Hybrid 4-Aminoquinoline-1,3,5-triazine Derivatives: Design, Synthesis, Characterization, and Antibacterial Evaluation

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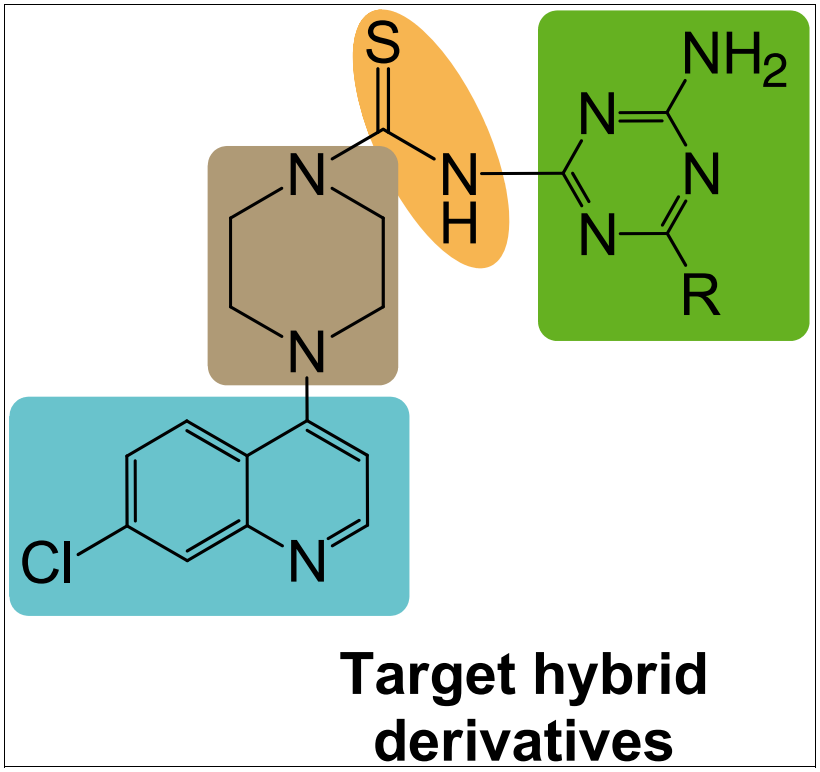

A series of novel 4-aminoquinoline 1,3,5-triazine derivatives were synthesized and characterized by FTIR, ^{1}H-NMR, ^{13}C-NMR, MS, and elemental analysis. The antibacterial activities of synthesized compounds were tested against three Gram-positive bacteria, namely *Bacillus subtilis* (NCIM-2063), *Bacillus cereus* (NCIM-2156), and *Staphylococcus aureus* (NCIM-2079), and four Gram-negative bacteria, namely *Proteus vulgaris* (NCIM-2027), *Proteus mirabilis* (NCIM-2241), *Escherichia coli* (NCIM-2065), and *Pseudomonas aeruginosa* (NCIM-2036), using ciprofloxacin as reference standard drug. Results showed compound **9a** and **9e** as potent antibacterial agents against all bacterial strains except *Bacillus cereus* (NCIM-2156).

INTRODUCTION

For many years after the discovery of penicillin by Sir Alexander Fleming some eight decades ago, the phenomenon of microbial resistance was largely ignored [1,2]. Now, however, the prevalence of antibiotic-resistant microorganisms, in both the community and the hospital, has reached a level that compromised the treatment regime, which results in inefficiency in therapeutic applications [3–5]. New, more potent agents have been introduced, but resistant microorganisms hold its supremacy and continue to be selective enriched. Therefore, development of cost-effective and potent new chemical entity would serve the purpose, especially because many pharmaceutical companies have abandoned efforts to find and develop new antimicrobials.

Concerning the immense pharmacological activity of 1,3,5-triazine [6–10] and in follow up of our previous studies toward the development of novel antibacterial compounds derived from 1,3,5-triazine, here we wish to report a novel class of hybrid 4-aminoquinoline 1,3,5-triazine [11–16].

RESULTS AND DISCUSSION

Chemistry. The synthesis of target hybrid 4-aminoquinoline 1,3,5-triazine derivatives **9a–j** was accomplished via a five-step synthetic protocol. The first step corresponds to the synthesis of 7-chloro-4-(piperazin-1-yl)quinoline (**3**), which was achieved by the substitution of piperazine (**2**) with 4-chloro of 4, 7-dichloroquinoline (**1**) in presence of isopropyl alcohol. In the second step, the synthesis of mono-substituted 1,3,5-triazines, namely 4,6-dichloro-1,3,5-triazin-2-amine (**6**), was accomplished by nucleophilic substitution of the Cl atom of the 2,4,6-trichloro-1,3,5-triazine (**4**) with excess amount of ammonia gas. The third step leads to the synthesis of di-substituted 1,3,5-triazine derivatives **7a–j** by the nucleophilic substitution of one of the Cl atoms of mono-substituted 1,3,5-triazine (**6**) with different primary and secondary amines **a–j** (Scheme 1). Furthermore, the fourth step is the synthesis of tri-substituted 1,3,5-triazine derivatives **8a–j** by the nucleophilic substitution of the remaining Cl atom of di-substituted 1,3,5-triazine derivatives **7a–j** with potassium thiocynate in presence of few pieces of tin granules as catalyst. In the fifth step, the title compounds **9a–j** were synthesized by incorporating tri-substituted 1,3,5-triazine derivatives **8a–j** with 7-chloro-4-(piperazin-1-yl)quinoline (**3**).

