ISSN: 0975-766X
CODEN: IJPTFI
Available Online through *Research Article*
www.ijptonline.com

SYNTHESIS AND *IN VITRO* ANTI-BREAST CANCER EVALUATION OF SOME NOVEL BENZIMIDAZOLE –PYRIDINE CONJUGATES

Eman M. Mohi El-Deen
Department of Therapeutical Chemistry, National Research Centre, Dokki, Cairo, Egypt, 12311.
Email: e.mohi.2010@live.com

Received on 25-12-2015 *Accepted on 23-01-2016*

Abstract

A new series of benzimidazole bearing pyridine derivatives were synthesized by condensation of 1,2-phenylenediamine with 2-oxo-1,2-dihydro-pyridine-3-carboxylic acid derivative **2** in phosphoric acid to give 3-(1H-benzo[d]imidazol-2-yl)-pyridin-2(1*H*)-one derivative **3**. Compound **3** on further reaction with a mixture of phosphorus oxychloride and phosphorus pentachloride gave 2-(2-chloro-) pyridin-3-yl)-1H-benzo[d] imidazole derivative **4**. Moreover, **4** were reacted with different amines to give 3-(1H-benzo[d]imidazol-2-yl)-N-(substituted) pyridin-2-amine derivatives **5a-c.** Meanwhile, N-(N-(substituted) sulfamoyl) pyridin-3-yl)-1H-benzo[d]imidazole-1-carboxamide derivatives **7a,b** were synthesized by reaction of **5c** with chlorosulfonyl isocyanate to give 2-(pyridin-3-yl)-1H-benzo [d]imidazole-1-carbonyl) sulfamoyl chloride derivative followed by reaction of the latter with different amines. On the other hand, upon treatment compound **4** with hydrazine hydrate gave the hydrazide derivative **8**. The reaction of compound **8** with different alkyl/arylisothiocyanates gave the corresponding thiosemicarbazide derivatives **9a-d,** while their cyclo condensation reaction with ethyl chloro acetate gave the corresponding thiazolidin-4-one derivatives **10a-c.** Moreover, condensation of **10a,c** with aromatic aldehydes afforded the corresponding 5-arylidine-thiazolidin-4-one derivatives **11a,b.** Cytotoxic evaluation of some of the newly synthesized compounds against human breast carcinoma cell line MCF-7 revealed that, these compounds showed promising activity compared with Doxorubicin as positive control.

Keywords:

Benzimidazole; Pyridine; Sulfamoyl carboxamide; Thiazolidin-4-one; MCF-7 cells.

Introduction

Benzimidazoles are nitrogen-containing heterocyclic compounds, well known for their diverse biological and therapeutical properties. Many of them display important pharmacological effects, including antimicrobial [1-3], antiviral [4, 5], analgesic [6], anti-inflammatory [7, 8], antioxidant [9, 10], antidiabetic [11] activities. Also, benzimidazole derivatives have potential cytotoxic activity against different human cancer cell lines [12-15]. Furthermor, several benzimidazole -pyridine conjugates have been reported to possess remarkable anticancer activity [16-18].

Breast cancer is the most common cancer in women and it is the second leading cause of cancer-related deaths [19]. There are considerable challenges in treating breast cancer, including treatment resistance, disease recurrence, and metastasis [20]. Therefore, the discovery and development of anticancer drugs, especially cytotoxic agents, is one of the significant targets in medicinal chemistry.

Based on these findings, the present work includes synthesis of new series of benzimidazole-pyridine compounds incorporating with different side chains and heterocyclic moities which was reported to possess potential anticancer activity such as; N-substituted sulfamoyl carboxamide, thiosemicarbazide, thiazolidin-4-one, 5-arylidine-thiazolidin-4-one [21-24]. Then, twelve of the new compounds were selected as a representative examples to evaluate their cytotoxic activity against human breast carcinoma cell line MCF-7.

Experimental

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 ±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. ^{1}H 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.

2-Oxo-6-phenyl-4-(thiophen-2-yl)-1, 2-dihydropyridine-3-carboxylic acid (2)

A solution of the carbonitrile derivative **1** (0.1 mol) in concentrated sulphuric acid (30 mL) was heated in water bath at 80 °C for 1 h, then allowed to cool. The reaction solution was poured onto ice/water and stirred for 15 min. at room temperature. The obtained solid was collected by filtration, washed with cold water and recrystallized from DMF/EtOH to give the carboxylic acid derivative **2**.

Yield: 84 %, m.p. = 277-278 °C. IR (KBr) ν, cm^{-1}: 3428-3266 (br., OH), 3230 ((NH), 3068 ($CH_{aromatic}$), 1671 (C═O, acid), 1632 (C═O, pyridone). ^{1}H NMR spectrum (500 MHz, d6-DMSO, δ ppm): 6.65-7.87 (m, 9H, Ar-H), 8.21 (br. s ,1H, NH, D_2O exchangeable). ^{13}C NMR (125 MHz, d6-DMSO, δ ppm): 105.7, 123.7, 126.9, 127.8, 128.3, 129.4, 130.6, 134.0, 139.0, 141.0, 150.2, 154.3 (aromatic-C), 161.7 (ring C=O), 168.4 (carboxylic C=O). MS m/z: $M^{+\cdot}$297 (77 %). Anal. Calcd. For $C_{16}H_{11}NO_3S$ (297.33): C, 64.63; H, 3.73; N, 4.71; S, 10.78 %; found: C, 64.96; H, 4.02; N, 5.01; S, 10.45 %.

3-(1H-benzo[d]imidazol-2-yl)-6-phenyl-4-(thiophen-2-yl) pyridin-2(1*H*)-one (3)

A mixture of the carboxylic acid **2** (0.1 mol) and 1,2-phenylenediamine (0.1 mol) in phosphoric acid (200 mL) was heated at 160-180°C for 1 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/H_2O to give compound **3**.

Yield: 72 %, m.p. = 198 °C. IR (KBr) ν, cm^{-1}: 3334, 3262 (NH), 3102, 3020 ($CH_{aromatic}$), 1636 (C=O). ^{1}H NMR spectrum (500 MHz, d6-DMSO, δ ppm): 6.92-8.01 (m, 13H, Ar-H), 8.16 (s ,1H, NH, D_2O exchangeable), 11.63 (br. s ,1H, NH, D_2O exchangeable). ^{13}C NMR (125 MHz, d6-DMSO, δ ppm): 103.6, 112.9, 124.1, 126.4, 126.9, 127.6, 128.0, 128.5, 129.3, 129.9, 130.4, 140.6, 141.0, 146.4, 147.1 (aromatic-C), 165.4 (C=O). MS m/z: $M^{+\cdot}$ 369 (73 %). Anal. Calcd. for $C_{22}H_{15}N_3OS$ (369.44): C, 71.52; H, 4.09; N, 11.37; S, 8.68 %; found: C, 71.91; H, 4.46; N, 11.65; S, 8.96 %.

2-(2-Chloro-6-phenyl-4-(thiophen-2-yl) pyridin-3-yl)-1*H*-benzo[d]imidazole (4)

A suspension of compound **3** (0.01 mol) in phosphorus oxychloride (10 mL) in presence of phosphorous pentachloride (0.01 mole) was refluxed for 8 h. The reaction mixture was cooled, poured slowly onto crushed ice and neutralized with aqueous ammonia (28 %) to pH 7. The obtained solid was filtered off, washed with water and recrystallized from ethanol/H_2O to give the chloro derivative **4**.

Yield: 70 %, m.p = 139 °C. IR (KBr) ν, cm^{-1}: 3217(NH), 3132, 3042 ($CH_{aromatic}$), 1627 (C=N), 765 (C–Cl). ^1H NMR spectrum (500 MHz, d6-DMSO, δ ppm): 6.55-8.04 (m, 13H, Ar-H), 11.71 (s, 1H, NH, D_2O exchangeable). MS m/z: $M^{+\cdot}$387 (69 %). Anal. Calcd. for $C_{22}H_{14}ClN_3S$ (387.88): C, 68.12; H, 3.64; N, 10.83; S, 8.27 %; found: C, 68.56; H, 4.02; N, 10.41; S, 8.59 %.

