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Synthesis and in vitro antimicrobial studies of medicinally important novel *N*-alkyl and *N*-sulfonyl derivatives of 1-[bis(4-fluorophenyl)-methyl]piperazine

J. N. Narendra Sharath Chandra, C. T. Sadashiva, C. V. Kavitha and K. S. Rangappa*

Department of Studies in Chemistry, University of Mysore, Manasagangothri, Mysore 570006, India

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Abstract—A series of novel substituted 1-[bis(4-fluorophenyl)-methyl]piperazine derivatives (**4a–g**) and (**5h–m**) have been synthesized. The synthesized compounds were characterized by IR and ^{1}H NMR. All the synthesized compounds were evaluated in vitro for their efficacy as antimicrobial agents against representative strains of Gram-positive (*Staphylococcus aureus* ATCC 25953, *Streptococcus pneumoniae* ATCC 49619, *Bacillus cereus* 11778, and *Bacillus subtilis* 6051) and Gram-negative bacteria (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 2853, *Proteus vulgaris* ATCC 2853, and *Salmonella typhi* ATCC 9484) by paper disc diffusion and microdilution methods. Among the newly synthesized compounds **4e**, **5l**, and **5m** showed potent antimicrobial activities, when compared to the standard drug.

1. Introduction

Microbial diseases are now more frequent than they used to be during first half of the century, and are still difficult to be diagnosed clinically. In order to combat them, a number of several synthetic and semi-synthetic antibacterial drugs are used in clinical practice such as sulfonamides, nitrofuranes, penicillines, cephalosporins, tetracycline's macrolides, and oxazolidinones. There has been much progress in this field.[1,2] In spite of many significant improvements in antibacterial therapy, many problems remain to be solved for most antimicrobial drugs available. For instance, appearance of multidrug resistance Gram-positive bacteria, in particular, methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant *Enterococci* is a serious menace. The use of amphotericin B known as the 'gold standard' is limited by nephrotoxicity.[3,4] These problems highlight the need for the advent of safe, novel, and effective antibacterial compounds. Diphenyl piperazine derivatives possess broad pharmacological action on central nervous system (CNS), especially on dopaminergic neurotransmission.[5] Piperazine and substituted piperazine are important pharmacophores that can be found in many marketed drugs, such as the HIV protease inhibitor crixivan[6–8] and the other drugs under development. Piperazinyl-linked ciprofloxacine dimers are reported to be potent antibacterial agents against resistant strains, a novel class of mixed D_2/D_4 receptor antagonists, dual calcium antagonist, antimalarial agents, and potential antipsychotic agents.[9] Recently, piperazine derivatives containing tetrazole nucleus have been reported as antifungal agents.[10]

Sulfonamides are among the most widely used antibacterial agents in the world, chiefly because of their low cost, low toxicity, and excellent activity against common bacterial disease. The synergetic action of sulfonamides with trimethoprim has brought about enormous resurgence of sulfonamide usage everywhere over the last decade and the most common side effects of this class of drugs are nausea, vomiting, diarrhea, anorexia, and hypersensitivity reaction.[11] Piperazine sulfonamides exhibit diverse pharmacological activity such as MMP-3 inhibition, antibacterial activity, and carbonic anhydrase inhibition.[12] This prompted us to synthesize new derivatives of piperazine sulfonamides (**5h–m**) with a view to find the efficacy of these piperazine sulfonamides as antibacterial. Diphenyl-attached piperazine is expected to increase antimicrobial activity probably by

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* Corresponding author. Tel.: +91 821 2419661; fax: +91 821 2412191; e-mail addresses: rangappaks@gmail.com; rangappaks@chemistry.uni-mysore.ac.in

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enhancing lipophilicity of molecule, and fluoro-attached diphenyl ring is smaller substituent (as compared to hydrogen) on aromatic diphenyl ring, that decreases molecular refractivity (MR).[13] Recently, we have reported the synthesis and antimicrobial studies of bioactive amides[14], N-alkylation of heterocyclic compounds.[15] In continuation of our research on the synthesis of N-alkylation of bioactive heterocycles herein, we have described the synthesis of *N*-alkyl and *N*-sulfonyl derivatives of 1-[bis(4-fluorophenyl)-methyl]piperazine along with their in vitro antimicrobial activity by paper disc diffusion and microdilution methods.

