Original Article

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SYNTHESIS, CHARACTERISATION AND ANTIMICROBIAL SCREENING OF SOME 1- SUBSTITUTED BENZIMIDAZOLE DERIVATIVES

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Abstract

The 1-substituted benzimidazoles (7a-j) were prepared by mannich reaction with benzimidazole derivatives (5), formaldehyde and different aromatic amines (6a-j). The pthalic anhydride (1) condensation with glycine (2) gives acetyl isoindoline derivatives (3), it cyclized with ortho phenylene diamine (4) gives benzimidazole derivatives (5). The yield of the synthesized benzimidazoles ranged from 52-76%. The structures of the synthesized compounds were characterized and confirmed by IR spectroscopy and physical analysis. All the compounds were screened for their in vitro antimicrobial activity against standard pathological bacterial and fungal strains shows moderate to good effective zone of inhibitions. All the compounds, exhibited a MIC values ranging between 12.8 to 102.4 μg for bacterial species. For fungal species, MIC values ranging between 12.8 to 25.6μg against *Aspergillus* strain. The results obtained suggest marked antibacterial and antifungal activity of the benzimidazoles at the MIC levels examined. The compounds 7d and 7i had better activity against the tested bacterial and fungal strains. The compound was showed comparatively strong activity against fungal strain than the tested bacterial *species*.

Keywords: Benzimidazoles, Antibacterial activity and Antifungal activity.

Introduction

Benzimidazoles and its derivatives represent one of the most biologically active class of compounds, possessing a wide spectrum of activities and these are well-documented in literature. They show selective neuropeptides YY1 receptor antagonists[1], potent inhibitors of TIE-2 and VEGFER-2 tyrosine kinase receptors [2], antitumor agents[3], gamma-amino butyric acid agonists and 5-HT_3 antagonists[4]. Substituted benzimidazole derivatives have found commercial application in veterinarian medicine as anthelmintic agents and in diverse human therapeutic areas such as treatment of ulcers and antihistaminic.[5]

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Similarly, the general synthesis of benzimidazoles is by the condensation reaction of 1,2-phenylenediamine with carboxaldehydes, carboxylic acids[6, 7] or their derivatives[8,9] such as, chlorides, nitriles and orthoesters, under strong acidic conditions, with high temperatures. Benzimidazoles have also been prepared on a solid phase to prove a combinatorial approach[10] The most popular strategies for their synthesis utilize N-alkylation of an unsubstituted benzimidazoles[11] ammonium salts are inexpensive, commercially available reagents for few organic transformation reactions such as halogenation of aromatic compounds and synthesis of 3, 4-dihydropyrimidine-2(1H)-ones[12]. However, there are no reports of the use of ammonium salts as catalysts for the synthesis of benzimidazoles. In continuation on the synthesis of heterocycles[13,14] and on the development of synthetic methodologies[15–17]. Some reports state that benzimidazoles possess anti-anxiety activity and anti-HIV activity[18-19]. Benzimidazoles are potent inhibitors of β- tubulin polymerisation in many

species of fungi and have been used for plant disease control since the late 1960's, although direct interactions with β- tubulin seem to be the predominant process for this chemical class, interactions with other forms of tubulin as well as differential interactions with tubulin in the free and polymerized states also have been reported. They have significant antibacterial activities also reported[20-22]. Benzimidazoles fungicides control a remarkably broad spectrum of plant pathogenic fungi. But they do not control oomycete organisms that are responsible for downy mildew disease of many crops and late blight, an important disease of solanaceous crops. Of interests is the recent identification of benzamide fungicides that selectively affect tubulin and microtubule interactions in oomycetes without significant inhibitory effects in true fungi[23-24] and some benzimidazoles derivatives shows potential antifungal activities on fungi species[25-26]. We herein report a facile method for the synthesis of benzimidazoles by the condensation of 1, 2-phenylenediamine with carboxylic acid compounds, it gives very good yields and we decided to evaluate the antimicrobial activity of their mannich bases.