General procedure for synthesis of 5a-c

A mixture of the chloro derivative **4** (0.001 mol) and different amines namely: ethanolamine, furan-2-amine, 1-methylpiperazine (0.001 mol) in DMF (10 mL) was heated under reflux for 12 h. The reaction mixture was concentrated under reduced pressure, poured onto cold water. The obtained solid was filtered, dried and recrystallized from DMF/EtOH to get the desired derivatives **5a-c**, respectively.

2-((3-(1H-benzo[d]imidazol-2-yl)-6-phenyl-4-(thiophen-2-yl)pyridin-2-yl)amino)ethan-1-ol (5a)

Yield: 69 %, m.p. = 169-170 °C. IR (KBr) ν, cm^{-1}: 3418 (OH), 3298, 3267 (NH), 3084 ($CH_{aromatic}$), 2919, 2849 ($CH_{aliphatic}$). ^1H NMR spectrum (500 MHz, d6-DMSO, δ ppm): 3.67 (t, 2H, *J* = 6.7 Hz, NCH_2), 3.89 (t, 2H, *J* = 6.7 Hz, OCH_2), 5.01 (s, 1H, OH, D_2O exchangeable), 5.78 (br. s ,1H, NH, D_2O exchangeable), 6.55-8.14 (m, 13H, Ar-H), 11.48 (br. s , 1H, NH, D_2O exchangeable). MS m/z: $M^{+\cdot}$412 (34 %). 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, 70.16; H, 4.58; N, 13.89; S, 8.13 %.

3-(1H-benzo[d]imidazol-2-yl)-N-(furan-2-yl)-6-phenyl-4-(thiophen-2-yl)pyridin-2-amine (5b)

Yield: 71%, m.p. = 157 °C. IR (KBr) ν, cm^{-1}: 3380, 3272 (NH), 3063 ($CH_{aromatic}$). ^1H NMR spectrum (500 MHz, d6-DMSO, δ ppm): 6.42-8.01 (m, 16H, Ar-H), 10.01; 11.63 (2s, 2H, 2NH, D_2O exchangeable). MS m/z: $M^{+\cdot}$ 434 (28 %). Anal. Calcd. for $C_{26}H_{18}N_4OS$ (434.52): C, 71.87; H, 4.18; N, 12.89; S, 7.38 %; found: C, 71.49; H, 4.52; N, 13.18; S, 7.02 %.

2-(2-(4-Methylpiperazin-1-yl)-6-phenyl-4-(thiophen-2-yl)pyridin-3-yl)-1H-benzo[d]imidazole (5c)

Yield: 73 %, m.p. = 161 °C. IR (KBr) ν, cm^{-1}: 3264 (NH), 3023 ($CH_{aromatic}$), 2919, 2849 ($CH_{aliphatic}$). ^1H NMR spectrum (500 MHz, d6-DMSO, δ ppm): 2.11 (s, 3H, N-CH_3), 2.95 (t, 4H, *J* = 5.5 Hz, 2N-CH_2), 3.61 (t, 4H, *J* = 5.5 Hz, 2N-CH_2), 6.55-8.41 (m, 13 Ar-H), 11.52 (br. s ,1H, NH, D_2O exchangeable).

^{13}C NMR (125 MHz, d6-DMSO, δ ppm): 46.4 (N-CH_3), 47.8 (2N-CH_2), 54.9 (2N-CH_2), 104.5, 104.8, 112.2, 114.2, 114.9, 116.3, 123.6, 127.5, 128.7, 129.4, 129.9, 130.1, 141.1, 148.3, 151.8, 160.8 (aromatic-C). MS m/z: $M^{+\cdot}$ 451 (36 %).

Anal. Calcd. for $C_{27}H_{25}N_5S$ (451.59): C, 71.81; H, 5.58; N, 15.51; S, 7.10 %; found: C, 71.49; H, 5.98; N, 15.21; S, 6.77 %.

(2-(2-(4-Methylpiperazin-1-yl)-6-phenyl-4-(thiophen-2-yl)pyridin-3-yl)-1H-benzo[d]imidazole-1-carbonyl) sulfamoyl chloride (6)

A stirred suspension of **5c** (0.001 mol) in dichloromethane (10 mL) was cooled to 0-5° C and chlorosulfonyl isocyanate (CSI) (0.001 mol) in (5 mL) of dry dichloromethane was added dropwise. Following the addition, the reaction mixture was stirred for 2 h at 0-10° C. After reaction completion, the solvent was evaporated under reduced pressure and the residue was solidified by the addition of petroleum ether (20 mL).The obtained solid was filtered, dried and recrystallized from $CHCl_3$/ pet.ether to give the sulfamoyl chloride derivative **6**.

Yield: 66 %, m.p = 192 °C. IR (KBr) ν, cm^{-1}: 3429 (NH), 3028 ($CH_{aromatic}$), 2919, 2851 ($CH_{aliphatic}$), 1644 (C=O), 1342, 1183 (SO_2), 693 (C–Cl). ^{1}H NMR spectrum (500 MHz, d6-DMSO, δ ppm): 2.19 (s, 3H, N-CH_3), 2.97 (t, 4H, *J* = 6.2 Hz, 2N-CH_2), 3.64 (t, 4H, *J* = 6.2 Hz, 2N-CH_2), 6.60-8.36 (m, 13H, Ar-H), 11.52 (br s ,1H, NH, D_2O exchangeable).

^{13}C NMR (125 MHz, d6-DMSO, δ ppm): 46.7 (N-CH_3), 48.1 (2N-CH_2), 55.0 (2N-CH_2), 103.6, 110.9, 114.2, 114.9, 116.3, 123.6, 127.6, 127.9, 128.7, 129.3, 130.4, 134.6, 139.8, 140.7, 145.6, 148.9, 160.2 (aromatic-C), 164.0 (C=O). MS m/z: $M^{+\cdot}$593 (25 %). Anal. Calcd. for $C_{28}H_{25}ClN_6O_3S_2$ (593.12): C, 56.70; H, 4.25; N, 14.17; S, 10.81 %; found: C, 57.11; H, 4.58; N, 14.49; S, 11.13 %.

General procedure for synthesis of 7a,b.

To a solution of the sulfamoyl chloride derivative **6** (0.002 mol) in dry 1,4-dioxane (10 mL) containing few drops of triethylamine, the appropriate primary amine namely: furan-2-amine and 3-bromoaniline (0.002 mol) was added. The reaction mixture was stirred at room temperature for 6 h, and then poured onto ice/water. The obtained solid was collected by filtration and recrystallized from DMF/H_2O to give the N-substituted sulfamoyl derivatives **7a,b**.

N-(N-(furan-2-yl)sulfamoyl)-2-(2-(4-methylpiperazin-1-yl)-6-phenyl-4-(thiophen-2-yl)pyridin-3-yl)-1H-benzo[d]imidazole-1-carboxamide (7a)

Yield: 69 %, m.p. = 175 °C. IR (KBr) ν, cm^{-1}: 3425, 3245 (NH), 3057 ($CH_{aromatic}$), 2924, 2857 ($CH_{aliphatic}$), 1640 (C=O), 1340, 1174 (SO_2). ^{1}H NMR spectrum (500 MHz, d6-DMSO, δ ppm): 2.15 (s, 3H, N-CH_3), 2.96 (t, 4H, , *J* = 7.8 Hz, 2N-CH_2), 3.66 (t, 4H, , *J* = 7.8, Hz, 2N-CH_2), 6.30-8.29 (m, 16 Ar-H), 12.24 (br s, 2H, 2NH, D_2O exchangeable).

