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Anticancer properties of some triazolo[3,4-*b*][1,3,4]thiadiazoles

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In the present work, we presented an efficient synthesis and anticancer activity evaluation of some new 3-R-6-(5-arylfuran-2-yl-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazoles. We have shown that the proposed synthetic protocols provided the possibility to design triazolothiadiazoles diversity with a considerable chemical novelty. The structures of target substances were confirmed by using ^{1}H NMR spectroscopy, mass spectrometry and elemental analysis. The synthesized compounds were selected by the National Cancer Institute Developmental Therapeutic Program for the *in vitro* cell line screening. Among all the substances tested, three compounds exhibited significant cytotoxic activity.

1. Introduction

Cancer disorders are one of the most dangerous medical, biological and social problems today and are the cause of up to 12% of all deaths and inferior to this indicator only to cardiovascular diseases.[1] Despite the rapid development of modern organic, pharmaceutical and medicinal chemistry, the effectiveness of antitumor drugs remains low. This is largely due to the nonspecificity of their action, the resistance of tumors, insufficient study of the mechanisms of disease pathogenesis. In addition, the treatment of serious diseases is expensive, for example, the therapy of stage III cancer costs 25-30 thousand dollars, and oncohematology - 50 thousand dollars. Nevertheless, even with full funding, the effectiveness of treatment is no more than 50%. Most of the obtained compounds are not clinically used in consequence to their high toxicity, poor solubility in water, indiscriminate action and a number of other side effects.[2] Therefore, the problem of investigating new, more effective drugs remains relevant. To overcome these limitations, the search for new effective and safe anticancer drugs continues around the world.

One of the promising methods to solve this problem is the screening of potential antitumor agents among new synthesized compounds. Nitrogen-based heterocyclic analogues are an extremely important class of organic substances that are widely used in medicinal chemistry, as more than 60% of drugs and more than 85% of biologically active substances described in the literature contain a nitrogen-containing heterocycle.[3] In recent years, there has been an increasing interest in condensed nitrogen-containing heterocyclic systems, as many of them exhibit different types of pharmacological activity.[4-6] [1,2,4]Triazolo[3,4-*b*][1,3,4]thiadiazoles are one of the little-studied and hard-to-reach representatives of this class of

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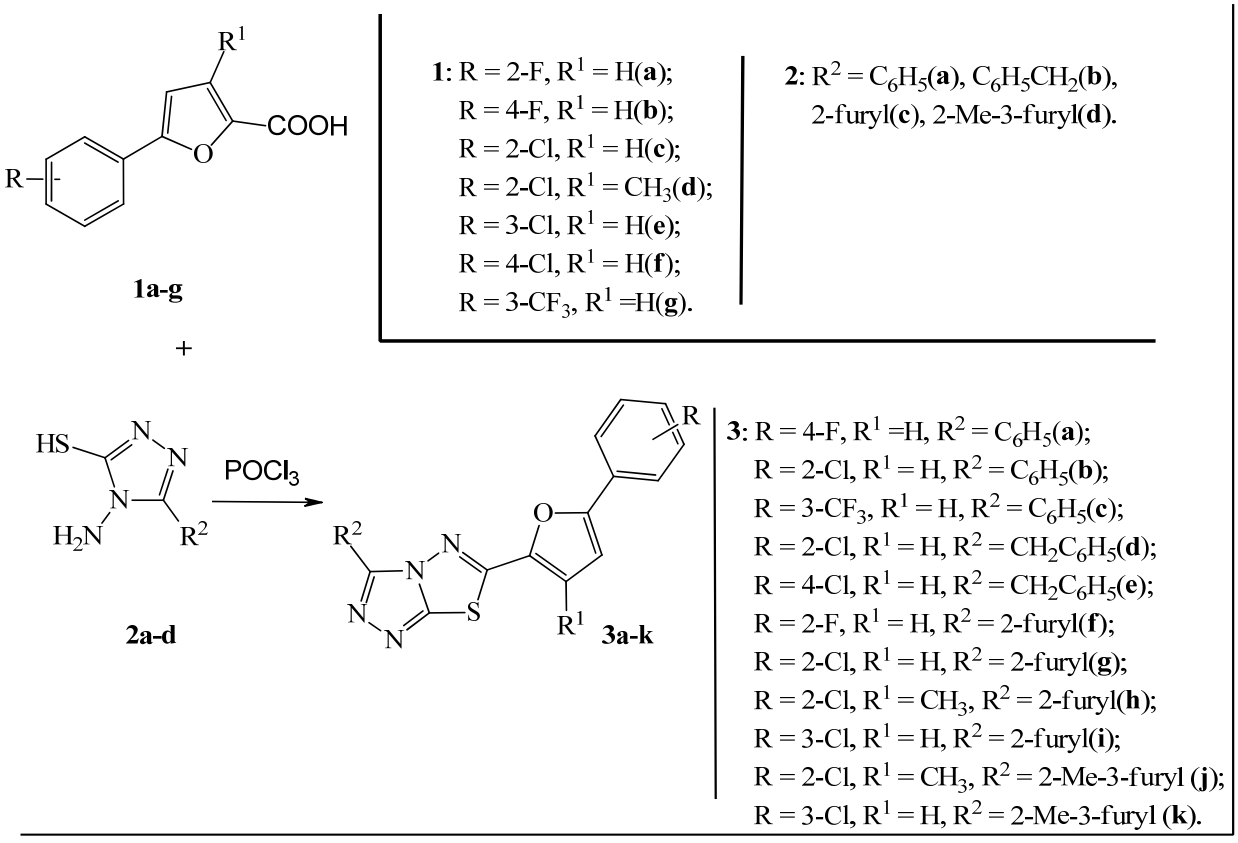
compounds.[7] The presence of considerable material on the chemistry and biological properties of this class of compounds allows us to consider them as one of the promising classes of biologically active compounds with a wide range of action. Among the triazolothiadiazole derivatives, there have been identified compounds that possess antitumor,[8-10] antimicrobial,[11-13] antidipressant,[13] anticonvulsant[14] and other activities. They are inhibitors of viral helicase,[15] cholinesterases,[16] carbonic anhydrases,[17] c-Met kinases.[18] Considering mentioned above, the search for new antitumor agents among this class of compounds is an interesting and relevant direction.

