Fluorinated *s*-Triazinyl Piperazines as Antimicrobial Agents

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A series of 1,3,5-triazine derivatives that contain 4-amino-2-trifluoromethyl-benzonitrile, 8-hydroxyquinoline, and different piperazines as substituents at the carbon atoms of the triazine ring have been synthesized by a simple and efficient synthetic protocol. The chemical structures of the compounds were elucidated with the aid of IR, ^{1}H NMR and ^{13}C NMR spectroscopy, and elemental analysis. The antimicrobial activity of the compounds was tested against seven 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) and four fungi (*Aspergillus niger* MTCC 282, *Aspergillus fumigatus* MTCC 343, *Aspergillus clavatus* MTCC 1323, *Candida albicans* MTCC 183). The results indicate that some of the novel *s*-triazines have noteworthy activity in minimum inhibitory concentration as well as agar diffusion tests.

Key words: 2,4,6-Trichloro-1,3,5-triazine, 4-Amino-2-trifluoromethyl-benzonitrile, Piperazines

Introduction

Control of deadly infectious diseases is threatened by emergence of multidrug resistance and dissemination of resistant pathogenic microbes. In the developing countries, this problem is especially alarming. Furthermore, increased numbers of immunocompromised HIV patients may further increase the burden of antimicrobial resistance (Nathan, 2004).

As a part of our endeavour towards the development of new and efficient antibacterial and antifungal agents, we have synthesized some novel 1,3,5-triazines. The advent of 1,3,5-triazines, associated with diverse biological activities such as antimicrobial (Zhou *et al*., 2008; Srinivas *et al*., 2006), antiprotozoal (Alessandro *et al*., 2005), anticancer (Menicagli *et al*., 2004), antimalarial, (Melato *et al*., 2008) and antiviral (Xiong *et al*., 2008) activity, respectively, accelerated the rate of progress in synthesizing new 1,3,5-triazine derivatives. Likewise, 4-amino-2-trifluoromethyl-benzonitrile is also a useful pharmacophore found in the anticancer drug bicalutamide as a structural unit. A literature survey revealed that piperazines and substituted piperazines are an important family of heterocyclic compounds attracting significant interest in medicinal chemistry (Kerns *et al*., 2003; Ryckebusch *et al*., 2003; Upadhayaya *et al*., 2004). The favourable antimicrobial properties of 8-hydroxyquinoline prompted us to combine this structural component with the *s*-triazine nucleus (Ritu *et al*., 2010; Block and Beale, 2005; Okide *et al*., 2000).

Material and Methods

Chemicals

2,4,6-Trichloro-1,3,5-triazine and 8-hydroxyquinoline were purchased from Sigma Aldrich (Mumbai, India). 4-Amino-2-trifluoromethyl-benzonitrile was a gift from Ramdev Chemicals (Boisar, India). Acetone, tetrahydrofuran, and 1,4-dioxane of HPLC grade were purchased from Rankem (Surat, India). The TLC plates (silica gel 60 F254) were obtained from Merck (Darmstadt, Germany). Substituted piperazine derivatives were gifts from Catapharma (Nasik, India), Enzal Chemicals Pvt. Ltd. (Mumbai, India), Dr. Prem's Molecules Pvt. Ltd. (Vadodara, India), Ami Organics Pvt. Ltd. (Surat, India), Modepro India Pvt. Ltd. (Mumbai, India), Siddharth Interchem Pvt. Ltd. (Ankleshwar, India), and Mahrshee Laboratories (Bharuch, India).

Analytical methods and instruments

Melting points were determined in open capillaries on a Veego electronic apparatus VMP-D

(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 x 7.5 cm) coated with silica gel-G, and spots were visualized under UV light. NMR spectra were recorded on a Varian 400 MHz spectrometer (Varian India Pvt. Ltd., Mumbai, India) using DMSO (dimethyl sulfoxide) as solvent and TMS (tetramethylsilane) as internal standard. Elemental analyses (C, H, N) were performed using a Heraeus Carlo Erba 1180 CHN analyzer (Hanau, Germany). All of the novel compounds gave C, H, and N analyses within a 0.05 range from the theoretical values.

Agar diffusion method

The synthesized *s*-triazinyl derivatives **5a**–**k** 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) and fungi (*Aspergillus niger* MTCC 282, *Aspergillus fumigatus* MTCC 343, *Aspergillus clavatus* MTCC 1323, *Candida albicans* MTCC 183) using the agar diffusion test (Cruickshank *et al*., 1975). 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 test compounds were dissolved in DMSO 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 DMSO were placed on the solidified nutrient agar medium that had been inoculated with the respective microorganism; then the plates were incubated for 24 h at (37 ± 1) °C. A control disc impregnated with an equivalent amount of DMSO 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. Each assay was performed in triplicate.

