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Synthesis and Antimicrobial Activity of New Benzimidazole derivatives Bearing Five-Membered Heterocyclic Moieties

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ABSTRACT

A new series of 2-substituted-1*H*-benzimidazol as potentially antimicrobial agents were designed, synthesized and characterized by IR, ^{1}H-NMR and Mass spectra. The synthesized compounds were in vitro screened against *Escherichia coli*, *Klebsiella spp.* (gram negative), *Staphylococcus aureus*, *Staphylococcus epidermidis* (gram positive) as well as *Candida albicans*. The in vitro biological evaluation revealed that some of the target compounds exerted good antimicrobial activity. Noticeably, compound 11 exhibited promising activity against *Staphylococcus aureus* and *Klebseilla SPP* at 1 mg/mL concentration.

KEYWORDS: benzimidazol, pyrazoline, isoxazoline, antimicrobial.

الخلاصة

تم تحضير سلسلة جديدة من substituted-1*H*-benzoimidazol-2 كمضادات محتملة للميكروبات ، وتم تحضيرها وتشخيصها بواسطة طيف الأشعة تحت الحمراء ، طيف الرنين النووي المغناطيسي ومطياف الكتلة. تم فحص المشتقات المحضرة في المختبر ضد *Escherichia coli*, *Klebsiella spp.*, *Staphylococcus aureus*, *S.espidermi*. بالاضافة الى *Candida albicans*. أظهر التقييم البيولوجي في المختبر أن بعض المركبات المستهدفة تمارس نشاطًا جيدًا كمضاد للميكروبات. بشكل ملحوظ، أظهر المركب 11 نشاطًا واعدًا ضد بكتيريا *S. aureus* و *Klebseilla SPP* عند 1 مجم/مل.

INTRODUCTION

Pathogenic bacterial infection diseases, such as tuberculosis (TB) among the top 10 causes of death worldwide according to the world health organization (WHO) report [1]. Fortunately, since the introduction of penicillin, as an active antibacterial agent in the 1940s, the deployment of a multitude of natural and synthetic antibiotics has provided an immeasurable benefit to human health [2]. However, pathogenic bacteria have developed resistance against countless antibacterial agents, rapidly resulting in the widespread emergence of bacterial resistance, thereby severely limiting treatment options [3]. Benzimidazoles, a fused imidazole ring with a benzene in the 4 and 5 position, are useful intermediates/subunits for the development of small molecules with biological activity. The imidazole moiety, a five membered ring, is a common moiety found integrated in a large number of natural products as well as pharmacologically active compounds [4–7]. Due to this biological activity, scientists get attracted in developing synthetic methods to obtain benzimidazole compounds. It was demonstrated that benzimidazoles could be used in the clinic as antibacterial drugs [8, 9]. According to research results, the antibacterial mechanism of benzimidazoles is related to their structural similarity to purine. It is well known that purine plays an important role in the biosynthesis of nucleic acids and proteins in the bacterial cell wall. As competitive inhibitors, benzimidazoles can replace purine, thereby blocking the biosynthesis of key components, terminating or inhibiting the growth of bacteria [10]. Furthermore, benzimidazoles exhibit significant biological activity as anti-inflammatory [11], anticancer [12], antiparasitic [13], antiprotozoal agents [14], HIV [15] and as antioxidants [16]. In our continuous attempt to exploring novel antimicrobial agents, the current research including the synthesis of new benzimidazoles with five-membered heterocyclic moiety followed by the in vitro activity against

several pathogenic bacteria as well as *Candida albicans*.

MATERIALS AND METHODS

1-Materials

All starting materials and solvents were purchased from Sigma-Aldrich and Fluka and used without further purification. Melting points were determined on an electro-thermal capillary apparatus and are uncorrected; FT-IR measurements were recorded on a Shimadzu model FTIR-8400S. Mass spectra were recorded on a Shimadzu GCMS-QP2010 Ultra apparatus. ^{1}H NMR spectra were obtained with a Bruker spectrometry model ultra-shield at 300 MHz in DMSO-d6.

