ISSN 1814-9758. Ukr. Bioorg. Acta, 2020, Vol. 15, N 1
UDC 547.789+615.277.3
DOI: hpps://doi.org/10.15407/bioorganica2020.01.002

Ukrainica Bioorganica Acta
www.bioorganica.org.ua

UBA

RESEARCH ARTICLE

Anticancer evaluation of di- and trifunctional substituted 1,3-thiazoles

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Abstract: Anticancer activity of a series of polyfunctional substituted 1,3-thiazoles has been studied within the international scientific program "NCI-60 Human Tumor Cell Lines Screen". Screening was performed *in vitro* on 60 cell lines of lungs, kidneys, CNS, ovaries, prostate, and breast cancer, epithelial cancer, leukemia, and melanoma. The most effective compounds were those with a piperazine substituent at C^2 of the 1,3-thiazole cycle: 1-(4-((4-methylphenyl)sulfonyl)-2-phenyl-1,3-thiazol-5-yl)piperazine (average lg GI_{50} = -5.87, lg TGI = -5.54, lg LC_{50} = -5.21), 1-(2-(3,5-dimethyl-1*H*-pyrazol-1-yl)-4-((4-methylphenyl)sulfonyl)-1,3-thiazol-5-yl)piperazine (average lg GI_{50} = -5.66, lg TGI = -5.26, lg LC_{50} = -4.83), and 1-(2,4-bis((4-methylphenyl)sulfonyl)-1,3-thiazol-5-yl)piperazine (average lg GI_{50} = -5.67, lg TGI = -5.21, lg LC_{50} = -4.67).

Keywords: 1,3-thiazole; anticancer activity; growth inhibitor; cytostatic activity; cytotoxic activity.

Introduction

Derivatives of 1,3-thiazoles play an important role in basic and applied research. It has been demonstrated that 1,3-thiazoles are widely used for creation of dyes, insecticides, herbicides, and pharmaceuticals. Di- and tri-substituted 1,3-thiazole are effective anti-inflammatory, anthelmintic, antiviral, and bactericidal agents [1-6]. The nature of the chemical groups in the heterocycle can significantly affect their pharmacological properties. Despite of wide range of thiazole libraries, many of trisubstituted thiazoles stays unavailable due to multistep and complicated pathways for its synthesis.

The purpose of this work was to synthesize and evaluate the antitumor activity of di- and tri- substituted 1,3-thiazole. S_NAr reactions were convenient for direct introduction of substituents into proper positions of heterocycle. Well known that substitution of halogen atom in C^4 or C^5 position demands high temperatures and Pd catalysis. Present of EWG makes liable of halogen atom in position 5 of thiazole ring. That allows to modify one by introducing different O, N and substituents. Such reactions pass in mild conditions and with high level of regioselectivity, that's why yields of desired products was pretty fine.

Received: 20.03.2020
Revised: 03.04.2020
Accepted: 10.04.2020
Published online: 30.06.2020

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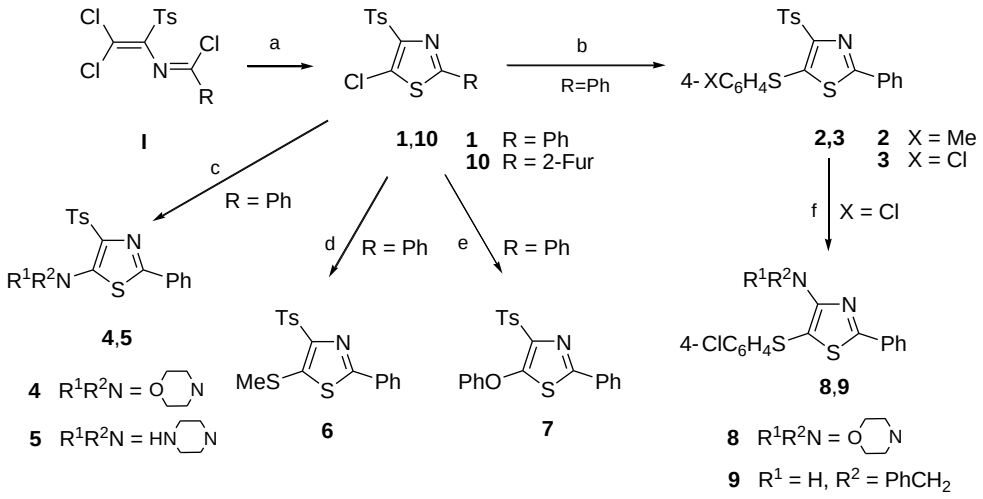
Results and Discussion

Chemistry

Syntheses of compounds **1-23** are presented in Schemes 1 and 2. 1-*R*-3-Tosyl-1,4,4-trichloro-2-aza-1,3-butadienes **I** and 1-tosyl-2,2-dichloroethenylisothiocyanate **II** were used as starting compounds. Imidoyl chlorides **I** react with thiourea to give 2-*R*-4-tosyl-5-chloro-1,3-thiazoles **1, 10**. 5-Chloro-1,3-thiazole **1** reacts with *N*-, *O*- and *S*-nucleophiles to eliminate the chlorine anion and form the corresponding 1,3-thiazole derivatives **2-7**. Heating of compound **3** with hydrogen peroxide in acetic acid followed by the reaction of the obtained product with morpholine or benzylamine yields 4-aminosubstituted 5-((4-chlorophenyl) sulfanyl)-2-phenyl-1,3-thiazoles **8, 9**.

