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Research Article

SYNTHESIS AND CHARACTERIZATION OF NOVEL MANNICH BASES OF BENZIMIDAZOLE DERIVATIVES FOR ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY

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ABSTRACT

Objectives: A series of mannich bases of benzimidazole derivatives were synthesized from o-phenylenediaamine in two steps via benzimidazole intermediates

Methods:The synthesized Compounds 3a-c were characterized by IR, 1H NMR, mass and elemental analysis and were evaluated for their anti fungal activity against a panel of two pathogenic fungal strains namely, *Aspergillus niger, and Candida albicans* by two fold serial dilution method, antibacterial activity against *B.subtilis, S .aureus, E. Coli, S. Typhi*

Results: All the compounds showed significant inhibitory activity against the microbes with the 100µg/ml which produces 100% inhibition against the microorganism. Anti bacterial activity was determined using Ciprofloxacin as a standard and antifungal activity was determined using standard Ketoconazole,

Conclusions: Out of the synthesized compounds 3a, 3b and 3c shows excellent antibacterial activity and compound 3a showed good antifungal activity than others.

Keywords: Benzimidazole derivatives, Anti fungal activity, Antibacterial activity

INTRODUCTION

The benzimidazole is an important pharmacophore in modern drug discovery. Literature review reveals that Manich bases of benzimidazole derivatives exhibits diverse pharmacological activities like, antimicrobial[1], analgesic[2], anti-inflammatory[3], anthelmintic[4], antiviral as well as antitumor[5], etc. In addition benzimidazoles are very important intermediates in organic reactions. Research in this area is still unexplored and is directed towards the synthesis of compounds with enhanced biological activity. Based on the above observation it is worthwhile to prepare newer novel Mannich bases of benzimidazoles with enhanced antimicrobial activity.

MATERIALS AND METHODS

The melting points were determined in open glass capillaries and are uncorrected. IR spectra were recorded on Shimadzu FT-IR 8400-S spectrophotometer by KBr pellet technique. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on AMX-400 NMR spectrophotometer at 400 MHz using DMSO-d_6 as the solvent and tetra methyl silane (TMS) as internal standard. The chemical shifts are expressed in δ ppm. The splitting patterns were designated as follows; s: singlet; d: doublet; q: quartet; m: multiplet. LCMS were recorded by using Shimadzu LCMS-2010A instrument by ESI. Molecular ion (M^+) value in m/z units is provided along with percent relative abundance in parenthesis. Synthesis of the intermediate and target compounds was accomplished according to the steps depicted in **Scheme 1.**

Reagents and conditions: i) formic acid and 10%NaoH ii) p-aminobenzoic acid, P-Dimethyl amino benzaldehyde and ethanol iii) p-aminobenzoic acid, p-hydroxy benzaldehyde and ethanol iv) p-aminobenzoic acid, P-methoxy benzaldehyde and ethanol

General method for the preparation of Synthesis of benzimidazole(2)

O-Phenylenediaamine**(1)** (1.08 g, 10.0 mmol) was placed in a 100 ml flask and it was added 0.7 g (0.52ml, 13.6 mmol) of 85% formic acid. The mixture was heated on a water bath for 2 hr at 100 ^{0}C, cooled and 10%NaOH solution was added slowly with constant rotation of the flask, until the mixture was just alkaline to litmus. Crude benzimidazole was filtered off at the pump, washed with ice cooled water. The crude product was dissolved in boiling water, discoloring carbon was added to it, and the mixture was digested for 15 minutes. Filtered rapidly at the pump though a preheated bucker funnel, the filtered was cooled to about 10 ^{0}C, the product was filtered off, washed with cold water and dried to give pure benzimidazole.

General procedure for the synthesis of Mannich bases of benzimidazole derivatives(3a-c)

To a solution containing benzimidazole(2) (1.18g,10 mmol), in 20 ml of ethanol, 1.40g(10 mmol) of aromatic aldehyde and 1.37g(10 mmol) of 4-aminobenzoic acid were added with constant stirring for 1 hr. the reaction mixture was reflux for 10 hr .On ice cooling the product formed was filtered, dried in vacuum and recrystallized with ethanol

4-[1H-Benzimidazole-yl(p-dimethylaminobenzal)methyl-amino] benzoic acid(3a)

UV λ_{max} (DMSO): 279 nm; IR (KBr) ν_{max}: 1591 (C =C, Ar), 3252 (C-H, Ar), 3259 (C-H,s), 1410 (C-H, b), 945 (C-C, Ring), 1352 (C-N, s), 1681 (C=N, s), 3429 (C-N, 3^{0}amine), 2360 (COOH,s), 1681 (C=O, s); ^{1}H NMR(DMSO- d_6) δ: 7.26 (Ar-H, m), 0.86 (-CH_3,m) 2.36, 2.00 (-NH, d), 11(OH, COOH, s); LC-MS: *m/z* 446.58. (M^+); Mol. formula $C_{27}H_{34}N_4O_2$. m.p.190 -191 ^{0}C, Yield 73%.