^{13}C NMR (125 MHz, d6-DMSO, δ ppm): 47.1 (N-CH_3), 48.5 (2N-CH_2), 56.2 (2N-CH_2), 103.4, 107.5, 111.0, 114.3, 115.1, 117.2, 123.6, 127.6, 127.8, 128.0, 129.2, 129.4, 130.3, 130.6, 139.2, 140.7, 141.4, 145.5, 160.1, 161.4 (aromatic-C), 164.1 (C=O). MS m/z: $M^{+\cdot}$639 (22 %).

Anal. Calcd. for $C_{32}H_{29}N_7O_4S_2$ (639.75): C, 60.08; H, 4.57; N, 15.33; S, 10.02 %; found: C, 60.36; H, 4.30; N, 15.61; S, 10.34 %.

N-(N-(3-bromophenyl)sulfamoyl)-2-(2-(4-methylpiperazin-1-yl)-6-phenyl-4-(thiophen-2-yl)pyridin-3-yl)-1H-benzo [d] imidazole-1-carboxamide (7b)

Yield: 64 %, m.p. = 163 °C. IR (KBr) ν, cm^{-1}: 3428, 3258 (NH), 3065 ($CH_{aromatic}$), 2928, 2855 ($CH_{aliphatic}$), 1646 (C=O), 1343, 1146 (SO_2), 678 (C–Br). ^{1}H NMR spectrum (500 MHz, d6-DMSO, δ ppm): 2.17 (s, 3H, N-CH_3), 3.0 (t, 4H, *J* = 6.8 Hz, 2N-CH_2), 3.71 (t, 4H, *J* = 6.8 Hz, 2N-CH_2), 6.30-8.29 (m, 17H, Ar-H), 12.36 (br s, 2H, 2NH, D_2O exchangeable). MS m/z: $M^{+\cdot}$ 728 (18 %). Anal. Calcd. for $C_{34}H_{30}BrN_7O_3S_2$ (728.68): C, 56.04; H, 4.15; N, 13.46; S, 8.80 %; found: C, 56.36; H, 4.52; N, 13.11; S, 8.43 %.

2-(2-Hydrazinyl-6-phenyl-4-(thiophen-2-yl) pyridin-3-yl)-1H-benzo[d]imidazole (8)

A mixture of the chloro derivative **4** (0.01 mol) and hydrazine hydrate 100 % (0.03 mol) in absolute ethanol (30 mL) was refluxed for 8 h. After reaction completion, the reaction solution was concentrated, poured onto ice/water. The obtained solid was filtered, dried and recrystallized from ethanol/H_2O to give the hydrazide derivative **8**.

Yield: 78 %, m.p = 176°C. IR (KBr) ν, cm^{-1}: 3362, 3255 (NH), 3063 ($CH_{aromatic}$), 1633 (C=N). ^{1}H NMR spectrum (500 MHz, d6-DMSO, δ ppm): 4.62 (br. s, 2H, NH_2), 6.53-7.83 (m, 13H, Ar-H), 8.64; 11.68 (2s, 2H, 2NH, D_2O exchangeable). MS m/z: $M^{+\cdot}$383 (49 %). Anal. Calcd. for $C_{22}H_{17}N_5S$ (383.47): C, 68.91; H, 4.47; N, 18.26; S, 8.36 %; found: C, 68.65; H, 4.88; N, 18.62; S, 8.71 %.

General procedure for synthesis of 9a-d.

A mixture of the hydrazide derivatives **8** (0.003 mol) and different alkyl/arylisothiocyanates namely: methylisothiocyanate, phenylisothiocyanate, 4-bromophenylisothiocyanate, 4-methoxy- phenylisothiocyanate (0.003 mol) in absolute ethanol (20 mL) was heated under reflux for 8 h. The reaction solution was poured onto ice/water and the obtained solid was collected by filtration and crystallized from EtOH/H_2O to give the N-substituted thiosemicarbazide derivatives **9a-d**, respectively.

1-(3-(1H-benzo[d]imidazol-2-yl)-6-phenyl-4-(thiophen-2-yl) pyridin-2-yl)-N-methyl thiosemicarbazid (9a)

Yield: 69 %, m.p. = 189 °C. IR (KBr) ν, cm^{-1}: 3438, 3298, 3232 (NH), 3058 ($CH_{aromatic}$), 2942, 2837 ($CH_{aliphatic}$), 1606 (C=N), 1238 (C=S). ^1H NMR spectrum (500 MHz, d6-DMSO, δ ppm): 2.61 (s, 3H, NCH_3), 6.72-7.88 (m, 13H, Ar-H), 8.16; 9.57; 10.26; 11.89 (4s, 4H, 4NH, D_2O exchangeable). MS m/z: $M^{+\cdot}$456 (23 %). Anal. Calcd. for $C_{24}H_{20}N_6S_2$ (456.59): C, 63.13; H, 4.42; N, 18.41; S, 14.04 %; found: C, 63.41; H, 4.12; N, 18.78; S, 14.37 %.

1-(3-(1H-benzo[d]imidazol-2-yl)-6-phenyl-4-(thiophen-2-yl) pyridin-2-yl)-N-phenyl thiosemicarbazid (9b)

Yield: 60 %, m.p. = 125-126 °C. IR (KBr) ν, cm^{-1}: 3416, 3287, 3211 (NH), 3072 ($CH_{aromatic}$), 1620 (C=N), 1239 (C=S). ^1H NMR spectrum (500 MHz, d6-DMSO, δ ppm): 6.78-8.13 (m, 18H, Ar-H), 8.48; 9.61; 10.97; 11.96 (4s, 4H, 4NH, D_2O exchangeable). MS m/z: $M^{+\cdot}$518 (34 %).

Anal. Calcd. for $C_{29}H_{22}N_6S_2$ (518.66): C, 67.16; H, 4.28; N, 16.20; S, 12.36 %; found: C, 66.79; H, 4.52; N, 16.59; S, 12.11 %.

1-(3-(1H-benzo[d]imidazol-2-yl)-6-phenyl-4-(thiophen-2-yl)pyridin-2-yl)-N-(4-bromophenyl)thiosemicarbazide(9c)

Yield: 65 %, m.p.= 163-164 °C. IR (KBr) ν, cm^{-1}: 3432, 3277, 3215 (NH), 3059 ($CH_{aromatic}$), 1611 (C=N), 1235 (C=S). ^1H NMR spectrum (500 MHz, d6-DMSO, δ ppm): 6.65-8.21 (m, 17H, Ar-H), 8.55; 9.91; 10.89; 12.02 (4s, 4H, 4NH, D_2O exchangeable). MS m/z: $M^{+\cdot}$597 (19 %).

Anal. Calcd. for $C_{29}H_{21}BrN_6S_2$ (597.55): C, 58.29; H, 3.54; N, 14.06; S, 10.73 %; found: C, 58.01; H, 3.82; N, 14.43; S, 10.41 %.

1-(3-(1H-benzo[d]imidazol-2-yl)-6-phenyl-4-(thiophen-2-yl)pyridin-2-yl)-N-(4-methoxyphenyl)thiosemicarbazide (9d)

Yield: 71 %, m.p. = 158-159 °C. IR (KBr) ν, cm^{-1}: 3424, 3291, 3231 (NH), 3081 ($CH_{aromatic}$), 2943, 2858 ($CH_{aliphatic}$), 1615 (C=N), 1240 (C=S). ^1H NMR spectrum (500 MHz, d6-DMSO, δ ppm): 3.70 (s, 3H, OCH_3), 6.74-8.03 (m, 17H, Ar-H), 8.32; 9.57; 10.93; 11.89 (4s, 4H, 4NH, D_2O exchangeable).

