Evaluation of Biological Activity of some Benzimidazole Derivatives as Antifungal

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ABSTRACT

In this study, a series of benzimidazole derivatives was synthesized by using a simple, inexpensive and rapid method, which use ammonium salt. Some of these derivatives were exclusively isolated and characterized with other derivatives and tested for anti-fungal activity. The biological activity of these compounds as fungicides was tested against three commercially known fungicides(C.albicans, patient isolate C.glabrata and C.krusei). Most of the obtained compounds exhibit anti-fungal activity, especially compound VI_B, VI_D and VI_H that showed significant activity when compared with that used as standard drug.

KEYWORDS: Benzimidazole, Derivatives, Anti-fungal agent, Biological activity.

1. INTRODUCTION

The study of biological activity of benzimidazoles and its derivatives gives considerable importance characterized by the terms of their use in many different areas of our live. Various studies have shown many of the uses of these compounds, especially as antagonists[1], potent inhibitors of tyrosine kinase[2],antitumor agents[3], gamma-amino butyric acid (GABA) agonists, and 5-HT3 potent agonist [4]. Benzimidazole derivatives have found commercial application in medicine as antihistaminic[5]. It is known that the synthesis of benzimidazole derivatives carried out by the condensation of 1,2-phenylenediamine with carboxylic acids or carboxaldehydes [6,7] or by the method shown in [8,9],undergoing condensation of nitriles, chlorides and orthoesters, using strong acidic conditions with high temperature. Benzimidazole derivatives also have been synthesized on the base of a solid phase to prove a combinatorial approach as anthelmintic agent

and in diverse human therapeutic area [10].One of the most popular methods to synthesize these compounds utilize N-alkylation of their unsubstituted benzimidazoles [11]. Ammonium salts were employed by researchers for transformation such as halogenations of aromatic compounds, which result of synthesis 3,4-dihydropyrimidines-2(1H)-ones[12]. Ammonium salts are inexpensive, commercially available reagents for many organic reactions. Based on the progress of research, there are no reports on the use of ammonium salts as catalysts for the synthesis of benzimidazole derivatives. Study of the direct synthesis of heterocyclices [13,14] on the basis of synthetic methodologies[15-17],have shown that substituted of benzimidazole derivatives posess diversified pharmacological activity[18].Benzimidazole derivatives shown potential for applications in a variety of pharmacological targets, they have attracted a wide interest in clinical applications because of their diverse activity[19]. Most of these compounds such as 2- substituted benzimidazoles have been found to posess antifungal[20], antispasmodic[21],antihistaminic[22], antimicrobial[23], antitumor [24], anticancer[25], cycloxygenaseinhibitors activities[26]. Benzimidazole derivatives have also been investigated for their analgesic[27],and they have shown antitubercular activity[28].The broad range of benzimidazole derivative applications especially as antifungal agents has encouraged us to perform this work. In a country where agriculture is the main base of economy, the development of benzimidazole derivatives can be used as fungicides in agriculture and home gardens. In this paper simple and rapid procedure for the synthesis of (8) benzimidazole derivatives and their spectral characterization have been described.

2.MATERIALS AND METHODS

All chemical reagents, that used in this study were verificated of purity purchased from Aldrich (Milwaukee, MI,USA) and E. Merich (Darmstadet,Germany). compound (VI_H) was used as a crude product for further reaction.

All used solvents were purified according to standard procedures. Initial attempts for the reaction 1,2-Phenylenediamine with carboxylic acids, carboxaldehydes and β-ketoesters with different ammonium salts such as NH_4Cl and under different solvent (DMF,CH_3CN, MeOH and ether) at room temperature, produced 2- phenyl-1H-benizimidazoles obtained in small amounts that didn't exceed 50% .Reaction was

