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NEW THIOPYRANO[2,3-*d*][1,3]THIAZOLE DERIVATIVES AS POTENTIAL ANTIVIRAL AGENTS

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*A series of novel thiopyrano[2,3-d][1,3]thiazole derivatives were synthesized and evaluated for their antiviral activity in vitro within AACF NIAID programme. Compounds were studied towards Dengue Virus Type 2, Venezuelan Equine Encephalitis Virus, Respiratory Syncytial Virus, SARS Coronavirus, Rift Valley Fever Virus, Tacaribe Virus, Influenza Virus Types A and B. Among the tested thiopyrano[2,3-d][1,3]thiazoles, alkyl rel-(5R,5aR,11bS)-2,6-dioxo-3,5a,6,11b-tetrahydro-2H,5H-chromeno[4',3':4,5]thiopyrano[2,3-d][1,3]thiazole-5-carboxylates **8** and **11** were found to be the most active and showed significant antiviral activity against Influenza Virus Types A H3N2 and H5N1.*

K e y w o r d s: thiopyrano[2,3-d][1,3]thiazoles, screening, antiviral activity.

Viruses are known as common pathogens that cause a variety of diseases. Search for new efficient antiviral agents is an important worldwide problem among scientists and clinicians, because of rapid emergence of drug resistant strains. Even influenza virus may cause life-threatening events in high-risk patients. To overcome the drawbacks of the current antiviral drugs and to obtain more efficacious drugs, new antiviral drugs with a novel mode of action should be developed. Thiopyrano[2,3-*d*][1,3]thiazoles have become a promising area of research because of their diverse biological activities, such as anticancer, antitrypanosomal, antimycobacterial, anti-inflammatory and antioxidant [1-7] (Fig. 1).

In the last two decades, there has been an increase in the number of studies on 4-thiazolidinone derivatives as potential antiviral agents [8-13]. Thiopyrano[2,3-*d*][1,3]thiazoles could be of special interest as cyclic mimetics of biologically active (including antiviral) 4-thiazolidinones. The aim of present study was to estimate the antiviral activity of new thiopyrano[2,3-*d*][1,3]thiazole derivatives.

Materials and Methods

The synthesis of 2-oxo-3,7-dihydro-2*H*-thiopyrano[2,3-*d*]thiazole-6-carboxylic acids (**1-5**)[15], *rel*-(5*R*,5a*R*,11b*S*)-2,6-dioxo-3,5a,6,11b-tetrahydro-2*H*,5*H*-chromeno[4′,3′:4,5]thiopyrano[2,3-*d*][1,3]thiazole-5-carboxylic acids derivatives (**6-13**)[16], 6-carboxymethylene-2-oxo-3,5,6,7-tetrahydro-2*H*-thiopyrano[2,3-*d*][1,3]thiazole-6-carboxylic acids (**14-17**)[3], 2,6-dioxo-3,5a,6,11b-tetrahydro-2*H*,5*H*-cromeno[4′,3′:4,5]thiopyrano[2,3-*d*]thiazol-5a-yl] acetic acids (**18**,**19**)[3,14], 7′-(R-phenyl)-1-(R[1]-phenyl)-3′,7′-dihydro-2*H*,2′*H*,5*H*-spiro[pyrolidin-3,6′-thiopyrano[2,3-d]thiazol]-2,2′,5-triones (**20-24**)[3] and their characteristics were described in our previous reports. The structure of compounds involved in the study is presented in Fig. 2.

The antiviral activity screening of the compounds was performed at the National Institute of Allergic and Infectious Diseases of the National Institute of Health (Bethesda, MD, USA) within AACF (Antimicrobial Acquisition and Coordinating Facility (http://www.niaid-aacf.org) programme. Antiviral activity was determined against Dengue Virus Type 2 (New Guinea C strain, Vero 76 cell line), Venezuelan Equine Encephalitis Virus (TC-83 strain, Vero cell line), Respiratory Syncytial Virus (A2 strain, MA 104 cell line), SARS Coronavirus (Urbani strain, Vero 76 cell line), Influenza Virus Type A H_5N_1 (Vietnam/1203/2004H strain, MDCK cell line), Rift Valley Fever Virus (MP-12 strain, Vero 76 cell line), Tacaribe Virus (TRVL-11573 strain, Vero cell line), Influenza Virus Type A H_3N_2 (Perth/16/2009 strain, MDCK cell line), Influenza Virus Type A H_1N_1 (California/07/2009 strain, MDCK cell line), Influenza Virus Type B (Florida/4/2006 strain, MDCK cell line) using standart AACF screening assay protocols [17-19].

