LITERATURE CITED

1. V. P. Fedotov, I. S. Komolov, N. V. Sadovnikova, et al., Probl. Endokrinol., No. 4, 57-60 (1978).
2. V. P. Fedotov, N. V. Sadovnikova, V. I. Gudoshnikov, et al., Byull. Éksp. Biol. Med., No. 6, 729-731 (1984).
3. Yu. P. Shvachkin and L. A. Kolomeitseva, Khim. Prir. Soedin., No. 3, 397-398 (1983).
4. N. Ahmad, S. Sharma, A. Rastogi, et al., Acta Diabretol. Lat., 21, 361-364 (1984).
5. J. Okabayashi, M. Otsuki, A. Ohki, et al., Endocrinology, 113, 2210-2215 (1983).
6. J. F. Rehfeld, Li. Larsson, N. K. Goltermann, et al., Nature, 284, 33-37 (1980).

SYNTHESIS AND BIOLOGICAL ACTIVITY OF AMIDINOMERCAPTIC ACIDS AND RELATED COMPOUNDS

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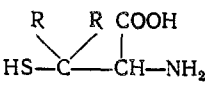
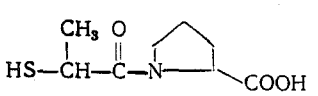
One of the new and promising areas of research on antihypertensive agents is the synthesis and study of compounds which inhibit angiotensin and dipeptidyl transferase I, dipeptidyl carboxypeptidase (DCP: EC 3.4.15.1) which catalyzes the formation of the octapeptide, angiotensin II, which has pressor properties, and which also catalyzes the inactivation of the depressor peptide bradykinin. A series of data has now been compiled on the structural features of this enzyme's active center and the requirements for making inhibitors of the enzyme [6, 11]. At the same time there is still some question about the relationship between the inhibiting and antihypertensive effects of DCP since there have been cases where no correlation between those activities was found [13].

The present work is concerned with the synthesis and investigation of the inhibiting activity of a new group of chemical compounds — the amidinomercaptic acids and related substances. A basic prerequisite to this investigation was the study made of the inhibiting action and antihypertensive activity of a series of thiol compounds, primarily L-cysteine (I) and D-penicillamine (II) [4, 5]. The amino acids (I and II), just as the known drug Captopril, contain a terminal mercapto group that is capable of bonding with Zn^{2+} at DCP's active center but do not have a water-repellent cyclic polymethylene chain that is so essential to the biological activity of III and its analogs [6].



I: R = H; II: R = CH_3.



III

In that connection, we thought it would be of interest to introduce fragments of amino acids I and II into molecules that have saturated azaheterocyclic structures. To accomplish that purpose amino acids I and II were reacted with lactim esters (IV) and lactan acetals (V). The latter are rather strong alkylating agents and are readily esterified upon reacting with acids [8]. Nevertheless, we were able to select conditions whereby esterification did not accompany the reaction and the target amidomercapto acids (VI) could be obtained at a good yield.

In addition to the hydrophobic polymethylene chain, the synthesized compounds also have a carboxyl and mercapto group as well as a C=N fragment that in principle are capable of bonding with the groups of DCP's active center.

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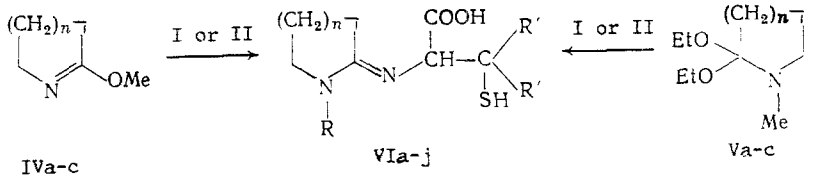
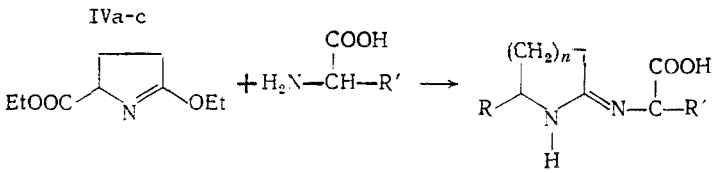