4-[1H-Benzimidazole-yl-(p-hydroxybenzal) methyl-amino]benzoic acid(3b)

UV λ_{max} (DMSO): 278 nm; IR (KBr) ν_{max}: 1609 (C =C, Ar), 3217 (C-H, Ar), 3196 (C-H,s), 1384 (C-H, b), 941 (C-C, Ring), 1348 (C-N, s), 1665 (C=N, s), 3390 (C-N, 3^{0}amine),3639(OH, s), 2804 (COOH,s), 1635 (C=O, s); ^{1}H NMR(DMSO- d_6) δ: 7.26 (Ar-H, m), 6.67(-CH_2, m), 4.00 (-NH, d), 11(OH, COOH, s), 5.00 (OH, Ar, s); LC-MS: *m/z* 434.58 (M^+); Mol. formula $C_{26}H_{32}N_3O_3$. m.p.195 -196 ^{0}C, Yield 75%.

4-[1H-Benzimidazole-yl-(p-methoxybenzal) methyl-amino] benzoic acid(3c)

UV λ_{max} (DMSO): 279 nm; IR (KBr) ν_{max}: 1512 (C =C, Ar), 3394 (C-H, Ar), 3394 (C-H,s), 1458 (C-H, b), 996 (C-C, Ring), 1350 (C-N, s), 1600 (C=N, s), 3454 (C-N, 3^{0}amine), 2350 (COOH,s), 1688 (C=O, s), 1253(OCH_3, s); ^{1}H NMR(DMSO- d_6) δ: 7.26 (Ar-H, m), 6.67(-CH_2, m),

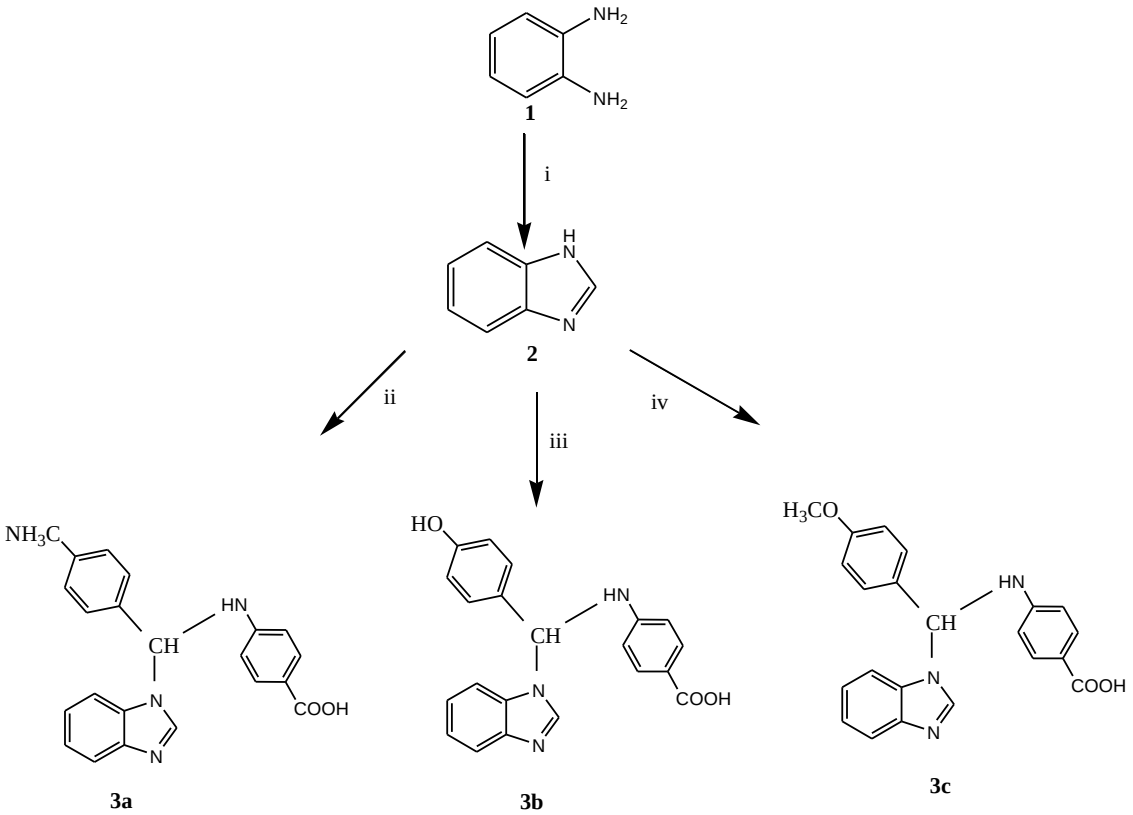
4.00 (-NH, d), 11(OH, COOH, s), 6.65 (-OCH_3, s); LC-MS: *m/z* 448.68 (M^+); Mol. formula $C_{27}H_{34}N_3O_3$. m.p.158 -160 ^{0}C, Yield 83%.