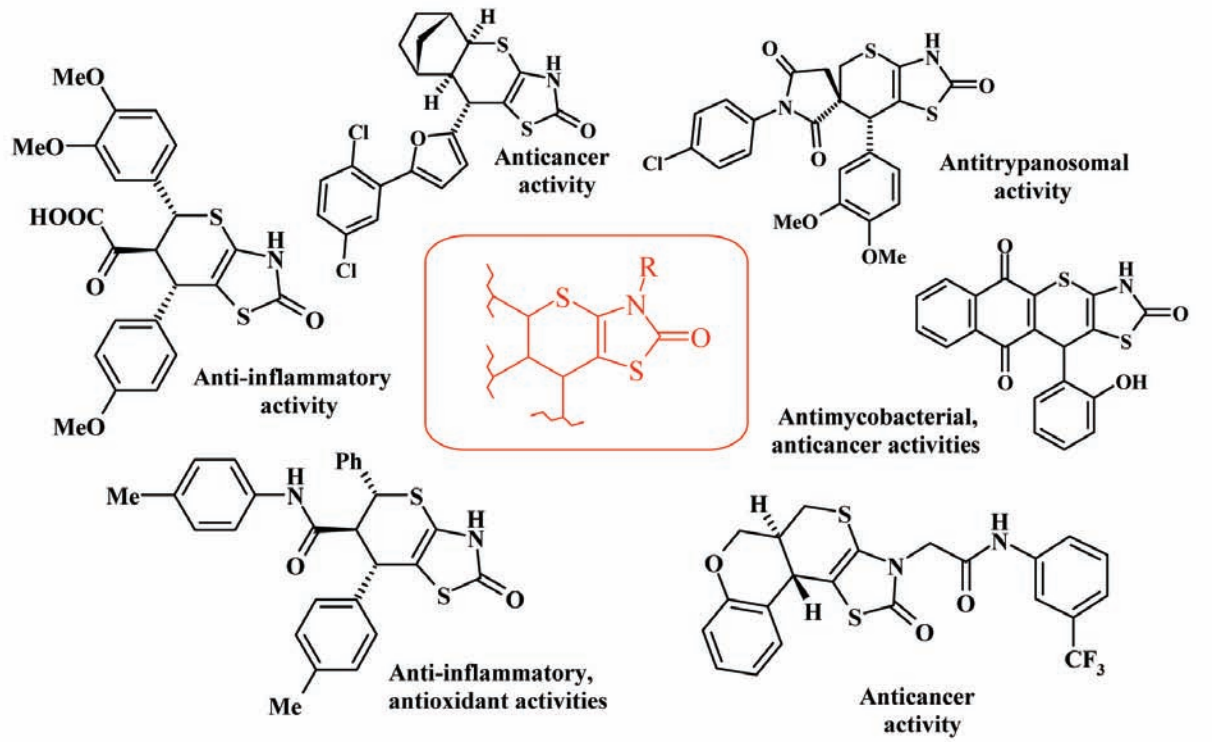

Fig. 1. Biologically active compounds among thiopyrano[2,3-d][1,3]thiazoles

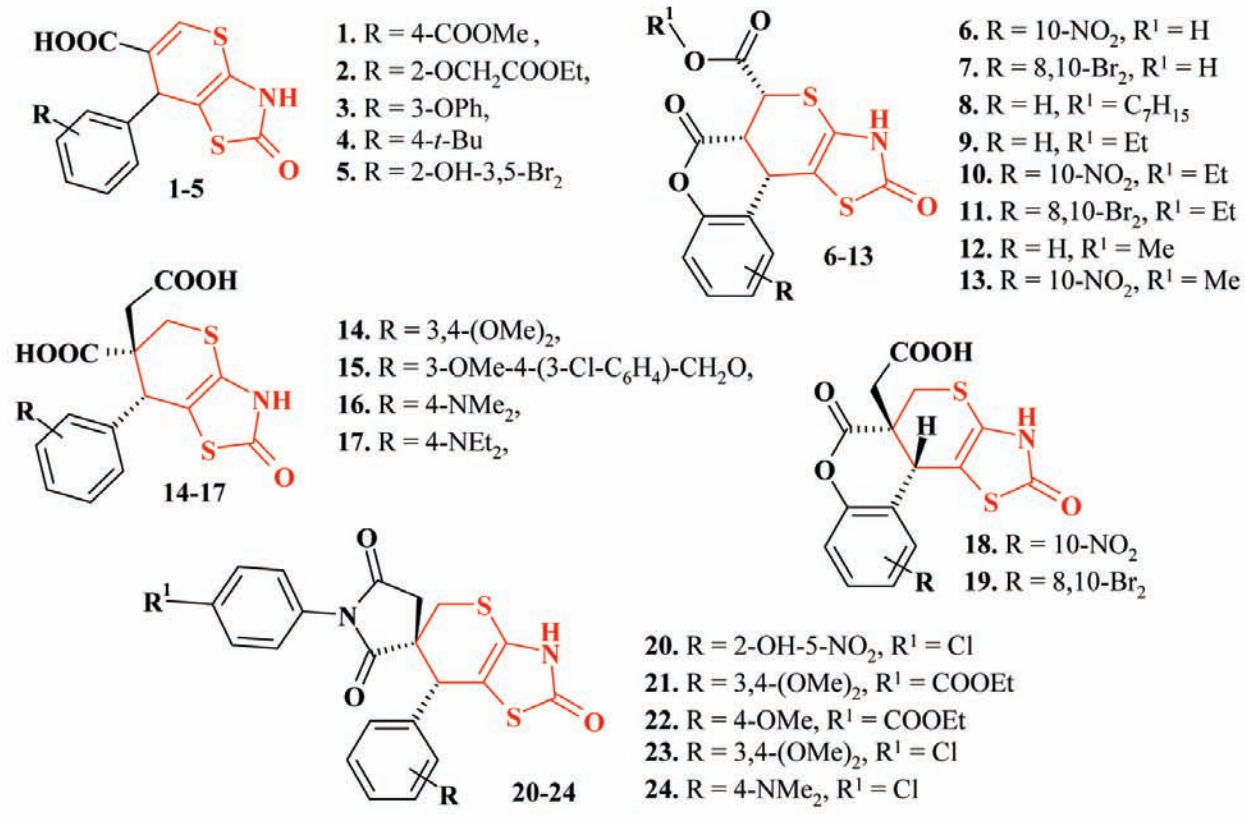

Fig. 2. Compounds tested for antiviral activity

Antiviral assay. Antiviral assay was performed at a virus's panel with a protocol of the NIAID's antimicrobial acquisition and coordinating [17]. Results for each tested compound were reported as virus-inhibitory concentration; 50% endpoint (EC_{50}) and cell-inhibitory concentration, 50% endpoint (CC_{50}) were determined. A general selectivity index (SI) was calculated as a ration of (EC_{50})/(CC_{50}). An SI 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 log10 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 Ribavirin was evaluated in parallel with test drugs in each test. 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.

Cytotoxicity. In the CPE inhibition tests, two wells of uninfected cells treated with each concentration of tested compounds was run in parallel with the infected, treated wells. At the time CPE was determined microscopically. The toxicity control cells were also examined microscopically for any changes in cell appearance compared to normal control cells run in the same plate. These changes may be enlargement, granularity, cells with ragged edges, filmy appearance, rounding, detachment from the surface of the well, or other changes. These changes were given a designation of T (100% toxic), PVH (partially toxic–very heavy – 80%), PH (partially toxic–heavy – 60%), P (partially toxic – 40%), Ps (partially toxic–slight–20%), or 0 (no toxicity – 0%), conforming to the degree of cytotoxicity seen. A 50% cell inhibitory (cytotoxic) concentration (CC_{50}) was determined by regression analysis of these data.

