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RESEARCH ARTICLE

A new class of 2-(4-cyanophenyl amino)-4-(6-bromo-4-quinolinyloxy)-6-piperazinyl (piperidinyl)-1,3,5-triazine analogues with antimicrobial/antimycobacterial activity

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Abstract

This study presents the synthesis and *in vitro* pharmacological evaluations of novel 2-(4-cyanophenyl amino)-4-(6-bromo-4-quinolinyloxy)-6-piperazinyl (piperidinyl)-1,3,5-triazines. The title compounds were assayed for their *in vitro* antimicrobial activity against eight bacteria (*Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Salmonella typhi*, *Proteus vulgaris*, *Shigella flexneria*) and four fungi (*Aspergillus niger*, *Aspergillus fumigatus*, *Aspergillus clavatus*, *Candida albicans*) using paper disc diffusion and agar streak dilution method as well as against *Mycobacterium tuberculosis* H37Rv strain using BACTEC MGIT and Lowenstein-Jensen MIC method. The bioassay results indicate that nine compounds namely 5d, 5h, 5n, 5p, 5q, 5r, 5s, 5t and 5u could be considered as possible potential agents with dual antimicrobial and antimycobacterial activities. The structures of the compounds were elucidated with the aid of IR, ^{1}H NMR, ^{13}C NMR, ^{19}F NMR spectroscopy and CHN analysis.

Keywords: 2,4,6-trichloro-1,3,5-triazin, 6-bromo-4-hydroxyquinoline, piperazine, antimicrobial activity, antimycobacterial activity

Introduction

Bacterial infections have been most deleterious to the human health due to pathogenic microbes which continuously evolve resistance to currently used antibacterial agents. In the developing countries, this problem is especially alarming. Furthermore, increased numbers of patients with impaired immunity may further increase the burden of antimicrobial resistance[1]. Thus development of novel antimicrobial drugs is crucial need to combat with the multidrug-resistant infections. However, another most worrisome trend in recent years is the increase in multidrug-resistant tuberculosis strains due to the quiescent form of mycobacterium tuberculosis strains with an estimated 2 million deaths each year[2–4]. The WHO has estimated that, According to the stop TB partnership's global plan to stop TB, 2006–2015, 1.3 million MDR-TB cases will need to be treated in the 27 high MDR-TB burden countries between 2010 and 2015. In 2008, there were an estimated 8.9–9.9 million incident cases of TB, 9.6–13.3 million prevalent cases of TB, 1.1–1.7 million deaths from TB among HIV-negative people and an additional 0.45–0.62 million TB deaths among HIV-positive people (classified as HIV deaths in the International Statistical Classification of Diseases), with best estimates of 9.4 million, 11.1 million, 1.3 million and 0.52 million, respectively[5]. Hence, there is an urgent need to identify new regimens involving novel mechanism of action and synthetically feasible to address resistance crisis as well as the global health emergency.

As part of our ongoing studies in developing new active s-triazinyl analogues[6–8], here in we report the synthesis and pharmacological activities of novel 2-(4-cyanophenyl amino)-4-(6-bromo-4-quinolinyloxy)-6-piperazinyl (piperidinyl)-1,3,5-triazines. 1,3,5-Triazine

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analogues constitute an important class of the realm of heterocycles, which has attracted much synthetic interest due to their wide range of biological activities such as antimicrobial[9–11], anticancer[12, 13], antimalarial[14], and antiviral[15] activity. A literature survey revealed that piperazines and substituted piperazines are an important family of heterocyclic compounds attracting significant interest in medicinal chemistry[16–20]. Recently several *s*-triazine derivatives bearing morpholine, piperidine and some piperazine moieties are reported to possess potent antimycobacterial activity[21], while quinoline-based anti-TB compound TMC207 is currently in Phase II clinical trials with very promising activity against MDR-TB[22,23]. Recently, several scaffolds-based on bromo-substituted quinoline moiety and some piperazine derivatives are reported to possess potent pharmacological activities[24–26]. This prior literature survey encouraged us to envisage the combination of these separate pharmacophoric groups of similar activity in a compact system to identify new candidates that may be of value in designing new, potent, selective and less toxic biologically active agents.

Materials and methods

Melting points were determined in open capillaries on a Veego electronic apparatus VMP-D (Veego Instrument Corporation, Mumbai, India) and are uncorrected. IR spectra (4000–400 cm^{-1}) of synthesized compounds were recorded on a Shimadzu 8400-S FT-IR spectrophotometer (Shimadzu India Pvt. Ltd., Mumbai, India) using KBr pellets. Thin layer chromatography was performed on object glass slides (2 × 7.5 cm) coated with silica gel-G and spots were visualized under UV irradiation. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian 400 MHz model spectrometer (Varian India Pvt. Ltd., Mumbai, India) using DMSO as a solvent and TMS as internal standard with ^{1}H resonant frequency of 400 MHz and ^{13}C resonant frequency of 100 MHz. ^{19}F NMR spectra were obtained on the same spectrometer using $CDCl_3$ as a solvent and $CFCl_3$ as an external standard, positive for downfield shift with ^{19}F resonant frequency of 400 MHz. The ^{1}H NMR, ^{13}C NMR and ^{19}F NMR chemical shifts were reported as parts per million (ppm) downfield from TMS (Me_4Si) and $CFCl_3$ and were performed at centre for excellence, Vapi, India. The splitting patterns are designated as follows; s, singlet; br s, broad singlet; d, doublet; m, multiplet. Elemental analyses (C, H, N) were performed using a Heraeus Carlo Erba 1180 CHN analyzer (Hanau, Germany).

4-[4,6-Dichloro-1,3,5-triazin-2-ylamino]-benzonitrile (1) was synthesized according to reported literature[27].

4-(4-(6-bromoquinolin-4-yloxy)-6-chloro-1,3,5-triazin-2-ylamino)-benzonitrile (3).

To a stirred solution of 6-bromo-4-hydroxyquinoline (8 g, 0.035 mol) in anhydrous THF (150 mL) 60% NaH (0.84 g, 0.035 mol) was added at room temperature during 1 h and **1** (9.31 g, 0.035 mol) was then added to the mixture. Stirring was continued for another 20 h at 45°C. Progress of the reaction was monitored by TLC using toluene: acetone (7:3) as eluent. The mixture was treated with crushed ice, filtered and dried to afford 3[28]. M.P. 282–283°C, yield 80%, IR (KBr) cm^{-1}, 2221 (C≡N), 1261 (C-O-C). (see Figures 1 and 2).

General procedure for preparation of compounds 5a-u

To a solution of 3 (0.01 mol) in 1,4-dioxane (30 mL), the respective substituted piperazine derivative was added and the reaction mixture was refluxed for 13–25 h. Potassium carbonate (0.01 mol) was used for neutralization of the reaction mixture. Progress of the reaction was monitored by TLC using toluene: acetone (8:2) as eluent. The mixture was then treated with crushed ice and neutralized by dilute HCl. The precipitate thus obtained was filtered off, dried and recrystallized from THF to afford the desired compounds 5a-u.

5a M.P. 242–244°C, yield 88% (found: C 55.59, H 3.96, N 21.61, $C_{24}H_{21}BrN_8O$, calc: C 55.71, H 4.09, N 21.66%). IR (KBr) cm^{-1}, 3280 (-NH), 2224 (C≡N), 1247 (C-O-C), 839 (*s*-triazine C-N str.); ^{1}H NMR (400 MHz, DMSO-d_6, δ) ppm, 9.13 (s, 1H, -N<u>H</u>, s-triazine to amino-benzonitrile linkage), 8.78 (d, J = 1.8 Hz, 1H, C_5 proton of quinoline), 8.58 (d, J = 8.3 Hz, 1H, -N=CH-, C_2 proton of quinoline), 8.46 (s, 1H, C_3 proton of quinoline), 8.26 (d, J = 7.5 Hz, 1H, C_8 proton of quinoline), 8.02 (dd, J = 7.7, 1.8 Hz, 1H), 7.54–7.35 (m, 6H, Ar-H), 3.79 (br s, 4H, piperazine), 3.56 (br s, 4H, piperazine), 1.98 (s, 1H, N-C<u>H</u>$_3$); ^{13}C-NMR (100 MHz, DMSO-d_6, δ) ppm, 177.1 (1C, C-6, *s*-triazine, <u>C</u>-N at piperazine linkage), 165.9 (1C, C-4, *s*-triazine, <u>C</u>-O-C at quinoline linkage), 164.4 (1C, C-2, *s*-triazine, <u>C</u>-NH at benzonitrile moiety), 154.2, 151.1 (2C, 1C of -<u>C</u>$_2$H=N-C_9 and 1C of -C_2H=N-<u>C</u>$_9$, quinoline), 146.1–120.7 (12C, aromatic carbon atoms), 105.1 (1C, <u>C</u>≡N), 99.1 (1C, -<u>C</u>-C≡N), 48.7, 44.1 (4C, piperazine), 21.3 (1C, -<u>C</u>H$_3$).

