Research Article

Synthesis of Novel Benzimidazole Derivatives with Expected Antitumor Activities

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Accepted on: 30-10-2015; **Finalized on:** 30-11-2015.

ABSTRACT

Refluxing a mixture of chloroacetic acid with 3,4-diaminobenzophenone to give 2-chloromethyl imidazole**1**. When mercaptoacetic acid was reacted with compound 1,5-benzoyl-imidazole, compound **2** was obtained. Also, thiazino-benzimidazole**3** was obtained by the reaction of compound**2** with acetic anhydride/pyridine. Thiazinobenzimidazolone -dioxide**4** was yielded upon stirring compound**3** in glacial acetic acid/hydrogen peroxide (Scheme 1). Stirring a mixture of acetyl acetone and chloromethylimidazol**1** in absolute alcohol gave benzoyl-benzoimidazolyl**5**. When compound **5** was refluxed with a solution of urea and/or thiourea, pyrimidine derivatives **6a**,**b** were obtained. Whereas, the reaction mixture of **6b** with methylisocyanate and/or phenyl isocyanate, yielded dimethyl-pyrimidine derivatives **7a**,**b**. Refluxing a mixture of compound **5** and 2-cyanoacetamide and/or 2-cyanothioacetamide resulted in pyridine carbonitrile derivatives **9a**,**b**. Also when compounds **6b** and/or **9b** were reacted with methyl iodide, pyrimidine **8** and nicotinonitrile **10** were obtained respectively (Scheme 2). The reaction mixture of compound **1** with5-chlorobenzo[d]oxazole-2-thiol, 2-(methylthio)-1*H*-benzo[d]imidazole, 5-methyl-2-methylthio-1*H*-benzo[d]imidazole, 2-(methylthio)-1*H*-pyrimidine or 2-(furan-2-yl)-1*H*-benzo[d]imidazole was stirred to yield imidazolyl-methanone derivatives **11-15**(Scheme 3). In the present investigation, the newly synthesized products were screened (using the MTT colorimetric assay) for their *in vitro* inhibition capacity in two human cancer cell lines (hepatocellular carcinoma (HEPG2) and breast cancer (MCF-7)) in comparison to the known anticancer drugs: 5-Flurouracil and Doxorubicin. The anticancer activity results indicated that the synthesized products **6a**, **9b** and **10** showed growth inhibition activity against HEPG2 cell line and synthesized products **2**, **9a**, **9b**, and **12** showed growth inhibition activity against MCF-7, but with varying intensities extents in comparison to the known anticancer drugs, 5-Flurouracil and Doxorubicin.

Keywords: Imidazole, pyrimidine, pyridine-carbonitrile, cytotoxicity, anticancer activity, human cancer cell lines.

INTRODUCTION

Deaths due to cancer are on increase. According to global cancer statistics in 2002, there are 10.9 million new cancer cases and 6.7 million deaths[1] worldwide, whereas in 2008 this number increased to 12.66 million new cases and more than 7.5 million deaths. New drugs to fight cancer are constantly needed. Cancer is the leading cause of death in developed countries and the second leading cause of death in developing countries.[2] Although surgical resection is potentially curative, the risk of recurrence remains very high. Besides surgical resection, treatment strategies for high risk patients are still mainly based on adjuvant or neoadjuvant use of chemotherapeutic agents alone or in combination with radiotherapy. Unfortunately, the use of the above standard therapeutic protocols only results in a moderate decline in mortality and the risk of sustaining a recurrence of disease remains high. Thus, it is urgent to develop novel chemotherapeutic agents for the treatment of cancer. Heterocyclic compounds are considered as an extremely important class of compounds which play a key role in health care and pharmaceutical drug design.[3] Currently, a number of heterocyclic compounds are available commercially as anti-cancer drugs.

Heterocyclic benzimidazole derivatives are the important class of nitrogen containing heterocycles with a wide range of medicinal properties such as serotoninergic 5-HT3 and 5-HT4 receptors in the CNS,[4] antihistamine,[5] anticancer,[6] antibacterial,[7] antifungal,[8] anti-inflammatory, antianalgesic,[9] antioxidant,[10] antidiabetic,[11] selective neuropeptide YY1 receptor antagonists,[12] antimalerial, antitubercular,[13] antiulcer,[14] antitumor/antiproliferative/ anticancer activity.[15,16] where moiety plays the role of 'Master Key'.[17] Therefore, it is an imperative anchor for development of new therapeutic drugs.

Herein we report the synthesis and *in vitro* growth inhibition characterization of new derivatives. The *in vitro* antiproliferative activity of each compound in the study has been determined using the MTT colorimetric assay[18-21] in hepatocellular carcinoma cell line HEPG2 and breast carcinoma cell line MCF-7. The cytotoxic potency of the selected products was studied in comparison to two known anticancer drugs, 5-Flurouracil (5-FU) and Doxorubicin (DOX).

MATERIALS AND METHODS

Chemistry

Melting points were determined with an open capillary tube on an Electrothermal (variable heater, Stuart, UK) melting point apparatus and were uncorrected. IR spectra

were recorded on a JASCO FT-IR 6100 using KBr disc (JASCO, Japan). ^{1}HNMR spectra were measured in DMSO-d^6 using Joel EX 270 MHz spectrophotometer, spectra were recorded with trimethylsilane (TMS) as internal standard. Chemical shifts (δ) are given in ppm. The mass spectra were recorded at 70 eV on an MS-50 Kratos (A.E.I.) spectrometer (Kratos, UK) provided with a data system. Elemental analyses were carried out at the Microanalysis Laboratory, Cairo University, Cairo, Egypt, using elementary Analyses system GmbH-vario EL III Element Analyzer, Germany.

General procedure for the preparation of (2-(chloromethyl)-1H-benzo[d]imidazol-5-yl)(phenyl)methanone (1)

Cholroacetic acid (60 mmol) in 50mL of 4N HCl was refluxed for 30min. Then 3,4-diaminobenzophenone (40mmol) was added to the reaction mixture. The reaction mixture was refluxed for 20 h and then left to cool at room temperature. The separated solid was filtered off, washed with petroleum ether and recrystallized from ethanol to give compound **1** as brown powder.

Yield (60%). m.p. 187-189^{o}C; IR (cm^{-1}): 3330 (NH), 1685 (C=O) and 1645 (C=N). ^{1}H NMR (DMSO-d^6, δppm): 4.85 (s, 2H, *CH*$_2$), 7.58-7.70 (m, 5H, Ph-*H*), 7.95-8.00 (m, 2H, benzimidazol-*H*), 8.05 (s, 1H, benzimidazol-*H*) and 10.08 (br, 1H, N*H*, D$_2$O-exchangeable). MS (m/z, %): 270 (M$^+$, 70). Anal.Calcd.for $C_{15}H_{11}ClN_2O$ (270.71): C, 66.55; H, 4.10; N, 10.35. found: C, 66.50; H, 4.00; N.10.29.

Synthesis of 2-((5-benzoyl-1H-benzo[d]imidazol-2-yl)methylthio)acetic acid (2)

A mixture of mercaptoacetic acid (10mmol) in 15 mL of DMF and few drops of triethylamine was stirred for 30 min, then a solution of compound **1** (10mmol) in DMF (15 mL) was added. The reaction mixture was stirred for 6h at room temperature and then heated at 90^{o}C for 6h. The reaction mixture was poured onto crushed ice. The resulting solid was filtered off, dried and recrystallized from ethanol to give compound **2** as brown powder.