1-Tosyl-2,2-dichloroethenylisothiocyanate **II** was used for the synthesis of trifunctionally substituted 1,3-thiazoles **11-23**. Compound **II** was treated with thiophenols or alkyl mercaptans in the presence of pyridine. A cyclization took place to form intermediate 2-aryl(alkyl)sulfanyl-4-tosyl-5-



Scheme 1. Synthesis of 4,5-difunctional substituted 1,3-thiazoles **1**-**10**. Reagents and conditions: (a) $(H_2N)_2C=S$ (excess), MeCN, reflux, 2 h; (b) 4-MeC_6H_4SH or 4-ClC_6H_4SH, Et_3N, MeCN, reflux, 2 h; (c) morpholine or piperazine (excess), dioxane, 100 °C, 24 h; (d) NaSH (excess), THF, 60 °C, 3 h; MeI, MeONa, MeOH, 20 °C, 5 h; (e) PhONa, THF, 20 °C, 24 h; (f) H_2O_2 (excess), CF_3CO_2H, reflux, 3 h; morpholine or $PhCH_2NH_2$ (excess), dioxane, 100 °C, 24 h.

chloro-1,3-thiazoles, which were oxidized with hydrogen peroxide to 1,3-thiazoles **III** containing at position C^2 an arylsulfonyl or an alkylsulfonyl and at position C^4 the tosyl group. Substitution of the chlorine atom at position C^5 of compounds **III** with 4-chlorothiophenol followed by the oxidation of the sulfanyl group under the action of hydrogen peroxide in trifluoroacetic acid yields 1,3-thiazole derivatives **IV** with three different sulfonyl groups. 1,3-Thiazoles **11-16** were obtained under the treatment of compounds **IV** by *N*- and *O*-nucleophiles. The reaction of 1,3-thiazoles **IV** with dimethylamine, benzylamine, or ammonia in a molar ratio of 1:2 at 20 °C yields 2-amino-1,3-thiazole derivatives **11-13** as a result of replacing a sulfonyl group at position C^2. Treatment of thiazoles **IV** with the excess of an amine or sodium 4-chlorophenolate, leads to the substitution of the two arylsulfonyl groups yielding compounds **14-16**. 1,3-Thiazole **17** was obtained from reagent **II**, hydrazine hydrate, and acetylacetone. It gives when heated with morpholine or piperazine, the corresponding 5-amino-2-pyrazolyl-4-tosyl-1,3-thiazoles **18, 19** and, when treated with sodium hydrogen sulfide followed by propyl iodide, 1,3- thiazole **20**.

5-Piperazino-substituted 1,3-thiazoles **21, 22** were prepared by the nucleophilic substitution of the chlorine atom in compounds **III** for piperazine in boiling ethanol. 1,3-Thiazole **23** was obtained from isothiocyanate **II**, methyl mercaptan, piperazine, and hydrochloric acid.

Structures of synthesized compounds shown in Table 1 were confirmed by ^1H NMR spectra and elemental analysis.

Biological Evaluation

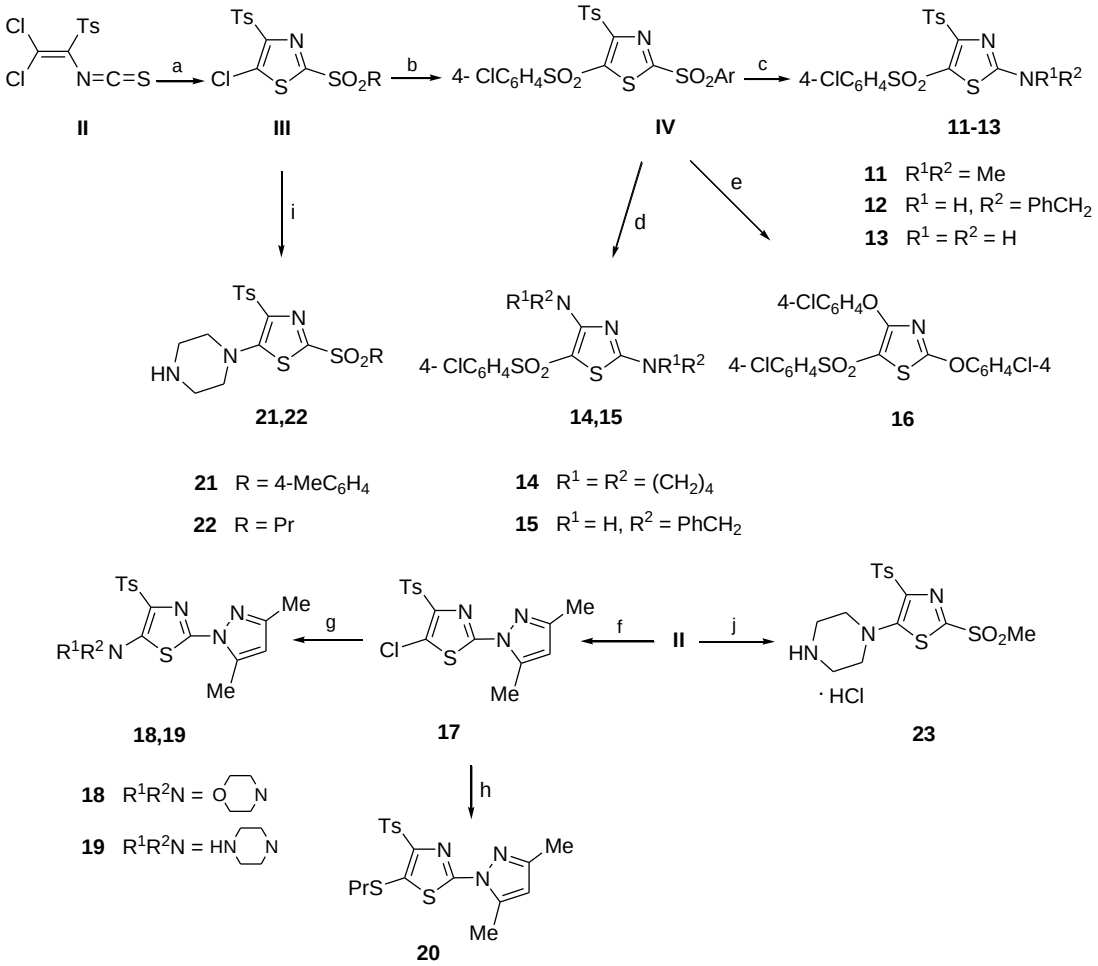
Anticancer activity of the synthesized compounds was studied within an international scientific program of the US National Institutes of Health. The screening was performed *in vitro* on 60 cell lines of lungs, kidneys, CNS, ovaries, prostate, and breast cancer, epithelial cancer, leukemia, and melanoma at a substance concentration of 10^{-5} M. Growth percentage (GP) of cancer cells compared to the control (in the absence of a chemical substance, 100%) was determined [7-10]. Synthesized 1,3-thiazole derivatives have been shown to be active against several types of cancer cells (Table 1).