To determine the minimum inhibitory concentration (MIC), a stock solution of each synthesized compound (100 μg/mL) in DMSO was prepared, and graded quantities of the test compounds were incorporated in a specified quantity of molten sterile agar, *i.e*. nutrient agar for evaluation of antibacterial activity and Sabouraud dextrose agar for evaluation of 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 DMSO; the plates were incubated at (37 ± 1) °C for 24 h (bacteria) or 48 h (fungi). The lowest concentration of a substance that prevents the development of visible growth is considered to be its MIC value.

*4-(4,6-Dichloro-1,3,5-triazin-2-ylamino)-2-trifluoromethyl-benzonitrile (**1**)*

To a stirred solution of 2,4,6-trichloro-1,3,5-triazine (10 g, 0.054 mol) in anhydrous THF (150 mL) 4-amino-2-trifluoromethyl-benzonitrile (10.09 g, 0.054 mol) was dropwise added at 0–5 °C. The resulting reaction mixture was stirred at this temperature for 2 h. Then triethyl amine (5.48 g, 0.054 mol) was added and stirring was continued for another 5 h. The reaction mixture was then treated with crushed ice, followed by neutralization with dilute HCl, and filtered, dried, and recrystallized from acetone to afford **1**. – M.p. 259.5 °C (dec.). – FT-IR (KBr): $\bar{\nu}$ = 2223 cm^{-1} (CN).

*4-[4-Chloro-6-(quinolin-8-yloxy)-1,3,5-triazin-2-ylamino]-2-trifluoromethyl-benzonitrile (**3**)*

To a stirred solution of 8-hydroxyquinoline (8 g, 0.055 mol) in anhydrous THF (150 mL) 60% NaH (1.32 g, 0.055 mol) was added at room temperature during 1 h, and **1** (18.41 g, 0.055 mol) was then added to the mixture. Stirring was continued for another 16 h at 45 °C. Progress of the reaction was monitored by TLC using toluene/acetone (95:5, v/v) as eluent. The mixture was treated with crushed ice, filtered, and dried to afford **3** (Xiong *et al*., 2009). – M.p. 267.7 °C (dec.). – FT-IR (KBr): $\bar{\nu}$ = 2223 cm^{-1} (CN), 1255–1257 cm^{-1} (C-O-C).

*General procedure for preparation of compounds **5a–5k***

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 8–10 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 (95:5, v/v) 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–k**.

*4-[4-(4-Methyl-piperazin-1-yl)-6-(quinolin-4-yloxy)-1,3,5-triazin-2-ylamino]-2-trifluoromethyl-benzonitrile (**5a**):* Yield: 78%. – M.p. 273 °C (dec.). – IR (KBr): $\tilde{\nu}$ = 3100–3300 (-NH), 2221 (CN), 1255 (C-O-C), 814 cm^{-1} (*s*-triazine C-N str.). – ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.84 (1H, s, -NH), 7.55–7.62 (3H, m, quinoline), 7.15–7.45 (6H, m, Ar-H), 3.84 (8H, br s, piperazine), 2.40 (3H, s, -CH_3). – ^{13}C NMR (100 MHz, DMSO-d_6): δ = 176.21 (C-3, *s*-triazine, C-N at piperazine linkage), 168.99 (C-1, *s*-triazine, C-O-C at quinoline linkage), 164.11 (C-5, *s*-triazine, C-NH at benzonitrile moiety), 149.34–113.08 (15C, -Ar. C including C-CF_3 at 130.50 and CF_3 at 125.00), 104.66 (C-32, CN), 97.08 (C-11, C-CN), 50.35, 46.98 (4C, piperazine ring carbon atoms), 14.03 (C-31, N-CH_3). – $C_{25}H_{21}F_3N_8O$ (506.48 g/mol): calcd. C 59.28, H 4.18, N 22.12; found C 59.27, H 4.19, N 22.10.

