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SYNTHESIS AND ANTICANCER ACTIVITY OF ISATIN, OXADIAZOLE AND 4-THIAZOLIDINONE BASED CONJUGATES

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Abstract. Following the *N*-alkylation reaction of starting 2-chloro-*N*-(5-aryl-1,3,4-oxadiazol-2-yl)-acetamides **1a-c** with 2,4-thiazolidinedione or 5-sudstituted isatins the corresponding non-condensed oxadiazole derivatives with thiazolidine **2a-c** or isatin **4a-h** fragments were synthesized. The obtained compounds have been used in Knoevenagel condensation with 5*R*-isatin (for **2a-c**) or 4-thiazolidinone derivatives (for **4a-h**) for synthesis of the appropriate 5-ylidenederivatives **3a-g, 5a-k** and **6a-d**. Anticancer activity of eight synthesized compounds was evaluated toward 60 human tumor cell lines panel in National Cancer Institute.

Keywords: 4-thiazolidinone, isatin, 1,3,4-oxadiazole, alkylation, Knoevenagel condensation, anticancer activity.

1. Introduction

1*H*-indole-2,3-dione [1] and thiazolidine [2] are the privileged scaffolds in the modern medicinal chemistry that have a broad spectrum of the biological activity including antimicrobial [7], antioxidant [4], antimycobacterial [5], antidepressant [6], anticonvulsant [6] effects. A considerable interest has been focused on the anticancer activity of isatin [7] and thiazolidine [8, 9] derivatives. It is known that the combination of different bioactive fragments with complementary pharmacophoric functions or with different mechanisms of the action often showed synergistic effects. Thus, among isatin based conjugates with thiazolidinone [10-14], oxadiazole [15] or benzothiazole [16, 17] the promising anticancer agents were identified.

Combination of 4-thiazolidinone and isatin templates with oxadiazole moiety is a perspective approach for drug-like molecules build-up, considering that various oxadiazoles have a wide spectrum of the pharmacological activities. Furthermore, certain 1,3,4-oxadiazole derivatives were reported to posses anti-inflammatory [18], antimicrobial [19], antimycobacterial [20], immunosuppressive [21] and anticancer [22-24] activities. Therefore, in the present work we planned to conjugate the oxadiazole ring with isatin and 4-thiazolidinone scaffolds to combine the benefits of their effects and to give a compact structure with expected anticancer activity. The structural variations were explored by attachment the oxadiazole moiety to the thiazolidine *N*3 and/or isatin *N*1 positions (Fig. 1).

2. Experimental

2.1. Materials and Methods

All starting materials were purchased from Merck and Sigma-Aldrich. Melting points were measured in open capillary tubes on BUCHI B-545 melting point apparatus and are uncorrected. The elemental analyses (C, H, N) were performed using the Perkin-Elmer 2400 CHN analyzer and were within ± 0.4 % of the theoretical values. ^{1}H NMR spectra were recorded on Varian Gemini spectrometer at 300 MHz using a mixture of DMSO-d_6+CCl_4 as a solvent and tetramethylsilane (TMS) as an internal standard. Chemical shifts values are reported in ppm units with use of δ scale.

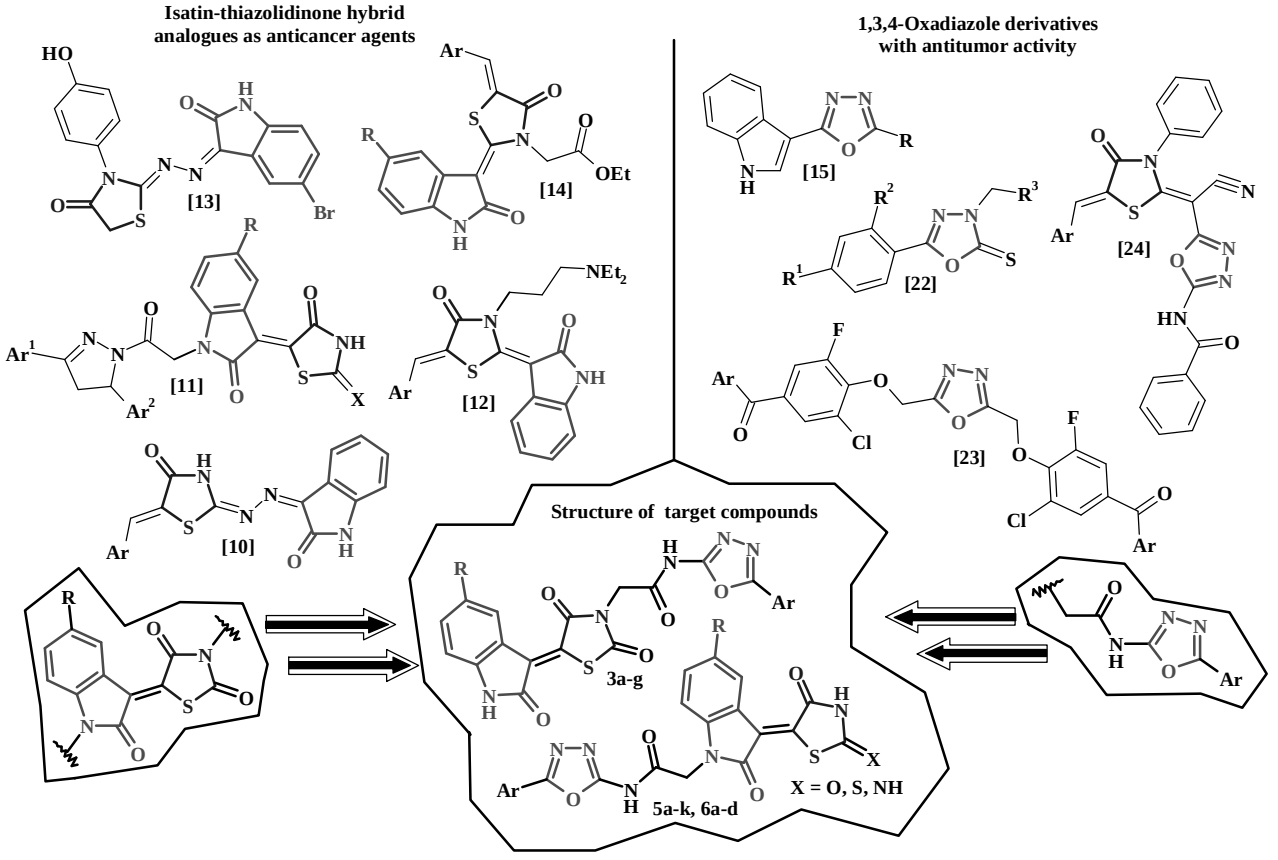


Fig. 1. Structures of anticancer isatin-based 4-thiazolidinones and 1,3,4-oxadiazole derivatives and background for target compound synthesis