monitored by (TLC, eluent hexane/ethyl acetate 30/70).On the other hand , carrying the reaction out with ammonium salts such as NH_4Cl, NH_4Br, NH_4F, NH_4NO_3, $(NH_4)_2CO_3$,$(NH_4)_2SO_4$ in the presence of CH_3Cl as shown in (scheme 1), rapid, reaction proceeds with good yields to get 2-phenyl- 1H-benzimidazole.As can be seen using NH_4Cl at room temperature gives the highest percent's yield. Results using different ammonium salts are listed in (Table 1). The antifungal activities of the samples were measured by cup plate method, each of the plant pathogenic strains on potato dextrose agar (pda)medium. potato dextrose agar (pda) medium contained potato 250g,dextrose 25g,agar 25g,and 0.5 litre of water. One week old cultures were employed. The compounds to be tested were suspended 1500 ppm in a potato dextrose agar (pda) medium and autoclaved at 100 C^O for 30 minutes at 1 atmosphere pressure. These medium were poured into sterile Petri plate and organisms were inoculated after cooling the Petri plate. The percentage inhabitation for fungi was calculated after one week using the formula: Percentage inhabitation=100(X-Y) /X. Where, X: area of colony in control plate, Y: area of colony in test plate .The fungicidal of compounds was tested at 1500ppm concentration in *vitro* plant pathogenic organisms, results shown in Tab.(2).Indicates that many compounds are suitable to be a toxic for fungi especially compounds VI_B, VI_D and VI_H. Compounds almost inhibit the fungi about 85 % .Hence compounds can be employed as fungicide in private agricultural fields. Search could evolve in the future to find other uses for this type of compounds. Also can be considered that the work has achieved the desired goal.

Table 1. conditions used for synthesis of 2-phenyl benzimidazole by the condensation of 1,2-phenylenediamine,with benzaldehyde, using various ammonium salts in $CHCl_3$, at R.T.

NH_4X	Time (hours)	Yield (%)
NH_4Br	4	85
NH_4Cl	4	90
NH_4F	6	75
$(NH_4)_2SO_4$	4	78
$(NH_4)_2CO_3$	6	80

Reaction carried out with molar ratio benzaldehyde -1,2-phenylene-diamine(1:1)using (5mmol) NH_4Cl in 10 ml $CHCl_3$ for 10 minutes at room temperature.

