European Journal of Medicinal Chemistry 46 (2011) 6066–6074

Contents lists available at SciVerse ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Synthesis and biological activity of novel *N*-cycloalkyl-(cycloalkylaryl)-2-[(3-R-2-oxo-2*H*-[1,2,4]triazino[2,3-*c*]quinazoline-6-yl)thio]acetamides

Galyna G. Berest [a,*], Olexii Yu. Voskoboynik [a], Sergiy I. Kovalenko [a], Olexii M. Antypenko [a], Inna S. Nosulenko [a], Andrii M. Katsev [b], Olena S. Shandrovskaya [b]

[a] *Pharmaceutical Chemistry Department, Zaporozhye State Medical University, 26, Mayakovsky ave., 69035 Zaporozhye, Ukraine*
[b] *Department of Pharmacy, Crimean State Medical University, 95006 Simferopol, Ukraine*

ARTICLE INFO

Article history:
Received 7 June 2011
Received in revised form
7 October 2011
Accepted 11 October 2011
Available online 20 October 2011

Keywords:
N-cycloalkyl-(cycloalkylaryl)-2-[(3-R-2-oxo-2*H*-[1,2,4]triazino[2,3-*c*]quina-zoline-6-yl) thio]acetamides
Anticancer
Antibacterial
Bioluminescence inhibition

ABSTRACT

In this paper the novel *N*-cycloalkyl-(cycloalkylaryl)-2-[(3-R-2-oxo-2*H*-[1,2,4]triazino[2,3-*c*]quinazoline-6-yl)thio]acetamides synthesis by aminolysis of activated by thionyl chloride or carbonyldiimidazole [(3-R-2-oxo-2*H*-[1,2,4]triazino[2,3-*c*]quinazolin-6-yl)-thio]acetic acids and alkylation of the 3-R-6-thio-6,7-dihydro-2*H*-[1,2,4]triazino[2,3-*c*]quinazoline-2-ones potassium salts with *N*-cycloalkyl-(cycloalkylaryl)-2-chloroacetamides are proposed. The structures of compounds are determined by ^{1}H, ^{13}C NMR, LC-MS and EI-MS analysis. The *in vitro* anticancer, antibacterial activity and *Photobacterium leiognathi* Sh1 bioluminescence inhibition of synthesized compounds were revealed. SAR results were discussed. Compound **4.10** was found to be the most anticancer active one, selectively influenced on the non-small cell lung and CNS cancer cell lines, especially on the HOP-92 (log $GI_{50} = -6.01$) and U251 (log $GI_{50} = -6.00$).

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1. Introduction

Nowadays heterocyclic compounds are intensively studied to enhance the range of anticancer agents [1]. During the last decade lots of quinazoline anticancer drugs were discovered [2–13]. Among them, “Iressa” (**1**), “Erlotinib”, its hydrochloride (**2**) and “Vandetanib” (**3**) are already being used. In spite of the fact, that the mentioned compounds are derivatives of 4-aminoquinazoline, the other substituted quinazolines also have antiproliferative activity, which is not limited only by the indicated pharmacophore [2–13]. One of the perspective antitumor heterocycles are azino- and azolo-annelated quinazolines [14–16]. Recently, group of Slovak’s scientists have found that [1,2,4]triazolo[4,3-*c*]quinazoline derivatives (**4**) possessed considerable *in vitro* antitumor activity against cell line HeLa and B16 [14]. Also anticancer activity is characteristic for 6-cyanobenzimidazole[1,2-*c*]quinazolines (**5**), indolo[2′,3′:3,4] pyrido[2,1-*b*]quinazolin-5(7*H*)-ones (**6**) [15,16]. The latter substances inhibited breast (MCF7/ADR), CNS (U251), colon (SW620), lung (H522), melanoma (UACC62), ovarian (SKOV3), prostate (DU145) and renal (ACHN) cancer cell lines in concentration 1–8 μM *in vitro* [16] Fig. 1.

Furthermore, several biological activities have been found for [1,2,4]triazino[*c*]quinazolines [17–32]. 3-R-6-thio-6,7-dihydro-2*H*-[1,2,4]triazino[2,3-*c*]quinazolin-2-ones and their potassium salts, that were synthesized by us, have already shown antibacterial properties against *Escherichia coli*, *Aspergillus niger*, *Mycobacterium luteum* and antifungal activity against *Candida albicans* and *Candida tenuis* [31,32]. Additionally, combination of such pharmacological fragments in our compounds as 1-adamantylamine, [4-(1-adamantyl)phenyl]amine, (3-ethylcyclo[2.2.1]hept-2-yl)amine and triazinoquinazoline skeleton, might cause the appearance of other biological activities. Therefore, “structure–activity” relationship for substances **7**–**10** has already demonstrated that the adamantyl group is essential in maintaining anticancer activity as an oxygen-sensitive hypoxia-inducible factor-1, which is found in many human tumours resistant to radiation treatment and in animal models associated with increased tumour growth, vascularization and metastasis inhibitor [33] Fig. 2.

So, the aim of this work was the development of synthesis methods for novel *N*-cycloalkyl-(cycloalkylaryl)-2-[(3-R-2-oxo-2*H*-[1,2,4]triazino[2,3-*c*]quinazolin-6-yl)thio]acetamides, investigation of their physico–chemical properties, cytotoxicity, antitumor and

* Corresponding author. Tel.: +38 097 687 68 89; fax: +38 0612 34 15 71.
E-mail address: kovalenkosergiy@gmail.com (G.G. Berest).

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doi:10.1016/j.ejmech.2011.10.022

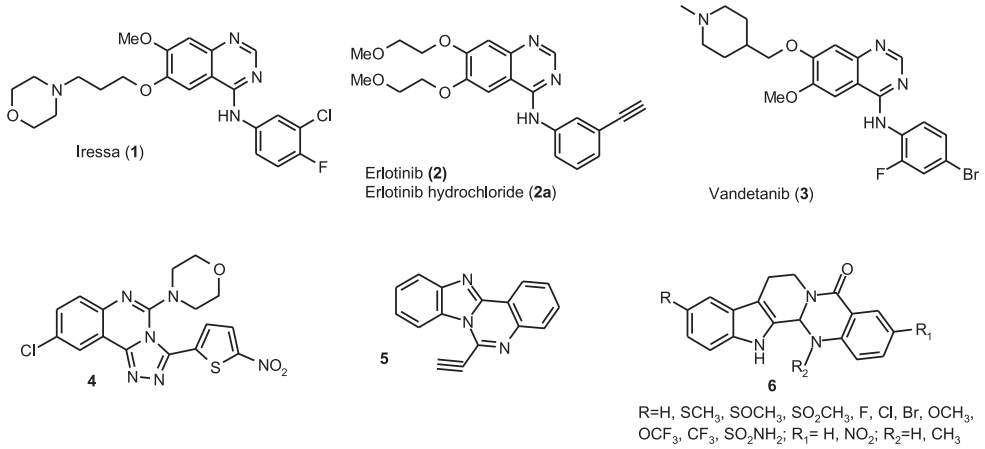


Fig. 1. Structures of quinazoline-based compounds.

antibacterial activities. The obtained results could serve as a powerful tool for the further rational anticancer drug design.

2. Results and discussion

Synthesis of the 3-R-6-thio-6,7-dihydro-2H-[1,2,4]triazino[2,3-*c*]quinazoline-2-ones potassium salts (**2.1**–**2.4**) was carried out by two approaches. First, by treatment of 6-R-3-(2-aminophenyl)-1,2,4-triazin-5-ones (**1.1**–**1.4**) with carbon disulfide in ethanol in presence of potassium hydroxide (Method A). The second one, by interaction of compounds **2** with potassium ethylxanthogenate in the propanol-2 (Method B, Scheme 1) [31,32]. Subsequent alkylation of the potassium salts **2.1**–**2.4** with chloroacetic acid and their derivatives in presence of basic reagents in aqueous-propanol-2 solution lead to the corresponding acids **3.1**–**3.4** (Scheme 1) [33].

