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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and anticancer activity evaluation of new 1,2,3-triazole-4-carboxamide derivatives

Nazariy Pokhodylo · Olga Shyyka · Vasyl Matiychuk

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Abstract Anticancer screening of several novel 1,2,3-triazoles has been performed. The 1,2,3-triazole derivatives were synthesized from available starting materials according to the convenient synthetic procedures using a multicomponent reaction which gave a wide access to triazole derivatives production. The synthesized compounds were tested for their anticancer activity in NCI60 cell lines. It was observed that some compounds showed remarkable anticancer activity. Two of them possessed a significant activity on leukemia, melanoma, non-small cell lung, CNS, ovarian, renal, and breast cancer. 5-Amino-1-p-tolyl-1*H*-[1,2,3]triazole-4-carboxylic acid (2,5-dichloro-phenyl)-amide showed a significant correlation in COMPARE analysis.

Keywords Anticancer activity · 1,2,3-Triazole-4-carboxamide · COMPARE analysis · Molecular docking

Introduction

Triazoles and their derivatives are of great importance in medicinal chemistry and can be used for the synthesis of numerous heterocyclic compounds with different biological activities. It is well known that triazoles are very attractive targets for combinatorial library synthesis because of their broad range of pharmaceutical activities. They have been reported to be inhibitors of glycogen synthase kinase-3 (Olesen *et al*., 2003), antagonists of GABA receptors (Bascal *et al*., 1996; Biagi *et al*., 1993), agonists of muscarinic receptors (Moltzen *et al*., 1994), neuroleptic (Chakrabarti *et al*., 1989), as well as compounds showing anti-HIV-1 (Alvarez *et al*., 1994), cytotoxic (Sanghvi *et al*., 1990), antihistaminic (Buckle *et al*., 1986), and antiproliferative activities (Hupe *et al*., 1991). Moreover, the triazole fragment includes an antibiotic Cefatricin (Fass and Prior, 1978; Bohm and Karow, 1981) and anticonvulsant drug Rufinamide (Portmann *et al*., 1998). Thus, design and synthesis of novel triazole derivatives are a perspective direction of medicinal chemistry for the scientists working in this field.

Epidermal growth factor receptor (EGFR) is the first growth factor receptor to be proposed as a target for cancer therapy (Ciardiello and Tortora, 2008). The development of specific antagonists targeting the EGFR has proved to be a promising therapeutic concept. Although EGFR plays a few roles in human body, its overexpression or overactivity leads to a number of cancers as well (Zhang *et al*., 2007). Actually, the majority of human epithelial cancers, including lung cancer, breast cancer, and ovarian cancer, are marked by functional activation of growth factors and receptors of the EGFR family. Therefore, selective blockade of EGFR has been shown to be an effective therapeutic approach against multiple epithelial cancers (Pao and Chmielecki, 2010). EGFR plays an important role in carcinogenesis and is therefore an intriguing target for cancer therapy. The present study is devoted to the synthesis and evaluation of anticancer activity of new 1,2,3-triazole-4-carboxamides. The aim of the research was to perform a molecular docking of some 1,2,3-triazoles into EGFR tyrosine kinase active site for purposeful searching of EGFR tyrosine kinase inhibitors as potential anticancer agents.

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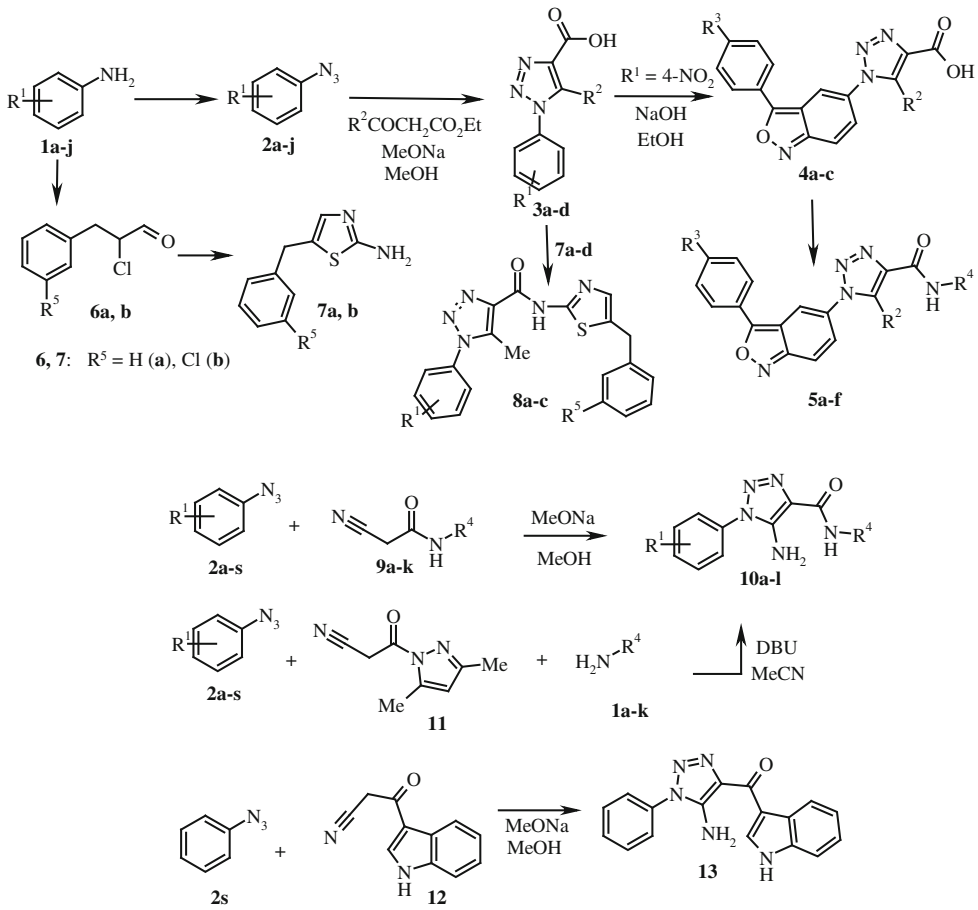
Scheme 1 Synthesis of 1,2,3-triazole-4-carboxamide derivatives



Results and discussion

Chemistry

The compounds presented in the article were obtained in a simple and convenient synthetic path (Scheme 1). The 1*H*-1,2,3-triazole 4-carboxylic acids **3a–d** were synthesized by base-catalyzed cyclocondensation of aryl azides **2a–c** (prepared from sodium azide and diazonium salts obtained from anilines **1a–c**) with ethyl acetoacetate according to the procedure described in the literature (Pokhodylo *et al*., 2009c). It was found that the reaction of 1*H*-1,2,3-triazolyl-4-nitroarenes **3a–d** with arylacetonitriles in ethanol in the presence of an excess of sodium hydroxide formed compounds **4a–c** in good yields. The 1*H*-1,2,3-triazole amides **5a–f** were synthesized by in situ simple activation of acids **3a–d** using CDI, carbodiimides (EDC and DCC) or ready conversion to the corresponding chlorides with thionyl or oxalyl chloride, in the presence of a tertiary amine, in dichloromethane or acetonitrile used as the reaction solvent. Overall yield for the amides **5a–f** was 51–73 % that allowed quickly and without chromatographic purification creating a library of such compounds.

It has previously been shown that 3-aryl-2-chloropropanals **6a**, **b** were easily obtained by the Meerwein reaction of arenediazonium chlorides with acrolein. Such reactions were carried out in aqueous acetone solution in the presence of $CuCl_2$ as catalyst. Aldehydes **6a**, **b** were subjected to heterocyclization with thiourea on heating in alcohol to yield corresponding 2-amino-5-benzyl-1,3-thiazoles **7a**, **b** (Obushak *et al*., 2004). The reaction of 2-amino-5-benzyl-1,3-thiazoles **7a**, **b** with 1*H*-1,2,3-triazole acid chlorides led to the corresponding amides **8a–c** in good yields.

The reaction of azides with acetonitriles with activated methylene group is a convenient method for 5-amino-1*H*-1,2,3-triazoles synthesis. Using cyanacetic acid amides and aryl azides, we obtained a series of new 5-amino-1*H*-1,2,3-triazole-4-carboxamides **10a–l** in good yields. Moreover, recently, we have proposed a new multicomponent strategy for 1-(R^1-phenyl)-5-methyl-*N*-R^2-1*H*-1,2,3-triazole-4-carboxamides in high yields by the reaction of arylazides with dicetene and appropriate amines produced (Pokhodylo *et al*., 2009a). In the current study, we have found that multicomponent methods were suitable for preparation of 5-amino-1*H*-1,2,3-triazole-4-carboxamides **10a–l** from arylazides, amines, and 3-(3,5-dimethyl-1*H*-pyrazol-1-yl)-3-oxopropanenitrile as donor of cyanoacetic acid fragment.

Using commercially available or easily prepared azides and amines, 5-amino-1*H*-1,2,3-triazole-4-carboxamides **10a–l** were obtained in a one-pot system in high yields and in short time. The 5-aminotriazole **13** was prepared by the cycloaddition reactions of arylazides with cyanacetyl indoles (Pokhodylo *et al*., 2009b).