2. Results and Discussion

2.1 Chemistry

The purpose of this work, which is a continuation of our research on the synthesis and analysis of biologically active substances[19-29], is the synthesis of 3-R-6-(5-arylfuran-2-yl-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole derivatives and the study of their anticancer activity. Cyclization of carboxylic acids with 5-substituted 4-amino-4*H*-1,2,4-triazolo-3-thioles is one of the most common methods for the synthesis of [1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole derivatives.[7] Various aliphatic and aromatic carboxylic acids have been studied quite well in this cyclization. However, carboxylic acids of the heterocyclic series in the synthesis of [1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazoles have been insufficiently researched. In this work, we carried out the synthesis of 3-R-6-(5-arylfuran-2-yl-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazoles (**3a-k**) derivatives. For this purpose, we studied the interaction of 5-arylfuran-2-carboxylic acids **1a-g** with 5-substituted 4-amino-4*H*-1,2,4-triazole-3-thioles **2a-d**. It was found that when the above reagents are heated in phosphorus oxochloride, cyclization takes place with the closure of the thiadiazole cycle and the formation of [1,2,4]triazolo [3,4-*b*][1,3,4]thiadiazole system (**Scheme 1**).

Methods of quantitative elemental analysis and ^{1}H NMR spectroscopy were used to confirm the structure and individuality of the synthesized substances. Interpretation of the spectra revealed that the signals for protons of all structural units were observed in their characteristic ranges.



Scheme 1. Synthesis of some triazolo[3,4-*b*][1,3,4]thiadiazoles

2.2 Anticancer activity

The anticancer activity of the synthesized compounds was evaluated within the framework of an international cooperation program with the National Cancer Institute (Bethesda, Maryland, USA) DTP NCI (Developmental Therapeutic Program). The 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.[30-33] The quantitative criterion of the compounds activity was the percentage of the cancer cell lines growth (GP,%)

in comparison with the control. The results of the anticancer activity study are presented in Table 1. It was found that the synthesized compounds showed anticancer activity at different levels. The most active were compounds **3d** and **3e** with values of average mitotic activity of 48.95% and 49.39%. A feature of their structure is the presence of a benzyl radical in position 3 of the [1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazoles cycle. It should also be noted the high cytotoxic effect of compound **3e** relative to COLO 205 Colon Cancer -21.37%, MALME-3M Melanoma -9.92%, SK-MEL-5 Melanoma -7.64% and compound **3j** relative to TK-10 Renal Cancer -21.22% and ACHN Renal Cancer -11.46%.