2.2. Chemistry

General procedure for synthesis of 2-(2,4-dioxothiazolidin-3-yl)-N-(5-aryl-1,3,4-oxadiazol-2-yl)-acetamides (**2a-c**). The reaction mixture of 2,4-thiazolidinedione (20 mmol), potassium hydroxide (20 mmol) in ethanol medium (20 ml) was refluxed for 15 min. The appropriate 2-chloro-*N*-(5-aryl-1,3,4-oxadiazol-2-yl)-acedamide **1a-c** and a few crystals of a potassium iodide as a catalyst were added to a reaction mixture and the final solution was refluxed for 5 h. The product was obtained as a precipitate after cooling of the reaction mixture, filtering off and recrystallized with DMF-ethanol (1:2) mixture.

2-(2,4-Dioxothiazolidin-3-yl)-N-(5-(4-methoxyphenyl)-1,3,4-oxadiazol-2-yl)-acetamide (**2a**). Yield 73 %, mp 494–495 K. ^{1}H NMR (300 MHz, DMSO-d_6+CCl_4): δ 12.27 (br.s, 1H, NH); 7.83 (d, 2H, J = 8.5 Hz, arom); 7.12 (d, 2H, J = 8.5 Hz, arom); 4.47 (s, 2H, CH_2CO); 3.83 (s, 3H, OCH_3). Calcd. for $C_{14}H_{12}N_4O_5S$: C, 48.27; H, 3.47; N, 16.08; Found: C, 47.88; H, 3.61; N, 16.33 %.

2-(2,4-Dioxothiazolidin-3-yl)-N-(5-(4-chlorophenyl)-1,3,4-oxadiazol-2-yl)-acetamide (**2b**). Yield 78 %, mp 507–508 K. ^{1}H NMR (300 MHz, DMSO-d_6+CCl_4): δ 12.47 (br.s, 1H, NH); 7.94 (d, 2H, J = 7.3 Hz, arom); 7.55 (d, 2H, J = 6.9 Hz, arom); 4.46 (s, 2H, CH_2CO); 4.26 (s, 2H, CH_2-thiaz.). Calcd. for $C_{13}H_9ClN_4O_4S$: C, 44.26; H, 2.57; N, 15.88; Found: C, 44.45; H, 2.43; N, 16.04 %.

2-(2,4-Dioxothiazolidin-3-yl)-N-(5-(3,4-dimethoxyphenyl)-1,3,4-oxadiazol-2-yl)-acetamide (**2c**). Yield 69 %, mp 470–471 K. Calcd. for $C_{15}H_{14}N_4O_6S$: C, 47.62; H, 3.73; N, 14.81; Found: C, 47.43; H, 3.57; N, 15.06 %.

General procedure for synthesis of 2-[5-(5R-2-oxo-1,2-dihydroindol-3-ylidene)-2,4-dioxothiazolidin-3-yl]-N-(5-aryl-1,3,4-oxadiazol-2-yl)-acetamides (**3a-g**). The mixture of the compound **2a-c** (5 mmol), anhydrous sodium acetate (5 mmol), of appropriate isatine (5.5 mmol) and 10 ml of glacial acetic acid was heated under reflux for 5 h. The precipitate was filtered off and recrystallized with DMF-AcOH (1:1) mixture.

2-[5-(5-Chloro-2-oxo-1,2-dihydroindol-3-ylidene)-2,4-dioxothiazolidin-3-yl]-N-(5-(4-methoxyphenyl)-1,3,4-oxadiazol-2-yl)-acetamide (**3a**). Yield 72 %, mp 555–556 K. ^{1}H NMR (300 MHz, DMSO-d_6+CCl_4): δ 12.29 (br.s, 1H, NH); 11.43 (s, 1H, NH); 9.04 (s, 1H, isat.); 7.84

(d, 2H, *J* = 8.1 Hz, arom); 7.69 (d, 1H, *J* = 6.6 Hz, isat.); 7.15 (d, 2H, *J* = 8.5 Hz, arom); 6.97 (d, 1H, *J* = 8.1 Hz, isat.); 4.71 (s, 2H, CH_2CO); 3.86 (s, 3H, OCH_3). Calcd. for $C_{22}H_{14}ClN_5O_6S$: C, 51.62; H, 2.76; N, 13.68; Found: C, 51.38; H, 2.59; N, 13.85 %.

2-[5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidene)-2,4-dioxothiazolidin-3-yl]-N-(5-(4-methoxyphenyl)-1,3,4-oxadiazol-2-yl)-acetamide (**3b**). Yield 68 %, mp 556–557 K. ^{1}H NMR (300 MHz, DMSO-d_6+CCl_4): δ 12.44 (br.s, 1H, NH); 11.45 (s, 1H, NH); 8.91 (s, 1H, isat.); 7.86 (d, 2H, *J* = 8.1 Hz, arom); 7.61 (d, 1H, *J* = 6.6 Hz, isat.); 7.13 (d, 2H, *J* = 8.5 Hz, arom); 6.94 (d, 1H, *J* = 8.1 Hz, isat.); 4.73 (s, 2H, CH_2CO); 3.84 (s, 3H, OCH_3). Calcd. for $C_{22}H_{14}BrN_5O_6S$: C, 47.50; H, 2.54; N, 12.59; Found: C, 47.73; H, 2.43; N, 12.72 %.

2-[5-(2-Oxo-1,2-dihydroindol-3-ylidene)-2,4-dioxothiazolidin-3-yl]-N-(5-(4-chlorophenyl)-1,3,4-oxadiazol-2-yl)-acetamide (**3c**). Yield 68 %, mp 540–541 K. ^{1}H NMR (300 MHz, DMSO-d_6+CCl_4): δ 12.38 (br.s, 1H, NH); 11.28 (s, 1H, NH); 8.91 (s, 1H, isat.); 7.96 (d, 2H, *J* = 8.3 Hz, arom); 7.55 (d, 2H, *J* = 8.1 Hz, arom); 7.42 (t, 1H, *J* = 7.5 Hz, isat.); 7.18 (t, 1H, *J* = 7.6 Hz, isat.); 7.01 (d, 1H, *J* = 7.9 Hz, isat.); 4.78 (s, 2H, CH_2CO). Calcd. for $C_{21}H_{12}ClN_5O_5S$: C, 52.34; H, 2.51; N, 14.53; Found: C, 52.58; H, 2.63; N, 14.38 %.

