Acta Poloniae Pharmaceutica – Drug Research, Vol. 69 No. 3 pp. 423–432, 2012 ISSN 0001-6837
Polish Pharmaceutical Society

MICROWAVE ASSISTED SYNTHESIS AND DETERMINATION OF *IN-VITRO* ANTIMICROBIAL EFFICACY OF WELL CHARACTERIZED S-TRIAZINYL PIPERAZINES AND PIPERIDINES

RAHUL V. PATEL[1]*, PREMLATA KUMARI[1] and KISHOR H. CHIKHALIA[2]

[1] Applied Chemistry Department, S.V. National Institute of Technology, Surat 395 007, India
[2] Department of Chemistry, School of Science, Gujarat University, Ahmedabad 380025, India

Abstract: An easy and convenient microwave-assisted synthesis of a library of s-triazinyl piperazines and piperidines, which, in addition to 4-aminobenzonitrile contain 8-hydroxyquinoline is described. The newly synthesized analogues were then subjected to determine their efficacy against some human pathogenic bacterial and fungal strains as 3 Gram negative bacteria (*K. pneumoniae*, *S. typhi*, *P. vulgaris*), 1 Gram positive bacteria (*B. cereus*) and 2 fungal species (*A. clavatus*, *A. fumigatus*) with an intent to develop novel class of antimicrobial agents. Microwave irradiation method was adopted for the final nucleophilic reactions, facilitates the condensation of piperazine and piperidine substituents to the s-triazine core. The results of bioassay showed that some of the newly synthesized s-triazines emerged as lead molecules with excellent MIC (mg/mL) values against the full array of bacterial and fungal pathogens comparable to the commercial antibiotics. The structure of final scaffolds has been affirmed on the basis of IR, ^{1}H NMR, ^{13}C NMR, ^{19}F NMR and elemental analyses.

Keywords: 2,4,6-trichloro-1,3,5-triazine, 8-hydroxyquinoline, 4-aminobenzonitrile, piperazines and piperidines, antimicrobial activity

Rapid development of bacterial resistance to most of the known antibiotics is a serious health problem worldwide, which leads to an increase of incidence of infection caused by the human pathogenic bacteria and fungi (1–4). The key factor responsible for such mutations in microbial genomes is the incorrect use of antibiotics, which lead to the development of resistant genotypes (5–7). Furthermore, an increased numbers of immunocompromised patients attributable to the ongoing HIV epidemic can further increase the burden of antimicrobial resistance in human civilization by facilitating the spread of resistant pathogens (8). Significant impact of the affliction of infectious disease in developing countries due to multidrug resistant posed by bacteria has driven us to examine the newly synthesized derivatives against the representative panel of bacterial and fungal strains in order to reduce drug resistance to the pathogenic strains and in order to maintain a pool of new bioactive candidates at all times.

The advent of 1,3,5-triazines, associated with diverse biological activities such as antimicrobial (9, 10), antiprotozoal (11), anticancer (12), antimalarial (13) and antiviral (14) activity accelerated the rate of progress of 1,3,5-triazinyl derivatives. 2,4,6-Trichloro-1,3,5-triazine is an inexpensive, commercially available reagent and the different reactivities of the substituent chlorine atoms, which are controlled by temperature, makes its use more attractive. In a view of its adaptable chemistry, sequential introduction of various piperazine and piperidine substituents into the 1,3,5-triazine ring has been done. Piperazines and piperidins occupied a unique place in the realm of pharmacological activities (15–17). For instance, linezolid, eperezolid 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 the pharmaceuticals such as paroxetine, risperidone, methylphenidate, raloxifene, minoxidil and thioridazine. Besides, considerable evidence has been accumulated in the past few years concerning the efficiency of 8-hydroxyquinoline with antimicrobial (18–20), antimalarial (21, 22), antitubercular (23, 24), anticancer (25, 26), antileishmanial (27), anticalculus and antiplaque (28) activity. Hence in

* Corresponding author: e-mail: rahul.svnit11@gmail.com

the present paper, an efficient and green approach was developed to prepare novel s-triazinyl piperazines and piperidines bearing 4-aminobenzonitrile as well as 8-hydroxyquinoline entities having potent clinical importance (Scheme 1). For this purpose, 2,4,6-trichloro-1,3,5-triazine was reacted with 4-aminobenzonitrile and the resulting compound was then condensed with 8-hydroxyquinoline followed by coupling reactions with various piperazine and piperidine derivatives with the aim of obtaining more potent pharmacologically active compounds (**5a–j**). It has been demonstrated that the adoption of microwave irradiation method in chemical reactions has taken an incontestable place as it can dramatically increase the product purity and yields, reduce the reaction time and estimate a precise control of the reaction conditions as well as convenient operation. Conventional heating method can be altered using microwave radiation technique as it utilizes the ability of liquids or solids to transform electromagnetic energy into heat furnishing reduced reaction time.

EXPERIMENTAL

2,4,6-Trichloro-1,3,5-triazine and 8-hydroxyquinoline were purchased from Sigma Aldrich. Acetone, tetrahydrofuran and 1,4-dioxane were used of HPLC grade and were purchased from Labort Fine Chem, India. The TLC plates (silica gel 60 F254 grade, Germany) were obtained from Merck, India. Substituted piperazine and piperidine derivatives were obtained from Catapharma, Enzal Chemicals Pvt. Ltd., Dr. Prem's molecules Pvt. Ltd., Ami organics Pvt. Ltd., Modepro (India) Pvt. Ltd., Siddharth Interchem Pvt. Ltd. and Mahrshee Laboratories, India.