*5b*M.P. 235–236°C, yield 87% (found: C 56.41, H 4.21, N 20.97, $C_{25}H_{23}BrN_8O$, calc: C 56.50, H 4.36, N 21.09%). IR (KBr) cm^{-1}, 3301 (-NH), 2222 (C≡N), 1256 (C-O-C), 831 (*s*-triazine C-N str.); ^{1}H NMR (400 MHz, DMSO-d_6, δ) ppm, 9.21 (s, 1H, -N<u>H</u>, s-triazine to amino-benzonitrile linkage), 8.74 (d, J = 1.6 Hz, 1H, C_5 proton of quinoline), 8.66 (d, J = 8.9 Hz, 1H, -N=CH-, C_2 proton of quinoline), 8.53 (s, 1H, C_3 proton of quinoline), 8.31 (d, J = 7.7 Hz, 1H, C_8 proton of quinoline), 8.11 (dd, J = 7.8, 1.2 Hz, 1H), 7.49–7.36 (m, 4H, Ar-H), 3.77 (br s, 4H, piperazine), 3.51 (br s, 4H, piperazine), 2.35 (q, J = 7.3 Hz, 2H, N-C<u>H</u>$_2$), 1.88 (t, J = 6.7 Hz, 3H, CH_2-C<u>H</u>$_3$); ^{13}C-NMR (100 MHz, DMSO-d_6, δ) ppm, 176.6 (1C, C-6, *s*-triazine, <u>C</u>-N at piperazine linkage), 167.1 (1C, C-4, *s*-triazine, <u>C</u>-O-C at quinoline linkage), 164.1 (1C, C-2, *s*-triazine, <u>C</u>-NH at benzonitrile moiety), 153.2, 148.0 (2C, 1C of -<u>C</u>$_2$H=N-C_9 and 1C of -C_2H=N-<u>C</u>$_9$, quinoline), 144.8–121.9 (12C, aromatic carbon atoms), 105.8 (1C, <u>C</u>≡N), 95.7 (1C, -<u>C</u>-C≡N), 49.8, 42.3, 38.9 (5C, 4C of piperazine and 1C of N-<u>C</u>H$_2$-CH_3), 19.9 (1C, CH_2-<u>C</u>H$_3$).

5c M.P. 265–267°C, yield 82% (found: C 56.87, H 3.49, N 18.16, $C_{29}H_{22}BrClN_8O$, calc: C 56.74, H 3.61, N 18.25%). IR (KBr) cm^{-1}, 3280 (-NH), 2221 (C≡N), 1258 (C-O-C), 832 (*s*-triazine C-N str.), 756 (C-Cl); ^{1}H NMR (400 MHz,

DMSO-d_6, δ) ppm, 9.11 (s, 1H, -NH, s-triazine to amino-benzonitrile linkage), 8.81 (d, J = 2.2, 1H, C_5 proton of quinoline), 8.68 (d, J = 8.3 Hz, 1H, -N=CH-, C_2 proton of quinoline), 8.49 (s, 1H, C_3 proton of quinoline), 8.26 (d, J = 7.5 Hz, 1H, C_8 proton of quinoline), 8.16 (dd, J = 7.7, 1.4 Hz, 1H), 7.58–7.35 (m, 8H, Ar-H), 3.83 (br s, 4H, piperazine), 3.46 (br s, 4H, piperazine); ^{13}C-NMR (100 MHz, DMSO-d_6, δ) ppm, 177.1 (1C, C-6, *s*-triazine, C-N at piperazine linkage), 166.7 (1C, C-4, *s*-triazine, C-O-C at quinoline linkage), 163.5 (1C, C-2, *s*-triazine, C-NH at benzonitrile moiety), 152.9, 148.2 (2C, 1C of -C_2H=N-C_9 and 1C of -C_2H=N-C_9, quinoline), 146.3–119.9 (18C, aromatic carbon atoms), 106.1 (1C, C≡N), 97.7 (1C, -C-C≡N), 49.2, 47.5 (4C, piperazine).

5d M.P. 271–273°C, yield 81% (found: C 53.63, H 3.18, N 17.34, $C_{29}H_{21}BrCl_2N_8O$, calc: C 53.72, H 3.26, N 17.28%). IR (KBr) cm^{-1}, 3291 (-NH), 2224 (C≡N), 1253 (C-O-C), 830 (*s*-triazine C-N str.), 749 (C-Cl); ^{1}H NMR (400 MHz, DMSO-d_6, δ) ppm, 9.18 (s, 1H, -NH, s-triazine to amino-benzonitrile linkage), 8.86 (d, J = 1.8, 1H, C_5 proton of quinoline), 8.62 (d, J = 8.2 Hz, 1H, -N=CH-, C_2 proton of quinoline), 8.47 (s, 1H, C_3 proton of quinoline), 8.29 (d, J = 7.5 Hz, 1H, C_8 proton of quinoline), 8.09 (dd, J = 7.6, 1.4 Hz, 1H), 7.61–7.37 (m, 7H, Ar-H), 3.85 (br s, 4H, piperazine), 3.51 (br s, 4H, piperazine); ^{13}C-NMR (100 MHz, DMSO-d_6, δ) ppm, 175.9 (1C, C-6, *s*-triazine, C-N at piperazine linkage), 165.9 (1C, C-4, *s*-triazine, C-O-C at quinoline linkage), 164.6 (1C, C-2, *s*-triazine, C-NH at benzonitrile moiety), 153.1, 148.7 (2C, 1C of -C_2H=N-C_9 and 1C of -C_2H=N-C_9, quinoline), 145.3–117.3 (18C, aromatic carbon atoms), 105.5 (1C, C≡N), 98.1 (1C, -C-C≡N), 50.2, 42.8 (4C, piperazine).

5e M.P. 206–208°C, yield 77% (found: C 57.45, H 4.09, N 19.37, $C_{24}H_{20}BrN_7O$, calc: C 57.38, H 4.01, N 19.52%). IR (KBr) cm^{-1}, 3297 (-NH), 2222 (C≡N), 1256 (C-O-C), 835 (*s*-triazine C-N str.); ^{1}H NMR (400 MHz, DMSO-d_6, δ) ppm, 9.20 (s, 1H, -NH, s-triazine to amino-benzonitrile linkage), 8.80 (d, J = 1.7, 1H, C_5 proton of quinoline), 8.66 (d, J = 8.2 Hz, 1H, -N=CH-, C_2 proton of quinoline), 8.50 (s, 1H, C_3 proton of quinoline), 8.33 (d, J = 7.3 Hz, 1H, C_8 proton of quinoline), 8.15 (dd, J = 7.6, 1.4 Hz, 1H), 7.52–7.40 (m, 4H, Ar-H), 3.81 (t, J = 4.9 Hz, 4H, piperidine), 3.63 (t, J = 5.7 Hz, 4H, piperidine), 1.55–1.51 (m, 2H, piperidine); ^{13}C-NMR (100 MHz, DMSO-d_6, δ) ppm, 176.4 (1C, C-6, *s*-triazine, C-N at piperidine linkage), 166.7 (1C, C-4, *s*-triazine, C-O-C at quinoline linkage), 163.6 (1C, C-2, *s*-triazine, C-NH at benzonitrile moiety), 152.5, 151.1 (2C, 1C of -C_2H=N-C_9 and 1C of -C_2H=N-C_9, quinoline), 148.2–125.2 (12C, aromatic carbon atoms), 104.9 (1C, C≡N), 96.4 (1C, -C-C≡N), 54.9, 47.7, 38.6 (5C, piperidine).

