SYNTHESIS AND ANTIPROLIFERATIVE ACTIVITY OF SPIROCYCLIC CYCLODIPEPTIDES, DERIVATIVES OF 1-AMINO-1-CYCLOBUTANECARBOXYLIC ACID

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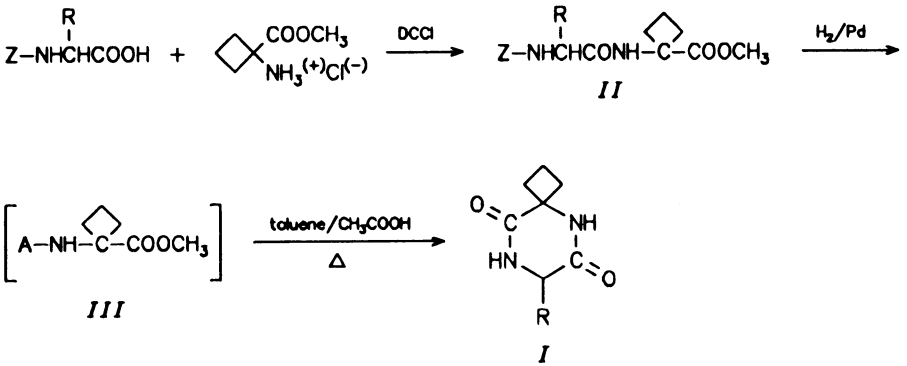
A series of chiral spirocyclic cyclodipeptides of the general formula *I* was synthesized; the aim was to determine how the substitution of cyclobutane for cyclopentane in cyclo(-alanyl-1-amino-1-cyclopentanecarbonyl-) would influence the inhibition of the proliferative activity of the caudal morphogenetic system (CMS) of chick embryos. Spirocyclic cyclodipeptides *Ia – Il* were obtained by cyclization of linear dipeptides *IIa – IIf*, prepared by condensation of protected amino acids by DCCI method. The inhibition was investigated by the Chick Embryotoxicity Screening Test. The results show generally lower activity in the tested series, as compared with derivatives containing 1-amino-1-cyclopentanecarboxylic acid.

The derivatives of 2,5-piperazinedione containing 1-amino-1-cyclopentanecarboxylic acid have been reported[1–3]. One member of this series, the spirocyclic dipeptide cyclo-(-alanyl-1-amino-1-cyclopentanecarbonyl-) (VUFB-15754; ALAPTIDE) represents a drug which significantly influences biological systems in several clinical applications (nootropic activity, therapy of skin and mucous membrane lesions)[4]. To map the relation between their structure and biological activity we have synthesized a series of spirocyclic cyclodipeptides, derivatives of 2,5-piperazinedione containing 1-amino-1-cyclobutanecarboxylic acid (Acb)*. The synthesis was carried out in solution using the carbodiimide method.

* The symbols for amino acids and peptides are according to the suggestions of the IUPAC-IUB Commission of Biochemical Nomenclature[5]. Acb, 1-amino-1-cyclobutanecarboxylic acid; Acp, 1-amino-1-cyclopentanecarboxylic acid; DMSO, dimethyl sulfoxide; DCCI, *N,N'*-dicyclohexylcarbodiimide.

The synthesis started from chiral *N*-benzyloxycarbonyl protected amino acids and methyl 1-amino-1-cyclobutanecarboxylate, prepared from 1-aminocyclobutanecarboxylic acid[6] by esterification, after Brenner[7]. The corresponding linear dipeptide methyl esters were prepared by routine methods of peptide synthesis in which *N*-carbobenzoxy derivative of an amino acid was condensed with 1-aminocyclobutane methyl ester in the presence of *N,N'*-dicyclohexylcarbodiimide. The protected dipeptides *IIa – IIl* were then hydrogenated in presence of 5% palladium on carbon. Free dipeptides *IIIa – IIII* easily cyclized to the corresponding piperazinediones, especially when heated in boiling toluene[8], as shown in Scheme 1.



