UDC 547.856'873:615.277.3.014]-047.37

O. Yu. Voskoboynik, I. S. Nosulenko, G. G. Berest, S. I. Kovalenko

Zaporizhzhia State Medical University

6-MONO- AND 6,6-DISUBSTITUTED 3-R-6,7-DIHYDRO-2*H*-[1,2,4]TRIAZINO[2,3-*c*]QUINAZOLINE-2-ONES – PROMISING CLASS OF ANTICANCER AGENTS

*Anticancer activity of novel 6-mono- i 6,6-disubstituted 3-R-6,7-dihydro-2H-[1,2,4]triazino[2,3-c]quinazolin-2-ones was described in presented manuscript. It was shown that 10-bromo-6-isobutyl-3-(4-fluorophenyl)-6,7-dihydro-2H-[1,2,4]triazino[2,3-c]quinazolin-2-one (***1.3***) and 6-(methoxyphenyl)-8-methyl-3-phenyl-6,7-dihydro-2H-[1,2,4]triazino[2,3-c]quinazolin-2-one (***1.4***) reveals high non-selective anticancer activity (mean growth -10.53% and 46.24 % correspondingly) against 60 cancer cell lines. Substantial dose-depended iv vitro study on 60 cancer cell lines for compound* **1.3** *showed, that it effectively inhibits growth of SR (lg GI_{50} = -6.17) of leukemia, NCI-H460 (lg GI_{50} = -5.79) of non-small lung cancer, HCT-116 (lg GI_{50} = -5.80), HCT-15 (lg GI_{50} = -5.78) of colon cancer, SNB-75 (lg GI_{50} = -5.88), U-251 (lg GI_{50} = -5.81) of CNS cancer, UACC-257 (lg GI_{50} = -5.83), UACC-62 (lg GI_{50} = -5.81) of melanoma, A498 (lg GI_{50} = -5.80), UO-31 (lg GI_{50} = -5.81) of renal cancer and MDA-MB-231/ATCC (lg GI_{50} = -5.81), MDA-MB-468 (lg GI_{50} = -5.80) of breast cancer cell lines. "Structure-biological activity" relationships for described compounds were discussed.*

Key words: 6-mono- i 6,6-disubstituted 3-R-6,7-dihydro-2*H*-[1,2,4]triazino[2,3-*c*]quinazoline-2-ones; in vitro screening; anticancer activity

INTRODUCTION

Improvement of the approaches to creation of massive combinatorial libraries of potential bioactive compounds as well as development of novel virtual screening and high-throughput screening allowed to create the series of innovative drugs. In spite of the mentioned above successes, some diseases are still incurable, what is serious challenge for specialists in medicinal chemistry. One of the greatest problems of modern medicine is tumor diseases that are on the top mortality positions in almost of all countries. Among the reasons, that caused the low effectiveness of oncological pathology treating: are imperfect methods of early diagnostics, high quantity of cell lines with their metabolism features what causes the impossibility of creation of universal antitumor agents and high toxicity of existing anticancer drugs. Considering the mentioned above facts investigations that aimed to creation of novel compounds with anticancer activity are one of the most urgent problems of modern science. Among the objects of investigation that aimed to the creation of the novel antitumor agent one of the most interesting are heterocyclic compounds including quinazolines and their condensed analogues [1-6, 9, 10, 12, 14-16, 18, 19]. Those attention of specialist in medicinal chemistry caused as by the high biological activity of heterocyclic compounds, so by wide possibility of their chemical modification. Mentioned research programs allowed to create several classes of high effective antitumor drugs that contain quinazoline fragment [20], however their potential is not exhausted. Considering the mentioned above facts it was decided to conduct the search of the novel antitumor agents among the 6-mono- i 6,6-disubstituted 8-R_1-10-R_2-3-R-6,7-dihydro-2*H*-[1,2,4]triazino[2,3-*c*]-quinazolin-2-ones, evaluate "structure-antitumor activity" relationships and recommend the most active for further studies.

MATERIALS AND METHODS

Novel 6-mono- and 6,6-disubstituted 8-R_1-10-R_2-3-R-6,7-dihydro-2*H*-[1,2,4]triazino[2,3-*c*]-quinazolin-2-ones (**1.1-1.9**, **2.1-2.6, 3.1-3.3** end **4.1-4.19**), that were synthesized at the department of organic and bioorganic chemistry of Zaporizhzhia state medical university (Head of the department Prof. Kovalenko S. I.) were studied for their anticancer activity. General structures of mentioned above compounds are presented on Fig. 1.

In vitro screening for anticancer activity on 60 cell lines. Studying of anticancer activity was conducted at the National Cancer Institute (Bethesda, Maryland, USA) according to the DTP (Development Therapeutic Program) protocol [7, 8, 11, 13, 17]. Screening of anticancer activity (I phase) conducted for 36 promising compounds and consist of their testing on 60 cell lines of human cancer in 10.00 μM concentration. Lines covers all basic tumor diseases including leukemia (CCRF-CEM, HL-60(TB), K-562, MOLT-4, RPMI-8226, SR), non-small cell lung cancer

(A549/ATCC, EKVX, HOP-62, HOP-92, NCI-H226, NCI-H23, NCI-H322M, NCI-H460, NCI-H522), colon cancer (COLO 205, HCC-2998, HCT-116, HCT-15, HT29, KM12, SW-620), CNS cancer (SF-268, SF-295, SF-539, SNB-19, SNB-75, U251), melanoma (LOX IMVI, MALME-3M, M14, MDA-MB-435, SK-MEL-2, SK-MEL-5, SK-MEL-28, UACC-62, UACC-257), ovarian cancer (IGROV-1, OVCAR-3, OVCAR-4, OVCAR-5, OVCAR-8, NCI/ADR-RES, SK-OV-3), renal cancer (786/0, A498, ACHN, CAKI-1, RXF 393, SN12C, UO-31), prostate cancer (PC-3, DU-145) and breast cancer (MCF-7, MDA-MB-231/ATCC, HS 578T, BT-549, T-47D, MDA-MB-468). 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. End point of determinations were made with a protein binding dye, sulforhodamine B (SRB). Results for each tested agent were reported as the percent growth of the treated cells comparing to the untreated control cells.

