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SYNTHESIS AND ANTIBACTERIAL EVALUATION OF SOME NOVEL MANNICH BASES OF BENZIMIDAZOLE DERIVATIVES

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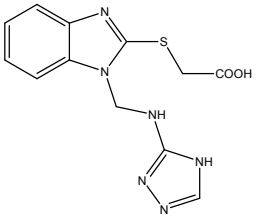
ABSTRACT. Substituted benzimidazoles are known for their chemotherapeutic importance and many pharmacological properties. In this paper, we synthesized some novel Mannich bases of benzimidazole derivatives. The synthesized compounds were characterized by their physical and spectral data and in vitro antibacterial activity of these compounds tested against *Bacillus subtilis*, *Bacillus pumilus*, *Escherichia coli* and *Pseudomonas aeruginosa* organisms. The potency of the synthesized compounds was determined against standard drug Ciprofloxacin by measuring the zone of inhibition.

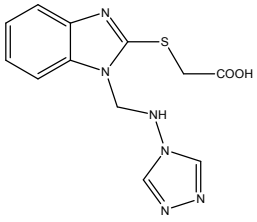
KEY WORDS: Benzimidazole, Mannich base, Antimicrobial activity

INTRODUCTION

Infectious microbial diseases remain pressing problems worldwide, because resistance to a number of antimicrobial agents among variety of clinically significant species of microorganisms has become an important global health problem. One way to battle with this challenge is the conscious usage of the currently marketed antibiotics; the other is the development of novel antimicrobial agents [1-8]. Hence, there will always be a vital need to discover new chemotherapeutic agents to avert the emergence of resistance and ideally shorten the duration of therapy. Benzimidazole nucleus is an important heterocyclic ring. Substituted benzimidazole have received considerable attention during last decades as they are endowed with variety of biological activities and have wide range of therapeutic properties. A literature survey indicates that benzimidazole derivatives possess different pharmacological and biological properties like antibacterial, anti-inflammatory, antifungal, anti-tubercular, anticancer, anthelmintic activity, etc. Combination of two or more active moieties into one is a common procedure of manipulation and this can possibly results in augmenting the activity, removal of untoward side effects and particularly prevent the development of resistance by the infectious micro-organisms [9-14].



III



VI

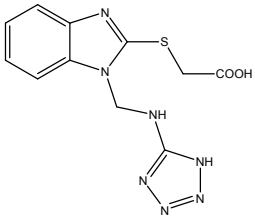


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Figure 1. The target compounds for synthesizing.

By considering the above facts, we plan to synthesize a bi heterocyclic system comprising of benzimidazole nucleus and biologically important heterocyclic systems like triazoles and tetrazoles system (Figure 1). We have also planned to evaluate the synthesized compounds for anti-bacterial activity.

EXPERIMENTAL

Material and equipments

All of chemicals and solvents were purchased from Merck (Darmstadt, Germany) and Sigma-Aldrich chemical Co. (USA). Melting points (uncorrected) were determined with a digital Electrothermal melting point apparatus (model 9100, Electrothermal Engineering Ltd., Essex, UK). ^{1}H and ^{13}C-NMR spectra were recorded with a Bruker 300 MHz (model AMX, Karlsruhe, Germany) spectrometer (internal reference: TMS). IR spectra were recorded with a Thermo Nicolet FT-IR (model Nexus-870, Nicolet Instrument Corp, Madison, Wisconsin, USA) spectrometer. Mass spectra were recorded with an Agilent Technologies 5973, Mass Selective Detector (MSD) spectrometer (Wilmington, USA).

Preparations (Scheme 1)

*2-Mercapto benzimidazole (**I**)*. A mixture of *o*-phenylenediamine (0.1 mol), potassium hydroxide (0.1 mol), and carbon disulfide (0.1 mol), 100 mL of 95% ethanol and 15 mL of water were refluxed for 3 h. Then warm water was added and acidified with dilute acetic acid. The product was filtered and dried and is recrystalized by ethanol [15]. White needle crystals; yield 73%; m.p. 300-302 ^{o}C.

*(1H-benzimidazol-2-ylthio) acetic acid (**II**)*. A mixture containing 2-mercaptobenzimidazole (0.013 mol), 20 mL of ethanol, potassium hydroxide (0.016 mol) was refluxed for 1 h. After cooling the resulting solution to 30 ^{o}C added chloroacetic acid (0.012 mol). After stirring at 25-30 ^{o}C for 18 h, the reaction mixture was added to 100 g of ice-water and stirred for 30 min at 0-10 ^{o}C. The obtained precipitate was collected by filtration, washed with water until free of chloride, air dried at 50 ^{o}C and recrystalized from water [16-18]. White needle crystals; yield 75%; m.p. 214-216 ^{o}C. IR (KBr) (cm^{-1}): 3153 (OH), 3114 (NH), 2980, 2917 (Ar-CH=CH), and 1675 (C=O absorption band of COOH). H-NMR (δ, ppm): 12.7 (1H, bs, 1H of benzimidazole NH), 12.5 (1H, s, 1H of OH of COOH), 7.0-7.43 (4H, m, 4H of Ar-H), 4.12 (2H, s, 2H of CH_2 of CH_2COOH). $C_9H_8N_2O_2S$. MS: m/z (regulatory intensity): 208 (100), 209 (8), 210 (4).

