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MICROWAVE ASSISTED SYNTHESIS OF (2-SUBSTITUTED-1H-BENZIMIDAZOL-1-YL) ACETATE AND ITS EVALUATION AS POTENTIAL ANTIMICROBIAL AGENTS

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ABSTRACT

Some derivatives of 2-substituted-1H benzimidazole were synthesized by microwave assisted reaction between different acids and o-phenylenediamine. Nucleophilic substitution of 2-substituted-1H benzimidazole results in formation of ethyl (2-substituted-1H-benzimidazol-1-yl) acetate. The structures of the synthesized compounds were evaluated by spectral and elemental methods of analyses. All the synthesized compounds were screened for their antimicrobial activities. All of the derivatives showed good activity towards bacteria. Some of the synthesized compounds showed moderate activity against tested fungi.

1. INTRODUCTION

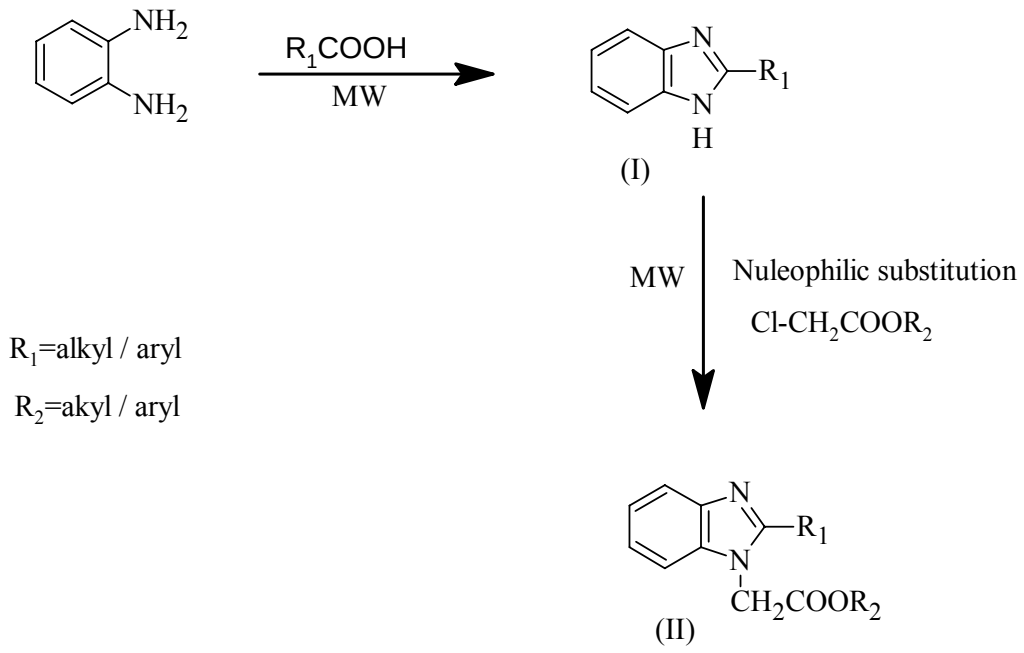
Recently, Microwave heating has emerged as a powerful technique to promote a variety of chemical reactions [1]. Microwave reactions under solvent-free conditions are attractive in offering reduced pollution, low cost and offer high yields together with simplicity in processing and handling [2]. The recent introduction of single-mode technology [3] assures safe and reproducible experimental procedures and microwave synthesis has gained acceptance and popularity among the synthetic chemist community. The application of microwave irradiation to organic synthesis has been the focus of considerable attention in recent years and is becoming an increasingly popular technology [4]. Microwave irradiation has been also applied to carry out synthesis in open vessel [5], using organic solvents such as ethanol, N,N–Dimethylformamide (DMF), 1,2–Dichloroethane (DCE), 1,2–dichlorobenzene etc. as energy transfer media which absorb microwave energy efficiently through dipole rotation. The salient features of microwave approach are shorter reaction times, simple reaction conditions and enhancements in chemical yields [6, 7]. The increase in bacterial resistance has attracted considerable interest in the discovery and development of new classes of antibacterial agents [8]. Despite numerous attempts to develop new structural prototype in the search for more effective antimicrobials, the benzimidazoles still remain as one of the most versatile class of compounds against microbes [9]. Benzimidazoles are remarkably effective compounds both with respect to their inhibitory activity and their favorable selectivity ratio. Extensive biochemical and pharmacological studies have confirmed that benzimidazole molecules are effective against various strains of microorganisms [10-17]. Benzimidazoles are regarded as a promising class of bioactive heterocyclic compounds that exhibit a range of biological activities. Specifically, this nucleus is a constituent of vitamin-B12 [18]. This ring system is present in numerous antioxidant [19–21], antiparasitic [22, 23], antihelmintics [24], antiproliferative [25], anti-HIV [26], anticonvulsant [27], anti-inflammatory [28–31], antihypertensive [32, 33], antineoplastic [34, 35], and antitrichinellosis [36] activities. Efforts have been made from time to time to generate libraries of these compounds and screened them for potential biological activities.Recently, the chemistry and the biological profiles of various pharmacophores of 1-N substituted and 2-substituted benzimidazole derivatives have been worked out in detail. These findings prompted us to synthesize (2-substituted-1H-benzimidazol-1-yl) acetate derivatives. Each of the benzimidazole analogues prepared has been tested for their antimicrobial activities and results are reported in this paper.