Yield (65%). m.p. 205-207 ^{o}C; IR (cm^{-1}): 3500-3360 (OH, NH), 1690, 1685 (2C=O) and 1635 (C=N). ^{1}H NMR (DMSO-d^6, δppm): 3.40 (s, 2H, C*H*$_2$CO), 4.00 (s, 2H, C*H*$_2$S), 7.55-7.72 (m, 5H, Ph-*H*), 7.95-8.00 (m, 2H, benzimidazol-*H*), 8.03 (s, 1H, benzimidazol-*H*), 10.55 (br, 1H, N*H*, D$_2$O-exchangeable) and 12.00 (br, 1H, O*H*, D$_2$O-exchangeable). MS (m/z, %): 326 (M$^+$, 12). Anal.Calcd.for $C_{17}H_{14}N_2O_3S$ (326.37): C, 62.56; H, 4.32; N, 8.58; S, 9.82. found: C, 62.50; H, 4.29; N, 8.54; S, 9.77.

Synthesis of 8-benzoyl-1H-[1,4]thiazino[4,3-a]benzimidazol-4(3H)-one (3)

A mixture of compound **2** (20mmol) in acetic anhydride (10 mL) and dry pyridine (5 mL) was heated at 90^{o}C for 8 h. The reaction mixture was evaporated under reduced pressure. The resulting residue was washed with petroleum ether and recrystallized from acetone to give compound **3** as black powder.

Yield (50%). m.p>300 ^{o}C; IR (cm^{-1}): 3040 (CH-aram.), 2900 (CH-aliph.), 1698, 1690 (2C=O) and 1640 (C=N). ^{1}H NMR (DMSO-d^6, δppm): 3.50 (s, 2H, C*H*$_2$CO), 4.42 (s, 2H, C*H*$_2$S), 7.57-7.75 (m, 5H, Ph-*H*), 7.96-8.01 (m, 2H, benzimidazol-*H*) and 8.04 (s, 1H, benzimidazol-*H*). MS (m/z, %): 308 (M$^+$, 27). Anal.Calcd.for $C_{17}H_{12}N_2O_2S$ (308.35): C, 66.22; H, 3.92; N, 9.08; S, 10.40. found: C, 65.19; H, 3.87; N, 9.01; S, 10.37.

Synthesis of 8-benzoyl-1H-[1,4]thiazino[4,3-a]benzimidazol-4(3H)-one 2,2-dioxide (4)

A mixture of compound **3** (5mmol) in glacial acetic acid (15 mL) and hydrogen peroxide (15 mL, 30%) was stirred for 72h at room temperature. The resulting residue, filtered, washed with petroleum ether and recrystallized from ethanol to give compound **4** as black powder.

Yield (56%). m.p>300 ^{o}C; IR (cm^{-1}): 3045 (CH-aram.), 2910 (CH-aliph.), 1695, 1685 (2C=O), 1645 (C=N) and 1560, 1460 (SO_2). ^{1}H NMR (DMSO-d^6, δppm): 4.30 (s, 2H, C*H*$_2$CO), 4.80 (s, 2H, C*H*$_2$S), 7.50-7.62 (m, 5H, Ph-*H*), 7.92-7.97 (m, 2H,benzimidazol-*H*) and 8.03 (s, 1H, benzimidazol-*H*). MS (m/z, %): 340 (M$^+$, 18). Anal.Calcd.for $C_{17}H_{12}N_2O_4S$ (340.35): C, 59.99; H, 3.55; N, 8.23; S, 9.42. found: C, 59.94; H, 3.50; N, 8.18; S, 9.38.

Synthesis of 3-((5-benzoyl-1H-benzo[d]imidazol-2-yl)methyl)pentane-2,4-dione (5)

Acetyl acetone (10 mmol) was added to 40 mL of absolute ethanol containing sodium metal (10 mmol) and the mixture was stirred for 4 h at room temperature. Compound **1** (10 mmol) was added and the reaction mixture was stirred at 60^{o}C for 4 h, the reaction was cooled at room temperature and poured onto cold water (100 mL) containing drops of dil. HCl. The precipitated solid product was filtered off, dried and recrystallized from ethanol to give compound **5** as black powder.

Yield (60%). m.p. 270-273 ^{o}C; IR (cm^{-1}): 3360 (NH), 3100 (CH-aram.), 2920 (CH-aliph.), 1700, 1685, 1670 (3C=O) and 1630 (C=N). ^{1}H NMR (DMSO-d^6, δppm): 1.95 (s, 3H, *Me*), 2.10 (s, 3H, *Me*), 3.83 (s, 2H, C*H*$_2$), 4.03 (s, 1H, C*H*), 7.54-7.65 (m, 5H, Ph-*H*), 7.97-8.01 (m, 2H, benzimidazol-*H*), 8.03 (s, 1H, benzimidazol-*H*) and 10.20 (br, 1H, N*H*, D$_2$O-exchangeable). MS (m/z, %): 335 (M$^+$ + 1, 27). Anal.Calcd.for $C_{20}H_{18}N_2O_3$ (334.13): C, 71.84; H, 5.43; N, 8.38. Found: C, 71.80; H, 5.39; N, 8.33.

General procedure for synthesis of (6a) and (6b)

A solution of urea and/or thiourea (10 mmol) in (50 mL) absolute ethanol containing (10 mmol) sodium metal was stirred at room temperature for 2h. Compound **5** (10 mmol) was added and the reaction mixture was refluxed for 10h. The reaction mixture was followed by TLC.

The solvent was evaporated under reduced pressure, washed with cold water containing drops of dil. HCl. The

precipitated solid product was filtered off, dried and crystallized from appropriate solvent.

5-((5-benzoyl-1H-benzo[d]imidazol-2-yl)methyl)-4,6-dimethylpyrimidin-2(1H)-one (6a)

Crystallized from ethanol as brown powder. Yield (45%). m.p. 180-182°C; IR (cm^{-1}): 3300, 3120 (2NH), 3040 (CH-arom.), 2910 (CH-aliph.), 1710, 1690 (2C=O) and 1665, 1660 (2C=N). ^{1}H NMR (DMSO-d^6, δppm): 1.95 (s, 3H, *Me*), 2.20 (s, 3H, *Me*), 3.85 (s, 2H, C*H*$_2$), 7.54-7.65 (m, 5H, Ph-*H*), 8.05-8.10 (m, 2H, benzimidazol-*H*), 8.15 (s, 1H, benzimidazol-*H*), 9.60 (br, 1H, N*H*, D_2O- exchangeable) and 10.20 (br, 1H, N*H*, D_2O-exchangeable). MS (m/z, %): 359 (M^+ + 1, 38). Anal.Calcd.for $C_{21}H_{18}N_4O_2$ (358.14): C, 70.38; H, 5.06; N, 15.63. found: C, 70.32; H, 5.00; N, 15.60.

