Novel s-Triazinyl piperazines: Design, synthesis, characterization and anti-microbial activity

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ABSTRACT

Eleven compounds have been synthesized in a series of [1, 3, 5] triazine analogues which, in addition to 4-amino benzonitrile, contain 8-hydroxy quinoline and different piperazines as well as piperidine substituents on the C-6 position of s-triazine ring. The title compounds were then evaluated for their in vitro microbial activity against 2 gram –Ve bacteria (E.coli, P. aeruginosa), 2 gram +Ve bacteria (S. aureus, B. subtilis) and 2 fungal species (C. albicans and A. niger). The most of the synthesized compounds have shown promising antimicrobial activity. All the final compounds were structurally elucidated on the basis of IR, 1H NMR, 13C NMR and elemental analysis.

Keywords: Cyanuric chloride, 8-hydroxy quinoline, 4-amino benzonitrile, anti microbial activity.

INTRODUCTION

The increasing number of multidrug resistant pathogens has led us to screen the newly synthesized derivatives against the representative panel of bacterial and fungal strains. As multidrug-resistant bacterial strains proliferate, the design and synthesis of novel antimicrobial molecules has been of enormous interest in recent years.

Over the past few decades [1, 3, 5] triazine compounds have been grabbing the attention of the synthetic chemists for their wide gamut of biological activities such as anti microbial [1, 2], anti protozoal [3], anti cancer [4], anti malarial [5] and anti viral [6] activity. Cyanuric chloride, the starting material is an inexpensive, commercially available reagent and the different reactivities of the substituent chlorine atoms, which are controlled by the temperature makes its use more attractive. In a view of its adaptable chemistry, we are promoted for sequential introduction of various piperazine and piperidine substituent into the [1, 3, 5] triazine ring. Literature survey reveals that piperazine ring is important for biological activity [7-9]. For instance, linezolid,
eperezolid, AZD2563 and itraconazole, which are currently important antibiotics used for the treatment of microbial infections, contain a piperazine ring in their structures. The piperidine structure is found in paroxetine, risperidone, methylphenidate, raloxifene, minoxidil and thioridazine pharmaceuticals. Due to appealing diverse biological properties among all the hydroxyquinoline derivatives, the adorable chemistry of 8-hydroxy quinoline has gained much importance such as anti microbial [10-12], anti malarial [13, 14], anti tuberculosis [15, 16], anti cancer [17, 18], anti leishmanial [19], anti calculus and anti plaque [20].

MATERIALS AND METHODS

All the melting points were determined in open capillary on Veego (Model: VMP-D) electronic apparatus and are uncorrected. The IR spectra (4000-400 cm\(^{-1}\)) of synthesized compounds were recorded on Shimadzu 8400-S FT-IR spectrophotometer with KBr pellets. Thin layer chromatography was performed on microscopic glass slides (2x7.5 cm) coated with silica gel-G, using appropriate mobile phase system and spots were visualized under UV radiation. Nuclear magnetic resonance spectra were recorded on Varian 400 MHz model spectrometer using DMSO as a solvent and TMS as internal standard (Chemical shifts in \(\delta\) ppm). All new compounds were subjected to elemental analysis and the results were in acceptable range.

General Experimentation

2.1- 4-(4,6-Dichloro-[1,3,5]triazin-2-ylamino)-benzonitrile (1).

To a stirred solution of cyanuric chloride (10 g, 0.054 mole) in anhydrous THF (150 ml) was added 4-amino benzonitrile (6.41 g, 0.054 mole) drop wise at 0-5 \(\circ\)C. The resulting reaction mixture was stirred at this temperature for 2 h, then triethyl amine (5.48 g, 0.054 mole) was added in the reaction mixture and stirring was continued for another 2 h. The resulted reaction mixture was then treated with crushed ice, followed by neutralization by dilute HCl and then filtered, dried and recrystallized from acetone to afford (1), M.p. 248.7 \(\circ\)C (dec.). FT-IR (KBr): 2223 cm\(^{-1}\) (CN).

2.2- 4-[4-Chloro-6-(quinolin-8-yloxy)-[1, 3, 5]triazin-2-ylamino]-benzonitrile (3).