Experimental

Step 1:-

Synthesis of 2-glycyl-isoindole-1,3 dione (3)

Weigh 9.86gm of pthallic anhydride (1) and 5gm of glycine (2) were taken in a test tube and kept in a heated sand bath (180-185°C). The mixture was stirred continuously during the first five minutes and any pthallic anhydride which sublimed into the melted reaction mixture till there was complete fusion. The melted mixture was kept undisturbed for 5minutes when the liquid mass solidified. The solid (3) obtained was then recrystallised from 10% ethanol.

Step 2:-

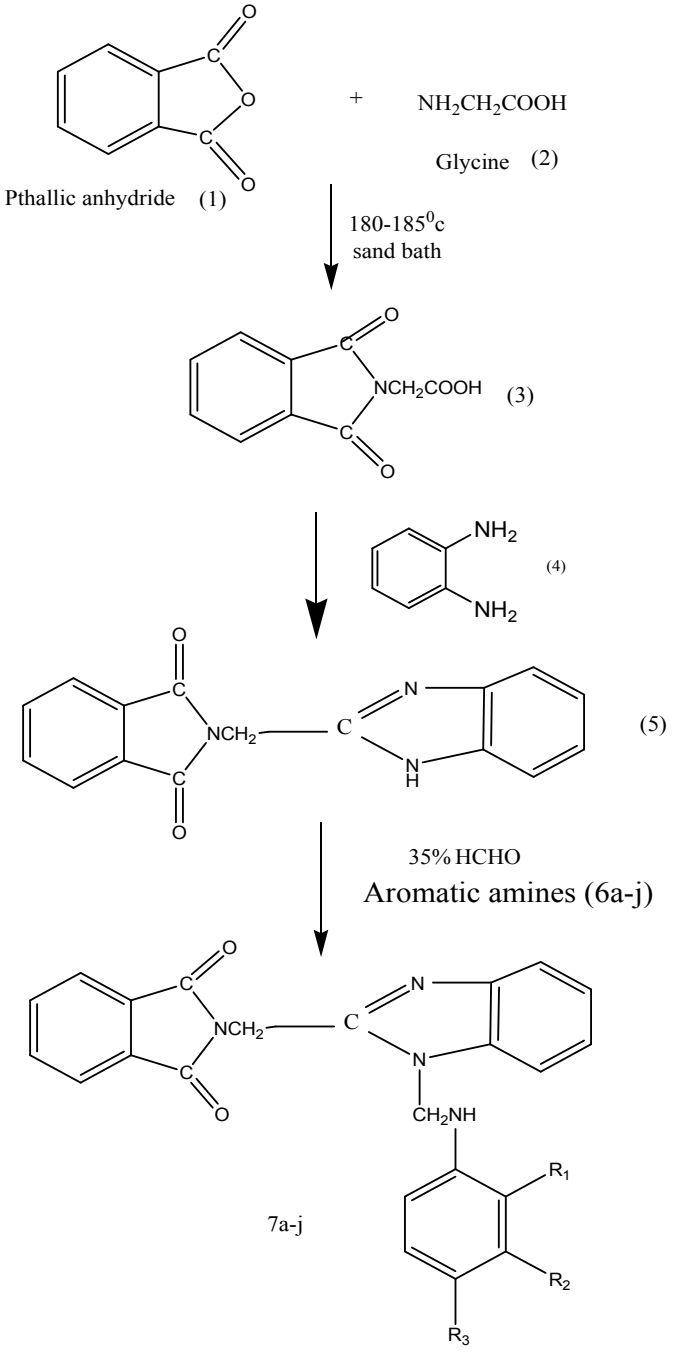
synthesis of 2-methyl (benzimidazolyl)-isoindole-1, 3-dione (5)

6gm of (3) and 3.60gm of orthophenylenediamine (4) were refluxed in 50 ml of 4N HCl for one and half hours. The solution on cooling gave a precipitate (5) which was filtered, dried and recrystallised from ethanol.

