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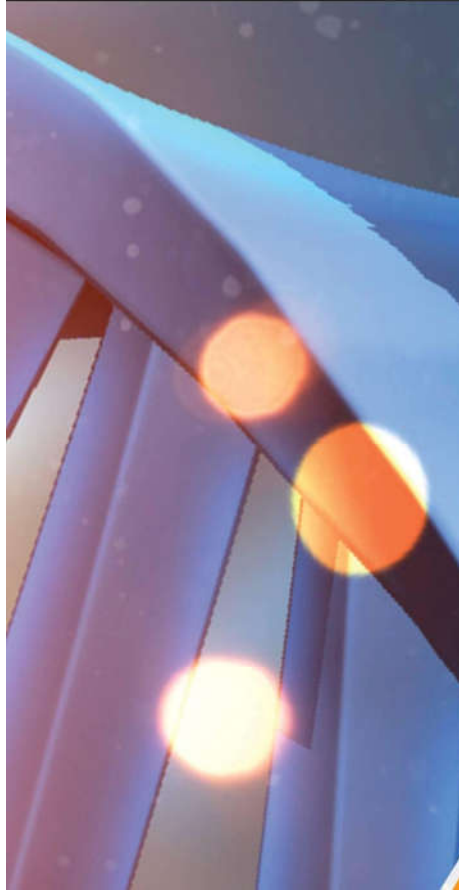

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VETERINARY SCIENCE AND PHARMACY

[(3-R-2-OXO-2*H*-[1,2,4]TRIAZINO[2,3-*C*]QUINAZOLIN-6-YL)THIO]ACETAMIDES WITH THE FRAGMENTS OF CARCASE AMINES – EFFECTIVE SARS CORONAVIRUS INHIBITORS

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Abstract. *It was shown, that N-cycloalkyl-(cycloalkaryl)-2-[(3-R-2-oxo-2H-[1,2,4]triazino-[2,3-c]quinazolin-6-yl)thio]acetamides revealed antiviral activity against SARS Coronavirus in visual test for V (inhibition of viral cytopathic effect) and NR (increase in NR dye uptake). Results for each tested compound were reported as virus-inhibitory concentration, 50 % endpoint (EC_{50} μg/ml), or 90 % effective concentration (EC_{90} μg/ml) and cell-inhibitory concentration, 50 % endpoint (CC_{50} μg/ml) were determined. The high inhibiting activity of compounds (EC_{50} 1.0-33.0 μg/ml) was estimated. It was noted, that antiviral effect of compound 1.4 was comparable with test drug. It was shown, that planar [1,2,4]triazino[2,3-c]quinazoline system caused antiviral activity, additionally established by carcase amines fragment and depend on the nature of substituent in 3rd position.*

Keywords: *[(3-R-2-oxo-2H-[1,2,4]triazino[2,3-c]quinazolin-6-yl)thio]acetamides, carcase amines, SARS Coronavirus, antiviral activity, virus-inhibitory concentration, cell-inhibitory concentration*

Introduction. To date, there are more than 2000 types of viruses, but it is assumed that there are millions of them. Many viruses are infectious agents. That may strike human cells to cause different diseases, such as encephalitis, hepatitis, influenza, herpes, poliomyelitis, AIDS (HIV), etc.

Recent publication describe the strategies of antiviral drug creation based on chemical modification of aliphatic polycyclic systems (adamantane, norborane, bicyclo[2.2.0]hexane, bicyclo[2.2.1]heptane, pentacycloundecane, etc) by introduction of pharmacophore groups or their combinations with cyclic, aromatic or heterocyclic fragments [1-8]. Following to the mentioned strategy, we decided to combine carcass amine fragment with planar [1,2,4]triazino[2,3-*c*]quinazoline system, what, as we consider, yielded the compounds with high antiviral activity. To evaluate antiviral activity, we selected compounds with known "pharmacophoric" fragments – *N*-cycloalkyl-(cycloalkaryl)-2-[(3-R-2-oxo-2*H*-[1,2,4]triazino[2,3-*c*]quinazolin-6-yl)thio]acetamides.

Experimental part. The [(3-R-2-oxo-2*H*-[1,2,4]triazino[2,3-*c*]quinazolin-6-yl)thio]acetamides derivatives with the fragments of carcase amines (1.1-1.10) were previously discussed for structure and high antiviral activity against Flu A H_1N_1, Flu A H_3N_2, Flu A H_5N_1, Flu B strains [9].