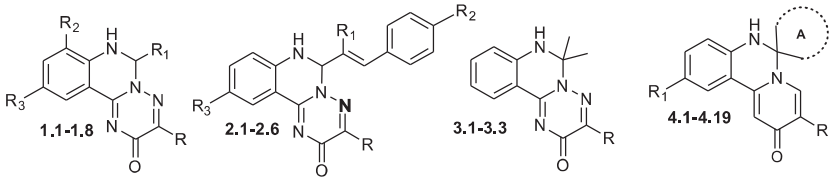
Dose-depended antitumor activity (phase 2) was conducted for most active according phase 1 studies compounds (**1.3**, **1.4**). Dose-dependency was studied at five concentrations in ten-fold dilution (100-0.01 μM) at 57-59 lines of 9 cancer cell types [7, 8, 11, 13, 17]. Experimental data allows calculation of 3 parameters 1) GI_{50} – concentration of compound that inhibit cell line growth on 50 %; 2) TGI – concentration of compound that inhibit cell line growth on 100 %; 3) LC_{50} – concentration of compound that resulted 50 % cell death, GI_{50} shows effective inhibition level, TGI – cytostatic effect, LC_{50} – cytotoxic effect. If logarithmic values of studied parameters (lg GI_{50}, lg TGI and lg LC_{50}) were less, then -4.00, compounds were considered as active. For each of parameters average values were calculated (mean graph midpoints, MG_MID).

RESULTS AND DISCUSSION

Experimental data showed, that only 6 of the 36 compounds revealed high antitumor activity (**1.3**, **1.4**, **2.6**, **4.2**, **4.10**, **4.11**, Fig. 1, Tab. 1). Mentioned above compounds inhibited growth of cancer cell lines (mean growth, %) in range of 46.24-79.06 %, and compound **1.3** (-10.53 %) revealed high antiproliferative activity against almost all cell lines (Fig. 1, Tab. 1).

We found that combination of substituents in positions 3 and 6 of 8-R_1-10-R_2-6-alkyl-(R-aryl-)-3-R-6,7-dihydro-2*H*-[1,2,4]triazino[2,3-*c*]qinazoline-2-ones (**1.1-1.8**) molecules was determinative for revealing of cytotoxic activity against cancer cell lines (Fig. 1, 2). Thus, presence of phenyl (**1.4**), 4-fluorphenyl (**1.3**) 4-methoxyphenyl (**1.2**) fragments in position 3 caused increasing of the cytotoxicity, but was not essential for occurrence of mentioned activity (**1.8**). More substantial increasing of studied compounds cytotoxicity against cancer cell lines depends on the nature of substituent in 6th position. Thus, introducing of the 6 *iso*-propyl- (**1.2**), *iso*-butyl- (**1.3**), 4-methoxyphenyl- (**1.4**), 2-hydroxyphenyl- (**1.8**) led to the increasing of activity comparing to other compounds. However, the most significant for presence of anticancer activity was the nature of substituent in benzene ring of quinazoline system, namely methyl group in position 8 (**1.4**) and bromine in position 10 (**1.3**) (Tab. 1). Thus the highest cytotoxicity was revealed by compound **1.3**, that was active against almost all cell lines. (Tab. 1).

Among 10-R^3-6-[(*E*)-2-R^1-2-(4-R^2-phenyl)vinyl]-3-R-6,7-dihydro-2*H*-[1,2,4]triazino[2,3-*c*]quinazoline-2-ones (**2.1-2.6**) the similar "structure-biological activity" pattern was observed. Thus, the highest levels of activity were revealed by compounds, that contains in 3rd position phenyl moiety, and, what important, bromine in po-



1.1 R=4-FC_6H_4, R_1=i-C_3H_7, R_2=R_3=H; **1.2** R=4-$CH_3OC_6H_4$, R_1=*i*-C_3H_7, R_2=R_3=H; **1.3** R=4-FC_6H_4, R_1=*i*-C_4H_9, R_2=H, R_3=Br; **1.4** R=C_6H_5, R_1=4-$CH_3OC_6H_4$, R_2=CH_3, R=H; **1.5** R=4-$CH_3C_6H_4$, R_1=2-$CH_3OC_6H_4$, R_2=R_3=H; **1.6** R=4-FC_6H_4, R_1=2-$CH_3OC_6H_4$, R_2=R_3=H; **1.7** R=4-FC_6H_4, R_1=3,4-$(CH_3O)_2C_6H_3$, R_2=R_3=H; **1.8** R=4-FC_6H_4, R_1=2,3-$Cl_2C_6H_3$, R_2=R_3=H;
2.1 R=4-$CH_3C_6H_4$, R_1=R_2=R_3=H; **2.2** R=4-*i*-$C_3H_7C_6H_4$, R_1=R_2=R_3=H; **2.3** R=4-$CH_3C_6H_4$, R_1=CH_3, R_2=R_3=H; **2.4** R=4-*i*-$C_3H_7C_6H_4$, R_1=CH_3, R_2=R_3=H; **2.5** R=C_6H_5, R_1=CH_3, R_2=H, R_3=Br; **2.6** R=4-$CH_3C_6H_4$, R_1=Cl, R_2=NO_2, R_3=H;
3.1 R=CH_3; **3.2** R=4-$C_2H_5C_6H_4$; **3.3** R=thien-2-yl;
4.1 R=CH_3, A=cyclopentane, R_1=H; **4.2** R=4-$C_2H_5OC_6H_4$, A=cyclopentane, R_1=H; **4.3** R=4-FC_6H_4, A=cyclopentane, R_1=H; **4.4** R=thien-2-yl, A=cyclopentane, R_1=H; **4.5** R=CH_3, A=4-*tert*-butylcyclohexane, R_1=H; **4.6** R=C_6H_5, A=4-*tert*-butylcyclohexane, R_1=H; **4.7** R=4-$CH_3C_6H_4$, A=4-*tert*-butylcyclohexane, R_1=H; **4.8** R=4-$CH_3OC_6H_4$, A=4-*tert*-butylcyclohexane, R_1=H; **4.9** R=4-FC_6H_4, A=4-*tert*-butylcyclohexane, R_1=H; **4.10** R=thien-2-yl, A=4-*tert*-butylcyclohexane, R_1=H; **4.11** R=C_6H_5, A=4-*tert*-butylcyclohexane, R_1=Br; **4.12** R=C_6H_5, A=1-methylpiperidine, R_1=H; **4.13** R=4-$CH_3C_6H_4$, A=1-methylpiperidine, R_1=H; **4.14** R=4-$C_2H_5C_6H_4$, A=1-methylpiperidine, R_1=H; **4.15** R=4-$CH_3OC_6H_4$, A=1-methylpiperidine, R_1=H; **4.16** R=4-$C_2H_5C_6H_4$, A=1-methylpiperidine, R_1=H; **4.17** R=4-FC_6H_4, A=1-methylpiperidine, R_1=H; **4.18** R=C_6H_5, A=1-methylpiperidine, R_1=Br; **4.19** R=4-FC_6H_5, A=1-methylpiperidine, R_1=Br