Step 3:-

synthesis of 1H-substituted benzimidazolyl isoindole 1, 3 dione (7a-j):-

4.6gm of (5) was dissolved in 8ml of 35% formaldehyde mixed in ethanol. To this equimolar quantity of aromatic amines (6a-j) was added and the mixture was refluxed for 4-5 hrs on a water bath. The solution was left over night in a freezer. The solid obtained were filtered, dried and recrystallized from ethanol.



Melting points were determined using an open capillary tube melting point apparatus. Infra Red spectra were recorded (in KBr) on FTIR 8300 Shimadzu spectrophotometer.

Spectral Data

2-((1-((4-sulfonicacid phenylamino) methyl)-1-benzoimidazol-2-yl) methyl) isoindoline-1, 3-dione (Compound7a)

IR (wave number in cm^{-1}):- 3476.8 (O-H str), 3373.9-3267.4 (N–H, str), 3065.2 (C–H, str , aromatic), 2913.2 (C–H str, aliphatic), 1674.5 (C=N str, imines), 1628.9 (C=O, str), 1590.8-1456.2 (C=C, str, aromatic).

2-((1-((4-nitrophenylamino)methyl)-1-benzoimidazol-2-yl)methyl)isoindoline-1,3-dione (Compound7b)

IR(wave number in cm^{-1}):- 3367.3 (N–H, str), 3039.4 (C–H, str, aromatic), 2970.5 (C–H, str, aliphatic) 1588.3-1497.5 (C=C str, aromatic), 1630.4 (C=O, str), 1678.6 (C=N str, imines), 1510.2-1455.6 (NO_2 str).

2-((1-((2-nitrophenylamino)methyl)-1-benzoimidazol-2-yl)methyl)isoindoline-1,3-dione (Compound7c)

IR(wave number in cm^{-1}):- 3358.4 (N–H, str), 3025.3 (C–H str, aromatic), 2964. 6(C–H str , aliphatic) 1578.2-1487.7 (C=Cstr, aromatic), 1640.7 (C=O, str), 1678.3 (C=N str, imines), 1527.8-1445.2 (NO_2 str).

2-((1-((4-fluorophenylamino)methyl)-1-benzoimidazol-2-yl)methyl)isoindoline-1,3-dione (Compound7d)

IR (wave number in cm^{-1}):- 3362.8 (N–H str), 3075.3 (C–H str, aromatic), 2917.5 (C–H str, aliphatic), 1610.8 (C=O str), 1680.7 (C=N str, imines), 1602.7-1499.5 (C=C str, aromatic), 1218.6 (C–F str).

2-((1-((2-aminophenylamino)methyl)-1-benzoimidazol-2-yl)methyl)isoindoline1,3-dione (Compound7e)

IR (wave number in cm^{-1}):- 3407.2-3342.5 (N–H, str), 3005.2-2992.8 (C–H, str aliphatic), 3067.2 (C–H, str, aromatic), 1589.4-1447.1 (C=C str, aromatic), 1617.5(C=O, str), 1691.8 (C=N, str, imines).

2-((1-((4-chlorophenylamino)methyl)-1-benzoimidazol-2-yl)methyl)isoindoline-1,3-dione (Compound7f).

IR (wave number in cm^{-1}):- 3338.2 (N–H str), 3042.7 (C–H str, aromatic), 2909.2 (C–H str, aliphatic), 1634.1 (C=O str), 1672.6 (C=N str, imines), 1598.2-1492.2 (C=C str, aromatic), 1014.8 (C–Cl str).

2-((1-((3-chlorophenylamino)methyl)-1-benzoimidazol-2-yl)methyl)isoindoline-1,3-dione (Compound7g).

IR (wave number in cm^{-1}):- 3347.1-3264.0 (N–H str), 3051.9 (C–H str, aromatic), 2885.4 (C–H str, aliphatic), 1639.7 (C=O str), 1679.4 (C=N str, imines), 1586.0-1498.2 (C=C str, aromatic), 1027.5 (C–Cl str).

2-((1-((2-chlorophenylamino)methyl)-1-benzoimidazol-2-yl)methyl)isoindoline-1,3-dione (Compound7h).

