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Synthesis, Molecular Docking and Cytotoxicity Evaluation of Novel 1,2-Disubstituted Benzimidazole Derivatives Against Liver and Breast Cancer Cell Lines.

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ABSTRACT

A series of novel 1, 2-disubstituted benzo[d]imidazole derivatives was synthesized, which bearing at position-1 different bioactive side chains or heterocyclic moieties. While, they are linked to 2,4,6-trisubstituted pyridine ring at position-2. *In vitro* Cytotoxic evaluation of all the new synthesized benzimidazole compounds (**3-15a,b**) against hepatocellular carcinoma (HepG-2) and breast carcinoma (MCF-7) cell lines showed that most of them gave significant cytotoxicity. Moreover, compounds **7**, **8**, and **14a,b** revealed more potent anticancer activity than 5-fluorouracil as a reference drug and their IC₅₀ values ranged from 12.30 to 24.50 μ M. Additionally, molecular docking study was carried out for all the derivatives to estimate the binding mode of these compounds into the active site of c-kit tyrosine kinase. The tested compounds showed good binding affinity with low docking score values compatible with their cytotoxic activity.

Keywords: benzimidazole derivatives; 2,4,6-trisubstituted pyridine, HepG-2 cells; MCF-7 cells; molecular docking

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INTRODUCTION

Recently, the synthesis of novel benzimidazole derivatives became a target for many researchers and scientists in the field of drug discovery as a result of the diverse biological properties of these compounds, such as antimicrobial [1-4], antiviral [5, 6], antidiabetic [7, 8], analgesic [9, 10], anti-inflammatory [11], antioxidant [12, 13], treating Alzheimer's disease [14], and antihypertensive [15, 16] activities. Moreover, many of benzimidazole derivatives have shown potential cytotoxic activity against different human carcinoma cell lines [17-20]. In addition, several promising anticancer agents with benzimidazole ring system are performed their activity by acting mainly as protein kinase inhibitors [21-23]. Whereas, recent advances have involved the role of tyrosine kinases (TK) in the pathophysiology of cancer. It has been found that, the activation of cancer cells can be obstructed by selective tyrosine kinase inhibitors (TKI), so they assumed to be a promising target for anticancer drug discovery [24-26].

On the other hand, pyridine derivatives have shown significant biological properties and they are found in the structure of several natural products that have therapeutic relevance [27]. Also, recent studies showed that 2,4,6-trisubstituted pyridine compounds were exhibited remarkable anticancer activity [28-30].

In spite of the rapid development that is being made in the treatment of cancer, but it is still a leading cause of death worldwide [31]. In particular breast cancer which is the most frequent malignant tumor in women, statistics indicate that between each eight women one diagnosed with breast cancer [32]. At the same time, liver cancer which known as Hepatocellular carcinoma (HCC) is the sixth most prevalent malignancy and it is the elevation cause of the cancer related mortality [33]. Thus, many efforts must be made to overcome the increased risk of cancer, it could be achieved through the development of the existing anticancer drugs or by discovering new drugs have more potent activity and less side effects.

Based on the above, the present study aims to synthesize novel 1,2-disubstituted benzimidazole derivatives with expected anticancer activity. The target compounds incorporated at position-2 to 2,4,6-trisubstituted pyridine ring. While, position-1 of the benzimidazole nucleus linked to different chains or five membered heterocyclic ring such as: amide; N-aryl sulfonyl; acetohydrazide; semicarbazide, thiosemicarbazide, 1,3,4-thiadiazole, pyrazol-3-one, 1,2,4-triazol-3-one, and 1,2,4-triazol-3-thione moieties, which have been reported to possess potent cytotoxic activity against different human carcinoma cell lines [34-43]. The *in vitro* cytotoxic activity of all the new benzimidazole derivatives was evaluated against hepatocellular carcinoma (HepG-2) and breast carcinoma (MCF-7) cell lines.

The c-kit is an influential member of tyrosine kinase family, which plays a key role in tumor occurrence and progression, and it is an appropriate target for the development of drugs used in the treatment of many human malignancies [44, 45]. Thus, the molecular docking study of the new synthesized compounds was carried out to explore their binding affinity to c-kit kinase which could be a measure of the strength of cancer inhibition.

MATERIALS AND METHODS

All melting points are uncorrected and were recorded on an open glass capillary tubes using an Electrothermal IA9100 digital melting point apparatus. Elemental microanalyses were carried out at Micro analytical Unit and were found within $\pm 0.5\%$ of the theoretical values. Infrared spectra were recorded on a Jasco FT/IR-6100, Fourier transform, Infrared spectrometer (Japan) at cm^{-1} scale using KBr disc technique. ^1H NMR and ^{13}C NMR spectra were recorded on a Jeol-Ex-500 NMR spectrometer (JEOL, Tokyo, Japan) in the presence of TMS as internal standard. The mass spectra were measured using mass spectrometer Shimadzu QP-2010 plus mass spectrometer. Follow up of the reactions and checking the purity of the compounds were made by TLC on silica gel aluminium sheets (Type 60, F 254, Merck, Darmstadt, Germany) and the spots were detected by exposure to UV analysis lamp at λ 254/366 nm for few seconds and by iodine vapor. The chemical names given for the prepared compounds are according to the IUPAC system.

4-(4-Methoxyphenyl)-2-oxo-6-phenyl-1,2-dihydropyridine-3-carboxylic acid (2)

A solution of the carbonitrile derivative **1** (0.1 mol) in concentrated sulphuric acid (35 mL) was heated in water bath at 80 °C for 2 h, then allowed to cool. The reaction solution was poured onto ice/water and

stirred for 1 h at room temperature. The obtained solid was collected by filtration, washed with iced water and recrystallized from DMF/EtOH to give the carboxylic acid derivative **2**.

Yield: 79 %, m.p. = 260 °C. IR (KBr) ν , cm^{-1} : 3415-3224 (br., OH), 3187 ((NH), 3098 ($\text{CH}_{\text{aromatic}}$), 2922 ($\text{CH}_{\text{aliphatic}}$), 1685 (C=O, acid), 1639 (C=O, pyridone). ^1H NMR spectrum (500 MHz, d_6 -DMSO, δ ppm): 3.80 (s, 3H, OCH_3), 6.65-7.87 (m, 10H, Ar-H), 8.21 (br. s, 1H, NH, D_2O exchangeable). MS m/z : M^+ 321 (48 %). Anal. Calcd. For $\text{C}_{19}\text{H}_{15}\text{NO}_4$ (321.33): C, 71.02; H, 4.71; N, 4.36 %; found: C, 71.36; H, 5.08; N, 4.69 %.

3-(1H-benzo[d]imidazol-2-yl)-4-(4-methoxyphenyl)-6-phenylpyridin-2(1H)-one (3)

A mixture of the carboxylic acid **2** (0.2 mol) and 1,2-phenylenediamine (0.2 mol) in phosphoric acid (300 mL) was heated at 160-180°C for 2 h, the reaction solution was poured onto ice/water and the medium was neutralized by addition of 4N NaOH solution. The obtained solid was filtered, washed with water and recrystallized from EtOH to give compound **3**.

Yield: 69 %, m.p. = 175 °C. IR (KBr) ν , cm^{-1} : 3385, 3256 (NH), 3121 ($\text{CH}_{\text{aromatic}}$), 2924 ($\text{CH}_{\text{aliphatic}}$), 1638 (C=O), 1606 (C=N). ^1H NMR spectrum (500 MHz, d_6 -DMSO, δ ppm): 3.78 (s, 3H, OCH_3), 6.52-7.83 (m, 14H, Ar-H), 9.85 (s, 1H, NH, D_2O exchangeable), 11.62 (br. s, 1H, NH, D_2O exchangeable). ^{13}C NMR (125 MHz, d_6 -DMSO, δ ppm): 55.79 (OCH_3), 104.42, 112.15, 114.68, 116.23, 127.54, 128.69, 129.21, 129.88, 130.01, 135.22, 142.13, 146.25, 148.17, 148.23, 151.47, 160.65 (aromatic-C), 164.31 (C=O). MS m/z : M^+ 393 (33 %). Anal. Calcd. for $\text{C}_{25}\text{H}_{19}\text{N}_3\text{O}_2$ (393.44): C, 76.32; H, 4.87; N, 10.68 %; found: C, 76.71; H, 4.58; N, 11.02 %.

2-(2-Chloro-4-(4-methoxyphenyl)-6-phenylpyridin-3-yl)-1H-benzo[d]imidazole (4)

A mixture of compound **3** (0.02 mol) and phosphorous pentachloride (0.02 mol) in phosphorus oxychloride (20 mL) was refluxed for 6 h. The reaction mixture was cooled, poured slowly onto ice/water and neutralized with 2N NaOH solution to pH 7. The obtained solid was filtered off, washed with water and recrystallized from DMF/ H_2O to give the chloro derivative **4**.

Yield: 73 %, m.p. = 205 °C. IR (KBr) ν , cm^{-1} : 3408 (NH), 3144, 3042 ($\text{CH}_{\text{aromatic}}$), 2928, 2857 ($\text{CH}_{\text{aliphatic}}$), 1607 (C=N), 763 (C-Cl). ^1H NMR spectrum (500 MHz, d_6 -DMSO, δ ppm): 3.77 (s, 3H, OCH_3), 6.74-8.23 (m, 14H, Ar-H), 11.52 (s, 1H, NH, D_2O exchangeable). MS m/z : M^+ 411 (44 %). Anal. Calcd. for $\text{C}_{25}\text{H}_{18}\text{ClN}_3\text{O}$ (411.88): C, 72.90; H, 4.40; N, 10.20 %; found: C, 72.53; H, 4.71; N, 10.55 %.