To a stirred solution of 8-hydroxy quinoline (8 g, 0.055 mole) in anhydrous THF (150 ml) was added 60% NaH (1.32 g, 0.055 mole) at room temperature for 1 h and 1 (14.67 g, 0.055 mole) was added into the reaction mixture. Stirring was continued for another 4 h at 45 \(\circ\)C. After the completion of the reaction, it was treated with crushed ice, filtered and dried by THF to afford (3) [21], M.p. 285.38 (dec.). FT-IR (KBr): 2223 cm\(^{-1}\) (CN), 1255 cm\(^{-1}\) (C-O-C).

2.3- General procedure for preparation of compounds (5a-k).

To a solution of 3 (0.01 mole) in 1, 4-Dioxane (20 ml), added different substituted piperazine and piperidine derivatives and the reaction mixture was refluxed for 6 to 10 h. potassium carbonate was used for the neutralization of the reaction mixture. After the completion of the reaction, it was treated with crushed ice, neutralized by dilute HCl. The precipitates thus obtained was filtered, dried and recystallized from THF to afford desired compounds 5a-k.

2.4- Characterization of synthesized compounds (5a-k).

4-[4-(4-Methyl-piperazin-1-yl)-6-(quinolin-8-yloxy)-[1, 3, 5]triazin-2-ylamino]-benzonitrile (5a).

Yield: 86%; m.p. 277 \(\circ\)C (dec.); IR (KBr, cm\(^{-1}\)): 3100-3300 (-NH), 3060 (C-H), 2220-2225 (CN), 1558, 1303, 840 (C-N, C\(_3\)N\(_3\)), 1255 (C-O-C), 814 (s-triazine C-N str.); \(^1\)H NMR (400 MHz, DMSO- \(d_6\)) \(\delta\) 3.84 (8H, t, Piperazine), 2.40 (3H, s, -CH3), 8.84 (1H, s, -NH), 7.15-7.35 (7H, m, Ar-H), 7.55(3H, m, Quinoline); \(^13\)C NMR (400 MHz, DMSO- \(d_6\)) \(\delta\) 169.21 (C-3, s-triazine, C-N...
at piperazine linkage), 168.59 (C-1, s-triazine, C-O-C at quinoline linkage), 161.91 (C-5, s-triazine, C-NH at benzonitrile moiety), 145.34-102.08 (16C, quinoline and benzonitrile ring carbons), 52.35, 46.98 (4C, piperazine ring carbons), 46.03 (C-31, N-CH₃ linkage); Anal. Calcd. for C₂₄H₂₂N₈O: C, 65.74; H, 5.06; N, 25.55; Found: C, 65.73; H, 5.08; N, 25.54.

4-[4-(4-Ethyl-piperazin-1-yl)-6-(quinolin-8-yloxy)-[1, 3, 5]triazin-2-ylamino]-benzonitrile (5b).
Yield: 80%; m.p. 240°C (dec.); IR (KBr, cm⁻¹): 3100-3300 (-NH), 3068 (C-H), 2220-2225 (CN), 1572, 1313, 839 (C-N, C₃N₃), 1255 (C-O-C, S-triazine), 806 (s-triazine C-N str.); ¹H NMR (400 MHz, DMSO-d₆) δ 2.1 (3H, t, CH₃), 2.40 (2H, q, -CH₂), 3.82 (8H, m, Piperazine), 7.2-7.5 (7H, m, Ar-H), 7.55 (3H, m, Quinoline), 8.85 (1H, s, -NH); ¹³C NMR (400 MHz, DMSO-d₆) δ 170.08 (C-3, s-triazine, C-N at piperazine linkage), 168.66 (C-1, s-triazine, C-O-C at quinoline linkage), 161.88 (C-5, s-triazine, C-NH at benzonitrile moiety), 147.34-102.08 (16C, quinoline and benzonitrile ring carbons), 51.62, 50.29, 45.68 (4C, piperazine ring carbons and 1C at C-31, N-CH₃-), 12.39 (C-32); Anal. Calcd. for C₂₅H₂₄N₈O: C, 66.36; H, 5.35; N, 24.76; Found: C, 66.37; H, 5.35; N, 24.73.