In vitro antifungal activity[6-7]

In vitro anti fungal activity of the synthesized compounds was evaluated by two fold serial dilution method. Media used was Potato Dextrose Broth (PDB). Initially, the stock culture of *Aspergillus niger*, and *Candida albicans* were revived by inoculating in broth media and grown at 37^{0}C for 48 hrs. The tubes of the above media PDB (5 ml) were prepared and each tube was added with compounds (100 µg) and inoculated with 100 µl of 48 hr old cultures. The control tubes with Ketoconazole and DMSO were also prepared. All the tubes were incubated at 37^{0}C for 48 h with constant shaking and the absorbance of biomass were measured 660 nm against autoclaved, uninoculated media as blank. The result of i*n vitro* anti fungal activity is expressed as Minimum Inhibitory concentration (MIC) and is given in **Table 1.**

Antibacterial activity[8-10]

All the compounds were screened in- vitro for their antibacterial activity against *Staphylococcus aureus*, *Bacilllus subtilis*, *Solmonella typhi*, *Escherichia coli* by cup- plate method at 100µg/ml concentration using Ciprofloxacin as standard and DMSO as solvent control. After 24 hr of incubation at 37^{0}C, the MIC was measured. The results are tabulated in **Table-2**



Scheme 1: Synthesis of mannich bases of benzimidazole derivatives

Table 1: In vitro antifungal activity of mannich bases of benzimidazole derivatives

Compound	Concentration (µg/ml)	Zone of inhibition (mm)	
		Candida albicans	*Aspergillus niger*
3a	100	11	10
3b	100	9	9
3c	100	8	8
Standard (Ketoconazole)	100	23	22
Standard (DMSO)	-	-	-

Table 2: In vitro Antibacterial activity of mannich bases of benzimidazole derivatives

Compound	Concentration (µg/ml)	Zone of inhibition (mm)			
		Gram positive		Gram negative	
		B.subtilis	*S .aureus*	*E. coli*	*S. typhi*
3a	100	13	12	8	13
3b	100	12	12	13	14
3c	100	13	10	10	12
Standard (Ciprofloxacin)	100	24	22	21	22
Control (DMSO)	-	-	-	-	-

RESULTS AND DISCUSSION

The melting points of all synthesized compounds were found in open capillary tubes and readings were uncorrected. The results of IR spectra were given in spectral detail heading which showed absorption bands for aromatic C-H,N-H,C-N,C=N,C=O,OH,C-Cl, and O-CH_3 groups. The results of the ^1H NMR spectra given under spectral detail heading showed that the numbers of hydrogen atoms present in all the synthesized compounds were exact when compared to the number of hydrogen atoms in the expected compounds. the molecular mass of the synthesized compounds was nearer to the molecular mass of the expected compounds

For all three compounds antibacterial activity was determined using standard Ciprofloxacin, results were tabulated in **Table-2**. All the compounds showed significant inhibitory activity against the microbes with the 100µg/ml which produces 100% inhibition against the microorganism. Depending on the functional group present in the aromatic ring, different MIC values were obtained. Out of the synthesized compounds 3a, 3b and 3c shows excellent antibacterial activity. The compounds substituted with Methoxy and hydroxyl group **(3b & 3c)** showed higher antibacterial activity compared to others, for all three compounds antifungal activity was determined using standard Ketoconazole, results were tabulated in **Table-1.** out of three compounds, compound and **3a** showed good antifungal activity than others.

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