Results and discussions

Antiviral activity assay of synthesized compounds allowed us to identify some highly active thiopyrano[2,3-*d*]thiazoles, which demonstrated certain sensitivity profile towards, Influenza Virus Types A H_3N_2 and H_5N_1, as well Dengue Virus. The obtained results are summarized in Table.

Among 2-oxo-3,7-dihydro-2*H*-thiopyrano[2,3-*d*]thiazole-6-carboxylic acids (**1-5**) compound **1** showed a weak activity against Dengue Virus (EC_{50} = 71 μg/ml, SI > 1.4), and derivatives **3-5** exhibited an efficiency against Venezuelan Equine Encephalitis Virus (EC_{50} = 21÷45 μg/ml, SI = 1,1÷1,7), Dengue Virus (EC_{50} = 18÷32 μg/ml, SI = 1,0÷1,1), Respiratory Syncytial Virus (EC_{50} = 26 μg/ml, SI = 1,1÷> 3,8), SARS Coronavirus (SI = 1,2÷2,3). The substituents in the positions 3, 4 and 5 of aryl frgment contributed to increase of antiviral activity for a above-mentioned group of compounds. Moderate effect against influenza virus A (H_5N_1, Vietnam strain) is identified for derivative **4** (EC_{50} = 3.6 ÷ 6.8 μg/ml and SI = 5.0÷8.9).

According to the results of study compounds **14-17** haven't shown an antiviral effect, in the same time tetracyclic derivatives **18** and **19** pocessed a weak activity against influenza virus A (H_5N_1, Vietnam strain). It has been identified compound **20** among 7′,-(R-phenyl)-1-(R[1]-phenyl)-3′,7′,-dihydro-2*H*,2′,*H*,5*H*-spiro[pyrolidin-3,6′,-thiopyrano[2,3-*d*] thiazol]-2,2′,5-triones (**20-24**), which had some activity against Dengue virus (EC_{50} = 8÷18 μg/ml, SI = 4,7÷> 13.0). Other viruses were resistant to the action of compounds **20-24**. The SAR study revealed that combination of a chlorine atom in the *para*-position of the 1-aryl substituent and 2-hydroxy-5-nitrophenyl (**20**), 4-methoxyphenyl (**23**), and 4-dimethylaminophenyl (**24**) fragments in position 7 of basic heterocycle are important for new antiviral thiopyrano[2,3-*d*]thiazoles design.

Derivatives of *rel*-(5*R*,5a*R*,11b*S*)-2,6-dioxo-3,5a,6,11b-tetrahydro-2*H*,5*H*-chromeno[4′,3′:4,5] thiopyrano[2,3-*d*][1,3]thiazole-5-carboxylic acids (**6-13**) belong to the most promising group of compounds. The substituents in the positions 8 and 10 of the basic tetracyclic heterosystem and the ester group in position 5 are desirable for antiviral activity. It was found, the increase of the alkyl moiety length for alkyl *rel*-(5*R*,5a*R*,11b*S*)-2,6-dioxo-3,5a,6,11b-tetrahydro-2*H*,5*H*-chromeno[4′,3′:4,5] thiopyrano[2,3-*d*][1,3]thiazole-5-carboxylates (**8-13**)

T a b l e . Antiviral activity of the synthesized compounds[a]