Molecular docking

Research was performed by the method of molecular docking as a computer approach in the search of molecules with affinity to certain biotargets. Docking studies were conducted with OpenEye Scientific Software program package that includes Fred Receptor, Vida, Flipper, Babel3, Omega2, and Fred2. Chrystallographic model of EGFR tyrosine kinase was obtained from Protein Data Bank (www.rcsb.org). The obtained 1,2,3-triazole derivatives and well-known selective EGFR inhibitors, such as Erlotinib, Lapatinib, Gefitinib, and others, were selected as research objects. The obtained seven scoring function values (Chemgauss2, Chemscore, PLP, Screenscore, Shapegauss, Zapbind, and Consensus) were used for in silico estimation of EGFR tyrosine kinase-compound binding. Consensus (cumulative) scoring function ranking allowed us to select compounds, which could prospectively be selective EGFR tyrosine kinase inhibitors at the level of erlotinib for future (in-depth) pharmacological studies as well as could be used as templates for the synthesis of various related analogs. With the help of Fred receptor, the active site (biotarget) of EGFR tyrosine kinase was obtained from crystallographic model for performing molecular docking.

Molecular docking studies include the following stages:

1. Generating R-, S-, and cys-trans isomers of ligands using program Flipper.
2. 3D optimization of isomers using program Hyper Chem 7.5 (www.hyper.com) (molecular mechanics method MM+ and semi-empirical quantum-mechanical method PM3).
3. Conformers generating (Omega2). Further program Fred2 will choose minimum energy conformation for each molecule.
4. 3D molecular docking (Fred2).

As a result, we obtained values of seven scoring functions (Chemgauss2, Chemscore, PLP, Screenscore, Shapegauss, Zapbind, and Consensus). Consensus scoring function was selected to analyze the results because of its property to result in ranking (compound ranking), which includes values of all scoring functions.

Analysis and ranking of the molecular docking results were obtained using the selected compounds and crystallographic model of EGFR tyrosine kinase with cumulative scoring function (consensus) that allowed selecting 22 compounds for further evaluation of in vitro anticancer activity. The interactions between EGFR tyrosine kinase-active site and the most active compounds **10d** and **10h** are shown in the Fig. 1.

Evaluation of anticancer activity in vitro

The synthesized 1,2,3-triazoles (**5a–f**, **8a–c**, **10a–l**, and **13**) were submitted and evaluated at the single concentration of 10^{-5} M toward panel of the ~60 cancer cell lines. The human tumor cell lines were derived from the nine different cancer types: leukemia, non-small cell lung, colon, CNS, melanoma, ovarian, renal, prostate, and breast cancers. Primary anticancer assays were performed according to the US NCI protocol, as described elsewhere (http://dtp.nci.nih.gov; Monks *et al*., 1991; Boyd and Paull, 1995; Boyd, 1997; Shoemaker, 2006). The results for each compound are reported as the percent growth (GP) (Table 1). Range of growth (%) shows the lowest and the highest growths that were found among different cancer cell lines.

The synthesized 1,2,3-triazoles (**5a–f**, **8a–c**, **10a–l**, **13**) displayed moderate (**5b**, **c**, **d**, **8b**, **10a**, **d**, **i**, **h**) or low (**5a**, **e**, **f**, **8a–c**, **10b**, **c**, **e–g**, **j–l**) activity in the in vitro screen on tested cell lines. However, a selective influence of some compounds on several cancer cell lines was observed

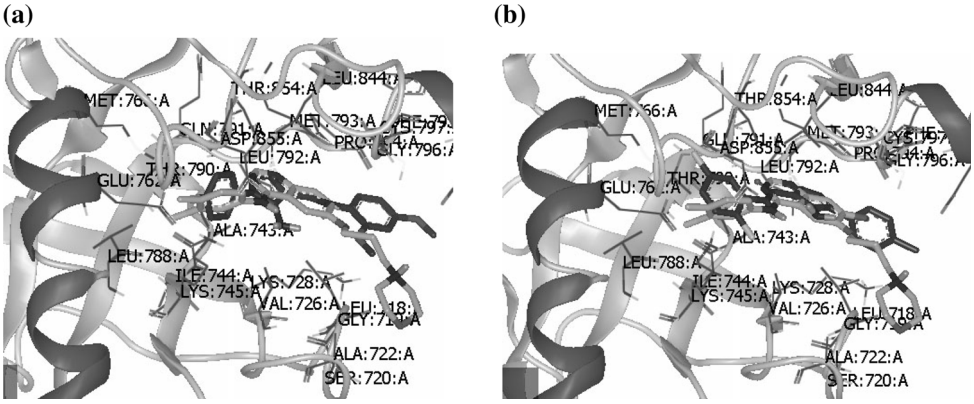


Fig. 1 Compounds **10h** (**a**) and **10d** (**b**) docked in the active site of EGFR tyrosine kinase in comparison with selective inhibitor Gefitinib

Table 1 Anticancer screening data in concentration 10^{-5} M

Compound	Mean growth (%)	Range of growth (%)	The most sensitive cell lines	Growth of the most sensitive cell lines
5a	103.19	78.54–131.66	UO-31 (renal cancer)	78.54
			K-562 (leukemia)	89.82
5b	80.29	46.61–108.20	SNB-75 (CNS Cancer)	46.61
			IGROV1 (ovarian cancer)	49.91
			SF-539 (CNS cancer)	54.10
			MDA-MB-468 (breast cancer)	56.59
			HOP-92 (non-small cell lung cancer)	61.49
			NCI-H522 (non-small cell lung cancer)	62.03
5c	89.85	52.03–121.05	UO-31 (renal cancer)	52.03
			A498 (renal cancer)	56.21
			UACC-62 (melanoma)	64.20
			RPMI-8226 (leukemia)	65.30
			MDA-MB-468 (breast cancer)	68.04
5d	97.13	58.37–138.35	HOP-92 (non-small cell lung cancer)	58.37
			PC-3 (prostate cancer)	60.62
			HOP-62 (non-small cell lung cancer)	74.23
			RPMI-8226 (leukemia)	76.16
5e	103.81	77.59–160.15	SR (leukemia)	77.59
			MCF7 (breast cancer)	81.74
			UO-31 (renal cancer)	82.59
5f	102.87	67.83–116.41	UO-31 (renal cancer)	67.83
			UACC-62 (melanoma)	83.61
			SR (leukemia)	85.12
			NCI-H522 (non-small cell lung cancer)	88.90
8a	100.04	80.00–124.71	CAKI-1 (renal cancer)	80.00
			A549/ATCC (non-small cell lung cancer)	80.30
8b	92.49	53.93–138.01	SNB-75 (CNS Cancer)	53.93
			HOP-62 (non-small cell lung cancer)	54.81
			OVCAR-8 (Ovarian Cancer)	68.04
			UO-31 (renal cancer)	73.35
			SF-268 (CNS Cancer)	75.49
			U251 (CNS Cancer)	75.63
8c	92.82	62.67–113.87	RPMI-8226 (leukemia)	62.67
			CCRF-CEM (leukemia)	63.27
			UO-31 (renal cancer)	70.75
			EKVX (non-small cell lung cancer)	74.94
			UACC-62 (melanoma)	76.62
10a	86.31	55.94–143.12	SK-MEL-5 (melanoma)	55.94
			EKVX (non-small cell lung cancer)	56.67
			RPMI-8226 (leukemia)	59.59
			T-47D (breast cancer)	60.87
10b	102.26	68.23–154.14	SR (leukemia)	68.23
			MOLT-4 (leukemia)	75.78
10c	96.20	69.40–149.48	HOP-92 (non-small cell lung cancer)	69.40
			PC-3 (Prostate Cancer)	74.28

Table 1 continued

Compound	Mean growth (%)	Range of growth (%)	The most sensitive cell lines	Growth of the most sensitive cell lines
10d	53.33	−13.42–98.42	RXF 393 (renal cancer)	−13.42
			UO-31 (renal cancer)	−7.62
			HOP-92 (non-small cell lung cancer)	−3.42
			SNB-75 (CNS Cancer)	1.32
			786-0 (renal cancer)	2.96
10e	95.78	71.45–119.00	HOP-92 (non-small cell lung cancer)	71.45
			SNB-75 (CNS Cancer)	76.15
			T-47D (breast cancer)	77.40
10f	106.99	78.93–154.29	SF-539 (CNS Cancer)	78.93
			MCF7 (breast cancer)	83.26
			SR (leukemia)	83.60
10 g	95.24	64.01–140.52	CCRF-CEM (leukemia)	64.01
			SR (leukemia)	66.68
			EKVX (non-small cell lung cancer)	75.89
			HOP-92 (non-small cell lung cancer)	76.75
			A498 (renal cancer)	78.69
10h	64.36	−27.30–126.14	SNB-75 (CNS Cancer)	−27.30
			U251 (CNS Cancer)	−9.89
			SF-539 (CNS Cancer)	−4.64
			T-47D (breast cancer)	−2.61
			786-0 (renal cancer)	−1.64
10i	88.03	56.31–139.48	SK-MEL-5 (melanoma)	56.31
			RPMI-8226 (leukemia)	60.93
			MDA-MB-468 (breast cancer)	62.57
10j	104.92	87.03–145.64	NCI-H522 (non-small cell lung cancer)	87.03
			A498 (renal cancer)	89.06
10k	98.82	81.40–127.82	RPMI-8226 (leukemia)	81.40
			A498 (renal cancer)	81.69
			MCF7 (breast cancer)	82.49
10l	100.31	71.71–135.21	K-562 (leukemia)	71.71
			HOP-92 (non-small cell lung cancer)	77.35
			HL-60(TB) (leukemia)	78.38
13	98.00	73.67–126.74	MCF7 (breast cancer)	73.67
			MDA-MB-468 (breast cancer)	78.51

