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Synthesis and Biological Screening of Some New Substituted 1-Acetyl Benzimidazol-2-yl Methyl Isoindoline-1,3-Dione Analogs as Anti-Microbial Agents

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ABSTRACT

*The present work deals with the synthesis of 2-(1-acetyl-1H-benzimidazol -2-yl) methyl) isoindoline-1,3-dione **(3)**, used as a key synthons to synthesize a series of some new heterocyclic compounds incorporating benzimidazole moiety. The newly synthesized compounds are characterized by their elemental analysis, IR, ^{1}H, ^{13}C-NMR and mass spectral data and assessed for their antimicrobial activity.*

Keywords: Amino acids; Phthalimide; Benzimidazole; Heterocyclic compounds; Antimicrobial; Anticancer activities

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INTRODUCTION

Over the past years, several benzimidazole derivatives have been synthesized and widely screened for their biological activities. These classes of hetrocycles have found applications in diverse pharmacological areas such as antibacterial, antibiotics [1,2], anticancer [3], anti-inflammatory [4], antifungal [5], antidiabetic [6], enzyme inhibitors [7,8], anti-hepatitis C [9], cytotoxicity [10] and antihypertensive [11]. More over these derivatives were applied as antiparasitic, elastase inhibitors, anti-stress, antioxidant, antiviral (anti-HIV). Because of this wide range of biological and pharmaceutical activities and industrial applications, benzimidazole moiety has received much attention in developing new therapeutic agents. In this present study, a series of benzimidazoles were synthesized and screened for their antimicrobial and anticancer activities.

EXPERIMENTAL SECTION

All melting points for the prepared derivatives were measured in capillary tubes using a Gallen-Kamp apparatus and were uncorrected. The FT-IR spectra were recorded on a Perkin- Elmer 1650 spectrophotometer (KBr pellets). The 1H,13CNMR spectra were measured in dimethyl sulphoxide-*d*6 as a solvent using a Varian Gemini 180 spectrometer operating at 400 MHz for ^{1}H and 75 MHz for ^{13}C NMR. TMS was used as an internal standard and the chemical shifts were reported as δppm. The FAB mass spectra were recorded on a JEOL SX 102/DA-6000 mass spectrometer.

General Methods

Synthesis of 2-(1,3-dioxo-1,3-dihydroisoindolin-2-yl)acetic acid (1):

A mixture of phthalic anhydride (0.01 mol) and glycine (0.01 mol) were added in Pyrex flask. The mixture was heated under solvent free and microwave irradiation conditions for the time needed to complete the reaction (Monitored by TCL). The reaction mixture cooled to room-temp, quenched with 20 cm^3 H_2O. The pure product separated, filtered off and recrystallized from ethanol to give 1.

$C_{10}H_7NO_4$ (MW205): yellow powder; 73% yield; m. p 185-187 ^oC. Anal Calc: C, 58.54; H, 3.44; N, 6.83; Found: C, 58.36; H, 3.35; N, 6.6. FT-R (KBr cm^{-1}) υ max: 1723; 1773(C=O); 2934(CH-aliphatic); 3066(CH-aromatic); 3479(OH). MS: *m/z* (%) 205 (6.26 M^+); 63.03(100).

Synthesis of 2-(1H-benzo[d]imidazol-2-yl]methyl)isoindoline-1,3-dione(2):

2-(1,3-dioxoisoindolin-2-yl)acetic acid **1** (0.01 mol), 15 ml (4 N HCl), O-phenylenediamine (0.01 mol) were added in Pyrex flask. The mixture was heated under solvent free and microwave irradiation conditions for the time needed to complete the reaction (Monitored by TCL). The reaction mixture cooled to room-temp, quenched with 20 cm^3 H_2O. The pure product separated, filtered off and recrystallized from ethanol to give 2.

$C_{16}H_{11}N_3O_2$ (M W 277): white powder; 70% yield; m. p › 320^oC. Anal Calc: C, 69.31; H, 4.00; N, 15.15; Found: C, 69.23; H, 3.86; N, 15.01. FT-IR (KBr cm^{-1}) υ max: 1597(C=N); 1640(CO); 2920-2850(CH-aliphatic); 3064(CH-aromatic); 3431 (NH). ^{1}H NMR (δppm) (DMSO*d*6): δ=4.29(s, 2H, CH_2); 7.18-8.12(m, 8H, Ar–H); 9.75(s, 1H, NH).

Synthesis of 2-((1-acetyl-1H- benzimidazol-2-yl)methyl)isoindoline- 1,3-dione (3):

2-(1H-benzimidazol-2-yl) methyl) isoindoline-1,3-dione **2** (0.01mol) was heated under reflux in acetic anhydride (25 ml) for 2 hrs, cooled to room-temp, poured onto ice water. The solid that separated filtered off, washed with water and recrystallized from benzene to give 3.

$C_{18}H_{13}O_3N_3$ (M W 319): yellow crystals; 62% yield; m. p. 177-180^oC. Anal Calc: C,67. 71; H, 4.10; N, 13.16; Found: C, 67.65; H, 3.87; N, 13.06. FT-IR (KBr cm^{-1})υmax: 1615(C=N); 1736, 1761(CO); 2921(CH-aliphatic);3055 (CH- aromatic).^{1}H NMR (δppm) (DMSO*d*6): δ = 2.51 (s, 3H, $COCH_3$); 3.33 (s, 2H, CH_2); 7.25-7.99 (m, 8H, AR–H). 13C- NMR (400MHz, DMSO-*d*6), 26.95 (CH_3); 40.39 (CH_2); 115.71, 120.06, 122.72, 125.32, 130.97, 132.17, 132.46 (aromatic >C=C<); 142.16(hetero aromatic >C=N–); 157.06, 161.16, 170.06 (3C=O).