2-[5-(5-Chloro-2-oxo-1,2-dihydroindol-3-ylidene)-2,4-dioxothiazolidin-3-yl]-N-(5-(4-chlorophenyl)-1,3,4-oxadiazol-2-yl)-acetamide (**3d**). Yield 74 %, mp 548–549 K. ^{1}H NMR (300 MHz, DMSO-d_6+CCl_4): δ 12.27 (br.s, 1H, NH); 11.20 (s, 1H, NH); 8.84 (s, 1H, isat.); 7.97 (d, 2H, *J* = 8.3 Hz, arom); 7.56 (d, 2H, *J* = 8.2 Hz, arom); 7.35 (d, 1H, *J* = 7.9 Hz, isat.); 6.97 (d, 1H, *J* = 8.0 Hz, isat.); 4.73 (s, 2H, CH_2CO). Calcd. for $C_{21}H_{11}Cl_2N_5O_5S$: C, 48.85; H, 2.15; N, 13.56; Found: C, 49.04; H, 2.19; N, 13.73 %.

2-[5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidene)-2,4-dioxothiazolidin-3-yl]-N-(5-(4-chlorophenyl)-1,3,4-oxadiazol-2-yl)-acetamide (**3e**). Yield 69 %, mp 544–545 K. Calcd. for $C_{21}H_{11}BrClN_5O_5S$: C, 44.98; H, 1.98; N, 12.49; Found: C, 45.18; H, 2.07; N, 12.34 %.

2-[5-(5-Chloro-2-oxo-1,2-dihydroindol-3-ylidene)-2,4-dioxothiazolidin-3-yl]-N-(5-(3,4-dimethoxyphenyl)-1,3,4-oxadiazol-2-yl)-acetamide (**3f**). Yield 74 %, mp 548–549 K. ^{1}H NMR (300 MHz, DMSO-d_6+CCl_4): δ 12.42 (br.s, 1H, NH); 11.43 (s, 1H, NH); 8,96 (s, 1H, isat.); 7.93 (s, 1H, arom); 7.43-7.50 (m, 2H, arom); 7.06-7.21 (m, 2H, arom); 4.72 (s, 2H, CH_2CO); 3.88 (s, 6H, 2*OCH_3). Calcd. for $C_{23}H_{16}ClN_5O_7S$: C, 50.98; H, 2.98; N, 12.92; Found: C, 51.21; H, 2.86; N, 12.76 %.

2-[5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidene)-2,4-dioxothiazolidin-3-yl]-N-(5-(3,4-dimethoxyphenyl)-1,3,4-oxadiazol-2-yl)-acetamide (**3g**). Yield 67 %, mp 485–486 K. Calcd. for $C_{23}H_{16}BrN_5O_7S$: C, 47.11; H, 2.75; N, 11.94; Found: C, 47.34; H, 2.68; N, 12.13 %.

General procedure for synthesis of 1-[2-(5-aryl-1,3,4-oxadiazol-2-yl)-2-oxoethyl]-1H-indole-2,3-diones (**4a-h**). A mixture of isatin or 5-chloro(bromo)isatin (50 mmol), appropriate 2-chloro-*N*-(5-aryl-1,3,4-oxadiazol-2-yl)-acedamide **1a-c** (60 mmol), and potassium carbonate (125 mmol) was stirred in anhydrous DMF (30 ml) at room temperature during 12 h. Afterwards the reaction mixture was poured into cold water. A solid product was filtered, washed with water, ethanol, dried, and recrystallized with DMF:ethanol (1:2) mixture.

1-{2-[5-(4-Methoxyphenyl)-1,3,4-oxadiazol-2-yl]-2-oxoethyl}-1H-indole-2,3-dione (**4a**). Yield 62 %, mp 477–478 K. ^{1}H NMR (300 MHz, DMSO-d_6+CCl_4): δ 7.75 (d, 2H, *J* = 8.0 Hz, arom); 7.54–7.62 (m, 2H, arom); 7.03–7.10 (m, 4H, arom); 4.32 (s, 2H, CH_2CO); 3.81 (s, 3H, OCH_3). Calcd. for $C_{19}H_{14}N_4O_5$: C, 60.32; H, 3.73; N, 14.81; Found: C, 60.58; H, 3.81; N, 14.69 %.

1-{2-[5-(4-Methoxyphenyl)-1,3,4-oxadiazol-2-yl]-2-oxoethyl}-5-chloro-1H-indole-2,3-dione (**4b**). Yield 67 %, mp 494–495 K. ^{1}H NMR (300 MHz, DMSO-d_6+CCl_4): δ 7.82 (s, 1H, isat.); 7.46–7.59 (m, 3H, arom); 7.09–7.16 (m, 3H, arom); 4.37 (s, 2H, CH_2CO); 3.84 (s, 3H, OCH_3). Calcd. for $C_{19}H_{13}ClN_4O_5$: C, 55.28; H, 3.17; N, 13.57; Found: C, 55.04; H, 3.31; N, 13.72 %.

1-{2-[5-(3,4-Dimethoxyphenyl)-1,3,4-oxadiazol-2-yl]-2-oxoethyl}-1H-indole-2,3-dione (**4c**). Yield 63 %, mp 479–480 K. ^{1}H NMR (300 MHz, DMSO-d_6+CCl_4): δ 7.42–7.57 (m, 4H, arom); 7.05–7.19 (m, 3H, arom); 4.39 (s, 2H, CH_2CO); 3.88 (s, 6H, 2*OCH_3). Calcd. for $C_{20}H_{16}N_4O_6$: C, 58.82; H, 3.95; N, 13.72; Found: C, 59.08; H, 3.84; N, 13.87 %.

1-{2-[5-(3,4-Dimethoxyphenyl)-1,3,4-oxadiazol-2-yl]-2-oxoethyl}-5-chloro-1H-indole-2,3-dione (**4d**). Yield 57 %, mp 476–477 K. ^{1}H NMR (300 MHz, DMSO-d_6+CCl_4): δ 7.39–7.57 (m, 2H, arom); 6.97–7.16 (m, 4H, arom); 4.36 (s, 2H, CH_2CO); 3.84 (s, 6H, 2*OCH_3). Calcd. for $C_{20}H_{15}ClN_4O_6$: C, 54.25; H, 3.41; N, 12.65; Found: C, 54.49; H, 3.28; N, 12.84 %.