(Table 1). The compound **10d** was highly active on renal cancer RXF 393 cell line (GP = −13.42 %), while the compound **10h** was highly active on the CNS cancer SNB-75 cell line (GP = −27.30 %). Compounds **10a**, **10i** were quite active on the melanoma SK-MEL-5 cell line (GP = 55.94 % and GP = 56.31 %, respectively) and leukemia RPMI-8226 cell line (GP = 59.59 % and GP = 60.93 %, respectively). Compounds **5b**, **8b** were quite active on the CNS cancer SNB-75 cell line (GP = 46.61 % and GP = 53.93 %, respectively). The majority of the tested compounds displayed growth inhibition on non-small cell lung cancer cell line HOP-92 (**5b**, **d**, **10c**, **d**, **e**, **g**, **l**) and different cell lines of Leukemia (**5a**, **c**, **e**, **f**, **8c**, **10a**, **b**, **i**, **f**, **k**, **l**). Finally, compounds **10d**, **10h** were selected for in vitro testing against a full panel of about 60 tumor cell lines at tenfold dilutions of five concentrations (100, 10, 1, 0.1, and 0.01 μM). Based on the cytotoxicity assays, three antitumor activity dose–response parameters were calculated for each experimental agent against each cell line: GI_{50}—molar concentration of the compound that inhibits 50 % net cell growth; TGI—molar concentration of the compound leading to total inhibition;

and LC_{50}—molar concentration of the compound leading to 50 % net cell death. Values were calculated for each of these parameters if the level of activity was reached; however, if the effect was not reached or was exceeded, the value was expressed as greater or less than the maximum or minimum concentration tested. Mean graph midpoints (MG_MID) were calculated for each of the parameters, giving an averaged activity parameter over all the cell lines for each compound. For the calculation of the MG_MID, insensitive cell lines were included with the highest concentration tested.

The most potent inhibition of human tumor cells was found for compounds **10d** and **10h** (Table 2) (MG_MID GI_{50} −4.52 and −4.12, respectively). The tested compounds showed a broad spectrum of growth inhibition activity against human tumor cells, as well as some distinctive patterns of selectivity. In general, compounds **10d**, **10h** selectively inhibited the growth of CNS cancer cell lines. We found that the compounds **10d** possessed significant activity on non-small cell lung cancer cell line HOP-92 (Log $GI_{50} = -6.23$); ovarian cancer cell lines, OVCAR-4 and SK-OV-3 (Log $GI_{50} = -5.79$ and Log $GI_{50} = -5.55$); and melanoma cell line MALME-3 M (Log $GI_{50} = -5.61$). The compound **10h** demonstrated high growth inhibition on breast cancer line MDA-MB-231/ATCC (Log $GI_{50} = -5.19$) and on ovarian cancer line SK-OV-3 (Log GI50 = −4.86). Furthermore, these compounds **10d**, **10h** appeared to be the most active against CNS cancer cell lines SNB-75 (Log $GI_{50} = -5.86$ and Log $GI_{50} = -5.41$, respectively), and the compound **l** possessed high activities on SF-539 and U251 cell lines (CNS) (Log $GI_{50} = -4.75$ and Log $GI_{50} = -4.93$, respectively). The most potent and selective cytotoxic activities against separate tumor cell lines are shown in Table 3 and Fig. 2.

COMPARE analysis

NCI's COMPARE algorithm (Paull *et al*., 1989; Zaharevitz *et al*., 2002; http://dtp.nci.nih.gov/docs/compare/compare.html; Weinstein *et al*., 1997) allows us to suppose biochemical mechanisms of action of novel compounds on the basis of their in vitro activity profiles compared to those of standard agents. Similarity of pattern to that of the seed is expressed quantitatively as a Pearson correlation coefficient (PCC). The results obtained with the COMPARE algorithm indicate that compounds high in this ranking may possess a mechanism of action similar to that of the seed compound. We used accessible online tool—NCI COMPARE analysis—to discover the similarity of compounds**10d**, **10h** with the seed one (Table 4). Correlations with PCC >0.6 were selected as significant. The compound **10h** did not yield any significant activity correlation with any of the standard agents. This may indicate that it has a unique mode of anticancer action. The compound **10d** showed a significant correlation with nitroestrone and some

Table 3 The influences of compounds **10d**, **10h** on the growth of individual tumor cell lines

Compound	Disease	Cell line	Log GI50	Log TGI
10d	Leukemia	K-562	−5.11	−4.00
	Leukemia	SR	−5.60	−4.22
	Non-small cell lung cancer	HOP-92	−6.23	−5.23
	CNS cancer	SF-268	−5.26	−4.00
	CNS cancer	SF-295	−5.30	−4.00
	CNS cancer	SF-539	−5.37	−4.00
	CNS cancer	SNB-75	−5.86	−5.22
	CNS cancer	U25	−5.22	−4.00
	Melanoma	MALME-3M	−5.61	−4.00
	Ovarian cancer	OVCAR-4	−5.79	−4.00
	Ovarian cancer	SK-OV-3	−5.55	−4.00
	Renal cancer	RXF 393	−5.46	−4.30
	Renal cancer	TK-10	−5.23	−4.00
	Breast cancer	HS 578T	−5.35	−4.00
	Breast cancer	MDA-MB-231/ATCC	−5.21	−4.00
10h	CNS cancer	SF-539	−4.75	−4.00
	CNS cancer	SNB-75	−5.41	−4.00
	CNS cancer	U25	−4.93	−4.00
	Melanoma	MALME-3M	−4.89	−4.00
	Ovarian cancer	SK-OV-3	−4.86	−4.00
	Renal cancer	RXF 393	−4.43	−4.00
	Renal cancer	TK-10	−4.52	−4.00
	Breast cancer	MDA-MB-231/ATCC	−5.19	−4.00

Table 2 Summary of anticancer screening data at dose-dependent assay

Compound	*N*	Log GI_{50}			Log TGI		
		N_1	Range	MG_MID	N_2	Range	MG_MID
10d	59	20	−6.23 to −4.05	−4.52	4	−5.22 to −4.22	−4.05
10h	59	8	−5.41 to −4.01	−4.12	0	–	−4.00

N number of human tumor cell lines tested at the 2nd stage assay, N_i number of sensitive cell lines (parameters Log GI_{50} and Log TGI <−4.00)

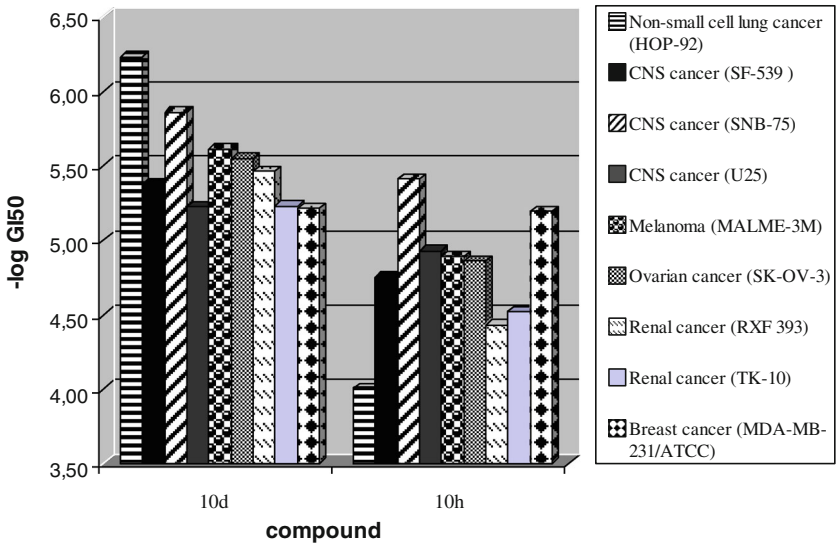


Fig. 2 Anticancer activities of the most active compounds **10d**, **10h**

Table 4 COMPARE analysis results for the tested compounds

Compound	PCC	Target	Target vector NSC	Count common cell lines	Seed Stdev	Target Stdev	Target mechanism of action
10d	0.832	Nitroestrone	S321803	55	0.232	0.065	Inhibits estrogen sulfotransferase (EST), a progesterone-induced secretory endometrial enzyme which affects estrogen receptor levels
	0.685	Flavoneacetic acid	S347512	56	0.23	0.088	Flavone acetic acid exhibits an antiproliferative effect on endothelial cells as a result of a superoxide-dependent mechanism, which induces changes in permeability of the vasculature of the tumor
	0.663	Piperazine alkylator	S344007	56	0.23	0.146	Alkylating agent, alkylate DNA at the N7 position of guanine
	0.635	Spirohydantoin mustard	S172112	46	0.252	0.186	Bifunctional alkylating agent
10h	0.336	Nitroestrone	S321803	54	0.331	0.265	Inhibits estrogen sulfotransferase (EST), a progesterone-induced secretory endometrial enzyme which affects estrogen receptor levels

alkylating agents. This molecular target should be considered as the first priority and be explored for the hit-to-lead optimization.