Table 1. Cytotoxicity of compounds **3a-k** at a concentration of 10^{-5} M on 60 cancer cell lines.

Compound	Average mitotic activity on 60 lines,%	Mitotic activity range on 60 lines,%	Most sensitive cell line (cancer line/type) GP, %
3a	99.41	61.16 - 143.26	UO-31 Renal Cancer 61.16
3b	77.63	16.01 - 136.42	NCI-H460 (Non-Small Cell Lung Cancer) 16.01 HCT-15 Colon Cancer 39.80 HCT-116 Colon Cancer 40.54 SF-295 CNS Cancer 42.12 K-562 Leukemia 47.04
3c	93.03	61.09 - 119.38	UO-31 Renal Cancer 61.09 PC-3 Prostate Cancer 62.54
3d	48.95	5.86 – 93.33	NCI-H460 Non-Small Cell Lung Cancer 9.82 CAKI-1 Renal Cancer 5.86 LOX IMVI Melanoma 6.04 HCT-116 Colon Cancer 28.92 HCT-15 Colon Cancer 22.54 HT29 Colon Cancer 25.85 SW-620 Colon Cancer 29.63
3e	49.39	-21.37 - 95.14	COLO 205 Colon Cancer -21.37 MALME-3M Melanoma -9.92 SK-MEL-5 Melanoma -7.64 M14 Melanoma 5.65 HCC-2998 Colon Cancer 13.55 NCI-H460 Non-Small Cell Lung Cancer 14.61 NCI-H23 Non-Small Cell Lung Cancer 15.62
3f	93.60	55.08 - 127.49	A498 Renal Cancer 55.08 UO-31 Renal Cancer 61.42
3g	61.37	5.91 - 109.78	NCI-H460 Non-Small Cell Lung Cancer 5.91 HCT-116 Colon Cancer 24.93 HCT-15 Colon Cancer 24.80 U251 CNS Cancer 34.61 LOX IMVI Melanoma 32.42 IGROV1 Ovarian Cancer 34.06 RXF 393 Renal Cancer 32.18
3h	88.99	49.61 - 118.42	HCT-15 Colon Cancer 49.61 U251 CNS Cancer 60.36 SK-MEL-5 Melanoma 61.25
3i	95.96	65.86 - 132.86	K-MEL-5 Melanoma 65.86
3j	59.10	-21.22 - 94.47	TK-10 Renal Cancer -21.22 ACHN Renal Cancer -11.46 OVCAR-4 Ovarian Cancer 12.77 RPMI-8226 Leukemia 16.57
3k	107.98	89.90 - 126.29	SR Leukemia 21.36 CAKI-1 Renal Cancer 89.90

According to the standard NCI procedure Compounds **3d**, **3e** and **3j** were selected for the secondary phase of the study, which consisted of testing them on 60 cancer cell lines at five concentrations at 10-fold dilution (100μM, 10μM, 1μM, 0.1μM and 0.01μM. Based on experimental data of thorough in vitro screening of compounds, three dose-dependent parameters were calculated:

GI_{50} - concentration that causes inhibition of 50% of cell lines growth;
TGI - the concentration of the compound that leads to a complete inhibition of growth;
LC_{50} is the concentration of a substance that leads to 50% cell death.
GI_{50} is interpreted as an effective level of inhibition, TGI as a cytostatic effect, and LC_{50} is a lethal concentration that characterizes the cytotoxic effect. If the studied parameters values (GI_{50}, TGI and LC_{50}) are less than 100μM, the compounds are considered to be active (relative to this line).

The obtained results of thorough *in vitro* screening of compounds are shown in table 2. The most active compound was **3d** for which MG-MID GI_{50} was 3.410. In contrast, the activity of compounds **3e** and **3j** was an order of magnitude lower. For them, the MG-MID GI_{50} were 27.280 and 52.176, respectively. Of particular note are the sensitivity of CCRF-CEM, CNS U251, epithelial bowel cancer HCT-116 and HCT-15, and LOX IMVI melanoma lines. These compounds caused

inhibition of the growth of 50% of the line cells at concentrations below 0.01μM. Cytotoxic effect was not observed in most lines of malignant tumors. NCI-H460 line exception (5.89 E-5) Non-small cell lung cancer, HCC-2998 7.10E-5 and KM12 5.66 E-5 Epithelial bowel cancer, UACC-62 9.85 E-5 Melanoma, OVCAR-3 5.16 E-5 Ovarian cancer in the case of compound **3d**. A similar effect was observed for the SK-MEL-5 8.58E-6 Melanoma line in the case of compound **3e**.