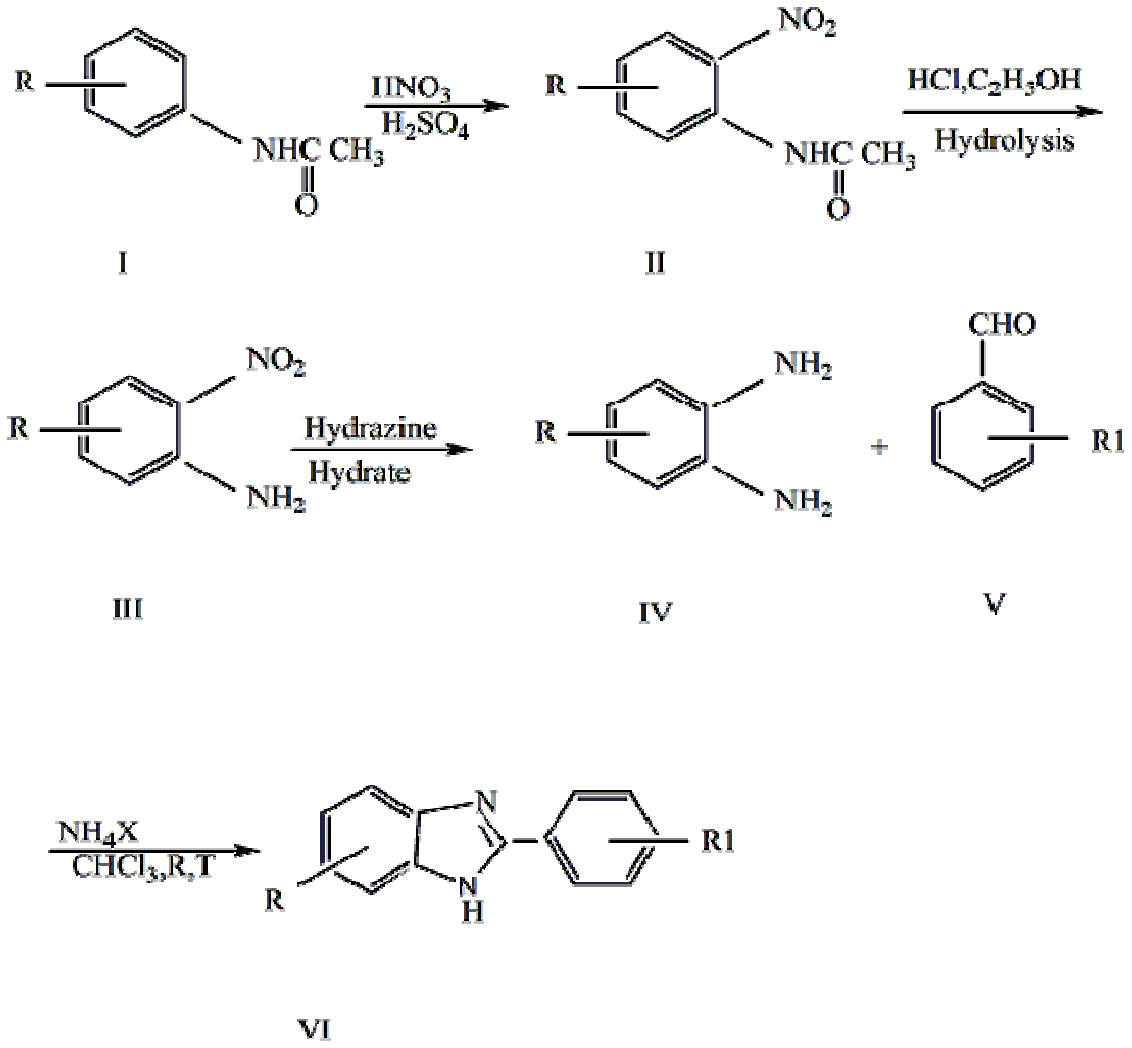
3.Experimental Part

3.1.General Method

All of the synthesized benzimidazole derivatives were analyzed by Mass, IR, and NMR spectroscopy. Mass spectra measured on Liquid Chromatography-Mass Spectroscopy (LCMS) Agilent mass spectrometer.IR spectra were recorded on Nicolet 740 Fourier transform infrared (FTIR) spectrometer.1H NMR-spectra were recorded on a Varian

Gemini 200and 300 MHz instrument in $CDCl_3$ and DMSO-d6 using Tetramethylsilane (TMS) as an internal standard. Melting Points measured using a Buchi-510 apparatus and were uncorrected. All instrumental analyses were performed at Bin Hayyan Laboratory (Aqaba Special Economic Zone, Jordan).

Scheme 1. Preparation Route of compounds in this study.



R= -H, $-NO_2$, $-CH_3$.
R_1= -H, $-NO_2$, $-CH_3$, -OH, $-OCH_3$.

3.2.General Experimental Procedure for the Synthesis of Benzimidazoles

(1mmol) from benzaldehyde derivatives (V),where (R_1=-H, $-NO_2$, $-CH_3$, -OH, $-OCH_3$) was added in batches to (1mmol) a stirred of 1,2-phenylene-diamine (IV) and (5mmol)NH_4Cl in 10 ml $CHCl_3$ for 10 minutes at room temperature (scheme 1) .Blend stirring was continued for 4-6 hours. After completed of

reaction inferred by (TLC, eluent hexane/ethyl acetate30:70),the solvent was removed under reduced pressure and extracted with ethyl acetate (50 ml),then organic layer was washed in 25ml of water. After that layers were separated and the organic layer was dried over sodium sulfate. The solvent was removed under reduced pressure and the crude product was subjected to column chromatography using petroleum ether [$E_t OA_C$ (10:1)],which gave the compound 2-phenyl-1H-benzimidazole (VI_A) as a solid in 90% yield.

4. Spectral Data for benzimidazole derivatives synthesized in this study:

2-phenyl-1H-benzimidazole (VI_A) :

Solid; Molecular formula: $C_{13}H_{10}N_2$, Yield 90%, m.p-240-242 ℃; ^{1}H NMR: δ 6.06 (bs, 1H, NH), 6.82 (d, 2H, aromatic), 6.98 (d,2H, aromatic), 7.06 (t,1H, aromatic),7.28 (m, 2H, aromatic), 7.52 (m, 2H, aromatic), IR (KBr): 3426(-NH), 3042(Ar-CH), 1742, 1631(-C = N) cm^{-1}; Mass (LCMS): *m/z*195 (M^+ + H).

