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SYNTHESIS OF 3*S*-SUBSTITUTED TRIAZINO[*5,6 - b*]INDOLES AND 4-THIAZOLIDINONE-TRIAZINO[*5,6 - b*]INDOLE HYBRIDS WITH ANTITUMOR ACTIVITY

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Abstract. The synthesis and antitumor activity screening of 1,2,4-triazino[*5,6-b*]indoles based conjugates were performed. Reaction between 3-mercapto-1,2,4-triazino[*5,6-b*]indoles and several *N*-arylchloroacetamides yielded 3*S*-substituted 1,2,4-triazino[*5,6-b*]indoles. Based on 3-hydrazine-1,2,4-triazino[*5,6-b*]indoles the new 4-thiazolidinones have been synthesized. Seven synthesized compounds were tested for their anticancer activity in NCI60 cell lines.

Keywords: synthesis, triazinoindoles, 4-thiazolidinones, anticancer activity.

1. Introduction

The chemistry of isatin derivatives is particularly interesting because of their variety of biological activities and potential application in medicinal chemistry [1]. The synthesis of 1,2,4-triazino[*5*,6-*b*]indoles is a promising direction of isatin modification considering their antifungal [2], antiviral [3, 4], and antihypertensive [5] properties. Recently, the antitumor activity evaluation has become a privileged direction of the mentioned compounds investigation [6]. Thus, antitumor agent *Inauhzin* was identified as an inhibitor of SIRT1 activity and suppressor of tumour growth through activation of p53 [7] (Fig. 1).

On the other hand our previous studies allowed us to identify the high antitumor activity of 4-thiazolidinone conjugates with pyrazoline, benzothiazole cycles [8-16] as well as indole-thiazolidinone hybrids [17, 18].

These observations have prompted us to synthesized new 3*S*-substituted triazino[*5,6-b*]indoles and thiazolidinone-triazinoindole hybrids with the hope of discovering active compounds that would elicit anticancer activity.

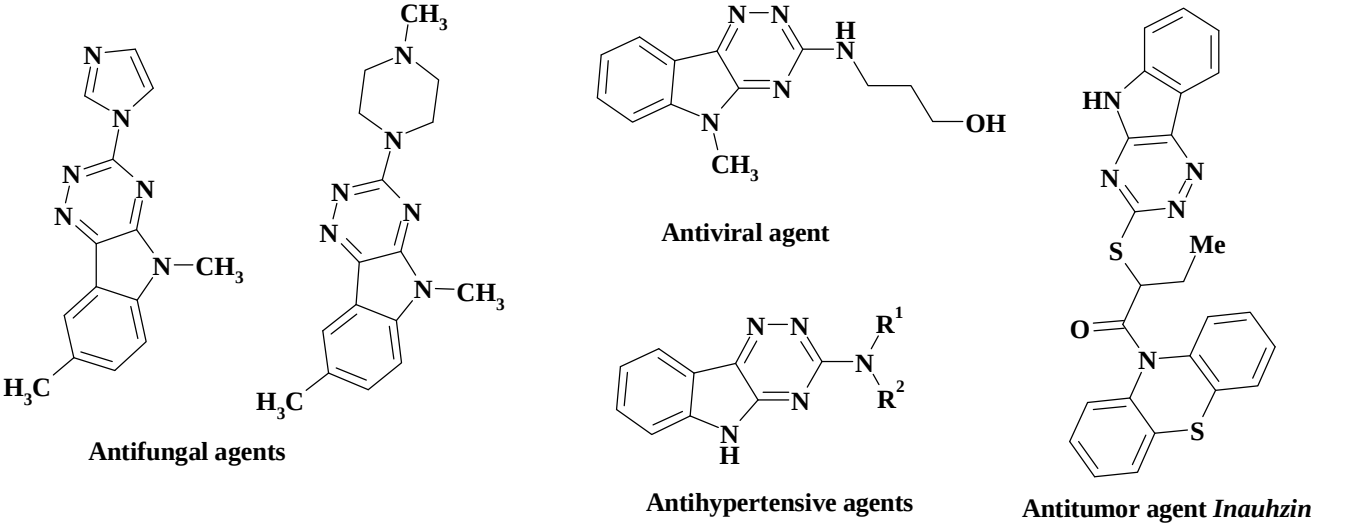


Fig. 1. Biological activity of 1,2,4-triazino[*5,6-b*]indoles

2. Experimental

2.1. Materials and Methods

The starting 3-mercapto-1,2,4-triazino[*5,6-b*]indoles [3-5] were obtained according to the methods described previously.

Melting points were measured in open capillary tubes on a BŰCHI B-545 melting point apparatus and are uncorrected. The elemental analyses (C, H, N) were performed using the Perkin-Elmer 2400 CHN analyzer. Analyses indicated by the symbols of the elements or functions were within ± 0.4 % of the theoretical values. The ^{1}H NMR spectra were recorded on Varian Gemini 300 MHz in DMSO-d_6 or DMSO-d_6+CCl_4 mixture using tetramethylsilane (TMS) as an internal standard. Chemical shifts are reported in ppm units with the use of δ scale.

2.2. Chemistry

General procedure for synthesis of 3S-substituted 1,2,4-triazino[5,6-b]indoles (**2.1**-**2.14**). A suspension of compound **1.1**-**1.3** (3 mmol) and potassium hydroxide (3 mmol) was stirred at r.t. during 5 min, later ethyl chloroacetate or appropriate 2-chloro-*N*-arylacetamide (3.3 mmol) was added and the mixture was refluxed for 5 h in EtOH (10 ml). The obtained powders were filtered off, washed with ethanol and recrystallized with DMF : ethanol (1:2) mixtures.

(1,2,4-Triazino[5,6-b]indole-3-ylsulfanyl)-acetic acid ethyl ester (**2.1**). Yield 78 %, mp 515–517 K. ^{1}H NMR (300 MHz, DMSO-d_6+CCl_4): *d* 12.48 (s, 1H, NH), 8.28 (d, 1H, *J* = 7.7 Hz, arom), 7.61 (t, 1H, *J* = 7.5 Hz, arom), 7.53 (d, 1H, *J* = 7.9 Hz, arom), 7.37 (d, 1H, *J* = 7.4 Hz, arom), 4.10 (s, 2H, SCH_2), 3.73 (br.s, 2H, OCH_2), 2.97 (br.s, 3H, $CH_2\underline{CH_3}$). Calc. for $C_{13}H_{12}N_4O_2S$: C, 54.15; H, 4.20; N, 19.43; Found: C, 54.38; H, 4.41; N, 19.64 %.

