SYNTHESIS AND BIOLOGICAL ACTIVITY OF NOVEL SUBSTITUTED AND FUSED PYRIMIDINE DERIVATIVES

THESIS

SUBMITTED TO

NATIONAL INSTITUTE OF TECHNOLOGY

WARANGAL

FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

IN

CHEMISTRY

By

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WARANGAL-506 004, (T.S), INDIA

AUGUST-2016

DECLARATION

I hereby declare that the research work presented in the thesis has been carried out by me under the supervision of **Prof. G. V. P. Chandramouli,** Professor, Department of Chemistry, National Institute of Technology Warangal. I also affirm that this work is original and has not been submitted in part or full, for any degree or diploma to this or any other university or Institution.

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CERTIFICATE

The research work presented in this thesis entitled "**SYNTHESIS AND BIOLOGICAL ACTIVITY OF NOVEL SUBSTITUTED AND FUSED PYRIMIDINE DERIVATIVES"** submitted by **Mr. L. SURESH** for the degree of **Doctor of Philosophy in Chemistry** under my guidance and supervision. This work is original and has not been submitted for any other degree or diploma to this or any other university.

(G. V. P. Chandramouli)
Research Supervisor

Date:

Place:

ACKNOWLEDGEMENTS

It gives me immense pleasure and pride to express my deep sense of gratitude for my teacher and supervisor ***Prof. G. V. P. Chandramouli***, Professor, Department of Chemistry, National Institute of Technology Warangal for his evergreen expertise and astute guidance. His unabated enthusiasm, which stems from his absolute command over the subject, has been a constant source of inspiration to me to work hard and the outcome is expressed in the form of this thesis. I consider myself fortunate in that it would have been impossible to achieve this goal without his support and care.

I express my sincere thanks to ***Prof. T. Srinivasa Rao***, Director, National Institute of Technology, Warangal, for having given me the opportunity to carry out the work and allowing me to submit in the form of thesis.

I wish to express my thanks to present and former Heads of our Department, ***Prof. V. Rajeswar Rao, Prof.(Ms.) B. Rajitha*** and ***Prof. K. Laxma Reddy*** for providing laboratory facilities to carry out the research work.

I express my thanks to Doctoral Scrutiny Committee (DSC) members, ***V. Rajeswar Rao,*** **Dr.** ***P. V. Srilakshmi***, Department of Chemistry and ***Prof. Y. Pydisetty*** Department of Chemical Engineering for their support and encouragement.

I would like to express my special thanks to ***Prof. B. V. Appa Rao***, ***Prof. A. Ramachandraiah***, ***Prof. I. Ajitkumar Reddy***, ***Prof. P. Nageswar Rao***, ***Dr. K. V. Gobi***, ***Dr. Venkatathri Narayanan***, ***Dr. Vishnu Shanker***, ***Dr. D. Kasinath***, ***Dr. B. Srinivas*** and ***Dr. K. Hari Prasad*** for their valuable advice and encouragement.

I take this opportunity to express my thanks to ***Dr. C. Ganesh Kumar*** and **P. Onkara**, for testing Biological Activities and thanks to ***Dr. N. Jagadeesh*** **babu** interpretation of Single-Crystal X-ray diffraction data.

I wish to thank ***The Directors***, Indian Institute of Chemical Technology (IICT)-Hyderabad, University of Hyderabad and Sophisticated Test and Instrumentation Centre (STIC), Cochin University of Science &Technology-Kerala for providing library and instrumental facilities.

I wish to express my special thanks to ***Head***, ***Dept. of Biotechnology***, ***NIT Warangal***, for providing biological activities and molecular docking studies.

I acknowledge with gratitude the keen and helpful guidance of ***Dr. M. Amaravathi*** throughout the research work for her constant encouragement.

I would like to express my sincere thanks to ***Dr. D. Mohan Rao, Managing Director, Symed Labs Ltd., Hyderabad***, for providing industrial project.

I thank all my seniors, ***Dr. B. Prasanna***, **Dr. *P. Naveen kumar***, ***Dr. S. Kanakaraju*** and **P. Sagar Vijay Kumar** for their valuable advice and encouragement throughout my research work.

With all happiness I acknowledge the cheerful assistance rendered by all my research colleagues, ***M. Narsihma Reddy***, ***K. Koteshwara Reddy***, ***G. Srinivasa Rao***, ***K. Vimal Kumar***, ***M. Nookaraju***, ***K. Chaitanya Kumar***, ***V. Ravibabu***, ***G. Rajitha***, ***G. Ramesh***, ***V. Krishnaiah***, **G. *Malikarjun***, ***P. Sreenu***, ***R. Rajkumar***, ***Tewodros Birhanu***, ***T. Surendar***, ***B. Thirupaiah***, ***M. Venkanna***, ***B. Paplol***, ***R. Hithavani***, ***S. Nagaraju***, ***B. Santosh Kumar***, ***A. Rajini***, ***T. Ramesh***, ***M Satyanarayana***, ***A. Ajay Kumar***, ***K. Yugender***, ***E. Rajesh***, ***V. Amarnath***, ***Mayuri***, ***Varun***, ***Parameshwara chary***, ***Srikanth***, ***Sowmya***, ***N. V. Bharath***, ***Vinay***, ***Sujatha***, **Sanjay**, ***Satyanarayana***, ***Sunil***, ***Suman***, ***Suresh***, ***Ambedtkar Babji, Vijendher Reddy*** and ***Ramaiah*** and other co-research scholars for their unstinted co-operation

My heart goes to my beloved ***Father and Mother*** who with all their patience, prayers and faith in the Almighty, waited all these long years to see me reaching this stage. Their blessings and care always gives me new zeal and enthusiasm to do something more with perfection.

I always remember and cherish the encouragement and inspiration provided by all my friends and well wishers during the course of my research work.

(L. SURESH)

CONTENTS

ABBREVIATIONS

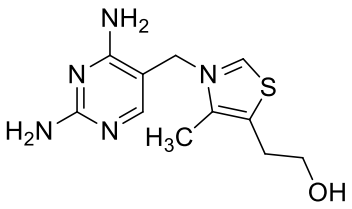
Ac	:	Acetate
AcOH	:	Acetic acid
AcONa	:	Sodium acetate
anhyd.	:	Anhydrous
aq.	:	Aqueous
Anal	:	Analysis
Bn	:	Benzyl
[Bmim]BF_4	:	1-Butyl-3-methyl imidazolium tetrafluoroborate
[Bmim]Br	:	1-Butyl-3-methyl imidazolium bromide
[Bmim]Cl	:	1-Butyl-3-methyl imidazolium chloride
[Bmim]PF_6	:	1-Butyl-3-methylimidazoliumhexafluorophosphate
[Bmim]HSO_4	:	1-Butyl-3-methylimidazolium hydrogen sulphate
[Bmim]$FeCl_4$	:	1-Butyl-3-methylimidazolium tetrachloroferrate
Calcd	:	Calculated
Ca	:	Candida albicans
$CDCl_3$	:	Deuterated chloroform
CeO_2	:	Cerium dioxide
DABCO	:	1,4- Diazabicyclo[2,2,2]octane
DBU	:	1,8-Diazabicycloundec-7-ene
DCM	:	Dichloromethane
DHP	:	Dihydropyrimidine
DMAP	:	4-(Dimethylamino) pyridine
DMF	:	N,N-Dimethylformamide
DMS	:	Dimethyl sulphide
DMSO	:	Dimethyl sulfoxide
DMSO-d_6	:	Dueterated dimethyl sulfoxide
EtOAc	:	Ethyl acetate
h	:	Hour(s)
HCl	:	Hydrogenchloride
^{1}H NMR	:	Proton Nuclear Magnetic Resonance
Hz	:	Hertz

IL	:	Ionic liquid
IR	:	Infrared
KBr	:	Potassium bromide
K_2CO_3	:	Potassium carbonate
MBC	:	Minimum Bactericidal Concentration
MFC	:	Minimum Fungicidal Concentration
MeOH	:	Methanol
m.p.	:	Melting point
mL	:	Milli litre
MS	:	Mass spectra
min	:	Minute
mmol	:	Millimole
mM	:	Milli Molar
MHz	:	Mega Hertz
MW	:	Microwave
µM	:	Micro Molar
NaH	:	Sodium hydride
NaOH	:	Sodium hydroxide
Na_2CO_3	:	Sodium carbonate
NMR	:	Nuclear Magnetic Resonance
p	:	*para*
Ph	:	Phenyl
ppm	:	Parts per million
*p*TSA or TsOH	:	*p*-Toluenesulfonic acid
r.t. or RT	:	Room Temperature
ROS	:	Reactive Oxygen Species
TEA or Et_3N	:	Triethylamine
THF	:	Tetrahydrofuran
5-AT	:	5-Amino Tetrazole
TLC	:	Thin Layer Chromatography
TMSCl	:	Trimethylsilylchloride
$TMSN_3$	:	Trimethylsilylazide
TMS	:	Trimethylsilan

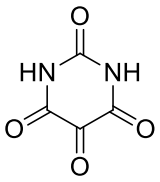
INTRODUCTION

1. PYRIMIDINES

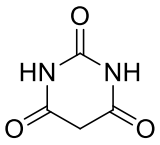
Nitrogen-containing heterocycles are widely distributed in nature and are essential to life, playing a vital role in the metabolism of all living cells. Among these, pyrimidines represent one of the most prevalent group of heterocycles found in natural products such as amino acid derivatives (willardiine, tingitanine), antibiotics (bacimethrin, sparsomycin, bleomycin), alkaloids (heteromines, crambescins, manzacidins, variolins, meridianins, psammopemmins *etc*.) and toxins.[1-7] The pyrimidine ring system has wide occurrence in nature as substituted or ring fused compounds, including the nucleotides, thiamine (vitamin B1) (**1**) and alloxan (**2**).[8-10] The system is also found in many of the synthetic compounds such as barbituric acid (**3**) and in the HIV drug, zidovudine (**4**).[11-12] The first synthetic bioactive pyrimidine was prepared by Gabriel and Colman in 1900, from barbituric acid to 2,4,6-trichloropyrimidine followed by reduction with zinc dust in hot water.[13-16]



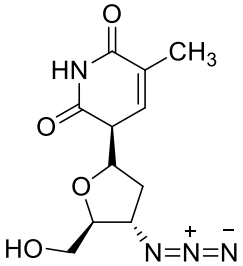
Thiamine (1)



Alloxan (2)

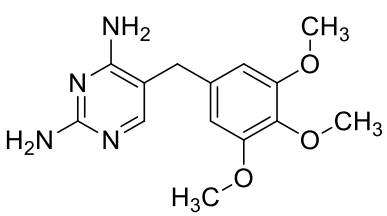


Barbituric acid (3)

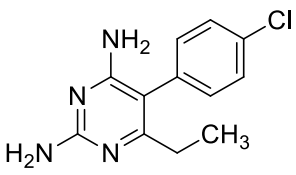


Zidovudine (4)

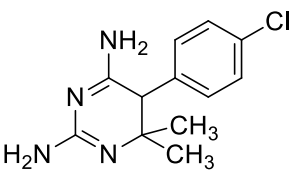
The pyrimidine system is present in some of the dihydrofolate reductase inhibitors like Trimethoprim (**5**), Pyrimethamine (**6**), Cycloguanil (**7**), Acide folique (**8**), Methotrexate (**9**) and Trimetrexate (**10**). In addition to above, pyrimethamine in combination with sulfadiazine (**11**) is used for the treatment of *toxoplasma gondii* infections in immunocompromised patients, such as HIVpositive individuals. Similarly, trimetrexate has been used with folinic acid (leucovorin) (**12**) in treating pneumocystis pneumonia. It has been investigated for use in treating leiomyosarcoma.[17-27]



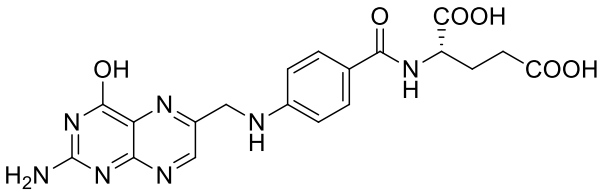
Trimethoprim (5)



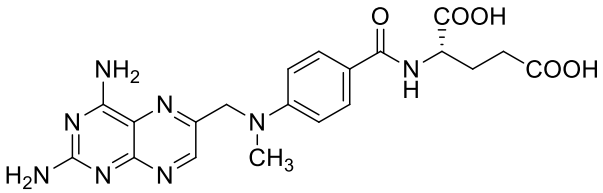
Pyrimethamine (6)



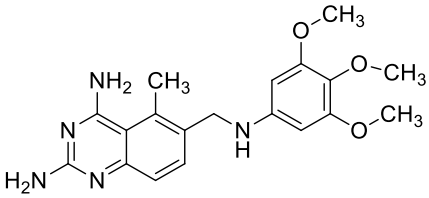
Cycloguanil (7)



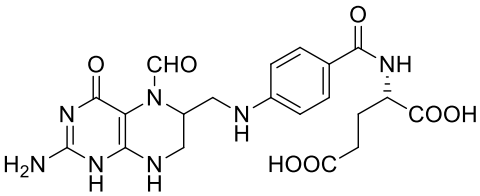
Acide folique (8)



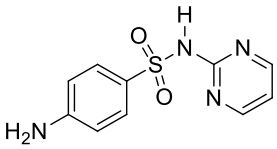
Methotrexate (9)



Trimetrexate (10)

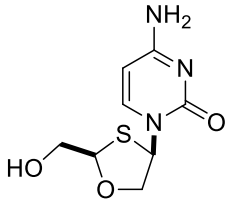


Folicc acid (11)

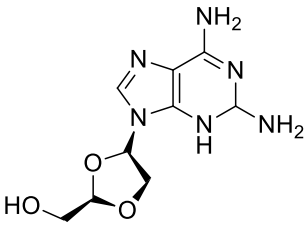


Sulfaadiazine (12)

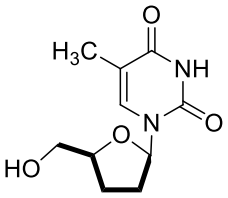
The discovery of AZT inspired many of the researchers in the discovery of several potent anti-HIV drugs like abacavir, emtricitabine, lamivudine, didanosine, apricitabine, stampidine, elvucitabine, racivir, amdoxovir, stavudine, zalcitabine, festinavir and tenofovir (**Fig. 1**).[28-33]



Apricitabine (13)



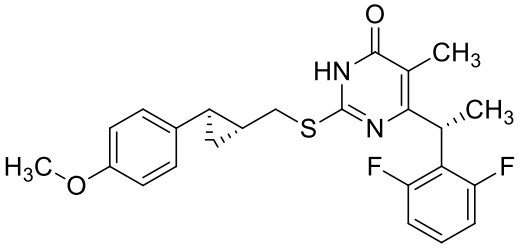
Amdoxovir (14)



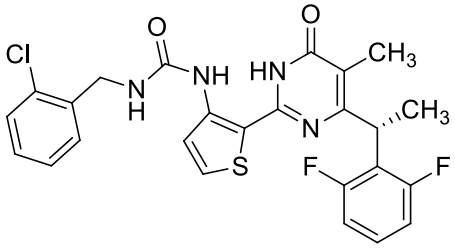
Stavudine (15)

Figure. 1

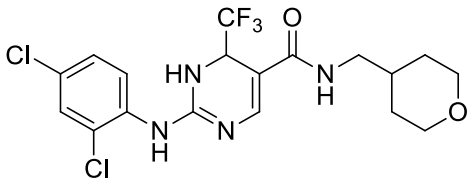
Over the years, the pyrimidine system turned out to be an important pharmacophore endowed with druglike properties and a wide range of pharmacological activities depending on the decoration of the scaffold. A few illustrative examples of pyrimidine derivatives which are active as inhibitors of HIV, HCV, CDK, CB2, VEGFR and Adenosine A1/A2a/A313 are represented in (**Fig. 2**). Due to the long-lasting interest in pyrimidine derivatives as potential drugs, the synthetic community has dedicated many efforts to the investigation of new approaches to those derivatives.[34-39]



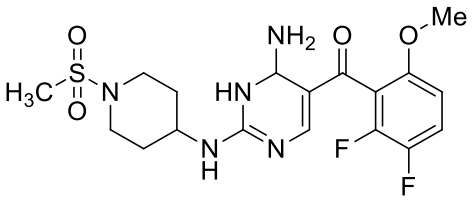
HIV-RT Inhibitor (16)



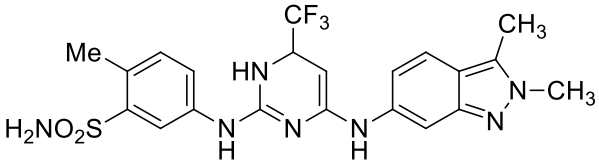
HCV-inhibitor (17)



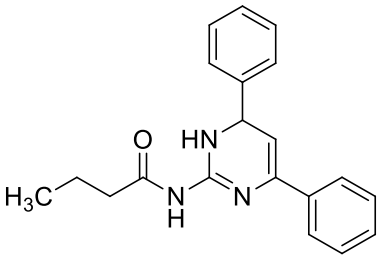
CB2-inhibitor (18)



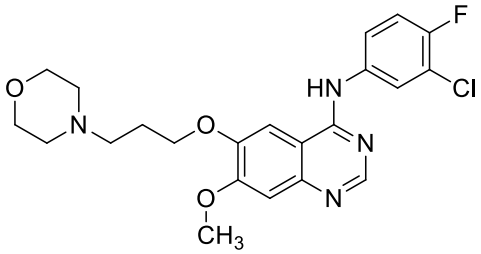
CDK-inhibitor (19)



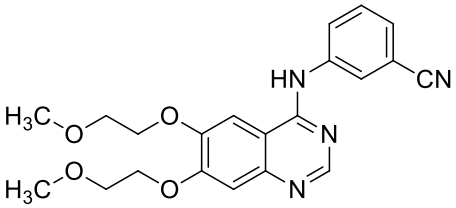
VEGFR-inhibitor (20)



A1-inhibitor (21)



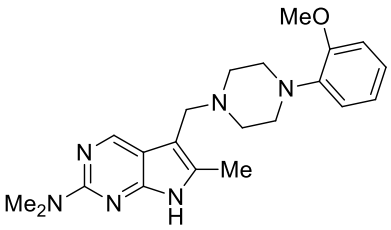
Gefitinib (22)



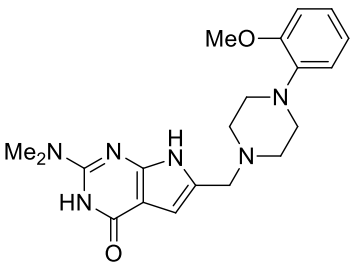
Erlotinib (23)

Figure. 2

Linz *et al*.[40] disclosed that a series of pyrrolo[2,3-*d*] and pyrrolo[3,2-*d*]pyrimidines act as selective dopamine D4-ligands (**24**, **25** and **26**).



(**24**)



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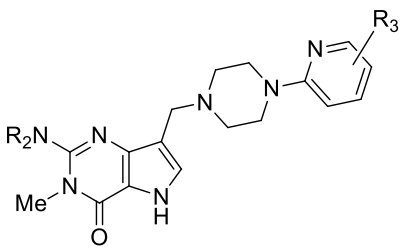
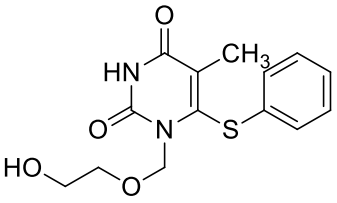
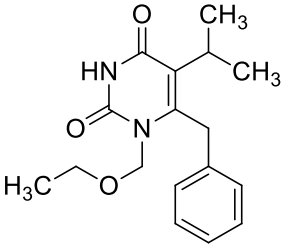


(26)

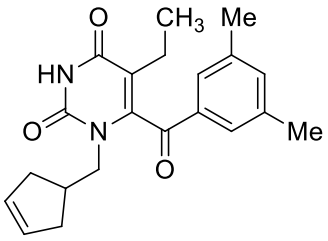
Among many of the pyrimidine analogues synthesized in the last decade the important compounds are Emivirine 9, GCA-186 (**27**) and SJ-3366 (**28**) which exhibited persuasive activity against HIV-1 (**29**). Compounds acting on high blood pressure are prazosin (**30**), doxazosin (**31**) and terazosin (**32**) (**Fig. 3**).[41-50]



(27)



(28)



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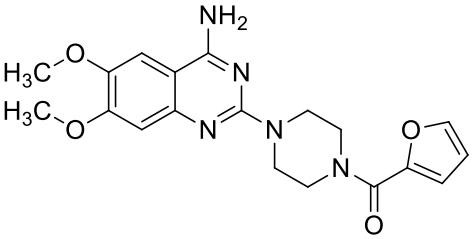
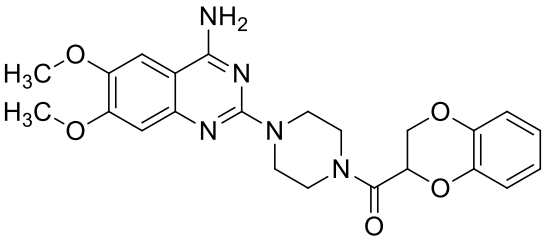
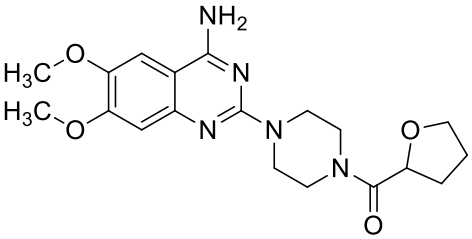


(Prazosin) (30)



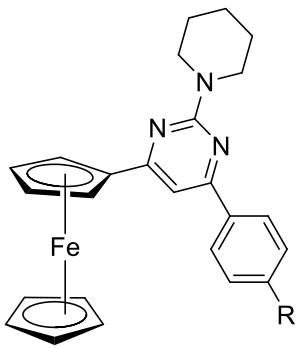
(Doxazosin) (31)



(Terazosin) (32)

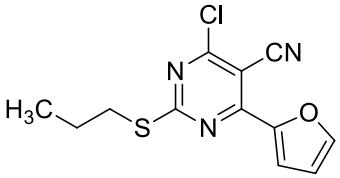
Figure. 3

Parveen *et al*.[51] synthesized and examined the *in vitro* antiamoebic activity of the iron complex compounds (**33**) using HM1: IMSS strain of *E. histolytica.*



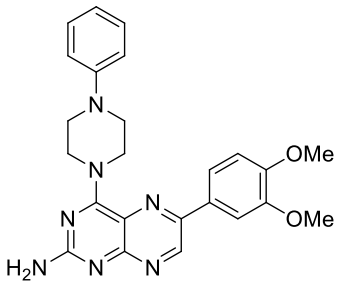
(33)

Agarwal and co-workers[52] prepared a series of chloropyrimidines and demonstrated their *in vitro* antitubercular and antifungal activity of the compound (**34**).



(**34**)

Jonghe *et al*.[53] disclosed that a series of pteridine analogueso of compound (**35**) act as potential immunosuppressive as well as antiinflammatory agents.

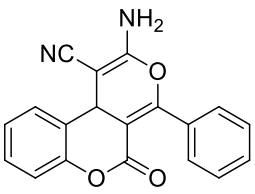


(**35**)

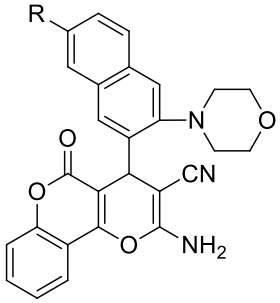
1.1. IMPORTANT TYPES OF SUBSTUTED AND FUSED PYRIMIDINES

A) PYRANO PYRIMIDINES

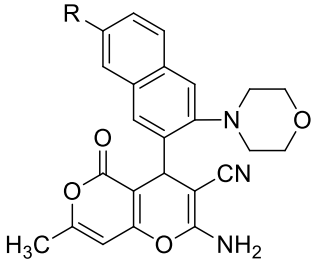
Among the different types of chromene systems, tetrahydrobenzo[*b*]pyrans (**36**) are of considerable interest because of their wide range of biological properties,[54-57] such as spasmolytic, diuretic, anticoagulant, anticancer, and antianaphylactic activities. They can also be used for curing huntington's disease, parkinson's disease, AIDS associated dementia, and down's syndrome.[58-60] Similar compounds of type (**37** and **38**), are the first class of compounds which have shown fully selective inhibition of the human excitatory amino acid transporter subtype 1 (EAAT 1). Many of the other similar compounds are (**39**), (**40**) and (**41**) which exhibit wide therapeutic and pharmacological properties of pyrano pyrimidines.[61-63]



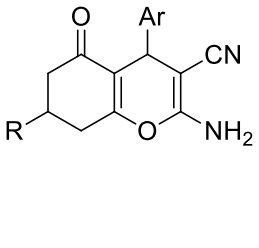
(**36**)



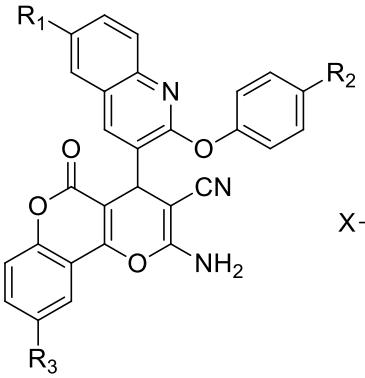
(**37**)



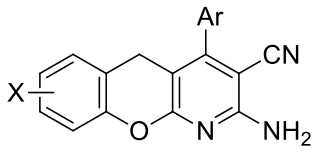
(**38**)



(39)



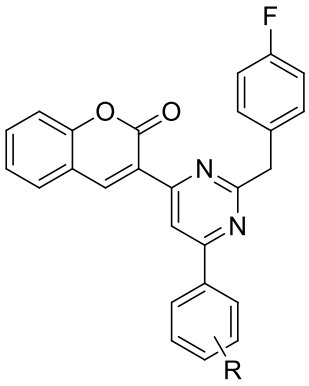
(40)



(41)

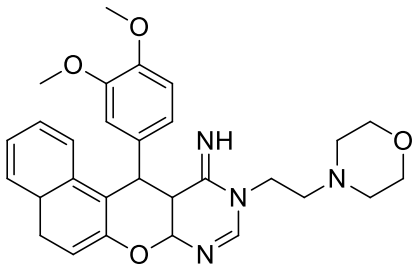
The pyranopyrimidine derivatives are known to be important synthetic bioactive compounds which have gained renewed interest in the area of medicinal chemistry.[64-72]

Recently**, Kallappa *et al.***[73] reported the Microwave-assisted synthesis of new fluorinated coumarin–pyrimidine hybrids and studied their potent anticancer activity.



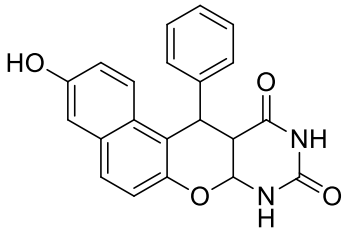
(42)

McCoy and *co-workers*[74] reported the synthesis of selective modulation of Gq/Gs pathways for naphtho pyrano pyrimidines which act as antagonists of the neuropeptide S receptor (**43**)



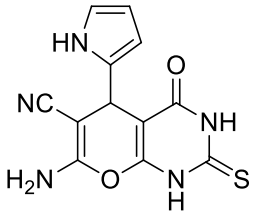
(**43**)

Khurana *et al.*[75] have studied the synthesis and antimicrobial activity of a new class of pyrano pyrimidine derivatives (**44**)



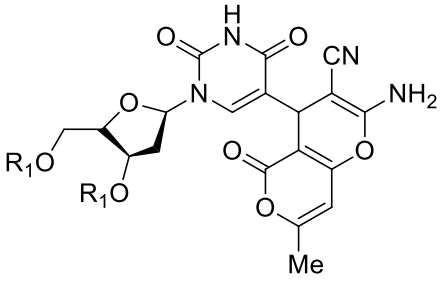
(**44**)

Afzal *et al.*[76] reported the synthesis and antimicrobial activity of a new class of pyrano pyrimidine derivatives (**45**)



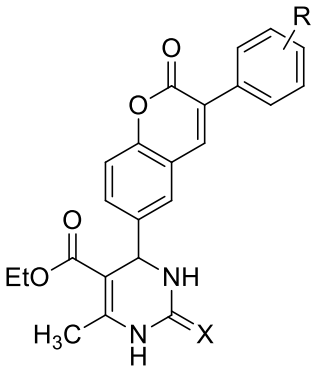
(**45**)

Fan *et al*.[77] have described an efficient synthesis of pyrano[4,3-*b*]pyran and their hybrids (**46**).



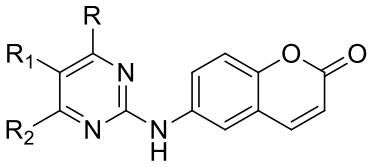
(**46**)

Emami *et al.*[78] designed and synthesized new coumarinemonastrol hybrids (**47**) which act as anticancer agents.



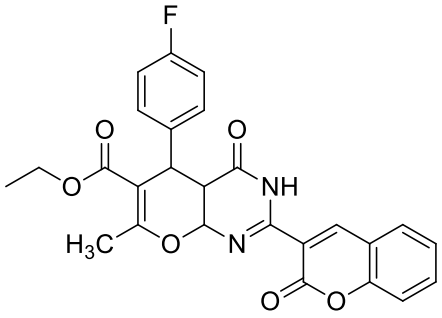
(47)

Kamilia *et al.*[79] reported the synthesis and vasorelaxant evaluation of novel coumarin pyrimidine hybrids (**48**)



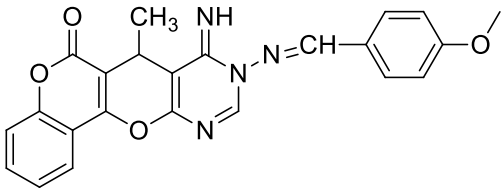
(48)

Ghashang *et al.*[80] published the synthesis of substituted chromeno[2,3-*d*]pyrimidinone derivatives and their antimicrobial activity (**49**).



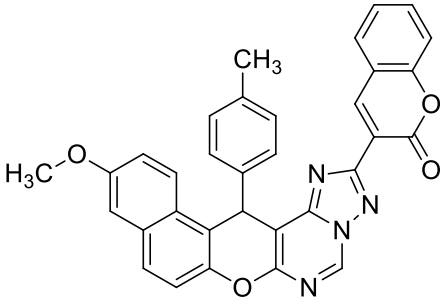
(49)

Bedair *et al.*[81] described the 4-hydroxycoumarin in the synthesis and antimicrobial activity of new pyrano[2,3-*d*]pyrimidine (**50**).



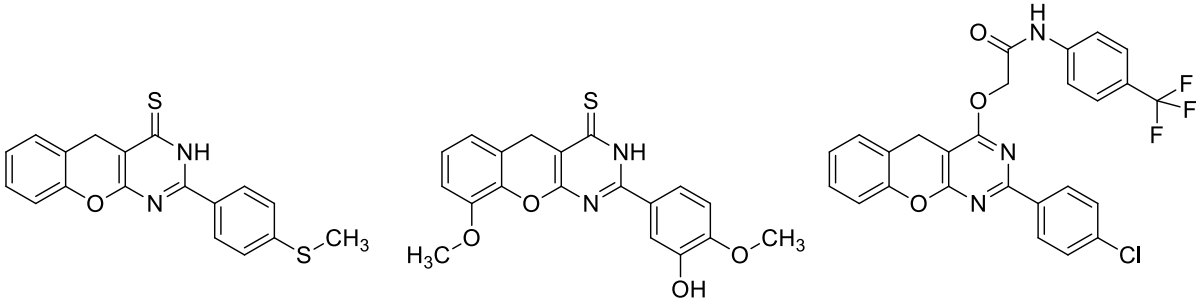
(50)

F. A. Eid and *co-workers*[82] synthesized pyrano pyrimidines (**51**) and evaluated their *invitro* antimicrobial activity.



(51)

Borisov *et al*,[83] Developed a improved conditions for the reaction of 2-iminocoumarin-3-(thio)carboxoamides with aromatic aldehydes, leading to different substituted benzopyrano [2,3-*d*]pyrimidine scaffolds like (**52**, **53** and **54**) (**Fig. 4**) and studied their biological activity.

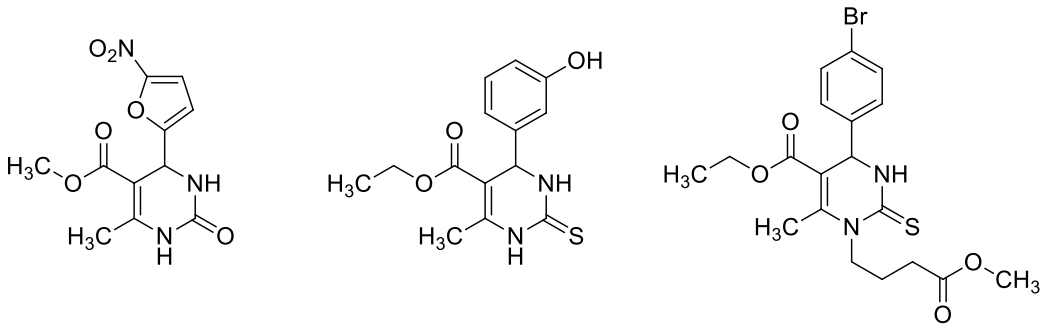


(52) **(53)** **(54)**

Figure. 4

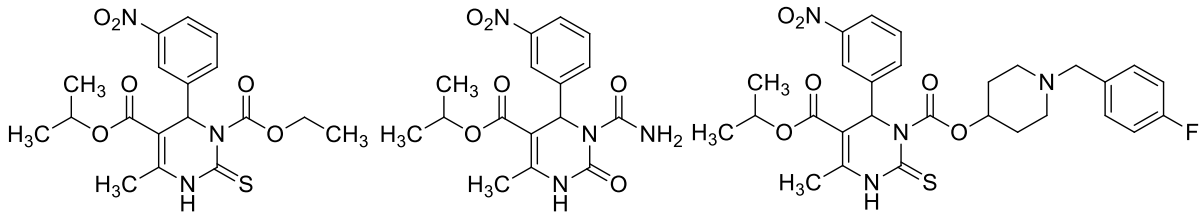
B) DIHYDROPYRIMIDIN-2(5*H*)-ONES

In recent times, dihydropyrimidinone derivatives have increasingly attracted the attention of chemists, because of their broad biological activities such as calcium channel blockers, antitumor, antiviral, antihypertensive and anti- inflammatory activities.[84-86] **Nitractin (55)** was first reported in the 1960's as an agent against the trachoma group of viruses. **Monastrol (56)** is known as a specific inhibitor of mitotic kinesin Eg5. In addition pyrimidine-5-carboxamide of type (**57)** was claimed to have anticarcinogenic activity.[87-88]



(Nitractin) (55) **(Monastrol) (56)** **(SW02) (57)**

Further modification of the substituent at N3 of DHPMs led to the development of orally active long- lasting antihypertensive agents, such as DHPMs (**58**) **(SQ 32926)**, (**59**) **(SQ 32547)** and (**60**) (**Fig. 5**).[89-93]

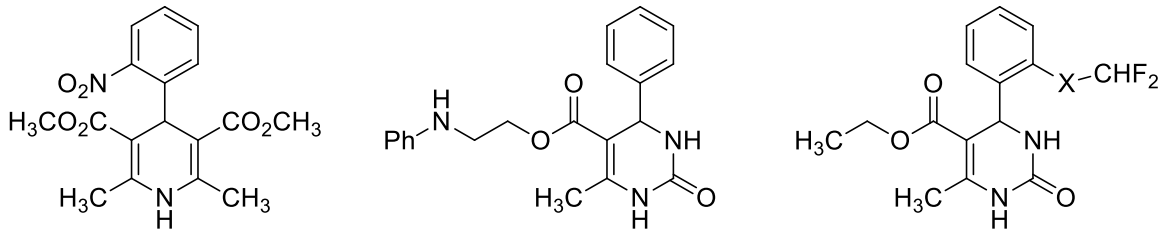


(58) **(59)** **(60)**

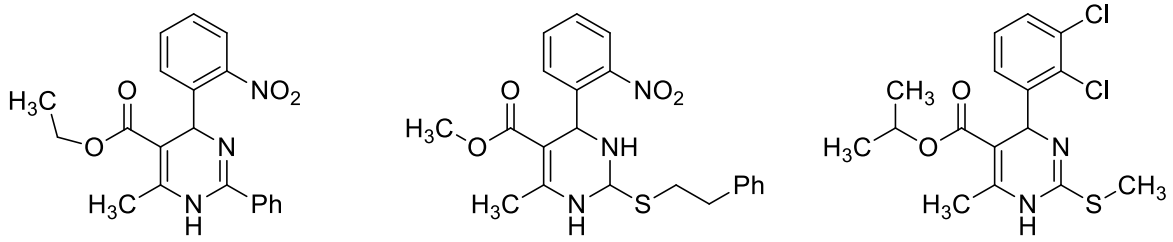
Figure 5. Structures of DHPM calcium channel modulators.

Dihydropyrimidin-2(1*H*)-thione core is an important pharmacophore useful as intermediate for the preparation biologically potent thiazolopyrimidine and thiazoloquinazoline derivatives.[94-96]

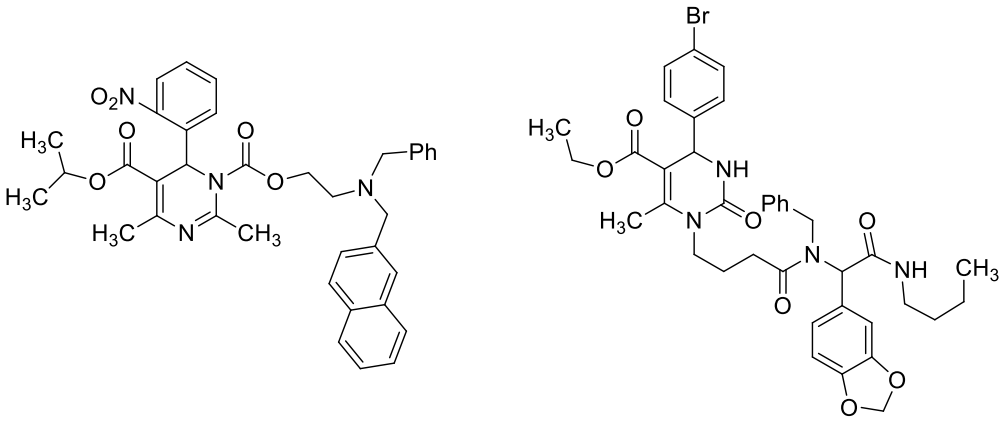
Khanina and co-workers reported *β*-aminoethyl esters of type (**61** and **62**) that exhibit hypotensive activity and coronary dilatory properties. During the mid 1980's, interest was focused on 4-aryl-1,4-dihydropyrimidine-5-carboxylate calcium channel blockers which closely mimic the dihydropyridine (DHP) scaffold, *e.g.* compounds (**63–68**) (**Fig. 6**).[97-101]



(61) **(62)** **(63)**



(64) **(65)** **(66)**

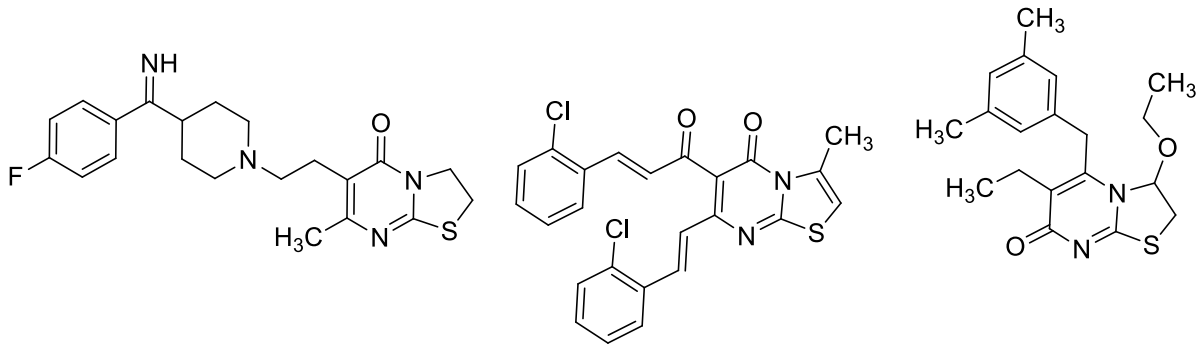


(67) **(68)**

Figure. 6

C) THIAZOLO[4,5-*d*]PYRIMIDINES

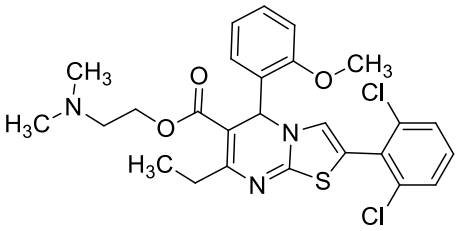
Compounds having a thiazolo[3,2-*a*]-pyrimidine ring have shown a wide variety of biological activities (e.g., antituberculotic, anti-tumour, bronchodilators, central nervous system (CNS) depressants, analgesic, anti-human immunodeficiency virus (HIV)-1, anti-inflammatory, anti-microbial, anti-diuretic and CNSactive agents) (**Fig. 7**).[102-109]



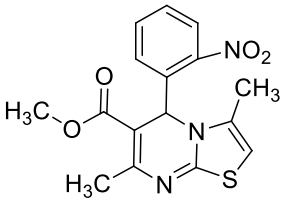
(69) **(70)** **(71)**

Figure. 7

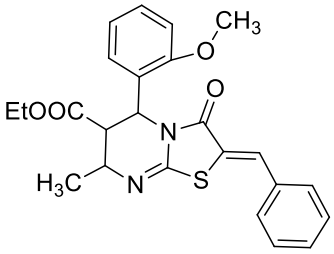
J. Wichmann *et al.*[110-111] reported that a series of 5*H*-thiazolo[3,2-*a*]pyrimidine derivatives (**72-74**) act as potent calcium channel modulators and antiinflammatory agents respectively.



(72)

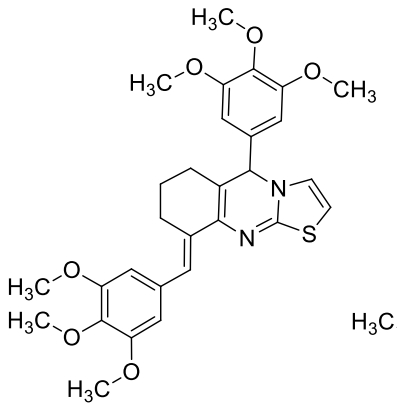


(73)

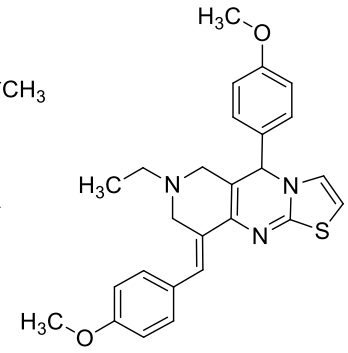


(74)

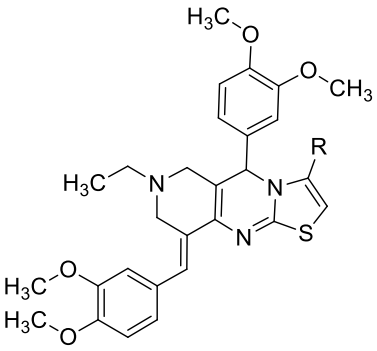
M. Mohamed and co-workers[112] designed and synthesized a novel series of thiazolo[2,3-*b*] quinazolines, pyrido[4,3-*d*]thiazolo[3,2-*a*]pyrimidine derivatives (**75**, **76** & **77**) and evaluated for there *in vitro* antitumor activity on 60 cell lines panel assay. Similarly, thiazolo[3,2-*a*]pyrimidine derivatives **(Fig. 8)** work against different human tumor cell lines. The activity results revealed that the pyrimidine moiety fused to *N*-methylpipredine ring is essential for antitumor activity.[112]



(75)



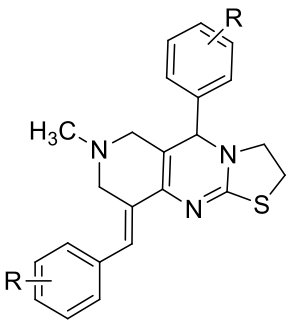
(76)



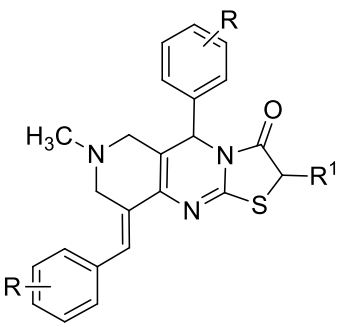
(77)

Figure. 8

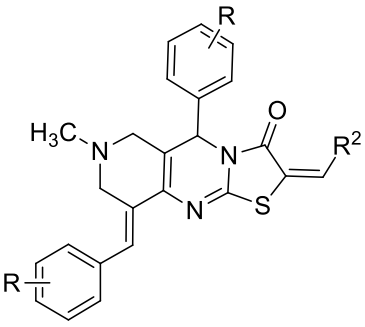
A. Basiri *et al.*[113] reported the synthesis of novel thiazolopyrimidine derivatives (**78**-**80**) *via* microwave assisted, domino cascade methodology in ionic liquid and evaluated their *invitro* acetylcholinesterase (AChE).



(78)

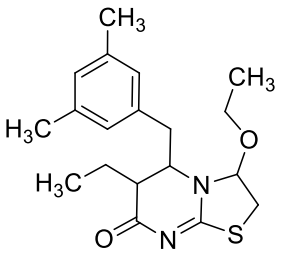


(79)



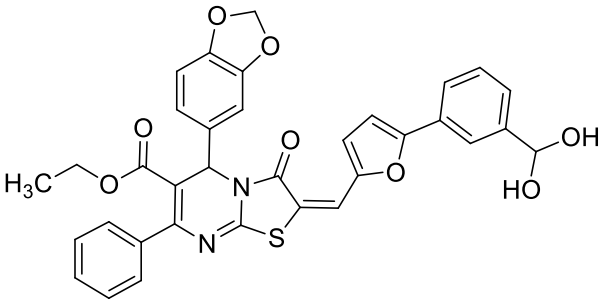
(80)

K. Danel *et al.*[113] reported the synthesis and anti HIV-1 activity of 2,3-dihydro-7*H*-thiazolo[3,2-*a*] pyrimidin-7-one derivatives (**81**).



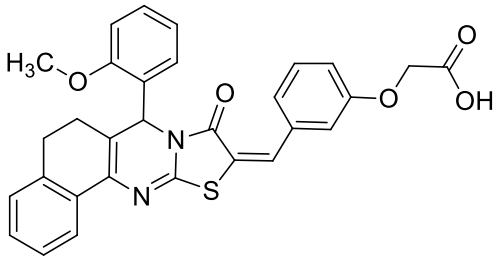
(81)

Z. Qin *et al.*[114] reported that thiazolo[3,2-*a*]pyrimidinones **(82)** have displayed bactericidal effects on both planktonic and biofilm cells of *S.epidermidis*.



(82)

Y. Feng *et al.*[115] discovered a series of quinazoline-2(1*H*)-thione derivatives of type (**83**) as new antiapoptotic Bcl-2 family protein (Bcl-xL, Bcl-2 and Mcl-1) inhibitors.

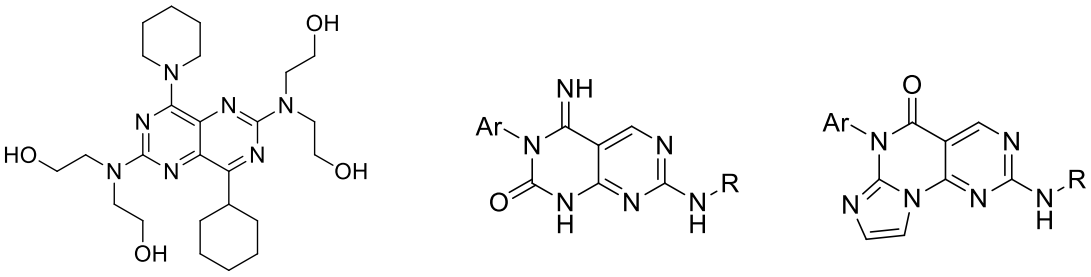


(83)

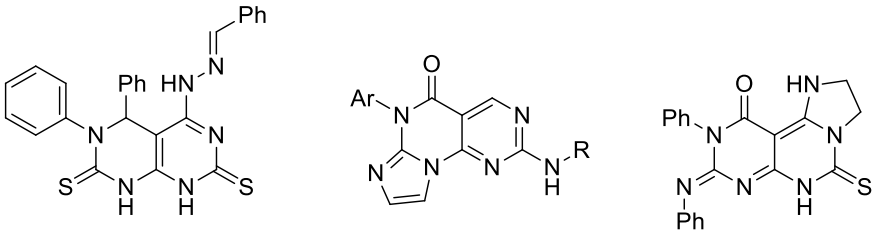
D) PYRIMIDO PYRIMIDINES

Pyrimido[4,5-*d*]pyrimidines are form an important class of annulated uracils with biological significance because of their similarities with purines and pteridine frameworks.[116] Fused pyrimidinedione derivatives (**84-89**) are important pharmaceuticals due to their biological

activities which find applications in diseases such as hypertension, diabetes, and immunosuppression as shown in (**Fig. 9**).[117-118]



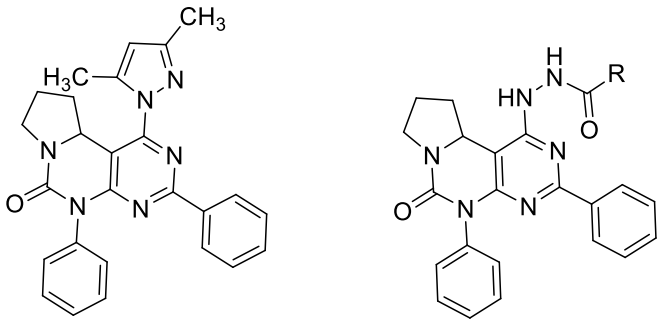
Dipyridamole (84) **(85)** **(86)**



(87) **(88)** **(89)**

Figure. 9

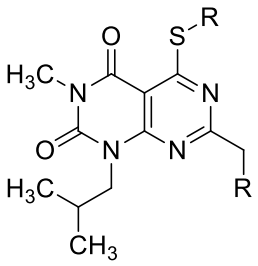
M.M. Hanna *et al*.[119] fused pyrimidines together which constitutes an important skeleton (**90** and **91**) for compounds with diverse biological activities including anti-inflammatory and analgesic activities (**Fig. 10**).



(90) **(91)**

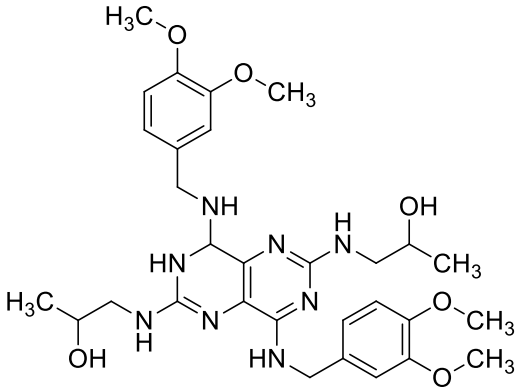
Figure. 10

H. Wang *et al*.[120] explored general synthetic methods to prepare 1,3,5,7-tetrasubstituted pyrimido[4,5-*d*]pyrimidines (**92**) and extensively investigated their MCT1 inhibitory activity.

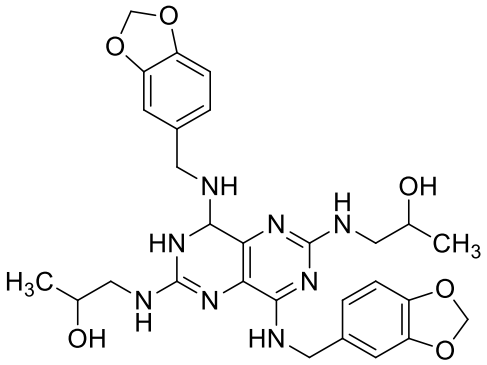


(92)

Kappusamy *et al.*[121] observed that 4,8-dibenzylaminopyrimidopyrimidines (**93** and **94**) retained NT-inhibitory activity in cancer cell lines.

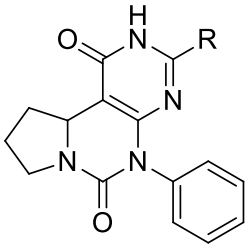


(93)



(94)

Sanghvi *et al.*[122] synthesized new pyrimido[5,4-*e*]pyrrolo[1,2-*c*]pyrimidines (**95**) and studied their antiinflammatory and analgesic activities.

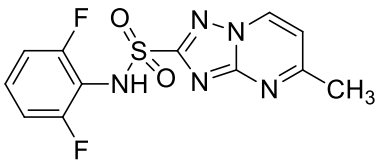


(**95**)

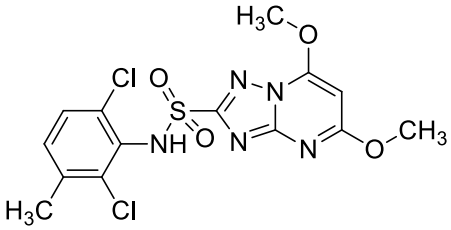
E) TRIAZOLOPYRIMIDINES

Triazolo[1,5-*a*]pyrimidine heterobicycles constitute well established scaffolds that are frequently encountered in highly significant bioactive molecules, pharmaceuticals and agrochemicals.[123-126] Triazolo[1,5-*a*]pyrimidine constitute the main part of flumetsulam **96** and metosulam **97** which are well known herbicides effective for controlling various broadleaf and grass weed species at low doses in corns and cereals respectively. On the other

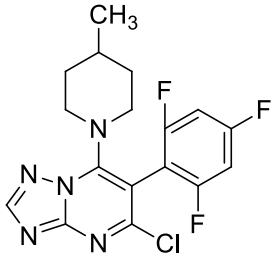
hand, 7-amino-triazolo[1,5-*a*]pyrimidines such as BAS600 **98** and TTI-237 **99** act as fungicides in plants and as potent anticancer agents (**Fig. 11**).[127-128]



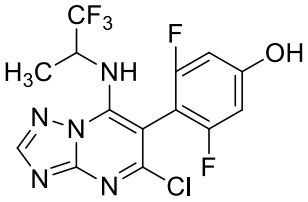
Flumetsulam (96)



Metosulam (97)



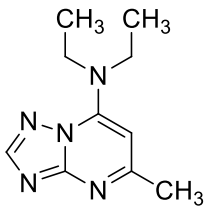
BAS600 (98)



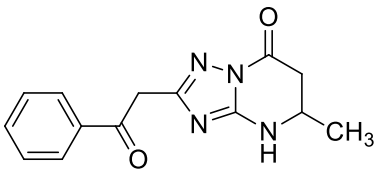
TTI-237 (99)

Figure .11

These triazolo[1,5-*a*]pyrimidines (TPs), which are analogues of purine bases have been identified to possess multifaceted pharmacological properties, including antihypertensive, cardiac stimulant, antimalarial, antifungal, anti-HBV, antimicrobial, anticancer and herbicidal activities.[129-132] The most widely known derivative is the simple molecule of Trapidil (**100**), a clinically used vasodilator, Recently, a new antibiotic substance named Essramycin (**101**), the first isolated 1,2,4-triazolo[1,5-*a*]pyrimidine natural product, which was reported to exhibit broad-spectrum antibacterial properties.[133-134]

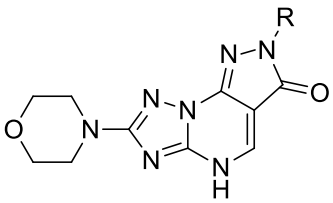


Trapidil (100)

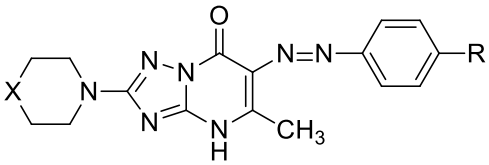


Essramycin (101)

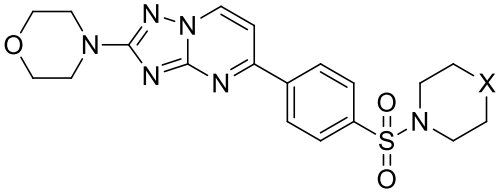
On the other hand, triazolopyrimidines bearing pyrazole and morpholine moietie (**102** and **105**) were evaluated for their antitumor activity.[135-138]



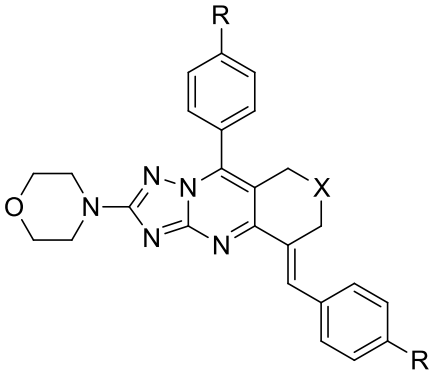
(**102**)



(**103**)

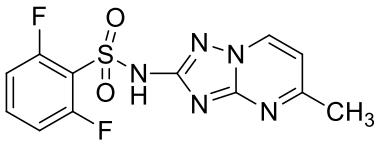


(**104**)

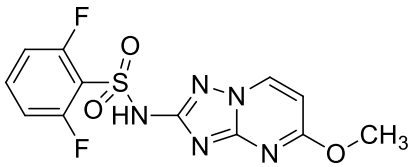


(**105**)

C. N. Chen *et al.*[139] identified that triazolopyrimidines possess high herbicidal activity (**106**) and (**107**).



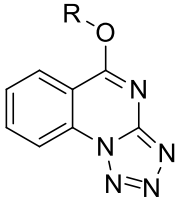
Flumetsulam (**106**)



Y6610 (**107**)

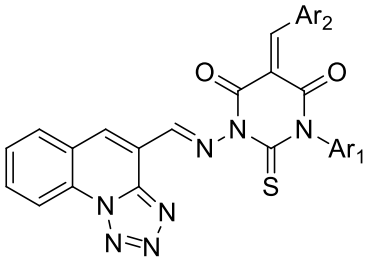
F) TETRAZOLO PYRIMIDINES

Wang *et al.* [140] synthesized several derivatives of 5- alkoxy-tetrazolo[1,5-*a*]quinazoline (**108**) by reacting 2,4-dichloroquinazoline which display a weak anticonvulsant activity.s

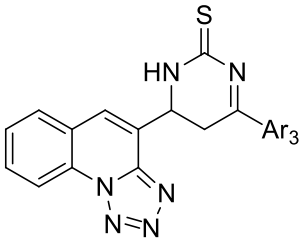


(108)

Bekhit *et al*.[141] synthesized a series of tetrazolo[1,5-*a*]quinoline derivatives (**109** and **110**) and were evaluated for their antiinflammatory and antimicrobial activities.

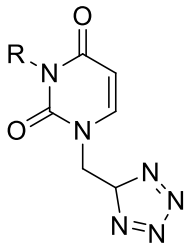


(109)



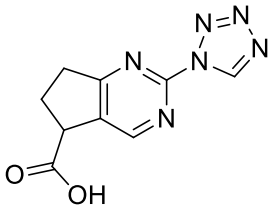
(110)

S. Kristafor *et al.*[142] Reported the synthesis of a novel series of the biologically active acyclic pyrimidine nucleosides, a novel class of tetrazole-containing N-acyclic pyrimidine nucleoside analogues (**111**).



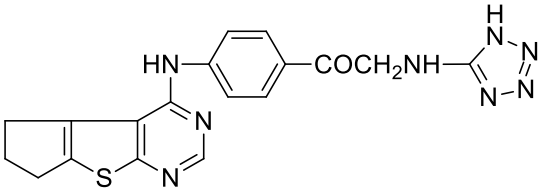
(111)

Peter Mullens *et al.*[143] developed a cost effective method to prepare tetrazolo-pyrimidines (**112**) up to 100grm level.



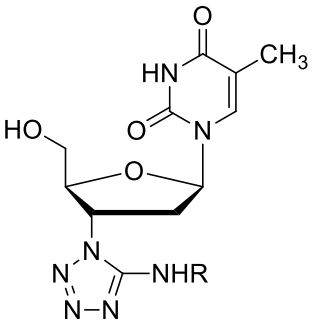
(112)

A series of novel thieno[2,3-*d*]pyrimidine derivatives containing tetrazole ring (**113**) were synthesized by **Salahuddin *et al.***[144] and their antibacterial activity was evaluated.



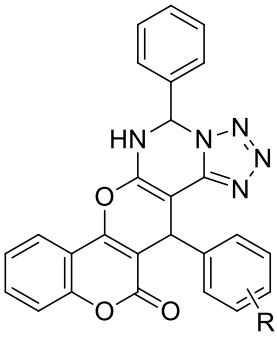
(**113**)

The synthesis of derivatives of 3'-(5-amino-1,2,3,4-tetrazol-4-yl)-3'-deoxythymidines[145] (**114**), which exhibit activity against the human immune deficiency virus, was developed by **Bayer AG**.

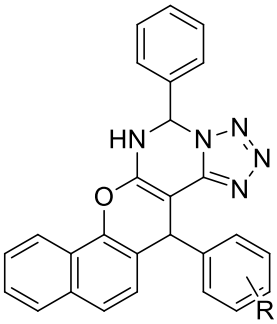


(**114**)

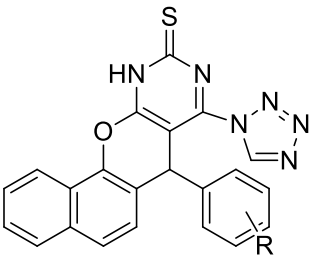
Chandramouli *et al.*[146-147] reported the synthesis of three different series of pyrimidines derivatives bearing tetrazoles (**115**, **116**, **117** and **118**) and evaluated their antimicrobial activity (**Fig. 12**).



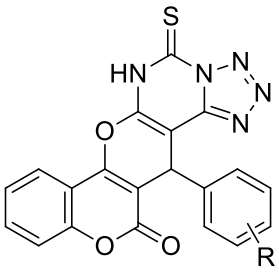
(115)



(116)



(117)

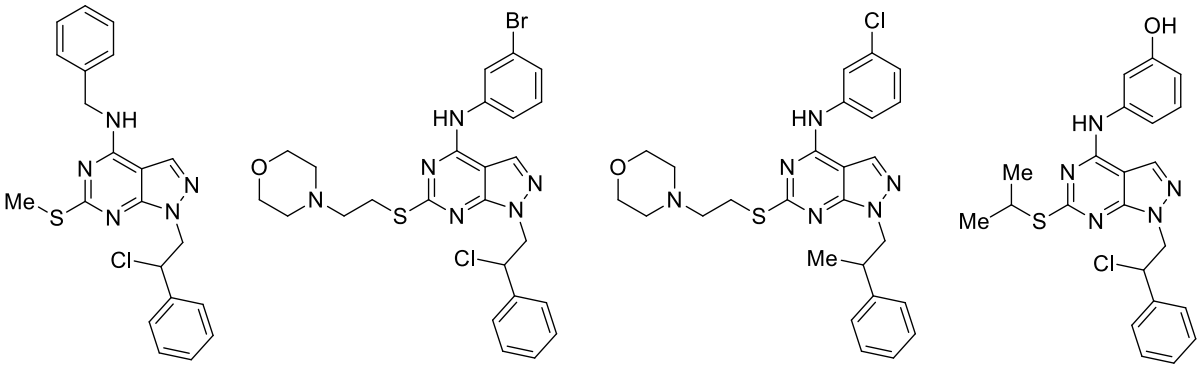


(118)

Figure. 12

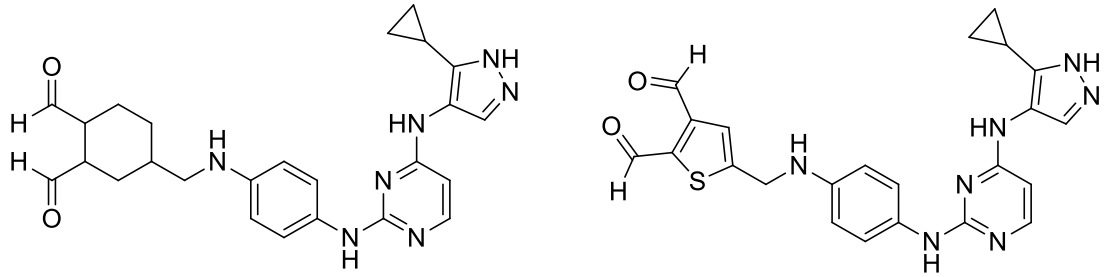
G) PYRAZOLO PYRIMIDINE

F. Carraro, *et al.*[148-155] reported that pyrazolo[3,4-*d*]pyrimidines (**119-122**) at as potential anticancer drugs.



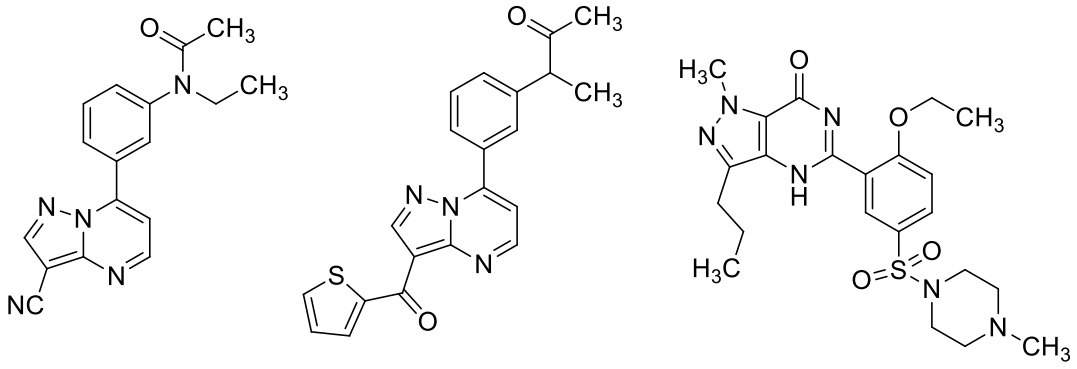
(119) **(120)** **(121)** **(122)**

C. S. MartI *et al.*[156] described the biological activity and synthesis of new pyrazolo pyrimidines (**123** & **124**).



(123) **(124)**

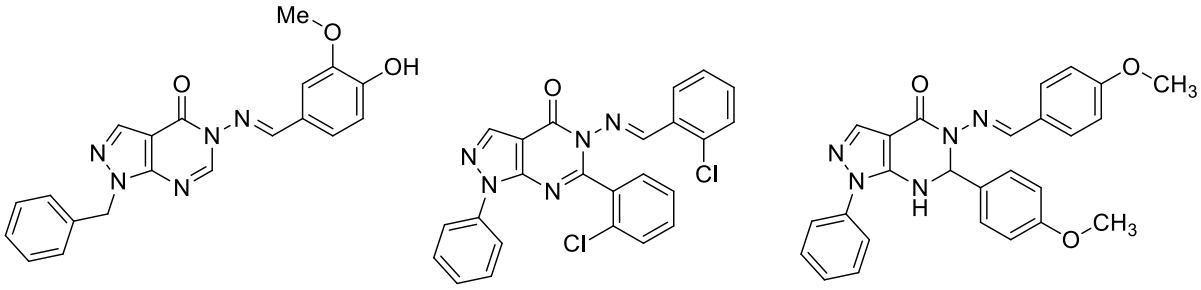
A number of different compounds consisting of pyrazolopyrimidines as central core were synthesized which exhibit biological activities such as the pyrazolopyrimidine antibiotics that represent a class of modified nucleosides containing the unusual C-riboside link.[157-162] Mahajan and Mahajan prepared derivatives of pyrazolo[3,4-*d*]pyrimidines. Pyrazolopyrimidins consist of various isomeric forms like pyrazolo[3,4-*d*]pyrimidines (**125-127**) (**Fig. 13**).[163-167]



Zalepion (125) **Indipion (126)** **Sildenafil (127)**

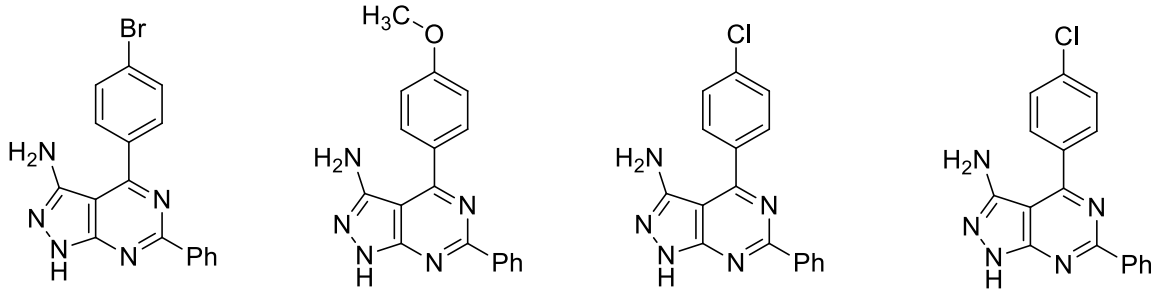
Figure. 13

M. Chauhan *et al.* [168] reported synthesis and mechanism action of pyrazolopyrimidinones as anticancer agents and also elevated ROS levels (**128-130**).



(128) **(129)** **(130)**

Rostamizadeh *et al.* [169] generated a library of pyrazolo[3,4-*d*]pyrimidine derivatives and elaborated their anti-bacterial activity (**131-134**) (**Fig. 14**).



(131) **(132)** **(133)** **(134)**

Figure. 14

1.2. PRESENT WORK

Inspired by the broad spectrum of Biological activities exhibited by various pyrimidine compounds, the present research program was designed to synthesis new pyrimidines. As past our ongoing program of synthesizing interest in the systems of new and novel heterocylic system, we have taken up the synthesis of new pyrimidines contains other heterocylic system as either fused or as substituted in the pyrimidine frame work.

We have also chosen sustainable and mostly green chemical path ways either by using ionic liquids or by using nano-particles, for the synthesis of novel pyrimidines like pyrano pyrimidines, dihydrothiochromeno pyrimidines, fused thiazolothiochromeno pyrimidines, pyrazolo-pyrimido pyrimidine, fused triazolo and tetrazolo[1,5-*a*]pyrimidines and pyrazolo phthalazine pyrimidines. Almost all the new synthesized molecules were evaluated for any one of the Biological activities like antimicrobial activity or antidibetic activity. The contents of the research work embodied in the thesis were presented under different chapters showed below.

TITLE: Synthesis and evolution of biological activity of novel substituted and fused pyrimidine derivatives.

CHAPTER-II: Synthesis of novel pyrano[2,3-*d*]pyrimidines using ionic liquid.

CHAPTER-III: This chapter is sub divided into Section A and Section B.

***SECTION-A*:** Synthesis of fused dihydro-1*H*-thiochromeno[4,3-*d*]pyrimidines under green conditions

***SECTION-B*:** Green chemical synthesis of novel fused thiazolo[4,3-*d*]thiochromeno[4,3-*d*]pyrimidine derivatives.

CHAPTER-IV: Synthesis of novel pyrazolo-pyrimido[4,5-*d*]pyrimidine derivatives using [Bmim]$FeCl_4$ ionic liquid.

CHAPTER-V: One-pot three-component synthesis of new type of fused triazolo[1,5-*a*] and tetrazolo[1,5-*a*]pyrimidines.

CHAPTER-VI: Synthesis of new pyrazolo[1,2-*b*]phthalazino pyrimidines derivatives by using [BSO_3Hmim]HSO_4.

CHAPTER-VII: Evolution of Biological activity.

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AN EFFICIENT ONE-POT THREE-COMPONENT SYNTHESIS OF NOVEL PYRANO[2,3-*d*]PYRIMIDINES USING IONIC LIQUID

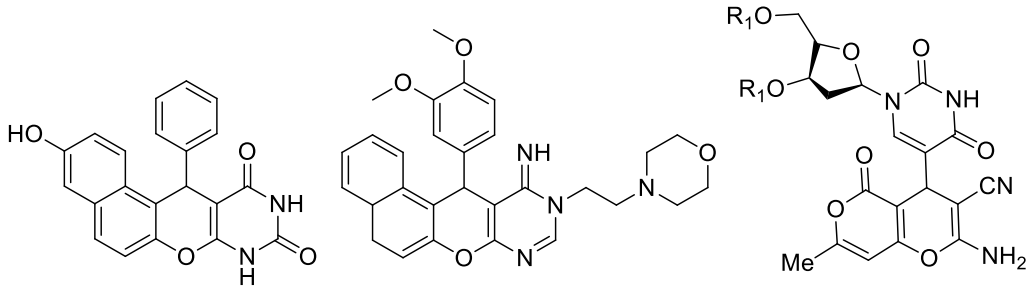
2.1. INTRODUCTION

Multicomponent reactions (MCRs) are always resource effective, environmentally acceptable when compared to step wise reactions. In recent years, multicomponent reactions (MCRs) have emerged as powerful tools in the synthesis of diverse and complex compounds as well as small and drug like heterocycles,[1] because of their productivity, simple procedures, convergence, facile execution, and atom economy.[2] Expeditious domino and multicomponent reactions (MCRs) have also emerged as powerful strategies for sustainable organic synthesis.[3]MCRs using ILs will be one of the novel approaches, which will meet the requirements of green chemistry as well as for developing libraries of medicinal scaffolds.[4]

In the recent years, sustainable chemistry emerged into research areas focussing on the development of efficient, economical and environmentally friendly organic synthesis.[5] Organic solvents are responsible for causing pollution problems particularly in synthetic organic processes.[6] Considerable efforts have been made to eliminate hazardous organic solvents in different areas of synthetic organic chemistry.[7] This has resulted in a paradigm shift towards the use of eco-friendly green protocols in all phases of chemical constructions which can be appreciated by creative research that widely addresses the issues of atom economy, economy of steps, and avoidance of pollution.[8] Ionic liquids have become inevitable media for the selective formations of new bonds during synthesis of small molecules,[9] because of their specific properties.[10-11]

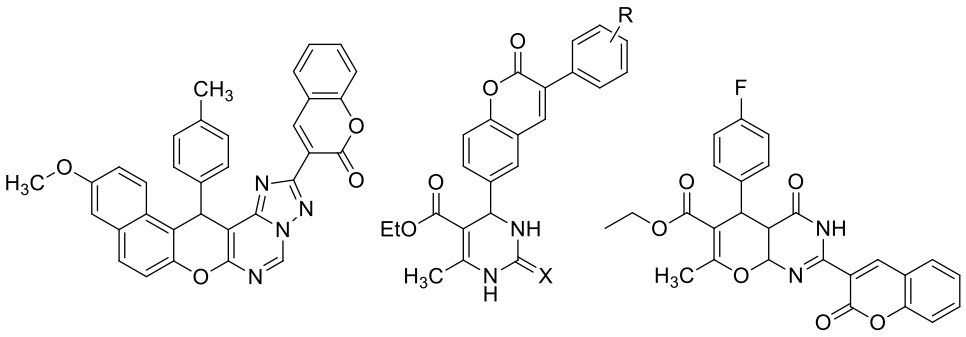
In the recent years, the synthesis of novel pyrimidine derivatives has gained considerable interest in the area of medicinal chemistry for their diverse range of biological activities including antimicrobial,[12] antimalarial,[13] anti-inflammatory,[14] anti-viral,[15] anti-platelet,[16] anti-tumor,[17] anti-histamine,[18] anti-thrombotic[19] and antigenic properties.[20-21]

Pyrans and coumarins are heterocyclic compounds which constitute an important group of natural products having varied biological activities such as antitumor,[22] antiinflammatory,[23] antioxidant,[24] and anti-coagulant[25] and anti-tubercular activities.[26] The different substituents in the coumarin nucleus strongly influence the biological activity of the resulting derivatives. Some of the well-known pyranopyrimidine derivatives (**1**, **2** and **3 Fig. 1**)[27] were reported as antimicrobial agents. Coumarins possess a variety of therapeutic and pharmacological properties.[28] Chromenopyrano pyrimidine derivatives (**4**, **5** and **6 Fig. 2**)[29] were evaluated for their *invitro* antimicrobial activity against various bacterial stains.



(1) **(2)** **(3)**

Figure 1. Pyrano[2,3-*d*]pyrimidins based biologically active molecules

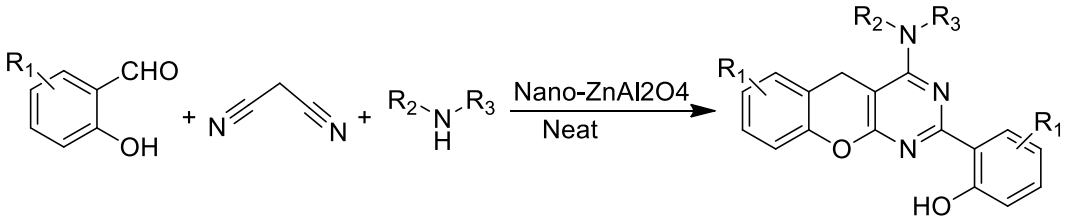


(4) **(5)** **(6)**

Figure 2. Chromenopyrano[2,3-*d*]pyrimidine based biologically active molecules

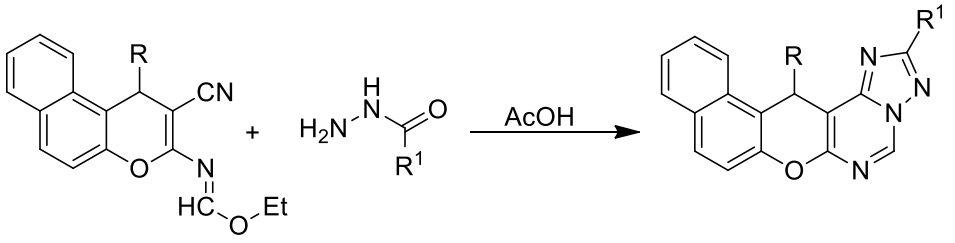
2.1.1. EARLIER APPROACHES FOR THE SYNTHESIS OF PYRANO PYRIMIDINES AND CHROMENO PYRIMIDINES

Umamahesh *et al.*[30] reported the synthesis of a novel, facile, rapid, solvent free protocol for the one pot green synthesis of chromeno[2,3-d]pyrimidines using reusable nano ZnAl2O4 – a NOSE approach (**Scheme 1**)



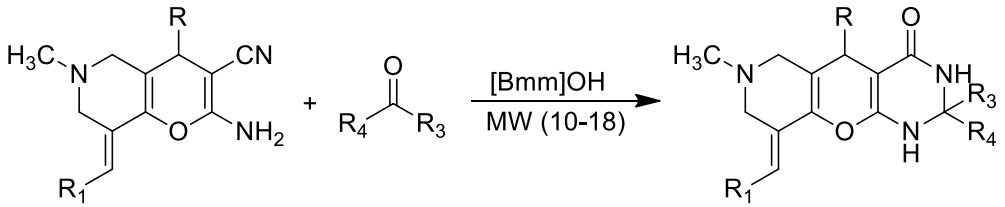
(Scheme 1)

Fakher *et al.*[31] have studied the synthesis and antigenotoxic activity of some naphtho[2,1-*b*] pyrano[3,2-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine derivatives (**Scheme 2**).



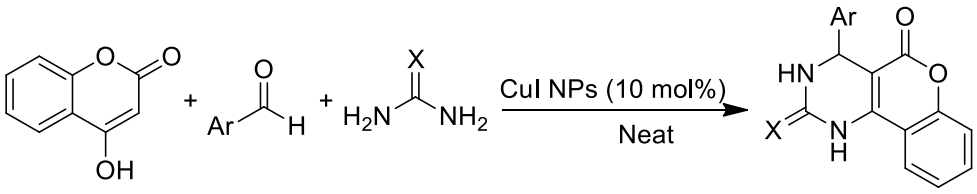
(Scheme 2)

Siddiqui *et al.*[32] conducted a microwave accelerated facile and efficient synthesis of piperido[3′,4′:5,6]pyrano[2,3-*d*] pyrimidinones catalyzed by basic ionic liquid [Bmim]OH **(Scheme 3).**



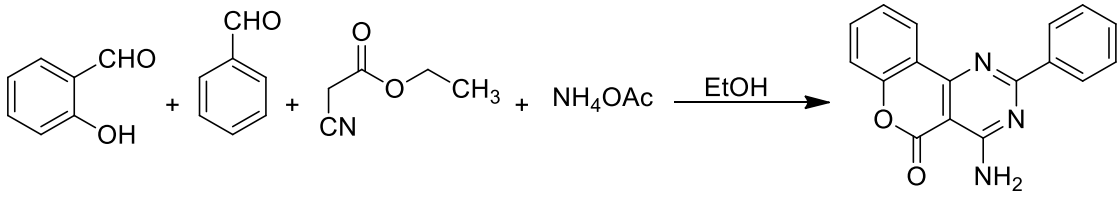
(Scheme 3)

Abdolmohammadi *et al.*[33] reported the rapid and mild synthesis of chromeno[*d*]pyrimidinones using nanocrystalline copper (I) iodide under solvent-free conditions (**Scheme 4**).



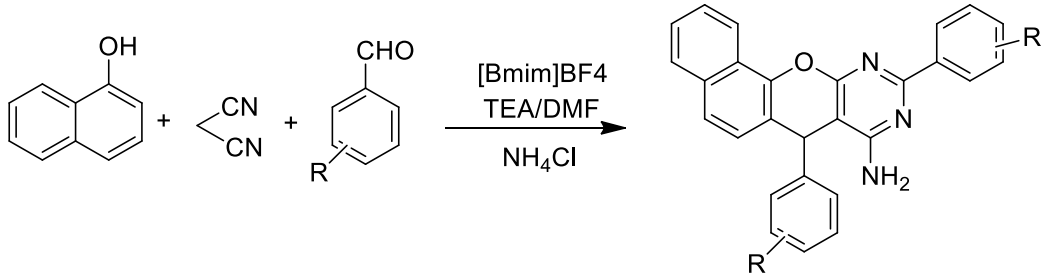
(Scheme 4)

Wu *et al.*[34] described a method for the synthesis of disubstituted 2-phenyl-benzopyranopyrimidine derivatives (**Scheme 5**).



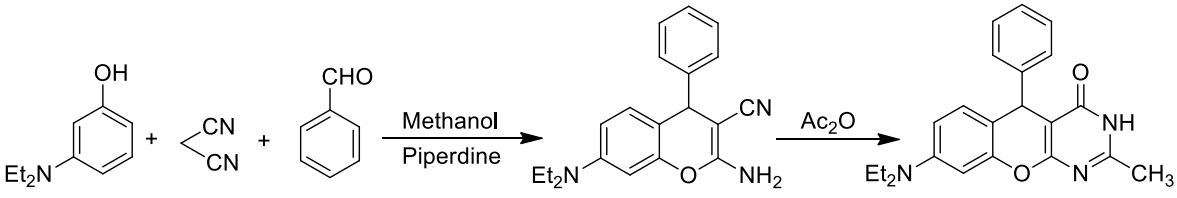
(Scheme 5)

Kanakaraju et al.[35] have developed the Ionic liquid catalyzed one-pot multi-component synthesis of novel chromeno[2,3-*d*]pyrimidin-8-amine derivatives (**Scheme 6**).



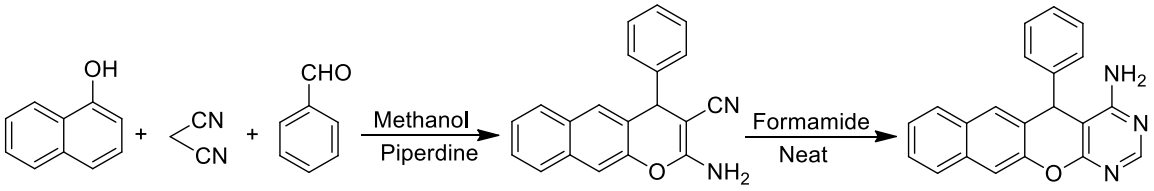
(**Scheme 6)**

Sabry et al.[36] synthesized 4*H*-chromene, coumarin, 12*H*-chromeno[2,3-*d*]pyrimidine derivatives and studied their antimicrobial and cytotoxicity activities (**Scheme 7**)



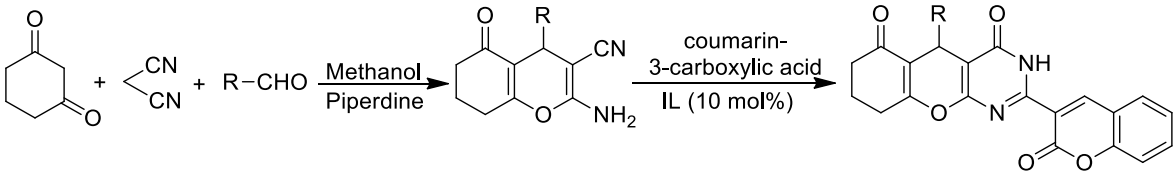
(Scheme 7)

McCoy *et al.*[37] synthesized napthopyranopyrimidines and demonstrated selective modulation of Gq/Gs pathways (**Scheme 8**).



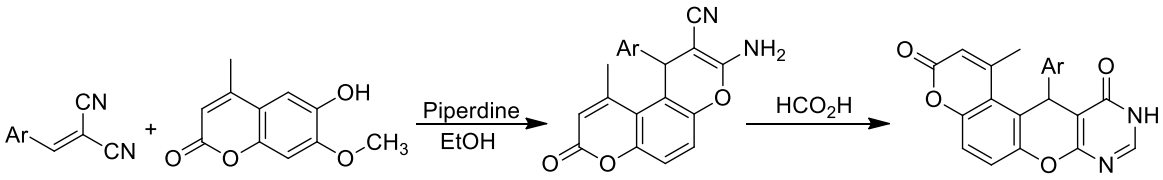
(Scheme 8)

Janardhan *et al.*[38] described the synthesis and antimicrobial activity of chromeno pyrimidinone derivatives (**Scheme 9**)



(**Scheme 9)**

Ouf and group.[39] designed the synthesis of pyranochromene and pyranopyrimidine derivatives from substituted natural coumarin isolated from Ammi majus L (**Scheme 10**).



(Scheme 10)

2.2. PRESENT WORK

In view of the diverse activities shown by the above two heterocyclic compounds (pyrimidine and coumarin) and as a continuation of our search for biologically active heterocyclic molecules, it was planned to construct a system, which contains both these moieties in a single molecular frame work and to explore the additive effects towards their biological activities. We report herein the synthesis of new chromenopyrano[2,3-*d*]pyrimidine and pyrano[2,3-*d*]pyrimidin derivatives. Even though the literature reported methods for the synthesis of pyrimidines were quite useful, they have one or more limitations such as:

(a) Use of homogeneous Lewis acid catalyst like $ZnBr_2$ which could not be recycled from the reaction mixture

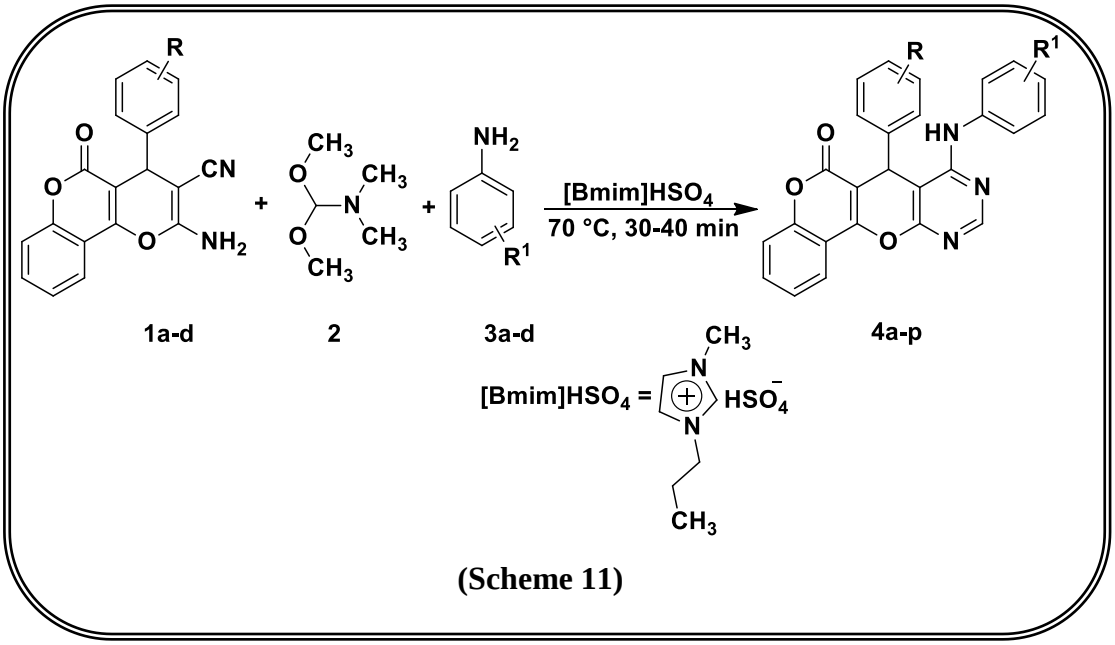
(b) Usage of expensive reagents, toxic solvents, and toxic metals

(c) Low yield, harsh reaction conditions

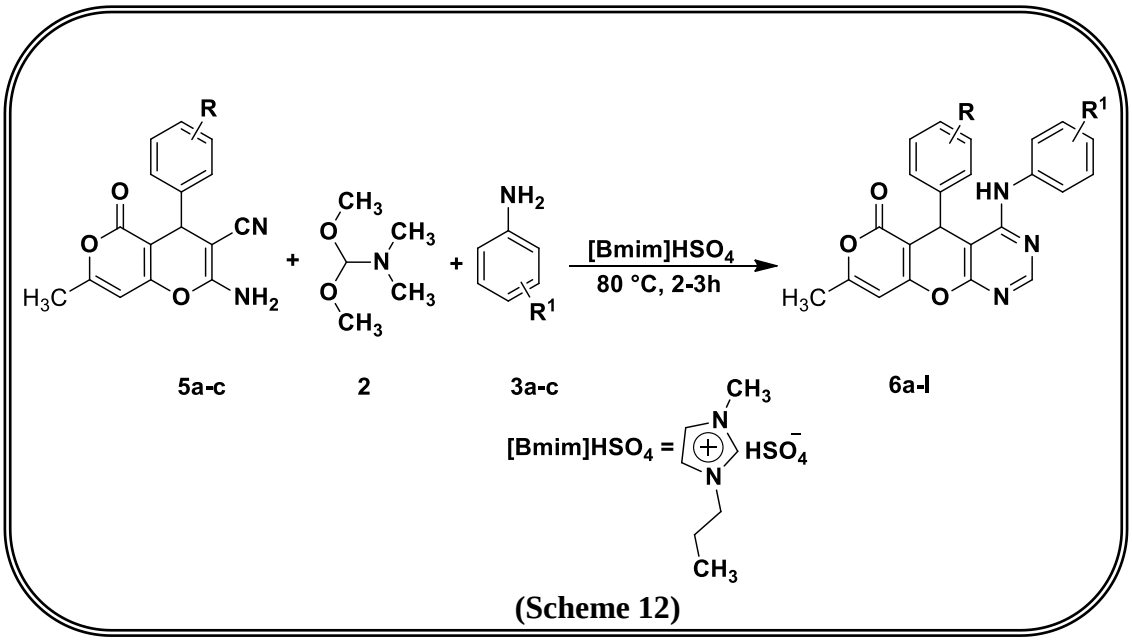
(d) In situ generation of hydrazoic acid which is highly toxic and explosive.[40]

Thus, there is a need to develop convenient and safe methods for the preparation of new pyrimidine derivatives. It was thought interesting for investigating green chemical methods. The use of ionic liquids (ILs) as solvents for these chemical reactions offer several advantages from the environmental perspective,[41] as well as addressing some of the above problems. In this context, ionic liquids, especially acidic and based on the 1,3- dialkylimidazolium salts, are of great promise as attractive alternatives to conventional solvents. They possess the advantages of high thermal stability, negligible vapour pressure, immiscibility with a number of organic solvents, and recyclability.[42] Butylimidazolium salts have already been demonstrated as efficient catalysts and solvents for various organic transformations.[43]

Our literature survey revealed that, till now there was no report in the literature on the synthesis of chromenopyrano[2,3-*d*]pyrimidine and pyrano[2,3-*d*]pyrimidin derivatives *via* one-pot three component reaction using ionic liquids. As part of our ongoing research work on the developments of new routes to heterocyclic system in ionic liquids, we tried a simple and efficient procedure for one-pot three component synthesis of novel chromenopyrano[2,3-*d*]pyrimidine and pyrano[2,3-*d*]pyrimidin derivatives. The starting materials used in the present study (**Scheme 11-12)** were prepared based on literature methods and they were identified by comparison of physical data with the sited procedures.[44] Later the 2-amino-5-oxo-4-phenyl-4,5-dihydropyrano[3,2-*c*]chromene-3-carbontrile/2-amino-7-methyl-5-oxo-4,5-dihydropyrano[4,3*b*]pyran-3-carbonitriles, *N*,*N*-dimethylacetaldehyde dimethyl acetal and aromatic amines were reacted in the presence of [Bmim]HSO_4 ionic liquid.



(Scheme 11)



(Scheme 12)

These reactions are very fast, smooth and the work up is easy. The ionic liquid is regenerated and used again for all the remaining condensations.

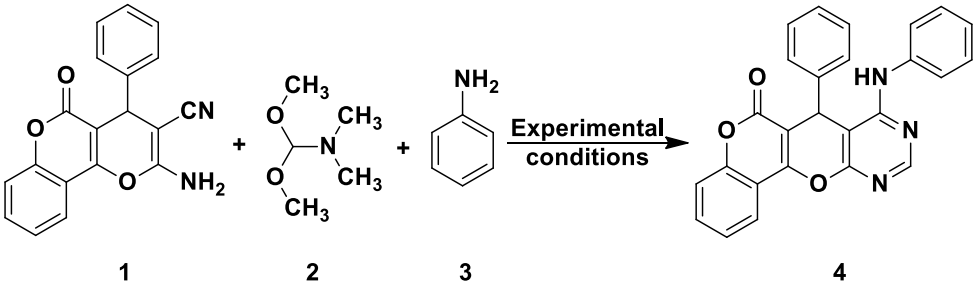
2.3. RESULTS AND DISCUSSION

In order to explore the possibility of the preliminary experiment, we have investigated the reaction conditions for optimization. Initially, we have chosen the reaction of 2-amino-3-carbonitrile 4-phenyl-4,5-dihydropyrano[3,2-*c*]chromene **1a** (1 mmol) with N,N-dimethylformamide dimethyl acetal **2** (1mmol) and aromatic

aniline **3a** (1 mmol) as a model reaction. We have performed the reaction employing neat condition and the reaction did not produce any desired product even after 24 h (Table 1, entry 1). Later the reaction was carried out in common organic solvents at 70 °C (Table 1, entries 2-5) but the yields were not good. The reaction was also carried out using typical ionic liquids such as [Bmim]Cl, [Bmim]Br, [Bmim]NO_3, [Bmim]PF_6, [Bmim]$ZnCl_3$, [Bmim]BF_4 and [Bmim]HSO_4 as a reaction media at 70 °C (Table 1, entries 6-12). Among the screened ionic liquids, we observed that [Bmim]HSO_4 was the most effective ionic liquid for the selective formation of desired product. It was also observed that [Bmim]HSO_4 showed outstanding activity in the formation of required product, as compared to other ionic liquids at low reaction time and temperature and afforded the desired product in 95% yield (Table 1, entry 12). Ascertaining [Bmim]HSO_4 as the right catalytic medium for the experiment, we then focussed our attention on designing and also generalizing the favourable conditions for the reaction. To investigate the effect of the temperature, systemic studies were carried out in the presence of [Bmim]HSO_4. Further it was observed that the optimum temperature was 70 °C and the optimum time limit was 30 min (Table 1, entries 13-15).

In case of pyranopyrimidine also the same model reaction was carried out using various 2-amino-7-methyl-5-oxo-4,5-dihydropyrano[4,3-*b*]pyran-3-carbonitriles **5a-c** (1 mmol), DMF-DMA **2** (1.2 mmol) and aniline **3a-c** (1 mmol) was performed in the presence of various solvents and the ionic liquids under different temperatures. The results in this regard are depicted in (Table 2, entry 1-10**)**. From these results, it was observed that the efficiency and the yield of the reaction using [Bmim]HSO_4 at 80 °C (Table 2, entry 6) was higher than those obtained in other solvents, such as acetonitrile, ethanol, acetic acid (Table 2, entry 1-3) and other ionic liquids like [Bmim]BF_4, [Bmim]Br and [Bmim]PCl_5 (Table 2, entries 8-10). It was inferred from the above results that the ionic liquid medium is an essential and crucial factor for promoting the reaction.

Table 1. Optimization of Reaction Conditions for the Synthesis of **4a**[a]



1 2 3 4

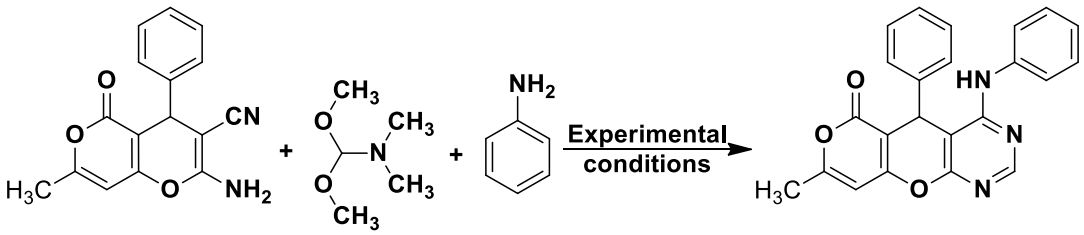
Entry[a]	Solvent	Conditions (°C)	Time	Yield[b] (%)
1	neat	Reflux	24h	NR
2	acetone	70	6h	16
3	acetonitrile	70	6h	12
4	ethanol	70	6h	20
5	acetic acid	70	6h	34
6	[Bmim]Cl	70	1h	70
7	[Bmim]Br	70	1h	55
8	[Bmim]NO_3	70	1h	50
9	[Bmim]PF_6	70	1h	72
10	[Bmim]BF_4	70	1h	82
11	[Bmim]$ZnCl_3$	70	1h	90
12	**[Bmim]HSO_4**	**70**	**30min**	**95**
13	[Bmim]HSO_4	rt	30min	52
14	[Bmim]HSO_4	60	30min	70
15	[Bmim]HSO_4	80	30min	95

[a]Reaction conditions: 2-Amino-3-carbonitrile-4-phenyldihydropyrano[3,2-*c*]chromeno (1 mmol), dmf-dma (1 mmol), aniline (1 mmol) and solvent (1 mL). [b]Isolated yields.

