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Synthesis, antitumor evaluation and molecular modeling study of novel benzimidazoles and pyrazinobenzimidazoles

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ABSTRACT

Some novel benzimidazole and pyrazinobenzimidazole derivatives **5-8** was designed for evaluation of their *in vitro* cytotoxicity studies using MTT-based assay against three cancer cell lines namely, human hepatoma cell line (HepG2), human breast cancer cell line (MCF-7) and kidney of African green monkey (Vero B). Compounds **5a** and **5c-e** exhibit the highest and broad spectrum activities against all of the three cell lines tested when compared with reference drug 5-Fluorouracil (5-FU). 1-(1*H*-Benzimidazol-2-yl)-3-phenylprop-2-en-1-one **5a** showed superior and great potency and lethal effect against HepG2, MCF-7 and Vero B cell lines with IC_{50} values of 2, 1.8 and 3.5µg/ml, respectively, comparable to 5-FU (IC_{50} values of 62, 12 and 13µg/ml, respectively). Moreover, compound **7b** showed potent activity against MCF-7 and Vero B cell lines with IC_{50} values of 2 and 2.5µg/ml, respectively. Docking study of compounds 5a and 7b into the ATP binding site of epidermal growth factor receptor (EGFR) revealed comparable binding manner to an EGFR inhibitor, Erlotinib.

INTRODUCTION

Cancer is the result of uncoordinated and uncontrolled growth of cells. The major cause of death from cancer is metastasis (Vogelstein *et al*., 2013, Su *et al*., 2015)where cancer cells detach themselves from the parent neoplasm, invade the circulation system and spread to other body sites through several pathways (Bagi, 2002, Balmer and Valley, 2002). Cancer is continuing to be a major threat to public health and the severity of the problem, led to an impressive progress in discovering potent anticancer agents. In the scope of recognising different chemical compounds which may act as a lead for designing new antitumor agents, we are interested in this study with benzimidazole derivatives. Considerable attention has been focused on benzimidazole family as one of the bioactive heterocyclic compounds that exhibited a range of biological and clinical applications. They are structural isosters of purine based nucleic acids and can interact with biological macromolecules such as protein, enzymes and receptors (Bansal and Silakari, 2012). Thus benzimidazole nucleus has been confirmed as an important pharmacophore in drug discovery of new antitumor agents (Hida *et al*., 1994, Neff *et al*., 2007, Styskala *et al*., 2008, Shaharyar *et al*., 2010, Hranjec *et al*., 2011, Abdel-Mohsen *et al*., 2010, Rahman and Siddiqui, 2010, Luo *et al*., 2011, Ramla *et al*., 2007, Ng *et al*., 2007, Zhong *et al*., 2009, Refaat, 2010, Ramla *et al*., 2006, Thimmegowda *et al*., 2008, Gowda *et al*., 2009). It is well known that small molecules such as pyridopyrimidines (Cockerill, *et al*., 2001), aminoquinazolines (El-Azab *et al*., 2010, Hamed *et al*., 2013) and benzimidazoles (Soni *et al*., 2012, Boschelli *et al*., 1999) are potent epidermal growth factor receptor-tyrosine kinase (EGFR-TK) inhibitors. Tyrosine kinase is an enzyme that can transfer a phosphate group from ATP to a protein and this is an important mechanism in regulating cell activity such as cell division. The epidermal growth factor receptors (EGFR) are over-expressed in a considerable number of human cancer cells (Cockerill and Lackey, 2002, Wakeling, 2002).

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Therefore, inhibition of EGFR-TK represented a rational approach to cancer therapy. In view of the previous findings, our research aimed at the synthesis of different substituted benzimidazolechalcones and pyrazinobenzimidazole derivatives in an attempt to reach an active antitumor agent with potentiated activity and selectivity toward cancerous cells. Computer docking methodology plays an effective role in the drug design and in the mechanistic examination by putting a molecule into the binding site of the target receptor in a noncovalent manner (Kontoyianni *et al*., 2004). So, the most active compounds were docked into the ATP binding site of EGFR to estimate if these compounds have comparable binding manner to an EGFR inhibitor, Erlotinib (Stamos *et al*., 2002).

EXPERIMENTAL PROTOCOLS

Chemistry

Melting points were recorded using Fisher-Johns apparatus and are uncorrected. IR spectra (KBr) were determined on Mattson 5000 FT-IR spectrometer. Proton magnetic resonance (^{1}HNMR) spectra were recorded on FT-NMR spectrometer (200 MHz) Gemini Varian using $DMSOd_6$ relative to tetramethylsilane (TMS) as internal standard (chemical shifts in δ ppm). Mass spectra (MS) were measured on JEOL JMS-600H spectrometer. Elemental analysis was carried out for C, H and N at the Microanalytical Centre of Cairo University. All reagents were purchased from the Aldrich Chemical Company.

The well-known compounds, 2-(1-hydroxyethyl)-1*H*-benzimidazole (**2**) (Pool *et al*., 1937) and 2-acetyl-1*H*-benzimidazole (**3**) (Selvakumar *et al*., 2012) were prepared according to the reported procedures.

*General procedure for synthesis of 1-(1H-benzimidazol-2-yl)-3-arylprop-2-en-1-ones **(5a-f)**(Scheme 1)*

A mixture of 2-acetyl benzimidazole**3** (1.6 g, 0.01mol) and the appropriate aldehyde **4a-f** (0.01mol) in ethanol (30 ml) and water (30 ml) was stirred for 2 hours. Then 10% sodium hydroxide (3 ml) was added drop-wise to the reaction mixture with continuous stirring for further 6 hours and the separated solid was filtered, dried and crystallized from aqueous ethanol to give compounds **(5a-f).**

*1-(1H-Benzimidazol-2-yl)-3-phenylprop-2-en-1-one**(5a)***

yellow powder; Yield 92%; mp 193–194 °C; ^{1}H NMR ($DMSOd_6$, 200 MHz):δ = 13.66 (1H, s, NH, D_2O exchangeable), 8.53-7.39 (11H, m, Ar-H, HC=CH); Anal. Calcd for $C_{16}H_{12}N_2O$ (%): C, 77.40; H, 4.87; N, 11.28. Found: C, 77.60; H, 5.00; N, 11.58.