*4-[4-(4-Ethyl-piperazin-1-yl)-6-(quinolin-4-yloxy)-1,3,5-triazin-2-ylamino]-2-trifluoromethyl-benzonitrile (**5b**):* Yield: 83%. – M.p. 247 °C (dec.). – IR (KBr): $\tilde{\nu}$ = 3100–3300 (-NH), 2223 (CN), 1255 (C-O-C), 806 cm^{-1} (*s*-triazine C-N str.). – ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.85 (1H, s, -NH), 7.56 - 7.63 (3H, m, quinoline), 7.22–7.54 (6H, m, Ar-H), 3.82 (8H, br s, piperazine), 2.40 (2H, q, -CH_2), 2.13 (3H, t, CH_3). – ^{13}C NMR (100 MHz, DMSO-d_6): δ = 176.58 (C-3, *s*-triazine, C-N at piperazine linkage), 169.36 (C-1, *s*-triazine, C-O-C at quinoline linkage), 163.79 (C-5, *s*-triazine, C-NH at benzonitrile moiety), 150.34–113.44 (15C, -Ar. C including C-CF_3 at 129.90 and CF_3 at 125.61), 104.99 (C-37, CN), 97.08 (C-11, C-CN), 51.62, 50.29, 45.68 (4C, piperazine ring carbon atoms and 1C at C-31, N-CH_2-), 12.39 (C-32, CH_2-CH_3). – $C_{26}H_{23}F_3N_8O$ (520.51 g/mol): calcd. C 59.99, H 4.45, N 21.53; found C 59.97, H 4.44, N 21.53.

*[4-[4-(3-Chloro-phenyl)-piperazin-1-yl]-6-(quinolin-4-yloxy)-1,3,5-triazin-2-ylamino]-2-trifluoromethyl-benzonitrile (**5c**):* Yield: 79%. – M.p. 262 °C (dec.). – IR (KBr): $\tilde{\nu}$ = 3100–3300 (-NH), 2223 (CN), 1257 (C-O-C), 813 cm^{-1} (*s*-triazine C-N str.), 754 (C-Cl). – ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.86 (1H, s, -NH), 7.71–7.78 (3H, m, quinoline), 7.13–7.40 (10H, m, Ar-H), 3.85 (8H, br s, piperazine). – ^{13}C NMR (100 MHz, DMSO-d_6): δ = 176.18 (C-3, *s*-triazine, C-N at piperazine linkage), 169.18 (C-1, *s*-triazine, C-O-C at quinoline linkage), 164.21 (C-5, *s*-triazine, C-NH at benzonitrile moiety), 151.33–112.86 (21C, -Ar. C including C-CF_3 at 130.52 and CF_3 at 125.09), 104.86 (C-38, CN), 98.08 (C-11, C-CN), 47.88, 46.73 (4C, piperazine ring carbon atoms). – $C_{30}H_{22}ClF_3N_8O$ (603.00 g/mol): calcd. C 59.76, H 3.68, N 18.58; found C 59.75, H 3.66, N 18.59.

*4-[4-[4-(2,3-Dichloro-phenyl)-piperazin-1-yl]-6-(quinolin-4-yloxy)-1,3,5-triazin-2-ylamino]-2-trifluoromethyl-benzonitrile (**5d**):* Yield: 71%. – M.p. >300 °C (dec.). – IR (KBr): $\tilde{\nu}$ = 3100–3300 (-NH), 2221 (CN), 1255 (C-O-C), 806 cm^{-1} (*s*-triazine C-N str.), 754 (C-Cl). – ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.86 (1H, s, -NH), 7.53–7.59 (3H, m, quinoline), 7.23–7.45 (9H, m, Ar-H), 3.83 (8H, br s, piperazine). – ^{13}C NMR (100 MHz, DMSO-d_6): δ = 177.00 (C-3, *s*-triazine, C-N at piperazine linkage), 169.27 (C-1, *s*-triazine, C-O-C at quinoline linkage), 164.73 (C-5, *s*-triazine, C-NH at benzonitrile moiety), 152.05–111.98 (21C, -Ar. C including C-CF_3 at 129.88 and CF_3 at 125.25), 105.15 (C-43, CN), 97.59 (C-11, C-CN), 48.13, 46.46 (4C, piperazine ring carbon atoms). – $C_{30}H_{21}Cl_2F_3N_8O$ (637.44 g/mol): calcd. C 56.53, H 3.32, N 17.58; found C 56.51, H 3.31, N 17.57.

*4-[4-Piperidin-1-yl-6-(quinolin-4-yloxy)-1,3,5-triazin-2-ylamino]-2-trifluoromethyl-benzonitrile (**5e**):* Yield: 80%. – M.p. 288 °C (dec.). – IR (KBr): $\tilde{\nu}$ = 3100–3300 (-NH), 2223 (CN), 1256 (C-O-C), 817 cm^{-1} (*s*-triazine C-N str.). – ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.87 (1H, s, -NH), 7.64–7.69 (3H, m, quinoline), 7.22–7.39 (6H, m, Ar-H), 3.91 (4H, t, piperidine), 3.70 (6H, t, piperidine). – ^{13}C NMR (100 MHz, DMSO-d_6): δ = 176.89 (C-3, *s*-triazine, C-N at piperazine linkage), 169.16 (C-1, *s*-triazine, C-O-C at quinoline link-

age), 163.97 (C-5, *s*-triazine, C-NH at benzonitrile moiety), 151.31–112.73 (15C, -Ar. C including C-CF_3 at 130.83 and CF_3 at 125.08), 105.15 (C-31, CN), 96.99 (C-11, C-CN), 67.84, 29.85 (5C, C-26 to C-30, piperidine ring carbon atoms). – $C_{25}H_{20}F_3N_7O$ (491.47 g/mol): calcd. C 61.10, H 4.10, N 19.95; found C 61.11, H 4.09, N 19.93.