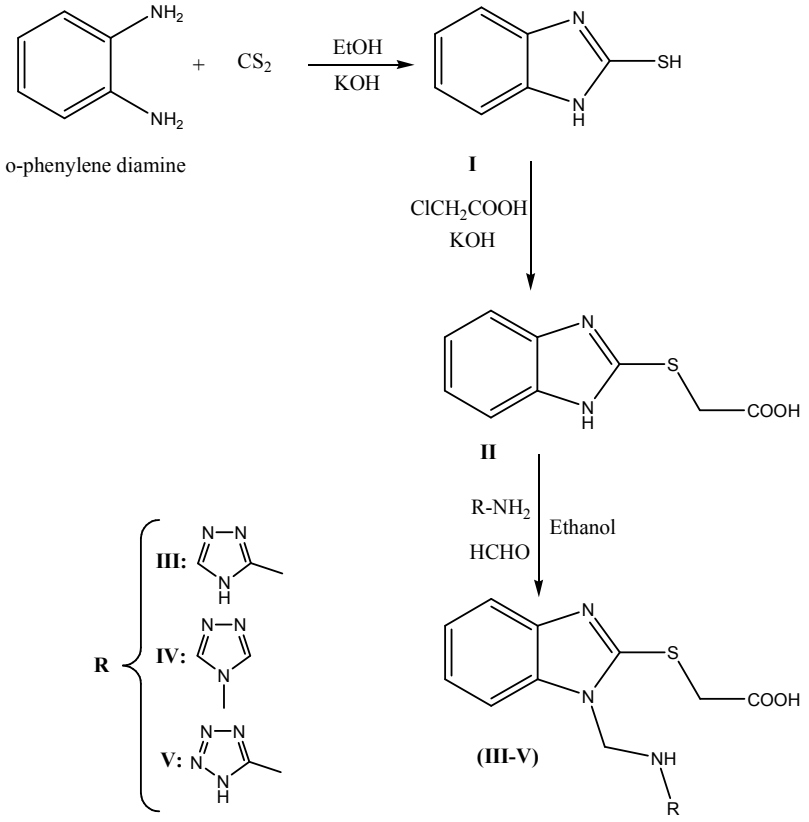
*General procedure for the synthesis of Mannich Bases (**III-V**)*. Benzimidazolylthioacetic acid (**II**) (0.002 mol) dissolved in ethanol and 3-4 drops of conc. HCl was added and reaction mixture was kept for stirring. To the stirring reaction mixture, formaldehyde (0.002 mol) was added drop wise and stirring was continued for 10 min. Meanwhile 0.002 mol appropriate amine ($R\text{-}NH_2$) was dissolved in ethanol and was added into the above reaction mixture drop wise with continuous stirring. After stirring the reaction, mixture was refluxed for 12 h. The mixture was allowed to cool at room temperature. The solid thus separated was filtered and dried. The obtained products (**III-V**) were re-crystallized from ethanol [19, 23].

*{1-[(4H-[1,2,4]Triazol-3-ylamino)-methyl]-1H-benzimidazol-2-ylsulfanyl}-acetic acid (**III**)*. White needle crystals; yield 65 %; m.p. 254-256 ^{o}C. IR (KBr) (cm^{-1}): 3180 (NH), 2950, 2870 (Ar-CH=CH), and 1678 (C=O absorption band of COOH). H-NMR (δ, ppm): 9.6 (1H,s, 1H of OH of COOH), 7.6 (1H,s, 1H of NH of triazole), 6.4-7.3 (6H, m, 4H of Ar-H, 1H of CH of triazole, 1H of NH), 4.8 (2H, d, 2H of $N\text{-}CH_2\text{-}NH$-), 3.9-4 (2H, d, 2H of CH_2COOH). Anal.

calcd. for $C_{12}H_{12}N_6O_2S$; C, 47.36; H, 3.97; N, 27.62%. Found: C, 47.19; H, 3.79; N, 27.54%. MS: m/z (regulatory intensity): 304 (100), 305 (13.6), 306 (5).