*1-(1H-Benzimidazol-2-yl)-3-(4-methoxyphenyl)prop-2-en-1-one **(5b)***

White powder; Yield 93%; mp 171 – 172 °C; IR ν(cm^{-1}); 3255 (N-H), 1664 (C=O); ^{1}H NMR ($DMSOd_6$, 200 MHz):δ = 13.42 (1H, s, NH, D_2O exchangeable), 8.1-7.9 (3H, m, Ar-H), 7.80 (1H, d, C=CH, *J*= 15 Hz), 7.50-7.37 (1H, m, Ar-H), 7.20-7.04 (4H, m, Ar-H), 6.80 (1H, d, CH=CH,J = 15 Hz), 3.84 (3H, s,OCH_3); MS m/z (%) 279.10 (M^++1), 5.15), 278.10 (M^+, 11.34), 160.90 (4.12), 136.10 (12.37), 107.05 (25.77), 104 (19.07), 67 (100); Anal. Calcd for $C_{17}H_{14}N_2O_2$ (%): C, 73.37; H, 5.07; N, 10.07. Found: C, 73.21; H, 4.88; N, 9.81.

*1-(1H-Benzimidazol-2-yl)-3-(4-(trifluoromethyl)phenyl)prop-2-en-1-one **(5c)***

White powder; Yield 87%; mp 210 – 211 °C; ^{1}H NMR ($DMSOd_6$, 200 MHz):δ = 13.57 (1H, s, NH, D_2O exchangeable), 8.34-7.25 (10H, m, Ar-H, HC=CH); MS m/z (%) 317.95 (M^++2, 1.51), 316.95 (M^++1, 12.28), 316 (M^+, 51.77), 315.05 (M^+-1, 5.70), 287.05 (100), 174.05 (0.10), 160 (0.09), 146 (0.64), 117.15 (7.70); Anal. Calcd for $C_{17}H_{11}F_3N_2O$ (%): C, 64.56; H, 3.51; N, 8.86. Found: C, 64.20; H, 3.37; N, 8.61.

*1-(1H-Benzimidazol-2-yl)-3-(4-fluorophenyl)prop-2-en-1-one **(5d)***

White powder; Yield 91%; mp 188–189 °C; IR ν(cm^{-1}); 3267 (N-H), 1653 (C=O); ^{1}H NMR ($DMSOd_6$, 200 MHz):δ = 13.44 (1H, s, NH, D_2O exchangeable), 8.17-6.91 (10H, m, Ar-H, HC=CH); MS m/z (%) 267.95 (M^++2, 1.11), 266.95 (M^++1, 7.34), 265.95 (M^+, 38.62), 265.05 (M^+-1, 2.57), 246 (100), 185.05 (1.82), 158 (1.05), 148.95 (22.53), 117 (5.70); Anal. Calcd for $C_{16}H_{11}FN_2O$ (%): C, 72.17; H , 4.16; N, 10.52. Found: C, 72.37; H , 4.33; N, 10.22.

*1-(1H-Benzimidazol-2-yl)-3-(pyridin-3-yl)prop-2-en-1-one **(5e)***

White powder; Yield 89%; mp 168–169 °C; ^{1}H NMR ($DMSOd_6$, 200 MHz):δ = 13.53 (1H, s, NH, D_2O exchangeable), 9.00 (1H, s, Ar-H), 8.70 (1H, m, Ar-H), 8.30 (1H, d, CH=CH, J = 16 Hz), 8.20-8.00 (2H, m, Ar-H), 7.9 (1H, d, CH=CH, J = 16 Hz), 7.6 -6.7 (4H, m, Ar-H); MS m/z (%) 251 (M^++2, 2.43), 250 (M^++1, 8.02), 249 (M^+, 33.31), 248.05 (M^+-1, 3.18), 158 (1.51), 144 (1.90), 132.05 (9.34), 117.05 (5.32), 55 (100), Anal. Calcd for $C_{15}H_{11}N_3O$ (%): C, 72.28; H, 4.45; N, 16.86. Found: C, 71.98; H, 4.21; N, 16.79.

*1-(1H-Benzimidazol-2-yl)-3-(pyridin-4-yl)prop-2-en-1-one **(5f)***

White powder; Yield 83%; mp 203–204 °C; IR ν(cm^{-1}); 3269 (N-H), 1672 (C=O); ^{1}H NMR ($DMSOd_6$, 200 MHz):δ = 13.27 (1H, s, NH, D_2O exchangeable), 8.80-6.95 (10H, m, Ar-H, HC=CH); MS m/z (%) 250.95 (M^++2, 1.31), 249.90 (M^++1, 3.37), 248.95 (M^+, 12.86), 247.95 (M^+-1, 1.20), 157.95 (0.21), 132 (8.70), 118 (100), 117.05 (6.10), 91 (30.49); Anal. Calcd for $C_{15}H_{11}N_3O$ (%): C, 72.28; H, 4.45; N, 16.86. Found: C, 72.48; H, 4.25; N, 16.99.

*General procedure for synthesis of 1-{1-[2-(4-substitutedphenyl)-2-oxoethyl]-1H-benzimidazol-2-yl}-3-arylprop-2-en-1-ones **(7a-j)** (Scheme 2)*

A mixture of chalcone derivatives **5a-f** (0.006 mol), the appropriate α-bromophenacyl derivative **6a,b** (0.006 mol) and anhydrous potassium carbonate (0.007 mol) in acetone (100 ml)

was stirred at room temperature for 6 hours. The solvent was evaporated under reduced pressure and the residue was triturated with water and filtered. The raw product was crystallized from aqueous ethanol to give compounds **7a–j**.

*1-{1-[2-(4-Chlorophenyl)-2-oxoethyl]-1H-benzimidazol-2-yl}-3-(4-methoxyphenyl)prop-2-en-1-one **(7a).***

White powder; Yield 84%; mp 188–189 °C; IR ν(cm^{-1}); 1692, 1660 (C=O), 1599,1450 (C=N, C=C); ^{1}H NMR ($DMSOd_6$, 200 MHz):δ = 8.36-6.72 (14H, m, Ar-H, CH=CH), 6.07 (2H, s, CH_2CO), 3.66 (3H, s, OCH_3); MS m/z (%) 432.80 (M^++2, 0.37), 431.80 (M^++1, 0.28), 430.24 (M^+, 0.24), 400.80 (0.24), 325.10 (1.68), 320.10 (0.36), 57.05 (100); Anal. Calcd for $C_{25}H_{19}ClN_2O_3$ (%): C, 69.69; H, 4.44; N, 6.50. Found: C, 69.51; H,4.27; N, 6.33.

*1-{1-[2-(4-Bromophenyl)-2-oxoethyl]-1H-benzimidazol-2-yl}-3-(4-methoxyphenyl)prop-2-en-1-one **(7b)***

White powder; Yield 89%; mp 201–202 °C; ^{1}H NMR ($DMSOd_6$, 200 MHz):δ = 8.33-6.58 (14H, m, Ar-H, CH=CH), 5.73 (2H, s, CH_2CO), 3.76 (3H, s, OCH_3); MS m/z (%) 475.25 (M^+, 0.05), 474.10 (M^+-1, 0.04), 369.15 (1.86), 278 (3.47), 248.05 (3.71), 199 (0.92), 138.95 (100); Anal. Calcd for $C_{25}H_{19}BrN_2O_3$ (%): C, 63.17; H, 4.03; N, 5.89. Found: C, 62.91; H, 4.21; N, 5.68.