In the present article, new derivatives of 1,2,3-triazole-4-carboxamides were described. Molecular docking was performed to search molecules with affinity to EGFR tyrosine kinase as potent target for cancer therapy. In vitro anticancer activity for the synthesized compounds was evaluated. These preliminary results allowed identifying the most active compounds: 22 of the synthesized compounds were tested, and two of them, **10d**, **10h**, displayed considerable antitumor activity against leukemia, melanoma, lung, CNS, ovarian, renal, and breast cancer cell lines. In particular, the compound **10d** can be defined as a prospective antitumor agent with the values of $\log GI_{50}$ and logTGI being −6.23 and −5.23, respectively, for NSCLC cell line HOP-92. The obtained results of antitumor activities of such derivatives can be interesting with the promise to obtain more selective and active anticancer agents among 1,2,3-triazoles and prove the necessity of further investigation.

Experimental

Materials and methods

^{1}H NMR spectra were recorded on a Varian Mercury 400 instrument (400 MHz for ^{1}H and 100 MHz for ^{13}C) with TMS or deuterated solvent as an internal reference. Mass spectra were run using Agilent 1100 series LC/MSD with an API-ES/APCI ionization mode. Satisfactory elemental

analyses were obtained for new compounds (C ± 0.17, H ± 0.21, and N ± 0.19). All compounds are in the form of white solids. In NMR spectra data, the abbreviations Bis means 2,1-benzisoxazole cycle; Tr—triazole; Tz—thiazole; Td—thiadiazole; and Fur—furane correspondently.

Chemistry

*General procedure for the synthesis of 5-alkyl-1H-1,2,3-triazole-4-carboxamides **5a–f** and **8a–c***

To the suspension of 1*H*-1,2,3-triazole-4-carboxylic acid (1 mmol) in dry dichloromethane (DCM, 15 mL), oxalyl chloride (0.17 mL, 2 mmol) and two drops of DMF were added at 0 °C. The mixture was allowed to be stirred for 15 min at 0 °C and for ~3 h at room temperature, until no gas was observed. The solvent and the excess of oxalyl chloride were then distilled off to dryness. The obtained acyl chloride, without further purification, was dissolved in dry DCM (20 mL), cooled at 0 °C, and Et_3N (0.42 mL, 3 mmol) was added under stirring followed by addition of appropriate amines (1 mmol) in DCM (5 mL). The resulting mixture was allowed to stir at room temperature for an appropriate time, until complete conversion, as indicated by TLC. The solution was washed with water, a 5 % hydrocloric acid solution, sat. solution of sodium hydrocarbonate, and brine, dried over anhydrous Na_2SO_4, concentrated in vacuo to yield carboxamides **5a–f** and **8a–c**.

*5-Methyl-1-(3-phenyl-2,1-benzisoxazol-5-yl)-N-[3-(trifluoromethyl)phenyl]-1H-1,2,3-triazole-4-carboxamide (**5a**)* Yield 94 %, mp 167–168 °C. IR [cm^{-1}]: 1665 (CO); 3330 (NH). ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.93 (s, 1H, NH), 8.51 (s, 1H, H_{Bis}-4), 8.38 (s, 1H, H_{Ar}-2), 8.16 (d, J = 7.6 Hz, 2H, H_{Ph}-2,6), 8.12 (d, J = 9.0 Hz, 1H, H_{Bis}-7), 7.93 (d, J = 9.0 Hz, 1H, H_{Bis}-6), 7.67–7.53 (m, 5H, H_{Ph}-3,4,5 + H_{Ar}-5,6), 7.43 (d, J = 7.6 Hz, 1H, H_{Ar}-3), 2.62 (s, 3H, H_{Me}). ^{13}C NMR (100 MHz, DMSO-d_6): δ 167.2 (C_{Bis}-3), 160.6 (CO), 157.1 (C_{Bis}-7a), 140.2 (C_{ArNH}-1), 139.6 (C_{Tr}-4), 138.4 (C_{Tr}-5), 132.5 (C_{ArNH}-3), 132.1 (C_{Bis}-5), 131.9 (CH_{Bis}-6), 130.5 (CH_{ArNH}-5), 130.4 (2×CH_{Ph}-2,6 + C_{Ph}-4), 127.5 (2×CH_{Ph}-3,5), 127.4 (C_{Ph}-1), 126.0 (d, J = 274.3 Hz, CF_3), 124.7 (CH_{ArNH}-6), 120.7 (C_{Bis}-3a), 120.1 (CH_{ArNH}-4), 117.7 (CH_{ArNH}-2), 117.2 (C_{Bis}-4), 113.6 (C_{Bis}-7), 10.2 (C_{Me}). MS *m/z* 464 $(M+H)^+$. Calcd. for $C_{24}H_{16}F_3N_5O_2$: C, 62.20; H, 3.48; N, 15.11; Found: C, 62.21; H, 3.56; N, 15.18 %.

*1-(3-(4-Chlorophenyl)benzo[c]isoxazol-5-yl)-N-(3-methoxyphenyl)-5-methyl-1H-1,2,3-triazole-4-carboxamide (**5b**)* Yield 91 %, mp 209–210 °C. IR [cm^{-1}]: 1675 (CO); 3347 (NH). ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.29 (s, 1H, NH), 8.47 (d, J = 0.9 Hz, 1H, H_{Bis}-4), 8.20 (d, J = 8.1 Hz, 2H, H_{Ar}-2,6), 7.88 (dd, J = 9.4, 0.8 Hz, 1H, H_{Bis}-7), 7.68–7.55 (m, 4H, H_{Ar}-3,5 + H_{Bis}-6 + H_{ArNH}-2), 7.45 (d, J = 8.1 Hz, 1H, H_{ArNH}-6), 7.19 (t, J = 8.1 Hz, 1H, H_{ArNH}-5), 6.61 (d, J = 8.1 Hz, 1H, H_{ArNH}-4), 3.79 (d, J = 0.9 Hz, 3H, H_{MeO}), 2.70 (d, J = 0.8 Hz, 3H, H_{Me}). ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.4 (C_{Bis}-3), 160.1 (C_{ArNH}-3), 160.0 (CO), 157.1 (C_{Bis}-7a), 141.7 (C_{Tr}-4), 140.2 (C_{Ar}-1), 139.3 (C_{Tr}-5), 132.3 (C_{Bis}-5), 131.9 (CH_{Bis}-6), 134.5 (C_{Ar}-4), 129.4 (2×CH_{Ar}-2,6), 129.6 (CH_{ArNH}-5), 126.7 (2×CH_{Ar}-3,5), 126.4 (C_{Ar}-1), 120.7 (C_{Bis}-3a), 117.6 (CH_{Bis}-4), 113.5 (CH_{Bis}-7), 112.7 (CH_{ArNH}-6), 109.7 (CH_{ArNH}-4), 106.3 (CH_{ArNH}-2), 55.4 (C_{MeO}), 10.4 (C_{Me}). MS *m/z* 460, 462 $(M+H)^+$. Calcd. for $C_{24}H_{18}ClN_5O_3$: C, 62.68; H, 3.95; N, 15.23; Found: C, 62.61; H, 3.92; N, 15.32 %.

