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Synthesis, characterization and biological evaluation of fused thiazolo[3,2-*a*]pyrimidine derivatives†

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A series of fused thiazolo[3,2-*a*]pyrimidines (**7a–g**, **8a–f**, **11a–g** and **12a,b**) have been synthesized in good yields by reaction of fused 3,4-dihydropyrimidin-2(1*H*)-thiones (**4a–g**) with phenacyl bromides (**5**,**6**)/3-(2-bromoacetyl)coumarins (**9**,**10**) under conventional heating in acetic acid. Analytical and spectral studies as well as single crystal X-ray diffraction data on the representative compound **8e** confirmed the structure of all the reaction products. All the synthesized compounds were screened for their antibacterial, antioxidant and DNA cleavage activities. The compound **7e** against *Escherichia coli*, **8a** and **8c–e** against *Pseudomonas aeruginosa* have shown prominent antibacterial activity compared to the standard drug Penicillin with MIC 9.375 μg mL^{-1}, whereas the compounds **11c**, **12a** and **12b** have shown very good antioxidant activity compared to the standard drug Trolox with IC_{50} values 12.36, 11.12 and 13.88 μM respectively. Compounds **11f** and **12b** have completely cleaved the DNA even at 50 μg mL^{-1} concentration and the remaining compounds have partially cleaved the DNA.

Introduction

Treatment of bacterial infections still remains an important and challenging therapeutic problem, due to the emergence of bacteria resistant to current therapeutic agents.[1] Reactive oxygen species (ROS) are causing damage to nucleic acids, proteins, carbohydrates and lipids in many types of cells including macrophages,[2] eventually leading to many chronic diseases.[3] Antioxidants exert their effects by preventing the generation of ROS, which can protect the formation of free radicals and retard the progress of diseases.[3] These are also useful to extend the shelf life of food and pharmaceuticals during processing, storage and transportation.[4] Deoxyribonucleic acid (DNA) is the primary target for most anticancer and antiviral therapies.[5] Therefore, the investigation towards the development of new antibiotics, antioxidants and molecules that can exhibit DNA binding and cleaving properties have become a necessary endeavor.

Dihydropyrimidine (DHPM) derivatives are known to possess diverse pharmacological properties including calcium channel modulators, mitotic kinesin inhibitors, adrenergic receptor antagonists, antibacterial and antiviral agents.[6] Hence, much attention has been focused on the modification of their core synthon. One such modification was observed when the reaction of dihydropyrimidin-thiones is carried out with 1,2-dielectrophiles such as 2-bromo ketones,[7] chloroacetyl chloride,[8] chloroacetic acid,[9] methyl chloroacetate[10] and 1,2-dichloroethane[11] to form ring annulation products, thiazolo[3,2-*a*]pyrimidine derivatives. The synthesis of thiazolo[3,2-*a*]-pyrimidines have recently gained considerable interest due to the broad spectrum of biological activities that include anti-inflammatory,[9a,12] antihypertensive,[13] antifungal,[7a] antibiofilm, antibacterial,[9b] antiviral,[9c] antioxidant,[14] anticancer,[15] anti-tumor,[16] anti-HIV,[17] calcium antagonists[7b] and group 2 metabotropic glutamate receptor antagonists.[18] They also serve as inhibitors of CDC25B phosphatase,[8] acetylcholinesterase (AChE) enzymes[19] and Bcl-2 family proteins.[20] The structures of thiazolo[3,2-*a*]pyrimidine derivatives and their pharmacological activities are given in Fig. 1.[6]

In continuation of our research towards the synthesis of biologically potent heterocyclic compounds,[21] herein we report an efficient method for the synthesis of fused thiazolo[3,2-*a*]-pyrimidine derivatives and to evaluate their antibacterial, antioxidant and DNA cleavage activities.

Results and discussion

Fused 3,4-dihydropyrimidin-2(1*H*)-thiones (**4a–g**) were prepared in good yields *via* a modified Biginelli reaction on 1-tetralone

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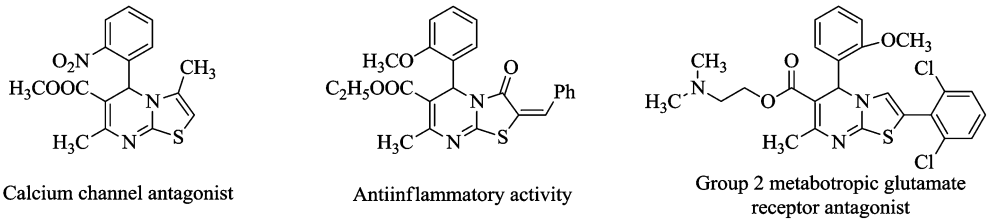


Fig. 1 Biologically potent thiazolo[3,2-*a*]pyrimidine derivatives.

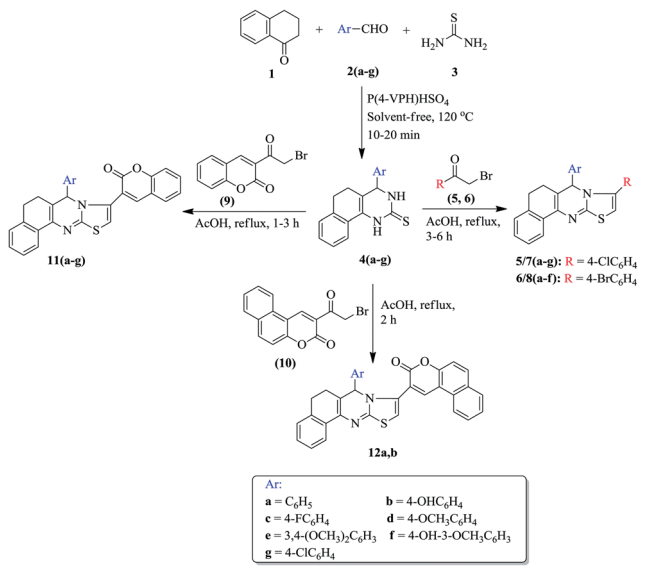
(**1**), aromatic aldehydes (**2a–g**) and thiourea (**3**) in the presence of poly(4-vinylpyridinium)hydrogen sulfate [P(4-VPH)HSO_4] as a catalyst under solvent-free conditions at 120 °C.[22] The title compounds, fused thiazolo[3,2-*a*]pyrimidines (**7a–g**, **8a–f**, **11a–g** and **12a,b**) were obtained by cyclization of **4a–g** with 4-chlorophenacyl bromide (**5**)/4-bromophenacyl bromide (**6**)/3-(2-bromoacetyl)-2*H*-chromen-2-one (**9**)/2-(2-bromoacetyl)-3*H*-benzo[*f*]chromen-3-one (**10**) under reflux in acetic acid (Scheme 1, Table 1).

The formation of title compounds **7a–g**, **8a–f**, **11a–g** and **12a,b** from fused 3,4-dihydropyrimidin-2(1*H*)-thiones (**4a-g**) were characterized by IR, ^{1}H NMR, ^{13}C NMR and mass spectral data. From the FTIR spectra, the disappearance of absorption bands ranging from 3144–3274 cm^{-1} for thioamide (–N–H) in the starting material and the appearance of a weak band ranging from 1598–1611 cm^{-1} in **7a–g** and **8a–f**, and 1628–1636 cm^{-1} in **11a–g** and **12a,b** of imine (–C=N–) group was indicative of formation of the products. Compounds **11a–g** and **12a,b** were also shown a strong band ranging from 1710–1740 cm^{-1} confirm the presence of lactone carbonyl (–O–C=O) group. From the ^{1}H NMR spectra, the absence of singlets at 8.98–9.47 ppm and 9.66–9.81 ppm for thioamide (–N–H) in the starting material, and from ^{13}C NMR the disappearance of a signal at 173.2–174.3 ppm of cyclic thiourea carbon (–C=S), and the presence of a signal at 160.9–163.3 ppm of imine carbon (–C=N) confirmed the formation of the products. In addition, the molecular ion peak from the mass spectra, elemental analyses data and single crystal X-ray diffraction structure of a compound **8e** are further evidences for the formation of products.