2-(1,2,4-Triazino[5,6-b]indole-3-ylsulfanyl)-N-phenylacetamide (**2.2**). Yield 83 %, mp 573–575 K. ^{1}H NMR (300 MHz, DMSO-d_6+CCl_4): *d* 12.63 (s, 1H, NH, indole), 10.40 (s, 1H, CONH), 8.30 (d, 1H, *J* = 7.7 Hz, arom), 7.57-7.73 (m, 4H, arom), 7.45 (t, 1H, *J* = 7.8 Hz, arom), 7.33 (t, 2H, *J* = 8.2 Hz, arom), 7.07 (t, 1H, *J* = 7.3 Hz, arom), 4.29 (s, 2H, SCH_2). Calc. for $C_{17}H_{13}N_5OS$: C, 60.88; H, 3.91; N, 20.88; Found: C, 67.11; H, 4.12; N, 21.09 %.

2-(1,2,4-Triazino[5,6-b]indole-3-ylsulfanyl)-N-(2-trifluoromethylphenyl)acetamide (**2.3**). Yield 86 %, mp 527–529 K. Calc. for $C_{18}H_{12}F_3N_5OS$: C, 53.60; H, 3.00; N, 17.36; Found: C, 53.94; H, 3.34; N, 17.68 %.

2-(1,2,4-Triazino[5,6-b]indole-3-ylsulfanyl)-N-(4-acetylphenyl)acetamide (**2.4**). Yield 76 %, mp 523–525 K. ^{1}H NMR (300 MHz, DMSO-d_6+CCl_4): *d* 12.60 (s, 1H, NH, indole), 10.74 (s, 1H, CONH), 8.29 (d, 1H, *J* = 7.7 Hz, arom), 7.95 (d, 2H, *J* = 8.4 Hz, arom), 7.77 (d, 2H, *J* = 8.4 Hz, arom), 7.68 (t, 1H, *J* = 7.7 Hz, arom), 7.57 (d, 1H, *J* = 7.9 Hz, arom), 7.42 (t, 1H, *J* = 7.3 Hz, arom), 4.34 (s, 2H, SCH_2), 2.54 (s, 3H, CH_3). Calc. for $C_{19}H_{15}N_5O_2S$: C, 60.47; H, 4.01; N, 18.56; Found: C, 60.68; H, 4.23; N, 18.78 %.

2-(1,2,4-Triazino[5,6-b]indole-3-ylsulfanyl)-N-(4-sulfamoylphenyl)acetamide (**2.5**). Yield 80 %, mp 567–569 K. ^{1}H NMR (300 MHz, DMSO-d_6+CCl_4): *d* 12.62 (s, 1H, NH, indole), 10.75 (s, 1H, CONH), 8.30 (d, 1H, *J* = 7.6 Hz, arom), 7.79 (br.s, 4H, arom), 7.69 (t, 1H, *J* = 7.2 Hz, arom), 7.58 (d, 1H, *J* = 8.1 Hz, arom), 7.43 (t, 1H, *J* = 7.2 Hz, arom), 7.29 (s, 2H, NH_2), 4.32 (s, 2H, SCH_2). Calc. for $C_{17}H_{14}N_6O_3S_2$: C, 49.27; H, 3.40; N, 20.28; Found: C, 49.03; H, 3.18; N, 20.03 %.

2-(8-Bromo-1,2,4-triazino[5,6-b]indole-3-ylsulfanyl)acetamide (**2.6**). Yield 88 %, mp > 613 K. Calc. for $C_{11}H_8BrN_5OS$: C, 39.07; H, 2.38; N, 20.71; Found: C, 39.41; H, 2.54; N, 20.95 %.

2-(8-Bromo-1,2,4-triazino[5,6-b]indole-3-ylsulfanyl)-N-(2-methoxyphenyl)acetamide (**2.7**). Yield 85 %, mp 533–535 K. ^{1}H NMR (400 MHz, DMSO-d_6+CCl_4): *d* 12.85 (s, 1H, NH, indole), 9.66 (s, 1H, CONH), 8.48 (s, 1H, arom), 8.06 (d, 1H, *J* = 8.2 Hz, arom), 7.84 (d, 1H, *J* = 8.5 Hz, arom), 7.54 (d, 1H, *J* = 8.5 Hz, arom), 7.00-7.06 (m, 2H, arom), 6.90 (t, 1H, *J* = 8.0 Hz, arom), 4.29 (s, 2H, SCH_2), 3.77 (s, 3H, OCH_3). Calc. for $C_{18}H_{14}BrN_5O_2S$: C, 48.66; H, 3.18; N, 15.76; Found: C, 48.42; H, 2.96; N, 15.53 %.

2-(8-Bromo-1,2,4-triazino[5,6-b]indole-3-ylsulfanyl)-N-(3-methylphenyl)acetamide (**2.8**). Yield 88 %, mp 585–587 K. ^{1}H NMR (300 MHz, DMSO-d_6+CCl_4): *d* 12.76 (br.s, 1H, NH, indole), 10.29 (s, 1H, CONH), 8.45 (s, 1H, arom), 7.82 (d, 1H, *J* = = 8.6 Hz, arom), 7.54 (d, 1H, *J* = 8.6 Hz, arom), 7.46 (s, 1H, arom), 7.39 (d, 1H, *J* = 8.3 Hz, arom), 7.20 (t, 1H, *J* = 7.7 Hz, arom), 6.88 (d, 1H, *J* = 7.5 Hz, arom), 4.28 (s, 2H, SCH_2), 2.27 (s, 3H, CH_3). Calc. for $C_{18}H_{14}BrN_5OS$: C, 50.48; H, 3.29; N, 16.35; Found: C, 50.74; H, 3.52; N, 16.54 %.

2-(8-Bromo-1,2,4-triazino[5,6-b]indole-3-ylsulfanyl)-N-(4-chlorophenyl)acetamide (**2.9**). Yield 77 %, mp 597–599 K. ^{1}H NMR (300 MHz, DMSO-d_6+CCl_4): *d* 12.75 (s, 1H, NH, indole), 10.54 (s, 1H, CONH), 8.45 (s, 1H, arom), 7.82 (d, 1H, *J* = 8.6 Hz, arom), 7.65 (d, 2H, *J* = 8.8 Hz, arom), 7.53 (d, 1H, *J* = 8.6 Hz, arom), 7.38 (d, 2H, *J* = 8.8 Hz, arom), 4.29 (s, 2H, SCH_2). Calc. for $C_{17}H_{11}BrClN_5OS$: C, 45.50; H, 2.47; N, 15.61; Found: C, 45.18; H, 2.16; N, 15.29 %.

