SUBSTANCES WITH ANTINEOPLASTIC ACTIVITY. LIII.* N-{δ-(4-PYRROLO[2,3-*d*]PYRIMIDINYLTHIO)VALERYL}AMINO ACIDS AND ANALOGOUS DERIVATIVES OF DI- AND TRIGLYCINE

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Received July 11th, 1972

δ-(4-Pyrrolo[2,3-*d*]pyrimidinylthio)valeric acid was prepared by condensation of 4-mercaptopyrrolo[2,3-*d*]pyrimidine with δ-bromovaleric ester and by subsequent saponification. Ethyl esters of N-{δ-(4-pyrrolo[2,3-*d*]pyrimidinylthio)valeryl}amino acids *IIa* and *IVa* and analogous derivatives of di- and triglycine *IIIa* and *Va* were prepared by condensation of 4-mercaptopyrrolo[2,3-*d*]pyrimidine with the ethyl esters of the corresponding N-(δ-bromovaleryl)amino acids, -diglycine and -triglycine, esters *VIa*—*VIIIa* by the chloride method from acid *I* and the ethyl ester of the corresponding amino acid. The compounds prepared show no pronounced antineoplastic activity in animals with transplantable tumours.

The subject of the present communication is the synthesis of δ-(4-pyrrolo[2,3-*d*]-pyrimidinylthio)valeric acid (*I*), of its esters *Ia* and *Ib*, N-{δ-(4-pyrrolo[2,3-*d*]-pyrimidinylthio)valeryl}amino acids *II*, *IV*, *VI*—*VIII*, their ethyl esters *IIa*, *IVa*, *VIa*—*VIIIa*, and analogous derivatives of diglycine *III* and *IIIa*, and of triglycine *V* and *Va* (Table I) where antineoplastic activity was assumed for animals with transplantable tumours.

In the earlier papers of this series[1,2] it was found that some N-{δ-(6-purinylthio)valeryl}amino acids, -dipeptides, -tripeptides and their esters with high affinity for the tissues of certain organs display a selective antineoplastic activity for mice and rats with a given transplantable tumour. These compounds represent the transport form of the sulfide-bound 6-mercaptopurine. We were interested to see in what way the replacement of the 6-mercaptopurine residue in the molecule of the above compounds with 4-mercaptopyrrolo[2,3-*d*]pyrimidine (7-desaza-6-mercaptopurine), *i.e.* the replacement of nitrogen in position 7 of the purine ring with the methine group, would affect the antineoplastic activity. In the molecules of *I*—*VIII* and of their esters, two structural principles of antimetabolite character merge: the 6-mercaptopurine one, present in δ-(6-purinylthio)valeric acid[3],** and the pyrrolo[2,3-*d*]pyrimidine one, present *e.g.* in the cancerostatically active antibiotics tubercidin, toyokamycin and sangivamycin[4,5].

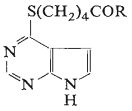
The desired compounds were prepared in principle by using the previously described methods[1,2,6]: ethyl esters of N-{δ-(4-pyrrolo[2,3-*d*]pyrimidinylthio)valeryl}amino

* Part LII: This Journal *38*, 1091 (1973).

** δ-(6-Purinylthio)valeric acid is produced for pharmaceutical purposes under the name Cytogran-Spofa.

acids (*IIa*, *IVa*) and analogous derivatives of diglycine and triglycine (*IIIa*, *Va*) were obtained by condensation of 4-mercaptopyrrolo[2,3-*d*]pyrimidine[7] with ethyl esters of the corresponding N-(δ-bromovaleryl)amino acids, -diglycine and -triglycine in an aqueous ethanolic solution in the presence of 1·1 molar equivalents of sodium hydroxide (method *A*). The triglycine ester *Va* was also prepared by condensation of the diglycine derivative *III* with the glycine ethyl ester by using N,N-carbonyldiimidazol (method *B*). Esters *VIa – VIIIa* were prepared by the chloride method from acid *I*. The chloride of acid *I* was prepared by a reaction of acid *I* with thionyl chloride in the presence of a catalytic amount of dimethylformamide and was processed further without isolation. Acids *I – VIII* were prepared by saponification of esters *Ia – VIIIa* with 2 – 3 molar equivalents of an aqueous solution of sodium hydroxide at room temperature.