2-Methods

Synthesis of chalcone derivatives (1-2)

These compounds were prepared according to the procedure described in reference [17]. To a mixture of 1*H*-benzimidazole-2-carbaldehyde (1.46g, 0.01 mol) and equimolar amount of corresponding acetophenone dissolved in a minimum amount of ethanol, aqueous NaOH solution (0.25 mol, 40%) was added drop wise. The reaction crude was stirred at room temperature until the completion of the reaction (monitored by TLC using DCM: MeOH;97:3). The precipitate formed after the acidifying the reaction mixture was filtered off, washed thoroughly with cold distilled water then recrystallized from ethanol.

1-(2-Amino-phenyl)-3-(1H-benzoimidazol-2-yl)-propenone (1)

Orange powder, yield 91%, m.p 220-222 ˚C; IR ($\bar{\upsilon}$cm^{-1}): 3481, 3319 (NH_2), 3433 (NH), 3057 (aromatic C-H), 2939, 2899 (aliphatic C-H), 1645 (C=O), 1614 (CH=CH), 1579 (C=N), 1531 (aromatic C=C). ^{1}H-NMR (300MHz, DMSO-d6) δ (ppm): 6.62-6.65 (m, 1H, Ar-H), 6.63(d, 1H, Ar-H), 7.23-7.33 (m, 3H, NH_2, Ar-H), 7.49-7.67 (m, 5H, CH-chalcone, Ar-H), 7.96(d, 1H, Ar-H), 8.25(d, 1H, CH chalcone), 12.99(s, 1H, NH benzoimidazol). GCMS (NCI) m/z: 264 M^+ For $C_{16}H_{13}N_3O$.

1-(4-Amino-phenyl)-3-(1H-benzoimidazol-2-yl)-propenone (2)

dark yellow powder, yield 92%, m.p 282-284 ˚C; IR ($\bar{\upsilon}$cm^{-1}): 3529, 3323 (NH_2), 3400 (NH-benzoimidazol), 3005 (aromatic C-H), 2931, 2889 (aliphatic C-H), 1649 (C=O), 1604 (CH=CH), 1579 (C=N), 1554 (aromatic C=C). ^{1}H-NMR (300MHz, DMSO-d6) δ (ppm): 6.28 (s, 2H, NH_2), 6.66(d, 2H, Ar-H), 7.24-7.25(m, 2H, Ar-H),7.50(d, 1H, CH-chalcone), 7.61-7.62(m, 2H, Ar-H), 7.88(d, 2H, Ar-H), 8.16(d, 1H, CH-chalcone), 12.96(s, 1H, NH benzoimidazol) . LC-MASS m/z: 263.8 M^+ (10.48 min) 96%, For $C_{16}H_{13}N_3O$.

Synthesis of 3, 5-disubstitutedarylpyrazoline (3-4)

These compounds was prepared according to the procedure described in reference [18]. To an ethanolic solution of chalcone derivative (0.1mol), few drops of glacial acetic acid was added and the mixture stirring at 25 ºC for 15 min. Then, hydrazine hydrate (0.25 mol, 80 %) was added drop wise and the reaction mixture was further stirred for 15 minute. TLC using dichloromethane checked the completion of the reaction and methanol as eluent (94:6), the solid products were filtered off, washed thoroughly with diethyl ether, and recrystallized from absolute ethanol.

4-[5-(1H-Benzoimidazol-2-yl)-4,5-dihydro-1H-pyrazol-3-yl]-phenylamine(3)

White powder, yield 83%, m.p 232-234 ˚C; IR ($\bar{\upsilon}$cm^{-1}): 3529, 3323 (NH_2), 3444 (NH-pyrazoline), 3304 (NH-benzoimidazol) 3086 (aromatic C-H), 1635 (C=N pyrazine), 1606 (C=N benzoimidazol), 1587 (aromatic C=C). ^{1}H-NMR (300MHz, DMSO-d6) δ (ppm): 3.13-3.19 (m, 1H, Ha-pyrazoline), 3.37-3.44(m, 2H, Hb-pyrazoline, NH-pyrazoline),4.88-4.93(m, 1H, Hx-pyrazoline), 6.53(s, 2H, NH_2), 7.09-7.11 (m, 3H, NH-benzoimidazol, Ar-H), 7.31-7.47(m, 6H, Ar-H. LC-MASS (NCI) m/z: 276 M+(9.23min) 84.5%, For $C_{16}H_{15}N_5$.