2-(8-Bromo-1,2,4-triazino[5,6-b]indole-3-ylsulfanyl)-N-(2-chloro-5-trifluorophenyl)acetamide

(**2.10**). Yield 75 %, mp 531–533 K. Calc. for $C_{18}H_{10}BrClF_3N_5OS$: C, 41.84; H, 1.95; N, 13.55; Found: C, 41.52; H, 1.62; N, 13.18 %.

2-(8-Chloro-1,2,4-triazino[5,6-b]indole-3-ylsulfanyl)-N-(2-methoxyphenyl)acetamide (**2.11**). Yield 78 %, mp 529–531 K. ^{1}H NMR (300 MHz, DMSO-d_6+CCl_4): *d* 12.90 (s, 1H, NH, indole), 9.69 (s, 1H, CONH), 8.35 (s, 1H, arom), 8.07 (d, 1H, *J* = 7.0 Hz, arom), 7.71 (d, 1H, *J* = 8.6 Hz, arom), 7.59 (d, 1H, *J* = 8.5 Hz, arom), 7.00-7.06 (m, 2H, arom), 6.91 (t, 1H, *J* = 6.4 Hz, arom), 4.30 (s, 2H, SCH_2), 3.78 (s, 3H, OCH_3). Calc. for $C_{18}H_{14}ClN_5O_2S$: C, 54.07; H, 3.53; N, 17.51; Found: C, 54.38; H, 3.77; N, 17.84 %.

2-(8-Chloro-1,2,4-triazino[5,6-b]indole-3-ylsulfanyl)-N-(3-methylphenyl)acetamide (**2.12**). Yield 85 %, mp 541–543 K. ^{1}H NMR (300 MHz, DMSO-d_6+CCl_4): *d* 12.80 (br.s, 1H, NH, indole), 10.33 (s, 1H, CONH), 8.33 (s, 1H, arom), 7.71 (d, 1H, *J* = 8.7 Hz, arom), 7.58 (d, 1H, *J* = 8.7 Hz, arom), 7.48 (s, 1H, arom), 7.41 (d, 1H, *J* = 8.2 Hz, arom), 7.20 (t, 1H, *J* = 7.6 Hz, arom), 6.88 (d, 1H, *J* = 6.7 Hz, arom), 4.29 (s, 2H, SCH_2), 2.29 (s, 3H, CH_3). Calc. for $C_{18}H_{14}ClN_5OS$: C, 56.32; H, 3.68; N, 18.24; Found: C, 56.53; H, 3.94; N, 18.57 %.

2-(8-Chloro-1,2,4-triazino[5,6-b]indole-3-ylsulfanyl)-N-(4-chlorophenyl)acetamide (**2.13**). Yield 81 %, mp 553–555 K. ^{1}H NMR (300 MHz, DMSO-d_6+CCl_4): *d* 12.76 (s, 1H, NH, indole), 10.52 (s, 1H, CONH), 8.31 (s, 1H, arom), 7.57-7.67 (m, 4H, arom), 7.36-7.39 (m, 2H, arom), 4.29 (s, 2H, SCH_2). Calc. for $C_{17}H_{11}Cl_2N_5OS$: C, 50.51; H, 2.74; N, 17.32; Found: C, 50.74; H, 2.95; N, 17.53 %.

2-(8-Chloro-1,2,4-triazino[5,6-b]indole-3-ylsulfanyl)-N-(4-sulfamoylphenyl)acetamide (**2.14**). Yield 86 %, mp 563–565 K. ^{1}H NMR (300 MHz, DMSO-d_6+CCl_4): *d* 12.81 (s, 1H, NH, indole), 10.77 (s, 1H, CONH), 8.32 (s, 1H, arom), 7.56-7.80 (m, 6H, arom), 7.30 (s, 2H, NH_2), 4.33 (s, 2H, SCH_2). Calc. for $C_{17}H_{13}ClN_6O_3S_2$: C, 45.49; H, 2.92; N, 18.72; Found: C, 45.24; H, 2.65; N, 18.51 %.

General procedure for synthesis 3-(1,2,4-triazino[5,6-b]indol-3-ylamino)-2-thioxothiazolidin-4-ones (**4.1-4.2**). A mixture of 50 mmol (1,2,4-triazino[5,6-b]indol-3yl)hydrazine **3.1** or **3.2** and 50 mmol trithiocarbonyl diglycolic acid was refluxed in 30 ml of ethanol during 5 h. After cooling the reaction mixture was poured into cold water and the solid mass which separated out was filtered, dried and recrystallized in turn with AcOH.

3-(1,2,4-Triazino[5,6-b]indol-3-ylamino)-2-thioxothiazolidin-4-ones (**4.1**). Yield 66 %, mp > 473 K. Calc. for $C_{12}H_8N_6OS_2$: C, 45.56; H, 2.55; N, 26.56; Found: C, 45.79; H, 2.32; N, 26.70 %.

3-(8-Chloro-1,2,4-triazino[5,6-b]indol-3-ylamino)-2-thioxothiazolidin-4-ones (**4.2**). Yield 59 %, mp > 473 K. Calc. for $C_{12}H_7ClN_6OS_2$: C, 41.09; H, 2.01; N, 23.96; Found: C, 40.88; H, 2.25; N, 24.12 %.

General procedure for synthesis of 5-ylidene-3-(1,2,4-triazino[5,6-b]indol-3-ylamino)-2-thioxothiazolidin-4-ones (**5.1-5.10**, **6.1-6.4**). A mixtures of compound **4.1** or **4.2** (3 mmol), appropriate aldehyde or isatin (3.3 mmol) and anhydrous sodium acetate (3 mmol) were refluxed for 2 h in glacial acetic acid (10 ml). The obtained powders were filtered off, washed with methanol and recrystallized with DMF:ethanol (1:2) mixtures.

5-(3-Bromobenzylidene)-3-(1,2,4-triazino[5,6-b]indol-3-ylamino)-2-thioxothiazolidin-4-one (**5.1**). Yield 78 %, mp 593–595 K. Calc. for $C_{19}H_{11}BrN_6OS_2$: C, 47.21; H, 2.29; N, 17.39; Found: C, 47.54; H, 2.58; N, 17.67 %.

