Acta Poloniae Pharmaceutica – Drug Research, Vol. 69 No. 5 pp. 879–891, 2012 ISSN 0001-6837
Polish Pharmaceutical Society

MICROWAVE-ASSISTED SYNTHESIS OF COUMARIN BASED 1,3,5-TRIAZINYL PIPERAZINES AND PIPERIDINES AND THEIR ANTIMICROBIAL ACTIVITIES

DIVYESH PATEL[1], RAHUL PATEL[1], PREMLATA KUMARI[1]* and NAVIN PATEL[2]

[1]Applied Chemistry Department, S.V. National Institute of Technology, Surat-395007, India
[2]Department of Chemistry, V.N. South Gujarat University, Surat-395007, India

Abstract: A series of 1,3,5-triazine derivatives that contain aniline, 4-hydroxycoumarin and 7-hydroxy-4-methylcoumarin and different piperazines and piperidines as substituents on the carbon atoms of the triazine ring has been synthesized by a simple and efficient synthetic protocol. Comparative studies were performed on above compounds, which were synthesized with conventional and microwave heating method. The microwave method was observed to be more beneficial as it provides an increase of yield and 90–95% reduction time. The antimicrobial activity of the compounds was tested against four bacteria (*Staphylococcus aureus* MTCC 96, *B. subtilis MTCC 441*, *Escherichia coli* MTCC 739, *Pseudomonas aeruginosa* MTCC 741) and two fungi (*Aspergillus niger* MTCC 282, *Candida albicans* MTCC 183). The preliminary *in vitro* evaluation studies revealed that some of the compounds have promising antimicrobial activities.

Keywords: *s*-triazine, coumarins, piperazines-piperidines, microwave irradiation, antimicrobial activity

Despite significant progress in antimicrobial therapy, infectious diseases caused by bacteria and fungi remain a major health concern due to the development of resistance to existing antimicrobial drugs. The increasing incidence of bacterial resistance to large number of antibacterial agents such as glycopeptides, sulfonamides, β-lactams, nitroimidazoles, quinolones, tetracyclins, chloramphenicol and macrolides is becoming a major concern (1, 2). In particular, the emergence of multiple drug resistant Gram-positive and Gram-negative bacteria has caused life-threatening infectious diseases in many countries around the world (3).

As a part of our endeavor towards new and efficient antibacterial and antifungal agents, we have synthesized some novel 1,3,5-triazines. The advent of 1,3,5-triazines, associated with diverse biological activities such as antimicrobial (4–6), antiprotozoal (7), anticancer (8), antimalarial (9) and antiviral (10) activity accelerated the rate of progress of 1,3,5-triazine derivatives. Coumarin derivatives were reported with diverse structural features and versatile biological properties such as antibiotics (11, 12), fungicides (13, 14), anti-inflammatory (15, 16), anticoagulant (17), antitumor (18) and antileucemic (19). Regarding their high fluorescence ability, they are also used as fluorescent probes in biology and medicine (20). More recently, coumarin derivatives have been evaluated in the treatment of human immunodeficiency virus, due to their ability to inhibit human immunodeficiency virus' integrase enzyme (21). Among the various coumarin derivatives, 4-hydroxy substituted coumarins are important group of coumarin derivatives showing various biological activities (22, 23). Hydroxy derivatives of 4-methylcoumarin are important group of coumarin derivatives showing medicinal as well as other applications (24). For example 7-hydroxy-4-methylcoumarin is used as spasmolytic drug in several European countries (25). Literature survey informs that piperazines and substituted piperazines are important family of heterocyclic compounds as they have attracted significant interest in medicinal chemistry (26–28) and also some piperidine derivatives are important due to their antimicrobial properties (29).

The molecular manipulation of promising lead compounds is still a major line of approach to develop new drugs. It involves an effort to combine the separate pharmacophoric groups of similar activ-

* Corresponding author: premlatakumari1@gmail.com

ity into one compound, thus making structural changes in the biological activity. The present paper deals with synthesis both by conventional and microwave method, characterization and antimicrobial activity of novel s-triazinyl piperazines and piperidines bearing aniline as well as 4-hydroxycoumarin and 7-hydroxy-4-methylcoumarin entities having potent clinical importance.

EXPERIMENTAL

Microwave assisted reactions were carried out by a microwave reactor (model-Roto Synth Terminal-640). All the melting points were determined in open capillary on a Veego (Model: VMP-D) electronic apparatus and are uncorrected. The IR spectra (4000–400 cm^{-1}) of synthesized compounds were recorded on a Shimadzu 8400-S FT-IR spectrophotometer using KBr discs. 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. Nuclear magnetic resonance spectra were recorded on a Varian 400 MHz model spectrometer using $CDCl_3$ as a solvent and TMS as an internal standard (chemical shifts in δ ppm). All new compounds were subjected to elemental analysis using a Heraeus Carlo Erba 1180 CHN analyzer.

(4,6-Dichloro-1,3,5-triazin-2-yl)phenylamine (1).

To a stirred solution of 2,4,6-trichloro-1,3,5-triazine (15 g, 0.081 mol) in anhydrous THF (150 mL) and aniline (7.97 g, 0.081 mol) were added dropwise at 0–5°C. The resulting reaction mixture was stirred at this temperature for 2 h, then triethylamine (8.28 g, 0.081 mol) was added to 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 diluted HCl and then filtered, dried and recrystallized from acetone to afford pure 16.99 g (yield 87%) of **1** as white colored amorphous solid, m.p. 191–194°C; IR (KBr, cm^{-1}): 3296.45 cm^{1} (NH).

4-(4-Chloro-6-phenylamino-1,3,5-triazin-2-yl-oxy)chromen-2-one (3a)

To a stirred solution of 4-hydroxycoumarin (8 g, 0.050 mol) and 60% NaH (1.19 g, 0.050 mol) in anhydrous THF (150 mL), compound **1** (11.89 g, 0.050 mol) was added and the mixture was stirred for 2 h at room temperature and then stirring was continued for another 14 h at 45–50°C. After completion of the reaction, it was treated with crushed ice, filtered, dried and recrystallized from acetone to afford pure 15.04g (yield 82%) of **3a** as white colored amorphous solid, mp 250–253°C; IR (KBr, cm^{-1}): 1254–1257 cm^{-1} (C-O-C), 1697.21 (C=O).

7-(4-Chloro-6-phenylamino-1,3,5-triazin-2-yl-oxy)-4-methylchromen-2-one (3b)

To a stirred solution of 7-hydroxy-4-methylcoumarin (8 g, 0.045 mol) and 60% NaH (1.05 g, 0.045 mol) in anhydrous THF (150 mL), compound **1** (10.95 g, 0.045 mol) was added and the reaction mixture was stirred for 2 h at room temperature and then stirring was continued for another 14 h at 45–50°C. After completion of the reaction, the mixture was treated with crushed ice, filtered, dried and recrystallized from acetone to afford pure 14.02 g (yield 82%) of **3b** as white colored amorphous solid, mp 264–267°C; IR (KBr, cm^{-1}): 1255–1257 cm^{-1} (C-O-C), 1697.40 (C=O), 1465.70 (CH_3).

General procedures for preparation of compounds (5a–j) and (6a–j)

Conventional method

To a solution of **3a** (3.68 g, 0.01 mol) in 1,4-dioxane (30 mL), N-benzylpiperazine **4a** (1.78 g, 0.01 mol) was added and the reaction mixture was refluxed for 10 to 15 h with TLC monitoring. Potassium carbonate was used for neutralization of the reaction mixture. After completion of the reaction, the mixture was treated with crushed ice and neutralized by diluted HCl. The precipitate thus obtained was filtered, dried and recrystallized from THF (15 mL) to give 3.34 g (yield 66%) of **5a**, as white colored compound. The same procedure was utilized for synthesis of compounds **5b–j** and **6a–j** using differently substituted piperazines and pyrimidines (**4b–j**).

Microwave method

In order to testify whether microwave irradiation speeds up the final nucleophilic substitution reactions, the same reactions were carried out in the same scale and the same solvent i.e., 1,4-dioxane under microwave irradiation. The reaction time was found to be dramatically reduced for each substitution from 10 to 15 h (conventional heating method) to 2–6 min under microwave irradiation. Microwave assisted reactions were conducted in septum-sealed reaction vessels in microwave reactor. The final condensation of compound **3** with various substituted piperazines and piperidines was carried out under microwave irradiation. For example, compound **3b** (3.89 g, 0.01 mol) in 1 equivalent of K_2CO_3 (1.41 g) was condensed with 4-benzylpiperidine **4b** (1.69 g,

Table 1. Spectral data of synthesized compounds.