^{13}C NMR (125 MHz, d6-DMSO, δ ppm): 56.1 (O-CH_3), 114.1, 114.4, 114.8, 117.1, 123.9, 124.5, 125.9, 127.4, 127.8, 128.7, 129.2, 129.6, 130.1, 131.4, 132.8, 139.5, 144.7, 145.6, 156.8, 157.3, 161.4 (aromatic-C), 187.2 (C=S). MS m/z: $M^{+\cdot}$548 (21 %). Anal. Calcd. for $C_{30}H_{24}N_6OS_2$ (548.68): C, 65.67; H, 4.41; N, 15.32; S, 11.69 %; found: C, 66.01; H, 4.76; N, 15.03; S, 11.33 %.

General procedure for synthesis of 10a-c.

A mixture of the thiosemicarbazide derivatives **9a,c,d** (0.001 mol), ethyl chloroacetate (0.001 mol) and anhydrous sodium acetate (0.003 mol) in absolute ethanol (20 mL) was heated under reflux for 12 h. The reaction mixture was concentrated, poured onto cold water, the obtained solid was collected by filtration and crystallized from dil. ethanol to give the thiazolidin-4-one derivatives **10a-c,** respectively.

2-(2-(3-(1H-benzo[d]imidazol-2-yl)-6-phenyl-4-(thiophen-2-yl) pyridin-2-yl) hydrazono)-3-methylthiazolidin-4-one (10a)

Yield: 61 %, m.p. = 154°C. IR (KBr) ν, cm^{-1}: 3415, 3281 (NH), 3028 ($CH_{aromatic}$), 2928, 2848 ($CH_{aliphatic}$), 1739 (C=O), 1618 (C=N). ^{1}H NMR spectrum (500 MHz, d6-DMSO, δ ppm): 3.42 (s, 3H, NCH_3), 4.10 (s, 2H, CH_2S, thiazolidinone), 6.53-7.98 (m, 13H, Ar-H), 8.20; 11.97 (2s, 2H, 2NH, D_2O exchangeable). MS m/z: $M^{+\cdot}$496 (26 %). Anal. Calcd. for $C_{26}H_{20}N_6OS_2$ (496.61): C, 62.88; H, 4.06; N, 16.92; S, 12.91 %; found: C, 62.52; H, 4.39; N, 17.33; S, 12.54 %.

2-(2-(3-(1H-benzo[d]imidazol-2-yl)-6-phenyl-4-(thiophen-2-yl)pyridin-2-yl)hydrazono)-3-(4-bromophenyl) thiazolidin-4-one (10b)

Yield: 63 %, m.p.= 148-149 °C. IR (KBr) ν, cm^{-1}: 3412, 3294 (NH), 3100, 3025 ($CH_{aromatic}$), 2916, 2851 ($CH_{aliphatic}$), 1732 (C=O), 1634 (C=N). ^{1}H NMR spectrum (500 MHz, d6-DMSO, δ ppm): 4.25 (s, 2H, SCH_2, thiazolidinone), 6.57-8.22 (m, 17H, Ar-H), 8.35; 11.97 (2s , 2H, 2NH, D_2O exchangeable). MS m/z: $M^{+\cdot}$637 (16 %). Anal. Calcd. for $C_{31}H_{21}BrN_6OS_2$ (637.57): C, 58.40; H, 3.32; N, 13.18; S, 10.06 %; found: C, 58.76; H, 3.62; N, 12.88; S, 10.43 %.

2-(2-(3-(1H-benzo[d]imidazol-2-yl)-6-phenyl-4-(thiophen-2-yl)pyridin-2-yl)hydrazono)-3-(4-methoxyphenyl) thiazolidin-4-one (10c)

Yield: 67 %, m.p.= 121-123 °C. IR (KBr) ν, cm^{-1}: 3426, 3229 (NH), 3114 ($CH_{aromatic}$), 2959, 2846 ($CH_{aliphatic}$), 1737 (C=O), 1622 (C=N). ^{1}H NMR spectrum (500 MHz, d6-DMSO, δ ppm): 3.78 (s, 3H, OCH_3), 4.20 (s, 2H, CH_2S, thiazolidinone), 6.78-8.07 (m, 17H, Ar-H), 8.31; 11.87 (2s , 2H, 2NH, D_2O exchangeable). ^{13}C NMR (125 MHz, d6-DMSO, δ ppm): 34.1 (CH_2S, thiazolidinone), 55.7 (O-CH_3), 114.1, 114.4, 115.1, 117.6, 123.6, 123.9, 125.6, 127.5, 127.8, 128.9, 129.1, 129.3, 130.7, 132.8, 140.8, 141,3 145.5, 148.8, 156.7, 157.1, 162.1 (S-C-N thiazolidinone, aromatic-C), 169.3 (C=O, thiazolidinone). MS m/z: $M^{+\cdot}$588 (27 %). Anal. Calcd. for $C_{32}H_{24}N_6O_2S_2$ (588.70): C, 65.29; H, 4.11; N, 14.28; S, 10.89 %; found: C, 65.62; H, 3.87; N, 14.71; S, 11.23 %.

General procedure for synthesis of 11a,b.

A mixture of the thiazolidin-4-one derivative **10a** (0.001 mol), 4-methoxybenzaldehyde and/or 4-methylbenzaldehyde (0.001 mol) and anhydrous sodium acetate (0.002 mol) in glacial acetic acid (20 mL) was heated under reflux for 8 h. The reaction mixture was concentrated under reduced pressure and poured onto cold water. The obtained solid was collected by filtration and crystallized from ethanol/H_2O to give the 5-arylidine-thiazolidin-4-one derivatives **11a;b,** respectively.

2-(2-(3-(1H-benzo[d]imidazol-2-yl)-6-phenyl-4-(thiophen-2-yl)pyridin-2-yl)hydrazono)-5-((E)-4-methoxy benzylidene)-3-methylthiazolidin-4-one (11a)

Yield: 68 %, m.p. = 166-167 °C. IR (KBr) ν, cm^{-1}: 3428, 3272 (NH), 3098, 3063 ($CH_{aromatic}$), 2919, 2852 ($CH_{aliphatic}$), 1748 (C=O), 1633 (C=N), 1598 (C=C). ^{1}H NMR spectrum (500 MHz, d6-DMSO, δ ppm): 3.55 (s, 3H, NCH_3), 3.86 (s, 3H, OCH_3), 6.58-8.17 (m, 18H, CH olefinic; Ar-H), 10.03; 12.14 (2s, 2H, 2NH, D_2O exchangeable). ^{13}C NMR (125 MHz, d6-DMSO, δ ppm): 31.7 (N-CH_3), 56.8 (O-CH_3), 114.2, 114.5, 115.3, 115.6, 117.4, 124.2, 125.6, 127.5, 127.7, 129.3, 129.6, 129.9, 130.7, 131.3, 132.5, 141.3, 143.5, 144.6, 148.9, 156.6, 157.5, 159.3, 162.7 (N-C-S thiazolidinone, olefinic-C, and aromatic-C), 167.4 (C=O, thiazolidinone). MS m/z: $M^{+\cdot}$614 (21 %). Anal. Calcd. for $C_{34}H_{26}N_6O_2S_2$ (614.74): C, 66.43; H, 4.26; N, 13.67; S, 10.43 %; found: C, 66.87; H, 4.58; N, 13.99; S, 10.12 %.

2-(2-(3-(1H-benzo[d]imidazol-2-yl)-6-phenyl-4-(thiophen-2-yl)pyridin-2-yl)hydrazono)-3-methyl-5-((E)-4-methyl benzylidene) thiazolidin-4-one (11b)

Yield: 64 %, m.p.= 189-191°C. IR (KBr) ν, cm^{-1}: 3429, 3269 (NH), 3097, 3056 ($CH_{aromatic}$), 2917, 2853 ($CH_{aliphatic}$), 1749 (C=O), 1638 (C=N), 1598 (C=C). ^{1}H NMR spectrum (500 MHz, d6-DMSO, δ ppm): 2.38 (s, 3H, CH_3-Ar), 3.60 (s, 3H, NCH_3), 6.47-8.17 (m, 18H, CH olefinic; Ar-H), 9.93; 12.13 (2s, 2H, 2NH, D_2O exchangeable). MS m/z: $M^{+\cdot}$598 (14 %). Anal. Calcd. for $C_{34}H_{26}N_6OS_2$ (598.74): C, 68.21; H, 4.38; N, 14.04; S, 10.71 %; found: C, 68.59; H, 4.82; N, 14.38; S, 10.43 %.

