SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF BENZIMIDAZOLE CARBOXYLATE DERIVATIVES

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Abstract : A new series of 2-{3-[(substituted carbonyl) amino]-2-methyl benzy1)-1H-benzimidazole-1- carboxylates derivatives were synthesized by reaction of o-phenylene diamine and 2-methyl-3-nitrophenylacetic acid as starting materials Chemical structures of synthesized monomers were confirmed by elemental analyses and spectroscopic methods. The structures of synthesized compounds were confirmed by FT-IR, ^{1}H-NMR, ^{13}C NMR and Mass spectroscopy. The synthesized compounds were tested for their antimicrobial potency against two gram +ve and two gram –ve bacteria Staphylococcus aureus, Escherichia coli, Bacillus subtilis, Salmonella typhosa and four fungi Aspergillus niger, Candida albicans, Cryptococcus neoformans, Thielaviopsis paradoxa strains using cup plate method. Compound 6b and 6c shows more potent antimicrobial activity by comparing with Ciproflaxacin and Meconazole as standard drug for bacteria and fungi respectively. Compound 6b and 6c shows a zone of inhibition of 11 – 14 mm against all tested strain expect B.subtilis which was 15 – 8 mm.

Keywords: Benzimidazole, o-phenylenediamine, antimicrobial, Ciproflaxacin and Meconazole.

I.INTRODUCTION

Benzimidazole derivatives are of wide interest because of their diverse biological activity and clinical applications, and are remarkably effective compounds both with respect to their inhibitory activity and their favorable selectivity ratio. [1-3] These derivatives are found to exhibit various biological activites such as anticancer [4], antihypertensive [5], anthelmenthic [6-8], antiprotozoal [9-10], antimicrobial [11-16], antioxidant [17-18], anti-inflammatory [19-20] and analgesic [21] activity. Different synthetic methods are reported for the synthesis of benzimidazole and its derivatives which includes processes like coupling of o-phenylenediamine with carbonyl compounds in presence of various catalysts like ZrCl4, SnCl4, BF3, polyethylene glycol, ceric ammonium nitrate [22] etc. The present study utilizes the same coupling phenomenon of o-phenylenediamine with substituted organic acids in presence of ring closing agents like HCl to form 2-substituted derivatives followed by their antibacterial screening. [23-24]

II.MATERIAL AND METHODS

Experimental

All chemicals and reagents were used are of analytical grade. Melting points were determined on an electro thermal apparatus using open capillaries and are uncorrected. Thin-layer chromatography was accomplished on 0.2-mm precoated plates of silica gel G60 F254 (Merck). Visualization was made with UV light (254 and 365nm) or with an iodine vapor. IR spectra were recorded on a Shimadzu-Fourier transform infra-red **(FTIR)-8400 Spectrophotometer** using **KBr** disc. 1H NMR spectra were recorded on a **Bruker DPX-400 MHz** spectrometer. Chemical shifts are expressed in δ ppm downfield from TMS as an internal standard. The mass spectral data were obtained with a **SHIMADZU-LCMS**

