DOI: 10.15415/jptrm.2015.31005

Synthesis,Characterization and Biological studies on Mannich Bases of 2-Substituted Benzimidazole Derivatives

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Received: January 30, 2015| Revised: March 26, 2015| Accepted: May 4, 2015

Published online: May 20, 2015

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Abstract In the present study novel derivatives of 2-substituted benzimidazoles were prepared via Mannich reaction and evaluated for their in vitro antimicrobial activity against two gram negative strains (*Escherichia coli and Pseudomonas aeruginosa)*, two gram positive strains (*Bacillus subtilis and Staphylococcus aureus*) and fungal strains (*Candida albicans and Aspergillus niger*).The synthesized compounds were also screened for antioxidant activity.The newly synthesized compounds were characterized by spectral and analytical techniques.The results revealed that all the synthesized compounds have a significant antioxidant and biological activity against the tested microorganisms.

Keywords: Mannich Bases, antimicrobial activity, antioxidant activity.

1. INTRODUCTION

Mannich bases are the end products of mannich reaction and are known as beta amino ketone carrying compounds. Mannich reaction is a carbon carbon bond forming neucleophilic addition reaction. This reaction is useful for synthesizing N-methyl derivatives and many drug molecules. Mannich reaction has been studied by several groups of workers in the field of medicinal

Journal of Pharmaceutical Technology, Research and Management
Volume 3, No. 1,
May 2015
pp. 57–64

chemistry, mainly because of the various pharmacological properties of the Mannich Bases so formed. A variety of Mannich Bases have been reported to possess analgesic (Malinka *et al*, 2005), anti-inflammatory (Kalluraya *et al*, 2005; Koksal *et al*, 2007), local anaesthetic, anticancer (Ivanova *et al,* 2007; Gul *et al*, 2000), anticonvulsant (Vashishtha *et al*, 2004), antipsychotic (Scott *et al*,1992), antiviral (Edwards *et al*,1983), anthelmintic (Bennet-Jenlins *et al*, 1996) antimalarial (Barlin *et al*, 1990), antibacterial (Ashok *et al*, 2007; Pandeya *et al*, 2000) antifungal (Pandeya *et al*, 2000; Singh *et al*, 2007) and several other activities.

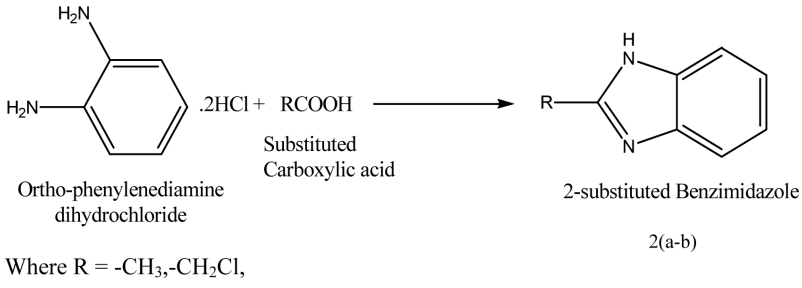
Microbial resistance to antimicrobial agents is of grave concern in the medical community. Hence the development of novel, potent and unique antimicrobial agents are the preeminent way to overcome microbial resistance and develop effective therapies. 2-substituted benzimidazole derivatives have attracted considerable attention for the past few decades due to their diverse pharmacological properties. Literature is flooded with benzimidazole-containing compounds showing biological activities such as anti-allergic agents, PARP inhibitors- as anticancer agents (White *et al*, 2000) and as cytomegalovirus (HCMV) inhibitors (Zhu *et al*, 2000).They are also reported as anthelmintic agents and in diverse human therapeutic areas such as treatment of ulcers, anti inflammatory agents and as antihistaminics. Because of their diverse uses, medicinal chemists classify them as "privileged sub structures" for drug design (Evans et al, 1988; Mason *et al*, 1999).

Antioxidants are nutrients that help to protect cells from a normal but damaging physiological process known as oxidative stress (Mandal *et al*, 2009). It has been determined that active oxygen molecules such as superoxide,hydroxyl and peroxyl radicals play an important role in oxidative stress related to the pathogenesis of many diseases such as Alzheimer, Parkinson and DNA damage leads to carcinogenesis (Kamil et al, 2013).

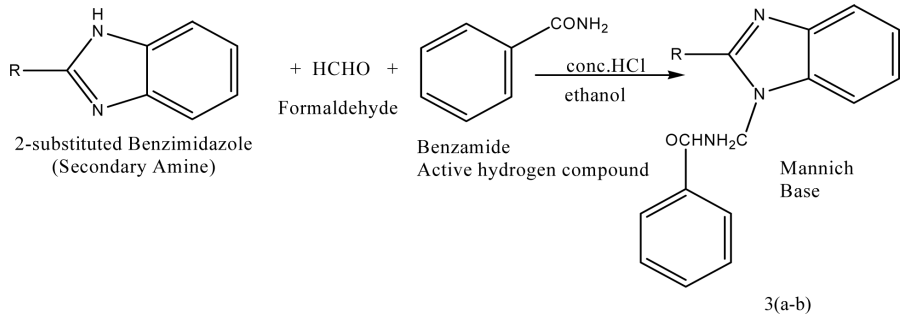
Inspired by the above facts, we hereby report the synthesis, antimicrobial and antioxidant activity of 2-substituted benzimidazole derivatives. The structures of all the compounds were confirmed by elemental and spectral analysis.