5-((5-benzoyl-1H-benzo[d]imidazol-2-yl)methyl)-4,6-dimethylpyrimidin-2(1H)-thione (6b)

Crystallized from ethanol as dark brown powder. Yield (60%). m.p. 210-213 °C; IR (cm^{-1}): 3310, 3100 (2NH), 3040 (CH-arom.), 2900 (CH-aliph.), 1690 (C=O), 1662, 1660 (2C=N) and 1263 (C=S). ^{1}H NMR (DMSO-d^6, δppm): 1.97 (s, 3H, *Me*), 2.24 (s, 3H, *Me*), 3.82 (s, 2H, C*H*$_2$), 7.55-7.68 (m, 5H, Ph-*H*), 8.07-8.14 (m, 2H, benzimidazol-*H*), 8.17 (s, 1H, benzimidazol-*H*) and 10.80 (br, 2H, 2N*H*, D_2O-exchangeable). MS (m/z, %): 374 (M^+, 41). Anal.Calcd.for $C_{21}H_{18}N_4OS$ (374.46): C, 67.36; H, 4.85; N, 14.96; S, 8.56. found: C, 67.31; H, 4.80; N, 14.92; S, 8.53.

General procedure for synthesis of (7a) and (7b)

A solution of **6b** (10mmol) in 50 mL absolute ethanol containing (10 mmol) of sodium metal was stirred at room temperature for 3h. Methylisocyanate and/or phenyl isocyanate (10mmol) was added. The reaction mixture was refluxed for 8h and followed by TLC. The reaction was lefted to cool, poured onto crushed ice containing drops of dil. HCl. The solid product was filtered off and crystallized from suitable solvent to give compound **7a**, **7b**.

5-((5-benzoyl-1H-benzo[d]imidazol-2-yl)methyl)-4,6-dimethylpyrimidin-2-yl methylcarbamothioate (7a)

Recrystallized from benzene as brown powder. Yield (45%). m.p. >300 °C; IR (cm^{-1}): 3300, 3080 (2NH), 3045 (CH-arom.), 2895 (CH-aliph.), 1780, 1695 (2C=O) and 1669, 1665 (2C=N). ^{1}H NMR (DMSO-d^6, δppm): 2.35 (s, 3H, *Me*), 2.45 (s, 3H, *Me*), 2.84 (s, 3H, NH-*Me*), 4.10 (s, 2H, C*H*$_2$), 7.10 (br-s, 1H, MeN*H*, D_2O-exchangeable), 7.55-7.67 (m, 5H, Ph-*H*), 7.95-8.02 (m, 2H, benzimidazol-*H*), 8.15 (s, 1H, benzimidazol-*H*) and 10.35 (br, 1H, N*H*, D_2O-exchangeable). MS (m/z, %): 433 (M^+ + 2, 38). Anal.Calcd.for $C_{23}H_{21}N_5O_2S$ (431.14): C, 64.02; H, 4.91; N, 16.23; S, 7.43. found: C, 64.00; H, 4.86; N, 16.20; S, 7.39.

5-((5-benzoyl-1H-benzo[d]imidazol-2-yl)methyl)-4,6-dimethylpyrimidin-2-yl phenylcarbamothioate (7b)

Crystallized from ethanol as brown powder. Yield (56%). m.p. 232-234 °C; IR (cm^{-1}): 3310, 3060 (2NH), 1710, 1698 (2C=O) and 1665, 1590 (2C=N). ^{1}H NMR (DMSO-d^6, δppm): 2.32 (s, 3H, *Me*), 2.35 (s, 3H, *Me*), 4.39 (s, 2H, C*H*$_2$), 7.20-7.95 (m, 10H, Ph-*H*), 7.98-8.04 (m, 2H, benzimidazol-*H*), 8.15 (s, 1H, benzimidazol-*H*) and 9.20 (br, 2H, 2N*H*, D_2O-exchangeable). MS (m/z, %): 493 (M^+, 47). Anal.Calcd.for $C_{28}H_{23}N_5O_2S$ (493.58): C, 68.13; H, 4.70; N, 14.19; S, 6.50. found: C, 68.09; H, 4.65; N, 14,14; S, 6.41.

General procedure for synthesis of (9a) and (9b)

A mixture of compound **5** (10 mmol) in (50 mL) absolute ethanol containing catalytic drops of piperdine and 2-cyano acetamide and/or 2-cyanothio acetamide (10mmol). The reaction mixture was heated under reflux for 7 h. The reaction was followed by TLC, the reaction mixture was left to cool and poured with stirring onto cold water containing drops of dil. HCl. The precipitate solid product was filtered off, dried and recrystallied from suitable solvent.

5-((5-benzoyl-1H-benzo[d]imidazol-2-yl)methyl)-4,6-dimethyl-2-oxo-1,2-dihydropyridine-3-carbonitrile (9a)

Crystallized from petroleum ether/ethyl acetate as brown powder. Yield (47%). m.p. 255-257°C; IR (cm^{-1}): 3358 (NH-benzamdazol), 3303 (NH-pyridine), 3030 (CH-arom.), 2920 (CH-aliph.), 2227 (CN), 1670, 1660 (2C=O) and 1630 (C=N). ^{1}H NMR (DMSO-d^6, δppm): 2.38 (s, 3H, *Me*), 2.44 (s, 3H, *Me*), 3.95 (s, 2H, C*H*$_2$), 7.53-7.61 (m, 5H, Ph-*H*), 7.70-7.74 (m, 2H, benzimidazol-*H*), 7.90 (s, 1H, benzimidazol-*H*), 9.00 (br, 1H, N*H*-pyridine, D_2O-exchangeable) and 10.10 (br, 1H, N*H*, D_2O-exchangeable). MS (m/z, %): 384 (M^+ + 2, 15). Anal.Calcd.for $C_{23}H_{18}N_4O_2$ (382.41): C, 72.24; H, 4.74; N, 14.65. found: C, 72.16; H, 4.70; N, 14.59.

5-((5-benzoyl-1H-benzo[d]imidazol-2-yl)methyl)-4,6-dimethyl-2-thioxo-1,2-dihydropyridine-3-carbonitrile (9b)

Crystallized from petroleum ether/ethyl acetate as brown powder. Yield (54%). m.p. 222-225 °C; IR (cm^{-1}): 3370 (NH-benzamdazol), 3303 (NH-pyridine), 2215 (CN), 1668 (C=O), 1625 (C=N) and 1260 (C=S). ^{1}H NMR (DMSO-d^6, δppm): 2.21 (s, 3H, *Me*), 2.43 (s, 3H, *Me*), 4.10 (s, 2H, C*H*$_2$), 7.54-7.63 (m, 5H, Ph-*H*), 7.72-7.77 (m, 2H, benzimidazol-*H*), 8.01 (s, 1H, benzimidazol-*H*), 8.98 (br, 1H, N*H*-pyridine, D_2O- exchangeable) and 10.00 (br, 1H, N*H*-benzimidazol, D_2O-exchangeable). MS (m/z, %): 399 (M^+ + 1, 41). Anal.Calcd.for $C_{23}H_{18}N_4OS$ (398.48): C, 69.32; H, 4.55; N, 14.06; S, 8.05. found: C, 69.24; H, 4.50; N, 14.00; S, 8.00.

