American Journal of Organic Chemistry 2016, 6(2): 39-53
DOI: 10.5923/j.ajoc.20160602.01

Synthesis, Characterization and *in vitro* Cytotoxic Evaluation of Some Novel Heterocyclic Compounds Bearing the Indole Ring

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Abstract Reactions of 1*H*-indole-3-carboxaldhyde **1** with thiosemicarbazide derivatives give thiosemicarbazone derivatives **2a,b**. Cyclization of thiosemicarbazone **2a** with HCl, Ac_2O, phenacyl bromides and chloroacetic acid afforded the corresponding 1,2,4-triazole-3-thiol **3**, diacetyl derivative **4**, 1,3-thiazole derivative **5** and 1,3-thiazolidin-4-one derivative **6**, respectively. Compound **6** undergoes a series of heterocyclization reactions to give new heterocyclic compounds. Structures of the newly synthesized compounds have been confirmed by elemental analysis and spectral data. The newly synthesized compounds were evaluated for in vitro cytotoxic activity against three human cancer cell lines, including human liver cancer (Hep G2), human colon cancer (HT-29) and human breast cancer (MCF-7) using MTT assays.

Keywords Thiosemicarbazone, 1.3-Thiazole, 1,3-Thiazoldinone, Pyrazolo [3,4-d]1,3-thiazole, Cytotoxic activity, MTT assay

1. Introduction

Thiosemicarbazones has been used as intermediates for the preparation of many heterocyclic compounds. In the literature, manyresearchers have reported the S/N regioselective nucleophilic completion in the synthesis of heterocyclic compounds by intramolecular cyclization reactions. Changes in reaction conditions can induce S-attack or N-attack to eventually afford different cyclic products from a single starting material. Moreover, thiosemicarbazones bearing an aromatic heterocyclic moiety seem to possess enhanced biological activities [1, 2]. On other hand, heterocyclic compounds containing the indole ring are of major importance due to their therapeutic and pharmacological activities [3-7]. In view of the above and in continuation of our studies on the synthesis of heterocyclic compounds exhibiting biological activity, we report herein the successful reaction of *1H*-indol-3-carboxaldehyde with thiosemicarbazide derivatives to afford the corresponding thiosemicarbazones derivatives. Subsequent cyclization by different reagents and different conditions gave novel heterocyclic compounds bearing the indole moiety which were then investigated for potential cytotoxic activities.

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Published online at http://journal.sapub.org/ajoc

2. Experiment

2.1. General

Melting points were measured on a Gallenkamp apparatus and are uncorrected. IR spectra were recorded on Shimadzu FT-IR 8101 PC infrared spectrophotometer (υ_{max} in cm^{-1}). The ^{1}H-NMR and ^{13}C NMR spectra were determined in DMSO-d6 at 300 MHz on a Varian Mercury VXR-300 NMR spectrometer using TMS as an internal standard. Mass spectra were measured on a GCMS-QP1000 EX spectrometer at 70 Ev. Elemental analyses were carried out at the Microanalyticalcenter of Cairo University and the main chemical warfare laboratories.

2.2. Chemistry

2.2.1. General Procedure for the Preparation of Thiosemicarbazones 2a,b

An equimolar mixture of2-(4-bromophenyl)-1*H*-indole-3 -carboxaldehyde **1** and the selected thiosemicarbazide such as 4-(4-methylphenyl)-thiosemicarbazide or 4-(4- phenyl-1, 3-thiazol-2-yl) thiosemicarbazide (0.01 mol) were refluxed in absolute ethanol (20 mL) in the presence of 2-3 drops of glacial acetic acid for 3h. The reaction mixture was cooled to room temperature, the product separated and filtered, washed with cold water, dried and recrystallized from the appropriate solvent to give **2a,b**.

1-[2-(4-Bromophenyl)-*1H*-indol-3-ylmethylene]-N-(4-methylphenyl)thiosemicarbazone (2a)

Yellow powder. Yield 80%, m.p. 249-250°C (ethanol-DMF). FT-IR (KBr, υ_{max}/cm^{-1}): 3135, 3317 (NH), 3042, 2975, 2857 (CH), 1246 (C=S). ^{1}H-NMR (DMSO-d6) δ ppm: 2.32 (s, 3H, CH_3), 7.17-7.28 (m, 4H, Ar-H), 7.45-7.52 (m, 4H, Ar-H), 7.60 (d, 1H, indole proton), 7.77-7.95 (m, 2H, indole proton), 8.33 (d, 1H, indole proton), 8.55 (s, 1H, =CH), 10.01 (s, 1H, NH exchanged by D_2O), 11.46 (s, 1H, NH exchanged by D_2O), 11.99 (s, 1H, NH exchanged by D_2O). MS: m/z (%): 463 (M^+, 0.3), 357 (1.3), 313 (0.8), 298 (72.5), 284 (10), 271 (100), 216 (23.3), 192 (16.3). Anal. calcd for $C_{23}H_{19}BrN_4S$ (463.39): C, 59.61; H, 4.13; Br, 17.24; N, 12.09; S, 6.92.Found: C, 59.41; H, 4.00; Br, 17.04; N, 12.00; S, 6.72.

1-[2-(4-Bromophenyl)-*1H*-indol-3-ylmethylene]-N-(4-phenyl-1,3-thiazol-2-yl)-thiosemicarbazone (2b)

Yellow powder. Yield 60%, m.p. 140-142°C (ethanol). FT-IR (KBr, υ_{max}/cm^{-1}): 3223, 3161, 3125 (NH), 3039, 2967, 2864 (CH), 1237 (C=S). ^{1}H-NMR (DMSO-d6) δ ppm: 6.95-7.30 (m, 10H, Ar-H and H-5 thiazole), 7.32 (d, 1H, indole proton), 7.69-7.87 (m, 2H, indole proton), 8.19 (d, 1H, indole proton), 8.22 (s, 1H, N=CH), 8.47 (s, 1H, NH exchanged by D_2O), 8.90 (s, 1H, NH exchanged by D_2O), 12.44 (s, 1H, NH exchanged by D_2O). Anal. calcd for $C_{25}H_{18}BrN_5S_2$ (532.48): C, 56.39; H, 3.41; Br, 15.01; N, 13.15; S, 12.04. Found: C, 56.19; H, 3.21; Br, 14.89; N, 13.00; S, 11.89.

2.2.2. 5-[2-(4-Bromophenyl)-1*H*-indol-3-yl]-4-(4- methyl phenyl)-*4H*-1,2,4–triazole-3-thiol (3)

A solution of thiosemicarbazonederivative **2a** (0.01 mol) in absolute ethanol (15 mL) containing a few drops of HCl was refluxed for 2h. After cooling and dilution with water, the solid formed were filtered off, washed with water, air dried and recrystallized from ethanol to give **3** as green powder. Yield 62%, m.p. 336–338°C (ethanol). FT-IR (KBr, υ_{max}/cm^{-1}): 3166 (NH), 3097, 2951, 2919 (CH), 1606 (C=N). ^{1}H-NMR (DMSO-d6) δ ppm: 2.08 (s, 3H, CH_3), 7.16-7.48 (m, 8H, Ar-H), 7.78 (d, 1H, indole proton), 7.65- 7.94 (m, 2H, indole proton), 8.43 (d, 1H, indole proton), 4.33 (s, 1H, SH exchanged by D_2O), 12.05 (s, 1H, NH exchanged by D_2O). ^{13}C NMR (DMSO-d6) δ ppm: 20.44 (CH_3), 154.84, 154.98 (2XC=N), 162.17 (C-S), 106.49, 111.47, 120.92, 121.00, 122.43, 122.70, 123.10, 125.81, 130.24, 131.14, 131.32, 131.93, 136.51, 141.73. Anal. calcd for $C_{23}H_{17}BrN_4S$ (461.38): C, 59.87; H, 3.71; Br, 17.32; N, 12.14; S, 6.95. Found: C, 59.57; H, 3.51; Br, 17.22; N, 12.04; S, 6.85.

2.2.3. *N*-[4-Acetyl-5-(2-(4-bromophenyl)-1*H*-indol-3-yl)-4,5-dihydro-1,3,4-thiadiazol-2-yl]-N-(4-methylphenyl) acetamide(4)

A solution of the thiosemicarbazone derivative **2a** in acetic anhydride (12 mL) was heated under reflux for 5h with continuous stirring and then allowed to attain room temperature. The reaction mixture was slowly added to 400 mL of ice-cold water and then stirred at room temperature for 1h. The separated product was collected by filtration, washed with water, dried, and recrystallized from ethanol and DMF (2:1) to give **4** as orange powder, yield 55%, m.p. 180-182°C. FT-IR (KBr, υ_{max}/cm^{-1}): 3419 (NH), 3044, 2986, 2919 (CH), 1750, 1688 (2XC=O). ^{1}H-NMR (DMSO-d6) δ ppm: 2.16 (s, 3H, CH_3), 2.19 (s, 3H, CH_3) , 2.26 (s, 3H, CH_3), 6.93-7.28 (m, 9H, Ar-H and H-5, thiadiazole ring), 7.34 (d, 1H, indole proton), 7.51-7.82 (m, 2H, indole proton), 8.40 (d, 1H, indole proton), 11.55 (s, 1H, NH exchanged by D_2O). MS: m/z, (%): 547 (M^+, 0.5), 517 (0.3), 502 (0.2), 489 (1.2), 446 (0.23), 358 (79.7), 276 (1.6), 271 (100), 77 (80.3). Anal. calcd for $C_{27}H_{23}BrN_4O_2S$ (547.47): C, 59.23; H, 4.23; Br, 14.60; N, 10.23; S, 5.86. Found: C, 59.03; H, 4.03; Br, 14.40; N, 10.03; S, 5.56.