Working scheme

Experimental

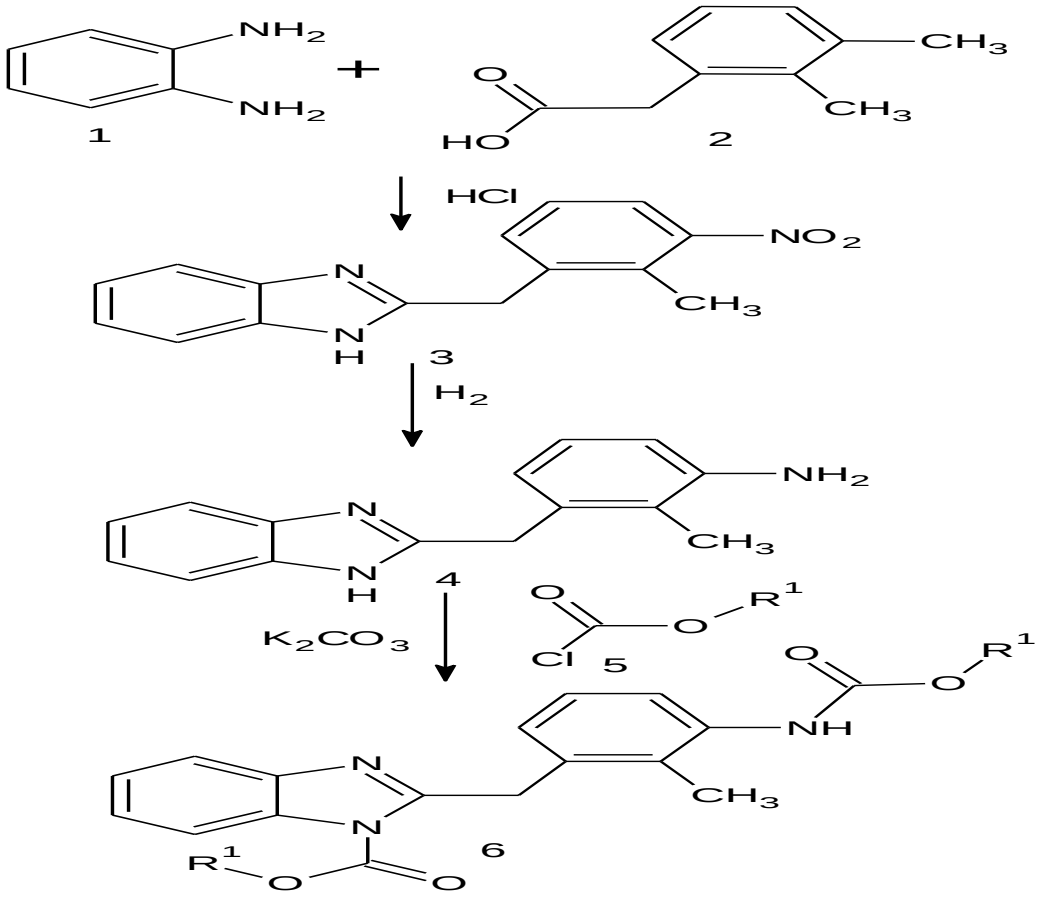
General method of preparation

1. Preparation of 2-(2-methyl-3-nitrobenzyl)-1H-benzimidazole (3)

4N HCl solution (50 cm^3) was added to a round-bottom flask equipped with a magnetic stirring bar and reflux condenser. o-Phenylenediamine (1, 4.0 g, 0.036 mol) and 2-methyl-3-nitrophenylacetic acid (2, 6.6 g, 0.036 mol) were added and the solution was stirred for 30 minutes. The reaction mass was reflux at temperature 85°C for 3 hour. The product was precipitated by adding ammonia solution. The resulting precipitated solid was filtered and washed with water. The compound was re- crystallized from water and ethanol.

2. Preparation of 3-(1H-benzimidazole-2yl-methyl)-2-methyl aniline (4)

2-(2-Methyl-3-nitrobenzy1)-lH-benzimidazole (3, 10 g, 0.03 mol) and methanol (100 cm') were added to 80 cm^3 closed vessel flask equipped with a magnetic stirring bar. Ammonium formate (10 g, 0.16 mol) and 5% palladium on carbon 50% wet (1 g, 0.1 volume) were added to the reaction mass and the flask was closed. The reaction mass was reflux at temperature 90 C-100°C for 2 hour After completion of the reaction, the catalyst was filtered off by using celite bed and the solvent was removed under vacuum below 60°C. The product obtained was dried in a desiccator to afford 3-(1H-benzimidazo1e-2-ylmethyl)-2- methylaniline (4).



