SUBSTITUTED ω-(4-OXO-3,4-DIHYDRO-5-PYRIMIDINYL) ALKANOIC ACIDS, THEIR DERIVATIVES AND ANALOGUES*

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Condensation of guanidine with dimethyl 2-formylbutanedioate (*XI*), diethyl 2-cyanobutanedioate (*XII*), diethyl 2-cyanoheptanedioate (*XIII*), ethyl 6,6-dicyanohexanoate (*XIV*), or diethyl 6,6-dicyanoundecanedioate (*XV*), followed by hydrolysis of the esters formed, afforded the respective substituted ω-(2-amino-5-pyrimidinyl)alkanoic acids (*II*, *V*, *VI*, *IX* and *X*). Analogously, condensation of the ester *XIII* with urea or thiourea gave acids *VII* and *VIII*. The acid *II* was converted into its ethyl and butyl ester (*III* and *IV*, respectively). The compounds prepared showed no significant antineoplastic effects in mice with experimental tumours.

In a previous paper[1] of this series we described the syntheses and some biological properties (mainly antineoplastic) of ω-(2-amino-6-hydroxy-4-oxo-3,4-dihydro--5-pyrimidinyl)alkanoic acid. Of greatest interest proved to be 5-(2-amino-6-hydroxy--4-oxo-3,4-dihydro-5-pyrimidinyl)pentanoic acid (damvar) (*I*), now undergoing the first phase of clinical tests. The present paper deals with the preparation of some compounds structurally close to damvar, *viz.* substituted ω-(4-oxo-3,4-dihydro--5-pyrimidinyl)alkanoic acids and their derivatives *II*–*VIII*, 5-(2,4,6-triamino--5-pyrimidinyl)pentanoic acid (*IX*) and 5,5-bis(4-carboxybutyl)-2,6-diamino-4-imino--4,5-dihydropyrimidine (*X*). The compounds prepared were tested for antineoplastic activity in mice with transplantable tumours.

The compounds *II*–*X* (Table I) were obtained by the methods previously described[1]: 2-aminopyrimidines *II*, *V*, *VI*, *IX* and *X* by condensation of guanidine with dimethyl 2-formylbutanedioate (*XI*), diethyl 2-cyanobutanedioate (*XII*) diethyl 2-cyanoheptanedioate (*XIII*), ethyl 6,6-dicyanohexanoate (*XIV*) or diethyl 6,6-dicyanoundecanedioate (*XV*) in the presence of sodium methoxide at room temperature (compounds *V*, *VI*, *IX* and *X*) or at the boiling point of the reaction mixture (compound *II*), followed by hydrolysis of the esters formed. 2-Hydroxypyrimidine *VII* and 2-mercaptopyrimidine *VIII* were prepared analogously by condensation of *XIII* with urea or thiourea in a boiling mixture containing sodium ethoxide. The esters *III* and *IV* were obtained by esterification of the acid *II* using the thionyl chloride me-

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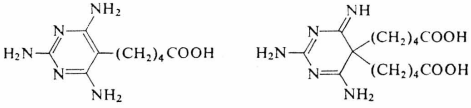
thod[1]. Hydrochloride of the acid *VI* and sodium salt of the acid *II* were prepared in the usual way. Ethyl 6,6-dicyanohexanoate (*XIV*) and diethyl 6,6-dicyanoundecanedioate (*XV*), needed for syntheses of the acids *IX* and *X*, were prepared by condensation of malonodinitrile with ethyl 5-bromopentanoate in the presence of sodium ethoxide. The esters *XIV* and *XV* were isolated from the reaction mixture by fractional distillation *in vacuo*. The ester *XIV*, being unstable, rapidly decomposed, so that it could not be characterized. Its identity was demonstrated by a successful preparation of compound *IX*. Compounds *XI* (ref.[2]), *XII* (ref.[3]) and *XIII* (ref.[4]), needed to synthetize compounds *II* and *V–VIII*, are known and were prepared by the methods described.

I *II–VI* *VII–VIII*



IX *X*

Substituents R, X, Y of compounds *II–VIII* see Table I.