1-{2-[5-(3,4-Dimethoxyphenyl)-1,3,4-oxadiazol-2-yl]-2-oxoethyl}-5-bromo-1H-indole-2,3-dione (**4e**). Yield 56 %, mp 480–481 K. ^{1}H NMR (300 MHz, DMSO-d_6+CCl_4): δ 7.44–7.57 (m, 2H, arom); 7.01–7.21 (m, 4H, arom); 4.38 (s, 2H, CH_2CO); 3.85 (s, 6H, 2*OCH_3). Calcd. for $C_{20}H_{15}BrN_4O_6$: C, 49.30; H, 3.10; N, 11.50; Found: C, 49.51; H, 3.27; N, 11.69 %.

1-{2-[5-(4-Chlorophenyl)-1,3,4-oxadiazol-2-yl]-2-oxoethyl}-1H-indole-2,3-dione (**4f**). Yield 61 %, mp 486–487 K. ^{1}H NMR (300 MHz, DMSO-d_6+CCl_4): δ 7.83-7.89 (m, 3H, arom); 7.42–7.50 (m, 4H, arom); 6.99 (d, 1H, *J* = 8.0 Hz, isat.); 4.36 (s, 2H, CH_2CO). Calcd. for $C_{18}H_{11}ClN_4O_4$: C, 56.48; H, 2.90; N, 14.64; Found: C, 56.71; H, 3.04; N, 14.46 %.

1-{2-[5-(4-Chlorophenyl)-1,3,4-oxadiazol-2-yl]-2-oxoethyl}-5-chloro-1H-indole-2,3-dione (**4g**). Yield 54 %,

mp 490–491 K. ^{1}H NMR (300 MHz, DMSO-d_6+CCl_4): δ 7.84–7.92 (m, 3H, arom); 7.48–7.57 (m, 4H, arom); 4.48 (s, 2H, CH_2CO). Calcd. for $C_{18}H_{10}Cl_2N_4O_4$: C, 51.82; H, 2.42; N, 13.43; Found: C, 52.11; H, 2.51; N, 13.57 %.

1-{2-[5-(4-Chlorophenyl)-1,3,4-oxadiazol-2-yl]-2-oxoethyl}-5-bromo-1H-indole-2,3-dione (**4h**). Yield 56 %, mp 500–501 K. ^{1}H NMR (300 MHz, DMSO-d_6+CCl_4): δ 7.87–8.01 (m, 3H, arom); 7.42–7.58 (m, 4H, arom); 4.42 (s, 2H, CH_2CO). Calcd. for $C_{18}H_{10}BrClN_4O_4$: C, 46.83; H, 2.18; N, 12.14; Found: C, 46.72; H, 2.32; N, 12.35 %.

General procedure for synthesis of 5-{1-[2-(5-aryl-1,3,4-oxadiazol-2-yl)-2-oxoethyl]-2-oxo-1,2-dihydroindole-3-ylidene}-2-(thi)oxothiazolidin-4-ones **(5a-k)** *and 2-amino-4-thiazolones* **(6a-d)**. A mixture of the compound **4a-h** (5 mmol), appropriate 4-thiazolidinone (5.5 mmol) and anhydrous sodium acetate (5 mmol) was refluxed for 3 h in a glacial acetic acid (10 ml). Obtained powder was filtered off, washed with water and ethanol, dried and recrystallized with DMF-ethanol (1:1) mixture.

5-{1-[2-(5-(4-Methoxyphenyl)-1,3,4-oxadiazol-2-yl)-2-oxoethyl]-2-oxo-1,2-dihydroindole-3-ylidene}-2,4-dioxothiazolidine (**5a**). Yield 72 %, mp 494–495 K. ^{1}H NMR (300 MHz, DMSO-d_6+CCl_4): δ 12.28 (br.s, 1H, NH); 9.06 (d, 1H, J = 7.5 Hz, isat.); 7.85–7.96 (m, 3H, arom); 7.08–7.18 (m, 4H, arom); 4.78 (s, 2H, CH_2CO); 3.83 (s, 3H, OCH_3). Calcd. for $C_{22}H_{15}N_5O_6S$: C, 55.34; H, 3.17; N, 14.67; Found: C, 55.18; H, 3.28; N, 14.53 %.

5-{1-[2-(5-(4-Methoxyphenyl)-1,3,4-oxadiazol-2-yl)-2-oxoethyl]-2-oxo-1,2-dihydroindole-3-ylidene}-2-thioxothiazolidin-4-one (**5b**). Yield 78 %, mp 537–538 K. ^{1}H NMR (300 MHz, DMSO-d_6+CCl_4): δ 12.34 (br.s, 1H, NH); 8.96 (d, 1H, J = 7.5 Hz, isat.); 7.87 (d, 2H, J = 8.1 Hz, arom); 7.49 (t, 1H, J = 7.6 Hz, isat.); 7.07–7.14 (m, 4H, arom, isat.); 4.81 (s, 2H, CH_2CO); 3.88 (s, 3H, OCH_3). Calcd. for $C_{22}H_{15}N_5O_5S_2$: C, 53.54; H, 3.06; N, 14.19; Found: C, 53.39; H, 3.15; N, 14.32 %.

5-{1-[2-(5-(4-Methoxyphenyl)-1,3,4-oxadiazol-2-yl)-2-oxoethyl]-2-oxo-5-chloro-1,2-dihydroindole-3-ylidene}-2-thioxothiazolidin-4-one (**5c**). Yield 75 %, mp 531–532 K. ^{1}H NMR (300 MHz, DMSO-d_6+CCl_4): δ 12.28 (br.s, 1H, NH); 8.91 (d, 1H, J = 7.5 Hz, isat.); 7.82 (d, 2H, J = 8.2 Hz, arom); 7.37 (d, 1H, J = 7.5 Hz, isat.); 7.17 (d, 2H, J = 7.9 Hz, arom); 7.03 (d, 1H, J = 7.7 Hz, isat.); 4.79 (s, 2H, CH_2CO); 3.86 (s, 3H, OCH_3). Calcd. for $C_{22}H_{14}ClN_5O_5S_2$: C, 50.05; H, 2.67; N, 13.26; Found: C, 50.18; H, 2.76; N, 13.14 %.