2.2.4. 2-(4-Bromophenyl)-3-[3-(4-methylphenyl)-4- phenyl-1,3-thiazol-2(3H)-ylidene]hydrazonomethyl-*1H*-indole (5)

To a solution of thiosemicarbazone derivative **2a** (0.01 mol) in absolute ethanol (20 mL) was added equimolar amounts of phenacyl bromide and anhydrous sodium acetate. The reaction mixture was heated under reflux for 6h with continuous stirring, then partially concentrated under reduced pressure and left to cool. The separated solid product was filtered off and recrystallized from ethanol to give **5** as a yellow powder. Yield 60%, m.p. 280-282°C. FT-IR (KBr, υ_{max}/cm^{-1}): 3122 (NH), 3028, 2947, 2826 (CH). ^{1}H-NMR (DMSO-d6) δ ppm: 2.27 (s, 3H, CH_3), 6.58 (s, 1H, H-5 thiazole ring), 7.14-7.28 (m, 13H, Ar-H), 7.44 (d, 1H, indole proton), 7.54-7.75 (m, 2H, indole proton), 8.30 (d, 1H, indole proton), 8.41 (s, 1H, N=CH), 11.85 (s, 1H, NH exchanged by D_2O). MS: m/z, (%): 563 (0.33), 430 (0.3), 367 (0.32), 354 (0.36), 291 (0.96), 270 (0.56), 252 (60.32), 134 (35.86), 61(100). Anal. calcd for $C_{31}H_{23}BrN_4S$ (563.51): C, 66.07; H, 4.11; Br, 14.18; N, 9.94; S, 5.69. Found: C, 65.97; H, 4.00; Br, 14.00; N, 9.64; S, 5.49.

2.2.5. 2-(4-Bromophenyl)-3-[3-(4-methylphenyl)–4 -oxo-1,3-thiazolidin-2-ylidene]-hydrazonomethyl-*1H* - indole (6)

A mixture of thiosemicarbazone derivative **2a** (0.01 mol), chloroacetic acid (0.01 mol), and anhydrous sodium acetate (0.01 mol) in glacial acetic acid (20 mL) was heated under reflux for 8h with continuous stirring. The reaction mixture was left to cool and poured into ice-cold water, and the separated solid was filtered off, washed with water, dried, and recrystallized from DMF to give **6** as a yellow powder. Yield 70%, m.p. 340-342°C; FT-IR (KBr, υ_{max}/ cm^{-1}): 3273 (NH), 3044, 2959, 2861 (CH), 1703 (C=O), 1601 (C=N). ^{1}H-NMR (DMSO-d6) δ ppm: 2.36 (s, 3H, CH_3), 4.09 (s, 2 H, CH_2), 7.19-7.31 (m, 8H, Ar-H), 7.45 (d, 1H, indole proton), 7.52-7.75 (m, 2H, indole proton), 7.90 (s, 1H, CH=N), 8.35(d, 1H, indole proton), 11.04 (s, 1H, NH exchanged by D_2O). ^{13}C-NMR (DMSO-d6) δ ppm: 20.65 (CH_3), 32.16

(CH_2), 152 (N=CH), 162 (N=C), 172 (C=O), 108.37, 111.78, 121.16, 122.42, 123.22, 125.76, 128.01, 129.40, 129.5, 129.61, 130.96, 131.13, 131.85, 132.51, 136.48, 138.06, 141.55. Anal. calcd for $C_{25}H_{19}BrN_4OS$ (503.41): C, 59.65; H, 3.80; Br, 15.87; N, 11.13; S, 6.37. Found: C, 59.35; H, 3.50; Br, 15.67; N, 11.00; S, 6.27.

2.2.6.2-(4-Bromophenyl)-3-[5-benzylidene-3-(4-methyl-phenyl)-4-oxo-1,3-thiazolidin-2-ylidene]hydrazonom ethyl -*1H*-indole (7)

To a solution of compound **6** (0.01 mol) and anhydrous sodium acetate (0.015 mol) in glacial acetic acid (10 mL) was added the benzaldehyde (0.01 mol). The mixture was heated under reflux for 6h with continuous stirring. The reaction mixture was left to cool and poured onto crushed ice with stirring. The separated solid was filtered off, washed with water, dried, and recrystallized from ethanol and DMF (2:1) to give **7** as orange powder. Yield 60%, m.p. 210-212°C. FT-IR (KBr) υ_{max}/cm^{-1}): 3292 (NH); 3029, 2942, 2842 (CH), 1683 (C=O). ^{1}H-NMR (DMSO-d6) δ ppm: 2.36 (s, 3H, CH_3), 7.25-7.32 (m, 14H, Ar-H and olefinic CH=), 7.51-7.77 (m, 3H, indole proton), 8.38 (d, 1H, indole proton), 8.42 (s, 1H, CH=N), 12.14 (s, 1H, NH exchanged by D_2O). ^{13}C-NMR (DMSO-d6) δ ppm: 20.68 (CH_3), 142.00 (C=CH), 153.00 (N=CH), 156.00 (N=C), 165.00 (C=O), 108.12, 111.97, 122.27, 122.59, 125.72, 127.83, 128.09, 129.44, 129.60, 129.81, 131.06, 131.25, 131.80, 132.29, 133.84, 136.53, 138.38. Anal. calcd for $C_{32}H_{23}BrN_4OS$ (591.52): C, 64.98; H, 3.92; Br, 13.51; N, 9.47; S, 5.42. Found: C, 64.68; H, 3.72; Br, 13.31; N, 9.27; S, 5.22.

2.2.7. 2-(4-Bromophenyl)-3-[6-(4-methyl phenyl)-2,3-diphenyl-2,3,3a,6-tetrahydro-*5H*-pyrazolo-[3,4-d]-1,3 - thiazol-5-ylidene]-hydrazonomethyl-*1H*-indole (8)

A mixture of compound **7** (0.01mol) and phenyl hydrazine (0.01 mol) was refluxed in ethanol (50 mL) in the presence of a few drops of acetic acid for 4h. The reaction mixture was cooled, and the solid separated was filtered off, washed with water and recrystallized from aqueous ethanol to give compound **8** as an orange powder. Yield 55%, m.p. 102-104°C. FT-IR (KBr, υ_{max}/cm^{-1}): 3229 (NH), 3054, 2936, 2857 (CH), 1605 (C=N). ^{1}H-NMR (DMSO-d6) δ ppm: 2.24 (s, 3H, CH_3), 4.09 (d, 1H, H-pyrazole), 6.67 (d, 1H, H-pyrazole), 7.08-7.22 (m, 18H, Ar-H), 7.24-7.94 (m, 3H, indole proton), 8.19 (d, 1H, indole proton), 8.52 (s, 1H, CH=N), 12.20 (s, 1H, NH exchanged by D_2O). MS: m/z (%): 681 (M^+, 0.1), 666 (0.4), 510 (3.2), 537 (2.2), 271 (100), 165 (73.5), 77 (30.9). Anal. calcd for $C_{38}H_{29}BrN_6S$ (681.65): C, 66.96; H, 4.29; Br, 11.72; N, 12.33; S, 4.70. Found: C, 66.76; H, 4.09; Br, 11.52; N, 12.03; S, 4.50.

2.2.8.2-(4-Bromophenyl)-3-[3-phenyl-6-(4-methylphenyl)-3 ,3a-dihydro-1,3-thiazolo[4,5-c]isoxazol-5-ylidene]-hy drazonomethyl-1H-indole (9)

A mixture of compound **7** (0.01 mol), hydroxylamine hydrochloride (0.012 mol), sodium acetate (0.012 mol) was refluxed in ethanol (30 mL) in the presence of a few drops of acetic acid for 1h and kept overnight. Excess solvent was distilled off under reduced pressure and the remainder was then poured into water. The solid obtained was recrystallized from ethanol to give **9** as a white powder. Yield 65%; m.p. 170-172°C. FT-IR (KBr, υ_{max}/cm^{-1}): 3173 (NH); 3057, 2922, 2859 (CH). ^{1}H-NMR (DMSO-d6) δ ppm: 2.17 (s, 3H, CH_3), 4.35 (d,1H, H-isoxazole), 5.55 (d, 1H, H-isoxazole), 6.65-8.27 (m, 17 H, Ar-H and indole proton), 8.38 (s, 1H, CH= N), 11.57 (s, 1H, NH exchanged by D_2O). MS: m/z (%): 606 (M^+, 0.33), 530 (0.25), 488 (0.34), 324 (26.67), 297 (100), 271 (8.89). Anal. calcd for $C_{32}H_{24}BrN_5OS$ (606.53): C, 63.37; H, 3.99; Br, 13.17; N, 11.55; S, 5.29. Found: C, 63.17; H, 3.79; Br, 13.00; N, 11.35; S, 5.09.

2.2.9. 2-[2-(4-Bromophenyl)-1H-indol-3-ylmethylidene -hydrazono]-4-chloro-3-(4-methylphenyl)-2,3-dihydr o-1,3-thiazole-5-carboxaldehyde (10)

To the Vilsmeier-Haack complex prepared from DMF (10 mL) and $POCl_3$ (0.02 mol) at 0°C was added the 1,3-thiazolidin-4-one derivative **6** (0.004 mol) and the reaction mixture was stirred at 60-65°C for 4h. The reaction mixture was kept overnight and it was then slowly added to crushed ice. The product separated on neutralization with $NaHCO_3$, was filtered off and recrystallized from ethanol to give **10** as a yellow powder. Yield 70%; m.p. 150-152°C. FT-IR (KBr, υ_{max}/cm^{-1}): 3216 (NH), 3031, 2956, 2781 (CH), 1600 (C=N), 1675 (C=O). ^{1}H-NMR (DMSO-d6) δ ppm: 2.08 (s, 3H, CH_3), 7.19-8.22 (m, 12H, Ar-H and indole proton), 8.36 (s, 1H, CH=N), 9.95 (s, 1H, CHO), 12.45 (s, 1H, NH exchanged by D_2O). ^{13}C-NMR (DMSO-d6) δ ppm: 20.56 (CH_3), 135.89 (C-Cl), 151 (N=CH), 156 (N=C), 164(C=O), 105.37, 121.01, 123.44, 125.71, 127.99, 129.40, 129.51, 131.20, 131.29, 131.32, 131.40, 131.88. Anal. calcd for $C_{26}H_{18}BrClN_4OS$ (549.87): C, 56.79; H, 3.30; Br, 14.53; Cl, 6.45; N, 10.19; S, 5.83. Found: C, 56.59; H, 3.00; Br, 14.33; Cl, 6.25; N, 10.00; S, 5.53.