2. MATERIALS AND METHODS

The purity of the synthesized compounds were ascertained by thin layer chromatography on silica gel G in various solvent systems using iodine vapours as detecting agent.Melting points were determined by the melting point determination apparatus (TEMPO) in open capillary tubes and are uncorrected. Elemental analysis were done using Eager Xperience CHN analyzer. Infra red spectra were recorded on Perkin



Scheme 1: Preparation of 2-substituted benzimidazoles.



Scheme 2: Preparation of Mannich base of 2- substituted benzimidazoles.

Elmer Spectrum FTIR spectrophotometer in KBR phase. Proton NMR spectra were recorded on Bruker Avance II 400 NMR Spectrometer using DMSO-d_6 as a solvent and tetra methyl silane as internal standard. Chemical shift value is expressed in delta parts per million (δ ppm).

2.1 Chemistry

Mannich Bases of 2-substituted benzimidazole were synthesized by the reaction of 2-substituted benzimidazole(secondary amine),formalin and benzamide (active hydrogen compound).(3a-b). 2-substituted benzimidazoles (2a-b). were synthesized by the reaction of ortho phenylene diamine and 2-substituted carboxylic acid.

General scheme for the synthesis of compounds

STEP-1 Synthesis of 2 substituted benzimidazole.
Where R = -CH_3,-CH_2Cl,
STEP-2 Synthesis of mannich base of benzimidazole and benzamide

Table 1: Physical data of mannich bases of 2-substituted benzimdazoles.

S. No	(Comp code)Name of compound	Molecular Formula	%age yield & Rf value	M.P	Elemental Analysis calculated (%found)
1)	(3a) N-(2-methyl-benzimidazol-1-yl methyl)-benzamide	$C_{16}H_{15}N_3O$	80%, 0.69	180°C	C=72.43(64.84), H=5.7 (5.54), N=15.84(12.69)
2)	(3b) N-(2-chloromethyl-benzimidazol-1-yl methyl)-benzamide	$C_{16}H_{14}ClN_3O$	82% 0.59	190°C	C=64.11(67.06), H=4.7 (5.41), N=13.5(13.92)

2.2 The title compounds were prepared by the following steps

2.2.1 Synthesis of 2-substituted benzimidazoles [2(a-b)]

0.1 mole of o-phenylene diamine dihydrochloride, 0.03 mole of substituted carboxylic acid, 20 ml of water was taken and refluxed for 4-5 hrs.Then cooled reaction mixture was made distinctly basic by gradual addition of conc. ammonia solution.Collected the precipitated product and re-crystallized it from 10 percent ethanol.

2.2.2 Synthesis of mannich base of 2-substituted benzimidazoles with benzamide [3(a-b)]

To the ethanolic solution of benzamide (0.01mole), benzimidazole (0.01 mole) was added. Then formaldehyde (37%) (0.01 mole) was added. The reaction mixture was then adjusted to the pH of 3.5 with conc.HCl. Then it was refluxed with stirring at 80°C for 10-12 hrs. Formalin solution was added to it in portions in order to complete the reaction. Completion of reaction was monitored by TLC. Product was collected and washed with water and recrystallized from ethanol. Solvent system- $CHCl_3$:CH_3OH, 9.5:0.5

By adopting similar type of procedures and applying equimolar quantities of reactants, 2 compounds were synthesized.Physical and analytical data of synthesized compounds is given in Table 1.Synthetic pathway for preparation of title compounds is shown in scheme 1 and scheme 2.

2.3 Spectral Data

2.3.1 N-(2-methyl-benzimidazol-1ylmethyl)benzamide (3a)

IR (KBr,cm^{-1}) N-H streching for sec amine (3308) C-N stretching(1000-1350) C=C stretching of aromatic ring (1526) C=O streching(1634),CH bending for aromatic rings (675-870)

^{1}H NMR (300 MHz, DMSO-d_6,δ ppm) 7.3-7.9(m,9H,ArH),4.89(s,2H,NCH_2NH), 8.8(s, 1H, NH) 2.54(3H,s,CH_3)
Anal:Calculated(%found) for $C_{16}H_{15}N_3O$: C=72.43(69.84),H=5.7(5.54),N=15.84(15.69)

