SYNTHESIS AND BIOLOGICAL ACTIVITY OF N-SUBSTITUTED 5-FLUOROURACIL-1-ACETAMIDES

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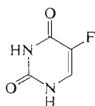
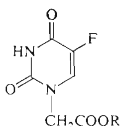
Reaction of 5-fluorouracil (*I*) with sodium chloroacetate in the presence of sodium hydride afforded a mixture of 5-fluorouracil-1-acetic acid (*II*) and 5-fluorouracil-3-acetic acid (*III*). *p*-Nitrophenyl 5-fluorouracil-1-acetate (*IV*) reacted with amines in methanol or dimethylformamide to give N-substituted 5-fluorouracil-1-acetamides *V*. The following amides were prepared: diethylamide *Vc*, n-hexylamide *Vd*, cyclohexylamide *Ve*, adamantylamide *Vf*, anilide *Vg*, benzylamide *Vh*, benzhydrylamide *Vi*, pyrrolidide *Vj*, piperidide *Vk*, morpholide *Vl*, 3-dimethylaminopropylamide *Vm*, 3-aminopropylamide *Vn*, 2-hydroxyethylamide *Vo*, bis(2-hydroxyethyl)amide *Vp* and tris(hydroxymethyl)methylamide *Vq*. Compounds *Vc*—*Vq* do not inhibit the growth of *Escherichia coli* in concentrations up to 1 mg/ml. Compounds *Vf* and *Vh* have a weak, compound *Vq* a significant, inhibition effect on the growth of L-1210 mouse leukemic cells *in vitro* in concentrations $1{\cdot}5 \,.\, 10^{-5}$ mol l^{-1}.

The marked cytostatic properties of 5-fluorouracil (*I*) and its nucleosides made these compounds the subject of many studies[1-4]. 5-Fluorouracil and its N-tetrahydro-2-furanyl derivative (Ftorafur, USSR) found clinical use in chemotherapy of cancer. Since 5-fluorouracil nucleosides undergo *in vivo* a facile cleavage to give the compound *I*, their application is of limited practical significance in comparison with the compound *I* itself. Recently, attention has been paid to N^1-substituted 5-fluorouracil derivatives with the aim to find compounds which would exhibit the desired biological effect without undergoing the *in vivo* cleavage to compound *I*. We have found such compounds *e.g.* among some 5-fluorouracil nucleosides with modified sugar moiety: these derivatives show bacteriostatic activity and are not cleaved into the compound *I* under *in vitro* conditions[5]. In our previous communications we described the preparation of 5-fluorouracil-1-acetic acid[6] (*II*) and its *p*-nitrophenyl ester *IV*. The latter compound enables an advantageous bonding of the compound *II* to proteins as well as a facile preparation of the low-molecular amides *Va* and *Vb* (ref.[7]).

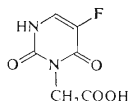
With the aim to subject to a detailed biological investigation some derivatives of the compound *II*, we prepared a series of N-substituted amides *V* of various pharmacokinetic parameters, determined by introduction of typical lipophilic, hydrophilic, aromatic or aliphatic groups. The antibacterial activity of these compounds was studied with *Escherichia coli* B in synthetic glucose-containing medium (ref.[5]); simultaneously, their effect upon growth of L-1210 mouse leukemic cells *in vitro* was investigated. The inhibition of eukaryotic cell growth by 5-fluorouracil and its nucleosides was employed in the study of mechanism of its action[8]. Although



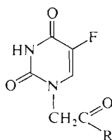
I



II, R = H
IV, R = C_6H_4—NO_2—4



III



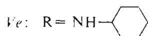
V

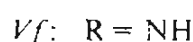
Va: R = NH_2

Vb: R = $NH(CH_2)_5COOH$

Vc: R = $N(C_2H_5)_2$

Vd: R = NH-*n*-C_6H_{13}

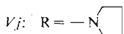
Ve: 

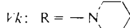
Vf: 

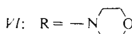
Vg: R = NHC_6H_5

Vh: R = $NHCH_2C_6H_5$

Vi: R = $NHCH(C_6H_5)_2$

Vj: 

Vk: 

Vl: 