Table 2. *In vitro* study of compounds **3d**, **3e** and **3j** anticancer activity on 60 cancer cell lines at a concentration gradient (10^{-4}-10^{-8}M).

Cancer cell line	Anticancer activity in vitro, μM					
	3d		3e		3j	
	GI_{50}	TGI	GI_{50}	TGI	GI_{50}	TGI
1	2	3	4	5	6	7
Leucemia						
CCRF-CEM	< 0.01	> 100	> 100	> 100	> 100	> 100
HL-60 (TB)	0.630	82.5	> 100	> 100	> 100	> 100
K-562	0.0608	> 100	> 100	> 100	7.12	> 100
MOLT-4	0.721	> 100	> 100	> 100	> 100	> 100
RPMI-8226	2.50	> 100	43.0	> 100	> 100	> 100
SR	0.0851	> 100	17.1	> 100	4.14	> 100
Non-Small Cell Lung Cancer						
A549/ATCC	0.194	> 100	6.22	> 100	.	> 100
EKVX	1.25	59.6	3.57	> 100	.	> 100
HOP-62	10.2	> 100	15.8	> 100	11.6	> 100
HOP-92	7.81	45.8	3.84	> 100	3.94	> 100
NCI-H226	5.67	52.5	4.91	> 100	2.14	> 100
NCI-H23	1.26	> 100	3.51	> 100	> 100	> 100
NCI-H322M	.	> 100	9.69	> 100	> 100	> 100
NCI-H460	0.0628	11.8	3.17	> 100	4.47	> 100
NCI-H522	0.585	52.4	5.33	> 100	6.28	> 100
CNS Cancer						
SF-268	0.208	79.1	> 100	> 100	8.41	> 100
SF-295	0.0684	12.6	2.30	-	4.87	> 100
SF-539	11.1	47.2	2.61	-	> 100	> 100
SNB-19	0.362	> 100	-	-	> 100	> 100
SNB-75	10.7	> 100	2.33	> 100	4.83	> 100
U251	< 0.01	52.6	-	> 100	4.22	> 100
Colon cancer						
COLO 205	0.472	47.8	2.63	7.08	> 100	> 100
HCC-2998	0.120	20.4	2.74	6.92	> 100	> 100
HCT-116	< 0.01	85.4	3.69	> 100	5.58	> 100
HCT-15	< 0.01	> 100	2.58	> 100	5.00	> 100
HT29	0.218	> 100	3.97	> 100	.	> 100
KM12	0.211	17.8	4.74	> 100	.	> 100
SW-620	0.469	65.6	6.79	> 100	.	> 100
Melanoma						
LOX IMVI	< 0.01	> 100	5.77	> 100	4.11	> 100
MALME-3M	3.33	54.3	2.09	5.88	> 100	> 100
M14	10.3	> 100	2.77	> 100	> 100	> 100
MDA-MB-435	0.307	41.1	3.95	> 100	.	> 100
SK-MEL-2	11.8	34.6	2.94	.	47.8	> 100
SK-MEL-28	8.46	> 100	5.66	> 100	> 100	> 100
SK-MEL-5	0.0741	19.3	1.71	3.84	3.37	> 100
UACC-257	3.95	56.9	4.05	> 100	> 100	> 100
UACC-62	1.06	27.1	3.54	> 100	> 100	> 100
Ovarian Cancer						
IGROV1	0.764	> 100	9.80	> 100	> 100	> 100
OVCAR-3	1.13	19.7	> 100	> 100	8.13	> 100
OVCAR-4	5.20	> 100	5.55	> 100	-	-
OVCAR-5	21.0	> 100	> 100	> 100	> 100	> 100
OVCAR-8	0.218	> 100	> 100	> 100	5.27	> 100
NCI/ADR-RES	2.03	> 100	> 100	> 100	.	> 100
SK-OV-3	10.7	> 100	4.62	> 100	5.46	> 100
Prostate Cancer						
PC-3	0.151	> 100	5.41	> 100	4.92	> 100
DU-145	0.507	63.6	6.40	> 100	> 100	> 100
Renal Cancer						
786-0	2.78	> 100	> 100	> 100	3.93	> 100
A498	11.4	44.65	3.54	> 100	> 100	> 100
ACHN	0.487	> 100	4.42	> 100	2.72	.
CAKI-1	0.0251	12.5	6.32	> 100	> 100	> 100
RXF 393	4.95	34.7	70.5	> 100	3.79	> 100
SN12C	3.14	> 100	6.77	> 100	> 100	> 100
TK-10	18.9	> 100	7.56	> 100	2.60	.
UO-31	0.608	34.0	5.89	> 100	.	> 100
Breast Cancer						
MCF7	0.237	> 100	5.82	> 100	> 100	> 100
MDA-MB-231/ATCC	12.2	83.5	> 100	> 100	> 100	> 100
HS 578T	10.2	62.3	> 100	> 100	6.55	> 100
BT-549	11.3	64.9	3.17	> 100	> 100	> 100
T-47D	4.33	> 100	7.30	> 100	> 100	> 100
MDA-MB-468	2.79	35.6	4.43	> 100	3.51	> 100

