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

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ABSTRACT

Reaction of 1*H*-indole-3-carboxaldhyde (**1**) with thiosemicarbazide derivatives to 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-ones derivative (**6**), respectively. Compound **6** undergoes a series of heterocyclization reactions to give new heterocyclic compounds. The structures of newly synthesized compounds had been confirmed by elemental analysis and spectral data. Some of newly synthesized compounds were evaluated for *in vitro* cytotoxic activity against three human cancer cell lines, including human liver cancer (Hep G2), humam colon cancer (HT-29) and human breast cancer (MCF-7) using MTT assay.

KEYWORDS

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

INTRODUCTION

Thiosemicarbazones have been used as intermediate for the preparation of many heterocyclic compounds.In the literature, many researchers 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 afforded different cyclic products from a singlet starting material. Moreover, thiosemicarbazones bearing an aromatic heterocyclic moiety seem to possess enhanced biological activities [1,2]. On other hand, heterocyclic compounds containing indoles ring major importance due to its 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 here reaction of 1*H*-indol-3-carboxaldehyde with thiosemicarbazide derivatives to afforded the corresponding thiosemicarbazones derivatives, then cyclization by different reagents and different conditions to give some novel heterocyclic compounds bearing indole moiety and study cytotoxic activities as such derivatives.

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EXPERIMENTAL

Melting points were measured on a Gallenkamp apparatus and are uncorrected. IR spectra were recorded on Shimadzu FT-IR 8101 PC infrared spectrophotometer (ν_{max} in cm^{-1}). The ^1H NMR and ^{13}C NMR spectra were determined in DMSO-d_6 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 Microanalytical center of Cairo University and the main chemical warfare laboratories.

General procedure for the preparation of thiosemicarbazones 2a,b: An equimolecular mixture of 2-(4-bromo phenyl)-1*H*-indole-3-carboxaldehyde (**1**) and the selected thiosmicarbazide 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 the 3 h. The reaction mixture was cooled to room temperature and separated product was filtered off, 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-methyl phenyl)thiosemicarbazone (2a): Yellow solid. Yield 80 %, m.p.: 249-250 °C (ethanol-DMF). FT-IR (KBr, ν_{max}, cm^{-1}): 3317, 3135 (NH), 3042, 2975, 2857 (CH), 1246 (C=S). ^1H NMR (DMSO-d_6) 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 $C_{23}H_{19}N_4SBr$ (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-Bromo phenyl)-1*H*-indol-3-ylmethylene]-N-(4-phenyl-1,3-thiazol-2-yl)-thiosemicarbazone (2b): Yellow solid. Yield 60 %, m.p.: 140-142 °C (ethanol). FT-IR (KBr, ν_{max}, cm^{-1}): 3223, 3161, 3125, (NH), 3039, 2967, 2864 (CH), 1237 (C=S). ^1H NMR (DMSO-d_6) 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 $C_{25}H_{18}N_5S_2Br$ (532.48): C, 56.39; H, 3.41; Br, 15.01; N, 13.15; S, 12.04. Found (%): C, 56.19; H, 3.21; B r,14.89; N,13.00; S,11.89.

5-[2-(4-Bromo phenyl)-1*H*-indol-3-yl]-4-(4-methyl phenyl)-4*H*-1,2,4–triazole-3-thiol (3): A solution of thiosemicarbazone derivative (**2a**) (0.01 mol) in absolute ethanol (15 mL) containing few drops of HCl was refluxed for 2 h. After cooling and dilution with water, the solid formed were filtered off, washed with water, air dried and recrystallized from ethanol to give compound **3** as green powder. Yield 62 %; m.p.: 336-338 °C (ethanol). FT-IR (KBr, ν_{max}, cm^{-1}): 3166 (NH), 3097, 2951, 2919 (CH), 1606 (C=N). ^1H NMR (DMSO-d_6) 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-d_6) δ ppm: 20.44 (CH_3), 154.84, 154.98 (2C=N), 162.17 (C-S), 106.49, 111.47, 120.92, 121, 122.43, 122.70, 123.10, 125.81, 130.24, 131.14, 131.32, 131.93, 136.51,141.73. Anal. calcd. (%) for $C_{23}H_{17}N_4SBr$ (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.

***N*-[4-acetyl-5-(2-(4-bromo phenyl)-1*H*-indol-3-yl)-4,5-dihydro-1,3,4-thiadiazol-2-yl]-N-(4-methyl phenyl)acetamide (4):** A solution of the thiosemicarbazone derivative (**2a**) in acetic anhydride (12 mL) was heated under reflux for 5 h with continuous stirring and then allowed to attain room temperature. The reaction mixture was slowly added to 400 mL of ice-cooled water and then stirred at room temperature for 1 h. 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, ν_{max}, cm^{-1}): 3419 (NH), 3044, 2986, 2919 (CH), 1750, 1688 (2C=O). ^1H NMR (DMSO-d_6) 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 $C_{27}H_{23}N_4O_2SBr$ (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-(4-Bromo phenyl)-3-[3-(4-methyl phenyl)-4-phenyl-1,3-thiazol-2(3*H*)-ylidene]hydrazono-methyl-*1H*-indole (5): To a solution of thiosemicarbazone derivative (**2a**) (0.01 mol) in absolute ethanol (20 mL) were added equimolar amounts of the phenacyl bromides and anhydrous sodium acetate. The reaction mixture was heated under reflux for 6 h 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 yellow solid. Yield 60 %, m.p.: 280-282 °C. FT-IR (KBr, ν_{max}, cm^{-1}): 3122 (NH), 3028, 2947, 2826 (CH). ^1H NMR (DMSO-d_6) 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 $C_{31}H_{23}N_4SBr$ (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-(4-Bromo phenyl)-3-[3-(4-methyl phenyl)-4-oxo-1,3-thiazolidin-2-ylidene]hydrazonomethyl-1*H*-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 8 h 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 yellow powder. Yield 70 %, m.p.: 340-342 °C; FT-IR (KBr, ν_{max} cm^{-1}): 3273 (NH),