***In Vitro* Anticancer Screening**

Human breast carcinoma cell line MCF-7 was available at the National Cancer Institute, Cairo, Egypt. The antitumor activity of the newly synthesized compounds was measured using the sulfo-rhodamine-B stain (SRB) assay using the method of Shekhan P. *et. al.* [25]. The cell lines were grown in RPMI 1640 medium containing 10% fetal bovine serum and 2 mM L-glutamine.

The cells were plated in 96-multiwell plate (104 cells/well) for 24 h before treatment with compounds to allow the attachment of the cells to the wall of the plate. Different concentrations of the compounds under test (5, 12.5, 25 and 50 µg/mL) were added to the cell monolayer triplicate wells which were prepared for each individual dose. Monolayer cells were incubated with the compounds for 48 h at 37°C and in atmosphere of 5 % CO_2. After 48h, Cultures fixed with trichloroacetic acid and stained for 30 minutes with 0.4% (wt/vol) sulforhodamine B (SRB) dissolved in 1% acetic acid. Unbound dye was removed by four washes with 1% acetic acid, and protein-bound dye was extracted with Tris-EDTA buffer. Color intensity was measured in an ELISA reader. Measurements were done six times (n = 6) and averaged. The relationship between surviving fraction and drug concentration was plotted to get the survival curve of each compound and the concentration that causes 50 % inhibition of cellular viability (IC50 value) was calculated (Table 1).

Results and Discussion

Chemistry

The synthesis of novel benzimidazole-linked pyridine derivatives were prepared as outlined in Schemes 1 and 2. The structures of all of the newly synthesized compounds were confirmed by elemental analyses and spectral data (IR, ^{1}H-NMR, MS). Moreover, ^{13}C-NMR spectra were recorded for some of the compounds. The starting material, 2-oxo-6-phenyl-4-thiophen-2-yl-1,2-dihydro-pyridine-3-carbonitrile (**1**) was prepared according to the reported methode [26]. The cyano group of the nitrile derivative **1** was hydrolyzed to carboxyl group by heating in concentrated sulphuric acid to give, the key intermediate, 2-pyridone-3-carboxylic acid derivative **2.** IR spectrum of compound **2** exhibited the disappearance of C≡N absorption band at 2211 cm^{-1} of the parent nitrile and represented broad absorption band in the region 3428-3266 cm^{-1} and strong band at 1671 cm^{-1} corresponding to carboxyl O-H and carboxyl C=O groups, respectively. Also, ^{13}C NMR spectrum of **2** exhibited, besides the expected carbon signals of the molecule, two signals at δ 161.7, 168.4 ppm corresponding to (pyridone ring C=O) and (carboxylic C=O), respectively. Moreover, mass spectrum of **2** showed the molecular ion peak which in agreement with its molecular formula. In order to synthesize the target benzimidazole -pyridine conjugates, cyclocondensation of the carboxylic acid derivative **2** with 1,2-phenylenediamine was carried out by utilizing phosphoric acid at 160-180°C as a condensing agent to give 3-(1H-benzo[d]imidazol-2-yl)-6-phenyl-4-(thiophen-2-yl) pyridin-2(1*H*)-one (**3**). IR spectrum of compound **3** exhibited the disappearance of two absorption bands corresponding to carboxyl O-H and carboxyl C=O groups. ^{1}H NMR spectrum of **3** represented a

multiplet signal of the aromatic protons at δ 6.92-8.01 ppm. Also, ^{13}C NMR spectrum of compound **3** revealed, besides the expected carbon signals of the molecule, the disappearance of the signal at 168.4 ppm of the carboxyl C=O carbon. In addition, mass spectrum of **3** displayed the molecular ion peak which in agreement with its molecular formula. Further reaction of compound **3** with $PCl_5/POCl_3$ mixture as a chlorinating reagent afforded the conversion of pyridone moiety to chloropyridine to give 2-(2-Chloro-pyridin-3-yl)-1*H*-benzo[d]imidazole derivative **4.** IR spectrum of compound **4** exhibited the disappearance of the C=O absorption band and represented an absorption band at 765 cm^{-1} of the C–Cl bond. Also, mass spectrum of **4** exhibited the molecular ion peak which in agreement with its molecular formula. Nucleophilic substitution of the chloro derivative **4** with various primary/secondary amines afforded the N-substituted amino derivatives **5a-c**. The IR spectra of compounds **5a-c** displayed the expected absorption bands of the molecules at the expected regions. For example, IR spectrum of **5a** showed two absorption bands at 3418 and 3298 cm^{-1} corresponding to OH and NH groups of ethanolamine moiety, respectively. Also, ^{1}H NMR spectra of **5a-c** derivatives confirmed their chemical structures.

For example, ^{1}H NMR spectrum of **5c** represented the new 1-methylpiperazine functionality as a singlet signal at δ 2.11 ppm for the three protons of NC*H*$_3$ and two triplet at δ 2.95, 3.61 ppm representing the 4N-C*H*$_2$, in addition to the parent signals of the molecule. Moreover, ^{13}C NMR spectrum of **5c** showed signal at δ 46.4 ppm for N-*C*H$_3$, two signals at δ 47.8, 54.9 ppm corresponding to 4N-*C*H$_2$ and sixteen signals at the region δ 104.5-160.8 of the aromatic-C. The molecular ion peaks represented by mass spectra of **5a-c** confirmed the molecular formulae of the compounds. Moreover, chlorosulfonyl isocyanate (CSI) has been found to be a versatile reagent in organic synthesis with great interest in heterocyclic chemistry [27, 28].