*1-{1-[2-(4-Chlorophenyl)-2-oxoethyl]-1H-benzimidazol-2-yl}-3-(4-(trifluoromethyl)phenyl)prop-2-en-1-one **(7c)***

White powder; Yield 78%; mp 196–197 °C; IR ν(cm^{-1}); 1694, 1657 (C=O), 1595,1448 (C=N, C=C); ^{1}H NMR ($DMSOd_6$, 200 MHz):δ = 8.32-7.22 (14H, m, Ar-H, CH=CH), 6.06 (2H, s, CH_2CO); MS m/z (%) 468.30 (M^+, 1.14), 467.30 (M^+-1, 3.42), 366.30 (1.05), 316.30 (0.55), 154.05 (1.69), 57.05 (100); Anal. Calcd for $C_{25}H_{16}ClF_3N_2O_2$ (%): C, 64.04; H, 3.44; N, 5.97. Found: C, 63.91; H, 3.40; N, 5.78.

*1-{1-[2-(4-Bromophenyl)-2-oxoethyl]-1H-benzimidazol-2-yl}-3-(4-(trifluoromethyl)phenyl)prop-2-en-1-one **(7d)***

White powder; Yield 80%; mp 246–247 °C; ^{1}H NMR ($DMSOd_6$, 200 MHz):δ = 7.93-7.06 (12H, m, Ar-H), 7.84 (1H, d, J= 16 Hz), 7.08 (1H, d, J= 16 Hz), 6.12 (2H, s, CH_2CO); MS m/z (%) 514.95 (M^++2, 2.74), 513.95 (M^++1, 9.20), 512.95 (M^+, 8.77), 511.90 (M^+-1, 26.90), 395 (3.84), 369 (1.87), 197.95 (2.69), 182.90 (100), 116.05 (5.75); Anal. Calcd for $C_{25}H_{16}BrF_3N_2O_2$ (%): C, 58.50; H, 3.14; N, 5.46. Found: C, 58.21; H, 3.07; N, 5.66.

*1-{1-[2-(4-Chlorophenyl)-2-oxoethyl]-1H-benzimidazol-2-yl}-3-(4-fluorophenyl)prop-2-en-1-one **(7e)***

White powder; Yield 86%; mp 231–232 °C; ^{1}H NMR ($DMSOd_6$, 200 MHz):δ = 7.98-6.88 (14H, m, Ar-H, CH=CH), 6.22 (s, 2H, s, CH_2CO); MS m/z (%) 419.10 (M^++1, 4.07), 418.20 (M^+, 4.07), 400.20 (0.38), 290 (0.62), 267.20 (0.62), 69.05 (100); Anal. Calcd for $C_{24}H_{16}ClFN_2O_2$ (%): C, 68.82; H, 3.85; N, 6.69. Found: C, 69.02; H, 3.45; N, 6.31.

*1-{1-[2-(4-Bromophenyl)-2-oxoethyl]-1H-benzimidazol-2-yl}-3-(4-fluorophenyl)prop-2-en-1-one **(7f)***

White powder; Yield 81%; mp 256–257 °C; IR ν(cm^{-1}); 1693, 1655 (C=O), 1599,1450 (C=N, C=C); ^{1}H NMR ($DMSOd_6$, 200 MHz):δ = 8.18-6.93 (12H, m, Ar-H), 7.22 (1H, d, CH=CH,J = 15 Hz), 7.05 (1H, d, CH=CH,J = 15 Hz), 5.66 (2H, s, CH_2CO); MS m/z (%) 463.80 (M^++1, 1.51), 462.80 (M^+, 0.82), 461.90 (M^+-1, 1.69), 307.90 (1.89), 265.05 (8.12), 149.05 (15.79), 118.05 (100); Anal. Calcd for $C_{24}H_{16}BrFN_2O_2$ (%): C, 62.22; H, 3.48; N, 6.05. Found: C, 62.40; H, 3.21; N, 6.39.

*1-{1-[2-(4-Chlorophenyl)-2-oxoethyl]-1H-benzimidazol-2-yl}-3-(pyridin-3-yl)prop-2-en-1-one **(7g)***

White powder; Yield 81%; mp 175–176 °C; ^{1}H NMR ($DMSOd_6$, 200 MHz):δ = 8.62-6.91 (14H, m, Ar-H, CH=CH), 6.28 (2H, s, CH_2CO); MS m/z (%) 404.20 (M^++2, 0.08), 403.20 (M^++1, 0.14), 402.20 (M^+, 0.17), 401.20 (M^+-1, 0.17), 366.85 (0.63), 324.90 (0.09), 290.90 (0.20), 105 (100); Anal. Calcd for $C_{23}H_{16}ClN_3O_2$ (%): C, 68.74; H, 4.01; N, 10.46. Found: C, 68.53; H, 4.39; N, 10.18.

*1-{1-[2-(4-Bromophenyl)-2-oxoethyl]-1H-benzimidazol-2-yl}-3-(pyridin-3-yl)prop-2-en-1-one **(7h)***

White powder; Yield 86%; mp 267–268 °C; ^{1}H NMR ($DMSOd_6$, 200 MHz):δ = 7.91-6.92 (m, 12H, m, Ar-H), 7.78 (1H, d, CH=CH, J= 16 Hz), 7.08 (1H, CH=CH, d, J = 16 Hz), 6.27 (2H, s, CH_2CO); MS m/z (%) 447.80 (M^++2, 3.04), 446.80 (M^++1, 3.61), 445.80 (M^+, 2.92), 369.10 (0.16), 291.10 (0.40), 249.10 (0.12), 1.85 (3.81), 105 (100); Anal. Calcd for $C_{23}H_{16}BrN_3O_2$ (%): C, 61.90; H, 3.61; N, 9.42. Found: C, 62.03; H, 3.30; N, 9.78.

*1-{1-[2-(4-Chlorophenyl)-2-oxoethyl]-1H-benzimidazol-2-yl}-3-(pyridin-4-yl)prop-2-en-1-one **(7i)***

White powder; Yield 79%; mp 228–229 °C; ν(cm^{-1}); 1691, 1657 (C=O), 1563,1453 (C=N, C=C); ^{1}H NMR ($DMSOd_6$, 200 MHz):δ = 8.49-6.82 (14H, m, Ar-H, CH=CH), 6.29 (2H, s, CH_2CO); MS m/z (%) 404.10 (M^++2, 0.51), 403.10 (M^++1, 0.43), 402.10 (M^+, 0.74), 401.10 (M^+-1, 0.36), 367.10 (0.58), 325.10 (0.61), 291.10 (0.84), 55 (100); Anal. Calcd for $C_{23}H_{16}ClN_3O_2$ (%): C, 68.74; H, 4.01; N, 10.46. Found: C, 68.66; H, 3.77; N, 10.23.