*4-[4-Morpholin-4-yl-6-(quinolin-4-yloxy)-1,3,5-triazin-2-ylamino]-2-trifluoromethyl-benzonitrile (**5f**):* Yield: 90%. – M.p. 275 °C (dec.). – IR (KBr): $\bar{\nu}$ = 3100–3300 (-NH), 2221 (CN), 1375 (morpholine C-O-C str.), 1258 (C-O-C), 806 cm^{-1} (*s*-triazine C-N str.). – ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.84 (1H, s, -NH), 7.56–7.62 (3H, m, quinoline), 7.15–7.45 (6H, m, Ar-H), 3.83 (4H, t, morpholine), 3.85 (4H, t, morpholine). – ^{13}C NMR (100 MHz, DMSO-d_6): δ = 177.99 (C-3, *s*-triazine, C-N at piperazine linkage), 169.66 (C-1, *s*-triazine, C-O-C at quinoline linkage), 164.07 (C-5, *s*-triazine, C-NH at benzonitrile moiety), 150.31–113.93 (15C, -Ar. C including C-CF_3 at 130.73 and CF_3 at 125.28), 104.86 (C-35, CN), 96.59 (C-11, C-CN), 67.84, 46.85 (4C, morpholine ring carbon atoms). – $C_{24}H_{18}F_3N_7O_2$ (493.44 g/mol): calcd. C 58.42, H 3.68, N 19.87; found C 58.40, H 3.69, N 19.85.

*4-[4-(4-Phenyl-piperazin-1-yl)-6-(quinolin-4-yloxy)-1,3,5-triazin-2-ylamino]-2-trifluoromethyl-benzonitrile (**5g**):* Yield: 81%. – M.p. 256 °C (dec.). – IR (KBr): $\bar{\nu}$ = 3100–3300 (-NH), 2223 (CN), 1256 (C-O-C), 815 cm^{-1} (*s*-triazine C-N str.). – ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.82 (1H, s, -NH), 7.57–7.60 (3H, m, quinoline), 7.09–7.39 (11H, m, Ar-H), 3.89 (8H, br s, piperazine). – ^{13}C NMR (100 MHz, DMSO-d_6): δ = 177.36 (C-3, *s*-triazine, C-N at piperazine linkage), 169.50 (C-1, *s*-triazine, C-O-C at quinoline linkage), 164.64 (C-5, *s*-triazine, C-NH at benzonitrile moiety), 151.33–114.00 (21C, -Ar. C including C-CF_3 at 130.30 and CF_3 at 124.89), 105.61 (C-41, CN), 97.17 (C-11, C-CN), 49.49, 47.89 (4C, piperazine ring carbon atoms). – $C_{30}H_{23}F_3N_8O$ (568.55 g/mol): calcd. C 63.38, H 4.08, N 19.71; found C 63.38, H 4.06, N 19.70.

*4-[4-(4-Acetyl-piperazin-1-yl)-6-(quinolin-4-yloxy)-1,3,5-triazin-2-ylamino]- 2-trifluoromethyl-benzonitrile (**5h**):* Yield: 84%. – M.p. 238 °C (dec.). – IR (KBr): $\bar{\nu}$ = 3100–3300 (-NH), 2223 (CN), 1700 (-C=O), 1475 (-CH_3), 1255 (C-O-C), 819 cm^{-1} (*s*-triazine C-N str.). – ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.80 (1H, s, -NH), 7.60–7.66 (3H, m, quinoline), 7.13–7.30 (6H, m, Ar-H), 3.85 (8H, br s, piperazine), 2.39 (3H, S, -CH_3). – ^{13}C NMR (100 MHz, DMSO-d_6): δ = 176.10 (C-3, *s*-triazine, C-N at piperazine linkage), 169.29 (C-1, *s*-triazine, C-O-C at quinoline linkage), 167.49 (C-38, acetyl C=O), 164.39 (C-5, *s*-triazine, C-NH at benzonitrile moiety), 150.65–112.41 (15C, -Ar. C including C-CF_3 at 130.03 and CF_3 at 125.18), 105.56 (C-32, CN), 97.77 (C-11, C-CN), 48.04, 46.45 (4C, piperazine ring carbon atoms), 22.32 (C-31, CO-CH_3). – $C_{26}H_{21}F_3N_8O_2$ (534.49 g/mol): calcd. C 58.43, H 3.96, N 20.96; found C 58.41, H 3.95, N 20.95.