	IR (KBr, cm^{-1}), ^{1}H NMR ($CDCl_3$, δ, ppm), ^{13}C NMR ($CDCl_3$, δ, ppm)
5a:	IR: 3298.38 (N-H str.), 1697.41 (C=O of coumarin), 1261.49 (C-O-C); ^{1}H NMR: 10.17 (s, 1H, -NH at aniline linkage), 7.77 (d, 1H, 1H at C-19 of coumarin), 7.71–7.31 (m, 10H, Ar-H), 6.63–7.17 (m, 3H, 3H of coumarin), 5.91 (s, 1H, 1H at C-22 of coumarin), 3.72 (s, 2H, -CH_2 at piperazine linkage), 3.57 (br s, 8H, piperazine ring); ^{13}C NMR: 167.31 (C-2, C-O-C at coumarin linkage), 165.16 (C-4, C-NH at aniline linkage), 158.59 (C-21, C=O at coumarin), 152.84 (C-23, C-O-C at coumarin), 115.05 (C-22), 117.61 (C-18), 117.75 (C-7), 117.80 (C-11), 120.15 (C-16), 121.30 (C-17), 123.11 (C-10), 123.30 (C-14), 124.04 (C-19), 128.69 (C-9), 132.23 (C-15), 139.49 (C-8), 151.87 (C-12), 124.08, 125.79, 127.77, 128.45, 132.99, 137.74 (6C, Ar-C, phenyl ring carbons at piperazine), 96.33 (C-22, C-C=O of coumarin), 62.80 (C-32, N-CH_2 at piperazine linkage), 43.16, 49.42 (4C, piperazine ring carbons).
5b:	IR: 3296.57 (N-H str.), 1692.13 (C=O of coumarin), 1258.32 (C-O-C); ^{1}H NMR: 10.19 (s, 1H, -NH at aniline linkage), 7.83 (d, 1H, 1H at C-19 of coumarin), 7.78–7.36 (m, 10H, Ar-H), 7.21–6.93 (m, 3H, 3H of coumarin), 5.96 (s, 1H, 1H at C-22 of coumarin), 3.90 (t, 4H, piperidine), 3.60 (t, 4H, piperidine), 2.54 (d, 2H, -CH_2 at piperidine linkage), 1.77 (t, 1H, -CH- piperidine); ^{13}C NMR: 167.35 (C-2, C-O-C at coumarin linkage), 165.11 (C-4, C-NH at aniline linkage), 158.54 (C-21, C=O at coumarin), 152.80 (C-23,C-O-C at coumarin), 115.92 (C-22), 117.55 (C-18), 117.62 (C-7), 117.73 (C-11), 120.01 (C-16), 122.25 (C-17), 123.44 (C-10), 123.90 (C-14), 124.11 (C-19), 128.54 (C-9), 134.50 (C-15), 139.03 (C-8), 151.60 (C-12), 128.52, 128.80, 128.85, 129.13, 129.20, 140.49 (6C, Ar-C, phenyl ring carbons at piperazine), 96.25 (C-22, C-C=O of coumarin), 42.50 (C-32,CH_2 at piperidine linkage), 33.10, 41.42, 45.69 (5C, piperidine ring carbons).
5c:	IR: 3292.43 (N-H str.), 1694.11 (C=O of coumarin), 1454.12 (-CH_3) 1260.21 (C-O-C); ^{1}H NMR: 10.17 (s, 1H, -NH at aniline linkage), 7.71 (d, 1H, 1H at C-19 of coumarin), 7.65–7.22 (m, 5H, Ar-H), 7.18–6.87 (m, 3H, 3H of coumarin), 5.92 (s, 1H, 1H at C-22 of coumarin), 3.66-3.71 (m, 4H, piperidine), 2.45 (br s, 2H, -CH_2 piperidine), 1.99 (q, 2H, piperidine), 1.87 (6H, d, 2-CH_3); ^{13}C NMR: 167.31 (C-2, C-O-C at coumarin linkage), 165.20 (C-4, C-NH at aniline linkage), 158.46 (C-21, C=O at coumarin), 152.85 (C-23,C-O-C at coumarin), 149.60 (C-12), 112.92 (C-22), 116.10 (C-18), 118.78 (C-7), 118.86 (C-11), 120.25 (C-16), 122.95 (C-17), 123.90 (C-10), 124.10 (C-14), 124.17 (C-19), 128.82 (C-9), 133.90 (C-15), 140.08 (C-8), 96.12 (C-22, C-C=O of coumarin), 33.25,45.42, 46.54 (5C, piperidine ring carbons), 22.54 (C-32,C-33, -CH_3).
5d:	IR: 3296.19 (N-H str.), 1694.24 (C=O of coumarin), 1261.56 (C-O-C); ^{1}H NMR: 10.21 (s, 1H, -NH at aniline linkage), 7.89 (d, 1H, 1H at C-19 of coumarin), 7.79–7.37 (m, 15H, Ar-H), 7.23–6.94 (m, 3H, 3H of coumarin), 4.57 (s, 1H, N-CH at piperazine linkage) 5.88 (s, 1H, 1H at C-22 of coumarin), 3.54 (br s, 8H, piperazine ring); ^{13}C NMR: 167.29 (C-2, C-O-C at coumarin linkage), 165.13 (C-4, C-NH at aniline linkage), 158.58 (C-21, C=O at coumarin), 152.80 (C-23, C-O-C at coumarin), 115.02 (C-22), 118.72 (C-18), 118.80 (C-7), 118.84 (C-11), 119.90 (C-16), 123.14 (C-10), 123.45 (C-14), 124.20 (C-17), 126.05 (C-19), 128.39 (C-9), 141.42 (C-15), 139.30 (C-8), 151.68 (C-12), 126.11, 128.11, 128.22, 128.45, 128.69, 128.75, 129.02, 129.15, 129.16, 129.45, 134.44, 134.95 (12C, Ar-C, phenyl ring carbons at piperazine), 96.41 (C-22, C-C=O of coumarin), 72.83 (C-32,N-CH at piperazine linkage), 43.10,49.51 (4C, piperazine ring carbons).
5e:	IR: 3297.08 (N-H str.), 1696.18 (C=O of coumarin), 1265.20 (C-O-C), 808.21 (-Cl); ^{1}H NMR: 10.21 (s, 1H, -NH at aniline linkage), 7.87 (d, 1H, 1H at C-19 of coumarin), 7.83–7.33 (m, 14H, Ar-H), 7.27–7.03 (m, 3H, 3H of coumarin), 4.67 (s, 1H, N-CH at piperazine linkage), 5.90 (s, 1H, 1H at C-22 of coumarin), 3.57 (br s, 8H, piperazine ring); ^{13}C NMR: 167.34 (C-2, C-O-C at coumarin linkage), 165.17 (C-4, C-NH at aniline linkage), 158.60 (C-21, C=O at coumarin), 152.83 (C-23,C-O-C at coumarin), 115.05 (C-22), 118.61 (C-18), 118.77 (C-7), 118.80 (C-11), 119.50 (C-16), 122.92 (C-17), 123.40 (C-9), 123.97 (C-14), 126.17 (C-19), 128.20 (C-10), 133.94 (C-15), 139.42 (C-8), 151.94 (C-12), 126.20, 127.89, 128.16, 128.41, 128.49, 128.66, 129.11, 129.14, 129.22, 129.23, 134.80, 139.95 (12C, Ar-C, phenyl ring carbons at piperazine), 96.41 (C-22, C-C=O of coumarin), 73.08 (C-32,N-CH at piperazine linkage), 44.09, 50.32(4C, piperazine ring carbons).
5f:	IR: 3298.10 (N-H str.), 1697.12 (C=O of coumarin), 1266.03 (C-O-C), 1154.29 (C-F); ^{1}H NMR: 10.24 (s, 1H, -NH at aniline linkage), 7.75 (d, 1H, 1H at C-19 of coumarin), 7.70–7.37 (m, 9H, Ar-H), 7.20–6.93 (m, 3H, 3H of coumarin), 5.95 (s, 1H, 1H at C-22 of coumarin), 3.60 (br s, 8H, piperazine ring); ^{19}F NMR –121.67 (s, 1F); ^{13}C NMR: 167.14 (C-2, C-O-C at coumarin linkage), 165.19 (C-4, C-NH at aniline linkage), 158.62 (C-21, C=O at coumarin), 152.88 (C-23, C-O-C at coumarin), 115.78 (C-22), 117.77 (C-18), 118.72 (C-7), 118.80 (C-11), 119.87 (C-16), 121.10 (C-17), 123.51 (C-10), 123.90 (C-14), 125.50 (C-19), 128.80 (C-9), 134.49 (C-15), 140.12 (C-8), 157.14 (C-12), 121.30, 124.20, 128.48, 141.88, 141.92, 143.54 (6C, Ar-C, phenyl ring carbons at piperazine), 96.30 (C-22, C-C=O of coumarin), 46.09, 50.26 (4C, piperazine ring carbons).