2-(2-Chloro-4-(4-methoxyphenyl)-6-phenylpyridin-3-yl)-1-ethyl-1H-benzo[d]imidazole (5)

To a stirred solution of compound **4** (0.002 mol) in 5 % (w/v) ethanolic NaOH solution (20 mL), ethyl iodide (0.004 mol) was added. The reaction mixture was stirred at room temperature for 5 h, the obtained solid was filtered off, washed with water and recrystallized from EtOH to give compound **5**.

Yield: 73 %, m.p. = 180 °C. IR (KBr) ν , cm^{-1} : 3036 ($\text{CH}_{\text{aromatic}}$), 2929, 2846 ($\text{CH}_{\text{aliphatic}}$), 1604 (C=N), 767 (C-Cl). ^1H NMR spectrum (500 MHz, d_6 -DMSO, δ ppm): 1.31 (t, 3H, $J = 7.5$ Hz, NCH_2CH_3), 3.78 (s, 3H, OCH_3), 4.04 (q, 2H, $J = 7.5$ Hz, NCH_2CH_3), 6.57-8.13 (m, 14H, Ar-H). MS m/z : M^+ 439 (32 %). Anal. Calcd. for $\text{C}_{27}\text{H}_{22}\text{ClN}_3\text{O}$ (439.94): C, 73.71; H, 5.04; N, 9.55 %; found: C, 73.46; H, 5.37; N, 10.03 %.

General procedure for synthesis of 6 and 7.

A mixture of the chloro derivatives **5** or **4** (0.001 mol) and the appropriate amine namely: morpholine and 1-methylpiperazine (0.005 mol) was heated with stirring at 100 °C for 10 h. The excess amine was evaporated under reduced pressure, the obtained residue was dissolved in *N,N*-dimethylformamide (10 mL) and poured onto ice/water. The obtained solid was filtered off, washed with water and recrystallized from EtOH to give the amino derivatives **6** and **7**, respectively.

4-(3-(1-Ethyl-1H-benzo[d]imidazol-2-yl)-4-(4-methoxyphenyl)-6-phenylpyridin-2-yl) morpholine (6)

Yield: 68 %, m.p. = 220 °C. IR (KBr) ν , cm^{-1} : 3048 ($\text{CH}_{\text{aromatic}}$), 2923, 2854 ($\text{CH}_{\text{aliphatic}}$), 1606 (C=N). ^1H NMR spectrum (500 MHz, d_6 -DMSO, δ ppm): 1.38 (t, 3H, $J = 5.8$ Hz, CH_2CH_3), 3.62 (t, 4H, $J = 3.5$ Hz, 2NCH_2 ,

morpholine), 3.77 (s, 3H, OCH₃), 3.89 (t, 4H, *J* = 3.5 Hz, 2OCH₂, morpholine), 4.07 (q, 2H, *J* = 5.8 Hz, CH₂CH₃), 6.56-8.21 (m, 14H, Ar-H). MS *m/z*: M⁺490 (37 %). Anal. Calcd. for C₃₁H₃₀N₄O₂ (490.61): C, 75.89; H, 6.16; N, 11.42 %; found: C, 75.51; H, 6.50; N, 11.77 %.

2-(4-(4-Methoxyphenyl)-2-(4-methylpiperazin-1-yl)-6-phenylpyridin-3-yl)-1H-benzo[d]imidazole (7)

Yield: 71 %, m.p. = 270 °C. IR (KBr) ν , cm⁻¹: 3387 (NH), 3033 (CH_{aromatic}), 2929, 2843 (CH_{aliphatic}), 1605 (C=N). ¹H NMR spectrum (500 MHz, d₆-DMSO, δ ppm): 2.12 (s, 3H, NCH₃), 2.23 (t, 4H, *J* = 3.5 Hz, 2NCH₂), 3.61 (t, 4H, *J* = 3.5 Hz, 2NCH₂), 3.78 (s, 3H, OCH₃), 6.85-8.14 (m, 14H, Ar-H), 10.26 (br. s, 1H, NH, D₂O exchangeable). ¹³C NMR (125 MHz, d₆-DMSO, δ ppm): 46.42 (NCH₃), 49.25 (2NCH₂), 54.99 (2NCH₂), 55.81 (OCH₃), 104.56, 112.17, 114.69, 114.88, 116.26, 127.53, 128.82, 129.24, 129.98, 130.11, 135.30, 142.18, 146.42, 148.35, 148.50, 151.73, 160.91 (aromatic-C). MS *m/z*: M⁺475 (41 %). Anal. Calcd. for C₃₀H₂₉N₅O (475.60): C, 75.76; H, 6.15; N, 14.73 %; found: C, 75.42; H, 6.49; N, 14.32 %.

2-(4-(4-Methoxyphenyl)-2-(4-methylpiperazin-1-yl)-6-phenylpyridin-3-yl)-N-phenyl-1H-benzo[d]imidazole-1-carboxamide (8)

A mixture of compound **7** (0.001 mol) and phenylisocyanate (0.001 mol) in *N,N*-dimethylformamide (10 mL) was refluxed for 8 h. Then, the solvent was evaporated under reduced pressure and the residue was solidified by addition of cold water (30 mL). The obtained solid was filtered off, and recrystallized from EtOH/H₂O to give the amide derivative **8**.

Yield: 68 %, m.p. = 140 °C. IR (KBr) ν , cm⁻¹: 3327 (NH), 3131, 3050 (CH_{aromatic}), 2925, 2854 (CH_{aliphatic}), 1643 (C=O, amide), 1601 (C=N). ¹H NMR spectrum (500 MHz, d₆-DMSO, δ ppm): 2.14 (s, 3H, NCH₃), 2.25 (t, 4H, *J* = 3.9 Hz, 2NCH₂), 3.59 (t, 4H, *J* = 3.9 Hz, 2NCH₂), 3.78 (s, 3H, OCH₃), 6.87-8.33 (m, 19H, Ar-H), 9.85 (s, 1H, NH, D₂O exchangeable). MS *m/z*: M⁺594 (32 %). Anal. Calcd. for C₃₇H₃₄N₆O₂ (594.72): C, 74.73; H, 5.76; N, 14.13 %; found: C, 74.45; H, 5.43; N, 14.52 %.

General procedure for synthesis of 9a,b.

A mixture of compound **7** (0.001 mol) and the appropriate arylsulfonyl chloride namely: benzenesulfonyl chloride and 4-methylbenzenesulfonyl chloride (0.001 mol) in dry acetone (20 mL) containing few drops of triethylamine, was refluxed with stirring for 8 h. After reaction completion, the solvent was evaporated under reduced pressure and the obtained solid was collected and crystallized from CHCl₃/pet.ether to give the substituted sulfonyl derivatives **9a**; **9b**, respectively.

2-(4-(4-Methoxyphenyl)-2-(4-methylpiperazin-1-yl)-6-phenylpyridin-3-yl)-1-(phenylsulfonyl)-1H-benzo[d]imidazole (9a)

Yield: 65 %, m.p. = 130 °C. IR (KBr) ν , cm⁻¹: 3068 (CH_{aromatic}), 2942, 2854 (CH_{aliphatic}), 1603 (C=N), 1395, 1177 (SO₂). ¹H NMR spectrum (500 MHz, d₆-DMSO, δ ppm): 2.13 (s, 3H, NCH₃), 2.25 (t, 4H, *J* = 3.2 Hz, 2NCH₂), 3.64 (t, 4H, *J* = 3.2 Hz, 2NCH₂), 3.78 (s, 3H, OCH₃), 6.77-8.39 (m, 19H, Ar-H). MS *m/z*: M⁺615 (30 %). Anal. Calcd. for C₃₆H₃₃N₅O₃S (615.75): C, 70.22; H, 5.40; N, 11.37; S, 5.21 %; found: C, 70.54; H, 5.71; N, 11.74; S, 4.89 %.

2-(4-(4-Methoxyphenyl)-2-(4-methylpiperazin-1-yl)-6-phenylpyridin-3-yl)-1-(4-methylphenylsulfonyl)-1H-benzo [d] imidazole (9b)

Yield: 61 %, m.p. = 166 °C. IR (KBr) ν , cm⁻¹: 3042 (CH_{aromatic}), 2942, 2848 (CH_{aliphatic}), 1610 (C=N), 1396, 1173 (SO₂). ¹H NMR spectrum (500 MHz, d₆-DMSO, δ ppm): 2.12 (s, 3H, NCH₃), 2.21 (t, 4H, *J* = 3.4 Hz, 2NCH₂), 2.84 (s, 3H, SO₂-C₆H₄-CH₃), 3.69 (t, 4H, *J* = 3.4 Hz, 2NCH₂), 3.78 (s, 3H, OCH₃), 6.85-8.41(m, 18H, Ar-H). MS *m/z*: M⁺629 (26 %). Anal. Calcd. for C₃₇H₃₅N₅O₃S (629.78): C, 70.57; H, 5.60; N, 11.12; S, 5.09 %; found: C, 70.22; H, 5.99; N, 10.88; S, 4.77 %.

Ethyl 2-(2-(4-(4-methoxyphenyl)-2-(4-methylpiperazin-1-yl)-6-phenylpyridin-3-yl)-1H-benzo[d]imidazol-1-yl) acetate (10)

To a stirred suspension of compound **7** (0.01 mol) in absolute ethanol (50 mL), a solution of sodium ethoxide (0.23 g of sodium in 10 mL absolute ethanol) was added dropwise, and the stirring was continued at room temperature for 1 h. Then, ethyl chloroacetate (0.01 mol) was added and the reaction mixture was refluxed at 100 °C for 3 h. After reaction completion, the solvent was evaporated under reduced pressure, the residue was treated with cold water. The obtained solid was filtered off and recrystallized from DMF/H₂O to give the ester derivative **10**.

