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Synthesis, reactions, structure-activity relationship of 2-benzimidazole analogs as anticancer agents and study their molecular docking

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ABSTRACT

*A new series of benzimidazolederivatives ,**1,2,3** are reported herein. Their reactions with some nucleophiles gave **4** and **6.**The reaction of **3** with sodium azide gave **5**and with primary amines gave**7a-c.** Treatment of **7c** with acrylonitrile, cinnamaldehyde, phenylisothiocyanate, gave **8,9**and **10.** Treatment of **10** with chloroacetic acid and/or malonic acid gave **11** and **12**. The benzimidazol-2-thione derivative **2** reacted with acetylacetone to give the dicarbonyl derivative **13** which on hydrazinolysis gave the pyrazole derivative **14.** Reaction of **2** with benzalacetophenone derivative, anthranilic acid, sodium nitrite and/or thiourea gave **15,16,17**and **18** respectively. The formation of the 2-benzimidazolyl pyrimidine thione derivative **19** was also investigated. Some of the new synthesized derivatives were screened for their antitumor activities and theirmolecular docking and the results were encouraging.*

Keywords: Benzimidazolederivatives , Biological activity , Synthesis, Antitumor activity

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INTRODUCTION

Benzimidazole derivatives represent one of the most biologically active classof compounds, possessing a wide spectrum of activities and these are well-documented in literature asthey show selective neuropeptides YY, receptor antagonists**[1]**, potent inhibitors of TiE-2 and VEGFER_2 tyrosine kinase receptor**[2]**, gamma-amino butyric acid (GABA) agonists and 5-HT3 antagonists**[3]**. They have been showing promising activities in the treatment of several diseases like epilepsy, diabetes, and antifertility**[4]**. For these reasons, they gained much attention as important pharmacophore and privileged structure in medicinal chemistry**[5]** encompassing a diverse range of biological activities**[6]**. The benzimidazole derivatives have found commercial application in veterinarian medicine as anthelmintic agents and diverse human therapeutic areas**[7].**

Structures containing benzimidazole moiety, well-known to have a wide range of biological properties, have commercial applications in various realms of therapy, including antiulcerative, anti-hypertensive, antiviral, antifungal, anti-tumorand antihistaminic agents, and antihelminthic agents in veterinary medicine**[8]**. Furthermore, these heterocycles are considered to be privileged structures by medicinal chemists.

The preparation of benzimidazoles has gained considerable attention in recent years. Thebenzimidazole is found in a variety of naturally occurring compounds and is of significantimportance in medicinal chemistry**[9]**.

The present investigation deals with synthesis of some benzimidazol derivatives, study some of their behavior towards some nucleophilic as well as electrophilic reagents in the hope of obtaining new derivatives of biological interest and potential target compounds. The substituted benzimidazoles were tested for their cytotoxic activity and their structure activity relationship were examined.

MATERIALS AND METHODS

2.1.Chemistry

All melting points are uncorrected. IR spectra (KBr) were recorded with a Perkin Elmer Spectrum RXIFT-IR system. ^{1}HNMR were measured with a Varian Gemini 200 MHz instrument using TMS as internal standard and mass spectra were measured with a Shimadzu GC–MS–QP 100 EX mass spectrometer.

2.2.General procedure preparation of compounds

*2.2.1.Synthesis of 1H-benzo[d]imidazol-2(3H)-one**1** and 1H-benzo[d]imidazole-2(3H)-thione**2***

Both of 1H-benzo[d]imidazol-2(3H)-one**1** and 1H-benzo[d]imidazole-2(3H)-thione**2** were prepared according to L.Srikanthet.al**[10].**

Analysis of **1** $C_7H_6N_2O$(134)(%)calcd: C 62.68 , H 4.47 , N 20.89. Found: C 62.70 , H 4.50 , N 20.90.
Analysis of **2** $C_7H_6N_2S$(150)(%)calcd: C 56.00 , H 4.00 , N 18.66. Found: C 56.02 , H 4.02 , N 18.70.

*2.2.1.1.Synthesis of 2-chloro-1H-benzo[d]imidazole**3***

A mixture of **1** (0.01 mol), phosphorus oxychloride (3 ml) and phosphorus pentachloride (0.5 gm) was heated on a steam bath for 2 hours. It was poured into crushed ice and the solid that separated was filtered off, washed well and crystallized from ethanol as yellowish white crystals **3**of60% yield and m.p. 147˚C.

Analysis of **3** $C_7H_5N_2Cl$ (152.5)(%) calcd: C 55.08 , H 3.28 , N 18.36 , Cl 23.28 . Found: C 55.16 , H 3.32 , N 18.43 , Cl 23.32

*2.2.1.1.1.Synthesis of 2-hydrazinyl-1H-benzo[d]imidazole**4***

A mixture of **3** (0.01 mol) and hydrazine hydrate (0.01 mol) in 30 ml of ethanol was refluxed for 6 hours. After concentration and cooling the solid obtained was crystallized from ethanol white crystals **4**of 75%yield andm.p 190 ˚C.

Analysis of **4** $C_7H_8N_4$ (148)(%) calcd: C 56.76 , H 5.41 , N 37.84 . Found: C 56.82 , H 5.47 , N 37.91. Found: C 56.82 , H 5.38 , N 37.90

*2.2.1.1.2.Synthesis of[1,2-d]tetrazol-4H-benzimidazole**5***

A mixture of **3** (0.01mol) , sodium azide (0.02 mol,1.3gm)dissolved in 5 ml of water and dimethylformamide (20 ml) was refluxed for 2 hours and cooled. The solid obtained upon dilution with water was filtered off and recrystallized from ethanol to give **5** as greenish crystals of 60% yield and m.p178 ˚C.

Analysis of **5**$C_7H_5N_5$ (159)(%) calcd: C 52.83 , H 3.14 , N 44.02. Found: C 52.90 , H 3.00 , N 44.00

*2.2.1.1.3.Synthesisof4-(1H-benzo[d]imidazol-2-ylamino) – N-(4-aminophenyl)benzenesulfonamide**6***

A mixture of **3** (0.01 mol) and sulphaniline (0.01 mol) in ethanol (50 ml) was refluxed for 10 hours , the solid product obtained after evaporating the solvent was crystallized from ethanol to give **6** as orange crystals of 85% yield and m.p 276 ˚C.

Analysis of**6**$C_{19}H_{17}N_5SO_2$ (379)(%) calcd: C 60.14 , H 4.52 , N 18.46 , S 8.45. Found: C 60.00 , H 4.48 , N 18.52 , S 8.53

*2.2.1.1.4.Reaction of 2-chloro-1H-benzo[d]imidazole**3** with amines: Formation of **7 a-c***

A mixture of **3** (0.01 mol) anddifferent amines namely 2-amino pyridine , benzyl amine and/or p-phenylenediamine (0.01 mol) in (30 ml) ethanol was refluxed for 6 hours. After concentration and cooling the solid obtained was crystallized from ethanol to give N-(pyridin-2-yl)-1H-benzo[d]imidazol-2-amine**7a** as white crystals of 60% yield

and m.p 324 ˚C, N-benzyl-1H-benzo[d]imidazol-2-amine **7b** as yellowish crystals of 65% yield and m.p 300 ˚C and N^1-(1H-benzo[d]imidazol-2-yl)benzene-1,4-diamine **7c** as brownish crystals of 75% yield and m.p 297 ˚C.

Analysis of **7a** $C_{12}H_{10}N_4$ (210)(%) calcd: C 68.57 , H 4.76 , N 26.66. Found: C 68.60 , H 4.80 , N 26.70. Analysis of**7b** $C_{14}H_{13}N_3$ (223)(%) calcd: C 75.33 , H 5.82 , N 18.83. Found: C 75.00 , H 5.78 , N 18.80. Analysis of**7c** $C_{13}H_{12}N_4$ (224)(%) calcd: C 69.64 , H 5.35 , N 25.00. Found: C 69.60 , H 5.80 , N 25.30

*2.2.1.1.4.1. Reaction of 7c with acrylonitrile: Formation ofN-(4-(1H-pyrazol-1-yl)phenyl)-1H-benzo[d]imidazol-2-amine **8***

To (0.01 mol) of **7c**wasadded (0.02 mol) of acrylonitrile in 20 ml pyridine , the mixture was heated under reflux for 6 hours. After cooling , it was poured into ice and HCl mixture and the product that separated was filtered off , washed well with water , dried and recrystallized from ethanol to give **8** as brown crystals of 60% yield and m.p 218 ˚C.