3044, 2959, 2861 (CH), 1703 (C=O), 1601 (C=N). ^{1}H NMR (DMSO-d_6) 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-d_6) 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 $C_{25}H_{19}N_4OSBr$ (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-(4-Bromo phenyl)-3-[5-benzylidene-3-(4-methyl phenyl)-4-oxo-1,3-thiazolidin-2-ylidene]hydrazonomethyl-1*H*-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 6 h 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) ν_{max}, cm^{-1}): 3292 (NH); 3029, 2942, 2842 (CH), 1683 (C=O). ^{1}H NMR (DMSO-d_6) 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 (C=CH), 153(N=CH), 156 (N=C), 165 (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 $C_{32}H_{23}N_4OSBr$ (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-(4-Bromo phenyl)-3-[6-(4-methyl phenyl)-2,3-diphenyl-2,3,3a,6-tetrahydro-*5H*-pyrazolo[3,4-d]1,3-thiazol-5-ylidene]hydrazonomethyl-1*H*-indole (8): A mixture of compound **7** (0.01 mol) and phenyl hydrazine (0.01 mol) was refluxed in ethanol (50 mL) in presence of few drops of acetic acid for 4 h. 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 orange powder. Yield 55 % yield; m.p.: 102-104 °C. FT-IR (KBr, ν_{max}, cm^{-1}): 3229 (NH), 3054, 2936, 2857 (CH), 1605 (C=N). ^{1}H NMR (DMSO-d_6) δ ppm: 2.24 (s, 3H, CH_3), 4.09 (d, 1H, CH-pyrazole), 6.67 (d, 1H, CH-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 $C_{38}H_{29}N_6SBr$ (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-(4-Bromo phenyl)-3-[3-phenyl-6-(4-methyl phenyl)-3,3a-dihydro-1,3-thiazolo [4,5-c]isoxazol-5-ylidene]hydrazonomethyl-1*H*-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 presence of few drops of acetic acid for 10 h and kept overnight. Excess of 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 white powder. Yield 65 %; m.p.: 170-172 °C. FT-IR (KBr, ν_{max}, cm^{-1}): 3173 (NH); 3057, 2922, 2859 (CH). ^{1}H NMR (DMSO-d_6) δ ppm: 2.17 (s, 3H, CH_3), 4.35 (d, 1H, CH-isoxazole), 5.55 (d, 1H, CH-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 $C_{32}H_{24}N_5OSBr$ (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-(4-Bromo phenyl)-1*H*-indol-3-ylmethylidene-hydrazono]-4-chloro-3-(4-methyl phenyl)-2,3-dihydro-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 4 h. 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 and recrystallized from ethanol to give **10** as yellow powder. Yield 70 %; m.p.: 150-152 °C. FT-IR (KBr, ν_{max}, cm^{-1}): 3216 (NH), 3031, 2956, 2781 (CH), 1600 (C=N), 1675 (C=O). ^{1}H NMR (DMSO-d_6) 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-d_6) 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 $C_{26}H_{18}N_4OSBrCl$ (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-(4-Bromo phenyl)-3-[6-(4-methyl phenyl)-1,6-dihydro-*5H*-pyrazolo[3,4-d]-1,3-thiazol-5-ylidene]hydrazono-methyl-1*H*-indole (11): A mixture of compound **10** (0.01 mol) and hydrazine hydrate (0.01 mol) was refluxed in ethanol (50 mL) for 4 h. The reaction mixture was cooled and the precipitate was filtered off and recrystallized from ethanol to gives **11** as yellow powder. Yield 64 %, m.p.: 300-302 °C. FT-IR (KBr, ν_{max}, cm^{-1}): 3380, 3176 (NH), 3052, 2966, 2864 (CH), 1604 (C=N). ^{1}H NMR (DMSO-d_6) 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 $C_{26}H_{19}N_6SBr$ (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.

N′-{2-[2-(4-Bromo phenyl)-1*H*-indol-3-yl-methylene-hydrazono]-4-chloro-3-(4-methyl phenyl)-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 2 h. The precipitate formed after cooling was filtered off, washed with cold ethanol, dried and recrystallized from xylene to give **12** as orange powder. Yield 50 %; m.p.:

230-232 °C. FT-IR (KBr, ν_{max}, cm^{-1}): 3327 (NH), 2920, 2853 (CH), 1668 (C=O), 2196 (CN). ^{1}H NMR (DMSO-d_6) 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 $C_{29}H_{21}N_7OSBrCl$ (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-(4-Bromo phenyl)-1*H*-indol-3-ylmethylene-hydrazono]-3-(4-methyl phenyl)-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 triethylamine in absolute ethanol (30 mL) was heated under reflux for 8 h. The precipitate formed after cooling was filtered off, washed with cold ethanol, dried and recrystallized from ethanol to give **13** as orange powder. Yield 67 %; m.p.: 250-252 °C. FT-IR (KBr, ν_{max}, cm^{-1}): 3337 (NH), 3055, 2923, 2865 (CH). ^{1}H NMR (DMSO-d_6) 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 $C_{32}H_{23}N_6SBr$ (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-(4-Bromophenyl)-1*H*-indol-3-ylmethylenehydrazono]-3'-(4-methyl phenyl)-3-phenyl-2,5'-bi-1,3-thiazolidin-2'-ylidene-4,4'-dione (16): To a stirred solution of 0.56 g KOH (0.01 mol) in 20 mL DMF, 1,3-thiazolidin-4-one **6** (0.10 mol) was added. After stirring for 0.5 h, phenyl isothiocyanate (0.01 mol) was added to the resulting mixture. After complete addition, stirring of the reaction mixture at room temperature for 12 h. Then ethyl chloroacetate (0.01 mol) was added to the reaction mixture and stirred for 6 h. The reaction mixture was poured into crushed ice. The resulting precipitate was filtrated off, dried and recrystallized from xylene to give **16** as orange powder. Yield, 60 %, m.p.: 290-292 °C. FT-IR (KBr, ν_{max}, cm^{-1}): 3267 (NH), 3042, 2964, 2919 (CH), 1702 (C=O), 1600 (C=N). ^{1}H NMR (DMSO-d_6) 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-d_6) 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 $C_{34}H_{24}N_5O_2S_2Br$ (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-Oxo-2-phenylethyl{2-[2-(4-bromo phenyl)-1*H*-indol-3-ylmethylenehydrazono]-3-(4-methyl phenyl)-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 (0.01 mol) carbon disulfide was added slowly over the course of 10 min. After complete addition, stirring of the reaction mixture was continued for 6 h. Then phenacyl bromide (0.01 mol) was added to the mixture and stirring continued for 3 h, 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 red powder. Yield, 60 %; m.p.: 200-202 °C. FT-IR (KBr, ν_{max}, cm^{-1}): 3274 (NH), 3056, 2967, 2861 (CH), 1702 (C=O), 1241 (C=S). ^{1}H NMR (DMSO-d_6) δ ppm: 2.37 (s, 3H, CH_3), 4.09 (s, 2H, CH_2), 4.76 (s, 1H, H-5 thiazolidinone), 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-d_6) 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, 111.22, 114.03, 122.12, 124.60, 128.51, 130.52, 130.6, 130.65, 130.84, 130.85, 132.92, 133.08, 149.01. Anal. calcd. (%) for $C_{34}H_{25}N_4O_2S_2Br$ (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.

