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Research Article

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SYNTHESIS AND EVALUATION OF SOME NEW BENZIMIDAZOLE DERIVATIVES AS POTENTIAL ANTIMICROBIAL AGENTS

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ABSTRACT

As part of ongoing studies in developing new antimicrobials, a novel series of N'-(substituted benzylidene)-2-[2-(substituted phenyl)-1*H*-benzimidazol-1-yl] acetohydrazide **4a-e** were synthesized and evaluated for their in vitro antimicrobial activity. Among synthesized compounds, the compound with a 3-nitro group **4b** on the aromatic ring possessing azomethine linkage showed good antibacterial activity, however lower than standard, ciprofloxacin and the compound with 4-chloro group **4e** showed better antifungal activity; almost similar to that of standard, fluconazole thus they could be promising candidates for novel drugs. The novel heterocycles were characterized by melting point, R_f value and spectral analyses.

Keywords: Benzimidazole, Schiff base, *o*-phenylenediamine, Azomethine, Antimicrobial activity.

INTRODUCTION

The emergence of resistance to the major classes of antibacterial agents is recognized as a serious health concern. Particularly, the emergence of multidrug resistant strains of Gram-positive bacterial pathogens is a problem of ever increasing significance. In order to prevent this serious problem, the elaboration of new drugs is a very actual task. 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 activities including antimicrobial[1-4], antiproliferative[5,6], anti-inflammatory[7,8], sunscreen[9], antidiabetic[10], spasmolytic[11], antihypertensive[12,13], antitubercular[14] and antiviral[15]. Benzimidazole nucleus is also found in a variety of naturally occurring compounds such as, vitamin B_{12} 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[16], Albendazole, Mebendazole, Thiabendazole; antihelmintic[17], Domperidone; antidopaminergic[18], Pimozide; antipsychotic[19], Pimobendan; ionodilator[20] and Rifaximin; anticancer[21]. Since azomethine linkage has also shown antimicrobial activity[22].

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 five new of N'-(substituted benzylidene)-2-[2-(substituted phenyl)-1*H*-benzimidazol-1-yl] acetohydrazide derivatives **4a–e** and evaluated for their *in-vitro* antimicrobial activities against Gram positive and Gram negative bacteria and fungi.

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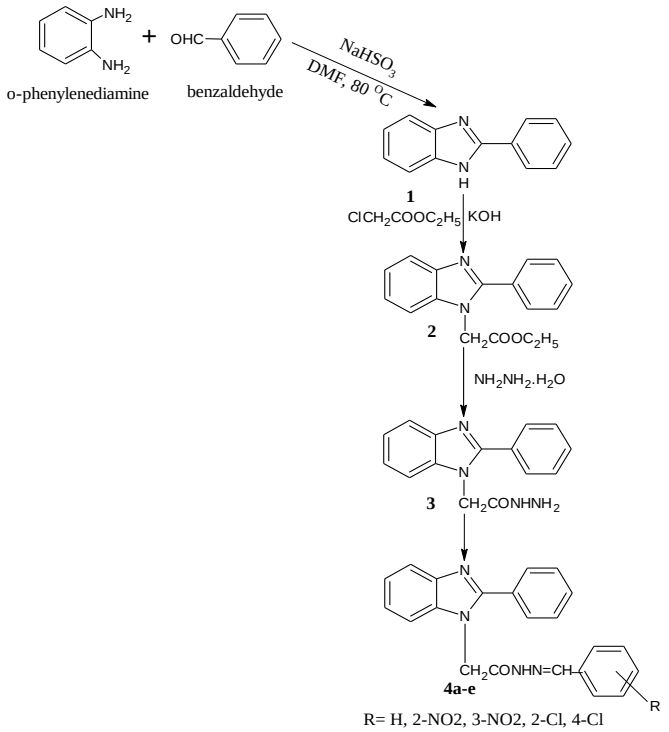
CHEMISTRY