Fig. 1. *General structure of 6-mono- and 6,6-disubstituted 3-R-6,7-dihydro-2H-[1,2,4]triazino[2,3-c]-quinazolin-2-ones ((1.1-1.9, 2.1-2.6, 3.1-3.3 and 4.1-4.19).*

Table 1

CYTOTOXIC ACTIVITY OF 6-MONO-, 6,6-DISUBSTITUTED 8-R_1-10-R_2-3-R-6,7-DIHYDRO-2*H*-[1,2,4]TRIAZINO[2,3-*c*]QUINAZOLIN-2-ONES IN 10.00 MM CONCENTRATION

Compound	Mean growth, %	Range of growth, %	Studied cell lines/ sensitive cell lines*	Most sensitive cell line growth, %**
1.1	101.14	78.57-131.17	56/2	78.57 (T-47D/BC)
1.2	95.55	70.86-130.58	55/7	70.86 (SR/L), 75.05 (HCT-15/ColC)
1.3	-10.53	-76.33-78.36	58/58	-76.33 (HCT-116/ColC), -66.99 (SF-539/CNSC)
1.4	46.24	-14.12-86.52	56/55	-7.81 (SK-MEL-2/M), -14.12 (CAKI-1/RC)
1.5	99.93	74.97-122.44	56/1	74.97 (UO-31/RC)
1.6	100.44	83.05-112.36	59/1	83.05 (SNB-75/CNSC)
1.7	98.56	75.97-116.66	56/2	75.97 (UO-31/RC)
1.8	96.42	77.55-115.36	56/7	77.55 (UO-31/RC), 78.19 (MDA-MB-231/ATCC/BC)
2.1	100.72	66.96-121.37	59/1	66.96 (UO-31/RC)
2.2	98.48	73.86-118.91	56/5	78.98 (SR/L), 78.31 (CAKI-1/RC), 73.86 (UO-31/RC)
2.3	98.68	60.36-118.09	58/3	60.36 (UO-31/RC)
2.4	96.22	72.10-125.61	56/3	76.04 (SNB-75/CNSC), 72.10 (UO-31/RC)
2.5	94.95	72.15-115.76	57/7	72.86 (SNB-75/CNSC), 72.15 (T-47D/BC)
2.6	79.06	44.93-114.65	59/36	44.93 (SF-295/CNSC)
3.1	102.55	81.79-126.21	57/2	81.79 (SNB-75/CNSC)
3.2	100.52	59.16-121.87	57/2	59.16 (SR/L)
3.3	96.37	69.43-115.82	57/3	69.43 (RPMI-8226/L)
4.1	97.09	75.41-113.33	57/3	77.60 (UACC-257/M), 75.41 (UO-31/RC)
4.2	62.29	-45.03-100.91	57/52	0.95 (SR/L), -45.03 (OVCAR-4/OV)
4.3	101.85	82.88-116.70	57/1	82.88 (UO-31/RC)
4.4	91.85	61.26-116.24	57/13	73.19 (SR/L), 61.26 (UO-31/RC)
4.5	96.47	72.36-111.45	59/4	72.36 (UO-31/RC), 78.58 (PC-3/PC)
4.6	98.40	79.32-119.36	59/2	79.70 (SK-MEL-2/M), 79.32 (UO-31/RC)
4.7	99.20	74.73-118.02	59/3	74.73 (HOP-62/nscLC)
4.8	97.37	73.37-115.98	58/4	77.76 (HOP-62/nscLC), 73.37 (UO-31/RC)
4.9	98.58	74.91-114.37	59/7	74.91 (SR/L), 77.06 (UO-31/RC)
4.10	68.36	-35.50-100.94	59/47	9.96 (SR/L), -35.50 (HCT-116/ColC)
4.11	68.36	-35.50-100.94	59/17	9.96 (SR/L), -35.50 (HCT-116/ColC)
4.12	97.30	54.70-115.33	59/3	54.70 (UO-31/RC)
4.13	95.31	65.23-111.96	55/5	74.26 (CAKI-1/RC), 65.23 (UO-31/RC)
4.14	98.87	75.01-116.85	57/2	75.01 (UO-31/RC)
4.15	98.88	78.39-116.28	57/1	78.39 (SNB-75/CNSC)
4.16	100.15	74.71-116.45	57/3	74.71 (SR/L)
4.17	95.37	67.78-130.91	57/4	67.78 (UO-31/RC)
4.18	94.18	53.54-113.55	59/8	60.80 (SR/L), 53.54 (UO-31/RC)
4.19	94.77	70.89-108.62	57/3	77.21 (SR/L), 70.89 (UO-31/RC)

* – Compounds with mean growth ≤ 85 % were considered as sensitive; ** – L – leukemia; nscLC – non-small cell lung cancer; CC – colon cancer; CNSC – CNS cancer; M – melanoma; OV – ovarian cancer; RC – renal cancer; PC – prostate cancer; BC – breast cancer.

sition 10 (**2.5**). Experimental data showed, that compounds with 4-methylphenyl-(**2.1**, **2.3**) or 4-isopropylphenyl moieties (**2.2**, **2.4**) were inactive. The exception was compound **2.6**, that in addition to 4-methylphenyl fragment in 3rd position contains 1-chloro-2-(4-nitrophenyl) vinyl moiety in position 6, what was, as we consider, the key factor of high cytotoxic activity presence. Mentioned above compound reveals high activity against 36 cell lines (Tab. 1).