These optimized conditions were then applied for all further experiments, which afforded a library of chromenopyrano[2,3-*d*]pyrimidine derivatives (**4a-p**) and (**6a-l**) in decent yields and the results are summarized in Table 3. Subsequently, we examined the scope and efficiency of the reaction with respect to various aldehydes and amines under optimal conditions (Table 3, entry 1-28). It was observed that a

variety of aromatic aldehydes and amines bearing either electron withdrawing or electron donating substitutions at the *ortho*-, *meta*- and *para*-positions participated well in this reaction. All the synthesized compounds were confirmed by their spectral data (IR, Mass, ^{1}H NMR, and ^{13}C NMR) and elemental analysis. Spectral data for all the compounds were in full agreement with the proposed structures. Further, the structure of compound **4i** was unambiguously confirmed by single crystal X-ray diffraction analysis (**Fig. 3**) (CCDC 1440717). The compounds **4i** crystallized in the centrosymmetric monoclinic P21/n space group with one molecule in the asymmetric unit. The structure of compound **6e** was further confirmed by single crystal X-ray diffraction analysis (**Fig. 4**) (CCDC 1440717). A plausible mechanism for the formation of synthesized compounds, *i.e* chromeno pyrano[2,3-*d*]pyrimidin-6(5*H*)-one derivatives, **4a-p** and **6a-l** is proposed in **Scheme 13**.[4-5] 2-Amino-7-methyl-5-oxo-4,5-dihydropyrano[4,3-*b*]pyran-3-carbonitrile / 2-Amino-3-carbonitrile dihydropyrano [3,2-*c*]chromeno upon condensation with DMF-DMA formed the key intermediate (**A**). Then the *in situ* formed activated imine attacked the aromatic amine to give intermediate (**B**). By the elimination of dimethylamine the intermediate (**C**) was formed. The proton of the imidazolium group (C2-H) formed a hydrogen bond with the nitrogen of the nitrile group which increased its electrophilicity for the intramolecular nucleophilic attack, followed by proton transformation from (**B**) to (**C**). The hydrolysis of the pyrimidine ring yielded the complex (**D**), which upon intramolecular cyclization and subsequent dehydration produced the corresponding chromeno pyrano[2,3-*d*] pyrimidin-6(5*H*)-one derivatives **4a-p** and **6a-l**.

Table 2. Optimization of reaction parameters for the synthesis of **6a**

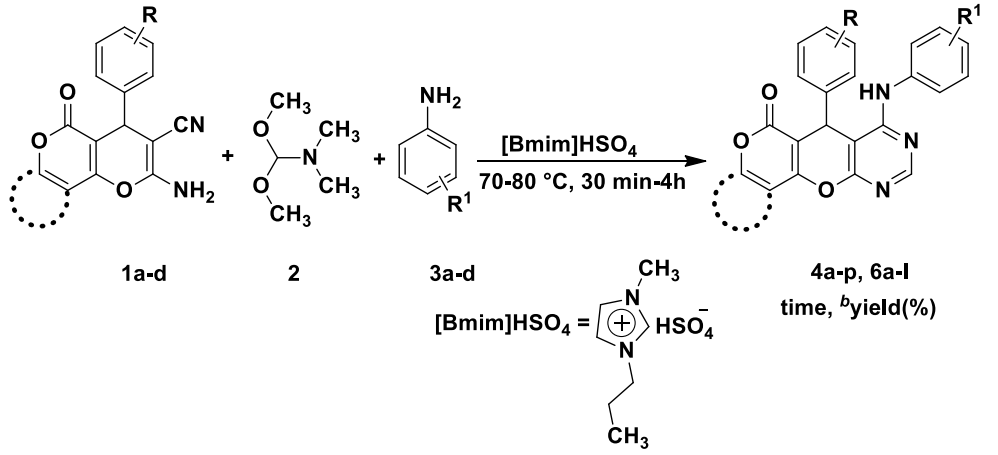


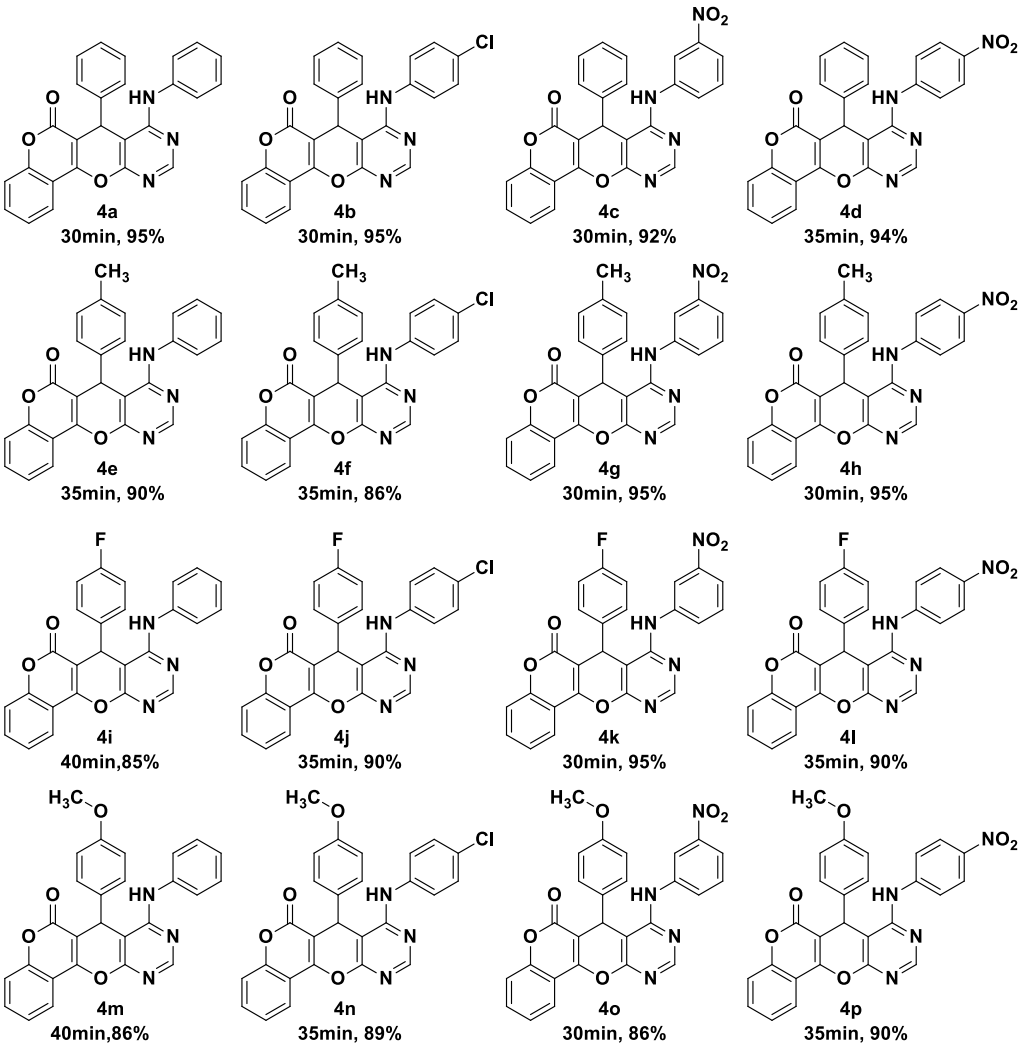
5 **2** **3** **6**

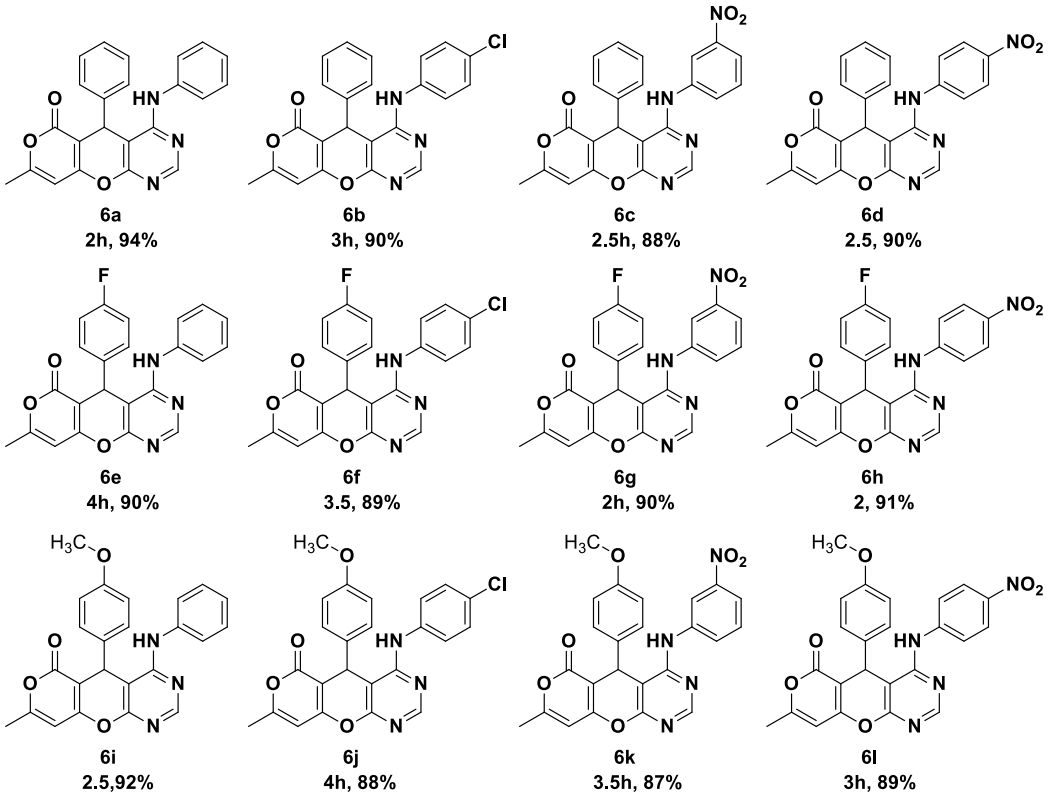
Entry[a]	Solvent	Temp (°C)	Time (h)	Yield[b] (%)
1	Ethanol	Reflux	10	28
2	Acetonitrile	Reflux	10	24
3	AcOH	Reflux	8	44
4	[Bmim]HSO_4	r.t.	2	48
5	[Bmim]HSO_4	60	2	76
6	**[Bmim]HSO_4**	**80**	**2**	**94**
7	[Bmim]HSO_4	100	2	92
8	[Bmim]BF_4	80	2	82
9	[Bmim]Br	80	2	74
10	[Bmim]PCl_5	80	2	62

[a]Reaction conditions: 2-Amino-7-methyl-5-oxo-4-phenyl-4,5-dihydropyrano[4,3-*b*]pyran-3-carbonitriles (1 mmol), DMF-DMA (1.2 mmol), Aniline (1 mmol), [Bmim]HSO_4 (2 mL), 80 °C, 2 h; [b]Isolated yields after purification.

Table 3. Synthesis of chromenopyrano[2,3-*d*]pyrimidine derivatives (**4a**–**p**) and (**6a-l**) in [Bmim]HSO_4 ionic liquid

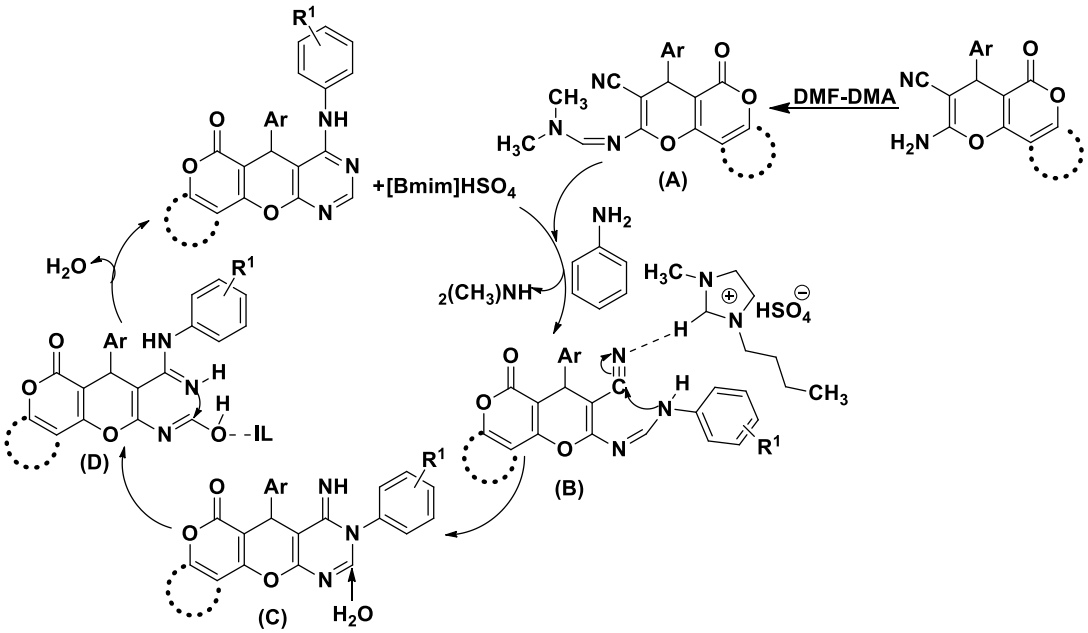






[a]Reaction conditions: 2-Amino-3-carbonitrile dihydropyrano[3,2-*c*]chromeno /2-Amino-7-methyl-5-oxo-4-phenyl-4,5-dihydropyrano[4,3-*b*]pyran-3-carbonitriles (1 mmol), dmf-dma (1 mmol), aromatic amines (1 mmol) and ionic liquid [Bmim]HSO_4 (2 mL). [b]Yields of the isolated product.

In terms of green chemistry, efficient recovery and reusability of the ionic liquid is also a very important aspect. Therefore, the recovery and recyclability of [Bmim]HSO_4 was also examined. After the completion of the reaction, 20 mL of diethyl ether was added to the reaction mixture. It was shaken well and the ethereal layer was decanted before washing the crude reaction mixture with ether (4 × 10 mL). The residue was dried under vacuum at 45 °C for 3 h, yielding the ionic liquid in its activated form. This was then subjected directly to another reaction, affording again the desired product in high yield. The ethereal layer was evaporated, washed with water and the product was crystallized from ethanol. Even after recycling for four times, the activity of the IL remained the same and almost quantitative yield was obtained each time (**Fig. 5**).



Scheme 13. Proposed mechanism for the formation of chromono pyrano[2,3-*d*]pyrimidine derivatives

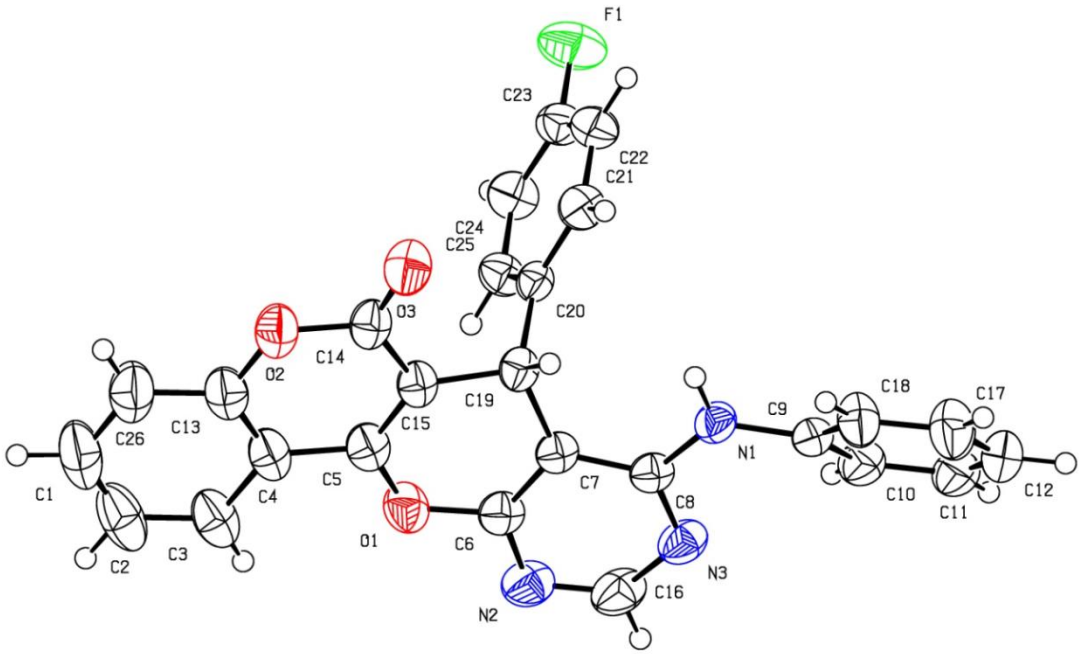


Figure 3. ORTEP representation of compound **4i** (CCDC 1440717). The thermal ellipsoids are drawn at 50% probability level.

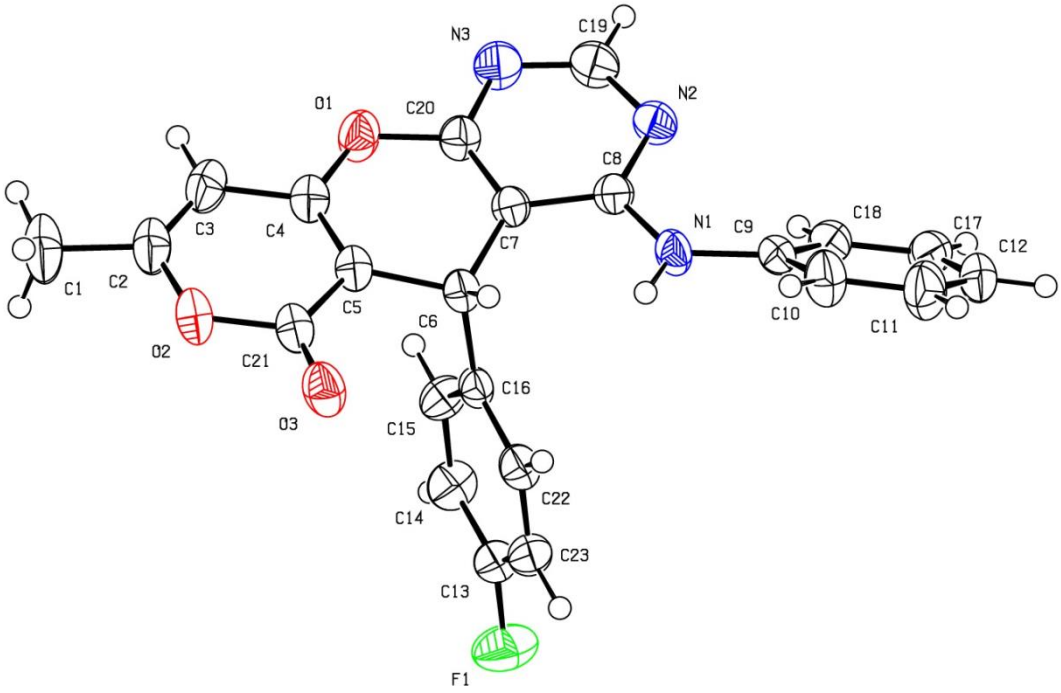


Figure 4. ORTEP representation of compound **6e** (CCDC 1401827). The thermal ellipsoids are drawn at 50% probability level.

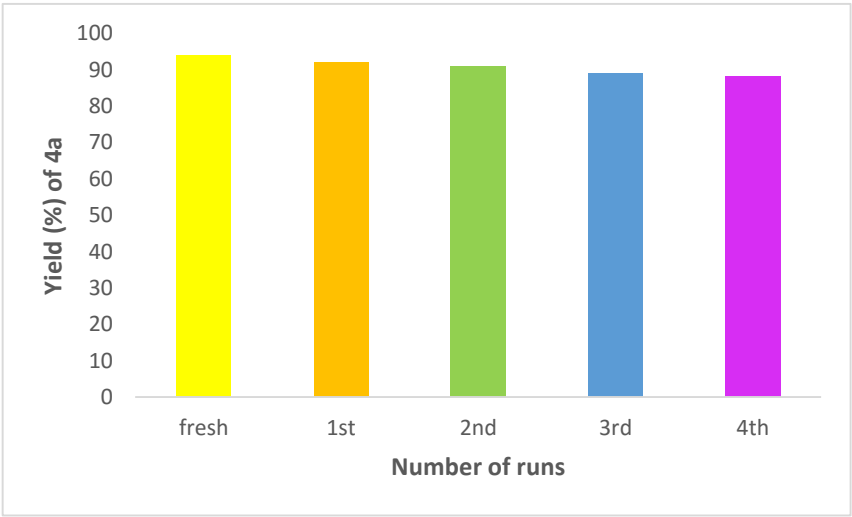


Figure 5. Recycling of the [Bmim]HSO_4 in the synthesis of compounds **4a**

2.4. EXPERIMENTAL

Melting points were recorded on a Stuart SMP30 melting point apparatus and were uncorrected. Column chromatography was performed using silica gel (60–120 mesh size) purchased from Thomas Baker and Thin layer chromatography (TLC) was carried out using aluminium sheets pre-coated with silica gel $60F_{254}$ purchased from Merck. IR spectra (KBr) were taken on Bruker WM-4 (X) spectrometer (577 model). ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker WM-400 spectrometer at 400 MHz and 100 MHz, respectively, in DMSO-d_6 with TMS as an internal standard. The chemical shifts were reported in ppm (δ). Mass spectra (ESI) were carried out on a Jeo1 JMSD-300 spectrometer. CHN analysis was carried out using Carlo Erba EA 1108 automatic elemental analyzer. All the chemicals and solvents were of analytical or synthetic grade and were used devoid of further purification unless otherwise stated.

2.4.1. SPECTRAL DISCUSSION

IR: In all the compounds **4a–p** and **6a–l**, the formation of pyrimidine was confirmed by the disappearance of band around 2200 cm^{-1} due to (–CN) group and appearance of –NH group around 3316-3443 cm^{-1}. The lactone (C=O) group was observed at 1711-1729 cm^{-1} and the other carbonyl (C=O) was appeared around 1664-1684 cm^{-1}.

^{1}H NMR: In ^{1}H NMR, the –NH signal was observed at δ 4.64-5.16 ppm. In all the compounds, the singlet protons of pyrimidine (CH) group were observed as singlets at δ 8.41-8.68 ppm. All other aromatic and aliphatic protons appeared at expected regions.

^{13}C NMR: In ^{13}C NMR, the signal appeared at δ 150.12-155.18 ppm can be attributed to pyrimidine carbon. The signal observed at δ 157.84-158.94 ppm was assigned to lactone (C=O) carbon in all the compounds.

Mass: The structures of all synthesized compounds were further confirmed by its mass spectra. The mass spectra detected the expected molecular ion signals (M + 1) corresponding to respective molecular weight of the synthesized compounds.

2.4.2. General procedure for the synthesis of chromeno pyrano[2,3-*d*]pyrimidine derivatives (4a-p) and (6a-l)

A dry 50 mL flask was charged with 2-Amino-3-carbonitrile dihydropyrano[3,2-*c*]chromeno/2-amino-7-methyl-5-oxo-4-phenyl-4,5-dihydropyrano[4,3-*b*]pyran-3 carbonitriles **1** (1 mmol), DMF-DMA **2** (1.2 mmol) and ionic liquid [Bmim]HSO_4 (2 mL). The reaction mixture was stirred at 70-80 °C for 30 min for 4h. The progress of the reaction was monitored by TLC and after completion of the reaction (single spot on TLC), aromatic amine **3** (1 mmol) was added and the reaction was continued for an additional 60–150 min. The progress of the reaction was monitored by TLC (eluent = n-hexane/ethyl acetate : 8/2). After completion of the reaction, the reaction mixture was cooled to RT and poured into ice cold water, the solid separated was filtered, washed with water, dried and purified by column chromatography using silicagel (ethylacetate/n-hexane: 2/8) to afford title compounds **4a-p** and **6a-l** in good yields.

2.4.3. PHYSICAL AND SPECTRAL DATA

7-Phenyl-8-(phenylamino)chromeno[3,4,5,6]pyrano[2,3-d]pyrimidin-6-(7H)-one (**4a**). White solid: mp 262-264 °C; **IR** (KBr) υ_{max}(cm^{-1}) 3376, 2918, 1693, 1548, 1566, 1386; **^{1}H NMR** (300 MHz, DMSO-d_6) δ 5.73 (s, 1H), 7.03-7.16 (m, 1H, ArH), 7.18-7.27 (m, 1H, ArH), 7.31 (d, *J* = 7.2 Hz, 4H, ArH), 7.48-7.52 (m, 6H, ArH), 7.52-7.8.02 (m, 1H, ArH), 8.05 (s, 1H, ArH), 8.42 (s, 1H, ArH), 8.87 (s, 1H, NH); **^{13}C NMR** (75 MHz, DMSO-d_6) δ 163.58, 162.41, 161.24, 159.25, 158.74, 156.20, 144.66, 142.88, 138.53, 135.28, 133.91, 131.50, 130.26, 129.04, 127.65, 125.37, 124.74, 122.92, 121.22, 102.54, 98.68, 98.07, 32.55; **ESI-MS**: *m/z* 420 (M + 1). Anal. Calcd. For $C_{26}H_{17}N_3O_3$: C, 74.45; H, 4.09; N, 10.02; Found: C, 74.37; H, 4.05; N, 10.19.

8-[4-Chlorophenyl)amino]-7-phenylchromeno[3,4,5,6]pyrano[2,3-d]pyrimidin-6-(7H)-one (**4b**). White solid: mp 268-270 °C; **IR** (KBr) υ_{max}(cm^{-1}) 3374, 2932, 1696, 1603, 1510, 1386; **^{1}H NMR** (300 MHz, DMSO-d_6) δ 5.56 (s, 1H), 7.17-7.62 (m, 6H, ArH), 7.73 (t, *J* = 7.8 Hz, 4H, ArH), 7.89 (d, *J* = 7.8 Hz, 1H, ArH), 8.03-8.55 (m, 1H, ArH), 8.58 (s, 1H, ArH), 8.63 (s, 1H, ArH), 9.32 (s, 1H, NH); **^{13}C NMR** (75MHz, DMSO-d_6) δ 163.58, 162.41, 161.24, 159.25, 158.74, 156.20,

144.66, 142.88, 138.53, 135.28, 133.91, 131.50, 130.26, 129.04, 127.65, 125.37, 124.74, 122.92, 121.22, 102.54, 98.68, 98.07, 32.55; **ESI-MS**: *m/z* 455 (M + 1); Anal. Calcd. For $C_{26}H_{16}ClN_3O_3$: C, 68.80; H, 3.55; N, 9.26; Found: C, 68.72; H, 3.51; N, 9.37.

8-[3-Nitrophenyl)amino]-7-phenylchromeno[3,4,5,6]pyrano[2,3-d]pyrimidin-6-(7H)-one (**4c**). White powder; mp 272-274 °C; **IR** (KBr) υ_{max}(cm^{-1}) 3401, 3070, 1716, 1569, 1527, 134; **^{1}H NMR** (300 MHz, DMSO-d_6) δ 5.55 (s, 1H), 7.25-7.35 (m, 7H, ArH), 7.47 (t, *J* = 7.8 Hz, 3H, ArH), 7.72-8.22 (m, 2H, ArH), 8.25 (s, 1H, ArH), 8.57 (s, 1H, ArH), 9.25 (s, 1H, NH); **^{13}C NMR** (75 MHz, DMSO-d_6) δ 164.33, 162.49, 161.95, 159.28, 158.00, 156.12, 144.09, 142.47, 138.78, 135.66, 134.38, 132.23, 131.16, 129.63, 127.26, 126.95, 124.61, 122.75, 120.93, 102.80, 99.36, 98.86, 32.89; **ESI-MS**: *m/z* 465 (M + 1); Anal. Calcd. For $C_{26}H_{16}N_4O_5$: C, 67.24; H, 3.47; N, 12.06; Found: C, 67.16; H, 3.43; N, 12.23.

8-[4-Nitrophenyl)amino]-7-phenylchromeno[3,4,5,6]pyrano[2,3-d]pyrimidin-6-(7H)-one (**4d**). White powder; mp: 256-259 °C; **IR** (KBr) υ_{max} (cm^{-1}): 3374, 3081, 1696, 1570, 1327; **^{1}H NMR** (400 MHz, DMSO-d_6): δ 5.57 (s, 1H), 6.83 (d, *J* = 7.8 Hz, 3H), 6.88 (d, *J* = 7.8 Hz, 3H), 7.40-7.43 (m, 1H), 7.47-7.53 (m, 2H), 7.70-7.75 (m, 2H), 8.00-8.02 (m, 2H), 8.34 (s, 1H), 8.70 (s, 1H); **^{13}C NMR** (100 MHz, DMSO-d_6): δ 165.12, 161.34, 160.39, 159.36, 158.22, 155.36, 142.60, 141.30, 137.49, 135.21, 134.18, 131.14, 130.11, 129.13, 127.19, 126.25, 125.78, 121.90, 120.12, 103.19, 98.28, 97.78, 39.19; **ESI-MS**: *m/z* 465 (M + 1); Anal. Calcd. For $C_{26}H_{16}N_4O_5$: C, 67.24; H, 3.47; N, 12.06; Found: C, 67.18; H, 3.40; N, 12.26.

8-[Phenylamino)-7-(p-tolyl]chromeno[3,4,5,6]pyrano[2,3-d]pyrimidin-6-(7H)-one (**4e**). White powder; mp 280-282 °C; **IR** (KBr) υ_{max}(cm^{-1}) 3359, 3023, 1696, 1649, 1565, 1385; **^{1}H NMR** (300 MHz, DMSO-d_6) δ 2.18 (s, 3H, CH_3), 5.68 (s, 1H), 7.06 (t, *J* = 8.7 Hz, 3H, ArH), 7.30 (t, *J* = 8.7 Hz, 2H, ArH), 7.38 (d, *J* = 8.7 Hz, 2H, ArH), 7.50 (t, *J* = 8.7 Hz, 2H, ArH), 7.54 (d, *J* = 8.7 Hz, 2H, ArH), 7.58-8.01 (m, 1H, ArH), 8.03 (s, 1H, ArH), 8.42 (s, 1H, ArH), 8.83 (s, 1H, NH); **^{13}C NMR** (75 MHz, DMSO-d_6) δ 165.07, 163.97, 162.94, 160.09, 157.31, 155.92, 141.18, 136.85, 134.48, 131.32, 130.21, 129.18, 127.60, 126.82, 125.04, 123.72, 121.41, 103.07, 99.75, 98.71, 31.30, 19.65; **ESI-MS**: *m/z* 434 (M + 1); Anal.

Calcd. For $C_{27}H_{19}N_3O_3$: C, 74.81; H, 4.42; N, 9.69; Found: C, 74.72; H, 4.58; N, 9.52.

8-[4-Chlorophenyl)amino)-7-(p-tolyl]chromeno[3,4,5,6]pyrano[2,3-d]pyrimidin-6-(7H)-one (**4f**). White powder; mp 286-288 °C; **IR** (KBr) υ_{max}(cm^{-1}) 3395, 2923, 1728, 1564, 1511, 1378; **^{1}H NMR** (300 MHz, DMSO-d_6) δ 2.18 (s, 3H, CH_3), 5.68 (s, 1H), 7.05-7.17 (m, 2H, ArH), 7.35 (t, *J* = 8.4 Hz, 1H, ArH), 7.47-7.54 (m, 3H, ArH), 7.66 (d, *J* = 8.4 Hz, 3H, ArH), 7.71-8.01 (m, 2H, ArH), 8.03 (s, 1H, ArH), 8.44 (s, 1H, ArH), 8.95 (s, 1H, NH); **^{13}C NMR** (75 MHz, DMSO-d_6) δ 164.33, 162.22, 161.21, 159.63, 158.59, 156.50, 147.22, 142.04, 140.72, 136.60, 134.71, 133.23, 130.58, 129.63, 128.55, 127.29, 125.18, 123.14, 107.05, 105.26, 103.20, 99.25, 98.00, 31.43, 19.25; **ESI-MS**: *m/z* 469 (M + 1); Anal. Calcd. For $C_{27}H_{18}ClN_3O_3$: C, 69.31; H, 3.88; N, 8.98; Found: C, 69.19; H, 3.84; N, 8.72.

8-[3-Nitrophenyl)amino]-7-(p-tolyl)chromeno[3,4,5,6]pyrano[2,3-d]pyrimidin-6-(7H)-one (**4g**). White powder; mp 296-298 °C; **IR** (KBr) υ_{max}(cm^{-1}) 3375, 2923, 1721, 1634, 1529, 1350; **^{1}H NMR** (300 MHz, DMSO-d_6) δ 2.17 (s, 3H, CH_3), 5.75 (s, 1H, CH), 7.05-7.15 (m, 3H, ArH), 7.36-7.47 (m, 1H, ArH), 7.49 (d, *J* = 8.4 Hz, 2H, ArH), 7.52 (s, 1H, ArH), 7.54 (d, *J* = 8.4 Hz, 1H, ArH), 7.62-7.74 (m, 1H, ArH), 7.88-8.04 (m, 1H, ArH), 8.13 (t, *J* = 8.4 Hz, 1H, ArH), 8.57 (s, 1H, ArH), 8.65 (s, 1H, ArH), 9.32 (s, 1H, NH); **^{13}C NMR** (75 MHz, DMSO-d_6) δ 165.07, 163.97, 162.94, 160.09, 157.31, 155.92, 141.18, 136.85, 134.48, 131.32, 130.21, 129.18, 127.60, 126.82, 125.04, 123.72, 121.41, 103.07, 99.75, 98.71, 31.30, 19.65; **ESI-MS**: *m/z* 480 (M + 1); Anal. Calcd. For $C_{27}H_{18}N_4O_5$: C, 67.78; H, 3.79; N, 11.71; Found: C, 67.86; H, 3.75; N, 11.56.

8-[4-Nitrophenyl)amino]-7-(p-tolyl)chromeno[3,4,5,6]pyrano[2,3-d]pyrimidin-6-(7H)-one (**4h**). White powder; mp 287-289 °C; **IR** (KBr) υ_{max}(cm^{-1}) 3327, 2906, 1774, 1634, 1578, 1310; **^{1}H NMR** (300 MHz, DMSO-d_6) δ 1.19 (s, 3H, CH_3), 5.53 (s, 1H, CH), 7.41 (d, *J* = 7.8 Hz, 3H, ArH), 7.47-7.57 (m, 2H, ArH), 7.60 (d, *J* = 7.8 Hz, 2H, ArH), 7.73 (t, *J* = 7.8 Hz, 1H, ArH), 7.90 (t, *J* = 7.8 Hz, 1H, ArH), 8.01-8.54 (m, 3H), 8.65 (s, 1H, ArH), 9.29 (s, 1H, ArH); **^{13}C NMR** (75 MHz, DMSO-d_6) δ 167.08, 164.57, 161.79, 160.19, 156.31, 154.34, 141.12, 137.34, 135.45, 132.45, 131.67, 129.34, 127.90, 125.98, 125.16, 123.56, 123.40, 104.09,

99.30, 98.80, 31.40; **ESI-MS**: *m/z* 480 (M + 1); Anal. Calcd. For $C_{27}H_{18}N_4O_5$: C, 67.78; H, 3.79; N, 11.71; Found: C, 67.80; H, 3.73; N, 11.54.

7-[4-Flurophenyl)-8-(phenylamino]chromeno[3,4,5,6]pyrano[2,3-d]pyrimidin-6-(7H)-one (**4i**). White powder; mp 282-286 °C; **IR** (KBr) υ_{max}(cm^{-1}) 3366, 3067, 1694, 1649, 1566, 1387; **^{1}H NMR** (300 MHz, DMSO-d_6) δ 5.74 (s, 1H, CH), 7.04-7.13 (m, 3H, ArH), 7.30 (t, *J* = 7.8 Hz, 2H, ArH), 7.48-7.57 (m, 6H, ArH), 7.72-8.01 (m, 1H, ArH), 8.04 (s, 1H, ArH), 8.42 (s, 1H, ArH), 8.87 (s, 1H, NH); **^{13}C NMR** (75 MHz, DMSO-d_6) δ 163.97, 162.62, 161.92, 160.09, 158.74, 156.63, 142.94, 137.65, 136.93, 135.58, 133.11, 131.71, 129.57, 128.10, 127.50, 126.06, 123.21, 121.13, 102.49, 99.36, 98.28, 32.99; **ESI-MS**: *m/z* 438 (M + 1); Anal. Calcd. For $C_{26}H_{16}FN_3O_3$: C, 71.39; H, 3.69; N, 9.61; Found: C, 71.49; H, 3.64; N, 9.82.

8-[4-Chlorophenyl)amino)-7-(-4-fluorophenyl]chromeno[3,4,5,6]pyrano[2,3-d]pyrimidin-6-(7H)-one (**4j**). White powder; mp 276-278 °C; **IR** (KBr) υ_{max}(cm^{-1}) 3364, 2922, 1701, 1649, 1564, 1387; **^{1}H NMR** (300 MHz, DMSO-d_6) δ 5.74 (s, 1H, CH), 7.10 (t, *J* = 8.7 Hz, 2H, ArH), 7.13-7.50 (m, 2H, ArH), 7.52-7.56 (m, 4H, ArH), 7.63 (d, *J* = 8.7 Hz, 2H, ArH), 7.72-8.01 (m, 1H, ArH), 8.03 (s, 1H, ArH), 8.46 (s, 1H, ArH), 8.98 (s, 1H, NH); **^{13}C NMR** (75 MHz, DMSO-d_6) δ 165.53, 164.00, 163.76, 161.44, 160.69, 159.63, 158.37, 155.73, 141.57, 139.52, 138.14, 135.62, 133.84, 131.50, 130.73, 128.10, 123.72, 121.41, 116.48, 115.72, 102.31, 98.93, 97.39, 32.13; **ESI-MS**: *m/z* 473 (M + 1); Anal. Calcd. For $C_{26}H_{15}ClFN_3O_3$: C, 66.18; H, 3.20; N, 8.91; Found: C, 66.36; H, 3.15; N, 8.74.

7-[-4-Fluorophenyl)-8-((3-nitrophenyl)amino]chromeno[3,4,5,6]pyrano[2,3-d]pyrimidin-6-(7H)-one (**4k**). White powder; mp 302-304 °C; **IR** (KBr) υ_{max}(cm^{-1}) 3319, 3057, 1720, 1657, 1527, 1382; **^{1}H NMR** (300 MHz, DMSO-d_6) δ 5.80 (s, 1H, CH), 7.10 (t, *J* = 8.7 Hz, 2H, ArH), 7.49-7.60 (m, 5H, ArH), 7.62 (s, 1H, ArH), 7.76 (t, *J* = 8.7 Hz, 1H, ArH), 7.88-7.8.10 (m, 2H, ArH), 8.56 (s, 1H, ArH), 8.62 (s, 1H, ArH), 9.34 (s, 1H,NH); **^{13}C NMR** (75 MHz, DMSO-d_6) δ 164.55, 163.79, 162.72, 161.65, 159.28, 158.80, 156.69, 155.09, 141.92, 139.88, 138.58, 135.91, 133.84, 131.71, 130.93, 127.26, 123.32, 121.45, 117.80, 115.64, 102.80, 98.60,

97.54, 32.55; **ESI-MS**: *m/z* 483 (M + 1); Anal. Calcd. For $C_{26}H_{15}FN_4O_5$: C, 64.73; H, 3.13; N, 11.61; Found: C, 64.58; H, 3.18; N, 11.89.

7-[-4-Fluorophenyl)-8-((4-nitrophenyl)amino]chromeno[3,4,5,6]pyrano[2,3-d]pyrimidin-6-(7H)-one (**4l**). White powder; mp 306-308 °C; **IR** (KBr) $\upsilon_{max}(cm^{-1})$ 3312, 3068, 1724, 1623, 1512, 1334; **^{1}H NMR** (300 MHz, DMSO-d_6): δ 5.55 (s, 1H), 7.09 (t, *J* = 8.7 Hz, 3H), 7.34 (d, *J* = 8.7 Hz, 3H), 7.42-7.46 (m, 3H), 7.60 (d, *J* = 8.7 Hz, 3H), 8.39 (s, 1H), 8.86 (s, 1H); **^{13}C NMR** (75 MHz, DMSO-d_6) δ 164.12, 162.94, 162.12, 162.34, 159.18, 157.80, 156.19, 155.04, 142.97, 139.58, 138.18, 134.94, 132.84, 131.61, 130.56, 127.23, 123.37, 121.78, 117.80, 116.57, 101.59, 98.56, 96.67, 33.16; **ESI-MS**: *m/z* 483 (M + 1); Anal. Calcd. For $C_{26}H_{15}FN_4O_5$: C, 64.73; H, 3.13; N, 11.61; Found: C, 64.52; H, 3.119; N, 11.86.

7-[-4-Meyhoxyphenyl)-8-(phenylamino]chromeno[3,4,5,6]pyrano[2,3-d]pyrimidin-6-(7H)-one (**4m**). White powder; mp 220-322 °C; **IR** (KBr) $\upsilon_{max}(cm^{-1})$ 3393, 3005, 1701, 1650, 1566, 1384; **^{1}H NMR** (300 MHz, DMSO-d_6) δ 3.65 (s, 3H, CH_3), 5.66 (s, 1H, CH), 6.81-7.08 (m, 2H, ArH), 7.16-7.26 (m, 1H, ArH), 7.28-7.39 (m, 3H, ArH), 7.42-7.52 (m, 3H, ArH), 7.57 (t, *J* = 8.7 Hz, 2H, ArH), 7.71-8.04 (m, 1H, ArH), 8.04 (s, 1H, ArH), 8.41 (s, 1H, ArH), 8.82 (s, 1H, NH); **^{13}C NMR** (75 MHz, DMSO-d_6) δ 161.99, 160.91, 158.37, 157.56, 155.23, 154.48, 151.10, 133.84, 132.78, 131.71, 130.21, 128.62, 128.62, 126.82, 125.04, 124.00, 122.42, 116.48, 115.50, 114.46, 112.89, 105.68, 99.22, 98.17, 56.09, 31.30; **ESI-MS**: *m/z* 451 (M + 1); Anal. Calcd. For $C_{27}H_{19}N_3O_4$: C, 72.15; H, 4.26; N, 9.35; Found: C, 72.38; H, 4.31; N, 9.48.

8-[4-Chlorophenyl)amino)-7-(-4-meyhoxyphenyl)chromeno[3,4,5,6]pyrano[2,3-d]pyrimidin-6-(7H)-one (**4n**). White powder; mp: 266-268 °C; **IR** (KBr) $\upsilon_{max}(cm^{-1})$ 3374, 3066, 1695, 1649, 1566, 1386; **^{1}H NMR** (300 MHz, DMSO-d$_6$) δ 3.65 (s, 3H, CH_3), 5.66 (s, 1H, CH), 6.82 (d, *J* = 8.7 Hz, 2H, ArH), 7.34-7.50 (m, 4H, ArH), 7.53 (d, *J* = 8.7 Hz, 2H, ArH), 7.66 (d, *J* = 8.7 Hz, 2H, ArH), 7.71-8.01 (m, 1H, ArH), 8.03 (s, 1H, ArH), 8.44 (s, 1H, ArH), 8.93 (s, 1H, NH); **^{13}C NMR** (75 MHz, DMSO-d_6) δ 162.72, 161.44, 158.59, 157.31, 155.48, 154.25, 151.94, 134.35, 132.78, 131.71, 130.97, 129.41, 127.90, 126.31, 124.52, 124.00, 117.56, 115.72, 114.17, 112.13, 105.68, 99.75, 98.17, 56.37, 32.13; **ESI-MS**: *m/z* 485 (M +

1); Anal. Calcd. For $C_{27}H_{18}ClN_3O_4$: C, 67.02; H, 3.75; N, 8.68; Found: C, 67.19; H, 3.70; N, 8.46.

7-[-4-Meyhoxyphenyl)-8-((3-nitrophenyl)amino]chromeno[3,4,5,6]pyrano[2,3-d]pyrimidin-6-(7H)-one (**4o**). White powder; mp: 262-264 °C; **IR** (KBr) υ_{max}(cm^{-1}) 3375, 2923, 1721, 1657, 1529, 1350, 1260; **^{1}H NMR** (300 MHz, DMSO-d_6) δ 3.64 (s, 3H, CH_3), 5.73 (s, 1H, CH), 6.81 (s, 1H, ArH), 6.83-7.54 (m, 2H, ArH), 7.59 (t, *J* = 8.1 Hz, 1H, ArH), 7.74 (t, *J* = 8.1 Hz, 3H, ArH), 7.89 (d, *J* = 8.1 Hz, 1H, ArH), 8.02 (d, *J* = 8.1 Hz, 1H, ArH), 8.11 (d, *J* = 8.1 Hz, 2H, ArH), 8.54 (s, 1H, ArH), 8.65 (s, 1H,ArH), 9.30 (s, 1H, NH); **^{13}C NMR** (75 MHz, DMSO-d_6) δ 163.04, 160.15, 158.08, 157.31, 155.73, 155.00, 151.94, 134.35, 132.23, 131.50, 130.21, 128.10, 126.31, 125.27, 124.74, 122.42, 117.30, 116.01, 115.21, 112.13, 105.38, 99.98, 98.93, 56.56, 32.55; **ESI-MS**: *m/z* 496 (M + 1); Anal. Calcd. For $C_{27}H_{18}N_4O_6$: C, 65.59; H, 3.67; N, 11.33; Found: C, 65.47; H, 3.63; N, 11.50.

7-[-4-Meyhoxyphenyl)-8-((3-nitrophenyl)amino]chromeno[3,4,5,6]pyrano[2,3-d]pyrimidin-6-(7H)-one (**4p**). White powder; mp: 275-277 °C; **IR** (KBr) υ_{max}(cm^{-1}) 3363, 2997, 1705, 1637, 1504, 1364, 1245; **^{1}H NMR** (300 MHz, DMSO-d_6) δ 3.64 (s, 3H, CH_3), 5.73 (s, 1H, CH), 6.81 (s, 1H, ArH), 6.83-7.54 (m, 2H, ArH), 7.59 (t, *J* = 8.1 Hz, 1H, ArH), 7.74 (t, *J* = 8.1 Hz, 3H, ArH), 7.89 (d, *J* = 8.1 Hz, 1H, ArH), 8.02 (d, *J* = 8.1 Hz, 1H, ArH), 8.11 (d, *J* = 8.1 Hz, 2H, ArH), 8.54 (s, 1H, ArH), 8.65 (s, 1H,ArH), 9.30 (s, 1H, NH); **^{13}C NMR** (75 MHz, DMSO-d_6) δ 163.04, 160.15, 158.08, 157.31, 155.73, 155.00, 151.94, 134.35, 132.23, 131.50, 130.21, 128.10, 126.31, 125.27, 124.74, 122.42, 117.30, 116.01, 115.21, 112.13, 105.38, 99.98, 98.93, 56.56, 32.55; **ESI-MS**: *m/z* 496 (M + 1); Anal. Calcd. For $C_{27}H_{18}N_4O_6$: C, 65.59; H, 3.67; N, 11.33; Found: C, 65.47; H, 3.63; N, 11.50.

8-Methyl-5-phenyl-4-(phenylamino)pyrano[3,4,5,6]pyrano[2,3-d]pyrimidin-6(5H)-one (**6a***). White powder; mp: 262-264 °C; **IR** (KBr) υ_{max} (cm^{-1}): 3352, 3086, 1694, 1597, 1571, 1398, 1260; **^{1}H NMR** (400 MHz, DMSO-d_6): δ 2.24 (s, 3H), 5.55 (s, 1H), 6.52 (s, 1H), 7.03 (t, 1H), 7.17 (t, 1H), 7.25-7.29 (m, 4H), 7.41 (d, 2H), 7.54 (d, 2H), 8.35 (s, 1H), 8.74 (s, 1H)); **^{13}C NMR** (100 MHz, DMSO-d_6): δ 19.36, 32.27, 98.63, 102.23, 121.65, 123.48, 127.22, 128.21, 138.98, 142.08,

156.32, 159.29, 160.92, 161.70, 162.87 ; **ESI-MS**: *m/z* 384 $(M + 1)^+$; Anal. Calcd. For $C_{23}H_{17}N_3O_3$: C, 72.05; H, 4.47; N, 10.96; Found: C, 72.11; H, 4.42; N, 10.91.

4-((4-Chlorophenyl)amino-8-methyl-5-phenylpyrano[3,4,5,6]pyrano[2,3-d]pyrimidin-6(5H)-one (**6b**). Yellow powder; mp: 272-275 °C; **IR** (KBr) υ_{max} (cm^{-1}): 3372, 3086, 1699, 1606, 1567, 1439, 1262; **^{1}H NMR** (400 MHz, DMSO-d_6): δ 2.24 (s, 3H), 5.54 (s, 1H), 6.53 (s, 1H), 7.17 (t, 1H), 7.26 (t, 2H), 7.33 (d, 2H), 7.40 (t, 2H), 7.61 (d, 2H), 8.38 (s, 1H), 8.86 (s, 1H); **^{13}C NMR** (100 MHz, DMSO-d_6): δ 19.38, 21.05, 32.23, 98.38, 98.61, 102.10, 122.97, 125.59, 127.07, 128.11, 128.07, 128.14, 128.32, 128.45, 128.75, 138.02, 142.01, 156.31, 159.25, 161.32, 162.95; **ESI-MS:** *m/z* 418 $(M + 1)^+$; Anal. Calcd. For $C_{23}H_{16}ClN_3O_3$: C, 66.11; H, 3.86; N, 10.06; Found: C, 66.06; H, 3.89; N, 10.13.

8-Methyl-4-((3-nitrophenyl)amino)-5-phenylpyrano[3,4,5,6]pyrano[2,3-d]pyrimidin-6(5H)-one (**6c**). White powder; mp: 268-270 °C; **IR** (KBr) υ_{max} (cm^{-1}): 3394, 3005, 1702, 1650, 1565, 1384, 1248; **^{1}H NMR** (400 MHz, DMSO-d_6): δ 2.25 (s, 3H), 5.62 (s, 1H), 6.55 (s, 1H), 7.16 (t, 1H), 7.26 (t, 2H), 7.41 (d, 2H), 7.57 (t, 1H), 7.88 (t, 1H), 8.06 (d, 1H), 8.48 (s, 1H), 8.60 (s, 1H), 9.24 (s, 1H)); **^{13}C NMR** (100 MHz, DMSO-d_6): δ 19.39, 32.22, 98.61, 100.09, 102.24, 115.00, 117.58, 127.12, 127.31, 128.26, 129.80, 140.38, 141.39, 147.84, 156.33, 158.51, 159.36, 161.29, 161.73, 163.02; **ESI-MS**: *m/z* 430 $(M + 1)^+$; Anal. Calcd. For $C_{23}H_{16}N_4O_5$: C, 64.48; H, 3.76; N, 13.08; Found: C, 64.40; H, 3.71; N, 13.02.

8-Methyl-4-((4-nitrophenyl)amino)-5-phenylpyrano[3,4,5,6]pyrano[2,3-d]pyrimidin-6(5H)-one (**6d**). White powder; mp: 273-275 °C; **IR** (KBr) υ_{max} (cm^{-1}): 3374, 3081, 1696, 1608, 1570, 1438, 1247; **^{1}H NMR** (400 MHz, DMSO-d_6): δ 2.25 (s, 3H), 5.68 (s, 1H), 6.55 (s, 1H), 7.13-7.18 (m, 1H), 7.25 (t, 2H), 7.39 (d, 2H), 7.91 (d, 2H), 8.17 (d, 2H), 8.53 (s, 1H), 9.39 (s, 1H)); **^{13}C NMR** (100 MHz, DMSO-d_6): δ 20.92, 36.46, 100.91, 101.94, 112.24, 119.24, 124.73, 125.67, 129.80, 137.90, 142.34, 147.02, 153.26, 156.45, 159.68, 162.62, 164.27, 165.34, 174.64; **ESI-MS**: *m/z* 429 $(M + 1)^+$; Anal. Calcd. For $C_{23}H_{16}N_4O_5$: C, 64.48; H, 3.76; N, 13.08; Found: C, 64.39; H, 3.71; N, 13.01.

5-(4-Fluorophenyl)-8-methyl-4-(phenylamino)pyrano[3,4,5,6]pyrano[2,3-d]pyrimidin-6(5H)-one (**6e**). White powder; mp: 285-287 °C; **IR** (KBr) υ_{max} (cm^{-}

^{1}): 3370, 3065, 1691, 1599, 1498, 1397, 1260; **^{1}H NMR** (400 MHz, DMSO-d_6): δ 2.24 (s, 3H), 5.55 (s, 1H), 6.52 (s, 1H), 7.04-7.12 (m, 3H), 7.28 (t, 2H), 7.43-7.47 (m, 2H), 7.52 (d, 2H), 8.36 (s, 1H), 8.74 (s, 1H); **^{13}C NMR** (100 MHz, DMSO-d_6): δ 19.41, 31.66, 98.69, 98.81, 102.09, 115.09, 155.30, 121.83, 123.65, 128.48, 130.13, 130.21, 138.21, 138.96, 156.47, 158.90, 159.19, 159.99, 160.94, 161.76, 162.41, 163.03; **ESI-MS**: *m/z* 402 $(M + 1)^+$; Anal. Calcd. For $C_{23}H_{16}FN_3O_3$: C, 68.82; H, 4.02; N, 10.47; Found: C, 68.75; H, 4.09; N, 10.42.

4-((4-Chlorophenyl)amino)-5-(4-fluorophenyl)-8-methylpyrano[3,4,5,6]pyrano [2,3-d]pyrimidin-6(5H)-one (**6f**). Light yellow powder; mp: 280-282 °C; **IR** (KBr) υ_{max} (cm^{-1}): 3352, 3083, 1694, 1667, 1505, 1490, 1229; **^{1}H NMR** (400 MHz, DMSO-d_6): δ 2.24 (s, 3H), 5.55 (s, 1H), 6.52 (s, 1H), 7.09 (t, 2H), 7.34 (d, 2H), 7.42-7.46 (m, 2H), 7.60 (d, 2H), 8.39 (s, 1H), 8.86 (s, 1H); **^{13}C NMR** (100 MHz, DMSO-d_6): δ 19.38, 31.57, 98.62, 99.17, 102.04, 115.18, 123.09, 123.65, 125.59, 127.18, 128.15, 128.33, 130.05, 130.13, 138.05, 156.41, 159.34, 161.00, 162.37, 163.03; **ESI-MS**: *m/z* 436 $(M + 1)^+$; Anal. Calcd. For $C_{23}H_{15}ClFN_3O_3$: C, 63.38; H, 3.47; N, 9.64; Found: C, 63.29; H, 3.41; N, 9.69.

5-(4-Fluorophenyl)-8-methyl-4-((3-nitrorophenyl)amine)pyrano [3,4,5,6]pyrano [2,3-d]pyrimidin-6(5H)-one (**6g**)**.** Yellow powder; mp: 281-283 °C; **IR** (KBr) υ_{max} (cm^{-1}): 3325, 3012, 1637, 1609, 1518, 1424, 1238; **^{1}H NMR** (400 MHz, DMSO-d_6): δ 2.05 (s, 3H), 5.62 (s, 1H), 6.62 (s, 1H), 7.02 (t, 2H), 7.56-7.34 (m, 2H), 7.68 (t, 2H), 7.92 (d, 1H), 8.04 (d, 1H), 8.56 (s, 1H), 8.43 (s, 1H); **^{13}C NMR** (100 MHz, DMSO-d_6): δ 20.91, 36.06, 98.60, 102.92, 109.23, 112.20, 113.92, 115.46, 123.63, 130.42, 130.62, 143.30, 148.73, 153.26, 158.24, 159.93, 162.23, 162.65, 163.28, 174.48; **ESI-MS**: *m/z* 447 $(M + 1)^+$; Anal. Calcd. For $C_{23}H_{15}FN_4O_5$; C, 61.88; H, 3.39; N, 12.55; Found: C, 61.79; H, 3.34; N, 12.48

5-(4-Fluorophenyl)-8-methyl-4-((4-nitrorophenyl)amine)pyrano[3,4,5,6]pyrano [2,3-d]pyrimidin-6(5H)-one (**6h**). Yellow powder; mp: 289-291 °C; **IR** (KBr) υ_{max} (cm^{-1}): 3334, 3092, 1691, 1601, 1509, 1438,1257; **^{1}H NMR** (400 MHz, DMSO-d_6): δ 2.25 (s, 3H), 5.63 (s, 1H), 6.55 (s, 1H), 7.09 (t, 2H), 7.47-7.43 (m, 2H), 7.57 (t, 1H), 7.88 (d, 1H), 8.05 (d, 1H), 8.49 (s, 1H), 8.59 (s, 1H), 9.23 (s, 1H); **^{13}C**

NMR (100 MHz, DMSO-d_6): δ 20.92, 36.45, 100.92, 101.94, 115.42, 124.73, 130.48, 137.98, 147.08, 156.4, 158.92, 159.93, 162.69, 163.23; **ESI-MS**: *m/z* 447 $(M + 1)^+$; Anal. Calcd. For $C_{23}H_{15}FN_4O_5$; C, 61.88; H, 3.39; N, 12.55; Found: C, 61.79; H, 3.34; N, 12.48.

5-(4-Methoxyphenyl)-8-methyl-4-(phenylamino)pyrano[3,4,5,6]pyrano[2,3-d]pyrimidin -6(5H)-one (**6i**). White powder; mp: 263-265 °C; **IR** (KBr) υ_{max} (cm^{-1}): 3394, 3005, 1702, 1650, 1565, 1446, 1248; **^{1}H NMR** (400 MHz, DMSO-d_6): δ 2.22 (s, 3H), 3.65 (s, 3H), 5.66 (s, 1H), 6.82 (d, 2H), 7.05 (t, 1H), 7.30 (t, 2H), 7.41 (d, 1H), 7.54-7.47 (d, 1H), 7.59 (d, 1H), 7.76-7.71 (m, 1H), 8.04 (m, 1H), 8.41 (s, 1H), 8.80 (s, 1H); **^{13}C NMR** (100 MHz, DMSO-d_6): δ 20.98, 36.87, 55.8, 100.92, 101.24, 112.26, 114.21, 117.86, 122.40, 129.52, 130.02, 134.67, 140.43, 153.29, 157.64, 158.32, 162.48, 164.69, 167.06, 173.38; **ESI-MS**: *m/z* 414 $(M + 1)^+$; Anal. Calcd. For $C_{24}H_{19}N_3O_4$; C, 69.72; H, 4.63; N, 10.16; Found: C, 69.64; H, 4.67; N, 10.09.

4-((4-Chlorophenyl)amino)-5-(4-methoxyphenyl)-8-methylpyrano[3,4,5,6]pyrano[2,3-d]pyrimidin-6(5H)-one (**6j**)**.** Yellow powder; mp: 267-269 °C; **IR** (KBr) υ_{max} (cm^{-1}): 3369, 2931, 1696, 1649, 1566, 1490, 1255; **^{1}H NMR** (400 MHz, DMSO-d_6): δ 1.91 (s, 3H), 3.65 (s, 3H), 5.66 (s, 1H), 6.82 (d, 2H), 7.41-7.39 (m, 2H), 7.47-7.53 (m, 1H), 7.67 (d, 2H), 7.73 (t, 1H), 8.02 (d, 1H), 8.44 (s, 1H), 8.92 (s, 1H);); **^{13}C NMR** (100 MHz, DMSO-d_6): δ 20.14, 36.50, 55.89, 100.92, 101.90, 114.26, 118.45, 119.49, 122.17, 129.60, 130.03, 134.69, 139.06, 148.05, 157.65, 158.33, 159.87, 162.33, 167.09, 175.56; **ESI-MS**: *m/z* 448 $(M + 1)^+$; Anal. Calcd. For $C_{24}H_{18}ClN_3O_4$; C, 64.36; H, 4.05; N, 9.38; Found: C, 64.29; H, 4.11; N, 9.28.

5-(4-Methoxyphenyl)-8-methyl-4-((3-nitrophenyl)amino)pyrano[3,4,5,6]pyrano[2,3-d]pyrimidin-6(5H)-one (**6k**). Yellow powder; mp: 272 -274 °C; **IR** (KBr) υ_{max} (cm^{-1}): 3363, 3078, 1711, 1609, 1570, 1442, 125; **^{1}H NMR** (400 MHz, DMSO-d_6): δ 1.91 (s, 3H), 3.65 (s, 3H), 5.73 (s, 1H), 7.41 (d, 2H), 7.53-7.40 (m, 1H), 7.59 (t, 1H), 7.73 (t, 1H), 7.90 (t, 1H), 8.02 (d, 1H), 8.12 (d, 1H), 8.54 (s, 1H), 8.65 (s, 1H), 9.29 (s, 1H); **^{13}C NMR** (100 MHz, DMSO-d_6): δ 21.01, 32.09, 54.99, 100.19, 105.79, 113.32, 113.96, 115.07, 116.61, 117.63, 122.61, 124.97, 127.16, 129.40, 129.82, 132.97, 133.63, 140.40, 147.86, 152.09, 154.21, 156.28, 158.44, 158.53,

159.90, 161.04, 172.12; **ESI-MS**: *m/z* 459 $(M + 1)^+$; Anal. Calcd. For $C_{24}H_{18}N_4O_6$; C, 62.88; H, 3.96; N, 12.22; Found: C, 62.95; H, 3.90; N, 12.12.

5-(4-Methoxyphenyl)-8-methyl-4-((4 nitrophenyl)amino)pyrano[3,4,5,6]pyrano [2,3-d]pyrimidin-6(5H)-one (**6l**). Yellow powder; mp: 278 -281 °C; **IR** (KBr) υ_{max} (cm^{-1}): 3361, 3046, 1702, 1602, 1568, 1418, 1278; **^{1}H NMR** (400 MHz, DMSO-d_6): δ 2.24 (s, 3H), 3.68 (s, 3H), 5.64 (s, 1H), 7.41 (d, 2H), 7.53-7.40 (m, 1H), 7.59 (t, 1H), 7.73 (t, 1H), 7.90 (t, 1H), 8.02 (d, 1H), 8.12 (d, 1H), 8.54 (s, 1H), 8.65 (s, 1H), 9.29 (s, 1H); **^{13}C NMR** (100 MHz, DMSO-d_6): δ 20.23, 34.06, 55.46, 100.92, 101.78, 112.20, 114.20, 114.39, 119.29, 124.70, 124.72, 130.00, 134.13, 137.93, 147.85, 153.07, 157.60, 158.34, 162.34, 164.26, 167.05, 175.34; **ESI-MS**: *m/z* 459 $(M + 1)^+$; Anal. Calcd. For $C_{24}H_{18}N_4O_6$; C, 62.88; H, 3.96; N, 12.22; Found: C, 62.95; H, 3.90; N, 12.12.

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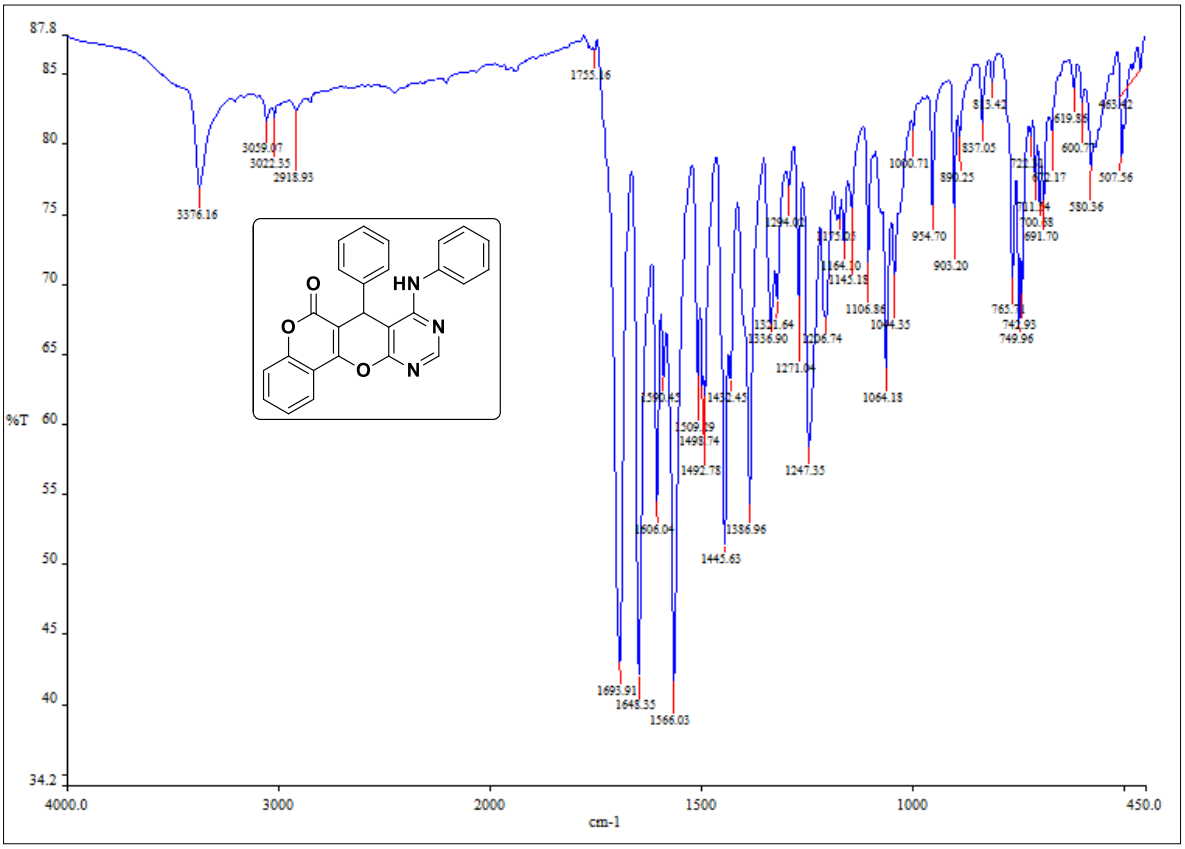
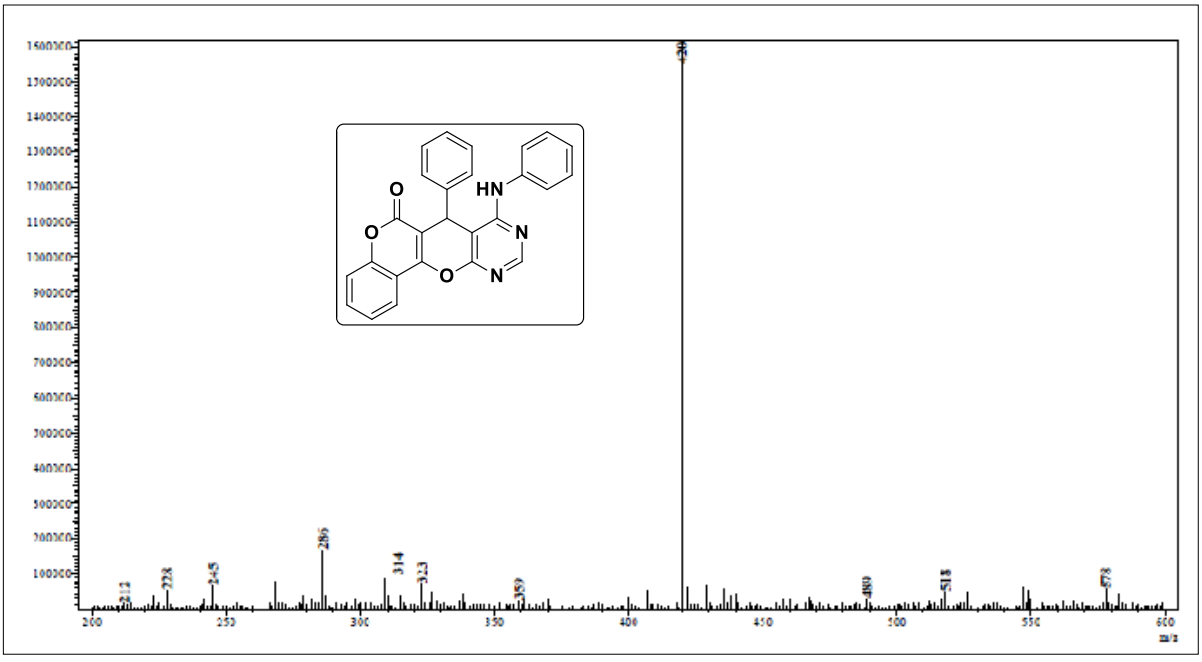
SOME SELECTED SPECTRA



IR Spectrum of compound **4a**



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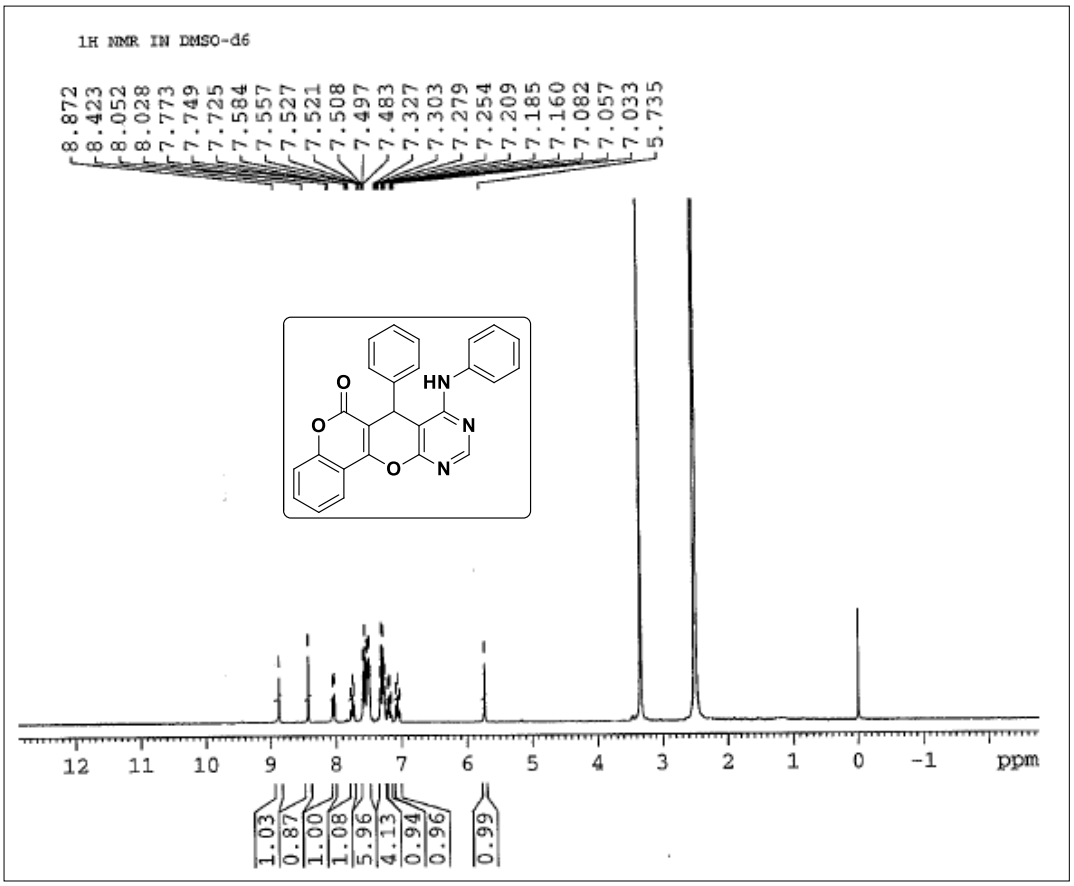

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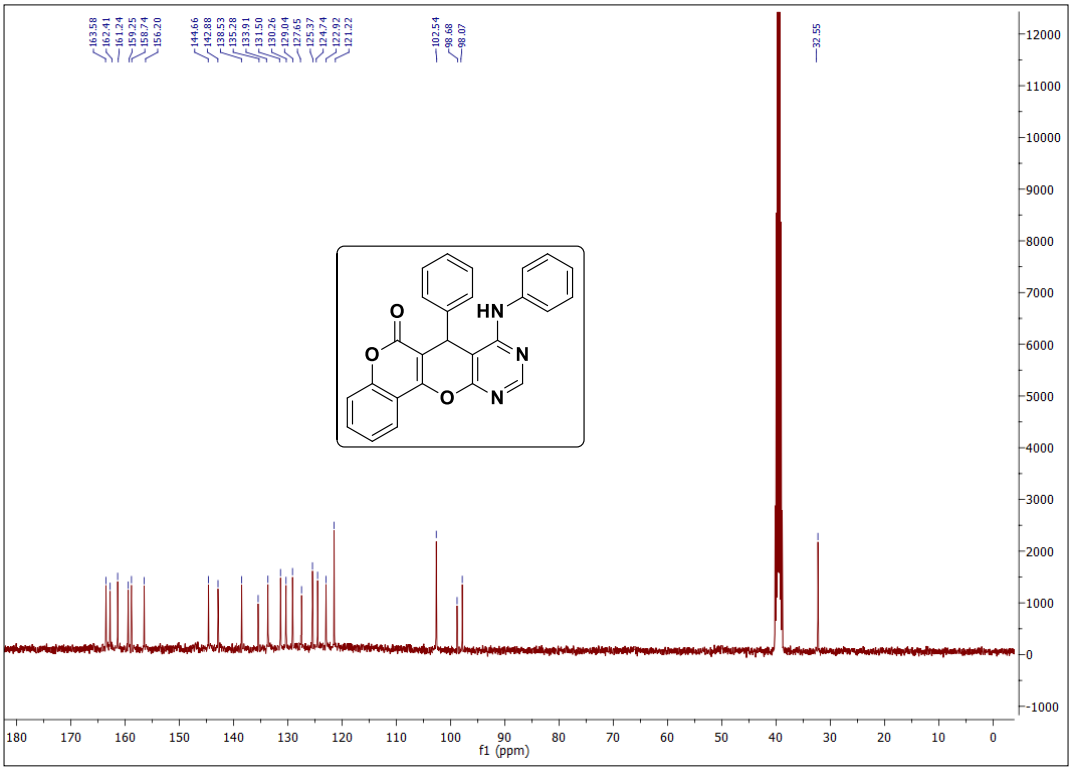

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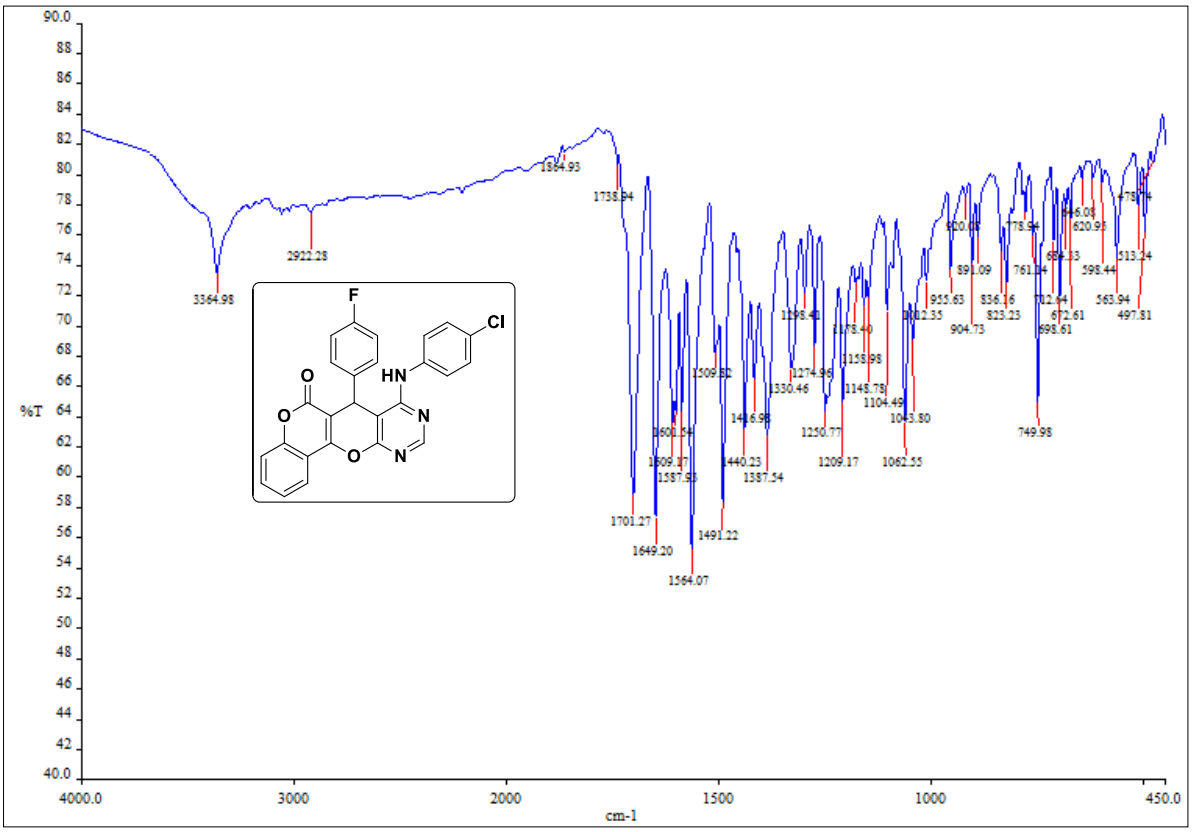

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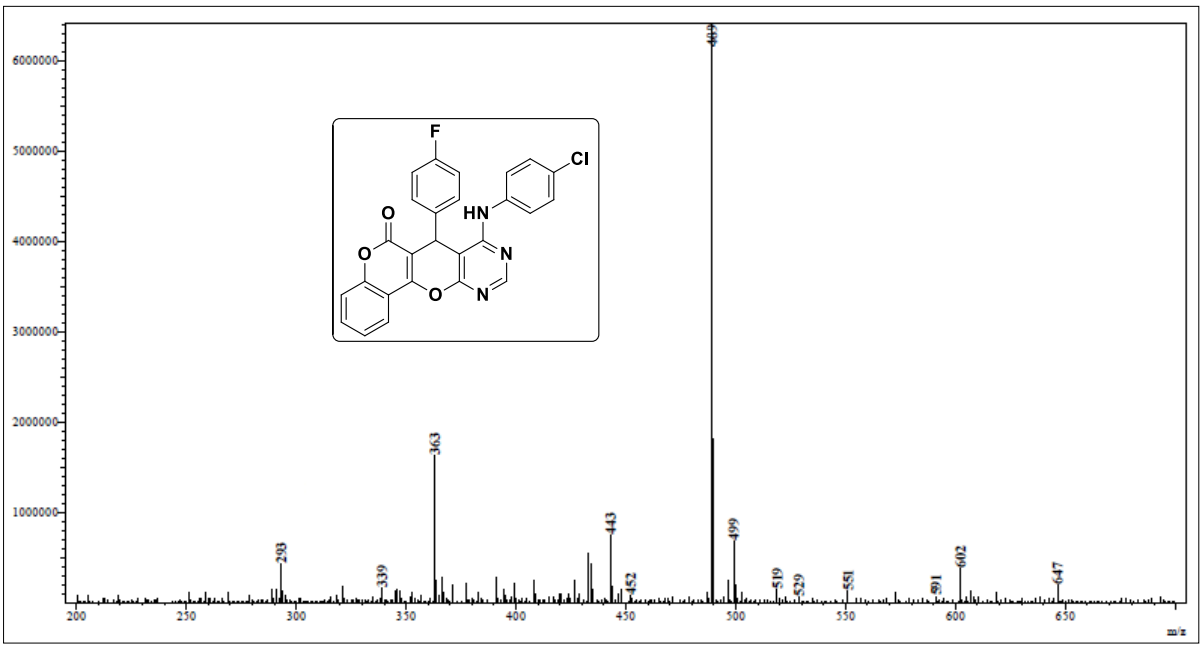

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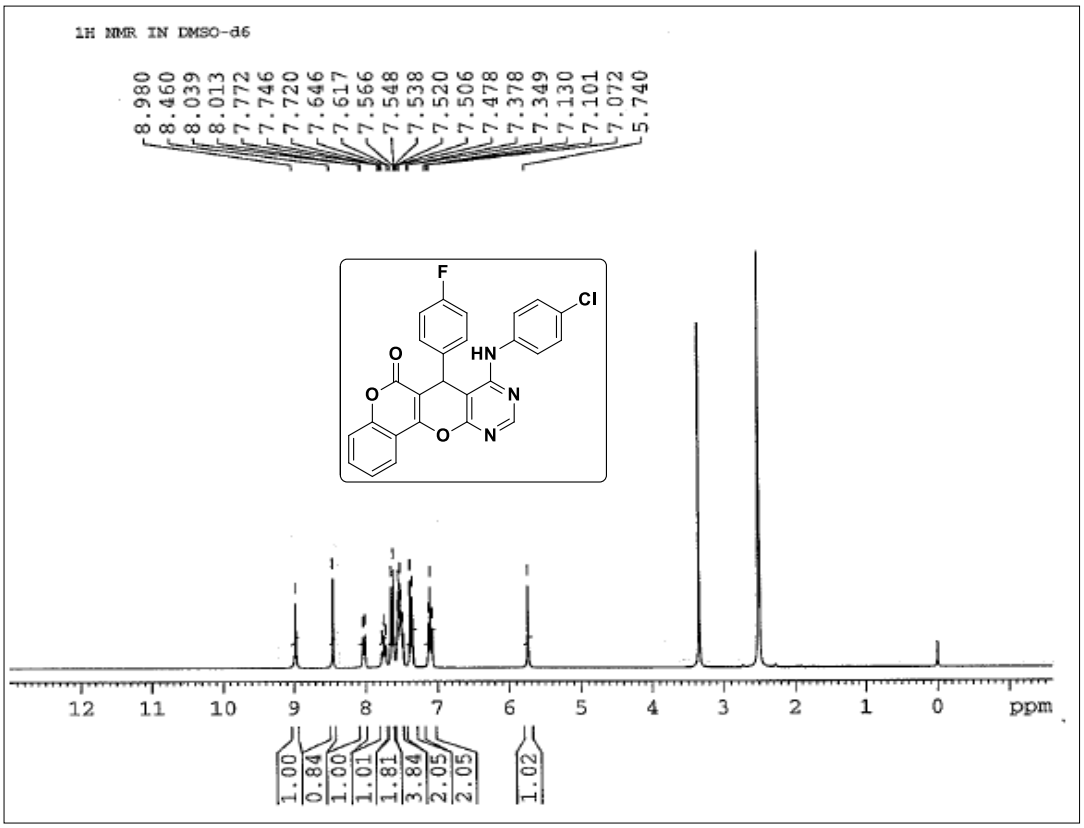

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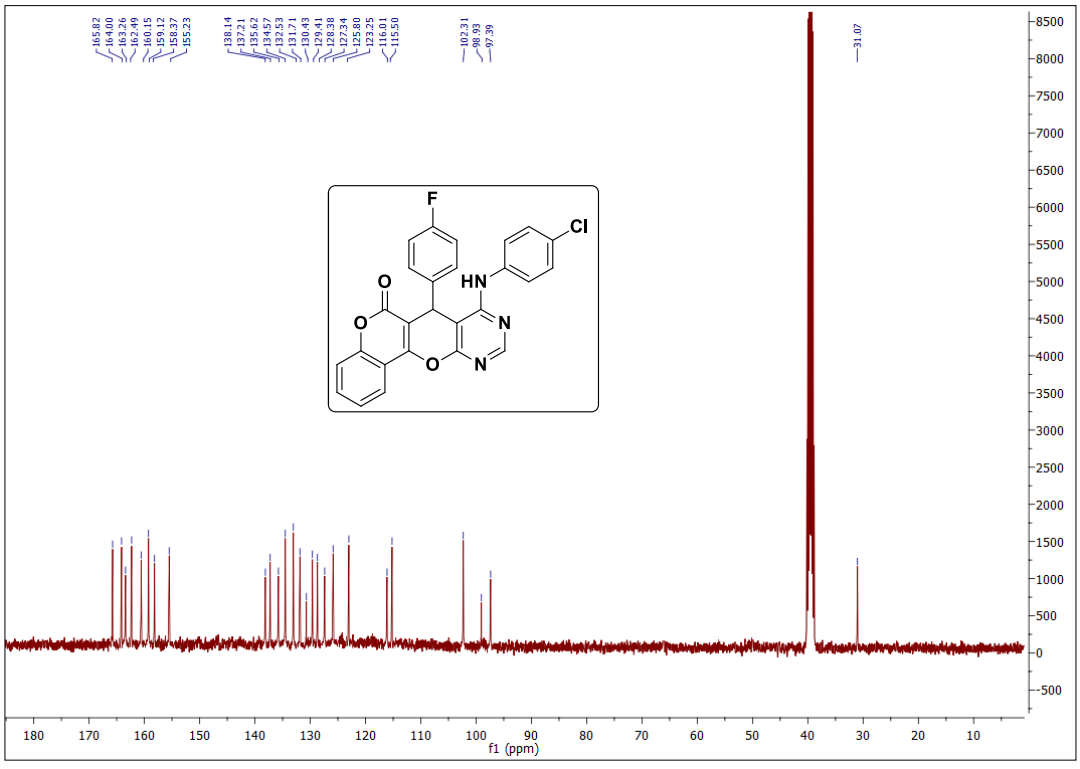

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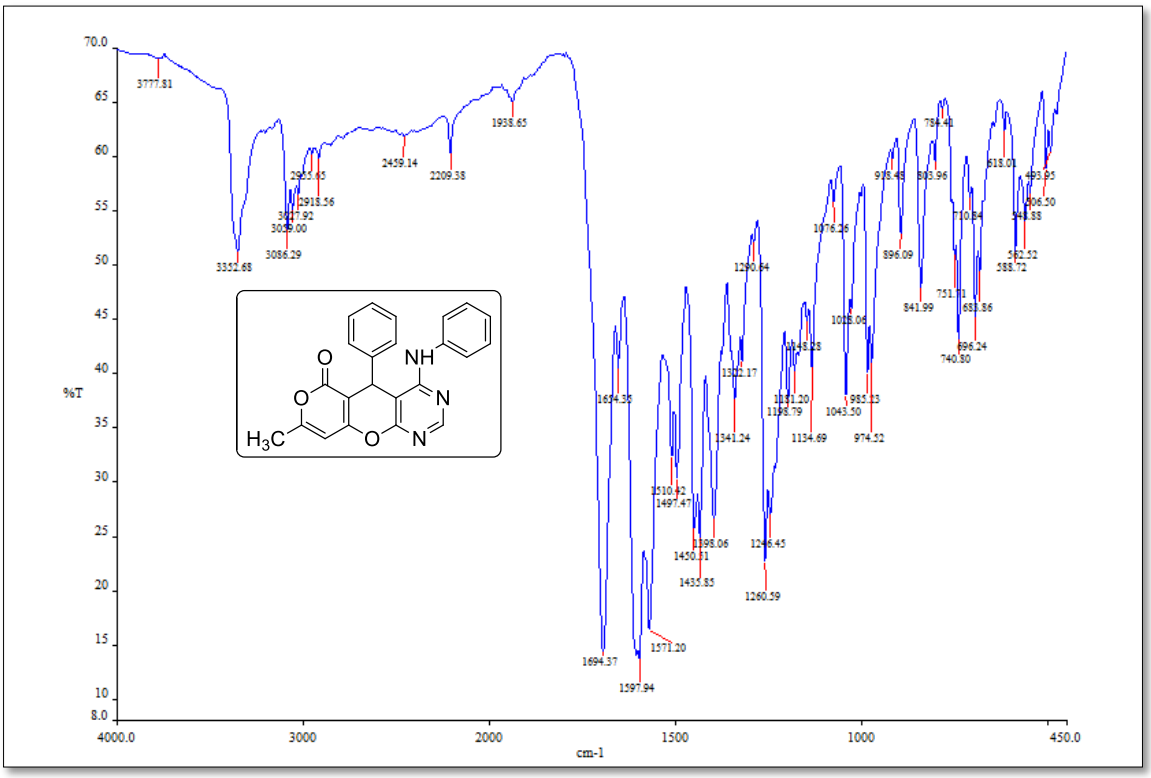

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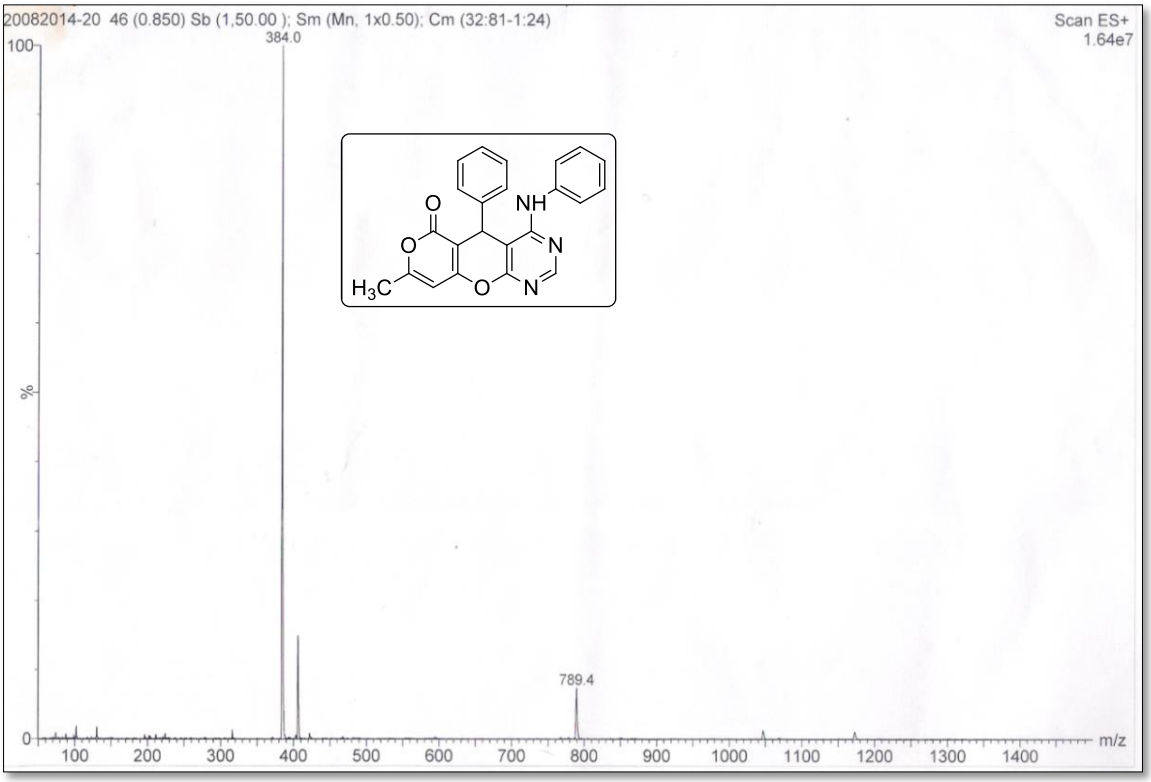

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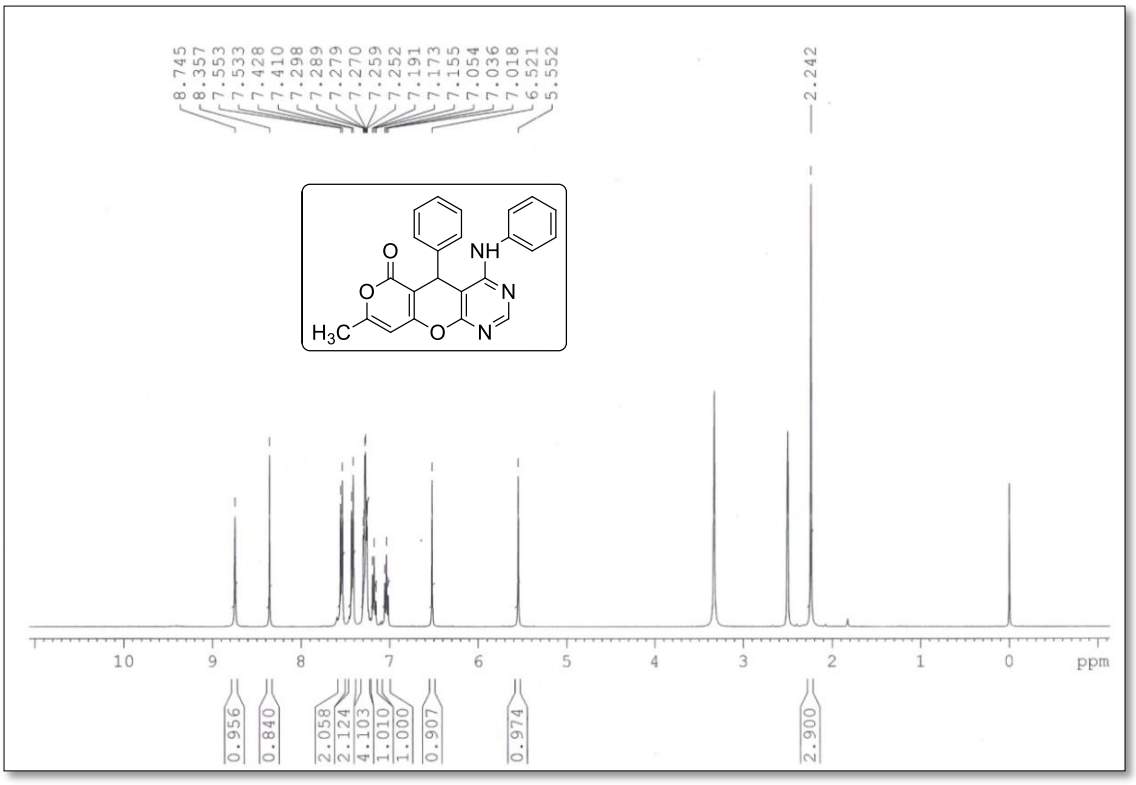

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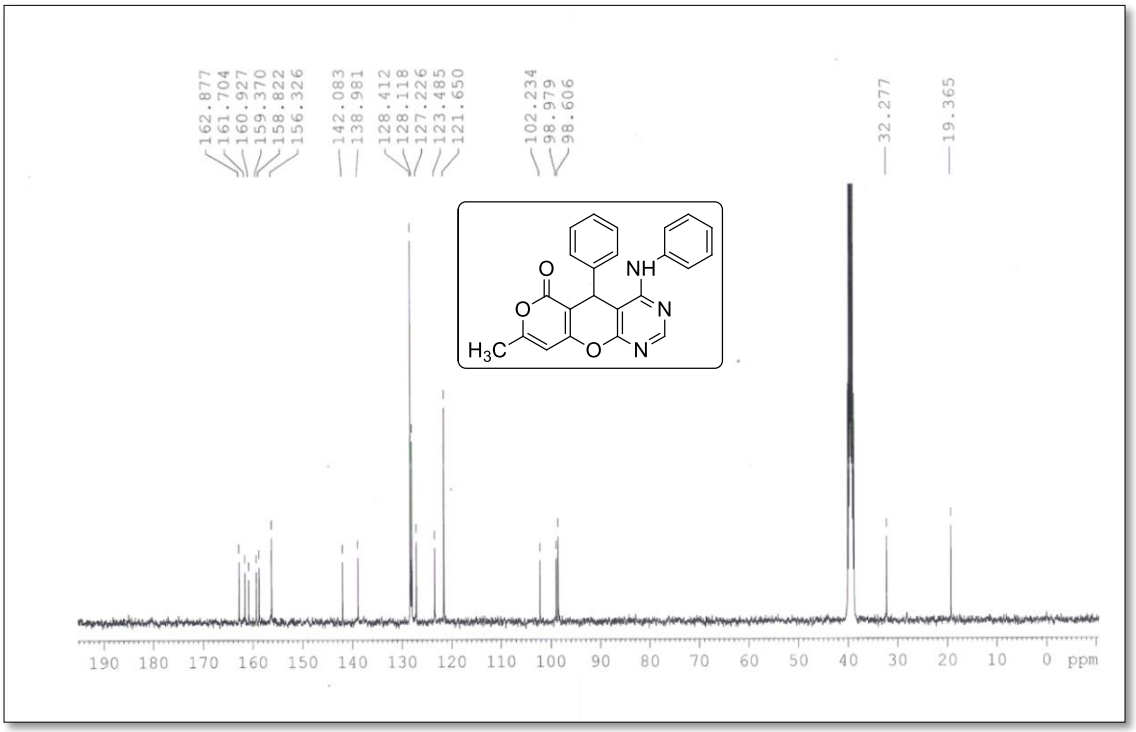
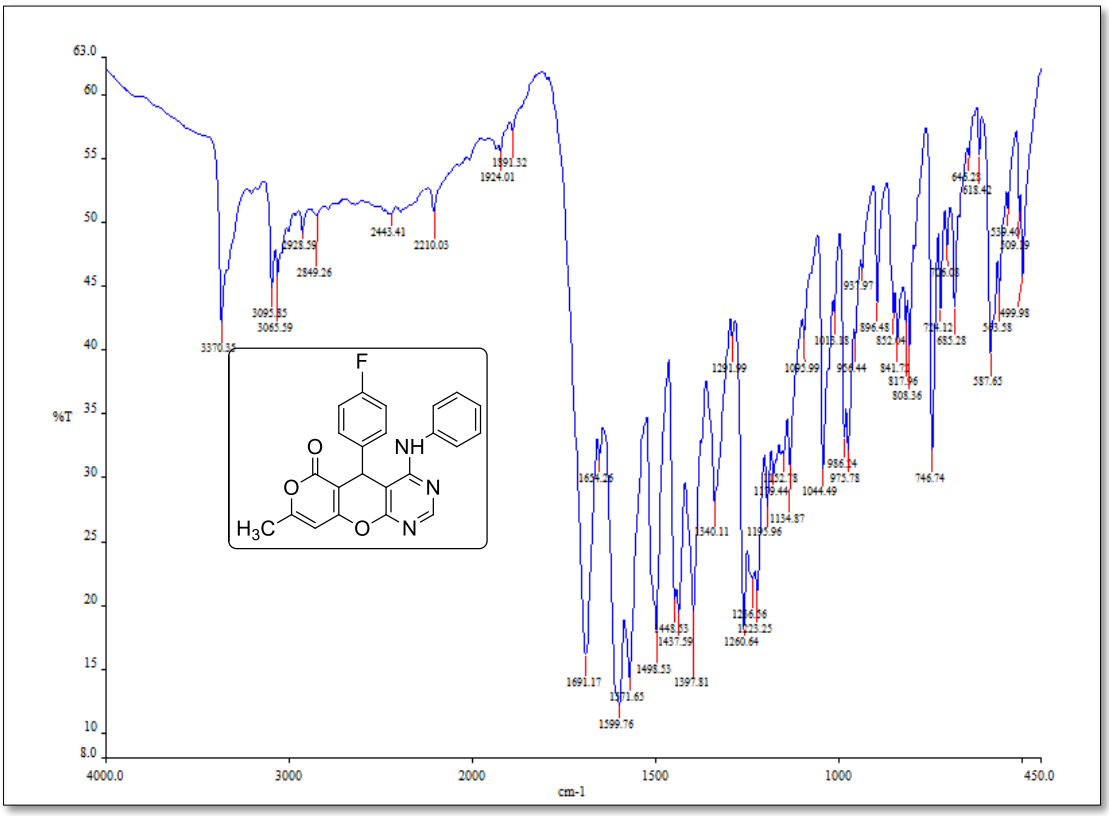


^{13}C NMR Spectrum of compound **6a**



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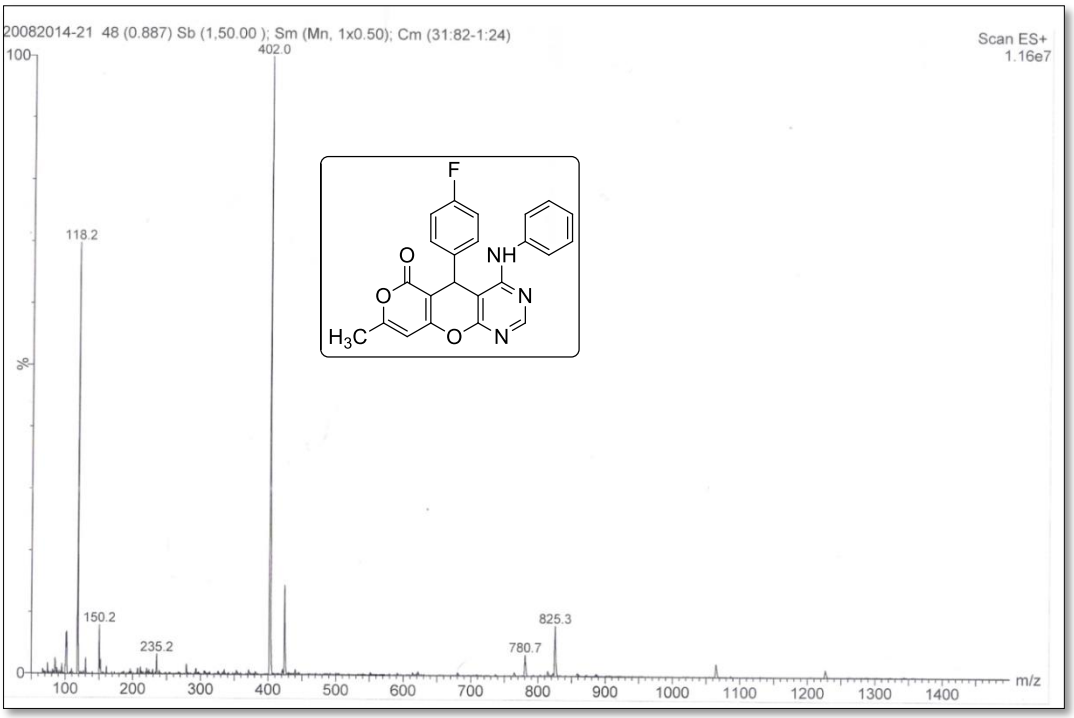
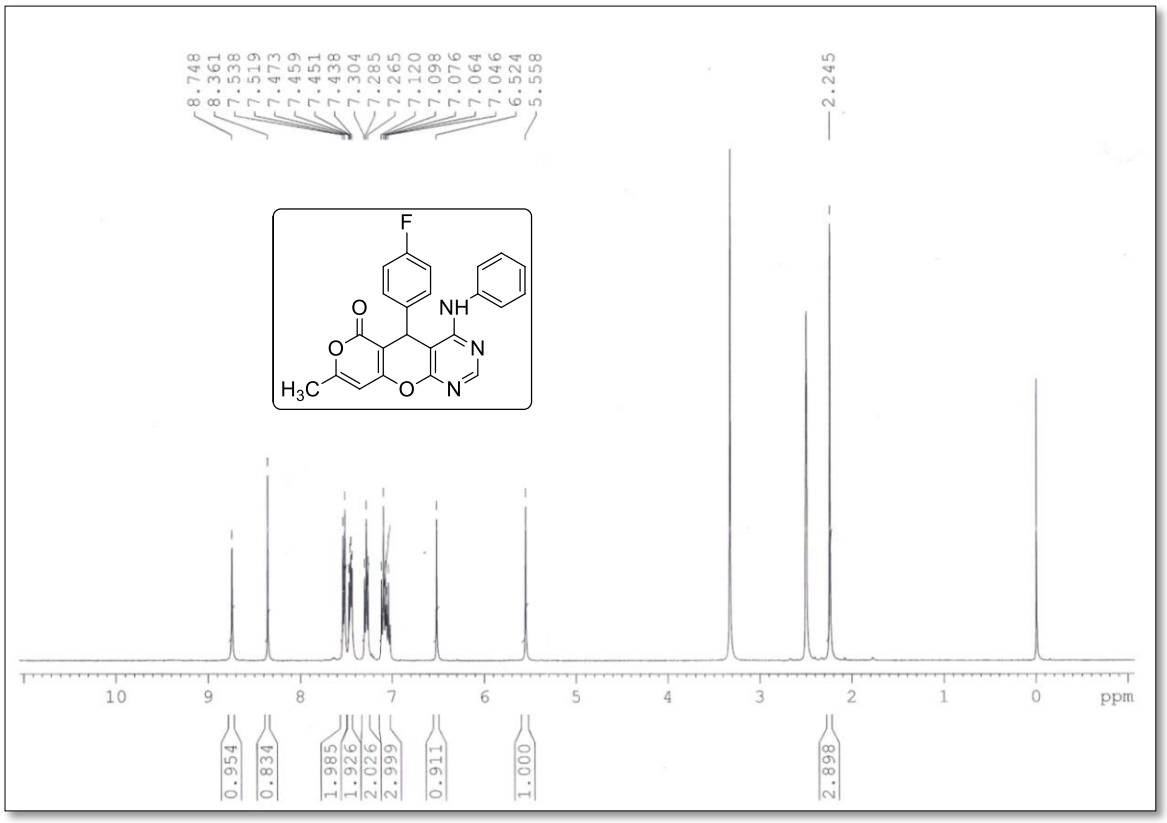
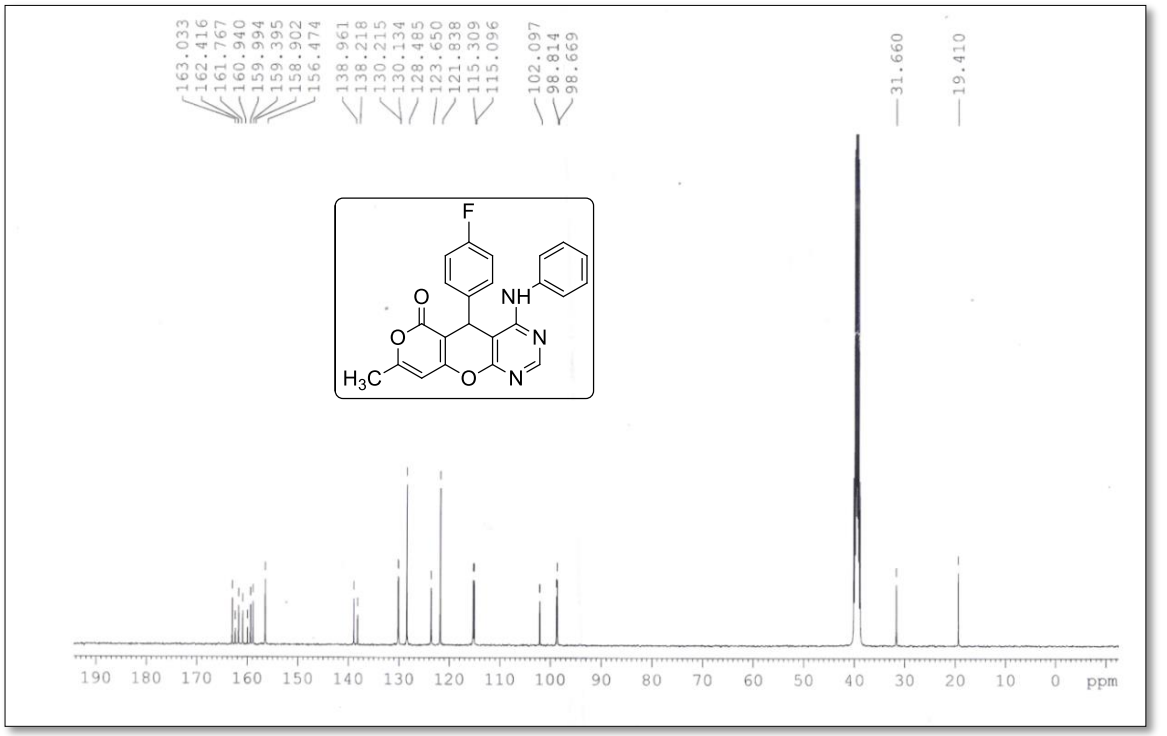


Mass Spectrum of compound **4e**



^{1}H NMR Spectrum of compound **4e**

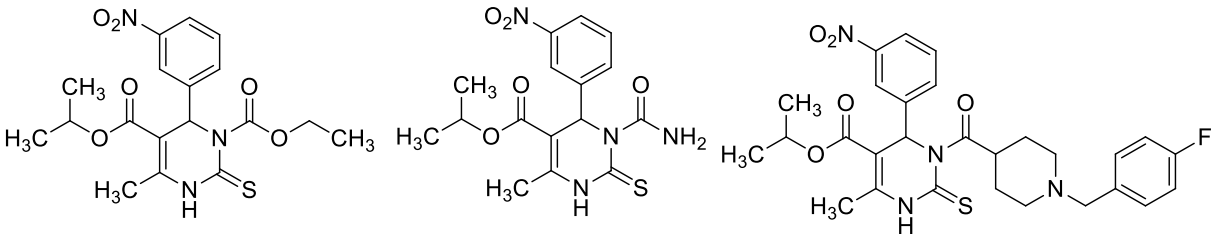


^{13}C NMR Spectrum of compound **4e**

SYNTHESIS OF FUSED DIHYDRO-1*H*-THIOCHROMENO[4,3-*d*]PYRIMIDINES UNDER GREEN CONDITIONS

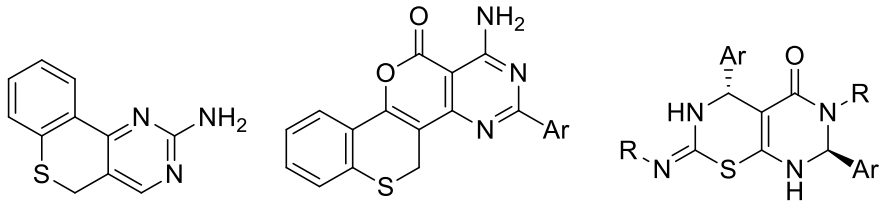
3.1. INTRODUCTION

Dihydropyrimidin-2(1*H*)-one (DHPM) derivatives have received considerable interest from the pharmaceutical industry due to their wide range of biological and therapeutic properties such as calcium channel modulators, antihypertensive agents, mitotic kinesin inhibitors, α1A-antagonists and neuropeptide Y(NPY) antagonists.[1] DHPMs are also known to possess interesting biological activities such as antiallergic, antibacterial, antifungal, antioxidant, antitumor, anti-inflammatory, antiviral and anticancer activities.[2] Most notable among them are batzelladine alkaloids, which have been found to be potent HIVgp-120-CD4 inhibitors.[3] Recently, 2-sulfanyl-6-methyl-1,4-dihydropyrimidine derivatives were reported as potent antifilarial agents.[4] 3,4-Dihydropyrimidin-2(1*H*)-thione core is an important pharmacophore useful as an intermediate for the preparation biologically potent thiazolopyrimidine and thiazoloquinazoline derivatives.[5] The most potent derivatives of N3 substituted DHPMs are thiourea derivatives.[6] Although the calcium channel blocking activity of this DHPM was comparable to DHPs, it was devoid of antihypertensive activity *in vivo*.[7] Further modification of the substituent at N3 subsequently led to the development of orally active long-lasting antihypertensive agents, such as DHPMs 1, 2 and 3. Compound 3 was shown to be both more potent and longer acting than nifedipine as an antihypertensive agent the *in vivo*. Similar pharmacological properties were established for the basic analog (**Fig. 1: 1, 2** and **3**).[8] In the present circumstances, the pyrimidine derivatives play a prominent role in organic and medicinal chemistry.[9] They exist in a wide variety of natural products and in rationally designed pharmaceutical agents.[10] Pyrimidines have become integral units to a large number of drug substances with activities including antimicrobial,[11] antioxidants,[12] anticonvulsant,[13] antidepressant,[14] anticancer,[15] and anti-biofilm.[16] In view of the diverse pharmacological properties of these compounds some of the well-known biologically potent thiochromen pyrimidine scaffolds (**4, 5** and **6**) are shown in (**Fig. 2**).[17]



(1) **(2)** **(3)**

Figure 1. Structures of DHPM calcium channel modulators



(4) **(5)** **(6)**

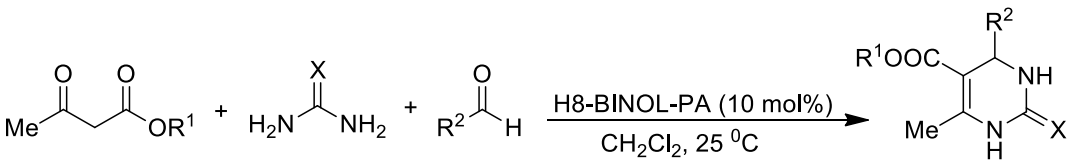
Figure 2. Biologically potent dihydrothiochromen pyrimidine scaffolds.