2.2.10. 2-(4-Bromophenyl)-3-[6-(4-methyl phenyl)-1,6-dihydro-*5H*-pyrazolo-[3,4-d]-1,3-thiazol-5-ylidene]-hydrazonomethyl-1*H*-indole (11)

A mixture of compound **10** (0.01mol) and hydrazine hydrate (0.01 mol) was refluxed in ethanol (50 mL) for 4h. The reaction mixture was cooled, and the precipitate was filtered off and recrystallized from ethanol to give **11** as a yellow powder. Yield 64%, m.p. 300-302°C. FT-IR (KBr, υ_{max}/cm^{-1}): 3380, 3176 (NH), 3052, 2966, 2864 (CH), 1604 (C=N). ^{1}H-NMR (DMSO-d6) δ ppm: 2.36 (s, 3H, CH_3), 6.93-7.24 (m, 9H, Ar-H and H-3 pyrazole), 7.29 (d, 1H, indole proton), 7.54-7.85 (m, 2H, indole proton), 8.41 (d, 1H, indole proton), 8.90 (s, 1H, CH=N), 4.28 (s, 1H, NH exchanged by D_2O), 12.03 (s, 1H, NH exchanged by D_2O). MS: m/z (%): 527 (M^+, 0.95), 567 (0.99), 281 (3.33), 254 (1.11), 248 (35.86), 118 (100). Anal. calcd for $C_{26}H_{19}BrN_6S$ (527.44): C, 59.21; H, 3.63; Br, 15.15; N, 15.93; S, 6.08. Found: C, 59.00; H, 3.43; Br, 15.00; N, 15.63; S, 6.00.

2.2.11. N'-{2-[2-(4-Bromophenyl)-*1H*-indol-3-ylmethyl-enehydrazono]-4-chloro-3-(4-methylphenyl)-2,3-dihydro-1,3-thiazol-5-ylmethylene}-2-cyanoacetohydrazide (12)

An equimolar mixture of **10** (0.02 mol) and cyanoacetic acid hydrazide (0.02 mol) in absolute ethanol (30 mL) was heated under reflux for 2h. The precipitate formed after cooling was filtered off, washed with cold ethanol, dried and recrystallized from xylene to give **12** as an orange powder. Yield 50%; m.p. 230-232°C. FT-IR (KBr, υ_{max}/ cm^{-1}): 3327 (NH), 2920, 2853 (CH), 2196 (CN), 1668 (C=O). ^{1}H-NMR (DMSO-d6) δ ppm: 2.36 (s, 3H, CH_3), 4.22 (s, 2H, CH_2), 7.15-7.27 (m, 8H, Ar-H), 7.38-7.92 (m, 3H, indole proton), 8.18 (d, 1H, indole proton), 8.29 (s, 1H, CH=N), 8.36 (s, 1H, CH=N), 11.33(s, 1H, NH exchanged by D_2O), 11.49 (s, 1H, NH exchanged by D_2O). MS: m/z (%): 630 (M^+, 0.87), 538 (1.19), 383 (8.04), 348 (1.32), 270 (88.70), 295(100). Anal.calcd for $C_{29}H_{21}BrClN_7OS$ (630.95): C, 55.20; H, 3.35; Br, 12.66; Cl, 5.62; N, 15.54; S, 5.08. Found: C, 55.00; H, 3.15; Br, 12.46; Cl, 5.52; N, 15.34; S, 5.00.

2.2.12. 2-[2-(4-Bromophenyl)-*1H*-indol-3-ylmethylene hydrazono]-3-(4-methylphenyl)-3,4–dihydro- 1,3-thiazolo-[4,5-b]-1,5-benzodiazepine (13)

An equimolar mixture of compound **10** (0.02 mol), *o*-phenylenediamine (0.02 mol) and 0.2 mL TEA in absolute ethanol (30 mL) was heated under reflux for 8h. The precipitate formed after cooling was filtered off, washed with cold ethanol, dried, and recrystallized from ethanol to give **13** as an orange powder. Yield 67%; m.p. 250-252°C. FT-IR (KBr, $\upsilon_{max/}$ cm^{-1}): 3337 (NH), 3055, 2923, 2865 (CH). ^{1}H-NMR (DMSO- d6) δ ppm: 2.27 (s, 3H, CH_3), 7.22-8.37 (m, 17 H, Ar-H and benzodiazepine), 8.90 (s, 1H, CH= N), 12.03 (s, 1H, NH exchanged by D_2O), 12.31(s, 1H, NH exchanged by D_2O). MS: m/z (%): 603 (0.98), 504 (0.32), 334 (3.89), 316 (1.21), 308 (74.55), 281 (16.62), 245 (3.05), 77 (100). Anal. Calcd for $C_{32}H_{23}BrN_6S$ (603.53): C, 63.68; H, 3.84; Br, 13.24; N, 13.92; S, 5.31. Found: C, 63.48; H, 3.54; Br, 13.14; N, 13.62; S, 5.11.

2.2.13. 2'-[2-(4-Bromophenyl)-*1H*-indol-3-ylmethylene hydrazono]-3'-(4-methylphenyl)-3-phenyl-2,5'–bis-1, 3- thiazolidin-2'-ylidene-4,4'-dione 16

To a stirred solution of 0.56g KOH (0.01mol) in 20 mL DMF, 1,3-thiazolidin-4-one **6** (0.10 mol) was added. After stirring for 30 min, phenyl isothiocyanate (0.01mol) was added to the resulting mixture and the reaction mixture stirred at room temperature for 12h. Then, ethyl chloroacetate (0.01 mol) was added to the reaction mixture and stirred for 6h. The reaction mixture was poured into crushed ice. The resulting precipitate was filtrated off, dried, and recrystallized from xylene to give **16** asanorange powder. Yield, 60%, m.p. 290-292°C. FT-IR (KBr, υ_{max}/cm^{-1}): 3267 (NH), 3042, 2964, 2919 (CH), 1702 (C=O), 1600 (C=N). ^{1}H-NMR (DMSO-d6) δ ppm: 2.36 (s, 3H, CH_3), 4.09 (s, 2H, CH_2), 7.07-7.32 (m, 13H, Ar-H), 7.45-7.55 (m, 2H, indole proton), 7.72 (d, 1H, indole proton), 8.33 (s, 1H, CH= N), 8.35 (d, 1H, indole proton), 12.02 (s, 1H, NH exchanged by D_2O). ^{13}C-NMR (DMSO-d6) δ ppm: 20.75 (CH_3), 32.16 (CH_2), 152.65 (CH=N), 157.15 (C=N), 162.38 (C=O), 164.72 (C=O), 99.43, 108.37, 110.45, 111.82, 114.23, 122.42, 125.70, 126.87, 129.66, 129.94, 130.66, 130.74, 130.98, 131.96, 136.49, 138.06, 141.55, 149.95. Anal. Calcd for $C_{34}H_{24}BrN_5O_2S_2$ (678.62): C, 60.18; H, 3.56; Br, 11.77; N, 10.32; S, 9.45. Found: C, 60.00; H, 3.36; Br, 11.57; N, 10.22; S, 9.25.

2.2.14. 2-Oxo-2-phenylethyl{2-[2-(4-bromophenyl)-*1H*-indol-3-ylmethylenehydrazono]-3-(4-methylphenyl)-4- oxo}-1,3-thiazolidine-5-carbodithioate (18)

To a stirred suspension of finely powdered potassium hydroxide (0.02 mol) in dry DMF (20 mL), 1,3-thiazolidin-4-one **6** (0.01 mol) was added. The resulted mixture was cooled at 10°C in an ice bath; then (0.01mol) carbon disulfide was added slowly over the course of 10 min. After complete addition, stirring of the reaction mixture was continued for 6h. Then, phenacyl bromide (0.01mol) was added to the mixture and stirring continued for 3h, then the mixture was poured into crushed ice and HCl. The resulting precipitate was filtrated off, dried, and recrystallized from xylene to give **18** as a red powder. Yield, 60%; m.p. 200-202°C. FT-IR (KBr, υ_{max}/cm^{-1}): 3274 (NH), 3056, 2967, 2861 (CH), 1702 (C=O), 1241 (C=S). ^{1}H-NMR (DMSO- d6) δ ppm: 2.37 (s, 3H, CH_3), 4.09 (s, 2H, CH_2), 4.76 (s, 1H , H-5thiazolidinone), 7.13-7.75 (m, 13H, Ar-H), 7.39-7.97 (m, 3H, indole proton), 8.22 (d, 1H, indole proton), 8.38 (s, 1H, CH=N), 12.09 (s, 1H, NH exchanged by D_2O). ^{13}C-NMR (DMSO-d6) δ ppm: 10.36 (CH_3), 30.01 (CH_2), 147.39 (CH=N), 150.43 (C=N), 164.36 (C=O), 164.73 (C=O), 185.40 (C=S), 107.37, 110.00, 111.22, 114.03, 122.12, 124.60, 128.51, 130.52, 130.60, 130.65, 130.84, 130.85, 132.92, 133.08, 149.01. Anal. Calcd for $C_{34}H_{25}$ $BrN_4O_2S_2$ (665.62): C, 61.35; H, 3.79; Br, 12.00; N, 8.42; S, 9.63. Found: C, 61.15; H, 3.59; Br, 11.89; N, 8.22; S, 9.43.

2.3. In Vitro Cytotoxic Screening (MTT assay)

In vitro cytotoxicity of newly synthesized compounds **1, 2a, 6** and **11** were evaluated against human liver cancer cell (Hep G2), human colon cancer cell (HT-29) and human breast cancer cell (MCF-7) cell line using a standard MTT assay. The monolayer cells were detached with trypsin-ethylenediaminetetra-acetic acid (EDTA) to make singlet cell suspensions and viable cells were counted using a hemocytometer, then diluted with the fetal bovine serum (FBS) medium with 5% FBS to give final density of 2×10^5 cells/mL.). One hundred microliters per well of cell suspension were seeded into 96-well plates at a plating density of 10,000 cells/well and incubated to allow for cell attachment at 37°C, 5% CO_2, 95% air and 100% relative humidity.

