Synthesis and Antimicrobial activity of some novel N-substituted-2-substituted benzimidazole derivatives

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INTRODUCTION

The incorporation of an imidazole nucleus, a biologically accepted pharmacophore, in the benzimidazole molecule has made it a versatile heterocycle possessing wide spectrum of biological activity including antimicrobial [1-4], antiproliferative [5,6], anti-inflammatory [7,8], sunscreen [9], antidiabetic [10], spasmyloytic [11], antihypertensive [12], and antiviral [13] activities. Benzimidazole nucleus is also found in a variety of naturally occurring compounds such as, vitamin B12, and its derivatives, and it is structurally similar to purine bases. Benzimidazoles are widely used as drugs such as, Omeprazole, Pantoprazole, Lansoprazole; proton pump inhibitor [14], Albenzazole, Mebendazole, Thiabendazole; antihelmintic [15], Domperidone; antiobsergic [16], Pimozide; antipsychotic [17], Pimobendan; ionodilator [18] and Rifaximin; anticancer [19]. Since azomethine linkage has also shown antimicrobial activity [20]. There is very scarce recent literature data on antimicrobial potential of benzimidazoles containing azomethine linkage that should combine favorable structural properties of both azomethine and benzimidazole moiety. Therefore, we have prepared a set of six new of N’-(substituted benzylidene)-2-[2-(substituted phenyl)-1H-benzimidazol-1-yl] acetoxyhydrizes 10-15 were synthesized and evaluated for their in vitro antimicrobial activity. The compounds with nitro group 11, 12, 13 and the compound with p-chloro group 15 on aromatic ring possessing azomethine linkage showed better antibacterial and antifungal activity respectively. The novel heterocycles were characterized by melting point range, Rf value, IR, NMR and mass spectral analysis.

MATERIALS AND METHODS

Chemistry

The synthetic pathway for preparation of different title compounds is shown in Scheme 1. 2-aryl-1H-benzimidazoles (1-3) were prepared via the oxidative condensation of o-phenylenediamine with appropriate benzaldehydes in presence of sodium bisulphate. Treatment of these compounds with ethyl chloroacetate in presence of KOH/K2CO3 yielded N-alkylated products (4-6). Reaction of these products with hydrazine hydrate yielded desired hydrazide compounds (7-9). Schiff base derivatives (10-15) were obtained upon the reaction of hydrazides with corresponding aromatic aldehydes in presence of glacial acetic acid in alcohol. The purity of the compounds was monitored by TLC and the structures of all the derivatives were assigned by IR, 1H NMR and mass spectrosocopic data, which are consistent with the proposed molecular structures.

Scheme 1

[Diagram showing the chemical structures and reaction pathways]

Antimicrobial activity

The synthesized compounds (10-15) were tested in vitro for antibacterial activity against Gram positive S. aureus (MTCC 80) and Gram negative E. coli (MTCC 40) by cup-plate agar diffusion method in nutrient agar medium with an incubation of 24 h at 37 °C. The zone of inhibition was measured in millimeters using 12.5, 25, 37.5, and 50 µg/ml concentrations of synthesized compounds. Ciprofloxacin was used as reference and DMF was used both as a solvent and as a control. The antifungal activity of the compounds was

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assayed against *C. albicans* (MTCC-183) and *A. niger* (MTCC-281) by cup-plate method in Sabouraud’s dextrose agar media with an incubation of 48 h at 28 °C. The zone of inhibition was measured in millimeters using 12.5, 25, 37.5, and 50 μg/ml concentrations of synthesized compounds. Fluconazole was used as reference and DMF was used both as a solvent and as a control.