To objectively interpret the data of the anticancer activity study, the selectivity index (SI) of the effect of compounds at the level of effective inhibition was calculated, which is the ratio of the mean MID GI_{50} for all cancer cell lines to the mean for a particular disease. The value of the selectivity index in the range of 3-6 is interpreted as moderate selectivity,

the value of SI> 6 indicates a high selectivity of the anticancer effect. The activity parameters of the test compounds are shown in table 3. In the case of compound **3d**, high selectivity was observed for leukemia SI = 50.15, epithelial bowel cancer SI = 15.787 and prostate cancer SI = 10.36. Instead, compound **3e** selectively acted on the line of epithelial bowel cancer SI = 7.036 and melanoma SI = 7.559. Compound **3j** did not possess any selectivity.

Table 3. Compounds **3d**, **3e** and **3j** selectivity of action on certain types of cancer at the GI50 level.

Compound	Parameter	Type of cancer								
		L	NCLC	CNS	CC	M	OC	PC	RC	BC
3d	GI_{50}	0.668	3.379	0.216	3.741	4.366	5.863	5.286	0.329	6.843
	SI	50.15	1.009	15.787	0.912	0.781	0.582	0.645	10.36	0.498
3e	GI_{50}	76.683	6.227	3.877	26.810	3.609	59.996	25.625	5.905	36.787
	SI	0.356	4.381	7.036	1.018	7.559	0.455	1.065	4.620	0.742
3j	GI_{50}	68.543	32.633	52.645	37.055	69.410	43.772	44.720	52.460	68.343
	SI	0.7612	1.599	0.991	1.408	0.752	1.192	1.167	0.995	0.763

*** L – Leucemia, NCLC – Non-Small Cell Lung Cancer, CNS – Central nervous system, CC – Colon cancer, M – Melanoma, OC – Ovarian Cancer, PC – Prostate Cancer, RC – Renal Cancer, BC – Breast Cancer.**

The next step in the investigation of anticancer activity was to compare the results of compounds **3d**, **3e** and **3j** with known drugs - 5-Fluorouracil (5-FU), Cisplatinum, as well as a natural anticancer substance - Curcumin. As can be seen in table 4, the anticancer activity of compound **3d** at the GI level is significantly higher than 5-FU and is comparable to Cisplatinum and Curcumin. At the same time, the activity of compounds **3e** and **3j** was commensurate with 5-Fluorouracil and significantly lower than the activity of Cisplatinum and Curcumin.

Table 4. Comparison of compounds **3d**, **3e** and **3j** anticancer activity with 5-Fluorouracil (5-FU), Cisplatinum and Curcumin.

Compound	Type of cancer									
	L	NCLC	CNS	CC	M	OC	PC	RC	BC	MG-MID
3d	0.668	3.379	0.216	3.741	4.366	5.863	5.286	0.329	6.843	3.410
3e	76.683	6.227	3.877	26.810	3.609	59.996	25.625	5.905	36.787	27.280
3j	68.543	32.633	52.645	37.055	69.410	43.772	44.720	52.460	68.343	52.176
5-FU	15.1	>100	8.4	72.1	70.6	61.4	45.6	22.7	76.4	52.5
Cisplatinum	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

* L – Leucemia, NCLC – Non-Small Cell Lung Cancer, CNS – Central nervous system, CC – Colon cancer, M – Melanoma, OC – Ovarian Cancer, PC – Prostate Cancer, RC – Renal Cancer, BC – Breast Cancer.