Synthesis of 2-[1-*(*(3-phenyl-acryloyl) -1H-benzimidazol-2-yl*(*methyl]isoindoline -1,3-dione (4):

To ethanolic solution of **3** (0.01 mol), benzaldehyde (0.01 mol) was added. The reaction mixture was heated under reflux in the presence of 5 mL of 10% aqueous NaOH for 6-8 hrs. The solvent was concentrated, left to cool, acidified with acetic acid. The solid that separated filtered off, recrystallized from ethanol to give 4.

$C_{25}H_{17}N_3O_3$ (M W 407): orange powder; 65% yield; m.p.›220^oC. Anal Calc: C, 73.70; H, 4.21; N, 10.31; Found: C, 73.55; H, 4.11; N, 10.12. FT-IR (KBr cm^{-1}) υ max: 1587(C=N); 1616, 1670(CO); 2920-2850(CH-aliphatic); 3064(CH-aromatic). ^{1}H NMR (δppm) (DMSO*d*6): δ = 4.50(s, 2H, CH_2); 6.85 (d, 1H, CH=<u>CH</u>CO); 7.19 (d, 1H, <u>CH</u>=CHCO); 7.53-8.12 (m, 13H, Ar–H). MS: *m/z* (%) 407(1.62 M^+); 83.29(100).

Synthesis of 2- [1-(5- phenyl-4,5- dihydro-1H-pyrazol-3-yl)-1H –benzimidazol-2-yl methyl]- isoindoline-1,3-dione (5):

A mixture of 2-[1-(3-phenyl-acryloyl)-1H-benzimidazol-2-yl)methyl]isoindoline -1,3-dione 4 (0.01 mol), hydrazine hydrate (0.01 mol) in absolute ethanol (30 mL) was heated under reflux for 6 hrs. The solvent was concentrated, cooled to room-temp. The solid that separated was collected, filtered off and recrystallized from acetic acid to give 5. $C_{25}H_{19}N_5O_2$ (M W 421): orange crystal; 70% yield; m.p.185-187ºC. Anal Calc: C, 71.25; H, 4.54; N, 16.62; Found: C, 71.03; H, 4.33; N, 16.41. FT-IR (KBr cm^{-1}) υ max: 1589(C=N); 1620 (CO); 3430(NH). ^{1}HNMR (δppm) (DMSO*d*6): δ = 1.87, 2.18 (dd, 2H, CH_2–pyrazole); 3.75(t, 1H, CH-pyrazole); 4.45 (s, 2H, CH_2); 7.21-7.70 (m, 13H, Ar–H); 8.41 (s, 1H, NH-pyrazole).

Synthesis of 2-[1-)(5- phenyl- 4,5-dihydroisoxazol- 3- yl)- 1H- benzimidazol-2-yl*)* methyl] isoindoline- 1,3-dione (6):

2- [1- ((3 – phenyl –acryloyl) -1H -benzimidazol- 2- yl)methyl] isoindoline – 1,3 dione 4 (0.01 mol), hydroxyl amine hydrochloride (0.01 mol) in acetic acid was heated under reflux for 6 hrs. The reaction mixture was cooled to room-temp, poured onto ice water. The solid separated was filtered off, dried and recrystallized from ethanol to give 6.

$C_{25}H_{18}N_4O_3$ (M W 422): dark brown crystal; 55% yield; m.p.›220^oC. Anal Calc: C, 71.08; H, 4.29; N, 13.26; Found: C, 70.53; H, 4.08; N, 13.14. FT-IR (KBr cm^{-1}) υ max: 1630(C=O); 2922 (CH-aliphatic); 3062(CH-aromatic). ^{1}HNMR (δppm) (DMSO*d*6): δ = 1.95 (dd, 2H, CH_2-isoxazole); 4.05 (t, 1H, CH-isoxazole); 4.85 (s,2H, CH_2) and 7.21-7.82(m,13H, Ar-H).

Synthesis of 2-[1-(2-oxo-6-phenyl-1,2,5,6-tetrahydropyrimidin-4-yl)-1H-benzimidazol-2-yl)methyl]isoindoline-1,3-dione (7a) and 2-[1-(2-thioxo-6-phenyl-1,2,5,6-tetrahydro-pyrimidin-4-yl)-1H-benzoimidazol-2-yl)methyl]isoindoline-1,3-dione (7b):

A mixture of 2- [1-((3-phenyl-acryloyl)-1H-benzimidazol-2-yl)methyl] isoindoline-1,3 dione 4 (0.01 mol), urea and /or thiourea (0.01 mol) in absolute ethanol (30 mL) containing sodium ethoxide (prepared from 0.2 gm of sodium metal dissolved in 5 mL absolute ethanol) was heated under reflux for 6 hrs. The solid produced after evaporation of solvent was collected, filtered off, washed well with water, and recrystallized from ethanol to give (7a, 7b).

7a: $C_{26}H_{17}N_5O_3$ (M W 477): orange powder; 70% yield; m.p.›220^oC. Anal Calc: C, 69.79; H, 3.83; N, 15.65; Found: C, 69.55; H, 3.65; N, 15.42. FT-IR (KBr cm^{-1}) υ max: 1599(C=N); 1640(CO); 2922(CH-aliphatic); 3060(CH-aromatic); 3431(NH). ^{1}H NMR (δppm) (DMSO*d*6): δ = 2.08(d, 2H, CH_2-pyrimidine); 3.45(t, 1H, CH-pyrimidine); 4.55(s, 2H, CH_2); 7.12-8.45 (m, 14H, Ar–H, NH).