Table 1. cont.

	IR (KBr, cm^{-1}), ^1H NMR ($CDCl_3$, δ, ppm), ^{13}C NMR ($CDCl_3$, δ, ppm)
5g:	IR: 3298.15 (N-H str.), 1697.21 (C=O of coumarin), 1267.22 (C-O-C), 1160.12 (C-F); ^1H NMR: 10.21 (s, 1H, -NH at aniline linkage), 7.87 (d, 1H, 1H at C-19 of coumarin), 7.78–7.39 (m, 9H, Ar-H), 7.29–6.98 (m, 3H, 3H of coumarin), 5.86 (s, 1H, 1H at C-22 of coumarin), 3.56 (br s, 8H, piperazine ring); ^{19}F NMR –121.25 (s, 1F); ^{13}C NMR: 167.18 (C-2, C-O-C at coumarin linkage), 165.31 (C-4, C-NH at aniline linkage), 158.67 (C-21, C=O at coumarin), 152.80 (C-23, C-O-C at coumarin), 115.71 (C-22), 117.71 (C-18), 118.66 (C-7), 118.75 (C-11), 119.90 (C-16), 121.02 (C-17), 123.46 (C-10), 123.96 (C-14), 125.41 (C-19), 128.84 (C-9), 134.45 (C-15), 139.92 (C-8), 157.20 (C-12), 121.38, 124.28, 128.55, 141.60, 141.87, 143.66 (6C, Ar-C, phenyl ring carbons at piperazine), 96.39 (C-22, C-C=O of coumarin), 46.31, 50.22 (4C, piperazine ring carbons).
5h:	IR: 3297.22 (N-H str.), 1697.10 (C=O of coumarin), 1264.31 (C-O-C), 1161.78 (C-F); ^1H NMR: 10.18 (s, 1H, -NH at aniline linkage), 7.80 (d, 1H, 1H at C-19 of coumarin), 7.74–7.35 (m, 9H, Ar-H), 7.24–6.90 (m, 3H, 3H of coumarin), 5.96 (s, 1H, 1H at C-22 of coumarin), 3.58 (br s, 8H, piperazine ring); ^{19}F NMR –64.70 (s, 3F); ^{13}C NMR: 167.25 (C-2, C-O-C at coumarin linkage), 165.36 (C-4, C-NH at aniline linkage), 158.59 (C-21, C=O at coumarin), 152.87 (C-23, C-O-C at coumarin), 113.17 (C-22), 116.60 (C-18), 118.81(C-7), 118.83 (C-11), 120.03 (C-16), 123.28 (C-10), 123.49 (C-14), 123.92 (C-17), 124.15 (C-19), 128.80 (C-9), 135.50 (C-15), 140.09 (C-8), 152.17 (C-12), 115.60, 116.10, 128.54, 129.70, 131.42, 141.24 (6C, Ar-C, phenyl ring carbons at piperazine), 123.90 (C-38,CF_3), 96.42 (C-22, C-C=O of coumarin), 47.79, 50.85(4C, piperazine ring carbons).
5i:	IR: 3297.25 (N-H str.), 1696.21 (C=O of coumarin), 1265.19 (-OCH_3), 1264.20 (C-O-C); ^1H NMR: 10.17 (s, 1H, -NH at aniline linkage), 7.74 (d, 1H, 1H at C-19 of coumarin), 7.71–7.26 (m, 7H, Ar-H), 7.21–6.83 (m, 3H, 3H of coumarin), 6.05 (s, 1H, 1H at C-22 of coumarin), 3.75 (s, 2H, N-CH_2 at piperazine linkage), 3.83, 3.72, 3.69-3.71 (s, 9H, 3-OCH_3), 3.54 (br s, 8H, piperazine ring); ^{13}C NMR: 168.09 (C-2, C-O-C at coumarin linkage), 165.40 (C-4, C-NH at aniline linkage), 158.62 (C-21, C=O at coumarin), 107.25 (C-22), 118.72 (C-18), 118.81 (C-7), 118.86 (C-11), 120.05 (C-16), 123.40 (C-10), 123.56 (C-14), 123.90 (C-17), 124.39 (C-19), 128.82 (C-9), 134.50 (C-15), 140.12 (C-8), 153.26 (C-12), 124.11, 128.77, 141.16, 141.39, 142.75, 143.48 (6C, Ar-C, phenyl ring carbons at piperazine), 153.05 (C-23, C-O-C at coumarin), 97.10 (C-22, C-C=O of coumarin), 62.83 (C-32,N-CH_2 at piperazine linkage), 56.12–59.97 (C-40, C-42, C-44, 3C, -OCH_3), 46.52, 50.20 (4C, piperazine ring carbons).
5j:	IR: 3297.11 (N-H str.), 1697.30 (C=O of coumarin), 1272.43 (-OCH_3), 1265.41 (C-O-C); ^1H NMR: 10.19 (s, 1H, -NH at aniline linkage), 7.83 (d, 1H, 1H at C-19 of coumarin), 7.77–7.31 (m, 9H, Ar-H), 7.23–6.87 (m, 3H, 3H of coumarin), 5.91 (ds 1H, 1H at C-22 of coumarin), 3.79 (s, 3H, -OCH_3), 3.57 (br s, 8H, piperazine ring); ^{13}C NMR: 167.95 (C-2, C-O-C at coumarin linkage), 165.31 (C-4, C-NH at aniline linkage), 158.59 (C-21, C=O at coumarin), 154.12 (C-23, C-O-C at coumarin), 115.19 (C-22), 117.42 (C-18), 118.71 (C-7), 118.82 (C-11), 119.90 (C-16), 123.49 (C-10), 123.91 (C-14), 124.22 (C-17), 126.76 (C-19), 128.89 (C-9), 134.50 (C-15), 139.92 (C-8), 115.50, 116.05, 128.85, 143.42, 151.60, 152.53 (6C, Ar-C, phenyl ring carbons at piperazine), 155.97 (C-12), 96.41 (C-22, C-C=O of coumarin), 56.10 (C-39, OCH_3), 46.14, 50.03 (4C, piperazine ring carbons).
6a:	IR: 3288.20 (N-H str.), 1700.21 (C=O of coumarin), 1448.59 (-CH_3), 1280.19 (C-O-C); ^1H NMR: 10.15 (s, 1H, -NH at aniline linkage), 7.85 (d, 1H, 1H at C-12 of coumarin), 7.77–7.32 (m, 10H, Ar-H), 7.16–6.85 (m, 2H, 2H of coumarin), 6.13 (s, 1H, 1H at C-15 of coumarin), 4.72 (s, 2H, -CH_2 at piperazine linkage), 3.57 (br s, 8H, piperazine ring), 2.47 (d, 3H, -CH_3 of coumarin); ^{13}C NMR: 166.42 (C-2, C-O-C at coumarin linkage), 162.10 (C-4, C-NH at aniline linkage), 160.31 (C-14, C=O at coumarin), 154.34 (C-8, C-O-C at coumarin), 116.15 (C-9), 116.62 (C-11), 118.20 (C-22), 118.43 (C-20), 118.71 (C-15), 118.86 (C-7), 123.49 (C-24), 124.85 (C-12), 128.12 (C-23), 128.26 (C-25), 140.01 (C-21), 142.47 (C-16), 154.24 (C-10), 127.14, 128.78, 128.80, 128.85, 138.56, 141.12 (6C, Ar-C, phenyl ring carbons at piperazine), 113.29 (C-15, C-C=O of coumarin), 62.75 (C-33, N-CH_2 at piperazine linkage), 43.16, 52.24 (4C, piperazine ring carbons), 21.43 (C-18, C-CH_3 of coumarin).
6b:	IR: 3291.35 (N-H str.), 1700.26 (C=O of coumarin), 1448.62 (-CH_3), 1282.21 (C-O-C); ^1H NMR: 10.21 (s, 1H, -NH at aniline linkage), 7.74 (d, 1H, 1H at C-12 of coumarin), 7.69–7.28 (m, 10H, Ar-H), 7.11–6.95 (m, 2H, 2H of coumarin), 6.24 (s, 1H, 1H at C-15 of coumarin), 4.02 (t, 4H, piperidine), 3.73 (t, 4H, piperidine), 2.75 (s, 2H, -CH_2), 2.47 (d, 3H, -CH_3 of coumarin), 1.92 (t, 1H, -CH, piperidine); ^{13}C NMR: 167.06 (C-2, C-O-C at coumarin linkage), 162.28 (C-4, C-NH at aniline linkage), 161.32 (C-14, C=O at coumarin), 154.49 (C-8, C-O-C at coumarin), 116.02 (C-9), 116.57 (C-11), 118.28 (C-22), 118.32 (C-20), 118.68 (C-15), 118.92 (C-7), 124.11 (C-24), 124.80 (C-12), 128.19 (C-23), 128.32 (C-25), 139.12 (C-21), 142.40 (C-16), 154.39 (C-10), 126.92, 128.82, 128.87, 128.92, 139.93, 141.41 (6C, Ar-C, phenyl ring carbons at piperazine), 115.46 (C-15, C-C=O of coumarin), 30.53, 41.37, 45.22 (5C, piperidine ring carbons), 43.47 (C-33, CH_2 at piperidine linkage).