2. Chemistry

The reaction of 1-[bis(4-fluorophenyl)-methyl]piperazine **1** with different alkyl halides (R-X) was carried out in presence of powdered potassium carbonate and *N*,*N*-dimethylformamide as solvent. Whereas the reaction of alkyl, aryl sulfonyl halides (R-SO_2-X) with **1** were carried out in presence of triethylamine and dichloromethane as solvent. The schematic diagram for the desired compounds (**4a**–**g**) and (**5h**–**m**) is depicted in Scheme 1. The chemical structures and physical data of all the synthesized compounds are given in Table 1.

3. Results and discussion

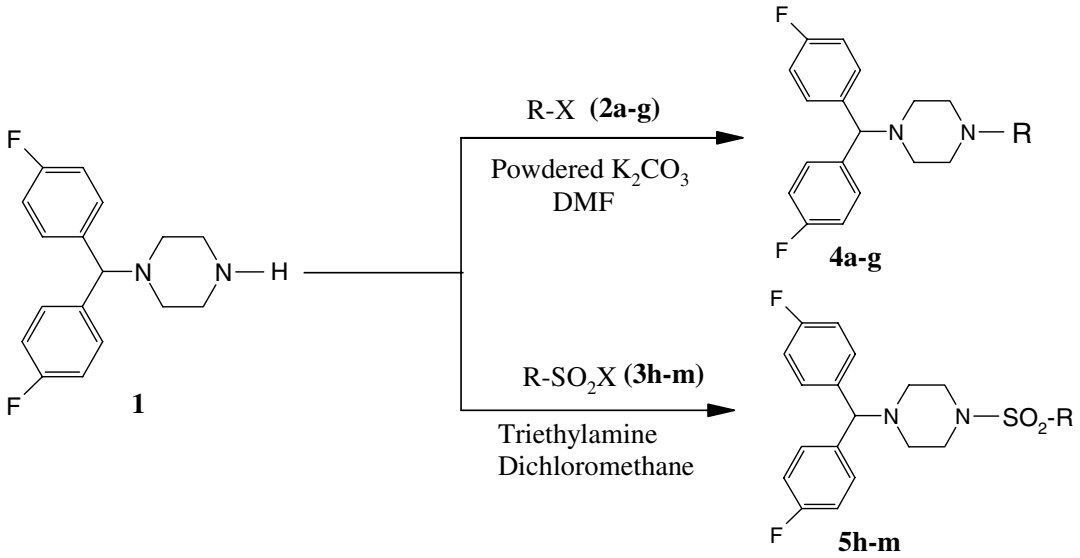
3.1. Chemistry

The nucleophilic substitution reaction of 1-[bis(4-fluorophenyl)-methyl]piperazine **1** with different alkyl halides (R-X) was carried out in presence of mild base potassium carbonate in different solvents (acetonitrile, ethanol, *N*, and *N*-dimethylformamide) to improve the yield. Among the different solvents used, *N*,*N*-dimethylformamide helps a better nucleophilic substitution reaction for the series of compounds (**4a**–**g**). The presence of N–H proton at 2.2 δ value in starting material piperazine **1** and the absence of this proton peak in proton NMR spectra confirm our products (**4a**–**g**). It is also confirmed by IR data, which showed terminal methyl group bending absorption at 1350 cm^{-1} and methylene group absorption at 1460 cm^{-1} (except for **4a**).

The alkyl and aryl sulfonamides (**5h**–**m**) were synthesized by the reaction of 1-[bis(4-fluorophenyl)-methyl]piperazine **1** with different alkyl and aryl sulfonyl chlorides (R-SO_2Cl) in presence of triethylamine in dichloromethane solvent. Unlike alkyl series (**4a**–**g**) in sulfonamide series (**5h**–**m**), we observed the disappearance of N–H proton peak in ^{1}H NMR. Apart from this, IR data showed asymmetric stretching of S=O at 1320 cm^{-1}, bending absorption at 1370 cm^{-1} for terminal methyl group (**5h**, **5j** and **5k**), and absorption at 1050 cm^{-1} for chloroaryl group (**5l** and **5m**). We obtained all the products (**4a**–**g**) and (**5h**–**m**) in good yield.

