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Synthesis of piperazine analogues of methylene-bis-salicylic acid as potential antibacterial agents

A. Nagaraj*, P. Ramesh Naik and S. Raghuveer
Department of Chemistry,
Telangana University, Nizamabad,
Telangana-503322 India

Abstract

A series of novel (2-methoxy-5-4-methoxy-3-[(4-arylpiperazino)carbonyl]benzylphenyl)(4-arylpiperazino)methanone **8(a-j)** have been synthesized and tested for their antibacterial activity against Gram-positive bacteria *viz. Bacillus subtilis*, *Staphylococcus aureus* and *Micrococcus luteus*, and Gram-negative bacteria *viz. Proteus vulgaris*, *Salmonella typhimurium*, and *Escherichia coli*. Antibacterial evaluation revealed that compounds **8(a-j)** were showed good antibacterial activity towards all the tested strains. Further, compounds containing 4-fluorophenyl (**8d**), 2,5-difluorophenyl (**8h**) and 4-methoxyphenyl (**8c**) moieties at 4-position of the piperazine ring exhibited potent inhibitory activity towards all the tested microorganisms.

Keywords: Methylene-bis-salicylic acid, Piperazine, Antibacterial Activity.

Introduction

The heterocyclic compounds participate in important biochemical processes, and are the constituents of main substances in living cells. The heterocyclic ring comprises the core of the active moiety or pharmacophore. An especially big attention is given to nitrogen containing heterocyclic compounds, as they possess a broad spectrum of biological activities, and are used in various fields of pharmacy[1-3]. Various biologically active synthetic compounds have six member, two nitrogen containing heterocyclic ring in their structures[4], such important class of compound is piperazine, which is of great significance to the rational drug design. This moiety can be found in various well known drugs with the desired therapeutic uses[5] (**Figure 1**) and shows considerable physiological effect such as antituberculosis[6],anthelmenitics[7], antianginals[8], anticancer[9], analgesic[10], antidepressant[11], antipsychotic[12], antidiabetic[13], antihistamines[14], hypolipidemic[15]. The modification of substitution pattern on the piperazine moiety facilitates a significance difference in the biological and pharmacological effect of the resultant molecules, and this moiety has encouraged the medicinal chemists to synthesize a large number of novel chemotherapeutic agents[16].

Further, in spite of a large number of antibiotics and chemotherapeutic agents available for medical use, but the treatment of infectious diseases still challenging problem[17] because of emerging infectious diseases[18] and the microbial pathogens have turning out to be more resistant towards antibiotics/chemotherapeutics[19], resulted a significant universal health failure[20]. Therefore, the broad spectrum potency is preferred for newly found antimicrobial agents, the recent efforts have been made towards the investigation of more potential antimicrobial agents[21]. Further, the

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synthesis of a variety of bis-heterocyclic compounds has received great attention[22] not only as main chain polymers but also because many biologically active natural[23] and synthetic products have molecular symmetry[24]. Thus, bis-heterocyclic compounds may eliminate the microbial resistance. In the synthesis of bis-heterocyclic, researchers tried to extend the existing method by using a broad range of protocols to improve the various scope and limitations regarding their yield, purity and mostly on the various biological applications[25].

Owing to the immense importance and varied biological activities exhibited by piperazines and in continuation of our ongoing research on biologically active bis-heterocyclics[26-30], it was considered to synthesize the bis-heterocyclic compounds containing two piperazine moieties in one molecule for enhancing biological activity. In the present study, the synthesis of new series of novel (2-methoxy-5-4-methoxy-3-[(4-arylpiperazino)carbonyl]benzylphenyl)(4-arylpiperazino)methanone **8(a-j)** with a view to explore their potential biological activity. The anti- bacterial activities of the compounds have also been evaluated.

