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SYNTHESIS, PHYSICOCHEMICAL AND BIOLOGICAL PROPERTIES OF 6-S- AND 6-N-SUBSTITUTED 3-R-2*H*-[1,2,4]TRIAZINO[2,3-*C*]QUINAZOLIN-2-ONES

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The present article describes the synthesis and biological activity of series of 6-S- and 6-N-substituted 3-R-2H-[1,2,4]triazino[2,3-c]quinazolin-2-ones. It is shown that 3-R-2H-[1,2,4]triazino[2,3-c]quinazoline-2,6(7H)-diones cannot be used as starting materials for the synthesis of the target compounds, whereas the modification of potassium 3-R-2-oxo-2H-[1,2,4]triazino[2,3-c]quinazolin-6-thiolates provides such an opportunity. The individuality of the synthesized compounds is proved by liquid chromatography–mass spectrometry and their structures by ^{1}H NMR, ^{13}C NMR and mass spectroscopy. The characteristic signals in ^{1}H NMR, ^{13}C NMR - spectra are described; the features of molecule fragmentation under electron impact are elucidated. The anticancer activity according to National Cancer Institute (USA) protocol is evaluated for some of the obtained compounds. The results of anticancer activity allow identifying a compound, which is capable of inhibiting the growth of ovarian cancer cells OVCAR-4 at 61.64%. The results of microbiological screening demonstrate that the synthesized compounds were inactive relative to *E. Coli*, show a moderate activity against *P. aeruginosa* and show very encouraging results in the study of antimicrobial activity against *S. aureus* and antifungal activity against *C. albicans*. The prospect of further research aimed at creating novel anticancer, antibacterial and antifungal agents based on the compounds described is herein discussed.

Keywords:triazines, quinazolines, anticancer action, antimicrobial action, antifungal action.

Introduction

Heterocyclic compounds for several decades are objects of investigations aimed to the creation of the novel drugs, in particular chemotherapeutic agents. Among the most perspective fields of research, we noted the elaborations related to studying of chemical transformations and biological activities of quinazoline derivatives, which became more intensive during last years. Mentioned fact is appreciably caused by discovery of ability of 4-anilinoquinazolines to inhibit activities of tyrosine kinases [1], which in turn allowed to createthe series of effective anticancer agents [2,3]. In spite of significant progress in studying of chemistry and pharmacological activities of quinazoline derivatives, publications devoted to the heterocondensed derivatives of mentioned above heterocyclic system are relatively few relatively few in spite of their high perspectives. Considering the mentioned facts and inspired by our previous works [4,5] which showed high potential of derivatives of [1,2,4]triazino[2,3-*c*]quinazoline system as anticancer agents, we decided to synthesize series of 6-S- and 6-N-substituted 3-R-2*H*-[1,2,4]triazino[2,3-*c*]quinazoline-2-ones and study their physicochemical and biological properties.

Results and discussion

Chemistry

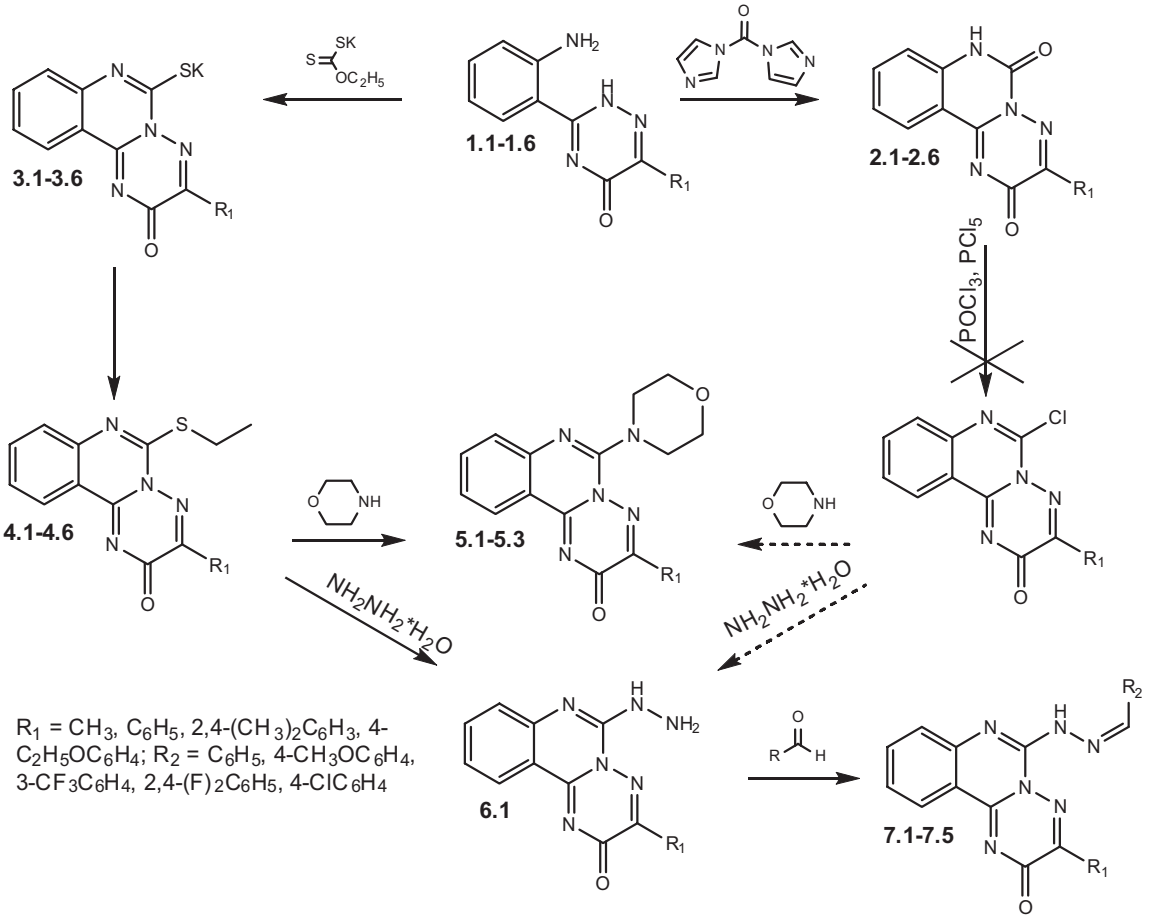
During planning of synthetic routes for target compounds we considered 6-chloro-3-R-2*H*-[1,2,4]-triazino[2,3-*c*]quinazoline-2-ones as most promising precursor with high potential for modification. Mentioned compounds planned to be obtained via chlorination of reported 3-R-2*H*-[1,2,4]triazino[2,3-*c*]quinazoline-2,6(7*H*)-diones – products of interaction between 3-(2-aminophenyl)-6-R-1,2,4-triazin-5(2*H*)-ones and *N*,*N*-carbonyldiimidazole [6]. Unfortunately, experimental data showed that chlorination of compounds **2.1–2.6** by phosphorus pentachloride or phosphorus oxochloride did not lead to the formation of target 6-chloroderivatives. Thus, polymerization processes occurs during the mentioned interaction, what, as we consider caused by the presence of second carbonyl fragment in the molecule. Considering described above, we decided to use another synthetic route for formation of target molecules, namely modification of potassium 3-R-2-oxo-2*H*-[1,2,4]triazino[2,3-*c*]quinazolin-6-thiolates (**3.1–3.6**), which also may be obtained via modification of corresponding 3-(2-aminophenyl)-6-R-1,2,4-triazine-5(2*H*)-ones [7]. Our results