General procedure for synthesis of (8) and (10)

A solution of compound **6b** and/or **9b** (10mmol) in (50 mL) dry acetone containing potassium carbonate (10 mmol) was stirred at room temperature for 3h. Methyl iodide (11mmol) was then added drop wisely and the reaction mixture was refluxed for 8h. Excess acetone was evaporated under reduced pressure, poured onto crushed ice. The precipitate was filtered off, dried and

crystallized from suitable solvent to give compounds **8**and **10.**

5-((5-benzoyl-1H-benzo[d]imidazol-2-yl)methyl)-4,6-dimethyl-2-(methylthio)pyrimidin (8)

Crystallized from ethanol as yellow powder. Yield (50%). m.p. 117-119 ^{o}C; IR (cm^{-1}): 3295 (NH), 3040 (CH-arom.), 2910 (CH-aliph.), 1697 (C=O) and 1670, 1658 (2C=N). ^{1}H NMR (DMSO-d^{6}, δppm): 2.25 (s, 3H, *Me*), 2.27 (s, 3H, *Me*), 2.57 (s, 3H, *Me*-S), 4.30 (s, 2H, C*H*$_2$), 7.56-7.62 (m, 5H, Ph-*H*), 7.97-8.02 (m, 2H, benzimidazol-*H*), 8.04 (s, 1H, benzimidazol-*H*) and 9.70 (br, 1H, N*H*, D_2O-exchangeable). MS (m/z, %): 389 (M^+ + 1, 25). Anal.Calcd.for $C_{22}H_{20}N_4OS$ (388.49): C, 68.02; H, 5.19; N, 14.42; S, 8.25. found: C, 67.97; H, 5.14; N, 14.39; S, 8.20.

5-((5-benzoyl-1H-benzo[d]imidazol-2-yl)methyl)-4,6-dimethyl-2-(methylthio)nicotino-nitrile (10)

Crystallized from petroleum ether/ethyl acetate as brown powder. Yield (59%). m.p. 114-116 ^{o}C; IR (cm^{-1}): 3360, (NH-benzamdazol), 2220 (CN), 1670 (C=O), 1628 (C=N, benzamdazol) and 1560 (C=N, pyridine). ^{1}H NMR (DMSO-d^{6}, δppm): 2.22 (s, 3H, *Me*), 2.42 (s, 3H, *Me*), 2.51 (s, 3H, *Me*-S), 4.11 (s, 2H, C*H*$_2$), 7.53-7.63 (m, 5H, Ph-*H*), 7.73-7.78 (m, 2H, benzimidazol-*H*), 8.02 (s, 1H, benzimidazol-*H*) and 9.20 (br, 1H, N*H*-benzimidazol, D_2O-exchangeable). MS (m/z, %): 412 (M^+, 11). Anal.Calcd.for. $C_{24}H_{20}N_4OS$ (412.51): C, 69.88; H, 4.89; N, 13.58; S, 7.77. found: C, 69.80; H, 4.85; N, 13.53; S, 7.74.

General procedure for synthesis of compounds (11-15)

To a well stirred solution of 5-chlorobenzo[d]oxazole-2-thiol, 2-(methylthio)-1*H*-benzo[d]imidazole, 5-methyl-2-(methylthio)-1*H*-benzo[d]imidazole, 2-(methylthio)-1*H*-perimidine or 2-(furan-2-yl)-1*H*-benzo[d]imidazole (10 mmol) and anhydrous K_2CO_3 (10 mmol) in dry acetone, compound **1** (0.01 mol) was added. The reaction mixture was stirred for 24 h at room temperature, the reaction mixture followed by TLC. Excess acetone was evaporated under reduced pressure and the reaction mixture was poured onto cold water and the precipitate obtained was filtered, dried and recrystallized from ethanol.

(2-((5-chlorobenzo[d]oxazol-2-ylthio)methyl)-1H-benzo[d]imidazol-5-yl)(phenyl)methanone (11)

Crystallized from ethanol as brown powder. Yield (65%). m.p. 266-268 ^{o}C; IR (cm^{-1}): 3380 (NH), 1696 (C=O) and 1660, 1655 (2C=N). ^{1}H NMR (DMSO-d^{6}, δppm): 4.72 (s, 2H, C*H*$_2$), 7.30-7.33 (m, 3H, Ar-*H*), 7.56-7.62 (m, 5H, Ph-*H*), 7.92-7.95 (m, 2H, benzimidazol-*H*), 7.97 (s, 1H, benzimidazol-*H*) and 9.80 (br, 1H, N*H*, D_2O-exchangeable). MS (m/z, %): 420 (M^+ + 1, 18). Anal.Calcd.for $C_{22}H_{14}ClN_3O_2S$ (419.88): C, 62.93; H, 3.36; N, 10.01; S, 7.64. found: C, 62.90; H, 3.32; N, 9.98; S, 7.61.

(2-((2-(methylthio)-1H-benzo[d]imidazol-1-yl)methyl)-1H-benzo[d]imidazol-5-yl)(phenyl)methanone (12)

Crystallized from ethanol as brown powder. Yield (55%). m.p. 145-147 ^{o}C; IR (cm^{-1}): 3410 (NH), 1689 (C=O) and 1650, 1641 (2C=N). ^{1}H NMR (DMSO-d^{6}, δppm): 2.37 (s, 3H, *Me*-S), 4.99 (s, 2H, C*H*$_2$), 7.47-7.53 (m, 4H, benzimidazol-*H*), 7.57-7.65 (m, 5H, Ph-*H*), 7.71-7.75 (m, 3H, benzimidazol-*H*) and 9.50 (br, 1H, N*H*, D_2O-exchangeable). MS (m/z, %): 399 (M^+ + 1, 51). Anal.Calcd.for $C_{23}H_{18}N_4OS$ (398.48): C, 69.32; H, 4.55; N, 14.06; S, 8.05. found: C, 69.29; H, 4.41; N, 14.00; S, 8.01.

(2-((5-methyl-2-(methylthio)-1H-benzo[d]imidazol-1-yl)methyl)-1H-benzo[d]imidazol-5-yl)(phenyl)methanone (13)

Crystallized from ethanol as brown powder. Yield (45%). m.p. 246-248 ^{o}C; IR (cm^{-1}): 3395 (NH), 1681 (C=O) and 1638, 1630 (2C=N). ^{1}H NMR (DMSO-d^{6}, δppm): 2.40 (s, 3H, *Me*), 2.43 (s, 3H, *Me*-S), 5.10 (s, 2H, C*H*$_2$), 7.48-7.52 (m, 3H, benzimidazol-*H*), 7.55-7.62 (m, 5H, Ph-*H*), 7.74-7.77 (m, 3H, benzimidazol-*H*) and 9.45 (br, 1H, N*H*, D_2O-exchangeable). MS (m/z, %): 412 (M^+, 30). Anal.Calcd.for $C_{24}H_{20}N_4OS$ (412.51): C, 69.88; H, 4.89; N, 13.58; S, 7.77. found: C, 69.85; H, 4.85; N, 13.55; S, 7.74.