3.2. Antimicrobial activity

N-Alkyl and *N*-sulfonyl piperazine derivatives (**4a**–**g**) and (**5h**–**m**) were synthesized and screened for their efficacy as antimicrobials against various pathogens in vitro by paper disc diffusion and microdilution methods. Streptomycin was used as a positive control against both Gram-positive and Gram-negative bacteria. The results

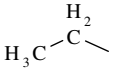
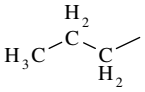
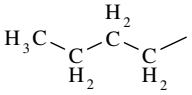
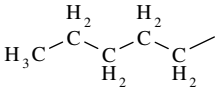
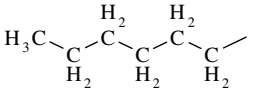
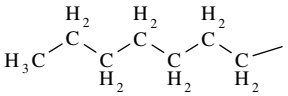
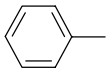
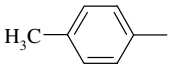
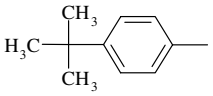
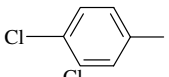
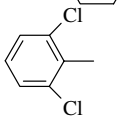


Where R-X= methyl iodide, **2a**; propyl bromide, **2c**; pentyl bromide, **2e**; heptyl bromide, **2g** ; ethyl iodide, **2b**; butyl bromide, **2d**; hexyl bromide, **2f**;

R-SO_2-X methane sulfonyl chloride, **3h**; benzene sulfonyl chloride, **3i;** 4-methyl benzenesulfonyl chloride, **3j;** 4-tert-butyl benzenesulfonyl chloride, **3k**; 4-chloro benzenesulfonyl chloride, **3l**; 2,6-dichloro benzenesulfonyl chloride, **3m**;

Scheme 1.

Table 1. Physical data of bioactive piperazine derivatives (**4a**–**g**) and (**5h**–**m**)

Compound	R	Yield (%)	Mp °C
4a		82	Oily
4b		78	Oily
4c		79	Oily
4d		83	Oily
4e		75	Oily
4f		82	Oily
4g		76	Oily
5h		82	155–157
5i		83	163–165
5j		85	162–164
5k		78	138–141
5l		80	173–175
5m		82	178–181

of our antibacterial studies of all the synthesized compounds are depicted in Tables 2 and 3.

Among the alkyl piperazines (**4a**–**g**), compound **4e** showed significant inhibitory activity against both Gram-positive (zone of inhibition 14–20 mm) and Gram-negative (zone of inhibition 16–20 mm) bacteria. Compound **4a** showed less activity (zone of inhibition 5–7 mm) against Gram-positive bacteria and Gram-negative bacteria (5–8 mm inhibition zone). Similarly, other derivatives **4b**, **4c**, and **4d** showed a moderate to good activity. Compound **4f** showed least activity and **4g** showed no antibacterial activity at all. From the results obtained, it reveals that the antibacterial activity increases proportionately as the length of the carbon chain increases up to 5 atoms, beyond which antibacterial activity decreases dramatically. This could be due to bulkiness of the carbon chain, which render the molecule unable to penetrate through the cell wall of the bacteria. The tested compounds (**4a**–**g**) showed relatively better activity against Gram-negative as compared to Gram-positive bacteria. This is probably due to the lipophilic alkyl chain that helps the molecule to penetrate through the lipid cell membrane of Gram-negative bacteria. Compound **4e** showed observable antibacterial activity in least concentration of 48 μg/mL against Gram-negative bacteria *Pseudomonas aeruginosa.*

Alkyl and aryl sulfonyl piperazines (**5h**–**m**) showed antibacterial activity relatively better than alkyl piperazine (**4a**–**g**) against both Gram-positive and Gram-negative bacteria. Compounds **5l** and **5m** showed significant antibacterial activity (zone of inhibition 20–28, 20–29 mm, respectively) as compared to standard streptomycin (zone of inhibition 20–24 mm) and rest of the derivatives (**5h**, **5i**, **5j**, and **5k**) which showed moderate to good activity. Significant antibacterial activity of **5l** and **5m** could be attributed to chloroaryl moiety. In general sulfonamides are effective, probably because of sulfonyl linkage as supported by many active sulfonamide drugs. Compound **5j** which is the most potent among sulfonamide series (**5h**–**m**) showed observable antibacterial activity against *Escherichia coli* at 98 μg/mL, *P. aeruginosa* at 46 μg/mL, and *Proteus vulgaris* at 54μg/mL. The structures of the potent antibacterials are shown in Figure 1.

