Synthesis, some reactions, cytotoxic evaluation and antioxidant study of novel benzimidazole derivatives

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ABSTRACT

A series of novel benzimidazole derivatives incorporating chalcone (3), pyrazolines(4),(5), (16), oxazoline(6), pyrimidines(7-13),(17) and oxirane(15) derivatives were synthesized and some of their reactions with some electrophiles and/or nucleophiles were studied. Some of the new derivatives were biologically evaluated as antioxidants and the results showed that the presence of the epoxide ring in (15) as well as the pyrimidine thione in (7) are responsible for their reactivity, rather than the presence of the pyrazoline ring system as in (4a). On the other hand, some of the synthesized compounds were tested as for their cytotoxic activity and the results were encouraging. All the synthesized compounds were characterized by elemental analysis as well as spectral data such as IR, \(^1\)H-NMR and Mass spectra.

Key words: Benzimidazole derivatives, Reactions with nucleophiles, Biological activity, Antioxidant and Cytotoxic activity.

INTRODUCTION

Benzimidazoles are an important group of heterocyclic compounds that are biologically active and of significant importance in medicinal chemistry. The benzimidazole ring is an important pharmacophore in modern drug discovery. The chemistry and pharmacology of benzimidazoles have been of great interest to medicinal chemistry because its derivatives possessed various biological activities such as antioxidant, antimicrobial, antihelmintic, anticancer, antihypertensive, antineoplastic, anti-inflammatory, anti-analgesic, antiprotozoal, anti-hepatitis B virus, antiulcer, antiviral, antifungal, and anticonvulsant activity.

On the other hand, chalcones are very well known important starting materials for the synthesis of various classes of heterocyclic compounds such as pyrazolines, isoxazolines, pyrimidines and/or benzodiazepines as well. Most of these compounds were found to be highly bioactive and were widely used in pharmaceutics. The bacteriostatic as well as bactericidal activity in the chalcone system is attributed to the presence of the enone function –C=\(\cdot\)C=O.

Hence it was thought interesting to prepare a benzimidazole moiety incorporating the enone function. In view of the above findings and in continuation of our research program to find effective new antimicrobial and/or antitumor agents for the treatment of infectious diseases, the present study focused on the synthesis and biological evaluation of some benzimidazolo incorporating chalcone, pyrazoles, oxazole, pyrimidines and oxirane moieties in order to
throw a more precise information about their chemical reactivity and study on benzimidazole chalcone combined molecule, and its biological activity as antioxidant and/or antitumor as well.

**MATERIALS AND METHODS**

Melting points are determined on a Gallen Kemp melting point apparatus and are uncorrected. Elemental analysis (% C,H,N) is carried out by a Perkin-Elmer 2400 CHN analyser. IR spectra of compounds have been recorded on a Thermo-Nicolet FT-IR200 spectrometer in KBr disc (cm$^{-1}$). $^1$H-NMR are spectra recorded on Bruker DRX(300 MHz) spectrophotometer using DMSO-d$_6$as solvent and TMS as internal standard. Chemical shifts are reported in $\delta$ppm. Mass spectra of synthesized compounds were carried out using Shimadzu GC–MS(Shimadzu 2010 plus) mass spectrometer direct probe method.

![Scheme 1](image-url)
Scheme 2
Synthesis of (1H-benzo[d]imidazol-2-yl)-3-(5-chloro-3-methyl-1-phenyl-1H-pyrazol-5-yl)prop-2-en-1-one (3)
A mixture of 2-acetyl benzimidazole (1)(0.04 mol) and the aldehyde (2)(0.1 mol) were dissolved in ethanol (20 ml) the reaction mixture was stirred in ice bath ,then add 5ml of 10% aqueous NaOH drop wise through 30 min .The solid obtained was filtered off, washed well with dilute ethanol followed by crystallization from ethanol to give 3 as dark green crystals ; yield 75% ;m.p 189°C. IR :3231 cm⁻¹ (√NH), 3130, 3060, 2980, 2929, 2860 cm⁻¹ (√C-H), 1667 cm⁻¹ (√C=O),1604 cm⁻¹ (√C=N ). The ¹H-NMR( 300 MHz ,DMSO-d₆): δ ppm at 10.50(s,1H,NH), 6.86-8.39 (m, 9H,Ar-H), 3.80(d,1H,CHα), 2.50(d,1H, CHβ),2.23(s,3H,CH3) . MS: 361 △+.(M-1)(12.1%), 191.5(2%), 145(2.2%), 76(8.5%) ,50(100%). Anal. of C₂₀H₁₅N₄O Cl(362.5) (%) calcd: C, 66.20; H, 4.13; N, 15.44; Cl, 9.79. Found:C, 66.21; H, 4.21; N, 15.45Cl, 9.88.