Analysis of **8** $C_{16}H_{13}N_5$ (275)(%) calcd: C 69.81 , H 4.72 , N 25.45. Found: C 69.84 , H 4.69 , N 25.42

*2.2.1.1.4.2.Reaction of 7c with cinnamaldehyde: Formation of (N^4E)-N^1-(1H-benzo[d]imidazol-2-yl)-N^4-(3-phenylallylidene)benzene-1,4-diamine asShiff's base **9***

A mixture (0.01 mol) of **7c** in (30 ml) absolute ethyl alcohol and (0.05 mol) of sodium ethoxide (prepared by dissolving sodium metal (0.02 mol) in 20 ml of absolute ethanol) and cinnamaldehyde (0.01 mol) wasstirrered for 2 hours. Filter the precipitate and recrystallize it from ethanol as yellowish powder **9** in 48% yield and m.p 205˚C.

Analysis of **9** $C_{22}H_{18}N_4$ (338)(%) calcd: C 78.10 , H 5.32 , N 16.56. Found: C 78.09 , H 5.29 N 16.52

*2.2.1.1.4.3.Reaction of 7c with phenylisothiocyanate: Formation of 1-(4-(1H-benzo[d]imidazol-2-ylamino)phenyl)-3-phenylthiourea **10***

A mixture of **7c** (0.01 mol) and phenylisothiocyanate (0.013 mol) in 20 ml of pyridine was refluxed for 6 hours cooled , then poured into ice – HCl mixture. The solid that separated was filtered off , washed well with water and recrystallized from ethanol to give **10** as yellow crystals in 80% yield and m.p 203˚C.

Analysis of **10**$C_{20}H_{17}N_5S$ (359)(%) calcd: C66.85 , H 4.73 , N 19.49 , S 8.91. Found: C 66.90 , H 4.70 , N 19.52 , S 9.00

*2.2.1.1.4.3.1.Reaction of 10 with chloroacetic acid: Formation of N-(3-(4-(1H-benzo[d]imidazol-2-ylamino)phenyl)-4-oxothiazolidin-2-yl)benzamide**11***

A mixture of **10** (0.01 mol) ,chloroacetic acid (0.03 mol) and dry acetone (50 ml) in the presence of anhydrous potassium carbonate (0.03 mol) was heatedon a water bath for 24 hours. After filtration while hot and removing the excess of solvent , the separated product was recrystallized from ethanol to give **11** as yellow crystals in 87% yield and m.p318 ˚C.

Analysis of **11**$C_{23}H_{19}N_5O_2S$ (429)(%) calcd: C 64.33, H 4.42 , N 16.31 , S7.45. Found: C 64.30 , H 4.35 , N 16.29 , S 7.50

*2.2.1.1.4.3.2.Reaction of 10 with malonic acid: Formation of 1-(4-(1H-benzo[d]imidazol-2-ylamino)phenyl)-3-phenyl-2-thioxodihydropyrimidine-4,6(1H,5H)-dione**12***

A mixture of **10** (0.01 mol) and malonic acid (0.01 mol) was heated on a steam – bath for 2 hours, then left to cool and poured onto ice while stirring . The product was collected and recrystallized from ethanol as white crystals **12**of 60% yield and m.p267 ˚C.

Analysis of **12** $C_{23}H_{17}N_5O_2S$ (427)(%) calcd: C 64.63 , H 3.98 , N 16.39 , S 7.49. Found: C 64.68 , H 4.00 , N 16.46 , S 7.52

2.2.2.Reaction of 2 with acetylacetone: Formation of 3-(1H-benzo[d]imidazol-2(3H)-ylidene)pentane-2,4-dione ***13***
To a solution of **2** (0.01 mol) in ethanol (50 ml) , acetyl acetone (0.01 mol) was added and the reaction mixture was refluxed for 6 hours. The solid product obtained after concentration and cooling was crystallized from ethanol to give **13** as pale white ppt .in 75% yield and m.p 176 ˚C.

Analysis of **13** $C_{12}H_{12}N_2O_2$ (216)(%) calcd: C 66.66 , H 5.55 , N 12.96. Found: C 66.70 , H 5.60 , N 13.00

2.2.2.1.Reaction of 13 with hydrazine hydrate: Formation of 2-(3,5-dimethyl-4H-pyrazol-4-ylidene)-2,3-dihydro-1H-benzo[d]imidazole ***14***
To a solution of **13** (0.01 mol) in ethanol (50 ml), was added hydrazine hydrate (0.01 mol) and the reaction mixture was refluxed for 6 hours. The solid product obtained after cooling was recrystallized from ethanol to give **14** as white crystals in 70% yield and m.p300 ˚C.

Analysis of **14** $C_{12}H_{12}N_4$ (212)(%) calcd: C 67.92 , H 5.66 , N 26.41. Found: C 68.00 , H 5.70 , N 26.00

*2.2.2.2.Reaction of 2 with benzalacetophenone: Formation of 3-(1H-benzo[d]imidazol-2-ylthio)-1,3-diphenylpropan-1-one****15***
To a solution of **2** (0.01 mol) in ethanol (50 ml) was addedbenzalacetophenone (0.01 mol) and the reaction mixture was refluxed for 6 hours. The solid product obtained after concentration and cooling was crystallized from ethanol to give **15** as yellow crystals in 65% yield and m.p 277 ˚C.

Analysis of **15** $C_{22}H_{18}N_2OS$ (358)(%) calcd: C 73.74 , H 5.02 , N 7.82 , S 8.93. Found: C 73.82 , H 5.00 , N 8.00 , S 9.00

*2.2.2.3.Reaction of 2 with anthranilic acid: Formation of 2-(1H-benzo[d]imidazol-2-yl)-3H-indol-3-one****16***
A mixture of **2** (0.01 mol) and anthranilic acid (0.01 mol)was heated in (30 ml)butanol under reflux for 6 hours, the product obtained after cooling and evaporation of excess solvent under reduced pressure was crystallized from ethanol to give **16** as dark brown ppt of 62% yield and m.p 303 ˚C.

Analysis of **16** $C_{14}H_{11}N_3O$ (237)(%) calcd: C 70.87 , H 4.64 , N 17.72. Found: C 71.00 , H 4.60 , N 17.70

*2.2.2.4.Reaction of 2 with sodium nitrite: Formation of1,2-di(1H-benzo[d]imidazol-2-yl)disulfane****17***
A mixture of **2** (0.01 mol) in ethanol (20 ml), sodium nitrite (0.01 mol) and acetic acid (3 ml) was stirred at room temperature for 4 hours. The solid product which separated out was crystallized from ethanol to give **17** as brownish ppt. in 50% yield and m.p 273 ˚C.

Analysis of **17**$C_{14}H_{10}N_4S_2$ (298)(%) calcd: C 56.37 , H 3.35 , N 18.79 , S 21.47. Found: C 56.42 , H 3.40 , N 19.00 , S 21.50

2.2.2.5.Reaction of 2 with thiourea: Formation of 1-(1H-benzo[d]imidazol-2-yl)thiourea ***18***
To a solution of**2** (0.01 mol) in dimethylformamide (30 ml) was added thiourea (0.01 mol) and the reaction mixture was refluxed for 16 hours. The solid product obtained after cooling was crystallized from ethanol as pale yellow crystals **18** in 80%yield withm.p 188 ˚C.