showed, that alkylation of compounds **3.1−3.6** by ethyl iodide in propanol-2 led to the formation of 6-(ethylthio)-3-R-2*H*-[1,2,4]triazino[2,3-*c*]quinazoline-2-ones with high yields. Mentioned compounds interesting as potential bioactive agents and initial compound for synthesis of various 6-N substituted 3-R-2*H*-[1,2,4]triazino[2,3-*c*]quinazoline-2-ones. One of the most effective approaches for chemical optimization of [1,2,4]triazino[2,3-*c*]quinazoline-2-one system is introduction of morpholine moiety in mentioned heterocylicfragment, particularly, in view of recent publications which described its grate role as pharmacophore [8,9]. Synthesis of 3-R-6-morpholino-2*H*-[1,2,4]triazino-[2,3-*c*]quinazoline-2-ones **5.1−5.3** wasconducted via refluxingof compounds **4.2, 4.4, 4.6** in morpholine during 12 hours. In what follows**,** we conducted research aimed to the synthesis of derivatives of6-hydrazinyl-3-methyl-2*H*-[1,2,4]triazino[2,3-c]-quinazoline-2-one as promising antimicrobial and antifungal. Compound **6.1** was prepared by interaction of **4.1** with five fold excess of hydrazine hydrate in propanol-2. Experimental data showed that reaction of compound **6.1** with equimolar amount of aromatic aldehydes in presence of hydrochloric acid yielded corresponding derivatives **7.1−7.5.**

Purity of synthesized compounds proved by LC-MS (APCI) method, their structure by complex of physicochemical methods including ^{1}H and ^{13}C NMR, IR-, mass-(EI) − spectrometry.

^{1}H NMR-spectrums of compounds **4.1−4.6** were characterized by presence of AM-system which consists of doublet of doublets at 1.45−1.50 ppm and triplet at 3.26−3.31 ppm and corresponds to SCH_2CH_3 fragment. In^{1}H NMRspectraof compounds **5.1−5.3** two four-proton broad singlets, which were caused be presence of morpholine moiety were observed at 3.84−3.85 and 3.67−3.68 ppm. Two broad singlets caused by the presence of hydrazine fragment at 9.27 and 4.61 ppm were characteristic signals for compound **6.1**.

Spectral data which were obtained for compounds **7.1−7.5** also corresponded to the proposed structure. Thus, for the compounds **7.1, 7.3−7.5** signals of NH-protonswere observed as broad singlets at 11.35−11.57 ppm. Singlets of N=CH− fragment at 8.57−8.84 ppm as well as signals of aromatic moiety in position 6 were also present in ^{1}H NMR spectra of mentioned compounds.In 'aromatic" part of the spectrum of compounds **4.1−4.6, 5.1−5.3, 6.1, 7.1−7.5** signals of benzene fragment of triazinoquinazoline system as ABCD-system and substituents in position 3 were also observed [10].^{13}C



Scheme 1. Synthetic routes for 6-S − and 6-N − substituted 3-R-2*H*-[1,2,4]triazino[2,3-*c*]quinazoline-2-ones

NMR spectrum of compound **4.2** additionally proved the structure which was proposed.

