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Piperazine bridged 4-aminoquinoline 1,3,5- triazine derivatives: Design, Synthesis, characterization and antibacterial evaluation

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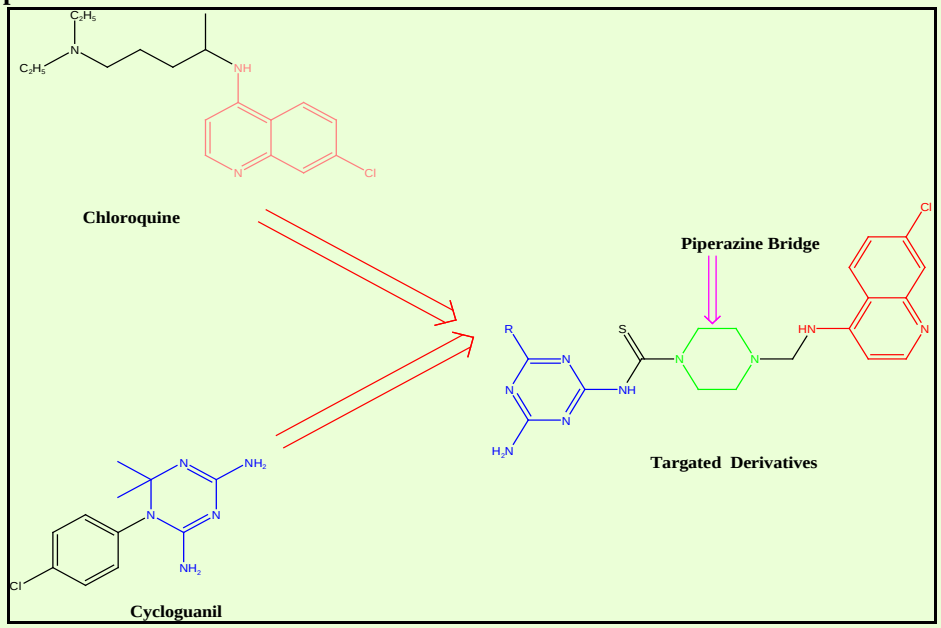
Abstract : Graphical Abstract



A series of novel 4-aminoquinoline 1,3,5-triazine derivatives were synthesized via Six step reactions. All synthesized compounds were characterized by FT-IR, ^{1}HNMR, ^{13}CNMR And Mass spectrometry. The antibacterial activity of 10 synthesized compounds were tested against three gram positive bacteria *Bacillus subtilis* (NCIM-2063), *Bacillus cereus* (NCIM-2156), *Staphylococcus aureus* (NCIM-2079) and four gram negative bacteria *Proteus vulgaris* (NCIM- 2027), *Proteus mirabilis* (NCIM-2241), *Escherichia coli* (NCIM-2065), *Pseudomonas aeruginosa* (NCIM-2036) by using ciprofloxacin as reference standard drug. Compound 11i and 11j were found most potent among synthesized derivatives, against all bacterial strains.

Keywords: 4-Aminoquinoline, *s*-triazines, Antibacterial activity.

Introduction

The development and construction of highly nitrogen-rich DNA- interactive new heterocyclic compounds continue to be an essential aspect for producing bioactive products and their structural analogues [1-3]. Synthetic chemistry represents a modular approach toward the synthesis of low as well as high molecular weight compounds that access only the most practical transformations to make connections with excellent fidelity [4-5]. A pharmacologically active hetero cycle is the quinoline ring that occurs in several natural products and displays a broad range of biological activity including antitumor [6], Antihistaminic [7], Antihypertensive [8] and Anticarcinogenic [9] properties, etc. Recently, 6-fluoroquinolones are considered as potential antibacterial agents and some of the promising fluoro quinolones are namely Norfloxacine, Ciprofloxacine, Ofloxacine and so on. Now, however, the prevalence of antibiotic resistant microorganisms, both in the community and the hospital, has reached a level that compromised the treatment regime which results inefficiency in therapeutic application. New, more potent agents have been introduced, but resistant microorganisms hold its supremacy and continue to be selective enriched [10-12].

Pertaining to the enormous pharmacological activity of hybrid 4-aminoquinoline analogues [13-16] and in follow up of previous studies towards the development of novel antibacterial agents derived from hybrid 4-aminoquinoline1.3.5 triazine. Here we wish to report a novel class of hybrid 4-aminoquinoline 1.3.5 triazine derivatives [17-20].

Result and discussion

Chemistry

The synthesis of target 4- amino conjugated 1,3,5-traizine derivatives were constructed through six step procedure. The first step involve the synthesis of Piperazin-1-yl-methylamine was achieved by the substitution of piperazine with chloro-methylamine in presence triethylamine. Whereas 7-Chloro-quinolin-4-yl)-piperazin-1-ylmethyl-amine construction was achieve by substitution of Piperazin-1-yl-methylamine with chlorine atom on 4^{th} position of 4,7-dichloroquinoline in presence of isopropyl alcohol. The third step involved synthesis of mono-substituted 1,3,5-triazine accomplished via nucleophilic substitution reaction where Cl atom, which was attached on second carbon position, removed by passing of huge amount of ammonia gas. The fourth step leads to designing of di-substituted 1,3,5-triazine analogues through the nucleophilic substitution reaction at one of the Cl atom of 4,6-Dichloro-[1,3,5]triazin-2-ylamine was substituted with list of different primary and secondary amines. Additional in fifth synthetic protocol tri-substituted 1,3,5-triazine derivatives constructed by the nucleophilic substitution of the remained Cl atom of di-substituted 1,3,5-triazine derivatives with potassium thiocynate in presences of few pieces of silver granules as catalyst. even as, in final synthetic protocol the title compounds were synthesized by incorporating trisubstituted 1,3,5-triazine derivatives with 7-Chloro-quinolin-4-yl)-piperazin-1-ylmethyl-amine through the rearrangement of the hydrogen atom. All the titled derivatives were synthesized with efficient yields. The structure confirmation of all the compounds was done by spectral analysis like FTIR, ^{1}H NMR, ^{13}C NMR, Mass spectroscopy and elemental analysis. FTIR spectra of the compounds 11a-j were exhibit the peaks in the range of 3523-3209 cm^{-1} which was signify to N-H $_{stretch}$. Whereas 3150 - 2850 cm^{-1} explored C-H stretching for Aromatic carbon atom. A strong stretching band arise 1379-1280 cm^{-1} showed C-N $_{stretch}$ for Aromaticity. Whereas C=S group appear 1249- 1076 cm^{-1}. The strong absorption bands at 1084–650 cm^{-1} confirm the existence of aromatic skeleton.