Microwave assisted reactions were carried out by using rotative solid phase microwave reactor (RotoSYNTH, Milestone GmbH, 50–60 Hz). The rotation of the rotor, irradiation time and power were monitored with the ''Easy Control-640'' software package. The melting points of the products 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. To monitor the reactions, as well as, to establish the identity and purity of reactants and products, thin layer chromatography was performed on microscopic glass slides (2 × 7.5 cm) coated with silica gel-G, using appropriate mobile phase system and spots were visualized under UV radiation. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian 400 MHz model spectrometer using DMSO as a solvent and TMS as an internal standard with ^{1}H resonanse frequency of 400 MHz and ^{13}C resonance frequency of 100 MHz. ^{19}F NMR spectra were obtained on the same spectrometer using $CDCl_3$ as a solvent and $CFCl_3$ as an external standard, positive for downfield shift with ^{19}F resonant frequency of 400 MHz. The ^{1}H NMR, ^{13}C NMR and ^{19}F NMR chemical shifts were reported as parts per million (ppm) downfield from TMS (Me_4Si) and $CFCl_3$. The splitting patterns are designated as follows; s, singlet; d, doublet; m, multiplet. Elemental analyses (C, H, N) were done on a CHN rapid analyzer. All the new compounds gave C, H and N analysis within 0.05% of the theoretical values, i.e., in acceptable range.

4-[(4, 6-Dichloro-1, 3, 5-triazin-2-yl) amino]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 benzonitrile (6.41 g, 0.054 mole) was added dropwise at 0–5°C. The resulting reaction mixture was stirred at this temperature for 2 h, then triethylamine (5.48 g, 0.054 mol) was added in the reaction mixture and stirring was continued for another 4 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°C (dec.). FT-IR (KBr): 2223 cm^{-1} (CN).

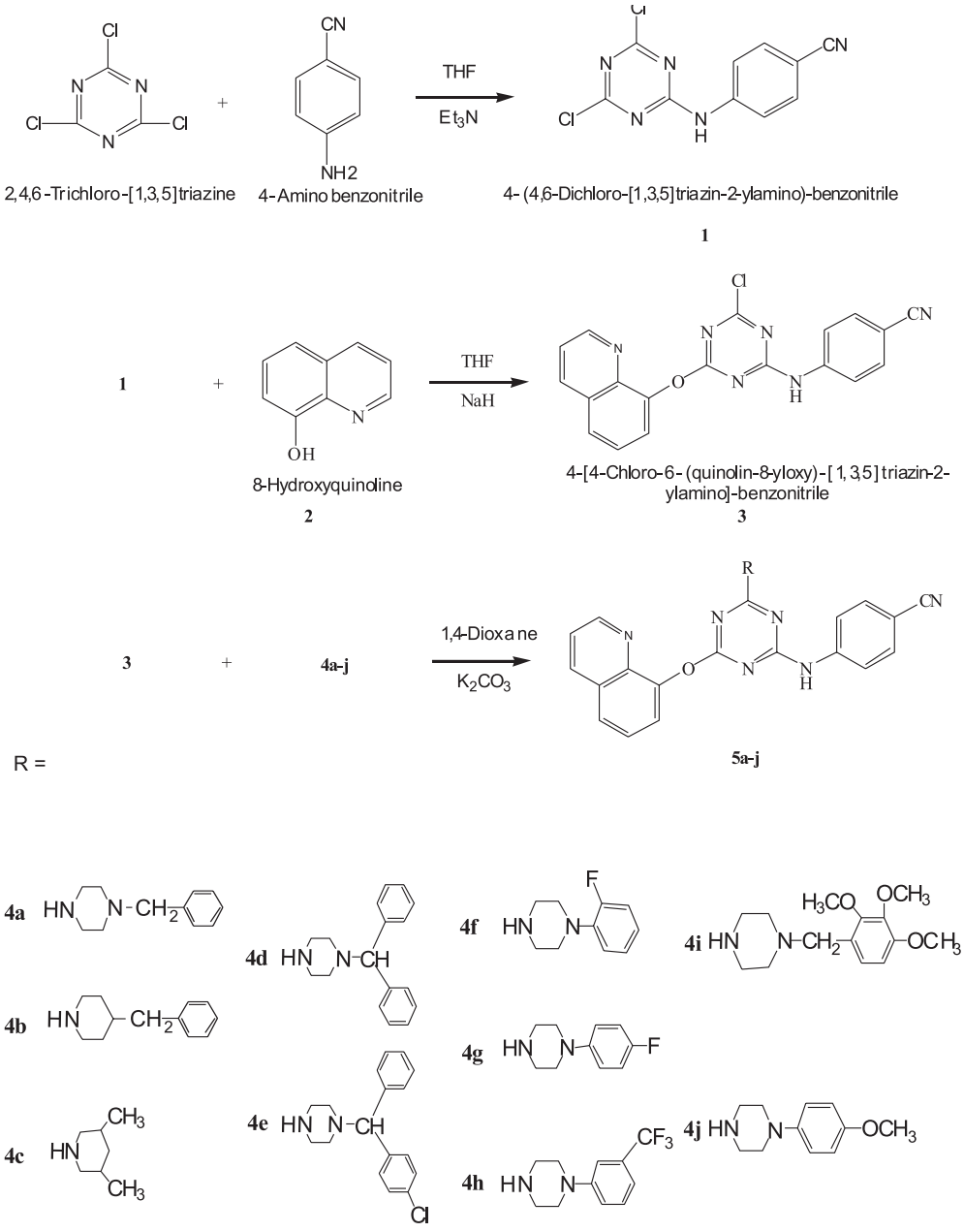
4-[4-Chloro-6-(quinolin-8-yloxy)-1,3,5-triazin-2-ylamino]-benzonitrile (3)

To a stirred solution of 8-hydroxyquinoline (8 g, 0.055 mol) in anhydrous THF (150 mL) 60% NaH (1.32 g, 0.055 mole) was added at room temperature for 2 h and **1** (14.67 g, 0.055 mole) was added into the reaction mixture. Stirring was contunied for another 5 h at 45°C. After the completition of the reaction, the reaction mixture was treated with crushed ice, filtered and dried to afford **3** (29), m.p. 285.3°C (dec.). FT-IR (KBr): 2223 cm^{-1} (CN), 1255 cm^{-1} (C-O-C).