5f M.P. 223–224°C, yield 80% (found: C 54.63, H 3.62, N 19.31, $C_{23}H_{18}BrN_7O_2$, calc: C 54.77, H 3.60, N 19.44%). IR (KBr) cm^{-1}, 3288 (-NH), 2221 (C≡N), 1423 (Morpholine C-O-C str.), 1261 (C-O-C), 832 (*s*-triazine C-N str.); ^{1}H NMR (400 MHz, DMSO-d_6, δ) ppm, 9.17 (s, 1H, -NH, s-triazine to amino-benzonitrile linkage), 8.82 (d, J = 1.8, 1H, C_5 proton of quinoline), 8.59 (d, J = 8.3 Hz, 1H, -N=CH-, C_2 proton of quinoline), 8.53 (s, 1H, C_3 proton

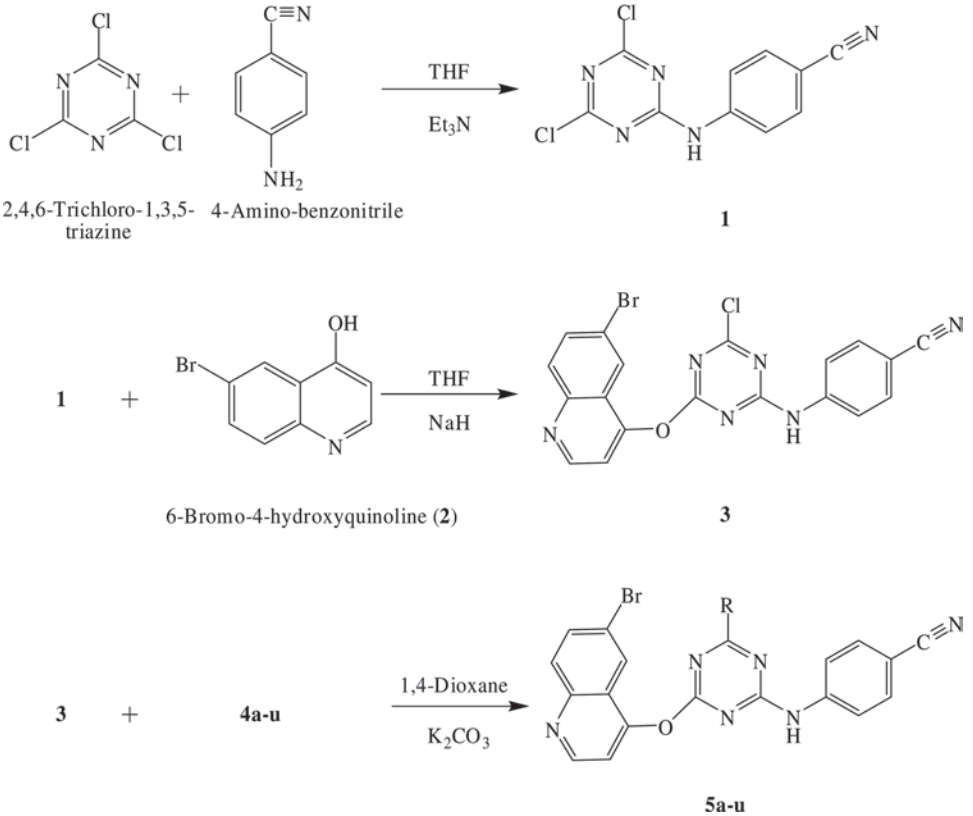

Figure 1. Schematic diagram for the synthesis of 2-(4-cyanophenyl amino)-4-(6-bromo-4-quinolinyloxy)-6-piperazinyl (piperidinyl)-1,3,5-triazines.

of quinoline), 8.30 (d, J = 7.5 Hz, 1H, C_8 proton of quinoline), 8.13 (dd, J = 7.8, 1.3 Hz, 1H), 7.55–7.41 (m, 4H, Ar-H), 2.42–2.35 (m, 4H, -CH_2, morpholine), 1.72–1.67 (m, 2H, -CH_2, morpholine), 1.24–1.20 (m, 2H, -CH_2, morpholine); ^{13}C-NMR (100 MHz, DMSO-d_6, δ) ppm, 177.1 (1C, C-6, *s*-triazine, C-N at morpholine linkage), 165.7 (1C, C-4, *s*-triazine, C-O-C at quinoline linkage), 164.4 (1C, C-2, *s*-triazine, C-NH at benzonitrile moiety), 154.3, 150.9 (2C, 1C of -C_2H=N-C_9 and 1C of -C_2H=N-C_9, quinoline), 149.2–123.1 (12C, aromatic carbon atoms), 105.2 (1C, C≡N), 98.8 (1C, -C-C≡N), 57.1, 53.3 (4C, morpholine ring carbon atoms).

5g M.P. 282–283°C, yield 82% (found: C 60.24, H 3.92, N 19.46, $C_{29}H_{23}BrN_8O$, calc: C 60.11, H 4.00, N 19.34%). IR (KBr) cm^{-1}, 3285 (-NH), 2221 (C≡N), 1251 (C-O-C), 831 (*s*-triazine C-N str.); ^{1}H NMR (400 MHz, DMSO-d_6, δ) ppm, 9.15 (s, 1H, -NH, s-triazine to amino-benzonitrile linkage), 8.77 (d, J = 1.8, 1H, C_5 proton of quinoline), 8.64 (d, J = 8.4 Hz, 1H, -N=CH-, C_2 proton of quinoline), 8.47 (s, 1H, C_3 proton of quinoline), 8.28 (d, J = 7.6 Hz, 1H, C_8 proton of quinoline), 8.16 (dd, J = 7.2, 1.4 Hz, 1H), 7.54–7.30 (m, 9H, Ar-H), 3.80 (br s, 4H, piperazine), 3.53 (br s, 4H, piperazine); ^{13}C-NMR (100 MHz, DMSO-d_6, δ) ppm, 176.7 (1C, C-6, *s*-triazine, C-N at piperazine linkage), 166.9 (1C, C-4, *s*-triazine, C-O-C at quinoline linkage), 164.0 (1C, C-2, *s*-triazine, C-NH at benzonitrile moiety), 153.3, 149.8 (2C, 1C of -C_2H=N-C_9 and 1C of -C_2H=N-C_9, quinoline), 145.9–119.3 (18C, aromatic carbon atoms), 104.9 (1C, C≡N), 97.2 (1C, -C-C≡N), 52.0, 48.2 (4C, piperazine).

5h M.P. 234–235°C, yield 77% (found: C 55.15, H 3.81, N 20.43, $C_{25}H_{21}BrN_8O_2$, calc: C 55.06, H 3.88, N 20.55%). IR (KBr) cm^{-1}, 3281 (-NH), 2223 (C≡N), 1709 (-C=O), 1489 (-CH_3), 1258 (C-O-C), 828 (*s*-triazine C-N str.); ^{1}H NMR (400 MHz, DMSO-d_6, δ) ppm, 9.22 (s, 1H, -NH, s-triazine to amino-benzonitrile linkage), 8.79 (d, J = 1.6, 1H, C_5 proton of quinoline), 8.65 (d, J = 8.2 Hz, 1H, -N=CH-, C_2 proton of quinoline), 8.49 (s, 1H, C_3 proton of quinoline), 8.32 (d, J = 7.5 Hz, 1H, C_8 proton of quinoline), 8.08 (dd, J = 7.6, 1.4 Hz, 1H), 7.47–7.34 (m, 4H, Ar-H), 3.84 (br s, 4H, piperazine), 3.48 (br s, 4H, piperazine), 2.39 (s, 3H, COCH$_3$); ^{13}C-NMR (100 MHz, DMSO-d_6, δ) ppm, 176.9 (1C, C-6, *s*-triazine, C-N at piperazine linkage), 169.1,