The synthesized samples were dissolved in 1 mL dimethylsulfoxide (DMSO) and further diluted in serum free

medium to produce six concentrations starting from 1 to 10^{-6} mg/mL. About 500-10,000 cells in 200 µl media per well were incubated at 37°C and 5% CO_2 overnight to allow the cells to attach to the wells. 100 µl from each dilution of tested samples was added to each well, mixed by shaking at 150 rpm for 5 minutes, incubated at 37°C and 5% CO_2 for 48h. 20 µl of MTT (5 mg/mL) in phosphate buffered saline (PBS) was added to each well plate and mixed by shaking at 150 rpm for 5 min and incubated at 37°C and 5% CO_2 for 5h to allow the MTT to be metabolized. The medium with MTT was then flicked off and the formed formazan crystals (MTT metabolic product) were solubilized in 200 µl of DMSO and then absorbance was measured at 560 nm using a micro plate reader [8]. The viability of treated cells was calculated in reference to the untreated control cells by using the following formula:

Cell viability (%) = [100 × (Sample Abs)/(Control Abs)].

3. Results and Discussion

3.1. Chemistry

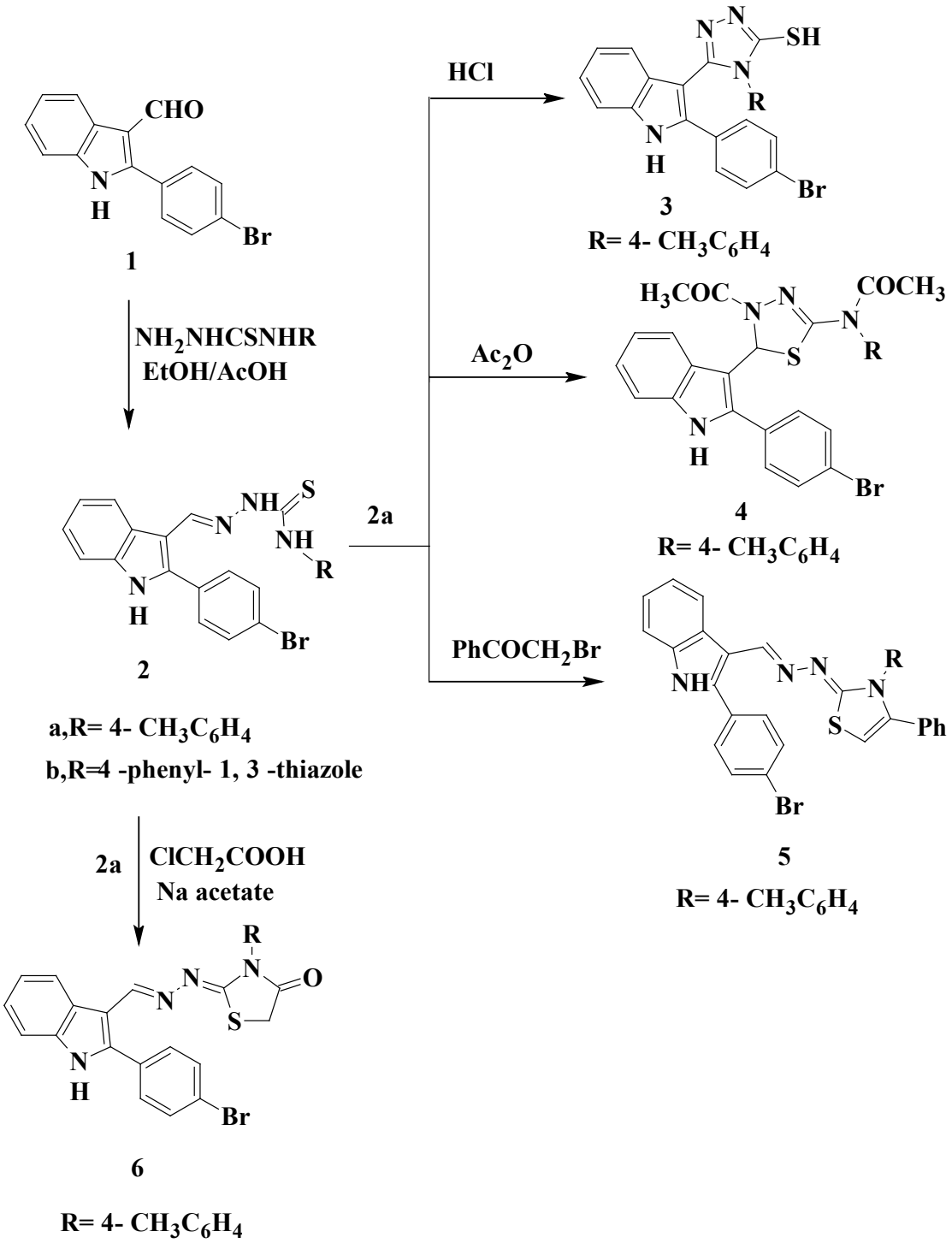
The synthetic procedures adopted to obtain the target compounds are outlined in Schemes 1-3. The key intermediate 1-[*1H*-indol-3-ylmethylene]thiosemicarbazone derivatives **2a,b** were prepared by the reaction of *1H*-indole-3-carboxaldehyde **1** with thiosemicarbazide derivatives such as 4-(4-methylphenyl)thiosemicarbazide or 4-(4-phenyl- 1,3-thiazol-2-yl)thiosemicarbazide in refluxing ethanol containing acetic acid [9] (Scheme 1). The structures of compounds **2a** and **2b** were based on analytical and spectral data. The ^{1}H-NMR spectra of **2a** displayed three D_2O- exchangeable NH proton signals at δ 10.01, δ 11.46 and δ 11.99 ppm and a singlet signal at δ 2.32 ppm for the CH_3 protons.

Cyclization of thiosemicarbazone derivative **2a** depended on the cyclizing agent and the reaction conditions. Thus, thiosemicarbazone derivative **2a**, which underwent ring closure in acidic media [10], afforded 5-[*1H*-indol-3-yl] -4*H*-1,2,4-triazole-3-thiol derivative **3** (Scheme 1). The 1H-NMR spectra of **3** displayed D_2O-exchangeable signals at δ 4.33 ppm and δ 12.05 ppm for SH and NH protons, respectively. The ^{13}C NMR spectra of **3** showed signals at δ 20.44, 154.84, 154.98 and 162.17 ppm for a CH_3, 2XC=N and C-S groups, respectively.

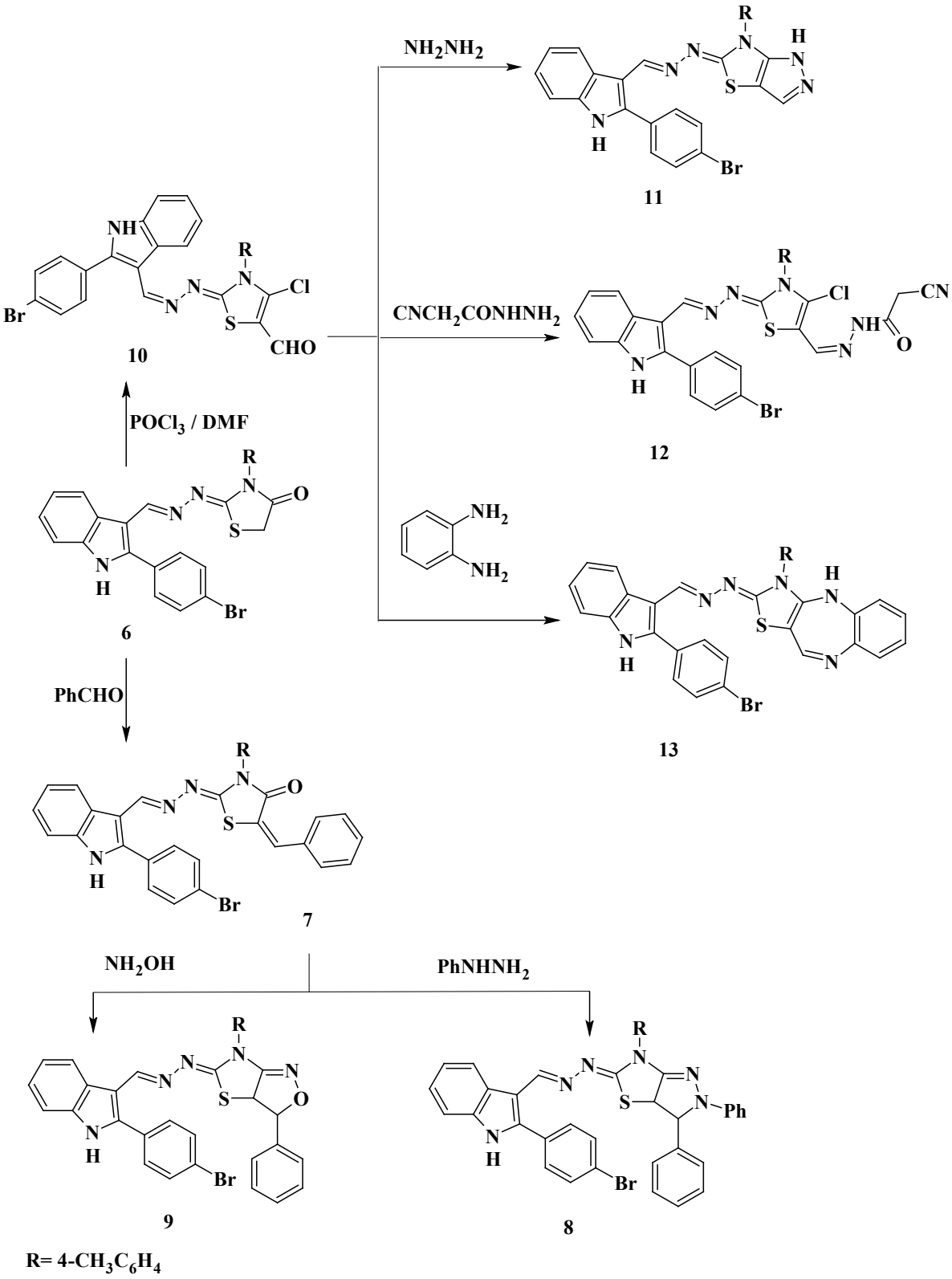
Whereas, heterocyclization of thiosemicarbazonederivea-tive**2a** in the presence of acetic anhydride gives *N*-[4-acetyl-5-(*1H*-indol-3-yl)-1,3,4-thiadiazol-2-yl]acetamide **4** (Scheme 1). A plausible mechanism for the reaction of compound **2a** with acetic anhydride is shown in Figure 1. In this reaction, the resonance effects between NH and the phenyl group may reduce the nucleophilicity of NH while the steric effect of the phenyl group on the NH retards nucleophilic substitution with acetic anhydride. Therefore, the initial monoacetyl substituted products are gradually converted to the diacetyl substituted thiadiazoline [11] derivative **4**. The ^{1}H-NMR spectrum of compound **4** showed signals at δ 2.16, δ 2.19 and δ 2.26 ppm corresponding to three CH_3 groups and a multiple at δ 6.93-7.28 ppm for the aromatic protons and CH-5 of 1,3,4-thiadiazoline ring. The mass spectrum of compound **4** showed the molecular ion peak at m/z 547 corresponding to the molecular formula $C_{27}H_{23}Br\ N_4O_2\ S$.