5-{1-[2-(5-(3,4-Dimethoxyphenyl)-1,3,4-oxadiazol-2-yl)-2-oxoethyl]-2-oxo-1,2-dihydroindole-3-ylidene}-2,4-dioxothiazolidine (**5d**). Yield 73 %, mp 532–533 K. ^{1}H NMR (300 MHz, DMSO-d_6+CCl_4): δ 12.33 (br.s, 1H, NH); 8.92 (s, 1H, isat.); 7.95 (s, 1H, arom); 7.38-7.51 (m, 2H, arom); 7.07-7.15 (m, 3H, arom); 4.83 (s, 2H, CH_2CO); 3.90 (s, 6H, 2*OCH_3). Calcd. for $C_{23}H_{17}N_5O_7S$: C, 54.44; H, 3.38; N, 13.80; Found: C, 54.63; H, 3.45; N, 13.67 %.

5-{1-[2-(5-(3,4-Dimethoxyphenyl)-1,3,4-oxadiazol-2-yl)-2-oxoethyl]-2-oxo-1,2-dihydroindole-3-ylidene}-2-thioxothiazolidin-4-one (**5e**). Yield 79 %, mp 540–541 K. ^{1}H NMR (300 MHz, DMSO-d_6+CCl_4): δ 12.42 (br.s, 1H, NH); 8.97 (s, 1H, isat.); 7.94 (s, 1H, arom); 7.44-7.52 (m, 2H, arom); 7.12-7.19 (m, 2H, arom); 7.03 (d, 1H, J = = 7.4 Hz, isat.); 4.78 (s, 2H, CH_2CO); 3.88 (s, 6H, 2*OCH_3). Calcd. for $C_{23}H_{17}N_5O_6S_2$: C, 52.77; H, 3.27; N, 13.38; Found: C, 52.63; H, 3.37; N, 13.51 %.

5-{1-[2-(5-(3,4-Dimethoxyphenyl)-1,3,4-oxadiazol-2-yl)-2-oxoethyl]-2-oxo-5-chloro-1,2-dihydroindole-3-ylidene}-2,4-dioxothiazolidine (**5f**). Yield 71 %, mp 514–515 K. Calcd. for $C_{23}H_{16}ClN_5O_7S$: C, 50.98; H, 2.98; N, 12.92; Found: C, 51.21; H, 3.07; N, 13.14 %.

5-{1-[2-(5-(3,4-Dimethoxyphenyl)-1,3,4-oxadiazol-2-yl)-2-oxoethyl]-2-oxo-5-chloro-1,2-dihydroindole-3-ylidene}-2-thioxothiazolidin-4-one (**5g**). Yield 76 %, mp 520–521 K. ^{1}H NMR (300 MHz, DMSO-d_6+CCl_4): δ 12.14 (br.s, 1H, NH); 8.96 (s, 1H, isat.); 7.93 (s, 1H, arom); 7.43–7.50 (m, 2H, arom); 7.06–7.21 (m, 2H, arom); 4.83 (s, 2H, CH_2CO); 3.88 (s, 6H, 2*OCH_3). Calcd. for $C_{23}H_{16}ClN_5O_6S_2$: C, 49.51; H, 2.89; N, 12.55; Found: C, 49.73; H, 2.98; N, 12.44 %.

5-{1-[2-(5-(4-Chlorophenyl)-1,3,4-oxadiazol-2-yl)-2-oxoethyl]-2-oxo-1,2-dihydroindole-3-ylidene}-2,4-dioxothiazolidine (**5h**). Yield 78 %, mp 495–496 K. ^{1}H NMR (300 MHz, DMSO-d_6+CCl_4): δ 12.39 (br.s, 1H, NH); 8.85 (d, 1H, J = 7.6 Hz, isat.); 7.94 (d, 2H, J = 8.2 Hz, arom); 7.58 (d, 2H, J = 8.0 Hz, arom); 7.39 (t, 1H, J = 7.5 Hz, isat.); 7.18 (t, 1H, J = 7.6 Hz, isat.); 7.04 (d, 1H, J = 7.5 Hz, isat.); 4.85 (s, 2H, CH_2CO). Calcd. for $C_{21}H_{12}ClN_5O_5S$: C, 52.34; H, 2.51; N, 14.53; Found: C, 52.62; H, 2.64; N, 14.44 %.

5-{1-[2-(5-(4-Chlorophenyl)-1,3,4-oxadiazol-2-yl)-2-oxoethyl]-2-oxo-1,2-dihydroindole-3-ylidene}-2-thioxothiazolidin-4-one (**5i**). Yield 79 %, mp 547–548 K. ^{1}H NMR (300 MHz, DMSO-d_6+CCl_4): δ 12.22 (br.s, 1H, NH); 8.92 (d, 1H, J = 7.5 Hz, isat.); 7.95 (d, 2H, J = 7.9 Hz, arom); 7.55 (d, 2H, J = 8.1 Hz, arom); 7.44 (t, 1H, J = 7.6, 7.5 Hz, isat.); 7.14 (t, 1H, J = 7.6 Hz, isat.); 7.07 (d, 1H, J = 7.6 Hz, isat.); 4.82 (s, 2H, CH_2CO). Calcd. for $C_{21}H_{12}ClN_5O_4S_2$: C, 50.66; H, 2.43; N, 14.06; Found: C, 50.49; H, 2.54; N, 13.95 %.

5-{1-[2-(5-(4-Chlorophenyl)-1,3,4-oxadiazol-2-yl)-2-oxoethyl]-2-oxo-5-chloro-1,2-dihydroindole-3-ylidene}-2-thioxothiazolidin-4-one (**5j**). Yield 74 %, mp 537–538 K. ^{1}H NMR (300 MHz, DMSO-d_6+CCl_4): δ 12.25 (br.s, 1H, NH); 8.87 (s, 1H, isat.); 7.91 (d, 2H, J = 7.9 Hz, arom); 7.57 (d, 2H, J = 8.1 Hz, arom); 7.38 (d, 1H, J = 7.5 Hz, isat.); 6.98 (d, 1H, J = 7.6 Hz, isat.); 4.81 (s, 2H, CH_2CO). Calcd. for $C_{21}H_{11}Cl_2N_5O_4S_2$: C, 47.38; H, 2.08; N, 13.15; Found: C, 47.53; H, 2.17; N, 13.27 %.