7b: $C_{26}H_{17}N_5O_2S$ (M W 463): orange powder; 65% yield; m.p.›240^oC. Anal Calc: C, 67.37; H, 3.70; N, 15.11; S, 6.92; Found: C, 67.21; H, 3.67; N, 15.00; S, 6.76. FT-IR (KBr cm^{-1}) υ max: 1222(C=S); 1558(C=N); 1643(CO); 2534(S–H); 2920 (CH-aliphatic); 3047(CH-aromatic); 3336(NH). ^{1}H NMR (δppm) (DMSO*d*6): δ = 2.49(d, 2H, CH_2-thiopyrimidine); 3.45(t, 2H, CH -thiopyrimidine); 4.25(s, 2H, CH_2); 7.19-8.35(m, 14H, CH-Ar, NH).

Synthesis of 2-[1-(5-phenyl-2-thioxo-3,5-dihydro-2H-[1,3,4]thiadiazolo [3,2a] pyrimidin-7-yl)-1H-benzoimidazol-2-yl methyl] isoindoline-1,3dione (8):

To ethanolic solution of 2-[1- (3-phenyl -acryloyl) -1H -benzimidazol- 2 - yl methyl] isoindoline -1,3 dione 4 (0.01 mol) containing sodium ethoxide (prepared from 0.2 gm of sodium dissolved in 5 ml absolute ethanol), 2-mercapto-5-aminothiadiazole (0.01 mol) was added. The reaction mixture was heated under reflux for 8 hrs. The reaction solution was concentrated, cooled to room temp. The solid product was collected, filtered off, washed well with water and recrystallized from ethanol to give 8.

$C_{27}H_{18}N_6O_2S_2$ (M W 522): orange crystal; 50% yield; m.p.248-250^oC. Anal Calc: C, 62.05; H, 3.47; N, 16.08; S; 12.27; Found: C, 61.85; H, 3.34; N, 15.90; S, 12.00; FT-IR (KBr cm^{-1}) υ max: 1588(C=N); 1620(C=O); 2664 (C-SH); 3437(NH). ^{1}HNMR (δppm) (DMSO*d*6): δ = 1.80-2.08(d, 1H, CH-pyrimidine); 3.80 (d, 1H, CH-pyrimidine); 4.55(s, 2H, CH_2); 7.20-8.09(m, 13H, Ar–H) and 13.40(s, 1H, NH-thiadiazole).

Synthesis of 4-[2-(1,3-dioxo-1,3-dihydroisoindolin-2-yl*)*methyl)-benzimidazol-1-yl]-2,4-dioxo-butyric acid ethyl ester (9):

To ethanolic solution of 2-((1-acetyl-1H- benzimidazol -2 –yl) methyl) isoindoline -1,3-dione **3**(0.01 mol) containing sodium ethoxide, diethyl oxalate (0.01 mol) was added drop wise through 30 min. The reaction mixture was stirred in ice bath for 1hr, heated under reflux for 10 hrs. The solid obtained after concentration was filtered off, washed well with water, dried and recrystallized from ethanol to give 9.

$C_{22}H_{17}N_3O_6$ (M W 419): beige crystals; 83% yield; m. p. 138-140^oC. Anal Calc: C, 63.01; H, 4.09; N, 10.02; Found: C, 62.96; H, 4.00; N, 9.82. FT-IR (KBr cm^{-1}) υmax: 1543(C=N); 1632(CO imide);1717(COester).^{1}H NMR (δppm) (DMSO*d*6) : δ = 0.99 (t, 3H, CH_3-ester); 4.15 (q, 2H,CH_2-ester); 4.95-5.00(s, 4H, 2x CH_2); 7.18-7.87 (m, 8H, Ar–H) and 12.85(s,1H,CH_2CO,CH=C-OH). 13C- NMR (400MHz, DMSO-*d*6), 14.07 (CH_3); 39.33(CO$\underline{CH_2}$CO); 40.59(CH_2); 61.23 (CH_2-ester); 110.00-138.07((aromatic>C=C<); 139.42(heteroaromatic>C=N); 151.12, 151.76, 161.03, 168.26 and 169.80(5CO).

Synthesis of 5-[2-((1,3-dioxoisoindolin-2-yl (methyl)–benzimidazol–1–yl]-2H-pyrazole-3-carbohydrazide (10):

A mixture of 4-[2-((1,3-dioxo-1,3 dihydroisoindolin-2-yl)methyl)-benzimidazol-1-yl]-2,4-dioxobutyric acid ethyl ester 9 (0.01 mol) and hydrazine hydrate (0.02 mol) was fused at 180°C for 2 hrs. The reaction mixture cooled to room-temp, poured onto ice water. The solid produced was filtered off, washed with water, dried and recrystallized from acetic acid to give 10.

$C_{20}H_{15}N_7O_3$ (M W 401): brown powder; 79% yield; m.p.240-242^oC. Anal Calc: C, 59.85; H, 3.77; N, 24.43; Found: C, 59.66; H, 3.53; N, 24.25; FT-IR (KBr cm^{-1}) υ max: 1612(C=N); 1660(C=O); 3327-3275, 3426($NHNH_2$). ^{1}HNMR (δppm) (DMSO*d*6): δ = 4.17(br, 2H, NH_2); 4.69 (s, 2H, CH_2); 7.20-7.96 (m, 9H, Ar–H) and 9.70 (s, 1H, NH-pyrazole).