General procedure for preparation of final compounds (5a–j)

Conventional method

To a solution of **3** (0.01 mol) in 1,4-dioxane (20 mL), appropriate different substituted piperazine and piperidine derivatives were added and the reaction mixture was refluxed for 10 to 12 h. Potassium carbonate was used for neutralization of the reaction



Scheme 1. Synthesis of final s-triazinyl piperazine and piperidine derivatives where **4a-j** (**R**) = various substituted piperazine and piperidine derivatives used as coupling agents

mixture. After the completition of the reaction, it was treated with crushed ice and neutralized by dilute HCl. The precipitates thus obtained were filtered, dried and recrystallized from THF to afford desired compounds **5a–j.**

Microwave method

In order to testify whether microwave irradiation speeds up the final nucleophilic substitution reactions, the same reactions were carried out in 1,4-dioxane solvent as using conventional heating method. In fact, the reaction time was dramatically reduced for each substitution from 10–12 h (conventional heating method) to 3–5 min using the microwave irradiation technique. Microwave assisted reactions were conducted in septum-sealed reaction vessels in rotative solid phase microwave reactor. The subsequent condensation of compound **3** with various

substituted piperazines and piperidines was carried out under microwave irradiation using 1,4-dioxane as a solvent and the reaction progress was monitored after every 1 min of irradiation by TLC system. A mixture of compound **3** in 1 equivalent of K_2CO_3 and each piperazine and piperidine derivative was exposed to microwave irradiation at 400 W power and 80°C temperature. After the completion of the reaction, the solvent was removed by using vacuum solvent recovery module, the resulting precipitates were treated with crushed ice, neutralized by dil. HCl, filtered and dried to afford final scaffolds. The optimization of reaction conditions along with reaction time and yield is described in Table 1 for each final nucleophilic reaction. Interestingly, remarkable rate acceleration was observed in the final coupling reactions.

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

Yield: 75%; m.p. 277°C (dec.); IR (KBr, cm^{-1}): 3100–3300 (-NH), 2225 (CN), 1475 (-CH_2), 1255 (C-O-C), 810 (s-triazine C-N str.); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm) 8.86 (s, 1H, -NH), 7.60–7.64 (m, 3H, quinoline), 7.22–7.57 (m, 12H, Ar-H), 3.83 (br s, 8H, piperazine), 2.40 (s, 2H, -CH_2); ^{13}C NMR (400 MHz, DMSO-d_6, δ, ppm): 169.33 (C-3, s-triazine, C-N at piperazine linkage), 168.88 (C-1, s-triazine, C-O-C at quinoline linkage), 162.37 (C-5, s-triazine, C-NH at benzonitrile moiety), 146.46–102.01 (22C, aromatic ring carbons), 64.44 (C-31, N-CH_2), 50.77, 45.79 (4C, piperazine ring carbons); Analysis: calcd. for $C_{30}H_{26}N_8O$: C, 70.02; H, 5.09; N, 21.78%; found: C, 70.03; H, 5.07; N, 21.78%.

4-[4-(4-Benzylpiperidin-1-yl)-6-(quinolin-8-yloxy)-1,3,5-triazin-2-ylamino]-benzonitrile (5b)

Yield: 81%; m.p. 267°C (dec.); IR (KBr, cm^{-1}): 3100–3300 (-NH), 2223 (CN), 1475 (-CH_2), 1257 (C-O-C), 816 (s-triazine C-N str.); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 8.83 (s, 1H, -NH), 7.56–7.63 (m, 3H, quinoline), 7.26–7.39 (m, 9H, Ar-H), 3.83 (t, 4H, piperidine), 3.66 (t, 4H, piperidine), 2.41 (s, 2H, -CH_2), 1.69 (t, 1H, -CH, piperidine); ^{13}C NMR (400 MHz, DMSO-d_6, δ, ppm): 169.24 (C-3, s-triazine, C-N at piperazine linkage), 168.55 (C-1, s-triazine, C-O-C at quinoline linkage), 161.93 (C-5, s-triazine, C-NH at benzonitrile moiety), 146.36–101.41 (20C, quinoline and benzonitrile ring carbons), 46.07, 43.08, 36.49, 30.77 (6C, piperazine ring carbons and –CH_2); Analysis: calcd. for $C_{29}H_{24}N_8O$: C, 69.58; H, 4.83; N, 22.39%; found: C, 69.58; H, 4.82; N, 22.37%.

4-[4-(3,5-Dimethylpiperidin-1-yl)-6-(quinolin-8-yloxy)-1,3,5-triazin-2-ylamino]-benzonitrile (5c)

Yield: 77%; m.p. 292°C (dec.); IR (KBr, cm^{-1}): 3100–3300 (-NH), 2221 (CN), 1256 (C-O-C), 808 (s-triazine C-N str.); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 8.87 (s, 1H, -NH), 7.52–7.57 (m, 3H, quinoline), 7.17–7.39 (m, 7H, Ar-H), 3.70–3.77 (m, 4H, piperidine), 2.49 (br s, 2H, -CH_2, piperidine), 1.94 (q, 2H, piperidine), 1.42 (d, 6H, 2-CH_3); ^{13}C NMR (400 MHz, DMSO-d_6, δ, ppm): 169.69 (C-3, s-triazine, C-N at piperazine linkage), 168.67 (C-1, s-triazine, C-O-C at quinoline linkage), 162.22 (C-5, s-triazine, C-NH at benzonitrile moiety), 146.35–102.16 (16C, quinoline and benzonitrile ring carbons), 52.46, 40.70, 29.19 (5C, C-26 to C-30, piperidine), 19.18 (2C, 2CH_3); Analysis: calcd. for $C_{26}H_{25}N_7O$: C, 69.16; H, 5.58; N, 21.71%; found: C, 69.14; H, 5.58; N, 21.69%.