For example, 2-phenyl-4-tosyl-5-chloro-1,3-thiazole (**1**) considerably inhibits growth of cells of leukemia (K-562, GP = 30.07% and SR, GP = 13.60%), lung cancer (NCI-H522, GP = 44.91%), melanoma (M14, GP = 37.06% and MDA-MB-435, GP = 1.37%), and breast cancer (MDA-MB-468, GP = 0.73%).

The replacement of the chlorine atom in compound **1** with p-tolylsulfanyl group (compound **2**) results in a significant 70% reduction of the inhibitory activity towards leukemia and lung cancer cells and in the full extinction of the activity towards melanoma cells MDA-MB-435 (GP = 106.38%) and M14 (GP = 102.19%).

The substitution of the chlorine atom by methylsulfanyl group (compound **6**) results in a uniform decrease of the inhibitory activity towards leukemia and lung cancer cells and does not change melanoma and breast cancer cells inhibition. The substitution of the chlorine atom in compound **1** with morpholine (compound **4**) as well as phenoxy group (compound **7**) also does not change leukemia and melanoma cells growth inhibition. In summary, any replacement of the chlorine atom results in a decrease of inhibitory activity in relation to parent compound **1**.

1,3-Thiazoles **8, 9** containing the 4-chlorophenylsulfonyl group at position C^5 and a substituted amino group at position C^4 are somewhat more active than compound **4** with the morpholino group at position C^5. Thus, 1,3-thiazole



Scheme 2. Synthesis of 2,4,5-trifunctional substituted 1,3-thiazoles **11**-**23**. Reagents and conditions: (a) ArSH or PrSH, Py, benzene, 15 °C, 8 h; H_2O_2 (excess), AcOH, reflux, 4 h; (b) 4-ClC_6H_4SH, Et_3N, THF, 5 °C, 30 h; H_2O_2 (excess), CF_3CO_2H, reflux 4 h; (c) Me_2NH or $PhCH_2NH_2$, or NH_3, THF, 20 °C, 24 h; (d) piperidine or $PhCH_2NH_2$ (excess), THF, 60 °C, 48 h; (e) 4-ClC_6H_4ONa (excess), THF, 20 °C, 24 h; (f) NH_2NH_2 H_2O (excess), THF, 20 °C, 5 h; Ac_2CH_2 (excess), AcOH, reflux, 10 h; (g) morpholine or piperazine (excess), BuOH, reflux, 20 h; (h) NaSH (excess), MeOH, 20-25 °C, 20 h; PrI, MeONa, MeOH, reflux, 3 h; (i) piperazine (excess), EtOH, reflux, 1 h; (j) MeSH, Py, benzene, 20-25 °C, 5 h; H_2O_2 (excess), AcOH, reflux, 4 h; piperazine (excess), EtOH, reflux, 2 h; HCl (excess), 4 °C, 24 h.

8 was active against leukemia HL-60(TB) (GI = 49.92% and K-562 (GP = 72.30%) as well as breast cancer MDA-MB-468 (GP = 72.05%) cells. Compound **9** showed appreciable inhibitory activity only against lung cancer cells NCI-H522 (GI = 59.56%).

1,3-Thiazole **17** which bears a pyrazole ring instead of the benzene one at C^2 showed lower inhibitory activity compared with that of the parent compound **1**. It showed only minor activity towards breast cancer cells BT-549 (GP = 84.33%). On the other hand, the substitution of the chlorine atom in compound **17** with the propylsulfanyl group results in compound **20**, which inhibitory activity towards lung cancer cells HOP-92 is significantly higher (GP = 20.91%). The activity of compound **20** against the other cancer types remains at the level of compound **17**. The replacement of the chlorine atom in compound **17** with the morpholine cycle does not increase the inhibitory activity. Compound **18** proved to be practically inactive towards all types of cancer cells.

Entering a furan cycle in position C^2 of the 1,3-thiazole leads to a significant increase in the anticancer activity. Thus, synthesized 5-chloro-2-(furan-2-yl)-4-tosyl-1,3-thiazole (**10**) was active against leukemia (K-562, GP = 18.83% and SR, GP = 8.28%), lung cancer (NCI-H522, GP = 18.49%), melanoma (M14, GP = 32.33% and MDA-BM-435, GP = 4.90%), and breast cancer (MDA-MB-468, GP = -22.87%).

The most active were 1,3-thiazoles containing a piperazine ring in position C^5. Thus, compound **19** was active against epithelial cancer cells HCC-2998 (GP = -88.55%) and HT29 (GP = -33.93%), almost all melanoma lines (MALME-3M, GP = -55.98%; M14, GP = -89.83%; MDA-MB-435, GP = -2,08%; SK-MEL-28, GP = -83.71%; SK-MEL-5, GP = 80.68%; UACC-257, GP = -79.19%; UACC-62, GP = 72.05%), and breast cancer as well (T-47D, GP = -29.33%; MDA-MB-468, GP = -55.79%).

1,3-Thiazole **21** with two tosyl groups and C^5 linked piperazine also showed high anticancer activity (average GP = -5.77%). The most effect was observed on leukemia HL-60 (TB) (GP = -45.35%), lung cancer NCI-H522 (GP = -44.16%) and NCI-H460 (GP = -38.88%), colon cancer COLO 205 (GP = -32.92%), CNS cancer SF-539

(GP = -15.93%), melanoma LOX IMVI (GP = -41.57%), M14 (GP = -49.08%) and MDA-MB-435 (GP = -43.55%), prostate cancer DU-145 (GP = -35.29%), breast cancer HS 578T (GP = -21.17%) and MDA-MB-468 (GP = -21.44%) cells.

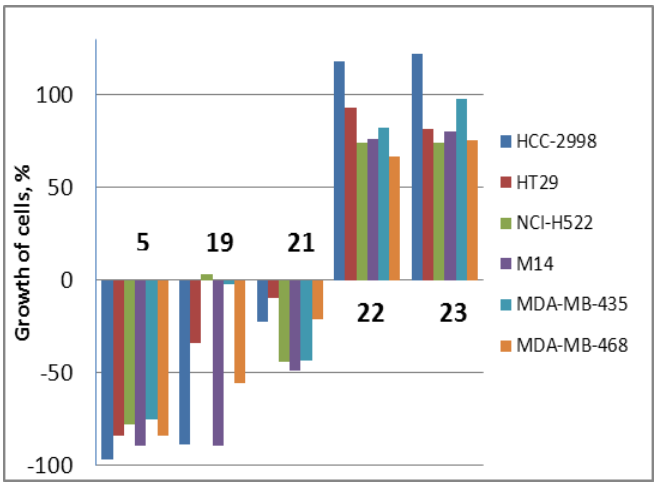


Figure 1. Antitumor activity of 5-(piperazin-1-yl)-4-tosyl-1,3-thiazoles **5, 19, 21-23**.