Synthesis of 2-(1-(2-((1,3-dioxoisoindolin-2-yl)methyl)-1H-benzimidazol-1-yl)ethylidene)hydrazine-1-carbothioamide (11):

To ethanolic solution of 2-((1-acetyl-1H- benzimidazol -2-yl) methyl) isoindoline- 1,3-dione 3 (0.01 mol), thiosemicarbazide (0.01 mol) was added.The reaction mixture was heated under reflux for 4 hrs. The solid obtained after evaporation of ethanol was collected, filtered off, and recrystallized from benzene to give 11.

$C_{19}H_{16}N_6O_2S$ (M W 392): brown crystal; 75% yield; m.p.140-142^oC. Anal Calc: C, 58.15; H, 4.11; N, 21.42;S, 8.17; Found: C, 58.02; H, 4.00; N, 21.25;S, 8.05; FT-IR(KBr cm^{-1})υmax: 1222(C=S);1580(C=N); 1620-1708(CO); 2673(S–H); 2981 (CH-aliphatic); 3051(CH-aromatic); 3167-3360,3440($NHNH_2$). ^1H NMR (δppm) (DMSO*d*6): δ = 2.45(s, 3H, CH_3); 4.95(s, 2H, CH_2); 7.20 (br, 2H, NH_2); 7.56-7.8.12(m, 8H, Ar–H) and 13.00 (s, 1H, NH). MS: *m/z* (%) 392(2.58 M^+); 57.21(100).

Synthesis of N-(4-acetyl-5-(2-((1,3-dioxoisoindolin-2-yl)methyl)-1H-benzimid azol-1-yl)-5-methyl-4,5-dihydro-1,3,4-thiadiazol-2-yl)acetamide (12):

A solution of 2-(1-(2-((1,3-dioxoisoindolin -2-yl) methyl)-1H-benzimidazol-1-yl) ethylidene) hydrazine-1-carbothioamide 11(0.01 mol) in acetic anhydride (25 mL) was heated under reflux for 2 hrs, cooled to room-temp, poured onto ice water. The resulting solid was filtered off, washed with water, dried and purified by recrystallization from benzene to give 12.

$C_{23}H_{20}N_6O_4S$ (M W 476): brown crystal; 42% yield; m.p.142-143^oC. Anal Calc: C, 57.97; H, 4.23; N, 17.64; S, 6.73; Found: C, 57.63; H, 4.73; N, 17.74; S, 6.24; FT-IR (KBr cm^{-1}) υ max: 1536(C=N); 1611-1642(CO imide); 1719 (C=O keton); 3424 (N-H). ^1H NMR (δppm) (DMSO*d*6): δ = 1.90, 2.50 (s, 6H, 2xCO CH_3); 2.80(s, 3H, CH_3-thiadiazole); 4.95(s, 2H, CH_2); 7.29-7.74 (m, 8H, Ar–H) and 12.90 (s, 1H, NH). MS: *m/z* (%) 476(1.64 M^+); 191(100).

Synthesis of 6-amino-4-[2-((1,3-dioxo-1,3-dihydroisoindolin-2- yl)methyl) benzimidazol- 1-yl]-3,4-dimethyl-2,4-dihydropyrano [2, 3-c] pyrazole-5-carbonitrile (13a) and 3,6-Diamino-4-[2-((1,3-dioxoisoindolin-2-yl)methyl) benzimidazol-1-yl]-4-methyl-1,4-dihydropyrano [2,3-c] pyrazole-5-carbonitrile (13b):

A mixture of 2-((1-acetyl-1H- benzimidazol-2-yl)methyl)isoindoline- 1,3-dione **3** (0.01 mol) and malononitril (0.01 mol) was fused at 180^o C for 30 min., ethyl aceto acetate (0.01 mol) or ethylcyanoacetate (0.01 mol) and hydrazine hydrate (0.01 mol) in 50 mL dioxane were added. The reaction mixture was heated under reflux for 10 hrs, cooled to room-temp, acidified by acetic acid. The solid formed was filtered off, washed with dilute ethanol and purified by recrystallization from ethanolto give (13a, b).

13a. $C_{25}H_{19}N_7O_3$ (M W 465): beige crystals; 53% yield; m. p. 240^oC. Anal Calc: C, 64.51; H, 4.11; N, 21.06; Found: C, 64.26; H, 4.22; N, 20.84. FT-IR (KBrcm^{-1}) υmax: 1543(C=N); 1667 (CO imides); 2195 (CN); 3325(NH); 3116-3197(NH_2). ^1H NMR (δppm) (DMSO*d*6): δ = at 1.91- 2.08(s, 6H, 2x CH_3); 7.00 (br, s, 2H, NH_2); 5.00 (s, 2H, CH_2); 7.26-8.12 (m, 8H, Ar–H); 11.25 (s, 1H, NH).

13b. $C_{24}H_{18}N_8O_3$ (M W 466): pale brown; 67% yield; m. p. 252^oC. Anal Calc: C, 61.80; H, 3.89; N, 24.02; Found: C, 61.66; H, 3.80; N, 23.95. FT-IR (KBr cm^{-1}) υ max: 1543(C=N); 1640 (CO imide); 2195 (CN), 3444(NH), 333394-3352 (NH_2). MS: *m/z* (%) 465(4.13M^+); 66.08(100).