Table 1. cont.

	IR (KBr, cm^{-1}), ^1H NMR ($CDCl_3$, δ, ppm), ^{13}C NMR ($CDCl_3$, δ, ppm)
6c:	IR: 3292.46 (N-H str.), 1698.15 (C=O of coumarin), 1448.69 (-CH_3 of coumarin), 1288.67 (C-O-C); ^1H NMR: 10.19 (s, 1H, -NH at aniline linkage), 7.70 (d, 1H, 1H at C-12 of coumarin), 7.62–7.25 (m, 5H, Ar-H), 7.16–6.98 (m, 2H, 2H of coumarin), 6.19 (s, 1H, 1H at C-15 of coumarin), 3.81–3.90 (m, 4H, piperidine), 2.78 (br s, 2H, -CH_2, piperidine), 2.47 (d, 3H, -CH_3 of coumarin), 2.11 (q, 2H, piperidine), 1.90 (d, 6H, 2-CH_3); ^{13}C NMR: 166.97 (C-2, C-O-C at coumarin linkage), 163.08 (C-4, C-NH at aniline linkage), 160.57 (C-14, C=O at coumarin), 154.52 (C-8,C-O-C at coumarin), 116.92 (C-9), 118.51 (C-11), 118.77 (C-22), 118.79 (C-20), 118.81 (C-15), 118.84 (C-7) ,123.54 (C-24), 124.85 (C-12), 128.80 (C-23), 128.85 (C-25), 139.41 (C-21), 141.45 (C-16), 153.92 (C-10), 115.32 (C-15, C-C=O of coumarin), 31.43, 44.51, 46.66 (5C, piperidine ring carbons), 21.97 (C-33,C-34, -CH_3).
6d:	IR: 3284.88 (N-H str.), 1700.31 (C=O of coumarin), 1448.59 (-CH_3 of coumarin), 1278.85 (C-O-C); ^1H NMR: 10.20 (s, 1H, -NH at aniline linkage), 7.86 (d, 1H, 1H at C-12 of coumarin), 7.79–7.33 (m, 15H, Ar-H), 7.23–7.06 (m, 2H, 2H of coumarin), 6.21 (s, 1H, 1H at C-15 of coumarin), 4.52 (s, 1H, N-CH at piperazine linkage), 3.34 (br s, 8H, piperazine ring), 2.48 (d, 3H, -CH_3 of coumarin); ^{13}C NMR: 167.31 (C-2, C-O-C at coumarin linkage), 162.06 (C-4, C-NH at aniline linkage), 161.99 (C-14, -C=O at coumarin), 155.59 (C-8, C-O-C at coumarin), 117.50 (C-9), 117.75 (C-11), 118.82 (C-22), 118.54 (C-20), 118.85 (C-15), 118.87 (C-7), 123.68 (C-24), 124.63 (C-12), 128.69 (C-23), 128.78 (C-25), 139.99 (C-21), 153.01 (C-16), 153.87 (C-10), 126.32 126.52, 127.77, 130.19, 132.23, 132. 99, 137.74, 139.49, 142.09, 148.37, 149.52, 152.84 (12C, Ar-C, phenyl ring carbons at piperazine), 117.61 (C-15, O=C-C of coumarin), 43.14, 52.24 (4C, piperazine ring carbons), 21.48 (C-18, C-CH_3 of coumarin).
6e:	IR: 3290.46 (N-H str.), 1698.19 (C=O of coumarin), 1447.89 (-CH_3 of coumarin), 1278.82 (C-O-C), 808.12 (-Cl); ^1H NMR: 10.21 (s, 1H, -NH at aniline linkage), 7.82 (d, 1H, 1H at C-12 of coumarin), 7.75–7.36 (m, 14H, Ar-H), 7.27–7.11 (m, 2H, 2H of coumarin), 6.17 (s, 1H, 1H at C-15 of coumarin), 3.39 (br s, 8H, piperazine ring), 2.45 (d, 3H, -CH_3 of coumarin); ^{13}C NMR: 167.45 (C-2, C-O-C at coumarin linkage), 162.96 (C-4, C-NH at aniline linkage), 162.10 (C-14, -C=O at coumarin), 155.63 (C-8, C-O-C at coumarin), 115.02 (C-9), 117.69 (C-11), 118.56 (C-22), 118.68 (C-20), 118.89 (C-15), 118.94 (C-7), 123.71 (C-24), 124.58 (C-12), 128.51 (C-23), 128.87 (C-25), 139.26 (C-21), 153.12 (C-16), 153.80 (C-10), 126.44, 126.62, 127.83, 129.80, 132.14, 132.96, 137.45, 139.73, 141.95, 148.46, 149.58, 153.03 (12C, Ar-C, phenyl ring carbons at piperazine), 116.70 (C-15, O=C-C of coumarin), 43.39, 52.31 (4C, piperazine ring carbons), 21.42 (C-18, C-CH_3 of coumarin).
6f:	IR: 3288.24 (N-H str.), 1700.21 (C=O of coumarin), 1447.91 (-CH_3 of coumarin), 1278.87 (C-O-C), 1156.77 (C-F); ^1H NMR: 10.23 (s, 1H, -NH at aniline linkage), 7.78 (d, 1H, 1H at C-12 of coumarin), 7.71–7.30 (m, 9H, Ar-H), 7.22–7.13 (m, 2H, 2H of coumarin), 6.19 (s, 1H, 1H at C-15 of coumarin), 3.51 (br s, 8H, piperazine ring), 2.41 (d, 3H, -CH_3 of coumarin); ^{19}F NMR –120.58 (s, 1F); ^{13}C NMR: 166.49 (C-2, C-O-C at coumarin linkage), 162.48 (C-4, C-NH at aniline linkage), 162.15 (C-14, -C=O at coumarin), 116.51 (C-9), 117.14 (C-11), 118.10 (C-22), 118.55 (C-20), 118.81 (C-15), 118.85 (C-7), 123.49 (C-24), 124.85 (C-12), 128.77 (C-23), 128.83 (C-25), 140.01 (C-21), 121.13, 125.50, 141.79, 141.83, 141.92, 142.10 (6C, Ar-C, phenyl ring carbons at piperazine), 143.24 (C-16), 158.27 (C-10), 154.33 (C-8, C-O-C at coumarin), 115.62 (C-15, O=C-C of coumarin), 46.52, 49.71 (4C, piperazine ring carbons), 21.48 (C-18, C-CH_3 of coumarin).
6g:	IR: 3292.67 (N-H str.), 1700.36 (C=O of coumarin), 1447.87 (-CH_3 of coumarin), 1278.91 (C-O-C), 1159.18 (C-F); ^1H NMR: 10.21 (s, 1H, -NH at aniline linkage), 7.73 (d, 1H, 1H at C-12 of coumarin), 7.65–7.26 (m, 9H, Ar-H), 7.10–6.96 (m, 2H, 2H of coumarin), 6.31 (s, 1H, 1H at C-15 of coumarin), 3.49 (br s, 8H, piperazine ring), 2.39 (d, 3H, -CH_3 of coumarin); ^{19}F NMR –119.77 (s, 1F); ^{13}C NMR: 167.03 (C-2, C-O-C at coumarin linkage), 162.51 (C-4, C-NH at aniline linkage), 162.20 (C-14, -C=O at coumarin), 116.43 (C-9), 117.20 (C-11), 118.21 (C-22), 118.39 (C-20), 118.73 (C-15), 118.92 (C-7), 123.45 (C-24), 124.51 (C-12), 128.54 (C-23), 128.73 (C-25), 139.99 (C-21), 143.21 (C-16), 158.14 (C-10), 121.56, 125.67, 141.45, 141.66, 141.87, 142.04 (6C, Ar-C, phenyl ring carbons at piperazine), 154.30 (C-8, C-O-C at coumarin), 115.49 (C-15, O=C-C of coumarin), 46.57, 49.68 (4C, piperazine ring carbons), 21.53 (C-18, C-CH_3 of coumarin).