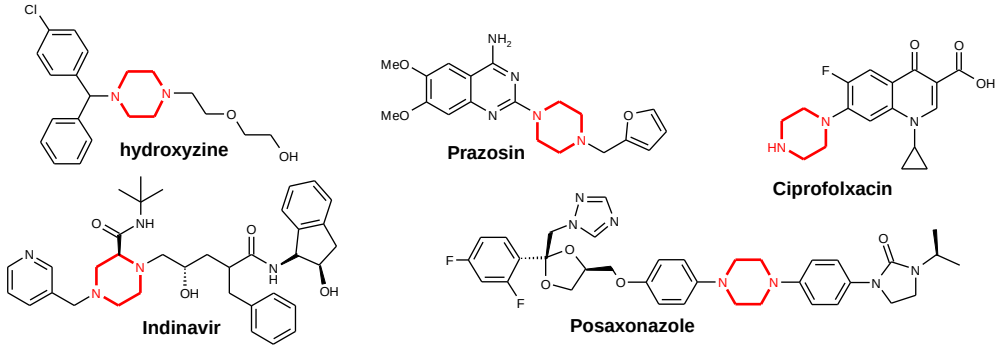

Figure 1: clinically used drugs containing piperazine ring

Experimental

Material and Methods: All the chemical and solvent were of commercial grade and were used as supplied or were prepared according to procedures described in literature. Reactions were monitored by thin-layer chromatography (TLC) on pre-coated silica gel F_{254} plates from Merck, and compounds visualized either by exposure to UV light. Melting points were determined in open capillary tube on a Fisher–Johns apparatus and are uncorrected. IR spectra were recorded using KBr disk on a Perkin–Elmer FTIR spectrometer. The NMR spectra were recorded on a Varian Gemini spectrometer at 300 MHz for ^{1}H and 75 MHz for ^{13}C. Mass spectra were recorded on a VG micro mass 7070H spectrometer. The structure of all the synthesized compounds was assigned on basis of their analytical Data and spectral data.

General procedure for the synthesis of 1-arylpiperazine 3(a-j):

The mixture of substituted aniline (10g, 78.4 mmol), compound **2** (14.7 g, 82.4 mmol), and *p*-TsOH (0.5, 3%) in xylene (44 mL) was heated to reflux at 140-145 ^{o}C for 12-24 h. When the reaction was completed, the mixture was cooled to room temperature to crystallize. The crystal was filtered and recrystallized in the distilled water.

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1-Phenylpiperazine (3a): IR (KBr) υ_{max}: 3398, 3032, 2982 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ 2.39-2.43 (m, 4H, CH_2-N of piperazine), 3.10-3.16 (m, 4H, CH_2-N of piperazine), 4.37 (bs, 1H, NH of piperazine), 6.80-6.90 (m, 3H, ArH), 7.19 (d, 2H, J = 8.1 Hz, ArH); MS: *m/z* 162 (M^+).

Preparation of 5-(3-carboxy-4-hydroxybenzyl)-2-hydroxybenzoic acid (5):

A mixture of salicylic acid **4** (0.23 mol), formaldehyde (0.13 mol), and 50% H_2SO_4 (180 g) was gently boiled for 10 h under reflux. After completion of the reaction it was filtered, washed with cold water and finally several times with boiling water to remove any unreacted salicylic acid, collected the product and dried. The compound **4** was obtained in 82% yield as white solid, having strong bitter taste; m.p. 238-240 ^oC (dec), IR (KBr) υ_{max}: 3410, 3062, 1702 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz): δ 3.99 (s, 2H, CH_2), 6.92 (d, J = 9.1 Hz, 2H, ArH), 7.38 (d, J = 9.1 Hz, 2H, ArH), 7.42 (s, 2H, ArH), 9.87 (s, 2H, OH), 10.90 (s, 2H, COOH); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 44.7, 111.2, 123.8, 132.6, 133.9, 137.9, 161.3, 176.7; MS: *m/z* 288 (M^+).

Preparation of 5-(3-carboxy-4-methoxybenzyl)-2-methoxybenzoic acid (6):

To a solution of **5** (0.01 mol) and K_2CO_3 (0.04 mol) in DMF (16 mL), MeI (0.03 mol) was added. The reaction mixture was stirred for 12 h at room temperature (TLC, EtOAc: Pet-ether, 2:1). The mixture was poured in water (30 mL), and extracted with Et_2O (3 x 20 mL). Washing the organic phase with 2*N* NaOH solution, dried over Na_2SO_4 and evaporation of solvent gave compound **6** in 74% yield as white solid; mp 194-196 ^oC, IR (KBr) υ_{max}: 3300-3200, 3037, 1698, 1070 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz): δ 3.82 (s, 6H, OCH_3), 3.91 (s, 2H, CH_2), 6.60 (d, J = 8.7 Hz, 2H, ArH), 7.72 (d, J = 8.7 Hz, 2H, ArH), 7.87 (s, 2H, ArH), 10.7 (s, 2H, COOH); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 41.2, 54.2, 122.0, 122.8, 131.9, 133.0, 134.3, 156.1, 170.1; MS *m/z*: 316 (M^+).

