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Synthesis and Evaluation of Mannich Bases of β-Resorcylic Acid for Antiinflammatory and Antimicrobial Properties

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Mannich bases of β-resorcylic acid were synthesized using different secondary amines. They were characterized by analytical and spectral analysis. Antiinflammatory and antimicrobial activity of synthesized compounds was evaluated and some compounds showed greater anti-inflammatory activity when compared to parent compound. Almost all compounds showed antimicrobial activity against tested organisms.

Key Words: Synthesis, Mannich Base, β-Resorcylic acid, Anti-inflammatory and antimicrobial properties.

INTRODUCTION

β-Resorcylic acid (2,4-dihydroxybenzoic acid) and its esters are reported to possess antimicrobial activity while its 3,5-substituted derivatives were found to posses antiinflammatory activity[1–3]. Since β-resorcylic acid possesses active hydrogen atoms and is therefore liable to undergo Mannich reaction[4]. The Mannich bases of some NSAIDs have been synthesized with the claims to have greater activities and lesser side effects[5, 6]. The Mannich bases of β-resorcylic acid may possess similar properties to the basic nucleus or modified. To exploit these vistas Mannich bases of β-resorcylic acid were synthesized using secondary amines and tested for its antiinflammatory activity and antimicrobial activity.

EXPERIMENTAL

All chemicals used were of general reagent and fine chemicals grade. TLC was performed on silica gel G using sulphuric acid as detecting agent and KBr phase was used for IR on Shimadzu IR-47 spectrophotometer. Melting points of synthesized compounds were determined by Toshniwal melting point determination apparatus in open capillaries and are uncorrected. ^{1}H NMR spectra were recorded on Bruker at 200 MHz and mass spectra on Perkin-Elmer, U.S.A. using electrospray ionization technique.

Mannich bases of β-resorcylic acid were synthesized using secondary amines **(Scheme-1).** Mannich bases were synthesized by reaction β-resorcylic acid (1 M)

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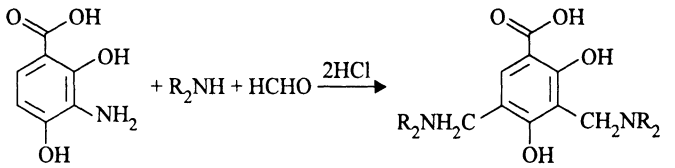
in 10 mL of ethanol and added secondary amines (2.2 M) dropwise with constant stirring. After a period of 5 min the mixture was treated with formaldehyde (4 M) dropwise. The above mixture was shaken for 45 min and pH of 4.5 was maintained using concentrated HCl. The reaction mixture was allowed to stand at room temperature for 24 h and then refluxed for 1.5 h at 50–60°C with stirring. The resulting mixture was allowed to cool and kept overnight in a refrigerator. The solid base was filtered, collected and washed with ethanol and dried.



Scheme-1

where R is —CH_3 (Compound 1), —C_2H_5 (Compound 2), —C_2H_4OH (Compound 3), —C_3H_7 (Compound 4), —isoC_3H_7 (Compound 5), —C_4H_9 (Compound 6).

Compound 1 (3,5-Bis-dimethylaminomethyl-2,4-dihydroxybenzoic acid hydrochloride): Synthesis was performed as per general procedure using dimethylamine. Yield: 65%; m.p. : 158°C; TLC (benzene : methanol : glacial acetic acid 79 : 14 : 7); R_f value: 0.25; Anal. (Calcd.) Found (%): C (54.99) 55.06; H (6.71) 7.02; N (11.66); 11.88, IR (KBr, cm^{-1}): 3400 ν(phenolic OH stretch band), 2700 ν(stretch of COOH), 1700 ν(C=O stretch of COOH), 1600 ν(C=C stretch of aromatic ring), 1475 ν(CH_3, —CH_2 deformation vibration), 1360 ν(OH deformation), 1090 ν(C—N stretch), 1010, 940 ν(penta-substitution in aromatic ring); ^{1}H NMR: δ 2.74 (12H, s, $N(CH_3)_2$), δ 5.2 (2H, s, —OH), δ 6.31 (1H, s, —ArCH), δ 10.68 (1H, —COOH); Mass: m/z 241.01.

Compound 2 (3,5-Bis-diethylaminomethyl-2,4-dihydroxy-benzoic acid hydrochloride): Synthesis was performed as per general procedure using diethylamine. Yield: 75%; m.p.: 180°C; TLC (benzene : methanol : glacial acetic acid 79 : 14 : 7); R_f value: 0.30; Anal. (Calcd.) Found (%): C (60.79) 61.12; H (8.16) 8.44; N (9.45) 9.58; IR (KBr, cm^{-1}): complies; ^{1}H NMR: δ 1.25 (12H, m, —CH_2—CH_3), δ 3.14 (8H, m, NCH_2CH_3), δ 5.3 (2H, s, —OH), δ 6.23 (1H, s, —ArCH), δ 10.76 (1H, —COOH); Mass: m/z 297.22.

