Synthesis and antiviral activity of 2-amino-2-thiazolinium and cyclohexylammonium salts of alkylthiocarboxylic acids

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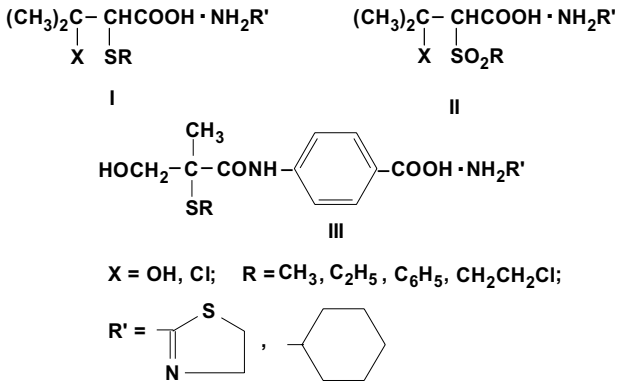
The synthesis of 2-amino-2-thiazolinium and cyclohexylammonium salts of alkylthiocarboxylic acids was performed by treating 2-amino-2-thiazoline in acetone or ethanol, or cyclohexylamine in ether with the appropriate acid. In this series, the 2-amino-2-thiazolinium salt of 2-methylthio-3-methyl-3-oxybutanoic acid was effective enough against *Herpes simplex* virus and vaccinia.

Key words: alkylthiocarboxylic acids, 2-amino-2-thiazolinium and cyclohexylammonium salts, antiviral activity

We have recently reported that some 2-amino-2-thiazolinium salts of alkylcarboxylic and alkylsulfonic acids possess radioprotective activity [1].

In continuation of search for new antiviral compounds, we synthesized in water soluble 2-amino-2-thiazolinium and cyclohexylammonium salts of alkylthiocarboxylic acids (**I–III**).



3-Chloro-2-methyl-(ethyl-, phenyl-, 2-chloroehtyl)-thiocarboxylic acids (**I, III**) were obtained by addition of methyl-, ethyl-, phenyl- or 2-chloroethylsulphenylchlorides to 3-methylbuten-2-oic acid and to 4-carboxyanilide-2-methylpropen-2-oic acid [2, 3, 4]. Hydrolysis of 3-chloro-2-methyl-(ethyl-, phenyl-) thiobutanoic acid in aqueous acetone at 60 °C led to the formation of the corresponding oxyderivatives I (a–d) [3, 5]. 3-Chloro-(oxy-)-2-methyl-(phenyl-)thio-3-metylbutanoic acid by treatment with excess H_2O_2 in the mixture of glacial acetic acid and acetic anhydride was oxidized to 3-chloro-(oxy-)-3-methyl-(phenyl)-sulfonyl-3-methylbutanoic acid **II** (a–c) [3, 5]. 2-Amino-2-thiazolinium and cyclohexylammonium salts of alkylthiocarboxylic acids (**I–III**) were obtained by treating 2-amino-2-thiazoline in acetone or ethanol or cyclohexylamine in ether with the appropriate acid. The structure of new synthesized salts (**I–III**) was determined by NMR spectra and elemental analysis. (Table 1).

Antiviral activity was studed with the series synthesized salts of substituted 2-alkyl- and phenylthio-(sulfonyl)-3-methylbutanoic (**I** a, b, d, e, g, h, **II** a, b) and 4-carboxyanilide 2-alkylthio-2-methylpropanoic **III** (a, c) acids with 2-amino-2-thiazoline and cyclohexylamine (Table 2). It was estimated that 2-amino-2-thiazolinium salt of 3-methyl-2-methylthio-3-oxybutanoic acid **I** a was effective enough against *Herpes simplex* and vaccinia viruses. Modification of the structure of this molecule: methylsulfonylgroup instead of methylthio (**II** a), or 2-amino-2-thiazoline instead of cyclohexylamine in amino addition salts (**II** b) led to the disappearance of this activity. However, its antiviral activity was shown against vesicular stomatitis and rota-viruses, respectively. Compounds **I** d and **I**

Table 1. **Constants, elemental analysis and spectral data of 2-amino-2-thiazolinium and cyclohexylammonium salts of alkylthiocarboxylic acids (I - III)**

