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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Design, synthesis, characterization, and in vitro antimicrobial action of novel trisubstituted *s*-triazines

Paresh K. Patel · Rahul V. Patel · Dharmesh H. Mahajan · Parimal A. Parikh · Girish N. Mehta · Kishor H. Chikhalia

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Abstract In this article, synthesis of a library of trisubstituted *s*-triazines, which, in addition to 4-amino-benzonitrile, contain 4-hydroxy-*N*-methylquinolin-2(1*H*)-one as well as substituted aliphatic amines condensed to C-6 position of *s*-triazinyl core is discussed. The newly synthesized analogues were then subjected to determine their efficacy against some bacterial and fungal strains as two gram-positive bacteria (*Staphylococcus aureus* MTCC 96 and *Bacillus cereus* MTCC 619), six gram-negative bacteria (*Escherichia coli* MTCC 739, *Pseudomonas aeruginosa* MTCC 741, *Klebsiella pneumoniae* MTCC 109, *Salmonella typhi* MTCC 733, *Proteus vulgaris* MTCC 1771, and *Shigella flexneria* MTCC 1457) and two fungal species (*Aspergillus niger* MTCC 282 and *Candida albicans* MTCC 183) with an intent to develop novel class of antimicrobial agents. The results of bioassay showed that some of the newly synthesized *s*-triazine congeners emerged with noteworthy antimicrobial activity. The structure of final scaffolds has been assigned on the basis of IR, ^{1}H NMR, ^{13}C NMR, mass spectroscopy and elemental analysis.

Keywords *s*-Triazine · Quinoline · Antimicrobial activity

P. K. Patel · R. V. Patel · G. N. Mehta
Department of Applied Chemistry, Sardar Vallabhbhai National Institute of Technology (SVNIT), Surat 395 007, Gujarat, India
e-mail: kruparesh@yahoo.com

R. V. Patel
e-mail: rahul.svnit.11@gmail.com

G. N. Mehta
e-mail: girish17in@yahoo.co.in

D. H. Mahajan
Department of Chemistry, KBS Commerce & NATARAJ Professional Science College, Vapi 396 195, Gujarat, India
e-mail: mahajan_dh@yahoo.com

P. A. Parikh
Department of Chemical Engineering, Sardar Vallabhbhai National Institute of Technology (SVNIT), Surat 395 007, Gujarat, India
e-mail: parimal_svr@yahoo.co.uk

K. H. Chikhalia (✉)
School of Science, Department of Chemistry, Gujarat University, Ahmedabad 380 009, Gujarat, India
e-mail: chikhalia_kh@yahoo.com

Introduction

A higher incidence of opportunistic microbial infections caused by various bacteria and fungi due to the evolution and spread of multidrug-resistant microorganisms has become a widespread medical problem. Such infections most commonly cause severe morbidity and mortality in debilitated and immunocompromised patients (Chu *et al.*, 1996; Overbye and Barrett, 2005; Walsh, 2000). Many research groups have been working to solve the antibacterial resistance problems. One possible long-term solution is in the development of agents that act on unexploited antibacterial target (Niccolai *et al.*, 1997). The *s*-triazine-based derivatives demonstrate a wide range of biological activities. They are found to be effective as antibacterial (Srinivas *et al.*, 2006; Solankee *et al.*, 2010; Patel *et al.*, 2010a, b, c; Zhou *et al.*, 2008), antifungal (Mishra *et al.*, 2000; Sareen *et al.*, 2006), antitubercular (Sunduru *et al.*, 2010), antimalarial (Agarwal *et al.*, 2005; Manohar *et al.*, 2010), anticancer (Menicagli *et al.*, 2004; Saczewski *et al.*, 2006; Saczewski and Bulakowska, 2006), antiprotozoals (Baliani

et al., 2005), estrogen receptor modulators (Rodriguez *et al.*, 2004), and anti-HIV(Xionga *et al.*, 2009; Xionga *et al.*, 2008; Ludovici *et al.*, 2001; Mahajan *et al.*, 2009; Patel *et al.*, 2007). Derivatives of *s*-triazine have been used in the treatment of depression (Whitten *et al.*, 1996) and as bridging agents to synthesize herbicides (Seffernick *et al.*, 2002). Further, substituted *s*-triazines have been used as NLO materials, which have a wide range of applications in optoelectronics and telecommunications (Thalladi *et al.*, 1998; Zhu and Wu, 2001). 2,4,6-trichloro-1,3,5-triazine is an inexpensive, commercially available reagent, and the different reactivities of the chlorine atoms, which are controlled by temperature, make its use more attractive. Quinolines are the effective moieties in a large number of natural and synthetic heterocyclic compounds that exhibit significant antibiotic activity found in the structure of many well-known antimicrobial drugs like ciprofloxacin, norfloxacin, ofloxacin, pefloxacin, rufloxacin, enrofloxacin, etc. In addition, condensations of substituted aromatic amines to any pharmacophore play an important role leading to the distinctive modification in their biological profiles of the resultant systems. In the design of new compounds, the developments of hybrid molecule through the combination of different pharmacophores in one structure may lead to increased antimicrobial activity. These observations prompted us to adopt the sequential introduction of the substituted aliphatic amines to the *s*-triazine core involving ethereal and amine linkages to the *s*-triazine core by hydroxyl quinoline and aminobenzonitrile moieties, respectively, to maintain a pool of new bioactive candidates at all times.

Recently, our research group has reported antimicrobial activities of a series of new *s*-triazine-based analogues involving substitutions of 4-amino-benzonitrile and 8-hydroxyquinoline (Patel *et al.*, 2010a, b, c), 4-amino-benzonitrile, and 4-hydroxy-*N*-methylquinolin-2(1*H*)-one (Patel *et al.*, 2011a, b), as well as simple aniline and 4-hydroxycoumarin (Patel *et al.*, 2011a, b). It is suffice to mention here that we introduced piperazine or piperidine derivatives in all the cases that our previous study investigated and the compounds have shown promising antimicrobial activities, representing a promising lead for further optimization. To extend their structure–activity relationships (SARs), we have designed and synthesized a novel series based on the modification of a piperazinyl or piperidinyl structural unit to simple aliphatic amines. We have introduced the similar 4-amino-benzonitrile and 4-hydroxy-*N*-methylquinolin-2(1*H*)-one in this study to identify the differences between the biological profiles of the resultant series, in which activity was found to be increased significantly against most of the studied strains of bacteria and fungi in terms of MIC as well as inhibition zones.

Experimental

Materials and methods

2,4,6-Trichloro-1,3,5-triazine, 4-hydroxy-*N*-methylquinolin-2(1*H*)-one, and 4-amino-benzonitrile were purchased from Sigma-Aldrich Germany. Used solvent acetone was of laboratory grade purchased from Merck and Qualigens; tetrahydrofuran was of AR grade, purchased from Merck, used without further purification; and coupling-substituted aliphatic amines were purchased from Merck, Qualigens, Finar, National and Spectrochem. The melting points of the products were determined in open capillaries using Veego electronic apparatus (Model: VMP-D) and are uncorrected. The IR spectra (4000–400 cm^{-1}) of synthesized compounds were recorded on Shimadzu 8400-S FT-IR spectrophotometer using KBr pellets. To monitor the reactions, and to establish the purity of reactants and products, thin layer chromatography was performed on microscopic glass slides (2 × 7.5 cm) coated with silica gel-G, and spots were visualized under UV radiation. ^{1}H NMR and ^{13}C NMR spectra were recorded on Varian 400-MHz model spectrometer using DMSO-*d6* as a solvent and TMS as internal standard with ^{1}H resonant frequency of 400 MHz and ^{13}C resonant frequency of 100 MHz. The ^{1}H NMR and ^{13}C NMR chemical shifts were reported as δ parts per million (ppm) downfield from TMS (Me_4Si). The splitting patterns are designated as follows; s, singlet; d, doublet; and m, multiplet. The mass spectra were recorded on JOEL SX-102 (EI) model. Elemental analyses (C, H, and N) were performed using Heraeus Carlo Erba 1180 CHN analyzer (Hanau, Germany).