4-[4-(4-Isopropyl-piperazin-1-yl)-6-(quinolin-8-yloxy)-[1, 3, 5]triazin-2-ylamino]-benzonitrile (5c).
Yield: 88%; m.p. 297°C (dec.); IR (KBr, cm⁻¹): 3100-3300 (-NH), 3057 (C-H), 2220-2225 (CN), 1567, 1305, 840 (C-N, C₃N₃), 1100 (isopropyl), 1255 (C-O-C), 819 (s-triazine C-N str.), 1380 (isopropyl); ¹H NMR (400 MHz, DMSO-d₆) δ 3.84 (8H, t, Piperazine), 2.40 (6H, d, -CH₃), 2.44 (1H, m, -CH), 8.83 (1H, s, -NH), 7.12-7.39 (7H, m, Ar-H), 7.56 (3H, m, Quinoline); ¹³C NMR (400 MHz, DMSO-d₆) δ 169.29 (C-3, s-triazine, C-N at piperazine linkage), 168.56 (C-1, s-triazine, C-O-C at quinoline linkage), 161.99 (C-5, s-triazine, C-NH at benzonitrile moiety), 146.32-101.65 (16C, quinoline and benzonitrile ring carbons), 54.11, 52.88, 46.82 (4C, piperazine ring carbons and 1C at C-31, N-CH₂-), 12.39 (C-32); Anal. Calcd. for C₂₆H₂₆N₈O: C, 66.94; H, 5.62; N, 24.02; Found: C, 66.93; H, 5.60; N, 24.03.

4-[4-(4-Acetyl-piperazin-1-yl)-6-(quinolin-8-yloxy)-[1, 3, 5]triazin-2-ylamino]-benzonitrile (5d).
Yield: 77%; m.p. 229°C (dec.); IR (KBr, cm⁻¹): 3100-3300 (-NH), 3066 (C-H), 2220-2225 (CN), 1700 (–C=O), 1578, 1321, 833 (C-N, C₃N₃), 1475 (-CH₃), 7.1-7.3 (7H, m, Ar-H), 7.6 (3H, m, Quinoline); ¹H NMR (400 MHz, DMSO-d₆) δ 3.85 (8H, t, Piperazine), 2.39 (3H, S, -CH₃), 8.85 (1H, s, -NH), 7.1-7.3 (7H, m, Ar-H), 7.6 (3H, m, Quinoline); ¹³C NMR (400 MHz, DMSO-d₆) δ 169.89 (C-34, acetyl C=O), 169.13 (C-3, s-triazine, C-N at piperazine linkage), 168.56 (C-1, s-triazine, C-O-C at quinoline linkage), 161.99 (C-5, s-triazine, C-NH at benzonitrile moiety), 146.32-101.65 (16C, quinoline and benzonitrile ring carbons), 54.11, 52.88, 46.82 (4C, piperazine ring carbons and 1C at C-31, N-CH₂-), 21.03 (2C, 2CH₃); Anal. Calcd. for C₂₆H₂₆N₈O₂: C, 66.94; H, 5.62; N, 24.02; Found: C, 66.93; H, 5.60; N, 24.03.

4-[4-Morpholin-4-yl-6-(quinolin-8-yloxy)-[1, 3, 5]triazin-2-ylamino]-benzonitrile (5e).
Yield: 89%; m.p. 279°C (dec.); IR (KBr, cm⁻¹): 3100-3300 (-NH), 3052 (C-H), 2220-2225 (CN), 1700 (–C=O), 1578, 1321, 833 (C-N, C₃N₃), 1475 (-CH₃), 161.97 (C-5, s-triazine, C-NH at benzonitrile moiety), 145.34-102.08 (16C, quinoline and benzonitrile ring carbons), 54.11, 52.88, 46.82 (4C, piperazine ring carbons and 1C at C-31, N-CH₂-), 22.32 (C-31); Anal. Calcd. for C₂₅H₁₉N₇O₂: C, 64.37; H, 4.75; N, 24.02; Found: C, 64.36; H, 4.76; N, 24.03.