5-{1-[2-(5-(4-Chlorophenyl)-1,3,4-oxadiazol-2-yl)-2-oxoethyl]-2-oxo-5-bromo-1,2-dihydroindole-3-ylidene}-2-thioxothiazolidin-4-one (**5k**). Yield 76 %, mp > 593 K. ^{1}H NMR (300 MHz, DMSO-d_6+CCl_4): δ 12.32 (br.s, 1H, NH); 8.96 (s, 1H, isat.); 7.94 (d, 2H, J = 8.1 Hz, arom); 7.56 (d, 2H, J = 8.0 Hz, arom); 7.44 (d, 1H, J = 7.6 Hz, isat.); 7.04 (d, 1H, J = 7.5 Hz, isat.); 4.83 (s, 2H, CH_2CO). Calcd. for $C_{21}H_{11}BrClN_5O_4S_2$: C, 43.73; H, 1.92; N, 13.85; Found: C, 43.68; H, 2.04; N, 13.72 %.

5-{1-[2-(5-(4-Methoxyphenyl)-1,3,4-oxadiazol-2-yl)-2-oxoethyl]-2-oxo-1,2-dihydroindole-3-ylidene}-2-amino-4-thiazolone (**6a**). Yield 81 %, mp 517–518 K. ^{1}H NMR (300 MHz, DMSO-d_6+CCl_4): δ 9.05, 9.26, 9.49 (d, br.s, br.s, 2H, NH_2); 8.96 (d, 1H, J = 7.5 Hz, isat.); 7.88(d, 2H, J = 8.2 Hz, arom); 7.35 (t, 1H, J = 7.8 Hz, isat.); 7.07-7.14 (m, 4H, arom, isat.); 4.81 (s, 2H, CH_2CO); 3.84 (s, 3H, OCH_3). Calcd. for $C_{22}H_{16}N_6O_5S$: C, 55.46; H, 3.38; N, 17.64; Found: C, 55.28; H, 3.45; N, 17.82 %.

5-{1-[2-(5-(3,4-Dimethoxyphenyl)-1,3,4-oxadiazol-2-yl)-2-oxoethyl]-2-oxo-1,2-dihydroindole-3-ylidene}-2-amino-4-thiazolone (**6b**). Yield 77 %, mp 531–532 K. ^{1}H NMR (300 MHz, DMSO-d_6+CCl_4): δ 9.04, 9.24, 9.43 (d, br.s, br.s, 2H, NH_2); 8.98 (s, 1H, isat.); 7.92 (s, 1H, arom); 7.44–7.56 (m, 2H, arom); 7.04–7.16 (m, 3H, arom); 4.82 (s, 2H, CH_2CO); 3.87 (s, 6H, 2*OCH_3). Calcd. for $C_{23}H_{18}N_6O_6S$: C, 54.54; H, 3.58; N, 16.59; Found: C, 54.69; H, 3.67; N, 16.51 %.

5-{1-[2-(5-(3,4-Dimethoxyphenyl)-1,3,4-oxadiazol-2-yl)-2-oxoethyl]-2-oxo-5-chloro-1,2-dihydroindole-3-ylidene}-2-amino-4-thiazolone (**6c**). Yield 81 %, mp 522–523 K. ^{1}H NMR (300 MHz, DMSO-d_6+CCl_4): δ 9.05, 9.23, 9.46 (d, br.s, br.s, 2H, NH_2); 8.96 (s, 1H, isat.); 7.91 (s, 1H, arom); 7.41–7.52 (m, 2H, arom); 7.06–7.19 (m, 2H, arom); 4.83 (s, 2H, CH_2CO); 3.88 (s, 6H, 2*OCH_3). Calcd. for $C_{23}H_{17}ClN_6O_6S$: C, 51.07; H, 3.17; N, 15.54; Found: C, 50.89; H, 3.25; N, 15.68 %.

5-{1-[2-(5-(4-Chlorophenyl)-1,3,4-oxadiazol-2-yl)-2-oxoethyl]-2-oxo-1,2-dihydroindole-3-ylidene}-2-amino-4-thiazolone (**6d**). Yield 79 %, mp 541–542 K. ^{1}H NMR (300 MHz, DMSO-d_6+CCl_4): δ 9.03, 9.21, 9.44 (d, br.s, br.s, 2H, NH_2); 8.97 (s, 1H, J = 7.6 Hz, isat.); 7.97 (d, 2H, J = 7.9 Hz, arom); 7.56 (d, 2H, J = 8.2 Hz, arom); 7.37 (t, 1H, J = 7.5 Hz, isat.); 7.12 (t, 1H, J = 7.7 Hz, isat.); 7.03 (d, 1H, J = 7.4 Hz, isat.); 4.83 (s, 2H, CH_2CO). Calcd. for $C_{21}H_{13}ClN_6O_4S$: C, 52.45; H, 2.72; N, 17.48; Found: C, 52.64; H, 2.64; N, 17.57 %.

2.3. Primary Anticancer Assay

Primary anticancer assay was performed according to the US National Cancer Institute (NCI) protocol, and described elsewhere [25, 26]. The compounds were added at a single concentration and the cell culture was incubated for 48 h. End point determination was made with a protein binding dye, sulforhodamine B. The results for each compounds are reported as the percent growth of treated cells when compared to untreated control cells. The percentage growth was evaluated spectrophotometrically versus controls not treated with test agents.