Furthermore, treatment of thiosemicarbazone derivative **2a** with phenacyl bromides in boiling ethanol in the presence of anhydrous sodium acetate [12] yielded the corresponding 3-[1,3-thiazol-2(*3H*)-ylidene]hydrazonomethyl-1*H*-indole derivative **5**. The^{1}H-NMR spectrum of **5** showed a signal at δ6.58 ppm corresponding to CH-5 of thiazole ring and a signal at δ 8.30 ppm for an N=CH proton. The mass spectrum of compound **5** showed the molecular ion peak at *m/z* 563 corresponding to the molecular formula $C_{31}H_{23}\ BrN_4S$.

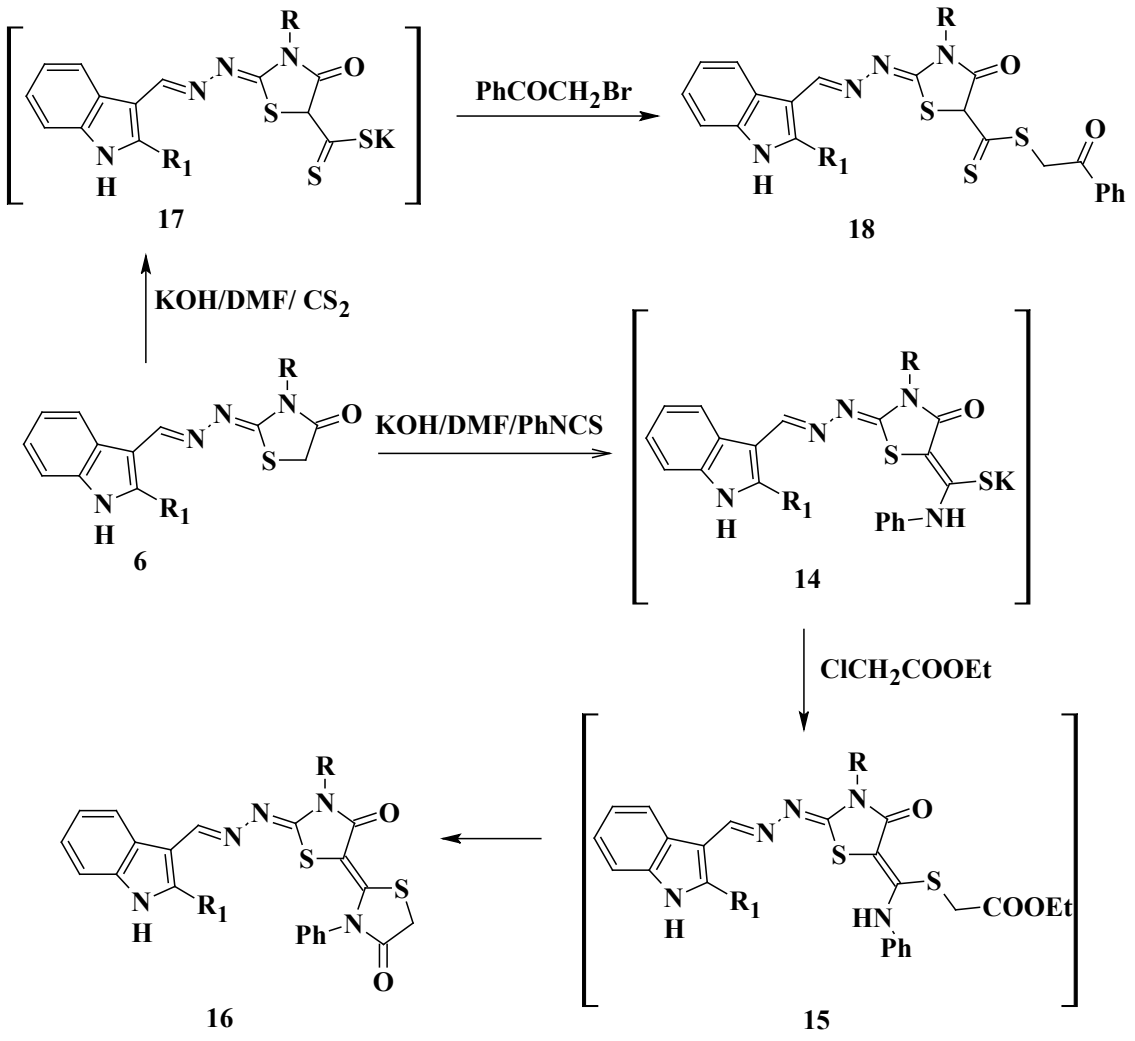
Refluxing thiosemicarbazone derivative **2a** with chloroacetic acid in the presence of anhydrous sodium acetate in glacial acetic acid [13] afforded 1,3-thiazolidin-4-one derivative **6** (Scheme 1). The IR spectrum of **6** showed the disappearance of NH bands of the substituted thiosemicarbazone moiety and the presence of a new band at 1703 cm^{-1} attributed to a carbonyl group of thiazolidin-4-one. The ^{1}H-NMRspectrum of **6** showed a new signal at δ 4.09 ppm attributedto the CH_2 proton of the thiazolidinone ring. The ^{13}C NMR spectrum of **6** showed signals at δ 20.65, 32.16, 152, 162 and 172 ppm for CH_3, CH_2, N=CH, N=C, and C=O groups, respectively. Condensation of 1,3-thiazolidin-4-one derivative **6** with benzaldehyde in the presence of freshly fused sodium acetate in boiling glacial acetic acid [14] yielded the corresponding arylidene derivative **7** (Scheme 2). The analytical and spectral data of compound **7** was consistent with the proposed structure. Thus, the 1H-NMR spectrum of compound **7** showed no evidence of thiazolo-methylene protons and showed a multiple signal at δ 7.25-7.32 for the aromatic protons and olefinic CH=proton. The ^{13}C NMR spectra of **7** showed signals at δ 20.68, 142, 153, 156 and 165 ppm corresponding to CH_3, C=CH, N=CH, N=C and C=O groups, respectively. Compound **7** was used as the starting material for further syntheses of other heterocyclic compounds. Thus, the reaction of compound **7** withphenylhydrazine [15] afforded 3-(pyrazolo[3,4-d]1, 3-thiazol-5-ylidene)hydrazonomethyl-1*H*-indole **8**. The 1H-NMR spectrum of **8** showed a doublet signals at δ 4.09 and δ 6.67 due to the 2XCH protons of pyrazoline. The mass spectrum of compound **8** showed the molecular ion peak at *m/z* 681 corresponding to the molecular formula $C_{38}H_{29}BrN_6S$. On the other hand, cyclocondensation **7** with hydroxylamine hydrochloride in presence of sodium acetate [16] afford 3-[1,3-thiazolo[4,5-c]isoxazol-5-ylidene] hydrazonomethyl-1*H*-indole **9** (Scheme 2).



Scheme 1. Synthesis of compounds 2-6



Scheme 2. Synthesis of compounds **7-13**



Scheme 3. Synthesis of compounds **16** and **18**

The 1H-NMR spectrum of **9** showed doublet signals at δ 4.53 and δ 6.67 due to the 2XCH protons of isoxazole. The mass spectrum of compound **9** showed the molecular ion peak at *m/z* 606 corresponding to the molecular formula $C_{32}H_{24}BrN_5OS$.

Moreover, chloroformylation of 1,3-thiazolidin-4-one derivative **6** using the *Vilsmeier-Haack* reagent ledto 4-chloro-1,3-thiazole-5-carboxaldehyde **10**. The most probable reaction [17] involves initial formation of intermediates **A-C** that underwent further chlorination and hydrolysis to yield compound **10** (Figure 2). The IR spectrum of compound **10** showed a band at 1675 cm^{-1} due to C=O group stretching. The 1H-NHR of compound **10** revealed a new signal at δ 9.95 ppm assigned to CHO proton and disappearance of a signal at δ 4.09 ppm attributed to CH_2thiazolidinone. The ^{13}C-NMR spectra of **10** showed a new signal at δ135.89 ppm due to a C-Cl group. The reaction of 4-chloro-1,3-thiazole-5-carboxaldehyde **10** with hydrazine hydrate [18] afforded the corresponding pyrazolo-[3,4-d]-1,3-thiazole derivative **11** (Scheme 2). The chemical structure of the compound **11** was elucidated on the basis of elemental analysis and spectral data. The IR spectrum of compound **11** was characterized by the presence of strong bands at 3380, 3176 cm^{-1} due to two N-H stretches. The massspectrum of compound **11** showed the molecular ion peak at *m/z* 527 corresponding to the molecular formula $C_{26}H_{19}BrN_6S$.

Furthermore, reaction 4-chloro-1,3-thiazole-5-carboxaldehyde **10** with cyanoacetic acid hydrazide [19] afforded the corresponding cyanoacetohydrazide derivative **12**. The 1H-NMR of compound **12** showed D_2O-exchangeable signals at δ11.33 and 11.49 ppm due to two NH protons and singlet signals at δ 8.29 ppm, 8.36 ppm and 4.22 ppm due to 2 CH=N and CH_2 protons, respectively. The reaction of 4-chloro-1,3-thiazole-5-carbaldehyde **10** with *o*-phenylenediamine in ethanol solution containing triethylamine (TEA) as catalyst afforded 1,3-thiazolo [4,5-b]1,5-benzodiazepine derivative **13** (Scheme 2). The ^{1}H- NMR spectrum of compound **13** showed D_2O-exchangeable signals at δ 12.03 ppm and 12.31 ppm due to 2

NH protons. The mass spectrum of compound **13** showed the molecular ion peak at *m/z* 603 corresponding to the molecular formula $C_{32}H_{23}BrN_6S$.

