Breast Cancer | |
| MCF7 | 20.12 |
| MDA-MB-231/ATCC | 72.08 |
| HS 578T | 63.48 |
| BT-549 | 60.76 |
| T-47D | 46.47 |
| MDA-MB-468 | 85.98 |
| Mean | 62.78 |
| Delta | 72.08 |
| Range | 121.00 |

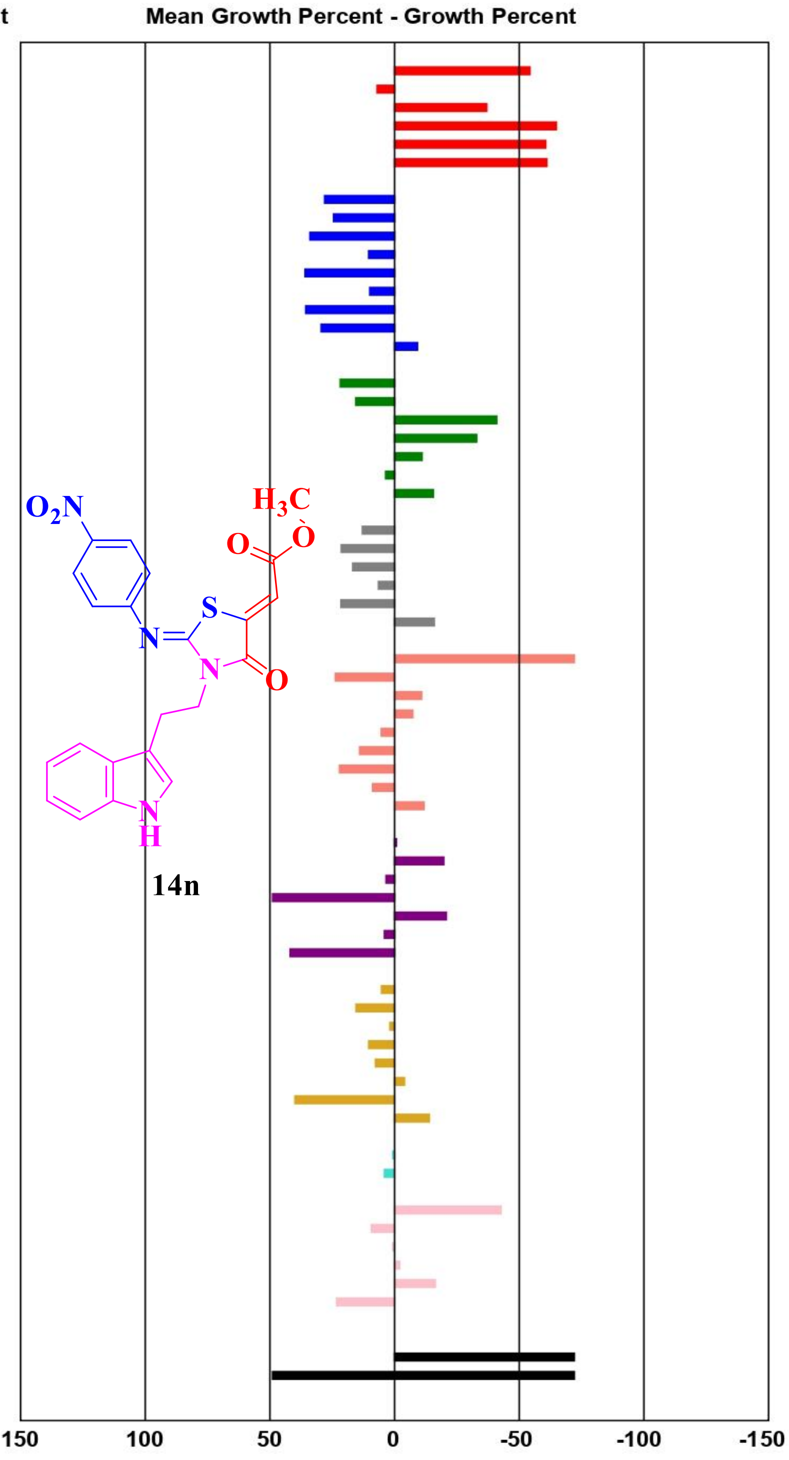
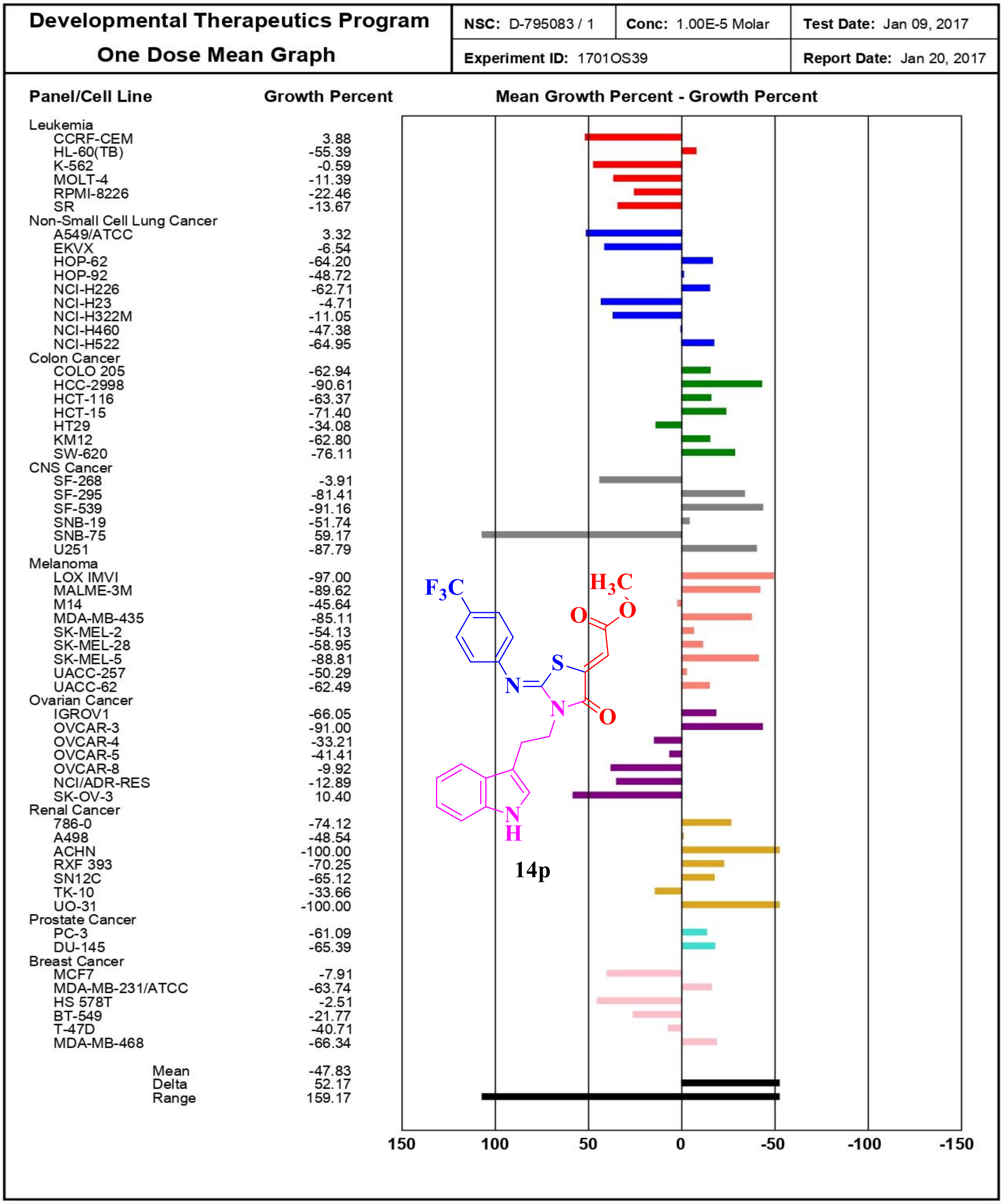


**Table-11** One dose results of 14p



**Developmental Therapeutics Program**

**One Dose Mean Graph**

**NSC:** D-795083 / 1 | **Conc:** 1.00E-5 Molar | **Test Date:** Jan 09, 2017

**Experiment ID:** 1701OS39 | **Report Date:** Jan 20, 2017

| Panel/Cell Line | Growth Percent |
|---|---|
| Leukemia | |
| CCRF-CEM | 3.88 |
| HL-60(TB) | -55.39 |
| K-562 | -0.59 |
| MOLT-4 | -11.39 |
| RPMI-8226 | -22.46 |
| SR | -13.67 |
| Non-Small Cell Lung Cancer | |
| A549/ATCC | 3.32 |
| EKVX | -6.54 |
| HOP-62 | -64.20 |
| HOP-92 | -48.72 |
| NCI-H226 | -62.71 |
| NCI-H23 | -4.71 |
| NCI-H322M | -11.05 |
| NCI-H460 | -47.38 |
| NCI-H522 | -64.95 |
| Colon Cancer | |
| COLO 205 | -62.94 |
| HCC-2998 | -90.61 |
| HCT-116 | -63.37 |
| HCT-15 | -71.40 |
| HT29 | -34.08 |
| KM12 | -62.80 |
| SW-620 | -76.11 |
| CNS Cancer | |
| SF-268 | -3.91 |
| SF-295 | -81.41 |
| SF-539 | -91.16 |
| SNB-19 | -51.74 |
| SNB-75 | 59.17 |
| U251 | -87.79 |
| Melanoma | |
| LOX IMVI | -97.00 |
| MALME-3M | -89.62 |
| M14 | -45.64 |
| MDA-MB-435 | -85.11 |
| SK-MEL-2 | -54.13 |
| SK-MEL-28 | -58.95 |
| SK-MEL-5 | -88.81 |
| UACC-257 | -50.29 |
| UACC-62 | -62.49 |
| Ovarian Cancer | |
| IGROV1 | -66.05 |
| OVCAR-3 | -91.00 |
| OVCAR-4 | -33.21 |
| OVCAR-5 | -41.41 |
| OVCAR-8 | -9.92 |
| NCI/ADR-RES | -12.89 |
| SK-OV-3 | 10.40 |
| Renal Cancer | |
| 786-0 | -74.12 |
| A498 | -48.54 |
| ACHN | -100.00 |
| RXF 393 | -70.25 |
| SN12C | -65.12 |
| TK-10 | -33.66 |
| UO-31 | -100.00 |
| Prostate Cancer | |
| PC-3 | -61.09 |
| DU-145 | -65.39 |
| Breast Cancer | |
| MCF7 | -7.91 |
| MDA-MB-231/ATCC | -63.74 |
| HS 578T | -2.51 |
| BT-549 | -21.77 |
| T-47D | -40.71 |
| MDA-MB-468 | -66.34 |
| Mean | -47.83 |
| Delta | 52.17 |
| Range | 159.17 |

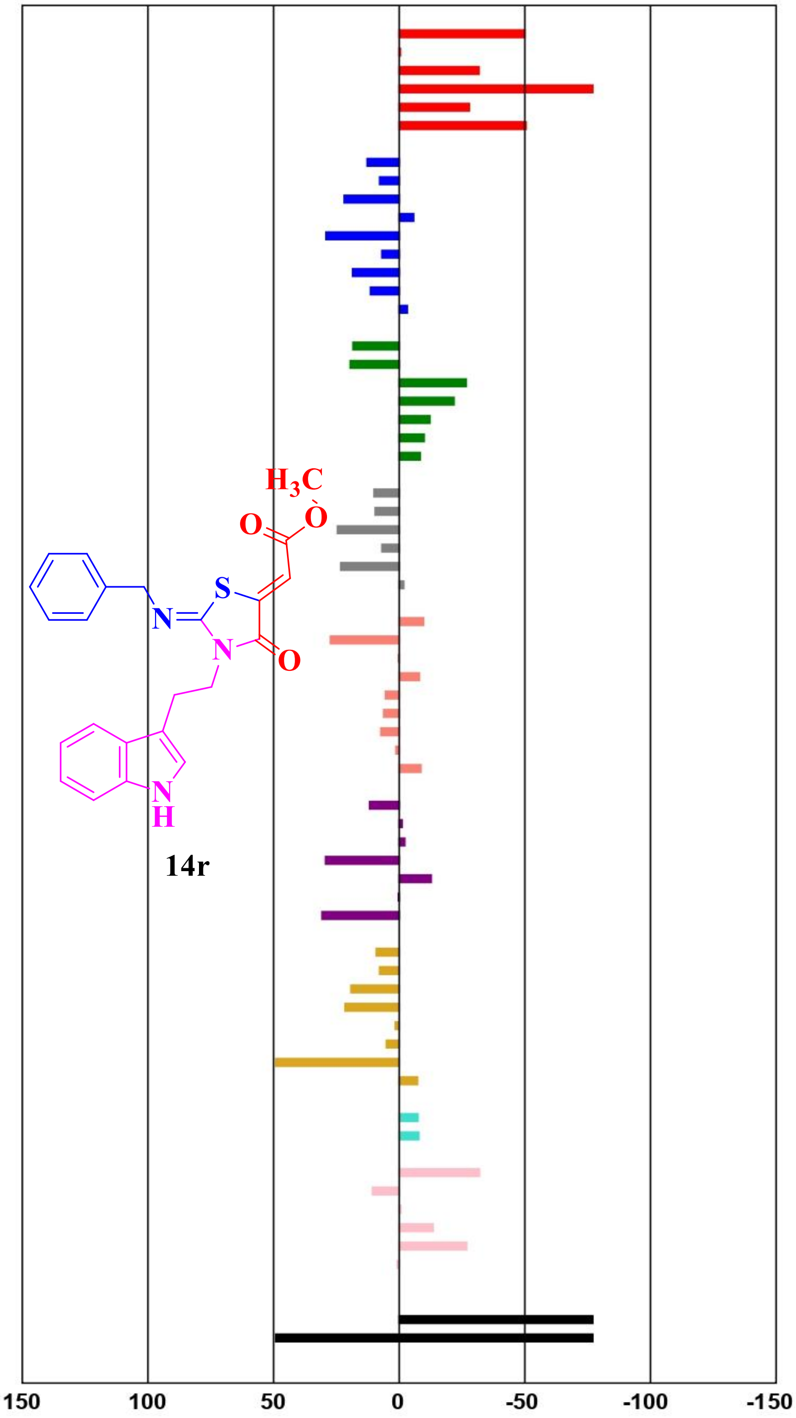
**14p**

**Table-12** One dose results of 14r

| Developmental Therapeutics Program One Dose Mean Graph | NSC: D-797227 / 1 | Conc: 1.00E-5 Molar | Test Date: Apr 10, 2017 |
|---|---|---|---|
| | Experiment ID: 1704OS85 | | Report Date: Apr 26, 2017 |

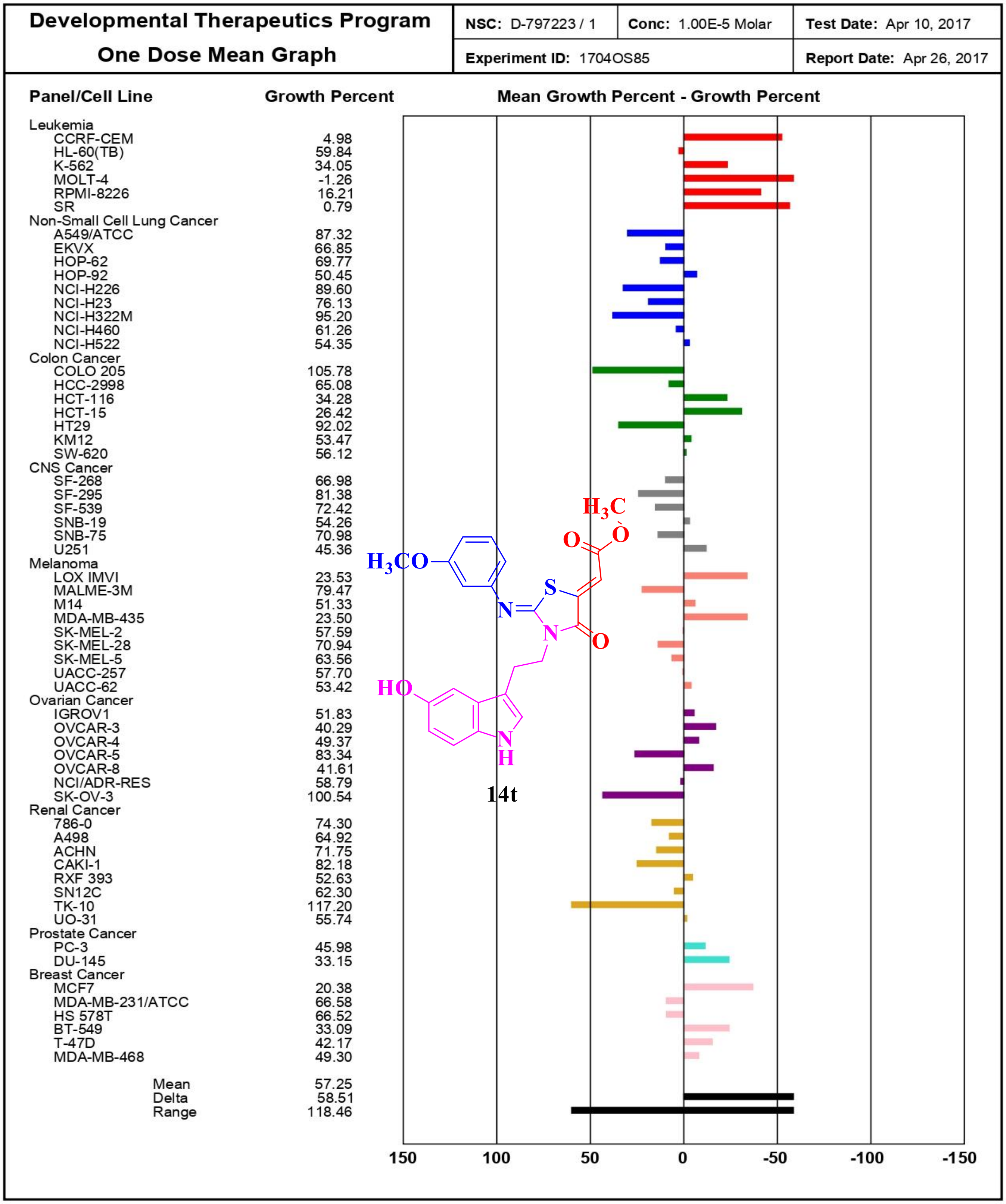
| Panel/Cell Line | Growth Percent |
|---|---|
| Leukemia | |
| CCRF-CEM | 30.31 |
| HL-60(TB) | 79.85 |
| K-562 | 48.54 |
| MOLT-4 | 3.29 |
| RPMI-8226 | 52.41 |
| SR | 29.83 |
| Non-Small Cell Lung Cancer | |
| A549/ATCC | 93.08 |
| EKVX | 88.10 |
| HOP-62 | 102.21 |
| HOP-92 | 74.58 |
| NCI-H226 | 109.46 |
| NCI-H23 | 87.21 |
| NCI-H322M | 98.87 |
| NCI-H460 | 91.76 |
| NCI-H522 | 77.11 |
| Colon Cancer | |
| COLO 205 | 98.71 |
| HCC-2998 | 99.82 |
| HCT-116 | 53.64 |
| HCT-15 | 58.51 |
| HT29 | 68.07 |
| KM12 | 70.40 |
| SW-620 | 71.97 |
| CNS Cancer | |
| SF-268 | 90.32 |
| SF-295 | 89.88 |
| SF-539 | 104.91 |
| SNB-19 | 87.19 |
| SNB-75 | 103.59 |
| U251 | 78.53 |
| Melanoma | |
| LOX IMVI | 70.63 |
| MALME-3M | 107.67 |
| M14 | 80.50 |
| MDA-MB-435 | 72.29 |
| SK-MEL-2 | 85.79 |
| SK-MEL-28 | 86.47 |
| SK-MEL-5 | 87.59 |
| UACC-257 | 81.62 |
| UACC-62 | 71.68 |
| Ovarian Cancer | |
| IGROV1 | 92.12 |
| OVCAR-3 | 79.14 |
| OVCAR-4 | 78.10 |
| OVCAR-5 | 109.64 |
| OVCAR-8 | 67.57 |
| NCI/ADR-RES | 80.60 |
| SK-OV-3 | 111.03 |
| Renal Cancer | |
| 786-0 | 89.43 |
| A498 | 88.10 |
| ACHN | 99.52 |
| CAKI-1 | 101.86 |
| RXF 393 | 81.85 |
| SN12C | 85.43 |
| TK-10 | 129.46 |
| UO-31 | 73.02 |
| Prostate Cancer | |
| PC-3 | 72.91 |
| DU-145 | 72.53 |
| Breast Cancer | |
| MCF7 | 48.41 |
| MDA-MB-231/ATCC | 90.97 |
| HS 578T | 79.72 |
| BT-549 | 66.87 |
| T-47D | 53.48 |
| MDA-MB-468 | 81.05 |
| Mean | 80.32 |
| Delta | 77.03 |
| Range | 126.17 |

Mean Growth Percent - Growth Percent



14r

**Table-13** One dose results of 14t



14t

**Developmental Therapeutics Program**

**One Dose Mean Graph**

**NSC:** D-797223 / 1

**Conc:** 1.00E-5 Molar

**Test Date:** Apr 10, 2017

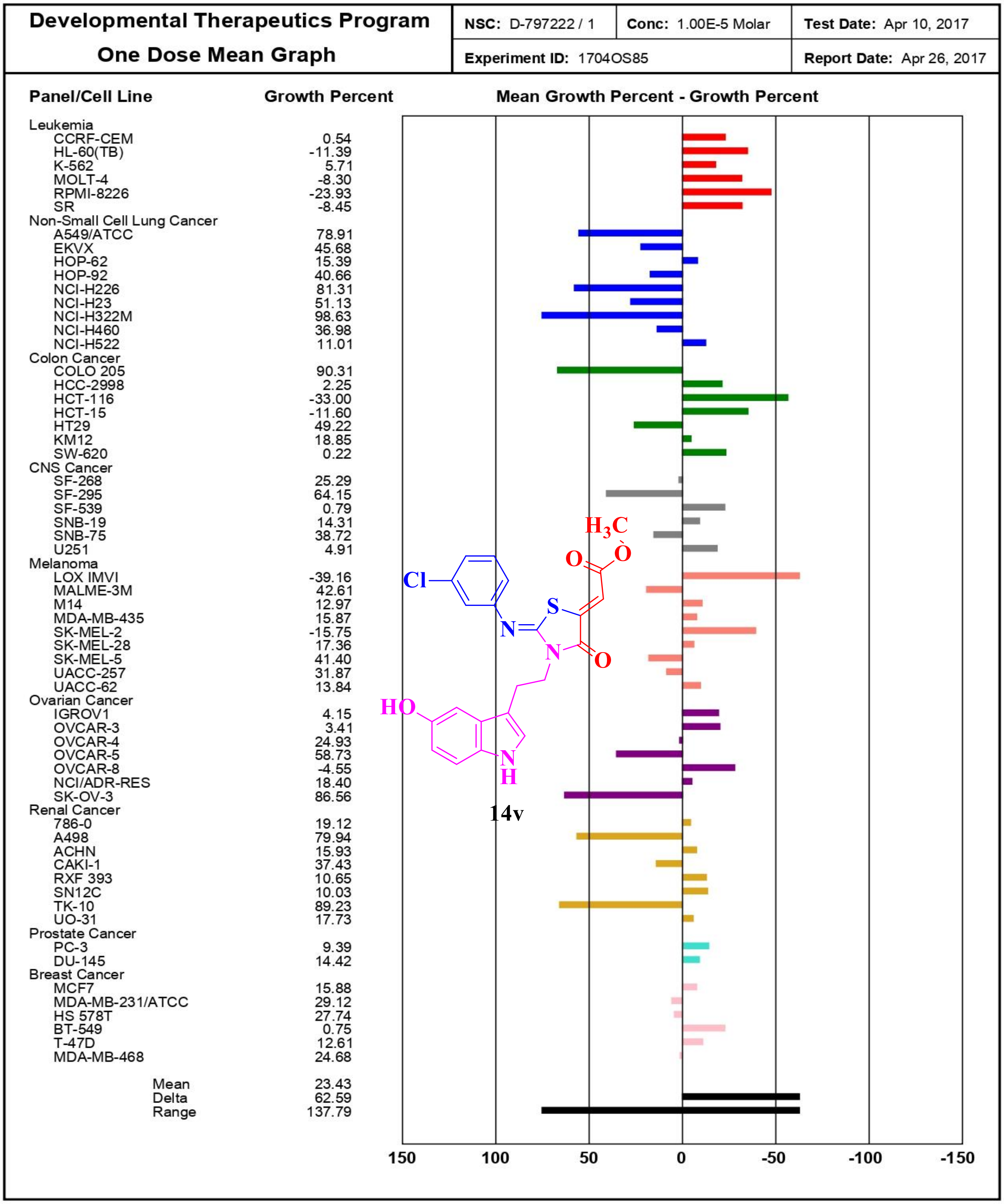
**Experiment ID:** 1704OS85

**Report Date:** Apr 26, 2017

**Mean Growth Percent - Growth Percent**

| Panel/Cell Line | Growth Percent |
|---|---|
| Leukemia | |
| CCRF-CEM | 4.98 |
| HL-60(TB) | 59.84 |
| K-562 | 34.05 |
| MOLT-4 | -1.26 |
| RPMI-8226 | 16.21 |
| SR | 0.79 |
| Non-Small Cell Lung Cancer | |
| A549/ATCC | 87.32 |
| EKVX | 66.85 |
| HOP-62 | 69.77 |
| HOP-92 | 50.45 |
| NCI-H226 | 89.60 |
| NCI-H23 | 76.13 |
| NCI-H322M | 95.20 |
| NCI-H460 | 61.26 |
| NCI-H522 | 54.35 |
| Colon Cancer | |
| COLO 205 | 105.78 |
| HCC-2998 | 65.08 |
| HCT-116 | 34.28 |
| HCT-15 | 26.42 |
| HT29 | 92.02 |
| KM12 | 53.47 |
| SW-620 | 56.12 |
| CNS Cancer | |
| SF-268 | 66.98 |
| SF-295 | 81.38 |
| SF-539 | 72.42 |
| SNB-19 | 54.26 |
| SNB-75 | 70.98 |
| U251 | 45.36 |
| Melanoma | |
| LOX IMVI | 23.53 |
| MALME-3M | 79.47 |
| M14 | 51.33 |
| MDA-MB-435 | 23.50 |
| SK-MEL-2 | 57.59 |
| SK-MEL-28 | 70.94 |
| SK-MEL-5 | 63.56 |
| UACC-257 | 57.70 |
| UACC-62 | 53.42 |
| Ovarian Cancer | |
| IGROV1 | 51.83 |
| OVCAR-3 | 40.29 |
| OVCAR-4 | 49.37 |
| OVCAR-5 | 83.34 |
| OVCAR-8 | 41.61 |
| NCI/ADR-RES | 58.79 |
| SK-OV-3 | 100.54 |
| Renal Cancer | |
| 786-0 | 74.30 |
| A498 | 64.92 |
| ACHN | 71.75 |
| CAKI-1 | 82.18 |
| RXF 393 | 52.63 |
| SN12C | 62.30 |
| TK-10 | 117.20 |
| UO-31 | 55.74 |
| Prostate Cancer | |
| PC-3 | 45.98 |
| DU-145 | 33.15 |
| Breast Cancer | |
| MCF7 | 20.38 |
| MDA-MB-231/ATCC | 66.58 |
| HS 578T | 66.52 |
| BT-549 | 33.09 |
| T-47D | 42.17 |
| MDA-MB-468 | 49.30 |
| Mean | 57.25 |
| Delta | 58.51 |
| Range | 118.46 |

**Table-14** One dose results of 14v



**Developmental Therapeutics Program**

**One Dose Mean Graph**

**NSC:** D-797222 / 1

**Conc:** 1.00E-5 Molar

**Test Date:** Apr 10, 2017

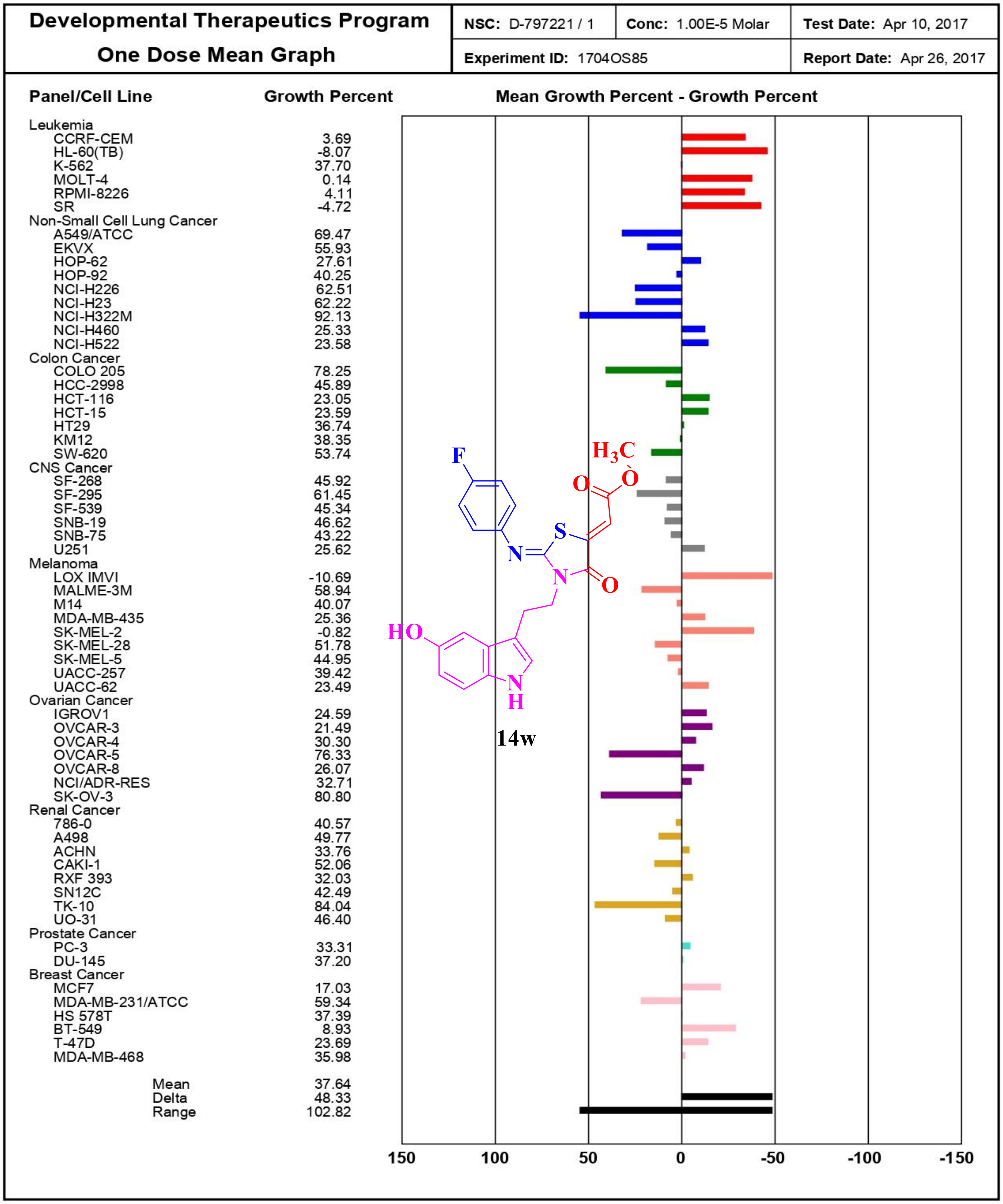
**Experiment ID:** 1704OS85

**Report Date:** Apr 26, 2017

| Panel/Cell Line | Growth Percent |
|---|---|
| Leukemia | |
| CCRF-CEM | 0.54 |
| HL-60(TB) | -11.39 |
| K-562 | 5.71 |
| MOLT-4 | -8.30 |
| RPMI-8226 | -23.93 |
| SR | -8.45 |
| Non-Small Cell Lung Cancer | |
| A549/ATCC | 78.91 |
| EKVX | 45.68 |
| HOP-62 | 15.39 |
| HOP-92 | 40.66 |
| NCI-H226 | 81.31 |
| NCI-H23 | 51.13 |
| NCI-H322M | 98.63 |
| NCI-H460 | 36.98 |
| NCI-H522 | 11.01 |
| Colon Cancer | |
| COLO 205 | 90.31 |
| HCC-2998 | 2.25 |
| HCT-116 | -33.00 |
| HCT-15 | -11.60 |
| HT29 | 49.22 |
| KM12 | 18.85 |
| SW-620 | 0.22 |
| CNS Cancer | |
| SF-268 | 25.29 |
| SF-295 | 64.15 |
| SF-539 | 0.79 |
| SNB-19 | 14.31 |
| SNB-75 | 38.72 |
| U251 | 4.91 |
| Melanoma | |
| LOX IMVI | -39.16 |
| MALME-3M | 42.61 |
| M14 | 12.97 |
| MDA-MB-435 | 15.87 |
| SK-MEL-2 | -15.75 |
| SK-MEL-28 | 17.36 |
| SK-MEL-5 | 41.40 |
| UACC-257 | 31.87 |
| UACC-62 | 13.84 |
| Ovarian Cancer | |
| IGROV1 | 4.15 |
| OVCAR-3 | 3.41 |
| OVCAR-4 | 24.93 |
| OVCAR-5 | 58.73 |
| OVCAR-8 | -4.55 |
| NCI/ADR-RES | 18.40 |
| SK-OV-3 | 86.56 |
| Renal Cancer | |
| 786-0 | 19.12 |
| A498 | 79.94 |
| ACHN | 15.93 |
| CAKI-1 | 37.43 |
| RXF 393 | 10.65 |
| SN12C | 10.03 |
| TK-10 | 89.23 |
| UO-31 | 17.73 |
| Prostate Cancer | |
| PC-3 | 9.39 |
| DU-145 | 14.42 |
| Breast Cancer | |
| MCF7 | 15.88 |
| MDA-MB-231/ATCC | 29.12 |
| HS 578T | 27.74 |
| BT-549 | 0.75 |
| T-47D | 12.61 |
| MDA-MB-468 | 24.68 |
| Mean | 23.43 |
| Delta | 62.59 |
| Range | 137.79 |

14v

**Table-15** One dose results of 14w



| Developmental Therapeutics Program<br>One Dose Mean Graph | **NSC:** D-797221 / 1 | **Conc:** 1.00E-5 Molar | **Test Date:** Apr 10, 2017 |
|---|---|---|---|
| | **Experiment ID:** 1704OS85 | | **Report Date:** Apr 26, 2017 |

| **Panel/Cell Line** | **Growth Percent** |
|---|---|
| Leukemia | |
| CCRF-CEM | 3.69 |
| HL-60(TB) | -8.07 |
| K-562 | 37.70 |
| MOLT-4 | 0.14 |
| RPMI-8226 | 4.11 |
| SR | -4.72 |
| Non-Small Cell Lung Cancer | |
| A549/ATCC | 69.47 |
| EKVX | 55.93 |
| HOP-62 | 27.61 |
| HOP-92 | 40.25 |
| NCI-H226 | 62.51 |
| NCI-H23 | 62.22 |
| NCI-H322M | 92.13 |
| NCI-H460 | 25.33 |
| NCI-H522 | 23.58 |
| Colon Cancer | |
| COLO 205 | 78.25 |
| HCC-2998 | 45.89 |
| HCT-116 | 23.05 |
| HCT-15 | 23.59 |
| HT29 | 36.74 |
| KM12 | 38.35 |
| SW-620 | 53.74 |
| CNS Cancer | |
| SF-268 | 45.92 |
| SF-295 | 61.45 |
| SF-539 | 45.34 |
| SNB-19 | 46.62 |
| SNB-75 | 43.22 |
| U251 | 25.62 |
| Melanoma | |
| LOX IMVI | -10.69 |
| MALME-3M | 58.94 |
| M14 | 40.07 |
| MDA-MB-435 | 25.36 |
| SK-MEL-2 | -0.82 |
| SK-MEL-28 | 51.78 |
| SK-MEL-5 | 44.95 |
| UACC-257 | 39.42 |
| UACC-62 | 23.49 |
| Ovarian Cancer | |
| IGROV1 | 24.59 |
| OVCAR-3 | 21.49 |
| OVCAR-4 | 30.30 |
| OVCAR-5 | 76.33 |
| OVCAR-8 | 26.07 |
| NCI/ADR-RES | 32.71 |
| SK-OV-3 | 80.80 |
| Renal Cancer | |
| 786-0 | 40.57 |
| A498 | 49.77 |
| ACHN | 33.76 |
| CAKI-1 | 52.06 |
| RXF 393 | 32.03 |
| SN12C | 42.49 |
| TK-10 | 84.04 |
| UO-31 | 46.40 |
| Prostate Cancer | |
| PC-3 | 33.31 |
| DU-145 | 37.20 |
| Breast Cancer | |
| MCF7 | 17.03 |
| MDA-MB-231/ATCC | 59.34 |
| HS 578T | 37.39 |
| BT-549 | 8.93 |
| T-47D | 23.69 |
| MDA-MB-468 | 35.98 |
| Mean | 37.64 |
| Delta | 48.33 |
| Range | 102.82 |

14w

**Table-16** One dose results of 14x

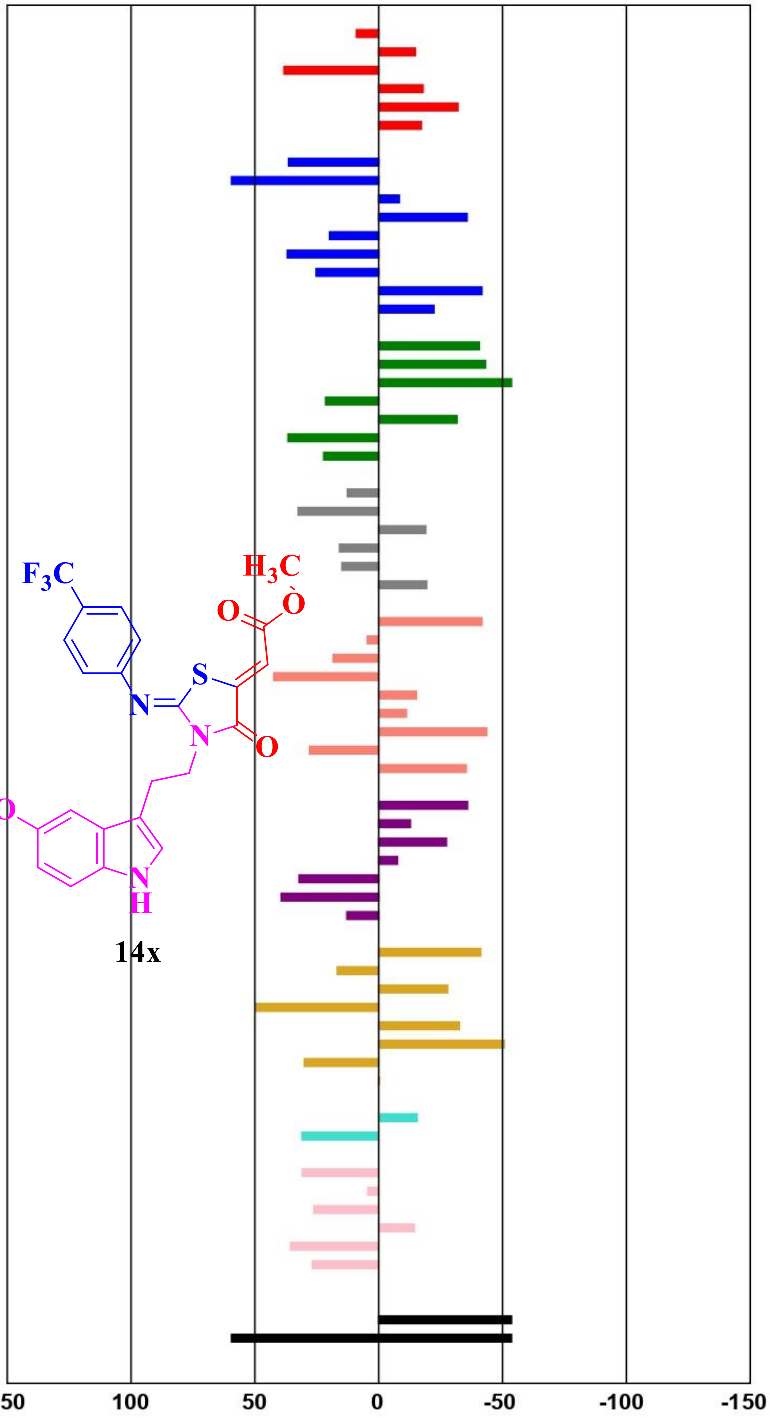
## Developmental Therapeutics Program

## One Dose Mean Graph

**NSC:** D-797220 / 1
**Conc:** 1.00E-5 Molar
**Test Date:** Apr 10, 2017
**Experiment ID:** 1704OS85
**Report Date:** Apr 26, 2017

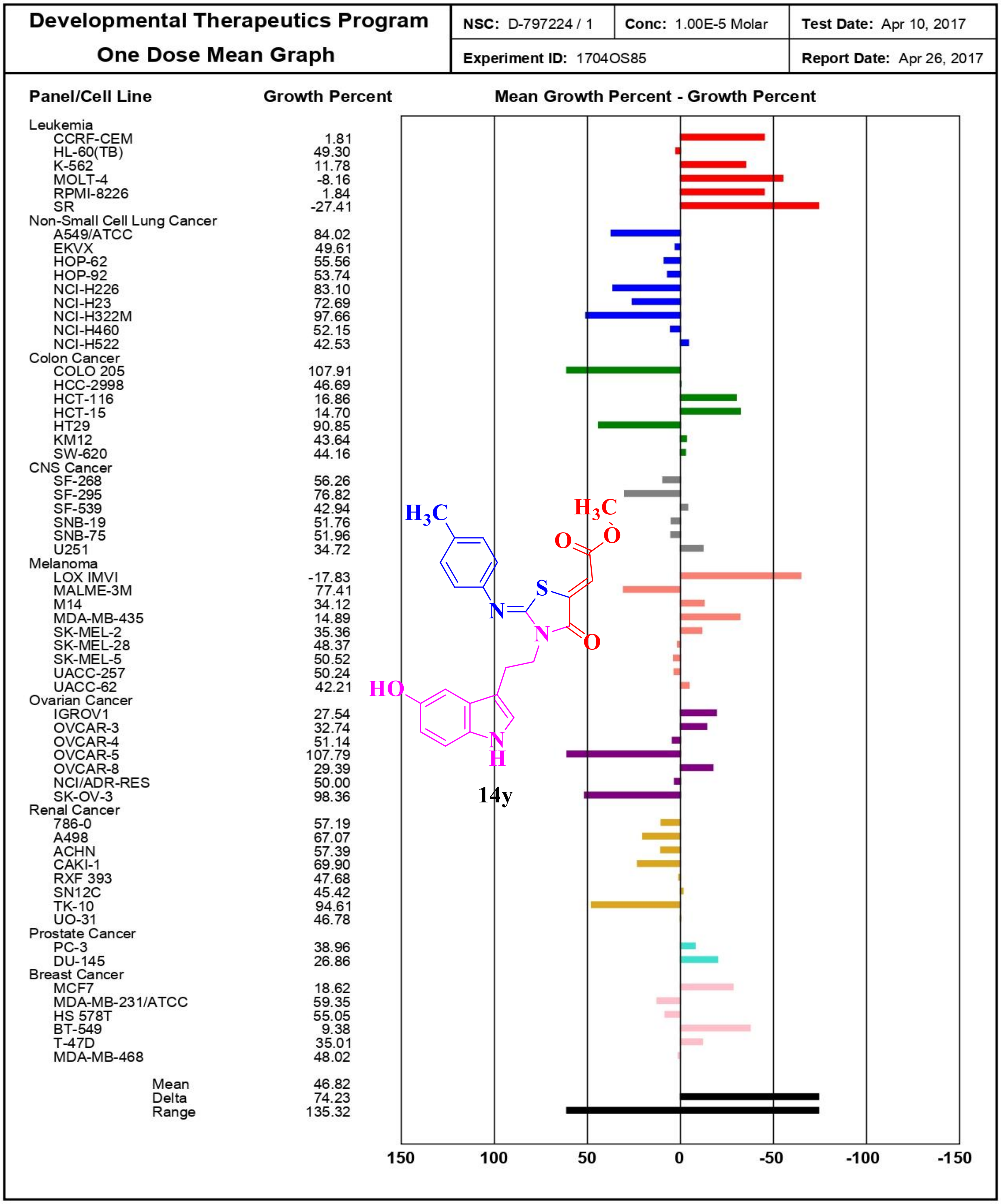
| Panel/Cell Line | Growth Percent |
|---|---|
| Leukemia | |
| CCRF-CEM | -9.32 |
| HL-60(TB) | -33.12 |
| K-562 | 19.92 |
| MOLT-4 | -36.20 |
| RPMI-8226 | -50.29 |
| SR | -35.50 |
| Non-Small Cell Lung Cancer | |
| A549/ATCC | 18.09 |
| EKVX | 41.12 |
| HOP-62 | -26.61 |
| HOP-92 | -53.98 |
| NCI-H226 | 1.54 |
| NCI-H23 | 18.60 |
| NCI-H322M | 6.96 |
| NCI-H460 | -59.96 |
| NCI-H522 | -40.60 |
| Colon Cancer | |
| COLO 205 | -58.93 |
| HCC-2998 | -61.40 |
| HCT-116 | -71.91 |
| HCT-15 | 3.12 |
| HT29 | -49.91 |
| KM12 | 18.27 |
| SW-620 | 3.90 |
| CNS Cancer | |
| SF-268 | -5.68 |
| SF-295 | 14.19 |
| SF-539 | -37.29 |
| SNB-19 | -2.49 |
| SNB-75 | -3.46 |
| U251 | -37.66 |
| Melanoma | |
| LOX IMVI | -59.97 |
| MALME-3M | -13.75 |
| M14 | 0.07 |
| MDA-MB-435 | 24.02 |
| SK-MEL-2 | -33.49 |
| SK-MEL-28 | -29.48 |
| SK-MEL-5 | -61.91 |
| UACC-257 | 9.58 |
| UACC-62 | -53.63 |
| Ovarian Cancer | |
| IGROV1 | -54.15 |
| OVCAR-3 | -31.10 |
| OVCAR-4 | -45.63 |
| OVCAR-5 | -25.88 |
| OVCAR-8 | 13.85 |
| NCI/ADR-RES | 21.02 |
| SK-OV-3 | -5.47 |
| Renal Cancer | |
| 786-0 | -59.48 |
| A498 | -1.57 |
| ACHN | -46.10 |
| CAKI-1 | 31.38 |
| RXF 393 | -50.86 |
| SN12C | -68.96 |
| TK-10 | 11.67 |
| UO-31 | -18.46 |
| Prostate Cancer | |
| PC-3 | -33.76 |
| DU-145 | 12.67 |
| Breast Cancer | |
| MCF7 | 12.51 |
| MDA-MB-231/ATCC | -13.92 |
| HS 578T | 7.87 |
| BT-549 | -32.74 |
| T-47D | 17.26 |
| MDA-MB-468 | 8.44 |
| Mean | -18.31 |
| Delta | 53.60 |
| Range | 113.03 |

**Mean Growth Percent - Growth Percent**



14x

**Table-17** One dose results of 14y



**Developmental Therapeutics Program**

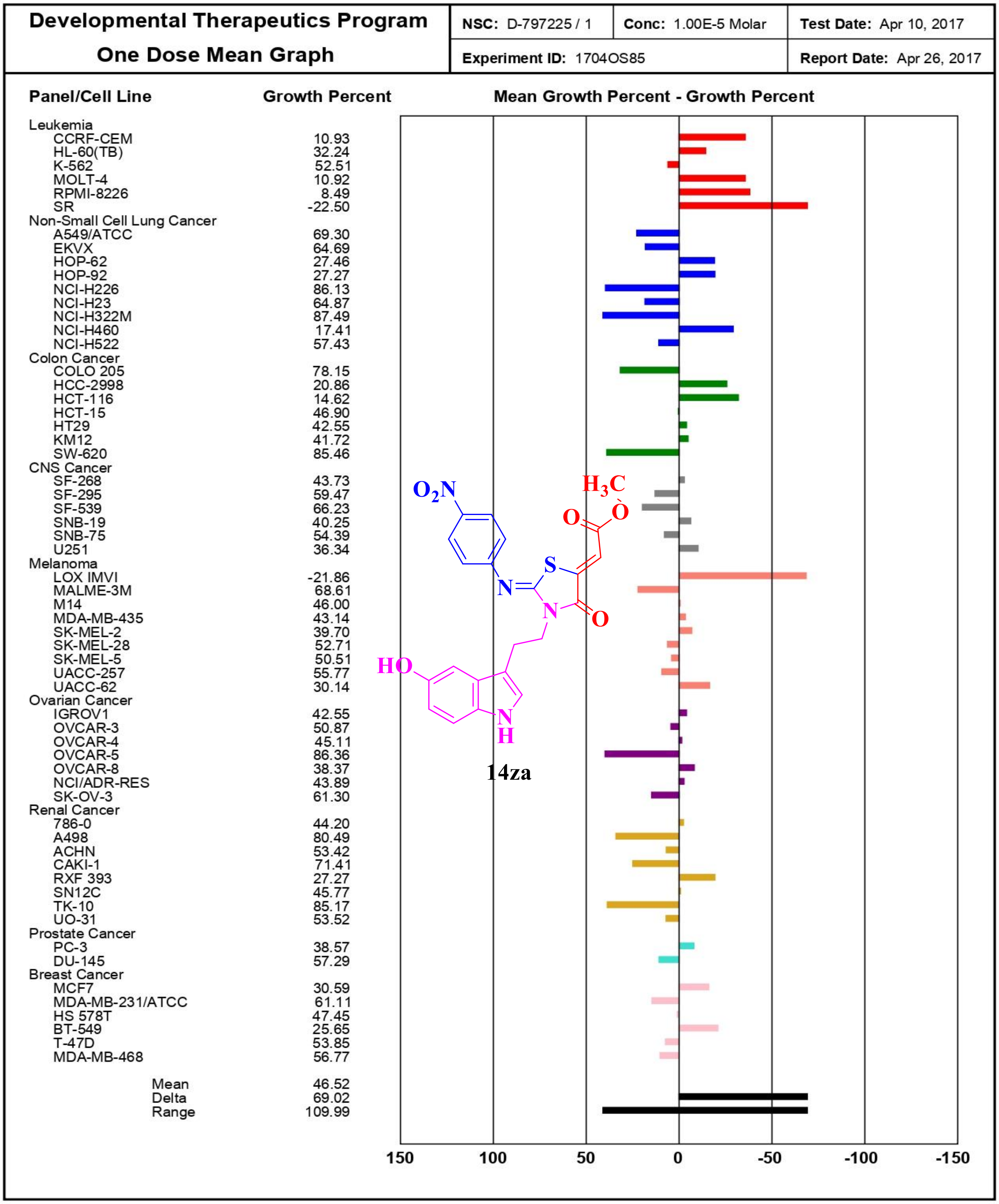
**One Dose Mean Graph**

**NSC:** D-797224 / 1 **Conc:** 1.00E-5 Molar **Test Date:** Apr 10, 2017

**Experiment ID:** 1704OS85 **Report Date:** Apr 26, 2017

| Panel/Cell Line | Growth Percent |
|---|---|
| Leukemia | |
| CCRF-CEM | 1.81 |
| HL-60(TB) | 49.30 |
| K-562 | 11.78 |
| MOLT-4 | -8.16 |
| RPMI-8226 | 1.84 |
| SR | -27.41 |
| Non-Small Cell Lung Cancer | |
| A549/ATCC | 84.02 |
| EKVX | 49.61 |
| HOP-62 | 55.56 |
| HOP-92 | 53.74 |
| NCI-H226 | 83.10 |
| NCI-H23 | 72.69 |
| NCI-H322M | 97.66 |
| NCI-H460 | 52.15 |
| NCI-H522 | 42.53 |
| Colon Cancer | |
| COLO 205 | 107.91 |
| HCC-2998 | 46.69 |
| HCT-116 | 16.86 |
| HCT-15 | 14.70 |
| HT29 | 90.85 |
| KM12 | 43.64 |
| SW-620 | 44.16 |
| CNS Cancer | |
| SF-268 | 56.26 |
| SF-295 | 76.82 |
| SF-539 | 42.94 |
| SNB-19 | 51.76 |
| SNB-75 | 51.96 |
| U251 | 34.72 |
| Melanoma | |
| LOX IMVI | -17.83 |
| MALME-3M | 77.41 |
| M14 | 34.12 |
| MDA-MB-435 | 14.89 |
| SK-MEL-2 | 35.36 |
| SK-MEL-28 | 48.37 |
| SK-MEL-5 | 50.52 |
| UACC-257 | 50.24 |
| UACC-62 | 42.21 |
| Ovarian Cancer | |
| IGROV1 | 27.54 |
| OVCAR-3 | 32.74 |
| OVCAR-4 | 51.14 |
| OVCAR-5 | 107.79 |
| OVCAR-8 | 29.39 |
| NCI/ADR-RES | 50.00 |
| SK-OV-3 | 98.36 |
| Renal Cancer | |
| 786-0 | 57.19 |
| A498 | 67.07 |
| ACHN | 57.39 |
| CAKI-1 | 69.90 |
| RXF 393 | 47.68 |
| SN12C | 45.42 |
| TK-10 | 94.61 |
| UO-31 | 46.78 |
| Prostate Cancer | |
| PC-3 | 38.96 |
| DU-145 | 26.86 |
| Breast Cancer | |
| MCF7 | 18.62 |
| MDA-MB-231/ATCC | 59.35 |
| HS 578T | 55.05 |
| BT-549 | 9.38 |
| T-47D | 35.01 |
| MDA-MB-468 | 48.02 |
| Mean | 46.82 |
| Delta | 74.23 |
| Range | 135.32 |

**Table-18** One dose results of 14za



**Developmental Therapeutics Program**

**One Dose Mean Graph**

**NSC:** D-797225 / 1

**Conc:** 1.00E-5 Molar

**Test Date:** Apr 10, 2017

**Experiment ID:** 1704OS85

**Report Date:** Apr 26, 2017

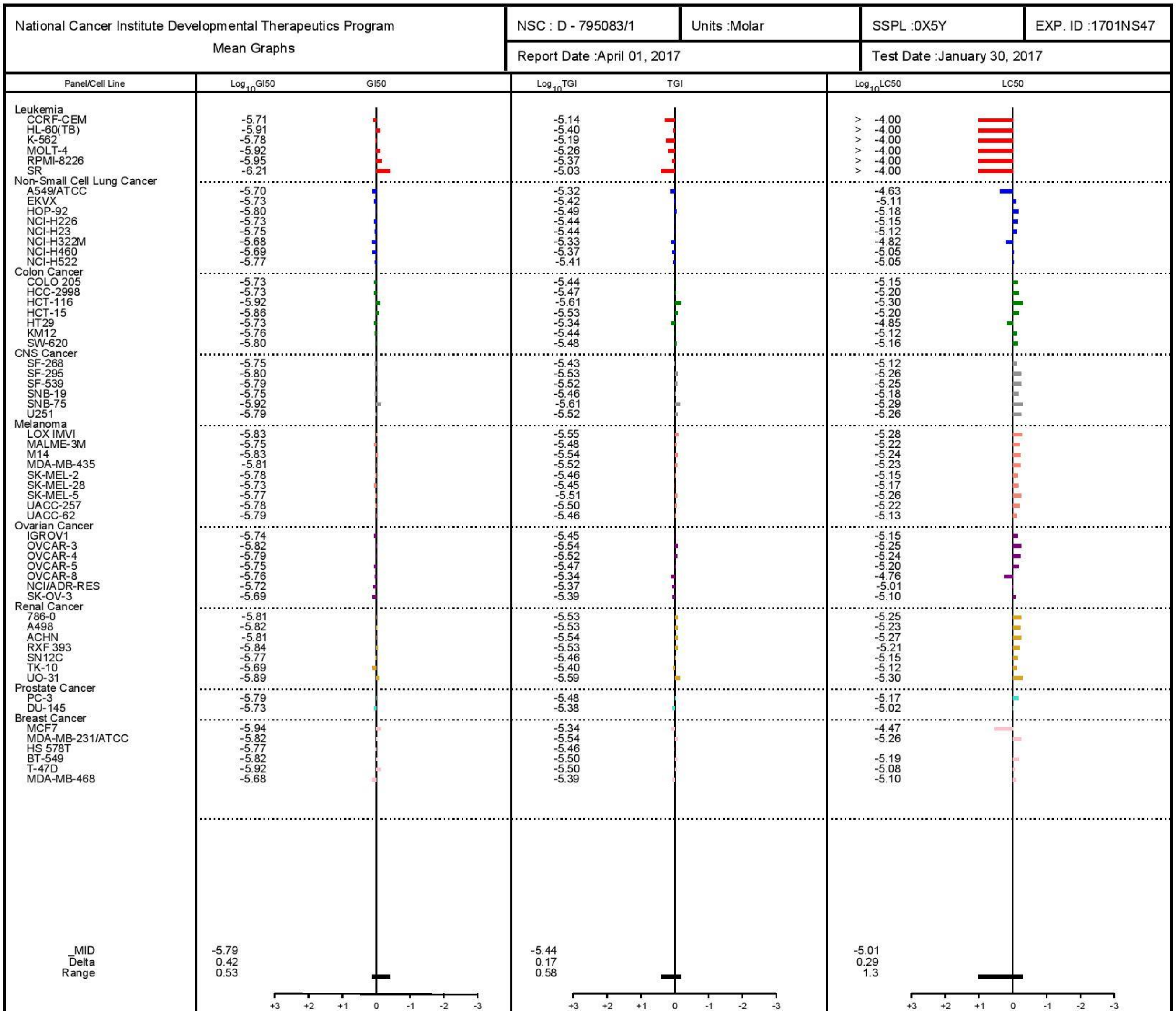
| Panel/Cell Line | Growth Percent |
|---|---|
| Leukemia | |
| CCRF-CEM | 10.93 |
| HL-60(TB) | 32.24 |
| K-562 | 52.51 |
| MOLT-4 | 10.92 |
| RPMI-8226 | 8.49 |
| SR | -22.50 |
| Non-Small Cell Lung Cancer | |
| A549/ATCC | 69.30 |
| EKVX | 64.69 |
| HOP-62 | 27.46 |
| HOP-92 | 27.27 |
| NCI-H226 | 86.13 |
| NCI-H23 | 64.87 |
| NCI-H322M | 87.49 |
| NCI-H460 | 17.41 |
| NCI-H522 | 57.43 |
| Colon Cancer | |
| COLO 205 | 78.15 |
| HCC-2998 | 20.86 |
| HCT-116 | 14.62 |
| HCT-15 | 46.90 |
| HT29 | 42.55 |
| KM12 | 41.72 |
| SW-620 | 85.46 |
| CNS Cancer | |
| SF-268 | 43.73 |
| SF-295 | 59.47 |
| SF-539 | 66.23 |
| SNB-19 | 40.25 |
| SNB-75 | 54.39 |
| U251 | 36.34 |
| Melanoma | |
| LOX IMVI | -21.86 |
| MALME-3M | 68.61 |
| M14 | 46.00 |
| MDA-MB-435 | 43.14 |
| SK-MEL-2 | 39.70 |
| SK-MEL-28 | 52.71 |
| SK-MEL-5 | 50.51 |
| UACC-257 | 55.77 |
| UACC-62 | 30.14 |
| Ovarian Cancer | |
| IGROV1 | 42.55 |
| OVCAR-3 | 50.87 |
| OVCAR-4 | 45.11 |
| OVCAR-5 | 86.36 |
| OVCAR-8 | 38.37 |
| NCI/ADR-RES | 43.89 |
| SK-OV-3 | 61.30 |
| Renal Cancer | |
| 786-0 | 44.20 |
| A498 | 80.49 |
| ACHN | 53.42 |
| CAKI-1 | 71.41 |
| RXF 393 | 27.27 |
| SN12C | 45.77 |
| TK-10 | 85.17 |
| UO-31 | 53.52 |
| Prostate Cancer | |
| PC-3 | 38.57 |
| DU-145 | 57.29 |
| Breast Cancer | |
| MCF7 | 30.59 |
| MDA-MB-231/ATCC | 61.11 |
| HS 578T | 47.45 |
| BT-549 | 25.65 |
| T-47D | 53.85 |
| MDA-MB-468 | 56.77 |
| Mean | 46.52 |
| Delta | 69.02 |
| Range | 109.99 |