The direct aminolysis of carboxylic acids and their esters is well-known method of amides synthesis, but, unfortunately, [(3-R-2-oxo-6,7-dihydro-2H-[1,2,4]triazino[2,3-*c*]quinazolin-6-yl)thio]acetic acids **3.1**–**3.4** and their esters appeared to be inert to ammonium and primary amines. It was necessary to activate carboxylic group of acids **3.1**–**3.4** for further successful amidation. The thionyl chloride or *N*,*N*′-carbonyldiimidazole (CDI) were selected as activating agents. The experiments showed that synthesis with appropriate acid chlorides (Method A) or acid imidazolides (Method B) underwent quite easily with good yields. It is important to mention that reactive capacity of the *N*-nucleophilic reagents was sufficient for reaction and only two conditions were necessary for the whole transformation to appropriate amides **4.1**–**4.10**: anhydrous dioxane and refluxing during 2–3 h. Besides, we have developed an alternative way of amides synthesis **4.1**–**4.10**, namely, alkylation of potassium salts **2.1**–**2.4** by *N*-cycloalkyl(cycloalkylaryl)-2-chloroacetamides (Method C) in aqueous-propanol-2 mixture, propanol-2 or dioxane (Scheme 2). The results of the experiment showed that the last mentioned synthetic method was a preparative one and had advantages such as short duration of synthesis (60–90 min), high yielding and purity of the obtained substances.

Elemental analysis, IR, ^{1}H and ^{13}C NMR, LS-MS and EI-MS data evaluated the structure and purity of synthesized substances. In the LS-MS spectra compounds **4.1**–**4.10** were characterized by positive ions [M + 1] and [M + 3], which simultaneously proved the structure and characterized isotopic type of Sulfur.

The two stretching bands of associated NH group were detected in the range of 3516–3058 cm^{-1} in the IR spectra. Besides, compounds **4.1**–**4.10** showed stretching vibrations of ν_{CO}-group (band "Amide I") at 1678–1633 cm^{-1} and mixed stretching–deformation vibration of the band N–H and C–N ("Amide II") at 1597–1510 cm^{-1}. Substances **4.1**–**4.10** had low intensity stretching vibrations of C=C bond of aromatic ring at 1589–1468 cm^{-1}, out-of-plane deformation stretching at 850–666 cm^{-1} and strong peak at 2960–2850 cm^{-1} of the symmetric and antisymmetric stretchings of CH_3– and CH_2– moieties.

In ^{1}H NMR spectra of amides **4.1**–**4.10** S-triazinoqunazoline carcass was characterized by sub-spectra that had appropriate signals and chemical shifts [29,31,32]. Two one-proton triplets of H-10 and H-9 resonated at 7.69–7.66 ppm and 8.01–7.95 ppm, two one-proton doublets of H-8 and H-11 – at 7.76–7.70 ppm and 8.49–8.45 ppm. The last one was considerably shifted in the low

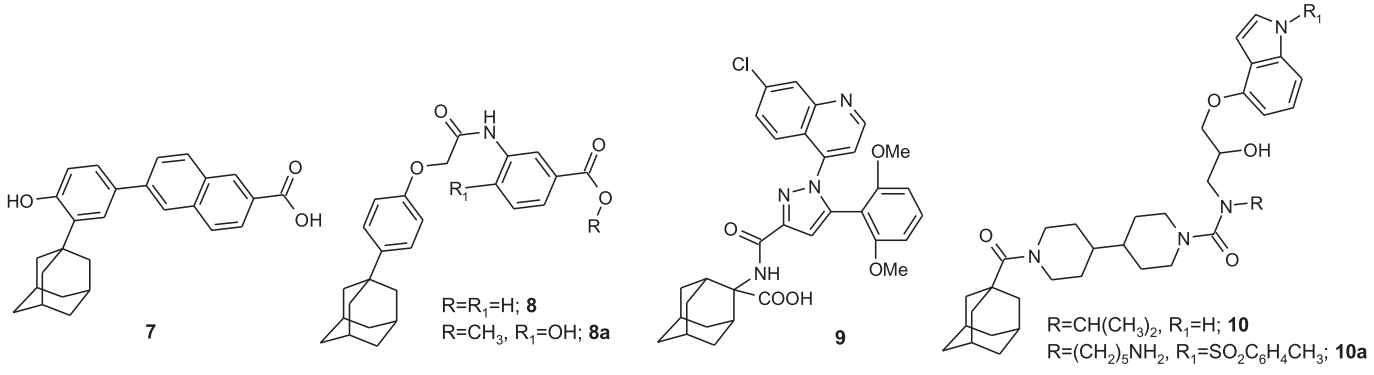
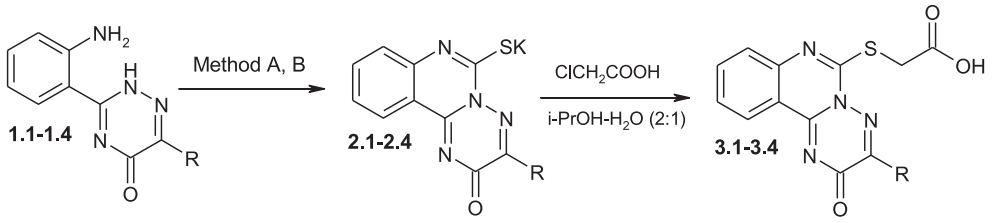


Fig. 2. Structures of adamantyl-based compounds.



Method A: CS_2, KOH, EtOH; Метод B: $KSC(S)OC_2H_5$, i-PrOH;
1.1, 2.1, 3.1 R=Me; **1.2, 2.2, 3.2** R=C_6H_5; **1.3, 2.3, 3.3** R= 4-MeC_6H_4, **1.4, 2.4, 3,4** R=4-$MeOC_6H_4$

Scheme 1. Synthesis of the 3-R-6-thio-6,7-dihydro-2H-[1,2,4]triazino[2,3-c]guinazoline-2-ones Potassium salts (**2.1**–**2.4**) and [(3-R-2-oxo-2*H*- [1,2,4]triazino[2,3-c]quinazoline-6-yl)thio]acetic acids (**3.1**–**3.4**).

field due to the electron-acceptive influence of Nitrogen. Characteristic signals of compounds **4.1**–**4.10** were two-proton singlet of SCH_2– group at 4.25–3.94 ppm and one-proton singlet of C(O)NH-group at 10.40–7.74 ppm. It is important to notice that singlet of amide's **4.4**–**4.7** C(O)NH-group, which contained 4-(1-adamantyl) phenyl substituent, resonated in strong field and for amides **4.1**–**4.3**, **4.8**–**4.10** with donor substituents it was observed in "aromatic region" of spectra. The protons of cycloalkyl substituents of compounds **4.1**–**4.10** were observed in the strong field. The adamantyl fragment exhibited six two-proton singlets at 1.73–1.62 ppm (H-4′, H-6′, H-10′) and at 1.98–1.84 ppm (H-2′, H-8′, H-9′), that was characteristic for the near bridge Carbon type protons [34,35]. The three proton singlet at 2.05–2.01 ppm (H-3′, H-5′, H-7′) characterized protons near nodal atom of Carbon. More complicated situation was observed in ^{1}H NMR spectra of compounds **4.8**–**4.10**, whose axial and equatorial protons have formed multiples at 1.51–0.92 ppm, 2.16–2.09 ppm, 2.67–2.60 ppm and 3.55–3.39 ppm.

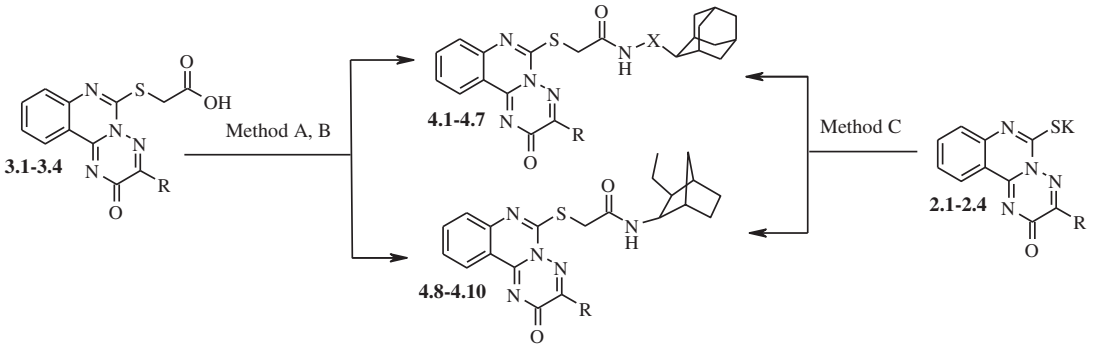
In ^{13}C NMR spectra signals of Carbon C-6, C-2 and C(O)NH (**4.1**, **4.3**, **4.8**) were the most shifted ones and appeared at 155.20–155.16 ppm, 161.02–160.13 ppm and 166.39–166.17 ppm appropriately. The additional confirmation of structure and S-regioselectity of alkylation was the position of the Carbon of SCH_2–group that resonated at 36.79–35.78 ppm. The signals of nodal atoms of adamantan's Carbon of amides **4.1**, **4.3** appeared at 36.49 ppm (C-4′, C-6′, C-10′) and at 41.43 ppm (C-2′, C-8′, C-9′), while near bridge type Carbon was located at 29.30–29.23 ppm [34]. sp^3-hybridizated Carbon atoms of the 3-ethylbicyclo[2.2.1] hepthyl substituent (**4.8**) were observed in the strong field according to the suggested structure.