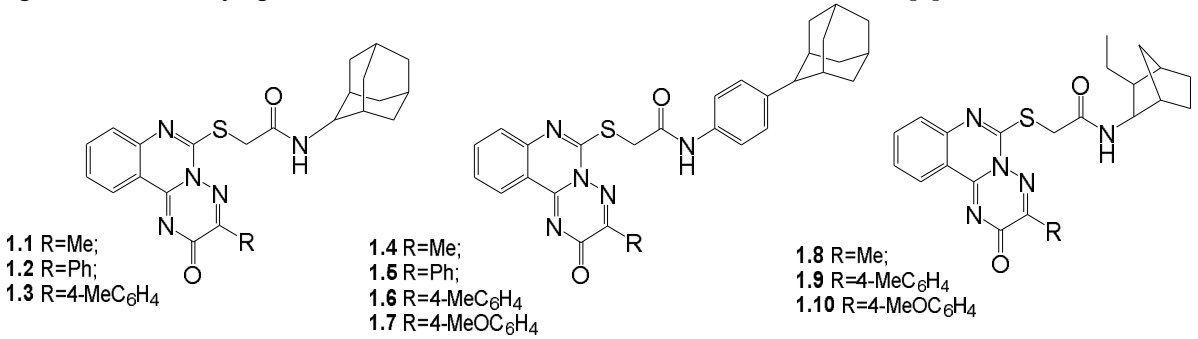

Fig. 1. General structure of [(3-R-2-oxo-2H-[1,2,4]triazino[2,3-c]quinazolin-6-yl)thio]acetamides with the fragments of carcase amines (1.1-1.10)

Methods for assay of antiviral activity. Primary antiviral assay was performed at a SARS Coronavirus (Urbani/Vero 76) with a protocol of the NIAID's Antimicrobial Acquisition and Coordinating [10, 11]. Results for each tested compound were reported as virus-inhibitory concentration, 50 % endpoint (EC_{50} μg/ml), or 90 % effective concentration (EC_{90} μg/ml) and cell-inhibitory concentration, 50 % endpoint (CC_{50} μg/ml) were determined. A general selectivity index (SI_{50}) was calculated as a ration of (EC_{50})/(CC_{50}). An SI_{50} of 3 or greater indicates that confirmatory testing is needed.

Inhibition of Viral Cytopathic Effect (CPE). This test, run in 96 well flat-bottomed microplates, was used for the initial antiviral evaluation of compounds. In this CPE inhibition test,

four log_{10} dilutions of each test compound (e.g. 1000, 100, 10, 1 μg/ml) were added to 3 cups containing the cell monolayer; within 5 min. On the next step, the virus was added and the plate was sealed and incubated at 37°C. CPE read microscopically when untreated infected controls develop a 3 to 4+ CPE (approximately 72 to 120 hr). A known positive control drug was evaluated in parallel with test drugs in each test. This drug was «$M_{12}8_{533}$» for SARS Coronavirus virus [11]. The data are expressed as 50 % effective concentrations (EC_{50}).

Increase in Neutral Red (NR) Dye Uptake. This test was run to validate the CPE inhibition seen in the initial test, and utilized the same 96-well micro plates after the CPE has been read. When neutral red was added to the medium cells that were not damaged by virus take up a greater amount of dye, which is desplayed on a computerized microplate autoreader. An EC_{50} was determined from this dye uptake.

Decrease in Virus Yield Assay (VYR-test). Compounds considered active by CPE inhibition and by NR dye uptake were re-tested on reduction of virus yield by assaying frozen and thawed eluates from each cup for virus titer by serial dilution onto monolayers of susceptible cells. Development of CPE in these cells is the indication of presence of infectious virus. The same as in the initial tests, a known active drug were run in parallel as a positive control. The 90 % effective concentration (EC_{90}), which is drug concentration that inhibits virus yield by 1 log_{10}, was determined from these data.

Results and discussion. Mean values of antiviral activity basic parameters, namely EC_{50}, μg/ml, CC_{50}, μg/ml and SI_{50} are presented in the Table.