Synthesis of 4-(4,6-dichloro-1,3,5-triazin-2-ylammino)benzonitrile (3)

Mixture of 2,4,6-trichloro-1,3,5-triazine chloride **1** (1.85 g, 0.01 mol) and anhydrous potassium carbonate (K_2CO_3) (1.38 g, 0.01 mol) in 25 ml acetone was stirred at 0–5°C for 20 min. 4-amino-benzonitrile **2** (1.84 g, 0.01 mol) was added portion wise at the same temperature to the above mixture; after complete addition, the reaction mixture was stirred for 4–5 h at the same temperature. Progress of the reaction was monitored by TLC using toluene/acetone (8:2, v/v) solvent system as an eluent. After completion of the reaction, resultant mixture was poured on crushed ice. The solid product obtained was filtered, washed with distilled water, dried, and purified by column chromatography using toluene/acetone solvent system as an eluent. Yield: 90%; m.p. >300°C (dec.); IR KBr cm: 3277 (N–H), 2228 (C≡N), 833 (C_3N_3, *s*-triazine); ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.50 (s, 1H, –N*H* proton of *s*-triazine to

aminobenzonitrile linkage), 7.86–7.78 (m, 4H, Ar–*H* aromatic proton); ^{13}C NMR (100 MHz, DMSO-d_6): δ 173.81 (2C, *C*–Cl of *s*-triazine), 164.37 (1C, *C*–NH of *s*-triazine to aminobenzonitrile linkage), 141.99–119.32 (6C, Ar–*C* of aromatic), 106.84 (1C, –*C*≡N of aminobenzonitrile moiety).

Synthesis of 4-(4-chloro-6-(1-methyl-2-oxo-1,2-dihydroquinilin-4-yloxy)-1,3,5-triazin-2-ylamino)-benzonitrile (5)

Mixture of 4-hydroxy-*N*-methylquinolin-2(1*H*)-one **4** (1.75 g, 0.01 mol) and anhydrous potassium carbonate (K_2CO_3) (1.38 g, 0.01 mol) in 50 ml acetone was stirred at room temperature for 30 min. Then, compound **3** (2.66 g, 0.01 mol) was added portion wise at room temperature with stirring; after complete addition, the reaction mixture was stirred 6–7 h at 40–45°C in water bath. Progress of the reaction was monitored by TLC using toluene/acetone (7:3, v/v) solvent system as an eluent. After completion of the reaction, resultant mixture was poured on crushed ice. The solid product was obtained, filtered, washed with distilled water, dried, and purified by column chromatography using toluene/acetone solvent system as an eluent. Yield: 88%; m.p. 243–246°C; IR KBr cm: 3281 (N–H), 2225 (C≡N), 831(C_3N_3, *s*-triazine); 1256 (C–O–C); ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.25 (s, 1H, –N*H* proton of *s*-triazine to aminobenzonitrile linkage), 7.78–7.25 (m, 8H, Ar–*H* aromatic proton), 6.71 (s, 1H, –C*H*–(C=O)–N proton of quinoline moiety), 3.66 (s, 3H, –N–CH_3 proton of quinoline moiety); ^{13}C NMR (100 MHz, DMSO-d_6) δ 175.78 (1C, *C*–Cl of *s*-triazine), 170.45 (1C, *C*–O–C, of *s*-triazine to 4-HMQ linkage), 169.71 (1C, *C*–NH of *s*-triazine to aminobenzonitrile linkage), 160.69 (1C, –*C*=O of quinoline moiety), 143.40–127.63 (13C, Ar–*C* of aromatic), 105.94 (1C, –*C*≡N of aminobenzonitrile moiety), 102.92 (1C, –*C*–(C=O)–N of quinoline moiety), 29.76 (1C, –N–*C*H_3 of quinoline moiety); ESI–MS (*m*/*z*): 404.95 (M^+, 100).

General procedure for the preparation of 4-(substituted aliphaticamine)-6-(4-cyanophenylamino)-1,3,5,-truiazine-2-yloxy)-1-methylquinolin-2(1*H*)-one (6a–o)

Mixture of compound **5** (1.01 g, 0.0025 mol), anhydrous potassium carbonate (K_2CO_3) (0.35 g, 0.0025 mol), and substituted aliphatic amine **a**–**o** (0.0025 mol) was stirred in 50 ml tetrahydrofuran. The reaction mixture was refluxed for 5–24 h in water bath; progress of the reaction was monitored by TLC using toluene/acetone (7:3, v/v) solvent system as an eluent. After the completion of the reaction, resultant mixture was poured on crushed ice. The solid product was obtained, filtered, washed with distilled water, and dried and purified by column chromatography using toluene/acetone solvent system as an eluent.

4-(4-(1-Methyl-2-oxo-1,2-dihydroquinolin-4-yloxy)-6-(methylamino)-1,3,5-triazin-2-ylamino)benzonitrile (**6a**)

Yield: 82%; m.p. 166°C; IR (KBr cm^{-1}): 3271 (N–H), 2909 (C–H in N–CH_3), 2222 (C≡N), 1647 (C=O), 1305 (C–N in 2^o aromatic amine), 1246 (C–O–C), 1109 (C–N in aliphatic amine), 833 (C_3N_3 s-triazine); ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.22 (s, 1H, –N*H* of aminobenzonitrile linkage), 9.99 (s, 1H, –N*H*–CH_3 of methyl amine), 8.04–7.28 (m, 8H, Ar. –*H*), 6.59 (s, 1H, –C*H*–(C=O)–N of quinoline), 3.67 (s, 3H, –N–CH_3 of quinoline); 3.67 (s, 3H, –N–CH_3 of methyl amine); ^{13}C NMR (100 MHz, DMSO-d_6): δ 174.12 (1C, –*C*–NH–CH_3, of *s*-triazine to methyl amine) 172.68 (1C, –*C*–O–C, *s*-triazine to 4-HMQ linkage), 168.28 (1C, –*C*–NH of aminobenzonitrile), 163.38 (1C, –*C*=O of quinoline), 159.21 (1C, –*C*–O–C, 4-HMQ linkage to *s*-triazine) 151.86–115.99 (12C, –Ar. C atoms), 104.91 (1C, –*C*N), 101.95 (1C, –*C*–(C=O)–N of quinoline), 32.19 (1C, –N–*C*H_3 of quinoline), 26.12 (1C, –NH–*C*H_3 of methyl amine); Anal. Calcd. For $C_{21}H_{17}N_7O_2$: C, 63.15; H, 4.29; N, 24.55% Found: C, 63.09; H, 4.20; N, 24.63%; ESI–MS (*m*/*z*): 400.38 (M^+, 100%).

4-(4-(Dimethylamino)-6-(1-methyl-2-oxo-1,2-dihydroquinolin-4-yloxy)-1,3,5-triazin-2-ylamino)benzonitrile (**6b**)

Yield: 81%; m.p. 248–249°C; IR (KBr cm^{-1}): 3274 (N–H), 2890 (C–H in N–CH_3), 2224 (C≡N), 1654 (C=O), 1309 (C–N in 2^o aromatic amine), 1249 (C–O–C), 1121 (C–N in aliphatic amine), 830 (C_3N_3 s-triazine); ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.29 (s, 1H, –N*H* of aminobenzonitrile linkage), 8.18–7.32 (m, 8H, Ar. –*H*), 6.42 (s, 1H, –C*H*–(C=O)–N of quinoline), 3.60 (s, 3H, –N–CH_3 of quinoline); 3.06 (s, 6H, –N–(CH_3)$_2$ of dimethyl amine); ^{13}C NMR (100 MHz, DMSO-d_6): δ 175.21 (1C, –*C*–O–C, *s*-triazine to 4-HMQ linkage), 171.85 (1C, –*C*–N(CH_3)$_2$, of *s*-triazine to dimethyl amine), 166.58 (1C, –*C*–NH of aminobenzonitrile), 162.88 (1C, –*C*=O of quinoline), 156.11 (1C, –*C*–O–C, 4-HMQ linkage to *s*-triazine) 147.09–114.12 (12C, –Ar. C atoms), 104.25 (1C, –*C*N), 102.08 (1C, –*C*–(C=O)–N of quinoline), 40.56 (2C, –N(*C*H_3)$_2$), 32.19 (1C, –N–*C*H_3 of quinoline); Anal. Calcd. For $C_{22}H_{19}N_7O_2$ C, 63.91; H, 4.63; N, 23.72% Found: C, 63.85; H, 4.55; N, 23.80%; ESI–MS (*m*/*z*): 414.40 429.39 (M^+, 100%).