Vm: R = $NHCH_2CH_2CH_2\overset{(+)}{N}H(CH_3)_2Cl^{(-)}$

Vn: R = $NH(CH_2)_3NH_2$

Vo: R = $NHCH_2CH_2OH$

Vp: R = $N(CH_2CH_2OH)_2$

Vq: R = $NHC(CH_2OH)_3$

TABLE I

Substituted amides of 5-fluorouracil-1-acetic acid *V*

Compound Yield, %	M.p., °C (solvent[a])	R_F			E_{Up}[c]	Formula (mol.weight)	Calculated/Found			
		S1	S2	S3			C	H	F	N
Vc	178—182	0·32	0·73	0·64	0·59	$C_{10}H_{14}FN_3O_3$	49·37	5·80	7·81	17·28
75	(A)					(243·2)	50·21	6·28	8·12	17·31
Vd	212—214[b]	0·34	0·70	0·80	0·28	$C_{12}H_{18}FN_3O_3$	53·12	6·68	7·00	15·49
82	(B)					(271·3)	52·94	6·94	6·88	15·74
Ve	267—269	0·30	0·76	0·78	0·38	$C_{12}H_{16}FN_3O_3$	53·52	5·99	7·06	15·60
70	(A)					(269·3)	53·85	5·84	6·79	15·72
Vf	274—277[b]	0·33	0·85	0·81	0·26	$C_{16}H_{20}FN_3O_3$	59·80	6·27	5·91	13·08
68	(B)					(321·4)	59·47	6·54	5·95	12·99
Vg	185—186[b]	0·31	0·82	0·62	0·61	$C_{12}H_{10}FN_3O_3$	54·75	3·83	7·22	15·97
65	(A)					(263·2)	54·28	3·63	7·40	15·94
Vh	250—253[b]	0·26	0·81	0·72	0·38	$C_{13}H_{12}FN_3O_3$	56·31	4·36	6·85	15·16
85	(C)					(277·3)	56·86	4·59	6·93	15·08
Vi	180—182	0·42	0·76	0·60	0·56	$C_{19}H_{16}FN_3O_3$	64·58	4·56	5·38	11·89
76	(A)					(353·4)	64·86	4·50	5·52	12·22
Vj	265—267[b]	0·39	0·70	0·58	0·52	$C_{10}H_{12}FN_3O_3$	49·79	5·02	7·88	17·42
70	(A)					(241·2)	49·96	5·39	8·11	17·95
Vk	242—244[b]	0·40	0·72	0·65	0·55	$C_{11}H_{14}FN_3O_3$	51·76	5·53	7·44	16·47
72	(A)					(255·2)	52·19	5·85	7·40	16·34
Vl	240—241[b]	0·25	0·54	0·56	0·54	$C_{10}H_{12}FN_3O_4$	46·69	4·70	7·39	16·34
80	(C)					(257·2)	46·98	4·58	7·63	16·60
Vm	234—238	0	0·05	0·62	—0·10	$C_{11}H_{18}ClFN_4O_3$	42·79	5·88	6·15	18·15
70	(A)					(308·8)[d]	41·85	6·25	6·26	18·46
Vn	192—196	0	0	0·40	—0·10	$C_9H_{13}FN_4O_3$	44·26	5·36	7·78	22·95
80	(D)					(244·2)	44·33	4·86	8·13	23·13
Vo	245—247	0·05	0·27	0·55	0·55	$C_8H_{10}FN_3O_5$	41·56	4·36	8·22	18·18
85	(B)					(231·2)	41·33	4·80	8·13	18·14
Vp	92—96	0	0·24	0·58	0·48	$C_{10}H_{14}FN_3O_5$	43·63	5·13	6·90	15·27
85	(A)					(275·2)	43·38	5·11	6·52	15·34
Vq	180—182	0	0·15	0·52	0·49	$C_{10}H_{14}FN_3O_6$	41·24	4·84	6·52	14·43
60	(A)					(291·2)	41·71	4·82	6·91	13·78

TABLE I
(*Continued*)

Compound Yield, %	M.p., °C (solvent[a])	R_F			E_{Up}[c]	Formula (mol.weight)	Calculated/Found			
		S1	S2	S3			C	H	F	N
I	—	—	—	0·57	0·62	—	—	—	—	—
II	—	—	—	0·43	1·25	$C_6H_5FN_2O_4$ (188·1)	38·30 38·52	2·68 2·74	10·10 10·24	14·89 15·05
III	—	—	—	0·51	0·94	$C_6H_5FN_2O_4$ (188·1)	38·30 38·12	2·68 2·70	10·10 9·83	14·89 14·92
IV	—	0·53	0·81	—	—	—	—	—	—	—

[a] Solvents: (A) ethanol–light petroleum; (B) 50% aqueous ethanol; (C) ethanol; (D) methanol–ether; [b] melting preceded by sublimation; [c] electrophoretic mobility in E1 referred to uridine 3'-phosphate; [d] calculated: 11·49% Cl; found: 11·42% Cl.

it was shown recently that some of the L-1210 cell lines are relatively insensitive towards 5-fluorouracil[9], the cell line used in our experiments exhibited LD_{50} for 5-fluorouracil at about $1{\cdot}5 \cdot 10^{-5}$ mol l^{-1}.