The methods of preparation of *I – VIII*, *Ia – VIIIa* and *Ib*, some of their physicochemical properties and yields in which they were obtained are summarized in Table I. The preparation of the required N-(δ-bromovaleryl)amino acids and of their esters has been described earlier[6].



An informative evaluation of the compounds as to their antineoplastic activity in animals with transplantable tumours has been done at this institute by Dr V. Jelínek and Dr H. Veselá with coworkers. Mice of the H strain with S 37 sarcoma and Wistar rats with an ascitic Yoshida sarcoma Y were used; in some cases also the same strain of mice with a S 180 sarcoma, with a mammary gland adenocarcinoma, with an Ehrlich ascites tumour and a Krebs ascites carcinoma (Kr 2). The compounds were applied *per os* in a daily dose of 200 mg/kg; the mice received the dose for 12 days with the exception of Sunday, the rats for 5 days, beginning on the third or second day after tumour transplantation, respectively. Details on the method and evaluation of results in ref.[8]. Of the compounds studied, *IVa* inhibited the growth of Kr 2 by 22%, *VIIa* by 30%, without simultaneous pronounced effect on the survival of the experimental animals. (The tumour weight of the control group of animals, as well as their lifetime, were set equal to 100%). Compound *VIIa* extended the survival of the treated rats with Y sarcome by 38%, *VIIIa* by 26%. Other compounds were not antineoplastically interesting.

In summary, it may be said that replacement of nitrogen in position 7 of the molecule of N-[δ-(6-purinylthio)valeryl]amino acids and the same derivatives of diglycine and triglycine, and of their ethyl esters, with a methine group, does not result in any antineoplastically more active compounds.

TABLE I
Yields and Some Properties of Compounds Prepared

Compound R	Yield %	M.p., °C (solvent)	Formula (m. w.)	Calculated/Found % C	% H	% N	% S	UV-Spectra λ_{max}, nm (log ε) 0·1M-HCl	0·1M-NaOH
I									
—OH	97	198—200[a]	$C_{11}H_{13}N_3O_2S$ (251·3)	52·60 52·67	5·21 5·27	16·72 16·85	12·74 12·70	312 (4·052) 258 (3·978) 220 (4·334)	292 (4·127) 248 (3·930) 221 (4·308)
Ia									
—OCH_3	95	100—102 (acetone–hexane)	$C_{12}H_{15}N_3O_2S$ (265·3)	54·31 54·45	5·70 5·95	15·84 15·75	12·08 12·34	311 (3·968) 258 (3·890) 220 (4·302)	294 (4·053) 249 (3·897) 222 (4·282)
Ib									
—OC_2H_5	84	113—114 (acetone–hexane)	$C_{13}H_{17}N_3O_2S$ (279·3)	55·89 56·00	6·13 6·29	15·04 15·18	11·47 11·28	311 (3·978) 258 (3·886) 220 (4·302)	294 (4·064) 249 (3·908) 222 (4·305)
II									
—$NHCH_2COOH$	90	237—239[a]	$C_{13}H_{16}N_4O_3S$ (308·3)	50·63 50·62	5·23 5·52	18·17 17·96	10·40 10·48	310 (4·048) 257 (4·025) 218 (4·373)	292 (4·156) 246 (3·990) 220 (4·365)
IIa									
—$NHCH_2COOC_2H_5$	58	147—148 (aqueous ethanol)	$C_{15}H_{20}N_4O_3S$ (336·4)	53·55 53·39	5·99 5·84	16·66 16·57	9·53 9·83	311 (4·022) 258 (3·940) 220 (4·350)	294 (4·103) 249 (3·940) 222 (4·328)
III									
$(-NHCH_2CO)_2OH$	82	215—217 (water)	$C_{15}H_{19}N_5O_4S$ (365·4)	49·30 49·58	5·24 5·52	19·17 19·28	8·78 8·85	310 (4·048) 257 (4·008) 218 (4·342)	292 (4·130) 248 (3·954) 220 (4·322)