4-[4-(4-Benzhydrylpiperazin-1-yl)-6-(quinolin-8-yloxy)-1,3,5-triazin-2-ylamino]-benzonitrile (5d)

Yield: 89%; m.p. 250°C (dec.); IR (KBr, cm^{-1}): 3100–3300 (-NH), 2225 (CN), 1255 (C-O-C), 813 (s-triazine C-N str.); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 8.85 (s, 1H, -NH), 7.51–7.55 (m, 3H, quinoline), 7.25–7.49 (m, 16H, Ar-H), 3.75 (br s, 8H, piperazine), 2.40 (s, 1H, -CH), 2.23 (s, 1H, -CH); ^{13}C NMR (400 MHz, DMSO-d_6, δ, ppm): 169.29 (C-3, s-triazine, C-N at piperazine linkage), 168.77 (C-1, s-triazine, C-O-C at quinoline linkage), 161.98 (C-5, s-triazine, C-NH at benzonitrile moiety), 147.37–102.00 (28C, aromatic ring carbons), 77.17 (C-31, -CH), 50.54, 46.99 (4C, piperazine ring carbons); Analysis: calcd. for $C_{36}H_{30}N_8O$: C, 73.20; H, 5.12; N, 18.97%; found: C, 73.22; H, 5.13; N, 18.97%.

4-[4-{4-[(4-Chlorophenyl)-phenylmethyl]-piperazin-1-yl}-6-(quinolin-8-yloxy)-1,3,5-triazin-2-ylamino]-benzonitrile (5e)

Yield: 81%; m.p. 279°C (dec.); IR (KBr, cm^{-1}): 3100–3300 (-NH), 2223 (CN), 1256 (C-O-C), 809 (s-triazine C-N str.); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 8.82 (s, 1H, -NH), 7.62–7.68 (m, 3H, quinoline), 7.11–7.47 (m, 16H, Ar-H), 3.84 (br s, 8H, piperazine), 2.23 (s, 1H, -CH); ^{13}C NMR (400 MHz, DMSO-d_6, δ, ppm): 169.22 (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), 146.46–101.43 (28C, aromatic ring carbons), 76.76 (C-31, -CH), 50.24, 47.93 (4C, piperazine ring carbons); Analysis: calcd. for $C_{36}H_{29}ClN_8O$: C, 69.17; H, 4.68; N, 17.93%; found: C, 69.18; H, 4.66; N, 17.92%.

4-{4-[4-(2-Fluorophenyl)-piperazin-1-yl]-6-(quinolin-8-yloxy)-1,3,5-triazin-2-ylamino]-benzonitrile (5f)

Yield: 72%; m.p. 279°C (dec.); IR (KBr, cm^{-1}): 3100–3300 (-NH), 2224 (CN), 1256 (C-O-C), 814 (s-triazine C-N str.); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 8.87 (s, 1H, -NH), 7.61–7.70 (m, 3H, quinoline); 7.17–7.44 (m, 11H, Ar-H), 3.83 (br s, 8H, piperazine); ^{13}C NMR (400 MHz, DMSO-d_6, δ, ppm): 169.27 (C-3, s-triazine, C-N at piperazine linkage), 168.59 (C-1, s-triazine, C-O-C at quinoline linkage), 162.17 (C-5, s-triazine, C-NH at benzonitrile moiety), 158.59 (C-36, C-F), 146.78–101.77 (21C, quinoline and benzonitrile ring carbons), 48.88, 47.57 (4C, piperazine ring carbons); ^{19}F NMR (400 MHz, $CDCl_3$, δ, ppm): –120.17 (1F, s, 2-F-Ar); Analysis: calcd. for $C_{29}H_{23}FN_8O$: C, 67.17; H, 4.47; N, 21.61%; found: C, 67.15; H, 4.49; N, 21.60%.

4-{4-[4-[(4-Fluorophenyl)-piperazin-1-yl]-6-(quinolin-8-yloxy)]-1,3,5-triazin-2-ylamino}-benzonitrile (5g)

Yield: 85%; m.p. 288°C (dec.); IR (KBr, cm^{-1}): 3100–3300 (-NH), 2223 (CN), 1255 (C-O-C), 810 (s-triazine C-N str.); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 8.86 (s, 1H, -NH), 7.54–7.61 (m, 3H, quinoline), 7.13–7.39 (m, 11H, Ar-H), 3.80 (br s, 8H, piperazine); ^{13}C NMR (400 MHz, DMSO-d_6, δ, ppm): 169.23 (C-3, s-triazine, C-N at piperazine linkage), 168.59 (C-1, s-triazine, C-O-C at quinoline linkage), 162.22 (C-5, s-triazine, C-NH at benzonitrile moiety), 157.99 (C-34, C-F), 145.88–102.22 (21C, quinoline and benzonitrile ring carbons), 49.49, 47.73 (4C, piperazine ring carbons); ^{19}F NMR (400 MHz, $CDCl_3$, δ, ppm): –117.34 (1F, s, 4-F-Ar); Analysis: calcd. for $C_{29}H_{23}FN_8O$: C, 67.17; H, 4.47; N, 21.61%; found: C, 67.16; H, 4.46; N, 21.63%.

4-{4-(Quinolin-8-yloxy)-6-[4-(3-trifluoromethylphenyl)-piperazin-1-yl]-1,3,5-triazin-2-ylamino}-benzonitrile (5h).

Yield: 80%; m.p. > 300°C (dec.); IR (KBr, cm^{-1}): 3100–3300 (-NH), 2222 (CN), 1255 (C-O-C), 811 (s-triazine C-N str.); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 8.79 (s, 1H, -NH), 7.62–7.66 (m, 3H, quinoline), 7.09–7.42 (m, 11H, Ar-H), 3.83 (br s, 8H, piperazine); ^{13}C NMR (400 MHz, DMSO-d_6, δ, ppm): 169.13 (C-3, s-triazine, C-N at piperazine linkage), 168.46 (C-1, s-triazine, C-O-C at quinoline linkage), 162.10 (C-5, s-triazine, C-NH at benzonitrile moiety), 146.33–102.02 (23C, quinoline, benzonitrile ring and trifluorophenyl ring carbons), 49.50, 48.08 (4C, piperazine ring carbons); ^{19}F NMR (400 MHz, $CDCl_3$, δ, ppm): –64.02 (6F, s, 2-CF_3); Analysis: calcd. for $C_{30}H_{23}F_3N_8O$: C, 63.38; H, 4.08; N, 19.71%; found: C, 63.39; H, 4.06; N, 19.70.