2-[5-(1H-Benzoimidazol-2-yl)-4,5-dihydro-1H-pyrazol-3-yl]-phenylamine(4)

White powder, yield 90%, m.p 220-222 ˚C; IR ($\bar{\upsilon}$cm^{-1}): 3414 (NH-pyrazoline), 3369 (NH-benzoimidazol) 3057 (aromatic C-H), 1620 (C=N pyrazoline), 1606 (C=N benzoimidazol), 1587 (aromatic C=C). ^{1}H-NMR (300MHz, DMSO-d6) δ (ppm): 3.38-3.46 (m, 2H, Ha-pyrazoline, NH-pyrazoline), 3.59-3.70 (m, 1H, Hb-pyrazoline,), 4.94-5.02 (m, 1H, Hx-pyrazoline), 6.55-6.76 (m, 4H, NH_2 , Ar-H), 7.06-7.18 (m, 4H, Ar-H), 7.47-7.64 (m, 2H, Ar-H), 12.46(s, 1H, NH benzoimidazol).GCMS (NCI) m/z: 277 M^+ For $C_{16}H_{15}N_5$.

Synthesis of 4-[5-(1H-Benzoimidazol-2-yl)-4,5-dihydro-isoxazol-3-yl]-phenylamine (5).

This compound was prepared according to the procedure described in reference [19].To a solution of chalcone compound 1 (0.1 mol) in a minimum amount of ethanol with few drops of glacial acetic acid, hydroxylamine hydrochloride (0.6 mol) was added at room temperature and the mixture stirred overnight . The progress of the reaction was checked by TLC using dichloromethane and methanol as eluent (92:8) The obtained precipitate was filtered off, washed with diethyl ether, dried and recrystallized from ethanol.

Off white powder, yield 72%, m.p 320-322 ˚C; IR ($\bar{\upsilon}$ cm^{-1}): 3421, 3365 (NH_2), 3402 (NH benzoimidazol), 3053 (aromatic C-H), 1645 (C=N benzoimidazol), 1600(C=N isoxazoline), 1556 (C=C aromatic) . ^{1}H-NMR (300MHz, DMSO-d6) δ (ppm): 3.2-3.5 (m, 2H, CH_2-isoxazoline), 5.35-5.45(m, 3H, NH_2, CHx-isoxazoline), 6.49- 6.52 (d, 1H, Ar-H), 6.65-6.67(d, 1H, Ar-H), 7.02-7.06 (d, 1H, Ar-H), 7.17-7.20(m, 2H, Ar-H), 7.47-7.50(m, 2H, Ar-H), 11.55(s, 1H, NH benzoimidazol). GCMS (NCI) m/e: 278.3 M^+ For $C_{16}H_{14}N_4O$.

Synthesis of 4-(2-Amino-phenyl)-6-(1H-benzoimidazol-2-yl)-1,2-dihydro-pyrimidine-2-thiol (6)

This compound was prepared according to the procedure described in reference [20]. The (1mmol) from compound 2 was dissolved in (10 mL) absolute ethanol with 7 drops of glacial acetic acid, the solution stirring for 30 min then (2 mmol) thiourea was added to solution and Reflux for 24 h. The procures of the reaction was checked by TLC using dichloromethane and methanol as eluent (6%). The solid product filters off, washed with diethyl ether and recrystallized from EtOH.