Com-pound	Virus / strain / cell line	Method[b]	EC_{50} [c], μg/ml	CC_{50} [d], μg/ml	SI [e]
1	Dengue Virus Type 2 / New Guinea C / Vero 76	NR	71	>100	>1.4
3	Venezuelan Equine Encephalitis Virus / TC -83 / Vero	NR	35	59	1.7
	Dengue Virus Type 2 / New Guinea C / Vero 76	NR	32	34	1.1
		V	18	32	1.8
	Flu B / Florida /4/2006/ MDCK	NR	32	39	1.2
	Respiratory Syncytial Virus / A2 / MA 104	V	32	>100	>3.1
	SARS CoV / Urbani / Vero 76	NR	26	33	1.3
		V	56	>100	>1.8
4	Venezuelan Equine Encephalitis Virus / TC -83 / Vero	NR	24	35	1.5
		V	21	28	1.3
	Flu A (H_5N_1) / Vietnam /1203/2004H / MDCK	NR	6.8	34	5.0
		V	3.6	32	8.9
	Flu B / Florida /4/2006/ MDCK	NR	30	41	1.4
		V	32	>100	>3.1
	Rift Valley Fever Virus / MP -12 / Vero 76	NR	28	41	1.5
	SARS CoV / Urbani / Vero 76	NR	32	38	1.2
5	Venezuelan Equine Encephalitis Virus / TC -83 / Vero	NR	45	50	1.1
	Dengue Virus Type 2 / New Guinea C / Vero 76	NR	32	36	1.1
		V	32	32	1.0
	Tacaribe Virus / TRVL -11573 / Vero	NR	40	53	1.3
	Rift Valley Fever Virus / MP -12 / Vero 76	NR	26	36	1.4
	Respiratory Syncytial Virus / A2 / MA 104	NR	26	>100	>3.8
		V	37	>100	2.7
	Flu A (H_3N_2) / Perth /16/2009 / MDCK	NR	32	43	1.3
	SARS CoV / Urbani / Vero 76	NR	27	63	2.3
		V	32	68	2.1
6	Venezuelan Equine Encephalitis Virus / TC -83 / Vero	NR	41	58	1.4
	Flu B / Florida /4/2006/ MDCK	NR	29	>100	>3.4
	Flu A (H_1N_1) / California /07/2009/ MDCK	NR	54	>100	>1.9
		V	100	>100	>1.0

T a b l e . Continuation

7	Venezuelan Equine Encephalitis Virus / TC -83 / Vero	NR	24	65	2.7
		V	27	42	1.6
	Dengue Virus Type 2 / New Guinea C / Vero 76	V	32	42	1.3
	Flu A (H_5N_1) / Vietnam /1203/2004H / MDCK	NR	3.1	45	15
		V	10	32	3.2
	Flu B / Florida /4/2006/ MDCK	NR	8.8	18	2.0
		V	13	24	1.8
	Flu A (H_1N_1) / California /07/2009/ MDCK	NR	31	37	1.2
	Flu A (H_3N_2) / Perth /16/2009 / MDCK	NR	35	49	1,4
		V	32	>100	3.1
	SARS CoV / Urbani / Vero 76	V	42	>100	>2.4
8	Flu A (H_5N_1) / Vietnam /1203/2004H / MDCK	NR	32	>100	3,1
		V	32	>100	3,1
	Flu B / Florida /4/2006/ MDCK	NR	32	>100	3.1
		V	32	>100	3.1
	Tacaribe Virus / TRVL -11573 / Vero	NR	57	60	1.1
	Flu A (H_3N_2) / Perth /16/2009 / MDCK	NR	0.6	>100	>170
		V	2,5	>100	>40
	SARS CoV / Urbani / Vero 76	NR	56	>100	>1.8
		V	23	28	1.2
10	Venezuelan Equine Encephalitis Virus / TC -83 / Vero	NR	77	>100	>1.3
	Flu A (H_5N_1) / Vietnam /1203/2004H / MDCK	NR	8.0	>100	>13
	Tacaribe Virus / TRVL -11573 / Vero	NR	50	>100	>2.0
	Rift Valley Fever Virus / MP -12 / Vero 76	NR	34	>100	>2.9
	SARS CoV / Urbani / Vero 76	NR	48	>100	>2.1
11	Flu A (H_5N_1) / Vietnam /1203/2004H / MDCK	NR	0.31	>100	>320
		V	0.32	>100	>310
	Flu B / Florida /4/2006/ MDCK	NR	34	>100	>2.9
		V	32	>100	>3.1
	Flu A (H_1N_1) / California /07/2009/ MDCK	NR	32	>100	>3.1
		V	32	>100	>3.1
	Flu A (H_3N_2) / Perth /16/2009 / MDCK	NR	45	>100	>2.2
13	Flu A (H_5N_1) / Vietnam /1203/2004H / MDCK	NR	32	>100	>3.1
		V	32	>100	>3.1
	Flu B / Florida /4/2006/ MDCK	NR	32	>100	>3.1
		V	32	>100	>3.1
	Flu A (H_1N_1) / California /07/2009/ MDCK	NR	27	>100	>3.7
		V	32	100	3.1
	Flu A (H_3N_2) / Perth /16/2009 / MDCK	NR	30	>100	>3.3
		V	42	>100	>2.4