4-{4-[Quinolin-8-yloxy]-6-[4-(2,3,4-trimethoxybenzyl)-piperazin-1-yl]-1,3,5-triazin-2-ylamino}-benzonitrile (5i)

Yield: 79%; m.p. 289°C (dec.); IR (KBr, cm^{-1}): 3100–3300 (-NH), 2223 (CN), 1479 (-CH_2), 1255 (C-O-C), 811 (s-triazine C-N str.); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 8.82 (s, 1H, -NH), 7.52–7.56 (m, 3H, quinoline), 7.11–7.47 (m, 11H, Ar-H), 3.84 (t, 8H, piperazine), 2.56 (s, 9H, -OCH_3); ^{13}C NMR (400 MHz, DMSO-d_6, δ, ppm): 169.33 (C-3, s-triazine, C-N at piperazine linkage), 168.58 (C-1, s-triazine, C-O-C at quinoline linkage), 163.33 (C-5, s-triazine, C-NH at benzonitrile moiety), 156.68–101.51 (22C, aromatic ring carbons), 60.59, 60.31, 57.17 (4C, 3OCH_3 and -CH_2), 50.52, 46.48 (4C, piperazine ring carbons); Analysis: calcd. for $C_{33}H_{32}N_8O_4$: C, 65.55; H, 5.33; N, 18.53%; found: C, 65.57; H, 5.33; N, 18.53%.

4-{4-[4-(4-Methoxyphenyl)-piperazin-1-yl]-6-(quinolin-8-yloxy)-1,3,5-triazin-2-ylamino}-benzonitrile (5j)

Yield: 90%; m.p. 247°C (dec.); IR (KBr, cm^{-1}): 3100–3300 (-NH), 2222 (CN), 1255 (C-O-C), 813 (s-triazine C-N str.); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 8.87 (s, 1H, -NH), 7.81 (m, 3H, quinoline), 7.17–7.44 (m, 11H, Ar-H), 3.83 (br s, 8H, piperazine), 2.45 (s, 3H, -OCH_3); ^{13}C NMR (400 MHz, DMSO-d_6, δ, ppm): 169.20 (C-3, s-triazine, C-N at piperazine linkage), 168.88 (C-1, s-triazine, C-O-C at quinoline linkage), 163.33 (C-5, s-triazine, C-NH at benzonitrile moiety), 145.45–101.49 (22C, aromatic ring carbons), 56.07 (C-38, -OCH_3), 50.50, 47.72 (4C, piperazine ring carbons); Analysis: calcd. for $C_{30}H_{26}N_8O_2$: C, 67.91; H, 4.94; N, 21.12%; found: C, 67.92; H, 4.92; N, 21.13%.

Antimicrobial activity

All the newly synthesized s-triazinyl piperazine and piperidine scaffolds (**5a-j**) were examined for antimicrobial efficacy against several human pathogenic bacterial and fungal strains as: one Gram positive bacteria (*Bacillus cereus* MTCC 619), three Gram negative bacteria (*Klebsiella pneumoniae* MTCC 109, *Salmonella typhi* MTCC 733, *Proteus vulgaris* MTCC 1771) and two fungal species (*Aspergillus fumigatus* MTCC 343, *Aspergillus clavatus* MTCC 1323) using disc diffusion sensitivity test (30). Mueller-Hinton agar media were sterilized (autoclaved at 120°C for 30 min) and allowed

to pour at uniform depth of disc to be solidified and access of suspension was decanted, which was then inoculated (1 mL/100 mL of medium) with the suspension (10^5 CFU/mL) and turbidity of all the bacterial cultures was adjusted to 0.5 McFarland Nephelometery Standard, which was streaked over the surface of media using a sterile cotton swab (15 min at 180°C) to ensure pronominal growth of microorganisms. The sterile plates previously soaked in a known concentration of the test compounds in DMSO were placed on the solidified nutrient agar medium, which was previously inoculated with pathogenic bacterial suspension for the sole purpose of producing zones of inhibition in millimeter in the bacterial lawn at the end of an incubation period of 24 h at 37 ± 1°C if any, around the disc. An additional control disc impregnated with an equivalent amount of solvent (DMSO) was also

Table 1. Optimization of final reaction step by conventional and microwave irrediation methods.

Entry	Solvent	R	Microwave method[b]		Conventional method	
			Reaction time (min)	Yield[a] (%)	Reaction time (h)	Yield[a] (%)
1	1,4,-Dioxane	N-Benzylpiperazine	3	80	11	75
2	1,4,-Dioxane	N-Benzylpiperidine	4	82	12	81
3	1,4,-Dioxane	3,5-Dimethylpiperidine	3	79	10	77
4	1,4,-Dioxane	Benzhydrylpiperazine	5	89	10	89
5	1,4,-Dioxane	4-Chlorobenzhydrylpiperazine	5	85	12	81
6	1,4,-Dioxane	1-(2-Fluorophenyl)piperazine	3	75	12	72
7	1,4,-Dioxane	1-(4-Fluorophenyl)piperazine	3	88	12	85
8	1,4,-Dioxane	1-(3-Trifluoromethylphenyl) piperazine	4	83	12	80
9	1,4,-Dioxane	1-(2,3,4-Trimethoxybenzyl) piperazine	5	80	11	79
10	1,4,-Dioxane	1-(4-Methoxyphenyl)piperazine	3	90	12	90

[a]Yields refer to pure products. [b]Pulsed irradiated at 400 W.

Table 2. Antibacterial activity of the synthesized compounds.