Table 1 Synthesis of fused thiazolo[3,2-*a*]pyrimidine derivatives

Product[a]	Ar	Time (h)	Yield[b] (%)	M.P. (°C)
7a	C_6H_5	5	82	319–320
7b	4-OHC_6H_4	4	80	292–294
7c	4-FC_6H_4	5	79	301–303
7d	4-$OCH_3C_6H_4$	4	82	260–262
7e	3,4-$(OCH_3)_2C_6H_3$	3	85	233–236
7f	4-OH-3-$OCH_3C_6H_3$	3	84	271–273
7g	4-ClC_6H_4	5	82	317–318
8a	C_6H_5	5	80	322–324
8b	4-OHC_6H_4	5	79	294–296
8c	4-FC_6H_4	6	80	298–300
8d	4-$OCH_3C_6H_4$	5	81	261–263
8e	3,4-$(OCH_3)_2C_6H_3$	3	86	242–244
8f	4-OH-3-$OCH_3C_6H_3$	4	84	280–282
11a	C_6H_5	1	88	290–291
11b	4-OHC_6H_4	3	82	293–294
11c	4-FC_6H_4	3	80	277–279
11d	4-$OCH_3C_6H_4$	2	87	275–277
11e	3,4-$(OCH_3)_2C_6H_3$	1	89	267–269
11f	4-OH-3-$OCH_3C_6H_3$	1	88	261–263
11g	4-ClC_6H_4	2	83	280–282
12a	C_6H_5	2	80	310–312
12b	4-OHC_6H_4	2	77	264–266

[a] Reaction conditions: fused 3,4-dihydropyrimidin-2(1*H*)-thiones (**4a–g**, 1 mmol), 4-chlorophenacyl bromide/4-bromophenacyl bromide/3-(2-bromoacetyl)-2*H*-chromen-2-one/2-(2-bromoacetyl)-3*H*-benzo[*f*]chromen-3-one (1 mmol), acetic acid (10 mL), reflux. [b] Isolated yields.



Scheme 1 Synthesis of fused 3,4-dihydropyrimidin-2(1*H*)-thiones and thiazolo[3,2-*a*]pyrimidines.

Single crystal X-ray diffraction analysis of compound **8e** was carried out on a crystal obtained from the reaction mixture. The compound was crystallizes in the triclinic space group $P\bar{1}$ with one completely protonated **8e**, one partially protonated **8e**, one Br^-, one partially proton transferred HBr and three molecules of acetic acid in the asymmetric unit. The ORTEP representation of the molecular structure of **8e** is shown in Fig. 2. A summary of the crystallographic data, structure refinement details, the geometrical parameters of hydrogen bonds and packing diagram is given in the supporting file.

All the synthesized compounds were screened for their *in vitro* antibacterial activity against *Bacillus subtilis*, *Staphylococcus aureus*, *Staphylococcus epidermidis* (Gram-positive),

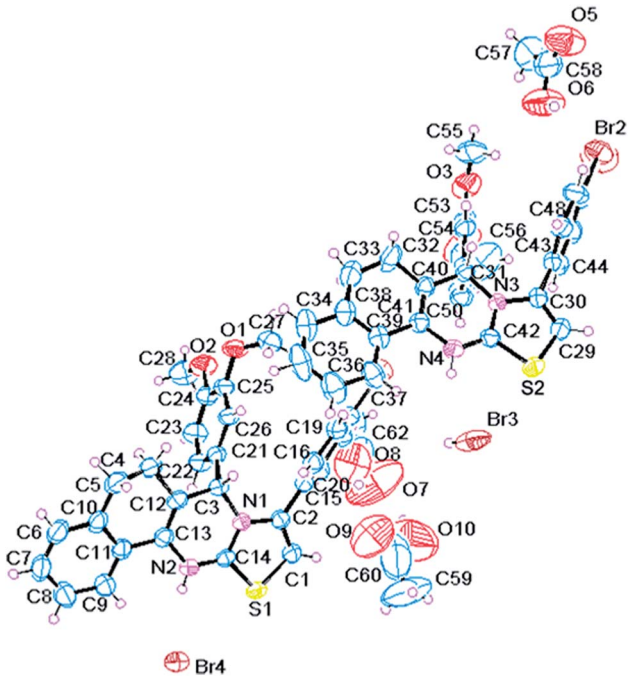


Fig. 2 ORTEP representation of compound **8e**. Thermal ellipsoids are drawn at 50% probability level.

Escherichia coli, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* (Gram-negative) bacterial strains by micro dilution method recommended by CLSI standard protocol[23] using Penicillin and Streptomycin as standard drugs. The results are tabulated in Table 2.

Evaluation of the antibacterial screening data revealed that, the compound **7e** against *Escherichia coli*, **8a** and **8c–e** against *Pseudomonas aeruginosa* have shown prominent activity than the standard drug Penicillin (MIC: 12.5 μg mL^{-1}) with MIC 9.375 μg mL^{-1} (~1.33 fold potent than Penicillin, Table 2). The potential activity may be attributed due to the presence of pharmacologically active moieties like phenyl, 4-fluoro phenyl, 4-methoxy phenyl and 3,4-dimethoxy phenyl groups on pyrimidine core and 4-chloro phenyl/4-bromo phenyl group on thiazole core. Among all the compounds, **8d** is active against all the tested bacterial strains. Remaining compounds derived from 4-chlorophenacyl bromide and 4-bromophenacyl bromide has shown weak to moderate activity. Compound **11a** (bearing pyranone moiety) has shown weak activity against Gram-positive bacterial strains whereas inactive against Gram-negative bacterial strains, rest of the compounds (**11b–g** and **12a,b**) were inactive against all the tested bacterial strains.

All the synthesized compounds were also screened for free radical scavenging activity in terms of hydrogen donating or radical scavenging ability by DPPH method.[24] Methanol (95%), DPPH solution and standard compounds (Trolox, Curcumin, Quercetin and Ascorbic acid) were used as blank, control and reference respectively. The IC_{50} values of the compounds under study for antioxidant results are shown in Table 3.

There is mounting evidence for the radical scavenging capacity of phenol and coumarin derivatives.[25] The free radical scavenging ability of compounds in present study results have also exhibited good scavenging ability for compounds bearing 4-fluoro phenyl and 2*H*-chromen-2-one (**11c**, IC_{50} value 12.36 μM), and phenyl/4-hydroxy phenyl and 3*H*-benzo[*f*]chromen-3-one (**12a**, IC_{50} value 11.12 μM and **12b**, IC_{50} value 13.88 μM). These on comparison with the standard Trolox with IC_{50} value of 14.22 μM found to be active by ~1.15 fold for **11c**, ~1.27 fold

Table 2 Antibacterial activity of fused thiazolo[3,2-*a*]pyrimidine derivatives[a]

	MIC (μg mL^{-1})					
Compound	Bs	Sa	Se	Ec	Pa	Kp
7b	75	75	75	75	18.75	75
7d	—	—	—	—	—	150
7e	75	—	—	9.375	37.5	75
7f	—	—	—	—	75	75
7g	—	—	—	—	—	150
8a	—	18.75	—	18.75	9.375	37.5
8b	75	37.5	75	37.5	37.5	75
8c	—	9.375	—	18.75	9.375	37.5
8d	18.75	18.75	37.5	18.75	9.375	18.75
8e	—	150	—	150	9.375	37.5
8f	75	150	75	37.5	75	150
11a	75	150	75	—	—	—
Penicillin	1.562	1.562	3.125	12.5	12.5	6.25
Streptomycin	6.25	6.25	3.125	6.25	1.562	3.125

[a] Gram-positive bacterial strains:-Bs: *Bacillus subtilis*; Sa: *Staphylococcus aureus*; Se: *Staphylococcus epidermidis*; Gram-negative bacterial strains:-Ec: *Escherichia coli*; Pa: *Pseudomonas aeruginosa*; Kp: *Klebsiella pneumoniae*. "-" Indicates the concentration >150 μg mL^{-1}.