Preparation of 5(3-chlorocarbonyl-4-methoxybenzyl)-2-methoxy-1-benzenecarbonylchloride (7):

To a dried compound **6** (0.002 mol), thionylchloride (5 mL) was added. The mixture was refluxed for 4 h, and then the resultant was removed with simple distillation to give simple **7** in 48% yields. The compound can be used directly without purification. IR (KBr) ν_{max}: 3041, 2967, 1714, 1072, 689 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz): δ 3.87 (s, 6H, OCH_3), 4.98 (s, 2H, CH_2), 6.96 (d, J = 7.1 Hz, 2H, ArH), 7.26 (d, J = 7.1 Hz, 2H, ArH), 7.67 (s, 2H, ArH); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 41.9, 59.2, 118.7, 120.9, 133.9, 134.2, 137.6, 162.3, 170.2; MS: *m/z* 353 (M^+).

General procedure for synthesis of (2-methoxy-5-4-methoxy-3-[(4-arylpiperazino)carbonyl]-benzylphenyl)(4-arylpiperazino)methanone 8(a-j):

To a mixture of compound **7** (0.001 mol), corresponding arylpiperazine derivatives **3** (0.0022 mol) and potassium carbonate (0.003 mol), a mixture of solvent containing acetone (25 mL) and ethyl alcohol (25 mL) was added. The mixture was refluxed for 8 h, after completion of the reaction the solvent was evaporated. The resultant compounds **8(a-j)** were purified by chromatography using EtOAc/hexane.

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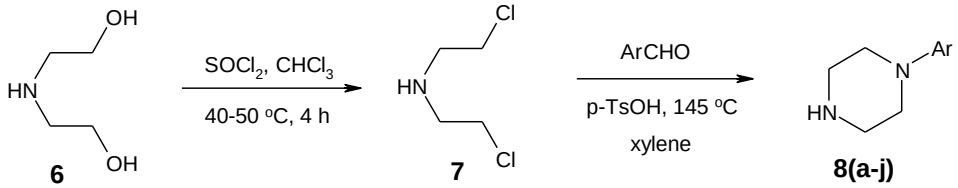
(2-methoxy-5-4-methoxy-3-[(4-phenylpiperazino)carbonyl]benzylphenyl)(4-phenylpiperazino)-methanone (8a): IR (KBr) υ_{max}: 3032, 2921, 1711, 1469, 1232, 1037, 756 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz): δ 3.38-3.43 (m, 8H, CH_2 of piperazine), 3.55-3.60 (m, 8H, CH_2 of piperazine), 3.92 (s, 6H, OCH_3), 4.28 (s, 2H, CH_2), 6.90-7.00 (m, 8H, ArH), 7.10-7.20 (m, 6H, ArH), 7.62 (d, J = 7.3 Hz, 2H, ArH); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 40.7, 42.4, 54.9, 58.3, 119.0, 119.4, 122.3, 123.3, 126.4, 130.5, 131.1, 132.6, 152.3, 159.0, 170.8; MS: *m/z* 604 (M^+).

Results and Discussion

The chlorination of diethanolamine **1** by thionyl chloride at 40-50 ^{0}C in chloroform for 4 h, led to the formation of *N*,*N*-di(2-chloroethyl)amine **2**, which on cyclo-condensation with various arylamines in the presence of *p*-TsOH in xylene at reflux temperature for 12-24 h, to afford 1-arylpiperazine **3(a-j)** (**Scheme 1**). The IR spectrum of compound **3a** showed absorption bands at 3398 and 3032 cm^{-1} due to O-H and Ar-H. Its proton NMR spectra showed the signals corresponding to the aromatic protons at δ 6.80-6.90 and 7.19 ppm as multiplet for three protons and doublet for two protons respectively. The proton corresponding to the -NH group of piperazine ring appeared at δ 4.37as a broad singlet, the protons of piperazine ring appeared at δ 2.39-2.43 and δ 3.10-3.16 ppm as multiplets.