Yield: 75 %, m.p. = 180 °C. IR (KBr) ν , cm⁻¹: 3061 (CH_{aromatic}), 2922, 2857 (CH_{aliphatic}), 1746 (C=O), 1606 (C=N). ¹H NMR spectrum (500 MHz, d₆-DMSO, δ ppm): 1.22 (t, 3H, *J* = 5.5 Hz, OCH₂CH₃), 2.11 (s, 3H, NCH₃), 2.23 (t, 4H, *J* = 3.5 Hz, 2NCH₂), 3.63 (t, 4H, *J* = 3.5 Hz, 2NCH₂), 3.77 (s, 3H, OCH₃), 3.88 (s, 2H, NCH₂C=O), 4.21 (q, 2H, *J* = 5.5 Hz, OCH₂CH₃), 6.91-8.35 (m, 14H, Ar-H). ¹³C NMR (125 MHz, d₆-DMSO, δ ppm): 14.59 (OCH₂CH₃), 46.44 (NCH₃), 49.21 (2NCH₂), 54.99 (2NCH₂), 55.82 (OCH₃), 58.76 (NCH₂C=O), 60.01 (OCH₂CH₃), 104.54, 111.23, 112.12, 114.74, 114.87, 116.11, 116.28, 127.54, 128.82, 129.24, 129.97, 130.12, 135.19, 136.01, 146.40, 148.39, 148.50, 151.75, 160.92 (aromatic-C), 166.30 (C=O). MS *m/z*: M⁺ 561 (33 %). Anal. Calcd. for C₃₄H₃₅N₅O₃ (561.69): C, 72.71; H, 6.28; N, 12.47 %; found: C, 72.44; H, 6.61; N, 12.08 %.

2-(2-(4-(4-Methoxyphenyl)-2-(4-methylpiperazin-1-yl)-6-phenylpyridin-3-yl)-1H-benzo[d]imidazol-1-yl) acetohydrazide (11)

A mixture of ester derivative **10** (0.001 mol) and hydrazine hydrate (0.002 mol) in absolute ethanol (10 mL) was refluxed for 12 h. Then, the reaction mixture was poured onto ice/water, the obtained solid was filtered, dried and recrystallized from ethanol to get the acetohydrazide derivative **11**.

Yield: 69 %, m.p. = 280 °C. IR (KBr) ν , cm⁻¹: 3398, 3260 (NH), 3054 (CH_{aromatic}), 2922, 2855 (CH_{aliphatic}), 1648 (C=O), 1607 (C=N). ¹H NMR spectrum (500 MHz, d₆-DMSO, δ ppm): 2.13 (s, 3H, NCH₃), 2.26 (t, 4H, *J* = 3.4 Hz, 2NCH₂), 3.61 (t, 4H, *J* = 3.4 Hz, 2NCH₂), 3.77 (s, 3H, OCH₃), 4.02 (s, 2H, NCH₂C=O), 5.15 (s, 2H, NH₂, D₂O exchangeable), 6.89-8.31 (m, 14H, Ar-H), 9.91 (s, 1H, NH, D₂O exchangeable). MS *m/z*: M⁺ 547 (22 %). Anal. Calcd. for C₃₂H₃₃N₇O₂ (547.66): C, 70.18; H, 6.07; N, 17.90 %; found: C, 70.55; H, 5.79; N, 17.61 %.

4-(2-(4-(4-Methoxyphenyl)-2-(4-methylpiperazin-1-yl)-6-phenylpyridin-3-yl)-1H-benzo[d]imidazol-1-yl)-2,4-dihydro-3H-pyrazol-3-one (12)

A mixture of the acetohydrazide derivative **11** (0.001 mol) and triethyl orthoformate (0.002 mol) in glacial acetic acid (10 mL) was refluxed for 8 h. After cooling, the reaction mixture was poured onto ice/water, the obtained solid was filtered, dried and recrystallized from ethanol to give the pyrazol-3-one derivative **12**.

Yield: 62 %, m.p. = 220 °C. IR (KBr) ν , cm⁻¹: 3426 (NH), 3064 (CH_{aromatic}), 2923, 2859 (CH_{aliphatic}), 1672 (C=O), 1610 (C=N). ¹H NMR spectrum (500 MHz, d₆-DMSO, δ ppm): 2.12 (s, 3H, NCH₃), 2.27 (t, 4H, *J* = 3.9 Hz, 2NCH₂), 3.67 (t, 4H, *J* = 3.9 Hz, 2NCH₂), 3.78 (s, 3H, OCH₃), 4.63 (d, 1H, *J* = 4.3 Hz, CHC=O, pyrazolone), 6.87-8.35 (m, 15H, Ar-H), 11.61 (br. s, 1H, NH, D₂O exchangeable). MS *m/z*: M⁺ 557 (21 %). Anal. Calcd. for C₃₃H₃₁N₇O₂ (557.66): C, 71.08; H, 5.60; N, 17.58 %; found: C, 71.42; H, 5.13; N, 17.91 %.

5-((2-(4-(4-Methoxyphenyl)-2-(4-methylpiperazin-1-yl)-6-phenylpyridin-3-yl)-1H-benzo[d]imidazol-1-yl) methyl)-1,3,4-thiadiazol-2-amine (13)

A mixture of the ester derivative **10** (0.001 mol) and thiosemicarbazide (0.001 mol) in phosphorus oxychloride (10 mL) was heated in water bath at 100 °C for 6 h. The reaction mixture was poured onto ice/water and the pH of the medium was adjusted to 7 by addition of ammonia solution. The obtained solid was collected by filtration and recrystallized from acetone to give the 1,3,4-thiadiazole derivative **13**.

Yield: 59 %, m.p. = 205 °C. IR (KBr) ν , cm⁻¹: 3306, 3165 (NH), 3041 (CH_{aromatic}), 2924, 2850 (CH_{aliphatic}), 1629 (C=N). ¹H NMR spectrum (500 MHz, d₆-DMSO, δ ppm): 2.13 (s, 3H, NCH₃), 2.25 (t, 4H, *J* = 3.2 Hz, 2NCH₂, piperazine), 3.65 (t, 4H, *J* = 3.2 Hz, 2NCH₂, piperazine), 3.78 (s, 3H, OCH₃), 4.55 (s, 2H, NCH₂), 6.22 (br. s, 2H,

NH₂, D₂O exchangeable), 6.87-8.38 (m, 14H, Ar-H). MS m/z: M⁺ 588 (24 %). Anal. Calcd. for C₃₃H₃₂N₈OS (588.73): C, 67.32; H, 5.48; N, 19.03; S, 5.45 %; found: C, 67.73; H, 5.81; N, 19.34; S, 5.12 %.

General procedure for synthesis of 14a,b.

A mixture of compound **10** (0.001 mol) and semicarbazide hydrochloride or thiosemicarbazide (0.001 mol) in absolute ethanol (20 mL) containing sodium acetate anhydrous (0.002 mol), was refluxed with stirring for 6 h. The reaction mixture was concentrated under reduced pressure, poured onto ice/water. The obtained solid was filtered, dried and recrystallized from ethanol to give the semicarbazide and the thiosemicarbazide derivatives **14a**; **b**, respectively.

1-(2-(2-(4-(4-Methoxyphenyl)-2-(4-methylpiperazin-1-yl)-6-phenylpyridin-3-yl)-1H-benzo[d]imidazol-1-yl)acetyl) semicarbazide (14a)

Yield: 65 %, m.p. = 150 °C. IR (KBr) ν , cm⁻¹: 3358, 3262, 3187 (NH), 3062 (CH_{aromatic}), 2921, 2853 (CH_{aliphatic}), 1680, 1642 (2C=O), 1603 (C=N). ¹H NMR spectrum (500 MHz, d₆-DMSO, δ ppm): 2.12 (s, 3H, NCH₃), 2.24 (t, 4H, *J* = 3.5 Hz, 2NCH₂), 3.61 (t, 4H, *J* = 3.5 Hz, 2NCH₂), 3.78 (s, 3H, OCH₃), 4.21 (s, 2H, NCH₂C=O), 6.09 (s, 2H, NH₂, D₂O exchangeable), 6.87-8.29 (m, 14H, Ar-H), 9.87, 11.24 (2s, 2H, 2NH, D₂O exchangeable). MS m/z: M⁺ 590 (37 %). Anal. Calcd. for C₃₃H₃₄N₈O₃ (590.69): C, 67.10; H, 5.80; N, 18.97 %; found: C, 67.43; H, 5.46; N, 18.64 %.

1-(2-(2-(4-(4-Methoxyphenyl)-2-(4-methylpiperazin-1-yl)-6-phenylpyridin-3-yl)-1H-benzo[d]imidazol-1-yl)acetyl) thiosemicarbazide (14b)

Yield: 69 %, m.p. = 160 °C. IR (KBr) ν , cm⁻¹: 3374, 3217, 3173 (NH), 3064 (CH_{aromatic}), 2921, 2858 (CH_{aliphatic}), 1647 (C=O), 1234 (C=S), 1607 (C=N). ¹H NMR spectrum (500 MHz, d₆-DMSO, δ ppm): 2.13 (s, 3H, NCH₃), 2.25 (t, 4H, *J* = 3.4 Hz, 2NCH₂), 3.63 (t, 4H, *J* = 3.4 Hz, 2NCH₂), 3.78 (s, 3H, OCH₃), 4.26 (s, 2H, NCH₂C=O), 6.49 (br. s, 2H, NH₂, D₂O exchangeable), 6.83-8.35 (m, 14H, Ar-H), 8.87, 10.77 (2s, 2H, 2NH, D₂O exchangeable). MS m/z: M⁺ 606 (31 %). Anal. Calcd. for C₃₃H₃₄N₈O₂S (606.75): C, 65.33; H, 5.65; N, 18.47; S, 5.28 %; found: C, 65.64; H, 5.98; N, 18.11; S, 4.99 %.