Table 3 Antioxidant activity of fused thiazolo[3,2-*a*] pyrimidines

Compound	IC_{50} (μM)
7a	82.58
7b	80.50
7c	25.79
7d	80.91
7e	66.75
7f	41.29
7g	29.67
8a	44.75
8b	46.57
8c	26.01
8d	74.86
8e	63.32
8f	28.76
11a	64.34
11b	18.25
11c	12.36
11d	53.22
11e	47.54
11f	28.45
11g	47.26
12a	11.12
12b	13.88
Trolox	14.22
Curcumin	10.32
Quercetin	9.84
Ascorbic acid	3.8

for **12a** and ~1.02 fold for **12b**. Compound possessing 4-hydroxy phenyl/4-fluoro phenyl/4-hydroxy-3-methoxy phenyl/4-chloro phenyl on pyrimidine core, and 4-chloro phenyl/4-bromo phenyl/2H-chromen-2-one on thiazole core (**7c**, **7g**, **8c**, **8f**, **11b** and **11f**) have shown moderate scavenging ability with IC_{50} values 25.79, 29.67, 26.01, 28.76, 18.25 and 28.45 μM respectively. Remaining compounds have shown weak scavenging ability/capacity with IC_{50} values ranging from 41.29–82.58 μM.

The DNA cleavage activity of the titled compounds (**7a–g**, **8a–f**, **11a–g** and **12a,b**) was determined using agarose gel electrophoresis method.[26] DNA cleavage is controlled by relaxation of supercoiled circular form into nicked circular form and linear form. When circular plasmid DNA is subjected to electrophoresis, relatively fast migration will be observed for the intact supercoiled form. If one strand is cleaved, the supercoils will relax to produce a slower moving open circular form. If both strands are cleaved, a linear form will be generated and migrates between supercoiled and open circular forms. The pictures of the gels are presented in Fig. 3.

The gel after electrophoresis clearly revealed that, the plasmid pUC18 having three forms of DNA, whereas in case of standard $FeSO_4$ (10 mg/1 mL) complete DNA cleavage was observed. Compared to standard $FeSO_4$, compound **11f** and **12b** have completely cleaved the DNA even at 50 μg mL^{-1}, whereas **7c**, **7f**, **8b** and **8e** have shown complete cleavage at 100 μg mL^{-1}. Remaining compounds have partially cleaved the DNA. From these results we deduce that, the presence of pyranone moiety on thiazole core, 4-hydroxy phenyl and 4-hydroxy-3-methoxy phenyl groups on pyrimidine core has enhanced the DNA cleavage activity.

Conclusion

In conclusion, we have synthesized a series of fused thiazolo[3,2-*a*]pyrimidines under conventional method in good yields, and evaluated their antibacterial, antioxidant and DNA cleavage activities. The compound **7e** against *E.coli*, **8a** and **8c–e** against *P.aeruginosa* are shown marked antibacterial activity and the compounds **11c**, **12a** and **12b** are shown very good antioxidant activity. Similarly, the compound **11f** and **12b** are shown complete DNA cleavage even at 50 μg mL^{-1}. These results are suggesting that the synthesized compounds can be better candidates for future investigations.

Experimental

All the reagents and solvents were purchased from Aldrich/Merck and used without further purifications. Melting points were determined in open capillaries using Stuart SMP30 apparatus (Bibby Scientific Ltd. United Kingdom) and are uncorrected. The progress of the reactions as well as purity of compounds was monitored by thin layer chromatography with F_{254} silica-gel precoated sheets (Merck, Darmstadt, Germany) using hexane/ethyl acetate 8/2 as eluent; UV light and iodine vapours were used for detection. IR spectra were recorded on a Perkin-Elmer 100S spectrometer (Perkin-Elmer Ltd. United

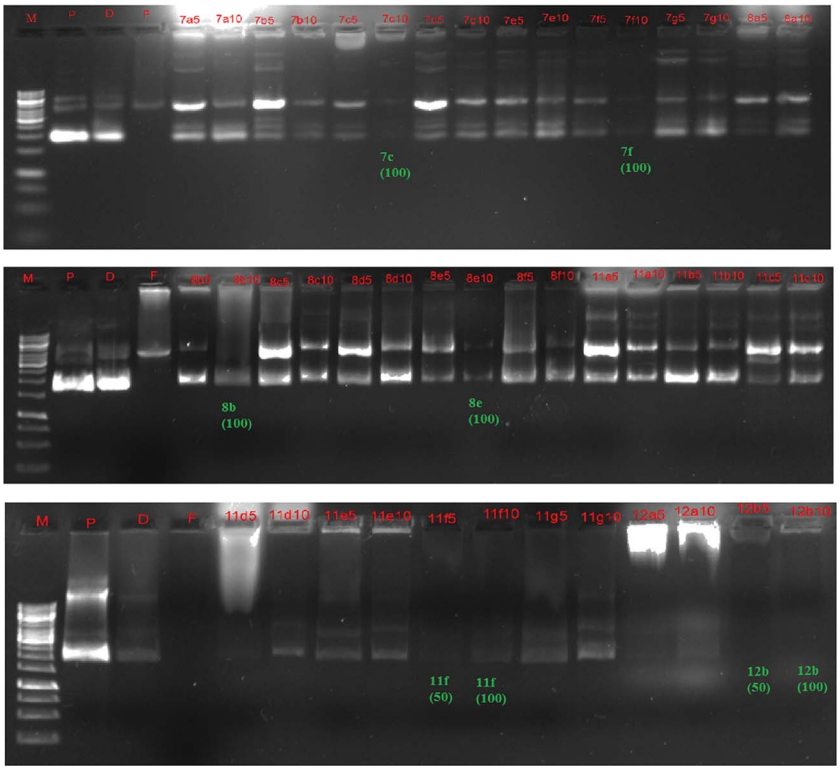


Fig. 3 DNA cleavage activity of fused thiazolo[3,2-*a*]pyrimidines (**7a–g**, **8a–f**, **11a–g** and **12a,b**) at 50 and 100 μg mL^{-1}. Where, M-Marker; P-Plasmid pUC18; D-DMSO treated plasmid; F–$FeSO_4$ (5 mM) treated plasmid; 5–50 μg mL^{-1}; 10–100 μg mL^{-1}.

Kingdom) utilizing KBr pellets; values are expressed in cm^{-1}. ^1H NMR and ^{13}C NMR spectra were obtained at 400 MHz and 100 MHz respectively on Bruker spectrometer (Bruker Corporation Ltd., Germany) using DMSO-d_6 as solvent and TMS as internal standard, chemical shifts are expressed in parts per million. Elemental analyses were performed on a Carlo-Erba model EA1108 analytical unit (Triad Scientific Ltd., New Jersey, USA) and the values are ± 0.4% of theoretical values. Mass spectra were recorded on a Jeol JMSD-300 spectrometer (Jeol Ltd., Tokyo, Japan).

Synthesis of 4-aryl-3,4,5,6-tetrahydrobenzo[*h*]quinazoline-2(1*H*)-thiones (4a–g)

To a clean and dry round bottom flask were added 1-tetralone (**1**, 1 mmol), aromatic aldehyde (**2a–g**, 1 mmol), thiourea (**3**, 1.2 mmol) and P(4-VPH)HSO_4 (0.015 g). The resulting mixture was heated at 120 °C under solvent-free conditions for 10–20 min. After completion of the reaction (monitored by TLC), water (5 mL) was added and the mixture was stirred at room temperature for an additional 10 min. The solid separated out was filtered, washed with water, dried and the crude product was crystallized from ethanol to afford the pure product. The aqueous layer containing catalyst was recovered under reduced pressure, washed with dichloromethane, dried and reused for subsequent runs.

