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Research Article

SYNTHESIS OF NOVEL SUBSTITUTED BENZIMIDAZOLE DERIVATIVES AS POTENTIAL ANTIMICROBIAL AGENTS

R. Walia[1*], K. Dhamija[1], Vandana, Md J. Akhtar[1] and HS. Lamba[2]

[1]HIMT College of Pharmacy, Gr Noida, Uttar Pradesh, India.
[2] H R Institute of pharmaceutical Sciences, Ghaziabad, Uttar Pradesh, India.

ABSTRACT

Benzimidazole derivatives play important role in medical field with manypharmacological activities such as antimicrobial, antiviral, antidiabetic and anticanceractivity. The potency of these clinically useful drugs in the treatment of microbial infections andother activities encouraged the development of some more potent and significantcompounds. Benzimidazoles are remarkably effective compounds against variousstrains of microorganisms. This research article is summarized to know about the chemistry ofdifferent derivatives of substituted benzimidazoles along with their pharmacologicalactivities. In the present research the new N-(2-(1H-benzo[d]imidazol-2-yl)phenyl)-N-phenylbenzamide derivatives were synthesised. The reaction was carried out between o-phenylenediamine with substituted anthranilic acids. The resultant compounds were than refluxed with benzoyl chloride in the presence of pyridine to yield the product.The compounds were synthesised in normal yield and their structures were confirmed by IR, H^1-NMR spectral data. The antimicrobial activity was evaluated against bacteria and fungi were studied.

Key words: Benzimidazole, Anthranilic acid, antibacterial, antifungal.

INTRODUCTION

Benzimidazole isa heterocyclic aromatic organic compound which is an important pharmacophore and a privileged structure in medicinal chemistry. This compound is bicyclic in nature which consists of the fusion of benzene and imidazole. Nowadays it is a moiety of choice which possesses many pharmacological properties. The most prominent benzimidazole compound in natureis *N*-ribosyl-dimethylbenzimidazole, which serves as an axial ligand for cobalt in vitaminB12.[1]As Infectious microbial diseases are causing problems world-wide now adays , because of resistance to number of antimicrobial agents (β-lactam antibiotics, macrolides, quinolones, and vancomycin). In 1991 benzimidazole derivatives were synthesized by derivatization at N-H of benzimidazole by electron donating group and substitution with long chain of propyl, acetamido, thio, thiazole-amino, tetramethylpiperidine on pyridine resulting in good antiulcer activity.[2, 3]. Some widely used antibacterial drugs such asfuracilin, furazolidone and

ftivazide areknown to contain benzimidazole group.[4]A variety of clinicallysignificant species of microorganisms hasbecome an important health problemglobally.[5]One way to fight with this challengeis the appropriate usage of the available resources or by synthesizing new moieties bearing a chromophore to producenovel anti-microbial agents. [6,7]

One such approach is used in the present research. We used the process of synthesis of 2-substituted benzimidazoles from the substituted anthranillic acid.

MATERIAL AND METHODS

Materials

The melting points of the synthetic compounds were determined in open glass capillaries using melting point apparatus and are uncorrected. All the Infra-red **(IR)** spectra were recorded in KBr pellets on the IR Affinity shimadzu FTIR-spectrophotometer.Proton magnetic resonance spectra were recorded on Bruker model DRX-400 MHz NMR spectrometer in DMSO-d_6 and $CDCl_3$ using tetramethylsilane as the internal reference.

Methods

Step-I General procedure for step-1

To 0.1mol of o-phenylenediamine was added 0.1mol of substituted anthranilic acid **(Table-1)**in a round-bottomed flask. The mixture was heated on a water bath at 100^o C for 1hr. The mixture was than refluxed for 3 hrs to complete the reaction. To check the completeness of the reaction the monitoring was done on TLC. After completion sodium hydroxide solution was added slowly with constant stirring until the mixture was alkaline to litmus. The crude crystals were filtered at the pump, the precipitates were washed with cold water, and the precipitates were recrystallized using boiled water. Solvent system used for monitoring of TLC was Toluene: Ethyl acetate: Formic Acid (5:4:1).

Step-II General Procedure for step-2

A known amount of compound 0.1molfrom step-1and equivalent amount of benzoyl chloride (0.1mol) was refluxed with pyridine (40 ml) for 3 hrs. The reaction mixture was cooled, treated with cold ice and neutralized with conc. HCl. The crude residue was separated which was further filtered and washed with ice cold water. The product was recrystallized from ethanol. Solvent system used for monitoring of TLC was Toluene: Ethyl acetate: Formic Acid (5:4:1).