Synthesis of 2-(5-(5-chloro-3-methyl-1-phenyl-1H-pyrazol-4-yl)-4,5-dihydro-1H-pyrazol-3-yl)-1H-benzo[d]imidazole(4a),2-(5-(5-chloro-3-methyl-1-phenyl-1H-pyrazol-4-yl)-1-phenyl-4,5-dihydro-1H-pyrazol-3-yl)-1H-benzo[d]imidazole(4b)
A mixture of 3(0.01 mol),and hydrazines, namely hydrazine hydrate  and/or phenylhydrazine (0.01 mol) in ethanol (30 ml) was refluxed for 6 hrs. After concentration and cooling the solid obtained was crystallized from ethanol to give 4a and/or 4b respectively .4a as dark green crystals ; yield 68% ; m.p 200°C,IR spectrum of 4a:3232 cm⁻¹ (√NH), 3200 cm⁻¹ (√NH), 3126, 3060, 2980, 2929,2890, 2860 cm⁻¹ (√C-H), 1626 cm⁻¹ (√C=N ),1601 cm⁻¹ (√C=C) . ¹H-NMR( 300 MHz ,DMSO-d₆): δ ppm at 11.20(s,1H,NH), 10.59(s,1H,NH), 6.88-7.86 (m, 9H,Ar-H), 3.28(t,1H,CHCH₂), 2.99(d,2H, CH₂CH),1.31(s,3H,CH₃) . MS: 375 △+ (M-1)(2.04%), 191.5(2%),224(0.5%), 167(3.9%),153(0.3%) 144(1%), 94(2.1%),55(100%). Anal. C₂₀H₁₇N₆Cl(376.5) (%) calcd: C, 63.74; H, 4.25; N, 22.30 Cl, 9.88. Found:C, 63.75; H, 4.26; N, 22.32; Cl, 9.42. 4b as black crystals ; yield 56% ; m.p 215°C . IR :3275 cm⁻¹ (√NH), 3200 cm⁻¹ (√NH), 3126, 3050, 2980, 2929,2890 cm⁻¹ (√C-H), 1613cm⁻¹ (√C≡N ),1599cm⁻¹ (√C=C) . ¹H-NMR( 300 MHz ,DMSO-d₆): δ ppm at 11.2(s,1H,NH), 6.12-8.7 (m, 14H,Ar-H), 2.98(t,1H,CHCH₂), 2.27(d,2H, CH₂CH),1.46(s,3H,CH₃) . MS: 452 △+(M+1)( 4.11%), Anal. C₂₆H₂₁N₆Cl(452.5) (%) calcd: C, 68.95; H, 4.64; N, 18.56; Cl, 7.84. FoundC, 68.99; H, 4.65; N, 18.53; Cl, 7.82.
Synthesis of 3-(1H-benzo[d]imidazol-2-yl)-5-(5-chloro-4-methyl-1-phenyl-1H-pyrazol-4-yl)-4,5-dihydro-1H-pyrazole-1-carbothioamide(5)

A mixture of 3(0.01 mol),thiosemicarbazide(0.01 mol;0.8 gms dissolved in 1 ml water containing sodium acetate(0.01 mol,0.8 g) in ethanol (30 ml)was refluxed for 6 hrs. After concentration and cooling the product that obtained was collected, washed well with dilute ethanol followed by crystallization from ethanol to give 5 as green crystals ; yield 69% :m.p 202°C:IR : 3399,3232 cm⁻¹(νN-H), 3162,3136, 3067, 2959,2927, 2881 cm⁻¹(ν C-H), 1613 cm⁻¹(ν C=N ),1597 cm⁻¹(ν C=C),1249 cm⁻¹(ν C=S) .¹H-NMR( 300 MHz :DMSO-d₆): δppm at 12.20(s,1H,NH), 11.2(s,2H,NH₂),6.84-8.58 (m, 9H,Ar-H), 2.99(t,1H,CH₂CH₃), 2.47(d,2H, CH₂CH₃),1.23(s,3H,CH₃) . MS: 435 ¹(M)ⁿ ( 60.12%), 365(100%),192(2%), 155(3%) ,129(5%) 144(1%) ,77(49.6%) . Anal.C₉H₂N₅S Cl(343.5) (%) calcd: C, 57.86; H, 4.13; N, 22.50; S, 7.34; CI, 8.15 Found:C, 57.90; H, 4.15; N, 22.48; S, 7.35;Cl, 8.12.

Synthesis of 3-(1H-benzo[d]imidazol-2-yl)-5-(5-chloro-3-methyl-1-phenyl-1H-pyrazol-4-yl)-4,5-dihydropyrazole(6)

A mixture of 3(0.01 mol),hydroxylamine hydrochloride(0.01 mol) in pyridine (30 ml)was refluxed for 6 hrs. The reaction mixture was poured into ice and HCl and the solid filtered off , washed well with dilute ethanol followed by crystallization from ethanol to give (3) as dark brown crystals ; yield 65% :m.p 215°C:IR : 3376cm⁻¹(νN-H), 3130, 3060,2960, 2920, 2880cm⁻¹(ν C-H), 1598 cm⁻¹(ν C=N ±),1H-NMR( 300 MHz :DMSO-d₆): δppm at 11.44(s,1H,NH), 6.85-7.86 (m, 9H,Ar-H). MS: 377¹(M)ⁿ ( 4.0%),239(58.1%), 192(3%),141(40.6%), 131(11.4%) ,82(2%) 72(1%), 63(100%),56(70.8%) . Anal.C₂₀H₁₈N₅Cl(377.5) (%) calcd: C, 63.57; H, 4.23; N, 18.54; Cl, 9.40 Found:C, 63.62; H, 4.29; N, 18.53; Cl, 9.35.