I	R	*II, III*	A
a	H	*a*	Gly
b	CH_3	*b*	L-Ala
c	CH_3	*c*	D-Ala
d	CH_2OH	*d*	L-Ser
e	CH_2OH	*e*	D-Ser
f	$CH(CH_3)_2$	*f*	L-Val
g	$CH(CH_3)_2$	*g*	D-Val
h	$CH_2CH(CH_3)_2$	*h*	L-Leu
i	$CH_2CH(CH_3)_2$	*i*	D-Leu
j	C_6H_5	*j*	D-Pgl
k	$CH_2C_6H_5$	*k*	L-Phe
l	$CH_2C_6H_5$	*l*	D-Phe

SCHEME 1

TABLE I
Properties of protected linear dipeptides *II*

Compound	M.p., °C Yield, %	$[\alpha]_D$, deg c^a	Formula M.w.	Calculated/Found		
				% C	% H	% N
IIa[b]	83 – 86 42	–	–	–	–	–
IIb	92 – 94 74	−14.9 0.8	$C_{17}H_{22}N_2O_5$ 334.4	61.07 60.89	6.63 6.39	8.38 8.56
IIc	92 – 93 72	+12.7 0.8	$C_{17}H_{22}N_2O_5$ 334.4	61.07 61.18	6.63 6.50	8.38 8.47
IId	89 – 91 49	−11.2 0.2	$C_{17}H_{22}N_2O_6$ 350.4	58.28 58.20	6.33 6.39	8.00 7.78
IIe	88 – 90 61	+10.1 0.8	$C_{17}H_{22}N_2O_6$ 350.4	58.28 58.65	6.33 6.27	8.00 8.16
IIf	159 – 163 74	−9.9 0.7	$C_{19}H_{26}N_2O_5$ 362.4	63.00 63.04	7.23 7.39	7.73 8.05
IIg	158 – 162 82	+10.8 1.0	$C_{19}H_{26}N_2O_5$ 362.4	63.00 63.11	7.23 7.47	7.73 8.11
IIh	108 – 111 31	−13.5 1.0	$C_{20}H_{28}N_2O_5$ 376.5	63.81 63.72	7.50 7.39	7.44 7.52
IIi	97 – 99 32	+13.8 1.2	$C_{20}H_{28}N_2O_5$ 376.5	63.81 63.66	7.50 7.58	7.44 7.81
IIj	174 – 176 84	−76.1 0.2	$C_{22}H_{24}N_2O_5$ 396.4	66.65 66.42	6.10 6.34	7.07 7.09
IIk	115 – 117 60	−5.8 1.1	$C_{23}H_{26}N_2O_5$ 410.5	67.30 67.46	6.38 6.24	6.82 6.85
IIl	111 – 112 63	+7.1 1.2	$C_{23}H_{26}N_2O_5$ 410.5	67.30 67.51	6.38 6.46	6.82 6.70

[a] Methanol. [b] Refs[1,12,13].

TABLE II
Properties of spirocyclic cyclodipeptides *I*

Compound	M.p.[a], °C Yield, %	$[\alpha]_D$, deg *c*	Formula M.w.	Calculated/Found		
				% C	% H	% N
Ia[d]	277 – 278 74	–	–	–	–	–
Ib	274 – 275 34	+8.0 1.3[b]	$C_8H_{12}N_2O_2$ 168.2	57.13 57.05	7.19 7.28	16.65 16.39
Ic	274 – 275 34	–8.5 0.9[b]	$C_8H_{12}N_2O_2$ 168.2	57.13 57.18	7.19 7.09	16.65 16.50
Id	216 – 218 70	+17.5 0.5[c]	$C_8H_{12}N_2O_3$ 184.2	52.17 51.90	6.57 6.81	15.21 15.03
Ie	216 – 218 72	+17.4 0.2[c]	$C_8H_{12}N_2O_3$ 184.2	52.17 51.93	6.57 6.79	15.21 15.20
If	297 – 299 14	+12.0 3.5[b]	$C_{10}H_{16}N_2O_2$ 196.3	61.20 61.38	8.22 8.28	14.27 14.49
Ig	292 – 293 47	–12.6 0.9[b]	$C_{10}H_{16}N_2O_2$ 196.3	61.20 61.05	8.22 8.18	14.27 14.30
Ih	248 –249 60	+19.8 0.9[b]	$C_{11}H_{18}N_2O_2$ 210.3	62.83 62.64	8.62 8.56	13.32 13.15
Ii	241 – 244 36	–20.1 1.2[b]	$C_{11}H_{18}N_2O_2$ 210.3	62.83 62.91	8.62 8.70	13.32 13.43
Ij	265 – 267 30	–37.9 0.2[c]	$C_{13}H_{14}N_2O_2$ 230.3	67.81 67.50	6.13 6.09	12.16 12.42
Ik	285 – 288 41	+55.7 1.2[b]	$C_{14}H_{16}N_2O_2$ 244.3	68.83 68.56	6.60 6.73	11.47 11.30
Il	286 – 288 42	–59.4 1.0[b]	$C_{14}H_{16}N_2O_2$ 244.3	68.83 68.98	6.60 6.86	11.47 11.64