4-Phenyl-3,4,5,6-tetrahydrobenzo[*h*]quinazoline-2(1*H*)-thione (4a)

Yellow solid; yield: 92%; Mp.: 258–259 °C; IR (KBr) υ_{max} (cm^{-1}): 3159 (NH), 1194 (C═S); ^1H NMR (400 MHz, DMSO-d_6): δ 1.80–1.88 (m, 1H), 2.15–2.23 (m, 1H), 2.55–2.62 (m, 1H), 2.69–2.77 (m, 1H), 4.95 (s, 1H), 7.17–7.22 (m, 3H), 7.31–7.41 (m, 5H), 7.68 (d, J = 7.2 Hz, 1H), 9.08 (s, 1H), 9.76 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 174.2, 142.8, 135.4, 128.6, 127.9, 127.7, 127.5, 126.9, 126.6, 126.3, 121.6, 111.1, 58.4, 27.3, 23.6; MS (ESI) m/z: 293 $[M + H]^+$; anal. calcd for $C_{18}H_{16}N_2S$: C, 73.94; H, 5.52; N, 9.58; found: C, 73.67; H, 5.71; N, 9.64.

4-(4-Hydroxyphenyl)-3,4,5,6-tetrahydrobenzo[*h*]quinazoline-2(1*H*)-thione (4b)

White solid; yield: 88%; Mp.: 270–272 °C; IR (KBr) υ_{max} (cm^{-1}): 3445 (OH), 3263, 3144 (NH), 1171 (C═S); ^1H NMR (400 MHz, DMSO-d_6): δ 1.79–1.87 (m, 1H), 2.09–2.18 (m, 1H), 2.57–2.63 (m, 1H), 2.67–2.73 (m, 1H), 4.82 (s, 1H), 6.75 (d, J = 8.4 Hz, 2H), 7.11 (d, J = 8.4 Hz, 2H), 7.15–7.22 (m, 3H), 7.67 (d, J = 7.2 Hz, 1H), 8.98 (s, 1H), 9.47 (s, 1H), 9.67 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 173.8, 157.1, 135.4, 133.3, 128.3, 127.8, 127.6, 127.5, 126.4, 126.3, 121.6, 115.3, 111.6, 58.0, 27.3, 23.6; MS (ESI) m/z: 309 $[M + H]^+$; anal. calcd for $C_{18}H_{16}N_2OS$: C, 70.10; H, 5.23; N, 9.08; found: C, 70.25; H, 5.17; N, 9.22.

4-(4-Fluorophenyl)-3,4,5,6-tetrahydrobenzo[*h*]quinazoline-2(1*H*)-thione (4c)

White solid; yield: 91%; Mp.: 252–254 °C; IR (KBr) υ_{max} (cm^{-1}): 3179 (NH), 1220 (C–F), 1190 (C═S); ^1H NMR (400 MHz, DMSO-d_6): δ 1.79–1.87 (m, 1H), 2.14–2.22 (m, 1H), 2.58–2.64 (m, 1H), 2.69–2.73 (m, 1H), 4.99 (s, 1H), 7.17–7.24 (m, 5H), 7.34–7.37 (m, 2H), 7.69 (d, J = 7.2 Hz, 1H), 9.09 (s, 1H), 9.78 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 174.2, 162.9, 139.1, 135.4, 129.0, 128.9, 127.8, 127.5, 126.7, 126.3, 121.7, 115.6, 115.4, 110.9, 57.6, 27.3, 23.5; MS (ESI) m/z: 311 $[M + H]^+$; anal. calcd for $C_{18}H_{15}FN_2S$: C, 69.65; H, 4.87; N, 9.03; found: C, 69.52; H, 4.95; N, 9.16.

4-(4-Methoxyphenyl)-3,4,5,6-tetrahydrobenzo[*h*]quinazoline-2(1*H*)-thione (4d)

Yellow solid; yield: 93%; Mp.: 211–212 °C; IR (KBr) υ_{max} (cm^{-1}): 3274, 3196 (NH), 1253 (C–O–C), 1172 (C═S); ^1H NMR (400 MHz, DMSO-d_6): δ 1.79–1.87 (m, 1H), 2.12–2.20 (m, 1H), 2.55–2.63 (m, 1H), 2.68–2.74 (m, 1H), 3.74 (s, 3H), 4.88 (s, 1H), 6.93–6.95 (m, 2H), 7.15–7.24 (m, 5H), 6.67 (d, J = 7.2 Hz, 1H), 9.00 (s, 1H), 9.69 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 173.9, 158.9, 135.4, 134.9, 128.2, 127.7, 127.6, 127.5, 126.5, 126.3, 121.6, 114.0, 111.4, 57.8, 55.1, 27.3, 23.6; MS (ESI) m/z: 323 $[M + H]^+$; anal. calcd for $C_{19}H_{18}N_2OS$: C, 70.78; H, 5.63; N, 8.69; found: C, 70.89; H, 5.54; N, 8.58.

4-(3,4-Dimethoxyphenyl)-3,4,5,6-tetrahydrobenzo[*h*]-quinazoline-2(1*H*)-thione (4e)

Yellow solid; yield: 94%; Mp.: 207–209 °C; IR (KBr) υ_{max} (cm^{-1}): 3196 (NH), 1258 (C–O–C), 1138 (C═S); ^1H NMR (400 MHz, DMSO-d_6): δ 1.87–1.93 (m, 1H), 2.16–2.21 (m, 1H), 2.61–2.65 (m, 1H), 2.69–2.75 (m, 1H), 3.73 (s, 6H), 4.89 (s, 1H), 6.82–6.85 (m, 1H), 6.92–6.97 (m, 2H), 7.16–7.23 (m, 3H), 7.66 (d, J = 7.2 Hz, 1H), 8.98 (s, 1H), 9.71 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 174.0, 148.7, 148.5, 135.4, 135.2, 127.8, 127.7, 127.6, 126.6, 126.3, 121.6, 119.0, 111.9, 111.3, 111.0, 58.1, 55.6, 55.5, 27.4, 23.6; MS (ESI) m/z: 353 $[M + H]^+$; anal. calcd for $C_{20}H_{20}N_2O_2S$: C, 68.16; H, 5.72; N, 7.95; found: C, 68.02; H, 5.88; N, 8.06.

4-(4-Hydroxy-3-methoxyphenyl)-3,4,5,6-tetrahydrobenzo[*h*]quinazoline-2(1*H*)-thione (4f)

Yellow solid; yield: 90%; Mp.: 230–232 °C; IR (KBr) υ_{max} (cm^{-1}): 3347 (OH), 3177 (NH), 1272 (C–O–C), 1195 (C═S); ^1H NMR (400 MHz, DMSO-d_6): δ 1.85–1.93 (m, 1H), 2.12–2.21 (m, 1H), 2.59–2.74 (m, 2H), 3.73 (s, 3H), 4.85 (s, 1H), 6.71–6.89 (m, 3H), 7.71–7.22 (m, 3H), 7.67 (d, J = 7.2 Hz, 1H), 8.97 (s, 1H), 9.06 (s, 1H), 9.69 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 173.9, 147.5, 146.4, 135.4, 133.8, 127.8, 127.6, 127.5, 126.5, 126.3, 121.6, 119.4, 115.5, 111.5, 58.2, 55.6, 27.4, 23.6; MS (ESI) m/z: 339 $[M + H]^+$; anal. calcd for $C_{19}H_{18}N_2O_2S$: C, 67.43; H, 5.36; N, 8.28; found: C, 67.31; H, 5.49; N, 8.35.