4-(4-(Ethylamino)-6-(1-methyl-2-oxo-1,2-dihydroquinolin-4-yloxy)-1,3,5-triazin-2-ylamino)benzonitrile (**6c**)

Yield: 55%; m.p. 118–122°C; (KBr cm^{-1}): 3273 (N–H), 2960, 2892 (C–H in CH_2 and CH_3), 2228 (C≡N), 1650 (C=O), 1326 (C–N in 2^o aromatic amine), 1254 (C–O–C), 1128 (C–N in aliphatic amine), 835 (C_3N_3 s-triazine); ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.20 (s, 1H, –N*H* of aminobenzonitrile linkage), 9.90 (s, 1H, –N*H*–CH_2–CH_3 of ethy amine), 8.11–7.39 (m, 8H, Ar. –*H*), 6.50 (s, 1H, –C*H*–(C=O)–N of quinoline), 3.63–3.52 (m, 5H, –NH–CH_2–CH_3 of ethyl amine and –N–CH_3 quinoline); 1.24 (t, $J = 6.48$ Hz, 3H, of –N–CH_2–CH_3 of ethyl amine); ^{13}C NMR (100 MHz, DMSO-d_6): δ 173.78 (1C, –*C*–NH–CH_2–CH_3, of *s*-triazine to ethyl amine) 171.08 (1C, –*C*–O–C, *s*-triazine to 4-HMQ linkage), 167.49 (1C, –*C*–NH of aminobenzonitrile), 161.89 (1C, –*C*=O of quinoline), 155.01 (1C, –*C*–O–C, 4-HMQ linkage to *s*-triazine) 149.06–114.56 (12C, –Ar. C atoms), 103.90 (1C, –*C*N), 100.09 (1C, –*C*–(C=O)–N of quinoline), 46.89 (1C, –*C*H_2–CH_3 of ethyl amine), 31.09 (1C, –N–*C*H_3 of quinoline), 15.12 (1C, –N–CH_2–*C*H_3 of ethyl amine); Anal. Calcd. For $C_{22}H_{19}N_7O_2$: C, 63.91; H, 4.63; N, 23.72% Found: C, 63.95; H, 4.68; N, 27.65%; ESI–MS (*m*/*z*): 414.38 (M^+, 100%).

4-(4-(Diethylamino)-6-(1-methyl-2-oxo-1,2-dihydroquinolin-4-yloxy)-1,3,5-triazin-2-ylamino)benzonitrile (**6d**)

Yield: 73%; m.p. 84–87°C; IR (KBr cm^{-1}): 3278 (N–H), 2955, 2880 (C–H in CH_2 and CH_3), 2221 (C≡N), 1648 (C=O), 1315 (C–N in 2^o aromatic amine), 1250 (C–O–C), 1120 (C–N in aliphatic amine), 830 (C_3N_3 s-triazine); ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.19 (s, 1H, –N*H* of aminobenzonitrile linkage), 8.16–7.29 (m, 8H, Ar. –*H*), 6.41 (s, 1H, –C*H*–(C=O)–N of quinoline), 3.63 (s, 3H, –N–CH_3 of quinoline); 3.22 (q, $J = 6.40$ Hz, 4H, –N–(CH_2–CH_3)$_2$ of dimethyl amine), 1.12 (t, $J = 6.20$ Hz, 6H, –N–(CH_2–CH_3)$_2$ of dimethyl amine); ^{13}C NMR (100 MHz, DMSO-d_6): δ 173.99 (1C, –*C*–O–C, *s*-triazine to 4-HMQ linkage), 170.81 (1C, –*C*–NH of aminobenzonitrile), 169.88 (1C, –*C*–N(CH_2–CH_3)$_2$, of *s*-triazine to diethyl amine), 162.01 (1C, –*C*=O of quinoline), 154.19 (1C, –*C*–O–C, 4-HMQ linkage to *s*-triazine) 149.69–115.03 (12C, –Ar. C atoms), 105.65 (1C, –*C*N), 101.68 (1C, –*C*–(C=O)–N of quinoline), 43.16 (2C, –N(*C*H_2–CH_3)$_2$ of diethyl amine), 34.29 (1C, –N–*C*H_3 of quinoline), 13.94 (2C, –N(CH_2–*C*H_3)$_2$ of diethyl amine); Anal. Calcd. For $C_{24}H_{23}N_7O_2$: C, 65.29; H, 5.25; N, 22.21% Found: C, 65.20; H, 5.15; N, 22.15%; ESI–MS (*m*/*z*): 442.52 (M^+, 100%).

4-(4-(Isopropylamino)-6-(1-methyl-2-oxo-1,2-dihydroquinolin-4-yloxy)-1,3,5-triazin-2-ylamino)benzonitrile (**6e**)

Yield: 81%; m.p. 116–118°C; IR (KBr cm^{-1}): 3274 (N–H), 2968, 2888 (C–H in CH_2 and CH_3), 2222 (C≡N), 1652 (C=O), 1320 (C–N in 2^o aromatic amine), 1248 (C–O–C), 1117 (C–N in aliphatic amine), 836 (C_3N_3 s-triazine); ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.21 (s, 1H, –N*H* of aminobenzonitrile linkage), 9.97 (s, 1H, –N*H*–CH(CH_3)$_2$ of isopropyl amine), 8.13–7.31 (m, 8H, Ar. –*H*), 6.39 (s, 1H, –C*H*–(C=O)–N of quinoline), 4.14 (m, $J = 5.8$ Hz, 1H, -CH), 3.59 (s, 3H, –N–CH_3 of quinoline), 1.23 (dd, $J = 6.1, 1.7$ Hz, 6H, 2CH3); ^{13}C NMR (100 MHz, DMSO-d_6): δ 172.01 (1C, –*C*–NH–CH(CH_3)$_2$ of *s*-triazine to isopropyl amine), 170.99 (1C, –*C*–O–C, *s*-triazine to 4-HMQ linkage), 167.07 (1C, –*C*–NH of aminobenzonitrile), 161.01 (1C, –*C*=O of y quinoline), 153.39 (1C, –*C*–O–C, 4-HMQ linkage to *s*-triazine), 146.69–114.93 (12C, –Ar. C atoms), 103.86 (1C, –*C*N), 100.98 (1C, –*C*–(C=O)–N of quinoline), 42.45 (1C, –NH–*C*H(CH_3)$_2$ of *s*-triazine to isopropyl amine), 32.87 (1C, –N–*C*H_3 of quinoline), 20.99 (2C, –NH–CH(*C*H_3)$_2$ of *s*-triazine to isopropyl amine); Anal. Calcd. For $C_{23}H_{21}N_7O_2$: C, 64.63; H, 4.95; N, 22.94% Found: C, 64.72; H, 4.91; N, 23.01%; ESI–MS (*m*/*z*): 428.39 (M^+, 100%).

4-(4-(Cyclopropylamino)-6-(1-methyl-2-oxo-1,2-dihydroquinolin-4-yloxy)-1,3,5-triazin-2-ylamino)benzonitrile (**6f**)

Yield: 46%; m.p. 234–236°C; IR (KBr cm^{-1}): 3279 (N–H), 2953, 2880 (C–H in CH_2), 2225 (C≡N), 1650 (C=O), 1318 (C–N in 2^o aromatic amine), 1246 (C–O–C), 1110 (C–N in aliphatic amine), 838 (C_3N_3 s-triazine); ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.19 (s, 1H, –N*H* of aminobenzonitrile linkage), 9.91 (s, 1H, –N*H*–CH(CH_2)$_2$ of cyclopropyl amine), 8.11–7.36 (m, 8H, Ar. –*H*), 6.31 (s, 1H, –C*H*–(C=O)–N of quinoline), 3.51 (s, 3H, –N–CH_3 of quinoline), 3.08 (p, $J = 9.8$ Hz, 1H, –NH–C*H*(CH_2)$_2$ of cyclopropyl amine), 0.98–0.41 (m, 4H, –NH–CH(*C*H_2)$_2$ of cyclopropyl amine); ^{13}C NMR (100 MHz, DMSO-d_6): δ 174.23 (1C, –*C*–O–C, *s*-triazine to 4-HMQ linkage), 170.81 (1C, –*C*–NH–CH(CH_2)$_2$ of *s*-triazine to cyclopropyl amine), 166.88 (1C, –*C*–NH of aminobenzonitrile), 161.91 (1C, –*C*=O of quinoline), 151.26 (1C, –*C*–O–C, 4-HMQ linkage to *s*-triazine), 145.29–113.01 (12C, –Ar. C atoms), 104.15 (1C, –*C*N), 101.56 (1C, –*C*–(C=O)–N of quinoline), 32.16 (1C, C-25, –N–*C*H_3 of quinoline), 25.01 (1C, –NH–*C*H(CH_2)$_2$ of *s*-triazine to cyclopropyl amine), 13.35 (2C, –NH–CH(*C*H_2)$_2$ of *s*-triazine to cyclopropyl amine); Anal. Calcd. For $C_{23}H_{19}N_7O_2$: C, 64.93; H, 4.50; N, 23.05%

Found: C, 64.85; H, 4.53; N, 22.96%; ESI–MS (*m*/*z*): 426.43 (M^+, 100%).