Analysisof**18** $C_8H_8N_4S$ (192)(%) calcd: C 50.00 , H 4.16 , N 29.16 , S 16.66. Found: C 49.85 , H 4.20 , N 29.30 , S 17.00

2.2.2.5.1. Reaction of 18 with malonic acid: Formation of 1-acetyl-3-(1H-benzo[d]imidazol-2-yl)-2-thioxodihydropyrimidine-4,6(1H,5H)-dione ***19***
A mixture of **18** (0.01 mol) , malonic acid (0.01 mol) and acetyl chloride (1 ml) was heated on a steam – bath for 2 hours, left to cool then poured onto ice while stirring . The product was collected and recrystallized from ethanol as white crystals**19**of 60% yield and m.p 278 ˚C.

Analysis of **19** $C_{13}H_{10}N_4O_3S$ (302)(%) calcd: C 51.65 , H 3.31 , N 18.54. Found: C 51.70, H 3.29 , N 18.50

RESULTS AND DISCUSSION

The new derivatives were prepared following the reaction sequences depicted in Schemes **1** and **2**.

Thus benzimidazole derivatives **1** and **2** which are prepared according to the literature**[10].** These compounds werefurther proved by spectroscopic analysis . Compound **1** under infrared spectrum showed absorption bands at 1708 cm^{-1} , 1627.8 cm^{-1} , 2806.4 cm^{-1}- 3023.2 cm^{-1} and 3200 cm^{-1} attributable to √ C=O , √ C=N , √ C-H and √ NH. ^{13}C-NMR showed signals at δ ppm, 155.1 , 129.5 , 120.3 and 108.3 corresponding to C=O , C-N , CH-C and CH-CH Compound **2** showed absorption bands of infrared spectrum at 1209.4 cm^{-1} , 1596.9 cm^{-1} , 2808.9-3026.2 cm^{-1} and 3205.09 cm^{-1} attributable to √ C=S , √ C=N , √ C-H and √ NH. The ^{1}H-NMR spectrum showed signals δppm at 6.39-7.50(m,4H,Ar-H), 9.20(s,1H,NH) and 9.34(s,1H,NH). The mass spectrum of compound **2** showed the molecular ion peak at m/z 150 (1.35%) and main ion peak m/z 53 (100%).

The behavior of compound **1** towards nucleophilic reagent like $POCl_3$ gave 2-chloro-1H-benzo[d]imidazole **3** which showed absorption bands of infrared spectrum at 1624.8 cm^{-1} , 1577.2 cm^{-1} , 2850-3057.4 cm^{-1} and 3150 cm^{-1} attributable to √ C=N , √ C=C , √ C-H and √ NH. The ^{1}H-NMR showed signal bands at δppm 6.91-8.51(m,4H,Ar-H) and 10.56(s,1H,NH). The mass spectrum of **3** showed molecular ion peak at m/z 79 (100%)and m/z 154 (3.91%).

Reaction of 2-chloro-1H-benzo[d]imidazole **3** with hydrazine hydrate in ethanol gave 2-hydrazinyl-1H-benzo[d]imidazole **4**. The infrared spectrum of **4** showed absorption bands at 1614.4 cm^{-1} , 1576.4 cm^{-1} , 2849.7-3010 cm^{-1} , 3200 cm^{-1} and 3310 cm^{-1} attributable to √ C=N , √ C=C , √ C-H , √ NH and √ NH_2. ^{13}C-NMR(DMSO-d_6) of **4** showed signals at δ ppm , 141 138 , 136 , 123 , 125 , 121.6 and 119.1 attributable to N=C-NH , C-NH , C-NH , CH-C-N , CH-CNH , CH=CH and CH=CH

A large number of tetrazole derivatives are reported to exhibit antifungal**[11]** , antiproliferative , antimicrobial**[12]** activities and as antiglacomaophthalmicagents**[13]**. This promoted the author to synthesize a new tetrazolobenzimidazole derivative through the reaction of **3** with sodium azide to give tetrazolobenzimidazole derivative **5**. The infrared spectrum of **5** showed absorption bands at 1624.9 cm^{-1} , 1576.6 cm^{-1} ,2851.4-3056.4 cm^{-1} and 3200 cm^{-1} attributable to √ C=N , √ C=C , √ C-H and √ VH. The ^{1}H-NMR showed signal bands δppm at 12.93(s,1H,NH) and 6.91-8.53(m,4H,Ar-H). The mass spectrum of **5** showed molecular ion peak at m/z 55 (100%) and m/z 156 (3.04%)

Similarly,the reaction of 2- chloro-1H-benzo [d] imidazole **3** with sulphaniline gave 4-(1H-benzo[d]imidazole-2-ylamino)-N-(4-aminophenyl)benzene sulfonamide **6.** The infrared spectrum of **6** displayed absorption bands at 1625.7 cm^{-1} , 1576.4 cm^{-1} , 2849.7-3050 cm^{-1} , 3357.2 cm^{-1} , 3261.6 cm^{-1} and 2615 cm^{-1} attributable to √ C=N , √ C=C , √ C-H , √ NH_2 , √ NH and √ S=O. ^{13}C-NMRof**6** showed signals δ ppm at 151.8 , 130.05 , 127.3 , 125.08 , 112.3-123.9 attributable to N=C-NH , =C-NH_2 , NH-C=CH , =C-SO_2 , NH and CH=CH.

2-Chloro-1H-benzo[d]imidazole **3** reacts with primary amines namely, 2-aminopyridine , benzyl amine and/or p-phenylenediamine in ethanol giving N-(pyridin-2-yl)-1H-benzo[d]imidazol-2-amine **7a** , N-benzyl-1H-benzo[d]imidazol-2-amine **7b**, N^{1}-(1H-benzo[d]imidazol-2-yl)benzene-1,4-diamine **7c**respectively .

The infrared spectrum of **7a** showed absorption bands at 1623.8 cm^{-1} , 1575.4 cm^{-1} , 2850.6-3057.3 cm^{-1} , 3200 cm^{-1} attributable to √ C=N , √ C=C , √ C-H and √ NH. The mass spectrum of **7a** at m/z 208(100%) as M-2H$^{\bullet}$ and m/z 55(69%).

The infrared spectrum of **7b** showed absorption bands at 1626.3 cm^{-1} , 1579 cm^{-1} , 2852.9-3056.9 cm^{-1} and 3271.4 cm^{-1} attributable to √ C=N , √ C=C , √ C-H and √ NH. The ^{1}H-NMR showed signalsδppm at 6.91-8.52(m,9H,Ar-H), 11.6(s,1H,NH), 3.31(d,2H,CH_2) and 6.55-6.58(t,1H,NH-CH_2).^{13}C-NMR of **7b** showed signals δ ppm at 141.4 ,128.3 , 128.2 , 127.0 ,121.5-123.9 , 109.4-118.1 and 43.19 attributable to N=C-NH , C-NH , HN-CH_2 , C-N=C , CH-C ,CH-CH and CH_2-C.

The infrared spectrum of **7c** showed absorption bands at 1624.9 cm^{-1} , 1577.4 cm^{-1} , 2918.9-3010.7 cm^{-1} , 3198.5 cm^{-1} and 3311.6 cm^{-1} attributable to √ C=N , √ C=C , √ C-H , √ NH and √ NH_2. ^{13}C-NMRof **7c** showed signals δ ppm at 141 , 138 , 136 , 125.9 , 102-125.9 attributable to N=C-NH , H_2N-C , N-C , C-NH and CH.

Treatment of **7c** with acrylonitrile in boiling pyridine afforded the corresponding N-(4-(1H-pyrazol-1-yl)phenyl)-1H-benzo[d]imidazole-2-amine **8**. The infrared spectrum of **8** showed absorption bands at 1608.2 , 1575.3 , 2818-3042.1 cm^{-1} and 3160 attributable to √ C=N , √ C=C ,√ C-H and √ NH and devoidof √—C≡N.The ^{1}H-NMR showed signal bands δppmat 6.57-8.17(m,8H,Ar-H), 11.5(s,1H,NH), 6.54(s,1H,NH), 3.33, 3.51(d,1H,CH_a), 2.65,2.57(d,1H,CH_c) and 2.49,2.503,2.508,2.515(2d,1H,CH_b). The mass spectrum of **8** showed molecular ion peak at m/z 277(27%) and m/z 202(100%).