[a] Sealed capillary. [b] Acetic acid. [c] Methanol. d Refs[1,12,13].

The yields and analytical constants of the Z-protected linear dipeptides are listed in Table I, the constants of final products are in Table II.

The results of the CHEST I test show generally lower inhibition of proliferation in comparison with cyclopentane analogues and relatively low activity (toxicity) in the D-series of amino acids (Table III). Similarly, hydrophobic amino acids greatly increase toxicity, for example both antipodes of phenylalanine [cyclo(-L-Phe-Acb-) (*Ik*) and cyclo(-D-Phe-Acb-) (*Il*)].

Reliable conclusions concerning the relationships between chemical structure and biological response can only be made when the results of supplementary tests available. These other biological results are under our study as well.

EXPERIMENTAL

The melting points were determined on a Kofler micro hot-stage and are uncorrected. The melting points of subliming compounds were determined in a sealed tube. IR spectra were recorded in KBr pellets using a Perkin–Elmer model 577 spectrometer. Elemental analyses were performed on an automatic elemental analyzer (Laboratorní přístroje, Prague). Optical rotations were measured on a Polamat A (Zeiss, Jena) polarimeter in a 10 cm tube at 25 °C. The measurement of the inhibition of proliferative activity was read using a stereomicroscope (Wild M8, Heerbrugg, Switzerland). Dimethyl sulfoxide of analytical grade was subjected to vacuum fractional distillation before use.

TABLE III
Antiproliferative activity of cyclic dipeptides. Length of embryo trunk 24 h after treatment (means ± SE)

Compound	Length, mm		
	$1 . 10^{-3}$ [a]	$1 . 10^{-4}$ [a]	$1 . 10^{-5}$ [a]
Ia	2.0 ± 0.5	2.46 ± 0.4	2.5 ± 0.3
Ib	1.9 ± 0.5	2.23 ± 0.3	2.45 ± 0.2
Ic	1.9 ± 0.6	1.93 ± 0.6	2.23 ± 0.5
Id	2.6 ± 0.1	2.78 ± 0.15	2.8 ± 0.2
Ie	2.55 ± 0.3	2.35 ± 0.25	2.55 ± 0.2
If	1.8 ± 0.6	1.8 ± 0.6	2.4 ± 0.4
Ig	1.75 ± 0.7	2.1 ± 0.5	2.5 ± 0.4
Ih	1.7 ± 0.6	2.3 ± 0.3	2.5 ± 0.25
Ii	1.7 ± 0.6	2.0 ± 0.5	2.25 ± 0.6
Ij	2.6 ± 0.3	2.3 ± 0.35	2.5 ± 0.2
Ik	1.4 ± 0.8	1.8 ± 0.8	2.3 ± 0.1
Il	1.0 ± 0.7	1.4 ± 0.95	1.6 ± 0.9

[a] Mol l^{-1}.

