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SYNTHESIS OF DERIVATIVES OF 2-PHENYLTHIAZOLIDINE-4-CARBOXYLIC ACID AND A STUDY OF THEIR RADIOPROTECTIVE PROPERTIES

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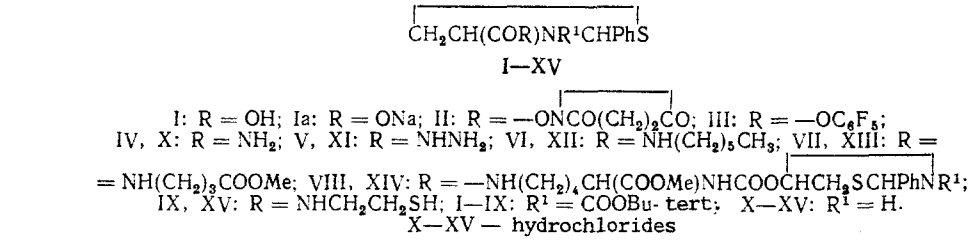
There has been a recent increase in the number of publications concerned with the synthesis and study of the biological activity of various thiazolidines and their derivatives. Found among the compounds containing a thiazolidine ring were not only highly active radioprotective substances [10] and biologically active substances [1, 7], but also herbicides [11] pesticides [12], and plant growth hormones [4].

The insertion of thiazolidine-4-carboxylic acid into the peptide chain of biologically active peptides in place of cysteine, in some cases led to a pronounced increase in activity, such as in the case of oxytocin [8].

The present study is concerned with the synthesis of 2-phenylthiazolidine-4-carboxylic acid esters and amides that have not been described in the literature, and the study of their toxic and radioprotective properties.



The starting product, 2-phenylthiazolidine-4-carboxylic acid, was obtained by reacting benzaldehyde with cysteine [1]. A tert-butyloxycarbonyl group (BOC-group) was selected to protect the NH-function. The literature describes an unsubstituted N-BOC-thiazolidine-4-carboxylic acid which was synthesized by the use of tert-butyloxycarbonylazide in a Schotten–Baumann reaction [9]. We have shown that it is more convenient to introduce a BOC-group into thiazolidine compounds by the action of di-tert-butylpyrocarbonate in an anhydrous medium in the presence of a tertiary amine [5]. This procedure completely eliminates any side reactions associated with the scission of the thiazolidine ring as a result of hydrolysis, and th yield of the N-BOC-derivative (I) is significantly increased (up to 93%). The carboxyl group of compound I was activated by conversion to a corresponding N-oxysuccinimide ester (II). The procedure which we developed earlier to obtain N-oxysuccinimide esters by the action of bisuccinimide oxalate [3] results in a higher yield of compounds II (81%) than can be obtaine by the use of dicyclohexylcarbodiimide for the same purpose (about 70%). In addition to the N-oxysuccinimide ester of II, the pentafluorophenyl ester of 2-phenylthiazolidine-4-carboxyli acid (III) was obtained by the method in [2].

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By reacting compounds of II with ammonia, hydrazine, and hexylamine, we obtained high yields of the corresponding amide (IV), hydrazide (V), and hexylamide (VI). The resultant N-oxysuccinimide was easily separated from the basic products since it precipitated in the salt form with the amine component which was taken in excess. The methyl ester of γ-aminobutyric acid and the methyl ester of L-lysine were also used as the amine components. Aminolysis of II proceeded smoothly with the formation of the methyl ester of N-BOC-2-phenylthiazolidine-4-carbonyl-N'-γ-aminobutyric acid (VII) and the methyl ester of N,N'-α,ε-bis(N-BOC-2-phenylthiazolidine-4-carbonyl)-L-lysine (VIII).

The molecular model demonstrated that the reaction center of compound II was shielded by the BOC-group on one side and by a closely approaching phenyl ring on the other side. Nevertheless, the α-groups as well as the ε-groups of lysine managed to react with II.

The pentafluorophenyl ester of III also underwent aminolysis smoothly in which case β-mercaptoethylamine, and β-mercaptoethylamide-phenyl-N-BOC-thiazolidine-4-carboxylic acid were used as the amine component, resulting in a quantitative yield of (IX). In order to synthesize the hydrochlorides of compounds X-XV, the BOC-protective group was removed by the action of a 4 N solution of HCl cooled to 10°C in dioxane. No side reactions were observed.

The structure of the obtained compounds was confirmed by element analysis and IR-spectroscopy. The yields of the obtained compounds and certain physicochemical characteristics are given in Table 1.