Studying of mass-spectra of compounds **4.2** and **5.2** allowed to evaluate features of fragmentation of molecular ions.Thus, mass-spectrum (EI) of compound **4.2** characterized by absence of molecular ion, basic directions of fragmentation caused by cleavage of C2–C3 and N4–N5 bonds (m/z 232) followed by elimination of ethyl (m/z 199) moiety. We noted that most intensive signals in spectrum correspond to products of deep destruction of heterocyclic fragment. In mass-spectrum of compound **5.2** low intensive signal of molecular ion was observed. In contrast to previously reported [1,2,4]triazino[2,3-*c*]quinazoline-2-ones[11] in spectrum of compounds **5.2** signal of fragmentary ion caused by cleavage of C2–C3 and N4–N5 bonds with m/z 256 was extremely low intensive (9.5%), what, as we considered, may be explained by presence of labile morpholine moiety. Namely by fragmentation of morpholine fragment caused series of high intensive signals in particular with m/z 211 (cleavage of O1–C2 and N4–C5 bonds of morpholine cycle), m/z 240 (cleavage of O1–C2 and O1–C6 bonds of morpholine cycle) and m/z 225 (cleavage of O1–C2 and C5–C6 bonds).

Biological activity

Anticancer action

Compounds **4.1, 4.2, 4.6, 5.1** were selected by the National Cancer Institute (NCI) Developmental Therapeutic Program (www.dtp.nci.nih.gov) for the *in vitro* cell line screening to investigate their anticancer activity. Anticancer assays were performed according to the US NCI protocol, which was described elsewhere [12–14]. Results for each tested agent were reported as the percent growth of the treated cells comparing to the untreated control cells. The screening results are shown in Table 1.

Experimental data showed that compounds **4.1, 4.2, 5.1** did not reveal significant anticancer activity. Thus, range of growth for **4.1** was 86.41–121.98% (mean growth 101.86%), for **4.2** 67.44–110.07% (mean growth 91.44%), and for **5.1** 77.63–117.80% (mean growth 98.90%). In the same time compound **4.6** was able to inhibit growth of ovarian cancer OVCAR-4 cell lineon 61.64% (mean growth 38.36–118.41) Considering the structure activity relationships, we noted that introduction of thioethane fragment led to the expansion of activity spectrum.

Antimicrobial and antifungal action

Results of microbiological screening showed that compounds **4.1, 4.4, 6.1, 7.1, 7.1–7.4** were relatively inactive against *E. Coli* (MIC 50–100 μg/ml, MBC 50–200 μg/ml). In the same time mentioned compounds revealed moderate action against to *P. aeruginosa* (MIC 25–50 μg/ml, MBC 50–100 μмg/ml) which was comparable to activity of reference substance Trimethoprim (MIC 62.5 μg/ml), but significantly inferior to Nitrofural (MIC 6.25 μg/ml). The most encouraging results were obtained during the studying of antibacterial activity against *S. aureus*and antifungal activity against *C. albicans*. Thus, it was evaluated that compounds **4.1, 6.1, 7.1** and **7.5** exhibit significant inhibiting activity against *S. aureus*(MIC 6.25 μg/ml). Also we detected high antifungal activity for series of 6-(2-arylilidenhydrazinil)-3-methyl-2H-[1,2,4]triazino[2,3-c]quinazoline-2-ones. Thus, compounds **7.1–7.3** revealed antifungal action on the same level as Nitrofural (MIC 25 μg/ml) significantly exceeding the actionof Trimethoprim (MIC 62.5 μg/ml). We

Table 1

Percentage of *in vitro* tumor cell lines growth at 10 μM for synthesized compounds (4.1, 4.2, 4.6, 5.1)

Compd.	Mean growth, %	Range of growth, %	Cell line growth, %*
4.1	101.86	86.41–121.98	86.41 (HCT-116/ColC),
4.2	91.44	67.44–110.07	79.28 (CCRF-CEM/L), 82.69 (SR/L), 81.77 (A549/ATCC/nscLC), 77.79 (HOP-92/nscLC), 85.49 (NCI-H226/nscLC), 82.64 (HCT-116/ColC), 79.29 (HT29/ColC), 82.86 (SF-295/CNSC), 80.25 (SNB-75/CNSC), 78.51 (SK-MEL-5/M), 81.98 (UACC-62/M), 83.68 (OVCAR-4/OV), 80.36 (786-0/RC), 81.95 (ACHN/RC), 79.49 (RXF 393/RC), 78.56 (UO-31/RC), 83.10 (MCF7/BC), 82.25 (BT-549/BC), **67.44 (T-47D/BC)**
4.6	94.53	38.36–118.41	75.10 (SR/L), 82.38 (A549/ATCC/nscLC), 84.31 (HOP-92/nscLC), 76.49 (NCI-H460/nscLC), 76.58 (HCT-116/ColC), 79.31 (HT29/ColC), 86.24 (KM12/ColC), **38.36 (OVCAR-4/OV)**, 78.81 (ACHN/RC), 86.36 (T-47D/BC)
5.1	98.90	77.63–117.80	86.81 (SK-MEL-5/M), 84.12 (OVCAR-8/OV), 86.63 (SK-OV-3/OV), 86.51 (CAKI-1/RC), 82.74 (UO-31/RC), 81.57 (MCF7/BC), 82.55 (BT-549/BC), 77.63 (T-47D/BC)

Note: *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.