(2-((2-(methylthio)-1H-perimidin-1-yl)methyl)-1H-benzo[d]imidazol-5-yl)(phenyl) methanone (14)

Crystallized from ethanol as brown powder. Yield (40%). m.p. >300 ^{o}C; IR (cm^{-1}): 3325 (NH), 1697 (C=O)amd 1665, 1657 (2C=N). ^{1}H NMR (DMSO-d^{6}, δppm): 2.47 (s, 3H, *Me*-S), 4.99 (s, 2H, C*H*$_2$), 7.37-7.42 (m, 6H, Ar-*H*), 7.57-7.69 (m, 5H, Ph-*H*), 7.93-7.96 (m, 3H, benzimidazol-*H*) and 9.40 (br, 1H, N*H*, D_2O-exchangeable). MS (m/z, %): 449 (M^+ + 1, 28). Anal.Calcd.for $C_{27}H_{20}N_4OS$ (448.54): C, 72.30; H, 4.49; N, 12.49; S, 7.15. found: C, 72.27; H, 4.45; N, 12.45; S, 7.11.

(2-((2-(furan-2-yl)-1H-benzo[d]imidazol-1-yl)methyl)-1H-benzo[d]imidazol-5-yl)(phenyl)methanone (15)

Crystallized from ethanol as brown powder. Yield (40%). m.p. 278-281 ^{o}C; IR (cm^{-1}): 3390 (NH), 1690 (C=O) and 1660, 1654 (2C=N). ^{1}H NMR (DMSO-d^{6}, δppm): 4.71 (s, 2H, C*H*$_2$), 6.69 (d, 1H, furyl-*H*), 7.15 (d, 1H, furyl-*H*), 7.49-7.54 (m, 4H, benzimidazol-*H*), 7.60-7.67 (m, 5H, Ph-*H*), 7.90 (d, 1H, furyl-*H*), 7.92-7.95 (m, 2H, benzimidazol-*H*), 7.98 (s, 1H, benzimidazol-*H*) and 9.20 (br, 1H, N*H*, D_2O-exchangeable). MS (m/z, %): 418 (M^+, 38). Anal.Calcd.for $C_{26}H_{18}N_4O_2$ (418.45): C, 74.63; H, 4.34; N, 13.39. found: C, 74.60; H, 4.30; N, 13.46.

Determination of anticancer activities

Cell culture

All the following procedures were done in a sterile area using a Laminar flow cabinet biosafety class II level (Baker, SG403INT, and Sanford, ME, USA). Human hepatocellular carcinoma HEPG2 and breast cancer MCF-7 cells were obtained from National Cancer Institute, Cairo University. The cells were grown in DMEM supplemented with 10% heat inactivated fetal bovine serum (FBS), 100 U/mL of penicillin, and 100 µg of streptomycin/mL in a humidified incubator with 5% CO_2 at 37°C.

In vitro cell proliferation and cell viability assay-Trypan blue exclusion assay

Trypan blue exclusion assay was performed to assess the effect of newly synthesized products on viability of HEPG2 and MCF7 cells. Approximately 0.75×10^5cells/mL was seeded in a six well tissue culture plate and different concentrations of compounds were added after 24 h. For the determination of growth rate, smaller aliquots were collected in a 0.5 mL tubes, trypan blue (0.4%) was added to the cell suspension, and the number of cells (viable-unstained and non-viable-blue) was counted using a haemocytometer. The media was not changed during the induction period. Each experiment was repeated a minimum of three times and the results are presented as graphs.

MTT assay

The synthesized products were subjected to a screening system for evaluation of their anticancer activity against hepatocellular carcinoma HEPG2 cell line and breast carcinoma MCF-7 cell line in comparison to the known anticancer drugs: 5-FU and DOX.

Cell survival was further assessed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) dyereductionassay[22] which is based on the ability of viable cells to metabolize a yellow tetrazolium salt to violet form azan product that can be detected spectrophotometrically. Exponentially growing cells (HEPG2 and MCF-7) were plated in triplicate in 96-well sterilized plates at a density of 1×10^4 cells/well. After 24 h, cells were treated with escalating doses of the compound under investigation and incubated in 5% CO_2 atmosphere with high humidity. After 48 and 72 h of compound exposure, the cells were incubated with MTT (0.5 mg/mL) for another4 h at 37°C. The blue MTT formazan precipitate was then, solubilized in detergent (50% final concentration of N,N-dimethylformamide and 10% of sodium dodecyl sulphate) and incubated for an additional 2 h. Absorbance was measured at 570 nm on a multi-well ELISA plate reader.

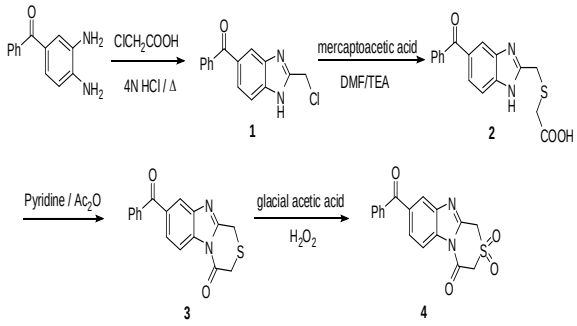
The mean absorbance of medium control was the blank and was subtracted. IC_{50} values (concentration of compound causing 50% inhibition of cell growth) were estimated after72 h exposure of compound.

The absorbance of control cells was taken as 100% viability and the values of treated cells were calculated as a percentage of control.

The 5-fluorouracil and doxorubicin anticancer drugs were used as positive control, and cells without samples were used as negative control. The relation between surviving fraction and drug concentration is plotted to get the survival curve of both cancer cell lines with the specified compound. A statistical significance was tested between samples and negative control (cells with vehicle) using independent t-test by SPSS 11 program. DMSO is the vehicle used for dissolution of products.

RESULTS AND DISCUSSION

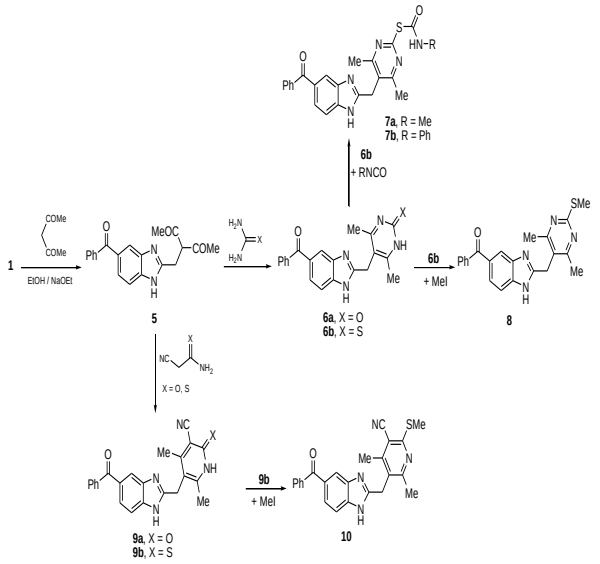
Benzimidazole[23-28] moiety came in pictures in the 1950s after the news that 5,6-dimethyl-l-(a-D-ribofuranosyl) benzimidazole was an integral part of the structure of the vitamin B12.[29] In the present work, a synthesis of 2-substituted benzimidazole derivatives **1** has been explored by reacting a mixture of cholroacetic acid and 3,4-diaminobenzophenonein 4N hydrochloric acid to give (2-(chloromethyl)-1*H*-benzo[d]imidazol-5-yl)(phenyl)methanone**1**. When a solution of compound **1** was reacted with a mixture of mercaptoacetic acid in DMF in the presence of a few drops of triethylamine,2-((5-benzoyl-1*H*-benzo[d]imidazol-2-yl)methylthio)acetic acid **2** was obtained. Also, 1,2-disubstitutedbenzimidazole derivatives **3,4** had been obtained by heating compound **2** in acetic anhydride and dry pyridine to obtain *8*-benzoyl-1*H*-[1,4]thiazino[4,3-a]benzimidazol-4(3*H*)-one **3**. Stirring compound **3** in glacial acetic acid and hydrogen peroxide afforded 8-benzoyl-1*H*-[1,4]thiazino[4,3-a]benzimidazol-4(3*H*)-one 2,2-dioxide**4** (Scheme1).