The first described synthesis of 5-fluorouracil-1-acetic acid (*II*) was carried out by fluorination[6] of uracil-1-acetic acid, easily accessible from uracil[10]. As an alternative, we tried the reaction of 5-fluorouracil with sodium chloroacetate. Since the presence of fluorine atom affects the electron distribution in the 5-fluorouracil anion(s) and enhances the amount of the N^3-isomer *III* relative to the reaction of uracil, we compared the ratio of the isomers *II* and *III* formed from the monoanion or dianion of the compound *I*. We found that although the overall reaction of the dianion with 1 equivalent of chloroacetate was almost quantitative, the isomers *II* and *III* arose in practically equimolar amounts. Reaction of the monoanion was more advantageous, leading predominantly (in higher absolute yield) to the desired N^1-isomer *II*. It seems thus that this method of preparation of the compound *II* can compete with the synthesis from uracil-1-acetic acid, particularly if 5-fluorouracil is more easily accessible than fluorination reagents.

The N-substituted amides of the general formula *V* were prepared from the *p*-nitrophenyl ester *IV* (ref.[7]): its reaction with amines (or their salts in the presence of a tertiary base) in methanol or dimethylformamide afforded smoothly the compounds *V* (together with *p*-nitrophenol) which were purified by chromatography on silica gel or by crystallization.

All prepared compounds *V* (Table I) were chromatographically pure and free of both 5-fluorouracil (*I*) and compound *II*. Their electrophoretic mobility corresponded to that of 5-fluorouracil; the only exceptions were the compounds *Vm* and *Vn* which in slightly alkaline medium exist in the form of betains.

In addition to low-molecular amines, AH-Sepharose was also subjected to the reaction: it reacted smoothly with the compound *IV* under formation of a modified polymer, containing bound moieties of the compound *II*. Their presence was unequivocally proved by UV-spectrum of the modified polymer. Use of such polymers in affinity chromatography still awaits an experimental check.

Antibacterial activity tests of the compounds *V* on *Escherichia coli* B were negative. Since the strain employed is highly sensitive toward 5-fluorouracil[5], this result at the same time shows that the tested derivatives did not contain even traces of this compound.

Most of the compounds investigated did not affect the proliferation of L-1210 mouse leukemic cells *in vitro*. As seen from Table II, at concentrations $0{\cdot}6-0{\cdot}8 \,.\, 10^{-3}$ mol l^{-1} neither of the compounds *V* matched the activity of compound *I*; a low activity was found with the benzylamide *Vh* and the adamantylamide *Vf* (25% and 22% inhibition, respectively, at $1{\cdot}5 \,.\, 10^{-5}$ mol l^{-1}; under these conditions *I* shows 46% inhibition). Of all the studied series only the tris(hydroxymethyl)methylamide *Vq* appears to be interesting: its inhibition effect is 33% under comparable conditions. However, it remains to be proved that the compound *Vq* does not liberate 5-fluorouracil by catabolic reactions, although such reaction is not very probable.

TABLE II

Cell growth inhibition (% of control) of L-1210 leukemic cells *in vitro* (Conditions, *cf.* Experimental)

Compound	At 2 µg/ml[a]	At 1·5 . 10^{-5}M[b]	Compound	At 2 µg/ml[a]	At 1·5 . 10^{-5}M[b]
I	53·7	53·7	*Vj*	86	—
II	86	—	*Vk*	94	—
Vc	98	—	*Vl*	94	—
Vd	117	—	*Vm*	84	—
Ve	115	—	*Vn*	107	—
Vf	79	78	*Vo*	92	—
Vg	92	—	*Vp*	98	—
Vh	73	75	*Vq*	75	67
Vi	90	—			

[a] 48 h incubation time; [b] 72 h incubation time.