Table 2

Antibacterial and antifungal activity of synthesized compounds (μg/ml)

Comp. №	Studied strains							
	E. coli		S. aureus		P. aeruginosa		C. albicans	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MFC
4.1	100	200	**6.25**	100	50	100	50	50
4.4	100	100	25	200	50	100	50	100
6.1	50	100	**6.25**	50	25	50	50	100
7.1	100	200	**6.25**	50	50	50	**25**	**25**
7.2	50	100	12.5	200	50	100	**25**	50
7.3	100	100	25	100	50	100	**25**	50
7.4	100	100	50	100	50	100	50	50
7.5	100	100	6.25	200	50	100	50	100
Trimethoprim	50	50	31,2	62,5	62,5	125	62,5	125
Nitrofural	1,5	–	6,25	–	6,25	–	25,0	–

noted that introduction of fluorine atom (compounds **7.4, 7.5**) in molecule of mentioned class of compounds led to the decreasing of antifungal action.

Conclusions

Results of our work showed that potassium 3-R-2-oxo-2*H*-[1,2,4]trizino[2,3-*c*]quinazoline-6-thiolatesin contrast to 3-R-2*H*-[1,2,4]triazino[2,3-*c*]quinazololine-2,6(7*H*)-dionesmay be used as initial compounds for synthesis of 6-S- and 6-N-substituted 3-R-2*H*-[1,2,4]triazino[2,3-*c*]quinazolin-2-ones. Evaluation of biological activity of mentioned above compounds allowed to claim that they are perspective objects for investigations, aimed to creation of novel anticancer and antifungal drugs.

Experimental part

Melting points were determined in open capillary tubes and were uncorrected. The elemental analyses (C, H, N, S) were performed using the ELEMENTAR vario EL Cube analyzer (USA). Analyses were indicated by the symbols of the elements or functions within ±0.3% of the theoretical values. ^{1}H NMR spectra (400 MHz) and ^{13}C NMR spectra (100 MHz): were recorded on a Varian-Mercury 400 (Varian Inc, Palo Alto, CA, USA) spectrometers 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, USA).

Compounds **1.1−1.6, 2.1−2.6** and **3.1−3.6** were obtained according to the described synthetic protocols [6,7,15].

General synthetic protocolfor 3-R-6-(ethylthio)-2*H*-[1,2,4]triazino[2,3-*c*]quinazolin-2-ones (**4.1-4.6**) To suspension of 5 мМ of corresponding 3-R-2-oxo-2*H*-[1,2,4]triazino[2,3-*c*]quinazolin-6-thiolates (**3.1-3.6**) in 20 ml of propanol-2 5 мМ of ethyl iodide was added. Mixture was refluxed during 2 hours and cooled. Formed solid was filtered off, washed by propanol-2 and dried.

6-(ethylthio)-3-methyl-2*H*-[1,2,4]triazino[2,3-*c*]quinazolin-2-one (**4.1**) Yield 89,3%; M.p. 220−222^{0}C, ^{1}H NMR (400 MHz, dmso-d6+ccl4) δ 8.52 (d, *J*=7.8 Hz, 1H, H-11), 7.89 (t, *J*=7.7 Hz, 1H, H-9), 7.70 (d, *J*=7.9 Hz, 1H, H-8), 7.61 (t, *J*==7.8 Hz, 1H, H-10), 3.26 (dd, J=14.4, 7.1 Hz, 2H, S$\underline{CH_2}$CH$_3$, 2.42 (s, 3H, CH$_3$), 1.46 (t, *J*=7.2 Hz, 3H, SCH$_2\underline{CH_3}$; LC-MS m/z=273.0 [M+l]; Anal. calcd. for $C_{13}H_{12}N_4OS$: C, 57.34; H, 4.44; N, 20.57; S, 11.77 Found: C, 57.37; H, 4.49; N, 20.61; S, 11.82.

6-(ethylthio)-3-phenyl-2*H*-[1,2,4]triazino[2,3-*c*]quinazolin-2-one (**4.2**) Yield 99.3%; M.p. 199−201^{0}C, ^{1}H NMR (400 MHz, dmso-d6+ccl4) δ 8.53 (d, *J*=7.7 Hz, 1H, H-11), 8.33 (d, *J*=7.0Hz, 2H, 3 Ph H-2, 6), 7.94 (t, *J*=7.6 Hz, 1H, H-9), 7.75 (d, *J*=7.7 Hz, 1H, H-8), 7.65 (t, *J*=7.6 Hz, 1H, H-10), 7.62−7.47 (m, 3H, 3 Ph H-3,4,5), 3.31 (dd, *J*=13.9, 6.9 Hz, 2H, $\underline{CH_2}$CH$_3$), 1.49 (t, J=7.0 Hz, 3H, CH$_2\underline{CH_3}$); ^{13}C NMR (100 MHz, DMSO) δ 159.59, 155.09, 150.80, 149.45, 144.31, 135.71, 131.94, 131.76, 129.81, 128.56, 126.79, 126.06, 117.95, 25.62, 14.11; EI-MS (m/z (I rel. %)) 232 (12.5), 231 (6.5), 217 (10.6), 204 (7.7), 200 (7.6), 199 (51.2), 172 (24.4), 171 (8.2), 162 (24.8), 161 (9.8), 156 (9.7), 146 (9.1), 145 (9.3), 144 (33.9), 143 (6.2), 134 (31.1), 133 (5.4), 130 (16.9), 129 (74.4), 128 (7.2), 122 (6.3), 117 (7.1), 116 (6.9), 107 (5.7), 104 (11.4), 103 (100), 102 (86.7), 101 (6.4), 91 (6.1), 90 (61.3), 89 (24.9), 88 (8.9), 77 (21.4), 76 (77.2), 75 (38.3), 69 (6.7%), 64 (13.9), 63 (34.9), 62 (12.1), 60 (10.5), 52 (6.8), 51 (12.8); LC-MS m/z=345.0 [M+l]; Anal. calcd. for $C_{18}H_{14}N_4OS$: C, 64.65; H, 4.22; N, 16.75; S, 9.59; Found: C, 64.65; H, 4.22; N, 16.75; S, 9.59.