*4-[4-(4-Isopropyl-piperazin-1-yl)-6-(quinolin-4-yloxy)-1,3,5-triazin-2-ylamino]-2-trifluoromethyl-benzonitrile (**5i**):* Yield: 67%. – M.p. 287 °C (dec.). – IR (KBr): $\bar{\nu}$ = 3100–3300 (-NH), 2222 (CN), 1380 (isopropyl), 1257 (C-O-C), 819 cm^{-1} (*s*-triazine C-N str.). – ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.83 (1H, s, -NH), 7.67–7.70 (3H, m, quinoline), 7.12–7.42 (6H, m, Ar-H), 3.79 (8H, br s, piperazine), 2.44 (1H, q, -CH), 2.40 (6H, d, -CH_3). – ^{13}C NMR (100 MHz, DMSO-d_6): δ = 177.11 (C-3, *s*-triazine, C-N at piperazine linkage), 168.96 (C-1, *s*-triazine, C-O-C at quinoline linkage), 164.29 (C-5, *s*-triazine, C-NH at benzonitrile moiety), 149.32–114.05 (15C, -Ar. C including C-CF_3 at 129.33 and CF_3 at 125.48), 105.16 (C-38, CN), 96.97 (C-11, C-CN), 54.11, 52.88, 46.82 (4C, piperazine ring carbon atoms and 1C at C-31, N-CH-), 21.03 (2C, 2CH_3). – $C_{27}H_{25}F_3N_8O$ (534.54 g/mol): calcd. C 60.67, H 4.71, N 20.96; found C 60.68, H 4.69, N 20.96.

*4-[4-(4-Pyridin-2-yl-piperazin-1-yl)-6-(quinolin-4-yloxy)-1,3,5-triazin-2-ylamino]-2-trifluoromethyl-benzonitrile (**5j**):* Yield: 80%. – M.p. 291 °C (dec.). – IR (KBr): $\bar{\nu}$ = 3100–3300 (-NH), 2223 (CN), 1256 (C-O-C), 806 cm^{-1} (*s*-triazine C-N str.). – ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.84 (1H, s, -NH), 7.60–7.64 (4H, m, 3H of quinoline and 1H of pyridyl), 7.19–7.35 (9H, m, Ar-H), 3.84 (8H, br s, piperazine). – ^{13}C NMR (100 MHz, DMSO-d_6): δ = 176.48 (C-3, *s*-triazine, C-N at piperazine linkage), 169.68 (C-1, *s*-triazine, C-O-C at quinoline linkage), 164.79 (C-5, *s*-triazine, C-NH at benzonitrile moiety), 161.49 (C-31, N-C at piperazine-pyridine linkage), 151.03–110.98 (19C, -Ar. C including C-CF_3 at 130.02 and CF_3 at 125.36), 104.08 (C-37, CN), 96.67 (C-11, C-CN), 47.98, 46.71 (4C, piperazine ring carbon atoms). – $C_{29}H_{22}F_3N_9O$ (569.54 g/mol):

