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Synthesis and radioprotective activity of some *threo*-D,L-phenylserine derivatives

Nijolė Dirvianskytė*

Institute of Biochemistry, Mokslininkų 12, LT-08662 Vilnius, Lithuania

Laima Leonavičienė,

Rūta Bradūnaitė

Institute of Experimental and Clinical Medicine of Vilnius University, Žygimantų 9, LT-01102 Vilnius, Lithuania

The synthesis of 2-aminothiazolinium, 2-mercaptoethylamine and 2-amino-1-phenylethanol salts of *N*-acyl-*threo*-D,L-phenylserine was accomplished. Fourteen derivatives were investigated for their toxicity and radioprotective effect on mice. 2-aminothiazolinium salt of *N*-formyl-*threo*-D,L-phenylserine at a dose of 250 mg/kg was the most potent radioprotector.

Key words: *N*-acyl-*threo*-D,L-phenylserine derivatives, radioprotective activity

INTRODUCTION

Radioprotector is a chemical or biological compound capable of modifying the normal response of a biological system to radiation-induced toxicity or lethality [1, 2]. Any chemical agent that can improve the tolerance of a normal tissue to radiation is of paramount interest [3]. The search of more effective and less toxic radioprotectants is of great importance in the development of different compounds [4].

Our earlier studies [5–8] have shown a broad spectrum of the biological activity of *threo*-D,L-phenylserine derivatives. It has been revealed that they can regulate inflammatory and autoimmune processes in the animal models of arthritis and can be useful for the development of new non-steroidal anti-inflammatory drugs (NSAIDs) that possess the anti-inflammatory activity.

On the basis of previous studies [6, 9–12] we have synthesized fourteen nonproteinogenic amino acid *threo*-D,L phenylserine derivatives (**I–XIV**) and investigated their possible radioprotective effect in mice exposed to various doses of irradiation.

RESULTS AND DISSCUSSION

The starting *threo*-D,L-phenylserine esters (**I, II, XIII, XIV**) were synthesized from their commercially available corresponding acids (Chemapol, Prague, Czech Republic) and ethanol under a reflux in the presence of gaseous HCl, octanol or 1-tetradecanol in the presence of p-toluenesulfonic acid in benzene, respectively.

N-acylphenylserines were obtained by condensating corresponding acyl chlorides with *threo*-D,L-phenylserine in aqueous alkaline solution [6, 11].

2-aminothiazolinium bromide has been reported to be a moderate radioprotector, while the corresponding 2-aminothiazoline is almost nonprotective [9]. In continuation of the search for an effective modification of known radioprotective agents, the synthesis of 2-aminothiazolinium salts of *N*-acyl-*threo*-D,L-phenylserine (**III, IV, V, VI**) was developed.

2-mercaptoethylamine salt of *N*-propionyl-*threo*-D,L-phenylserine (**VII**) and 2-amino-1-phenylethanol salts of *N*-propionyl-, *N*-butyryl-*threo*-D,L-phenylserine (**VIII, IX**) were synthesized in analogical way. Their structure was determined by the elemental analysis, IR and ^{1}H NMR spectra (Table 1).

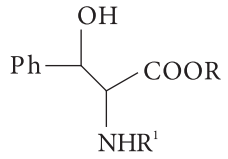
N-(*N*^{1}-Arylsuccinoyl)-*threo*-D,L-phenylserine ethyl esters (**X–XII**) were synthesized from *threo*-D,L-phenylserine ethyl ester hydrochloride (**I**) and *N*-substituted butanedioic acid amide or *N*-2-fluorenylsulfonyl-β-alanine in anhydrous pyridine and dicyclohexylcarbodiimide (DCC) at 0 °C [13]. *N*-(Fluoren(on)-2-sulfonyl)-*threo*-D,L-phenylserine octyl esters (**XIII,XIV**) were synthesized from the *threo*-D,L-phenylserine octyl ester p-toluenesulfonate and fluorene or 9-fluorenone-2-sulfonylchloride in the presence of chloroform and triethylamine [10, 12].

EXPERIMENTAL

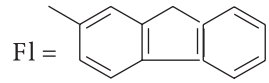
The melting points were determined in open capillaries and are uncorrected. IR spectra were recorded on a Specord IR-75 (Germany) in KBr pellets. ^{1}H NMR spectra – on a Hitachi R-22 spectrometer (90 MHz, Japan) using HMDS as an internal

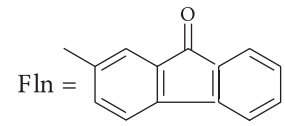
* Corresponding author. E-mail: biochemija@bchi.lt