6-(ethylthio)-3-(4-isopropylphenyl)-2*H*-[1,2,4]triazino[2,3-*c*]quinazolin-2-one (**4.3**) Yield 80,7%; M.p. 182−184^{0}C; ^{1}H NMR (400 MHz,

dmso_d_6+ccl_4) δ 8.55 (d, *J*=7.9 Hz, 1H, H-11), 8.28 (d, *J*=7.8 Hz, 2H, 3 Ph H-2,6), 7.90 (t, *J*=7.8 Hz, 1H, H-9), 7.73 (d, *J*=7.9 Hz, 1H, H-8), 7.63 (t, *J*=7.8 Hz, 1H, H-10), 7.36 (d, *J*=7.8 Hz, 2H, 3 Ph H-3,5), 3.30 (dd, *J*=14.2, 7.0 Hz, 2H,$\underline{CH_2}CH_3$), 3.12−2.81 (m, 1H, $CH(CH_3)_2$), 1.49 (t, *J*=7.1 Hz, 3H, $CH_2\underline{CH_3}$), 1.32 (d, *J=7.0* Hz, 6H, $CH(CH_3)_2$);LC-MS m/z − 377.0 [M+l]; Anal. calcd. for $C_{21}H_{20}N_4OS$: C, 67.00; H, 5.35; N, 14.88; S, 8.52; Found: C, 67.04; H, 5.39; N, 14.92; S, 8.56.

3-(3,4-dimethylphenyl)-6-(ethylthio)-2*H*-[1,2,4]triazino[2,3-*c*]quinazolin-2-one (**4.4**) Yield 85,9%; M.p. 200−201^{0}C, ^{1}H NMR (400 MHz, dmso_d_6+ccl_4) δ 8.55 (d, J=7.9 Hz, 1H, H-11), 8.12 (c, 1H, 3 Ph H-2), 8.08 (d, J=7.8 Hz, 1H, 3 Ph H-6), 7.91 (t, *J*=7.8 Hz, 1H, H-9), 7.73 (d, *J*= =7.9 Hz, 1H, H-8), 7.63 (t, *J*=7.8 Hz, 1H, H-10), 7.25 (d, *J*=7.7 Hz, 1H, 3 Ph H-5), 3.30 (dd, У=14.4, 7.1 Hz, 2H, SCH_2CH_3), 2.39 (s, 3H, 4-CH_3), 2.36 (s, 3H, 3-CH_3), 1.50 (t, J=7.2 Hz, 3H, $SCH_2\underline{CH_3}$); LC-MS m/z=363.0 [M+l]; Anal. calcd. for $C_{20}H_{18}N_4OS$: C, 66.28; H, 5.01; N, 15.46; S, 8.85, Found: C, 66.31; H, 5.04; N, 15.51; S, 8.91.

6-(ethylthio)-3-(4-fluorophenyl)-2*H*-[1,2,4]-triazino[2,3-*c*]quinazolin-2-one*(**4.5**)* Yield 78.4%; M.p. 219−221^{0}C; ^{1}H NMR (400 MHz, dmso-d6+ccl4) δ 8.54 (d, *J*=7.7 Hz, 1H, H-11), 8.5 (dd, 2H, 3 Ph H-2,6), 7.91 (t, *J*=7.7 Hz, 1H, H-9), 7.73 (d, *J*=7.8 Hz, 1H, H-8), 7.63 (t, *J*=7.8 Hz, 1H, H-10), 7.26 (t, *J*=7.8 Hz, 2H, 3 Ph H-3,5), 3.30 (dd, *J*=14.1, 6.9 Hz, 2H, $\underline{CH_2}CH_3$), 1.50 (t, J=7.0 Hz, 3H, $CH_2\underline{CH_3}$); LC-MS m/z=353.0 [M+l]; Anal. calcd. for $C_{18}H_{13}FN_4OS$: C, 61.35;. H, 3.72; N, 15.90; S, 9.10; Found: C, 61.39; H, 3.77; N, 15.93; S, 9.14.

3-(4-ethoxyphenyl)-6-(ethylthio)-2*H*-[1,2,4]-triazino[2,3-*c*]quinazolin-2-one (**4.6**) Yield 82.4%; M.p. 218−220^{0}C, ^{1}NMR (400 MHz, dmso-d_6+ccl_4) δ 8.52 (d, *J*=7.8 Hz, 1H, H-11), 8.39 (d, *J*=8.0Hz, 2H, 3 Ph H-2,6), 7.91 (t, *J*=7.8 Hz, 1H, H-9), 7.73 (d, *J*=7.9 Hz, 1H, H-8), 7.63 (t, *J*=7.8 Hz, 1H, H-10), 7.01 (d, *J*=8.0Hz, 2H, 3 Ph H-3,5), 4.15 (dd, *J*=13.2, 6.9Hz, 2H, $\underline{CH_2}CH_3$), 3.30 (dd, *J*=14.2, 6.9 Hz, 2H, $S\underline{CH_2}CH_3$, 1.50 (t, *J*=7.0Hz, 3H, $OCH_2\underline{CH_3}$), 1.45 (t, *J*=6.6Hz, 3H, SCH_2CH_3); LC-MS m/z=379.0 [M+l]; Anal. calcd. for $C_{20}H_{18}N_4O_2S$: C, 63.47; H, 4.79; N, 14.80; S, 8.47, Found: C, 63.49; H, 4.81; N, 14.87; S, 8.52.