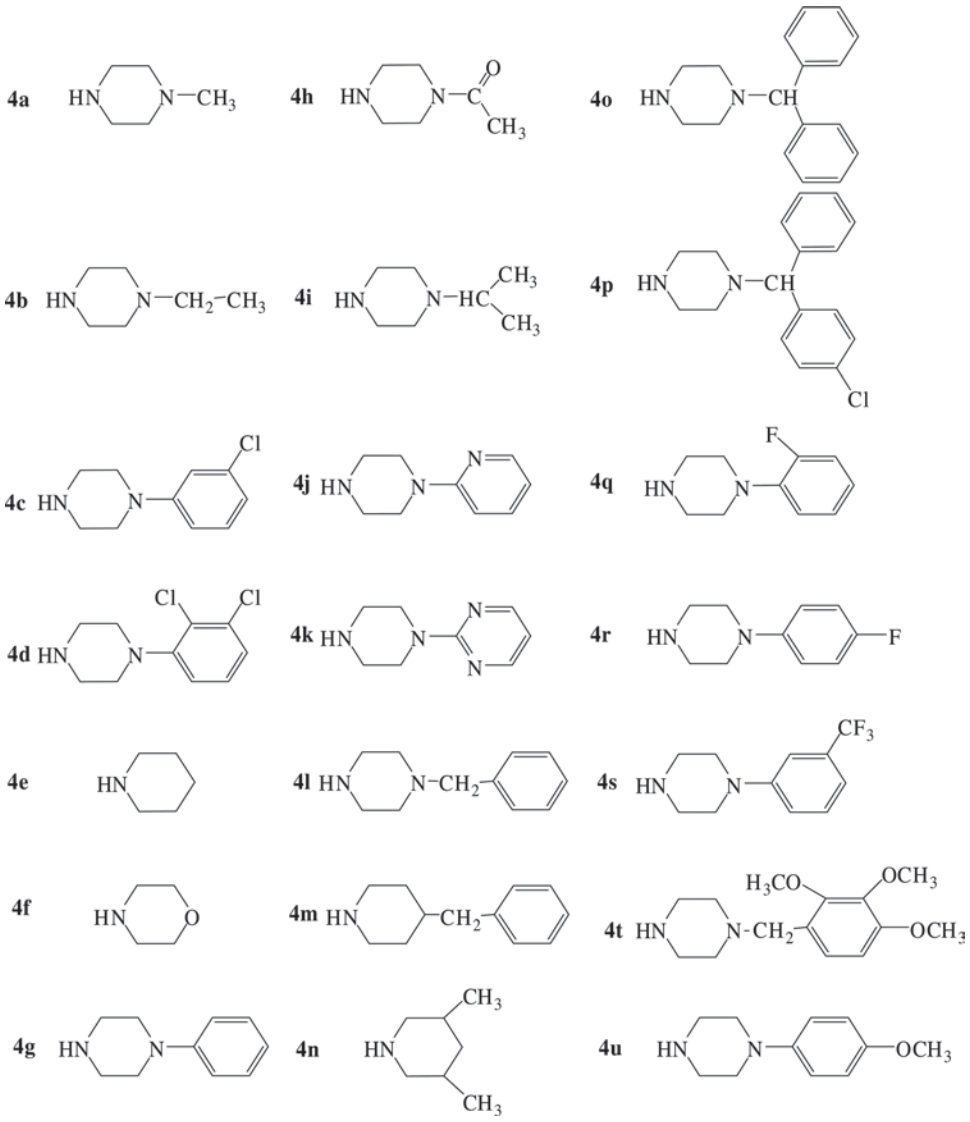

Figure 2. [4(a-u), R] = Piperazine and piperidine bases coupled to compound 3.

167.2 (2C, 1C at C-4, *s*-triazine, C-O-C at quinoline linkage and 1C at N-COCH$_3$), 163.3 (1C, C-2, *s*-triazine, C-NH at benzonitrile moiety), 155.2, 151.2 (2C, 1C of -C$_2$H=N-C$_9$ and 1C of -C$_2$H=N-C$_9$, quinoline), 147.2–128.1 (12C, aromatic carbon atoms), 104.9 (1C, C≡N), 99.4 (1C, -C-C≡N), 48.6, 44.6 (4C, piperazine), 23.1 (1C, COCH$_3$).

5i M.P. 256–257°C, yield 75% (found: C 57.11, H 4.56, N 20.61, $C_{26}H_{25}BrN_8O$, calc: C 57.25, H 4.62, N 20.54%). IR (KBr) cm^{-1}, 3282 (-NH), 2221 (C≡N), 1399 (isopropyl), 1255 (C-O-C), 836 (*s*-triazine C-N str.); ^{1}H NMR (400 MHz, DMSO-d$_6$, δ) ppm, 9.23 (s, 1H, -NH, s-triazine to amino-benzonitrile linkage), 8.84 (d, *J* = 1.8, 1H, C$_5$ proton of quinoline), 8.61 (d, *J* = 8.6 Hz, 1H, -N=CH-, C$_2$ proton of quinoline), 8.51 (s, 1H, C$_3$ proton of quinoline), 8.34 (d, *J* = 7.9 Hz, 1H, C$_8$ proton of quinoline), 8.04 (dd, *J* = 7.6, 1.4 Hz, 1H), 7.51–7.36 (m, 4H, Ar-H), 3.88 (br s, 4H, piperazine), 3.44 (br s, 4H, piperazine), 2.81–2.83 (m, 1H, N-CH), 1.96 (d, *J* = 6.9 Hz, 6H, -2CH$_3$); ^{13}C-NMR (100 MHz, DMSO-d$_6$, δ) ppm, 177.2 (1C, C-6, *s*-triazine, C-N at piperazine linkage), 166.4 (1C, C-4, *s*-triazine, C-O-C at quinoline linkage), 164.3 (1C, C-2, *s*-triazine, C-NH at benzonitrile moiety), 152.7, 148.9 (2C, 1C of -C$_2$H=N-C$_9$ and 1C of -C$_2$H=N-C$_9$, quinoline), 146.4–119.9 (12C, aromatic carbon atoms), 106.2 (1C, C≡N), 96.7 (1C, -C-C≡N), 53.2, 50.3, 39.2 (5C, 4C of piperazine and 1C of N-CH), 23.1 (2C, 2CH$_3$).

5j M.P. 254–256°C, yield 72% (found: C 57.85, H 3.69, N 21.83, $C_{28}H_{22}BrN_9O$, calc: C 57.94, H 3.82, N 21.72%). IR (KBr) cm^{-1}, 3309 (-NH), 2218 (C≡N), 1252 (C-O-C), 831 (*s*-triazine C-N str.); ^{1}H NMR (400 MHz, DMSO-d$_6$, δ) ppm, 9.19 (s, 1H, -NH, s-triazine to amino-benzonitrile linkage), 8.82 (d, *J* = 1.9, 1H, C$_5$ proton of quinoline), 8.69 (d, *J* = 8.2 Hz, 1H, -N=CH-, C$_2$ proton of quinoline), 8.47 (s, 1H, C$_3$ proton of quinoline), 8.25 (d, *J* = 7.5 Hz, 1H, C$_8$ proton of quinoline), 8.14 (dd, *J* = 7.6, 1.4 Hz, 1H), 8.02 (dd, *J* = 7.3, 1.8 Hz, 1H, pyridyl), 7.53–7.34 (m, 7H, Ar-H), 3.83 (br s, 4H, piperazine), 3.46 (br s, 4H, piperazine); ^{13}C-NMR (100 MHz, DMSO-d$_6$, δ) ppm, 175.4 (1C, C-6, *s*-triazine, C-N at piperazine linkage), 165.8 (1C, C-4, *s*-triazine, C-O-C at quinoline linkage), 164.2 (1C, C-2, *s*-triazine, C-NH at benzonitrile moiety), 158.8, 158.1, 154.7, 151.1 (4C, 2C of -C$_2$H=N-C$_9$ or 1C of -C$_2$H=N-C$_9$ of Quinoline and 2C of N$_{pip}$-C-N$_{py}$-CH or 1C of N$_{pip}$-C-N$_{py}$-CH), 147.2–117.7 (15C, aromatic carbon atoms), 104.9 (1C, C≡N), 97.2 (1C, -C-C≡N), 52.2, 48.6 (4C, piperazine).