4-[4-(4-Acetyl-piperazin-1-yl)-6-(quinolin-8-yloxy)-[1, 3, 5]triazin-2-ylamino]-benzonitrile (5f).
Yield: 89%; m.p. 279°C (dec.); IR (KBr, cm⁻¹): 3100-3300 (-NH), 3066 (C-H), 2220-2225 (CN), 1700 (–C=O), 1578, 1321, 833 (C-N, C₃N₃), 1475 (-CH₃), 806 (s-triazine C-N str.); ¹H NMR (400 MHz, DMSO-d₆) δ 3.85 (8H, t, Piperazine), 2.39(3H, S, -CH₃), 8.85 (1H, s, -NH), 7.1-7.3 (7H, m, Ar-H), 7.6 (3H, m, Quinoline); ¹³C NMR (400 MHz, DMSO-d₆) δ 169.89 (C-34, acetyl C=O), 169.13 (C-3, s-triazine, C-N at piperazine linkage), 168.56 (C-1, s-triazine, C-O-C at quinoline linkage), 162.39 (C-5, s-triazine, C-NH at benzonitrile moiety), 146.32-101.41 (16C, quinoline and benzonitrile ring carbons), 48.04, 45.45 (4C, piperazine ring carbons), 22.32 (C-31); Anal. Calcd. for C₂₅H₁₀N₉O₂: C, 64.93; H, 4.51; N, 23.05; Found: C, 64.94; H, 4.51; N, 23.03.
4-[4-Piperidin-1-yl-6-(quinolin-8-yloxy)-[1,3,5]triazin-2-ylamino]-benzonitrile (5f). Yield: 80%; m.p. 282\(^\circ\)C (dec.); IR (KBr, cm\(^{-1}\)) : 3100-3300 (-NH), 3065 (C-H), 2220-2225 (CN), 1558, 1303, 840 (C-N, C\(_3\)N\(_3\)), 1255 (C-O-C), 817 (s-triazine C-N str.); \(^1\)H NMR (400 MHz, DMSO-\(d_6\)) \(\delta\) 3.85(10H, t, Piperidine), 8.85 (1H, s, -NH), 7.2-7.35 (7H, m, Ar-H), 7.56 (3H, m, Quinoline); \(^13\)C NMR (400 MHz, DMSO-\(d_6\)) \(\delta\) 169.26 (C-3, s-triazine, C-N at piperazine linkage), 168.54 (C-1, s-triazine, C-O-C at quinoline linkage), 162.37 (C-5, s-triazine, C-NH at benzonitrile moiety), 145.56-101.41 (16C, quinoline and benzonitrile ring carbons), 46.68, 25.11, 22.99 (5C, C-26 to C-30, piperidine ring carbons); Anal. Calcd. for C\(_{24}\)H\(_{21}\)N\(_7\)O: C, 68.07; H, 5.00; N, 23.15%; Found: C, 68.07; H, 5.01; N, 23.14%.

4-[4-(4-Phenyl-piperazin-1-yl)-6-(quinolin-8-yloxy)-[1,3,5]triazin-2-ylamino]-benzonitrile (5g). Yield: 84%; m.p. 215\(^\circ\)C (dec.); IR (KBr, cm\(^{-1}\)) : 3100-3300 (-NH), 3060 (C-H), 2220-2225 (CN), 1560, 1315, 832 (C-N, C\(_3\)N\(_3\)), 1255 (C-O-C), 815 (s-triazine C-N str.); \(^1\)H NMR (400 MHz, DMSO-\(d_6\)) \(\delta\) 3.85(8H, t, Piperazine), 8.82 (1H, s, -NH), 7.1-7.3 (12H, m, Ar-H), 7.54 (3H, m, Quinoline); \(^13\)C NMR (400 MHz, DMSO-\(d_6\)) \(\delta\) 169.36 (C-3, s-triazine, C-N at piperazine linkage), 168.50 (C-1, s-triazine, C-O-C at quinoline linkage), 161.94 (C-5, s-triazine, C-NH at benzonitrile moiety), 151.33-101.44 (22C, quinoline and benzonitrile ring carbons and phenyl ring at piperazine), 49.49, 47.89 (4C, piperazine ring carbons); Anal. Calcd. for C\(_{29}\)H\(_{24}\)N\(_8\)O: C, 69.58; H, 4.83; N, 22.39%; Found: C, 69.56; H, 4.81; N, 22.40%.