Data for Intermediate compounds (Step-1)

Compound-1 (1H-benzo[d]imidazol-2-yl)-N-phenylaniline

Yield 10 gm (76%), m.p.301^{o}C.IR (KBr) (cm^{-1}): 3278(N-H str), 3154-2988(aromatic CH_3 str), 2854-2988(aliphatic CH_3 str), 1653-1340(C-C Ar str) 1310(C-N str); ^{1}H NMR (DMSO-d_6) δ(ppm): 5.4 (s, 1H, benzimidazole-NH),5.2 (s, 1H, Ar-NH), 7.24-7.59(s, 4H, benzimidazole), 6.70-7.60(m, 4H, Ar-H), 6.81-7.30(m, 5H, Ar- NH).

Compound-3N-(2-(1H-benzo[d]imidazol-2-yl) phenyl)acetamide

Yield 20 gm (71%), m.p.421 ^{o} C.IR (KBr) (cm^{-1}): 3278(N-H str), 3154-2988(aromatic CH_3 str), 2854-2988(aliphatic CH_3 str), 1329 (C-N str), 1670 (C=O str),1653-1340(C-C Ar str) ; ^{1}H NMR (DMSO-d_6) δ(ppm): 5.4 (s, 1H, benzimidazole-NH),5.2 (s, 1H, Ar-NH), 7.24-7.59(s, 4H, benzimidazole), 6.70-7.60(m, 4H, Ar-H), 6.81-7.30(m, 5H, Ar- NH).

Compound-5 2-amino-3-(1H-benzo[d]imidazol-2-yl) phenol

Yield 20 gm (70%), m.p.415 ^{o} C. IR (KBr) (cm^{-1}): 3408(O-H str), 3278(N-H str), 3154-2988(aromatic CH_3 str), 2854-2988(aliphatic CH_3 str), 1329 (C-N str), 1685 (C=O str),1653-1340(C-C Ar str) ; ^{1}H NMR (DMSO-d_6) δ(ppm):) 5.30 (s, 1H, Ar-OH),5.4 (s, 1H, benzimidazole-NH),6.2 (s, 1H, Ar-NH), 7.24-7.59 (s, 4H, benzimidazole), 6.66 (m, 1H, Ar-H), 6.70 (m, 1H, Ar-H), 7.10 (m, 1H, Ar-H).

Compound-7 2-(1H-benzo[d]imidazol-2-yl)-4-nitroaniline

Yield 22gm (73%), m.p.480 ^{o} C. IR (KBr) (cm^{-1}): 3278(N-H str), 3154-2988(aromatic CH_3 str), 2854-2988(aliphatic CH_3 str), 1329 (C-N str), 1685 (C=O str),1653-1340(C-C Ar str), 1525(Ar NO_2 str), 854(C-

N str), ; ^{1}H NMR (DMSO-d_6) δ(ppm):) 5.4 (s, 1H, benzimidazole-NH),6.28 (s, 1H, Ar-NH_2), 7.26-7.59 (s, 4H, benzimidazole), 6.96 (m, 1H, Ar-H), 7.97 (m, 1H, Ar-H), 8.10 (m, 1H, Ar-H).

Compound-9
2-(1H-benzo[d]imidazol-2-yl)-5-chloroaniline
Yield 22gm (74%), m.p 435^{o}C.IR (KBr) (cm^{-1}): 3178(N-H str), 3054-2988(aromatic CH_3 str), 2854-2921(aliphatic CH_3 str), 1420 (C-N str), 1653-1340(C-C Ar str), 780(C-Cl str); ^{1}H NMR (DMSO-d_6) δ(ppm): 5.0 (s, 1H, benzimidazole-NH),6.27 (s, 1H, Ar-NH_2), 7.22-7.59 (s, 4H, benzimidazole), 6.86 (m, 1H, Ar-H), 6.91 (m, 1H, Ar-H), 7.50 (m, 1H, Ar-H).

Data for Final compounds (Step-2)
Compound-2N-(2-(1H-benzo[d]imidazol-2-yl)phenyl)-N-phenylbenzamide
12 gm (81%), m.p.550 ^{o} C.IR (KBr) (cm^{-1}): 3269 (N-H str), 3054-2988(aromatic CH_3 str), 2854-2988(aliphatic CH_3 str), 1653-1340(C-C Ar str) 1654(C=O), 1599(C=N str), 1309(C-N str); ^{1}H NMR (DMSO-d_6) δ(ppm): 5.4 (s, 1H, benzimidazole-NH), 7.22-7.59(s, 4H, benzimidazole), 7.25(m, 1H, Ar-H), 7.39(m, 1H, Ar-H), 7.77(m, 1H, Ar-H), 7.87(m, 1H, Ar-H), 7.19-7.50 (m, 5H, Ar- N-C=O), 7.63-8.03 (m, 5H, Ar- C=O).