General synthetic prototocol for 3-R-6-morpholino-2*H*-[1,2,4]triazino[2,3-*c*]quinazolin-2-ones (**5.1−5.3**). Mixture 3 mM of corresponding 6-(ethylthio)-3-R-2*H*-[1,2,4]triazino[2,3-*c*]quinazoline-2-ones (**4.2, 4.4, 4.6**) and 10 ml of morpholine was refluxed during 12 hours and cooled. The formed precipitate was filtered off and cooled. For analysis obtained compounds were crystallized from water-dioxane mixture (1:1).

6-morpholino-3-phenyl-2*H*-[1,2,4]triazino-[2,3-*c*]quinazolin-2-one (5.1) Yield 79.9%; M.p. 223−226^{0}C, ^{1}H NMR (400 MHz, dmso-d_6+ccl_4) δ 8.45 (d, *J*=7.9 Hz, 1H, H-11), 8.26 (d, *J*=7.1 Hz, 2H, 3 Ph H-2,6), 7.82 (t, *J*=7.9 Hz, 1H, H-9), 7.65−7.36 (m, 5H, H-8,10, 3 Ph H-3,4,5), 3.84 (bs, 4H, morpholineH-2,2'6,6'), 3.67 (bs, 4H, morpholine H-3,3'5,5') LC-MS m/z=360.0 [M+l]; Anal. calcd. for $C_{20}H_{17}N_5O_2$: C, 66.84; H, 4.77; N, 19.49; Found: C, 66.89; H, 4.82; N, 19.53.

3-(3,4-dimethylphenyl)-6-morpholino-2*H*-[1,2,4]triazino[2,3-*c*]quinazolin-2-one (5.2) Yield 58.4%; M.p. 257−258^{0}C ^{1}H NMR (400 MHz, dmso-d_6+ccl_4) δ 8.45 (d, *J*=7.8 Hz, 1H, H-11), 8.05 (s, 1H, 3 Ph H-2), 8.03 (d, *J*=8.0 Hz, 1H, 3 Ph H-6), 7.81 (t, *J*=7.7 Hz, 1H, H-9), 7.57 (d, *J*=8.0 Hz, 1H, H-8), 7.47 (t, *J*=7.9 Hz, 1H, H-10), 7.26 (d, *J*=7.9 Hz, 1H, 3 Ph H-5), 3.85 (bs, 4H, morpholine H-2,2'6,6'), 3.68 (bs, 4H, morpholine H-3,3'5,5'), 2.37 (s, 3H, 4-CH_3), 2.36 (s, 3H, 3-SH_3); EI-MS (m/z (I rel.%)) 388 (3.2), 387 (23.2), , 256 (9.5), 255 (16.7), 241 (18.2), 225 (31.9), 212 (11.6), 211 (78.5), 186 (5.4), 185 (10.4), 184 (5.3), 172 (6.7), 155 (6.8), 149 (11.3), 143 (40.5), 142 (5.1), 131 (32.8), 130 (36.7), 129 (52.4), 125 (8.4), 124 (6.2), 123 (8.2), 118 (6.4), 117 (19.9), 116 (66.2), 99 (7.4), 98 (7.1), 97 (18.8), 96 (9.2), 95 (14.2), 93 (8), 91 (18.1), 90 (27.9), 89 (24.8), 88 (10.0), 86 (37.3), 70 (12.2), 69 (32.6), 68 (10.2), 67 (14.6), 65 (5.8), 57 (100), 55 (45.4), 51 (5.7), 49 (39.7), 41 (7.2); LC-MS m/z=388.0 [M+l]; Anal, calcd. for $C_{22}H_{21}N_5O_2$: C, 68.20; H, 5.46; N, 18.08; Found: C, 68.23; H, 5.49; N, 18.11.

3-(4-ethoxyphenyl)-6-morpholino-2*H*-[1,2,4]triazino[2,3-*c*]quinazolin-2-one (5.3) Yield 93,4%; M.p. 237−239^{0}C, ^{1}H NMR (400 MHz, dmso_d_6+ccl_4) δ 8.44 (d, *J*=8.0 Hz, 1H, H-11), 8.31 (d, *J*=8.0 Hz, 2H, 3 Ph H-2,6), 7.80 (t, *J*=7.9 Hz, 1H, H-9), 7.56 (d, *J*=8.0 Hz, 1H, H-8), 7.46 (t, *J*=7.9 Hz, 1H, H-10), 7.01 (d, *J*=7.9 Hz, 2H, 3 Ph H-3,5), 4.14 (dd, *J*=13.2, 6.7 Hz, 2H, $O\underline{CH_2}CH_3$ 3.84 (bs, 4H, morpholine H-2,2'6,6'), 3.66 (bs, 4H, morpholineH-3,3'5,5'), 1.45 (t, *J*=6.6 Hz, 3H, $OCH_2\underline{CH_3}$); LC-MS m/z=404.0 [M+l]; Anal. calcd. for $C_{22}H_{21}N_5O_3$: C, 65.50; H, 5.25; N, 17.36; Found: C, 65.55; H, 5.28; N, 17.39.