EI-MS spectra of substances **4.5** and **4.8** were characterized by absence of the molecular ion causes by its low stability. Main pathways of $[M+]^{\bullet}$ fragmentation were caused by the cleavage of the amide bond (*m*/*z* 347 and *m*/*z* 285) and C(2)–C(3) and N(3)–N(4) bonds of triazine system (*m*/*z* 244), that had the strongest intensity in spectrum. Fragmentary ion with *m*/*z* 244 further eliminated fragments of CO, SCH_2 and CNO. Additionally for the substance **3.5** fragmentation of the adamantane substituent was revealed.

3. Pharmacology

3.1. Bioluminescence inhibition test

The results of the bioluminescence inhibition of luminescent bacteria *Photobacterium leiognathi* strain Sh1 showed that synthesized acids **3.1**–**3.4** and amides **4.1**–**4.10** possessed inhibition properties in the acute and chronic action tests in the majority of experiments (Table 1) [36]. Compound **1.1** showed cytotoxicity in concentration of 0.1 and 0.25 mg/mL in acute action test. Replacement of methyl substituent of substance **3.1** by aryl for substances **3.2**–**3.4** lead to the reduction of their cytotoxicity. Compound **3.2** revealed the 50% of inhibition at 0.25 mg/mL. Similar results were observed for substances **3.1**–**3.4** in chronic action test. Inhibition of bacteria's bioluminescence by compounds **3.1**, **3.2** and **3.3** was demonstrated only in concentration of 0.25 mg/mL in the mentioned test. While compound **3.4** inhibited bacteria bioluminescence in concentration of 0.25 mg/mL at 45% versus control. Amide **4.1** demonstrated inhibition properties with the increasing of concentration in the both acute and chronic action tests. Replacement of the 3-methyl group of **4.1** by phenyl for **4.2** leads to a considerable reduction of inhibition properties as well as introduction of the methoxyphenyl fragment in the structure of compound **4.3** in chronic action test. Moderate inhibition activity in



Method A: $SOCl_2$, dioxane, NH_2R_1; Method B: CDI, dioxane or DMF, NH_2R_1; Method C: propanol-2 or dioxane, $ClCH_2C(O)NHR_1$;
R=Me, Ph, 4-MePh, 4-MeOPh; X=H, C_6H_4; R_1=1-adamantyl; 4-(1-adamantyl)phenyl; (3-ethylbicyclo[2.2.1]hept-2-yl

Scheme 2. Synthesis of the N-cycloalkyl-(cycloalkylaryl)-2-[(3-R-2-oxo-2*H*-[1,2,4]-triazino[2,3-c]quinazolin-6-yl)thio]acetamides (**4.1**–**4.10**).

Table 1
Bioluminescence intensity, %.

Compd.	Control	Acute action test, mg/mL			Chronic action test, mg/mL		
		0.025	0.1	0.25	0.025	0.1	0.25
DMSO	100.0	100.0	100.0	100.0	100.0	100.0	100.0
3.1	100.0	66.7	0	0	36.4	61.5	0
3.2	100.0	100.0	75.0	50.0	34.0	40.9	3.6
3.3	100.0	91.7	20.0	0	93.3	255.3	0
3.4	100.0	105.0	80.0	0	88.9	191.5	45.0
4.1	100.0	36.00	0	0	25.00	1.88	0
4.2	100.0	86.96	80.43	56.52	97.14	85.71	78.57
4.3	100.0	85.6	87.5	83.3	127.3	107.7	116.7
4.4	100.0	90.00	65.71	32.86	86.96	94.57	50.00
4.5	100.0	102.94	80.88	54.41	118.75	111.25	66.25
4.6	100.0	84.62	96.15	50.00	170.00	140.00	16.00
4.7	100.0	76.60	59.57	59.57	48.72	34.62	76.92
4.8	100.0	91.67	104.17	62.50	90.28	93.06	86.11
4.9	100.0	110.00	96.00	120.00	100.00	120.00	44.00
4.10	100.0	38.5	84.0	90.9	85.3	144.0	80.0
Tetracycline	100.0	80.7	9.1	0	0	0	0

Table 2
Antimicrobial activity of synthesized compounds.

Compd.[a]	Conc, mg/ml	The inhibitory zones of the investigated compounds, mm				
		E. coli	*S. aureus*	*M. luteum*	*C. tenuis*	*A. niger*
4.3	0.5	0	9	0	0	18[b]
	0.1	0	0	0	0	0
4.4	0.5	0	0	0	0	21[b]
	0.1	0	0	0	0	0
4.7	0.5	0	7	0	0	0
	0.1	0	0	0	0	0
4.10	0.5	0	0	0	0	18[b]
	0.1	0	0	0	0	0
Vancomicin	0.01	16	18	58	0	0
Nystatin	0.01	0	11	15	24	25
Oxacillin	0.01	0	21	0	0	0

[a] Compounds **3.1**, **3.2**, **3.3**, **3.4**, **4.1**, **4.2**, **4.5**, **4.6**, **4.8** and **4.9** at concentration of 1.0 and 5.0 mg/ml did not inhibit investigated bacteria.
[b] Zone of late spore formation of *Aspergillus niger* (without inhibition of fungus mycelium).

the acute and chronic action tests was characteristic for compounds **4.4**–**4.7**, where adamantyl fragment was replaced by 4-(1-adamantyl)phenyl substituent. It is interesting to mention that compounds **4.4**–**4.7** showed effect of hormesis in chronic action test. Amides **4.8**–**4.10** with 3-ethylcyclo[2.2.1]heptyl substituent moderately inhibited bioluminescence of bacteria in concentration of 0.25 mg/mL in chronic action test.

The SAR study elucidated that: (1) the most cytotoxic compounds against luminescent bacteria *P. leiognathi* strain Sh1 in acute and chronic test are acids **3.1**–**3.4**; (2) cytotoxicity of the compounds (**3.1**–**3.4**) increases in such order of derivatives PhMeO > Ph > PhMe > Me; (3) functionalization of acids **3.1**–**3.4** to amides **4.1**–**4.10** leads to the decrease of cytotoxicity and bioluminescence inhibition; (4) it's important that, the similar relationship of the cytotoxicity from the type of substituent at 3 position is observed for amides **4.1**–**4.10**.

3.2. Antimicrobial and antifungal activities

The results of antimicrobial screening showed that substances **3.1**–**3.4** and **4.1**–**4.10** had moderate antimicrobial activity (Table 2). The highest bactericidal data were estimated for substances **4.3** and **4.7** against *S. aureus*. While other cultures of bacteria revealed to be insensitive to synthesized compounds in investigated concentrations. It is interesting to mention, that compounds **4.3**, **4.4** and **4.10** showed considerable fungicidal activity against *A. niger*, and in concentration of 0.5 mg/mL caused the late spore formation against mentioned fungus at 18–23 mm.

The SAR study revealed that: (1) substances **3.1**–**3.4** and **4.1**–**4.10** show moderate antimicrobial and antifungal activity, with most active ones – amides **4.3**, **4.4**, **4.7** and **4.10**; (2) amides **4.3** and **4**.7 with adamantyl and aryl substituents at 3 position possess antimicrobial activity against *Staphylococcus aureus*; (3) antifungal activity of amides **4.3**, **4.4** and **4.10** against *A. niger* is also determined by cycloalkyl-(cycloalkylaryl-) substituent in thioacetic fragment and aryl moiety at 3 position.

3.3. Anticancer assay for preliminary in vitro testing

The moderate cytotoxicity of the synthesized substances against luminescent bacteria *P. leiognathi* strain Sh1 and antimicrobial activity against *E. coli*, *S. aureus*, *M. luteum*, *C. tenuis* and *A. niger* appeared to be the reason for the *in vitro* cell line anticancer activity screening in National Cancer Institute (NCI) under Developmental Therapeutic Program (www.dtp.nci.nih.gov). Compounds **3.1**–**3.4**, **4.3**, **4.10** were submitted and evaluated according to the US NCI protocol, which was described elsewhere [38–42]. The compounds were first evaluated at one dose primary anticancer assay towards or approximately 60 cell lines (concentration 10^{-5} M). The human tumor cell lines were derived from nine different cancer types: leukemia, melanoma, lung, colon, CNS, ovarian, renal, prostate and breast cancers. In the screening protocol, each cell line was inoculated and preincubated for 24–48 h on a microtiter plate. Test agents were then added at a single concentration and the culture was incubated for further 48 h. The end point determinations were made with a protein binding dye, sulforhodamine B (SRB). Results for each test agent were reported as the percent growth of the treated cells when compared to the untreated control cells. The preliminary screening results are shown in Table 3.