**Experimental section**

**General**
Reagents, instrumentation and measurements: all reagents, solvents and catalysts were of analytical grade and used directly. Melting points were determined in open glass capillary tubes and are uncorrected. The completion of reaction was monitored by thin layer chromatography (TLC) using silica gel-G coated glass plates and visualization was done using iodine/uv lamp. IR spectra (υmax in cm⁻¹) were recorded on a Bruker alpha-T spectrophotometer using ATR technique. 1H NMR spectra were recorded on a Bruker Avance II 400 NMR spectrometer in CDCl₃, or DMSO-d₆ as the solvent and TMS as an internal standard. All chemical shifts were reported as δ (ppm) values. Mass spectra were recorded on Quattro II and Q-TOF MS ES Micromass spectrometer.

**Synthesis**

**General procedure for the synthesis of 2-aryl-1H-benzimidazoles (1-3)**
Equimolar amounts of o-phenylenediamine (0.1 mol) and appropriate benzaldehydes (0.1 mol) were mixed in 100 ml of DMF, sodium bisulphate (0.03 mol) was added and the mixture was stirred at 80°C until the reaction was complete according to the TLC data. The mixture was cooled and added dropwise to cold water under vigorous stirring. The product separated was collected, washed with water, dried, and recrystallized from DMF to afford the desired compounds (1-3).

1. **2-(nitrophenyl)-1H-benzimidazole (1).** Yield: 50.29%. Mp 240-242°C. δ H NMR (DMSO-d₆): 8.42 (s, 1H, -NH), 2.05 (s, 2H, -NH₂), 12.37 (s, 1H, -NH), 7.62-7.15 (m, 6H, ArH); m/z: 231.2 (100%).

2. **2-(2-nitrophenyl)-1H-benzimidazole (2).** Yield: 75.44%. Mp 180-182°C. δ H NMR (KBr): 3030, 1600-1450, 870-781 (aromatic ring), 1620 (C=N in ring), 3300 (-NH), 1530,1350 (NO₂) cm⁻¹; 1H NMR (DMSO-d₆): δ 8.87 (d, 1H, ArH), 7.96-7.94 (d, 1H, ArH), 12.37 (s, 1H, -NH), 7.62-7.15 (m, 6H, ArH); m/z: 239.2 (100%).

3. **2-(3-nitrophenyl)-1H-benzimidazole (3).** Yield: 53.68%. Mp 210-213°C. δ H NMR (KBr): 3074, 1650-1460, 920-694 (aromatic ring), 1630 (-C=N in ring), 1748 (-C=O of amide), 3392-3328 (-NHNH₂) cm⁻¹; 1H NMR (DMSO-d₆): δ 8.46-8.44 (d, 1H, ArH), 7.96-7.94 (d, 1H, ArH), 8.21-8.20 (d, 1H, ArH), 7.96-7.94 (d, 1H, ArH), 8.41 (s, 1H, ArH); m/z: 511.3 (100%).

**General procedure for the synthesis of 2-(substituted phenyl)-1H-benzimidazole-1-y1 acetohydrazide (7-9)**
Hydrazine hydrate (0.01 mol, 0.5 ml) and related esters (0.01 mol) in appropriate solvent were refluxed on water bath for about 7 h. The reaction mixture was cooled and poured into cold water under vigorous stirring. The crude product obtained was filtered off, dried and recrystallized from appropriate solvent to afford the desired compounds (7-9).

1. **2-(nitrophenyl)-1H-benzimidazole-1-y1 acetohydrazide (7).** Yield: 65.44%. Mp 235-237°C. δ H NMR (KBr): 3066, 1652-1457, 952-721 (aromatic ring), 1630 (-C=N in ring), 1748 (-C=O of amide), 3382-3328 (-NHNH₂) cm⁻¹; 1H NMR (DMSO-d₆): δ 8.09 (s, 2H, -N=CH), 8.11 (s, 1H, -NH), 2.10 (s, 2H, -NH₂), 8.23-8.21 (d, 1H, ArH), 7.98-7.96 (d, 1H, ArH), 7.61-7.15 (m, 6H, ArH); m/z: 511.1 (100%).