Synthesis of 6-hydrazinyl-3-methyl-2*H*-[1,2,4]triazino[2,3-*c*]quinazolin-2-one (**6.1**). To suspension of 30 mM (8.16 g) of 6-(ethylthio)-3-methyl-2*H*-[1,2,4]triazino[2,3-*c*]quinazoline-2-one and 50 ml propanol-2 150 mM (7.59 g) of hydrazine hydrate was added. Mixture was refluxed during 2 hours and cooled. Formed solid was filtered off, washed by water and dried.

6-hydrazinyl-3-methyl-2*H*-[1,2,4]triazino[2,3-*c*]quinazolin-2-one (6.1) Yield 52.7%; M.p. 210−212^{0}C, ^{1}H NMR (400 MHz, dmso-d_6+ccl_4) δ 9.27 (s, 1H, −$\underline{NH}NH_2$), 8.26 (d, 1H, *J*=7.9, H-11), 7.74

(t, 1H, *J*=7.7, H-9), 7.44 (d, 1H, *J*=7.7, H-8), 7.31 (t, 1H, *J*=7.7, H-10), 4.61 (s, 2H, −NH<u>NH</u>$_2$), 2.34 (s, 3H, 3-SH_3), LC-MS m/z=243.0 [M+l]; Anal. calcd. for $C_{11}H_{10}N_6O$: C, 54.54; H, 4.16; N, 34.69; O, 6.60 Found: C, 54.57; H, 4.20; N, 34.72; O, 6.63.

General synthetic protocol for 6-(2-arylylidenehydrazinyl)-3-methyl-2*H*-[1,2,4]triazino[2,3-c]quinazolin-2-ones (7.1−7.5) To suspension of 3 mM (0.73 g) 6-(ethylthio)-3-methyl-2*H*-[1,2,4]-triazino[2,3-*c*]quinazolin-2-one (6.1) in 20 ml of propanol-2 3 mM of corresponding aromatic aldehyde and 1 drop of concentrated hydrochloric acid were added. Mixture was refluxed during 2 hours and cooled. Formed solid was filtered off, washed by water and cooled.

6-(2-benzylidenehydrazinyl)-3-methyl-2*H*-[1,2,4]triazino[2,3-*c*]quinazolin-2-one (7.1) Yield 99,3%; M.p. 278−280^{0}C; ^{1}H NMR (400 MHz, dmso-d_6+ccl_4) δ 11.35 (b.s, 1H, NH), 8.63 (s, 1H, N=<u>CH</u>−), 8.43 (d, *J*=7.7 Hz, 1H, H-11), 7.82 (d, *J=7.2* Hz, 2H, 6 Ph H-2,6), 7.78 (t, *J*=7.8 Hz, 1H, H-9), 7.63 (d, *J*=7.8 Hz, 1H, H-8), 7.52−7.34 (m, 4H, H-10, 6 Ph H-3, 4, 5), 2.53 (s, 3H, CH_3); LC-MS m/z=331.0 [M+l]; Anal. calcd. for $C_{18}H_{14}N_6O$: C, 65.44; H, 4.27; N, 25.44, Found: C, 65.48; H, 4.31; N, 25.47.

6-(2-(4-methoxybenzylidene)hydrazinyl)-3-methyl-2*H*-[1,2,4]triazino[2,3-*c*]quinazolin-2-one (7.2) Yield 98,7%; M.p. 239−242^{0}C; ^{1}H NMR (400 MHz, dmso-d_6+ccl4) δ 8.57 (s, 1H, N=<u>CH</u>−), 8.38 (d, *J*=7.9 Hz, 1H, H-11), 7.79 (d, *J*=8.3 Hz, 2H, 6 Ph H-2,6), 7.74 (t, *J*=7.9 Hz, 1H, H-9), 7.64 (d, *J*=7.9 Hz, 1H, H-8), 7.34 (t, *J*=7.9 Hz, 1H, H-10), 6.96 (d, *J*=8.3 Hz, 2H, 6 Ph H-3,5), 3.85 (s, 3H, OCH_3), 2.46 (s, 1H, CH_3); LC-MS m/z=361.0 [M+l]; Anal. calcd. for$S_{19}H_{16}N_4O_2$: C, 63.33; H, 4.48; N, 23.32, Found: C, 63.38; H, 4.53; N, 23.38.

6-(2-(4-chlorobenzylidene)hydrazinyl)-3-methyl-2*H*-[1,2,4]triazino[2,3-*c*]quinazolin-2-one (7.3) Yield 93,4%; M.p. 274−276^{0}C; ^{1}H NMR (400 MHz, dmso-d_6+ccl_4) δ 11.40 (s, 1H, NH), 8.60 (s, 1H, N=<u>CH</u>−), 8.41 (d, *J*=8.0 Hz, 1H, H-11), 7.84 (d, *J*=7.3 Hz, 2H, 6 Ph H-2,6), 7.77 (t, *J*= =8.0 Hz, 1H, H-9), 7.62 (d, *J*=8.0 Hz, 1H, H-8), 7.44 (d, *J*=7.3 Hz, 2H, 6 Ph H-3,5), 7.38 (t, *J*= =8.0 Hz, 1H, H-10), 2.46 (s, 3H, CH_3); LC-MS m/z=365.0 [M+l]; Anal. calcd. for $C_{18}H_{13}ClN_6O$: C, 59.27; H, 3.59; N, 23.04; Found: C, 59.31; H, 3.63; N, 23.08.