5-(4-Bromobenzylidene)-3-(1,2,4-triazino[5,6-b]indol-3-ylamino)-2-thioxothiazolidin-4-one (**5.2**). Yield 82 %, mp > 613 K. ^{1}H NMR (300 MHz, DMSO-d_6+CCl_4): *d* 12.33 (s, 1H, NH, indole), 11.90 (s, 1H, NH), 8.19-8.21 (m, 2H, =CH, arom), 7.66 (br.s, 4H, arom), 7.56 (t, 1H, *J* = 7.6 Hz, arom), 7.49 (d, 1H, *J* = 7.8 Hz, arom), 7.35 (t, 1H, *J* = 7.4 Hz, arom). Calc. for $C_{19}H_{11}BrN_6OS_2$: C, 47.21; H, 2.29; N, 17.39; Found: C, 47.42; H, 2.41; N, 17.52 %.

5-(4-Chlorobenzylidene)-3-(1,2,4-triazino[5,6-b]indol-3-ylamino)-2-thioxothiazolidin-4-one (**5.3**). Yield 82 %, mp > 613 K. ^{1}H NMR (300 MHz, DMSO-d_6+CCl_4): *d* 12.27 (s, 1H, NH, indole), 11.81 (s, 1H, NH), 8.18-8.23 (m, 2H, =CH, arom), 7.73 (d, 2H, *J* = 8.1 Hz, arom), 7.49-7.57 (m, 4H, arom), 7.36 (t, 1H, *J* = 7.3 Hz, arom). Calc. for $C_{19}H_{11}ClN_6OS_2$: C, 51.99; H, 2.53; N, 19.15; Found: C, 52.23; H, 2.77; N, 19.34 %.

5-(4-Fluorobenzylidene)-3-(1,2,4-triazino[5,6-b]indol-3-ylamino)-2-thioxothiazolidin-4-one (**5.4**). Yield 75 %, mp > 613 K. ^{1}H NMR (300 MHz, DMSO-d_6+CCl_4): *d* 12.25 (s, 1H, NH, indole), 11.74 (s, 1H, NH), 8.26 (s, 1H, =CH), 8.20 (d, 1H, *J* = 7.6 Hz, arom), 7.75-7.79 (m, 2H, arom), 7.57 (t, 1H, *J* = 7.5 Hz, arom), 7.49 (d, 1H, *J* = 7.7 Hz, arom), 7.27-7.38 (m, 3H, arom). Calc. for $C_{19}H_{11}FN_6OS_2$: C, 54.02; H, 2.62; N, 19.89; Found: C, 54.36; H, 2.95; N, 20.09 %.

5-(4-Methoxybenzylidene)-3-(1,2,4-triazino[5,6-b]indol-3-ylamino)-2-thioxothiazolidin-4-one (**5.5**). Yield 80 %, mp 553–555 K. Calc. for $C_{20}H_{14}N_6O_2S_2$: C, 55.29; H, 3.25; N, 19.34; Found: C, 55.48; H, 3.57; N, 19.58 %.

5-(4-Dimethylaminobenzylidene)-3-(1,2,4-triazino[5,6-b]indol-3-ylamino)-2-thioxothiazolidin-4-one (**5.6**). Yield 69 %, mp 563–565 K. ^{1}H NMR (300 MHz, DMSO-d_6+CCl_4): *d* 12.48 (s, 1H, NH, indole),

11.16 (s, 1H, NH), 8.23 (br.s, 1H, =CH), 7.84 (br.s, 1H, arom), 7.39-7.59 (m, 5H, arom), 6.89 (br.s, 2H, arom), 3.09 (s, 6H, 2*CH_3). Calc. for $C_{21}H_{17}N_7OS_2$: C, 56.36; H, 3.83; N, 21.91; Found: C, 56.58; H, 4.03; N, 22.13 %.

5-(2,6-Dichlorobenzylidene)-3-(1,2,4-triazino[5,6-b]indol-3-ylamino)-2-thioxothiazolidin-4-one (**5.7**). Yield 85 %, mp 593–594 K. ^{1}H NMR (300 MHz, DMSO-d_6+CCl_4): *d* 12.31 (s, 1H, NH, indole), 12.08 (s, 1H, NH), 8.43 (br.s, 1H, =CH), 8.21 (d, 1H, *J* = 7.7 Hz, arom), 7.37-7.61 (m, 6H, arom). Calc. for $C_{19}H_{10}Cl_2N_6OS_2$: C, 48.21; H, 2.13; N, 17.75; Found: C, 47.98; H, 1.87; N, 17.53 %.

5-(4-Bromobenzylidene)-3-(8-chloro-1,2,4-triazino[5,6-b]indol-3-ylamino)-2-thioxothiazolidin-4-one (**5.8**). Yield 72 %, mp 618–620 K. ^{1}H NMR (300 MHz, DMSO-d_6+CCl_4): *d* 12.44 (s, 1H, NH, indole), 11.95 (s, 1H, NH), 8.23 (br.s, 1H, =CH), 8.21 (s, 1H, arom), 7.66 (br.s, 4H, arom), 7.58 (d, 1H, *J* = 8.5 Hz, arom), 7.50 (d, 1H, *J* = 8.5 Hz, arom). Calc. for $C_{19}H_{10}BrClN_6OS_2$: C, 44.07; H, 1.95; N, 16.23; Found: C, 44.28; H, 2.17; N, 16.56 %.

5-(4-Chlorobenzylidene)-3-(8-chloro-1,2,4-triazino[5,6-b]indol-3-ylamino)-2-thioxothiazolidin-4-one (**5.9**). Yield 70 %, mp > 623 K. ^{1}H NMR (300 MHz, DMSO-d_6+CCl_4): *d* 12.44 (s, 1H, NH, indole), 11.96 (s, 1H, NH), 8.24 (br.s, 1H, =CH), 8.20 (s, 1H, arom), 7.73 (d, 2H, *J* = 8.4 Hz, arom), 7.48-7.56 (m, 4H, arom). Calc. for $C_{19}H_{10}Cl_2N_6OS_2$: C, 48.21; H, 2.13; N, 17.75; Found: C, 48.52; H, 2.45; N, 18.02 %.

5-(4-Nitrobenzylidene)-3-(8-chloro-1,2,4-triazino[5,6-b]indol-3-ylamino)-2-thioxothiazolidin-4-one (**5.10**). Yield 82 %, mp 613–616 K. ^{1}H NMR (300 MHz, DMSO-d_6+CCl_4): *d* 12.53 (br.s, 1H, NH, indole), 12.24 (br.s, 1H, NH), 8.33 (d, 2H, *J* = 5.0 Hz, arom), 8.29 (br.s, 1H, =CH), 7.93-7.96 (m, 2H, arom), 7.69 (d, 1H, *J* = 8.3 Hz, arom), 7.49 (d, 1H, *J* = 8.7 Hz, arom). Calc. for $C_{19}H_{10}ClN_7O_3S_2$: C, 47.16; H, 2.08; N, 20.26; Found: C, 46.96; H, 1.82; N, 20.01 %.