2.3.2 N-(2-chloromethyl-benzimidazol-1-yl methyl)-benzamide (3b)

IR (KBr,cm^{-1}) N-H streching for sec amine(3309)C-N stretching (1000-1350) C=C stretching of aromatic ring(1536)C=O streching(1638),CH bending for aromatic rings(675-870)
^{1}HNMR (300MHz, DMSO-d_6,δppm), 7.4-8.1(m,9H,ArH), 4.92 (s,2H,NCH_2NH), 9.0 (s,1H,NH), 2.54(2H,s,CH_2)
Anal:Calculated(%found)for $C_{16}H_{14}ClN_3O$:C=64.11(65.06),H=4.71(4.41),N=13.5(13.9)

3. ANTIMICROBIAL EVALUATION

The synthesized compounds were evaluated for their in vitro antimicrobial activity against gram positive bacteria:*Staphylococcus aureus* (MTCC 7443),*Bacillus subtilis* (MTCC 1790)Gram negative *Escherichia coli* (MTCC 82), *Pseudomonas aeruginosa*(MTCC 7814) and fungal strain: *Candida albicans* (MTCC 4748) and *Aspergillus niger* (MTCC 2208). Antimicrobial activity was assessed by serial two fold dilution technique. Ciprofloxacin was used as standard drug for antibacterial activity and clotrimazole was used as standard drug for antifungal activity. All the compounds were dissolved in DMSO to give concentration of 100µg/ml.Two fold dilutions of test and standard compounds were prepared in double strength nutrient broth I.P.(bacteria) and Sabouraud dextrose broth I.P.(fungi).The stock solution was serially diluted to give concentrations of 50-1.56µg/ml.The tubes were incubated at 37±1ºC for 24 hrs (bacteria) and 25 ºC for 48 hrs (fungi). After that the inoculated culture tubes were macroscopically examined for turbidity.The culture tube showing turbidity (lower concentration)and the culture tube showing no turbidity (higher concentration) gave the minimum inhibitory concentration of the compound. The MIC for antibacterial is given in Table 2 and MIC for antifungal is given in Table 3.

Antimicrobial activity of synthesized compounds

4. ANTIOXIDANT ACTIVITY (FREE RADICAL SCAVENGING ACTIVITY)

The free radical scavenging activity of the synthesized compounds were measured by 1, 1-biphenyl-2-picryl-hydrazyl radical (DPPH).

Table 2: In vitro antibacterial activity of the title compounds (3a-b) Minimum inhibitory concentration (μg/ml).

comp	E .coli	P.aeruginosa	S.aureus	B.subtilis
3a	12.5	12.5	12.5	12.5
3b	3.125	6.25	3.125	3.125
ciprofloxacin	6.25	6.25	3.125	3.125

Table 3: In vitro antifungal activity of the title compounds (3a-b) Minimum inhibitory concentration (μg/ml).

comp	C.albicans	A.niger
3a	12.5	12.5
3b	3.125	3.125
clotrimazole	1.56	1.56

Table 4: Antioxidant activity of synthesized compounds (3a-b).

S. No.	Compound	100 μg/ml Avg. ± SD	300 μg/ml Avg. ± SD	500 μg/ml Avg. ± SD	1000 μg/ml Avg. ± SD
1	3a	28.27± 1.00	29.13± 0.49	33.73± 0.80	47.20± 0.53
2	3b	30.30± 0.95	33.47± 0.46	55.03± 0.50	86.50± 0.66
Std.	Ascorbic acid	75.67± 0.76	83.17± 0.40	89.30± 0.57	92.77± 0.38

Stock solution of DPPH (33mg in 1L) was prepared in methanol.5ml of this stock solution was added to 1ml of test solution at diff.conc. (100,300,500,1000μg/ml).After 30 min. absorbance was measured at 517nm and compared with std at diff.conc. (100,300,500,1000μg/ml).Ascorbic acid was used as std compound.

%anti-radical activity = Control Absorbance-Sample absorbance/Control absorbance x100

The antioxidant activity of synthesized compounds is given in Table 4.

5. RESULTS AND DISCUSSION

In this study 2 novel compounds incorporating the scaffold of benzimidazole were synthesized and evaluated for antimicrobial and antioxidant activity.

Synthesis of the compounds were carried out as outlined in the scheme **1** and scheme **2**.Benzamide (active hydrogen compound) was reacted with secondary amine (2-substituted benzimidazole) in the presence of formalin and conc. hydrochloric acid to furnish the Mannich bases. These were characterized on the basis of their elemental and spectral analysis. Data obtained were found to be in good agreement with the calculated values of the proposed structure. All the synthesized compounds showed significant antimicrobial activity against bacterial strains and fungal strains. Ciprofloxacin was used as standard drug for antibacterial activity and clotrimazole was used as standard drug for antifungal activity. Compound 3b was found to be active against gram positive, gram negative bacteria and fungal strains. It also showed significant antioxidant activity as compared to Ascorbic acid.

ACKNOWLEDGEMENT

The authors are thankful to Director, Chitkara College of Pharmacy, Chitkara University, Punjab for providing necessary facilities to carry out this work.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests.

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