Compounds **3.1**–**3.4** showed cytotoxicity against cancer cell lines of leukemia, CNS cancer, renal cancer and prostate cancer. More evident cytotoxic activity against tumor cell lines of CCRF-CEM, HL-60(TB), K-562, MOLT-4 and SR was characteristic for compound **3.4** with *p*-methoxyphenyl substituent. While compounds **3.1**–**3.3** demonstrated higher antitumor activity against CNS cancer cell lines SNB-75, U251, renal cancer UO-31 and prostate cancer PC-3. It is important that compound **4.3**, opposite to substances **3.1**–**3.4**, inhibited practically all tumor cell lines in the range of 54–90% versus control (Table 3). Compound **4.3** inhibited growth of leukemia SR cell line, non-small cell lung cancer EKVX, SNB-75 of the CNS cancer, A498, CAKI-1, UO-31 of the renal cancer, PC-3 of the prostate cancer and MDA-MB-231/ATCC, T-47D of the breast cancer. While compound **4.10** appeared to be the more active cytotoxic agent comparing to compound **4.3**. So, substance **4.10** was effective against tumor cell lines of leukemia (K-562, MOLT-4, RPMI-8226, SR), non-small cell lung cancer (A549/ATCC, EKVX, HOP-92, NCI-H23, NCI-H322M, NCI-H460), colon cancer (COLO 205, HCT-116, HCT-15, HT29, KM12, SW-620), CNS cancer (SF-268, SF-295, SNB-19, SNB-75), melanoma (LOX IMVI, M14, MDA-MB-435, SK-MEL-2, SK-MEL-28, SK-MEL-5, UACC-257), ovarian cancer (IGROV1, OVCAR-8, NCI/ADR-RES), renal cancer (786-0, CAKI-1, RXF 393, RXF-393, SN12C, TK-10), prostate cancer (PC-3), and breast cancer (MCF7, MDA-MB-231/ATCC, BT-549). It is interesting to mention that substance **4.10** exhibited noticeable antiproliferative effect against non-small cell lung cancer (HOP-62, NCI-H522), ovarian cancer (OVCAR-3), (OVCAR-4, SK-OV-3), renal cancer (ACHN, UO-31), and breast cancer (HS 578T, T-47D, MDA-MB-468).

Substance **4.10** was subsequenly investigated for dose-dependent action in 5 concentrations (10^{-4}–10^{-8} M). In Table 4

Table 3
Cytotoxic activity of the compounds in concentration 10^{-5} M against 60 cell cancer lines.

Test compounds	Mean growth, %	Range of growth, %	Most sensitive cell line growth, %*
3.1	109.80	83.45–142.51	93.33 (CCRF-CEM/L), 83.45 (SNB-75/CNS), 96.05 (CAKI-1/RC), 87.14 (UO-31/RC), 84.45 (PC-3/PC)
3.2	105.33	82.53–127.73	92.52 (CCRF-CEM/L), 91.97 (K-562/L), 91.87 (SR/L), 82.53 (A549/ATCC/nscLC), 86.57 (SNB-75/CNSC), 91.36 (U251/CNSC), 96.84 (M14/M), 93.24 (UACC-257/M), 96.97 (SK-OV-3/OC), 89.01 (UO-31/RC), 90.76 (PC-3/PC)
3.3	103.01	74.22–123.43	95.57 (CCRF-CEM/L), 94.48 (K-562/L), 84.86 (MOLT-4/L), 68.04 (SR/L), 96.51 (A549/ATCC/nscLC), 94.95 (EKVX/nscLC), 84.35 (SF-295/CNSC), 74.22 (SNB-75/CNSC), 96.04 (SK-MEL-5/M), 95.09 (UACC-257/M), 89.89 (SK-OV-3/OV), 82.80 (CAKI-1/RC), 85.32 (UO-31/RC), 86.48 (PC-3/PC)
3.4	105.05	63.47–145.27	90.66 (CCRF-CEM/L), 63.47 (HL-60(TB)/L), 80.90 (K-562/L), 84.78 (MOLT-4/L), 65.78 (SR/L), 95.86 (A549/ATCC/nscLC), 94.90 (NCI-H522/nscLC), 94.68 (HCC-2998/CC), 93.93 (SNB-75/CNSC), 86.92 (LOX IMVI/M), 95.40 (SK-MEL-5/M), 91.41 (CAKI-1/RC), 91.64 (UO-31/RC), 91.74 (PC-3/PC), 89.86 (MDA-MB-231/ATCC/BC)
4.3	85.70	49.65–121.05	85.05 (RPMI-8226/L), 69.62 (SR/L), 62.96 (EKVX/nscLC), 74.21 (HOP-92/nscLC), 79.88 (NCI-H322M/nscLC), 82.14 (HCT-116/ColC), 85.84 (SF-268/CNSC), 81.45 (SF-295/CNSC), 85.79 (SNB-19/CNSC), 63.03 (SNB-75/CNSC), 82.93 (LOX IMVI/M), 74.60 (OVCAR-3/OV), 83.19 (OVCAR-4/OV), 81.61 (SK-OV-3/OV), 49.65 (A498/RC), 56.92 (CAKI-1/RC), 83.27 (SN12C/RC), 51.06 (UO-31/RC), 54.14 (PC-3/PC), 76.88 (MCF7/BC), 64.80 (MDA-MB-231/ATCC/BC), 82.53 (BT-549/BC), 70.68 (T-47D/BC)
4.10	31.39	−98.94–110.28	39.23 (K-562/L), 11.38 (MOLT-4/L), 47.38 (RPMI-8226/L), 7.67 (SR/L), 50.38 (A549/ATCC/nscLC), 25.61 (EKVX/nscLC), −19.46 (HOP-62/nscLC), 56.97 (HOP-92/nscLC), 62.84 (NCI-H23/nscLC), 65.58 (NCI-H322M/nscLC), 56.05 (NCI-H460/nscLC), −49.92 (NCI-H522/nscLC), 28.64 (COLO 205/ColC), 64.33 (HCT-116/ColC), 67.94 (HCT-15/ColC), 42.76 (HT29/ColC), 68.27 (KM12/ColC), 45.93 (SW-620/ColC), 66.33 (SF-268/CNSC), 69.86 (SF-295/CNSC), 39.82 (SNB-19/CNSC), 4.94 (SNB-75/CNSC), −98.94 (U251/CNSC), 70.53 (LOX IMVI/M), −29.17 (MALME-3M/M), 44.16 (M14/M), 58.58 (MDA-MB-435/M), 56.52 (SK-MEL-2/M), 68.65 (SK-MEL-28/M), 59.80 (SK-MEL-5/M), 55.64 (UACC-257/M), 16.96 (IGROV1/OV), −69.71 (OVCAR-3/OV), −58.54 (OVCAR-4/OV), 53.06 (OVCAR-8/OV), 73.65 (NCI/ADR-RES/OV), −5.12 (SK-OV-3/OV), 77.65 (786-0/RC), −17.15 (ACHN/RC), 36.10 (CAKI-1/RC), 9.46 (RXF 393/RC), 9.46 (RXF-393/RC), 57.78 (SN12C/RC), 13.78 (TK-10/RC), −12.73 (UO-31/RC), 55.08 (PC-3/PC), −31.96 (DU-145/PC), 48.17 (MCF7/BC), 22.47 (MDA-MB-231/ATCC/BC), −9.67 (HS 578T/BC), 90.86 (BT-549/BC), −11.26 (T-47D/BC), −59.54 (MDA-MB-468/BC)

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

results of log GI_{50}, log TGI, log LC_{50} are shown. (GI_{50} – molar concentration of the compound that inhibits 50% net cell growth; TGI – molar concentration of the compound leading to total inhibition of cell growth; LC_{50} – molar concentration of the compound leading to 50% net cell death). It is important to mention that substance **4.10** had strong anticancer effect only in the concentration of 10^{-4} and 10^{-5} M (Table 4).

Table 4
The influence of compounds **4.10** on the growth of individual tumor cell lines (log GI50 ≤ −5.40).

