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Synthesis and antitumor activity of 2-cyanocinnamic acid amides and their indole analogues

Yuliia Matiichuk[a], Iryna Drapak[a], Serhii Kramarenko[a], Yana Drapak[a], Liybov Kostyshyn[a], Nataliya Shtoyko[a] and Vasyl Matiychuk[b*]

[a]*Danylo Halytsky Lviv National Medical University, 69, Pekarska St., Lviv, Ukraine*
[b]*Ivan Franko National University of Lviv, 6 Kyryla and Mefodia, Lviv, Ukraine*

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By the reaction of substituted benzaldehydes and indol-3-carbaldehydes (2*E*)-2-cyano-*N*-[5-(R-benzyl)-1,3-thiazol-2-yl]-3-(R^1-phenyl)prop-2-enamides 3-(1-R^1-1*H*-indol-3-yl)-2-cyano-*N*-(5-(R-benzyl)-1,3-thiazol-2-yl)prop-2-enamides were synthesised. The antitumor activity of prepared compounds were investigated. 3-(1-Benzyl-1*H*-indol-3-yl)-2-cyano-*N*-(5-(2-chlorobenzyl)-1,3-thiazol-2-yl)prop-2-enamide (**7b**) has been identified as hit compound with values of MG-MID GI_{50} = 3.903 μM, TGI = 29.10 μM, LC_{50} = 57.54 μM.

1. Introduction

It has been reported that 2-arylacrylonitrile derivatives have shown promising anticancer activity.[1-4.] These researchers developed a model pharmacophore that includes an extended conjugated system spanning (spanning) two terminal aromatic rings, one of which contains an electron-withdrawing group that is critical for cytotoxicity. The central core of this conjugation is an acrylonitrile fragment, which also acts as a Michael acceptor (see **Fig. 1**).

* Corresponding author
E-mail address v_matiychuk@ukr.net (V. Matiychuk)

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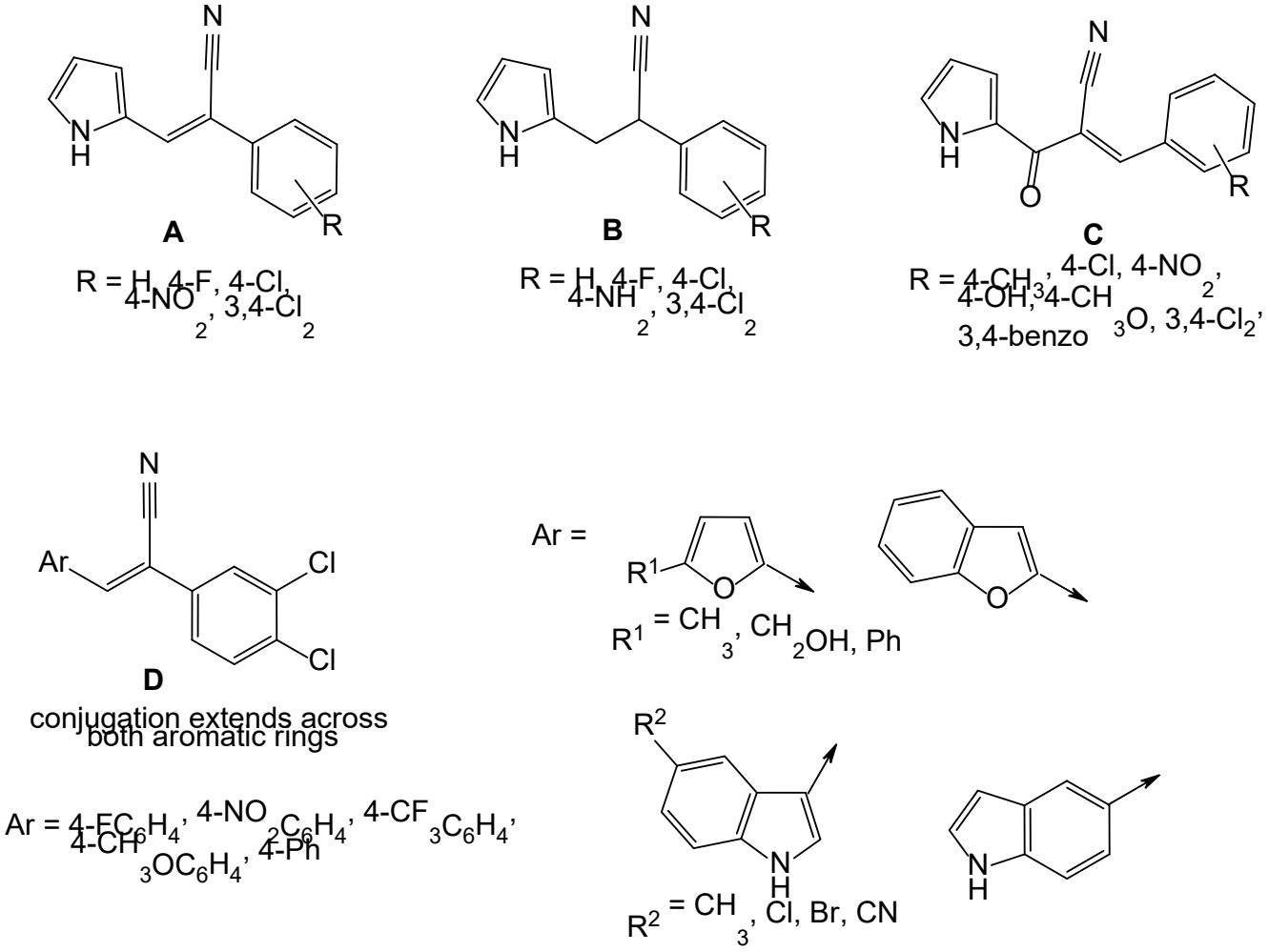

Fig. 1. Structure of acrylonitrile derivatives with anticancer activity.