4. Conclusion

In conclusion, a series of novel 1-[bis(4-fluorophenyl)-methyl]piperazine derivatives (**4a**–**g**) and (**5h**–**m**) were synthesized and their antimicrobial activities have been evaluated. Compounds **4e**, **5l**, and **5m** demonstrated potent inhibition against all the strains tested. Further research in this area is in progress in our laboratory.

5. Experimental

The melting point was recorded on a SELACO-650 hot stage apparatus and is uncorrected. IR (KBr) spectra were recorded on a Jasco FT/IR-4100 Fourier transform infrared spectrometer, ^{1}H NMR were recorded on a Shimadzu AMX spectrometer by using $CDCl_3$ as solvent and TMS as an internal standard (Chemical shift in ppm). TLC was conducted on 0.25 mm silica gel plates ($60F_{254}$, Merck). Visualization was made with ultraviolet light. All extracted solvents were dried over anhydrous Na_2SO_4 and evaporated with a BUCHI rotary evaporator. Reagents were obtained commercially and used as received.

5.1. General procedure for the synthesis of 1-[bis(4-fluorophenyl)-methyl]piperazine derivatives (4a–g)

To a solution of 1-[bis(4-fluorophenyl)-methyl]piperazine **1** (1 g, 1 equiv) in DMF, were added alkyl halides (R-X) (1.2 equiv) and powdered potassium carbonate

Table 2. Inhibition zone (diameter) in mm of synthesized compounds against tested bacterial strains by paper disc diffusion method

Compound	Gram-positive bacteria				Gram-negative bacteria			
	S. aureus ATCC 25953	*S. pneumoniae* ATCC 49619	*B. cereus* 11778	*B. subtilis* 6051	*E. coli* ATCC 25922	*P. aeruginosa* ATCC 2853	*P. vulgaris* ATCC 2853	*S. typhi* ATCC 9484
4a	6	4	5	7	7	6	5	8
4b	8	5	6	3	9	10	12	4
4c	11	13	12	14	12	14	13	15
4d	15	12	14	11	17	18	16	14
4e	18	15	16	14	20	19	17	16
4f	3	4	2	3	4	6	7	5
4g	—	—	—	—	—	—	—	—
5h	14	11	13	10	15	16	17	14
5i	12	14	11	12	13	15	18	10
5j	16	13	12	15	18	17	13	17
5k	19	17	18	14	17	15	16	15
5l	25	20	22	21	28	23	25	20
5m	28	24	22	20	29	26	21	24
Streptomycin	24	21	22	23	22	20	23	22

Zone of inhibition in mm.

Table 3. Minimum inhibitory concentration (MIC) in μg/mL of the synthesized compounds against tested bacterial strains by microdilution method

Compound	Gram-positive bacteria				Gram-negative bacteria			
	S. aureus ATCC 25953	*S. pneumoniae* ATCC 49619	*B. cereus* 11778	*B. subtilis* 6051	*E. coli* ATCC 25922	*P. aeruginosa* ATCC 2853	*P. vulgaris* ATCC 2853	*S. typhi* ATCC 9484
4a	180	202	132	210	174	110	39	152
4b	159	174	78	125	58	92	96	69
4c	98	108	99	104	167	154	48	180
4d	139	162	105	140	94	71	100	—
4e	30	35	40	38	56	48	51	64
4f	330	340	405	400	438	451	418	428
4g	—	—	—	—	—	—	—	—
5h	106	171	184	167	122	103	133	120
5i	169	155	193	185	84	135	62	135
5j	201	143	119	123	98	46	54	84
5k	128	139	129	115	120	20	84	104
5l	146	158	144	165	112	84	36	169
5m	154	165	150	148	110	108	20	139
Streptomycin	157	210	234	134	154	178	147	195

MIC in μg/mL.