5k M.P. 267–268°C, yield 76% (found: C 55.62, H 3.56, N 23.95, $C_{27}H_{21}BrN_{10}O$, calc: C 55.77, H 3.64, N 24.09%). IR (KBr) cm^{-1}, 3294 (-NH), 2220 (C≡N), 1257 (C-O-C), 840 (*s*-triazine C-N str.); ^{1}H NMR (400 MHz, DMSO-d$_6$, δ) ppm, 9.22 (s, 1H, -NH, s-triazine to amino-benzonitrile linkage), 8.84 (d, *J* = 2.0, 1H, C$_5$ proton of quinoline), 8.62 (d, *J* = 8.2 Hz, 1H, -N=CH-, C$_2$ proton of quinoline), 8.51 (s, 1H, C$_3$ proton of quinoline), 8.47–8.43 (m, 2H, pyrimidyl), 8.22 (d, *J* = 7.5 Hz, 1H, C$_8$ proton of quinoline), 8.19 (dd, *J* = 7.7, 1.5 Hz, 1H), 7.47–7.35 (m, 4H, Ar-H), 6.79 (t, *J* = 6.8 Hz, 1H, pyrimidyl), 3.79 (br s, 4H, piperazine), 3.42 (br s, 4H, piperazine); ^{13}C-NMR (100 MHz, DMSO-d$_6$, δ) ppm, 176.9 (1C, C-6, *s*-triazine, C-N at piperazine linkage), 165.9 (1C, C-4, *s*-triazine, C-O-C at quinoline linkage), 163.8 (1C, C-2, *s*-triazine, C-NH at benzonitrile moiety), 161.2, 159.5, 157.6, 152.4 (5C, 2C of -C$_2$H=N-C$_9$ or 1C of -C$_2$H=N-C$_9$ of Quinoline and 3C of pyrimidyl ring), 148.4–120.1 (13C, aromatic carbon atoms), 105.4 (1C, C≡N), 98.7 (1C, -C-C≡N), 48.7, 42.1 (4C, piperazine).

5l M.P. 255–257°C, yield 74% (found: C 60.58, H 4.28, N 18.74, $C_{30}H_{25}BrN_8O$, calc: C 60.71, H 4.25, N 18.88%). IR (KBr) cm^{-1}, 3290 (-NH), 2221 (C≡N), 1257 (C-O-C), 833 (s-triazine C-N str.); ^{1}H NMR (400 MHz, DMSO-d$_6$, δ) ppm, 9.17 (s, 1H, -NH, s-triazine to amino-benzonitrile linkage), 8.82 (d, *J* = 1.7, 1H, C$_5$ proton of quinoline), 8.69 (d, *J* = 8.2 Hz, 1H, -N=CH-, C$_2$ proton of quinoline), 8.48 (s, 1H, C$_3$ proton of quinoline), 8.29 (d, *J* = 7.6 Hz, 1H, C$_8$ proton of quinoline), 8.13 (dd, *J* = 7.8, 1.5 Hz, 1H), 7.51–7.29 (m, 9H, Ar-H), 3.84 (br s, 4H, piperazine), 3.51 (br s, 4H, piperazine), 2.72 (s, 2H, N$_{pip}$-CH$_2$); ^{13}C-NMR (100 MHz, DMSO-d$_6$, δ) ppm, 177.1 (1C, C-6, *s*-triazine, C-N at piperazine linkage), 166.9 (1C, C-4, *s*-triazine, C-O-C at quinoline linkage), 164.1 (1C, C-2, *s*-triazine, C-NH at benzonitrile moiety), 153.1, 148.6 (2C, 1C of -C$_2$H=N-C$_9$ and 1C of -C$_2$H=N-C$_9$, quinoline), 145.1–119.7 (18C, aromatic carbon atoms), 105.7 (1C, C≡N), 97.9 (1C, -C-C≡N), 65.3 (1C, N-CH$_2$), 49.1, 46.2 (4C, piperazine).

5m M.P. 239–241°C, yield 75% (found: C 62.80, H 4.53, N 16.67, $C_{31}H_{26}BrN_7O$, calc: C 62.84, H 4.42, N 16.55%). IR (KBr) cm^{-1}, 3296 (-NH), 2221 (C≡N), 1247 (C-O-C), 832 (s-triazine C-N str.); ^{1}H NMR (400 MHz, DMSO-d$_6$, δ) ppm, 9.26 (s, 1H, -NH, s-triazine to amino-benzonitrile linkage), 8.79 (d, *J* = 1.8, 1H, C$_5$ proton of quinoline), 8.61 (d, *J* = 8.4 Hz, 1H, -N=CH-, C$_2$ proton of quinoline), 8.53 (s, 1H, C$_3$ proton of quinoline), 8.26 (d, *J* = 7.5 Hz, 1H, C$_8$ proton of quinoline), 8.05 (dd, *J* = 7.6, 1.4 Hz, 1H), 7.63–7.36 (m, 9H, Ar-H), 3.88 (4H, t, *J* = 6.8 Hz, piperidine), 3.67 (4H, t, *J* = 8.8 Hz, piperidine), 2.43 (2H, s, -CH$_2$), 1.83 (1H, t, *J* = 7.4 Hz, -CH, piperidine); ^{13}C-NMR (100 MHz, DMSO-d$_6$, δ) ppm, 176.5 (1C, C-6, *s*-triazine, C-N at piperidine linkage), 166.4 (1C, C-4, *s*-triazine, C-O-C at quinoline linkage), 163.9 (1C, C-2, *s*-triazine, C-NH at benzonitrile moiety), 151.7, 150.3 (2C, 1C of -C$_2$H=N-C$_9$ and 1C of -C$_2$H=N-C$_9$, quinoline), 145.9–117.8 (18C, aromatic carbon atoms), 104.9 (1C, C≡N), 98.4 (1C, -C-C≡N), 46.3, 40.2, 36.1, 29.9 (6C, 5C of piperidine and 1C of -CH$_2$).

5n M.P. 284–285°C, yield 74% (found: C 58.91, H 4.43, N 18.36, $C_{26}H_{24}BrN_7O$, calc: C 58.87, H 4.56, N 18.48%). IR (KBr) cm^{-1}, 3299 (-NH), 2221 (C≡N), 1258 (C-O-C), 828 (s-triazine C-N str.); ^{1}H NMR (400 MHz, DMSO-d$_6$, δ) ppm, 9.19 (s, 1H, -NH, s-triazine to amino-benzonitrile linkage), 8.83 (d, *J* = 1.7, 1H, C$_5$ proton of quinoline), 8.63 (d, *J* = 8.0 Hz, 1H, -N=CH-, C$_2$ proton of quinoline), 8.50 (s, 1H, C$_3$ proton of quinoline), 8.25 (d, *J* = 7.7 Hz, 1H, C$_8$ proton of quinoline), 8.10 (dd, *J* = 7.6, 1.4 Hz, 1H), 7.47–7.37 (m, 4H, Ar-H), 3.69 (dd, *J* = 11.9, 7.2 Hz, 2H, piperidine), 2.91 (dd, *J* = 12.0, 7.7 Hz, 2H, piperidine), 1.79–1.71 (m, 4H, piperidne), 1.41 (d, *J* = 6.5 Hz, 6H, 2CH$_3$); ^{13}C-NMR (100 MHz, DMSO-d$_6$, δ) ppm, 175.8 (1C, C-6, *s*-triazine, C-N at piperidine linkage), 167.1 (1C, C-4, *s*-triazine, C-O-C at quinoline linkage), 164.4 (1C, C-2, *s*-triazine,

C-NH at benzonitrile moiety), 153.8, 149.5 (2C, 1C of -C_2H=N-C_9 and 1C of -C_2H=N-C_9, quinoline), 147.0–121.4 (12C, aromatic carbon atoms), 105.9 (1C, C≡N), 96.5 (1C, -C-C≡N), 52.1, 36.6, 31.9 (5C, piperidine), 22.7 (2C, 2CH_3).