*1-[3-(4-Chlorophenyl)-2,1-benzisoxazol-5-yl]-N-(furan-2-ylmethyl)-5-methyl-1H-1,2,3-triazole-4-carboxamide (**5c**)* Yield 89 %, mp 144–145 °C. IR [cm^{-1}]: 1688 (CO); 3371 (NH). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.84 (t, J = 6.0 Hz, 1H, NH), 8.41 (d, J = 0.8 Hz, 1H, H_{Bis}-4), 8.18 (d, J = 8.7 Hz, 2H, H_{Ar}-2,6), 7.86 (dd, J = 9.4, 0.8 Hz, 1H, H_{Bis}-7), 7.60 (d, J = 8.7 Hz, 2H, H_{Ar}-3,5), 7.57 (dd, J = 9.4, 1.7 Hz, 1H, H_{Bis}-6), 7.45 (dd, J = 1.8, 0.8 Hz, 1H, H_{Fur}-5), 6.33 (dd, J = 3.2, 1.8 Hz, 1H, H_{Fur}-4), 6.24 (d, J = 3.2 Hz, 1H, H_{Fur}-3), 4.49 (d, J = 6.0 Hz, 2H, CH_2), 2.64 (s, 3H, H_{Me}). ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.4 (C_{Bis}-3), 160.1 (CO), 157.1 (C_{Bis}-7a), 149.7 (C_{Fur}-2), 141.7 (C_{Tr}-4), 141.3 (CH_{Fur}-5), 139.3 (C_{Tr}-5), 132.3 (C_{Bis}-5), 131.9 (CH_{Bis}-6), 134.5 (C_{Ar}-4), 129.4 (2×CH_{Ar}-2,6), 126.7 (2×CH_{Ar}-3,5), 126.4 (C_{Ar}-1), 120.7 (C_{Bis}-3a), 117.6 (CH_{Bis}-4), 113.5 (CH_{Bis}-7), 110.5 (CH_{Fur}-4), 108.7 (CH_{Fur}-3), 37.2 (C_{MeO}), 10.4 (C_{Me}). MS *m/z* 434, 436 $(M+H)^+$. Calcd. for $C_{22}H_{16}ClN_5O_3$: C, 60.91; H, 3.72; N, 16.14; Found: C, 60.99; H, 3.75; N, 16.21 %.

*N-(2-chlorophenyl)-5-ethyl-1-(3-phenyl-2,1-benzisoxazol-5-yl)-1H-1,2,3-triazole-4-carboxamide (**5d**)* Yield 85 %, mp 120–121 °C. IR [cm^{-1}]: 1662 (CO); 3354 (NH). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.79 (s, 1H, NH), 8.47 (s, 1H, H_{Bis}-4), 8.39 (d, J = 7.9 Hz, 1H, H_{Ar}-2), 8.16 (d, J = 7.0 Hz, 2H, H_{Ph}-2,6), 7.90 (d, J = 9.5 Hz, 1H, H_{Bis}-7), 7.64–7.48 (m, 5H, H_{Ph}-3,4,5 + H_{Bis}-6 + H_{Ar}-6), 7.37 (t, J = 7.4 Hz, 1H, H_{Ar}-5), 7.17 (t, J = 7.4 Hz, 1H, H_{Ar}-4), 3.13 (q, J = 7.4 Hz, 2H, CH_2), 1.18 (t, J = 7.4 Hz, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ 167.3 (C_{Bis}-3), 160.3 (CO), 157.1 (C_{Bis}-7a), 144.2 (C_{Tr}-4), 138.1 (C_{Tr}-5), 140.8 (C_{Ar}-1), 132.4 (C_{Bis}-5), 132.3 (CH_{Bis}-6), 130.7 (C_{Ph}-4), 130.5 (2×CH_{Ph}-2,6), 129.6 (CH_{Ar}-3), 129.1 (2×CH_{Ph}-3,5), 128.1 (C_{Ph}-1), 127.4 (C_{Ar}-5), 120.9 (C_{Bis}-3a), 120.5 (C_{Ar}-2), 119.7 (CH_{Ar}-4), 117.7 (CH_{Bis}-4), 114.3 (CH_{Ar}-6), 113.5 (CH_{Bis}-7), 17.2 (CH_2), 13.4 (CH_3). MS *m/z* 444, 446 $(M+H)^+$. Calcd. for $C_{24}H_{18}ClN_5O_2$: C, 64.94; H, 4.09; N, 15.78; Found: C, 64.99; H, 4.13; N, 15.61 %.

*N-(3-chlorophenyl)-5-ethyl-1-(3-phenyl-2,1-benzisoxazol-5-yl)-1H-1,2,3-triazole-4-carboxamide (**5e**)* Yield 85 %, mp 193–194 °C. IR [cm^{-1}]: 1672 (CO); 3367 (NH). ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.61 (s, 1H, NH), 8.45 (s, 1H, H_{Bis}-4), 8.22–8.07 (m, 3H, H_{Ph}-2,6 + H_{Ar}-2), 7.89 (d, J = 9.6 Hz, 1H, H_{Bis}-7), 7.83 (d, J = 7.9 Hz, 1H, H_{Ar}-6), 7.67–7.49 (m, 4H, H_{Ph}-3,4,5 + H_{Bis}-6), 7.30 (t, J = 7.5 Hz, 1H, H_{Ar}-5), 7.07 (d, J = 8.0 Hz, 1H, H_{Ar}-4), 3.13 (q, J = 7.2 Hz, 2H, CH_2), 1.18 (t, J = 7.2 Hz, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ 167.3 (C_{Bis}-3), 160.2 (CO), 157.1 (C_{Bis}-7a), 144.2 (C_{Tr}-4), 138.1 (C_{Tr}-5), 139.2 (C_{Ar}-1), 134.8 (C_{Ar}-3), 132.4 (C_{Bis}-5), 132.3 (CH_{Bis}-6), 130.7 (C_{Ph}-4), 130.5 (2×CH_{Ph}-2,6), 129.1 (2×CH_{Ph}-3,5), 128.1 (C_{Ph}-1), 130.0 (C_{Ar}-5), 123.4 (CH_{Ar}-4), 120.9 (C_{Bis}-3a), 118.6 (CH_{Ar}-2), 117.7 (CH_{Bis}-4), 116.5 (CH_{Ar}-6), 113.5 (CH_{Bis}-7), 17.2 (CH_2), 13.4 (CH_3). MS *m/z* 444, 446 $(M+H)^+$. Calcd. for $C_{24}H_{18}ClN_5O_2$: C, 64.94; H, 4.09; N, 15.78; Found: C, 65.11; H, 4.18; N, 15.83 %.

*N-(4-chlorophenyl)-5-ethyl-1-(3-phenyl-2,1-benzisoxazol-5-yl)-1H-1,2,3-triazole-4-carboxamide (**5f**)* Yield 91 %, mp 190–191 °C. IR [cm^{-1}]: 1624 (CO); 3395 (NH). ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.73 (s, 1H, NH), 8.53 (s, 1H, H_{Bis}-4), 8.15 (s, J = 7.6 Hz, 2H, H_{Ph}-2,6), 7.88-7.96 (m, 3H, H_{Bis}-7 + H_{Ar}-2,6), 7.65-7.55 (m, 4H, H_{Ph}-3,4,5 + H_{Bis}-6), 7.37 (d, J = 8.4 Hz, 2H, H_{Ar}-3,5), 3.03 (q, J = 7.6 Hz, 2H, CH_2), 1.05 (t, J = 7.6 Hz, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ 167.3 (C_{Bis}-3), 160.0 (CO), 157.1 (C_{Bis}-7a), 144.4 (C_{Tr}-4), 138.3 (C_{Tr}-5), 138.1 (C_{Ar}-1), 132.5 (C_{Bis}-5), 132.1 (CH_{Bis}-6), 130.5 (C_{Ph}-4), 130.3 (2×CH_{Ph}-2,6), 129.1 (2×CH_{Ph}-3,5), 128.1 (C_{Ph}-1), 127.6 (2×CH_{Ar}-3,5), 127.3 (C_{Ar}-4), 122.7 (2×CH_{Ar}-2,6), 120.8 (C_{Bis}-3a), 117.7 (CH_{Bis}-4), 113.6 (CH_{Bis}-7), 17.2 (CH_2), 13.5 (CH_3). MS *m/z* 444, 446 $(M+H)^+$. Calcd. for $C_{24}H_{18}ClN_5O_2$: C, 64.94; H, 4.09; N, 15.78; Found: C, 64.87; H, 4.11; N, 15.75 %.

*N-(5-benzyl-1,3-thiazol-2-yl)-5-methyl-1-phenyl-1H-1,2,3-triazole-4-carboxamide (**8a**)* Yield 92 %, mp 198–199 °C. IR [cm^{-1}]: 1677 (CO); 3315 (NH). ^{1}H NMR (400 MHz, DMSO-d_6): 11.88 (s, 1H, NH), 7.82–7.49 (m, 5H, H_{Ph}), 7.37–7.16 (m, 6H, H_{PhC} + H_{Tz}), 4.11 (s, 2H, CH_2), 2.62 (s, 3H, H_{Me}). ^{13}C NMR (100 MHz, DMSO-d_6): δ 159.0 (CO), 156.7 (C_{Tz}-2), 140.1 (C_{PhC}-1), 138.4 (C_{Tr}-4), 137.2 (C_{Tr}-5), 135.5 (C_{Ph}-1), 134.9 (C_{Tz}-5), 131.6 (C_{Tz}-5), 130.1 (CH_{Ph}-4), 129.8 (2×CH_{Ph}-2,6), 128.6 (2×CH_{PhC}-2,6), 128.6 (2×CH_{PhC}-3,5), 126.6 (CH_{PhC}-4), 125.5 (2×CH_{Ph}-3,5), 32.7 (CH_2), 9.8 (C_{Me}). MS *m/z* 376 $(M+H)^+$. Calcd. for $C_{20}H_{17}N_5OS$: C, 63.98; H, 4.56; N, 18.65; Found: C, 64.08; H, 4.79; N, 18.59 %.