2-Phenyl-5-(piperazin-1-yl)-4-tosyl-1,3-thiazole (**5**) has been a prominent anticancer agent. It significantly decreased the growth of ovarian cancer cells (IGROV-1, GP = 31.39% and OVCAR, GP = 49.75%), and destroyed, with the average GP of -70%, almost all cell lines of leukemia, melanoma, and colon, CNS, kidney, and breast cancer. The most significant are the data reflecting the almost complete destruction of the following cell lines: colon cancer HCC2998 (GP = -96.68%), CNS U251 (GP = -91.74%), melanoma SK-MEL-28 (GP = -97.03%) and SK-MEL-5 (GP = -98.77%), kidney cancer TK-10 (GP = -88.46%), breast cancer MCF7 (GP = -84.29%) and MDA-MB-468 (GP = -84.34%).

Advanced *in vitro* study of compounds **5, 19, 21** at five concentrations of the 10-fold dilution (10^{-4}-10^{-8} M) was also performed towards 60 human cancer cell lines, the set of which was identical to that for the pre-screening stage (Table 2). High antitumor potential of compound **21** has been confirmed by a significant level of inhibition (average lg GI_{50} = -5.67), as well as cytostatic (average lg TGI = -5.21) and cytotoxic (average lg LC_{50} = -4.67) effects. The highest data were found for compound **5**: average lg GI_{50} = -5.87, lg TGI = -5.54, and lg LC_{50} = -5.21.

It is of interest that among the 5-piperazino-substituted 1,3-thiazoles **5, 19, 21-23** compounds **22, 23** containing a C^2 linked alkylsulfonyl group exhibit the lowest level of antitumor activity. This is readily illustrated by Figure 1 with some selected cell lines. The average activity value of compounds **22** and **23** was 95.48% and 98.61%, respectively (Table 1).

Conclusions

The study of the antitumor activity of di- and trifunctionally substituted 1,3-thiazoles towards the NCI 60 human cancer cell lines revealed "leader compounds" – 5-(piperazin-1-yl)-4-tosyl-1,3-thiazoles. Therein, the nature of the substituent at C^2 of the 1,3-thiazole cycle critically affects the level of activity. Most preferred is the presence of phenyl, tosyl or 3,5-dimethyl-1*H*-pyrazol-1-yl substituent in this position.

Table 1. Mitotic activity of the 1,3-thiazole derivatives towards NCI 60 cell lines at the 10^{-5} M concentration.

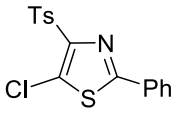
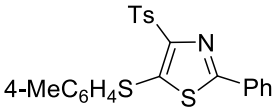
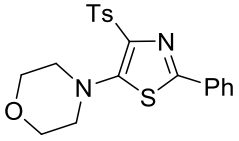
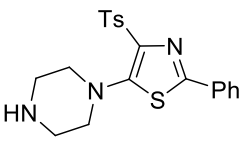
Compd	Structure	Average GP	The most sensitive cell lines (GP)
1		85.92	*leukemia* K-562 (30.07), SR (13.60); *lung cancer* NCI-H522 (44.91); *melanoma* M-14 (37.06), MDA-MB-435 (1.37); *breast cancer* MDA-MB-468 (0.73)
2		97.48	*leukemia* K-562 (90.20), SR (88.33); *lung cancer* NCI-H522 (83.67); *melanoma* M-14 (109.28), MDA-MB-435 (119.03); *kidney cancer* CAKI-1 (71.89)
4		100.29	*leukemia* K-562 (103.76), SR (88.33); *lung cancer* NCI-H522 (75.91); *melanoma* MDA-MB-435 (106.38); *breast cancer* T-47D (70.39)
5		-52.89	*colon cancer* HCC-2998 (-96.68); *melanoma* SK-MEL-5 (-98.77); *CNS cancer* U251 (-91.74); *breast cancer* MDA-MB-468 (-84.34)

Table 1. (*Contd.*)