3-R-6,6-dimethyl-6,7-dihydro-2*H*-[1,2,4]triazino [2,3-*c*]quinazolin-2-ones (**3.1-3.3**) were low-active as cytostatic. Their activity depends on the nature of substituent in position 3, the most active was the compound **3.3** with thiophene fragment.

Among 3′-R-spiro[cyclopentan-1,6′-[1,2,4]triazino[2,3-*c*] qinazolin]-2′(7′*H*)-ones (**4.1-4.4**) the most active were compounds **4.2** and **4.4** with 4-ethoxyphenyl and thiophen-2-yl moiety. Thus, compound **4.2** inhibited growth of 52 cancer cell lines (Tab. 1). Replacing of cyclopentan fragment (**4.1-4.4**) on 4-*tert*-butylcyclohexane (**4.5-4.11**) substituent in position 6 insignificantly effect on antitumor activity. We noted, that the highest growth inhibiting

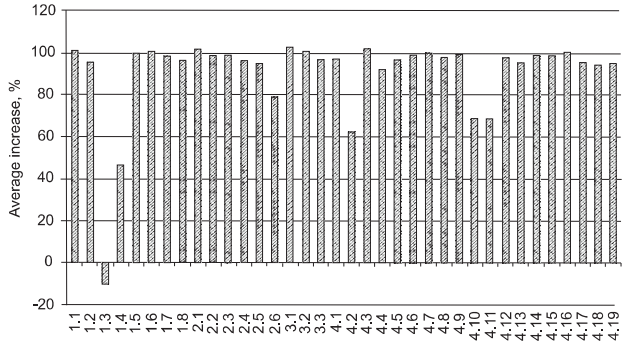


Fig. 2. *Cytotoxic activity (men growth of cancer cell lines, %) of 6-mono-, 6,6-disubstituted 8-R_1-10-R_2-3-R-6,7-dihydro-2H-[1,2,4]triazino[2,3-c]quinazolin-2-ones (1.1-1.9, 2.1-2.6, 3.1-3.3 and 4.1-4.19) in 10.00 μM concentration.*

Table 2

RESULTS OF SUBSTANTIAL (DESE-DEPENDED) STUDY OF SYNTHESIZED COMPOUNDS ANTICANCER ACTIVITY IN FIVE CONCENTRATION (100 μM, 10 μM, 1,0 μM, 0,1 μM, 0,01 μM)

Cancer cell lines*	Compounds/Effects range value (for values GI_{50} ≤-5.00; lg TGI < -4.00; lg LC_{50} < -4.00)					
	1.3			**1.4**		
	lg GI_{50}	lg TGI	lg LC_{50}	lg GI_{50}	lg TGI	lg LC_{50}
1	2	3	4	5	6	7
MG_MID	-5.72	-5.35	-4.86	-5.44	-4.14	-4.0
Leukemia						
CCRF-CEM	-5.63	-5.18	-4.00	-5.35	>-4.00	>-4.00
HL-60(TB)	-5.44	-4.65	>-4.00	-5.61	>-4.00	>-4.00
K-562	-5.71	-5.38	-5.06	-5.51	>-4.00	>-4.00
MOLT-4	-5.68	-5.38	-5.07	-5.39	>-4.00	>-4.00
RPMI-8226	-5.69	-5.28	-4.43	-5.51	>-4.00	>-4.00
SR	-6.17	-5.70	-5.35	-5.51	-5.06	>-4.00
Non small cell lung						
A549/ATCC	-5.73	-5.39	-5.04	-5.46	>-4.00	>-4.00
HOP-62	-5.76	-5.42	-5.09	-5.34	>-4.00	>-4.00
HOP-92	-5.77	-5.39	-5.01	-5.42	>-4.00	>-4.00
NCI-H226	-5.69	-5.24	-4.13	-5.43	>-4.00	>-4.00
NCI-H322M	-5.59	-5.09	>-4.00	-5.41	>-4.00	>-4.00
NCI-H460	-5.79	-5.53	-5.26	-5.50	>-4.00	>-4.00
NCI-H522	-5.78	-5.49	-5.20	-5.63	-5.13	>-4.00
Colon cancer						
COLO 205	-5.66	-5.30	-4.79	-5.38	>-4.00	>-4.00
HCC-2998	-5.59	-5.24	-4.59	-5.25	>-4.00	>-4.00
HCT-116	-5.80	-5.53	-5.26	-5.45	>-4.00	>-4.00
HCT-15	-5.78	-5.50	-5.21	-5.56	>-4.00	>-4.00
HT29	-5.73	-5.48	-5.22	-5.47	>-4.00	>-4.00
KM12	-5.39	>-4.00	>-4.00	-5.47	>-4.00	>-4.00
SW-620	-5.76	-5.47	-5.19	-5.46	>-4.00	>-4.00
CNS cancer						
SF-295	-5.78	-5.49	-5.21	-5.41	>-4.00	>-4.00
SF-539	-5.76	-5.50	-5.25	-5.54	>-4.00	>-4.00
SNB-19	-5.63	-5.05	>-4.00	-5.07	>-4.00	>-4.00
SNB-75	-5.88	-5.53	-5.17	-5.56	>-4.00	>-4.00
U251	-5.81	-5.53	-5.26	-5.40	>-4.00	>-4.00
Melanoma						
LOX IMVI	-5.75	-5.48	-5.20	-5.49	>-4.00	>-4.00