Result and Discussion

Antibacterial chemistry

All the synthesized compounds were tested for their *in vitro* antibacterial activity by the broth dilution technique in terms of MIC (Table 1). The present study included the Ciprofloxacin as reference antibacterial agent. MIC data of synthesized compounds on bacterial strain showed following conclusion. Compound **11a** having P-aminophenol substitution on 1,3,5 triazine shows potent activity against *S. Aureus, E. Coli, P. vulgaris,* while moderate activity against *P. Subtilis, P. aeruginosa and P. Mirabilis* and mild action on *B. Cereus.* Compound with o- toluidine substitution **11b** shows potent activity against *S. Aureus, B. Cereus, P. Aeruginosa, E. coli and P. Mirabilis* while moderate activity against *B. Subtilis and P. vulgaris.* Compound **11c**

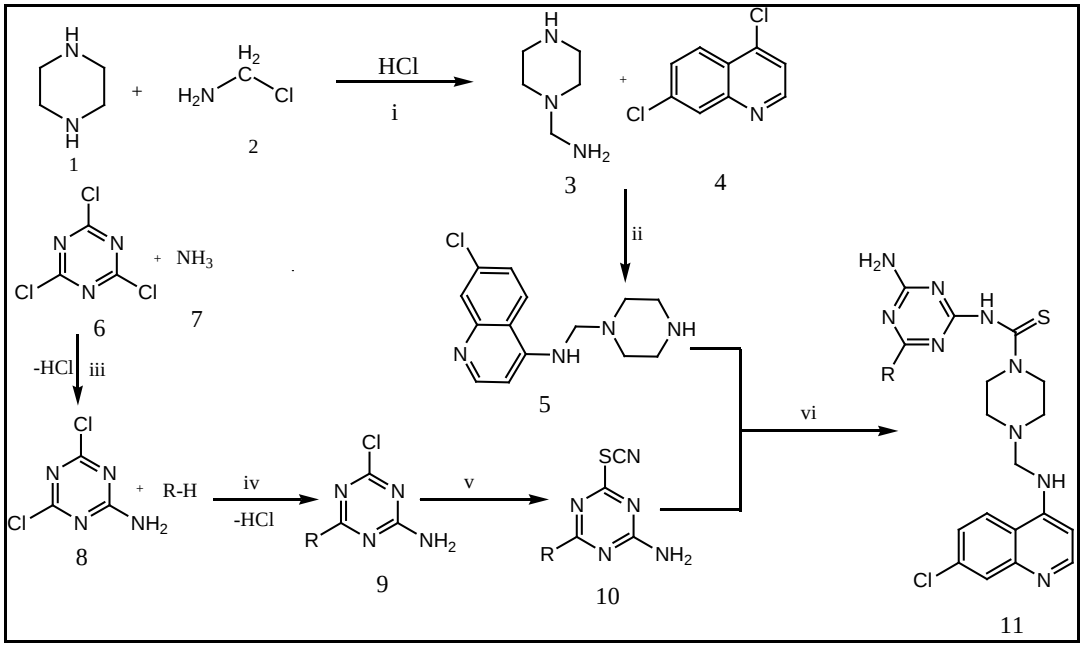
bearing 4- methoxyaniline substitution exhibit potent activity against *P. mirabilis* and moderate activity against *B. Cereus, P. aurogenosa,* and *P. vulgaris.* Replacement of 4-methoxyaniline with Piperazine ring 11d exhibited higher activity against all bacterial strain except than B. subtilis. Further replacement of piperazine with 4-Nitro-phenylamine 11e showed higher activity against S. aureus, *P. vulgaris, B. Subtilis* while mild to moderate activity exhibited on *P. aurogenosa, E. Coli,* and *P. mirabilis.* Again further replacement of aromatic amine with aliphatic amine like thioacetamide compound 11f showed potent activity against S. Aureus *P. vulgaris,* while mild activity against *B. Subtilis P. aurogenosa, E. Coli,* and *P. mirabilis.* Bridged derivative 11g having 1,3 diamino propane substitution exhibited potent activity against *P. vulgaris P. aurogenosa S. Aureus, P. mirabilis* and *B. cereus* and moderate activity against *B. subtilis.* Compound 11h having marpholine substitution showed potent activity against all bacterial strain except *S. aureus.* Semicarbazide derivative 11i showed higher activity against *B. subtilis, B. cereus. P. aurogenosa. E. coli, P. mirabilis* and *P. vulgaris*, while moderate antibacterial efficiency seen on *S. aureus*. Compound 11j were obtained via thiosemicarbazide substitution, the derivative exhibited most potent antibacterial activity among all the synthesized derivative in term of bacterial strains it maximise the inhibition of *S. aureus, B. cereus, P. aerugenosa, E. coli* and *P. vulgaris* at 3.125 μg mL^{-1} of MIC while B. subtilis growth was inhibited at 6.50 μg mL^{-1} of MIC and P. mirabilis MIC was obtained at 12.5 μg mL^{-1}. Result shows that most of the compounds inhibited the growth of bacterial strain with MIC ranging from 3.125 μg mL^{-1} to 100 μg $mL^{-}1$, From structure activity relationship (SAR) of the synthesized compounds having aliphatic substitutation on 1,3,5-triazine such as **11f**, **11g**, **11i** and **11j** exhibit highest activity against *B. cereus* and *P. vulgaris* . Whatever aromatic substitutation on 1,3,5-triazine *viz*. **11a**, **11b**, **11c**, **11d**, **11e** and **11h** exhibit highest activity against *S. Aureus, P. aurogenosa. P. mirabilis* and *P. vulgaris*.