2. **2-(2-nitrophenyl)-1H-benzimidazole-1-y1 acetohydrazide (8).** Yield: 83.68%. Mp 210-213°C. δ H NMR (KBr): 3074, 1652-1420, 920-694 (aromatic ring), 1630 (-C=N in ring), 1748 (-C=O of amide), 3392-3322 (-NHNH₂) cm⁻¹; 1H NMR (DMSO-d₆): δ 8.40 (s, 2H, -N=CH), 8.31 (s, 1H, -NH), 2.05 (s, 2H, -NH₂), 7.60-7.16 (m, 5H, ArH), 8.21-8.20 (d, 1H, ArH), 7.96-7.94 (d, 1H, ArH), 8.41 (s, 1H, ArH); m/z: 511.3 (100%).

3. **2-(2-chlorophenyl)-1H-benzimidazole-1-y1 acetohydrazide (9).** Yield: 68%. Mp 242-244°C. δ H NMR (KBr): 3100, 1650-1460, 902-694 (aromatic ring), 1632 (-C=N in ring), 1748 (-C=O of amide), 3400-3300 (-NH₂) cm⁻¹; 1H NMR (DMSO-d₆): δ 5.17 (s, 2H, -N=CH), 8.42 (s, 1H, -NH), 2.01 (s, 2H, -NH₂), 7.60-7.21 (m, 6H, ArH), 8.22-8.20 (d, 1H, ArH), 7.96-7.94 (d, 1H, ArH); m/z: 511.1 (100%).

**General procedure for the synthesis of N’-(substituted benzylidene)-2-[2-(substituted phenyl)-1H-benzimidazol-1-yl] acetohydrazide (10-15)**
A mixture of related hydrazides (7-9) (0.01 mol), corresponding aldehydes (0.01 mol) and 2-3 drops of glacial acetic acid in alcohol (100 ml) was refluxed on water bath for about 7 h. After completion of reaction (monitored by TLC), reaction mixture was cooled and poured into cold water with continuous stirring. The solid thus obtained was filtered, dried and recrystallized from appropriate solvent to afford the desired compounds (10-15).

N’-(2-nitrobenzylidene)-2-[2-(nitrophenyl)-1H-benzimidazol-1-yl] acetohydrazide (10). Yield: 70.42%. Mp 270-272°C. δ H NMR (KBr): 3120, 1698-1445, 971-783 (aromatic ring), 1557 (-C=N in ring), 1748 (-C=O of amide), 3428 (-NH₂), 1523, 1347 (-NO₂), 1628 (-N=CH) cm⁻¹; 1H NMR (CDCl₃): δ 8.6 (s, 2H, -NCHR), 7.63 (s, 1H, -NH), 8.35 (s, 1H, -N=CH) 7.85-7.71 (m, 4H, ArH), 8.51-7.95 (dd, dd 4H, ArH), 7.54-7.23 (m, 4H, ArH); m/z: 444.3 (100%).
N’-(3-nitrobenzylidene)-2-[2-(nitrophenyl)-1H-benzimidazol-1-yl] acetohydrazide (11). Yield: 70.42%. Mp 277-279° C; Rf 0.69, IR (KBr): 3100, 1698-1445, 972-784 (aromatic ring), 1557 (>C=N of amide), 3410 (-NH), 1523, 1347 (-N02), 1630 (-N=CH) cm⁻¹; 1H NMR (CDCl3): δ 5.37 (s, 2H, -NCH2), 7.63 (s, 1H, -NH), 8.75-7.21 (m, 7H, ArH), 8.41-7.95 (dd, dd 4H, ArH), 8.74-7.23 (m, 3H, ArH), 8.88 (s, 1H, ArH); m/z: 444.1 (100%).

N’-(3-nitrobenzylidene)-2-[2-(chlorophenyl)-1H-benzimidazol-1-yl] acetohydrazide (12). Yield: 76.45%. Mp 244-246° C; Rf 0.70, IR (KBr): 3080, 1698-1440, 972-784 (aromatic ring), 1718 (>C=O of amide), 3400 (-NH), 1523, 1347 (-N02), 1632 (-N=CH) cm⁻¹; 1H NMR (CDCl3): δ 5.37 (s, 2H, -NCH2), 7.63 (s, 1H, -NH), 8.65 (s, 1H, -N=CH), 7.42-7.50 (m, 7H, ArH), 8.41-7.95 (dd, dd 4H, ArH), 8.76 (s, 1H, ArH); m/z: 444.9 (100%).