In addition to the antitumor effect, other types of biological activity have been observed for this type of compound. Entacapone **E** is a commercial 4-dihydroxy-5-nitrobenzylidine derivative – a novel potent inhibitor of catechol-O-methyltransferase (COMT2).[5] COMT acts as the main metabolic enzyme for 3,4-dihydroxyphenyl-L-alanine (L-dopa) together with aromatic amino acid decarboxylase. L-Dopa is a precursor to dopamine. Deficient levels of this neurotransmitter are observed in the brains of patients with Parkinson's disease. Also have been reported to be non-peptide inverse agonists of the ghrelin receptor (GHSR)[6] (see **Fig. 2**).

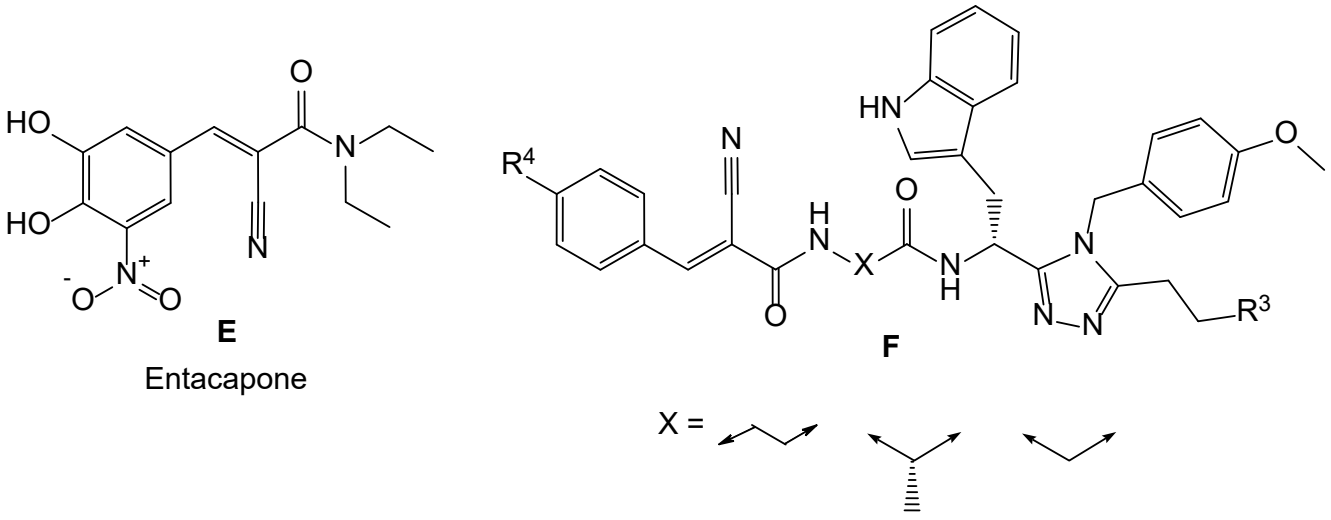

Fig. 2. Structure of acrylonitrile derivatives with biological activity.

2. Results and Discussion

In this work we described the synthesis and antitumor activity of (2*E*)-2-cyano-*N*-[5-(R-benzyl)-1,3-thiazol-2-yl]-3-(R^1-phenyl)prop-2-enamides and 3-(1-R^1-1*H*-indol-3-yl)-2-cyano-*N*-(5-(R-benzyl)-1,3-thiazol-2-yl)prop-2-enamides. This type of compound also has an acrylonitrile group and can be effectively conjugated through the enol form, which is stabilized by the formation of a hydrogen bond (see **Fig. 3**).

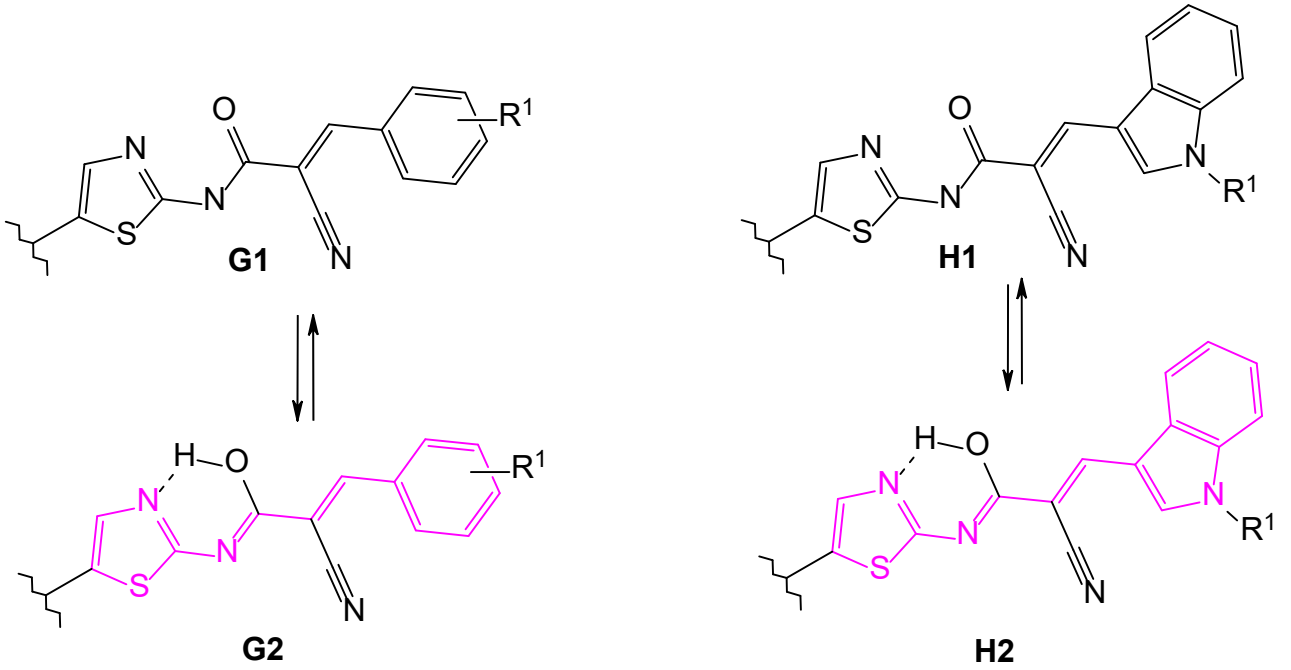

Fig. 3. The method of formation of conjugated π-system in compounds.