Compound-4N-(2-(1H-benzo[d]imidazol-2-yl)phenyl)-N-acetylbenzamide
Yield 14 gm (82%), m.p.550 ^{o} C. IR (KBr) (cm^{-1}): 3269 (N-H str), 3054-2988(aromatic CH_3 str), 2854-2988(aliphatic CH_3 str), 1653-1340(C-C Ar str) 1654(C=O), 1599(C=N str), 1309(C-N str); ^{1}H NMR (DMSO-d_6) δ(ppm): 2.2 (s, 3H, CH_3), 5.1 (s, 1H, benzimidazole-NH), 7.22-7.59(s, 4H, benzimidazole), 7.25(m, 1H, Ar-H), 7.39(m, 1H, Ar-H), 7.77(m, 1H, Ar-H), 7.87(m, 1H, Ar-H), 7.65-8.05(m, 5H, Ar-C=O-N).

Compound-6N-(2-(1H-benzo[d]imidazol-2-yl)-6-hydroxyphenyl)benzamide
Yield 14 gm (82%), m.p. - 555 ^{o} C. IR (KBr) (cm^{-1}): 3420(O-H str), 3269 (N-H str), 3054-2988(aromatic CH_3 str), 2854-2988(aliphatic CH_3 str), 1653-1340(C-C Ar str),1654(C=O), 1599(C=N str), 1309(C-N str); ^{1}H NMR (DMSO-d_6) δ(ppm): 5.1 (s, 1H, benzimidazole-NH), 7.20-7.59(s, 4H, benzimidazole), 6.89(m, 1H, Ar-H),7.05(m, 1H, Ar-H), 7.34(m, 1H, Ar-H), 7.63(m, 2H, Ar-H), 7.70(m, 1H, Ar-H), 8.05(m, 2H, Ar-C=O-N), 9.15(s, 1H, C=O-NH).

Compound-8N-(2-(1H-benzo[d]imidazol-2-yl)-4-nitrophenyl)benzamide
Yield 14 gm(82%), m.p. 485 ^{o} C. IR (KBr)(cm^{-1}): 3269 (N-H str), 3054-2988(aromatic CH_3 str), 2854-2988(aliphatic CH_3 str), 1653-1340(C-C Ar str),1654(C=O), 1599(C=N str), 1535(Ar NO_2 str), 1309(C-N str),880(C-N str),; ^{1}H NMR (DMSO-d_6) δ(ppm): 5.1(s, 1H, benzimidazole-NH), 7.20-7.59(s, 4H, benzimidazole), 8.13(m, 1H, Ar-H),8.20(m, 1H, Ar-H), 8.63(m, 1H, Ar-H), 7.63-8.05(m, 5H, Ar-H), 9.20(s, 1H, C=O-NH).

Compound-10N-(2-(1H-benzo[d]imidazol-2-yl)-5-chlorophenyl)benzamide
Yield 10 gm (71%), m.p. - 555 ^{o} C. IR (KBr) (cm^{-1}): 3278(N-H str), 3054-2988(aromatic CH_3 str), 2854-2988(aliphatic CH3 str), 1400 (C-N str), 1670 (C=O str due to amide),1653-1340(C-C Ar str) 780(C-Cl str); ^{1}H NMR (DMSO-d_6) δ(ppm): 5.15 (s, 1H, benzimidazole-NH), 7.21-7.59(s, 4H, benzimidazole), 7.30(m, 1H, Ar-H),7.71(m, 1H, Ar-H), 8.05(m, 1H, Ar-H), 7.65(m, 2H, Ar-H), 7.70(m, 1H, Ar-H), 8.05(m, 2H, Ar-C=O-N), 9.15(s, 1H, C=O-NH).

ANTIMICROBIAL ACTIVITY
The synthesized final compounds **2, 4, 6, 8 ,** and **10,** were screened for antimicrobial activity against both gram positive S. aureus and gram negative E. coli bacteria and antifungal activity against C. albicans according to cup-plate agar diffusion method[8] with some minor modifications at a concentration 50 µg and 100 µg, respectively. In this method a slug is removed by means of a sterile cork.[9] Ciprofloxacin and Fluconazole as in were used as standard for comparison of antibacterial and antifungal activity[10] accordingly with the standard methods.[11,12]. Dimethyl sulphoxide (DMSO) was used as control. The results of screening are given in Table 2.