3.1.1. VARIOUS IMPORTANT APPROACHES FOR THE SYNTHESIS OF DIHYDROPYRIMIDINE-THIONES

The first synthetic method for the preparation of 3,4-dihydropyrimidine-2(1*H*)-ones (DHPMs) was reported by Biginelli. It involves the one-pot three component condensation of *β*-dicarbonyl compounds, aldehydes, and urea or thiourea in ethanol under strongly acidic conditions.[18]

Xiao-Hua Chen *et al.*[19] reported H_8-binol-based phosphoric acids catalyzed single step and environmentally friendly process for the highly enantioselective organocatalytic Biginelli reaction **(Scheme 1)**.

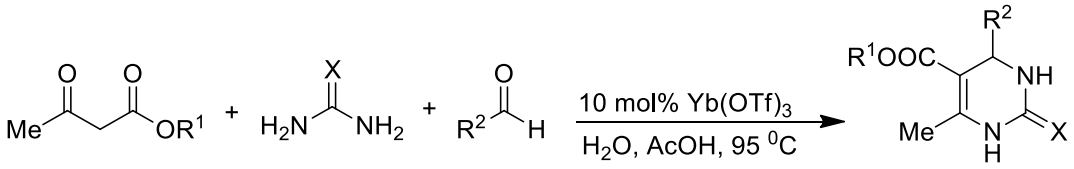
.



(Scheme 1)

Damkaci *et al.*[20] conducted Biginelli reaction with multiple unknowns under different reaction conditions **(Scheme 2)**.

.



(Scheme 2)

V. D. Vijay *et al.*[21] developed a simple and efficient method for the synthesis of 4-(substituted phenyl)-3,4-dihydro-1*H*-indeno[1,2-*d*]pyrimidine-2,5-diones **(Scheme 3)**.

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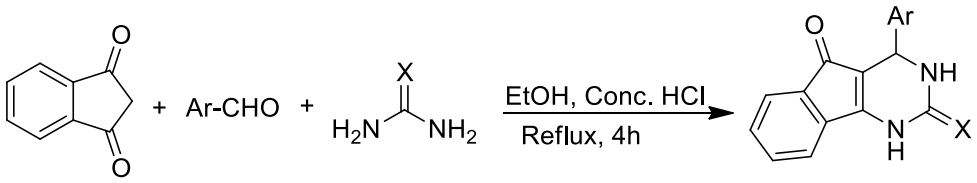
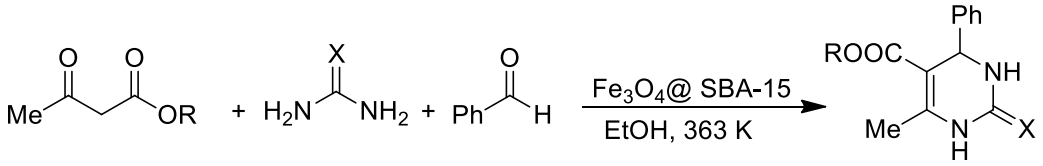


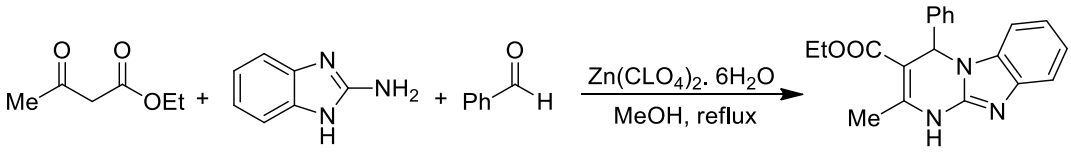
(Scheme 3)

J. Mondal *et al.*[22] described a novel and efficient Fe_3O_4 mesoporous SBA-15 as a robust and magnetically recoverable catalyst for one-pot synthesis of 3, 4-dihydropyrimidin-2(1 H)-ones via the Biginelli reaction **(Scheme 4)**.



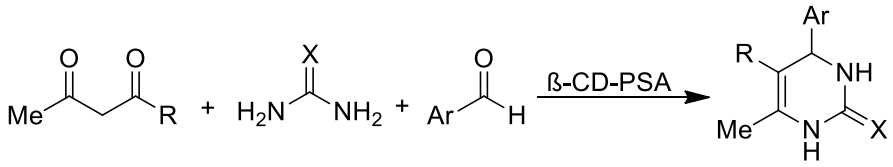
(Scheme 4)

Kaur *et al.*[23] described a novel and efficient one-pot method for the preparation of tricyclic dihydropyrimidine derivatives **(Scheme 5)**.



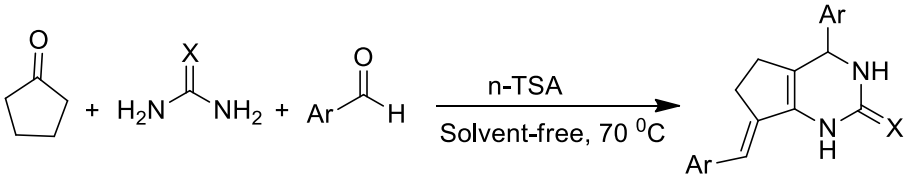
(Scheme 5)

Gong and co-workers[24] reported a β-Cyclodextrin-propyl sulfonic acid as a new and eco-friendly catalyst for one-pot multi-component synthesis of 3, 4-dihydropyrimidones via Biginelli reaction **(Scheme 6)**.



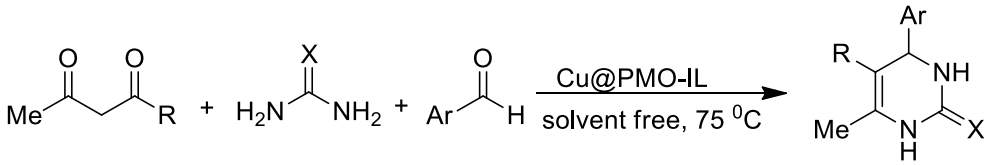
(Scheme 6)

Rahman *et al.*[25] used a nano titania-supported sulfonic acid for conducting same reaction under solvent free condition **(Scheme 7)**.



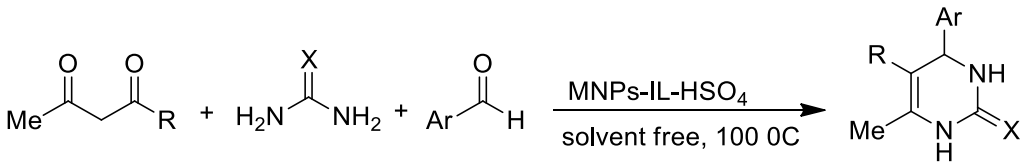
(Scheme 7)

Elhamifar and co-workers[26] described ionic liquid-based ordered mesoporous organosilica-supported copper as a novel and efficient nanocatalyst for the one-pot synthesis of Biginelli products **(Scheme 8)**.



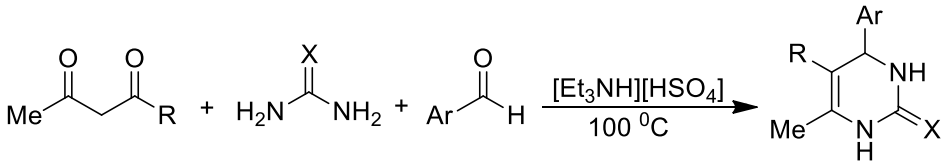
(Scheme 8)

Javad Safar and co-workers[27] developed simple and efficient Brønsted acidic ionic liquid based magnetic nanoparticles for the Biginelli synthesis of 3, 4-dihydropyrimidin-2 (1 H)-ones/thiones **(Scheme 9)**.



(Scheme 9)

H. Khabazzadeh *et al.*[28] described the synthesis of 3,4-dihydropyrimidin-2(1*H*)-ones in good to excellent yields by employing a simple ammonium salt of sulfuric acid in molten state as a cheap and mild acidic ionic liquid **(Scheme 10)**.

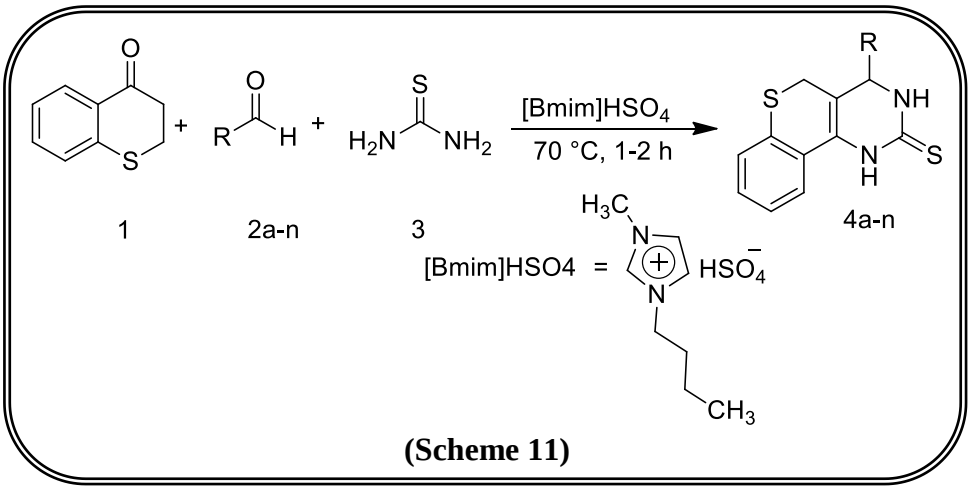


(Scheme 10)

3.2. PRESENT WORK

Recently, the interest in the synthesis of 3,4-dihydropyrimidin-2(1*H*)-ones and their derivatives is increasing tremendously because of their pharmacological and therapeutic properties. Many of the reported methods suffer from one or several drawbacks such as low yield, long reaction time, tedious workup, harsh reaction conditions and use of large quantity of expensive reagents and unsatisfactory yields.[29] Hence, it became essential to study and develop a simple, cost effective, eco- friendly and improved method for the synthesis of DHPMs. In the context of sustainable chemistry,[30] ionic liquids (ILs) have gained considerable attention in several branches of the chemical industry as potential "green" substitutes for conventional organic solvents.[31] The green aspect of ILs is mainly due to non-flammability and reduced air pollution.[32] The introduction of structural functionalities on the cationic or anionic part has made it possible to design new ILs with targeted properties.[33] In the recent years, ionic liquids (ILs) especially the acidic types have gained a renewed interest in the area of heterocyclic synthesis.[34] Nowadays, ILs are being used in multicomponent reactions (MCRs) and their properties have also been investigated in organic synthesis.[35] Keeping in view of all there aspects, we have developed simple, efficient and eco-friendly method for

the synthesis of fused dihydro-1*H*-thiochromeno[4,3-*d*]pyrimidine-2(5*H*)-thione under green chemical method **(Scheme 11)**.



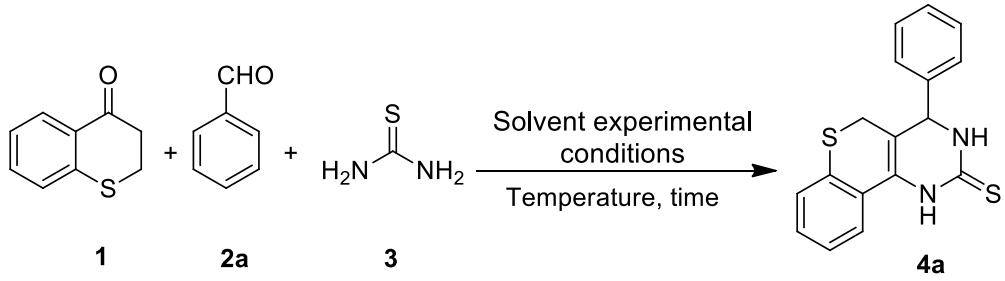
(Scheme 11)

3.3. RESULTS AND DISCUSSION

Highly volatile and toxic organic solvents were replaced by a green medium namely [Bmim]HSO_4 in the present research program. Thiochromeno[3,4-*d*]pyrimidine derivatives (**4a-n**) were obtained in good yields. The conditions were optimized for the designed protocol based on the reaction of thiochroman-4-one **1** (1 mmol), aromatic aldehyde **2a-n** (1 mmol) and thiourea **3** (1 mmol) in the presence of [Bmim]HSO_4. Initially, a one-pot three component reaction with thiochroman-4-one **1**, benzaldehyde **2a** and thiourea **3** was taken up as a model reaction to optimize the reaction conditions and the results are summarized in Table 1. When the reaction was carried out under neat conditions it did not give the required products even after 24 h (Table 1 entry 1). Later the reaction was carried out with organic solvents such as MeOH, EtOH, MeCN and DMF which resulted in poor yields (Table 1, entries 2-6). It was found that when acetic acid was applied, the acidic mixture of all the reactants under reflux conditions gave **4a** in 35% yields (Table 1, entry 6). While, studying the scope and efficiency of the reaction with different ILs in this MCR, it was observed that almost all the investigated ILs such as [Bmim]BF_4, [Bmim]Br, [Bmim]PF_6 and [Bmim]HSO_4 were capable of promoting the synthesis of desired compound **4a**. Good results were achieved by carrying out the reaction using [Bmim]HSO_4 ionic liquid and the derived product was obtained in good isolated yields when compared to the results

obtained using ionic liquid analogous tetrafluoroborate, bromide and hexaflurophosphate (Table 1, entries 7-9). The yield of product **4a** was improved and the reaction time was shortened as the temperature was increased from room temperature to 70 °C (Table 1, entries 10-14). No further improvement was observed up to 80 °C (Table 1, entry 15). Therefore, 70 °C was considered as the most suitable optimal reaction temperature for all these reactions. It was inferred from the above results that the ionic liquid medium is an essential and crucial factor for promoting the reaction. To demonstrate the generality of this method, the reaction was investigated under optimized conditions, and the results are illustrated in (**Table 2)**. It was observed that a wide range of aromatic aldehydes (**2a-n**) bearing either electron-donating or electron-withdrawing groups could be employed as coupling partners with thiochroman-4-one **1** and thiourea **3** and they were smoothly transformed to the corresponding thiochromeno[3,4-*d*]pyrimidines (**4a-n**) in good to moderate yields.

Table 1. Optimization of reaction parameters for the synthesis of compound **4a**



1 **2a** **3** **4a**

Entry[a]	Solvent	Temp (°C)	Time (h)	Yield[b](%)
1	Neat	70	24	NR
2	MeOH	70	12	08
3	EtOH	70	12	12
4	CH_3CN	70	10	06
5	DMF	70	14	0
6	AcOH	70	8	35
7	[Bmim]BF_4	70	2	70
8	[Bmim]Br	70	2	68
9	[Bmim]PF_6	70	3	52

10	**[Bmim]HSO_4**	**70**	**1**	**92**
11	[Bmim]HSO_4	rt	3	38
12	[Bmim]HSO_4	40	3	48
13	[Bmim]HSO_4	50	3	62
14	[Bmim]HSO_4	60	2	84
15	[Bmim]HSO_4	80	1	92

[a] Reaction conditions: thiochroman-4-one **1** (1 mmol), benzaldehyde **2a** (1 mmol) and thiourea **3** (1 mmol) and solvent (2 mL). [b] Yields of the isolated products.

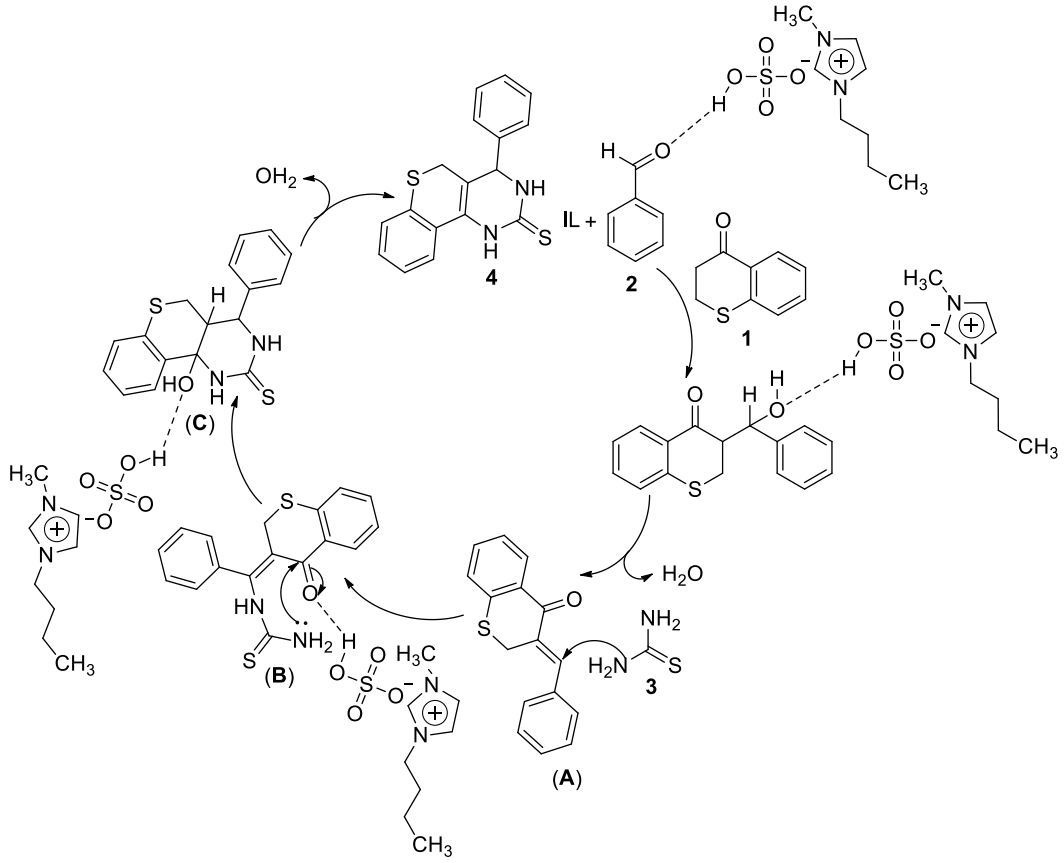
. All para, meta, and ortho-substituted aldehydes were easily converted into the desired products, indicating that steric bulk did not have any significant impact on the reactivity. Aldehydes bearing pyrazole group furnished the corresponding product in good yields. However, functional groups such as chloro, fluoro, nitro, methyl, methoxy slightly retarded the transformation. Several pharmaceutically relevant scaffolds could be easily generated with the help of this green protocol.

Table 2. Synthesis of thiochromeno[3,4-*d*]pyrimidine derivatives (**4a-n**)[a]

Product	R	Time (h)	Yield[b] (%)
4a	C_6H_5	1	92
4b	4-Me- C_6H_4	1	90
4c	4-OMe- C_6H_4	1	92
4d	4-Cl- C_6H_4	1	92
4e	4-F- C_6H_4	1.5	88
4f	4-NO_2- C_6H_4	1	90
4g	3-NO_2- C_6H_4	1	86
4h	2-Cl- C_6H_4	1.5	84
4i	$C_{10}H_7$	1.5	86
4j	6-OMe-$C_{10}H_6$	1.5	90
4k	Pyrazole	2	84
4l	4-NO_2-Pyrazole	2	84
4m	4-Cl-Pyrazole	2	84
4n	4-OCH_3-Pyrazole	2	84

[a]Reaction conditions: thiochroman-4-one **1** (1 mmol), aromatic aldehyde **2a-n** (1 mmol) and thiourea **3** (1 mmol) and [Bmim]HSO_4 (2 mL). [b] Yields of the isolated products.

A mechanism is proposed for the formation of compound **4** in (**Scheme 12**). In the first step, a hydrogen bond formation between the hydrogen atom of [Bmim]HSO_4 and carbonyl group of benzaldehyde **2** produce a complex which upon condensation with thiochroman-4-one **1** gives the α,β-unsaturated intermediate (**A)**. The formation of intermediate (**B)** takes place by a condensation between α,β-unsaturated intermediate **A** and thiourea **3**. Subsequently, intermediate **B** undergoes intramolecular cyclization to form another intermediate (**C**). In this last step, the intermediate **C** undergoes dehydration affording the final product **4**. Ionic liquid recyclability is one of the most necessary features and thus making it useful for commercial applications. Consequently, we studied the recyclability of the [Bmim]HSO_4 ionic liquid in the above model reaction. After completion of the reaction, the mixture was transferred into ice cold water and stirred thoroughly. The solid product thus obtained was isolated by filtration, and the filtrate having ionic liquid was extracted with ethyl acetate (2 × 20 mL) to eliminate the non-ionic organic impurities. Then the water was evaporated under reduced pressure and the recovered ionic liquid was dried under vacuum and reused for four times in consequent reactions without evident changes in the product yields.



Scheme 12. Proposed mechanism for the formation of compound **4a**

In conclusion, we have successfully developed a new and efficient route for the synthesis of biologically important thiochromeno[3,4-*d*]pyrimidine derivatives in [Bmim]HSO_4 ionic liquid. The significant advantages of the methodology are higher yields, milder reaction condition, shorter reaction time, convenient procedure, and environmentally friendly green protocols.

3.4. EXPERIMENTAL

Chemicals were purchased from Merck and Aldrich chemical companies. The ^{1}H NMR and ^{13}C NMR spectra were recorded in DMSO-d_6 solvent on Bruker WM-300 spectrometer using TMS as internal standard. The chemical shifts were reported in ppm (δ). Mass spectra (ESI) were carried out on a JEOL JMSD-300 spectrometer. CHN analysis was carried out using Carlo Erba EA 1108 automatic elemental analyser. Melting points were determined in open capillary tubes and were recorded on a Stuart SMP30 melting point apparatus.

3.4.1. SPECTRAL DISCUSSION

IR: In all the compounds **4a–n** the formation of pyrimidine was confirmed due to appearance of –NH group around 3316-3443 cm^{-1} and the other carbonyl (C=s) was appeared around 1664-1694 cm^{-1}.

^{1}H NMR: In ^{1}H NMR, the –NH signal was observed at δ 9.64-8.16 ppm. In all the compounds, the singlet protons of pyrimidine (CH) group were observed at δ 5.41-5.68 ppm. All other aromatic and aliphatic protons appeared at expected regions.

^{13}C NMR: In ^{13}C NMR, the signal appeared at δ 31.12-30.18 ppm can be attributed to CH_2 carbon. The signal observed at δ 62.84-60.94 ppm was assigned to (CH) carbon.

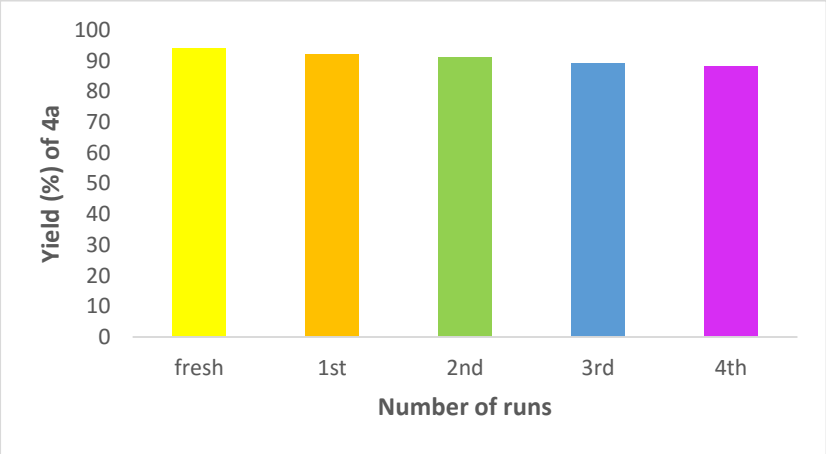
Mass: The structures of all synthesized compounds were further confirmed by its mass spectra. The mass spectra detected the expected molecular ion signals (M + 1) corresponding to respective molecular weight of the synthesized compounds.

3.4.2. General procedure for the synthesis of thiochromeno[3,4-*d*]pyrimidine derivatives (4a-n)

A dry 50 mL flask was charged with thiochroman-4-one **1** (1 mmol), aromatic aldehyde **2 a-n** (1 mmol), thiourea **3** and [Bmim]HSO_4 ionic liquid (2 mL). The reaction mixture was stirred at 70 °C for 1 h. The progress of the reaction was monitored by TLC (eluent = n-hexane/ethyl acetate: 8/2, v/v). After completion of the reaction, the reaction mixture was cooled to RT and poured into ice cold water, the solid separated was filtered, washed with water, dried and purified by column chromatography using silica gel (ethyl acetate/n-hexane: 2/8, v/v) to afford title compounds **4a-n.**

3.4.3. Reusability study of [Bmim]HSO_4 ionic liquid: One of the important issues in green synthesis is recovery and reusability. To further investigate the recyclability of the IL [Bmim]HSO_4, After completion of the reaction, the mixture was diluted with 20 mL of water and stirred thoroughly.The solid product was isolated by filtaration, and washed twice with distilled water (2 × 10 mL). The residue (water) containing dissolved Iionic liquid was extracted with ethyl acetate (2 × 20 mL) to remove the non-ionic organic impurities. Then aqueous layer was evaporated and the ionic liquid was dried at 60-70 °C under vacuum and the results

have shown that the [Bmim]HSO_4 ionic liquid could be used at least for four times without any significant loss in the yield of the product.



Reusability of [Bmim]HSO_4 in the synthesis of title compounds **4a**.

3.4.4. PHYSICAL AND SPECTRAL DATA

4-Phenyl-3,4-dihydro-1H-thiochromeno[4,3-d]pyrimidine-2(5H)-thione (4a). Pale yellow solid; mp: 262- 264 °C; **IR** (KBr) υ_{max} (cm^{-1}): 3352, 1694, 1597, 1435; **^{1}H NMR** (400 MHz, DMSO-d_6): δ 3.02 (dd, *J* = 16 Hz, 1H), 3.52 (dd, *J* = 16 Hz, 1H), 5.05 (s, 1H), 7.20-7.24 (m, 2H), 7.31 (t, *J* = 8 Hz, 2H), 7.41 (d, *J* = 8 Hz, 2H), 7.48 (d, *J* = 8 Hz, 2H), 7.63 (t, *J* = 8 Hz, 1H), 9.25 (s, 1H), 9.86 (s, 1H); **^{13}C NMR** (100 MHz, DMSO-d_6): 174.8, 143.0, 134.7, 133.9, 132.3, 130.8 129.9, 128.6, 127.9, 127.1, 126.8, 126.2, 121.3, 111.4, 61.0, 31.7; **ESI-MS**: *m/z* 312 $(M + 1)^+$; Anal. Calcd. For $C_{17}H_{14}N_2S_2$; C, 65.77; H, 4.55; N, 9.02 ; Found :C, 65.68; H, 4.49; N, 9.16.

4-(p-Tolyl)-3,4-dihydro-1H-thiochromeno[4,3-d]pyrimidine-2(5H)-thione (4b). White solid; mp : 303- 305 °C; **IR** (KBr) υ_{max} (cm^{-1}): 3186, 1676, 1572, 1471; **^{1}H NMR** (400 MHz, DMSO-d_6): δ 2.30 (s, dd, *J* = 16 Hz, 1H), 2.99 (dd, *J* = 16 Hz, 1H), 3.53 (d, *J* = 8 Hz, 3H), 4.98 (s, 1H), 7.20-7.23 (m, 4H), 7.30 (t, *J* = 8 Hz, 3H), 7.63-7.65 (m, 1H), 9.20 (s, 1H), 9.76 (s, 1H); **^{13}C NMR** (100 MHz, DMSO-d_6): 174.1, 139.4, 134.9, 134.3, 132.5, 130.9, 130.2, 128. 8, 128.0, 127.3, 126.8, 126.3,

121.3, 115.5, 111.5, 61.0, 33.3, 19.2; **ESI-MS**: *m/z* 325 $(M + 1)^+$; Anal. Calcd. For $C_{18}H_{16}N_2S_2$; C, 66.63; H, 4.97; N, 8.76 ; Found : C, 66.73; H, 4.91; N, 8.58.

4-(4-Methoxyphenyl)-3,4-dihydro-1H-thiochromeno[4,3-d]pyrimidine-2(5H)-thione (4c). White solid; mp: 270- 272 °C; **IR** (KBr) υ_{max} (cm^{-1}): 3180, 3220, 1673, 1572, 1470 713; **^{1}H NMR** (400 MHz, DMSO-d_6): δ 2.97 (dd, *J* = 16 Hz, 1H), 3.51 (dd, *J* = 16 Hz, 1H), 3.74 (s, 3H), 4.96 (s, 1H), 6.95 (d, *J* = 8 Hz, 2H), 7.21 (t, *J* = 8 Hz, 2H), 7.28-7.32 (m, 3H), 7.62-7.64 (m, 1H), 9.17 (s, 1H), 9.78 (s, 1H); **^{13}C NMR** (100 MHz, DMSO-d_6): 174.1, 158.7, 139.4, 135.1, 134.4, 132.6, 130.9, 130.0, 128.7, 127.9, 127.3, 126.2, 121.4, 115.8, 111.5, 61.7, 36.4, 33.5; **ESI-MS**: *m/z* 341 $(M + 1)^+$; Anal. Calcd. For $C_{18}H_{16}N_2OS_2$; C, 63.50; H, 4.74; N, 8.23 ; Found : C, 63.64; H, 4.69; N, 8.37.

4-(4-Chlorophenyl)-3,4-dihydro-1H-thiochromeno[4,3-d]pyrimidine-2(5H)-thione (4d). Yellow solid; mp: 260- 262 °C; **IR** (KBr) υ_{max} (cm^{-1}): 3182, 1674, 1574, 1487; **^{1}H NMR** (400 MHz, DMSO-d_6): δ 3.01 (dd, *J* = 16 Hz, 1H), 3.54 (dd, *J* = 16 Hz, 1H), 5.02 (s, 1H), 7.20- 7.22 (m, 2H), 7.28-7.35 (m, 2H), 7.40 (d, *J* = 8 Hz, 3H), 7.63-7.65 (m, 1H), 9.23 (s, 1H), 9.82 (s, 1H); **^{13}C NMR** (100 MHz, DMSO-d_6): 174.0, 146.0, 135.4, 134.6, 131.1, 130.2, 128.2, 127.9, 127.3, 126.4, 121.6, 116.2, 111.7, 111.6, 61.8, 33.6; **ESI-MS**: *m/z* 346 $(M + 1)^+$; Anal. Calcd. For $C_{17}H_{13}ClN_2S_2$; C, 59.20; H, 3.80; N, 8.13 ; Found : C, 59.11; H, 3.75; N, 8.32.

4-(4-Fluorophenyl)-3,4-dihydro-1H-thiochromeno[4,3-d]pyrimidine-2(5H)-thione (4e). White solid; mp: 284- 286 °C; **IR** (KBr) υ_{max} (cm^{-1}): 3185, 1665, 1550, 1470; **^{1}H NMR** (400 MHz, DMSO-d_6): δ 2.89 (dd, *J* = 16 Hz,1H), 3.46 (dd, *J* = 16 Hz, 1H), 5.49 (s, 1H), 7.22 (t, *J* = 8 Hz, 2H), 7.28-7.42 (m, 3H), 7.47-7.50 (m, 2H), 7.63-7.65 (m, 1H), 9.16 (s, 1H), 9.89 (s, 1H); **^{13}C NMR** (100 MHz, DMSO-d_6): 174.4, 162.9, 139.3, 134.8, 134.0, 132.4, 130.7, 130.0, 128.7, 127.9, 127.2, 126.7, 126.2, 121.2, 111.4, 60.9, 33.1; **ESI-MS**: *m/z* 330 $(M + 1)^+$; Anal. Calcd. For $C_{17}H_{13}FN_2S_2$; C, 62.17; H, 3.99; N, 8.53 ; Found : C, 62.39; H, 3.94; N, 8.32.

4-(4-Nitrophenyl)-3,4-dihydro-1H-thiochromeno[4,3-d]pyrimidine-2(5H)-thione (4f). Yellow solid; mp: 256- 258 °C; **IR** (KBr) υ_{max} (cm^{-1}): 3183, 1675, 1571, 1523; **^{1}H NMR** (400 MHz, DMSO-d_6): δ 3.06 (dd, *J* = 16 Hz, 1H), 3.56 (dd, *J* = 16 Hz, 1H), 5.24 (s, 1H), 7.21- 7.24 (m, 2H), 7.30 (d, *J* = 8 Hz, 1H), 7.67 (d, *J* = 8 Hz,

3H), 8.29 (d, *J* = 8 Hz, 2H), 9.37 (s, 1H), 9.99 (s, 1H); **^{13}C NMR** (100 MHz, DMSO-d_6): 173.8, 139.4, 135.2, 134.3, 132.5, 130.7, 130.1, 128.2, 127.7, 127.0, 126.1, 121.1, 115.9, 111.4, 61.7, 33.5; **ESI-MS**: *m/z* 356 $(M + 1)^+$; Anal. Calcd. For $C_{17}H_{13}N_3O_2S_2$; C, 57.45; H, 3.69; N, 11.82 ; Found : C, 57.26; H, 3.63; N, 11.68.

4-(3-Nitrophenyl)-3,4-dihydro-1H-thiochromeno[4,3-d]pyrimidine-2(5H)-thione (4g). Pale yellow solid; mp: 275- 277 °C; **IR** (KBr) υ_{max} (cm^{-1}): 3412, 1674, 1579, 1526; **^{1}H NMR** (400 MHz, DMSO-d_6): δ 3.09 (dd, *J* = 16 Hz, 1H), 3.57 (dd, *J* = 16 Hz, 1H), 5.28 (s, 1H), 7.23-7.31 (m, 3H), 7.65-7.74 (m, 2H), 7.87 (s, 1H), 8.22-8.28 (m, 2H), 9.36 (s, 1H), 9.97 (s, 1H); **^{13}C NMR** (100 MHz, DMSO-d_6): 173.5, 146.9, 135.2, 134.4, 132.6, 130.9,128.3, 127.8, 127.0, 126.2, 121.3, 116.1, 111.6, 61.29, 34.0; **ESI-MS**: *m/z* 356 $(M + 1)^+$; Anal. Calcd. For $C_{17}H_{13}N_3O_2S_2$; C, 57.45; H, 3.69; N, 11.82 ; Found : C, 57.32; H, 3.65; N, 11.66.

4-(2-Chlorophenyl)-3,4-dihydro-1H-thiochromeno[4,3-d]pyrimidine-2(5H)-thione (4h). Yellow solid; mp: 250- 252 °C; **IR** (KBr) υ_{max} (cm^{-1}): 3164, 1672, 1566, 1491; **^{1}H NMR** (400 MHz, DMSO-d_6): δ 2.65 (dd, *J* = 16 Hz, 1H), 3.05 (dd, *J* = 16 Hz, 1H), 5.94 (s, 1H), 7.01 (d, *J* = 8 Hz, 1H), 7.23 (d, *J* = 8 Hz, 2H), 7.55 (d, *J* = 8 Hz, 1H), 8.12-8.34 (m, 4H), 9.16 (s, 1H), 9.63 (s, 1H); **^{13}C NMR** (100 MHz, DMSO-d_6): 172.0, 147.0, 136.4, 134.6, 132.1, 130.2, 128.2, 127.4, 127.0, 125.8, 121.2, 117.2, 112.7, 111.8, 62.8, 33.1; **ESI-MS**: *m/z* 346 $(M + 1)^+$; Anal. Calcd. For $C_{17}H_{13}ClN_2S_2$; C, 59.20; H, 3.80; N, 8.12 ; Found : C, 59.36; H, 3.85; N, 38.

4-(Napthalen-1-yl)-3,4-dihydro-1H-thiochromeno[4,3-d]pyrimidine-2(5H)-thione (4i). Brown solid ; mp: 298- 300 °C; **IR** (KBr) υ_{max} (cm^{-1}): 3170, 1674, 1565, 1472; **^{1}H NMR** (400 MHz, DMSO-d_6): δ 2.84 (dd, *J* = 16 Hz, 1H), 3.48 (dd, *J* = 16 Hz, 1H), 5.92 (s, 1H), 7.24-7.29 (m, 3H), 7.57-7.69 (m, 5H), 7.93-7.99 (m, 2H), 8.39 (d, *J* = 8 Hz, 1H), 9.24 (s, 1H), 9.91 (s, 1H); **^{13}C NMR** (100 MHz, DMSO-d_6): 176.8, 142.0, 134.7, 133.6, 132.8, 131.4, 130.6 129.7, 129.2, 128.4, 127.7, 127.1, 126.8, 126.2, 126.0, 122.5, 113.4, 62.0, 30.4; **ESI-MS**: *m/z* 362 $(M + 1)^+$; Anal. Calcd. For $C_{21}H_{16}N_2S_2$; C, 69.97; H, 4.47; N, 7.77 ; Found : C, 69.78; H, 4.42; N, 7.41.

4-(6-Methoxynapthalen-1-yl)-3,4-dihydro-1H-thiochromeno[4,3-d]pyrimidine-2(5H)-thione (4j). Pale yellow solid; mp: 271- 273 °C; **IR** (KBr) υ_{max} (cm^{-1}): 3384, 1628, 1527, 1482; **^{1}H NMR** (400 MHz, DMSO-d_6): δ 3.06 (dd, *J* = 16 Hz, 1H), 3.20 (dd, *J* = 16 Hz, 1H), 3.68 (s, 3H), 5.16 (s, 1H), 7.23-7-88 (m, 10H), 9.30 (s, 1H), 9.86 (s, 1H); **^{13}C NMR** (100 MHz, DMSO-d_6): 174.8, 143.0, 134.7, 133.9, 132.3, 130.8 129.9, 128.6, 127.9, 127.1, 126.8, 126.2, 121.3, 111.4, 61.0, 31.7; **ESI-MS**: *m/z* 392 $(M + 1)^+$; Anal. Calcd. For $C_{22}H_{18}N_2OS_2$; C, 67.66; H, 4.65; N, 7.17 ; Found : C, 67.88; H, 4.70; N, 7.29.

4-(1,3-Dipheny-lH-pyrazol-4-yl)-3,4-dihydro-1H-thiochromeno[4,3-d]

Pyrimidine-2(5H)-thione (4k). Yellow solid; mp: 328- 330 °C; **IR** (KBr) υ_{max} (cm^{-1}): 3191, 1663, 1597, 1538; **^{1}H NMR** (400 MHz, DMSO-d_6): δ 2.95 (dd, *J* = 16 Hz, 1H), 3.13 (dd, *J* = 16 Hz, 1H), 5.38 (s, 1H), 7.20-7.50 (m, 8H), 7.57-7.70 (m, 3H), 7.88 (t, *J* = 8 Hz, 3H), 8.51 (s, 1H), 9.22 (s, 1H), 9.70 (s, 1H); **^{13}C NMR** (100 MHz, DMSO-d_6): δ 181.7, 150.3, 141.2, 138.3, 132.7, 131.2, 130.0, 128.7, 128.1, 127.5, 126.8, 125.0, 123.4, 119.3, 118.3, 114.3, 104.8, 66.0, 29.5; **ESI-MS**: *m/z* 453 $(M + 1)^+$; Anal. Calcd. For $C_{26}H_{20}N_4S_2$; C, 69.00; H, 4.45; N, 12.38 ; Found : C, 69.24; H, 4.39; N, 12.08.

4-(3-(4-Nitrophenyl)-1-phenyl-lH-pyrazol-4-yl)-3,4-dihydro-1H-thiochromeno

[4,3-d]pyrimidine-2(5H)-thione (4l). White solid; mp: 262- 264 °C; **IR** (KBr) υ_{max} (cm^{-1}): 3181, 1674, 1593, 1523; **^{1}H NMR** (400 MHz, DMSO-d_6): δ 3.06 (dd, *J* = 16 Hz, 1H), 3.20 (dd, *J* = 16 Hz, 1H), 5.46 (s, 1H), 7.17- 7.22 (m, 2H), 7.26-7.38 (m, 2H), 7.45 (t, *J* = 8 Hz, 1H), 7.50-7.60 (t, *J* = 8 Hz, 3H), 7.89-8.00 (d, *J* = 8 Hz, 2H), 7.27 (t, *J* = 8 Hz, 2H), 8.35-8.40 (m, 1H), 8.62 (s, 1H), 9.07 (s, 1H), 9.72 (s, 1H); **^{13}C NMR** (100 MHz, DMSO-d_6): δ 181.6, 150.18, 146.4, 141.0, 138.0, 132.5, 131.2, 129.9, 128.7, 128.1, 127.5, 127.3, 126.5, 124.8, 123.2, 118.2, 118.2, 114.1, 104.6, 65.7, 29.3; **ESI-MS**: *m/z* 499 $(M + 1)^+$; Anal. Calcd. For $C_{26}H_{19}N_5O_2S_2$; C, 62.76; H, 3.85; N, 14.07 ; Found : C, 62.45; H, 3.80; N, 14.38.

4-(3-(4-Chlorophenyl)-1-phenyl-lH-pyrazol-4-yl)-3,4-dihydro-1Hthiochromeno

[4,3-d]pyrimidine-2(5H)-thione (4m). White solid; mp: 262- 264 °C; **IR** (KBr) υ_{max} (cm^{-1}): 3234, 1674, 1540, 1502; **^{1}H NMR** (400 MHz, DMSO-d_6): δ 3.06 (dd, *J*

= 16 Hz, 1H), 3.80 (dd, *J* = 16 Hz, 1H), 5.38 (s, 1H), 7.22-7.88 (m, 13H), 8.54 (s, 1H) 9.23 (s, 1H), 9.71 (s, 1H); **^{13}C NMR** (100 MHz, DMSO-d_6): δ 182.2, 152.4, 145.4, 142.4, 137.5, 131.8, 131.0, 129.5, 128.4, 128.0, 127.4, 127.0, 126.2, 125.8, 123.6, 117.6, 116.8, 114.3, 104.4, 64.6, 31.2; **ESI-MS**: *m/z* 488 $(M + 1)^+$; Anal. Calcd. For $C_{26}H_{19}ClN_4S_2$; C, 64.12; H, 3.93; N, 11.50 ; Found : C, 64.32; H, 3.88; N, 11.76.

4-(1-Phenyl-3-(p-tolyl)-lH-pyrazol-4-yl)-3,4-dihydro-1H-thiochromeno[4,3 d]pyrimidine-2(5H)-thione (4n). Yellow solid; mp: 290-392 °C; **IR** (KBr) υ_{max} (cm^{-1}): 3382, 1695, 1572, 1509; **^{1}H NMR** (400 MHz, DMSO-d_6): δ 2.29 (dd, *J* = 16 Hz, 1H), 3.53 (dd, *J* = 16 Hz, 1H), 3.79 (s, 3H), 5.94 (s, 1H), 7.01 (d, *J* = 8 Hz, 3H), 7.21 (d, *J* = 8 Hz, 3H), 7.57 (d, *J* = 8 Hz, 2H), 8.15-8.34 (m, 6H), 8.93 (s, 1H) 9.21 (s, 1H); **^{13}C NMR** (100 MHz, DMSO-d_6): δ 181.9, 150.4, 146.6, 141.2, 138.3, 131.4, 128.8, 128.3, 127.8, 127.5, 126.8, 125.1, 123.6, 119.1, 118.4, 114.3, 104.8, 66.1, 36.5, 29.5; **ESI-MS**: *m/z* 468 $(M + 1)^+$; Anal. Calcd. For $C_{27}H_{22}N_4S_2$; C, 69.50; H, 4.75; N, 12.01; Found : C, 69.29; H, 4.81; N, 12.32.

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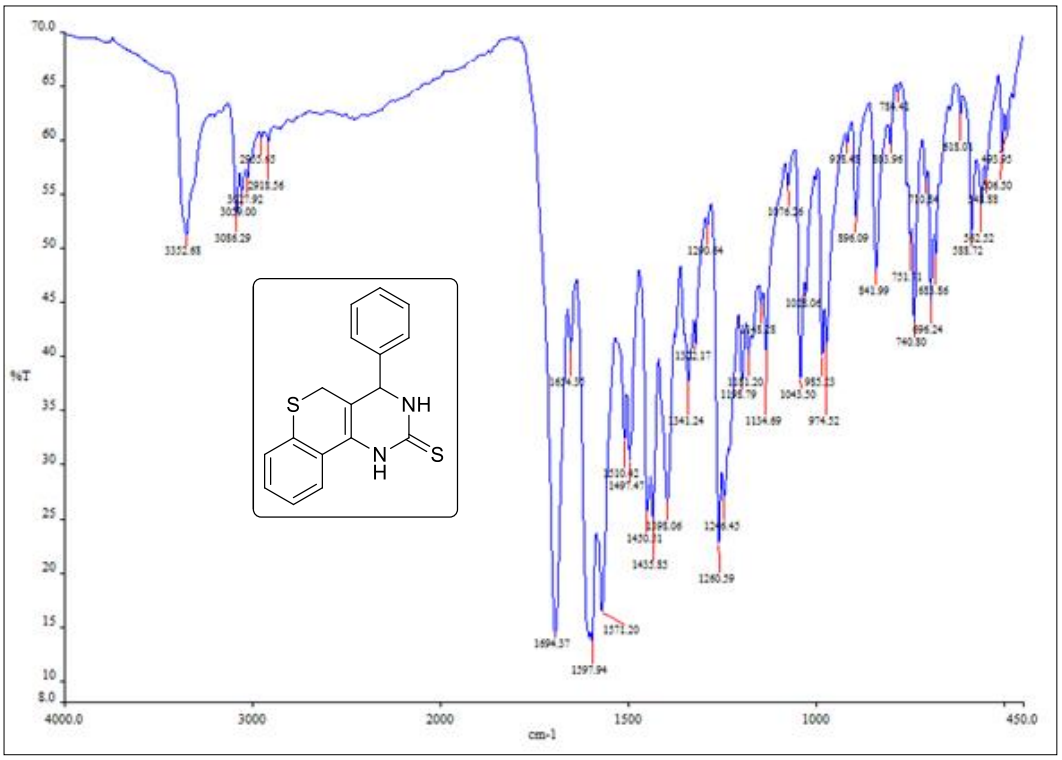
SELECTED SPECTRA



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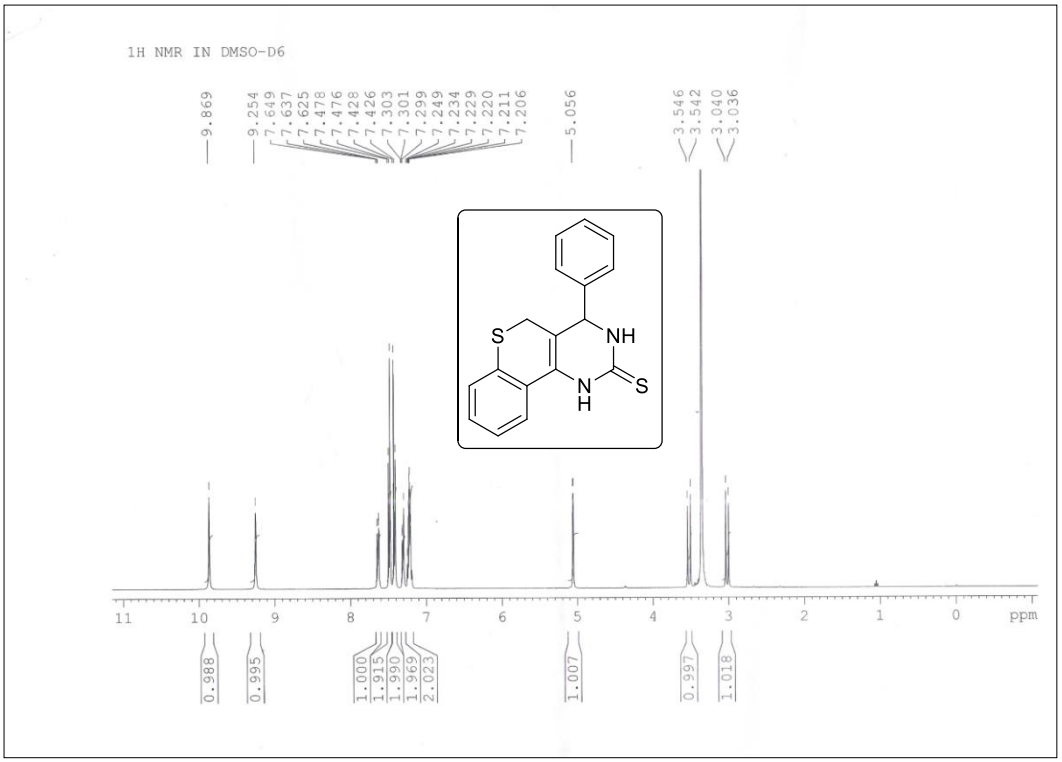
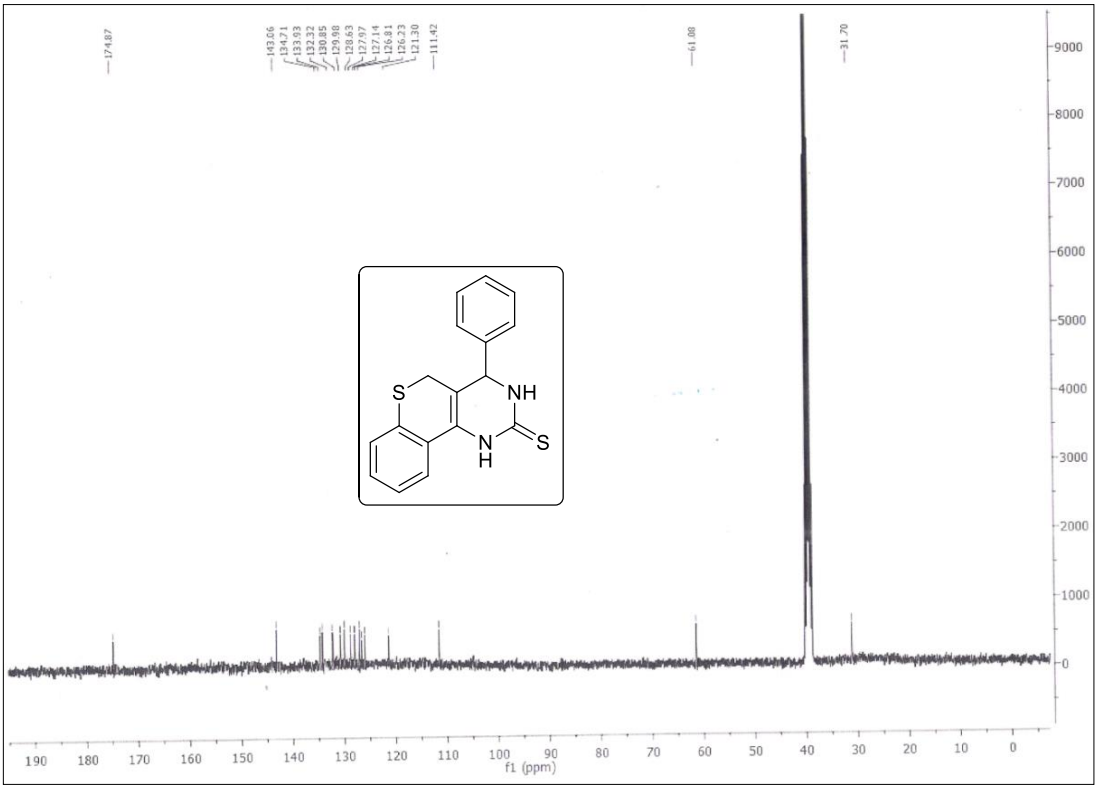


^{1}H NMR Spectrum of compound **4a**



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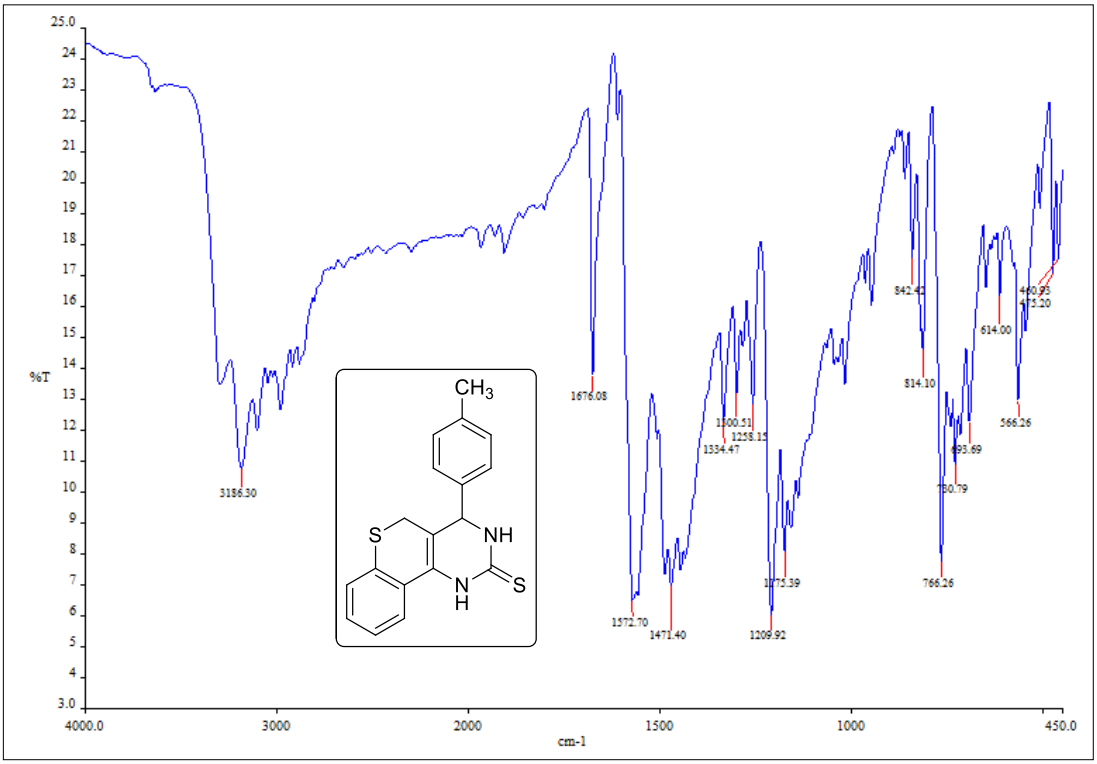

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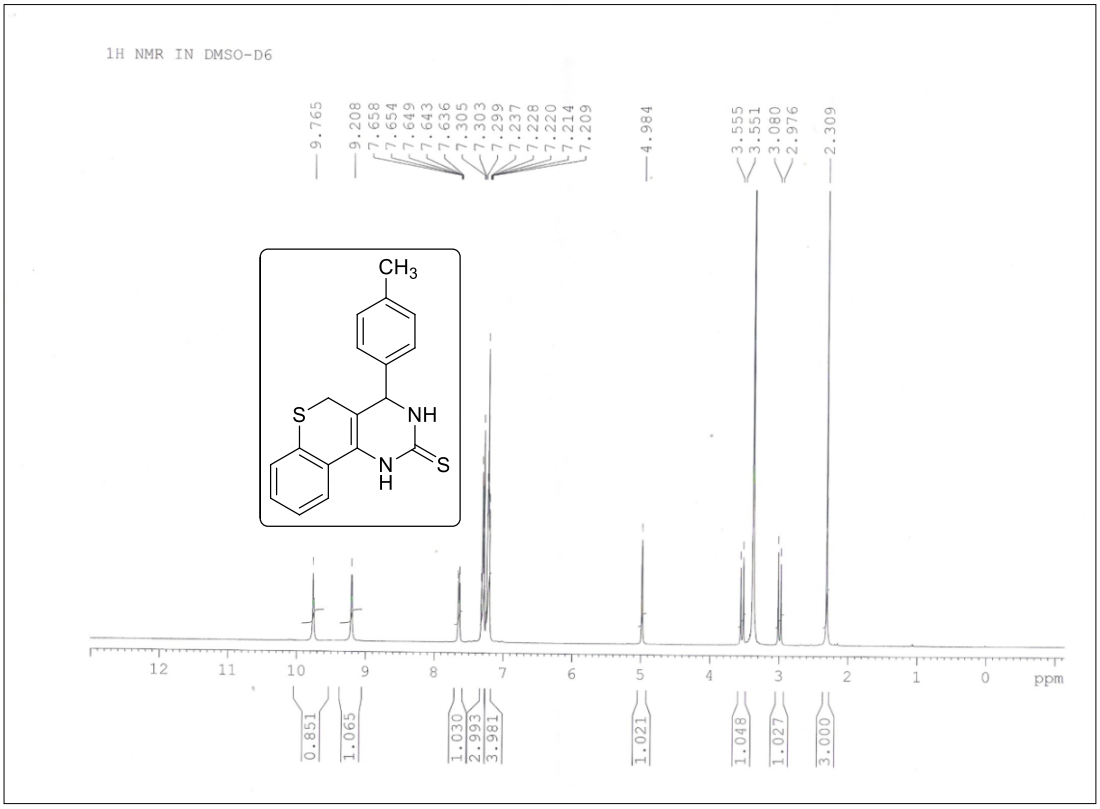

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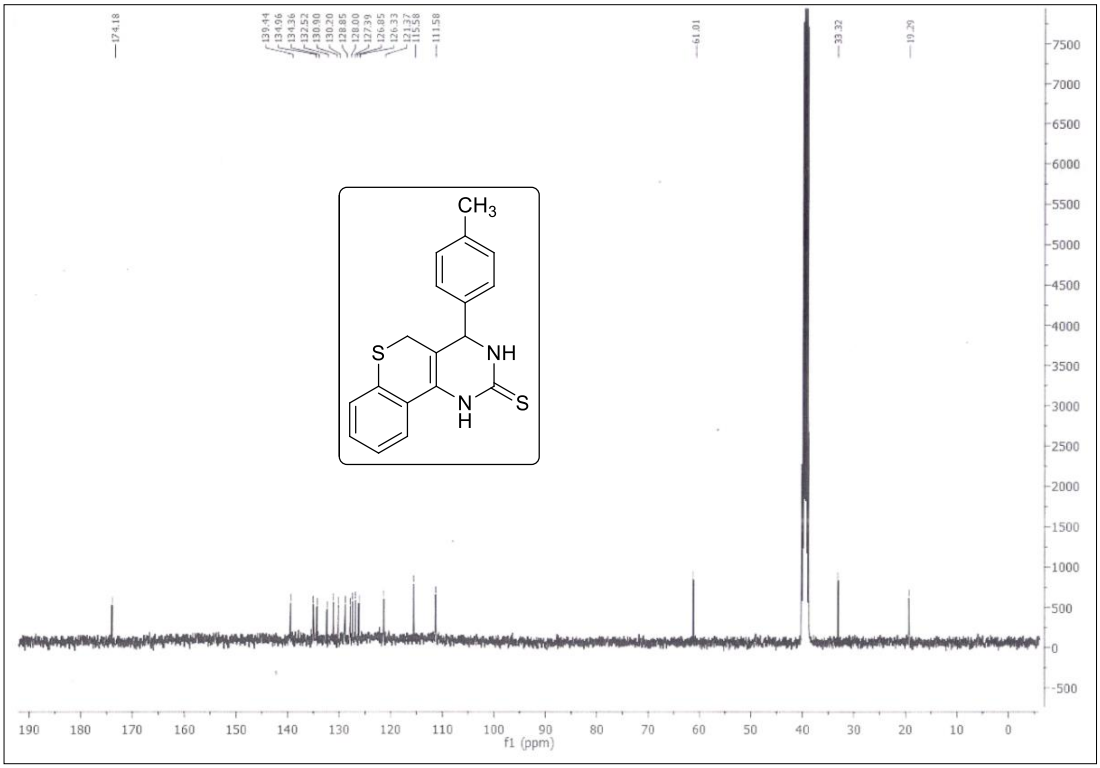

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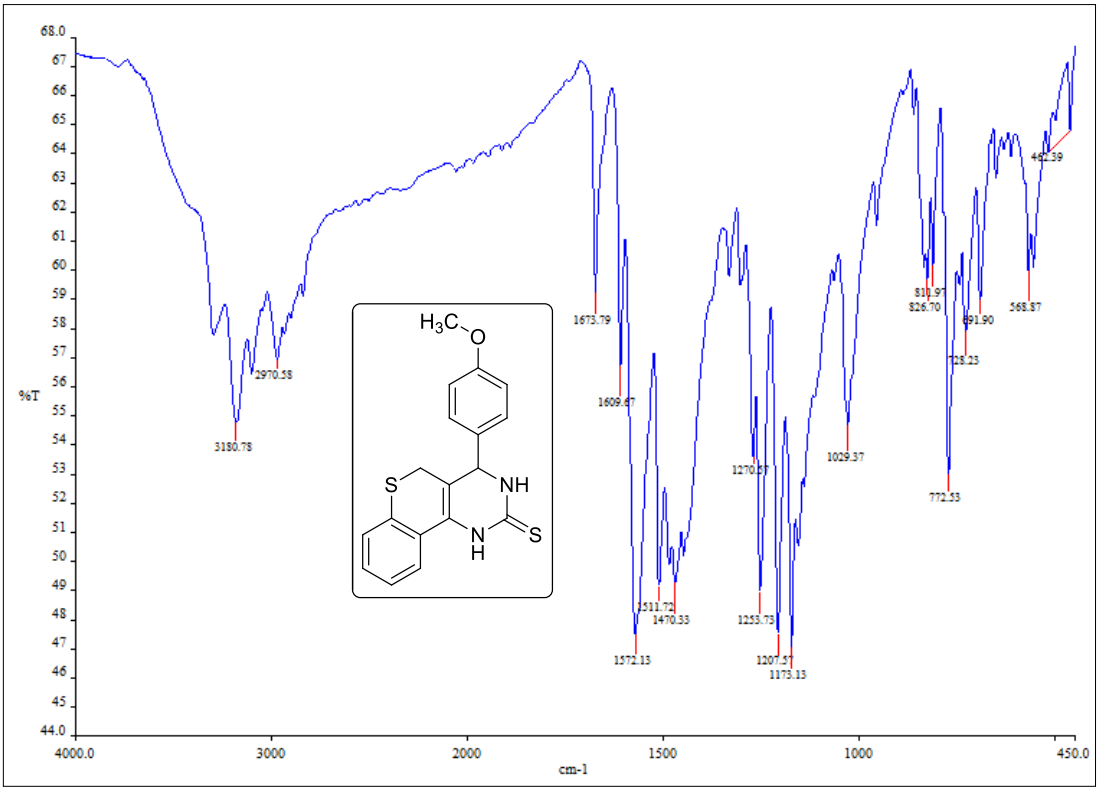

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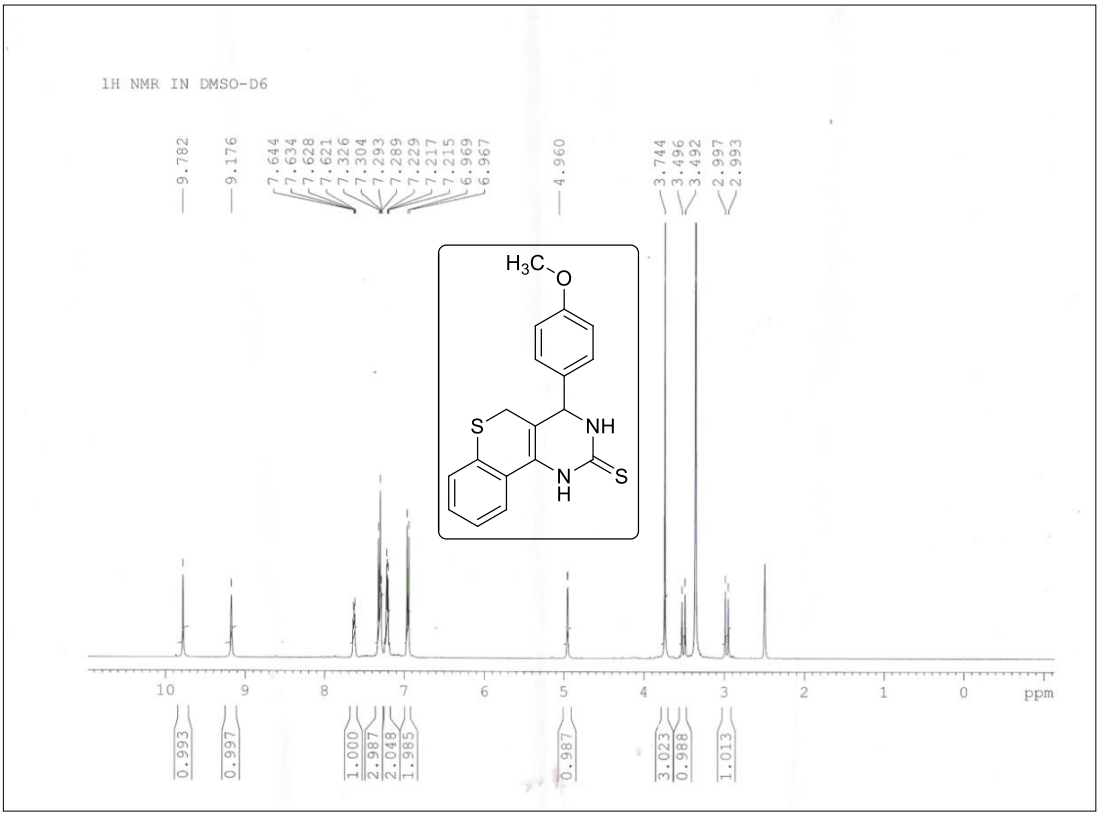
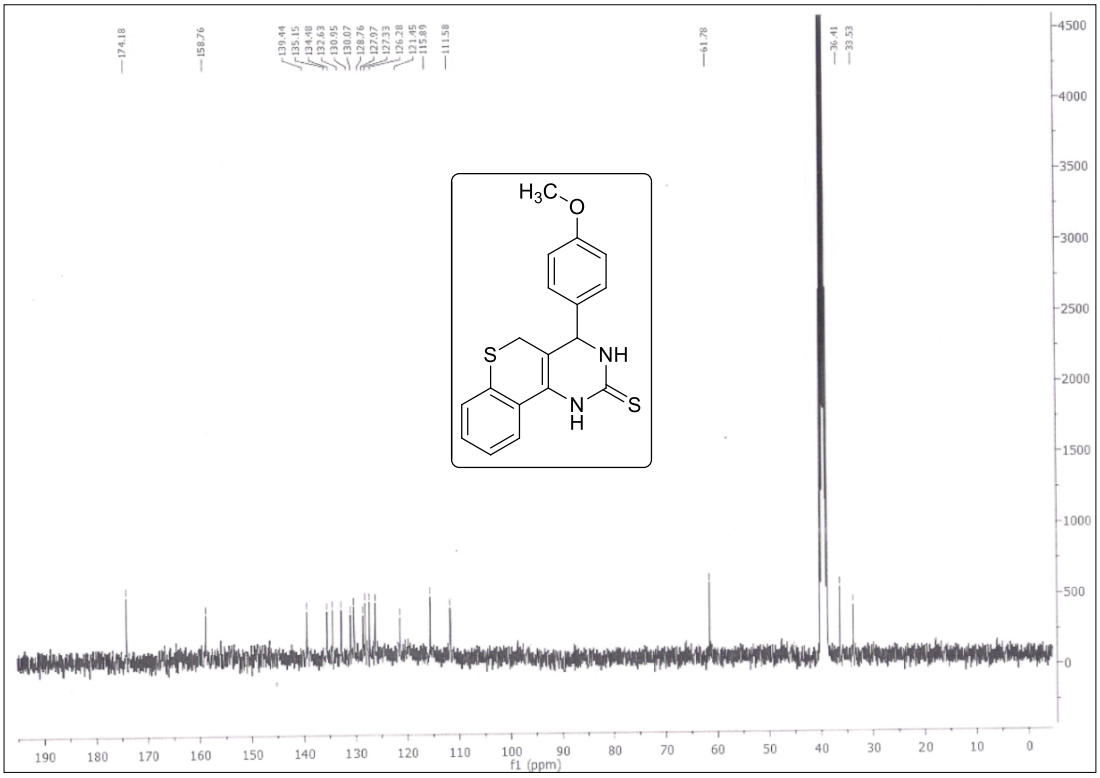


^{1}H NMR Spectrum of compound **4c**

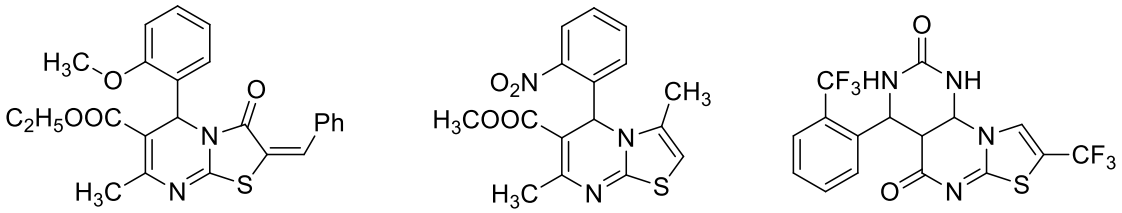


^{13}C NMR Spectrum of compound **4c**

GREEN CHEMICAL SYNTHESIS OF NOVEL FUSED THIAZOLO[4,3-*d*]THIOCHROMENO[4,3-*d*]PYRIMIDINE DERIVATIVES

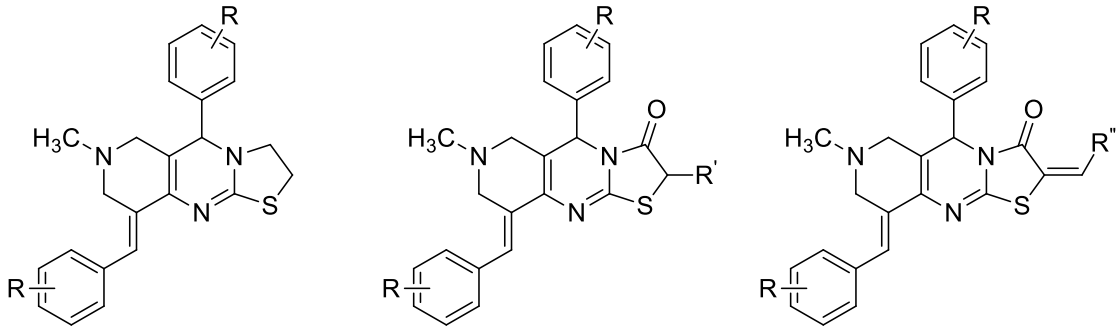
3.1. INTRODUCTION

Pyrimidines represent one of the most biologically and pharmaceutically active class of compounds.[1] When the pyrimidine moiety is fused with different heterocycles, it results in hybrid scaffolds with improved activity.[2] Thiazolo[3,2-*a*]pyrimidines are considered to be pyrimidine based hybrid scaffolds, which have attracted considerable attention in view of their broad biological and medicinal applications such as antimicrobial,[3] anti-biofilm,[4] anti-inflammatory,[5] antioxidant,[6] anticonvulsant,[7] anticancer[8] and anti-Parkinsonian activities.[9] They also serve as inhibitors of Bcl-2 family proteins and CDC25B phosphatases.[10] Prominently, the thiazolo[3,2-*a*]pyrimidines (**Fig. 1**) are potent calcium modulators (**1**), antiviral agents against human hepatitis C virus (HCV) (**2**) and antitumor activity (**3**).[11] Recently F. A. M. Al-Omary *et al.*[12] designed and synthesized a novel series of thiazolo[2,3-*b*] quinazolines, pyrido[4,3-*d*]thiazolo[3,2-*a*]pyrimidine derivatives and evaluated for *in vitro* antitumor activity for 60 cell lines panel assay. Compounds (**4**), **(5)** and (**6**) have shown remarkable broad-spectrum antitumor activity **(Fig. 2)**.



1 **2** **3**

Figure 1. Biologically active thiazolo[4,3-*d*]pyrimidine derivatives

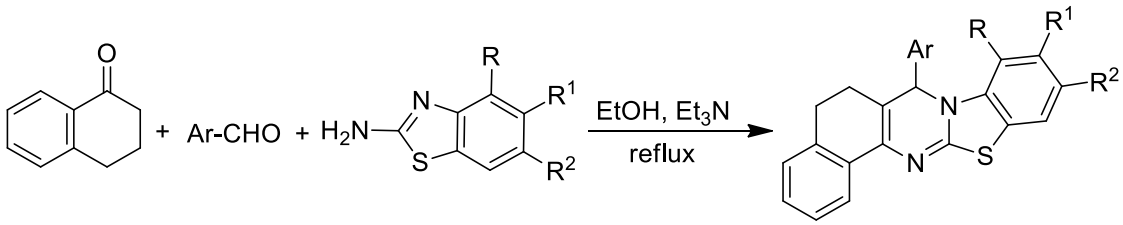


4 **5** **6**

Figure 2. Thiazolo pyrimidine derivatives fused to *N*-methylpipredine antitumor activity.

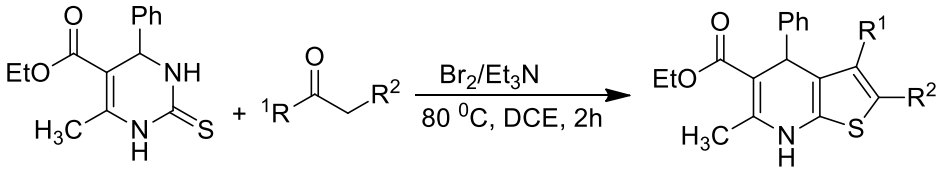
3.1.1. VARIOUS IMPORTANT APPROACHES FOR THE SYNTHESIS OF THIAZOLO[3,2-*a*]PYRIMIDINES

Kumar. M and co-workers[13] reported the synthesis of annulated benzothiazolo quinazolines by a diversity oriented simple method involving one-pot three-component reaction of substituted 2-aminobenzothiazoles, 1-tetralone and aromatic/heteroaromatic aldehydes in ethanol in the presence of catalytic amount of triethylamine (**Scheme 1**).



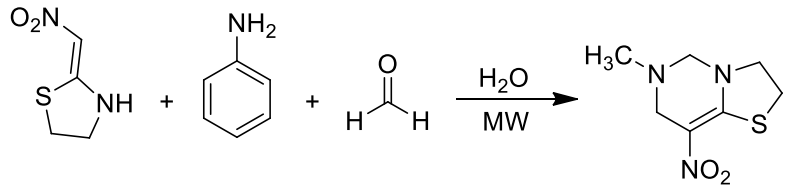
(**Scheme 1)**

Singh *et al.*[14] describe a convenient method for synthesis of thiazolo [3,2-*a*]pyrimidine derivatives in a one-pot procedure (S**cheme 2**).



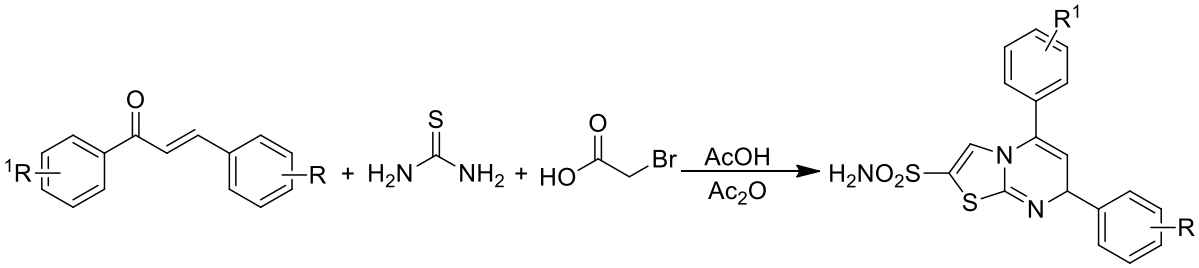
(**Scheme 2)**

Yıldırım *et al*,[15] designed a rapid and efficient protocol for the synthesis of novel nitrothiazolo [3,2-*c*]pyrimidines via microwave-mediated Mannich cyclisation (**Scheme 3**).



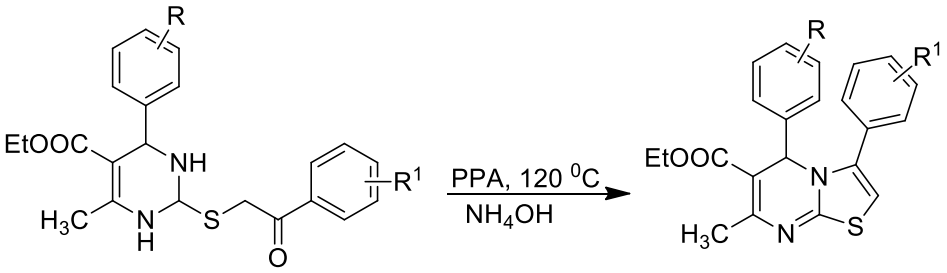
(**Scheme 3)**

Zhao *et al*,[16] synthesized a series of novel halogenated thiazolo [3,2-*a*] pyrimidin-3-one carboxylic acid derivatives (**Scheme 4**).



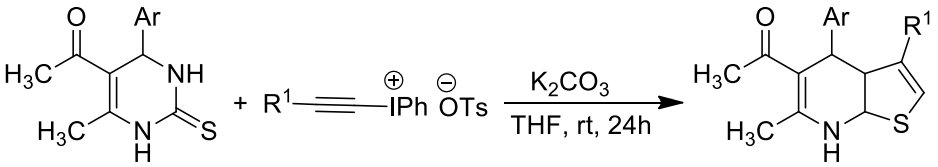
(**Scheme 4)**

S. G. Abdel moty *et al.*[17] synthesized a series of thiazolo[3,2-*a*]pyrimidine derivatives by heating 2-phenacylthio-dihydropyrimidine hydrobromides with freshly prepared polyphosphoric acid, followed by neutralization with ammonia (**Scheme 5**).



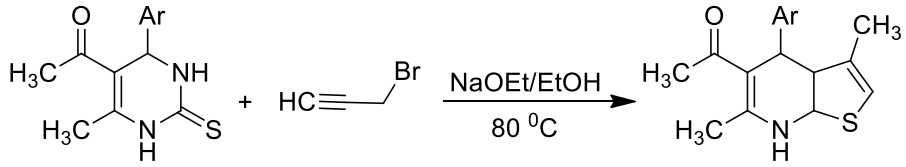
(**Scheme 5)**

A. V. Shelke *et al.*[18] synthesized a series of biologically active thiazolo[3,2-*a*]pyrimidine derivatives by the cyclocondensation of 3,4-dihydropyrimidin-2(1*H*)-thiones with alkynyl(aryl)iodonium tosylates (**Scheme 6**).



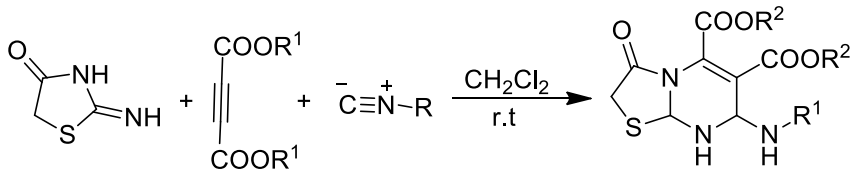
(**Scheme 6)**

S. Fatima *et al.*[19] reported an efficient one-pot synthesis of multifunctional 5*H* thiazolo[3,2-*a*]pyrimidines by the reaction of 4-aryl dihydrothiopyrimidines with propargyl bromide in the presence of inorganic base (**Scheme 7**).



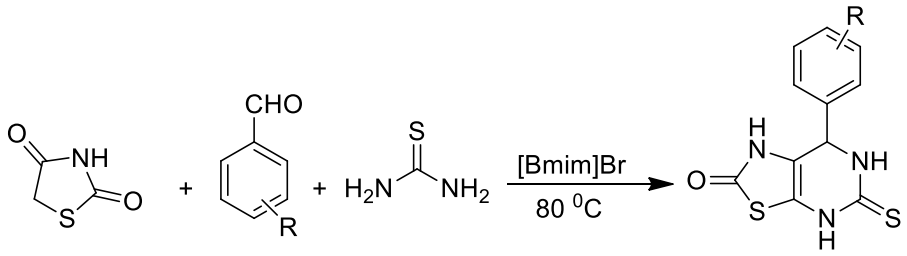
(**Scheme 7)**

Esmaeili *et al*[20] developed a simple and efficient method for the synthesis of an efficient regioselactive synthesis of highly functionalized 3-oxo-2,3-dihydro-5*H*-thiazolo [3,2-*a*] pyrimidines via an isocyanide-based three-component reaction (**Scheme 8**).



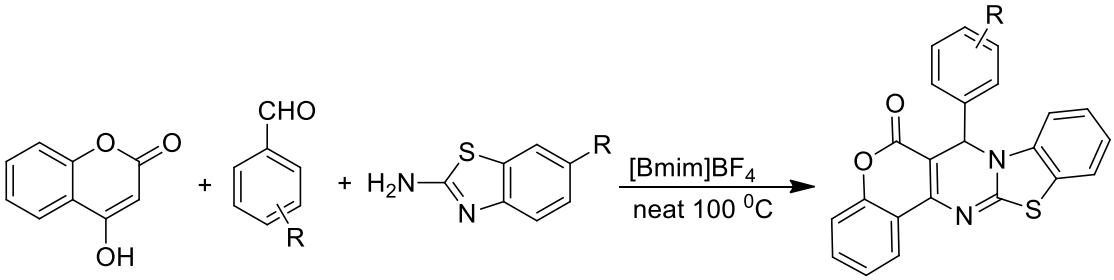
(**Scheme 8)**

P. Singh *et al*.[21] developed an efficient Ionic liquid catalyzed synthesis of 7-phenyl-1, 4, 6, 7-tetrahydro-thiazolo [5,4-*d*]pyrimidine-2,5-diones (**Scheme 9**).



(**Scheme 9)**

A. V. S. Reddy *et al*.[22] conducted a highly efficient and facile synthesis of densely functionalized thiazolo [3,2-*a*] chromeno [4,3-*d*] pyrimidin-6 (7*H*)-ones using [Bmim]BF_4 as a reusable catalyst (**Scheme 10**).



(**Scheme 10)**

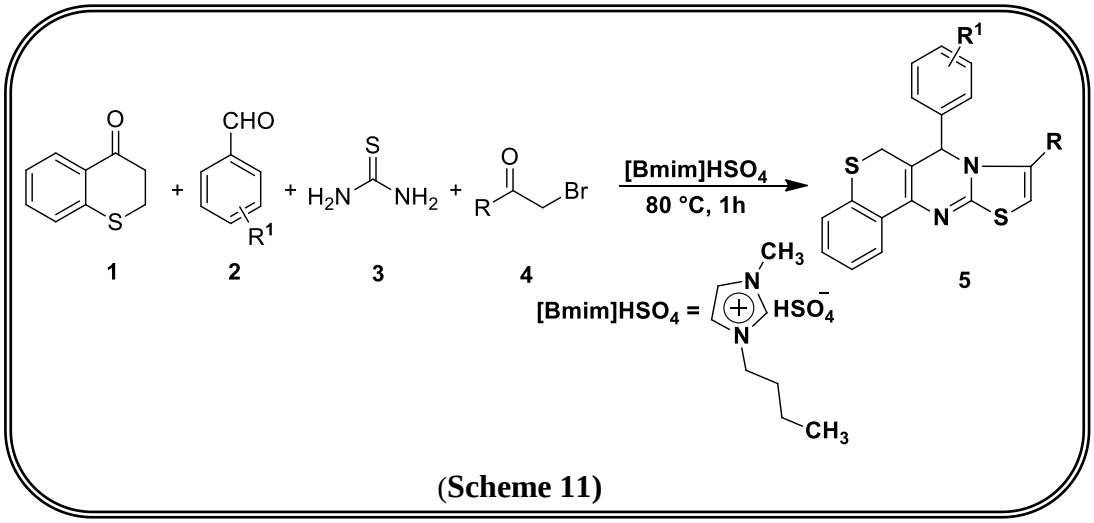
Multicomponent synthesis has recently been recognized as an important area in medicinal chemistry.[23-24] It allows the assembly of complex molecules in one pot, thus maximizing synthetic efficiency and reducing the costs.[25] In the last few decades there has been a growing emphasis on sustainable chemistry due to the global push to improve green credentials.[26-27] Ionic liquids have shown greater promise as an attractive alternatives to conventional organic solvents.[28-29] These reliable and inexpensive methods offer not only a

powerful platform to access molecular diversity but provide manipulative simplicity, enhanced reaction rates, improved product selectivity and cleaner products.[30]

3.2. PRESENT WORK

Compared with the conventional organic reactions, MCRs are advantageous in being highly convergent and to achieve structural complexity.[31-32] ILs can be considered as more than just alternative "green" solvents.[33] They differ from molecular solvents by their unique ionic character, as well as their "structure and organization", which can lead to specific effects, making them tunable and multipurpose materials.[34]

In view of the high degree of bio-activity shown by thiazolo[3,2-*a*]thiochromeno[4,3-*d*]pyrimidines, we have focused on the design of a novel structural entity that incorporates these three structural moieties thiazoles into a single molecular scaffold.[35,36] In the cause of present scheme are designed multicomponent green chemical method for the synthesis of thiazolo[3,2-*a*]thiochromeno[4,3-*d*]pyrimidines by using [Bmim]HSO_4 (**Scheme 11**).



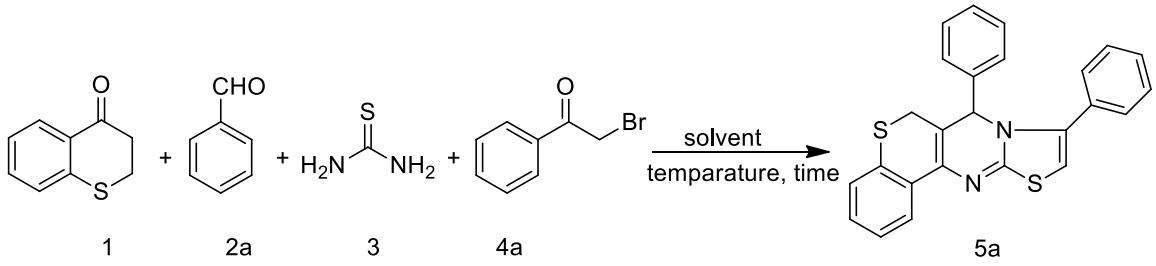
(**Scheme 11**)

3.3. Results and discussion

In order to optimize the reaction conditions for the four-component reaction, equimolar mixture of thiochroman-4-one **1**, benzaldehyde **2**, thiourea **3** and phenacyl bromides **4** was taken. Our initial effort was focused to study the effect of different solvents for the synthesis of thiazolo[3,2-*a*]thiochromeno[4,3-*d*]pyrimidines. The results of the model reaction carried out using different solvents are summarized in Table 1. When the reaction was carried out under neat conditions, the reaction did not proceed at all (Table 1, entry 1). Further the reaction was carried out in water and the yield of product was also low (Table 1, entry 2). It was observed that the yield was low, when the reaction was carried out using common

organic solvents (Table 1, entry 3-6). When the reaction was attempted in the presence of ionic liquids such as [Bmim]Cl, [Bmim]Br, [Bmim]$ZnCl_3$, [Bmim]BF_4, [Bmim]PF_6 and [Bmim]HSO_4 at 80 ºC, the product was obtained in 60%, 56%, 65%, 82%, 50% and 90% yields, respectively (Table 1, entry 7-12). However in terms of yield and reaction time, the best result was obtained with [Bmim]HSO_4 indicating that it was the most suitable reaction medium for the above transformation. To study the effect of temperature on the reaction, the model reaction was carried out at different temperatures (Table 1, entry 13-16). These results showed that with an increase in temperature, the yield of reaction varied significantly up to 80 ºC.