3-[3-(1,2,4-Triazino[5,6-b]indol-3-ylamino)-4-oxo-2-thioxothiazolidin-5-ylidene]-1,3-dihydroindol-2-one (**6.1**). Yield 73 %, mp > 633 K. ^{1}H NMR (300 MHz, DMSO-d_6+CCl_4): *d* 13.42 (br.s, 1H, NH, isatin), 12.61 (br.s, 1H, NH, indole), 11.23 (br.s, 1H, NH), 8.27 (d, 1H, *J* = 7.8 Hz, arom), 7.55-7.64 (m, 3H, arom), 7.32-7.43 (m, 2H, arom), 7.13 (t, 1H, *J* = 7.6 Hz, arom), 6.97 (d, 1H, *J* = 7.7 Hz, arom). Calc. for $C_{20}H_{11}N_7O_2S_2$: C, 53.92; H, 2.49; N, 22.01; Found: C, 54.21; H, 2.71; N, 22.28 %.

3-[3-(1,2,4-Triazino[5,6-b]indol-3-ylamino)-4-oxo-2-thioxothiazolidin-5-ylidene]-5-bromo-1,3-dihydroindol-2-one (**6.2**). Yield 78 %, mp > 633 K. ^{1}H NMR (300 MHz, DMSO-d_6+CCl_4): *d* 13.35 (br.s, 1H, NH, isatin), 12.61 (br.s, 1H, NH, indole), 11.36 (br.s, 1H, NH), 8.26 (d, 1H, *J* = 7.5 Hz, arom), 7.55-7.68 (m, 3H, arom), 7.48 (d, 1H, *J* = 8.4 Hz, arom), 7.39 (t, 1H, *J* = 8.1 Hz, arom), 6.92 (d, 1H, *J* = 8.4 Hz, arom). Calc. for $C_{20}H_{10}BrN_7O_2S_2$: C, 45.81; H, 1.92; N, 18.70; Found: C, 46.03; H, 2.12; N, 18.96 %.

3-[3-(1,2,4-Triazino[5,6-b]indol-3-ylamino)-4-oxo-2-thioxothiazolidin-5-ylidene]-5-chloro-1,3-dihydroindol-2-one (**6.3**). Yield 82 %, mp > 633 K. ^{1}H NMR (300 MHz, DMSO-d_6+CCl_4): *d* 13.37 (br.s, 1H, NH, isatin), 12.58 (br.s, 1H, NH, indole), 11.33 (br.s, 1H, NH), 8.27 (d, 1H, *J* = 7.5 Hz, arom), 7.55-7.67 (m, 3H, arom), 7.50 (br.s, 1H, arom), 7.34-7.43 (m, 2H, arom), 6.97 (d, 1H, *J* = 8.1 Hz, arom). Calc. for $C_{20}H_{10}ClN_7O_2S_2$: C, 50.05; H, 2.10; N, 20.43; Found: C, 50.36; H, 2.43; N, 20.76 %.

3-[3-(1,2,4-Triazino[5,6-b]indol-3-ylamino)-4-oxo-2-thioxothiazolidin-5-ylidene]-5-chloro-1,3-dihydroindol-2-one (**6.4**). Yield 75 %, mp > 623 K. ^{1}H NMR (300 MHz, DMSO-d_6+CCl_4): δ 13.40 (br.s, 1H, NH, isatin), 12.59 (br.s, 1H, NH, indole), 11.14 (br.s, 1H, NH), 8.27 (d, 1H, *J* = 7.6 Hz, arom), 7.64 (t, 1H, *J* = 7.6 Hz, arom), 7.57 (d, 1H, *J* = 7.6 Hz, arom), 7.39-7.44 (m, 2H, arom), 7.14 (d, 1H, *J* = 7.6 Hz, arom), 6.85 (d, 1H, *J* = 7.9 Hz, arom), 2.34 (s, 3H, CH_3). Calc. for $C_{21}H_{13}N_7O_2S_2$: C, 54.89; H, 2.85; N, 21.34; Found: C, 55.12; H, 3.02; N, 21.56 %.

2.3. Primary Anticancer Assay

Primary anticancer assay was performed at approximately sixty human tumor cell lines panel derived from nine neoplastic diseases, in accordance with the protocol of the Drug Evaluation Branch, National Cancer Institute, Bethesda [20-22]. The tested compounds were added to the culture at a single concentration (10^{-5} M) and the cultures were incubated for 48 h. End point determinations were made with a protein binding dye, sulforhodamine B (SRB). Results for each tested compound were reported as the percent of growth of the treated cells when compared to the untreated control cells. The percentage growth was evaluated spectrophotometrically versus controls not treated with test agents.

3. Results and Discussion

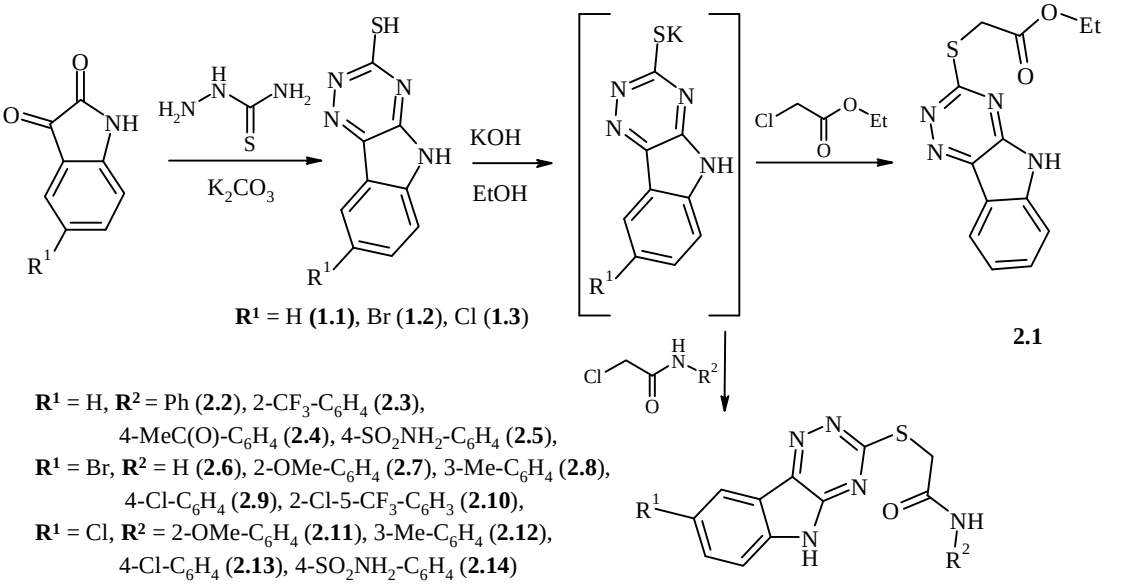
3.1. Chemistry

The general methods for synthesis of target *S*-substituted triazino[*5,6-b*]indoles and thiazolidinone-triazinoindole conjugates are depicted in Scheme 1 and 2.