light pink powder, yield 73%, m.p 195-197˚C; IR ($\bar{\upsilon}cm^{-1}$): 3420(NH), 3338, 3234 (NH_2), 3300(NH), 3146(aromatic C-H), 2922, 2825(aliphatic C-H), 2613(SH), 1689(NH bend), 1633(C=N benzoimidazol), 1606(C=N pyrimidine-2-thiol), 1587 (C=C aromatic)**.** ^{1}H-NMR (300MHz, DMSO-d_6) δ (ppm**):** 3.40 (s, 1H, SH), 6.54(s, 2H, NH_2), 6.93-6.98 (m, 2H, Ar-H), 7.25 – 7.28(m, 4H, Ar-H), 7.41 (d, 1H, Ar-H), 7.53-7.63(m, 2H, Ar-H, CH Pyrimidine-2-thiol), 7.77(s, 1H, CH Pyrimidine-2-thiol), 10.20(s, 1H, NH Pyrimidine-2-thiol), 12.76(s, 1H, NH benzoimidazol).GCMS (NCI) m/z: 325 M^+ For $C_{17}H_{19}N_5S$.

Synthesis of {2-[5-(1H-Benzoimidazol-2-yl)-4,5-dihydro-1H-pyrazol-3-yl]-phenyl}-benzylidene-amine (7)

These Schiff base compound was synthesized according to the procedure in reference [21]. The mixture of (1.5mmol) benzaldehyde was dissolved in 1,4-dioxane with 2 drops of concentration H_2SO_4 and mixed for 15 min at R.T. the (1.5 mmol) 0f (3,4) compound was added to the mixture and stir for 2h. the procures of reaction was checked by TLC using n-hexan and ethyl acetate as eluent (6:1).the solid product filters off, dry and washed with distilled water then with diethyl ether.

Brown powder, yield 57%, m.p 214-216˚C; IR ($\bar{\upsilon}cm^{-1}$): 3445 (NH-pyrazoline), 3410 (NH-benzoimidazol) 3095 (aromatic C-H), 1640(C=N pyrazoline), 1618(C=N benzoimidazol), 1600(CH=N), 1573 (aromatic C=C). ^{1}H-NMR (300MHz, DMSO-d_6) δ (ppm): 3.52-3.56 (m, 2H, Ha-pyrazoline, NH pyrazoline), 3.87-3.93(m, 1H, Hb-pyrazoline,), 5.25-5.36(m, 1H, Hx-pyrazoline) 6.57-8.53(m, 15H, Ar-H, CH=N, NH benzoimidazol). GCMS (NCI) m/z: 365.4M^+ For $C_{23}H_{19}N_5$.

Synthesis of thiourea derivatives (8-9)

These compounds were synthesized according to the procedure reported in reference [22]. A mixture of amino derivative (3) (1mmol) and an equimolar amount of the corresponding phenyl isothiocyanate derivatives in absolute ethanol (10 ml) was reflux for 14 hrs at 100 C˚. The progress of the reaction was checked by TLC using dichloromethane and methanol as eluent (96:4) after the completion of the reaction, the mixture was added to ice water, filters off and recrystallized from EtOH.

1-{4-[5-(1H-Benzoimidazol-2-yl)-4,5-dihydro-1H-pyrazol-3-yl]-phenyl}-3-(4-nitro-phenyl)-thiourea (8)

Off white powder, yield 73%, m.p 195-197 ˚C; IR ($\bar{\upsilon}cm^{-1}$): 3338(NH), 3295(NH), 3244(NH), 3099(C-H aromatic), 1635(C=N pyrazoline), 1610(C=N benzoimidazol), 1595 (N-C=S), 1542 (C=C aromatic). ^{1}H-NMR (300MHz, DMSO-d6) δ (ppm):3.36-3.49(m, 1H, Ha-pyrazoline), 3.58(s, 1H, NH- pyrazoline), 3.75-3.83 (m, 1H, Hb-pyrazoline), 4.02-4.12(m, 1H, Hx-pyrazoline,), 6.37-6.42(m, 2H, Ar-H), 6.74-6.77(m, 2H, Ar-H), 7.49-7.52(m, 2H, Ar-H), 7.74-7.79 (m, 4H, NH, NH benzoimidazol, Ar-H), 8.03-8.06(d, 2H, Ar-H),

8.24-8.27(d, 2H, Ar-H), 10.64(s, 1H, NH). GCMS (NCI) m/z: 457M+ For $C_{23}H_{19}N_7O_2S$.