Continuation Table 2

1	2	3	4	5	6	7
MALME-3M	-5.73	-5.42	-5.11	-5.18	>-4.00	>-4.00
M14	-5.73	-5.44	-5.15	-5.27	>-4.00	>-4.00
MDA-MB-435	-5.74	-5.42	-5.10	-5.69	-5.27	>-4.00
SK-MEL-2	-5.73	-5.47	-5.21	-5.63	-5.24	>-4.00
SK-MEL-28	-5.74	-5.43	-5.11	-5.10	>-4.00	>-4.00
SK-MEL-5	–	–	–	-5.60	>-4.00	>-4.00
UACC-257	-5.83	-5.49	-5.16	-5.34	>-4.00	>-4.00
UACC-62	-5.81	-5.52	-5.23	-5.50	>-4.00	>-4.00
Ovarian cancer						
IGROV1	-5.56	-5.07	-4.01	-5.38	>-4.00	>-4.00
OVCAR-3	-5.71	-5.45	-5.16	-5.48	>-4.00	>-4.00
OVCAR-4	-5.77	-5.41	-5.05	-5.45	>-4.00	>-4.00
OVCAR-5	-5.78	-5.49	-5.20	–	>-4.00	>-4.00
OVCAR-8	-5.72	-5.42	-5.11	-5.36	>-4.00	>-4.00
NCI/ADR-RES	-5.66	-5.27	-4.35	-5.45	>-4.00	>-4.00
SK-OV-3	-5.73	-5.32	-4.30	-5.52	-5.02	>-4.00
Renal cancer						
786-0	-5.77	-5.49	-5.22	-5.32	>-4.00	>-4.00
A498	-5.80	-5.47	-5.14	-5.62	-5.10	>-4.00
ACHN	-5.76	-5.49	-5.21	-5.62	>-4.00	>-4.00
CAKI-1	-5.58	-5.04	>-4.00	-5.57	>-4.00	>-4.00
RXF 393	-5.78	-5.47	-5.16	-5.52	>-4.00	>-4.00
SN12C	-5.73	-5.37	-5.02	-5.40	>-4.00	>-4.00
UO-31	-5.81	-5.50	-5.18	-5.60	>-4.00	>-4.00
Prostate cancer						
PC-3	-5.67	-5.26	-4.14	-5.47	>-4.00	>-4.00
DU-145	-5.53	-5.07	>-4.00	-5.29	>-4.00	>-4.00
Breast cancer						
MCF7	-5.72	-5.36	-5.01	-5.47	>-4.00	>-4.00
MDA-MB-231/ ATCC	-5.81	-5.50	-5.19	-5.59	>-4.00	>-4.00
HS 578T	-5.75	-5.31	-4.03	-5.50	>-4.00	>-4.00
BT-549	-5.55	-5.11	-4.13	-5.07	>-4.00	>-4.00
T-47D	-5.76	-5.41	-5.06	-5.48	>-4.00	>-4.00
MDA-MB-468	-5.80	-5.48	-5.16	-5.57	>-4.00	>-4.00

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

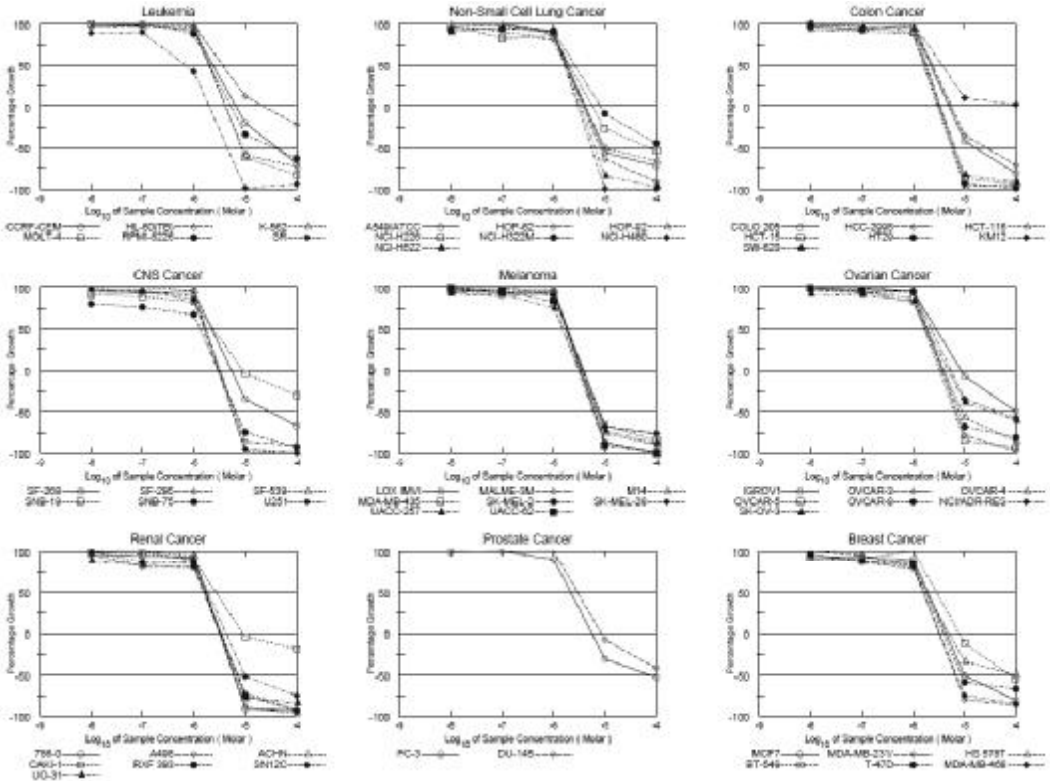


Fig. 3. *Results of substantial study of anticancer activity of compound 1.3 in five concentrations.*