EXPERIMENTAL CHEMICAL PART

A set of "Eugon" type atomic models were used to construct molecular models. IR-spectra were read on a UR-20 spectrophotometer (GDR). Melting point was determined on a Koefler star TLC was performed on Silufol plates in the following systems: 1) ethyl acetate–pyridine–AcOH–water (5:4:5:5); 2) chloroform–methanol–benzene (85:10:5); 3) ethyl acetate–hexane (1:3) and 4) chloroform–acetone–AcOH (80:40:1).

N-tert-Butyloxycarbonyl-2-phenylthiazolidine-4-carboxylic Acid (I). A 2.09 g (0.01 mole portion of 2-phenylthiazolidine-4-carboxylic acid [1] was suspended in 20 ml of DMFA to which 2.02 g (0.02 mole) of Et_3N was added. A 2.5 ml (0.011 mole) portion of di-tert-butylpyrocarbonate was added and the resultant solution was mixed at room temperature for 2 h. It was then evaporated, and the oily residue was dissolved in ethyl acetate. The solution was first washed with a 5% aqueous solution of $KHSO_4$ and then with a saturated NaCl solution. After drying, the solution was evaporated and the residue was crystallized. IR-spectra, ν_{max}, cm^{-1} 2600-2400 (OH), 1720 (C=O), 1630 (C=O), 1420 (COOH), 1255 and 910 [$-C(CH_3)_3$].

Sodium N-tert-Butyloxycarbonyl-2-phenylthiazolidine-4-carboxylate (Ia). A 3.1 g portion (0.01 mole) of I was dissolved in 50 ml of MeOH to which 20 ml (0.01 mole) of 0.5 N NaOH was added. The solution was then evaporated until dry, and the residue was recrystallized from ethyl alcohol. Yield of Ia 2.9 g.

N-Oxysuccinimide N'-tert-Butyloxycarbonyl-2-phenylthiazolidine-4-carboxylate (II). A 4.3 g (0.015 mole) portion of di-N-oxysuccinimide oxalate was added to a solution of 3.1 g (0.01 mole) of I and 2.4 g (0.03 mole) of pyridine in abs. DMFA. The reaction mixture was mixed for 2 h at room temperature and left overnight. The precipitate was filtered off and the solution was evaporated to the point of an oily residue which was dissolved in ethyl acetate. This was washed with a 5% solution of $NaHCO_3$ and a NaCl solution, then dried and evaporated, and then recrystallized from propanol. IR-spectra, ν_{max}, cm^{-1}: 1820 (C=O), 1790 (C=O), 1750 (C=O), 1690 (C=O), 1100 (C=O).

Pentafluorophenyl N-tert-Butyloxycarbonyl-2-phenylthiazolidine-4-carboxylate (III). A 6.08 g (0.033 mole) portion of pentafluorophenol and 2.28 g (0.011 mole) of dicyclohexylcarbodiimide were added, while stirring, to a solution of 3.10 g (0.01 mole) of I in 25 ml of DMFA, cooled to 0°C. The reaction mixture was stirred for 2 h and left in a refrigerator overnight. The residue was filtered off and the solution was vacuum evaporated, and then recrystallized from isopropanol. IR-spectra, ν_{max}, cm^{-1}: 1800 (C=O), 1700 (C=O), 1530-1520 (C_6F_5), 1000 (C–F).

N-tert-Butyloxycarbonyl-2-phenylthiazolidine-4-carboxylic Acid Amide (IV). A 3 g portion (0.0074 mole) of II was dissolved in 100 ml of abs. MeOH cooled to 0°C and 10 ml abs. containing 0.25 g (0.015 mole) of ammonia were added. The solution was kept at room temperature for 1,5 h in a sealed vial. The precipitate was then filtered off and the solvent was evaporated. The crystal-