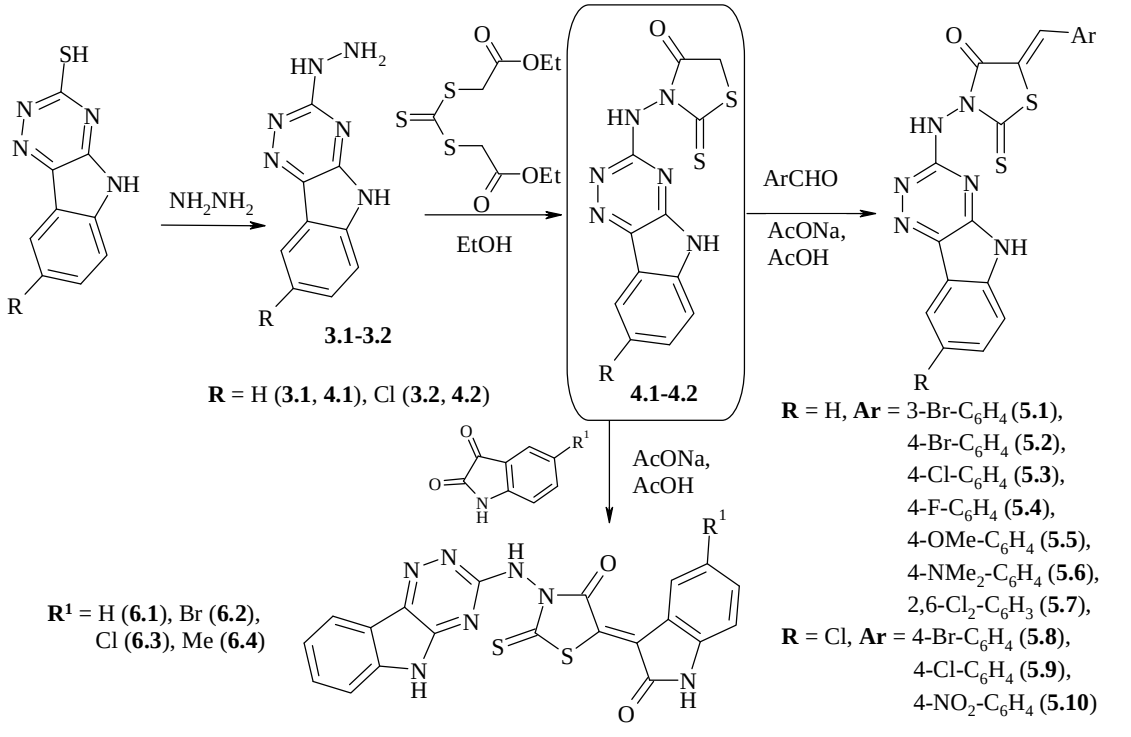
Synthesis of 3-mercapto-1,2,4-triazino[*5,6-b*]indoles **1.1-1.3** was performed *via* the reaction of 5-*R*-

isatins and thiosemicarbazide at the presence of K_2CO_3 in water medium [3-5]. Compounds **1.1-1.3** were utilized in *S*-alkylation reaction with ethyl chloroacetate or several *N*-arylchloroacetamides, thus the corresponding 1,2,4-triazino[*5,6-b*]indole derivatives **2.1-2.14** have been obtained (Scheme 1).

3-Mercapto-1,2,4-triazino[*5,6-b*]indoles were utilized in reaction with hydrazine producing corresponding **3.1-3.2** as precursors for synthesis of new 4-thiazolidinones with 1,2,4-triazino[*5,6-b*]indole moiety in 3 position (**4.1-4.2**). Synthesized methylene active derivatives **4.1-4.2** readily reacted with aromatic aldehydes and isatins to produce 5-ylidenederivatives **5.1-5.5** and **6.1-6.4** *via* Knoevenagel condensation procedure (medium – acetic acid, catalyst – fused sodium acetate).



Scheme 1



Scheme 2

The characterization data of synthesized *S*-substituted 1,2,4-triazino[*5,6-b*]indoles and novel heterocyclic substituted thiazolidones are presented in experimental part. Analytical and spectral data (^{1}H NMR) confirmed the structure of the synthesized compounds.

The protons of the methylene group (CH_2CO) in the ^{1}H NMR spectra of synthesized compounds **2.1-2.14** appear as singlet at δ~4,30 ppm, NH proton of indole cycle shows the broad singlet at δ~12,43-12,84 ppm. The chemical shift for the methylidene group of 5-arylidenederivatives **5.1-5.10** is insignificantly displaced in a weak magnetic field, *δ*~8.22 ppm and clearly indicated that only *Z*-isomers were obtained in Knoevenagel reaction of indolotriazine substituted thiazolidinones with aromatic aldehydes [19].

3.2. Evaluation of Anticancer Activity *in vitro*

Some new indolotriazine derivatives (**2.2, 2.4-2.9**) were submitted and evaluated at single concentration of 10^{-5} M towards panel of approximately sixty cancer cell lines. The human tumor cell lines were derived from nine different cancer types: leukemia, melanoma, lung, colon, CNS, ovarian, renal, prostate, and breast cancers [20-22]. The compounds were added at a single concentration and the cell culture was incubated for 48 h. End point determinations were made with a protein binding dye, sulforhodamine B (SRB). The results for each compound are reported as the percent growth (GP, %) of treated cells when compared to untreated control cells (Table 1). The range of growth % shows the lowest and the highest growth % found among different cancer cell lines.

The tested compounds displayed low antitumor activity with average values GP from 86.14 (**2.7**) to 98.97 (**2.5**), excepted **2.8** (GP = 55.41 %), which demonstrated cytostatic effect (GP ≤ 50 %) on 23 cell lines (Table 1).