IR (wave number in cm^{-1}):- 3315.6-3284.2 (N–H str), 3055.8 (C–H str, aromatic), 2820.3 (C–H str, aliphatic), 1612.5 (C=O str), 1669.2 (C=N str, imines), 1593.5-1486.2 (C=C str, aromatic), 1022.1 (C– Cl str).

2-((1-((4-bromophenylamino)methyl)-1-benzoimidazol-2-yl)methyl)isoindoline-1,3-dione (Compound7i).

IR (wave number in cm^{-1}):- 3230.2-3215.4 (N–H str), 3056.2 (C–H str, aromatic), 2917.5 (C–H str, aliphatic), 1624.2 (C=O str), 1678.5 (C=N str, imines), 1582.2-1494.1 (C=C str, aromatic), 914.3-800.6 (C–Br str).

2-((1-((2-bromophenylamino)methyl)-1-benzoimidazol-2-yl)methyl)isoindoline-1,3-dione (Compound7j).

IR (wave number in cm^{-1}):- 3327.7-3265.9 (N–H str), 3051.5 (C–H str, aromatic), 2902.7 (C–H str, aliphatic), 1633.8 (C=O str), 1659.6 (C=N str, imines), 1594.8-1477.8 (C=C str, aromatic), 926.5-818.2 (C–Br str).

Table 01: Physical constants of synthesized benzimidazoles

Compounds	Molecular Formula	R_1	R_2	R_3	Melting Point (°c)	% Yield
7a	$C_{23}H_{18}O_5N_4S$	H	H	SO3H	382-384	58
7b	$C_{23}H_{17}N_5O_4$	H	H	NO_2	286	52
7c	$C_{23}H_{17}N_5O_4$	NO_2	H	H	283-284	48
7d	$C_{23}H_{17}N_4O_2F$	H	H	F	365-366	76
7e	$C_{23}H_{19}O_2N_5$	NH_2	H	H	297-298	64
7f	$C_{23}H_{17}N_4O_2Cl$	H	H	Cl	341-342	72
7g	$C_{23}H_{17}N_4O_2Cl$	H	Cl	H	356-357	65
7h	$C_{23}H_{17}N_4O_2Cl$	Cl	H	H	330-332	60
7i	$C_{23}H_{17}N_4O_2Br$	H	H	Br	313-314	62
7j	$C_{23}H_{17}N_4O_2Br$	H	Br	H	304-306	57

Antimicrobial activity

Micro-organisms

Three strains of bacteria used were Bacterium BACILLUS SUBTILIS, (MTCC- 441), STAPHYLOCOCCUS AUREUS (MTCC-2940) *and Escherichia coli* (MTCC-739). These standard strains were obtained from Microbial Type Culture Collection and gene bank (MTCC); Institute of Microbial Technology, Chandigarh, India. Fungal strain isolates from the same source were used, being *Aspergillus niger* (MTCC-277). All bacterial strains were cultivated in Mueller Hinton Agar (MHA) media and fungi were cultivated in Sabouraud Dextrose Agar (SDA) media. The stock culture was maintained on agar slant at 4 °C. These strains were subculture on a fresh appropriate agar plate 24 h prior to any antimicrobial test.

Agar diffusion assay

The antimicrobial activity was undertaken agar disc diffusion test according to the National Committee for Clinical Laboratory Standards(NCCLS,2001)[27] using 100 µl of suspension of the tested microorganisms, containing 2.0 × 10^6 CFU/ml for bacteria and 2.0 ×10^5 CFU /ml spore for fungal strains.The suspension was standardised by adjusting the optical density to 0.1 at 600 nm. This was used to inoculate by flooding the surface of MHA and SDA plates for bacteria and fungi respectively. Then sterilized discs were prepared at 50µg/disc for synthesized compounds and 50µg/disc for standard antibiotics (ciprofloxacin and clotrimazole) separately. A disc prepared with only the corresponding volume of DMSO was used as negative control. The petri plates were then incubated and antimicrobial activity was evaluated by measuring the diameter of the zones of inhibition around the disc.