*N-(5-benzyl-1,3-thiazol-2-yl)-1-(2-methoxyphenyl)-5-methyl-1H-1,2,3-triazole-4-carboxamide (**8b**)* Yield 92 %, mp 210–211 °C. IR [cm^{-1}]: 1689 (CO); 3393 (NH). ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.72 (s, 1H, NH), 7.61 (t, J = 7.9 Hz, 1H, H_{Ar}-5), 7.42 (d, J = 7.6 Hz, 1H, H_{Ar}-4), 7.33 –7.20 (m, 7H, H_{PhC} + H_{Ar}-6 + H_{Tz}), 7.16 (t, J = 7.7 Hz, 1H, H_{Ar}-3), 4.11 (s, 2H, H_{CH2}), 3.85 (s, 3H, H_{MeO}), 2.42 (s, 3H, H_{Me}). ^{13}C NMR (100 MHz, DMSO-d_6) δ 159.0 (CO), 156.6 (C_{Tz}-2), 153.8 (C_{Ar}-2), 140.1 (C_{Ph}-1), 140.0 (C_{Tr}-4), 136.5 (C_{Tr}-5), 134.9 (C_{Tz}-5), 132.4 (CH_{Ar}-4), 131.6 (C_{Tz}-4), 128.6 (2×CH_{Ph}-2,6), 128.6 (2×CH_{Ph}-3,5), 128.6 (CH_{Ar}-6), 126.6 (CH_{Ph}-4), 123.8 (C_{Ar}-1), 121.1 (CH_{Ar}-5), 112.9 (CH_{Ar}-3), 56.1 (CH_3O), 32.7 (CH_2), 9.2 (C_{Me}). MS *m/z* 406 $(M+H)^+$. Calcd. for $C_{21}H_{19}N_5O_2S$: C, 62.21; H, 4.72; N, 17.27; Found: C, 62.22; H, 4.79; N, 17.33 %.

*N-[5-(3-chlorobenzyl)-1,3-thiazol-2-yl]-5-methyl-1-(4-methylphenyl)-1H-1,2,3-triazole-4-carboxamide (**8c**)* Yield 92 %, mp 210–211 °C. IR [cm^{-1}]: 1655 (CO); 3392 (NH). ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.26 (s, 1H, NH), 7.49 (d, J = 8.2 Hz, 2H, H_{Ar}-2,6), 7.41 (d, J = 8.1 Hz, 2H, H_{Ar}-3,5), 7.37–7.29 (m, 3H, H_{ArC} + H_{Tz}), 7.29–7.21 (m, 2H, H_{ArC}), 4.11 (s, 2H, H_{CH2}), 2.46 (s, 3H, H_{Me}), 2.38 (s, 3H, H_{MeAr}). ^{13}C NMR (100 MHz, DMSO-d_6) δ 159.9 (CO), 157.3 (C_{Tz}-2), 143.6 (C_{Ar}-1), 140.7 (C_{ArC}-1), 139.3 (C_{ArC}-3), 137.4 (C_{Tr}-4), 135.4 (C_{Tr}-5), 133.8 (C_{Tz}-5), 133.3 (C_{Ar}-4), 131.6 (C_{Tz}-4), 131.2 (C_{ArC}-2), 130.8 (2×CH_{Ar}-3,5), 128.9 (C_{ArC}-2), 127.8 (C_{ArC}-6), 127.2 (C_{ArC}-4), 125.9 (2×CH_{Ar}-2,6), 32.1 (CH_2), 21.4 (C_{MeAr}), 10.2 (C_{Me}). MS *m/z* 424 $(M+H)^+$. Calcd. for $C_{21}H_{18}ClN_5OS$: C, 59.50; H, 4.28; N, 16.52; Found: C, 59.53; H, 4.49; N, 16.62 %.

*General procedure for the synthesis of amides **10a**, **c–e**, **g–i**, **k**, **l***

An appropriate cyanacetamide **9** (1 mmol) was added to the solution of sodium methoxide (54 mg, 1 mmol) in dry methanol (10 mL). The solution of appropriate arylazide **2** (10.0 mmol) in dry methanol (5 mL) was added dropwise. The mixture was stirred for 1 h. The resulting suspension was filtered, and the solid product was washed with water and methanol to give triazole **10** as a white solid.

*General procedure for the synthesis of amides **10b**, **f**, **j***

An appropriate amine **1** (1 mmol), arylazide **1** (1 mmol), and DBU (0.15 mL, 1 mmol) were added to the solution of 3-(3,5-dimethyl-1*H*-pyrazol-1-yl)-3-oxopropanenitrile (1 mmol) in dry acetonitrile (20 mL). The mixture was heated under reflux during 30 min. Then, it was cooled to room temperature, and the solid started to sediment. The product was filtered and washed with methanol to give triazole **10** as a white solid.

*5-Amino-N-(2,4-dichlorophenyl)-1-(2-methylphenyl)-1H-1,2,3-triazole-4-carboxamide (**10a**)* Yield 88 %, mp

138–139 °C. IR [cm^{-1}]: 1671 (CO), 3028, 3335 (NH). ^1H NMR (400 MHz, DMSO-d_6): δ 9.29 (s, 1H, NH), 8.39 (d, $J = 8.9$ Hz, 1H, H_{ArNH}-6), 7.55–7.39 (m, 4H, H_{Ar}), 7.37–7.31 (m, 2H, H_{Ar}), 6.35 (s, 2H, NH_2), 2.16 (s, 3H, H_{Me}). ^{13}C NMR (100 MHz, DMSO-d_6): δ 160.2 (CO), 146.2 (C_{Tr}-5), 135.9 (C_{Ar}-1), 134.2 (C_{Ar}-2), 133.4 (C_{ArNH}-1), 131.4 (CH_{Ar}-3), 130.5 (CH_{Ar}-6), 128.8 (CH_{Ar}-4), 128.0 (C_{Tr}-4), 127.8 (2×CH_{ArNH}-3,5), 127.2 (CH_{Ar}-5), 124.0 (CH_{ArNH}-4), 122.6 (CH_{ArNH}-6), 120.6 (C_{ArNH}-2), 17.6 (C_{Me}). MS *m/z* 362, 363, 364 $(M+H)^+$. Calcd. for $C_{16}H_{13}Cl_2N_5O$: C, 53.05; H, 3.62; N, 19.33; Found: C, 53.15; H, 3.56; N, 19.37 %.

5-Amino-N-benzyl-1-(4-methylphenyl)-1H-1,2,3-triazole-4-carboxamide (***10b***) Yield 97 %, mp 197–198 °C. IR [cm^{-1}]: 1678 (CO), 3010, 3334 (NH). ^1H NMR (400 MHz, DMSO-d_6): δ 8.60 (t, $J = 6.3$ Hz, 1H, NH), 7.46 (d, $J = 8.4$ Hz, 2H, H_{Ar}-2,6), 7.37 (d, $J = 8.4$ Hz, 2H, H_{Ar}-3,5), 7.34 (d, $J = 7.2$ Hz, 2H, H_{Ph}-2,6), 7.28 (t, $J = 7.2$ Hz, 2H, H_{Ph}-3,5), 7.20 (t, $J = 7.2$ Hz, 1H, H_{Ph}-4), 6.23 (s, 2H, NH_2), 4.47 (d, $J = 6.3$ Hz, 2H, CH_2), 2.44 (s, 3H, H_{Me}). ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.1 (CO), 144.8 (C_{Tr}-5), 139.8 (C_{Ar}-1), 138.5 (C_{Ph}-1), 132.5 (C_{Ar}-4), 130.9 (2×CH_{Ar}-2,6), 128.4 (2×CH_{Ph}-3,5), 127.5 (2×CH_{Ph}-2,6), 127.1 (CH_{Ph}-4), 125.1 (2×CH_{Ar}-3,5), 124.5 (C_{Tr}-4), 43.5 (CH_2), 21.20 (C_{Me}). MS *m/z* 308 $(M+H)^+$. Calcd. for $C_{17}H_{17}N_5O$: C, 66.43; H, 5.58; N, 22.79; Found: C, 66.52; H, 5.67; N, 22.73 %.