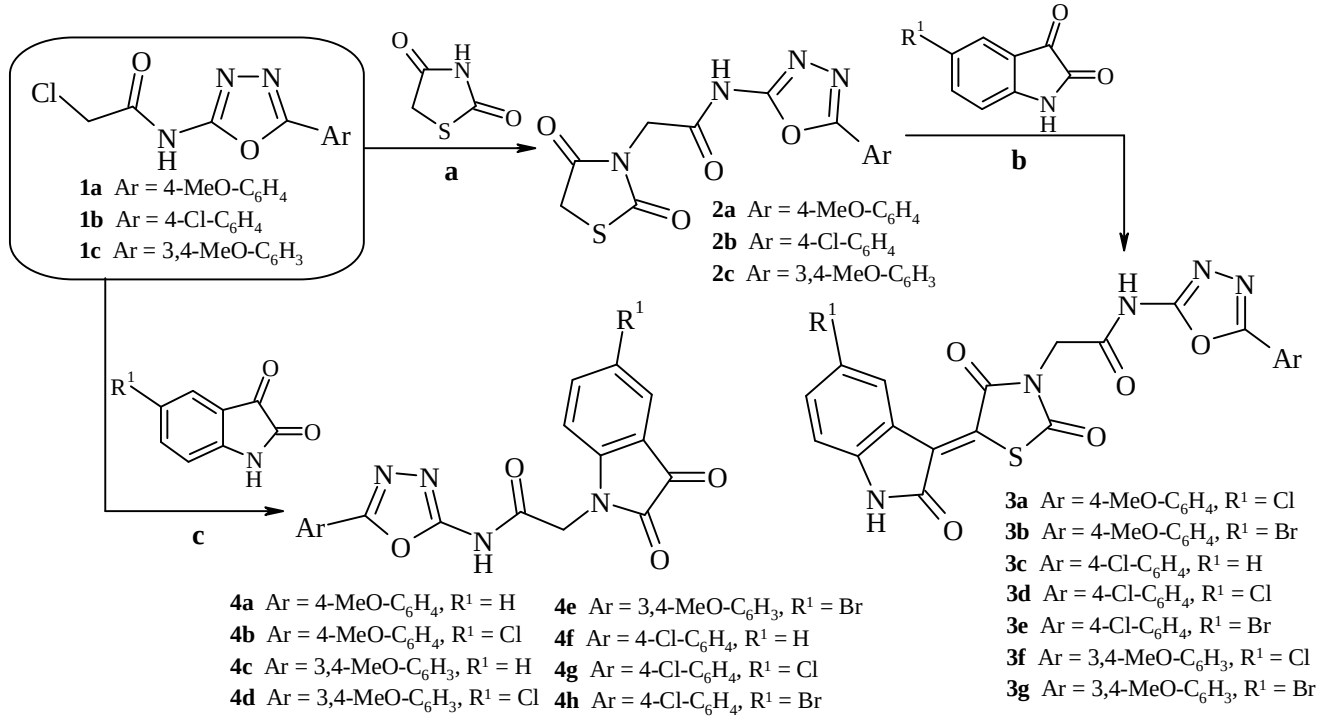
3. Results and Discussion

3.1. Chemistry

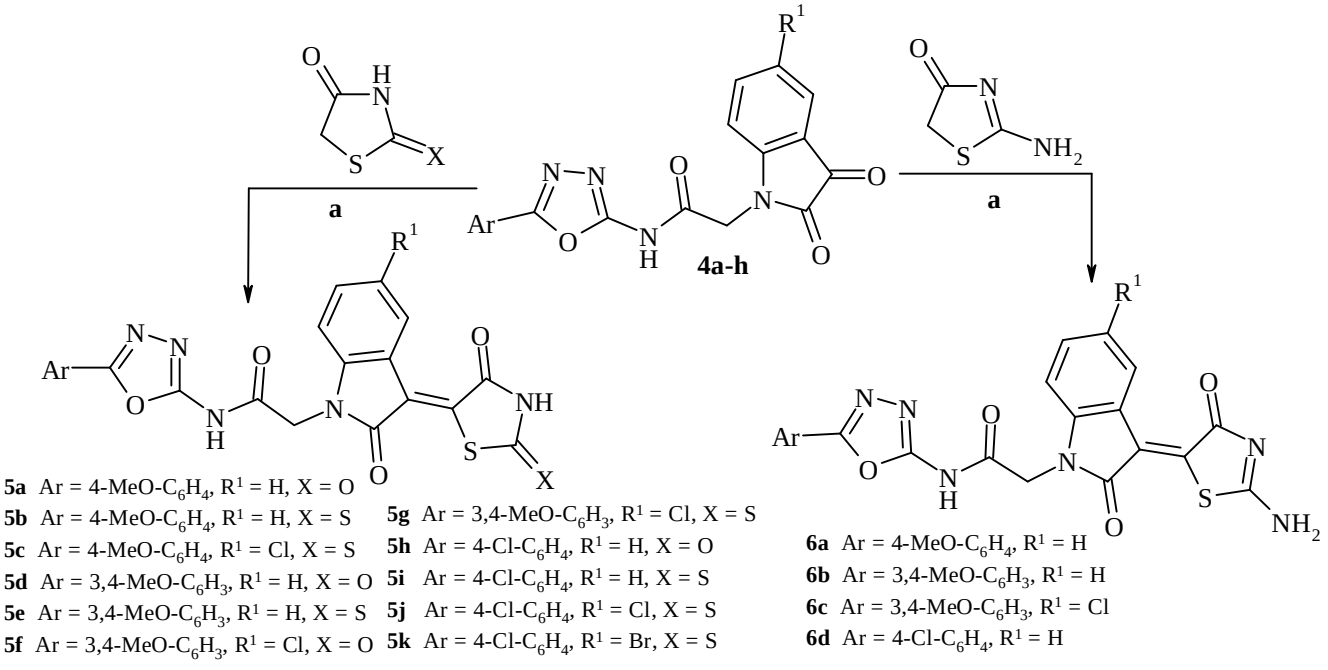
The starting 2-chloro-*N*-(5-aryl-1,3,4-oxadiazol-2-yl)-acetamides **1a-c** were obtained using the reaction of the chloroacetyl chloride and appropriate 2-amino-5-aryl-1,3,4-oxadiazole in dioxane medium. Synthesis of new 4-thiazolidinones with oxadiazole moiety in *N3* position (**2a-c**) was performed based on the alkylation reaction of 2,4-thiazolidinedione potassium salt, generated *in situ*, and 2-chloro-*N*-(5-aryl-1,3,4-oxadiazol-2-yl)-acedamides **1a-c**. Considering the fact, that the presence and nature of the moiety in thiazolidinone *C5* position are critical for realization and character of the pharmacological effects [2, 11, 27], the following modification was directed to the methylene group of synthesized compounds **2a-c**. Thus, the synthesis of new non-condensed systems with 4-thiazolidinone, 1,3,4-oxadiazole, and indoline moieties **3a-g** were performed, using standard Knoevenagel reaction procedure (medium – acetic acid, catalyst – fused sodium acetate). 2-Chloro-*N*-(5-aryl-1,3,4-oxadiazol-2-yl)-acetamides **1a-d** were tested as alkylating agents in the reaction with isatin, 5-chloroisatin and 5-bromoisatin in DMF medium at room temperature [28]. The corresponding 1-[2-(5-aryl-1,3,4-oxadiazol-2-yl)-2-oxoethyl]-1*H*-indole-2,3-diones **4a-h** have been obtained according to Scheme 1.

1,3,4-Oxadiazole-indolines **4a-h** were successfully utilized as oxocompounds in the Knoevenagel reaction with 2,4-thiazolidinedione, 2-thioxo-4-thiazolidinone, and 2-amino-4-thiazolone. Following the mentioned reaction the new 4-thiazolidinone conjugates with oxadiazole and isatin **5a-k** and **6a-d** were synthesized (Scheme 2).