*1-{1-[2-(4-Bromophenyl)-2-oxoethyl]-1H-benzimidazol-2-yl}-3-(pyridin-4-yl)prop-2-en-1-one **(7j)***

White powder; Yield 84%; mp 278–279 °C; ^{1}H NMR ($DMSOd_6$, 200 MHz):δ = 8.46-6.96 (12H, m, Ar-H), 7.94 (1H, d, CH=CH, J = 17 Hz), 7.38 (1H, d, CH=CH, J = 17 Hz), 6.30 (2H, s, CH_2CO); MS m/z (%) 446.90 (M^++1, 0.94), 445.90 (M^+, 0.72), 444.90 (M^+-1, 0.86), 316.10 (0.89), 248.10 (0.83), 133.10 (3.55), 55 (100); Anal. Calcd for $C_{23}H_{16}BrN_3O_2$ (%): C, 61.90; H, 3.61; N, 9.42. Found: C, 62.16; H, 3.97; N, 9.23.

*General procedure for synthesis of 1-(2-arylvinyl)-3-(4-substitutedphenyl)pyrazino [1,2-a]benzimidazole derivatives **(8a-j)** (Scheme 2)*

A mixture of compound **7a-j**(0.003 mol) and ammonium acetate (0.03 mol) in 50 ml of acetic acid was refluxed for 1 hour. The reaction mixture was cooled, poured into ice water and neutralized with sodium carbonate solution. The formed precipitate was filtered, dried and crystallized from aqueous ethanol to give compounds **8a-j**.

*1-(2-(4-Methoxyphenyl)vinyl)-3-(4-chlorophenyl)pyrazino[1,2-a]benzimidazole **(8a)***

White powder; Yield 74%; mp 198–199 °C; IR ν(cm^{-1}); 1630, 1453 (C=N, C=C); ^{1}H NMR ($DMSOd_6$, 200 MHz):δ = 8.82 (1H, s, Ar-H), 8.18-6.68 (14H, m, Ar-H, CH=CH), 3.46 (3H, s, OCH_3); MS m/z (%) 413.20 (M^++2, 0.32), 412.20 (M^++1, 0.41), 411.20 (M^+, 0.86), 410.20 (M^+-1, 0.32), 381.20 (0.74), 306.10 (0.67), 301.10 (0.57), 57.05 (100); Anal. Calcd for $C_{25}H_{18}ClN_3O$ (%): C, 72.90; H, 4.40; N, 10.20. Found: C, 73.16; H, 4.07; N, 9.98.

*1-[2-(4-Methoxyphenyl)vinyl]-3-(4-bromophenyl)pyrazino[1,2-a]benzimidazole **(8b)***

White powder; Yield 79%; mp 186–187 °C; ^{1}H NMR ($DMSOd_6$, 200 MHz):δ = 8.72 (1H, s, Ar-H), 8.36-6.72 (14H, m, Ar-H, CH=CH), 3.64 (3H, s, OCH_3); MS m/z (%) 458 (M^++2, 6.62), 457 (M^++1, 17.25), 455.95 (M^+, 42.16), 454.95 (M^+-1, 39.72), 377 (1.39), 352.05 (1.57), 301 (30.14), 63 (100); Anal. Calcd for $C_{25}H_{18}BrN_3O$ (%): C, 65.80; H, 3.98; N, 9.21. Found: C, 65.69; H, 3.81; N, 9.07.

*1-[2-(4-Trifluoromethylphenyl)vinyl]-3-(4-chlorophenyl)pyrazino [1,2-a]benzimidazole **(8c)***

White powder; Yield 81%; mp 213–214 °C; ν(cm^{-1}); 1631,1446 (C=N, C=C); ^{1}H NMR ($DMSOd_6$, 200 MHz):δ = 8.68 (1H, s, Ar-H), 8.16-7.18 (14H, m, Ar-H, CH=CH); MS m/z (%) 451.90 (M^++2, 7.74), 450.59 (M^++1, 6.75), 449.90 (M^+, 19.38), 448.95 (M^+-1, 2.40), 415.20 (0.51), 339.10 (1.60), 305.10 (0.41), 57 (100);Anal. Calcd for $C_{25}H_{15}ClF_3N_3$ (%): C, 66.75; H, 3.36; N, 9.34. Found: C, 66.37; H, 3.01; N, 9.12.

*1-[2-(4-Trifluoromethylphenyl)vinyl]-3-(4-bromophenyl)pyrazino [1,2-a]benzimidazole **(8d)***

White powder; Yield 84%; mp 233–234 °C; ^{1}H NMR ($DMSOd_6$, 200 MHz):δ = 8.81 (1H, s, Ar-H), 7.85-7.12 (14H, m, Ar-H, CH=CH); MS m/z (%) 494.10 (M^+, 22.55), 493.10 (M^+-1, 67.65), 464.10 (72.55), 324.10 (76.47), 291.10 (64.71), 167.10 (100); Anal. Calcd for $C_{25}H_{15}BrF_3N_3$ (%): C, 60.75; H, 3.06; N, 8.50. Found: C, 61.02; H, 3.02; N, 8.02.

*1-[2-(4-Fluorophenyl)vinyl]-3- (4-chlorophenyl) pyrazino[1,2-a]benzimidazole **(8e)***

White powder; Yield 80%; mp 207–208 °C; ^{1}H NMR ($DMSOd_6$, 200 MHz):δ = 8.76 (1H, s, Ar-H), 8.24-6.93 (14H, m, Ar-H, CH=CH); MS m/z (%) 401.20 (M^++2, 0.25), 400.20 (M^++1, 0.32), 399.20 (M^+, 0.23), 381.10 (0.25), 365.10 (0.26), 289.10 (2.13), 55 (100); Anal. Calcd for $C_{24}H_{15}ClFN_3$ (%): C, 72.09; H, 3.78; N, 10.51. Found: C, 71.97; H, 3.67; N, 10.38.

*1-[2-(4-Fluorophenyl)vinyl]-3-(4-bromophenyl)pyrazino[1,2-a]benzimidazole **(8f)***

White powder; Yield 76%; mp 168–169 °C; ν(cm^{-1}); 1630,1454 (C=N, C=C); ^{1}H NMR ($DMSOd_6$, 200 MHz): δ = 8.92 (1H, s, Ar-H), 8.18-6.83 (14H, m, Ar-H, CH=CH); MS m/z (%) 445.90 (M^++2, 0.42), 444.90 (M^++1, 0.51), 443.90 (M^+, 0.53), 350.20 (0.47), 347.20 (0.31), 289.10 (0.51), 169.10 (1.78), 122.10 (4.06), 57 (100); Anal. Calcd for $C_{24}H_{15}BrFN_3$ (%): C, 64.88; H, 3.40; N, 9.46. Found: C, 64.66; H, 3.67; N, 9.18.