***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.

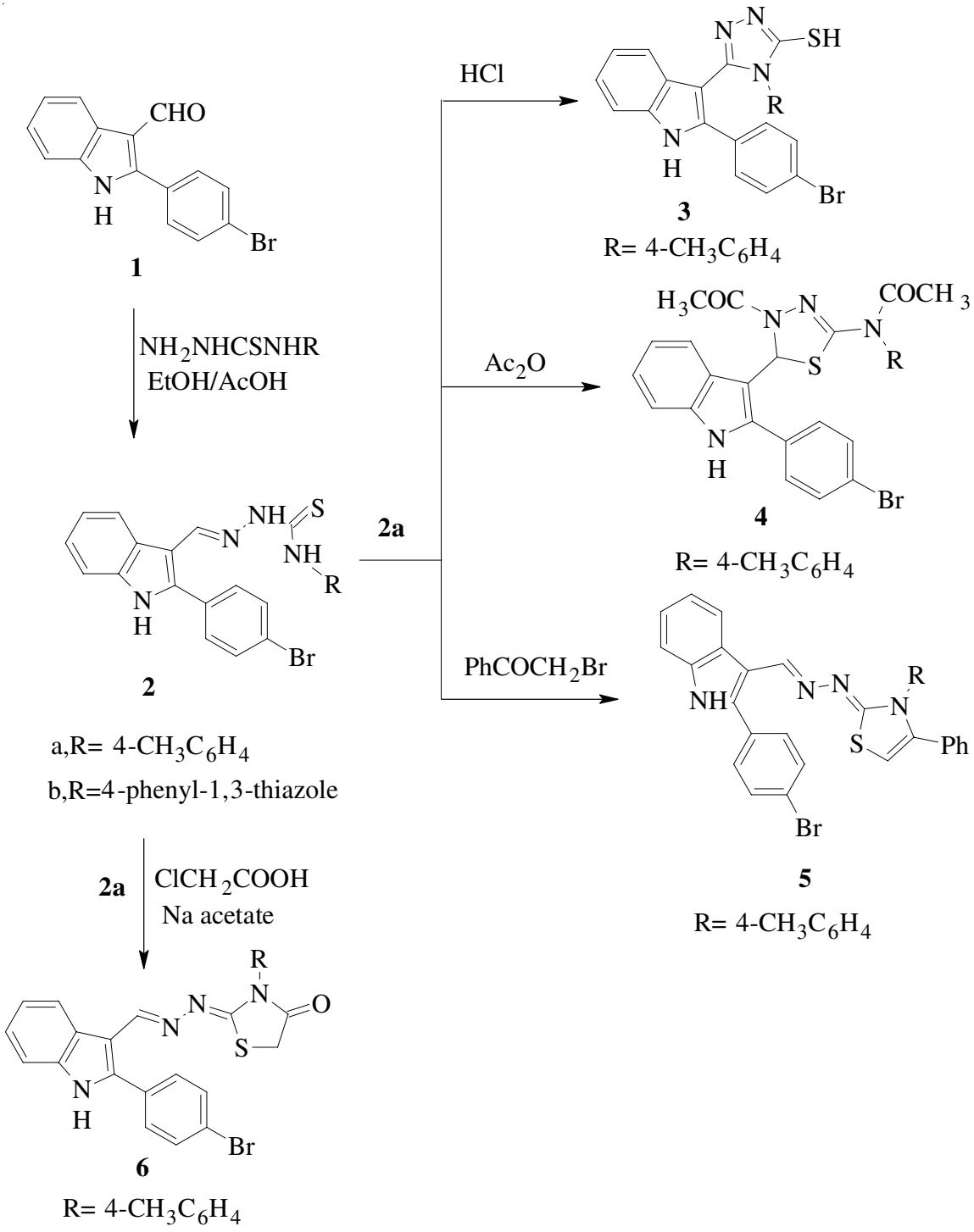
The monolayer cells were detached with trypsin-ethylene-diaminetetra-acetic acid (EDTA) to make singlet cell suspensions and viable cells were counted using a hemocytometer, then diluted with the fetal bovine serum (FBS) medium with 5 % FBS to give final density of 2×10^5 cells/mL. One hundred microlitres per well of cell suspension were seeded into 96-well plates at 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 dimethyl sulfoxide (DMSO) and further diluted in serum free medium to produce six concentration starting from 1 mg/mL to 10^{-6}. 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, mix by shaking at 150 rpm for 5 min, incubate at 37 °C and 5 % CO_2 for 48 h. 20 µL of MTT (5 mg/mL) in phosphate buffered saline (PBS) was added to each well plate and mix by shaking at 150 rpm for 5 min and incubate at 37 °C and 5 % CO_2 for 5 h 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 micro plate reader [8]. Viability of treated cells was calculated in reference to the untreated control cells by using the following formula:

$$\text{Cell viability (\%)} = \frac{\text{Sample absorbance}}{\text{Control absorbance}} \times 100$$