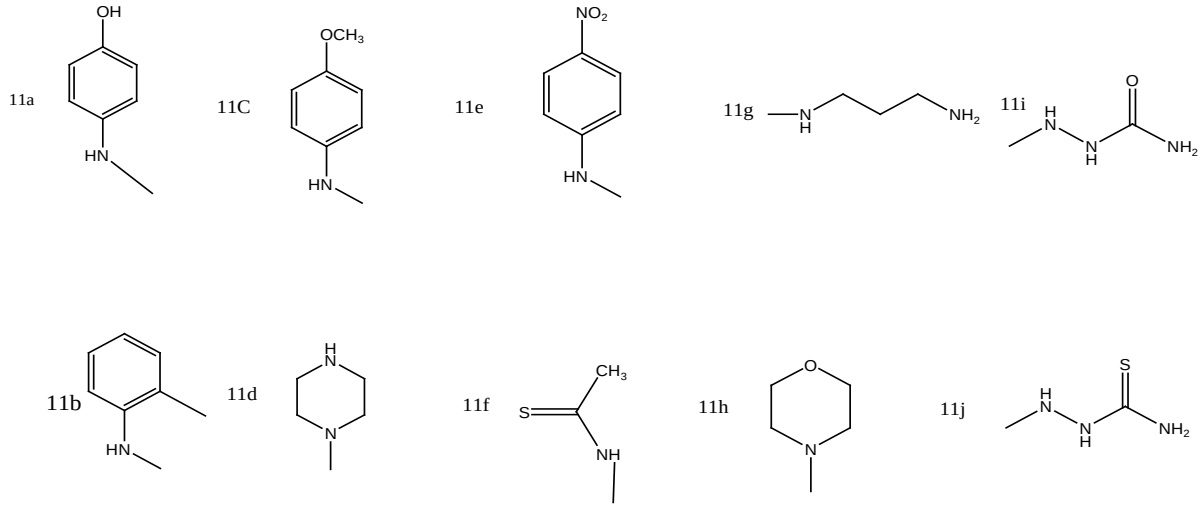
Table: 1 MIC (μg mL^{-1}) results of the tested compounds. Minimum Inhibitory Concentration (μg mL^{-1})

Compounds	***Gram +ve***			***Gram –ve***			
	S. aureus	**B. subtilis**	**B. cereus**	**P. aeruginosa**	**E. coli**	**P. mirabilis**	**P. vulgaris**
11a	3.125	12.5	100	12.5	3.125	12.5	3.125
11b	6.25	12.5	6.25	6.25	6.25	3.125	12.5
11c	100	50	12.5	12.5	25	6.25	12.5
11d	3.125	6.25	50	3.125	3.125	3.125	6.25
11e	3.125	3.125	6.25	12.5	100	25	6.25
11f	6.25	25	12.5	25	50	50	3.125
11g	12.5	25	12.5	6.25	100	12.5	3.125
11h	50	6.25	6.25	3.125	6.25	12.5	12.5
11i	50	3.125	6.25	6.25	3.125	3.125	12.5
11j	3.125	6.25	3.125	3.125	3.125	12.5	3.125
Ciprofloxacin (Standard)	6.25	12.5	3.125	25	6.25	25	12.5

Experimental

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 (2.0 cm-1, flat, smooth, abex) were recorded on Perkin Elmer RX-I Spectrophotometer. 1H NMR spectra were recorded on Bruker Avance II 400 NMR and 13C NMR spectra on Bruker Avance II 100 NMR spectrometer in CDCl3-d6 using TMS as internal standard. Mass spectra were obtained on VGAUTOSPEC spectrometer equipped with electrospray ionization (ESI) sources. Elemental analysis was carried out on Vario EL-III CHNOS elemental analyzer.





Scheme 1: ***Reagents and Conditions:*** **R-H (a-j) various amines (i) K_2CO_3, isopropyl alcohol, Triethyl amine, reflux at 80-85^{0}C for 12 h (ii) K_2CO_3, isopropyl alcohol, reflux at 80-85^{0}C for 36 h (iii) Acetone, stir 3 h at 0-5^{0}C, $NaHCO_3$ (iv) Acetone, stir 5 h at 40-45^{0}C reflux, $NaHCO_3$ (v) KSCN, 1,4-dioxane, reflux at 100^{0}C for 6-7 h, K_2CO_3, silver granules (vi) dry acetone, reflux for 8-9 h at 45^{0}C.**

Piperazin-1-yl-methylamine(3)

A mixture of A mixture of Piperazine (**1**) (0.01 mole), Chloro-methylamine (**2**) (0.01 mole), triethylamine (0.001mole) and anhydrous Potassium carbonate (0.01 mole) in 100 ml of isopropyl alcohol were refluxed with continuous stirring for 12 hr at 80 -85^{0}C. The reaction mixture was concentrated under reduced pressure. The resulted residue was dissolved in dichloromethane, washed with brine and dried over Na_2SO4. 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.