**Mean Growth Percent - Growth Percent**

**14za**

# Five dose results of 14p

## Table-19

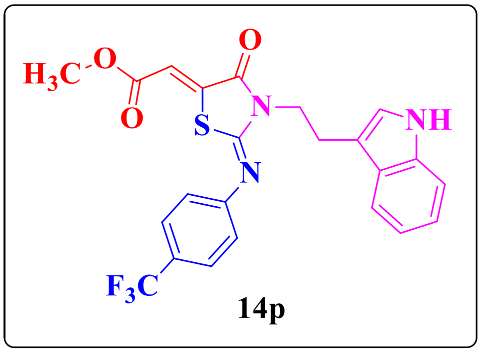


National Cancer Institute Developmental Therapeutics Program
Mean Graphs

NSC : D - 795083/1 | Units :Molar | SSPL :0X5Y | EXP. ID :1701NS47

Report Date :April 01, 2017 | Test Date :January 30, 2017

| Panel/Cell Line | $Log_{10}$GI50 | $Log_{10}$TGI | $Log_{10}$LC50 |
|---|---|---|---|
| Leukemia | | | |
| CCRF-CEM | -5.71 | -5.14 | > -4.00 |
| HL-60(TB) | -5.91 | -5.40 | > -4.00 |
| K-562 | -5.78 | -5.19 | > -4.00 |
| MOLT-4 | -5.92 | -5.26 | > -4.00 |
| RPMI-8226 | -5.95 | -5.37 | > -4.00 |
| SR | -6.21 | -5.03 | > -4.00 |
| Non-Small Cell Lung Cancer | | | |
| A549/ATCC | -5.70 | -5.32 | -4.63 |
| EKVX | -5.73 | -5.42 | -5.11 |
| HOP-92 | -5.80 | -5.49 | -5.18 |
| NCI-H226 | -5.73 | -5.44 | -5.15 |
| NCI-H23 | -5.75 | -5.44 | -5.12 |
| NCI-H322M | -5.68 | -5.33 | -4.82 |
| NCI-H460 | -5.69 | -5.37 | -5.05 |
| NCI-H522 | -5.77 | -5.41 | -5.05 |
| Colon Cancer | | | |
| COLO 205 | -5.73 | -5.44 | -5.15 |
| HCC-2998 | -5.73 | -5.47 | -5.20 |
| HCT-116 | -5.92 | -5.61 | -5.30 |
| HCT-15 | -5.86 | -5.53 | -5.20 |
| HT29 | -5.73 | -5.34 | -4.85 |
| KM12 | -5.76 | -5.44 | -5.12 |
| SW-620 | -5.80 | -5.48 | -5.16 |
| CNS Cancer | | | |
| SF-268 | -5.75 | -5.43 | -5.12 |
| SF-295 | -5.80 | -5.53 | -5.26 |
| SF-539 | -5.79 | -5.52 | -5.25 |
| SNB-19 | -5.75 | -5.46 | -5.18 |
| SNB-75 | -5.92 | -5.61 | -5.29 |
| U251 | -5.79 | -5.52 | -5.26 |
| Melanoma | | | |
| LOX IMVI | -5.83 | -5.55 | -5.28 |
| MALME-3M | -5.75 | -5.48 | -5.22 |
| M14 | -5.83 | -5.54 | -5.24 |
| MDA-MB-435 | -5.81 | -5.52 | -5.23 |
| SK-MEL-2 | -5.78 | -5.46 | -5.15 |
| SK-MEL-28 | -5.73 | -5.45 | -5.17 |
| SK-MEL-5 | -5.77 | -5.51 | -5.26 |
| UACC-257 | -5.78 | -5.50 | -5.22 |
| UACC-62 | -5.79 | -5.46 | -5.13 |
| Ovarian Cancer | | | |
| IGROV1 | -5.74 | -5.45 | -5.15 |
| OVCAR-3 | -5.82 | -5.54 | -5.25 |
| OVCAR-4 | -5.79 | -5.52 | -5.24 |
| OVCAR-5 | -5.75 | -5.47 | -5.20 |
| OVCAR-8 | -5.76 | -5.34 | -4.76 |
| NCI/ADR-RES | -5.72 | -5.37 | -5.01 |
| SK-OV-3 | -5.69 | -5.39 | -5.10 |
| Renal Cancer | | | |
| 786-0 | -5.81 | -5.53 | -5.25 |
| A498 | -5.82 | -5.53 | -5.23 |
| ACHN | -5.81 | -5.54 | -5.27 |
| RXF 393 | -5.84 | -5.53 | -5.21 |
| SN12C | -5.77 | -5.46 | -5.15 |
| TK-10 | -5.69 | -5.40 | -5.12 |
| UO-31 | -5.89 | -5.59 | -5.30 |
| Prostate Cancer | | | |
| PC-3 | -5.79 | -5.48 | -5.17 |
| DU-145 | -5.73 | -5.38 | -5.02 |
| Breast Cancer | | | |
| MCF7 | -5.94 | -5.34 | -4.47 |
| MDA-MB-231/ATCC | -5.82 | -5.54 | -5.26 |
| HS 578T | -5.77 | -5.46 | |
| BT-549 | -5.82 | -5.50 | -5.19 |
| T-47D | -5.92 | -5.50 | -5.08 |
| MDA-MB-468 | -5.68 | -5.39 | -5.10 |
| MID | -5.79 | -5.44 | -5.01 |
| Delta | 0.42 | 0.17 | 0.29 |
| Range | 0.53 | 0.58 | 1.3 |



**14p**

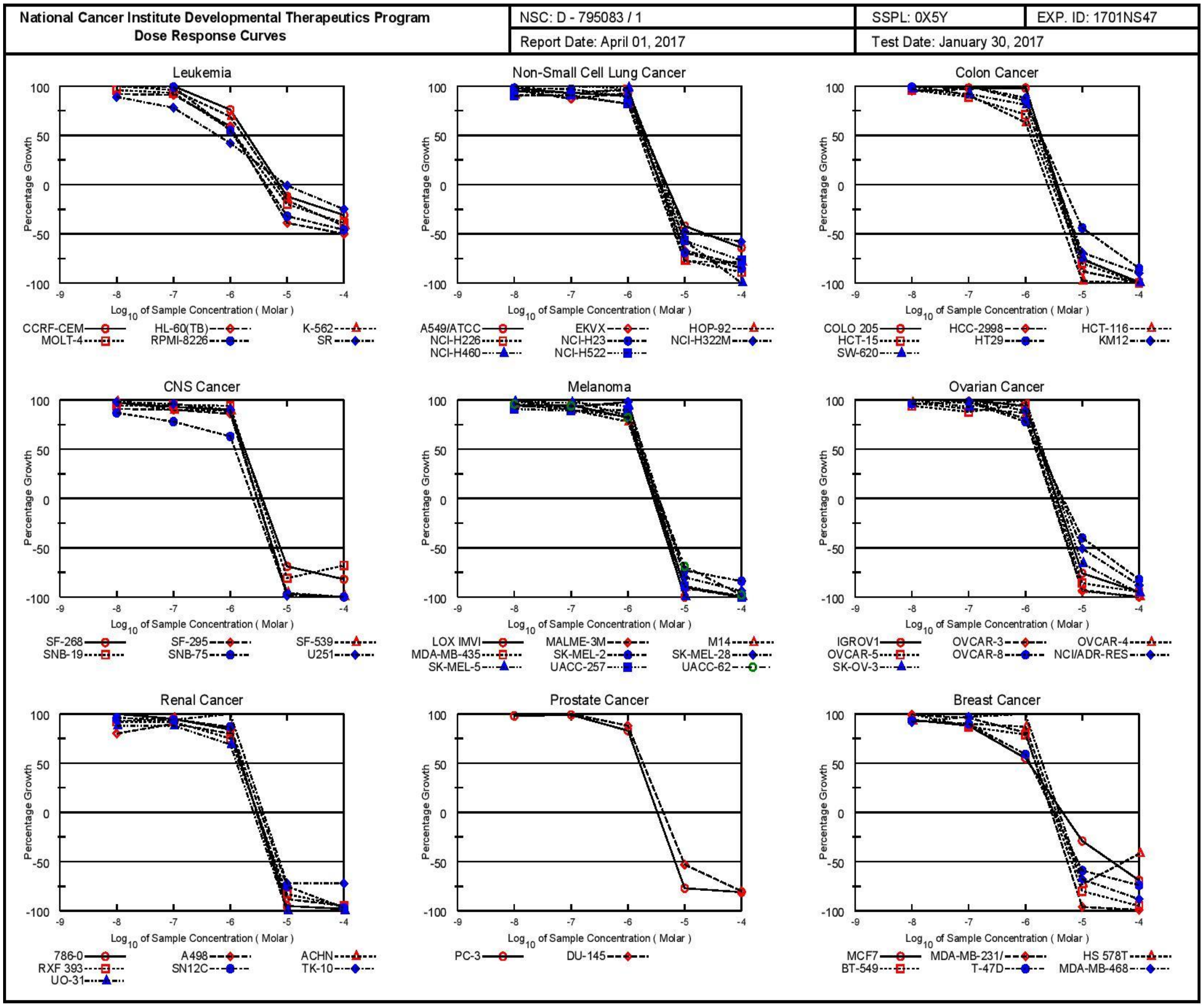
## Table-20

### National Cancer Institute Developmental Therapeutics Program
### In-Vitro Testing Results

| | | | |
|---|---|---|---|
| NSC : D - 795083 / 1 | Experiment ID : 1701NS47 | Test Type : 08 | Units : Molar |
| Report Date : April 01, 2017 | Test Date : January 30, 2017 | QNS : | MC : |
| COMI : TRY-4TF-DM | Stain Reagent : SRB Dual-Pass Related | SSPL : 0X5Y | |

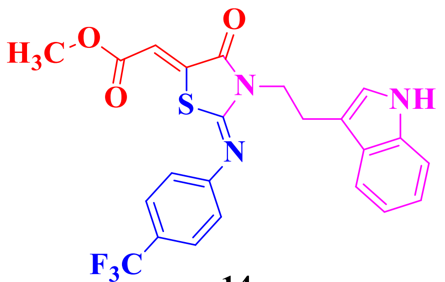
| | | | Log10 Concentration | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Time | | Mean Optical Densities | | | | | Percent Growth | | | | | | | | | |
| Panel/Cell Line | Zero | Ctrl | -8.0 | -7.0 | -6.0 | -5.0 | -4.0 | -8.0 | -7.0 | -6.0 | -5.0 | -4.0 | GI50 | TGI | LC50 |
| Leukemia | | | | | | | | | | | | | | | |
| CCRF-CEM | 0.623 | 2.911 | 3.005 | 2.944 | 2.352 | 0.546 | 0.430 | 104 | 101 | 76 | -12 | -31 | 1.95E-6 | 7.22E-6 | > 1.00E-4 |
| HL-60(TB) | 0.842 | 2.959 | 2.795 | 2.774 | 2.088 | 0.512 | 0.423 | 92 | 91 | 59 | -39 | -50 | 1.23E-6 | 3.98E-6 | > 1.00E-4 |
| K-562 | 0.226 | 2.337 | 2.336 | 2.257 | 1.679 | 0.189 | 0.132 | 100 | 96 | 69 | -16 | -42 | 1.66E-6 | 6.42E-6 | > 1.00E-4 |
| MOLT-4 | 0.803 | 3.153 | 3.057 | 2.977 | 2.130 | 0.645 | 0.492 | 96 | 93 | 56 | -20 | -39 | 1.22E-6 | 5.51E-6 | > 1.00E-4 |
| RPMI-8226 | 0.767 | 2.677 | 2.784 | 2.666 | 1.813 | 0.525 | 0.413 | 106 | 99 | 55 | -32 | -46 | 1.13E-6 | 4.30E-6 | > 1.00E-4 |
| SR | 0.367 | 1.251 | 1.154 | 1.055 | 0.743 | 0.362 | 0.274 | 89 | 78 | 42 | -1 | -25 | 6.13E-7 | 9.31E-6 | > 1.00E-4 |
| Non-Small Cell Lung Cancer | | | | | | | | | | | | | | | |
| A549/ATCC | 0.524 | 2.614 | 2.578 | 2.460 | 2.406 | 0.304 | 0.191 | 98 | 93 | 90 | -42 | -64 | 2.01E-6 | 4.80E-6 | 2.33E-5 |
| EKVX | 0.747 | 2.333 | 2.276 | 2.132 | 2.226 | 0.244 | 0.138 | 96 | 87 | 93 | -67 | -82 | 1.86E-6 | 3.81E-6 | 7.80E-6 |
| HOP-92 | 1.233 | 1.978 | 1.911 | 1.906 | 1.843 | 0.267 | 0.261 | 91 | 90 | 82 | -78 | -79 | 1.58E-6 | 3.24E-6 | 6.65E-6 |
| NCI-H226 | 1.055 | 2.270 | 2.215 | 2.185 | 2.235 | 0.245 | 0.119 | 95 | 93 | 97 | -77 | -89 | 1.87E-6 | 3.62E-6 | 7.01E-6 |
| NCI-H23 | 0.661 | 1.933 | 1.904 | 1.900 | 1.792 | 0.202 | 0.098 | 98 | 97 | 89 | -69 | -85 | 1.76E-6 | 3.64E-6 | 7.54E-6 |
| NCI-H322M | 1.063 | 2.460 | 2.393 | 2.361 | 2.400 | 0.552 | 0.443 | 95 | 93 | 96 | -48 | -58 | 2.08E-6 | 4.63E-6 | 1.53E-5 |
| NCI-H460 | 0.337 | 3.163 | 3.202 | 3.163 | 3.116 | 0.144 | -0.007 | 101 | 100 | 98 | -57 | -100 | 2.04E-6 | 4.28E-6 | 8.98E-6 |
| NCI-H522 | 1.256 | 2.871 | 2.708 | 2.732 | 2.585 | 0.544 | 0.286 | 90 | 91 | 82 | -57 | -77 | 1.71E-6 | 3.91E-6 | 8.95E-6 |
| Colon Cancer | | | | | | | | | | | | | | | |
| COLO 205 | 0.670 | 2.492 | 2.506 | 2.451 | 2.448 | 0.161 | 0.004 | 101 | 98 | 98 | -76 | -99 | 1.88E-6 | 3.65E-6 | 7.08E-6 |
| HCC-2998 | 0.631 | 2.298 | 2.208 | 2.251 | 2.314 | 0.077 | -0.012 | 95 | 97 | 101 | -88 | -100 | 1.86E-6 | 3.43E-6 | 6.31E-6 |
| HCT-116 | 0.243 | 2.047 | 2.135 | 1.887 | 1.387 | 0.005 | -0.040 | 105 | 91 | 63 | -98 | -100 | 1.21E-6 | 2.47E-6 | 5.03E-6 |
| HCT-15 | 0.225 | 1.682 | 1.619 | 1.521 | 1.260 | 0.045 | -0.041 | 96 | 89 | 71 | -80 | -100 | 1.38E-6 | 2.95E-6 | 6.33E-6 |
| HT29 | 0.362 | 2.348 | 2.325 | 2.385 | 2.041 | 0.204 | 0.055 | 99 | 102 | 85 | -44 | -85 | 1.86E-6 | 4.56E-6 | 1.42E-5 |
| KM12 | 0.508 | 2.732 | 2.821 | 2.836 | 2.462 | 0.156 | 0.052 | 104 | 105 | 88 | -69 | -90 | 1.74E-6 | 3.62E-6 | 7.54E-6 |
| SW-620 | 0.331 | 2.193 | 2.132 | 2.042 | 1.833 | 0.082 | -0.009 | 97 | 92 | 81 | -75 | -100 | 1.57E-6 | 3.29E-6 | 6.89E-6 |
| CNS Cancer | | | | | | | | | | | | | | | |
| SF-268 | 0.709 | 2.323 | 2.268 | 2.214 | 2.159 | 0.219 | 0.127 | 97 | 93 | 90 | -69 | -82 | 1.78E-6 | 3.67E-6 | 7.58E-6 |
| SF-295 | 0.631 | 2.320 | 2.174 | 2.157 | 2.082 | 0.020 | -0.042 | 91 | 90 | 86 | -97 | -100 | 1.57E-6 | 2.95E-6 | 5.54E-6 |
| SF-539 | 0.873 | 2.531 | 2.493 | 2.367 | 2.341 | 0.036 | -0.047 | 98 | 90 | 89 | -96 | -100 | 1.62E-6 | 3.02E-6 | 5.64E-6 |
| SNB-19 | 0.758 | 2.556 | 2.441 | 2.459 | 2.440 | 0.147 | 0.241 | 94 | 95 | 94 | -81 | -68 | 1.78E-6 | 3.45E-6 | 6.67E-6 |
| SNB-75 | 0.792 | 1.730 | 1.608 | 1.522 | 1.385 | 0.024 | -0.044 | 87 | 78 | 63 | -97 | -100 | 1.21E-6 | 2.48E-6 | 5.09E-6 |
| U251 | 0.534 | 2.385 | 2.341 | 2.316 | 2.196 | 0.007 | -0.051 | 98 | 96 | 90 | -99 | -100 | 1.63E-6 | 2.99E-6 | 5.51E-6 |
| Melanoma | | | | | | | | | | | | | | | |
| LOX IMVI | 0.364 | 2.691 | 2.574 | 2.580 | 2.263 | -0.005 | -0.061 | 95 | 95 | 82 | -100 | -100 | 1.49E-6 | 2.81E-6 | 5.30E-6 |
| MALME-3M | 0.701 | 1.167 | 1.141 | 1.136 | 1.158 | 0.063 | -0.018 | 94 | 93 | 98 | -91 | -100 | 1.79E-6 | 3.30E-6 | 6.06E-6 |
| M14 | 0.514 | 1.896 | 1.820 | 1.765 | 1.590 | 0.050 | 0.005 | 95 | 90 | 78 | -90 | -99 | 1.46E-6 | 2.90E-6 | 5.76E-6 |
| MDA-MB-435 | 0.454 | 2.594 | 2.599 | 2.374 | 2.239 | 0.047 | -0.022 | 100 | 90 | 83 | -90 | -100 | 1.56E-6 | 3.03E-6 | 5.89E-6 |
| SK-MEL-2 | 1.090 | 2.538 | 2.545 | 2.532 | 2.306 | 0.296 | 0.177 | 101 | 100 | 84 | -73 | -84 | 1.65E-6 | 3.43E-6 | 7.15E-6 |
| SK-MEL-28 | 0.836 | 2.199 | 2.259 | 2.109 | 2.177 | 0.165 | 0.048 | 104 | 93 | 98 | -80 | -94 | 1.87E-6 | 3.55E-6 | 6.77E-6 |
| SK-MEL-5 | 0.816 | 3.202 | 3.151 | 3.127 | 3.063 | 0.004 | -0.058 | 98 | 97 | 94 | -100 | -100 | 1.69E-6 | 3.06E-6 | 5.55E-6 |
| UACC-257 | 1.087 | 2.397 | 2.281 | 2.250 | 2.268 | 0.116 | -0.003 | 91 | 89 | 90 | -89 | -100 | 1.67E-6 | 3.18E-6 | 6.04E-6 |
| UACC-62 | 0.929 | 2.854 | 2.781 | 2.744 | 2.498 | 0.285 | 0.021 | 96 | 94 | 82 | -69 | -98 | 1.62E-6 | 3.47E-6 | 7.44E-6 |
| Ovarian Cancer | | | | | | | | | | | | | | | |
| IGROV1 | 0.481 | 2.059 | 2.138 | 2.095 | 1.969 | 0.118 | 0.024 | 105 | 102 | 94 | -76 | -95 | 1.82E-6 | 3.59E-6 | 7.07E-6 |
| OVCAR-3 | 0.459 | 1.780 | 1.819 | 1.768 | 1.523 | 0.029 | -0.050 | 103 | 99 | 81 | -94 | -100 | 1.50E-6 | 2.90E-6 | 5.61E-6 |
| OVCAR-4 | 0.710 | 1.522 | 1.498 | 1.452 | 1.416 | 0.051 | -0.031 | 97 | 91 | 87 | -93 | -100 | 1.61E-6 | 3.05E-6 | 5.78E-6 |
| OVCAR-5 | 0.789 | 1.905 | 1.835 | 1.772 | 1.863 | 0.112 | 0.036 | 94 | 88 | 96 | -86 | -95 | 1.79E-6 | 3.38E-6 | 6.36E-6 |
| OVCAR-8 | 0.593 | 2.562 | 2.478 | 2.516 | 2.138 | 0.357 | 0.109 | 96 | 98 | 78 | -40 | -82 | 1.74E-6 | 4.60E-6 | 1.75E-5 |
| NCI/ADR-RES | 0.529 | 1.846 | 1.889 | 1.868 | 1.700 | 0.258 | 0.065 | 103 | 102 | 89 | -51 | -88 | 1.89E-6 | 4.31E-6 | 9.80E-6 |
| SK-OV-3 | 1.180 | 2.539 | 2.539 | 2.439 | 2.574 | 0.401 | 0.044 | 100 | 93 | 103 | -66 | -96 | 2.05E-6 | 4.06E-6 | 8.03E-6 |
| Renal Cancer | | | | | | | | | | | | | | | |
| 786-0 | 0.745 | 2.658 | 2.655 | 2.560 | 2.361 | 0.037 | 0.016 | 100 | 95 | 84 | -95 | -98 | 1.56E-6 | 2.95E-6 | 5.61E-6 |
| A498 | 1.461 | 2.200 | 2.050 | 2.128 | 2.050 | 0.169 | 0.079 | 80 | 90 | 80 | -88 | -95 | 1.50E-6 | 2.98E-6 | 5.91E-6 |
| ACHN | 0.385 | 1.655 | 1.653 | 1.591 | 1.475 | -0.009 | -0.057 | 100 | 95 | 86 | -100 | -100 | 1.56E-6 | 2.90E-6 | 5.38E-6 |
| RXF 393 | 0.888 | 1.511 | 1.463 | 1.461 | 1.353 | 0.153 | 0.040 | 92 | 92 | 75 | -83 | -95 | 1.43E-6 | 2.98E-6 | 6.19E-6 |
| SN12C | 0.554 | 2.314 | 2.235 | 2.202 | 2.086 | 0.140 | 0.015 | 96 | 94 | 87 | -75 | -97 | 1.69E-6 | 3.45E-6 | 7.03E-6 |
| TK-10 | 0.939 | 1.892 | 1.828 | 1.832 | 1.945 | 0.265 | 0.261 | 93 | 94 | 106 | -72 | -72 | 2.06E-6 | 3.94E-6 | 7.53E-6 |
| UO-31 | 0.610 | 1.851 | 1.707 | 1.704 | 1.467 | -0.005 | -0.041 | 88 | 88 | 69 | -100 | -100 | 1.30E-6 | 2.56E-6 | 5.06E-6 |
| Prostate Cancer | | | | | | | | | | | | | | | |
| PC-3 | 0.671 | 2.759 | 2.714 | 2.736 | 2.413 | 0.158 | 0.129 | 98 | 99 | 83 | -77 | -81 | 1.62E-6 | 3.32E-6 | 6.83E-6 |
| DU-145 | 0.393 | 1.821 | 1.893 | 1.881 | 1.655 | 0.184 | 0.079 | 105 | 104 | 88 | -53 | -80 | 1.87E-6 | 4.21E-6 | 9.48E-6 |
| Breast Cancer | | | | | | | | | | | | | | | |
| MCF7 | 0.440 | 2.342 | 2.222 | 2.111 | 1.492 | 0.313 | 0.137 | 94 | 88 | 55 | -29 | -69 | 1.16E-6 | 4.53E-6 | 3.37E-5 |
| MDA-MB-231/ATCC | 0.663 | 2.066 | 2.049 | 2.005 | 1.806 | 0.024 | 0.008 | 99 | 96 | 81 | -96 | -99 | 1.50E-6 | 2.87E-6 | 5.49E-6 |
| HS 578T | 1.019 | 2.016 | 1.950 | 1.916 | 1.886 | 0.272 | 0.594 | 93 | 90 | 87 | -73 | -42 | 1.70E-6 | 3.49E-6 | . |
| BT-549 | 1.227 | 2.094 | 2.109 | 1.979 | 1.915 | 0.251 | 0.063 | 102 | 87 | 79 | -80 | -95 | 1.53E-6 | 3.16E-6 | 6.51E-6 |
| T-47D | 0.976 | 2.108 | 2.024 | 1.990 | 1.646 | 0.400 | 0.249 | 93 | 90 | 59 | -59 | -74 | 1.19E-6 | 3.17E-6 | 8.39E-6 |
| MDA-MB-468 | 0.982 | 1.798 | 1.733 | 1.774 | 1.840 | 0.316 | 0.120 | 92 | 97 | 105 | -68 | -88 | 2.08E-6 | 4.05E-6 | 7.89E-6 |

# Table-21



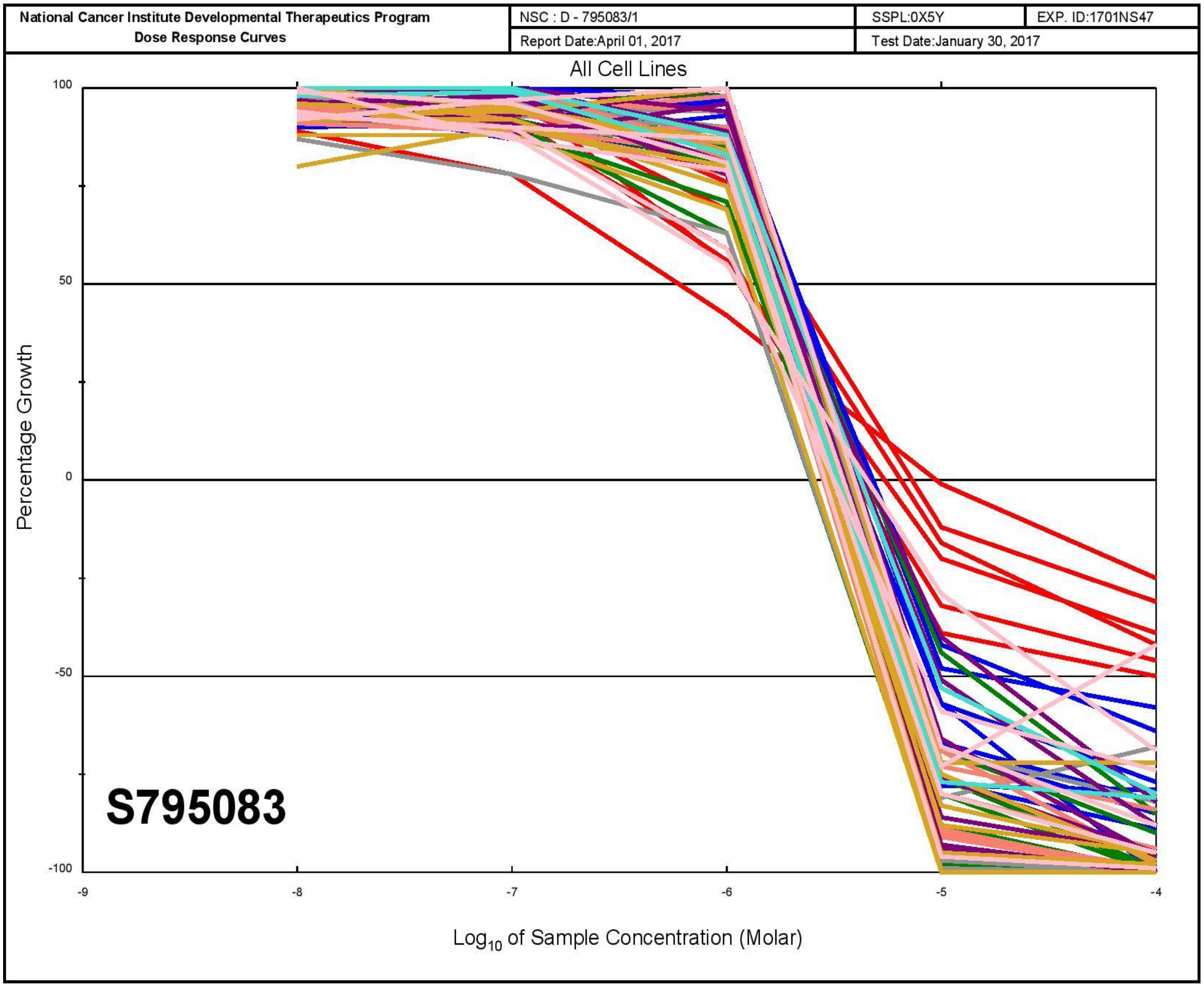
National Cancer Institute Developmental Therapeutics Program
Dose Response Curves

NSC: D - 795083 / 1
SSPL: 0X5Y
EXP. ID: 1701NS47
Report Date: April 01, 2017
Test Date: January 30, 2017

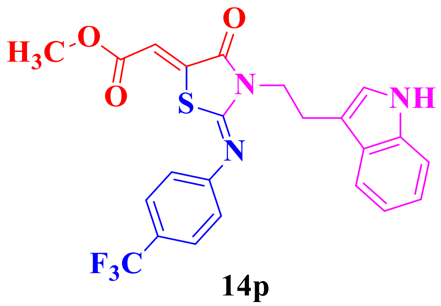


**14p**

## Table-22



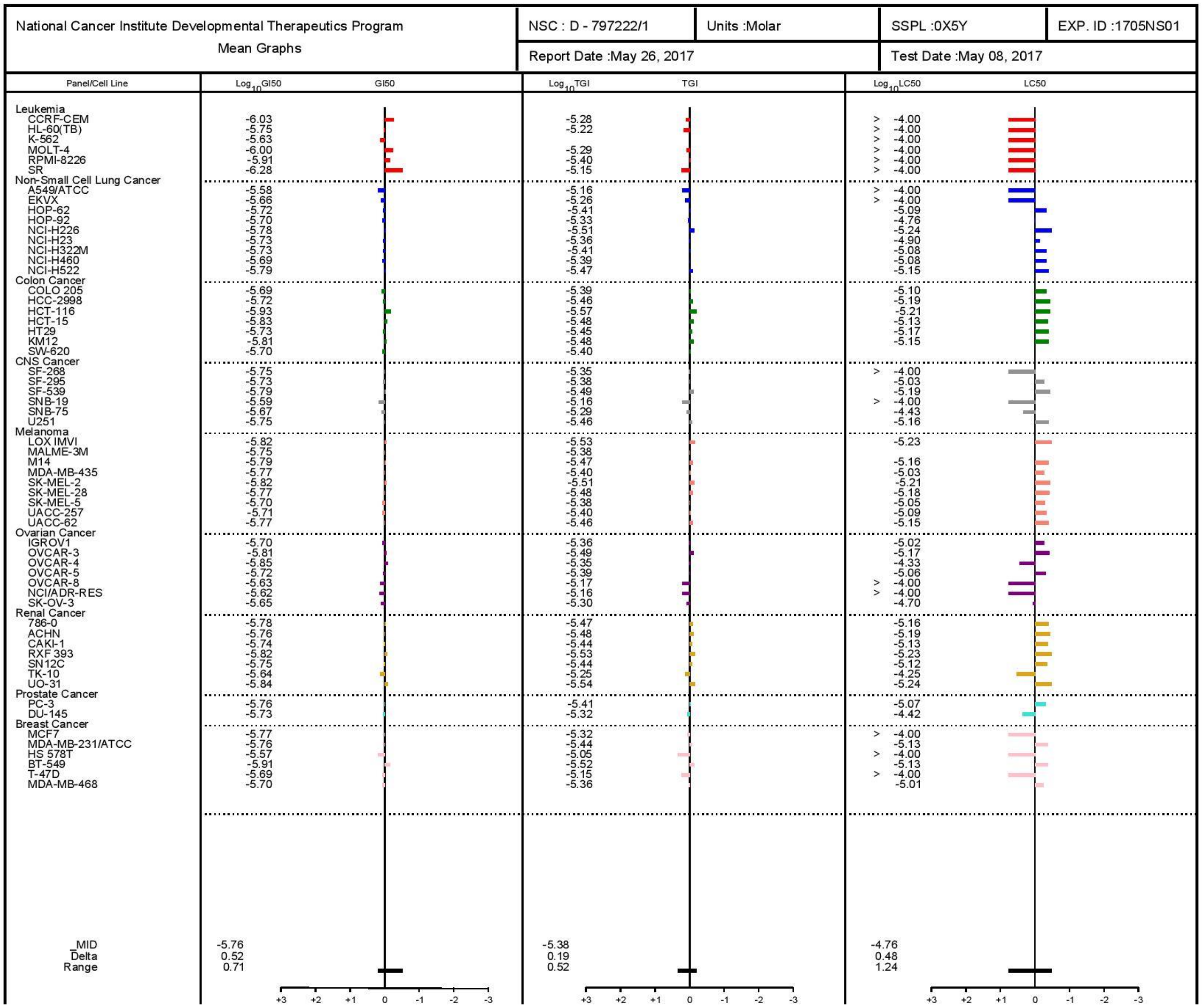
| National Cancer Institute Developmental Therapeutics Program<br>Dose Response Curves | NSC : D - 795083/1 | SSPL:0X5Y | EXP. ID:1701NS47 |
|---|---|---|---|
| | Report Date:April 01, 2017 | Test Date:January 30, 2017 | |



**14p**

# Five dose results of 14v

## Table-23

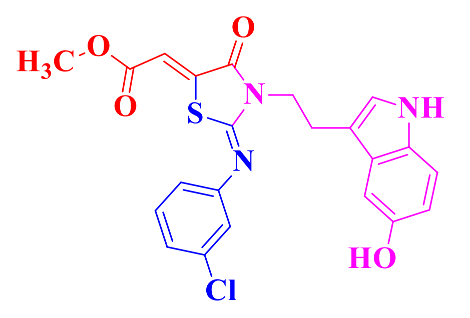


National Cancer Institute Developmental Therapeutics Program
Mean Graphs

NSC : D - 797222/1 | Units :Molar | SSPL :0X5Y | EXP. ID :1705NS01

Report Date :May 26, 2017 | Test Date :May 08, 2017

| Panel/Cell Line | $Log_{10}$GI50 | $Log_{10}$TGI | $Log_{10}$LC50 |
|---|---|---|---|
| Leukemia | | | |
| CCRF-CEM | -6.03 | -5.28 | > -4.00 |
| HL-60(TB) | -5.75 | -5.22 | > -4.00 |
| K-562 | -5.63 | | > -4.00 |
| MOLT-4 | -6.00 | -5.29 | > -4.00 |
| RPMI-8226 | -5.91 | -5.40 | > -4.00 |
| SR | -6.28 | -5.15 | > -4.00 |
| Non-Small Cell Lung Cancer | | | |
| A549/ATCC | -5.58 | -5.16 | > -4.00 |
| EKVX | -5.66 | -5.26 | > -4.00 |
| HOP-62 | -5.72 | -5.41 | -5.09 |
| HOP-92 | -5.70 | -5.33 | -4.76 |
| NCI-H226 | -5.78 | -5.51 | -5.24 |
| NCI-H23 | -5.73 | -5.36 | -4.90 |
| NCI-H322M | -5.73 | -5.41 | -5.08 |
| NCI-H460 | -5.69 | -5.39 | -5.08 |
| NCI-H522 | -5.79 | -5.47 | -5.15 |
| Colon Cancer | | | |
| COLO 205 | -5.69 | -5.39 | -5.10 |
| HCC-2998 | -5.72 | -5.46 | -5.19 |
| HCT-116 | -5.93 | -5.57 | -5.21 |
| HCT-15 | -5.83 | -5.48 | -5.13 |
| HT29 | -5.73 | -5.45 | -5.17 |
| KM12 | -5.81 | -5.48 | -5.15 |
| SW-620 | -5.70 | -5.40 | |
| CNS Cancer | | | |
| SF-268 | -5.75 | -5.35 | > -4.00 |
| SF-295 | -5.73 | -5.38 | -5.03 |
| SF-539 | -5.79 | -5.49 | -5.19 |
| SNB-19 | -5.59 | -5.16 | > -4.00 |
| SNB-75 | -5.67 | -5.29 | -4.43 |
| U251 | -5.75 | -5.46 | -5.16 |
| Melanoma | | | |
| LOX IMVI | -5.82 | -5.53 | -5.23 |
| MALME-3M | -5.75 | -5.38 | |
| M14 | -5.79 | -5.47 | -5.16 |
| MDA-MB-435 | -5.77 | -5.40 | -5.03 |
| SK-MEL-2 | -5.82 | -5.51 | -5.21 |
| SK-MEL-28 | -5.77 | -5.48 | -5.18 |
| SK-MEL-5 | -5.70 | -5.38 | -5.05 |
| UACC-257 | -5.71 | -5.40 | -5.09 |
| UACC-62 | -5.77 | -5.46 | -5.15 |
| Ovarian Cancer | | | |
| IGROV1 | -5.70 | -5.36 | -5.02 |
| OVCAR-3 | -5.81 | -5.49 | -5.17 |
| OVCAR-4 | -5.85 | -5.35 | -4.33 |
| OVCAR-5 | -5.72 | -5.39 | -5.06 |
| OVCAR-8 | -5.63 | -5.17 | > -4.00 |
| NCI/ADR-RES | -5.62 | -5.16 | > -4.00 |
| SK-OV-3 | -5.65 | -5.30 | -4.70 |
| Renal Cancer | | | |
| 786-0 | -5.78 | -5.47 | -5.16 |
| ACHN | -5.76 | -5.48 | -5.19 |
| CAKI-1 | -5.74 | -5.44 | -5.13 |
| RXF 393 | -5.82 | -5.53 | -5.23 |
| SN12C | -5.75 | -5.44 | -5.12 |
| TK-10 | -5.64 | -5.25 | -4.25 |
| UO-31 | -5.84 | -5.54 | -5.24 |
| Prostate Cancer | | | |
| PC-3 | -5.76 | -5.41 | -5.07 |
| DU-145 | -5.73 | -5.32 | -4.42 |
| Breast Cancer | | | |
| MCF7 | -5.77 | -5.32 | > -4.00 |
| MDA-MB-231/ATCC | -5.76 | -5.44 | -5.13 |
| HS 578T | -5.57 | -5.05 | > -4.00 |
| BT-549 | -5.91 | -5.52 | -5.13 |
| T-47D | -5.69 | -5.15 | > -4.00 |
| MDA-MB-468 | -5.70 | -5.36 | -5.01 |
| _MID | -5.76 | -5.38 | -4.76 |
| Delta | 0.52 | 0.19 | 0.48 |
| Range | 0.71 | 0.52 | 1.24 |



**14v**

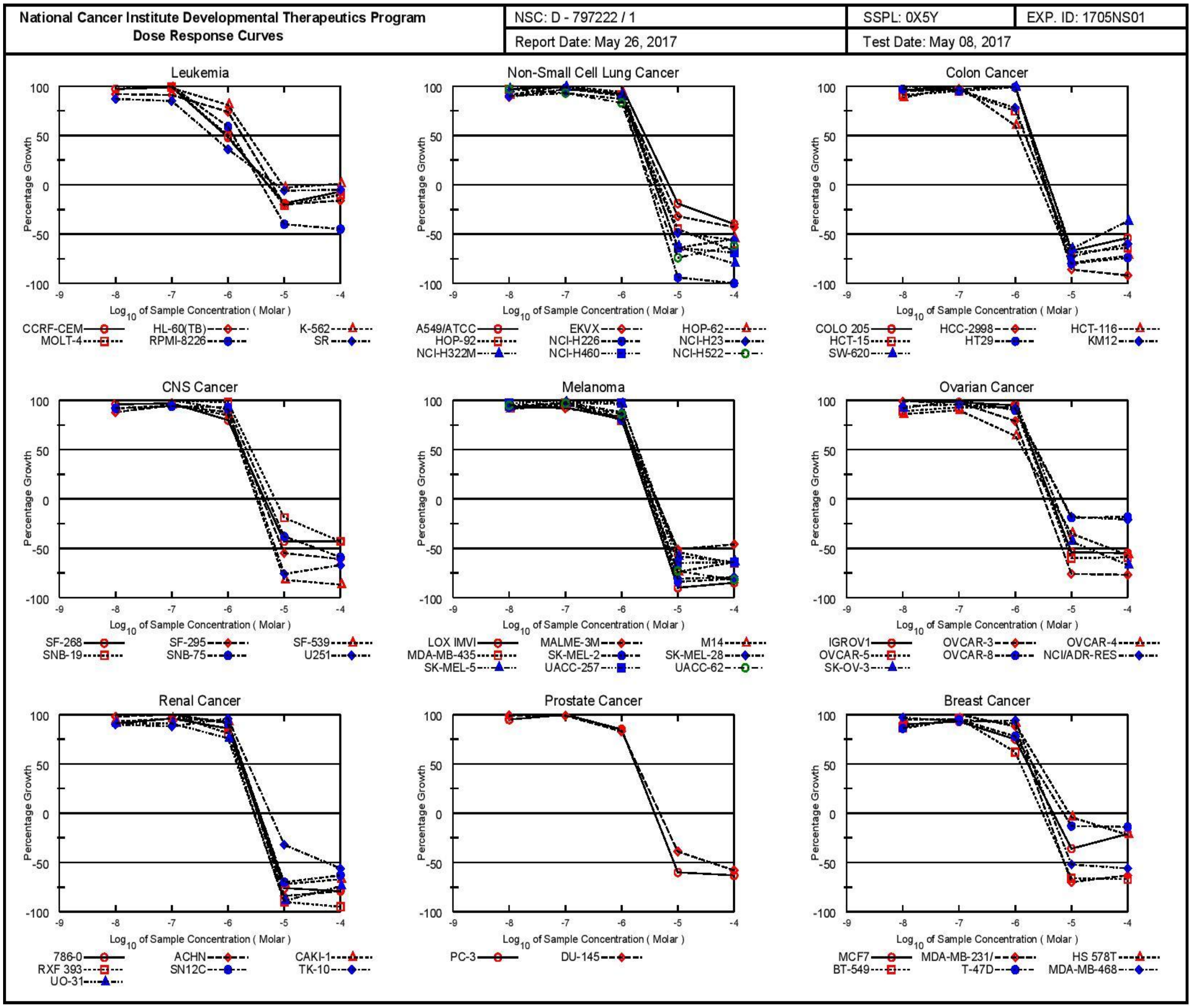
# Table-24

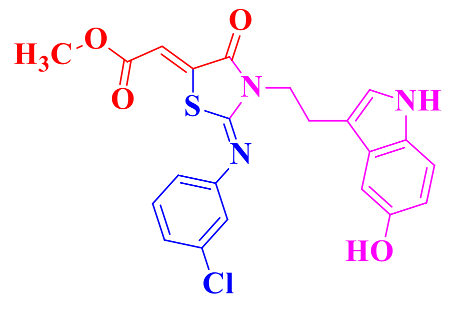
## National Cancer Institute Developmental Therapeutics Program In-Vitro Testing Results

| | | | |
|---|---|---|---|
| NSC : D - 797222 / 1 | Experiment ID : 1705NS01 | Test Type : 08 | Units : Molar |
| Report Date : May 26, 2017 | Test Date : May 08, 2017 | QNS : | MC : |
| COMI : Ser-3Chloro-DM | Stain Reagent : SRB Dual-Pass Related | SSPL : 0X5Y | |

| | | | Log10 Concentration | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Time | | Mean Optical Densities | | | | | Percent Growth | | | | | | | |
| Panel/Cell Line | Zero | Ctrl | -8.0 | -7.0 | -6.0 | -5.0 | -4.0 | -8.0 | -7.0 | -6.0 | -5.0 | -4.0 | GI50 | TGI | LC50 |
| Leukemia | | | | | | | | | | | | | | | |
| CCRF-CEM | 0.541 | 2.318 | 2.263 | 2.309 | 1.402 | 0.440 | 0.505 | 97 | 99 | 48 | -19 | -7 | 9.31E-7 | 5.27E-6 | > 1.00E-4 |
| HL-60(TB) | 0.638 | 3.009 | 2.823 | 2.803 | 2.388 | 0.509 | 0.535 | 92 | 91 | 74 | -20 | -16 | 1.79E-6 | 6.09E-6 | > 1.00E-4 |
| K-562 | 0.262 | 2.074 | 2.167 | 2.030 | 1.731 | 0.255 | 0.283 | 105 | 98 | 81 | -3 | 1 | 2.35E-6 | . | > 1.00E-4 |
| MOLT-4 | 0.503 | 2.165 | 2.235 | 2.154 | 1.336 | 0.399 | 0.452 | 104 | 99 | 50 | -21 | -10 | 1.00E-6 | 5.10E-6 | > 1.00E-4 |
| RPMI-8226 | 0.873 | 2.366 | 2.384 | 2.432 | 1.759 | 0.528 | 0.482 | 101 | 104 | 59 | -40 | -45 | 1.24E-6 | 3.98E-6 | > 1.00E-4 |
| SR | 0.241 | 0.953 | 0.859 | 0.848 | 0.498 | 0.226 | 0.230 | 87 | 85 | 36 | -6 | -5 | 5.22E-7 | 7.13E-6 | > 1.00E-4 |
| Non-Small Cell Lung Cancer | | | | | | | | | | | | | | | |
| A549/ATCC | 0.418 | 1.964 | 1.920 | 2.025 | 1.972 | 0.339 | 0.251 | 97 | 104 | 101 | -19 | -40 | 2.65E-6 | 6.95E-6 | > 1.00E-4 |
| EKVX | 0.754 | 2.040 | 1.894 | 2.000 | 1.941 | 0.511 | 0.430 | 89 | 97 | 92 | -32 | -43 | 2.19E-6 | 5.51E-6 | > 1.00E-4 |
| HOP-62 | 0.550 | 1.673 | 1.568 | 1.706 | 1.606 | 0.197 | 0.250 | 91 | 103 | 94 | -64 | -55 | 1.90E-6 | 3.93E-6 | 8.13E-6 |
| HOP-92 | 1.034 | 1.502 | 1.490 | 1.513 | 1.458 | 0.571 | 0.346 | 97 | 102 | 91 | -45 | -67 | 2.00E-6 | 4.67E-6 | 1.74E-5 |
| NCI-H226 | 0.909 | 2.671 | 2.663 | 2.639 | 2.489 | 0.057 | -0.014 | 100 | 98 | 90 | -94 | -100 | 1.65E-6 | 3.08E-6 | 5.78E-6 |
| NCI-H23 | 0.652 | 2.313 | 2.155 | 2.201 | 2.093 | 0.331 | 0.286 | 90 | 93 | 87 | -49 | -56 | 1.86E-6 | 4.34E-6 | 1.26E-5 |
| NCI-H322M | 0.600 | 1.640 | 1.612 | 1.627 | 1.560 | 0.223 | 0.119 | 97 | 99 | 92 | -63 | -80 | 1.87E-6 | 3.93E-6 | 8.26E-6 |
| NCI-H460 | 0.238 | 2.195 | 2.243 | 2.311 | 2.210 | 0.087 | 0.074 | 102 | 106 | 101 | -64 | -69 | 2.04E-6 | 4.10E-6 | 8.26E-6 |
| NCI-H522 | 0.985 | 2.441 | 2.397 | 2.343 | 2.197 | 0.261 | 0.373 | 97 | 93 | 83 | -74 | -62 | 1.63E-6 | 3.40E-6 | 7.08E-6 |
| Colon Cancer | | | | | | | | | | | | | | | |
| COLO 205 | 0.464 | 1.684 | 1.631 | 1.713 | 1.721 | 0.154 | 0.215 | 96 | 102 | 103 | -67 | -54 | 2.05E-6 | 4.04E-6 | 7.96E-6 |
| HCC-2998 | 1.045 | 3.106 | 2.993 | 3.048 | 3.143 | 0.143 | 0.089 | 95 | 97 | 102 | -86 | -92 | 1.88E-6 | 3.48E-6 | 6.41E-6 |
| HCT-116 | 0.166 | 1.353 | 1.209 | 1.366 | 0.880 | 0.036 | 0.047 | 88 | 101 | 60 | -79 | -72 | 1.18E-6 | 2.71E-6 | 6.22E-6 |
| HCT-15 | 0.314 | 1.963 | 1.822 | 1.885 | 1.556 | 0.097 | 0.114 | 91 | 95 | 75 | -69 | -64 | 1.50E-6 | 3.32E-6 | 7.36E-6 |
| HT29 | 0.263 | 1.631 | 1.588 | 1.557 | 1.613 | 0.053 | 0.069 | 97 | 95 | 99 | -80 | -74 | 1.87E-6 | 3.57E-6 | 6.80E-6 |
| KM12 | 0.325 | 1.691 | 1.765 | 1.642 | 1.389 | 0.088 | 0.131 | 105 | 96 | 78 | -73 | -60 | 1.53E-6 | 3.29E-6 | 7.05E-6 |
| SW-620 | 0.297 | 1.984 | 2.032 | 2.067 | 1.971 | 0.104 | 0.187 | 103 | 105 | 99 | -65 | -37 | 1.99E-6 | 4.02E-6 | . |
| CNS Cancer | | | | | | | | | | | | | | | |
| SF-268 | 0.502 | 1.661 | 1.617 | 1.622 | 1.434 | 0.285 | 0.284 | 96 | 97 | 80 | -43 | -43 | 1.76E-6 | 4.47E-6 | > 1.00E-4 |
| SF-295 | 0.687 | 2.292 | 2.104 | 2.208 | 2.105 | 0.312 | 0.269 | 88 | 95 | 88 | -55 | -61 | 1.85E-6 | 4.15E-6 | 9.29E-6 |
| SF-539 | 0.811 | 2.240 | 2.121 | 2.166 | 2.031 | 0.146 | 0.102 | 92 | 95 | 85 | -82 | -87 | 1.63E-6 | 3.24E-6 | 6.44E-6 |
| SNB-19 | 0.439 | 1.738 | 1.782 | 1.806 | 1.709 | 0.356 | 0.251 | 103 | 105 | 98 | -19 | -43 | 2.57E-6 | 6.89E-6 | > 1.00E-4 |
| SNB-75 | 0.714 | 1.735 | 1.654 | 1.670 | 1.659 | 0.441 | 0.293 | 92 | 94 | 93 | -38 | -59 | 2.12E-6 | 5.10E-6 | 3.68E-5 |
| U251 | 0.277 | 1.226 | 1.284 | 1.308 | 1.140 | 0.067 | 0.093 | 106 | 109 | 91 | -76 | -67 | 1.76E-6 | 3.51E-6 | 6.99E-6 |
| Melanoma | | | | | | | | | | | | | | | |
| LOX IMVI | 0.436 | 2.684 | 2.530 | 2.523 | 2.267 | 0.043 | 0.065 | 93 | 93 | 81 | -90 | -85 | 1.52E-6 | 2.98E-6 | 5.84E-6 |
| MALME-3M | 0.632 | 1.390 | 1.342 | 1.326 | 1.260 | 0.310 | 0.339 | 94 | 92 | 83 | -51 | -46 | 1.76E-6 | 4.16E-6 | . |
| M14 | 0.436 | 1.512 | 1.419 | 1.468 | 1.327 | 0.112 | 0.156 | 91 | 96 | 83 | -74 | -64 | 1.62E-6 | 3.36E-6 | 6.99E-6 |
| MDA-MB-435 | 0.353 | 1.753 | 1.664 | 1.716 | 1.477 | 0.163 | 0.124 | 94 | 97 | 80 | -54 | -65 | 1.68E-6 | 3.96E-6 | 9.34E-6 |
| SK-MEL-2 | 1.246 | 2.459 | 2.376 | 2.419 | 2.218 | 0.201 | 0.241 | 93 | 97 | 80 | -84 | -81 | 1.53E-6 | 3.08E-6 | 6.22E-6 |
| SK-MEL-28 | 0.629 | 1.860 | 1.771 | 1.835 | 1.718 | 0.121 | 0.129 | 93 | 98 | 88 | -81 | -79 | 1.69E-6 | 3.33E-6 | 6.58E-6 |
| SK-MEL-5 | 0.953 | 2.077 | 1.998 | 2.060 | 2.037 | 0.396 | 0.345 | 93 | 98 | 96 | -58 | -64 | 1.99E-6 | 4.19E-6 | 8.81E-6 |
| UACC-257 | 1.164 | 2.208 | 2.176 | 2.221 | 2.182 | 0.412 | 0.420 | 97 | 101 | 97 | -65 | -64 | 1.96E-6 | 3.99E-6 | 8.12E-6 |
| UACC-62 | 0.739 | 2.552 | 2.470 | 2.505 | 2.301 | 0.197 | 0.135 | 95 | 97 | 86 | -73 | -82 | 1.68E-6 | 3.47E-6 | 7.13E-6 |
| Ovarian Cancer | | | | | | | | | | | | | | | |
| IGROV1 | 0.421 | 1.677 | 1.706 | 1.657 | 1.611 | 0.196 | 0.188 | 102 | 98 | 95 | -54 | -55 | 2.00E-6 | 4.35E-6 | 9.46E-6 |
| OVCAR-3 | 0.385 | 1.375 | 1.369 | 1.354 | 1.166 | 0.092 | 0.090 | 99 | 98 | 79 | -76 | -77 | 1.54E-6 | 3.23E-6 | 6.79E-6 |
| OVCAR-4 | 0.539 | 1.163 | 1.079 | 1.103 | 0.942 | 0.350 | 0.230 | 86 | 90 | 64 | -35 | -57 | 1.40E-6 | 4.44E-6 | 4.69E-5 |
| OVCAR-5 | 0.650 | 1.453 | 1.362 | 1.397 | 1.399 | 0.262 | 0.268 | 89 | 93 | 93 | -60 | -59 | 1.92E-6 | 4.07E-6 | 8.64E-6 |
| OVCAR-8 | 0.422 | 1.685 | 1.700 | 1.716 | 1.555 | 0.343 | 0.345 | 101 | 102 | 90 | -19 | -18 | 2.32E-6 | 6.72E-6 | > 1.00E-4 |
| NCI/ADR-RES | 0.584 | 2.023 | 1.931 | 1.962 | 1.904 | 0.481 | 0.459 | 94 | 96 | 92 | -18 | -21 | 2.41E-6 | 6.89E-6 | > 1.00E-4 |
| SK-OV-3 | 1.067 | 2.126 | 2.044 | 2.125 | 2.127 | 0.609 | 0.356 | 92 | 100 | 100 | -43 | -67 | 2.24E-6 | 5.01E-6 | 1.99E-5 |
| Renal Cancer | | | | | | | | | | | | | | | |
| 786-0 | 0.550 | 2.267 | 2.118 | 2.196 | 2.029 | 0.133 | 0.113 | 91 | 96 | 86 | -76 | -79 | 1.67E-6 | 3.40E-6 | 6.93E-6 |
| ACHN | 0.415 | 1.648 | 1.626 | 1.673 | 1.550 | 0.066 | 0.087 | 98 | 102 | 92 | -84 | -79 | 1.73E-6 | 3.33E-6 | 6.39E-6 |
| CAKI-1 | 0.533 | 2.394 | 2.273 | 2.325 | 2.271 | 0.150 | 0.178 | 93 | 96 | 93 | -72 | -67 | 1.83E-6 | 3.67E-6 | 7.37E-6 |
| RXF 393 | 1.057 | 1.501 | 1.503 | 1.556 | 1.415 | 0.109 | 0.049 | 100 | 112 | 81 | -90 | -95 | 1.51E-6 | 2.97E-6 | 5.85E-6 |
| SN12C | 0.438 | 1.727 | 1.723 | 1.741 | 1.606 | 0.132 | 0.163 | 100 | 101 | 91 | -70 | -63 | 1.79E-6 | 3.67E-6 | 7.51E-6 |
| TK-10 | 0.816 | 1.784 | 1.687 | 1.670 | 1.746 | 0.558 | 0.359 | 90 | 88 | 96 | -32 | -56 | 2.29E-6 | 5.65E-6 | 5.66E-5 |
| UO-31 | 0.545 | 2.033 | 1.903 | 1.902 | 1.671 | 0.059 | 0.144 | 91 | 91 | 76 | -89 | -74 | 1.43E-6 | 2.88E-6 | 5.78E-6 |
| Prostate Cancer | | | | | | | | | | | | | | | |
| PC-3 | 0.568 | 1.931 | 1.864 | 1.938 | 1.723 | 0.230 | 0.209 | 95 | 100 | 85 | -60 | -63 | 1.74E-6 | 3.87E-6 | 8.59E-6 |
| DU-145 | 0.400 | 1.469 | 1.463 | 1.457 | 1.283 | 0.246 | 0.167 | 99 | 99 | 83 | -39 | -58 | 1.86E-6 | 4.81E-6 | 3.82E-5 |
| Breast Cancer | | | | | | | | | | | | | | | |
| MCF7 | 0.901 | 3.254 | 3.018 | 3.088 | 2.676 | 0.574 | 0.715 | 90 | 93 | 75 | -36 | -21 | 1.69E-6 | 4.73E-6 | > 1.00E-4 |
| MDA-MB-231/ATCC | 0.625 | 1.533 | 1.549 | 1.557 | 1.423 | 0.186 | 0.232 | 102 | 103 | 88 | -70 | -63 | 1.74E-6 | 3.59E-6 | 7.44E-6 |
| HS 578T | 0.868 | 1.639 | 1.599 | 1.597 | 1.568 | 0.830 | 0.679 | 95 | 95 | 91 | -4 | -22 | 2.68E-6 | 8.99E-6 | > 1.00E-4 |
| BT-549 | 0.896 | 1.811 | 1.694 | 1.771 | 1.465 | 0.304 | 0.294 | 87 | 96 | 62 | -66 | -67 | 1.24E-6 | 3.05E-6 | 7.49E-6 |
| T-47D | 0.751 | 1.601 | 1.479 | 1.560 | 1.415 | 0.651 | 0.648 | 86 | 95 | 78 | -13 | -14 | 2.03E-6 | 7.15E-6 | > 1.00E-4 |
| MDA-MB-468 | 0.774 | 1.196 | 1.182 | 1.168 | 1.169 | 0.373 | 0.338 | 97 | 93 | 94 | -52 | -56 | 1.99E-6 | 4.40E-6 | 9.71E-6 |