Compound	*In vitro* antibacterial activity – Zone of inhibition in mm (MIC in µg/mL)			
	Gram positive	Gram negative		
	B. cereus	*K. pneumoniae*	*S. typhi*	*P. vulgaris*
5a	20 (100)	18 (100)	20 (100)	19 (100)
5b	21 (100)	18 (100)	21 (100)	18 (100)
5c	**25 (6.25)**	20 (100)	20 (100)	23 (25)
5d	21 (100)	**24 (25)**	22 (50)	24 (12.5)
5e	24(12.5)	23 (50)	**25 (12.5)**	23 (25)
5f	22 (25)	23 (50)	19 (100)	22 (50)
5g	24 (12.5)	21 (100)	21 (100)	**25 (6.25)**
5h	**25 (6.25)**	**24 (12.5)**	24 (25)	23 (25)
5i	**25 (6.25)**	**24 (25)**	**25(12.5)**	22 (50)
5j	22 (25)	3 (50)	**25 (25)**	21 (100)
Ciprofloxacin	**29 (3.125)**	**33 (3.125)**	**30 (3.125)**	**31 (3.125)**
DMSO	–	–	–	–

The MIC values were evaluated at concentration range, 3.125–100 µg/mL. The table shows the corresponding zone of inhibition in millimeters and MIC values in µg/mL.

Table 3. Antifungal activity of the synthesized compounds.

Compound	*In vitro* antifungal activity – Zone of inhibition in mm (MIC in µg/mL)	
	A. fumigatus	*A. clavatus*
5a	22 (100)	22 (100)
5b	17 (100)	23 (100)
5c	20 (100)	26 (25)
5d	23 (25)	23 (100)
5e	**25 (12.5)**	25 (50)
5f	24 (25)	26 (25)
5g	24 (**12.5**)	26 (25)
5h	24 (25)	26 (12.5)
5i	23 (25)	26 (**6.25**)
5j	21 (100)	**27 (6.25)**
Ketoconazole	**29 (3.125)**	**31 (3.125)**
DMSO	**-**	**-**

Note: The MIC values were evaluated at concentration range, 3.125–100 µg/mL. The table shows the corresponding zone of inhibition in millimeters and MIC values in µg/mL.

used in the assay without any sample, which did not reveal any inhibition. Ciprofloxacin and ketoconazole were used as standard control drugs for antibacterial and antifungal activity, respectively, at 100 µg/disc. Ciprofloxacin is active against both Gram-positive and Gram-negative bacteria by functioning inhibition of DNA gyrase, a type II topoisomerase and topoisomerase IV, which is the necessary enzyme to separate bacterial DNA, thereby inhibiting cell division by binding these enzymes and prevent them from decatenating replicating DNA (31). Therefore, in our present study, ciprofloxacin is used as standard drug control because it is a broad spectrum antibiotic. The results of this activity evaluation are mentioned in Tables 1 and 2.

To determine minimum inhibitory concentration, a stock solution of the synthesized compound (100 µg/mL) in DMSO was prepared and appropriate respective quantities of the test compounds were incorporated in the specified quantity of molten sterile agar, in which, nutrient agar and Sabouraud dextrose agar medium was used for antibacterial and antifungal activity evaluation, 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. Microorganism suspension was prepared having approximately 10^5 CFU/mL and applied to plates with diluted compounds in DMSO and incubated at 37 ± 1°C for 24 h for MIC determination for pathogenic bacteria and 48 h for fungi. The lowest concentration of the substance, on the plate to be tested, preventing the development of visible growth of inoculated bacteria and fungi was recorded to represent MIC expressed in µg/mL.

RESULTS

Optimization of final reaction step by conventional and microwave irrediation methods, antibacterial activity of the synthesized compounds and antifungal activity of the synthesized compounds are presented in Tables 1, 2 and 3, respectively.

DISCUSSION AND CONCLUSION

The synthesis of compounds **5a–j** was undertaken as shown in Scheme 1. Two basic synthetic approaches, conventional heating and microwave irradiation were applied for the final nucleophilic substitution reactions, resulted in significant decrease in reaction time. It was observed that there was no vast difference in the reaction yields (Table 1) corresponding to both the methods applied. The final condensation reactions were carried out by microwave irradiation method at different power values and at different reaction times and the average of final optimum values of reaction time and

power are summarized in Table 1. The disubstituted s-triazine intermediate **3** was obtained in 75–80% by the reaction between 4-[(4,6-dichloro-1,3,5-triazin-2-yl)amino]benzonitrile **1** and 8-hydroxyquinoline in the presence of 60% NaH at 45–50°C in which the nucleophilic substitution of first chlorine atom produced **1** in 90% yield from cyanuric chloride and 4-aminobenzonitrile using triethylamine. Condensation of **3** with appropriate piperazine and piperidine substituents in 1,4-dioxane at 70–80°C provided the target compounds **5a–j**. C_3N_3 stretching frequency in *s*-triazine ring was observed between 810–820 cm^{-1}. Compound **1** displayed absorption band at 2220–2225 cm^{-1} confirming the presence of cyano group and a strong band in the region varied between 3250–3290 cm^{-1} appearing due to the presence of secondary -NH functional group as well as confirmed the replacement of the first reactive chlorine atom of s-triazine system. Moreover, characteristic IR frequency absorption band appeared nearby 1255 cm^{-1} corresponding with the C-O-C linkage by disappearing stretching peak at 3610–3613 cm^{-1} of O-H belonging to the 8-hydroxyquinoline gave proof to the formation of intermediate **3** and selective replacement of the second chlorine atom. The absence of C-Cl stretching band at 700–760 cm^{-1} confirmed the formation of final compounds with third chlorine atom replacement at reflux temperature. In the ^{1}H NMR spectra the synthesis of final compounds **5a–j** was confirmed on the basis of the fact that the piperazine proton revealed the signal between δ 3.75–3.85 ppm, signal varying from δ values 8.79–8.86 ppm for –NH and the proton corresponding to quinoline moiety resonated at δ values between 7.66–7.74 ppm. ^{13}C NMR spectral data interpretation clearly indicates that the carbon signals in the range 168.46–168.88, 169.13–169.69 and δ 161.93–163.33 ppm confirmed the selective replacement of chlorine atoms at C-1, C-3 and C-5 positions of triazinyl ring by 4-aminobenzonitrile, 8-hydroxyquinoline and piperazines as well as piperidine constituents, respectively, whereas, carbon atoms corresponding to the piperazine ring revealed signals in the range of δ 46–50 ppm in ^{13}C NMR spectra, whereas, carbon of CN appeared to resonate around δ 105 ppm. ^{19}F NMR spectra for the analogues **5f** and **5g** confirmed the presence of fluorine atom in the *ortho* and *para* position of the phenyl ring, respectively, attached to the amino nitrogen of piperazine coupling agent by giving the corresponding peaks resonating around δ –120 and –117 ppm, respectively, whereas, another fluorine NMR spectra obtained for the compound **5h** gave singlet at δ –64.8 ppm corresponding to trifluoromethyl functional group present in the moiety. In ^{19}F NMR spectral assignment the internal standard $CFCl_3$ assigned a shift of zero and its presence will not have any influence upon a compounds' chemical shifts, plus, its observed signals lies substantially downfield of most signals derived from carbon bound fluorine. Therefore, the fluorine chemical shifts analyzed here (δ ppm) are negative in value. Assignments of the structure of final scaffolds (**5a–j**) are based on correct elemental analysis, which was found to be within ± 0.05% limits.