Antibacterial activity

All the synthesized compounds were screened for invitro antibacterial activity by filter paper disc diffusion method against gram positive Bacterium bacillus subtilis , staphylococcus aureus and gram negative bacterium Escherichia coli using ciprofloxacin 50 µg discs as a standard drug. The sterilized Mueller Hinton agar medium was heated on a water bath to melt the media, when the media was lukewarm, the organisms were inoculated separately and 15ml was poured aseptically in to sterile petridishes and allowed to solidify. The synthesized benzimidazoles were dissolved and diluted with dimethylsulphoxide then sterilized discs were soaked in synthesized compounds with 50 µg/disc concentrations separately. The zone of inhibition was compared with the standard drug after 24 hours of incubation at 37°c.

Antifungal Activity

The synthesized compounds were screened for their invitro antifungal activity against aspergillus niger species at a concentration 50gm using clotrimazole 50 discs as a standard drug, by filter paper disc method. Fungi species were inoculated into sabouraud dextrose agar petriplates separately by using sterile inoculation loop. The standard drug 50 µg/disc was placed on the media. The synthesized benzimidazoles were dissolved and diluted with dimethyl sulphoxide. The sterile whatmann no-2 filter disc were soaked in synthesized compounds 50 µg/disc concentrations separately and then kept on the media surface. The zone of inhibition was compared with the standard drug after 24 hours of incubation at 30°c. The observed zone of inhibition is presented in table -2.

Broth micro-dilution assay

The minimum inhibitory concentration (MIC) and minimum bactericidal or fungicidal concentration (MBC/MFC) were determined by twofold serial micro broth dilution method (NCCLS, 1999)[28] in Mueller Hinton or Sabouraud broth. The synthesized compounds and standard antibiotics (ciprofloxacin and clotrimazole) were dissolved in 1% DMSO aqueous solution at concentration of 10 mg/ml. These solutions were used in the determination of the antimicrobial activity against the reference strains. The compounds were diluted two fold concentrations from 0.2 to 102.4 µg/ml and the starting inoculums of 2.0 $\times 10^7$ CFU/ ml was used. Solvent (DMSO) blanks were included. The test tubes were incubated at 37°C for bacteria and 30°C for fungi. The lowest concentration showing no visible growth was considered as the MIC. The results of Minimum Inhibitory Concentrations of 1H substituted Benzimidazole derivatives compared with standard antibiotics (µg/disc) were displayed in Table-3.

Table 02: Antimicrobial activity (mm)* of the synthesized compounds series 7a-j and reference antibiotics determined by the agar disc diffusion assay.

Compounds	Inhibition zone diameters of the test substances (mm) *			
	Bacillus Subtilis (MTCC- 441)	Staphylococcus Aureus (MTCC-2940)	Escheria Coli (MTCC-739)	Aspergillus Niger (MTCC-277)
7a	6.5±0.2	6.3±0.2	5.5±0.3	7.1±0.2
7b	5.6±0.2	5.3±0.3	5.0±0.2	7.0±0.3
7c	4.7±0.3	4.5±0.2	3.8±0.2	6.1±0.3
7d	9.2±0.2	8.7±0.3	8.2±0.1	10.0±0.2
7e	5.2±0.2	5.5±0.3	4.8±0.3	6.5±0.3
7f	6.3±0.2	6.8±0.2	5.2±0.3	7.5±0.2
7g	6.6±0.4	5.5±0.3	4.6±0.2	6.5±0.3
7h	4.8±0.3	4.8±0.1	4.5±0.4	6.2±0.2
7i	9.0±0.1	8.0±0.1	8.0±0.1	9.2±0.2
7j	5.0±0.2	5.2±0.3	4.4±0.3	6.1±0.3
Clotrimazole	–	–	–	13.2±0.2
Ciprofloxacin	12.6±0.3	11.5±0.3	11.8±0.3	–

Values are given as mean ± SD (n = 3). (–): Not tested, Standard antibiotic concentration= 50µgm/ disc. Tested benzimidazole concentration= 50µgm/ disc.