T a b l e . Continuation

17	Tacaribe Virus / TRVL -11573 / Vero	NR	28	38	1.4
18	Flu A (H_5N_1) / Vietnam /1203/2004H / MDCK	NR	50	>100	>2.0
	Flu A (H_1N_1) / California /07/2009/ MDCK	NR	32	>100	>3.1
		V	32	>100	>3.1
19	Venezuelan Equine Encephalitis Virus / TC -83 / Vero	NR	67	>100	>1.5
	Flu A (H_5N_1) / Vietnam /1203/2004H / MDCK	NR	31	>100	>3.2
20	Dengue Virus Type 2 / New Guinea C / Vero 76	NR	18	84	4.7
		V	8	>100	>13
	Flu A (H_5N_1) / Vietnam /1203/2004H / MDCK	NR	30	43	1.4
	Tacaribe Virus / TRVL -11573 / Vero	NR	26	56	2.2
	Respiratory Syncytial Virus / A2 / MA 104	NR	46	>100	>2.2
23	Flu A (H_5N_1) / Vietnam /1203/2004H / MDCK	V	32	>100	>3.1
	Flu B / Florida /4/2006/ MDCK	NR	32	>100	>3.1
	Flu A (H_1N_1) / California /07/2009/ MDCK	NR	27	>100	>3.7
		V	32	100	3.1
24	Flu A (H5N1) / Vietnam /1203/2004H / MDCK	V	32	>100	3.1
	Rift Valley Fever Virus / MP -12 / Vero 76	NR	23	68	3.0
	Respiratory Syncytial Virus / A2 / MA 104	NR	23	50	2.2
	SARS CoV / Urbani / Vero 76	NR	43	>100	>2.3
Riba-virin	Respiratory Syncytial Virus/ A2 / MA 104	NR	6.1	> 250	> 41
	Rift Valley Fever Virus / MP -12 / Vero 76	NR	8.9	> 1000	> 110
		V	9.1	> 1000	> 110
	Tacaribe Virus / TRVL -11573 / Vero	NR	5.4	> 1000	> 190
		V	5.6	> 1000	> 180
	Flu A (H_1N_1) / California /07/2009/ MDCK	NR	4.9	> 100	> 20
		V	3.2	> 100	> 31
	Flu A (H_3N_2) / Perth /16/2009 / MDCK	NR	>11.0	> 100	> 9,1
		V	>11.0	> 100	> 9.1
	Flu A (H_5N_1) / Vietnam /1203/2004H / MDCK	NR	2.3	> 100	>43.0
		V	5.3	> 100	> 19
	Flu B / Florida /4/2006/ MDCK	NR	2.4	> 100	> 42
		V	1.7	> 100	> 59

[a] Compounds **2**, **9**, **12**, **14-16**, **21** and **22** did not show any antiviral activity. [b]NR – neutral red, V – visual [c]EC_{50} – compound concentration that reduces viral replication by 50% [d]CC_{50} – compound concentration that reduces cell viability by 50% [e]SI – CC_{50}/EC_{50}.

contributes for antiviral activity increasing (Fig. 3). Moreover, two the most active hits **8** and **11** belong to the above-mentioned group of thiopyrano[2,3-*d*] [1,3]thiazoles. Compound **8** showed a higher activity against Influenza Virus Type A (H_3N_2, Perth strain) with EC_{50} = 0.6÷2.5 μg/ml and SI = 40.0÷>170.0, and derivative **11** – against Influenza Virus Type A (H_5N_1, Vietnam strain) with EC_{50} = 0.31÷0.32 μg/ml and SI = >310.0÷>320.0.