Antibacterial assay

Method used

Cup-plate diffusion method-The test compounds were prepared in different concentrations using dimethylsulfoxide. Solutions of the test compounds were prepared by dissolving 5 mg each in 5 mL of dimethylsulfoxide at a concentration of 1000 µg/mL. Volumes of 0.05 mL and 0.1 mL of each compound were used for testing. Nutrient broth agar (45 – 50°C), poured into sterile Petri-dishes and left to solidify. After that petridishes were streaked with organisms. Then cup-shape wells (10 mm diameter) were made in each plate using sterile cork-borer (No. 9).[9] The solutions of each test compound (0.05 and 0.1 mL) were added separately in the cups and petri dishes were subsequently incubated. A reference standard for both gram positive and gram negative bacteria was made by dissolving accurately weighed quantity of Ciprofloxacin (50 and 100 µg/mL, respectively) in sterile distilled water, separately. The incubation was carried out at 37ºC for 24h. All the experiments were carried out in triplicate. Simultaneously, controls were maintained by employing 0.1 mL of dimethylsulfoxide which did not reveal any inhibition. Zones of inhibition produced by each compound was measured in mm. The results of antibacterial studies are given in **Table 2**.

Anti fungal activity

All those compounds screened for antibacterial activity were also tested for their antifungal activity using potato-dextrose-agar (PDA) medium by same cup plate method against Candida albicans using Fluconazole as reference standard.

RESULTS AND DISCUSSION

From the results of antibacterial screening given in the table**(Table-2)**, it is evident that most of the compounds are weakly active and few are moderately active against *S. aureus*and *E. coli* but compounds 8,10 possess very good activity against *S. aureus*and *E. coli* at concentration of 100µg.Similarly from the results of antifungal screening, it is evident that the compounds 6,8 and 10 possess good activity against fungi *Candida albicans.* All other compounds possess *less*activityagainst bacteria and fungi tested.

CONCLUSION

According to the results and discussion it was found that the activity of substituted benzimidazoles changed with difference in electronegativity. As the results indicate the zone of inhibition is more in compounds possessing the electron withdrawing groups and thus the activity is more in that compounds.

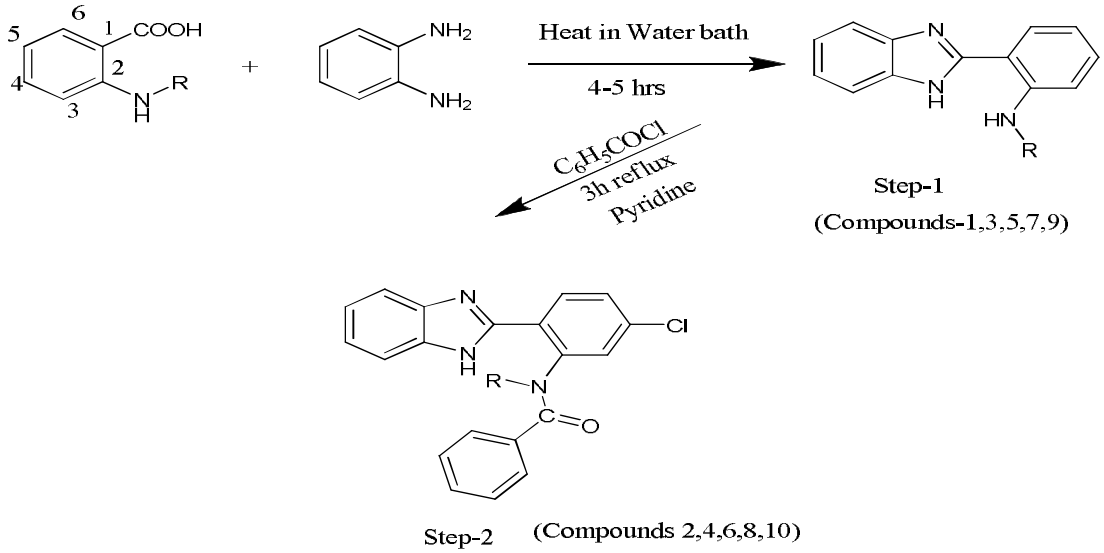

Fig. 1:

Table 1: Subtituents at various positions of anthranilic acid

Substituted anthranilic acids.	R	Other Substituent
1	C_6H_5	----
2	$COCH_3$	----
3	H	3-OH
4	H	$5NO_2$
5	H	4-Cl

Table 2: Antibacterial and antifungal activity of synthesised compounds

Compunds	Zone of inhibition (in mm) of synthesised compounds					
	Antibacterial activity				Antifungal activity	
	Escherichia coli		Staphylococcus aureus		Candida albicans	
	50 µg	100 µg	50 µg	100 µg	50 µg	100 µg
2	14	18	8	10	12	13
4	12	17	9	11	16	18
6	15	19	17	14	17	18
8	18	21	17	20	17	20
10	19	25	18	28	15	19
Ciprofloxacin	27	32	28	34	--	--
Fluconazole	--	--	--	--	25	28

- -- Indicates the activity not performed

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