Disease	Cell line	log GI50	log TGI	Log LC50
Leukemia	CCRF-CEM	−5.70	>−4.30	>−4.30
	MOLT-4	−5.79	>−4.30	>−4.30
	RPMI-8226	−5.53	−4.42	>−4.30
	SR	−5.87	−5.13	>−4.30
Non-small cell lung cancer	EKVX	−5.71	>−4.30	>−4.30
	HOP-62	−5.61	−4.94	−4.44
	HOP-92	−6.01	−5.40	−4.59
	NCI-H522	−5.87	−5.10	−4.41
Colon cancer	COLO 205	−5.46	>−4.30	>−4.30
	HT29	−5.49	>−4.30	>−4.30
CNS cancer	SF-295	−5.46	−4.66	>−4.30
	SNB-19	−5.46	−4.65	>−4.30
	SNB-75	−5.51	−4.94	−4.51
	U251	−6.00	−5.64	−5.24
Melanoma	LOX IMVI	−5.45	−4.74	>−4.30
	MALME-3M	−5.41	−4.61	>−4.30
	UACC-62	−5.45	−4.58	>−4.30
Ovarian cancer	IGROV1	−5.57	−4.44	>−4.30
	OVCAR-3	−5.83	−5.25	−4.55
	OVCAR-4	−5.78	−5.20	−4.36
Renal cancer	786−0	−5.52	−4.80	>−4.30
	ACHN	−5.72	>−4.30	>−4.30
	CAKI-1	−5.43	−4.41	>−4.30
	RXF 393	−5.83	−5.38	−4.76
Prostate cancer	PC-3	−5.55	−4.61	>−4.30
	DU-145	−5.50	−4.43	>−4.30
Breast cancer	MDA-MB-231/ATCC	−5.51	−4.68	>−4.30
	HS 578T	−5.41	−4.70	>−4.30
	T-47D	−5.43	>−4.30	>−4.30
	MDA-MB-468	−5.87	−5.40	−4.52

The SAR study revealed that: (1) newly synthesized 6-substituted 3-R-2-oxo-2*H*-[1,2,4]triazino[2,3-*c*]quinazolines are previous unknown anticancer agents; (2) anticancer activity among compounds **3.1**–**3.4** is determined by substituent at 3 position and increases in such order Me > Ph > 4-MePh > 4-MeOPh; (3) functionalization of the carboxylic group of thioacetic moiety at 6 position by introduction of 1-aminoadamantyl (**4.3**) or (3-ethylbicyclo[2.2.1]hept-2-yl)amine (**4.10**) leads to the increasing of anticancer activity; (4) further modification of carboxylic group by aryl-(aralkyl-, heteryl-)amides is a perspective way for modification of the compounds for further elucidation of their mechanism of action.

4. Conclusion

In the present paper, synthesis of novel [(3-R-2-oxo-2*H*-[1,2,4]triazino[2,3-c]quinazoline-6-yl)thio]acetic acids and *N*-cycloalkyl-(cycloalkylaryl)-2-[(3-R-2-oxo-2*H*-[1,2,4]triazino[2,3-*c*]quinazoline-6-yl)thio]acetamides were described. The antibacterial, antifungal and bioluminiscence inhibition activities of the synthesized substances against *E. coli*, *S. aureus*, *M. luteum*, *C. tenuis*, *A. niger* and *P. leiognathi* and were investigated and determinated as moderate. Six compounds (**3.1**-**3.4**, **4.3**, **4.10**) were tested for anticancer activity against leukemia, melanoma, lung, colon, CNS, ovarian, renal, prostate and breast cancers cell lines. The most potent anticancer compound appeared to be **4.10** selectively influencing the non-small cell lung and CNS cancer cell lines, especially the HOP-92 (log GI_{50} = −6.01) and U251 (log GI_{50} = −6.00). The obtained results have proved the necessity of the further investigations of the antitumor potential of [1,2,4]triazino[2,3-*c*]quinazoline derivatives.

5. Experimental protocols

5.1. Chemistry

Melting points were determined in open capillary tubes and were uncorrected. Elemental analyses (C, H, N) performed at the ELEMENTAR vario EL Cube analyzer (USA) and were within ±0.3% from the theoretical values. IR spectra (4000–600 cm^{-1}) were recorded on a Bruker ALPHA FT–IR spectrometer (Bruker Bioscience, Germany) using a module for measuring attenuated total reflection (ATR). ^1H NMR spectra (400 MHz) and ^{13}C NMR spectra (100 MHz): were recorded on a Varian-Mercury 400 (Varian Inc., Palo Alto, CA, USA) spectrometer with TMS as internal standard in DMSO-d_6 solution. LC-MS were recorded using chromatography/mass spectrometric system which consists of high performance liquid chromatograph "Agilent 1100 Series" (Agilent, Palo Alto, CA, USA) equipped with diode-matrix and mass-selective detector "Agilent LC/MSD SL" (atmospheric pressure chemical ionization – APCI). Electron impact mass spectra (EI-MS) were recorded on a Varian 1200 L instrument at 70 eV (Varian Inc., Palo Alto, CA, USA).

Substances **2.1**–**2.4** and **3.1**–**3.4** were synthesized according to the reported procedures [31,32]. Other starting materials and solvents were obtained from commercially available sources and used without additional purification.

5.1.1. General procedure for synthesis of N-cycloalkyl-(cycloalkaryl)-2-[(3-R-2-oxo-2H-[1,2,4]triazino[2,3-c]quinazolin-6-yl)thio] acetamides (4.1–4.10)

Method A. The thionyl chloride (1.2 g, 0.011 mol) was added to the solution of proper acid (**3.1**–**3.4**) (0.01 mol) in 10 mL of anhydrous dioxane with subsequent adding of the 2–3 drops of DMF. Mixture was heated at the water bath at 60–80°C till full elimination of hydrochloric acid. Then proper amine (0.01 mol) was added to the resulting mixture with stirring and refluxing for 2–3 h. The mixture was poured in the water, neutralized to pH 6–7 by acetic acid. The precipitate was filtered, dried and recrystallized from dioxane–water (1:1).

Method B. N,N′-carbonyldiimidazole (1.95 g, 0.011 mol) was added to a solution of proper acid (**3.1**–**3.4**) (0.01 mol) in 10 mL of anhydrous dioxane or DMF and heated at the water bath at 60-80°C for 1 h with calcium chloride tube. The proper amine (0.01 mol) was added with stirring to the resulting mixture and refluxed for 2–3 h. The mixture was poured in the water, neutralized to pH 6–7 by acetic acid. The precipitate was filtered, dried and recrystallized from the dioxane–water (1:1).

Method C. N-cycloalkyl-(cycloalkaryl)-2-chloroacetamides (0.011 mol) were added to a suspension of Potassium salt of 3-R-6-thio-6,7-dihydro-2*H*-[1,2,4]triazino[2,3-*c*]quinazolin-2-ones (**2.1**–**2.6**) (0.01 mol) in 20 ml of propanol-2, water-propanol-2 (1:2), or dioxane and refluxed for 60–90 min. The mixture was cooled and poured in the water. The precipitate was filtered, dried and recrystallized from the dioxane–water (1:1).

5.1.2. N-1-Adamantyl-2-[(3-methyl-2-oxo-2H-[1,2,4]triazino[2,3-c] quinazolin-6-yl)thio]acetamides (**4.1**)

Yield: Method A, 48.4%; Method B, 64.3%; Method C, 89.2%; M.p. 160–162 °C; IR (cm^{-1}): 3516, 3358, 3259, 3077, 2907, 2849, 1676, 1663, 1585, 1558, 1505, 1470, 1425, 1388, 1360, 1344, 1306, 1286, 1265, 1210, 1165, 1139, 1103, 1045, 998, 956, 885, 862, 813, 773, 687, 633, 608; ^1H NMR (400 MHz) δ: 1.62 (s, 6H, H-4′, 6′, 10′ Ad), 1.96 (s, 6H, H-2′, 8′, 9′ Ad), 2.01 (s, 3H, H-3′, 5′, 7′ Ad), 2.39 (s, 3H, 3-CH_3), 3.94 (s, 2H, -S-CH_2-), 7.67 (t, 1H, J = 7.7 Hz, H-10), 7.74 (d, 1H, J = 7.9 Hz, H-8), 7.84 (s, 1H, –NHC(O)), 7.98 (t, 1H, J = 7.7 Hz, H-9), 8.46 (d, 1H, J = 7.9 Hz, H-11); ^{13}C NMR (100 MHz) δ: 18.18 (CH_3), 29.23 (3′, 5′, 7′ Ad), 36.49 (4′, 6′, 10′ Ad), 36.68 (–SCH_2), 41.43 (2′, 8′, 9′ Ad), 51.72 (1′ Ad), 118.55 (11a), 126.05 (8), 126.65 (10), 127.88 (11), 136.03 (9), 144.24 (11b), 151.94 (3), 154.94 (7a), 155.16 (6), 161.02 (2), 166.17 (CONH); LC-MS, m/z = 436 [M + 1], 438 [M + 3]; Anal. calcd. for $C_{23}H_{25}N_5O_2S$: C, 63.43; H, 5.79; N, 16.08; S, 7.36; Found: C, 63.42; H, 5.79; N, 16.08; S, 7.34.