N-Carbobenzoxydipeptide Methyl Esters *II*. General Procedure

To the suspension of methyl 1-amino-1-cyclobutanecarboxylate hydrochloride (0.497 g, 3 mmol) in methylene chloride (15 ml) was added 1-ethylpiperidine (0.42 ml, 3 mmol), followed by 5 min of stirring with a *Z*-amino acid (3 mmol). After cooling at −15 °C, DCCI (0.673 g, 3.3 mmol) were added. The mixture was stirred for 1 h with cooling (−15 °C) and 24 h at 4 °C, then glacial acetic acid (0.2 ml) was added. The *N,N'*-dicyclohexylurea was removed by filtration and filtrate was diluted by methylene chloride (30 ml). The filtrate was evaporated in a vacuum on a water bath (< 40 °C). The semi-solid residue was dissolved in ethyl acetate (40 ml) and the solution filtered to remove the excess of dicyclohexylurea. The filtrate was successively washed with 5% hydrochloric acid (10 ml), 5% sodium carbonate (10 ml), and water (10 ml) and dried over sodium sulfate. The solution was evaporated to dryness, recrystallized from an ethyl acetate–petroleum ether solvent system.

In IR spectra following characteristic vibrations were found: 3 320 (NH), 1 740 (CO, ester), 1 645 (CO, *Z*), 1 650 (CO, amide I), 1 540 (CO, amide II).

Spirocyclic Cyclodipeptides *I*. General Procedure

N-Carbobenzoxydipeptide methyl ester *II* (3 mmol) was dissolved in methanol and 5% Pd/C (0.5 g) was added. This solution was hydrogenated in an autoclave at 2 MPa for 2 h at room temperature. The catalyst was removed by filtration and the filtrate evaporated to dryness. A crude oily product was cyclized in 120 ml of dry toluene with anhydrous acetic (0.2 ml) followed by continuous removal of toluene by distillation for 2 h. The crude products were crystallized from 2-propanol.

In IR spectra following characteristic vibrations were found: 3 180, 3 090 (NH), 3 040 (NH, cis amide), 1 660 – 1690 (CO, amide I), 1 440 – 1450 (CO, amide II), 1 305 – 1340 (amide III).

Antiproliferative Activity Tests

All experiments were performed on chick embryos of White Leghorn stock (breeding station Dobřenice, The Czech Republic) incubated at 37.5 °C and 60 – 70% relative humidity in a forced-draft thermostatic oven. A standard technique – the Chicken Embryotoxicity Screening Test (CHEST I, refs[9,10]) – was employed with a view towards ultimate comparison of the antiproliferative effects of specific (single) newly synthesized spirocyclic dipeptides.

The spirocyclic dipeptides, diluted in 10% water–dimethyl sulfoxide (which has no effect on the embryonic development) in decimal geometric series from $1 . 10^{-3}$ to $1 . 10^{-5}$ mol l^{-1}, were injected in 3 μl volumes into the subgerminal cavity of embryos incubated for 38 – 42 h (developmental stages[11] IIII 10 – 11), using the usual window technique. Each dose was applied to eight to ten embryos under a stereomicroscope. After 24 h of further incubation, the length of the newly proliferating part of the trunk between the vitelline arteries and the tip of the tail was measured at linear magnification × 13 with a measuring ocular eyepiece[9]. The projections of the lengths obtained in this way were converted to the millimetres. The lower limit of the growth norm of the caudal morphogenetic system within 24 h is 2.3 mm. A decrease of the mean value of the trunk length below the norm mentioned is considered to be a manifestation caused by an interference of the drug tested with the function of the caudal morphogenetic system. That condition is termed as the growth retardation, i. e. the antiproliferative effect. The means and SE for single doses of the cyclic dipeptides investigated are presented in Table IV.

TABLE IV
Comparison of length (mm) of trunk 24 h after treatment in a series of cyclodipeptides cyclo(-A-Acb-) *I* and cyclo(-A-Acp-) in concentration $1 . 10^{-3}$ mol l^{-1}

A	Cyclo(-A-Acp-)	Cyclo(-A-Acb-)
Gly	1.6	2.0
L-Ala	2.4	1.9
D-Ala	1.2	1.9
L-Val	1.9	1.8
D-Val	1.4	1.75
L-Leu	1.5	1.7
D-Leu	1.4	1.7
D-Phe	1.1	1.0
L-Phe	[a]	1.4
L-Ser	1.6	2.6
D-Ser	–	2.55
D-Pgl	–	2.4

[a] Insoluble.

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