The structures of compounds *II–X* were studied (Table I) with the aid of IR spectra (KBr discs) and UV spectra in acid (A), alkaline (B) and neutral (C) media, with the ester *XV* we measured the ^{1}H NMR spectrum, and with the compound *VI–VIII* the values of pK_a in 80% aqueous dimethyl sulphoxide. The spectral data of compounds *II–VI* are not at variance with the assumption of structures that would be analogous to that of compound *I* (damvar) and would have different degrees of inner ionization[1], excepting esters *III* and *IV*, in which no inner ionization can be expected. The assumption that compound *VII* exists in the dioxo form is also consistent with the spectral data. With compound *VIII* the IR spectra are indicative of the thio form rather than the thiol form. Compound *IX* can be expected in the amino form, supposing that only one of the amino groups participated in the inner ionization. Compound *X*, being disubstituted at the 5-position, must be supposed to have an imine group at position 4 and, like the above-mentioned compounds, to form inner salts. The pK_a values of compound *VI–VIII* are also in accordance with the structures assumed.

TABLE I

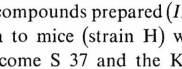
Substituted ω-(4-oxo-3,4-dihydro-5-pyrimidinyl)alkanoic acids, their derivatives and analogues

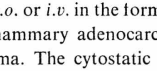
Number	R, X Y	M.p., °C (yield, %)	Formula (mol.mass)	Calculated/Found			UV spectra[a] λ_{max} (log ε)		
				% C	% H	% N	A	B	C
II	H, NH_2 CH_2COOH	317–319 (90)	$C_6H_7N_3O_3$ (169·1)	42·61 42·33	4·17 4·28	24·84 24·38	261 (3·92) 221 (4·02)	281 (3·86) 231 (3·98)	289 (3·81)
III	H, NH_2 $CH_2COOC_2H_5$	217–218 (73)	$C_8H_{11}N_3O_3$ (197·2)	48·73 48·94	5·62 5·83	21·31 21·70	261 (3·95) 221 (4·06)	281 (3·89) 231 (3·96)	292 (3·89)
IV	H, NH_2 $CH_2COO(CH_2)_3CH_3$	217–218 (88)	$C_{10}H_{15}N_3O_3$ (225·3)	53·32 53·41	6·71 6·92	18·66 19·00	261 (3·94) 221 (4·06)	281 (3·90) 233 (3·97)	292 (3·90)
V	NH_2, NH_2 CH_2COOH	[b] (88)	$C_6H_8N_4O_3$ (184·2)	39·13 39·45	4·38 4·42	30·42 30·37	270 (4·24)	271 (4·02) 226 (3·96)	278 (4·13)
VI[c]	NH_2, NH_2 $(CH_2)_4COOH$	267–268 (78)	$C_9H_{14}N_4O_3$ (226·2)	47·78 47·75	6·24 6·50	24·77 24·95	274 (4·27)	272 (4·06) 243 (3·78)	278·5 (4·18) 241 (3·73)

VII[c]	NH_2, O $(CH_2)_4COOH$	293—295 (65)	$C_9H_{13}N_3O_4$ (227·2)	47·57 47·52	5·77 5·72	18·49 18·62	274 (4·32) 226·5 (3·80)	277·5 (4·25)	276 (4·27)
VIII[c,d]	NH_2, S $(CH_2)_4COOH$	288—290 (74)	$C_9H_{13}N_3O_3S$ (243·3)	44·43 44·64	5·39 5·60	17·27 17·41	285 (4·33) 244 (3·93)	298 (4·19) 265 (4·07) 242 (4·24)	298 (4·18) 265 (4·18) 242 (4·24)
IX	—	276—278 (54)	$C_9H_{15}N_5O_2$ (225·3)	47·99 47·71	6·71 6·66	31·09 30·80	283 (4·23) 218 (4·29)	276 (4·05)	276 (4·06)
X	—	304—306 (48)	$C_{14}H_{23}N_5O_4$ (325·4)	51·68 51·42	7·13 7·33	21·53 21·32	282 (4·07) 242 (4·39)	286 (3·89) 234 (4·29)	283 (4·01) 242 (4·39)

[a] A–C see Experimental. [b] It resisted melting up to 350°C. [c] pK_a values in 80% aqueous dimethyl sulphoxide: *VI* 8·55, 13·55; *VII* 8·70, 11·28; *VIII* 9·17. [d] Calculated: 13·18% S; found: 12·99% S.

All the compounds prepared (*II* – *X*) were applied *p.o.* or *i.v.* in the form of a microsuspension to mice (strain H) with sarcoma Sak, mammary adenocarcinoma HK, ascitic sarcome S 37 and the Krebs ascitic carcinoma. The cytostatic effects were not significant.