Synthesis of 6-(1H-benzo[d]imidazol-2-yl)-4-(5-chloro-3-methyl-1-phenyl-1H-pyrazol-4-yl)-5,6-dihydropyrimidine-2(1H)-thione(7a),4-(1H-benzo[d]imidazol-2-yl)-6-(5-chloro-3-methyl-1-phenyl-1H-pyrazol-4-yl)-5,6-dihydropyrimidin-2(1H)-one (7b)

A mixture of 3(0.01 mol),withthiourea and/orurea(0.01 mol) in absolute ethanol (30 ml) containing sodium ethoxide ( prepared from 0.2 gm sodium dissolved in 5 ml absolute ethanol) was refluxed for 6 hrs. After concentration and cooling the product that obtained was collected, washed well with dilute ethanol followed by crystallization from ethanol to give 7a and/or/7b. 7a as green crystals ; yield 76% :m.p 235°C:IR spectrum of 7a: 3402cm⁻¹(νN-H), 3160, 3020, 3009,2981, 2926cm⁻¹(ν C-H),2660(C-SH), 1622 cm⁻¹(ν C=N),1594 cm⁻¹(ν C=C),1249 cm⁻¹( ν C=S).The ¹H-NMR ( 300 MHz :DMSO-d₆): showed signal bands δppm at 11.45,11.02 (2xs,2x1H,2xNH), 7.07-8.00 (m, 9H,Ar-H), 2.56(t,1H,CH₂CH₃), 2.19(d,2H, CH₂CH₃),1.23(s,3H,CH₃) . MS: 418¹(M-2)( 0.16%), 345(14%), 303(100%), 282(7%) ,238(12%) ,193(11.5%), 152(31.9%), 123(84.4%),78(58%). Anal.C₂₀H₁₈N₄S(418.5) (%) calcd: C, 59.92; H, 4.04; N, 19.97; S, 7.62;CI, 8.44 Found:C, 59.99; H, 4.05; N, 19.95; S, 7.59;Cl, 8.40.

7b as green crystals ; yield 64% :m.p 220°C:IR :showed absorption bands at 3401cm⁻¹(νN-H), 3130, 3069,3004,2980, 2925, 2828cm⁻¹(ν C-H), 1677 cm⁻¹(ν C=O), 1629 cm⁻¹(ν C=N),1594 cm⁻¹(ν C=C).¹H-NMR ( 300 MHz :DMSO-d₆): showed signal bands δppm at 11.44,9.57(2xs,2x1H,2xNH), 6.83-7.88 (m, 9H,Ar-H), 2.51(t,1H,CH₂CH₃), 2.21(d,2H, CH₂CH₃),1.29(s,3H,CH₃) . MS: 406¹(M+1)( 4.0%), 386(50%),364(59%), 272(77%) ,239(37%) ,120(18%), 63(100%). Anal.C₂₀H₁₇N₄OCl(406.5) (%) calcd: C, 62.29; H, 4.20; N, 20.76;Cl, 8.77. Found:C, 62.35; H, 4.22; N, 20.75;Cl, 8.75.

Synthesis of N-((4-(1H-benzo[d]imidazol-2-yl)-6-(5-chloro-3-methyl-1-phenyl-1H-pyrazol-4-yl)-5,6-dihydropyrimidin-2-yl)thio)(p-methoxyphenyl)methyl)-2-nitroaniline (8)

A mixture of 7a(0.01 mol),and Schiff base namelyN-(4-methoxybenzylidene)-2-nitroaniline prepared from refluxing (0.01 mol) of o-nitro aniline and 4- methoxybenzaldehyde in 15 ml of absolute ethanol ) in 30 ml of absolute ethanol was refluxed for 6 hrs. After concentration and cooling the product that obtained was collected, washed well with dilute ethanol followed by crystallization from ethanol to give 8 as dark orange crystals ; yield 76% :m.p 245°C: IR : 3461cm⁻¹(νN-H),3069, 2987, 2921, 2850, 1612 cm⁻¹(ν C=C),1591 cm⁻¹(ν C=C),¹H-NMR( 300 MHz :DMSO-d₆): δppm at 11.39,9.87 (2xs,2x1H,2xNH), 6.88-7.86 (m, 9H,Ar-H), 3.76(s,3H,CH₃),3.37(t,1H,CH₂CH₃), 2.44(d,2H, CH₂CH₃),1.64(s,3H,CH₃) . MS: 675¹(M²-2)( 3.16%), 380(0.06%),321(0.21%), 282(7%), 220(0.12%),193(0.1%), 152(31.9%). Anal.C₂₂H₁₉N₅O₂Cl(675) (%) calcd: C, 62.08; H, 4.28; N, 16.55; S, 4.73;Cl, 5.24 Found:C, 62.11; H, 4.31; N, 16.61; S, 4.76;Cl, 5.25.

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Synthesis of ethyl 2-[(1H-benzo[d]imidazol-2-yl)-4-(5-chloro-3-methyl-1-phenyl-1H-pyrazol-4-yl)-5,6-dihydropyrimidin-2-ylthio)acetohydrazide(11)

A mixture of 7a(0.01 mol), and ethylacetocetate (0.01 mol) in presence of dry aceton containing anhydrous K2CO3 was refluxed for 2 hrs. After evaporation of the excess solvent, the reaction mixture was poured into ice/10%HCl(15 ml) filtered off dried and, crystallized from ethanol to give 9 as dark yellow crystals; yield 66%: m.p 2600°C. IR : 3273cm⁻¹(ν(NH)), 3069, 2921, 2853, cm⁻¹ (ν C-H), 1725 cm⁻¹ (ν C=O ester), 1617 cm⁻¹ (ν C=N). ¹H-NMR(300 MHz, DMSO-d₆): δppm at 9.87 (s,1H,NH), 6.85-8.81 (m, 9H,Ar-H), 3.45 (s,3H,CH₃). 13C-NMR(75 MHz, DMSO-d₆): δppm at 145.04 (C=O amide), 158.6 cm⁻¹ (C=N). MS: 478(M+1) 10%, 465(9.9%) 382(12%), 262(14%), 140(19%), 128(25%), 111(30.1%), 97(36%), 85(26%), 75(39%), 65(41%). Found: C, 57.80; H, 4.26; N, 22.74; S, 6.49; Cl, 7.20.