5o M.P. 286–288°C, yield 72% (found: C 64.67, H 4.25, N 16.68, $C_{36}H_{29}BrN_8O$, calc: C 64.58, H 4.37, N 16.74%). IR (KBr) cm^{-1}, 3307 (-NH), 2218 (C≡N), 1255 (C-O-C), 836 (s-triazine C-N str.); ^{1}H NMR (400 MHz, DMSO-d_6, δ) ppm, 9.24 (s, 1H, -NH, s-triazine to amino-benzonitrile linkage), 8.85 (d, *J* = 1.8, 1H, C_5 proton of quinoline), 8.62 (d, *J* = 8.3 Hz, 1H, -N=CH-, C_2 proton of quinoline), 8.51 (s, 1H, C_3 proton of quinoline), 8.33 (d, *J* = 7.6 Hz, 1H, C_8 proton of quinoline), 8.15 (dd, *J* = 7.7, 1.4 Hz, 1H), 7.62–7.28 (m, 14H, Ar-H), 4.41 (s, 1H, N-CH), 3.83 (br s, 4H, piperazine), 3.49 (br s, 4H, piperazine); ^{13}C-NMR (100 MHz, DMSO-d_6, δ) ppm, 177.3 (1C, C-6, *s*-triazine, C-N at piperazine linkage), 166.6 (1C, C-4, *s*-triazine, C-O-C at quinoline linkage), 164.4 (1C, C-2, *s*-triazine, C-NH at benzonitrile moiety), 152.6, 150.2 (2C, 1C of -C_2H=N-C_9 and 1C of -C_2H=N-C_9, quinoline), 146.5–116.9 (24C, aromatic carbon atoms), 105.6 (1C, C≡N), 97.1 (1C, -C-C≡N), 76.3 (1C, -N_{pip}-CH), 49.5, 46.1 (4C, piperazine).

5p M.P. 274–275°C, yield 74% (found: C 61.34, H 4.14, N 15.83, $C_{36}H_{28}BrClN_8O$, calc: C 61.42, H 4.01, N 15.92%). IR (KBr) cm^{-1}, 3294 (-NH), 2224 (C≡N), 1254 (C-O-C), 839 (s-triazine C-N str.); ^{1}H NMR (400 MHz, DMSO-d_6, δ) ppm, 9.12 (s, 1H, -NH, s-triazine to amino-benzonitrile linkage), 8.84 (d, *J* = 1.9, 1H, C_5 proton of quinoline), 8.59 (d, *J* = 8.3 Hz, 1H, -N=CH-, C_2 proton of quinoline), 8.47 (s, 1H, C_3 proton of quinoline), 8.24 (d, *J* = 7.5 Hz, 1H, C_8 proton of quinoline), 8.04 (dd, *J* = 7.5, 1.5 Hz, 1H), 7.59–7.25 (m, 13H, Ar-H), 4.02 (s, 1H, N-CH), 3.81 (br s, 4H, piperazine), 3.52 (br s, 4H, piperazine); ^{13}C-NMR (100 MHz, DMSO-d_6, δ) ppm, 176.4 (1C, C-6, *s*-triazine, C-N at piperazine linkage), 166.9 (1C, C-4, *s*-triazine, C-O-C at quinoline linkage), 164.1 (1C, C-2, *s*-triazine, C-NH at benzonitrile moiety), 155.1, 150.1 (2C, 1C of -C_2H=N-C_9 and 1C of -C_2H=N-C_9, quinoline), 148.2–118.4 (24C, aromatic carbon atoms), 106.2 (1C, C≡N), 98.3 (1C, -C-C≡N), 75.6 (1C, -N_{pip}-CH), 47.8, 44.2 (4C, piperazine).

5q M.P. 235–237°C, yield 81% (found: C 58.18, H 3.63, N 18.64, $C_{29}H_{22}BrFN_8O$, calc: C 58.30, H 3.71, N 18.76%). IR (KBr) cm^{-1}, 3288 (-NH), 2221 (C≡N), 1253 (C-O-C), 834 (s-triazine C-N str.); ^{1}H NMR (400 MHz, DMSO-d_6, δ) ppm, 9.26 (s, 1H, -NH, s-triazine to amino-benzonitrile linkage), 8.74 (d, *J* = 1.7, 1H, C_5 proton of quinoline), 8.61 (d, *J* = 8.5 Hz, 1H, -N=CH-, C_2 proton of quinoline), 8.45 (s, 1H, C_3 proton of quinoline), 8.23 (d, *J* = 7.6 Hz, 1H, C_8 proton of quinoline), 8.06 (dd, *J* = 7.7, 1.3 Hz, 1H), 7.51–7.32 (m, 11H, Ar-H), 6.95 (dd, *J* = 12.5, 7.2 Hz, 2H), 6.77–6.73 (m, 1H), 6.52 (dd, *J* = 12.7, 6.9 Hz, 1H), 3.85 (br s, 4H, piperazine), 3.40 (br s, 4H, piperazine); ^{13}C-NMR (100 MHz, DMSO-d_6, δ) ppm, 177.1 (1C, C-6, *s*-triazine, C-N at piperazine linkage), 165.8 (1C, C-4, *s*-triazine, C-O-C at quinoline linkage), 164.3 (1C, C-2, *s*-triazine, C-NH at benzonitrile moiety), 151.6, 150.4, 148.4 (3C, 2C of -C_2H=N-C_9 and 1C of -C_2H=N-C_9, quinoline and 1C of C-F), 146.2–119.5 (17C, aromatic carbon atoms), 104.9 (1C, C≡N), 97.2 (1C, -C-C≡N), 51.1, 42.5 (4C, piperazine); ^{19}F NMR (400 MHz, $CDCl_3$, δ) ppm, -122.1 (1F, s, C-F).

5r M.P. 272–273°C, yield 79% (Found: C 58.22, H 3.61, N 18.70, $C_{29}H_{22}BrFN_8O$, calc: C 58.30, H 3.71, N 18.76%). IR (KBr) cm^{-1}, 3296 (-NH), 2223 (C≡N), 1258 (C-O-C), 832 (s-triazine C-N str.); ^{1}H NMR (400 MHz, DMSO-d_6, δ) ppm, 9.20 (s, 1H, -NH, s-triazine to amino-benzonitrile linkage), 8.77 (d, *J* = 2.0, 1H, C_5 proton of quinoline), 8.63 (d, *J* = 8.5 Hz, 1H, -N=CH-, C_2 proton of quinoline), 8.53 (s, 1H, C_3 proton of quinoline), 8.26 (d, *J* = 7.5 Hz, 1H, C_8 proton of quinoline), 8.13 (dd, *J* = 7.6, 1.3 Hz, 1H), 7.57–7.31 (m, 11H, Ar-H), 7.19 (dd, *J* = 12.8, 7.7 Hz, 2H), 6.68–6.66 (m, 1H), 6.59 (dd, *J* = 12.7, 6.1 Hz, 1H), 3.89 (br s, 4H, piperazine), 3.50 (br s, 4H, piperazine); ^{13}C-NMR (100 MHz, DMSO-d_6, δ) ppm, 177.6 (1C, C-6, *s*-triazine, C-N at piperazine linkage), 165.9 (1C, C-4, *s*-triazine, C-O-C at quinoline linkage), 163.3 (1C, C-2, *s*-triazine, C-NH at benzonitrile moiety), 152.2, 150.4, 147.1 (3C, 2C of -C_2H=N-C_9 and 1C of -C_2H=N-C_9, quinoline and 1C of C-F), 144.7–120.1 (17C, aromatic carbon atoms), 104.9 (1C, C≡N), 96.8 (1C, -C-C≡N), 47.7, 43.9 (4C, piperazine); ^{19}F NMR (400 MHz, $CDCl_3$, δ) ppm, -117.9 (1F, s, C-F).

5s M.P. 294–295°C, yield 76% (found: C 55.72, H 3.31, N 17.19, $C_{30}H_{22}BrF_3N_8O$, calc: C 55.65, H 3.42, N 17.31%). IR (KBr) cm^{-1}, 3307 (-NH), 2223 (C≡N), 1256 (C-O-C), 837 (s-triazine C-N str.); ^{1}H NMR (400 MHz, DMSO-d_6, δ) ppm, 9.21 (s, 1H, -NH, s-triazine to amino-benzonitrile linkage), 8.81 (d, *J* = 1.8, 1H, C_5 proton of quinoline), 8.63 (d, *J* = 8.2 Hz, 1H, -N=CH-, C_2 proton of quinoline), 8.46 (s, 1H, C_3 proton of quinoline), 8.27 (d, *J* = 7.5 Hz, 1H, C_8 proton of quinoline), 8.13 (dd, *J* = 7.6, 1.4 Hz, 1H), 7.83 (t, *J* = 7.9 Hz, 1H), 7.56–7.31 (m, 7H, Ar-H), 3.82 (br s, 4H, piperazine), 3.42 (br s, 4H, piperazine); ^{13}C-NMR (100 MHz, DMSO-d_6, δ) ppm, 175.5 (1C, C-6, *s*-triazine, C-N at piperazine linkage), 167.1 (1C, C-4, *s*-triazine, C-O-C at quinoline linkage), 164.2 (1C, C-2, *s*-triazine, C-NH at benzonitrile moiety), 151.8, 150.3 (2C, 1C of -C_2H=N-C_9 and 1C of -C_2H=N-C_9, quinoline), 147.2–117.7 (19C, aromatic carbon atoms C-CF_3 at 130.3 & CF_3 at 125.6), 105.8 (1C, C≡N), 95.2 (1C, -C-C≡N), 49.1, 46.4 (4C, piperazine); ^{19}F NMR (400 MHz, $CDCl_3$, δ) ppm, -63.7 (3F, s, -CF_3).