RESULTS AND DISCUSSION

The synthetic procedures adopted to obtain the target compounds are outlined in **Schemes I-III**. The key intermediate 1-[1*H*-indol-3-ylmethylene]thiosemicarbazone derivatives

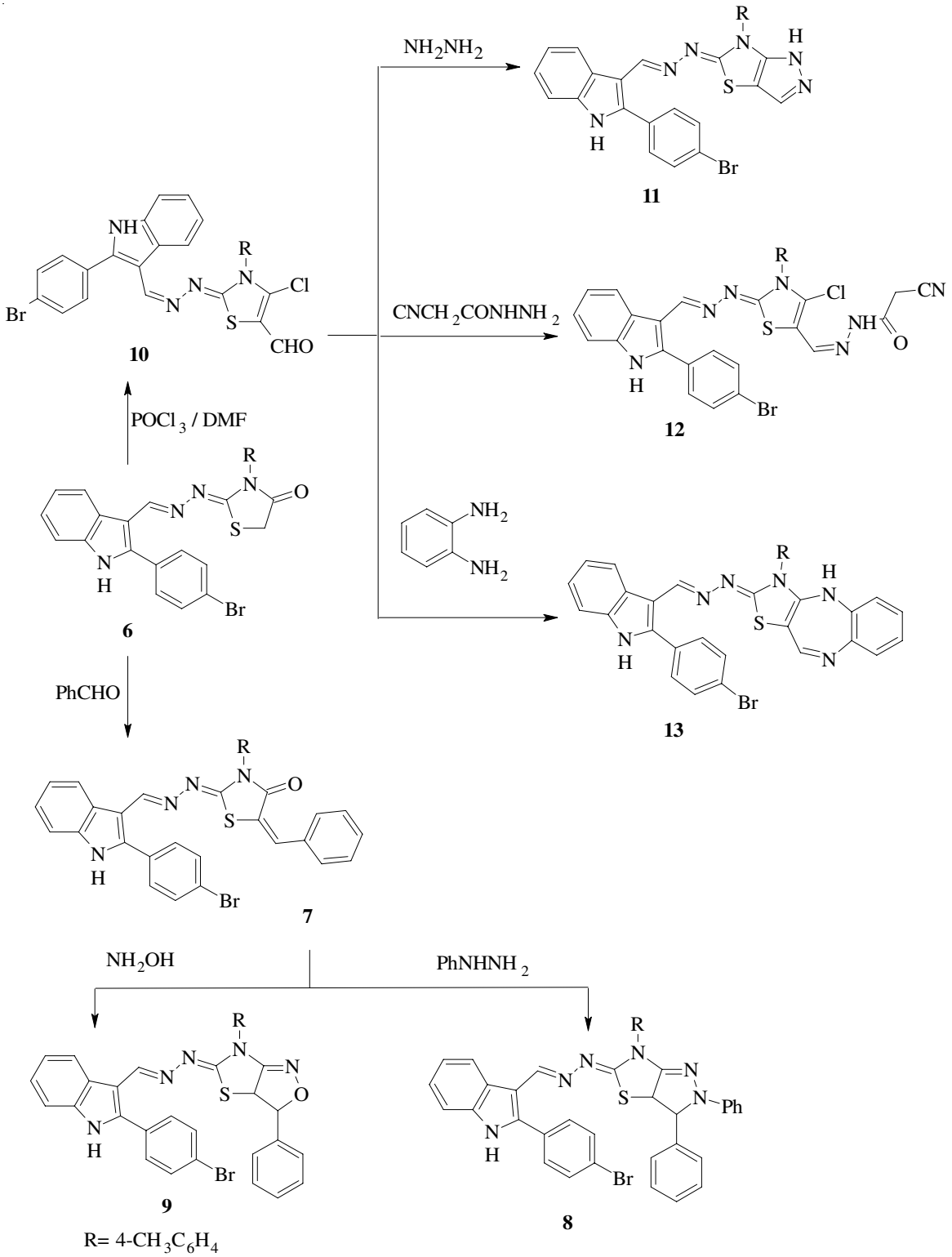


Scheme-I: Synthesis of compounds **2-6**

2a,b were prepared by reaction 1*H*-indole-3-carboxaldehyde (**1**) with thiosemicarbazide derivatives such as 4-(4-methyl phenyl)thiosemicarbazide or 4-(4-phenyl-1,3-thiazol-2-yl)-thiosemicarbazide in refluxing ethanol containing acetic acid [9] (**Scheme-I**). The structure of compound **2a, b** were based on analytical and spectral data. The ^{1}H NMR spectra of **2a** displayed D_2O-exchangeable signals at δ 10.01, δ 11.46, δ 11.99 ppm of three NH protons and singlet signal at δ 2.32 ppm for CH_3 proton.

Cyclization of thiosemicarbazone derivative **2a** depend on cyclizing agent and conditions of reaction. Thus, thiosemicarbazones derivative (**2a**) which may undergo to ring closure by acid medium [10] afforded 5-[1*H*-indol-3-yl]-4*H*-1,2,4-triazole-3-thiol derivative (**3**) (**Scheme-I**). ^{1}H NMR spectra of **3** displayed D_2O-exchangeable signals at δ 4.33 ppm and δ 12.05 ppm of SH and NH protons respectively. ^{13}C NMR spectra of **3** showed signals at δ 20.44,154.84,154.98 and 162.17 ppm to CH_3, 2 C=N and C-S, respectively.