In general, it should be noted that compounds **6**, **7**, **8**, **10**, **11**, **13** have the specific antiviral activity against influenza viruses.

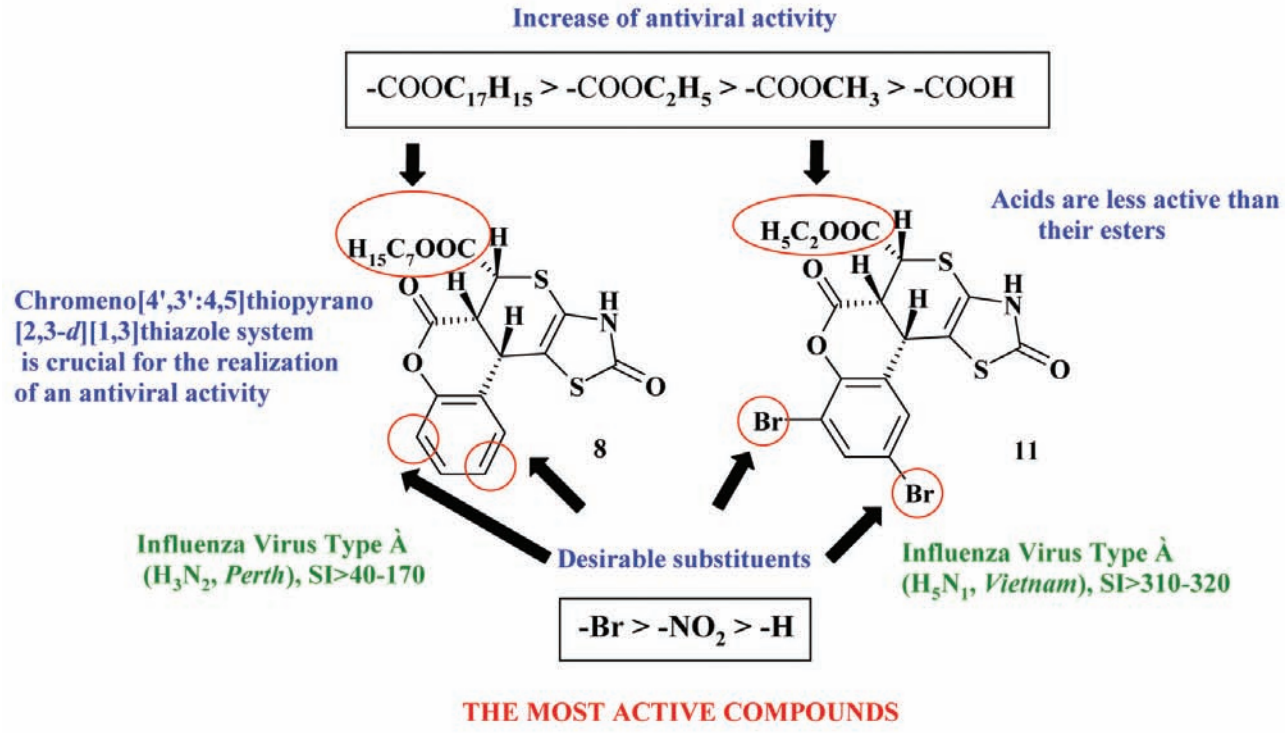

Fig. 3. SAR study of the thiopyrano[2,3-d][1,3]thiazoles.

The screening of 24 thiopyrano[2,3-*d*][1,3] thiazole derivatives against a wide range of viruses has been carried out. Some compounds possess moderate levels of the antiviral activity. However, the preliminary results of antiviral activity allowed to identify the active compounds **8** and **11**, which have shown the significant antiviral activity against Influenza Virus Type A H_3N_2 (Perth strain) and Influenza Virus Type A H_5N_1 (Vietnam strain).

Thus, derivatives bearing thiopyrano[2,3-*d*] [1,3]thiazole fragment could be considered as promising basis for further modification in searching for new antiviral agents.

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