Table 1. Optimization of reaction conditions for the synthesis of **5a**



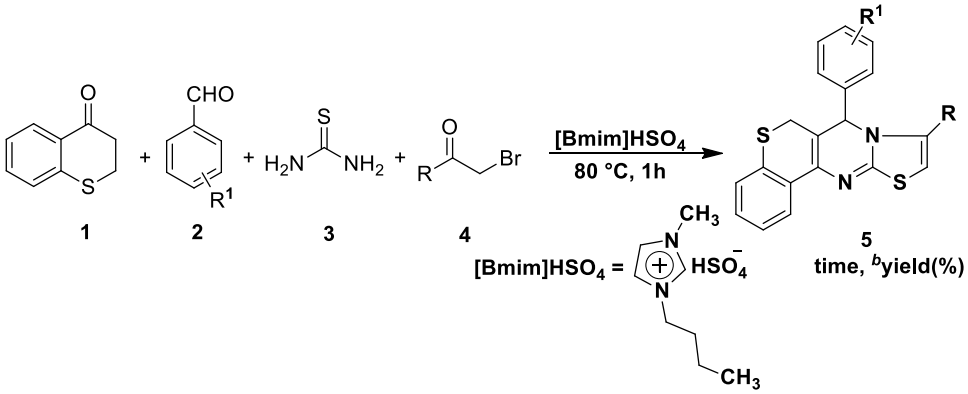
Entry[a]	Solvent	T (^{0}C)	Time (h)	Yield (%)[b]
1	neat	80	12	0
2	H_2O	Reflux	12	25
3	EtOH	Reflux	12	30
4	MeCN	reflux	12	20
5	DMF	80	12	30
6	AcOH	80	12	36
7	[Bmim]Cl	80	1	60
8	[Bmim]Br	80	1	56
9	[Bmim]$ZnCl_2$	80	1	65
10	[Bmim]BF_4	80	1	82
11	[Bmim]PF_6	80	1	50
12	**[Bmim] HSO_4**	**80**	**1**	**90**
13	[Bmim] HSO_4	r.t.	12	53
14	[Bmim] HSO_4	40	1	70
15	[Bmim] HSO_4	60	1	81
16	[Bmim] HSO_4	100	1	90

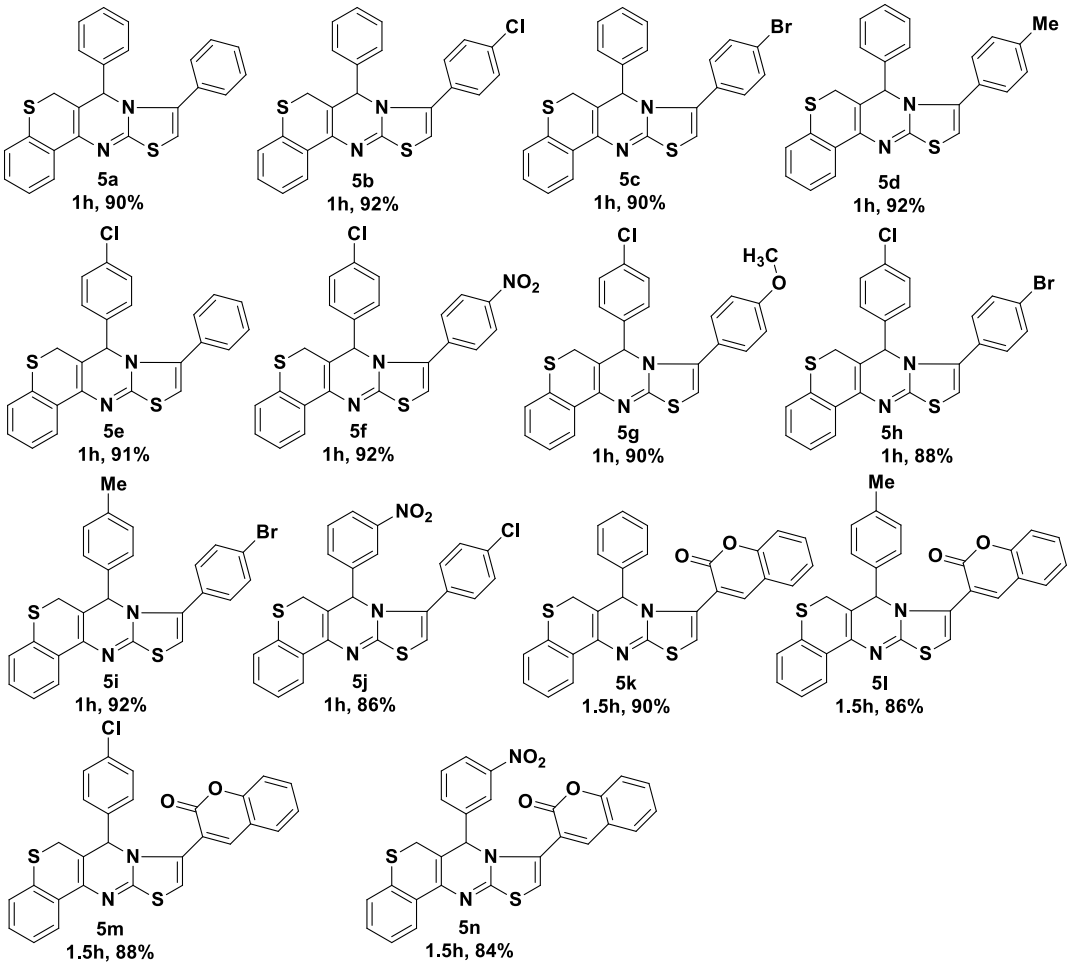
[a] Reaction conditions: thiochroman-4-one **1** (1 mmol), benzaldehyde **2a** (1 mmol)**,** thiourea **3** (1 mmol), phenacyl bromide **4a** (1 mmol) and solvent (1 mL). [b]Isolated yield.

No significant increase was observed in yield even at higher temperatures as compared to 80 ºC. Therefore the best optimal temperature condition observed for this reaction was 80 ºC. Subsequently, the reactions with diversely substituted aromatic aldehydes having electron releasing and electron-withdrawing functional groups also proceeded smoothly to yield various novel thiazolo[3,2-*a*]thiochromeno[4,3-*d*]pyrimidine derivatives (Table 2).

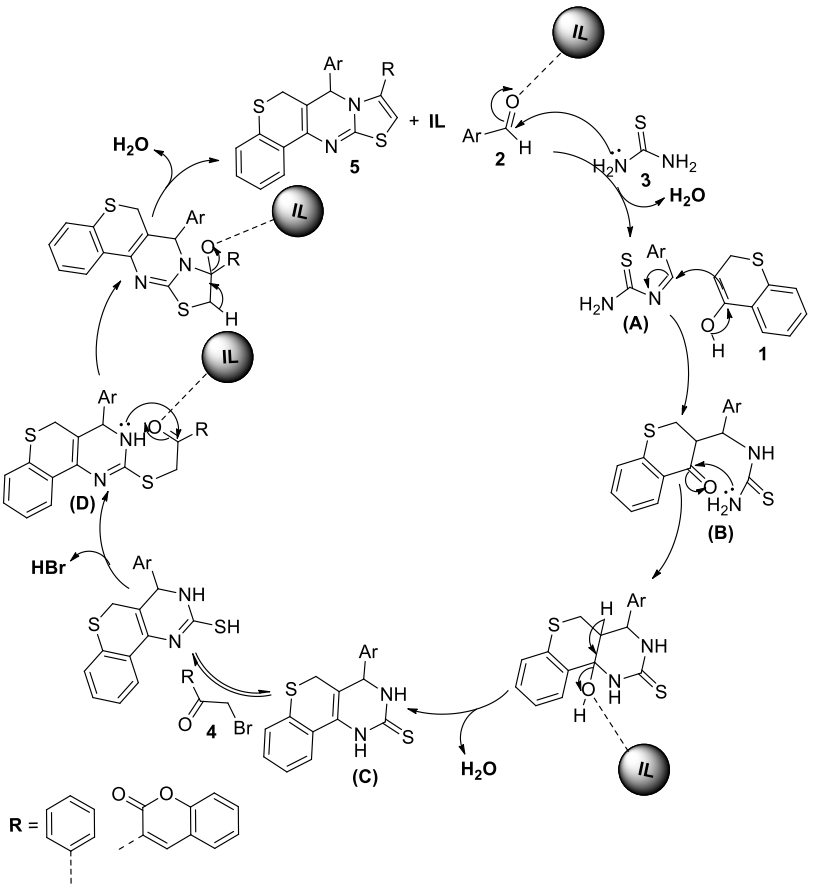
A speculative mechanistic explanation for this reaction is provided in **Scheme 11**. The formation of compound **5** proceeds *via* initial condensation between the benzaldehyde **1** and thiourea **2** to form the intermediate (**A**). This intermediate is attracted by the thiochroman-4-one **3** resulting in the intermediate (**B**) followed by elimination of water producing a double electrophilic pyrimidine-2(5*H*)-thione intermediate (**C**). Subsequently, the intermediate (**C**) undergoes intermolecular proton exchange and attack by the phenacyl bromide *via* elimination of hydrogen bromide to generate intermediate (**D**). Finally, the expected product (**5**) was obtained by intermolecular cyclization followed by the elimination of water from the intermediate (**D**).

Table 2. Synthesis of thiazolo[3,2-*a*]thiochromeno[4,3-*d*]pyrimidine derivatives (5a-n)[a]



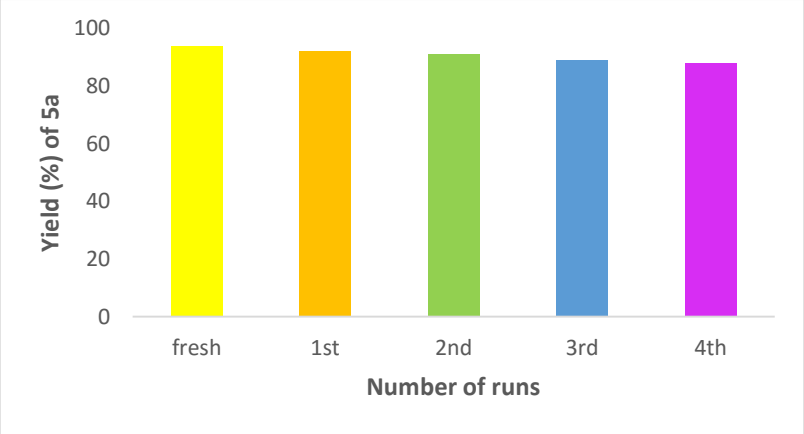


[a]Reaction conditions: thiochroman-4-one **1** (1 mmol), benzaldehyde **2a** (1 mmol)**,** thiourea **3** (1 mmol), phenacyl bromides **4** (1 mmol) and ionic liquid [Bmim]HSO_4 (1 mL). [b]Yields of the Isolated products.



Scheme 1. Proposed mechanism for the formation of thiazolo[3,2-*a*]thiochromeno[4,3-*d*]pyrimidines **5**.

We have also examined the recyclability of the reaction medium, i.e. [Bmim]HSO_4 ionic liquid, during the synthesis of thiazolo[3,2-*a*]thiochromeno[4,3-*d*]pyrimidine derivatives (**5a-n**). After completion, the reaction mixture was poured into ice cold water. The solid product obtained was isolated by filtration and the filtrate containing the ionic liquid [Bmim]HSO_4 was extracted with ethyl acetate to remove the non-ionic organic impurities. The ionic liquid was recovered from water under reduced pressure. Further, *tert*-butanol was added to [Bmim]HSO_4 to eliminate the bromide impurities in the form of volatile *tert*-butyl bromide. Finally, the ionic liquid was dried at 60-70 ºC and reused for subsequent reactions for an additional four cycles. It was observed that there was a slight decrease in its activity in terms of product yields, wherever the ionic liquid was used beyond the fourth cycle.



Reusability of [Bmim]HSO_4 in the synthesis of title compounds **5a**.

We have demonstrated an ionic liquid mediated novel protocol for the synthesis of thiazolo[3,2-*a*]thiochromeno[4,3-*d*]pyrimidines *via* the four component condensation reaction of thiochroman-4-one, benzaldehyde**,** thiourea and phenacyl bromide using [Bmim]HSO_4 as a green solvent medium. This procedure offers several notable advantages including shorter reaction times, excellent yields, simple workup procedure and ease of separation and recyclability of the ionic liquid.

3.4. EXPERMENTAL

Chemicals were purchased from Merck and Aldrich chemical companies. The ^{1}H NMR and ^{13}C NMR spectra were recorded in DMSO-d_6 solvent on Bruker WM-300 spectrometer using TMS as internal standard. The chemical shifts were reported in ppm (δ). Mass spectra (ESI) were carried out on a JEOL JMSD-300 spectrometer. CHN analysis was carried out using Carlo Erba EA 1108 automatic elemental analyser. Melting points were determined in open capillary tubes and were recorded on a Stuart SMP30 melting point apparatus.

3.4.1. SPECTRAL DISCUSSION

IR: In all the compounds **4a–n** the formation of pyrimidine was confirmed due to appearance of C=N group around 1554-1518 cm^{-1}

^{1}H NMR: In ^{1}H NMR, the singlet protons of (CH) group were observed as a singlet at δ 5.25-5.68 ppm. The formation of thiazole was conformed due to appearance of (=C-H) at 7.75-7.78 ppm of all the compounds.

^{13}C NMR: In ^{13}C NMR, the signal appeared at δ 31-30.18 ppm can be attributed to CH_2 carbon. The signal observed at δ 62.29-61.44 ppm was assigned to (CH) group were observed as a singlet at carbon and in all the compounds.

Mass: The structures of all synthesized compounds were further confirmed by its mass spectra. The mass spectra detected the expected molecular ion signals (M + 1) corresponding to respective molecular weight of the synthesized compounds.

3.4.2. X-ray crystallographic study

X-ray data for the compound **5a** were collected at room temperature using a Bruker Smart Apex CCD diffractometer with graphite monochromated MoKα radiation (λ=0.71073Å) with ω-scan method.[37] Preliminary lattice parameters and orientation matrices were obtained from four sets of frames. Unit cell dimensions were determined using 7488 reflections. Integration and scaling of intensity data were accomplished using SAINT program.[37] The structure was solved by Direct Methods using SHELXS97[38] and refinement was carried out by full-matrix least-squares technique using SHELXL-2014/7.[38] Anisotropic displacement parameters were included for all non-hydrogen atoms. H bound to N atom was located from the difference Fourier map. All other H atoms were positioned geometrically and treated as riding on their parent C atoms with C-H distances of 0.93--0.97 Å, and with $U_{iso}(H) = 1.2U_{eq}$ (C) or $1.5U_{eq}$ for methyl atoms. The sulfur atom of thiochromene ring is found to be disordered over two sites; 0.534 site occupancy for S1 atom (representing the major component) and 0.466 site occupancy for S1D atom (minor component). PART and FVAR instructions were used for modeling the sulfur atom disorder and DFIX instruction for restraining the C-S bond distance to 1.70 Å with e.s.d value of 0.01Å.

Crystal data for 5a: The compound crystallized as a salt in the presence of strong HBr acid. The asymmetric unit consists of a protonated form of 5a as cation and bromide anion in 1:1 stoichiometric ratio with the molecular formula $C_{25}H_{19}N_2S_2^+.Br^-$, M = 491.45, crystal dimensions 0.32 x 0.21 x 0.15 mm^3, monoclinic, space group $P2_1/n$ (No. 14), a = 10.2834(19), b = 9.0889(17), c = 23.455(4) Å, β = 99.254(1)°, V = 2165.7(2) $Å^3$, Z = 4, D_c = 1.507 g/cm^3, F_{000} = 1000, CCD area detector, MoKα radiation, λ = 0.71073Å, T = 293(2)K, $2\theta_{max}$ = 52.4°, 18881 reflections collected, 4277 unique (R_{int} = 0.038), Final *GooF* = 1.05, $R1$ = 0.0381, $wR2$ = 0.1007, R indices based on 4277 reflections with $I > 2\sigma(I)$ (refinement on F^2), 285 parameters, μ = 2.106 mm^{-1}, Min and Max Resd. Dens. = -0.39 and 0.78 e/$Å^3$. CCDC **1451972** contains the supplementary crystallographic data for this paper which can be obtained free of charge at https://summary.ccdc.cam.ac.uk/structure-summary-form or from the Cambridge Crystallographic Data Centre (CCDC), 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44(0) 1223 336 033; email: deposit@ccdc.cam.ac.uk.

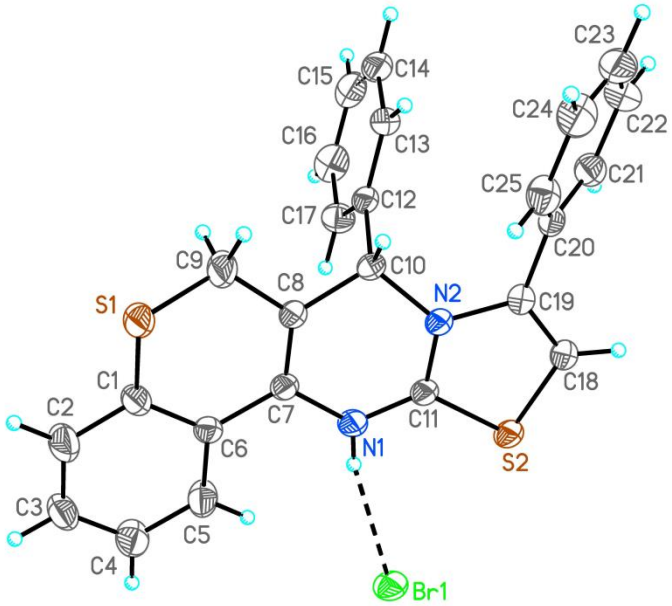


Figure 2. ORTEP representation of compound **5a**. The thermal ellipsoids are drawn at 50% probability level and H atoms are shown as small spheres of arbitrary radius (CCDC 1451972).

3.4.3. General procedure for the synthesis of thiazolo[3,2-*a*]thiochromeno[4,3-*d*]pyrimidines derivatives (5a-n)

A mixture of thiochroman-4-one **1** (1 mmol), benzaldehyde **2** (1 mmol), thiourea **3** (1 mmol) was stirred in presence of ionic liquid, [Bmim]HSO_4 (1 mL) at 80 °C for 20 min followed by the addition of phenacyl bromides **4** (1 mmol) and the resulting mixture was refluxed for 40 min (silicon oil bath temperature). The progress of reaction was monitored by TLC. After completion of the reaction, the mixture was diluted with 20 mL of water, 2 × 20 mL of EtOAc was added and shaken vigorously. The organic layer was separated from the solution. The aqueous layer was evaporated, the ionic liquid was dried at 60-70 °C under vacuum and then reused. The results have shown that the [Bmim]HSO_4 ionic liquid could be reused at least for four times without any significant loss in the yield. The product was purified by column chromatography on neutral alumina (100-200 mesh), eluted with a solvent system of ethyl acetate-hexane (20:80 v/v) to afford the title compounds **5a-n**.

3.4.4. PHYSICAL AND SPECTRAL DATA

7,9-Diphenyl-6,7-dihydrothiazolo[3,2-a]thiochromeno[4,3-d]pyrimidine (5a). Brown powder; mp: 254-256 °C; **IR** (KBr) υ_{max} (cm^{-1}): 2889, 1542, 1523, 1108; **^{1}H NMR** (400 MHz, DMSO-d_6): δ 2.98 (dd, *J* = 16 Hz, 1H), 3.62 (dd, *J* = 16 Hz, 1H), 6.28 (s, 1H), 6.84 (d, *J* = 8 Hz, 2H), 7.16 (d, *J* = 8 Hz, 2H), 7.22 (d, *J* = 8 Hz, 3H), 7.32 (d, *J* = 8 Hz, 1H), 7.38 (t, *J* = 8 Hz, 5H), 7.41 (d, *J* = 8 Hz, 1H), 7.51 (s, 1H); **^{13}C NMR** (100 MHz, DMSO-d_6): 162.09,

140.16, 138.68, 133.16, 130.55, 130.09, 130.04, 129.59, 129.17, 128.97, 128.30, 128.12, 127.45, 126.58, 124.55, 110.12, 108.85, 62.29, 25.29; **ESI-MS**: *m/z* 410 $(M + 1)^+$; Anal. Calcd. For $C_{25}H_{18}N_2S_2$; C, 73.14; H, 4.42; N, 6.82; Found: C, 73.04; H, 4.47; N, 6.68.

9-(4-Chlorophenyl)-7-phenyl-6,7-dihydrothiazolo[3,2-a]thiochromeno[4,3 d]pyrimidine (5b). Brown powder; mp: 258-260 °C; **IR** (KBr) υ_{max} (cm^{-1}): 2930, 1740, 1554, 1074; **^{1}H NMR** (400 MHz, DMSO-d_6): δ 2.98 (dd, *J* = 16 Hz, 1H), 3.56 (dd, *J* = 16 Hz, 1H), 6.30 (s, 1H), 6.87 (d, *J* = 8 Hz, 2H), 7.23 (t, *J* = 8 Hz, 4H), 7.34-7.50 (m, 5H), 7.53 (d, *J* = 8 Hz, 2H), 7.70 (s, 1H); **^{13}C NMR** (100 MHz, DMSO-d_6): 162.28, 148.59, 137.72, 137.43, 134.42, 133.22, 131.59, 130.20, 129.64, 129.04, 128.62, 128.30, 126.59, 124.69, 123.98, 122.82, 108.37, 61.49, 25.14; **ESI-MS**: *m/z* 445 $(M + 1)^+$; Anal. Calcd. For $C_{25}H_{17}N_2S_2$; C, 67.48; H, 3.85; N, 6.30; Found: C, 67.40; H, 3.89; N, 6.42.

9-(4-Bromophenyl)-7-phenyl-6,7-dihydrothiazolo[3,2-a]thiochromeno[4,3-d]pyrimidine (5c). Brown powder; mp: 250-252 °C; **IR** (KBr) υ_{max} (cm^{-1}): 2760, 1551, 1524, 1066; **^{1}H NMR** (400 MHz, DMSO-d_6): δ 2.99 (dd, *J* = 16 Hz, 1H), 3.59 (dd, *J* = 16 Hz, 1H), 6.25 (s, 1H), 6.90 (d, *J* = 8 Hz, 2H), 7.16-7.19 (m, 3H), 7.24 (t, *J* = 8 Hz, 2H), 7.27-7.59 (m, 4H), 7.72 (d, *J* = 8 Hz, 2H), 7.75 (s, 1H); **^{13}C NMR** (100 MHz, DMSO-d_6): 162.15, 138.93, 133.14, 132.08, 131.92, 130.11, 129.68, 129.25, 128.30, 127.51, 126.59, 126.17, 124.57, 124.26, 108.76, 62.25, 25.62; **ESI-MS**: *m/z* 490 $(M + 1)^+$; Anal. Calcd. For $C_{25}H_{17}BrN_2S_2$; C, 61.35; H, 3.50; N, 5.72; Found: C, 61.23; H, 3.56; N, 5.92.

7-Phenyl-9-(p-tolyl)-6,7-dihydrothiazolo[3,2-a]thiochromeno[4,3-d]pyrimidine (5d). Brown powder; mp: 241-243 °C; **IR** (KBr) υ_{max} (cm^{-1}): 2895, 1542, 1526, 1046; **^{1}H NMR** (400 MHz, DMSO-d_6): δ 2.19 (s, 3H), 2.97 (dd, *J* = 16 Hz, 1H), 3.60 (dd, *J* = 16 Hz, 1H), 6.23 (s, 1H), 6.70 (d, *J* = 8 Hz, 2H), 6.95 (d, *J* = 8 Hz, 2H), 7.26 (d, *J* = 8 Hz, 3H), 7.36-7.44 (m, 5H), 7.52 (d, *J* = 8 Hz, 1H), 7.73 (s, 1H); **^{13}C NMR** (100 MHz, DMSO-d_6): 161.97, 140.11, 139.15, 135.78, 133.15, 130.57, 130.04, 129.72, 129.01, 128.29, 128.13, 127.41, 126.57, 126.04, 124.60, 109.13, 62.00, 25.35, 21.18; **ESI-MS**: *m/z* 425 $(M + 1)^+$; Anal. Calcd. For $C_{26}H_{20}N_2S_2$; C, 73.55; H, 4.75; N, 6.60; Found: C, 73.39; H, 4.79; N, 6.41.

7-(4-Chlorophenyl)-9-phenyl-6,7-dihydrothiazolo[3,2-a]thiochromeno[4,3-d]pyrimidine (5e). Brown powder; mp: 254-256 °C; **IR** (KBr) υ_{max} (cm^{-1}): 2930, 1552, 1528, 1086; **^{1}H NMR** (400 MHz, DMSO-d_6): δ 2.98 (dd, *J* = 16 Hz, 1H), 3.56 (dd, *J* = 16 Hz, 1H), 6.30 (s, 1H), 6.87 (d, *J* = 8 Hz, 2H), 7.23 (t, *J* = 8 Hz, 4H), 7.34-7.50 (m, 5H), 7.53 (d, *J* = 8 Hz, 2H), 7.70 (s, 1H); **^{13}C NMR** (100 MHz, DMSO-d_6): 162.48, 147.33, 136.72, 137.13, 134.63,

132.60, 131.59, 130.13, 129.79, 129.28, 128.69, 128.60, 126.56, 124.69, 123.68, 122.83, 108.36, 61.46, 25.27; **ESI-MS**: *m/z* 446 $(M + 1)^+$; Anal. Calcd. For $C_{25}H_{17}ClN_2S_2$; C, 67.48; H, 3.85; N, 6.30; Found: C, 67.32; H, 3.89; N, 6.56.

7-(4-Chlorophenyl)-9-(4-nitrophenyl)-6,7-dihydrothiazolo[3,2-a]thiochromeno[4,3-d]pyrimidine (5f). Brown powder; mp: 258-260 °C; **IR** (KBr) υ_{max} (cm^{-1}): 2938, 1554, 1529, 1085; **^{1}H NMR** (400 MHz, DMSO-d_6): δ 2.99 (dd, *J* = 16 Hz, 1H), 3.55 (dd, *J* = 16 Hz, 1H), 6.34 (s, 1H), 6.94 (d, *J* = 8 Hz, 2H), 7.25 (d, *J* = 8 Hz, 1H), 7.35 (d, *J* = 8 Hz, 2H), 7.40 (d, *J* = 8 Hz, 2H), 7.58 (d, *J* = 8 Hz, 2H), 7.75 (d, *J* = 8 Hz, 1H), 8.25 (d, *J* = 8 Hz, 2H), 8.37 (s, 1H); **^{13}C NMR** (100 MHz, DMSO-d_6): 162.28, 148.59, 147.78, 137.72, 137.43, 134.42, 133.40, 133.22, 131.59, 130.44, 130.20, 129.64, 129.37, 129.04, 128.62, 128.30, 126.59, 124.69, 123.98, 122.82, 108.37, 61.49, 25.14; **ESI-MS**: *m/z* 491 $(M + 1)^+$; Anal. Calcd. For $C_{25}H_{16}ClN_3O_2S_2$; C, 61.28; H, 3.29; N, 8.58; Found: C, 61.45; H, 3.34; N, 8.28.

7-(4-Chlorophenyl)-9-(4-methoxyphenyl)-6,7-dihydrothiazolo[3,2-a]thiochromeno[4,3-d]pyrimidine (5g). Brown powder; mp: 264-266 °C; **IR** (KBr) υ_{max} (cm^{-1}): 2938, 1712, 1594, 1012; **^{1}H NMR** (400 MHz, DMSO-d_6): δ 2.99 (dd, *J* = 16 Hz, 1H), 3.55 (dd, *J* = 16 Hz, 1H), 3.66 (s, 3H), 6.35 (s, 1H), 6.94 (d, *J* = 8 Hz, 2H), 7.25 (d, *J* = 8 Hz, 2H), 7.37 (t, *J* = 8 Hz, 4H), 7.58 (d, *J* = 8 Hz, 1H), 7.76 (d, *J* = 8 Hz, 1H), 8.25 (d, *J* = 8 Hz, 2H), 8.37 (s, 1H); **^{13}C NMR** (100 MHz, DMSO-d_6): 161.93, 161.04, 139.94, 137.80, 134.22, 133.24, 131.64, 130.13, 129.41, 129.20, 128.32, 126.58, 124.57, 120.15, 114.47, 108.32, 61.45, 55.93, 25.18; **ESI-MS**: *m/z* 476 $(M + 1)^+$; Anal. Calcd. For $C_{26}H_{19}ClN_2OS_2$; C, 65.74; H, 4.03; N, 5.90; Found: C, 65.61; H, 4.09; N, 5.74.

9-(4-Bromophenyl)-7-(4-chlorophenyl)-6,7-dihydrothiazolo[3,2-a]thiochromeno[4,3-d]pyrimidine (5h). Red powder; mp: 245-247 °C; **IR** (KBr) υ_{max} (cm^{-1}): 2926, 1552, 1525, 1015; **^{1}H NMR** (400 MHz, DMSO-d_6): δ 2.99 (dd, *J* = 16 Hz, 1H), 3.56 (dd, *J* = 16 Hz, 1H), 6.29 (s, 1H), 6.95 (d, *J* = 8 Hz, 2H), 7.21 (d, *J* = 8 Hz, 2H), 7.25 (d, *J* = 8 Hz, 2H), 7.33 (d, *J* = 8 Hz, 4H), 7.37 (d, *J* = 8 Hz, 1H), 7.63 (d, *J* = 8 Hz, 1H), 8.38 (s, 1H); **^{13}C NMR** (100 MHz, DMSO-d_6): 162.15, 138.74, 137.67, 134.31, 133.22, 132.13, 132.00, 130.14, 129.45, 129.25, 128.31, 127.29, 126.58, 124.63, 124.36, 108.30, 61.44, 25.13; **ESI-MS**: *m/z* 525 $(M + 1)^+$; Anal. Calcd. For $C_{26}H_{16}BrClN_2S_2$; C, 57.31; H, 3.08; N, 5.35; Found: C, 57.52; H, 3.14; N, 5.58.

9-(4-Bromophenyl)-7-(p-tolyl)-6,7-dihydrothiazolo[3,2-a]thiochromeno[4,3-d]pyrimidine (5i). Brown powder; mp: 274-276 °C; **IR** (KBr) υ_{max} (cm^{-1}): 2754, 1562, 1550, 1067; **^{1}H**

NMR (400 MHz, DMSO-d_6): δ 2.21 (s, 3H), 2.98 (dd, *J* = 16 Hz, 1H), 3.58 (dd, *J* = 16 Hz, 1H), 6.20 (s, 1H), 6.77 (d, *J* = 8 Hz, 2H), 6.70 (d, *J* = 8 Hz, 2H), 7.21 (d, *J* = 8 Hz, 2H), 7.33-7.41 (m, 4H), 7.63 (d, *J* = 8 Hz, 2H), 7.73 (s, 1H); **^{13}C NMR** (100 MHz, DMSO-d_6): 162.07, 139.29, 138.92, 135.75, 133.14, 132.08, 131.98, 130.10, 129.81, 128.31, 127.40, 126.59, 126.00, 124.54, 124.28, 109.06, 61.96, 25.30, 21.20; **ESI-MS**: *m/z* 504 $(M + 1)^+$; Anal. Calcd. For $C_{26}H_{19}BrN_2S_2$; C, 62.02; H, 3.80; N, 5.56; Found: C, 62.27; H, 3.86; N, 5.89.

9-(4-Chlorophenyl)-7-(3-nitrophenyl)-6,7-dihydrothiazolo[3,2-a]thiochromeno[4,3-d]pyrimidine (5j). Brown powder; mp: 268-270 °C; **IR** (KBr) υ_{max} (cm^{-1}): 2926, 1557, 1526, 1085; **^{1}H NMR** (400 MHz, DMSO-d_6): δ 3.01 (dd, *J* = 16 Hz, 1H), 3.54 (dd, *J* = 16 Hz, 1H), 6.46 (s, 1H), 7.21 (d, *J* = 8 Hz, 2H), 7.37 (d, *J* = 8 Hz, 4H), 7.44 (d, *J* = 8 Hz, 2H), 7.52 (s, 1H), 7.57 (d, *J* = 8 Hz, 2H), 7.74 (d, *J* = 8 Hz, 1H), 8.13 (s, 1H); **^{13}C NMR** (100 MHz, DMSO-d_6): 166.83, 158.13, 153.55, 148.12, 143.51, 139.88, 136.04, 134.66, 133.85, 133.03, 132.13, 130.23, 129.03, 128.28, 126.56, 125.38, 124.89, 124.06, 122.93, 118.30, 115.85, 95.18, 61.36, 24.97; **ESI-MS**: *m/z* 491 $(M + 1)^+$; Anal. Calcd. For $C_{25}H_{16}ClN_3O_2S2$; C, 61.28; H, 3.29; N, 8.58; Found: C, 61.12; H, 3.35; N, 8.39.

3-(7-Phenyl-6,7-dihydrothiazolo[3,2-a]thiochromeno[4,3-d]pyrimidin-9-yl)-2H-chromen-2-one (5k). Brown powder; mp: 283-285 °C; **IR** (KBr) υ_{max} (cm^{-1}): 2901, 1713, 1553, 1083; **^{1}H NMR** (400 MHz, DMSO-d_6): δ 2.99 (dd, *J* = 16 Hz, 1H), 3.58 (dd, *J* = 16 Hz, 1H), 6.32 (s, 1H), 7.22 (t, *J* = 8 Hz, 4H), 7.37 (d, *J* = 8 Hz, 2H), 7.40 (d, *J* = 8 Hz, 3H), 7.49 (d, *J* = 8 Hz, 2H), 7.75 (d, *J* = 8 Hz, 2H), 7.90 (s, 1H), 8.32 (s, 1H); **^{13}C NMR** (100 MHz, DMSO-d_6): 162.16, 158.91, 154.00, 147.35, 138.97, 134.21, 133.66, 133.22, 129.96, 129.48, 128.29, 127.75, 126.57, 125.60, 124.65, 118.43, 116.79, 116.24, 108.23, 62.26, 25.12; **ESI-MS**: *m/z* 479 $(M + 1)^+$; Anal. Calcd. For $C_{28}H_{18}N_2O_2S2$; C, 70.27; H, 3.79; N, 5.85; Found: C, 70.13; H, 3.85; N, 5.61.

3-(7-p-Tolyl)-6,7-dihydrothiazolo[3,2-a]thiochromeno[4,3-d]pyrimidin-9-yl)-2H-chromen-2-one (5l). Red powder; mp: 250-252 °C; **IR** (KBr) υ_{max} (cm^{-1}): 2885, 1740, 1554, 1074; **^{1}H NMR** (400 MHz, DMSO-d_6): δ 2.15 (s, 3H), 2.98 (dd, *J* = 16 Hz, 1H), 3.57 (dd, *J* = 16 Hz, 1H), 6.28 (s, 1H), 6.98 (d, *J* = 8 Hz, 2H), 7.09 (d, *J* = 8 Hz, 2H), 7.35-7.41 (m, 3H), 7.46 (t, *J* = 8 Hz, 1H), 7.51 (d, *J* = 8 Hz, 1H), 7.68 (d, *J* = 8 Hz, 1H), 7.75 (t, *J* = 8 Hz, 2H), 7.89 (s, 1H), 8.20 (s, 1H); **^{13}C NMR** (100 MHz, DMSO-d_6): 162.06, 159.00, 153.98, 147.21, 139.58, 136.08, 134.18, 133.74, 133.23, 130.12, 130.00, 129.88, 128.30, 127.79, 126.56, 125.59, 124.65, 118.51, 116.80, 116.26, 108.39, 62.12, 25.14, 21.15; **ESI-MS**: *m/z* 493 $(M + 1)^+$;

Anal. Calcd. For $C_{29}H_{20}N_2O_2S2$; C, 70.71; H, 4.09; N, 5.69; Found: C, 70.62; H, 4.14; N, 5.43.

3-(7-(4-Chlorophenyl)-6,7-dihydrothiazolo[3,2-a]thiochromeno[4,3-d]pyrimidin-9-yl)-2H-chromen-2-one (5m). Brown powder; mp: 264-266 °C; **IR** (KBr) υ_{max} (cm^{-1}): 2926, 1711, 1570, 1031; **^{1}H NMR** (400 MHz, DMSO-d_6): δ 3.01 (dd, *J* = 16 Hz, 1H), 3.56 (dd, *J* = 16 Hz, 1H), 6.34 (s, 1H), 7.28 (d, *J* = 8 Hz, 2H), 7.39 (d, *J* = 8 Hz, 3H), 7.50 (t, *J* = 8 Hz, 2H), 7.70-7.76 (m, 5H), 7.98 (s, 1H), 8.33 (s, 1H); **^{13}C NMR** (100 MHz, DMSO-d_6): 162.15, 158.91, 154.02, 147.57, 142.78, 138.03, 134.64, 134.26, 133.60, 133.41, 133.27, 131.38, 130.14, 129.97, 129.51, 129.72, 128.28, 126.56, 125.63, 125.29, 124.75, 118.43, 116.84, 116.16, 107.80, 61.37, 25.03; **ESI-MS**: *m/z* 514 $(M + 1)^+$; Anal. Calcd. For $C_{28}H_{17}N_2O_2S2$; C, 65.55; H, 3.34; N, 5.46; Found: C, 65.37; H, 3.39; N, 5.78.

3-(7-(3-Nitrophenyl)-6,7-dihydrothiazolo[3,2-a]thiochromeno[4,3-d]pyrimidin-9-yl)-2H-chromen-2-one (5n). Brown powder; mp: 276-278 °C; **IR** (KBr) υ_{max} (cm^{-1}): 2927, 1723, 1524, 1068; **^{1}H NMR** (400 MHz, DMSO-d_6): δ 2.92 (dd, *J* = 16 Hz, 1H), 3.39 (dd, *J* = 16 Hz, 1H), 6.00 (s, 1H), 7.09 (d, *J* = 8 Hz, 4H), 7.34-7.39 (m, 1H), 7.43-7.49 (m, 1H), 7.55-7.63 (m, 1H), 7.76 (d, *J* = 8 Hz, 2H), 7.85 (t, *J* = 8 Hz, 1H), 7.95 (d, *J* = 8 Hz, 1H), 8.12 (s, 1H), 8.34 (s, 1H), 8.41 (s, 1H); **^{13}C NMR** (100 MHz, DMSO-d_6): 166.87, 159.18, 158.13, 154.32, 153.55, 146.79, 143.51, 139.89, 136.04, 135.21, 133.85, 133.64, 130.81, 130.22, 128.28, 126.55, 125.38, 124.38, 124.05, 123.35, 119.24, 118.30, 115.85, 111.25, 95.17, 58.17, 24.98; **ESI-MS**: *m/z* 524 $(M + 1)^+$; Anal. Calcd. For $C_{28}H_{17}N_3O_4S2$; C, 64.23; H, 3.27; N, 8.03; Found: C, 64.39; H, 3.33; N, 8.28.

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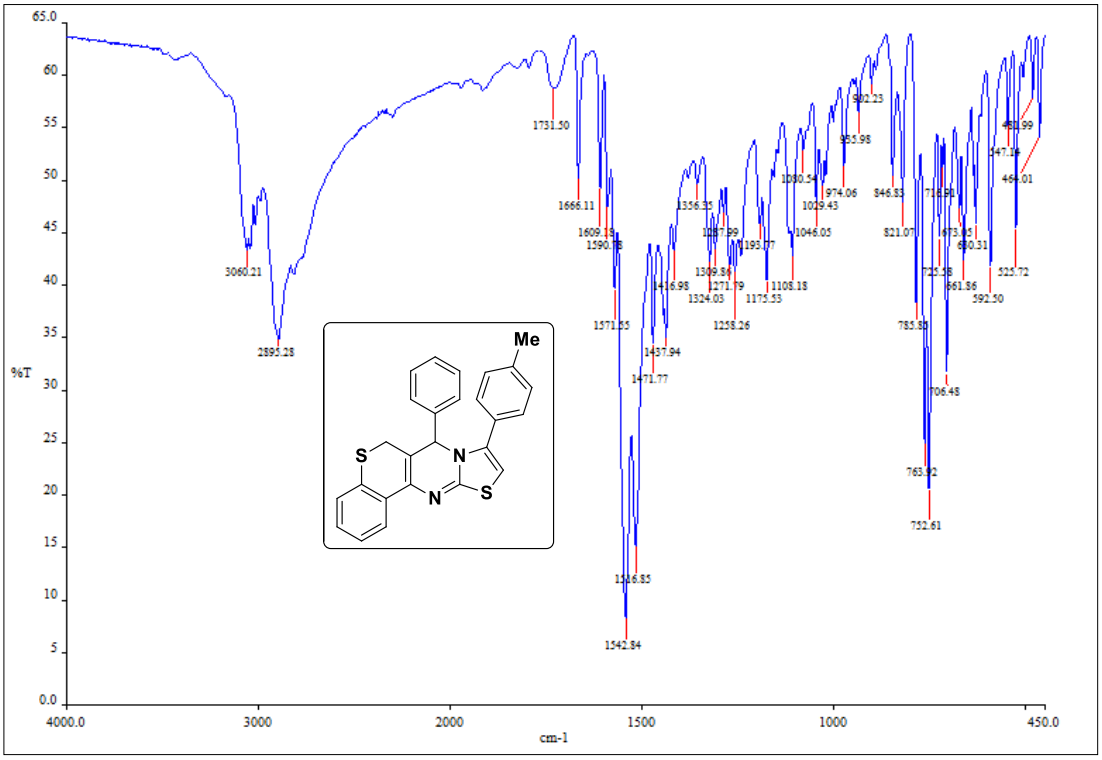
SELECTED SPECTRA



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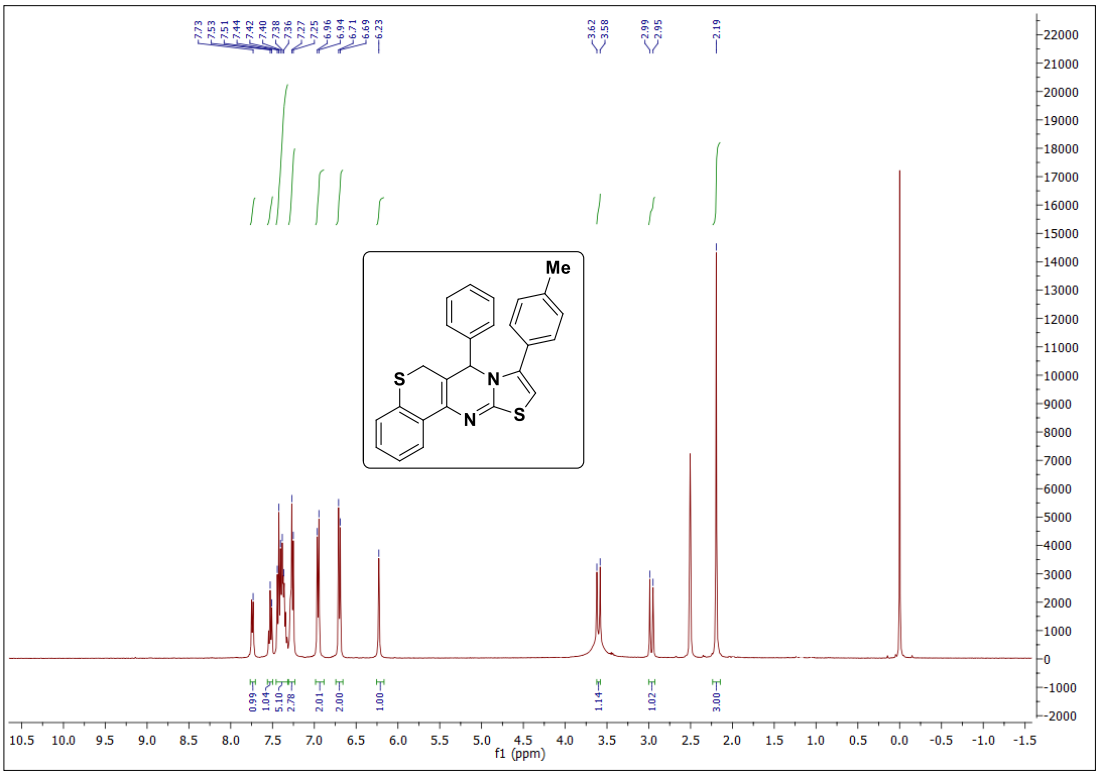

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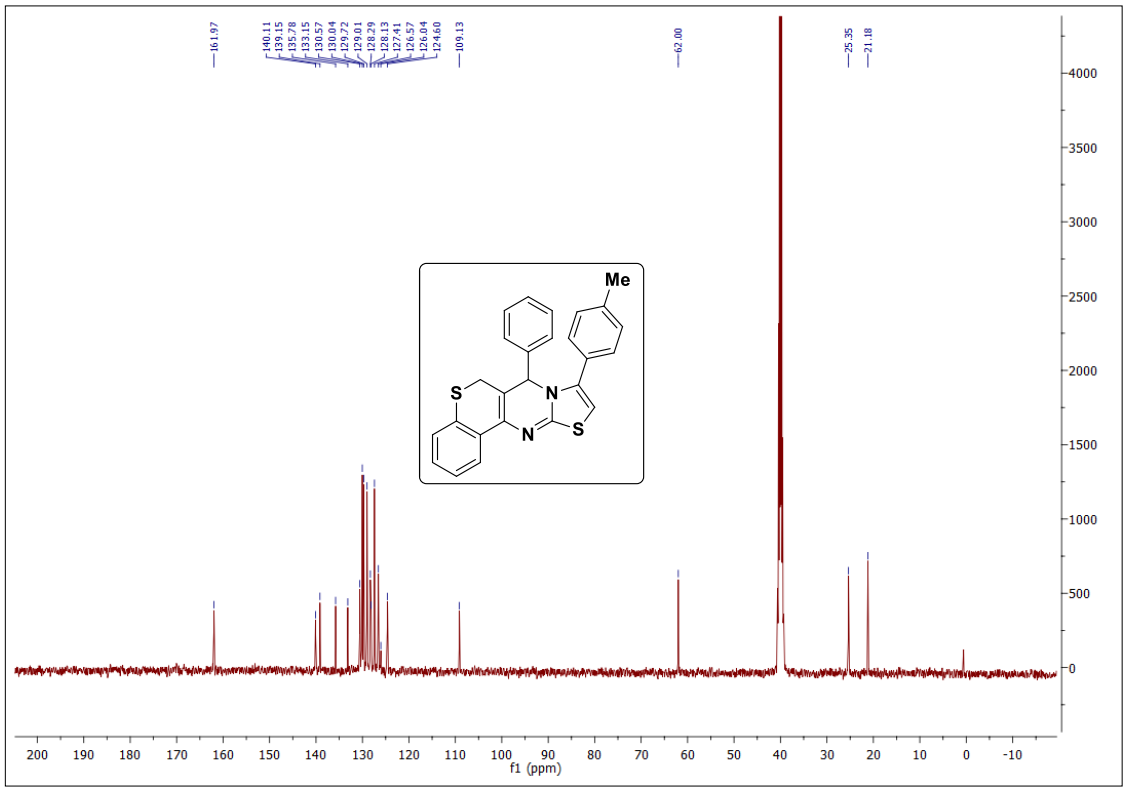
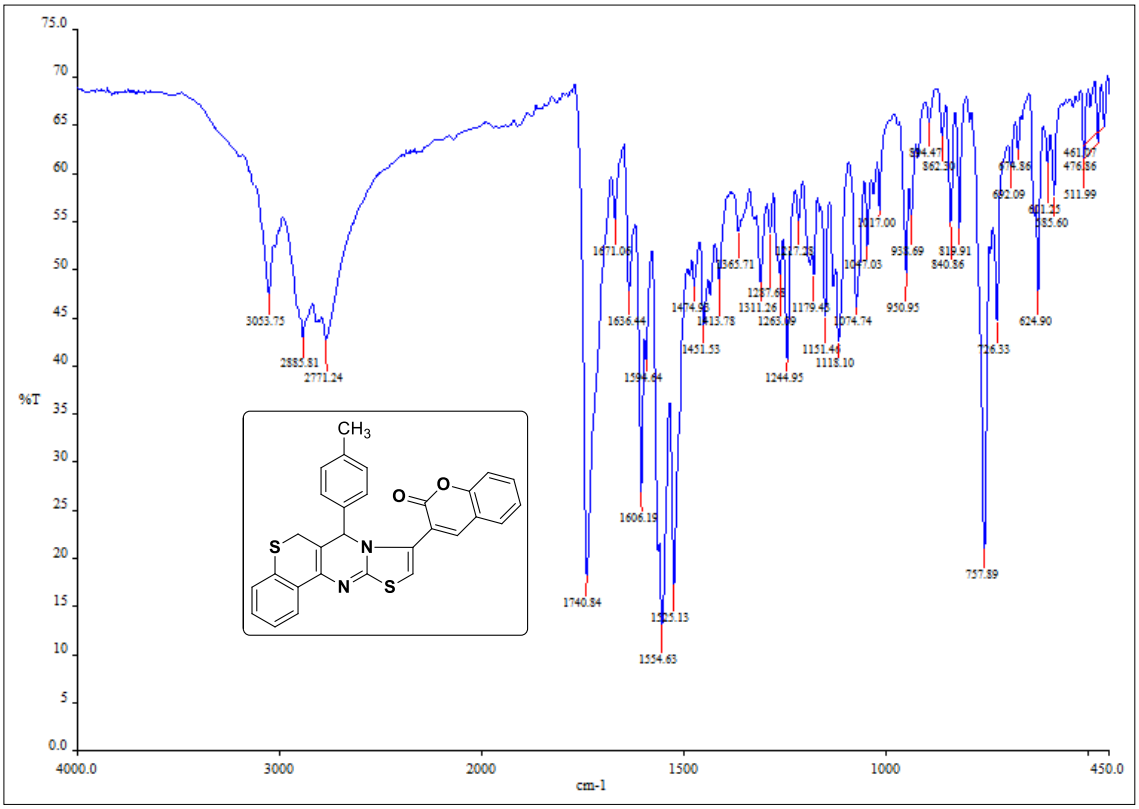
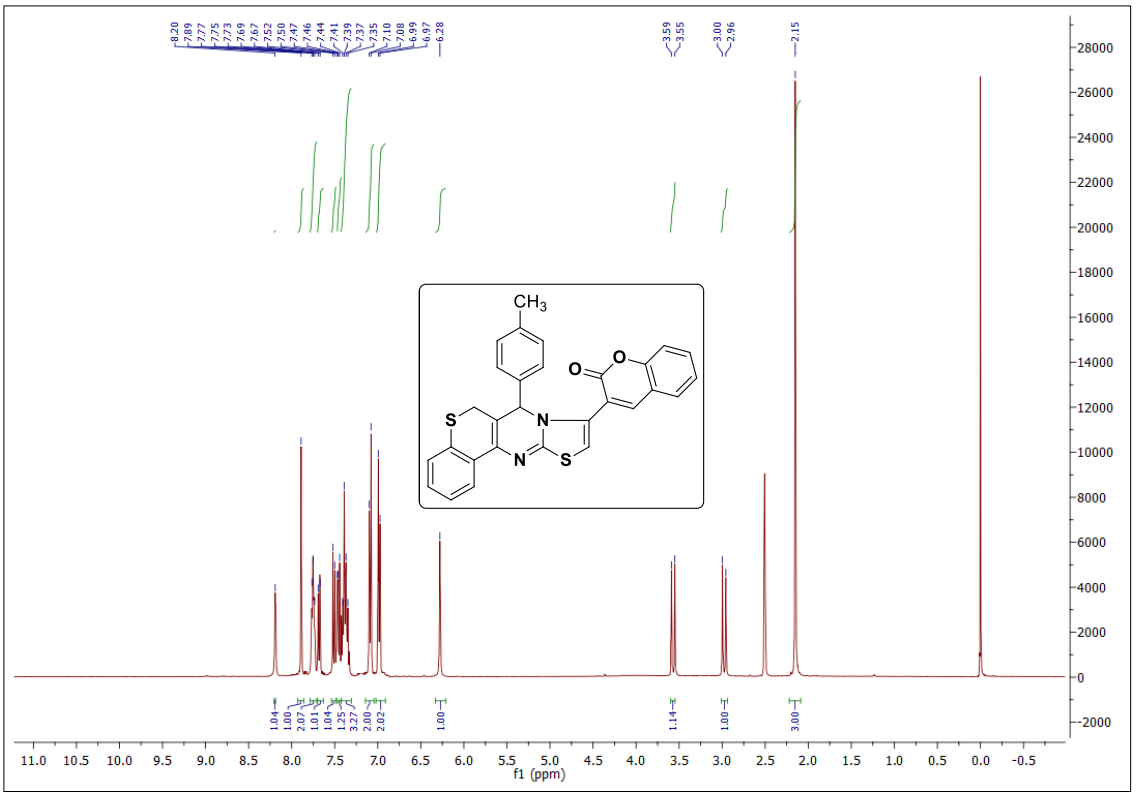


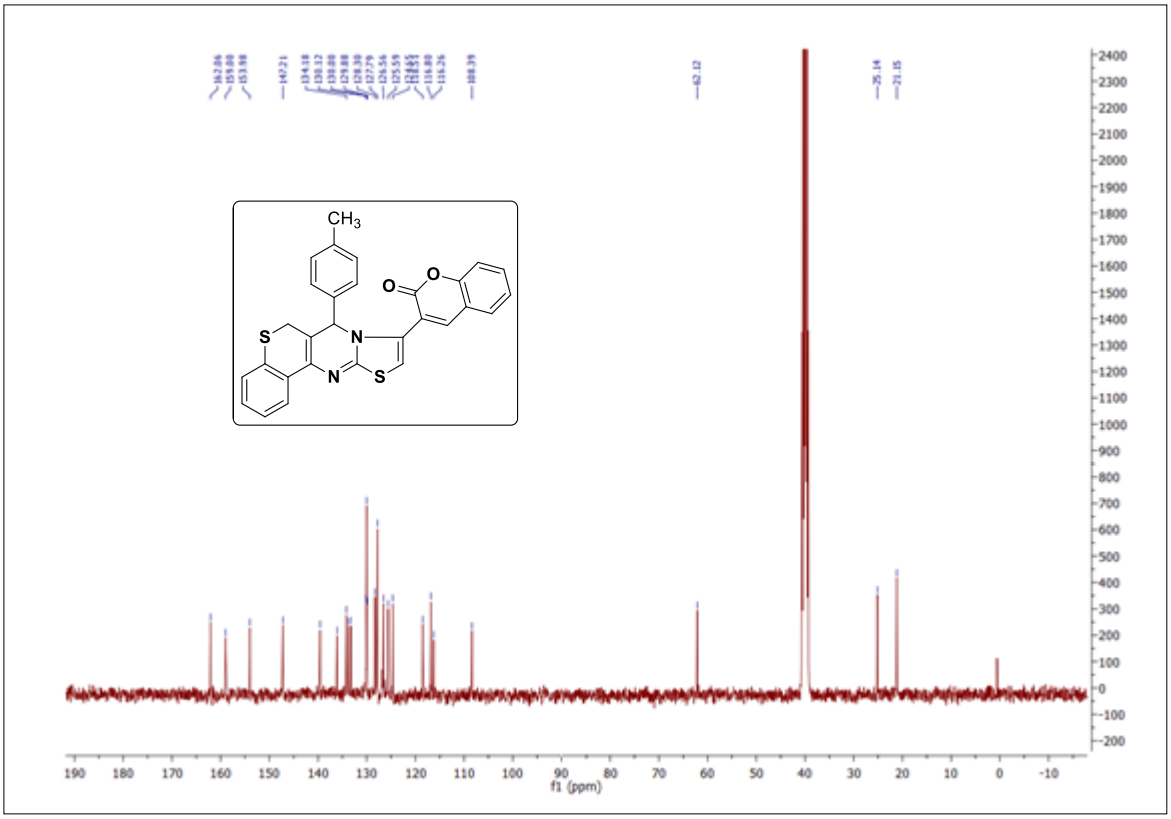
^{13}C NMR Spectrum of compound **5d**



IR Spectrum of compound **5l**



^{1}H NMR Spectrum of compound **5l**



^{13}C NMR Spectrum of compound **5l**

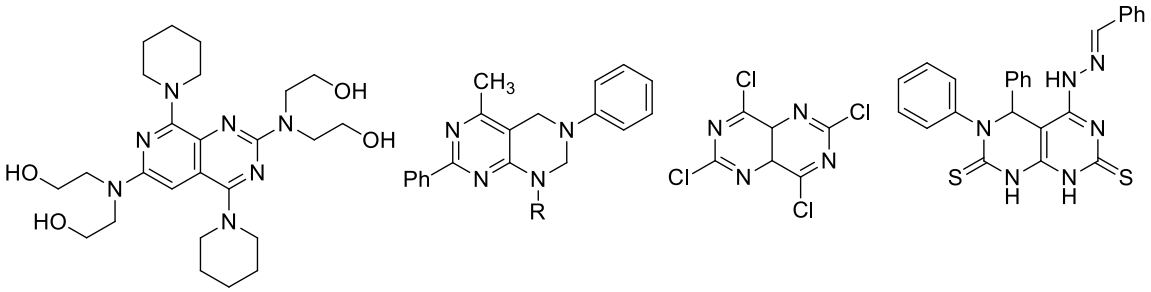
SYNTHESIS OF NOVEL PYRAZOLO-PYRIMIDO[4,5-*d*]PYRIMIDINE DERIVATIVES USING [Bmim]$FeCl_4$ IONIC LIQUID

4.1. INTRODUCTION

Functionalized nitrogen-heterocycles play a prominent role in medicinal chemistry and they have been intensively used as scaffolds for drug development. In this context pyrimidines are of particular interest because of their pharmacological profile. Pyrimidines represent an important class of heterocyclic compounds with wide ranging applications.[1] The synthesis of novel pyrimidine derivatives and investigation of their chemical and biological behaviour has gained more importance in recent decades for biological and pharmaceutical reasons.[2] Their derivatives were reported to possess broad spectrum of biological activity in both medicinal and pharmaceutical areas such as antibacterial, antiHIV, anticancer, immunosuppressive, antiulcer, antinociceptive, antifungal, antiinflammatory, antiproliferative, antiallergic and analgesic activities.[3] On the other hand pyrimido pyrimidine hybrid scaffold was the base of many bioactive molecules.[4] Over many years, pyrazolo pyrimido[4,5-*d*]pyrimidine derivatives have been reported to exhibit diverse biological functions like antimalarial,[5] antibacterial,[6] fungicidal,[7] antibiofilm,[8] anticancer[9] and kinase 2 inhibitor activities.[10-11] Further, pyrimido pyrimidine containing numerous bioactive compounds were reported in literature (**Fig. 1: 1**, **2**, **3** and **4**).[12] several reports on pyrimido pyrimidine scaffolds especially for the treatment of anti-inflammatory and analgesic diseases are available (**Fig. 2: 5**, **6**, **7** and **8**).[13] Inspired by the above facts, the synthesis of molecules containing both pyrazole and pyrimido-pyrimidine ring was taken up in the present investigation using the multicomponent reaction approach.

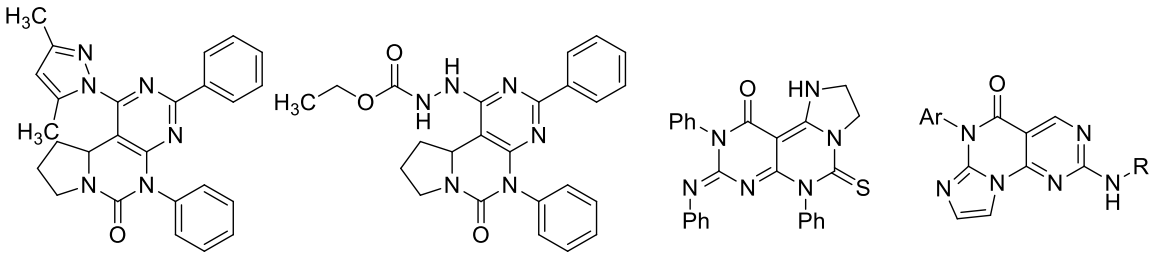
Multicomponent reactions (MCRs) are a special type of organic reactions in which three or more different starting materials react to produce a final product, in a one pot procedure.[14-16] In the recent past, ionic liquids have gained a renewed attention in view of their strong solvating ability, catalytic behavior and recyclability.[17] MCRs using ionic liquids (ILs) have gained much attention as

efficient synthetic method for synthesis of complex novel molecules/hybrids in the realm of green chemistry.[18-19] We have taken up the synthesis of pyrazolo pyrimido[4,5-*d*]pyrimidine derivatives by a four-component condensation process.



(1) **(2)** **(3)** **(4)**

Figure. 1

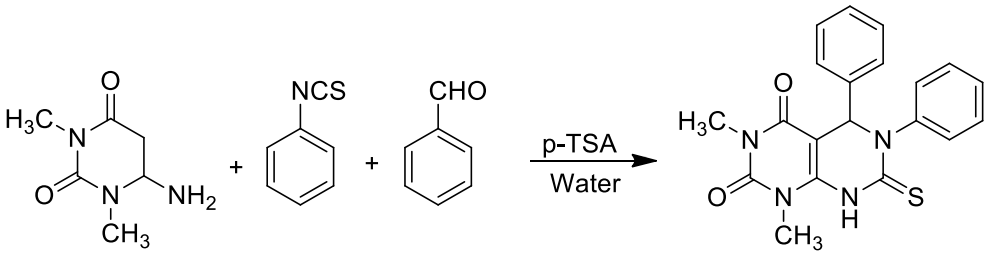


(5) **(6)** **(7)** **(8)**

Figure. 2

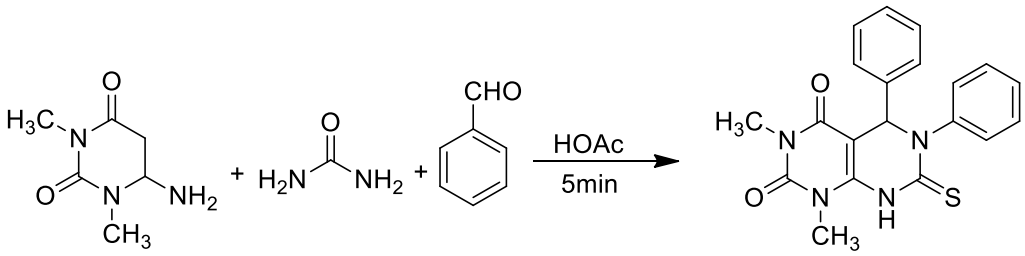
4.1.1. VARIOUS IMPORTANT APPROACHES FOR THE SYNTHESIS OF PYRIMIDO PYRIMIDINE

S. Majumder *et al.*[20] developed an efficient and regioselective one-pot multi-component synthesis of pyrimido [4,5-*d*] pyrimidine derivatives in water (**Scheme 1**).



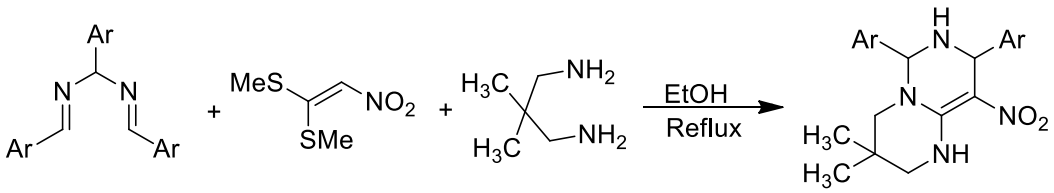
Scheme 1

M. Dabiri *et al.*[21] reported a novel and efficient synthesis of pyrimido[4,5-*d*] pyrimidine-2,4,7-trione and pyrido[2,3-*d*:6,5-*d*]dipyrimidine-2,4,6,8-tetrone derivatives (**Scheme 2**).



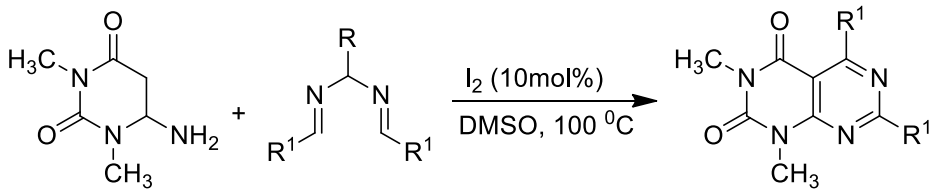
Scheme 2

A. Alizadeh *et al.*[22] describe the Synthesis of the novel pyrimido[1,6-*a*]pyrimidine and imidazo [1, 2-*c*]pyrimidine derivatives based on heterocyclic ketene aminals (**Scheme 3**).



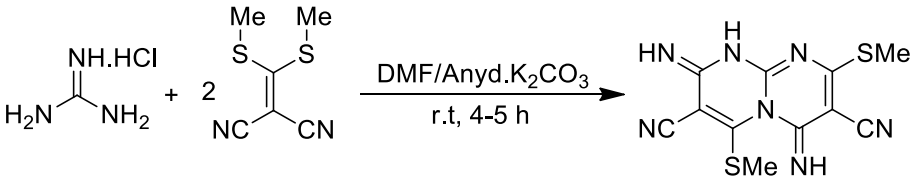
Scheme 3

F. M. Moghaddam *et al.*[23] reported highly efficient synthesis of pyrimido [4,5-*d*] pyrimidine-2,4-dione derivatives catalyzed by iodine (**Scheme 4**).



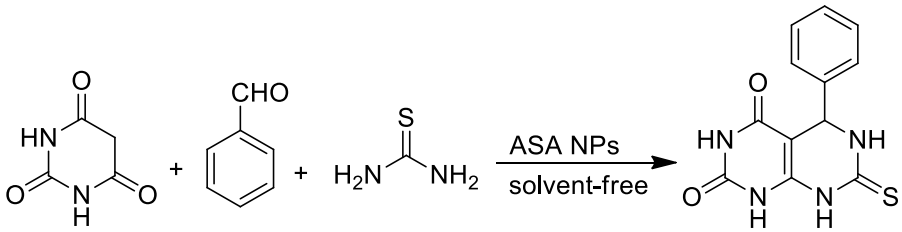
Scheme 4

B. D. Kalyankar *et al.*[24] developed a convenient route for synthesis of bis diimino benzothiazolo pyrimido pyrimidines (**Scheme 5**).



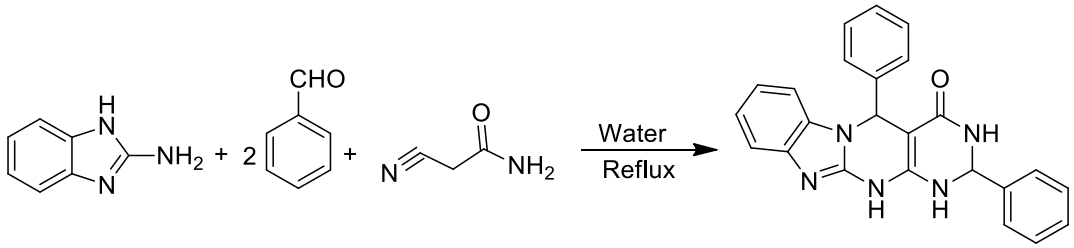
Scheme 5

M. Nasr-Esfahani *et al.*[25] used aluminatesulfonic acid nanoparticles as recyclable nanocatalyst for the synthesis a new pyrimidopyrimidines (**Scheme 6**).



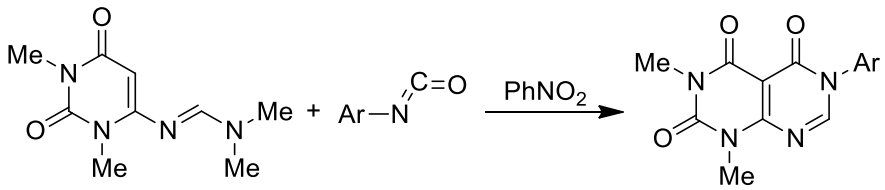
Scheme 6

J. Liu. *et al.*[26] reported a catalyst-free reaction in water for the synthesis of benzo[4,5] imidazo [1,2-*a*] pyrimido [4,5-*d*] pyrimidin-4(1*H*)-one derivatives (**Scheme 7**).



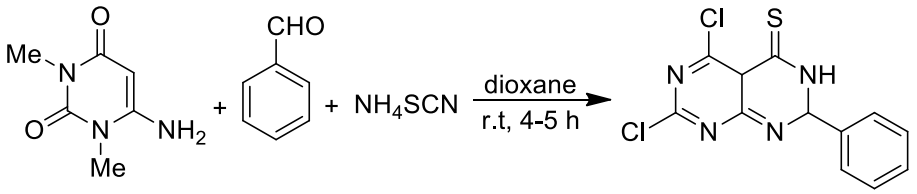
Scheme 7

D. Prajapati *et al.*[27] studied a facile one-pot synthesis of novel pyrimido [4,5-*d*] pyrimidine derivatives using 6-[(dimethylamino) methylene] aminouracil (**Scheme 8**).



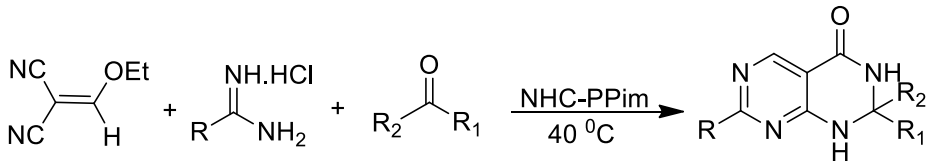
Scheme 8

P. Sharma *et al.*[28] developed an expedient synthesis of novel, fused pyrimido[4,5-*d*] pyrimidine and pyrimido [5,4-*e*][1,2,4] triazolo [4,3-*c*] pyrimidine analogues from 4-amino-2, 6-dichloro-pyrimidine (**Scheme 9**).



Scheme 9

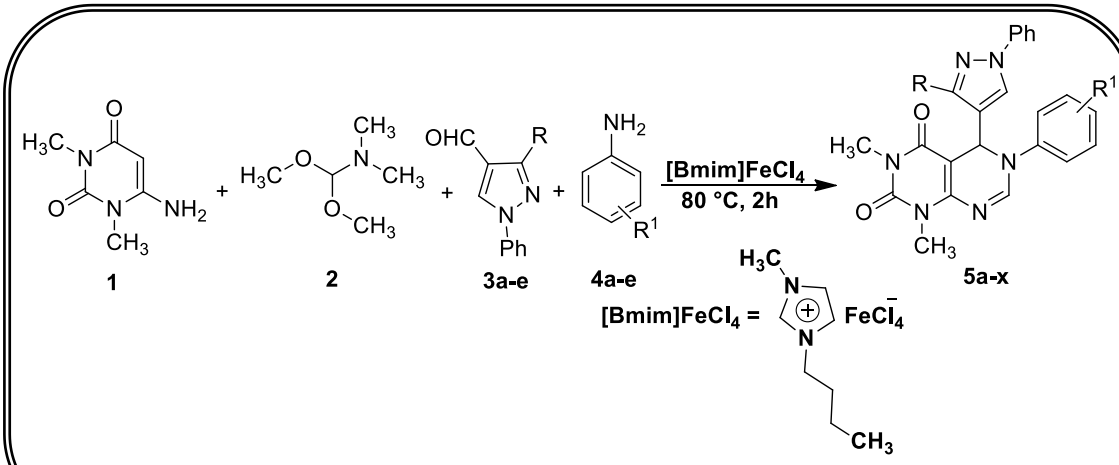
M. Liu *et al.*[29] described one-pot NHC-assisted access to 2, 3-dihydropyrimido [4, 5-*d*] pyrimidin-4 (1 H)-ones (**Scheme 10**).



Scheme 10

4.2. PRESENT WORK

The above mentioned facts, generated innovative ideas that these active pharmacophores, if linked together would generate novel molecular templates which many likely exhibit interesting biological properties. Hence, in continuation of our interest in the synthesis of biologically active heterocycles, by using green chemical MCRs, we designed and executed the synthesis of some new heterocyclic compounds like pyrazolo pyrimido[4,5-*d*]pyrimidine derivatives (**Scheme 11**). This combination was taken up as an attempt to investigate the influence of such structure on the antibacterial, antibiofilm and protein leakage biological activities, hoping to add some synergistic biological significance to the target molecules.

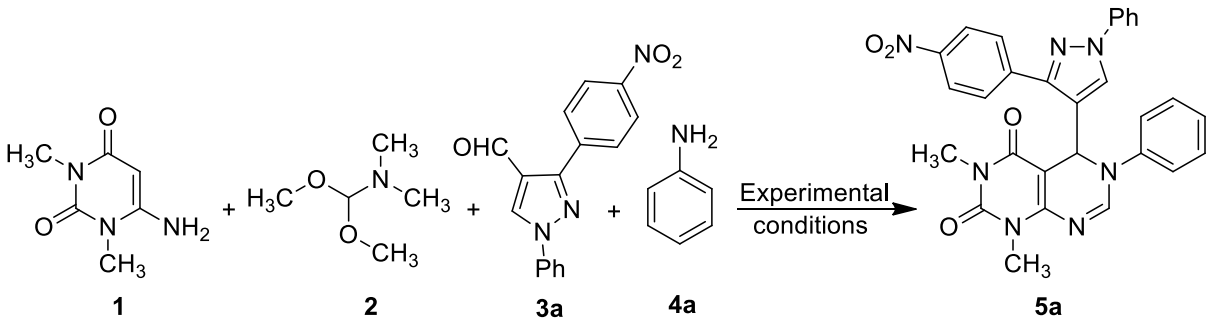


(Scheme 11)

4.3. RESULTS AND DISCUSSION

In the present investigation an efficient, four-component sequential protocol for the synthesis of pyrazolo pyrimido[4,5-*d*]pyrimidines **5** was developed by the reaction of 6-amino-1,3-dimethyluracil **1**, N,N-dimethylformamide dimethyl acetal **2**, pyrazole aldehydes **3a** and aromatic anilines **4a**, employing ionic liquid [Bmim]$FeCl_4$ as a reaction medium (Table 1), with a view to develop green transformations with unique reactivity and selectivity. Initially, it was observed that the reaction under neat conditions did not give the required product (Table 1, entry 1). When the reaction was carried out in the presence of water, it failed to afford the product (Table 1, entry 2). Our next endeavor was to enhance the yield of the reaction by employing various solvents (Table 1, entries 3–8). We achieved a slight improvement in the yield of the product, but the results were not very encouraging. Interestingly, when we performed the same reaction in the presence of different typical ionic liquids, the yields were better (Table 1, entries 9-12). It was interesting to note that when [Bmim]$FeCl_4$ was used the reactivity was much improved. Subsequently, the effect of temperature was examined, no further improvement in the product yield was observed (Table 1, entries 13-15) for [Bmim]$FeCl_4$ at temperatures above and below 80 °C.

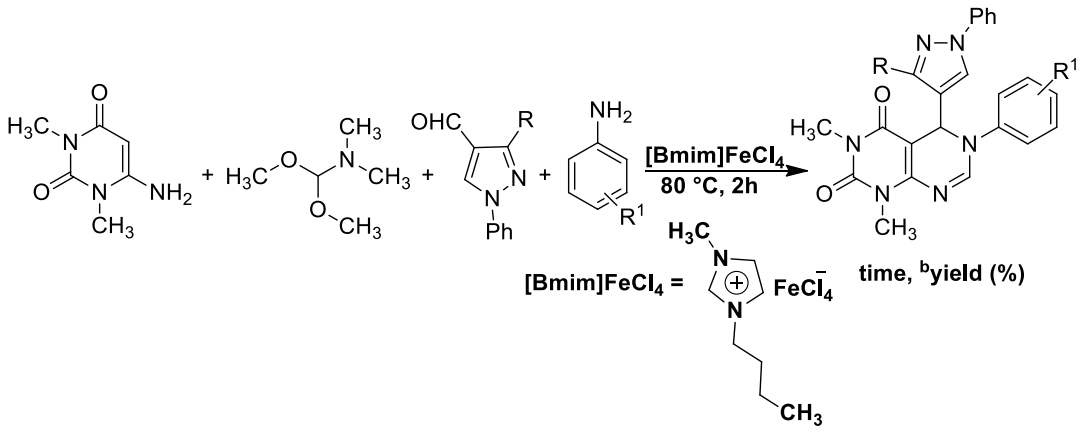
Table 1. Optimization studies for the synthesis of pyrazolo pyrimido[4,5-*d*]pyrimidines **5a**

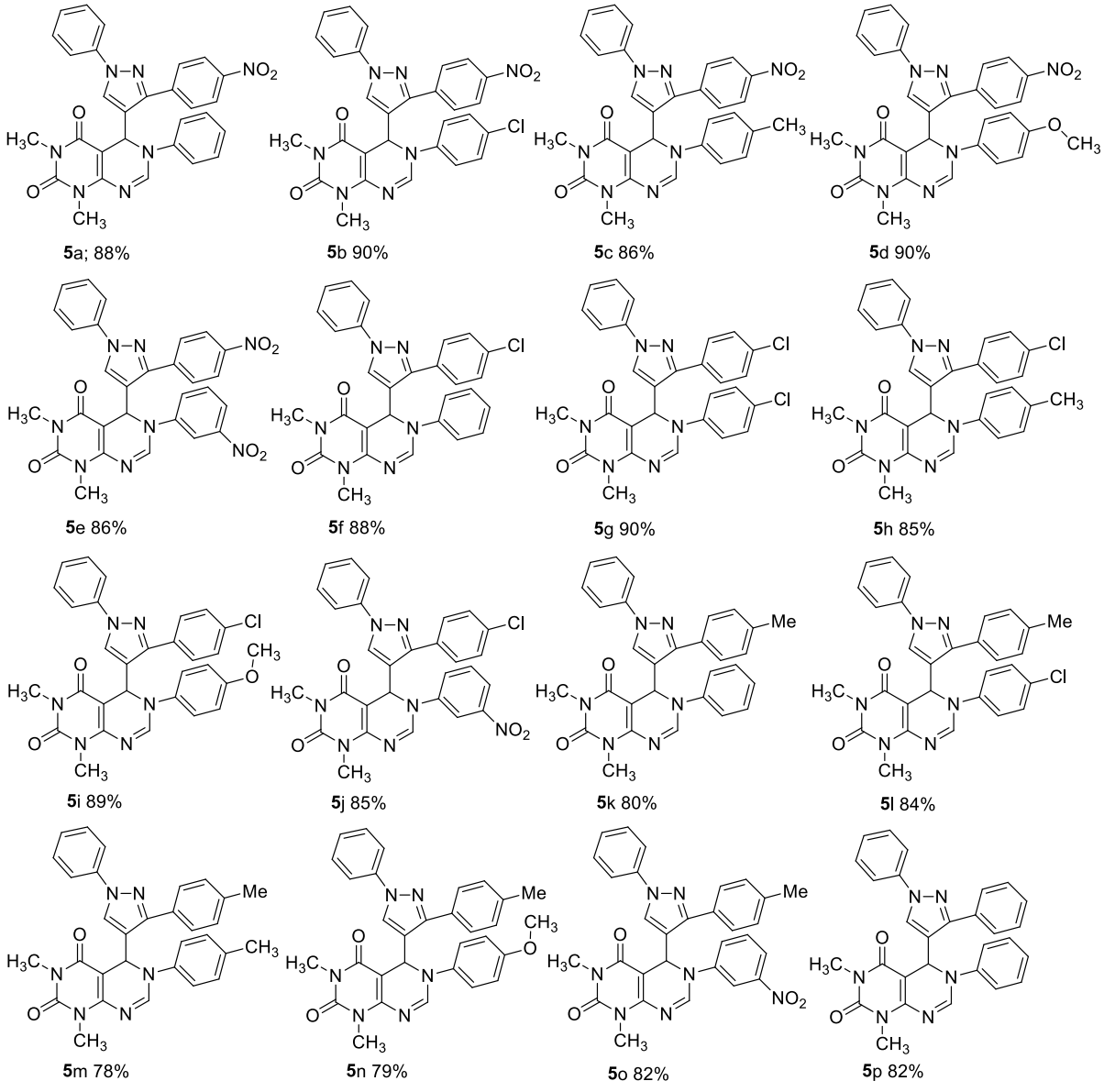


Entry[a]	Solvent	Temp (°C)	Time (h)	Yield[b] (%)
1	neat	80	24	-[c]
2	Water	reflux	12	-[c]
4	Ethanol	reflux	12	10
5	Acetic acid	reflux	12	12
6	DMF	reflux	12	25
7	$PhNO_2$	reflux	12	32
8	Toluene	reflux	12	36
9	[Bmim]Br	80	4	56
10	$[Bmim]PF_6$	80	4	48
11	$[Bmim]BF_4$	80	3	60
12	**$[Bmim]FeCl_4$**	**80**	**2**	**90**
13	$[Bmim]FeCl_4$	rt	2	34
14	$[Bmim]\ FeCl_4$	60	2	72
15	$[Bmim]\ FeCl_4$	100	2	90

[a]Reaction conditions: 6-Amino-1,3-dimethyluracil **1** (1 mmol), N,N-dimethylformamide dimethyl acetal **2** (1 mmol), pyrazole aldehyde **3** (1 mmol) and aniline **4** (1 mmol), and solvent (2 mL). [b]Yields of the isolated products:
[c]Reaction failed to occur.

Table 2. Synthesis of pyrazolo pyrimido[4,5-*d*]pyrimidine derivatives[a]



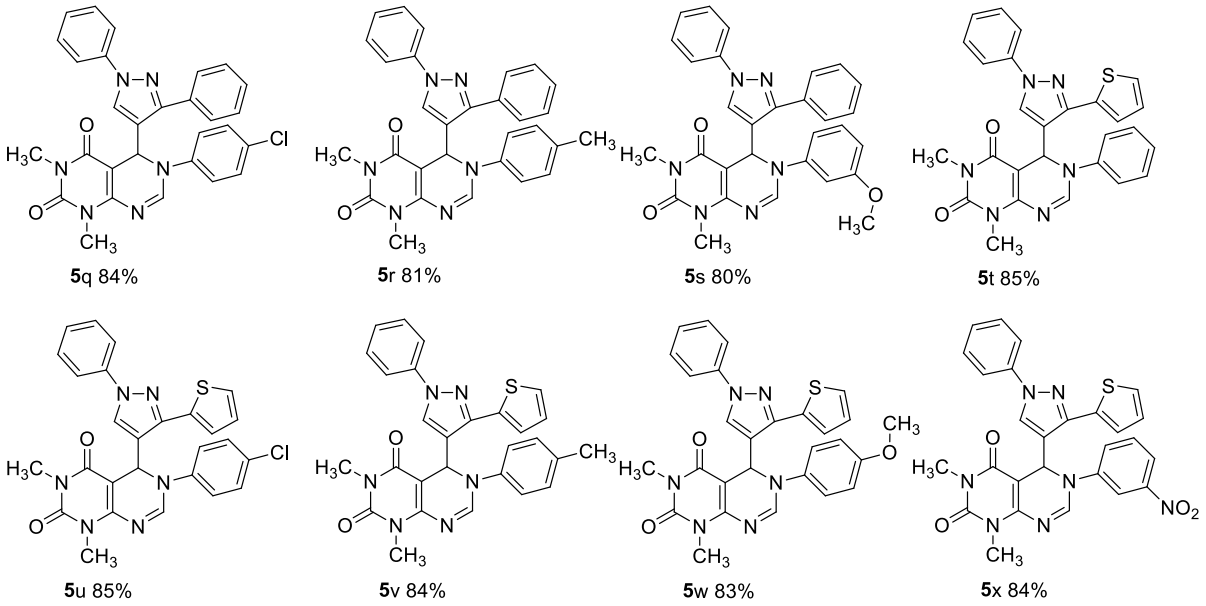


5a; 88% **5**b 90% **5**c 86% **5**d 90%

5e 86% **5**f 88% **5**g 90% **5**h 85%

5i 89% **5**j 85% **5**k 80% **5**l 84%

5m 78% **5**n 79% **5**o 82% **5**p 82%

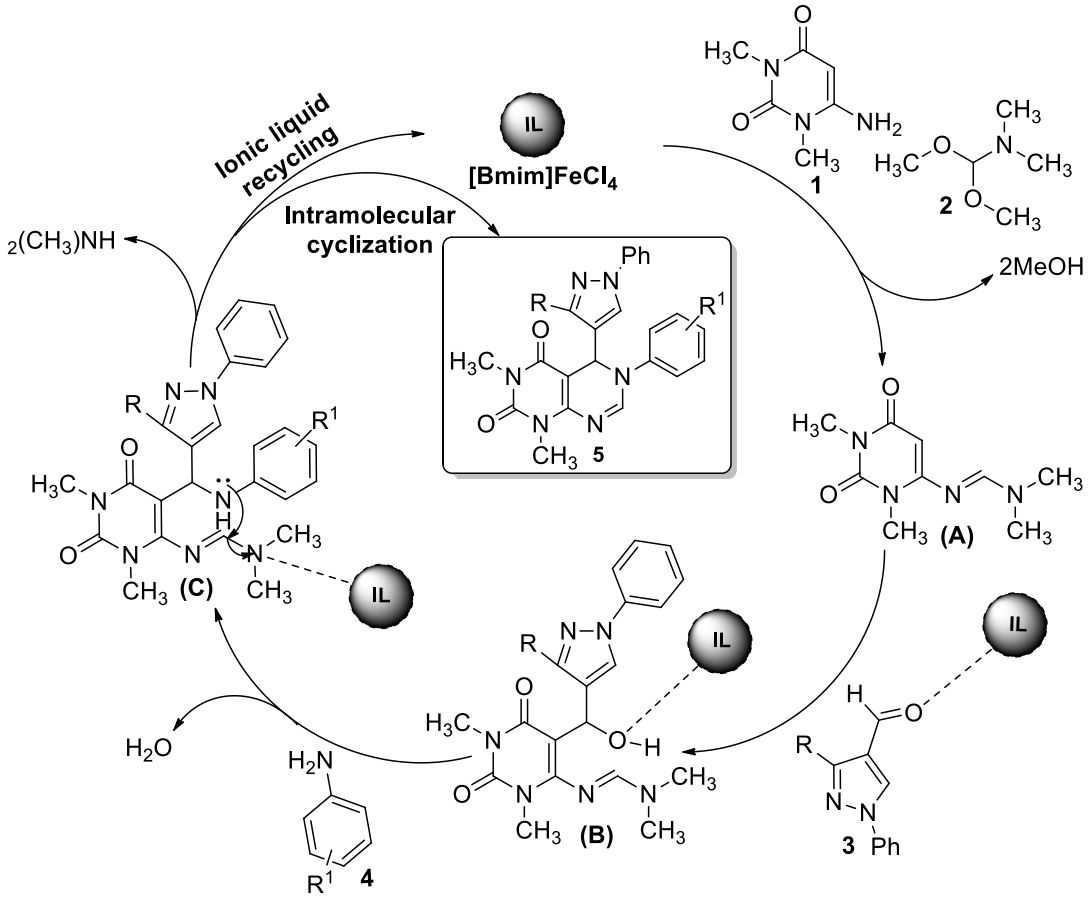


[a]Reaction conditions: 6-Amino-1,3-dimethyluracil **1** (1 mmol), N,N-dimethylformamide dimethyl acetal **2** (1 mmol), pyrazole aldehyde **3** (1 mmol) and aromatic amine **4** (1 mmol), and [Bmim]$FeCl_4$ (l mL).

[b]Isolated yield.

Having optimized the reaction conditions, we explored its applicability for preparation of a library, employing various pyrazole aldehydes **3 (a-e)** and aryl amines **4 (a-e)** (**Table 2**). The reaction occurred well in all these cases affording good yields of 84-90% (Table 2). Moreover, with the available variations we noted an interesting trend in the reaction with respect to the substitutions in the pyrazole aldehyde **3** and aromatic aniline **4** (Table 2, **5 (a-x)**) by varying electron-withdrawing and electron-donating groups. In the case of electron-withdrawing groups on pyrazole aldehydes and aromatic amines, the reaction underwent smoothly, and the desired products were obtained in a good range of yields, i.e. 85-90%. Good to moderate yield (78-84%) was observed when electron-donating groups were present on the aromatic ring of the pyrazole aldehydes and aromatic amines. The structures of all the newly synthesized pyrazolo pyrimido[4,5-*d*]pyrimidine derivatives were confirmed by their IR, ^{1}H NMR, ^{13}C NMR and EI-HRMS analysis data.

The structures of the representative compounds **5f** have been further confirmed by X-ray diffraction analysis shown in (**Fig. 4**). The sequential reactions presumably proceeded through the formation of the observed product by a rationalized mechanism pathway as depicted in (**Scheme 12**). Initially the reaction of 6-amino-1,3-dimethyluracil **1** with N,N-dimethylformamide dimethyl acetal **2** afforded the amidine intermediate **A**. It was proposed that there was simultaneous reaction of pyrazole aldehyde **3** with intermediate **A** which resulted in the formation of an intermediate **B**. The intermediate **B** presumably may interact with the aromatic amine *via* dehydration leading to the formation of intermediate **C**. Finally, the intermediate **C** undergoes a subsequent nucleophilic attack on the imino carbon atom eliminating dimethylamine followed by cyclization to generate the pyrazolo pyrimido[4,5-*d*]pyrimidine **5**.



Scheme 1. Proposed mechanism for the synthesis of compound **5**.

Additionally, we also investigated the recyclability of [Bmim]$FeCl_4$ for four consecutive cycles (fresh + 3 cycles) during the synthesis of pyrazolo pyrimido[4,5-*d*]pyrimidine **5a**. After completion of the reaction, 20 mL of diethyl ether was added to the reaction mixture. It was then shaken well and the ethereal layer was decanted before washing the crude reaction mixture with ether (3 × 10 mL). The residue was dried under vacuum at 45 °C for 2 h, yielding the ionic liquid in its activated form. This was then subjected directly to another reaction, affording again the desired product in high yield. The ethereal layer was evaporated and washed with water, followed by ethanol, to obtain the pure product. Even after recycling the ionic liquid for four times, the activity of the ionic liquid remained the same and almost quantitative yields were obtained each time (**Fig. 3**).

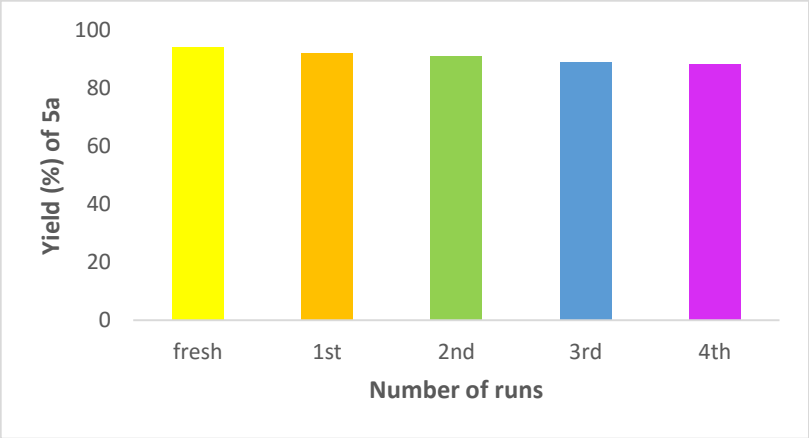


Figure 3. Recycling of the [Bmim]$FeCl_4$ in the synthesis of compound **5a**.

We achieved a novel four-component reaction leading to highly functionalized pyrazolo pyrimido[4,5-*d*]pyrimidines derivatives starting from simple and readily available inputs under green conditions without any activation or modification. The ionic liquid was reused for the next four cycles effectively. This approach may be of value to researchers seeking novel synthetic fragments with unique properties for medicinal chemistry programs.

4.4. EXPERMENTAL

Chemicals were purchased from Merck and Aldrich chemical companies. The ^1H NMR and ^{13}C NMR spectra were recorded in DMSO-d_6 solvent on Bruker WM-300 spectrometer using TMS as internal standard. The chemical shifts were

reported in ppm (δ). Mass spectra (ESI) were carried out on a JEOL JMSD-300 spectrometer. CHN analysis was carried out using Carlo Erba EA 1108 automatic elemental analyser. Melting points were determined in open capillary tubes and were recorded on a Stuart SMP30 melting point apparatus.

4.4.1. SPECTRAL DISCUSSION

IR

In all the compounds **5a–x** the formation of pyrimidine was confirmed due to appearance of carbonyl group around 1620-1656 cm^{-1} all the compounds.

^{1}H NMR

In ^{1}H NMR, the (CH) signal was observed at δ 6.64-6.16 ppm. In all the compounds, the singlet protons of pyrazole (CH) was observed at 8.68-8.23 ppm and pyrimidine (CH) group were observed as a singlet at δ 8.21-7.68 ppm. All other aromatic and aliphatic protons appeared at expected regions.

^{13}C NMR

In ^{13}C NMR, the signal appeared at δ 67.12-60.18 ppm can be attributed to CH carbon. The signal observed at δ 162.84-160.94 ppm was assigned to (C=O) group were observed all the compounds.

Mass

The structures of all synthesized compounds were further confirmed by its mass spectra. The mass spectra detected the expected molecular ion signals (M + 1) corresponding to respective molecular weight of the synthesized compounds.

4.4.2. X-ray crystallographic study

X-ray data for the compound **5f** were collected at room temperature using a Bruker Smart Apex CCD diffractometer with graphite monochromated MoKα radiation (λ=0.71073Å) with ω-scan method.[30] Preliminary lattice parameters and orientation matrices were obtained from four sets of frames. Unit cell dimensions were determined using 7488 reflections. Integration and scaling of intensity data were accomplished using SAINT program.[30] The structure was solved by Direct Methods using SHELXS97[31] and refinement was carried out by full-matrix least-squares technique using SHELXL-2014/7.[31] Anisotropic displacement parameters were included for all non-hydrogen atoms. H bound to N atom was located from the difference Fourier map. All other H atoms were positioned geometrically and

treated as riding on their parent C atoms with C-H distances of 0.93--0.97 Å, and with $U_{iso}(H) = 1.2U_{eq}$ (C) or $1.5U_{eq}$ for methyl atoms. The sulfur atom of thiochromene ring is found to be disordered over two sites; 0.534 site occupancy for S1 atom (representing the major component) and 0.466 site occupancy for S1D atom (minor component). PART and FVAR instructions were used for modeling the sulfur atom disorder and DFIX instruction for restraining the C-S bond distance to 1.70 Å with e.s.d value of 0.01Å.

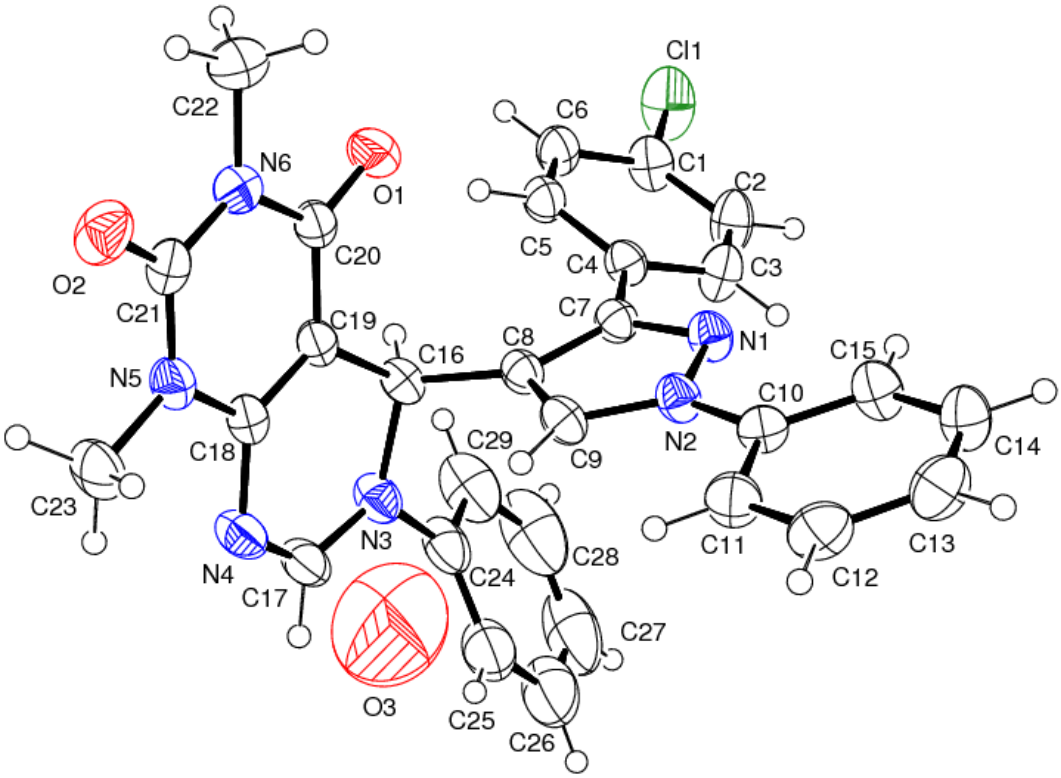


Figure 4. ORTEP representation of compound **5f** (CCDC 1487732). The thermal ellipsoids are drawn at 50% probability level.

4.4.3. General procedure for the synthesis of thiazolo[3,2-*a*]thiochromeno[4,3-*d*]pyrimidines derivatives (5a-x)

To a mixture of 6-amino-1 3-dimethyluracil **1** (1 mmol), N,N-dimethylformamide dimethyl acetal **2** (1 mmol) was added in ionic liquid [Bmim]$FeCl_4$ (1 mL). The resulting mixture was stired at 80 °C for 10 min. After completion of the reaction as indicated by TLC, aromatic anilines **3** (1 mmol) and pyrazole aldehydes **4** (1 mmol) were added to the reaction mixture through a syringe and then allowed to

stir at the same temperature until completion of the reaction (as indicated by TLC analysis). The product was purified by column chromatography (100-200 mesh), eluted with a solvent system of ethyl acetate-hexane (20:80 v/v) to afford the title compounds **5a-x**.

4.4.4. PHYSICAL AND SPECTRAL DATA

1,3-Dimethyl-5-(3-(4-nitrophenyl)-1-phenyl-1H-pyrazol-4-yl)-6-phenyl-5,6 dihydro pyrimido [4,5-d]pyrimidine-2,4(1H,3H)-dione (**5a).** Yields 88%; mp: 334–336 °C; IR(KBr): ν 3074.78, 2942.61, 1697.17, 1641.16, 1532.79, 1471.28 cm^{-1}; ^{1}H NMR (400 MHz, DMSO): δ 3.17 (s, 3H), 3.48 (s, 3H), 6.39 (s, 1H), 7.06 (t, *J* = 8.0 Hz, 3H), 7.49 (t, *J* = 8.0 Hz, 2H), 7.73 (t, *J* = 8.0 Hz, 3H), 7.86 (s, 1H), 8.23 (d, *J* = 8.0 Hz, 2H), 8.29 (t, *J* = 8.0 Hz, 2H), 8.31 (d, *J* = 8.0 Hz, 1H), 8.38 (d, *J* = 8.0 Hz, 1H), 8.69 (s, 1H); ^{13}C NMR (100 MHz, DMSO): δ 160.91, 153.00, 152.02, 151.16, 149.49, 148.14, 141.94, 139.62, 132.76, 131.85, 131.00, 129.86, 128.64, 128.30, 126.90, 123.41, 122.43, 119.69, 118.69, 89.51, 49.69, 29.69, 27.96; MS-ESIMS: m/z 534 $(M + H)^{+}$; HR ESIMS: m/z calcd for $C_{29}H_{23}N_7O_4$: 534.1812; found: 534.1810.

6-(4-Chlorophenyl)-1,3-dimethyl-5-(3-(4-nitrophenyl)-1-phenyl-1H-pyrazol-4-yl)-5,6dihydropyrimido[4,5-d]pyrimidine-2,4(1H,3H)-dione (5b). Yields 90%; mp: 308–310 °C; IR(KBr): ν 3072.48, 2942.59, 1685.99, 1634.49, 1548.20, 1468.19 cm^{-1}; ^{1}H NMR (400 MHz, DMSO): δ 3.69 (s, 3H), 3.76 (s, 3H), 6.31 (s, 1H), 6.87 (d, *J* = 8.0 Hz, 1H), 7.28 (d, *J* = 8.0 Hz, 1H), 7.49 (t, *J* = 8.0 Hz, 2H), 7.52-7.68 (m, 3H), 7.92-799 (m, 3H), 8.04 (d, *J* = 8.0 Hz, 2H), 8.37 (s, 1H), 8.45 (d, *J* = 8.0 Hz, 1H), 8.67 (s, 1H); ^{13}C NMR (100 MHz, DMSO): δ 162.34, 154.96, 152.20, 150.84, 149.59, 148.12, 141.00, 138.39, 133.20, 132.10, 131.02, 129.39, 128.90, 127.38, 125.50, 123.39, 122.49, 119.30, 117.49, 89.20, 48.49, 29.28,

27.38; MS-ESIMS: m/z 568 $(M + H)^+$; HR ESIMS: m/z calcd for $C_{29}H_{22}ClN_7O_4$: 567.1422; found: 567.1426.