XI, $R^1 = CH_3$, $R^2 = CHO$, n = 1
XII, $R^1 = C_2H_5$, $R^2 = CN$, n = 1
XIII, $R^1 = C_2H_5$, $R^2 = CN$, n = 4

XIV, $R^3 = H$
XV, $R^3 = (CH_2)_4COOC_2H_5$

EXPERIMENTAL

The melting points, determined on the Kofler hot stage, are not corrected. The analytical samples were dried over phosphorus pentoxide *in vacuo* (at a pressure of 27 Pa) and at temperatures proportional to their melting points. The ultraviolet spectra of *c.* 0·001% solutions (λ_{max}, nm/logε) were measured, employing a spectrophotometer Unicam SP 8000, in 0·1M-HCl in 50% methanol (A), 0·1M-NaOH in 50% methanol (B), and in 50% methanol (C). The infrared spectra (ν, cm^{-1}) in KBr discs were recorded with an apparatus Unicam SP 200 P and the ^1H NMR spectrum with an apparatus Tesla BS 487 C. Purity of the compounds was monitored by thin-layer chromatography on silica gel with a luminiscent indicator (Silufol UV_{254}, Kavalier) in a system chloroform–methanol–2-propanol–ammonia (1 : 1 : 1 : 1). The spots were detected in UV light at 254 nm. The pK_a values of compounds *VI* to *VIII* were determined in 80% aqueous dimethyl sulphoxide.

ω-(4-Oxo-3,4-dihydro-5-pyrimidinyl)alkanoic Acids (*II*, *V*, *VIII*),
5-(2,4,6-Triamino-5-pyrimidinyl)pentanoic Acid (*IX*)
and 5,5-Bis(4-carboxybutyl)-2,6-diamino-4-imino-4,5-dihydropyrimidine (*X*)

To a solution of 2·76 g of sodium (0·12 mol) in 80 ml of methanol was added 7·64 g (0·08 mol) of guanidine hydrochloride. Stirring for 10 min was followed by the addition of 6·07 g (0·04 mol) of *XI* (preparation of *II*), or 7·97 g (0·04 mol) of *XII* (preparation of *V*), or 9·64 g (0·04 mol) of *XIII* (preparation of *VI*), or 7·76 g (0·04 mol) of *XIV* (preparation of *X*). In preparing compound *VII* the methylate solution was treated with urea (7·2 g, 0·12 mol) and *XIII* (14·46 g, 0·06 mol), in preparing compound *VIII* with thiourea (9·13 g, 0·12 mol) and *XIII* (14·46 g, 0·06 mol). The mixture was stirred at room temperature for 4 h (preparation of *V*, *VI*, *IX*, *X*), or at the boiling points for 3 h (preparation of *II*, *VII*, *VIII*), then left standing overnight at room temperature. The solvent was distilled off *in vacuo*, the residue was taken into 60 ml of water with 1·7 g (0·04 mol) of sodium hydroxide, the mixture was stirred for 2 h at room temperature and filtered. The filtrate was brought to pH 4·2 with conc. hydrochloric acid and cooled. The separated solid was collected on a filter and recrystallized either from water (*V* to *X*) or from a mixture of dimethyl sulphoxide, ethanol and water (*II*). The melting points and other data of compounds *II* and *V*–*X* are given in Table I. IR spectra (KBr discs): *II* 1 695 (COOH), 1 660 (lactam), 3 390 (NH_2), 3 080 (NH); *V* 1 650 (lactam), 3 140, 1 360 (NH_3^+), 1 710 (COOH), 3 400 (NH_2); *VI* 1 705 (COOH), 1 665 (lactam), 3 510, 3 380 (NH_2), 3 140, 1 360 (NH_3^+), 1 620 (NH_2), 1 590 (COO^-); *VII* 3 200, 1 380 (NH_3^+), 1 590 (COO^-), C=O (1 715), NH (3 320);

VIII 1 555 (S=C—N), 1 640 (lactam), 1 690 (COOH), 3 460 (NH_2), 3 200 (NH); *IX* 1 638 (NH_2), 3 100 (NH_3^+), 3 450 (NH_2), 3 360 (NH_2), 1 520, 1 560 (pyridine ring), 1 690 (COOH); *X* 1 700 (COOH), 1 590 (COO^-), 3 080 (NH_3^+), 3 370 (=NH,NH_2), 1 650 (NH_2).