1-{4-[5-(1H-Benzoimidazol-2-yl)-4,5-dihydro-1H-pyrazol-3-yl]-phenyl}-3-phenyl-thiourea (9)

Yellow powder, yield 68%, m.p 242-244 ˚C; IR ($\bar{\upsilon}cm^{-1}$): 3465(NH), 3300(NH), 3315(NH), 3053(C-H aromatic), 1660(C=N pyrazoline), 1620(C=N benzoimidazol), 1591 (N-C=S), 1508 (C=C aromatic). ^{1}H-NMR (300MHz, DMSO-d6) δ (ppm): 3.37(s, 1H, NH- pyrazoline), 3.42-3.49(m, 1H, Ha-pyrazoline), 3.86-3.96 (m, 1H, Hb-pyrazoline), 6.11-6.16(m, 1H, Hx-pyrazoline,), 6.62-6.65(d, 2H, Ar-H), 7.14-7.21(m, 2H, Ar-H), 7.48-7.52(m, 2H, Ar-H), 7.59-7.62 (m, 2H, Ar-H), 8.03-8.06(d, 2H, Ar-H), 7.68-7.72(m, 2H, Ar-H), 7.99- 8.02 (d, 1H, Ar-H), 10.01(s, 1H, NH), 10.20(s, 1H, NH), 12.45(s, 1H, NH benzoimidazol). GCMS (NCI) m/z: 412M^+ For $C_{23}H_{20}N_6S$.

Synthesis of amides derivatives(10-11)

These compounds were obtained according to a modified procedure depicted in reported work [23]. To a cooled solution (0-5) ˚C of amino derivative (3,4) no. of mmole in 1,4-dioxane, chloroacetyl chloride (1mmol) was added with continues stirring for 10 minutes then the temperature arise to room temperature. Furthermore, the reaction mixture stirred for approximately one hour and the reaction progress was checked by TLC using dichloromethane and methanol as eluent (95:5). The precipitate formed was filtered, washed with diethyl ether

N-{2-[5-(1H-Benzoimidazol-2-yl)-4,5-dihydro-1H-pyrazol-3-yl]-phenyl}-2-chloro-acetamide (10)

Yellow powder, yield 86%, m.p 340-342 ˚C; IR ($\bar{\upsilon}cm^{-1}$): 3440(NH), 3431(NH), 3365(NH), 3040(C-H aromatic), 2928, 2883(C-H aliphatic), 1690(C=O), 1643(C=N pyrazoline), 1606(C=N benzoimidazol), 1535 (C=C aromatic). ^{1}H-NMR (300MHz, DMSO-d6) δ (ppm): 3.83-3.95 (m, 1H, Ha-pyrazoline), 4.17-4.28(m, 1H, Hb-pyrazoline,), 4.59-4.87(m, 2H, NH-benzoimidazol, NH-pyrazoline), 5.03(s, 2H, CH_2-Cl), 6.06-6.10(m, 1H, Hx-pyrazoline), 6.94-6.97(d, 1H, Ar-H), 7.2-7.3(m, 1H, Ar-H), 7.52-7.64(m, 3H, Ar-H), 7.78-7.82(m, 2H, Ar-H), 8.29-8.32(d, 1H, Ar-H), 10.68(s, 1H, NH amide). GCMS (NCI) m/z: 352 M^+ For $C_{18}H_{16}ClN_5O$.

N-{4-[5-(1H-Benzoimidazol-2-yl)-4,5-dihydro-1H-pyrazol-3-yl]-phenyl}-2-chloro-acetamide (11)

Yellow powder, yield 76%, m.p 333-335 ˚C; IR ($\bar{\upsilon}cm^{-1}$): 3498(NH), 3433(NH), 3414(NH), 3030(C-H aromatic), 2928, 2883(C-H aliphatic), 1670(C=O), 1649(C=N pyrazoline), 1606(C=N benzoimidazol), 1535 (C=C aromatic). ^{1}H-NMR (300MHz, DMSO-d6) δ (ppm): 3.73-3.79 (m, 1H, Ha-pyrazoline), 3.98-4.02(m, 1H, Hb-pyrazoline,), 4.36(s, 2H, CH_2-Cl), 4.73-4.86(m, 2H, NH-benzoimidazol, NH-pyrazoline), 5.94-5.96(m, 1H, Hx-pyrazoline), 7.35-7.38(m, 2H, Ar-H), 7.66-7.69(m, 2H, Ar-H), 7.78-7.85(m, 4H, Ar-H), 10.87(s, 1H, NH amide). GCMS (NCI) m/z: 350M^+ For $C_{18}H_{16}ClN_5O$.