3. Preparation of substituted benzimidazole carboxylate (6)

Acetone (15 cm^3) was added to a round-bottom glass flask equipped with a magnetic stirring bar. Potassium carbonate (8.28 g, 0.06 mol) was added to the reaction mass followed by 3-(lH-benzimidazo1e-2- y1 methyl)-2-methylaniline (4, 11.3 g, 0.048 mol). Substituted chloroformate (7, 0.048 mol) was then added maintaining the temperature at 0 - 5°C. The precipitated solid was removed by vacuum filtration. The filtrate was collected and the acetone was removed under vacuum leaving the crude product. The compounds (6) were recrystallized from water and ethanol.

Methyl-2-{3-[(methoxy carbonyl) amino]-2-methylbenzy1}-1H benzimidazole-l- carboxylate

IR (KBr, cm^{-1}): 3443.3 (C-H aromatic), 3193.5-2959 (-NH str.), 1763.6 and 1726.0 (-O.CO.N str.), 1455.0, 1443.5, 1357.6, 1239.0, 1214.0, 1126.2, 1098.3, 1038.5, 778.1, 762.7, 749.2 (C-C aromatic).; ^{1}H NMR (DMSO-d_6): 2.10 (s, 3H, -CH3), 3.64 (s, 3H, CO.00H3), 4.01 (s, 3H,-CO.00H3), 4.50 (s, 2H, -CH2), 6.83-7.22 (m, 4H, Ar-H), 7.30-7.63 (m, 3H, Ar-H), 8.92 (s, 1H, -NH).; ^{13}C NMR (DMSO-d_6):13.84 (CH_3), 34.80 (CH3), 51.61 (OCH_3), 54.55 (OCH_3), 114.84 (ArC), 119.30 (ArC), 124.15 (ArC), 124.38 (ArC), 125.39 (ArC), 126.09 (ArC), 131.39 (ArC), 132.64 (ArC), 136.32 (ArC), 136.45 (ArC), 141.82 (ArC), 150.46 (ArC), 154.23 (C=0), 155.09 (C=0).; ES-MS (m/z): [M+1]: 353.9.

Ethyl-2-{3-[(ethoxycarbonypamino]-2-methylbenzyl}-1H-benzimidazole-l-carboxylate.

IR (KBr, cm^{-1}): 3429.8 (C-H aromatic), 3211.9- 2978.5 (C=N str.), 1744.3 and 1724.0 (-0.CO.N str.), 1474.3, 1456.0, 1376.0, 1358.6, 1334.5, 1283.4 1235.2, 1176.4, 1132.0, 1097.3, 1047.2, 1010.5, 762.7, 749.2 (C-C aromatic).; ^{1}H NMR (DMSO-d_6): 1.21-1.24 (t, 3H, -CH3), 1.32-1.36 **(t,**3H,-CH3), 2.11 (s, 3H, -CH3), 4.06-4.12 (q, 2H, -CH2), 4.42-4.8 (q, 2H, -CH2), 4.50 (s, 2H, -CH2), 6.78-7.96 (m, 7H, Ar-H), 8.88 (s,1H, -NH).; ^{13}C NMR (DMSO-d_6): 13.74 (CH3), 13.84 (CH3), 14.60 (CH3), 34.93 (CH3), 60.07 (OCH3), 64.05 (OCH3), 114.83 (ArC), 119.31 (ArC), 124.04 (ArC), 124.12 (ArC), 124.41 (ArC), 125.34 (ArC), 125.78 (ArC), 131.29 (ArC), 132.72 (ArC), 136.41 (ArC), 136.51

(ArC), 141.85 (ArC), 149.81 (ArC), 154.17 (C=0), 154.64 (C=0). MS (m/z): [M+1]: 381.9.

2-methyl propy1-2-{3-[(2 methylpropoxycarbonyl) amino]-2-methyl benzyl} -1H-benzimidazole-l-carboxylate.