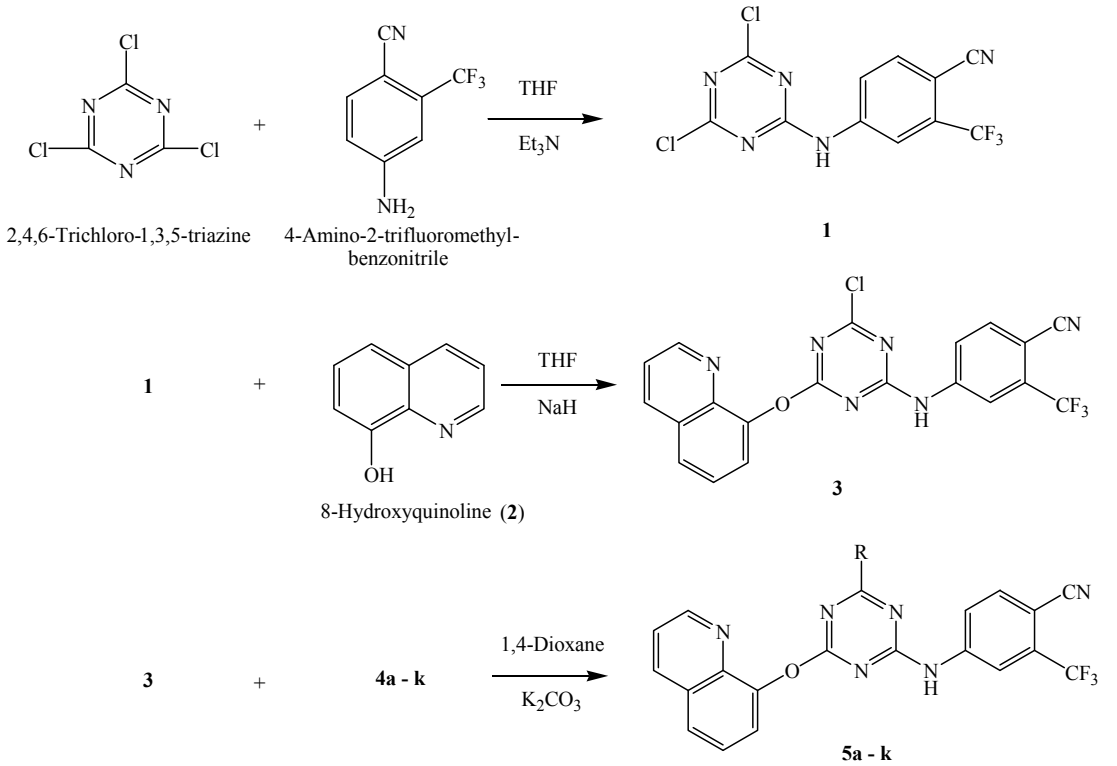

Fig. 1. Schematic diagram for the synthesis of the target fluorinated *s*-triazinyl piperazines.

calcd. C 61.16, H 3.89, N 22.13; found C 61.17, H 3.89, N 22.15.

*4-[4-(4-Pyrimidin-2-yl-piperazin-1-yl)-6-(quinolin-4-yloxy)-1,3,5-triazin-2-ylamino]-2-trifluoromethyl-benzonitrile (**5k**):* Yield: 85%. – M.p. 283 °C (dec.). – IR (KBr): $\tilde{\nu}$ = 3100–3300 (-NH), 2222 (CN), 1255 (C-O-C), 806 cm^{-1} (*s*-triazine C-N str.). – ^{1}H NMR (400 MHz, DMSO-d$_6$) δ = 8.90 (1H, s, -NH), 7.98–8.05 (5H, m, 3H of quinoline and 2H of pyrimidyl), 7.27–7.35 (7H, m, Ar-H), 3.84 (8H, br s, piperazine). – ^{13}C NMR (100 MHz, DMSO-d$_6$) δ = 177.01 (C-3, *s*-triazine, C-N at piperazine linkage), 169.78 (C-1, *s*-triazine, C-O-C at quinoline linkage), 164.09 (C-5, *s*-triazine, C-NH at benzonitrile moiety), 160.59 (C-31, N-C at piperazine-pyridine linkage), 157.57 (2C, C-33 and C-35), 150.93 – 111.78 (16C, -Ar. C including C-CF$_3$ at 130.34 and CF$_3$ at 125.16), 105.33 (C-37, CN), 97.37 (C-11, C-CN), 47.98, 46.71 (4C, piperazine ring carbon atoms). – $C_{28}H_{21}F_3N_{10}O$ (570.53 g/mol): calcd. C 58.95, H 3.71, N 24.55; found C 58.93, H 3.70, N 24.56.

Results and Discussion

Compounds **5a**–**5k** were synthesized according to Fig. 1. Nucleophilic substitution of one chlorine atom produced 4-(4,6-dichloro-1,3,5-triazin-2-ylamino)-2-trifluoromethyl-benzonitrile (**1**) in good yield from 2,4,6-trichloro-1,3,5-triazine and 4-amino-2-trifluoromethyl-benzonitrile. The disubstituted *s*-triazine intermediate **3** was obtained in good yield by the reaction between **1** and 8-hydroxyquinoline in the presence of 60% NaH at 45–50 °C. Condensation of **3** with appropriate piperazine and piperidine substituents (Fig. 2) in 1,4-dioxane at 70–80 °C provided the target compounds **5a**–**5k**. A C_3N_3 stretching band in the *s*-triazine ring was observed between 810–820 cm^{-1}. Compound 4-(4,6-dichloro-1,3,5-triazin-2-ylamino)-2-trifluoromethyl-benzonitrile (**1**) displayed an absorption band at 2220–2224 cm^{-1}, confirming the presence of a cyano group, and a strong band near 3250 cm^{-1}, due to the presence of an –NH group. Moreover, a character-