# Table-25


National Cancer Institute Developmental Therapeutics Program
Dose Response Curves
NSC: D - 797222 / 1
SSPL: 0X5Y
EXP. ID: 1705NS01
Report Date: May 26, 2017
Test Date: May 08, 2017
Leukemia
Non-Small Cell Lung Cancer
Colon Cancer
CNS Cancer
Melanoma
Ovarian Cancer
Renal Cancer
Prostate Cancer
Breast Cancer
Percentage Growth
100
50
0
-50
-100
-9
-8
-7
-6
-5
-4
Log10 of Sample Concentration ( Molar )
CCRF-CEM
HL-60(TB)
K-562
MOLT-4
RPMI-8226
SR
A549/ATCC
EKVX
HOP-62
HOP-92
NCI-H226
NCI-H23
NCI-H322M
NCI-H460
NCI-H522
COLO 205
HCC-2998
HCT-116
HCT-15
HT29
KM12
SW-620
SF-268
SF-295
SF-539
SNB-19
SNB-75
U251
LOX IMVI
MALME-3M
M14
MDA-MB-435
SK-MEL-2
SK-MEL-28
SK-MEL-5
UACC-257
UACC-62
IGROV1
OVCAR-3
OVCAR-4
OVCAR-5
OVCAR-8
NCI/ADR-RES
SK-OV-3
786-0
ACHN
CAKI-1
RXF 393
SN12C
TK-10
UO-31
PC-3
DU-145
MCF7
MDA-MB-231/
HS 578T
BT-549
T-47D
MDA-MB-468


H3C
O
O
O
S
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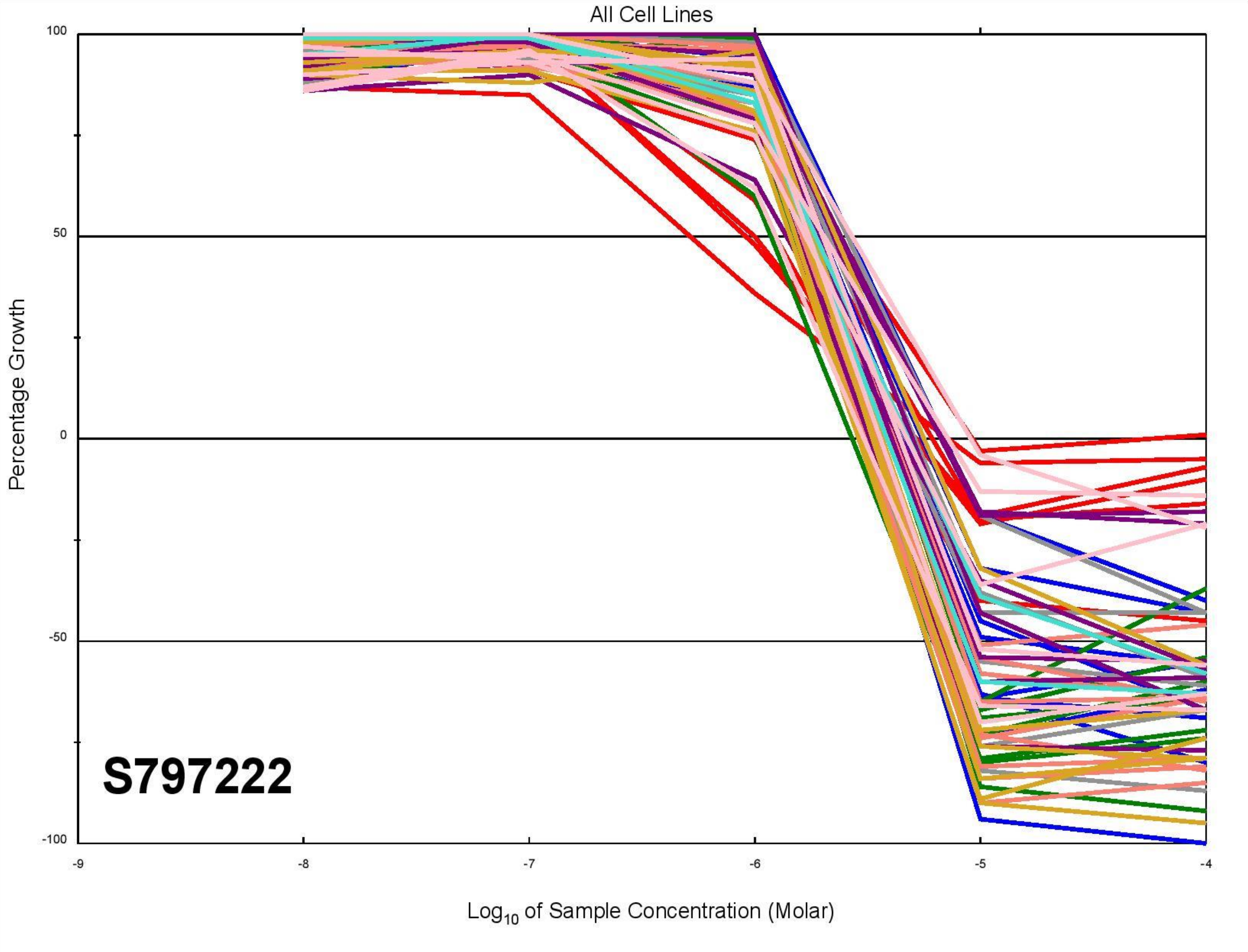
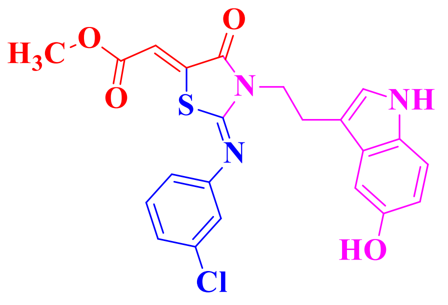
Cl

**14v**

## Table-26

| National Cancer Institute Developmental Therapeutics Program<br>Dose Response Curves | NSC : D - 797222/1 | SSPL:0X5Y | EXP. ID:1705NS01 |
|---|---|---|---|
| | Report Date:May 26, 2017 | Test Date:May 08, 2017 | |

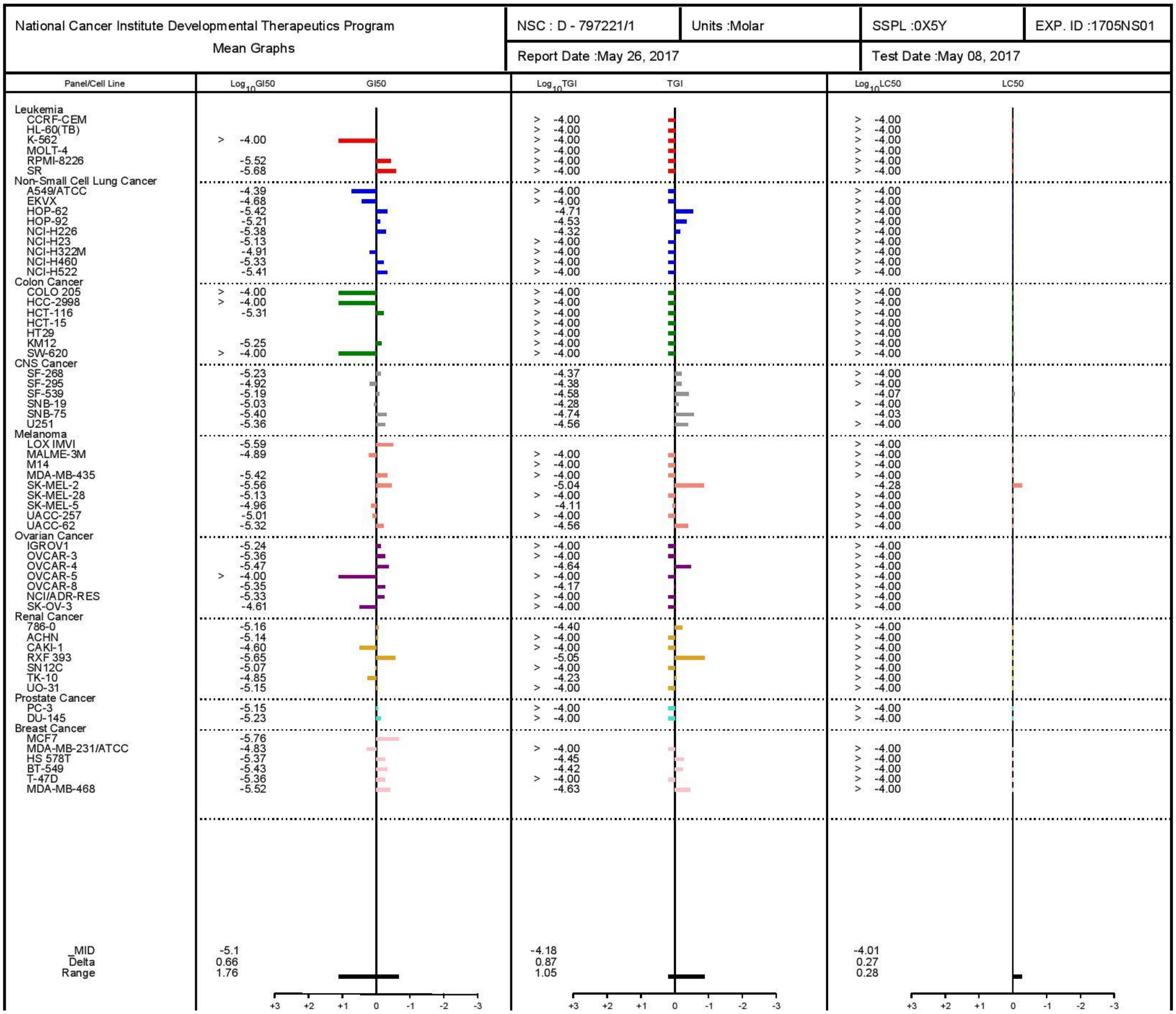




**14v**

## Five dose results of 14w

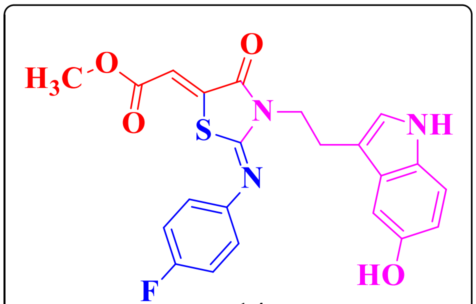
## Table-27



National Cancer Institute Developmental Therapeutics Program
Mean Graphs

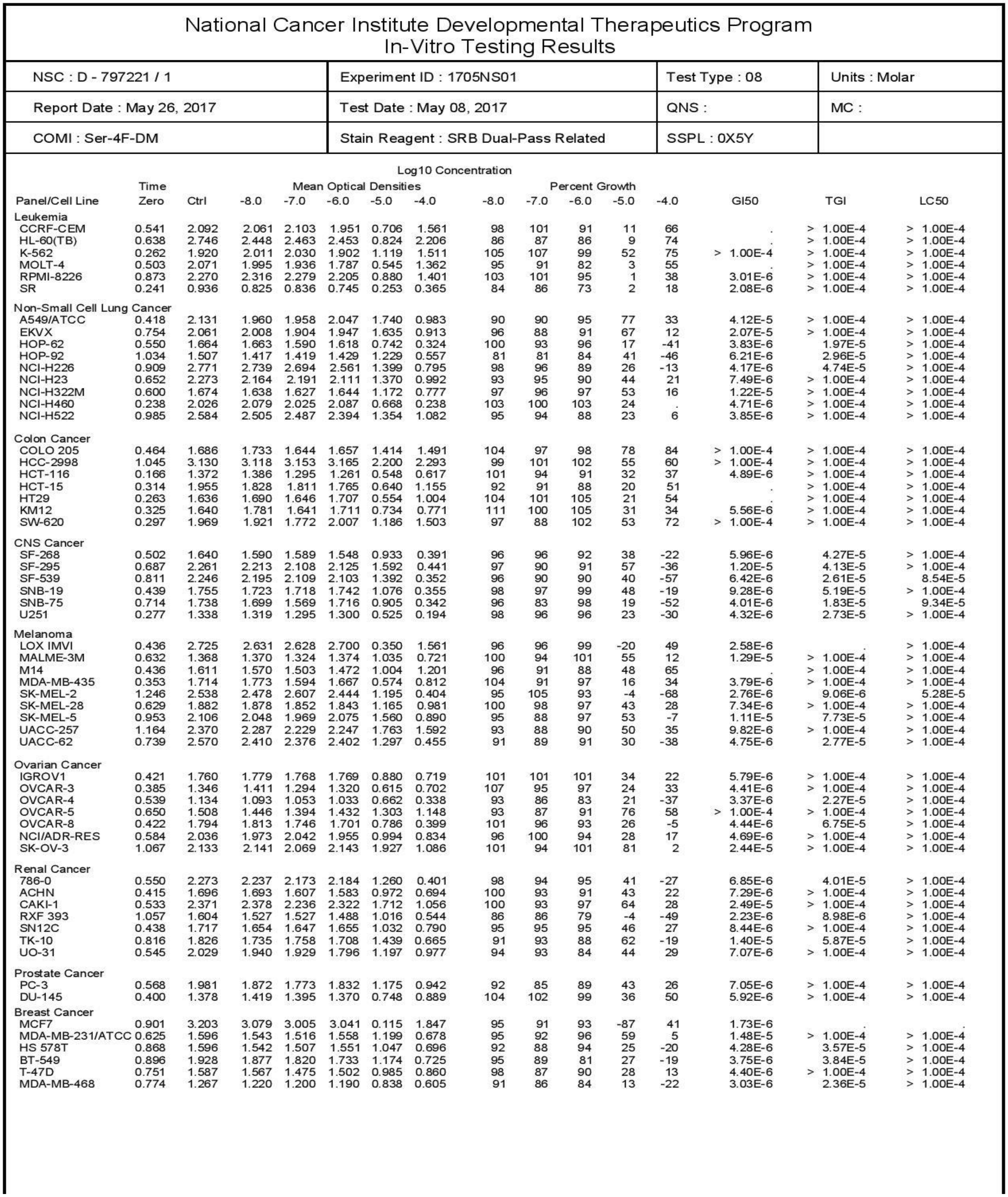
NSC : D - 797221/1 | Units :Molar | SSPL :0X5Y | EXP. ID :1705NS01
Report Date :May 26, 2017 | Test Date :May 08, 2017

| Panel/Cell Line | Log10 GI50 | Log10 TGI | Log10 LC50 |
|---|---|---|---|
| Leukemia | | | |
| CCRF-CEM | | > -4.00 | > -4.00 |
| HL-60(TB) | | > -4.00 | > -4.00 |
| K-562 | > -4.00 | > -4.00 | > -4.00 |
| MOLT-4 | | > -4.00 | > -4.00 |
| RPMI-8226 | -5.52 | > -4.00 | > -4.00 |
| SR | -5.68 | > -4.00 | > -4.00 |
| Non-Small Cell Lung Cancer | | | |
| A549/ATCC | -4.39 | > -4.00 | > -4.00 |
| EKVX | -4.68 | > -4.00 | > -4.00 |
| HOP-62 | -5.42 | -4.71 | > -4.00 |
| HOP-92 | -5.21 | -4.53 | > -4.00 |
| NCI-H226 | -5.38 | -4.32 | > -4.00 |
| NCI-H23 | -5.13 | > -4.00 | > -4.00 |
| NCI-H322M | -4.91 | > -4.00 | > -4.00 |
| NCI-H460 | -5.33 | > -4.00 | > -4.00 |
| NCI-H522 | -5.41 | > -4.00 | > -4.00 |
| Colon Cancer | | | |
| COLO 205 | > -4.00 | > -4.00 | > -4.00 |
| HCC-2998 | > -4.00 | > -4.00 | > -4.00 |
| HCT-116 | -5.31 | > -4.00 | > -4.00 |
| HCT-15 | | > -4.00 | > -4.00 |
| HT29 | | > -4.00 | > -4.00 |
| KM12 | -5.25 | > -4.00 | > -4.00 |
| SW-620 | > -4.00 | > -4.00 | > -4.00 |
| CNS Cancer | | | |
| SF-268 | -5.23 | -4.37 | > -4.00 |
| SF-295 | -4.92 | -4.38 | > -4.00 |
| SF-539 | -5.19 | -4.58 | -4.07 |
| SNB-19 | -5.03 | -4.28 | > -4.00 |
| SNB-75 | -5.40 | -4.74 | -4.03 |
| U251 | -5.36 | -4.56 | > -4.00 |
| Melanoma | | | |
| LOX IMVI | -5.59 | | > -4.00 |
| MALME-3M | -4.89 | > -4.00 | > -4.00 |
| M14 | | > -4.00 | > -4.00 |
| MDA-MB-435 | -5.42 | > -4.00 | > -4.00 |
| SK-MEL-2 | -5.56 | -5.04 | -4.28 |
| SK-MEL-28 | -5.13 | > -4.00 | > -4.00 |
| SK-MEL-5 | -4.96 | -4.11 | > -4.00 |
| UACC-257 | -5.01 | > -4.00 | > -4.00 |
| UACC-62 | -5.32 | -4.56 | > -4.00 |
| Ovarian Cancer | | | |
| IGROV1 | -5.24 | > -4.00 | > -4.00 |
| OVCAR-3 | -5.36 | > -4.00 | > -4.00 |
| OVCAR-4 | -5.47 | -4.64 | > -4.00 |
| OVCAR-5 | > -4.00 | > -4.00 | > -4.00 |
| OVCAR-8 | -5.35 | -4.17 | > -4.00 |
| NCI/ADR-RES | -5.33 | > -4.00 | > -4.00 |
| SK-OV-3 | -4.61 | > -4.00 | > -4.00 |
| Renal Cancer | | | |
| 786-0 | -5.16 | -4.40 | > -4.00 |
| ACHN | -5.14 | > -4.00 | > -4.00 |
| CAKI-1 | -4.60 | > -4.00 | > -4.00 |
| RXF 393 | -5.65 | -5.05 | > -4.00 |
| SN12C | -5.07 | > -4.00 | > -4.00 |
| TK-10 | -4.85 | -4.23 | > -4.00 |
| UO-31 | -5.15 | > -4.00 | > -4.00 |
| Prostate Cancer | | | |
| PC-3 | -5.15 | > -4.00 | > -4.00 |
| DU-145 | -5.23 | > -4.00 | > -4.00 |
| Breast Cancer | | | |
| MCF7 | -5.76 | | |
| MDA-MB-231/ATCC | -4.83 | > -4.00 | > -4.00 |
| HS 578T | -5.37 | -4.45 | > -4.00 |
| BT-549 | -5.43 | -4.42 | > -4.00 |
| T-47D | -5.36 | > -4.00 | > -4.00 |
| MDA-MB-468 | -5.52 | -4.63 | > -4.00 |
| MID | -5.1 | -4.18 | -4.01 |
| Delta | 0.66 | 0.87 | 0.27 |
| Range | 1.76 | 1.05 | 0.28 |



**14w**

# Table-28

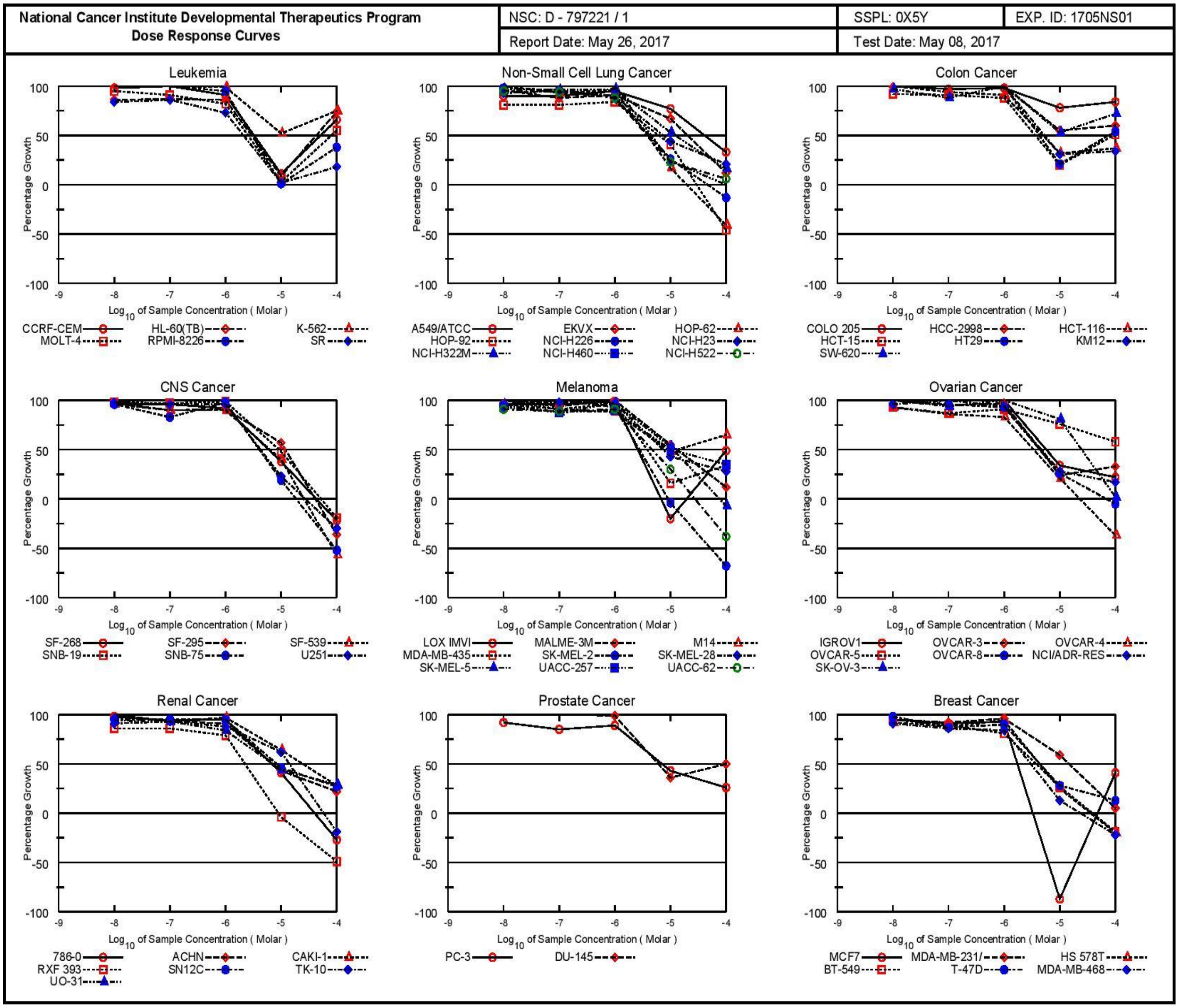


## National Cancer Institute Developmental Therapeutics Program
## In-Vitro Testing Results

| | | | |
|---|---|---|---|
| NSC : D - 797221 / 1 | Experiment ID : 1705NS01 | Test Type : 08 | Units : Molar |
| Report Date : May 26, 2017 | Test Date : May 08, 2017 | QNS : | MC : |
| COMI : Ser-4F-DM | Stain Reagent : SRB Dual-Pass Related | SSPL : 0X5Y | |

| | | | Log10 Concentration | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Time | | Mean Optical Densities | | | | | Percent Growth | | | | | | | | |
| Panel/Cell Line | Zero | Ctrl | -8.0 | -7.0 | -6.0 | -5.0 | -4.0 | -8.0 | -7.0 | -6.0 | -5.0 | -4.0 | GI50 | TGI | LC50 |
| Leukemia | | | | | | | | | | | | | | | |
| CCRF-CEM | 0.541 | 2.092 | 2.061 | 2.103 | 1.951 | 0.706 | 1.561 | 98 | 101 | 91 | 11 | 66 | . | > 1.00E-4 | > 1.00E-4 |
| HL-60(TB) | 0.638 | 2.746 | 2.448 | 2.463 | 2.453 | 0.824 | 2.206 | 86 | 87 | 86 | 9 | 74 | . | > 1.00E-4 | > 1.00E-4 |
| K-562 | 0.262 | 1.920 | 2.011 | 2.030 | 1.902 | 1.119 | 1.511 | 105 | 107 | 99 | 52 | 75 | > 1.00E-4 | > 1.00E-4 | > 1.00E-4 |
| MOLT-4 | 0.503 | 2.071 | 1.995 | 1.936 | 1.787 | 0.545 | 1.362 | 95 | 91 | 82 | 3 | 55 | . | > 1.00E-4 | > 1.00E-4 |
| RPMI-8226 | 0.873 | 2.270 | 2.316 | 2.279 | 2.205 | 0.880 | 1.401 | 103 | 101 | 95 | 1 | 38 | 3.01E-6 | > 1.00E-4 | > 1.00E-4 |
| SR | 0.241 | 0.936 | 0.825 | 0.836 | 0.745 | 0.253 | 0.365 | 84 | 86 | 73 | 2 | 18 | 2.08E-6 | > 1.00E-4 | > 1.00E-4 |
| Non-Small Cell Lung Cancer | | | | | | | | | | | | | | | |
| A549/ATCC | 0.418 | 2.131 | 1.960 | 1.958 | 2.047 | 1.740 | 0.983 | 90 | 90 | 95 | 77 | 33 | 4.12E-5 | > 1.00E-4 | > 1.00E-4 |
| EKVX | 0.754 | 2.061 | 2.008 | 1.904 | 1.947 | 1.635 | 0.913 | 96 | 88 | 91 | 67 | 12 | 2.07E-5 | > 1.00E-4 | > 1.00E-4 |
| HOP-62 | 0.550 | 1.664 | 1.663 | 1.590 | 1.618 | 0.742 | 0.324 | 100 | 93 | 96 | 17 | -41 | 3.83E-6 | 1.97E-5 | > 1.00E-4 |
| HOP-92 | 1.034 | 1.507 | 1.417 | 1.419 | 1.429 | 1.229 | 0.557 | 81 | 81 | 84 | 41 | -46 | 6.21E-6 | 2.96E-5 | > 1.00E-4 |
| NCI-H226 | 0.909 | 2.771 | 2.739 | 2.694 | 2.561 | 1.399 | 0.795 | 98 | 96 | 89 | 26 | -13 | 4.17E-6 | 4.74E-5 | > 1.00E-4 |
| NCI-H23 | 0.652 | 2.273 | 2.164 | 2.191 | 2.111 | 1.370 | 0.992 | 93 | 95 | 90 | 44 | 21 | 7.49E-6 | > 1.00E-4 | > 1.00E-4 |
| NCI-H322M | 0.600 | 1.674 | 1.638 | 1.627 | 1.644 | 1.172 | 0.777 | 97 | 96 | 97 | 53 | 16 | 1.22E-5 | > 1.00E-4 | > 1.00E-4 |
| NCI-H460 | 0.238 | 2.026 | 2.079 | 2.025 | 2.087 | 0.668 | 0.238 | 103 | 100 | 103 | 24 | . | 4.71E-6 | > 1.00E-4 | > 1.00E-4 |
| NCI-H522 | 0.985 | 2.584 | 2.505 | 2.487 | 2.394 | 1.354 | 1.082 | 95 | 94 | 88 | 23 | 6 | 3.85E-6 | > 1.00E-4 | > 1.00E-4 |
| Colon Cancer | | | | | | | | | | | | | | | |
| COLO 205 | 0.464 | 1.686 | 1.733 | 1.644 | 1.657 | 1.414 | 1.491 | 104 | 97 | 98 | 78 | 84 | > 1.00E-4 | > 1.00E-4 | > 1.00E-4 |
| HCC-2998 | 1.045 | 3.130 | 3.118 | 3.153 | 3.165 | 2.200 | 2.293 | 99 | 101 | 102 | 55 | 60 | > 1.00E-4 | > 1.00E-4 | > 1.00E-4 |
| HCT-116 | 0.166 | 1.372 | 1.386 | 1.295 | 1.261 | 0.548 | 0.617 | 101 | 94 | 91 | 32 | 37 | 4.89E-6 | > 1.00E-4 | > 1.00E-4 |
| HCT-15 | 0.314 | 1.955 | 1.828 | 1.811 | 1.765 | 0.640 | 1.155 | 92 | 91 | 88 | 20 | 51 | . | > 1.00E-4 | > 1.00E-4 |
| HT29 | 0.263 | 1.636 | 1.690 | 1.646 | 1.707 | 0.554 | 1.004 | 104 | 101 | 105 | 21 | 54 | . | > 1.00E-4 | > 1.00E-4 |
| KM12 | 0.325 | 1.640 | 1.781 | 1.641 | 1.711 | 0.734 | 0.771 | 111 | 100 | 105 | 31 | 34 | 5.56E-6 | > 1.00E-4 | > 1.00E-4 |
| SW-620 | 0.297 | 1.969 | 1.921 | 1.772 | 2.007 | 1.186 | 1.503 | 97 | 88 | 102 | 53 | 72 | > 1.00E-4 | > 1.00E-4 | > 1.00E-4 |
| CNS Cancer | | | | | | | | | | | | | | | |
| SF-268 | 0.502 | 1.640 | 1.590 | 1.589 | 1.548 | 0.933 | 0.391 | 96 | 96 | 92 | 38 | -22 | 5.96E-6 | 4.27E-5 | > 1.00E-4 |
| SF-295 | 0.687 | 2.261 | 2.213 | 2.108 | 2.125 | 1.592 | 0.441 | 97 | 90 | 91 | 57 | -36 | 1.20E-5 | 4.13E-5 | > 1.00E-4 |
| SF-539 | 0.811 | 2.246 | 2.195 | 2.109 | 2.103 | 1.392 | 0.352 | 96 | 90 | 90 | 40 | -57 | 6.42E-6 | 2.61E-5 | 8.54E-5 |
| SNB-19 | 0.439 | 1.755 | 1.723 | 1.718 | 1.742 | 1.076 | 0.355 | 98 | 97 | 99 | 48 | -19 | 9.28E-6 | 5.19E-5 | > 1.00E-4 |
| SNB-75 | 0.714 | 1.738 | 1.699 | 1.569 | 1.716 | 0.905 | 0.342 | 96 | 83 | 98 | 19 | -52 | 4.01E-6 | 1.83E-5 | 9.34E-5 |
| U251 | 0.277 | 1.338 | 1.319 | 1.295 | 1.300 | 0.525 | 0.194 | 98 | 96 | 96 | 23 | -30 | 4.32E-6 | 2.73E-5 | > 1.00E-4 |
| Melanoma | | | | | | | | | | | | | | | |
| LOX IMVI | 0.436 | 2.725 | 2.631 | 2.628 | 2.700 | 0.350 | 1.561 | 96 | 96 | 99 | -20 | 49 | 2.58E-6 | . | > 1.00E-4 |
| MALME-3M | 0.632 | 1.368 | 1.370 | 1.324 | 1.374 | 1.035 | 0.721 | 100 | 94 | 101 | 55 | 12 | 1.29E-5 | > 1.00E-4 | > 1.00E-4 |
| M14 | 0.436 | 1.611 | 1.570 | 1.503 | 1.472 | 1.004 | 1.201 | 96 | 91 | 88 | 48 | 65 | . | > 1.00E-4 | > 1.00E-4 |
| MDA-MB-435 | 0.353 | 1.714 | 1.773 | 1.594 | 1.667 | 0.574 | 0.812 | 104 | 91 | 97 | 16 | 34 | 3.79E-6 | > 1.00E-4 | > 1.00E-4 |
| SK-MEL-2 | 1.246 | 2.538 | 2.478 | 2.607 | 2.444 | 1.195 | 0.404 | 95 | 105 | 93 | -4 | -68 | 2.76E-6 | 9.06E-6 | 5.28E-5 |
| SK-MEL-28 | 0.629 | 1.882 | 1.878 | 1.852 | 1.843 | 1.165 | 0.981 | 100 | 98 | 97 | 43 | 28 | 7.34E-6 | > 1.00E-4 | > 1.00E-4 |
| SK-MEL-5 | 0.953 | 2.106 | 2.048 | 1.969 | 2.075 | 1.560 | 0.890 | 95 | 88 | 97 | 53 | -7 | 1.11E-5 | 7.73E-5 | > 1.00E-4 |
| UACC-257 | 1.164 | 2.370 | 2.287 | 2.229 | 2.247 | 1.763 | 1.592 | 93 | 88 | 90 | 50 | 35 | 9.82E-6 | > 1.00E-4 | > 1.00E-4 |
| UACC-62 | 0.739 | 2.570 | 2.410 | 2.376 | 2.402 | 1.297 | 0.455 | 91 | 89 | 91 | 30 | -38 | 4.75E-6 | 2.77E-5 | > 1.00E-4 |
| Ovarian Cancer | | | | | | | | | | | | | | | |
| IGROV1 | 0.421 | 1.760 | 1.779 | 1.768 | 1.769 | 0.880 | 0.719 | 101 | 101 | 101 | 34 | 22 | 5.79E-6 | > 1.00E-4 | > 1.00E-4 |
| OVCAR-3 | 0.385 | 1.346 | 1.411 | 1.294 | 1.320 | 0.615 | 0.702 | 107 | 95 | 97 | 24 | 33 | 4.41E-6 | > 1.00E-4 | > 1.00E-4 |
| OVCAR-4 | 0.539 | 1.134 | 1.093 | 1.053 | 1.033 | 0.662 | 0.338 | 93 | 86 | 83 | 21 | -37 | 3.37E-6 | 2.27E-5 | > 1.00E-4 |
| OVCAR-5 | 0.650 | 1.508 | 1.446 | 1.394 | 1.432 | 1.303 | 1.148 | 93 | 87 | 91 | 76 | 58 | > 1.00E-4 | > 1.00E-4 | > 1.00E-4 |
| OVCAR-8 | 0.422 | 1.794 | 1.813 | 1.746 | 1.701 | 0.786 | 0.399 | 101 | 96 | 93 | 26 | -5 | 4.44E-6 | 6.75E-5 | > 1.00E-4 |
| NCI/ADR-RES | 0.584 | 2.036 | 1.973 | 2.042 | 1.955 | 0.994 | 0.834 | 96 | 100 | 94 | 28 | 17 | 4.69E-6 | > 1.00E-4 | > 1.00E-4 |
| SK-OV-3 | 1.067 | 2.133 | 2.141 | 2.069 | 2.143 | 1.927 | 1.086 | 101 | 94 | 101 | 81 | 2 | 2.44E-5 | > 1.00E-4 | > 1.00E-4 |
| Renal Cancer | | | | | | | | | | | | | | | |
| 786-0 | 0.550 | 2.273 | 2.237 | 2.173 | 2.184 | 1.260 | 0.401 | 98 | 94 | 95 | 41 | -27 | 6.85E-6 | 4.01E-5 | > 1.00E-4 |
| ACHN | 0.415 | 1.696 | 1.693 | 1.607 | 1.583 | 0.972 | 0.694 | 100 | 93 | 91 | 43 | 22 | 7.29E-6 | > 1.00E-4 | > 1.00E-4 |
| CAKI-1 | 0.533 | 2.371 | 2.378 | 2.236 | 2.322 | 1.712 | 1.056 | 100 | 93 | 97 | 64 | 28 | 2.49E-5 | > 1.00E-4 | > 1.00E-4 |
| RXF 393 | 1.057 | 1.604 | 1.527 | 1.527 | 1.488 | 1.016 | 0.544 | 86 | 86 | 79 | -4 | -49 | 2.23E-6 | 8.98E-6 | > 1.00E-4 |
| SN12C | 0.438 | 1.717 | 1.654 | 1.647 | 1.655 | 1.032 | 0.790 | 95 | 95 | 95 | 46 | 27 | 8.44E-6 | > 1.00E-4 | > 1.00E-4 |
| TK-10 | 0.816 | 1.826 | 1.735 | 1.758 | 1.708 | 1.439 | 0.665 | 91 | 93 | 88 | 62 | -19 | 1.40E-5 | 5.87E-5 | > 1.00E-4 |
| UO-31 | 0.545 | 2.029 | 1.940 | 1.929 | 1.796 | 1.197 | 0.977 | 94 | 93 | 84 | 44 | 29 | 7.07E-6 | > 1.00E-4 | > 1.00E-4 |
| Prostate Cancer | | | | | | | | | | | | | | | |
| PC-3 | 0.568 | 1.981 | 1.872 | 1.773 | 1.832 | 1.175 | 0.942 | 92 | 85 | 89 | 43 | 26 | 7.05E-6 | > 1.00E-4 | > 1.00E-4 |
| DU-145 | 0.400 | 1.378 | 1.419 | 1.395 | 1.370 | 0.748 | 0.889 | 104 | 102 | 99 | 36 | 50 | 5.92E-6 | > 1.00E-4 | > 1.00E-4 |
| Breast Cancer | | | | | | | | | | | | | | | |
| MCF7 | 0.901 | 3.203 | 3.079 | 3.005 | 3.041 | 0.115 | 1.847 | 95 | 91 | 93 | -87 | 41 | 1.73E-6 | . | . |
| MDA-MB-231/ATCC | 0.625 | 1.596 | 1.543 | 1.516 | 1.558 | 1.199 | 0.678 | 95 | 92 | 96 | 59 | 5 | 1.48E-5 | > 1.00E-4 | > 1.00E-4 |
| HS 578T | 0.868 | 1.596 | 1.542 | 1.507 | 1.551 | 1.047 | 0.696 | 92 | 88 | 94 | 25 | -20 | 4.28E-6 | 3.57E-5 | > 1.00E-4 |
| BT-549 | 0.896 | 1.928 | 1.877 | 1.820 | 1.733 | 1.174 | 0.725 | 95 | 89 | 81 | 27 | -19 | 3.75E-6 | 3.84E-5 | > 1.00E-4 |
| T-47D | 0.751 | 1.587 | 1.567 | 1.475 | 1.502 | 0.985 | 0.860 | 98 | 87 | 90 | 28 | 13 | 4.40E-6 | > 1.00E-4 | > 1.00E-4 |
| MDA-MB-468 | 0.774 | 1.267 | 1.220 | 1.200 | 1.190 | 0.838 | 0.605 | 91 | 86 | 84 | 13 | -22 | 3.03E-6 | 2.36E-5 | > 1.00E-4 |

# Table-29



National Cancer Institute Developmental Therapeutics Program
Dose Response Curves

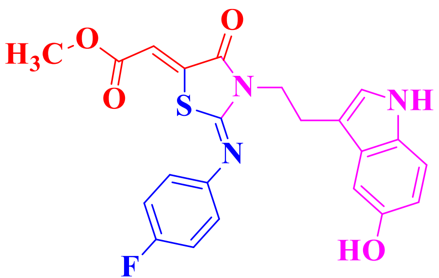
NSC: D - 797221 / 1

SSPL: 0X5Y

EXP. ID: 1705NS01

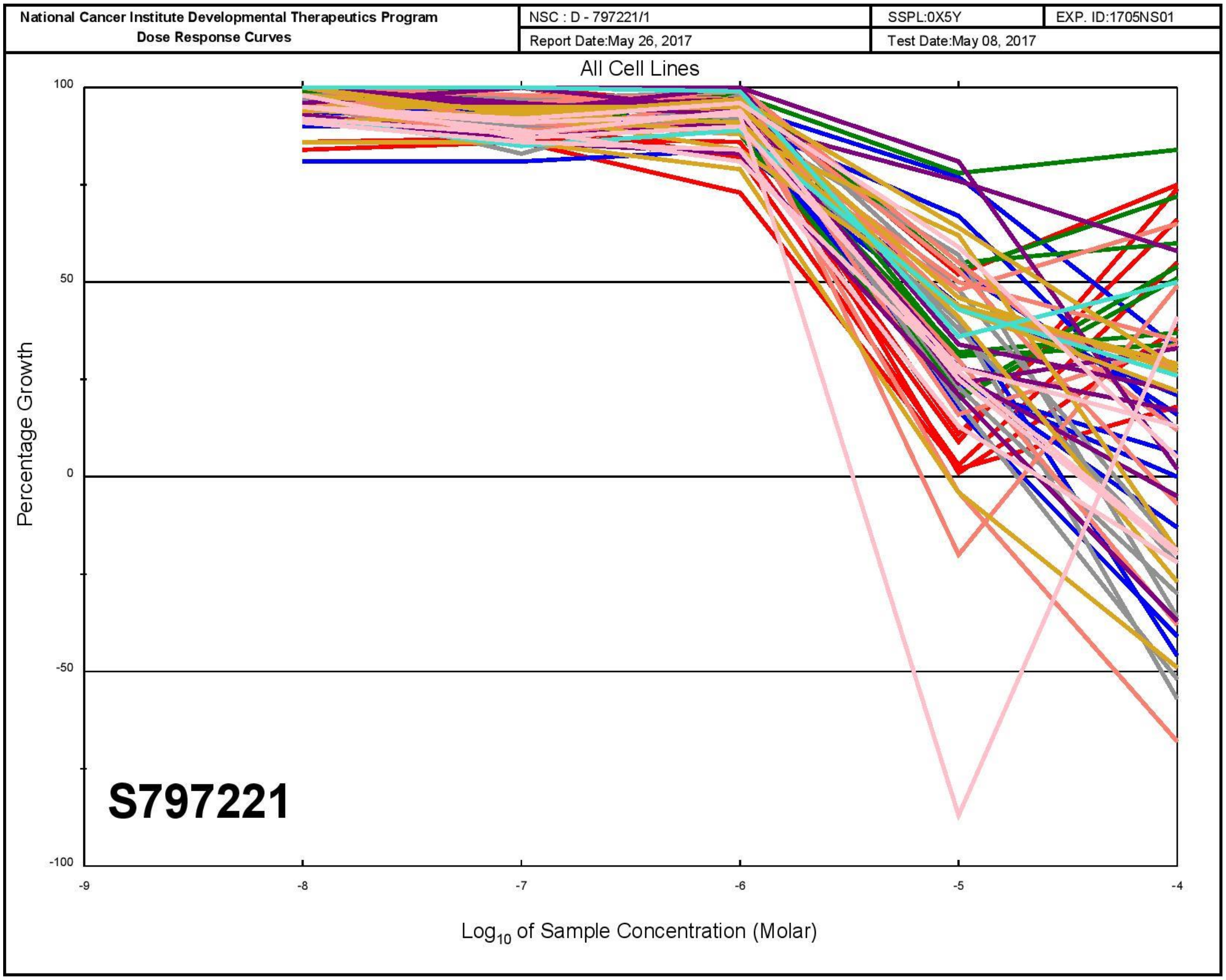
Report Date: May 26, 2017

Test Date: May 08, 2017



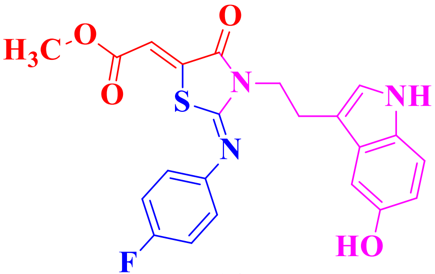
**14w**

# Table-30



National Cancer Institute Developmental Therapeutics Program
Dose Response Curves

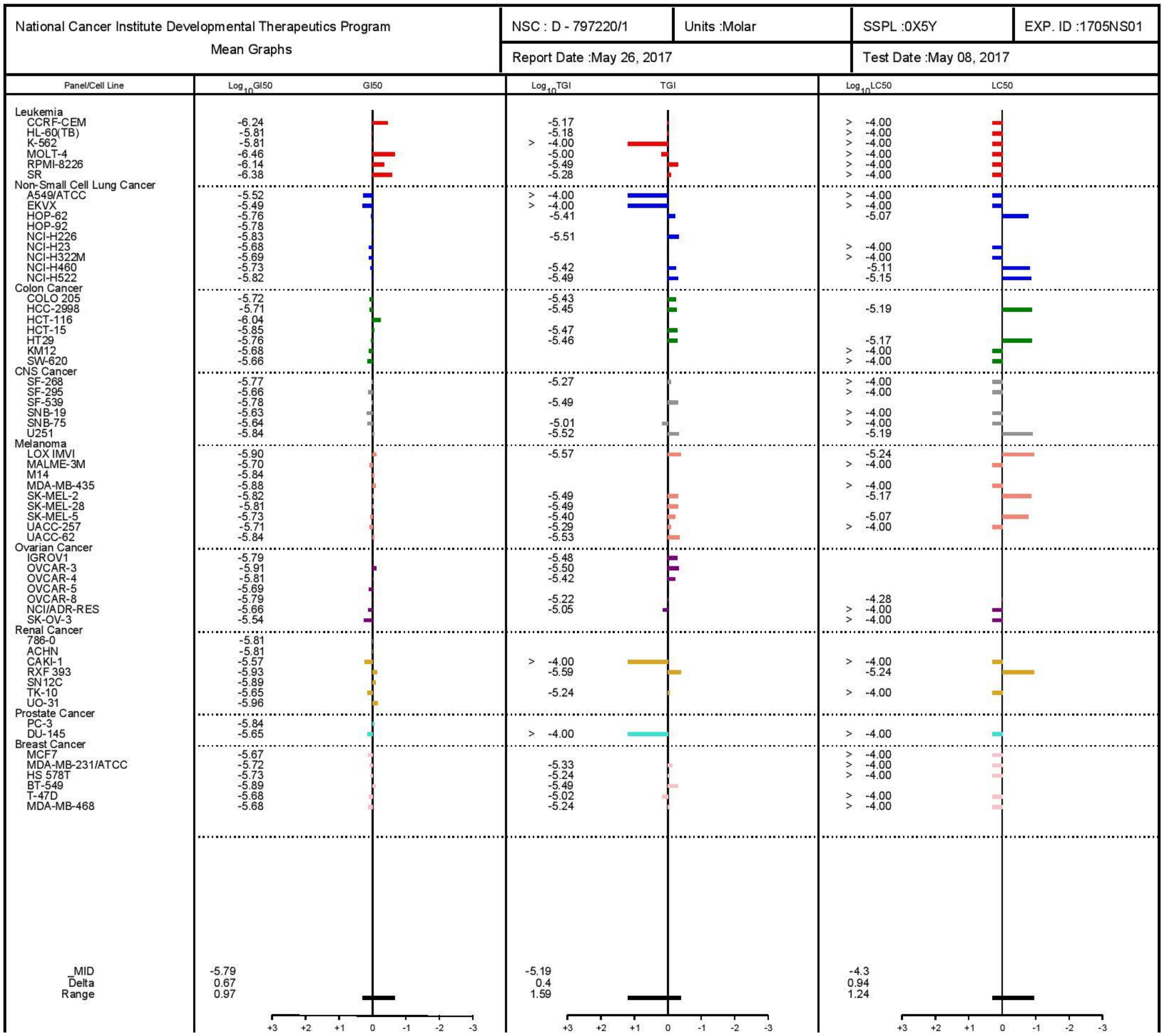
NSC : D - 797221/1
SSPL:0X5Y
EXP. ID:1705NS01
Report Date:May 26, 2017
Test Date:May 08, 2017



**14w**

## Five dose results of 14x

## Table-31

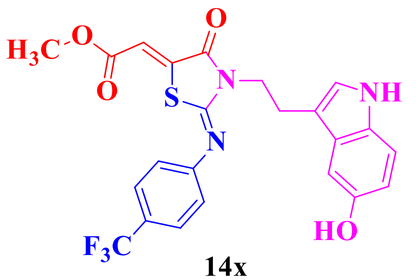


National Cancer Institute Developmental Therapeutics Program
Mean Graphs

NSC : D - 797220/1 | Units :Molar | SSPL :0X5Y | EXP. ID :1705NS01

Report Date :May 26, 2017 | Test Date :May 08, 2017

| Panel/Cell Line | $Log_{10}$GI50 | $Log_{10}$TGI | $Log_{10}$LC50 |
|---|---|---|---|
| Leukemia | | | |
| CCRF-CEM | -6.24 | -5.17 | > -4.00 |
| HL-60(TB) | -5.81 | -5.18 | > -4.00 |
| K-562 | -5.81 | > -4.00 | > -4.00 |
| MOLT-4 | -6.46 | -5.00 | > -4.00 |
| RPMI-8226 | -6.14 | -5.49 | > -4.00 |
| SR | -6.38 | -5.28 | > -4.00 |
| Non-Small Cell Lung Cancer | | | |
| A549/ATCC | -5.52 | > -4.00 | > -4.00 |
| EKVX | -5.49 | > -4.00 | > -4.00 |
| HOP-62 | -5.76 | -5.41 | -5.07 |
| HOP-92 | -5.78 | | |
| NCI-H226 | -5.83 | -5.51 | |
| NCI-H23 | -5.68 | | > -4.00 |
| NCI-H322M | -5.69 | | > -4.00 |
| NCI-H460 | -5.73 | -5.42 | -5.11 |
| NCI-H522 | -5.82 | -5.49 | -5.15 |
| Colon Cancer | | | |
| COLO 205 | -5.72 | -5.43 | |
| HCC-2998 | -5.71 | -5.45 | -5.19 |
| HCT-116 | -6.04 | | |
| HCT-15 | -5.85 | -5.47 | |
| HT29 | -5.76 | -5.46 | -5.17 |
| KM12 | -5.68 | | > -4.00 |
| SW-620 | -5.66 | | > -4.00 |
| CNS Cancer | | | |
| SF-268 | -5.77 | -5.27 | > -4.00 |
| SF-295 | -5.66 | | > -4.00 |
| SF-539 | -5.78 | -5.49 | |
| SNB-19 | -5.63 | | > -4.00 |
| SNB-75 | -5.64 | -5.01 | > -4.00 |
| U251 | -5.84 | -5.52 | -5.19 |
| Melanoma | | | |
| LOX IMVI | -5.90 | -5.57 | -5.24 |
| MALME-3M | -5.70 | | > -4.00 |
| M14 | -5.84 | | |
| MDA-MB-435 | -5.88 | | > -4.00 |
| SK-MEL-2 | -5.82 | -5.49 | -5.17 |
| SK-MEL-28 | -5.81 | -5.49 | |
| SK-MEL-5 | -5.73 | -5.40 | -5.07 |
| UACC-257 | -5.71 | -5.29 | > -4.00 |
| UACC-62 | -5.84 | -5.53 | |
| Ovarian Cancer | | | |
| IGROV1 | -5.79 | -5.48 | |
| OVCAR-3 | -5.91 | -5.50 | |
| OVCAR-4 | -5.81 | -5.42 | |
| OVCAR-5 | -5.69 | | |
| OVCAR-8 | -5.79 | -5.22 | -4.28 |
| NCI/ADR-RES | -5.66 | -5.05 | > -4.00 |
| SK-OV-3 | -5.54 | | > -4.00 |
| Renal Cancer | | | |
| 786-0 | -5.81 | | |
| ACHN | -5.81 | | |
| CAKI-1 | -5.57 | > -4.00 | > -4.00 |
| RXF 393 | -5.93 | -5.59 | -5.24 |
| SN12C | -5.89 | | |
| TK-10 | -5.65 | -5.24 | > -4.00 |
| UO-31 | -5.96 | | |
| Prostate Cancer | | | |
| PC-3 | -5.84 | | |
| DU-145 | -5.65 | > -4.00 | > -4.00 |
| Breast Cancer | | | |
| MCF7 | -5.67 | | > -4.00 |
| MDA-MB-231/ATCC | -5.72 | -5.33 | > -4.00 |
| HS 578T | -5.73 | -5.24 | > -4.00 |
| BT-549 | -5.89 | -5.49 | |
| T-47D | -5.68 | -5.02 | > -4.00 |
| MDA-MB-468 | -5.68 | -5.24 | > -4.00 |
| MID | -5.79 | -5.19 | -4.3 |
| Delta | 0.67 | 0.4 | 0.94 |
| Range | 0.97 | 1.59 | 1.24 |