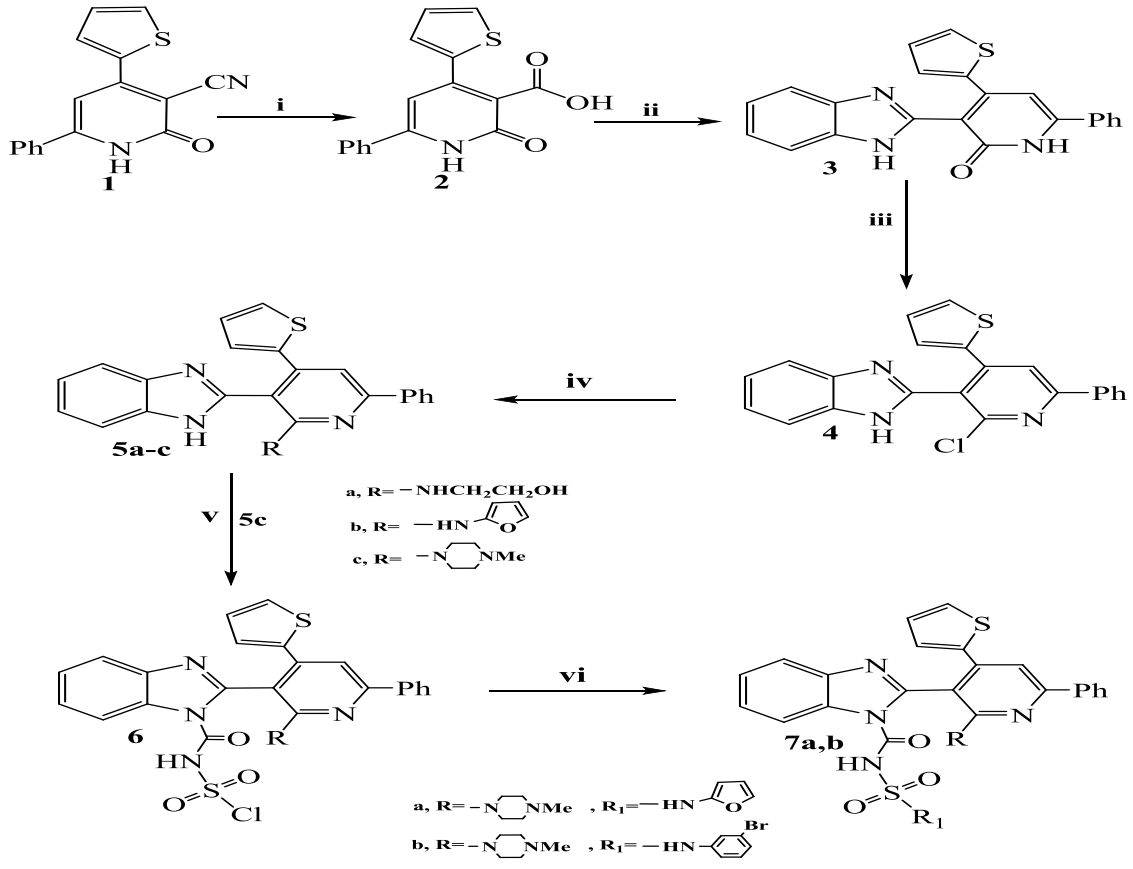
In the reactions of secondary amines with CSI, the isocyanate portion generally reacts prior to the chlorosulfonyl group through nucleophilic addition at the carbon-nitrogen double bond to give the N-chlorosulfonyl carboxamide derivative [29-31]. Thus, compound **5c** was treated with CSI in dry dichloromethane to give (2-(2-(4-methylpiperazin-1-yl)- pyridin-3-yl)-1H-benzo[d]imidazole-1-carbonyl)sulfamoyl chloride derivative **6** via nucleophilic addition of the benzimidazole NH group to the isocyanate portion of CSI. IR spectrum of compound **6** represented an absorption band of the amidic C=O group at 1644 cm^{-1}, two absorption bands at 1342, 1183 cm^{-1} due to the SO_2 group and an absorption band at 693 cm^{-1} corresponding to the C–Cl bond. ^{13}C NMR spectrum of **6** exhibited, besides the expected signals of aromatic carbons

of the molecule, signal at δ 164.0 ppm corresponding to C=O carbon. Also, Mass spectrum of compound **6** showed the molecular ion peak which confirmed its molecular formula.

Furthermore, nucleophilic substitution reaction of N-chlorosulfonyl carboxamide derivatives with amines afforded the synthesis of N-substituted sulfamoyl derivatives [32]. In this work, further reaction of compound **6** with different primary amines (furan-2-amine and 3-bromoaniline) in presence of triethylamine gave N-(N-(substituted)sulfamoyl)-1H-benzo[d]imidazole-1-carboxamide derivatives **7a;b,** respectively. IR spectra of the obtained derivatives **7a,b** revealed the appearance of two absorption bands at the regions 3428-3425 and 3258-3245 cm^{-1} referring to two NH groups and an absorption band at 678 cm^{-1} corresponding to the C–Br bond (compound **7b**). Moreover, ^{1}H NMR spectra of the compounds **7a,b** displayed the expected signals of the protons of the molecules at the expected regions. ^{13}C NMR spectrum of **7a** displayed twenty signals at the region δ 103.4-161.4 ppm representing the aromatic-C, in addition to the parent signals. Also, mass spectra of **7a,b** were in agreement with their molecular formulae (Scheme 1).

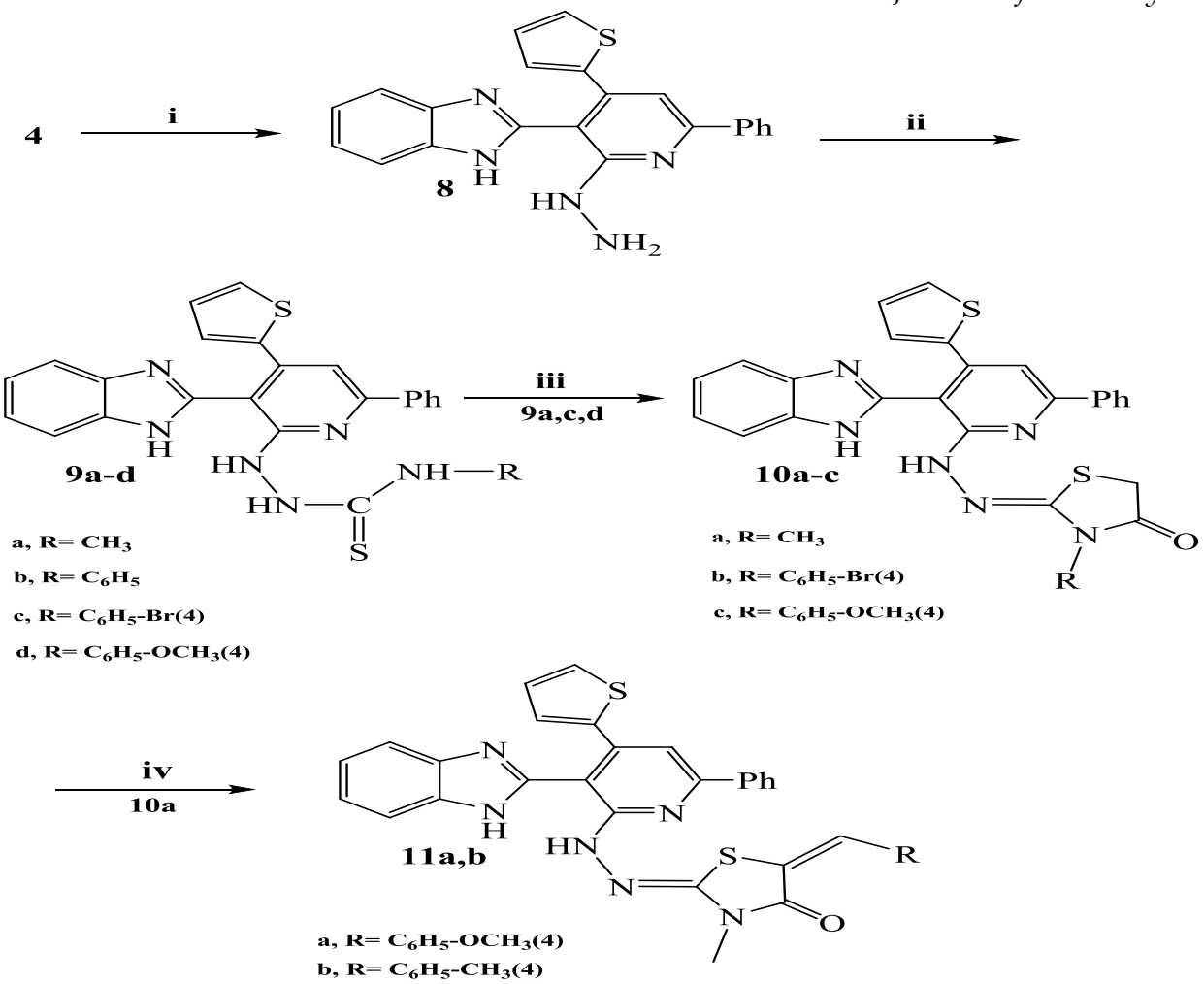
On the other hand, condensation of chloro derivative 4 with hydrazine hydrate in boiling ethanol afforded the hydrazide derivative 2-(2-hydrazinyl-6-phenyl-4-(thiophen-2-yl)pyridin-3-yl)-1H-benzo[d]imidazole (**8**). IR spectrum of compound **8** displayed two absorption bands at 3362, 3255 cm^{-1} due to the (2NH) groups and the disappearance of the C–Cl absorption band at 693 cm^{-1}. Also, mass spectrum of **8** exhibited the molecular ion peak which in agreement with its molecular formula. Nucleophilic addition of the formed hydrazide derivative **8** to different alkyl/arylisothiocyanates in ethanol under reflux, gave the thiosemicarbazides derivatives **9a-d.** The IR spectra of compounds **9a-d** displayed the expected absorption bands of the thiosemicarbazide moiety at the expected regions. For example, IR spectrum of **9a** showed three absorption bands at 3438, 3298 and 3232 cm^{-1} corresponding to (3NH) groups and an absorption band at 1238 cm^{-1}corresponding to C=S group . For example, ^{1}H NMR spectrum of **9d** revealed singlet signal due to OCH_3 at δ 3.70 ppm and four singlet signals at 8.32; 9.57; 10.93; 11.89 ppm for (4NH) protons that exchange with D_2O. Also,^{13}C NMR spectrum of **9d** displayed signal at 56.1 ppm and signal at 187.2 ppm representing the (O-CH_3) and C=S groups of the N-(4-methoxyphenyl)thiosemicarbazide moiety, respectively. In addition, Mass spectra of the compounds represented the molecular ion peaks which were in agreement with their molecular formulae. Further cyclocondensation reaction of the thiosemicarbazide derivatives **9a,c,d** with ethyl chloroacetate in presence of anhydrous sodium acetate in refluxing ethanol afforded thiazolidin-4-one derivatives **10a-c.** IR spectra of the obtained derivatives revealed an absorption bands