4-(1H-benzimidazol-2-yl) phenol (VI_B) :

Solid; Molecular formula:$C_{13}H_{10}N_2O$, Yield 78%, m.p-268-270 ℃; ^{1}H NMR: δ 6.06 (bs, 1H, NH), 6.82 (d, 2H, aromatic), 6.98 (d,2H, aromatic), 7.21 (d,2H, aromatic),7.52 (d, 2H, aromatic), IR (KBr): 3379(-NH), 3211(-OH), 3078(-Ar-CH), 1461(C = N) cm-1; Mass (LCMS): *m/z* 211 (M^+ + H).

2-(4-methoxyphenyl)-1H-benzimidazole (VI_C) :

Solid; Molecular formula:$C_{14}H_{12}N_2O$, Yield 84%, m.p-286-288 ℃; ^{1}H NMR: δ 3.70 (d,3H, OCH_3), 6.12 (bs, 1H, NH), 6.94 (d, 2H, aromatic), 6.98(d,2H, aromatic), 7.20 (d,2H, aromatic), 7.58 (d,2H, aromatic), IR (KBr): 3294(-NH), 3103(Ar-CH), 1184 (-OCH_3), 1588(-C=N) cm-1; Mass (LCMS): *m/z* 225 (M^+ +H).

2-(4-methylphenyl)-1H-benzimidazole (VI_D) :

Solid; Molecular formula:$C_{13}H_{12}N_2$, Yield 80%, m.p-255-257 ℃; ^{1}H NMR: δ 2.54 (d, 3H, CH_3),6.06 (bs, 1H, NH), 6.84 (d, 2H, aromatic), 6.96 (d,2H, aromatic), 7.18 (d,2H, aromatic),7.58 (d, 2H, aromatic), IR (KBr): 3346(-NH), 3024(Ar-CH), 2923(-CH_3), 1575(-C=N) cm-1; Mass (LCMS): *m/z* 209 (M^+ +H).

4-(6-nitro-1H-benzimidazol-2-yl) phenol (VI_E) :

Solid; Molecular formula:$C_{13}H_9N_3O_3$, Yield 82%, m.p-292-294 ℃; ^{1}H NMR: δ 6.08 (bs, 1H, NH), 6.74 (d, 2H, aromatic), 6.84 (d, 2H, aromatic), 7.45 (d,1H, aromatic), 8.02 (d,1H, aromatic),8.32 (s, 1H, aromatic), IR (KBr): 3737(-OH),3432(-NH), 3103(Ar-CH), 1562(C=N), 1532(-NO_2) cm-1; Mass (LCMS): *m/z* 256 (M^+ +H).

2-(4-methylphenyl)-6-nitro-1H-benzimidazole (VI_F) :

Solid; Molecular formula:$C_{14}H_{14}N_3O_2$, Yield 76%, m.p-260-262 ℃; ^{1}H NMR: δ 2.56 (d, 3H, CH_3),6.10 (bs, 1H, NH), 6.80 (d, 2H, aromatic), 6.86 (d,2H, aromatic), 7.54 (d,1H, aromatic),8.08 (d, 2H, aromatic), 8.44

(s, 1H, aromatic), IR (KBr): 3402(-NH), 3054(Ar-CH), 1534(-C=N), 1524(-NO_2) cm-1; Mass (LCMS): *m/z* 254 (M^+ +H).

6-nitro-2-phenyl-1H-benzimidazole (VI_G) :

Solid; Molecular formula:$C_{13}H_9N_3O_2$, Yield 68%, m.p-280-282 ℃; ^{1}H NMR: δ 6.08 (bs, 1H, NH), 6.90 (d, 2H, aromatic), 6.96 (d,2H, aromatic), 7.05 (t,1H, aromatic), 7.54 (d,1H, aromatic),8.12 (d, 1H, aromatic), 8.44 (s, 1H, aromatic), IR (KBr): 3211(-NH), 2984(Ar-CH), 1552(-NO_2), 1529 (-C=N) cm^{-1}; Mass (LCMS): *m/z* 240 (M^+ +H).