4-(4-Chlrophenyl)-3,4,5,6-tetrahydrobenzo[*h*]quinazoline-2(1*H*)-thione (4g)

White solid; yield: 94%; Mp.: 262–264 °C; IR (KBr) υ_{max} (cm^{-1}): 3269, 3194 (NH), 1195 (C═S), 775 (C–Cl); ^1H NMR (400 MHz, DMSO-d_6): δ 1.79–1.87 (m, 1H), 2.15–2.22 (m, 1H), 2.56–2.64 (m, 1H), 2.69–2.77 (m, 1H), 4.98 (s, 1H), 7.18–7.23 (m, 3H), 7.33 (d, J = 8.4 Hz, 2H), 7.46 (d, J = 8.4 Hz, 2H), 7.68 (d, J = 7.2 Hz, 1H), 9.10 (s, 1H), 9.81 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 174.3,

141.7, 135.4, 132.4, 128.8, 128.7, 127.8, 127.6, 126.9, 126.3, 121.7, 110.7, 57.6, 27.2, 23.4; MS (ESI) *m*/*z*: 327 $[M + H]^+$; anal. calcd for $C_{18}H_{15}ClN_2S$: C, 66.15; H, 4.63; N, 8.57; found: C, 66.24; H, 4.51; N, 8.69.

Synthesis of 9-(4-chlorophenyl)-7-aryl-6,7-dihydro-5*H*-benzo[*h*]thiazolo[2,3-*b*] quinazolines (7a–g)

4-Aryl-3,4,5,6-tetrahydrobenzo[*h*]quinazoline-2(1*H*)-thione (**4a–g**, 1 mmol) and 4-chloro phenacyl bromide (**5**, 1 mmol) were dissolved in 5 mL of glacial acetic acid and stirred at its reflux temperature for 3–5 h. After completion of the reaction (monitored by TLC), the mixture was kept a room temperature for 4 h, the solid separated out was filtered and quenched with cold acetic acid. The crude products obtained were purified by recrystallization from acetic acid.

9-(4-Chlorophenyl)-7-phenyl-6,7-dihydro-5*H*-benzo[*h*]thiazolo-[2,3-*b*]quinazoline (7a)

White solid; IR (KBr) υ_{max} (cm^{-1}): 1604 (C=N), 824 (C–Cl); ^1H NMR (400 MHz, DMSO-d_6): δ 1.76 (t, J = 7.6 Hz, 1H), 2.33 (t, J = 8.0 Hz, 1H), 2.57–2.79 (m, 2H), 6.16 (s, 1H), 6.84 (d, J = 6.4 Hz, 2H), 7.17–7.46 (m, 11H), 7.65 (d, J = 6.8 Hz, 1H); MS (ESI) *m*/*z*: 427 $[M + H]^+$; anal. calcd for $C_{26}H_{19}ClN_2S$: C, 73.14; H, 4.49; N, 6.56; found: C, 73.26; H, 4.59; N, 6.45.

4-(9-(4-Chlorophenyl)-6,7-dihydro-5*H*-benzo[*h*]thiazolo[2,3-*b*]-quinazolin-7-yl)phenol (7b)

Yellow solid; IR (KBr) υ_{max} (cm^{-1}): 3244 (OH), 1609 (C=N), 836 (C–Cl); ^1H NMR (400 MHz, DMSO-d_6): δ 1.75–1.82 (m, 1H), 2.26–2.32 (m, 1H), 2.61 (t, J = 8.0 Hz, 1H), 2.78 (t, J = 7.6 Hz, 1H), 6.04 (s, 1H), 6.52 (d, J = 8.4 Hz, 2H), 6.60 (d, J = 8.4 Hz, 2H), 7.25–7.40 (m, 6H), 7.50 (d, J = 8.0 Hz, 2H), 7.62 (d, J = 7.2 Hz, 1H), 9.63 (s, 1H); MS (ESI) *m*/*z*: 443 $[M + H]^+$; anal. calcd for $C_{26}H_{19}ClN_2OS$: C, 70.50; H, 4.32; N, 6.32; found: C, 70.37; H, 4.50; N, 6.44.

9-(4-Chlorophenyl)-7-(4-fluorophenyl)-6,7-dihydro-5*H*-benzo-[*h*]thiazolo[2,3-*b*]quinazoline (7c)

White solid; IR (KBr) υ_{max} (cm^{-1}): 1604 (C=N), 1236 (C–F), 837 (C–Cl); ^1H NMR (400 MHz, DMSO-d_6): δ 1.70–1.77 (m, 1H), 2.31 (t, J = 8.4 Hz, 1H), 2.59–2.64 (m, 1H), 2.75–2.79 (m, 1H), 6.20 (s, 1H), 6.89–7.03 (m, 4H), 7.25 (d, J = 8.4 Hz, 3H), 7.32–7.48 (m, 5H), 7.64 (t, J = 6.8 Hz, 1H); MS (ESI) *m*/*z*: 445 $[M + H]^+$; anal. calcd for $C_{26}H_{18}ClFN_2S$: C, 70.18; H, 4.08; N, 6.30; found: C, 70.09; H, 4.23; N, 6.44.

9-(4-Chlorophenyl)-7-(4-methoxyphenyl)-6,7-dihydro-5*H*-benzo[*h*]thiazolo[2,3-*b*]quinazoline (7d)

Yellow solid; IR (KBr) υ_{max} (cm^{-1}): 1607 (C=N), 1246 (C–O–C), 822 (C–Cl); ^1H NMR (400 MHz, DMSO-d_6): δ 1.75–1.82 (m, 1H), 2.28–2.35 (m, 1H), 2.60 (t, J = 8.4 Hz, 1H), 2.75–2.83 (m, 1H), 3.67 (s, 3H), 6.09 (s, 1H), 6.69–6.75 (m, 4H), 7.26–7.40 (m, 6H), 7.49 (d, J = 8.4 Hz, 2H), 7.63 (d, J = 7.6 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 161.3, 159.5, 138.5, 135.3, 134.9, 131.3, 130.6, 128.8, 128.5, 128.2, 128.1, 126.7, 123.7, 121.3, 114.0, 112.4, 110.1, 62.1, 55.1, 26.7, 23.2; MS (ESI) *m*/*z*: 457 $[M + H]^+$; anal. calcd for $C_{27}H_{21}ClN_2OS$: C, 70.96; H, 4.63; N, 6.13; found: C, 70.84; H, 4.72; N, 6.27.

9-(4-Chlorophenyl)-7-(3,4-dimethoxyphenyl)-6,7-dihydro-5*H*-benzo[*h*]thiazolo[2,3-*b*] quinazoline (7e)

Pale yellow solid; IR (KBr) υ_{max} (cm^{-1}): 1609 (C=N), 1245 (C–O–C), 834 (C–Cl); ^1H NMR (400 MHz, DMSO-d_6): δ 1.77–1.82 (m, 1H), 2.31 (t, J = 7.6 Hz, 1H), 2.59–2.79 (m, 2H), 3.47 (s, 3H), 3.67 (s, 3H), 6.13 (s, 1H), 6.56–6.58 (m, 1H), 6.81 (d, J = 8.4 Hz, 1H), 7.25–7.40 (m, 7H), 7.50 (d, J = 8.4 Hz, 2H), 7.63 (d, J = 7.6 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 161.1, 148.9, 147.9, 138.6, 135.3, 134.9, 131.5, 131.1, 128.8, 128.5, 128.1, 126.9, 126.7, 121.3, 119.5, 112.4, 112.1, 110.9, 109.8, 62.4, 55.4, 54.9, 26.7, 23.1; MS (ESI) *m*/*z*: 488 $[M + H]^+$; anal. calcd for $C_{28}H_{23}ClN_2O_2S$: C, 69.05; H, 4.76; N, 5.75; found: C, 69.30; H, 4.68; N, 5.81.