**14x**

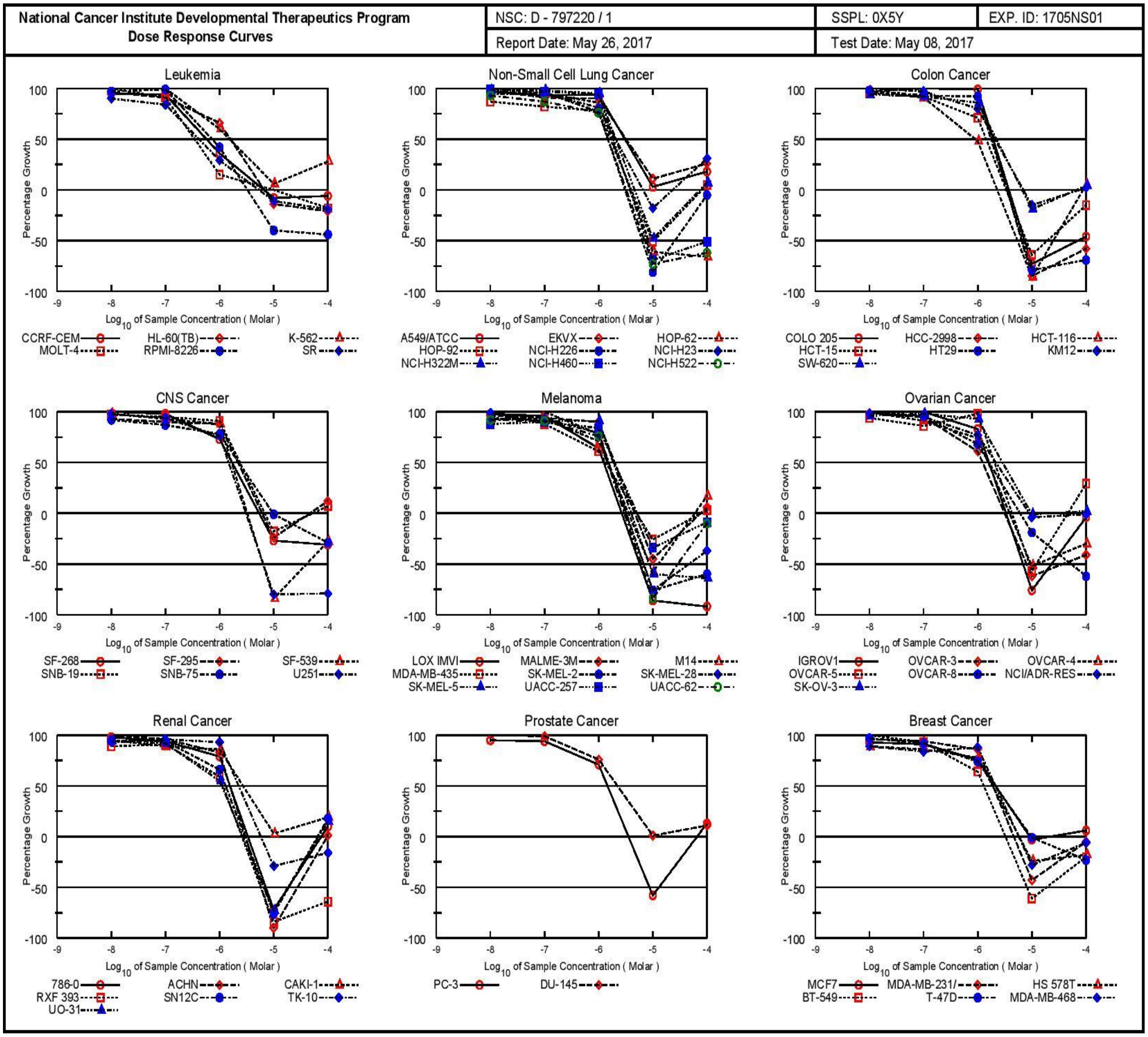
## Table-32

### National Cancer Institute Developmental Therapeutics Program
### In-Vitro Testing Results

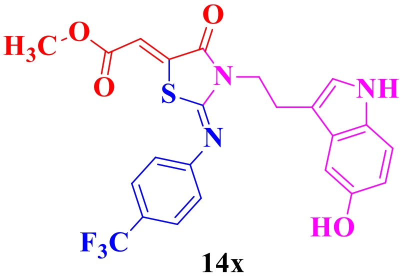
| | | | |
|---|---|---|---|
| NSC : D - 797220 / 1 | Experiment ID : 1705NS01 | Test Type : 08 | Units : Molar |
| Report Date : May 26, 2017 | Test Date : May 08, 2017 | QNS : | MC : |
| COMI : Ser-4TF-DM | Stain Reagent : SRB Dual-Pass Related | SSPL : 0X5Y | |

| | | | | | | | | Log10 Concentration | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Time | | | | Mean Optical Densities | | | | | | Percent Growth | | | | | | |
| Panel/Cell Line | Zero | Ctrl | -8.0 | -7.0 | -6.0 | -5.0 | -4.0 | -8.0 | -7.0 | -6.0 | -5.0 | -4.0 | | GI50 | TGI | LC50 |
| Leukemia | | | | | | | | | | | | | | | | |
| CCRF-CEM | 0.541 | 2.312 | 2.225 | 2.200 | 1.176 | 0.500 | 0.509 | 95 | 94 | 36 | -8 | -6 | | 5.69E-7 | 6.69E-6 | > 1.00E-4 |
| HL-60(TB) | 0.638 | 2.765 | 2.679 | 2.563 | 2.032 | 0.548 | 0.504 | 96 | 91 | 66 | -14 | -21 | | 1.57E-6 | 6.65E-6 | > 1.00E-4 |
| K-562 | 0.262 | 2.255 | 2.266 | 2.288 | 1.467 | 0.379 | 0.824 | 101 | 102 | 60 | 6 | 28 | | 1.55E-6 | > 1.00E-4 | > 1.00E-4 |
| MOLT-4 | 0.503 | 2.253 | 2.365 | 2.102 | 0.769 | 0.505 | 0.415 | 106 | 91 | 15 | . | -18 | | 3.49E-7 | 1.01E-5 | > 1.00E-4 |
| RPMI-8226 | 0.873 | 2.448 | 2.404 | 2.426 | 1.535 | 0.525 | 0.493 | 97 | 99 | 42 | -40 | -44 | | 7.22E-7 | 3.26E-6 | > 1.00E-4 |
| SR | 0.241 | 1.020 | 0.939 | 0.898 | 0.466 | 0.214 | 0.195 | 90 | 84 | 29 | -11 | -19 | | 4.16E-7 | 5.25E-6 | > 1.00E-4 |
| Non-Small Cell Lung Cancer | | | | | | | | | | | | | | | | |
| A549/ATCC | 0.418 | 2.006 | 1.955 | 1.888 | 1.915 | 0.464 | 0.703 | 97 | 93 | 94 | 3 | 18 | | 3.05E-6 | > 1.00E-4 | > 1.00E-4 |
| EKVX | 0.754 | 1.988 | 1.960 | 1.878 | 1.859 | 0.894 | 1.072 | 98 | 91 | 90 | 11 | 26 | | 3.20E-6 | > 1.00E-4 | > 1.00E-4 |
| HOP-62 | 0.550 | 1.593 | 1.594 | 1.541 | 1.442 | 0.217 | 0.185 | 100 | 95 | 86 | -61 | -66 | | 1.75E-6 | 3.85E-6 | 8.47E-6 |
| HOP-92 | 1.034 | 1.518 | 1.455 | 1.431 | 1.413 | 0.507 | 1.058 | 87 | 82 | 78 | -51 | 5 | | 1.65E-6 | . | . |
| NCI-H226 | 0.909 | 2.770 | 2.685 | 2.705 | 2.340 | 0.172 | 0.863 | 95 | 96 | 77 | -81 | -5 | | 1.48E-6 | 3.07E-6 | . |
| NCI-H23 | 0.652 | 2.317 | 2.224 | 2.238 | 2.008 | 0.533 | 1.170 | 94 | 95 | 81 | -18 | 31 | | 2.07E-6 | . | > 1.00E-4 |
| NCI-H322M | 0.600 | 1.713 | 1.685 | 1.690 | 1.654 | 0.311 | 0.676 | 98 | 98 | 95 | -48 | 7 | | 2.05E-6 | . | > 1.00E-4 |
| NCI-H460 | 0.238 | 2.152 | 2.131 | 2.093 | 2.045 | 0.075 | 0.118 | 99 | 97 | 94 | -69 | -51 | | 1.87E-6 | 3.79E-6 | 7.68E-6 |
| NCI-H522 | 0.985 | 2.503 | 2.397 | 2.309 | 2.143 | 0.269 | 0.371 | 93 | 87 | 76 | -73 | -62 | | 1.50E-6 | 3.25E-6 | 7.04E-6 |
| Colon Cancer | | | | | | | | | | | | | | | | |
| COLO 205 | 0.464 | 1.604 | 1.636 | 1.599 | 1.593 | 0.124 | 0.250 | 103 | 100 | 99 | -73 | -46 | | 1.93E-6 | 3.76E-6 | . |
| HCC-2998 | 1.045 | 3.054 | 3.003 | 3.086 | 3.147 | 0.156 | 0.444 | 97 | 102 | 105 | -85 | -58 | | 1.94E-6 | 3.56E-6 | 6.53E-6 |
| HCT-116 | 0.166 | 1.370 | 1.340 | 1.268 | 0.748 | 0.023 | 0.225 | 97 | 91 | 48 | -86 | 5 | | 9.13E-7 | . | . |
| HCT-15 | 0.314 | 1.915 | 1.842 | 1.785 | 1.449 | 0.114 | 0.266 | 95 | 92 | 71 | -64 | -15 | | 1.43E-6 | 3.36E-6 | . |
| HT29 | 0.263 | 1.695 | 1.672 | 1.600 | 1.576 | 0.054 | 0.083 | 98 | 93 | 92 | -79 | -69 | | 1.75E-6 | 3.43E-6 | 6.73E-6 |
| KM12 | 0.325 | 1.627 | 1.694 | 1.592 | 1.369 | 0.276 | 0.358 | 105 | 97 | 80 | -15 | 2 | | 2.07E-6 | . | > 1.00E-4 |
| SW-620 | 0.297 | 1.998 | 1.900 | 1.855 | 1.753 | 0.242 | 0.365 | 94 | 92 | 86 | -19 | 4 | | 2.19E-6 | . | > 1.00E-4 |
| CNS Cancer | | | | | | | | | | | | | | | | |
| SF-268 | 0.502 | 1.665 | 1.668 | 1.638 | 1.350 | 0.366 | 0.345 | 100 | 98 | 73 | -27 | -31 | | 1.69E-6 | 5.36E-6 | > 1.00E-4 |
| SF-295 | 0.687 | 2.272 | 2.156 | 2.116 | 2.080 | 0.526 | 0.876 | 93 | 90 | 88 | -24 | 12 | | 2.19E-6 | . | > 1.00E-4 |
| SF-539 | 0.811 | 2.251 | 2.227 | 2.154 | 2.079 | 0.133 | 0.587 | 98 | 93 | 88 | -84 | -28 | | 1.67E-6 | 3.26E-6 | . |
| SNB-19 | 0.439 | 1.775 | 1.731 | 1.706 | 1.651 | 0.358 | 0.535 | 97 | 95 | 91 | -18 | 7 | | 2.36E-6 | . | > 1.00E-4 |
| SNB-75 | 0.714 | 1.855 | 1.762 | 1.703 | 1.607 | 0.706 | 0.508 | 92 | 87 | 78 | -1 | -29 | | 2.27E-6 | 9.68E-6 | > 1.00E-4 |
| U251 | 0.277 | 1.280 | 1.259 | 1.225 | 1.036 | 0.055 | 0.060 | 98 | 94 | 76 | -80 | -79 | | 1.46E-6 | 3.05E-6 | 6.39E-6 |
| Melanoma | | | | | | | | | | | | | | | | |
| LOX IMVI | 0.436 | 2.669 | 2.625 | 2.577 | 1.872 | 0.062 | 0.034 | 98 | 96 | 64 | -86 | -92 | | 1.25E-6 | 2.68E-6 | 5.78E-6 |
| MALME-3M | 0.632 | 1.396 | 1.363 | 1.348 | 1.318 | 0.349 | 0.682 | 96 | 94 | 90 | -45 | 6 | | 1.97E-6 | . | > 1.00E-4 |
| M14 | 0.436 | 1.554 | 1.473 | 1.478 | 1.226 | 0.183 | 0.626 | 93 | 93 | 71 | -58 | 17 | | 1.45E-6 | . | . |
| MDA-MB-435 | 0.353 | 1.797 | 1.792 | 1.623 | 1.228 | 0.261 | 0.402 | 100 | 88 | 61 | -26 | 3 | | 1.32E-6 | . | > 1.00E-4 |
| SK-MEL-2 | 1.246 | 2.349 | 2.360 | 2.418 | 2.108 | 0.301 | 0.504 | 101 | 106 | 78 | -76 | -60 | | 1.52E-6 | 3.22E-6 | 6.79E-6 |
| SK-MEL-28 | 0.629 | 1.933 | 1.922 | 1.845 | 1.663 | 0.151 | 0.398 | 99 | 93 | 79 | -76 | -37 | | 1.54E-6 | 3.24E-6 | . |
| SK-MEL-5 | 0.953 | 2.170 | 2.101 | 2.065 | 2.058 | 0.378 | 0.340 | 94 | 91 | 91 | -60 | -64 | | 1.86E-6 | 3.99E-6 | 8.54E-6 |
| UACC-257 | 1.164 | 2.274 | 2.138 | 2.163 | 2.092 | 0.774 | 1.061 | 88 | 90 | 84 | -34 | -9 | | 1.94E-6 | 5.17E-6 | > 1.00E-4 |
| UACC-62 | 0.739 | 2.582 | 2.429 | 2.420 | 2.131 | 0.122 | 0.666 | 92 | 91 | 76 | -84 | -10 | | 1.45E-6 | 2.98E-6 | . |
| Ovarian Cancer | | | | | | | | | | | | | | | | |
| IGROV1 | 0.421 | 1.720 | 1.715 | 1.724 | 1.502 | 0.101 | 0.405 | 100 | 100 | 83 | -76 | -4 | | 1.62E-6 | 3.33E-6 | . |
| OVCAR-3 | 0.385 | 1.397 | 1.386 | 1.344 | 1.005 | 0.146 | 0.226 | 99 | 95 | 61 | -62 | -41 | | 1.23E-6 | 3.13E-6 | . |
| OVCAR-4 | 0.539 | 1.208 | 1.211 | 1.151 | 1.031 | 0.258 | 0.377 | 100 | 91 | 74 | -52 | -30 | | 1.54E-6 | 3.84E-6 | . |
| OVCAR-5 | 0.650 | 1.513 | 1.462 | 1.395 | 1.496 | 0.282 | 0.898 | 94 | 86 | 98 | -57 | 29 | | 2.04E-6 | . | . |
| OVCAR-8 | 0.422 | 1.665 | 1.679 | 1.614 | 1.273 | 0.341 | 0.160 | 101 | 96 | 68 | -19 | -62 | | 1.62E-6 | 6.04E-6 | 5.23E-5 |
| NCI/ADR-RES | 0.584 | 2.030 | 2.006 | 1.952 | 1.700 | 0.562 | 0.578 | 98 | 95 | 77 | -4 | -1 | | 2.16E-6 | 8.96E-6 | > 1.00E-4 |
| SK-OV-3 | 1.067 | 2.076 | 2.038 | 2.051 | 2.006 | 1.054 | 1.090 | 96 | 98 | 93 | -1 | 2 | | 2.86E-6 | . | > 1.00E-4 |
| Renal Cancer | | | | | | | | | | | | | | | | |
| 786-0 | 0.550 | 2.262 | 2.234 | 2.166 | 1.907 | 0.157 | 0.719 | 98 | 94 | 79 | -72 | 10 | | 1.56E-6 | . | . |
| ACHN | 0.415 | 1.663 | 1.713 | 1.617 | 1.451 | 0.044 | 0.425 | 104 | 96 | 83 | -90 | 1 | | 1.55E-6 | . | . |
| CAKI-1 | 0.533 | 2.449 | 2.346 | 2.245 | 2.178 | 0.593 | 0.900 | 95 | 89 | 86 | 3 | 19 | | 2.71E-6 | > 1.00E-4 | > 1.00E-4 |
| RXF 393 | 1.057 | 1.628 | 1.567 | 1.576 | 1.396 | 0.170 | 0.384 | 89 | 91 | 59 | -84 | -64 | | 1.16E-6 | 2.59E-6 | 5.79E-6 |
| SN12C | 0.438 | 1.732 | 1.667 | 1.656 | 1.288 | 0.104 | 0.670 | 95 | 94 | 66 | -76 | 18 | | 1.29E-6 | . | . |
| TK-10 | 0.816 | 1.735 | 1.672 | 1.699 | 1.672 | 0.576 | 0.684 | 93 | 96 | 93 | -29 | -16 | | 2.25E-6 | 5.76E-6 | > 1.00E-4 |
| UO-31 | 0.545 | 1.995 | 1.908 | 1.884 | 1.348 | 0.148 | 0.758 | 94 | 92 | 55 | -73 | 15 | | 1.10E-6 | . | . |
| Prostate Cancer | | | | | | | | | | | | | | | | |
| PC-3 | 0.568 | 1.924 | 1.858 | 1.845 | 1.526 | 0.237 | 0.741 | 95 | 94 | 71 | -58 | 13 | | 1.45E-6 | . | . |
| DU-145 | 0.400 | 1.475 | 1.514 | 1.466 | 1.215 | 0.410 | 0.517 | 104 | 99 | 76 | 1 | 11 | | 2.21E-6 | > 1.00E-4 | > 1.00E-4 |
| Breast Cancer | | | | | | | | | | | | | | | | |
| MCF7 | 0.901 | 3.186 | 3.016 | 2.981 | 2.637 | 0.874 | 1.031 | 93 | 91 | 76 | -3 | 6 | | 2.13E-6 | . | > 1.00E-4 |
| MDA-MB-231/ATCC | 0.625 | 1.612 | 1.570 | 1.552 | 1.477 | 0.359 | 0.597 | 96 | 94 | 86 | -43 | -5 | | 1.91E-6 | 4.67E-6 | > 1.00E-4 |
| HS 578T | 0.868 | 1.595 | 1.516 | 1.491 | 1.434 | 0.657 | 0.708 | 89 | 86 | 78 | -24 | -18 | | 1.87E-6 | 5.78E-6 | > 1.00E-4 |
| BT-549 | 0.896 | 1.862 | 1.859 | 1.795 | 1.514 | 0.350 | 0.713 | 100 | 93 | 64 | -61 | -20 | | 1.29E-6 | 3.25E-6 | . |
| T-47D | 0.751 | 1.535 | 1.510 | 1.472 | 1.329 | 0.742 | 0.575 | 97 | 92 | 74 | -1 | -23 | | 2.07E-6 | 9.64E-6 | > 1.00E-4 |
| MDA-MB-468 | 0.774 | 1.309 | 1.250 | 1.224 | 1.242 | 0.557 | 0.726 | 89 | 84 | 88 | -28 | -6 | | 2.11E-6 | 5.72E-6 | > 1.00E-4 |

## Table-33

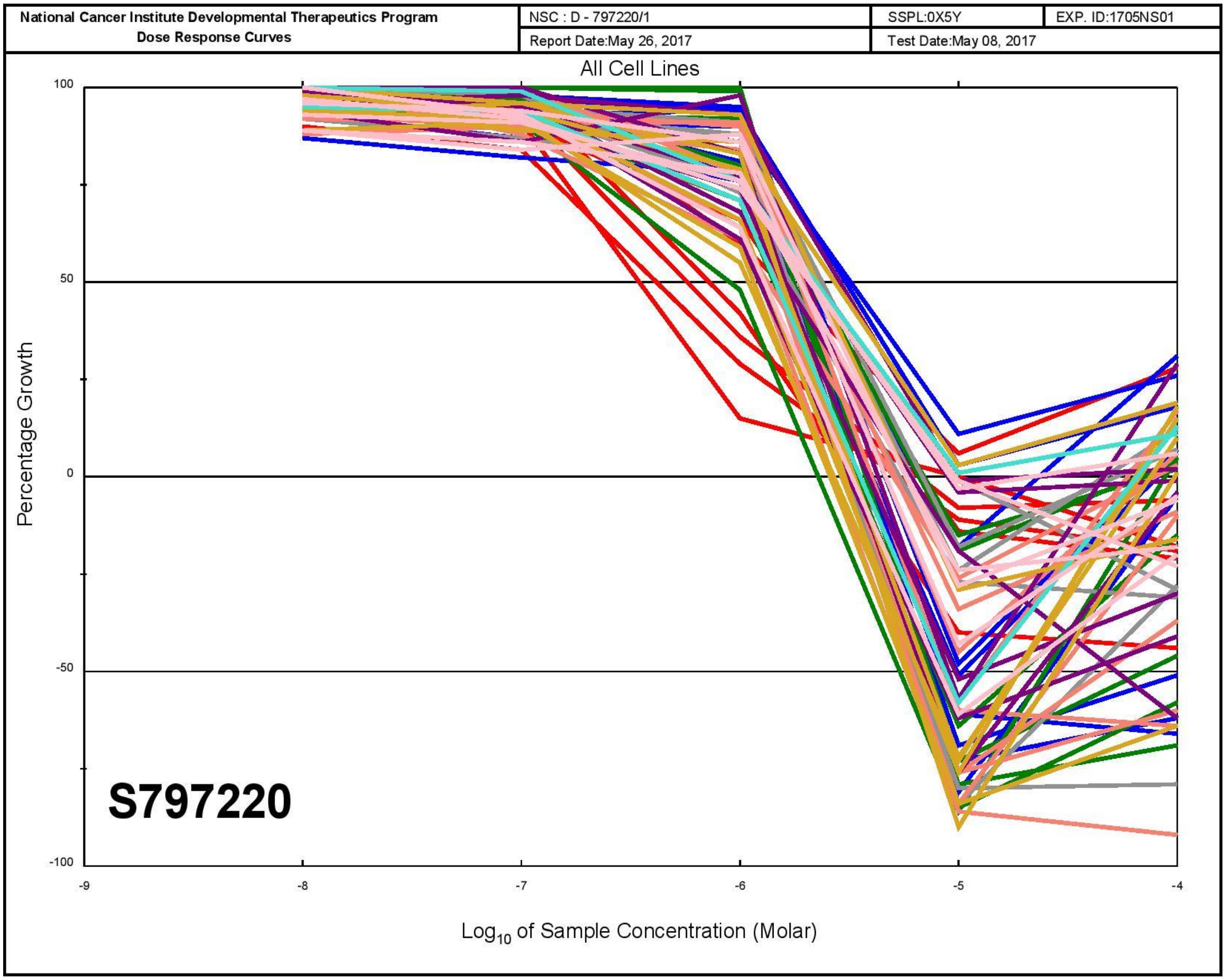


| National Cancer Institute Developmental Therapeutics Program<br>Dose Response Curves | NSC: D - 797220 / 1 | SSPL: 0X5Y | EXP. ID: 1705NS01 |
|---|---|---|---|
| | Report Date: May 26, 2017 | Test Date: May 08, 2017 | |

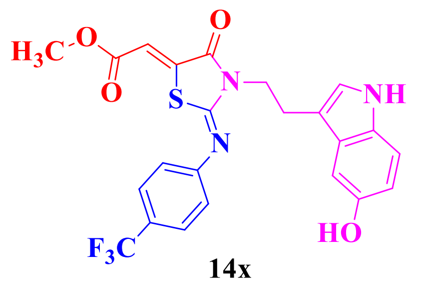


**14x**

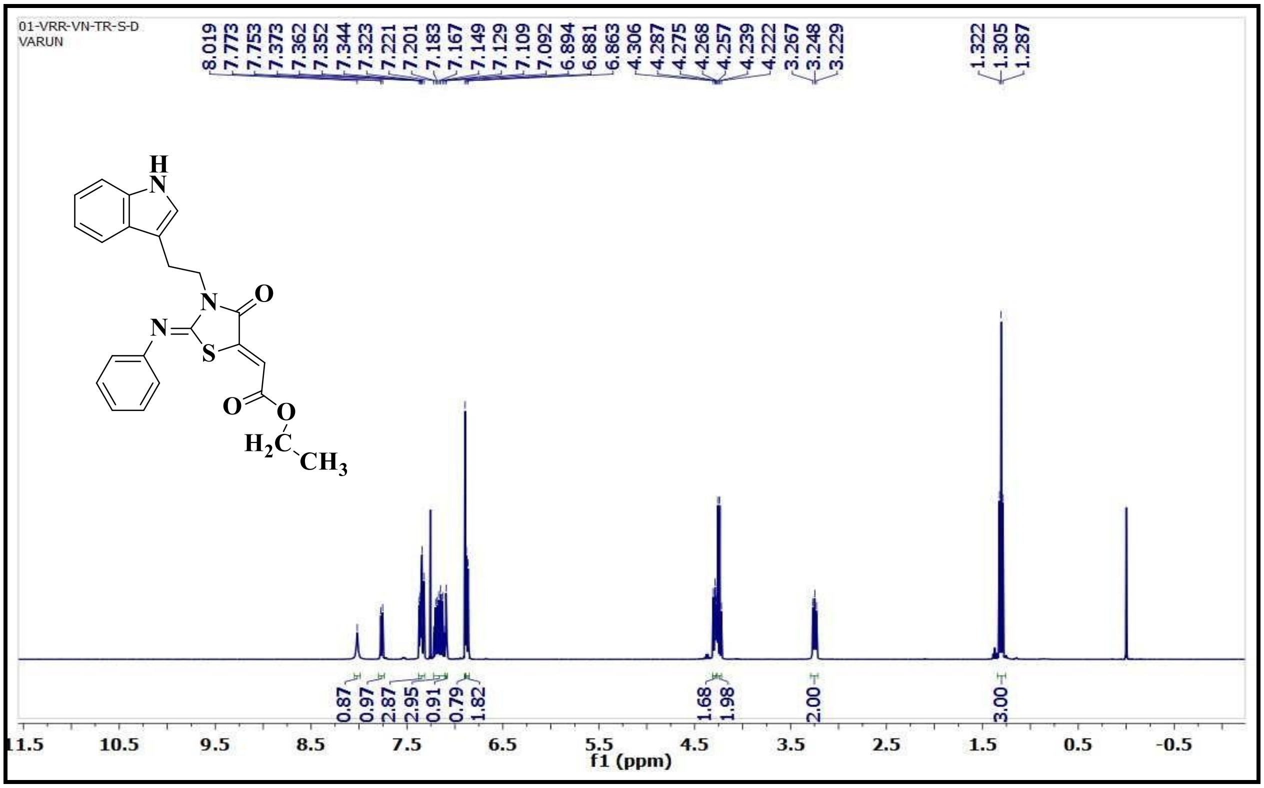
# Table-34



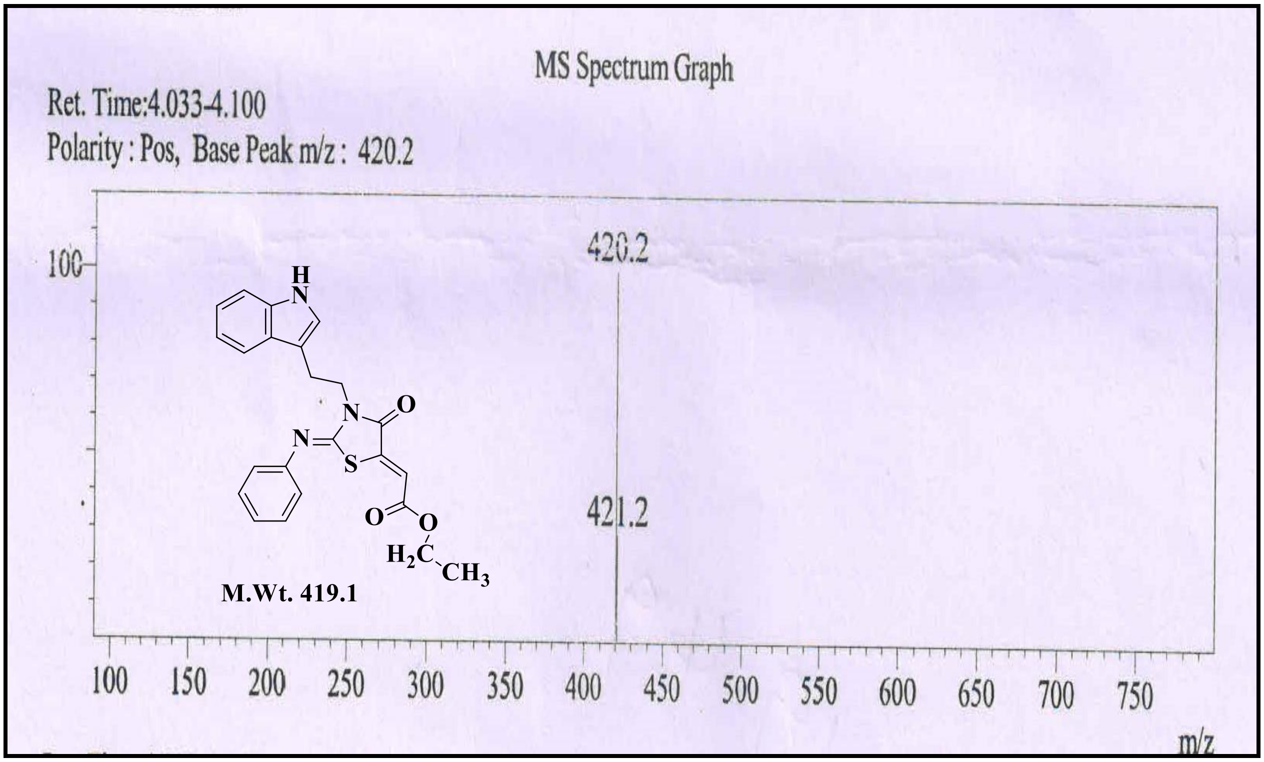
| National Cancer Institute Developmental Therapeutics Program<br>Dose Response Curves | NSC : D - 797220/1 | SSPL:0X5Y | EXP. ID:1705NS01 |
|---|---|---|---|
| | Report Date:May 26, 2017 | Test Date:May 08, 2017 | |



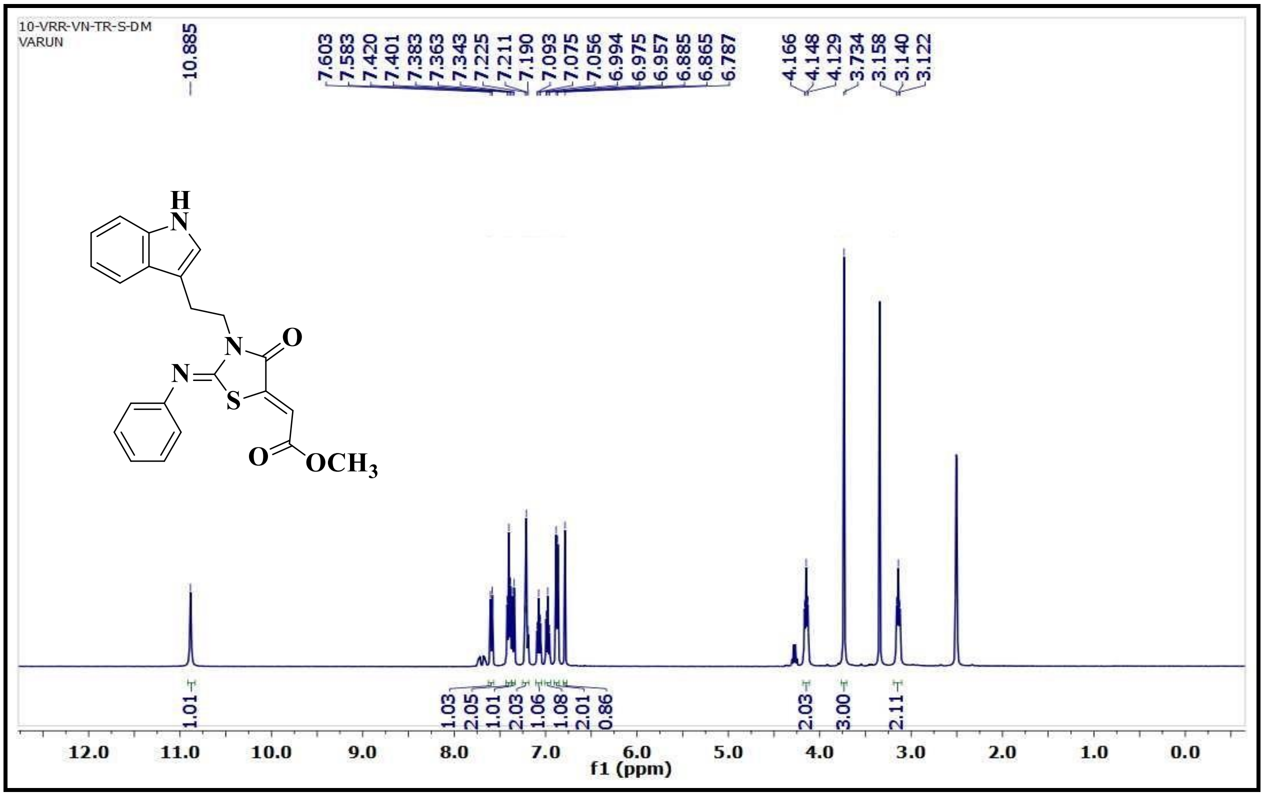
**14x**



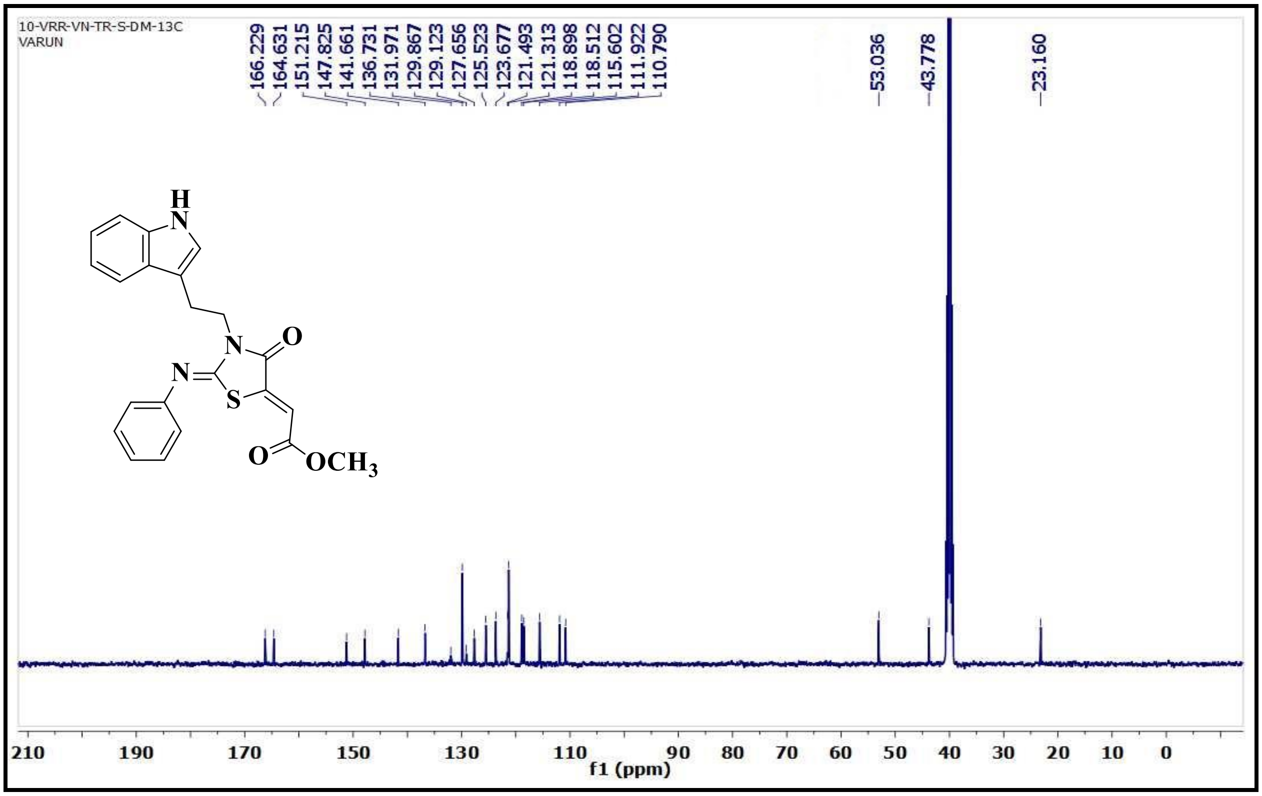
$^1$H NMR Spectrum of compound **14a**



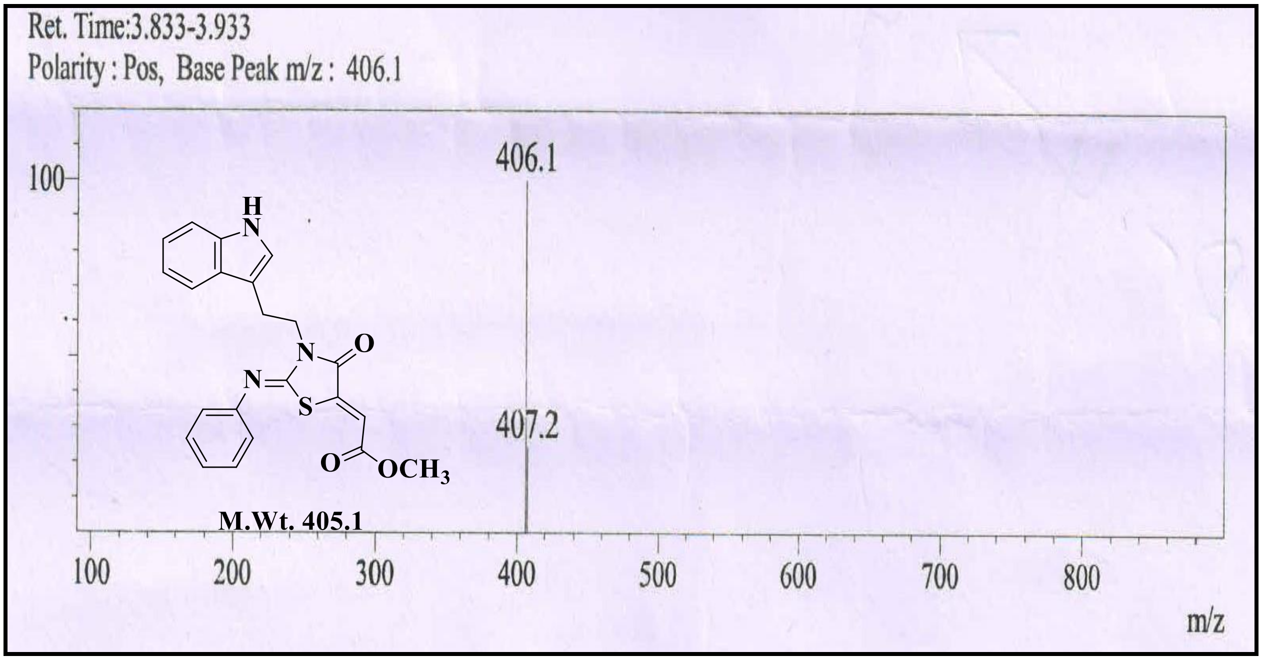
Mass Spectrum of compound **14a**



$^{1}$H NMR Spectrum of compound **14b**



$^{13}$C NMR Spectrum of compound **14b**



Mass Spectrum of compound **14b**

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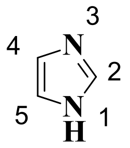
# CHAPTER-VI

# ONE-POT THREE COMPONENT SYNTHESIS OF 2-((1,4-DIPHENYL-1*H*-IMIDAZOL-2-YL)THIO)-1-PHENYLETHANONES AND THEIR BIOLOGICAL EVALUATION: ANTICANCER, ANTIMICROBIAL AND ANTIVIRAL ACTIVITY AND SYNTHESIS AND ANTICANCER ACTIVITY OF 2-(((1,4-DIPHENYL-1*H*-IMIDAZOL-2-YL)THIO)METHYL)-3,5-DIMETHYLPYRIDINES

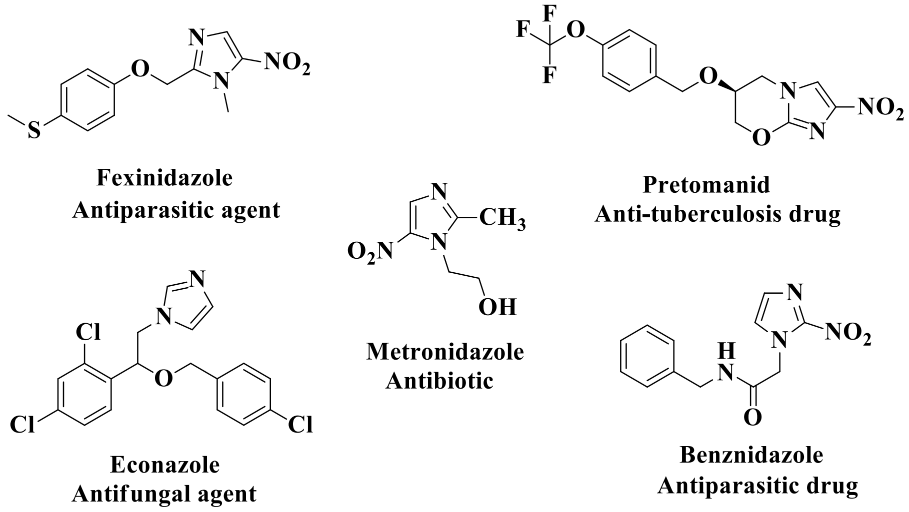
## INTRODUCTION

Imidazole is a five-membered aromatic heterocyclic ring with the molecular formula $C_3H_4N_2$. In which two nitrogen atoms are arranged at one and three positions (**1**) and it is called “1,3-diazole”. It can act as a base and as a weak acid. Generally, imidazole is classified as an alkaloid. This type of ring system is present in some important biological building blocks like histamine, histidine, biotin, nucleic acid, and alkaloids.



**(1)**

Imidazole ring has been of great interest for organic chemists due to its biological and pharmacological properties and it plays an important role in biochemical processes.[1] It shows antibacterial,[2] antifungal,[3-6] anti-inflammatory, fungicidal, herbicidal[7,] antitumor,[8] and plant growth, regulator[9] properties. They act as inhibitors of cyclooxygenase-2 (COX-2),[10] B-Raf kinase,[11] transforming growth factor b1 (TGF-b1) type 1 active in receptor-like kinase (ALK5),[12] p38 MAP kinase,[13] biosynthesis of interleukin-1 (IL-1)[14] and also substituted imidazoles are also extensively used as glucagon receptors[15] and CB1 cannabinoid receptor antagonists,[16] modulators of P-glycoprotein (P-gp)-mediated multidrug resistance (MDR).[17] Imidazoles as ionic liquids and imidazoles related N- heterocyclic carbenes are well known green solvents.



**Fexinidazole**
**Antiparasitic agent**

**Pretomanid**
**Anti-tuberculosis drug**

**Metronidazole**
**Antibiotic**

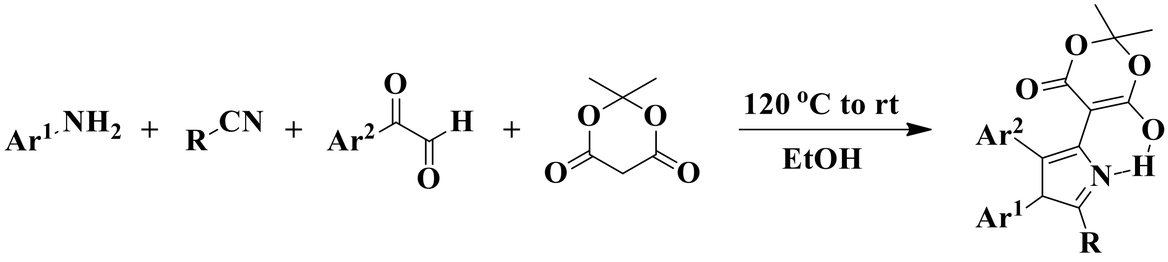
**Econazole**
**Antifungal agent**

**Benznidazole**
**Antiparasitic drug**

**Fig. 1** Some of the imidazole ring-containing drug molecules.

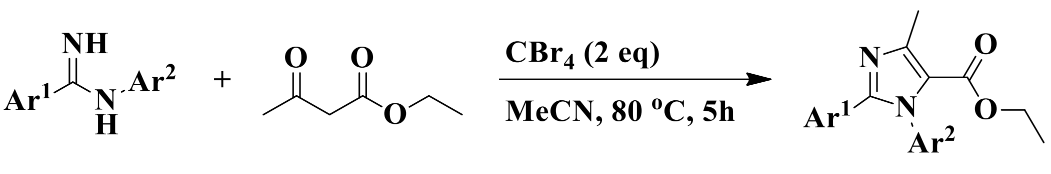
The following is a brief literature review on the synthesis of imidazoles.

Mehrabi[18] *et al.* described the synthesis of 1,2,4,5-tetrasubstituted imidazoles **(2)** through the reaction of arylamines, benzonitriles, arylglyoxals, and meldrum's acid in presence of ethanol.



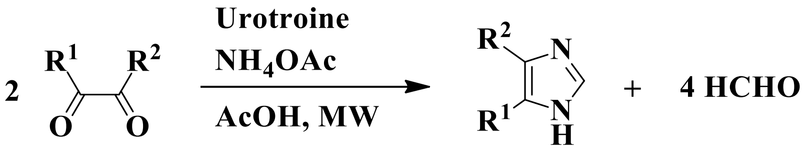
**2**

A metal-free synthesis of 1,2,4,5-tetrasubstituted imidazole derivatives **(3)** was reported by Zhang[19] *et al.* by the reaction of amidines with ethyl acetoacetate via oxidative cyclization in the presence of bromide.



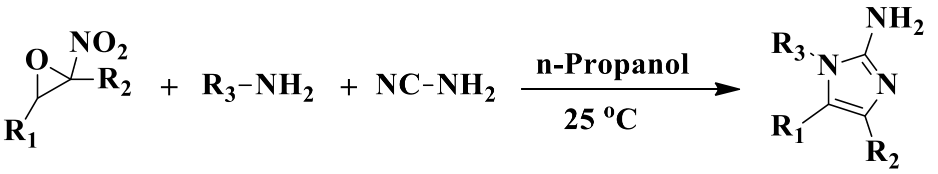
**3**

Bratulescu[20] described the microwave-assisted synthesis of 4,5-substituted imidazoles **(4)** starting from 1,2-diketones and urotropine in presence of ammonium acetate.



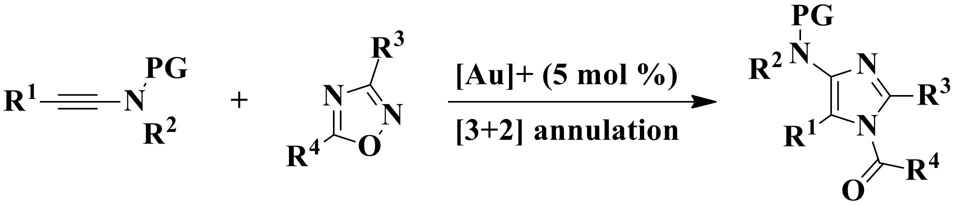
**4**

Guo[21] *et al.* reported the synthesis of 2-aminoimidazoles **(5)** under mild conditions via a one-pot three-component reaction of α-nitroepoxides with amines and cyanamide in presence of propanol.



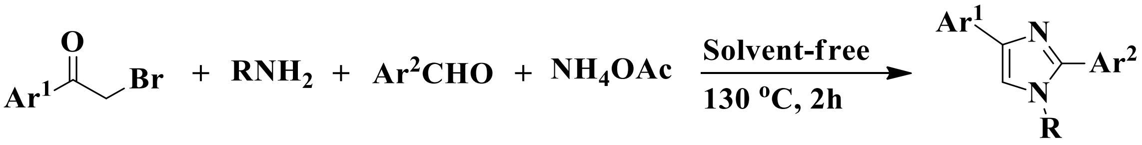
**5**

The synthesis of substituted 4-aminoimidazoles **(6)** was reported by Zeng[22] *et al.* via gold-catalyzed selective [3 + 2]annulation of 1,2,4-oxadiazoles with ynamides. The reaction proceeds with atom economy and high yields.



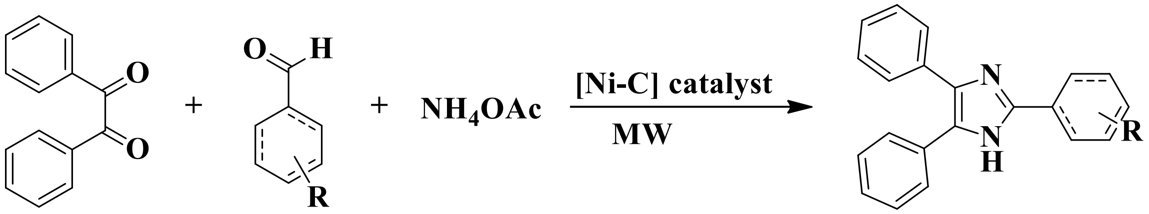
**6**

Adib[23] *et al.* described the synthesis of *N*-substituted 2,4-diarylimidazoles **(7)** from the four-component reaction of 2-bromoacetophenone, with an aldehyde, primary amine, and ammonium acetate under solvent-free conditions.



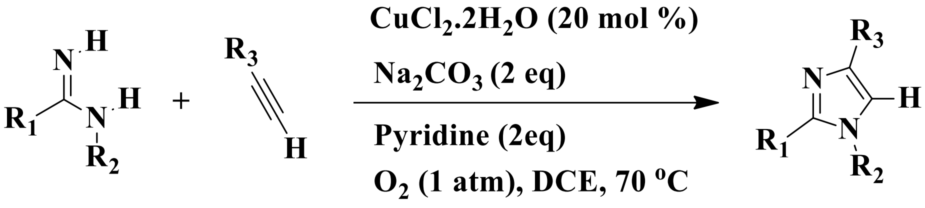
**7**

Chundawat[24] *et al.* reported the microwave-assisted synthesis of 2,4,5-trisubstituted imidazole derivatives **(8)** by the reaction of various aldehydes with benzil and ammonium acetate in presence of the Ni-C catalyst.



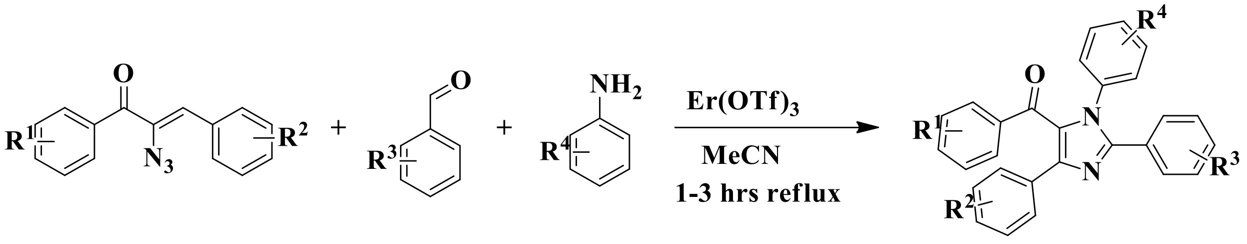
**8**

Li and Neuville[25] developed the copper-catalyzed synthesis of 1,2,4-trisubstituted imidazoles **(9)** from amidines and terminal alkynes. In this reaction oxidative diamination of terminal alkynes by amidines occurs to give the title compounds.



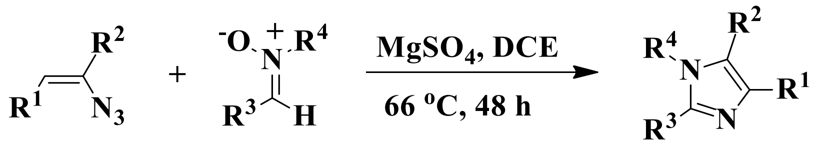
**9**

Rajaguru[26] *et al.* reported the synthesis of highly substituted imidazole derivatives **(10)** from different α-azido chalcones, aryl aldehydes, and anilines in the presence of erbium triflate catalyst.



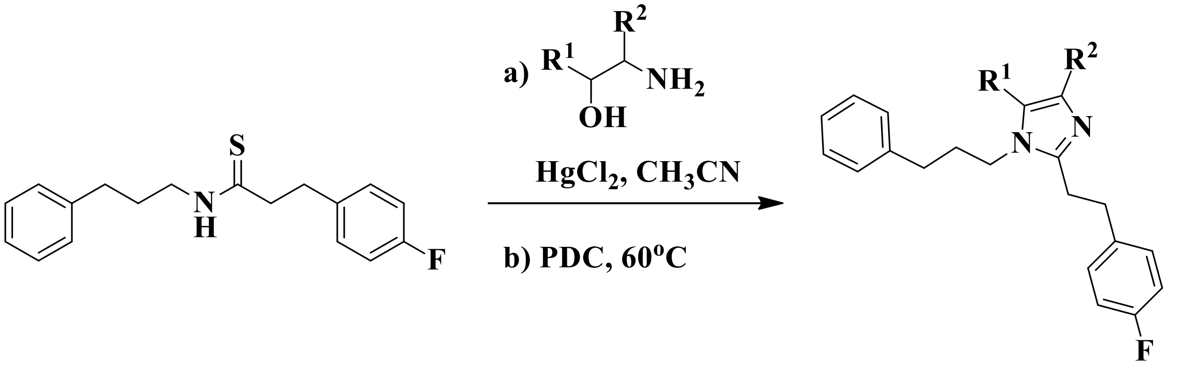
**10**

Hu[27] *et al.* developed a method for synthesis of 1,2,4,5-tetrasubstituted imidazoles **(11)** from the reaction of 2-azido acrylates and nitrones.



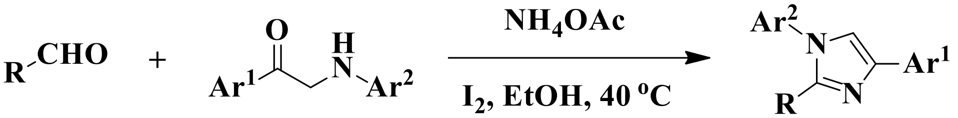
**11**

Paone and Shaw[28] developed reliable regiospecific incorporation of four alkyl substituents on imidazole ring **(12)**. In this reaction an addition of substituted amino alcohol to a thioamide and subsequent oxidation with PDC takes place.



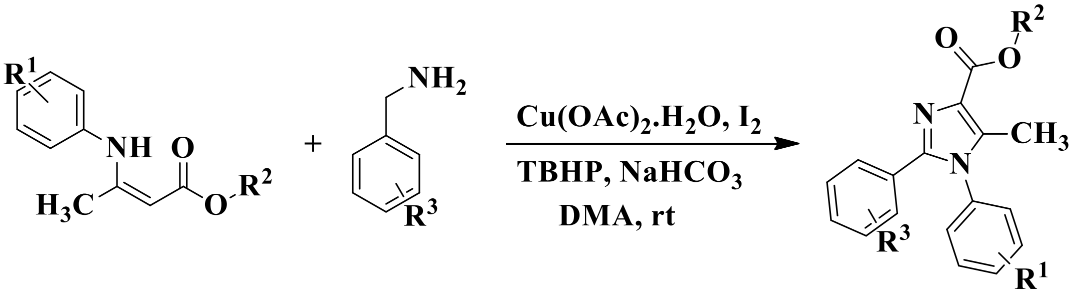
**12**

Tang[29] *et al.* developed the synthesis of 1,2,4-trisubstituted imidazole derivatives **(13)** via three-component [3+1+1] cycloaddition reaction of aldehydes, α-amino carbonyl compounds and ammonium acetate in ethanol using $I_2$ as the catalyst.



**13**

Pandya and Agrawal[30] reported a simple and concise route for the synthesis of highly substituted imidazole derivatives **(14)** by the reaction of (Z)-ethyl 3-(phenylamino) but-2-enoate and in benzylamine presence of Cu catalyst.



**14**

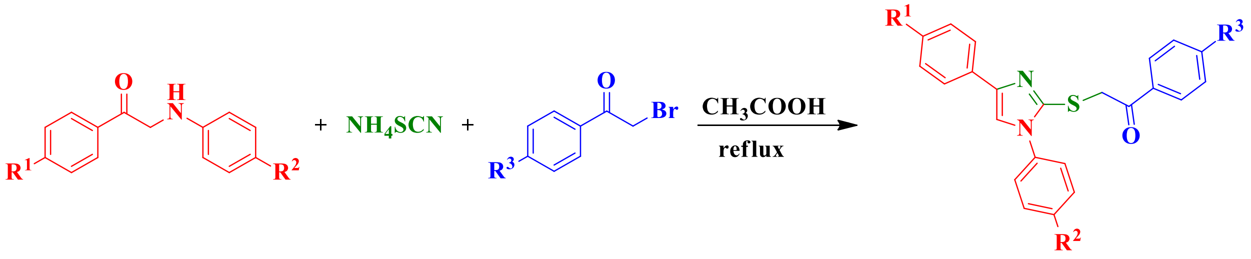
# CHAPTER-VI (SECTION-A)

## ONE-POT THREE COMPONENT SYNTHESIS OF 2-((1,4-DIPHENYL-1*H*-IMIDAZOL-2-YL)THIO)-1-PHENYLETHANONES AND THEIR BIOLOGICAL EVALUATION: ANTICANCER, ANTIMICROBIAL AND ANTIVIRAL ACTIVITY

# SECTION-A

## PRESENT WORK

In the present work, we have synthesized an efficient one-pot, three component novel 2-((1,4-diphenyl-1*H*-imidazol-2-yl)thio)-1-phenylethanones and characterized by their analytical and spectral data. The title compounds were synthesized by the reaction of various substituted 1-aryl-2-(arylamino)ethan-1-ones (**15**), ammonium thiocyanate (**16**) and substituted phenacyl bromide (**17**) in acetic acid under reflux condition, which is shown in **scheme-1.**

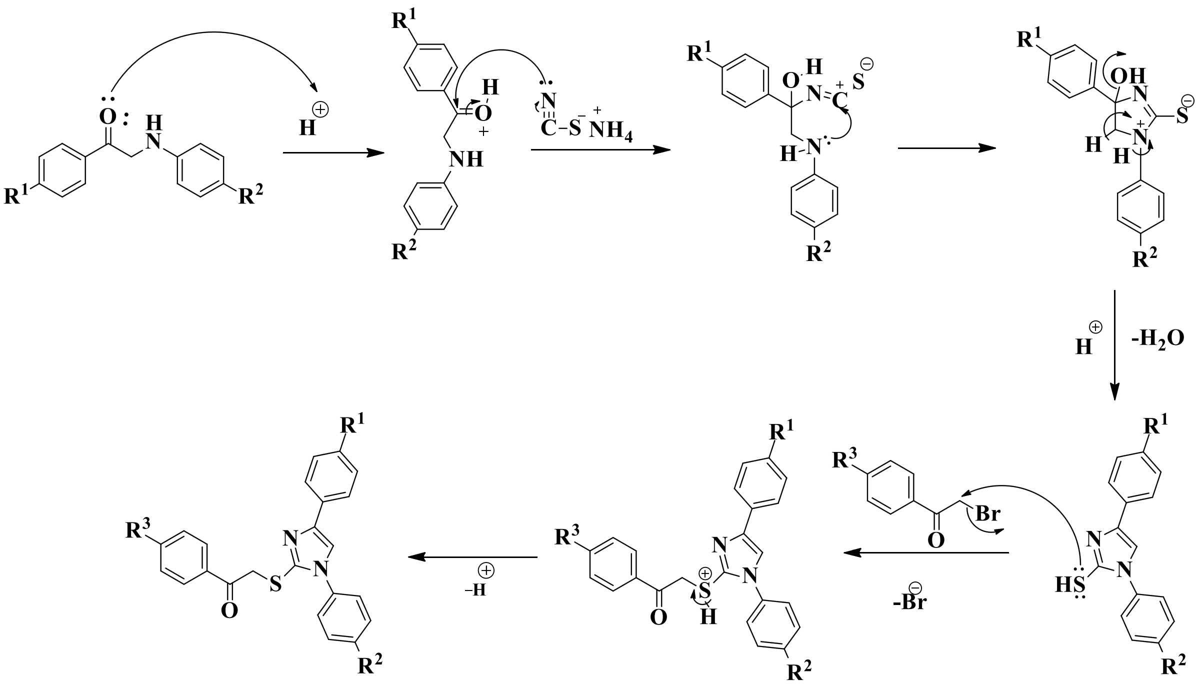


**15 (a-h)** **16** **17 (a-h)** **18 (a-v)**

**Scheme-1.** Synthesis of 2-((1,4-diphenyl-1*H*-imidazol-2-yl)thio)-1-phenylethanones **18 (a-v)**.

### Mechanism

The *N*-substituted imidazole thiones are synthesized using Markwald synthesis. The mechanism for the formation of **18 (a-v)** can be explained as shown in **scheme-2**. The 1-aryl-2-(arylamino)ethan-1-ones on treatment with acid gives the corresponding protonated derivatives. These undergo heterocyclization with ammonium thiocyanate to yield intermediate 2-mercaptoimidazole derivatives. Subsequently, the more nucleophilic thiol group of imidazole derivatives replace the bromine atom of 2-bromo-1-phenylethanone to give final compounds **18 (a-v)**.



**Scheme-2:** plausible mechanism for the formation of **18 (a-v)**

**Table-1:** Reaction time for the formation of product **18 (a-v)**

| S. No. | Compound | $R^1$ | $R^2$ | $R^3$ | Reaction time (h) |
|---|---|---|---|---|---|
| 1 | 18a | Br | $OCH_3$ | $CH_3$ | 2.5 |
| 2 | 18b | Br | $OCH_3$ | Ph | 3.5 |
| 3 | 18c | Br | $OCH_3$ | H | 3 |
| 4 | 18d | Cl | Cl | Br | 5 |
| 5 | 18e | Cl | $OCH_3$ | Cl | 3.5 |
| 6 | 18f | Br | Cl | Br | 5 |
| 7 | 18g | Cl | Cl | Ph | 4.5 |
| 8 | 18h | $CH_3$ | Cl | Br | 3.5 |
| 9 | 18i | H | Cl | $OCH_3$ | 2.5 |
| 10 | 18j | H | Cl | $CH_3$ | 3 |
| 11 | 18k | H | Cl | Ph | 3.5 |
| 12 | 18l | H | Cl | Br | 3.5 |
| 13 | 18m | H | Cl | H | 2.5 |
| 14 | 18n | H | Cl | Cl | 3 |
| 15 | 18o | H | Cl | $NO_2$ | 4.5 |

| | | | | | |
|---|---|---|---|---|---|
| 16 | 18p | $OCH_3$ | Cl | H | 3 |
| 17 | 18q | $OCH_3$ | Cl | $CH_3$ | 2.5 |
| 18 | 18r | $OCH_3$ | Cl | $OCH_3$ | 2 |
| 19 | 18s | $OCH_3$ | Cl | F | 3.5 |
| 20 | 18t | $OCH_3$ | Cl | Cl | 3 |
| 21 | 18u | $OCH_3$ | Cl | Br | 3 |
| 22 | 18v | $OCH_3$ | Cl | $NO_2$ | 4 |

All the newly synthesized compounds were well characterized by their physical and analytical techniques like melting point, NMR, CMR and Mass spectral data. The $^1H$ NMR spectrum of compound **18a** exhibited characteristic peaks at $\delta$ 2.40 (s, 3H, methyl), 3.83 (s, 3H, methoxy), 4.78 (s, 2H, $-S-CH_2$), the aromatic protons appeared in the range of $\delta$ 7.10-8.04 ppm. The $^{13}C$ NMR spectrum of **18a** exhibited characteristic peaks at $\delta$ 21.15 ppm which represents methyl carbon, $\delta$ 40.55 ppm corresponds to $S-CH_2$ carbon, 55.53 ppm represents methoxy carbon and $\delta$ 193.31ppm represents the carbonyl carbon. The mass spectrum of compound **18a** exhibited 493.0572 $[M+H]^+$

## EXPERIMENTAL SECTION

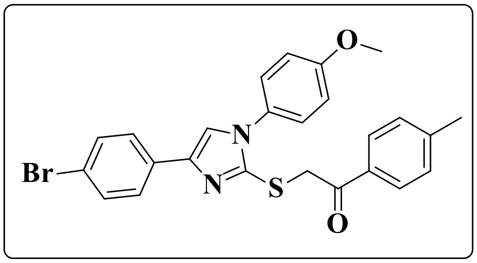
The starting materials 1-aryl-2-(arylamino)ethan-1-ones **15 (a-h)** were prepared as per the procedure[31] by utilizing substituted phenacyl bromides and substituted anilines in presence of ethanol and sodium bicarbonate.

### General procedure for the synthesis of 18 (a-v)

1-Aryl-2-(arylamino)ethan-1-one (1 mmol), excess of ammonium thiocyanate (3 mmol), and phenacyl bromide (1 mmol) were taken in acetic acid and refluxed for the specified time as shown in the table-1, the progress of the reaction was monitored by TLC using ethyl acetate and hexane (1:1) as mobile phase. After completion of the reaction, reaction mixture was cooled to room temperature, the solid separated was filtered, washed with water, dried and recrystallized from ethanol.