(5 equiv). The reaction mixture was stirred at room temperature for about 10–15 h (completion of the reaction was monitored by TLC using chloroform/methanol = 4.5:0.5). After the completion of the reaction, the reaction mass was quenched with distilled water and extracted with ethylacetate (3× 15 mL). Finally, the combined organic layer was washed with distilled water again and dried over anhydrous Na_2SO_4. After removal of the solvent in vacuo, the oily residue was purified by column chromatography.

5.2. General procedure for the synthesis of 1-[bis(4-fluorophenyl)-methyl]piperazine derivatives (5h–m)

To a solution of 1-[bis(4-fluorophenyl)-methyl]piperazine **1** (1 g, 1 equiv) and triethylamine (3 equiv) in dry dichloromethane at 0 °C were added alkyl and aryl sulfonyl chlorides (1.2 equiv). The reaction mixture was stirred at 0 °C for about 2 h and the stirring was continued at room temperature for about 4–5 h (completion of the reaction was monitored by TLC). After the completion of the reaction, the reaction mass was quenched with distilled water and extracted with dichloromethane (3× 15 mL). Finally, the combined organic layer was washed with distilled water again and dried over anhydrous Na_2SO_4. After removal of the solvent in vacuo, the residue was purified by recrystallization.

5.2.1. Synthesis of 1-[bis(4-fluorophenyl)-methyl]-4-methyl piperazine (4a). The general synthetic method described above affords **4a**, as a pale yellowish oil from piperazine **1** (1 g, 3.47 mmol) and methyl iodide **2a** (0.5913 g, 4.17 mmol).

IR ν_{max} (KBr): 1600, 1223, 1350 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz,): 7.1 (dd, 4H, J = 1.9 Hz, Ar–H); 7.3 (dd, 4H, J = 1.9 Hz, Ar–H); 4.4 (s, 1H, –CH–); 2.4 (s, 8H, $–CH_2–$); 2.2(s, 3H, $–N–CH_3$).

5.2.2. Synthesis of 1-[bis-(4-fluorophenyl)-methyl]-4-ethyl piperazine (4b). The general synthetic method described above affords **4b**, as a pale yellowish oil from piperazine **1** (1g, 3.47 mmol) and ethyl iodide **2b** (0.6499 g, 4.17 mmol).

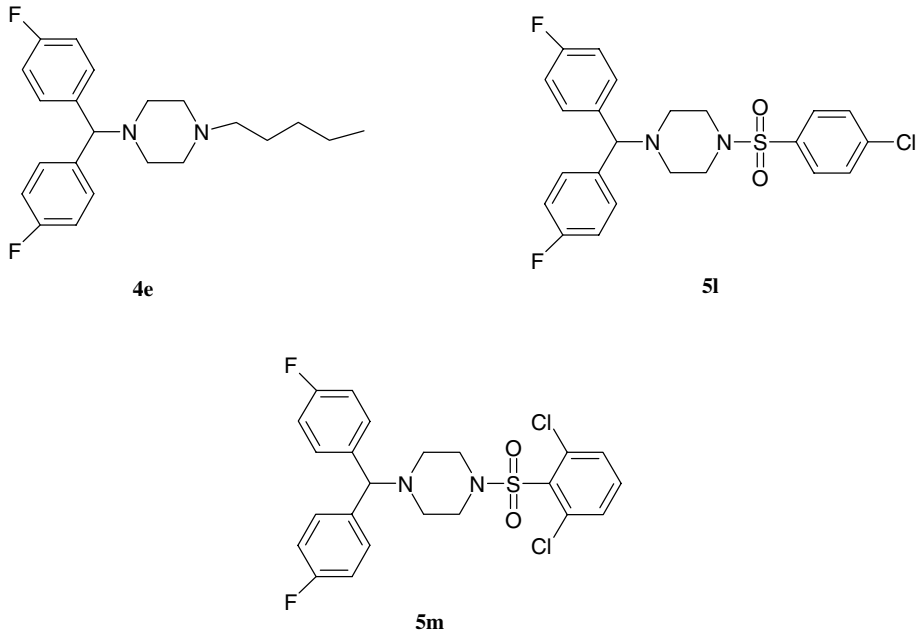


Figure 1. The structures of the potent antibacterials.