5.1.3. N-1-Adamantyl-2-[(3-phenyl-2-oxo-2H-[1,2,4]triazino[2,3-c] quinazolin-6-yl)thio]acetamides (**4.2**)

Yield: Method A, 56.3%; Method B, 77.4%; Method C, 91.3%; M.p. 214–216 °C; IR (cm^{-1}): 3287, 3067, 2904, 2848, 1674, 1644, 1590, 1555, 1509, 1487, 1469, 1455, 1359, 1337, 1310, 1286, 1269, 1242, 1183, 1161, 1136, 1102, 1020, 988, 939, 878, 844, 811, 782, 773, 753, 688, 668, 653, 615; ^1H NMR (400 MHz) δ: 1.63 (s, 6H, H-4′, 6′, 10′ Ad), 1.97 (s, 6H, H-2′, 8′, 9′ Ad), 2.01 (s, 3H, H-3′, 5′, 7′ Ad), 3.99 (s, 2H, –S–CH_2–), 7.64–7.57 (m, 3H, H-3′, 4′, 5′ 3-Ph), 7.69 (t, 1H, J = 7.7 Hz, H-10), 7.76 (d, 1H, J = 7.8 Hz, H-8), 7.88 (s, 1H, –NHC(O)), 8.01 (t, 1H, J = 7.7 Hz, H-9), 8.28 (d, 2H, J = 8.1 Hz, H-2′, 6′ 3-Ph), 8.49 (d, 1H, J = 7.9 Hz, H-11); LC-MS, m/z = 498 [M + 1], 500 [M + 3]; Anal. calcd. for $C_{28}H_{27}N_5O_2S$: C, 67.58; H, 5.47; N, 14.07; S, 6,44; Found: C, 67.58; H, 5.47; N, 14.09; S, 6.46.

5.1.4. N-1-Adamantyl-2-[(3-(4-methylphenyl)-2-oxo-2H-[1,2,4] triazino[2,3-c]quinazolin-6-yl)thio]acetamides(**4.3**)

Yield: Method A, 72.6%; Method C, 94.2%; M.p. 218–220 °C; IR (cm^{-1}): 3318, 3065, 3037, 2972, 2905, 2849, 1682, 1658, 1588, 1562, 1538, 1496, 1470, 1453, 1399, 1377, 1359, 1339, 1321, 1304, 1285, 1269, 1240, 1182, 1164, 1154, 1139, 1119, 1089, 1045, 989, 939, 876, 831, 810, 784, 770, 710, 701, 685, 653, 641, 625; ^1H NMR (400 MHz) δ: 1.63 (s, 6H, H-4′, 6′, 10′ Ad), 1.98 (s, 6H, H-2′, 8′, 9′ Ad), 2.01 (s, 3H, H-3′, 5′, 7′ Ad), 2.42 (s, 3H, 3-(4-CH_3Ph)), 3.99 (s, 2H, –S–CH_2–), 7.39 (d, 2H, H-3′, 5′ 3-(4-CH_3Ph)), 7.68 (t, 1H, J = 7.7 Hz, H-10), 7.82–7.74 (m, 2H, H-8, –NHC(O)), 7.98 (t, 1H, J = 7.7 Hz, H-9), 8.24 (d, 2H, H-2′, 6′ 3-(4-CH_3Ph)), 8.48 (d, 1H, J = 7.9 Hz, H-11); ^{13}C NMR (100 MHz): δ: 21.66 (CH_3), 29.30 (3′, 5′, 7′ Ad), 36.49 (4′, 6′, 10′ Ad), 36.79 (–SCH_2), 41.43 (2′, 8′, 9′ Ad), 51.74 (1′ Ad), 118.25 (11a), 126.09 (8), 126.73 (10), 127.93 (11), 129.32 (1′ Ph), 129.53 (2′, 6′ Ph), 129.78 (3′, 5′ Ph), 135.99 (9), 142.20 (4′ Ph), 144.20 (3), 149.29 (11b), 150.80 (7a), 155.20 (6), 160.13 (2), 166.17 (CONH); Anal. calcd. for $C_{29}H_{29}N_5O_2S$: C, 68.08; H, 5.71; N, 13.69; S, 6.27; Found: C, 69.00; H, 5.71; N, 13.70; S, 6.27.

5.1.5. N-[4-(1-Adamantyl)phenyl]-2-[3-methyl-2-oxo-2H-[1,2,4] triazino[2,3-c]quinazolin-6-yl thio]acetamides (**4.4**)

Yield: Method A, 72.0%; Method B, 81.2%; Method C, 97.3%; M.p. 225–227 °C; IR (cm^{-1}): 3251, 3184, 2897, 2845, 1655, 1583, 1558, 1503, 1466, 1449, 1433, 1405, 1393, 1361, 1332, 1315, 1286, 1259, 1220, 1206, 1190, 1163, 1131, 1101, 1046, 1016, 969, 955, 832, 807, 772, 715, 699, 685, 630, 607; ^1H NMR (400 MHz) δ: 1.73 (s, 6H, H-4′, 6′, 10′ Ad), 1.84 (s, 6H, H-2′, 8′, 9′ Ad), 2.05 (s, 3H, H-3′, 5′, 7′ Ad), 2.39 (s, 3H, 3-CH_3–), 4.21 (s, 2H, –S–CH_2–), 7.30 (d, 2H, J = 8.5 Hz, H-3′, 5′ –NHC_6H_4-Ad), 7.55 (d, 2H, J = 8.5 Hz, H-2′, 6′ –NHC_6H_4-Ad), 7.66 (t, 1H, J = 7.7 Hz, H-10), 7.70 (d, 1H, J = 7.9 Hz, H-8), 7.95 (t, 1H, J = 7.7 Hz, H-9), 8.45 (d, 1H, J = 7.9 Hz, H-11), 10.36 s, 1H, –NHC(O); LC-MS, m/z = 512 [M + 1], 514 [M + 3]; Anal. calcd. for $C_{29}H_{29}N_5O_2S$: C, 68.08; H, 5.71; N, 13.69; S, 6.27; Found: C, 68.10; H, 5.71; N, 13.70; S, 6.29.

5.1.6. N-[4-(1-Adamantyl)phenyl]-2-[3-phenyl-2-oxo-2H-[1,2,4] triazino[2,3-c]quinazolin-6-yl thio]acetamides (**4.5**)

Yield: Method A, 78.4%; Method C, 94.5%; M.p. 314–318 °C; IR (cm^{-1}): 3349, 3047, 2982, 2899, 2849, 1678, 1657, 1596, 1587, 1564, 1546, 1519, 1498, 1486, 1470, 1444, 1407, 1380, 1341, 1331, 1311, 1287, 1263, 1246, 1237, 1187, 1171, 1138, 1100, 1076, 1033, 1017, 1002, 988, 963, 941, 895, 877, 848, 836, 810, 779, 768, 751, 706, 687, 665, 654, 636; ^1H NMR (400 MHz) δ: 1.73 (s, 6H, H-4′, 6′, 10′ Ad), 1.84 (s, 6H,