Biological Activity Assay

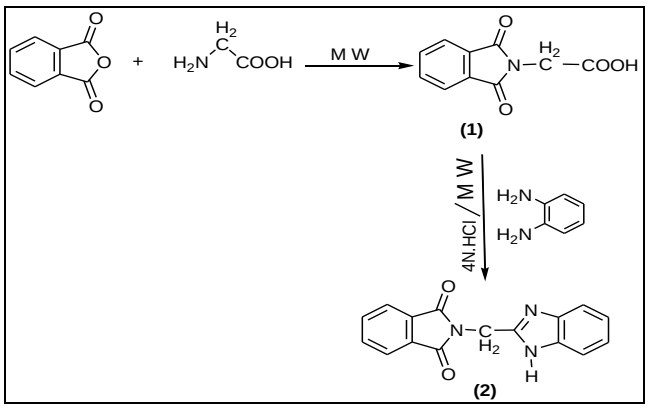
Compounds **(**2, 5b, 11, 17a, 22, 23 and 24) were evaluated *in vitro* for antimicrobial activity against the following four organisms: [*Staphylococcus aureus(Staph. Aur)* and *Bacillis subtilis(Bacil. Sub)*] as an example of Gram-positive bacteria and [*Escherichia coli (E. coli)* and *Salmonell SP (S.SP.)***)**] 9027 as Gram-negative bacteria and *Candida albicans* (RCMB 05036), *Aspergillus Fumigatus(RCMB2568)* as yeast-like fungi have been studied by using the cup plate agar diffusion method. The nutrient agar broth prepared by the usual was dispensed in 50 mL quantities in different conical flasks. Then, the 0.5 Ml culture of each bacteria (*Saphlococcus aureus* (RCMB010010) and *Bacillus subtilis* (RCMB 010067), gram-negative bacteria such as *Escherichia coli* (RCMB010052) and *Salmonella. SP***.** (RCMB010043) in nutrient agar broth was added and inoculated at 37ºC for 24h. The zone of inhibition of growth in the form of diameter in mm was measured.

RESULTS AND DISCUSSION

Chemistry

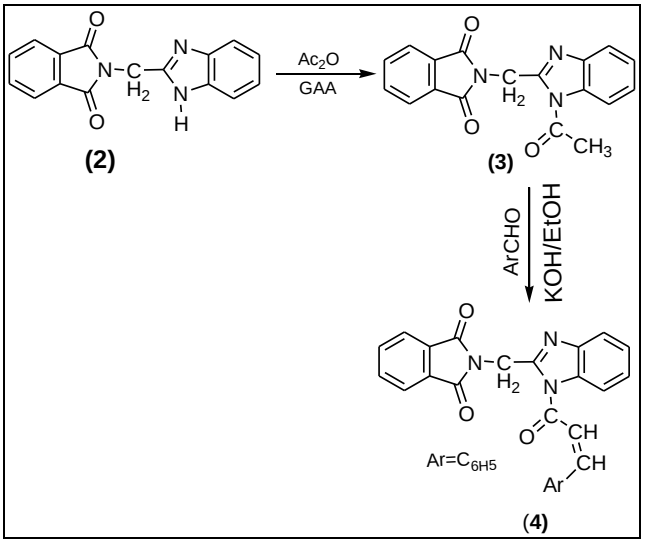
In this investigation we have studied to prepare a series of some new hetrocycles incorporating benzimidazole moiety with protected glycine. In the first step, Phthalic anhydride was reacted with glycine under solvent free and microwave irradiation reaction conditions to protect the amino group and converted to N-phthaloyl acetic acid (Scheme 1). The structure of compound (1) was elucidated on the basis of elemental analysis, spectral data and chemical transformation. FT-IR spectrum showed an absorption bands at 1723, 1773, 3479cm^{-1} attributable to CO and OH groups. Its mass spectrum revealed molecular ion peak *m/z* at 205 (6.26 M^+) with base peak *m/z* at 63.03(100%).

In the second step, N-phthaloyl acetic acid (1) was reacted with o pheylenediamine under microwave irradiation in the presence of 4N.dil HCl to afford the corresponding 2-(1H-benzoimidazol-2-yl]methyl)isoindoline-1,3 dione(2) (Scheme 1). The latter compound was used as starting material to synthesize some new heterocycles incorporating benzimidazole moiety. FT-IR spectrum of compound (2) showed characteristic band at 3431cm^{-1} for NH group and disappearance of the characteristic band for OH group. Its ^{1}H-NMR spectrum showed CH_2 protons and NH proton as a singlet in the 4.29, 9.75 ppm range.



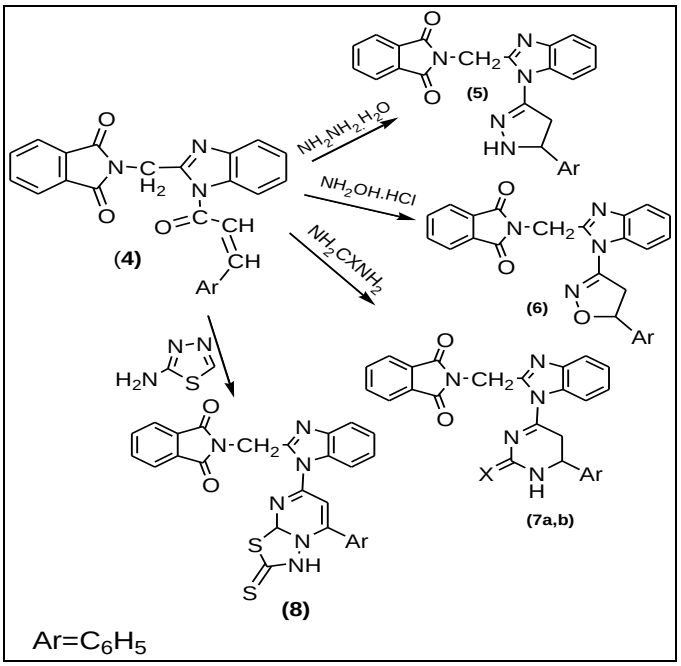
Scheme 1: Synthetic path for benezimidazole

Acylation of compound(2) in the presence of acetic anhydride and glacial acetic acid afforded the desired compound 2-(1-acetyl-1H-benzimidazol -2-yl) methyl) isoindoline-1,3-dione (3) [12]. The structure of (3) was inferred from its analytical and spectral data (Scheme 2). Its IR spectrum showed characteristic absorption bands at 1736, 1761(ketone CO), 1615 cm^{-1}(C=N) and disappearance of NH group. The ^{1}H-NMR spectrum exhibited singlet at 2.51ppm for $COCH_3$ protons and devoid NH proton. The ^{13}C-NMR spectrum chosen as a prototype showed (CH_3), (CH_2), (3C=O) peaks at 26.95, 40.39, 157.06, 161.16, 170.06ppm.