IR ν_{max} (KBr): 1600, 1223, 1460, 1350 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz,): 7.1 (dd, 4 H, J = 1.9Hz, Ar–H); 7.3 (dd, 4H, J = 1.9Hz, Ar–H); 4.4 (s, 1H, –CH–); 2.4 (s, 8H, $–CH_2–$); 2.3–2.4 (q, 2H, $–N–CH_2–$); 0.9–1.0 (t, 3H, $–CH_3$).

5.2.3. Synthesis of 1-[bis-(4-fluorophenyl)-methyl]-4-propyl piperazine (4c). The general synthetic method described above affords **4c**, as a pale yellowish oil from piperazine **1** (1 g, 3.47 mmol) and propyl bromide **2c** (0.5124 g, 4.17 mmol).

IR ν_{max} (KBr): 1600, 1223, 1460, 1350 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz,): 7.1 (dd, 4H, J = 1.9 Hz, Ar–H); 7.3 (dd, 4H, J = 1.9 Hz, Ar–H); 4.4 (s, 1H, –CH–); 2.4 (s, 8H, $–CH_2–$); 2.3–2.4 (t, 2H, $–N–CH_2–$); 1.1–1.5 (m, 2H, $–CH_2–$); 0.8–1.0 (t, 3H, $–CH_3$).

5.2.4. Synthesis of 1-[bis-(4-fluorophenyl)-methyl]-4-butyl piperazine (4d). The general synthetic method described above affords **4d**, as a pale yellowish oil from piperazine **1** (1 g, 3.47 mmol) and butylbromide **2d** (0.5704 g, 4.17 mmol).

IR ν_{max} (KBr): 1600, 1223, 1460, 1350 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz,): 7.1 (dd, 4H, J = 1.9 Hz, Ar–H); 7.3 (dd, 4H, J = 1.9 Hz, Ar–H); 4.4 (s, 1H, –CH–); 2.4 (s, 8H, $–CH_2–$); 2.3–2.4 (t, 2H, $–N–CH_2–$); 1.3–1.6 (m, 2H, $–CH_2–$); 1.1–1.3 (m, 2H, $–CH_2–$); 0.8–1.0 (t, 3H, $–CH_3$).

5.2.5. Synthesis of 1-[bis-(4-fluorophenyl)-methyl]-4-pentyl-piperazine (4e). The general synthetic method described above affords **4e**, as a pale yellowish oil from piperazine **1** (1 g, 3.47 mmol) and pentylbromide **2e** (0.6292 g, 4.17 mmol).

IR ν_{max} (KBr): 1600, 1223, 1460, 1350, 1720 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz,): 7.1 (dd, 4H, J = 1.9 Hz, Ar–H); 7.3 (dd, 4H, J = 1.9 Hz, Ar–H); 4.4 (s, 1H, –CH–); 2.4 (s, 8H, $–CH_2–$); 2.3–2.4 (t, 2H, $–N–CH_2–$); 1.3–1.6 (m, 4H, $–CH_2–$); 1.1–1.3 (m, 2H, $–CH_2–$); 0.8–0.9 (t, 3H, $–CH_3$).

5.2.6. Synthesis of 1-[bis-(4-fluorophenyl)-methyl]-4-hexyl-piperazine (4f). The general synthetic method described above affords **4f**, as a pale yellowish oil from piperazine **1** (1 g, 3.47 mmol) and hexylbromide **2f** (0.6874 g, 4.17 mmol).

IR ν_{max} (KBr): 1600, 1223, 1460, 1350, 1720 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz,): 7.1 (dd, 4 H, J = 1.9 Hz, Ar–H); 7.3 (dd, 4H, J = 1.9 Hz, Ar–H); 4.4 (s, 1H, –CH–); 2.4 (s, 8H, $–CH_2–$); 2.3–2.4 (t, 2H, $–N–CH_2–$); 1.3–1.5 (m, 4H, $–CH_2–$); 1.1–1.25 (m, 4H, $–CH_2–$); 0.8–0.95 (t, 3H, $–CH_3$).