Faint yellow crystals; Yield: 54 %; M.p: 110-111 °C; MW: 115.18 ; R*f*: 0.48**; FTIR (ν_{max}; cm-1 KBr):)**, 3320.61 (N-H $_{broad}$), 2054.32 (C–H $_{stretch}$), 1586011 (N-H $_{stretch}$); **^{1}H NMR (400MHz, $CDCl_3$, TMS) δ ppm:** 8.13 (s, 2H, NH_2), 3.86-2.98 (m, 8H, 4XCH_2, Piperazine), 1.86 (br,s, 1H, NH); **^{13}C NMR (100MHz, DMSO-d_6) δ ppm:** 150.3, 135.7, 129.9, 118.6, 112.8; **Mass:** 115.11 $(M+H)^+$; **Elemental Analysis** for $C_5H_{13}N_3$: **Calculated:** C, 52.14; H, 11.38; N, 36.48; **Found:** C, 51.23; H, 10.86; N, 35.97

(7-Chloro-quinolin-4-yl)-piperazin-1-ylmethyl-amine (5)

A solution of Piperazin-1-yl-methylamine(0.002 mole) (3), 4,7 –dichloroquinoline (4) (0.001 mole) and potassium carbonate (0.001 mole) was refluxed for 36 hrs at 80-85^{0}C. The mixture was cooled and then reheated to distill the solvent under reduced pressure. Water (120 ml) was added into the reaction mixture and the aqueous layer was extracted twice with dichloromethane (80 ml). The combined layer was concentrated and it was prolonged under low pressure. Hexane (25 ml) was added into the reaction mass and t was stirred for hour at room temperature, which afforded a white crystalline solid. The content was filtered and washed with hexane and dried at 50-60^{0}C under vacuum for few hours. . The completion of reaction was monitored by TLC using methanol: ethyl acetate (2:8) as mobile phase.

White crystals; Yield: 72 %; M.p: 115-116 °C; MW: 276.76 ; R*f*: 0.32; **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 (400MHz, $CDCl_3$, 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, 105 quinoline), 6.83 (d, 1H *J*=5.1 Hz, quinoline), 3.18-2.96 (m, 8H, 4xCH2, Piperazine), 1.86 (br, s, 1H, NH); **^{13}C NMR (100MHz, 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; **Mass:** 276.16 $(M+H)^+$; **Elemental Analysis** for $C_{14}H_{17}ClN_4$: **Calculated:** C, 60.76; H, 6.19; N, 20.24; Cl, 12.81 **Found:** C, 60.87; H, 6.73; N, 20.87**.**

4,6-dichloro-1,3,5-triazin-2-amine (8)

Strong ammonia solution (**7**) (0.1 mol) was added into 25 mL of acetone contain 2,4,6-trichloro-1,3,5-triazine (**6**) (0.1 mol) maintaining temperature 0–5^{0}C. The resulting mixture was then stirred for 3h followed by drop wise addition of $NaHCO_3$ solution to take care that reaction mixture 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.

White crystals; Yield: 64%; M.p: 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; **Mass:** 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 9 (a-j)

Various distinguished amines (**a-j**) (0.1 mol) were added into 100 30 mL of acetone maintaining temperature 40–45^{0}C. The solution of mono subtituted-1,3,5-triazine (**8**) (0.1 mol) in 25 mL acetone was added constantly, stirred for 5h followed by drop-wise addition of $NaHCO_3$ solution (0.1 mol) taking care that 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 **9**(**a-j**).

General procedure for the synthesis of tri-substituted 1,3,5- triazine derivatives. 10 (a-j)

A solution of di-substituted 1,3,5-triazine compounds **9 (a-j)** (0.01 mol), potassium thiocynate (0.01 mole) and K_2CO3 (0.01 mole) in 1,4-dioxane was refluxed for 6-7 h in presence of tin granules which 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 **10** (**a-j**).

General procedure for the synthesis of titled compounds. 11(a-j)

A solution of (7-Chloro-quinolin-4-yl)-piperazin-1-ylmethyl-amine (**5**) (0.01 mol.) and desired trisubstituted 1,3,5-triazine compounds **10**(**a-j**) (0.01 mol.) in dry acetone was stirred at 40-45 oC 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 anhydrous Na_2SO4. The dried solution was concentrated under reduced pressure to obtain the titled compounds **11**(**a-j**).

4-[(7-Chloro-quinolin-4-ylamino)-methyl]-piperazine-1-carbothioic acid [4-amino-6-(4-hydroxy-phenylamino)-[1,3,5]triazin-2-yl]-amide(11a) Black crystals; Yield: 82%; M.p: 270-272 °C; MW:537.04 ; R*f*: 0.94; **FTIR (ν_{max} ; cm^{-1} KBr):** 3312.21 (N-H $_{broad}$, $-NH_2$), 2926.55 (C-C $_{stretch}$), 2057.64 (C-H $_{stretch}$, Ar), 1611.93 (N-H $_{stretch}$, Sec amine), 1582.21 (NH $_{stretch}$, NH_2), 1513.13 (NH $_{stretch}$, NH_2), 1450.96 (C-H $_{stretch}$, NH_2), 1371.56 (CN $_{stretch,}$ Ar NH), 1336.30 (N-H $_{Stretch}$, Sec amine), 1242.98 (OH $_{stretch}$, Phenol), 1165.09 (C=S $_{stretch}$), 832.91 (C-H $_{stretch}$); **^{1}H NMR (400 MHz, $CDCl_3$, TMS) δ ppm:** 8.540 (s, 1H, quinolyl), 7.962 (s, 1H, NH), 7.665 (s, 1H, Ar), 7.378 (s, 1H, quinolyl), 6.906 (s, 1H, quinolyl), 6.386 (s, 1H, quinolyl), 4.03-2.98 (m, 8H, 4xCH_2, piperazine), 3.703 (br, s, 1H, NH), 3.310 (s, 1H, quinolyl), 2.942 (s, 1H, NH), 1.549, 1.253 (s, 1H, CH); **^{13}C NMR (100MHz, DMSO-d_6) δ ppm:** 151.50, 149.95, 127.31 ,125.21, 124.07, 123.65, 122.62, 115.71, 115.49, 115.32, 98.45, 78.81, 78.48, 78.15, 40.60, 40.16, 39.95, 39.74, 39.54, 39.33, 39.12, 38.91, 30.49; **Mass:** 539.8 $(M+H)^+$; **Elemental analysis** for $C_{24}H_{25}ClN_{10}OS$: **Calculated:** C, 53.68; H, 4.69; Cl,6.60; N, 26.08; O,2.98; S,5.97; **Found:** C, 53.01; H, 4.38; N, 25.83.