Scheme 2: Synthesis of compound 3 and Chalcone 4

The latter compound is useful intermediate for the synthesis of a series of some new heterocyclic compounds. Thus, Chalcone (4) was prepared under Claisen Schmidt conditions [13-17] by the reaction of compound (3) with benzaldehyde in aqueous ethanolic KOH in good yield. The ^{1}H-NMR spectrum showed disappearance of CH_3protons and indicated the presence of two doublet within the 7.19, 6.85 ppm range corresponding to CH=CHCO protons. The reaction sequence for the synthesis of the desired heterocyclic compounds is outlined in (Schemes 3 and 4).



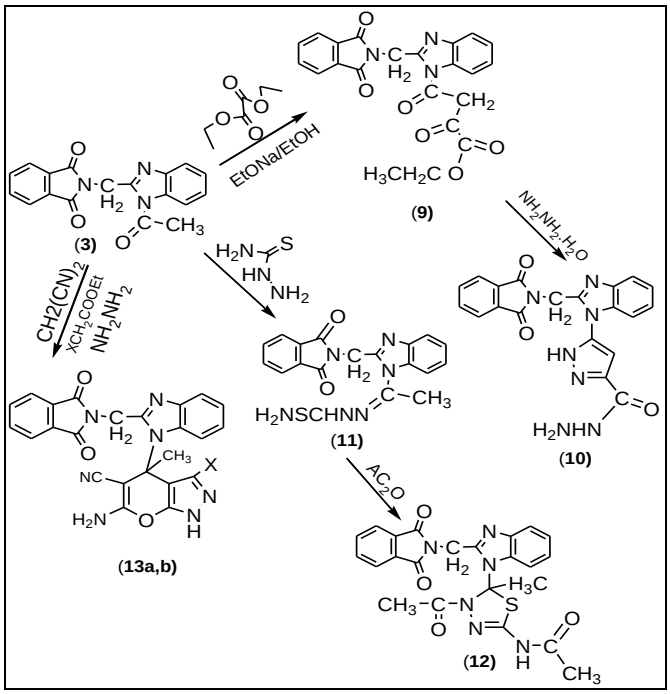
Scheme 3: Synthetic path for compounds 5, 6, 7a, b and 8

Compound (4) was converted into the corresponding 2-[1-(5-phenyl-4,5-dihy dro-1H -pyrazol-3-yl)-1H-benzimidazol-2-yl)methyl)]isoindoline-1,3-dione(5) by treatment with hydrazine hydrate in boiling ethanol [18-20]. Its ^{1}H- NMR spectrum showed a doublet of doublets within the 1.87- 2.18 range corresponding to H4, H4' of the pyrazoline ring, where a multiplet at 3.75 ppm corresponding to H5. In addition to a singlet signal corresponding to the NH proton was observed in the 8.41 ppm range.

Cyclization of 2-[1-(3-phenylacryloyl)-1H-benzimidazol-2-yl]methyl) isoindoline -1,3-dione (4) in presence of hydroxylamine hydrochloride afforded 2-[1-(5-phenyl-4,5-dihydroisoxazol-3-yl)-1H-benzimidazol-2-yl) methyl] isoindoline-1,3-dione (6) [21,22]. Its ^{1}H-NMR spectrum showed two doublet signals within the ranges 1.95 –2.15 corresponding to the (H4, H4') and one triplet signal at 4.05 ppm for H5 of the isoxazoline ring, respectively.

Cyclocondensation of compound (4) with urea, thiourea and /or 2-aminothia diazole afforded the corresponding pyrimidine derivatives (7a,b) and (8), respectively [23-27]. In ^{1}H-NMR spectra of compounds 7a,b showed the appearance of signals in the range 2.08 2.49, 3.45 ppm corresponding to CH_2,CH of pyrimidine ring and 8.45, 8.35 ppm corresponding to NH proton. The proton NMR spectrum of compound (8) showed two doublet signals for the CH protons of the pyrimidine ring at 1.80-2.08, 3.80 ppm and one singlet corresponding to NH proton of thiadiazole ring at 13.40 ppm, respectively.