0.01 mol) using 1,4-dioxane (30 mL) as a solvent under microwave irradiation at 180 W power for 2–8 min and the reaction was heated until completion determined by TLC analysis. After completion of the reaction, the solvent was recovered by using vacuum solvent recovery module, the remaining reaction mixture was treated with crushed ice, neutralized by dil. HCl and the precipitate thus obtained was filtered by Buchner funnel by applying vacuum, dried and recrystallized from THF to give 3.22 g

Table 1. cont.

	IR (KBr, cm^{-1}), ^1H NMR ($CDCl_3$, δ, ppm), ^{13}C NMR ($CDCl_3$, δ, ppm)
6h:	IR: 3296.32 (N-H str.), 1698.20 (C=O of coumarin), 1448.54 (-CH_3 of coumarin), 1277.78 (C-O-C), 1162.41 (CF_3); ^1H NMR: 10.23 (s, 1H, -NH at aniline linkage), 7.80 (d, 1H, 1H at C-12 of coumarin), 7.74–7.32 (m, 9H, Ar-H), 7.22–7.11 (m, 2H, 2H of coumarin), 6.28 (s, 1H, 1H at C-15 of coumarin), 3.54 (br s, 8H, piperazine ring), 2.40 (d, 3H, -CH_3 of coumarin); ^{13}C NMR: 167.21 (C-2, C-O-C at coumarin linkage), 163.39 (C-4, C-NH at aniline linkage), 162.26 (C-14, -C=O at coumarin), 116.43 (C-9), 116.52 (C-11), 118.13 (C-22), 118.28 (C-20) 118.75 (C-15), 118.82 (C-7), 123.29 (C-24), 123.58 (C-12), 128.82 (C-23), 128.88 (C-25), 117.21, 120.25, 129.77, 130.12, 131.40, 139.36 (6C, Ar-C, phenyl ring carbons at piperazine), 140.07 (C-21), 151.14 (C-16), 158.14 (C-10), 155.13 (C-8, C-O-C at coumarin), 123.45 (C-39, CF_3), 116.17 (C-15, O=C-C of coumarin), 47.71, 50.36 (4C, piperazine ring carbons), 22.17 (C-18, C-CH_3 of coumarin).
6i:	IR: 3292.43 (N-H str.), 1697.22 (C=O of coumarin), 1447.82 (-CH_3 of coumarin), 1278.65 (C-O-C), 1278.12 (OCH_3); ^1H NMR: 10.21 (s, 1H, -NH at aniline linkage), 7.77 (d, 1H, 1H at C-12 of coumarin), 7.69–7.25 (m, 7H, Ar-H), 7.18–7.03 (m, 2H, 2H of coumarin), 6.34 (s, 1H, 1H at C-15 of coumarin), 3.77, 3.71, 3.66 (s, 9H, $(OCH_3)_3$), 3.57 (br s, 8H, piperazine ring), 2.41 (d, 3H, -CH_3 of coumarin); ^{13}C NMR: 167.40 (C-2, C-O-C at coumarin linkage), 162.12 (C-4, C-NH at aniline linkage), 161.60 (C-14, -C=O at coumarin), 154.19 (C-8, C-O-C at coumarin), 116.23 (C-9), 116.30 (C-11), 118.17 (C-22), 118.23 (C-20), 118.65 (C-15), 118.80 (C-7), 123.32 (C-24), 124.42 (C-12), 128.80 (C-23), 128.83 (C-25), 140.01 (C-21), 143.49 (C-16), 153.92 (C-10), 117.29, 123.54, 124.85, 139.91, 141.12, 141.20 (6C, Ar-C, phenyl ring carbons at piperazine), 115.23 (C-15, O=C-C of coumarin), 62.77 (C-33, N-CH_2 at piperazine linkage), 55.92–59.74 (C-41, C-43, C-45, 3C, -OCH_3), 46.80, 50.10 (4C, piperazine ring carbons), 21.58 (C-18, C-CH_3 of coumarin).
6j:	IR: 3296.12 (N-H str.), 1702.35 (C=O of coumarin), 1448.85 (-CH_3 of coumarin), 1278.71 (C-O-C), 1271.62 (OCH_3); ^1H NMR: 10.18 (s, 1H, -NH at aniline linkage), 7.85 (d, 1H, 1H at C-12 of coumarin), 7.72–7.35 (m, 9H, Ar-H), 7.25–7.14 (m, 2H, 2H of coumarin), 6.23 (s, 1H, 1H at C-15 of coumarin), 3.72 (s, 3H, -OCH_3), 3.44 (br s, 8H, piperazine ring), 2.43 (d, 3H, -CH_3 of coumarin); ^{13}C NMR: 167.42 (C-2, C-O-C at coumarin linkage), 162.08 (C-4, C-NH at aniline linkage), 161.17 (C-14, -C=O at coumarin), 154.16 (C-8, C-O-C at coumarin), 116.10 (C-9), 116.25 (C-11), 118.10 (C-22), 118.44 (C-20), 118.69 (C-15), 118.85 (C-7), 123.47 (C-24), 123.71 (C-12), 128.65 (C-23), 128.89 (C-25), 139.86 (C-21), 117.23, 117.54, 129.05, 143.22, 151.49, 151.53 (6C, Ar-C, phenyl ring carbons at piperazine) 152.14 (C-16), 153.45 (C-10), 115.38 (C-15, O=C-C of coumarin), 56.70 (C-40, -OCH_3), 47.12, 49.74 (4C, piperazine ring carbons), 22.13 (C-18, C-CH_3 of coumarin).

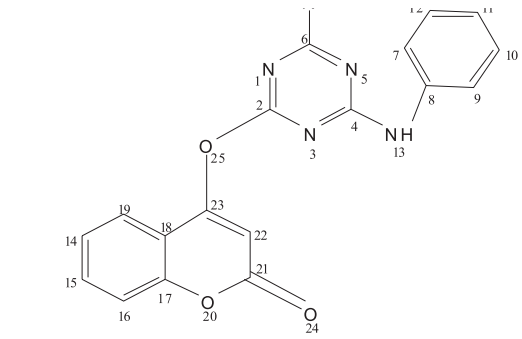
(yield 62%) of **6b** as white colored compound. The same procedure was utilized for synthesis of other final compounds also. The optimized reaction conditions in respect of reaction time and yield are presented in Table 5 for each final nucleophilic reaction.

Antimicrobial activity

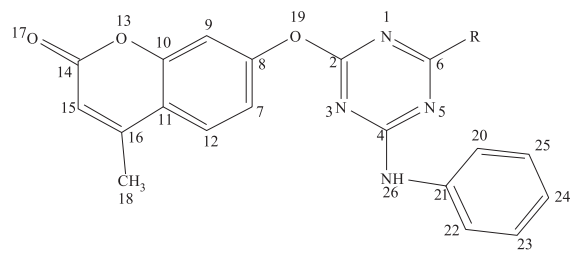
All the novel synthesized s-triazinyl piperazine and piperidine compounds (**5a–j**) and (**6a–j**) were examined for antimicrobial activity against several pathogenic bacterial and fungal strains as 2 Gram-negative bacteria (*MTCC 739 E. coli, MTCC 741 P. aeruginosa*), 2 Gram-positive bacteria (*MTCC 96 S. aureus, MTCC 441 B. subtilis*) and 2 fungal species (*MTCC 183 C. albicans and MTCC 282 A. niger*) using disc diffusion sensitivity test (30). The Mueller-Hinton agar media were sterilized (autoclaved at 120°C for 30 min) and allowed to pour 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 (15 min at 180°C) to ensure even growth of the organisms. The tested compounds were dissolved in dimethyl sulfoxide to get a solution of 3–100 µg/mL concentration. Sterile filter paper discs measuring 6.25 mm in diameter (Whatman no. 1 filter paper), previously soaked in a known concentration of the test compounds in dimethyl sulfoxide were placed on the solidified nutrient agar medium that had been inoculated with the microbials and the plates were incubated for 24 h at 37 ± 1°C. A control disc impregnated with an equivalent amount of dimethyl sulfoxide without any sample was also used and did not reveal any inhibition. Ciprofloxacin and ketoconazole (100 µg/disc) were used as control drugs for antibacterial and antifungal activity, respectively.

Table 2. Characteristic of newly prepared compounds.