5t M.P. 294–295°C, yield 72% (found: C 58.06, H 4.69, N 16.32, $C_{33}H_{31}BrN_8O_4$, calc: C 57.98, H 4.57, N 16.39%). IR (KBr) cm^{-1}, 3291 (-NH), 2222 (C≡N), 1490 (-CH_2), 1248 (C-O-C), 839 (s-triazine C-N str.); ^{1}H NMR (400 MHz, DMSO-d_6, δ) ppm, 9.16 (s, 1H, -NH, s-triazine to amino-benzonitrile linkage), 8.77 (d, *J* = 1.8, 1H, C_5 proton of quinoline), 8.68 (d, *J* = 8.3 Hz, 1H, -N=CH-, C_2 proton of quinoline), 8.46 (s, 1H, C_3 proton of quinoline), 8.33 (d, *J* = 7.6 Hz, 1H, C_8 proton of quinoline), 8.14 (dd, *J* = 7.6, 1.4 Hz, 1H), 7.53–7.33 (m, 6H, Ar-H), 6.86 (d, *J* = 7.5 Hz, 1H), 6.69 (d, *J* = 7.6 Hz, 1H), 3.89 (br s, 4H, piperazine), 3.71 (s, 9H, 3OCH_3), 3.50 (br s, 4H, piperazine); ^{13}C-NMR (100 MHz, DMSO-d_6, δ) ppm, 177.1 (1C, C-6, *s*-triazine,

C-N at piperazine linkage), 166.1 (1C, C-4, *s*-triazine, C-O-C at quinoline linkage), 164.6 (1C, C-2, *s*-triazine, C-NH at benzonitrile moiety), 154.2, 149.5 (2C, 1C of -C_2H=N-C_9 and 1C of -C_2H=N-C_9, quinoline), 147.2–116.3 (18C, aromatic carbon atoms), 106.0 (1C, C≡N), 98.4 (1C, -C-C≡N), 66.8, 63.1, 54.4 (4C, 3C of 3OCH_3 and 1C of N_{pip}-CH_2), 47.9, 41.8 (4C, piperazine).

5u M.P. 258–260°C, yield 78% (found: C 58.98, H 4.17, N 18.46, $C_{30}H_{25}BrN_8O_2$, calc: C 59.12, H 4.13, N 18.39%). IR (KBr) cm^{-1}, 3293 (-NH), 2224 (C≡N), 1254 (C-O-C), 827 (s-triazine C-N str.); ^{1}H NMR (400 MHz, DMSO-d_6, δ) ppm, 9.22 (s, 1H, -NH, s-triazine to amino-benzonitrile linkage), 8.80 (d, *J* = 1.7, 1H, C_5 proton of quinoline), 8.67 (d, *J* = 8.1 Hz, 1H, -N=CH-, C_2 proton of quinoline), 8.49 (s, 1H, C_3 proton of quinoline), 8.21 (d, *J* = 7.3 Hz, 1H, C_8 proton of quinoline), 8.15 (dd, *J* = 7.6, 1.4 Hz, 1H), 7.51–7.35 (m, 6H, Ar-H), 7.23 (d, *J* = 8.3 Hz, 1H), 6.65 (d, *J* = 7.2 Hz, 1H), 4.21 (s, 3H, OCH_3), 3.79 (br s, 4H, piperazine ring), 3.49 (br s, 4H, piperazine ring); ^{13}C-NMR (100 MHz, DMSO-d_6, δ) ppm, 176.6 (1C, C-6, *s*-triazine, C-N at piperazine linkage), 167.2 (1C, C-4, *s*-triazine, C-O-C at quinoline linkage), 163.3 (1C, C-2, *s*-triazine, C-NH at benzonitrile moiety), 155.0, 152.1 (2C, 1C of -C_2H=N-C_9 and 1C of -C_2H=N-C_9, quinoline), 149.0–119.8 (18C, aromatic carbon atoms), 105.5 (1C, C≡N), 97.9 (1C, -C-C≡N), 57.2 (1C, -OCH_3), 46.2, 44.8 (4C, piperazine).

In vitro evaluation of antimicrobial activity

The synthesized *s*-triazinyl derivatives 5a-u were examined for antimicrobial activity against several bacteria (*Staphylococcus aureus* MTCC 96, *Bacillus cereus* MTCC 619, *Escherichia coli* MTCC 739, *Pseudomonas aeruginosa* MTCC 741, *Klebsiella pneumoniae* MTCC 109, *Salmonella typhi* MTCC 733, *Proteus vulgaris* MTCC 1771, *Shigella flexneria* MTCC 1457) and fungi (*Aspergillus niger* MTCC 282, *Aspergillus fumigatus* MTCC 343, *Aspergillus clavatus* MTCC 1323, *Candida albicans* MTCC 183) species using paper disc diffusion technique[29]. The Mueller-Hinton agar media were sterilized (autoclaved at 120°C for 30 min), poured at uniform depth of 5 mm and allowed to solidify. The microbial suspension (10^5 CFU/mL; 0.5 McFarland Nephelometery Standards) was streaked over the surface of media using a sterile cotton swab to ensure even growth of the organisms. The tested compounds were dissolved in dimethyl sulfoxide to give solutions of 3.12–100 μg/mL. Sterile filter paper discs measuring 6.25 mm in diameter (Whatman no. 1 filter paper), previously soaked in a known concentration of the respective test compound in dimethyl sulfoxide were placed on the solidified nutrient agar medium that had been inoculated with the respective microorganism and the plates were incubated for 24 h at (37 ± 1)°C. A control disc impregnated with an equivalent amount of dimethyl sulfoxide without any sample was also used and did not produce any inhibition. Ciprofloxacin and ketoconazole (100 μg/disc) were used as control drugs for antibacterial and antifungal activity, respectively.

MIC of the compound was determined by agar streak dilution method[30]. A stock solution of the synthesized compound (100 μg/mL) in dimethyl sulfoxide was prepared and graded quantities of the test compounds were incorporated in a specified quantity of molten sterile agar, that is, nutrient agar for evaluation of antibacterial and sabouraud dextrose agar for antifungal activity, respectively. The medium containing the test compound was poured into a Petri dish at a depth of 4–5 mm and allowed to solidify under aseptic conditions. A suspension of the respective microorganism of approximately 10^5 CFU/mL was prepared and applied to plates with serially diluted compounds with concentrations in the range of 3.12–100 μg/mL in dimethyl sulfoxide and incubated at (37 ± 1)°C for 24 h (bacteria) or 48 h (fungi). The lowest concentration of the substance that prevents the development of visible growth is considered to be the MIC value.

In vitro evaluation of antimycobacterial activity

The preliminary antimycobacterial assessment for the final synthesized compounds was carried out using BACTEC MGIT method. The Mycobacterial Growth Indicator Tubes (MGIT) containing 4 ml of modified Middle brook 7H9 Broth Base were numbered as per the title compounds to be tasted for antimycobacterial efficacy by means of various concentrations prepared. The suspension was allowed to sit for 20 min and the tubes were centrifuged at 3000 rpm for 15 min. After that prepared suspension of 10^4 to 10^7 CFU/mL of H37 R_V *M. tuberculosis* strain was added in the medium to be incubated and 0.1 ml of egg-based medium (L. J.) was also added. The MGIT tubes were then tightly recapped, mixed well and incubated into BACTEC MGIT instrument at (37 ± 1)°C until positivity is observed. The readings were measured daily starting from the second day of incubation. Positive cultures were usually detected within 10 days. For reading the actual results, the MGIT tubes were removed from incubator and placed on the UV light next to a positive control tube and an uninoculated tube (negative control). Bright fluorescence detected by the corresponding MGIT tube was noticed in the form of bright orange colour in the bottom of the tube and also an orange reflection on the meniscus.[31] The primary screening was conducted at concentration of 6.25 μg/ml against *M. tuberculosis* H37 Rv in BACTEC MGIT system. Compounds demonstrating 99% inhibition in the primary screen were described as most potent compounds. All the other compounds to be tasted were re-examined for their actual MIC by using Lowenstein-Jensen MIC method. The MIC was defined as the lowest concentration inhibiting 99% of the inoculum.