Thus, the studied 3-R-6-(5-arylfuran-2-yl-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazoles possess a pronounced selective anticancer activity, which gives grounds to consider this condensed systems as a promising molecular framework for the design of potential anticancer agents.

3. Conclusions

In summary, we presented efficient synthetic approaches to a number of 3-R-6-(5-arylfuran-2-yl-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazoles for their anticancer activity evaluation. We have shown that the proposed synthetic protocols provided the possibility to design triazolothiadiazoles diversity with a considerable chemical novelty. The obtained results of the performed biological activity evaluation have shown that synthesized compounds have expressive anticancer properties. Further optimization of the structure to improve biological activity is currently in progress.

4. Experimental

4.1 Chemistry

All chemicals were of analytical grade and commercially available. When performing the synthetic part of the work, the reagents of the company Merck (Germany) and Sigma-Aldrich (USA) were used. All reagents and solvents were used without further purification and drying. All the melting points were determined in an open capillary and are uncorrected. ^{1}H-NMR spectra were recorded on a Varian Mercury 400 (Agilent Technologies, San Francisco, USA) instrument with TMS or deuterated solvent as an internal reference. Mass spectra were run using Agilent 1100 series LC/MSD (Agilent Technologies, San Francisco, USA) with an API–ES/APCI ionization mode. Elemental analysis was performed on an Elementar Vario L cube instrument (Elementar Analysen systeme GmbH, Hanau, Germany). Satisfactory elemental analyses were obtained for new compounds (C±0.17, H±0.21, N±0.19).

*General procedure for the preparation of 3-R-6-(5-arylfuran-2-yl-[1,2,4]triazolo[3,4-b][1,3,4] thiadiazoles **3a-k**.* To mixture consisting of equimolar amounts (5 mmol) of the corresponding 5-arylfuran-2-carboxylic acids **1a-g** and 5-

substituted 4-amino-4*H*-1,2,4-triazole-3-thiolaes **2a-d** 7 ml of $POCl_3$ were added. The resulting mixture was boiled until HCl ceased to be released and further cooled to a room temperature over 3 hours and poured onto 100 g of crushed ice. During cooling, an aqueous solution of ammonia was added up to pH 8. The precipitate was filtered off, washed on the filter with warm water, air dried and recrystallized from a mixture of alcohol-DMF.

*6-[5-(4-Fluoro-phenyl)-furan-2-yl]-3-phenyl-[1,2,4]triazolo[3,4b][1,3,4]thiadiazole **3a**.*

Yield: 69%; mp 194–195 ºC; ^{1}H NMR: δ_H= 8.27 (d, *J* = 7.0 Hz, 2H), 7.97 – 7.91 (m, 2H), 7.74 – 7.70 (m, 1H), 7.62 (t, *J* = 7.3 Hz, 2H), 7.57 (d, *J* = 7.3 Hz, 1H), 7.41–7.30 (m, 3H). ESI-MS: m/z 363 [M+H]+; anal. calcd. $C_{19}H_{11}FN_4OS$: C, 62.97 H, 3.06; N, 15.46. Found: C, 63.05; H, 2.98; N, 15.51.

*6-[5-(2-{Chloro-phenyl)-furan-2-yl]-3-phenyl-[1,2,4]triazolo[3,4b][1,3,4]thiadiazole **3b**.*

Yield: 81%; mp 198–199 °C; ^{1}H NMR: δ_H= : 8.25 (d, *J* = 7.6 Hz, 2H), 7.93 (d, *J* = 7.6 Hz, 1H), 7.72 (d, *J* = 3.7 Hz, 1H), 7.64–7.59 (m, 3H), 7.58–7.48 (m, 2H), 7.45 (t, *J* = 7.6 Hz, 1H), 7.41 (d, *J* = 3.7 Hz, 1H). ESI-MS: m/z 379 [M+H]+; anal. calcd. $C_{19}H_{11}ClN_4OS$: C, 60.24 H, 2.93; N, 14.79. Found: C, 60.26; H, 2.90; N, 14.75.

