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Design, Synthesis and Antimicrobial Evaluation of Mannich Bases of Novel Isoindole Analogues

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ABSTRACT

The most significant synthesis of isoindoles (phthalimides) is the dehydrative condensation of phthalic anhydride at high temperature with primary amines (adenine). Novel isoindole analogues with adenine can be synthesized through condensation of phthalic anhydride or substituted phthalic anhydride and purine (adenine). In this study only one type of isoindole analogues (phthalimides) were synthesized using mono bromo phthalic anhydride with primary amine (adenine). Different Mannich bases of isoindole analogues were synthesized using various types of aliphatic amines with variable yields (60- 80%). These Mannich bases were identified and confirmed by FT-IR, NMR and elemental analysis. The antimicrobial assay of these synthesized analogues was tested against *H. pylori, E. coli, P. auroginosa, S. typhi, B. subtilis, B. threogensis, S. aureus, MRSA, A. niger*, and were found to be active at Minimum Inhibitory Concentration [MIC] range of 125-1000 μg/ml. The most active compounds in the series was S-3 which has shown antibacterial activity against *H. pylori* at MIC of 125 μg/ml. This compound was identified as lead compounds and further molecular modification is in progress.

Keywords: Isoindole (Phthalimide), Mannich bases, Antimicrobial activity.

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INTRODUCTION

A number of phthalimide derivatives occur vary widely in nature and are essential to life. Phthalimides have been known for a long time for their wide applications and pharmacological properties such as antimicrobial[1,2,3], anxiolytic activity[4], hypoglycemic activity[5], antipsychotics[6], anti-inflammatory[7], anticovulsants[8], α-glucosidase inhibitor[9], anti-inflammatory[10], and plant growth regulators[11,12,13]. Phthalimide is an imide containing two carbonyl groups bonded to nitrogen that is not very basic or nucleophilic because of resonance involving both carbonyl groups[14,15]. Substitution in phthalimide ring might be mono, di, tri and tetra but the most potent site was mono substitution on position 4 of phthalimide ring. The exact mechanism of action of phthalimide is under investigation but it is assumed that it disrupts the function of microbe's m-RNA. Amine part in phthalimide moiety is adenine that binds with riboswitches and these riboswitches act as a nobel target for development of new antimicrobial agent. Riboswitches are unique among RNAs that serve as drug targets in that they have evolved to form structured and highly selective receptors for small drug-like metabolites**.** A riboswitch is a part of an mRNA molecule that can directly bind a small target molecule, and whose binding of the target affects the gene's activity. Purine riboswitches bind purines to regulate purine metabolism and transport[16].

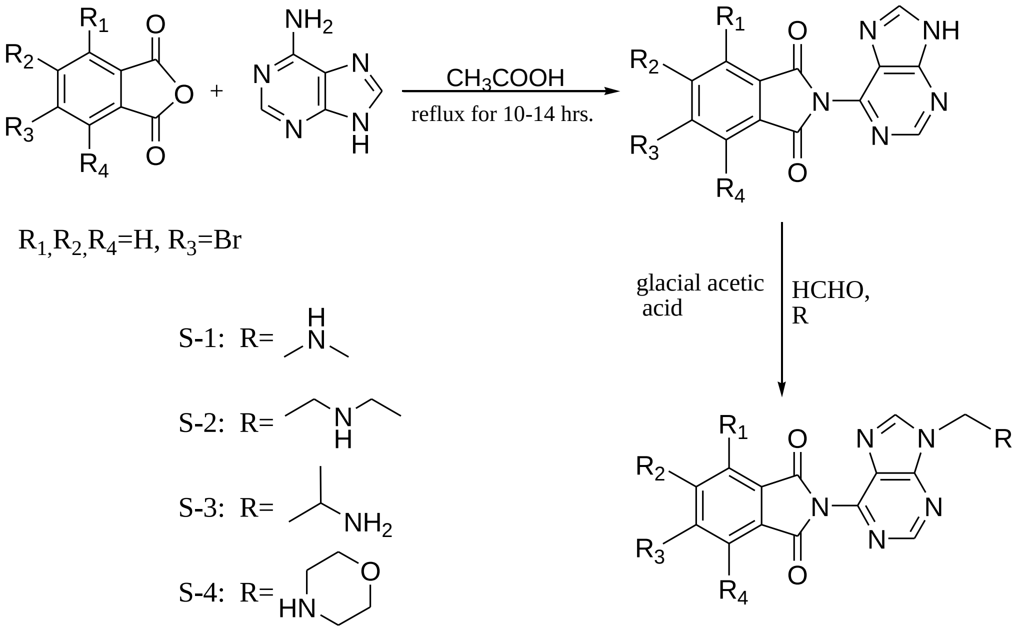
Several reports in the literature demonstrated the antimicrobial potential of phthalimide derivatives that encourage us to design the N-Mannich bases of phthalimide derivatives. Mono bromo N-substituted phthalimides were synthesized by the addition reaction between mono bromo phthalic anhydride and adenine in the presence of glacial acetic acid. The Mannich Bases of N-substituted phthalimide were synthesized by reacting mono bromo phthalic anhydride with different types of 1° and 2° amine and formaldehyde in the presence of glacial acetic acid. The purity of synthesized compounds was analysed by thin layer chromatography (TLC) and elemental analysis and the structures were identified by spectral data. The newly synthesized Mannich bases were tested for their antimicrobial activities against Gram +ve strains (such as *B. subtilis, B. threogensis, S. aureus, MRSA*) and Gram -ve strains (*H. pylori, E.coli, P. aeruginosa, S. typhi*) as well as fungal strain (such as *A. niger*).