Cyanoacetylaminothiazoles **3a-c** were used as the starting substances (materials) for the synthesis of the target products. They were obtained by the reaction of 2-amino-5-arylmethylthiazoles **1** with 3-(3,5-dimethyl-pyrazol-1-yl)-3-oxo-propionitrile **2** in boiling benzene according to the procedure described in the articles.[7,8] (see **Fig. 4**).

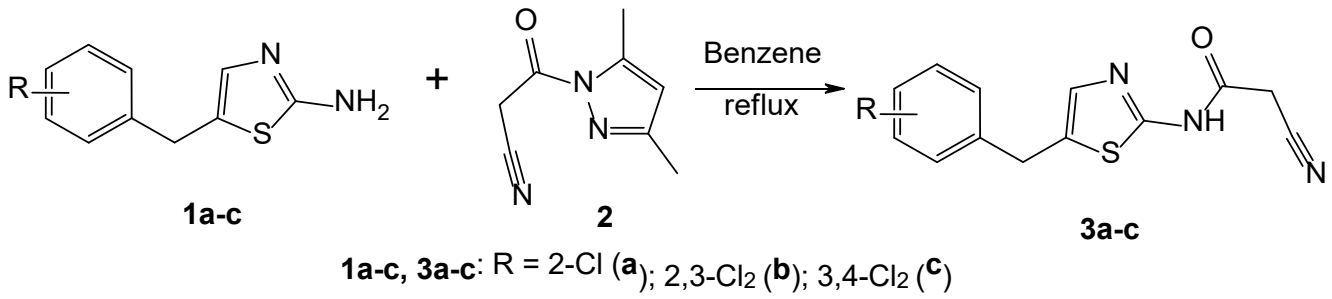

Fig. 4. Synthesis of cyanacetamides.

The obtained methylene-active derivatives **3a-c** in reaction with aromatic aldehydes **4a-d** or 1-R^1-1*H*-indole-3-carbaldehydes **5a, b** using homogeneous Knoevenagel reaction[9] were converted into the target (2*E*)-2-cyano-*N*-[5-(R-benzyl)-1,3-thiazol-2-yl]-3-(R^1-phenyl)prop-2-enamides **6a-d** and 3-(1-R^1-1*H*-indol-3-yl)-2-cyano-*N*-(5-(R-benzyl)-1,3-thiazol-2-yl)prop-2-enamides **7a-c**. This reaction was carried out in boiling ethanol in the presence of an aqueous solution of sodium hydroxide. The products **6a-d** and **7a-c** after filtration and recrystalization from ethanol-DMFA were isolated in 85-93% yields which are nearly quantitative preparative (see **Fig. 5**).