Finally, compound **2.8** was selected in advanced assay against a panel of approximately sixty tumor cell

Table 1

Anticancer screening data in concentration 10^{-5}M

Comp	60 cell lines assay in 1 dose 10^{-5} M conc				Active (selected for 5-dose 60 cell lines assay)
	Mean growth, %	Range of growth, %	The most sensitive cell lines	Growth of the most sensitive cell lines, %	
2.2	88.89	55.46 to 112.91	UO-31 (renal cancer)	55.46	Inactive
2.4	93.30	63.05 to 117.24	T-47D (breast cancer)	63.05	Inactive
2.5	98.97	69.89 to 133.44	UO-31 (renal cancer)	69.89	Inactive
2.6	90.87	37.67 to 114.65	MDA-MB-468 (breast cancer)	37.67	Inactive
2.7	86.14	47.61 to 108.61	T-47D (breast cancer)	47.61	Inactive
2.8*	55.41	3.74 to 103.13	SR (leukemia) A549/ATCC (lung cancer) HOP-62 (lung cancer) NCI-H226 (lung cancer) NCI-H23 (lung cancer) HCT-116 (colon cancer) SF-295 (CNS cancer) SNB-75 (CNS cancer) U251 (CNS cancer) LOX IMVI (melanoma) OVCAR-3 (ovarian cancer) OVCAR-4 (ovarian cancer) OVCAR-8 (ovarian cancer) SK-OV-3 (ovarian cancer) 786-0 (renal cancer) ACHN (renal cancer) CAKI-1 (renal cancer) RXF 393 (renal cancer) TK-10 (renal cancer) UO-31 (renal cancer) PC-3 (prostate cancer) MDA-MB-231/ATCC (breast cancer) T-47D (breast cancer)	34.94 37.57 9.23 39.69 49.13 39.09 43.27 33.01 36.06 3.74 45.51 26.86 34.73 39.22 31.77 35.81 38.93 30.44 42.63 33.68 30.48 47.07 34.10	Active
2.9	88.98	57.09 to 116.11	HCT-116 (colon cancer)	57.09	Inactive

Note: * the most sensitive cell lines with GP value ≤ 50 % are presented

Table 2

The influence of compound 2.8 on the growth of individual tumor cell lines

Disease	Cell line	pGI_{50}	pTGI[a]
MG_MID		5.24	4.65
Leukemia	CCRF-CEM	4.83	NA
Leukemia	HL-60 (TB)	4.58	NA
Leukemia	K-562	4.83	4.39
Leukemia	MOLT-4	4.77	4.18
Leukemia	RPMI-8226	5.07	4.11
Leukemia	SR	5.10	4.47
NSC lung cancer	A549/ATCC	5.43	4.91
NSC lung cancer	HOP-62	5.63	5.26
NSC lung cancer	HOP-92	5.75	5.19
NSC lung cancer	NCI-H226	5.34	4.54
NSC lung cancer	NCI-H322	5.07	NA
NSC lung cancer	NCI-H460	5.16	4.63
NSC lung cancer	NCI-H522	5.17	4.35
Colon cancer	COLO 205	5.11	4.56
Colon cancer	HCC-2998	4.94	NA
Colon cancer	HCT-116	5.46	4.50
Colon cancer	HCT-15	5.01	4.43
Colon cancer	HT29	4.93	4.57
Colon cancer	KM12	5.01	NA
Colon cancer	SW-620	4.94	4.09
CNS cancer	SF-268	5.33	4.77
CNS cancer	SF-295	5.45	4.82
CNS cancer	SF-539	5.30	4.71
CNS cancer	SNB-19	5.16	4.57
CNS cancer	SNB-75	5.70	5.28
CNS cancer	U251	5.50	5.13
Melanoma	LOX IMVI	5.71	5.36
Melanoma	MALME-3M	5.45	4.95
Melanoma	M14	4.96	4.51
Melanoma	MDA-MB-435	4.98	4.54
Melanoma	SK-MEL-2	5.32	4.80
Melanoma	SK-MEL-28	5.33	4.79
Melanoma	SK-MEL-5	5.45	4.61
Melanoma	UACC-257	5.11	4.49
Melanoma	UACC-62	4.97	4.57
Ovarian cancer	IGROV1	5.11	4.68
Ovarian cancer	OVCAR-3	5.10	4.68
Ovarian cancer	OVCAR-4	5.69	5.28
Ovarian cancer	OVCAR-8	5.43	4.82
Ovarian cancer	NCI/ADR-RES	4.94	4.48
Ovarian cancer	SK-OV-3	5.51	5.04
Renal cancer cer	786-0	5.43	4.84
Renal cancer	A498	5.73	5.08
Renal cancer	ACHN	5.48	4.88
Renal cancer	CAKI-1	5.17	4.62
Renal cancer	RXF 393	5.45	4.87
Renal cancer	SN12C	5.18	4.69
Renal cancer	UO-31	5.63	5.16
Prostate cancer	PC-3	5.35	4.74
Prostate cancer	DU-145	5.27	4.74
Breast cancer	MCF7	4.99	4.57
Breast cancer	MDA-MB-231/ATCC	5.59	4.94
Breast cancer	MDA-MB-468	4.97	4.49
Breast cancer	HS 578T	5.52	4.81
Breast cancer	T-47D	5.37	4.75