The synthetic pathway for preparation of different title compounds is shown in **Scheme 1**. Condensation of o-phenylenediamine with benzaldehyde afforded 2-phenyl-1*H*- benzimidazole **(1).** Compound **1** on chloroesterification in presence of catalytical amount of potassium hydroxide afforded ethyl (2-phenyl-1*H*-benzimidazol-1-yl) acetate **(2)**. The formation of compound **2** was evidenced by appearance of a singlet at δ 4.89 and a multiplet at δ 7.86-7.26 ppm due to NCH_2 and Ar-H, respectively. In the ^{1}H NMR spectra of compound **2,** the peak at δ 4.29-4.23 ppm was observed due to CH_2 and a peak at δ 1.28-1.24 ppm was due to the CH_3 group of compound **2**. Furthermore, in the IR spectra, the bands at 1743 cm^{-1} (>C=O of ester), 2960-2880 cm^{-1} (-CH_2, CH_3) and 1250 cm^{-1} (C-O-C) also confirmed the formation of compound **2**. The amination of compound **2** with hydrazine hydrate yielded 2-(2-phenyl- 1*H*-benzimidazol-1-yl) acetohydrazide **(3)**. The formation of compound **3** was evidenced by appearance of a signal at δ 8.14 and 2.11 ppm due to –NH and –NH_2, respectively. The IR spectral bands at 3352-3302 cm^{-1} (–$NHNH_2$) also confirmed the formation of compound **3**. Compound **3** on treatment with selected aromatic aldehydes in presence of glacial acetic acid in ethanol as a reaction mediator afforded N'-(substituted benzylidene)-2-[2-(substituted phenyl)-1*H*-benzimidazol-1-yl] acetohydrazide derivatives **(4a-e)**. The purity of the compounds was monitored by TLC and the structures of all the derivatives were assigned by IR, ^{1}H NMR and mass spectroscopic data, which are consistent with the proposed molecular structures.

Scheme 1. Synthetic pathway for preparation of N'-(substituted benzylidene)-2-[2-(substituted phenyl)-1*H*-benzimidazol-1-yl]



ANTIMICROBIAL ACTIVITY

The synthesized compounds **4a–e** 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 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.

RESULTS AND 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.

Table 1. Antibacterial activity of synthesized compounds

Compound	Zone of inhibition (mm)							
	S. aureus				*E. coli*			
	Concentration (ppm)				Concentration (ppm)			
	12.5	25	37.5	50	12.5	25	37.5	50
4a	11	13	14	15	10	12	14	15
4b	13	15	16	17	12	15	16	16
4c	08	10	11	12	07	09	11	12
4d	09	11	12	13	08	10	12	13
4e	13	14	15	15	12	14	15	16
Ciprofloxacin	26				25			

The minimum activity was shown by the compound **4c** having unsubstituted aromatic rings. When substitution was made in the aromatic ring, activity started increasing. Among synthesized compounds, compounds **4b** and **4e** showed good activity against both the strains. Compounds **4a** and **4d** were less active against both the strains. The screening results showed that aromatic ring having substitution at m-position by nitro group or at p-position by chloro group showed an increase in activity in comparison to o-substitution by chloro or nitro group.

The results of antifungal activity of the test compounds **4a–e** were found to be quite different from their antibacterial activity. Sensitivity of the selected fungal pathogens to synthetic compounds **4a–e** was determined in vitro at four concentrations (12.5, 25, 37.5 and 50 µg/ml). The compounds exhibited zone of inhibition of 07-25 mm in diameter whereas standard, Fluconazole showed a zone of inhibition of 27 and 26 mm in diameter against *C. albicans* and *A. niger* at 4 ppm concentration respectively. The antifungal screening results presented in **Table 2**, it is evident from the screening data that substitution at o-position by chloro group retained the activity whereas substitution by *p*-chloro group resulted in an increase in activity and it was found to be approximately equivalent to or slightly lower than Fluconazole. Substitution in the aromatic ring by 2-nitro group decreases the activity. Compounds **4c** and **4d** were found to be equipotent against both the strains. Compound **4b** showed moderate activity against *C. albicans* and *A. niger* whereas compound **4a** was found to be least active against both the strains. Among the tested compounds, compound **4e** showed better activity against both the strains.

Table 2. Antifungal activity of synthesized compounds

Compound	Zone of inhibition (mm)							
	C. albicans				*A. niger*			
	Concentration (ppm)				Concentration (ppm)			
	12.5	25	37.5	50	12.5	25	37.5	50
4a	12	14	15	16	11	13	15	16
4b	13	14	16	17	13	15	16	17
4c	19	21	23	24	18	19	22	23
4d	19	21	23	24	18	19	22	23
4e	21	23	24	25	19	20	23	24
Fluconazole	27				26			

CONCLUSION

In conclusion, 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 **4c** showed minimum activity and compounds **4b** and **4e** showed better activity. Thus it was concluded that among all benzimidazole derivatives, antibacterial activity decreases when there is o-substitution and it increases with p-substitution showing maximum activity by 3-nitro group on aromatic ring. From the antifungal activity study, it was observed that compound **4e** showed better activity against both the strains whereas compound **4a** was found to be least active. Thus it was concluded that the compound having p-chloro substituent is the most potent followed by o-chloro, unsubstituted, 3-nitro and 2-nitro group.