The series of title hybrid analogues were synthesized with good yields. On the other hand, the structure of the intermediate as well as target molecules was ascertained on the basis of spectroscopic analysis. FTIR spectra of compounds **9a–j** were in the range of 2900–3300 cm^{-1}, which was attributable to aromatic $N–H_{stretch}$, whereas 2900–3100 cm^{-1} showed aromatic C–H stretching. Other peaks such as 1016–1115 cm^{-1} were attributable to the C═S group, whereas aromatic C–N group appears at 1380 cm^{-1}. ^{1}H-NMR spectra of all the derivatives showed a singlet in the range of δ 8.0–8.1 attributable to (CH)N proton, while aromatic protons of the benzene molecule appeared as doublets at δ 7.60 and δ 7.20, each integrating for two protons, respectively. A singlet at 3.85 integrating for one proton corresponding to NH attached with 1,3,5-triazine. ^{1}H-NMR spectrum also showed the five quinoline protons at 8.20–6.44 ppm and the aliphatic protons at 3.90–1.02 ppm. The ^{13}C-NMR spectrum showed the nine quinoline carbons signals at the region of 150–90.5 ppm and the aliphatic carbons signals at 51.5–31.0 ppm. ^{13}C-NMR showed between 151.80 and 156.0 corresponding to carbon atom of the triazine ring.

Antibacterial activity. All the synthesized compounds were tested for their *in vitro* antibacterial activity against Gram-positive bacteria (*Staphylococcus aureus*, *Bacillus subtilis*, and *Bacillus cereus*) and Gram-negative bacteria (*Pseudomonas aeruginosa*, *Escherichia coli*, *Proteus mirabilis*, and *Proteus vulgaris*) by the broth dilution technique in terms of MIC, and the results were presented in Table 1. The present study included ciprofloxacin as reference antibacterial agent. Compound **9a** bearing *p*-aminophenol showed higher activity against *S. aureus*, *P. aeruginosa*, *E. coli*, and *P. vulgaris*, equipotent activity against *B. subtilis*, and moderate activity against *B. cereus*. The compound with *N*,*N*-dimethylbenzene-1,4-diamine substitution on 1,3,5-triazine **9b** exhibited higher activity against *S. aureus*, *B. subtilis*, and *P. vulgaris*, equipotent

Scheme 1. Reagents and conditions: R–H (a–j) various amines (i) K_2CO_3, isopropyl alcohol, refluxed at 80–85°C for 36 h; (ii) acetone, stirred for 3 h at 0–5°C, $NaHCO_3$; (iii) acetone, stirred for 5 h at 40–45°C reflux, $NaHCO_3$; (iv) KSCN, 1,4 dioxane, refluxed at 100°C for 6–7 h, K_2CO_3, tin granules; (v) dry acetone, refluxed for 8–9 h at 40–45°C.

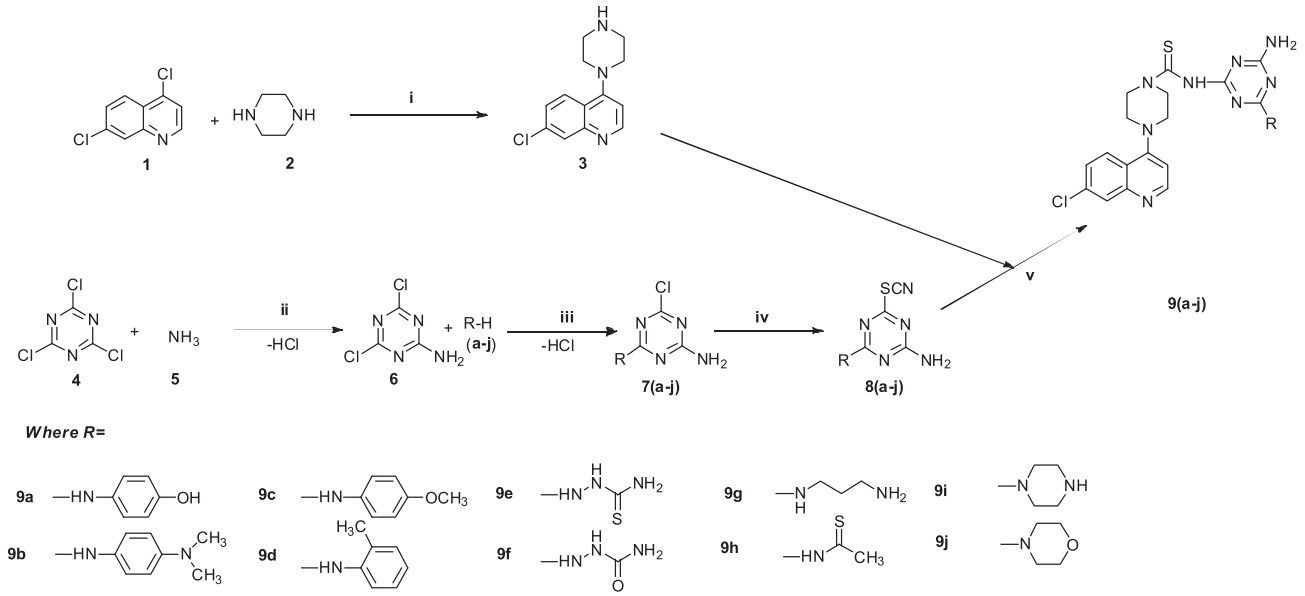

Table 1

Antibacterial activity of target hybrid derivatives **9a–j**.