MATERIALS AND METHODS

General procedure: Melting points were taken with an electrothermal melting point apparatus (SMP3 Bibby Stuart Scientific) in open capillary tubes and are uncorrected. Infrared spectra (KBr disc) were run on a FTIR-4000 Perkin Elmer Rx1 and the frequencies were expressed in cm^{-1}. ^{1}H NMR spectra were scanned on Bruker DRX-300 (300MHz) NMR spectrometer using DMSO-d$_6$ as solvent. Chemical shifts were expressed in ppm (parts per million) with respect to tetramethylsilane in (CDRI Lucknow). Elemental analysis (C, H, and N) were performed on Elementar Vario EL III analyzer and were within ±0.4 of theoretical value (CDRI lucknow). logP values were calculated using CHEM DRAW ultra 8.0 software.

1. Scheme: Synthesis of various Mannich bases of mono bromo N- substituted phthalimide

Equimolar quantity of mono bromo phthalic anhydride and adenine were taken in round bottom flask and dissolved in glacial acetic acid. The reaction mixture was refluxed at 50-60^0 C for 2-3 hrs. Then it was cooled, separated the crystals and recrystallized with methanol. Equimolar amount of mono bromo N-substituted phthalimide, formaldehyde and different types of primary and secondary amines were dissolved in glacial acetic acid within round bottom flask and the reactant mixtures were refluxed at 50-60^{0}C for 1 hrs. The reaction mixtures were cooled then separated the crystals and recrystallized with methanol. (Scheme 1)



Scheme 1 Various Mannich bases of mono bromo N- substituted phthalimide

Biological Evaluation

In vitro antimicrobial activity:

All newly synthesized compounds were screened for their in-vitro antimicrobial activities by disc diffusion method using Mueller-Hilton agar medium against *H. pylori, K. pneumoniae, P.auroginosa, S. typhi, B. subtilis, B. threogensis, S. aureus, MRSA* and *A. niger.*

In disc diffusion method agar plates were prepared using mueller-Hilton and 4 mm sterilized filter paper discs were placed at different places upon the plate. 5 mg of each tested compounds were dissolved in saline and dimethyl sulfoxide (1 % in final soln.) and volume was made up to 5 ml. This dilution containing 1000 µg/ml of drug concentration and was used for the testing of synthesized compounds. 10-15 microlitre of each test drug was applied upon discs. Inhibition zones were measured in milimeters at the end of an incubation period of 24 hrs at 37˚C. Ciprofloxacin and Norfloxacin in Dimethyl sulfoxide (1mg/ml) were used as standard drug for antimicrobial activity, Fluconazole in Dimethyl sulfoxide (1mg/ml) was used as standard drug for antifungal activity and Dimethyl sulfoxide as control.