TABLE 1. Derivatives of 2-Phenylthiazolidine-4-carboxylic Acid

Compound	Yield, %	mp, °C	$[\alpha]_D^{20}$	R_f (system)	Found, %					Empirical formula	Calculated, %				
					C	H	Cl	N	S		C	H	Cl	N	S
I	93	175—7	103 (C 1, DMFA)	0,8 (1)	58,20	6,27	—	4,44	10,93	$C_{15}H_{19}NO_4S$	58,23	6,19	—	4,53	10,37
Ia	88	131—5			54,32	5,71	—	4,75		$C_{15}H_{18}NO_4SNa$	54,37	5,47	—	4,23	
II	81	122—5	52 (C 1, DMFA)	0,89 (2)	55,61	5,70	—	6,94	8,25	$C_{19}H_{22}N_2O_6S$	56,14	5,45	—	6,89	7,89
III	73,5	95—7	93,2 (C 1, Dioxane	0,9 (3)	52,91	4,28	—	3,44	6,44	$C_{21}H_{18}F_5NO_4S$	53,05	3,82	—	2,95	6,74
IV	85	148—50	110 (C 1, DMFA)	0,7 (2)	58,42	6,69	—	8,98	10,46	$C_{15}H_{20}N_2O_3S$	58,42	6,54	—	9,09	10,40
V	79	117—9	121 (C 1, DMFA)	0,4 (4)	55,71	6,59	—	12,53	10,17	$C_{15}H_{21}N_3O_3S$	55,71	6,55	—	12,99	9,91
VI	91		59,6 (C 3, MeOH)	0,88 (2)	64,36	8,24	—	7,01	7,81	$C_{21}H_{32}N_2O_3S$	64,23	8,24	—	7,14	8,17
VII	91		31,5 (C 1,6, MeOH)	0,8 (2)	58,38	7,04	—	6,46	7,53	$C_{20}H_{28}N_2O_5S$	58,80	6,91	—	6,86	7,85
VIII	83	158—9	80,8 (C 3, DMFA)	0,85 (2)	59,50	6,83	—	7,68	8,64	$C_{37}H_{50}N_4O_8S_2$	59,81	6,78	—	7,54	8,63
IX	97			0,8 (3)	55,19	6,32	—	7,81	14,40	$C_{17}H_{24}N_2O_3S_2$	55,41	6,56	—	7,60	14,40
X·HCl	85	153—6	—90 (C 1, MeOH)	0,4 (2)	48,72	5,38	14,01	11,57	13,31	$C_{10}H_{13}ClN_2SO$	49,07	5,35	14,49	11,45	13,10
XI·HCl	73	205—8 (with decomp.)	—121,3 (C 1, MeOH)	0,4 (2)	46,24	5,44	14,00	16,17	12,60	$C_{10}H_{14}ClN_3SO$	46,24	5,43	13,65	16,18	12,34
XII·HCl	87	122—3		0,6 (2)	58,11	7,76	11,01	8,63	9,69	$C_{16}H_{25}ClN_2SO$	58,43	7,66	10,78	8,52	9,75
XIII·HCl	67	110—3		0,46 (2)	52,40	6,51	10,61	8,28	9,22	$C_{15}H_{21}ClN_2SO_3$	52,24	6,14	10,28	8,12	9,30
XIV·HCl	99	120—3	—80,6 (C 3, MeOH)	0,38 (2)	52,81	5,96	11,62	8,87	10,22	$C_{27}H_{36}Cl_2N_4S_2O$	52,67	5,90	11,52	9,10	10,42
XV·HCl	86	109—12	—61,9 (C 1, MeOH)	0,3 (2)	47,55	5,60	11,33	9,12	21,12	$C_{12}H_{17}Cl_2N_3S_2O$	47,28	5,62	11,63	9,19	21,03

Note. $N^{20}D$ for VI, VII, and IX is 1.5238, 1.5318, and 1.5280, respectively.

TABLE 2. Toxic and Radioprotective Properties of 2-Phenylthiazolidine-4-carboxylic Acid Derivatives

Compound	LD_{50}, mg/kg	Dose administered, mg/kg	Survival rate, %	Longevity, days
Ia	750	250	0	11,7
II	50	15	0	9,0
IV	1000	300	0	5,7
V	50	15	0	12,4
X	500	160	20,0	9,6
XI	250	80	0	10,7
XII	800	260	0	8,2
XIII	600	200	7,0	13,0
XIV	300	100	0	9,8
XV	300	100	0	8,6
Control			0	8—9

line residue was dissolved in ethyl acetate and washed with a 5% $NaHCO_3$ solution and water. The residue was then dried, evaporated, and recrystallized from a 2:1 mixture of ethyl acetate and hexane. IR-spectra, ν_{max}, cm^{-1}: 3400-3100 (NH), 1710 (C=O), 1670 (amide I), 1640 (amide II).

N-tert-Butyloxycarbonyl-2-phenylthiazolidine-4-carboxylic Acid Hydrazide (V). A tenfold excess of 3.2 g (0.01) of anhydrous hdyrazine was added to a solution of 4.06 g (0.01 mole) of II in 60 ml of ethyl acetate. The mixture was stirred for 2 h and left overnight at room temperture. The precipitate was filtered off. The raction mixture was then diluted with ethyl acetate to 150 ml and repeatedly washed with water. After drying, the residue was recrystallized from a 2:1 mixture of ethyl acetate and hexane. IR-spectra, ν_{max}, cm^{-1}: 3400-3100 (NH), 1680 (C=O), 1630 (amide I), 1540 (amide II).