General procedure for synthesis of 15a,b.

A solution of compounds **14a**, **b** (0.001 mol) in 1N NaOH solution (20 mL) was refluxed with stirring for 5 h. The reaction mixture was poured onto ice/water and the medium was neutralized by addition of cold 1N HCl solution. The obtained solid was filtered, dried and recrystallized from DMF to give the 1,2,4-triazol-3-one and the 1,2,4-triazole-3-thione derivatives **15a**; **b**, respectively.

5-((2-(4-(4-Methoxyphenyl)-2-(4-methylpiperazin-1-yl)-6-phenylpyridin-3-yl)-1H-benzo[d]imidazol-1-yl)methyl)-2,4-dihydro-3H-1,2,4-triazol-3-one (15a)

Yield: 74 %, m.p. = 299 °C. IR (KBr) ν , cm⁻¹: 3426, 3298 (NH), 3068 (CH_{aromatic}), 2923, 2855 (CH_{aliphatic}), 1657 (C=O), 1611 (C=N). ¹H NMR spectrum (500 MHz, d₆-DMSO, δ ppm): 2.14 (s, 3H, NCH₃), 2.27 (t, 4H, *J* = 4.1 Hz, 2NCH₂, piperazine), 3.66 (t, 4H, *J* = 4.1 Hz, 2NCH₂, piperazine), 3.78 (s, 3H, OCH₃), 4.38 (s, 2H, NCH₂), 5.66 (br. s, 1H, NH, D₂O exchangeable), 6.84-8.37 (m, 14H, Ar-H), 11.83 (s, 1H, NH, D₂O exchangeable). MS m/z: M⁺ 572 (25 %). Anal. Calcd. for C₃₃H₃₂N₈O₂ (572.67): C, 69.21; H, 5.63; N, 19.57 %; found: C, 68.87; H, 5.98; N, 19.88 %.

5-((2-(4-(4-Methoxyphenyl)-2-(4-methylpiperazin-1-yl)-6-phenylpyridin-3-yl)-1H-benzo[d]imidazol-1-yl)methyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione (15b)

Yield: 76 %, m.p. = 294 °C. IR (KBr) ν , cm⁻¹: 3434, 3288 (NH), 3069 (CH_{aromatic}), 2925, 2858 (CH_{aliphatic}), 1230 (C=S), 1609 (C=N). ¹H NMR spectrum (500 MHz, d₆-DMSO, δ ppm): 2.11 (s, 3H, NCH₃), 2.24 (t, 4H, *J* = 3.2 Hz, 2NCH₂, piperazine), 3.64 (t, 4H, *J* = 3.2 Hz, 2NCH₂, piperazine), 3.78 (s, 3H, OCH₃), 4.39 (s, 2H, NCH₂), 6.85-8.37 (m, 14H, Ar-H), 11.14, 12.80 (2s, 2H, 2NH, D₂O exchangeable). MS m/z: M⁺ 588 (38 %). Anal. Calcd. for C₃₃H₃₂N₈OS (588.73): C, 67.32; H, 5.48; N, 19.03; S, 5.45 %; found: C, 67.01; H, 5.86; N, 18.78; S, 5.17 %.

Cytotoxicity assay

Cancer cells from different cancer cell lines human breast adenocarcinoma (MCF7), and human hepatocellular carcinoma (HEPG2), were purchased from American type Cell Culture collection (ATCC, Manassas, USA) and grown on the appropriate growth medium Dulbecco's modified Eagle's medium (DMEM) or Roswell Park Memorial Institute medium (RPMI 1640) supplemented with 100 mg/mL of streptomycin, 100 units/ mL of penicillin and 10% of heat-inactivated fetal bovine serum in a humidified, 5% (v/v) CO₂ atmosphere at 37 °C.

Exponentially growing cells from different cancer cell lines were trypsinized, counted and seeded at the appropriate densities (2000-1000 cells/0.33 cm² well) into 96-well microtiter plates. Then, the cells were incubated in a humidified atmosphere at 37°C for 24 hours, and were exposed to five different concentrations of the tested compounds (0.1, 1, 10, 100, 1000 µM) for 72 hours. The viability of treated cells were determined using MTT (Methyl Thiazol Tetrazolium) assay as follows: Media were removed; cells were incubated with 200 µL of 5% MTT solution/well (Sigma Aldrich, MO) and were allowed to metabolize the dye into a colored-insoluble formazan crystals for 2 hours. The remaining MTT solution were discarded from the wells and the formazan crystals were dissolved in 200 µL/well acidified isopropanol for 30 min, covered with aluminum foil and with continuous shaking using a MaxQ 2000 plate shaker (Thermo Fisher Scientific Inc, MI) at room temperature. Absorbance were measured at 570 nm using a Stat Fax^R 4200 plate reader (Awareness Technology, Inc., FL). The cell viability were expressed as percentage of control and the test was done 3 times for each concentration and the results were expressed as mean ± standard deviation (SD). The concentration that induces 50% of maximum inhibition of cell proliferation (IC₅₀) were determined using Graph Pad Prism version 5 software (Graph Pad software Inc, CA) [46, 47]. Then, the IC₅₀ values of the tested compound and the reference drug (5-fluorouracil) were listed in Table (1).

Molecular docking studies

All the molecular modeling calculations and docking simulation studies were performed using Molecular Operating Environment (MOE[®]), version 2008.10, Chemical Computing Group Inc., Montreal, Quebec, Canada. All the interaction energies and different calculations were automatically calculated.

Target compounds optimization

The target compounds were constructed into a 3D model using the builder interface of the MOE program. After checking their structures and the formal charges on atoms by 2D depiction, the following steps were carried out: the target compounds were subjected to a conformational search. All conformers were subjected to energy minimization, all the minimizations were performed with MOE until a RMSD gradient of 0.01 Kcal/mole and RMS distance of 0.1 Å with MMFF94X force-field and the partial charges were automatically calculated. The obtained data base was then saved as MDB file to be used in the docking calculations.

Optimization of the enzymes active site

The X-ray crystallographic structure of c-kit tyrosine kinase receptor complexed with 4-(4-methyl-piperazin-1-ylmethyl)-N-[4-methyl-3-(4-pyridin-3-yl-pyrimidin-2-ylamino)-phenyl]- benzamide, **STI-571** (PDB ID: 1T46) [48] was obtained from the Protein Data Bank through the internet. The enzyme was prepared for docking studies by removing the ligand molecule, **STI-571** from c-kit tyrosine kinase receptor active site. Hydrogen atoms were added to the system with their standard geometry. The atoms connection and type were checked for any errors with automatic correction. Selection of the receptor and its atoms potential were fixed. MOE Alpha Site Finder was used for the active site search in the enzyme structure using all default items. Dummy atoms were created from the obtained alpha Spheres. Re-docking of co-crystalline ligand to the receptor active sites to insure the docking method was efficient and the active pocket was saved as MOE file to be used for docking simulation of the selected compounds.

Docking of all benzimidazole derivatives (**3-15a,b**) to the receptor active sites

Docking of the conformation database of the target compounds was done using MOE-Dock software. The following methodology was generally applied via loading of the enzyme active site file and the Dock tool was initiated. The program specifications were adjusted to:

- Dummy atoms as the docking site.
- Triangle matcher as the placement methodology to be used.
- London dG as Scoring methodology to be used and was adjusted to its default values.

The MDB file of the ligand to be docked was loaded and Dock calculations were run automatically. The obtained poses were studied and the poses showed best ligand-enzyme interactions were selected and stored for energy calculations. The 2D interaction and stereo view for compounds (**3-15a,b**) inside the active site of c-kit tyrosine kinase were obtained and saved as both MOE and photo files.

- From the docking analysis, the binding mode of compounds (**3-15a,b**) based on binding energy was calculated and listed in Table (2).