Synthesis of 2-[(1H-benzo[d]imidazol-2-yl)-6-(5-chloro-3-methyl-1-phenyl-1H-pyrazol-4-yl)-5,6-dihydropyrimidin-2-ylthio)acetic acid(10)

A suspension of 9 in ethanolic sodium hydroxide solution (prepared from dissolving 0.01 mol of sodium hydroxide in 1ml of water then mixed with 15ml of absolute ethanol) was refluxed for 2 hr. then evaporated to half its volume, acidified with dilute HCl. The solid that separated was collected, washed with dilute ethanol and recrystallized from ethanol to give 10 as yellow crystals; yield 61%; m.p 245 °C. IR : 3302,3275cm⁻¹(ν(NH)), 3145, 3025, 2936, 2853 cm⁻¹ (ν C-H), 1710 cm⁻¹ (ν C=O, of an acid), 1605 cm⁻¹ (ν C=O amide), 1586 cm⁻¹ (C=N). ¹H-NMR(300 MHz, DMSO-d₆): δppm at 11.9, 11.6, 11.4 (3xs,4H,2x1H,2xNH, NH₂), 7.2-8.9 (m, 16H,Ar-H), 3.45 (s,2H,CH₂), 2.50(t,3H, CH₃). 13C-NMR(75 MHz, DMSO-d₆): δppm at 162.05 (C=O amide), 155.3 (C=N), 140.3 (C=O). MS: 492(M+1) 4.05%, 360.9(12.1%), 123(11.6%), 118.7(12.2%), 74.9(13.8%), 58(13.8%), and the base peak at 50.5(100%). Anal.C₂₁H₂₃N₃O₅S Cl(492.5) (%) calcd: C, 56.04; H, 4.26; N, 22.49; Cl, 7.20. Found:C, 56.10; H, 4.31; N, 22.69; S, 6.50; Cl, 7.30.

Reaction of 11 with carbonyl compounds :Synthesis of 2-(4-((1H-benzo[d]imidazol-2-yl)-6-(5-chloro-3-methyl-1-phenyl-1H-pyrazol-4-yl)-5,6-dihydropyrimidin-2-ylthio-N(propan-2-ylidene)acetohydrazide(12a)

A suspension of 11(0.01 mol) in dry aceton (30 ml) was heated on a water bath for 6 hrs. After concentration and cooling the residue was collected, washed well with dilute ethanol and recrystallized from ethanol to give 12a as darkorange crystals; yield 71%: m.p 255 °C. IR : 3276 cm⁻¹ (ν(NH)), 3109,3001, 2959, 2926,2845, cm⁻¹ (ν C-H), 1678 cm⁻¹ (ν C=O amide), 1614 cm⁻¹ (ν C=N),1537 cm⁻¹ (ν C=C). ¹H-NMR(300 MHz, DMSO-d₆): δppm at 10.65,10.05 (2xs,2H,2xNH), 6.73-8.72 (m, 16H,Ar-H), 3.29 (s,3H,CH₃). 13C-NMR(75 MHz, DMSO-d₆): δppm at 145.04 (C=O amide), 158.6 cm⁻¹ (C=N). MS: 352.9(M+1) (20.1%), 302.5(10.9%), 195(7.5%), 193(7.3%), 148(25.7%), 127(8.3%), 120(35.9%), 82(5.5%), 77(2.5%), 55(100%). Anal.C₂₁H₂₃N₃O₅S Cl(492.5) (%) calcd: C, 58.58; H, 4.73; N, 21.02; S, 6.49; Cl, 6.02. Found:C, 58.50; H, 4.75; N, 21.04; S, 6.50; Cl, 6.05.

Reaction of 11 with anilidazole :Formation of 42-(4-((1H-benzo[d]imidazol-2-yl)-6-(5-chloro-3-methyl-1-phenyl-1H-pyrazol-4-yl)-5,6-dihydropyrimidin-2-ylthio-N(4-methoxy benzylidene)acetohydrazide(12b)

A mixture of 11(0.01 mol) and anilidazole (0.01 mol) in 30 ml of absolute ethanol containing sodium ethoxide (formed by dissolving 0.4 gm of sodium metal in 5 ml of absolute ethanol) was refluxed for 6 hrs. After concentration and cooling the product was collected, washed well with dilute ethanol and recrystallized from ethanol to give 12b as darkorange crystals; yield 51%: m.p 300 °C. IR : 3396, 2583 cm⁻¹ (ν(NH)), 3111,3050, 2850 cm⁻¹ (ν C-H), 1671 cm⁻¹ (ν C=O amide), 1614 cm⁻¹ (ν C=N),1586 cm⁻¹ (ν C=C). ¹H-NMR(300 MHz, DMSO-d₆): δppm at 10.39,10.02 (2xs, 2H, 2xNH), 7.12-8.87 (m, 13H,Ar-H), 3.29 (s,1H,N=CH₂), 2.51(s,2H,CH₂), 2.70(s,2H,CH₂), 2.50(t,1H,CH₂), 2.30(d,2H,CH₂), 1.06(s,3H,CH₃). MS: 610(M)(10%),465(9.9%) 382(12%), 262(14%), 140(19%), 125(23%) 111(30.1%), 97(36%), 85(26%), 75(39%), 57(100%).
A suspension of 12 (0.01 mol) in 15 ml acetic anhydride was heated on a water bath for 2 hrs. After evaporation of solvent (under reduced pressure), the product was neutralized with dilute HCl, then with dilute ethanol and recrystallized from ethanol to give 14 as green crystals; yield 75%; m.p 220. C 60.93; H 4.42; N 18.21; S 5.31; Cl 5.82.