Compounds	MIC ($\mu g\,mL^{-1}$)						
	Gram-positive			Gram-negative			
	Staphylococcus aureus	*Bacillus subtilis*	*Bacillus cereus*	*Pseudomonas aeruginosa*	*Escherichia coli*	*Proteus mirabilis*	*Proteus vulgaris*
9a	3.125	12.5	100	12.5	3.125	12.5	3.125
9b	3.125	3.125	6.25	25	100	25	6.25
9c	100	50	12.5	12.5	25	6.25	12.5
9d	6.25	12.5	6.25	6.25	6.25	3.125	12.5
9e	3.125	6.25	6.25	3.125	3.125	12.5	3.125
9f	50	3.125	6.25	6.25	6.25	6.25	50
9g	50	50	50	6.25	100	12.5	50
9h	6.25	50	12.5	25	50	50	3.125
9i	3.125	6.25	50	3.125	3.125	3.125	6.25
9j	50	6.25	6.25	3.125	6.25	12.5	12.5
Ciprofloxacin	6.25	12.5	3.125	25	6.25	25	12.5

activity against *P. aeruginosa* and *P. mirabilis*, and moderate activity against *B. cereus and E. coli*. On the other hand, introduction of 4-methoxyaniline substitution on 1,3,5-triazine **9c** exhibited higher activity against *P. mirabilis* and *P. aeruginosa*, equipotent activity against *P. vulgaris*, and moderate activity against *S. aureus*, *B. subtilis*, *B. cereus*, and *E. coli*. Replacement of 4-methoxyaniline by *o*-toluidine **9d** showed higher activity against *P. aeruginosa* and *P. mirabilis*, equipotent activity against *S. aureus*, *B. subtilis*, *E. coli*, and *P. vulgaris*, and moderate activity against the *B. cereus*. Further replacement of *o*-toluidine to aliphatic amine (thiosemicarbazide) in the case of compound **9e** showed higher activity against *S. aureus*, *P. vulgaris*, *E. coli*, *P. aeruginosa*, *B. subtilis*, and *P. mirabilis* and renders compounds active against *B. cereus*. Compound **9f** bearing semicarbazide showed higher activity against *P. aeruginosa* and *P. mirabilis*, equipotent activity against *E. coli*, and moderate activity against *S. aureus*, *B. cereus*, and *P. vulgaris*. Moderate activity was observed in the case of compounds **9g** having 1,3-diaminopropane as substituent on 1,3,5-triazine, toward all Gram-positive and Gram-negative strains except *P. aeruginosa* and *P. mirabilis*. Replacement of 1,3-diaminopropane with ethanethioamide in the case of compound **9h** showed moderate activity against all Gram-positive and Gram-negative strains except *P. vulgaris*. However, higher activity was observed in the case of analogue having piperazine **9i** against all Gram-positive and Gram-negative strains except *B. cereus*. Finally, replacement of piperazine with morpholine in the case of compound **9j** showed higher activity against *B. subtilis*, *P. aeruginosa*, *E. coli*, and *P. mirabilis*, equipotent activity against *P. vulgaris*, and moderate activity against *S. aureus* and *B. cereus*.

Compounds **9a**, **9e**, **9b**, and **9i** bearing *p*-aminophenol, thiosemicarbazide, N^1,N^1-dimethylbenzene-1,4-diamine, and piperazine substituents on 1,3,5-triazine exhibited highest activity against *S. aureus* at 3.125 $\mu g\,mL^{-1}$ of MIC. Compounds **9c**, **9f**, **9g**, and **9j** bearing 4-methoxyaniline, semicarbazide, 1,3-diaminopropane, and morpholine substituents on 1,3,5-triazine exhibited moderate activity against *S. aureus* at 100 and 50 $\mu g\,mL^{-1}$ of MIC. Compounds **9d** and **9h** bearing *o*-toluidine and ethanethioamide have the same activity at 6.25 $\mu g\,mL^{-1}$ of MIC.

Structure–activity relationship analysis showed that compounds having aliphatic substitution on 1,3,5-triazine such as **9e**, **9h**, and **9f** exhibited highest activity against *S. aureus*, *P. vulgaris*, and *B. subtilis*, while aromatic substitution on 1,3,5-triazine, namely **9a**, **9b**, **9c**, **9d**, **9i**, and **9j**, exhibited highest activity against *S. aureus*, *P. aeruginosa*, and *P. mirabilis* and no activity against *B. cereus*. More pronounced activity was disclosed by the analogues having the hydroxyl, methoxy, and dimethylamine substitution on the phenyl ring connected to 1,3,5-triazine against *P. aeruginosa*, *P. mirabilis*, and *P. vulgaris* and no activity against *B. cereus*. On the other hand, piperazine and morpholine substitution on the 1,3,5-triazine revealed highest activity against *B. subtilis*, *P. aeruginosa*, *P. mirabilis*, *P. vulgaris*, and *E. coli* and no activity against *B. cereus*.

ANTIBACTERIAL SCREENING

Minimum inhibitory concentration. All synthesized compounds were screened for determination of their MIC ($\mu g\,mL^{-1}$) against selected Gram-positive organisms, namely *B. subtilis* (NCIM-2063), *B. cereus* (NCIM-2156), and *S. aureus* (NCIM-2079), and Gram-negative organisms, namely *P. aeruginosa* (NCIM-2036), *E. coli* (NCIM-2065), *P. mirabilis* (NCIM-2241), and *P. vulgaris* (NCIM-2027), by the broth dilution method as recommended by the National Committee for Clinical Laboratory Standards with minor modifications. Ciprofloxacin was used as standard antibacterial agent. Solutions of the test compounds and reference drug were prepared in DMSO at concentrations of 100, 50, 25, 12.5, 6.25, and 3.125 $\mu g\,mL^{-1}$. Eight tubes were prepared in