IVa-c VIa-j Va-c

IVa: $n = 1$; IVb: $n = 2$; IVc: $n = 3$. Va: $n = 1$; Vb: $n = 2$; Vc: $n = 3$.
VIa: $R' = H$, $R = H$, $n = 1$; VIb: $R' = H$, $R = Me$, $n = 1$; VIc: $R' = H$, $R = Me$, $n = 2$;
VId: $R' = H$, $R = H$, $n = 3$; VIe: $R' = H$, $R = Me$, $n = 3$; VIf: $R' = Me$, $R = H$, $n = 1$;
VIg: $R' = Me$, $R = Me$, $n = 1$; VIh: $R' = Me$, $R = H$, $n = 2$; VIi: $R' = Me$, $R = Me$, $n = 2$; VIj: $R' = Me$, $R = Me$, $n = 3$.

In order to ascertain the role played by individual groups in DCP inhibition and the antihypertensive action produced by the reaction between the lactim esters IV and VII and certain amino acids (I, II, and VIII), we synthesized a number of other amidine acids (IX) (including the earlier described acid IXe [12]) that either do or do not have a terminal mercapto group.



VII I, II, VIIIa-c IXa-f

VIIIa: $R' = (CH_2)_2SMe$; VIIIb: $R' = H$; VIIIc: $R' = (CH_2)_2CONH_2$. IXa: $R = COOEt$, $R' = CH_2SH$, $n = 1$; IXb: $R = COOEt$, $R' = CMe_2SH$, $n = 1$; IXc: $R = H$, $R' = (CH_2)_2SMe$, $n = 1$; IXd: $R = R' = H$, $n = 1$; IXe: $R = R' = H$, $n = 3$; IXf: $R = H$, $R' = (CH_2)_2CONH_2$, $n = 1$.

The structure of the resultant amidine acids was confirmed by element analysis, IR- and mass spectra. The IR-spectra of the synthesized compounds have CO and C=N group absorption bands in the 1600-1700 cm^{-1} region and the mass spectra exhibit molecular ionic peaks whose basic fragmentation paths are related to the splitting of the saturated rings and the removal of the carboxyl group. In the case of the mercapto derivatives that fragmentation is associated with the removal of the SH and H_2S groups.

Earlier [2] we suggested that compound III, its analogs, and other DCP inhibitors that contain amino acid residues, can be inactivated in vivo by peripheral decarboxylation and that their antihypertensive action can be amplified and prolonged by combining these preparations with decarboxylase inhibitors. In actual experimental conditions the hydrazides of the acids did in fact enhance and prolong the antihypertensive action of compound III [2]. Inasmuch as the compounds synthesized in our study are amino acid derivatives, an examination of that problem was only natural. At the same time this would enable us to esetablish the probable acid function contribution to the biological activity of the synthesized substances. The results of our study of the inhibiting activity and antihypertensive action of compounds VI and IX, and how their activity is affected by peripheral decarboxylation inhibitors are presented in the experimental pharmacological part.

EXPERIMENTAL (CHEMICAL)

2-(N-1-Carboxy-2-mercaptoethylimino)pyrrolidine (VIa). A 1.19 g (0.012 mole) portion of compound IVa in 5 ml of methanol was added dropwise at 20-25°C to a suspension of 1.09 g (0.01 mole) of compound I in 25 ml of methanol. The mixture was stirred at that temperature for 16 h and then vacuum evaporated. A 100 ml portion of absolute ether was added to the residue. The resultant precipitate was filtered off and the yield of compound VIa was 1.64 g. Compounds VIc, VIf, VIi, and VIj were obtained in a similar fashion. The physical constants, reaction conditions, yields, and analytical characteristics of the amidinomercaptic acid derivatives are given in Table 1.