2. RESULT AND DISCUSSION

2.1 Chemistry

The reaction sequence for different title compounds is outlined in Scheme 1. The starting material 2-substituted-1H-benzimidazole **I** was prepared according to a reported procedure through the reaction of o-phenylenediamine with appropriate carboxylic acid [37].

Structure of compound **I** was confirmed by comparison of its physical and spectral data with the reported ones [38]. Nucleophilic substitution of compound **I** yielded ethyl (2-substituted-1H-benzimidazol-1-yl) acetate **II**. The structure of compound **II** was confirmed by its IR, ^{1}H NMR as well as elemental analysis. The IR spectra of compound **IIa** showed broad band at 1733.89 cm^{-1} (–C=O ester) and at 1669.28 cm^{-1} (–C=N). ^{1}HNMR spectra revealed the multiplet at δppm 7.1756–7.4426 corresponding to the four aromatic protons. Mass spectrum of compound **IIa** revealed the molecular ion peak M^+ at m/z 218, corresponding to molecular mass of this compound. The purity of synthesized compound was monitored by TLC. Physical and analytical data of compounds **IIa-d** are shown in Table 2.



Scheme 1: Synthesis of benzimidazole derivatives

Table 1: Various substitution of synthesized benzimidazole derivatives

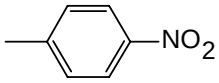
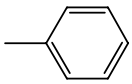
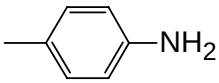
Compound	R_1	R_2
IIa	$-CH_3$	$-C_2H_5$
IIb		$-C_2H_5$
IIc		$-C_2H_5$
IId		$-C_2H_5$

Table 2: Physical and analytical data of compound IIa-d

Product	Colour & shape	Melting point (^{0}C)	Yield (%)	Rf value	Molecular formula (Mol. Wt.)	Found (Calculated) (%)		
						C	H	N
IIa	Cream white crystals	183-186	79.12	0.61	$C_{12}H_{14}N_2O_2$ (218)	66.05 (66.04)	6.42 (6.41)	12.84 (12.83)
IIb	Brown crystals	224-227	75.09	0.64	$C_{17}H_{15}N_3O_4$ (325)	62.76 (62.75)	4.61 (4.60)	12.92 (12.90)
IIc	Yellowish crystals	199-201	80.12	0.60	$C_{17}H_{16}N_2O_2$ (280)	72.85 (72.83)	5.71 (5.72)	10.0 (9.9)
IId	Brown crystals	243-245	74.18	0.67	$C_{17}H_{17}N_3O_2$ (295)	69.15 (69.14)	5.76 (5.77)	14.23 (14.22)

3. Biological activities

The compounds **IIa-d** were screened for their antimicrobial activity against *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 29213) bacterial strains. Ciprofloxacin was used as a reference standard and the antifungal activity of the compounds **IIa-d** was assayed by using the cup-plate agar diffusion method against *Aspergillus niger* fungal strain using Miconazole as standard. The results of the antimicrobial activity screening of the tested compounds are summarized in Table 3, 4 and 5. The synthesized compound **IIc** and **IId** have good antibacterial activity against *S. aureus* and *E. coli* and compound **IIb** and **IId** showed good antifungal activity against *A. niger* as compared with standard.