EXPERIMENTAL

Melting points were determined on a Kofler block and are uncorrected. Unless otherwise stated, the solutions were taken down at 40°C/2 kPa and the compounds were dried over phosphorus pentoxide at 13 Pa. Thin-layer chromatography on silica gel was carried out on Silufol UV 254 sheets (Kavalier, Czechoslovakia) in the solvent systems: S 1 chloroform–methanol (9 : 1), S 2 chloroform–methanol (75 : 25), paper chromatography on a paper Whatman No 1 in the system S 3 2-propanol–conc. aqueous ammonia–water (7 : 1 : 2). Paper electrophoresis was performed on a paper Whatman No 3 at 20 V/cm (1 h) in 0·1M triethylammonium hydrogen carbonate (pH 7·5). UV spectra were taken on a Specord UV-VIS spectrophotometer (Carl Zeiss, Jena) in aqueous solutions. Preparative chromatography on silica gel was carried out on 30 × 16 × 0·5 cm preparative silica gel plates, containing a fluorescence indicator (Merck), preparative chromatography on cellulose on a column (80 × 4 cm) of microcrystalline cellulose (Macherey and Nagel) in the system S 3 (20 ml/h), detection with a Uvicord (LKB, Sweden) instrument.

Reaction of 5-Fluorouracil (*I*) with Sodium Chloroacetate

a) *With one equivalent of sodium hydride.* Sodium hydride (5 mmol) was added to a suspension of 5-fluorouracil (5 mmol) in dimethyl sulfoxide (5 ml). After stirring for 30 min sodium chloroacetate (5 mmol) was added. The mixture was stirred overnight at room temperature under exclusion of moisture, taken down at 80°C/13 Pa, the residue was codistilled with dimethylformamide (2 × 5 ml) under the same conditions and dissolved in water (20 ml), The solution was applied on a column (100 ml) of Dowex 50X8 (H^+ form), the UV-absorbing aqueous eluate taken down *in vacuo*, the residue codistilled with water (3 × 20 ml) and dissolved in water (20 ml). After adjusting to pH 9 with ammonia, the solution was applied on a column of Dowex 1X2 (acetate form; 100 ml). The column was eluted with water (200 ml) and then with 0–1M formic acid (linear gradient, 2 l each). The 0·30–0·40M eluate contained pure 5-fluorouracil (S 3, E 1), the 0·60–0·70M eluate contained 0·27 g (28·5%) of compound *II*, identical (S 3, E 1) with the authentic material. UV spectrum (pH 2): λ_{max} 271 nm, (pH 12) 275. For analysis see Table I. The 0·70–0·75M eluate afforded 60 mg (6·5%) of compound *III* which, according to its UV spectrum, was the 3-isomer. (pH 2: λ_{max} 270 nm, pH 12: λ_{max} 299 nm). For analysis see Table I.

b) *With two equivalents of sodium hydride.* The reaction was performed with 10 mmol of sodium hydride in the same way as described under *a*). After 18 h the reaction was almost quantitative (S 3, E 3). The work-up procedure according to *a*) afforded in the 0·70–0·80M eluate a mixture of *II* and *III* (0·40 g; 42% based on *I*), containing 58% of *II* (24·4% based on *I*) and 42% of *II* (17·6% based on *I*).This percentage was determined spectrophotometrically from spots, eluted after chromatography in S 3. It was not possible to separate the two compounds even by repeated chromatography in formic acid (gradient 0–0·5M; 2 l each) on the same column of ion exchange resin.

Preparation of N-Substituted 5-Fluorouracil-1-acetamides *V* (General Procedure)

a) The corresponding amine (1·3 mmol/1 mmol *IV*) was added at room temperature to a stirred suspension of the compound *IV* (ref.[7]) in methanol (3 ml/1 mmol) and the resulting yellow solution was set aside at room temperature overnight. After addition of methanol (20 ml/1 mmol *IV*), Dowex 50X8 (H^+ form) was added under stirring until the yellow colour disappeared. The mixture was filtered, the solid washed with methanol and the filtrate was taken down. The residue was chromatographed on silica gel plates (two for 1 mmol of *IV*) in a 9 : 1 mixture of chloro-

form and ethanol. The UV-absorbing product bands were eluted with methanol, the eluate was taken down *in vacuo* and the product was crystallized. Yields, physical constants, crystallization conditions and analyses of the products *V* are given in Table I.