*3-Phenyl-6-[5-(3-trifluoromethyl-phenyl-furan-2-yl]-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole **3c***. Yield: 77%; mp 179–180°C;^{1}H NMR: δ_H = 8.27 (d, *J* = 8.1 Hz, 2H), 8.22 – 8.15 (m, 2H), 7.78–7.75 (m, 3H), 7.67–7.54 (m, 4H). m/z 412 [M+H]+; anal. calcd. $C_{20}H_{11}F_3N_4OS$: C, 58.25 H, 2.69; N, 13.59. Found: C, 58.33; H, 2.55; N, 13.64.

*3-Benzyl-6-[5-(2-{chloro-phenyl-furan-2-yl]-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole **3d**.*

Yield: 74%; mp 185–186°C; ^{1}H NMR: δ_H= 7.89 (d, *J* = 7.8 Hz, 1H), 7.63–7.58 (m, 2H), 7.50 (t, *J* = 7.5 Hz, 1H), 7.44 (t, *J* = 7.6 Hz, 1H), 7.38–7.28 (m, 5H), 7.24 (t, *J* = 6.8 Hz, 1H), 4.43 (s, 2H). m/z 394 [M+H]+; anal. calcd. $C_{20}H_{13}ClN_4OS$: C, 61.15 H, 3.34; N, 14.26. Found: C, 61.21; H, 3.30; N, 14.32.

*3-Benzyl-6-[5-(4-{chloro-phenyl)-furan-2-yl]-[1,2,4]triazolo[3,4-b][1,3,4] thiadiazole **3e**.*

Yield: 72%; mp 202–203 °C; ^{1}H NMR: δ_H= 7.85 (d, *J* = 7.1 Hz, 2H), 7.63–7.53 (m, 3H), 7.41–7.27 (m, 5H), 7.25 (s, 1H), 4.43 (s, 2H). m/z 394 [M+H]+; anal. calcd. $C_{20}H_{13}ClN_4OS$: C, 61.15 H, 3.34; N, 14.26. Found: C, 61.16; H, 3.26; N, 14.29.

*6-[5-(2-Fluoro-phenyl)-furan-2-yl]-3-furan-2-yl-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole **3f**.*

Yield: 68%; mp 171–172 °C; ^{1}H NMR: δ_H= 8.01 (s, 1H), 7.90 (t, *J* = 7.6 Hz, 1H), 7.69 (d, *J* = 3.6 Hz, 1H), 7.51–7.43 (m, 1H), 7.41–7.33 (m, 2H), 7.30 (d, *J* = 3.0 Hz, 1H), 7.15 (s, 1H), 6.79 (s, 1H). m/z 352 [M+H]+; anal. calcd. $C_{17}H_9FN_4O_2S$: C, 57.95 H, 2.57; N, 15.90. Found: C, 57.80; H, 2.66; N, 15.85.

*6-[5-(2-Chloro-phenyl)-furan-2-yl]-3-furan-2-yl-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole **3g***. Yield: 77 %; mp 183–184 °C; ^{1}H NMR: δ_H= 7.99 (s, 1H), 7.92 (d, *J* = 7.4 Hz, 1H), 7.68 (d, *J* = 3.3 H Hz, 1H), 7.60 (d, *J* = 7.5 Hz, 1H), 7.50 (d, *J* = 7.2 Hz, 1H), 7.44 (t, *J* = 11.5 Hz, 1H), 7.39 (d, *J* = 3.2 Hz, 1H), 7.30 (s, 1H), 6.78 (s, 1H). m/z 369 [M+H]+; anal. calcd. $C_{17}H_9ClN_4O_2S$: C, 55.37 H, 2.46; N, 15.19. Found: C, 55.30; H, 2.52; N, 15.23.

*6-[5-(2-Chloro-phenyl)-3-methyl-furan-2-yl]-3-furan-2-yl[1,2,4]triazolo[3,4b][1,3,4] thiadiazole **3h***. Yield: 71%; mp 188-189 °C; ^{1}H NMR: δ_H= 8.02 (s, 1H), 7.92 (d, *J* = 8.2 Hz, 2H), 7.62 (d, *J* = 7.6 Hz, 1H), 7.56–7.49 (m, 1H), 7.49–7.42 (m, 1H), 7.40 (s, 1H), 7.27 (s, 1H), 6.81 (s, 1H), 3.30 (s, 3H). m/z 383 [M+H]+; anal. calcd. $C_{18}H_{11}ClN_4O_2S$: C, 56.47 H, 2.90; N, 14.63. Found: C, 56.41; H, 2.97; N, 14.56.