5-Amino-N-(4-methoxyphenyl)-1-(4-methylphenyl)-1H-1,2,3-triazole-4-carboxamide (***10c***) Yield 74 %, mp 219–220 °C. IR [cm^{-1}]: 1639 (CO), 3038, 3345 (NH). ^1H NMR (400 MHz, DMSO-d_6): δ 9.85 (s, 1H, NH), 7.72 (d, $J = 9.1$ Hz, 2H, H_{Ar}-2,6), 7.48 (d, $J = 8.4$ Hz, 2H, H_{ArNH}-2,6), 7.38 (d, $J = 8.4$ Hz, 2H, H_{ArNH}-3,5), 6.82 (d, $J = 9.1$ Hz, 2H, H_{Ar}-3,5), 6.34 (s, 2H, NH_2), 3.75 (s, 3H, H_{MeO}), 2.45 (s, 3H, H_{Me}). ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.1 (CO), 157.1 (C_{ArNH}-4), 144.8 (C_{Tr}-5), 139.8 (C_{Ar}-1), 132.7 (C_{ArNH}-1), 132.5 (C_{Ar}-4), 130.9 (2×CH_{Ar}-2,6), 122.1 (2×CH_{ArNH}-2,6), 124.5 (C_{Tr}-4), 125.1 (2×CH_{Ar}-3,5), 114.5 (2×CH_{ArNH}-3,5), 55.3 (C_{MeO}), 21.20 (C_{Me}). MS *m/z* 324 $(M+H)^+$. Calcd. for $C_{17}H_{17}N_5O_2$: C, 63.15; H, 5.30; N, 21.66; Found: C, 63.23; H, 5.31; N, 21.72 %.

5-Amino-N-(2,5-dichlorophenyl)-1-(4-methylphenyl)-1H-1,2,3-triazole-4-carboxamide (***10d***) Yield 79 %, mp 233–234 °C. IR [cm^{-1}]: 1646 (CO), 3057, 3305 (NH). ^1H NMR (400 MHz, DMSO-d_6): δ 9.49 (s, 1H, NH), 8.24 (s, 1H, H_{ArNH}-6), 7.56 (d, $J = 8.5$ Hz, 1H, H_{ArNH}-3), 7.45 (d, $J = 8.2$ Hz, 2H, H_{Ar}-3,5), 7.39 (d, $J = 8.2$ Hz, 2H, H_{Ar}-2,6), 7.22 (d, $J = 8.5$ Hz, 1H, H_{ArNH}-4), 6.57 (s, 2H, NH_2), 2.38 (s, 3H, H_{Me}). ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.8 (CO), 146.0 (C_{Tr}-5), 139.8 (C_{ArN}-1), 132.7 (C_{ArNH}-1), 132.5 (C_{Ar}-4), 131.4 (CH_{ArNH}-3), 130.9 (2×CH_{Ar}-2,6), 129.7 (CH_{ArNH}-5), 125.6 (C_{Tr}-4), 125.1 (2×CH_{Ar}-3,5), 123.7 (CH_{ArNH}-4), 123.0 (CH_{ArNH}-6), 116.1 (C_{ArNH}-2), 21.2 (C_{Me}). MS *m/z* 362, 363, 364 $(M+H)^+$. Calcd. for $C_{16}H_{13}Cl_2N_5O$: C, 53.05; H, 3.62; N, 19.33; Found: C, 53.01; H, 3.67; N, 19.25 %.

5-Amino-N-(5-ethyl-1,3,4-thiadiazol-2-yl)-1-(4-methylphenyl)-1H-1,2,3-triazole-4-carboxamide (***10e***) Yield 78 %, mp 259–260 °C. IR [cm^{-1}]: 1628 (CO), 3025, 3341 (NH). ^1H NMR (400 MHz, DMSO-d_6): δ 7.46 (d, $J = 7.9$ Hz, 2H, H_{Ar}-3,5), 7.37 (d, $J = 7.9$ Hz, 2H, H_{Ar}-2,6), 6.79 (s, 2H, NH_2), 2.79 (q, $J = 7.6$ Hz, 2H), 2.36 (s, 3H, H_{Me}), 1.21 (t, $J = 7.6$ Hz, 3H, H_{Me}). ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.4 (C_{Td}-1), 165.2 (C_{Td}-2), 161.5 (CO), 144.1 (C_{Tr}-5), 138.6 (C_{ArN}-1), 134.0 (C_{Ar}-4), 130.7 (2×CH_{Ar}-2,6), 124.0 (2×CH_{Ar}-3,5 + C_{Tr}-4), 24.2 (C_{Et}), 21.4 (C_{Me}), 15.0 (C_{Et}). MS *m/z* 330 $(M+H)^+$. Calcd. for $C_{14}H_{15}N_7OS$: C, 51.05; H, 4.59; N, 29.77; Found: C, 51.17; H, 4.51; N, 29.83 %.

5-Amino-1-(3,5-dimethylphenyl)-N-(1-methylethyl)-1H-1,2,3-triazole-4-carboxamide (***10f***) Yield 85 %, mp 182–183 °C. IR [cm^{-1}]: 1667 (CO), 3030, 3321 (NH). ^1H NMR (400 MHz, DMSO-d_6): δ 7.60 (d, $J = 8.3$ Hz, 1H, NH), 7.17 (s, 2H, H_{Ph}-2,6), 7.10 (s, 1H, H_{Ph}-4), 6.22 (s, 2H, NH_2), 4.14 (dq, $J = 13.2$, 6.5 Hz, 1H, H_{CH}), 2.40 (s, 6H, H_{Me}), 1.22 (d, $J = 6.6$ Hz, 6H, H_{Me}). ^{13}C NMR (100 MHz, DMSO-d_6) δ 159.7 (CO), 146.2 (C_{Tr}-5), 139.5 (C_{ArN}-1), 139.8 (2×C_{Ar}-3,5), 130.3 (CH_{Ar}-4), 124.4 (C_{Tr}-4), 123.1 (2×CH_{Ar}-2,6), 41.5 (CH), 23.2 (2×C_{Me}), 21.20 (2×C_{Me}). MS *m/z* 274 $(M+H)^+$. Calcd. for $C_{14}H_{19}N_5O$: C, 61.52; H, 7.01; N, 25.62; Found: C, 61.54; H, 7.11; N, 25.58 %.

5-Amino-N-(2,6-dimethylphenyl)-1-(4-fluorophenyl)-1H-1,2,3-triazole-4-carboxamide (***10g***) Yield 74 %, mp 206–207 °C. IR [cm^{-1}]: 1657 (CO), 3061, 3338 (NH). ^1H NMR (400 MHz, DMSO-d_6): δ 9.46 (s, 1H, NH), 7.65 (dd, $J = 8.8$, 4.8 Hz, 2H, H_{Ar}-2,6), 7.37 (t, $J = 8.8$ Hz, 2H, H_{Ar}-3,5), 7.06 (br.s, 3H, H_{ArNH}-3,4,5), 6.35 (s, 2H, NH_2), 2.24 (s, 6H, H_{Me}). ^{13}C NMR (100 MHz, DMSO-d_6) δ 162.5 (d, $J = 247.5$ Hz, C_{Ar}-4), 160.81 (CO), 146.5 (C_{Tr}-5), 135.4 (2×C_{ArNH}-2,6), 134.2 (C_{ArNH}-1), 130.7 (C_{Ar}-1), 128.0 (2×CH_{ArNH}-3,5), 127.7 (d, $J = 9.0$ Hz, 2×CH_{Ar}-2,6), 122.0 (C_{Tr}-5), 117.1 (d, $J = 23.4$ Hz, 2×CH_{Ar}-3,5), 124.5 (CH_{ArNH}-4), 21.20 (C_{Me}), 18.4 (2×C_{Me}). MS *m/z* 326 $(M+H)^+$. Calcd. for $C_{17}H_{16}FN_5O$: C, 62.76; H, 4.96; N, 21.53; Found: C, 62.82; H, 4.93; N, 21.67 %.

5-Amino-N-(2,4-dimethoxyphenyl)-1-(4-fluorophenyl)-1H-1,2,3-triazole-4-carboxamide (***10h***) Yield 94 %, mp 216–217 °C. IR [cm^{-1}]: 1664 (CO), 3009, 3359 (NH). ^1H

NMR (400 MHz, DMSO-d_6): 8.92 (s, 1H), 8.18 (dd, $J = 8.8$, 1.2 Hz, 1H), 7.64 (ddd, $J = 8.8$, 4.8, 1.3 Hz, 2H), 7.38 (td, $J = 8.8$, 1.3 Hz, 2H), 6.61 (dd, $J = 2.4$, 1.3 Hz, 1H), 6.52–6.39 (m, 3H), 3.95 (d, $J = 1.2$ Hz, 3H), 3.79 (d, $J = 1.3$ Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 162.5 (d, $J = 247.5$ Hz, C_{Ar}-4), 159.9 (CO), 156.2 (C_{ArNH}-4), 149.5 (C_{ArNH}-2), 145.2 (C_{Tr}-5), 130.7 (C_{Ar}-1), 127.5 (d, $J = 9.0$ Hz, 2×CH_{Ar}-2,6), 122.2 (C_{Tr}-4), 121.1 (C_{ArNH}-1), 120.2 (C_{ArNH}-6), 117.3 (d, $J = 23.4$ Hz, 2×CH_{Ar}-3,5), 104.1 (C_{ArNH}-5), 98.7 (C_{ArNH}-3), 56.1 (C_{MeO}), 55.4 (C_{MeO}). MS m/z 358 $(M+H)^+$. Calcd. for $C_{17}H_{16}FN_5O_3$: C, 57.14; H, 4.51; N, 19.60; Found: C, 57.21; H, 4.47; N, 19.70 %.