Compd	X	R	R'	M. p. °C	Calc., % N	Found, % N	Formula	1H NMR (δ, ppm)
1	2	3	4	5	6	7	8	9
I a	OH	CH_3		110–112	10.51	10.45	$C_9H_{18}N_2O_3S$	1.30 (6H, s,$(CH_3)_2C$), 2.15 (3H, s, SCH_3), 3.03 (1H, s, CH), 4.02 (2H, t, CH_2S), 4.40 (2H, t, CH_2N)
I b	OH	C_2H_5		108–109	9.99	10.17	$C_{10}H_{20}N_2O_3S_2$	1.31 (6H, s,$(CH_3)_2C$), 1.23 (3H, t, $\mathbf{CH_3}CH_2S$), 2.71, 2.41 (2H, q, J=7Hz $CH_3\mathbf{CH_2}S$), 3.06 (1H, s, CH), 3.51 (2H, t, CH_2 s), 3.70 (2H, t, CH_2N)
I c	OH	C_2H_5		117–119	5.05	5.02	$C_{13}H_{27}NO_3S$	1.31 (6H, s, $(CH_3)_2C$), 1.23 (3H, t, $\mathbf{CH_3}CH_2S$), 2.53, 2.68 (2H, q, J=7Hz CH_3 $\mathbf{CH_2}S$), 3.06 (1H, s, CH), 1.01–2.14 (10H, m, C_6H_{10}), 2.97 (1H, m, CH)
I d	OH	C_6H_5		106–108	4.30	4.43	$C_{17}H_{27}NO_3S$	1.35 (6H, s, $(CH_3)_2$ C), 3.86 (1H, s CH), 1.03–2.14 (10H, m, C_6H_{10}), 3.01 (1H, m, CH)
I e	Cl	CH_3		103–105	4.97	4.78	$C_{12}H_{24}ClNO_2S$	1.64, 1.72 (6H, 2s, $(CH_3)_2C$)2.16 (3H, s, SCH_3), 5.61 (1H, s, CH), 0.99–2.08 (10H, m, C_6H_{10}), 2.90 (1H, m, CH)
I f	Cl	CH_3		106–108	9.83	9.94	$C_9H_{17}ClN_2O_2S$	1.62,170 (6H, 2s, $(CH_3)_2C$) 2.16 (3H,s, SCH_3), 5.60 (1H, s, CH), 3.24 (2H, t, CH_2S), 3.83 (2H, t, CH_2N)
I g	Cl	C_6H_5		117–118	4.07	4.20	$C_{17}H_{26}ClNO_2S$	1.75, 1.83 (6H, 2s, $(CH_3)_2C$), 5.95 (1H, s, CH), 0.89–2.00 (10H, m, C_6H_{10}), 2.90 (1H, m, CH)
I h	Cl	CH_2CH_2Cl		197–199	8.40	8.28	$C_{10}H_{18}Cl_2N_2O_2S$	1.62, 1.70 (6H, 2s, $(CH_3)_2C$), 2.91 (2H, t, CH_2S), 3.65 (2H, t, CH_2Cl), 5,72 (2H, s, CH), 3.45 (2H, t, CH_2S), 3.73 (2H,t, CH_2N)
I i	Cl	CH_2CH_2Cl		106–108	4.24	4.32	$C_{13}H_{25}Cl_2NO_2S$	1.67, 1.75 (6H,2s, $(CH_3)_2C$) 2.93 (2H, t, SCH_2), 3.67 (2H, t, CH_2Cl), 5.73 (1H, s, CH), 0,81–2.09 (10H, m, C_6H_{10}), 2.88 (1H, m, CH)

1	2	3	4	5	6	7	8	9
II a	OH	CH_3		178–179	9.38	9.27	$C_9H_{18}N_2O_5S$	1.43, 1.48 (6H, 2s, $(CH_3)_2C$), 3,67 (1H, s, CH), 3,64 (2H, t, CH_2S), 3,94 (2H, t, CH_2 N)
II b	OH	CH_3		117–119	4.74	4.68	$C_{12}H_{25}NO_5S$	1.35, 1.44 (6K, 2s, $(CH_3)_2C$, 3.64 (1H, s, CH), 0.84–2.14 (10H, m, C_6H_{10}), 3.04 (1H, m, CH)
III a	OH	CH_3		175–177	11.31	11.50	$C_{15}H_{21}N_3O_4S_2$	1.42 (3H s, CH_3), 1.80 (3H, s, SCH_3), 3.74 (2H, d, J=8Hz), 3.43 (2H, t, CH_2S), 3.85 (2H, t, CH_2N)
III b	Cl	C_2H_5		126–128	4.64	4.90	$C_{13}H_{16}ClNO_3S$	1.88 (3H, s, CH_3), 1.19 (3H, t, $\mathbf{CH_3}CH_2S$), 2.53 2H, t. $CH_3\mathbf{CH_2}S$).3.02, 3.17 (2H, q, J=6H_z), 8.60 (1H, s, NH)
III c	Cl	C_2H_5		107–109	6.99	6.82	$C_{19}H_{29}ClN_2O_{3S}$	1.78 (3H, s, CH_3), 1.11 (3H, t, $\mathbf{CH_3}CH_2S$), 2.53 (2H, t, $CH_3\mathbf{CH_2}S$), 3.19, 3.50 (2H, q, J=10Hz, CH_2), 0.95–1.95 (10H, m, C_6H_{10}), 2.87 (1H, m, CH)

Table 2. **Antiviral activity of compounds (I–III)**

Compd.	MTC, mkg/ml	Antiviral activity						
		HSV	VV	FPV	RSV	VSV	VEEV	RV
I a	50	++++	++++	–	++	–	–	–
I b	100	–	n. t.	++	–	–	–	–
I d	50	–	n. t.	++	–	–	–	–
I e	n. t.	–	n. t.	–	–	–	–	–
I g	n. t.	–	n. t.	–	–	–	–	–
I h	100	–	n. t.	–	–	–	–	–
II a	25	–	++	–	–	+++	–	–
II b	100	–	n. t.	–	–	–	–	+++
III a	25	+	–	–	–	–	–	–
III c	n. t.	–	–	–	–	–	–	–

"++++", "+++", "++", "–" – strong, middle, weak and no activity respectively; n. t. – no tested

c showed a week antiviral activity in influenza virus infected cell culture experiments. The other study compounds did not demonstrate antiviral activity. The antiviral activity of compounds **I** a, **II** a and **I** c may be associated with possibility to inhibit some main enzymes of the indicated virus [8].