4-(9-(4-Chlorophenyl)-6,7-dihydro-5*H*-benzo[*h*]thiazolo[2,3-*b*]-quinazolin-7-yl)-2-methoxyphenol (7f)

White solid; IR (KBr) υ_{max} (cm^{-1}): 3306 (OH), 1605 (C=N), 1242 (C–O–C), 821 (C–Cl); ^1H NMR (400 MHz, DMSO-d_6): δ 1.78–1.84 (m, 1H), 2.26–2.34 (m, 1H), 2.59–2.65 (m, 1H), 2.75–2.81 (m, 1H), 3.47 (s, 3H), 6.09 (s, 1H), 6.42–6.45 (m, 1H), 6.63 (d, J = 8.4 Hz, 2H), 7.25–7.40 (m, 6H), 7.51 (d, J = 8.4 Hz, 2H), 7.66 (d, J = 7.6 Hz, 1H), 9.20 (s, 1H); MS (ESI) *m*/*z*: 473 $[M + H]^+$; anal. calcd for $C_{27}H_{21}ClN_2O_2S$: C, 68.56; H, 4.48; N, 5.92; found: C, 68.71; H, 4.36; N, 5.98.

7,9-Bis(4-chlorophenyl)-6,7-dihydro-5*H*-benzo[*h*]thiazolo[2,3-*b*]quinazoline (7g)

White solid; IR (KBr) υ_{max} (cm^{-1}): 1611 (C=N), 826 (C–Cl); ^1H NMR (400 MHz, DMSO-d_6): δ 1.72–1.79 (m, 1H), 2.31 (t, J = 8.0 Hz, 1H), 2.59–2.64 (m, 1H), 2.75–2.79 (m, 1H), 6.19 (s, 1H), 6.89 (d, J = 8.4 Hz, 2H), 7.23–7.35 (m, 7H), 7.39 (d, J = 7.6 Hz, 1H), 7.47 (d, J = 8.4 Hz, 2H), 7.62 (d, J = 7.6 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 161.5, 138.3, 137.5, 135.3, 135.0, 133.5, 131.4, 128.9, 128.7, 128.5, 128.1, 126.7, 121.4, 111.5, 110.1, 61.8, 26.6, 23.0; MS (ESI) *m*/*z*: 462 $[M + H]^+$; anal. calcd for $C_{26}H_{18}Cl_2N_2S$: C, 67.68; H, 3.93; N, 6.07; found: C, 67.51; H, 4.05; N, 6.19.

Synthesis of 9-(4-bromophenyl)-7-aryl-6,7-dihydro-5*H*-benzo-[*h*]thiazolo[2,3-*b*]quinazolines (8a–f)

A mixture of 4-aryl-3,4,5,6-tetrahydrobenzo[*h*]quinazoline-2(1*H*)-thione (**4a–f**, 1 mmol) and 4-bromo phenacyl bromide (**6**, 1 mmol) were taken in 5 mL of glacial acetic acid and stirred at its reflux temperature for 3–6 h. After completion of the reaction shown by TLC, the mixture was kept a room temperature for 4 h, the solid separated out was filtered, washed with cold acetic acid and recrystallized from acetic acid to afford the pure product.

9-(4-Bromophenyl)-7-phenyl-6,7-dihydro-5*H*-benzo[*h*]thiazolo-[2,3-*b*]quinazoline (8a)

White solid; IR (KBr) υ_{max} (cm^{-1}): 1603 (C=N), 578 (C–Br); ^1H NMR (400 MHz, DMSO-d_6): δ 1.72–1.80 (m, 1H), 2.28–2.34 (m,

1H), 2.59 (t, J = 8.0 Hz, 1H), 2.75–2.81 (m, 1H), 6.15 (s, 1H), 6.84 (d, J = 7.6 Hz, 2H), 7.15–7.40 (m, 9H), 7.58 (d, J = 8.4 Hz, 3H); MS (ESI) m/z: 472 $[M + H]^+$; anal. calcd for $C_{26}H_{19}BrN_2S$: C, 66.24; H, 4.06; N, 5.94; found: C, 66.15; H, 4.23; N, 5.78.

4-(9-(4-Bromophenyl)-6,7-dihydro-5*H*-benzo[*h*]thiazolo[2,3-*b*]-quinazolin-7-yl)phenol (8b)

Yellow solid; IR (KBr) υ_{max} (cm^{-1}): 3253 (OH), 1609 (C=N), 631 (C–Br); ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.76–1.84 (m, 1H), 2.26–2.34 (m, 1H), 2.58–2.65 (m, 1H), 2.76–2.84 (m, 1H), 6.05 (s, 1H), 6.52 (d, J = 8.4 Hz, 2H), 6.58 (d, J = 8.4 Hz, 2H), 7.20–7.38 (m, 6H), 7.61–7.69 (m, 3H), 9.61 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 161.2, 157.8, 138.6, 135.3, 131.5, 131.4, 128.9, 128.8, 128.2, 127.1, 126.7, 126.6, 123.6, 123.4, 121.3, 115.3, 112.6, 110.0, 62.3, 26.7, 23.2; MS (ESI) m/z: 487 $[M]^+$; anal. calcd for $C_{26}H_{19}BrN_2OS$: C, 64.07; H, 3.93; N, 5.75; found: C, 63.95; H, 4.04; N, 5.85.

9-(4-Bromophenyl)-7-(4-fluorophenyl)-6,7-dihydro-5*H*-benzo-[*h*]thiazolo[2,3-*b*]quinazoline (8c)

White solid; IR (KBr) υ_{max} (cm^{-1}): 1604 (C=N), 1227 (C–F), 632 (C–Br); ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.72–1.78 (m, 1H), 2.27–2.34 (m, 1H), 2.59–2.64 (m, 1H), 2.75–2.81 (m, 1H), 6.19 (s, 1H), 6.89–7.03 (m, 5H), 7.16–7.40 (m, 6H), 7.61 (d, J = 8.4 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 163.1, 161.5, 138.4, 135.3, 134.9, 131.5, 131.4, 129.2, 128.9, 128.1, 126.9, 126.7, 123.7, 121.2, 115.6, 115.4, 111.7, 61.8, 26.6, 23.0; MS (ESI) m/z: 490 $[M + H]^+$; anal. calcd for $C_{26}H_{18}BrFN_2S$: C, 63.81; H, 3.71; N, 5.72; found: C, 63.90; H, 3.87; N, 5.56.

9-(4-Bromophenyl)-7-(4-methoxyphenyl)-6,7-dihydro-5*H*-benzo[*h*]thiazolo[2,3-*b*]quinazoline (8d)

White solid; IR (KBr) υ_{max} (cm^{-1}): 1606 (C=N), 1245 (C–O–C), 634 (C–Br); ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.75–1.81 (m, 1H), 2.27–2.35 (m, 1H), 2.56–2.64 (m, 1H), 2.75–2.81 (m, 1H), 3.67 (s, 3H), 6.09 (s, 1H), 6.72 (d, J = 8.0 Hz, 4H), 7.19–7.40 (m, 7H), 7.63 (d, J = 8.4 Hz, 2H); MS (ESI) m/z: 502 $[M + H]^+$; anal. calcd for $C_{27}H_{21}BrN_2OS$: C, 64.67; H, 4.22; N, 5.59; found: C, 64.52; H, 4.45; N, 5.68.

9-(4-Bromophenyl)-7-(3,4-dimethoxyphenyl)-6,7-dihydro-5*H*-benzo[*h*]thiazolo[2,3-*b*]quinazoline (8e)

Pale yellow solid; IR (KBr) υ_{max} (cm^{-1}): 1598 (C=N), 1266 (C–O–C), 630 (C–Br); ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.77–1.83 (m, 1H), 2.31 (t, J = 8.0 Hz, 1H), 2.57–2.65 (m, 1H), 2.75–2.83 (m, 1H), 3.47 (s, 3H), 3.67 (s, 3H), 6.12 (s, 1H), 6.57–6.59 (m, 1H), 6.81 (d, J = 8.4 Hz, 2H), 7.21–7.40 (m, 7H), 7.64 (d, J = 8.8 Hz, 2H); MS (ESI) m/z: 532 $[M + H]^+$; anal. calcd for $C_{28}H_{23}BrN_2O_2S$: C, 63.28; H, 4.36; N, 5.27; found: C, 63.36; H, 4.49; N, 5.06.