Note: [a] NA (not active) – value of pTGI is less than 4.00

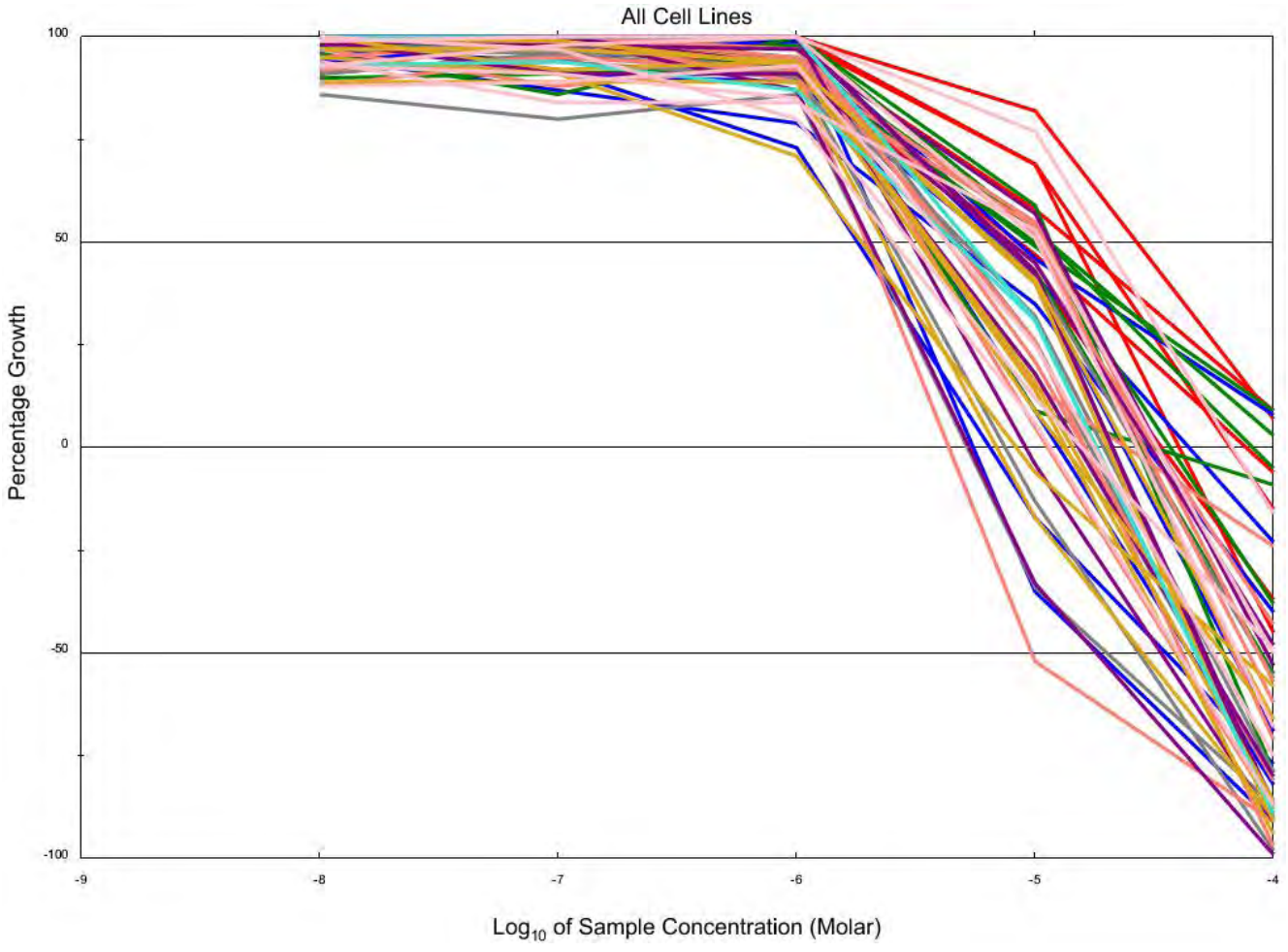


Fig. 2. The influence of compound **2.8** on individual tumor cell lines

lines at 10-fold dilutions of five concentrations (μM): 100, 10, 1, 0.1 and 0.01 [20-22]. Based on the cytotoxicity assays, the antitumor activity dose-response parameters were calculated for experimental agents against each cell line: GI_{50} – molar concentration of the compound that inhibits 50 % net cell growth; TGI – molar concentration of the compound leading to total inhibition (both are presented in Table 2); and LC_{50} – molar concentration of the compound leading to 50 % net cell death.

The tested compound **2.8** showed significant inhibition activity against 43 ($pGI_{50} > 5$) from 55 human tumor cells with average pGI_{50} / pTGI values 5.24 / 4.65 (Table 2). Compound **2.8** demonstrates the highest influence ($pGI_{50} \geq 5.50$) on individual cell lines: HOP-62 and HOP-92 (NSC lung cancer), SNB-75 and U251 (CNS cancer), LOX IMVI (Melanoma), OVCAR-4 and SK-OV-3 (Ovarian cancer), A498 and UO-31 (Renal cancer), as well as MDA-MB-231/ATCC and HS 578T (Breast cancer).

The influence of compound **2.8** on individual tumor cell lines at 10-fold dilutions of five concentrations (μM): 100, 10, 1, 0.1 and 0.01) is depicted in Fig. 2.

Table 3

Anticancer selectivity pattern of the most active compound 2.8 at the GI_{50} (μM) and TGI (μM) levels

Disease	GI_{50}	SI[a]	TGI	SI[b]
Leukemia	13.80	0.4	64.56	0.4
NSC lung cancer	4.37	1.4	19.95	1.3
Colon cancer	8.71	0.7	48.98	0.5
CNS Cancer	3.89	1.6	13.18	1.9
Melanoma	5.62	1.1	18.20	1.4
Ovarian Cancer	5.01	1.2	14.79	1.7
Renal Cancer	3.63	1.7	13.18	1.9
Prostate Cancer	4.89	1.3	18.20	1.4
Breast Cancer	5.13	1.2	19.50	1.3

Notes: [a] selectivity index at the GI_{50} level; [b] selectivity index at the TGI level.

The selectivity index (SI) obtained by dividing the full panel MG-MID (μM) of the compound **2.8** by its individual subpanel MG-MID (μM) was considered as a measure of compound's selectivity. Ratios between 3 and 6 refer to moderate selectivity, ratios greater than 6 indicate high selectivity toward the corresponding cell line, while compounds not meeting either of these criteria are rated non-selective [23]. In this context, the active compound **2.8** do not have selectivity toward any subpanel at both the GI_{50} and TGI levels (selectivity indexes are less than 2.0) (Table 3).

4. Conclusions

In the present paper *S*-substituted 1,2,4-triazino[*5,6-b*]indoles and 4-thiazolidinone based conjugates with triazinoindole moiety are described. Antitumor activity assay of seven synthesized compounds allowed us to identify a highly active compound **2.8** which demonstrated significant inhibition activity against 43 ($pGI_{50} > 5$) out of 55 human tumor cells with average pGI_{50} / pTGI values 5.24 / 4.65.

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СИНТЕЗ 3*S*-ЗАМІЩЕНИХ ТРІАЗИНО[*5,6-B*]ІНДОЛІВ ТА 4-ТІАЗОЛІДИНОН-ТРІАЗИНО[*5,6-b*]ІНДОЛЬНИХ ГІБРИДНИХ МОЛЕКУЛ З ПРОТИПУХЛИННОЮ АКТИВНІСТЮ

Анотація. *Здійснено синтез та скринінг протипухлинної активності 1,2,4-тріазино[5,6-b]індолів. При взаємодії 3-меркапто-1,2,4-тріазино[5,6-b]індолів та N-арилхлороацетамідів одержано 3S-заміщені 1,2,4-тріазино[5,6-b]індоли. На основі 3-гідразино-1,2,4-тріазино[5,6-b]індолів отримано групу нових похідних 4-тіазолідинону. Вивчення протипухлинної активності семи синтезованих сполук здійснено на 60 лініях ракових клітин згідно протоколу NCI.*

Ключові слова: *синтез, тріазиноіндоли, 4-тіазолідинони, протипухлинна активність.*