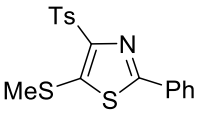
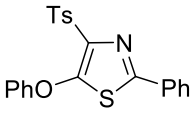
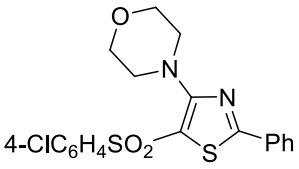
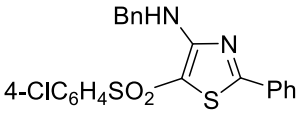
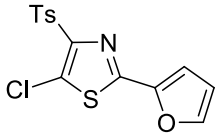
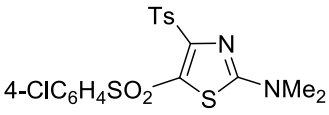
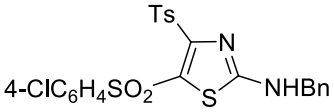
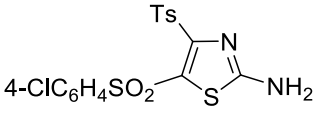
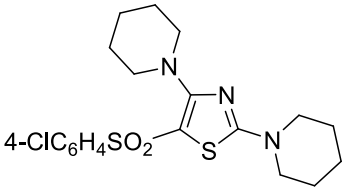
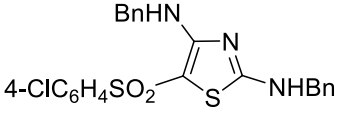
Compd	Structure	Average GP	The most sensitive cell lines (GP)
6		89.71	*leukemia* K-562 (58.49), SR (49.84); *lung cancer* NCI-H522 (74.11); *melanoma* M-14 (94.70), MDA-MB-435 (36.78); *breast cancer* MDA-MB-468 (65.56)
7		90.45	*leukemia* SR (95.44); *lung cancer* NCI-H522 (85.13); *melanoma* M-14 (101.29), MDA-MB-435 (107.97); *breast cancer* T-47D (54.11)
8		94.46	*leukemia* HL-60(TB) (49.92), SR (92.70); *lung cancer* NCI-H522 (83.12); *melanoma* M-14 (101.29), MDA-MB-435 (107.63); *breast cancer* MDA-MB-468 (72.05)
9		92.98	*leukemia* K-562 (88.04), SR (77.65); *lung cancer* NCI-H522 (59.56); *melanoma* M-14 (100.66), MDA-MB-435 (107.82); *breast cancer* MDA-MB-468 (96.90)
10		71.98	*leukemia* K-562 (18.83), SR (8.28); *lung cancer* NCI-H522 (18.49); *melanoma* M-14 (32.33), MDA-MB-435 (4.90); *breast cancer* MDA-MB-468 (-22.87)
11		84.17	*leukemia* HL-60(TB) (52.69); *lung cancer* A549/ATCC (56.18); *melanoma* UACC62 (59.80); *breast cancer* MDA-MB-468 (66.25)
12		107.47	*CNS cancer* SNB-75 (64.32); *melanoma* UACC-62 (79.75)
13		91.75	*leukemia* CCRF-CEM (72.45), HL-60(TB) (72.65), K-562 (73.24), MOLT (64.64); *lung cancer* HOP-92 (47.03)
14		100.46	*leukemia* K-562 (114.19), SR (100.78); *lung cancer* NCI-H522 (91.60); *melanoma* M-14 (106.27); *breast cancer* MDA-MB-468 (94.07); *CNS cancer* SNB-75 (73.04)
15		84.40	*leukemia* HL-60(TB) (81.31), K-562 (85.08); *lung cancer* A549/ATCC (44.72), NCI-H522 (49.57); *melanoma* M-14 (77.99), MDA-MB-435 (114.47); *breast cancer* MDA-MB-231/ATCC (42.35)

Table 1. (*Contd.*)

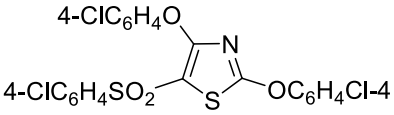
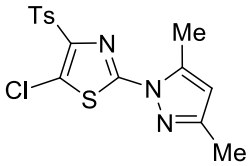
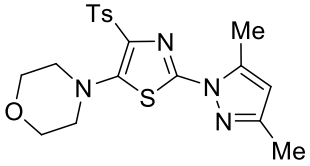
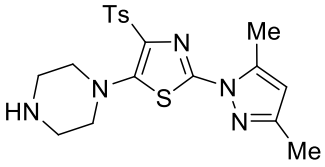
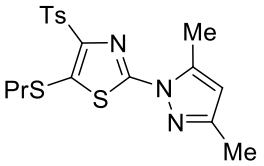
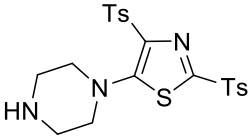
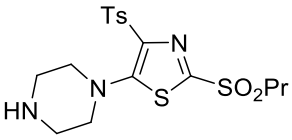
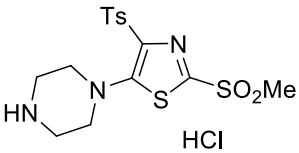
Compd	Structure	Average GP	The most sensitive cell lines (GP)
16		98.55	*leukemia* HL-60(TB) (71.46), K-562 (96.63), SR (43.24); *lung cancer* NCI-H522 (88.25); *melanoma* M-14 (99.61), MDA-MB-435 (96.32); *breast cancer* MCF7 (76.99)
17		102.42	*leukemia* K-562 (92.44); *lung cancer* NCI-H522 (89.59); *melanoma* M-14 (111.85), MDA-MB-435 (107.95); *breast cancer* BT-549 (84.33)
18		89.56	*leukemia* MOLT-4 (81.01), K-562 (81.84); *lung cancer* HOP-92 (20.91), NCI-H522 (78.32); *CNS cancer* SNB-19 (77.87); *breast cancer* MDA-MB-231/ATCC (68.26)
19		23.20	*leukemia* SR (-8.89); *colon cancer* HCC-2998 (-88.55); *melanoma* SK-MEL-28 (-83.71); *breast cancer* MDA-MB-468 (-55.79)
20		89.56	*leukemia* MOLT-4 (81.01), K-562 (92.44); *lung cancer* HOP-92 (20.91), NCI-H322M (88.60); *CNS cancer* SNB-19 (98.94); *breast cancer* MDA-MB-468 (117.20)
21		-5.77	*leukemia* HL-60(TB) (-45.35); *lung cancer* NCI-H522 (-44.16); *melanoma* M-14 (-49.08); *ovarian cancer* OVCAR-3 (-40.27); *prostate cancer* DV-145 (-35.29)
22		95.48	*leukemia* SR (64.37); *colon cancer* SW-620 (134.85); *melanoma* M-14 (76.23); *ovarian cancer* OVCAR-3 (136.53); *breast cancer* MDA-MB-468 (66.81)
23		98.61	*colon cancer* HCC-2998 (-88.55); *CNS cancer* SNB-19 (98.94); *renal cancer* RXF-393 (115.77); *breast cancer* MCF-7 (82.11), MDA-MB-468 (75.35)

Table 2. Parameter values (lg) of the anticancer activity of compounds **5, 19, 21** against the NCI 60 human cancer cell lines (five-dose assay).