Synthesis of 2-substituted phenylamino-4H-thiozol-5-one derivatives (12, 13)

These derivatives were synthesized as described in the published work [24]. To a solution of the corresponding amide (10, 11) (1 mmol) in a minimum amount of absolute ethanol, KSCN (1.5 mmol) was added then the reaction mixture was stirred for 24 hrs at 100 C˚. the reaction progress was checked by TLC using dichloromethane and methanol as eluent (92:8) The solid precipitate was collected by filtration, dried and recrystallized from water/ethanol (3:7).

2-{2-[5-(1H-Benzoimidazol-2-yl)-4,5-dihydro-1H-pyrazol-3-yl]-phenylamino}-4H-thiazol-5-one (12)

Yellow powder, yield 63%, m.p 127-129 ˚C; IR ($\bar{\upsilon}cm^{-1}$): 3483(NH), 3423(NH), 3338(NH), 3034(C-H aromatic), 2937, 2870(C-H aliphatic), 1695(C=O), 1685(C=N pyrazoline), 1610(C=N benzoimidazol), 1595 (C=N thiazol),1531 (C=C aromatic). ^{1}H-NMR (300MHz, DMSO-d6) δ (ppm): 3.37(s, 2H, CH_2-thiazol), 3.69-3.80 (m, 1H, Ha-pyrazoline), 3.91-4.02(m, 1H, Hb-pyrazoline,), 4.06 (s, 1H, NH- pyrazoline), 4.19(s, 1H, NH-benzoimidazol), 4.53-4.70(m, 1H, Hx-pyrazoline), 7.12-7.21(m, 2H, Ar-H), 7.43-7.79(m, 4H, Ar-H), 7.87-8.01(m, 2H, Ar-H,), 10.70(s, 1H, NH). GCMS (NCI) m/z: 376.2M^+ For $C_{19}H_{16}N_6OS$.

2-{4-[5-(1H-Benzoimidazol-2-yl)-4,5-dihydro-1H-pyrazol-3-yl]-phenylamino}-4H-thiazol-5-one (13)

Off white powder, yield 59%, m.p 110-112 ˚C; IR ($\bar{\upsilon}cm^{-1}$): 3480(NH), 3440(NH), 3320(NH), 3040(C-H aromatic), 2991, 2856(C-H aliphatic),

1666(C=O), 1651(C=N pyrazoline), 1604(C=N benzoimidazol), 1595 (C=N thiazol), 1531 (C=C aromatic). ^{1}H-NMR (300MHz, DMSO-d6) δ (ppm): 1.18-1.24 (m, 1H, Ha-pyrazoline), 3.43-3.50(m, 1H, Hb-pyrazoline,), 4.05(s, 2H, CH_2-thiazol), 4.20 (s, 1H, NH-benzoimidazol), 4.60(s, 1H, NH-pyrazoline), 5.89-5.94(m, 1H, Hx-pyrazoline), 7.32-7.35(dd, 2H, Ar-H), 7.64-7.67(dd, 2H, Ar-H), 7.75-7.78(d, 2H, Ar-H), 7.86-7.89(d, 2H, Ar-H), 10.81(s, 1H, NH). GCMS (NCI) m/z: 376.4M^+ For $C_{19}H_{16}N_6OS$.

