



SYNTHESIS, SPECTRAL CHARACTERIZATION AND ANTIMICROBIAL STUDIES OF NOVEL BENZIMIDAZOLE DERIVATIVES

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ABSTRACT

2-Chloromethyl-1H-benzimidazole derivatives were prepared by condensing 2-Chloromethyl-1H-benzimidazole with different heterocycles. The synthesized derivatives were characterized by IR, ¹H NMR, ¹³C NMR (1D, 2D NMR) and elemental analysis. The compounds were screened for *in vitro* and microbial activity against panel of selected gram positive and gram negative bacterial strains using Ciprofloxacin as standard.

Key words: 2-Chloromethyl-1H-benzimidazole, Antimicrobial activity.

INTRODUCTION

The benzimidazole has been an important pharmacophore and privileged structure in medicinal chemistry. It is a benz annulated ring system in which benzene ring is fused with five membered ring system having heteroatom at 1 and 3 position. The properties of benzimidazole and its analogues have been studied since hundred years. The most prominent benzimidazole compound in nature is N-ribosyl-dimethyl benzimidazole, which serve as an axial ligand for cobalt in vitamin B12. Substituted benzimidazole derivatives have found various pharmacological activities such as antitumor^{1,2}, antiulcer³, antiinflammatory⁴, antiviral^{5,6}, antihemietic^{7,8}, antibacterial^{9,10} and antifungal^{11,12} properties. Optimization of substituent around the benzimidazole nucleus has resulted in many drugs like albendazole, mebendazole, thiabendazole as anthelmintics; omeprazole, lansoprazole, pantoprazole as proton pump inhibitors and many lead compounds in a wide range of other therapeutic areas¹³. Though, all seven position in the benzimidazole nucleus can be substituted with variety of chemical entities, but most of the biologically active benzimidazole based compounds bear functional groups at 1, 2 and/or 5 (or 6). Based on the above observations,

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we have planned to synthesize a novel series of benzimidazole derivatives derived from 2-chloromethyl-1H-benzimidazole followed by their *in vitro* antibacterial, antifungal activities¹⁴. As an inception, various 2-chloromethyl-1H-benzimidazole were synthesized and characterized by FT-IR, 1DNMR, 2D NMR (¹H, ¹³C), mass, CHN analysis and the antimicrobial activities were screened.

EXPERIMENTAL

Materials and methods

Characterization techniques

Melting point (M.P.) were determined by open capillary method and are uncorrected. IR spectra were recorded by Jasco FTS 3000 HX (KBr pellets). ¹H NMR spectra were recorded on Bruker Advance III NMR spectrometer (500 MHz) using TMS as internal standard (Chemical shifts in ppm). ¹³C NMR spectra were recorded on the same instrument at 125.76 MHz and are referenced using the central line of the solvent signal (DMSO -d₆ septet at S = 39.5 ppm). Mass spectra were recorded with JOEL ac MATE II instrument. Elemental analysis (C, H and N) were performed with a Perkin Elmer 2400 series II CHN Analyzer.

Table 1:

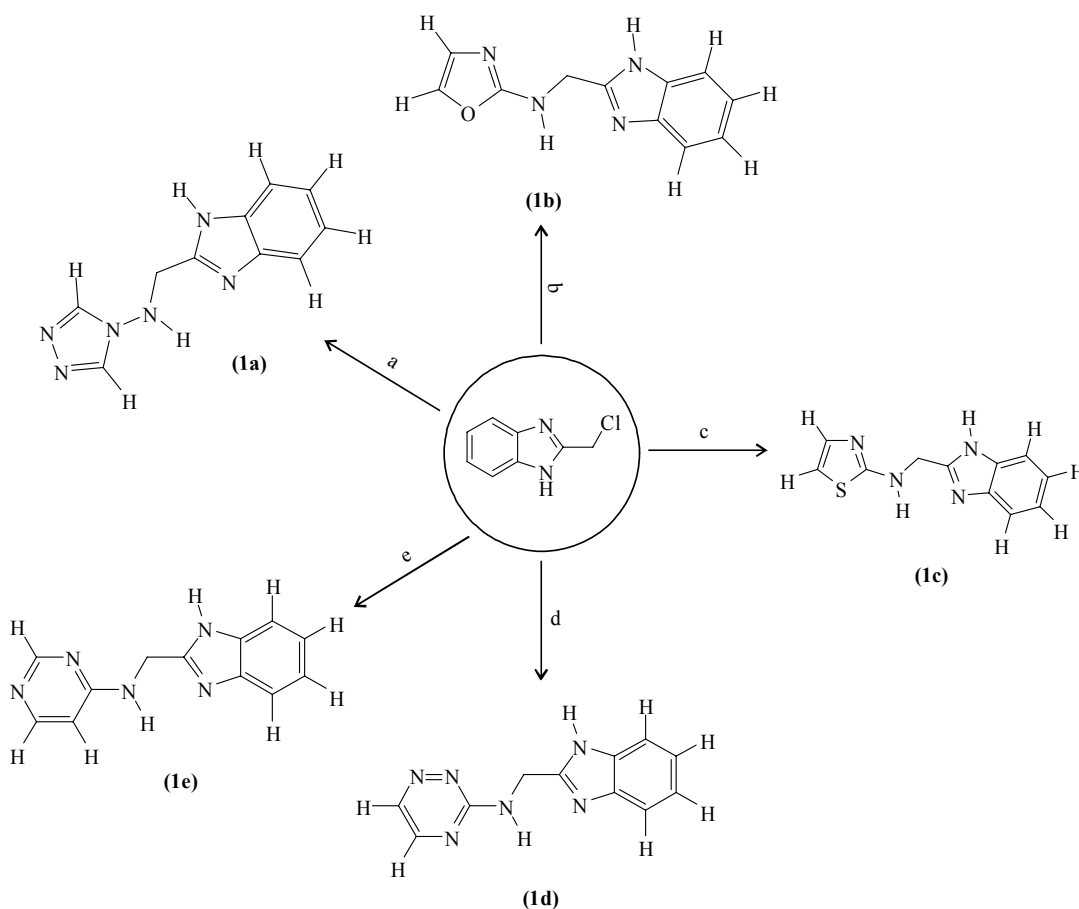
Product code	M.P. (°C)	Reaction time (h)	Elemental analysis (%)						Molecular weight	Molecular formula
			Calculated			Found				
			C	H	N	C	H	N		
1a	310	15	56.07	4.71	39.23	56.01	4.69	39.21	214.23	C ₁₀ H ₁₀ N ₆
1b	342	16	61.67	4.71	26.15	61.50	4.70	26.01	214.22	C ₁₁ H ₁₀ N ₄ O
1c	398	16	57.37	4.38	24.33	57.30	4.30	24.23	230.29	C ₁₁ H ₁₀ N ₄ S
1d	455	14	58.40	4.46	37.15	58.38	4.50	37.20	226.24	C ₁₁ H ₁₀ N ₆
1e	395	16	63.99	4.92	31.09	64.00	4.99	31.21	225.25	C ₁₂ H ₁₁ N ₅

General procedure for synthesis of compounds 1a-1e¹⁴

N-((1H-benzo[d]imidazol-2-yl)methyl)-4H-1,2,4-triazol-4-amine (1a)

2-Chloromethyl-1H-benzimidazole (1.665 g, 0.01 mol) and K₂CO₃ (0.02 mol, 2.76 g) were stirred at room temperature in dimethyl formamide (25 mL) for half an hour and pinch of KI was added. After that 4H-1,2,4-triazole-4-amine (0.840 g, 0.01 mol) was added to