duplicate with the second set being used as MIC reference controls (16–24 h visual). After sample preparation, the controls were placed in a 37°C incubator and read for macroscopic growth (clear or turbid) the next day. Into each tube, 0.8 mL of nutrient broth was pipette (tubes 2–7), tube 1 (negative control) received 1.0 mL of nutrient broth, and tube 8 (positive control) received 0.9 mL of nutrient. Tube 1, the negative control, did not contain bacteria or antibiotic. The positive control, tube 8, received 0.9 mL of nutrient broth because it contained bacteria but not antibiotic. The test compound was dissolved in DMSO (100 $\mu g\,mL^{-1}$), 0.1 mL of increasing concentration of the prepared test compounds that are serially diluted from tube 2 to tube 7 from highest (100 $\mu g\,mL^{-1}$) to lowest (3.125 $\mu g\,mL^{-1}$) concentration (tube 2–7 containing 100, 50, 25, 12.5, 6.25, and 3.125 $\mu g\,mL^{-1}$). After this process, each tube was inoculated with 0.1 mL of the bacterial suspension whose concentration corresponded to 0.5 McFarland scale (9×10^8 cells mL^{-1}), and each bacterium was incubated at 37°C for 24 h at 150 rpm. The final volume in each tube was 1.0 mL. The incubation chamber was kept humid. At the end of the incubation period, MIC values were recorded as the lowest concentration of the substance that gave no visible turbidity, that is, no growth of inoculated bacteria [18].

CONCLUSION

In this study, we reported a convenient route for the synthesis of new hybrid compounds incorporating 4-aminoquinoline nucleus with substituted 1,3,5-triazine as potent antibacterial agent. The study suggests that compound **9a** with the *P*-aminophenol substitution on 1,3,5-triazine nucleus is a more potent antibacterial agent than the **9g** derivative with the 1,3-diaminopropane group. Our study is in progress toward the development of new derivatives of this skeleton and will be reported subsequently.

EXPERIMENTAL

Materials and methods. All commercially available solvents and reagents were of analytical grade and used without further purification. Melting points were determined on a Veego MPI melting point apparatus, and FTIR spectra (2.0 cm^{-1}, flat, smooth, abex) were recorded on Perkin Elmer RX-I spectrophotometer (USA). ^{1}H-NMR spectra were recorded in $CDCl_3$ using Bruker Avance II 400 NMR and ^{13}C-NMR spectra on Bruker Avance II 100 NMR spectrometer in DMSO-d_6 using TMS as internal standard (Fallanden, Switzerland). Mass spectra were obtained on VG-AUTOSPEC spectrometer equipped with ESI sources (Fisons Instruments, Manchester, UK). Elemental analysis was carried out on Vario EL-III CHNOS elemental analyzer (Elementar Analysensysteme, Hanau, Germany).

Synthesis

7-Chloro-4-piperazin-1-yl-quinoline (3). A mixture of 4,7 dichloroquinoline (0.01 mol), piperazine (0.01 mol), and anhydrous potassium carbonate (0.01 mol) in 100 mL of isopropyl alcohol was refluxed for 36 h at 80–85°C. The resulting reaction mixture was then concentrated under reduced pressure. The resulted residue was dissolved in dichloromethane, washed with brine, and dried over Na_2SO_4. The dried solution was concentrated under reduced pressure to obtain the title compound. The completion of reaction was monitored by TLC using methanol/ethyl acetate (2:8) as mobile phase.

White crystals; yield: 79%; mp: 112–113°C; MW: 247.72; R_f: 0.36; FTIR (ν_{max}; cm^{-1} KBr): 3287.48 (N–H secondary), 3058.63 (C–H broad), 1549.16–1442.28 (aromatic C═N), 1642 (C═C), 1257 (C–N); ^{1}H-NMR (400 MHz, $CDCl_3$, TMS) δ ppm: 8.71 (d, 1H $J = 5.2$ Hz, quinoline), 8.05(d, 1H $J = 2.3$ Hz, quinoline), 7.92 (d, 1H $J = 8.7$ Hz, quinoline), 7.40 (d, 1H $J = 2.4$ Hz, quinoline), 6.83 (d, 1H $J = 5.1$ Hz, quinoline), 3.18–2.96 (m, 8H, 4×CH_2, piperazine), 1.86 (br s, 1H, NH); ^{13}C-NMR (100 MHz, DMSO-d_6) δ ppm: 159.4, 153.2, 150.3, 135.7, 129.9, 128.6, 126.1, 122.7, 118.4, 52.8, 45.7; MS: 248.76 $(M + H)^+$; elemental analysis for $C_{13}H_{14}ClN_3$: Calcd.: C, 63.03; H, 5.70; N, 16.96. Found: C, 62.87; H, 5.73; N, 16.87.

4,6-Dichloro-1,3,5-triazin-2-amine (6). Strong ammonia solution (**5**) (0.1 mol) was added into 25 mL of acetone containing 2,4,6-trichloro-1,3,5-triazine (**4**) (0.1 mol) maintaining temperature 0–5°C. The resulting mixture was then stirred for 3 h followed by dropwise addition of $NaHCO_3$ solution (0.1 mol) taking care that the reaction mixture does not become acidic. The completion of reaction was monitored by TLC using benzene/ethyl acetate (9:1) as mobile phase. The product was filtered and washed with cold water and recrystallized with ethanol to afford pure products **6**.

White crystals; yield: 64%; mp: 243–246°C; MW:164.98; R_f: 0.53; FTIR (ν_{max}; cm^{-1} KBr): 3487.48 (N–H primary), 1498 (C═N), 1257 (C–N), 768 (C–Cl); ^{1}H-NMR (400 MHz, $CDCl_3$, TMS) δ ppm: 6.86 (br s, 2H, NH_2); ^{13}C-NMR (100 MHz, DMSO-d_6) δ ppm: 186.4, 168.9; MS: 165.87 $(M + H)^+$; elemental analysis for $C_3H_2Cl_2N_4$: Calculated: C, 21.84; H, 1.22; N, 33.96. Found: C, 22.01; H, 1.21; N, 33.89.