*1-[2-(3-Pyridyl)vinyl]-3-(4-chlorophenyl)pyrazino[1,2-a]benzimidazole **(8g)***

White powder; Yield 69%; mp 236–237 °C; ^{1}H NMR ($DMSOd_6$, 200 MHz):δ = 8.62 (1H, s, Ar-H), 8.32-6.94 (14H, m, Ar-H, CH=CH); MS m/z (%) 384.30 (M^++2, 0.58), 383.20 (M^++1, 1.55), 382.30 (M^+, 2.03), 381.15 (M^+-1, 2.39), 350.20 (0.42), 293.20 (0.71), 195 (1.57), 57 (100)' Anal. Calcd for $C_{23}H_{15}ClN_4$ (%): C, 72.16; H, 3.95; N, 14.63. Found: C, 72.46; H, 3.67; N, 15.00.

*1-[2-(3-Pyridyl)vinyl]-3-(4-bromophenyl)pyrazino[1,2-a]benzimidazole **(8h)***

White powder; Yield 73%; mp 223–224 °C; ^{1}H NMR ($DMSOd_6$, 200 MHz):δ = 8.64 (1H, s, Ar-H), 6.94-8.12 (14H, m, Ar-H, CH=CH); MS m/z (%) 428.20 (M^++1, 0.10), 427.20 (M^+, 0.08), 426.20 (M^+-1, 0.09), 351.20 (0.09), 272.10 (0.16), 195.10 (0.43), 69.05 (100)' Anal. Calcd for $C_{23}H_{15}BrN_4$ (%): C, 64.65; H, 3.54; N, 13.11. Found: C, 64.38; H, 3.37; N, 13.01.

*1-[2-(4-Pyridyl)vinyl]-3-(4-chlorophenyl)pyrazino[1,2-a]benzimidazole **(8i)***

White powder; Yield 80%; mp 218–219 °C; IRν(cm^{-1}); 1630,1450 (C=N, C=C); ^{1}H NMR ($DMSOd_6$, 200 MHz):δ = 8.67 (1H, s, Ar-H), 8.18-7.11 (14H, m, Ar-H, CH=CH); MS m/z (%) 383.10 (M^++1, 62.25), 382.10 (M^+, 58.16), 349.10 (2.13), 271.10 (18.44), 244.10 (7.80), 60 (100); Anal. Calcd for $C_{23}H_{15}ClN_4$ (%): C, 72.16; H, 3.95; N, 14.63. Found: C, 72.38; H, 3.57; N, 14.22.

*1-[2-(4-Pyridyl)vinyl]-3-(4-bromophenyl)pyrazino[1,2-a]benzimidazole **(8j)***

White powder; Yield 72%; mp 243–244 °C; ^{1}H NMR ($DMSOd_6$, 200 MHz):δ = 8.68 (1H, s, Ar-H), 8.50-6.80 (14H, m, Ar-H, CH=CH); MS m/z (%); 428.90 (M^++1, 39.51), 427.80 (M^+, 44.70), 426.90 (M^+-1, 47.80), 370.20 (45.85), 254.10 (85.37), 192.10 (42.39), 127.10 (100); Anal. Calcd for $C_{23}H_{15}BrN_4$ (%):C, 64.65; H, 3.54; N, 13.11. Found: C, 64.35; H, 3.42; N, 12.88.

BIOLOGICAL EVALUATION

Cytotoxicity screening

The cytotoxicity screening was done by employing tetrazolium salt MTT assay (Mosmann *et al*., 1983, Denizot and Lang, 1986).Serial dilutions (60 µL) of the test compounds and 5-florouracil dissolved in 0.05% DMSO were given to 120 µL of the suspended cells (50,000 cells/mL) in wells of 96-well plates. The viability of the cells was measured by the colorimetric MTT assay after 5 days of incubation according to the reported procedures. The absorbance of the purple formazan solution was measured at λ_{540} nm by microplate reader (ELx800 Absorbance Microplate Reader, BioTek) against DMSO as a negative control. The cytotoxicity of the tested compounds was expressed as the concentration that resulted in a 50% inhibition of growth (IC_{50}) compared to the untreated cells (DMSO without the tested compounds) and 5- FU. Qualitative morphological study by MTT assay was done for compounds **5c** and **7i** against MCF-7 and HepG2, respectively using high dose of 37 µg/ml and low dose of 4 µg/ml for compound **5c** and doses of 37, 12 and 4 µg/ml for compound **7i**. Images were taken using Gx microscope (GXMGXD202 Inverted Microscope) (10x Eyepiece) and DMSO was used as a negative control.

MOLECULAR DOCKING METHODOLOGY

Docking studies have been carried out using MOE 2008.10 (MOE 2008.10 of Chemical Computing Group. Inc.).The crystal structure of EGFR with Erlotinib (*Tarceva*™) (PDB code: 1M17) were obtained from protein data bank (PDB). Docking study was proceeded according to the standard official procedure of MOE 2008.10 (MOE 2008.10 of Chemical Computing Group. Inc, Halgren, 1996)and the MOE's Pose Viewer utility (El-Azab *et al*., 2010).

RESULTS and DISCUSSION

Chemistry

The reaction sequence used to synthesize the desired compounds is depicted in Schemes 1 and 2.

Synthesis of compounds 2–5 (Scheme 1)

The reported 2-(1-hydroxyethyl)-1*H*- benzimidazole**2** was obtained in a high yield via cyclization of *o*-phenylenediamine with lactic acid in 4N-HCl. Oxidation of 2-(1-hydroxyethyl)-1*H*-benzimidazole **2** with potassium dichromate in concentrated sulphuric acid gave 2-acetyl-1*H*-benzimidazole **3**. Claisen-Schmidt condensation is the most convenient method for preparation of α,β-unsaturated carbonyl compounds in which an equimolar quantity of aromatic aldehyde and aliphatic aldehyde or ketone was condensed in the presence of 10-60% aqueous alcoholic alkali. Hence, condensation of 2-acetyl-1*H*- benzimidazole**3** and a variety of aromatic aldehydes **4a-f** in aqueous ethanolic solution of sodium hydroxide 10% gave 1-(1*H*-benzimidazol-2-yl)-3-arylprop-2-en-1-ones **5a-f**. The structures of the prepared compounds were substantiated by elemental and spectral analyses. For example, in ^{1}H NMR spectrum of compounds **5b** and **5e**, the vinylic protons resonated as two doublets within the aromatic region with *J* value 15 Hz and 16 Hz, respectively and this observation indicated the trans configuration (Silverstein *et al*.,1991).