at the range 1739-1732 cm^{-1} due to C=O of the new formed thiazolidin-4-one ring. ^{1}H NMR spectra of **10a-c** confirmed their chemical structures. They exhibited, besides the parent protons, a new singlet signal at the range δ 4.10-4.25 ppm representing (CH_2S, thiazolidin-4-one). In addition, mass spectra of the compounds **10a-c** were in consistent with molecular structures of the compounds. The synthesis of 5-arylidine-thiazolidin-4-one derivatives **11a,b** was achieved by reaction of 2-(2-(3-(1H-benzo[d]imidazol-2-yl)-6-phenyl-4-(thiophen-2-yl)pyridin-2-yl)hydrazono)-3-methylthiazolidin-4-one (**10a**) with aromatic aldehydes namely: 4-methoxybenzaldehyde and 4-methylbenzaldehyde , respectively. ^{1}H NMR spectra of **11a, b** exhibited the disappearance of singlet signal at δ 4.10 ppm representing the two protons of CH_2 of thiazolidin-4-one ring in the parent compound. Moreover, ^{1}H NMR spectrum of **11a**, exhibited singlet signal at δ 3.86 ppm corresponding to OCH_3 of 4-methoxybenzylidene moiety. Also, ^{13}C NMR spectrum of **11a** displayed signal at 56.8 ppm representing the O*C*H$_3$ group besides the expected signals of aromatic carbons of the molecule. Mass spectra of the derivatives **11a, b** showed the molecular ion peaks of the compounds which were in agreement of their molecular formulae (Scheme 2).



Scheme1:

i) Conc. H_2SO_4 , 80 °C, 1 h ; ii) 1,2-phenylenediamine, H_3PO_4, 160-180 °C, 1 h ; iii) $POCl_3$, PCl_5, reflux for 8 h ; iv) different amines, DMF, reflux for 12 h ; v) CSI, CH_2Cl_2, 0-10 °C, stirring for 2 h ; vi) different amines, Et_3N, 1,4-dioxane, stirring for 6 h.



Scheme2:

i) Hydrazine hydrate, absolute ethanol, reflux for 8 h; ii) different alkyl/arylisothiocyanates, absolute ethanol, reflux for 8 h; iii) ethyl chloroacetate, anhydrous sodium acetate, absolute ethanol, reflux for 12 h; iv) aromatic aldehyde, anhydrous sodium acetate, glacial acetic acid, reflux for 8 h.

Biological Results and Discussion

On the basis that the main target of this work is the synthesis of novel derivatives of potent anti-breast cancer activity, twelve of the newly prepared compounds were chosen as representative examples to examine their anticancer activity against human breast carcinoma cell line MCF-7 in comparison to doxorubicin as a reference drug. The tested compounds are **2, 3, 4, 5c, 6, 7a, 8, 9a, 9d, 10a, 10c, 11a**. According to the resultant data (Table 1), the key intermediate 2-pyridone-3- carboxylic acid derivative **2** revealed moderate growth inhibitory activity with IC50 = 84.1 µM. While, the target compound **3** (benzimidazole bearing 2-pyridone derivative) showed an observable increase in the activity with IC50 = 61.5 µM. Conversion of 2-pyridone moiety of compound **3** to chloropyridine as compound **4** led to enhance the activity exhibting IC50 = 56.5 µM. Further increase in the potency was achieved upon replacement of the Cl group of pyridine

moiety with 1-methylpiprazine as compound **5c** with IC50 = 52.9 µM. The most potent compound in this study was the sulfamoyl chloride derivative **6** with IC50 = 18.9 µM, it was found to be nearly as potent as the positive control Doxorubicin with IC50 = 18.3 µM. A noticeable reduction in the growth inhibitory activity has occurred upon reaction of compound **6** with furan-2-amine to give the N-(furan-2-yl)sulfamoyl)- carboxamide derivative **7a** with IC50 = 39.1 µM.

On the other hand, similar potency was obtained upon substitution of Cl group of compound **4** with hydrazine group as the hydrazide derivative **8** with IC50 = 56.3 µM. The thiosemicarbazide derivatives **9a**, **9d** revealed drop in the potency with IC50 = 73.6, 69.1 µM, respectively.

An observable increase in the activity was atchived by cyclocondensation reaction of The thiosemicarbazide derivatives to form thiazolidin-4-one derivatives as compounds **10a**, **10c** with IC50 = 46.3, 25.1 µM, respectively. In addition, 5-arylidine-thiazolidin-4-one derivative **11a** exhibited further increase in breast carcinoma inhibitory potency with IC50 = 20.3 µM.

The above results revealed that benzimidazole -pyridine conjugates showed promising anticancer activities and could be rich source for further research and study.

Table-1: IC50 values of the tested compounds against human breast carcinoma cell line MCF-7.

Compound No.	IC_{50} (µg/mL)	IC_{50} (µM)
2	25.0	84.1
3	22.7	61.5
4	21.9	56.5
5c	23.9	52.9
6	11.2	18.9
7a	25.0	39.1
8	21.6	56.3
9a	33.6	73.6
9d	37.9	69.1
10a	23.0	46.3
10c	14.8	25.1
11a	12.5	20.3
Doxorubicin	9.9	18.3

Conclusion

This work includes the synthesis of new series of benzimidazole bearing pyridine derivatives carrying different biologically active moieties such as, sulfamoyl chloride, substituted thiosemicarbazides, thiazolidin-4-one and 5-arylidine-thiazolidin-4-one. Some of the newly synthesized compounds were selected as representative examples to evaluate their anticancer activity against human breast carcinoma cell line MCF-7. It was found that , the most potent compounds were sulfamoyl chloride derivative 6, thiazolidin-4-one derivative 10c and 5-arylidine-thiazolidin-4-one derivative 11a, they showed significant anti-proliferative activities compared with the reference standard drug doxorubicin.

These results indicate that benzimidazole-pyridine compounds are promising candidates as anticancer agents and they encourage further structural variations to obtain more potent derivatives.

Acknowledgment: The author is grateful to National Research Centre for its support of this work.