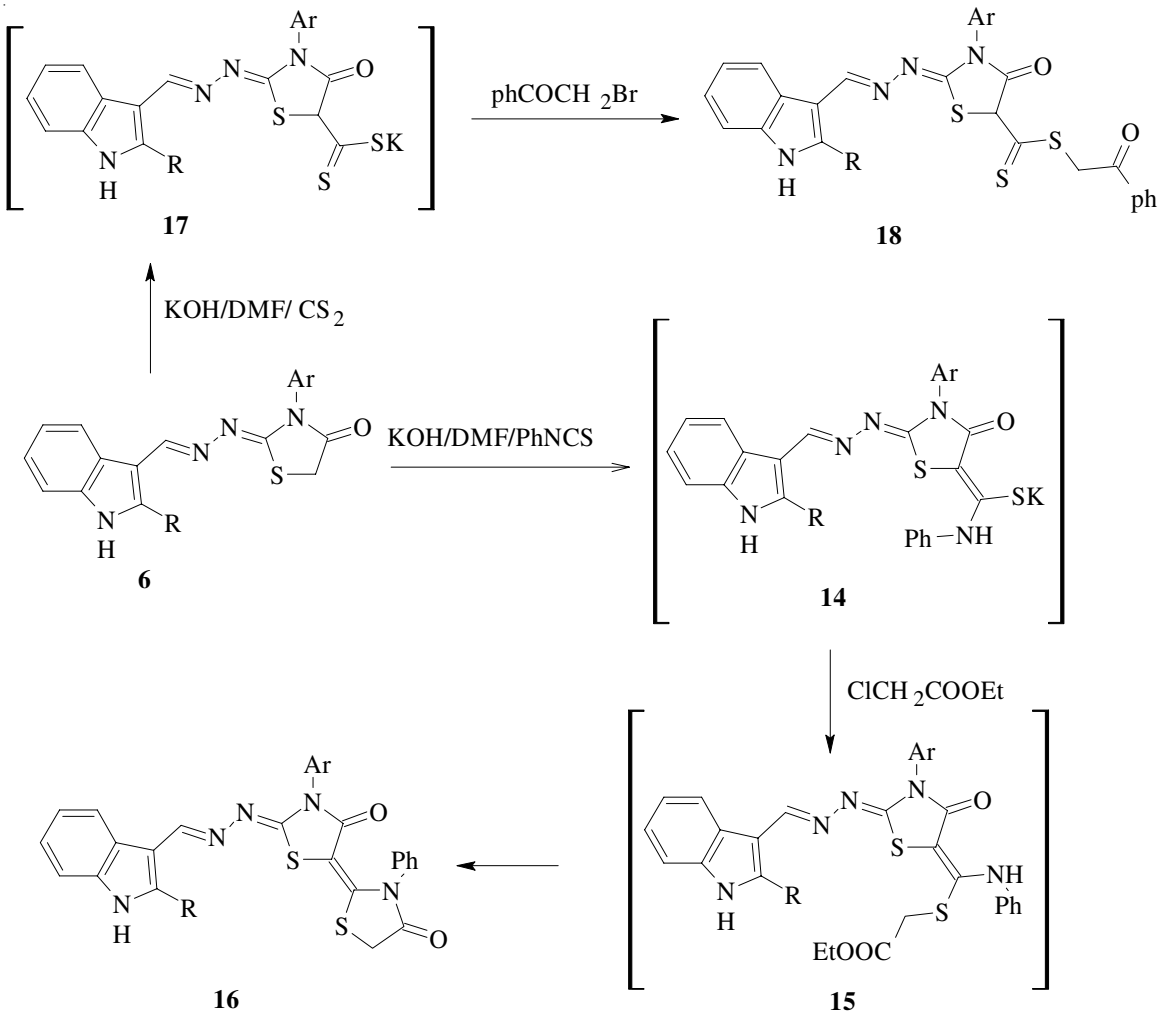
While, heterocyclization of thiosemicarbazone derivative (**2a**) in the presence of acetic anhydride gives *N*-[4-acetyl-5-(1*H*-indol-3-yl)-1,3,4-thiadiazol-2-yl]acetamide (**4**) (**Scheme-I**). It is suggested that the mechanism of the reaction compound



Scheme-II: Synthesis of compounds **7-13**

2a with acetic anhydride follows Fig. 1. Reaction compound **2a** with acetic anhydride, the resonance effects between NH and the phenyl group may reduce the nucleophilicity of NH and the steric effect of phenyl group on the NH retards nucleophilic substitution with acetic anhydride. Therefore, the initial monoacetyl substituted products are gradually converted to diacetyl substituted thiadiazoline [11] derivative (**4**). ^{1}H NMR spectrum of compound **4** showed a signal at δ 2.16, δ 2.19, δ 2.26 ppm corresponding to three CH_3 groups and multiplet signal 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 $C_{27}H_{23}N_4O_2SBr$.

Furthermore, treatment of thiosemicarbazone derivative **2a** with phenacyl bromides in boiling ethanol in the presence of anhydrous sodium acetate [12] yielded the corresponding