Scheme 1

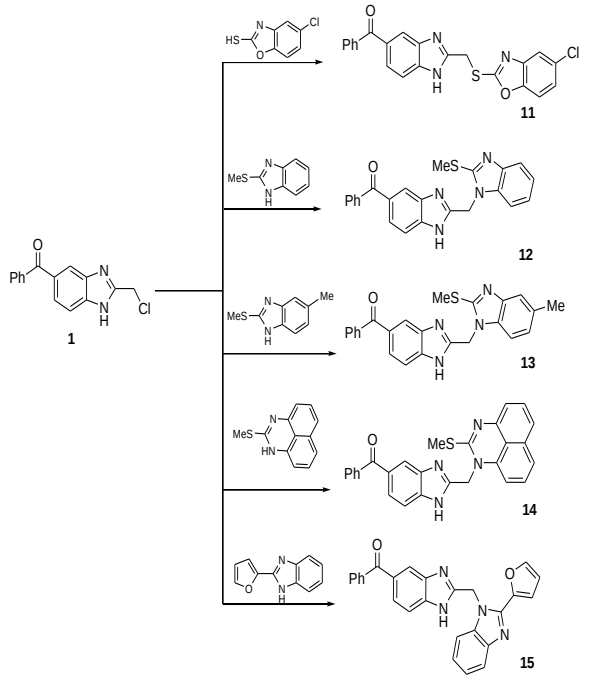
Recently, dihydropyrimidines[30,31] (DHPs) occupied a distinct and unique place in the medicinal field, due to the importance of DHPs, various methods for synthesis of these compounds have been developed. We had reported the synthesis of pyrimidine derivatives**5-8**, using acetyl acetone in absolute ethanol containing sodium metal with compound **1** to afford 3-((5-benzoyl-1*H*-benzo[d]imidazol-2-yl)methyl)pentane-2,4-dione **5**, then a solution of urea and/or thiourea in absolute ethanol containing sodium metal was reacted with compound **5** to give 5-((5-benzoyl-1*H*-benzo[d]imidazol-2-yl)methyl)-4,6-dimethylpyrimidin-2(1*H*)-one/thione respectively **6a**,**b.** Also, 5-((5-benzoyl-1*H*-benzo[d]imidazol-2-yl)methyl)-4,6-dimethylpyrimidin-2-yl methyl/phenylcarbamthioate respectively **7a,b** were obtained by refluxing **6b** in absolute ethanol containing sodium metal with methylisocyanate and/or phenyl isocyanate. A solution of compound **6b** in dry acetone containing potassium carbonate was stirred with methyl iodide to give 5-((5-benzoyl-1*H*-benzo[d]imidazol-2-yl)methyl)-4,6-dimethyl-2-(methylthio)pyrimidine**8**.
Meanwhile, refluxing of a mixture of compound **5** in absolute ethanol containing piperdine and 2-cyanoacetamide and/or 2-cyanothioacetamide was afforded 5-((5-benzoyl-1*H*-benzo[d]imidazol-2-yl)methyl)-4,6-dimethyl-2-oxo/thioxo-1,2-dihydropyridine-3-

carbonitrile derivatives respectively **9a,b**. Also,5-((5-benzoyl-1*H*-benzo[d]imidazol-2-yl)methyl)-4,6-dimethyl-2-(methylthio) nicotinonitrile**10** was obtained by the reaction of a solution of compound **9b** in dry acetone containing potassium carbonate with methyl iodide(Scheme 2).



Scheme 2

Imidazole and its derivatives had been an important class of heterocyclic compounds that exhibited a wide range of biological and pharmacological activities. So, further novel benzimidazole were presented, at the stirring mixture of compound **1** and/or 5-chlorobenzo[d]oxazole-2-thiol/ 2-(methylthio)-1*H*-benzo[d]imidazole/5-methyl-2-(methylthio)-1*H*-benzo[d]imidazole/2-(methylthio)-1*H*-pyrimidine or 2-(furan-2-yl)-1*H*-benzo[d]imidazole in anhydrous K_2CO_3 and dry acetone, to give benzo[d]imidazolmethanonederivatives **11-15**(Scheme3).



Scheme 3

Bioactivity

Anticancer Activity

Cancer and other chronic diseases share some common pathogenic mechanisms, such as DNA damage, oxidative stress, and chronic inflammation.

These diseases can be controlled by resistant to mutagens/carcinogens and/or to inhibit progression of the disease by administering chemopreventive agents.[32] Chemotherapy and surgery are standard methods for treatment of these diseases, although not been fully effective.