3: Ar = a) C_6H_5; b) 4-CH_3-C_6H_4; c) 4-OCH_3-C_6H_4; d) 4-F-C_6H_4; e) 4-Br-C_6H_4; f) 3-NO_2-C_6H_4; g) 4-NO_2-C_6H_4; h) 2,5-$(F)_2$- C_6H_3; i) 3-OH-C_6H_5; j) 4-OH-C_6H_4

Scheme 1

The condensation of salicyclic acid **4** with formaldehyde in the presence of 50% sulphuric acid at reflux for 10 h gave 5-(3-carboxy-4-hydroxybenzyl)-2-hydroxybenzoic acid **5** in 82% yield. The compound 5 on methylation with methyl iodide in the presence of potassium carbonate in DMF at room temperature for 12 h gave 5-(3-carboxy-4-methoxybenzyl)-2-methoxybenzoic acid **6** in 74% yield, which was reacted with thionylchloride at reflux for 4h led to the formation of 5(3-chloro-carbonyl-4-methoxybenzyl)-2-methoxy-1-benzenecarbonylchloride **7** in 48% of yields. Further, the condensation of compound **7** with the corresponding arylpiperazine derivatives **3(a-j)** in the presence of potasium carbonate, in a mixture of solvent containing acetone and ethyl alcohol (1:1) at reflux for 8 h, resulted the corresponding compounds (2-methoxy-5-4-methoxy-3-[(4-arylpiperazino)carbonyl]benzylphenyl)(4-arylpiperazino)methanone **8(a-j)** (**Scheme 2**). The structures of compounds were confirmed by its EI mass, IR, ^1H NMR and ^{13}C NMR spectral data.

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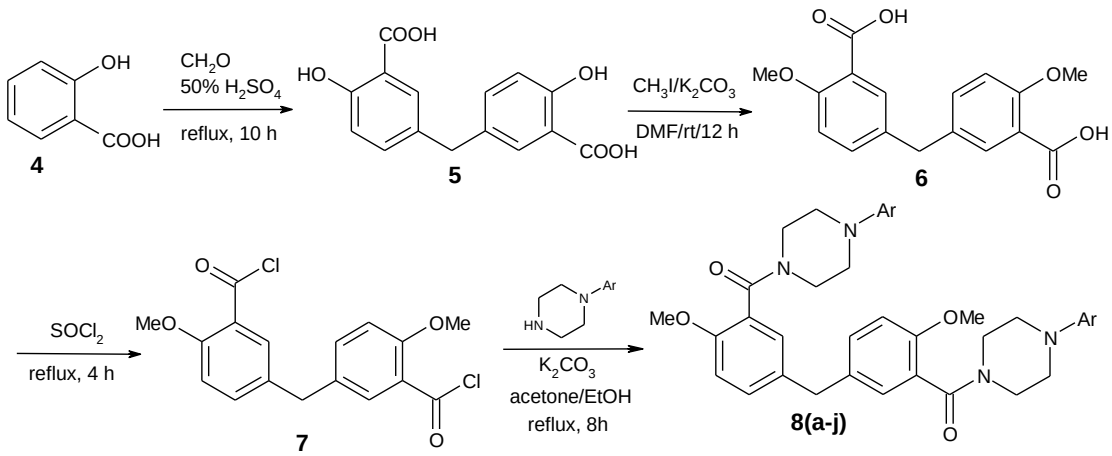

3: Ar = a) C_6H_5; b) 4-CH_3-C_6H_4; c) 4-OCH_3-C_6H_4; d) 4-F-C_6H_4; e) 4-Br-C_6H_4; f) 3-NO_2-C_6H_4; g) 4-NO_2-C_6H_4; h) 2,5-$(F)_2$- C_6H_3; i) 3-OH-C_6H_5; j) 4-OH-C_6H_4

Scheme 1

The IR spectrum of **5** showed the absorption band at 1702 due to C=O, and a broad band at 3410 cm^{-1}, due to O-H. Its protons NMR spectrum showed resonences at δ 10.9 as singlet for two protons and at δ 9.87 as singlet for two protons corresponding to hydroxyl and carboxylic protons, at δ 7.42 as singlet for two protons, at δ 7.38 as doublet with *J* value 9.1 Hz for two protons and at δ 6.92 as doublet with *J* value 9.1 Hz for two protons corresponding to aromatic protons, at δ 3.99 as singlet for two protons corresponding to the methylene protons. Its mass spectrum showed a signal at *m/z* 288 corresponding to molecular ion. The IR spectrum of **6** showed the absorption band at 1698 due to C=O, and a broad band in the range of 3300-3200 cm^{-1}, due to COOH. Its protons NMR spectrum showed resonences at δ 10.7 as singlet for two protons corresponding to carboxylic protons, at δ 7.87 as singlet for two protons, at δ 7.22 as doublet with *J* value 8.7 Hz for two protons and at δ 6.60 as doublet with *J* value 8.7 Hz for two protons corresponding to aromatic protons, at δ 3.82 as singlet for six protons corresponding to methoxyl protons and at δ 3.91 as singlet for two protons for methylene. Its mass spectrum showed a signal at *m/z* 316 corresponding to molecular ion.