N-tert-Butyloxycarbonyl-2-phenylthiazolidine-4-carboxylic Acid Hexylamide (VI). A 0.57 g portion (0.0056 mole) of hexylamide was added to a solution of 1.1 g (0.0028 mole) of II in 15 ml of ethyl acetate. After the mixture was heated the resultant white crystalline precipitate was filtered off, and the solution was diluted with ethyl acetate and thoroughly washed with water. After drying and evaporation, product VI was obtained in the form of a colorless oil. IR-spectra, ν_{max}, cm^{-1}: 3300 broad (NH), 1680 broad (C=O, amide I), 1550 (amide II).

Methyl N-tert-Butyloxycarbonyl-2-phenylthiazolidine-4-carbonyl-N'-γ-aminomethyl Butyrate (VII). A mixture of 3.7 g (0.02) of methyl γ-aminobutyrate hydrochloride and 2 g (0.02 mole) of N-methylmorpholine in 15 ml of ethyl acetate was added to a solution of 3.2 g (0.008 mole of II in 30 ml of ethyl acetate. The mixture was left overnight at room temperature, and then diluted with ethyl acetate and washed with water, a 5% $KHSO_4$ solution, and water. After drying and evaporation, VII was obtained in the form of a colorless oil. IR-spectra, ν_{max}, cm^{-1}: 3340 (NH), 1730 (C=O), 1690 broad (C=O, amide I), 1550 (amide II).

Methyl N,N'-α,ε-bis(N-tert-Butyloxycarbonyl-2-phenylthiazolidine-4-carbonyl)-L-lysine (VIII). A mixture of 4.66 g (0.02 mole) of methyl L-lysine bishydrochloride and 4 g (0.04 mole) of N-methylmorpholine in 25 ml of DMFA was added to a solution of 4.06 g (0.01 mole) of II in 20 ml of abs. DMFA. The reaction mixture was stirred for 5 h and left overnight at room temperature. The precipitate was filtered off, the solution was evaporated to dryness, and the resultant residue was dissolved in 100 ml of ethyl acetate and repeatedly washed with an aqueous NaCl solution. After drying and evaporation, the residue was recrystallized from acetone. IR-spectra, ν_{max}, cm^{-1}: 3360 (NH), 1740 (C=O), 1690 (C=O), 1680 and 1660 (amide I of both carboxyl groups), 1540 and 1520 (amide II).

N-tert-Butyloxycarbonyl-2-phenylthiazolidine-4-carboxylic Acid β-Mercaptoethylamide (IX). A mixture of 1 g (0.0088 mole) of β-mercaptoethylamine hydrochloride and 0.89 g (0.0088 mole) of N-methylmorpholine in 10 ml of DMFA was added to a solution of 2 g (0.0042 mole) of III in 10 ml of DMFA. The reaction mixture was stirred for 4 h and left overnight at room temperature. The precipitate was filtered off, and the solution was evaporated, and the residue was dissolved in 100 ml of ethyl acetate and thoroughly washed with water, a 5% $KHSO_4$ solution, and water. After drying and evaporation, product IX was obtained in the form of a colorless oil. IR-spectra, ν_{max}, cm^{-1}: 3300-3000 (NH), 2700-2550 (SH), 1680 broad (C=O, amide I), 1540 (amide II).

General Method for Removing the N-tert-Butyloxycarbonyl Group. A 0.1 mole sample of the compound (IV, V, VI, VII, VIII*, IX) was placed into a solution of abs. dioxane, cooled to 10°C and containing 0.3 g mole of dry hydrogen chloride. The mixture was then agitated until the substance was completely dissolved. The end of the reaction was tracked by TLC. After approximately 30 min the solution was decanted into dry ether, the precipitate was then filtered off and thoroughly washed with dry ether. After vacuum drying, the residue was recrystallized from an ether-MeOH mixture.

2-Phenylthiazolidine-4-carboxylic Acid Amide, HCl (X). IR-spectra, ν_{max}, cm^{-1}: 3400-3100 (NH), 2700-2250 ($N^{+}H_2$), 1695 (amide I), 1610 (amide II).

2-Phenylthiazolidine-4-carboxylic Acid Hydrazide, HCl (XI). IR-spectra, ν_{max}, cm^{-1}: 3400 (NH), 2700-2400 ($N^{+}H_2$), 1690 (amide I), 1575 (amide II).