Table 1: Antibacterial activity of synthesized compounds:

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<th>Compound</th>
<th>S.aureus 12.5 µg/ml (mm)</th>
<th>S.aureus 25 µg/ml (mm)</th>
<th>S.aureus 37.5 µg/ml (mm)</th>
<th>S.aureus 50 µg/ml (mm)</th>
<th>E.coli 12.5 µg/ml (mm)</th>
<th>E.coli 25 µg/ml (mm)</th>
<th>E.coli 37.5 µg/ml (mm)</th>
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REFERENCES


DISCUSSION

Antibacterial screening results (the zone of inhibition), presented in Table 1, revealed that all compounds tested showed some degree of antibacterial activity. The compounds exhibited zone of inhibition of 07-24 mm in diameter whereas standard, Ciprofloxacin showed a zone of inhibition of 26 and 25 mm in diameter against S. aureus and E. coli. At 4 ppm concentration respectively. The minimum activity was shown by the compound 14 having 2-Cl and 2-NO₂ group on aromatic rings. Except compounds 10, 14 and 15, all the compounds were found to be more active against S. aureus than E. coli. Compounds 11 and 12 were found to be equipotent against both the strains. Among synthesized compounds, compounds 11, 12 and 13 showed good activity against both the strains. Compounds 10 and 15 showed moderate activity while compounds 14 were less active against S. aureus. From the above discussion, it is evident that compound 13 emerged as the most active antibacterial benzimidazoles.

The results of antifungal activity of the test compounds 10-15 were found to be quite different from their antibacterial activity. Sensitivity of the selected fungal pathogens to some synthetic compounds 10-15 was determined in vitro at four concentrations (12.5, 25, 37.5 and 50 µg/ml). The antifungal screening results presented in Table 2, it is evident from the screening data that compound 15 was more effective against C.albicans and A.niger compared with the other derivatives. Compounds 13 and 14 showed moderate activity whereas compounds 11 and 12 showed weak antifungal activity against both the fungal species. The minimum activity was shown by the compound 10 having 2-NO₂ group on both the aromatic rings.

CONCLUSION

Summarizingly, a series of benzimidazole derivatives have been synthesized successfully in appreciable yields and screened for their in vitro antimicrobial activity. From the antibacterial activity study, it was observed that compound 14 having 2-Cl and 2-NO₂ group on aromatic rings showed minimum activity. Compound having substitution at m-position by nitro group showed an increase in the activity in comparison to o-substitution by chloro and nitro group. Thus, it was concluded that among all benzimidazole derivatives, antibacterial activity decreases when there is an o-substitution and it increases with m- or p-substitution showing maximum activity by 3-nitro group attached to m-position of both the aromatic rings.

From the antibacterial activity study, it was observed that compound 10 showed very weak activity against both the fungal strains used. Substitution by 3-NO₂ or 2-Cl group resulted in an increase in activity. It was concluded that compound 15 having both 3-NO₂ and 2-Cl group showed overall maximum antifungal activity against both the strains.

Table 2: Antifungal activity of synthesized compounds:

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<th>Compound</th>
<th>C.albicans 12.5 µg/ml (mm)</th>
<th>C.albicans 25 µg/ml (mm)</th>
<th>C.albicans 37.5 µg/ml (mm)</th>
<th>C.albicans 50 µg/ml (mm)</th>
<th>A.niger 12.5 µg/ml (mm)</th>
<th>A.niger 25 µg/ml (mm)</th>
<th>A.niger 37.5 µg/ml (mm)</th>
<th>A.niger 50 µg/ml (mm)</th>
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