Table 1. Antiviral activity of synthesized compounds against SARS Coronavirus (Urbani/Vero 76)

Compd.	Conc. Range, (μg/ml)	Assay	EC_{50}, μg/ml	EC_{90}	CC_{50}, μg/ml	SI_{50}	SI_{90}
1.1	0.1-100	PV	2.8		32	11	
	0.1-100	PNR	1.8		49	27	
	0.032-100	SV	>49		49	0	
	0.032-100	SNR	>13		13	0	
1.2	0.1-100	PV	6.8		32	4.7	
	0.1-100	PNR	3		>100	>33	
	0.032-100	SV	>49		49	0	
	0.032-100	SNR	96		>100	>1	
1.3	0.1-100	PV	8.1		32	4	
	0.1-100	PNR	23		49	2.1	
1.4	0.1-100	PV	2.8		24	8.6	
	0.1-100	PNR	1.6		37	23	
	0.032-100	SV	8.3		39	4.7	
	0.032-100	SNR	6.9		42	6.1	
	0.032-100	TNR	6.9		42	6.1	
	0.032-100	TVYR/NR		11.5	42		>3.7
1.5	0.1-100	PV	>28		28	0	
	0.1-100	PNR	65		>100	1.5	
1.6	0.1-100	PV	23		28	1.2	
	0.1-100	PNR	19		>100	>5.3	
1.7	0.1-100	PV	6.1		28	4.6	
	0.1-100	PNR	11		58	5.3	
1.8	0.1-100	PV	13		28	2.2	
	0.1-100	PNR	>54		54	0	
1.9	0.1-100	PV	>28		28	0	
	0.1-100	PNR	26		37	1.4	
1.10	0.1-100	PV	22		24	1.1	
	0.1-100	PNR	12		39	3.3	
$M_{12}8_{533}$	0.1-100	PV	<0.1		>100	>1000	
	0.1-100	PNR	<0.13		>100	>770	
	0.032-100	SV	1.7		>100	>59	
	0.032-100	SNR	0.38		>100	>260	
	0.032-100	TNR	1.7		>100	>59	
	0.032-100	TVYR/NR		12.6	>100		>7.9

N-cycloalkyl-(cycloalkaryl)-2-[(3-R-2-oxo-2*H*-[1,2,4]triazino[2,3-*c*]quinazolin-6-yl)thio]-acetamides (11.1-11.10) show significant antiviral activity. The general index of selectivity (SI_{50}) of compounds 11.1-11.10 is from 1.0 to 33. The highest antiviral activity in relation to the SARS

Coronavirus in the primary visual test (PV) showed compounds 1.1, 1.2 and 1.4. Additional tests (SV, SNR and TNR) and VYR-test (decrease in virus yield assay) showed that «$M_{12}8_{533}$» is more effective antiviral agent. So, EC_{90} for «$M_{12}8_{533}$» was 12.6 μg/ml (SI_{90}>7.9), while EC_{90} level for most active among studied compound 1.4 was 11.5 μg/ml (SI_{90}>3.7, Table).

Thus, the presented results allowed to suggest, that presence of planar 3-R-2*H*-[1,2,4]-triazino[2,3-*c*]quinazoline system determined the antiviral activity against SARS Coronavirus. Conducted *SAR*-analysis showed, that it was also influenced by the presence of lipophilic carcase amines fragment and the nature of substituents in the 3rd position. The most active were compounds in the next series: 4-MeC_6H_4<Ph<Me. To our mind, the promising approaches for further modification of planar *as*-triazino[2,3-*c*]quinazoline system, for purposeful synthesis of novel antiviral agents are: functionalization of substituent in position 3 and introduction of halogens, trifluoromethyl- and alkoxy- groups to positions 8, 9, 10, 11, aimed to increase the lipophilicity.

Conclusions. In this paper, the results of the study of the antiviral activity of the synthesized compounds are presented. Antiviral activity against SARS Coronavirus in visual test for V (inhibition of viral cytopathic effect) and NR (increase in NR dye uptake) is revealed. Ten compounds (1.1-1.10) were tested for antiviral activity against SARS Coronavirus (Urbani/Vero 76). Results for each tested compound were reported as virus-inhibitory concentration, 50 % endpoint (EC_{50} μg/ml), or 90 % effective concentration (EC_{90} μg/ml) and cell-inhibitory concentration, 50 % endpoint (CC_{50} μg/ml) were determined. The high inhibiting activity of compounds (EC_{50} 1.0-33.0 μg/ml) was estimated. The most potent antiviral compound appeared to be 1.4. Antiviral effect of compound 1.4 was found to be comparable to the test drug.

So, the planar [1,2,4]triazino[2,3-*c*]quinazoline system caused antiviral activity, additionally established by carcase amines fragment and depend on the nature of substituent in 3rd position.

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