IR (KBr, cm^{-1}): 3444.2 (C-H aromatic), 3284.2- 2875.3 (-NH str.), 1745 and 1694.2 (-0.CO.N str.), 1472.4, 1455.0, 1295.9, 1246.8, 1204.3, 1121.4, 1047.2, 982.6, 763.7, 742.5 (C-C aromatic); ^{1}H NMR (DMSO-d_6): 0.919-0.964 (d, 6H, -C(CH3)2), 0.971- 0.976 (d, 6H,-C(CH3)2), 1.87 -1.93 (m, 1H,-CH), 2.05-2.10 (m, 1H, -CH), 3.83-3.85 (d, 2H, -CH2), 4.23-4.26 (d, 2H, -CH2), 4.51 (s, 2H, -CH2), 6.80-7.96 (m, 7H, Ar-H), 8.89 (s, 1H, -NH).; ^{13}C NMR (DMSO-d_6): 13.03 (CH3), 13.83 (CH3), 18.84 (CH3), 18.91 (CH3), 27.14 (CH), 27.66 (CH), 35.00 (CH3), 70.03 (OCH_2), 73.69 (OCH2), 114.66 (ArC), 119.38 (ArC), 124.14. (ArC), 124.44 (ArC), 125.34 (ArC), 125.97 (ArC) 131.42 (ArC), 132.74 (ArC), 136.31 (ArC), 136.50 (ArC), 141.86 (ArC),150.00 (ArC), 154.22 (C=0), 154.78 (C=0). MS (m/z): 438.0.

Phenyl 2- {3-[(phenoxycarbonyl)amino]-2-methyl benzy1}-1H-benzimidazole-l carboxylate

IR (KBr, cm^{-1}): 3444.2 (C-H aromatic), 3295.8-2955 (-NH str.), 1748.3 and 1691.2 (-O.CO.N str.), 1533.1, 1455.0, 1391.4, 1339.3, 1299.8, 1203.4, 1121.4, 1086.7, 1040.4, 969.1, 764.6, 742.5, 697.1 (C-C aromatic); ^{1}H NMR (DMSO-d_6): 2.06 (s, 3H, -CH3), 4.91(s, 2H, -CH2), 5.14 (s, 2H, -OCH2), 5.4 (s, 2H, -OCH2), 6.78-7.91 (m, 17H, Ar-H), 9.05 (s, 1H, -NH).

^{13}C NMR (DMSO-d_6): 13.80 (CH2), 34.93 (CH2), 65.66 (OCH2), 69.26 (OCH2), 114.76 (ArC), 119.39 (ArC), 124.03 (ArC), 124.21 (ArC), 124.46 (ArC) 125.41 (ArC), 125.93 (ArC), 127.86 (ArC), 128.40 (ArC), 128.65 (ArC), 131.31 (ArC), 132.67 (ArC), 134.62 (ArC), 136.35 (ArC), 136.39 (ArC), 136.92 (ArC), 141.87 (ArC), 149.76 (ArC), 154.19 (C=0), 154.54 (C=0).; MS (m/z): 506.

The physiochemical characterization of synthesized derivatives is presented in Table 1.

Antibacterial Evaluation

All the synthesized benzimidazoleamide derivatives 6a-6d were screened for their in vitro antibacterial activity against Staphylococcus aureus, Bacillus subtilis, Escherichia coli, and Salmonella typhosa and antifungal activity against Aspergillus niger, Candida albicans, Cryptococcus neoformans, Thielaviopsis paradoxa using agar diffusion method (cup plate method).[25] Ciprofloxacin was used as standard drugs for antibacterial activity and Miconazole was used as standard drugs for antifungal activity. Three different concentrations (2mg and 5mg) of synthesized derivatives were used to evaluate antimicrobial potential, and the results have been summarized in Table 2 and Table 3.

III.RESULT AND DISCUSSION

In the current invention, we are reporting the synthesis of some novel 2-benzyl substituted benzimidazole derivatives by convensional method. In this study, a series of 2-benzyl substituted benzimidazole derivatives have been synthesized from o-phenylenediamine (1) and 2-methy1-3- nitrophenylacetic acid (2) as starting materials.

In the present work, 2-(2-methyl-3-nitrobenzy1)-1H benzimidazole (3) was obtained in good yields by the condensation of o-phenylenediamine (1) and 2-methyl-3-nitrophenylacetic acid

(2) in presence of aqueous hydrochloric acid solution under reflux.