1-Methyl-2-(N-1-carboxy-2-mercaptoethylimino(pyrrolidine (VIb). A 1.3 g (0.009) portion of Va in 5 ml of methanol was added dropwise at 10-15°C to a suspension of 0.7 g (0.006 mole) of compound I in 25 ml of methanol. The mixture was stirred for 4 h at 20-25°C, and then filtered. The filtrate was evaporated, the residue was washed two to three times with absolute ether (in 30 ml portions). The residue was then triturated in absolute ether and fil-

TABLE 1. Characteristics and Reactions of Synthesized Compounds

Compound	mp, °C (solvent)	Yield, %	Molar ratio of reagents	Reaction time, h	Found %				Empirical formula	Calculated, %			
					C	H	N	S		C	H	N	S
VIa	175–6 (Propanol-2)	87	10 (I) : 12 (IVa)	16	44,21	6,56	14,80	16,89	$C_7H_{22}N_2O_2S$	44,68	6,38	14,89	17,02
VIb*	183–6 (ethanol)	43	6 (I) : 9 (Va)	4	45,09	6,53	12,94	15,05	$C_8H_{14}N_2O_2S \cdot 2/3H_2O$	44,86	7,01	13,08	14,95
VIc	154–6 (methanol)	93	10 (I) : 12 (Vb)	4	49,95	7,47	12,92	14,74	$C_9H_{16}N_2O_2S$	50,00	7,41	12,96	14,81
VId	175–7 (Propanol-2)	80	10 (I) : 15 (IVc)	10	50,16	7,40	12,86	. . .	$C_9H_{16}N_2O_2S$	50,00	7,41	12,96	. . .
VIe	169–73 (Propanol-2)	87	10 (I) : 12 (Vc)	6	52,56	8,00	12,03	13,88	$C_{10}H_{18}N_2O_2S$	52,17	7,83	12,17	13,91
VIf	182–5 (methanol)	88	10 (II) : 15 (IVa)	8	50,02	7,27	12,95	15,00	$C_9H_{16}N_2O_2S$	50,00	7,41	12,96	14,81
VIg	150–2 (Propanol-2)	66	5 (II) : 7 (Va)	2	51,90	8,00	12,14	13,80	$C_{10}H_{18}N_2O_2S$	52,17	7,83	12,17	13,91
VIh	150–1 (Propanol-2)	48	5 (II) : 6 (IVb)	10	52,11	7,86	12,12	13,99	$C_{10}H_{18}N_2O_2S$	52,17	7,83	12,17	13,91
VIi	178–80 (ethanol)	90	5 (II) : 5,3 (Vb)	5**	53,70	8,50	11,12	12,95	$C_{11}H_{20}N_2O_2S$	54,10	8,20	11,48	13,11
VIj	183–5 (Propanol-2)	78	5 (II) : 6 (Vc)	8	56,03	8,40	10,96	12,32	$C_{12}H_{22}N_2O_2S$	55,81	8,53	10,85	12,40
IXa	175–7 (Propanol-2)	86	10 (I) : 10 (VII)	6	46,08	6,14	10,81	12,47	$C_{10}H_{16}N_2O_4S$	46,15	6,15	10,77	12,31
IXb	181–3 (Propanol-2)	80	5 (II) : 5 (VII)	4	50,20	7,30	. . .	11,31	$C_{12}H_{20}N_2O_4S$	50,00	6,94	. . .	11,11
IXc	184–6***	69	20 (VIIIa) : 22 (IVa)	9	49,96	6,90	13,27	15,08	$C_9H_{16}N_2O_2S$	50,23	6,97	13,02	14,88
IXd	245–7***	85	20 (VIII) : 26 (IVa)	4	50,43	6,80	19,86	. . .	$C_6H_{10}N_2O_2$	50,70	7,04	19,72	. . .
IXf	214–6***	78	10 (VIII) : 11 (IVa)	2,5	50,87	7,18	19,85	. . .	$C_9H_{15}N_3O_3$	50,70	7,04	19,72	. . .