The active methylene in 1,3-thiazolidin-4-one derivative **6** was allowed to react with phenyl isothiocyanate in dry dimethylformamide (DMF) containing a catalytic amount of potassium hydroxide to give the non-isolable potassium salt **14**. Then, ethyl chloroacetate [20] was added to afford 2'-[1*H*-indol-3-ylmethylenehydrazono]-2,5'-bis-1,3-thiazolidin-2'-ylidene-4,4'-dione **16.** Thereaction mechanism is assumed to proceed *via* S-alkylation of **14** to give the intermediate **15** which was cyclized to **16**. Elemental analyses and spectral data support these proposed 1,3-thiazolidinone structures. The 1H-NMR spectrum of compound **16** showed a singlet signal at *δ*4.09 ppm corresponding to CH_2 protons on the thiazolidinone ring. The ^{13}C NMR spectrum of **16** showed signals at δ 20.75, 32.16, 152.65, 157.15, 162.38 and 164.72 ppm to CH_3, CH_2, N=CH, C=N and 2XC=O groups, respectively.

Furthermore, the reaction of 1,3-thiazolidin-4-one derivative **6** with carbon disulfide in boiling DMF containing a catalytic amount of potassium hydroxide afforded non-isolable intermediate potassium sulfide salts **17**. Then, phenacyl bromide [21] was added to afford 2-oxo-2-phenylethyl-{2-[1*H*-indol-3-ylmethyl-enehydrazono]}-1,3-thiazolidine-5-carbodithioate **18** (Scheme 3). The chemical structure of compound **18** was elucidated on the basis of elemental analysis and spectral data. Compound **18** was characterized by the presence of a strong band at 1241 cm^{-1} (C=S) in the IR spectrum. The 1H-NMR spectrum of **18** showed a singlet at δ 4.09 ppm corresponding to CH_2 and a singlet signal at δ 4.76 ppm for an H-5thiazolidinone proton. The ^{13}C NMR spectra of **18** showed signals at δ 10.36, 30.01, 147.39, 150.43, 164.36, 164.73 and 185.40 ppm to CH_3, CH_2, CH=N, C=N, 2XC=O and C=S groups, respectively.

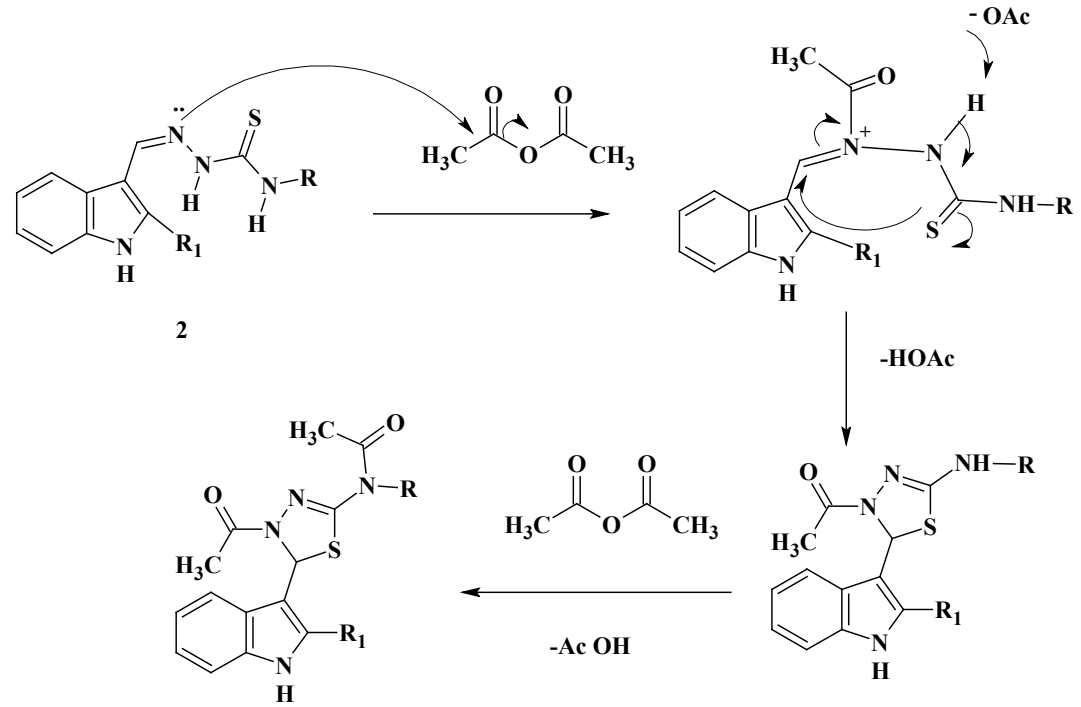


Figure 1. Proposed mechanism for the formation of compound 4

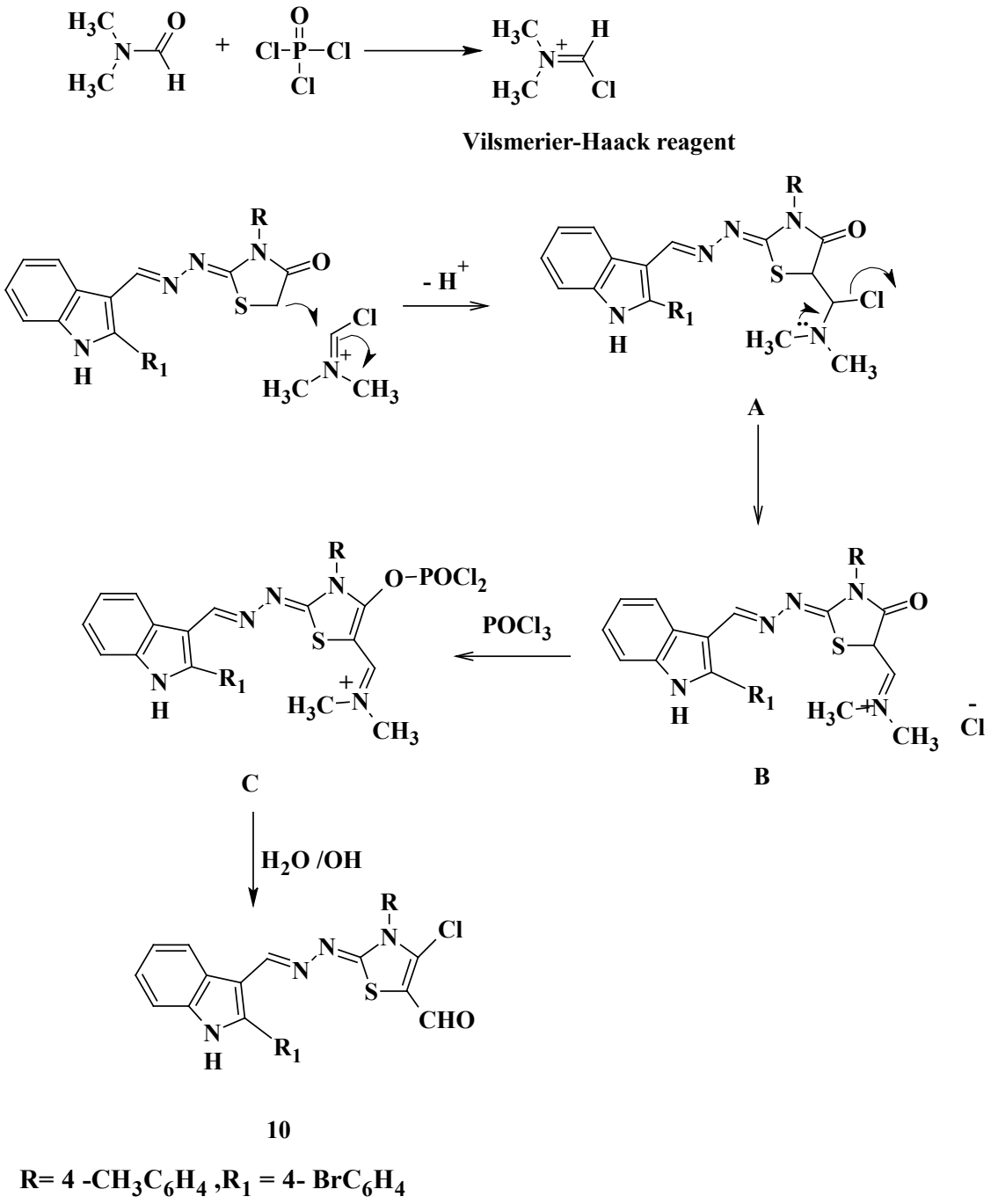


Figure 2. Proposed mechanism for the formation of compound 10

3.2. In Vitro Cytotoxicity Screening

The newly synthesized compounds **1, 2a, 6** and **11** were evaluated for their in vitro cytotoxic effects against human liver cancer (Hep G2) cell line, human colon cancer (HT-29) cell line and human breast cancer (MCF-7) cell line by the standard MTT (3- (4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay [22, 23].

The method is based on the ability of a mitochondrial dehydrogenase from viable cells to cleave the tetrazolium rings of the pale yellow MTT and form purple formazan crystals which are impermeable to cell membranes (Scheme 4). The crystals can be solubilized by detergents. The number of living cells is directly proportional to the level of formed formazan which can be quantified photometrically. When the amount of purple formazan produced by cells treated with an agent is compared with the amount of formazan produced by untreated control cells, the effectiveness of the agent in causing death of cells can be deduced (see Figure 3).

An MTT assay to determine the drug concentration required to inhibit the growth of human cancer cells by 50% (IC_{50}) was conducted. The results of the MTT assay percentage viability and IC_{50} values are shown in Tables 1

and 2 and Figures 4 –7.