Reaction of 11 with CS₂ in KOH: Formation of 2-(4-(1H-benzo[d]imidazol-2-yl)-5-(chloro-3-methyl-1-phenyl-1H-pyrazol-4-yl)-5,6-dihydropyrimidin-2(1H)-thione (15)

A suspension of 11 (0.01 mol) in acetone (30 ml) and methanol (15 ml) was treated with 8% aqueous sodium hydroxide (12 ml) while cooling for 2 hrs. After concentration and cooling the product was collected and treated with petroleum ether (b.p. 40-60° C), then with dilute ethanol and recrystallized from ethanol to give 15 as light brown crystals; yield 75%; m.p 250. C 63.40; H 3.96; N 14.79; Cl 9.21; Found: C 63.40; H 3.96; N 14.79; Cl 9.21.

Reaction of 11 with H₂O in alkaline NaOH: Synthesis of 2-(3-(5-chloro-3-methyl-1-phenyl-1H-pyrazol-4-yl)-oxiran-2-yl)-1H-benzo[d]imidazole (16)

A suspension of 3 (0.01 mol) in acetone (30 ml) and methanol (15 ml) was treated with 8% aqueous sodium hydroxide (12 ml) while cooling and stirring then hydrogen peroxide (15 ml, 30%) was added (on portions). The solution was stirred while cooling for 2 hrs., then left side overnight. The product was collected, washed well with dilute ethanol and then recrystallized from ethanol to give 16 as orange crystals; yield 81%; m.p 200°C. IR : 3420 cm⁻¹ (C=O ketone COCH=N), 1598 cm⁻¹ (C=N), 1290 cm⁻¹ (C=S), 1530 cm⁻¹ (M-1) (2.9%), 375 (13.8%), 302 (8.7%), 209 (10.1%), 165 (16%), 121 (46.1%), 106 (31.3%), 91 (45%), 81 (31%), 65 (32.3%), 55 (100%). Anal. C₁₅H₁₂N₂O₂ Cl (392.5) (%) calcld: C, 56.09; H, 3.62; N, 18.94; Cl, 8.63; Found: C, 56.04; H, 3.55; N, 18.91; Cl, 8.58.

Reaction of 15 with hydrazine hydrate: Synthesis of 3-(1H-benzo[d]imidazol-2-yl)-5-(5-chloro-3-methyl-1-phenyl-1H-pyrazol-4-yl)-4,5-dihydro-1H-pyrazol-4-ol (17a) and 4-(1H-benzo[d]imidazol-2-yl)-6-(5-chloro-3-methyl-1-phenyl-1H-pyrazol-4-yl)-5-hydroxy-5,6-dihydropyrimidin-2(1H)-thione (17b)

A mixture of 15 (0.01 mol), hydrazine hydrate (0.01 mol) in absolute ethanol (30 ml) was refluxed for 6 hrs. After concentration and cooling the product was collected and washed well with dilute ethanol and recrystallized from

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The ppm for the aromatic protons and signals of the methyl protons at 1.31, while the signals of medium . Treatment of chalcone molecular ion peaks at 453 and the base peak at m/z 365.

Addition of compound 17a and/or 17b respectively. 17a as yellow crystals; yield 78%: m p 265°C. IR: 3400 cm⁻¹ (C=O), 3300 cm⁻¹ (OH), 3069, 2987, 2921, 2850 cm⁻¹ (C-H). MS: 435(M⁺)(3.69%), 382(5.6%), 328(2%), 213(4%), 192(2%), 186(2%), 157(5%), 113(8.4%), 116(9.4%), 96(1.2%), 77(16.2%), 57(100%). CMR: 78.51. (d, 1H, NH), 11.11 (s, 1H, NH), 6.83-7.98 (m, 9H, Ar-H), 3.41 (d, 1H, -CH-OH), 2.11 (d, 1H, -CH-NH), 1.03 (s, 3H, CH₃). MS: 435(M⁺)(4.11%), 372(4.1%), 214(2.3%), 192(6.7%), 186(7.5%), 96(3.7%), 77(65%), 57(100%). Anal.C₆H₇NO₂Cl(420.5) (%) calcd: C, 57.73; H, 3.89; N, 19.24; S, 7.31; Cl, 8.20.