H-2′, 8′, 9′ Ad), 2.05 (s, 3H, H-3′, 5′, 7′ Ad), 4.25 (s, 2H, −S−CH_2−), 7.31 (d, 2H, J = 8.5 Hz, H-3′, 5′ −NHC$_6H_4$-Ad), 7.56 (d, 2H, J = 8.5 Hz, H-2′, 6′ −NHC$_6H_4$-Ad), 7.77–7.59 (m, 5H, H-3′, 4′, 5′ 3-Ph, H-8, 10), 7.98 (t, 1H, J = 7.7 Hz, H-9), 8.30 (d, 2H, J = 7.3, H-2′, 6′ 3-Ph), 8.49 (d, 1H, J = 7.9 Hz, H-11), 10.40 (s, 1H, −NHC(O); EI-MS, m/z (I_{rel}, %) = 396 (6.3), 395 (5.7), 348 (16.9), 347 (73.5), 346 (49.1), 345 (16.1), 339 (7.2), 316 (5.4), 307 (8.7), 302 (15.9), 301 (64.8), 269 (16.0), 268 (5.2), 258 (5.1), 254 (13.8), 253 (57.9), 252 (10.8), 246 (7.0), 245 (16.1), 244 (99.9), 243 (26.4), 240 (5.4), 228 (5.8), 227 (49.5), 226 (8.0), 218 (25.8), 217 (29.8), 216 (58.8), 213 (8.9), 212 (7.1), 211 (7.0), 210 (16.6), 197 (9.5), 196 (55.8), 195 (5.6), 189 (5.4), 188 (9.0), 186 (7.2), 185 (20.9), 184 (11.2), 183 (7.0), 182 (8.6), 180 (6.1), 179 (8.3), 178 (43.2), 170 (42.5), 161 (5.0), 160 (5.6), 159 (17.8), 158 (9.6), 157 (9.0), 156 (20.0),155 (8.7), 154 (9.4), 153 (12.0), 152 (9.6), 148 (21.9), 145 (8.0), 144 (7.8), 143 (16.9), 142 (7.5), 141 (6.0), 135 (22.4), 134 (8.4), 133 (24.1), 132 (32.9), 131 (12.6), 130 (16.0), 129 (22.9), 128 (18.2), 127 (12.5), 119 (10.4), 118 (21.9), 117 (22.4), 116 (18.3), 115 (12.5), 106 (5.3), 105 (7.2), 104 (14.4), 103 (80.9), 101 (12.8), 95 (7.0), 94 (27.6), 93 (34.4), 92 (12.9), 90 (12.7), 89 (10.5), 86 (13.8), 79 (26.3), 77 (10.2), 75 (5.4), 67 (16.5), 66 (7.4), 65 (21.6), 64 (14.3), 63 (15.4), 56 (14.9), 55 (19.1), 53 (6.1), 47 (9.2), 43 (15.2), 41 (21.1)LC-MS, m/z = 574 [M + 1], 575 [M + 2], 576 [M + 3]; Anal. calcd. for $C_{34}H_{31}N_5O_2S$: C, 71.18; H, 5.45; N, 12.21; S, 5.59; Found: C, 71.19; H, 5.45; N, 12.23; S, 5.61.

5.1.7. *N-[4-(1-Adamantyl)phenyl]-2-[3-(4-methylphenyl)-2-oxo-2H-[1,2,4]triazino[2,3-c]quinazolin-6-yl thio]acetamides (**4.6**)*

Yield: Method B, 84.3%; Method C, 99.9%; M.p. 266–268 °C; IR (cm^{-1}): 3344, 2982, 2915, 2898, 2882, 2849, 1675, 1655, 1584, 1562, 1542, 1517, 1490, 1469, 1451, 1406, 1368, 1340, 1329, 1308, 1285, 1264, 1237, 1189, 1139, 1125, 1106, 1071, 1036, 1017, 989, 961, 942, 893, 875, 834, 807, 779, 768, 708, 686, 665, 643, 624; ^{1}H NMR (400 MHz) δ: 1.73 (s, 6H, H-4′, 6′, 10′ Ad), 1.84 (s, 6H, H-2′, 8′, 9′ Ad), 2.05 (s, 3H, H-3′, 5′, 7′ Ad), 2.42 (s, 3H, 3-(4-CH_3Ph)), 4.25 (s, 2H, −S−CH_2−), 7.31 (d, 2H, J = 8.5, H-3′, 5′ −NHC$_6H_4$-Ad), 7.41 (d, 2H, J = 7.9 Hz, H-3′, 5′ 3-(4−CH$_3$Ph)), 7.56 (d, 2H, J = 8.5 Hz, H-2′, 6′ −NHC$_6H_4$-Ad), 7.68 (t, 1H, J = 7.7 Hz, H-10), 7.74 (d, 1H, J = 7.8 Hz, H-8), 7.99 (t, 1H, J = 7.7 Hz, H-9), 8.25 (d, 2H, J = 7.9 Hz, H-2′, 6′ 3-(4-CH$_3$Ph)), 8.49 (d, 1H, J = 7.9 Hz, H-11), 10.39 (s, 1H, −NHC(O); LC-MS, m/z = 588 [M + 1], 590 [M + 3]; Anal. calcd. for $C_{35}H_{33}N_5O_2S$: C, 71.53; H, 5.66; N, 11.92; S, 5.46; Found: C, 71.53; H, 5.66; N, 11.92; S, 5.44.

5.1.8. *N-[4-(1-Adamantyl)phenyl]-2-[3-(4-methoxyphenyl)-2-oxo-2H-[1,2,4]triazino[2,3-c]quinazolin-6-yl thio]acetamides (**4.7**)*

Yield: Method B, 64.7%; Method C, 93.2%; M.p. 259–262 °C; IR (cm^{-1}): 3346, 2982, 2898, 2849, 1678, 1654, 1597, 1563, 1538, 1518, 1504, 1490, 1469, 1452, 1406, 1370, 1341, 1315, 1304, 1255, 1244, 1177, 1140, 1117, 1050, 1036, 1016, 989, 942, 879, 843, 808, 780, 769, 722, 701, 687, 665, 643, 621; ^{1}H NMR (400 MHz) δ: 1.73 (s, 6H, H-4′, 6′, 10′ Ad), 1.84 (s, 6H, H-2′, 8′, 9′ Ad), 2.05 (s, 3H, H-3′, 5′, 7′ Ad), 3.88 (s, 3H, 3-(4-CH_3OPh)), 4.25 (s, 2H, −S−CH_2−), 7.17 (d, 2H, J = 8.3, H-3′, 5′ 3-(4−CH$_3$OPh)), 7.31 (d, 2H, J = 8.5 Hz, H-3′, 5′−NHC$_6$H$_4$-Ad), 7.56 (d, 2H, J = 8.5 Hz, H-2′, 6′ −NHC$_6$H$_4$-Ad), 7.68 (t, 1H, J = 7.7 Hz, H-10), 7.74 (d, 1H, J = 7.8 Hz, H-8), 7.97 (t, 1H, J = 7.7 Hz, H-9), 8.39 (d, 2H, J = 8.3, H-2′, 6′ 3-(4-CH$_3$OPh)), 8.49 (d, 1H, J = 7.9 Hz, H-11), 10.40 (s, 1H, −NHC(O); LC-MS, m/z = 604 [M + 1], 607 [M + 3]; Anal. calcd. for $C_{35}H_{33}N_5O_3S$: C, 69.63; H, 5.51; N, 11.60; S, 5,31; Found: C, 69.64; H, 5.51; N, 11.61; S, 5.32.

5.1.9. *N-(3-ethylbicyclo[2.2.1]hept-2-yl)-2-[3-methyl-2-oxo-2H-[1,2,4]triazino[2,3-c]quinazolin-6-yl thio]acetamides (**4.8**)*

Yield: Method A, 36.9%; Method B, 81.3%; Method C, 84.6%; M.p. 217–219 °C; IR (cm^{-1}): 3287, 2944, 2866, 1667, 1636, 1584, 1558, 1538, 1509, 1469, 1454, 1418, 1386, 1360, 1337, 1287, 1261, 1208, 1173, 1133, 1102, 1045, 955, 880, 856, 768, 698, 685, 630, 607; ^{1}H NMR (400 MHz) δ: 1.48–0.94 (m, 11H, 3-CH_3CH$_2$-, 3-CH$_3$CH_2-, 5, 5′, 6, 6′, 7 bicyclo[2.2.1]heptyl), 2.16–2.09 (m, 2H, H-3′, 4′ bicyclo[2.2.1]heptyl), 2.38 (s, 3H, 3-CH_3), 2.66 (m, 1H, H-1′ bicyclo[2.2.1]heptyl), 3.50–3.39 (m, 1H, H-2′ bicyclo[2.2.1]heptyl), 3.96 (s., 2H, −S−CH_2−), 7.66 (t, 1H, J = 7.7 Hz, H-10), 7.73 (d, 1H, J = 7.9 Hz, H-8), 7.97 (t, 1H, J = 7.7 Hz, H-9), 8.05 (d, 1H, J = 8.5 Hz, −NHC(O)−), 8.46 (d, 1H, J = 7.9 Hz, H-11); ^{13}C NMR (100 MHz): δ: 18.17 (CH$_3$), 18.79 (CH$_3$CH$_2$), 20.70 (6′), 28.87 (CH$_3$CH$_2$), 30.33 (5′), 35.78 (−SCH$_2$), 36.33 (7′), 36.85 (1′), 38.95 (4′), 48.51 (3′), 49.37 (2′), 118.50 (11a), 125.99 (8), 126.70 (10), 127.90 (11), 135.91 (9), 144.21 (3), 151.92 (11b), 154.74 (7a), 155.16 (6), 160.98 (2), 166.39 (CONH); EI-MS, m/z (I_{rel}, %) = 303 (7.2), 302 (45.1), 285 (31.8), 246 (7.9), 245 (24.1), 244 (100.0), 143 (16.3), 218 (5.6), 217 (17.7), 216 (51.5), 188 (4.9), 179 (6.3), 170 (7.9), 148 (12.6), 143 (7.7), 129 (7.0), 123 (5.9), 122 (9.0), 95 (24.4), 93 (14.0), 91 (5.3), 90 (8.6), 81 (11.8), 67 (20.2), 57 (8.7), 56 (9.4), 55 (12.9); LC-MS, m/z = 424 [M+1], 426 [M+3]; Anal. calcd. for $C_{22}H_{25}N_5O_2S$: C, 62.39; H, 5.95; N, 16.54; S, 7.57; Found: C, 62.40; H, 5.95; N, 16.54; S, 7.58.