Reaction of **7c** with cinnamaldehyde in ethanol in presence of sodium ethoxide afforded the corresponding (N^4E)-N^1-(1H-benzo[d]imidazol-2-yl)-N^4-(3- phenylallylidene) benzene -1,4- diamine**9**. The infrared spectrum of **9** showed absorption bands at1605 cm^{-1}, 1553 cm^{-1} , 2923.9-3028.2 cm^{-1} and 3296.6 cm^{-1} attributable to √ C=N , √ C=C , √ CH and √ NH.The^{1}H-NMR showed signal bands δppm at 6.42-8.50(m,13H,Ar-H),11.45(s,1H,NH), 2.08,2.15,2.26,2.49(2d,1H,CH_b), 6.36(s,1H,NH), 3.40(d,1H,N=CH_a) and 2.502-2.507(d,1H,CH_c-ph). The mass spectrum of **9** showed molecular ion peak at m/z 338(9.02%) and m/z 284(100%).

Treatment of **7c** with phenylisothiocyanate in presence of pyridine afforded the corresponding 1-(4-(1H-benzo[d]imidazol-2-ylamino)phenyl)-3-phenylthiourea **10**. The infrared spectrum of **10** showed absorption bands at 1626.7 cm^{-1},1594.3 cm^{-1} 2853.5-3038.1 cm^{-1} and 3206.7 cm^{-1} attributable to √ C=N , √ C=C , √ C-H and √ NH. ^{13}C-NMRof **10** showed signal bands δ ppm at 179, 141, 140, 139, 137, 134, and 123.5-128.3 attributable to –C=S-NH, N=C-NH, NH-C=CH, -C-N=C, -C-N, NH-C=CH and CH=CH

Treatment of **7c** with chloroacetic acid in dry acetone and anhydrous potassium carbonate afforded the correspondig N-(3-(4-(1H-benzo[d]imidazol-2-ylamino)phenyl)-4-oxothiazolidin-2-yl)benzamide **11**. The infrared red spectrum of **11** showed absorption bands at 1619.7 cm^{-1}, 1714.2 cm^{-1}, 2873.9-3020 cm^{-1} and 3145.5 cm^{-1} attributable to √ C=N, √ C=O, √ CH and √ NH. ^{13}C-NMR of **11** showed signals at 141.5, 131.7, 171.2, 31.0, 167 and 117.7-123 attributable to NH-C=N, CH-CN, C=O, HC-S, NH-CO-CH and CH=CH.

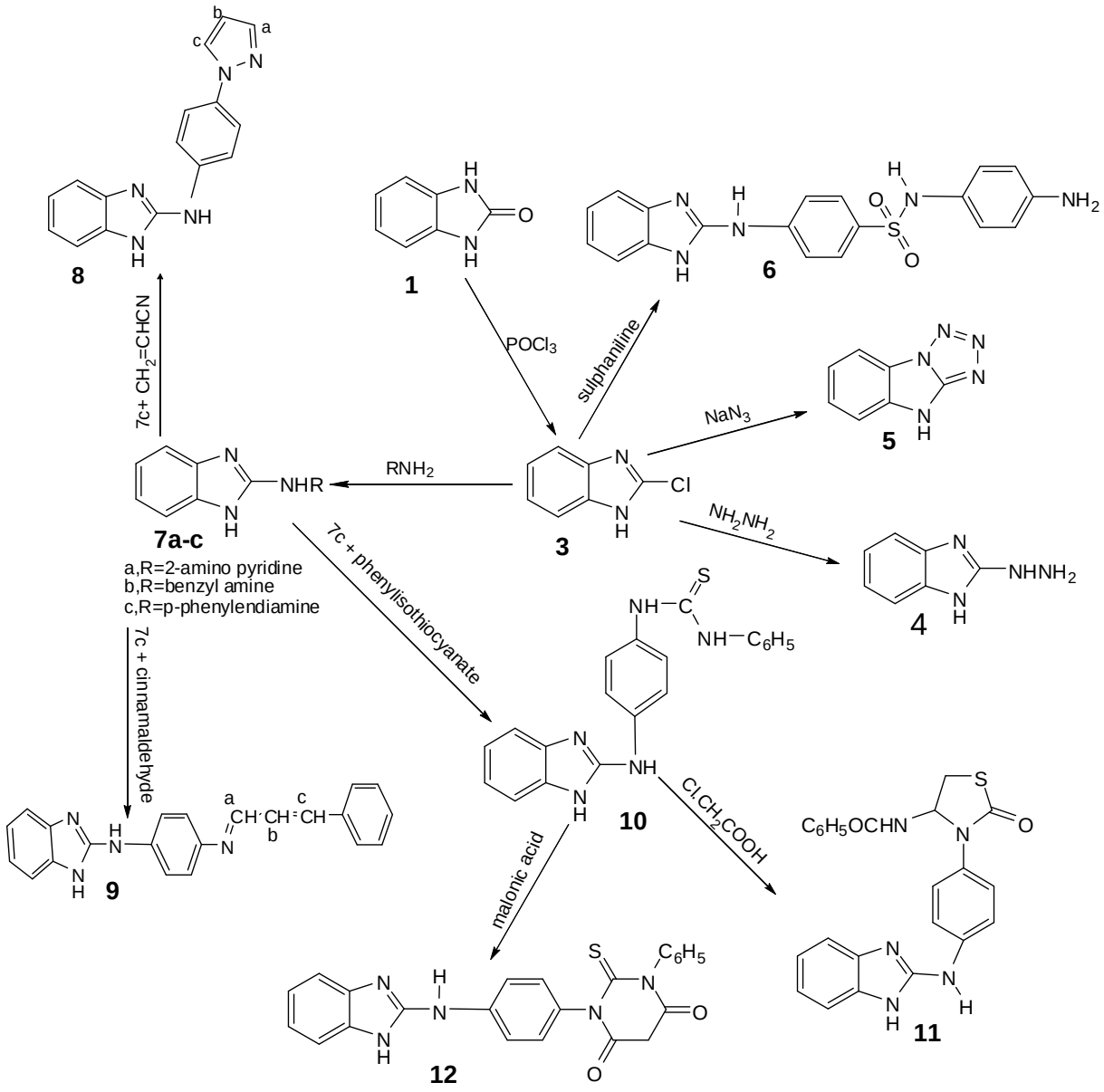
A large number of pyrimidine derivatives are reported to exhibit anti-inflammatory**[14]**,anti-microbial**[14,15],**anti-cancer**[16,17]**,radioprotective**[17]**,anti-angiogen**[18],**antitumor**[19]**,anti-proliferative**[20]**,anti-histaminic**[21]**, antiviraland antibacterialactivities**[22]**.

This prompted the author to synthesize a new pyrimidine derivative through the reaction of **7c** with malonic acid to give the corresponding 1-(4-(1H-benzo[d]imidazol-2-ylamino)phenyl) – 3-phenyl-2-thioxodihydropyrimidine-4,6(1H,5H)-dione**12**. The infrared spectrum of **12** showed of **12** absorption bands at 1678.9 cm^{-1}, 1628.3 cm^{-1}, 1570 cm^{-1}, 12852.6-3050 cm^{-1} and 3300 cm^{-1} attributable to √ C=O, √ C=N, √ C=C, √ C-H and √ NH. ^{13}C-NMR of **12** showed signalsδppm at 179 (C=S), 167 (C=O), 141 (N=C-NH)(benzimidazole), 134 (NH-C=CH), 119.1-128.9 (CH=CH), 103.05 (CH=C), 101(CH) and 40.3 (CH_2).

Reaction of compound **2** with acetylacetone afforded the corresponding diketo compound 3-(1H-benzo[d]imidazol-2(3H)-ylidene)pentane-2,4-dione **13**. The infrared spectrum of **13** showed absorption bands at 1708.4 cm^{-1}, 1629.3 cm^{-1}, 2806.3-3021.5 cm^{-1} and 3400 cm^{-1}(broad) attributable to √ C=O, √ C=N, √ CH and √ NH. ^{13}C-NMR showed of **13** signal δ ppm at 166 (C=C), 154 (C=O), 198.4(C-CO-CH_3), 203.5 (C-CO-CH_3), 29.4 (CH_3), 29.4 (CH_3) and 40.9 alkyl CH_3.