3-methyl-6-(2-(3-(trifluoromethyl)benzylidene)hydrazinyl)-2*H*-[1,2,4]triazino[2,3-*c*]-quinazolin-2-one (7.4) Yield 63,6%; M.p. 236−238^{0}C, ^{1}H NMR (400 MHz, dmso-d_6+ccl_4) δ 11.51 (b.s, 1H, NH), 8.69 (s, 1H, N=<u>CH</u>−, 8.39 (d, *J*= =8.0 Hz,1H, H-11), 8.10 (s, 1H, 6 Ph H-2), 7.75(t, *J*=8.0 Hz,1H, H-9), 7.66 (m, 4H, H-8, 3 Ph H-4,5,6), 7.36 (t, *J*=8.0 Hz,1H, H-10), 2.47 (s, 3H, CH_3); LC-MS m/z=399.0 [M+l]; Anal. calcd. for $C_{19}H_{13}F_3N_6O$: C, 57.29; H, 3.29; N, 21.10; Found: C, 57.32; H, 3.34; N, 21.14.

6-(2-(2,4-difluorobenzylidene)hydrazinyl)-3-methyl-2*H*-[1,2,4]triazino[2,3-*c*]quinazolin-2-one (7.5) Yield 96,5%; M.p. 273−275^{0}C, ^{1}NMR (400 MHz, dmso-d_6+ccl_4) δ 11.57 (bs, 1H, NH), 8.84 (s, 1H, N=CH−), 8.41 (d, *J*=7.7 Hz, 1H, H-11), 8.23 (d, *J*=7.7 Hz,1H, 6 Ph H-6), 7.77 (t, *J*=7.7 Hz, 1H, H-9), 7.62 (d, *J*=7.7 Hz, 1H, H-8), 7.38 (t, *J*=7.7 Hz,1H, H-10), 7.21−6.92 (m, 2H, 6 Ph H-3,5), 2.47 (s, 3H,CH_3); LC-MS m/z=367.0 [M+l]; Anal. calcd. for $C_{18}H_{12}F_2N_6O$: C, 59.02; H, 3.30; F, 10.37; N, 22.94, Found: C, 59.04; H, 3.35; F, 10.40; N, 23.00.

Cytotoxic activity against malignant human tumor cells

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 [8−10]. 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.

Antimicrobial and antifungal test

Assay was conducted on Mueller-Hinton medium by two-fold serial dilution of compound in 1 ml, after that 0.1 ml of microbial seeding (106 cells/ml) was add. Minimal inhibit concentration of compound was determined by absence of visual growth in test tube with minimal concentration of substance, minimal bactericide/ fungicide concentration was determined by absence of growth on agar after inoculation of microorganism from transparent test-tubes. Dimethylsulfoxide was used as a solvent, initial solution concentration was 1 mg/ml For preliminary screening the mentioned ahead standard test cultures were used: *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *Candida albicans* ATCC 885-653. All test strains were received from bacteriological laboratory Zaporizhzhya Regional Laboratory Center of State Sanitary and Epidemiological Service of Ukraine. Nitrofualan Trimetoprim were used as reference compound with proved antibacterial/antifungal activity. Additional quality control of culture medium and solvents was conducted by commonly used methods.

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SYNTHESIS, PHYSICOCHEMICAL AND BIOLOGICAL PROPERTIES OF 6-S- AND 6-N-SUBSTITUTED 3-R-2H-[1,2,4]TRIAZINO[2,3-C]QUINAZOLIN-2-ONES

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The present article describes the synthesis and biological activity of series of 6-S- and 6-N-substituted 3-R-2H-[1,2,4]-triazino[2,3-c]quinazolin-2-ones. It is shown that 3-R-2H-[1,2,4]-triazino[2,3-c]quinazoline-2,6(7H)-diones cannot be used as starting materials for the synthesis of the target compounds, whereas the modification of potassium 3-R-2-oxo-2H-[1,2,4]triazino[2,3-c]-quinazolin-6-thiolates provides such an opportunity. The individuality of the synthesized compounds is proved by liquid chromatography–mass spectrometry and their structures by ^{1}H NMR, ^{13}C NMR and

mass spectroscopy. The characteristic signals in ^{1}H NMR, ^{13}C NMR–spectra are described; the features of molecule fragmentation under electron impact are elucidated. The anticancer activity according to National Cancer Institute (USA) protocol is evaluated for some of the obtained compounds. The results of anticancer activity allow identifying a compound, which is capable of inhibiting the growth of ovarian cancer cells OVCAR-4 at 61.64%. The results of microbiological screening demonstrate that the synthesized compounds were inactive relative to E. Coli*, show a moderate activity against* P. aeruginosa *and show very encouraging results in the study of antimicrobial activity against* S. aureus *and antifungal activity against* C. albicans. *The prospect of further research aimed at creating novel anticancer, antibacterial and antifungal agents based on the compounds described is herein discussed.*

Keywords: triazines, quinazolines, anticancer action, antimicrobial action, antifungal action.

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