reaction mixture, which was refluxed for 15 hrs until TLC showed completion of of reaction. The reaction mixture was poured into water (20 mL) and the mixture was extracted with ethylacetate. The organic extracts was washed with water, dried over anhydrous sodium sulphate and concentrated to obtain crude product. The residue was crystallised from diethyl ether to give pure compound at room temperature. Physical data of compounds (**1a-1e**) are presented in Table 1. Lemon solid, Yield (45%); mp 310°C (dec); IR (KBr) 3408 (NH Str), 3057 (-CH Str), 2925 (aliphatic, C-H) 1591 (N-N), 1286 (C-N) cm^{-1} ; ^1H NMR (500 MHz, DMSO-*d*6) δ : 12.232 (s, 1H), 8.29 (s, 2H), 7.569 (m, 2H), 7.30 (m, 2H) 6.944 (s, 1H), 3.745 (s, 2H); ^{13}C NMR (125.76 MHz, DMSO-*d*6) δ : 145.640, 142.049, 137.428, 137.398, 123.015, 123.70, 115.577, 115.359, 47.971; Mass (m/z): 214.10.



Scheme 1

Reaction condition: DMF/ K_2CO_3 reflux for 17 hr, **Reagents used:** (a) 4H-1,2,4-triazol-4-amine (b) oxazol-2-amine (c) Thiazol-2-amine (d) 1,2,4-triazin-3-amine (e) pyrimidin-4-amine

N-((1H-benzo[d] imidazol-2-yl) methyl) oxazol-2-amine (1b)

2-Chloromethyl-1H-benzimidazole (1.665 g, 0.01 mol) and K_2CO_3 (0.02 mol, 2.76 g) were stirred at room temperature in dimethyl formamide (25 mL) for half an hour and pinch of KI was added. After that oxazol-2-amine (0.840 g, 0.01 mol) was added to reaction mixture which was refluxed for 15 hrs until TLC showed completion of reaction. The reaction mixture was poured into water (20 mL) and the mixture was extracted with ethylacetate. The organic extracts was washed with water, dried over anhydrous sodium sulphate and concentrated to obtain crude product. The residue was crystallised from diethyl ether to give pure compound at room temperature. Yellow solid, Yield (55%); mp 342°C (dec); IR (KBr) 3367 (NH Str), 3025 (-CH Str), 2925 (aliphatic, C-H), 1243 (C-N) cm^{-1} ; 1H NMR (500 MHz, DMSO-*d*6) δ : 12.167 (s, 1H), 8.212 (s, 1H), 7.62 (d, 1H), 7.56 (m, 2H), 7.28 (m, 2H), 7.16 (d, 1H), 4.375 (s, 2H); ^{13}C NMR (125.76 MHz, DMSO-*d*6) δ : 164.88, 141.45, 137.75, 137.67, 127.88, 123.09, 116.15, 43.47; Mass (m/z): 214.22.

N-((1H-benzo[d] imidazol-2-yl) methyl) thiazol-2-amine (1c)

2-Chloromethyl-1H-benzimidazole (1.665 g, 0.01 mol) and K_2CO_3 (0.02 mol, 2.76 g) were stirred at room temperature in dimethyl formamide (25 mL) for half an hour and pinch of KI was added. After that thiazole-2-amine (1.001, 0.01 mol) was added to reaction mixture which was refluxed for 15 hrs until TLC showed completion of reaction. The reaction mixture was poured into water (20 mL) and the mixture was extracted with ethylacetate. The organic extracts was washed with water, dried over anhydrous sodium sulphate and concentrated to obtain crude product. The residue was crystallised from diethyl ether to give pure compound at room temperature. Light yellow solid, Yield (65%); mp 398°C (dec); IR (KBr) 3375 (NH Str), 3049 (-CH Str), 2925 (aliphatic, C-H) cm^{-1} ; 1H NMR (500 MHz, DMSO-*d*6) δ : 12.272 (s, 1H), 8.191 (s, 1H), 7.78 (d, 1H), 7.56 (m, 2H), 7.37 (m, 2H), 6.964 (d, 1H), 4.848 (s, 2H); ^{13}C NMR (125.76 MHz, DMSO-*d*6) δ : 163.84, 141.45, 137.75, 137.67, 123.48, 123.09, 115.82, 115.75, 113.00, 44.18. Mass (m/z): 230.29.