It was stated that pyrazole derivatives showed anticancer**[23-25]**, anti-inflammatory,antimicrobial and antioxidant**[26,27]**, antioxidant**[28]**, antimicrobial**[29,30],**molluscicidal**[28]** , anti-angiogenic**[29]** , antitumor**(30)** activities and as a novel carrier for nitric oxide**[31]** .

This prompted the author to synthesize pyrazole derivative, through the reaction of **13** with hydrazine hydrate to give 2-(3,5-dimethyl-4H-pyrazol-4-ylidene)-2,3-dihydro-1H-benzo[d]imidazole **14**. The infrared spectrum of **14** showed absorption bands for √ C=N ,√ NH at 1582 cm^{-1} and 3441 cm^{-1}(broad) and devoid of √ C=O respectively.The^{1}H-NMR showed signal bands δppm at 11.04,11.16(2xs,2x1H,2XNH),6.67-7.87(m,4H,Ar-H)and1.23,1.26(2xs,2x3H,2xCH_3). The mass spectrum of **14** showed molecular ion peak at m/z 79(100%) and m/z 205(14.90%) and m/z 208(1.18%) as M-41$^{\bullet}$.

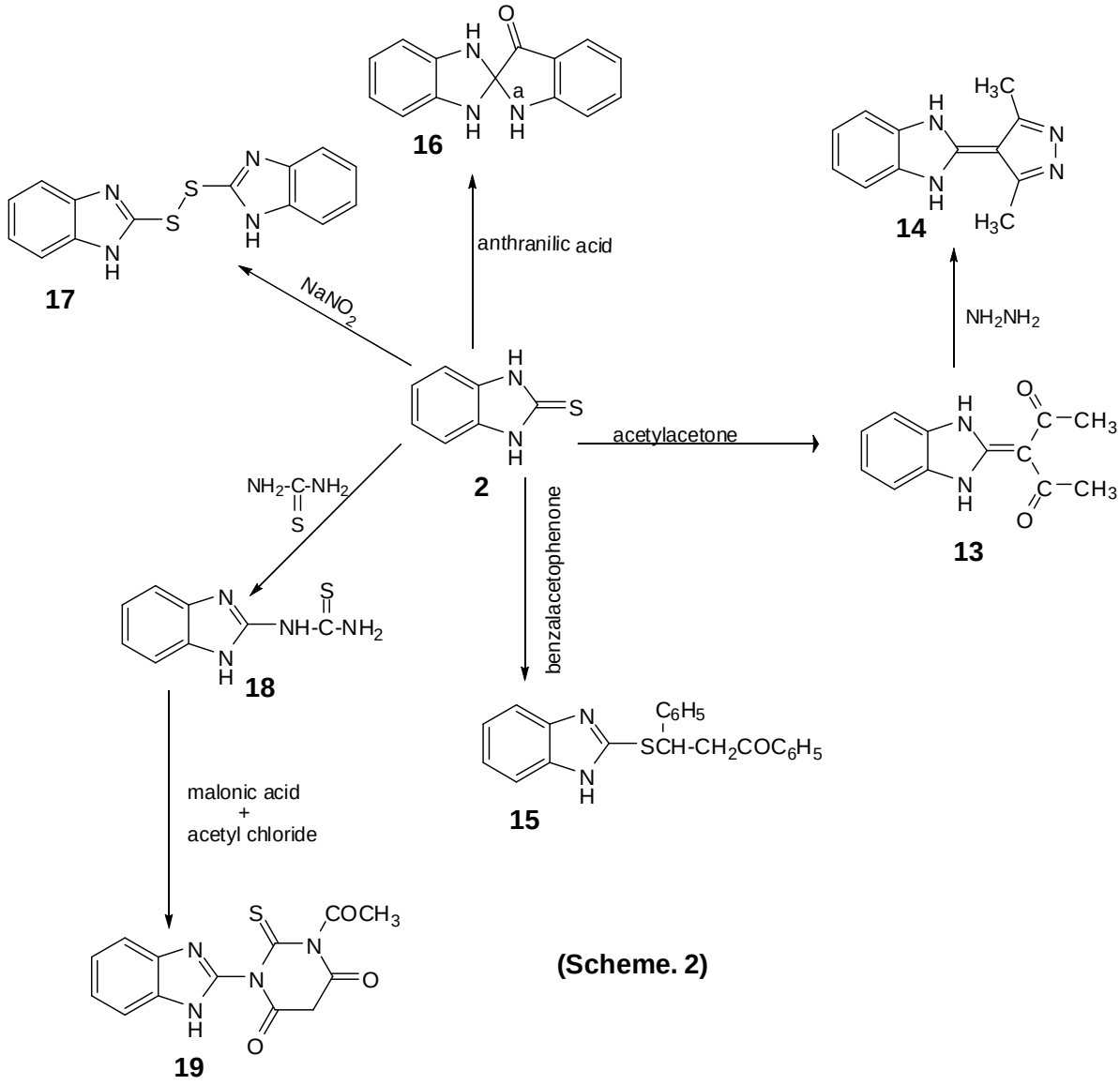


(Scheme.1)

Reaction of**2** with benzalacetophenone gave the adduct 3-(1H-benzo[d]imidazol-2-ylthio)-1,3-diphenylpropan-1-one **15**. The infrared spectrum showed absorption bands at 1677.1 cm^{-1}, 1602.9 cm^{-1}, 3437.9 cm^{-1}, 2850.5-3044.1 cm^{-1} and 1449.7 cm^{-1} attributable to √ C=O, √ C=N, √ NH, √ CH and √ CH_2. ^{13}C-NMR showed signal δ ppm at 40.3 (CH-aliphatic), 47.5 (CH_2), 199.8 (-C=O), 141.5 (CH-benzimidazole), 133.1 (CH-benzene) and 111.2-129.5 (CH=CH).

Reaction of **2** with anthranilic acid afforded 1,3-dihydrospiro[benzo[d]imidazole-2,2'-indolin]-3'-one**16.** The infrared spectrum of **16** exhibited bands for √ C=O at 1675 cm^{-1},√ C=N at 1617.8 cm^{-1} and √ NH at 3148.07 cm^{-1}.The ^{1}H-

NMR showed signal bands δppm at 12.51(s,1H,NH_a), 8.50,8.10(2xs,2x1H,2x NH) and 7.09-8.09(m,8H,Ar-H). The mass spectrum of **16** showed molecular ion peak m/z at 239 (13.60%) as $M+2\dot{1}^{\bullet}$ and m/z 178(100%).
Compound **2** was oxidized on treatment with nitrous acid to the disulfide derivative 1,2-di(1H-benzo[d]imidazol-2-yl)disulfane**17**. The infrared spectrum showed the bands of √ CN at 1529.03 cm^{-1}, √ S-S at 500 cm^{-1}, √ CH at 3087.7 cm^{-1} and √ NH 3246.2 cm^{-1}. The mass spectrum of **17** showed molecular ion peak at m/z 298(5.45%) and m/z 108(100%).



(Scheme. 2)

Treatment of the thione derivative **2** with thiourea in boiling DMF gave 1-(1H-benzo[d]imidazol-2-yl)thiourea**18** through the nucleophilic attack of the nitrogen of thiourea to the carbon of the thione moiety accompanied by elimination of one molecule of H_2S. The infrared spectrum of **18**exhibited absorption bands at 1583.8 cm^{-1} for √ C=N, 3151.7-3318.1 for √ NH_2, NH, √ C=S at 1210.4 cm^{-1} and 2874.8-3109.3 cm^{-1} for √ CH. The ^{1}H-NMR showed signal bands at 12.49 (s,2H,NH_2), 6.91-7.31(m,4H,Ar-H), 6.0(s,1H,NH-C=S) and 5.2(s,1H,NH cyclic). The ^{1}H-NMR spectrum signal bands δppm at 10.1 (s,1H,NH), 6.12-8.52 (m,4H,Ar-H), 3.54(s,2H,CH_2) and 2.64 (s,3H,$COCH_3$). The mass spectrum of **18** showed molecular ion peak at m/z 195(6.52%) as M^{+3} and m/z 54(100%).