*5-Amino-N-(4-ethylphenyl)-1-(4-methoxyphenyl)-1H-1,2,3-triazole-4-carboxamide (**10i**)* Yield 92 %, mp 183–184 °C. IR [cm^{-1}]: 1655 (CO), 3017, 3360 (NH). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.79 (s, 1H, NH), 7.69 (d, $J = 8.4$ Hz, 2H, H_{ArNH}-3,5), 7.48 (d, $J = 8.8$ Hz, 2H, H_{ArNH}-3,5), 7.13–7.04 (m, 4H, H_{Ar}), 6.28 (s, 2H, NH_2), 3.86 (s, 3H, H_{MeO}), 2.59 (q, $J = 7.6$ Hz, 2H, H_{Et}), 1.21 (t, $J = 7.6$ Hz, 3H, H_{Et}). ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.8 (CO), 159.9 (C_{ArNH}-4), 145.4 (C_{Tr}-4), 138.5 (C_{ArN}-4), 136.9 (C_{ArNH}-1), 128.0 (C_{Ar}-1), 127.7 (2×CH_{Ar}-2,6), 126.0 (2×CH_{ArNH}-2,6), 122.0 (C_{Tr}-5), 120.3 (2×CH_{Ar}-3,5), 114.9 (2×CH_{ArNH}-3,5), 55.7 (C_{MeO}), 28.2 (C_{Et}), 16.1 (C_{Et}). MS *m/z* 338 $(M+H)^+$. Calcd. for $C_{18}H_{19}N_5O_2$: C, 64.08; H, 5.68; N, 20.76; Found: C, 64.11; H, 5.75; N, 20.85 %.

*5-Amino-N-cyclopentyl-1-[3-(trifluoromethyl)phenyl]-1H-1,2,3-triazole-4-carboxamide (**10j**)* Yield 93 %, mp 152–153 °C. IR [cm^{-1}]: 1679 (CO), 3026, 3348 (NH). ^{1}H NMR (400 MHz, DMSO-d_6): 7.95–7.87 (m, 2H, H_{Ar}), 7.85–7.78 (m, 3H, H_{Ar} + NH), 6.50 (s, 2H, NH_2), 4.25 (h, $J = 7.3$ Hz, 1H, CH), 2.00–1.83 (m, 2H, CH_2), 1.81–1.68 (m, 2H, CH_2), 1.67–1.50 (m, 4H, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ 159.9 (CO), 145.2 (C_{Tr}-5), 135.8 (C_{Ar}-1), 131.3 (q, $J = 32.5$ Hz, C_{Ar}-3), 131.0 (CH_{Ar}-5), 127.8 (CH_{Ar}-6), 125.6 (2×CH_{Ar}-4), 123.7 (q, $J = 271.3$ Hz, CF_3), 122.2 (C_{Tr}-4), 120.8 (CH_{Ar}-2), 50.5 (CH), 32.7 (2×CH_2), 24.2 (2×CH_2). MS m/z 340 $(M+H)^+$. Calcd. for $C_{15}H_{16}F_3N_5O$: C, 53.10; H, 4.75; N, 20.64; Found: C, 53.19; H, 4.63; N, 20.53 %.

*5-Amino-N-(4-fluorophenyl)-1-[3-(trifluoromethyl)phenyl]-1H-1,2,3-triazole-4-carboxamide (**10k**)* Yield 95 %, mp 211–212 °C. IR [cm^{-1}]: 1668 (CO), 3022, 3383 (NH). ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.19 (s, 1H, NH), 8.07–7.65 (m, 6H, H_{Ar}), 7.04 (t, $J = 8.8$ Hz, 2H, H_{ArNH}-3,5), 6.69 (s, 2H, NH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ 159.68 (CO), 159.7 (d, $J = 250.1$ Hz, C_{ArNH}-4), 145.19 (C_{Tr}-5), 135.8 (C_{Ar}-1), 131.3 (q, $J = 32.7$ Hz, C_{Ar}-3), 131.0 (CH_{Ar}-5), 127.9 (C_{ArNH}-1), 121.5 (d, $J = 9.0$ Hz, 2×CH_{Ar}-2,6), 127.8 (CH_{Ar}-6), 125.6 (2×CH_{Ar}-4), 123.7 (q, $J = 272.1$ Hz, CF_3), 122.2 (C_{Tr}-4), 120.8 (CH_{Ar}-2), 115.8 (d, $J = 24.2$ Hz, 2×CH_{Ar}-3,5). MS *m/z* 366 $(M+H)^+$. Calcd. for $C_{16}H_{11}F_4N_5O$: C, 52.61; H, 3.04; N, 19.17; Found: C, 52.69; H, 3.22; N, 19.11 %.

*5-Amino-N-(2,4-dimethoxyphenyl)-1-[3-(trifluoromethyl)phenyl]-1H-1,2,3-triazole-4-carboxamide (**10l**)* Yield 86 %, mp 163–164 °C. IR [cm^{-1}]: 1694 (CO), 3013, 3397 (NH). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.93 (s, 1H, NH), 8.19 (d, $J = 8.8$ Hz, 1H, H_{ArNH}-5), 7.91 (br.s, 2H, H_{ArNH}), 7.87–7.77 (m, 2H, H_{Ar}), 6.69–6.55 (m, 3H, H_{Ar} + NH_2), 6.47 (d, $J = 8.7$ Hz, 1H, H_{ArNH}-6), 3.96 (s, 3H, H_{MeO}), 3.79 (s, 3H, H_{MeO}). ^{13}C NMR (100 MHz, DMSO-d_6) δ 159.7 (CO), 156.2 (C_{ArNH}-4), 149.5 (C_{ArNH}-2), 145.2 (C_{Tr}-5), 135.8 (C_{Ar}-1), 131.3 (q, $J = 33.0$ Hz, C_{Ar}-3), 131.0 (CH_{Ar}-5), 127.8 (CH_{Ar}-6), 125.6 (2×CH_{Ar}-4), 123.7 (q, $J = 272.9$ Hz, CF_3), 122.2 (C_{Tr}-4), 121.1 (C_{ArNH}-1), 120.8 (CH_{Ar}-2), 120.2 (C_{ArNH}-6), 104.1 (C_{ArNH}-5), 98.8 (C_{ArNH}-3), 56.1 (C_{MeO}), 55.4 (C_{MeO}). MS *m/z* 408 $(M+H)^+$. Calcd. for $C_{18}H_{16}F_3N_5O_3$: C, 53.07; H, 3.96; N, 17.19; Found: C, 53.02; H, 3.87; N, 17.29 %.

Pharmacology

A primary anticancer assay was performed at ~60 human tumor cell lines panel derived from nine neoplastic diseases, in accordance with the protocol of the Drug Evaluation Branch, National Cancer Institute, Bethesda. The tested compounds were added to the culture at a single concentration (10^{-5} M). and the cultures were incubated for 48 h. End-point determinations were made with a protein binding dye, sulforhodamine B (SRB). Results for each tested compound were reported as the percent of growth of the treated cells compared with the untreated control cells. The percentage growth was evaluated spectrophotometrically versus controls not treated with test agents. The cytotoxic and/or growth inhibitory effects of the most active selected compounds were tested in vitro against the full panel of about 60 human tumor cell lines at tenfold dilutions of five concentrations ranging from 10^{-4} to 10^{-8} M. The 48-h continuous drug exposure protocol was followed, and an SRB protein assay was used to estimate cell viability or growth.

Using the seven absorbance measurements [time zero, (T_z), control growth in the absence of drug, (C), and test growth in the presence of drug at the five concentration levels (T_i)], the percentage growth was calculated at each of the drug concentration levels. Percentage growth inhibition was calculated as

$[(T_i - T_z)/(C - T_z)] \times 100$ for concentrations for which $T_i \geq T_z$

$[(T_i - T_z)/T_z] \times 100$ for concentrations for which $T_i < T_z$.

Three dose–response parameters were calculated for each compound. Growth inhibition of 50 % (GI_{50}) was calculated from $[(T_i - T_z)/(C - T_z)] \times 100 = 50$, which is the drug concentration resulting in a 50 % lower net protein increase in the treated cells (measured by SRB staining) compared to the net protein increase seen in the control cells. The drug concentration resulting in total growth inhibition (TGI) was calculated from $T_i = T_z$. The LC_{50} (concentration of drug resulting in a 50 % reduction in the measured protein at the end of the drug treatment compared to that at the beginning) indicating a net loss of cells following treatment was calculated from $[(T_i - T_z)/T_z] \times 100 = -50$. Values were calculated for each of these three parameters if the level of activity was reached; however, if the effect was not reached or was exceeded, the value for that parameter was expressed as more or less than the maximum or minimum concentration tested. The log-GI_{50}, logTGI, and logLC_{50} were then defined as the mean of the logs of the individual GI_{50}, TGI, and LC_{50} values, respectively. The lowest values were obtained with the most sensitive cell lines. Compounds having these values ≤ 4 were declared to be active.

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