SUBSTANCES WITH ANTINEOPLASTIC ACTIVITY. L.*

N-[δ-(2-AMINO-6-PURINYLTHIO)VALERYL]AMINO ACIDS, THEIR ETHYL ESTERS AND ANALOGOUS DERIVATIVES OF DI- AND TRIGLYCINE

A. ČERNÝ, R. KOTVA and M. SEMONSKÝ

Research Institute of Pharmacy and Biochemistry, Prague 3

Received July 27th, 1971

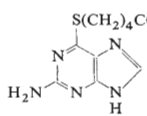
N-[δ-(2-Amino-6-purinylthio)valeryl] derivatives of glycine, diglycine, triglycine, DL-valine, L-leucine, L-aspartic acid and L-glutamic acid were synthesized in the form of ethyl esters as well as of free acids. Their *in vivo* antineoplastic effect was studied for orientation purposes.

It was described[1,2] that N-[δ-(6-purinylthio)valeryl]amino acids, dipeptides, tripeptides and their esters show a selective antineoplastic efficiency toward mice and rats bearing certain transplantable tumours, due to their high affinity for certain tissues. These compounds represent the transport form of the 6-mercaptopurine bound in them by a sulfide bond. It was of interest to establish the antineoplastic effect of replacing the 6-mercaptopurine residue in the molecule of these compounds by the antineoplastically active, but much more toxic, 6-thioguanine[3].

The compounds were prepared in principle by the methods described earlier[1,2,4]; the ethyl esters of N-[δ-(2-amino-6-purinylthio)valeryl]amino acids and analogous derivatives of diglycine and triglycine (*Ia–Va*) were obtained by condensation of 6-thioguanine with the corresponding ethyl esters of N-(δ-bromovaleryl)amino acids, diglycine and triglycine[4] in an aqueous–ethanolic medium, in the presence of 1·1 molar equivalents of sodium hydroxide (method A). The derivative of triglycine *IIIa* was obtained by the method described in a relatively low yield (43%). For its preparation it was of advantage to employ the condensation of the diglycine *II* derivative with the glycine ethyl ester using N,N′-carbonyldiimidazol (method B) (76% of ester *IIIa*). Acids *VI* and *VII* were prepared by condensation of the sodium salt of 6-thioguanine with N-(δ-bromovaleryl)-L-aspartic acid or with N-(δ-bromovaleryl)-L-glutamic acid, in the presence of 2·2 molar equivalents of triethylamine, in an aqueous medium at 30–35°C (method C); the acids *I–III* were prepared by saponification of esters *Ia–IIIa* with 2 molar equivalents of an aqueous solution of sodium hydroxide at room temperature (method D). Some physico-

* Part XLIX: This Journal *37*, 2077 (1972).

chemical properties and yields of compounds *I*–*III*, *VI*, *VII* and *Ia*–*Va* are summarized in Table I.



I, R = —CH_2COOH

II, R = —$CH_2CONHCH_2COOH$

III, R = —$(CH_2CONH)_2CH_2COOH$

IVa, R = —$CH(CH_3)_2$ | $CHCOOC_2H_5$

Va, R = —$CH_2CH(CH_3)_2$ | $CHCOOC_2H_5$

VI, R = —CH_2COOH | $CHCOOH$

VII, R = —CH_2CH_2COOH | $CHCOOH$

Compounds *Ia*, *IIa* and *IIIa* are the corresponding ethyl esters.

The homogeneity of the compounds prepared was examined by paper and silica gel chromatography and by spectrophotometry in the UV region of the spectrum in 0·1M-HCl and 0·1M-NaOH in 50% methanol. The preparation of N-(δ-bromovaleryl)amino acids and of their esters was described earlier[4]. The antineoplastic efficiency of the compounds *in vivo* was tested by Dr V. Jelínek and Dr H. Veselá of this Institute. The methods used are described in ref.[5]. Of the compounds tested the greatest promise was shown by *IIIa* applied to H strain mice with a transplantable S 37 tumour: at a dose of 200 mg/kg per os daily, applied continually for 12 days, beginning on the second day after tumour transplantation, the compound brings about a survival of the treated animals by 78%, without a clear effect on the tumour size (100% = survival of the control, untreated group with the same tumour). Compound *I* decreases with the same dosage the size of mammary adenocarcinome of H-strain mice by 65%, with a practically identical survival as with the control, untreated group of animals (100% corresponds to the average tumour size in the untreated group of animals). More details on the results of testing of the compounds prepared will be published elsewhere.