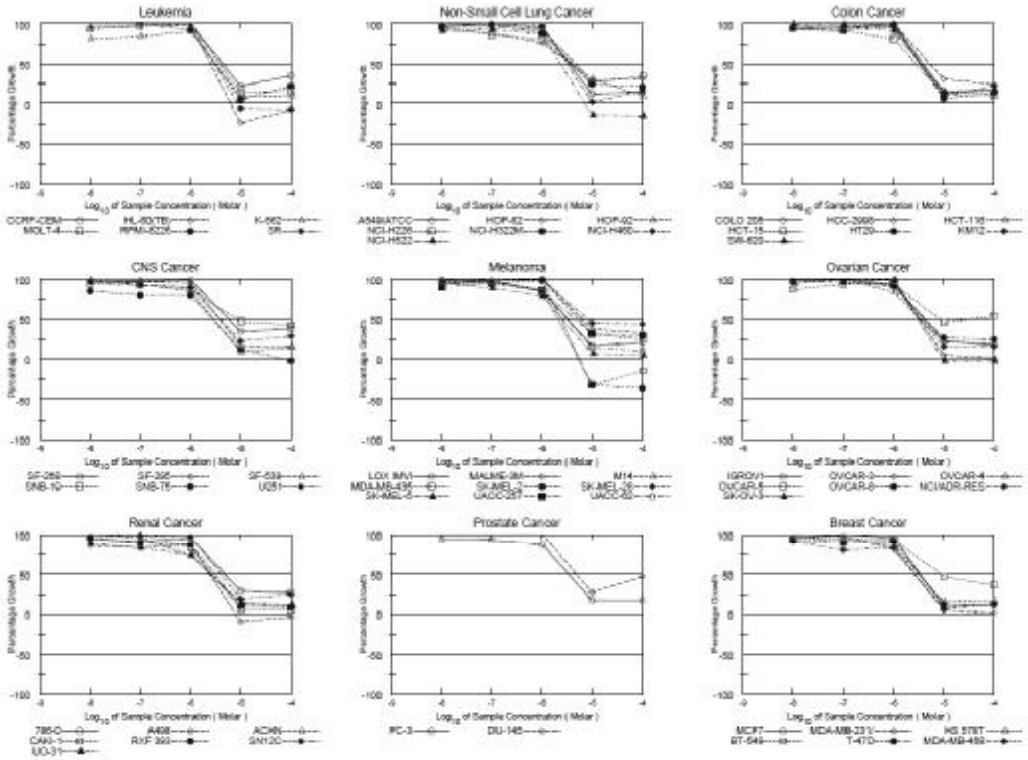


Fig. 4. *Results of substantial study of anticancer activity of compound 1.4 in five concentrations.*

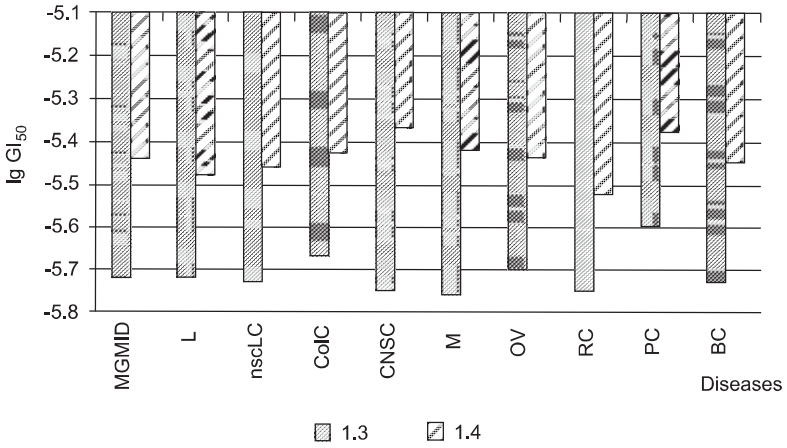


Fig. 5. *Antitumor activity of compounds 1.3 and 1.4 against oncological diseases.*

activity was revealed by compound **4.10**, that contain thiophen-2-yl substituent in 3rd (Tab. 1). Mentioned above compound inhibits growth of 47 cancer cell lines. Besides that, high activity (active against 17 cancer cell lines) is revealed by compound **4.11**, that contains the phenyl moiety at position 3 and bromine atom at position 10, what proves the significant effect of halogen on anticancer activity level (Tab. 1). Moreover, compound **4.6** with phenyl moiety in position 3 reveals moderate anticancer activity. Further modification of molecule by introduction of 6-spirocondensed 1-methylpiperidine fragment (compounds **4.12-4.19**) leads to the moderate anticancer activity (Tab. 1). The broadest spectrum of anticancer activity (against 8 cell lines) has been revealed by **4.18**, that as compound **4.11,** contains phenyl group at position 3 and bromine atom at position 10.

Thus, conducted study of anticancer activity allowed to obtain a number of substantiated evidences that 6-mono- i 6,6-disubstituted 3-R-6,7-dihydro-2*H*-[1,2,4]triazino[2,3-*c*]-quinazolne-2-ones are promising bioactive agents and to select 2 compounds (**1.3** and **1.4**) with high activity for next phase of study. Analysis of substantial *in vitro* studies (in range of the doses 100-0.01 μM) proved (Tab. 2, Fig. 3, 4), that compounds **1.3** and **1.4** reveals high anticancer activity. The highest level of growth inhibiting activity was revealed by compound **1.3**, that by the value of mean growth (MG_MID) lg GI_{50} -5.72 exceeds compound **1.4** (MG_MID lg GI_{50} – -5.44).

It is important, that compound **1.3** has significant level of inhibiting activity (lg GI_{50} = -5.44 – -6.17), high cytostatic (lg TGI = -4.00 – -5.70) and cytotoxyc (lg LC_{50} = -4.00 – -5.70) effects against all cancer cell lines (Tab. 2). Compound **1.3** were effective against cell lines of SR (lg GI_{50} = -6.17, lg TGI = -5.70) of leukemia; lines NCI-H460 (lg GI_{50} = -5.79, lg TGI = -5.53) of non-small lung cancer; lines HCT-116 (lg GI_{50} = -5.80, lg TGI = -5.53), HCT-15 (lg GI_{50} = -5.78, lg TGI = -5.50) of colon cancer; lines SNB-75 (lg GI_{50} = -5.88, lg TGI = -5.53), U-251 (lg GI_{50} = -5.81, lg TGI = -5.53) of CNS cancer; lines UACC-257 (lg GI_{50} = -5.83, lg TGI = -5.49), UACC-62 (lg GI_{50} = -5.81, lg TGI = -5.52) of melanoma; lines A498 (lg GI_{50} = -5.80, lg TGI = -5.47), UO-31 (lg GI_{50} = -5.81, lg TGI = -5.50) of renal cancer; lines MDA-MB-231/ATCC (lg GI_{50} = -5.81, lg TGI = -5.50), MDA-MB-468 (lg GI_{50} = -5.80, lg TGI = -5.48) of breast cancer (Tab. 2).

It is important, that compound **1.4** reveals high anticancer activity not only against singe lines, but is active against groups of oncological diseases. Thus mentioned compound is effective against CNS cancer (lg GI_{50} = -5.75, lg TGI = -5.40), melanoma (lg GI_{50} = -5.76, lg TGI = -5.46), and colon cancer (lg GI_{50} = -5.75, lg TGI = -5.40, Fig. 5).