Table No 03: Minimum Inhibitory Concentrations of 1H substituted Benzimidazole derivatives compared with standard antibiotics (µg/disc)

Compounds	Minimum Inhibitory Concentrations (µg/disc)			
	Bacillus Subtilis (MTCC- 441)	Staphylococcus Aureus (MTCC-2940)	Escheria Coli (MTCC-739)	Aspergillus Niger (MTCC-277)
7a	25.6	25.6	51.2	25.6
7b	25.6	25.6	51.2	25.6
7c	51.2	51.2	102.4	25.6
7d	12.8	12.8	12.8	12.8
7e	25.6	25.6	51.2	25.6
7f	25.6	25.6	51.2	25.6
7g	25.6	25.6	51.2	25.6
7h	51.2	51.2	102.4	25.6
7i	12.8	25.6	25.6	12.8
7j	25.6	25.6	51.2	25.6
Clotrimazole	-	-	-	12.8
Ciprofloxacin	12.8	12.8	12.8	-

Results and Discussion

Our aim of present study is to synthesize their substituted 1-H benzimidazole derivatives by mannich reaction of isoindoline derivatives of 1-H benzimidazole (5), formaldehyde with different amines namely sulphanilic acid (6a), para nitro aniline (6b), ortho nitro aniline (6c), para fluoro aniline (6d) , ortho diamino benzene (6e), para chloro aniline (6d), meta chloro aniline (6d), ortho chloro aniline (6d), para bromo aniline (6d) and ortho bromo aniline (6d) in presence of ethanol to yield respective 1-H substituted benzimidazoles (7a-j).

Infra red studies of the compounds shows that N–H, stretching ranging from 3407.2-3264.0 cm^{-1}., C–H stretching aromatic ranging from 3025.3-3075.3 cm^{-1}, C–H, stretching, aliphatic ranging from 2820.3-3005.2, C=O, stretching ranging from 1610.8-1640.7 cm^{-1}, C=N, stretching ,imines ranging from 1659.6-1691.8cm^{-1}), C=C, stretching aromatic ranging from 1447.1-1602.7 cm^{-1}.

All the synthesized compounds were in conformity with the structures envisaged and were characterized by spectral data. All the synthesized compounds were screened for invitro antibacterial and antifungal activity at a concentration and the results are shown in table -2.

Results indicate all the compounds were less effective against E.coli. The compounds 7a, 7b, 7e, 7f, 7g and 7j have moderate activity against B.subtilis, S.aureus at 50 µgm concentration. Compounds 7d and 7i shows good activity against B.subtilis, S.aureus at 50µgm concentration. Compounds 7c and 7h shows less effective against B.subtilis, S.aureus at 50µgm concentration.

All the compounds were showing moderate to good effective zone of inhibition against A.niger. Compound 7d and 7i showed good activity against all the above mentioned tested microorganisms. These results are displayed in table-2. The minimum inhibitory concentration results were shown in table-3. All the compounds minimum inhibitory concentration values ranging from 12.8-51.2µgm/ disc against B.subtilis and S.aureus . All the compounds have shown minimum inhibitory concentration value ranging from 12.8-102.4µgm/ disc. for E.coli and tested compounds shows minimum inhibitory concentration values ranging from 12.8-25.6µgm/ disc for A.niger.

Conclusion

All the tested compounds are showing significant antimicrobial activity against B.subtilis, S.aureus and A.niger. The most of the compounds showing non-significant results against tested E.coli strain, except 7d and 7i. It was observed that compounds 7d and 7i is active against tested bacterial and fungal strains.

These observations prove that presence of electro negative fluorine, bromo, chloro, nitro and sulphonic acid groups increase the antimicrobial activity. The presence of more electronegative fluorine and bromo group at the para position also slightly increases the activity. The meta substituted chloro compound shows more potent than ortho substituted chloro compound. The ortho substituted bromo, chloro, nitro and amino products it not significantly enhance the antimicrobial activity. So it proves that para substituted derivatives of benzimidazoles were showing more antimicrobial action than that of ortho substituted benzimidazoles.

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