4-[4-[4-(2-Chloro-phenyl)-piperazin-1-yl]-6-(quinolin-8-yloxy)-[1,3,5]triazin-2-ylamino]-benzonitrile (5h). Yield: 78%; m.p. 257\(^\circ\)C (dec.); IR (KBr, cm\(^{-1}\)) : 3100-3300 (-NH), 3059 (C-H), 2220-2225 (CN), 1556, 1313, 847 (C-N, C\(_3\)N\(_3\)), 1255 (C-O-C), 754 (C-Cl), 813 (s-triazine C-N str.), 767 (-Cl str.); \(^1\)H NMR (400 MHz, DMSO-\(d_6\)) \(\delta\) 3.85(8H, t, Piperazine), 7.13-7.40 (11H, m, Ar-H), 7.78 (3H, m, Quinoline), 8.86 (1H, s, -NH); \(^13\)C NMR (400 MHz, DMSO-\(d_6\)) \(\delta\) 169.29 (C-3, s-triazine, C-N at piperazine linkage), 168.54 (C-1, s-triazine, C-O-C at quinoline linkage), 161.33-101.44 (22C, quinoline and benzonitrile ring carbons and phenyl ring at piperazine), 48.88, 47.73 (4C, piperazine ring carbons); Anal. Calcd. for C\(_{29}\)H\(_{23}\)ClN\(_8\)O: C, 65.10; H, 4.33; N, 20.94%; Found: C, 65.11; H, 4.33; N, 20.91%.

4-[4-[4-(2,3-Dichloro-phenyl)-piperazin-1-yl]-6-(quinolin-8-yloxy)-[1,3,5]triazin-2-ylamino]-benzonitrile (5i). Yield: 79%; m.p. 297-300\(^\circ\)C (dec.); IR (KBr, cm\(^{-1}\)) : 3100-3300 (-NH), 3066 (C-H), 2220-2225 (CN), 1556, 1313, 847 (C-N, C\(_3\)N\(_3\)), 1255 (C-O-C), 813 (s-triazine C-N str.), 754 (C-Cl), 806 (s-triazine C-N str.), 746 (-Cl str.); \(^1\)H NMR (400 MHz, DMSO-\(d_6\)) \(\delta\) 3.83(8H, t, Piperazine), 8.86 (1H, s, -NH), 7.23-7.45 (10H, m, Ar-H), 7.73 (3H, m, Quinoline); \(^13\)C NMR (400 MHz, DMSO-\(d_6\)) \(\delta\) 169.29 (C-3, s-triazine, C-N at piperazine linkage), 168.5 (C-1, s-triazine, C-O-C at quinoline linkage), 161.99 (C-5, s-triazine, C-NH at benzonitrile moiety), 151.33-101.44 (22C, quinoline and benzonitrile ring carbons and phenyl ring at piperazine), 49.93, 47.77 (4C, piperazine ring carbons); Anal. Calcd. for C\(_{29}\)H\(_{22}\)Cl\(_2\)N\(_8\)O: C, 65.10; H, 4.33; N, 20.94%; Found: C, 65.11; H, 4.43; N, 20.91%.

4-[4-(4-Pyridin-2-yl-piperazin-1-yl)-6-(quinolin-8-yloxy)-[1,3,5]triazin-2-ylamino]-benzonitrile (5j). Yield: 87%; m.p. 287\(^\circ\)C (dec.); IR (KBr, cm\(^{-1}\)) : 3100-3300 (-NH), 3062 (C-H), 2220-2225 (CN), 1558, 1303, 840 (C-N, C\(_3\)N\(_3\)), 1255 (C-O-C), 806 (s-triazine C-N str.), 746 (-Cl str.); \(^1\)H NMR (400 MHz, DMSO-\(d_6\)) \(\delta\) 3.84(8H, t, Piperazine), 7.2-7.35 (11H, m, Ar-H), 7.55 (3H, m, Quinoline), 8.84 (1H, s, -NH); \(^13\)C NMR (400 MHz, DMSO-\(d_6\)) \(\delta\) 169.21 (C-3, s-triazine, C-N at piperazine linkage), 168.68 (C-1, s-triazine, C-O-C at quinoline linkage), 161.79 (C-5, s-triazine, C-NH at benzonitrile moiety), 160.79 (C-31), 151.33-101.44 (20C, quinoline and benzonitrile ring moieties).
carbons and phenyl ring at piperazine), 47.74, 47.71 (4C, piperazine ring carbons); Anal. Calcd. for C_{28}H_{23}N_{9}O: C, 67.05; H, 4.62%; N, 25.13; Found: C, 67.04; H, 4.62; N, 25.11%.