IIIa										
(—$NHCH_2CO)_2OC_2H_5$	72	172—174 (aqueous ethanol)	$C_{17}H_{23}N_5O_4S$ (393·5)	51·89 51·75	5·89 6·18	17·80 17·58	8·15 8·03	310 (4·056) 257 (3·973) 218 (4·297)	292 (4·117) 247 (3·924) 222 (4·292)
IV										
—$NHCHCOOH$ -D,L $\vert$ $CH(CH_3)_2$	81	154—156[a]	$C_{16}H_{22}N_4O_3S$ (350·4)	54·83 54·56	6·33 6·37	15·99 15·70	9·15 9·52	311 (4·028) 258 (3·992) 220 (4·354)	293 (4·106) 249 (3·964) 222 (4·350)
IVa										
—$NHCHCOOC_2H_5$ -D,L $\vert$ $CH(CH_3)_2$	95	95—97 (acetone–hexane)	$C_{18}H_{26}N_4O_3S$ (378·5)	57·11 57·22	6·93 6·91	14·80 14·78	8·47 8·67	312 (4·084) 257 (4·000) 219 (4·434)	291 (4·154) 246 (4·030) 221 (4·383)
V										
(—$NHCH_2CO)_3OH$	50	216—218 (water)	$C_{17}H_{23}N_6O_5S$ (422·5)	48·33 48·55	5·25 5·18	19·90 19·48	7·59 8·03	311 (4·108) 258 (4·029) 219 (4·359)	293 (4·164) 248 (4·004) 220 (4·328)
Va										
(—$NHCH_2CO)_3OC_2H_5$	78[b]	235—237 (aqueous ethanol)	$C_{19}H_{26}N_6O_5S$ (450·5)	50·65 50·44	5·81 5·96	18·66 18·36	7·12 7·13	308 (4·068) 256 (3·968) 219 (4·326)	294 (4·190) 250 (3·958) 222 (4·330)
VI										
—$NHCHCH_2COOH$[c] -L $\vert$ $COOH$	93	178—179[d]	$C_{15}H_{18}N_4O_5S.$ H_2O (384·4)[e]	46·86 46·74	5·25 5·38	14·57 14·41	8·34 8·56	310 (4·075) 257 (3·990) 219 (4·330)	292 (4·130) 247 (3·958) 219 (4·292)
VIa										
—$NHCHCH_2COOC_2H_5$[f] -L $\vert$ $COOC_2H_5$	86	128—129 (acetone–hexane)	$C_{19}H_{26}N_4O_5S$ (422·5)	54·01 54·08	6·20 6·28	13·26 13·52	7·59 7·83	312 (4·079) 258 (3·992) 220 (4·322)	292 (4·132) 245 (3·968) 219 (4·362)

TABLE I
(*Continued*)

Compound R	Yield %	M.p., °C (solvent)	Formula (m. w.)	Calculated/Found				UF-Spectra λ_{max}, nm (log ε)	
				% C	% H	% N	% S	0·1M-HCl	0·1M-NaOH
VII									
—NHCH(CH_2)$_2$COOH[g] -L COOH	80	192—193[d]	$C_{16}H_{20}N_4O_5S$ (380·4)	50·51 50·22	5·30 5·60	14·73 14·90	8·43 8·64	310 (4·064) 257 (3·996) 218 (4·334)	294 (4·097) 248 (3·903) 219 (4·387)
VIIa									
—NHCH(CH_2)$_2$COOC_2H_5[h] -L $COOC_2H_5$	97	110—111 (acetone–hexane)	$C_{20}H_{28}N_4O_5S$ (436·5)	55·02 55·18	6·46 6·60	12·83 13·11	7·34 7·60	311 (3·982) 258 (3·990) 220 (4·302)	294· (4·060) 249 (3·875) 222 (4·264)
VIII									
—NHCHCOOH[i] -L $CH_2CH(CH_3)_2$	30	182—184[a]	$C_{17}H_{24}N_4O_3S$ (364·5)	56·02 56·06	6·64 6·63	15·37 15·42	8·80 8·76	311 (3·982) 258 (3·886) 220 (4·273)	294 (4·053) 249 (3·852) 222 (4·292)
VIIIa									
—NHCHCOOC_2H_5[j] -L $CH_2CH(CH_3)_2$	87	87—89 (acetone–hexane)	$C_{19}H_{28}N_4O_3S$ (392·5)	58·13 57·84	7·19 7·39	14·27 14·23	8·17 8·34	311 (4·103) 257 (4·038) 217 (4·373)	292 (4·097) 246 (3·959) 219 (4·397)