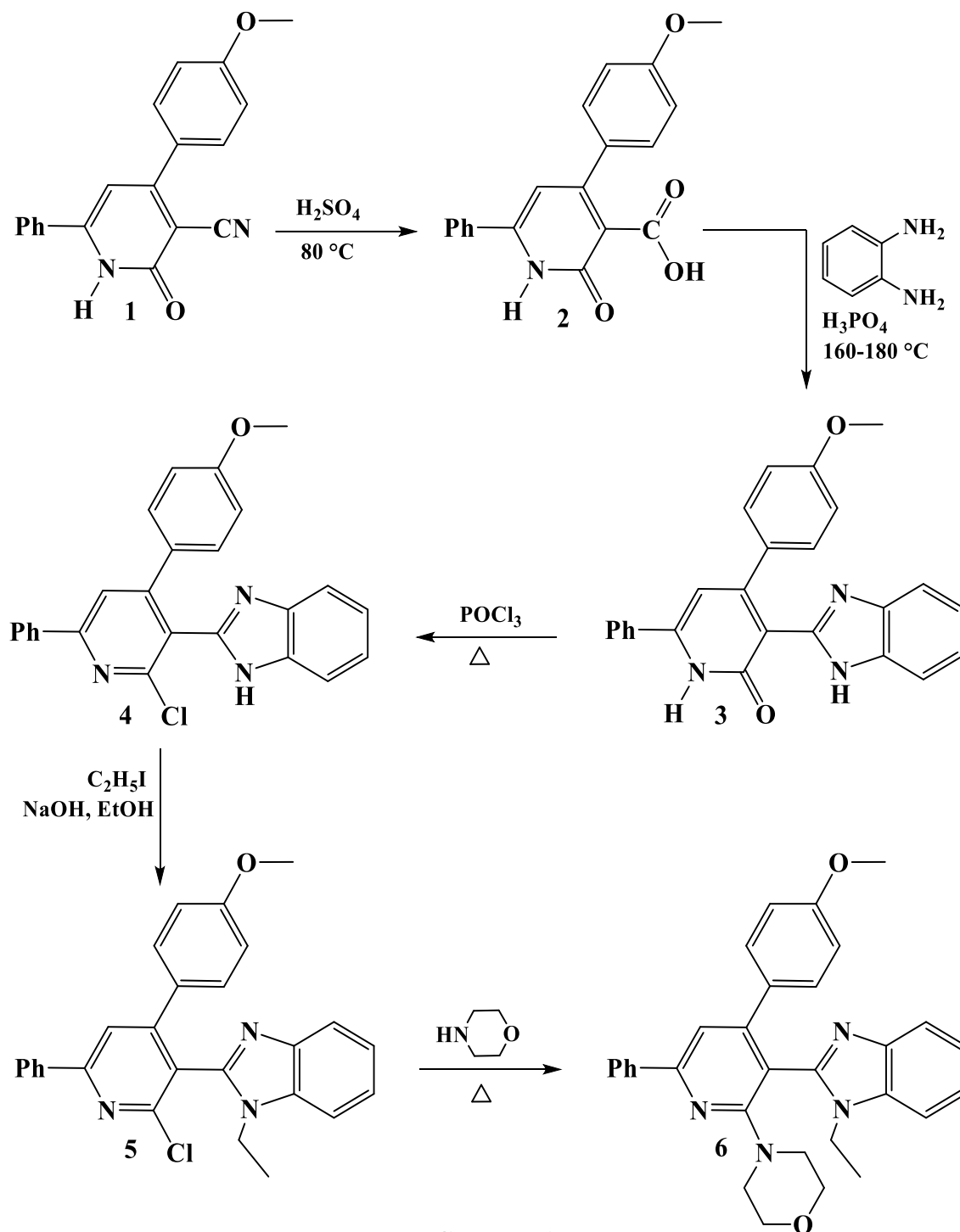
RESULTS AND DISCUSSION

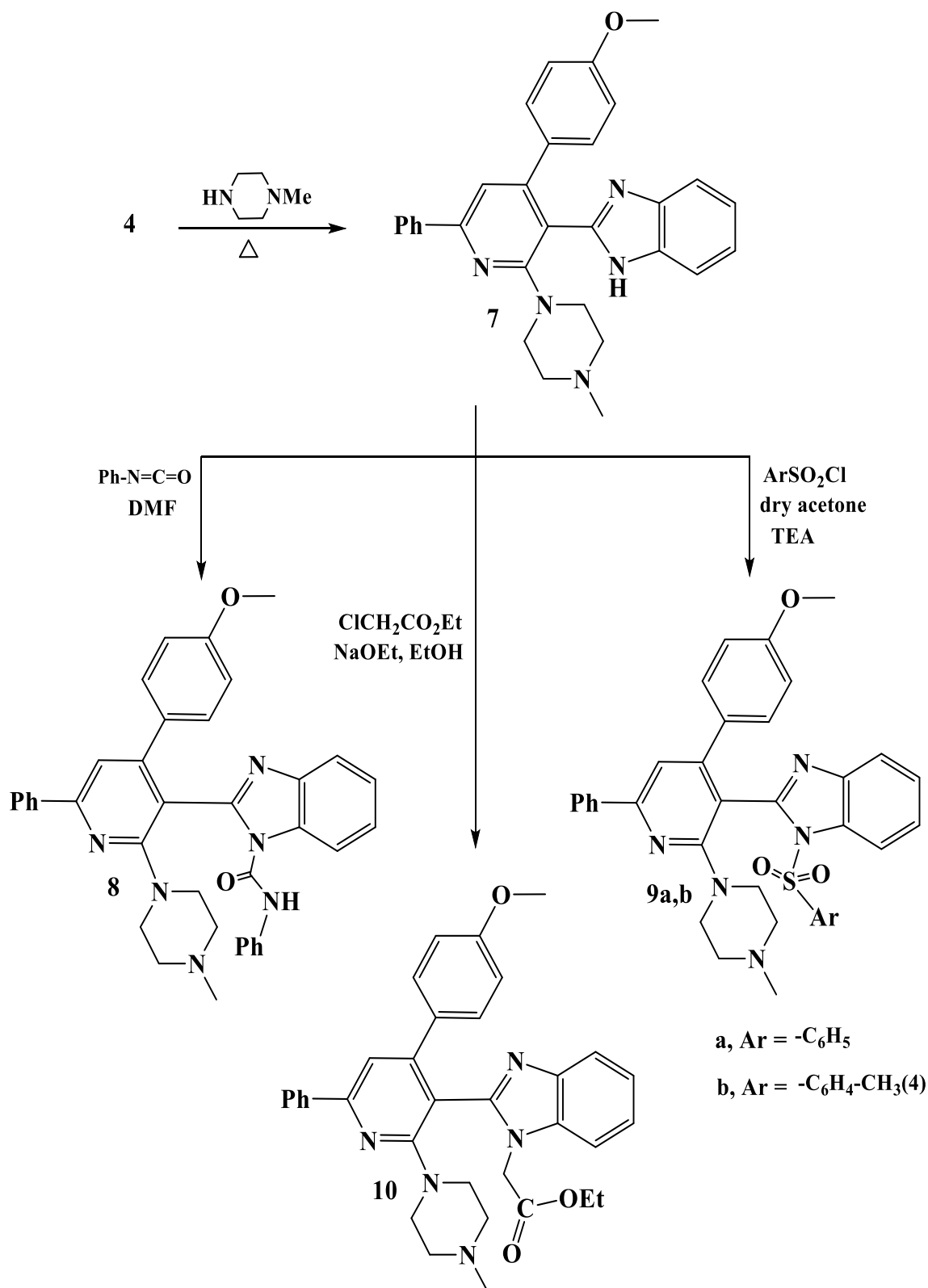
Chemistry

The synthetic approaches of the target 1,2-disubstituted benzimidazole derivatives have been shown in Schemes 1-3. Starting with 2-pyridone 3-carbonitrile derivative, 4-(4-methoxyphenyl)-2-oxo-6-phenyl-1,2-dihydropyridine-3-carbonitrile (**1**), which prepared as the reported method [49]. Upon heating of the nitrile derivative **1** in sulphuric acid, the cyano group underwent acid hydrolysis to give the new pyridone acid derivative **2** [50]. IR spectrum of compound **2** exhibited broad absorption band at 3415-3224 cm^{-1} and strong band at 1685 cm^{-1} corresponding to carboxyl O-H and carboxyl C=O groups, respectively. Cyclocondensation reaction of the pyridone acid derivative **2** with o-phenylenediamine via heating in phosphoric acid afforded the benzimidazole-pyridone conjugate **3**. IR spectrum of compound **3** revealed the disappearance of two absorption bands corresponding to O-H and C=O groups. Also, ^{13}C NMR spectrum of compound **3** represented sixteen signals at the range 104.42-160.65 due to the aromatic-C and a signal at 164.31 corresponding to pyridone C=O group. On further reaction of compound **3** with $\text{PCl}_5/\text{POCl}_3$ mixture, chloro-dehydroxylation reaction of the pyridone moiety was performed to give 2-(2-chloro-4-(4-methoxyphenyl)-6-phenylpyridin-3-yl)-1*H*-benzo[d]imidazole (**4**). Subsequent reaction of compound **4** with ethyl iodide in basic medium afforded alkylation at position-1 of the benzimidazole moiety to give, the target 1,2-disubstituted benzimidazole derivative, 2-(2-chloro-4-(4-methoxyphenyl)-6-phenylpyridin-3-yl)-1-ethyl-1*H*-benzo[d]imidazole (**5**). ^1H NMR spectrum of compound **5** revealed, in addition to the parent protons, a triplet signal at δ 1.31 and quartet signal at δ 4.04 ppm of NCH_2CH_3 moiety. Owing to the high therapeutic values of morpholine or piperazine ring [51], the chloro derivative **5** was subjected to nucleophilic substitution reaction through heating with morpholine to give 4-(3-(1-ethyl-1*H*-benzo[d]imidazol-2-yl)-4-(4-methoxyphenyl)-6-phenylpyridin-2-yl) morpholine (**6**). ^1H NMR spectrum of compound **6** displayed the protons of morpholine ring as two triplet signals at δ 3.62 and 3.89 ppm due to 2NCH_2 and 2OCH_2 , respectively (Scheme 1).