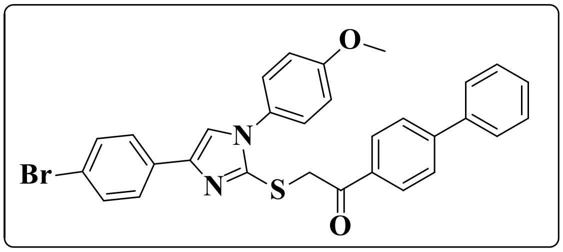
## SPECTRAL DATA

### 2-((4-(4-Bromophenyl)-1-(4-methoxyphenyl)-1*H*-imidazol-2-yl)thio)-1-(p-tolyl)ethanone (18a)



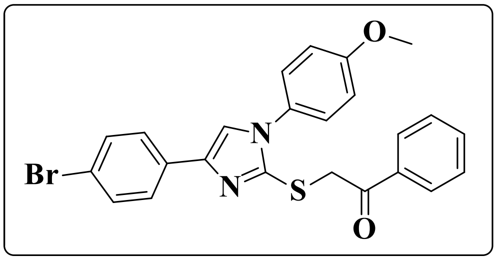
Color: white solid, mp: 222-224 ºC, yield: (0.439g, 89%), FT-IR (KBr, $\nu_{max}$ cm$^{-1}$): 1671 (C=O), 1606 (C=N), **$^1$H NMR (400MHz, DMSO-d$_6$, ppm):** $\delta$ 2.40 (s, 3H, methyl), 3.83 (s, 3H, methoxy), 4.78 (s, 2H, methylene), 7.10 (d, *J*=8.8Hz, 2H, Ar-H), 7.36 (d, *J*=8.4Hz, 2H, Ar-H), 7.44 (d, *J*=9.2Hz, 2H, Ar-H), 7.52 (d, *J*=8.8Hz, 2H, Ar-H), 7.63 (d, *J*=8.4Hz, 2H, Ar-H), 7.91 (d, *J*=8.4Hz, 2H, Ar-H), 8.04 (s, 1H, imidazole) ppm, **$^{13}$C NMR (DMSO-d$_6$):** $\delta$ 21.15, 40.55, 55.53, 114.63, 120.41, 126.42, 126.81, 128.47, 128.85, 129.25, 131.50, 132.96, 138.17, 141.73, 144.12, 159.47, 193.31 ppm; **MS (ESI) *m/z* (%):** 493.057 [M+H]$^+$; Anal. Calcd. for $C_{25}H_{21}BrN_2O_2S$; C, 60.89; H, 4.24; N, 5.63. Found: C, 60.85; H, 4.29; N, 5.68%

### 1-([1,1'-Biphenyl]-4-yl)-2-((4-(4-bromophenyl)-1-(4-methoxyphenyl)-1*H*-imidazol-2-yl)thio) ethanone (18b)



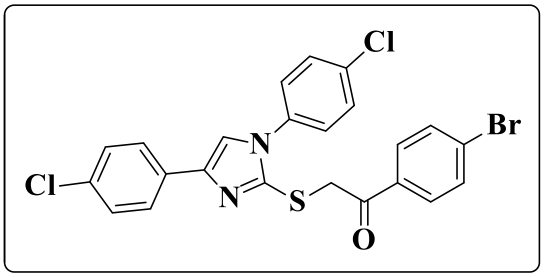
Color: white solid, mp: 247-249 ºC, yield: (0.505g, 91%), FT-IR (KBr, $\nu_{max}$ cm$^{-1}$): 1680 (C=O), 1602 (C=N), **$^1$H NMR (400MHz, DMSO-d$_6$, ppm):** $\delta$ 3.82 (s, 3H, methoxy), 4.83 (s, 2H, methylene), 7.11 (d, *J*=8.8Hz, 2H, Ar-H), 7.44-7.46 (m, 3H, Ar-H), 7.50-7.53 (m, 4H, Ar-H), 7.63 (d, *J*=8.8Hz, 2H, Ar-H), 7.78 (d, *J*=8.8Hz, 2H, Ar-H), 7.86 (d, *J*=8.4Hz, 2H, Ar-H), 8.07-8.10 (m, 3H, Ar-H) ppm, **$^{13}$C NMR (DMSO-d$_6$):** $\delta$ 40.80, 55.54, 114.39, 114.65, 120.43, 120.65, 126.37, 126.56, 126.88, 126.98, 128.47, 128.66, 129.07, 130.89, 131.58, 134.15, 137.60, 138.71, 141.66, 144.95, 159.59,193.31 ppm; **MS (ESI) *m/z* (%):** 555.0731 [M+H]$^+$; Anal. Calcd. for $C_{30}H_{23}BrN_2O_2S$; C, 64.87; H, 4.17; N, 5.04. Found: C, 64.83; H, 4.14; N, 5.12%

### 2-((4-(4-Bromophenyl)-1-(4-methoxyphenyl)-1*H*-imidazol-2-yl)thio)-1-phenylethanone (18c)



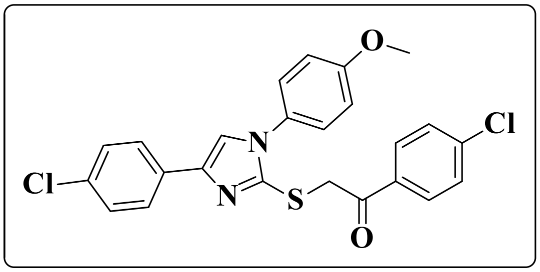
Color: light yellow solid, mp: 192-194 $^o$C, yield: (0.397g, 83%), FT-IR (KBr, $\nu_{max}$ cm$^{-1}$): 1674 (C=O), 1597 (C=N), **$^1$H NMR (400MHz, DMSO-d$_6$, ppm):** $\delta$ 3.83 (s, 3H, methoxy), 4.80 (s, 2H, methylene), 7.10 (d, *J*=9.2Hz, 2H, Ar-H), 7.44 (d, *J*=8.8Hz, 2H, Ar-H), 7.50 (d, *J*=8.4Hz, 2H, Ar-H), 7.54-7.61 (m, 5H, Ar-H), 7.67-7.71 (m, 1H, Ar-H), 8.00-8.03 (m, 2H, Ar-H) ppm, **$^{13}$C NMR (DMSO-d$_6$):** $\delta$ 40.59, 55.54, 113.89, 114.65, 120.14, 120.44, 126.09, 126.43, 126.80, 127.12, 128.35, 128.72, 131.51,131.76, 133.59, 135.49, 138.07, 141.72, 159.49, 193.85 ppm; Anal. Calcd. for $C_{24}H_{19}BrN_2O_2S$; C, 60.13; H, 3.99; N, 5.84. Found: C, 60.17; H, 3.92; N, 5.80%

**2-((1,4-Bis(4-chlorophenyl)-1*H*-imidazol-2-yl)thio)-1-(4-bromophenyl)ethanone (18d)**



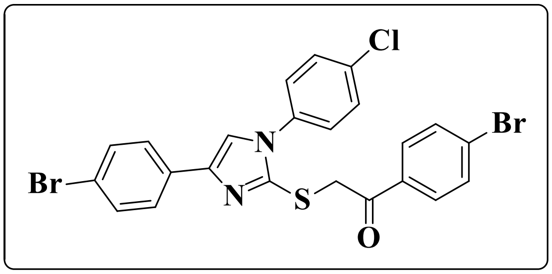
Color: light yellow solid, mp: 211-213 $^o$C, yield: (0.456g, 88%), FT-IR (KBr, $\nu_{max}$ cm$^{-1}$): 1683 (C=O), 1618 (C=N), **$^1$H NMR (400MHz, DMSO-d$_6$, ppm):** $\delta$ 4.77 (s, 2H, methylene), 7.39 (d, *J*=8.4Hz, 2H, Ar-H), 7.57 (d, *J*=8.8Hz, 2H, Ar-H), 7.65 (d, *J*=8.0Hz, 4H, Ar-H), 7.77 (d, *J*=8.4Hz, 2H, Ar-H), 7.93 (d, *J*=8.4Hz, 2H, Ar-H), 8.13 (s, 1H, imidazole) ppm, **$^{13}$C NMR (DMSO-d$_6$):** $\delta$ 40.61, 120.09, 126.16, 127.13, 127.13, 127.69, 128.62, 129.55, 130.35, 130.74, 131.77, 133.52, 134.48, 134.87, 138.64, 141.23, 193.19 ppm; Anal. Calcd. for $C_{23}H_{15}BrCl_2N_2OS$; C, 53.30; H, 2.92; N, 5.41. Found: C, 53.34; H, 2.95; N, 5.48%

**1-(4-Chlorophenyl)-2-((4-(4-chlorophenyl)-1-(4-methoxyphenyl)-1*H*-imidazol-2-yl)thio) ethanone (18e)**



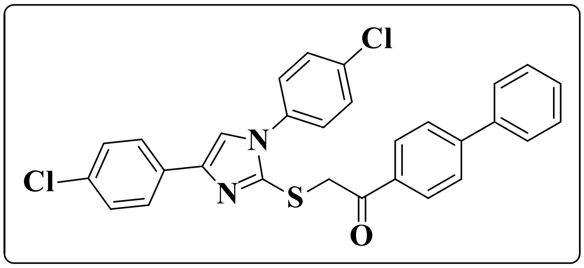
Color: white solid, mp: 203-205 $^o$C, yield: (0.398g, 85%), FT-IR (KBr, $\nu_{max}$ cm$^{-1}$): 1685 (C=O), 1589 (C=N), **$^1$H NMR (400MHz, DMSO-d$_6$, ppm):** $\delta$ 3.88 (s, 3H, methoxy), 4.68 (s, 2H, methylene), 6.99 (d, *J*=8.8Hz, 2H, Ar-H), 7.30-7.34 (m, 5H, Ar-H), 7.47 (d, *J*=8.4Hz, 2H, Ar-H), 7.63 (d, *J*=8.8Hz, 2H, Ar-H), 7.99 (d, *J*=8.8Hz, 2H, Ar-H) ppm, **$^{13}$C NMR (DMSO-d$_6$):** $\delta$ 40.62, 55.32, 114.02, 116.84, 126.02, 126.09, 126.57, 129.02, 129.65, 130.06, 134.07, 134.38, 135.41, 140.05, 142.60, 158.96, 193.08 ppm; Anal. Calcd. for $C_{24}H_{18}Cl_2N_2O_2S$; C, 61.41; H, 3.87; N, 5.97. Found: C, 61.45; H, 3.84; N, 5.92%

### 1-(4-Bromophenyl)-2-((4-(4-bromophenyl)-1-(4-chlorophenyl)-1*H*-imidazol-2-yl)thio) ethanone (18f)



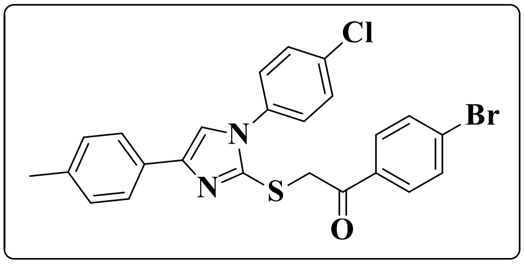
Color: white solid, mp: 245-247 °C, yield: (0.5g, 89%), FT-IR (KBr, $\nu_{max}$ $cm^{-1}$): 1682 (C=O), 1684 (C=N), **$^1$H NMR (400MHz, DMSO-$d_6$, ppm):** $\delta$ 4.75 (s, 2H, methylene), 7.49 (d, *J*=8.4Hz, 2H, Ar-H), 7.55-7.57 (m, 4H, Ar-H), 7.64 (d, *J*=8.4Hz, 2H, Ar-H), 7.77 (d, *J*=8.4Hz, 2H, Ar-H), 7.94 (d, *J*=8.4Hz, 2H, Ar-H), 8.07 (s, 1H, imidazole) ppm, **$^{13}$C NMR (DMSO-$d_6$):** $\delta$ 40.34, 45.75, 88.19, 119.54, 119.68, 126.16, 126.89, 127.55, 129.52, 130.36, 131.33, 131.76, 132.53, 133.08, 134.77, 135.25, 139.99, 141.18, 193.45 ppm; **MS (ESI) *m/z* (%):** 560.9031 $[M+H]^+$; Anal. Calcd. for $C_{23}H_{15}Br_2ClN_2OS$; C, 49.09; H, 2.69; N, 4.98. Found: C, 49.10; H, 2.65; N, 4.94%

### 1-([1,1'-Biphenyl]-4-yl)-2-((1,4-bis(4-chlorophenyl)-1*H*-imidazol-2-yl)thio)ethanone (18g)



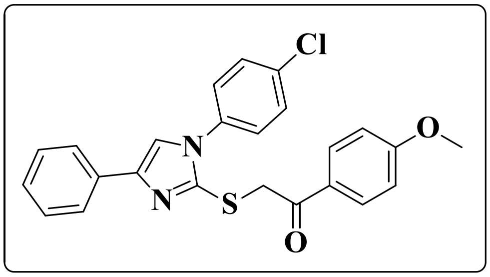
Color: white solid, mp: 208-210 °C, yield: (0.474g, 92%), FT-IR (KBr, $\nu_{max}$ $cm^{-1}$): 1680 (C=O), 1600 (C=N), **$^1$H NMR (400MHz, DMSO-$d_6$, ppm):** $\delta$ $\delta$ 4.73 (s, 2H, methylene), 7.23 (d, *J*=8Hz, 2H, Ar-H), 7.35-7.39 (m, 2H, Ar-H), 7.60-7.65 (m, 5H, Ar-H), 7.71-7.74 (m, 4H, Ar-H), 7.98 (s, 3H, Ar-H), 8.04 (d, *J*=8.4Hz, 2H, Ar-H) ppm; Anal. Calcd. for $C_{29}H_{20}Cl_2N_2OS$; C, 67.57; H, 3.91; N, 5.43. Found: C, 67.53; H, 3.95; N, 5.40%

### 1-(4-Bromophenyl)-2-((1-(4-chlorophenyl)-4-(p-tolyl)-1*H*-imidazol-2-yl)thio)ethanone (18h)



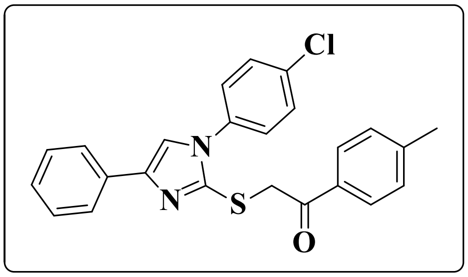
Color: white solid, mp: 234-236 $^{o}$C, yield: (0.403g, 81%), FT-IR (KBr, $\nu_{max}$ cm$^{-1}$): 1682 (C=O), 1595 (C=N), **$^{1}$H NMR (400MHz, DMSO-d$_{6}$, ppm):** $\delta$ 2.28 (s, 3H, methyl), 4.74 (s, 2H, methylene), 7.11 (d, *J*=8Hz, 2H, Ar-H), 7.50 (d, *J*=8Hz, 2H, Ar-H), 7.55 (d, *J*=8.4Hz, 2H, Ar-H), 7.63 (d, *J*=8.4Hz, 2H, Ar-H), 7.77 (d, *J*=8Hz, 2H, Ar-H), 7.95 (d, *J*=6.4Hz, 3H, Ar-H) ppm; **MS (ESI) *m/z* (%):** 499 [M+H+2]$^{+}$; Anal. Calcd. for $C_{24}H_{18}BrClN_{2}OS$; C, 57.90; H, 3.64; N, 5.63. Found: C, 57.96; H, 3.60; N, 5.60%

**2-((1-(4-Chlorophenyl)-4-phenyl-1*H*-imidazol-2-yl)thio)-1-(4-methoxyphenyl)ethanone (18i)**



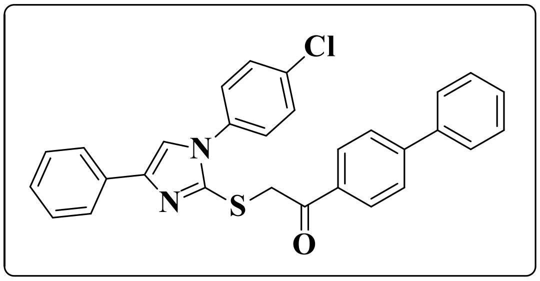
Color: light yellow solid, mp: 195-197 $^{o}$C, yield: (0.339g, 78%), FT-IR (KBr, $\nu_{max}$ cm$^{-1}$): 1676 (C=O), 1602 (C=N), **$^{1}$H NMR (400MHz, DMSO-d$_{6}$, ppm):** $\delta$ 3.86 (s, 3H, methoxy), 4.75 (s, 2H, methylene), 7.06 (d, *J*=8.4Hz, 2H, Ar-H), 7.21 (t, *J*=7.2Hz, 1H, Ar-H), 7.33 (t, *J*=7.2Hz, 2H, Ar-H), 7.58 (d, *J*=8.4Hz, 2H, Ar-H), 7.63-7.69 (m, 4H ,Ar-H), 7.98-8.02 (m, 3H, Ar-H) ppm, **$^{13}$C NMR (DMSO-d$_{6}$):** $\delta$ 40.06, 56.05, 114.38, 119.53, 124.75, 127.30, 127.44, 128.96, 129.97, 131.29, 133.44, 133.74, 135.89, 141.46, 141.68, 163.89, 192.86 ppm; **MS (ESI) *m/z* (%):** 434 [M+H]$^{+}$; Anal. Calcd. for $C_{24}H_{19}ClN_{2}O_{2}S$; C, 66.28; H, 4.40; N, 6.44. Found: C, 66.24; H, 4.47; N, 6.40%

**2-((1-(4-Chlorophenyl)-4-phenyl-1*H*-imidazol-2-yl)thio)-1-(p-tolyl)ethanone (18j)**



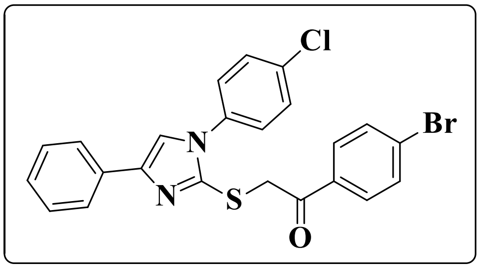
Color: white solid, mp: 182-184 $^{o}$C, yield: (0.36g, 86%), FT-IR (KBr, $\nu_{max}$ cm$^{-1}$): 1680 (C=O), 1602 (C=N), **$^{1}$H NMR (400MHz, DMSO-d$_{6}$, ppm):** $\delta$ 2.40 (s, 3H, methyl), 4.77 (s, 2H, methylene), 7.21 (t, *J*=7.2Hz, 1H, Ar-H), 7.29-7.36 (m, 4H ,Ar-H), 7.57 (d, *J*=8Hz, 2H, Ar-H), 7.66 (t, *J*=8Hz, 4H, Ar-H), 7.92 (d, *J*=7.6Hz, 2H, Ar-H), 8.01 (s, 1H, Ar-H) ppm, **$^{13}$C NMR (DMSO-d$_{6}$):** $\delta$ 21.67, 40.70, 119.52, 124.73, 127.30, 127.41, 128.98, 129.74, 129.98, 133.45, 133.61, 133.72, 135.87, 141.43, 141.67, 144.52, 194.07 ppm; **MS (ESI) *m/z* (%):** 419 [M+H]$^{+}$; Anal. Calcd. for $C_{24}H_{19}ClN_{2}OS$; C, 68.81; H, 4.57; N, 6.69. Found: C, 68.85; H, 4.53; N, 6.63%

### 1-([1,1’-Biphenyl]-4-yl)-2-((1-(4-chlorophenyl)-4-phenyl-1*H*-imidazol-2-yl)thio)ethanone (18k)



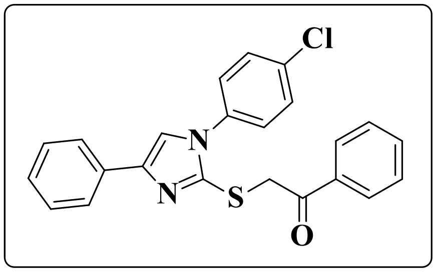
Color: white solid, mp: 209-213 °C, yield: (0.428g, 89%), FT-IR (KBr, $\nu_{max}$ cm$^{-1}$): 1682 (C=O), 1606 (C=N), **$^1$H NMR (400MHz, DMSO-$d_6$, ppm):** $\delta$ 4.82 (s, 2H, methylene), 7.19 (t, *J*=7.2Hz, 1H, Ar-H), 7.30 (t, *J*=7.2Hz, 2H, Ar-H), 7.45 (t, *J*=6.8Hz, 1H, Ar-H), 7.51-7.59 (m, 4H, Ar-H), 7.64-7.67 (m, 4H ,Ar-H), 7.78 (d, *J*=7.6Hz, 2H, Ar-H), 7.86 (d, *J*=8Hz, 2H, Ar-H), 8.03 (s, 1H, Ar-H), 8.11 (d, *J*=8Hz, 2H, Ar-H) ppm, **$^{13}$C NMR (DMSO-$d_6$):** $\delta$ 40.79, 119.64, 124.75, 127.35, 127.43, 127.50, 128.93, 129.61, 130.01, 133.48, 133.67, 134.99, 135.89, 139.33, 141.40, 141.57, 145.31, 194.12 ppm; **MS (ESI) *m/z* (%):** 481 [M+H]$^+$; Anal. Calcd. for $C_{29}H_{21}ClN_2OS$; C, 72.41; H, 4.40; N, 5.82; found: C, 72.41; H, 4.40; N, 5.82. Found: C, 72.46; H, 4.44; N, 5.86%

### 1-(4-Bromophenyl)-2-((1-(4-chlorophenyl)-4-phenyl-1*H*-imidazol-2-yl)thio)ethanone (18l)



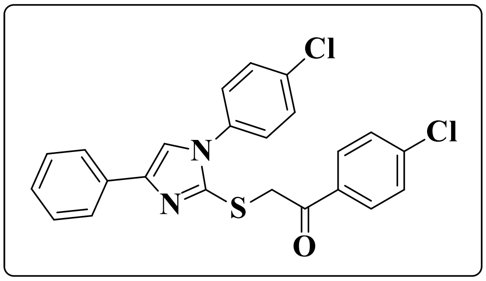
Color: white solid, mp: 195-197 °C, yield: (0.396g, 82%), FT-IR (KBr, $\nu_{max}$ cm$^{-1}$): 1682 (C=O), 1604 (C=N), **$^1$H NMR (400MHz, DMSO-$d_6$, ppm):** $\delta$ 4.75 (s, 2H, methylene), 7.21 (t, *J*=7.2Hz, 1H, Ar-H), 7.31 (t, *J*=7.2Hz, 2H, Ar-H), 7.55-7.65 (m, 6H, Ar-H), 7.77 (d, *J*=7.6Hz, 2H, Ar-H), 7.96 (d, *J*=8Hz, 2H, Ar-H), 8.02 (s, 1H, Ar-H) ppm; **MS (ESI) *m/z* (%):** 485 [M+H+2]$^+$; Anal. Calcd. for $C_{23}H_{16}BrClN_2OS$; C, 57.10; H, 3.33; N, 5.79. Found: C, 57.13; H, 3.30; N, 5.72%

### 2-((1-(4-Chlorophenyl)-4-phenyl-1*H*-imidazol-2-yl)thio)-1-phenylethanone (18m)



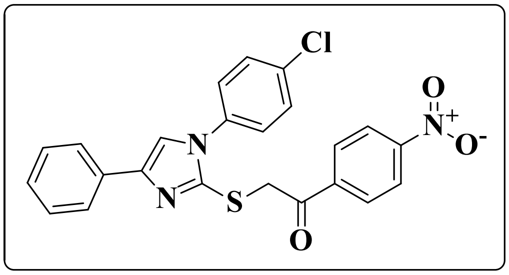
Color: white solid, mp: 169-171 °C, yield: (0.315g, 78%), FT-IR (KBr, $\nu_{max}$ cm$^{-1}$): 1680 (C=O), 1602 (C=N), **$^1$H NMR (400MHz, DMSO-$d_6$, ppm):** $\delta$ 4.81(s, 2H, methylene), 7.21 (t, *J*=7.6Hz, 1H, Ar-H), 7.31 (t, *J*=7.6Hz, 2H, Ar-H), 7.54-7.58 (m, 4H ,Ar-H), 7.64-7.70 (m, 5H, Ar-H), 8.03 (d, *J*=7.6Hz, 3H, Ar-H) ppm, **$^{13}$C NMR (DMSO-$d_6$):** $\delta$ 40.71, 119.51, 124.71, 127.29, 127.37, 128.85, 128.95, 129.20, 130.01, 133.97, 135.87, 136.17, 141.45, 141.67, 194.60 ppm; **MS (ESI) *m/z* (%):** 405 [M+H]$^+$; Anal. Calcd. for $C_{23}H_{17}ClN_2OS$; C, 68.22; H, 4.23; N, 6.92. Found: C, 68.26; H, 4.27; N, 6.97%

### 1-(4-Chlorophenyl)-2-((1-(4-chlorophenyl)-4-phenyl-1*H*-imidazol-2-yl)thio)ethanone (18n)



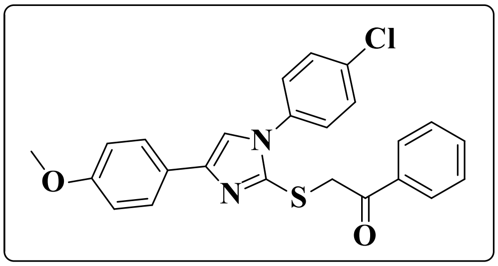
Color: white solid, mp: 213-215 °C, yield: (0.351g, 80%), FT-IR (KBr, $\nu_{max}$ cm$^{-1}$): 1685 (C=O), 1607 (C=N), **$^1$H NMR (400MHz, DMSO-d$_6$, ppm):** $\delta$ 4.76 (s, 2H, methylene), 7.21 (t, *J*=7.2Hz, 1H, Ar-H), 7.31 (t, *J*=7.2Hz, 2H, Ar-H), 7.57 (t, *J*=8.4Hz, 2H, Ar-H), 7.61-7.65 (m, 6H, Ar-H), 8.04 (d, *J*=9.6Hz, 3H, Ar-H) ppm; **MS (ESI) *m/z* (%):** 439 [M+H]$^+$; Anal. Calcd. for $C_{23}H_{16}Cl_2N_2OS$; C, 62.88; H, 3.67; N, 6.38. Found: C, 62.92; H, 3.64; N, 6.34%

### 2-((1-(4-Chlorophenyl)-4-phenyl-1*H*-imidazol-2-yl)thio)-1-(4-nitrophenyl)ethanone (18o)



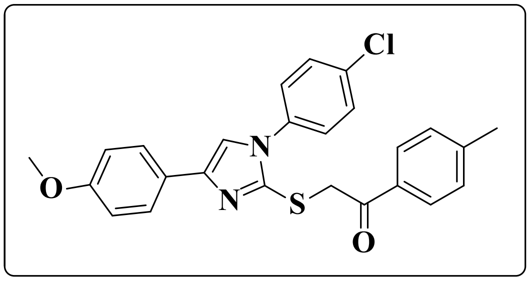
Color: yellow solid, mp: 231-233 °C, yield: (0.391g, 87%), FT-IR (KBr, $\nu_{max}$ cm$^{-1}$): 1689 (C=O), 1609 (C=N), **$^1$H NMR (400MHz, DMSO-d$_6$, ppm):** $\delta$ 4.80 (s, 2H, methylene), 7.20 (t, *J*=7.2Hz, 1H, Ar-H), 7.29 (t, *J*=7.2Hz, 2H, Ar-H), 7.55-7.64 (m, 6H, Ar-H), 8.03 (s, 1H, Ar-H) 8.24 (d, *J*=7.6Hz, 2H, Ar-H), 8.36 (d, *J*=7.6Hz, 2H, Ar-H) ppm, **$^{13}$C NMR (DMSO-d$_6$):** $\delta$ 40.77, 119.68, 124.21, 124.66, 127.34, 128.91, 130.00, 130.31, 133.48, 133.64, 135.79, 140.96, 141.01, 141.70, 150.38, 194.08 ppm; **MS (ESI) *m/z* (%):** 450 [M+H]$^+$; Anal. Calcd. for $C_{23}H_{16}ClN_3O_3S$; C, 61.40; H, 3.58; N, 9.34. Found: C, 61.44; H, 3.54; N, 9.30%

### 2-((1-(4-Chlorophenyl)-4-(4-methoxyphenyl)-1*H*-imidazol-2-yl)thio)-1-phenylethanone (18p)



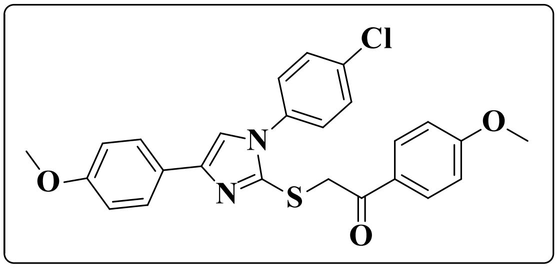
Color: white solid, mp: 171-173 °C, yield: (0.334g, 77%), FT-IR (KBr, $\nu_{max}$ cm$^{-1}$): 1682 (C=O), 1604 (C=N), **$^1$H NMR (400MHz, CDCl$_3$, ppm):** $\delta$ 3.81 (s, 3H, methoxy), 4.69 (s, 2H, methylene), 6.88 (d, *J*=8.8Hz, 2H, Ar-H), 7.33 (d, *J*=8.4Hz, 2H, Ar-H), 7.40-7.48 (m, 5H, Ar-H), 7.57-7.63 (m, 3H, Ar-H), 7.99 (d, *J*=7.6Hz, 2H, Ar-H) ppm, **$^{13}$C NMR (CDCl$_3$):** *δ* 41.11, 55.31, 114.01, 116.81, 126.07, 126.22, 126.62, 128.62, 128.71, 129.60, 133.57, 135.69, 158.92, 194.07 ppm; **MS (ESI) *m/z* (%):** 435 [M+H]$^+$; Anal. Calcd. for $C_{24}H_{19}ClN_2O_2S$; C, 66.28; H, 4.40; N, 6.44. Found: C, 66.24; H, 4.47; N, 6.47%

### 2-((1-(4-Chlorophenyl)-4-(4-methoxyphenyl)-1*H*-imidazol-2-yl)thio)-1-(p-tolyl)ethanone (18q)



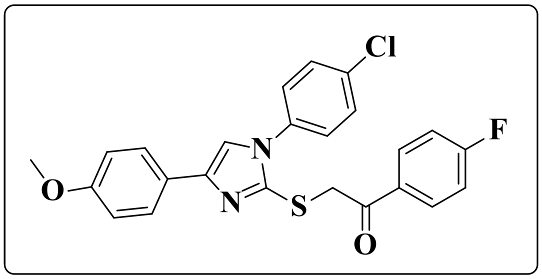
Color: white solid, mp: 187-189 °C, yield: (0.363g, 81%), FT-IR (KBr, $\nu_{max}$ cm$^{-1}$): 1680 (C=O), 1602 (C=N), **$^1$H NMR (400MHz, CDCl$_3$, ppm):** *δ* 2.42 (s, 3H, methyl), 3.82 (s, 3H, methoxy), 4.68 (s, 2H, methylene), 6.89 (d, *J*=8.8Hz, 2H, Ar-H), 7.26 (d, 3H, *J*=8Hz, Ar-H), 7.34 (d, *J*=8.8Hz, 2H, Ar-H), 7.42 (d, *J*=6.4Hz, 2H, Ar-H), 7.64 (d, *J*=8.8Hz, 2H, Ar-H), 7.89 (d, *J*=8.4Hz, 2H, Ar-H) ppm, **$^{13}$C NMR (CDCl$_3$):** *δ* 21.76, 41.17, 55.31, 114, 116.80, 126.09, 126.25, 126.66, 128.74, 129.38, 129.58, 133.15, 144.54, 158.91, 193.65 ppm; **MS (ESI) *m/z* (%):** 449 [M+H]$^+$; Anal. Calcd. for $C_{25}H_{21}ClN_2O_2S$; C, 66.88; H, 4.71; N, 6.24. Found: C, 66.84; H, 4.75; N, 6.27%

### 2-((1-(4-Chlorophenyl)-4-(4-methoxyphenyl)-1*H*-imidazol-2-yl)thio)-1-(4-methoxyphenyl) ethanone (18r)



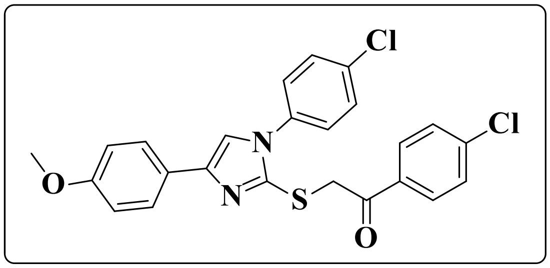
Color: white solid, mp: 212-214 °C, yield: (0.385g, 83%), FT-IR (KBr, $\nu_{max}$ cm$^{-1}$): 1674 (C=O), 1600 (C=N), **$^1$H NMR (400MHz, CDCl$_3$, ppm):** *δ* 3.83 (s, 3H, methoxy), 3.88 (s, 3H, methoxy), 4.66 (s, 2H, methylene), 6.89-6.94 (m, 4H, Ar-H), merging in CDCl$_3$ peak 7.26 (s, 1H, Ar-H), 7.34 (d, *J*=8.8Hz, 2H, Ar-H), 7.42 (d, *J*=8.4Hz, 2H, Ar-H), 7.65 (d, *J*=8.4Hz, 2H, Ar-H), 7.97 (d, J=8.8Hz,2H, Ar-H) ppm, **$^{13}$C NMR (CDCl$_3$):** *δ* 40.99, 55.31, 55.55, 113.85, 114.01, 116.81, 126.09, 126.25, 126.68, 128.62, 129.58, 131.00, 134.25, 142.55, 158.91, 163.91, 192.55 ppm; **MS (ESI) *m/z* (%):** 465 [M+H]$^+$; Anal. Calcd. for $C_{25}H_{21}ClN_2O_3S$; C, 64.58; H, 4.55; N, 6.02. Found: C, 64.53; H, 4.51; N, 6.10%

### 2-((1-(4-Chlorophenyl)-4-(4-methoxyphenyl)-1*H*-imidazol-2-yl)thio)-1-(4-fluorophenyl) ethanone (18s)



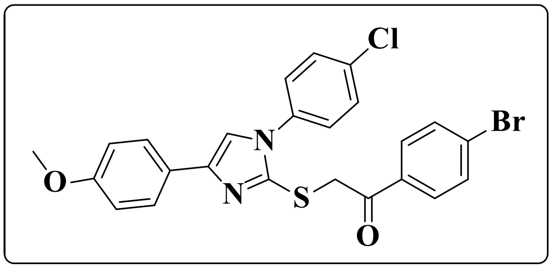
Color: white solid, mp: 189-191 $^{o}$C, yield: (0.366g, 81%), FT-IR (KBr, $\nu_{max}$ cm$^{-1}$): 1686 (C=O), 1610 (C=N), **$^{1}$H NMR (400MHz, CDCl$_3$, ppm):** $\delta$ 3.83 (s, 3H, methoxy), 4.66 (s, 2H, methylene), 6.89 (d, *J*=8.8Hz, 2H, Ar-H), 7.14 (d, *J*=8.4Hz, 2H, Ar-H), merging in CDCl3 peak 7.26 (s, 1H, Ar-H), 7.34 (d, *J*=8.4Hz, 2H, Ar-H), 7.43 (d, *J*=8.8Hz, 2H, Ar-H), 7.61 (d, *J*=8.8Hz, 2H, Ar-H), 8.02-8.06 (m, 2H, Ar-H) ppm, **$^{13}$C NMR (CDCl$_3$):** $\delta$ 40.76, 55.31, 114.02, 115.74, 115.96, 116.85, 126.03, 126.12, 126.60, 129.64, 131.32, 131.42, 135.43, 142.59, 158.96, 164.75, 167.29, 192.63 ppm; **MS (ESI)** ***m/z*** **(%):** 453 [M+H]$^+$; Anal. Calcd. for $C_{24}H_{18}ClFN_2O_2S$; C, 63.64; H, 4.01; N, 6.18. Found: C, 63.67; H, 4.12; N, 6.14%

### 1-(4-Chlorophenyl)-2-((1-(4-chlorophenyl)-4-(4-methoxyphenyl)-1*H*-imidazol-2-yl)thio) ethanone (18t)



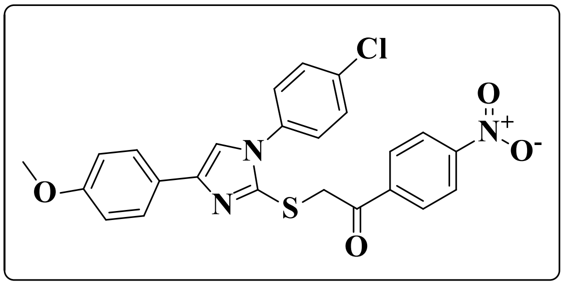
Color: white solid, mp: 231-233 $^{o}$C, yield: (0.398g, 85%), FT-IR (KBr, $\nu_{max}$ cm$^{-1}$): 1685 (C=O), 1598 (C=N), **$^{1}$H NMR (400MHz, CDCl$_3$, ppm):** $\delta$ 3.83 (s, 3H, methoxy), 4.63 (s, 2H, methylene), 6.89 (d, *J*=8.4Hz, 2H, Ar-H), peak merged in CDCl$_3$ (s, 1H, Ar-H), 7.33 (d, *J*=8.4 Hz, 2H, Ar-H), 7.42-7.45 (m, 4H, Ar-H), 7.59 (d, *J*=8.8 Hz, 2H, Ar-H), 7.95 (d, *J*=8.4Hz, 2H, Ar-H), 7.91 (d, *J*=8.4Hz, 2H, Ar-H) ppm, **$^{13}$C NMR (CDCl$_3$):** $\delta$ 40.62, 55.32, 114.02, 116.84, 126.02, 126.09, 126.57, 129.02, 129.65, 130.06, 134.07, 134.38, 135.41, 140.05, 142.60, 158.96, 193.08 ppm; **MS (ESI)** ***m/z*** **(%):** 469 [M+H]$^+$; Anal. Calcd. for $C_{24}H_{18}Cl_2N_2O_2S$; C, 61.41; H, 3.87; N, 5.97. Found: C, 61.45; H, 3.91; N, 5.94%

### 1-(4-Bromophenyl)-2-((1-(4-chlorophenyl)-4-(4-methoxyphenyl)-1*H*-imidazol-2-yl)thio) ethanone (18u)



Color: light yellow solid, mp: 247-249 °C, yield: (0.452g, 88%), FT-IR (KBr, $\nu_{max}$ cm$^{-1}$): 1682 (C=O), 1602 (C=N), **$^1$H NMR (400MHz, $CDCl_3$, ppm):** $\delta$ 3.83 (s, 3H, methoxy), 4.63 (s, 2H, methylene), 6.88 (d, *J*=8 Hz, 2H, Ar-H), 7.31 (s, 1H, imidazole), 7.34 (d, *J*=8 Hz, 2H, Ar-H), 7.43 (d, *J*=8 Hz, 2H, Ar-H), 7.57-7.62 (m, 4H, Ar-H), 7.87 (d, *J*=8 Hz, 2H, Ar-H) ppm, **$^{13}$C NMR ($CDCl_3$):** $\delta$ 40.51, 55.50, 114.32, 118.37, 126.02, 126.45, 127.29, 128.02, 129.96, 130.87, 132.23, 133.32, 135.27, 135.95, 140.76, 141.66, 158.74, 194.01 ppm; **MS (ESI) *m/z* (%):** 515 [M+H+2]$^+$; Anal. Calcd. for $C_{24}H_{18}BrClN_2O_2S$; C, 56.10; H, 3.53; N, 5.45. Found: C, 56.14; H, 3.57; N, 5.49%

**2-((1-(4-Chlorophenyl)-4-(4-methoxyphenyl)-1H-imidazol-2-yl)thio)-1-(4-nitrophenyl) ethanone (18v)**



Color: yellow solid, mp: 219-221 °C, yield: (0.379g, 80%), FT-IR (KBr, $\nu_{max}$ cm$^{-1}$): 1689 (C=O), 1612 (C=N), **$^1$H NMR (400MHz, $CDCl_3$, ppm):** $\delta$ 3.82 (s, 3H, methoxy), 4.66 (s, 2H, methylene), 6.86 (d, *J*=8 Hz, 2H, Ar-H), 7.31 (s, 1H, imidazole), 7.35 (d, *J*=7.6 Hz, 2H, Ar-H), 7.44 (d, *J*=7.6 Hz, 2H, Ar-H), 7.55 (d, *J*=8 Hz, 2H, Ar-H), 8.19 (d, *J*=8 Hz, 2H, Ar-H), 8.32 (d, *J*=8 Hz, 2H, Ar-H), **$^{13}$C NMR ($CDCl_3$):** $\delta$ 40.44, 55.30, 114.00, 116.96, 123.83, 125.93, 126.49, 129.69, 134.47, 135.27, 140.38, 142.62, 150.42, 158.99, 193.02 ppm; **MS (ESI) *m/z* (%):** 480 [M+H]$^+$; Anal. Calcd. for $C_{24}H_{18}ClN_3O_4S$; C, 60.06; H, 3.78; N, 8.76. Found: C, 60.12; H, 3.81; N, 8.72%

## BIOLOGICAL ACTIVITY

### *In vitro* anticancer assay

The *in vitro* anticancer activity experimental protocol is carried out as discussed in chapter-II.

### *In vitro* anticancer activity results and discussion

The newly synthesized compounds were screened for their *in vitro* anti-cancer activity against 60 human cancer cell lines at 10 µM concentration at single dose assay and the results are shown in table-2.

Among the tested compounds, compound **18a** showed moderate activity on non-small cell lung cancer NCI-H522 cell line (47.70% growth, 52.30% inhibition), CNS cancer SNB-75 cell line (45.96% growth, 54.04% inhibition).

Compound **18b** showed potent activity against ovarian cancer OVCAR-4 cell line (15.26% growth, 84.74% inhibition).

Compound **18c** showed moderate activity against ovarian cancer OVCAR-8 cell line (45.47% growth, 54.53% inhibition).

**Table-2**: *In vitro* anticancer activity of compounds.

| **Cancer cell line** | **18a** | **18b** | **18c** | **18d** | **18e** | **18g** | **18h** | **18o** | **18v** |
|---|---|---|---|---|---|---|---|---|---|
| **Non-small cell lung cancer** | | | | | | | | | |
| **HOP-62** | 58.94 | 81.82 | 92.21 | **48.29** | 85.84 | 82.02 | 66.68 | 96.21 | 50.14 |
| **HOP-92** | 83.07 | 67 | 86.79 | **43.93** | 67.43 | 81.28 | **39** | 87.47 | **28.02** |
| **NCI-H522** | **47.7** | 76.38 | 50.1 | 58.82 | 68.9 | 73.65 | 64.63 | 70.51 | 70.68 |
| **Colon cancer** | | | | | | | | | |
| **HCT-116** | 65.05 | 71.95 | 88.4 | **43.92** | 75.77 | **32.81** | 64.8 | 66.94 | 74.59 |
| **CNS cancer** | | | | | | | | | |
| **SF-268** | 72.66 | 78.84 | 63.23 | 78.09 | 76.78 | 73.85 | 71.63 | **-86.67** | 73.09 |
| SNB-75 | **45.96** | 76.7 | 85.94 | **44.38** | 80.04 | 99.06 | 81.41 | 93.81 | **38.97** |
| **U251** | 68.8 | 64.95 | 84.09 | **37.47** | 80.44 | 88.25 | 62.69 | 93.30 | 60.62 |
| **Ovarian cancer** | | | | | | | | | |
| **OVCAR-4** | 62.62 | **15.26** | 58.31 | **9.78** | 52.14 | 56.44 | 63.37 | 92.81 | **38.67** |
| **OVCAR-8** | 63.61 | 53.97 | **45.47** | **47.98** | 54.93 | 75.85 | 56.19 | 95.38 | 51.85 |
| **Renal cancer** | | | | | | | | | |
| **786-0** | 84.9 | 95.81 | 86.17 | **34.71** | 63.54 | 93.87 | 86.45 | 90.29 | 52.85 |
| **ACHN** | 85.01 | 64.02 | 86.46 | **22.35** | 78.93 | 101.72 | 83.06 | 95.09 | 81.51 |
| **UO-31** | 96.84 | 101.57 | 83.32 | **45.51** | 94.57 | 89.99 | 92.29 | 59.73 | 82.51 |

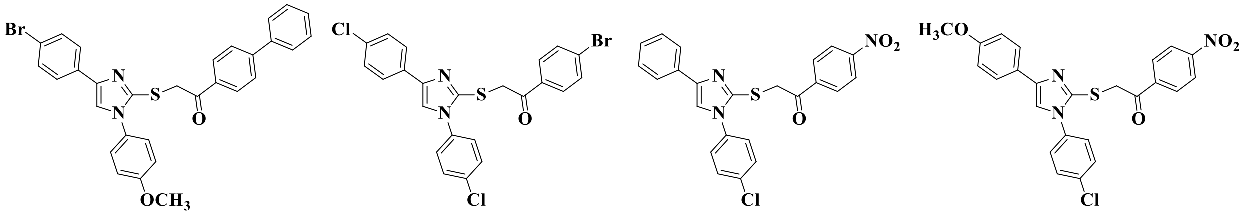
Compound **18d** showed potent activity against ovarian cancer OVCAR-4 cell line (9.78% growth, 90.22% inhibition), renal cancer ACHN cell line **(**22.35% growth, 77.65% inhibition), good activity on CNS cancer U251 cell line (37.47% growth, 62.53% inhibition), renal cancer 786-0 cell line (34.71% growth, 65.29% inhibition), moderate activity on non-small cell lung cancer HOP-62 cell line (48.29% growth, 51.71% inhibition), HOP-92 cell line (43.93% growth, 56.07% inhibition), colon cancer HCT-116 cell line (43.92% growth, 56.08% inhibition), CNS cancer SNB-75 cell line (44.38% growth, 55.62% inhibition), ovarian cancer OVCAR-8 cell line (47.98% growth, 52.02% inhibition), renal cancer UO-31 cell line (45.51% growth, 54.49% inhibition).

Compound **18g** showed significant activity on colon cancer HCT-116 cell line (32.81% growth, 67.19% inhibition).

Compound **18h** showed significant activity against non-small cell lung cancer HOP-92 cell line (39% growth, 61% inhibition).

Compound **18o** showed potent activity against CNS cancer SF-268 cell line (**-86.67**% growth).

Compound **18v** showed potent activity on non-small cell lung cancer HOP-62 cell line (28.02% growth, 71.98% inhibition),



**18b** **18d** **18o** **18v**

The compounds having potent anticancer activity

## *In vitro* antimicrobial activity

The *in vitro* antimicrobial assay protocol is carried out as discussed in chapter-III.

## *In vitro* antimicrobial activity results and discussion

Antimicrobial activity results of compounds 18 (a-o) were showed as in table-3 and table-4

**Table 3: *In vitro* antimicrobial primary screening at 32 µg/mL**

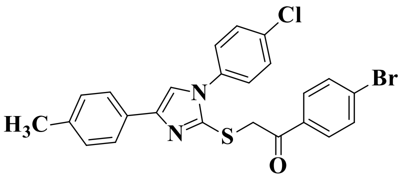
| **Compound** | **Antimicrobial activity at 32 µg/mL** | | | | | | |
|---|---|---|---|---|---|---|---|
| | ***Escherichia coli***<br>**ATCC 25922**<br>**FDA control** | ***Klebsiella pneumoniae***<br>**ATCC 700603**<br>**MDR** | ***Acinetobacter baumannii***<br>**ATCC 19606**<br>**Type strain** | ***Pseudomonas aeruginosa***<br>**ATCC 27853**<br>**Type strain** | ***Staphylococcus aureus***<br>**ATCC 43300**<br>**MRSA** | ***Candida albicans***<br>**ATCC 90028**<br>**CSLI Ref** | ***Cryptococcus neoformans***<br>**ATCC 20881**<br>**H99** |
| **18a** | I | I | I | I | I | I | I |
| **18b** | I | I | I | I | I | I | I |
| **18c** | I | I | I | I | I | I | I |
| **18d** | I | I | I | I | I | I | I |
| **18e** | I | I | I | I | I | I | I |
| **18f** | I | I | I | I | I | I | I |
| **18g** | I | I | I | I | I | I | I |
| **18h** | I | I | I | I | I | I | **A** |
| **18i** | I | I | I | I | I | **A** | **A** |
| **18j** | I | I | I | I | I | I | I |
| **18k** | I | I | I | I | I | **A** | I |
| **18l** | I | I | I | I | I | I | **A** |
| **18m** | I | I | I | I | I | I | **A** |
| **18n** | I | I | I | I | I | I | **A** |
| **18o** | I | I | I | I | I | **A** | **A** |

**Table 4: The *In vitro* antimicrobial activity (Hit- conformation)**

| Compound | MIC [µg/mL] | | | | | | | $CC_{50}$ [µg/mL] |
|---|---|---|---|---|---|---|---|---|
| | ***Escherichia coli*** **ATCC 25922 FDA control** | ***Klebsiella pneumoniae*** **ATCC 700603 MDR** | ***Acinetobacter baumannii*** **ATCC 19606 Type strain** | ***Pseudomonas aeruginosa*** **ATCC 27853 Type strain** | ***Staphylococcus aureus*** **ATCC 43300 MRSA** | ***Candida albicans*** **ATCC 90028 CSLI Ref** | ***Cryptococcus neoformans*** **ATCC 20881 H99** | **HEK293 ATCC CRL1573** |
| **18h** | >32 | >32 | >32 | >32 | >32 | >32 | **16** | >32 |
| **18i** | >32 | >32 | >32 | >32 | >32 | >32 | **16** | >32 |
| **18k** | >32 | >32 | >32 | >32 | >32 | >32 | 32 | **10.2** |
| **18l** | >32 | >32 | >32 | >32 | >32 | >32 | **16** | >32 |
| **18m** | >32 | >32 | >32 | >32 | >32 | >32 | 32 | >32 |
| **18n** | >32 | >32 | >32 | >32 | >32 | >32 | **16** | >32 |
| **18o** | >32 | >32 | >32 | >32 | >32 | >32 | **16** | >32 |

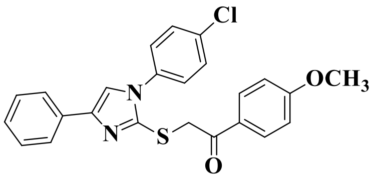
**Antimicrobial activity conclusion**

The *in vitro* primary antimicrobial study identified **18h, 18i, 18l, 18n, and 18o** compounds with inhibitory activity ≤ 16 µg/mL against *C. albicans* and *C. neoformans*. In Hit-conformation, compounds **18h**, **18i**, **18l**, **18n** and **18o** showed potent activity against *C. neoformans* with MIC value of **16** µg/mL, with all five compounds displaying no cytotoxicity against mammalian HEK293 cells.



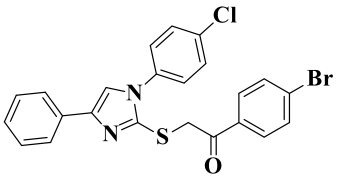
18h

*Cryptococcus neof ormans*
(MIC- 16 µg/mL)



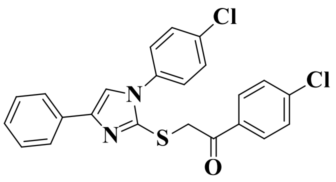
18i

*Cryptococcus neof ormans*
(MIC- 16 µg/mL)



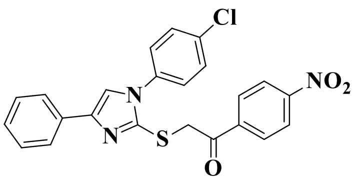
18l

*Cryptococcus neof ormans*
(MIC- 16 µg/mL)



18n

*Cryptococcus neof ormans*
(MIC- 16 µg/mL)



18o

*Cryptococcus neof ormans*
(MIC- 16 µg/mL)

Compounds with potent antimicrobial activity.

### *In vitro* antiviral activity assay

*In vitro* antiviral activity assay protocol is carried out as discussed in the chapter-III.

### *In vitro* antiviral activity results and discussion

The newly synthesized compounds were evaluated for their antiviral activity against various human viruses. The HEL cell culture was used to assess the compounds against *herpes simplex* virus type I (KOS) [HSV-1 KOS], *herpes simplex* virus type 2 (G) [HSV-2 G], *hepus simplex virus-1* TK$^-$KOS ACV, thymidine kinase-deficient *Herpes simplex* virus type 1 (HSV-1 TK- KOS ACV), *vaccinia* virus [VV] and *Adeno* virus type 2 (AV-2), HeLa cell culture was used for *vesicular stomatitis*, *coxsackie* virus B4, r*espiratory syncytial* virus, vero cell culture was used to assess the compounds against *parainfluenza* type 3 virus (PI-3V), *sindbis* virus (SV), *coxsackie* virus B4 (CV B4), *Punto toro* virus (PTV) and *yellow fever virus* (YFV). The CRFK cell culture medium was used for *feline corona* virus (FIPV) and *feline herpes* virus. The MDCK cell line was used to infect with *influenza* A virus type H1N1 (strain A/Ned/378/05) and type H3N2 (strain A/HK/7/87), and *influenza* B virus (strain B/Ned/537/05). Based on the microscopical analysis of the virus-induced cytopathicity (CPE), the antiviral potency of the compounds was determined and compared to different reference antiviral drugs (brivudin, cidofovir, acyclovir, ganciclovir, zalcitabine, and alovudine) and it is shown in t**able 5-9**.

## Table 5: Cytotoxicity and antiviral activity in HEL cell cultures

| Compound | Con. unit | Minimum cytotoxic con. | $EC_{50}$[b] | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Herpes simplex virus-1 (KOS) | Herpes simplex virus-2 (G) | Herpes simplex virus-1 $TK^-$KOS $ACV^r$ | Vaccinia virus | Adenovirus-2 | Human Corona virus (229E) |
| **18a** | µM | ≥20 | >100 | >100 | >100 | >100 | >100 | >100 |
| **18b** | µM | 100 | >100 | >100 | >100 | >100 | >100 | >100 |
| **18c** | µM | >100 | >100 | >100 | >100 | >100 | >100 | >100 |
| **18d** | µM | 100 | >100 | >100 | >100 | >100 | >100 | >100 |
| **18e** | µM | >100 | >100 | >100 | >100 | >100 | >100 | >100 |
| **18f** | µM | 4 | >100 | >100 | >100 | >100 | >100 | >100 |
| **18g** | µM | 20 | >100 | >100 | >100 | >100 | >100 | >100 |
| **18h** | µM | ≥4 | >100 | >100 | >100 | >100 | >100 | >100 |
| **18i** | µM | >100 | >100 | >100 | >100 | >100 | >100 | >100 |
| **18j** | µM | ≥20 | >100 | >100 | >100 | >100 | >100 | >100 |
| **18k** | µM | ≥20 | >100 | >100 | >100 | >100 | >100 | >100 |
| **18l** | µM | 20 | >100 | >100 | >100 | >100 | >100 | >100 |
| **18m** | µM | ≥20 | >100 | >100 | >100 | >100 | >100 | >100 |
| **18n** | µM | ≥20 | >100 | >100 | >100 | >100 | >100 | >100 |
| **18o** | µM | 20 | >100 | >100 | >100 | >100 | >100 | >100 |
| **Brivudin** | µM | >250 | 0.08 | 112 | 10 | 10 | - | - |
| **Cidofovir** | µM | >250 | 4.5 | 2 | 3.4 | 22 | 29 | - |
| **Acyclovir** | µM | >250 | 0.7 | 0.2 | 85 | >250 | - | - |
| **Zalcitabine** | µM | >250 | - | - | - | - | 19 | - |
| **Alovudine** | µM | >250 | - | - | - | - | 50 | - |
| **UDA** | µg/ml | >100 | - | - | - | - | - | 1.8 |
| **Ribavirin** | µM | >250 | - | - | - | - | - | 112 |

[a] Required to cause a microscopically detectable alteration of normal cell morphology.

[b] Required to reduce virus-induced cytopathogenicity by 50%

**Table 6: Cytotoxicity and antiviral activity in HeLa cell cultures**

| Compound | Con. unit | Minimum cytotoxic con[a] | $EC_{50}$[b] | | |
|---|---|---|---|---|---|
| | | | Vascular stomatitis virus | Coxsackie virus B4 | Respiratory syncytial virus |
| **18a** | µM | ≥100 | >100 | >100 | >100 |
| **18b** | µM | 100 | >100 | >100 | >100 |
| **18c** | µM | >100 | >100 | >100 | >100 |
| **18d** | µM | 20 | >100 | >100 | >100 |
| **18e** | µM | >100 | >100 | >100 | >100 |
| **18f** | µM | >100 | >100 | >100 | >100 |
| **18g** | µM | 100 | >100 | >100 | >100 |
| **18h** | µM | >100 | >100 | >100 | >100 |
| **18i** | µM | >100 | >100 | >100 | >100 |
| **18j** | µM | >100 | >100 | >100 | >100 |
| **18k** | µM | >100 | >100 | >100 | >100 |
| **18l** | µM | 100 | >100 | >100 | >100 |
| **18m** | µM | >100 | >100 | >100 | >100 |
| **18n** | µM | >100 | >100 | >100 | >100 |
| **18o** | µM | 100 | >100 | >100 | >100 |
| **DS-10.000** | µg/ml | >100 | 0.4 | 6.8 | 0.4 |
| **Ribavirin** | µM | >250 | 2.5 | 50 | 10 |

Table 7: Cytotoxicity and antiviral activity in Vero cell cultures

| Compound | Con. unit | Minimum cytotoxic con[a] | EC$_{50}$[b] | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Para-influenza-3 virus | Reovirus-1 | Sindbis virus | Coxsackie virus B4 | Punta Toro virus | Yellow Fever virus |
| **18a** | µM | ≥100 | >100 | >100 | >100 | >100 | >100 | >100 |
| **18b** | µM | 20 | >100 | >100 | >100 | >100 | >100 | >100 |
| **18c** | µM | ≥100 | >100 | >100 | >100 | >100 | >100 | >100 |
| **18d** | µM | ≥20 | >100 | >100 | >100 | >100 | >100 | >100 |
| **18e** | µM | >100 | >100 | >100 | >100 | >100 | >100 | >100 |
| **18f** | µM | 20 | >100 | >100 | >100 | >100 | >100 | >100 |
| **18g** | µM | 100 | >100 | >100 | >100 | >100 | >100 | >100 |
| **18h** | µM | ≥100 | >100 | >100 | >100 | >100 | >100 | >100 |
| **18i** | µM | >100 | >100 | >100 | >100 | >100 | >100 | >100 |
| **18j** | µM | >100 | >100 | >100 | >100 | >100 | >100 | >100 |
| **18k** | µM | >100 | >100 | >100 | >100 | >100 | >100 | >100 |
| **18l** | µM | 100 | >100 | >100 | >100 | >100 | >100 | >100 |
| **18m** | µM | 100 | >100 | >100 | >100 | >100 | >100 | >100 |
| **18n** | µM | 100 | >100 | >100 | >100 | >100 | >100 | >100 |
| **18o** | µM | 100 | >100 | >100 | >100 | >100 | >100 | **3.0** |
| **DS-10.000** | µg/ml | >100 | >100 | >100 | 100 | 45 | 2.3 | 0.8 |
| **Ribavirin** | µM | >250 | 146 | >250 | >250 | >250 | 125 | >250 |
| **Mycophenolic acid** | µM | ≥100 | 0.4 | 0.5 | 20 | 45 | 4.0 | 0.8 |

**Table 8: Cytotoxicity and antiviral activity in CRFK cell cultures**

| | | | $EC_{50}$[b] | |
|---|---|---|---|---|
| **Compound** | **Concentration unit** | $CC_{50}$[a] | **Feline Coronavirus (FIPV)** | **Feline Herpesvirus** |
| **18a** | µM | >100 | >100 | >100 |
| **18b** | µM | >100 | >100 | >100 |
| **18c** | µM | >100 | >100 | >100 |
| **18d** | µM | 13.7 | >100 | >100 |
| **18e** | µM | >100 | >100 | >100 |
| **18f** | µM | >100 | >100 | >100 |
| **18g** | µM | 53 | >100 | >100 |
| **18h** | µM | >100 | >100 | >100 |
| **18i** | µM | >100 | >100 | >100 |
| **18j** | µM | >100 | >100 | >100 |
| **18k** | µM | >100 | >100 | >100 |
| **18l** | µM | >100 | >100 | >100 |
| **18m** | µM | >100 | >100 | >100 |
| **18n** | µM | >100 | >100 | >100 |
| **18o** | µM | >100 | >100 | >100 |
| **HHA** | µg/ml | >100 | 9.4 | 2.3 |
| **UDA** | µg/ml | >100 | 5.7 | 2.0 |
| **Ganciclovir** | µM | >100 | >100 | 3.4 |

[a] 50% Cytotoxic concentration, as determined by measuring the cell viability with colometric formazan-based MTS assay.