The structure of synthesized 4-thiazolidinone derivatives was confirmed by NMR spectra. In ^{1}H NMR spectra of synthesized compounds the protons of the methoxy group show a singlet at $\delta \sim 3.81$–3.90 ppm. The protons of the methylene group CH_2CO appear as a singlet at $\delta \sim 4.32$–4.48 ppm (**2a-c, 4a-h**) and $\delta \sim 4.71$–4.88 ppm (**3a-g, 5a-k**, **6a-d**). For the protons of non-substituted isatin fragment two doublets and two triplets at $\delta \sim 6.99$–7.07 ppm, $\delta \sim 8.85$–9.06 ppm, $\delta \sim 7.14$–7.18 ppm and $\delta \sim 7.39$–7.44 ppm are observed, respectively. NH_2 protons of **6a-d** have been found as a doublet and two broad singlets at $\delta \sim 9.03$–9.05 ppm, $\delta \sim 9.19$–9.26 ppm, and $\delta \sim 9.42$–9.49 ppm. This could be explained by amino-imino tautomerism of these derivatives. In the ^{1}H NMR spectra NH proton of mentioned compounds have been found as a broad singlet at $\delta \sim 12.14$–12.47 ppm.



Scheme 1. Synthesis of 4-thiazolidinones with 1,3,4-oxadiazole and indoline moieties **3a-g** and *N*-substituted isatin derivatives **4a-h**. Reagents, conditions, and yields: KOH, KI, EtOH, reflux 4–5 h, 69–78 % (a); AcONa, AcOH, reflux 3–4 h, 62–74 %(b) and K_2CO_3, DMF, r.t. 12 h, 54–63 % (c)



Scheme 2. Synthesis of isatin-based 4-thiazolidinones and 2-amino-4-thiazolones ontaining 1,3,4-oxadiazole moiety. Reagents, conditions, and yields: AcONa, AcOH, reflux 3–4 h, 62–74 % (a)

Table 1

Anticancer screening data in concentration 10.00 μM

Compd.	60 cell lines assay in 1 dose 10.00 μM concentration			
	Mean growth, %	Range of growth, %	The most sensitive cell line	
			Cell line (Panel)	Growth, %
2a	103.56	74.37–119.69	A498 (Renal Cancer)	74.37
2b	99.34	74.16–122.65	UO-31 (Renal Cancer)	74.16
			MALME-3M (Melanoma)	82.28
2c	92.44	42.19–110.15	MALME-3M (Melanoma)	42.19
			A498 (Renal Cancer)	50.22
			CCRF-CEM (Leukemia)	54.35
			SK-MEL-2 (Melanoma)	57.23
			SF-295 (CNS Cancer)	63.43
			NCI-H226 (Non-Small Cell Lung Cancer)	71.85
			SF-539 (CNS Cancer)	73.09
4a	104.31	70.33–128.80	UO-31 (Renal Cancer)	70.33
			SR (Leukemia)	79.36
4c	99.76	70.06–114.06	UO-31 (Renal Cancer)	70.06
4e	102.02	84.99–123.95	UO-31 (Renal Cancer)	84.99
4g	89.90	55.54–119.79	PC-3 (Prostate Cancer)	55.54
			UO-31 (Renal Cancer)	61.78
			HCT-15 (Colon Cancer)	65.42
			IGROV1 (Ovarian Cancer)	70.66
			CAKI-1 (Renal Cancer)	72.66
			RPMI-8226 (Leukemia)	73.42
			OVCAR-3 (Ovarian Cancer)	73.44
			HL-60(TB) (Leukemia)	74.28
4h	96.16	67.86–116.61	PC-3 (Prostate Cancer)	67.86
			UO-31 (Renal Cancer)	70.09
			OVCAR-3 (Ovarian Cancer)	77.86

3.2. Evaluation of Anticancer Activity *in vitro*

Synthesized compounds **2a**, **2b**, **2c**, **4a**, **4c**, **4e**, **4g**, and **4h** were submitted and evaluated at the 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. Tested compounds displayed low antitumor activity in the *in vitro* screening on the tested cell lines with average values of GP = 89.90–104.31 %. It is noteworthy that the synthesized compounds demonstrated a selective influence on some cell lines of melanoma, leukemia, prostate and renal cancer (Table 1).Thus, compounds **2b** (GP = 74.16 %), **4a** (GP = = 70.33 %), **4c** (GP = 70.06 %), **4g** (GP = 61.78 %), and **4h** (GP = 70.09 %) were moderately active on a renal cancer UO-31 cell line. Futhermore, the compound **2c** was active on a melanoma MALME-3M and the renal cancer A498 cell lines (GP = 42.19 and 50.22 %, respectively) and **4g** – on a prostate cancer PC-3 cell line (GP = 55.54 %).

4. Conclusions

In the present paper, the synthesis of twenty two new 4-thiazolidinone derivatives with isatin and 1,3,4-oxadiazole moieties (**3a-g**, **5a-k**, **6a-d**) in molecules was described. Primary anticancer assay of eight synthesized compounds 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, Betesda). In general, the synthesized compounds displayed a moderate antitumor activity on the tested cell lines, as well as some distinctive patterns of selectivity on some cell lines of the melanoma, leukemia, prostate and renal cancer.

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СИНТЕЗ ТА ПРОТИПУХЛИННА АКТИВНІСТЬ КОН'ЮГАТІВ ІЗАТИНУ, ОКСАДІАЗОЛУ ТА 4-ТІАЗОЛІДИНОНУ

Анотація. *За реакцією N-алкілювання 2,4-тіазолідиндіону та 5-заміщених ізатинів дією 2-хлоро-N-(5-арил-1,3,4-оксадіазол-2-іл)-ацетамідів* ***1a-c*** *синтезовано неконденсовані похідні оксадіазолу з тіазолідиновим* ***2a-c*** *або ізатиновим* ***4a-h*** *фрагментами в молекулах. Одержані сполуки використані в реакції Кньовенагеля з 5R-ізатинами (для* ***2a-c****) та похідними 4-тіазолідинону (для* ***4a-h****) з метою синтезу відповідних 5-іліденпохідних* ***3a-g, 5a-k*** *та* ***6a-d****. Для 8 синтезованих сполук вивчено їх протиракову активність на 60 лініях пухлинних клітин в Національному Інституті Раку (США).*

Ключові слова: *4-тіазолідинон, ізатин, 1,3,4-оксадіазол, алкілювання, конденсація Кньовенагеля, протиракова активність.*