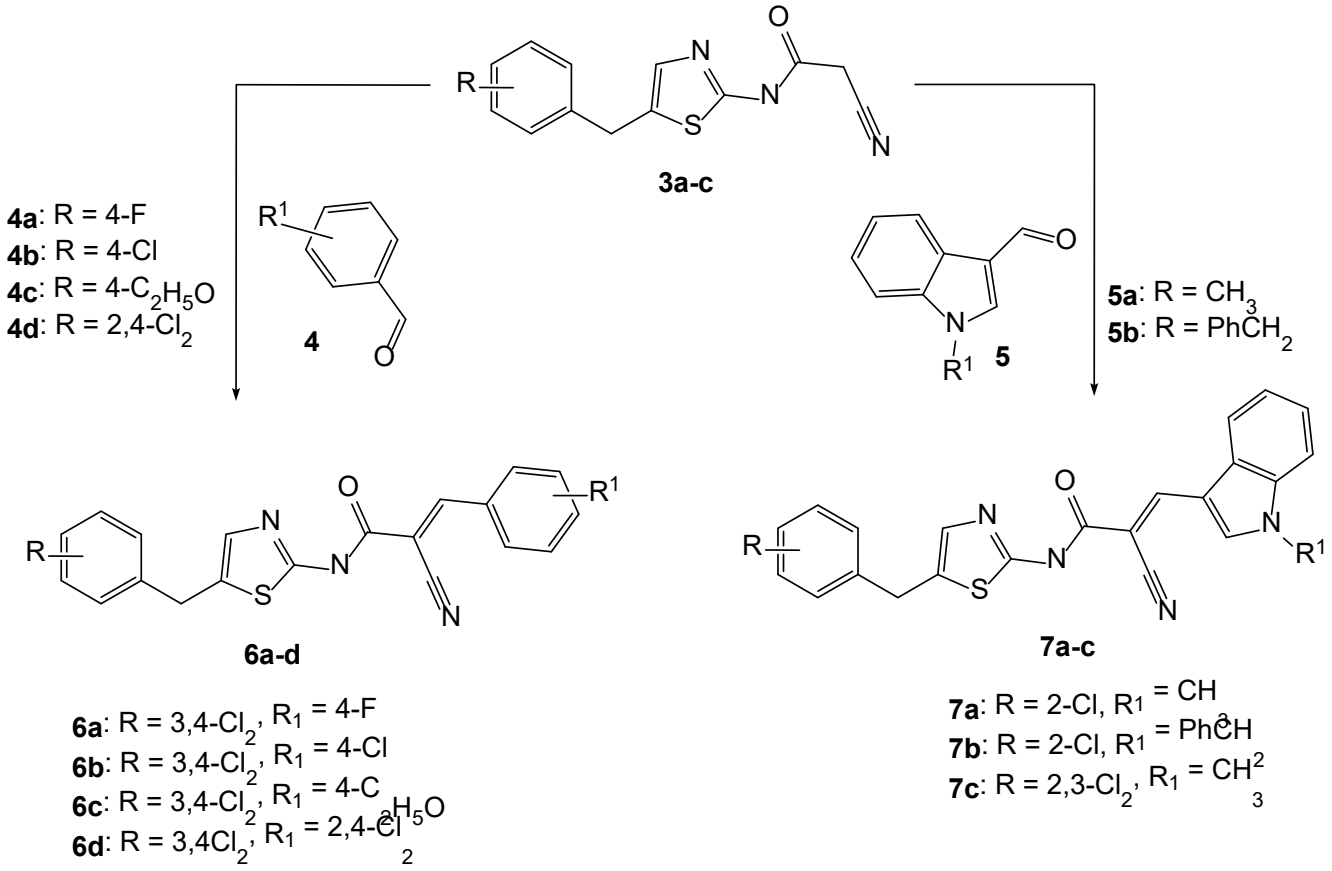

Fig. 5. Synthesis of target (2*E*)-2-cyano-*N*-[5-(R-benzyl)-1,3-thiazol-2-yl]-3-(R[1]-phenyl)prop-2-enamides **6a-d** and 3-(1-R[1]-1*H*-indole-3-yl)-2-cyano-*N*-(5-(R-benzyl)-1,3-thiazol-2-yl)prop-2-enamides **7a-c**.

The obtained compounds **6a-d** and **7a-c** are high-melting orange compounds, well soluble only in hot DMFA, DMSO, acetic acid and practically insoluble in water and classical non-polar solvents.

The structure of the obtained compounds **6a-d** and **7a-c** was proved by means of ^{1}H NMR spectroscopy. Signals of the NH group of the amide fragment were observed at 13.57–12.50 ppm. The CH= signal was 8.48–8.29 ppm for compounds **6a-d** and 8.76–8.73 ppm for compounds **7a-c**. The methylene group of the 5-arylmethyl fragment resonates in the region of 4.20–4.09 ppm.

It should be noted that derivatives with a similar structure (see **Fig. 6**) have significant antitumour potential [10]. The SAR analysis showed an important role of the cyanide group of the acrylonitrile fragment for biological activity. Attachment of the electron withdrawing group in position 2 of acrylonitrile fragment promotes cytotoxic potential. Bulky 2-aryl substituent of indole heterocycle is favorable for anti-cancer agent.

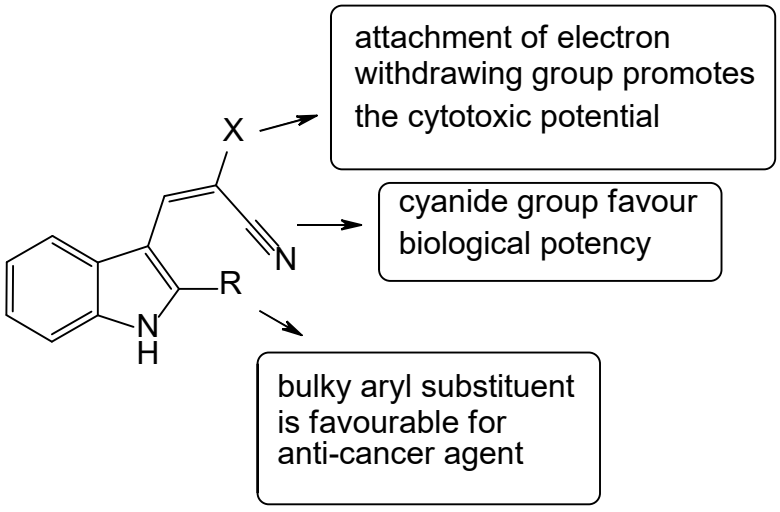

Fig. 6. Structural requirements of Knoevenagel-type indole derivatives for anticancer activity.[10]

The synthesized compounds were selected by the National Cancer Institute (NCI) Developmental Therapeutic Program[11] for the *in vitro* cell line screening to investigate their anticancer activity. Anticancer assays were performed according to the NCI protocol, which is described elsewhere.[12-15.] The screening results are shown in **Table 1**.

Table 1. Cytotoxic activity of the tested compounds in the concentration 10^{-5} M against 60 cancer cell lines

Compounds	Mitotic activity against 60 lines, GP %		Most sensitive lines (cancer line/type), GP %
	Average growth, %	Range of growth, %	
6a	91.15	50.41 – 107.67	ACHN (Renal Cancer) 61.12 K-562 (Leukemia) 59.17 UACC-62 (Melanoma) 58.54 LOX IMVI (Melanoma) 50.41
6b	80.77	22.7 – 111.70	HCT-15 (Colon Cancer) 38.53 LOX IMVI (Melanoma) 32.58 UACC-62 (Melanoma) 23.98 K-562 (Leukemia) 22.7
6c	85.94	35.15 – 110.54	LOX IMVI (Melanoma) 50.75 K-562 (Leukemia) 43.81 UACC-62 (Melanoma) 35.15
6d	82.21	25.47 – 113.37	ACHN (Renal Cancer) 47.73 HCT-15 (Colon Cancer) 37.68 UACC-62 (Melanoma) 35.15 LOX IMVI (Melanoma) 34.39 K-562 (Leukemia) 25.47
7a	101.46	79.50 – 119.83	UACC-62 (Melanoma) 79.50
7b	11.75	-74.64 – 93.14	OVCAR-4 (Ovarian Cancer) -74.64 ACHN (Renal Cancer) -58.39 NCI-H460 (Non-Small Cell Lung Cancer) -44.23 UACC-62 (Melanoma) -40.44 UACC-257 (Melanoma) -38.16 UO-31 (Renal Cancer) -34.32 CI-H226 (Non-Small Cell Lung Cancer) -33.58 NCI-H23 (Non-Small Cell Lung Cancer) -29.28 T-47D (Breast Cancer) -29.26 OVCAR-3 (Ovarian Cancer) -19.11 MDA-MB-435 (Melanoma) -15.10 MDA-MB-468 (Breast Cancer) -14.66 A498 (Renal Cancer) -13.25 CAKI-1 (Renal Cancer) -11.45 MALME-3M (Melanoma) -7.28 HOP-92 (Non-Small Cell Lung Cancer) -6.07 COLO 205 (Colon Cancer) -2.97 OVCAR-5 (Ovarian Cancer) -2.57 SR (Leukemia) 8.65
7c	89.92	41.43 – 111.93	SR (Leukemia) 55.37 MDA-MB-435 (Melanoma) 41.43