Compound 3 (3,5-Bis-[bis-(2-hydroxy-ethyl)amino]-2,4-dihydroxybenzoic acid hydrochloride): Synthesis was performed as per general procedure using diethanolamine. Yield: 70%, m.p.: 192°C; TLC (benzene : methanol : glacial acetic acid 79 : 14 : 7); R_f value: 0.22; Anal. (Calcd.) Found (%): C (64.74) 65.05; H (6.71) 7.05; N (7.77) 7.95; IR (KBr, cm^{-1}): complies; ^{1}H NMR: δ 2.15 (4H, t, CH_2—OH), δ 3.31 (8H, m,N —CH_2—CH_2—), δ 3.87 (8H, m, NCH_2CH_2), δ 5.24 (2H, s, —OH), δ 6.44 (1H, s, —ArCH), δ 10.87 (1H, —COOH); Mass: m/z 361.22.

Compound 4 (3,5-Bis-dipropylaminomethyl-2,4-dihydroxybenzoic acid hydrochloride): Synthesis was performed as per general procedure using

dipropylamine. Yield: 91%; m.p.: 178°C; TLC (benzene : methanol : glacial acetic acid 79 : 14 : 7); R_f value: 0.32; Anal. (Calcd.) Found (%): C (64.74) 65.05; H (9.15) 9.98; N (7.95) 8.05; IR (KBr, cm^{-1}): complies; ^1H NMR: δ 1.05 (12H, m, $—CH_3$), δ 1.88 (8H, m, N $—CH_2—CH_2—$), δ 3.54 (8H, m, NCH_2CH_2), δ 5.27 (2H, s, —OH), δ 6.66 (1H, s, —ArCH), δ 11.21 (1H, —COOH); Mass: m/z 353.45.

Compound 5 (3,5-Bis-diisopropylaminomethyl-2,4-dihydroxybenzoic acid hydrochloride): Synthesis was performed as per general procedure using diisopropylamine. Yield: 84%; m.p.: 205°C; TLC (benzene : methanol : glacial acetic acid 79 : 14 : 7); R_f value: 0.21; Anal. (Calcd.) Found (%): C (64.74) 65.24; H (9.15) 9.71; N (7.95) 8.11; IR (KBr, cm^{-1}): complies; ^1H NMR: δ 1.24 (24H, d, $—CH_3$), δ 3.04 (4H, m, $NCH_2(CH_3)_2$), δ 5.14 (2H, s, —OH), δ 6.73 (1H, s, —ArCH), δ 10.91 (1H, —COOH); Mass: m/z 353.87.

Compound 6 (3,5-Bis-dibutylaminomethyl-2,4-dihydroxy-benzoic acid hydrochloride): Synthesis was performed as per general procedure using dibutylamine. Yield: 90%; m.p.: 175°C; TLC (benzene : methanol : glacial acetic acid 79:14:7); R_f value: 0.26; Anal. (Calcd.) Found (%): C (67.61) 67.95; H (9.87) 10.14; N (6.86) 6.97; IR (KBr, cm^{-1}): complies; ^1H NMR: δ 1.05 (12H, t, $—CH_3$), δ 1.25 (8H, m, $—CH_2—CH_3$), δ 1.78 (8H, m, N $—CH_2—CH_2—$), δ 3.22 (8H, m, NCH_2CH_2), δ 5.08 (2H, s, —OH), δ 6.57 (1H, s, —ArCH), δ 11.17 (1H, —COOH); Mass: m/z 409.54.

Anti-inflammatory Activity: Anti-inflammatory activity was evaluated by carageenan induced rat paw edema method of winter *et al*[7]. Albino rats of either sex weighing between 150–200 g were randomly distributed in control and experimental group of six animals. At 0 h the test compounds and standard were administered orally at doses equimolar to standard. 1 h after this treatment edema was induced in hind paw of rat by injection of 0.1 mL of 1% carageenan in distilled water into plantar tissues of paw. The initial paw volume was measured by plethysmometer within 30 s of the injection. The relative increase in paw edema was found by remeasuring the paw volume after 3 h of carageenan injection.

Antibacterial Activity: Agar diffusion with paper disc method[8] was used to evaluate the antibacterial activity using norfloxacin as standard. The diameter of zone of inhibition in mm were measured and compared with standard.

RESULTS AND DISCUSSION

The synthesized compounds were purified by recrystallization process and characterized using melting point, TLC, elemental analysis, IR, NMR and mass spectroscopy studies to confirm their purity.

All the compounds synthesized possess both the anti-inflammatory and antimicrobial properties which are comparable to standard and to the parent compound (Table-1).

TABLE-1
ANTI-INFLAMMATORY AND ANTIMICROBIAL ACTIVITY
OF SYNTHESIZED COMPOUNDS

Compounds	Anti-inflammatory activity[a]	Antibacterial activity[b]			
		Bacillus subtilis	*Escherichia coli*	*Shigella dysenteriae*	*Klebsiella*
Control	—	—	—	—	—
Standard	41.55	14	12	11	15
Compound 1	40.25	18	17	16	20
Compound 2	50.64	19	20	19	19
Compound 3	53.24	20	22	21	24
Compound 4	40.25	17	16	18	16
Compound 5	48.05	19	17	19	20
Compound 6	53.24	22	23	24	22
Resorcylic acid	45.36	21	18	16	16

[a] % inhibition of edema at 4h, standard phenylbutazone.

[b] Values are zone of inhibition [mm, including the diameter of the bore 6 mm], standard norfloxacin.

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