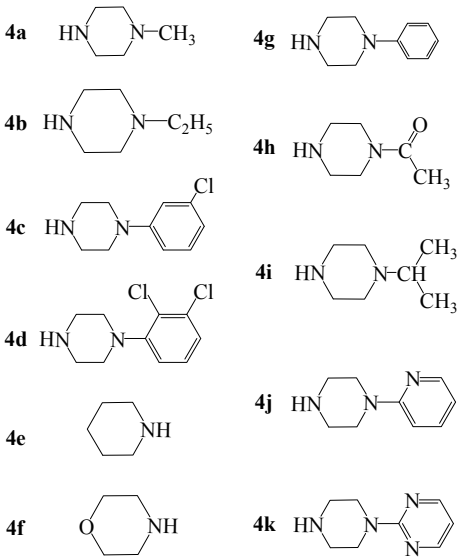

Fig. 2. Bases coupled to compound **3**.

istic band appeared at 1255 cm^{-1} corresponding to the C-O-C linkage, while disappearance of the –OH peak at 3610 cm^{-1}, belonging to 8-hydroxyquinoline, gave correction to the formation of intermediate **3**. Absence of a C-Cl stretching band at 700–760 cm^{-1} confirmed the formation of the final products by condensation of the piperazines and the *s*-triazine ring as all the chlorine atoms of the *s*-triazine ring were substituted by 4-amino-2-trifluoromethyl-benzonitrile, 8-hydroxyquinoline, and piperazines. The synthesis of **5a**–**5k** was confirmed on the basis of NMR spectra. The piperazine proton gave a signal at 3.79–3.89 ppm, the –NH group at 8.80–8.87 ppm, and the proton of the quinoline moiety resonated at 7.53–7.78 ppm. ^{13}C NMR spectra contained signals in the range 176.10–177.99, 168.96–169.78, and 163.79–164.79 ppm. The carbon atoms of the piperazine ring gave signals in the range of 46–50 ppm. The carbon atom of the CN group appeared to resonate around 105 ppm.

The antibacterial activity assays of the synthesized compounds **5a**–**5k** (Table I) revealed that attachment of *N*-methyl piperazine or *N*-ethyl piperazine to *s*-triazine resulted in moderate activity against all of the bacterial strains tested. The *N*-ethyl piperazine-bearing compound **5b** had a more potent inhibitory effect than the *N*-methyl piperazine-bearing compound **5a**. Compounds **5c** and **5d**, bearing halogen atom(s) in the phenyl ring of the piperazine moiety, had strong inhibitory activity against *S. typhi*, *B. cereus*, and *P. vulgaris*. Compound **5e**, bearing a piperidine substituent,

Table I. *In vitro* antibacterial activity.

Compound[a]	Zone of inhibition [mm (MIC in μg/mL)]						
	Gram (+)		Gram (–)				
	S. aureus	*B. cereus*	*E. coli*	*P. aeruginosa*	*K. pneumoniae*	*S. typhi*	*P. vulgaris*
5a (*N*-Methyl piperazine)	19 (100)	20 (100)	17 (100)	18 (100)	22 (50)	20 (100)	19 (100)
5b (*N*-Ethyl piperazine)	21 (100)	20 (100)	21 (100)	19 (100)	21 (100)	21 (100)	22 (50)
5c [1-(3-Chlorophenyl) piperazine]	24 (25)	25 (6.25)	24 (25)	24 (25)	25 (12.5)	25 (6.25)	23 (25)
5d [1-(2,3-Dichlorophenyl) piperazine]	25 (12.5)	26 (6.25)	25 (12.5)	24 (25)	24 (25)	24 (12.5)	25 (6.25)
5e (Piperidine)	21 (100)	23 (25)	27 (6.25)	17 (100)	22 (50)	19 (100)	16 (100)
5f (Morpholine)	19 (100)	18 (100)	22 (100)	17 (100)	27 (6.25)	19 (100)	17 (100)
5g (*N*-Phenyl piperazine)	21 (100)	18 (100)	22 (100)	17 (100)	21 (100)	17 (100)	16 (100)
5h (*N*-Acetyl piperazine)	23 (50)	20 (100)	28 (6.25)	20 (50)	27 (6.25)	22 (50)	21 (100)
5i (*N*-Isopropyl piperazine)	24 (25)	21 (50)	23 (50)	21 (50)	23 (25)	21 (100)	22 (50)
5j [1-(2-Pyridyl) piperazine]	26 (6.25)	23 (25)	25 (12.5)	27 (6.25)	27 (6.25)	23 (25)	21 (100)
5k [1-(2-Pyrimidyl) piperazine]	26 (6.25)	24 (12.5)	24 (25)	25 (12.5)	25 (12.5)	25 (6.25)	24 (12.5)
Ciprofloxacin (100 μg/disc)	29 (≤ 3)	29 (≤ 3)	32 (≤ 3)	33 (≤ 3)	33 (≤ 3)	30 (≤ 3)	31 (≤ 3)
DMSO	-	-	-	-	-	-	-

The MIC values were evaluated at the concentration range of 3.12–100 μg/mL. Each value is the mean of three independent experiments.