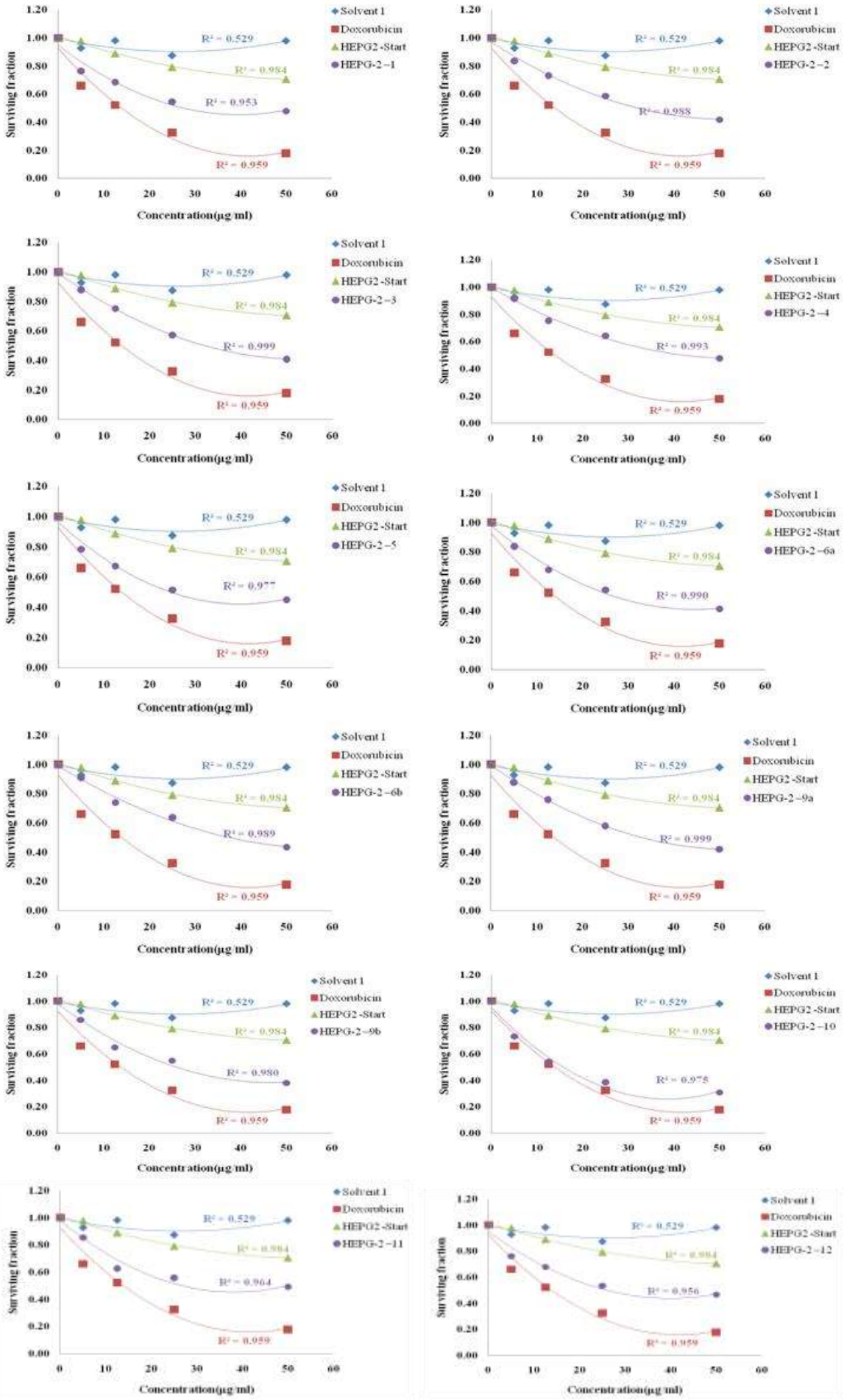
Most of the anti-tumor drugs currently used in chemotherapy are toxic to normal cells and cause toxicity for immune cells. So it is important to minimize curing doses to the least amount possible as well as trying to minimize the side effects of these drugs. Therefore, the identification of new anti-cancer drug with low side effects on immune system has become an essential goal in many studies of immunopharmacology.[33]

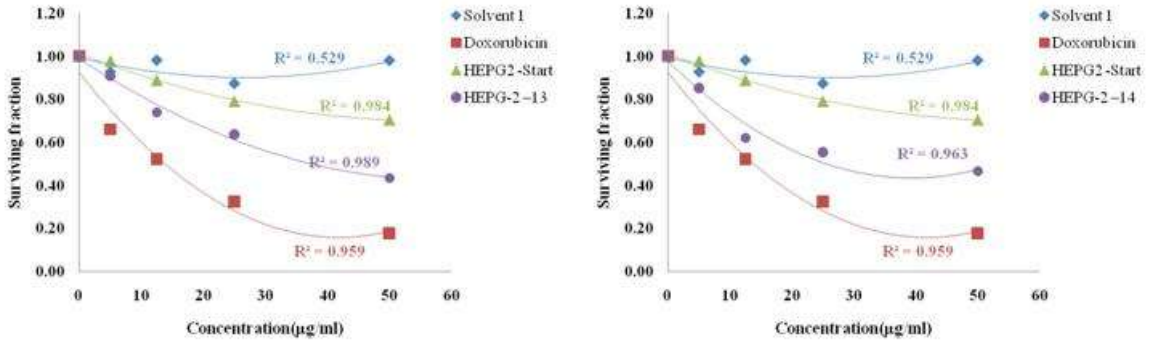
The newly synthesized products were evaluated for their *in vitro* cytotoxic activity against human hepatocellular carcinoma (HEPG2) and breast carcinoma (MCF-7) cell lines. Doxorubicin and 5-Fluorouracil, which are two of the most effective anticancer agents, were used as a reference drugs.

Our results showed that some of the newly synthesized products exhibited a moderate to strong growth inhibition activity on the tested cell lines between 0-50 µg/mL concentrations in comparison to the reference anticancer drugs. The relationship between surviving fraction and drug concentration was plotted to obtain the survival curve of the two cell lines. The response parameter calculated was the IC_{50} value, which corresponds to the concentration required for 50% inhibition of cell viability. Table 1 shows the *in vitro* cytotoxic activity of the synthesized compounds, where some compounds exhibited significant activity compared to the reference drugs.

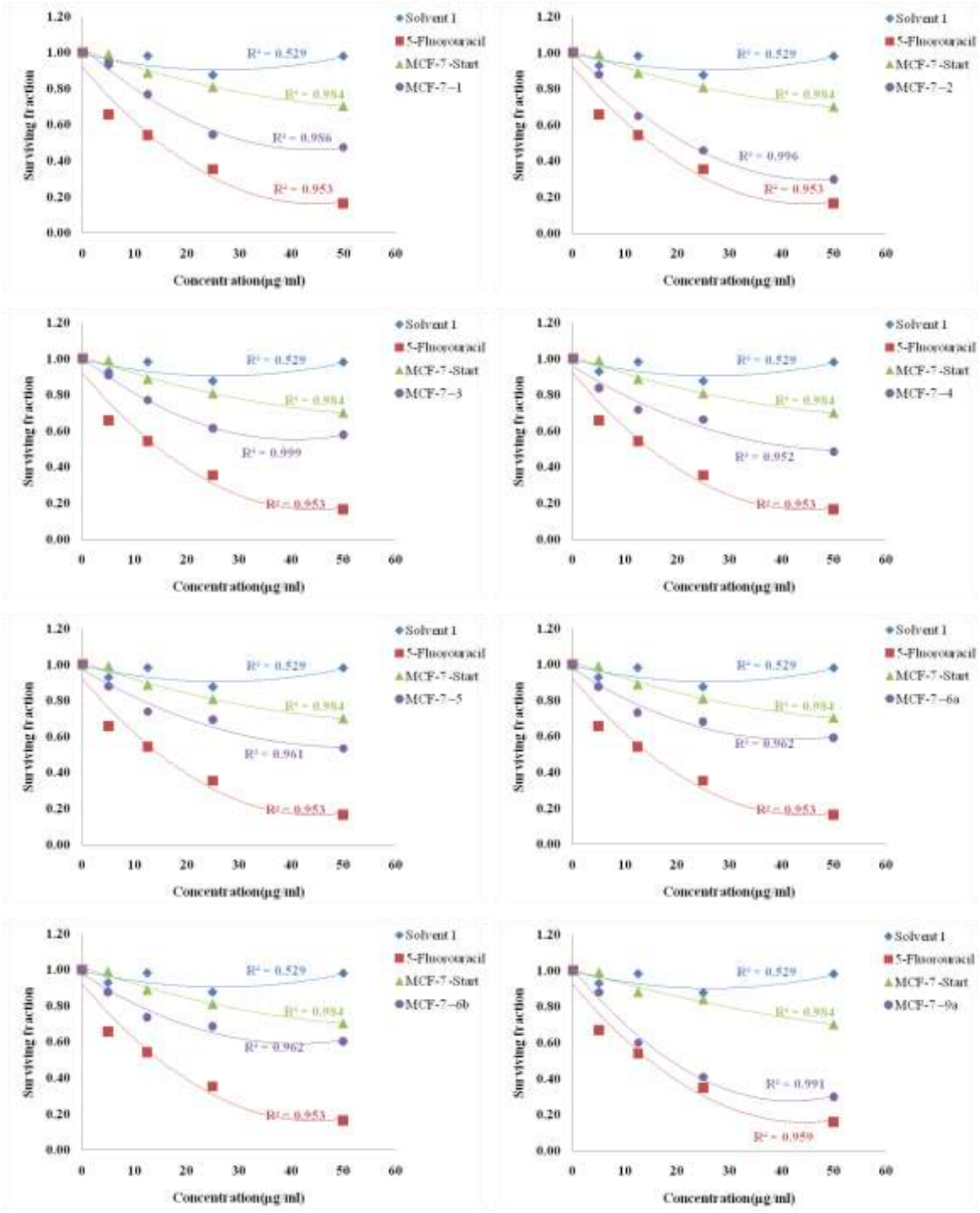
Figures (1-14) showed the cytotoxic activity of the synthesized products against breast HEPG2 cancer cell line and figures (15-28) showed the activity against MCF-7 cell line in comparison to the reference drugs: 5-FU and DOX. From the results obtained in Table 1, compounds **6a**, **9b** and **10** showed an *in vitro* cytotoxic activity with IC_{50} value of 20, 23 and 16 respectively for HEPG2 cell line.