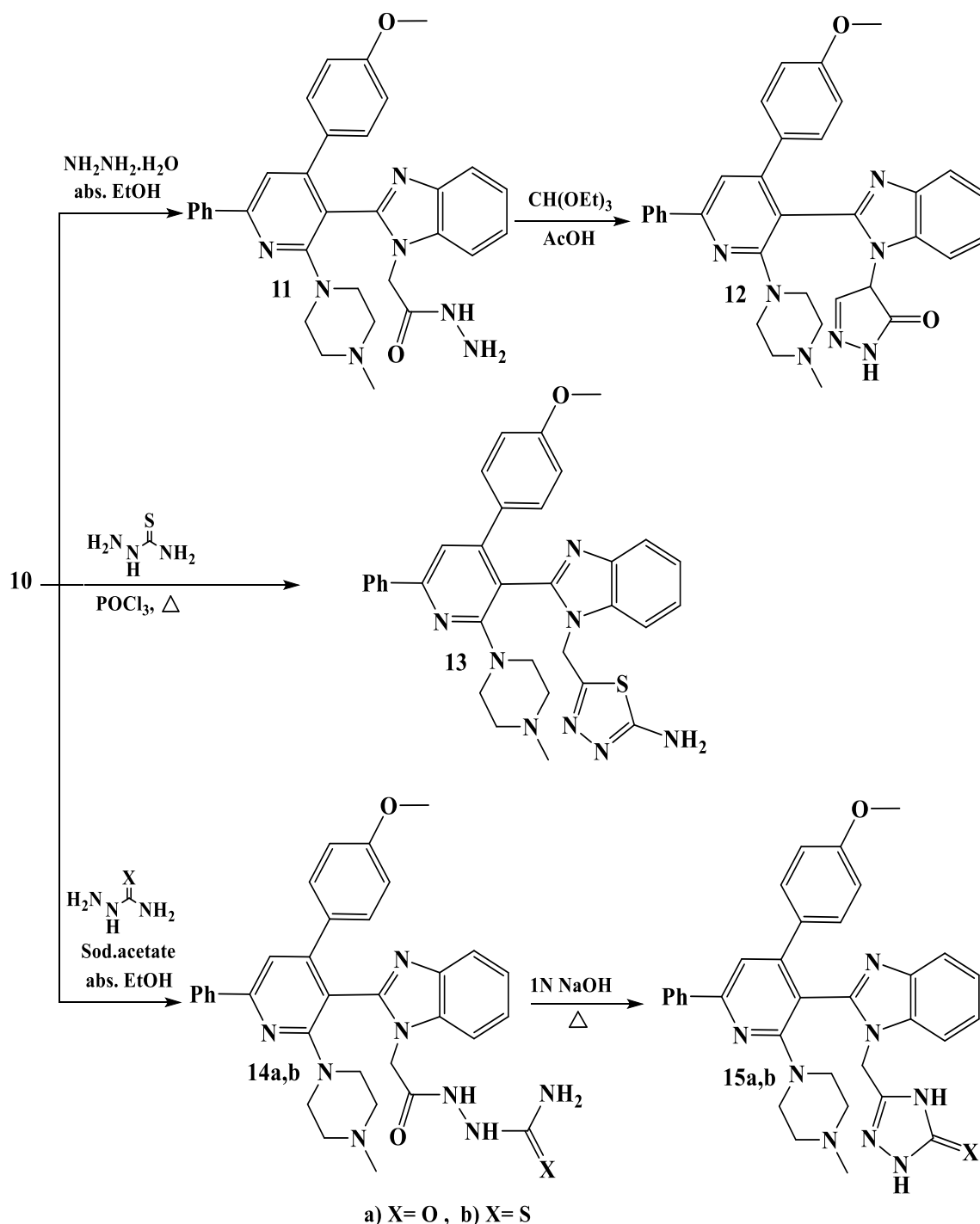
At the same time, the synthesis of 2-(4-(4-methoxyphenyl)-2-(4-methylpiperazin-1-yl)-6-phenylpyridin-3-yl)-1*H*-benzo[d]imidazole (**7**) was achieved via heating of the chloro derivative **4** with 1-methylpiperazine. ^{13}C NMR spectrum of compound **7** represented three signals at δ 46.42, 49.25, and 54.99 ppm corresponding to NCH_3 , 2NCH_2 , and 2NCH_2 of the methylpiperazine moiety, respectively. Then, compound **7** was utilized as a key intermediate for the synthesis of different 1,2-disubstituted benzimidazole derivatives as follows: in order to link position-1 of the benzimidazole nucleus with N-phenylcarboxamide moiety, compound **7** was reacted with phenylisocyanate in refluxing DMF to give 2-(4-(4-methoxyphenyl)-2-(4-methylpiperazin-1-yl)-6-phenylpyridin-3-yl)-*N*-phenyl-1*H*-benzo[d]imidazole-1-carboxamide (**8**). IR spectrum of **8** revealed an absorption band at 1643 cm^{-1} attributed to (C=O, amide). In addition, nucleophilic substitution reaction of compound **7** with benzenesulfonyl chloride and/or 4-methylbenzene sulfonyl chloride in refluxing dry acetone containing triethylamine afforded 2-(4-(4-methoxyphenyl)-2-(4-methylpiperazin-1-yl)-6-phenylpyridin-3-yl)-1-(phenylsulfonyl)-1*H*-benzo[d]imidazole (**9a**) and 2-(4-(4-methoxyphenyl)-2-(4-methylpiperazin-1-yl)-6-phenylpyridin-3-yl)-1-(4-methylphenylsulfonyl)-1*H*-benzo[d]imidazole (**9b**), respectively.

IR spectrum of compound **9a** revealed two absorption bands at 1395, and 1177 cm^{-1} related to (SO_2) stretching vibration. Also, ^1H NMR spectrum of compound **9b** displayed singlet signal at δ 2.84 ppm corresponding to the protons of CH_3 of tosyl moiety. Also, for the purpose of introducing the ethyl acetate moiety at position-1 of benzimidazole ring, compound **7** reacted with ethyl chloroacetate in sodium ethoxide solution to give ethyl 2-(2-(4-(4-methoxyphenyl)-2-(4-methylpiperazin-1-yl)-6-phenylpyridin-3-yl)-1*H*-benzo[d]imidazol-1-yl)acetate (**10**). IR spectrum of **10** showed an absorption band at 1746 cm^{-1} corresponding to ($\text{C}=\text{O}$, ester). ^1H NMR spectrum of compound **10** displayed a triplet signal at δ 1.22 ppm, singlet signal at δ 3.88 ppm, and quartet signal at δ 4.21 ppm pertaining to CH_3 , $\text{CH}_2\text{C}=\text{O}$, and CH_2O of the ethyl acetyl moiety, respectively (Scheme 2).





Scheme2



Scheme 3

Moreover, the condensation reaction of the 1,2-disubstituted benzimidazole ester derivative **10** with hydrazine hydrate in absolute ethanol yielded the acetohydrazone derivative **11**. IR spectrum of compound **11** exhibited an absorption band at 1648 cm^{-1} related to C=O group of the acetohydrazone moiety. Next, cyclocondensation reaction of compound **11** with triethyl orthoformate in glacial acetic acid afforded 4-(2-(4-(4-methoxyphenyl)-2-(4-methylpiperazin-1-yl)-6-phenylpyridin-3-yl)-1H-benzo[d]imidazol-1-yl)-2,4-dihydro-3H-pyrazol-3-one (**12**). IR spectrum of compound **12** showed an absorption band at 1672 cm^{-1} corresponding to C=O group of the pyrazolone ring. Additionally, ^1H NMR spectrum of **12** displayed doublet signal at δ 4.63 ppm due to (CHC=O, pyrazolone). Also, the ester derivative **10** was heated with thiosemicarbazide in phosphorus

oxychloride to give 5-((2-(4-(4-methoxyphenyl)-2-(4-methylpiperazin-1-yl)-6-phenylpyridin-3-yl)-1H-benzo[d]imidazol-1-yl)methyl)-1,3,4-thiadiazol-2-amine (**13**). IR spectrum of compound **13** showed the disappearance of the ester C=O band, and ¹H NMR spectrum revealed broad singlet signal at 6.22 ppm due to NH₂ group of the 1,3,4-thiadiazol-2-amine moiety, which exchanged with D₂O. Furthermore, the ester derivative **10** was condensed with semicarbazide hydrochloride and/or thiosemicarbazide in ethanol containing sodium acetate anhydrous to afford 1-(2-(2-(4-(4-methoxyphenyl)-2-(4-methylpiperazin-1-yl)-6-phenylpyridin-3-yl)-1H-benzo[d]imidazol-1-yl)acetyl)semicarbazid (**14a**) and 1-(2-(2-(4-(4-methoxyphenyl)-2-(4-methylpiperazin-1-yl)-6-phenylpyridin-3-yl)-1H-benzo[d]imidazol-1-yl)acetyl)thiosemicarbazide (**14b**), respectively. Then, the semicarbazide derivative **14a** and the thiosemicarbazide derivative **14b** were subjected to cyclocondensation reaction upon heating in basic medium (1N NaOH) to yield 5-((2-(4-(4-methoxyphenyl)-2-(4-methylpiperazin-1-yl)-6-phenylpyridin-3-yl)-1H-benzo[d]imidazol-1-yl)methyl)-2,4-dihydro-3H-1,2,4-triazol-3-one (**15a**) and 5-((2-(4-(4-methoxyphenyl)-2-(4-methylpiperazin-1-yl)-6-phenylpyridin-3-yl)-1H-benzo[d]imidazol-1-yl)methyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione (**15b**), respectively. IR spectrum of **15a** showed an absorption band at 1657 cm⁻¹ corresponding to C=O group of the 1,2,4-triazol-3-one ring. While, IR spectrum of **15b** displayed an absorption band at 1230 cm⁻¹ attributed to C=S group of the 1,2,4-triazol-3-thione ring (Scheme 3). The molecular structures of the new compounds (**2-15a,b**) have been validated, since they are in conformity with the elemental analysis values and spectral data (IR, NMR, MS) that have been performed for these compounds.