As can be seen from Table 1, the obtained amides of 2-cyanocinnamic acid **6a-d** showed moderate activity against leukemia cell line K-562 (GP = 25.47 – 59.17%) and melanoma cell lines LOX IMVI (GP = 32.58 – 50.75%) and UACC-62 (GP = 23.98 – 61.12%). For the above compounds, an increase in activity in the R^1 = 4-F → 4-C_2H_5O → 2,4-Cl_2 → 4-Cl series is observed.

For compounds **7a-c**, the antitumor effect significantly depends on the substituent in the first position of the indole ring. Compounds based on 1-methyl-3-indolecarbaldehyde **7a**, **c** were inactive. In contrast, the 1-benzyl-substituted derivative **7b** showed significant antitumor activity against the entire spectrum of malignant tumor lines, with cytotoxic effects observed for most cells. This compound **7b** was selected for an advanced assay against a panel of approximately sixty tumor cell lines at 10-fold dilutions of five concentrations: 100 μM, 10 μM, 1.0 μM, 0.1 μM and 0.01 μM (see **Table 2**).

It was found that compound **7b** exhibits a high level of anticancer activity with average values of MG-MID GI_{50} = 3.903 μM, TGI = 29.10 μM, LC_{50} = 57.54 μM. The most sensitive was the OVCAR-4 line of ovarian cancer with a value of GI_{50} = 0.393 μM, the NCI-H460 line of non-small cell lung cancer, GI_{50} = 0.530 μM and the ACHN line of renal cancer, GI_{50} = 0.823 μM.

Table 2. Results of an *in-vitro* in-depth study of the antitumor activity of compound **7b** at a concentration gradient (10^{-4}-10^{-8}M)

Cancer cell line	GI_{50}, μM	TGI, μM	LC_{50}, μM	Cancer cell line	GI_{50}, μM	TGI, μM	LC_{50} μM
Leukemia				**Melanoma**			
CCRF-CEM	2.51	>100	>100	LOX IMVI	4.61	48.8	>100
HL-60(TB)	>100	>100	>100	MALME-3M	1.78	4.14	9.62
K-562	3.03	>100	>100	M14	2.52	10.4	72.2
MOLT-4	2.49	>100	>100	MDA-MB-435	1.79	4.72	38.6
RPMI-8226	3.23	>100	>100	SK-MEL-2	2.05	4.85	14.6
SR	1.11	3.58	15.0	SK-MEL-28	2.36	6.74	55.8
Non-Small Cell Lung Cancer				SK-MEL-5	2.62	7.08	7.08
A549/ATCC	1.79	6.02	>100	UACC-257	3.30	>100	>100
EKVX	2.82	12.7	>100	UACC-62	1.52	3.16	6.58
HOP-62	2.52	10.4	37.2	**Ovarian Cancer**			
HOP-92	1.88	6.00	34.6	IGROV1	1.93	5.57	42.1
NCI-H226	1.70	5.67	>100	OVCAR-3	1.56	3.62	8.40
NCI-H23	1.71	4.08	9.72	OVCAR-4	0.393	1.61	4.80
NCI-H322M	2.74	>100	>100	OVCAR-5	1.70	4.59	>100
NCI-H460	0.530	1.99	5.92	OVCAR-8	1.35	3.13	7.23
NCI-H522	1.80	6.36	68.3	NCI/ADR-RES	2.68	13.3	>100
Colon Cancer				SK-OV-3	1.80	4.66	15.2
COLO 205	1.14	2.81	6.90	**Renal Cancer**			
HCC-2998	3.23	>100	>100	786-0	1.51	3.11	6.43
HCT-116	1.27	3.89	16.1	A498	1.08	2.87	7.62
HCT-15	2.83	>100	>100	ACHN	0.823	2.14	4.97
HT29	2.66	>100	>100	CAKI-1	1.43	3.75	9.81
KM12	1.89	6.62	>100	RXF 393	2.27	6.14	28.6
SW-620	1.52	4.20	>100	SN12C	1.57	3.88	9.64
CNS Cancer				TK-10	1.93	4.20	9.16
SF-268	2.79	23.4	>100	UO-31	1.46	3.18	6.91
SF-295	2.36	6.87	30.9	**Breast Cancer**			
SF-539	1.68	3.42	6.95	MCF7	3.01	>100	>100
SNB-19	3.07	17.8	99.2	MDA-MB-231/ATCC	1.93	4.79	23.2
SNB-75	1.50	4.54	35.4				
U251	1.36	2.92	6.28	HS 578T	1.93	7.08	>100
Prostate Cancer				BT-549	2.94	11.6	53.2
PC-3	2.01	7.48	>100	T-47D	1.69	4.15	26.8
DU-145	2.76	>100	>100	MDA-MB-468	1.84	4.68	21.3

To interpret the results of the anti-tumour activity screening, we calculated the selectivity index (SI) at the GI_{50} level of **7b**, which is equal to the ratio of the average MG-MID activity (μM) for all cell lines to the average value of the corresponding indicator for a particular type of cancer. An SI value in the range of 3-6 is considered to be moderately selective. A value of SI > 6 indicates high selectivity for the respective cell line. Compounds that do not meet any of the criteria are considered non-selective.[16] The calculated selectivity index of compound **7b** against the above-mentioned cell lines at the GI_{50} level was 9.931 against OVCAR-4 (Ovarian Cancer), 7.364 against NCI-H460 (Non-Small Cell Lung Cancer) and 4.742 against ACHN (Renal Cancer), indicating a high selective effect against these individual tumour lines. The selectivity of the action was observed at the TGI level in relation to ovarian cancer and renal cancer (see **Table 3**).