N-(1H-benzo[d] imidazol-2-yl) methyl)-1,2,4-triazin-3-amine (1d)

2-Chloromethyl-1 H-benzimidazole (1.665 g, 0.01 mol) and K_2CO_3 (0.02 mol, 2.76 g) were stirred at room temperature in dimethyl form amide (25 mL) for half an hour and pinch of KI was added. After that 1,2,4-triazin-3-amine (0.960 g, 0.01 mol) was added to reaction mixture which was refluxed for 15 hrs until TLC showed completion of reaction. The reaction mixture was poured into water (20 mL) and the mixture was extracted with ethyl acetate. The organic extracts was washed with water, dried over anhydrous sodium sulphate

and concentrated to obtain crude product. The residue was crystallized from diethyl ether to give pure compound. at room temperature. Pale yellow solid, Yield (68%); mp 455°C (dec); IR (KBr) 3315 (NH Str), 3040 (-CH Str), 2909 (aliphatic, C-H) cm^{-1} ; ^1H NMR (500 MHz, DMSO-*d*6) δ : 12.212 (s, 1H), 9.784 (s, 1H), 9.491 (s, 1H), 7.56 (m, 2H), 7.30 (m, 2H), 4.704 (s, 2H); ^{13}C NMR (125.76 MHz, DMSO-*d*6) δ : 160.41, 149.89, 141.45, 139.38, 138.53, 123.48, 123.09, 115.82, 115.75, 44.18 Mass (m/z): 226.24.

N-(1H-benzo[d]imidazol-2-yl) methyl) pyrimidin-4-amine (1e)

2-Chloromethyl-1H-benzimidazole (1.665 g, 0.01 mol) and K_2CO_3 (0.02 mol, 2.76 g) were stirred at room temperature in dimethyl form amide (25 mL) for half an hour and pinch of KI was added. After that pyrimidin-4-amine (0.950 g, 0.01 mol) was added to reaction mixture, which was refluxed for 15 hours until TLC showed completion of reaction. The reaction mixture was poured into water (20 mL) and the mixture was extracted with ethyl acetate. The organic extracts was washed with water, dried over anhydrous sodium sulphate and concentrated to obtain crude product. The residue was crystallized from diethyl ether to give pure compound at room temperature. Pale yellow solid, yield (68%); mp 455°C (dec); IR (KBr) 3411 (NH Str), 3073 (-CH Str), 2925 (aliphatic, C-H) cm^{-1} ; ^1H NMR (500 MHz, DMSO-*d*6) δ : 12.236 (s, 1H)8.770 (s, 1H), 8.29 (s, 1H), 7.53 (m, 2H), 7.26 (m, 2H), 4.754 (s, 2H); ^{13}C NMR (125.76 MHz, DMSO-*d*6) δ : 160.19, 159.89, 158.99, 149.85, 141.45, 139.38, 138.53, 123.48, 123.09, 115.82, 115.75, 107.90,44.1 Mass (m/z): 225.25.

Antimicrobial studies

The antimicrobial activities of the synthesized compounds against different pathogens were determined by Agar Well diffusion method. Using sterile inoculation loop, 20 pure colonies of the test organism are transferred to 5 mL of sterile nutrient broth and incubated at 37°C overnight for 18 hrs. The modified agar well diffusion method of Perez et al.¹⁵ was employed. Each selective medium was inoculated with the microorganism suspended in sterile water. Once the agar was solidified, it was punched with a six millimeters diameter wells and filled with 50 $\mu\text{g}/\text{mL}$ of the sample and blanks (ethanol and antibiotic). The test was carried out by triplicate. The plates were incubated at $35 \pm 2^\circ\text{C}$ for 24 hr. The antimicrobial activity was calculated by applying the expression in $\mu\text{g}/\text{mL}$. The antibacterial activities in terms of minimum inhibitory concentration (MIC) of compounds (**1a-1e**) are depicted in Table 2.

MIC's of compounds were compared with ciprofloxacin standard drug. MIC values in Table 2 revealed that compound **1a** exhibited excellent activity against *E. coli* at MIC 10 $\mu\text{g}/\text{mL}$ than other derivatives.