Cytotoxicity evaluation

Cytotoxicity results of the new benzimidazole derivatives (**3-15a,b**) against HepG-2 and MCF-7 cell lines, as shown in Table (1), revealed that many of them have promising anticancer activity and the IC₅₀ values of the compounds diverged according to their structure variations. The parent benzimidazole-pyridone compound **3** exhibited valuable cytotoxicity against both cell lines with IC₅₀ values 40.70 μM and 30.90 μM. The conversion of the pyridone moiety to pyridine ring as in compound **4** caused a dramatic decreasing in cytotoxicity to give IC₅₀ values 81.20 μM and >100 μM against HepG-2 and MCF-7 cell lines, respectively. Also, the target 1,2-disubstituted benzimidazole derivative **5**, which have an ethyl group at position-1 showed decreasing in activity against HepG-2 (IC₅₀ = 97.70 μM) and increasing in activity against MCF-7 (IC₅₀ = 81.30 μM). While, attaching morpholine ring to the pyridine moiety to give 4-(3-(1-ethyl-1H-benzo[d]imidazol-2-yl)-4-(4-methoxyphenyl)-6-phenylpyridin-2-yl)morpholine (**6**), led to a significant increase in anticancer activity, where the IC₅₀ values decreased to be (40.70 μM, 51.30 μM) for HepG-2 and MCF-7 cell lines, respectively. Similarly, the bioactive piperazine ring resulted in great improvement in the cytotoxic activity when it has been linked to the pyridine moiety to give compound **7**, which showed potent cytotoxic activity with IC₅₀ values (14.45 μM, 12.30 μM) compared with those of 5-fluorouracil (26.06 μM, 23.14 μM) against HepG-2 and MCF-7 cell lines, respectively.

Moreover, the target 1,2-disubstituted benzimidazoles (**8-15a,b**) gave cytotoxic activities characterized by the disparity in strength due to the variation of the substituent at position-1. The amide, N-phenyl-1H-benzo[d]imidazole-1-carboxamide derivative **8**, showed the most potent cytotoxicity among all the target compounds with equal IC₅₀ value (13.50 μM) against the two cell lines. While, 1-arylsulfonyl-1H-benzo[d]imidazole derivatives **9a,b** revealed weak cytotoxicity against both cell lines, where their IC₅₀ values exceeded 100 μM. Additionally, ethyl 2-(1H-benzo[d]imidazol-1-yl)acetate derivative **10** showed valuable anticancer activity, its IC₅₀ values against HepG-2 and MCF-7 cell lines were 28.80 μM and 32.30 μM, respectively. Then, upon conversion of the ethyl acetate moiety to acetohydrazide to yield 2-(1H-benzo[d]imidazol-1-yl)acetohydrazide derivative **11**, the cytotoxic activity decreased to great extent and the IC₅₀ values became above 100 μM. Furthermore, cyclization of the acetohydrazide derivative **11** to afford the 4-(1H-benzo[d]imidazol-1-yl)-2,4-dihydro-3H-pyrazol-3-one **12** resulted in the improvement of the anticancer activity and the IC₅₀ values were decreased to be 42.70 μM against HepG-2 and 69.00 μM against MCF-7. On the contrary, cyclization of ester derivative **11** to form 1,3,4-thiadiazole derivative **13**, led to significant decrease in the activity against the two cell lines with IC₅₀ values (>100 μM). Moreover, semicarbazide **14a** and thiosemicarbazide **14b** derivatives showed potent anticancer activity than that of the reference drug, they gave IC₅₀ values (24.50 μM, 20.40 μM) against HepG-2 and (24.00 μM, 21.40 μM) against MCF-7, respectively. Whereas, substantial drop in cytotoxicity was occurred upon cyclocondensation of compounds **14a,b** to give 1,2,4-triazol-3-one derivative **15a** and 1,2,4-triazol-3-thione derivative **15b**, the IC₅₀ values increased to be (40.70 μM, 75.80 μM) for **15a** and (49.00 μM, 97.70 μM) for **15b** against HepG-2 and MCF-7, respectively.

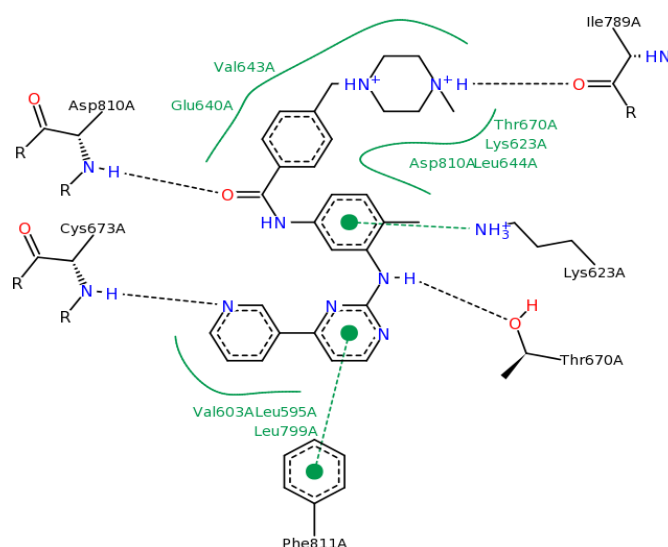
Table 1: Cytotoxic evaluation of the newly synthesized benzimidazole compounds against HepG-2 and MCF-7 cell lines.

Compound No.	IC ₅₀ (μM) HepG-2	IC ₅₀ (μM) MCF-7
3	40.70 ± 0.36	30.90 ± 0.22
4	81.20 ± 0.46	>100
5	97.70 ± 0.82	81.30 ± 0.42
6	40.70 ± 0.32	51.30 ± 0.38
7	14.45 ± 0.12	12.30 ± 0.09
8	13.50 ± 0.21	13.50 ± 0.15
9a	>100	>100
9b	>100	>100
10	28.80 ± 0.21	32.30 ± 0.29
11	>100	>100
12	42.70 ± 0.31	69.00 ± 0.51
13	>100	>100
14a	24.50 ± 0.15	24.00 ± 0.19
14b	20.40 ± 0.11	21.40 ± 0.19
15a	40.70 ± 0.35	75.80 ± 0.32
15b	49.00 ± 0.39	97.70 ± 0.52
5-FU	26.06 ± 0.43	23.14 ± 0.12

Molecular docking studies

Docking study using MOE 2008.10 program was performed to predict the binding modes and orientation of the new benzimidazole compounds (**3-15a,b**) at the ATP binding site of c-kit tyrosine kinase. The coordinates of the c-kit tyrosine kinase structure were obtained from the crystal structure of c-kit tyrosine kinase with its inhibitor (PDB ID: 1T46). The root mean square difference (RMSD) between the top docking pose and original crystallographic geometry of co-crystallized ligand **STI-571** was 0.8 Å°.

The ligand **STI-571** binds to c-Kit through hydrogen bonding as follows: one hydrogen bond between the backbone amide of c-Kit residue **Cys673** and the pyridine ring of the ligand at the N atom, the second hydrogen bond between the O atom of the side chain of the gatekeeper residue **Thr670** and the N atom of the amino group of the ligand aminopyrimidine moiety, and another hydrogen bond between the backbone amide of **Asp810** in the c-Kit **DFG** motif and the peptide bond of **STI-571**. While, there is no specific interactions between the piperazine ring of **STI-571** and the protein [48] (Fig. 1).


Fig. 1: 2D diagram showing the proposed binding mode of the ligand STI-571 in the active site of c-kit tyrosine kinase.

From Table (2), it was found that the compounds under study exhibited good fitting inside the binding site of the protein molecular surface with low binding energy ranged from (-5.17 to -8.43 kcal/mol) compared with that of the ligand **STI-571** (-6.38 kcal/mol). Obviously, compounds **7** and **8** showed the greater binding affinity with docking scores -7.24 and -8.43 kcal/mol, respectively. They formed hydrogen bond acceptors between nitrogen of piperazine and the backbone of **Ile789**. Additionally, Arene-cation interaction was appeared in compound **7** between the centroid of the phenyl group of the benzimidazole nucleus and **Arg791** (Fig. 2 and 3).

Table 2: Docking results of the benzimidazole derivatives (3-15a,b) with c-kit tyrosine kinase in comparison with the ligand STI-571, using MOE software version 2008.10.

Compd. NO.	Docking score (Kcal/mol)	Amino acid residues (bond length Å)	Atoms of compound	Type of bond
STI-571	-6.38	Lys623 Thr670; Cys673; Ile789; Asp810; Phe811	C ₆ H ₃ -4-CH ₃ H(NH) N(pyridine); HN ⁺ (piperazine); O(CO); Pyrimidine	Arene-cation HB HB HB HB Arene-Arene
3	-6.32	Glu640(2.8); His790	O(pyridone); imidazole	H-don Arene-Arene
4	-5.22	Ala636	Pyridine	Arene-Arene
5	-5.46	Ala636	Pyridine	Arene-Arene
6	-6.22	Ile571(2.6)	O(morphine)	H-don
7	-7.24	Ile789(3.2); Arg791	N(piperazine); C ₆ H ₄ (benzoimidazole)	H-acc Arene-cation
8	-8.43	Ile789(3)	N(piperazine)	H-acc
9a	-5.25	Ile808(2.8)	O(sulfonylbenzene)	H-don
9b	-5.17	Ile808(2.8)	O(sulfonylbenzene)	H-don
10	-6.14	Ile789(3.2)	N(piperazine)	H-acc
11	-5.48	Ile571(2.7)	H(NH ₂)	H-don
12	-6.39	Asp792(2.3)	O(pyrazolone)	H-don
13	-5.45	Ile571(2.4)	H(NH ₂)	H-don
14a	-6.98	Asp792(2.2); Asp810(2.2); Asp810(2.3)	H(NH ₂); H(NH ₂); H(NH)	H-don H-don H-don
14b	-7.20	Asp792(2); Asp810(1.5); Asp810(2.1)	H(NH ₂); H(NH ₂); H(NH)	H-don H-don H-don
15a	-6.26	Asp792	Triazole	Arene-Arene
15b	-6.75	Asp792	Triazole	Arene-Arene