Table 3. Anticancer selectivity pattern of the most active compound **7b** at GI_{50}, TGI and LC_{50} levels (C, μM)

Compound	Parameters	Subpanel tumor cell lines*								
		L	NSCLC	ColC	CNSC	M	OV	RC	PC	BC
7b	GI_{50}	18.73	1.943	2.077	2.127	2.506	1.630	1.509	2.385	2.223
	SI	0.208	2.009	1.879	1.835	1.557	2.394	2.586	1.636	1.756
	TGI	83.93	17.02	45.36	9.825	21.10	5.211	3.659	53.74	22.05
	SI	0.347	1.710	0.642	2.962	1.379	5.584	7.953	0.541	1.320
	LC_{50}	85.83	61.75	74.71	46.46	44.94	39.68	10.39	100	54.08
	SI	0.670	0.932	0.770	1.238	1.280	1.450	5.538	0.575	1.064

*L – leukemia, NSCLC – non-small cell lung cancer, ColC – colon cancer, CNSC – CNS cancer, M – melanoma, OV– ovarian cancer, RC – renal cancer, PC – prostate cancer, BC – breast cancer.

A comparison of the antitumor effect of compound **7b** with the classical organic antitumor agents – 5-Fluorouracil (5-FU) and Gefitinib, platinum complex – Cisplatin, and the natural antitumor substance Curcumin is shown in **Table 4**. It can be concluded that the investigated compound is more active than the mentioned reference drugs.

Table 4. Mean growth inhibitory concentration (GI_{50}, μM) of compound **7b** in comparison with 5-FU, Gefitinib, Cisplatin and Curcumin

Compound	Subpanel tumor cell lines									
	L	NSCLC	ColC	CNSC	M	OV	RC	PC	BC	MG-MID
7b	18.73	1.943	2.077	2.127	2.506	1.630	1.509	2.385	2.223	3.903
5-FU	15.1	>100	8.4	72.1	70.6	61.4	45.6	22.7	76.4	52.5
Gefitinib	3.54	7.81	7.02	8.14	5.28	6.63	2.67	1.65	7.81	3.24
Cisplatin	6.3	9.4	21.0	4.7	8.5	6.3	10.2	5.6	13.3	9.48
Curcumin	3.7	9.2	4.7	5.8	7.1	8.9	10.2	11.2	5.9	7.41

It should be noted that our results are correlated with the results presented in this article.[10] It should also be noted that the bulk substituent in the second position of the indole cycle was replaced by a benzyl substituent in the first position of the indole cycle. Due to the flexibility of this radical, the benzene ring probably takes a similar role in binding to the receptor as compound **7b** (see **Fig. 7**).

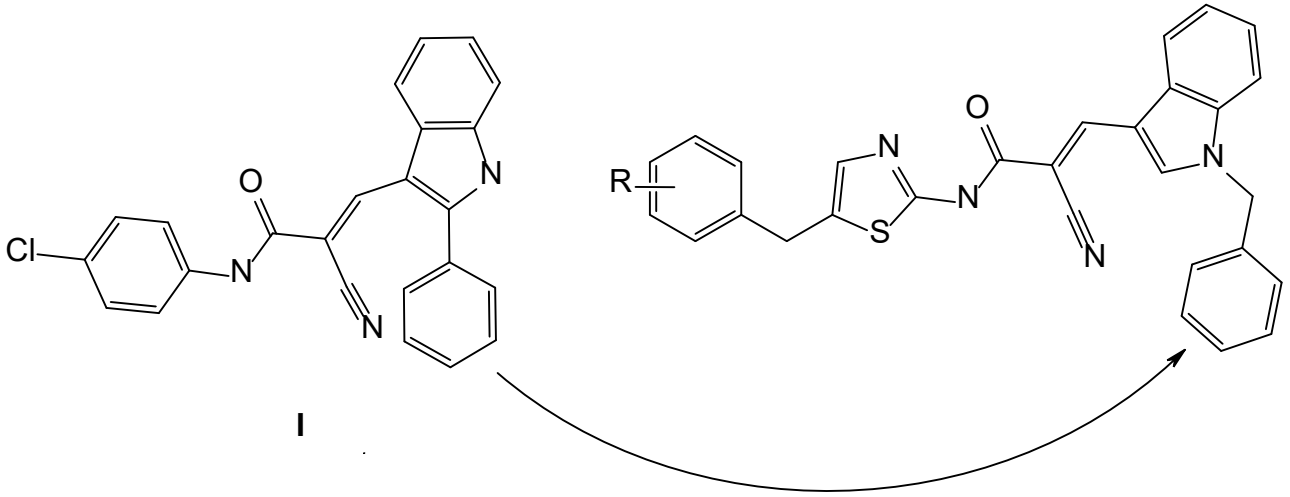

Fig. 7. Similarity of compounds **I** and **7b**.

3. Conclusions

As a result of this work, some new (2*E*)-2-cyano-*N*-[5-(R-benzyl)-1,3-thiazol-2-yl]-3-(R[1]-phenyl)prop-2-enamides and 3-(1-R[1]-1*H*-indol-3-yl)-2-cyano-*N*-(5-(R-benzyl)-1,3-thiazol-2-yl)prop-2-enamides were prepared and their anticancer activity was investigated. The synthesis method used in this work is preparatively simple and provides high yields of the target products. The structures of the obtained compounds were confirmed by ^{1}H and ^{13}C NMR spectroscopy and elemental analysis. 3-(1-Benzyl-1*H*-indol-3-yl)-2-cyano-*N*-(5-(2-chlorobenzyl)-1,3-thiazol-2-yl)prop-2-enamide (**7b**) was identified as hit compound with a high anti-tumour activity that exceeds the activity of the comparison drugs - 5-FU, Gefitinib, Cisplatin and Curcumin. The selectivity towards Subpanel tumour cell lines and individual cancer cell lines was calculated.