References

1. D. Joshi, K. Parikh, Synthesis and evaluation of novel benzimidazole derivatives as antimicrobial agents, Med. Chem. Res., 2014, **23**, 1290-1299.
2. F.A.S. Alasmary, A.M. Snelling, M.E. Zain, A.M. Alafeefy, A.S. Awaad, N. Karodia, Synthesis and evaluation of selected benzimidazole derivatives as potential antimicrobial agents, Molecules, 2015, **20** (8), 15206-15223.
3. Vasantha, G. Basavarajaswamy, M.V. Rai, P. Boja, V.R. Pai, N. Shruthi, M. Bhat, Rapid 'one-pot' synthesis of a novel benzimidazole-5-carboxylate and its hydrazone derivatives as potential anti-inflammatory and antimicrobial agents, Bioorg. Med. Chem. Lett., 2015, **25**(7), 1420-1426 .
4. C.W. Evans, C. Atkins, A. Pathak, B.E. Gilbert, J.W. Noah, Benzimidazole analogs inhibit respiratory syncytial virus G protein function, Antiviral Res., 2015, **121**, 31-38.
5. K. Ding, A. Wang, M.A. Boerneke, S.M. Dibrov, T. Hermann, Aryl-substituted aminobenzimidazoles targeting the hepatitis C virus internal ribosome entry site, Bioorg. Med. Chem. Lett., 2014, 24(14), 3113-3117.
6. A.D. Prasanna, A.L. Saleel, Design and synthesis of Mannich bases as benzimidazole derivatives as analgesic agents, Antiinflamm. Antiallergy Agents Med. Chem., 2015, **14**(1), 35-46.
7. M. Gaba, C. Mohan, Design; Synthesis and biological evaluation of novel 1, 2, 5-substituted benzimidazole derivatives as gastroprotective anti-inflammatory and analgesic agents, Med.Chem., 2015, **5**(2), 58-63.

8. U. Sahoo, A.K. Seth, R. Balaraman, R. Velmurugan, Design; synthesis of some novel thiazolidin-4-one derivatives bearing benzimidazole nucleus and biological evaluation of their possible in vitro antiinflammatory as cyclooxygenase inhibitors and antioxidant activity, Asian J. Chem., 2015, **27**(3), 961-968.
9. A.T. Mavrova, D. Yancheva, N. Anastassova, K. Anichina, J. Zvezdanovic, A. Djordjevic, D. Markovic, A. Smelcerovic, Synthesis; electronic properties; antioxidant and antibacterial activity of some new benzimidazoles, Bioorg. Med. Chem., 2015, 23(19), 6317-6326.
10. S.H. Nile, B. Kumar, S.W. Park, *In vitro* evaluation of selected benzimidazole derivatives as an antioxidant and xanthine oxidase inhibitors, Chem. Biol. Drug Des., 2013, **82**(3), 290-295.
11. P. Bathini, L. Kameshwari, N. Vijaya, Antidiabetic effect of 2 nitro benzimidazole in alloxan induced diabetic rats, Int. J. Basic Clin. Pharmacol., 2013, **2**(6), 814-818.
12. T.S. Reddy, H. Kulhari, V.G. Reddy, V. Bansal, A. Kamal, R. Shukla, Design; synthesis and biological evaluation of 1,3-diphenyl-1H-pyrazole derivatives containing benzimidazole skeleton as potential anticancer and apoptosis inducing agents, Eur. J. Med. Chem., 2015 **101**, 790-805.
13. K.S. Rangappa, R. Roopashree, C.D. Mohan, T.R. Swaroop, S. Jagadish, Synthesis; characterization and in vivo biological evaluation of novel benzimidazoles as potential anticancer agents, Asian J. Pharm. Clin. Res., 2014, **7**(5), 309-313.
14. K.B. Świątkiewicz, P. Olszewska, E.M. Olasik, Biological approach of anticancer activity of new benzimidazole derivatives, Pharmacol. Rep., 2014, **66**, 100-106.
15. B. Soni, M.S. Ranawat, A. Bhandari, R. Sharma, Synthesis and in vitro antitumor activity of benzimidazole derivatives, 2012, Int. J. Drug Res. Tech., **2**(7), 479-485.
16. H.S.A. Elzahabi**,** Synthesis; Characterization of some benzazoles bearing pyridine moiety: search for novel anticancer agents, Eur. J. Med. Chem., 2011, **46**, 4025-4034.
17. K. Starcević, M. Kralj, K. Ester, I. Sabol, M. Grce, K. Pavelić, Z.G. Karminski, Synthesis; antiviral and antitumor activity of 2-substituted-5-amidino-benzimidazoles, Bioorg. Med. Chem., 2007, **15**(13), 4419-4426.
18. J. Luo, W. Zhu, Y. Xuzong, Process for preparing 2-pyridyl-4-carbonyl-benzimidazole derivatives, Patent CN 1425663 A, Jun 25, 2003.

19. W. Wei, M.T. Lewis, Identifying and targeting tumor-initiating cells in the treatment of breast cancer, Endocr. Relat. Cancer, 2015, **22**(3), 135-155.
20. F. Cardoso, N. Harbeck, L. Fallowfield, S. Kyriakides, E. Senkus, Locally recurrent or metastatic breast cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up, Ann. Oncol., 2012, **23**(7), 11–19.
21. N.M.H. Taha, A.A.A. Boray, N.M. Saleh, S.A. Fouad, Synthesis and antitumor screening of some novel pyrrolo, pyrrolo[2,3-d]pyrimidinone and pyrrolo[2,3-b] pyridinone derivatives of sulfaquinoxaline, J. Appl. Sci. Res., 2013, **9**(4), 3108-3117.
22. S. Arora, S. Agarwal, S. Singhal, Anticancer activities of thiosemicarbazides /thiosemicarbazones: A review, Int. J. Pharm. Pharm. Sci., 2014, **6**(9), 34-41.
23. A. Deep, B. Narasimhan, K. Ramasamy, V. Mani, R.K. Mishra, A.B. Majeed, Synthesis; antimicrobial; anticancer evaluation and QSAR studies of Thiazolidin-4-ones clubbed with quinazolinone, Curr. Top. Med. Chem., 2013, **13**(16), 2034-2046.
24. M. Zhengyue, Z. Xinghua, B. Ligai, Z. Yajun, Y. Gengliang, Microwave-assisted synthesis of new 1,3-thiazolidin-4-ones and evaluation of their anticancer efficacy, Mod. Appl. Sci., 2011, **5**(3), 207-212.
25. P. Shekhan, R. Storenge, D. Scudiero, A. Monks, J. McMahon, D. Vistica, J.T. Warren, H. Bokesch, S. Kenney, M.R. Boyd, New colorimetric cytotoxicity assay for anticancer-drug screening, J. Natl. Cancer Inst., 1990, **82**(13), 1107-1112.
26. N. Latif, N. Mishriky, N.S. Girgis, Malononitriles and cyanoesters. 6.Synthesis of new biologically-active cyanopyridines, Indian J. Chem., Sect.B, 1981, **20**(2), 147-149.
27. A. Bendjeddou, Ryad.Djeribi, Z. Regainia , N. Aouf , N,N'-Substituted 1,2,5 thiadiazolidine 1,1-dioxides: synthesis; selected chemical and spectral proprieties and antimicrobial evaluation, Molecules, 2005, **10**, 1387-1398.
28. H. Taguchi, Organic syntheses utilizing the chemical properties of chlorosulfonyl isocyanate, TCIMAIL, 2014, No**.160**, 23-25.
29. M.R. Sarma, P.S. Goud, M. Sailaja, , P.R. Kumar, G.O. Reddy, S. Raju, Synthesis of tenidap: An improved process for the preparation of 5-chloro-2-oxindole-1-carboxamide, Org. Process. Res. Dev., 2001, **5**(1), 61.

30. N.D. Dhar, K. S. Murthy, Recent advances in the chemistry of chlorosulfonyl isocyanate, Synthesis, 1986, 6, 437-449.
31. D. Che, N. Corelli-Rennie, B. Guntoori, J. Faught, Process for the preparation of oxcarbazepine and related intermediates, Patent US 20050282797 A1, Dec 22, 2005.
32. C. Barbey, R. Bouasla, M. Berredjem, N. Dupont, P. Retailleau, N. Aouf, M. Lecouvey, Synthesis and structural study of new substituted chiral sulfamoyl oxazolidin-2-ones, Tetrahedron, 2012, **68**(44), 9125-9130.

Corresponding Author:
Eman M. Mohi El-Deen
Email: *e.mohi.2010@live.com*