Determination of Minimum Inhibitory Concentration [MIC]:

The MIC of newly synthesized compounds was determined by broth dilution method. Nutrient broth was dissolved in distilled water and was sterilized. 2 ml of this broth solution was pipette out and poured in a tube and 2 ml solution of synthesized compound was added. This was treated as stock solution. 2 ml of this stock solution was pipette out and poured in 2nd tube. This process was continued up to the second last tube (4000µg/ml to 31.25µg/ml). The same procedure was applied for standard drug. These serial dilutions were incubated at 37^0 C for 24 hrs. The turbidity of the tubes was compared with standard Mc Farland No. 1. The tube with clear solution contain a concentration was determined as MIC.

Zone of inhibition and minimum inhibitory concentration (MIC) are shown in the Table 2 and 3 respectively.

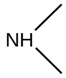
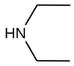
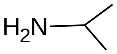
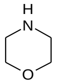
RESULT AND DISCUSSION

In the present study a series of Mannich bases of mono bromo substituted isoindole was synthesized and their physicochemical properties such as (Melting point, Rf value, logP value and % yield), zone of inhibition (in **mm**) and MIC values (in **µg**) of all the synthesized derivatives were determined and shown in Table 1, Table 2 and Table 3 respectively. All the synthesized compounds have shown antibacterial and antifungal activity against gram (-ve) bacteria (*H. pylori, E. coli, P.auroginosa, S. typhi*), gram (+ve) bacteria (*B. subtilis, B. threogensis, S. aureus, MRSA*) and a fungal strain (*A. niger*). Generally the antimicrobial activity was shown in the MIC range of 250-1000 µg/ml. Based on these MICs, the compounds were classified as least active, intermediate active and most active. The most active compound **S-3** which was active against *H. Pylori* at MIC of 125 µg/ml.

The compound **S-3** was identified as lead compound and further molecular modification is in progress.

All the synthesized derivatives were characterized by ^{1}HNMR, IR, Elemental analysis.

Table.1. Physicochemical Data of Compounds (S-1 – S-4):

S.No.	R	M.W.	M.P. (in Celcius)	Rf	% Yield	log P
S-1		401.21	78	0.56	70	2.29
S-2		429.27	90	0.68	78	2.97
S-3		415.24	94	0.83	68	2.57
S-4		443.25	64	0.87	80	1.89

Note: Rf values were determined in (chloroform:methanol 8:2)

Table 2. Zone Of Inhibition in (mm) of Compounds (S-1 to S-4)

Sr. No.	Code	Gram (-ve) bacteria				Gram (+ ve) bacteria				Fungal strain
		H. pylori	*E. coli*	*P.auroginosa*	*S.typhi*	*B. subtilis*	*B. threogensis*	*S. aureus*	*MRSA*	*A.niger*
1	S-1	-	12	7	8	8	11	-	10	8
2	S-2	-	14	8	-	-	-	8	12	-
3	S-3	17	12	12	10	7	8	-	6	11
4	S-4	12	8	8	-	-	9	-	-	7
5	Ciprofloxacin	32	-	29	28	37	30	38	32	-
6	Norfloxacin	39	27	25	33	36	32	38	30	-
7	Fluconazole	-	-	-	-	-	-	-	-	42

Table 3. MIC values in (µg) of the compounds (S-1 to S-4):

Compd. No.	Code	Gram (-ve) bacteria				Gram (+ ve) bacteria				Fungal strain
		H. pylori	*E. coli*	*P. auroginosa*	*S.typhi*	*B. subtilis*	*B. threogensis*	*S. aureus*	*MRSA*	*A.niger*
1	S-1	-	500	1000	1000	1000	500	-	500	1000
2	S-2	-	250	1000	-	-	-	1000	500	-
3	S-3	125	500	500	500	1000	1000	-	1000	500
4	S-4	500	1000	1000	-	-	1000	-	-	1000
5	Ciprofloxacin	3.91	-	7.8	7.8	0.98	7.8	0.98	3.91	-
6	Norfloxacin	0.98	15.63	15.63	3.91	1.95	3.91	0.98	7.8	-
7	Fluconazole	-	-	-	-	-	-	-	-	0.48

1 S-1: [5-bromo -2-(9-((dimethylamino) methyl)-9 H- purine- 6-yl) isoindoline-1,3-dione)]

IR (KBr) in cm^{-1}: 3000-3050 (C-H aromatic str.), 1601.9 (C=C aromatic str.), 1719.1 (C=O amide str.), 1665.8 (C=N str.), 1219 (C-N str.) 549.8 (C-Br str.)