*Found, %: H_2O 6.3. Calculated, %: H_2O 5.6.
**Reaction carried out in ethanol.
***Compunds IXc and d are dissolved in methanol for purification and precipitated out by abs. ether. Compound IXf was dissolved in ethanol. The solution was then boiled with charcoal, filtered, and evaporated.

tered off to yield 0.51 g of the final product VIb. Compounds VId, VIe, VIg, and VIh were obtained in a similar fashion.

2-(1-Carboxy-2-mercaptoethylimino)-5-ethoxycarbonyl Pyrrolidine (IXa). A mixture of 1.21 g (0.01 mole) of compound I, 1.85 g (0.01 mole) of VII [1], and 20 ml was stirred for 6 h at 50-60°C and then filtered. The filtrate was vacuum evaporated and the residue was triturated in absolute ether to yield 2.25 g of compound IXa. Compounds IXb and IXc were obtained by exposing the reagents to the same conditions at 20-25°C.

N-(Pyrrolidinylidene-2)glycine (IXd). A mixture of 2.48g (0.026 mole) of IVa and 1.5 g (0.02 mole) of VIIIc in 25 ml of methanol was stirred for 4 h at 20-25°C. The mixture was then cooled to 5-10°C and the precipitate was filtered off, resulting in a yield of 1.94 g. The filtrate was vacuum evaporated and the residue was triturated in abs. ether which resulted in an additional yield of 0.47 g of compound IXd.

N-(Pyrrolidinylidene-2)glutamine (IXf). A mixture of 1.1 (0.11 mole) of IVa and 1.46 g (0.01 mole) of VIIIc in 25 ml of methanol was kept at a boil for 2.5 h. The mixture was then cooled and the precipitate was filtered off, resulting in a yield of 1.1 g. The filtrate was vacuum evaporated and the residue was washed with abs. ether and triturated in abs. ethanol which resulted in an additional yield of 0.57 g of IXf.

EXPERIMENTAL (PHARMACOLOGICAL)

The derivatives of the amidinomercaptic acids (VI and IX) were tested for properties characteristic of DCP inhibitors (angiotensin of I-transfer enzyme), i.e., the ability to inhibit the indicated enzyme, the ability to enhance and prolong the effect of bradykinin (BK) and to exhibit an antihypertensive effect. The acute toxicity of the indicated compounds was also studied.

The effect the compounds had on the activity of ox renal DCP was assayed by the splitting of the tripeptide L-Phen-His-Leu which constitutes the C-end fragment of angiotensin I [3]. Subsequent to the enzyme-induced separation of the dipeptide His-Leu, the latter reacted with o-phthalic dialdehyde in an alkaline medium to form a product whose fluorescence was measured on an Opton spectrofluorometer (excitation at 370 nm, fluorescence at 500 nm). The His-Leu content in the tested samples was computed by the fluorescence of a standard His-Leu solution. DCP activity was expressed in μmoles of His-Leu/min. In the course of investigating the inhibiting effect that amidinomercaptic acids had on enzyme activity, 0.1 ml of the latter was preliminarily incubated in 1.9 ml of a sodium barbital buffer at pH 7.4 with each of the compounds for 15-20 min at 37°C after which 0.1 ml of a 1 mM solution of the L-Phen-His-Leu substrate was added. This was followed by fluorometry.

An isolated guinea pig lung preparation was used to examine the effect that the compounds had on the activity of pulmonary kininases which play an important role in kinin and angiotensin metabolism [14]. Pulmonary perfusion was accomplished through the pulmonary artery by a Krebs solution with or without amidinomercaptic acid derivatives (the tested concentrations ranged from $1 \cdot 10^{-7}$ to $1 \cdot 10^{-3}$ g/ml). The degree of kininase inhibition was determined by measuring the residual activity of BK which had passed through the vessels of the isolated lungs before and after a 5-minute perfusion of a potential inhibitor solution. BK activity in the perfusate backflow from the lungs was evaluated by the contractile magnitude of an isolated guinea pig ileum section. This was then compared to the response to BK (at the same concentration, i.e., 1-10 mg/ml) which was placed directly into the beaker with the isolated organ [7].