5.2.7. Synthesis of 1-[bis-(4-fluorophenyl)-methyl]-4-heptyl-piperazine (4 g). The general synthetic method described above affords **4g**, as a pale yellowish oil from piperazine **1** (1 g, 3.47 mmol) and heptylbromide **2g** (0.7462 g, 4.17 mmol).

IR ν_{max} (KBr): 1600, 1223, 1460, 1350 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz,): 7.1 (dd, 4H, J = 1.9 Hz, Ar–H); 7.3 (dd, 4H, J = 1.9 Hz, Ar–H); 4.4 (s, 1H, –CH–); 2.4 (s, 8H, $–CH_2–$); 2.3–2.4 (t, 2H, $–N–CH_2–$); 1.35–1.55 (m, 4H, $–CH_2–$); 1.1–1.3 (m, 6H, $–CH_2–$), 0.9–1.0 (t, 3H, $–CH_3$).

5.2.8. Synthesis of 1-[bis-(4-fluorophenyl)-methyl]-4-methane sulfonyl piperazine (5h). The general synthetic method described above affords **5h**, as colorless crystal-

line solid from piperazine **1** (1 g, 3.47 mmol) and methanesulfonyl chloride **3 h** (0.4770 g, 4.17 mmol).

IR ν_{max} (KBr): 1600, 1223, 1320, 1370, 3250 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz,): 7.1 (dd, 4H, $J = 1.9$ Hz, Ar–H); 7.3 (dd, 4H, $J = 1.9$ Hz, Ar–H); 4.4 (s, 1H, –CH–); 2.4 (s, 8H, $–CH_2–$); 2.6 (s, 3H, $–SO_2–CH_3$).

5.2.9. Synthesis of 1-benzenesulfonyl-4-[bis-(4-fluorophenyl)-methyl]piperazine (5i). The general synthetic method described above affords **5i**, as a colorless crystalline solid from piperazine **1** (1 g, 3.47 mmol) and benzenesulfonyl chloride **3i** (0.7354 g, 4.17 mmol).

IR ν_{max} (KBr): 1600, 1223, 1320, 3250 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz,): 7.1 (dd, 4H, $J = 1.9$ Hz, Ar–H); 7.3 (dd, 4H, $J = 1.9$ Hz, Ar–H); 4.4 (s, 1H, –CH–); 2.4 (s, 8H, $–CH_2–$); 7.8–7.9 (d, 2H, Ar–H); 7.5–7.6 (t, 2H, Ar–H); 7.2–7.3 (t, 1H, Ar–H).

5.2.10. Synthesis of 1-[bis-(4-fluorophenyl)-methyl]-4-toluene-4-sulfonyl)piperazine (5j). The general synthetic method described above affords **5j**, as a colorless crystalline solid from piperazine **1** (1 g, 3.47 mmol) and toluene-4-sulfonyl chloride **3j** (0.7943 g, 4.17 mmol).

IR ν_{max} (KBr): 1600, 1223, 1320, 1370, 3250 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz): 7.1 (dd, 4H, $J = 1.9$ Hz, Ar–H); 7.3 (dd, 4H, $J = 1.9$ Hz, Ar–H); 4.4 (s, 1H, –CH–); 2.25–2.55 (t, 8H, $–CH_2–$), 7.7–7.8 (d, 2H, Ar–H); 7.3–7.4 (t, 2H, Ar–H); 3.35 (s, 3H, $Ar–CH_3$).

5.2.11. Synthesis of 1-[bis-(4-fluorophenyl)-methyl]-4-(4-*tert*-butylbenzenesulfonyl)piperazine (5k). The general synthetic method described above affords **5k**, as a colorless crystalline solid from piperazine **1** (1 g, 3.47 mmol) and 4-*tert*-butylbenzenesulfonyl chloride **3k** (0.9666 g, 4.17 mmol).