Scheme-III: Synthesis of compounds **16** and **18**

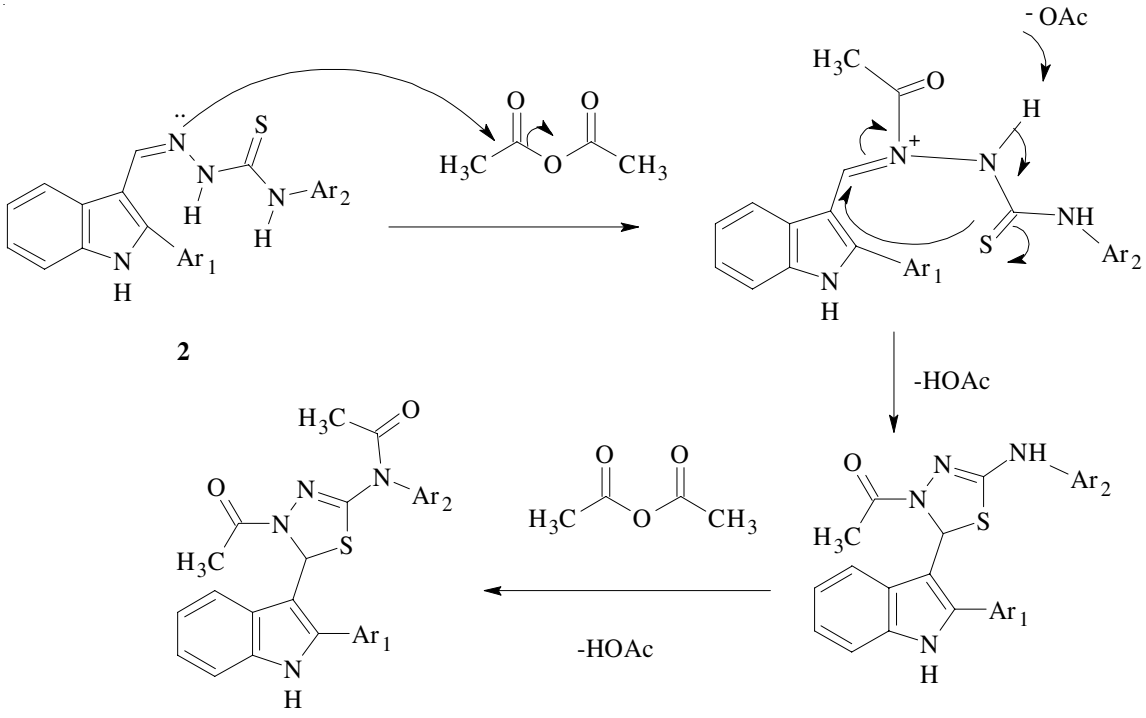


Fig. 1. Proposed mechanism formation of compound **4**

3-[1,3-thiazol-2(*3H*)-ylidene]hydrazonomethyl-1*H*-indole derivative **5.** ^{1}H NMR spectrum of **5** showed a signal at δ 6.58 ppm corresponding to CH-5 of thiazole ring and 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 $C_{31}H_{23}N_4SBr$.

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-I**). IR spectra of **6** showed the disappearance of NH bands of 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 NMR spectra of **6** showed a new signal at δ 4.09 ppm attributed to CH_2 proton of thiazolidinone ring. ^{13}C NMR spectra of **6** showed signals at δ 20.65, 32.16, 152, 162 and 172 ppm to CH_3, CH_2, N=CH, C=N and C=O respectively. Condensation 1,3-thiazolidin-4-one derivative **6** with benzaldehyde in the presence of freshly fused sodium acetate in boiling glacial acetic acid yielded the corresponding arylidene derivatives (**7**) [14] (**Scheme-II**). The analytical and spectral data of compound **7** was consistent with the proposed structure. Thus, ^{1}H NMR spectrum of compound **7** showed absence of thiazolo-methylene protons and showed a multiplet signal at δ 7.25-7.32 for the aromatic protons and olefinic CH= proton. ^{13}C NMR spectra of **7** showed signals at δ 20.68, 142, 153, 156 and 165 ppm to CH_3, C=CH, N=CH, N=C and C=O respectively. Compound **7** was used as starting material for further syntheses of other heterocyclic compounds. Thus, reaction compound **7** with phenyl hydrazine [15] afforded 3-(pyrazolo[3,4-d]1,3-thiazol-5-ylidene) hydrazono-methyl-1*H*-indole **8**. The ^{1}H NMR spectrum of **8** showed a doublet signals at δ 4.09 and δ 6.67 due to 2CH protons of pyrazoline. The mass spectrum of compound **8** showed the molecular ion peak at *m/z* 681corresponding to the molecular formula $C_{38}H_{29}N_6SBr$. On the other hand, cyclocondensation (**7**) with hydroxylamine hydrochloride in presence of sodium acetate [16] afforded 3-[1,3-thiazolo [4,5-c]isoxazol-5-ylidene]hydrazonomethyl-1*H*-indole **9** (**Scheme-II**).

The ^{1}H NMR spectrum of **9** showed a doublet signals at δ 4.53 and δ 6.67 due to 2CH protons of isoxazole. The mass spectrum of compound **9** showed the molecular ion peak at *m/z* 606 corresponding to the molecular formula $C_{32}H_{24}N_5OSBr$.

Moreover, chloroformylation of 1,3-thiazolidin-4-one derivative (**6**) using Vilsmeier-Haack reagent to 4-chloro-1,3-thiazole-5-carboxaldehyde (**10**). The most probable reaction involves initial formation of intermediate **A-C** that underwent further chlorination and hydrolysis to yield final products (**10**) [17] (Fig. 2). The IR spectrum of compound **10** showed bands at 1675 cm^{-1} due to C=O group. The ^{1}H NHR of compound **10** revealed a new signal at δ 9.95 ppm assigned to CHO proton and disappearance signal at δ 4.09 ppm attributed to CH_2 thiazolidinone. ^{13}C NMR spectra of **10** showed new signal at δ 135.89 ppm assigned for C-Cl group. Reaction 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-II**). The chemical structure of the compound **11** was elucidated on the basis of elemental analysis and spectral data. IR spectrum of compound **11** was characterized by the presence of strong bands at 3380, 3176 cm^{-1} due to two NH proton. The mass spectrum of compound **11** showed the molecular ion peak at *m/z* 547 corresponding to the molecular formula $C_{26}H_{19}N_6SBr$.

Furthermore, reaction 4-chloro-1,3-thiazole-5-carboxaldehyde (**10**) with a cyano acetic acid hydrazide [19] afforded the corresponding cyanoacetohydrazide derivative (**12**). ^{1}H NMR of compound **12** showed D_2O-exchangeable signal at δ11.33, 11.49 ppm due to 2 NH protons and singlet signals at δ 8.29, 8.36 ppm and 4.22 ppm due to 2 CH=N and CH_2 protons, respectively.

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-II**). The ^{1}H NMR spectrum of compound **13** showed D_2O-exchangeable signal at δ 12.03 and 12.31 ppm due to 2NH protons. The mass spectrum of compound **13** showed the molecular ion peak at *m/z* 603 corresponding to the molecular formula $C_{32}H_{23}N_6SBr$.