The experiments on isolated guinea pig ileum sections also tested the compounds' effect on bradykinin's spasmogenic effect ($1 \cdot 10^{-9}$-$1 \cdot 10^{-8}$ g/ml).

The compounds' effect on BK depressor effect was tested on anesthetized male rats with normal blood pressure. Arterial pressure was recorded in the common carotid by a mercury manometer. The BK (1 μg/kg) and the test compounds, dissolved in an isotonic NaCl solution, were administered through a catheter into the jugular vein [10].

The antihypertensive properties of the compounds were tested on nonanesthetized male rats weighing 140-160 g with a model of double kidney renal vascular hypertension.

Arterial pressure was measured exsanguinely in the caudal artery by a semiautomatic KN-209 gage (Natume, Japan) prior to and one and three hours after the test compounds were

TABLE 2. Effect of VI and IX on the Activity of Kininases, the Effects of Bradykinin and Arterial Pressure in Rats

Compound	Antihypertensive activity in rats with renal hypertension* - Arterial pressure reduction, mm Hg	DCP inhibition at a concentration of $1 \cdot 10^{-3}$ M, %	Retardation of BK-inactivation of lungs at a concentration of $1 \cdot 10^{-3}$ M, %	Amplification of BK depressor effect in rats at a dose of 10 mg/kg, %	LD_{50} for Mice in oral administration, mg/kg
VIa	40±12	70	74	27±7	1000
VIb	0	90	100	72±9	1000
VIc	31±10	91	47	42±7	1000
VId	35±8	89	65	61±12	1000
VIe	55±12	66	80	18±7	1000
VIf	8±3**	17	66	35±5	1000
VIg	10±5**	75	45	45±10	1000
VIh	51±10	35	43	120±14	1000
VIi	18±7	28	36	11±5**	1000
VIj	15±8**	0	0	75±15	1000
IXa	18±5	70	56	58±11	950
IXb	15±8**	35	44	35±15	1000
IXc	24±5	5	27	15±7**	1000
IXd	13±7**	33	35	28±12	1000
IXe	26±4	30	42	23±13	1000
IXf	33±11	17	22	0	900
Cysteine	19±6	90	70	140±12	1000
D-penicillamine	28±8	65	84	200±17	720
III	55±7*4	$1 \cdot 10^{-9}$***	$1 \cdot 10^{-8}$ (100%)	300±24	2500

*At an oral dose of 100 mg/kg.
**Differences fron control unreliable ($P \leq 0.05$).
***Concentration inhibiting 50% enzyme activity.
****At an oral dose of 10 mg/kg.

introduced into the stomach by a probe at a dose of 100 mg/kg. The action of the preparations was compared to the activity of D-penicillamine (100 mg/kg), cysteine (200 mg/kg), and compound III (10 mg/kg) which were administered in the same fashion.

The compounds' acute toxicity was assayed intragastrically on male mice weighing 16-17 g. The LD_{50} was computed graphically [9].

The experiments demonstrated that the derivatives of amidine and amidinomercaptic acids are only slightly toxic but that most of them inhibit DCP to various degrees as well as exhibit kinin-positive properties and the ability to lower arterial pressure in hypertensive rats (Table 2).

As a whole, the cyclic cysteine derivatives (compounds VIa-e) inhibited enzyme activity more than the D-penicillamine derivatives (VIe-j). Moreover, the addition of a carbethoxy-group to the pyrrolidine ring (compound IXa) did not alter its inhibiting activity, but reduced its antihypertensive action. Similar changes were observed in the activity of the corresponding D-penicillamine derivatives (compounds VIf-j and IXb).

The replacement of a thiol group in compound VIa by a CH_2SMe group (methionine derivative) resulted in a marked reduction in both the inhibitory effect on enzyme activity and the antihypertensive activity of compound IXc. Similar pharmacological property changes were noted when the CH_2SH group was removed (compounds VIa and IXd, and VId and IXe respectively) as well as when the SH group was replaced by CH_2CONH_2 (L-glutamine derivative as represented by IXf).