The secondary antimycobacterial screening for test compounds was obtained for *M. tuberculosis* H37 Rv, by using L. J. (Lowenstein and Jensen) MIC method[32,33]. Stock solutions of primary 1000, 500, 250 and secondary 200, 100, 62.5, 50, 25, 12.5, 6.25, 3.25 μg/mL dilutions of

each test compound in DMSO (dimethyl sulfoxide) were added in the liquid L. J. Medium and then media were sterilized by inspissation method. A culture of *M. tuberculosis* H37 Rv growing on L. J. medium was harvested in 0.85% saline in bijou bottles. These tubes were then incubated at 37°C for 24 h followed by streaking of *M. tuberculosis* H37 Rv (5×10^4 bacilli per tube). These tubes were then incubated at (37 ± 1)°C. Growth of bacilli was seen after 12 days, 22 days and finally 28 days of incubation. Tubes having the compounds were compared with control tubes where medium alone was incubated with *M. tuberculosis* H37 Rv. The concentration at which no development of colonies occurred or <20 colonies was taken as MIC concentration of test compound. The standard strain *M. tuberculosis* H37 Rv was tested with known drugs rifampicin, isoniazid, ethambutol and pyrazinamide.

Results and discussion

Investigation on antibacterial screening data (Table 1) showed some of the compounds were active against all the mentioned bacteria. Final *s*-triazinyl compounds 5h, 5n, 5p, 5s, 5t and 5u have the highest ability to inhibit *S. aureus*. Final analogues 5d, 5h, 5n, 5p, 5r, 5s, 5t showed higher effectiveness against *B. cereus*. The best activity was observed with compounds 5h, 5n, 5s, 5t and 5u against *E. coli* and *P. aeruginosa*. Inhibition of *K. pneumoniae* was also noted for *s*-triazine derivatives 5d, 5p, 5q, 5s and 5t. Compounds 5q, 5s and 5t had an effective action against *S. typhi* along with similar MIC profile of compounds 5h, 5n and 5p. Compounds 5d, 5h, 5q, 5s, 5t and 5u were superior in inhibiting the growth of *P. vulgaris*. Strong inhibitory effect was shown by final derivatives 5d, 5h, 5n, 5s and 5t against *S. flexneria*. All the remaining final *s*-triazine derivatives exerted good to moderate activity.

The antifungal results data (Table 2) revealed that, the synthesized compounds showed variable degree of inhibition against the tested fungi. Final *s*-triazine derivatives 5q, 5r, 5s and 5t displayed good antigrowth activity against *A. niger*. A significant inhibition was also observed for compounds 5d, 5p, 5s, 5t and 5u towards *A. fumigatus*. Compounds 5h, 5n, 5s, 5t and 5u act as the most potent inhibitors of the growth of *A. clavatus* fungi. Compounds 5q, 5r, 5s, 5t and 5u possessed the highest antifungal activity against *C. albicans*. All the remaining final *s*-triazine derivatives exerted good to moderate activity.

In vitro antimycobacterial activities of compounds 5a-u were assessed against *M. tuberculosis* H37Rv strain. The preliminary results observed from BACTEC MGIT method furnished in Table 3 indicated that final *s*-triazines 5n, 5t and 5u exhibited highest inhibition (99%) at a constant concentration level (6.25 μg/ml) against *M. tuberculosis* H37Rv. The secondary biological screening was performed using Lowenstein-Jensen MIC method and it is worthwhile to note that **5t** was the only compound displaying inhibition of *M. tuberculosis* H37Rv completely (99%) at the MIC of 3.12 μg/mL. Compounds 5p and 5s appeared with good inhibition effect in the term of MIC at 12.5 μg/mL, while all the remaining derivatives were found to exert MIC values ranging from 25–1000 μg/mL.

In conclusion, it is clear that the introduction of appropriate substituent on the *s*-triazine ring would lead to the more bioactive compounds. Bioassay results revealed that nine compounds (5d, 5h, 5n, 5p, 5q, 5r, 5s, 5t and 5u) out of the 21 studied displayed variable *in vitro* antibacterial, antifungal and antimycobacterial inhibitory effects. The presence of 6-bromo-4-hydroxyquinoline heteroatom ring in the title compounds significantly increase the biological potency of the resultant compounds compared to the presence of 8-hydroxyquinoline in our previous study. Higher inhibitory effects observed in this study appear to be dependent on the mono-chloro, di-chloro, acetyl linkage, dimethyl, monofluoro, trifluoromethyl, trimethoxy and mono-methoxy functionality to the nitrogen atom of piperazine bases condensed to the nucleus. Therefore, it is concluded that there exists ample scope for further study in this class of compounds in order to discover varied biological profiles such as anticancer activity or anti-HIV activity. The

Table 1. *In vitro* antimicrobial activity of newly synthesized compounds.

	MIC (μg/mL)							
	Gram-positive		Gram-negative					
Entry	*S. aureus*	*B. cereus*	*E. coli*	*P. aeruginosa*	*K. pneumoniae*	*S. typhi*	*P. vulgaris*	*S. flexneria*
5d	>6.25	6.25	>12.5	>12.5	25	>25	6.25	12.5
5h	6.25	6.25	12.5	12.5	>25	25	6.25	12.5
5n	6.25	6.25	12.5	12.5	>25	25	>6.25	12.5
5p	6.25	6.25	>12.5	>12.5	25	25	>6.25	>12.5
5q	>6.25	>6.25	>12.5	>12.5	25	25	6.25	>12.5
5r	>6.25	6.25	>12.5	>12.5	>25	>25	>6.25	>12.5
5s	6.25	6.25	12.5	12.5	25	25	6.25	12.5
5t	6.25	6.25	12.5	12.5	25	25	6.25	12.5
5u	6.25	>6.25	12.5	12.5	>25	>25	6.25	>12.5
Ciprofloxacin	1.0	1.0	1.0	1.0	1.0	1.0	1.0	≤3
DMSO	—	—	—	—	—	—	—	—

Table 2. *In vitro* antifungal activity of newly synthesized compounds.

	MIC (μg/mL)			
Entry	*A. niger*	*A. fumigatus*	*A. clavatus*	*C. albicans*
5d	>12.5	25	>25	>25
5h	>12.5	>25	25	>25
5n	>12.5	>25	25	>25
5p	>12.5	25	>25	>25
5q	12.5	>25	>25	25
5r	12.5	>25	>25	25
5s	12.5	25	25	25
5t	12.5	25	25	25
5u	>12.5	25	25	25
Ketoconazole	≤3	1.0	1.0	1.0
DMSO	—	—	—	—

Table 3. *In vitro* antimycobacterial activity of newly synthesized compounds.

	BACTEC MGIT method[a]		L. J MIC method[a]	
Entry	MIC (μg/mL)	% inhibition	MIC (μg/mL)	% inhibition
5d	>6.25	—	25	98
5h	>6.25	—	62.5	98
5n	6.25	99	6.25	98
5p	>6.25	—	12.5	99
5q	>6.25	—	50	99
5r	>6.25	—	25	99
5s	>6.25	—	12.5	99
5t	6.25	99	3.12	99
5u	6.25	99	6.25	99
Isoniazid	0.20	99%		
Refampicin	0.25	99%		
Ethambutol	3.12	99%		
Pyrazinamide	6.25	99%		

a: each value is the mean of two independent experiments.

study is currently under investigation and the results will be published in due course.

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Declaration of interest

The authors report no conflict of interest.

Supplementary material

Detailed discussion on the analytical and biological characterization is included within the supplementary information. Tables containing zone of inhibition and MICs for all the newly synthesized compounds are included within the supplementary information.

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