1,3-Dimethyl-5-(3-(4-nitrophenyl)-1-phenyl-1H-pyrazol-4-yl)-6-(p-tolyl)-5,6-dihydropyrimido[4,5-d]pyrimidine-2,4(1H,3H)-dione (5c). Yields 86%; mp: 318–320 °C; IR(KBr): ν 3065.29, 2937.22, 1695.46, 1624.24, 1522.24, 1434.53 cm^{-1}; ^1H NMR (400 MHz, DMSO): δ 2.34 (s, 3H), 3.69 (s, 3H), 3.76 (s, 3H), 6.31 (s, 1H), 6.87 (d, *J* = 8.0 Hz, 2H), 7.29 (s, 1H), 7.62 (d, *J* = 8.0 Hz, 2H), 7.67 (d, *J* = 8.0 Hz, 3H), 7.92-8.04 (m, 3H), 8.20 (d, *J* = 8.0 Hz, 2H), 8.45 (d, *J* = 8.0 Hz, 1H), 8.67 (s, 1H); ^{13}C NMR (100 MHz, DMSO): δ 160.85, 153.18, 151.99, 151.41, 149.73, 139.79, 139.68, 137.66, 132.21, 130.21, 129.86, 129.49, 129.41, 129.09, 128.49, 126.78, 126.51, 123.67, 118.65, 89.34, 49.82, 29.62, 27.92, 21.40; MS-ESIMS: m/z 548 $(M + H)^+$; HR ESIMS: m/z calcd for $C_{30}H_{25}N_7O_4$: 548.1968; found: 548.1973.

6-(4-Methoxyphenyl)-1,3-dimethyl-5-(3-(4-nitrophenyl)-1-phenyl-1H-pyrazol-4-yl)-5,6-dihydropyrimido[4,5-d]pyrimidine-2,4(1H,3H)-dione (5d). Yields 90%; mp: 324–326 °C; IR(KBr): ν 3045.32, 2939.25, 1689.25, 1658.23, 1527.56, 1454.30 cm^{-1}; ^1H NMR (400 MHz, DMSO): δ 3.17 (s, 3H), 3.47 (s, 3H), 3.63 (s, 3H), 6.27 (s, 1H), 6.59 (d, *J* = 8.0 Hz, 2H), 6.92 (d, *J* = 8.0 Hz, 2H), 7.47 (d, *J* = 8.0 Hz, 1H), 7.51 (d, *J* = 8.0 Hz, 2H), 7.58 (s, 1H), 7.65 (d, *J* = 8.0 Hz, 2H), 7.89 (d, *J* = 8.0 Hz, 2H), 8.19 (d, *J* = 8.0 Hz, 2H), 8.69 (s, 1H); ^{13}C NMR (100 MHz, DMSO): δ 160.98, 158.86, 153.97, 152.06, 150.14, 149.25, 147.22, 139.70, 139.46, 133.49, 130.34, 129.95, 129.54, 127.27, 126.81, 123.82, 118.90, 114.67, 88.62, 55.69, 50.28, 29.60, 27.90; MS-ESIMS: m/z 564 $(M + H)^+$; HR ESIMS: m/z calcd for $C_{30}H_{25}N_7O_5$: 564.5634; found: 564.5628.

1,3-Dimethyl-6-(3-nitrophenyl)-5-(3-(4-nitrophenyl)-1-phenyl-1H-pyrazol-4-yl)-5,6-dihydropyrimido[4,5-d]pyrimidine-2,4(1H,3H)-dione (5e). Yields 86%; mp: 332–334 °C; IR(KBr): ν 3013.35, 2943.58, 1681.53, 1624.52, 1524.63, 1483.56 cm^{-1}; ^{1}H NMR (400 MHz, DMSO): δ 3.21 (s, 3H), 3.50 (s, 3H), 6.57 (s, 1H), 7.35 (d, *J* = 8.0 Hz, 1H), 7.48 (d, *J* = 8.0 Hz, 1H), 7.51 (d, *J* = 8.0 Hz, 3H), 7.70 (d, *J* = 8.0 Hz, 2H), 7.81 (s, 1H), 7.87 (d, *J* = 8.0 Hz, 2H), 7.92 (d, *J* = 8.0 Hz, 1H), 8.01 (s, 1H), 8.19 (d, *J* = 8.0 Hz, 2H), 8.71 (s, 1H); ^{13}C NMR (100 MHz, DMSO): δ 163.18, 153.49, 151.39, 150.69, 149.20, 147.39, 142.05, 137.38, 134.29, 133.10, 131.40, 129.30, 128.47, 127.18, 125.30, 124.19, 122.58, 119.48, 118.50, 89.60, 48.20, 30.40, 26.40; MS-ESIMS: m/z 579 $(M + H)^{+}$; HR ESIMS: m/z calcd for $C_{29}H_{22}N_8O_6$: 579.5350; found: 579.5348.

5-(3-(4-Chlorophenyl)-1-phenyl-1H-pyrazol-4-yl)-1,3-dimethyl-6-phenyl-5,6 dihydropyrimido[4,5-d]pyrimidine-2,4(1H,3H)-dione (5f). Yields 88%; mp: 320–322 °C; IR(KBr): ν 3025.46, 2926.14, 1650.67, 1623.89, 1512.52, 1458.34 cm^{-1}; ^{1}H NMR (400 MHz, DMSO): δ 3.17 (s, 3H), 3.48 (s, 3H), 6.38 (s, 1H), 7.06 (t, *J* = 8.0 Hz, 3H), 7.50 (t, *J* = 8.0 Hz, 3H), 7.73 (t, *J* = 8.0 Hz, 3H), 7.85 (d, *J* = 8.0 Hz, 2H), 8.23 (d, *J* = 8.0 Hz, 2H), 8.32 (d, *J* = 8.0 Hz, 2H), 8.69 (s, 1H); ^{13}C NMR (100 MHz, DMSO): δ 160.90, 153.21, 152.00, 151.40, 149.75, 139.74, 133.00, 132.19, 129.88, 129.41, 128.58, 128.34, 126.87, 126.57, 123.72, 118.69, 89.31, 49.82, 29.64, 27.92; MS-ESIMS: m/z 523 (M + H)+; HR ESIMS: m/z calcd for $C_{29}H_{23}ClN_6O_2$: 523.9449; found: 523.9442.

6-(4-Chlorophenyl)-5-(3-(4-chlorophenyl)-1-phenyl-1H-pyrazol-4-yl)-1,3-dimethyl-5,6-dihydropyrimido[4,5-d]pyrimidine-2,4(1H,3H)-dione (5g). Yields 90%; mp: 343–345 °C; IR(KBr): ν 3025.56, 2926.63, 1658.87, 1624.34, 1524.14,

1435.38 cm^{-1}; ^{1}H NMR (400 MHz, DMSO): δ 3.17 (s, 3H), 3.47 (s, 3H), 6.27 (s, 1H), 7.03 (d, *J* = 8.0 Hz, 2H), 7.09-7.16 (m, 3H), 7.31 (t, *J* = 8.0 Hz, 1H), 7.41-7.49 (m, 5H), 7.71 (s, 1H), 7.84 (d, *J* = 8.0 Hz, 2H), 8.60 (s, 1H); ^{13}C NMR (100 MHz, DMSO): δ 160.89, 153.33, 151.98, 151.51, 149.91, 139.65, 138.38, 137.21, 133.10, 129.99, 129.88, 129.44, 128.72, 128.45, 128.28, 126.82, 124.22, 118.65, 89.26, 49.83, 29.59, 27.90, 20.88; MS-ESIMS: m/z 557 $(M + H)^{+}$; HR ESIMS: m/z calcd for $C_{29}H_{22}Cl_2N_6O_2$: 557.4300; found: 557.4312.

5-(3-(4-Chlorophenyl)-1-phenyl-1H-pyrazol-4-yl)-1,3-dimethyl-6-(p-tolyl)-5,6 dihydropyrimido[4,5-d]pyrimidine-2,4(1H,3H)-dione (5h). Yields 85%; mp: 346–348 °C; IR(KBr): ν 3059.58, 2928.27, 1626.28, 1628.43, 1524.28, 1408.74 cm^{-1} ; ^{1}H NMR (400 MHz, DMSO): δ 2.18 (s, 3H), 3.16 (s, 3H), 3.45 (s, 3H), 6.23 (s, 1H), 6.85 (t, *J* = 8.0 Hz, 3H), 7.36 (d, *J* = 8.0 Hz, 1H), 7.42 (d, *J* = 8.0 Hz, 2H), 7.46 (d, *J* = 8.0 Hz, 2H), 7.48 (d, *J* = 8.0 Hz, 3H), 7.65 (s, 1H), 7.84 (d, *J* = 8.0 Hz, 2H), 8.56 (s, 1H); ^{13}C NMR (100 MHz, DMSO): δ 160.99, 153.12, 152.01, 149.48, 148.93, 148.18, 147.27, 141.95, 139.40, 131.88, 131.26, 130.69, 129.94, 129.47, 127.39, 124.01, 122.48, 119.47, 118.98, 89.38, 49.59, 29.71, 27.97, 21.52; MS-ESIMS: m/z 538 $(M + H)^{+}$; HR ESIMS: m/z calcd for $C_{30}H_{25}ClN_6O_2$: 538.0115; found: 538.0112.

5-(3-(4-Chlorophenyl)-1-phenyl-1H-pyrazol-4-yl)-6-(4-methoxyphenyl)-1,3-dimethyl-5,6-dihydropyrimido[4,5-d]pyrimidine-2,4(1H,3H)-dione (5i). Yields 89%; mp: 338–340 °C; IR(KBr): ν 3068.28, 2922.37, 1613.81, 1651.24, 1514.47, 1426.25 cm^{-1} ; ^{1}H NMR (400 MHz, DMSO): δ 3.16 (s, 3H), 3.46 (s, 3H), 3.68 (s, 3H), 6.17 (s, 1H), 6.62 (d, *J* = 8.0 Hz, 2H), 6.91 (d, *J* = 8.0 Hz, 2H), 7.31 (t, *J* = 8.0 Hz, 1H), 7.38 (t, *J* = 8.0 Hz, 4H), 7.48 (d, *J* = 8.0 Hz, 2H), 7.57 (s, 1H), 7.86 (d, *J* =

8.0 Hz, 2H), 8.60 (s, 1H); ^{13}C NMR (100 MHz, DMSO): δ 160.92, 158.81, 153.83, 152.03, 150.29, 150.08, 139.59, 133.64, 133.09, 131.90, 130.28, 129.89, 129.74, 128.57, 126.94, 126.65, 124.15, 118.71, 114.61, 88.80, 55.72, 50.26, 29.59, 27.88; MS-ESIMS: m/z 554 $(M + H)^+$; HR ESIMS: m/z calcd for $C_{30}H_{25}ClN_6O_3$: 554.0109; found: 554.0103.

5-(3-(4-Chlorophenyl)-1-phenyl-1H-pyrazol-4-yl)-1,3-dimethyl-6-(3-nitro phenyl)-5,6-dihydropyrimido[4,5-d]pyrimidine-2,4(1H,3H)-dione (5j). Yields 85%; mp: 342-344 °C; IR(KBr): ν 3055.85, 2928.62, 1628.28, 1623.27, 1527.28, 1461.58 cm^{-1}; ^{1}H NMR (400 MHz, DMSO): δ 3.20 (s, 3H), 3.50 (s, 3H), 6.46 (s, 1H), 7.29 (d, *J* = 8.0 Hz, 2H), 7.37 (t, *J* = 8.0 Hz, 3H), 7.47 (t, *J* = 8.0 Hz, 3H), 7.67 (t, *J* = 8.0 Hz, 1H), 7.83 (d, *J* = 8.0 Hz, 2H), 7.98 (t, *J* = 8.0 Hz, 2H), 8.22 (s, 1H), 8.62 (s, 1H); ^{13}C NMR (100 MHz, DMSO): δ 160.49, 154.10, 152.20, 151.12, 148.59, 148.04, 143.20, 138.19, 134.30, 132.39, 132.00, 128.13, 127.90, 127.26, 124.50, 123.47, 121.30, 118.39, 117.69, 87.30, 47.39, 28.39, 26.29; MS-ESIMS: m/z 569 $(M + H)^+$; HR ESIMS: m/z calcd for $C_{29}H_{22}ClN_7O_4$: 568.9825; found: 568.9829.

1,3-Dimethyl-6-phenyl-5-(1-phenyl-3-(p-tolyl)-1H-pyrazol-4-yl)-5,6-dihydro pyrimido[4,5-d]pyrimidine-2,4(1H,3H)-dione (5k). Yields 80%; mp: 336–338 °C; IR(KBr): ν 3058.28, 2980.54, 1634.24, 1654.74, 1528.27, 1427.28 cm^{-1}; ^{1}H NMR (400 MHz, DMSO): δ 2.38 (s, 3H), 3.18 (s, 3H), 3.52 (s, 3H), 6.49 (s, 1H), 7.29 (d, *J* = 8.0 Hz, 2H), 7.02 (d, *J* = 8.0 Hz, 2H), 7.14 (d, *J* = 8.0 Hz, 3H), 7.39 (t, *J* = 8.0 Hz, 2H), 7.48 (d, *J* = 8.0 Hz, 2H), 7.59 (s, 1H), 7.69 (t, *J* = 8.0 Hz, 2H), 8.63 (s, 1H); ^{13}C NMR (100 MHz, DMSO): δ 160.47, 152.49, 151.38, 150.12, 149.67, 138.48, 137.64, 136.39, 131.39, 130.17, 129.98, 129.39, 129.05, 127.49, 126.80,

126.01, 124.59, 119.49, 86.39, 48.48, 30.64, 26.11, 20.94; MS-ESIMS: m/z 538 (M + H)$^+$; HR ESIMS: m/z calcd for $C_{30}H_{25}ClN_6O_2$: 538.0115; found: 538.0109.

6-(4-Chlorophenyl)-1,3-dimethyl-5-(1-phenyl-3-(p-tolyl)-1H-pyrazol-4-yl)-5,6 dihydro pyrimido[4,5-d]pyrimidine-2,4(1H,3H)-dione (5l). Yields 84%; mp: 330–332 °C; IR(KBr): ν 3058.28, 2962.28, 1628.25, 1625.22, 1525.27, 1435.25 cm^{-1}; ^{1}H NMR (400 MHz, DMSO): δ 2.37 (s, 3H), 3.17 (s, 3H), 3.46 (s, 3H), 6.26 (s, 1H), 7.01 (d, *J* = 8.0 Hz, 2H), 7.09 (d, *J* = 8.0 Hz, 2H), 7.16 (d, *J* = 8.0 Hz, 2H), 7.28 (t, *J* = 8.0 Hz, 3H), 7.45 (d, *J* = 8.0 Hz, 2H), 7.66 (s, 1H), 7.83 (t, *J* = 8.0 Hz, 2H), 8.54 (s, 1H); ^{13}C NMR (100 MHz, DMSO): δ 160.86, 153.19, 152.00, 151.40, 149.74, 139.75, 139.64, 137.67, 132.20, 130.17, 129.88, 129.41, 129.10, 128.47, 126.80, 126.51, 123.65, 118.62, 89.31, 49.78, 29.64, 27.93, 21.40; MS-ESIMS: m/z 538 (M + H)$^+$; HR ESIMS: m/z calcd for $C_{30}H_{25}ClN_6O_2$: 538.0115; found: 538.0119.

1,3-Dimethyl-5-(1-phenyl-3-(p-tolyl)-1H-pyrazol-4-yl)-6-(p-tolyl)-5,6-dihydro pyrimido[4,5-d]pyrimidine-2,4(1H,3H)-dione (5m). Yields 78%; mp: 358–360 °C; IR(KBr): ν 3066.28, 2927.41, 1628.55, 1625.54, 1524.05, 1422.52 cm^{-1}; ^{1}H NMR (400 MHz, DMSO): δ 2.19 (s, 3H), 2.36 (s, 3H), 3.16 (s, 3H), 3.45 (s, 3H), 6.22 (s, 1H), 6.87 (t, *J* = 8.0 Hz, 2H), 7.15 (d, *J* = 8.0 Hz, 2H), 7.31 (t, *J* = 8.0 Hz, 3H), 7.46 (t, *J* = 8.0 Hz, 3H), 7.65 (s, 1H), 7.83 (d, *J* = 8.0 Hz, 2H), 8.01 (d, *J* = 8.0 Hz, 1H), 8.53 (s, 1H); ^{13}C NMR (100 MHz, DMSO): δ 160.85, 153.35, 151.97, 151.50, 149.90, 139.67, 138.44, 137.56, 137.22, 130.29, 130.18, 129.99, 129.87, 129.61, 129.34, 129.00, 128.61, 126.75, 124.22, 119.67, 118.60, 89.30, 49.83, 29.59, 27.90, 21.39, 20.90; MS-ESIMS: m/z 517 (M + H)$^+$; HR ESIMS: m/z calcd for $C_{31}H_{28}N_6O_2$: 517.5930; found: 517.5937.

6-(4-Methoxyphenyl)-1,3-dimethyl-5-(1-phenyl-3-(p-tolyl)-1H-pyrazol-4-yl)-5,6 dihydropyrimido[4,5-d]pyrimidine-2,4(1H,3H)-dione (5n). Yields 79%; mp: 368–370 °C; IR(KBr): ν 3055.28, 2982.58, 1655.25, 1624.24, 1527.05, 1425.52 cm^{-1} ; ^{1}H NMR (400 MHz, DMSO): δ 2.16 (s, 3H), 3.08 (s, 3H), 3.42 (s, 3H), 3.68 (s, 3H), 6.34 (s, 1H), 6.39 (d, *J* = 8.0 Hz, 2H), 7.17 (d, *J* = 8.0 Hz, 2H), 7.36 (d, *J* = 8.0 Hz, 3H), 7.49 (t, *J* = 8.0 Hz, 3H), 7.67 (s, 1H), 7.98 (d, *J* = 8.0 Hz, 2H), 8.04 (d, *J* = 8.0 Hz, 1H), 8.48 (s, 1H); ^{13}C NMR (100 MHz, DMSO): δ 160.85, 154.35, 151.22, 150.62, 149.26, 138.67, 138.24, 137.56, 136.25, 130.33, 130.01, 129.22, 129.00, 128.61, 127.25, 127.00, 126.89, 126.20, 124.23, 119.65, 118.53, 89.12, 58.49, 48.03, 21.32, 20.82; MS-ESIMS: m/z 533 $(M + H)^+$; HR ESIMS: m/z calcd for $C_{31}H_{28}N_6O_3$: 533.5924; found: 533.5919.

1,3-Dimethyl-6-(3-nitrophenyl)-5-(1-phenyl-3-(p-tolyl)-1H-pyrazol-4-yl)-5,6 dihydropyrimido[4,5-d]pyrimidine-2,4(1H,3H)-dione (5o). Yields 82%; mp: 347–349 °C; IR(KBr): ν 3024.28, 2927.34, 1624.54, 1650.00, 1524.50, 1404.24 cm^{-1}; ^{1}H NMR (400 MHz, DMSO): δ 2.27 (s, 3H), 3.20 (s, 3H), 3.49 (s, 3H), 6.46 (s, 1H), 7.06 (d, *J* = 8.0 Hz, 2H), 7.18 (d, *J* = 8.0 Hz, 2H), 7.41-7.48 (m, 5H), 7.74 (s, 1H), 7.83 (d, *J* = 8.0 Hz, 2H), 7.91 (d, *J* = 8.0 Hz, 2H), 8.58 (s, 1H); ^{13}C NMR (100 MHz, DMSO): δ 161.33, 152.31, 152.05, 150.5, 149.48, 139.65, 139.67, 136.38, 133.64, 130.64, 129.38, 129.25, 129.83, 128.35, 126.73, 126.35, 122.34, 118.63, 89.86, 48.78, 26.52, 27.52, 20.34; MS-ESIMS: m/z 548 $(M + H)^+$; HR ESIMS: m/z calcd for $C_{30}H_{25}N_7O_4$: 548.5640; found: 548.5636.

5-(1,3-Diphenyl-1H-pyrazol-4-yl)-1,3-dimethyl-6-phenyl-5,6-dihydropyrimido[4-d]pyrimidine-2,4(1H,3H)-dione (5p). Yields 82%; mp: 341–343 °C; IR(KBr): ν 3025.35, 2924.20, 1625.28, 1625.44, 1514.24, 1415.27 cm^{-1}; ^{1}H NMR (400 MHz,

DMSO): δ 3.19 (s, 3H), 3.48 (s, 3H), 6.34 (s, 1H), 6.64 (t, *J* = 8.0 Hz, 1H), 6.73 (d, *J* = 8.0 Hz, 1H), 6.93 (d, *J* = 8.0 Hz, 1H), 7.42 (t, *J* = 8.0 Hz, 1H), 7.50 (t, *J* = 8.0 Hz, 3H), 7.59 (s, 1H), 7.68 (d, *J* = 8.0 Hz, 2H), 7.88 (d, *J* = 8.0 Hz, 3H), 8.22 (d, *J* = 8.0 Hz, 3H), 8.66 (s, 1H); ^{13}C NMR (100 MHz, DMSO): δ 160.97, 153.55, 152.08, 149.98, 149.10, 147.17, 139.65, 139.46, 130.45, 129.93, 129.44, 127.29, 123.99, 121.93, 119.21, 118.93, 113.55, 113.31, 88.73, 66.53, 29.63, 27.92; MS-ESIMS: m/z 489 $(M + H)^+$; HR ESIMS: m/z calcd for $C_{29}H_{24}N_6O_2$: 488.5399; found: 488.5393

6-(4-Chlorophenyl)-5-(1,3-diphenyl-1H-pyrazol-4-yl)-1,3-dimethyl-5,6 dihydro pyrimido[4,5-d]pyrimidine-2,4(1H,3H)-dione (5q). Yields 84%; mp: 378–380 °C; IR(KBr): ν 3085.28, 2927.28, 1639.28, 1621.74, 1558.52, 1438.77 cm^{-1}; ^{1}H NMR (400 MHz, DMSO): δ 3.18 (s, 3H), 3.36 (s, 3H), 6.28 (s, 1H), 7.00-7.08 (m, 4H), 7.37 (t, *J* = 8.0 Hz, 6H), 7.47 (t, *J* = 8.0 Hz, 2H), 7.66 (s, 1H), 7.85 (d, *J* = 8.0 Hz, 2H), 8.58 (s, 1H); ^{13}C NMR (100 MHz, DMSO): δ 160.89, 153.19, 151.99, 151.38, 149.73, 139.72, 133.00, 132.18, 129.87, 129.62, 129.40, 128.56, 128.33, 126.85, 126.53, 123.72, 118.67, 89.28, 49.80, 29.63, 27.93; MS-ESIMS: m/z 423 $(M + H)^+$; HR ESIMS: m/z calcd for $C_{29}H_{23}ClN_6O_2$: 523.9849; found: 523.9838.

5-(1,3-Diphenyl-1H-pyrazol-4-yl)-1,3-dimethyl-6-(p-tolyl)-5,6-dihydro pyrimido[4,5-d]pyrimidine-2,4(1H,3H)-dione (5r). Yields 81%; mp: 356–358 °C; IR(KBr): ν 3085.28, 2928.22, 1625.24, 1625.58, 1552.28, 1412.25 cm^{-1}; ^{1}H NMR (400 MHz, DMSO): δ 2.18 (s, 3H), 3.16 (s, 3H), 3.45 (s, 3H), 6.23 (s, 1H), 6.85 (t, *J* = 8.0 Hz, 3H), 7.35 (d, *J* = 8.0 Hz, 1H), 7.42 (d, *J* = 8.0 Hz, 3H), 7.45 (d, *J* = 8.0 Hz, 2H), 7.48 (d, *J* = 8.0 Hz, 3H), 7.65 (s, 1H), 7.84 (d, *J* = 8.0 Hz, 2H), 8.56 (s, 1H); ^{13}C NMR (100 MHz, DMSO): δ 160.90, 158.74, 153.74, 152.03, 151.60,

150.04, 139.69, 133.71, 133.07, 129.88, 129.45, 128.65, 128.46, 128.25, 126.81, 126.45, 124.16, 118.66, 114.69, 89.03, 55.76, 29.58, 27.88, 25.56; MS-ESIMS: m/z 503 $(M + H)^+$; HR ESIMS: m/z calcd for $C_{30}H_{26}N_6O_2$: 503. 5664; found: 503. 5652.

5-(1,3-Diphenyl-1H-pyrazol-4-yl)-6-(4-methoxyphenyl)-1,3-dimethyl-5,6 dihydro pyrimido [4,5-d]pyrimidine-2,4(1H,3H)-dione (5s). Yields 80%; mp: 320–322 °C; IR(KBr): ν 3028.59, 2984.28, 1629.28, 1658.88, 1528.25, 1448.58 cm^{-1}; ^1H NMR (400 MHz, DMSO): δ 3.16 (s, 3H), 3.45 (s, 3H), 3.67 (s, 3H), 6.18 (s, 1H), 6.60 (d, *J* = 8.0 Hz, 2H), 7.88 (d, *J* = 8.0 Hz, 2H), 7.30-7.39 (m, 6H), 7.41 (d, *J* = 8.0 Hz, 2H), 7.57 (s, 1H), 7.85 (d, *J* = 8.0 Hz, 2H), 8.57 (s, 1H); ^{13}C NMR (100 MHz, DMSO): δ 160.91, 158.76, 153.75, 144.38, 139.70, 133.73, 129.88, 128.66, 128.45, 126.47, 118.68, 114.71, 89.04, 55.77, 50.29, 34.66, 29.58; MS-ESIMS: m/z 519 $(M + H)^+$; HR ESIMS: m/z calcd for $C_{30}H_{26}N_6O_3$: 519.5658; found: 519.5663.

1,3-Dimethyl-6-phenyl-5-(1-phenyl-3-(thiophen-2-yl)-1H-pyrazol-4-yl)-5,6-di hydropyrimido [4,5-d]pyrimidine-2,4(1H,3H)-dione (5t). Yields 85%; mp: 348–350 °C; IR(KBr): ν 3052.58, 2928.58, 1631.27, 1680.51, 1558.48, 1454.28 cm^{-1}; ^1H NMR (400 MHz, DMSO): δ 3.14 (s, 3H), 3.48 (s, 3H), 6.41 (s, 1H), 7.11 (d, *J* = 8.0 Hz, 2H), 7.18 (t, *J* = 8.0 Hz, 4H), 7.48 (t, *J* = 8.0 Hz, 1H), 7.56 (t, *J* = 8.0 Hz, 2H), 7.58 (s, 1H), 7.72 (t, *J* = 8.0 Hz, 1H), 7.83 (d, *J* = 8.0 Hz, 3H), 8.62 (s, 1H); ^{13}C NMR (100 MHz, DMSO): δ 160.80, 153.45, 151.92, 149.60, 145.30, 141.22, 139.39, 134.17, 130.10, 129.92, 129.75, 127.92, 127.76, 127.26, 127.05, 126.90, 124.42, 124.08, 118.78, 89.80, 49.58, 29.64, 27.89; MS-ESIMS: m/z 495 $(M + H)^+$; HR ESIMS: m/z calcd for $C_{27}H_{22}N_6O_2S$: 495.5676; found: 495.5678.

6-(4-Chlorophenyl)-1,3-dimethyl-5-(1-phenyl-3-(thiophen-2-yl)-1H-pyrazol-4-yl)-5,6-dihydropyrimido[4,5-d]pyrimidine-2,4(1H,3H)-dione (5u). Yields 85%; mp: 352–354 °C; IR(KBr): ν 3028.28, 2958.55, 1652.28, 1668.28, 1558.02, 1427.27 cm^{-1}; ^{1}H NMR (400 MHz, DMSO): δ 3.14 (s, 3H), 3.48 (s, 3H), 6.41 (s, 1H), 7.12 (t, *J* = 8.0 Hz, 1H), 7.20-7.31 (m, 4H), 7.47 (d, *J* = 8.0 Hz, 1H), 7.52 (t, *J* = 8.0 Hz, 2H), 7.56 (t, *J* = 8.0 Hz, 2H), 7.77 (s, 1H), 7.83 (d, *J* = 8.0 Hz, 2H), 8.63 (s, 1H); ^{13}C NMR (100 MHz, DMSO): δ 160.78, 153.33, 151.92, 149.49, 145.27, 140.03, 139.38, 134.02, 132.18, 130.20, 129.91, 129.54, 128.39, 127.92, 127.26, 127.07, 126.93, 126.29, 124.07, 119.59, 118.77, 89.67, 49.65, 29.64, 27.90; MS-ESIMS: m/z 530 $(M + H)^{+}$; HR ESIMS: m/z calcd for $C_{27}H_{21}ClN_6O_2S$: 520.0126; found: 530.0132.

1,3-Dimethyl-5-(1-phenyl-3-(thiophen-2-yl)-1H-pyrazol-4-yl)-6-(p-tolyl)-5,6-dihydropyrimido[4,5-d]pyrimidine-2,4(1H,3H)-dione (5v). Yields 84%; mp: 330–332 °C; IR(KBr): ν 3028.21, 2958.75, 1625.23, 1615.20, 1558.16, 1452.22 cm^{-1}; ^{1}H NMR (400 MHz, DMSO): δ 2.28, (s, 3H), 3.49 (s, 3H), 3.59 (s, 3H), 6.64 (s, 1H), 7.05 (d, *J* = 8.0 Hz, 2H), 7.39 (d, *J* = 8.0 Hz, 2H), 7.75 (d, *J* = 8.0 Hz, 2H), 7.89 (t, *J* = 8.0 Hz, 2H), 8.02 (s, 1H), 7.72 (t, *J* = 8.0 Hz, 1H), 7.83 (d, *J* = 8.0 Hz, 3H),8.62 (s, 1H); ^{13}C NMR (100 MHz, DMSO): δ 160.80, 153.45, 151.92, 149.60, 145.30, 141.22, 139.39, 134.17, 130.10, 129.92, 129.75, 127.92, 127.76, 127.26, 127.05, 126.90, 124.42, 124.08, 118.78, 89.80, 49.58, 29.64, 27.89; MS-ESIMS: m/z 509 $(M + H)^{+}$; HR ESIMS: m/z calcd for $C_{28}H_{24}N_6O_2S$: 509.5942; found: 509.5938.

6-(4-Methoxyphenyl)-1,3-dimethyl-5-(1-phenyl-3-(thiophen-2-yl)-1H-pyrazol-4-yl)-5,6-dihydropyrimido[4,5-d]pyrimidine-2,4(1H,3H)-dione (5w). Yields 83%;

mp: 338–340 °C; IR (KBr): ν 3088.55, 2952.22, 1612.28, 1658.25, 1543.82, 1455.52 cm^{-1} ; ^{1}H NMR (400 MHz, DMSO): δ 3.18 (s, 3H), 3.36 (s, 3H), 3.65 (s, 3H), 6.38 (s, 1H), 6.85 (d, *J* = 8.0 Hz, 2H), 7.18 (t, *J* = 8.0 Hz, 3H), 7.48 (t, *J* = 8.0 Hz, 2H), 7.62 (t, *J* = 8.0 Hz, 2H), 7.86 (s, 1H), 7.93 (t, *J* = 8.0 Hz, 1H), 8.03 (d, *J* = 8.0 Hz, 3H), 8.62 (s, 1H); ^{13}C NMR (100 MHz, DMSO): δ 162.49, 151.45, 150.29, 149.48, 144.40, 140.22, 138.64, 133.65, 130.54, 129.24, 128.15, 127.82, 126.19, 125.06, 124.85, 124.05, 123.42, 123.08, 119.29, 88.50, 48.20, 30.40, 26.26; MS-ESIMS: m/z 525 $(M + H)^{+}$; HR ESIMS: m/z calcd for $C_{28}H_{24}N_6O_3S$: 525.5936; found: 525.5939.

1,3-Dimethyl-6-(3-nitrophenyl)-5-(1-phenyl-3-(thiophen-2-yl)-1H-pyrazol-4-yl)-5,6-dihydropyrimido[4,5-d]pyrimidine-2,4(1H,3H)-dione (5x). Yields 84%; mp: 208–210 °C; IR(KBr): ν 3059.34, 2935.28, 1650.63, 1686.51, 1555.52, 1492.13 cm^{-1}; ^{1}H NMR (400 MHz, DMSO): δ 3.14 (s, 3H), 3.48 (s, 3H), 6.41 (s, 1H), 7.11 (t, *J* = 8.0 Hz, 2H), 7.16 (t, *J* = 8.0 Hz, 3H), 7.48 (t, *J* = 8.0 Hz, 1H), 7.56 (t, *J* = 8.0 Hz, 2H), 7.57 (s, 1H), 7.59 (s, 1H), 7.84 (d, *J* = 8.0 Hz, 3H), 8.62 (s, 1H); ^{13}C NMR (100 MHz, DMSO): δ 160.79, 153.09, 151.95, 149.28, 148.30, 145.14, 142.17, 139.37, 133.82, 131.25, 131.08, 130.38, 129.91, 128.41, 127.95, 127.22, 126.92, 123.74, 122.36, 119.60, 119.37, 118.81, 89.88, 49.45, 29.71, 27.94; MS-ESIMS: m/z 540 $(M + H)^{+}$; HR ESIMS: m/z calcd for $C_{27}H_{21}N_7O_4S$: 540.5651; found: 540.5648.

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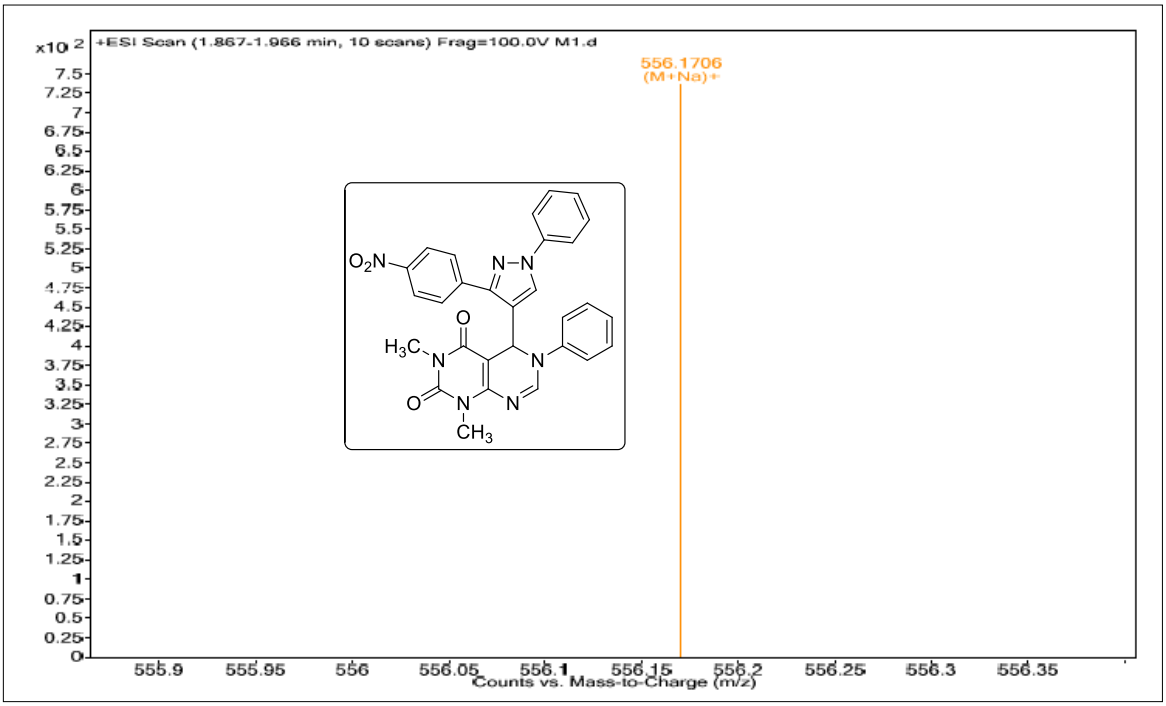
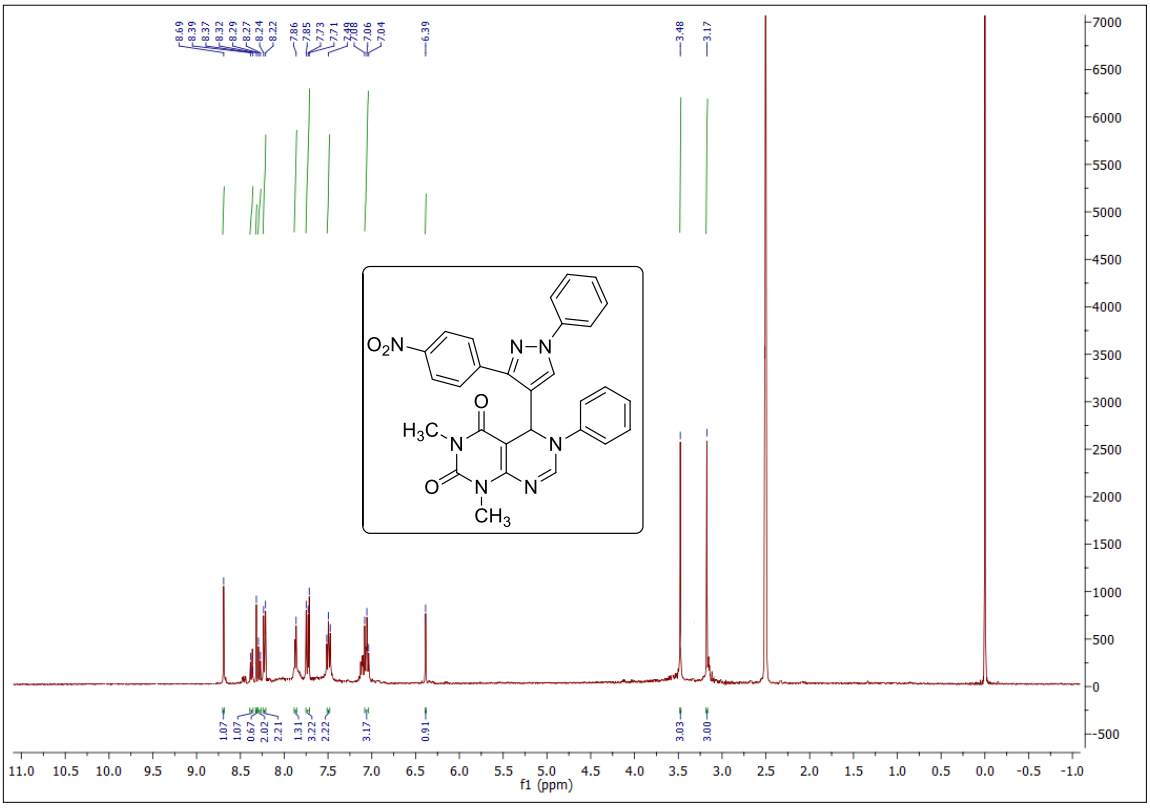
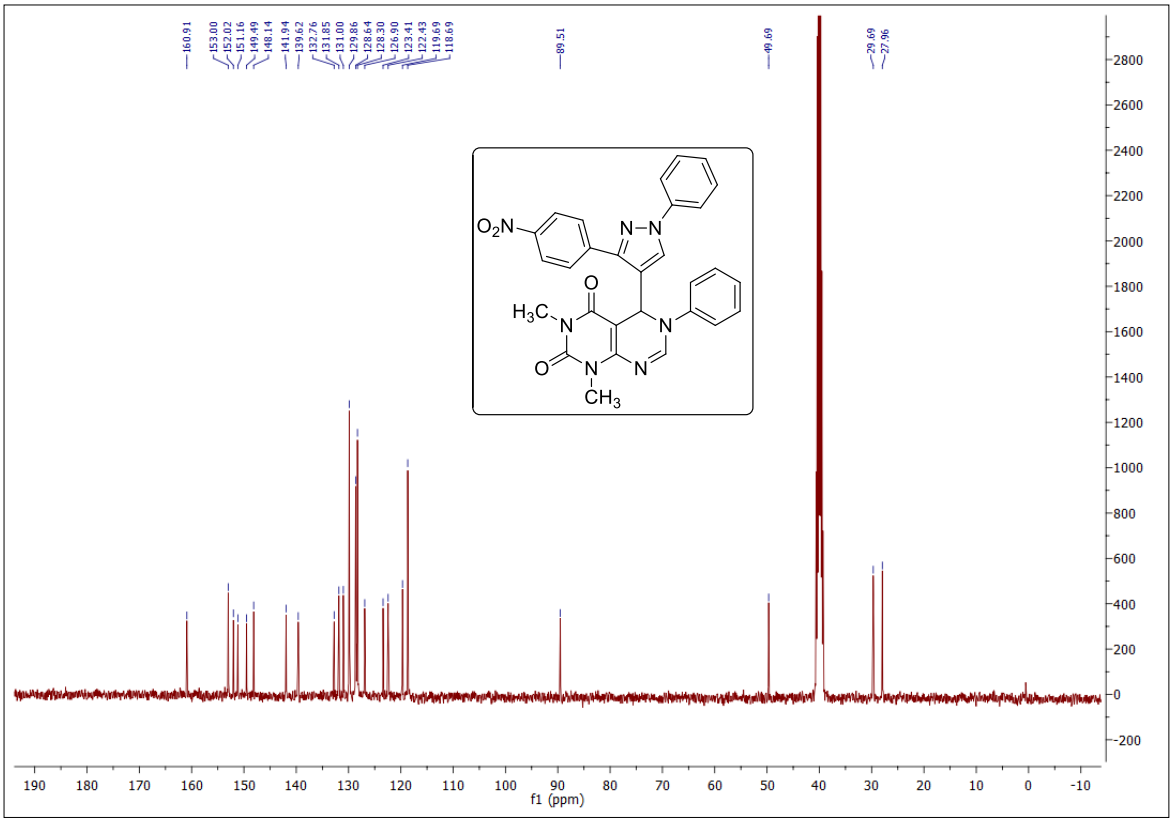
SELECTED SPECTRA



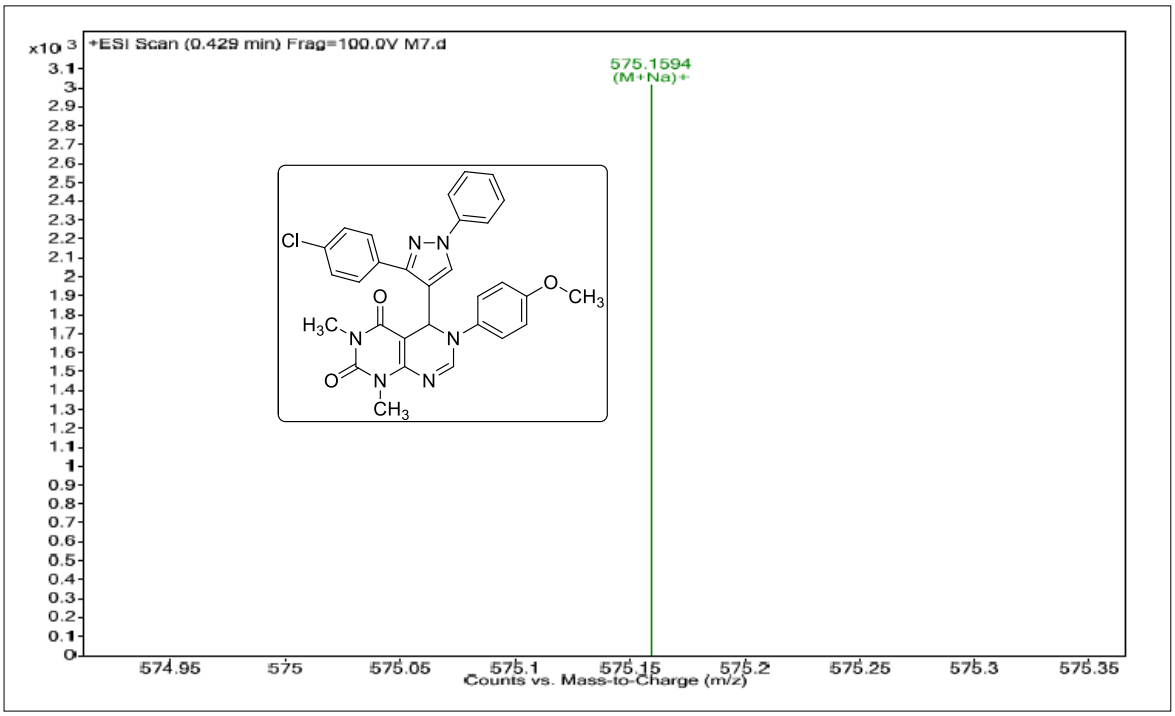
HR-MS Spectrum of compound **5a**



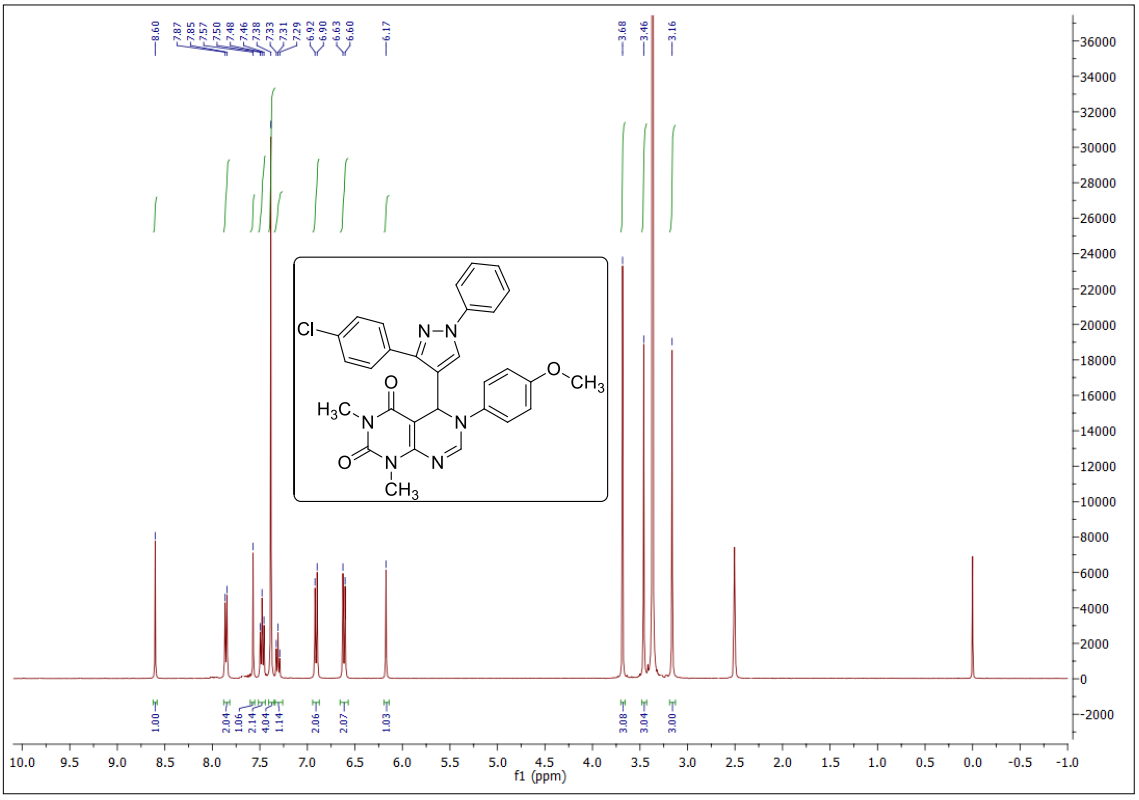
^{1}H NMR Spectrum of compound **5a**



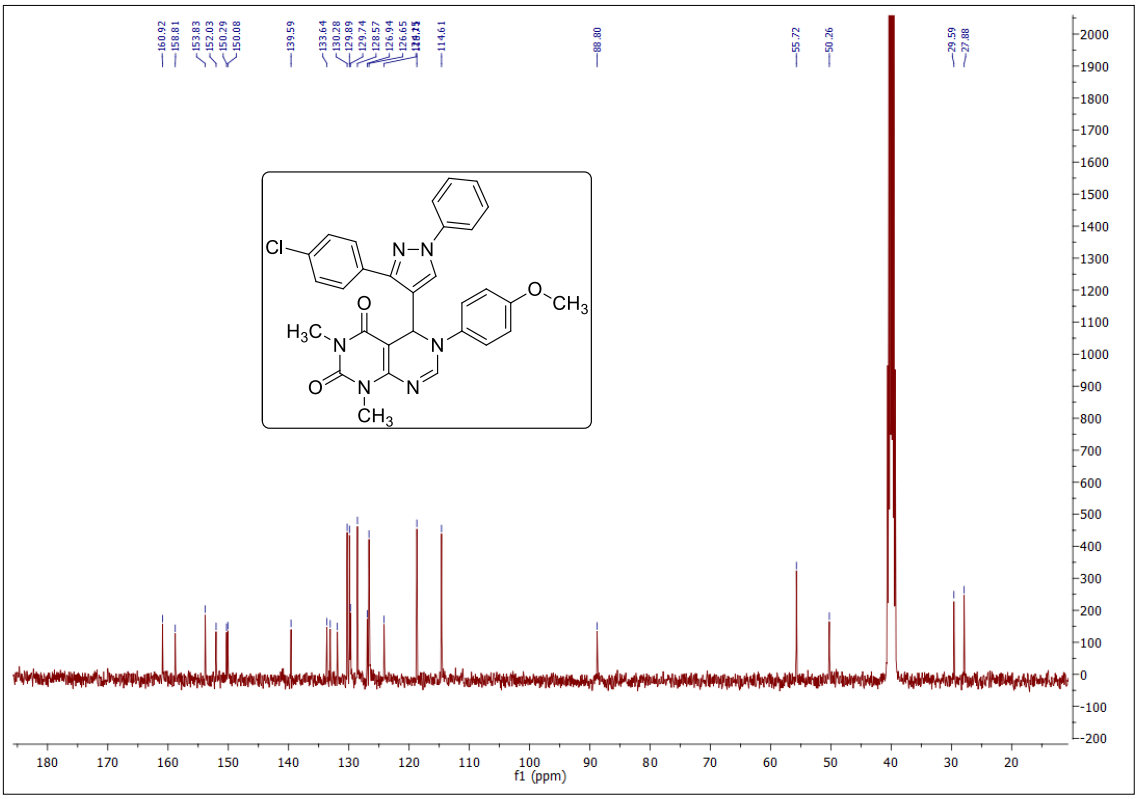
^{13}C NMR Spectrum of compound **5a**



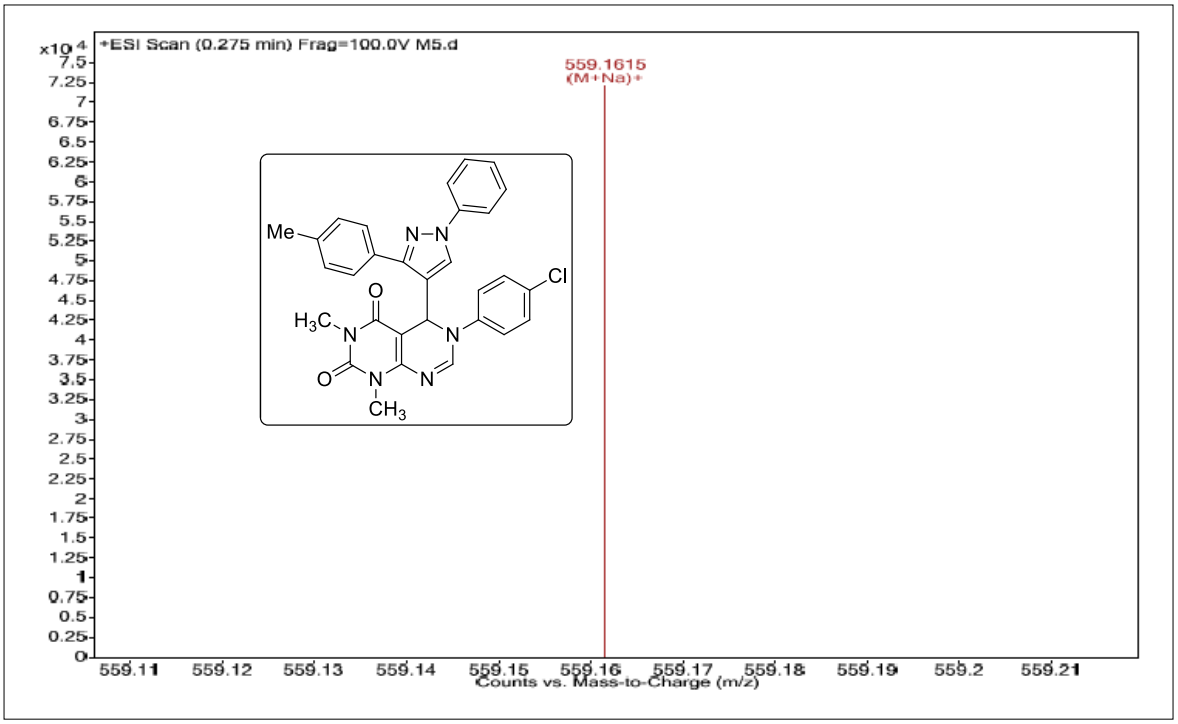
HR-MS Spectrum of compound **5i**



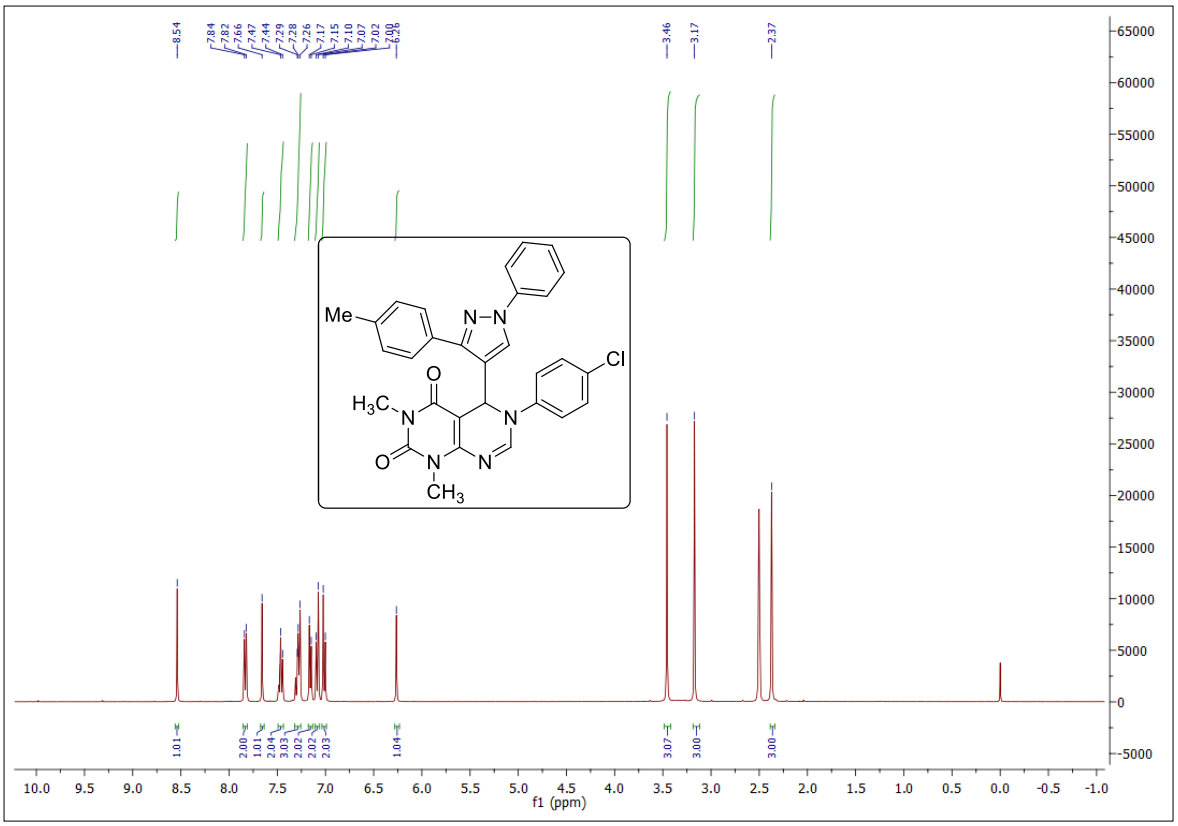
^{1}H NMR Spectrum of compound **5i**



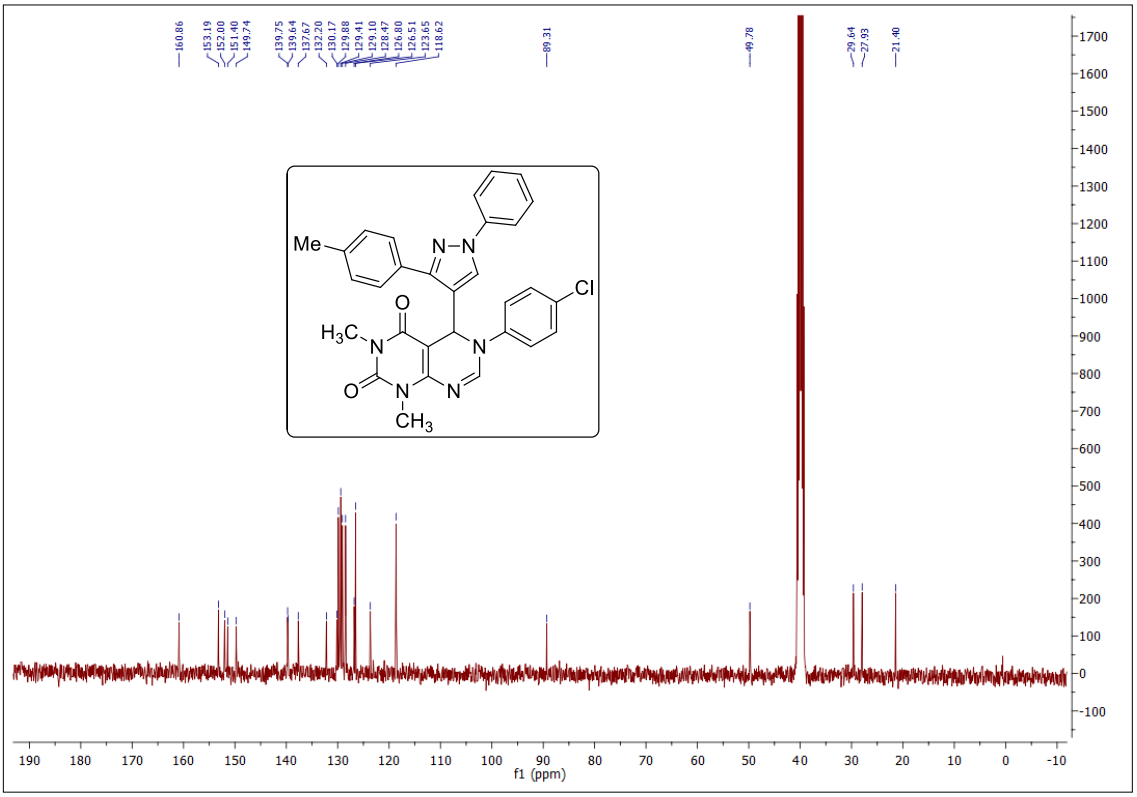
^{13}C NMR Spectrum of compound **5i**



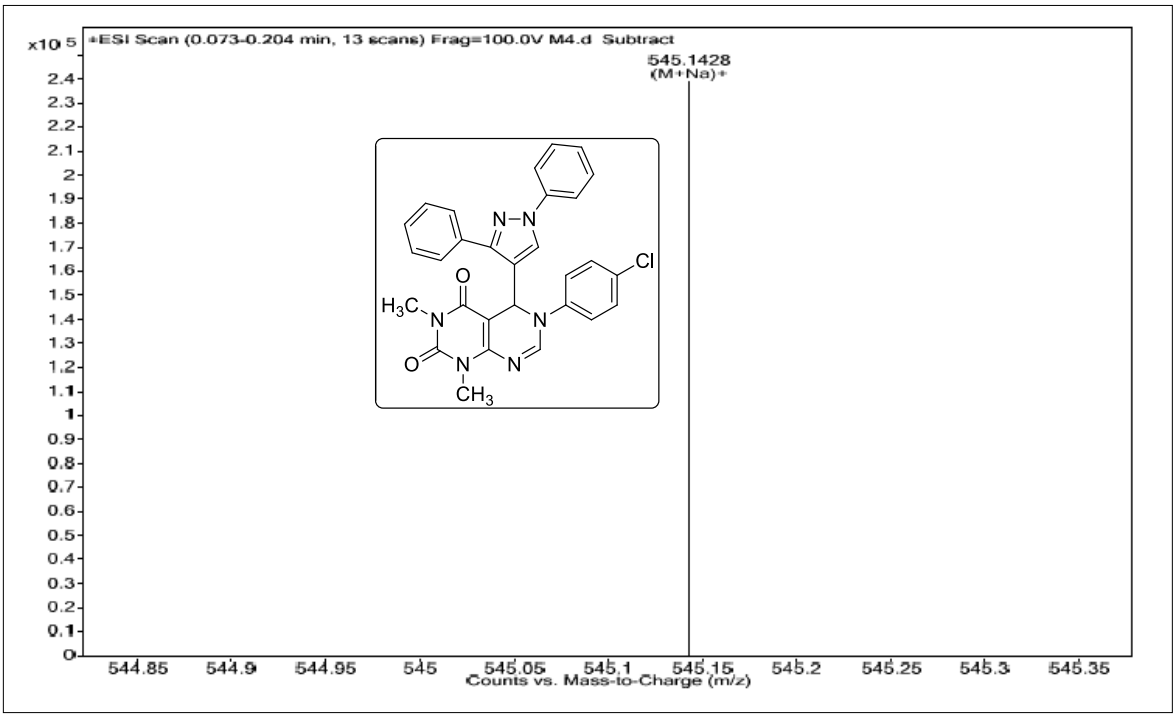
HR-MS Spectrum of compound **5i**



^{1}H NMR Spectrum of compound **5l**



^{13}C NMR Spectrum of compound **5l**



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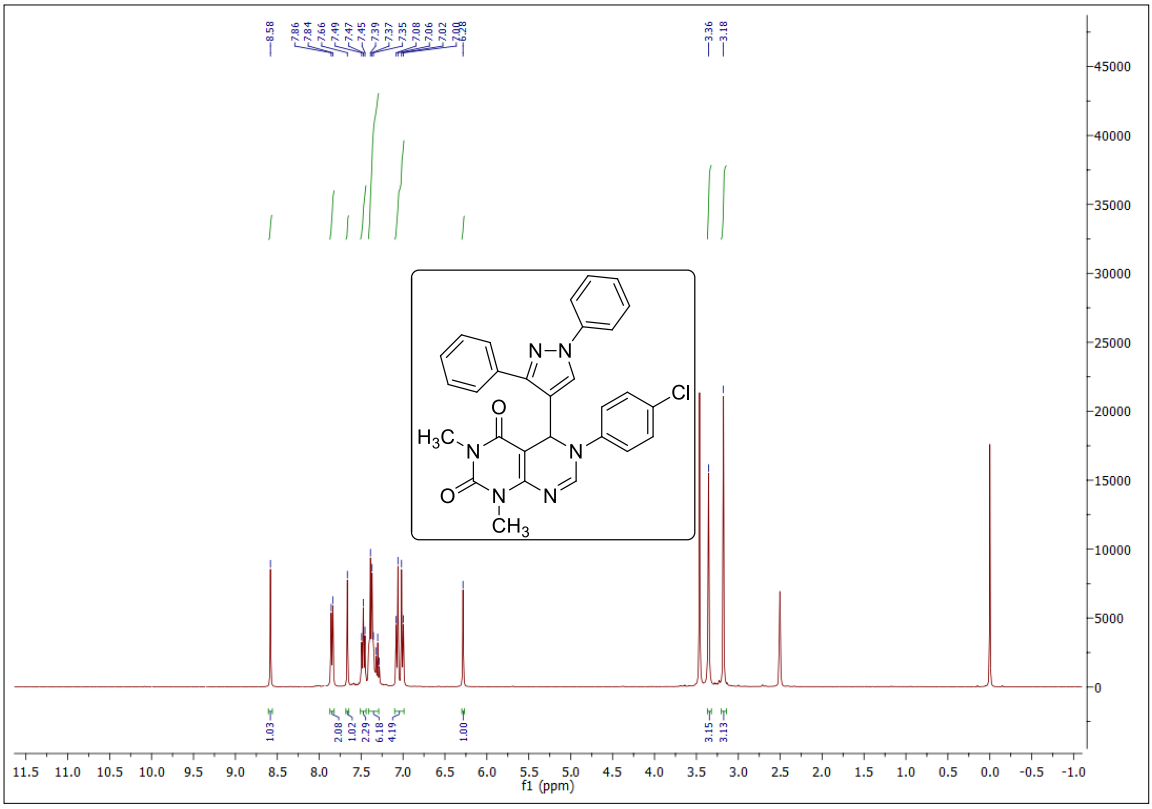
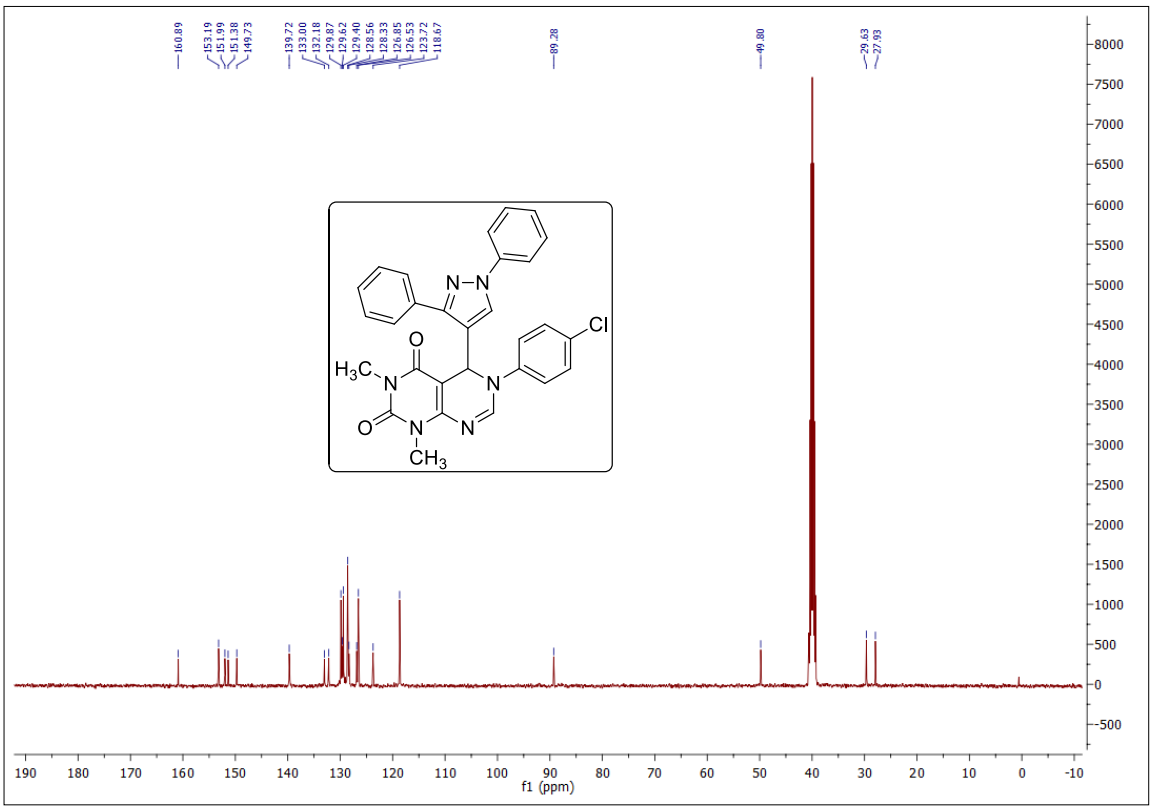
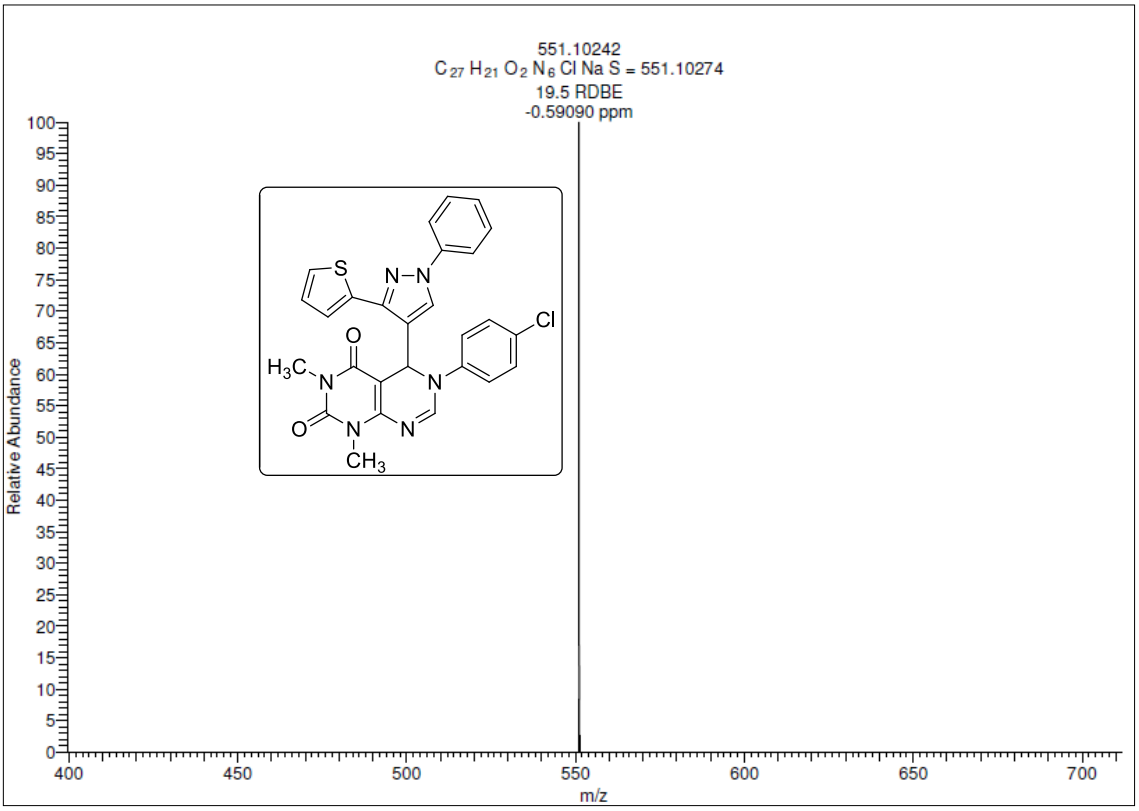


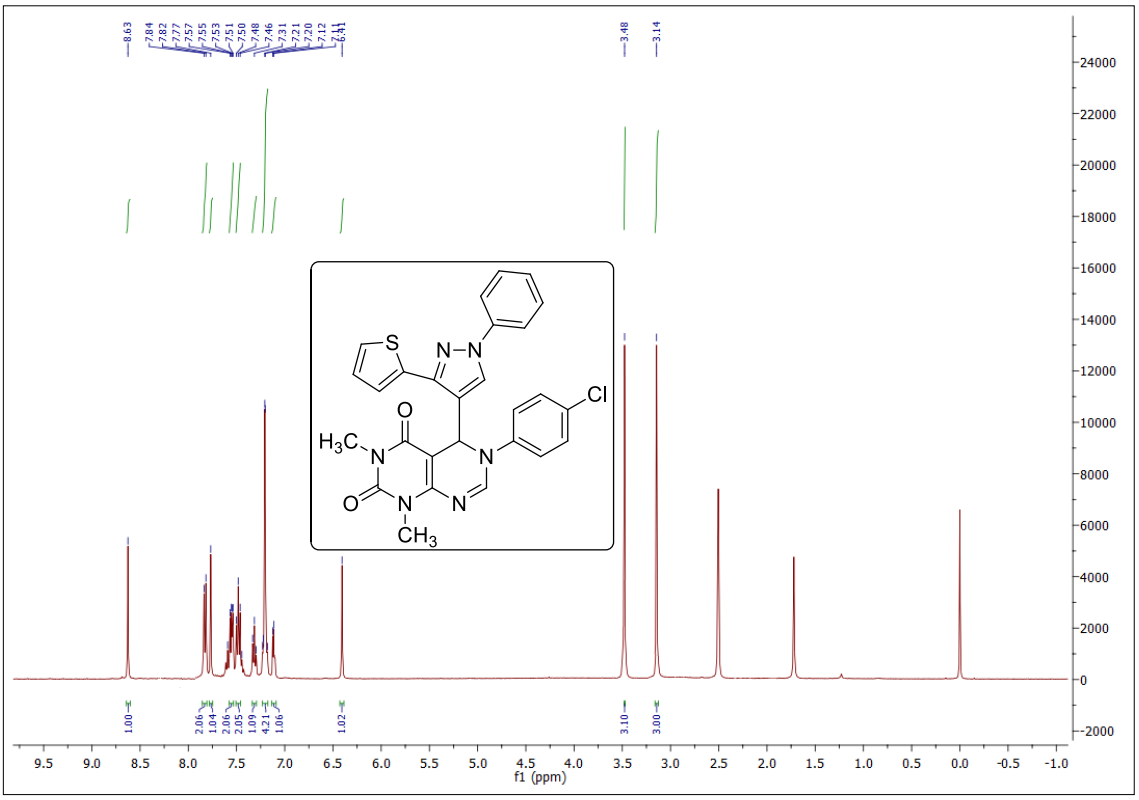
^{1}H NMR Spectrum of compound **5q**



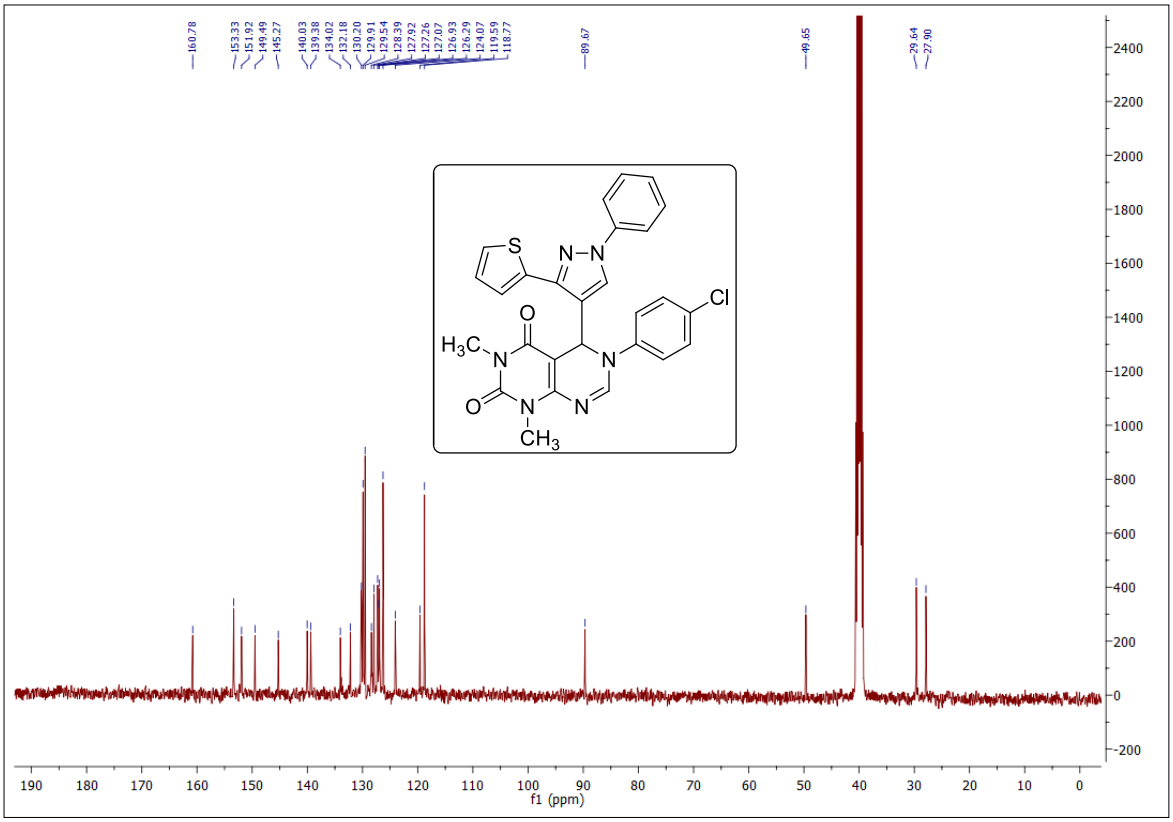
^{13}C NMR Spectrum of compound **5q**



HR-MS Spectrum of compound **5u**



^{1}H NMR Spectrum of compound **5u**



^{13}C NMR Spectrum of compound **5u**

ONE-POT THREE-COMPONENT SYNTHESIS OF NEW TYPE OF FUSED TRIAZOLO[1,5-*a*] AND TETRAZOLO[1,5-*a*] PYRIMIDINES

5.1. INTRODUCTION

Nowadays considerable attention has also been diverted towards the synthesis of simple heterocyclic and related N-containing heterocyclic derivatives such as purines, pteridines, quinazolines, triazolopyrimidines, tetrazolopyrimidines, pyrazolopyrimidines, furopyrimidines, pyridopyrimidines and pyrrolopyrimidines and all of them were found to possess remarkable pharmacological properties.[1] Pyrimidine ring with different heterocyclic scaffolds gives rise to a new class of hybrid heterocycles possessing improved activity.[2] The condensation of pyrimidine ring with another ring of triazole and tetrazole gives rise to the formation of bicyclic heterocycles known as fusedpyrimidines. Over the past few decades, there has been great interest in the synthesis of pyrimidine compounds due to their wide range of applications.[3] Pyrimidine with fused triazole or tetrazole derivatives are also known for their medicinal applications.[4] They exhibit antimicrobial,[5] anticancer,[6] anticonvulsant and antidepressant activities.[7] Some of these compounds are showing remarkable activity in the inhibition of tyrosin-kinases[8] and phosphodiesterases,[9] along with having antihypertensive[10] and antiviral activities.[11] From the standpoint of biological activity, fused hetero aromatic systems are often of much greater interest than the constituent monocyclic compounds. Some examples of derivatives of triazolo and tetrazolo[1,5-a]pyrimidine derivaties with their biological activities are shown below (**Fig. 1**).[12]

Multicomponent reactions (MCRs) are fast and effective methods for the sustainable and diversity oriented synthesis of heterocyclic scaffold, which provide, one of the most sustainable platform.[13] The approach of conducting the reaction in water will be one of the most suitable directions, which not only meets the necessities of green chemistry but for developing libraries of medicinal scaffolds.[14] Among the strategies used to construct the small molecule, multicomponent reactions are ideal synthetic tools to generate multiple molecular

scaffolds due to operational simplicity, resource and energy effectively from the viewpoint of atom-economy and sustainable technology.[15]

In view of the demands of organic synthesis, there is still need to develop new catalytic, environmentally benign and efficient protocols preferably by multicomponent approch.[16] The development of metal oxide nanoparticles has drawn much attention due to their operational simplicity, environmentally friendly nature, easy separation procedures, economical efficiency and recyclability.[17] Besides the network of hydrogen bonding capability, the high specific heat capacity, the large surface tension, the high cohesive energy are some of the exclusive properties of water that can intensely influence the conversion in this medium.

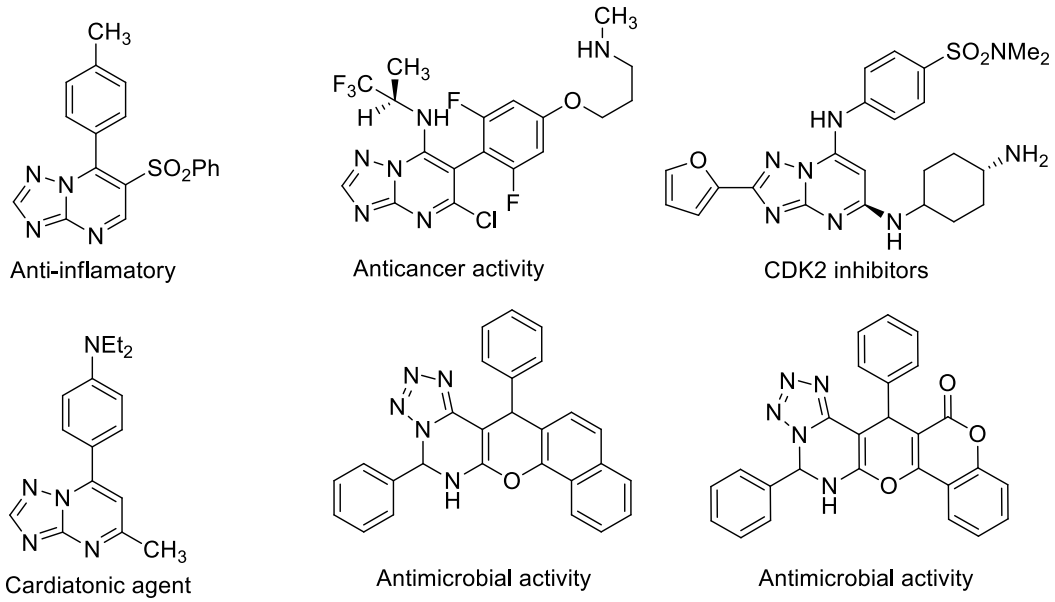
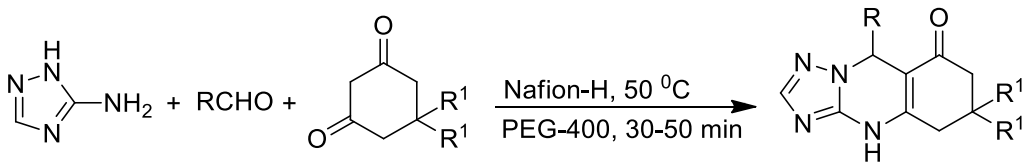


Figure 1. Triazolo and tetrazole pyrimidins based biologically active molecules

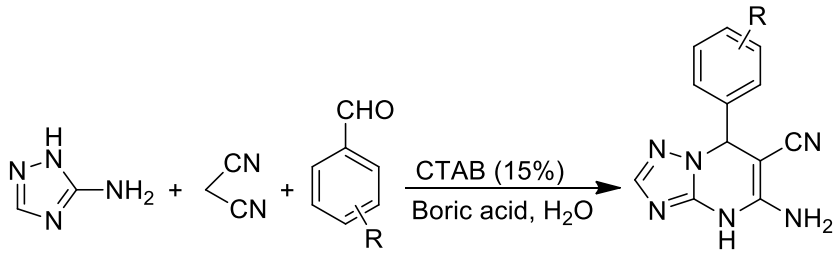
5.1.1. REPORTED SNTHETIC STRATAGE FOR TRIAZOLO & TETRAZOLO PYRIMIDINES

M. Kidwai et *al.*[18] Developed Nafion-H catalyzed efficient one-pot synthesis of triazolo [5,1-*b*] quinazolinones and triazolo [1,5-*a*] pyrimidines (**Scheme 1**).



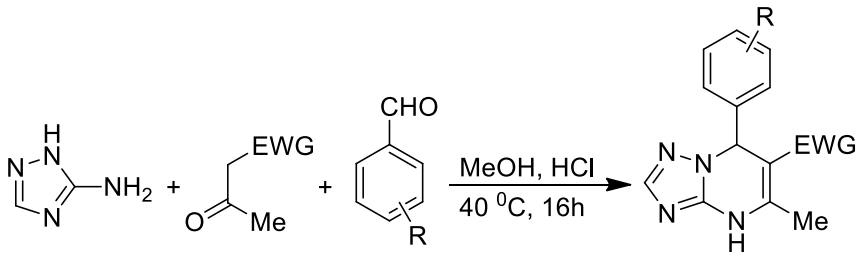
Scheme 1

M. Singh et *al.*[19] developed an efficient Boric acid aqueous micellar medium was an effective and recyclable catalytic system for the synthesis of aryl-7,8-dihydro [1,2,4]triazolo [4,3-*a*]pyrimidine-6-carbonitriles (**Scheme 2**).



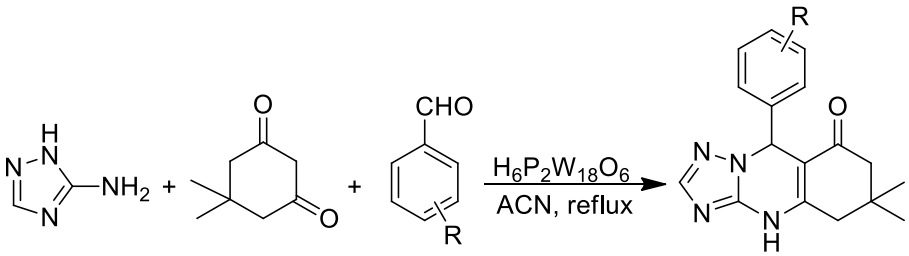
Scheme 2

Y. V. Sedash et *al.*[20] described the a three-component Biginelli-like condensations using 3-amino-1, 2,4-triazole as a 1,3-binucleophile (**Scheme 3**).



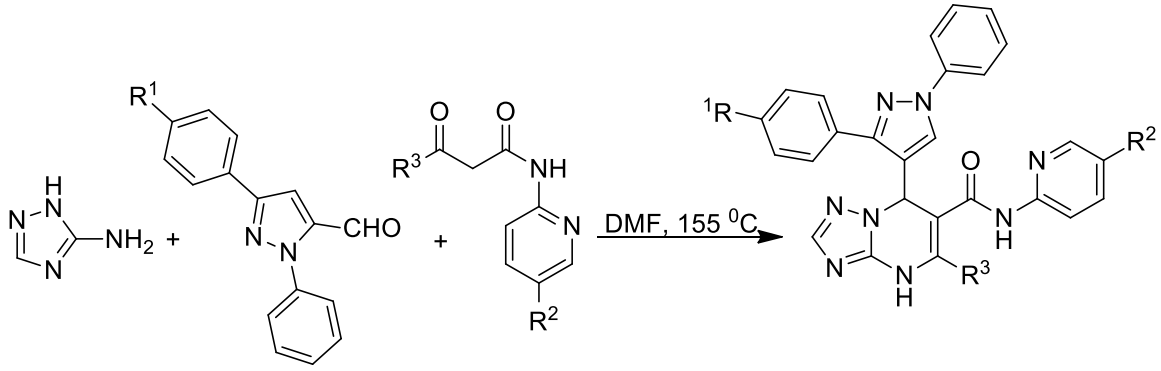
Scheme 3

J. Palaniraja et al.[21] reported synthesis and photophysical properties of unreported nitrogen ring junction quinazolines (**Scheme 4**).



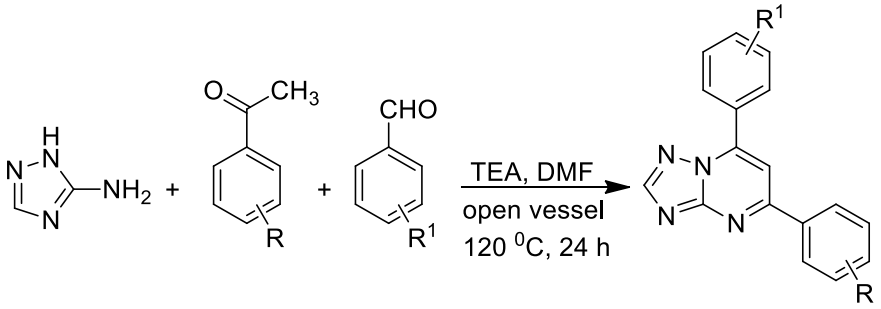
Scheme 4

J. D. Bhatt et *al.*[22] worked at the synthesis of Pyrazole clubbed triazolo [1,5-*a*] pyrimidine hybrids and on their anti-tubercular activity (**Scheme 5**).



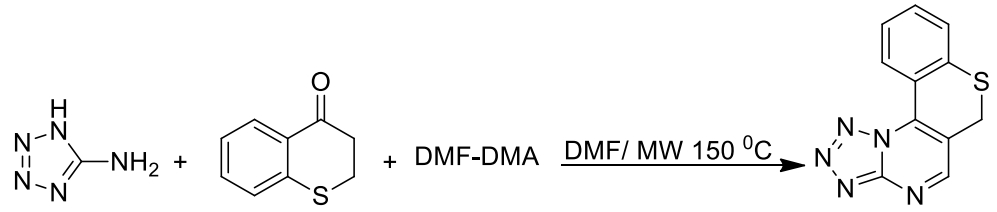
Scheme 5

X. He et *al.*[23] Described the synthesis of base-catalyzed one-step synthesis of 5,7-disubstituted-1,2,4-triazolo [1,5-*a*] pyrimidines (**Scheme 6**).



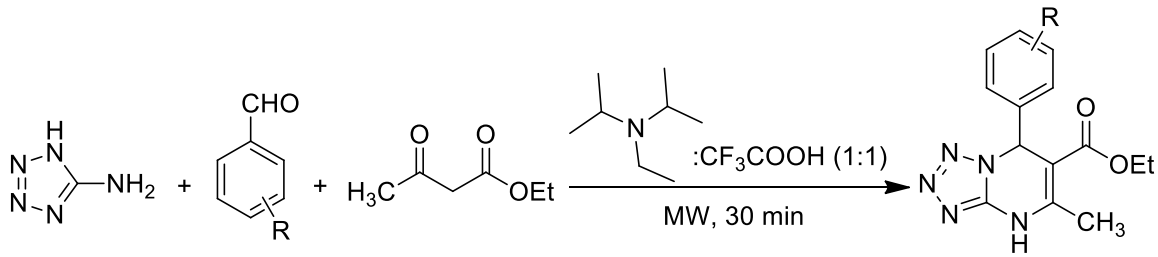
Scheme 6

E. M. Abbas et *al.*[24] reported an efficient multicomponent reaction for the synthesis of bioactive polyheterocyclic ring systems under controlled microwave irradiation (**Scheme 7**).



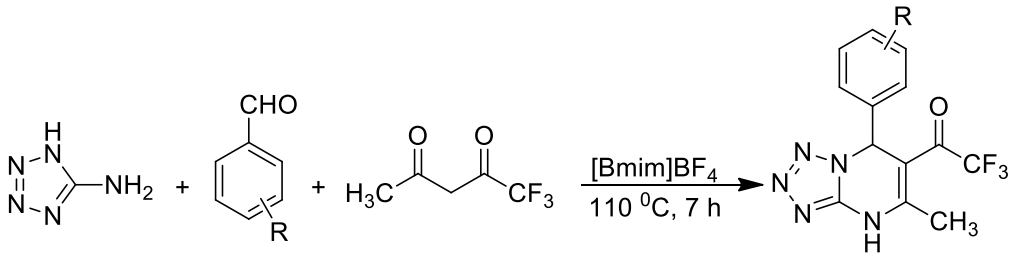
Scheme 7

C. Raju et *al.*[25] designed a simple synthesis of tetrazolo[1,5-*a*]pyrimidines by using diisopropylammonium trifluoroacetate mediated synthesis (**Scheme 8**).



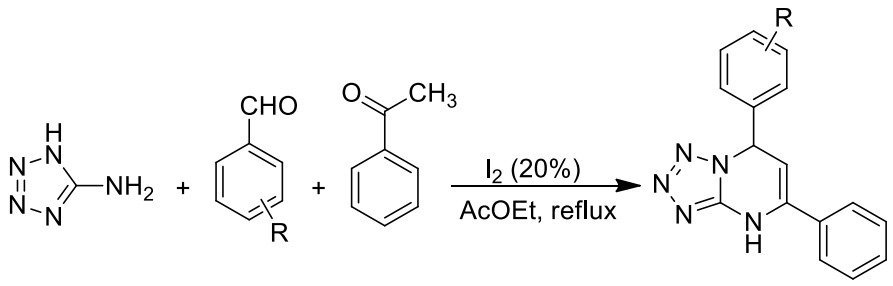
Scheme 8

T. J. Li et *al.*[26] developed an ionic liquid–mediated one-pot synthesis of 5-(Trifluoromethyl)-4,7-dihydrotetrazolo [1,5-*a*] pyrimidine Derivatives (**Scheme 9**).



Scheme 9

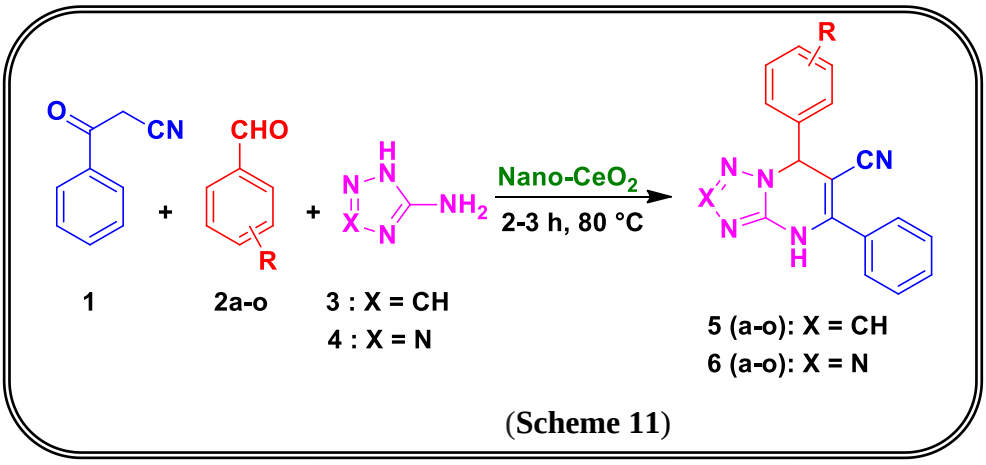
Y. L. Zeng et *al.*[27] used iodine catalyzed one-pot multicomponent synthesis for the production of a library of compounds containing tetrazolo [1,5-*a*] pyrimidine core (**Scheme 10**).



Scheme 10

5.2. PRESENT WORK

However, some flaws, like tedious workup procedures, poor yields, more reaction times and resource-wasting procedures, have occurred along with these multistage routines. Although the three component reactions of 3-aminotriazole/5-aminotetrazole with benzoylacetonitrile and aldehydes have been developed and improved the efficiency for the synthesis of triazolo or tetrazolo pyrimidines. But the high-temperatures, long reaction times, or low yields, as well as the stoichiometric amounts of corrosive protonic acid, used in these protocols would limit the practical use of their producers in a large scale as they do not meet the demand of "Green Chemistry".[28] Intrigued by these facts, we tried and achieved a convenient, effective, and eco-friendly process which yielded a library of tetrazolopyrimidines, which might result in an entirely new complementary biological activity (**Scheme 11**).

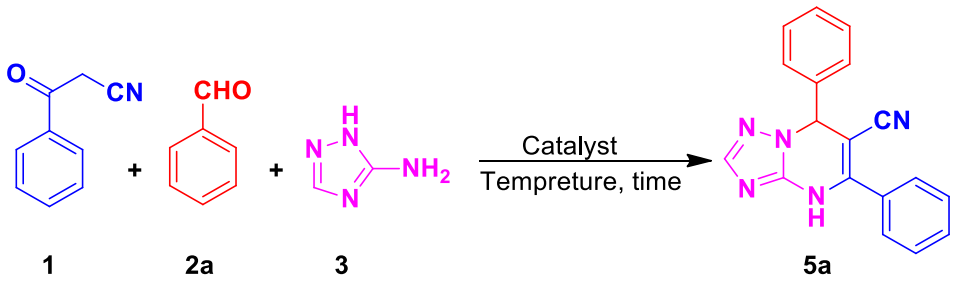


(**Scheme 11**)

5.3. RESULTS AND DISCUSSION

In perspective of a sustainable chemistry program for the synthesis of the triazolo and tetrazolo[1,5-*a*]pyrimidine derivatives using CeO_2 as catalyst, initially we explored the reaction of benzoylacetonitrile **1** (1 mmol), benzaldehyde **2a** (1 mmol) and 5-aminotriazole **3** (1 mmol) as a model substrate to investigate the feasibility of the strategy and to optimize the reaction conditions (Table 1). Initially, the above reaction was performed in neat condition which failed to yield the desired product even after 8h (Table 1, entry 1) and later the above reaction was conducted with various solvents such as ethanol, water, acetonitrile, toluene and dioxane (Table 1, entries 2-6). Among them, water was found to be the best solvent of choice. Further, we carried out the same reaction in the presence of different acids and bases such as p-TSA, AcOH, pyridine, triethylamine and piperidine, using water as a solvent and these catalysts did not promote the reaction efficiently (Table 1, entries 7–11). Later, the reaction was also done in the presence of different nano metaloxides, such as Fe_3O_4, CuO, ZnO, TiO_2 and CeO_2, (Table 1, entries 12–16).

Table 1. Optimization of reaction conditions for the synthesis of **5a**



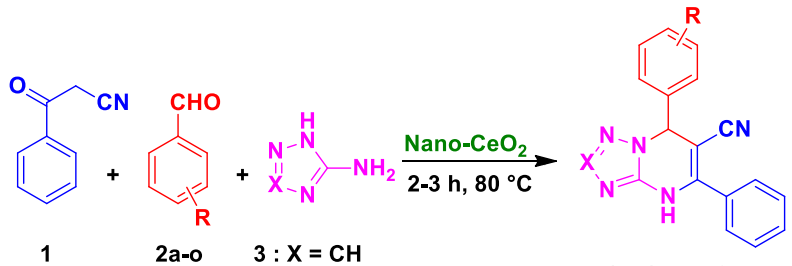
Entry[a]	Catalyst (mol %)	Solvent	Temp (°C)	Time (h)	Yield[b] (%)
1	----------	neat	80	12	none
2	----------	ethanol	80	16	20
3	----------	water	80	12	44
4	----------	acetonitrile	80	14	18

5	----------	toluene	80	24	12
6	----------	dioxane	80	24	10
7	p-TSA (10%)	water	80	6	54
8	Acetic acid (10%)	water	80	6	46
9	pyridine (10%)	water	80	8	40
10	Triethylamine (10%)	water	80	6	43
11	Piperidine (10%)	water	80	6	49
12	Fe_3O_4 (10%)	water	80	6	56
13	CuO (10%)	water	80	6	45
14	ZnO (10%)	water	80	6	62
15	TiO_2 (10%)	water	80	6	58
16	CeO_2 (10%)	water	80	3	82
17	**CeO_2 (20%)**	**water**	**80**	**2**	**90**
18	CeO_2 (30%)	water	80	2	86
19	CeO_2 (20%)	water	rt	2	60
20	CeO_2 (20%)	water	40	2	72
21	CeO_2 (20%)	water	60	2	84
22	CeO_2 (20%)	water	100	2	90

[a]Reaction conditions: benzoylacetonitrile **1** (1 mmol), benzaldehyde **2a** (1 mmol), 5-aminotriazole **3** (1 mmol), solvent (5 mL) and catalyst. [b]Isolated yield.

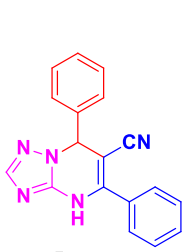
Among these, the CeO_2 nano particles were identified as the suitable catalyst for **5a** which was isolated is maximum amounts (90%). The yield of the desired product was also checked using different mol ratios of 20% and 30 mol% (Table 1, entries 17-18). The maximum yield was obtained when the 20 mol % catalyst was used (Table 1, entry 17). Besides, we also studied the effect of temperature on the above by conducting the reaction at different temperatures like room temperature, 40, 60, 80 and 100 °C (Table 1, entries 18-22). It was established that 80 °C was the optimum temperature to get the maximum yield of the title compounds.