One should note that none of the examined compounds exhibited a clear relationship between the structure of the cyclic radical (from a 5-membered to 7-membered radical) and the extent of the enzyme-inhibiting, kinin-positive, and antihypertensive properties. There was also no correlation between enzyme-inhibiting action and the degree of antihypertensive properties. Similar observations have been made by other investigators in their study of the relationship between the indicated properties of DCP inhibitors.

At the same time we found substances among the examined compounds that were two or three times more active than cysteine and D-penicillamine. However, all of them were less than

effective than compound III with respect to their enzyme-inhibiting action and antihypertensive activity.

We also examined the effect of isoniazid, a decarboxylase inhibitor, on the antihypertensive action of compound VIj, one of the amidine derivatives of D-penicillamine.

The experiments on rats with renal hypertension demonstrated that a preliminary oral isoniazid dose of 50 mg/kg potentiated the effect of VIj at an oral dose of 25 mg/kg on arterial pressure by raising it from 25 ± 8 to 72 ± 7 mm Hg, i.e., almost tripling the pressure. One might consequently assume that both natural amino acid derivatives and those of amidinomercaptic acids are affected by decarboxylating enzymes in the body, and that their effect can be modified by the concurrent administration of decarboxylation enzyme inhibitors.

Our chemical and pharmacological experiments have demonstrated that in a number of cases the transition from aminomercaptic acids (cysteine, D-penicillamine) to amidinomercaptic acids is accompanied by an increase in antihypertensive properties. Our experimental data allow us to hypothesize that the lowered arterial pressure brought about by these substances in hypertensive animals is associated with their inhibiting effect on DCP activity as well as with kinin-positive properties. It is significant that these substances should contain fragments of such functional groups as the mercapto group, a cyclic polymethylene chain, an amidine and carboxy groups in order for the indicated properties to be manifested. The importance of the carboxyl group in that respect was illustrated by compound VIj whose antihypertensive effect was significnatly potentiated when it was administered together with the peripheral decarboxylase inhibitor, isoniazid.

Thus, the results of our study allow us to conclude that the amidinomercaptic acids can be viewed as a new class of DCP inhibitors among which active antihypertensive agents with low toxicity might be found.

LITERATURE CITED

1. R. G. Glushkov, V. G. Granik, V. A. Volskova, et al., Khim-farm. Zh., No. 11, 14-16 (1973).
2. V. G. Granik, G. Ya. Shvarts, I. F. Faermark,e t al., Khim.-farm. Zh., No. 7, 778-782 (1985).
3. Yu. E. Eliseeva, V. N. Orekhovich, and L. V. Pavlikhina, Biokhimiya, 41, No. 3, 506-512 (1976).
4. G. Ya. Shvarts, Khim.-farm. Zh., No. 2, 7-19 (1979).
5. G. Ya. Shvarts, T. S. Paskhina, T. P. Egorova, et al., Khim.-farm. Zh., No. 8, 18-23 (1981).
6. G. Ya. Shvarts and V. G. Granik, Khim.-farm. Zh., No. 3, 271-282 (1984).
7. P. N. Aarsen and A. Zeegers, Br. J. Pharmacol., 45, 284-288 (1972).
8. T. T. Fitt and H. W. Gschwend, J. Org. Chem., 42, 2639-2641 (1977).
9. J. Litchfield and F. Wilcoxon, J. Pharmacol. Exp. Ther., 96, 99-113 (1949).
10. J. Möhring, Clin. Nephrol., 5, 167-174 (1975).
11. M. A. Ondetti and D. W. Cushman, Biochemical Regulation of Blood Pressure, ed. R. S. Soffer, New York (1981), pp. 166-204.
12. S. Petersen, and E. Tietze, Justus Liebigs Ann. Chem., 623, 166-176 (1959).
13. J. R. Prous, Annu. Drug Data, 4, 220, 1982.
14. J. W. Ryan and U. S. Ryan, Life Sci., 16, 812-816 (1975).