EXPERIMENTAL

^{1}H NMR spectra were measured on a Hitachi R-22 spectrometer operating at 90 MHZz (35 °C) with HMDSO (δ = 0.05 ppm) as the internal standard in CH_3OH-d_4 (**I** a–d, **II** a, b, **III** a), DMSO-d_6 (**I** e–i, **III** c), CCl_4 (**III** b). The J values are given in Hz. Signal characteristics are described using standard abbreviations: s – singlet, d – doublet, t – triplet, q – quartet, m – multiplet. The melting points were taken on a heated microscope stage (Boëtius block) and are uncorrected. Reactions were performed using solvents dried and purified by standard methods. Thin-layer chromatography (TLC) was performed on silufol UV-254 plates using chloroforme methanol as eluent. Spots were visualized in UV light or iodine vapour. The synthesis of compounds **I–III** was carried out as previously described [1–5], 2-amino-2-thiazoline as [6].

The general method for the synthesis of cyclohexylammonium and 2-amino-2-thiazolinium salts (I-III). To a stirred solution of appropriate alkylthiocarboxylic acid (**I–III**) (2 mmol) in 25 ml anhydrous ether, ethanol or acetone, a solution of cyclohexylamine (2 mmol) in 10 ml anhydrous ether or ethanol was added. The reaction mixture was kept for 24 h at room temperature. The solid was filtered off, washed with ether and recrystalized from ethyl acetate and hexane. The yields were 83–93%. The melting points, data of elemental analysis and ^{1}H NMR spectra are presented in Table 1.

BIOLOGICAL EXPERIMENTS

The antiviral activity of compounds was tested *in vitro*, using cell cultures infected with *Herpes simplex* (HSV, type I, strain 1C), vaccinia (VV, strain B-52), influenza (FPV/Rostock), respiratory syncytial (RSV, strain Long), vesicular stomatitis (VSV, strain Indiana), Venezuelan equine encephalitis (VEEV, strain VEE-230) or rota- (RV, strain SV-11) viruses. Experiments with RSV were executed in rabbit lung cells (RL-33), with rotavirus – in monkey kidney cells (MA-104), with the rest viruses – in primary fibroblasts of chicken embryos. The reduction of plaques or inhibition of their formation in screening test and plaque reduction assay was measured for each compound. The ratio maximal tolerated concentration (MTC) / minimal inhibiting concentration (MIC) of the compound was calculated. The MIC was determined as a concentration with the reduction of the titer of viruses compared with the untreated control by at least 1.25 lg PFU/ml). The more detailed reports of biological experiments and evaluation of results have been presented previously [7].

CONCLUSIONS

1. 2-Amino-2-thiazolinium and cyclohexylammonium salts of alkylthiocarboxylic acids were synthesized.

2. The 2-amino-2-thiazolinium salt of 2-methylthio-3-methyl-3-oxybutanoic acid was found to be effective enough against herpes virus and vaccinia.

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ALKILTIOKARBONO RŪGŠČIŲ 2-AMINO-2-TIAZOLINIO IR CIKLOHEKSILAMINO DRUSKŲ SINTEZĖ IR PRIEŠVIRUSINIS AKTYVUMAS

S a n t r a u k a

Susintetintos alkiltiokarbono rūgščių 2-amino-2-tiazolinio ir cikloheksilamino druskos veikiant 2-amino-2-tiazoliną arba cikloheksilaminą atitinkama rūgštimi etanolyje, acetone arba eteryje. Tirtoje eilėje 2-metiltio-3-metil-3-oksibutano rūgšties 2-amino-2-tiazolinio druska buvo aktyvi prieš herpes virusą ir raupų vakciną.

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СИНТЕЗ И ПРОТИВОВИРУСНАя АКТИВНОСТЬ СОЛЕЙ 2-АМИНО-2-ТИАЗОЛИНИя И ЦИКЛОГЕКСИЛАММОНИя АЛКИЛТИОКАРБОНОВЫХ КИСЛОТ

Р е з ю м е

Синтезированы 2-амино-2-тиазолиниевые и циклогексиламмониевые соли алкилтиокарбоновых кислот путем обработки 2-амино-2-тиазолина или циклогексиламина в этаноле, ацетоне или эфире. Соль 2-амино-2-тиазолиния 2-метилтио-3-метил-3-оксибутановой кислоты была наиболее активной из изученных солей против вирусов герпеса и оспенной вакцины.