General procedure for the synthesis of di-substituted 1,3,5-triazine derivatives 7a–j. Various distinguished amines **a–j** (0.1 mol) were added into 100 mL of acetone maintaining temperature 40–45°C. The solution of mono-subtituted-1,3,5-triazine (**6**) (0.1 mol) in 25 mL acetone was added constantly, stirred for 5 h, and followed by dropwise addition of $NaHCO_3$ solution (0.1 mol) taking care that the reaction mixture does not become acidic. The completion of reaction was monitored by TLC using benzene/ethyl acetate (9:1) as mobile phase. The product was filtered and washed with cold water and recrystallized with ethanol to afford pure products **7a–j** [17].

General procedure for the synthesis of tri-substituted 1,3,5-triazine derivatives 8a–j. A solution of di-substituted 1,3,5-triazine compounds **7a–j** (0.01 mol), potassium thiocynate (0.01 mol), and K_2CO_3 (0.01 mol) in 1,4-dioxane was refluxed for 6–7 h in presence of tin granules that act as catalyst. The completion of reaction was monitored by TLC using benzene/ethyl acetate (9:1) as mobile phase. The reaction mixture was filtered and concentrated under reduced pressure. The resulting residue was purified by ethanol to afford the desired product **8a–j** [13].

General procedure for the synthesis of titled compounds 9a–j. A solution of compound **3** (0.01 mol.) and desired tri-substituted 1,3,5-triazine compounds **8a–j** (0.01 mol) in dry acetone was stirred at 40–45°C for 8–9 h. The completion of

reaction was monitored by TLC using ethanol/acetone (1:1) as mobile phase. The reaction mixture was filtered and concentrated under reduced pressure. The resulting residue was dissolved in dichloromethane,washed with brine, and dried over anhydrious Na_2SO_4. The dried solution was concentrated under reduced pressure to obtain the titled compounds **9a–j** [13].

4-(7-Chloro-quinolin-4-yl)-piperazine-1-carbothioic acid [4-amino-6-(4-hydroxy-phenylamino)-[1,3,5]triazin-2-yl]-amide 9a. Black crystals; yield: 82%; mp: 260–262°C; MW: 508.00; R_f: 0.87; FTIR (ν_{max}; cm^{-1} KBr): 3414.65 (N–$H_{stretch}$, –NH_2), 2922.73 (C–$C_{stretch}$), 1607.74 (C–$H_{stretch}$), 1574.66 (N–$H_{stretch}$, sec. amine), 1512.6 (N–$H_{stretch}$, NH_2), 1425.65 (C–$H_{stretch}$), 1380.39 (C–$N_{stretch}$), 1236.16 ($OH_{stretch}$), 1163.29 (C═$S_{stretch}$), 872.39, 825.39 (C–$H_{stretch}$); ^{1}H-NMR (400 MHz, $CDCl_3$, TMS) δ ppm: 8.78 (d, 1H J = 5.1 Hz, quinoline), 8.08 (d, 1H J = 4.8 Hz, quinoline), 7.94 (d, 1H J = 4.2 Hz, quinoline), 7.40 (d, 1H J = 1.9 Hz, quinoline), 7.02 (s, 2H, NH_2), 6.980 (d, 1H J = 2.9 Hz, quinoline), 7.74–6.87(m, 4H, 4×CH, Ar–H), 5.46 (s, 1H, Ar–OH), 4.08–3.02 (m, 8H, 4×CH_2, piperazine), 3.70 (br s, 1H, NH), 3.52 (s, 1H, NH); ^{13}C-NMR (100 MHz, DMSO-d_6) δ ppm: 182.4, 181.8, 167.4, 159.3, 154.2, 150.8, 148.6, 135.7, 131.8, 130.3, 128.9, 126.1, 122,7, 121.8, 118.4, 116.8, 56.9, 52.7; MS: 509.8 $(M+H)^+$; elemental analysis for $C_{23}H_{22}ClN_9OS$: Calcd.: C, 54.38; H, 4.37; N, 24.82. Found: C, 55.01; H, 4.38; N, 24.83.

4-(7-Chloro-quinolin-4-yl)-piperazine-1-carbothioicacid[4-amino-6-(4dimethylamino-phenylamino)-[1,3,5]triazin-2-yl]-amide 9b. Dark blue crystals; yield: 71%; mp: 325–326°C; MW: 537.07; R_f: 0.83; FTIR (ν_{max}; cm^{-1} KBr): 3300.83 (N–H_{broad}, –NH_2), 3098.17 (C═C_{broad}), 1626.02 (C–$H_{stretch}$) 1574.56 (N–$H_{stretch}$, sec. amine), 1515.29 (C–$H_{stretch}$), 1472.49 (C–$H_{stretch}$), 1384.38 (C–$N_{stretch}$), 1166.09 (C═$S_{stretch}$), 871.40, 802.57(C–$H_{stretch}$); ^{1}H-NMR (400 MHz, $CDCl_3$, TMS) δ ppm: 8.47 (d, 1H J = 5.8 Hz, quinoline), 8.16 (d, 1H J = 4.2 Hz, quinoline), 7.87 (d, 1H J = 3.8 Hz, quinoline), 7.32 (d, 1H J = 1.5 Hz, quinoline), 6.98 (s, 2H, NH_2), 6.56 (d, 1H J = 2.3 Hz, quinoline), 6.61–6.47 (m, 4H, 4×CH, Ar–H), 3.02 (s, 6H, 2×CH_3), 4.03–2.98 (m, 8H, 4×CH_2, piperazine), 3.70 (br s, 1H, NH), 3.52 (s, 1H, NH); ^{13}C-NMR (100 MHz, DMSO-d_6) δ ppm: 183.3, 181.3, 165.7, 164.9, 159.2, 153.2, 151.6, 147.6, 134.8, 130.1, 128.5, 127.9, 126.2, 122.7, 118.7, 117.3, 114.1, 56.7, 53.9, 42.7; MS: 538.04 $(M+H)^+$; elemental analysis for $C_{25}H_{27}ClN_{10}S$: Calcd.: C, 56.12; H, 5.09; N, 26.18. Found: C, 56.14; H, 5.07; N, 26.21.