IR ν_{max} (KBr): 1600, 1223, 1320, 1370, 3250 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz): 7.1 (dd, 4H, $J = 1.9$ Hz, Ar–H); 7.3 (dd, 4H, $J = 1.9$ Hz, Ar–H); 4.4 (s, 1H, –CH–); 2.25–2.5 (t, 8H, $–CH_2–$); 7.7–7.8 (d, 2H, Ar–H); 7.3–7.4 (t, 2H, Ar–H); 1.4 (s, 9H, $–C(CH_3)_3$).

5.2.12. Synthesis of 1-[bis-(4-fluorophenyl)-methyl]-4-(4-chlorobenzenesulfonyl)piperazine (5l). The general synthetic method described above affords **5l**, as a colorless crystalline solid from piperazine **1** (1 g, 3.47 mmol) and 4-chlorobenzenesulfonyl chloride **3l** (0.8794 g, 4.17 mmol).

IR ν_{max} (KBr): 1600, 1223, 1320, 1050, 3250 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz,): 7.1 (dd, 4H, $J = 1.9$ Hz, Ar–H); 7.3 (dd, 4H, $J = 1.9$ Hz, Ar–H); 4.4 (s, 1H, –CH–); 2.25–2.55 (t, 8H, $–CH_2–$); 7.75–7.8 (d, 2H, Ar–H); 7.5–7.6 (d, 2H, Ar–H).

5.2.13. Synthesis of 1-[bis-(4-fluorophenyl)-methyl]-4-(2,6-dichlorobenzenesulfonyl piperazine (5m). The general synthetic method described above affords **5m**, as a colorless crystalline solid from piperazine **1** (1 g, 3.47 mmol) and 2,6-dichlorobenzenesulfonyl chloride **3 m** (1.022 g, 4.17 mmol).

IR ν_{max} (KBr): 1600, 1223, 1050, 1320 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz,): 7.1 (dd, 4 H, $J = 1.9$ Hz, Ar–H); 7.3 (dd, 4H, $J = 1.9$ Hz, Ar–H); 4.4 (s, 1H, –CH–); 2.25–2.5 (t, 8H, $–CH_2–$); 7.4–7.5 (t, 2H, Ar–H); 7.1–7.2 (t, 1H, Ar–H).

5.3. Microbiology: in vitro evaluation of antimicrobial activity

The standard strains were procured from the American Type Culture Collection (ATCC), Rockville, USA, and the pathological strains were procured from the Department of Microbiology, Madras Medical College and Research Institute, Chennai, India. The antibacterial activity of the synthesized compounds was screened against the following standard bacterial strains: *S. aureus* ATCC 25953, *Streptococcus pneumoniae* ATCC 49619, *Bacillus cereus* 11778, *Bacillus subtilis* 6051, *E. coli* ATCC 25922, *P. aeruginosa* ATCC 2853, *P. vulgaris* ATCC 2853, and *Salmonella typhi* ATCC 9484.

5.4. Paper disc diffusion method

Preliminary antibacterial screening was performed by the agar diffusion method using a paper disc. The sterilized (autoclaved at 120 °C for 30 min), liquified Mueller Hinton agar (40–50 °C) was inoculated (1 mL/100 mL of medium) with the suspension of the microorganism (matched to McFarland Barium sulfate standard) and poured into a Petri dish to give a depth of 3–4 mm. The paper discs impregnated with the test compounds (500 μg mL^{-1} in dimethylsulfoxide) were placed on the solidified medium. The plates were refrigerated at 4 °C for 2 h and then incubated at 37 °C for 24 h.

5.5. Minimum inhibitory concentration

A series of glass tubes containing different concentrations of the synthesized compounds (1–500 μg/mL in dimethylsulfoxide) with Mueller Hinton broth was inoculated with the required amount of the inoculum to obtain a suspension of microorganism, which contains 10^5 colony-forming units per milliliter. One growth control tube was prepared with the addition of the compound and one blank tube was prepared without the addition of microorganism. The tubes were incubated at 37 °C for 24 h. The turbidity produced in each tube was recorded by using a UV–visible spectrometer. The minimum inhibitory concentration (MIC—μg mL^{-1}) was considered to be the lowest concentration, which exhibited the same turbidity as the blank tube. The observed MICs (μg mL^{-1}) are presented in Table 3.

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