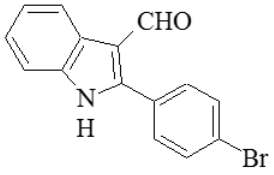
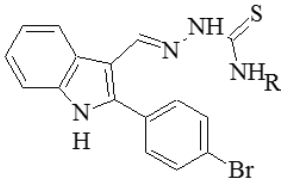
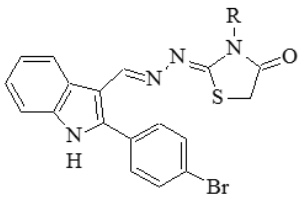
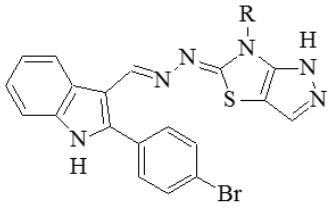
In order to investigate the structure-activity relationship of the indole ring, ring position 3 was reserved for a different substitutents. The obtained results from value of IC_{50} (Table 2 and Figure 7) revealed that:

1. Compound **11**, which has a pyrazolo[3,4-d]1,3-thiazol group at position-3 of the indole ring, is a more active cytotoxic agent against all three cancer cell lines; human liver cancer (Hep G2) cell line, human colon cancer (HT-29) cell line and human breast cancer (MCF -7) cell line.
2. Compound **1**, which has a CHO group at position-3 of the indole ring, is a more active cytotoxic agent against human liver cancer (Hep G2) cell line and human breast cancer (MCF-7) cell line, while only weakly cytotoxic against the colon cancer (HT-29) cell line.
3. Compound **2a**, which has a thiosemicarbazone group at position-3 of the indole ring, is a more active cytotoxic agent against the human liver cancer (Hep G2) cell line, while weakly cytotoxic against the colon cancer (HT-29) and human breast cancer (MCF-7) cell lines.
4. Compound **6**, which has a1,3-thiazolidine ring at position-3 of the indole ring is a more active cytotoxic agent against the human breast cancer MCF-7) cell line, but weakly cytotoxic against the human liver cancer (Hep G2) and human colon cancer (HT-29) cell lines.

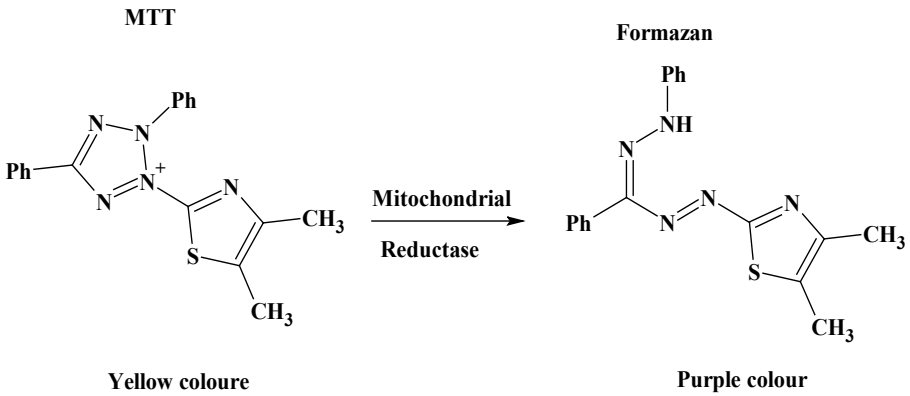
Table 1. *In vitro* cell viability % of test compounds **1**, **2a**, **6** and **11** with different concentrations (mg/mL) by MTT assay

Comp. No.	Dilution (mg/mL)	Cell viability %		
		Hep G2	HT-29	MCF-7
1	1.00000	15.05	13.72	17.35
	0.10000	21.14	19.28	22.64
	0.01000	31.54	28.75	30.56
	0.00100	32.25	55.88	36.22
	0.00010	56.63	84.31	64.15
	0.00001	76.34	100	87.92
2a	1.00000	21.14	19.28	20
	0.10000	21.86	21.24	29.05
	0.01000	28.67	27.77	42.26
	0.00100	33.69	62.09	62.26
	0.00010	58.87	84.31	89.81
	0.00001	81.72	98.03	100
6	1.00000	17.56	16.66	18.86
	0.10000	26.52	22.54	28.3
	0.01000	30.82	31.37	35.47
	0.00100	48.39	55.55	43.77
	0.00010	93.19	84.96	86.03
	0.00001	100	97.38	94.71
11	1.00000	21.86	19.93	17.35
	0.10000	26.88	25.49	21.5
	0.01000	28.32	30.06	26.41
	0.00100	35.12	39.86	35.47
	0.00010	72.04	65.68	50.56
	0.00001	100	96.07	72.07

Table 2. IC_{50} values (mg/mL) of the tested compounds **1**, **2a**, **6** and **11**

Compd. No.	Structure	IC_{50} (mg/mL)		
		Hep G2	HT-29	MCF-7
1		$8.83x10^{-5}$	$8.95x10^{-4}$	$7.79x10^{-5}$
2a		$8.49x10^{-5}$	$8.05x10^{-4}$	$8.03x10^{-4}$
6		$1.03x10^{-3}$	$9x10^{-4}$	$5.81x10^{-5}$
11		$6.94x10^{-5}$	$7.61x10^{-5}$	$9.89x10^{-5}$

IC_{50}: Concentration that causes a 50 % reduction of the cell growth



Scheme 4. Principle of MTT assay

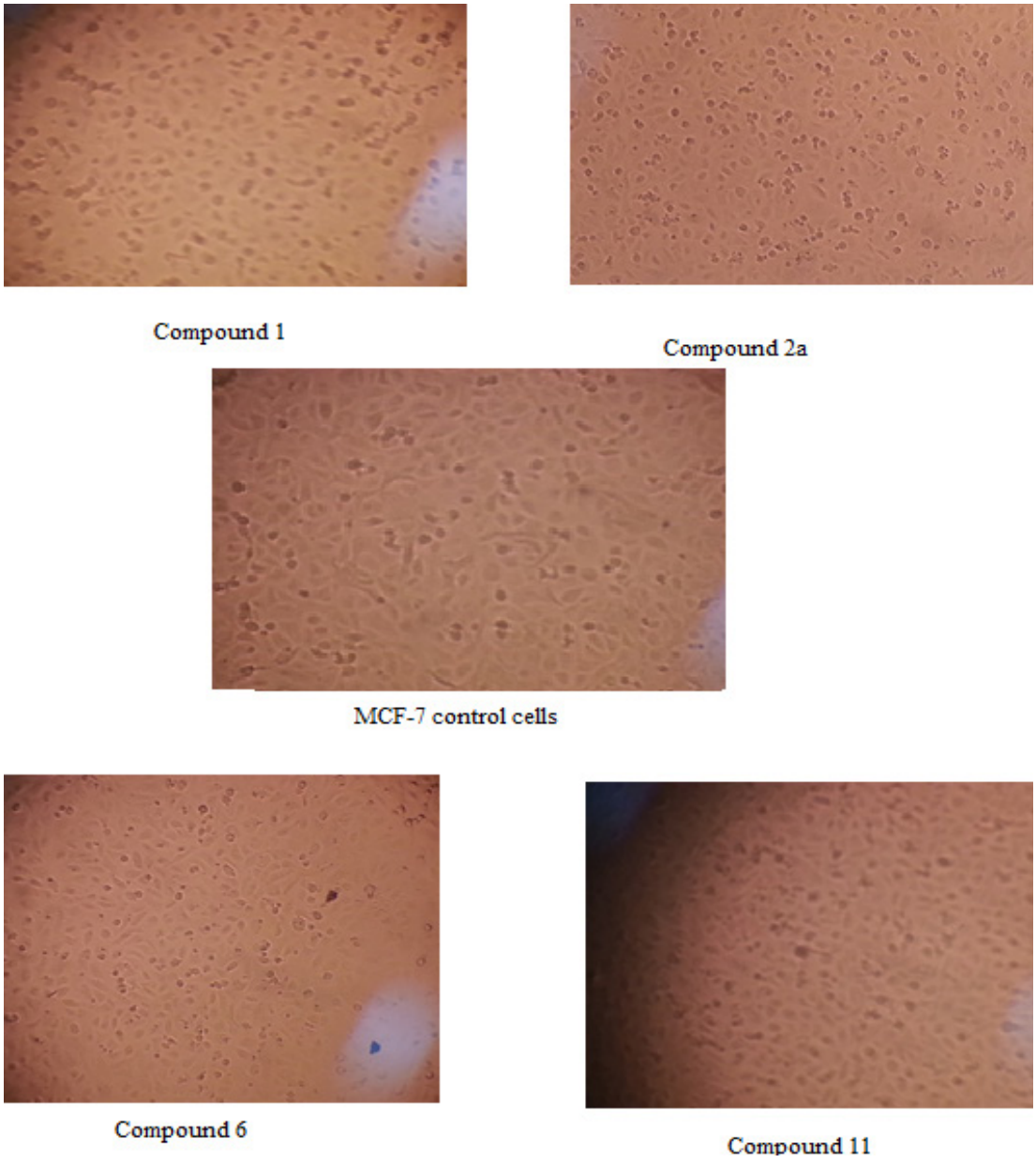


Figure 3. Pictorial view change in MCF-7 cell morphology after exposure to MTT

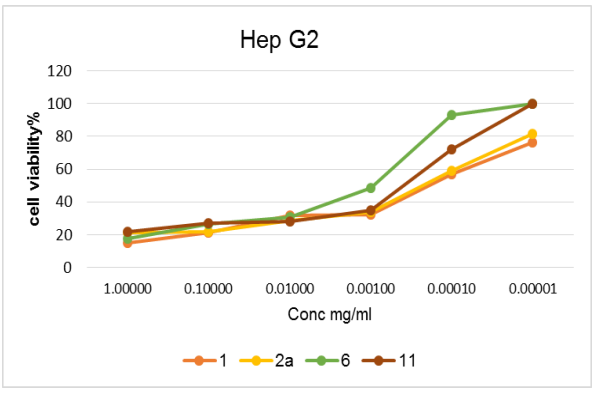


Figure 4. Cell viability % of Hep G2 with different concentrations of the tested compounds

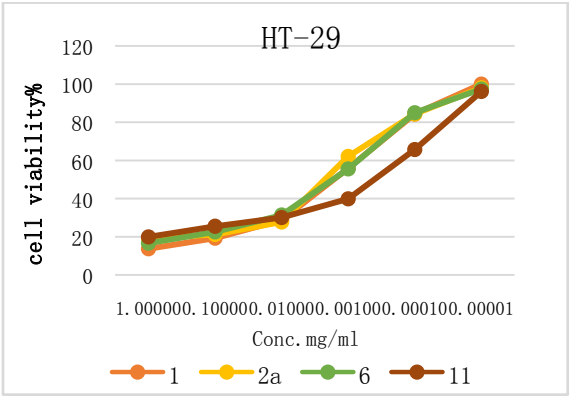


Figure 5. Cell viability % of HT-29 with different concentrations of the tested compounds