5.1.10. *N-(3-ethylbicyclo[2.2.1]hept-2-yl)-2-[3-(4-methylphenyl)-2-oxo-2H-[1,2,4]triazino[2,3-c]quinazolin-6-yl thio]acetamides (**4.9**)*

Yield: Method A, 67.3%; Method C, 87.3%; M.p. 248–250 °C; IR (cm^{-1}): 3285, 3078, 2950, 2868, 1668, 1633, 1589, 1562, 1549, 1501, 1468, 1454, 1391, 1372, 1335, 1308, 1270, 1239, 1183, 1135, 1104, 1019, 991, 939, 830, 782, 770, 712, 699, 684, 641, 626; ^{1}H NMR (400 MHz) δ: 1.51–0.94 (m, 11H, 3-CH_3CH$_2$−, 3-CH$_3$CH_2−, 5, 5′, 6, 6′, 7 bicyclo [2.2.1]heptyl, 2.16–2.09 (m, 2H, H-3′, 4′ bicyclo[2.2.1]heptyl), 2.42 (s, 3H, 3-(4-CH_3Ph), 2.67 (m, 1H, H-1′ bicyclo[2.2.1]heptyl), 3.60–3.41 (m, 1H, H-2′ bicyclo[2.2.1]heptyl), 4.00 (s., 2H, −S−CH_2−), 7.41 (d, 2H, J = 7.5 Hz, H-3, H-5 4−CH$_3$Ph), 7.68 (t, 1H, J = 7.7 Hz, H-10), 7.76 (d, 1H, J = 7.9 Hz, H-8), 7.98 (t, 1H, J = 7.7 Hz, H-9), 8.07 (d, 1H, J = 8.5 Hz, −NHC(O)−), 8.24 (d, 2H, J = 7.5 Hz, H-2, H-6 4−CH$_3$Ph), 8.48 (d, 1H, J = 7.9 Hz, H-11); LC-MS, m/z = 500 [M + 1], 502 [M + 3]; Anal. calcd. for $C_{28}H_{29}N_5O_2S$: C, 67.31; H, 5.85; N, 14.02; S, 6.42; Found: C, 67.31; H, 5.85; N, 14.02; S, 6.42.

5.1.11. *N-(3-ethylbicyclo[2.2.1]hept-2-yl)-2-{[3-(4-methoxyphenyl)-2-oxo-2H-[1,2,4]triazino[2,3-c]quinazolin-6-yl thio]acetamides (**4.10**)*

Yield: Method B, 73.8%; Method C, 89.6%; M.p. 230–233 °C; IR (cm^{-1}): 3274, 3067, 2945, 2866, 1668, 1643, 1590, 1563, 1545, 1500, 1469, 1454, 1421, 1372, 1340, 1317, 1306, 1288, 1272, 1259, 1238, 1174, 1137, 1105, 1049, 1016, 989, 965, 940, 878, 840, 810, 766, 723, 700, 684, 641, 622; ^{1}H NMR (400 MHz) δ: 1.47–0.92 (m, 11H, 3-CH_3CH$_2$-, 3-CH$_3$CH_2−, 5, 5′, 6, 6′, 7 bicyclo[2.2.1]heptyl), 2.16–2.11 (m, 2H, H-3′, 4′ bicyclo[2.2.1]heptyl), 2.60 (m, 1H, H-1′ bicyclo[2.2.1] heptyl), 3.55–3.43 (m, 1H, H-2′ bicyclo[2.2.1]heptyl), 3.87 (s, 3H, 3-(4-CH_3OPh), 4.00 (s, 2H, −S−CH_2−), 7.16 (d, 2H, J = 7.9 Hz, H-3, H-5 4−CH$_3$OPh), 7.68 (t, 1H, J = 7.7 Hz, H-10), 7.76 (d, 1H, J = 7.9 Hz, H-8), 7.98 (t, 1H, J = 7.7 Hz, H-9), 8.08 (d, 1H, J = 8.5 Hz, −NHC(O)−), 8.37 (d, 2H, J = 7.9 Hz, H-2, H-6 4−CH$_3$OPh), 8.48 (d, 1H, J = 7.9 Hz, H-11); LC-MS, m/z = 516 [M + 1], 518 [M + 3]; Anal. calcd. for $C_{28}H_{29}N_5O_3S$: C, 65.22; H, 5.67; N, 13.58; S, 6.22; Found: C, 65.24; H, 5.67; N, 13.56; S, 6.20.

5.2. *Biological assay*

5.2.1. *Bioluminescence inhibition test*

The marine luminescent bacteria *P. leiognathi* strain Sh1, isolated from the Azov Sea Shrimp, were used for the bioluminescence analysis [36]. Bacteria were cultivated on a nutrient environment containing (g/L): pepton − 5, yeast extract − 1.5, meat extract − 1.5, sodium chloride − 30, pH = 7.4. In the acute action test (inhibiting luminescence of bacteria), bacteria were diluted in the 3% sodium

chloride solution up to a concentration of 10^5 cells/mL. 5–50 lg/mL of the studied substances suspended in DMSO was mixed with 1 mL of the diluted bacterial suspension. Vials were incubating for 10 min at 25 °C, then the intensity of bioluminescence was measured in percent (%) relative to the control, which was prepared without the studied compounds. In the chronic action test (inhibiting growth and luminescence of bacteria), growth medium was added for potential breeding in a ratio of 1:50 and the mix was incubated for 16–18 h at 30 °C. Whereupon the intensity of bioluminescence was measured the same way as in acute action testing. Tetracycline was used as a reference. The bacterial luminescence was measured with a Bioluminometer BLM-8801 ("Science", Krasnoyarsk, Russia).

5.2.2. Antimicrobial and antifungal test

The investigation of antimicrobial and antifungal activity of compounds **1.1**–**1.4** and **.3.1**–**3.10** was carried out using the stiff plate agar diffusion method against *E. coli*, *S. aureus*, *M. luteum*, *C. tenuis* and *A. niger* [37]. The amount of microbial cells was 109 c.f.u./mL. Incubation period of bacteria was 24 h at 35 °C, yeast – 48–72 h at 28–30 °C. Antibiotics vancomicin, oxacillin, nystatin were used as standards. The bacterial cultures, standards and the obtained substances were streaked across grooves in 5 mg/mL concentration, and then allowed to diffuse in the agar nutrient plate. The antimicrobial effect and degree of activity of the tested compounds were evaluated by measuring of the inhibition zone diameters (not sensitive: 11–15 mm; sensitive: 16–25 mm; highly sensitive >25 mm). The results were compared with well-known drug standards vancomicin, nystatin, and oxacillin. All experiments were repeated three times.

5.2.3. Cytotoxic activity against malignant human tumor cells

Primary anticancer assay was performed at human tumor cell lines panel derived from nine neoplastic diseases, in accordance with the protocol of the Drug Evaluation Branch, National Cancer Institute, Bethesda [38–42]. 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 when compared to 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 10-fold dilutions of five concentrations ranging from 10^{-4} to 10^{-8} M. A 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 concentrations levels. Percentage growth inhibition was calculated as:

$[(Ti - Tz)/(C - Tz)] \times 100$ for concentrations for which $Ti \geq Tz$,

$[(Ti - Tz)]/Tz] \times 100$ for concentrations for which $Ti < Tz$.

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) as 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 as 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 greater or less than the maximum or minimum concentration tested. The log GI_{50}, log TGI, log LC_{50} were then determined, defined as the mean of the log's of the individual GI_{50}, TGI, LC_{50} values. The lowest values were obtained with the most sensitive cell lines.

Acknowledgements

The authors gratefully acknowledge "Enamine Ltd." (Kiev, Ukraine) for financial support of this work, and team of the Drug Synthesis and Chemistry Branch, National Cancer Institute, Bethesda, MD, USA, for *in vitro* evaluation of anticancer activity. We are grateful to Lviv Polytechnic National University for *in vitro* evaluation of antibaterial activity. The authors have declared no conflict of interest.

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