4-(4-(Butylamino)-6-(1-methyl-2-oxo-1,2-dihydroquinolin-4-yloxy)-1,3,5-triazin-2-ylamino)benzonitrile (**6g**)

Yield: 51%; m.p. 156–158°C; IR (KBr cm^{-1}): 3276 (N–H), 2932, 2892 (C–H in CH_2 and CH_3), 2221 (C≡N), 1655 (C=O), 1322 (C–N in 2^o aromatic amine), 1249 (C–O–C), 1121 (C–N in aliphatic amine), 839 (C_3N_3 s-triazine); ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.21 (s, 1H, –N*H* of aminobenzonitrile linkage), 9.95 (s, 1H, –N*H*–$(CH_2)_3$–CH_3 of n-butyl amine), 8.15–7.31 (m, 8H, Ar. –*H*), 6.34 (s, 1H, –C*H*–(C=O)–N of quinoline), 3.61 (s, 3H, –N–CH_3 quinoline); 3.31 (m, 2H, –N–CH_2–$(CH_2)_2$–CH_3 of n-butyl amine), 1.50–1.34 (m, 4H, –N–CH_2–$(CH_2)_2$–CH_3 of n-butyl amine), 0.94 (t, $J = 12.4$ Hz, 3H, –N–$(CH_2)_3$–CH_3 of n-butyl amine); ^{13}C NMR (100 MHz, DMSO-d_6): δ 173.51 (1C, –*C*–NH–$(CH_2)_3$–CH3 of *s*-triazine to n-butyl amine), 170.12 (1C, –*C*–O–C, *s*-triazine to 4-HMQ linkage), 165.09 (1C, –*C*–NH of aminobenzonitrile), 162.16 (1C, –*C*=O of quinoline), 155.13 (1C, –*C*–O–C, 4-HMQ linkage to *s*-triazine), 145.09–115.16 (12C, –Ar. C atoms), 105.18 (1C, –*C*N), 101.36 (1C, –*C*–(C=O)–N of quinoline), 43.17 (1C, –NH–*C*H_2– (CH_2)–CH_3 of n-butyl amine), 32.87 (2C, –N–*C*H_3 of quinoline and –NH–CH_2–*C*H_2–CH_2–CH_3 of n-butyl amine), 21.87 (1C, –NH–$(CH_2)_2$–*C*H_2–CH_3 of n-butyl amine), 13.12 (1C, –NH–$(CH_2)_3$–*C*H_3 of n-butyl amine); Anal. Calcd. For $C_{24}H_{23}N_7O_2$: C, 65.29; H, 5.25; N, 22.21% Found: C, 65.35; H, 5.33; N, 22.14%; ESI–MS (*m*/*z*): 442.50 (M^+, 100%).

4-(4-(Cyclohexylamino)-6-(1-methyl-2-oxo-1,2-dihydroquinolin-4-yloxy)-1,3,5-triazin-2-ylamino)benzonitrile (**6h**)

Yield: 96%; m.p. 135–138°C; IR (KBr cm^{-1}): 3279 (N–H), 2929, 2854 (C–H in CH_2), 2224 (C≡N), 1646 (C=O), 1319 (C–N in 2^o aromatic amine), 1246 (C–O–C), 1116 (C–N in aliphatic amine), 839 (C_3N_3 s-triazine); ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.18 (s, 1H, –N*H* of aminobenzonitrile linkage), 9.92 (s, 1H, –N*H*–$CH(CH_2)_5$ of cyclohexyl amine), 8.18–7.31 (m, 8H, Ar. –*H*), 6.29 (s, 1H, –C*H*–(C=O)–N of quinoline), 3.71 (s, 1H, –NH–C*H*$(CH_2)_5$ of cyclohexyl amine), 3.61 (s, 3H, –N–CH_3 quinoline); 1.98–1.21 (m, 10H,–NH–CH(CH_2)$_5$ of cyclohexyl amine); ^{13}C NMR (100 MHz, DMSO-d_6): δ 173.12 (1C, –*C*–O–C, *s*-triazine to 4-HMQ linkage), 170.76 (1C, –*C*–NH–$CH(CH_2)_5$ of *s*-triazine to cyclohexyl amine), 165.09 (1C, –*C*–NH of aminobenzonitrile), 162.11 (1C, –*C*=O of quinoline), 150.07 (1C, –*C*–O–C, 4-HMQ linkage to *s*-triazine), 148.89–115.17 (12C, –Ar. C atoms), 105.10 (1C, –*C*N), 101.16 (1C, –*C*–(C=O)–N of quinoline), 52.12 (1C, –NH–*C*H$(CH_2)_5$ of cyclohexyl amine), 35.12–25.08 (6C, –N–*C*H_3 of quinoline and –NH–CH(*C*H_2)$_5$ of cyclohexyl amine); Anal. Calcd. For $C_{26}H_{25}N_7O_2$: C, 66.79; H, 5.39; N, 20.97% Found: C, 66.71; H, 5.30; N, 20.91%; ESI–MS (*m*/*z*): 468.50 (M^+, 100%).

4-(4-(2-Chloroethylamino)-6-(1-methyl-2-oxo-1,2-dihydroquinolin-4-yloxy)-1,3,5-triazin-2-ylamino)benzonitrile (**6i**)

Yield: 53%; m.p. 187°C; IR (KBr cm^{-1}): 3271 (N–H), 2942, 2913 (C–H in CH_2), 2221 (C≡N), 1653 (C=O), 1317 (C–N in 2^o aromatic amine), 1250 (C–O–C), 1110 (C–N in aliphatic amine), 842 (C_3N_3 s-triazine); ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.28 (s, 1H, –N*H* of aminobenzonitrile linkage), 9.92 (s, 1H,–N*H*–$(CH_2)_2$–Cl of 2-chloroethyl amine), 8.18–7.29 (m, 8H, Ar. –*H*), 6.20 (s, 1H, –C*H*–(C=O)–N of quinoline), 3.75 (t, $J = 4.7$ Hz, 2H, –NH–CH_2–C*H*$_2$–Cl of 2-chloroethyl amine), 3.61(s, 3H, –N–CH_3 quinoline), 3.52 (t, $J = 4.7$ Hz, 2H, –NH–C*H*$_2$–CH_2–Cl 2-chloroethyl amine); ^{13}C NMR (100 MHz, DMSO-d_6): δ 173.12 (1C, –*C*–NH–$(CH_2)_2$–Cl, of *s*-triazine to 2-chloroethyl amine) 170.86 (1C, –*C*–O–C, *s*-triazine to 4-HMQ linkage), 165.88 (1C, –*C*–NH of aminobenzonitrile), 163.10 (1C, –*C*=O of quinoline), 155.12 (1C, –*C*–O–C, 4-HMQ linkage to *s*-triazine) 149.25–114.45 (12C, –Ar. C atoms), 104.16 (1C, –*C*N), 100.97 (1C, –*C*–(C=O)–N of quinoline), 44.98 (2C, –NH–(*C*H_2)$_2$–Cl, of 2-chloroethyl amine) 32.21 (1C, –N–*C*H_3 of quinoline); Anal. Calcd. For $C_{22}H_{18}ClN_7O_2$: C, 59.00; H, 4.05; N, 21.89% Found: C, 58.91; H, 4.13; N, 21.98%; ESI–MS (*m*/*z*): 448.85 (M^+, 100%).