[a] 50% Effective concentration, or concentration producing 50% inhibition of virus-induced cytopathic effect, as determined by measuring the cell viability with colometric formazan-based MTS assay.

CRFK cells: Crandell-Rees Feline Kidney cells

**Table 9: Cytotoxicity and antiviral activity in MDCK cell cultures**

| Compound | Con. unit | Cytotoxicity | | Antiviral $EC_{50}$[c] | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | $CC_{50}$[a] | Minimum cytotoxic con.[b] | Influenza A/H1N1 A/Ned/378/05 | | Influenza A/H3N2 A/HK/7/87 | | Influenza B B/Ned/537/05 | |
| | | | | Visual CPE score | MTS | Visual CPE score | MTS | Visual CPE score | MTS |
| **18a** | µM | >100 | ≥4 | >100 | >100 | >100 | >100 | >100 | >100 |
| **18b** | µM | >100 | 100 | >100 | >100 | >100 | >100 | >100 | >100 |
| **18c** | µM | >100 | 100 | >100 | >100 | >100 | >100 | >100 | >100 |
| **18d** | µM | >100 | 20 | >100 | >100 | >100 | >100 | >100 | >100 |
| **18e** | µM | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 |
| **18f** | µM | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 |
| **18g** | µM | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 |
| **18h** | µM | >100 | ≥100 | >100 | >100 | >100 | >100 | >100 | >100 |
| **18i** | µM | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 |
| **18j** | µM | >100 | 100 | >100 | >100 | >100 | >100 | >100 | >100 |
| **18k** | µM | 56.9 | 100 | >100 | >100 | >100 | >100 | >100 | >100 |
| **18l** | µM | >100 | ≥100 | >100 | >100 | >100 | >100 | >100 | >100 |
| **18m** | µM | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 |
| **18n** | µM | >100 | ≥100 | >100 | >100 | >100 | >100 | >100 | >100 |
| **18o** | µM | >100 | 100 | >100 | >100 | >100 | >100 | >100 | >100 |
| **Zanamivir** | µM | >100 | >100 | 0.4 | 0.1 | 0.8 | 0.1 | 0.5 | 0.4 |
| **Rebavirin** | µM | >100 | 100 | 8.9 | 4.4 | 4.0 | 1.0 | 8.9 | 8.4 |
| **Amantadine** | µM | >100 | >100 | 100 | >100 | 0.2 | 0.1 | >100 | >100 |
| **Remantadine** | µM | >200 | >200 | >200 | >200 | 0.02 | 0.01 | >200 | >200 |

[a] 50% Cytotoxic concentration, as determined by measuring the cell viability with colometric formazan-based MTS assay.

[b] Minimum compound concentration that causes microscopically detectable alteration of normal cell morphology.

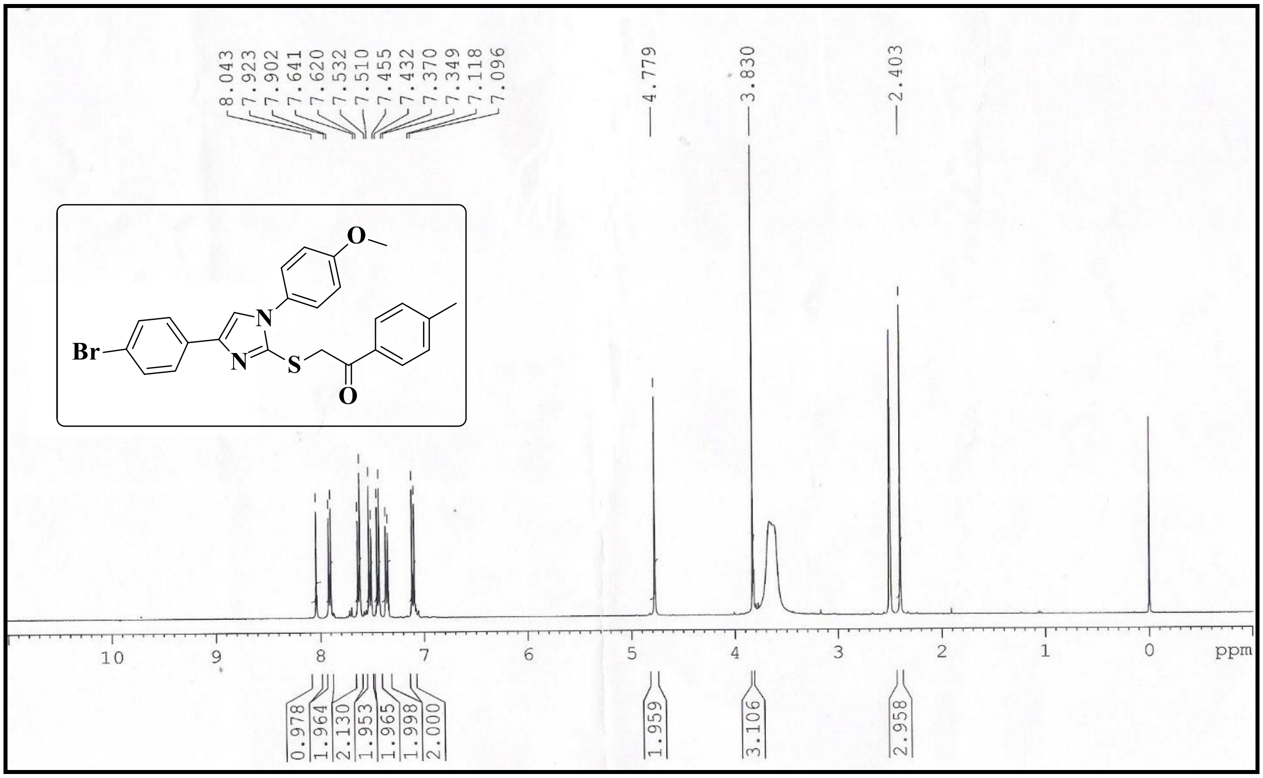
[c] 50% Effective concentration, or concentration producing 50% inhibition of virus-induced cytopathic effect, as determined by measuring the cell viability with colometric formazan-based MTS assay.

### Antiviral activity conclusion

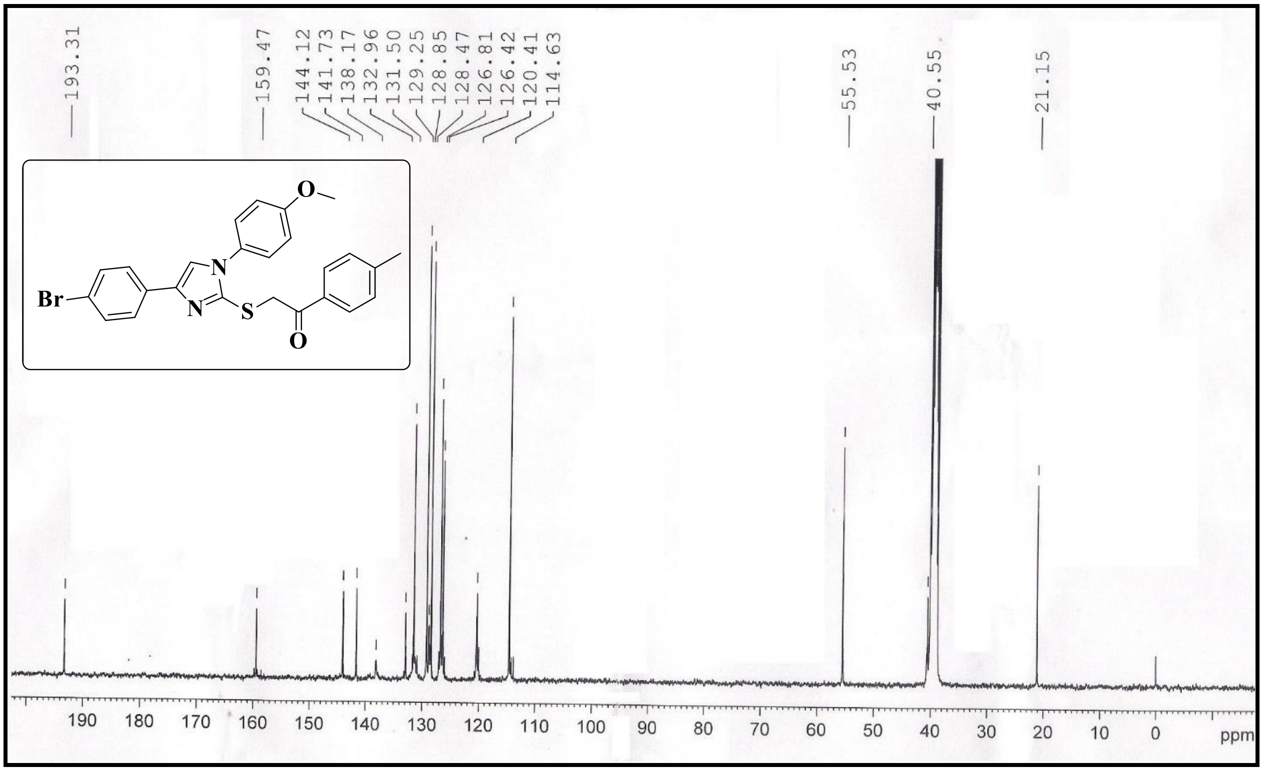
From the antiviral activity results, compound **18o** showed potent activity against the *yellow fever virus* with $EC_{50}$ value of **3** µM. The remaining compounds did not exhibit any potency against the tested viruses.

## SUMMARY

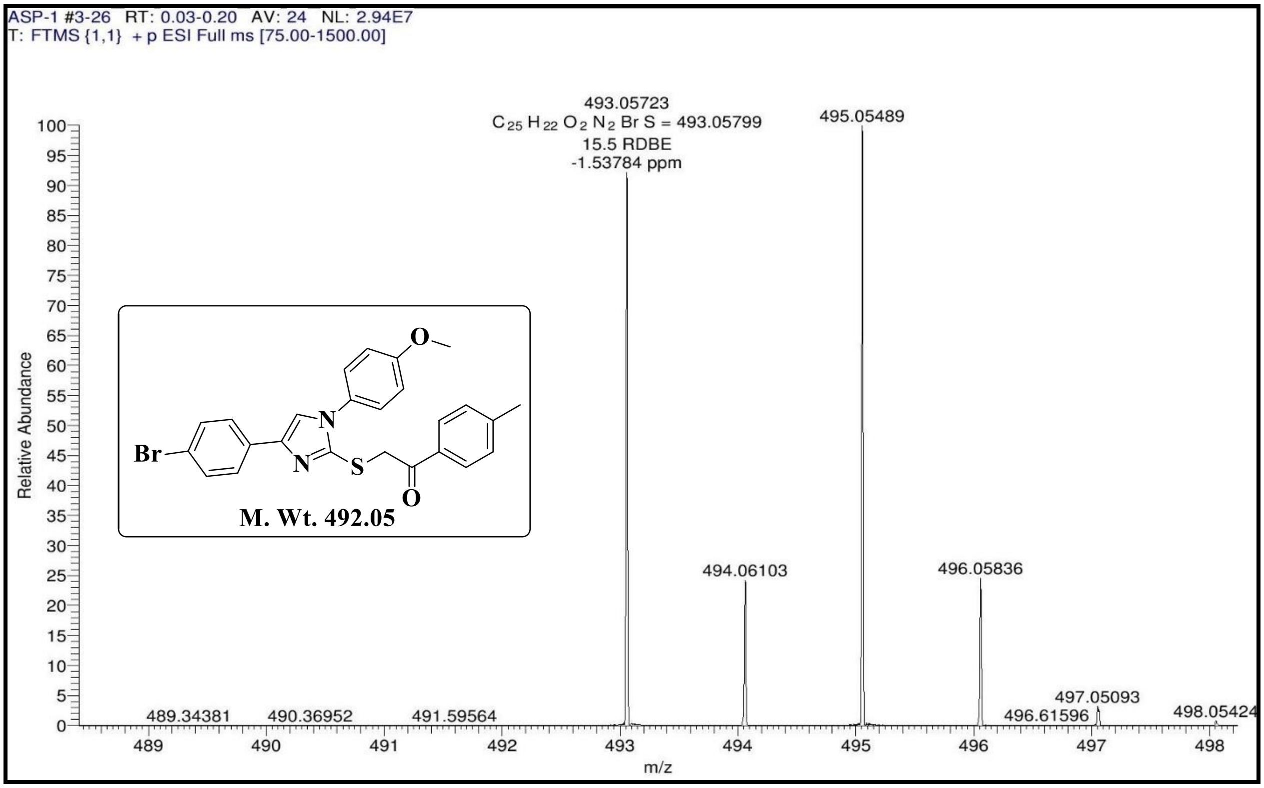
In summary, we have synthesized novel substituted imidazole derivatives via a one-pot MCR approach and some of the selected compounds were screened for their anti-cancer activity, compound **18o** showed potent anticancer activity against CNS cancer SF-268 cell line, **18b** and **18d** showed good anticancer activity. The *in vitro* anti-microbial study clearly reveals that compounds **18h, 18i, 18l, 18n, and 18o** showed potent antifungal activity. All the newly synthesized compounds were evaluated against different viruses, the compound **18o** showed potent activity against *yellow fever virus*.



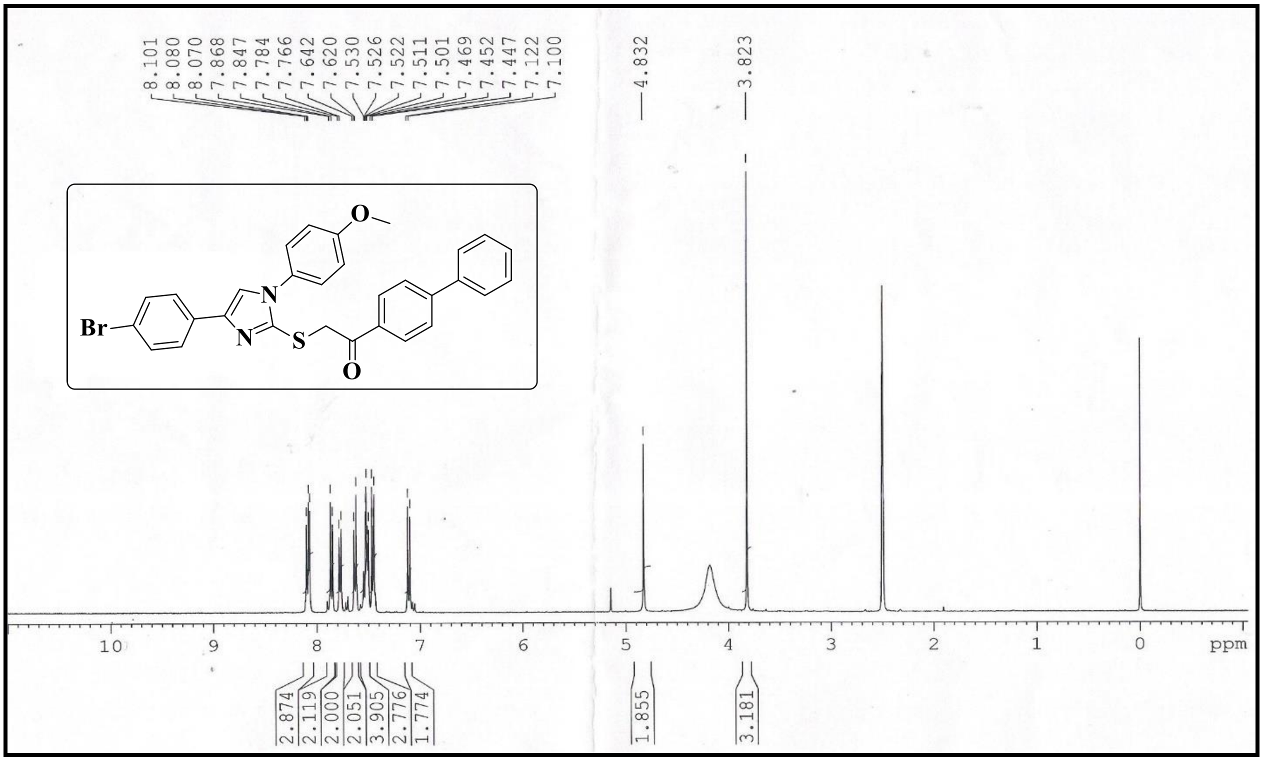
$^{1}$H NMR Spectrum of compound **18a**



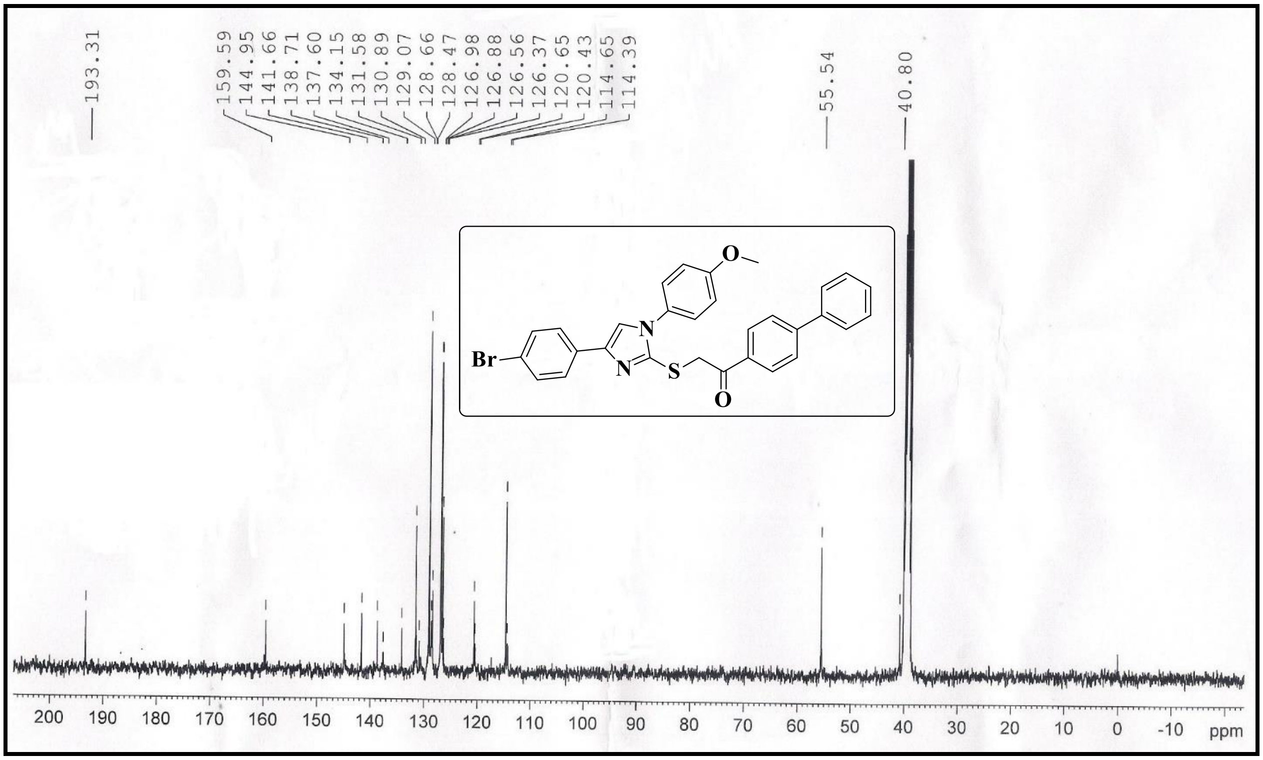
$^{13}$C NMR Spectrum of compound **18a**



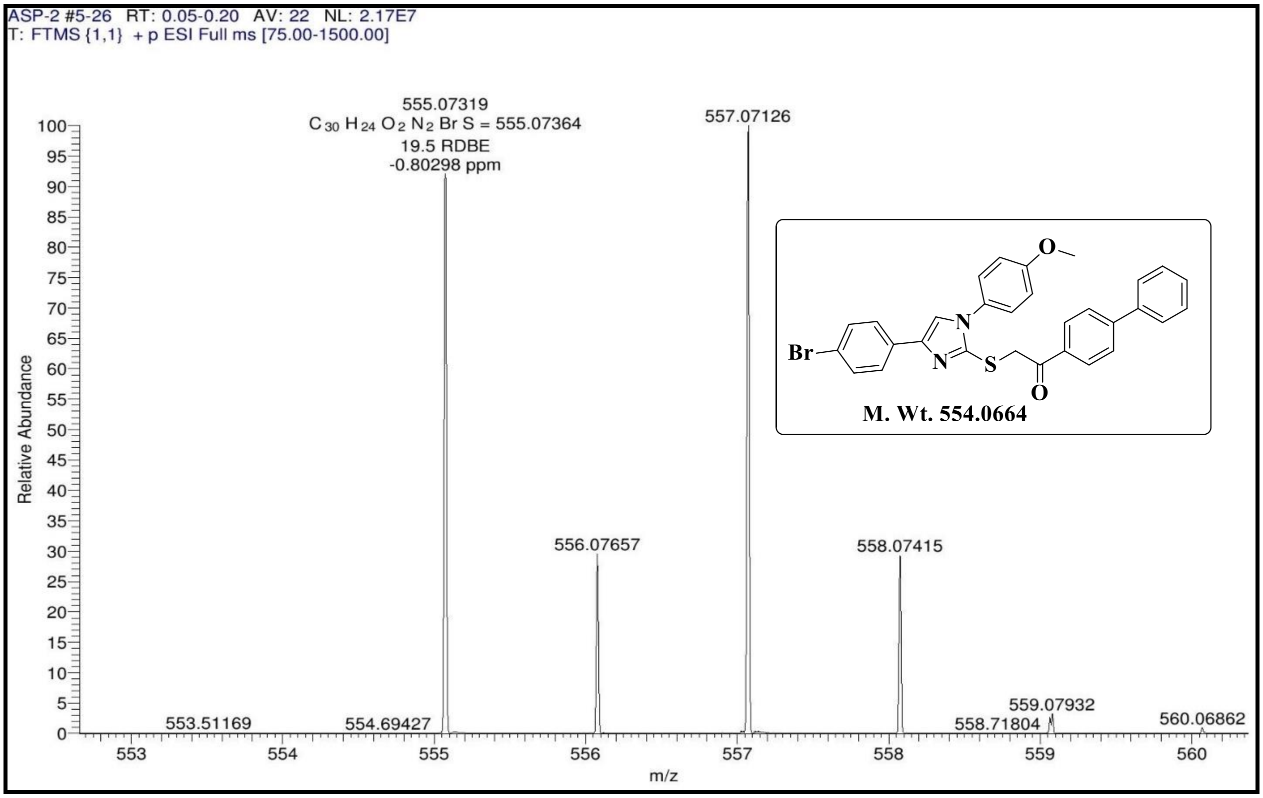
Mass Spectrum of compound **18a**



$^1$H NMR Spectrum of compound **18b**



$^{13}$C NMR Spectrum of compound **18b**



Mass Spectrum of compound **18b**

# CHAPTER-VI (SECTION-B)

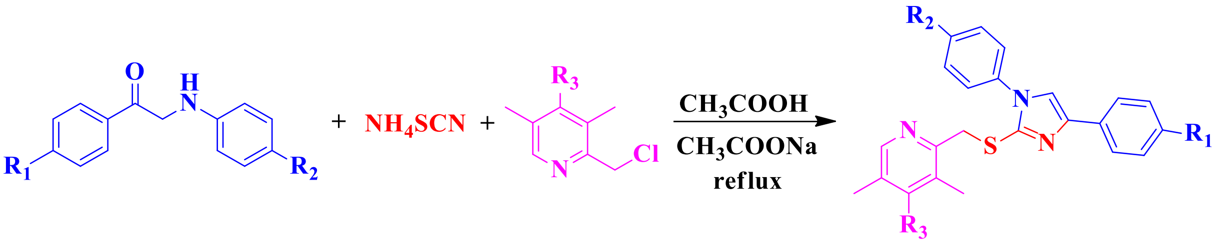
## SYNTHESIS AND ANTICANCER ACTIVITY OF 2-(((1,4-DIPHENYL-1*H*-IMIDAZOL-2-YL)THIO)METHYL)-3,5-DIMETHYLPYRIDINES

# SECTION-B

## PRESENT WORK

In the present work, we have synthesized novel substituted imidazole derivatives via a simple one-pot multicomponent reaction approach using 1-aryl-2-(arylamino)ethan-1-ones, ammonium thiocyanate and 2-(chloromethyl)-3,5-dimethylpyridine in presence of acetic acid and sodium acetate with good yields in a short reaction time as shown in scheme-3**.** All the newly synthesized imidazole derivatives were well characterized by analytical data like IR, NMR, CMR and Mass spectrometry. All the newly synthesized compounds were evaluated for their anticancer activity against 60 cancer cell lines at 10 µM concentration and some compounds were showed moderate activity.

Various 2-bromo-1-phenylethanones **19 (a-f)**, ammonium thiocyante **20** were procured from commercial source and used without any further purification. The preparation method for starting materials 1-aryl-2-(arylamino)ethan-1-ones is given in part-A of this chapter. 2-(chloromethyl)-3,5-dimethylpyridine **21 (a)**, 2-(chloromethyl)-4-methoxy-3,5-dimethylpyridine **21 (b**) were prepared according to literature procedure[1,2].

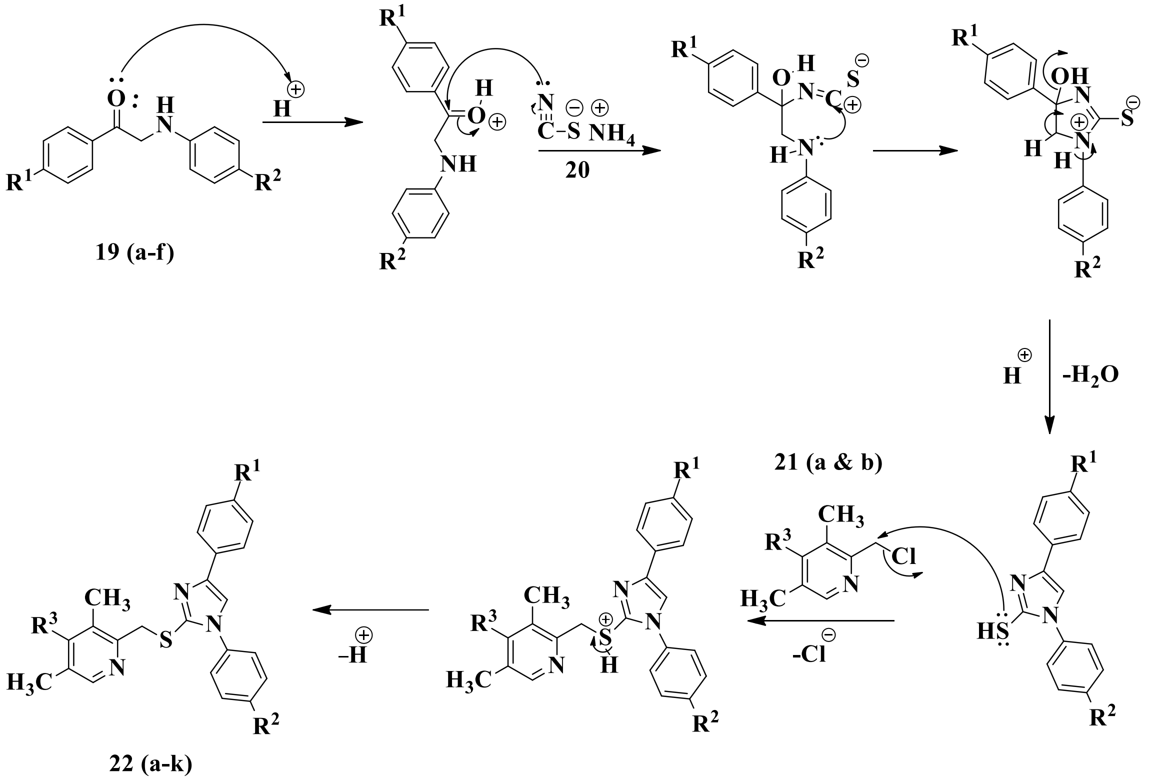


**19 (a-f)** **20** **21 (a & b)** **22 (a-k)**

**Scheme-3.** Synthesis of imidazole compounds **22 (a-k)**

### Mechanism

The *N*-substituted imidazole thiones are synthesized using Marckwald synthesis. The mechanism for the formation of **22 (a-k)** can be explained as shown in scheme-4. The 1-aryl-2-(arylamino)ethan-1-ones on treatment with acid give corresponding protonated derivatives. These undergo heterocyclization with ammonium thiocyanate to yield intermediate 2-mercaptoimidazole derivatives. Subsequently the more nucleophilic thiol group of imidazole derivatives replace the chlorine atom of 2-(chloromethyl)-3,5-dimethylpyridine to give final compounds **22 (a-k)**.



**Scheme-4** plausible mechanism for the formation of **22a-k**

**Table 10:** Reaction time of imidazole derivatives **22 (a-k)**

| S.No. | Compound | R$^1$ | R$^2$ | R$^3$ | Reaction time in hr |
|---|---|---|---|---|---|
| **1** | 22a | -H | -Cl | -H | 4 |
| **2** | 22b | -H | -Cl | -OCH$_3$ | 3.5 |
| **3** | 22c | -CH$_3$ | -Cl | -H | 4 |
| **4** | 22d | -CH$_3$ | -Cl | -OCH$_3$ | 3 |
| **5** | 22e | -OCH$_3$ | -Cl | -H | 3.5 |
| **6** | 22f | -F | -Cl | -H | 6 |
| **7** | 22g | -F | -Cl | -OCH$_3$ | 5.5 |
| **8** | 22h | -Cl | -Cl | -H | 5.5 |
| **9** | 22i | - Cl | -Cl | -OCH$_3$ | 5 |
| **10** | 22j | -Br | -OCH$_3$ | -H | 4 |
| **11** | 22k | -Ph | -Cl | -H | 5 |

The data of the designed molecules along with reaction time is presented in table 10 and analytical data like IR, $^1$H NMR and $^{13}$C NMR and mass spectral data supports the formation of the desired products. The $^1$H NMR spectrum of compound **22a** exhibited characteristic peaks δ

2.38 and δ 2.55 ppm corresponds to two-$CH_3$ protons, δ 4.63 ppm represents the,-S-$CH_2$ and δ 7.43- 8.15 ppm represents the aromatic protons. In $^{13}C$ NMR spectrum of compound **22a** methyl carbon attached to aromatic ring appears at δ 18.02 and 18.23 ppm, methylene carbon attached to 'S' atom which was directly attached to imidazole ring appears at δ 34.61 ppm. The mass spectral data of compound was confirmed by peak at m/z 407.23 as $(M+H)^+$ peak.

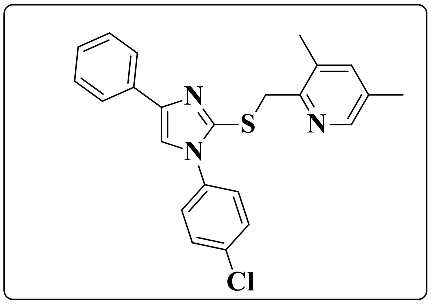
## EXPERIMENTAL SECTION

### General Procedure for synthesis of compounds 22 (a-k)

A mixture of 1-aryl-2-(arylamino)ethan-1-one (**19 a-f**, 1 mmol), ammonium isothiocyanate (**20**, 2.5 mmol) were dissolved in 5 mL of acetic acid. The reaction mixture was heated to 60 °C for about 1 hour. To this reaction mass sodium acetate (1.5 mmol) and substituted 2-(chloromethyl)-3,5-dimethylpyridine (**21 a-b**, 1 mmol) were added and the reaction mass was stirred at the same temperature and progress of reaction checked with TLC. After completion of the reaction, the reaction mixture was poured into ice cold water, isolated solid was filtered, washed with water and dried. The obtained solid was recrystallized with methanol to get the desired pure product.

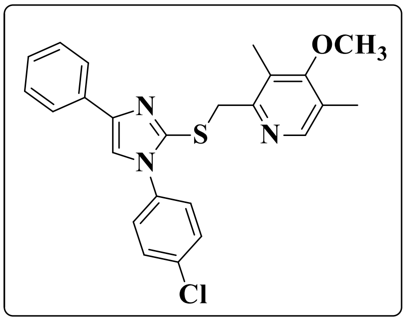
## SPECTRAL DATA

### 2-(((1-(4-Chlorophenyl)-4-phenyl-1*H*-imidazol-2-yl)thio)methyl)-3,5-dimethylpyridine (22a)



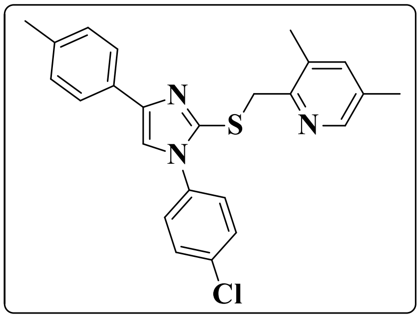
White solid, mp: 182-184 °C, yield: (0.316g, 78%), FT-IR (KBr, $\nu_{max}$ $cm^{-1}$): 1608 (C=N), 1561 (C=C), $^1H$ NMR (400MHz, $CDCl_3$, ppm): δ 2.38 (s, 3H, -$CH_3$), 2.55 (s, 3H, -$CH_3$), 4.63 (s, 2H, -S-$CH_2$), 7.36 (t, *J* = 7.6Hz, 2H, Ar-H), 7.43-7.52 (m, 6H, Ar-H), 7.65(d, *J* = 7.2Hz, 2H, Ar-H), 7.72 (s, 1H, Ar-H), 8.15 (s, 1H, Ar-H); $^{13}C$ NMR (100 MHz, $CDCl_3$): δ 18.02, 18.23, 34.61, 119.22, 124.78, 127.32, 128.62, 129.55, 132.96, 134.69, 135.19, 139.40, 140.85, 142.53, 144.31, 149.41 ppm; MS (ESI)*m/z*(%): 407.23 $[M+H]^+$; Anal. Calcd. for $C_{23}H_{20}ClN_3S$; C, 68.05; H, 4.97; N, 10.35. Found: C, 68.13; H, 4.93; N, 10.39%.

### 2-(((1-(4-Chlorophenyl)-4-phenyl-1*H*-imidazol-2-yl)thio)methyl)-4-methoxy-3,5-dimethyl pyridine (22b)



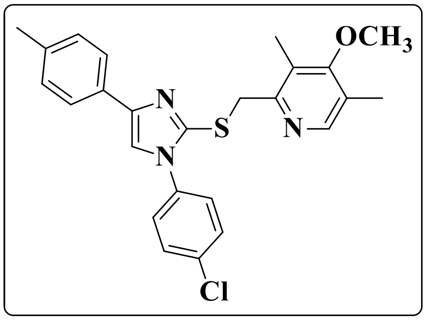
Color: light yellow solid, mp: 195-197 ºC, yield: (0.361g, 83%), FT-IR (KBr, $\nu_{max}$ cm$^{-1}$): 1603 (C=N), 1566 (C=C), $^1$H NMR (400MHz, CDCl$_3$, ppm): δ 2.21(s, 3H, -CH$_3$), 2.25(s, 3H, -CH$_3$), 3.71 (s, 3H, -OCH$_3$), 4.52 (s, 2H, -S-CH$_2$), 7.28 (d, *J* = 8.8Hz, 3H, Ar-H), 7.36-7.41 (m, 5H, Ar-H), 7.81 (d, *J* = 7.6Hz, 2H, Ar-H), 8.11 (s, 1H, Ar-H); $^{13}$C NMR (100 MHz, CDCl$_3$): δ 11.22, 13.29, 38.82, 59.91, 117.78, 124.85, 125.13, 125.34, 126.73, 127.13, 128.62, 129.40, 133.50, 134.11, 135.65, 142.29, 142.69, 149.22, 154.55, 163.96 ppm; MS (ESI)*m/z*(%): 437.33 [M+H]$^+$; Anal. Calcd. for $C_{24}H_{22}ClN_3OS$; C, 66.12; H, 5.09; N, 9.64. Found: C, 66.15; H, 5.16; N, 9.60%.

**2-(((1-(4-Chlorophenyl)-4-(p-tolyl)-1*H*-imidazol-2-yl)thio)methyl)-3,5-dimethylpyridine (22c)**



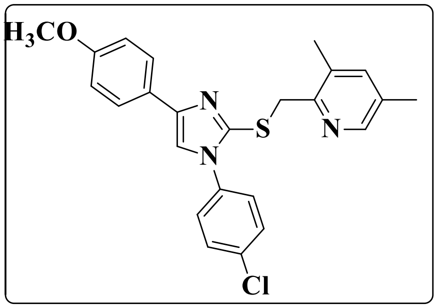
Color: white solid, mp: 208-210 ºC, yield: (0.336g, 80%), FT-IR (KBr, $\nu_{max}$ cm$^{-1}$): 1612 (C=N), 1564 (C=C), $^1$H NMR (400MHz, CDCl$_3$, ppm): δ 2.35 (s, 3H, -CH$_3$), 2.40 (s, 3H, -CH$_3$), 2.57 (s, 3H, -CH$_3$), 4.65 (s, 2H, -S-CH$_2$), 7.17 (d, *J* = 8Hz, 2H, Ar-H), 7.40 (s, 1H, Ar-H), 7.46 (d, *J* = 8.4Hz, 2H, Ar-H), 7.54 (d, *J* = 8.4Hz, 4H, Ar-H), 7.79 (s, 1H, Ar-H), 8.14 (s, 1H, Ar-H); $^{13}$C NMR (100 MHz, CDCl$_3$): δ 18.05, 18.19, 21.27, 34.02, 119.08, 124.78, 127.23, 129.34, 129.58, 129.70, 129.84, 134.80, 135.24, 135.92, 137.31, 138.61, 139.72, 142.37, 145.37, 148.90 ppm; MS (ESI)*m/z*(%): 420 [M]$^+$; Anal. Calcd. for $C_{24}H_{22}ClN_3S$; C, 68.64; H, 5.28; N, 10.01. Found: C, 68.69; H, 5.24; N, 10.15%.

**2-(((1-(4-Chlorophenyl)-4-(p-tolyl)-1*H*-imidazol-2-yl)thio)methyl)-4-methoxy-3,5-dimethylpyridine (22d)**



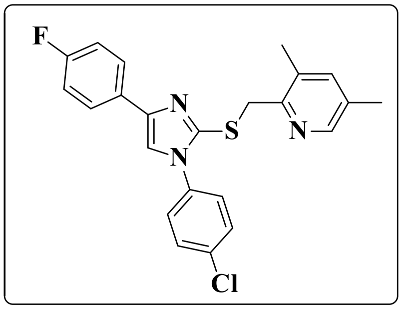
Color: white solid, mp: 225-227 ºC, yield: (0.355g, 79%), FT-IR (KBr, $\nu_{max}$ cm$^{-1}$): 1620 (C=N), 1554 (C=C), $^1$H NMR (400MHz, CDCl$_3$, ppm): δ 2.38 (s, 3H, -CH$_3$), 2.41 (s, 3H, -CH$_3$), 2.52 (s, 3H, -CH$_3$), 4.20 (s, 3H, -OCH$_3$), 4.72 (s, 2H, -S-CH$_2$), 7.27-7.28 (m, 2H, Ar-H), 7.58-7.61 (m, 3H, Ar-H), 7.82 (d, *J* = 8Hz, 2H, Ar-H), 7.92 (d, *J* = 8.4Hz, 2H, Ar-H), 8.09 (s, 1H, Ar-H); $^{13}$C NMR (100 MHz, CDCl$_3$): δ 12.04, 14.59, 21.43, 34.09, 61.56, 121.17, 126.43, 128.50, 129.93, 130.08, 131.49, 133.46, 135.97, 137.02, 138.26, 139.75, 140.19, 147.36, 171.52 ppm; MS (ESI)*m/z*(%): 450 $[M]^+$; Anal. Calcd. for $C_{25}H_{24}ClN_3OS$; C, 66.73; H, 5.38; N, 9.34. Found: C, 66.78; H, 5.34; N, 9.30%.

### 2-(((1-(4-Chlorophenyl)-4-(4-methoxyphenyl)-1*H*-imidazol-2-yl)thio)methyl)-3,5-dimethylpyridine (22e)



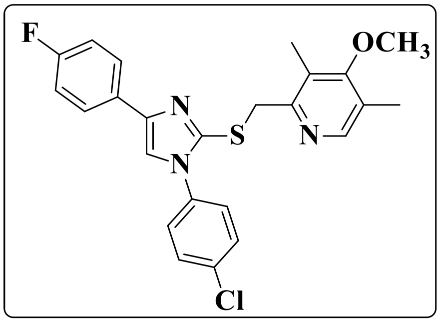
Color: white solid, mp: 191-193 $^{o}$C, yield: (0.327g, 75%), FT-IR (KBr, $\nu_{max}$ cm$^{-1}$): 1610 (C=N), 1566 (C=C), $^1$H NMR (400MHz, $CDCl_3$, ppm): δ 2.25 (s, 3H, $-CH_3$), 2.30 (s, 3H, $-CH_3$), 3.84 (s, 3H, $-OCH_3$), 4.50 (s, 2H, -S-$CH_2$), 6.94 (d, *J* = 8.8Hz, 2H, Ar-H), 7.20 (s, 1H, Ar-H), 7.24 (s, 2H, Ar-H), 7.27 (s,1H, Ar-H), 7.37 (d, *J* = 8.4Hz, 2H, Ar-H), 7.74 (d, *J* = 8.8Hz, 2H, Ar-H), 8.13 (s, 1H, Ar-H); $^{13}$C NMR (100 MHz, $CDCl_3$): δ 17.95, 18.47, 38.53, 55.32, 113.34, 114.03, 114.69, 116.80, 126.14, 126.40, 127.18, 129.31, 131.30, 132.04, 133.95, 135.73, 138.72, 141.89, 142.58, 147.18, 151.74, 158.90 ppm; MS (ESI)*m/z*(%): 437.27 $[M+H]^+$; Anal. Calcd. for $C_{24}H_{22}ClN_3OS$; C, 66.12; H, 5.09; N, 9.64. Found: C, 66.17; H, 5.15; N, 9.68%.

### 2-(((1-(4-Chlorophenyl)-4-(4-fluorophenyl)-1*H*-imidazol-2-yl)thio)methyl)-3,5-dimethyl pyridine (22f)



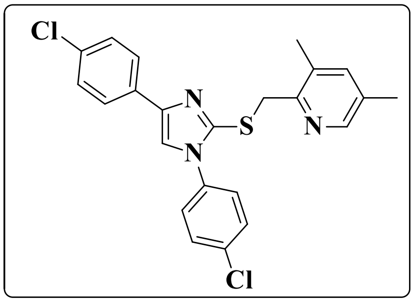
Color: white solid, mp: 213-215 $^{o}$C, yield: (0.350g, 81%), FT-IR (KBr, $\nu_{max}$ cm$^{-1}$): 1615 (C=N), 1557 (C=C), $^1$H NMR (400MHz, $CDCl_3$, ppm): δ 2.26 (s, 3H, $-CH_3$), 2.31 (s, 3H, $-CH_3$), 4.51 (s, 2H, -S-$CH_2$), 7.08 (t, *J* = 8.8Hz, 2H, Ar-H), 7.21 (s, 1H, Ar-H), 7.25-7.30 (m, 2H, Ar-H), 7.30 (s, 1H, Ar-H), 7.39 (d, *J* = 8.8Hz, 2H, Ar-H), 7.75-7.78 (m, 2H, Ar-H), 8.13 (s, 1H, Ar-H); $^{13}$C NMR (100 MHz, $CDCl_3$): δ 17.95, 18.48, 38.42, 115.40, 115.61, 116.56, 117.42, 126.43, 126.51, 129.40, 131.31, 132.13, 138.76, 141.84, 147.18, 151.58, 160.91, 163.35 ppm; MS (ESI)*m/z*(%): 424 $[M]^+$; Anal. Calcd. for $C_{23}H_{19}ClFN_3S$; C, 65.16; H, 4.52; N, 9.91. Found: C, 65.12; H, 4.58; N, 9.95%.

### 2-(((1-(4-Chlorophenyl)-4-(4-fluorophenyl)-1*H*-imidazol-2-yl)thio)methyl)-4-methoxy-3,5-dimethylpyridine (22g)



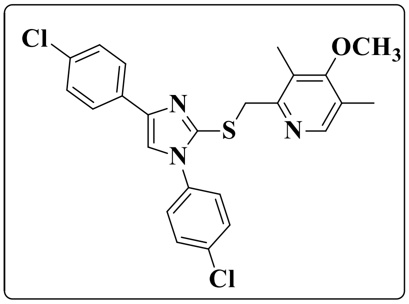
Color: white solid, mp: 227-229 °C, yield: (0.354g, 78%), FT-IR (KBr, $\nu_{max}$ $cm^{-1}$): 1610 (C=N), 1555 (C=C), $^1$H NMR (400MHz, $CDCl_3$, ppm): δ 2.41 (s, 3H, $-CH_3$), 2.50 (s, 3H, $-CH_3$), 4.20 (s, 3H, $-OCH_3$), 4.67 (s, 2H, $-S-CH_2$), 7.15 (t, *J* = 8.8 Hz, 2H, Ar-H), 7.60 (d, *J* = 6.4, 3H, Ar-H), 7.93-7.99 (m, 4H, Ar-H), 8.06 (s, 1H, Ar-H); ppm; MS (ESI)*m/z*(%): 454 $[M]^+$; Anal. Calcd. for $C_{24}H_{21}ClFN_3OS$; C, 63.50; H, 4.66; N, 9.26. Found: C, 63.54; H, 4.62; N, 9.21%.

**2-(((1,4-Bis(4-chlorophenyl)-1*H*-imidazol-2-yl)thio)methyl)-3,5-dimethylpyridine (22h)**



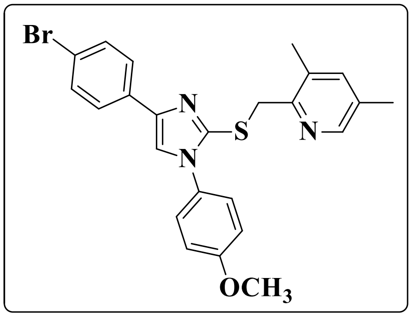
Color: white solid, mp: 229-231 °C, yield: (0.365g, 83%), FT-IR (KBr, $\nu_{max}$ $cm^{-1}$): 1614 (C=N), 1553 (C=C), $^1$H NMR (400MHz, $CDCl_3$, ppm): δ 2.42 (s, 3H, $-CH_3$), 2.59 (s, 3H, $-CH_3$), 4.63 (s, 2H, $-S-CH_2$), 7.32 (d, *J* = 8.4Hz, 2H, Ar-H), 7.44 - 7.48 (m, 3H, Ar-H), 7.53 - 7.58 (m, 4H, Ar-H), 7.84 (s, 1H, Ar-H), 8.16 (s, 1H, Ar-H); $^{13}$C NMR (100 MHz, $CDCl_3$): δ 18.07, 18.15, 33.70, 119.68, 126.03, 127.39, 128.76, 129.33, 129.61, 131.49, 132.89, 134.88, 135.18, 135.37, 135.99, 139.08, 139.45, 141.50, 145.60, 148.79 ppm; MS (ESI)*m/z*(%): 440 $[M]^+$; Anal. Calcd. for $C_{23}H_{19}Cl_2N_3S$; C, 62.73; H, 4.35; N, 9.54. Found: C, 62.76; H, 4.32; N, 9.50%.

**2-(((1,4-Bis(4-chlorophenyl)-1*H*-imidazol-2-yl)thio)methyl)-4-methoxy-3,5-dimethyl pyridine (22i)**



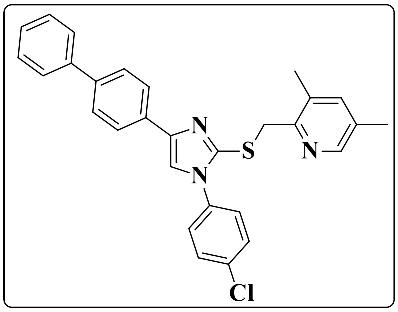
Color: white solid, mp: 241-243 °C, yield: (0.371g, 79%), FT-IR (KBr, $\nu_{max}$ $cm^{-1}$): 1610 (C=N), 1550 (C=C), $^1$H NMR (400MHz, $CDCl_3$, ppm): δ 2.21 (s, 3H, $-CH_3$), 2.26 (s, 3H, $-CH_3$), 3.72 (s, 3H, $-OCH_3$), 4.52 (s, 2H, $-S-CH_2$), 7.28 (d, *J* = 8.8Hz, 2H, Ar-H), 7.34-7.36 (m, 3H, Ar-H), 7.39 (d, *J* = 8.4Hz, 2H, Ar-H), 7.74 (d, *J* = 8.4Hz, 2H, Ar-H), 8.11 (s, 1H, Ar-H); $^{13}$C NMR (100 MHz, $CDCl_3$): δ 11.23, 13.30, 38.76, 59.92, 117.90, 125.12, 125.41, 126.08, 126.71, 128.77, 129.45, 132.05, 132.64, 134.27, 141.61, 149.21, 154.40, 163.99 ppm; MS (ESI)*m/z*(%): 470 $[M]^+$; Anal. Calcd. for $C_{24}H_{21}Cl_2N_3OS$: C, 61.28; H, 4.50; N, 8.93. Found: C, 61.33; H, 4.54; N, 8.89%.

### 2-(((4-(4-Bromophenyl)-1-(4-methoxyphenyl)-1*H*-imidazol-2-yl)thio)methyl)-3,5-dimethylpyridine (22j)



Color: white solid, mp: 247-249 °C, yield: (0.408g, 85%), FT-IR (KBr, $\nu_{max}$ cm$^{-1}$): 1610 (C=N), 1554 (C=C), $^1$H NMR (400MHz, $CDCl_3$, ppm): δ 2.41 (s, 3H, $-CH_3$), 2.51 (s, 3H, $-CH_3$), 4.21 (s, 3H, $-OCH_3$), 4.72 (s, 2H,$-S-CH_2$), 7.39-7.47 (m, 3H, Ar-H), 7.60 (d, *J* = 8.4Hz, 2H, Ar-H), 7.67 (s, 1H, Ar-H), 7.96 (d, *J* = 8Hz, 4H, Ar-H), 8.06 (s, 1H, Ar-H) ppm; $^{13}$C NMR (100 MHz, $CDCl_3$): δ 12.08, 14.62, 61.60, 121.79, 126.66, 128.64, 129.32, 130.14, 131.73, 133.26, 136.11, 137.30, 137.66, 139.54, 147.00, 171.67 ppm; Anal. Calcd. for $C_{24}H_{22}BrN_3OS$: C, 60.00; H, 4.62; N, 8.75. Found: C, 60.16; H, 4.57; N, 8.79%.

### 2-(((4-([1,1'-Biphenyl]-4-yl)-1-(4-chlorophenyl)-1*H*-imidazol-2-yl)thio)methyl)-3,5-dimethylpyridine (22k)



Color: white solid, mp: 252-254 °C, yield: (0.428g, 89%), FT-IR (KBr, $\nu_{max}$ cm$^{-1}$): 1622 (C=N), 1595 (C=C), $^1$H NMR (400MHz, $CDCl_3$, ppm): δ 2.26 (s, 3H, $-CH_3$), 2.33 (s, 3H, $-CH_3$), 4.54 (s, 2H, $-S-CH_2$), 7.21 (s, 1H, Ar-H), 7.28 (d, *J* = 8.8Hz, 2H, Ar-H), 7.32-7.40 (m, 4H, Ar-H), 7.45 (t, *J* =7.6Hz, 2H, Ar-H), 7.64 (d, *J* =7.6Hz, 4H, Ar-H), 7.88 (d, *J* = 8.4Hz, 2H, Ar-H) 8.14 (s, 1H, Ar-H); $^{13}$C NMR (100 MHz, $CDCl_3$): δ 17.96, 18.52, 38.46, 117.87, 125.03, 125.24, 126.74, 126.92, 127.20, 127.31, 127.90, 128.79, 129.39, 131.31, 132.07, 132.57, 134.13, 135.64, 138.72, 139.76, 140.89, 142.35, 142.50, 147.25, 151.71 ppm; MS (ESI)*m/z*(%): 482.29 $[M]^+$; Anal. Calcd. for $C_{29}H_{24}ClN_3S$; C, 72.26; H, 5.02; N, 8.72. Found: C, 72.22; H, 4.94; N, 8.76%.

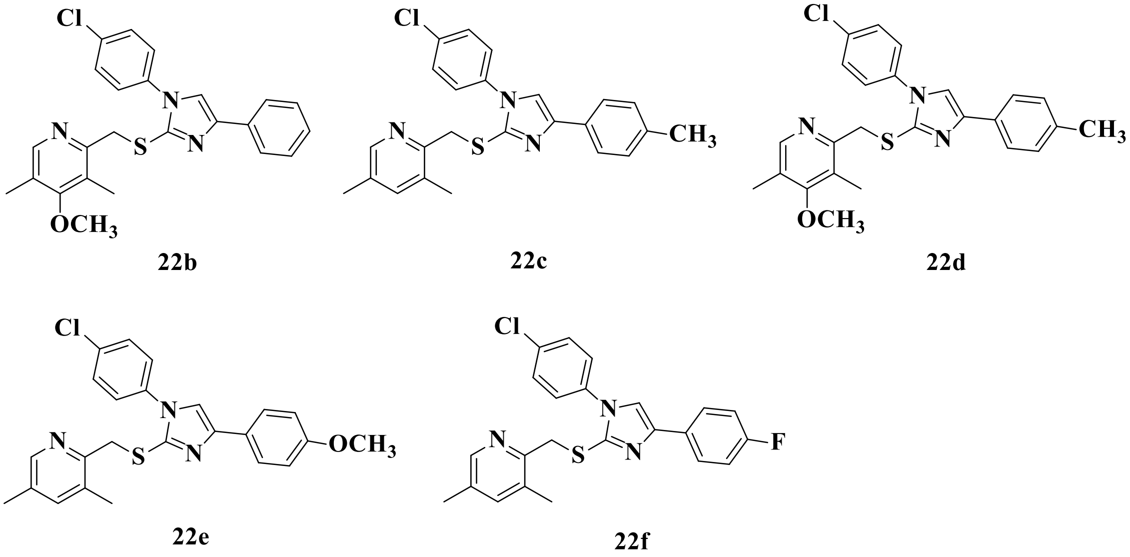
## BIOLOGICAL ACTIVITY

### *In vitro* anticancer assay

The *in vitro* anticancer activity was carried and the procedure is already given in chapter-II.

### *In vitro* anticancer activity results and discussion

Among all the synthesized compounds, 10 compounds **(table-11** to **table-20)** were selected for screening for *in vitro* anticancer activity against 60 human cancer cell lines at $10^{-5}$ M concentration and results are given as growth percent as shown in **table-21**. Among these compounds, compound **22b** showed moderate activity on breast cancer BT-549 (59.41% growth, 40.59% inhibition), compound **22c** has shown moderate activity on non-small cell lung cancer NCI-H522 (59.63% growth, 40.37% inhibition), compound **22d** showed moderate activity on non-small cell lung cancer NCI-H522 (55.88% growth, 44.12% inhibition), compound **22e** showed moderate activity on non-small cell lung cancer NCI-H522 (54.31% growth, 45.69% inhibition), moderate activity against colon cancer HT29 (59.75% growth, 40.25% inhibition). Compound **22f** showed moderate activity on non-small cell lung cancer NCI-H522 (58.61% growth, 41.39% inhibition).



**22b** **22c** **22d**

**22e** **22f**

The compounds that have anticancer activity among all the synthesized imidazole derivatives.

**Table 21:** *In vitro* anticancer activity of the title compounds

| **Cancer cell lines** | **Growth Percentage (%)** | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **22a** | **22b** | **22c** | **22d** | **22e** | **22f** | **22g** | **22h** | **22j** | **22k** |
| **Leukemia** | | | | | | | | | | |
| **MOLT-4** | 80.35 | 72.26 | 65.69 | 73.46 | 68.32 | 67.67 | 76.97 | 79.37 | 69.93 | 71.41 |
| **Non-small cell lung cancer** | | | | | | | | | | |
| **NCI-H522** | 67.41 | 72.94 | **59.63** | **55.88** | **54.31** | **58.61** | 69.32 | 77.26 | 64.48 | 68.95 |
| **Colon cancer** | | | | | | | | | | |
| **HCT-116** | 72.66 | 75.04 | 74.35 | 79.80 | 68.26 | 77.72 | 80.03 | 67.65 | 81.27 | 68.27 |
| **HT29** | 80.07 | 77.63 | 70.74 | 73.84 | **59.75** | **61.51** | 81.28 | 86.73 | 68.39 | 73.90 |
| **Renal cancer** | | | | | | | | | | |
| **UO-31** | 69.26 | 65.53 | 70.94 | **63.23** | 74.64 | 67.25 | **61.02** | 72.33 | 68.92 | 75.47 |
| **Prostate cancer** | | | | | | | | | | |
| **PC-3** | 85.85 | 67.01 | 88.94 | 81.75 | 85.01 | 86.02 | 68.92 | 90.59 | 67.67 | 82.14 |
| **Breast cancer** | | | | | | | | | | |
| **BT-549** | 101.66 | **59.41** | 104.23 | 102.49 | 97.14 | 108.34 | 100.47 | 105.91 | 108.37 | 108.09 |
| **T-47D** | **64.85** | 66.50 | 67.62 | 88.07 | **62.62** | 65.77 | 65.95 | 65.39 | **61.15** | 73.11 |

## Anticancer activity conclusion

Among all the tested compounds, compound **22b** exhibited moderate inhibitory activity on breast cancer BT-549 cell line, compound **22c** showed moderate inhibitory activity on non-small cell lung cancer NCI-H522, compound **22d** showed moderate inhibitory activity on non-small cell lung cancer NCI-H522, compound **22e** showed moderate inhibitory activity on non-small cell lung cancer NCI-H522 and colon cancer HT29 cell lines. Compound **22f** showed moderate inhibitory activity on non-small cell lung cancer NCI-H522 cell line.