Synthesis of compounds 7and 8 (Scheme 2)

N-Alkylation of benzimidazoles was successfully achieved via substitution reaction with alkyl halides. Elaboration to compounds **7a-j** was performed by the reaction of compounds **5a-f** with the appropriate α-bromophenacyl derivatives **6a,b** in dry acetone in the presence of anhydrous K_2CO_3. The structures of compounds **7a-j** were confirmed by IR that revealed the presence of two different carbonyl bands due to propenone and oxoethyl residues at about 1660-1655 cm^{-1} and 1694-1691 cm^{-1}, respectively. Also, ^{1}HNMR spectra were characterized by the presence of methylene protons which resonate in aliphatic area in the range of 6.30-5.66 ppm. Moreover, the vinylic protons appeared as two doublets within the aromatic region in the ^{1}H NMR spectra of compounds **7d**, **7f,7h** and **7j** with high values of *J* coupling constant. Hence, it can be concluded that the isomeric form of vinylic group is trans. Cyclization of the diketo compounds **7a-j** was achieved via refluxing with ammonium acetate in acetic acid to afford the corresponding pyrazino derivatives **8a-j**.The chemical structures of the isolated products **8a-j** were confirmed by their spectral and microanalytical data. In IR spectra, the notable feature was the disappearance of the stretching vibrations of the carbonyl groups.In addition, they were confirmed by MS and microanalytical data.

BIOLOGICAL ACTIVITY

In vitro cytotoxicity screening

The *in vitro* cytotoxicity studies of the newly synthesized compounds were performed on three different cancer cell lines, namely human hepatoma cell line (HepG2), human breast cancer cell line (MCF-7) and kidney of African green monkey (Vero B). The quantitative evaluation of the cytotoxicity screening was done by employing tetrazolium salt MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide) assay. MTT assay is applied to assess cytotoxicity and viability of the cells. It is a colorimetric metabolic activity assay that measures the ability of viable cells to reduce a yellow tetrazolium salt to purple insoluble formazan in the mitochondria. Hence, formation of the purple formazan depends on the metabolic activity of the cells and directly related to the number of viable cells. However, the cytotoxicity of the tested compounds affects the metabolic activity of the cells and in turn, decreases the production of formazan. By comparing the amount of the formazan resulted from cells treated with the tested compounds with the amount of formazan resulted from untreated control cells, the cytotoxic effects of the target compounds can be concluded. The coloured formazan solution was measured using a spectrophotometer and the correspondent

compound concentrations were calculated. Linear regression analysis was used to define dose-response curves and to compute the concentration of the tested compounds needed to reduce absorbance of the formazan by 50%. So, the cytotoxicity of the tested compounds was expressed as the concentration that resulted in a 50% inhibition of growth (IC_{50}) compared to the untreated cells (DMSO without the tested compounds) and 5- FU as shown in Table 1.

Table 1: *In vitro* antitumor activities of the designed compounds.

Compound No.	IC_{50} (µg/ml)[a]		
	HepG2[b]	MCF-7[c]	Vero B[d]
5a	2	1.8	3.5
5b	ND[e]	ND[e]	ND[e]
5c	5.5	2	2.5
5d	4	3	4
5e	7	5.5	9
5f	20	25	20
7a	Inactive[f]	20	Inactive[f]
7b	30	2	2.5
7c	ND[e]	ND[e]	ND[e]
7d	ND[e]	ND[e]	ND[e]
7e	30	10	10
7f	20	20	20
7g	30	20	20
7h	30	20	Inactive[f]
7i	20	7	10
7j	30	20	20
8a	Inactive[f]	18	20
8b	18	10	18
8c	ND[e]	ND[e]	ND[e]
8d	ND[e]	ND[e]	ND[e]
8e	20	10	20
8f	ND[e]	ND[e]	ND[e]
8g	Inactive[f]	Inactive[f]	Inactive[f]
8h	18	20	18
8i	Inactive[f]	Inactive[f]	Inactive[f]
8j	Inactive[f]	20	Inactive[f]
5-FU[g]	62	12	13

[a] IC_{50}: Compound concentration required to inhibit tumor cell proliferation by 50%
[b] Human Hepatocellular carcinoma cell line
[c] Human breast adenocarcinoma cell line
[d] Kidney of normal adult African green monkey
[e] Not determined due to solubility problem
[f] Inactive because the IC_{50} values were high
[g] 5-FU: 5-Fluorouracil

For the preliminary characterization of the expected cytotoxicity of the tested compounds, we first examined the changes in cell morphology induced by the treatment under a phase contrast microscope. The purple formazan should be visible inside the cells before dissolving it in isopropanol/HCl solution. A qualitative morphological study of cytotoxicity by MTT assay was applied for compounds **5c** and **7i** using high and low doses and compared with the untreated cells (negative control). Viable cells exhibited deep purple cell nucleus with rod shaped formazon whereas apoptotic cells lacked formazon growth in a dose dependent manner (Figures 1 and 2).

Among the tested compounds, compounds **5a** and **5c-e** showed the highest and broad spectrum activity against the three cancer cell lines. Compound **5a**, with unsubstituted phenyl ring, exploited great potency and lethal effect over HepG2, MCF-7 and Vero B cell lines with IC_{50} values of 2, 1.8 and 3.5 µg/ml, respectively. It is clear that compound **5a** (1.8-3.5µg/ml) is almost 7 to 18 folds as 5-FU (12-62 µg/ml). By comparing the most active compound **5a** with the other members in the same series, we concluded that, substitution of the phenyl ring or replacing it with a pyridyl ring relatively decreased the activity. With regard to the selectivity against individual cell lines, compound **5a** showed selective inhibition against HepG2 and MCF-7 cell lines. However, compound **5c** showed effectiveness against Vero B cell line with IC_{50} reached 2.5 µg/ml. The results of the cytotoxicity of compounds **7a,b**and **7e-j** revealed that there was a decrease in the activity when compared with N-unsubstituted compounds **5**. Compound **7b** showed promising antiproliferative activity against MCF-7 and Vero B cell lines at IC_{50} values of 2 and 2.5 µg/ml, respectively. In addition, both **7e** and **7i** showed good growth inhibition against MCF-7 and Vero B cell lines, while the rest of compounds among this series exerted moderate activity with IC_{50} ranging from 20-30 µg/ml. However, compound **7a** showed activity against only MCF-7 cell line, while compound **7h** gave its activity against only HepG2 and MCF-7 cell lines. A notable decrease in the inhibitory activity was observed in the cyclized pyrazino derivative **8b** against MCF-7 and Vero B cell lines at IC_{50} 10 and 18 µg/ml, respectively in comparison with the activity of its open chain analogue **7b** against the same cell lines (IC_{50} 2 and 2.5 µg/ml, respectively). On the other hand, a little or no change in the activity of most of the cyclized pyrazino derivatives was observed in comparison with that of the corresponding uncyclized α, β-unsaturated ketone derivatives **7**.