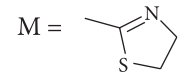
Scheme



threo–DL







Compound	R	R1	References
I	C_2H_5	H×HCl	–
II	$CH_2(CH_2)_{12}CH_3$	H×4-$CH_3C_6H_4SO_3H$	[10]
III	H×H_2N-M	CHO	"–"
IV	H×H_2N-M	$COCH_3$	"–"
V	H×H_2N-M	$COCH_2CH_3$	"–"
VI	H×H_2N-M	$COCH_2CH_2CH_3$	[11]
VII	H×$H_2NCH_2CH_2SH$	$COCH_2CH_3$	"–"
VIII	H×$CH_2(NH_2)CH(C_6H_5)OH$	$COCH_2CH_3$	"–"
IX	H×$CH_2(NH_2)CH(C_6H_5)OH$	$COCH_2CH_2CH_3$	"–"
X	C_2H_5	$COCH_2CH_2CONHC_6H_4$-4-Br	[13]
XI	C_2H_5	$COCH_2CH_2CONHC_6H_4$-4-CH_3	"–"
XII	C_2H_5	$COCH_2CH_2NHSO_2Fl$	"–"
XIII	CH_2(CH2)6CH3	SO_2Fl	[12]
XIV	$CH_2(CH_2)_6CH_3$	SO_2Fln	[10]

Table 1. **Constants and spectral data of salts III–V, VII–IX**

Comp.	Formula	Melting-point, °C	Calc / Found				IR cm^{-1}				^{1}H NMR(δ, ppm)	
			C	H	N	S	CONH	OH	N-H	COO–	CH_2S	CH_2N
III	$C_{13}H_{17}O_4N_3S$	188–9	50.15 50.18	5.50 5.45	13.49 13.64	10.29 9.74	1652	3230	3315	1560	3.45, t, *J* = 6Hz	3.79, t, *J* = 6Hz
IV	$C_{14}H_{19}O_4N_3S$	180–2	51.68 51.82	5.88 5.92	12.91 12.97	9.85 9.55	1640	3165	3325	1530	3.44, t, *J* = 6Hz	3.79, t, *J* = 6Hz
V	$C_{15}H_{21}O_4N_3S$	177–8	53.08 53.35	6.23 6.37	12.38 12.65*J*	9.44 9.11	1630	3230	3400	1533	3.46, t, *J* = 6Hz	3.81, t, *J* = 6Hz
VII	$C_{14}H_{22}O_4N_2S$	163–5	53.48 53.32	7.05 7.04	8.91 8.83	10.20 9.50	1625	3213	3366	1500	2.96, 3.11, m., 4CH,2CH_2(each)	
VIII	$C_{20}H_{26}O_5N_2$	163–5	64.16 64.14	6.99 6.96	7.48 7.46	–	1613	3133	3365	1530	2.6–2.9, m., CH_2CH	
IX	$C_{21}H_{28}O_5N_2$	155–7	64.93 64.65	7.26 7.30	–	–	1626	–	3300	1540	2.79–3.03, m., CH_2CH	

reference (δ = 0.05 ppm to TMS) in DMSO-d_6 solution. The multiplicities are marked as follows: *s* – singlet, *d* – doublet, *t* – triplet, *m* – multiplet, *q* – quadruplet (Table 1).

The general method for the synthesis of 2-aminothiazolinium, 2-mercaptoethylamine, 2-amino-1-phenylethanole salts of N-acyl-threo-D,L-phenylserine (**III–IX**).

A mixture of 20 mmol *N*-acylphenylserine and 22 mmol of amine was dissolved in 30 ml of absolute ethanol, immediately filtered, kept at room temperature for 1 hour and then overnight in a refrigerator. The formed precipitate was filtered off and washed with ether. The recrystallization from ethanol gave pure compounds. The melting points, the data of elemental analysis, IR and ^{1}H NMR data are given in Table 1. The yields of salts (**III–IX**) were 75–90%.

Compounds **I–XIV** were tested for toxicity and antiradiation activity in mice. The preparations were injected intraperitoneally (i. p.) 15–30 min before irradiation. The control mice were injected with a vehicle only. Toxic and radioprotective properties of the compounds are summarized in Table 2. Acute toxicity tests showed most compounds to be of low toxicity. For compounds **I, X–XII** LD_{50} was 1500 mg/kg or higher. The highest toxicity among the investigated compounds was revealed for preparations **II, VI** and **XIV**.

No radioprotective action was revealed for preparation **II**. Compounds **I** and **IV** showed only a weak radioprotective activity, and DRF for these compounds was 1.09 and 1.04, respectively.