As a point of interest compound **2** reacted with malonic acid and acetyl chloride to give 1-acetyl-3-(1H-benzo[d]imidazol-2-yl)-2-thioxodihydropyrimidine-4,6(1H,5H)-dione **19** which was proved previously by the author that it has highly biological activities**[32]**.The infrared spectrum of **19** showed absorption bands for √ C=O at 1758.7 cm^{-1}, √ $COCH_3$ at 1676.2 cm^{-1}, √ C=N at 1526.25 cm^{-1}, √ C=S at 1190.87 cm^{-1} and √ CH at2852.4-3061.4 cm^{-1}.

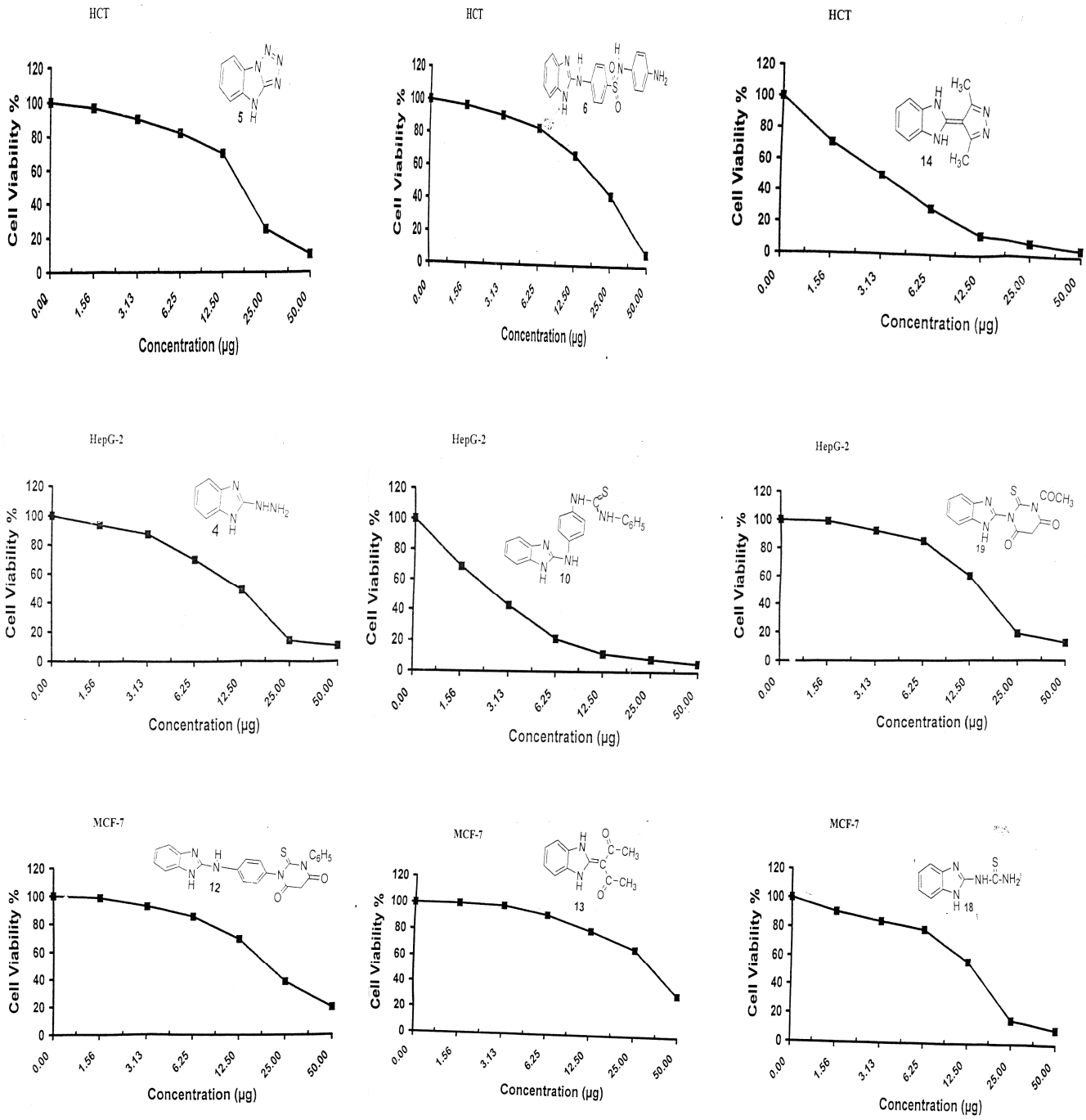


Fig.(1). Evaluation of cytotoxicity of 2-benzimidazole derivatives against HCT, HepG-2 and MCF-2

4. Cytotoxic assay

Cytotoxic assay was performed using the modified method as previously described**[33]**. Cancer cells were grown in Ham´s/F_{12} medium containing 2 m Ml-glutamine supplemented with 100 U/ml penicillin, streptomycin and 10% FBS.

Except Hep G-2 cell was grown in DMEM. Briefly , cell lines**Table(1)** suspended in RPMI- 1640 containing 10% FBD were seeded at $1x10^4$ cells(100 µL) per well ina96—well plate , and incubated in humidified atmosphere , 95% air 5% CO_2 at 37 ºC . After 24h, additional medium (100 µL) containing the test compound and vehicle was added to a final concentration of 50 µg/ml , 0.2% DMSO , and further incubated for 3 days. Cells were subsequently fixed with 95% EtOH, stained with crystal violet solution, and lysed with a solution of 0.1 N HCl in MeOH after which absorbance was measured at 550 nm whereas Hep G_2, HCT and MCF-7 cells were stained by MTT. IC_{50} values were determined as the drug and sample concentrations at 50% inhibition of cell growth**Fig.(1).**

Table 1: Cytotoxic activity of 2- benzimidazole derivatives (4-6 , 10 , 12 - 14 , 18 , 19)

Cell lines[a] IC_{50} (µg/ml)[b,c]										
	4	5	6	10	12	13	14	18	19	**Etoposide**
Hep G-2	12.1	NT	NT	2.7	NT	NT	3.2	NT	15.5	21.00
HCT	NT	18.1	21.6	NT	NT	NT	NT	NT	NT	30.00
MCF-7	NT	NT	NT	NT	20.3	37.5	NT	14.7	NT	6.00

NT: indicates not tested; [a]Cancer cell lines were hepatocellular carcinoma cell line (Hep G-2); colon carcinoma cell line (HCT); breast carcinoma cell line (MCF-7).; [b]When IC_{50} > 50 µg/ml denotes inactive compound.; [c] The assays were performed in triplicate. [d]Etoposide was used as a reference drug.

5. Cytotoxic activity:

Cytotoxic activity of benzimidazol analogs **4 -6 , 10 , 12 - 14 , 18 and 19** against three cancer lines using a modified method**[33].**