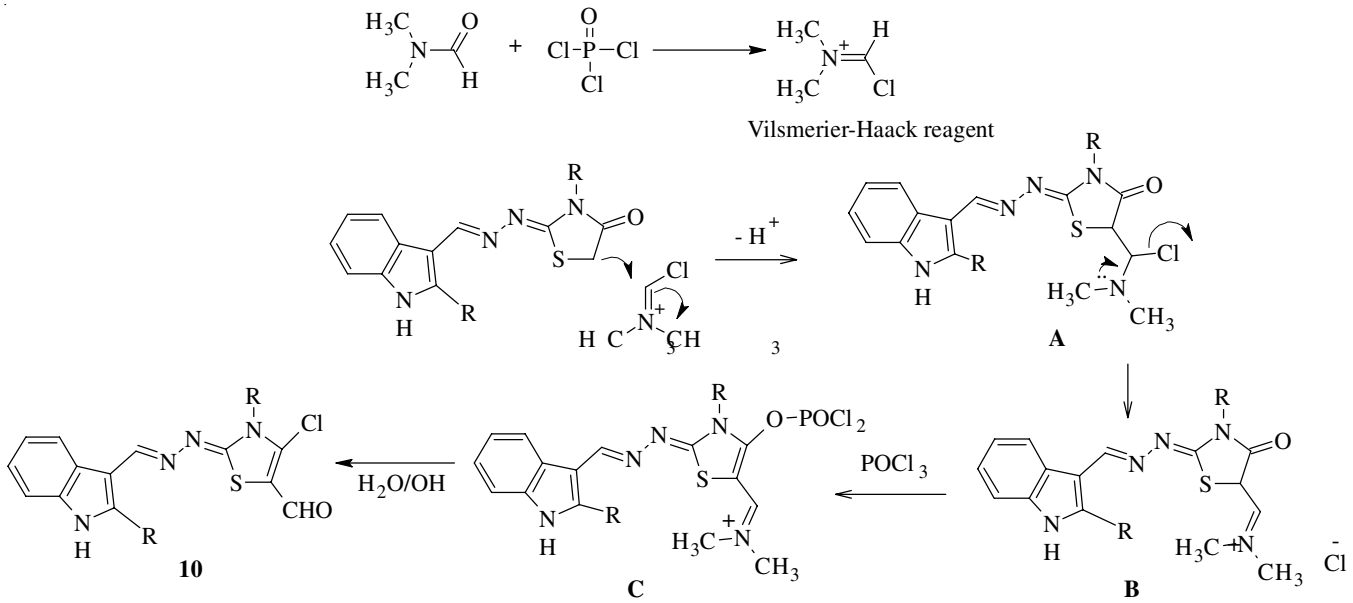


Fig. 2. Proposed mechanism formation of compound **10**

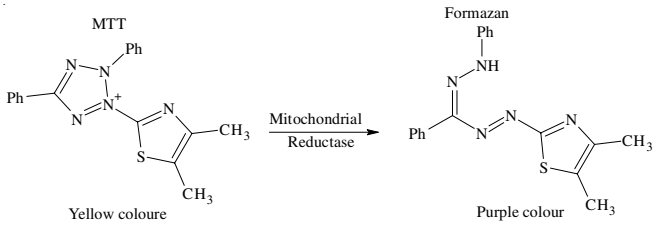
The active methylene in 1,3-thiazolidin-4-one derivative **6** was allowed to react with phenyl isothiocyanate in dry *N,N'*-dimethylformamide (DMF) containing catalytic amount of potassium hydroxide to give the non-isolable potassium salt **14** and then ethylchloroacetate [20] was added afforded 2'-[1*H*-indol-3-ylmethylenehydrazono]-2,5'-bi-1,3-thiazolidin-2'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

Furthermore, the reaction 1,3-thiazolidin-4-one derivative (**6**) with carbon disulfide in boiling DMF containing catalytic amount of potassium hydroxide afforded non-isolable intermediate potassium sulfide salts (**17**) then phenacyl bromide [21] was added afforded 2-oxo-2-phenylethyl {2-[1*H*-indol-3-ylmethylenehydrazono]}-1,3-thiazolidine-5-carbodithioate (**18**) (**Scheme-III**). The chemical structure of the 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. ^{1}H NMR spectrum of compound **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-5 thiazolidinone proton. ^{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, 2C=O, C=S respectively.

***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-di-methyl thiazol-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-IV**). 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 (Fig. 3).

MTT assay to determine the drug concentration required to inhibit the growth of human cancer cells by 50 % (IC_{50}).



Scheme-IV: Principle of MTT assay

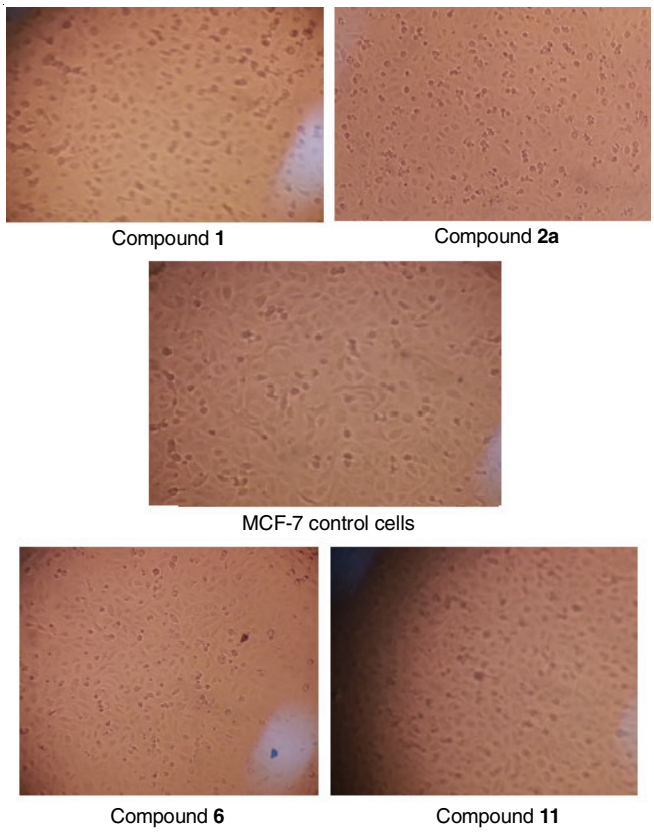


Fig. 3. Pictoral view change in MCF-7 cell morphology after exposure to MTT

The results of the MTT assay percentage viability and IC_{50} values are shown in Tables 1 and 2 and Figs. 4-7.