4-(4-(2-Hydroxyethylamino)-6-(1-methyl-2-oxo-1,2-dihydroquinolin-4-yloxy)-1,3,5-triazin-2-ylamino)benzonitrile (**6j**)

Yield: 49%; m.p. 198–201°C; IR (KBr cm^{-1}): 3460 (O–H in primary alcohol), 3273 (N–H), 2946, 2911 (C–H in CH_2), 2226 (C≡N), 1643 (C=O), 1346 (C–O–H in primary alcohol), 1305 (C–N in 2^o aromatic amine), 1261 (C–O–C), 1103 (C–N in aliphatic amine), 1031 (C–O in primary alcohol), 839 (C_3N_3 s-triazine); ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.19 (s, 1H, –N*H* of aminobenzonitrile linkage), 9.93 (s, 1H, –N*H*–$(CH_2)_2$–OH of ethanol amine), 8.19–7.33 (m, 8H, Ar. –*H*), 6.31 (s, 1H, –C*H*–(C=O)–N of quinoline), 4.68 (s, 1H, –NH–$(CH_2)_2$–O*H* of ethanol amine), 3.72 (t, $J = 4.4$ Hz, 2H, –NH–CH_2–C*H*$_2$–OH of ethanol amine), 3.63–3.48 (m, 5H, –N–CH_3 of quinoline and –NH–C*H*$_2$–CH_2–OH of ethanol amine); ^{13}C NMR (100 MHz, DMSO-d_6): δ 174.18 (1C, –*C*–NH–$(CH_2)_2$–OH, of *s*-triazine to ethanol amine), 172.91 (1C, –*C*–O–C,

s-triazine to 4-HMQ linkage), 168.09 (1C, –*C*–NH of aminobenzonitrile), 162.89 (1C, –*C*=O of quinoline), 154.21 (1C, –*C*–O–C, 4-HMQ linkage to *s*-triazine) 145.85–115.01 (12C, –Ar. C atoms), 103.78 (1C, –*C*N), 102.19 (1C, –*C*–(C=O)–N of quinoline), 65.65 (1C, –NH–CH_2–*C*H_2–OH of ethanol amine), 45.89 (1C, –NH–*C*H_2–CH_2–OH of ethanol amine) 31.68 (1C, –N–*C*H_3 of quinoline); Anal. Calcd. For $C_{22}H_{19}N_7O_3$: C, 61.53; H, 4.46; N, 22.83% Found: C, 61.60; H, 4.40; N, 22.74%; ESI–MS (*m*/*z*): 430.43 (M^+, 100%).

4-(4-(Benzylamino)-6-(1-methyl-2-oxo-1,2-dihydroquinolin-4-yloxy)-1,3,5-triazin-2-ylamino)benzonitrile (**6k**)

Yield: 96%; m.p. 154–155°C; IR (KBr cm^{-1}): 3278 (N–H), 2938, 2909 (C–H in CH_2), 2224 (C≡N), 1649 (C=O), 1321 (C–N in 2^o aromatic amine), 1248 (C–O–C), 1112 (C–N in aliphatic amine), 838 (C_3N_3 s-triazine); ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.22 (s, 1H, –N*H* of aminobenzonitrile linkage), 9.97 (s, 1H, –N*H*–CH_2–C_6H_5 of benzyl amine), 8.20–7.25 (m, 13H, Ar. –*H*), 6.15 (s, 1H, –C*H*–(C=O)–N of quinoline), 4.85 (s, 2H, –NH–CH_2–C_6H_5 of benzyl amine), 3.61(s, 3H, –N–CH_3 quinoline); ^{13}C NMR (100 MHz, DMSO-d_6): δ 175.12 (1C, –*C*–O–C, *s*-triazine to 4-HMQ linkage), 171.87 (1C, –*C*–NH–CH_2–C_6H_5 of *s*-triazine to cyclohexyl amine), 165.09 (1C, –*C*–NH of aminobenzonitrile), 163.19 (1C, –*C*=O of quinoline), 151.01 (1C, –*C*–O–C, 4-HMQ linkage to *s*-triazine), 147.81–114.69 (18C, –Ar. C atoms), 104.47 (1C, –*C*N), 103.14 (1C, –*C*–(C=O)–N of quinoline), 44.43 (1C, –NH–*C*H_2–C_6H_5 of cyclohexyl amine), 33.12 (1C, –N–*C*H_3 of quinoline); Anal. Calcd. For $C_{27}H_{21}N_7O_2$: C, 68.20; H, 4.45; N, 20.62% Found: C, 68.09; H, 4.51; N, 20.56%; ESI–MS (*m*/*z*): 476.47 (M^+, 100%).

4-(4-(1-Methyl-2-oxo-1,2-dihydroquinolin-4-yloxy)-6-(2-(thiophen-2-yl)ethylamino)-1,3,5-triazin-2-ylamino)benzonitrile (**6l**)

Yield: 95%; m.p. 138–141°C; IR (KBr cm^{-1}): 3274 (N–H), 2941, 2913 (C–H in CH_2), 2222 (C≡N), 1652 (C=O), 1319 (C–N in 2^o aromatic amine), 1270 (C–S in thiophine)1244 (C–O–C), 1119 (C–N in aliphatic amine), 833 (C_3N_3 s-triazine); ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.19 (s, 1H, –N*H* of aminobenzonitrile linkage), 9.92 (s, 1H, –N*H*–CH_2–CH_2–C_4H_3S of thiophen-2-ethyl amine), 8.18–6.98 (m, 11H, Ar. –*H*), 6.29 (s, 1H, –C*H*–(C=O)–N of quinoline), 3.63 (s, 3H, –N–CH_3 quinoline), 3.21 (d, $J = 18.8$ Hz, 4H, –NH–CH_2–CH_2–C_4H_3S); ^{13}C NMR (100 MHz, DMSO-d_6): δ 171.98 (1C, –*C*–NH–$(CH_2)_2$–C_4H_3S of *s*-triazine to thiophen-2-ethyl amine), 173.11 (1C, –*C*–O–C, *s*-triazine to 4-HMQ linkage), 167.56 (1C, –*C*–NH of aminobenzonitrile), 163.11 (1C, –*C*=O of quinoline), 152.76 (1C, –*C*–O–C, 4-HMQ linkage to *s*-triazine), 147.89–114.75 (16C, –Ar. C atoms), 104.68 (1C, –*C*N), 102.89 (1C, –*C*–(C=O)–N of quinoline), 41.24 (1C, –NH–*C*H_2–CH_2–C_4H_3S of thiophen-2-ethyl amine), 32.66 (2C, –N–*C*H_3 of quinoline and –NH–CH_2–*C*H_2–C_4H_3S of thiophen-2-ethyl amine); Anal. Calcd. For $C_{26}H_{21}N_7O_2S$: C, 63.02; H, 4.27; N, 19.79% Found: C, 63.11; H, 4.32; N, 19.72%; ESI–MS (*m*/*z*): 496.59 (M^+, 100%).

4-(4-(2-(5-Methoxy-1H-indol-3-yl)ethylamino)-6-(1-methyl-2-oxo-1,2-dihydroquinolin-4-yloxy)-1,3,5-triazin-2-ylamino)benzonitrile (**6m**)