Funding

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4. Experimental

4.1. Materials and Methods

Commercially available reagents and solvents were used for the synthetic part of the work. ^{1}H NMR spectra were obtained in pulsed Fourier transform mode on a Varian VXR-400 spectrometer (400 MHz) in DMSO-d_6, while ^{13}C NMR spectra of all compounds were recorded on a Bruker Avance DRX-500 spectrometer (125 MHz). Elemental analysis was carried out on a Perkin Elmer 2400 CHN analyser. All melting points were determined in an open capillary and left uncorrected.

4.2. General procedure

*General procedure for the synthesis of cyanacetamides **3a-c.*** To a solution of 0.01 mol of aminothiazole **1a-c** in 15 ml of benzene was added a solution of 0.01 mol of 2,5-dimethyl-1-cyanoacetylpyrazole. The mixture was refluxed for 15-30 min. Cooled, the resulting precipitate was filtered and recrystallised from alcohol.

*N-[5-(2-chlorobenzyl)-1,3-thiazol-2-yl]-2-cyanoacetamide (**3a**).* Yield 91%. M.p. 201–202°C. ^{1}H NMR (400 MHz, DMSO), δ, ppm: 12.33 (s, 1H, NH), 7.45 (dd, *J* = 7.4, 1.3 Hz, 1H, C_6H_4), 7.41 (dd, *J* = 7.3, 1.6 Hz, 1H, C_6H_4), 7.35–7.27 (m, 2H, C_6H_4), 7.25 (s, 1H, thiazole), 4.19 (s, 2H, CH_2), 3.98 (s, 2H, CH_2). Anal Calcd for $C_{13}H_{10}ClN_3OS$: C, 53.52; H, 3.45; N, 14.40; found C, 53.54; H, 3.30; N, 14.31%.

*N-[5-(2,3-dichlorobenzyl)-1,3-thiazol-2-yl]-2-cyanoacetamide (**3b**).* Yield 89%. M.p. 211–212°C. ^{1}H NMR (400 MHz, DMSO), δ, ppm: 12.45 (s, 1H, NH), 7.48 (dd, *J* = 7.8, 1.2 Hz, 1H, C_6H_3), 7.42–7.38 (m, 2H, C_6H_3), 7.29 (s, 1H, thiazole), 4.23 (s, 2H, CH_2), 3.99 (s, 2H, CH_2). Anal Calcd for $C_{13}H_9Cl_2N_3OS$: C, 47.87; H, 2.78; N, 12.88; found C, 47.85; H, 2.76; N, 12.85%.

*N-[5-(3,4-dichlorobenzyl)-1,3-thiazol-2-yl]-2-cyanoacetamide (**3c**).* Yield 87%. M.p. 214–215°C. ^{1}H NMR (400 MHz, DMSO), δ, ppm: 12.47 (s, 1H, NH), 7.57 (d, *J* = 3.8 Hz, 1H, C_6H_3), 7.41 (d, *J* = 7.8 Hz, 2H, C_6H_3), 7.34 (s, 1H, thiazole), 4.29 (s, 2H, CH_2), 4.01 (s, 2H, CH_2). Anal Calcd for $C_{13}H_9Cl_2N_3OS$: C, 47.87; H, 2.78; N, 12.88; found C, 47.84; H, 2.76; N, 12.90%.

*General procedure for the synthesis of (2E)-2-cyano-N-[5-(R-benzyl)-1,3-thiazol-2-yl]-3-(R^1-phenyl)prop-2-enamide **6a-d** and 3-(1-R^1-1H-indol-3-yl)-2-cyano-N-(5-(R-benzyl)-1,3-thiazol-2-yl)prop-2-enamide **7a-c**.* To a solution of 0.001 mol of *N*-[5-R-benzyl-1,3-thiazol-2-yl]-2-cyanoacetamide **3** in 5 ml of ethanol was added of 0.001 mol of aromatic aldehyde **4** or 1-R^1-1*H*-indole-3-carbaldehyde **5** and one drops of 10% water solution of NaOH was added. The mixture was refluxed for 30 min. The precipitate formed was filtered off, washed with ethanol, and the product was purified by recrystallization from a mixture of ethanol–DMFA.

*(2E)-2-cyano-N-[5-(3,4-dichlorobenzyl)-1,3-thiazol-2-yl]-3-(4-fluoro-phenyl)prop-2-enamide (**6a**).* Yield 87%. M.p. 250–251°C. ^{1}H NMR (400 MHz, DMSO), δ, ppm: 13.14 (s, 1H, NH), 8.35 (s, 1H, CH=), 8.07 (dd, *J* = 8.6, 5.5 Hz, 2H, 6H-C_6H_3), 7.59 (d, *J* = 3.7 Hz, 1H, 2H-C_6H_3), 7.58 (d, *J* = 6.2 Hz, 1H, 5H-C_6H_3), 7.43 (т, *J* = 8.8 Hz, 2H, C_6H_4), 7.37 (s, 1H, thiazole), 7.29 (dd, *J* = 8.3, 1.8 Hz, 1H, C_6H_4), 4.09 (s, 2H, CH_2). Anal Calcd for $C_{20}H_{12}Cl_2FN_3OS$: C, 55.57; H, 2.80; N, 9.72; found C, 53.46; H, 2.83; N, 9.69%.