TABLE-1
in vitro CELL VIABILITY % OF TEST COMPOUNDS **1**, **2a**, **6** AND **11** WITH DIFFERENT CONCENTRATIONS (mg/mL) BY MTT ASSAY

Compd. 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	100.00	87.92
2a	1.00000	21.14	19.28	20.00
	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	100.00
6	1.00000	17.56	16.66	18.86
	0.10000	26.52	22.54	28.30
	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	100.00	97.38	94.71
11	1.00000	21.86	19.93	17.35
	0.10000	26.88	25.49	21.50
	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	100.00	96.07	72.07

TABLE-2
IC_{50} VALUES (mg/mL) OF THE TESTED COMPOUNDS **1**, **2a**, **6** AND **11**

Compd. No.	IC_{50} (mg/mL)		
	Hep G2	HT-29	MCF-7
1	8.83×10^{-5}	8.95×10^{-4}	7.79×10^{-5}
2a	8.49×10^{-5}	8.05×10^{-4}	8.03×10^{-4}
6	1.03×10^{-3}	9.00×10^{-4}	5.81×10^{-5}
11	6.94×10^{-5}	7.61×10^{-5}	9.89×10^{-5}

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

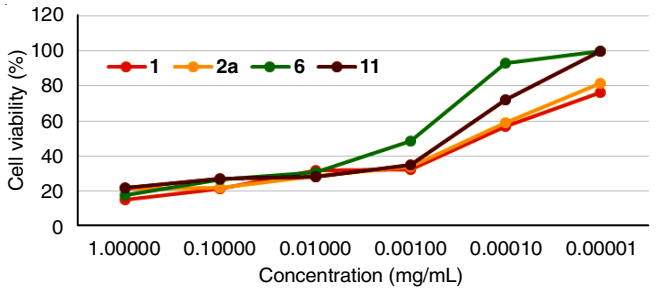


Fig. 4. Cell viability % of Hep G2 with different concentrations of the tested compounds

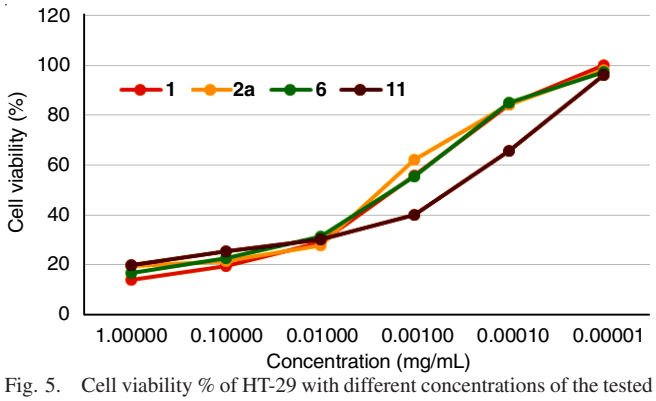


Fig. 5. Cell viability % of HT-29 with different concentrations of the tested compounds

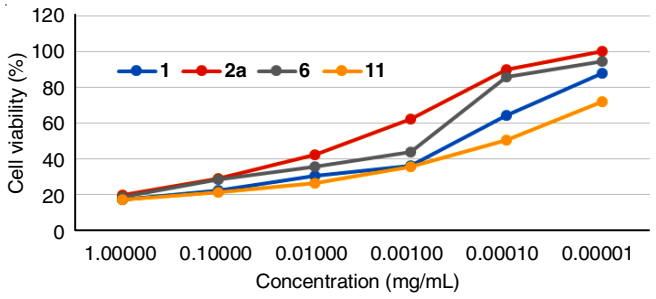


Fig. 6. Cell viability % of MCF-7 with different concentrations of the tested compounds

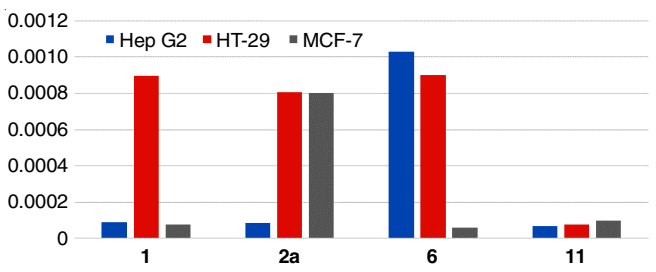


Fig. 7. Evaluation of IC_{50} of test compounds; Order activity of test compounds against human liver cancer (Hep G2) cell line: **11** > **2a** > **1** > **6**; Order activity of test compounds against human colon cancer (HT-29) cell line: **11** > **2a** > **1** > **6**; Order activity of test compounds against human breast cancer (MCF-7) cell line: **6** > **1** > **11** > **2a**

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

• Compound **11** having pyrazolo[3,4-d]1,3-thiazol at position-3 of indole ring a more active cytotoxic agent against all three cancer cell; human liver cancer (Hep G2) cell line, human colon cancer (HT-29) cell line and human breast cancer (MCF-7) cell line

• Compound **1** having CHO at position-3 of indole ring more active cytotoxic agent against human liver cancer (Hep G2) cell line and human breast cancer (MCF-7) cell line while week cytotoxic agent against colon cancer (HT-29) cell line.

• Compounds **2a** having thiosemicarbazone group at position-3 of indole ring more active cytotoxic agent against human liver cancer (Hep G2) cell line while week cytotoxic agent against colon cancer (HT-29) cell line and human breast cancer (MCF-7) cell line.

• Compound **6** having 1,3-thiazolidine ring at position-3 of indole ring more active cytotoxic agent against human breast cancer (MCF-7) cell line but week cytotoxic agent against human liver cancer (Hep G2) cell line and human colon cancer (HT-29) cell line.

Conclusion

In this work, varieties of heterocyclic system have been synthesized from the thiosemicarbazone derivatives. The new synthesis compounds **1**, **2a, 6** and **11** have been evaluated for the *in vitro* cytotoxic activity against human liver cancer (Hep G2), human colon cancer (HT-29) and human breast cancer (MCF-7) cell lines activity using MTT assay, compound **11** showed 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 indole ring. Compounds **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 CHO group at position-3 of indole ring. Compound **2a** also showed higher cytotoxic activities against the human liver cancer (Hep G2) cell line due to the presence of thiosemicarbazone group at position-3 of indole ring. Compound **6** also showed higher cytotoxic activities against the human breast cancer (MCF-7) cell line due to the presence of 1,3-thiazolidine ring at position-3 of 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.

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