Yield: 60%; m.p. 139–142°C; IR (KBr cm^{-1}): 3271 (N–H), 2945, 2909 (C–H in CH_2), 2821 (C–H in O–CH_3), 2225 (C≡N), 1648 (C=O), 1316 (C–N in 2^o aromatic amine), 1250 (C–O–C), 1120 (C–N in aliphatic amine), 838 (C_3N_3 s-triazine); ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.01 (s, 1H, –N*H* of 5-methoxy-1*H*-indole), 10.12 (1H, s, –N*H* of aminobenzonitrile linkage), 9.88 (s, 1H of –N*H* of 2-(5-methoxy-1*H*-indol-3-yl)ethylamino), 8.15–6.95 (m, 12H of Ar. –*H*), 6.33 (s, 1H, –C*H*–(C=O)–N of quinoline), 3.89 (s, 3H, –O–CH_3 of 2-(5-methoxy-1*H*-indol-3-yl)ethylamino), 3.60 (s, 3H, –N–CH_3 quinoline), 3.45 (t, $J = 7.8$ Hz, 2H, –NH–CH_2–CH_2– of 2-(5-methoxy-1*H*-indol-3-yl)ethylamino), 2.43 (t, $J = 7.8$ Hz, 2H, –NH–CH_2–CH_2– of 2-(5-methoxy-1*H*-indol-3-yl)ethylamino); ^{13}C NMR (100 MHz, DMSO-d_6): δ 174.02 (1C, –*C*–NH–CH_2–CH_2– of *s*-triazine to 2-(5-methoxy-1*H*-indol-3-yl)ethylamino), 172.43 (1C, –*C*–O–C, *s*-triazine to 4-HMQ linkage), 166.88 (1C, –*C*–NH of aminobenzonitrile), 162.56 (1C, –*C*=O of quinoline), 153.61 (1C, –*C*–O–C, 4-HMQ linkage to *s*-triazine), 146.12–107.83 (20C, –Ar. C atoms), 104.55 (1C, –*C*N), 101.26 (1C, –*C*–(C=O)–N of quinoline), 59.21 (1C, –O–*C*H_3 of 2-(5-methoxy-1*H*-indol-3-yl)ethylamino), 40.31 (1C, –NH–*C*H_2–CH_2– of 2-(5-methoxy-1*H*-indol-3-yl)ethylamino), 31.81 (1C, –N–*C*H_3 of quinoline), 23.44 (1C, –NH–CH_2–*C*H_2– of 2-(5-methoxy-1*H*-indol-3-yl)ethylamino); Anal. Calcd. For $C_{31}H_{26}N_8O_3$: C, 66.66; H, 4.69; N, 20.06% Found: C, 66.58; H, 4.64; N, 20.13%; ESI–MS (*m*/*z*): 559.60 (M^+, 100%).

2-(4-(4-Cyanophenylamino)-6-(1-methyl-2-oxo-1,2-dihydroquinolin-4-yloxy)-1,3,5-triazin-2-ylamino)acetic acid (**6n**)

Yield: 70%; m.p. 204–206°C; IR (KBr cm^{-1}): 3275 (N–H), 2880 (O–H in glycine), 2223 (C≡N), 1714 (C=O in glycine), 1648 (C=O), 1311 (C–N in 2^o aromatic amine), 1276 (C–O in glycine), 1248 (C–O–C), 1113 (C–N in aliphatic amine), 839 (C_3N_3 s-triazine); ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.52 (s, 1H, –NH–CH_2–(C=O)–O*H* of

glycine), 10.24 (s, 1H, –N*H* of aminobenzonitrile linkage), 9.92 (s, 1H, –NH–CH_2–(C=O)–OH of glycine), 8.28–7.15 (m, 8H, Ar. –*H*), 6.13 (s, 1H, –C*H*–(C=O)–N of quinoline), 3.87 (s, 2H, –NH–CH_2–(C=O)–OH of glycine), 3.60 (s, 3H, –N–CH_3 quinoline); ^{13}C NMR (100 MHz, DMSO-d_6): δ 175.43 (2C, –*C*–O–C, *s*-triazine to 4-HMQ linkage and –NH–CH_2–(*C*=*O*)–OH of glycine), 171.89 (1C, –*C*–NH–CH_2–(C=O)–OH of *s*-triazine to glycine), 167.26 (1C, –*C*–NH of aminobenzonitrile), 162.56 (1C, –*C*=O of quinoline), 151.69 (1C, –*C*–O–C, 4-HMQ linkage to *s*-triazine), 148.12–114.83 (12C, –Ar. C atoms), 105.26 (1C, –*C*N), 100.98 (1C, –*C*–(C=O)–N of quinoline), 48.39 (1C, –NH–*C*H_2–(C=O)–OH of glycine), 33.12 (1C, –N–*C*H_3 of quinoline); Anal. Calcd. For $C_{22}H_{17}N_7O_4$: C, 59.59; H, 3.86; N, 22.11% Found: C, 59.52; H, 3.91; N, 22.04%; ESI–MS (*m*/*z*): 444.38 (M^+, 100%).

Ethyl-2-(4-(4-cyanophenylamino)-6-(1-methyl-2-oxo-1,2-dihydroquinolin-4-yloxy)-1,3,5-triazin-2-ylamino)acetate (**6o**)

Yield: 78%; m.p. 240°C; IR (KBr cm^{-1}): 3278 (N–H), 2939, 2909 (C–H in CH_2 and CH_3), 2221 (C≡N), 1739 (C=O in glycineethyl ester), 1642 (C=O), 1309 (C–N in 2^o aromatic amine), 1243 (C–O–C), 1218 (C–O in glycineethyl ester), 1120 (C–N in aliphatic amine), 833 (C_3N_3 s-triazine); ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.14 (s, 1H, –N*H* of aminobenzonitrile linkage), 9.89 (s, 1H, –N*H*–CH_2–(C=O)–O–CH_2–CH_3 of glycine ethylester), 8.21–7.11 (m, 8H, Ar. –*H*), 6.09 (s, 1H, –C*H*–(C=O)–N of quinoline), 3.62 (s, 3H, –N–CH_3 quinoline), 3.31 (s, 2H, –NH–CH_2–(C=O)–O–CH_2–CH_3 of glycine ethylester), 2.23 (t, J = 8.3 Hz, 3H, –NH–CH_2–(C=O)–O–CH_2–CH_3 of glycine ethylester), 1.89 (q, J = 11.7 Hz, 2H, –NH–CH_2–(C=O)–O–CH_2–CH_3 of glycine ethylester); ^{13}C NMR (100 MHz, DMSO-d_6): δ 174.68 (1C, –*C*–O–C, *s*-triazine to 4-HMQ linkage and –NH–CH_2–(*C*=*O*)–OH of glycine), 170.99 (2C, –*C*–NH–*C*H_2–(C=O)–O–CH_2–CH_3 of *s*-triazine to glycine ethylester), 166.69 (1C, –*C*–NH of aminobenzonitrile), 161.10 (1C, –*C*=O of quinoline), 153.96 (1C, –*C*–O–C, 4-HMQ linkage to *s*-triazine), 146.86–115.03 (12C, –Ar. C atoms), 105.47 (1C, –*C*N), 101.56 (1C, –*C*–(C=O)–N of quinoline), 63.55 (1C, –NH–CH_2–(C=O)–O–*C*H_2–CH_3 of glycine ethylester), 46.96 (1C, –NH–*C*H_2–(C=O)–O–*C*H_2–CH_3 of glycine ethylester), 31.23 (1C, –N–*C*H_3 of quinoline), 12.76 (1C, –NH–CH_2–(C=O)–O–CH_2–*C*H_3 of glycine ethylester); Anal. Calcd. For $C_{24}H_{21}N_7O_4$: C, 61.14; H, 4.49; N, 20.80% Found: C, 61.22; H, 4.43; N, 20.93%; ESI–MS (*m*/*z*): 472.50 (M^+, 100%).

In vitro evaluation of antimicrobial activity

The synthesized *s*-triazinyl derivatives **6a**–**o** were examined for their 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 *Shigella Flexneria* MTCC 1457) and fungi (*Aspergillus niger* MTCC 282, and *Candida albicans* MTCC 183) species using paper disc diffusion technique (Gillespie, 1994). 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 dimethylsulfoxide to give solutions of 3.12–50 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 dimethylsulfoxide 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 dimethylsulfoxide without any sample was also used, and it did not produce any inhibition. Ciprofloxacin and ketoconazole (50 μg/disc) were used as control drugs for antibacterial and antifungal activities, respectively.