Compd. no.	Formula	M.w.	C H N analysis calc. (found) %			M.p. °C
			C	H	N	
5a	$C_{29}H_{26}N_6O_3$	506.56	68.76 (68.67)	5.17 (5.19)	16.59 (16.53)	240–242
5b	$C_{30}H_{27}N_5O_3$	505.57	71.27 (71.22)	5.38 (5.40)	13.85 (13.81)	217–220
5c	$C_{25}H_{25}N_5O_3$	443.50	67.70 (67.63)	5.68 (5.66)	15.79 (15.83)	253–256
5d	$C_{35}H_{30}N_6O_3$	582.65	72.15 (72.21)	5.19 (5.15)	14.42 (14.46)	237–241
5e	$C_{35}H_{29}ClN_6O_3$	617.10	68.12 (68.15)	4.74 (4.69)	13.62 (13.57)	248–250
5f	$C_{28}H_{23}FN_6O_3$	510.52	65.87 (65.92)	4.54 (4.50)	16.46 (16.43)	246–248
5g	$C_{28}H_{23}FN_6O_3$	510.52	65.87 (65.79)	4.54 (4.59)	16.46 (16.55)	252–255
5h	$C_{29}H_{23}F_3N_6O_3$	560.53	62.14 (62.06)	4.14 (4.21)	14.99 (14.91)	277–279
5i	$C_{32}H_{32}N_6O_6$	596.63	64.42 (64.47)	5.41 (5.45)	14.09 (14.16)	259–261
5j	$C_{29}H_{26}N_6O_4$	522.55	66.66 (66.73)	5.02 (5.05)	16.08 (16.17)	236–238
6a	$C_{30}H_{28}N_6O_3$	520.58	69.22 (69.24)	5.42 (5.42)	16.14 (16.22)	250–253
6b	$C_{31}H_{29}N_5O_3$	519.59	71.66 (71.60)	5.63 (5.68)	13.48 (13.42)	258–262
6c	$C_{26}H_{27}N_5O_3$	457.52	68.25 (68.34)	5.95 (5.98)	15.31 (15.20)	260–264
6d	$C_{36}H_{32}N_6O_3$	596.68	72.47 (72.54)	5.41 (5.30)	14.08 (14.12)	268–271
6e	$C_{36}H_{31}ClN_6O_3$	631.12	68.51 (68.45)	4.95 (4.88)	13.32 (13.37)	275–278
6f	$C_{29}H_{25}FN_6O_3$	524.55	66.40 (66.27)	4.80 (4.86)	16.02 (16.06)	273–276
6g	$C_{29}H_{25}FN_6O_3$	524.55	66.40 (66.30)	4.80 (4.83)	16.02 (16.09)	279–282
6h	$C_{30}H_{25}F_3N_6O_3$	574.55	62.71 (62.68)	4.39 (4.44)	14.63 (14.67)	292–295
6i	$C_{33}H_{34}N_6O_6$	610.66	64.91 (16.82)	5.61 (5.56)	13.76 (13.80)	254–257
6j	$C_{30}H_{28}N_6O_4$	536.58	67.15 (67.22)	5.26 (5.22)	15.66 (15.73)	284–288



A



B

Figure 1. Atoms designation for compounds **5a–j** (**A**) and **6a–j** (**B**)

Table 3. Antimicrobial activity of the final **5a–j** compounds.

Compound	*In vitro* antibacterial and antifungal activity – Zone of inhibition in mm (MIC in µg/mL)					
The final compounds containing 4-hydroxy -coumarin **5** (R)	Gram-positive bacterial strains		Gram-negative bacterial strains		Fungal strains	
	S. aureus MTCC96	*B. subtils MTCC441*	*E. coli MTCC739*	*P. aeruginosa MTCC 741*	*A. niger MTCC282*	*C. albicans MTCC183*
5a (*N*-Benzylpiperazine)	19 (50)	19 (50)	17 (50)	20 (50)	20 (50)	19 (50)
5b (4-Benzylpiperidine)	2 (25)	22 (3.12)	21 (50)	21 (25)	19 (50)	19 (50)
5c (3,5-Dimethylpiperidine)	23 (12.5)	23 (3.12)	23 (12.5)	22 (12.5)	19 (50)	20 (25)
5d (Benzhydryl piperazine)	22 (25)	22 (6.25)	20 (50)	22 (12.5)	20 (50)	22 (12.5)
5e (4-Chlorobenzhydryl piperazine)	24 (3.12)	22 (12.5)	22 (25)	24 (6.25)	21 (25)	22 (6.25)
5f (2-Fluorophenyl-piperazine)	23 (6.25)	20 (50)	22 (25)	23 (6.25)	22 (12.5)	20 (25)
5g (4-Fluorophenyl-piperazine)	23 (6.25)	21 (25)	21 (50)	24 (6.25)	22 (12.5)	22 (6.25)
5h (3-Trifluoromethyl-phenylpiperazine)	24 (6.25)	22 (6.25)	23 (12.5)	23 (6.25)	21 (6.25)	23 (6.25)
5i (2,3,4-Trimethoxylbenzyl-piperazine)	23 (12.5)	21 (25)	24 (6.25)	22 (12.5)	22 (6.25)	23 (3.12)
5j [1-(4-Methoxyphenyl)-piperazine)	21 (25)	21 (25)	23 (12.5)	20 (25)	22 (6.25)	22 (3.12)
Ciprofloxacin (100 µg/disc)	29 (≤ 3)	29 (≤ 3)	32 (≤ 3)	33 (≤ 3)	–	–
Ketoconazole (100 µg/disc)					30 (6.25)	33 (≤ 3)
DMSO	–	–	–	–	–	–

The MIC values were evaluated at concentration range 3–100 µg/mL. The table shows the corresponding zone of inhibition in millimeters and MIC values in µg/mL.

To determine the minimum inhibitory concentration (MIC), a stock solution of the synthesized compound (100 µg/mL) in dimethyl sulfoxide was prepared and graded quantities of the test compounds were incorporated in specified quantity of molten sterile agar, nutrient agar for antibacterial and Sabouraud dextrose agar for antifungal activity evaluation. 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. Suspension of the microorganism was prepared to contain approximately 10^5 CFU/mL and applied to plates with serially diluted compounds with required concentration of 3.12–100 µg/mL in dimethyl sulfoxide to be tested and incubated at 37 ± 1°C for 24 and 48 h for bacteria and fungi, respectively.

RESULTS

The results are presented in Tables 3–5.

DISCUSSION AND CONCLUSION

Synthesis of intermediates and target compounds was accomplished according to the steps illustrated in Scheme 1. A series of coumarins such as 4-hydroxycoumarin and 7-hydroxy-4-methylcoumarin based *s*-triazinyl piperazines and piperidines **5a–j** and **6a–j** were synthesized. Two basic synthetic approaches, conventional heating and microwave irradiation were applied for the final nucleophilic substitution reactions. The adoption of microwave irradiation resulted in significant decrease in reaction time. However, the analogues derived by both the methods i.e., conventional heating and microwave method were subjected to analytical characterization as IR, ^{1}H NMR, ^{13}C NMR, ^{19}F NMR and elemental analysis and the results obtained were almost similar for each and every compound for each analysis except some deviation of resonating frequencies of some protons and carbons corresponding to some analogues in the limit

Table 4. Antimicrobial activity of the final **6a–j** compounds.

Compound	*In vitro* antibacterial and antifungal activity – Zone of inhibition in mm (MIC in µg/mL)					
The final compounds containing 7-hydroxy -4-methylcoumarin **6** (R)	Gram-positive bacterial strains		Gram-negative bacterial strains		Fungal strains	
	S. aureus MTCC96	*B. subtils MTCC441*	*E. coli MTCC739*	*P. aeruginosa MTCC 741*	*A. niger MTCC282*	*C. albicans MTCC183*
6a (*N*-Benzylpiperazine)	20 (50)	21 (50)	19 (50)	22 (50)	21 (50)	19 (50)
6b (4-Benzylpiperidine)	21 (25)	23 (3.12)	20 (25)	23 (25)	21 (50)	19 (50)
6c (3,5-Dimethylpiperidine)	23 (12.5)	25 (3.12)	24 (12.5)	23 (12.5)	21 (50)	21 (25)
6d (Benzhydryl piperazine)	23 (25)	22 (6.25)	21 (50)	23 (12.5)	22 (25)	22 (12.5)
6e (4-Chlorobenzhydryl piperazine)	24 (3.12)	22 (12.5)	22 (25)	25 (6.25)	25 (12.5)	23 (6.25)
6f (2-Fluorophenyl-piperazine)	24 (6.25)	24 (6.25)	25 (6.25)	25 (6.25)	24 (12.5)	22 (6.25)
6g (4-Fluorophenyl-piperazine)	25 (3.12)	23 (6.25)	24 (6.25)	25 (6.25)	24 (12.5)	23 (6.25)
6h (3-Trifluoromethyl-phenyl piperazine)	24 (3.12)	24 (6.25)	26 (6.25)	23 (6.25)	24 (6.25)	24 (6.25)
6i (2,3,4-Trimethoxybenzyl-piperazine)	24 (6.25)	23 (12.5)	26 (6.25)	24 (12.5)	24 (6.25)	24 (3.12)
6j [1-(4-Methoxyphenyl) piperazine)	23 (12.5)	23 (12.5)	24 (6.25)	25 (6.25)	25 (6.25)	23 (3.12)
Ciprofloxacin (100 µg/disc)	29 (≤ 3)	29 (≤ 3)	32 (≤ 3)	33 (≤ 3)	–	
Ketoconazole (100 µg/disc)					30 (6.25)	33 (≤ 3)
DMSO	–	–	–	–	–	–

The MIC values were evaluated at concentration range, 3–100 µg/mL. The table shows the corresponding zone of inhibition in millimeters and MIC values in µg/mL.

of ± 0.2 ppm. The best optimum interpreted values of all the characterized analysis are discussed here. The difference in terms of yield were also observed (Table 5) corresponding to both the methods applied.