Table 3: Antimicrobial activity data for synthesized compounds IIa-d and ciprofloxacin against *S. aureus*

Compound	Bacteria along with zone of inhibition (mm)			
	S. aureus			
	25 µg/ml	50 µg/ml	100 µg/ml	200 µg/ml
IIa	-	-	-	11
IIb	-	13	11	20
IIc	-	13	19	23
IId	13	14	21	22
Ciprofloxacin	17	18	24	32

Table 4: Antimicrobial activity data for synthesized compounds IIa-d and ciprofloxacin against *E. coli*

Compound	Bacteria along with zone of inhibition (mm)			
	E. coli			
	25 µg/ml	50 µg/ml	100 µg/ml	200 µg/ml
IIa	-	-	-	11
IIb	-	13	11	20
IIc	11	17	22	25
IId	12	15	22	26
Ciprofloxacin	13	16	24	31

Table 5: Antifungal activity data for synthesized compounds IIa-d and miconazole against *A. niger*

Compound	Fungi along with zone of inhibition (mm)			
	A. niger			
	25 µg/ml	50 µg/ml	100 µg/ml	200 µg/ml
IIa	-	13	17	20
IIb	9	14	20	22
IIc	-	-	10	17
IId	-	17	20	23
Miconazole	14	19	24	27

4. CONCLUSION

It was discovered that microwave-assisted approach is highly efficient procedure for the preparation of various benzimidazole derivatives compared to conventional heating method. The reactions occurred remarkably fast, under mild condition using highly inexpensive reagents and a lab microwave oven as the irradiation source.

The benefits of this environmentally benign and safe protocol include a simple reaction set up, application of commercially available inexpensive reagents, easy work up, good product yield, short reaction time.

The *in vitro* antimicrobial activity of compounds showed that compound **IIc** and **IId** have good antibacterial activity against *S. aureus* and *E. coli.* Compound **IIb** and **IId** also showed good antifungal activity against *A. niger* as compared with standard. It showed that derivative having p-amino group possesses antibacterial activity and derivative having p-nitro group possesses antifungal activity.

5. EXPERIMENTAL PROTOCOL

All melting points were determined in open capillary tube and are uncorrected. Infrared spectra were recorded in KBr on FT-IR - 8400, Spectrophotometer. The ^{1}H NMR spectra were measured in dimethyl sulfoxide or $CDCl_3$ solutions on a BRUKER AVANCE II 400 NMR Spectrophotometer using TMS as an internal reference (chemical shift in δppm). The mass spectra were recorded on QP-2010 PLUS GC-MS system. All the synthesized compounds were microanalyzed satisfactorily for C, H and N by Elementar Vario EL III elemental analyzer.

5.1 METHOD OF SYNTHESIS

5.1.1 General procedure for synthesis of 2-Substituted-1H benzimidazole

Equimolar mixture of o-phenylenediamine dihydrochloride, 20 mL of water and acid was subjected to microwave irradiation at 350W for 25 minutes, reaction mixture was cooled and distinctly basic by gradual addition of concentrated ammonia solution, the precipitated product was collected and recrystallized from 10 % aqueous ethanol.

5.1.2 General procedure for synthesis of Ethyl (2-Substituted-1H-benzimidazol-1-yl) acetate

Ethylchloroacetate (0.01 mol) was added to the equimolar solution of 2- Substituted-1H-benzimidazole in dry acetone (20 mL). To that mixture, anhydrous K_2CO_3 (1 g) was added and the reaction mixture subjected to microwave irradiation at 420W for 35 minutes. Acetone was removed after completion of reaction and the obtained residue was recrystallized from ethanol.

5.1.3 2-methyl-1H-benzimidazole (Ia)

The overall yield was 70.12 %, the M.P. was 176-178ºC. IR (KBr): 2986.57, 2874.70 (-CH_3 stretching), 1621.06 (C=N stretching), 3049.25, 3061.78 (Aromatic C-H stretching), 3141.82 (NH stretching) cm^{-1}.

5.1.4 Ethyl (2-methyl-1H-benzimidazol-1-yl) acetate (IIa)

The overall yield was 79.12 %, the M.P. was 183–186ºC (Found: C, 66.05; H, 6.42; N, 12.84. Calculated for $C_{12}H_{14}N_2O_2$: C, 66.04; H, 6.41; N, 12.83). IR (KBr): 1733.89 (-C=O stretching), 1669.28 (C=N stretching), 3065.15 (Aromatic C-H stretching), 1602.74, 1473.51 (Aromatic C=C stretching) cm^{-1}. ^1H NMR (DMSO), δppm: 1.3232 (CH_3 methyl), 7.1756-7.4426 (Ar-H), 7.4600-7.7972 (Ar-H benzimidazole). Mass spectrum shows the formation of (M^+) peak at 218 m/z.

5.1.5 2-(4-nitrophenyl)-1H-benzimidazole (Ib)

The overall yield was 70.12 %, the M.P. was 176-178ºC. IR (KBr): 2986.57, 2874.70 (-CH_3 stretching), 1621.06 (C=N stretching), 3049.25, 3061.78 (Aromatic C-H stretching), 3141.82 (NH stretching) cm^{-1}.