RESULT AND DISCUSSION
Chemistry

The chalcone derivative (3) was prepared by the conventional method [23,24] from the interaction of the aceto [d] imidazol-2-yl)ethanone (1) with 5-chloro-3-methyl-1-phenyl-1H-pyrazole-4-carboxaldehyde (2) in alkaline ethanolic medium. Treatment of chalcone (3) with hydrazines namely hydrazine hydrate and/or phenyl hydrazine afforded the corresponding pyrazoline derivatives (4a,4b) while treatment of (3) with thiosemicarbazide afforded the corresponding 3-[(1H-benzo[d]imidazol-2-yl)-5-(5-chloro-3-methyl-1-phenyl-1H-pyrazol-4-yl)-4,5-dihydro-1H-pyrazole-1-carbothioamide (5). The assigned structures for (3), (4a,b) and (5) were confirmed on the basis of IR, H-NMR and mass spectral analysis. The IR spectra of (4a,b) showed bands in the region 3126-2860 cm⁻¹ for C=H, in the region 1626-1613 cm⁻¹ for C=N and in the region 1599-1601 cm⁻¹ for C=O. The 1H-NMR for (4a) showed the signals of NH as two signlets at δ 11.2, 10.59, the multiplet at 6.88-7.86 ppm for the aromatic protons and signals of the methyl protons at 1.31, while the signals of (4b) were signal at δ 11.2 ppm as signlet for NH, at 6.12-8.7 ppm for the 9 aromatic protons and methyl protons appeared as singlet at 1.46. The mass spectra of (4a) and (4b) showed the molecular ion peaks at m/z 375 and m/z 453 which are agreed well with the proposed structure. The IR spectrum of (5) revealed absorption bands at the region 3399, 3232 cm⁻¹ for NH, in the region 1612-1601 cm⁻¹ for C=N and in the region 1597 cm⁻¹ for C=O. The 1H-NMR revealed two singlets at δ 12.2, 11.2 for the NH, a multiplet at 6.8-8.5 ppm for the aromatic protons and singlet at 1.2 for the methyl protons. The mass spectrum showed the molecular ion peaks at 435 and the base peak at m/z 365.

Treatment of the chalcone (3) with hydroxyl amine hydrochloride affected 1,3 cyclocondensation to the corresponding 3,5 diaryl-1,2-oxazine derivative (6). The IR spectrum of (6) revealed absorption bands at the region 3376, 3130-2880 cm⁻¹ characteristic for NH and CH=O respectively. The 1H-NMR showed signals at δ 11.44 for the NH, a multiplet at 6.85-7.87 ppm for the 9 aromatic protons and singlet at 1.23 for the methyl protons. The chalcone derivative (3) on the treatment with thiourea and/or urea in refluxing ethanol afforded the corresponding 4,6-diaryl-5,6-dihydro pyrimidin-2(1H)-thione (7a) or its pyrimidin-2(1H)-one derivative (7b) respectively. The structures of compounds (7a,7b) were elucidated on the basis of elemental analysis as well as spectral data. The IR spectrum of (7a) showed bands in the region 3402, 3333 cm⁻¹ for NH, in the region 3160-2926 cm⁻¹ for C=H, in the region 1622, 1594 cm⁻¹ for C=N and a characteristic absorption band at 1249 for C=S. The 1H-NMR showed signals at δ 11.2, 11.02 for the 9 aromatic protons and a singlet of the methyl protons at 1.23 ppm. The mass spectrum of (7a) showed the molecular ion peaks at m/z 406 for M+1⁺ and the base peak at m/z 63.

Addition of compound (7a) to a Schiff base in the presence of sodium ethoxide affected alkylation of the active olefinic bond of the Schiff base to give the corresponding α-aryl amino benzylidene thiopyrimidine derivative (8).
The mass spectrum showed the molecular ion peaks at m/z 675 for M-2 for the spectrum of chloro-3-methyl-1-phenyl-1H-pyrazol-4-yl)-4,5-dihydropyrimidin-2-ylthio)acetate diaryl pyrimidin-2-yl thio-N-arylidene acetohydrazide.

Alkylation of (7a) with ethylchloroacetate in dry acetone containing anhydrous potassium carbonate occurred at the thiene group only and not in the NH group to give the corresponding ethyl 2-((6-(1H-benzol[d]imidazol-2-yl)-4-(5-chloro-3-methyl-1-phenyl-1H-pyrazol-4-yl)-4,5-dihydropyrimidin-2-ylthio)acetate(9). This was in agreement with the previous findings [25-27]. The IR spectrum of (9) showed absorption bands at 3273 cm⁻¹ for NH, in the region 3090-2853 cm⁻¹ for νC=H, at 1725 cm⁻¹ for νC=O ester, and 1617 cm⁻¹ for νC=N. The mass spectrum showed the molecular ion peaks at m/z 506 for M-1. The ¹H-NMR reveled a multiplet at 7.2-8.8 ppm for the 9 aromatic protons and three singlets at 9.67, 4.31 and 1.06 due to NH, COCH₂ and CH₃ protons respectively.

Refluxing (9) in alcoholic sodium hydroxide gave the corresponding thioacetic acid (10). The IR spectrum of (10) showed absorption bands at 3420 cm⁻¹ for νOH, 3363 cm⁻¹ for νNH, in the region 3090-2850 cm⁻¹ for νC=H at 1710 cm⁻¹ for νC=O acid, and 1605 cm⁻¹ for νC=N. The mass spectrum showed the molecular ion peak at m/z 478 for M-1. The ¹H-NMR revealed a multiplet at 7.21-8.87 ppm for the aromatic protons and four singlets at 1.03, 2.50, 9.67 and 10.39 due to CH₃, COCH₂, NH and OH protons respectively. Moreover, refluxing (9) with hydrazine hydrate in ethanol afforded the corresponding 5,6-dihydropyrimidin-2-thioacetohydrazide derivative (11). The IR spectrum of (11) showed absorption bands at 3300, 3275, 3178, 2851, 1667 and 1617 cm⁻¹ due to stretching absorption bands of νNH₂, νNH, νC=H, νC=O, and νC=N respectively. The ¹H-NMR showed the following signals at 11.9, 11.16 and 8.9 ppm for NH₂, and two NH 7.2-8.9 ppm respectively.