2-Phenylthiazolidine-4-carboxylic Acid Hexylamide, HCl (XII). IR-spectra, ν_{max}, cm^{-1}: 3300, 3230 (NH), 2700-2400 ($N^{+}H_2$), 1670 (amide I), 1550 (amide II).

Methyl 2-Phenylthiazolidine-4-carbonyl-N-γ-aminobutyrate, HCl (XIII). IR-spectra, ν_{max}, cm^{-1}: 3280, 3220 (NH), 2700-2400 ($N^{+}H_2$), 1730 (C=O), 1660 (amide I), 1550 (amide II).

Methyl N,N'-α,ε-bis(2-Phenylthiazolidine-4-carbonyl)-L-lysine, bis-Hydrochloride (XIV). IR-spectra, ν_{max}, cm^{-1}: 3200 (NH), 2700-2400 ($N^{+}H_2$), 1730 (C=O), 1670 broad (amide I, 1550 broad (amide II).

β-Mercaptoethylamide-2-phenylthiazolidine-4-carboxylic Acid, HCl (XV). IR-spectra, ν_{max}, cm^{-1}: 3200 (NH), 2700-2250 (SH and $N^{+}H_2$), 1680 (amide I), 1550 broad and 1520 (amide II and $N^{+}H_2$).

EXPERIMENTAL BIOLOGICAL PART

The toxic and radioprotective properties of the compounds were studied in male mice of line C57Bl/6, weighing 22-24 g. A total of 280 animals were used in the experiments.

The mice were irradiated on a gamma ray IGUR with an absolute lethal dose of 8 GY and dose rate of 1.2 cGy/sec. The compounds were administered intraperitoneally in the amount of 0.2 ml 15 min prior to irradiation. The dose of the substance was 1/3 of the LD_{50}. The same quantity of distilled water was administered to the control animals.

The radioprotective properties of the compounds were evaluated on the basis of a 30-day survival rate and average longevity (AL) of the perished animals. The results of the biological tests are given in Table 2.

The substances were administered at the LD_{50} dose in the clinical studies of acute intoxication. Almost all of the tested substances caused respiratory suppression and reduced tactile sensitivity.

If one compares the chemical structure of the tested thiazolidine derivatives to their toxicity, it is apparent that the determining factors are the nature of the substituent in the amino- and carboxyl groups, their structure, and the relative position of the radicals in the molecule. For example, compound V is five times more toxic than compound XI, but differs from the latter only by having a N-tert-butyloxycarbonyl group in position 3. However, little toxicity is exhibited by compound IV which, like V, has a N-tert-butyloxycarbonyl group, but is an amide instead of a hydrazide.

Our study of the radioprotective properties of 2-phenylthiazolidine-4-carboxylic acid derivatives has demonstrated that the substances tested under the described conditions do not exhibit anti-radiation activity. The only substance that provided a survival rate that was 20% greater than the control was compound X.

A comparison of our results with earlier obtained data [5] has shown that compounds that have aliphatic radicals instead of a phenyl radical in position 2 of the thiazolidine ring may be more effective.

*A solution containing 0.6 mole HCl should be taken for 0.1 mole of compound VIII.

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QUANTITATIVE INTERRELATIONSHIPS OF STRUCTURE AND BIOLOGICAL ACTIVITY FOR SUBSTITUTED AMINOGUANIDINES

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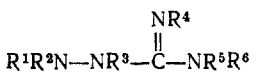
UDC 615.31:547.497.1].015.11

The successful development of studies in search of new biologically active substances with necessary properties requires a substantial degree of knowledge of the basic principles of the influence of structure on the biological action of compounds. In view of this, a complex approach to the consideration of the physical, chemical, and biological properties of potential pharmaceutical substances, in conjunction with methods of quantitative analysis of structure versus activity, is now taking on great importance for this whole field of research [4]. The methodology of such an approach consists of the following: the structural changes are considered on the basis of compounds with known properties (lead compounds, essential pharmacophore structures) by substitution within the limits of chemical possibilities. From these hypothetical subsystems, compounds that are subjected to biological and physicochemical investigation are selected and synthesized according to definite criteria.

The quantitative relationships of structure and activity obtained from the experimental data and various molecular parameters (substituent constants, experimental values for description of molecules, etc.), in the case of sufficient statistical significance, permit the prediction of the biological properties of not-yet investigated compounds of homologous and analogous series of active substances, and also suggest the synthesis of the corresponding compounds and directions for further investigations.

As is known from the literature, derivatives of aminoguanidine



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