On the other hand, compound (3) was allowed to react with diethyoxalate in the presence of ethanolic sodium ethoxide to afford 4[2-(1,3-dioxo-1,3-dihydro-isoindolin-2-yl) methyl] benzimidazol-1-yl)-2,4-dioxo-butyric acid ethyl ester (9) [28,29] (Scheme 4). The proposed structure was elucidated by correct analytical data and ^{1}H-NMR,^{13}C-NMR spectra. The proton NMR showed triplet signal in the 0.99 ppm range of CH_3 protons, quartet signal at 4.15 ppm for CH_2 protons, two singlet corresponding for four protons of $2CH_2$ at 4.95-5.00 and one singlet in the range 12.85 ppm due to (CH_2CO---CH=OH) proton, respectively. The ^{13}C-NMR spectrum chosen as a prototype showed (CH_3), ($3CH_2$) and (5C=O) peaks at 14.07, 39.33, 40.59, 61.23, 151.12, 151.76, 161.03, 168.26 and 169.80. Cyclization of 4[2-(1,3-dioxo-1,3-dihydro-isoindolin-2-yl) methyl] benzimidazol-1-yl)-2,4-dioxo-butyric acid ethyl ester (9) with excess of hydrazine hydrate in refluxing ethanol afforded the corresponding 5-[2-(1,3-dioxoisoindolin-2-yl-methyl)benzimidazol-1-yl].2H-pyrazole-3-carbohydrazide (10) [28,29] (Scheme 4). Its proton NMR spectrum showed broad signal corresponding to the exchangeable NH_2 protons in the 4.17 ppm range. In addition to a sjnglet signal corresponding to the exchangeable NH proton was observed in the 9.70 ppm range and devoid the CH_2CH_3 protons.



Scheme 4: Synthesis of compounds 9-13

Furthermore, condensation of the prepared compound (3) with thiosemicarbazide in boiling ethanol yielded 2-[1-(2-(1,3-dioxoisoindolin-2-yl) methyl]-1H-benzimi dazol-1-yl) ethylidene) hydrazine-1-carbothioamide (11) [30]. Its proton NMR spectrum showed the appearance of the broad signal corresponding to the exchangeable NH_2 protons in the 7.20 ppm range and D2O exchangeable signal at the range13.00 ppm, corresponding to NH proton.

Moreover, N-(4-acetyl-5-(2-(1,3-dioxo-isoindolin-2-yl)methyl)-1H-benzimidazol-1-yl)-5-methyl-4,5-dihydro-1,3,4-thiadiazol-2-yl) acetamide (12) [30] was synthesized by refluxing compound(11) with aceyic anhydride. The ^{1}H-NMR spectrum showed three singlet signals in the 1.90, 2.50, 2.80 ppm range for nine protons of $3CH_3$ and D2O exchangeable signal at the range12.90 ppm, corresponding to NH proton.

On the other hand, 6-(1-acetyl-1H-benzimidazol-2-yl) methyl] isoindoline-1,3-dione (3) was reacted with active methylene compounds namely, malonoitrile, ethylacetoacetate and/or ethylcyanoacetate in refluxing dioxane containing hydrazine hydrate [31] to give 6-anino-4-[2-(1,3-dioxo-1,3-dihydroisonidolin-2-yl) methyl) benzimidazol-1-yl)]-3,4-dimethyl-1-4,dihydroprano[2,3-c] pyrazol-5-carbonitrile (13a) and 3,6-diamino-4-[2-(1-3-dioxo-1,3-dihydroisoindolin-2-yl) methyl] benzimidazol-1-yl)-4-methyl-1,4-dihydropyrano[2,3-c) pyrazole-5-carbonitrile (13b). Their proton NMR spectra showed signals at1.91- 2.08 ppm corresponding to CH_3 protons, singlet broad in the 7.00,7.20 ppm range due to NH_2 protons and D_2O exchangeable signal at the range 10.65, 11.25 ppm, corresponding to NH protons, respectively.

Pharmacological Screening

Four test organisms representing different groups of microorganisms were used to evaluate the bioactivity of the designed products. The utilized test organisms were: [*Staphylococcus aureus (Staph. Aur)* and *Bacillis subtilis(Bacil. Sub)*] as an example of Gram-positive bacteria, [*Escherichia coli (E. coli)* and *Salmonell SP (S.SP.)*] 9027 as Gram-negative bacteria and *Candida albicans* (RCMB 05036), *Aspergillus Fumigatus (RCMB2568)* as yeast-like fungi. These strains were obtained from the Regional Center for Mycology and Biotechnology, Al-Azhar University, Cairo, Egypt. Ampicillin and Gentamycin were used as anti-bacterial references standard. The inhibition zone (IZ) is given in Table 1.

Table 1: *In vitro* antimicrobial and anti-fungal activity of the synthesized tested compounds and evaluation of the inhibition zone (IZ)

	Benzimidazole derivatives IZ(inhibition zone)							St.
	2	**5b**	**11**	**17a**	**22**	**23**	**24**	**IZ**
Gram positive bacteria								Ampicillin
Staph. aur.	11	12	11	11	10	12	13	23
Bacil. Sub.	NA	10	10	11	12	11	13	32
Gram Negative bacteria								Gentamycin
Salmonella. SP.	13	12	13	12	13	12	14	17
E.Coli	12	13	12	15	14	13	15	19
Amphotericin B								
***Aspergillus Fumigatus* (RCMB2568)**	NA	NA	12	11	11	12	11	23
***Candida albicans* (RCMB 05036)**	NA	NA	11	9	10	8	10	25

The antimicrobial screening data showed that the tested compounds (2, 5b, 11, 17a, 22, 23 and 24) exhibit antimicrobial properties. Some of the tested benzimidazole derivatives exhibit good activity against different strains of bacteria and fungus. The tested compounds showed activity against Gram +ve bacteria than Gram -ve bacteria this may be due to the peptide glycan layer is thinner in Gram -ve than Gram +ve bacteria and showed moderate to weak activity towards *Candida albicans* and *Aspergillus Fumigatus*. Significant activity against Gram -ve bacteria was observed in compounds (17, 22, 24) pyranopyrazole, hydrazinocarbothioamide and thiazole containing benzimidazole.