Compound **III** (a dose of 250 mg/kg) prior to the radiation exposure, results in positive benefits and significantly protects

Table 2. **Dose LD_{50} and radioprotective activity of *threo*-DL-phenylserine derivatives following intraperitoneal injection before the exposure to an acute whole-body irradiation with various doses of gamma rays**

Compound	LD_{50} (mg/kg)	Dose injected (mg/kg)	n	Time (min)[a]	Survival of mice after 30 days (%)					DRF[b] ($LD_{50/30}$)
					Dose of irradiation (Gy)					
					7	7.5	8	8.5	9	
I	1640	550	5	15	80	60	40			1.09
II	400	130	5	15	40	0	0			
III	750	250	5	15	100*	60*	60*			1.20*
IV	690	230	5	15	80	60	0			1.04
Control (solvent [c])			5	15	60	20	0			
VII	600	200	5	15		80	80	80	60	1.06
VIII	550	185	5	15		80	60	60	40	1.05
IX	700	230	5	15		60	60	60	0	1.04
X	1500	500	5	15		80	60	60	0	1.02
XI	>1500	500	5	15		80	80	20		1.05
XII	1500	500	5	15		80	80	60	40	1.08
Control (solvent [c])			5	15		50	30	10	0	
V	840	300 100	15	30				60 40		
VI	450	250 100	15	30				26 26		
XIII	>800	300 100	15	30				0 0		
XIV	400	250 100	15	30				40 20		
Control (solvent [c])			15					0		

Note: [a]Time interval between the injection of a compound and irradiation; [b]Dose reduction factor (DRF); [c]Sterile distilled water containing 0.1% Tween 80; n – number of mice in groups; * Significant difference compared to control.

from mortality. A thirty-day survival was 100% at a dose of 7 Gy and 60% at doses of 7.5 and 8 Gy. The percent of the survivors in the control group was by 40% lower than in the treated mice exposed to radiation by 7 and 7.5 Gy, and 100% lethality was observed by using the 8 Gy dose.

Pre-treatment of male (CBA × C57BL/6) F_1 hybrids with preparations **VII–XII** caused a higher percent of the survival in comparison with the control group after the exposure to various doses of gamma radiation (Table 2). The preparations showed some radioprotective effects and their DRFs were in a range of 1.02–1.08.

The survival results of the radioprotection studies of compounds **V, VI** and **XIII, XIV** investigated on male white-inbred mice showed the radioprotective activity of preparations **V** and **XIV**. No radioprotecting effect was revealed for compound **XIII** and only a weak radioprotective effect was observed for compound **VI** (26% of the irradiated animals survived by using both 100 mg/kg and 250 mg/kg doses of preparation). Detailed biological investigations and the obtained data are described in our article [14].

BIOLOGICAL EXPERIMENTS

Acute toxicity of the compounds and the LD_{50} value was determined on mice by the approved Litchfield and Wilcoxon [15] method. The survival of mice (5 in each group) orally receiving the graduated single dose levels of each compound dissolved in sterile distilled water containing 0.1% Tween 80 was observed for 7 days.

Whole-body irradiation was performed on the plant IGUR with 4 sources of ^{137}Cs with a dose rate of 58–60 cGy/min and 5.5 cGy/min in dose diapason 7–9 Gy. The radioprotective activity of the preparations was examined by measuring the survival of mice, receiving the compounds 15 or 30 minutes before the exposure to radiation and by calculating the dose reduction factor (DRF), which was determined by dividing the $LD_{50/30}$ values obtained from the radiation survival curve in the presence of a radioprotective agent by the $LD_{50/30}$ value obtained from a control radiation survival curve. The dosage of the compounds in most cases approximates to one-third of the toxic 50% lethal dose. The results were recorded for 30 days and compared with the results obtained for the controls. Antiradiation testing was performed in three experiments. **I–IV** derivatives were evaluated for their radioprotective effects against ^{137}Cs gamma rays using female C57BL/6 mice. Males (CBAxC57BL/6)F_1 were used for the investigation of compounds **III, VII–XII** and male white-outbred mice were pretreated with compounds **V, VI, XIII** and **XIV**.

CONCLUSIONS

1. 2-aminotiazolinium, 2-amino-1-phenylethanol, 2-mercaptoethylamine salts of *N*-acyl-*threo*-D,L-phenylserine acids were synthesized.

2. 2-aminothiazolinium salt of *N*-formyl-*threo*-D,L-phenylserine at a dose 250 mg/kg exhibited the highest radioprotective effect on mice.

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KAI KURIŲ *TREO*-D,L-FENILSERINO DARINIŲ SINTEZĖ IR RADIOPROTEKTORINIS AKTYVUMAS

S a n t r a u k a

Susintetintos *N*-acil-*treo*-D,L-fenilserino rūgščių 2-aminotiazolinio, 2-merkaptoetilamino, 2-amino-1-feniletanolio druskos. Iš keturiolikos ištirtų *treo*-D,L-fenilserino darinių *N*-formil-*treo*-D,L-fenilserino 2-aminotiazolinio druskos radioprotektorinis aktyvumas buvo didžiausias.