EXPERIMENTAL

The melting points were determined in Kofler's block and are not corrected. Samples for analysis were dried *in vacuo* (0·2 Torr) over phosphorus pentoxide at a temperature increased in proportion to their melting point. Some of the compounds contain crystal solvent and the dried substances are hygroscopic. The UV spectra of the compunds were recorded on a Unicam SP-700 spectrophotometer in 1 cm quartz cuvettes at a concentration of about 1 mg compound in 100 ml aqueous–methanolic (1 : 1) 0·1M-HCl or 0·1M-NaOH. The values of specific rotation refer to compounds free of the crystal solvent and were determined in a Perkin-Elmer type 141 polarimeter at an accuracy of ±1°. The purity of the compounds was followed by chromatography on thin layers of silica gel G (Merck) using chloroform–ethanol (9 : 1) or 2-propanol–ammonia–

TABLE I

N-[δ-(2-Amino-6-purinylthio)valeryl]amino Acids and Their Derivatives

No	Method (yield) %	M.p., °C (solvent)	Formula (M.w.)	Calc./Found C %	H %	N %	S %	UV Spectrum λ_{max} (log ε) 0·1M-HCl	0·1M-NaOH
I	D (90)	154—156 (water)	$C_{12}H_{16}N_6O_3S.$ .3 H_2O[a] (378·4)	38·09 38·07	5·86 5·60	22·21 22·65	8·47 8·54	315 (4·21) 275 (4·15) 238 (3·86)	311 (4·10) 224 (4·35)
Ia	A (70)	145—146 (water)	$C_{14}H_{20}N_6O_3S$ (352·4)	47·71 47·42	5·72 5·99	23·85 24·13	9·10 9·32	317 (4·22) 276 (4·12) 240 (3·96)	313 (4·09) 225 (4·39)
II	D (87)	230—232 (water)	$C_{14}H_{19}N_7O_4S$ (381·4)	44·09 43·89	5·02 5·02	25·71 25·46	8·41 8·69	318 (4·24) 275 (4·11) 240 (3·91)	315 (4·12) 227 (4·30)
IIa	A (92)	202—204 (aq. ethanol)	$C_{16}H_{23}N_7O_4S$ (409·5)	46·93 46·64	5·66 5·93	23·95 23·82	7·83 7·95	316 (4·25) 277 (4·13) 241 (3·73)	311 (4·11) 224 (4·34)
III	D (98)	241—243 (water)	$C_{16}H_{22}N_8O_5S$ (438·5)	43·82 43·89	5·06 5·40	25·56 25·20	7·31 7·50	316 (4·20) 274 (4·12) 236 (3·96)	314 (4·12) 226 (4·38)
IIIa	A, B (43, 76)	250—252 (aq. ethanol)	$C_{18}H_{26}N_8O_5S$ (466·5)	46·34 46·05	5·61 5·51	24·03 24·33	6·87 6·97	317 (4·21) 276 (4·12) 240 (3·96)	315 (4·08) 227 (4·33)
IVa	A (88)	100—102 (acetone–hexane)	$C_{17}H_{26}N_6O_3S.$ $^1/_2$ C_3H_6O[b] (423·6)	52·46 52·65	6·90 7·08	19·84 19·54	— —	317 (4·21) 276 (4·11) 240 (3·96)	315 (4·07) 227 (4·33)
Va	A (86)	118—122 (acetone–hexane)	$C_{18}H_{28}N_6O_3S$[c] (408·5)	52·92 52·89	6·91 7·18	20·57 20·47	7·85 7·91	318 (4·22) 278 (4·05) 240 (3·81)	312 (4·04) 227 (4·27)
VI	C (66)	153—155 (water)	$C_{14}H_{18}N_6O_5S.$ .2 H_2O[d,e] (418·4)	40·18 40·70	5·30 4·76	20·09 20·60	— —	317 (4·14) 276 (4·03) 242 (3·81)	313 (4·06) 226 (4·28)
VII	C (69)	176—177 (water)	$C_{15}H_{20}N_6O_5S$[f] (396·4)	45·44 45·30	5·09 5·30	21·20 21·20	8·09 8·06	318 (4·18) 275 (4·10) 240 (3·88)	312 (4·07) 228 (4·31)

[a] By drying at 100°C/0·2 Torr the compound lost 14·12% its weight; for $C_{12}H_{16}N_6O_3S.3\ H_2O$ (378·4) calculated: 14·29% H_2O. For $C_{12}H_{16}N_6O_3S$ (324·4) calculated: 44·43% C, 4·97% H, 25·91% N, 9·89% S; found: 44·23% C, 4·84 H, 25·73% N, 9·95% S. [b] The compound dried at room temperature loses at 100°C/0·2 Torr over P_2O_5 7·12% its weight (under simultaneous melting); for $C_{17}H_{26}N_6O_3S.{}^1/_2\ C_3H_6O$ (423·6) calculated 6·85% C_3H_6O. For $C_{17}H_{26}N_6O_3S$ (394·5) calculated: 51·76% C, 6·64% H, 21·31% N, 8·13% S; found: 51·64% C, 6·78% H, 21·17% N, 8·27% S. [c] $[\alpha]_D^{20}$ −16·0° (*c* 1, ethanol). [d] $[\alpha]_D^{20}$ +10·3° (*c* 1, 0·1M-NaOH). [e] By drying at 110°C/0·2 Torr over P_2O_5 the compound lost 8·2% its weight; for $C_{14}H_{18}N_6O_5S.2\ H_2O$ (418·4) calculated: 8·6% H_2O. The solvent-free compound melted at 162—165°C. For $C_{14}H_{18}N_6O_5S$ (382·4) calculated: 43·97% C, 4·75% H, 8·38% S; found: 43·70% C, 4·62% H, 8·10% S. [f] $[\alpha]_D^{20}$ +2° (*c* 1, 0·1M-NaOH).

water (10 : 1 : 1), detection with bromophenol blue[4] as well as paper chromatography using the solvent systems and types of detection described before[6], mostly in a system of 1-butanol–acetic acid–water (4 : 1 : 5), 1-butanol–pyridine–water (6 : 4 : 3) or in a mixture of benzene with chloroform (1 : 1) on a formamide-impregnated paper.