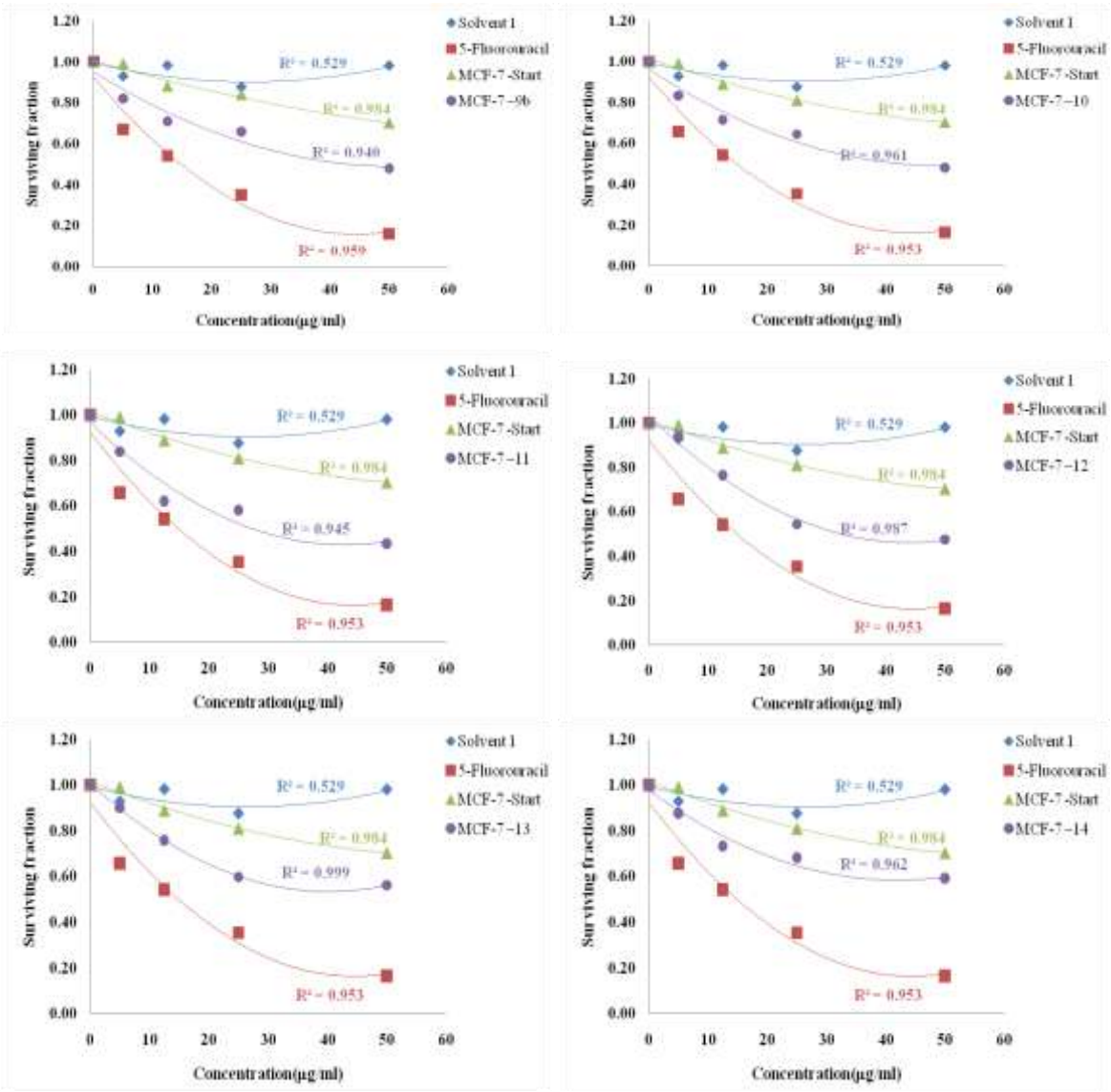
Compounds **2**, **9a**, **9b**, and **12** showed an *in vitro* cytotoxic activity with IC_{50} value of 22, 18, 18.5 and 24 respectively for MCF-7 cell line when the cells were subjected to different concentrations of the compound. It can be deduced from our results that products **2**, **6a**, **9a**, **9b**,**10**, and **12** were the most active and induced a reasonable growth inhibition, in a dose-dependent manner against both cell lines when compared to start material and to 5-FU and DOX (Table 1).





Figures 1-14: The cytotoxic activity of the synthesized products against human breast HEPG2 cancer cell line





Figures 15-28: The cytotoxic activity of the synthesized products against human breast MCF7 cancer cell line

Table 1: IC_{50} of the newly synthesized products against the two cell lines

Compound	HEPG-2	MCF7
Solvent	76	76
Start	49	54
1	29	32
2	33	22
3	32.5	42
4	42	40
5	24	50
6a	20	50
6b	37	50
9a	33	18
9b	23	18.5
10	16	50
11	26.5	25
12	27	24
13	38	38
14	30	50
5 flurouracil	12	13
Doxorubicin	11	14

CONCLUSION

In summary, we have reported the synthesis of a variety of benzo[d]imidazole derivatives containing different types of heterocyclic ring as pyridine, dihydropyridine, pyrimidine and benzo[d]oxazole. The newly synthesized compounds were screened for their *in vitro* inhibition capacity in two human cancer cell lines HEPG2 and MCF-7 using 5-Flurouracil and Doxorubicin as reference drugs. Some of newly synthesized products exhibited a moderate to good growth inhibition activity. Where compound **10** showed the highest activity against HEPG-2 cell, and compound **9a** showed the highest activity against MCF7 cell.

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Source of Support: Nil, **Conflict of Interest:** None.