Ethyl and n-Butyl 2-(2-Amino-4-oxo-3,4-dihydro-5-pyrimidinyl) Acetates (*III* and *IV*)

To 20 ml of ethanol (in preparation of *III*), or n-butanol (in preparation of *IV*), chilled to −40°C, was added dropwise 2·02 g (0·017 mol) of thionyl chloride, then, in portions and at the same temperature, 2·54 g (0·015 mol) of acid *II*. The mixture was stirred for 2 h at 40°C and for another 2 h at the boiling point, within which time it became homogeneous. The solvent was distilled off *in vacuo*, the residue was dissolved in 50 ml of water, the solution was filtered and alkalinized with solid sodium hydrogen carbonate. The crude product was recrystallized (*III* from ethanol, *IV* from n-butanol). The melting points and other data are given in Table I. IR spectrum (KBr disc): *III* 1 725 (ester), 1 660 (lactam), 3 340 (NH_2), 3 080 (NH); *IV* 1 725 (ester), 1 660 (lactam), 3 340 (NH_2), 3 080 (NH).

2,6-Diamino-4-oxo-5-(4-carboxybutyl)-3,4-dihydropyrimidine Hydrochloride

0·5 g of acid *VI* was dissolved in 3·5 ml of boiling concentrated hydrochloric acid and the solution was left standing for 4 h in a refrigerator to crystallize; yield 0·53 g (91%) of the hydrochloride, m.p. 264—268°C (decomp.); for $C_9H_{15}ClN_4O_3$ (262·7) calculated: 41·15% C, 5·75% H, 13·50% Cl; found: 40·82% C, 5·76% H, 13·47% Cl; UV spectrum (λ max, log ε) 273 (4·24) (A), 272 (4·12), 224 (3·76) (B), 276 (4·17) (C).

Sodium 2-(2-Amino-4-oxo-3,4-dihydro-5-pyrimidinyl)acetate

0·42 g (2·5 mmol) of acid *II* and 0·21 g (2·5 mmol) of sodium hydrogen carbonate were dissolved in 35 ml of boiling water, and the solution was cooled down. The sodium salt separated after an addition of ethanol; yield 0·39 g (81·5%), m.p. 260—265°C (decomp.). For $C_6H_7N_3O_3.H_2O$ calculated: 10·99% Na; found: 10·93% Na.

Ethyl 6,6-Dicyanohexanoate (*XIV*) and Diethyl 6,6-Dicyanoundecanedioate (*XV*)

To a solution of 12·7 g (0·55 mol) of sodium in 450 ml of ethanol pre-warmed to 40°C, was added 36·3 g (0·55 mol) of malonodinitrile in 30 ml of ethanol. After stirring for 10 min, 104·6 g (0·5 mol) of ethyl 5-bromopentanoate was added dropwise at the same temperature. The mixture was then boiled under a reflux condenser for 5 h and left standing overnight. The separated sodium bromide was removed by filtration, the filtrate was concentrated to a pasty consistence and diluted with 500 ml of water. The organic portion was taken into chloroform, the chloroform phase was washed with water and dried with sodium sulphate. The volatile components were removed *in vacuo* (water pump) and the residue was distilled. The hexanoate *XIV* was obtained in a range of 155—160°C at a pressure of 66 Pa, and the undecanedioate *XV* in a range of 200 to 210°C at 66 Pa. Redistillation gave 15·6 g (14·7%) of *XIV*, b.p. 130—133°C/40 Pa and 20·1 g (11·3%) of *XV*, b.p. 185—190°C/40 Pa. The hexanoate *XIV* was unstable and had to be kept in a refrigerator. Undecanedioate *XV*: For $C_{17}H_{26}N_2O_4$ (322·4) calculated: 63·33% C, 8·13% H, 8·69% N; found: 62·84% C, 8·28% H, 8·42% N. ^{1}H NMR spectrum (hexadeuteriodimethyl sulphoxide, δ, ppm): 7·49 (s, 1 H, C(6)—H), 6·80 (bs, 3 H, NH, NH_2), 4·02 (t, J = 6,5 Hz, 2 H, CO_2CH_2—), 3·21 (s, 2 H, CH_2), 1·50 (m, 4 H, —CH_2CH_2—), 0·90 (t, 3 H, —CH_3).

The elemental analyses were performed by Mrs J. Komancová and Mrs V. Šmídová (Analytical Department, head Dr J. Körbl). The antineoplastic effects of the compounds were assessed by Dr K. Řežábek.

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