## SUMMARY

In summary, we have described an efficient synthesis of substituted imidazoles via a one-pot multi-component approach. The method utilized for the synthesis of these compounds has an advantages such as shorter reaction time, easy workup and good yields. The newly synthesized compounds have shown moderate anticancer activates.

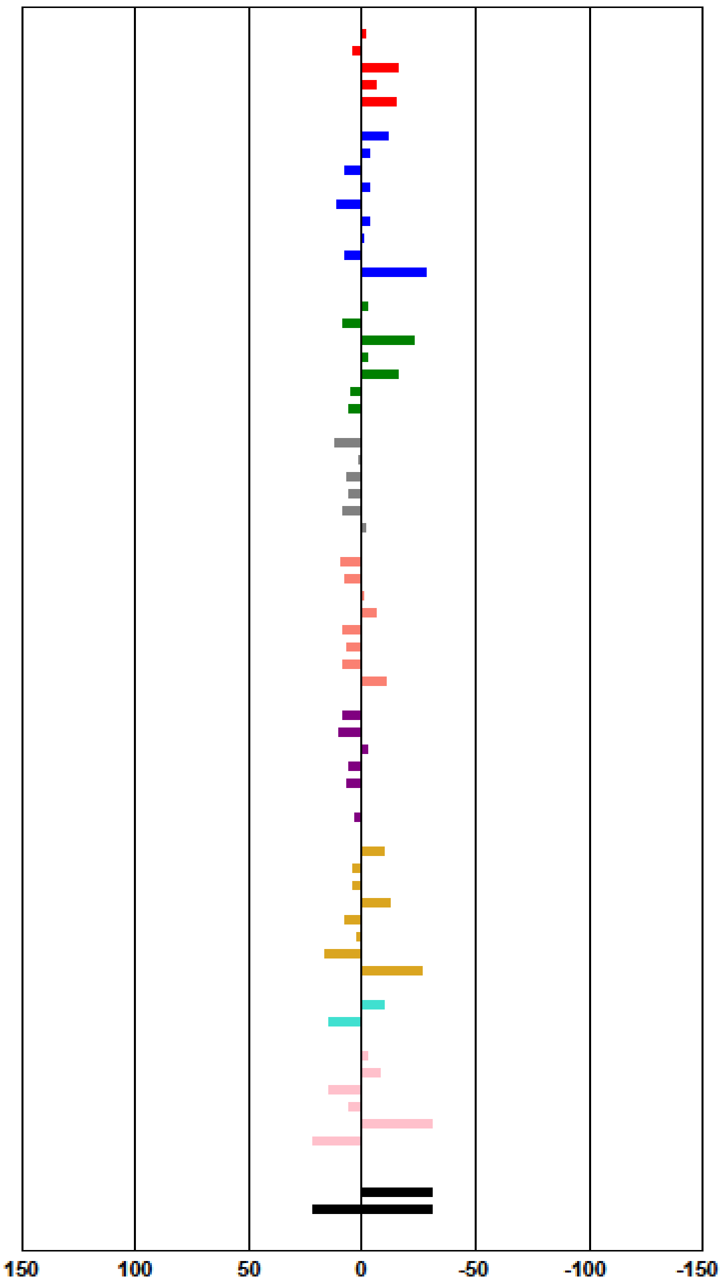
## Anticancer activity of compounds 22a to 22k

## Table-11 Compound 22a

| **Developmental Therapeutics Program**<br>**One Dose Mean Graph** | **NSC: D-798616 / 1** | **Conc: 1.00E-5 Molar** | **Test Date: Jun 12, 2017** |
|---|---|---|---|
| | **Experiment ID: 1706OS20** | | **Report Date: Jun 28, 2017** |

| Panel/Cell Line | Growth Percent |
|---|---|
| Leukemia | |
| CCRF-CEM | 94.32 |
| HL-60(TB) | 99.42 |
| MOLT-4 | 80.35 |
| RPMI-8226 | 89.56 |
| SR | 80.68 |
| Non-Small Cell Lung Cancer | |
| A549/ATCC | 84.69 |
| EKVX | 92.31 |
| HOP-62 | 102.82 |
| HOP-92 | 92.03 |
| NCI-H226 | 106.47 |
| NCI-H23 | 92.55 |
| NCI-H322M | 94.79 |
| NCI-H460 | 103.20 |
| NCI-H522 | 67.41 |
| Colon Cancer | |
| COLO 205 | 93.08 |
| HCC-2998 | 103.49 |
| HCT-116 | 72.66 |
| HCT-15 | 93.03 |
| HT29 | 80.07 |
| KM12 | 99.91 |
| SW-620 | 100.91 |
| CNS Cancer | |
| SF-268 | 107.53 |
| SF-295 | 96.97 |
| SF-539 | 101.87 |
| SNB-19 | 101.25 |
| SNB-75 | 104.20 |
| U251 | 94.10 |
| Melanoma | |
| LOX IMVI | 104.41 |
| MALME-3M | 102.80 |
| MDA-MB-435 | 95.22 |
| SK-MEL-2 | 89.58 |
| SK-MEL-28 | 103.70 |
| SK-MEL-5 | 102.39 |
| UACC-257 | 104.01 |
| UACC-62 | 85.60 |
| Ovarian Cancer | |
| IGROV1 | 104.30 |
| OVCAR-3 | 105.74 |
| OVCAR-4 | 93.43 |
| OVCAR-5 | 101.44 |
| OVCAR-8 | 101.77 |
| NCI/ADR-RES | 95.92 |
| SK-OV-3 | 98.98 |
| Renal Cancer | |
| 786-0 | 86.15 |
| A498 | 99.67 |
| ACHN | 99.48 |
| CAKI-1 | 83.59 |
| RXF 393 | 103.01 |
| SN12C | 97.43 |
| TK-10 | 112.11 |
| UO-31 | 69.26 |
| Prostate Cancer | |
| PC-3 | 85.85 |
| DU-145 | 109.98 |
| Breast Cancer | |
| MCF7 | 93.70 |
| MDA-MB-231/ATCC | 88.05 |
| HS 578T | 110.43 |
| BT-549 | 101.66 |
| T-47D | 64.85 |
| MDA-MB-468 | 116.70 |
| Mean | 95.53 |
| Delta | 30.68 |
| Range | 51.85 |

**Mean Growth Percent - Growth Percent**

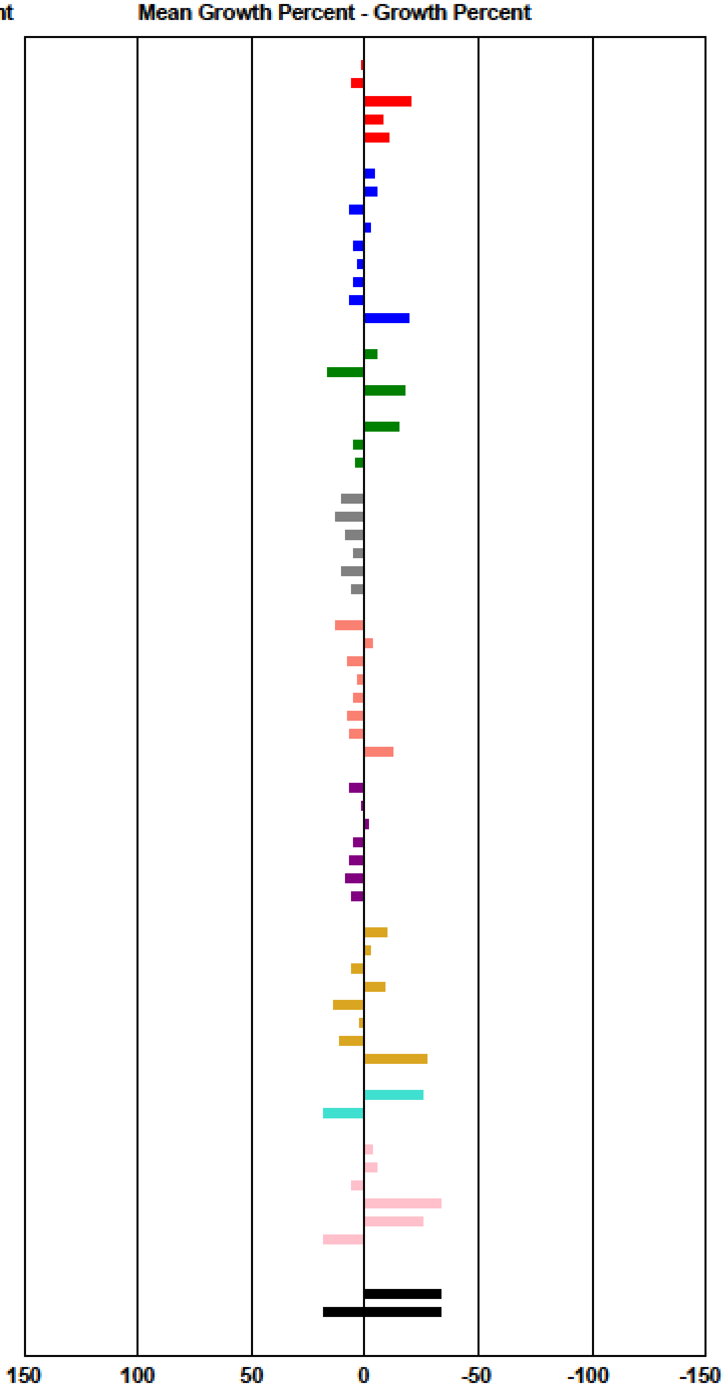


# Table-12: Compound 22b

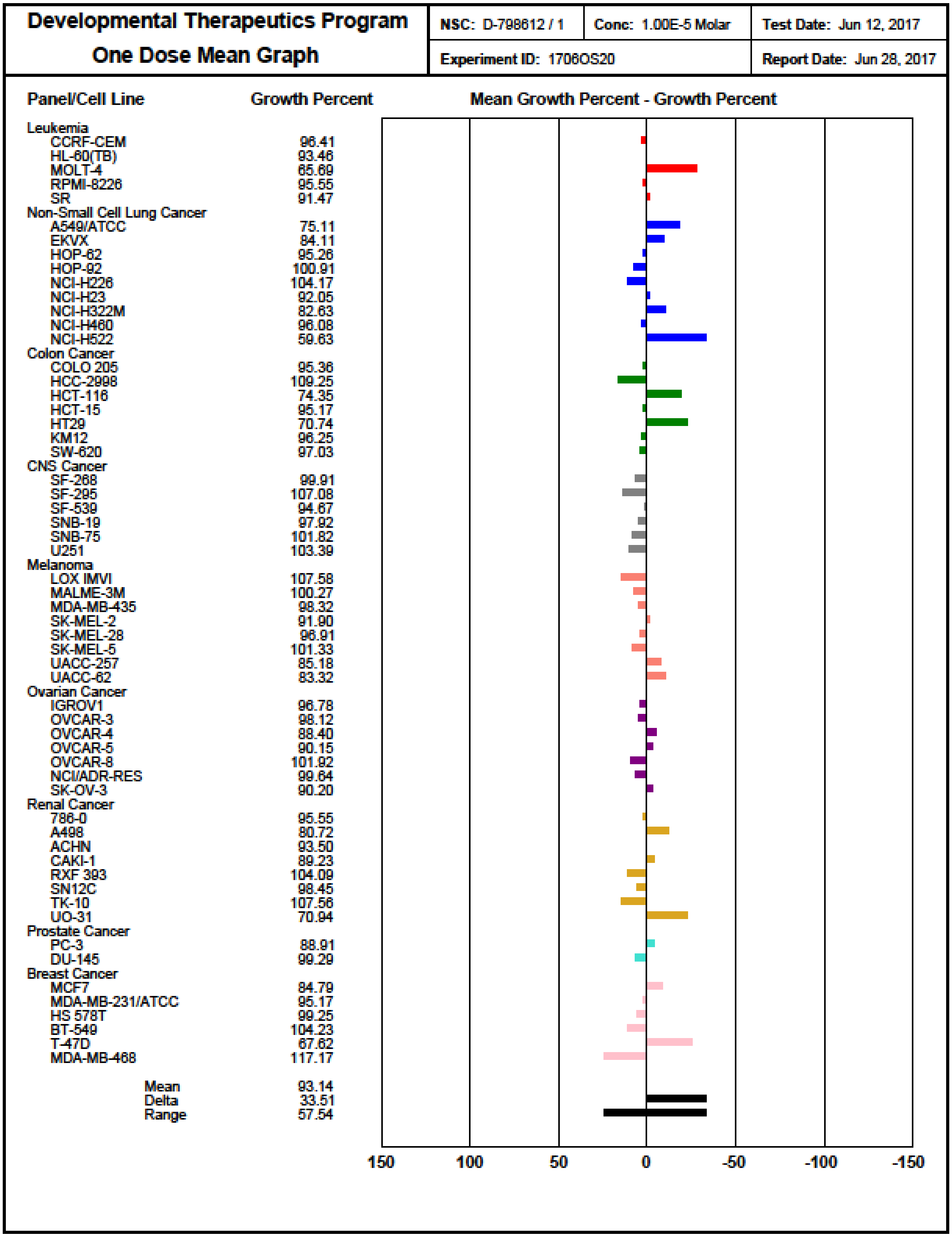
**Developmental Therapeutics Program**
**One Dose Mean Graph**

**NSC:** D-798615 / 1
**Conc:** 1.00E-5 Molar
**Test Date:** Jun 12, 2017
**Experiment ID:** 1706OS20
**Report Date:** Jun 28, 2017

| Panel/Cell Line | Growth Percent |
|---|---|
| Leukemia | |
| CCRF-CEM | 93.73 |
| HL-60(TB) | 98.19 |
| MOLT-4 | 72.26 |
| RPMI-8226 | 84.28 |
| SR | 81.64 |
| Non-Small Cell Lung Cancer | |
| A549/ATCC | 88.34 |
| EKVX | 86.82 |
| HOP-62 | 99.10 |
| HOP-92 | 89.65 |
| NCI-H226 | 97.31 |
| NCI-H23 | 94.91 |
| NCI-H322M | 97.09 |
| NCI-H460 | 99.03 |
| NCI-H522 | 72.94 |
| Colon Cancer | |
| COLO 205 | 86.95 |
| HCC-2998 | 108.68 |
| HCT-116 | 75.04 |
| HCT-15 | 92.48 |
| HT29 | 77.63 |
| KM12 | 96.97 |
| SW-620 | 96.13 |
| CNS Cancer | |
| SF-268 | 102.21 |
| SF-295 | 105.01 |
| SF-539 | 100.58 |
| SNB-19 | 96.62 |
| SNB-75 | 102.69 |
| U251 | 97.64 |
| Melanoma | |
| LOX IMVI | 105.23 |
| MALME-3M | 89.46 |
| MDA-MB-435 | 99.84 |
| SK-MEL-2 | 95.45 |
| SK-MEL-28 | 96.71 |
| SK-MEL-5 | 99.26 |
| UACC-257 | 99.03 |
| UACC-62 | 80.29 |
| Ovarian Cancer | |
| IGROV1 | 98.75 |
| OVCAR-3 | 93.12 |
| OVCAR-4 | 90.43 |
| OVCAR-5 | 96.96 |
| OVCAR-8 | 98.51 |
| NCI/ADR-RES | 100.36 |
| SK-OV-3 | 98.28 |
| Renal Cancer | |
| 786-0 | 82.80 |
| A498 | 89.82 |
| ACHN | 97.66 |
| CAKI-1 | 83.40 |
| RXF 393 | 106.22 |
| SN12C | 94.14 |
| TK-10 | 103.30 |
| UO-31 | 65.53 |
| Prostate Cancer | |
| PC-3 | 67.01 |
| DU-145 | 110.07 |
| Breast Cancer | |
| MCF7 | 89.41 |
| MDA-MB-231/ATCC | 87.00 |
| HS 578T | 97.93 |
| BT-549 | 59.41 |
| T-47D | 66.50 |
| MDA-MB-468 | 110.13 |
| Mean | 92.17 |
| Delta | 32.76 |
| Range | 50.72 |



# Table-13: Compound 22c



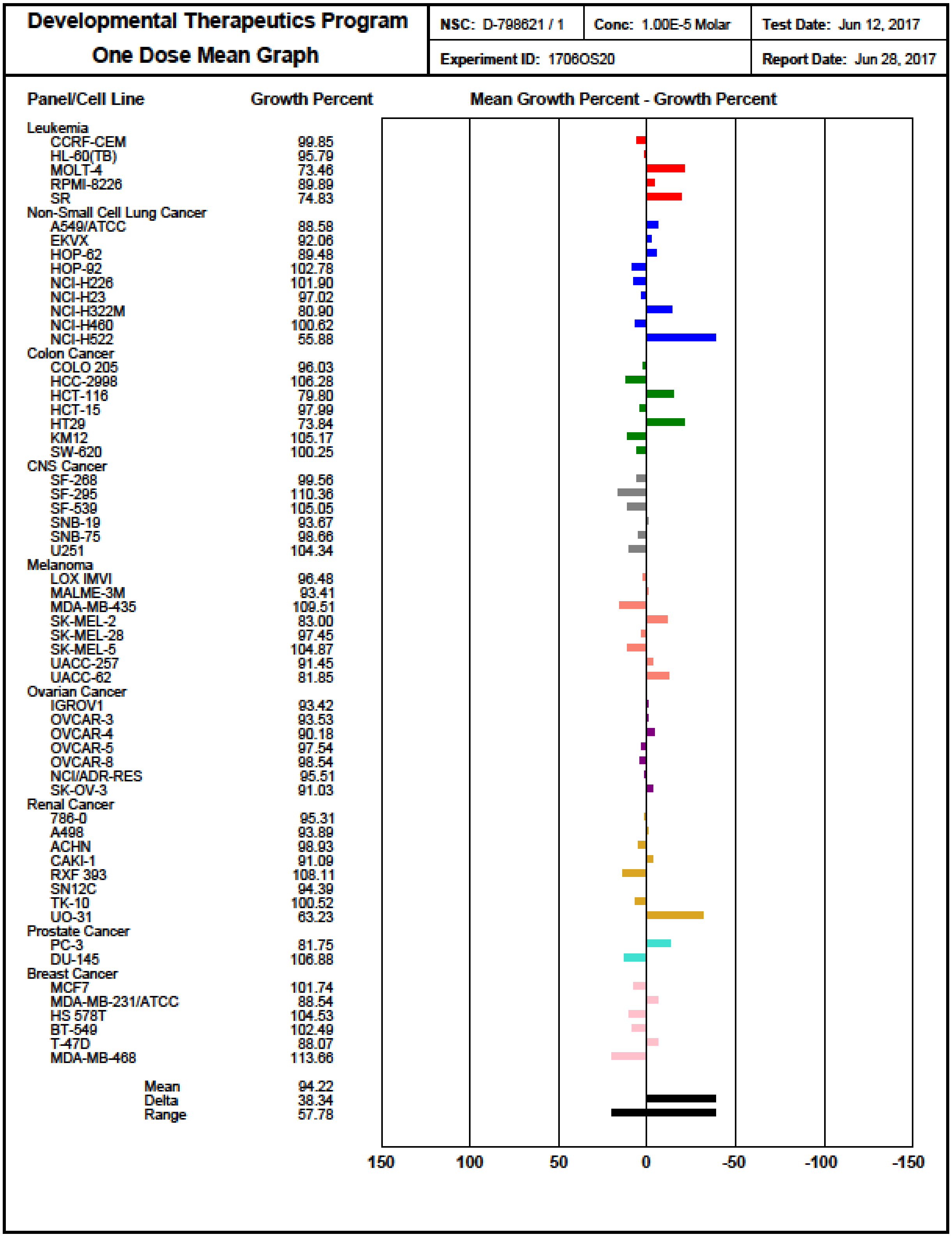
**Developmental Therapeutics Program**

**One Dose Mean Graph**

| | | |
|---|---|---|
| NSC: D-798612 / 1 | Conc: 1.00E-5 Molar | Test Date: Jun 12, 2017 |
| Experiment ID: 1706OS20 | | Report Date: Jun 28, 2017 |

| Panel/Cell Line | Growth Percent |
|---|---|
| Leukemia | |
| CCRF-CEM | 96.41 |
| HL-60(TB) | 93.46 |
| MOLT-4 | 65.69 |
| RPMI-8226 | 95.55 |
| SR | 91.47 |
| Non-Small Cell Lung Cancer | |
| A549/ATCC | 75.11 |
| EKVX | 84.11 |
| HOP-62 | 95.26 |
| HOP-92 | 100.91 |
| NCI-H226 | 104.17 |
| NCI-H23 | 92.05 |
| NCI-H322M | 82.63 |
| NCI-H460 | 96.08 |
| NCI-H522 | 59.63 |
| Colon Cancer | |
| COLO 205 | 95.36 |
| HCC-2998 | 109.25 |
| HCT-116 | 74.35 |
| HCT-15 | 95.17 |
| HT29 | 70.74 |
| KM12 | 96.25 |
| SW-620 | 97.03 |
| CNS Cancer | |
| SF-268 | 99.91 |
| SF-295 | 107.08 |
| SF-539 | 94.67 |
| SNB-19 | 97.92 |
| SNB-75 | 101.82 |
| U251 | 103.39 |
| Melanoma | |
| LOX IMVI | 107.58 |
| MALME-3M | 100.27 |
| MDA-MB-435 | 98.32 |
| SK-MEL-2 | 91.90 |
| SK-MEL-28 | 96.91 |
| SK-MEL-5 | 101.33 |
| UACC-257 | 85.18 |
| UACC-62 | 83.32 |
| Ovarian Cancer | |
| IGROV1 | 96.78 |
| OVCAR-3 | 98.12 |
| OVCAR-4 | 88.40 |
| OVCAR-5 | 90.15 |
| OVCAR-8 | 101.92 |
| NCI/ADR-RES | 99.64 |
| SK-OV-3 | 90.20 |
| Renal Cancer | |
| 786-0 | 95.55 |
| A498 | 80.72 |
| ACHN | 93.50 |
| CAKI-1 | 89.23 |
| RXF 393 | 104.09 |
| SN12C | 98.45 |
| TK-10 | 107.56 |
| UO-31 | 70.94 |
| Prostate Cancer | |
| PC-3 | 88.91 |
| DU-145 | 99.29 |
| Breast Cancer | |
| MCF7 | 84.79 |
| MDA-MB-231/ATCC | 95.17 |
| HS 578T | 99.25 |
| BT-549 | 104.23 |
| T-47D | 67.62 |
| MDA-MB-468 | 117.17 |
| Mean | 93.14 |
| Delta | 33.51 |
| Range | 57.54 |

## Table-14: Compound 22d



**Developmental Therapeutics Program**

**One Dose Mean Graph**

NSC: D-798621 / 1

Conc: 1.00E-5 Molar

Test Date: Jun 12, 2017

Experiment ID: 1706OS20

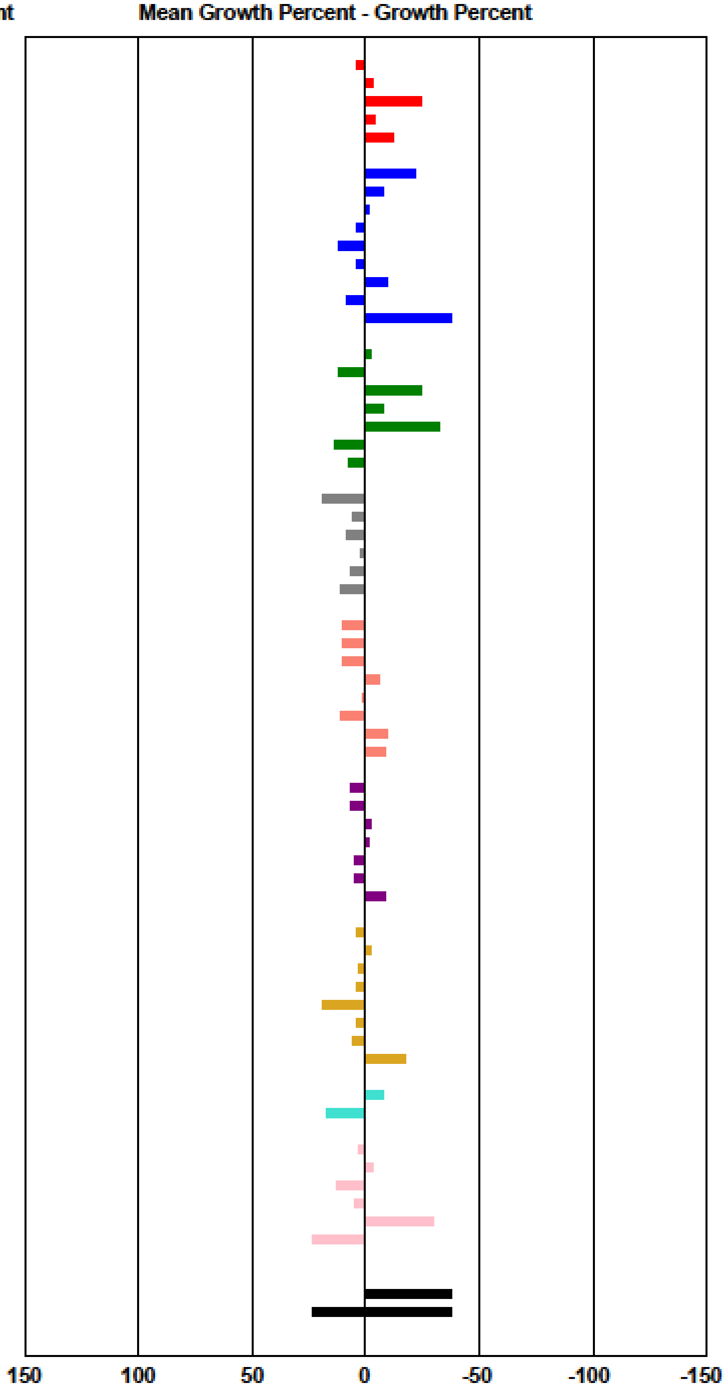
Report Date: Jun 28, 2017

| Panel/Cell Line | Growth Percent |
|---|---|
| Leukemia | |
| CCRF-CEM | 99.85 |
| HL-60(TB) | 95.79 |
| MOLT-4 | 73.46 |
| RPMI-8226 | 89.89 |
| SR | 74.83 |
| Non-Small Cell Lung Cancer | |
| A549/ATCC | 88.58 |
| EKVX | 92.06 |
| HOP-62 | 89.48 |
| HOP-92 | 102.78 |
| NCI-H226 | 101.90 |
| NCI-H23 | 97.02 |
| NCI-H322M | 80.90 |
| NCI-H460 | 100.62 |
| NCI-H522 | 55.88 |
| Colon Cancer | |
| COLO 205 | 96.03 |
| HCC-2998 | 106.28 |
| HCT-116 | 79.80 |
| HCT-15 | 97.99 |
| HT29 | 73.84 |
| KM12 | 105.17 |
| SW-620 | 100.25 |
| CNS Cancer | |
| SF-268 | 99.56 |
| SF-295 | 110.36 |
| SF-539 | 105.05 |
| SNB-19 | 93.67 |
| SNB-75 | 98.66 |
| U251 | 104.34 |
| Melanoma | |
| LOX IMVI | 96.48 |
| MALME-3M | 93.41 |
| MDA-MB-435 | 109.51 |
| SK-MEL-2 | 83.00 |
| SK-MEL-28 | 97.45 |
| SK-MEL-5 | 104.87 |
| UACC-257 | 91.45 |
| UACC-62 | 81.85 |
| Ovarian Cancer | |
| IGROV1 | 93.42 |
| OVCAR-3 | 93.53 |
| OVCAR-4 | 90.18 |
| OVCAR-5 | 97.54 |
| OVCAR-8 | 98.54 |
| NCI/ADR-RES | 95.51 |
| SK-OV-3 | 91.03 |
| Renal Cancer | |
| 786-0 | 95.31 |
| A498 | 93.89 |
| ACHN | 98.93 |
| CAKI-1 | 91.09 |
| RXF 393 | 108.11 |
| SN12C | 94.39 |
| TK-10 | 100.52 |
| UO-31 | 63.23 |
| Prostate Cancer | |
| PC-3 | 81.75 |
| DU-145 | 106.88 |
| Breast Cancer | |
| MCF7 | 101.74 |
| MDA-MB-231/ATCC | 88.54 |
| HS 578T | 104.53 |
| BT-549 | 102.49 |
| T-47D | 88.07 |
| MDA-MB-468 | 113.66 |
| Mean | 94.22 |
| Delta | 38.34 |
| Range | 57.78 |

# Table-15: Compound 22e

| Developmental Therapeutics Program<br>One Dose Mean Graph | NSC: D-798613 / 1 | Conc: 1.00E-5 Molar | Test Date: Jun 12, 2017 |
|---|---|---|---|
| | Experiment ID: 1706OS20 | | Report Date: Jun 28, 2017 |

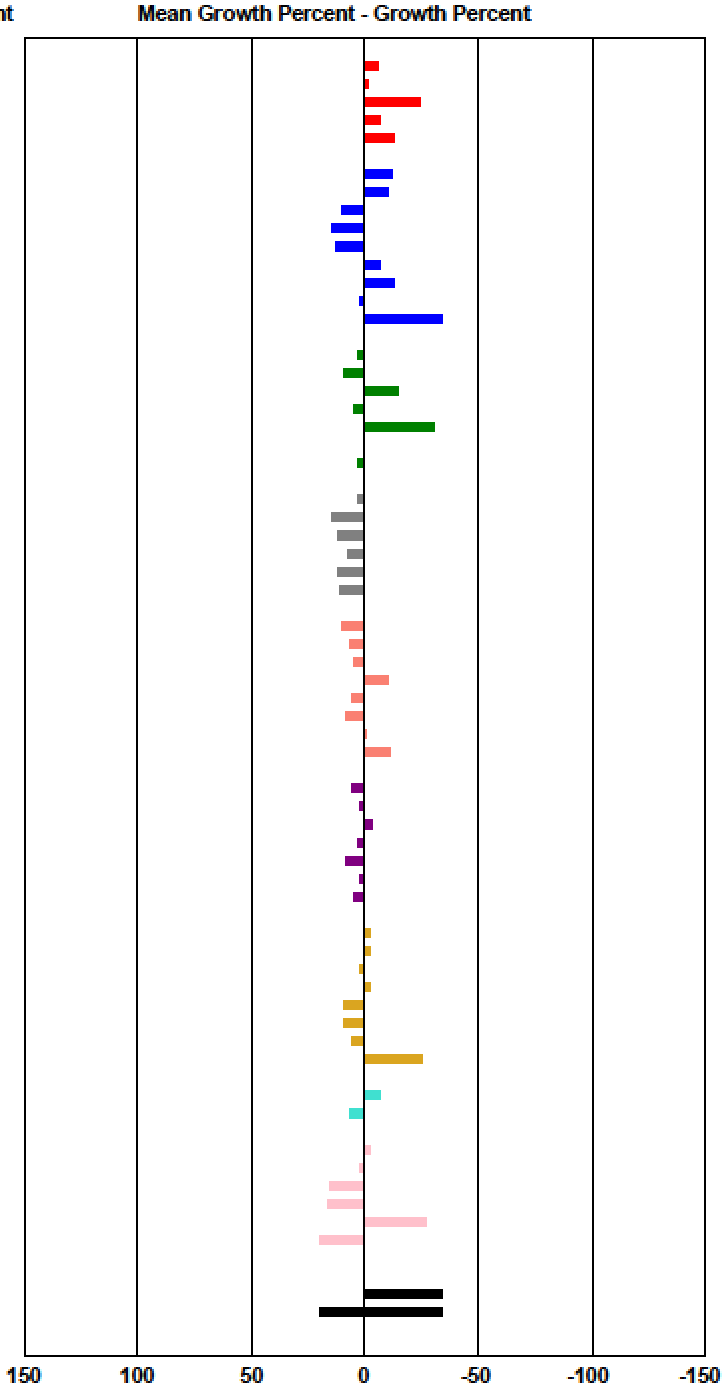
| Panel/Cell Line | Growth Percent |
|---|---|
| Leukemia | |
| CCRF-CEM | 95.80 |
| HL-60(TB) | 89.52 |
| MOLT-4 | 68.32 |
| RPMI-8226 | 88.17 |
| SR | 80.24 |
| Non-Small Cell Lung Cancer | |
| A549/ATCC | 70.27 |
| EKVX | 85.07 |
| HOP-62 | 90.85 |
| HOP-92 | 96.47 |
| NCI-H226 | 104.32 |
| NCI-H23 | 96.09 |
| NCI-H322M | 83.23 |
| NCI-H460 | 100.40 |
| NCI-H522 | 54.31 |
| Colon Cancer | |
| COLO 205 | 89.82 |
| HCC-2998 | 104.05 |
| HCT-116 | 68.26 |
| HCT-15 | 84.28 |
| HT29 | 59.75 |
| KM12 | 106.20 |
| SW-620 | 100.07 |
| CNS Cancer | |
| SF-268 | 110.92 |
| SF-295 | 97.82 |
| SF-539 | 100.61 |
| SNB-19 | 94.22 |
| SNB-75 | 98.45 |
| U251 | 102.93 |
| Melanoma | |
| LOX IMVI | 102.39 |
| MALME-3M | 101.94 |
| MDA-MB-435 | 102.47 |
| SK-MEL-2 | 86.76 |
| SK-MEL-28 | 93.94 |
| SK-MEL-5 | 102.87 |
| UACC-257 | 82.62 |
| UACC-62 | 83.61 |
| Ovarian Cancer | |
| IGROV1 | 98.44 |
| OVCAR-3 | 99.02 |
| OVCAR-4 | 90.25 |
| OVCAR-5 | 90.87 |
| OVCAR-8 | 96.72 |
| NCI/ADR-RES | 97.09 |
| SK-OV-3 | 84.22 |
| Renal Cancer | |
| 786-0 | 96.00 |
| A498 | 90.11 |
| ACHN | 95.03 |
| CAKI-1 | 96.47 |
| RXF 393 | 111.00 |
| SN12C | 96.38 |
| TK-10 | 98.36 |
| UO-31 | 74.60 |
| Prostate Cancer | |
| PC-3 | 85.01 |
| DU-145 | 109.25 |
| Breast Cancer | |
| MCF7 | 95.60 |
| MDA-MB-231/ATCC | 89.25 |
| HS 578T | 104.78 |
| BT-549 | 97.14 |
| T-47D | 62.62 |
| MDA-MB-468 | 115.56 |
| Mean | 92.26 |
| Delta | 37.95 |
| Range | 61.25 |



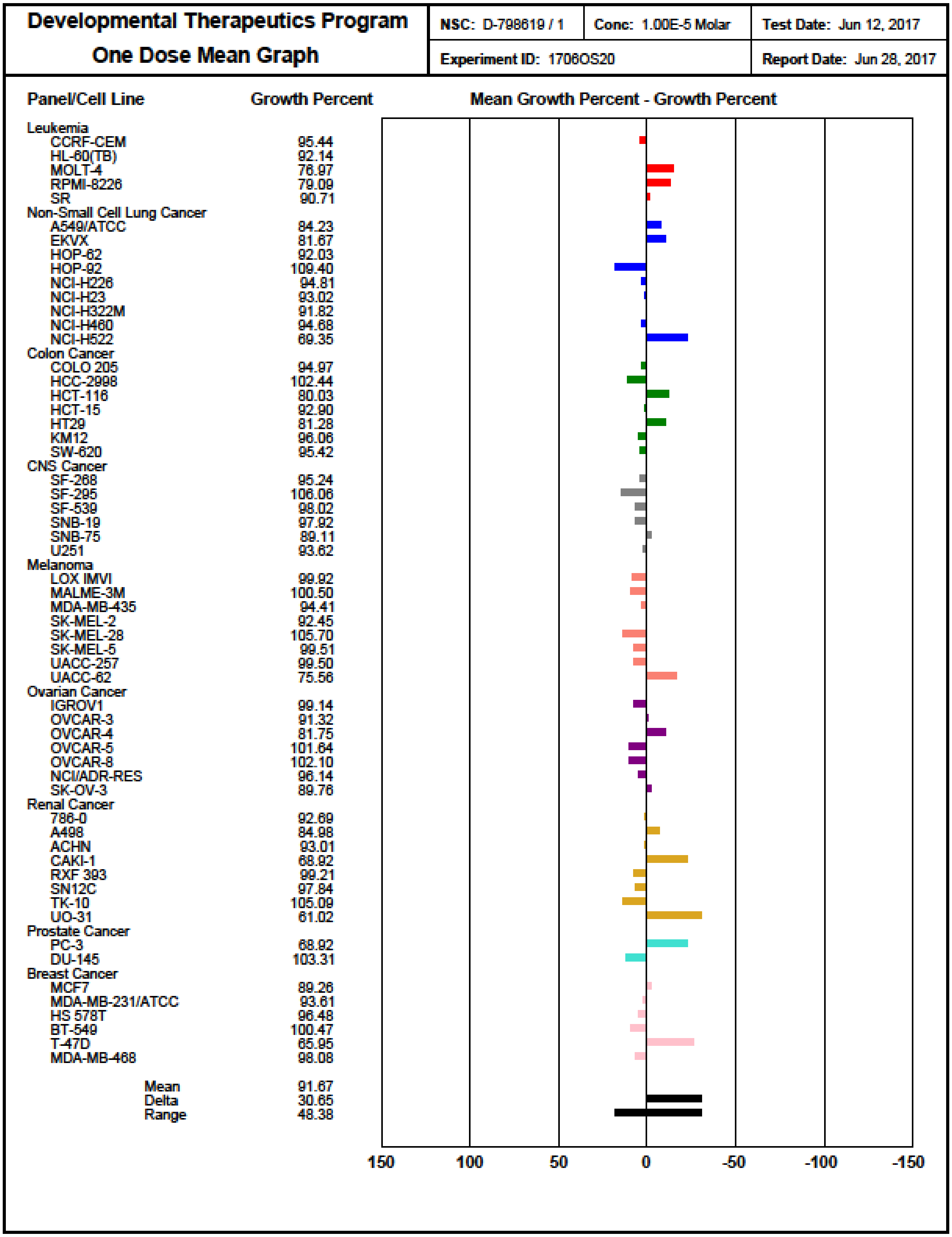
# Table-16: Compound 22f

| | | | |
|---|---|---|---|
| **Developmental Therapeutics Program** | **NSC: D-798620 / 1** | **Conc: 1.00E-5 Molar** | **Test Date: Jun 12, 2017** |
| **One Dose Mean Graph** | **Experiment ID: 1706OS20** | | **Report Date: Jun 28, 2017** |

| Panel/Cell Line | Growth Percent |
|---|---|
| Leukemia | |
| CCRF-CEM | 86.73 |
| HL-60(TB) | 91.08 |
| MOLT-4 | 67.67 |
| RPMI-8226 | 85.80 |
| SR | 79.49 |
| Non-Small Cell Lung Cancer | |
| A549/ATCC | 80.20 |
| EKVX | 82.35 |
| HOP-62 | 102.67 |
| HOP-92 | 107.01 |
| NCI-H226 | 104.77 |
| NCI-H23 | 86.07 |
| NCI-H322M | 79.32 |
| NCI-H460 | 94.26 |
| NCI-H522 | 58.61 |
| Colon Cancer | |
| COLO 205 | 95.93 |
| HCC-2998 | 101.31 |
| HCT-116 | 77.72 |
| HCT-15 | 97.31 |
| HT29 | 61.51 |
| KM12 | 92.71 |
| SW-620 | 95.55 |
| CNS Cancer | |
| SF-268 | 95.53 |
| SF-295 | 106.78 |
| SF-539 | 104.21 |
| SNB-19 | 100.16 |
| SNB-75 | 103.90 |
| U251 | 103.16 |
| Melanoma | |
| LOX IMVI | 102.75 |
| MALME-3M | 99.21 |
| MDA-MB-435 | 97.09 |
| SK-MEL-2 | 81.80 |
| SK-MEL-28 | 98.47 |
| SK-MEL-5 | 100.89 |
| UACC-257 | 91.90 |
| UACC-62 | 81.62 |
| Ovarian Cancer | |
| IGROV1 | 97.88 |
| OVCAR-3 | 94.30 |
| OVCAR-4 | 89.22 |
| OVCAR-5 | 95.82 |
| OVCAR-8 | 100.71 |
| NCI/ADR-RES | 94.43 |
| SK-OV-3 | 97.23 |
| Renal Cancer | |
| 786-0 | 90.11 |
| A498 | 89.96 |
| ACHN | 94.79 |
| CAKI-1 | 90.39 |
| RXF 393 | 101.69 |
| SN12C | 101.37 |
| TK-10 | 97.93 |
| UO-31 | 67.25 |
| Prostate Cancer | |
| PC-3 | 86.02 |
| DU-145 | 99.05 |
| Breast Cancer | |
| MCF7 | 89.95 |
| MDA-MB-231/ATCC | 94.63 |
| HS 578T | 107.70 |
| BT-549 | 108.34 |
| T-47D | 65.77 |
| MDA-MB-468 | 112.07 |
| Mean | 92.45 |
| Delta | 33.84 |
| Range | 53.46 |



# Table-17: Compound 22g



**Developmental Therapeutics Program**

**One Dose Mean Graph**

NSC: D-798619 / 1

Conc: 1.00E-5 Molar

Test Date: Jun 12, 2017

Experiment ID: 1706OS20

Report Date: Jun 28, 2017

| Panel/Cell Line | Growth Percent |
|---|---|
| Leukemia | |
| CCRF-CEM | 95.44 |
| HL-60(TB) | 92.14 |
| MOLT-4 | 76.97 |
| RPMI-8226 | 79.09 |
| SR | 90.71 |
| Non-Small Cell Lung Cancer | |
| A549/ATCC | 84.23 |
| EKVX | 81.67 |
| HOP-62 | 92.03 |
| HOP-92 | 109.40 |
| NCI-H226 | 94.81 |
| NCI-H23 | 93.02 |
| NCI-H322M | 91.82 |
| NCI-H460 | 94.68 |
| NCI-H522 | 69.35 |
| Colon Cancer | |
| COLO 205 | 94.97 |
| HCC-2998 | 102.44 |
| HCT-116 | 80.03 |
| HCT-15 | 92.90 |
| HT29 | 81.28 |
| KM12 | 96.06 |
| SW-620 | 95.42 |
| CNS Cancer | |
| SF-268 | 95.24 |
| SF-295 | 106.06 |
| SF-539 | 98.02 |
| SNB-19 | 97.92 |
| SNB-75 | 89.11 |
| U251 | 93.62 |
| Melanoma | |
| LOX IMVI | 99.92 |
| MALME-3M | 100.50 |
| MDA-MB-435 | 94.41 |
| SK-MEL-2 | 92.45 |
| SK-MEL-28 | 105.70 |
| SK-MEL-5 | 99.51 |
| UACC-257 | 99.50 |
| UACC-62 | 75.56 |
| Ovarian Cancer | |
| IGROV1 | 99.14 |
| OVCAR-3 | 91.32 |
| OVCAR-4 | 81.75 |
| OVCAR-5 | 101.64 |
| OVCAR-8 | 102.10 |
| NCI/ADR-RES | 96.14 |
| SK-OV-3 | 89.76 |
| Renal Cancer | |
| 786-0 | 92.69 |
| A498 | 84.98 |
| ACHN | 93.01 |
| CAKI-1 | 68.92 |
| RXF 393 | 99.21 |
| SN12C | 97.84 |
| TK-10 | 105.09 |
| UO-31 | 61.02 |
| Prostate Cancer | |
| PC-3 | 68.92 |
| DU-145 | 103.31 |
| Breast Cancer | |
| MCF7 | 89.26 |
| MDA-MB-231/ATCC | 93.61 |
| HS 578T | 96.48 |
| BT-549 | 100.47 |
| T-47D | 65.95 |
| MDA-MB-468 | 98.08 |
| Mean | 91.67 |
| Delta | 30.65 |
| Range | 48.38 |

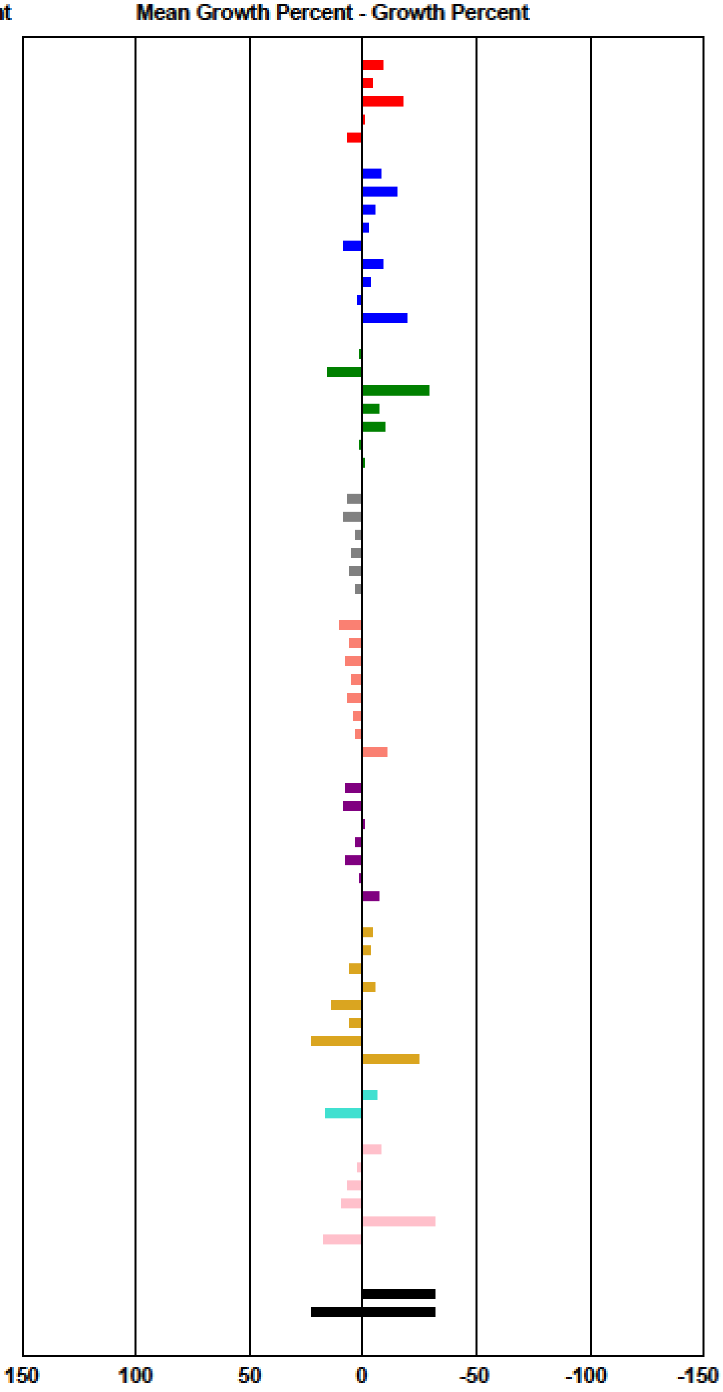
# Table-18: Compound 22h

**Developmental Therapeutics Program**

**One Dose Mean Graph**

| | | |
|---|---|---|
| **NSC:** D-798618 / 1 | **Conc:** 1.00E-5 Molar | **Test Date:** Jun 12, 2017 |
| **Experiment ID:** 1706OS20 | | **Report Date:** Jun 28, 2017 |

| Panel/Cell Line | Growth Percent |
|---|---|
| Leukemia | |
| CCRF-CEM | 87.67 |
| HL-60(TB) | 92.52 |
| MOLT-4 | 79.37 |
| RPMI-8226 | 98.01 |
| SR | 103.00 |
| Non-Small Cell Lung Cancer | |
| A549/ATCC | 89.06 |
| EKVX | 81.70 |
| HOP-62 | 91.54 |
| HOP-92 | 94.29 |
| NCI-H226 | 104.93 |
| NCI-H23 | 87.63 |
| NCI-H322M | 93.40 |
| NCI-H460 | 98.24 |
| NCI-H522 | 77.26 |
| Colon Cancer | |
| COLO 205 | 98.01 |
| HCC-2998 | 111.90 |
| HCT-116 | 67.65 |
| HCT-15 | 89.73 |
| HT29 | 86.73 |
| KM12 | 97.45 |
| SW-620 | 95.76 |
| CNS Cancer | |
| SF-268 | 103.12 |
| SF-295 | 104.54 |
| SF-539 | 99.64 |
| SNB-19 | 100.89 |
| SNB-75 | 102.55 |
| U251 | 99.17 |
| Melanoma | |
| LOX IMVI | 106.83 |
| MALME-3M | 102.36 |
| MDA-MB-435 | 104.15 |
| SK-MEL-2 | 101.65 |
| SK-MEL-28 | 102.98 |
| SK-MEL-5 | 100.70 |
| UACC-257 | 99.23 |
| UACC-62 | 86.58 |
| Ovarian Cancer | |
| IGROV1 | 104.17 |
| OVCAR-3 | 105.09 |
| OVCAR-4 | 96.19 |
| OVCAR-5 | 99.28 |
| OVCAR-8 | 103.65 |
| NCI/ADR-RES | 97.42 |
| SK-OV-3 | 89.87 |
| Renal Cancer | |
| 786-0 | 92.82 |
| A498 | 93.29 |
| ACHN | 102.07 |
| CAKI-1 | 91.31 |
| RXF 393 | 110.00 |
| SN12C | 101.75 |
| TK-10 | 119.20 |
| UO-31 | 72.33 |
| Prostate Cancer | |
| PC-3 | 90.59 |
| DU-145 | 112.75 |
| Breast Cancer | |
| MCF7 | 88.70 |
| MDA-MB-231/ATCC | 98.17 |
| HS 578T | 102.90 |
| BT-549 | 105.91 |
| T-47D | 65.39 |
| MDA-MB-468 | 113.19 |
| Mean | 96.45 |
| Delta | 31.06 |
| Range | 53.81 |



# Table-19: Compound 22j

**Developmental Therapeutics Program**

**One Dose Mean Graph**

**NSC:** D-798617 / 1

**Conc:** 1.00E-5 Molar

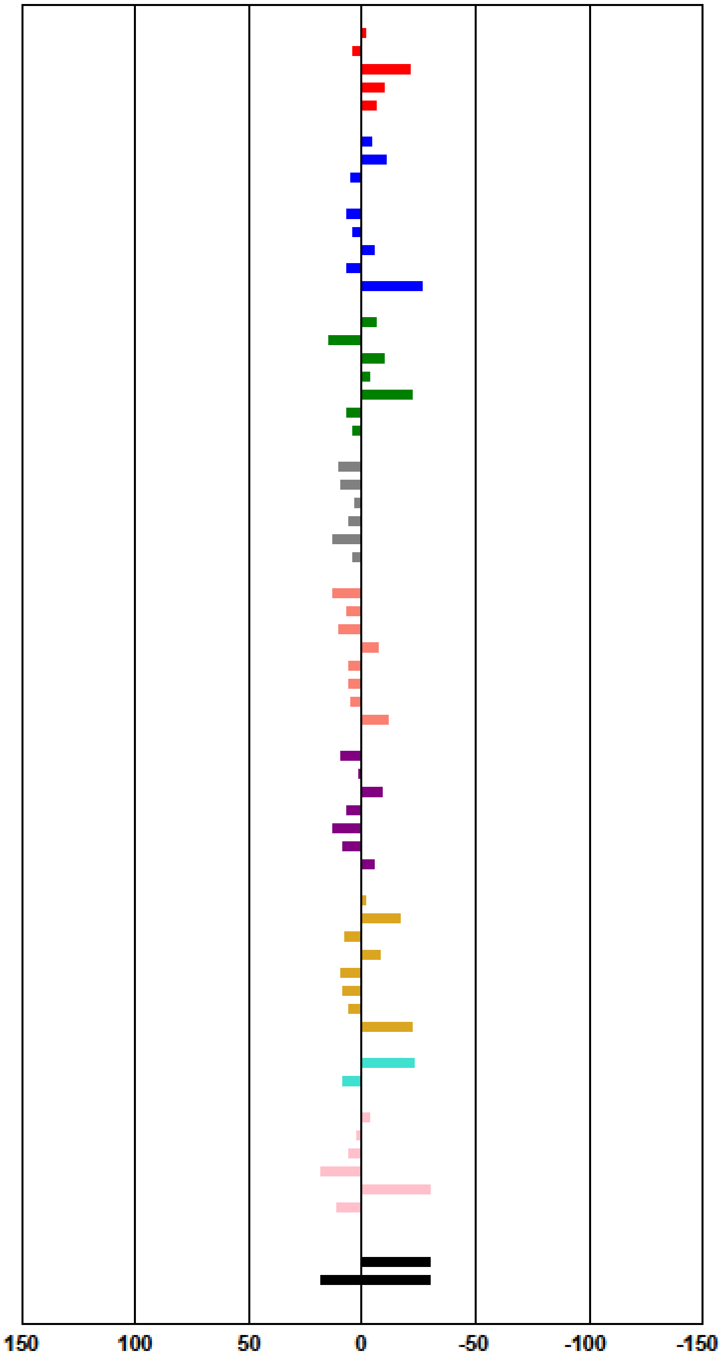
**Test Date:** Jun 12, 2017

**Experiment ID:** 1706OS20

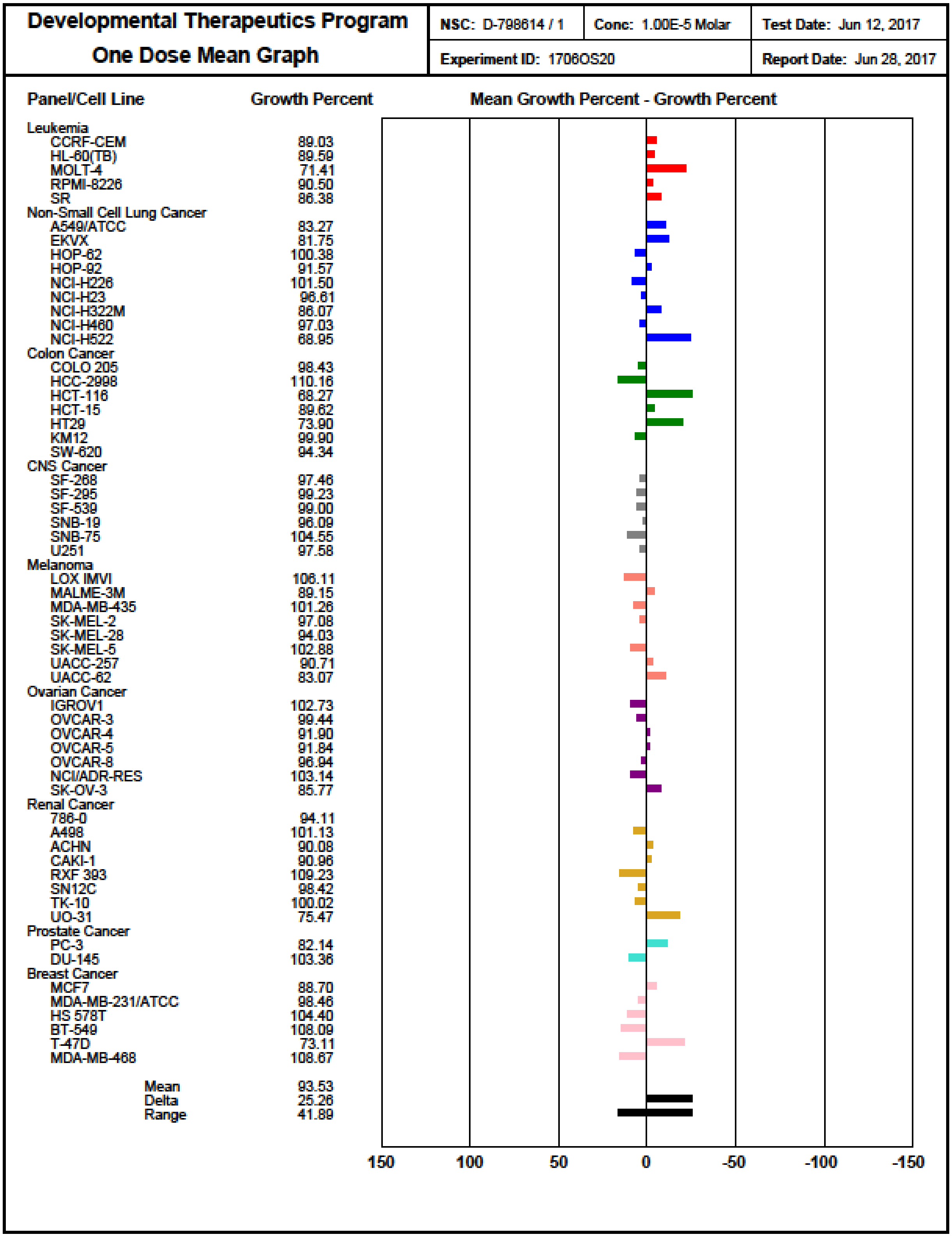
**Report Date:** Jun 28, 2017

| Panel/Cell Line | Growth Percent |
|---|---|
| Leukemia | |
| CCRF-CEM | 89.46 |
| HL-60(TB) | 94.58 |
| MOLT-4 | 69.93 |
| RPMI-8226 | 81.34 |
| SR | 84.66 |
| Non-Small Cell Lung Cancer | |
| A549/ATCC | 86.83 |
| EKVX | 79.90 |
| HOP-62 | 94.98 |
| HOP-92 | 90.89 |
| NCI-H226 | 96.89 |
| NCI-H23 | 94.48 |
| NCI-H322M | 85.15 |
| NCI-H460 | 96.75 |
| NCI-H522 | 64.48 |
| Colon Cancer | |
| COLO 205 | 84.60 |
| HCC-2998 | 104.68 |
| HCT-116 | 81.27 |
| HCT-15 | 87.07 |
| HT29 | 68.39 |
| KM12 | 96.98 |
| SW-620 | 94.61 |
| CNS Cancer | |
| SF-268 | 100.58 |
| SF-295 | 99.82 |
| SF-539 | 93.72 |
| SNB-19 | 95.98 |
| SNB-75 | 103.07 |
| U251 | 94.78 |
| Melanoma | |
| LOX IMVI | 103.04 |
| MALME-3M | 97.05 |
| MDA-MB-435 | 100.92 |
| SK-MEL-2 | 83.80 |
| SK-MEL-28 | 96.11 |
| SK-MEL-5 | 96.35 |
| UACC-257 | 95.59 |
| UACC-62 | 79.05 |
| Ovarian Cancer | |
| IGROV1 | 99.27 |
| OVCAR-3 | 91.36 |
| OVCAR-4 | 82.36 |
| OVCAR-5 | 97.41 |
| OVCAR-8 | 102.97 |
| NCI/ADR-RES | 98.76 |
| SK-OV-3 | 85.50 |
| Renal Cancer | |
| 786-0 | 89.12 |
| A498 | 74.49 |
| ACHN | 97.49 |
| CAKI-1 | 82.81 |
| RXF 393 | 99.99 |
| SN12C | 99.13 |
| TK-10 | 96.01 |
| UO-31 | 68.92 |
| Prostate Cancer | |
| PC-3 | 67.67 |
| DU-145 | 99.07 |
| Breast Cancer | |
| MCF7 | 87.39 |
| MDA-MB-231/ATCC | 92.62 |
| HS 578T | 96.03 |
| BT-549 | 108.37 |
| T-47D | 61.15 |
| MDA-MB-468 | 101.83 |
| Mean | 90.47 |
| Delta | 29.32 |
| Range | 47.22 |