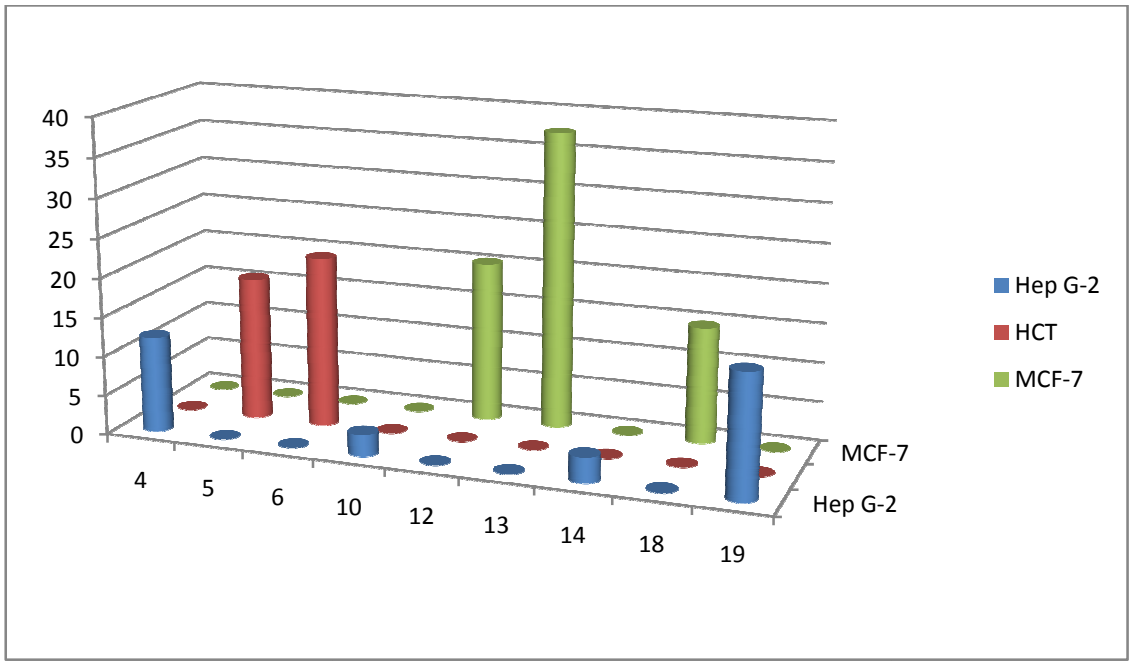


Figure (2): Cytotoxic activity of 2-benzimidazple derivatives(4-6,10.12-14,18,19)

The results which were listed in (Table 1) showed that compounds **10** and **14** have shown the least activity toward the tested cancer cell lines (Hep G-2) and (HCT) respectively with IC_{50} of 2.7 µg/ml and 3.2 µg/ml respectively. Compounds **12 , 13** and **18** exhibited cytotoxic activity against MCF-7 cell lines with the IC_{50} of 20.3 , 37.5 and 14.7

µg/ml. The benzimidazole derivatives **5**and **6**exerted activity against the colon carcinoma cell (HCT) with the IC_{50} of 18.1 and 21.6 µg/ml respectively. In addition ,benzimidazole derivatives **4** and **19** displayed activity against Hep G-2 cell line with the IC_{50} of 12.1 and 15.5 µg/ml respectively. It is notable that compound **13** is the most potent cytotoxic agent against MCF-7 with the IC_{50} of 37.5 µg/ml, this is presumably due to a high lipophilicityof dicarbonyl moiety which enhanced its absorption to the cancer cells. Similarly , the relative potency of compound **6** against HCT with the IC_{50} of 21.6µg/ml may also be related to the presence of ph-$NHSO_2$ph-NH_2 group with its hydrophobic nature, the data are represented in **Fig.(2).**

6. Molecular Docking:

All the molecular modeling studies were carried out on an Intel Pentium 1.6 GHz processor, 512 MB memory with Windows XP operating system using Molecular Operating Environment (MOE, 10.2008) software which provided by chemical computing group, Canada. Docking on the active site of cyclin-dependent kinase **2** (CDK2) enzyme was performed for synthesized compounds **6, 11, 12, 13 and 19**. All the minimizations were performed with MOE until a root mean standard deviation (RMSD) gradient of 0.05 k.cal/mol$Å^{-1}$ with MMFF94X force field and the partial charges were automatically calculated. A protein data bank file with the code 1FVV was selected for the X-ray crystallographic structure of CDK2 enzyme co-crystallized with a sulphone ligand by Davis et al**[34]**.The enzyme was prepared for docking studies where:

(i) Acting on only one chain of amino acids containing one molecule of the inhibitor.
(ii) 3D protonation for the amino acid side chain and the ligand.
(iii) Deleting all water of crystallization away from the active site.
(iv) Isolation of the active site and recognition of the amino acids.
(v) Studying the interaction of the ligand with the amino acids of the active site.

All of these procedures were taken and the 2D interactions of ligand with the amino acids of the active site are shown **Fig.(3).**In order to visualize these interactions in a better manner 3D interactions were illustrated in **Fig.(4).**Validation of the docking protocol indicates that the ligand is fitted in the active site pocket with S=-18.1741 Kcal/mol and rsmd=0.7723.

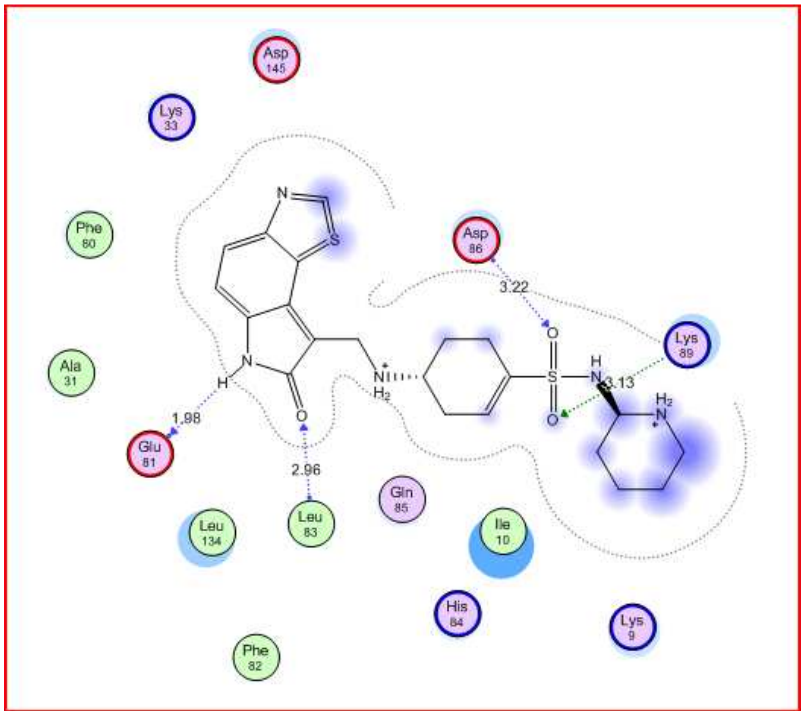


Figure (3) interactions of the ligand on the active site of CDK2

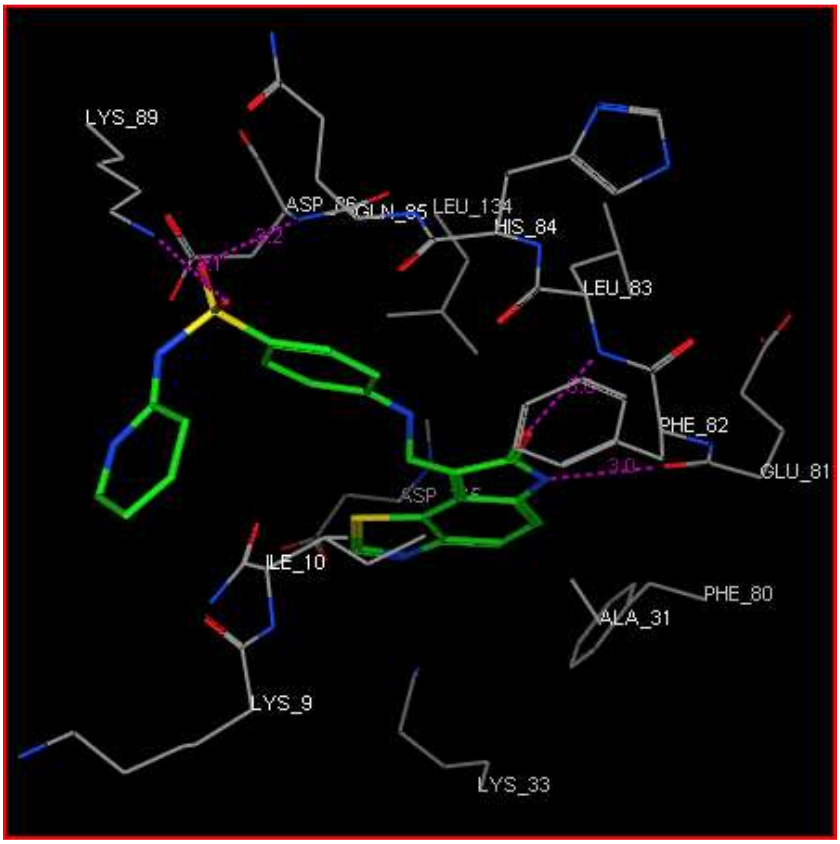


Figure (4) 3D interactions of the ligand on the active site of CDK2

From the above figure the ligand interacts with the active site of CDK2 by four interactions: the sulphonate group interacts with Lys 89 with a hydrogen bond of 3.13 Å & Asp 86 with a hydrogen bond of 3.22 Å on the other hand the carbonyl group of pyrolone interacts with Leu 83 with a hydrogen bond of 2.96 Å and finally the NH group of pyrolone interacts with Glu 81 with a hydrogen bond of 1.98 Å.