The first step comprises formation of intermediate **1** in very good yield by the nucleophilic displacement of one chlorine atom of *s*-triazine ring by aniline. Compound **1** was prepared by the reaction between 2,4,6-trichloro-[1,3,5]triazine and aniline in THF with the catalytic amount of triethylamine and stirred for 4 h at 0–5°C. Formation of the product was confirmed by a sharp band at 3296.45 cm^{-1} for -NH stretching in IR spectrum. The ^{1}H NMR data of compound **1** revealed signal between 6.97–7.70 ppm for aromatic protons and singlet at 10.17 ppm for -NH. The disubstituted *s*-triazine intermediate 4-(4-chloro-6-phenylamino-[1,3,5]triazin-2-yloxy)-chromen-2-one **3a** and 7-(4-chloro-6-phenylamino-[1,3,5]triazin-2-yloxy)-4-methyl-chromen-2-one **3b** were obtained by the reaction between (4,6-dichloro-[1,3,5]triazin-2-yl)-phenylamine **1** and 4-substituted coumarin derivatives (**2a** and **2b**) in the presence of 60% NaH at 45–50°C. Linkage of hydroxy group of coumarins with **1** was confirmed by appearance of C-O-C stretching band at 1254.21 cm^{-1} and also a strong band at 1697.21 cm^{-1} for C=O of coumarin. Further, confirmed by ^{13}C NMR spectrum, which showed C=O signals of coumarins around 158.61–162.06 ppm. Subsequent coupling of the so formed compounds (**3a** and **3b**) with the desired piperazines and piperidines under basic conditions in 1,4-dioxane solvent at 70–80°C formed the corresponding target compounds **5a–j** and **6a–j.**

^{19}F NMR spectra for the analogue **5f** and **5h** confirmed the presence of fluorine by giving the corresponding peaks by resonating around –121.67 and –64.70 ppm.

Bioassay indicated that halogenated benzhydryl piperazine bearing final compound with 4-hydroxycoumarin constituent is the most active

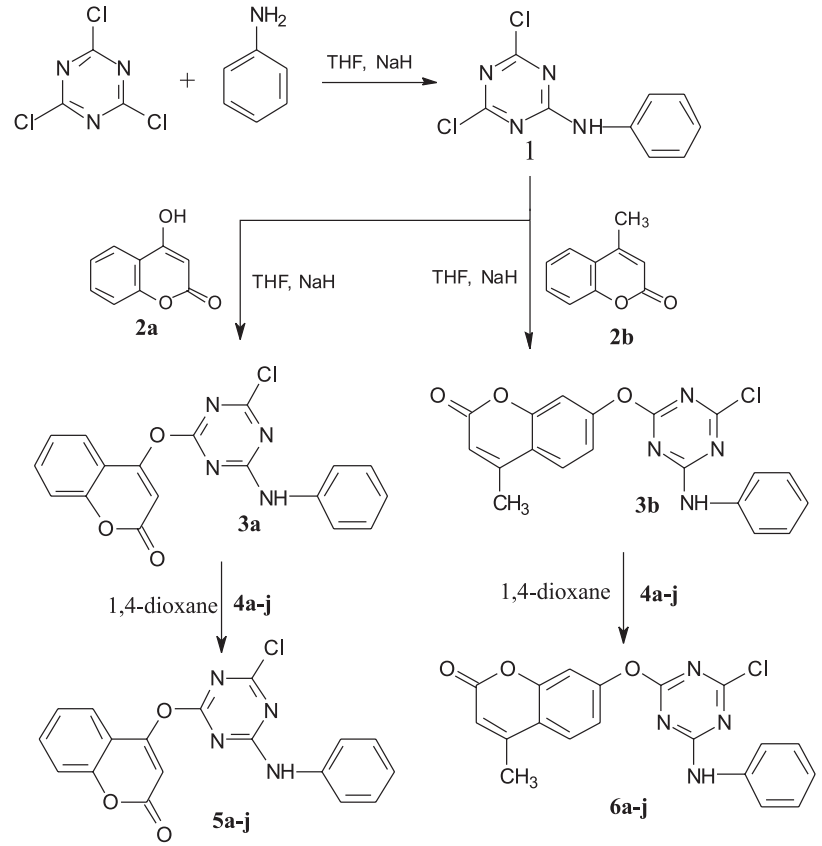
Table 5. Comparison data of microwave and conventional method

Compd. no.	R	Microwave method		Conventional method	
		Reaction time (min)	Yield[a] (%)	Reaction time (h)	Yield[a] (%)
5a	(N-Benzylpiperazine)	2	77	12	66
5b	(4-Benzylpiperidine)	3	84	10	71
5c	(3,5-Dimethylpiperidine)	3	76	13	64
5d	(Benzhydryl piperazine)	2	66	13	55
5e	(4-Chlorobenzhydryl piperazine)	3	81	15	72
5f	(2-Fluorophenylpiperazine)	4	87	15	75
5g	(4-Fluorophenylpiperazine)	3	82	15	74
5h	(3-Trifluoromethylphenyl- piperazine)	4	80	15	69
5i	(2,3,4-trimethoxylbenzyl- piperazine)	5	71	14	60
5j	[1-(4-Methoxyphenyl) piperazine]	4	92	12	83
6a	(N-Benzylpiperazine)	3	73	13	62
6b	(4-Benzylpiperidine)	2	62	12	51
6c	(3,5-Dimethylpiperidine)	3	71	13	65
6d	(Benzhydryl piperazine)	3	60	12	49
6e	(4-Chlorobenzhydryl piperazine)	2	82	14	70
6f	(2-Fluorophenylpiperazine)	3	90	15	79
6g	(4-Fluorophenylpiperazine)	3	88	14	81
6h	(3-Trifluoromethylphenyl piperazine)	5	68	15	59
6i	(2,3,4-trimethoxyl benzyl piperazine)	6	75	15	64
6j	[1-(4-Methoxyphenyl) piperazine)	3	97	13	86

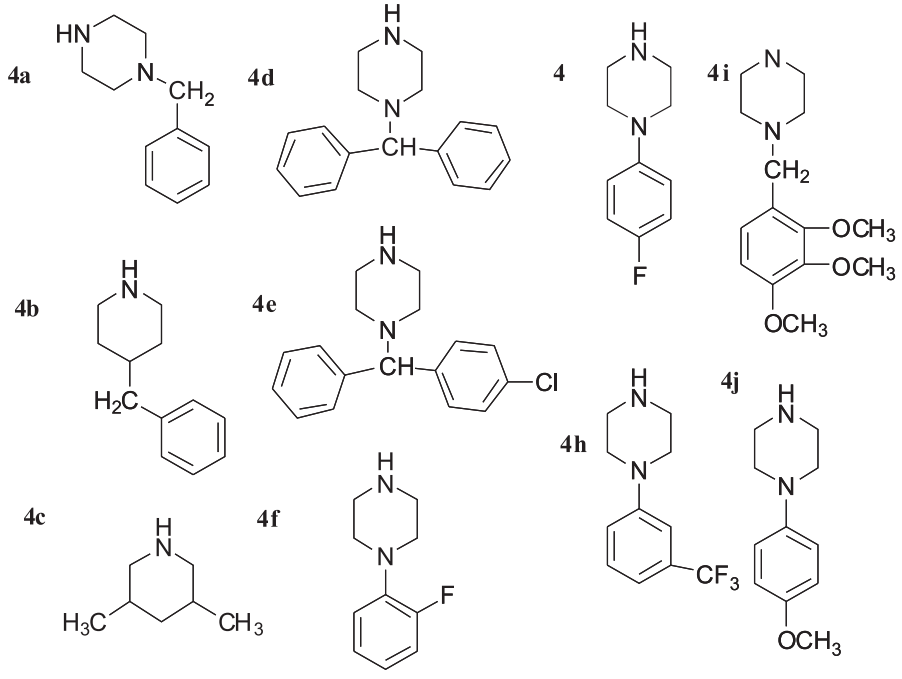
[a]Yields refer to pure products

compound to inhibit Gram-positive strain *S. aureus*. The analogue with trifluoromethyl group incorporation to the 3rd position of phenyl ring of piperazine moiety attached to *s*-triazine core contributes similar efficacy to inhibit Gram-positive *S. aureus* by means of zone of inhibition. On the other hand, when the position of hydroxyl group replaced to 7th position of coumarin ring, along with the presence of methyl group at the 4th position, it significantly increased the potency of 4-fluorophenylated piperazine bearing analogue **6g** against *S. aureus*.