Cell Line	Compd								
	5			**19**			**21**		
	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}
					Leukemia				
CCRF-CEM	-5.73	-5.28	-4.23	-5.57	-4.96	-4.21	-5.63	-5.05	-4.35
HL-60(TB)	-5.72	-5.43	-5.14	-5.62	-5.35	-5.07	-5.64	-5.32	-5.00
K-562	-6.26	-5.62	-5.11	-5.81	-5.39	-4.91	NT	NT	NT
MOLT-4	-5.74	-5.39	-5.03	-5.65	-5.31	-4.88	-5.52	-5.23	-4.53
RPMI-8226	NT	NT	NT	NT	NT	NT	-5.94	-5.01	-4.19
SR	-5.87	-5.47	-5.08	-5.64	-5.24	-4.49	-5.63	-5.31	-4.38
					Non-small cell lung cancer				
A549/ATCC	-5.74	-5.47	-5.20	-5.44	-4.89	-4.38	-5.64	-5.10	-4.48
EKVX	-5.87	-5.52	-5.16	-5.63	-4.90	-4.39	-5.49	-4.91	-4.40
HOP-62	-5.77	-5.51	-5.26	-5.71	-5.39	-5.07	-5.92	-5.39	-4.70
HOP-92	-6.46	-5.79	-5.37	-6.21	-5.50	-4.83	-5.45	-5.12	-4.57
NCI-H226	-5.79	-5.51	-5.23	-5.57	-5.00	-4.38	-5.67	-5.37	-5.06
NCI-H23	-5.84	-5.53	-5.23	-5.29	-4.71	-4.34	-5.63	-5.17	-4.55
NCI-H322M	NT	NT	NT	NT	NT	NT	NT	NT	NT
NCI-H460	-5.79	-5.52	-5.24	-5.74	-5.45	-5.16	-5.64	-5.20	-4.55
NCI-H522	-5.74	-5.46	-5.18	-5.67	-5.39	-5.10	NT	NT	NT
					Colon cancer				
COLO 205	-6.68	-6.33	-5.95	-5.78	-5.52	-5.25	-5.78	-5.45	-5.12
HCC-2998	-6.28	5.76	-5.34	-5.81	-5.52	-5.22	-5.59	5.22	-4.66
HCT-116	-5.93	-5.61	-5.29	-5.79	-5.49	-5.18	-5.75	-5.39	-5.04
HCT-15	-5.84	-5.54	-5.24	-5.67	-5.31	-4.86	-5.70	-5.07	-4.47
HT29	-6.12	-5.65	-5.24	-5.82	-5.40	-4.95	-5.56	-4.98	-4.30
KM12	-5.79	-5.51	-5.24	-5.74	-5.48	-5.22	-5.61	-5.18	-4.50
SW-620	-5.82	-5.54	-5.26	-5.77	-5.48	-5.19	-5.79	-5.44	-5.09
					CNS cancer				
SF-268	-5.80	-5.50	-5.20	-5.48	-4.92	-4.43	-5.58	-5.09	-4.46
SF-295	-5.77	-5.49	-5.21	-5.73	-5.43	-5.14	-5.60	-5.19	-4.59
SF-539	-5.81	-5.53	-5.24	-5.77	-5.46	-5.14	-5.56	-5.25	-4.83
SNB-19	-5.75	-5.36	-4.88	-5.16	-4.68	-4.30	-5.51	-4.95	-4.45
SNB-75	-5.97	-5.62	-5.27	-5.85	-5.39	-4.87	-5.59	-5.09	-4.54
U251	NT	NT	NT	NT	NT	NT	-5.55	-5.01	-4.45
					Melanoma				
LOX IMVI	-5.88	-5.57	-5.26	-5.75	-5.45	-5.15	-5.82	-5.39	-4.86
MALME-3M	-5.77	-5.50	-5.22	-5.33	-4.79	-4.36	-5.61	-5.34	-5.06
M14	-5.76	-5.47	-5.19	-5.67	-5.41	-5.16	-5.80	-5.48	-5.15
MDA-MB-435	-5.87	-5.55	-5.24	-5.75	-5.45	-5.16	-6.23	-5.65	-5.17
SK-MEL-2	-5.69	-5.42	-5.15	-5.65	-5.40	-5.15	NT	NT	NT
SK-MEL-28	-5.81	-5.54	-5.27	-5.76	-5.50	-5.24	-5.74	-5.44	-5.14
SK-MEL-5	-5.82	-5.55	-5.27	-5.78	-5.52	-5.26	-5.84	-5.55	-5.27
UACC-257	-5.80	-5.50	-5.19	-5.77	-5.48	-5.20	-5.79	-5.48	-5.17
UACC-62	-5.80	-5.53	-5.26	-5.77	-5.49	-5.22	-5.79	-5.28	-4.68
					Ovarian cancer				
IGROV1	-5.75	-5.44	-5.14	-5.74	-5.45	-5.17	-5.60	-5.13	-4.37
OVCAR-3	-5.77	-5.50	-5.23	-5.59	-5.15	-4.59	-5.61	-5.28	-4.83
OVCAR-4	-5.76	-5.46	-5.16	-5.38	-4.81	-4.40	-5.56	-4.92	-4.39
OVCAR-5	-5.80	-5.51	-5.22	-5.50	-4.95	-4.46	-5.44	-5.05	-4.51
OVCAR-8	-5.75	-5.46	-5.17	-5.64	-5.22	-4.47	-5.65	-4.99	-4.35
NCI/ADR-RES	-5.81	-5.49	-5.17	-5.50	-4.84	-4.32	-5.55	-4.92	-4.34
SK-OV-3	-5.76	-5.49	-5.23	-5.53	-5.11	-4.57	-5.50	-4.97	-4.43

Table 2. (*Contd.*)

Cell Line	Compd								
	5			**19**			**21**		
	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}
				Renal cancer					
786-0	-5.79	-5.48	-5.17	-5.66	-5.29	-4.77	-5.65	-5.37	-5.08
A498	-5.00	-5.66	-5.33	-5.91	-5.50	-5.08	-5.74	-5.34	-4.84
ACHN	-5.78	-5.50	-5.21	-5.46	-4.92	-4.45	-5.41	-4.91	-4.44
CAKI-1	-5.82	-5.50	-5.18	-5.68	-5.23	-4.65	-5.66	-5.34	-5.03
RXF 393	-5.90	-5.59	-5.29	-5.77	-5.45	-5.12	-5.80	-5.52	-5.24
SN12C	-5.80	-5.51	-5.23	-5.63	-5.15	-4.54	-5.57	-4.99	-4.47
TK-10	-5.76	-5.48	-5.20	-5.58	-5.19	-4.66	-5.42	-4.99	-4.46
UO-31	-5.94	-5.61	-5.29	-5.88	-5.54	-5.20	-5.49	-5.00	-4.45
				Prostate cancer					
PC-3	-5.81	-5.48	-5.15	-5.52	-4.99	-4.46	-5.51	-4.88	-4.41
DU-145	-5.79	-5.52	-5.25	-5.43	-4.88	-4.44	-5.66	-5.28	-4.76
				Breast cancer					
MCF7	-5.84	-5.55	-5.26	-5.76	-5.43	-5.10	-5.78	-5.21	-4.44
MDA-MB-231/ATCC	-5.83	-5.54	-5.25	-5.74	-5.44	-5.13	-5.54	-5.16	-4.62
HS 578T	-5.81	-5.45	-5.09	-5.68	-5.24	-4.25	-5.61	-5.14	-4.00
BT-549	-5.78	5.51	5.23	-5.47	-4.91	-4.43	-5.52	5.11	4.57
T-47D	-5.80	-5.50	-5.19	-5.76	-5.46	-5.16	-6.29	-5.18	-4.43
MDA-MB-468	-6.56	-6.01	-5.44	-5.92	-5.61	-5.30	-6.43	-5.77	-5.23