Antibacterial activity results investigated for newly synthesized scaffolds summarized in Table 2 revealed that the final analogue bearing N-benzylpiperazine as well as its piperidine entity constituents exhibited moderate activity against all the mentioned pathogenic Gram positive and Gram negative bacterial strains, while another piperidine constituent bearing two methyl functional groups showed strong inhibitory action against Gram positive *B. cereus*. The bioassay results revealed that incorporation of benzhydryl piperazine entity to the basic s-triazine core is essential for excellent antibacterial activity. In fact, the final scaffold bearing benzhydryl piperazine exhibited promising activity towards Gram negative *K. pneumoniae*, in addition, halogen atom insertion at 4th position in one of the phenyl rings in benzhydrylpiperazine entity attached to s-triazine proved much more beneficial to contribute promising activity against most of the mentioned bacterial strains compared to its parent benzhydrylpiperazine bearing scaffold, particularly inhibiting Gram negative *S. typhi*. The presence of fluorine atom(s) in the final scaffolds significantly enhances the net biomedical values, as the final scaffold bearing fluorine atom at 4th position of the phenyl ring of the piperazine moiety of the final system showed strong efficacy by inhibiting Gram negative *P. vulgaris*. The excellent activity results displayed in the present bioassay by the final scaffolds consist of trifluoromethyl group at 3rd position of piperazinyl phenyl ring inhibited both the Gram positive strain *B. cereus* as well as Gram negative *P. pneumoniae*. Similar efficacy results displayed by trimethoxy function group containing benzylpiperazine entity incorporated to s-triazine towards Gram positivee *B. cereus* and two Gram negative strains *K. pneumoniae* and *S. typhi*, whereas the presence of single methoxy functional group in the piperazine moiety showed good activity against Gram negative *S. typhi* with the similar efficacy compared to trimethoxy functional group bearing final scaffold.

The antifungal investigation results presented in Table 3 strongly indicate that all the final **5a-j** com-

pounds inhibited the spore germination of *A. fumigates* and *A. clavatus* fungi. Higher potency was observed in case of the final scaffolds bearing halogen atom(s) and methoxy functional group(s) in the piperazine ring attached to the basic s-triazine core. Fluorine has played a pivotal role in novel drug discovery as electronic effects within the molecule can be altered by incorporation of fluorine atom(s) or due to its higher electronegativity, thereby, enhancing chemical reactivity and pharmacological properties like lipophilicity, absorption, and transportation. Furthermore, lipophilicity of the fluorine atom is slightly higher than hydrogen, trifluoromethyl, one of the most lipophilic groups known, and is much more lipophilic than methyl or chloro substituent, which is the most significant factor in improving pharmacological activity of the molecule. For instance, fluorine is better leaving group than hydrogen, there is some potential for the formation of covalent bond between fluorinated molecule and enzyme at its active site by the loss of fluoride resulting in inhibition of enzyme's activity suitably proving the molecule potent antibacterial (32, 33) properties. The present antifungal bioasaay revealed that the insertion of electron withdrawing halogen atom like chlorine and fluorine to the *para* position of the piperazinyl phenyl ring contributed to excellent potency for inhibition of *A. fumigates* fungi. Moreover, insertion of electron releasing group(s) like methoxy functional group to the piperazinyl phenyl ring is much more beneficial to indicate strong inhibition of *A. clavatus* fungi.

In summary, a series of trisubstituted s-triazine derivatives has been successfully synthesized and tested for their antimicrobial activity. S-triazine nucleus is one of the active constituents present in many standard drugs, and is known to increase pharmacological activity of the molecules when its significant activity (34–38) is already reported. The presence of 8-hydroxyquinoline moiety is also an instrumental in contributing to the net biological activity. Herein, an effort was made to combine all these three potential units, namely s-triazine nucleus, 4-aminobenzonitrile, 8-hydroxyquinoline and various substituted piperazine and piperidine moieties in one core and report study of the biological behavior of the resultant systems. Hence, it is concluded that trisubstituted S-triazines are more active than mono and di-substituted S-triazines and thus, there is enough scope for further study in developing such compounds with a good lead activity. Overall conclusion for synthesized compounds is that most of them shown very promising activity as compared to standard drug for all representative panel of bacterial and fungal strains. In short, an attempt has been made to develop new s-triazinyl piperazines and piperidines by cost effective and environmentally friendly method.