^{1}H-NMR (DMSO) δ ppm: 1.14-1.19 (d, 6H, -N (C$\underline{H}_3$)$_2$), 4.73 (s, 2H, methylene –C$\underline{H}_2$), 7.86-8.30 (m, 3H, Ar-$\underline{H}$), 8.10-8.40 (m, 2H, purine-$\underline{H}$).

Elemental analysis: (Cal.) Found: C (47.90%) 47.87%, H (3.27%) 3.23%, N (20.95%) 20.98%.

2 S-2: [5-bromo-2-(9-((diethylamino) methyl)-9*H*- purine-6-yl)isoindoline -1, 3-dione)]

IR (KBr) in cm^{-1}: 3000-3050 (C-H aromatic str.), 1653.3 (C=C aromatic str.), 1753.4 (C=O str.), 1668 (C=N str.), 1250 (C-N str.), 599 (C-Br str.)

^{1}H-NMR (DMSO) δ ppm: 1.43 (t, 6H, -$(CH_3)_2$), 4.59 (q, 4H, -$(C\underline{H}_2\text{-}CH_3)_2$), 4.77 (s, 2H, methylene –$C\underline{H}_2$), 7.83-8.33 (m, 3H, Ar-$\underline{H}$), 8.15-8.39 (m, 2H, purine-$\underline{H}$).

Elemental analysis: (Cal.) Found: C (50.36%) 50.33%, H (3.99%) 3.95%, N (19.58%) 20.01%.

3 S-3: [5-bromo -2-(9-((isopropylamino) methyl)-9*H*- purine -6-yl) isoindoline-1,3-dione)]

IR (KBr) in cm^{-1}: 3000-3050 (C-H aromatic str.), 1625 (C=C aromatic str.), 1695 (C=O amide str.), 1644 (C=N str.), 1070 (C-N str.), 589 (C-Br str.).

^{1}H-NMR (DMSO) δ ppm: 1.38 (t, 6H, -$(CH_3)_2$), 4.55 (q, 4H, -$(C\underline{H}_2\text{-}CH_3)_2$), 4.72 (s, 2H, methylene –$C\underline{H}_2$), 7.70-8.25 (m, 3H, Ar-$\underline{H}$), 8.11-8.45 (m, 2H, purine-$\underline{H}$).

Elemental analysis: (Cal.) Found: C (49.17%) 49.18%, H (3.64%) 3.60%, N (20.24%) 20.26%.

4 S-4: [5-bromo-2-(9-(morpholino) methyl)-9*H*-purine-6-yl) isoindoline-1, 3-dione]

IR (KBr) in cm^{-1}: 3000-3050 (C-H aromatic str.), 1666 (C=C aromatic str.), 1640 (C=O amide str.), 1656 (C=N str), 1120 (C-N str.), 559 (C-Br str.).

^{1}H-NMR (DMSO) δ ppm: 1.37 (t, 6H, -$(C\underline{H}_3)_2$), 4.57 (q, 4H, -$(C\underline{H}_2\text{-}CH_3)_2$), 4.74 (s, 2H, methylene –$C\underline{H}_2$), 7.68-8.33 (m, 3H, Ar-$\underline{H}$), 8.10-8.41 (m, 2H, purine-$\underline{H}$).

Elemental analysis: (Cal.) Found: C (48.77%) 48.73%, H (3.41%) 3.38%, N (18.96%) 18.97%.

CONCLUSION:

We have shown that the above scheme can be used to synthesize Mannich bases of isoindole analogues. Contrary to our expectations, the isoindole analogues were found to be active but less potent as antimicrobials.

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