Experimental section

Chemistry

^{1}H NMR spectra were obtained on a Bruker Avance DRX 500 spectrometer. The melting points were estimated on a Fisher-Johns apparatus. The reaction progress was monitored by the TLC method on silica gel $60F_{254}$ Merck plates. All reagents and solvents were purchased from Aldrich and used without additional purification.

*5-Substituted 4-[(4-methylphenyl)sulfonyl]-2-phenyl-1,3-*thiazoles **1-4, 6-9** [11], *5-Chloro-2-(furan-2-yl)-4-((4-methylphenyl)sulfonyl)-1,3-thiazole* (**10**) [12], *2,4-Di-substituted 5-(4-chlorophenyl)sulfonyl-1,3-thiazoles* **11-16** [13], *2-(3,5-Dimethyl-1H-pyrazol-1-yl)-4-((4-methylphenyl)sulfonyl)-1,3-thiazoles* **17, 18** [14] were synthesized following the procedures described in the corresponding sources cited.

*1-(4-((4-Methylphenyl)sulfonyl)-2-phenyl-1,3-thiazol-5-yl)piperazine (**5**)* was synthesized similarly to compound **4**.

Yield 67%, mp 112-113 °C (EtOH). ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.45 (s, 3H, CH_3), 3.20-3.35 (m, 4H, $2CH_2$), 3.82-3.94 (m, 4H, $2CH_2$), 7.33-7.45 (m, 5H, Ar), 7.78 (d, *J* 7.5 Hz, 2H, Ar), 8.05 (d, *J* 7.5 Hz, 2H, Ar). Anal. Calcd. for $C_{20}H_{21}N_3O_2S_2$: C, 60.12; H, 5.30; N, 10.52; S, 16.05. Found: C, 59.98; H, 5.15; N, 10.32; S, 15.98.

*1-(2-(3,5-Dimethyl-1H-pyrazol-1-yl)-4-((4-methylphenyl)sulfonyl)-1,3-thiazol-5-yl)piperazine (**19**)* was synthesized similarly to compound **18**.

Yield 62%, mp 98-99 °C (EtOH). ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.17 (s, 3H, CH_3), 2.40 (s, 3H, CH_3), 2.44 (s, 3H, CH_3), 3.79-3.88 (m, 4H, $2CH_2$), 4.12-4.23 (m, 4H, $2CH_2$), 6.00 (s, 1H, CH), 7.38 (d, *J* 7.9 Hz, 2H, Ar), 7.82 (d, *J* 7.9 Hz, 2H, Ar). Anal. Calcd. for $C_{19}H_{23}N_5O_2S_2$: C, 54.65; H, 5.55; N, 16.77; S, 15.36. Found: C, 54.48; H, 5.49; N, 16.54; S, 15.12.

*2-(3,5-Dimethyl-1H-pyrazol-1-yl)-4-((4-methylphenyl)-sulfonyl)-5-(propylsulfanyl)-1,3-thiazole (**20**).*

To a suspension of 0.00015 mol of compound **17** in 10 ml of methanol, 0.00075 mol of sodium hydrosulfide was added. The mixture was stirred for 20 h at 20 °C, the precipitate was filtered off, and the filtrate was evaporated in vacuo. To the residue, 5 ml of water followed by 1 ml of concd hydrochloric acid was added to precipitate a solid, which was filtered off. To a suspension of this solid in 5 ml of methanol, 0.00015 mol of sodium methylate followed by 0.0002 mol of propyl bromide was added. The mixture was refluxed for 3 h then cooled to 20 °C, the precipitate was filtered off and recrystallized from ethanol. Yield 65%, mp 142-144 °C (EtOH). Anal. Calcd. for $C_{18}H_{21}N_3O_2S_3$: C, 53.04; H, 5.19; N, 10.31; S, 23.60. Found: C, 52.91; H, 5.00; N, 10.18; S, 23.54.

*General procedure for preparation of compounds (**21, 22**).*

To a solution of 0.08 mol of compound **II** [15] in 150 ml of benzene cooled to 0 °C, 0.08 mol of 4-methyl-benzenethiol or propanethiol followed by 0.08 mol of pyridine was added. The mixture was stirred for 8 h at 15 °C, the solvent was removed in vacuo, the residue was

washed with water, and dissolved in 200 ml of acetic acid. To this solution, 30 ml of a 30% aqueous hydrogen peroxide solution was added, the reaction mixture was boiled for 4 h then cooled to 10 °C. The precipitate was filtered off and dissolved in 50 ml of ethanol. To this solution, 0.25 mol of piperazine was added, the reaction mixture was boiled for 1 h then evaporated in vacuo. To the residue, 20 ml of water was added to precipitate a solid, which was filtered off and recrystallized from ethanol.

*1-(2,4-Bis((4-methylphenyl)sulfonyl)-1,3-thiazol-5-yl)-piperazine (**21**).*

Yield 72%, mp 132-133 °C (EtOH). ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.43 (s, 3H, CH_3), 2.47 (s, 3H, CH_3), 3.80-3.89 (m, 4H, $2CH_2$), 4.15-4.30 (m, 4H, $2CH_2$), 7.29 (d, *J* 7.6 Hz, 2H, Ar), 7.35 (d, *J* 7.9 Hz, 2H, Ar), 7.38 (d, *J* 7.6 Hz, 2H, Ar), 7.86 (d, *J* 7.9 Hz, 2H, Ar). Anal. Calcd. for $C_{21}H_{23}N_3O_4S_3$: C, 52.70; H, 4.78; N, 8.80; S, 20.14. Found: C, 52.81; H, 4.85; N, 8.63; S, 20.04.