The reaction of the hydrazine derivative (11) with carbonyl compounds was investigated. Thus, (11) reacted with acetone and/or anisaldehyde to give the hydrazide derivatives (12a) and/or (12b). The IR spectrum of (12a) showed absorption bands at 3300, 3003-2850, 1655 and 1605 cm⁻¹ due to absorption bands of νNH, νC=H, νC=O, and νC=N respectively. The ¹H-NMR showed signals at 6.10, 8.10, 28 ppm corresponding to the 9 aromatic protons and at 1.34, 1.36 ppm due to two singlets for the protons of the two methyl groups. The IR spectrum of (12b) showed absorption bands at 3252, 3396 cm⁻¹ for νOH, 1614 cm⁻¹ for νC=N. On the other hand, interaction of compound 4, 6-diaryl pyrimidin-2-ylthio-N-arylidene acetohydrazide (12b) with acetic anhydride affected rearrangement, followed by cyclization to the corresponding 1,3,4-oxadiazole derivative (13). The IR spectrum of (13) showed absorption bands at 3204 cm⁻¹ for νNH, in 1713 cm⁻¹ for νC=O ketone, and 1613 cm⁻¹ for νC=N. The ¹H-NMR of (13) revealed the NH protons as a singlet at 11.68 ppm, a multiplet at 7.2-8.9 ppm for the aromatic protons, methoxy protons COCH₃, and as singlet at 3.45, 1.91, 1.03 respectively. On the other hand, treatment of the hydrazine derivative (11) with carbon disulphide in the presence of aqueous potassium hydroxide affected cyclization to the corresponding 1,3,4-oxadiazole-5-thione derivative (14). The IR spectrum of (14) agreed well with the proposed structure, it revealed absorption bands at 3394, 3001-2850, 1667 and 1627, 1190 cm⁻¹ due to νOH, νC=H, νC=N, and νC=O respectively. The ¹H-NMR of (14) showed signals at 8.89, 8.77 as two singlets for the two NH cyclic, a singlet at 2.51 for –SCH₂, a multiplet at 6.68-8.03 ppm for the 9 aromatic protons. The mass spectrum of (14) showed the molecular ion peaks at m/z 534 for M⁺ and the base peak m/z 55 for CH₃N⁺N⁻.

The reaction of chalcone (3) with hydrogen peroxide in alkaline medium affected the formation of the corresponding oxirane derivative (15). This was in agreement with the previous findings [29]. The IR spectrum of (15) revealed the presence of stretching absorption bands of νOH, νC=H, νC=O, νC=N, and νC=Cl at 3235, 3107-2820, 1690, 1598, 608 cm⁻¹ and the epoxy linkage at 1290 cm⁻¹. The ¹H-NMR showed signals at 11.42 as singlet for the NH, 6.88-7.87 as multiplet for the aromatic protons, the system of the oxirane ring as a doublet at 3.89-4.01 ppm. The mass spectrum of (15) showed the molecular ion peaks at m/z 378 for M⁺ and the base peak m/z 57 for CH₃CH₂N⁻N₂.

The reaction of (15) with hydrazine hydrate in refluxing ethanol yielded the corresponding 4-hydroxy pyrazol derivative. The IR spectrum of (16) revealed the presence of absorption bands of νOH, νNH, νC=H, νC=O, and νC=Cl at 3420, 3265, 3200, 3061-2852, 1612 and 1530 cm⁻¹ respectively. The ¹H-NMR showed signals at 12.43 as a singlet for the OH and a doublet for the NH at the pyrazole ring and at 11.02 ppm a singlet for the cyclic NH, 6.88-
The reaction of (15) with urea and/or thiourea in refluxing ethanol affected the cyclization to the corresponding 4-hydroxy pyrimidin-2-one (17a) and/or 4-hydroxy pyrimidin-2-thione (17b) via opening of the epoxide ring followed by recyclization. The IR spectrum of (17a) revealed the presence of absorption bands at 3400, 3330, 3069-2850, 1655, 1612 and 1603 cm⁻¹ corresponding to νOH, νNH, νC-H, νC=O (amide), νC=N, and νC=S respectively. The ¹H-NMR showed signals at 12.49, 12.45, 11.43 as two doublet and a singlet, 6.88-7.86 as multiplet for the aromatic protons. The mass spectrum of (17a) showed the molecular ion peaks at m/z 423 for M+2, and the base peak m/z 57 for C₂H₅N₂⁺. The IR spectrum of (17b) revealed the presence of absorption bands at 3353 cm⁻¹ for νNH, 3069-2820 for νC-H, 1615 for νC=N and 1249 cm⁻¹ for νC=S respectively. The ¹H-NMR showed signals at 12.05, 11.11 as two singlets, 6.83-7.93 as multiplet for the aromatic protons. The mass spectrum of (17b) showed the molecular ion peaks at m/z 435 for M⁻, and the base peak m/z 57 for C₂H₅N₂⁻.

Antioxidant Testing:
The newly prepared compounds 4a, 7a, 15 are tested for antioxidant property by DPPH method.

Reduction of 1,1-diphenyl-2-picrylhydrazyl (DPPH) Free Radical (DPPH method) The nitrogen centered stable free radical DPPH has often been used to characterize antioxidant. It is reversibly reduced and the odd electron in the DPPH free radical gives strong absorption maximum at 517 nm which is purple in color. This property makes it suitable for spectrophotometric studies. A radical scavenging antioxidant reacts with DPPH stable free radical and converts into 1,1-diphenyl-2-picrylhydrazine. The resulting decolorization is stoichiometric with respect to the number of electrons captured. The change in the absorbance produced by this reaction has been used to measure the antioxidant properties of the tested newly derivatives. The solutions of the tested compounds (in ethanol) (100µM) were added to DPPH (100µM, in ethanol). The tubes were kept at an ambient temperature for 25 minutes and the absorbance was measured at 517 nm. The difference between the test and the control experiments (Ascorbic acid) was taken and expressed as the percentage scavenging of the DPPH radical.