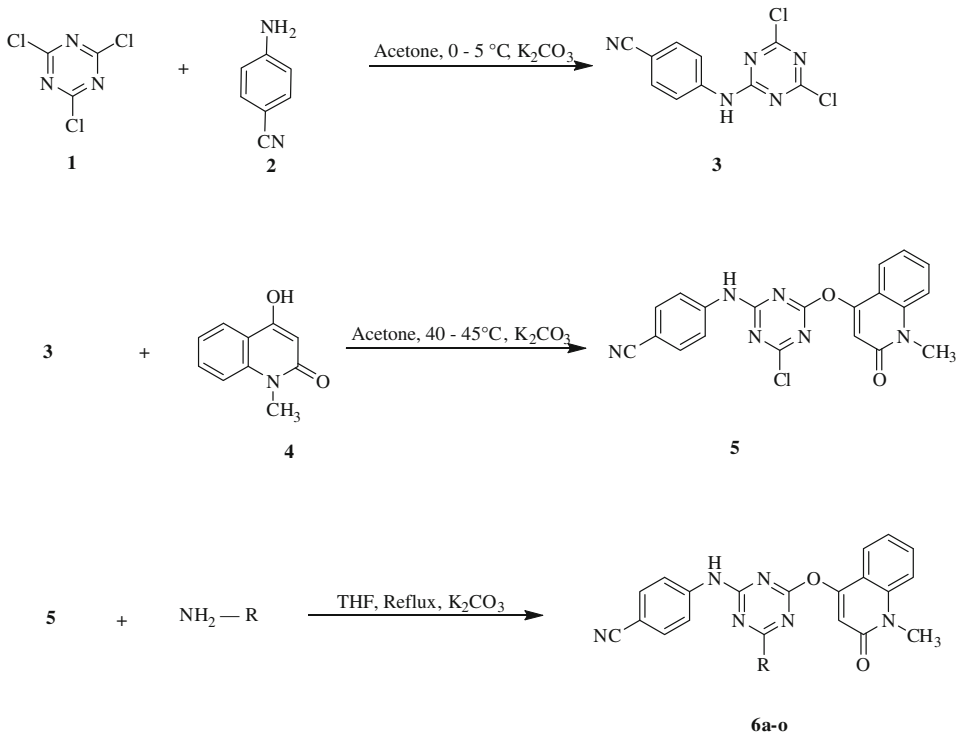
MIC of the compound was determined by agar streak dilution method (Hawkey and Lewis, 1994). A stock solution of the synthesized compound (50 μg/ml) in dimethylsulfoxide was prepared, and graded quantities of the test compounds were incorporated in a specified quantity of molten sterile agar, i.e., nutrient agar for evaluation of antibacterial activity, and sabouraud dextrose agar for 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 3.12–50 μg/ml in dimethylsulfoxide 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.

Results and discussion

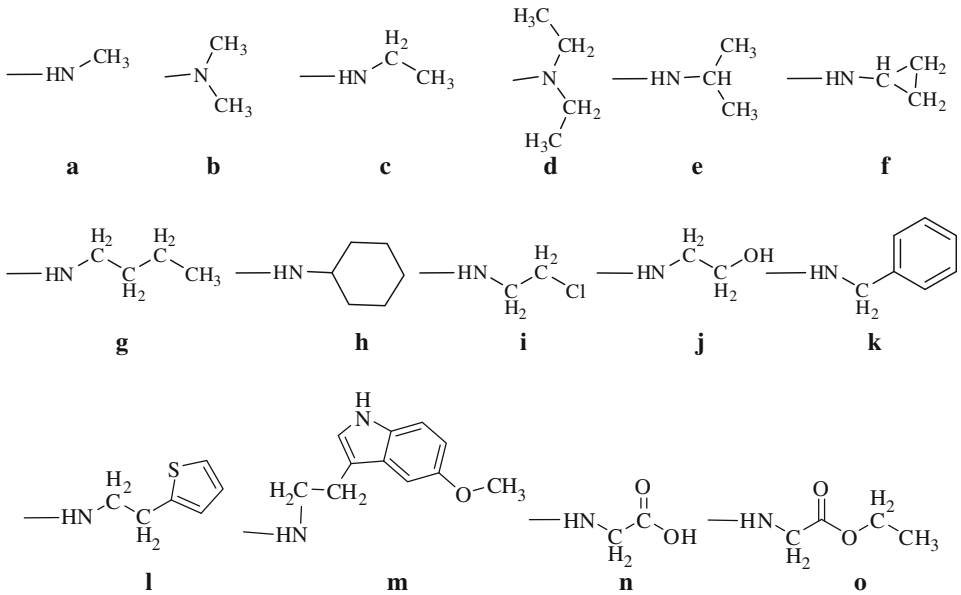
Chemistry

The designed target compounds were obtained as outlined in Scheme 1. The first step comprises formation of intermediate **3** in very good Yield: 90% by the nucleophilic displacement of one chlorine atom of *s*-triazine ring by 4-amino-benzonitrile. In FT-IR analysis, a C_3N_3 stretching

Scheme 1 Schematic diagram for the synthesis of final *s*-triazine derivatives

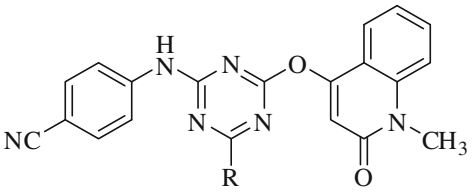


Where: –R =



in the *s*-triazine ring was observed at 833 cm^{-1}. In the IR spectrum of compound **1**, absorption band seen at 2228 cm^{-1} was assigned to C≡N group, and a strong band near 3277 cm^{-1} further confirmed the presence of a –NH group. The synthesis of disubstituted *s*-triazine intermediate **5** was achieved at Yield: 88% by the reaction between 4-(4,6-dichloro-1,3,5-triazin-2-ylammino)benzonitrile **3** and 4-hydroxy-*N*-methylquinolin-2(1*H*)-one **4** in the presence of potassium carbonate at 40–45°C, and the presence of C–O–C linkage in the proposed structures of compound **5** was indicated by the appearance of stretching bands at 1256 cm^{-1} in its IR spectra, while disappearance of the –OH peak at 3640 cm^{-1}, corresponding to the 4-hydroxy-*N*-methylquinolin-2(1*H*)-one, gave correction to the formation of the said intermediate. Subsequent coupling of the so-formed intermediate with the preferred substituted

Table 1 In-vitro antimicrobial activity



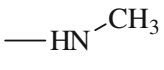
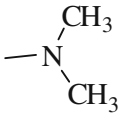
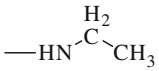
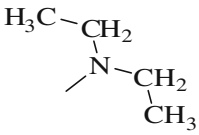
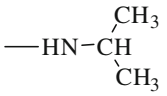
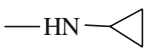
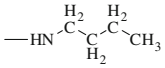
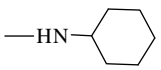
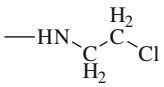
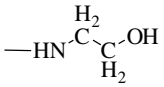
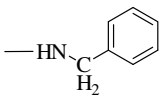
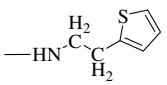
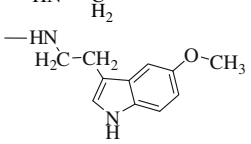
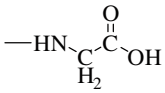
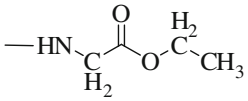
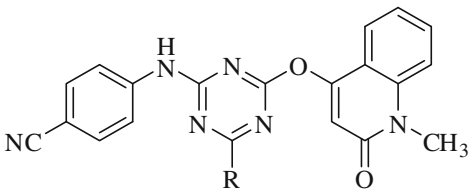
Entry	R	log *p*	Zone of inhibition [mm (MIC in μg/mL)]							
			S.a	B.c	E.c	P.a	K.p	S.t	P.v	S.f
6a		3.64	11 (50)	07 (50)	<5 (50)	14 (6.25)	(50)	<5 (50)	08 (50)	08 (50)
6b		4.43	12 (50)	14 (12.5)	10 (50)	08 (50)	(50)	07 (50)	09 (50)	<5 (50)
6c		3.98	10 (50)	<5 (50)	08 (50)	12 (12.5)	(50)	09 (50)	<5 (50)	07 (50)
6d		5.10	13 (25)	16 (12.5)	11 (50)	10 (50)	(50)	10 (50)	<5 (50)	10 (50)
6e		4.29	08 (50)	13 (25)	09 (50)	<5 (50)	(50)	06 (50)	08 (50)	08 (50)
6f		3.93	<5 (50)	11 (25)	07 (50)	06 (50)	11 (25)	<5 (50)	08 (50)	<5 (50)
6g		4.88	12 (50)	08 (50)	<5 (50)	11 (25)	(50)	06 (50)	<5 (50)	07 (50)
6h		5.19	14 (6.25)	10 (50)	08 (50)	<5 (50)	13 (12.5)	<5 (50)	07 (50)	09 (50)
6i		4.35	12 (25)	11 (50)	15 (25)	07 (50)	(50)	13 (25)	08 (50)	<5 (50)
6j		3.12	<5 (50)	<5 (50)	<5 (50)	09 (50)	(50)	09 (50)	07 (50)	07 (50)
6k		5.37	13 (12.5)	10 (50)	09 (50)	07 (50)	15 (12.5)	<5 (50)	<5 (50)	06 (50)
6l		5.46	15 (6.25)	13 (25)	10 (50)	10 (50)	10 (50)	12 (50)	07 (50)	14 (25)
6m		5.06	15 (6.25)	13 (25)	09 (50)	09 (50)	10 (50)	<5 (50)	06 (50)	12 (50)