4-[4-(4-Pyrimidin-2-yl-piperazin-1-yl)-6-(quinolin-8-yloxy)-[1,3,5]triazin-2-ylamino]-benzonitrile (5k).
Yield: 87%; m.p. 276^0C (dec.); IR (KBr, cm^{-1}): 3100-3300 (-NH), 3062 (C-H), 2220-2225 (CN), 1558, 1303, 840 (C-N, C_{3}N_{3}), 1255 (C-O-C), 806 (s-triazine C-N str.); ^1H NMR (400 MHz, DMSO- d_{6}) \delta 3.84(8H, t, Piperazine), 7.2-7.35(10H, m, Ar-H), 7.55 (3H, m, Quinoline), 8.84 (1H, s, -NH); ^13C NMR (400 MHz, DMSO- d_{6}) \delta 169.24 (C-3, s-triazine, C-N at piperazine linkage), 168.56 (C-1, s-triazine, C-O-C at quinoline linkage), 162.37 (C-5, s-triazine, C-NH at benzonitrile moiety), 158.27-101.44 (16C, quinoline and benzonitrile ring carbons and phenyl ring at piperazine), 47.72, 47.71 (4C, piperazine ring carbons); Anal. Calcd. for C_{27}H_{22}N_{10}O: C, 64.53; H, 4.41%; N, 27.87; Found: C, 64.54; H, 4.41; N, 27.85%.

2.5- Antimicrobial activity
All the newly synthesized compounds were tested for their in vitro antibacterial and antifungal activity (MIC-minimum inhibition concentration) by broth dilution method [22] with two gram positive bacteria S. aureus and B. subtilis, two gram negative bacteria E. coli, P. aeruginosa and fungi species like C. albicans, A. niger organisms. Ciprofloxacin, ampicillin, chloramphenicol, norfloxacin, flucanazole, griseofulvin, and Nystatin were used in assay as a standard control drug. Muller Hinton broth was used as nutrient medium to grow and dilute the drug suspension for test. DMSO was used as a diluent which is ineffective to the growth of microbes.

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### Table 1 various substituted piperazines and piperidine derivatives used as coupling agents

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### RESULTS AND DISCUSSION

Conventional methods were used to carry out all the reaction steps. The final key intermediate 4-[4-Chloro-6-(quinolin-5-yloxy)-[1,3,5]triazin-2-ylamino]-benzonitrile 3 was prepared by the reaction between 4-(4,6-Dichloro-[1,3,5]triazin-2-ylamino)-benzonitrile 1 and 8-hydroxy quinoline in which the primary intermediate 1 was obtained from cyanuric chloride and 4-amino quinoline.
benzonitrile using triethyl amine. C$_3$N$_3$ stretching frequency in s-triazine ring was observed at 821 cm$^{-1}$. The IR spectra of compound 1 revealed a strong band at 2220-2225 cm$^{-1}$ confirming the presence of cyan group and a strong band at 3290 cm$^{-1}$ confirmed the presence of $^2$-NH group. Moreover, characteristic peak of C-O-C appeared at 1255 cm$^{-1}$ by disappearing stretching peak at 3610 cm$^{-1}$ of O-H belonging to the 8-hydroxy quinoline in the final intermediate 3. Absence of C-Cl stretching band at 700-750 cm$^{-1}$ confirmed the formation of final compounds. The $^1$H NMR data of final compounds revealed signal between 3.82-3.85 δ ppm for piperazines, signal between 8.83-8.86 δ ppm for –NH and signal at 7.55 δ ppm for multiplet of quinoline moiety. $^{13}$C NMR spectral data interpretation clearly indicates that the signals in the range 168.54-168.89, 169.13-170.08 and 161.79-162.39 δ ppm confirmed the replacement of chlorine atoms at C-1, C-3 and C-5 positions of triazinyl ring by 4-amino benzonitrile, 8-hydroxy quinoline and piperazines as well as piperidine constituents respectively, whereas, carbon atoms present in the piperazine ring revealed signals in the range of 46-50 δ ppm in $^{13}$C-NMR spectra. In addition, elemental data mentioned above also confirmed the structure of the final molecules.