Table (1): Antioxidant Activity of the Target Compounds

<table>
<thead>
<tr>
<th>Sample conc. µg</th>
<th>DPPH scavenging % inhibition at 100µM</th>
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<tbody>
<tr>
<td></td>
<td>Tested compounds</td>
</tr>
<tr>
<td></td>
<td>4a 17a 15 Sample conc. µg DPPH scavenging %</td>
</tr>
<tr>
<td>640</td>
<td>82.74 85.26 78.53 - -</td>
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<tr>
<td>320</td>
<td>74.53 77.05 76.84 35 80.21</td>
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<td>22.32 30.11 22.00 5 12.98</td>
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C = 100 µM

It is clear from the results that compounds 7a, 15 exhibit significant antioxidant property in DPPH method at 100 µM concentration (Table) when compared with the standard reference Ascorbic acid.
Cytotoxicity Evaluation

Some of the newly prepared compounds were tested to determine their cytotoxicity in the human hepatocellular liver carcinoma cell line HEPG-2, against breast carcinoma cells MCF-7 cell line and against colon carcinoma cell lines HCT, using a modified method \[27\]. Thus, compounds 6,7b,11,12b were tested against HEPG-2, compounds 4a,8,16b,17a against MCF-7 and compounds 5,9,13 and 14 against HCT cell line respectively. The cells were routinely cultured in Eagle’s minimum essential media supplemented with 10% foetal bovine serum, 1% L-glutamine solution, and a non-essential amino acid solution. The investigated compounds were dissolved in a very small amount of DMSO, and a small volume was added to the cell culture. The tested compounds were prepared in triplicates at concentration ranging from 1.56 to 50 µg. The following types of controls were included: determination of 100% viability and 0% viability (the cells were treated with 10% DMSO), no cell control, control for the determination of a possible interaction of the tested compounds with the reagent, control of the setting incubation medium and control of the toxicity of DMSO. The results are expressed as inhibitory concentration that reduces the cell viability to 50% of the maximal (control) viability (IC\(_{50}\)). The results (Table 2) showed that compound 4a was inactive toward the breast cancer cells (MCF-7) (> 50), while compounds 8,16b,17a exhibited cytotoxic activity against MCF-7 with IC\(_{50}\) of 2.4, 30.8,2.6 µg/ml. Compounds 6,7b,11,12b exerted activity against HEPG-2 cancer cells with IC\(_{50}\) of 33,19.3,33.8 respectively. Compounds 5,9,13,4 exhibited cytotoxic activity against HCT cancer cells with IC\(_{50}\) of 9.1,5,0.3,9.7,7 µg/ml. It is notable that 8 and 17a are the most potent cytotoxic agents against MCF-7 with IC\(_{50}\) of 2.4,2.6 µg/ml, while 11,7b are the most potent against HEPG-2 with IC\(_{50}\) of 9.1,9.3 µg/ml and compound 13,9,14 and 15 the most potent against HCT with IC\(_{50}\) of 3.9,8,0.7 and 9.1 µg/ml. This is presumably due to a high lipophilicity of the benzimidazole moiety which enhances its absorption to the
cancer cells. However, cytotoxic activity of 4a,17a has not reported in the literature. Hence, the benzimidazole analogs 8,17a,11,7b,5,9,13,14 are new series of cytotoxic agents.

Table (2): Cytotoxic activity of benzimidazole derivatives (4a,5,6,7b,8,9,12b,13,14,16b)

<table>
<thead>
<tr>
<th>Cell lines</th>
<th>IC50 (µg/ml)</th>
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<tbody>
<tr>
<td></td>
<td>4a 5 6 7b 8 9 11 12b 13 14 16b 17a</td>
</tr>
<tr>
<td>MCF-7</td>
<td>&gt;50 NT NT 2.4 NT NT NT NT NT 30.8 2.6</td>
</tr>
<tr>
<td>Hep G-2</td>
<td>NT NT 33 19.3 NT NT 9.0 33.8 NT NT NT NT</td>
</tr>
<tr>
<td>HCT</td>
<td>NT 9.1 NT NT NT 5.0 NT NT 3.9 7.7 NT NT</td>
</tr>
</tbody>
</table>

NT: indicates not tested

- Cancer cell lines were hepatocellular carcinoma cell line (Hep G-2); colon carcinoma cell line (HCT); breast carcinoma cell line (MCF-7).
- When IC50 > 50 µg/ml denotes inactive compound.
- The assays were performed in triplicate.
Structure – activity relationship

According to the results of the bio activities, it is noted that whereas compound 7b displays the highest antioxidant, it also displayed cytotoxic activity against HEPG-2 cell. This is presumably due to a hydrophobic effect of sterically hindered benzimidazolo-thiopyrimidine group that enhances the penetration of compound 7b to the cancer cell.

CONCLUSION

The synthesis of target novel chalcone (3), pyrazolo(4a,b,5), isoxazole(6), pyrimidines (6-14), oxirane (15), hydroxyl pyrazolines (16), and hydroxyl pyrimidines (17a,b) was achieved according to the steps indicated. These reactions are simple, easily carried under normal reaction conditions. Most of the newly synthesized substituents were found to exhibit significant activity as antioxidant or anticancer agents. The findings demonstrate a new potential for some derivatives as lead compounds for further development as medicinal agents.
REFERENCES