The inhibitors assessment results for *B. subtilis* showed that piperidine analogue is essential to contribute higher potency against this particular strain. The dimethyl substituted analogues **5c** and **6c** displayed promising inhibition against *B. subtilis* with similar value of MIC and increased value of zone of inhibition in case of **6c** containing 7-hydroxy-4-methylcoumarin constituents, whereas, the piperidine analogue **5b** and **6b** containing benzyl piperidine constituent exhibit good inhibitors effect against *B. subtilis*. In case of the potency of final compound analogue against both the Gram negative strains, the halogenated compound as well as methoxy functional group containing compounds proved much more beneficial to possess good activity. The presence of trifluoro or trimethoxy group to the phenyl ring of piperazine moiety with 4-hydroxycoumarin as well as 7-hydroxy-4-methylcoumarin condensation to the *s*-triazine core displayed excellent activities against *E. coli*. Moreover, compounds **6f**, **6g** and **6j** containing single fluorine or methoxy group substitution contribute good inhibition potency against *E. coli* by means of MIC values compared to most potent analogues containing 7-hydroxy-4-methylcoumarin incorporation to *s*-triazine nucleus. Compounds **5e** and **5g** containing fluorine or chlorine atom incorporation to the 4th position of phenyl ring attached to piperazine ring condensed with *s*-triazine nucleus bearing 4-hydroxycoumarin substituent showed better inhibitory action against *P. aeruginosa,* whereas, all the fluorinated analogues along with 4-



4a–j:



Scheme 1. Synthesis of intermediates and target compounds

methoxyphenylpiperazine bearing compound **6j** with 7-hydroxy-4-methylcoumarin condensation to *s*-triazine core proved to be the most potent compounds against *P. aeruginosa*. In addition, antifungal results obtained proved our expectation to inhibit both the fungal strains most potently as compared to standard drug among all the analogues tested as phenyl piperazine moiety functionalized with methoxy functional group(s) proved as an antifungal agents like terconazole. Compounds **5i** and **6i,** with three methoxy functional groups on phenyl ring at piperazine entity with 4-hydroxycoumarin as well as 7-hydroxy-4-methylcoumarin incorporation, respectively, significantly inhibit both the strains of analyzed fungi.

In conclusion, the objective of the present study was to synthesize and investigate the antimicrobial activities of a new series of coumarin based *s*-triazinyl piperazines and piperidines with the hope of discovering new structure leads serving as antimicrobial agents. The preparation procedure follows for these compounds by microwave method offers reduction in the reaction time, excellent yields without formation of undesirable products, operation simplicity, cleaner reaction and easy work-up. Hence, it is a viable and feasible method for performing the synthesis of drug, intermediates and chemicals. Antimicrobial studies showed that the antifungal and antibacterial potency was mainly influenced by the functional groups on phenyl ring attached at piperazine and piperidine ring. Higher inhibitory effects observed in this study appear to be dependent on the electron withdrawing group like chloro, fluoro and also on electron donating group like methyl, methoxy functionality to the phenyl ring at piperazine and piperidine moiety. Hence, there is enough scope for further study in developing such compounds as a good lead activity.

REFERENCES

1. Davies J.: Nature 383, 219 (1996).
2. Chu D.T.W., Plattner J.J., Katz L.: J. Med. Chem. 39, 3853 (1996).
3. Grare M., Mourer M., Fontanay S., Regnouf-de-Vains J.B., Finance C., Duval R.E.: J. Antimicrob. Chemother. 60, 575 (2007).
4. Zhou C., Min J., Zhigang L., Anne Y., Heather D., Tian G., Young-Tae C., Neville R.K.: Biorg. Med. Chem. Lett. 18, 1308 (2008).
5. Srinivas K., Srinivas U., Bhanuprakash K., Harakishore K., Murthy U.S.N., Jayathirtha R.V.: Eur. J. Med. Chem. 41, 1240 (2006).
6. Patel A.C., Mahajan D.H., Chikhalia K.H.: Phosphorus Sulfur 185, 368 (2010).
7. Alessandro B., Gorka J.B., Mhairi L.S., Vanessa Y., Reto B., Michael P.B., Ian H.G.: J. Med. Chem. 48, 5570 (2005).
8. Rita M., Simona S., Giovanni S., Francesca V., Lisa D.V.: J. Med. Chem. 47, 4649 (2004).
9. Sergio M., Davide P., Paolo C., Nicoletta B., Diego M.: ChemMedChem 3, 873 (2008).
10. Yuan-Zhen X., Fen-Er C., Jan B., Erik D.C., Christophe P.: Eur. J. Med. Chem. 43, 1230 (2008).
11. Braun A.E., Gonzalez A.G.: Nat. Prod. Rep. 14, 465 (1995).
12. Hussain M., Hussain M.T., Rama N.H., Hameed S., Malik A., Khan K.M.: Nat. Prod. Res. 17, 207 (2003).
13. Satyanarayana V.S., Sreevani P., Sivakumar A., Vijayakumar V.: Arkivoc XVII, 221 (2008).
14. Khalid M.K., Zafar S.S., Muhammad Z.K., Zia-Ullah, Iqbal Choudhary M., Atta-ur-Rahman, Shahnaz P., Zahid H.C., Claudiu T.S.: J. Enzym. Inhib. Med. Chem. 19, 373 (2004).
15. Emmanuel-Giota A.A., Fylaktakidou K.C., Hadjipavlou-Litina D.J., Litinas K.E., Nicolaides D.N.: J. Hetrocycl. Chem. 38, 717 (2001).
16. Khalid M.K., Nida A., Uzma R.M., Saima J., Shahnaz P., Iqbal Choudhary M.: Eur. J. Med. Chem. 45, 4058 (2010).
17. Manolov I., Danchev N.D.: Eur. J. Med. Chem. 30, 531 (1995).
18. Raev L., Voinov E., Ivanov I., Popov D.: Pharmazie 45, 696 (1990).
19. Kotali A., Lafazanis I.S., Papageorgiou A., Chrysogelou E., Lialiaris T., Sinakos Z.: Molbank m574, 1 (2008).
20. Haugland R.P.: Handbook of fluorescent probes and research products. 9 edn., Molcular Probes Inc., Eugene 2002
21. Kirkiacharian S., Thuy D., Sicsic S., Bakhchinian R., Kurkjian R., Tonnaire T.: Farmaco 57, 703 (2002).
22. Mahajan D., Pannecoque C., De Clercq E., Chikhalia K.: Arch. Pharm. 342, 281 (2009).
23. Kaswala P.B., Chikhalia K.H., Shah N.K., Patel D.P., Patel D.H., Mudaliar G.V.: Arkivoc XI, 326 (2009).
24. Tyagi B., Mishra M.K., Jasra R.V.: J. Mol. Catal. A-Chem. 286, 41 (2008).
25. Kultti A., Pasonen S., Jauhiainen M., Rilla K.J., Karna R., Pyöriä E., Tammi R., Tammi M.: Exp. Cell Res. 315, 1914 (2009).
26. Kerns R. J., Rybak M. J., Kaatz G. W., Vaka F., Cha R., Grucz R. G., Diwadkar V. U.: Biorg. Med. Chem. Lett. 13, 2109 (2003).

27. Ryckebusch A., Deprez-Poulain R., Maes L., Debreu-Fontaine M.A., Mouray E., Grellier P., Sergheraert C.: J. Med. Chem. 46, 542 (2003).
28. Ram Shankar U., Neelima S., Sanjay J., Nawal K., Ramesh C., Sudershan A.: Biorg. Med. Chem. 12, 2225 (2004).
29. Baikenova G.G., Abdulina G.A., Gazaliev A.M., Fazylov S.D., Kudaibergenova S.Z.: Pharm. Chem. J. 38, 19 (2004).
30. Cruickshank R., Duguid J.P., Marion B.P., Swain R.H.A.: Medical Microbiolgy. 12th edn., Churchil Livingstone, London 1975.

Received: 18. 05. 2011