4-(9-(4-Bromophenyl)-6,7-dihydro-5*H*-benzo[*h*]thiazolo[2,3-*b*]-quinazolin-7-yl)-2-methoxyphenol (8f)

White solid; IR (KBr) υ_{max} (cm^{-1}): 3306 (OH), 1600 (C=N), 1277 (C–O–C), 634 (C–Br); ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.76–1.84 (m, 1H), 2.25–2.33 (m, 1H), 2.59–2.65 (m, 1H), 2.75–2.81 (m, 1H), 3.48 (s, 3H), 6.07 (s, 1H), 6.44–6.46 (m, 1H), 6.63 (d, J = 8.0 Hz, 2H), 7.22–7.40 (m, 6H), 7.60–7.66 (m, 3H), 9.21 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 160.9, 146.9, 146.8, 138.6, 135.3, 131.6, 131.4, 129.5, 128.7, 128.1, 127.3, 126.7, 123.6, 123.5, 121.3, 119.7, 116.1, 112.6, 111.5, 109.8, 62.5, 55.1, 26.7, 23.2; MS (ESI) m/z: 518 $[M + H]^+$ anal. calcd for $C_{27}H_{21}BrN_2O_2S$: C, 62.67; H, 4.09; N, 5.41; found: C, 62.40; H, 4.25; N, 5.58.

Synthesis of 3-(7-aryl-6,7-dihydro-5*H*-benzo[*h*]thiazolo[2,3-*b*]-quinazolin-9-yl)-2*H*-chromen-2-one (11a–g)

To a mixture of 4-aryl-3,4,5,6-tetrahydrobenzo[*h*]quinazoline-2(1*H*)-thione (**4a–g**, 1 mmol) and 3-(2-bromoacetyl)-2*H*-chromen-2-one (**9**, 1 mmol) in 25 mL of round bottom flask; 5 mL of glacial acetic acid was added and refluxed for 1–3 h. The progress of the reaction was monitored by TLC. After completion of the reaction, the solid separated out was filtered and washed with hot acetic acid to afford the pure product.

3-(7-Phenyl-6,7-dihydro-5*H*-benzo[*h*]thiazolo[2,3-*b*]-quinazolin-9-yl)-2*H*-chromen-2-ne (11a)

Pale yellow solid; IR (KBr) υ_{max} (cm^{-1}): 1711 (C=O of lactone), 1630 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.76 (t, J = 6.4 Hz, 1H), 2.28–2.36 (m, 1H), 2.57–2.65 (m, 1H), 2.77–2.81 (m, 1H), 6.25 (s, 1H), 7.16–7.26 (m, 6H), 7.34–7.49 (m, 5H), 7.64–7.89 (m, 4H); MS (ESI) m/z: 461 $[M + H]^+$ anal. calcd for $C_{29}H_{20}N_2O_2S$: C, 75.63; H, 4.38; N, 6.08; found: C, 75.47; H, 4.49; N, 6.15.

3-(7-(4-Hydroxyphenyl)-6,7-dihydro-5*H*-benzo[*h*]thiazolo[2,3-*b*]quinazolin-9-yl)-2*H*-chromen-2-one (11b)

Pale yellow solid; IR (KBr) υ_{max} (cm^{-1}): 3430 (OH), 1727 (C=O of lactone), 1633 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.74–1.79 (m, 1H), 2.26 (t, J = 8.0 Hz, 1H), 2.62 (t, J = 8.0 Hz, 1H), 2.76–2.80 (m, 1H), 6.10 (s, 1H), 6.52 (d, J = 8.4 Hz, 2H), 6.92 (d, J = 8.4 Hz, 2H), 7.24–7.51 (m, 6H), 7.68–7.74 (m, 3H), 7.92 (s, 1H), 9.61 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 161.1, 158.5, 158.0, 153.5, 146.4, 135.4, 133.5, 133.4, 129.4, 129.1, 128.8, 128.5, 128.1, 126.6, 125.0, 121.4, 118.0, 116.2, 115.9, 115.5, 113.4, 112.0, 62.4, 26.7, 23.1; MS (ESI) m/z: 477 $[M + H]^+$; anal. calcd for $C_{29}H_{20}N_2O_3S$: C, 73.09; H, 4.23; N, 5.88; found: C, 73.15; H, 4.38; N, 5.60.

3-(7-(4-Fluorophenyl)-6,7-dihydro-5*H*-benzo[*h*]thiazolo[2,3-*b*]-quinazolin-9-yl)-2*H*-chromen-2-one (11c)

White solid; IR (KBr) υ_{max} (cm^{-1}): 1740 (C=O of lactone), 1634 (C=N), 1226 (C–F); ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.76 (t, J = 6.4 Hz, 1H), 2.30 (t, J = 8.0 Hz, 1H), 2.60–2.66 (m, 1H), 2.76–2.83 (m, 1H), 6.27 (s, 1H), 7.02 (t, J = 8.4 Hz, 2H), 7.23–7.26 (m, 3H), 7.32–7.50 (m, 5H), 7.63–7.75 (m, 3H), 7.93 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 163.3, 161.4, 160.9, 158.4, 153.5, 146.9, 135.4, 135.2, 133.6, 133.1, 129.5, 129.4, 128.9, 128.1, 126.6, 125.0, 124.1, 121.5, 117.9, 116.2, 115.9, 115.7, 113.6, 111.3, 61.8, 26.6, 22.9; MS (ESI) m/z: 479 $[M + H]^+$; anal. calcd for $C_{29}H_{19}FN_2O_2S$: C, 72.79; H, 4.00; N, 5.85; found: C, 72.54; H, 4.21; N, 5.72.

3-(7-(4-Methoxyphenyl)-6,7-dihydro-5*H*-benzo[*h*]thiazolo[2,3-*b*]-quinazolin-9-yl)-2*H*-chromen-2-one (11d)

Yellow solid; IR (KBr) υ_{max} (cm^{-1}): 1716 (C=O of lactone), 1608 (C=N), 1248 (C–O–C); ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.74–1.79 (m, 1H), 2.28 (t, J = 7.6 Hz, 1H), 2.61 (t, J = 8.0 Hz, 1H), 2.79 (t, J = 7.6 Hz, 1H), 3.58 (s, 3H), 6.18 (s, 1H), 6.69 (d, J = 8.4 Hz, 2H), 7.70 (d, J = 8.8 Hz, 2H), 7.24–7.51 (m, 6H), 7.66–7.87 (m, 4H); MS (ESI) m/z: 491 $[M + H]^+$; anal. calcd for $C_{30}H_{22}N_2O_3S$: C, 73.45; H, 4.52; N, 5.71; found: C, 73.27; H, 4.72; N, 5.63.

3-(7-(3,4-Dimethoxyphenyl)-6,7-dihydro-5*H*-benzo[*h*]thiazolo-[2,3-*b*]quinazolin-9-yl)-2*H*chromen-2-one (11e)

White solid; IR (KBr) υ_{max} (cm^{-1}): 1734 (C=O of lactone), 1636 (C=N), 1261 (C–O–C); ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.76–1.83 (m, 1H), 2.29 (t, J = 8.0 Hz, 1H), 2.61–2.66 (m, 1H), 2.76–2.80 (m, 1H), 3.26 (s, 3H), 3.59 (s, 3H), 6.15 (s, 1H), 6.68 (s, 1H), 6.75 (s, 2H), 7.25–7.50 (m, 6H), 7.66–7.65 (m, 3H), 7.88 (s, 1H); MS (ESI) m/z: 521 $[M + H]^+$; anal. calcd for $C_{31}H_{24}N_2O_4S$: C, 71.52; H, 4.65; N, 5.38; found: C, 71.39; H, 4.84; N, 5.47.