*(2E)-3-(4-chlorophenyl)-2-cyano-N-[5-(3,4-dichlorobenzyl)-1,3-thiazol-2-yl]prop-2-enamide (**6b**).* Yield 85%. M.p. 255–257°C. ^{1}H NMR (400 MHz, DMSO), δ, ppm: 13.24–12.75 (br. s, 1H, NH), 8.34 (s, 1H, CH=), 8.00 (d, *J* = 8.0 Hz, 2H, C_6H_4), 7.65 (d, *J* = 8.0 Hz, 2H, C_6H_4), 7.60–7.54 (m, 2H, C_6H_3), 7.36 (s, 1H, thiazole), 7.30 (d, *J* = 8.1 Hz, 1H, C_6H_3), 4.10 (s, 2H, CH_2). Anal Calcd for $C_{20}H_{12}Cl_3N_3OS$: C, 53.53; H, 2.70; N, 9.36; found C, 53.49; H, 2.61; N, 9.23%.

*(2E)-2-cyano-N-[5-(3,4-dichlorobenzyl)-1,3-thiazol-2-yl]-3-(4-ethoxy-phenyl)prop-2-enamide (**6c**).* Yield 91%. M.p. > 260°C. ^{1}H NMR (400 MHz, DMSO), δ, ppm: 13.07–12.63 (br. s, 1H, NH), 8.29 (s, 1H, CH=), 8.00 (d, *J* = 7.0 Hz, 2H, Ar), 7.58 (s, 2H, Ar), 7.34 (s, 1H, thiazole), 7.29 (d, *J* = 8.0 Hz, 1H, Ar), 7.12 (d, *J* = 7.1 Hz, 2H, Ar), 4.14 (k, *J* = 6.2 Hz, 2H, CH_2), 4.10 (s, 2H, CH_2), 1.35 (t, *J* = 6.2 Hz, 3H, CH_3). Anal Calcd for $C_{22}H_{17}Cl_2N_3O_2S$: C, 57.65; H, 3.74; N, 9.17; found C, 57.72; H, 3.69; N, 9.21%.

*(2E)-2-cyano-N-[5-(3,4-dichlorobenzyl)-1,3-thiazol-2-yl]-3-(2,4-dichloro-phenyl)prop-2-enamide (**6d**).* Yield 86%. M.p. > 260°C. ^{1}H NMR (400 MHz, DMSO), δ, ppm: 13.57–12.99 (br. s, 1H, NH), 8.48 (s, 1H, CH=), 8.10 (d, *J* = 8.5 Hz, 1H, C_6H_3), 7.86 (d, *J* = 1.7 Hz, 1H, C_6H_3), 7.65 (dd, *J* = 8.5, 1.7 Hz, 1H), 7.62–7.54 (m, 2H, C_6H_3), 7.39 (s, 1H, thiazole), 7.30 (dd, *J* = 8.2, 1.7 Hz, 1H), 4.09 (s, 2H, CH_2). Anal Calcd for $C_{20}H_{11}Cl_4N_3OS$: C, 49.71; H, 2.29; N, 8.70; found C, 48.78; H, 2.34; N, 8.66%.

*N-[5-(2-Chloro-benzyl)-thiazol-2-yl]-2-cyano-3-(1-methyl-1H-indol-3-yl)-acrylamide (**7a**)* Yield 87%. M.p. 238–239°C. ^{1}H NMR (400 MHz, DMSO), δ, ppm: 12.64 (s, 1H, NH), 8.73 (s, 1H, CH=), 8.50 (s, 1H, 2H-indol), 8.01 (d, *J* = 6.9 Hz, 1H, Ar), 7.61 (d, *J* = 7.7 Hz, 1H, Ar), 7.45 (t, *J* = 8.0 Hz, 2H, Ar), 7.29–7.35 (m, 4H, Ar), 4.19 (s, 2H, CH_2), 3.96 (s, 3H, CH_3). Anal Calcd for $C_{23}H_{17}ClN_4OS$: C, 63.81; H, 3.96; N, 12.94; found C, 63.79; H, 3.99; N, 12.89%.

*3-(1-Benzyl-1H-indol-3-yl)-2-cyano-N-(5-(2-chlorobenzyl)-1,3-thiazol-2-yl)prop-2-enamide (**7b**).* Yield 90%. M.p. > 260°C. ^{1}H NMR (400 MHz, DMSO), δ, ppm: 12.88–12.50 (br. s, 1H, NH), 8.76 (s, 1H, CH=), 8.67 (s, 1H, 2H-indol), 8.12–7.95 (m, 1H, Ar), 7.68–7.57 (m, 1H, Ar), 7.50–7.39 (m, 2H, Ar), 7.36–7.28 (m, 9H, Ar), 5.65 (s, 2H, CH_2), 4.19 (s, 2H, CH_2). ^{13}C NMR (125 MHz, DMSO), δ C, ppm: 142.98, 137.76, 137.07, 136.52, 133.96, 133.23, 131.41, 129.92, 129.24, 129.17, 128.60, 128.30, 128.06, 127.75, 124.11, 122.62, 119.40, 118.68, 112.14, 109.97, 50.55, 30.44. Anal Calcd for $C_{29}H_{21}ClN_4OS$: C, 68.43; H, 4.16; N, 11.01; found C, 68.36; H, 4.20; N, 11.17%.

*2-Cyano-N-[5-(2,3-dichloro-benzyl)-thiazol-2-yl]-3-(1-methyl-1H-indol-3-yl)-acrylamide (**7c**).* Yield 86%. M.p. 240–241°C. ^{1}H NMR (400 MHz, DMSO), δ, ppm: 12.64 (s, 1H, NH), 8.74 (s, 1H, CH=), 8.52 (s, 1H, 2H-indol), 8.02 (d, *J* = 7.8 Hz, 1H, Ar), 7.62 (d, *J* = 7.4 Hz, 1H, Ar), 7.57 (dd, *J* = 7.9, 1.6 Hz, 1H, Ar), 7.44 (dd, *J* = 7.7, 1.5 Hz, 1H, Ar), 7.30–7.39 (m, 4H, Ar), 4.20 (s, 2H, CH_2), 3.94 (s, 3H, CH_3). Anal Calcd for $C_{23}H_{16}Cl_2N_4OS$: C, 59.11; H, 3.45; N, 11.99; found C, 59.09; H, 3.41; N, 12.01%.

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