Table 2. Synthesis of triazolo, tetrazolo[1,5-*a*]pyrimidines (**5a-o** and **6a-o**)[a]

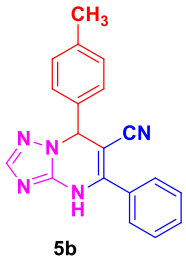


1 2a-o 3 : X = CH 4 : X = N

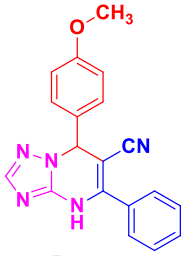
5 (a-o): X = CH
6 (a-o): X = N
time, [b]yield(%)



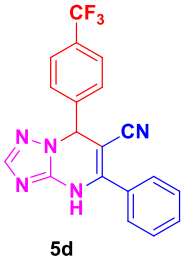
5a
2h, 90%



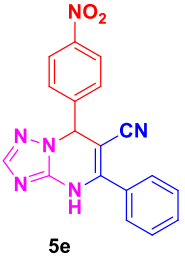
5b
2.5h, 88%



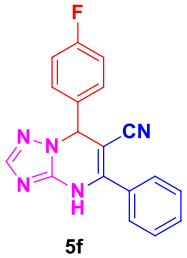
5c
2.5h, 88%



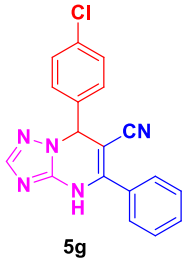
5d
2h, 89%



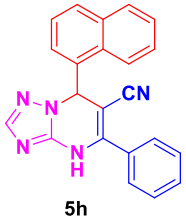
5e
2h, 92%



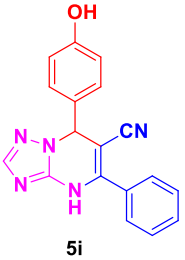
5f
2h, 89%



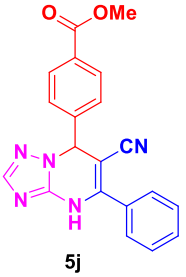
5g
2h, 92%



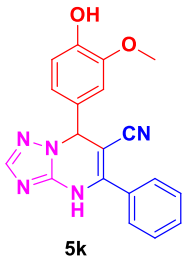
5h
3h, 88%



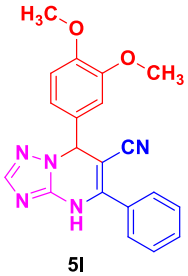
5i
3h, 86%



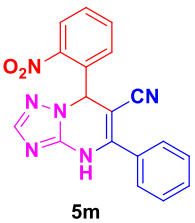
5j
2.5h, 88%



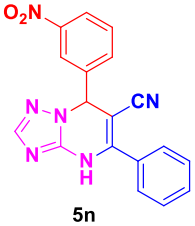
5k
2.5h, 84%



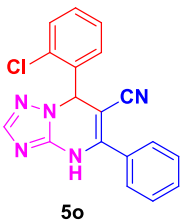
5l
2.5h, 82%



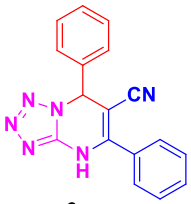
5m
2.5h, 86%



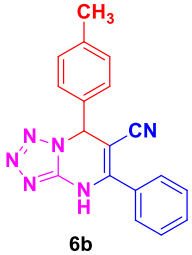
5n
2h, 89%



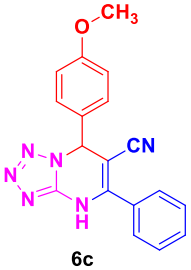
5o
2.5, 86%



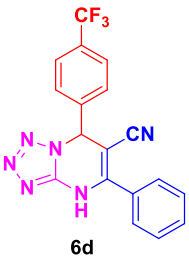
6a
2.5h, 86%



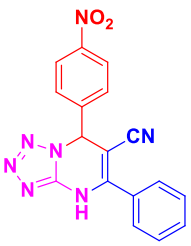
6b
3h, 84%



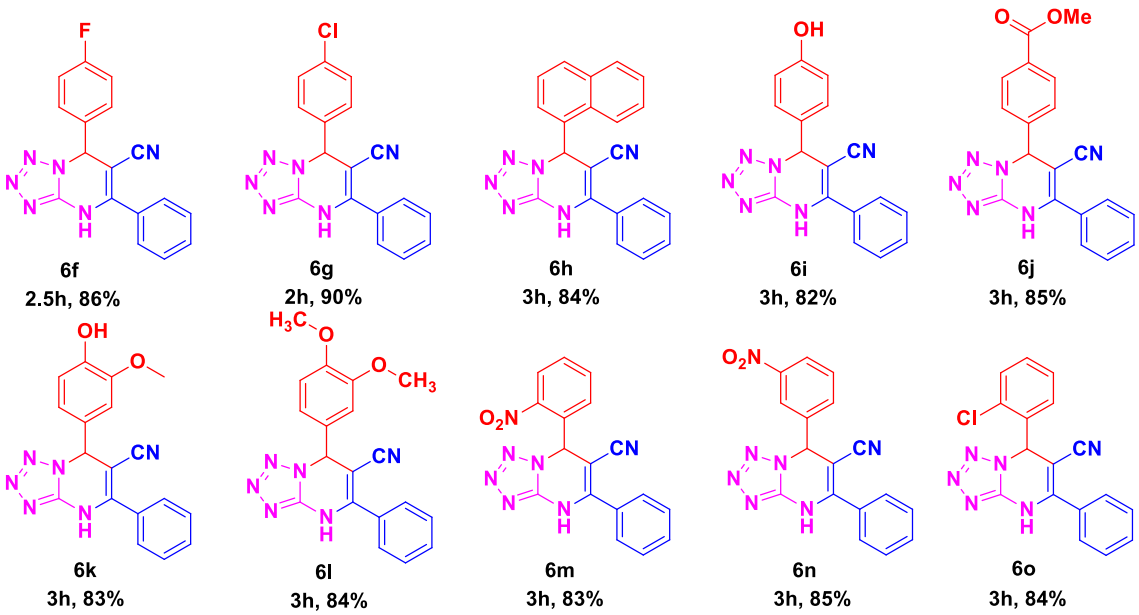
6c
3h, 85%



6d
2.5, 85%



6e
2h, 90%

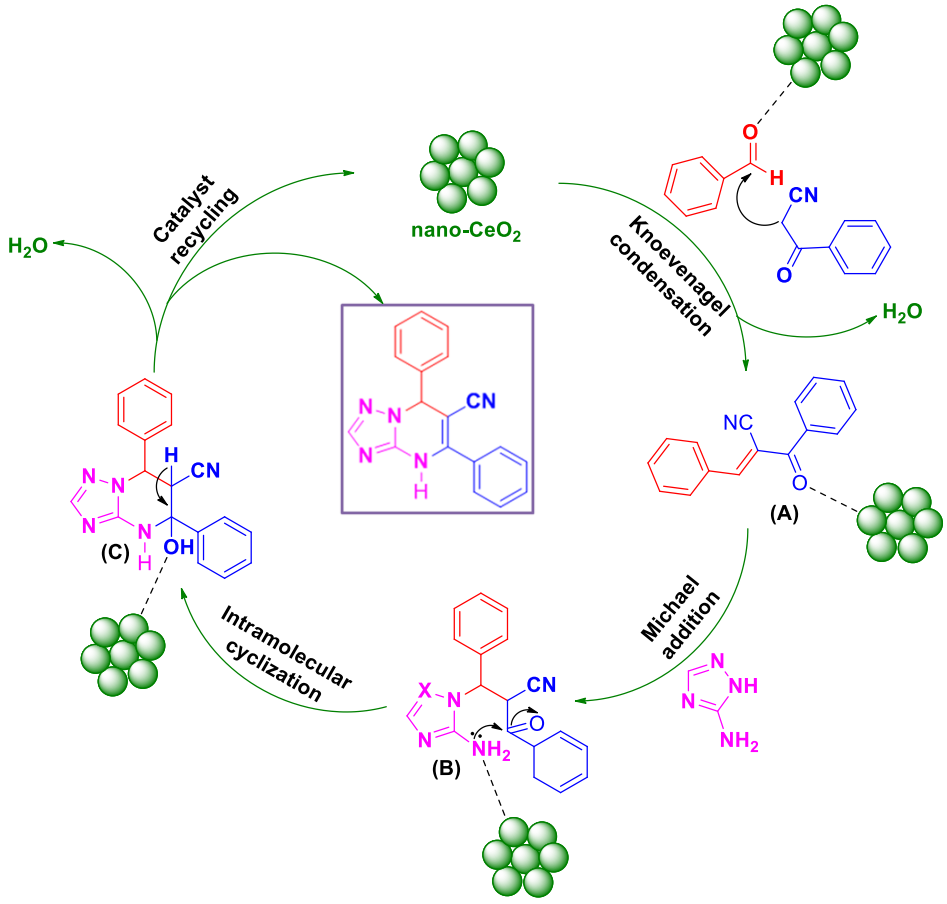


[a]Reaction conditions: benzoylacetonitrile **1** (1 mmol), aromatic aldehydes **2a-o** (1 mmol), 5-aminotriazole/ 5-aminotetrazole **3/4** (1 mmol), nano-CeO_2 (20 mol %) and water (5 mL). [b]Yields of the isolated products.

In all the studied experiments, the aromatic aldehydes reacted smoothly and obtained the corresponding triazolo and tetrazolo[1,5-*a*]pyrimidines in decent yields (Table 2, entries 1-30). Having optimized the reaction conditions, we then explored its applicability for a library of products employing various aromatic aldehydes. The reaction proceeded smoothly whether the aromatic aldehydes contains either electron donating or electron withdrawing groups, producing the corresponding title compounds in excellent yields.

Moreover, with comparing the available variations we noted an interesting fact that in the reaction with 5-aminotriazole **3** provided in excellent yields (Table 2, entries 1-15). But which 5-aminotetrazole **4** under similar reaction conditions desired product was obtained in moderate yields (Table 2, entries 16-30). After that, we examined the reaction with a diversity of substituted aromatic aldehydes and couldn't observe any remarkable differences in their reactivity and product yields even though the aromatic aldehydes contains different types of substituents on aromatic ring.

A plausible mechanism for the formation of triazolo and tetrazolo[1,5-*a*]pyrimidines using CeO_2 NPs is shown in (**Scheme 12**). Initially, the reaction may occur via a Knoevenagel condensation between aromatic aldehyde **2** and benzoylacetonitrile **1** to form the intermediate (**A**) with the eliminations of water in the presence of the ceria material, which makes the aldehydes more electrophilic. Consequently, by the Michael addition reaction of the intermediate (**A**) with 5-aminotriazole **3** resulting in the formation of intermediate (**B**). Later (**B**) which undergoes intermolecular cyclization to give intermediate (**C**). Finally, intermediate **C** undergoes intermolecular dehydrogenation to afford the title product. All the synthesized compounds were confirmed by their spectral data (IR, ESI-MS, ^{1}H NMR, and ^{13}C NMR). Spectral data for all the compounds were in full agreement with the proposed structures.



Scheme 12. Proposed mechanism for the formation of compound **5a**

With a view of testing green and sustainable protocols, the nanosized CeO_2 catalyst was employed for reusable studies for the synthesis of compound **5a** with benzoylacetonitrile **1**, benzaldehyde **2a** and 5-aminotriazole **3**. The results showed that the CeO_2 catalyst can be reused several times without a noticeable loss of catalytic activity. After each cycle, the reaction was followed by extraction of products and the catalysts reusability. The collected catalyst was washed with acetone for several times to remove organic substances and used for the next run. A chart of the catalytic activity of recycled CeO_2 material is provided in (**Fig. 2**).

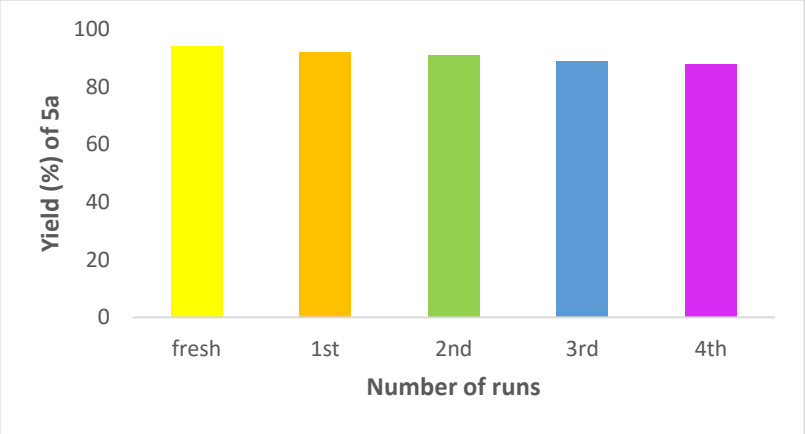


Figure. 2. Reusability studies of the nano-sized CeO_2 for the synthesis of compound **5a**

5.3.1. CHARACTERIZATION OF THE CATALYST

Powder-XRD: The XRD patterns of the synthesized ceria material which was calcined at 773 K are shown in Fig. 3(a). The main peaks (111), (200), (220) and (311) observed in this figure correspond to the fluorite structure of ceria and confirmed its formation. The average crystallite size was calculated using the intense peaks such as (111), (220), and (311) with the help of Sherrer equation, and the obtained value is around 8.9 nm. Fig. 3(b) shows the TEM image of ceria, from the figure it is clear that the average particle size of ceria is 7-9 nm, which is in good agreement with the XRD results.

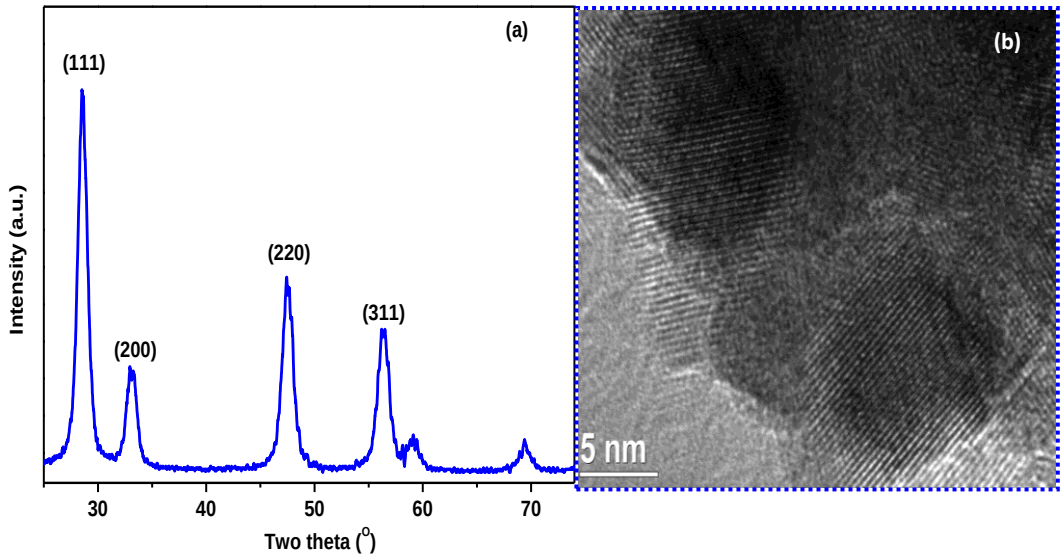
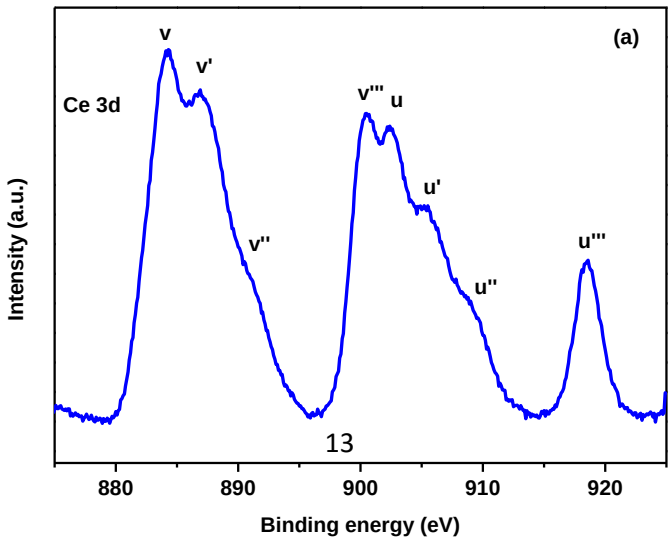


Figure. 3(a) Powder X-ray diffraction patterns of ceria nanoparticles (b) transmission electron microscopy image of ceria nanoparticles.

XPS: The oxidation state of a cerium on the surface of the cerium oxide can readily be determined by XPS, and in this context, the XPS analyses were performed on ceria sample. The results corresponding to Ce 3d, O1s is shown in **Fig. 4(a)** shows the Ce 3d XPS spectrum. The peaks designated as u, u″, u‴ and v, v″, v‴ can be assigned to Ce^{4+}, while the peaks u′ and v′ belong to Ce^{3+}. The O 1s core level XPS profile **(Fig. 4(b))** of the ceria sample is noted by a broad peak centered at 530.5 eV, which is attributed to the lattice oxygen (O_I). The peak centered at 532.5 eV, could be associated to adsorbed water and/or carbonates (O_{II}) From the XPS figure, it is clear that on the surface of the ceria material cerium exist in both 3+ and 4+ oxidation states along with the presence of two types of oxygen (lattice oxygen and adsorbed oxygen).[29]



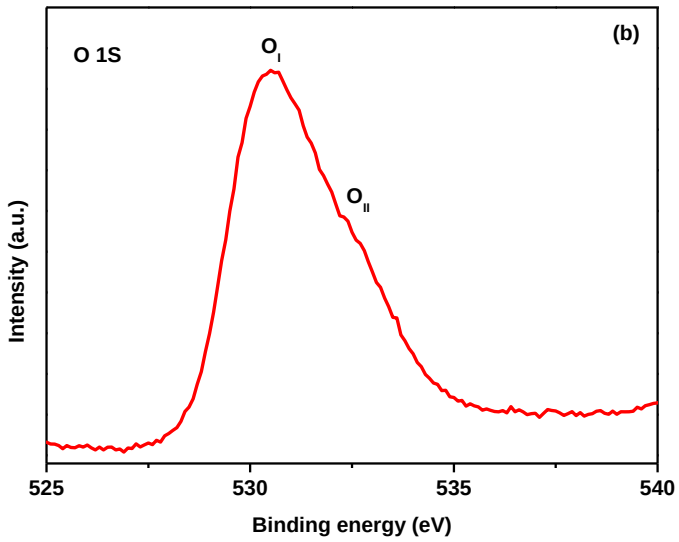


Figure. 4. XP spectra of ceria 3d and O 1S of ceria nano particles

5.3.2. SINGLE CRYSTAL X-RAY CRYSTALLOGRAPHIC STUDY

The structure of compound **5a** was further confirmed by single crystal X-ray diffraction analysis (**Fig. 5**, CCDC-1440718). The compound **5a** crystallizes in the centrosymmetric monoclinic C2/c space group with one molecule in the asymmetric unit gives the pertinent crystallographic data.

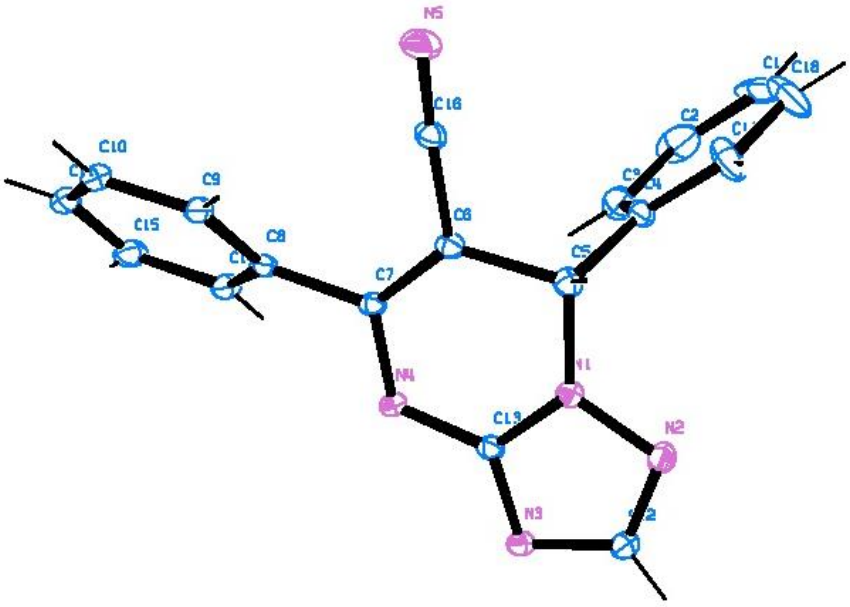


Figure. 5. PLATON representation of compound **5a (**CCDC 1440718). Thermal ellipsoids are drawn at 50% probability level.

5.3.3. SPECTRAL DISCUSSION

IR: In all the compounds **5a–o** and **6a–o**, the formation of pyrimidine was confirmed by the disappearance of band around 2200 cm^{-1} due to (–CN) group and appearance of –NH group around 3316-3443 cm^{-1}.

^{1}H NMR: In ^{1}H NMR, the –NH signal was observed at δ 11.55-9.16 ppm. In all the compounds, the singlet protons of pyrimidine (CH) group were observed as a singlet at δ 6.41-5.68 ppm. All other aromatic and aliphatic protons appeared at expected regions.

^{13}C NMR: In ^{13}C NMR, the signal appeared at δ 95-80.56 ppm can be attributed to nitrile carbon. The signal observed at δ 57.84-58.94 ppm was assigned to (CH) tertiary carbon and in all the compounds.

Mass: The structures of all synthesized compounds were further confirmed by its mass spectra. The mass spectra detected the expected molecular ion signals (M + 1) corresponding to respective molecular weight of the synthesized compounds.

5.4. EXPERIMENTAL RESULTS

5.4.1. MATERIALS AND METHOD

All reagents were procured from commercial sources and used without further purification. A Bruker WM-4 (X) spectrophotometer (577 model) was used for recording IR spectra (KBr). NMR spectra were recorded on a Bruker WM-500 spectrophotometer at 500 MHz (^{1}H), Bruker WM-400 spectrophotometer at 400 MHz (^{1}H) and 100 MHz (^{13}C) respectively, in DMSO-$d_6$with TMS as an internal standard. Elemental analysis was performed on a Carlo Erba EA 1108automatic elemental analyzer. Mass spectra (ESI) were carried out on a jeo1 JMSD-300 spectrometer.

Single crystal XRD: The single crystal X-ray diffraction data of the compound **5a** was crystallized from chloroform to yield prismatic crystals. X-ray data for the compounds were collected at room temperature using a Bruker Smart Apex CCD diffractometer with graphite monochromatedMoKα radiation (λ = 0.71073 Å) by the ω-scan method.[30] Preliminary lattice parameters and orientation matrices were obtained from four sets of frames. Integration and scaling of intensity data were accomplished using the SAINT program.[30] The structure was solved by direct

methods using SHELXS9735, and refinement was carried out by the full-matrix least-squares technique using SHELXL97.

Powder-XRD: Powder-XRD data was acquired in the 2θ range of 12–80° on a Rigaku Multiflex instrument using Cu Kα (λ = 1.5418 Å) radiation and a scintillation counter detector. Crystalline phases present in the samples were identified with the help of Powder Diffraction File-International Centre for Diffraction Data (PDF-ICDD). The average size of the crystalline domains (D) of the prepared materials were estimated with the help of the Scherrer equation (1) using the XRD data of all prominent lines.

$$D = K\,\lambda/\beta\,\cos\theta \qquad (1)$$

Where D denotes the crystallite size, λ is the X-ray wavelength (1.541 Å), K indicates the particle shape factor taken as 1, β represents the peak width (FWHM, full width at half maximum) in radians and θ is the Bragg diffraction angle.

TEM: TEM studies were made on a JEM-2010 (JEOL) instrument equipped with a slow-scan CCD camera at an accelerating voltage of 200 kV.

XPS: The XPS measurements were performed on a Shimadzu (ESCA 3400) spectrometer by using Al Kα (1486.7 eV) radiation as the excitation source. Charging effects of catalyst samples were corrected by using the binding energy of the adventitious carbon (C 1s) at 284.6 eV as internal reference. The XPS analysis was done at ambient temperature and pressures usually in the order of less than 10^{-8} Pa.

5.4.2. PREPARATION OF CERIA NANOPARTICLES

The CeO_2 nanoparticles were synthesized by a modified coprecipitation method using appropriate amounts of the corresponding $Ce(NO_3)_3{\cdot}6H_2O$ (Aldrich, AR grade) precursor. The desired amount of the precursor was dissolved in double-distilled water under mild stirring conditions. Dilute aqueous ammonia solution was added dropwise over a period until the pH of the solution reached ~8.5. The resulting pale yellow colored slurry was decanted, filtered, and washed several times with double distilled water. The obtained precipitate was oven-dried at 393 K for 12 h and calcined at 773 K for 5 h at a heating rate of 5 K min^{-1} in an air atmosphere.

5.4.3. General procedure for the synthesis of triazolo and tetrazolo[1,5-*a*]pyrimidine derivatives (5a-o and 6a-o). A dry 50 mL flask was charged with 3-oxo-3-phenylpropanenitrile (1 mmol), aromatic aldehydes (1 mmol), 5-aminotrizole/5-aminotetrazole (1 mmol) in water (5 mL) and nano-CeO_2 was added. The reaction mixture was stirred at 60 °C for 2-3h the progress of the reaction was monitored by TLC. After completion of the reaction the product was extracted with ethyl acetate and purified by column chromatography using silicagel (ethyl acetate: n-hexane 4:6) to afford pure compounds **5a-o** and **6a-o** in good yields.

5.4.4. PHYSICAL AND SPECTRAL DATA

5,7-Diphenyl-4,7-dihydro-[1,2,4]triazolo[1,5-a]pyrimidine-6-carbonitrile (5a). White powder; mp: 330-332 °C; **IR** (KBr) υ_{max} (cm^{-1}): 3128, 2922, 2346, 1552; **^{1}H NMR** (400 MHz, DMSO-d_6): δ 6.42 (s, 1H), 7.38-7.45 (m, 5H), 7.55 (d, *J* = 8 Hz, 3H), 7.66 (d, *J* = 8 Hz, 2H), 7.78 (s, 1H), 11.44 (s, 1H); **^{13}C NMR** (100 MHz, DMSO-d_6): δ 150.94, 149.91, 147.53, 140.09, 132.30, 131.47, 129.39, 129.12, 129.02, 127.83, 118.80, 81.01, 60.44; **ESI-MS**: *m/z* 300 $(M+1)^+$; Anal. Calcd. For $C_{18}H_{13}N_5$; C, 72.23; H, 4.38; N, 23.40; Found: C, 72.16; H, 4.33; N, 23.61.

5-Phenyl-7-(p-tolyl)-4,7-dihydro-[1,2,4]triazolo[1,5-a]pyrimidine-6-carbonitrile (5b). White powder; mp: 343-345 °C; **IR** (KBr) υ_{max} (cm^{-1}): 3158, 2992, 2327, 1558; **^{1}H NMR** (400 MHz, DMSO-d_6): δ 2.32 (s, 3H), 6.37 (s, 1H), 7.23-7.29 (m, 4H), 7.53-7.58 (m, 3H), 7.66 (d, *J* = 8 Hz, 2H), 7.76 (s, 1H), 11.40 (s, 1H); **^{13}C NMR** (100 MHz, DMSO-d_6): δ 150.83, 149.77, 147.46, 138.82, 137.25, 132.36, 131.41, 129.90, 129.10, 129.00, 118.78, 81.15, 60.23, 21.21; **ESI-MS**: *m/z* 314 $(M+1)^+$; Anal. Calcd. For $C_{19}H_{15}N_5$; C, 72.83; H, 4.82; N, 22.35; Found: C, 72.83; H, 4.87; N, 22.49.

7-(4-Methoxyphenyl)-5-phenyl-4,7-dihydro-[1,2,4]triazolo[1,5-a]pyrimidine-6-carbonitrile (5c). White powder; mp: 332-334 °C; **IR** (KBr) υ_{max} (cm^{-1}): 3134, 2984, 2356, 1558; **^{1}H NMR** (400 MHz, DMSO-d_6): δ 3.78 (s, 3H), 6.38 (s, 1H), 6.99 (d, *J* = 8 Hz, 1H), 7.27 (d, *J* = 8 Hz, 1H), 7.33 (d, *J* = 8 Hz, 1H), 7.56 (d, *J* = 8 Hz, 2H), 7.66 (d, *J* = 8 Hz, 2H), 7.75 (d, *J* = 8 Hz, 1H), 8.02 (t, *J* = 8 Hz, 1H), 8.85 (s, 1H), 11.37 (s, 1H); **^{13}C NMR** (100 MHz, DMSO-d_6): δ 160.09, 158.40, 150.78,

149.73, 147.36, 133.09, 132.39, 132.22, 131.40, 129.65, 129.23, 129.09, 129.00, 118.84, 114.49, 80.74, 59.93, 55.67; **ESI-MS**: *m/z* 330 $(M+1)^+$; Anal. Calcd. For $C_{19}H_{15}N_5O$; C, 69.29; H, 4.59; N, 21.26; Found: C, 69.43; H, 4.64; N, 21.09.

5-Phenyl-7-(4-(trifluoromethyl)phenyl)-4,7-dihydro-[1,2,4]triazolo[1,5-a]pyrimidine-6-carbonitrile (5d). White powder; mp: 326-328 °C; **IR** (KBr) υ_{max} (cm^{-1}): 3168, 2928, 2398, 1568; **^{1}H NMR** (400 MHz, DMSO-d_6): δ 6.60 (s, 1H), 7.57 (d, *J* = 8 Hz, 2H), 7.66 (t, *J* = 8 Hz, 4H), 7.83 (d, *J* = 8 Hz, 3H), 8.55 (s, 1H), 11.54 (s, 1H); **^{13}C NMR** (100 MHz, DMSO-d_6): δ 151.20, 150.41, 147.64, 144.26, 132.16, 131.55, 129.11, 129.04, 128.85, 126.43, 118.58, 80.25, 59.85; **ESI-MS**: *m/z* 368 $(M+1)^+$; Anal. Calcd. For $C_{19}H_{12}F_3N_5$; C, 62.13; H, 3.29; N, 19.07; Found: C, 62.27; H, 3.34; N, 19.32.

7-(4-Nitrophenyl)-5-phenyl-4,7-dihydro-[1,2,4]triazolo[1,5-a]pyrimidine-6-carbonitrile (5e). White powder; mp: 312-314 °C; **IR** (KBr) υ_{max} (cm^{-1}): 3169, 2955, 2386, 1556; **^{1}H NMR** (400 MHz, DMSO-d_6): δ 6.49 (s, 1H), 7.46 (d, *J* = 8 Hz, 2H), 7.51-7.57 (m, 5H), 7.68 (d, *J* = 8 Hz, 2H), 7.81 (s, 1H), 11.52 (s, 1H); **^{13}C NMR** (100 MHz, DMSO-d_6): δ 152.69, 144.58, 138.36, 133.86, 130.58, 130.68, 129.63, 129.06, 126.58, 126.39, 118.25, 90.58, 57.58; **ESI-MS**: *m/z* 345 $(M+1)^+$; Anal. Calcd. For $C_{18}H_{12}N_6O_2$; C, 62.79; H, 3.51; N, 24.41; Found: C, 62.61; H, 3.56; N, 24.62.

7-(4-Fluorophenyl)-5-phenyl-4,7-dihydro-[1,2,4]triazolo[1,5-a]pyrimidine-6-carbonitrile (5f). White powder; mp: 346-348 °C; **IR** (KBr) υ_{max} (cm^{-1}): 3196, 2983, 2338, 1568; **^{1}H NMR** (400 MHz, DMSO-d_6): δ 6.45 (s, 1H), 7.62 (t, *J* = 8 Hz, 3H), 7.74 (d, *J* = 8 Hz, 2H), 7.95-8.00 (m, 3H), 8.24 (t, *J* = 8 Hz, 1H), 8.27 (s, 1H), 9.26 (s, 1H); **^{13}C NMR** (100 MHz, DMSO-d_6): δ 154.27, 143.34, 135.69, 134.14, 131.58, 130.07, 129.41, 129.31, 129.02, 126.52, 125.91, 116.59, 93.35, 59.14; **ESI-MS**: *m/z* 318 $(M+1)^+$; Anal. Calcd. For $C_{18}H_{12}FN_5$; C, 68.13; H, 3.81; N, 22.07; Found: C, 68.24; H, 3.86; N, 22.27.

7-(4-Chlorophenyl)-5-phenyl-4,7-dihydro-[1,2,4]triazolo[1,5-a]pyrimidine-6-carbonitrile (5g). White powder; mp: 372-374 °C; **IR** (KBr) υ_{max} (cm^{-1}): 3128, 2998, 2384, 1523; **^{1}H NMR** (400 MHz, DMSO-d_6): δ 6.48 (s, 1H), 7.45 (d, *J* = 8

Hz, 2H), 7.51-7.57 (m, 4H), 7.67 (d, *J* = 8 Hz, 2H), 7.73-7.75 (m, 1H), 7.80 (s, 1H), 11.48 (s, 1H); **^{13}C NMR** (100 MHz, DMSO-d_6): δ 155.58, 147.58, 137.58, 135.55, 130.82, 129.25, 129.25, 127.39, 125.58, 118.58, 86.96, 55.58; **ESI-MS**: *m/z* 334 $(M+1)^+$; Anal. Calcd. For $C_{18}H_{12}ClN_5$; C, 64.77; H, 3.62; N, 20.98; Found: C, 64.56; H, 3.57; N, 20.81.

7-(Naphthalen-1-yl)-5-phenyl-4,7-dihydro-[1,2,4]triazolo[1,5-a]pyrimidine-6-carbonitrile (5h). White powder; mp: 384-386 °C; **IR** (KBr) υ_{max} (cm^{-1}): 3158, 2925, 2328, 1572; **^{1}H NMR** (400 MHz, DMSO-d_6): δ 6.53 (s, 1H), 7.29 (d, *J* = 8 Hz, 1H), 7.46 (s, 1H), 7.61 (t, *J* = 8 Hz, 2H), 7.72 (d, *J* = 8 Hz, 2H) 7.89 (d, *J* = 8 Hz, 2H), 7.95-8.00 (m, 2H), 8.26 (t, *J* = 8 Hz, 2H), 8.56 (s, 1H), 11.46 (s, 1H); **^{13}C NMR** (100 MHz, DMSO-d_6): δ 160.54, 156.38, 137.40, 136.44, 135.30, 133.60, 131.61, 129.72, 129.23, 128.24, 127.48, 125.68, 120.49, 117.63, 109.07, 106.94, 56.05; **ESI-MS**: *m/z* 350 $(M+1)^+$; Anal. Calcd. For $C_{22}H_{15}N_5$; C, 75.63; H, 4.33; N, 20.04; Found: C, 75.79; H, 4.38; N, 20.26.

7-(4-Hydroxyphenyl)-5-phenyl-4,7-dihydro-[1,2,4]triazolo[1,5-a]pyrimidine-6-carbonitrile (5i). White powder; mp: 330-332 °C; **IR** (KBr) υ_{max} (cm^{-1}): 3184, 2949, 2358, 1527; **^{1}H NMR** (400 MHz, DMSO-d_6): δ 6.38 (s, 1H), 6.89 (d, *J* = 8 Hz, 1H), 7.26 (d, *J* = 8 Hz, 1H), 7.38 (d, *J* = 8 Hz, 1H), 7.52 (d, *J* = 8 Hz, 2H), 7.64 (d, *J* = 8 Hz, 2H), 7.78 (d, *J* = 8 Hz, 1H), 8.14 (t, *J* = 8 Hz, 1H), 8.82 (s, 1H), 10. 32 (s, 1H), 11.48 (s, 1H); **^{13}C NMR** (100 MHz, DMSO-d_6): δ 156.55, 142.96, 138.35, 133.25, 132.94, 130.59, 129.59, 128.06, 127.58, 125.89, 117.28, 85.58, 54.82; **ESI-MS**: *m/z* 316 $(M+1)^+$; Anal. Calcd. For $C_{18}H_{13}N_5O$; C, 68.56; H, 4.16; N, 22.21; Found: C, 68.71; H, 4.21; N, 22.45.

Methyl4-(6-cyano-5-phenyl-4,7-dihydro-[1,2,4]triazolo[1,5-a]pyrimidin-7-yl) benzoate (5j). White powder; mp: 346-348 °C; **IR** (KBr) υ_{max} (cm^{-1}): 3126, 2978, 2394, 1581; **^{1}H NMR** (400 MHz, DMSO-d_6): δ 3.68 (s, 3H), 6.46 (s, 1H), 6.76 (d, *J* = 8 Hz, 2H), 7.18 (d, *J* = 8 Hz, 1H), 7.42 (d, *J* = 8 Hz, 1H), 7.56 (d, *J* = 8 Hz, 2H), 7.71 (d, *J* = 8 Hz, 2H), 7.84 (d, *J* = 8 Hz, 1H), 8.78 (s, 1H), 11.42 (s, 1H); **^{13}C NMR** (100 MHz, DMSO-d_6): δ 152.85, 147.58, 136.22, 135.58, 132.28, 130.58, 128.25, 127.25, 126.25, 119.25, 82.58, 63.45, 56.28; **ESI-MS**: *m/z* 358 $(M+1)^+$;

Anal. Calcd. For $C_{20}H_{15}N_5O_2$; C, 67.22; H, 4.23; N, 19.60; Found: C, 67.39; H, 4.28; N, 19.48.

7-(4-Hydroxy-3-methoxyphenyl)-5-phenyl-4,7-dihydro-[1,2,4]triazolo[1,5-a]pyrimidine-6-carbonitrile (5k). White powder; mp: 345-347 °C; **IR** (KBr) υ_{max} (cm^{-1}): 3159, 2915, 2314, 1586; **^{1}H NMR** (400 MHz, DMSO-d_6): δ 3.77 (s, 3H), 6.28 (s, 1H), 6.81 (t, *J* = 8 Hz, 2H), 6.96 (s, 1H), 7.57 (t, *J* = 8 Hz, 2H), 7.67 (d, *J* = 8 Hz, 2H), 7.76 (s, 1H), 8.01 (s, 1H), 8.86 (s, 1H), 9.23 (s, 1H); **^{13}C NMR** (100 MHz, DMSO-d_6): δ 150.70, 149.63, 148.14, 147.66, 147.30, 132.46, 131.38, 131.11, 129.68, 129.02, 120.53, 116.17, 112.15, 81.27, 60.28, 56.18; **ESI-MS**: *m/z* 346 $(M+1)^+$; Anal. Calcd. For $C_{19}H_{15}N_5O_2$; C, 66.08; H, 4.38; N, 20.28; Found: C, 66.23; H, 4.43; N, 20.03.

7-(3,4-Dimethoxyphenyl)-5-phenyl-4,7-dihydro-[1,2,4]triazolo[1,5-a]pyrimidine-6-carbonitrile (5l). White powder; mp: 362-364 °C; **IR** (KBr) υ_{max} (cm^{-1}): 3158, 2958, 2388, 1529; **^{1}H NMR** (400 MHz, DMSO-d_6): δ 3.64 (s, 3H), 3.76 (s, 3H), 6.48 (s, 1H), 6.68 (t, *J* = 8 Hz, 2H), 6.89 (s, 1H), 7.48 (t, *J* = 8 Hz, 1H), 7.85 (d, *J* = 8 Hz, 2H), 7.96 (d, *J* = 8 Hz, 1H), 8.18 (s, 1H), 8.44 (s, 1H), 11.23 (s, 1H); **^{13}C NMR** (100 MHz, DMSO-d_6): δ 150.93, 142.58, 137.58, 132.86, 130.58, 129.25, 128.28, 126.25, 124.56, 116.28, 82.25, 62.45, 59.86, 52.58; **ESI-MS**: *m/z* 360 $(M+1)^+$; Anal. Calcd. For $C_{20}H_{17}N_5O_2$; C, 66.84; H, 4.77; N, 19.49 Found: C, 66.68; H, 4.72; N, 19.67.

7-(2-Nitrophenyl)-5-phenyl-4,7-dihydro-[1,2,4]triazolo[1,5-a]pyrimidine-6-carbonitrile (5m). White powder; mp: 355-357 °C; **IR** (KBr) υ_{max} (cm^{-1}): 3199, 2958, 2353, 1559; **^{1}H NMR** (400 MHz, DMSO-d_6): δ 6.98 (s, 1H), 7.57 (d, *J* = 8 Hz, 1H), 7.66 (s, 1H), 7.70 (t, *J* = 8 Hz, 2H), 7.77 (t, *J* = 8 Hz, 1H), 7.86 (d, *J* = 8 Hz, 1H), 8.09 (t, *J* = 8 Hz, 2H), 8.11(d, *J* = 8 Hz, 1H), 9.26 (s, 1H), 11.57 (s, 1H); **^{13}C NMR** (100 MHz, DMSO-d_6): δ 155.14, 151.13, 149.32, 147.52, 134.38, 133.19, 132.41, 131.65, 130.18, 129.14, 128.94, 126.04, 125.32, 118.35, 78.93, 56.76; **ESI-MS**: *m/z* 345 $(M+1)^+$; Anal. Calcd. For $C_{18}H_{12}N_6O_2$; C, 62.79; H, 3.51; N, 24.41 Found: C, 62.58; H, 3.56; N, 24.63.

7-(3-Nitrophenyl)-5-phenyl-4,7-dihydro-[1,2,4]triazolo[1,5-a]pyrimidine-6-carbonitrile (5n). White powder; mp: 364-366 °C; **IR** (KBr) υ_{max} (cm^{-1}): 3169, 2929, 2358, 1548; **^{1}H NMR** (400 MHz, DMSO-d_6): δ 6.73 (s, 1H), 7.57 (d, *J* = 8 Hz, 3H), 7.69 (d, *J* = 8 Hz, 3H), 7.79 (t, *J* = 8 Hz, 2H), 7.91 (s, 1H), 8.29 (s, 1H), 11.58 (s, 1H); **^{13}C NMR** (100 MHz, DMSO-d_6): δ 151.30, 150.73, 148.49, 141.84, 134.57, 132.14, 131.58, 131.29, 129.11, 124.47, 122.74, 118.55, 79.89 59.44; **ESI-MS**: *m/z* 345 $(M+1)^+$; Anal. Calcd. For $C_{18}H_{12}N_6O_2$; C, 62.79; H, 3.51; N, 24.41 Found: C, 62.56; H, 3.55; N, 24.67.

7-(2-Chlorophenyl)-5-phenyl-4,7-dihydro-[1,2,4]triazolo[1,5-a]pyrimidine-6-carbonitrile (5o). White powder; mp: 312-314 °C; **IR** (KBr) υ_{max} (cm^{-1}): 3168, 2916, 2328, 1552; **^{1}H NMR** (400 MHz, DMSO-d_6): δ 6.64 (s, 1H), 7.68 (d, *J* = 8 Hz, 3H), 7.82 (d, *J* = 8 Hz, 3H), 7.85 (t, *J* = 8 Hz, 2H), 7.86 (t, *J* = 8 Hz, 1H), 8.38 (s, 1H), 11.38 (s, 1H); **^{13}C NMR** (100 MHz, DMSO-d_6): δ 156.58, 153.58, 144.35, 140.58, 132.58, 131.25, 130.92, 130.25, 129.18, 128.88, 127.63, 126.36, 117.26, 81.82, 53.35; **ESI-MS**: *m/z* 334 $(M+1)^+$; Anal. Calcd. For $C_{18}H_{12}ClN_5$; C, 64.77; H, 3.62; N, 20.98 Found: C, 64.88; H, 3.67; N, 20.83.

5,7-Diphenyl-4,7-dihydrotetrazolo[1,5-a]pyrimidine-6-carbonitrile (6a). White powder; mp: 386-388 °C; **IR** (KBr) υ_{max} (cm^{-1}): 3138, 2937, 2325, 1582; **^{1}H NMR** (400 MHz, DMSO-d_6): δ 5.76 (s, 1H), 7.63-7.69 (m, 4H), 7.87 (d, *J* = 8 Hz, 2H), 8.01 (d, *J* = 8 Hz, 2H), 8.16 (d, *J* = 8 Hz, 2H), 9.25 (s, 1H); **^{13}C NMR** (100 MHz, DMSO-d_6): δ 155.88, 154.45, 137.63, 134.69, 132.74, 129.14, 128.99, 119.37, 119.05, 112.32, 101.01, 57.71; **ESI-MS**: *m/z* 301 $(M+1)^+$; Anal. Calcd. For $C_{17}H_{12}N_6$; C, 67.99; H, 4.03; N, 27.98; Found: C, 67.84; H, 4.08; N, 27.78.

5-Phenyl-7-(p-tolyl)-4,7-dihydrotetrazolo[1,5-a]pyrimidine-6-carbonitrile (6b). White powder; mp: 379-381 °C; **IR** (KBr) υ_{max} (cm^{-1}): 3166, 2958, 2354, 1547; **^{1}H NMR** (400 MHz, DMSO-d_6): δ 2.09 (s, 3H), 6.48 (s, 1H), 7.64-7.81 (m, 2H), 7.85 (t, *J* = 8 Hz, 2H), 7.86 (d, *J* = 8 Hz, 2H), 8.10 (d, *J* = 8 Hz, 2H), 8.22 (d, *J* = 8 Hz, 1H), 9.26 (s, 1H); **^{13}C NMR** (100 MHz, DMSO-d_6): δ 158.15, 142.98, 138.45, 133.74, 131.74, 131.32, 130.19, 129.90, 129.77, 129.46, 129.04, 119.40, 83.60, 56.08, 21.35; **ESI-MS**: *m/z* 315 $(M+1)^+$; Anal. Calcd. For $C_{18}H_{14}N_6$; C, 68.78; H, 4.49; N, 26.74; Found: C, 68.94; H, 4.44; N, 26.87.

7-(4-Methoxyphenyl)-5-phenyl-4,7-dihydrotetrazolo[1,5-a]pyrimidine-6-carbonitrile (6c). White powder; mp: 396-398 °C; **IR** (KBr) υ_{max} (cm^{-1}): 3166, 2958, 2354, 1547; **^{1}H NMR** (400 MHz, DMSO-d_6): δ 3.89 (s, 3H), 6.47 (s, 1H), 7.18 (d, *J* = 8 Hz, 2H), 7.59 (t, *J* = 8 Hz, 2H), 7.71 (t, *J* = 8 Hz, 1H), 7.84 (d, *J* = 8 Hz, 2H), 8.12 (d, *J* = 8 Hz, 2H), 9.02 (s, 1H); **^{13}C NMR** (100 MHz, DMSO-d_6): δ 155.85, 136.55, 134.10, 133.50, 129.62, 129.17, 124.78, 117.85, 115.46, 107.21, 58.40, 56.27; **ESI-MS**: *m/z* 331 $(M+1)^+$; Anal. Calcd. For $C_{18}H_{14}N_6O$; C, 65.44; H, 4.27; N, 25.44; Found: C, 65.61; H, 4.22; N, 25.27.

5-Phenyl-7-(4-(trifluoromethyl)phenyl)-4,7-dihydrotetrazolo[1,5-a]pyrimidine-6-carbonitrile (6d). White powder; mp: 364-366 °C; **IR** (KBr) υ_{max} (cm^{-1}): 3168, 2938, 2318, 1527; **^{1}H NMR** (400 MHz, DMSO-d_6): δ 6.59 (s, 1H), 7.26-7.45 (m, 3H), 7.78 (t, *J* = 8 Hz, 2H), 7.92 (d, *J* = 8 Hz, 2H), 8.06 (d, *J* = 8 Hz, 2H), 9.44 (s, 1H); **^{13}C NMR** (100 MHz, DMSO-d_6): δ 149.55, 132.71, 131.69, 131.23, 130.19, 130.11, 129.96, 129.19, 128.94, 119.85, 116.55, 83.77, 57.69; **ESI-MS**: *m/z* 369 $(M+1)^+$; Anal. Calcd. For $C_{18}H_{11}F_3N_6$; C, 58.70; H, 3.01; N, 22.82 Found: C, 58.52; H, 3.06; N, 22.98.

7-(4-Nitrophenyl)-5-phenyl-4,7-dihydrotetrazolo[1,5-a]pyrimidine-6-carbonitrile (6e). White powder; mp: 347-349 °C; **IR** (KBr) υ_{max} (cm^{-1}): 3169, 2966, 2383, 1556; **^{1}H NMR** (400 MHz, DMSO-d_6): δ 6.27 (s, 1H), 7.38 (t, *J* = 8 Hz, 4H), 7.61-7.67 (m, 2H), 7.74 (d, *J* = 8 Hz, 1H), 7.86 (d, *J* = 8 Hz, 1H), 8.13 (d, *J* = 8 Hz, 1H), 9.03 (s, 1H); **^{13}C NMR** (100 MHz, DMSO-d_6): δ 150.25, 146.25, 138.28, 135.23, 132.82, 130.82, 130.25, 129.25, 127.54, 116.42, 115.71, 82.25, 54.58; **ESI-MS**: *m/z* 346 $(M+1)^+$; Anal. Calcd. For $C_{17}H_{11}N_7O_2$; C, 59.13; H, 3.21; N, 28.39 Found: C, 59.26; H, 3.26; N, 28.57.

7-(4-Fluorophenyl)-5-phenyl-4,7-dihydrotetrazolo[1,5-a]pyrimidine-6-carbonitrile (6f). White powder; mp: 372-374 °C; **IR** (KBr) υ_{max} (cm^{-1}): 3158, 2958, 2358, 1582; **^{1}H NMR** (400 MHz, DMSO-d_6): δ 6.78 (s, 1H), 7.33 (t, *J* = 8 Hz, 2H), 7.53 (t, *J* = 8 Hz, 4H), 7.64 (d, *J* = 8 Hz, 3H), 10.16 (s, 1H); **^{13}C NMR** (100 MHz, DMSO-d_6): δ 149.55, 140.78, 136.90, 132.71, 131.69, 131.23, 130.19, 130.11, 129.96, 129.19, 128.94, 119.85, 116.55, 83.77, 56.86; **ESI-MS**: *m/z* 319

$(M + 1)^+$; Anal. Calcd. For $C_{17}H_{11}FN_6$; C, 64.15; H, 3.48; N, 26.40 Found: C, 64.23; H, 3.43; N, 26.64.

7-(4-Chlorophenyl)-5-phenyl-4,7-dihydrotetrazolo[1,5-a]pyrimidine-6-carbonitrile (6g). White powder; mp: 338-340 °C; **IR** (KBr) υ_{max} (cm^{-1}): 3159, 2968, 2338, 1528; **^{1}H NMR** (400 MHz, DMSO-d_6): δ 6.64 (s, 1H), 7.48 (t, *J* = 8 Hz, 2H), 7.64 (t, *J* = 8 Hz, 2H), 7.82 (t, *J* = 8 Hz, 2H), 7.92 (d, *J* = 8 Hz, 3H), 10.54 (s, 1H); **^{13}C NMR** (100 MHz, DMSO-d_6): δ 156.56, 147.42, 134.28, 133.25, 132.58, 131.85, 130.57, 129.74, 128.17, 117.24, 114.17, 79.11, 55.77; **ESI-MS**: *m/z* 335 $(M+1)^+$; Anal. Calcd. For $C_{17}H_{11}ClN_6$; C, 60.99; H, 3.31; N, 25.10 Found: C, 60.84; H, 3.36; N, 25.29.

7-(Naphthalen-1-yl)-5-phenyl-4,7-dihydrotetrazolo[1,5-a]pyrimidine-6-carbonitrile (6h). White powder; mp: 369-371 °C; **IR** (KBr) υ_{max} (cm^{-1}): 3198, 2929, 2373, 1547; **^{1}H NMR** (400 MHz, DMSO-d_6): δ 6.64 (s, 1H), 7.34 (d, *J* = 8 Hz, 1H), 7.64 (t, *J* = 8 Hz, 2H), 7.84 (d, *J* = 8 Hz, 2H) 7.89 (d, *J* = 8 Hz, 2H), 7.91-8.12 (m, 3H), 8.32 (t, *J* = 8 Hz, 2H), 11.18 (s, 1H) **^{13}C NMR** (100 MHz, DMSO-d_6): δ 156.56, 147.42, 134.28, 133.25, 132.58, 131.85, 130.57, 129.74, 128.17, 117.24, 114.17, 79.11, 55.77; **ESI-MS**: *m/z* 351 $(M+1)^+$; Anal. Calcd. For $C_{21}H_{14}N_6$; C, 71.99; H, 4.03; N, 23.99.Found: C, 71.82; H, 4.09; N, 23.24.19.

7-(4-Hydroxyphenyl)-5-phenyl-4,7-dihydrotetrazolo[1,5-a]pyrimidine-6-carbonitrile (6i). White powder; mp: 356-358 °C; **IR** (KBr) υ_{max} (cm^{-1}): 3166, 2958, 2354, 1547; **^{1}H NMR** (400 MHz, DMSO-d_6): δ 6.24 (s, 1H), 7.47 (d, *J* = 8 Hz, 2H), 7.82 (t, *J* = 8 Hz, 2H), 7.91 (d, *J* = 8 Hz, 2H), 8.07 (t, *J* = 8 Hz, 2H), 8.14 (d, *J* = 8 Hz, 1H), 9.26 (s, 1H), 12.38 (s, 1H); **^{13}C NMR** (100 MHz, DMSO-d_6): δ 156.57, 137.47, 135.72, 134.17, 130.12, 129.47, 125.82, 119.57, 116.57, 92.68, 56.25; **ESI-MS**: *m/z* 317 $(M+1)^+$; Anal. Calcd. For $C_{17}H_{12}N_6O$; C, 64.55; H, 3.82; N, 26.57; Found: C, 64.73; H, 3.77; N, 26.79.

Methyl 4-(6-cyano-5-phenyl-4,7-dihydrotetrazolo[1,5-a]pyrimidin-7-yl)benzoate (6j). White powder; mp: 362-364 °C; **IR** (KBr) υ_{max} (cm^{-1}): 3149, 2935, 2327, 1582; **^{1}H NMR** (400 MHz, DMSO-d_6): δ 3.61 (s, 3H), 6.51 (s, 1H), 7.28 (d, *J* = 8 Hz, 2H), 7.67 (t, *J* = 8 Hz, 2H), 7.84 (d, *J* = 8 Hz, 2H), 8.02 (t, *J* = 8 Hz, 2H), 8.21

(d, *J* = 8 Hz, 1H), 11.21 (s, 1H); **^{13}C NMR** (100 MHz, DMSO-d_6): δ 155.65, 136.88, 134.58, 133.25, 129.62, 129.14, 124.24, 117.87, 115.14, 107.25, 62.58, 61.38, 56.25; **ESI-MS**: *m/z* 359 $(M+1)^+$; Anal. Calcd. For $C_{19}H_{14}N_6O_2$; C, 63.68; H, 3.94; N, 23.45; Found: C, 63.92; H, 3.88; N, 23.21.

7-(3-Hydroxy-4-methoxyphenyl)-5-phenyl-4,7-dihydro-[1,2,4]tetrazolo[1,5-a]pyrimidine-6-carbonitrile (6k). White powder; mp: 354-358 °C; **IR** (KBr) υ_{max} (cm^{-1}): 3158, 2954, 2324, 1522; **^{1}H NMR** (400 MHz, DMSO-d_6): δ 3.77 (s, 3H), 6.28 (s, 1H), 6.81 (d, *J* = 8 Hz, 2H), 6.95 (s, 1H), 7.56-7.76 (m, 4H), 8.00 (d, *J* = 8 Hz, 2H), 8.86 (s, 1H), 9.25 (s, 1H); **^{13}C NMR** (100 MHz, DMSO-d_6): δ 156.58, 134.56, 133.85, 132.28, 130.48, 128.58, 126.75, 118.28, 117.28, 89.27, 60.58, 55.58; **ESI-MS**: *m/z* 347 $(M+1)^+$; Anal. Calcd. For $C_{19}H_{15}N_5O_2$; C, 62.42; H, 4.07; N, 24.27; Found: C, 62.60; H, 4.12; N, 24.02.

7-(3,4-Dimethoxyphenyl)-5-phenyl-4,7-dihydrotetrazolo[1,5-a]pyrimidine-6-carbonitrile (6l). White powder; mp: 354-358 °C; **IR** (KBr) υ_{max} (cm^{-1}): 3158, 2954, 2324, 1522; **^{1}H NMR** (400 MHz, DMSO-d_6): δ 3.62 (s, 3H), 3.81 (s, 3H), 6.37 (s, 1H), 6.79 (d, *J* = 8 Hz, 2H), 7.49-7.74 (m, 4H), 8.02 (d, *J* = 8 Hz, 1H), 8.18 (s, 1H), 11.34 (s, 1H); **^{13}C NMR** (100 MHz, DMSO-d_6): δ 154.55, 144.58, 136.22, 133.85, 131.28, 129.58, 127.18, 115.57, 117.48, 86.77, 60.58, 58.48, 54.58; **ESI-MS**: *m/z* 361 $(M+1)^+$; Anal. Calcd. For $C_{19}H_{16}N_6O_2$; C, 63.32; H, 4.48; N, 23.32; Found: C, 63.17; H, 4.53; N, 23.15.

7-(2-Nitrophenyl)-5-phenyl-4,7-dihydrotetrazolo[1,5-a]pyrimidine-6-carbonitrile (6m). White powder; mp: 346-348 °C; **IR** (KBr) υ_{max} (cm^{-1}): 3187, 2981, 2328, 1589; **^{1}H NMR** (400 MHz, DMSO-d_6): δ 6.48 (s, 1H), 7.47 (d, *J* = 8 Hz, 1H), 7.58 (s, 1H), 7.72 (t, *J* = 8 Hz, 2H), 7.81 (t, *J* = 8 Hz, 1H), 7.90 (d, *J* = 8 Hz, 1H), 8.21 (t, *J* = 8 Hz, 2H), 8.32 (d, *J* = 8 Hz, 1H), 11.48 (s, 1H); **^{13}C NMR** (100 MHz, DMSO-d_6): δ 154.52, 150.85, 145.58, 144.55, 136.58, 132.58, 131.83, 130.28, 127.28, 124.58, 119.26, 84.82, 56.58; **ESI-MS**: *m/z* 346 $(M+1)^+$; Anal. Calcd. For $C_{17}H_{11}N_7O_2$; C, 59.13; H, 3.21; N, 28.39 Found: C, 59.31; H, 3.26; N, 28.53.

7-(3-Nitrophenyl)-5-phenyl-4,7-dihydrotetrazolo[1,5-a]pyrimidine-6-carbonitrile (6n). White powder; mp: 322-324 °C; **IR** (KBr) υ_{max} (cm^{-1}): 3158, 2965, 2318,

1573; **^{1}H NMR** (400 MHz, DMSO-d_6): δ 6.26 (s, 1H), 7.28 (d, *J* = 8 Hz, 3H), 7.42 (d, *J* = 8 Hz, 3H), 7.71 (t, *J* = 8 Hz, 2H), 8.26 (s, 1H), 11.58 (s, 1H); **^{13}C NMR** (100 MHz, DMSO-d_6): δ 152.29, 149.58, 145.51, 136.56, 134.58, 132.58, 130.58, 129.28, 128.58, 127.95, 125.28, 125.01, 118.58, 81.83, 53.55; **ESI-MS**: *m/z* 346 $(M+1)^+$; Anal. Calcd. For $C_{17}H_{11}N_7O_2$; C, 59.13; H, 3.21; N, 28.39 Found: C, 59.29; H, 3.26; N, 28.60.

7-(2-Chlorophenyl)-5-phenyl-4,7-dihydrotetrazolo[1,5-a]pyrimidine-6-carbonitrile (6o). White powder; mp: 346-348 °C; **IR** (KBr) υ_{max} (cm^{-1}): 3159, 2928, 2385, 1512; **^{1}H NMR** (400 MHz, DMSO-d_6): δ 6.37 (s, 1H), 7.58 (d, *J* = 8 Hz, 1H), 7.74 (s, 1H), 7.82 (t, *J* = 8 Hz, 2H), 7.96 (t, *J* = 8 Hz, 1H), 8.04 (d, *J* = 8 Hz, 1H), 8.32 (t, *J* = 8 Hz, 2H), 8.48 (d, *J* = 8 Hz, 1H), 11.10 (s, 1H); **^{13}C NMR** (100 MHz, DMSO-d_6): δ 149.77, 148.13, 145.27, 134.85, 132.75, 130.58, 130.58, 129.34, 128.58, 126.83, 125.28, 118.28, 87.47, 52.48; **ESI-MS**: *m/z* 335 $(M+1)^+$; Anal. Calcd. For $C_{17}H_{11}ClN_6$; C, 60.99; H, 3.31; N, 25.10 Found: C, 60.82; H, 3.37; N, 25.34.

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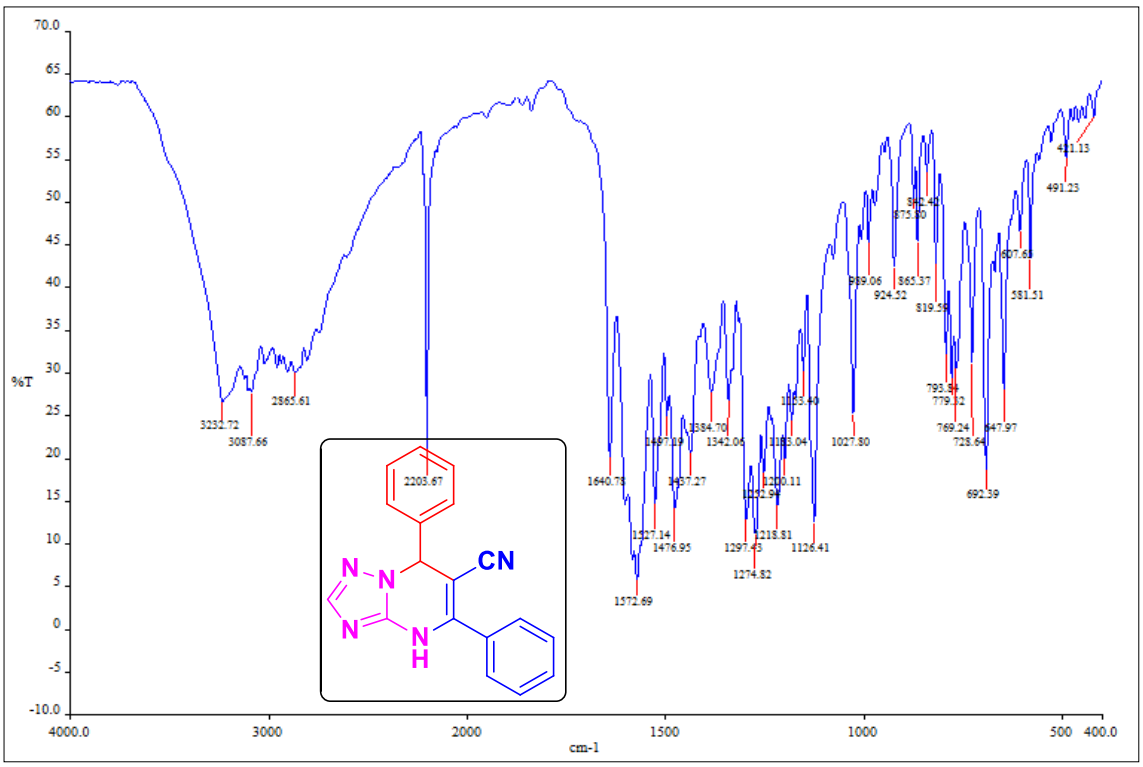
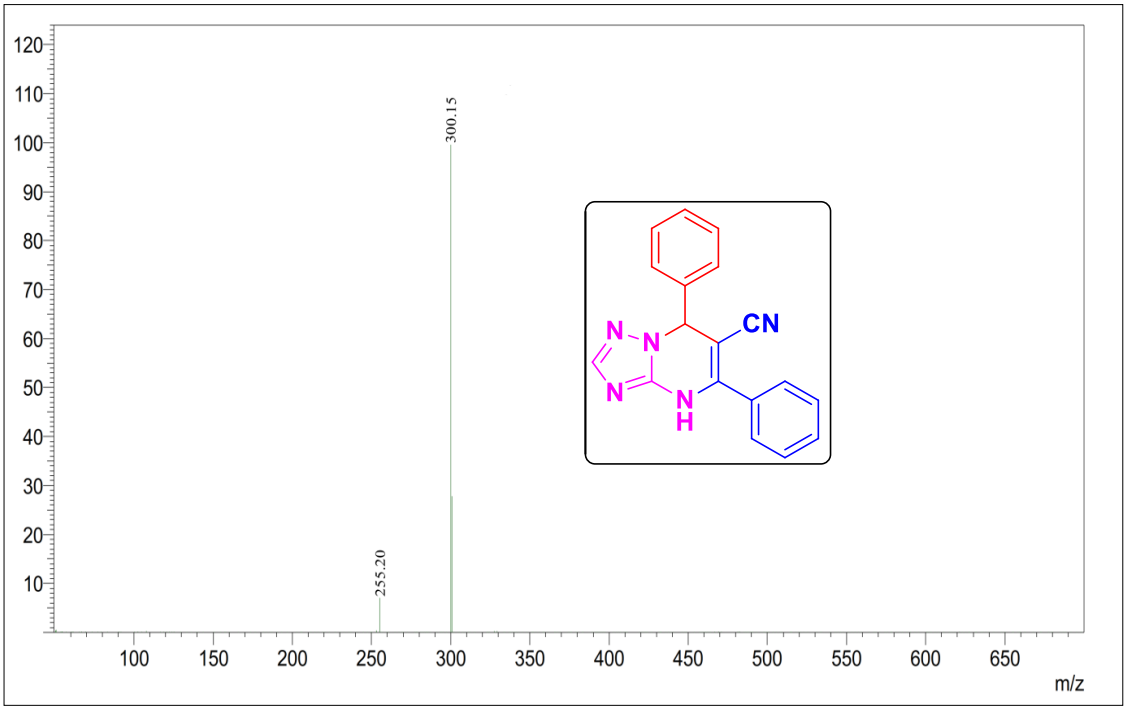
SOME SELECTED SPECTRA



IR Spectrum of compound **5a**



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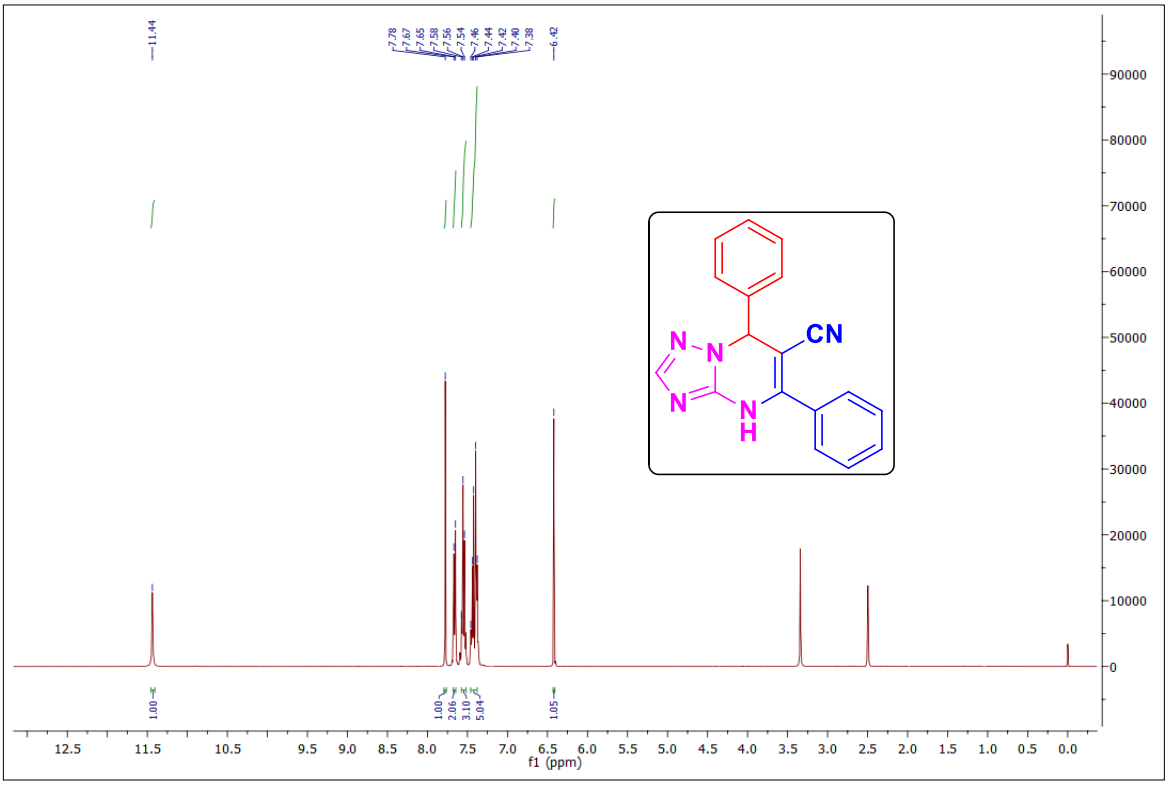

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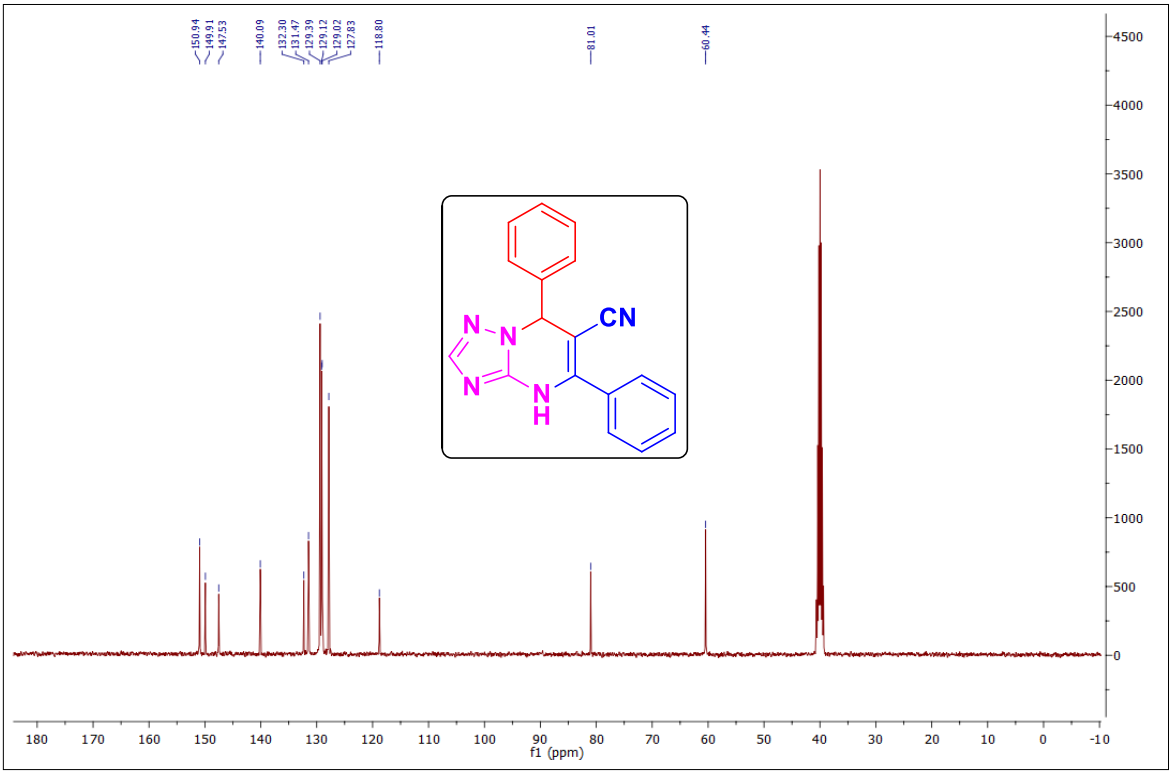

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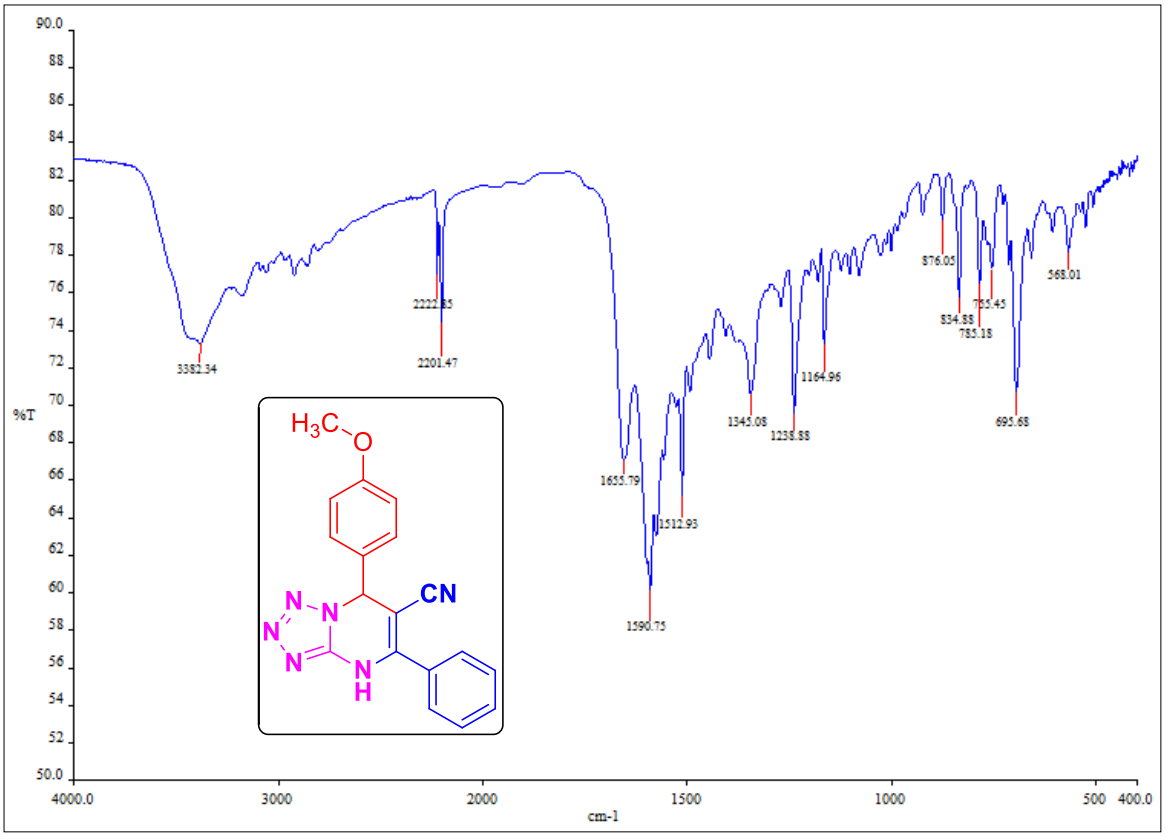

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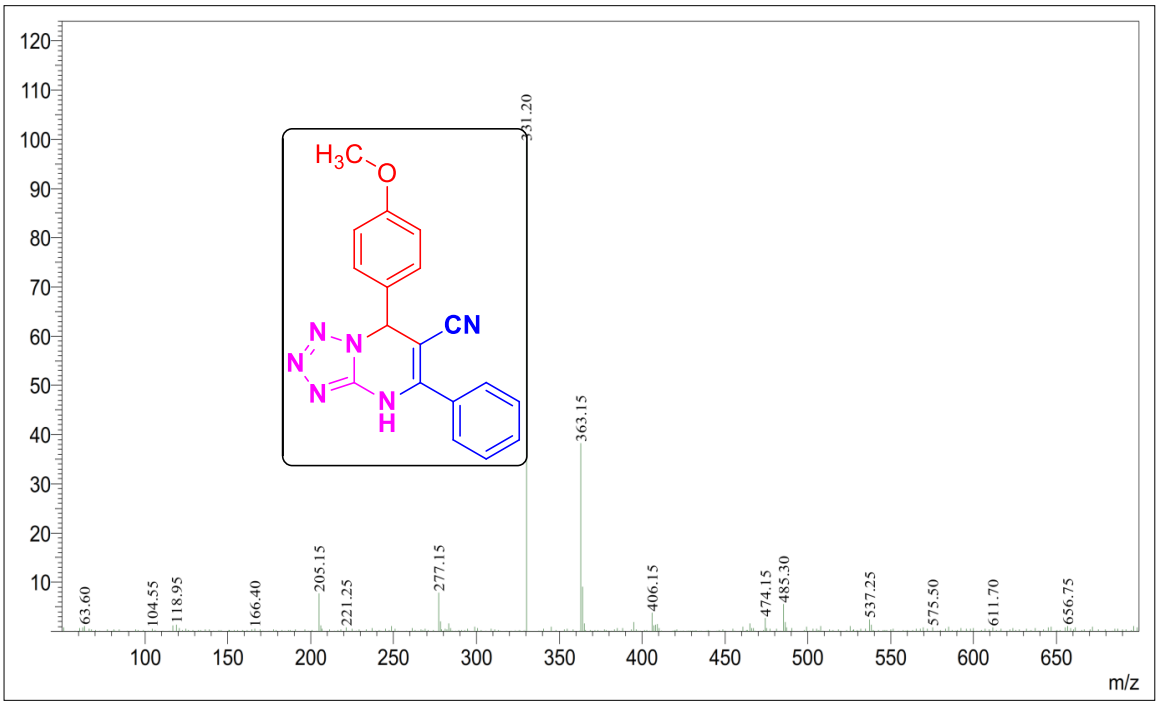
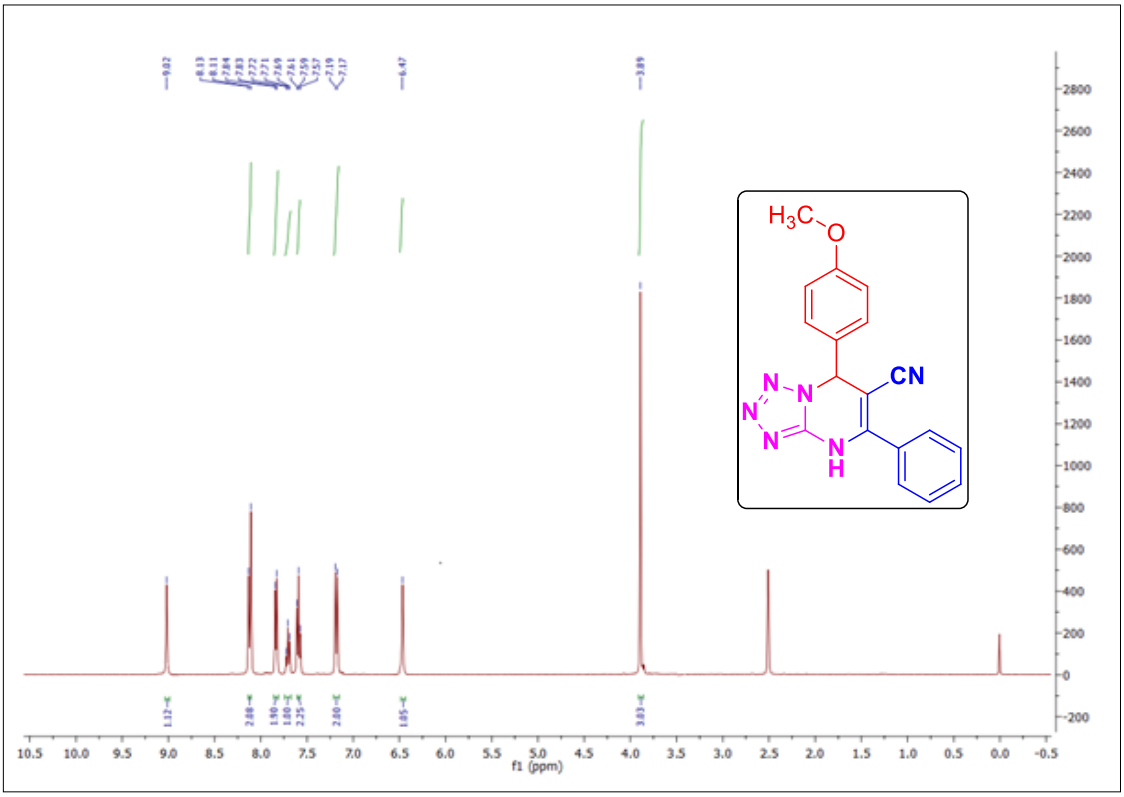
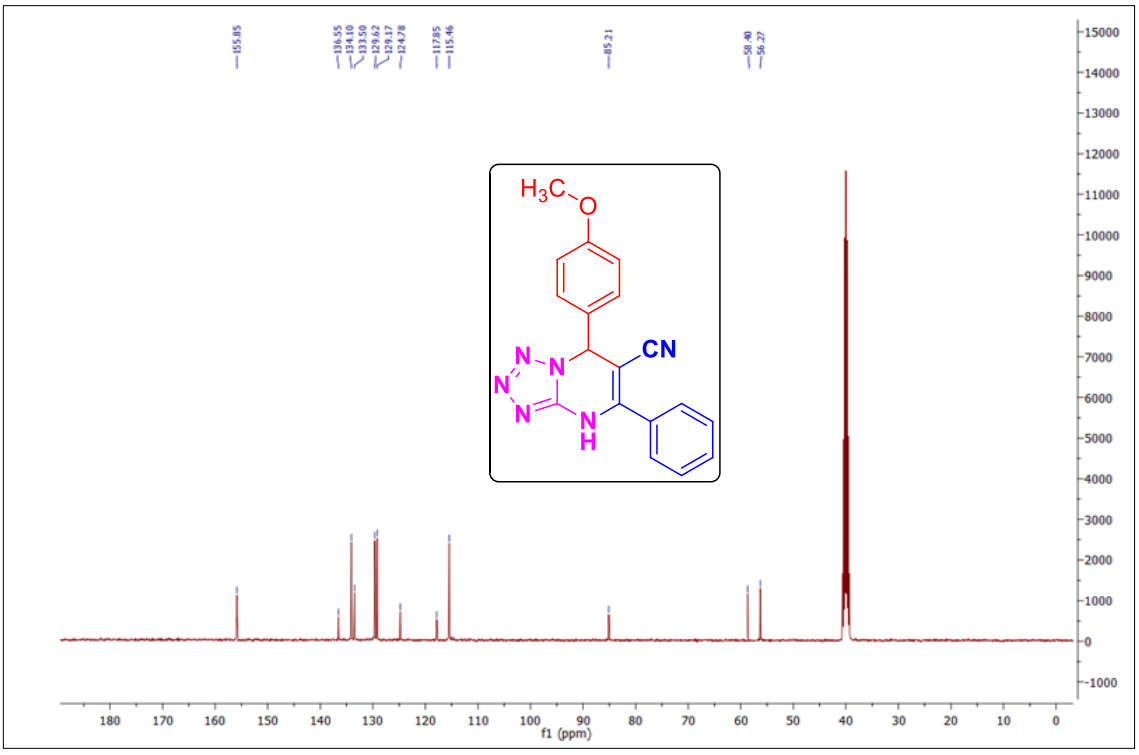


Mass Spectrum of compound **6c**



^{1}H NMR Spectrum of compound **6c**

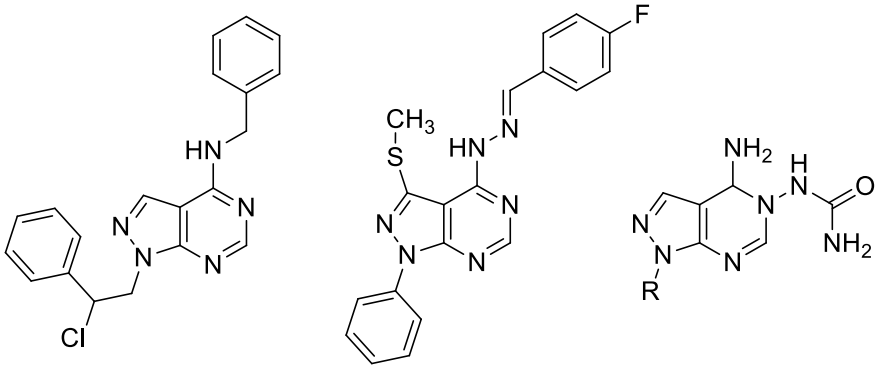


^{13}C NMR Spectrum of compound **6c**

SYNTHESIS OF NEW PYRAZOLO[1,2-*b*]PHALAZINO PYRIMIDINE DERIVATIVES BY USIING $[BSO_3Hmim]HSO_4$

6.1. INTRODUCTION

Pyrazoles constitute an important class of heterocyclic compounds for new drug development. They attracted much attention due to their broad spectrum of biological activities such as anticancer,[1] antiviral,[2] anti-inflammatory,[3] antifungal.[4] Pyrazole and its synthetic analoges have been found to exhibit antimicrobial,[5] antidepressant,[6] antidiabetic,[7] analgesic,[8] anticonvulsant[9] activities. They are also used as human acyl-cholesterol acyltransferase inhibitors.[10] Pyrazolopyrimidines have drawn considerable interest because of their profound biological activities like antihypertensive,[11] tuberculostatic[12] and antileishmanial,[13]anticancer[14] antiinflammatory,[15] antibacterial[16] and fungicidal activities.[17] For instance, this heterocyclic core is found as purine analogues and displays the role of antimetabolites in purine biochemical reactions.[18] Several compounds of this class are known for their interesting antitrypanosomal and antischistosomal activities.[19] Some of these derivatives indicated the path ways to develop as hA3AR antagonists scaffolds (**1**, **2** and **3**) (**Fig. 1**).

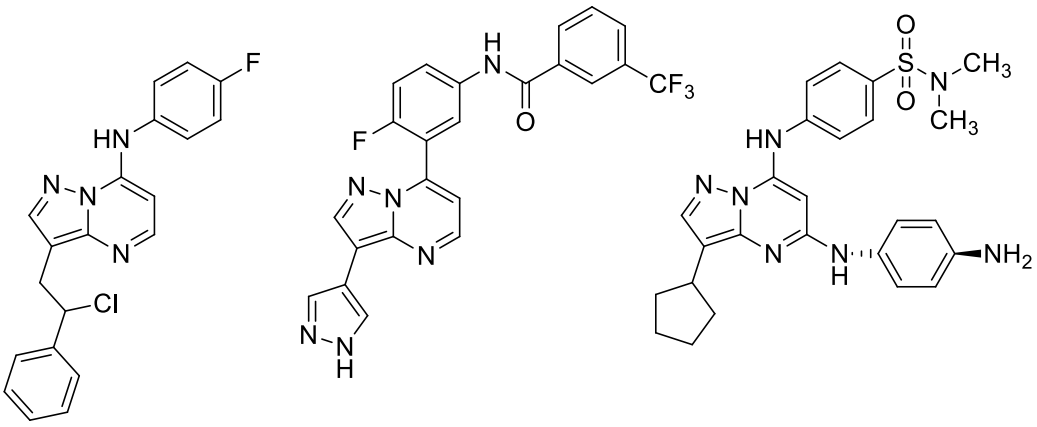


(1) **(2)** **(3)**

Figure. 1

Pyrazolo pyrimidine derivatives have drawn a great attention, since they are considered as an isostere to purine nucleus and different mechanisms were proposed to account for the cytotoxic effect of this class of compounds.[20] Its analogues have been extensively studied as kinase inhibitors and several compounds have been found active such as the B-Raf kinase inhibitors[21] or the cyclin dependent kinase inhibitors.[22] They were

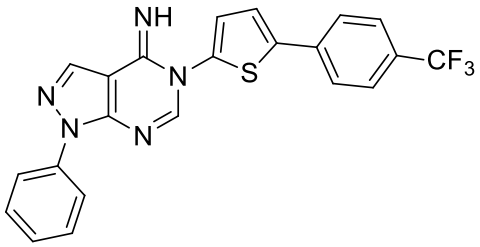
reported to act as glycogen synthase kinase (GSK) inhibitors,[23] cyclin dependent kinase (CDK) inhibitors,[24] dual src/Ab1 kinase inhibitors,[25] and epidermal growth factor receptor (EGFR) inhibitors (**4**-**6**) (**Fig. 2**).[26]



(4) **(5)** **(6)**

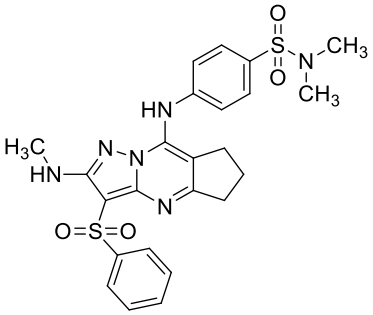
Figure. 2

Abed et al.,[27] have shown a simple approach to the synthesis of 3-fluoro pyrazolo [1,5-*a*] pyrimidine analogues and proved them as potent anticancer agents (**7**).



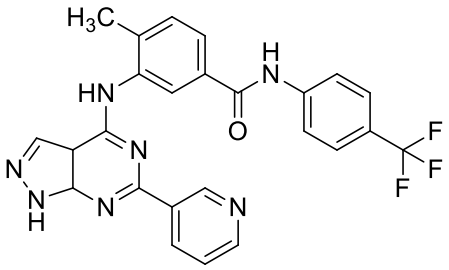
(7)

Kasiotis *et al*.,[28] described pyrazoles as potential anti-angiogenesis agents (**8**).



(8)

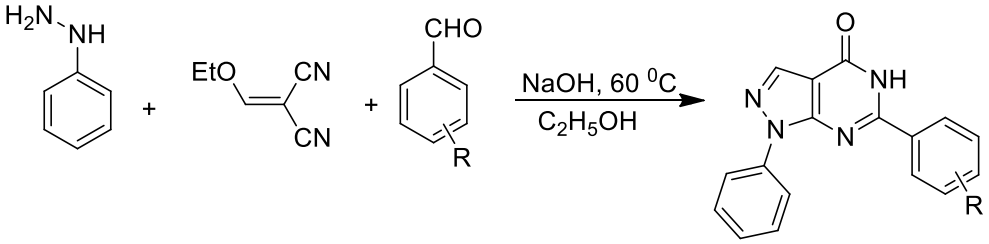
Zhao *et al*.,[29] reported the design and synthesis of novel pyrazolo [1,5-*a*] pyrimidine derivatives bearing nitrogen mustard moiety along with the evaluation of their antitumor activity both *in vitro* and *in vivo* (**9**).



(**9**)

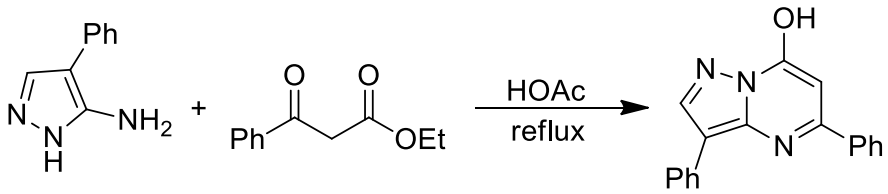
6.1.1. REPORTED SYNTHETIC STRATEGIES FOR BUILDING PYRAZOLES.

Liu *et al.*[30] proposed a convenient four-component one-pot strategy toward the synthesis of pyrazolo [3,4-*d*] pyrimidines (**Scheme 1)**.



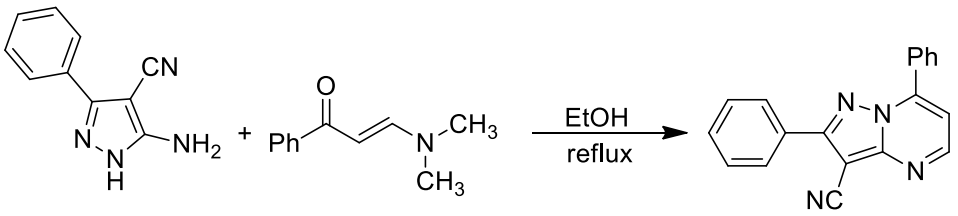
Scheme 1

Hassan *et al*.[31] described the construction of pyrazoles via enaminones synthesis and reported molecular docking studies of some novel heterocyclic compounds containing sulfonamide moiety (**Scheme 2**).



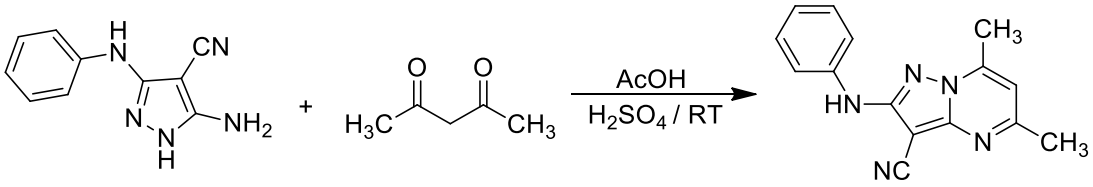
Scheme 2

Stepaniuk *et al*.[32] reported the synthesis of new pyrazolo [1,5-*a*]pyrimidines by reaction of β, γ-unsaturated γ-alkoxy-α-keto esters with N-unsubstituted 5-aminopyrazoles (**Scheme 3)**.



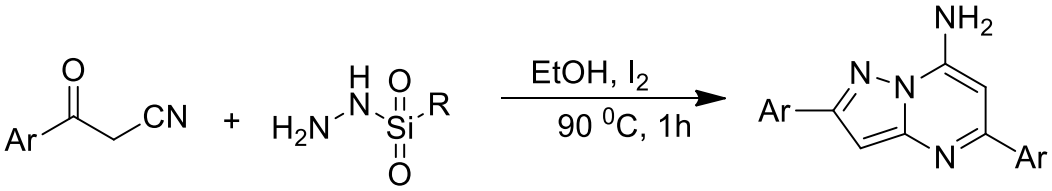
Scheme 3

Fathalla *et al*.[33] provided an efficient synthesis of new pyrazolo [1,5-*a*] pyrimidine derivatives (**Scheme 4**).



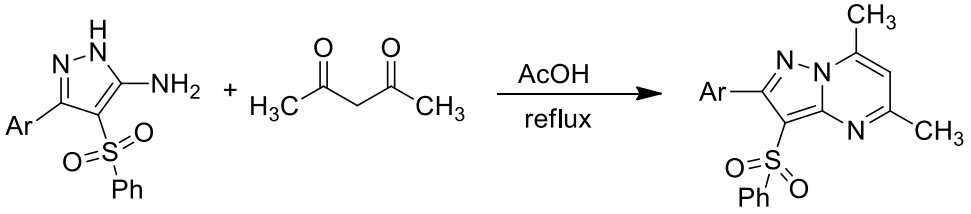
Scheme 4

Sun *et al*.[34] reported the I_2-catalyzed multicomponent reactions for accessing densely functionalized pyrazolo [1,5-*a*] pyrimidines and their disulphenylated derivatives (**Scheme 5**).



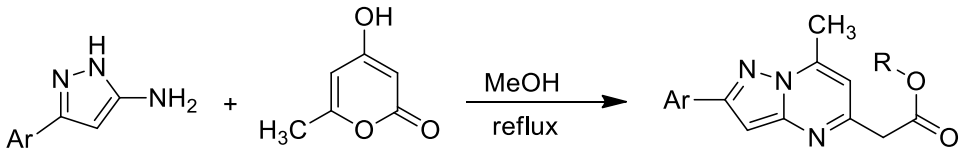
Scheme 5

Ivachtchenko *et al*.[35] found that a new series of substituted 5, 6-dimethyl-3-phenylsulfonyl-pyrazolo[1,5-*a*]pyrimidines can act as specific serotonin 5-HT 6 receptor antagonists (**Scheme 6**)



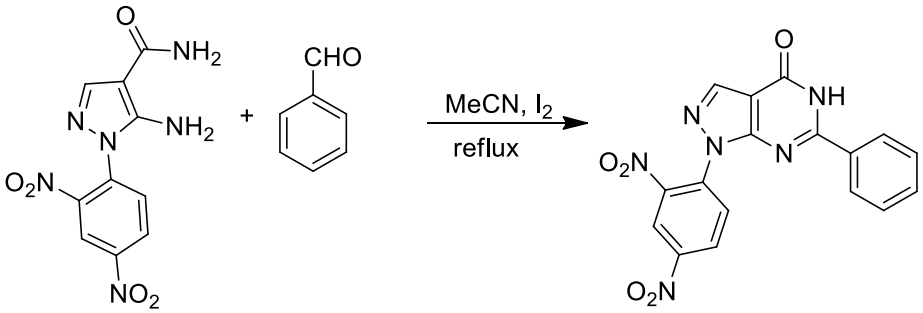
Scheme 6

Bassoude *et al.*[36] described an efficient one-step reaction leading to new pyrazolo [1,5-*a*] pyrimidinesby condensation of 2-pyrone with (3)-amino-3 (5)-arylpyrazoles (**Scheme 7**)



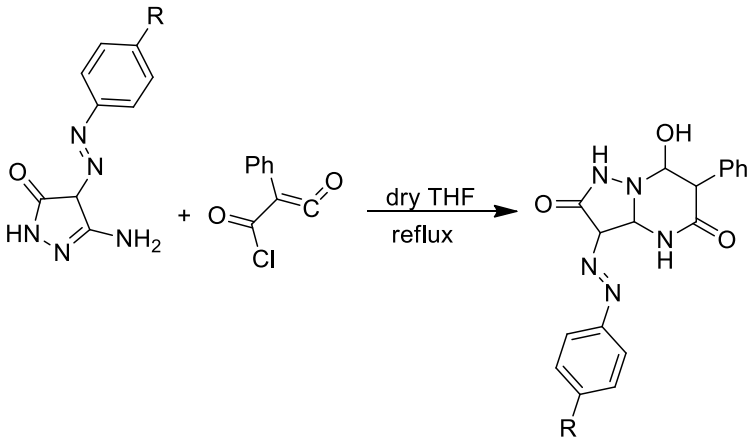
Scheme 7

Bakavoli *et al.*[37] reported the molecular iodine promoted synthesis of new pyrazolo [3,4-*d*] pyrimidine derivatives and their antibacterial activity (**Scheme 8**).



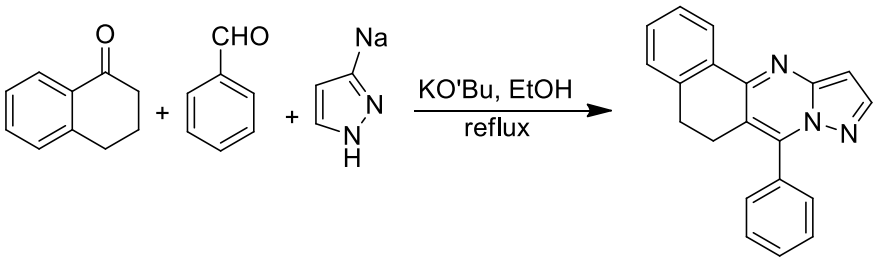
Scheme 8

Zahdifar *et al.*[38] conducted the reaction of (chloro carbonyl) phenyl ketene with 5-amino pyrazolones to synthesized a series of 7-hydroxy-6-phenyl-3-(phenyldiazenyl) pyrazolo [1, 5-*a*] pyrimidine-2, 5 (1*H*, 4*H*)-dione derivatives (**Scheme 9**).



Scheme 9

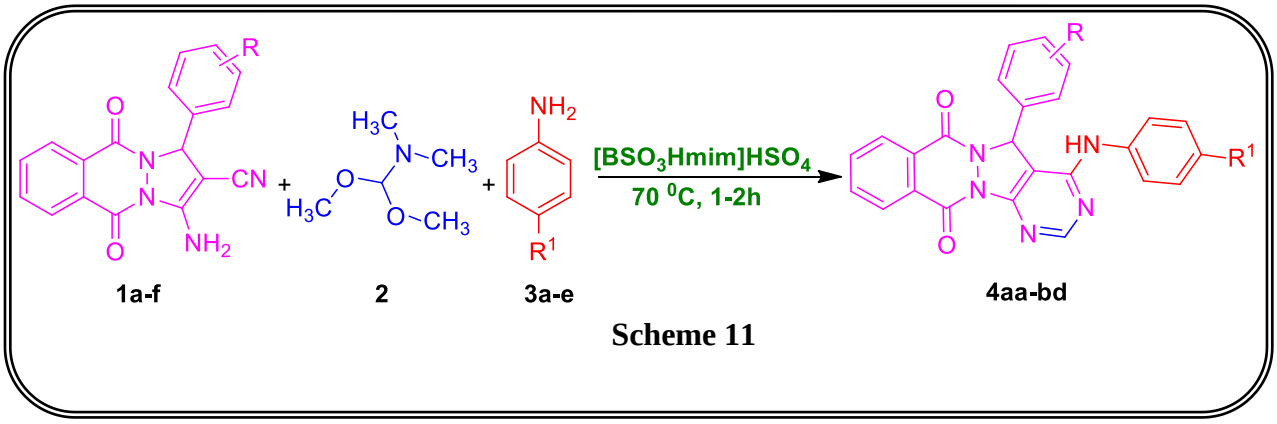
Saikia *et al.*[39] develped a facile one-pot synthesis of 7-substituted pyrazolo [1, 5-a] pyrimidines by base induced three-component reaction (**Scheme 10**)



Scheme 10

6.2. PRESENT WORK

As a part of our continuing research on development of new methodology for the synthesis of novel biologically active heterocyclic compounds by eco-friendly methods and also to develop new methods having superior atom economy, we have taken up the project of syntheisi of novel pyrazolo-pthalazino pyrimidines derivatives, in the present seheme. We have also taken up one-pot procedures wich do not need the isolation of intermediates, which are also associated with atom economy. MCRs have been broadly employed in the synthesis of heterocyclic compounds.[40] Therefore, the design and development of novel, efficient, and green MCRs focused on a target products is one of the most important challenges in organic synthesis. The use of ionic liquid as a solvent as well as catalyst for organic synthesis has recently attracted considerable attention. To the best of our knowledge, no synthetic methods leading to the formation of pyrimido pyrazolo[1,2-*b*]phthalazine have been disclosed in the literature so far. In the following, we will provide an efficient access to these valuable heteroaromatic scaffolds by using ionic liquid as an efficient medium on domino strategy (**scheme 11**).