Fig. (5)illustrates docking of co-crystallized ligand on the active site of CDK2 where the compound in green colour representing the original co-crystallized ligand and the compound in the red colour representing the docked ligand.

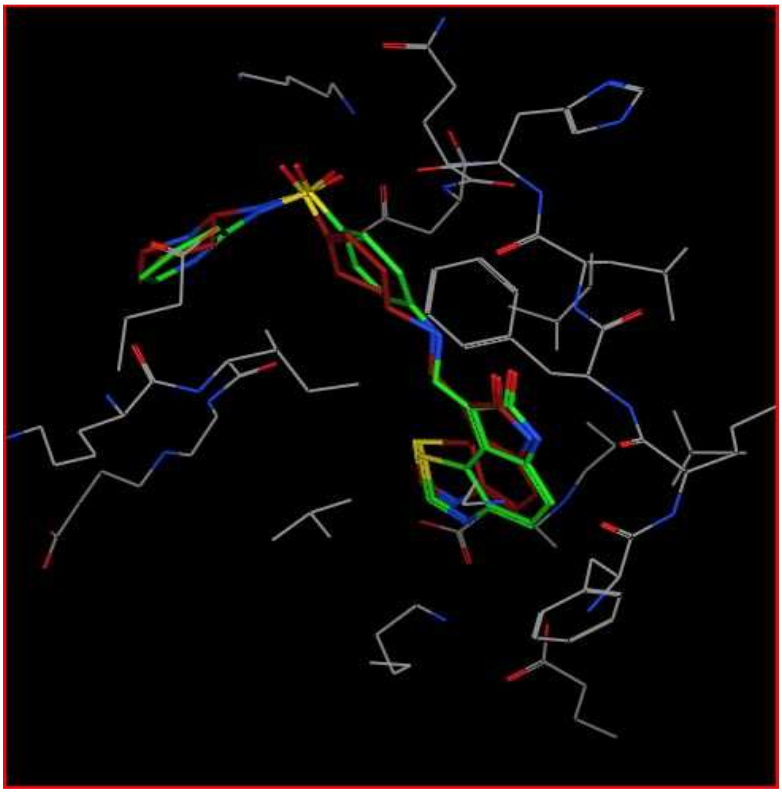


Figure (5) Validation of docking protocol on the active site of CDK2

Preparation of the synthesized compounds for docking was achieved via their 3D structure built by MOE. Certain procedures should be taken before docking which includes:

1-3D protonation of the structures.
2-Running conformational analysis using systemic search.
3-Selecting the least energetic conformer.
4-Applying the same docking protocol used with the ligand.

The previous measures were taken and docking for five compounds of the synthesized compounds was applied. Energy scoring (S) and amino acids interactions and the hydrogen bond lengths were measured and illustrated in the **table (2).**

Table(2): Energy scoring(S), amino acids interactions and the hydrogen bond tength

Comp.No.	(S) k.cal/mol	Amino acid length interactions	H-bond Å
6	-21.0970	Asp A86 Glu A8	2.74, 2.13 2.01
11	-24.4581	Glu A8 His A84 Leu A83	1.56 1.91 1.61
12	-17.0454	Lys A89 His A84 Leu A83	2.5 1.73 1.76
13	-16.2729	Gln A131	2.69
19	-16.4552	Lys A33	3.07

From the above table compounds **11** and **12** interact with the active site of CDK2 by three interactions for each. Compound **11** revealed energy score (S)=−24.46 kcal/mol and interacted with Glu A8, His A84 and Leu A83 with

three hydrogen bonds of 1.56, 1.91 and 1.61Å through –NH moiety ofthe benzimidazole ring, respectively **Fig.(5)**. While compound **12** revealed energy score (S)=−17.05 kcal/mol and interacted with Lys A89, His A84 and Leu A83 with three hydrogen bonds of 2.50, 1.73 and 1.76Å, respectively**Fig.(6).**

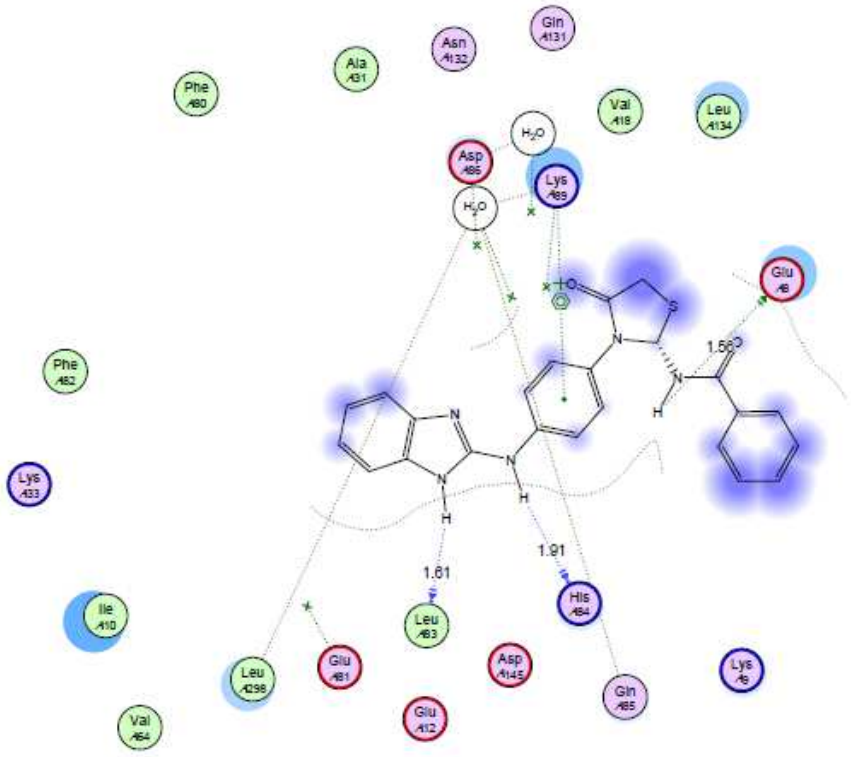


Fig (6) Compound 11 on the active site of the enzyme

CDKs are considered a potential target for anti-cancer medication. If it is possible to selectively interrupt the cell cycle regulation in cancer cells by interfering with CDK action, the cell will die.

Cyclin-dependent kinase 2, also known as cell division protein kinase 2, is a catalytic subunit of the cyclin-dependent kinase complex, whose activity is restricted to the G1-S phase of the cell cycle, and is essential for the G1/S transition, consequently arrests the cell cycle.

7. Structure Activity Relationship

According to the results of bioactivities, it is noted that compound 1-(4-(1H-benzo[d]imidazol-2-ylamino)phenyl)-3-phenyl-2-thioxodihydropyrimidine-4,6(1H,5H)-dione **12**provides a moderate cytotoxic activity against MCF-7 cell lines, it also showed that it has interacted with the active site of CDK2 by three interactions for each **Fig.(5)**. As CDK_S are considered a potential target for anti-cancer medication, if it is possible to selectively interrupt the cell cycle regulation in cancer cells by interfering with CDK action the cell will die. This is presumably due to the hydrophobic of sterically hindered NH moiety of the benzimidazole ring that enhances the penetration of compound **12** to the cancer cell. Up to this point, it could be concluded the imidazolyl-1,3-diphenyl-2-thioxo-4,6-dione can be considered as a promising cytotoxic agent.

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