EXPERIMENTAL

Chemistry

Reagents, instrumentation and measurements: all reagents, solvents and catalyst 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 (υ_{max} in cm^{-1}) were recorded on Bruker alpha-T spectrophotometer using A.T.R. technique. ^1H NMR spectra were recorded on a Brucker Avance II 400 NMR spectrometer in $CDCl_3$ or DMSO-d_6 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

Synthesis of 2-phenyl- 1*H*-benzimidazole (1): Equimolar amounts of o-phenylenediamine (0.1 mol) and benzaldehyde (0.1 mol) were mixed in 100 ml of DMF, 0.03 mol of sodium bisulphate was added and the mixture was stirred at 80°C until the reaction was complete according to the TLC data. The mixture was cooled to room temperature and added dropwise to cold water under vigorous stirring. The product separated as gummy material was extracted with ethyl acetate. The extract was washed with water, brine solution, dried over sodium sulphate and evaporated. The residue thus obtained was recrystallized from ethanol to afford compound **1**. Yield: 85.35%. m.p.: 280-282°C. R_f: 0.87. IR (KBr): 3100 (-NH), 3010, 1622-1400, 854-706 (aromatic ring), 1581 (-C=N), 1101 (C-N) cm^{-1}; ^1H NMR (DMSO-d_6): δ12.30 (s, 1H, NH), 7.94-7.19 (m, 9H, ArH); m/z: 195 (100%).

Synthesis of ethyl (2-phenyl-1*H*-benzimidazol-1-yl) acetate (2): Dimethylsulfoxide was added to potassium hydroxide (0.27 mol) (crushed pellets) and the mixture was stirred for 15 min and then compound **1** (0.067 mol) was added and the mixture was stirred for about 2h. Ethyl chloroacetate (0.27 mol) was added and the mixture was cooled briefly and stirred for further 2h. Water was added dropwise, the product separated as a free flowing solid was collected, dried and recrystallized from DMSO to furnish compound **2**.

Yield: 74.78%. m.p.: 105-107°C. R_f: 0.78. IR (KBr): 1473 (-NCH_2), 1743 (>C=O of ester), 2960-2880 (-CH_2, CH_3), 1250 (C-O-C) cm^{-1}; ^1H NMR ($CDCl_3$): δ 4.89 (s, 2H, N-CH_2), 4.29-4.23 (q, 2H, -CH_2-C), 1.28-1.24 (t, 3H, -C-CH_3); m/z: 281 (100%).

Synthesis of 2-(2-phenyl-1*H*-benzimidazol-1-yl)acetohydrazide (3): Hydrazine hydrate (2 ml, 0.035 mol) and compound **2** (0.035 mol) in ethanol were refluxed for 5h. The reaction mixture was cooled and poured into cold water. The crude product was filtered off, dried and recrystallized from ethanol to afford compound **3**. Yield: 77.97%. m.p.: 170-173°C. R_f: 0.54. IR (KBr): 1716 (>C=O of amide), 3352-3302 (-$NHNH_2$) cm^{-1}; ^1H NMR (DMSO-d_6): δ 7.93-7.20 (m, 9H, ArH), 8.14 (s, 1H, -NH), 4.62 (s, 2H, -NCH_2), 2.11 (s, 2H, -NH_2); m/z: 266.2 (100%).