4-[(7-Chloro-quinolin-4-ylamino)-methyl]-piperazine-1-carbothioic acid (4-amino-6-o-tolylamino-[1,3,5] triazin-2-yl)-amide (11b)

Dark white crystals; Yield: 62%; M.p: 265-267 °C; MW:535.07; R*f*: 0.79; **FTIR (ν_{max} ; cm^{-1} KBr):** 3322.54 (N-H $_{stretch,}$ NH_2), 2924.81 (C-H $_{stretch}$), 2053.94 (C-H $_{stretch}$, Ar), 1580.89 (N-H $_{stretch}$, Sec amine), 1537.85 (C-H $_{stretch}$, CH_3), 1503.77 (C-H $_{stretch}$, CH_3) , 1445.11, 1366.58 (C-N $_{stretch}$, Ar-NH), 1243.41 (C=S $_{stretch}$) 876.73, 809.91 (C-H $_{stretch}$); **^{1}H NMR (400MHz, $CDCl_3$, TMS) δ ppm:** 8.534-8.517 (d, 1H, *J*=5.1 Hz, quinolyl), 7.967 (s, 1H, NH), 7.883-7.857 (d, 1H, *J*=7.8 Hz, quinolyl), 7.882-7.857 (d, 1H, *J*=7.8 Hz, quinolyl), 7.883-7.857 (d, 1 H, *J* =8.1 Hz, quinolyl), 7.368-7.006 (m, 4H, CH_3), 6.715 (s, 1H, quinolyl), 6.398-6.380 (d, 1H, *J* =5.4Hz, quinolyl), 5.045 (s, 1H, quinolyl) 4.03-3.08 (m, 8H, 4xCH_2, piperazine) 4.899 (br, s, 1H, NH), 3.823 (s, 1H, quinolyl), 2.827-2.791 (d, 1H, *J* =10.3 Hz, Aromatic); **^{13}C NMR (100MHz, DMSO-d_6) δ ppm:** 167.10, 167.00, 165.20, 164.89, 150.61, 150.48, 147.71, 137.45, 137.36, 131.14, 131.04, 129.80, 126.55, 125.53, 124.61, 124.35, 123.90, 123.58, 123.44, 117.08, 98.49, 78.80, 78.47, 78.15, 55.58, 52.62, 42.51, 40.20, 39.99, 39.78, 39.57, 39.36, 39.15, 38.94, 30.50, 17.97; **Mass:** 539.8 $(M+H)^+$;**Elemental analysis** for $C_{25}H_{27}ClN_{10}S$: **Calculated:** C, 56.12; H, 5.09; Cl,6.63;N, 26.18;S, 5.99; **Found:** C, 56.01; H, 5.38; N, 26.83.

4-[(7-Chloro-quinolin-4-ylamino)-methyl]-piperazine-1-carbothioic acid [4-amino-6-(4-methoxy-phenyl amino) -[1,3,5]triazin-2-yl]-amide (11c)

Light violet crystals; Yield: 72%; M.p: 310-312 °C; MW: 551.07 ; R*f*: 0.81; **FTIR (ν_{max}; cm^{-1} KBr):** 3399.46 (N-H $_{stretch}$, -NH_2), 3062.79 (-C=C $_{broad}$, Ar), 2959.85 (C-H $_{stretch}$, Aromatic), 2836.66 (C-H $_{stretch}$, Aromatic), 2064.07 (C-H $_{stretch}$, Aromatic), 1740.37, 1711, 1710 (N-H $_{stretch}$, Sec amine), 1296.13 (C-N $_{stretch}$, Ar-NH), 1179.18 (C=S $_{stretch}$), 867.849 (C-H $_{stretch}$); **^{1}H NMR (400MHz, $CDCl_3$, TMS) δ ppm:** 7.423-7.396 (d, 1H, *J* =8.1 Hz, quinolyl), 7.257 (s, 1H, NH), 6.855-6.828 (d, 1H, *J* =8.1 Hz, quinolyl), 4.995 (s, 1H, quinolyl), 4.591 (br, s, 1H, NH), 4.02-2.96 (m, 8H, 4xCH_2, piperazine), 3.793 (s, 1H, quinolyl), 2.933 (s, 1H, NH), 1.831 (s, 1H, CH), 1.255 (s, 1H, CH); **^{13}C NMR (100MHz, DMSO-d_6) δ ppm:** 166.72, 164.34, 164.00, 154.48, 154.30, 132.93, 123.45, 121.95, 121.71, 113.24, 78.94, 78.61, 78.28, 66.33, 55.00, 40.06, 39.86, 39.65, 39.44, 39.23, 39.02, 38.01, 30.52; **Mass:** 550.8 $(M+H)^+$; **Elemental analysis** for $C_{25}H_{27}ClN_{10}S$: **Calculated:** C, 54.49; H, 4.94; Cl, 6.43; N, 25.42;S, 5.82; **Found:** C, 54.01; H, 4.22; N, 26.21.