Acknowledgments

The authors are thankful to Dr. P.D. Porey, Director and Dr. S Jauhari, Head of Applied Chemistry Department of S. V. National Institute of Technology, Surat for the scholarship, encouragement and facilities. Authors are also thankful to Catapharma, Enzal Chemicals Pvt. Ltd., Dr. Prem's molecules Pvt. Ltd., Ami Organics Pvt. Ltd., Modepro (India) Pvt. Ltd., Siddharth Interchem Pvt. Ltd. and Mahrshee Laboratories for providing valuable piperazine and piperidine derivatives.

REFERENCES

1. Chu D.T.W., Plattner J.J., Katz L.: J. Med. Chem. 39, 3853 (1996).
2. Beovic B.: Int. J. Food Microbiol. 112, 280 (2006).
3. Finch R., Hunter P.A.: J. Antimicrob. Chemother. 58, i3 (2006).
4. Suree N., Jung M.E., Clubb R.T.: Mini Rev. Med. Chem. 7, 991 (2007).
5. Rex J.H., Walsh T.J., Sobel J.D., Filler S.G., Pappas P.G., Dismukes W.E., Edwards J.E.: J. Clin. Infect. Dis. 30, 662 (2000).
6. Sanglard D., Odds F.C.: Lancet Infect. Dis. 2, 73 (2002).
7. Buzzini P., Arapitsas P., Goretti M., Branda E., Turchetti B., Pinelli P., Ieri F., Romani A.: Mini Rev. Med. Chem. 8, 1179 (2008).
8. Nathan C.: Nature 431, 899 (2004).
9. Zhou C., Min J., Liu Z.., Young A.., Deshazer H., Gao T., Chang Y-T., Kallenbach N.R.: Bioorg. Med. Chem. Lett. 18, 1308 (2008).
10. Srinivas K., Srinivas U., Bhanuprakash K., Harakishore K., Murthy U.S.N., Jayathirtha R.V.: Eur. J. Med. Chem. 41, 1240 (2006).
11. Baliani A., Bueno G.J.., Stewart M.L., Yardley V., Brun R., Barrett M.P., Gilbert I.H.: J. Med. Chem. 48, 5570 (2005).
12. Menicagli R., Samaritani S., Signore G., Vaglini F., Dalla Via L.: J. Med. Chem. 47, 4649 (2004).
13. Melato S., Prosperi D., Coghi P., Basilico N., Monti D.: ChemMedChem. 3, 873 (2008).
14. Xiong Y-Z.., Chen F-E., Balzarini J., De Clercq E., Pannecouque C.: Eur. J. Med. Chem. 43, 1230 (2008).

15. Mari S., Rossi M., Valenti P., Da Re P.: Farmaco 54, 411 (1999).
16. Wyrzykiewicz E., Wendzonka M., Kędzia B.: Eur. J. Med. Chem. 41, 519 (2006).
17. Foroumadi A., Ghodsi S., Emami S., Najjari S., Samadi S., Faramarzi M.A.., Beikmohammadi L. Et al.: Bioorg. Med. Chem. Lett. 16, 3499 (2006).
18. Ritu B. D., Satish F. V., Tarosh S. P., Chandresh L. J., Hiren V. D., Bharat C. D.: Appl. Organomet. Chem. 24, 408 (2010).
19. Okide G.B., Adikwu M.U., Esimone C.O.: Biol. Pharm. Bull. 23, 257 (2000).
20. Block J.H.,. Beale J.M.: Wilson and Giswold's Textbook of Organic Medicinal and Pharmaceutical Chemistry, 11th edn., Lippincott, Williams & Wilkins, Philadelphia, PA 2005.
21. Scheibel L.W., Adler A.: Mol. Pharmacol. 22, 140 (1982).
22. Scheibel L.W., Stanton G.G.: Mol. Pharmacol. 30, 364 (1986).
23. Crystal M.D., Carl F. N.: J. Antimicrob. Chemother. 65, 1424 (2010).
24. Urbanski T., Slopek S., Venulet J.: Nature 168, 29 (1951).
25. Madonna S., Béclin C., Laras Y.., Moret V., Marcovycz A., Lamoral-Theys D., Dubois J. et al.: Eur. J. Med. Chem. 45, 623 (2010).
26. Shaw A.Y.., Chang C-Y., Hsu M-Y., Lu P-J., Yang C-N., Chen H-L., Lo C-W. et al.: Eur. J. Med. Chem. 45, 2860 (2010).
27. Dardari Z., Lemrani M.., Bahloul A., Sebban A., Hassar M., Kitane S., Berrada M., Boudouma M.: Farmaco 59, 195 (2004).
28. Depalma P.D., Loux J.J., Hutchman J., Dolan M.M., Yankell S.L.: J. Dent. Res. 55, 292 (1976).
29. Xiong Y-Z.., Chen F-E., Balzarini J., De Clercq E., Pannecouque C.: Chem. Biodivers. 6, 561 (2009).
30. Cruickshank R., Duguid J.P., Marion B.P., Swain R.H.A.: Medicinal Microbiology. Vol. 2, 12th edn., Churchil Livingstone, London 1975.
31. Drlica K., Zhao X.: Microbiol. Mol. Biol. Rev. 61, 377 (1997).
32. Dolbier W.R. Jr.: J. Fluor. Chem. 126, 157 (2005).
33. Filler R, Saha R.: Fut. Med. Chem. 1, 777 (2009).
34. Mahajan D.H., Pannecouque C., De Clercq E., Chikhalia K.H.: Arch. Pharm. Chem. Life Sci. 342, 281 (2009).
35. Chikhalia K.H., Vashi D.B., Patel M.J.: J. Enzyme Inhib. Med. Chem. 24, 617 (2009).
36. Chikhalia K.H., Patel M.J.: J. Enzyme Inhib. Med. Chem. 24, 960 (2009).
37. Patel D.H., Chikhalia K.H., Shah N.K., Patel D.P., Kaswala P.B., Buha V.M.: J. Enzyme Inhib. Med. Chem. 25, 121 (2010).
38. Patel A. C., Mahajan D. H., Chikhalia K.H.: Phosphorus Sulfur Silicon Relat. Elem. 185, 368 (2010).

Received: 22. 02. 2011