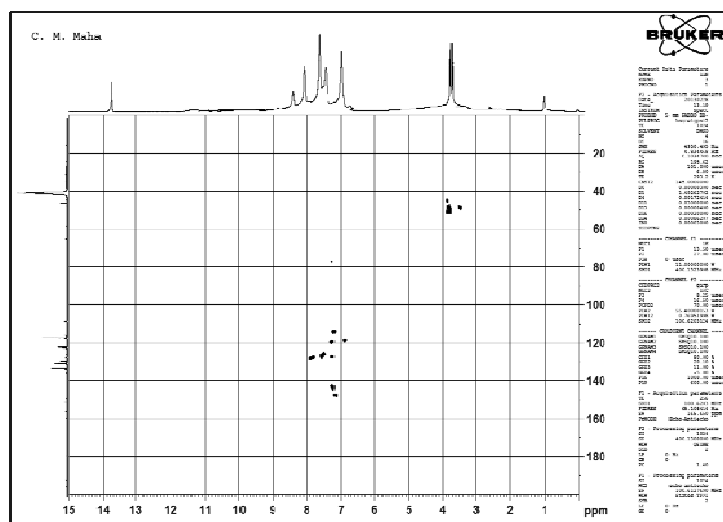
Table 2: Antibacterial activities of compounds (1a-1e), for bacterial strains in MIC ($\mu\text{g/mL}$)

Bacterial strains (MIC)	Compounds					
	Ciprofloxacin	1a	1b	1c	1d	1e
<i>K. pneumoniae</i>	15	20	50	50	50	30
<i>S. typhic</i>	10	15	50	50	50	30
<i>S. aureus</i>	5	15	-	35	25	20
<i>B. subtilis</i>	5	10	50	-	45	15
<i>P. auruginosa</i>	5	15	50	45	50	15
<i>E. coli</i>	5	10	35	50	35	15

Note:- No inhibition

RESULTS AND DISCUSSION

The synthesis of various benzimidazole derivatives were carried out as depicted in **Scheme 1**. A broad band at $3408 - 3273 \text{ cm}^{-1}$ is ascribed to N-H stretching frequency of the imidazole moiety. A strong band at 2925 cm^{-1} is due to the (C-H) stretching frequencies. Hence the IR data illustrate the formation of the 2-Chloromethyl-1H-benzimidazole derivatives.

**Fig. 1: HSQC spectrum of 1a**

In ^1H NMR spectrum of compound **1a-1c** and **2a-2c** show broad singlet in the region of 11.01-11.16 ppm and is assigned for free NH group present. A sharp singlet at 12-13 ppm is assignable to $-\text{NH}$ proton of benzimidazole and also peak at 4.7 ppm show the presence of CH_2 . On focusing the ^{13}C NMR spectral assignments, the signals at 164 is due to C-N of heterocyclic compounds (**1b-1e**). The regioselectivity and other structure features of compound **1c** were analysed by 1D NMR (^1H , ^{13}C) and 2D NMR (HSQC, HMBC) spectral techniques. In ^1H NMR spectrum, the doublet at 3.74 ppm show the presence of methylene group in benzimidazole moiety. Further, this assignment was substantiated by HSQC analysis (Fig. 1). In ^{13}C spectra, peaks at 47.1 and 142.6 ppm were unambiguously assigned to C-1 and C-10 carbons, respectively. In HSQC spectra, chemical shift at 47.1 ppm (C-10 carbon of benzimidazole). It shows one band correlation with signal at 4.7 ppm of methylene protons.

CONCLUSION

Five new 2-chloromethyl-1H-benzimidazole derivatives were synthesized in reasonably good yields. They were characterized by IR, ^1H , ^{13}C NMR (1D, 2DNMR), mass and elemental analysis. All the newly synthesized compounds were tested for antimicrobial activity by agar well diffusion method. Among the screened samples, compound **1a** exhibited as most active against *E. coli* compared to other synthesized compounds.

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