[1-([1,2,4]Triazol-4-ylaminomethyl)-1H-benzimidazol-2-ylsulfanyl]-acetic acid ***(IV)***. White needle crystals; yield 55%; m.p. 224-226 ^{o}C. IR (KBr) (cm^{-1}): 3200 (NH), 2943, 2861 (Ar-CH=CH), and 1652 (C=O absorption band of COOH). H-NMR (δ, ppm): 10.16 (1H, s, 1H of OH of COOH), 6.4-8.3 (6H, m, 4H of Ar-H, 2H of CH of triazole), 5.1 (2H, d, 2H of N-CH_2-NH-), 3.8-4.1 (2H, d, 2H of CH_2COOH). Anal. calcd. for $C_{12}H_{12}N_6O_2S$; C, 47.36; H, 3.97; N, 27.62%. Found: C, 47.33; H, 3.71; N, 27.63%. MS: m/z (regulatory intensity): 304 (100), 305 (13.5), 306 (5).

{1-[(1H-Tetrazol-5-ylamino)-methyl]-1H-benzimidazol-2-ylsulfanyl}-acetic acid ***(V)***. White needle crystals; yield 50%; m.p. 234-236 ^{o}C. IR (KBr) (cm^{-1}): 3340 (NH), 2932, 2856 (Ar-CH=CH), and 1702 (C=O absorption band of COOH). H-NMR (δ, ppm): 10.76 (1H, s, 1H of OH of COOH), 7.4-7.9 (4H, m, 4H of Ar-H), 5.4 (2H, d, 2H of N-CH_2-NH-), 3.87-4.15 (2H, d, 2H of CH_2COOH). Anal. calcd. for $C_{11}H_{11}N_7O_2S$; C, 43.27; H, 3.63; N, 32.11%. Found: C, 43.33; H, 3.71; N, 32.23%. MS: m/z (regulatory intensity): 305 (100), 306 (15), 307 (6).



Scheme 1. Scheme of synthesis for intermediates (**I** and **II**) and final compounds (**III-V**).

Antimicrobial activity

Antibacterial activity was carried out by disc diffusion method using *Bacillus subtilis*, *Bacillus pumilus*, *Escherichia coli* and *Pseudomonas aeruginosa* organisms for antibacterial activity. The potency of the synthesized compounds was determined against standard drug Ciprofloxacin by measuring the zone of inhibition [24-26].

RESULTS AND DISCUSSION

The starting compound 2-mercapto benzimidazole (**I**) was prepared from *o*-phenylene diamine, potassium hydroxide and carbon disulfide upon refluxing for 3 h in single step, respectively. The **I** was refluxed for 60 min with potassium hydroxide, followed by chloroacetic acid and stirred for 18 h to furnish (1*H*-benzimidazol-2-ylthio) acetic acid (**II**). The different Mannich bases (**III-V**) were synthesized by refluxing the appropriate substituted amines, formaldehyde with **II** in ethanolic medium for 12 h. The synthesized compounds were characterized by their physical and spectral data.

The resulted synthesized compounds (**III-V**) were screened for antibacterial activity studies at a concentration of 100 µg/mL using DMF as a control against *Bacillus subtilis*, *Bacillus pumilus*, *Escherichia coli* and *Pseudomonas aeruginosa* by disc diffusion method on agar nutrient media. Ciprofloxacin was used as standard drug for the comparison at the concentration of 100 µg/mL against Gram (+ve) and Gram (-ve) bacteria used for the study.

The data in the Table 1 indicates that the compounds were found to possess moderate to weak activity although several benzimidazoles were reported for good antibacterial activity. The compound **III** was active against *Pseudomonas aeruginosa*, and the compounds **III**, **IV** and **V** were active against *Bacillus subtilis*, whereas the compounds **IV** and **V** were active against *Bacillus pumilus*, rest of the compounds showed only weak activity when compared to the standard ciprofloxacin [26].

Table 1. Anti-bacterial activity of synthesized compounds (**III-VI**).

Entry	*Inhibition of zone diameter in mm			
	B. subtilis	*B. pumilus*	*E. coli*	*P. aeruginosa*
	100 µg/mL	100 µg/mL	100 µg/mL	100 µg/mL
III	17	12	8	16
IV	19	17	12	10
V	18	19	11	14
Ciprofloxacin	28	30	32	30
DMF	-	-	-	-

CONCLUSIONS

A series of some novel benzimidazoles were synthesized and evaluated for their potential antimicrobial activities. Based on results, it can be concluded that all the synthesized compounds showed good to moderate antimicrobial activities. The results indicated that new antimicrobial compounds could be prepared by changing of different substrates on various benzimidazole derivatives. These new compounds could be evaluated for further pharmacological activities in other studies.

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