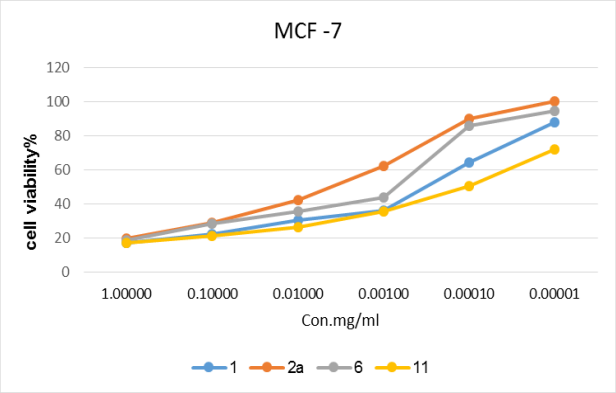


Figure 6. Cell viability % of MCF-7 with different concentrations of the tested compounds

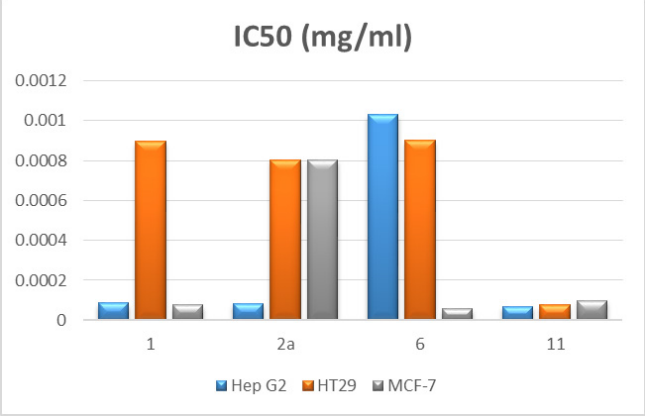


Figure 7. Evaluation of IC_{50} of test compounds

Order of activity for test compounds against human liver cancer (Hep G2) cell line: **11**>**2a**>**1**>**6**.

Order of activity for test compounds against human colon cancer (HT-29) cell line: **11**>**2a**>**1**>**6**.

Order of activity for test compounds against human breast cancer (MCF-7) cell line: **6**>**1**>**11**>**2a**.

4. Conclusions

In this work, a variety of heterocyclic systems have been synthesized from thiosemicarbazone derivatives. The newly synthesized compounds **1**, **2a**, **6** and **11** have been evaluated for in vitro cytotoxic activity against human liver cancer (Hep G2), human colon cancer (HT-29) and human breast cancer (MCF-7) cell lines using an MTT assay protocol. Compound **11** showed the best cytotoxic activity against all the three cancer cell lines due to the presence of pyrazolo [3,4-d]-1,3-thiazol group at position-3 of the in dole ring. Compound **1** also showed higher cytotoxic activities against the human liver cancer (Hep G2) and human breast cancer (MCF-7) cell line due to the presence of a CHO group at position-3 of the indole ring. Compound **2a** also showed higher cytotoxic activities against the human liver cancer (Hep G2) cell line due to the presence of a thio-semicarbazone group at position-3 of the indole ring. Compound **6** also showed higher cytotoxic activities against the human breast cancer (MCF-7) cell line due to the presence of a 1,3-thiazolidine ring at position-3 of the indole ring. Hence, it can be suggested that compound **1, 2a, 6** and **11** could be used as leads in the design and development of new anticancer drugs.

REFERENCES

[1] Kovala-Demertzi, D.; Yadav, P. N.; Demertzis, M. A.andColuccia, M. Synthesis, crystal structure, spectral properties and cytotoxic activity of platinum (II) complexes of 2-acetyl pyridine and pyridine-2-carbaldehydeN(4)-ethyl-thiosemicarbazones, J. Inorg. Biochem., 2000, 78, 347.

[2] Singh, S. B.; Ashok, K.; Galal, M. Z. and Amit, C. Synthesis and antibacterial studies of some semicarbazones of some carbonyl compounds. International Journal of Science and Research, 2015, 4(3), 389-392.

[3] Rao, V. K.; Rao, M.S.; Jain, N.; Panwar, J. and Kumar, A. Silver triflate catalyzed synthesis of 3-aminoalkylated indoles and evaluation of their antibacterial activities. Organic and Medicinal Chemistry Letters, 2011, 1(10), 1-7.

[4] Ismail, M. M.; Kamel, M. M.; Mohamed, L. W. and Fagga, S. I. Synthesis of new indole derivatives structurally related to donepezil and their biological evaluation as acetylcholinesterase inhibitors. Molecules, 2012, 17, 4811-4823.

[5] Chavan, R. S.; More, H. N.; and Bhosale, A. V. Synthesis and evaluation of analgesic and anti-inflammatory activities of a novel series of 3-(4,5-dihydropyrazolyl)indoles. International Journal of Pharmaceutical and Biomedical Research, 2010, 1(4), 135-143.

[6] Kumar, S.; Mehndiratta, S.; Nepali. K.; Gupta, M. K.; Koul, S.; Sharma, P. R.; Saxena, A. K. and Dhar, K. L. Novel indole-bearing combretastatin analogues as tubulin polymerization inhibitors. Organic and Medicinal Chemistry Letters, 2013, 3(3), 1-13.

[7] Zarghi, A.; Tahghighi, A.; Soleimani, Z.; Daraie, B.; Dadrass, G.O. and Hedayati, M.Design and synthesis ofsome 5-substituted-2-(4-(azido or methylsulfonyl) phenyl)-1*H*-indole derivatives as selective cyclooxygenase (cox-2) inhibitors. Sci. Pharm., 2008, 76, 361–376.

[8] Güze, Ö.; Karali, N. and Salman, A. Synthesis and anti-tuberculosis activity of 5-methyl/trifluoromethoxy-1H-indole -2,3-dione-3-thiosemicarbazone derivatives. Bioorganic& Medicinal Chemistry, 2008, 16, 8976 – 8987.

[9] Abdou, S.E. Reactions with visnaginone: Synthesis, cyclisation and microbial evaluation of some visnagin one thiosemicarbazone derivatives. Modern Applied Science, 2011, 5(5), 140-149.

[10] Shih, M. and Ling Wu, C. Efficient syntheses of thiadiazoline and thiadiazole derivatives by the cyclization of 3-aryl-4-formylsyndone thiosemicarbazones with acetic anhydride and ferric chloride, Tetrahedron, 2005, 61, 10917–10925.

[11] Abdel-Rahman, A.; El-Sayed, W.; El-Sayed, G.; Mohamed, A. and Faddad, A. Synthesis and antimicrobial activity of substituted[(pyrazol-4-yl)methylene]hydra-zono-2,3-dihydro

thia-zoles and their sugar derivatives. J. Heterocyclic Chem., 2012, 49, 93-101.

[12] Ahmed, O.; Sharma, P. and Singhvi, I. Synthesis and anti-hyperglycemic activity of some novel substituted thiazolidinone derivatives. Indo American Journal of Pharmaceutical Research, 2014, 4(2), 1008-1014.

[13] Prakasha K. C. and Channe, G. Synthesis of 5-arylidine - 2-(3,4,5-trimethoxyphenyl)-3-(4-phenyl-thiazol-2-yl)-thiazolidin-4-one derivatives as a novel class of antimicrobial agents. International Journal of Chemistry Research, 2011, 2(3), 10-14.

[14] Rao, M.; Sreeramulu, J.; Ravindranath, L. K.; Nagaraja Reddy, G.; Hanumanthurayudu, K.; Nageswara Reddy, G.; Jayaraju1, A. and Madhusudhan, P. Synthesis and biological screening of some pyridine and pyrrole derivatives of pyrazolo-[3,4-c]-pyrazoles. journal of Chemical and Pharmaceutical Research, 2012, 4(1), 272-278.

[15] Sailu, B.; Mahantia, S.; Satya Srinivas, A.; Balram, B.; Ram, B.; Taara, B. and Vasudh, B. Synthesis and antibacterial activity of novel isoxazoline derivatives. Der Pharma Chemica, 2012, 4 (5), 2036-2041.

[16] Thumar, N. J. and Patel, M. P. Synthesis, characterization, and in vitro microbial evaluation of some new 4*H*-chromene and quinoline derivatives of 1*H*-pyrazole. J. Heterocyclic Chem., 2012, 49, 1169.

[17] El-Rady, E. A. A convenient and facile synthesis of new thiazole derivatives. Phosphorus, sulfur and silicon, 2008, 183, 1659 - 1671.

[18] Bondock, S.; Khalif, W. and Faddad, A. Synthesis and antimicrobial evaluation of some new thiazole, thiazolidinone and thiazoline derivatives starting from 1-chloro-3,4-dihydro-naphthalene-2-carboxaldehyde. European Journal of Medicinal Chemistry, 2007, 42, 948-954.

[19] El-GamL, K. M. Synthesis and antimicrobial evaluation of new polyfunctionally substituted heterocyclic compounds derived from 2-cyano-N-(3-cyanoquinolin-2-yl) acetamide. American Journal of Organic Chemistry, 2015, 5(1), 1-9.

[20] Abdel-Aziz, H. A.; Gamal-Eldeen, A. M.; Hamdy, N. A. and Fakhr, I. M. I. Immunomodulatory and anti-cancer activity of some novel 2-substituted-6-bromo-3-methyl thiazolo[3,2-a] benzimidazole derivatives. Arch. Pharm., 2009, 342, 230–237.

[21] Mosmann, T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. Journal of Immunological Methods, 1983, 65, 55-63.

[22] Monks, A. Feasibility of high flux anticancer drug screen using a diverse panel of cultured human tumour cell lines. Journal of the National Cancer Society, 1991, 83, 757-766.

[23] Marshall, N. J.; Goodwin, C. J. and Holt, S. J. A critical assessment of the use of microculturetetrazolium assays to measure cell growth and function. Growth Regul., 1995, 5, 69–84.