4-(7-Chloro-quinolin-4-yl)-piperazine-1-carbothioicacid[4-amino-6-(3-methoxy-phenylamino)-[1,3,5]triazin-2-yl]-amide 9c. Light violet crystals; yield: 76%; mp: 310–312°C; MW: 522.03; R_f: 0.65; FTIR (ν_{max}; cm^{-1} KBr): 3414.65(N–$H_{stretch}$, NH), 3059.60(C–H_{broad}), 1735.72(C═$O_{stretch}$), 1689.27 (C–$H_{Stretch}$), 1575.06 (N–$H_{stretch}$, NH_2), 1486.51(C–$H_{stretch}$, CH_3), 1380.43 (C–$N_{stretch}$), 1115.37 (C═$S_{stretch}$), 870.28, 826.41 (C–$H_{stretch}$); ^{1}H-NMR (400 MHz, $CDCl_3$, TMS) δ ppm: 8.56 (d, 1H J = 5.9 Hz, quinoline), 8.27 (d, 1H J = 4.8 Hz, quinoline), 7.83 (d, 1H J = 3.9 Hz, quinoline), 7.38 (d, 1H J = 2.6 Hz, quinoline), 6.93 (s, 2H, NH_2), 6.48 (d, 1H J = 1.8 Hz, quinoline), 7.09–6.27 (m, 4H, 4×CH, Ar–H), 3.87 (s, 3H, OCH_3), 4.02–2.96 (m, 8H, 4×CH_2, piperazine), 3.88 (br s, 1H, NH), 3.52 (s, 1H, NH); ^{13}C-NMR (100 MHz, DMSO-d_6) δ ppm: 185.2, 182.7, 165.2, 164.5, 161.4, 159.4, 153.0, 150.9, 143.5, 135.7, 131.1, 130.3, 128.9, 126.0, 122.4, 118.3, 111.8, 110.1, 99.5, 56.8, 54.1, 53.4; MS: 523.07 $(M+H)^+$; elemental analysis for $C_{24}H_{24}ClN_9OS$: Calcd,: C, 55.22; H, 4.63; N, 24.15. Found: C, 55.24; H, 4.63; N, 24.17.

4-(7-Chloro-quinolin-4-yl)-piperazine-1-carbothioic acid (4-amino-6-o-tolylamino-[1,3,5]triazin-2-yl)-amide 9d. Dark violet crystals; yield: 69%; mp: 275–276°C; MW: 506.03; R_f: 0.77; FTIR (ν_{max}; cm^{-1} KBr): 3411.79 (N–$H_{stretch}$, NH_2), 2921.99 (C–H_{broad}), 2833.06 (C–$H_{stretch}$), 1689.27 (C–H), 1574.93 (N–$H_{stretch}$, sec. amine), 1498.77 (C═$C_{stretch}$), 1425.71 (C–$H_{stretch}$), 1382.43 (C–$N_{stretch}$), 1163.37 (C═$S_{stretch}$), 878.72, 810.01 (C–$H_{stretch}$); ^{1}H-NMR (400 MHz, $CDCl_3$, TMS) δ ppm: 8.86 (d, 1H J = 6.3 Hz, quinoline), 8.35 (d, 1H J = 5.2 Hz, quinoline), 7.87 (d, 1H J = 3.4 Hz, quinoline), 7.34 (d, 1H J = 2.1 Hz, quinoline), 6.95 (s, 2H, NH_2), 6.52 (d, 1H J = 1.7 Hz, quinoline), 7.15–6.54 (m, 4H, 4×CH, Ar–H), 4.15–3.04 (m, 8H, 4×CH_2, piperazine), 3.69 (br s, 1H, NH), 3.51 (s, 1H, NH), 2.32 (s, 3H, CH_3); ^{13}C-NMR (100 MHz, DMSO-d_6) δ ppm: 184.2, 181.8, 165.5, 164.9, 159.2, 154.1, 150.8, 142.4, 135.8, 131.8, 130.7, 129.3, 128.9, 126.8, 125.9, 123.6, 122.9, 121.7, 118.5, 56.7, 53.9, 18.2; MS: 508.03 $(M+H)^+$; elemental analysis for $C_{24}H_{24}ClN_9S$: Calcd.: C, 56.96; H, 4.78; N, 24.91. Found: C, 57.01; H, 4.77; N, 24.90.