4-[(7-Chloro-quinolin-4-ylamino)-methyl]-piperazine-1-carbothioic acid (4-amino-6-piperazin-1-yl-[1,3,5] triazin-2-yl)-amide (11d)

off white crystals; Yield: 64%; M.p: 250-252 °C; MW: 514.05; R*f*: 0.81; **FTIR (ν_{max} ; cm^{-1} KBr):** 3462.45 (N-H $_{stretch,}$ -NH2), 3338.63 (N-H $_{broad}$, Aromatic), 2920.93, 2851.11 (C-H $_{stretch}$), 2058.54 (C-H $_{stretch}$), 1738.07 (N-H $_{stretch}$, Sec- amine), 1583.54 (N-H $_{stretch}$, Sec- amine), 1480.89 (-C=C $_{stretch}$), 1432.41 (C-H $_{stretch}$, CH_3), 1367.65 (C-N $_{stretch}$, Ar-NH), 1135.09 (C=S $_{stretch}$), 996.80, 808.80 (C-H $_{stretch}$); **^{1}H NMR (400MHz, $CDCl_3$, TMS) δ ppm:** 8.535-8.518 (d ,1H, *J*=5.1Hz , quinolyl), 7.957 (s, 1H, quinolyl), 7.700-7.670 (d, 1H, *J* = 9.0Hz, NH), 7.400-7.37 (d, 1H, *J*=8.7 ,quinolyl), 6.381-6.364 (d, 1H, *J*=5.1Hz, quinolyl), 6.008 (s, 1H, piperazine), 3.312 (s, 1H, quinolyl), 2.515 (s, 1H, NH), 1.682, 1.253 (s, 1H, CH); **^{13}C NMR (100MHz, DMSO-d_6) δ ppm:** 151.54, 149.9, 148.73, 133.65, 127.39, 124.08, 123.08 , 117.24, 98.44, 78.71, 78.57, 78.38, 78.05, 66.36, 56.10,

53.85, 45.45, 40.15, 39.74, 39.53, 39.32, 39.11, 38.9, .30.49; **Mass:** 514.8 **(**$(M+H)^+$; **Elemental analysis** for $C_{22}H_{28}ClN_{11}S$: **Calculated:** C, 51.40; H, 5.49; Cl, 6.90; N, 29.97; S, 6.24; **Found:** C, 51.12; H, 5.21; N, 28.21.

4-[(7-Chloro-quinolin-4-ylamino)-methyl]-piperazine-1-carbothioic acid [4-amino-6-(4-nitro-phenyl amino) -[1,3,5]triazin-2-yl]-amide (11e)

Light brown crystals; Yield: 58%; M.p: 270-272 °C; MW: 565.15; R*f*: 0.82; **FTIR (ν_{max} ; cm^{-1} KBr):** 3445.59 (N-H $_{broad}$, NH_2), 2923.36 (-CH $_{broad}$), 2836.80 (-CH $_{stretch}$, Aromatic), 2053.35 (C-H $_{stretch}$, Aromatic), 1711.89, 1689.32 (N-H $_{stretch}$, Sec Amine), 1245.87 (C=S $_{stretch}$), 1084.76, 707.06 (C-H $_{stretch}$); **^{1}H NMR (400MHz, $CDCl_3$, TMS) δ ppm:** 7.588-7.561 (d. 1H, *J*=8.1, quinolyl), 7.484 (s, 1H, NH), 7.298-7.255(d, 1H, *J*=12.9 Hz, NH), 6.949-6.923 (d, 1H, *J*=7.8 Hz, quinolyl), 4.02-3.96 (m, 8H, 4xCH_2 , piperazine) 3.975 (br, s, 1H, NH), 3.636 (s, 1H, quinolyl), 1.601, 1.253 (s, 1H, CH); **^{13}C NMR (100MHz, DMSO-d_6) δ ppm:** 148.6, 129.18, 119.93, 110.83, 107.82, 77.80, 77.47, 77.15, 40.22, 40.01, 39.59, 39.39, 39.18, 38.87, 30.45, 29.06; **Mass:** 565.8 $(M+H)^+$; **Elemental analysis** for $C_{24}H_{24}ClN_{11}O_2S$: **Calculated:** C, 50.93; H, 4.27; Cl, 6.26; N, 27.22;S, 5.66,O,5.65; **Found:** C, 50.82; H, 4.11; N, 27.29.

4-[(7-Chloro-quinolin-4-ylamino)-methyl]-piperazine-1-carbothioic acid (4-amino-6-thioacetylamino-[1,3,5] triazin-2-yl)-amide (11f)

Grey color crystals; Yield: 68%; M.p: 295-297 °C; MW:504.03; R*f*: 0.86**; FTIR (ν_{max} ; cm^{-1} KBr):** 3301.06 (N-H $_{stretch}$, -NH_2), 3154.77 (N-H $_{broad,}$ -NH_2), 2852.69 (C-H $_{stretch}$ Ar), 2809.40 (-C=C $_{stretch}$, Aromatic), 1733.07 (N-H $_{stretch}$, Sec- amine), 1703.51 (N-H $_{stretch}$, Sec-amine), 1643.63 (N-H $_{stretch}$, Sec-amine), 1574.86 (C-H $_{stretch}$, CH_3), 1458.49 (C-H $_{stretch}$, CH_3), 1416.51 (C-N $_{stretch,}$ Ar-NH), 1265.19, 1179.28 (C=S $_{stretch}$), 870.84, 903.54 (C-H $_{stretch}$), 659.47, 691.16, 780.06 (C-H $_{rocking}$), **^{1}H NMR (400MHz, $CDCl_3$, TMS) δ ppm:** 8.539 (s, 1H, quinolyl), 7.966 (s, 1H, quinolyl), 7.692-7.663 (d, 1H, *J*=8.1, quinolyl), 7.405-7.378 (d, 1H, *J*=8.1 Hz, quinolyl), 7.256 (s, 1H, NH), 6.406-6.385 (d, 1H, *J*=6.3 Hz, quinolyl), 4.12-3.06 (m, 8H, 4xCH_2, piperazine) 4.723 (br, s, 1H, NH), 3.702 (s, 1H, quinolyl), 2.942 (s, 1H, NH), 1.558 (s, 1H, CH); **Mass:** 505.2 $(M+H)^+$; **Elemental** analysis for $C_{19}H_{22}ClN_{11}S_2$: **Calculated:** C, 45.28; H, 4.40; Cl, 7.03; N, 30.57;S, 12.72; **Found:** C, 46.26; H, 4.21; N, 29.91.