This procedure was employed for preparation of the diethylamide *Vc*, n-hexylamide *Vd*, pyrrolidide *Vj* and piperidide *Vk*.

b) The reaction was performed in the same manner as described under *a*), the mixture was taken down *in vacuo*, the residue mixed with water (15—20 ml/1 mmol *IV*) and Dowex 50X8 (H^+ form) was added until the colour disappeared. After filtration and washing the solid with water, the filtrate was extracted with ether. The aqueous layer was taken down and the residue chromatographed on silica gel plates as described under *a*). In this manner the following compounds were prepared: the morpholide *Vl*, N-(2-hydroxyethyl)amide *Vo* and N-bis(2-hydroxyethyl)amide *Vp*.

c) The reaction and work-up procedure were carried out according to *b*) and the residue after evaporation of the aqueous extract was chromatographed on silica gel in the system S 3. This procedure was used for preparation of the 3-dimethylaminopropylamide *Vm* (its hydrochloride was precipitated from the methanolic solution of *Vm* with ethanolic hydrogen chloride followed by an excess of light petroleum) and N-tris(2-hydroxymethyl)methylamide (*Vq*).

d) The reaction was performed as described under *b*). After neutralization the crystalline product was filtered, washed with water and dissolved in ethanol. The solution was taken down and the residue was further purified by chromatography on silica gel as described under *a*). By this procedure the cyclohexylamide *Ve*, benzylamide *Vh* and benzhydrylamide *Vi* were prepared.

Anilide *Vg*

Prepared from the compound *IV* (1 mmol), freshly distilled aniline (1 ml) and methanol (3 ml) by heating for 3 h to 50°C, standing overnight and work-up according to *a*).

Adamantylamide *Vf*

A mixture of *IV* (1 mmol), adamantylamine hydrochloride (1 mmol), dimethylformamide (4 ml) and triethylamine (140 μl) was stirred at room temperature overnight, taken down *in vacuo*, dissolved in ethanol (20 ml) and worked up according to procedure *a*).

N-(3-Aminopropyl)amide *Vn*

A solution of the compound *IV* (1·5 mmol) in dimethylformamide (4 ml) was added dropwise at room temperature in the course of 30 min to a solution of 1,3-diaminopropane (15 mmol) in dimethylformamide (10 ml). After stirring overnight at room temperature the mixture was taken down at 40°C/13 Pa and the residue was codistilled with dimethylformamide (2 × 10 ml) under the same conditions and dissolved in water. The solution was acidified with hydrochloric acid and extracted with ether (3 × 20 ml). The aqueous layer was applied on a column of Dowex 50X8 (H^+ form; 200 ml). The column was washed with water until the UV absorption disappeared and the Dowex was suspended in water (200 ml). The suspension was adjusted to pH 9·1 with ammonia, filtered, washed with water and the filtrate was taken down *in vacuo*, leaving 0·1 g of the pure product *Vn*. Further elution of the Dowex (in column) with 2·5% aqueous ammonia till disappearance of absorption afforded a further portion of the product which on rechromatography on a column of cellulose gave 0·19 g of the chromatographically pure (S 3, E 1) compound *Vn*.

ω-N-(5-Fluorouracil-1-yl)methylcarbonyl-AH-Sepharose

A solution of the compound *IV* (0·5 mmol) in dimethylformamide (2 ml) was added to a suspension of AH-Sepharose (Pharmacia, Sweden; 5 ml) in a mixture of 0·1M sodium tetraborate, pH 9·5 (10 ml) and dimethylformamide (5 ml). The suspension was shaken at room temperature overnight, poured into an empty column (1 cm diameter), washed with 0·1M sodium tetraborate, pH 9·5 (1 litre), and with water (1 litre) and transferred into a saturated sodium chloride solution. UV spectrum (20% aqueous glycerol): λ_{max} 274 nm.

Cell growth experiments were performed on L-1210 leukemic cells in RPMI 1640 Medium containing 5% calf fetal serum, penicillin (50 units/ml), streptomycin (50 μg/ml) and mercaptoethanol (10^{-6}M), using multiwell tissue culture plates (Nunc Product, Roskilde, Denmark). For measurement of the effect of drugs on cell growth, 0·15 ml of cell suspension (10^5 cells/ml) was placed in each well and a freshly prepared solution of the drug (50 μg) in physiological saline autoclaved for 20 min was added. Each sample was dispensed into 2 or 3 separate wells, and incubated in 10% CO_2 at 37°C in a humid atmosphere. After 72 hours cells were counted in a Bürker hemocytometer. During 72 hours the L-1210 cells in control wells underwent at least 3 mitotic cycles. The reproducibility was ±10%. Cell numbers were expressed as percentage of the number of cells in the control culture (Table II).

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