[a] Compounds **5a**–**5k** were tested at 100 μg/disc concentration in the radial diffusion assay.

Table II. *In vitro* antifungal activity.

Compound[a]	Zone of inhibition in [mm (MIC in μg/mL)]			
	A. niger	*A. fumigatus*	*A. clavatus*	*C. albicans*
5a (*N*-Methyl piperazine)	19 (100)	20 (50)	18 (100)	21 (100)
5b (*N*-Ethyl piperazine)	21 (50)	20 (50)	21 (50)	21 (100)
5c [1-(3-Chlorophenyl) piperazine]	23 (25)	23 (25)	24 (12.5)	25 (12.5)
5d [1-(2,3-Dichlorophenyl) piperazine]	23 (25)	25 (6.25)	25 (6.25)	25 (12.5)
5e (Piperidine)	23 (25)	21 (50)	21 (50)	23 (25)
5f (Morpholine)	26 (6.25)	20 (50)	23 (25)	23 (25)
5g (*N*-Phenyl piperazine)	20 (100)	18 (100)	20 (100)	22 (50)
5h (*N*-Acetyl piperazine)	24 (12.5)	23 (25)	27 (6.25)	19 (100)
5i (*N*-Isopropyl piperazine)	21 (50)	21 (50)	21 (50)	21 (100)
5j [1-(2-Pyridyl) piperazine]	24 (12.5)	24 (12.5)	22 (50)	28 (6.25)
5k [1-(2-Pyrimidyl) piperazine]	24 (12.5)	25 (6.25)	24 (12.5)	26 (6.25)
Ketoconazole (100 μg/disc)	30 (6.25)	29 (≤ 3)	31 (≤ 3)	33 (≤ 3)
DMSO	-	-	-	-

The MIC values were evaluated at the concentration range of 3.12–100 μg/mL. Each value is the mean of three independent experiments.

[a] Compounds **5a**–**5k** were tested at 100 μg/disc concentration in the radial diffusion assay.

was most effective against *E. coli*, whereas, the morpholine-bearing compound **5f** showed higher activity against *K. pneumoniae*. The compound, bearing an *N*-acetyl piperazine substituent (**5h**), showed good activity against *E. coli* and *K. pneumoniae*. The compounds **5j** and **5k**, containing the *N*-pyridyl piperazine and *N*-pyrimidyl piperazine moiety, respectively, proved most highly potent against most of the bacterial strains. Particularly, compound **5j** showed excellent inhibitory activity against *S. aureus*, *P. aeruginosa*, and *K. pneumoniae*, and compound **5k** showed strong inhibition against *S. aureus* and *S. typhi*.

All the final **5a**–**5j** compounds showed good to moderate inhibition of *A. niger*, *A. fumigatus*, *A. clavatus*, and *C. albicans* (Table II). Compound **5f** displayed good activity against *A. niger*. Compounds **5d** and **5k** were active against *A. fumigatus*, whereas compounds **5h** and **5j** showed greater potency against *A. clavatus* and *C. albicans*, respectively.

From the bioassay it is clear that the introduction of an appropriate substituent on the *s*-triazine ring would lead to more active antimicrobial derivatives. The variation of antimicrobial activity is related to the tested microorganisms as well as to the chemical structure of the test compounds. In the present study, higher potency has been observed with the final compounds bearing piperazine bases with halogen atom(s), methyl linker, and pyridine or pyrimidine ring containing nitrogen heteroatom(s). The fluorine atom is also found to enhance the biological activities of the compounds. Therefore, it was concluded that there exists ample scope for further study on this class of compounds with appropriate structural modification; for example the position of the hydroxy group at position 8 of the quinoline ring can be altered to the active position 4 of the quinoline ring as well as piperazine bases with a halogen atom like fluorine can be used. 4-Amino-2-trifluoromethyl-benzonitrile may be replaced by its non-fluorinated analogue 4-amino-benzonitrile in order to study the influence of presence and absence of the trifluoromethyl group on the biological profile of the resultant compounds. The study is currently under investigation and the results will be published in due course.

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