The active pyrazino compounds showed good IC_{50} in the range of 10-20 µg/ml. The increased inhibitory activity of compounds **8b**, **8h** and **8j** in comparison to compounds **8a**, **8g** and **8i**, respectively, may reflect the positive impact of the lipophilicity of the molecules resulted from replacement of Cl by Br atom. However, compounds **8g** and **8i** were completely inactive against all of the three cell lines tested.

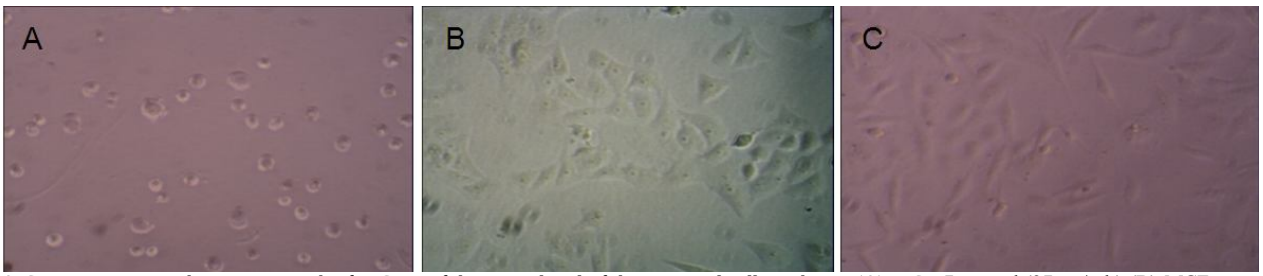


Fig. 1: A-C Representative phase micrograph of MCF-7 of the control and of those treated cells with 5c. (A) MCF-7 treated (37 µg/ml) (B) MCF-7 treated (4 µg/ml) and (C) Control cells, untreated MCF-7 cells.

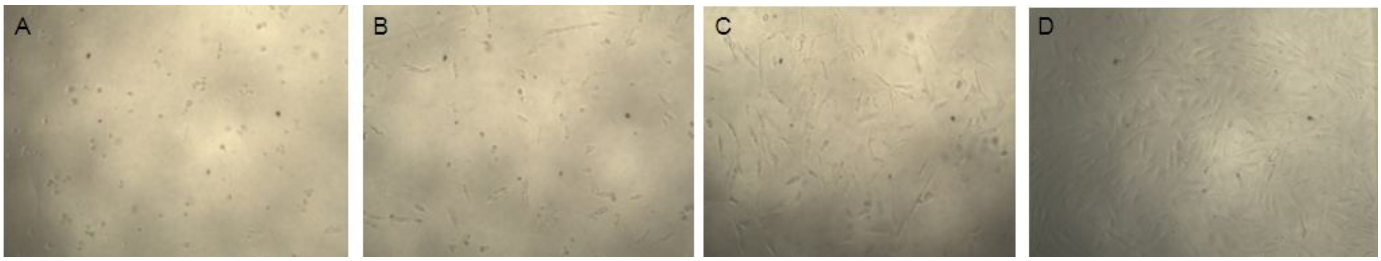


Fig. 2: D-G Representative phase micrograph of HepG2 of the control and of those treated cells with 7i. (D) HepG2 treated (37 µg/ml) (E) HepG2 treated (12 µg/ml) (F) HepG2 treated (4 µg/ml) and (G) Control cells, untreated HepG2 cells.

LIPINSKI'S RULE OF FIVE

Lipinski's rule of five provided very convenient and easily applied guidelines for the selection of compounds with a greater chance of yielding successful drugs that are orally active in human (Lipinski *et al*., 1997). If the rule of five score is greater than one, the compound is unlikely to be further pursued as a potential drug, because it would likely lack properties important in its ADME (Lipinski, 2004). The results showed that all investigated compounds obeyed these rules and should present good passive oral absorption (Table 2).

Table 2: Calculated Lipinski's rule of five for the biologically active compounds

Comp. No.	Parameter				
	M.wt[a]	log p[b]	HBD[c]	HBA[d]	No. of violations
5a	248.28	3.408	1	3	0
5c	316.28	4.304	1	3	0
5d	266.28	3.572	1	3	0
5e	249.27	1.992	1	4	0
5f	249.27	2.119	1	4	0
7a	430.88	5.358	0	5	1
7b	475.34	5.489	0	5	1
7e	418.86	5.465	0	4	1
7f	463.31	5.596	0	4	1
7g	401.85	3.885	0	5	0
7h	446.30	4.017	0	5	0
7i	401.85	4.012	0	5	0
7j	446.30	4.143	0	5	0
8a	411.88	6.927	0	4	1
8b	456.34	7.058	0	4	1
8e	399.85	7.034	0	3	1
8h	427.30	5.586	0	4	1
8j	427.30	5.713	0	4	1

[a] Molecular weight.
[b] An octanol-water partition coefficient.
[c] Number of hydrogen bond donors.
[d] Number of hydrogen bond acceptor.

DOCKING STUDIES

The promising antiproliferative activities of compounds **5a** and**7b** over breast cancer cells stimulated us to study the molecular docking of these compounds into the active site of EGFR, which is over expressed in breast cancer cells. The aim of this study is to reveal if these compounds have comparable binding manner to EGFR inhibitors such as Erlotinib. We assumed that the active target compounds **5a** and **7b** might demonstrate antiproliferative activity against MCF-7 cell line through inhibition of EGFR. The automated docking program of MOE 2008.10 was used to dock compounds **5a** and**7b** into the active binding site of EGFR along with the inhibitor Erlotinib. The calculated binding energies of the docked compounds **5a**, **7b** were -82.10, -71.22 and -114.13 kj/mol, respectively. The carbonyl group of compound **5a** showed two hydrogen bonds with Thr-766 (2.98Å) and HOH-10 (3.03Å) mediated hydrogen bonding interaction with Thr-830 side chain (3.33Å), Gln-767 side chain (3.42Å) and Thr-766 side chain (3.38Å) (Figure 3). Moreover, the benzimidazole ring of compound **5a** binds to a narrow hydrophobic pocket in the N-terminal domain of EGFR-TK similar to quinazoline ring of Erlotinib inhibitor. So, the results from the molecular docking study confirmed that the active compound **5a** may act on the same target receptor as Erlotinib.On the other hand compound**7b** bound with active binding site of EGFRin a different mode of interaction in which the carbonyl groups of both chalcone and phenacyl moieties showed one hydrogen bond with HOH-10 (2.66 and 3.81Å) mediated hydrogen bonding interaction with Thr-766 side chain (2.71Å) (Figure 3). . This different mode may be attributed to steric clash of the phenacyl moiety during docking procedure.