4-[(7-Chloro-quinolin-4-ylamino)-methyl]-piperazine-1-carbothioic acid [4-amino-6-(3-amino-propyl amino)-[1,3,5]triazin-2-yl]-amide (11g)

Sandy grey color crystals; Yield: ; M.p: 265-267 °C; MW: 502.04; R*f*: 0.89, **FTIR (ν_{max} ; cm^{-1} KBr):** 3296.46 (N-H $_{broad}$, NH_2), 2961.04, 2840.82 (-C=C $_{broad}$, Ar), 2057.25 (C-H $_{stretch}$, Aromatic), 1715.38 (N-H $_{stretch}$, NH_2), 1582.38, 1556.69 (N-H $_{stretch}$, Sec-amine), 1484.78, 1416.93, 1370.92 (C-N $_{stretch}$, Ar-NH_2), 1247.72 (C=S $_{stretch}$), 872.38-810.89 (C-H $_{stretch}$); **^{1}H NMR (400MHz, $CDCl_3$, TMS) δ ppm:** 8.520-8.503 (d, 1H, quinolyl), 7.947 (s, 1H, NH), 7.733-7.704 (d, 1H, J=8.7 Hz, CH), 7.399-7.294 (t, 2H, *J*=31.5 Hz, CH_2), 6.383-6.366 (d, 1H, *J*=5.1 Hz, quinolyl), 6.069 (s, 1H, quinolyl), 4.12-3.06 (m, 8H, 4xCH_2 , piperazine) 3.701 (s, 1H, NH), 3.413-3.322 (t, 2H, *J*=27.3 Hz, CH_2), 2.788-2.768 (d, 1H, quinolyl), 2.519 (s, 1H, NH), 1.255 (s, 1H, CH); **^{13}C NMR (100MHz, DMSO-d_6)δ ppm:** 178.20, 124.41, 77.92, 77.60, 77.27, 53.38, 48.42, 40.21, 40.00, 39.79, 39.58, 39.38, 39.17, 38.96, 30.45, **Mass:** 501.25 $(M+H)^+$; **Elemental analysis** for $C_{21}H_{28}ClN_{11}S$: **Calculated:** C, 50.24; H, 5.62; Cl, 7.06; N, 30.69;S, 6.39 ; **Found:** C, 48.19; H, 5.17; N, 29.95.

4-[(7-Chloro-quinolin-4-ylamino)-methyl]-piperazine-1-carbothioic acid (4-amino-6-morpholin-4-yl-[1, 3, 5] triazin-2-yl)-amide (11h)

Light yellow color crystals; Yield: ; M.p: 278-280 °C; MW: 515.03; R*f*: 0.85**; FTIR (ν_{max} ; cm^{-1} KBr):** 3209.49 (N-H $_{broad}$, NH_2), 3067.24 (N-H $_{stretch}$, NH_2), 2964.05, 2943.18, 2918.93 (C=C $_{stretch}$, Aromatic), 2851.47 (C=C $_{stretch}$, Aromatic), 2059.47 (C-H $_{stretch}$, Aromatic), 1584.65, 1545.05 (N-H $_{stretch}$, Sec-amine), 1330.15, 1280.57 (C-N $_{stretch}$, Ar-NH_2), 1249.10, 1199.27 (C=S $_{stretch}$), 910.38, 872.04 (C-H $_{stretch}$)**; ^{1}H NMR (400MHz, $CDCl_3$, TMS) δ ppm:** 8.522 (s, 1H, NH), 7.957 (s, 1H, quinolyl),7.393 (s, 1H, NH), 6.374 (s, 1H, quinolyl), 4.833 (br, s, 1H, NH), 4.12-3.06 (m, 8H, 4xCH_2 , piperazine) 3.739-3.701 (t, 2H, *J*=11.4 Hz, CH_2), 3.343-3.314 (t, 2H, *J*=8.7 Hz, CH_2), 2.954-2.939 (d, 1H, *J*=4.5 Hz, NH), 2.785-2.746 (m, 4H, C_2H_5), 2.526 (s, 1H, quinolyl),1.225 (s, 1H, CH); **^{13}C NMR (100MHz, DMSO-d_6) δ ppm:** 166.97, 165.27, 151.49, 149.64, 148.56, 133.9, 127.61, 124.36, 121.99, 117.04, 98.55, 77.87, 77.74, 66.19, 55.76, 53.53, 45.51, 42.94, 40.19, 39.99, 39.78, 39.57, 39.36, 38.94, 38.84, 30.45; **Mass:** 515.14 $(M+H)^+$; **Elemental analysis** for $C_{22}H_{27}ClN_{10}OS$ **Calculated:** 51.30; H, 5.28; Cl, 6.88; N, 27.20; O, 3.11; S, 6.23; **Found:** C, 50.24; H, 5.17; N, 29.28.