Antimicrobial Study

The synthesized benzoimidazol derivatives were tested for their antimicrobial activity against *Escherichia coli, Klebsiella SPP* (gram negative), *Staphylococcus aureus, S.espidermi*. (gram positive) as well as *C. albicans* using the well diffusion method (Table 1) [25]. The concentration for each compound was 1mg/mL. Plates were prepared by spreading approximately 105 CFU/ml culture broth of each indicator bacterial isolates on Muller Hinton agar surface using sterile cotton swabs. The agar plates were left for about 10 min before aseptically dispensing the 50µl of each compound into the agar wells already bored in the agar plates. Then the plates were incubated at 37C° for 24 hrs. Zones of inhibition were measured and recorded in millimeter diameter. The Dimethyl sulfoxide was used as control.

RESULTS AND DISCUSSION

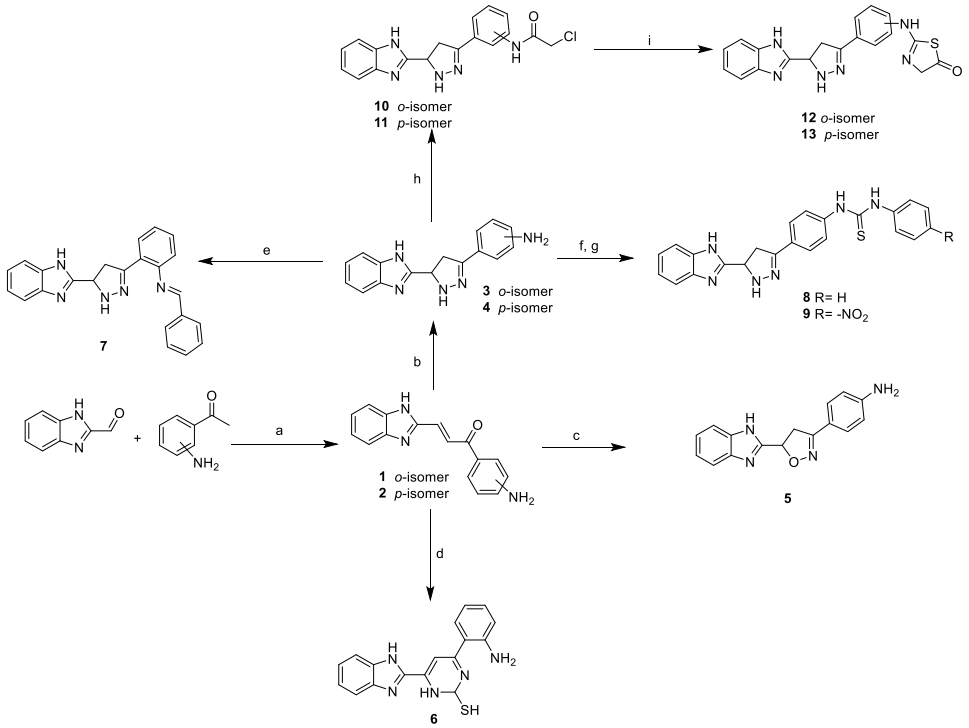
Synthesis of benzimidazole derivatives

Chalcone derivatives (1-2) were obtained via analogy method to Claisen-Schmidt condensation reaction [26]. In brief, substituted acetophenone was reacted with the corresponding 1*H*-benzimidazole-2-carbaldehyde in ethanol in the presence of aqueous sodium hydroxide.

The structures of obtained compounds were confirmed by spectral analysis. FT-IR spectrum for chalcone derivative 1 showed two absorption bands , the first at 1645 cm^{-1} and the second at 1604 cm^{-1} regions due to the stretching vibrations of the C=O and CH=CH groups, respectively. The ^{1}HNMR spectra show singlet signal at 6.28 ppm due to NH_2 with two doublet signals at 7.49 and 8.25 ppm due to CH=CH. The synthesized derivatives 3-6 were synthesized by cycloaddition reactions of the intermediate chalcone derivative (1,2) with hydrazine hydrtate, hydroxylamine hydrochloride and thiourea in acidic ethanolic solution as described in experimental section. The obtained derivatives were purified by recrystallization from suitable solvents. FT-IR spectrum showed disappearance of the C=O and CH=CH and appearance absorption bands at 1635,1620, 1600 and 1606 cm^{-1} due to C=N pyrazoline, C=N isoxazoline and C=N Pyrimidine-2-thiol for compound 3-6, respectively and at 2613 cm^{-1} due to SH for compound 6. ^{1}HNMR spectra showed singlet signals at 3.13-3.46 ppm related to NH proton for compounds 3,4 and singlet signal at 7.77-10.20 due to NH proton for compound 6 with appearance three multiplet signals at 3.13-3.46, 3.37-3.7 and 4.48-5.02 ppm due to CHa, CHb and CHx of pyrazoline derivative.