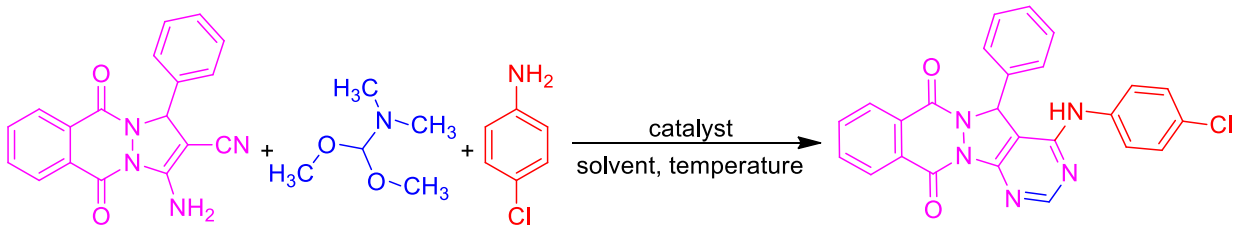


Scheme 11

6.3. RESULTS AND DISCUSSIONS

At the onset, we started investigating to access by exploring the condensation of 3-amino-5,10-dioxo-1-phenyl-5,10-dihydro-1*H*-pyrazolo[1,2-*b*]phthalazine-2-carbonitrile **1a** N,N dimethylformamide dimethyl acetal **2** with aromatic amines **3b** in the presence of $[BSO_3Hmim]HSO_4$ ionic liquid. Firstly the model reaction and was studied by employing a series of catalysts and solvents. Initially, we commenced the above three-component reaction in various solvents such as ethanol, acetonitrile, dioxane, toluene, DMF and water (Table 1, entries 1-6). Further screening indicated that using different Brønsted acid catalysts such as p-TSA and CH_3COOH exhibited a slight catalytic activity to give the product in a moderate yield (Table 1, entries 7–9) and also various ionic liquids (Table 1, entries 10-14) were tested. Finally, $[BSO_3Hmim]HSO_4$ ionic liquid was identified as the suitable reaction medium for **4ab** which is being isolated is maximum amounts (91%). In addition to this, we also studied the effect of temperature on the above reaction using different temperatures like room temperature, 40, 60, 80 °C (Table 1, entries 15 − 18). Based on the above results, the optimal condition was found to be that 70 °C improved the reaction yields.

Table 1. Optimized condition for the compound **4ab**



1a **2** **3b** **4ab**

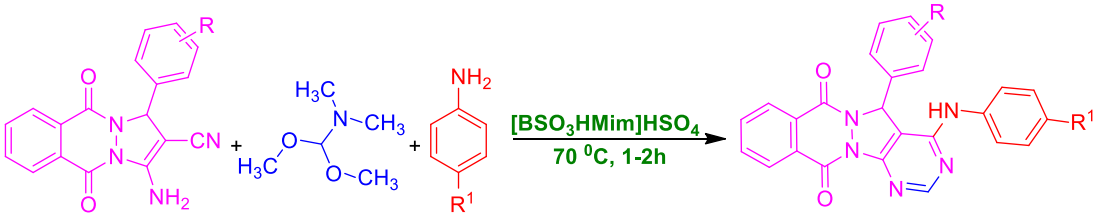
Entry[a]	Catalyst (mol %)	Temp (°C)	Time (h)	Yield[b] (%)
1	Ethanol	70	24	10
2	Acetonitrile	70	19	23
3	dioxane	70	11	41
4	Toluene	70	13	21
5	DMF	70	24	12
6	Water	70	11	14
7	p-TSA	70	8	30

8	Acetic acid(10%)	70	6	46
9	SA	70	6	54
10	[Bmim]Br	70	6	59
11	[Bmim]BF_4	70	6	46
12	[Bmim]PF_5	70	6	71
13	[Bmim]HSO_4	70	3	81
14	$[SO_3Hmim]HSO_4$	**70**	**2**	**91**
15	$[SO_3Hmim]HSO_4$	rt	2	90
16	$[SO_3Hmim]HSO_4$	40	2	80
17	$[SO_3Hmim]HSO_4$	60	2	73
18	$[SO_3Hmim]HSO_4$	80	2	51

[a]Reaction conditions: 3-amino-5,10-dioxo-1-phenyl-5,10-dihydro-1*H*-pyrazolo[1,2-*b*]phthalazine-2-carbonitrile (1mmol), DMF-DMA (1 mmol) and aromatic anilines (1 mmol), solvent (2 mL) and catalyst.[b]Yields of isolated products.

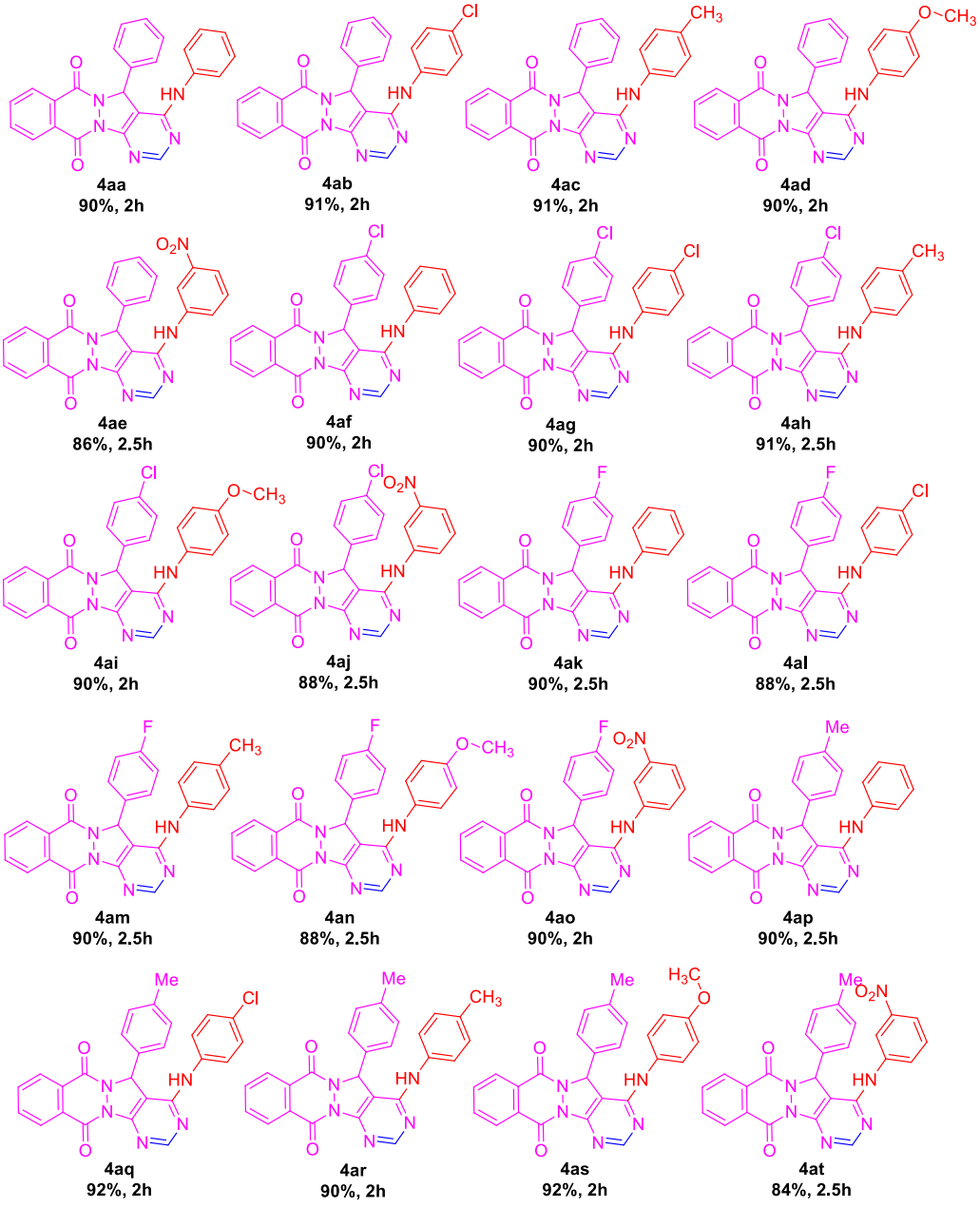
All these optimized conditions were then applied for all further experiments. Typically, a mixture of 3-amino-5,10-dioxo-1-phenyl-5,10-dihydro-1*H*-pyrazolo[1,2-*b*]phthalazine-2-carbonitrile **1a-f** (1mmol), N,N dimethylformamide dimethyl acetal **2** (1mmol) with aromatic amines **3a-e** (1mmol) and $[BSO_3Hmim]HSO_4$ at 70 °C for 1-2 h, afforded a library of 5-phenyl-4-(phenylamino)-5*H*-pyrimido[4',5':3,4]pyrazolo[1,2-b]phthalazine-7,12-diones **4aa-4bd** as shown in Table 2. The reaction was found to be compatible with various number of 3-amino-2-carbonitrile pyrazolo[1,2-*b*]phthalazines and aromatic anilines containing electron releasing as well as electron withdrawing groups in their aromatic rings. The product yield was not much influenced by electron donating or withdrawing groups present in the aromatic rings and the reaction proceeded smoothly to afford the desired products in good to excellent yields (Table 2).

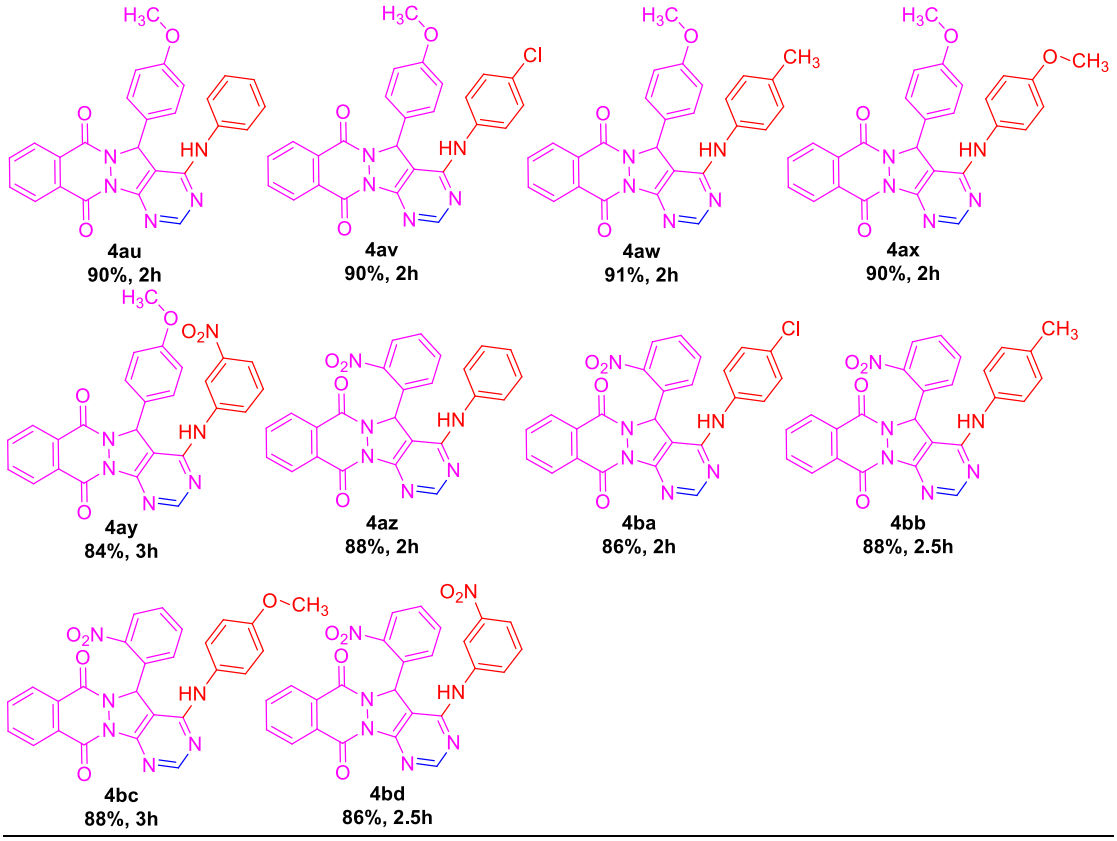
Table 2. synthesis of pyrazolo[1,2-*b*]phthalazino pyrimidine derivatives (**4aa-bd**)[a]



1a-f 2 3a-e 4aa-bd

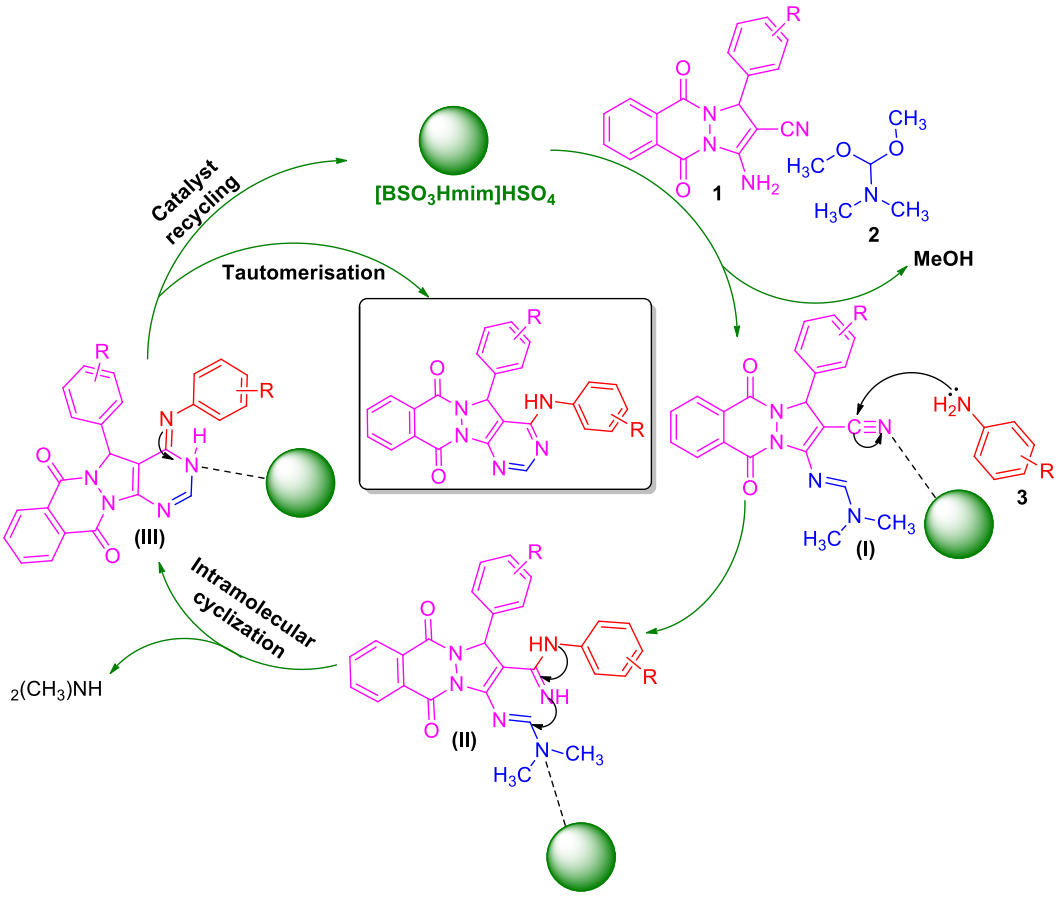
[b]yields (%), time





[a]Reaction conditions: 3-amino-5,10-dioxo-1-phenyl-5,10-dihydro-1*H*-pyrazolo[1,2-*b*]phthalazine-2-carbonitrile (1 mmol), DMF-DMA (1 mmol) aromatic amine (1 mmol) and $[SO_3Hmim]HSO_4$ (2mL).

Formation of the fused pyrazolo[1,2-*b*]phthalazine derivatives **4aa-4bd** can be explained by a plausible mechanism depicted in (**Scheme 12**). The condensation of 3-amino-5,10-dioxo-1-phenyl-5,10-dihydro-1*H*-pyrazolo[1,2-*b*]phthalazine-2-carbonitrile **1a-e** and N,N dimethylformamide dimethyl acetal loses methanol producing **I**. Further intermediate **I** reacts with aromatic anilines **3a-e** to form another intermediate **II** by the subsequently elimination of dimethyl amine. The intermediate **II** undergoes intramolecular cyclization to form the intermediate **III**. This intermediate undergoes intamolecular cyclization to afford the title compound **4aa-bd**. All the synthesized compounds **4** are stable solids whose structures were established by IR, ^1H, ^{13}C NMR spectroscopy, and elemental analysis.



Scheme 12. Proposed mechanism for the formation of pyrazolo[1,2-*b*]phthalazine derivatives **4aa-bd**

Finally, the possibility of recycling of ionic liquid [BSO_3Hmim]HSO_4 was examined by using the reaction of 3-amino-5,10-dioxo-1-phenyl-5,10-dihydro-1*H*-pyrazolo[1,2-*b*]phthalazine-2-carbonitrile **1a** (1mmol) N,N dimethylformamide dimethyl acetal **2** (1mmol) with aniline **3b** (1mmol) as model substrates under optimized conditions. After completion of the reaction the mixture was poured into water and stirred thoroughly. The solid product obtained was isolated by filtration, and the filtrate containing the ionic liquid was extracted with ethyl acetate (2 × 20 mL) to remove the non-ionic organic impurities. The water was then evaporated under reduced pressure and the recovered ionic liquid was dried under vacuum and reused four times in subsequent reactions without any evident change in the product yield (**Fig. 3**).

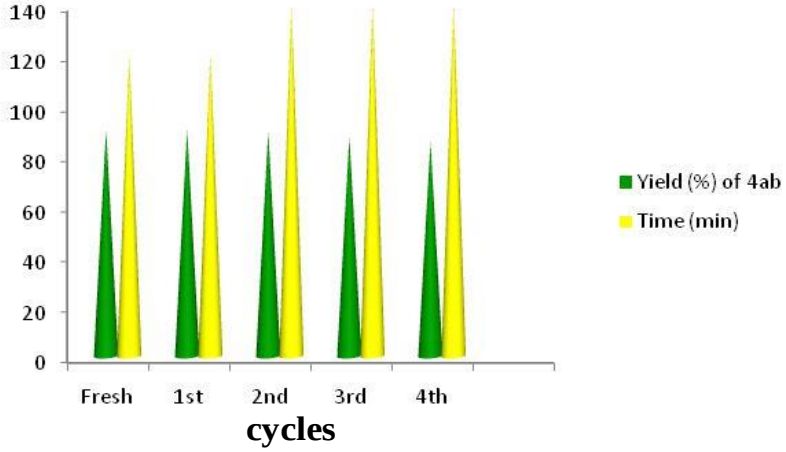


Figure 3. Reusability studies of the ionic liquid [BSO_3Hmim]HSO_4 catalyst for the synthesis of compound **4ab**.

6.4. SPECTRAL DISCUSSION

IR:

In all the compounds **4aa-bd** the formation of pyrimidine was confirmed by the disappearance of band around 2200 cm^{-1} due to (–CN) group and appearance of –NH group around 3316-3443 cm^{-1}. The carbonyl (C=O) group was observed at 1711-1729 cm^{-1}.

^{1}H NMR

In ^{1}H NMR, the –NH signal was observed at δ 4.64-5.16 ppm. In all the compounds, the singlet protons of pyrimidine (CH) group were observed as a singlet at δ 8.41-8.68 ppm. All other aromatic and aliphatic protons appeared at expected regions.

^{13}C NMR

In ^{13}C NMR, the signal appeared at δ 162.24-164.39 ppm can be attributed to (C=O) carbon. The signal observed at δ 157.84-158.94 ppm was assigned to lactone (C=O) carbon and in all the compounds.

Mass

The structures of all synthesized compounds were further confirmed by its mass spectra. The mass spectra detected the expected molecular ion signals (M + 1) corresponding to respective molecular weight of the synthesized compounds.

6.5. EXPERIMENTAL

Melting points were recorded on a Stuart SMP30 melting point apparatus and were uncorrected. Column chromatography was performed using silica gel (60–120 mesh size) purchased from Thomas Baker and Thin layer chromatography (TLC) was carried out using

aluminium sheets pre-coated with silica gel $60F_{254}$ purchased from Merck. IR spectra (KBr) were taken on Bruker WM-4 (X) spectrometer (577 model). ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker WM-400 spectrometer at 400 MHz and 100 MHz, respectively, in DMSO-d_6 with TMS as an internal standard. The chemical shifts were reported in ppm (δ). Mass spectra (ESI) were carried out on a Jeo1 JMSD-300 spectrometer. CHN analysis was carried out using Carlo Erba EA 1108 automatic elemental analyzer. All the chemicals and solvents were of analytical or synthetic grade and were used devoid of further purification unless otherwise stated.

6.5.1. General procedure for the synthesis of chromeno pyrano[2,3-*d*]pyrimidine derivatives (4aa-bd)

A dry 50 mL flask was charged with 3-amino-5,10-dioxo-1-phenyl-5,10-dihydro-1H-pyrazolo[1,2-*b*]phthalazine-2-carbonitrile **1a-f** (1 mmol), DMF-DMA **2** (1 mmol) and ionic liquid $[HSO_3Bmim]HSO_4$ (2 mL). The reaction mixture was stirred at 70 ℃ for 30 min. The progress of the reaction was monitored by TLC and after completion of the reaction (single spot on TLC), aromatic amine **3a-e** (1 mmol) was added and the reaction was continued for an additional 1.5-2 h. The progress of the reaction was monitored by TLC (eluent = n-hexane/ethyl acetate: 8/2). After completion of the reaction, the reaction mixture was cooled to RT and poured into ice cold water, the solid separated was filtered, washed with water, dried and purified by column chromatography using silicagel (ethylacetate / n-hexane: 2/8) to afford title compounds **4aa-bd** in good yields.

6.5.2. Physical and spectral data

5-Phenyl-4-(phenylamino)-5H-pyrimido[4',5':3,4]pyrazolo[1,2-b]phthalazine-7,12-dione (**4aa**). White solid: mp 358-360 °C; **IR** (KBr) $\upsilon_{max}(cm^{-1})$ 3302, 2939, 1720, 1620, 1504, 1386; **^{1}H NMR** (400 MHz, DMSO-d_6): δ 6.24 (s, 1H), 6.93 (d, *J* = 7.8 Hz, 3H), 7.28-7.69 (m, 4H), 7.69-7.95 (m, 4H), 8.16 (t, *J* = 7.8 Hz, 1H), 8.28 (t, *J* = 7.8 Hz, 1H), 8.78 (s, 1H), 9.08 (s, 1H); **^{13}C NMR** (100 MHz, DMSO-d_6): δ 55.53, 102.15, 114.28, 122.75, 127.22, 128.19, 129.18, 129.95, 134.15, 134.80, 138.23, 154.25, 155.01, 156.92, 158.96, 159.77; **ESI-MS**: *m/z* 420 (M + 1). Anal. Calcd. For $C_{25}H_{17}N_5O_2$: C, 74.45; H, 4.09; N, 10.02; Found: C, 74.73; H, 4.04; N, 10.28.

4-((4-Chlorophenyl)amino)-5-phenyl-5H-pyrimido[4',5':3,4]pyrazolo[1,2-b]phthalazine-7,12-dione. (**4ab**). White solid: mp 340-342 °C; **IR** (KBr) $\upsilon_{max}(cm^{-1})$ 3341, 2904, 1721, 1603, 1530, 1359; **^{1}H NMR** (400 MHz, DMSO-d_6): δ 6.28 (s, 1H), 7.14 (d, *J* = 7.8 Hz, 2H), 7.29

(d, *J* = 7.8 Hz, 3H), 7.69 (t, *J* = 7.8 Hz, 2H), 7.84-7.92 (m, 4H), 8.28 (t, *J* = 7.8 Hz, 1H), 8.32 (t, *J* = 7.8 Hz, 1H), 8.53 (s, 1H), 8.82 (s, 1H); **^{13}C NMR** (100 MHz, DMSO-d_6): δ 56.83, 113.29, 124.59, 126.36, 127.52, 128.99, 129.29, 132.36, 133.96, 134.36, 152.26, 156.58, 157.99, 158.28, 159.77, 160.58; **ESI-MS**: *m/z* 454 (M + 1); Anal. Calcd. For $C_{25}H_{16}ClN_5O_2$: C, 66.16; H, 3.55; Cl, 7.81; N, 15.43; Found: C, 66.32; H, 3.50; N, 15.69.

5-Phenyl-4-(p-tolylamino)-5H-pyrimido[4',5':3,4]pyrazolo[1,2-b]phthalazine-7,12-dione (**4ac**). White powder; mp 312-316 °C; **IR** (KBr) υ_{max}(cm^{-1}) 3324, 2948, 1728, 1525, 1349; **^{1}H NMR** (400 MHz, DMSO-d_6): δ 2.24 (s, 3H), 6.53 (s, 1H), 7.36 (d, *J* = 7.8 Hz, 2H), 7.83 (d, *J* = 7.8 Hz, 2H), 7.94 (t, *J* = 7.8 Hz, 3H), 8.02 (d, *J* = 7.8 Hz, 2H), 8.16 (d, *J* = 7.8 Hz, 2H), 8.21 (t, *J* = 7.8 Hz, 1H), 8.34 (t, *J* = 7.8 Hz, 1H), 8.47 (s, 1H), 8.56 (s, 1H); **^{13}C NMR** (100 MHz, DMSO-d_6): δ 24.28, 58.38, 117.39, 122.69, 125.39, 126.39, 127.56, 128.23, 130.68, 132.23, 136.69, 150.69, 152.32, 156.29, 157.69, 161.26; **ESI-MS**: *m/z* 434 (M + 1); Anal. Calcd. For $C_{26}H_{19}N_5O_2$: C, 72.04; H, 4.42; N, 16.16; Found: C, 72.28; H, 4.47; N, 16.47.

4-((4-Methoxyphenyl)amino)-5-phenyl-5H-pyrimido[4',5':3,4]pyrazolo[1,2-b]phthalazine-7,12-dione (**4ad**). White powder; mp: 368-370 °C; **IR** (KBr) υ_{max} (cm^{-1}): 3385, 2945, 1730, 1582, 1336; **^{1}H NMR** (400 MHz, DMSO-d_6): δ 3.64 (s, 3H), 6.69 (s, 1H), 7.18 (d, *J* = 7.8 Hz, 2H), 7.24 (d, *J* = 7.8 Hz, 2H), 7.68 (t, *J* = 7.8 Hz, 3H), 8.02 (d, *J* = 7.8 Hz, 2H), 8.12 (d, *J* = 7.8 Hz, 2H), 8.23 (t, *J* = 7.8 Hz, 1H), 8.30 (t, *J* = 7.8 Hz, 1H), 8.41 (s, 1H), 8.52 (s, 1H); **^{13}C NMR** (100 MHz, DMSO-d_6): δ 55.28, 57.99, 116.96, 122.85, 124.86, 125.28, 126.28, 129.29, 131.28, 134.28, 137.95, 152.58, 153.39, 155.58, 157.58, 162.29; **ESI-MS**: *m/z* 450 (M + 1); Anal. Calcd. For $C_{26}H_{19}N_5O_3$: C, 69.48; H, 4.26; N, 15.58; Found: C, 69.27; H, 4.21; N, 15.81.

4-((3-Nitrophenyl)amino)-5-phenyl-5H-pyrimido[4',5':3,4]pyrazolo[1,2-b]phthalazine-7,12-dione (**4ae**). White powder; mp 328-330 °C; **IR** (KBr) υ_{max}(cm^{-1}) 3289, 2935, 1717, 1649, 1353; **^{1}H NMR** (400 MHz, DMSO-d_6): δ 6.75 (s, 1H), 7.25 (d, *J* = 7.8 Hz, 2H), 7.34 (d, *J* = 7.8 Hz, 2H), 7.58 (t, *J* = 7.8 Hz, 2H), 7.73 (s, 1H), 7.93-8.03 (m, 3H), 8.16 (t, *J* = 7.8 Hz, 2H), 8.23 (t, *J* = 7.8 Hz, 1H), 8.38 (s, 1H), 8.54 (s, 1H); **^{13}C NMR** (100 MHz, DMSO-d_6): δ 58.59, 106.37, 118.53, 124.36, 128.92, 129.96, 129.69, 130.96, 130.28, 132.38, 136.96, 138.69, 152.26, 157.56, 158.58, 161.26; **ESI-MS**: *m/z* 465 (M + 1); Anal. Calcd. For $C_{25}H_{16}N_6O_4$: C, 64.65; H, 3.47; N, 18.10; Found: C, 74.51; H, 4.52; N, 9.73.

5-(4-Chlorophenyl)-4-(phenylamino)-5H-pyrimido[4',5':3,4]pyrazolo[1,2-b]phthalazine-7,12-dione (**4af**). White powder; mp 369-371 °C; **IR** (KBr) υ_{max}(cm^{-1}) 3325, 2925, 1720,

1588, 1325; **^{1}H NMR** (400 MHz, DMSO-d_6): δ 6.86 (s, 1H), 6.83 (d, *J* = 7.8 Hz, 2H), 6.96-7.18 (m, 4H), 7.26 (d, J = 7.8 Hz, 2H), 7.48 (t, *J* = 7.8 Hz, 1H), 7.73 (t, *J* = 7.8 Hz, 2H), 7.92 (d, *J* = 7.8 Hz, 2H), 8.24 (s, 1H), 8.62 (s, 1H); **^{13}C NMR** (100 MHz, DMSO-d_6): δ 52.25, 104.58, 119.92, 124.58, 127.39, 127.86, 128.25, 129.98, 132.89, 136.89, 151.55, 154.23, 157.93, 159.96, 160.89, 162.89; **ESI-MS**: *m/z* 454 (M + 1); Anal. Calcd. For $C_{25}H_{16}ClN_5O_2$: C, 66.16; H, 3.55; N, 15.43; Found: C, 66.02; H, 3.61; N, 15.73.

5-(4-Chlorophenyl)-4-((4-chlorophenyl)amino)-5H-pyrimido[4',5':3,4]pyrazolo[1,2-b]phthalazine-7,12-dione (**4ag**). White powder; mp 349-351 °C; **IR** (KBr) υ_{max}(cm^{-1}) 3358, 2925, 1718, 1634, 1358; **^{1}H NMR** (400 MHz, DMSO-d_6): δ 6.45 (s, 1H), 6.92 (d, *J* = 7.8 Hz, 2H), 7.15 (d, *J* = 7.6, 2H), 7.28 (d, *J* = 7.8 Hz, 2H), 7.33 (t, *J* = 7.8 Hz, 2H), 7.54 (t, *J* = 7.8 Hz, 2H), 7.72 (d, *J* = 7.8 Hz, 2H), 8.01 (s, 1H), 8.18 (s, 1H); **^{13}C NMR** (100 MHz, DMSO-d_6): δ 58.58, 109.58, 125.58, 126.59, 127.66, 128.59, 128.92, 130.86, 132.55, 151.83, 152.28, 154.58, 155.36, 161.58, 163.25; **ESI-MS**: *m/z* 488 (M + 1); Anal. Calcd. For $C_{25}H_{15}C_{l2}N_5O_2$: C, 61.49; H, 3.10; N, 14.34; Found: C, 61.76; H, 3.15; N, 14.58.

5-(4-Chlorophenyl)-4-(p-tolylamino)-5H-pyrimido[4',5':3,4]pyrazolo[1,2-b]phthalazine-7,12-dione (**4ah**). White powder; mp 345-347 °C; **IR** (KBr) υ_{max}(cm^{-1}) 3245, 2925, 1714, 1558, 1358; **^{1}H NMR** (400 MHz, DMSO-d_6) δ 1.19 (s, 3H), 6.53 (s, 1H), 7.41 (d, *J* = 7.8 Hz, 3H), 7.47-7.57 (m, 2H), 7.60 (d, *J* = 7.8 Hz, 2H), 7.73 (t, *J* = 7.8 Hz, 1H), 7.90 (t, *J* = 7.8 Hz, 1H), 8.01-8.34 (m, 3H), 8.52 (s, 1H), 8.96 (s, 1H); **^{13}C NMR** (100 MHz, DMSO-d_6) δ 22.34, 59.69, 106.56, 126.35, 127.59, 127.95, 128.25, 128.38, 130.58, 132.58, 150.19, 152.58, 155.58, 158.69, 160.58, 161.58; **ESI-MS**: *m/z* 468 (M + 1); Anal. Calcd. For $C_{26}H_{18}ClN_5O_2$: C, 66.74; H, 3.88; N, 14.97; Found: C, 66.97; H, 3.82; N, 14.76.

5-(4-Chlorophenyl)-4-((4-methoxyphenyl)amino)-5H-pyrimido[4',5':3,4]pyrazolo[1,2-b]phthalazine-7,12-dione (4ai). White powder; mp 345-347 °C; **IR** (KBr) υ_{max}(cm^{-1}) 3252, 2913, 1726, 1649, 1336; **^{1}H NMR** (400 MHz, DMSO-d_6) δ 3.69 (s, 3H), 6.58 (s, 1H), 7.58 (d, *J* = 7.8 Hz, 2H), 7.38 (d, *J* = 7.6 Hz, 2H), 7.58 (d, *J* = 7.8 Hz, 2H), 7.82 (t, *J* = 7.8 Hz, 2H), 7.58 (t, *J* = 7.8 Hz, 2H), 8.14 (d, *J* = 2H), 8.34 (s, 1H), 8.86 (s, 1H); **^{13}C NMR** (100 MHz, DMSO-d_6) δ 57.53, 61.89, 113.34, 125.59, 126.99, 127.53, 128.81, 128.56, 132.53, 134.58, 136.58, 151.66, 156.59, 158.58, 162.26, 163.58; **ESI-MS**: *m/z* 484 (M + 1); Anal. Calcd. For $C_{26}H_{18}ClN_5O_3$: C, 64.53; H, 3.75; N, 14.47; Found: C, 64.29; H, 3.70; N, 14.72.

5-(4-Chlorophenyl)-4-((3-nitrophenyl)amino)-5H-pyrimido[4',5':3,4]pyrazolo[1,2-b]phthalazine-7,12-dione (4aj). White powder; mp 358-360 °C; **IR** (KBr) υ_{max}(cm^{-1}) 3352,

2923, 1725, 1622, 1558, 1322; **^{1}H NMR** (400 MHz, DMSO-d_6) δ 5.74 (s, 1H), 7.10 (t, *J* = 7.8 Hz, 2H), 7.13-7.50 (m, 3H), 7.52-7.56 (m, 4H), 7.63 (d, *J* = 7.8 Hz, 2H), 7.72 (s, 1H), 8.12 (s, 1H), 8.68 (s, 1H); **^{13}C NMR** (100 MHz, DMSO-d_6) δ 60.19, 103.34, 119.30, 121.34, 133.03, 137.30, 149.02, 151.30, 155.34, 156.04, 162.93, 164.40, 165.53; **ESI-MS:** *m/z* 499 (M + 1); Anal. Calcd. For $C_{25}H_{15}ClN_6O_4$: C, 60.19; H, 3.03; N, 16.85; Found: C, 60.48; H, 3.09; N, 16.58.

5-(4-Fluorophenyl)-4-(phenylamino)-5H-pyrimido[4',5':3,4]pyrazolo[1,2-b]phthalazine-7,12-dione (4ak). Yellow powder; mp: 325-327 °C; **IR** (KBr) υ_{max} (cm^{-1}): 3358, 3027, 1716, 1658, 1455, 1246; **^{1}H NMR** (400 MHz, DMSO-d_6): δ 6.73 (s, 1H), 7.41 (d, *J* = 7.8 Mz, 2H), 7.53-7.40 (m, 2H), 7.59 (t, J = 7.8 Mz, 2H), 7.73 (t, *J* = 7.8 Mz, 2H), 7.90 (t, *J* = 7.8 Mz, 2H), 8.02 (d, *J* = 7.8 Mz, 2H), 8.12 (d, *J* = 7.8 Mz, 1H), 8.65 (s, 1H), 9.29 (s, 1H); **^{13}C NMR** (100 MHz, DMSO-d_6): δ 54.99, 100.19, 105.79, 115.07, 124.97, 125.38, 128.85, 129.16, 129.90, 30.87, 132.97, 133.63, 150.84, 152.09, 153.21, 156.28, 158.44, 158.53, 159.90, 161.04, 165.74; **ESI-MS**: *m/z* 438 $(M + 1)^+$; Anal. Calcd. For $C_{25}H_{16}FN_5O_2$; C, 68.64; H, 3.69; N, 16.01; Found: C, 68.31; H, 3.64; N, 16.34.

4-((4-Chlorophenyl)amino)-5-(4-fluorophenyl)-5H-pyrimido[4',5':3,4]pyrazolo[1,2-b]phthalazine-7,12-dione (4al). Yellow powder; mp: 358-361 °C; **IR** (KBr) υ_{max} (cm^{-1}): 3358, 2955, 1739, 1608, 1489, 1259; **^{1}H NMR** (400 MHz, DMSO-d_6): δ 6.64 (s, 1H), 7.41 (d, *J* = 7.8 Hz, 2H), 7.53-7.40 (m, 2H), 7.59 (d, *J* = 7.8 Hz, 1H), 7.73 (t, *J* = 7.8 Hz, 2H), 7.90 (d, *J* = 7.8 Hz, 1H), 8.02 (d, *J* = 7.8 Hz , 2H), 8.12 (d, *J* = 7.8 Hz, 2H), 8.54 (s, 1H), 8.65 (s, 1H); **^{13}C NMR** (100 MHz, DMSO-d_6): δ 55.46, 100.92, 119.29, 124.70, 124.72, 126.89, 127.93, 128.83, 129.54, 130.00, 134.13, 150.55, 153.07, 155.65, 157.60, 158.34, 160.32, 162.34, 163.29, 164.26 **ESI-MS**: *m/z* 472 $(M + 1)^+$; Anal. Calcd. For $C_{25}H_{15}ClFN_5O_2$; C, 63.63; H, 3.20; N, 14.84; Found: C, 63.41; H, 3.15; N, 14.63.

5-(4-Fluorophenyl)-4-(p-tolylamino)-5H-pyrimido[4',5':3,4]pyrazolo[1,2-b]phthalazine-7,12-dione (4am). Yellow powder; mp: 386-388 °C; **IR** (KBr) υ_{max} (cm^{-1}): 3247, 3028, 1736, 1625, 1570, 1256; **^{1}H NMR** (400 MHz, DMSO-d_6): δ 1.91 (s, 3H), 6.73 (s, 1H), 7.41 (d, *J* = 7.8 Mz, 2H), 7.53-7.40 (m, 2H), 7.59 (t, *J* = 7.8 Mz, 2H), 7.73 (d, *J* = 7.8 Mz, 1H), 7.90 (d, *J* = 7.8 Mz, 2H), 8.02 (d, *J* = 7.8 Mz, 1H), 8.12 (d, *J* = 7.8 Mz, 2H), 8.54 (s, 1H), 8.65 (s, 1H); **^{13}C NMR** (100 MHz, DMSO-d_6): δ 20.09, 54.99, 105.79, 113.32, 117.63, 122.61, 124.97, 126.98, 127.16, 128.38, 129.40, 129.82, 130.86, 132.97, 147.86, 152.09, 153.38, 154.21,

155.48, 156.28, 158.44, 159.90, 161.04, 162.83, 164.39; **ESI-MS**: *m/z* 452 $(M + 1)^+$; Anal. Calcd. For $C_{26}H_{18}FN_5O_2$; C, 69.17; H, 4.02; N, 15.51; Found: C, 69.48; H, 4.08; N, 15.78.

5-(4-Fluorophenyl)-4-((4-methoxyphenyl)amino)-5H-pyrimido[4',5':3,4]pyrazolo[1,2-b]phthalazine-7,12-dione (4an). Yellow powder; mp: 335-337 °C; **IR** (KBr) υ_{max} (cm^{-1}): 3309, 2919, 1700, 1599, 1367; **^{1}H NMR** (400 MHz, DMSO-d_6): δ 3.69 (s, 3H), 6.45 (s, 1H), 6.85 (d, *J* = 7.8 Hz, 2H), 7.36-7.39 (m, 4H), 7.95-7.97 (m, 2H), 8.13 (d, *J* = 7.8 Hz, 1H), 8.31 (d, *J* = 7.8 Hz, 1H), 8.66 (s, 1H), 8.98 (s, 1H); **^{13}C NMR** (100 MHz, DMSO-d_6): δ 55.53, 60.99, 102.15, 114.28, 122.75, 127.22, 128.19, 129.18, 129.95, 134.15, 134.80, 138.23, 154.25, 155.01, 156.92, 158.96, 159.77; **ESI-MS**: *m/z* 468 $(M + 1)^+$; Anal. Calcd. For $C_{26}H_{18}FN_5O_3$; C, 66.80; H, 3.88; F, 4.06; N, 14.98; Found: C, 66.69; H, 3.83; N, 14.75.

5-(4-Fluorophenyl)-4-((3-nitrophenyl)amino)-5H-pyrimido[4',5':3,4]pyrazolo[1,2-b]phthalazine-7,12-dione (4ao). Yellow powder; mp: 340-342 °C; **IR** (KBr) υ_{max} (cm^{-1}): 3358, 2988, 1713, 1638, 1558, 1285; ^{1}H NMR (400 MHz, DMSO-d_6): **^{1}H NMR** (400 MHz, DMSO-d_6): δ 6.58 (s, 1H), 6.58 (d, *J* = 7.8 Hz, 2H), 7.36 (d, *J* = 7.8 Hz, 2H), 7.53 (s, 1H), 7.93-8.12 (m, 3H), 8.23 (t, *J* = 7.8 Hz, 2H), 8.39 (d, *J* = 7.8 Hz, 2H), 8.66 (s, 1H), 8.98 (s, 1H); **^{13}C NMR** (100 Hz, DMSO-d_6): δ 60.99, 114.28, 122.75, 125.98, 127.22, 128.19, 129.18, 129.95, 130.15, 133.80, 150.34, 153.49, 155.01, 156.92, 158.96, 159.77, 160.23, 162.38; **ESI-MS:** m/z 483 $(M + 1)^+$; Anal. Calcd. For $C_{25}H_{15}FN_6O_4$; C, 62.24; H, 3.13; N, 17.42; Found: C, 62.46; H, 3.19; N, 17.73.

4-(Phenylamino)-5-(p-tolyl)-5H-pyrimido[4',5':3,4]pyrazolo[1,2-b]phthalazine-7,12-dione (4ap). Yellow powder; mp: 387-289 °C; **IR** (KBr) υ_{max} (cm^{-1}): 3258, 2918, 1712, 1602, 1525, 1258; **^{1}H NMR** (400 MHz, DMSO-d_6): δ 2.24 (s, 3H), 6.64 (s, 1H), 7.41 (d, *J* = 7.6 Hz, 2H), 7.53-7.40 (m, 3H), 7.59 (t, *J* = 7.6 Hz, 2H), 7.73 (d, *J* = 7.6 Hz, 2H), 7.90 (t, *J* = 7.6 Hz, 2H), 8.02 (d, *J* = 7.6 Hz, 1H), 8.12 (d, *J* = 7.6 Hz, 1H), 8.65 (s, 1H), 9.02 (s, 1H); **^{13}C NMR** (100 MHz, DMSO-d_6): δ 20.23, 57.46, 112.20, 119.29, 124.70, 126.34, 125.49, 126.45, 127.85, 128.74, 129.49,130.00, 134.13, 137.93, 150.34, 152.34, 157.60, 158.34, 162.34, 164.26, 167.05; **ESI-MS**: *m/z* 434 $(M + 1)^+$; Anal. Calcd. For $C_{26}H_{19}N_5O_2$; C, 72.04; H, 4.42; N, 16.16; Found: C, 72.32; H, 4.48; N, 16.46.

4-((4-Chlorophenyl)amino)-5-(p-tolyl)-5H-pyrimido[4',5':3,4]pyrazolo[1,2-b]phthalazine-7,12-dione (4aq). Yellow powder; mp: 345 -347 °C; **IR** (KBr) υ_{max} (cm^{-1}): 3325, 2976, 1719, 1618, 1556, 1295; **^{1}H NMR** (400 MHz, DMSO-d_6): δ 1.58 (s, 3H), 6.58 (s, 1H), 7.58 (d, *J* = 7.85 Mz, 2H), 7.58 (d, *J* = 7.8 Hz, 2H), 7.63 (d, *J* = 7.8 Hz, 2H), 7.69 (t, *J* = 7.8 Hz, 1H), 7.78

(d, *J* = 7.8 Hz, 2H), 7.95 (t, *J* = 7.8 Hz, 1H), 8.01 (d, *J* = 7.8 Hz, 2H), 8.45 (d, *J* = 7.8 Hz, 2H), 8.58 (s, 1H), 9.36 (s, 1H); **^{13}C NMR** (100 MHz, DMSO-d_6): δ 21.01, 54.57, 103.58, 113.56, 122.25, 124.58, 125.58, 127.26, 128.88, 129.28, 130.25, 132.28, 133.52, 152.28, 153.95, 154.29, 156.58, 156.58, 157.28, 159.28, 162.25, 163.95; **ESI-MS:** *m/z* 468 $(M + 1)^+$; Anal. Calcd. For $C_{26}H_{18}ClN_5O_2$; C, 66.74; H, 3.88; N, 14.97; Found: C, 66.52; H, 3.83; N, 14.78.

5-(p-Tolyl)-4-(p-tolylamino)-5H-pyrimido[4',5':3,4]pyrazolo[1,2-b]phthalazine-7,12-dion (4ar). Yellow powder; mp: 325-327 °C; **IR** (KBr) υ_{max} (cm^{-1}): 3286, 2964, 1725, 1628, 1585, 1288; **^{1}H NMR** (400 MHz, DMSO-d_6): δ 2.28 (s, 3H), 6.58 (s, 1H), 7.58 (d, *J* = 7.8 Hz, 2H), 7.58- 7.68 (m, 3H), 7.72 (t, *J* = 7.8 Hz, 2H), 7.87 (t, *J* = 7.8 Hz, 2H), 7.98 (t, *J* = 7.8 Hz, 2H), 8.12 (d, *J* = 7.8 Hz, 2H), 8.28 (d, *J* = 7.8 Hz, 2H), 8.63 (s, 1H), 8.93 (s, 1H); **^{13}C NMR** (100 MHz, DMSO-d_6): δ 20.23, 55.46, 112.20, 114.20, 124.70, 127.34, 128.93, 129.39, 130.00, 131.13, 132.34, 137.93, 150.34, 152.45, 157.60, 158.34, 160.23, 162.34, 164.26, 167.05; **ESI-MS**: *m/z* 459 $(M + 1)^+$; Anal. Calcd. For $C_{27}H_{21}N_5O_2$; C, 72.47; H, 4.73; N, 15.65; Found: C, 72.74; H, 4.78; N, 15.89.

***4-((4-Methoxyphenyl)amino)-5-(p-tolyl)-5H-pyrimido[4',5':3,4]pyrazolo[1,2-b]phthalazine-7,12-dione* (4as)**. Yellow powder; mp: 357-359 °C; **IR** (KBr) υ_{max} (cm^{-1}): 3354, 2945, 1715, 1658, 1212; **^{1}H NMR** (400 MHz, DMSO-d_6): δ 1.91 (s, 3H), 3.65 (s, 3H), 6.73 (s, 1H), 7.41 (d, *J* = 7.8 Hz, 2H), 7.53-7.40 (m, 4H), 7.59 (t, *J* = 7.8 Hz, 2H), 7.73 (t, *J* = 7.8 Hz, 2H), 7.90 (t, *J* = 7.8 Hz, 2H), 8.02 (d, *J* = 7.8 Hz, 2H), 8.12 (d, *J* = 7.8 Hz, 2H), 8.54 (s, 1H), 8.65 (s, 1H); **^{13}C NMR** (100 MHz, DMSO-d_6): δ 22.58, 55.58, 113.32, 22.61, 124.97, 125.93, 127.29, 129.38, 130.73, 132.97, 133.59, 150.34, 152.09, 154.58, 156.28, 158.58, 159.52, 160.58, 161.04, 164.23; **ESI-MS:** *m/z* 464 $(M + 1)^+$; Anal. Calcd. For $C_{27}H_{21}N_5O_3$; C, 69.97; H, 4.57; N, 15.11; Found: C, 69.76; H, 4.63; N, 15.37.

4-((3-Nitrophenyl)amino)-5-(p-tolyl)-5H-pyrimido[4',5':3,4]pyrazolo[1,2-b]phthalazine-7,12-dione (4at). Yellow powder; mp: 367-369 °C; **IR** (KBr) υ_{max} (cm^{-1}): 3385, 3088, 1709, 1668, 1536, 1225; **^{1}H NMR** (400 MHz, DMSO-d_6): δ 2.08 (s, 3H), 6.88 (s, 1H), 7.23 (d, *J* = 7.8 Hz, 2H), 7.62 (d, *J* = 7.8 Hz, 2H), 7.71 (t, *J* = 7.8 Hz, 1H), 7.78-7.84 (m, 1H), 7.96 (t, *J* = 7.8 Hz, 2H), 8.16 (d, *J* = 7.8 Hz, 2H), 8.12 (d, *J* = 7.8 Hz, 2H), 8.83 (s, 1H), 9.13 (s, 1H); **^{13}C NMR** (100 MHz, DMSO-d_6): δ 20.23, 58.55, 114.56, 125.39, 126.83, 127.83, 128.93, 129.93, 130.21, 131.19, 133.29, 149.39, 150.29, 153.34, 155.82, 157.60, 158.58, 162.58,

163.82, 164.66; **ESI-MS**: *m/z* 478 $(M + 1)^+$; Anal. Calcd. For $C_{26}H_{18}N_6O_4$; C, 65.27; H, 3.79; N, 17.56; Found: C, 65.56; H, 3.73; N, 17.78.

5-(4-Methoxyphenyl)-4-(phenylamino)-5H-pyrimido[4',5':3,4]pyrazolo[1,2-b]phthalazine-7,12-dion e (4au). Yellow powder; mp: 325-327 °C; **IR** (KBr) υ_{max} (cm^{-1}): 3358, 3028, 1719, 1658, 1228; **^{1}H NMR** (400 MHz, DMSO-d_6): δ 3.65 (s, 3H), 6.59 (s, 1H), 7.28 (d, *J* = 7.8 Hz, 2H), 7.34 (d, *J* = 7.8 Hz, 2H), 7.58 (t, *J* = 7.8 Hz, 2H), 7.82 (d, *J* = 7.8 Hz, 1H), 7.96 (t, *J* = 7.8 Hz, 2H), 8.02 (d, *J* = 7.8 Hz, 2H), 8.12 (d, *J* = 7.8 Hz, 2H), 8.54 (s, 1H), 8.65 (s, 1H); **^{13}C NMR** (100 MHz, DMSO-d_6): δ 54.58, 61.39, 114.56, 119.29, 124.58, 125.84, 126.55, 127.58, 129.58, 131.23, 134.55, 135.93, 151.45, 152.89, 153.88, 155.57, 161.58, 163.58, 165.58; **ESI-MS**: *m/z* 450 $(M + 1)^+$; Anal. Calcd. For $C_{26}H_{19}N_5O_3$; C, 69.48; H, 4.26; N, 15.58; Found: C, 69.75; H, 4.21; N, 15.94.

4-((4-Chlorophenyl)amino)-5-(4-methoxyphenyl)-5H-pyrimido[4',5':3,4]pyrazolo[1,2-b]phthalazine-7,12-dione (4av). Yellow powder; mp: 351-353 °C; **IR** (KBr) υ_{max} (cm^{-1}): 3358, 3059, 1723, 1626, 1259; **^{1}H NMR** (400 MHz, DMSO-d_6): δ 3.58 (s, 3H), 6.58 (s, 1H), 7.57 (d, *J* = 7.8 Hz, 2H), 7.43 (d, *J* = 7.8 Hz, 2H), 7.63 (d, *J* = 7.8 Hz, 1H), 7.73 (t, *J* = 7.8 Hz, 2H), 7.93 (t, *J* = 7.8 Hz, 1H), 8.15 (d, *J* = 7.8 Hz, 1H), 8.38 (d, *J* = 7.8 Hz, 1H), 8.62 (s, 1H), 8.9 (s, 1H); **^{13}C NMR** (100 MHz, DMSO-d_6): δ 57.58, 58.36, 115.58, 123.56, 125.58, 126.58, 127.58, 129.28, 130.58, 132.88, 150.33, 152.58, 153.58, 157.59, 158.58, 160.58, 164.95, 165.58; **ESI-MS**: *m/z* 484 $(M + 1)^+$; Anal. Calcd. For $C_{26}H_{18}ClN_5O_3$; C, 64.53; H, 3.75; N, 14.47; Found: C, 64.26; H, 3.70; N, 14.72.

5-(4-Methoxyphenyl)-4-(p-tolylamino)-5H-pyrimido[4',5':3,4]pyrazolo[1,2-b]phthalazine-7,12-dione (4aw). Yellow powder; mp: 332-334 °C; **IR** (KBr) υ_{max} (cm^{-1}): 3386, 2998, 1713, 1659, 1285; **^{1}H NMR** (400 MHz, DMSO-d_6): δ 2.18 (s, 3H), 3.71 (s, 3H), 6.68 (s, 1H), 7.28 (d, *J* = 7.8 Hz, 2H), 7.56 (d, *J* = 7.8 Hz, 2H), 7.63 (d, *J* = 7.8 Hz, 1H), 7.76 (t, *J* = 7.8 Hz,1H), 7.92 (t, *J* = 7.8 Hz, 2H), 8.16 (d, *J* = 7.8 Hz, 2H), 8.32 (d, *J* = 7.8 Hz, 1H), 8.31 (d, *J* = 7.8 Hz, 1H), 8.57 (s, 1H), 8.78 (s, 1H); **^{13}C NMR** (100 MHz, DMSO-d_6): δ 20.88, 57.58, 60.23, 119.58, 126.58, 127.58, 128.39, 129.38, 132.58, 132.24, 135.28, 152.58, 158.48, 159.84, 161.18, 163.58, 165.05; **ESI-MS**: *m/z* 464 $(M + 1)^+$; Anal. Calcd. For $C_{27}H_{21}N_5O_3$; C, 69.97; H, 4.57; N, 15.11; Found: C, 69.71; H, 4.51; N, 15.41.

5-(4-Methoxyphenyl)-4-((4-methoxyphenyl)amino)-5H-pyrimido[4',5':3,4]pyrazolo[1,2-b]phthalazine-7,12-dione (4ax). Yellow powder; mp: 322-324 °C; **IR** (KBr) υ_{max} (cm^{-1}): 3358, 2958, 1728, 1689, 1255; **^{1}H NMR** (400 MHz, DMSO-d_6): δ 3.68 (s, 3H), 3.72 (s, 3H),

6.68 (s, 1H), 7.52 (d, *J* = 7.8 Hz, 2H), 7.68 (d, *J* = 7.8 Hz, 2H), 7.78 (t, *J* = 7.8 Hz, 2H), 7.73 (t, *J* = 7.8 Hz, 2H), 7.90 (t, *J* = 7.8 Hz, 1H), 8.02 (d, *J* = 7.8 Hz, 2H), 8.12 (d, *J* = 7.8 Hz, 1H), 8.54 (s, 1H), 8.83 (s, 1H); **^{13}C NMR** (100 MHz, DMSO-d_6): δ 20.23, 34.06, 55.46, 100.92, 101.78, 112.20, 114.20, 114.39, 119.29, 124.70, 124.72, 130.00, 134.13, 137.93, 147.85, 153.07, 157.60, 158.34, 162.34, 164.26, 167.05, 175.34; **ESI-MS**: *m/z* 480 $(M + 1)^+$; Anal. Calcd. For $C_{27}H_{21}N_5O_4$; C, 67.63; H, 4.41; N, 14.61; Found: C, 67.87; H, 4.46; N, 14.92.

5-(4-Methoxyphenyl)-4-((3-nitrophenyl)amino)-5H-pyrimido[4',5':3,4]pyrazolo[1,2-b]phthalazine-7,12-dione (4ay). Yellow powder; mp: 358-361 °C; **IR** (KBr) υ_{max} (cm^{-1}): 3359, 2935, 1713, 1659, 1259; **^{1}H NMR** (400 MHz, DMSO-d_6): δ 3.68 (s, 3H), 6.59 (s, 1H), 7.56 (d, *J* = 7.8 Hz, 2H), 7.64 (d, *J* = 7.8 Hz, 2H), 7.62 (t, *J* = 7.8 Hz, 2H), 7.74 (s, 1H), 7.73 (t, *J* = 7.8 Hz, 1H), 7.93 (t, *J* = 7.8 Hz, 2H), 8.06 (d, *J* = 7.8 Hz, 2H), 8.65 (s, 1H), 9.09 (s, 1H); **^{13}C NMR** (100 MHz, DMSO-d_6): δ 20.23, 34.06, 55.46, 100.92, 101.78, 112.20, 114.20, 114.39, 119.29, 124.70, 124.72, 130.00, 134.13, 137.93, 147.85, 153.07, 157.60, 158.34, 162.34, 164.26, 167.05, 175.34; **ESI-MS**: *m/z* 459 $(M + 1)^+$; Anal. Calcd. For $C_{26}H_{18}N_6O_5$; C, 63.16; H, 3.67; N, 17.00; Found: C, 63.47; H, 3.62; N, 17.28.

5-(3-Nitrophenyl)-4-(phenylamino)-5H-pyrimido[4',5':3,4]pyrazolo[1,2-b]phthalazine-7,12-dione (4az). Yellow powder; mp: 346-348 °C; **IR** (KBr) υ_{max} (cm^{-1}): 3586, 2935, 1718, 1658, 1258; **^{1}H NMR** (400 MHz, DMSO-d_6): δ 1.88 (s, 3H), 6.45 (s, 1H), 7.25 (d, *J* = 7.8 Hz, 2H), 7.47 (m, 2H), 7.62 (t, *J* = 7.8 Hz, 2H), 7.75 (d, *J* = 7.8 Hz, 1H), 7.93 (t, *J* = 7.8 Hz, 2H), 8.02 (d, *J* = 7.8 Hz, 2H), 8.12 (d, *J* = 7.8 Hz, 2H), 8.16 (s, 1H), 8.59 (s, 1H); **^{13}C NMR** (100 MHz, DMSO-d_6): δ 21.01, 54.99, 113.96, 124.97, 125.83, 126.82, 127.58, 128.39, 129.58, 130.48, 132.58, 133.29, 152.09, 154.21, 155.35, 156.99, 157.58, 158.89, 159.89, 161.85, 163.39; **ESI-MS**: *m/z* 464 $(M + 1)^+$; Anal. Calcd. For $C_{25}H_{16}N_6O_4$; C, 64.65; H, 3.47; N, 18.10; Found: C, 64.89; H, 3.42; N, 18.43.

4-((4-Chlorophenyl)amino)-5-(3-nitrophenyl)-5H-pyrimido[4',5':3,4]pyrazolo[1,2-b]phthalazine-7,12-dione.(ba) Yellow powder; mp: 389-391 °C; **IR** (KBr) υ_{max} (cm^{-1}): 3353, 2969, 1713, 1656, 1288; **^{1}H NMR** (400 MHz, DMSO-d_6): δ 5.52 (s, 1H), 7.41 (d, *J* = 7.8 Hz, 2H), 7.58 (d, *J* = 7.8 Hz, 2H), 7.67 (t, *J* = 7.8 Hz, 1H), 7.86 (d, *J* = 7.8 Hz, 2H), 7.96 (d, *J* = 7.8 Hz, 1H), 8.13 (d, *J* = 7.8 Hz, 2H), 8.15 (d, *J* = 7.8 Hz, 2H) 8.72 (s, 1H), 9.06 (s, 1H); **^{13}C NMR** (100 MHz, DMSO-d_6): δ 55.85, 115.58, 123.58, 127.58, 128.49, 129.58, 132.25, 132.25, 134.28, 151.34, 154.28, 156.58, 159.85, 161.28, 164.58, 165.28; **ESI-MS**: *m/z* 499

$(M + 1)^+$; Anal. Calcd. For $C_{25}H_{15}ClN_6O_4$; C, 66.74; H, 3.88; N, 14.97; Found: C, 66.96; H, 3.83; N, 14.75.

5-(3-Nitrophenyl)-4-(p-tolylamino)-5H-pyrimido[4',5':3,4]pyrazolo[1,2-b]phthalazine-7,12-dione (4bb). Yellow powder; mp: 374-376 °C; **IR** (KBr) υ_{max} (cm^{-1}): 3358, 3036, 1717, 1688, 1256; **^{1}H NMR** (400 MHz, DMSO-d_6): δ 1.84 (s, 3H), 6.38 (s, 1H), 7.28 (d, *J* = 7.8 Hz, 2H), 7.57 (d, *J* = 7.8 Hz, 2H), 7.72 (t, *J* = 7.8 Hz, 2H), 7.94 (t, *J* = 7.8 Hz, 1H), 7.97 (t, *J* = 7.8 Hz, 2H), 8.13 (d, *J* = 7.8 Hz, 1H), 8.26 (d, *J* = 7.8 Hz, 2H), 8.62 (s, 1H), 8.84 (s, 1H); **^{13}C NMR** (100 MHz, DMSO-d_6): δ 56.58, 113.96, 122.28, 124.58, 125.45, 126.29, 127.58, 128.53, 132., 133.63, 152.09, 154.26, 156.58, 159.85, 160.55, 162.28, 163.28, 164.13; **ESI-MS**: *m/z* 479 $(M + 1)^+$; Anal. Calcd. For $C_{26}H_{18}N_6O_4$; C, 65.27; H, 3.79; N, 17.56; Found: C, 65.61; H, 3.73; N, 17.73.

4-((4-Methoxyphenyl)amino)-5-(3-nitrophenyl)-5H-pyrimido[4',5':3,4]pyrazolo[1,2-b]phthalazine-7,12-dione (4bc). Yellow powder; mp: 335-337 °C; **IR** (KBr) υ_{max} (cm^{-1}): 3358, 3055, 1720, 1658, 1258; **^{1}H NMR** (400 MHz, DMSO-d_6): δ 3.66 (s, 3H), 6.59 (s, 1H), 7.55 (d, *J* = 7.8 Hz, 2H), 7.58-7.78 (m, 2H), 7.82 (t, *J* = 7.8 Hz, 2H), 7.68 (d, *J* = 7.8 Hz, 1H), 7.91 (d, *J* = 7.8 Hz, 2H), 8.16 (d, *J* = 7.8 Hz, 1H), 8.24 (d, *J* = 7.8 Hz, 2H), 8.51 (s, 1H), 8.87 (s, 1H); **^{13}C NMR** (100 MHz, DMSO-d_6): δ 58.50, 114.58, 125.58, 126.58, 127.58, 128.82, 131.93, 152.35, 154.28, 157.25, 160.25, 162.58, 164.28, 165.28; **ESI-MS**: *m/z* 495 $(M + 1)^+$; Anal. Calcd. For $C_{26}H_{18}N_6O_5$; C, 63.16; H, 3.67; N, 17.00; Found: C, 63.38; H, 3.61; N, 17.26.

5-(3-Nitrophenyl)-4-((3-nitrophenyl)amino)-5H-pyrimido[4',5':3,4]pyrazolo[1,2-b]phthalazine-7,12-dione (4bd). Yellow powder; mp: 362-364 °C; **IR** (KBr) υ_{max} (cm^{-1}): 3358, 3058, 1715, 1658, 1256; **^{1}H NMR** (400 MHz, DMSO-d_6): δ 6.56 (s, 1H), 7.28 (d, *J* = 7.8 Hz, 2H), 7.61 (d, *J* = 7.8 Hz, 2H), 7.52 (t, *J* = 7.8 Hz, 2H), 7.83 (d, *J* = 7.8 Hz, 2H), 7.93 (t, *J* = 7.8 Hz, 1H), 8.02 (d, *J* = 7.8 Hz, 2H), 8.12 (d, *J* = 7.8 Hz, 1H), 8.54 (s, 1H), 8.65 (s, 1H); **^{13}C NMR** (100 MHz, DMSO-d_6): δ 54.55, 117.58, 124.28, 125.86, 126.56, 127.28, 128.58, 130.28, 132.28, 150.34, 152.09, 153.56, 155.58, 158.28, 159.85, 161.04, 162.65, 163.68, 165.04; **ESI-MS**: *m/z* 510 $(M + 1)^+$; Anal. Calcd. For $C_{25}H_{15}N_7O_6$; C, 58.94; H, 2.97; N, 19.25; Found: C, 58.71; H, 2.92; N, 19.54.

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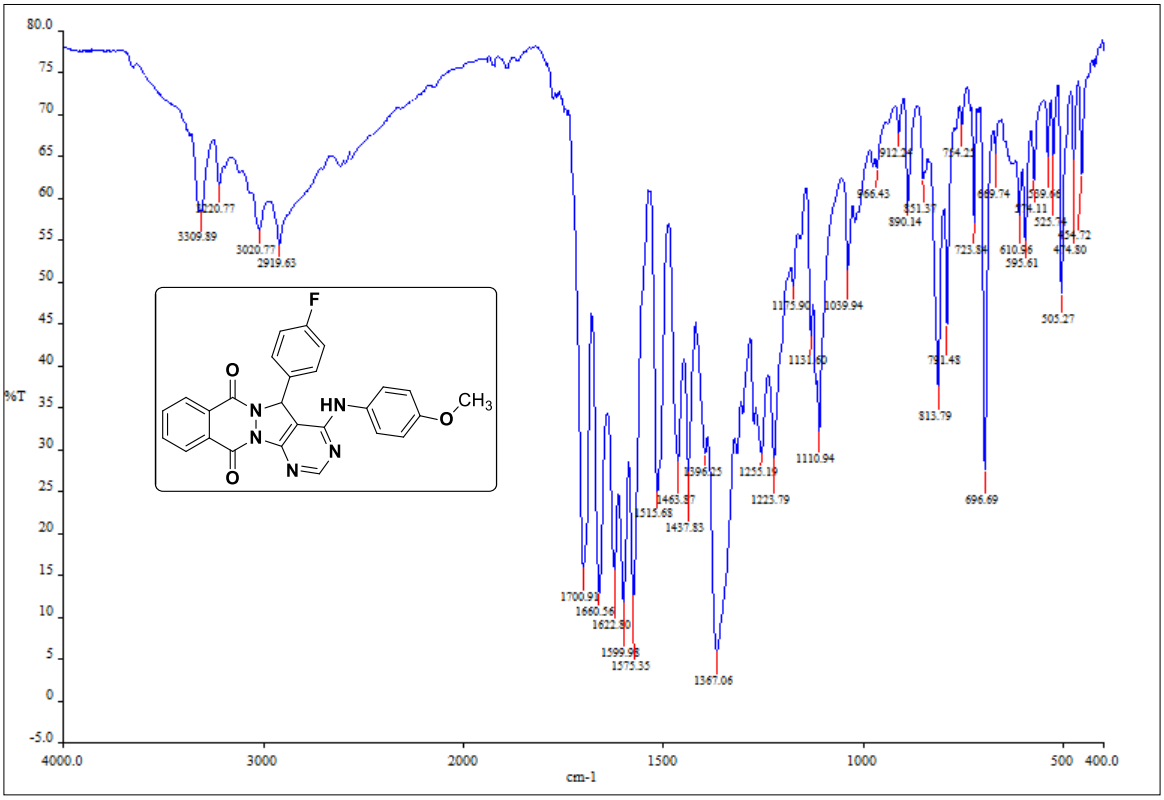
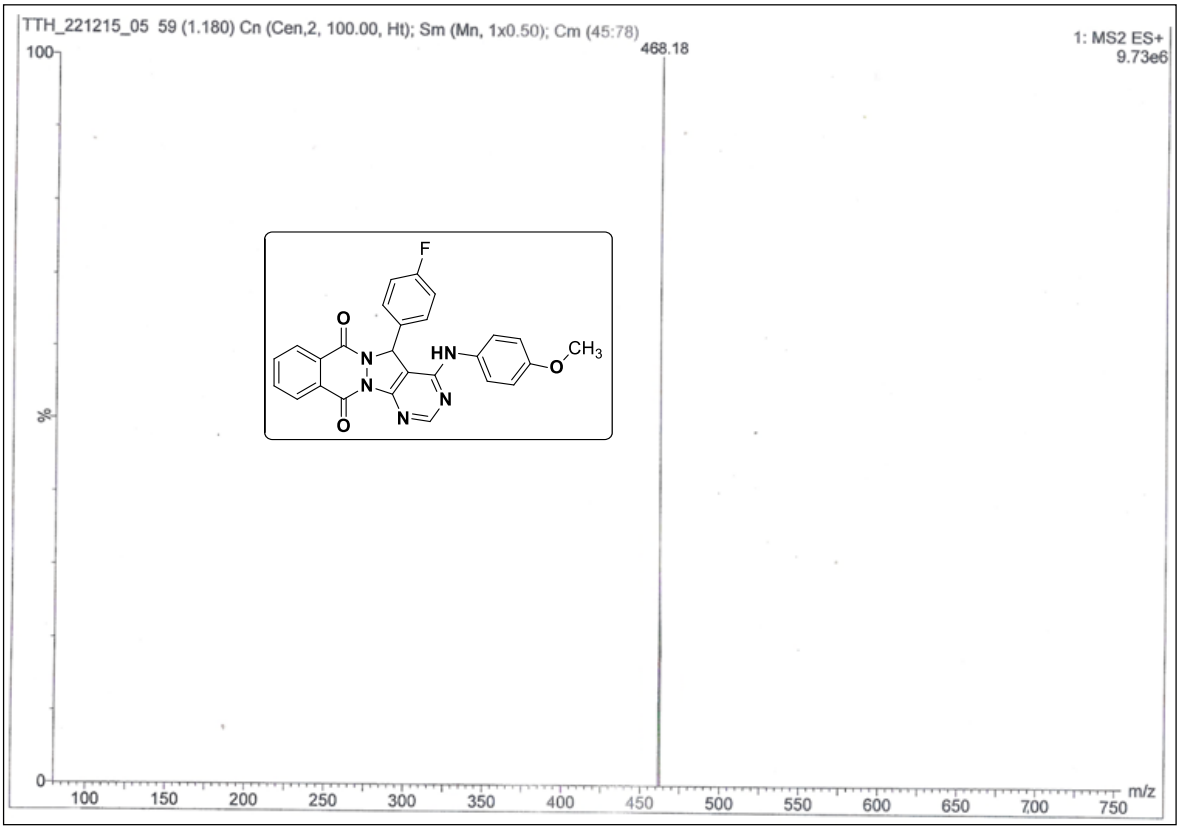
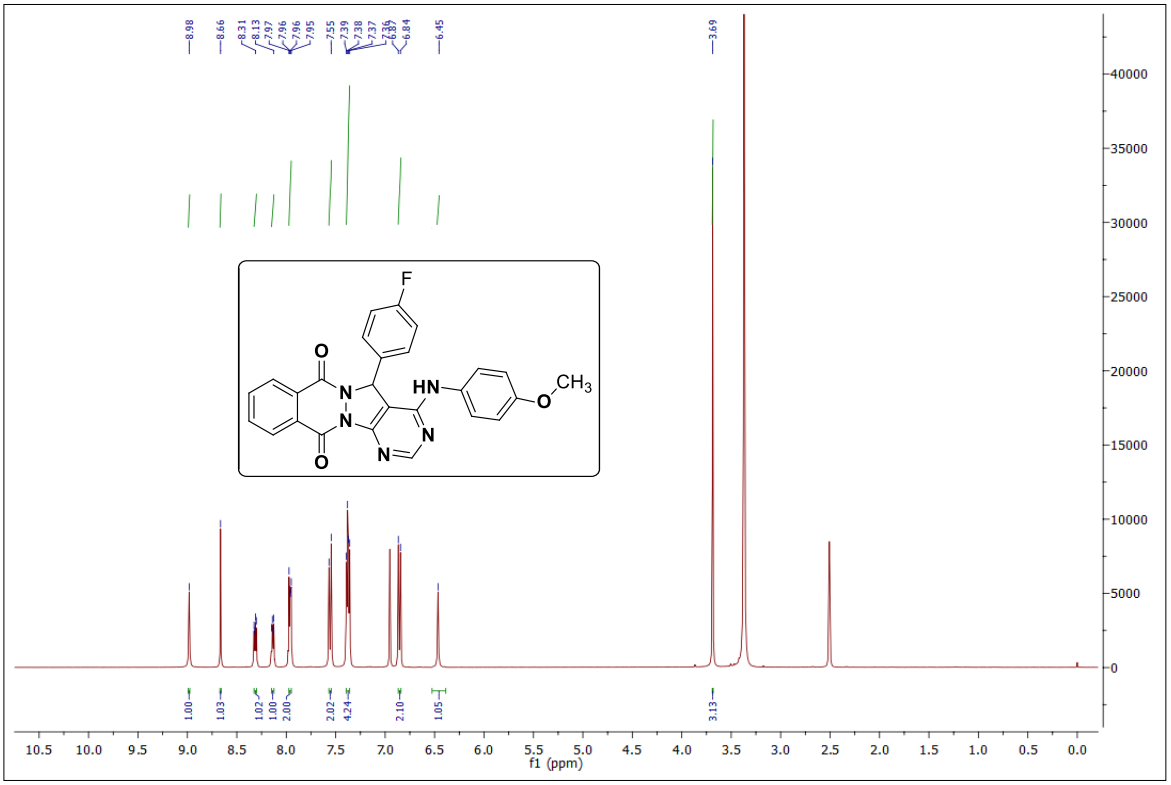


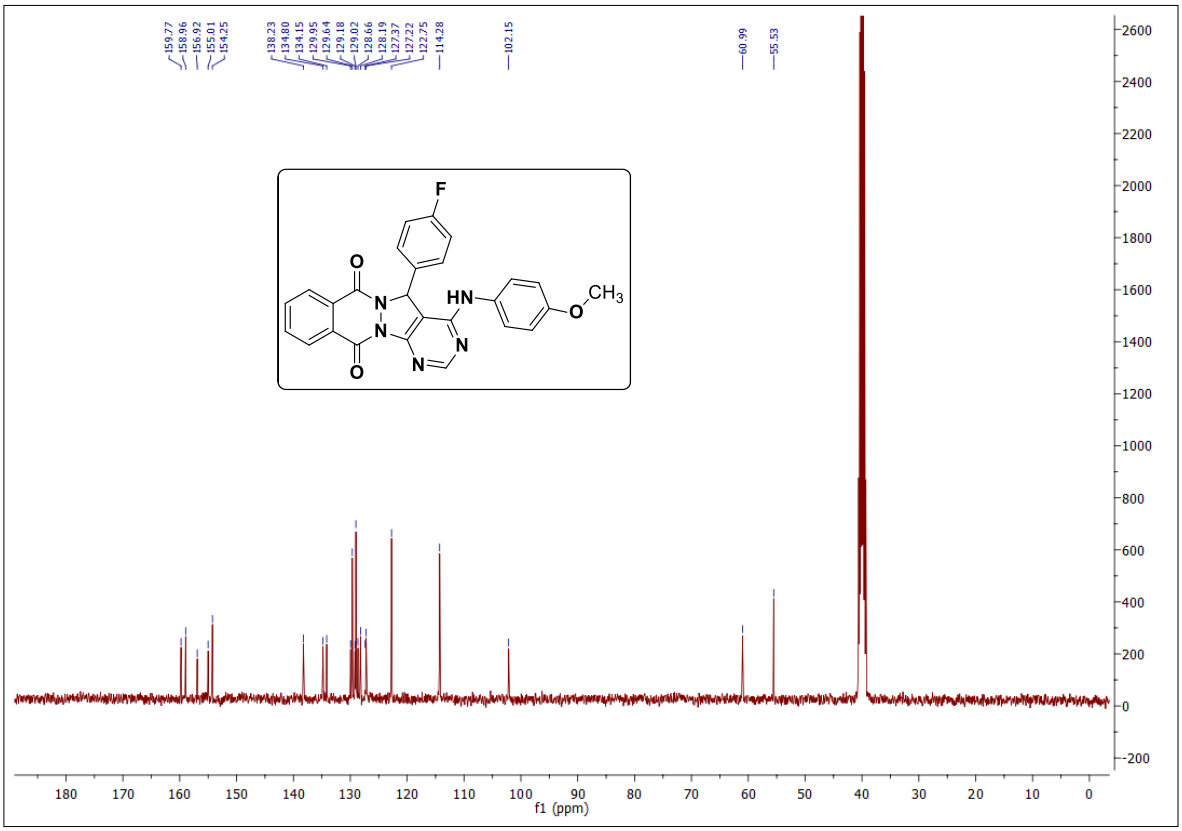
IR Spectrum of compound **4an**



Mass Spectrum of compound **4an**



^{1}H NMR Spectrum of compound **4an**



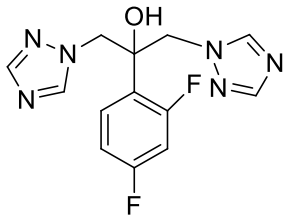
^{13}C NMR Spectrum of compound **4an**

EVOLUTION OF BIOLOGICAL ACTIVITY

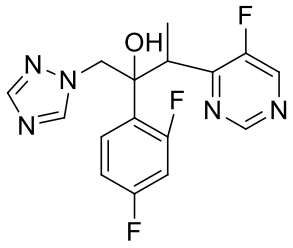
7. 1. INTRODUCTION

Biological activity of compounds represents pharmacological effects, physiological and biochemical mechanism by compound's interaction with biological system [1-2] In the recent years, antimicrobial resistance has gained renewed interest globally and has been a serious public health concern resulting in the incidence of various drugs-resistant microbial infections, such as community acquired infections like streptococcal infections, pneumonia, etc., or hospital-acquired infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin resistant enterococci (VRE) or extended spectrum beta-lactamase (BSLE) enzyme producing Gram-negative bacteria and azole-resistant *Candida* species.[3-4]

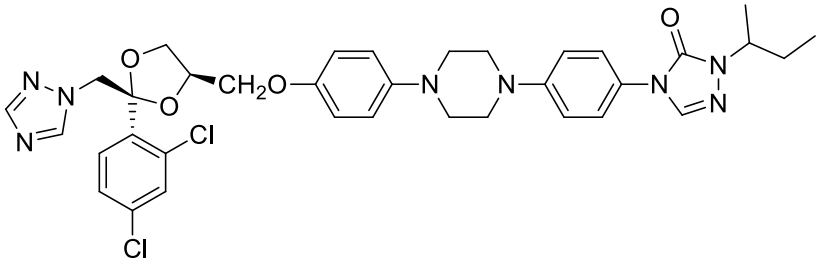
Eventually, the incidence of systemic fungal infections have increased significantly over the past three decades due to an increase in the number of immunocompromised hosts.[5-8] Fungal infections pose a continuous and serious threat to human health and life especially to immunocompromised patients.[9-14] Currently, fluconazole, itraconazole, and voriconazole (**Fig. 1**) are the most frequently used antifungals in the clinic.[15-16] These facts indicated interest is us to search for novel lead compounds with greater structural specificity towards fungal enzymes.



Fluconazole



Itraconazole



Voriconazole

Figure 1. Antifungal agents used in clinical therapy.

One of the internally manifested complaints in mankind is diabetes. Type 2 diabetes mellitus is the most common form of diabetes mellitus in which the body does not use insulin properly and the condition is called as insulin resistance. Improper use of insulin triggered by insulin resistance leads to abnormally high blood glucose levels called as hyper glycaemia. In human system, four enzymes namely salivary α-amylase, pancreatic α-amylase, α-glucosidase (also called as maltase–glucoamylase) and sucrase-isomaltase are known for the thorough breakdown of complex carbohydrates like starch into glucose, which is then absorbed into the blood stream by a specific transport system.[17-18]

From the literature it was observed that heterocycles containing pyrimidine ring system exhibit a wide spectrum of biological activities.[19-30] Among the pyrimidine and its derivatives have gained remarkable importance due to their widespread biological activities and their use in synthetic chemistry.[31-34]

7. 2. PRESENT WORK

The importance of biological applications of heterocyclic molecules attracted us to synthesize and to evaluate biological activities. Therefore, some of the newly synthesized compounds in the present program the synthesized compounds were screened for their antimicrobial (antibacterial, antifungal and antibiofilm) and antidibetic activities.

In the present study, some of the newly synthesized compounds which have already described in the earlier chapters (chapter II, IIIA, IIIB, IV, V and VI) were evaluated for antibacterial, antifungal, antibiofilm and antidibetic activities.

7.3. BIOLOGICAL PROTOCOL

The antibacterial activity of the synthesized compounds was tested against the gram-positive bacteria such as *Bacillus subtilis*, *Staphylococcus aureus*, and the gram-negative bacteria such as *Pseudomonas aeruginosa*, *Escherichia coli* using nutrient agar medium. The antifungal activity of the compounds was tested against *Candida albicans* and *Aspergillus niger* using Potato dextrose agar medium (PDA). The minimum inhibitory concentration (MIC) was carried out using microdilution susceptibility method.[35] Ciprofloxacin and fluconazole were used as standards in antibacterial and antifungal evaluation respectively. All the synthesized compounds

were evaluated for various biological activities such as antibacterial, minimum bactericidal concentration (MBC), anti-biofilm, antifungal, minimum fungicidal concentration (MFC), inhibition of ergosterol biosynthesis, reactive oxygen species (ROS) and protein leakage studies.

(a) *Antibacterial activity*

The antibacterial activity of the all the synthesized compounds was determined using well diffusion method[35] against different pathogenic bacterial strains procured from the Microbial Type Culture Collection and Gene Bank (MTCC), at CSIR-Institute of Microbial Technology, Chandigarh, India. The pathogenic reference strains were seeded on the surface of the Muller-Hinton agar Petri plates with 0.1 ml of previously prepared microbial suspensions individually containing 1.5×10^8cfu ml^{-1} (equal to 0.5 McFarland standard). Wells of 6.0 mm diameter were prepared in the media plates using a cork borer and the synthesized compounds were dissolved in 10% DMSO and at a dose range of 125 - 0.97 µg/mL they were added in each well under sterile conditions in a laminar air flow chamber. Standard antibiotic solutions of Ciprofloxacin at a dose range of 125 - 0.97 µg $well^{-1}$, served as positive control, while the well containing DMSO served as negative control. The plates were incubated for 24 h at 37 °C for the different bacterial strains. The well containing the least concentration showing the inhibition zone is considered as the minimum inhibitory concentration. All the experiments were carried out in duplicates and mean values are represented.

(b) *Minimum bactericidal concentration (MBC) assay*

Minimum bactericidal concentrationassay[36] was performed in sterile 2.0 mL microfuge tubes against a panel of pathogenic bacterial strains, including *Micrococcus luteus* MTCC 2470, *Staphylococcus aureus* MTCC 96, *Staphylococcus aureus* MLS-16 MTCC 2940, *Bacillus subtilis* MTCC 121, *Escherichia coli* MTCC 739, *Pseudomonas aeruginosa* MTCC 2453 and *Klebsiellaplanticola* MTCC 530, cultured overnight in Mueller Hinton broth. Serial dilutions of test compounds at different concentrations ranging from 0 to 125 µg mL^{-1} were prepared in Mueller Hinton broth. To the test compounds, 100 µL of overnight cultured bacterial suspensions were added to reach a final concentration of 1.5×10^8cfu mL^{-1} (equal to 0.5 McFarland standard) and incubated at 37 ºC for 24 h. After 24 h of incubation, the minimum bactericidal concentration (MBC) was determined by sampling 10 µL of suspension from the tubes onto Mueller Hinton agar plates and were incubated for 24 h at 37 ºC to observe the growth of test

organisms. MBC is the lowest concentration of test compound required to kill a particular bacterium strain. All the experiments were carried in duplicates and mean values are represented.

(c) *Biofilm inhibition assay*

The test compounds were screened in sterile 96 well polystyrene microtiter plates using the modified biofilm inhibition assay[37], against a panel of pathogenic bacterial strains including*Staphylococcus aureus* MTCC 96, *Staphylococcus aureus* MLS16 MTCC 2940, *Bacillus subtilis* MTCC121, *Pseudomonas aeruginosa* MTCC 2453, and *Klebsiellaplanticola* MTCC 530, which were cultured overnight in tryptone soy broth (supplemented with 0.5% glucose). The test compounds of predetermined concentrations ranging from 0 to 250 µg mL^{-1} were mixed with the bacterial suspensions having an initial inoculum concentration of 5×10^5 cfu mL^{-1}. Aliquots of 100 µL were distributed in each well and then incubated at 37 ºC for 24 h under static conditions. The medium was then discarded and washed with phosphate buffered saline to remove the non-adherent bacteria. Each well of the microtiter plate was stained with 100 µL of 0.1% crystal violet solution followed by 30 min incubation at room temperature. Later, the crystal violet solution from the plates was discarded, thoroughly washed with distilled water for 3 to 4 times and air dried at room temperature. The crystal violet stained biofilm was solubilized in 95% ethanol (100 µL) and the absorbance was recorded at 540 nm using TRIAD multimode reader (Dynex Technologies, Inc, Chantilly, VA, USA). Blank wells were employed as background check. The inhibition data were interpreted from the dose-response curves, where IC_{50} value is defined as the concentration of inhibitor required to inhibit 50% of biofilm formation under the above assay conditions. All the experiments were carried out in triplicates and the values are indicated as mean ± S.D.

(d) *Accumulation of Intracellular reactive oxygen species (ROS)*

The fluorometric assay using 2’’-7’-dichlorofluorescein diacetate (DCFH-DA) was used to measure the accumulation of ROS.[38] Briefly, the *Staphylococcus aureus* MLS16 (MTCC 2940) strain was cultured in 24-well microtitre plates. After biofilm formation, the mature biofilms were washed with 500 µL of 0.9% (w/v) NaCl and treated with different concentrations of compounds along with ciprofloxacin and incubated for 24 h at 35 °C. Later, the biofilms were incubated with 10 µM DCFH-DA for 24 h and the fluorescence was measured on an Infinite M200Pro (Tecan Trading AG, Switzerland) microtitre plate reader at an excitation and emission

wavelengths of 485 and 535 nm, respectively. In each biofilm the levels of ROS was quantified in duplicate. The entire content in each well was removed and the cells were separated from the supernatant by centrifugation at 4000 × g for 8 min. The fluorescence of the supernatant and of the resuspended sessile cells in PBS was measured separately to determine the generated fluorescence in either intracellular or extracellular environment.

(e) ***Antifungal activity***

The antifungal activity of the synthesized compounds was determined using well diffusion method[35] against different *Candida* strains such as *Candida albicans* MTCC 183, *C. albicans* MTCC 227, *C. albicans* MTCC 854, *C. albicans* MTCC 1637, *C. albicans* MTCC 3017, *C. albicans* MTCC 3018, *C. albicans* MTCC 3958, *C. albicans* MTCC 4748, *C. albicans*MTCC 7315, *C. parapsilosis* MTCC 1744, *C. aaseri*MTCC 1962,*C. glabrata*MTCC 3019, *C. krusei* MTCC 3020 and*Issatchenikahanoiensis* MTCC 4755 procured from the Microbial Type Culture Collection and Gene Bank (MTCC), CSIR-Institute of Microbial Technology, Chandigarh, India. The pathogenic reference strains were seeded on the surface of the Muller-Hinton agar Petri plates with 0.1 ml of previously prepared microbial suspensions individually containing 1.5 × 10^8cfu ml^{-1} (equal to 0.5 McFarland standard). Wells of 6.0 mm diameter were prepared in the media plates using a cork borer and the synthesized compounds dissolved in 10% DMSO at a dose range of 125 - 0.97 µg/mL were added in each well under sterile conditions in a laminar air flow chamber. Standard antibiotic solutions of Miconazole at a dose range of 125 - 0.97 µg $well^{-1}$, served as positive control, while the well containing DMSO served as negative control. The plates were incubated for 24 h at 30 °C for different *Candida* strains. The well containing the least concentration showing the inhibition zone is considered as the minimum inhibitory concentration. All the experiments were carried out in duplicates and mean values are represented.

(f) ***Minimum fungicidal concentration (MFC) assay***

Fungicidal assays were performed in sterile 2.0 ml microfuge tubes. Different *Candida* strains such as *Candida albicans* MTCC 183, *C. albicans* MTCC 227, *C. albicans* MTCC 854, *C. albicans* MTCC 1637, *C. albicans* MTCC 3017, *C. albicans* MTCC 3018, *C. albicans* MTCC 3958, *C. albicans* MTCC 4748, *C. albicans*MTCC 7315, *C. parapsilosis* MTCC 1744, *C. aaseri*MTCC 1962,*C. glabrata*MTCC 3019, *C. krusei* MTCC 3020 and*Issatchenikahanoiensis*

MTCC 4755, were cultured overnight in Sabouraud dextrose broth. Serial dilutions of test compounds in different concentrations ranging from 0 to 150 µg mL^{-1} were prepared in Sabouraud dextrose broth. To the test compounds, 100 µL of overnight cultured bacterial suspensions were added to reach a final concentration of 1.5×10^8cfu mL^{-1} (equal to 0.5 McFarland standard) and incubated at 30 ºC for 24 h. After 24 h incubation, the MFC was determined by sampling 10 µL of suspension from the tubes onto fresh plates of Sabouraud dextrose agar to observe the growth of the fungi. The plates were incubated for 24 h at 30 ºC. All the experiments were carried in the duplicates and mean values are represented, where MFC is the lowest concentration of compound required to kill a particular *Candida* strain.

(g) *Quantification of ergosterol content in* Candida albicans*MTCC 1637*

The total intracellular sterols from *Candidaalbicans*MTCC 1637 were extracted using the method of Breivik and Owades[39] with slight modifications. A single *C. albicans*colony cultured overnight in Sabouraud dextrose agar was inoculated with 50 ml of Sabouraud dextrose broth containing varying concentrations of the test compounds, including 0, 2, 4 and 16 µg mL^{-1}. The culture was incubated at 30 °C for 20 h with continuous shaking. The stationary phase cells were harvested by centrifugation at 8,000 rpm for 5 min and washed with sterile distilled water. The net wet weight of the cell pellet was determined. Three milliliters of 25% alcoholic potassium hydroxide solution were added to each pellet and vortexed for 1 min. The cell suspensions were transferred to sterile glass screw-cap tubes and were incubated in a water bath at 85 ºC for 1 h and then allowed to cool to room temperature. Sterols were then extracted by the addition of a mixture of sterile distilled water and *n*-heptane (1:3) followed by vigorous vortexing for 3 to 4 min. The heptane layer was transferred to a clean glass tube and stored at -20 °C for 24 h duration. An aliquot (20 µL) of the sterol extract diluted five-fold in 100% ethanol was scanned spectrophotometrically from 240 to 300 nm. The presence of ergosterol and 24(28)dehydroergosterol [24(28)DHE, a late sterol pathway intermediate] in the extracted sample exhibited a characteristic four-peaked curve. The absence of detectable ergosterol content in the extracts was indicated by a flat line. A dose-dependent decrease in the height of the absorbance peaks was evident which corresponded to the decreased ergosterol concentration. Ergosterol content was calculated as a percentage of the wet weight of the cell using the following equations:

$$\% \text{ Ergosterol} + \% \text{ 24(28) DHE} = [(A281.5/ 290) \times F]/\text{pellet weight}$$

% 24(28) DHE = [(*A*230/518) × *F*]/pellet weight, and

% Ergosterol = [% Ergosterol + %24 (28) DHE] - %24 (28) DHE,

Where *F* is the factor for dilution in ethanol and 290 and 518 are the *E* values (in percentages per centimeter) determined for crystalline ergosterol and 24 (28) DHE, respectively.

(h) *Protein leakage assay:*

The leakage of cellular material is one of the plausible mechanisms for the bacterial cell death.[40] The amount of protein leakage was assessed in *Micrococcus luteus* MTCC 2470 by using Bradford protein assay.[1] The bacterial cells (1.5×10^5 CFU/ml) were incubated with different concentrations of test compounds. Later, the cells were incubated in a shaking incubator at 37 ºC for 24 h. After incubation, 1 ml of culture sample was centrifuged at 4 ºC for 20 min at 1200 × g, and the supernatant was frozen at -20 ºC[2]. The supernatant was treated with Bradford reagent, and the O.D. was measured at 595 nm. The results were expressed as mean of three independent experiments.

(g) α-amylase assay

For determining activity of α-amylase, 400 µL of 0.5% (w/v) starch solution was added to 100 µL α-amylase, the substrate-enzyme mix was incubated at 37 ºC for 10 min, 250 µL of colouring reagent (sodium potassium tartrate and *Dinitrosalicylic acid*) was added and the mixture was boiled at 70 ºC upon a water bath for 15 min. Later 2.5 mL of deionised water was added to the assay mixture followed by measuring absorbance at 540 nm. For inhibition studies, enzyme was incubated with the synthesiszed compounds and acarbose (standard) for 30 min. before adding the substrate solution.[41]

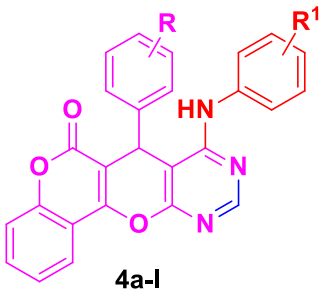
(h) α-glucosidase assay

The glycolytic activity of the enzyme was measured using 4-Nitrophenyl-α-D-Glucopyranoside (4-NGP) as substrate by the method described previously. A reaction mixture containing 25 µL of α-glucosidase (2 mg dissolved in 1 mL of 10 mM Tris-HCl at pH 7.0) and 25 µL of 4-NGP (substrate stock solution), 950 µL deionised H_2O were incubated at 37 °C for 10 min. The activity was determined by measuring the absorbance of the liberated 4-nitrophenol against an enzyme-free blank at 410 nm. For inhibition studies enzyme was incubated with synthesized compounds and acarbose (standard) for 30 min, before adding substrate solution. 1U

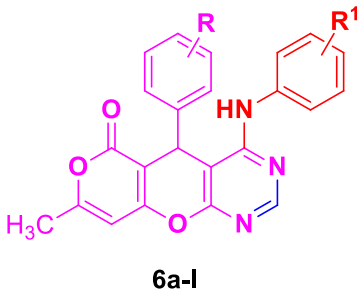
of glycolytic activity was defined as the amount of enzyme needed to release 1 µM of 4-nitrophenol per min, under standard assay conditions.[42]

7.4. RESULTS AND DISCUSSION

PYRANO[2,3-*D*]PYRIMIDINES (CHAPTER II)



4a-l



6a-l

(a) ***Antibacterial activity***

All the synthesized compounds (**4a-p** and **6a-l**) were screened for antibacterial activity *in vitro* against different Gram-positive and Gram-negative bacterial strains such as *Micrococcus luteus*MTCC 2470, *Staphylococcus aureus* MTCC 96, *Staphylococcus aureus* MLS-16 MTCC 2940, *Bacillus subtilis* MTCC 121, *Escherichia coli* MTCC 739, *Pseudomonas aeruginosa* MTCC 2453 and *Klebsiellaplanticola* MTCC 530. Among all the derivatives screened, compound **6l** showed promising activity (MIC values ranging between 3.9-15.6 µg/mL) against all the bacterial strains except *Pseudomonas aeruginosa* MTCC 2453; however, the compound **6h, 6i** and **4i** exhibited promising activity (MIC value 7.8 µg/mL and 12.6 µg/mL) specifically towards *Bacillus subtilis* MTCC 121and *Staphylococcus aureus*MTCC 96, respectively. Based on the structure-activity relationship of the synthesized derivatives, it was observed that the compound **6l** has a methoxy substituent attached to the basic pyranopyrimidine scaffold, which has an electron donating property which probably may be contributing to the antibacterial activity. In case of compound **6h**, a nitro substituent is attached to the basic pyranopyrimidine scaffold, while compound **6i** has a simple hydrogen atom attached to the basic pyranopyrimidine scaffold and **4i** has a simple hydrogen atom attached to the basic chromenopyrano[2,3-*d*]pyrimidine scaffold. The antibacterial activity results in this regard are tabulated in (**Table 1)**.

(b) ***Minimum bactericidal concentration (MBC)***

Based on the antibacterial activity results, the compounds **4a-4p** and **6a-6l** were screened for the minimum bactericidal concentration against all the bacterial strains except *Pseudomonas aeruginosa* MTCC 2453 in comparison to ciprofloxacin as standard. Compound **4i**, **4j** and **6l** consistently showed promising minimum bactericidal concentration, activity against all the tested bacterial strains. The activity data in this regard are shown in (**Table 2)**.

(c) ***Biofilm inhibition assay***

A biofilm is a structured consortium of bacteria embedded in a self-produced polymeric matrix consisting of polysaccharides, protein and DNA. Bacterial biofilms cause chronic infections in humans *via* hospital and community environments since they show increased tolerance to antibiotics and disinfectant chemicals as well as resisting phagocytosis and other components of the body's defence system. In the medical sector, bacteria colonizes through adhesion mechanism and result in biofilm formation on several biomedical implants such as stents, heart valves, vascular grafts and catheters. In this context, the novel compounds that can specifically target and inhibit the biofilm formation would be of great interest in comparison to the rational use of antibiotics and/or biocides. Considering these facts, a further step was undertaken to investigate whether these compounds exhibit a specific anti-biofilm activity or whether this observation was simply related to a general toxic effect on the Gram-positive bacterial strains. To this regard, the compounds **4a-4p** and **6a-6l** were screened for anti-biofilm activity against *Micrococcus luteus* MTCC 2470, *Staphylococcus aureus* MTCC 96, *Staphylococcus aureus* MLS16 MTCC 2940, *Bacillus subtilis* MTCC 121, *Escherichia coli* and *Klebsiellaplanticola* MTCC 530 which is common and important nosocomial pathogens having biofilm forming ability. The results summarized in **Table 3**, clearly reveal that not much information on the structure-activity relationship (SAR) can be highlighted at this stage; however, it was observed that compounds **6l** exhibited promising activity (IC_{50} values ranging between 2.5 – 11.5 μM) towards all the tested bacterial species, while compound **6i** showed specific activity towards *Micrococcus luteus* MTCC 2470, *Staphylococcus aureus* MLS16 MTCC 2940 and *Bacillus subtilis* MTCC 121 and compound **6f** showed specific activity towards *Micrococcus luteus* MTCC 2470, *Staphylococcus aureus* MTCC 96 and *Staphylococcus aureus* MLS16 MTCC 2940. Some of the compounds (**4i, 4j, 6g, 6h,** and **6j**) showed anti-biofilm

activity specifically towards *Staphylococcus aureus* MLS16 MTCC 2940 with IC_{50} values of 7.1, 8.1, 9.8, 8.6 and 4.9 μM, respectively. The basic chromenopyrano[2,3-*d*]pyrimidine and pyranopyrimidine scaffold of these compounds possesses different substituent which exhibit electron donating or electron withdrawing properties which antagonize the biofilm formation and probably may be contributing to the anti-biofilm activity. The activity data in this regard is shown in (**Table 3**).