The IR spectrum of compound **7** showed absorption bands appeared at 1714 (C=O) cm^{-1}. Its proton NMR spectrum showed a prominent signals corresponding to protons of methylene bridge appeared at δ 4.98 as a singlet for two protons, the proton of methoxy group appeared at δ 3.87 ppm as singlet, the other aromatic proton signals appear at expected region. Its ^{13}C NMR specta showed the signals corresponding to the carbons of acyl group appeared at δ 170.2 ppm. The IR spectra of **8a**, appearance of bands at 1711 (C=O) cm^{-1} and the absence of -OH (acid) and -NH (piperazine) stretching vibrations provided the evidence for condensation, involving piperazine-NH and OH of acid groups. Similarly, the absence of signals for the -OH and -NH protons in the ^1H NMR spectra followed by the presence of piperazine protons in the region of δ 3.38-3.43 and 3.55-3.60, the

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aromatic protons in the region of δ 6.90-7.00, 7.10-7.20 and 7.62 ppm, the methylene and methoxy protons appeared at δ 4.28 and 3.92 ppm respectively, well supported the structures. In the ^{13}C NMR spectra, the signals corresponding to the C=O were observed at δ 170.8. In summary all the newly synthesized compounds exhibited satisfactory spectral data consistent with their molecular structures.

Antibacterial Activity

All novel compounds **8(a-j)** were also assayed for their antibacterial activity against Gram-positive bacteria *viz. Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 6538p) and *Micrococcus luteus* (IFC 12708), and Gram-negative bacteria *viz*. *Proteus vulgaris* (ATCC 3851), *Salmonella typhimurium* (ATCC 14028), and *Escherichia coli* (ATCC 25922) by the broth dilution method[31]. The minimum inhibitory concentration (MIC, μg/mL), was determined for all the compounds and compared with the control. The MIC values of the assayed compounds are presented in Table 1. All assays include the solvent and reference controls. ampicillin was used as the standard drug. Antibacterial evaluation revealed that compounds **8(a-j)** were showed good antibacterial activity towards all the tested strains. Further, compounds containing 4-fluorophenyl (**8d**), 2,5-difluorophenyl (**8h**) and 4-methoxyphenyl (**8c**) moieties at 4-position of the piperazine ring exhibited potent inhibitory activity towards all the tested microorganisms.

Table 1. Antibacterial Activity of Compounds 8(a-j)

Compound	Minimum Inhibitory Concentration (MIC) in µg/mL					
	B. subtilis	S. aureus	M. luteus	P. vulgaris	S. typhimurium	E. coli
8a	12.5	25.0	25.0	--	25.0	**25.0**
8b	25.0	12.5	12.5	6.25	12.5	**25.0**
8c	6.25	6.25	12.5	6.25	3.12	**6.25**
8d	3.12	1.56	1.56	3.12	12.5	**6.25**
8e	12.5	6.25	12.5	25.0	--	**12.5**
8f	25.0	6.25	12.5	25.0	25.0	**25.0**
8g	6/25	12/5	12.5	25.0	12.5	**25.0**
8h	1.56	3.12	3.12	6.25	1.52	**3.56**
8i	12.5	12.5	25.0	--	--	**25.0**
8j	25.0	25.0	--	--	25.0	**12.5**
Ampicillin	1.56	1.56	1.56	3.12	3.12	**12.5**

— Indicates bacteria are resistant to the compound >50 µg/mL concentration. Standard deviation 0.05

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Conclusions

In conclusion, a new series of novel (2-methoxy-5-4-methoxy-3-[(4-arylpiperazino)carbonyl] benzylphenyl)(4-arylpiperazino)methanone **8(a-j)** has been synthesized and evaluated their antibacterial activity. Among the screened, the compounds **8c**, **8d** and **8h** showed more antibacterial activity compared to the standard drugs.

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