Table 1 continued

Entry	R	log *p*	Zone of inhibition [mm (MIC in μg/mL)]							
			S.a	B.c	E.c	P.a	K.p	S.t	P.v	S.f
6n		2.90	<5 (50)	07 (50)	<5 (50)	08 (50)	(50)	08 (50)	13 (25)	09 (50)
6o		3.51	06 (50)	09 (50)	07 (50)	<5 (50)	(50)	08 (50)	15 (12.5)	11 (50)
Cip.			17 (1.0)	16 (1.0)	18 (1.0)	15 (1.0)	16 (1.0)	16 (1.0)	17 (1.0)	17 (≤3)
DMSO			–	–	–	–	–	–	–	–

Cip. Ciprofloxacin, S.a *Staphylococcus aureus*, B.c *Bacillus cereus*, E.c *Escherichia coli*, P.a *Pseudomonas aeruginosa*, K.p *Klebsiella pneumoniae*, S.t *Salmonella typhi*, P.v *Proteus vulgaris*, S.f *Shigella flexneria*

Table 2 In-vitro antifungal activity



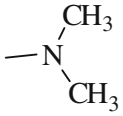
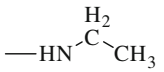
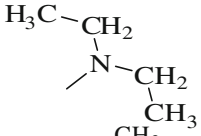
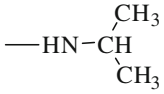
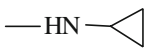
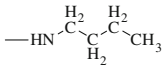
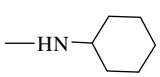
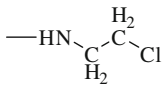
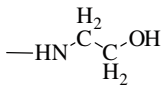
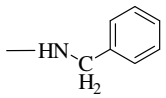
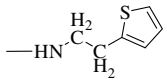
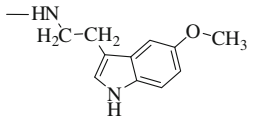
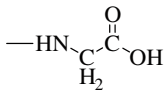
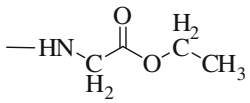
Entry	R	log *p*	Zone of inhibition [mm (MIC in μg/ml)]	
			A.n	C.a
6a		3.64	<5 (>50)	<5 (>50)
6b		4.43	<5 (>50)	<5 (>50)
6c		3.98	<5 (>50)	<5 (>50)
6d		5.10	09 (50)	09 (50)
6e		4.29	08 (50)	07 (50)
6f		3.93	10 (50)	08 (50)
6g		4.88	<5 (>50)	06 (50)
6h		5.19	09 (50)	10 (50)

Table 2 continued

Entry	R	log *p*	Zone of inhibition [mm (MIC in μg/ml)]	
			A.n	C.a
6i		4.35	08 (50)	09 (50)
6j		3.12	<5 (>50)	<5 (>50)
6k		5.37	<5 (>50)	<5 (>50)
6l		5.46	13 (25)	15 (25)
6m		5.06	12 (25)	15 (25)
6n		2.90	07 (50)	<5 (>50)
6o		3.51	09 (50)	<5 (>50)
Kit.			16 (≤3)	17 (1.0)
DMSO			–	–

Kit Ketoconazole, A.n *Aspergillus niger*, C.a *Candida albicans*

aliphatic amines under basic conditions in tetrahydrofuran solvent at 70–80°C formed the corresponding 1,3,5-triazines (**6a–o**). This reaction proceeded in good Yield: 46–96% and is general for different substituted aliphatic amines. The correctness of the synthesis of **6a–o** was confirmed on the basis of ^{1}H NMR, ^{13}C NMR, and Mass spectra of the synthesized compounds and the purity was ascertained by elemental analysis.

Biological activities

Biological assay summarized in Tables 1 and 2 revealed that all the newly synthesized compounds indicated good-to-excellent bioactivities against the different microorganisms studied. Final s-triazine analogues **6l** and **6m** bearing heterocyclic functionality to the coupling agent exhibited excellent activity against Gram-pistive *S. aureus* at 6.25 μg/mL of MIC and 15 mm of inhibition zone. In addition, compound **6h** with the substitution of cyclohexyl amine as coupling agent indicated similar MIC value as **6l** and **6m** by deviating slightly in the inhibition zone (14 mm), while compound **6k** involving phenyl ring appeared with half-fold MIC value (12.5 μg/mL) when compared to **6l** and **6m**. It is worthy to note that the said compound 6 l displayed potent inhibitory action against Gram-negative *S. flexneria* at 25 μg/mL of MIC and 15 mm of inhibitory zone. Furthermore, compounds **6d** and **6b** with the substitution of alkyl chain to the nitrogen atom of the coupling agent displayed significant growth inhibition of Gram-positive *B. cereus* at 12.5 μg/mL of MIC, 16 and 14 mm of growth inhibitory diameter's, respectively. Final analogue with halogen-substituted coupling amine (**6i**) displayed good activity against Gram-negative *E. coli* and *S. typhi* at 25 μg/mL of MIC, 15 and 13 mm of inhibition zones, respectively; however, all the remaining scaffolds displayed higher MICs against both the bacteria. Final scaffolds (**6a**, **6c**, and **6g**) with the alkyl amine chain as a coupling agent displayed activity in the decreasing order of the chain length in terms of MIC as 6.25 μg/mL, 12.5 μg/mL, and 25 μg/mL and inhibition zones of 14, 12, and 11 mm, respectively, against *P. aeruginosa*. Inhibition of Gram-negative bacteria *K. pneumoniae* was promisingly

shown by final *s*-triazine derivatives **6k** (MIC: 12.5 μg/mL; inhibition zone: 15 mm) with aromatic ring, **6h** (MIC: 12.5 μg/mL; inhibition zone: 13 mm) with cyclohexyl ring followed by **6f** (MIC: 25 μg/mL; inhibition zone: 11 mm) with cyclopropyl ring. Therefore, it can be stated that compound with more aromatic ring substitution enhances the biological activity against *K. pneumoniae*. Final derivatives with glycine coupler (**6n**) as well as its ethyl-ester form (**6o**) substitution exhibited diminished activities against *P. vulgaris* at 25 μg/mL and 12.5 μg/mL of MIC, and 13 and 15 mm of zones of inhibition; however, it can be said that conversion of carboxylic acid to its ester form may increase the activity.

The bioassay results obtained against the mentioned fungal strains revealed that two compounds, **5l** and **5m**, with heteroatom ring substitution displayed activities against both the fungi at 25 μg/mL of MIC and 12–15 mm of inhibition zone, while all the remaining derivatives were devoid of antifungal activities.

Conclusions

This study focused on the development of new *s*-triazines with broad therapeutic windows. Out of 15 compounds screened, majority of the compounds (14 compounds) exhibited promising in vitro antibacterial and antifungal inhibitory effects. In general, the compounds showed improved antibacterial activities when compared to their antifungal activities. From the results, it can be stated that the activities of the final scaffolds vary with the variation of the structural features of the coupling agents in the form of aliphatic amines. Furthermore, from the lipophilicity point of view, it can be concluded that compounds with greater lipophilicity (greater log *p* value) displayed higher activities in terms of lower MICs and higher inhibition zones due to their higher lipophilic nature. It clearly demonstrates the importance of lipophilicity for the antimicrobial activities of the resultant scaffolds. However, an opposite trend of this relationship of lipophilicity against MIC figures was observed in case of active compounds against *P. aeruginosa*. Finally, it can be said that *s*-triazine system with 4-amino-benzonitrile and 4-hydroxy-*N*-methylquinolin-2(1*H*)-one followed by the substitution of aliphatic amines that appeared with scaffolds containing significant activity profiles serves as a key role for further study on drug discovery.

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