(d) ***Accumulation of intracellular ROS in*** **Staphylococcus aureus** ***MTCC 96***

In order to elucidate whether oxidative stress was involved in the apoptotic cell death, the intracellular ROS accumulation within the cells of mature biofilms was measured using the fluorescent probe 2',7'-dichlorofluorescein-diacetate (DCFH-DA), a general ROS fluorescent probe. The DCFH-DA dye conversion mainly depends on the metabolically active cells in the biofilm. DCFH-DA is deacetylated in the cells where it can react quantitatively with intracellular radicals (mainly H_2O_2) to get converted to its fluorescent product (DCF), which is retained within the cells and thus provides an index of oxidation in the cell cytosol. In the present study, the accumulation of intracellular reactive oxygen species (ROS) in the mature biofilms of *Staphylococcus aureus* MTCC 96 culture was measured using DCFH-DA dye. The accumulation of intracellular ROS plays a major role in apoptotic mediated cell death. After treatment with compound **4i**, a significantly increased ROS levels were accumulated in the tested *Staphylococcus aureus* strain (**Fig 2**). At a concentration of 6 μg/mL, the compound **4i** treated biofilms showed increased levels of intracellular ROS accumulation which was closer to the standard drug ciprofloxacin, while at a concentration of 8 μg/mL, the intracellular ROS accumulation was equal to the standard drug ciprofloxacin. Some of the bactericidal drugs stimulate free radical formation via the Fenton reaction. Recent studies suggest that when bacteria is under oxidative stress, these free radicals contribute to arrest in cell growth or result in damage of the specific essential metabolic enzymes (cellular respiratory chain), disrupting cellular membrane, and DNA damage ultimately causing cell lysis and death.

(e) ***Antifungal activity***

Different *Candida* species are important opportunistic fungal pathogens and they frequently cause infections within immunocompromised patients undergoing cancer chemotherapy, broad-spectrum antibiotics and/or among HIV-infected individuals. Among the

many pathogenic *Candida* species, *Candida albicans* is the major fungal pathogen of utmost importance to humans. Due to its versatility, it can behave as a commensal organism posing a major problem from a clinical perspective resulting in chronic infections. Further, different *Candida* strains have the ability to produce extracellular polymeric substances (EPS) and get encased in this matrix to form biofilms, which are known to develop on the surfaces of prosthesis and medical devices. Considering these above facts, we screened, selected compounds such as **6a, 6b, 6c, 6d, 6h** and **6l** against different *Candida* strains and among them, the compounds **6l**and **6h** showed promising anti-*Candida* activity against many *Candida* strains with a MIC value of 7.8 μg/mealcomparable to the standard miconazole drug. While, the other compounds showed good to moderate activity (MIC values ranging between 7.8 – 62.5 μg/mL) against different *Candida* strains. The results of the antifungalactivity are tabulated in (**Table 4**).

(f) ***Minimum fungicidal concentration (MFC)***

Based on the antifungal activity results, the selected compounds **6a, 6b, 6c, 6d, 6h** and **6l** were further evaluated for minimum fungicidal concentrations (MFC) against different *Candida* strainsin comparison to the standard miconazole drug. All the compounds showed minimum fungicidal concentration (MFC) values ranging between 7.8 – 62.5 μg/mL. However, the standard miconazole drug exhibited MFC values ranging between 7.8 – 15.6 μg/mL.Among them, the compound **6l** proved promising against *Candida albicans* MTCC 1637 and *C. albicans*MTCC 4748 with a lower MFC value of 7.8 μg/mL. The MFC activity data in this regard is tabulated in (**Table 5**).

(g) ***Inhibition of ergosterol biosynthesis in*** **Candidaalbicans*MTCC 1637***

*Candida albicans*is now recognized as a major cause of hospital-acquired infections. Most of the antifungal drugs currently available to treat *Candida* infections target the ergosterol biosynthetic pathway or its end product ergosterol. In view of this fact, we further investigated the promising test compounds **6l** in comparison to the standard miconazole drugto delineate its mode of action in the ergosterol biosynthetic pathway for one of the susceptible strain of *C. albicans*MTCC 1637. In this regard, the UV spectral scans of the sterol profiles for the representative strain of *C. albicans*MTCC 1637 was determined and later the total ergosterol content was quantified from the data obtained on culturing the *C. albicans*MTCC 1637 strain with different concentrations (0, 2, 4, and 16 μg/mL) of the test compound **6l** and the standard

miconazole drug. Based on the results presented in **Fig 3**, it was observed that the ergosterol content decreased significantly with an increase in the concentration of test compounds **6l**. Similarly, a dose-dependent decrease in ergosterol content was observed when the *C. albicans*MTCC 1637 strain was cultured in the presence of miconazole. Our findings suggest that the pyranopyrimidine derivative **6l** altered the sterol profile which probably may be contributing to its antifungal activity through inhibition of ergosterol biosynthesis. The *Candida*-cidal activity of the compound **6l** might also be responsible for the direct damage of the cell membrane. However, the exact mechanism of action of this compound needs to be further elucidated.

Table 1. Antibacterial activity of the synthesized compound **4a-l** and **6a-l**

Test Compound	Minimum inhibitory concentration (µg/mL)						
	***Micrococcus luteus* MTCC 2470**	***Staphylococcus aureus* MTCC 96**	***Staphylococcus aureus* MLS-16 MTCC 2940**	***Bacillus subtilis* MTCC 121**	***Escherichia coli* MTCC 739**	***Pseudomonas aeruginosa* MTCC 2453**	***Klebsiella planticola* MTCC 530**
4a	>125	>125	>125	>125	>125	>125	>125
4b	>125	>125	>125	>125	>125	>125	>125
4c	>125	>125	>125	>125	>125	>125	>125
4d	>125	>125	>125	>125	>125	>125	>125
4e	>125	>125	>125	>125	>125	>125	>125
4f	>125	>125	>125	>125	>125	>125	>125
4g	>125	>125	>125	>125	>125	>125	>125
4h	>125	>125	>125	>125	>125	>125	>125
4i	15.6	15.6	15.6	>125	>125	>125	>125
4j	15.6	15.6	31.2	>125	>125	>125	>125
4k	>125	>125	>125	>125	>125	>125	>125
4l	>125	>125	>125	>125	>125	>125	>125

4m	>125	>125	>125	>125	>125	>125	>125
4o	>125	>125	>125	>125	>125	>125	>125
4p	>125	>125	>125	>125	>125	>125	>125
6a	>125	>125	31.2	>125	>125	>125	>125
6b	>125	>125	31.2	>125	>125	>125	>125
6c	>125	31.2	31.2	>125	>125	>125	>125
6d	15.6	15.6	31.2	>125	>125	>125	>125
6e	>125	15.6	>125	>125	>125	>125	>125
6f	>125	>125	>125	>125	>125	>125	>125
6g	>125	15.6	>125	>125	>125	>125	>125
6h	31.2	>125	15.6	7.8	>125	>125	>125
6i	>125	7.8	>125	>125	>125	>125	>125
6j	>125	>125	>125	>125	>125	>125	>125
6k	>125	>125	>125	>125	>125	>125	>125
6l	15.6	15.6	3.9	7.8	7.8	>125	7.8
Ciprofloxacin	0.9	0.9	0.9	0.9	0.9	0.9	0.9

Table 2. Minimum Bactericidal Concentration Assay (MBC) of the synthesized compounds **4a-4p** and **6a-6l**

Test Compound	Minimum bactericidal concentration (µg/mL)					
	***Micrococcus luteus* MTCC 2470**	***Staphylococcus aureus* MTCC 96**	***Staphylococcus aureus* MLS-16 MTCC 2940**	***Bacillus subtilis* MTCC 121**	***Escherichia coli* MTCC 739**	***Klebsiella planticola* MTCC 530**
4i	> 125	15.6	31.2	31.2	> 125	> 125
4j	> 125	15.6	31.2	31.2	> 125	> 125
6a	> 125	> 125	62.5	> 125	> 125	> 125
6b	> 125	> 125	62.5	> 125	> 125	> 125
6c	> 125	62.5	31.2	> 125	> 125	> 125
6d	31.2	31.2	62.5	> 125	> 125	> 125
6e	> 125	31.2	> 125	> 125	> 125	> 125
6g	> 125	31.2	> 125	> 125	> 125	> 125
6h	62.5	> 125	31.2	15.6	> 125	> 125
6i	> 125	15.6	> 125	> 125	> 125	> 125
6l	31.2	31.2	7.8	7.8	15.6	15.6
Ciprofloxacin	0.9	1.9	1.9	0.9	1.9	1.9

Table 3. Biofilm inhibition assay of the synthesized chromenopyrano[2,3-*d*]pyrimidine derivatives

Test Compound	IC_{50} values in (µM)					
	***Micrococcus luteus* MTCC 2470**	***Staphylococcus aureus* MTCC 96**	***Staphylococcus aureus* MLS-16 MTCC 2940**	***Bacillus subtilis* MTCC 121**	***Escherichia coli* MTCC 739**	***Klebsiella planticola* MTCC 530**
4i	-[a]	7.5 ± 0.26	-	-	-	-
4j	-	8.1 ± 0.18	-	-	-	-
6a	-	-	22.4 ± 0.52	-	-	-
6b	-	-	16.8 ± 0.44	-	-	-
6c	-	18.9 ± 0.38	15.6 ± 0.36	-	-	-
6d	10.2 ± 0.28	11.4 ± 0.26	22.4 ± 0.28	-	-	-
6e	-	9.8 ± 0.22	-	-	-	-
6g	-	8.6 ± 0.32	-	-	-	-
6h	17.4 ± 0.34	-	9.2 ± 0.24	4.5 ± 0.26	-	-
6i	-	4.9 ± 0.18	-	-	-	-
6l	11.5 ± 0.26	9.2 ± 0.24	2.5 ± 0.18	3.8 ± 0.26	4.2 ± 0.23	4.6 ± 0.18
Ciprofloxacin	0.5 ± 0.08	0.3 ± 0.11	0.4 ± 0.12	0.6 ± 0.08	0.4 ± 0.09	0.5 ± 0.10

Table 4. Antifungal activity of the synthesized pyrano[2,3-*d*]pyrimidine derivatives

Test Compound	Minimum inhibitory concentration (µg/mL)													
	***C. albicans* MTCC 183**	***C. albicans* MTCC 227**	***C. albicans* MTCC 854**	***C. albicans* MTCC 1637**	***C. albicans* MTCC 3017**	***C. albicans* MTCC 3018**	***C. albicans* MTCC 3958**	***C. albicans* MTCC 4748**	***C. albicans* MTCC 7315**	***C. parapsilosis* MTCC 1744**	***C. aaseri* MTCC 1962**	***C. glabrata* MTCC 3019**	***C. krusei* MTCC 3020**	***Issatchenkia hanoiensis* MTCC 4755**
6a	31.2	62.5	31.2	31.2	31.2	62.5	15.6	15.6	31.2	62.5	62.5	62.5	31.2	31.2
6b	62.5	62.5	31.2	31.2	31.2	62.5	31.2	15.6	31.2	31.2	62.5	31.2	62.5	62.5
6c	31.2	31.2	62.5	62.5	31.2	31.2	62.5	15.6	15.6	31.2	62.5	62.5	31.2	31.2
6d	31.2	31.2	15.6	15.6	31.2	31.2	15.6	62.5	31.2	15.6	7.8	15.6	15.6	7.8
6h	31.2	7.8	7.8	15.6	15.6	15.6	15.6	7.8	15.6	31.2	62.5	31.2	15.6	15.6
6l	7.8	15.6	7.8	7.8	7.8	15.6	7.8	7.8	15.6	31.2	15.6	7.8	15.6	7.8
Miconazole	7.8	7.8	7.8	7.8	7.8	7.8	7.8	7.8	7.8	7.8	7.8	7.8	7.8	7.8

Table 5. Minimum Fungicidal Concentration (MFC) of the synthesized pyrano[2,3-*d*]pyrimidine derivatives

Test Compound	Minimum inhibitory concentration (µg/mL)												
	***C. albicans* MTCC 183**	***C. albicans* MTCC 227**	***C. albicans* MTCC 854**	***C. albicans* MTCC 1637**	***C. albicans* MTCC 3018**	***C. albicans* MTCC 3958**	***C. albicans* MTCC 4748**	***C. albicans* MTCC 7315**	***C. parapsilosis* MTCC 1744**	***C. aaseri* MTCC 1962**	***C. glabrata* MTCC 3019**	***C. krusei* MTCC 3020**	***Issatchenkia hanoiensis* MTCC 4755**
6a	62.5	62.5	62.5	62.5	125	31.2	31.2	31.2	62.5	125	125	62.5	31.2
6b	62.5	125	62.5	62.5	125	62.5	15.6	62.5	62.5	62.5	62.5	125	125
6c	31.2	62.5	125	125	62.5	125	31.2	15.6	62.5	125	125	62.5	31.2
6d	62.5	62.5	31.2	15.6	62.5	31.2	125	62.5	15.6	15.6	31.2	31.2	15.6
6h	31.2	15.6	15.6	31.2	15.6	31.2	15.6	15.6	62.5	62.5	62.5	31.2	31.2
6l	15.6	15.6	15.6	7.8	31.2	15.6	7.8	15.6	62.5	31.2	15.6	15.6	15.6
Miconazole	15.6	7.8	7.8	15.6	15.6	7.8	15.6	7.8	7.8	7.8	15.6	7.8	7.8

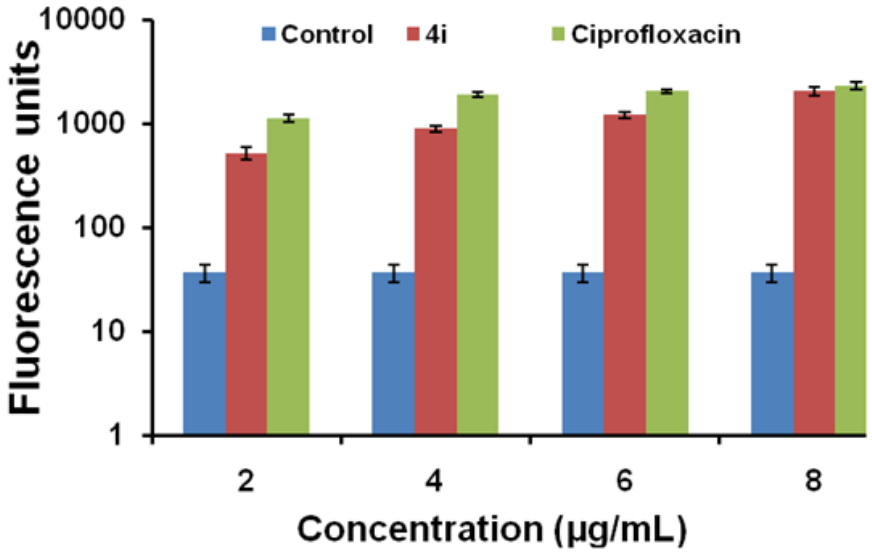


Figure 2. Intracellular ROS accumulation in *Staphylococcus aureus* MTCC 96 for the lead compound **4i**

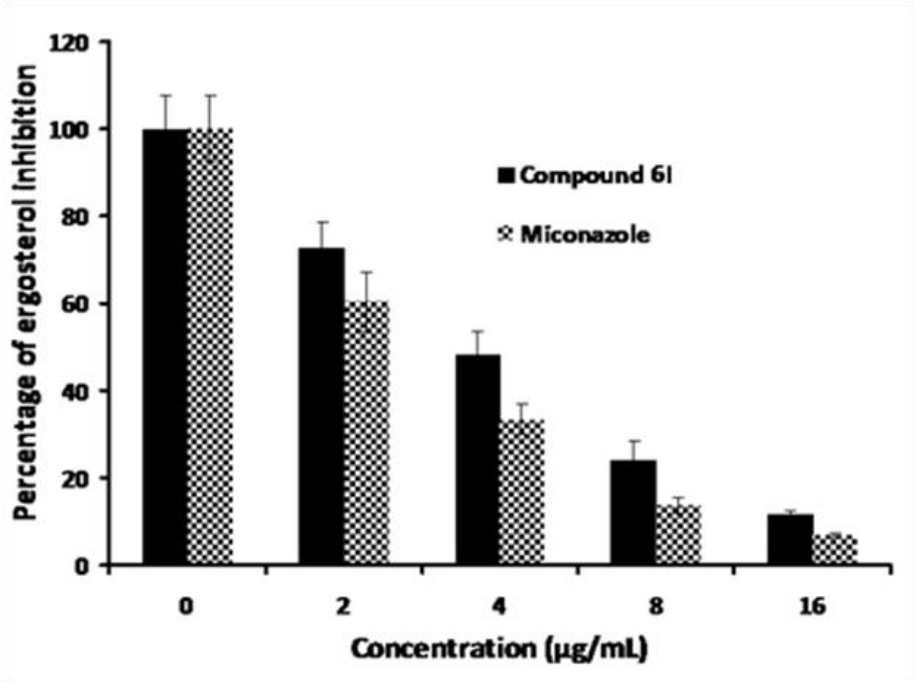
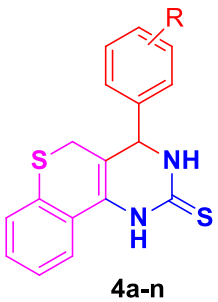
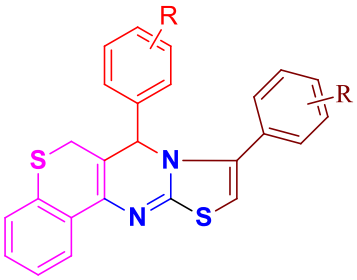


Figure 3. Effect of compound **6l** on the inhibition of ergosterol biosynthesis in *Candida albicans* MTCC 1637.

THIOCHROMENO[4,3-*d*]PYRIMIDINES AND THIAZOLO[4,3-*d*]THIOCHROMENO[4,3-*d*]PYRIMIDINE DERIVATIVES (CHAPTER III)



4a-n



5a-n

All the synthesized compounds were evaluated for various biological activities such as antibacterial, minimum bactericidal concentration (MBC), anti-biofilm and accumulation of intracellular ROS in *Staphylococcus aureus* MLS-16 MTCC 2940 biofilms.

(h) *Antibacterial activity*

Compounds **4a-n** and **5a-n** were screened for antibacterial activity *in vitro* against different Gram-positive and Gram-negative bacterial strains such as *Micrococcus luteus* MTCC 2470, *Staphylococcus aureus* MTCC 96, *Staphylococcus aureus* MLS-16 MTCC 2940, *Bacillus subtilis* MTCC 121, *Escherichia coli* MTCC 739, *Pseudomonas aeruginosa* MTCC 2453 and *Klebsiella planticola* MTCC 530. Among all the derivatives screened, compound **4c** and **5d** showed promising activity (MIC value of 3.9. 15.6 µg/mL against *Staphylococcus aureus* MTCC 96 and *Staphylococcus aureus*. However, all the other tested compounds (**4a, 4d, 4n, 5d, 5g** and **5k**) showed MIC values of >125 µg/mL against all the tested bacterial strains. Based on the structure-activity relationship of the synthesized derivatives, it was observed that the compound **4c** has a methoxy substituent attached to the basic thiochromeno[3,4-*d*]pyrimidine scaffold, which has an electron donating property which probably may be contributing to the antibacterial activity. In case of compound **4b**, a methyl substituent is attached to the basic thiochromeno[3,4-*d*]pyrimidine scaffold, having electron donating property is probably contributing to the antibacterial activity. Based on the structure-activity relationship of the synthesized derivatives, it was observed that the compound **5d** has a simple hydrogen atom attached to the basic thiazolo[3,2-*a*]thiochromeno[4,3- *d*]pyrimidines scaffold, which has neutral property which probably may be contributing to the antibacterial activity results to this regard are tabulated in (**Table 6**).

(i) ***Minimum bactericidal concentration (MBC)***

Based on the antibacterial activity results, the compounds **4b, 4c, 5d, 5g** and **5k** were screened for the minimum bactericidal concentration against *Staphylococcus aureus* MTCC 96, *Staphylococcus aureus* MLS16 MTCC 2940 and *Bacillus subtilis* MTCC 121 in comparison to ciprofloxacin as standard. Compounds **4b, 4c, 5d, 5g** and **5k** consistently showed promising minimum bactericidal concentration against all the tested bacterial strains. The activity data to this regard is shown in (**Table 7**).

(j) ***Biofilm inhibition assay***

A biofilm is a structured consortium of bacteria embedded in a self-produced polymeric matrix consisting of polysaccharides, protein and DNA. Bacterial biofilms cause chronic infections in humans *via* hospital and community environments since they show increased tolerance to antibiotics and disinfectant chemicals as well as resisting phagocytosis and other components of the body's defense system. In the medical sector, bacteria colonize through adhesion mechanism and result in biofilm formation on several biomedical implants such as stents, heart valves, vascular grafts and catheters. In this context, the novel compounds that can specifically target and inhibit the biofilm formation would be of significance in comparison to the rational use of antibiotics and/or biocides. Considering these facts, a further step was undertaken to investigate whether these compounds exhibit a specific anti-biofilm activity or whether this observation was simply related to a general toxic effect on the Gram-positive bacterial strains. To this regard, the compounds **4b**, **4c, 5d**, **5g** and **5k** were screened for anti-biofilm activity against *Staphylococcus aureus* MTCC 96, *Staphylococcus aureus* MLS16 MTCC 2940 and *Bacillus subtilis* MTCC 121, which are common and important nosocomial pathogens having biofilm forming ability. The results summarized in Table 8, clearly reveal that not much information on the structure-activity relationship (SAR) can be highlighted at this stage; however, it is observed that compound **4c** exhibited promising activity (IC_{50} values ranged between 2.1 – 8.1 μg/mL) towards all the tested bacterial species, while compound **4b** and **5d** showed good activity (IC_{50} values ranged between 8.8–11.3 and 1.9-5.3 μg/mL) against all the tested bacterial strains. The basic thiochromeno[3,4-*d*]pyrimidine scaffold of these compounds possesses different substituents which exhibit electron donating or electron withdrawing properties which antagonize the biofilm formation and probably may be contributing to the anti-biofilm activity. The basic chromenopyrano[2,3-*d*]pyrimidine scaffold in compound **5d** has different substituents which exhibit neutral or

electron donating properties which antagonize the biofilm formation and probably may be contributing to the anti-biofilm activity. The activity data to this regard is shown in (**Table 8**).

(k) ***Accumulation of intracellular ROS in*** **Staphylococcus aureus** ***MLS16 MTCC 2940 biofilms***

In order to elucidate whether oxidative stress in involved in the apoptotic cell death, the intracellular ROS accumulation within the cells of mature biofilms was measured using the fluorescent probe 2',7'-dichlorofluorescein-diacetate (DCFH-DA), a general ROS fluorescent probe. The DCFH-DA dye conversion mainly depends on the metabolically active cells in the biofilm. DCFH-DA is deacetylated in the cells where it can react quantitatively with intracellular radicals (mainly H_2O_2) to get converted to its fluorescent product (DCF), which is retained within the cells and thus provides an index of oxidation in the cell cytosol. In the present study, the accumulation of intracellular reactive oxygen species (ROS) in mature *Staphylococcus aureus* MLS16 biofilms was measured using DCFH-DA dye. The accumulation of intracellular ROS plays a major role in apoptotic mediated cell death. After treatment with compounds **4c** and **5d**, the ROS levels were significantly accumulated in the tested *Staphylococcus aureus* MLS16 strain (**Figure 4**) and *Staphylococcus aureus* strain (**Figure 5**). At a concentration of 4 µg/ml, the compounds **4c** and **5d** treated biofilms showed increased levels of intracellular ROS accumulation which was equal to the standard drug ciprofloxacin. The ROS measurements were also carried out separately in both sessile cells and in the supernatant. The ROS induced increase in fluorescence was observed only for the sessile cells, suggesting that the ROS accumulation is of intracellular origin. This accumulated ROS may be responsible for the bactericidal activity. Oxygen-containing free radical molecules and their precursors formed in biological systems are collectively termed as ROS, which include superoxides ($O_2^{\cdot -}$), peroxides (H_2O_2 and ROOH) and free radicals ($HO^{\cdot}$ and $RO^{\cdot}$). Some of the bactericidal drugs stimulate free radical formation via the Fenton reaction. Recent studies suggest that when bacteria is under oxidative stress, these free radicals contribute to arrest in the cell growth or bacterial mediated cell death by damaging the specific essential metabolic enzymes (cellular respiratory chain), disrupting cellular membrane, and DNA damage ultimately leading to cell lysis and death.

Table 6. Antibacterial activity of the synthesized compounds (**4a-n** and **5a-n**).

Test compound	Minimum inhibitory concentration (µg/mL)						
	Staphylococcus aureus **MTCC 96**	***Bacillus subtilis*** **MTCC 121**	***Staphylococcus aureus*** **MLS16 MTCC 2940**	***Micrococcus luteus*** **MTCC 2470**	***Klebsiella planticola*** **MTCC 530**	***Escherichia coli*** **MTCC 739**	***Pseudomonas aeruginosa*** **MTCC 2453**
4a	125	125	125	125	125	125	125
4b	7.8	15.6	7.8	125	125	125	125
4c	3.9	7.8	3.9	125	125	125	125
4d	125	125	125	125	125	125	125
4e	125	125	125	125	125	125	125
4f	125	125	125	125	125	125	125
4g	125	125	125	125	125	125	25
4h	125	125	125	125	125	125	125
4i	125	125	125	125	125	125	125
4j	125	125	125	125	125	125	125
4k	125	125	125	125	125	125	>125
4l	125	125	125	125	125	125	125
4m	125	125	125	125	125	125	125
4n	125	125	125	125	125	125	125
5a	31.2	>125	>125	>125	>125	>125	>125
5b	>125	>125	>125	>125	>125	>125	>125
5c	>125	>125	>125	>125	>125	>125	>125
5d	3.9	3.9	3.9	7.8	>125	>125	>125
5e	>125	>125	15.6	>125	>125	>125	>125
5f	>125	15.6	>125	15.6	>125	>125	>125
5g	7.8	62.5	15.6	>125	>125	>125	>125
5h	31.2	31.2	>125	>125	>125	>125	>125
5i	>125	>125	>125	>125	>125	>125	>125
5j	>125	>125	>125	>125	>125	>125	>125
5k	15.6	31.2	31.2	31.2	>125	>125	>125
5l	31.2	>125	31.2	>125	>125	>125	>125

5m	>125	>125	>125	>125	>125	>125	>125
5n	>125	>125	>125	>125	>125	>125	>125
Ciprofloxacin	0.9	0.9	0.9	0.9	0.9	0.9	0.9

Table 7. Minimum Bactericidal Concentration Assay (MBC) of the synthesized compounds (**4a-n** and **5a-n**)

Test compounds	**Minimum bactericidal concentration (MBC, µg/mL)**			
	***Staphylococcus aureus* MTCC 96**	***Bacillus subtilis* MTCC 121**	***Staphylococcus aureus* MLS16 MTCC 2940**	***Micrococcus luteus* MTCC 2470**
4b	15.6	31.2	15.6	-
4c	3.9	15.6	7.8	-
5d	7.8	3.9	7.8	15.6
5f	>125	31.2	>125	31.2
5g	15.6	62.5	31.2	>125
5h	62.5	31.2	>125	>125
5k	31.2	62.5	62.5	31.2
5l	62.5	>125	31.2	>125
Ciprofloxacin	1.9	0.9	0.9	1.9

Table 8. Biofilm inhibition assay of the synthesized compounds (**4a-n** and **5a-n**)

Test compounds	**IC_{50} values (µg/mL)**			
	***Staphylococcus aureus* MTCC 96**	***Bacillus subtilis* MTCC 121**	***Staphylococcus aureus* MLS16 MTCC 2940**	***Micrococcus luteus* MTCC 2470**
4b	8.8 ± 0.32	10.5 ± 0.24	11.3 ± 0.18	-
4c	2.1 ± 0.44	8.1 ± 0.29	4.5 ± 0.22	-
5d	2.1 ± 0.24	1.9 ± 0.42	2.4 ± 0.18	5.3 ± 0.16
Ciprofloxacin	0.6 ± 0.06	0.5 ± 0.09	0.4 ± 0.07	0.4 ± 0.06

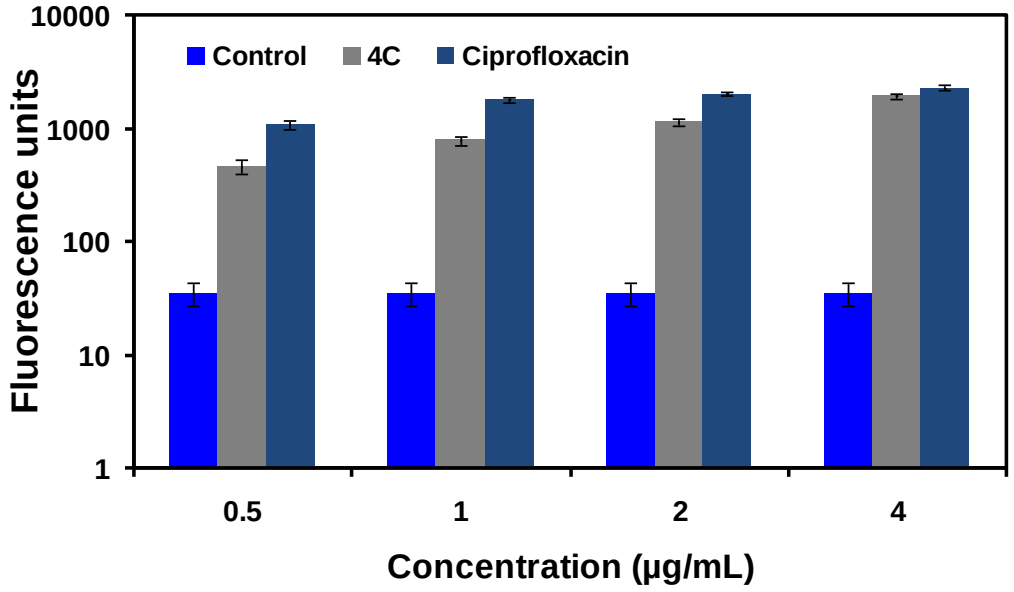


Figure 4. Intracellular Reactive Oxygen Species (ROS) accumulation in *Staphylococcus aureus* MLS16 (MTCC 2940) biofilms by the lead compound **4c**.

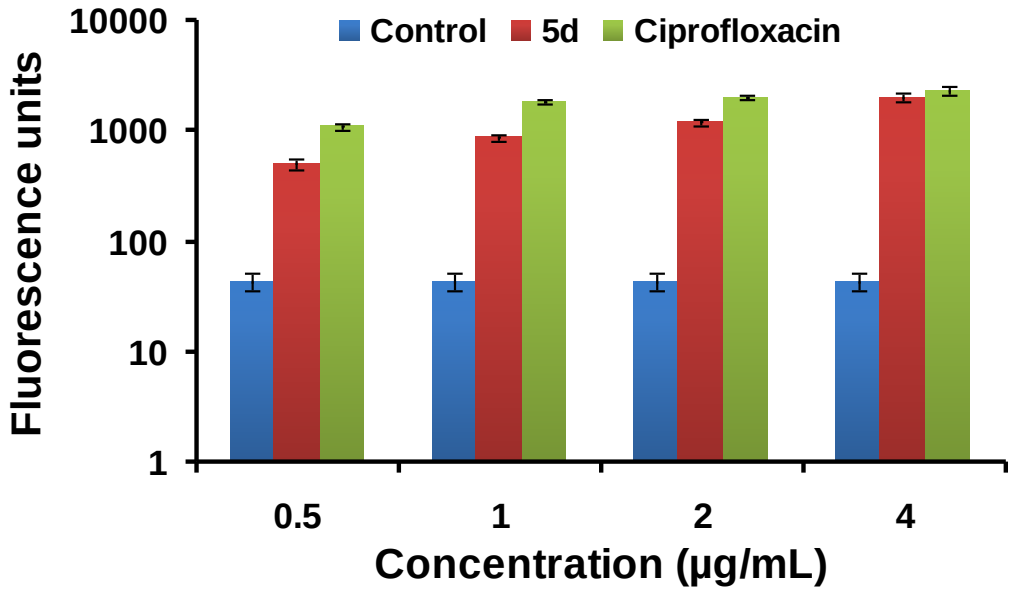
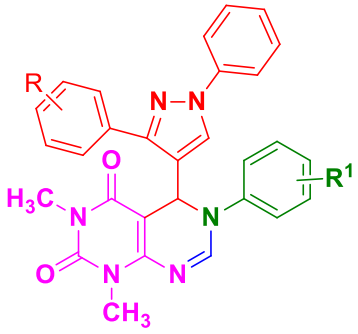


Figure 5. Intracellular ROS accumulation in *Staphylococcus aureus* MTCC 96 by the lead compound **5d**.

PYRAZOLO-PYRIMIDO PYRIMIDINE DERIVATIVES (CHAPTER IV)



5a-x

All the synthesized compounds were evaluated for various biological activities such as antibacterial, minimum bactericidal concentration (MBC), anti-biofilm, accumulation of intracellular ROS in *Micrococcus luteus* MTCC 2470 and Detection of protein leakage in *Micrococcus luteus* MTCC 2470 cells.

(l) *Antibacterial activity:* Compounds **5** were screened for antibacterial activity *in vitro* against different Gram-positive and Gram-negative bacterial strains such as *Micrococcus luteus* MTCC 2470, *Staphylococcus aureus* MTCC 96, *Staphylococcus aureus* MLS-16 MTCC 2940, *Bacillus subtilis* MTCC 121, *Escherichia coli* MTCC 739, *Pseudomonas aeruginosa* MTCC 2453 and *Klebsiella planticola* MTCC 530. Among all the screened derivatives, the compounds **5c**, **5i**, **5l** and **5m** were quite promising with MIC values ranging between 3.9-15.6 µg/mL, while compounds **5d**, **5t**, **5u**, **5v** and **5w** showed good activity with MIC values ranging between 15.6-31.2 µg/mL. All these compounds showed antibacterial activity against only Gram-positive bacteria such as *Staphylococcus aureus* MTCC 96, *Staphylococcus aureus* MLS16 MTCC 2940, *Bacillus subtilis* MTCC 121 and *Micrococcus luteus*. However, all the other compounds showed MIC value of >125 µg/mL, which was considered as not active against all the other bacterial strains. The antibacterial activity results to this regard are tabulated in (**Table 9**). Based on the structure-activity relationship of the synthesized pyrazolo-pyrimido[4,5-*d*]pyrimidines in that 5-

and 6-positions on the pyrazole and anilines gave significant activity, the compounds **5c**, **5i**, **5l** and **5m** having nitro, chloro methyl and methoxy substituents increased the activity considerably. It was observed that the compound **5f** has a simple hydrogen atom attached to the basic pyrazolo-pyrimido[4,5-*d*]pyrimidines scaffold, which has neutral property which probably may be contributing to the antibacterial activity. In case of compound **5l** methyl and chloro substituents is attached to the basic pyrazolo-pyrimido[4,5-*d*]pyrimidines scaffold, having electron releasing property which probably may be contributing to the antibacterial activity.

(m) ***Minimum Bactericidal Concentration (MBC)*****:** The minimum bactericidal concentration values were determined for only those compounds which showed antibacterial activity results. Based on the results, it was observed that the MBC values were 2-fold the antibacterial activity values. Among the screened derivatives, the compounds **5c**, **5i**, **5l**, **5m**, **5u**, **5v** and **5w** were quite promising with MBC values ranging between 7.8-31.2 µg/mL. The activity data in this regard is shown in (**Table 10**).

(n) ***Biofilm Inhibition Assay:*** Biofilms are structured communities of bacteria embedded in a self-produced, hydrated, polymeric matrix comprising of polysaccharides, protein and DNA. This polymeric matrix acts as a protective shield enabling the survival of microbes against various environmental stresses. Biofilms cause chronic infections in humans through hospital and community environments since they exhibit increased recalcitrance towards antibiotics and disinfectant chemicals. In the medical environments, several biomedical implants such as stents, heart valves, vascular grafts and catheters are employed during surgery and in case of contamination of these biomedical implants in post-surgical cases results in biofilm formation through adhesion mechanism. To address these issues, a systematic study was undertaken to identify the target compounds that can specifically inhibit biofilm formation. To this regard, the

compounds **5c**, **5l** and **5m** were screened for anti-biofilm activity against *Staphylococcus aureus* MTCC 96, *Staphylococcus aureus* MLS16 MTCC 2940, *Bacillus subtilis* MTCC 121 and *Micrococcus luteus* MTCC 2470 which are common nosocomial pathogens having the ability to form biofilms. The results summarized in Table 11, clearly reveal that not much information on the structure-activity relationship (SAR) can be highlighted at this stage; however, it was observed that compounds **5l** and **5m** exhibited promising activity with IC_{50} values ranging between 1.8 - 8.2 µg/mL towards all the tested pathogens. The basic pyrazolo-pyrimido[4,5-*d*]pyrimidines scaffold of these compounds possesses different substituents which exhibit neutral or electron donating properties which antagonize the biofilm formation and probably may be contributing to the anti-biofilm activity. The activity data to this regard is shown in (**Table 11**).

(o) ***Accumulation of intracellular ROS in Micrococcus luteus*** **MTCC 2470*:*** In order to elucidate whether oxidative stress was involved in the apoptotic cell death, the intracellular ROS accumulation within the cells of mature biofilms was measured using the fluorescent probe 2',7'-dichlorofluorescein-diacetate (DCFH-DA), a general ROS fluorescent probe. The DCFH-DA dye conversion mainly depends on the metabolically active cells in the biofilm. DCFH-DA is deacetylated in the cells where it reacts quantitatively with intracellular radicals (mainly H_2O_2) to get converted to its fluorescent product (DCF), which is retained within the cells and thus provides an index of oxidation in the cell cytosol. In the present study, the accumulation of intracellular reactive oxygen species (ROS) in the mature biofilms of *Micrococcus luteus* MTCC 2470 culture was measured using DCFH-DA dye. The accumulation of intracellular ROS plays a major role in apoptotic mediated cell death. After treatment with compound **5l**, a significantly increased ROS levels were accumulated in the tested strain of *Micrococcus luteus* MTCC 2470 (**Figure 6**). At a concentration of 0.5 µg/mL, the compound **5l** treated biofilms showed increased

levels of intracellular ROS accumulation which was closer to the standard drug ciprofloxacin, while at a concentration of 2 µg/mL, the intracellular ROS accumulation was equal to the standard drug ciprofloxacin. Further, the ROS measurements were also carried out separately in both sessile cells and in the supernatant. The ROS induced increase in fluorescence was observed only for the sessile cells, suggesting that the ROS accumulation is of intracellular origin. This accumulated ROS may be contributing to the bactericidal activity. Oxygen-containing free radical molecules and their precursors formed in biological systems are collectively termed as ROS, comprising of superoxides ($O_2^{\cdot -}$), peroxides (H_2O_2 and ROOH) and free radicals ($HO^{\cdot}$ and $RO^{\cdot}$). Some of the bactericidal drugs stimulate free radical formation via the Fenton reaction. Recent studies suggest that when bacteria is under oxidative stress, these free radicals contribute to arrest in cell growth or result in damage of the specific essential metabolic enzymes (cellular respiratory chain), disrupting cellular membrane, and DNA damage ultimately causing cell lysis and death.

(p) ***Detection of protein leakage in Micrococcus luteus MTCC 2470 cells***

The leakage of cytoplasmic content is a characteristic feature indicating the damage caused to the bacterial membrane. Due to the interaction of compound with cells, the cell membrane loses its integrity leading to the release of intracellular components such as nucleic acids and proteins by membrane disruption. Hence, the occurrence of these substances signifies the membrane damage that leads to the bacterial cell death. It was found that the test compound enhanced protein leakage by increasing the membrane permeability of *Micrococcus luteus* MTCC 2470 (**Figure 7**). Initially, the protein leakage from the membranes of *Micrococcus luteus* cells treated with test compound was almost the same as that from cells in the control group. At 12 h after incubation, protein leakage from cells treated with compound **5l** considerably increased; however, there was

no change in the amount of protein leakage from cells in the control group. Leakage from cells treated with compound **5l** was significantly higher than that observed from the cells in the control group. Hence, the results indicated the release of intracellular protein, an evidence for membrane damage resulting in bacterial cell death.

Table 9. Antibacterial activity of synthesized compounds (**5a-x**)

Test Compounds	Minimum inhibitory concentration (µg/ml)						
	Bacillus subtilis **MTCC 121**	***Staphylococcus aureus*** **MTCC 96**	***S. aureus MLS16*** **MTCC 2940**	***Micrococcus luteus*** **MTCC 2470**	***Klebsiella planticola*** **MTCC 530**	***Escherichia coli*** **MTCC 739**	***Pseudomonas aeruginosa*** **MTCC 2453**
5a	125	125	125	125	125	125	125
5b	125	125	125	125	125	125	125
5c	7.8	15.6	7.8	15.6	125	125	125
5d	31.2	31.2	31.2	15.6	125	125	125
5e	125	125	125	31.2	125	125	125
5f	125	125	125	125	125	125	125
5g	125	125	125	125	125	125	125
5h	125	125	125	15.6	125	125	125
5i	31.2	15.6	15.6	7.8	125	125	125
5j	125	125	125	125	125	125	125
5k	125	125	125	125	125	125	125
5l	7.8	7.8	7.8	3.9	125	125	125
5m	7.8	7.8	15.6	7.8	125	125	125
5n	125	125	125	125	125	125	125
5o	125	125	125	125	125	125	125
5p	62.5	31.2	15.6	7.8	125	125	125
5q	125	125	125	125	125	125	125
5r	125	125	125	125	125	125	125
5s	125	125	125	125	125	125	125
5t	31.2	31.2	15.6	15.6	125	125	125
5u	15.6	31.2	15.6	15.6	125	125	125
5v	31.2	15.6	15.6	15.6	125	125	125

5w	31.2	15.6	15.6	15.6	125	125	125
5x	125	125	125	125	125	125	125
Ciprofloxacin	0.9	0.9	0.9	0.9	0.9	0.9	0.9

Table 10. Minimum bactericidal concentration (MBC) of the synthesized compounds (**5a-x**)

Test compounds	Minimum bactericidal concentration (µg/ml)			
	Staphylococcus aureus **MTCC 96**	***Bacillus subtilis*** **MTCC 121**	***S. aureus MLS16*** **MTCC 2940**	***Micrococcus luteus*** **MTCC 2470**
5c	31.2	31.2	15.6	15.6
5d	62.5	31.2	31.2	15.6
5e	125	125	125	62.5
5h	125	125	125	31.2
5i	31.2	31.2	15.6	15.6
5l	7.8	7.8	31.2	15.6
5m	15.6	31.2	15.6	15.6
5n	62.5	62.5	31.2	15.6
5q	62.5	62.5	62.5	31.2
5u	31.2	31.2	31.2	31.2
5v	31.2	31.2	31.2	31.2
5w	31.2	31.2	31.2	15.6
Ciprofloxacin	1.9	0.9	0.9	1.9

Table 11. Biofilm inhibition assay of the synthesized compounds (**5a-x**)

Test compounds	IC_{50} values in (µg/mL)			
	Staphylococcus aureus **MTCC 96**	***Bacillus subtilis*** **MTCC 121**	***S. aureus*** **MLS16 MTCC 2940**	***Micrococcus luteus*** **MTCC 2470**
5c	12.6 ± 0.36	18.3 ± 0.44	9.5 ± 0.48	10.1 ± 0.26
5l	3.9 ± 0.32	4.1 ± 0.28	4.4 ± 0.18	1.8 ± 0.11
5m	4.2 ± 0.24	3.6 ± 0.34	8.2 ± 0.22	3.3 ± 0.16
Ciprofloxacin	0.6 ± 0.06	0.5 ± 0.09	0.4 ± 0.07	0.4 ± 0.06

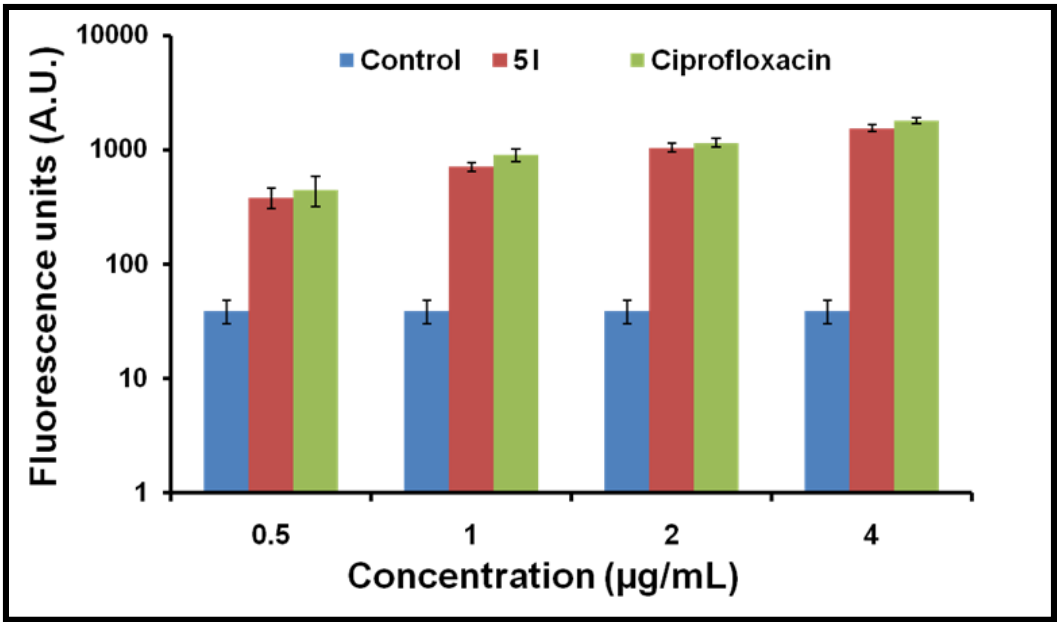


Figure 6. ROS accumulation measured in *Micrococcus luteus* MTCC 2470 when treated with compound **5l** and Miconazole (control). All the experiments were performed in triplicates and the results were expressed as mean ± S.D, (n=3).

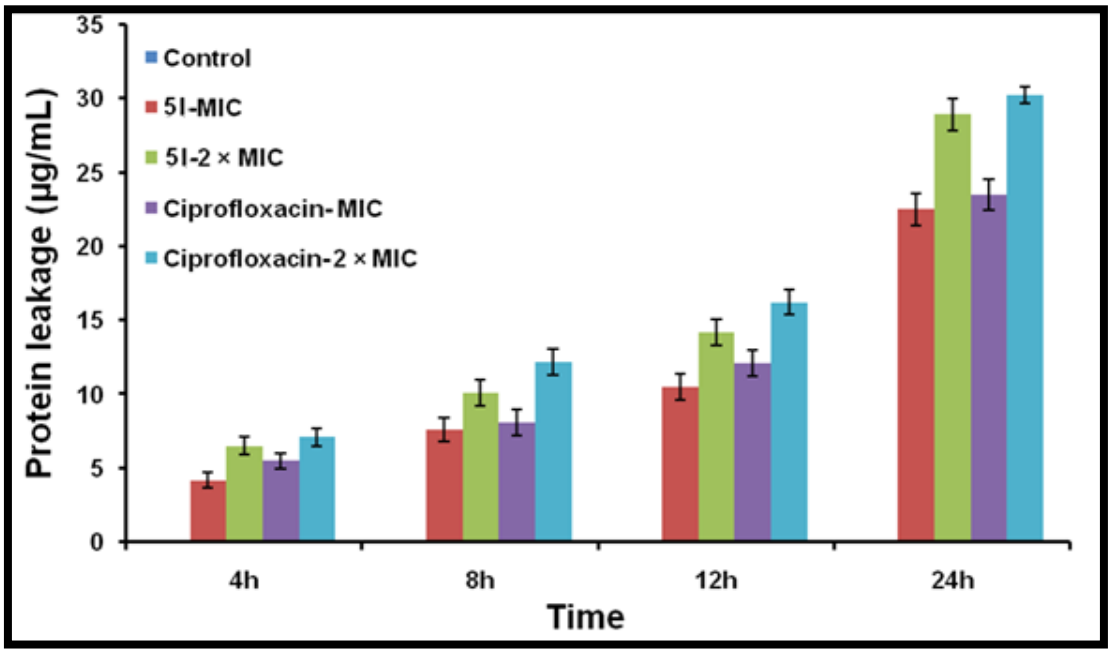
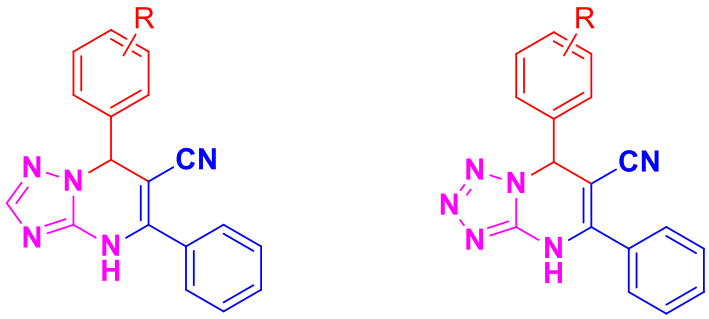


Figure 7. Leakage of protein from *Micrococcus luteus* MTCC 2470 cells when exposed to the test compound **5l**. The bacterial cells were treated with **5l** at two different concentrations i.e.

MIC and 2× MIC (in µg/mL). The untreated sample served as a control and ciprofloxacin run in parallel as a positive control. The assay was performed in triplicate and the results are expressed as mean ± S.D (n=3).

TRIAZOLO[1,5-*a*] AND TETRAZOLO[1,5-*a*]PYRIMIDINES (CHAPTER V)



(q) Antidibetic activity

The newly synthesized novel class of non-glycosidic triazolo and tetrazolopyrimidines (**5a-o** and **6a-o**) was tested for their α-glucosidase inhibitory activity by *in-vitro* enzyme assay. Homology modeling of the receptor (α-glucosidase). The ligand enzyme connections are in accordance with *in-vitro* results obtained in the wet lab. The results of inhibitory activity of the fused triazolo and tetrazolo[1,5-*a*]pyrimidine derivatives (**5a-o** and **6a-o**) at 5mM concentration against α-glucosidase are shown in (**Table 12** and **Table 13**). The tested molecules (**5a-o** and **6a-o**) have shown more selective inhibition towards α-glucosidase. The selective inhibition of α-glucosidase over α-amylase is greatly required, because, non-specific inhibition of α-amylase may lead to accumulation of non-digested carbohydrates, which in turn may result in abdominal cramping, diarrhoea and flatulence. The tested compounds (**5a-o** and **6a-o**) have shown varying degree of α-glucosidase inhibition with IC_{50} values ranging from 65.75 ± 2.95 µM to 272.33 ± 14.79 µM (Table 3). *In vitro* antidiabetic activity results indicated that compound **5d** (67.27 ± 2.94) and **6b** (65.75 ± 2.95µM) exhibited similar activity comparable with standard drug acarbose (51.76 µM).

The inhibitors **5d** and **6b** which showed top two minimal IC_{50} values against antidiabetic (α-glucosidase) activity were taken for further investigation. Different types of inhibition modes by different types of inhibitors were reported for α-glucosidase (antidiabetic) activity.

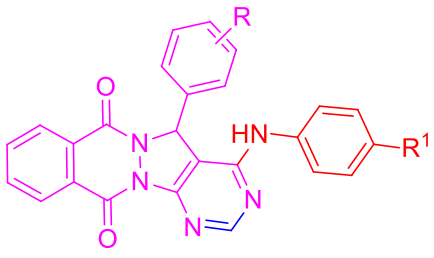
Table 12. α - glucosidase inhibition of the synthesized compounds **5a-o**

Test compounds	**IC_{50} values in (µM)**
5a	171.44 ± 2.69
5b	185.45 ± 5.19
5c	79.17 ± 5.92
5d	67.27 ± 2.94
5e	90.05 ± 2.06
5f	106.76 ± 2.98
5g	190.61 ± 1.42
5h	78.40 ± 2.72
5i	274.73 ± 5.70
5j	210.29 ± 5.91
5k	221.54 ± 2.06
5l	264.07 ± 4.49
5m	231.85 ± 2.86
5n	156.73 ± 3.25
5o	127.11 ± 9.25
Acarbose	**51.76**

Table 13. α - glucosidase inhibition of the synthesized compounds **6a-o**

Test compounds	IC_{50} values in ((µg/mL)
6a	260.72 ± 9.25
6b	65.75 ± 2.95
6c	272.33 ± 14.79
6d	243.89 ± 5.29
6e	221.83 ± 6.54
6f	255.82 ± 5.75
6g	280.13 ± 12.25
6h	446.56 ± 12.96
6i	185.93 ± 10.66
6j	255.77 ± 17.04
6k	91.49 ± 6.98
6l	267.07 ± 0.55
6m	124.33 ± 10.67
6n	174.22 ± 8.54
6o	104.73 ± 14.00
Acarbose	**51.76**

PYRAZOLO[1,2-*b*]PHTHALAZINE PYRIMIDINES (CHAPTER VI)



4aa-bd

(r) Antidibetic activity

All the synthesized fused pyrazolo[1,2-*b*]phthalazine pyrimidines were evaluated for antidibetic activity. Compounds **4aa-bd** was assayed for their antidibetic activity against α - glucosidase assay. The results were summarized in (**Table 14**). The structure activity relationship of the compounds showed that compounds with methoxy substituent on pyrimidine ring exhibited promising activity compared to acarbose standard. Among the tested compounds, compound **4bb** was found to be most active (IC_{50}: 30.98 µg/mL) exhibited more than standard drug acarbose (51.76±3.2 µM) whereas compounds **4ab**, **4an, 4ap, 4ax** and **4bd** potent activity (32.87, 49.88, 43.23, 46.33 and 49.09 µg/mL) compounds displayed promising activity. The inhibitors **4bb** which showed top two minimal IC_{50} values against antidiabetic (α-glucosidase) activity were taken for further investigation.

Table 14. α - glucosidase inhibition of the synthesized compounds **4aa-bd**

Test compounds	**IC_{50} values in (µg/mL)**	**Test compounds**	**IC_{50} values in (µg/mL)**
4aa	84.69	**4ap**	43.23
4ab	32.87	**4aq**	249.15
4ac	245.43	**4ar**	61.76

4ad	206.30	**4as**	82.44
4ae	50.85	**4at**	69.68
4af	189.97	**4au**	56.83
4ag	99.26	**4av**	398.66
4ah	125.71	**4aw**	59.86
4ai	68.73	**4ax**	314.56
4aj	54.09	**4ay**	63.71
4ak	206.44	**4ax**	46.33
4al	91.68	**4ba**	100.45
4am	120.55	**4bb**	30.97
4an	49.88	**4bc**	357.26
4ao	99.03	**4bd**	49.09
Acarbose	**51.76**	**Acarbose**	**51.76**

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The thesis entitled **"Synthesis and biological activity of novel substituted and fused pyrimidine derivatives"** is divided into seven chapters. The titles of all the chapters are given below.

CHAPTER-I: Introduction.

CHAPTER-II: Synthesis of novel pyrano[2,3-*d*]pyrimidines using ionic liquid.

CHAPTER-III: This chapter is sub divided into Section A and Section B.

***SECTION-A*:** Synthesis of fused dihydro-1*H*-thiochromeno[4,3-*d*]pyrimidines under green conditions

***SECTION-B*:** Green chemical synthesis of novel fused thiazolo[4,3-*d*]thiochromeno[4,3-*d*]pyrimidine derivatives.

CHAPTER-IV: Synthesis of novel pyrazolo-pyrimido[4,5-*d*]pyrimidine derivatives using [Bmim]$FeCl_4$ ionic liquid.

CHAPTER-V: One-pot three-component synthesis of new type of fused triazolo[1,5-*a*] and tetrazolo[1,5-*a*]pyrimidines.

CHAPTER-VI: Synthesis of new pyrazolo[1,2-*b*]phthalazine pyrimidines derivatives by using [BSO_3Hmim]HSO_4.

CHAPTER-VII: Evolution of Biological activity.

CHAPTER – I

INTRADUCTION

In this chapter a brief introduction on the importance of heterocycles in general and a few methods of synthesis of pyrimidine, pyrons, dihydrothiochromenes, thiazolothiochromenes, pyrazole, triazoles, tetrazoles and phthalazines was presented. Apart from this, the actual scope and objectives of the work were also mentioned.

The study of heterocyclic chemistry has evoked keen interest and considerable attention owing to the wide spread applications possessed by heterocycles. Amongst the heterocycles, nitrogen containing heterocycles are especially considered as "privileged" molecules for the synthesis and for development of new drugs, because nitrogen scaffold heterocycles are structural components of many bioactive natural products and natural drugs. Their wide range of biological and industrial applications have been the reason for the upsurge in interest and development of these heterocyclic compounds in general and pyrimidines and their substituted and fused derivatives like pyrano pyrimidines, dihydrothiochromenes, thiazolo thiochromeno, pyrazolo pyrimidines, triazolo, tetrazolo and phthalazines in particular. It is also interesting to note that these molecules invariably constitute the main lead molecules for several drugs.

PYRIMIDINE DERIVATIVES

Pyrimidines are a class of heterocyclic compounds, consisting of a six membered heterocyclic system analogous to benzene having two nitrogen atoms at 1 and 3 positions atom (**Figure 1**).

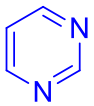


Figure 1. Pyrimidine

Pyrimidines are important heterocyclic compounds in medicinal chemistry; possess very interesting biological activities such as antimalarial, antibacterial, fungicidal, antibiofilm, anticancer, antiviral, anti-inflammatory, anti-platelet antioxidant. Pyrrolo-pyrimidine nucleoside derivatives act as potential anti-HCV (Hepatities C Virus) agent. Some of the aminopyrimidines are potent phosphodiesterase inhibitors. Fluroplex is used as an efficient cancer medicine. Piritrexim, minoxidil, Gleevec, Trimethoprim, 5-Flurouracil and

Sulfadiazine are well known chemotherapeutic agents and have a pyrimidine moiety in their structure (**Figure 2**).

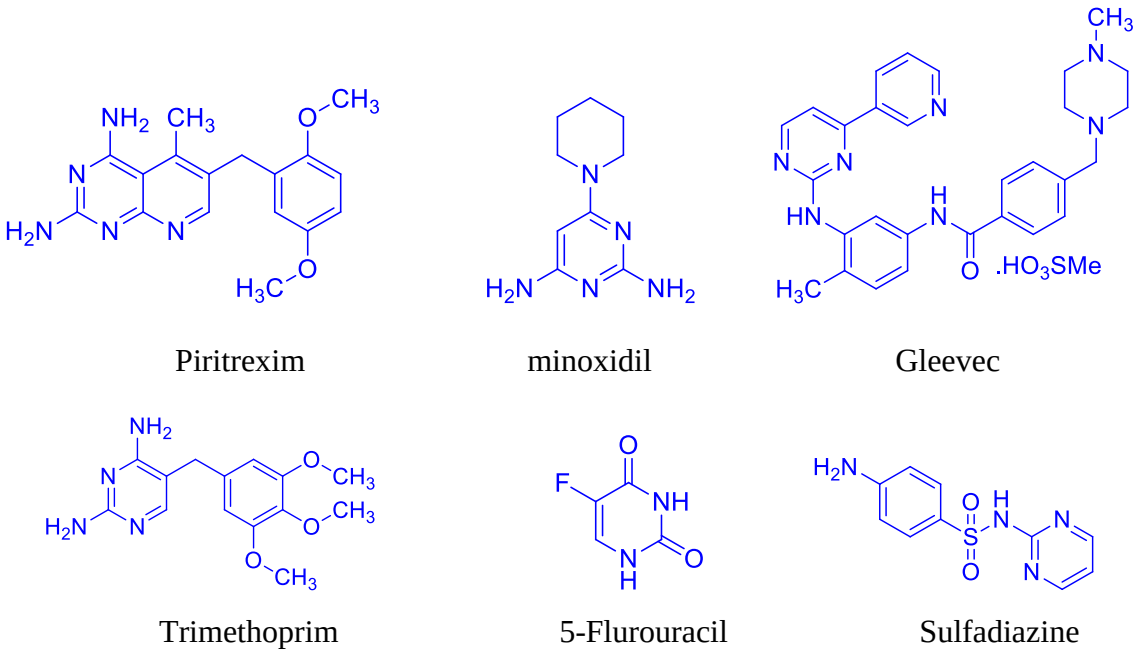


Figure 2. Structures of well known pyrimidine scaffold containing drugs

On the other hand, other heterocycles such as pyrans, dihydrothiochromenes, thiazolo thiochromenes, pyrazoles, triazoles and tetrazoles were also found to exhibit various biological properties.

Multicomponent reactions (MCRs) are powerful tools in the modern drug discovery which allow fast, automated, and high-output generation of organic compounds. In the recent past, ionic liquids have gained a renewed attention in view of their strong solvating ability, catalytic behaviour and recyclability. MCRs using ionic liquids (ILs) have gained much attention as efficient synthetic methods for synthesis of complex novel molecules/hybrids scaffolds. The beauty of the MCR is that it brings together divergent aspects of green chemistry within a single umbrella so that one can, execute and monitor organic synthesis.

The important biological applications of these above mentioned heterocycles prompted us to synthesize a series of novel substituted and fused pyrimidine derivatives incorporating those heterocyles using simple, convenient, elegant, green and well-versed methodologies and to evaluate the activity of the synthesized compounds.

CHAPTER – II

SYNTHESIS OF NOVEL PYRANO[2,3-*d*]PYRIMIDINES USING IONIC LIQUID

In this chapter a safe and an efficient approach for the synthesis of novel pyrano[2,3-*d*]pyrimidines *via* one-pot three component reaction of 2-amino-5-oxo-4-phenyl-4,5-dihydropyrano[3,2-*c*]chromene-3-carbontrile / 2-amino-7-methyl-5-oxo-4,5-dihydropyrano [4,3b]pyran-3-carbonitriles (**1a-c**/**5a-c**), N,N-dimethylformamide dimethyl acetal (**2**) and aromatic amines (**3**) by using [Bmim]HSO_4 ionic liquid was described (**Schemes 1-2**).

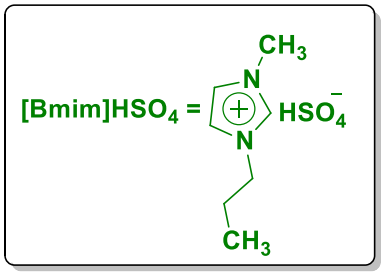
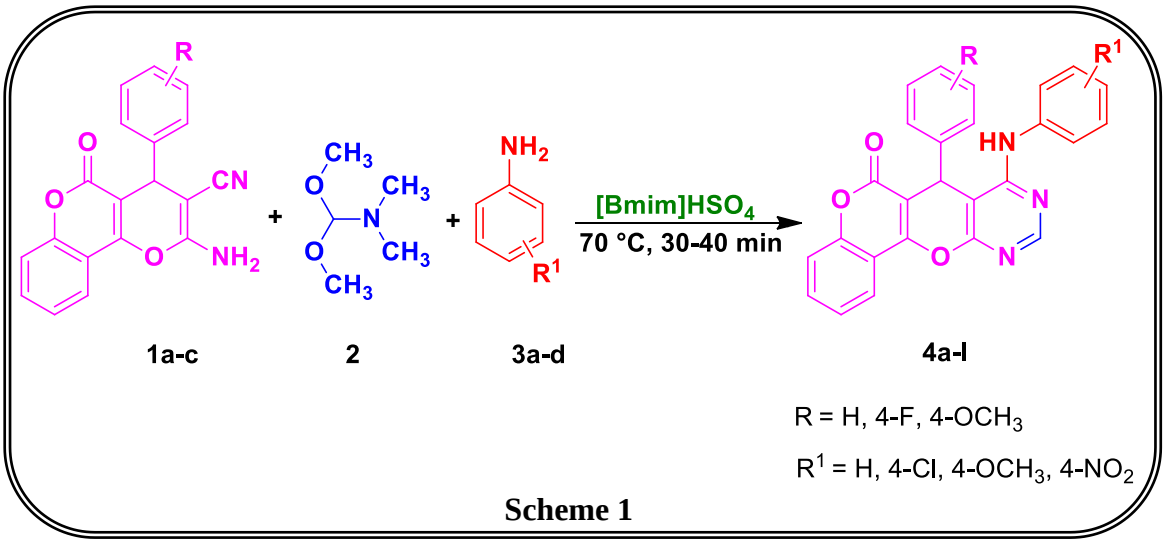
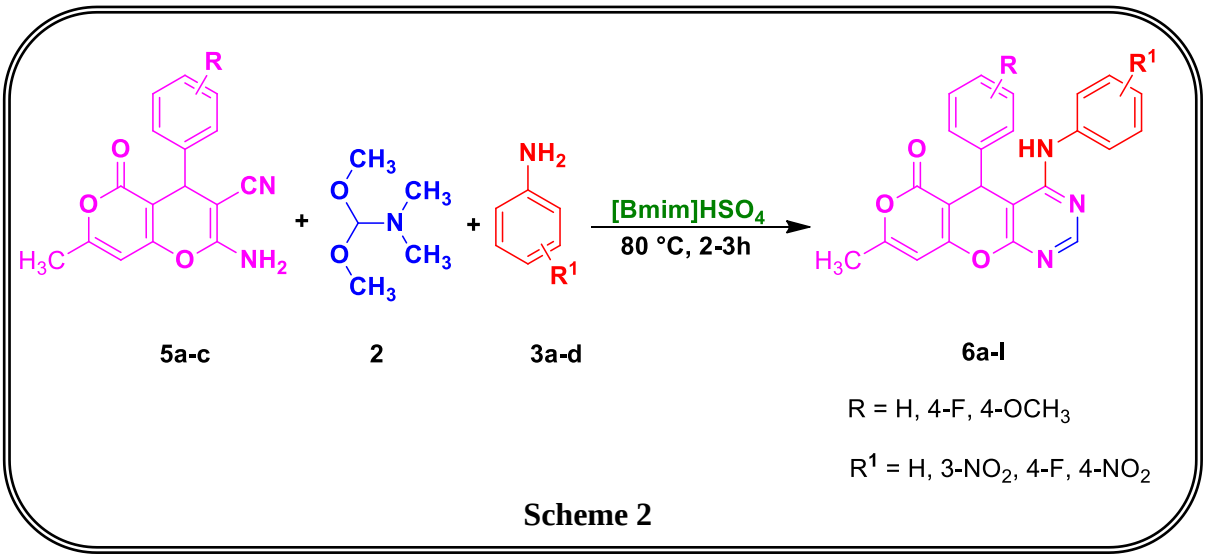


Figure 3. 1-Butyl-3-methylimidazolium hydrogen sulphate [Bmim]HSO_4



Scheme 1



Scheme 2

We have developed an efficient, practically convenient and environmentally safe method for the synthesis of novel pyrano[2,3-*d*]pyrimidines in good yield using [Bmim]HSO_4 ionic liquid. Structures of all the newly synthesized compounds were established by elemental analyses and spectral data. This protocol has the advantages of simple work-up, short reaction times, milder reaction conditions and good yields by using environmentally benign reusable solvent. The structure of **4i** and **6i** was also confirmed by single-crystal X-ray diffraction (**Figure 4** and **5**).

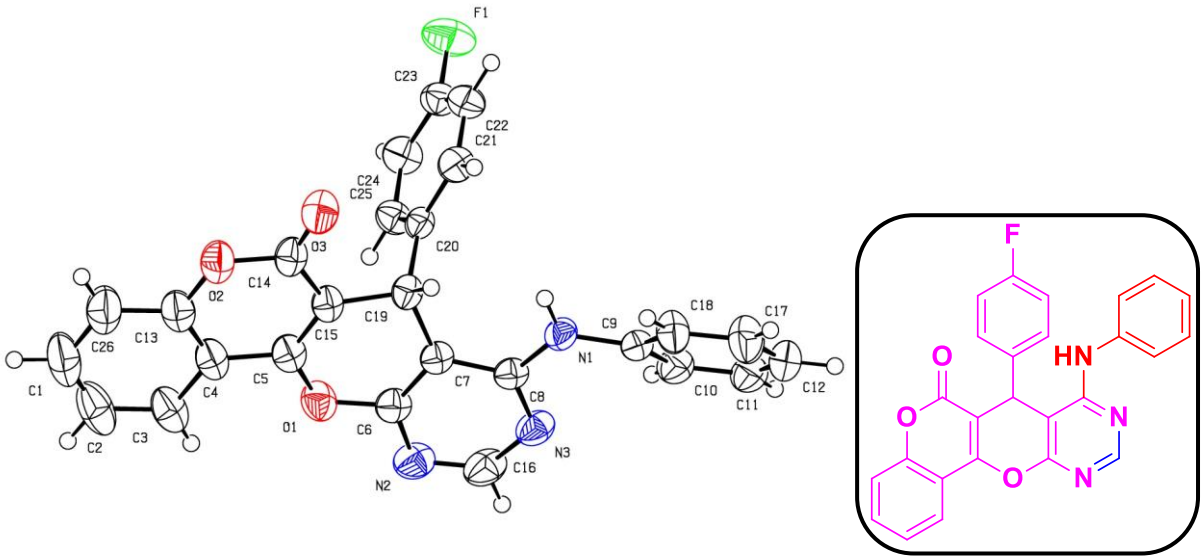


Figure 4. ***ORTEP representation of compound 4i (CCDC 1440717.Thermal ellipsoids are drawn at 50% probability level.***

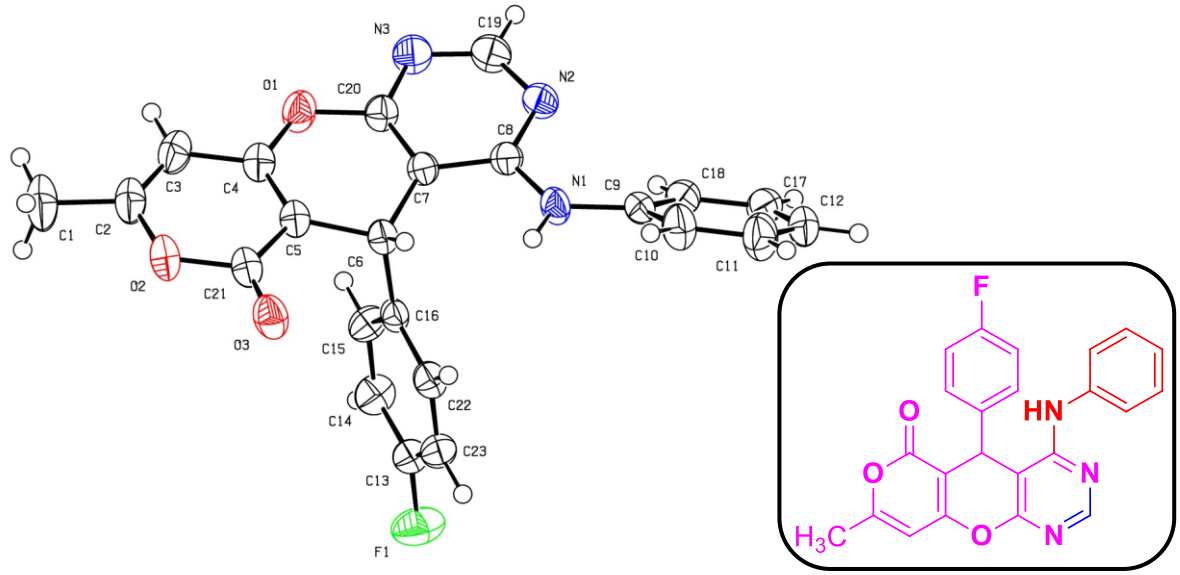
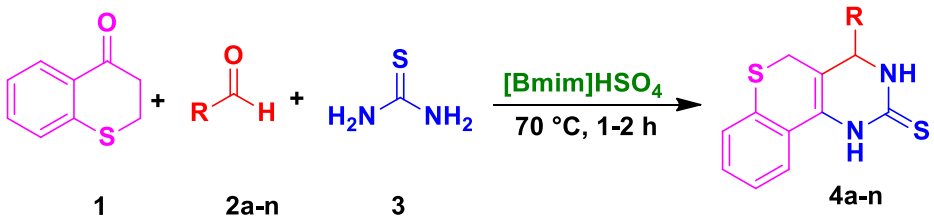


Figure 5. ***ORTEP representation of compound 6i (CCDC 1401827).Thermal ellipsoids are drawn at 50% probability level.***

CHAPTER – III (SECTION-A)

SYNTHESIS OF FUSED DIHYDRO-1*H*-THIOCHROMENO[4,3-*d*]PYRIMIDINES UNDER GREEN CONDITIONS

This section deals with a simple, efficient and eco-friendly method for the synthesis of fused dihydro-1*H*-thiochromeno[4,3-*d*]pyrimidine-2(5*H*)-thiones (**4a-n**) under green method. The conditions were optimized for the designed protocol based on the reaction of thiochroman-4-one **1**, aromatic aldehyde **2a-n** and thiourea **3** in the presence of [Bmim]HSO_4 (**Scheme 3**).



1 **2a-n** **3** **4a-n**

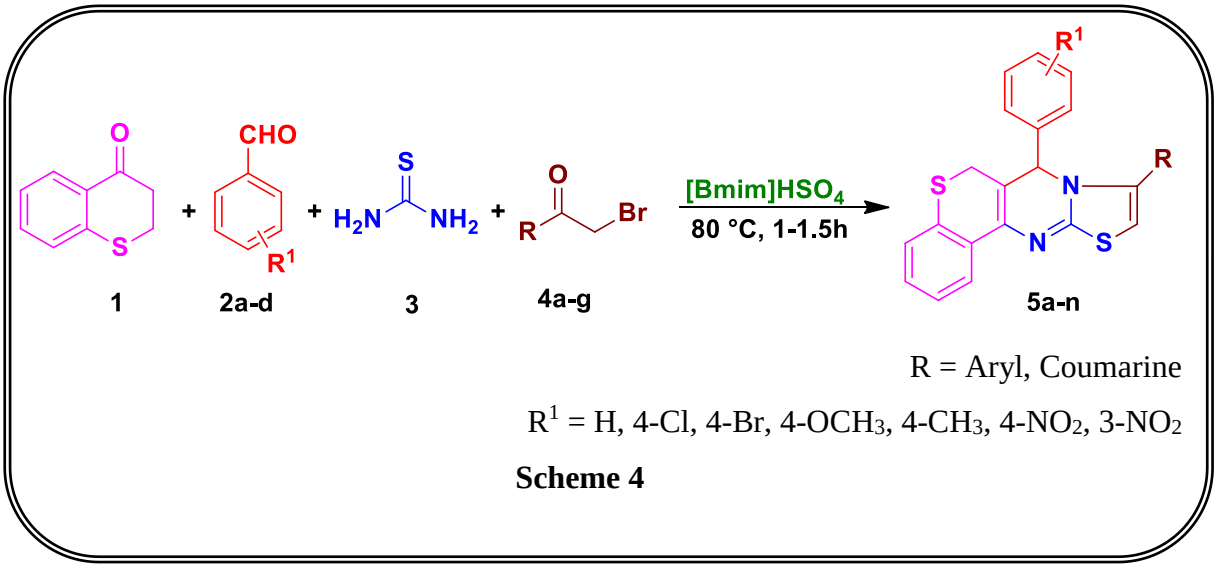
R = C_6H_5, 4-Me-C_6H_4, 4-OMe-C_6H_4, 4-Cl-C_6H_4, 4-F-C_6H_4, 4-NO_2-C_6H_4, 3-NO_2-C_6H_4, 2-Cl C_6H_4, $C_{10}H_7$, 6-OMe-$C_{10}H_6$, $C_{15}H_{12}N_2$, 4-NO_2-$C_{15}H_{12}N_2$, 4-Cl-$C_{15}H_{12}N_2$, $C_{15}H_{12}N_2$, 4-OCH_3-$C_{15}H_{12}N_2$

Scheme 3

CHAPTER – III (SECTION-B)

GREE CHEMICAL SYNTHESIS OF FUSED THIAZOLO[4,3-d] THIOCHROMENO [4,3-*d*]PYRIMIDINE DERIVATIVES

In this section, an efficient, simple and green procedure for the synthesis of thiazolo[4,3-*d*] thiochromeno[4,3-*d*]pyrimidine derivatives has been described by sequential condensation of equimolar mixture of thiochroman-4-one **1**, benzaldehyde **2**, thiourea **3** and phenacyl bromide **4** by using ionic liquid [Bmim]HSO_4 (**Scheme 4**).



R = Aryl, Coumarine

R^1 = H, 4-Cl, 4-Br, 4-OCH_3, 4-CH_3, 4-NO_2, 3-NO_2

Scheme 4

All the synthesized compounds were established by their spectral (IR, ^{1}H NMR, ^{13}C NMR and Mass) and elemental analysis data. The structure of **5a** was also confirmed by single-crystal X-ray diffraction (**Figure 6**).

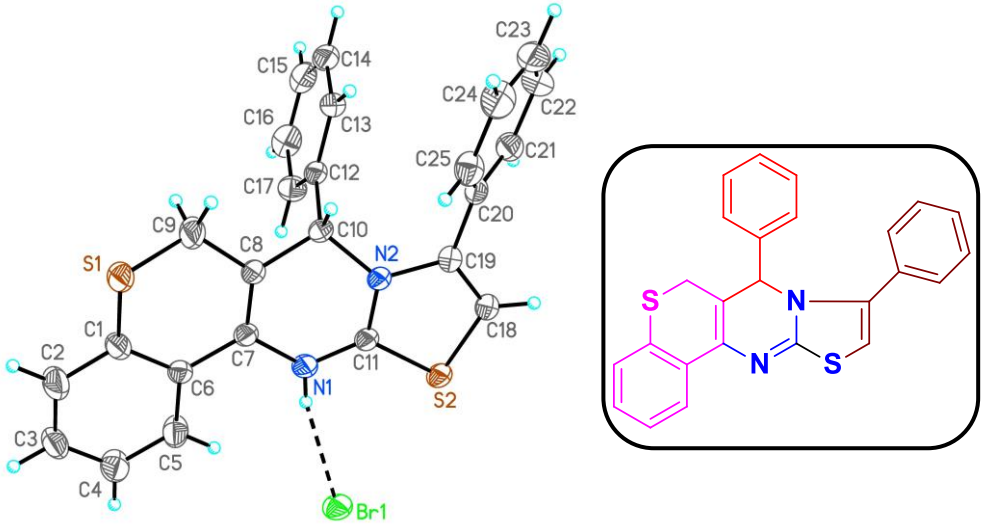


Figure 6. ***ORTEP representation of compound 5a (CCDC 1451972). Thermal ellipsoids are drawn at 50% probability level.***

CHAPTER – IV

SYNTHESIS OF NOVEL PYRAZOLO-PYRIMIDO[4,5-*d*]PYRIMIDINE DERIVATIVES USING [Bmim]$FeCl_4$ IONIC LIQUID

A series of novel pyrazolo-pyrimido[4,5-*d*]pyrimidine derivatives (**5a-x**), were synthesized by an efficient, four-component sequential protocol by the reaction of 6-amino-1,3-dimethyluracil **1**, N,N-dimethylformamide dimethyl acetal **2**, pyrazole aldehydes **3** and aromatic amines **4**, employing ionic liquid [Bmim]$FeCl_4$ as a reaction medium (**Scheme 5**).

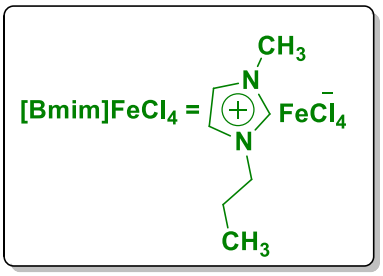
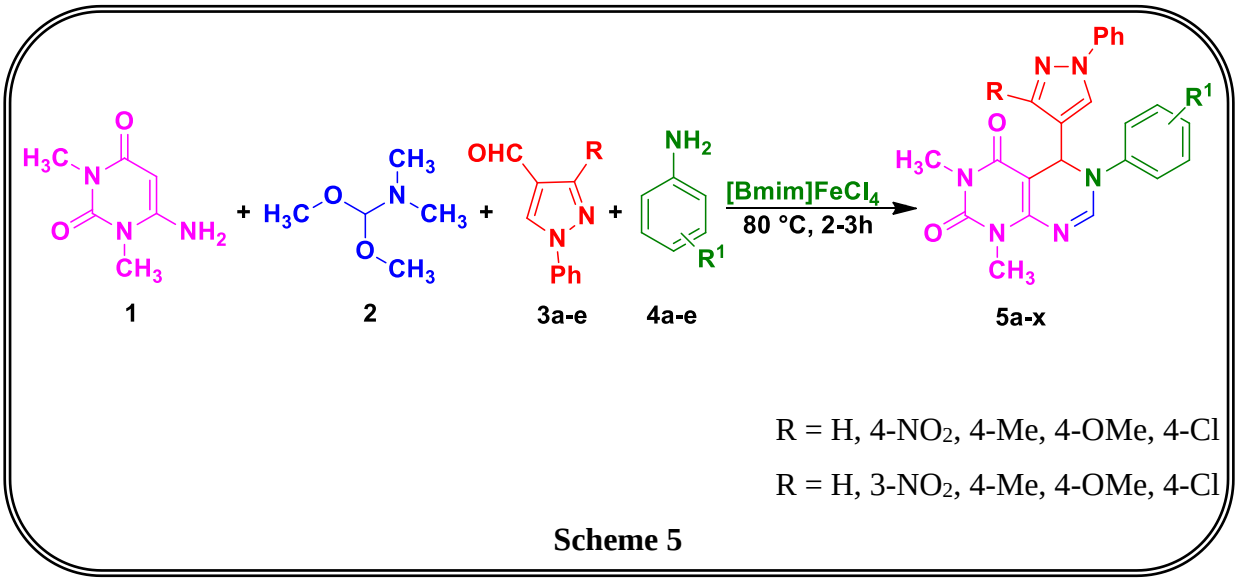


Figure 7. 1-Butyl-3-methylimidazolium tetrachloroferrate [Bmim]$FeCl_4$



Scheme 5

The structures of newly synthesized compounds **5a-x** were established on the basis of their elemental analyses, IR, ^{1}H NMR, ^{13}C NMR and mass spectral data. The structure of **5f** was also confirmed by single-crystal X-ray diffraction (**Figure 8**).

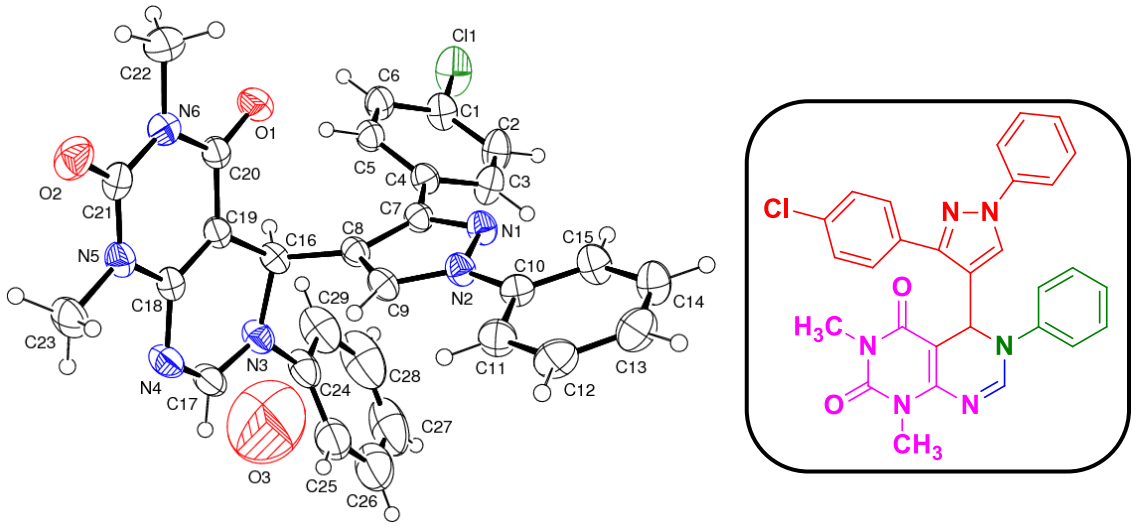
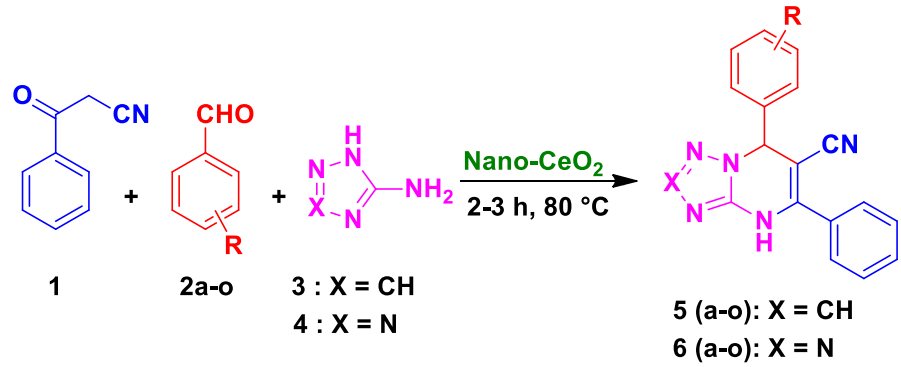


Figure 8. ***ORTEP representation of compound 5f (CCDC* 1487732*). Thermal ellipsoids are drawn at 50% probability level.***

CHAPTER – V

ONE-POT THREE COMPONENT SYNTHESIS OF NEW TYPE OF FUSED TRIAZOLO[1,5-*a*] AND TETRAZOLO[1,5-*a*]PYRIMIDINES

This chapter represents an efficient, simple and convenient method for the one-pot three-component synthesis of novel fused triazolo[1,5-*a*] and tetrazolo[1,5-*a*]pyrimidine derivatives. Nano CeO_2 catalysed reaction of benzoylacetonitrile **1**, benzaldehyde **2** and 5-aminotriazole or 5-aminotetrazole **3** in the presence of water (**Scheme 6**) produced the title compounds (**5a-o**) and (**6a-o**).



R = H, 4-F, 4-Cl, 4-COOMe, 4-CH_3, 4-OCH_3, 4-NO_2, 4-CF_3, 4-OH, 4-OH 3-OCH_3, 4-OCH_3- 3-OCH_3, 3-NO_2, 3-NO_2, 2-Cl, $C_{10}H_7$

Scheme 6

The newly synthesized compounds were characterized by IR, ^{1}H NMR, ^{13}C NMR, mass spectra, and elemental analysis. The structure of compound **5a** was further confirmed by single crystal X-ray diffraction analysis (**Figure 9**).

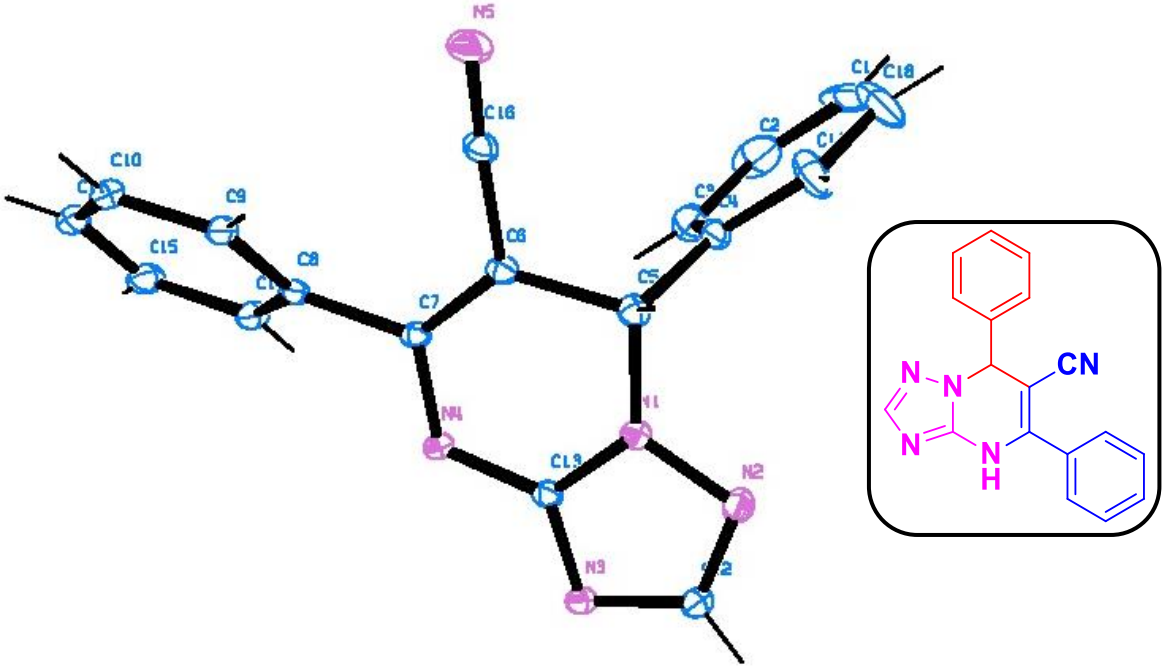


Figure 9. ***PLATON representation of compound 4a*** **(*CCDC-1440718). Thermal ellipsoids are drawn at 50% probability level.***

CHAPTER – VI

SYNTHESIS OF NEW PYRAZOLO[1,2-*b*]PHTHALAZINE PYRIMIDINES DERIVATIVES BY USING [BSO$_3$Hmim]HSO$_4$

In this chapter, we describe the synthesis of novel pyrazolo[1,2-*b*]phthalazine pyrimidine derivatives (**4az-bd**). As part of our ongoing research work concerned with the development of green chemical protocols for the synthesis of biologically active heterocycles from using ionic liquids, the reaction of 3-amino-5,10-dioxo-1-phenyl-5,10-dihydro-1*H*-pyrazolo[1,2-*b*]phthalazine-2-carbonitrile **1,** N,N dimethylformamide dimethyl acetal **2** with aromatic amines **3** in the presence of ionic liquid [BSO$_3$Hmim]HSO$_4$ resulted in the formation of the required pyrimidines (**Scheme 7)**.

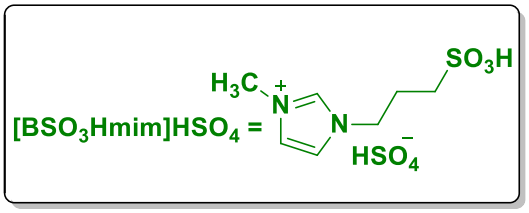
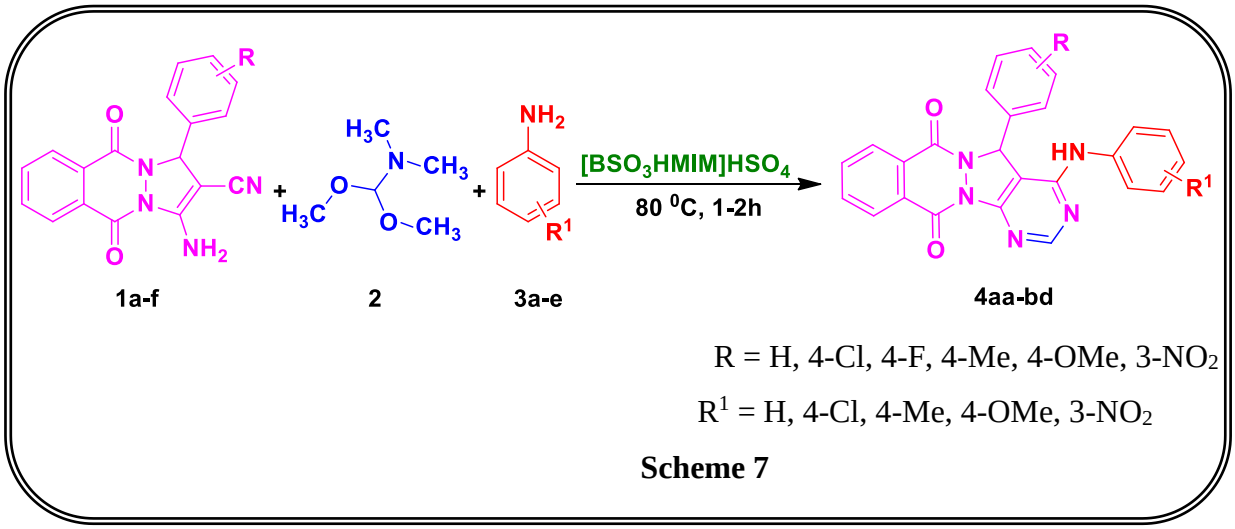


Figure 10. 1-(4-Sulfobutyl)-3-methylimidazolium Hydrogen Sulfate $[BSO_3Hmim]HSO_4$



Scheme 7

The structures of all the newly synthesized compounds were conformed on the basis of their spectral and elemental data. Spectral data of compounds were in full agreement with proposed structures.

CHAPTER – VII

EVALUTIOON OF BIOLOGICAL ACTIVITY

This chapter deals with the biological evaluation of newly synthesized compounds which were presented in Chapter– II, Chapter –III and Chapter–IV. These synthesized compounds were subjected to *in vitro* antibacterial, antifungal and biofilm inhibition testing against various pathogenic strains. Some of the synthesized compounds from Chapter V and Chapter VI were also evaluated for *in vitro* antidiabetic activity. Preliminary results indicate that some of them exhibited promising activities and they deserve more consideration as potential antimicrobial and antidiabetic agents.

Antimicrobial activity

The antibacterial activity of the synthesized compounds was determined using well diffusion method[20] against different pathogenic bacterial strains procured from the Microbial Type Culture Collection and Gene Bank (MTCC), CSIR-Institute of Microbial Technology,

Chandigarh, India. The pathogenic reference strains were seeded on the surface of the Muller-Hinton agar Petri plates with 0.1 ml of previously prepared microbial suspensions individually containing 1.5×10^8cfu ml^{-1} (equal to 0.5 McFarland standard). Wells of 6.0 mm diameter were prepared in the media plates using a cork borer and the synthesized compounds dissolved in 10% DMSO at a dose range of 125 - 0.97 µg/mL were added in each well under sterile conditions in a laminar air flow chamber. Standard antibiotic solutions of Ciprofloxacin at a dose range of 125 - 0.97 µg $well^{-1}$, served as positive control, while the well containing DMSO served as negative control. The plates were incubated for 24 h at 37 °C for the different bacterial strains. All the experiments were carried out in triplicates maintaining a control and a standard drug Ciprofloxacin for antibacterial and Miconazole for antifungal studies.

Biofilm inhibition assay

The test compounds were screened in sterile 96 well polystyrene microtiter plates using the modified biofilm inhibition assay[21], against a panel of pathogenic bacterial strains including*Staphylococcus aureus* MTCC 96, *Staphylococcus aureus* MLS16 MTCC 2940, *Bacillus subtilis* MTCC121, *Pseudomonas aeruginosa* MTCC 2453, and *Klebsiellaplanticola* MTCC 530, which were cultured overnight in tryptone soy broth (supplemented with 0.5% glucose). All the experiments were carried out in triplicates maintaining a control and a standard drug Ciprofloxacin.

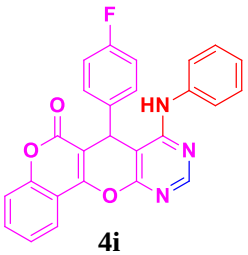
Antidiabetic activity

In vitro antidiabetc activity of some of the newly synthesized compounds was screened. All the experiments were carried out in triplicates maintaining a control and a standard drug Acarbose.

The detailed description of experimental protocols and results of evaluation of biological activity were presented in the same chapter of the thesis.

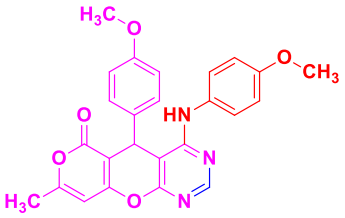
The structures of the molecules which were found to be biologically active which were synthesized under different schemes are shown below.

Pyrano[2,3-*d*]pyrimidine derivatives (chapter II)



4i

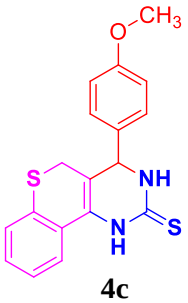
	MIC	**IC_{50}**
Antimicrobial activity	15.6 µg/mL	
Minimum Bactericidal Concentration Assay	15.6 µg/mL	
Biofilm inhibition assay		2.5 ± 0.18 µg/mL



6l

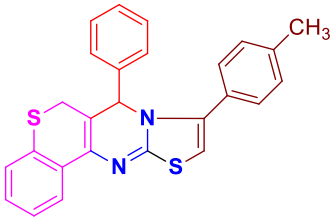
	MIC	**IC_{50}**
Antimicrobial activity	3.9 µg/mL	
Minimum Bactericidal Concentration Assay	7.8 µg/mL	
Antifungal activity	7.8 µg/mL	
Minimum Fungicidal Concentration	7.8 µg/mL	
Biofilm inhibition assay		2.5 ± 0.18 µg/mL

Dihydro-1*H*-thiochromeno[4,3-*d*]pyrimidines and thiazolo[4,3-*d*] thiochromeno[4,3-*d*]pyrimidine derivatives (chapter III)



4c **MIC** **IC_{50}**

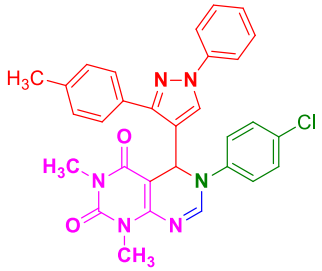
Antimicrobial activity	3.9 µg/mL	
Minimum Bactericidal Concentration Assay	3.9 µg/mL	
Biofilm inhibition assay		2.1 ± 0.44 µg/mL



5d **MIC** **IC_{50}**

Antimicrobial activity	3.9 µg/mL	
Minimum Bactericidal Concentration Assay	3.9 µg/mL	
Biofilm inhibition assay		1.9 ± 0.42 µg/mL

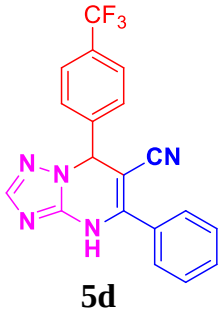
Pyrazolo-pyrimido[4,5-*d*]pyrimidine derivatives (chapter IV)



5l

	MIC	IC_{50}
Antimicrobial activity	3.9 µg/mL	
Minimum Bactericidal Concentration Assay	7.8 µg/mL	
Biofilm inhibition assay		1.8 ± 0.11 µg/mL

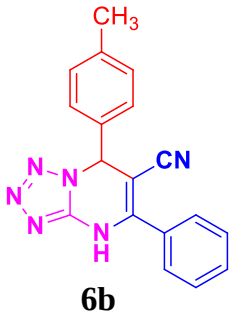
Triazolo[1,5-*a*] and tetrazolo[1,5-*a*]pyrimidine derivatives (chapter V)



5d

Antidiabetc activity

IC_{50} : 67.27 ± 2.94 µg/mL,

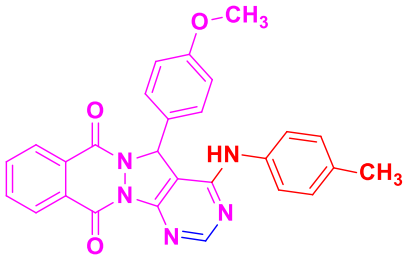


6b

Antidiabetc activity

IC_{50} : 65.75 ± 2.95 µg/mL

Pyrazolo[1,2-*b*]phthalazine pyrimidine derivatives (chapter VI)



4ab

Antidiabetc activity

IC_{50} : 32.87 ± 4.46 µg/mL