General procedure for synthesis of N'-(substituted benzylidene)- 2-[2-(substituted phenyl)-1*H*-benzimidazol-1-yl] acetohydrazide (4a–e): A mixture of compound **3** (0.01 mol), corresponding aldehyde (0.01 mol) and 2-3 drops of glacial acetic acid in ethanol (100 ml) was refluxed on water bath for about 7h. 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 DMF/DMSO to afford the desired compounds (**4a–e).**

N'- (2-nitrobenzylidene)-2- (2-phenyl- 1H-benzimidazol-1-yl) acetohydrazide (4a): Yield: 67.11%. m.p.: 277-279°C. R_f: 0.66. IR (KBr): 3100, 1657-1457, 861-745 (aromatic ring), 1630 (-C=N in ring), 1480 (-CH_2), 3428 (-NH), 1699 (>C=O of amide), 1632 (-CH=N), 1557, 1350 (NO_2) cm^{-1}; ^1H NMR (DMSO-d_6): δ 8.29-8.28 (d, 1H, ArH), 8.11-8.10 (d, 1H, ArH), 4.61 (s, 2H, - N-CH_2), 8.04 (s, 1H, -NH), 8.42 (s, 1H, -N=CH), 7.99-7.20 (m, 11H, ArH); m/z: 399 (100%).

N'- (3-nitrobenzylidene)-2- (2-phenyl- 1H-benzimidazol-1-yl) acetohydrazide (4b): Yield: 78.29%. m.p.: 270-272°C. R_f: 0.64. IR (KBr): 3080, 1650-1457, 860-745 (aromatic ring), 1630 (-C=N in ring), 1480 (-CH_2), 3402 (-NH-), 1699 (>C=O of amide), 1632 (-CH=N), 1557, 1350 (NO_2) cm^{-1}; ^1H NMR (DMSO-d_6): δ 8.29-8.28 (d, 1H, ArH), 8.42 (d, 1H, ArH), 8.60 (s, 1H, ArH), 4.61 (s, 2H, - N-CH_2), 8.04 (s, 1H, -NH), 8.11-8.10 (s, 1H, -N=CH), 7.99-7.20 (m, 10H, ArH); m/z: 399 (100%).

N'- benzylidene-2- (2-phenyl- 1H-benzimidazol- 1-yl) acetohydrazide (4c): Yield: 69.27%. m.p.: 151-153°C. R_f: 0.66. IR (KBr): 3100, 1652-1457, 804-741 (aromatic ring), 1620 (-C=N in ring), 1473 (-CH_2), 3382 (-NH-), 1716 (>C=O of amide), 1624 (-CH=N) cm^{-1}; ^1H NMR (DMSO-d_6): δ 7.65-7.41 (m, 5H, ArH), 7.18-7.15 (m, 5H, ArH), 7.99-7.96 (m, 2H, ArH), 7.24-7.20 (m, 2H, ArH), 4.55 (s, 2H, -N-CH_2), 8.11 (s, 1H, -NH), 8.29 (s, 1H, -N=CH); m/z: 354 (100%).

N'-(2-chlorobenzylidene)-2-(2-phenyl-1H-benzimidazol-1-yl) acetohydrazide (4d): Yield: 71.23%. m.p.: 202-204°C. R_f: 0.69. IR (KBr): 3066, 1652-1457, 854-783 (aromatic ring), 1626 (-C=N in ring), 1473 (-CH_2), 3392 (-NH-), 1716 (>C=O of amide), 1628 (-CH=N), 738 (C-Cl) cm^{-1}; ^1H NMR (DMSO-d_6): δ 8.60-8.59 (d, 1H, ArH), 8.42-8.41 (d, 1H, ArH), 7.99-7.96 (m, 2H, ArH), 7.65-7.20 (m, 9H, ArH), 4.55 (s, 2H, -N-CH_2), 8.11 (s, 1H, -NH), 8.29 (s, 1H, -N=CH); m/z: 388.5 (100%).

N'-(4-chlorobenzylidene)-2-(2-phenyl-1H-benzimidazol-1-yl) acetohydrazide (4e): Yield: 68.49%. m.p,: 250-252°C. R_f: 0.70. IR (KBr): 3101, 1652-1457, 854-795 (aromatic ring), 1626 (-C=N in ring), 1475 (-CH_2), 3402 (-NH-), 1716 (>C=O of amide), 1628 (-CH=N), 738 (C-Cl) cm^{-1}; ^1H NMR (DMSO-d_6): δ 8.29-8.28 (d, 1H, ArH), 8.11-8.10 (d, 1H, ArH), 8.76-8.75 (d, 1H, ArH), 8.61-8.60 (d, 1H, ArH), 7.99-7.20 (m, 9H, ArH), 4.61 (s, 2H, -N-CH_2), 8.04 (s, 1H, -NH), 8.42 (s, 1H, -N=CH); m/z: 388.3 (100%).

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