The mentioned anti bacterial results revealed that the compound 5j, bearing 1-(2-pyridyl) piperazine derivative to the basic s-triazine nucleus containing 8-hydroxy quinoline in addition to 4-amino benzonitrile linkage proved more beneficial compound compared to other analogues against Escherichia coli. The compounds 5c, 5i and 5k, bearing N-isopropyl piperazine, 1-(2,3-Dichlorophenyl) piperazine and 1-(2-pyrimidyl) piperazine substituent respectively have shown the highest sensitivity against P. aeruginosa.

Table 2 Antimicrobial study (MIC µg/mL) of synthesized compound 5a-k. Minimum inhibitory concentration

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<td>50</td>
<td>100</td>
</tr>
<tr>
<td>5d</td>
<td>N-Acetyl piperazine</td>
<td>50</td>
<td>100</td>
<td>25</td>
</tr>
<tr>
<td>5e</td>
<td>Morpholine</td>
<td>100</td>
<td>100</td>
<td>50</td>
</tr>
<tr>
<td>5f</td>
<td>Piperidine</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>5g</td>
<td>N-Phenyl piperazine</td>
<td>200</td>
<td>500</td>
<td>250</td>
</tr>
<tr>
<td>5h</td>
<td>1-(2-Chlorophenyl) piperazine</td>
<td>250</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>5i</td>
<td>1-(2,3-Dichlorophenyl) piperazine</td>
<td>100</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>5j</td>
<td>1-(2-Pyridyl) piperazine</td>
<td>25</td>
<td>100</td>
<td>50</td>
</tr>
<tr>
<td>5k</td>
<td>1-(2-Pyrimidyl) piperazine</td>
<td>50</td>
<td>50</td>
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<tr>
<td>Ampicillin</td>
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<td>Fluconazole</td>
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</table>

Compound 5d having N-acetyl piperazine substituent shown the best activity against S. aureus whereas final scaffolds 5e, 5h, 5i and 5k containing morpholine, 1-(3-chlorophenyl) piperazine, 1-(2,3-Dichlorophenyl) piperazine and 1-(2-pyrimidyl) piperazine constituents respectively proved as beneficial coupling agents to the final moiety for the best activity against B. subtilis. The biological screening results for fungal species revealed that compound 5j bearing 1-(2-pyridyl) piperazine constituent exhibited higher activity against C. albicans and the compound
having 1-(2-pyrimidyl) piperazine exhibited promising activity against *A. niger*. In short, we made an attempt to increase the biological activity by increasing the volume of the substituents attached to piperazine ring system led to different biological potency, depending on the nature, position and number of the atoms or groups introduced, whereas, high potency has been observed with the final scaffolds 5h, 5i, 5j and 5k bearing piperazine systems with halogen and heterocyclic entity such as pyridine and pyrimidine.

**CONCLUSION**

A series of trisubstituted s-triazine derivatives has been successfully synthesized and tested for their anti-microbial activity. S-triazine nucleus is one of the active constituents present in many standard drugs, and is known to increase the pharmacological activity of the molecules as we have already reported its significant activity [23-27]. The presence of 8-hydroxy quinoline moiety is also an instrumental in contributing the net biological activity. Herein, we have combined all these three potential unit, that is S-triazine nucleus, 4-amino benzonitrile moiety, 8-hydroxy quinoline in one core and studied biological behaviour of the resultant systems having various substituted piperazine derivatives and piperidine. All the other compounds were found to exhibit slight to moderate activity against mentioned organism. Hence, it is concluded that, trisubstituted S-triazine are more active than mono and di-substituted S-triazine and thus, there is enough scope for further study in developing such compounds as a good lead activity. Overall conclusion placed for synthesized compounds is that most of the compounds shown very good promising activity as compared to standard drug for all representative panel of bacterial and fungal strains.

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