*6-[5-(3-Chloro-phenyl)-furan-2-yl]-3-furan-2-yl-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole **3i***. Yield: 74%; mp 188–189 °C; ^{1}H NMR: δ_H= 8.02 (s, 1H), 7.92 (s, 1H), 7.81 (d, *J* = 7.3 Hz, 1H), 7.69 (d, *J* = 3.0 Hz, 1H), 7.52 (t, *J* = 7.7 Hz, 1H), 7.46 (d, *J* = 3.9 Hz, 2H), 7.32 (s, 1H), 6.80 (s, 1H). m/z 369 [M+H]+; anal. calcd. $C_{17}H_9ClN_4O_2S$: C, 55.37 H, 2.46; N, 15.19. Found: C, 55.44; H, 2.45; N, 15.16.

*6-[5-(2-Chloro-phenyl)-3-methyl-furan-2-yl]-3-(2-methyl-furan-3-yl)-[1,2,4] triazolo [3,4 -b] [1,3,4] thiadiazole **3j***. Yield: 73%; mp 183–184°C; ^{1}H NMR: δ_H= 7.90 (d, *J* = 7.7 Hz, 1H), 7.76 (s, 1H), 7.60 (d, *J* = 8.0 Hz, 1H), 7.51 (t, *J* = 7.4 Hz, 1H), 7.44 (t, *J* = 7.5 Hz, 1H), 7.38 (s, 1H), 7.05 (s, 3H), 2.66 (s, 1H), 2.51 (s, 3H). m/z 398 [M+H]+; anal. calcd. $C_{19}H_{13}ClN_4O_2S$: C, 57.50 H, 3.30; N, 14.12. Found: 57.52; H, 3.33; N, 14.20.

*6-[5-(3-Chloro-phenyl)-furan-2-yl]-3-(2-methyl-furan-3-yl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole **3k***. Yield: 75%; mp 196–197°C; ^{1}H NMR: δ_H= 7.88 (s, 1H), 7.79 (s, 1H), 7.73 (s, 1H), 7.62 (s, 1H), 7.52 (d, *J* = 4.8 Hz, 1H), 7.45 (s, 1H),

7.40 (s, 1H), 7.10 (s, 1H), 2.65 (s, 3H). m/z 398 [M+H]+; anal. calcd. $C_{19}H_{13}ClN_4O_2S$: C, 57.50 H, 3.30; N, 14.12. Found: C, 56.55; H, 2.87; N, 14.71.

4.2 Anticancer activity

During the first step (primary screening), test compounds were added at a concentration of 10^{-5} M to cell cultures and incubated for 48 hours. The end point was estimated using the dye-sulforodamine B. Results for each compound were expressed as Growth percent (GP%) of cells related to the control cells growth without test samples. During the secondary screening the study of compounds in 5 concentrations was executed. Human tumor cells from the screening panel were grown in RPMI 1640 medium containing 5% fetal bovine serum and 2 mm L-glutamine. A 0.4% solution of sulforodamine B (SRB) (100 µl) in 1% acetic acid was added to each hole, and the plates were incubated for 10 min at room temperature. After staining, the unbound dye was removed, and absorption was detected on an automated reader at a wavelength of 515 nm. The percentage of cell growth for each concentration was calculated based on 7 absorption measurements: zero time (Tz), growth in the control sample (C) and growth in the presence of the test substances at 5 concentrations (Ti). The percentage of growth inhibition was evaluated by the formulas: [(Ti-Tz) / (C-Tz)] × 100 for concentrations for which Ti> / = Tz [(Ti-Tz) / Tz] x 100 for concentrations for which Ti < As a result, 3 dose-dependent parameters were calculated for each test substance: 1) the concentration of the substance that causes growth inhibition of 50% of cells - GI_{50} (growth inhibition); 2) TGI (total growth inhibition) - the concentration of a substance that completely inhibits cell growth; 3) LC_{50} (lethal concentration) - the concentration that causes the death of 50% of tumor cells (http://dtp.nci.nih.gov).

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