Ethyl Esters of N-[δ-(2-amino-6-purinylthio)valeryl]amino Acids (*Ia*, *IVa*, *Va*), Diglycine (*IIa*) and Triglycine (*IIIa*) (Method A)

A solution of 6 mmol ethyl ester of N-(δ-bromovaleryl)amino acid in 5·5 ml ethanol (or a 55°C solution of 6 mmol ethyl ester of N-(δ-bromovaleryl)glycylglycine in 20 ml ethanol, or a 55°C solution of 6 mmol ethyl ester of N-(δ-bromovaleryl)triglycine in 325 ml 50% aqueous ethanol) was added to a solution of 0·84 g (5 mmol) 6-thioguanine in 5·5 ml (5·5 mmol) 1M-NaOH at 20°C, the mixture was stirred for 4 h at 20°C and left to stand at the same temperature overnight. In the case of esters *Ia* and *IIa* the precipitated product is filtered and purified by crystallization. In the case of *IIIa* most of the ethanol is distilled at reduced pressure and the precipitate is filtered. The precipitate is stirred for 15 min at 80°C with 1% aqueous solution of triethylamine, after cooling to room temperature the product is filtered and crystallized. In the case of esters *IVa* and *Va* the reaction mixture is extracted with 25 ml of a mixture of chloroform with ethanol (9 : 1), the organic fraction is washed with 0·1M-$NaHCO_3$ and water, dried (Na_2SO_4), the solvent is distilled off and the residue is purified by crystallization (Table I).

Ethyl Ester of N-[δ-(2-Amino-6-purinylthio)valeryl]glycylglycylglycine (*IIIa*) (Method B)

5·70 g (0·015 mol) derivative *II* dried at 95—100°C/0·2 Torr was added to a solution of 2·93 g (0·018 mol) N,N′-carbonyldiimidazol in 50 ml dimethylformamide at room temperature under stirring. After 1 h of stirring of the mixture, 3·10 g (0·03 mol) of freshly redistilled glycine ethyl ester is added to the imidazolide formed and, after 1 h of stirring at 20°C the mixture is left to stand overnight. After distilling off most of the dimethylformamide at reduced pressure at 60°C the residue is triturated with 80 ml 10% aqueous acetic acid and the precipitated ester *IIIa* is purified by crystallization.

N-[δ-(2-Amino-6-purinylthio)valeryl]amino Acids *VI* and *VII* (Method C)

A solution of 0·034 mol N-(δ-bromovaleryl)-L-aspartic acid or N-(δ-bromovaleryl)-L-glutamic acid in a mixture of 60 ml water and 6·75 g (0·067 mol) triethylamine at 0—5°C is added to a solution of 5 g (0·03 mol) 6-thioguanine in 60 ml (0·03 mol) 0·5M-NaOH at 30°C. The mixture is stirred for 10 h at 30—35°C and then left to stand overnight at room temperature. After filtering off a small amount of 6-thioguanine the filtrate is made acid with dilute hydrochloric acid to pH 3 and the precipitated oily product is purified first by dissolving in 1M-$NaHCO_3$ (60 ml) and by precipitating the compound by acidifying its filtered solution with dilute hydrochloric acid (to pH 3) followed by crystallization.

N-[δ-(2-Amino-6-purinylthio)valeryl] Oligoglycines *I—III* (Method D)

2 mmol of the corresponding ester (*Ia*, *IIa* or *IIIa*) are introduced into 8 ml (4 mmol) 0·5M-NaOH at room temperature, the mixture is stirred until the solid dissolves and then is left to stand at room temperature overnight. The reaction mixture is made acid with hydrochloric acid to pH 3 and the precipitated acid is recrystallized.

The analyses were carried out by Mr K. Havel, Mrs J. Komancová and Mrs V. Šmídová (direction Dr J. Kŏrbl) the testing of the compounds was done by Miss D. Dosedlová (direction: Dr V. Rábek). The UV spectra were recorded by Dr J. Vachek, all of this Institute.

REFERENCES

1. Semonský M., Černý A., Jelínek V.: Arzneimittel-Forsch. *20*, 316 (1970).
2. Černý A., Semonský M., Jelínek V.: This Journal, in press.
3. Clarke D. A., Elion G. B., Hitchings H. G., Stock C. C.: Cancer Res. *18*, 445 (1958).
4. Černý A., Semonský M., Jelínek V.: This Journal *36*, 2248 (1971).
5. Jelínek V.: Neoplasma *7*, 146 (1960).
6. Macek K.: J. Chromatog. *4*, 156 (1960).

Translated by A. Kotyk.