N-(4-Amino-6-(2-carbamothioylhydrazinyl)-1,3,5-triazin-2-yl)-4-(7-chloroquinolin-4-yl)piperazine-1-carbothioamide 9e. Light brown crystals; yield: 56%; mp: 320–321°C; MW: 490.01; R_f: 0.86; FTIR (ν_{max}; cm^{-1} KBr): 3331.64 (N–H_{broad}, –NH_2), 3212.08 (C–H_{broad}), 2058.37 (C–$H_{stretch}$), 1574.08 (N–$H_{stretch}$, sec. amine), 1379.90 (C–$N_{stretch}$), 1014.98 (C═$S_{stretch}$), 810.51 (C–$H_{stretch}$); ^{1}H-NMR (400 MHz, $CDCl_3$, TMS) δ ppm: 8.57 (d, 1H J = 6.2 Hz, quinoline), 8.53 (s, 2H, NH_2), 8.03 (d, 1H J = 4.6 Hz, quinoline), 7.83 (d, 1H J = 3.2 Hz, quinoline), 7.28 (d, 1H J = 2.3 Hz, quinoline), 6.91 (s, 2H, NH_2), 6.36 (d, 1H J = 1.8 Hz, quinoline), 3.70 (br s, 1H, NH), 3.52 (s, 1H, NH), 3.96–2.98 (m, 8H, 4×CH_2, piperazine), 1.95 (s, 1H, NH); ^{13}C-NMR (100 MHz, DMSO-d_6) δ ppm: 183.5, 182.4, 181.3 179.7, 168.3, 158.2, 152.8, 150.4, 135.3, 129.9, 128.7, 125.9, 122.8, 117.3, 56.9, 53.7; MS: 491.03 $(M+H)^+$; elemental analysis for $C_{18}H_{20}ClN_{11}S_2$: Calcd.: C, 44.12; H, 4.11; N, 31.44. Found: C, 44.14; H, 4.10; N, 31.43.

2-(4-Amino-6-(4-(7-chloroquinolin-4-yl)piperazine-1-carbothioamido)-1,3,5-triazin-2-yl)hydrazinecarboxamide 9f. White crystals; yield: 59%; mp: 225–226°C; MW: 473.94; R_f: 0.89; FTIR (ν_{max}; cm^{-1} KBr): 3466.20 (N–$H_{stretch}$, –NH_2), 3149.91 (C–H_{broad}), 2850 (C–$H_{stretch}$), 1727.43 (C═$O_{stretch}$), 1563 (N–H_{broad}, sec. amine), 1379.90 (C–$N_{stretch}$), 1016.78 (C═$S_{stretch}$), 811.00 (C–$H_{stretch}$); ^{1}H-NMR (400 MHz, $CDCl_3$, TMS) δ ppm: 8.56 (d, 1H J = 6.2 Hz, quinoline), 8.02 (d, 1H J = 4.6 Hz, quinoline), 7.83 (d, 1H J = 3.2 Hz, quinoline), 7.28 (d, 1H J = 2.3 Hz, quinoline), 6.94 (s, 2H, NH_2), 6.39 (d, 1H J = 1.6 Hz, quinoline), 5.93 (s, 2H, NH_2), 3.70 (br s, 1H, NH), 3.52 (s, 1H, NH), 3.96–3.04 (m, 8H, 4×CH_2, piperazine), 2.92 (s, 1H, NH); ^{13}C-NMR (100 MHz, DMSO-d_6) δ ppm: 182.6, 181.7 178.6, 169.5, 160.3, 157.5, 154.3, 150.7, 135.7, 130.2, 128.8, 127.1, 122.5, 118.4, 57.1, 53.8; MS: 474.95 $(M+H)^+$; elemental analysis for $C_{18}H_{20}ClN_{11}OS$: Calcd.: C, 45.62; H, 4.25; N, 32.51. Found: C, 45.64; H, 4.21; N, 32.50.

4-(7-Chloro-quinolin-4-yl)-piperazine-1-carbothioic acid [4-amino-6-(3-amino-propylamino)-[1,3,5]triazin-2-yl]-amide 9g. Brown crystals; yield: 70%; mp: 291–292°C; MW: 473; R_f: 0.84; FTIR (ν_{max}; cm^{-1} KBr): 3298.72 (N–H_{broad}, NH_2), 2928.12 (–C═C–$_{broad}$), 2053.58 (C–$H_{stretch}$) 1715.43 (C–$H_{stretch}$, CH_2), 1574.89 (N–$H_{stretch}$, sec. amine), 1379.15 (C–$N_{stretch}$), 1123.58 (C═$S_{stretch}$), 872.80, 810.33 (C–$H_{stretch}$); ^{1}H-NMR (400 MHz, $CDCl_3$, TMS) δ ppm: 8.62 (d, 1H J = 6.5 Hz, quinoline), 8.04 (d, 1H J = 5.3 Hz, quinoline), 7.87 (d, 1H J = 3.4 Hz, quinoline), 7.32 (d, 1H J = 2.8 Hz, quinoline), 6.87 (s, 2H, NH_2), 6.43 (d, 1H J = 1.9 Hz,

quinoline), 5.05 (s, 2H, NH_2), 3.98–3.04 (m, 8H, 4×CH_2, piperazine), 4.12 (br s, 1H, NH), 3.52 (s, 1H, NH), 3.28–1.92 (m, 6H, 3×CH_2); ^{13}C-NMR (100 MHz, DMSO-d_6) δ ppm: 183.3, 181.7, 165.3, 162.5, 159.1, 153.2, 150.7, 135.5, 130.1, 128.8, 126.1, 122.5, 118.4, 56.6, 53.8, 39.4, 31.2; MS: 474 $(M+H)^+$; elemental analysis for $C_{20}H_{25}ClN_{10}S$: Calcd.: C, 50.79; H, 5.33; N, 29.61. Found: C, 50.81; H, 5.32; N, 29.60.