Thus, the anticancer potential of novel 6-mono- i 6,6-disubstituted 3-R-6,7-dihydro-2*H*-[1,2,4]triazino[2,3-*c*]-quinazoline-2-ones was confirmed and "structure-activity" relationships were discussed. Obtained data may be considered as argument for further study of molecular mechanisms of anticancer effects by *in silico* approaches for rational design of bioactive compounds including COMPARE-analysis and molecular docking.

CONCLUSIONS

The anticancer activity of previously unknown 6-mono- and 6,6-disubstituted 3-R-6,7-dihydro-2*H*-[1,2,4]triazino[2,3-*c*]quinazolin-2-ones against individual cell lines was discovered and the most active compounds, namely 10-bromo-6-isobutyl-3-(4-fluorophenyl)-6,7-dihydro-2*H*-[1,2,4]triazino[2,3-*c*]quinazolin-2-one (**1.3**) and 6-(4-methoxyphenyl)-8-methyl-3-phenyl-6,7-dihydro-2*H*-[1,2,4]triazino[2,3-*c*]quinazolin-2-ones (**1.4**) were found, what as we consider is weighty argument for purposeful search of chemotherapeutic agents among studied class of the compounds. Determinant substituent, that according SAR-analysis effects on revealing of anticancer activity of synthesized compounds were identified. It was shown that presence of bromine atom at 10th position and phenyl, 4-fluorophenyl, 4-alkoxyphenyl or thiophen-2yl ad 3rd position significantly increases the anticancer activity of synthesized compounds. The "structure-biologi-

cal activity" relationships were discussed and strategy of further investigations, that based on usage of *in silico* screening methods was proposed.

REFERENCES

1. Берест Г. Г. 3-R-6-тіо-6,7-дигідро-2Н-[1,2,4]триазино[2,3-с]хіназолін-2-они: синтез, функціоналізація, фізико-хімічні та біологічні властивості: автореф. дис. ... канд. фарм. наук: спец. 15.00.02 «Фармацевтична хімія та фармакогнозія» / Г. Г. Берест. – Львів, 2012. – 23 с.
2. Воскобойнік О. Ю. Синтез, перетворення, фізико-хімічні та біологічні властивості [{2-R-(3Н)-хіназолін-4-іліден}гідразоно]карбонових кислот: Автореф. дис. … канд. фарм. наук: спец. 15.00.02 «Фармацевтична хімія та фармакогнозія» / О. Ю. Воскобойнік. – Львів, 2008. – 23 с.
3. Кривошей О. В. Синтез, фізико-хімічні властивості та протиракова активність амідів (3-оксо-3,4-дигідро-2Н-[1,2,4]триазино[4,3-с]хіназолін-4-іл) оцтової кислоти / О. В. Кривошей, С. І. Коваленко // Мед. хімія. – 2008. – Т. 10, № 4. – С. 109-117.
4. Barker A. J. PCT Int. Appl. WO 9633979 / A. J. Barker // Chem. Abstr. – 1997. – № 126 (3).
5. Berest G. G. Synthesis and biological activity of novel N-cycloalkyl-(cycloalkylaryl)-2-[(3-R-2-oxo-2H-[1,2,4]triazino[2,3-c]quinazoline-6-yl)thio]acetamides / [G. G. Berest, A. Yu. Voskoboynik, S. I. Kovalenko et al.] // Eur. J. of Med. Chem. – 2011. – Vol. 46, Issue 12. – P. 6066-6074.
6. Berest G. G. Synthesis of new 6-{[ω-(dialkylamino(heterocyclyl)alkyl]thio}-3-R-2H-[1,2,4]triazino[2,3-c]quinazoline-2-ones and evaluation of their anticancer and antimicrobial activities / [G. G. Berest, O. Yu. Voskoboynik, S. I. Kovalenko et al.] // Sci. Pharm. – 2012. – Vol. 80, Issue 1. – P. 37-65.
7. Boyd M. R. Some practical considerations and applications of the National Cancer Institute in vitro anticancer drug discovery screen / M. R. Boyd, K. D. Paull // Drug Dev. Res. – 1995. – № 34. – P. 91-109.
8. Boyd M. R. The NCI In Vitro Anticancer Drug Discovery Screen. Concept, Implementation and Operation / M. R. Boyd, B. A. Teicher // Cancer Drug Discovery and Development. – 1997. – Vol. 2. – P. 23-43.
9. Cockerill G. S. PCT Int. Appl. WO 9703069 / G. S. Cockerill, M. C. Carter, S. K. Mckeown et al. // Chem. Abstr. – 1997. – № 126 (15). – P. 199580n.
10. Denny W. A. The 4-anilinoquinazoline class of inhibitors of the erbB famyli of receptors tyrosine kinases / W. A. Denny // IL Farmaco. – 2001. – № 56. – P. 51-56.
11. Monks A. Feasibility of a high-flux anticancer drug screen using a diverse panel of cultured human tumor cell lines / [A. Monks, D. Scudiero, P. Skehan et al.] // J. Nat. Cancer Inst. – 1991. – № 83 (11). – P. 757-766.
12. http://dtp.nci.nih.gov/docs/compare/compare.html.
13. <http://dtp.nci.nih.gov>
14. Kidwai M. Chemotherapy and heterocyclic compounds / M. Kidwai, R. Venkataramanan, R. Mohan, P. Sapra // Curr. Med. Chem. – 2002. – № 9. – P. 1209-1228.
15. Kovalenko S. I. N-R-2-[(3-R-2-oxo-2H-[1,2,4]triazino[2,3-c]quinazoline-6-yl)thio]acetamides with thiazole and thiadiazole fragments in a molecules. Synthesis, physico-chemical properties, cytotoxicity research by bioluminescence inhibition, anticancer activity / [S. I. Kovalenko, I. S. Nosulenko, A. Yu. Voskoboynik et al.] // Sci. Pharm. – 2012. – Vol. 80. – P. 837-865.
16. Kovalenko S. I. Novel N-aryl(alkaryl)-2-[(3-R-2-oxo-2H-[1,2,4]triazino[2,3-c]quinazoline-6-yl)thio]-acetamides: synthesis, cytotoxicity, anticancer activity, compare analysis and docking. / [S. I. Kovalenko, I. S. Nosulenko, A. Yu. Voskoboynik et al.] // Med. Chem. Res. – 2013. – Vol. 22, Issue 6. – P. 2610-2632.
17. Shoemaker R. H. Application of high-throughput, molecular-targeted screening to anticancer drug discovery / R. H. Shoemaker, D. A. Scudiero, G. Melillo // Curr. Top. Med. Chem. – 2002. – № 2 (3). – P. 229-246.
18. Spirkova K. Synthesis and biological activity of some 2-substituted quinazolin-4-ones / K. Spirkova, S. Stankovsky, A. Mrvova, L. Cipak // Chem. Pap. – 1999. – № 53 (4). – P. 272-275.
19. Thomas A. P. PCT Int. Appl. WO 97 32,856 // A. P. Thomas, L. F. A. Hennequin, C. Johnstone // Chem. Abstr. – 1997. – № 127 (20). – P. 278209x.
20. www.drug.bank.org