4-(6-methyl-1H-benzimidazol-2-yl) phenol (VI_H) :

Solid; Molecular formula:$C_{14}H_{12}N_2O$, Yield 72%, m.p-275-277 ℃; ^{1}H NMR: δ 2.56 (d, 3H, CH_3), 6.48 (bs, 1H, NH), 6.78 (d, 2H, aromatic), 6.92 (d,2H, aromatic), 7.48 (d,1H, aromatic), 8.10 (d,1H, aromatic), 8.44 (s,1H, aromatic), IR (KBr): 3455(-OH), 3274(-NH), 3212(Ar-CH), 2898(-CH_3), 1534(-C=N) cm-1; Mass (LCMS): *m/z* 225 (M^+ +H).

5.RESULTS

Reaction between1,2-phenylenediamine and different aldehydes in the presence of ammonium salts gives the compounds (VI_A –VI_H) according to scheme(1).Synthesized compounds were confirmed by TLC, Mass, IR, and ^{1}HNMR spectral analysis. Melting Points(mp) and yields have been identified for each of these compounds. Further appearance of the molecular ion peak at 225 (m + 1) and 209 (m + 1) confirmed the structure of VI_H and VI_D. The titled compounds were confirmed by IR spectral data showing characteristic bands at 1384 – 3200 cm-1, indicating the presence of –NO_2 and –OH stretching; and sharp bands, ranging between 1680 – 1750 cm-1, indicating the presence of C = N. Compounds VI_A –VI_H were confirmed by stretching at 3500 cm-1, due to the presence of –NH. Compounds VI_A–VI_H were confirmed by ^{1}H NMR spectral analysis. The NMR proton peak at 6.00 – 6.50 ppm revealed the presence of –NH. The synthesized compounds (VI_B,VI_E, VI_F,VI_G, and $VI_{D)}$ were found to have potent anti-fungal activity. Compound (VI_D) exhibited more activity when compared to other prepared benzimidazoles.

6.DISCUSSION

It is well known that benzimidazole derivatives can be synthesized by using the reaction 1,2-Phenylenediamines with carbonyl compounds, under acidic conditions [6,7]. At the same time

reaction of 1,2-Phenylenediamine in the presence of β-ketoesters under neutral reflux conditions gives bevzodiazepin-2-ones,by elimination of alcohol and water [12]. Using acidic conditions also synthesized ethyl β-2-aminoaniline coronate, upon heating it gives 2-methyl-1H-benzoimidazole instead of benzodiazepine-2-ones,by elimination of ethyl acetate [13]. Depth study of synthesis benzimidazole derivatives made it necessary to find rapid, simple and inexpensive method for their synthesis. There are several studies on the synthesis of benzimidazole derivatives, using different techniques and different conditions, some of them have good yields and obtained compounds that have many uses in human life [29-32]. In this study synthesized benzimidazole derivatives using a rapid, and inexpensive method with good yields by utilizing ammonium salts. Method could be easily performed in laboratories conveniently .Studied the biological activity against fungi for the synthesis compounds.

7.CONCLUSION

Benzimidazole derivatives are known to have numerous properties and activates that can be used in different fields of life, they can be used in medicine, pharmacy, agriculture and others fields. In this study we synthesized some benzimidazole derivatives using rapid, simple and inexpensive method using ammonium salts as catalysts. The yields of synthesized compounds were in the range of 68-90%. The purity of these compounds were assessed by TLC and melting points. The assigned structures were further established by MS,IR and ^{1}HNMR spectral analysis. Some of the synthesized compounds (VI_A-VI_H) were found to have potent anti-fungal activity. Compounds (VI_B,VI_D and VI_H) exhibited more activity when compared to other benzimidazole derivatives. Hence, it can be concluded that the benzimidazole derivatives can potentially be developed into useful anti-fungal agents, which can prompt future researchers to synthesize a new series of benzimidazole derivatives containing a wide substituents, with the aim of producing a novel heterocyclic system, with enhanced activity.

Tab2. The in *vitro* antifungal activity of the prepared compounds in this study(MIC,µg/ml).
(MIC): Minimum Inhibority Concentration, expressed in µg/ml .

Compound	C.albicans	C.glabrata	C.krusei
VI_A	25	25	12.5
VI_B	12.5	6.25	6.25
VI_C	25	25	12.5
VI_D	12.5	12.5	6.25
VI_E	25	25	12.5
VI_F	12.5	12.5	6.25
VI_G	25	25	12.5
VI_H	12.5	12.5	6.25
Miconazole	12.5	3.125	3.125
Flocunazole	6.25	3.125	1.56

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