2-(2-Methyl-3-nitrobenzy1)-1H-benzimidazole (3) was reduced to its amine analogue by hydrogenation to afford compound **4.** The reaction was carried out in the presence of palladium catalyst and in situ generated hydrogen gas from ammonium formate under reflux.

3-(1H-Benzimidazole-2-y1 methyl)-2-methylaniline **(4)** was treated with allyl chloroformate / benzyl chloroformate **(5a-d)** in dichloromethane as solvent in presence of pyridine as base to afford compounds **(6a-d).**

The reaction monitoring and purity of compounds **(8a-d)** was confirmed by normal phase tic method using a mobile phase chloroform: methanol (9.8: 0.2) and was detected using uv fluorescence at maxima λ max 254.

The compound **6a** was synthesized by reacting 3-(1H-benzimidazole-2- yl methyl)-2-methylaniline **(4)** with methyl chloroformate **(5a).** The compound **6b** was synthesized by reacting 3-(1H-benzimidazole-2- yl methyl)-2-methylaniline **(4)** with ethyl chloroformate **(5b).** The compound 6c was synthesized by reacting 3-(1H-benzimidazole-2- yl methyl)-2-methylaniline (4) with isobutyl chloroformate (5c). The compound **6d** was synthesized by reacting 3-(1H-benzimidazole-2-yl methyl)-2-methylaniline **(4)** with benzyl chloroformate **(5d).**

The structures of compounds **(6a-d)** were confirmed by the above spectral data.

IV.CONCLUSION

A novel series of 2-{3-[(substituted carbonyl) amino]-2-methyl benzy1)-1H- benzimidazole-1- carboxylates 6a-d had been synthesized and characterized by IR, NMR, mass and elemental analysis. The final compounds were screened for in vitro antibacterial and antifungal activity against both Gram-positive and Gram-negative strains of bacteria and selected fungal strain by cup-plate method. Among the various derivatives, the compounds 6b-6c shows excellent inhibition of bacterial growth and 6b-d excellent inhibition of fungal growth as compared to standard drug ciprofloxacin and miconazole.

Table No. 1: Characterization of synthesized compounds

Compound	R^1	Molecular formula	M.P	% Yield	Elemental Analysis					
					Calculated			Found		
					%C	%H	%N	%C	%H	%N
6a	CH_3	$C_{19}H_{19}N_3O_4$	107	78	64.58	5.42	11.89	64.66	5.51	11.95
6b	CH_2CH_3	$C_{21}H_{25}N_3O_4$	115	75	66.13	6.08	11.02	66.33	6.4	11.33
6c	$CH_2C(CH_3)_2$	$C_{25}H_{31}N_3O_4$	90	70	68.63	7.14	9.60	68.70	7.32	9.89
6d	$CH_2C_6H_5$	$C_{31}H_{27}N_3O_4$	140	72	73.65	5.32	12.66	73.75	5.48	12.79

Table 2: Antibacterial activity of synthesized compound

Compound	S.aureus		E.coli		B.subtilis		S. typhosa	
	2mg	5mg	2mg	5mg	2mg	5mg	2mg	5mg
6a	-	-	-	+	-	-	-	+
6b	-	+	+	+	+	++	++	+
6c	+	+	-	+	+	+	+	+
6d	-	+	-	+	-	+	+	+
Ciprofloxacin	+	+	+	++	+	++	+	++

Inhibition zone diameter in mm: (+) 11-14mm, (++) 15-18mm

Table 3: Antifungal activity of synthesized compound

Compound	A.niger		C.albicans		C.neoformans		T.paradoxa	
	2mg	5mg	2mg	5mg	2mg	5mg	2mg	5mg
6a	-	+	-	+	--	-	-	+
6b	+	+	+	+	-	+	+	+
6c	+	+	-	+	+	+	+	+
6d	+	+	-	+	-	+	+	+
Miconazole	-	+	+	++	+	++	-	++

Inhibition zone diameter in mm: (+) 11-14mm, (++) 15-18mm

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