УДК 547.856'873:615.277.3.014]-047.37

О. Ю. Воскобойнік, І. С. Носуленко, Г. Г. Берест, С. І. Коваленко

6-МОНО- І 6,6-ДИЗАМІЩЕНІ 3-R-6,7-ДИГІДРО-2*H*-[1,2,4]ТРИАЗИНО[2,3-*c*]ХІНАЗОЛІН-2-ОНИ – ПЕРСПЕКТИВНИЙ КЛАС ПРОТИПУХЛИННИХ АГЕНТІВ

В представленій роботі вперше досліджена протипухлинна активність невідомих 6-моно- і 6,6-дизаміщених 3-R-6,7-дигідро-2*H*-[1,2,4]триазино[2,3-*c*]хіназолін-2-онів. Встановлено, що для 10-бромо-6-ізобутил-3-(4-флюорофеніл)-6,7-дигідро-2H-[1,2,4]триазино[2,3-*c*]хіназолін-2-ону (**1.3**) та 6-(4-метоксифеніл)-8-метил-3-феніл-6,7-дигідро-2*H*-[1,2,4]триазино[2,3-*c*]хіназолін-2-ону (**1.4**) характерна висока протипухлинна активність без значної селективності (середній приріст – 10,53 % та 46,24 % відповідно) щодо 60 ліній ракових клітин. Ґрунтовне *iv vitro* дослідження на 60 лініях ракових клітин у градієнті концентрацій (дозозалежність) для сполуки **1.3** показало, що вона ефективно інгібує ріст ліній SR (lg GI_{50} = -6,17) лейкемії, NCI-H460 (lg GI_{50} = -5,79) недрібноклітинного раку легень, HCT-116 (lg GI_{50} = -5,80), HCT-15 (lg GI_{50} = -5,78) епітеліальний рак товстої кишки, SNB-75 (lg GI_{50} = -5,88), U-251 (lg GI50 = -5,81) раку ЦНС, UACC-257 (lg GI50 = -5,83), UACC-62 (lg GI_{50} = -5,81) меланоми, A498 (lg GI_{50} = -5,80), UO-31 (lg GI_{50} = -5,81) раку нирок та MDA-MB-231/ATCC (lg GI_{50} = -5,81), MDA-MB-468 (lg GI_{50} = -5,80) раку молочної залози. Обговорені деякі закономірності (SAR-аналіз) «структура-активність» у досліджуваному ряду.

Ключові слова: 6-моно- і 6,6-дизаміщені 3-R-6,7-дигідро-2*H*-[1,2,4]-триазино[2,3-*c*]хіназолін-2-они; in vitro скринінг; протипухлинна активність

УДК 547.856'873:615.277.3.014]-047.37

А. Ю. Воскобойник, И. С. Носуленко, Г. Г. Берест, С. И. Коваленко

6-МОНО- И 6,6-ДИЗАМЕЩЕННЫЕ 3-R-6,7-ДИГИДРО-2*H*-[1,2,4]-ТРИАЗИНО[2,3-*c*]ХИНАЗОЛІН-2-ОНЫ – ПЕРСПЕКТИВНЫЙ КЛАСС ПРОТИВОРАКОВЫХ АГЕНТОВ

В представленной работе впервые исследована противораковая активность неизвестных 6-моно- и 6,6-дизамещенных 3-R-6,7-дигидро-2*H*-[1,2,4]триазино[2,3-*c*]хиназолин-2-онов. Установлено, что для 10-бромо-6-изобутил-3-(4-флюорофенил)-6,7-дигидро-2*H*-[1,2,4]триазино[2,3-*c*]хиназолин-2-она (**1.3**) и 6-(4-метоксифенил)-8-метил-3-фенил-6,7-дигидро-2*H*-[1,2,4]триазино-[2,3-*c*] хиназолин-2-она (*1.4*) характерна высокая противораковая активность без особой селективности (средний прирост – 10,53 % и 46,24% соответственно) к 60 линиям раковых клеток. Углубленное *iv vitro* исследование на 60 линиях раковых клеток в градиенте концентраций (дозозависимость) для соединения **1.3** показало, что оно эффективно ингибирует рост линий SR (lg GI_{50} = -6,17) лейкемии, NCI-H460 (lg GI_{50} = -5,79) немелкоклеточного рака легких, HCT-116 (lg GI_{50} = -5,80), HCT-15 (lg GI_{50} = -5,78) эпителиального рака прямой кишки, SNB-75 (lg GI_{50} = -5,88), U-251 (lg GI50 = -5,81) рака ЦНС, UACC-257 (lg GI50 = -5,83), UACC-62 (lg GI50 = -5,81) меланомы, A498 (lg GI_{50} = -5,80), UO-31 (lg GI_{50} = -5,81) рака почек и MDA-MB-231/ATCC (lg GI_{50} = -5,81), MDA-MB-468 (lg GI_{50} = -5,80) рака молочной железы. Обсуждены некоторые закономерности (SAR-анализ) «структура-активность» в исследуемом ряду.

Ключевые слова: 6-моно- и 6,6-дизамещенные 3-R-6,7-дигидро-2*H*-[1,2,4]триазино[2,3-*c*]хиназолин-2-оны; in vitro скрининг; противораковая активность

Адреса для листування:
69035, м. Запоріжжя, пр. Маяковського, 26.
Запорізький державний медичний університет

Надійшла до редакції 11.02.2016 р.

Мікробіологія

Рецензенти рубрики:

Філімонова Н. І.,
д. мед. н., професор