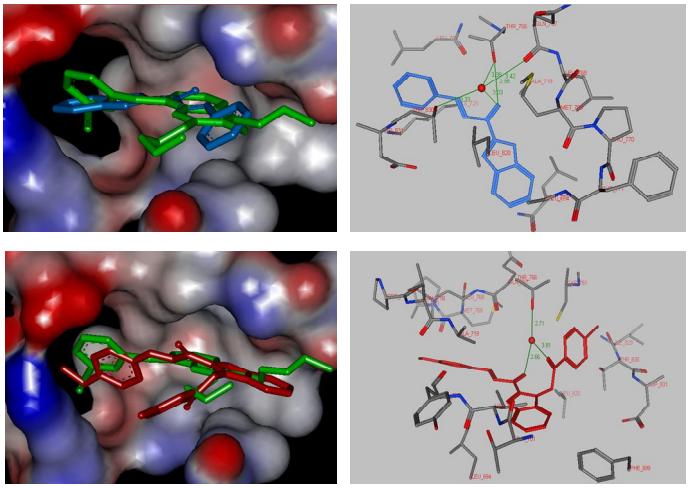
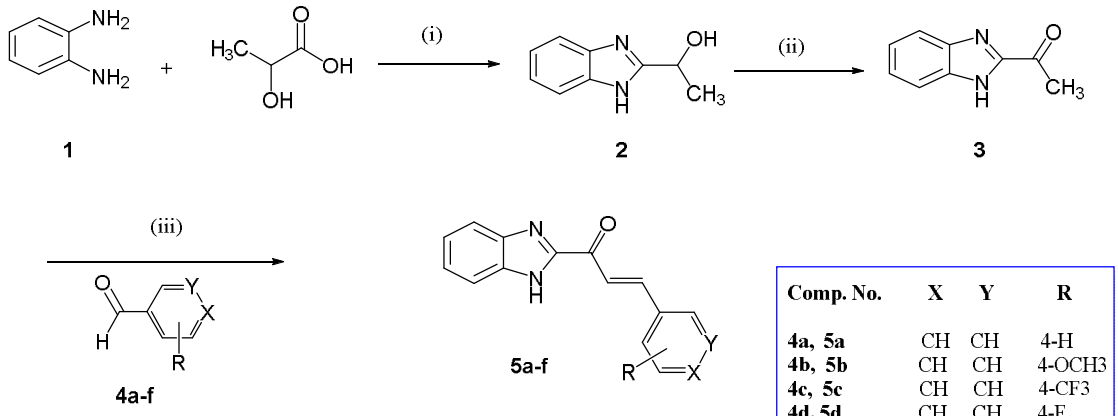
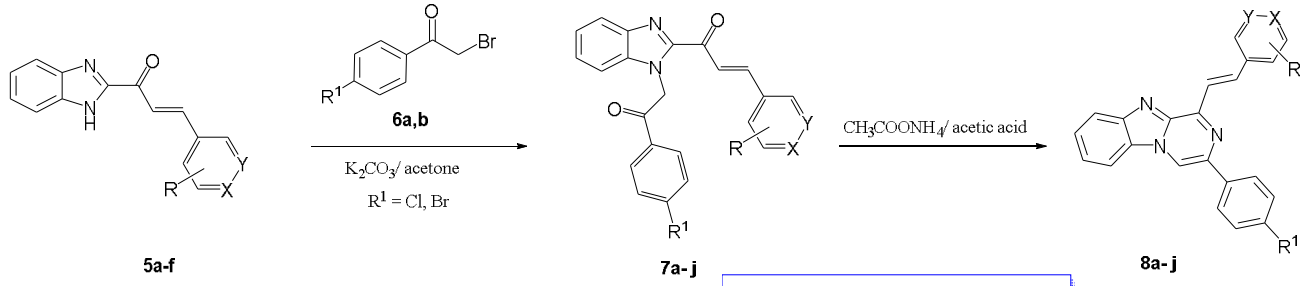


Fig. 3: The binding model of compounds 5a (Upper panel in blue) and 7b (Lower panel in red) with EGFR TK complex (PDB ID: 1M17). Left upper and lower panels showed overlay of the docked 5a and 7b with Erlotinib ligand (in green). The hydrogen bonds are shown in green lines.



Comp. No.	X	Y	R
4a, 5a	CH	CH	4-H
4b, 5b	CH	CH	4-OCH3
4c, 5c	CH	CH	4-CF3
4d, 5d	CH	CH	4-F
4e, 5e	CH	N	-
4f, 5f	N	CH	-

Scheme 1 Reaction protocol for the synthesis of **2, 3** and **5a-f**: (i) 4N HCl; (ii) $K_2Cr_2O_7/H_2SO_4$; (iii) Ethanol/H_2O, 10% NaOH



Comp.No.	X	Y	R	R^1
7a, 8a	CH	CH	4-OCH3	Cl
7b, 8b	CH	CH	4-OCH3	Br
7c, 8c	CH	CH	4-CF3	Cl
7d, 8d	CH	CH	4-CF3	Br
7e, 8e	CH	CH	4-F	Cl
7f, 8f	CH	CH	4-F	Br
7g, 8g	CH	N	-	Cl
7h, 8h	CH	N	-	Br
7i, 8i	N	CH	-	Cl
7j, 8j	N	CH	-	Br

Scheme 2 Reaction protocol for the synthesis of **7, 8a-j**: (i) K_2CO_3/acetone; (ii) CH_3COONH_4/acetic acid.

CONCLUSION

A series of substituted benzimidazole and pyrazinobenzimidazole derivatives were developed and three cancer cell lines including HepG2, MCF-7 and Vero B were used to evaluate the cytotoxic activity of these designed compounds. Among the tested compounds, Compounds **5a** and **5c-e** showed the highest and broad spectrum activity against the three cancer cell lines with IC_{50} range of 1.8-9 µg/ml, comparable to 5-FU with IC_{50} range of 12-62 µg/ml. Compound **5a**, with unsubstituted phenyl ring, free benzimidazole NH and α, β-unsaturated carbonyl moiety at the 2-position of benzimidazole nucleus, exploited great potency and lethal effect over HepG2, MCF-7 and Vero B cell lines with IC_{50} values of 2, 1.8 and 3.5 µg/ml, respectively. Molecular docking studies confirmed the strong cytotoxic activity of compounds **5a** and **7b** over MCF-7 and the postulation that these active compounds may act on the same enzyme target where EGFR inhibitor, Erlotinib, acts.

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