**Mean Growth Percent - Growth Percent**



## Table-20: Compound 22k



**Developmental Therapeutics Program**

**One Dose Mean Graph**

NSC: D-798614 / 1

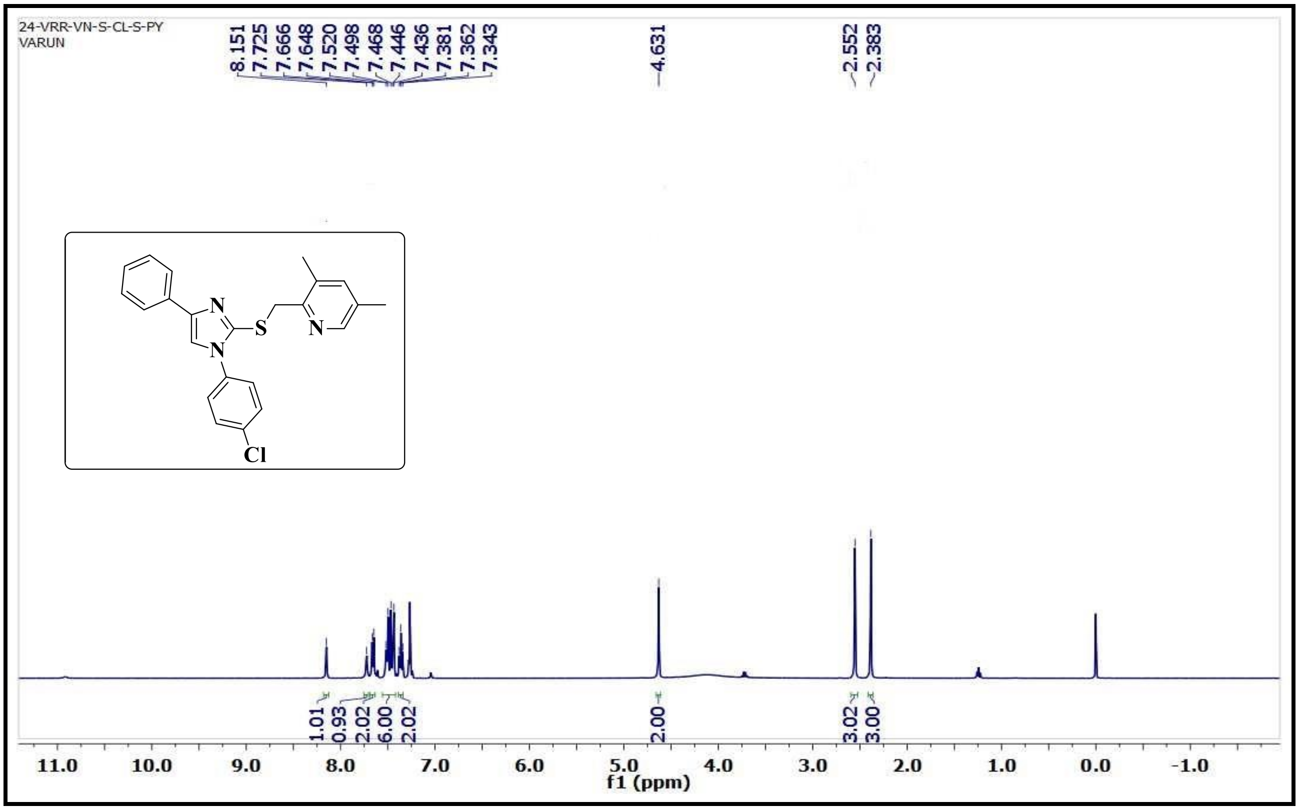
Conc: 1.00E-5 Molar

Test Date: Jun 12, 2017

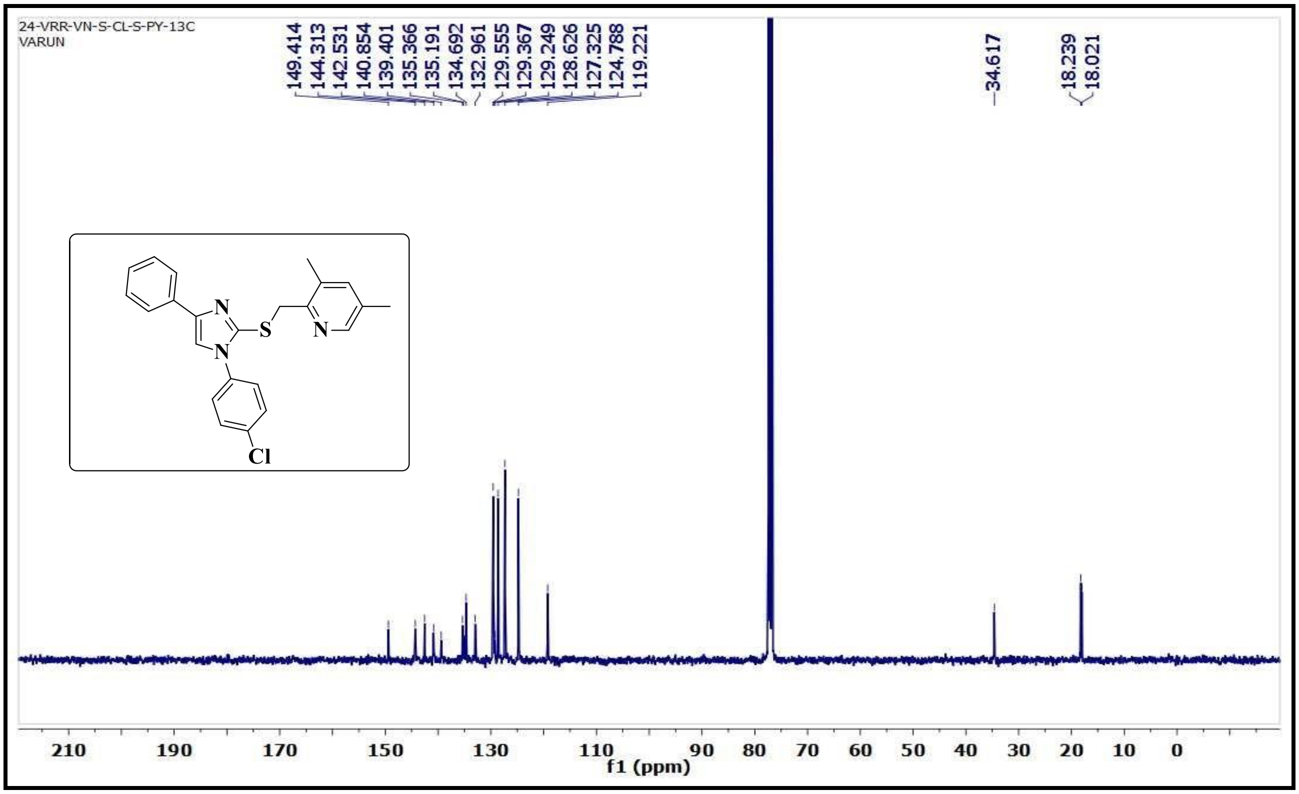
Experiment ID: 1706OS20

Report Date: Jun 28, 2017

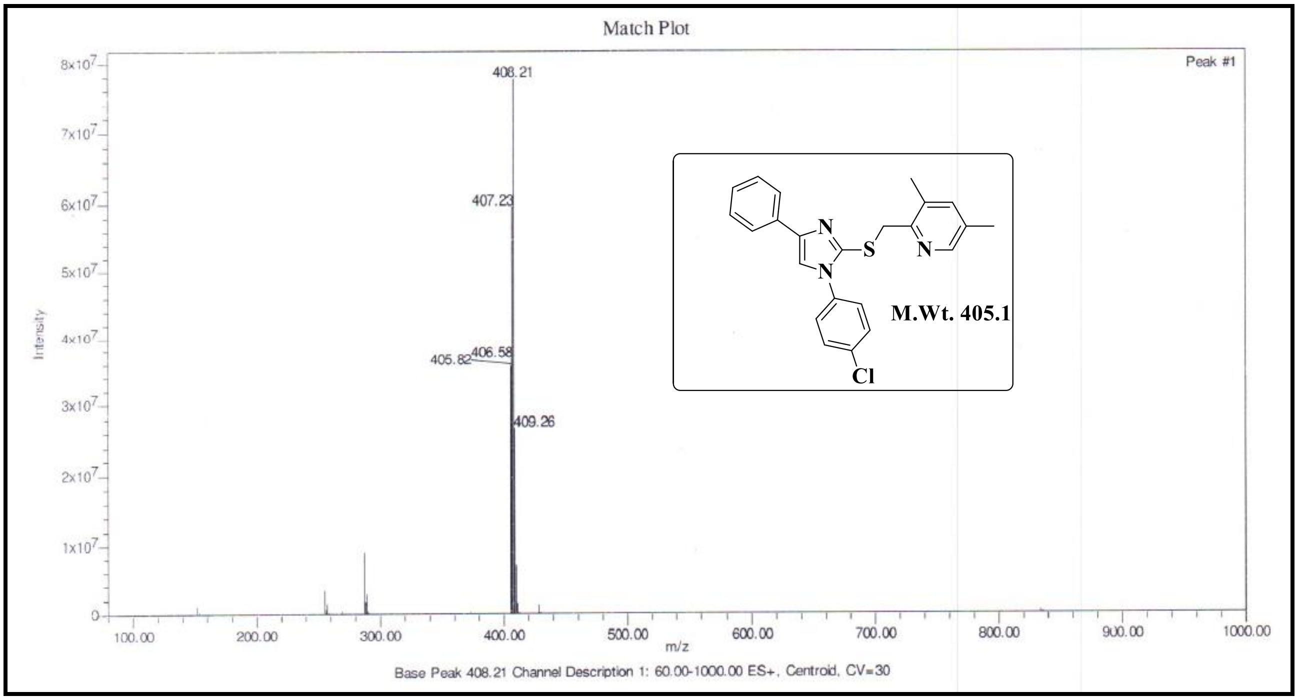
| Panel/Cell Line | Growth Percent |
|---|---|
| Leukemia | |
| CCRF-CEM | 89.03 |
| HL-60(TB) | 89.59 |
| MOLT-4 | 71.41 |
| RPMI-8226 | 90.50 |
| SR | 86.38 |
| Non-Small Cell Lung Cancer | |
| A549/ATCC | 83.27 |
| EKVX | 81.75 |
| HOP-62 | 100.38 |
| HOP-92 | 91.57 |
| NCI-H226 | 101.50 |
| NCI-H23 | 96.61 |
| NCI-H322M | 86.07 |
| NCI-H460 | 97.03 |
| NCI-H522 | 68.95 |
| Colon Cancer | |
| COLO 205 | 98.43 |
| HCC-2998 | 110.16 |
| HCT-116 | 68.27 |
| HCT-15 | 89.62 |
| HT29 | 73.90 |
| KM12 | 99.90 |
| SW-620 | 94.34 |
| CNS Cancer | |
| SF-268 | 97.46 |
| SF-295 | 99.23 |
| SF-539 | 99.00 |
| SNB-19 | 96.09 |
| SNB-75 | 104.55 |
| U251 | 97.58 |
| Melanoma | |
| LOX IMVI | 106.11 |
| MALME-3M | 89.15 |
| MDA-MB-435 | 101.26 |
| SK-MEL-2 | 97.08 |
| SK-MEL-28 | 94.03 |
| SK-MEL-5 | 102.88 |
| UACC-257 | 90.71 |
| UACC-62 | 83.07 |
| Ovarian Cancer | |
| IGROV1 | 102.73 |
| OVCAR-3 | 99.44 |
| OVCAR-4 | 91.90 |
| OVCAR-5 | 91.84 |
| OVCAR-8 | 96.94 |
| NCI/ADR-RES | 103.14 |
| SK-OV-3 | 85.77 |
| Renal Cancer | |
| 786-0 | 94.11 |
| A498 | 101.13 |
| ACHN | 90.08 |
| CAKI-1 | 90.96 |
| RXF 393 | 109.23 |
| SN12C | 98.42 |
| TK-10 | 100.02 |
| UO-31 | 75.47 |
| Prostate Cancer | |
| PC-3 | 82.14 |
| DU-145 | 103.36 |
| Breast Cancer | |
| MCF7 | 88.70 |
| MDA-MB-231/ATCC | 98.46 |
| HS 578T | 104.40 |
| BT-549 | 108.09 |
| T-47D | 73.11 |
| MDA-MB-468 | 108.67 |
| Mean | 93.53 |
| Delta | 25.26 |
| Range | 41.89 |



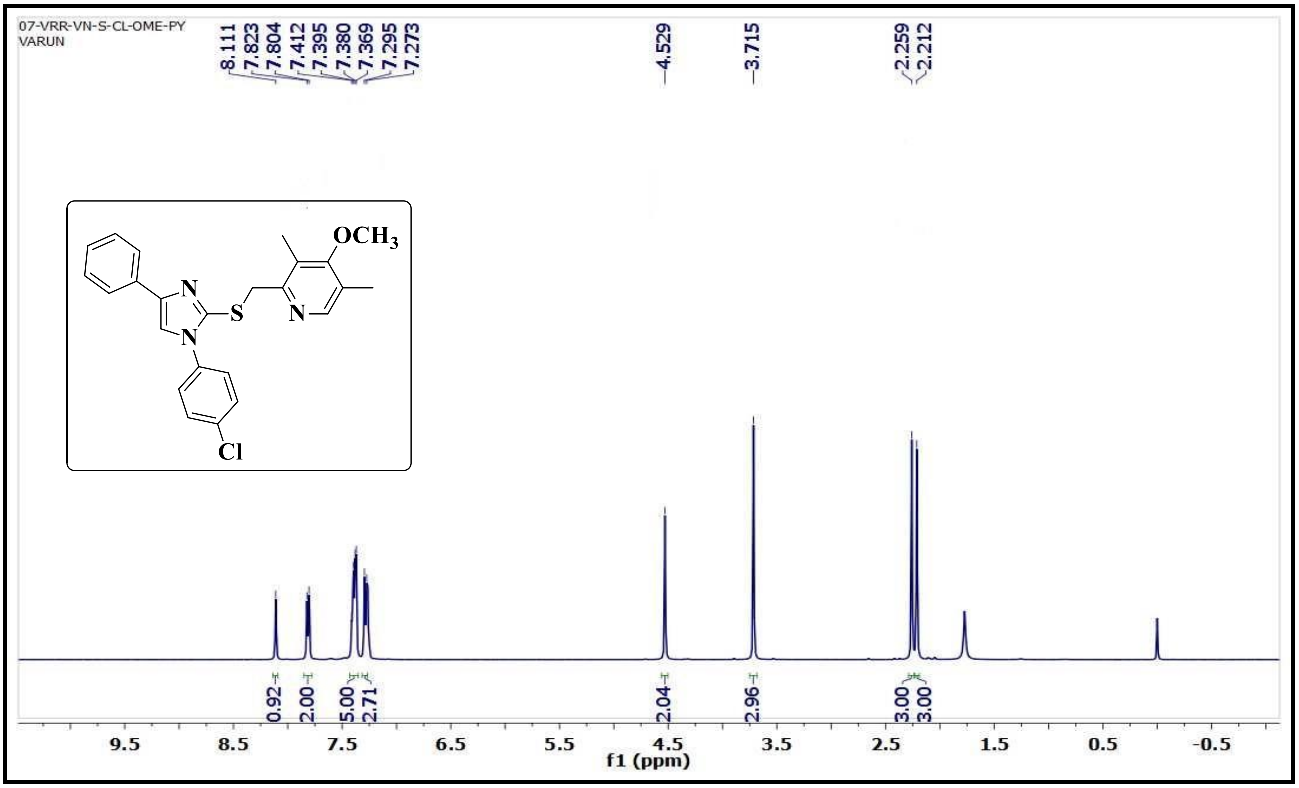
$^1$H NMR Spectrum of compound **22a**



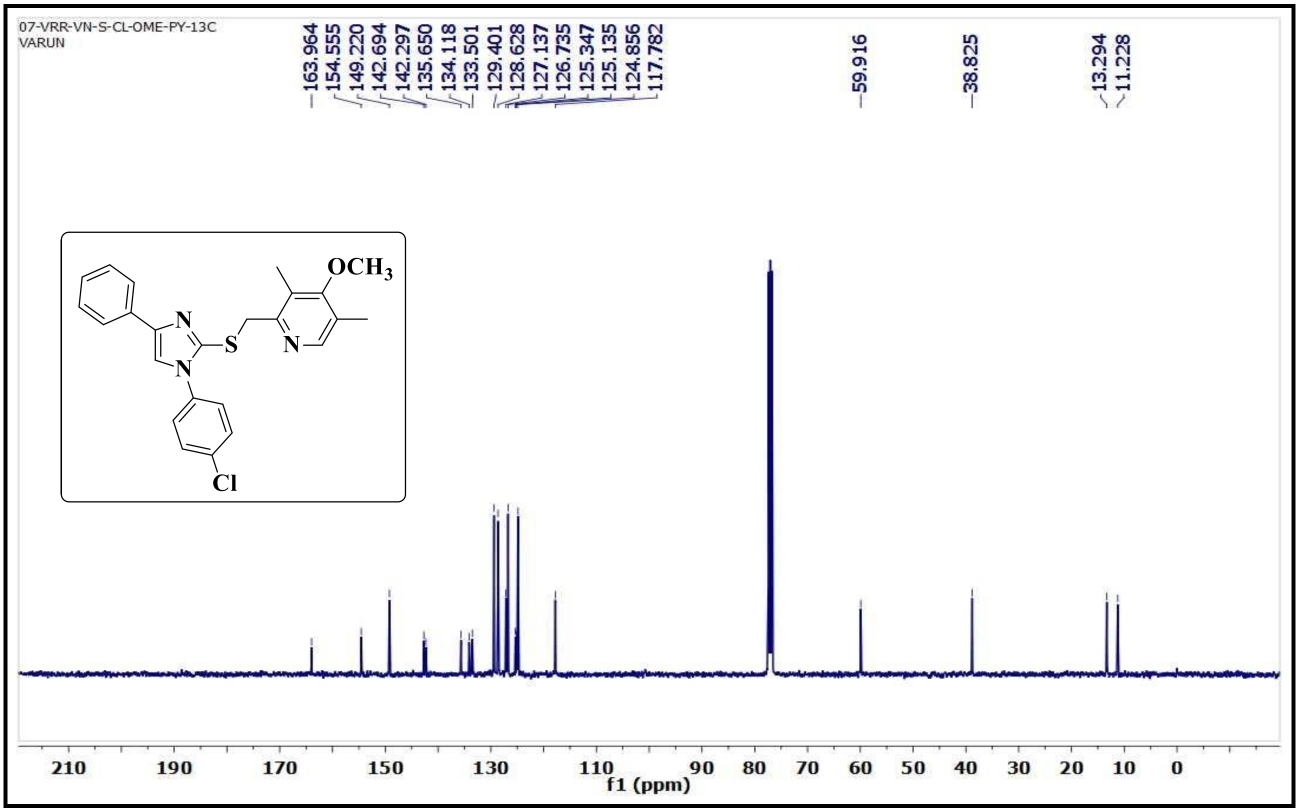
$^{13}$C NMR Spectrum of compound **22a**



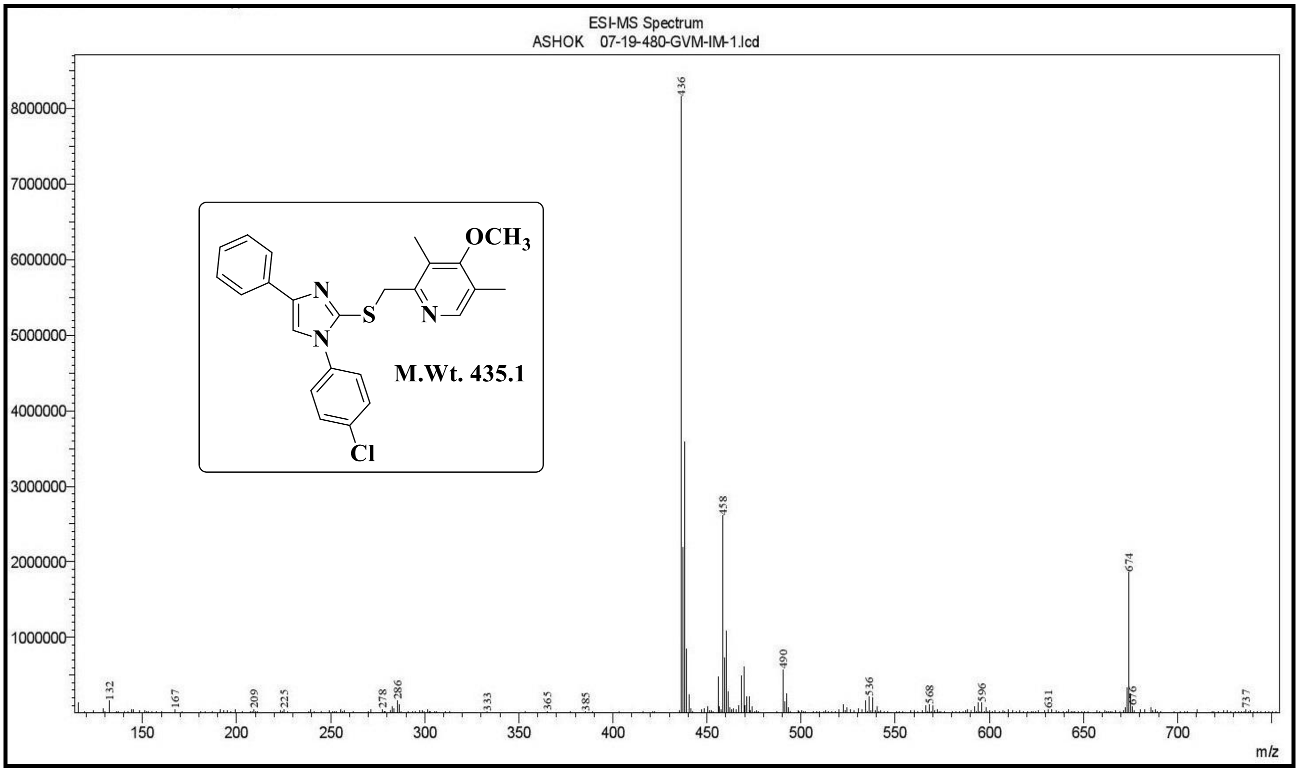
Mass Spectrum of compound **22a**



$^1$H NMR Spectrum of compound **22b**



$^{13}$C NMR Spectrum of compound **22b**



Mass Spectrum of compound **22b**

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# SUMMARY

The thesis entitled "**Synthesis of new heterocyclic compounds using multicomponent approach and their biological evaluation**" consists of six chapters. Chapter-I introduces multicomponent reactions, their applications in organic synthesis and recent literature on multicomponent reactions. The remaining five chapters describe the synthesis of new heterocyclic compounds and their biological evaluation.

## CHAPTER-I

### Introduction to multicomponent reactions

Chapter-I describes multicomponent reactions, their applications in various fields and synthesis of new heterocyclic compounds using multicomponent approach.

MCRs are organic chemical transformations in which three or more than three reactants combine to generate a single product in a one-pot reaction and in a single operation under mild reaction conditions, in an efficient way with high atom economy. Multicomponent reactions generate products with high chemical and structural diversity. MCRs have several advantages over conventional linear step synthesis[1]. MCRs are widely used for the synthesis of natural products[2-4], organic materials, polymers[5-7], bioactive molecules[8,9], medicinal chemistry[10-12], agro chemistry[13-15], and combinatorial chemistry[16-20]. Multicomponent reactions (MCRs) are the best synthetic methods to achieve the target molecule libraries and interest in MCRs has surged in recent years.

Molecular hybridization is useful to develop heterocyclic compounds with potential therapeutic values. Multicomponent approach[21-24] was the most efficient, and rapid synthetic route for the synthesis of molecularly diverse and therapeutic heterocyclic compounds. Keeping in view the importance of MCRs, we have used multicomponent approach for the synthesis of various new heterocyclic compounds.

4-Amino-4*H*-1,2,4-triazole 3,5-dithiol[25], aryl/hetryl chalcones[26], 1-(benzofuran-2-yl)-2-bromoethan-1-one[27], 1-aryl-2-(arylamino)ethan-1-ones[28] were prepared and used as starting materials for the synthesis of heterocyclic compounds.

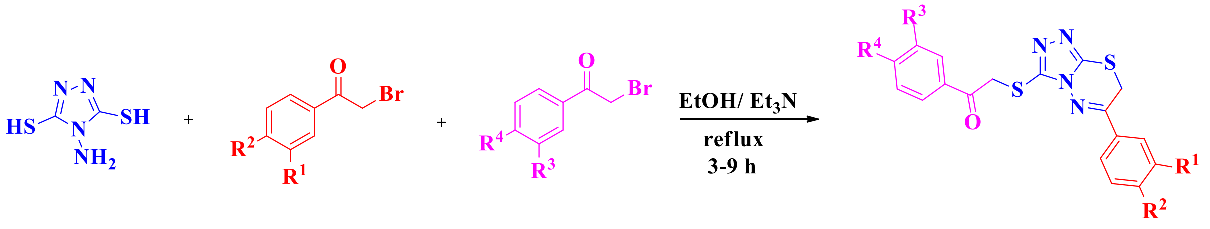
The objectives of the present work are mentioned and the outline of the work carried out in the present investigation is given.

# CHAPTER-II

This chapter deals with synthesis and investigation of anticancer activity of 1-phenyl-2-((6-phenyl-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-3-yl)thio)ethanones.

Earlier reports on synthesis and importance of aryl and heteryl triazolo thiadiazines have been reviewed briefly in the introduction.

1-Phenyl-2-((6-phenyl-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-3-yl)thio)ethanones **3 (a-l)** were synthesized via a multicomponent approach. Condensation of 4-amino-4*H*-1,2,4-triazole 3,5-dithiol **(1)**, with phenacyl bromides **2 (a-i)** and **2' (a-i)** in ethanol and $Et_3N$ resulted in the formation of title compounds (scheme-1).



**1** **2 (a-i)** **2' (a-i)** **3 (a-l)**

**yields: 80-91%**

**Scheme-1**

| Entry | Product | R$^1$ | R$^2$ | R$^3$ | R$^4$ |
|---|---|---|---|---|---|
| 1 | 3a | H | H | H | H |
| 2 | 3b | H | $CH_3$ | H | $CH_3$ |
| 3 | 3c | H | $OCH_3$ | H | $OCH_3$ |
| 4 | 3d | H | F | H | F |
| 5 | 3e | H | Cl | H | Cl |
| 6 | 3f | H | Br | H | Br |

| Entry | Product | R$^1$ | R$^2$ | R$^3$ | R$^4$ |
|---|---|---|---|---|---|
| 7 | 3g | Cl | Cl | Cl | Cl |
| 8 | 3h | H | Ph | H | Ph |
| 9 | 3i | H | $NO_2$ | H | $NO_2$ |
| 10 | 3j | H | H | H | F |
| 11 | 3k | H | $OCH_3$ | H | F |
| 12 | 3l | H | Cl | H | F |

The structures of all the newly synthesized compounds **3 (a-l)** were confirmed through their analytical and spectral data.

### *In vitro* anticancer activity

All the newly synthesized compounds **3 (a-l)** were screened for *in vitro* anticancer activity at National Cancer Institute, USA. The compounds were evaluated against 60 human cancer cell lines at 10µM concentration. Among these compounds, compounds **3d, 3f** and **3l** exhibited moderate inhibitory activity on renal cancer OU-31 cell line with growth percentage values of **47.42**, **46.76** and **48.14**, respectively. Compound **3l** exhibited moderate inhibitory activity on leukemia MOLT-4 with growth percentage value of **49.82**.

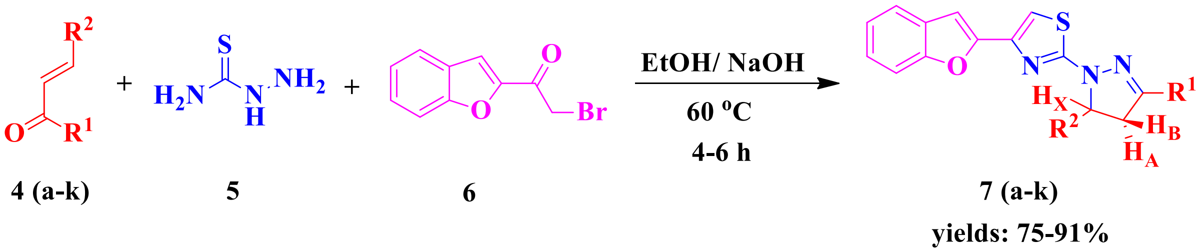
To summarize, the method has several advantages, such as low reaction time, good yields and easy workup. From anticancer activity results, the compounds **3d**, **3f**, and **3l** showed moderate activity.

## CHAPTER-III

This chapter contains two sections viz, section-A and section-B.

**Section-A** deals with an efficient one-pot synthesis of substituted 4-(benzofuran-2-yl)-2-(3-(aryl/heteryl)-5-(aryl/heteryl)-4,5-dihydro-1*H*-pyrazol-1yl)thiazoles.

The title compounds **7 (a-k)** were synthesized by the reaction of various chalcones **4 (a-k)**, thiosemicarbazide **(5)** and 1-(benzofuran-2-yl)-2-bromoethan-1-one **(6)** in presence alcoholic NaOH (scheme-2).



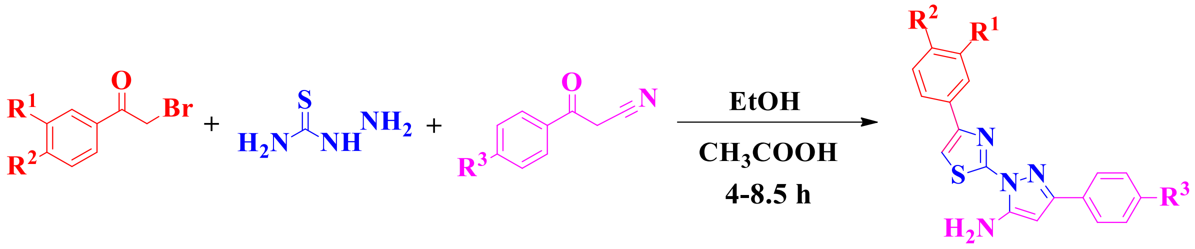
**Scheme-2**

| Entry | Product | R$^1$ | R$^2$ |
|---|---|---|---|
| 1 | 7a | Benzofuran-2-yl | 4-Bromophenyl |
| 2 | 7b | Benzofuran-2-yl | 4-Methoxyphenyl |
| 3 | 7c | 4-Fluorophenyl | Furan-2-yl |
| 4 | 7d | Thiophen-2-yl | 4-Chlorophenyl |
| 5 | 7e | Thiophen-2-yl | Phenyl |
| 6 | 7f | Thiophen-2-yl | 3,4,5-Trimethoxyphenyl |
| 7 | 7g | Thiophen-2-yl | 4-Bromophenyl |
| 8 | 7h | Thiophen-2-yl | 4-Fluorophenyl |
| 9 | 7i | 4-Methoxyphenyl | Furan-2-yl |
| 10 | 7j | Thiophen-2-yl | N,N-Dimethylaniline |
| 11 | 7k | Thiophen-2-yl | 4-Methoxyphenyl |

In brief, we have synthesized **7 (a-k)** via a facile one-pot base catalyzed multicomponent reaction with high yields by using aryl / hetryl chalcones, thiosemicarbazide and 1-(benzofuran-2-yl)-2-bromoethan-1-one in a short reaction time. This reaction not only has operational simplicity but it also involves construction of two potential heterocyclic rings (thiazole and dihydropyrazole) in one step reaction.

**Section-B** explains the synthesis of 3-phenyl-1-(4-phenylthiazol-2-yl)-1*H*-pyrazole-5-amines and their biological evaluation: antiviral, antimicrobial and anticancer activities.

In this section, we have synthesized 3-phenyl-1-(4-phenylthiazol-2-yl)-1*H*-pyrazole-5-amines **9 (a-n)** via a one-pot multicomponent approach. An equimolar mixture of phenacyl bromide **2 (a-h)**, thio-semicarbazide **(5)** and 3-oxo-3-phenylpropanenitrile **8 (a-b)** in ethanol containing catalytic amount of acetic acid under reflux to give corresponding title compounds **9 (a-n)** (scheme-3).



**2 (a-h)** **5** **8 (a-b)** **9 (a-n)**
**yields: 78-89%**

**Scheme-3**

| Entry | Product | $R^1$ | $R^2$ | $R^3$ |
|---|---|---|---|---|
| 1 | 9a | H | H | H |
| 2 | 9b | H | H | Cl |
| 3 | 9c | H | $CH_3$ | H |
| 4 | 9d | H | $CH_3$ | Cl |
| 5 | 9e | H | $OCH_3$ | H |
| 6 | 9f | H | $OCH_3$ | Cl |
| 7 | 9g | H | Cl | H |

| Entry | Product | $R^1$ | $R^2$ | $R^3$ |
|---|---|---|---|---|
| 8 | 9h | H | Cl | Cl |
| 9 | 9i | H | Br | H |
| 10 | 9j | H | Br | Cl |
| 11 | 9k | Cl | Cl | H |
| 12 | 9l | Cl | Cl | Cl |
| 13 | 9m | H | $NO_2$ | H |
| 14 | 9n | H | $NO_2$ | Cl |

### *In vitro* antiviral activity

The *in vitro* antiviral activity of newly synthesized compounds **9 (a-n)** were evaluated in CRFK cell cultures against different viral strains. Compound **9b** showed potent antiviral activity against *feline corona virus* with $EC_{50}$ value of 4.3 µM and against *feline herpes virus* with $EC_{50}$ value of 10.5 µM.

### *In vitro* antimicrobial activity

All the synthesized compounds **9 (a-n)** were screened for *in vitro* antimicrobial activity. Compounds **9c** and **9g** showed potent antifungal activity against *Candida albicans* strain with MIC values of 8 and 4 µg/mL, respectively. Compounds **9c, 9g, 9h, 9i, 9m** and **9n** exhibited potent antifungal activity against *Cryptococcus neoformans* with MIC values of 2, 1, 8, 4, 8, and 8 µg/mL, respectively.

### *In vitro* anticancer activity

All the newly synthesized compounds **9 (a-n)** were screened for *in vitro* anticancer activity at 10 µM concentration against 60 human cancer cell lines. Compound **9k** showed moderate activity against breast cancer MCF7 cell line with growth percentage value of 51.21.

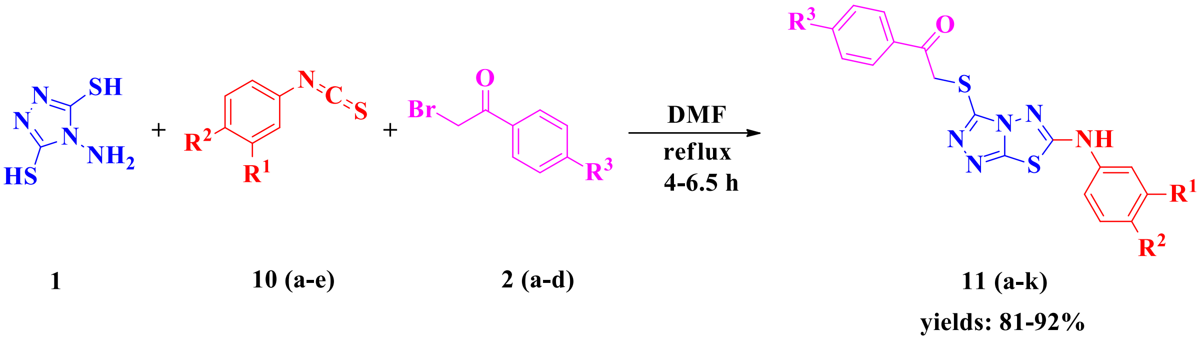
In summary, we have synthesized the above compounds through a one-pot three component reaction. From antiviral activity results, compound **9b** showed potent activity against *feline corona virus* and *feline herpes virus*. From antimicrobial activity results, compounds **9c, 9g, 9h, 9i, 9m** and **9n** showed potent antifungal activity against *Candida albicans*, *Cryptococcus neoformans* strains. From anticancer activity results, compound **9k** showed moderate activity against breast cancer MCF7 cell line.

## CHAPTER-IV

This chapter delineates the multicomponent synthesis of substituted 1-phenyl-2-((6-(phenylamino)-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazol-3-yl)thio)ethanones and their anticancer activity**.**

The introductory account deals with the synthesis and importance of triazolothiadiazoles.

Condensation of 4-amino-4*H*-1,2,4-triazole-3,5-dithiol **(1)**, phenyl isothiocyanates **10 (a-e)** and 2-bromo-1-phenylethanones **2 (a-d)** in DMF under reflux conditions afforded the title compounds **11 (a-k)** (sheme-4).



**Scheme-4**

| Entry | Product | $R^1$ | $R^2$ | $R^3$ |
|---|---|---|---|---|
| 1 | 11a | H | H | $CH_3$ |
| 2 | 11b | H | H | $OCH_3$ |
| 3 | 11c | $OCH_3$ | H | H |
| 4 | 11d | $OCH_3$ | H | $CH_3$ |
| 5 | 11e | $OCH_3$ | H | $OCH_3$ |
| 6 | 11f | H | F | $CH_3$ |

| Entry | Product | $R^1$ | $R^2$ | $R^3$ |
|---|---|---|---|---|
| 7 | 11g | Cl | H | $CH_3$ |
| 8 | 11h | Cl | H | $OCH_3$ |
| 9 | 11i | H | CN | H |
| 10 | 11j | H | CN | $CH_3$ |
| 11 | 11k | H | H | Cl |

### *In vitro* anticancer activity

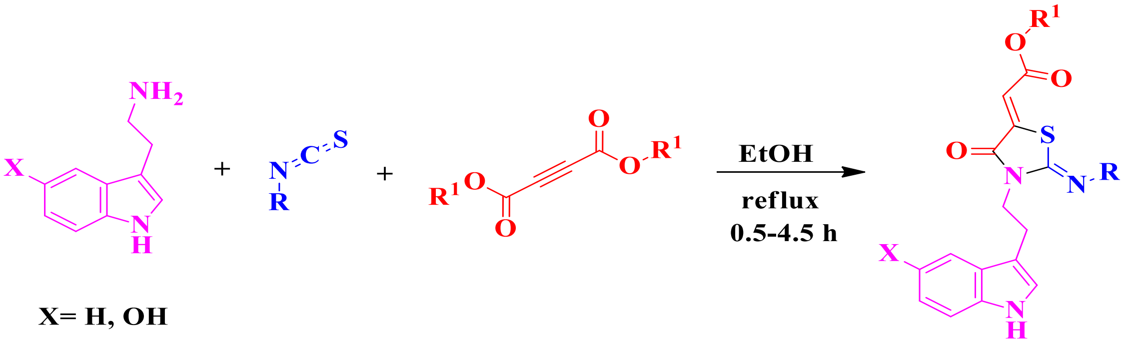
The *in vitro* anticancer activity of newly synthesized compounds were evaluated against 60 human cancer cell lines at 10µM concentration. Among all the tested compounds, compound 4**d** exhibited moderate inhibitory activity on renal cancer UO-31 with a growth percentage value of **58.74.**

In short, the advantage of the above multicomponent reaction is that it involves low reaction time, good yields and easy workup. From the anticancer activity results, compound **4d** showed moderate activity on renal cancer OU-31 cell line.

## CHAPTER-V

This chapter presents in detailone-pot multicomponent synthesis of (*Z*)-alkyl 2-((*Z*)-3-(2-(1*H*-indol-3-yl)ethyl)-4-oxo-2-(arylimino/benzylimino)thiazolidin-5-ylidene)acetates and their anticancer activity.

Tryptamine and its hydroxyl derivative **12 (a-b)** reacts with aryl isothiocyanates **10 (a-i)** and dialkylacetylene dicarboxylate **13 (a-b)** in ethanol under reflux conditions to give (*Z*)-alkyl 2-((*Z*)-3-(2-(1*H*-indol-3-yl)ethyl)-4-oxo-2-(phenylimino)thiazolidin-5-ylidene)acetates **14 (a-za)** (scheme-5).



**X= H, OH**

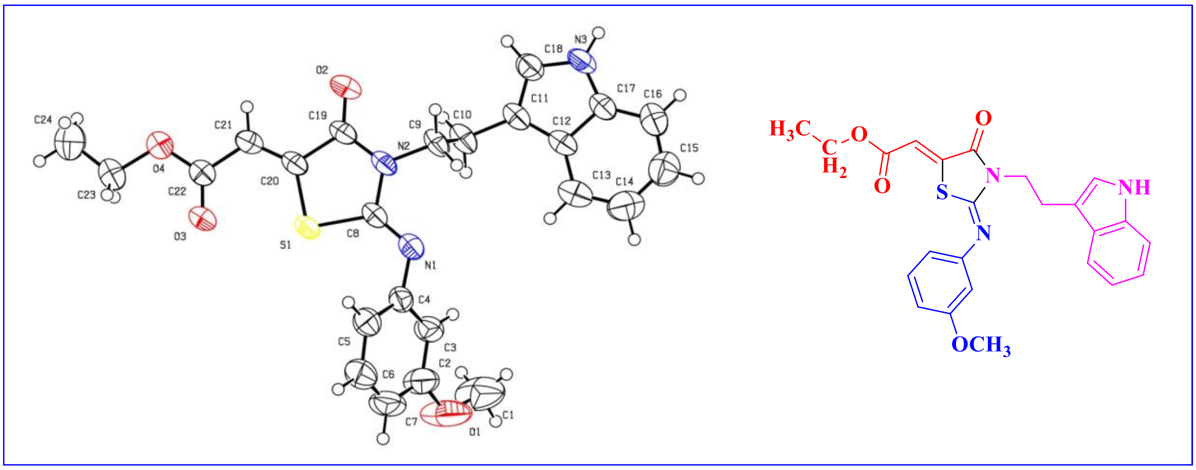
**12 (a, b)** **10 (a-i)** **13 (a-b)** **14 (a-za)**

**yields: 81-93%**

**Scheme-5**

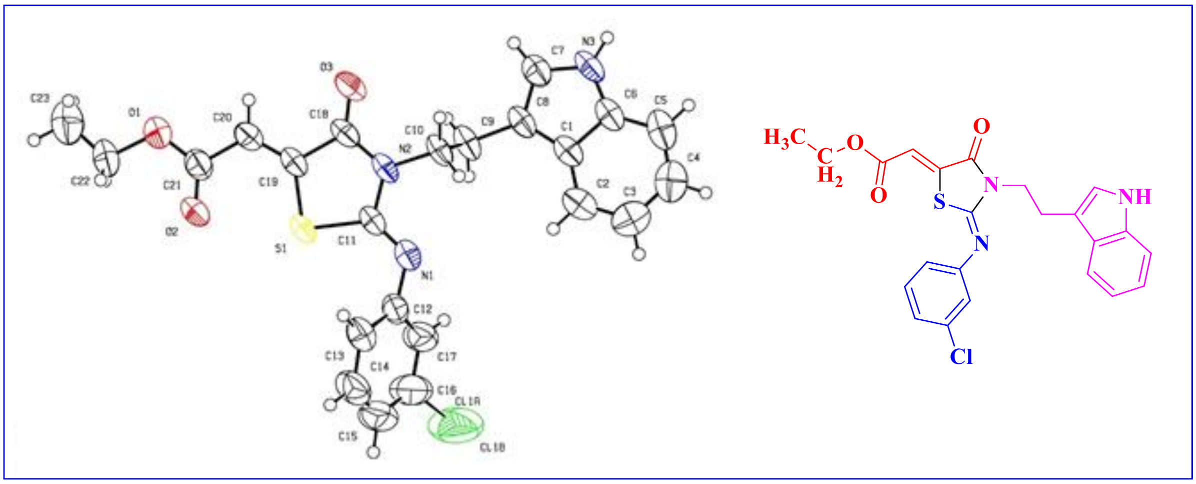
| Entry | Product | X | R | $R^1$ |
|---|---|---|---|---|
| 1 | 14a | H | phenyl | $OCH_2CH_3$ |
| 2 | 14b | H | phenyl | $OCH_3$ |
| 3 | 14c | H | 4-methyl phenyl | $OCH_2CH_3$ |
| 4 | 14d | H | 4-methyl phenyl | $OCH_3$ |
| 5 | 14e | H | 3-methoxy phenyl | $OCH_2CH_3$ |
| 6 | 14f | H | 3-methoxy phenyl | $OCH_3$ |
| 7 | 14g | H | 4-fluoro phenyl | $OCH_2CH_3$ |
| 8 | 14h | H | 4-fluoro phenyl | $OCH_3$ |
| 9 | 14i | H | 3-chloro phenyl | $OCH_2CH_3$ |
| 10 | 14j | H | 3-chloro phenyl | $OCH_3$ |
| 11 | 14k | H | 4-cyano phenyl | $OCH_2CH_3$ |
| 12 | 14l | H | 4-cyano phenyl | $OCH_3$ |
| 13 | 14m | H | 4-nitro phenyl | $OCH_2CH_3$ |
| 14 | 14n | H | 4-nitro phenyl | $OCH_3$ |
| 15 | 14o | H | 4-trifluoromethyl phenyl | $OCH_2CH_3$ |
| 16 | 14p | H | 4-trifluoromethyl phenyl | $OCH_3$ |
| 17 | 14q | OH | benzyl | $OCH_2CH_3$ |
| 18 | 14r | OH | benzyl | $OCH_3$ |
| 19 | 14s | OH | 3-methoxy phenyl | $OCH_2CH_3$ |
| 20 | 14t | OH | 3-methoxy phenyl | $OCH_3$ |
| 21 | 14u | OH | 3-chloro phenyl | $OCH_2CH_3$ |
| 22 | 14v | OH | 3-chloro phenyl | $OCH_3$ |
| 23 | 14w | OH | 4-fluoro phenyl | $OCH_3$ |
| 24 | 14x | OH | 4-trifluoromethyl phenyl | $OCH_3$ |
| 25 | 14y | OH | 4-methyl phenyl | $OCH_3$ |
| 26 | 14z | OH | 4-nitro phenyl | $OCH_2CH_3$ |
| 27 | 14za | OH | 4-nitro phenyl | $OCH_3$ |

**Crystal structure of compound 14e:** (Crystal system: Monoclinic)



ORTEP representation of compound 14e

**Crystal structure of compound 14i:** (Crystal system: Monoclinic)



ORTEP representation of compound 14i

### *In vitro* anticancer activity

From all the synthesized compounds, fifteen compounds were selected for their *in vitro* anticancer activity. From one dose anticancer activity results, compounds **14p**, **14v**, **14w** and **14x** exhibited excellent activity against NCI-60 cancer cell lines and were further selected for five dose assay from 10 nM to 100 µM concentration values. From the five dose level screening results, compounds **14p**, **14v**, **14w** and **14x** exhibited potent anticancer property in a concentration dependent manner.

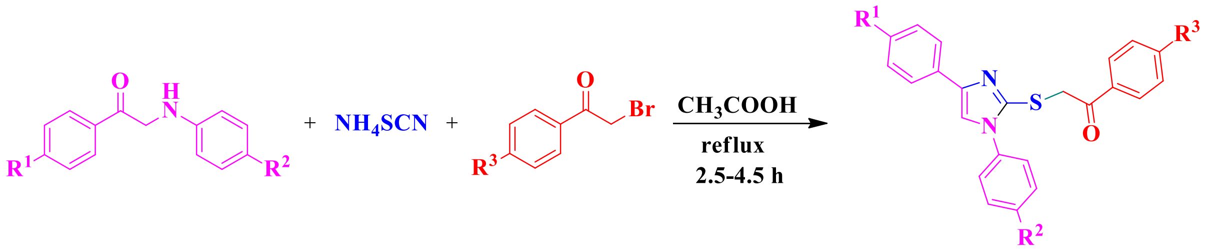
To summarize, the title compounds **14 (a-za)** were synthesized via one pot three component reaction. Among all synthesized compounds, some of the selected compounds were screened for their anticancer activity, and compounds **14p**, **14v**, **14w** and **14x** exhibited potent anticancer activity at five dose level.

## CHAPTER-VI

This chapter is divided into two sections viz, section-A and section-B

**Section-A** features one-pot three component synthesis of 2-((1,4-diphenyl-1*H*-imidazol-2-yl)thio)-1-phenylethanones and their biological evaluation: anticancer, antimicrobial and antiviral activity.

The title compounds **17 (a-v)** were synthesized by multicomponent reaction of 1-aryl-2-(arylamino)ethan-1-ones **16 (a-f)** with ammonium thiocyanate **(15)** and various substituted phenacyl bromides **2 (a-h)** in acetic acid under reflux conditions (scheme-6). All the synthesized compounds were screened for anticancer, antimicrobial and antiviral activity.



**15 (a-h)** **16** **2 (a-h)** **17 (a-v)**
**yields: 78-93%**

**Scheme-6**

| Entry | Product | $R^1$ | $R^2$ | $R^3$ |
|---|---|---|---|---|
| 1 | 17a | Br | $OCH_3$ | $CH_3$ |
| 2 | 17b | Br | $OCH_3$ | Ph |
| 3 | 17c | Br | $OCH_3$ | H |
| 4 | 17d | Cl | Cl | Br |
| 5 | 17e | Cl | $OCH_3$ | Cl |
| 6 | 17f | Br | Cl | Br |
| 7 | 17g | Cl | Cl | Ph |
| 8 | 17h | $CH_3$ | Cl | Br |
| 9 | 17i | H | Cl | $OCH_3$ |
| 10 | 17j | H | Cl | $CH_3$ |
| 11 | 17k | H | Cl | Ph |
| 12 | 17l | H | Cl | Br |
| 13 | 17m | H | Cl | H |
| 14 | 17n | H | Cl | Cl |
| 15 | 17o | H | Cl | $NO_2$ |
| 16 | 17p | $OCH_3$ | Cl | H |
| 17 | 17q | $OCH_3$ | Cl | $CH_3$ |
| 18 | 17r | $OCH_3$ | Cl | $OCH_3$ |
| 19 | 17s | $OCH_3$ | Cl | F |
| 20 | 17t | $OCH_3$ | Cl | Cl |
| 21 | 17u | $OCH_3$ | Cl | Br |
| 22 | 17v | $OCH_3$ | Cl | $NO_2$ |

The structures of all newly synthesized 2-((1,4-diphenyl-1*H*-imidazol-2-yl)thio)-1-phenylethanones **17 (a-v)** were confirmed by analytical and spectral data.

### *In vitro* anticancer activity

Some selected compounds were screened for their *in vitro* anticancer activity. Compounds **17b** and **17d** showed potent activity against ovarian cancer OVCAR-4 cell line with growth percentage values of 15.26 and 9.78, respectively. Compound **17d** showed good activity against renal cancer ACHN cell line with growth percentage value of 22.35. Compound **17v** showed good activity against non-small cell lung cancer HOP-92 cell line with a growth percentage value of 28.02.

### *In vitro* antiviral activity

The *In vitro* antiviral activity of newly synthesized compounds were evaluated in Vero cell cultures against various viral strains. Among all the tested compounds, compound **17o** showed potent activity against *yellow fever virus* with $EC_{50}$ value of 3.0 µM.

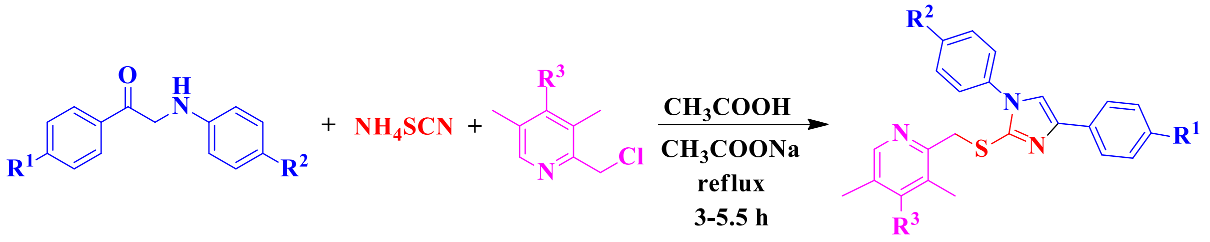
### *In vitro* antimicrobial activity

The synthesized compounds were screened for their *in vitro* antimicrobial activity. Compounds **17h**, **17i**, **17l**, **17n** and **17o** showed potent antifungal activity against *Cryptococcus neoformans* with MIC value of 16 µg/mL.

To sum up, the advantage of the above multicomponent reaction is that it involves less reaction time, good yields and easy workup. From the anticancer activity results, compounds **17b** and **17d** showed potent anticancer activity, **17v** showed good activity. From the antiviral activity results, compound **17o** showed potent activity against *yellow fever virus*. From the antimicrobial activity results, compounds **17h**, **17i**, **17l**, **17n** and **17o** showed potent antifungal activity against *Cryptococcus neoformans*.

**Section-B** outlines the synthesis and anticancer activity of 2-(((1,4-diphenyl-1*H*-imidazol-2-yl)thio)methyl)-3,5-dimethylpyridines.

Compounds **19 (a-k)** were synthesized through a one-pot multicomponent approach by utilizing 1-aryl-2-(arylamino)ethan-1-ones **16 (a-f)**, ammonium thiocyanate **(15)** and 2-(chloromethyl)-3,5-dimethylpyridine **18 (a & b)** in presence of acetic acid and sodium acetate with good yields in short reaction time (scheme-7). The newly synthesized compounds were obtained through a simple reaction workup.



**15 (a-f)** **16** **18 (a & b)** **19 (a-k)**

**yields: 75-89%**

**Scheme-7**

| Entry | Product | $R^1$ | $R^2$ | $R^3$ |
|---|---|---|---|---|
| 1 | 19a | H | Cl | H |
| 2 | 19b | H | Cl | $OCH_3$ |
| 3 | 19c | $CH_3$ | Cl | H |
| 4 | 19d | $CH_3$ | Cl | $OCH_3$ |
| 5 | 19e | $OCH_3$ | Cl | H |
| 6 | 19f | F | Cl | H |

| Entry | Product | $R^1$ | $R^2$ | $R^3$ |
|---|---|---|---|---|
| 7 | 19g | F | Cl | $OCH_3$ |
| 8 | 19h | Cl | Cl | H |
| 9 | 19i | Cl | Cl | $OCH_3$ |
| 10 | 19j | Br | $OCH_3$ | H |
| 11 | 19k | Ph | Cl | H |

All the newly synthesized Compounds **19 (a-k)** were characterized by analytical and spectral data.

### *In vitro* anticancer activity

Among all the synthesized compounds, few compounds were selected for their anticancer activity against 60-cancer cell lines at 10µM concentration. Compound **19b** showed moderate inhibitory activity on breast cancer BT-549 cell line. Compound **19c**, **19d** and **19f** showed moderate inhibitory activity on non-small cell lung cancer NCI-H522. Compound **19e** showed moderate inhibitory activity on non-small cell lung cancer NCI-H522 and colon cancer HT29 cell lines.

In summary, the advantage of the above multicomponent reaction is that it involves low reaction time and gives good yields. From anticancer activity results, compounds **19b**, **19c**, **19d**, **19e**, and **19f** showed moderate activity.

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# LIST OF PUBLICATIONS

## PUBLICATIONS

1. **Varun Arandkar**, Krishnaiah Vaarla and Rajeswar Rao Vedula*, Facile one pot multicomponent synthesis of novel 4-(benzofuran-2-yl)-2-(3-(aryl/heteryl)-5-(aryl/heteryl)-4,5-dihydro-1*H*-pyrazol-1yl)thiazole derivatives, *Synth. Commun.* **2018**, *48*, 1285-1290.

2. **Varun Arandkar** and Rajeswar Rao Vedula*, A facile one-pot expeditious synthesis of triazolothiadiazines and anticancer activity, *Phosphorus, Sulfur, and Silicon*, **2019**, *194*, 533-539

3. Krishnaiah Vaarla, Sreenu Pavurala, **Varun Arandkar**, Rajeswar Rao Vedula*, and Murali Krishna Toopurani, Solvent-Free One-Pot Tandem Multicomponent Synthesis of Triazolothiadiazinyl Coumarins and Their Antimicrobial Properties, *ChemistrySelect* **2019**, *4*, 5828-5834.

## PRESENTATION & PARTICIPATION IN SYMPOSIA

1. Presented a paper entitled **"One pot multicomponent synthesis of novel 4-(benzofuran-2-yl)-2-(3-(aryl/heteryl)-5-(aryl/heteryl)-4,5-dihydro-1H-pyrazol-1yl)thiazoles**" in National conference on **"Resent Developments in Chemical Sciences and Allied Technologies"** organized by Department of Chemistry, NIT-Warangal, held on **29th-30th June, 2017.**
2. Attended a National conference on **"Frontiers in Chemical Sciences and Technologies"** organized by Department of Chemistry, NIT-Warangal, held on **28th-29th January, 2016.**
3. Presented a paper entitled **"Multicomponent synthesis of substituted imidazole derivatives and their anticancer activity" in "Medicinal Chemistry Conference cum work shop (MED CHEM – 2015)"** held at IIT Madras, Chennai on **29 - 30th October 2015.**
4. Presented a paper entitled **"Synthesis and anticancer activity of 2-((1,4-diphenyl-1H-imidazol-2-yl)thio)-1-phenylethanones"** in an International conference on **" Nascent Developments in Chemical Sciences (NDCS - 2015)"** held at BITS-Pilani, Pilani, Rajasthan on **October 16 –18th October 2015.**
5. Attended a National conference on **"Recent Advances in Organic Synthesis"** organized by Department of Chemistry, NIT-Warangal, held on **29th January, 2016.**
6. Attended a National workshop on **"Applications of Mass Spectrometry"** at CSIR-IICT, Hyderabad held on **20th November, 2014.**
7. Attended a National workshop on **"Recent Advances in Synthesis and Applications of Heterocyclic Compounds"** at NIT-Warangal, held on **8th-10th November, 2013.**
8. Attended a National workshop on **"Primary Computational Methods for Chemical Research"** organized by Department of Chemistry, NIT-Warangal, held on **20th-22th September, 2013**.