2-[(4-Amino-6-(4-(7-chloroquinolin-4-yl) methyl] piperazine-1-carbothioamido)-1,3,5-triazin-2- yl) hydrazinecarboxamide (11i)

white color crystals; Yield: ; M.p: 120-122 °C; MW: 502.98; R*f*: 0.95**; FTIR (ν_{max} ; cm^{-1} KBr):** 3523.43, 3487.75 (N-H $_{stretch}$, -NH_2), 2965.05 (C-H $_{broad}$, Aromatic), 2929.80 (C=C $_{stretch}$, Aromatic), 2056.77 (C-H $_{stretch}$, Aromatic), 1564.42 (N-H $_{stretch}$, Sec-amine), 1379.90 (C-N $_{stretch}$, Ar-NH), 1076.94 (C=S $_{stretch}$), 849.05 (C-H $_{stretch}$); **^{1}H NMR (400MHz, $CDCl_3$, TMS) δ ppm:** 8.551-8.535 (d, 1H, *J*=4.8 Hz, NH), 7.969 (s, 1H, NH), 7.693-7.664 (d, 1H, *J*=8.7 Hz, quinolyl), 7.405-7.576 (d, 1H, *J*=8.7 Hz, quinolyl), 6.405-6.387 (d, 1H, *J*=5.4 Hz, quinolyl), 4.736 (br, s, 1H, NH), 4.12-3.06 (m, 8H, 4xCH_2 , piperazine) 3.819 (s, 1H, quinolyl), 3.355 (s, 1H, quinolyl), 2.832-2.794 (tr, 2H, *J*=11.4 Hz, CH_2), 1.625,1.25 (s, 1H, CH); **^{13}C NMR (100MHz, DMSO-d_6) δ ppm:** 206.38, 166.53, 122.79, 77.83, 77.70, 77.51, 77.18, 55.08, 42.44, 40.22, 40.01, 39.80, 39.59, 39.38, 39.17, 38.96, 38.45, 11.9**; Mass:** 504.2 $(M+H)^+$; **Elemental analysis** for $C_{19}H_{23}ClN_{12}OS$ **Calculated:** 45.37; H, 4.61; Cl, 7.05; N, 33.42; O, 3.18; S, 6.37; **Found:** C, 43.23; H, 5.07; N, 32.24.

N-(4-amino-6-(2-carbamothioylhydrazinyl)-1,3,5-triazin-2-yl)-4-[(7-chloroquinolin-4-yl)methyl]-piperazine-1-carbothioamide (11j)

Light brown color crystals; Yield: ; M.p: 195-197 °C; MW: 519.05; R*f*: 0.83**; FTIR (ν_{max} ; cm^{-1} KBr**): 3329.49 (N-H $_{broad}$, –NH_2), 2056.54 (C-H $_{stretch}$, Aromatic), 1583.69 (N-H $_{stretch}$, Sec-amine), 1371.58 (C-N $_{stretch}$, Aromatic), 810.44 (C-H $_{stretch}$); **^{1}H NMR (400MHz, $CDCl_3$, TMS) δ ppm:** 8.537-8.519 (d, 1H, *J*=5.1 Hz, NH), 7.956 (s, 1H, NH), 7.697-7.667 (d, 1H, *J*=9.0 Hz, NH), 7.599-7.370 (d, 1H, *J*=8.7 Hz, quinolyl), 7.257 (s, 1H, NH), 6.381-6.364 (d, 1H, *J*=5.1 Hz, quinolyl), 4.12-3.06 (m, 8H, 4xCH_2 , piperazine) 2.789-2.750 (t, 2H, *J*=11.7 Hz, CH_2), 1.654, 1.254 (s, 1H, CH); **^{13}C NMR (100MHz, DMSO-d_6) δ ppm:** 151.95, 149.92, 145.96, 127.45, 123.37, 117.61, 114.21, 98.51, 79.08, 78.75, 78.42, 52.99, 44.97, 40.18, 39.98, 39.77, 39.56, 39.35, 39.14, 38.93, 30.33; **Mass:** 518.2 **$(M+H)^+$; Elemental analysis** for $C_{19}H_{23}ClN_{12}S_2$ **Calculated:** 43.97; H, 4.47; Cl, 6.83; N, 32.38;S, 12.36; **Found:** C, 43.23; H, 4.07; N, 32.16.

Antibacterial Screening

Minimum Inhibitory Concentration

All synthesized compounds were screened for their minimum inhibitory concentration (MIC, μg/mL) against selected Gram positive organisms viz. *Bacillus subtilis* (NCIM-2063), *Bacillus cereus* (NCIM-2156), *S.aureus* (NCIM-2079) and Gram-negative organism viz. *Pseudomonas aeruginosa* (NCIM-2036), *Escherichia coli* (NCIM-2065), *P.mirabilis* (NCIM-2241), *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 dimethyl sulfoxide (DMSO) at concentrations of 100, 50, 25, 12.5, 6.25, 3.125 μg/mL. 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 (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 since it contained bacteria but not antibiotic. The test compound were dissolved in DMSO (100 μg/mL), 0.1 mL of increasing Concentration of the prepared test compounds which are serially diluted from tube 2 to tube 7 from highest (100 μg/mL) to lowest (3.125 μg/mL) concentration (tube 2–7 containing 100, 50, 25, 12.5, 6.25, 3.125 μg/mL). After this process, each tube was inoculated with 0.1 mL of the bacterial suspension whose concentration corresponded to 0.5 McFarland scales (9 × 108 cells/mL) and each bacterium was incubated at 37 °C for 24 h at 40 150 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, i.e. no growth of inoculated bacteria. Disc diffusion along with percentage of inhibition in comparison to standard The inoculum can be prepared by making a direct broth or saline suspension of isolated colonies of the same strain from 18 to 24 h Müeller-Hinton agar plate. The suspension is adjusted to match the 0.5 McFarland turbidity standard, using saline and a vortex mixer. Optimally, within 15 min after adjusting the turbidity of the inoculums suspension, a sterile cotton swab is dipped into the adjusted suspension and then afterwards the dried surface of an agar plate is inoculated by streaking the swab over the

entire sterile agar surface. Any excess surface moisture to be absorbed before applying the drug impregnated disk. Results were shown in table 1.

Conclusion

In present study, we reported a convenient route for the synthesis of new piperazine bridged 4-aminoquinoline incorporated nucleus with substituted *s*-triazine as potent antibacterial agent. The data show that derivatives **11i and 11j** with the semicarbazide and thiosemicarbazide substitutation on 1,3,5-triazine nucleus were more potent antibacterial agent than the other synthesize derivatives**.** Our study is in progress towards the development of new derivatives of this skeleton and will be reported in future.

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