*1-(4-((4-Methylphenyl)sulfonyl)-2-(propylsulfonyl)-1,3-thiazol-5-yl)piperazine (**22**).*

Yield 68%, mp 131-132 °C (EtOH). ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.91 (t, *J* 7.4 Hz, 3H, CH_3), 1.55-1.68 (m, 2H, CH_2), 2.45 (s, 3H, CH_3), 3.02-3.18 (m, 4H, $2CH_2$), 3.19-3.31 (m, 4H, $2CH_2$), 3.33-3.45 (m, 4H, $2CH_2$), 7.34 (d, *J* 7.5 Hz, 2H, Ar), 7.89 (d, *J* 7.5 Hz, 2H, Ar). Anal. Calcd. for $C_{17}H_{23}N_3O_4S_3$: C, 47.53; H, 5.03; N, 9.78; S, 22.39. Found: C, 47.45; H, 4.93; N, 9.65; S, 22.10.

*1-(4-((4-Methylphenyl)sulfonyl)-2-(methylsulfonyl)-1,3-thiazol-5-yl)piperazine (**23**).*

To a solution of 0.0026 mol of isothiocyanate **II** [15] and 00078 mol of pyridine in 10 ml of benzene, methanethiol was passed, obtained by the hydrolysis of 0.012 mol of *S*-methyl-isothiouronium sulfate. The mixture was stirred for 5 h, the precipitate was filtered off, and the filtrate was evaporated in vacuo. To the residue, 4 ml of acetic acid followed by 1.5 ml of 30% aqueous hydrogen peroxide solution was added. The reaction mixture was heated under reflux for 1.5 h then cooled to room temperature and the precipitate was separated. To a suspension of this solid in 5 ml of acetonitrile, 0.005 mol of piperazine was added. The mixture was stirred for 20 h and the precipitate was filtered off. To the filtrate, 0.5 ml of concd hydrochloric acid was added, the mixture was kept at 4 °C for 1 day, and the precipitate was separated. Yield 25%, mp 245-247 °C (dec.). ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.39 (s, 3H, CH_3), 3.28 (s, 4H, $2CH_2$), 3.36 (s, 3H, CH_3), 3.56 (s, 4H, $2CH_2$), 7.46 (d, *J* 8.0 Hz, 2H, Ar), 7.86 (d, *J* 8.1 Hz, 2H, Ar), 9.54 (s, 2H, NH, HCl). Anal. Calcd. for $C_{15}H_{20}ClN_3O_4S_3$: C, 41.13; H, 4.60; Cl, 8.09; N, 9.59; S, 21.96. Found: C, 41.24; H, 4.57; Cl, 8.17; N, 9.65; S, 21.85.

Biological tests

Anticancer *in vitro* screening methodology as well as data interpretation rules is described in details at the NCI Development Therapeutics Program site [16].

Notes

Acknowledgements. We would like to thank National Cancer Institute, Bethesda, MD, US for the anticancer activity investigations and Enamine Ltd. for the material and technical support.

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Оцінка протиракової активності ди- та трифункціональнозаміщених 1,3-тіазолів

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Резюме: Синтезовано ряд ди- та трифункціональнозаміщених 1,3-тіазолів з використанням в якості вихідних сполук 1-*R*-3-тозил-1,4,4-трихлоро-2-аза-1,3-бутадієнів або 1-тозил-2,2-дихлороетенілізотіоціанату. Скринінгові дослідження протиракової активності синтезованих сполук проведено *in vitro* на 60 лініях ракових клітин людини: лейкемії (лінії CCRF-CEM, HL-60 (TB), K-562, MOLT-4, RPMI-8226, SR), меланоми (лінії LOX IMVI, MALME-3M, M14, MDA-MB-435, SK-MEL-2, SK-MEL-28, SK-MEL-5 , UACC-257, UACC-62), раку легенів (лінії A549/ATCC, EKVX, HOP-62, HOP-92, NCI-H226, NCI-H23, NCI-H322M, NCI-H460, NCI-H522), товстої кишки (лінії COLO 205, HCC-2998, HCT-116, HCT-15, HT29, KM12, SW-620), мозку (лінії SF-268, SF-295, SF-539, SNB-19, SNB-75, U251), яєчників (лінії IGROV1, OVCAR-3, OVCAR-4, OVCAR-5, OVCAR-8, NCI/ADR-RES, SK-OV-3), нирок (лінії 786-0, A498, ACHN, CAKI-1, RXF 393, SN12C, TK-10, UO-31), простати (лінії PC-3, DU-145) і грудей (лінії MCF7, MDA-MB-231/ATCC, HS 578T, BT-549, T- 47D, MDA-MB-468) при концентрації $1 \cdot 10^{-5}$ M. В результаті визначено відсоток росту (GP) клітин ліній раку у порівнянні з контролем (контроль – 100%). Поглиблений *in vitro* скринінг сполук полягав у вивченні її протипухлинного ефекту в п`яти концентраціях при 10-кратному розведенні (10^{-4}-10^{-8} M). У результаті експерименту розраховано 3 дозозалежні параметри (GI_{50}, TGI, LC_{50}). Серед даних сполук 1-(4-((4-метилфеніл)сульфоніл)-2-феніл-1,3-тіазол-5-іл)піперазин (середні значення lg GI_{50} = -5.87, lg TGI = -5.54, lg LC_{50} = -5.21), 1-(2-(3,5-диметил)-1*H*-піразол-1-іл)-4-((4-метилфеніл)сульфоніл)-1,3-тіазол-5-іл)піперазин (середні значення lg GI_{50} = -5.66, lg TGI = -5.26, lg LC_{50} = -4.83) та 1-(2,4-біс((4-метилфеніл)сульфоніл)-1,3-тіазол-5-іл)піперазин (середні значення lg GI_{50} = -5.67, lg TGI = -5.21, lg LC_{50} = -4.67) виявили найвищу інгібуючу активність. Отримані результати свідчать про перспективність пошуку серед ди- та трифункціональнозаміщених похідних 1,3-тіазолу нових протиракових препаратів.

Ключові слова: 1,3-тіазол; протиракова активність; інгібітори росту; цитостатична активність; цитотоксична активність.