[a] Purified by precipitation from 1% NH_4OH with acetic acid. [b] Method C yielded 97%. [c] $[\alpha]_D^{20}$ +12·3° (*c* 1, 0·1M-NaOH). [d] Purified by precipitation from 1% NH_4OH with hydrochloric acid. [e] On drying at 132°C/0·2 Torr (P_2O_5) the compound lost 4·70% its weight, which corresponds to 1 mol crystal water; for $C_{15}H_{18}N_4O_5S.H_2O$ (384·4) calculated: 4·69% H_2O. For $C_{15}H_{18}N_4O_5S$ (366·4) calculated: 49·17% C, 4·95% H, 15·29% N, 8·75% S; found: 49·02% C, 5·25% H, 15·09% N, 8·96% S. [f] $[\alpha]_D^{26}$ −7·98° (*c* 1, ethanol). [g] $[\alpha]_D^{20}$ +2·5° (*c* 1, 0·1M-NaOH). [h] $[\alpha]_D^{26}$ −10·51° (*c* 1, ethanol). [i] $[\alpha]_D^{20}$ −29° (*c* 1, 0·1M-NaOH). [j] $[\alpha]_D^{20}$ −17·6° (*c* 1, ethanol).

EXPERIMENTAL

The melting points of the compounds were determined in Kofler's block and are not corrected. The samples for analysis were dried *in vacuo* at 0·2 Torr over P_2O_5 at a temperature raised in proportion to the melting point. The UV spectra were measured in a Unicam SP-700 spectrophotometer in 1 cm quartz cuvettes at a concentration of 1 mg/100 ml aqueous–methanolic (1 : 1) 0·1M-HCl or 0·1M-NaOH. The values of specific rotation refer to compounds free of crystal solvent and were determined in a Perkin-Elmer 141 polarimeter with an accuracy of ±1°. The purity was checked by paper chromatography[9] in 1-butanol–acetic acid–water (4 : 1 : 5), 1-butanol–pyridine–water (6 : 4 : 3), 2-propanol–ammonia–water (10 : 1 : 1), or in benzene, or a mixture of benzene with cyclohexane (7 : 3), on a formamide-impregnated paper. The yields shown in Table I refer to crude but relatively rather pure compounds.

Methyl and Ethyl Ester of δ-(4-Pyrrolo[2,3-*d*]pyrimidinylthio)valeric Acid (*Ia*, *Ib*)

A mixture of 15·12 g (0·1 mol) 4-mercaptopyrrolo[2,3-*d*]pyrimidine[7] in a solution of 4·40 g (0·11 mol) sodium hydroxide in 115 ml water and 19·50 g (0·1 mol) methyl ester of δ-bromovaleric acid in 120 ml methanol, or 20·91 g (0·1 mol) ethyl ester of δ-bromovaleric acid in 120 ml ethanol, was refluxed for 3 h. After distillation of most of the volatile fractions in water-pump vacuum, the residue was extracted with 250 ml 0·1M NaOH plus 250 ml chloroform, the organic phase was washed twice with 250 ml water, dried with Na_2SO_4 and the solvent was removed by distillation; crude ester *Ia* or *Ib*, was purified by crystallization (Table I).

δ-(4-Pyrrolo[2,3-*d*]pyrimidinylthio)valeric Acid (*I*)

0·1 mol ester *Ia* was combined with a solution of 8·8 g (0·22 mol) sodium hydroxide in 220 ml water at room temperature, the mixture was stirred until the solid dissolved and then left to stand for 2 days. After acidification with hydrochloric acid to pH 3, the precipitated acid was purified by crystallization (Table I).