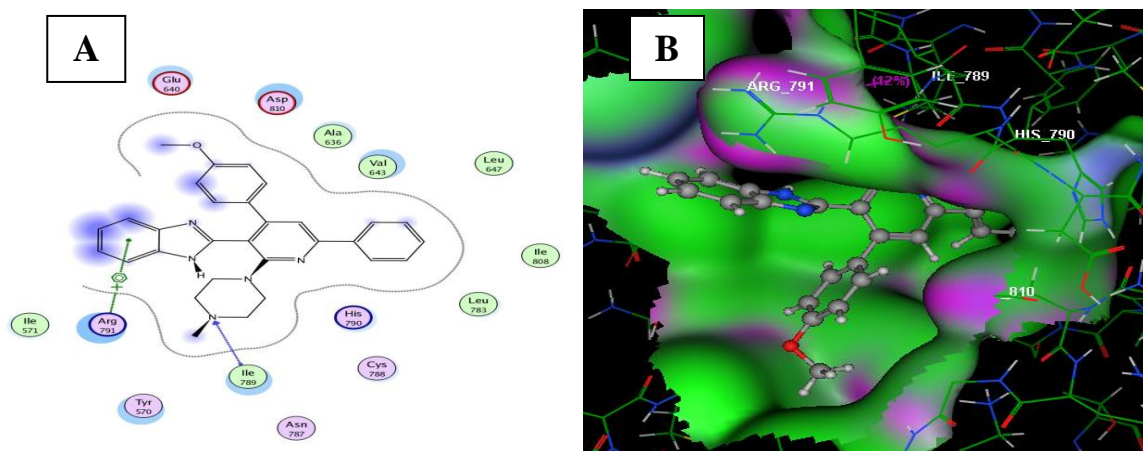


Fig. 2: The proposed binding mode of compound 7 docked in the active site of c-kit; A and B showing 2D and 3D ligand-receptor interactions (hydrogen bonds are illustrated as arrows; C atoms are colored gray, N blue and O red).

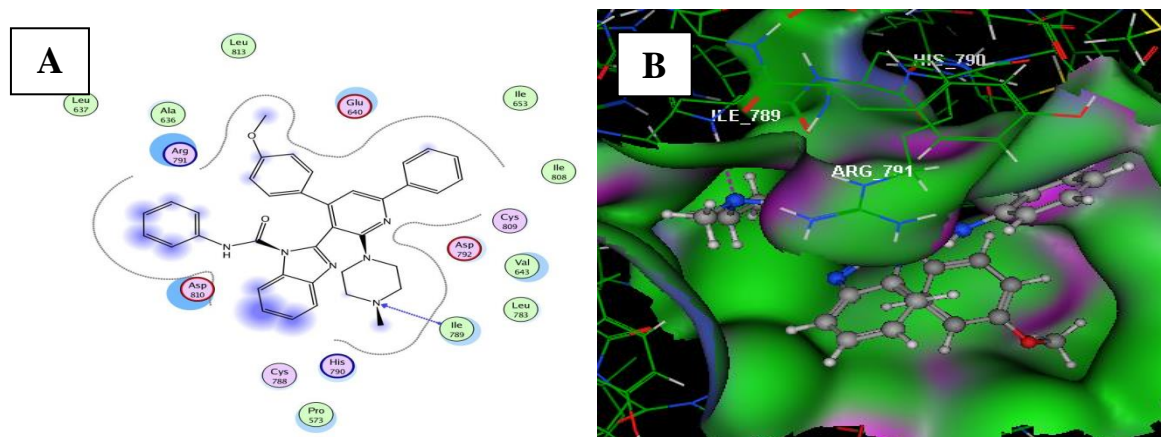


Fig.3: The proposed binding mode of compound 8 docked in the active site of c-kit; A and B showing 2D and 3D ligand-receptor interactions (hydrogen bonds are illustrated as arrows; C atoms are colored gray, N blue and O red).

Moreover, compounds **14a,b** showed good binding affinity with docking scores -6.98 and -7.20 kcal/mol, respectively. In both compounds, two H-bonds was linking NH₂ group as H-donors with side chains of **Asp792** and **Asp810**. Furthermore, **Asp810** is connected to NH of the side chain at position-1 of benzimidazole moiety by hydrogen bond donor (Fig. 4 and 5).

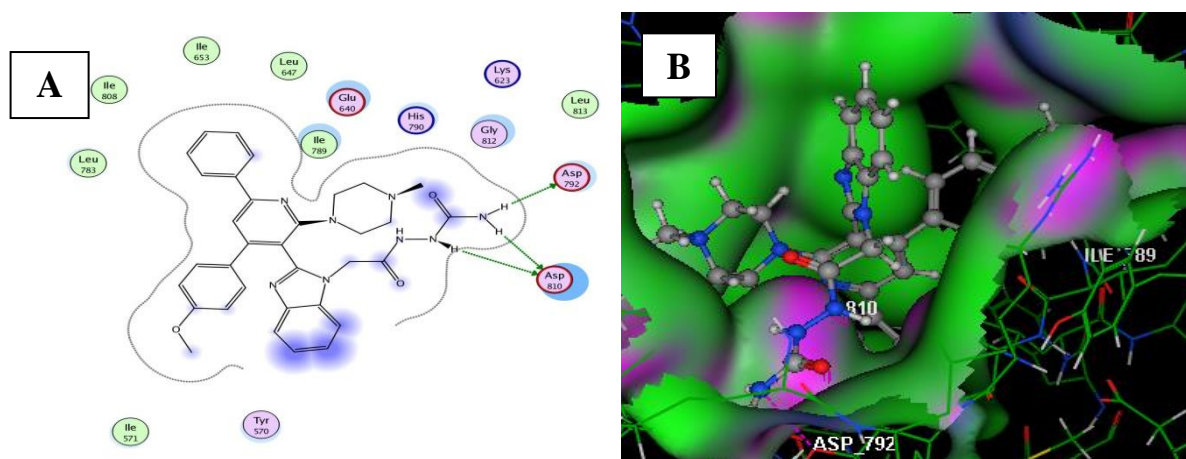


Fig. 4: The proposed binding mode of compound 14a docked in the active site of c-kit; A and B showing 2D and 3D ligand-receptor interactions (hydrogen bonds are illustrated as arrows; C atoms are colored gray, N blue and O red).

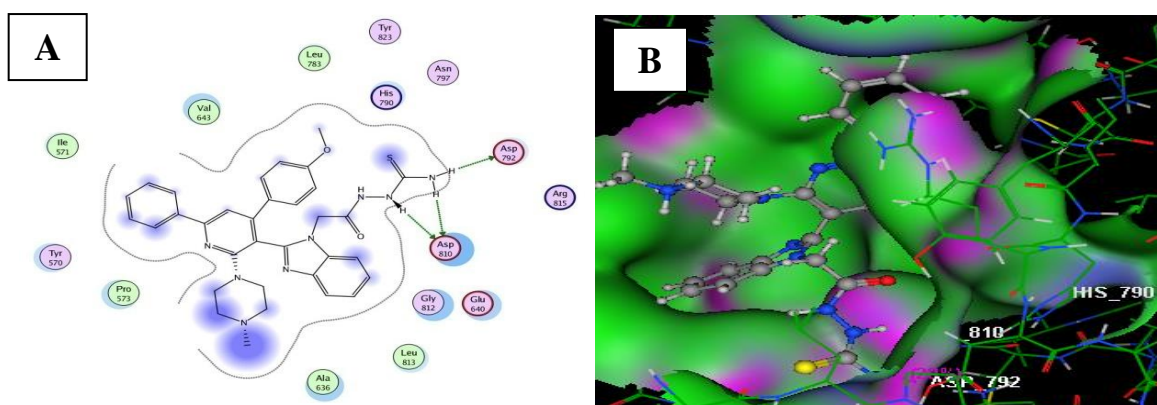


Fig. 5: The proposed binding mode of compound 14b docked in the active site of c-kit; A and B showing 2D and 3D ligand-receptor interactions (hydrogen bonds are illustrated as arrows; C atoms are colored gray, N blue and O red).

Furthermore, compounds **3**, **6**, **10**, **12** and **15a,b** showed reasonable docking scores ranged from -6.14 to -6.75 kcal/mol. While, compounds **4**, **5**, **9a;b**, **11** and **13** gave the lower binding affinity with docking scores ranged from -5.17 to -5.48 kcal/mol.

In this context, molecular modeling studies of the compounds are consistent with their pharmacological activity. As compounds (**7**, **8**, and **14a,b**), which gave the most potent cytotoxicity against HepG-2 and MCF-7 cell lines, showed lowest binding energy with c-Kit compared to all other compounds.

CONCLUSIONS

The purpose of the present work is the synthesis of new 1,2-disubstituted benzimidazole derivatives, which are projected to show potent anticancer activity. Thus, the parent 2-substituted benzimidazole derivative **3** was subjected to a series of various reactions to get the target compounds. Beside the 2,4,6-trisubstituted pyridine moiety at position-2, the target compounds were linked at position-1 to different pharmacological active chains or heterocyclic rings. *In vitro* cytotoxic evaluation of all the novel benzimidazole derivatives (**3-15a,b**) against hepatocellular carcinoma (HepG-2) and breast carcinoma (MCF-7) cell lines, showed that many of them have apparent cytotoxic activity against both cell lines and their cytotoxicity largely changed with structural variations. Moreover, compounds (**7**, **8** and **14a,b**) showed more potent cytotoxicity than that of the reference drug (5-FU). In addition, molecular docking results for all the new compounds revealed good binding affinity to the active site of c-kit tyrosine kinase compared with the ligand **STI-571**. Consequently, this new category of benzimidazole derivatives could be considered as a promising anticancer agents and need further research in order to prepare other derivatives that may have more potent cytotoxic activity.

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