5.1.6 Ethyl [2-(4-nitrophenyl)-1H-benzimidazol-1-yl] acetate (IIb)

The overall yield was 79.09 %, the M.P. was 224 – 227 ºC. IR (KBr): 1548.32, 1350.08 (NO_2 stretching), 1755.10, 1732.92 (-C=O stretching), 3082.04, 3078.18 (Aromatic C-H stretching), 1690.35, 1651.92 (C=N stretching) cm^{-1}. ^1H NMR (DMSO), δppm: 1.3110 (CH_3 methyl), 7.2169-7.6652 (Ar-H), 7.7114-8.2087 (Ar-H benzimidazole). Mass spectrum shows the formation of (M^+) peak at 324 m/z.

5.1.7 2-phenyl-1H-benzimidazole (Ic)

The overall yield was 78.23 %, the M.P. was 206-209ºC. IR (KBr): 3145.46 **(**NH stretching), 1683.74 (C=N stretching), 3071.43, 3004.89 (Aromatic C-H stretching) cm^{-1}.

5.1.8 Ethyl (2-phenyl-1H-benzimidazol-1-yl) acetate (IIc)

The overall yield was 80.12 %, the M.P. was 199-201ºC. IR (KBr): 3067.57, 3055.03 (Aromatic C-H stretching), 1595.02, 1464.83 (Aromatic C=C stretching), 1721.25 (-C=O stretching), 1689.53, 1622.99 (C=N stretching) cm^{-1}. ^1H NMR (DMSO), δppm: 1.3007 (CH_3 methyl), 7.2200-7.5538 (Ar-H), 7.6237-7.7768 (Ar-H benzimidazole). Mass spectrum shows the formation of (M^+) peak at 279 m/z.

5.1.9 2-(4-aminophenyl)-1H-benzimidazole (Id)

The overall yield was 76.28 %, the M.P. was 229-232ºC. IR (KBr): 3480.98, 3414.59 (NH_2 stretching), 1631.32, 1600.51 (C=N stretching), 3000.86 (Aromatic C-H stretching) cm^{-1}.

5.1.10 Ethyl [2-(4-aminophenyl)-1H-benzimidazol-1-yl] acetate (IId)

The overall yield was 74.18 %, the M.P. was 243 - 245 ºC. IR (KBr): 3050.86 (Aromatic C-H stretching), 3380.98 (NH_2 Asymmetric stretching), 3364.59 (NH_2 symmetric stretching), 1733.89 (C=O stretching), 1671.20, 1662.52 (C=N stretching) cm^{-1}. ^1H NMR (DMSO), δppm: 4.0002 (NH_2) 1.3337 (CH_3 methyl), 7.2232-7.6237 (Ar-H), 7.7553-7.7768 (Ar-H benzimidazole). Mass spectrum shows the formation of (M^+) peak at 295m/z.

5.2 Antimicrobial activity test

The antibacterial activity of synthesized compounds **IIa-d** was studied against *E. coli* and *S. aureus,* and antifungal activity was studied against *A. niger.* Ciprofloxacin and Miconazole were used as standard for antibacterial and antifungal activity respectively. The agar dilution method was performed using Nutrient agar medium for antibacterial activity and Saubroud's dextrose agar (Hi-Media) medium for antifungal activity. This method depends on the diffusion of drug from bore through the solidified agar layer of petri dish to an extent such that growth of the inoculated microorganism is prevented entirely in a circular area "zone" around the cup containing the solution of a compound under test.

The medium was sterilized by autoclaving at 15 lb pressure for 30 min. One loopful of the stock culture was inoculated at 10 mL of agar slant previously in sterilized test tubes, and incubated at 37 ^{0}C for 24 h to 72 h respectively for bacteria and fungi. About 3 mL of distilled water was added to the test tube and a suspension of the culture was obtained by shaking for few minutes.

Procedure

All the operations were carried out under aseptic conditions. Sterile medium was melted on water bath and kept at 45°C in constant temperature water bath. In each sterile petri dish molten medium was added so that thickness was approximately 8-10 mm and subcultured organism under study was inoculated. The inoculated dishes were allowed to set for 30 min at room temperature. Cups of 6 mm diameter were then made with the help of sterile stainless steel bore; 1ml of sample solution was added to each cup. Petri dishes were kept in refrigerator for 30 min. so as to allow diffusion of the solutions in the medium, and then incubated at 37 °C for 24 h for antibacterial activity and 72 h for antifungal activity. Zone of inhibition produced by test compounds were measured in mm.

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