Furthermore, the ^{1}HNMR spectrum of compound 6 showed four singlet signals at 3.40, 6.45, 7.77, 10.20 and 10.20 ppm due to SH, NH_2, CH and NH of Pyrimidine-2-thiol ring, respectively. The singlet signal appear at 12.6 related to HN of benzoimidazol system. Schiff base, thiourea and acetamide derivatives were obtained by the reaction of pyrazoline derivatives (3, 4) with benzaldehyde, phenyl isothiocyanate or chloroacetyl chloride respectively. The FT-IR spectra of other synthesized compounds (7-11) showed the absorption bands at 1600 cm^{-1} , 1591-1595 and 1670-1690 cm^{-1} regions due to the stretching vibrations of the CH=N , N-C=S and C=O groups, respectively as indicated in experimental section. The disappearance of the NH_2 stretching frequencies a good evidence of prepared target compounds. ^{1}HNMR spectra showed a singlet signal at range 7.77-8.53 ppm due to CH=N for Schiff bases and two singlet signals at 7.74-10.01 and 10.20-10.64 due to 2 NH groups for thoiurea derivatives. The two singlet signal at 4.36-5.03 and 10.68-10.87 ppm related to NH and CH_2Cl of acetamide derivatives. 1,3-thiozol-5-one derivatives (12,13) was synthesized by cyclization reaction of acetamide derivative with potassium thiocyanate in ethanolic solution. The FT-IR spectra of compounds showed the absorption bands at 1666-1695 and 1595 cm^{-1} regions due to the stretching vibrations of the C=O and C=N groups respectively. The disappearance of the NH_2 stretching frequencies a good evidence of prepared

target compounds. Furthermore, the ^{1}HNMR spectra showed singlet signal at 3.37-4.05 cm^{-1} due to CH_2 group (see experimental section).



Scheme 1. (a) NaOH 40%, ETOH (b) hydrazine hydrate, glacial acetic acid, ETOH (c) hydroxylamine hydrochloride, glacial acetic acid, ETOH (d) thiourea, glacial acetic acid, ETOH (e) benzaldehyde, H_2SO_4, 1,4-dioxane (f) phenyl isothiocyanate, ETOH (g) 4-Nitrophenyl isothiocyanate, ETOH (h) Chloroacetyl chloride, 1,4-dioxane (i) KSCN, ETOH.

Antimicrobial activity

The in vitro assay of the synthesized compounds (4-13) against several microbial species was achieved using 1mg /mL concentration as illustrated in Table 1. The tested derivatives exhibited promising activity against different species. Compounds 4 and 11 were the most potent agents against gram positive, gram negative as well as *Candida albicans*.

Table 1. In Vitro antimicrobial inhibition zone (mm) of the synthesized compounds.

Compound	Gram negative		Gram positive		fungi
(1mg /mL)	*S. aureus*	*S. espidermi*	*E.coli*	*Klebsiella spp.*	*C. albicans*
4	14	12	13	13	14
5	-	-	12	-	-
6	-	-	-	-	-
7	-	-	-	-	-
8	-	-	-	-	11
9	-	-	-	-	-
10	20	10	11	12	-
11	-	-	-	-	-
12	-	-	-	-	-
13	-	-	11	-	-
Amoxicillin	17	18	20	20	25

(-) exhibit no activity at the specified concentration.

CONCLUSION

The present work summarized the synthesis of 2-substituted-1H-benzimidazole derivatives containing a various five membered heterocyclic rings. The structure of synthesized derivatives was elucidated using spectral analysis. The new derivatives were examined for their antimicrobial activity against several bacterial species and some of them (compounds 4 and 11) exhibited promising results.

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