CONCLUSION

The objective of the present study was to synthesize and investigate the antibacterial and antifungal activity of a new series of heterocyclic compounds incorborating benzimidazole moiety in the hope of discovering new structural leads serving as antimicrobial agents. Some new benzimidazole derivatives have been prepared, and their physical properties were characterized. The biological activity of the compounds (2, 5b, 11, 17a, 22, 23 and 24) was evaluated by the agar diffusion method against *Escherichia coli*, *Salmonella. SP*., *Staphylococcus aurous* and *Candida albicans* [32]. The investigated compounds showed good to moderate activity against the test organisms *Escherichia coli* and *Pseudomonas aeruginosa* and moderate to weak activity towards *Candida albicans* and *Aspergillus Fumigatus.*

REFERENCES

[1] Y Özkay; Y Tunal; H Karaca; I Iskdag. *Arch Pharm Res* **2011**, 34, 1427-1435.
[2] A Mobinikhaledia; N Foroughifar; T Mosleh; A Hamta. *Iran J Pharm Res.* **2014**, 13, 873-879.
[3] A Gellis; H Kovacic; N Boufatah; P Vanelle. *Eur J Med Chem.* **2008**, 43, 1858-1864.
[4] TD Venu; SA Khanum; A Firdouse; BK Manuprasad; SH Shashikanth; R Mohamed; BS Vishwanth. *Bioorg Med Chem Lett.* **2008**, 18, 4409-4412.
[5] H Kucukbay; R Durmaz; E Orhan; S Gunal. *Farmaco.* **2003**, 58, 431-437
[6] RV Shingalapur; KM Hosamani; RS Keri; MH Hugar. *Eur J Med Chem*. **2010**, 45, 1753-1759.
[7] MB Wallace; J Feng; ZH Zhang; RJ Skene; L Shi; CHL Caster; DB Kassel; R Xu; SL Gwaltney. *Bioorg Med Chem Lett.* **2008**, 18, 2362-2367.
[8] J Charton; S Girault-Mizzi; MA Debreu-Fontaine; F Foufelle; I Hainault; JG Bizot-Espiard; DH Caignardc; CH Sergheraert. *Bioorg Med Chem.* **2006**, 14, 4490-4518.
[9] JR Hwua; R Singha; H Ching; YH Chang; AR Das; I Vliegen; E De Clercq; J Neyts. *Antiviral Res.* **2008** 77, 157-162.
[10] WY Mo; YJ Liang; Y Gu; LW Fu; HW He. *Bioorg Med Chem Lett.* **2011**, 21, 5975-5977.
[11] X Guo; L Shi; R Wang; X Liu; B Li; X Lu. *Bioorg Med Chem.* **2008**, 16, 10301-10310.
[12] MIB Sahar. *Turk J Chem*. **2011**, 35, 131-143.
[13] S Eddarir; N Cotelle; Y Bakkour; C Rolando. *Tetrahedron Lett.* **2003**, 44, 5359-5363.
[14] A Kumar; S Sharma; VD Tripathi; S Srivastava. *Tetrahedron*. **2010**, 66, 9445-9449.
[15] M Al-Masum; E Ng; MC Wai. *Tetrahedron Lett.* **2011**, 52, 1008-1010.
[16] ZN Siddiqui; PTN Shagufta; M Mohammed; A Anis; AU Khan. *J Enzym Inhib Med Chem*. **2012**, 27(1), 84-91.
[17] NTA Dawoud. *Chem Sci Trans*. **2017**, 6(1), 114-128.
[18] K Vashi; HB Naik. *Asian J Chem*. **2005**, 17, 409-414.
[19] S Bano; K Javed; S Ahmad; IG Rathish; S Singh; MS Alam. *Eur J Med Chem.* **2011**, 46, 5763-5768.

[20] R Bashir; S Ovais; S Yaseen; H Hamid; MS Alam; M Samim; S Singh; K Javed. *Bioorg Med Chem Lett*. **2011**, 21, 4301-4305
[21] A Solankee; K Patel; R Patel. *Indian J Chem Sect B*. **2013**, 52, 671-676.
[22] EO Kenandaa; LK Omosab. *Pharmacogn Commn*. **2017**, 7(1), 47-52.
[23] HM Shallal; WA Russu. *Eur J Med Chem*. **2011**, 46, 2043-2057.
[24] NB Patel; HR Patel. *Arabian J Chem.* **2012**, 5, 81-91.
[25] V Pathak; HK Maurya; SM Sharma; KM Srivastava; A Gupta. *Bioorg Med Chem Lett.* **2014**, 24, 2892-2896.
[26] BS Dawane; SG Konda; SB Zangade. *J Heterocycl Chem*. **2010**, 47, 1250-1254.
[27] SH Wafaa; AG Moustafa; SS Mamdouh; HB Marwa; HZ Hanafi. *J Adv Res*. **2013**, 4, 69-73.
[28] PJ Skinner; MC Cherrier; PJ Webb; Y Shin; T Gharbaoui; A Lindstrom; V Hong; SY Tamura; HT Dang; CC Pride; R Chen; JG Richman; DT Connolly; G Semple. *Bioorg Med Chem Lett*. **2007**, 17, 5620.
[29] BF Abdel-Wahab; HA Abdel-Aziz; EM Ahmed. *Arch Pharm.* **2008**, 341, 734.
[30] CK Urbain; AG Fernand; RM Christopher; HP Jacques. *J Chem Pharm Res*. **2014**, 6(10), 607-612.
[31] M Hamad; DK Khaled; YA Aisha; MH Eln. *Molecules*. **2010**, 15, 6619-6629.
[32] AC Scott. Laboratory control of antimicrobial therapy. In: Collee JG, Practicl Medical Microbiology, 13th edition, Edinburg: Churchill Livingstone, **1989**, 161-181.