N-(4-Amino-6-ethanethioamido-1,3,5-triazin-2-yl)-4-(7-chloroquinolin-4-yl)piperazine-1-carbothioamide 9h. White crystals; yield: 58%; mp: 257–258°C; MW: 474.01; R_f: 0.87; FTIR (ν_{max}; cm^{-1} KBr): 3303.53 (N–$H_{stretch}$, –NH_2), 2922.10 (–C═C–$_{broad}$), 2850.52 (C–$H_{stretch}$), 1574.56 (N–$H_{stretch}$, sec. amine), 1497.65 (C–$H_{stretch}$), 1381.07 (C–$N_{stretch}$), 1125.88 (C═$S_{stretch}$), 873.99, 823.16 (C–$H_{stretch}$); ^{1}H-NMR (400 MHz, $CDCl_3$, TMS) δ ppm: 8.68 (d, 1H J = 6.7 Hz, quinoline), 8.14 (d, 1H J = 5.8 Hz, quinoline), 7.82 (d, 1H J = 3.5 Hz, quinoline), 7.36 (d, 1H J = 2.9 Hz, quinoline), 6.97 (s, 2H, NH_2), 6.49 (d, 1H J = 2.1 Hz, quinoline), 4.08–3.01 (m, 8H, 4×CH_2, piperazine), 3.70 (br s, 1H, NH), 3.52 (s, 1H, NH), 1.15 (s, 3H, CH_3); ^{13}C-NMR (100 MHz, DMSO-d_6) δ ppm: 198.2, 182.5, 180.9, 168.4, 165.2, 159.2, 153.1, 149.8, 135.7, 130.3, 128.8, 126.2, 122.7, 118.2, 56.8, 53.8, 35.9; MS: 475.02 $(M+H)^+$; elemental analysis for $C_{19}H_{20}ClN_9S_2$: Calcd.: C, 48.14; H, 4.25; N, 26.59. Found: C, 48.15; H, 4.25; N, 26.60.

4-(7-Chloro-quinolin-4-yl)-piperazine-1-carbothioic acid (4-amino-6 piperazin-1-yl-[1,3,5]triazin-2-yl)-amide 9i. White crystals; yield: 62%; mp: 251–252°C; MW: 485.01; R_f: 0.83; FTIR (ν_{max}; cm^{-1} KBr): 3340.20 (N–$H_{stretch}$, –NH_2), 3212.5 (C–H_{broad}), 2850 (C–$H_{Stretch}$), 1537.87 (N–H_{broad}, sec. amine), 1367.10 (C–$N_{stretch}$), 1286.19 (piperazine), 1173.48 (C═$S_{stretch}$), 870.84, 808.80(C–$H_{stretch}$); ^{1}H-NMR (400 MHz, $CDCl_3$, TMS) δ ppm: 8.58 (d, 1H J = 6.2 Hz, quinoline), 8.09 (d, 1H J = 5.6 Hz, quinoline), 7.89 (d, 1H J = 3.9 Hz, quinoline), 7.36 (d, 1H J = 2.4 Hz, quinoline), 6.89 (s, 2H, NH_2), 6.52 (d, 1H J = 2.3 Hz, quinoline), 4.16–2.97 (m, 8H, 4×CH_2, piperazine), 4.06 (br s, 1H, NH), 3.09–2.89 (m, 8H, piperazine), 1.89 (s, 1H, NH); ^{13}C-NMR (100 MHz, DMSO-d_6) δ ppm:183.2, 181.7, 176.2, 168.6, 160.3, 154.2, 151.1, 137.2, 130.6, 128.5, 126.3, 122.5, 118.2, 58.4, 53.2, 49.6, 45.9; MS: 486.01 $(M+H)^+$; elemental analysis for $C_{21}H_{25}ClN_{10}S$: Calcd.: C, 52.00; H, 5.20; N, 28.88. Found: C, 52.02; H, 5.19; N, 28.88.

4-(7-Chloro-quinolin-4-yl)-piperazine-1-carbothioic acid(4-amino-6-morpholin-4-yl-[1,3,5]triazin-2-yl)-amide 9j. White crystals; yield: 68%; mp: 260–261°C; MW: 485.99; R_f: 0.86; FTIR (ν_{max}; cm^{-1} KBr): 3383.59 (N–$H_{stretch}$, NH_2), 3212.5 (C–H_{broad}), 2921.20 (N–H_{broad}, NH_2), 2850 (C–$H_{stretch}$) 1574.77 (N–$H_{broad,}$ sec. amine), 1367.10 (C–$N_{stretch}$), 1037.7 (C═$S_{stretch}$), 874.82 (C–$H_{stretch}$); ^{1}H-NMR (400 MHz, $CDCl_3$, TMS) δ ppm: 8.62 (d, 1H J = 6.7 Hz, quinoline), 8.12 (d, 1H J = 5.4 Hz, quinoline), 7.85 (d, 1H J = 3.7 Hz, quinoline), 7.28 (d, 1H J = 2.2 Hz, quinoline), 6.92 (s, 2H, NH_2), 6.42 (d, 1H J = 2.4 Hz, quinoline), 4.03–2.89 (m, 8H, 4×CH_2, piperazine), 3.73 (br s, 1H, NH), 3.76–3.65 (m, 8H, morpholine); ^{13}C-NMR (100 MHz, DMSO-d_6) δ ppm:182.5, 181.3, 176.2, 168.7, 159.4, 153.2, 150.6, 136.4, 131.2, 128.8, 126.3, 123.6, 119.7, 67.3, 57.2, 53.1, 48.9; MS: 486.06 $(M+H)^+$; elemental analysis for $C_{21}H_{24}ClN_9SO$: Calcd.: C, 51.90; H, 4.98; N, 25.94. Found: C, 51.91; H, 4.97; N, 25.93.

CONFLICT OF INTEREST

The authors have declared that there is no conflict of interest.

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