3-(7-(4-Hydroxy-3-methoxyphenyl)-6,7-dihydro-5*H*-benzo[*h*]-thiazolo[2,3-*b*]quinazolin-9-yl)-2*H*-chromen-2-one (11f)

Pale yellow solid; IR (KBr) υ_{max} (cm^{-1}): 3256 (OH), 1738 (C=O of lactone), 1638 (C=N), 1247 (C–O–C); ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.77–1.81 (m, 1H), 2.27 (t, J = 8.0 Hz, 1H), 2.60–2.66 (m, 1H), 2.77 (t, J = 7.6 Hz, 1H), 3.23 (s, 3H), 6.09 (s, 1H), 6.61 (d, J = 7.6 Hz, 3H), 7.24–7.50 (m, 6H), 7.61–7.74 (m, 3H), 7.93 (s, 1H), 9.18 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 161.0, 158.5, 153.5, 147.2, 146.4, 135.4, 133.6, 129.7, 129.4, 128.8, 128.1, 126.7, 125.0, 123.7, 121.4, 120.0, 118.0, 116.2, 112.0, 111.8, 62.7, 54.9, 26.7, 23.0; MS (ESI) m/z: 507 $[M + H]^+$; anal. calcd for $C_{30}H_{22}N_2O_4S$: C, 71.13; H, 4.38; N, 5.53; found: C, 71.02; H, 4.55; N, 5.41.

3-(7-(4-Chlorophenyl)-6,7-dihydro-5*H*-benzo[*h*]thiazolo[2,3-*b*]-quinazolin-9-yl)-2*H*-chromen-2-one (11g)

White solid; IR (KBr) υ_{max} (cm^{-1}): 1740 (C=O of lactone), 1636 (C=N), 840 (C–Cl); ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.73–1.78 (m, 1H), 2.27–2.35 (m, 1H), 2.60–2.66 (m, 1H), 2.76–2.80 (m, 1H), 6.27 (s, 1H), 7.22–7.26 (m, 5H), 7.34–7.51 (m, 5H), 7.64–7.75 (m, 3H), 7.94 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 161.5, 158.4, 153.5, 147.0, 137.8, 135.4, 133.9, 133.7, 133.0, 129.4, 129.0, 128.9, 128.1, 126.7, 125.0, 121.5, 117.9, 116.3, 115.7, 113.6, 111.1, 61.8, 26.6, 22.9; MS (ESI) m/z: 495 $[M + H]^+$; anal. calcd for $C_{29}H_{19}ClN_2O_2S$: C, 70.37; H, 3.87; N, 5.66; found: C, 70.50; H, 3.79; N, 5.74.

Synthesis of 2-(7-aryl-6,7-dihydro-5*H*-benzo[*h*]thiazolo[2,3-*b*]-quinazolin-9-yl)-3*H*-benzo[*f*]chromen-3-one (12a,b)

4-Aryl-3,4,5,6-tetrahydrobenzo[*h*]quinazoline-2(1*H*)-thione (**4a,b**, 1 mmol) and 2-(2-bromoacetyl)-3*H*-benzo[*f*]chromen-3-one (**10**, 1 mmol) were dissolved in 10 mL of glacial acetic acid and stirred at its reflux temperature for 2 h. After completion of the reaction (monitored by TLC), the solid separated out was filtered and washed with hot acetic acid to afford the pure product.

2-(7-Phenyl-6,7-dihydro-5*H*-benzo[*h*]thiazolo[2,3-*b*]-quinazolin-9-yl)-3*H*-benzo[*f*]chromen-3-one (12a)

Yellow solid; IR (KBr) υ_{max} (cm^{-1}): 1729 (C=O of lactone), 1637 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.72–1.79 (m, 1H), 2.27–2.34 (m, 1H), 2.61 (t, J = 8.0 Hz, 1H), 2.77–2.83 (m, 1H), 6.28 (s, 1H), 7.13–7.41 (m, 8H), 7.52 (s, 1H), 7.64–7.76 (m, 4H), 8.11 (d, J = 8.0 Hz, 1H), 8.31–8.34 (m, 2H), 8.72 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 161.4, 158.4, 153.8, 143.0, 138.7, 135.4, 135.1, 133.5, 129.9, 129.0, 128.8, 128.6, 128.1, 127.0, 126.7, 126.4, 122.4, 121.4, 116.4, 114.8, 113.5, 112.4, 111.4, 62.9, 26.6, 23.1; MS (ESI) m/z: 511 $[M + H]^+$; anal. calcd for $C_{33}H_{22}N_2O_2S$: C, 77.62; H, 4.34; N, 5.49; found: C, 77.74; H, 4.45; N, 5.33.

2-(7-(4-Hydroxyphenyl)-6,7-dihydro-5*H*-benzo[*h*]thiazolo[2,3-*b*]-quinazolin-9-yl)-3*H*-benzo[*f*]chromen-3-one (12b)

Yellow solid; IR (KBr) υ_{max} (cm^{-1}): 3358 (OH), 1710 (C=O of lactone), 1613 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.75–1.81 (m, 1H), 2.22–2.30 (m, 1H), 2.60–2.66 (m, 1H), 2.76–2.83 (m, 1H), 6.16 (s, 1H), 6.48 (d, J = 8.8 Hz, 2H), 6.91 (d, J = 8.8 Hz, 2H), 7.25–7.41 (m, 3H), 7.51 (s, 1H), 7.64–7.70 (m, 4H), 8.12 (d, J = 8.0 Hz, 1H), 8.33–8.41 (m, 2H), 8.82 (s, 1H), 9.56 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 161.0, 158.4, 158.0, 153.8, 142.7, 135.4, 135.0, 133.6, 129.9, 129.1, 128.9, 128.8, 128.7, 128.6, 128.1, 126.7, 126.4, 123.9, 122.5, 121.3, 116.4, 115.5, 115.0, 113.4, 112.5, 112.0, 62.6, 26.7, 23.2; MS (ESI) m/z: 527 $[M + H]^+$; anal. calcd for $C_{33}H_{22}N_2O_3S$: C, 75.27; H, 4.21; N, 5.32; found: C, 75.11; H, 4.43; N, 5.40.

Determination of minimum inhibitory concentration (MIC)

The MIC values for all the synthesized compounds as well as positive control drugs Penicillin and Streptomycin were determined against the three gram-positive and three gram-negative bacterial strains by micro dilution method recommended by CLSI Standard Protocol in liquid medium (Nutrient agar) distributed in 96-well plates. Serial dilutions of the test compounds as well as standards were performed at concentrations ranging from 150 to 0.97 μg mL^{-1} in a 200 μL culture medium final volume; afterwards each well was seeded with a 50 μL microbial suspension of 0.5 MacFarland density. In each test a microbial culture control and a sterility control (negative) were performed. The plates were incubated for 24 h at 37 °C. The lowest concentration which inhibited the visible microbial growth was considered the MIC.

Diphenylpicrylhydrazyl (DPPH) method

Free radical scavenging activity was done by DPPH method. Briefly, 0.004% w/v of DPPH radical solution was prepared in methanol and then 900 μL of this solution was mixed with 100 μL of solution containing 10 μg mL^{-1} of test compound. The absorbance was measured at 517 nm after 30 min of incubation.

Agarose gel electrophoresis method

Cleavage products were analyzed by agarose gel electrophoresis method. Test samples (10 mg mL^{-1}) was prepared in DMSO. The samples at two different concentrations (50 and 100 μg) were added to the isolated pUC18 (200 μg mL^{-1}) plasmid. The samples were incubated for 2 h at 37 °C and then 20 μL of DNA sample (mixed with bromophenol blue dye at 1 : 1 ratio) was loaded carefully into the electrophoretic chamber wells along with standard DNA marker containing TAE buffer (4.84 g Tris base, pH 8.0, 0.5 M EDTA/1 L) and finally loaded on agarose gel and constant 50 V of electricity was passed for around 30 min. Gel is removed and stained with 10 μg mL^{-1} ethidium bromide for 10–15 min and the bands were observed under UV transilluminator and photographed to determine the extent of DNA cleavage and the results were compared with standard $FeSO_4$.

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