Ethyl Esters of N-{δ-(4-Pyrrolo[2,3-*d*]pyrimidinylthio)valeryl}amino Acids, -diglycine and triglycine

Method A (esters *IIa*–*Va*): A solution of 0·012 mol ethyl ester of N-(δ-bromovaleryl)amino acid in 11 ml ethanol at 20°C (or 0·012 mol ethyl ester of N-(δ-bromovaleryl)diglycine in 240 ml 70% aqueous ethanol at 55°C, or 0·012 mol ethyl ester of N-(δ-bromovaleryl)triglycine in 325 ml 50% aqueous ethanol at 55°C) was added to a solution of 1·51 g (0·01 mol) 4-mercaptopyrrolo-[2,3-*d*]pyrimidine in 11 ml (0·011 mol) 1M-NaOH. The mixture was stirred for 4 h at 20°C and then left to stand overnight. In the case of esters *IIa* and *IIIa* the precipitated product was purified after filtration by crystallization. In the case of ester *Va*, most of the ethanol was removed from the mixture by distillation at reduced pressure and, after 3 h at 5°C, the product was filtered and crystallized. In the case of ester *IVa* the mixture was shaken with 25 ml of a mixture of chloroform with ethanol (4 : 1), the organic fraction was washed with 0·1M-$NaHCO_3$ and water; after drying with Na_2SO_4, the solvent was distilled off and the residue was purified by crystallization (Table I).

Method B (ester *Va*): 10·96 g (0·03 mol) acid *III*, dried at 95–100°C/0·2 Torr was combined under stirring at room temperature to a solution of 5·85 g (0·036 mol) N,N′-carbonyldiimidazol in 60 ml dimethylformamide. After 1 h of stirring, the mixture was combined with 6·20 g (0·06 mol) glycine ethyl ester and after 1 h of stirring at 20°C, the mixture was left to stand overnight. After distilling off most of the dimethylformamide at reduced pressure at 60°C, the residue was mixed with 160 ml 10% aqueous acetic acid and the precipitated ester *Va* was crystallized (Table I).

Method C (esters *VIa*–*VIIIa*): 1·3 g (0·011 mol) thionyl chloride was added dropwise under stirring at room temperature to a mixture of 2·51 g (0·01 mol) acid *I*, 50 ml dichloromethane

and 0·1 ml dimethylformamide. The mixture was refluxed for 2 h under stirring with exclusion of air moisture. After cooling, 0·011 mol amino acid ethyl ester hydrochloride was added and, at −5° to +5°C, it was combined with 4·04 g (0·04 mol) triethylamine. The mixture was left to stand overnight at room temperature and extracted with a mixture of 100 ml water and 20 ml ethanol. The organic fraction was washed with 1M-$NaHCO_3$ and water and after drying with Na_2SO_4 it was evaporated. The residue was crystallized (Table I).

N-{δ-(4-Pyrrolo[2,3-*d*]pyrimidinylthio)valeryl}amino Acids *II*–*VIII*

0·1 mol ester *IIa*, *IIIa*, *IVa*, *Va* or *VIIIa* was added to a solution of 8·8 g (0·22 mol) NaOH in 440 ml water at room temperature. The mixture was stirred until the solid dissolved and then left to stand at room temperature for 2 days. After acidification with HCl to pH 3, the acid was crystallized (Table I). In the case of acids *VI* and *VII* 0·1 mol esters *VIa* or *VIIa* were saponified with a solution of 13·2 g (0·33 mol) sodium hydroxide in 440 ml water. The conditions of ester saponification and processing of the reaction mixtures were the same as described above.

The analyses were done by Mr K. Havel, Mrs J. Komancová and Mrs V. Šmídová under the direction of Dr J. Körbl; evaluation of compounds by paper chromatography was done by Miss D. Dosedlová under the direction of Dr V. Rábek; the UV spectra were measured by Dr J. Vachek, all of this Institute.

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Translated by A. Kotyk.