SYNTHESIS OF NEW POTENTIAL BIS-INTERCALATORS BASED ON CHIRAL PYRIDINE-2,6-DICARBOXAMIDES

Abd El-Galil AMR[a], Osama I. ABD EL-SALAM[a], Abd El-Hamid ATTIA[a1] and Ivan STIBOR[b,*]

[a] *Department of Organic Chemistry, National Research Centre, Dokki, Cairo-12622, Egypt; e-mail: [1] aattia@nrc.sci.eg*

[b] *Department of Organic Chemistry, Prague Institute of Chemical Technology, 166 28 Prague 6, Czech Republic; e-mail: ivan.stibor@vscht.cz*

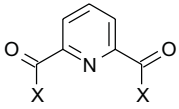
Received March 23, 1998
Accepted January 11, 1999

Dedicated to the memory of Dr Miroslav Protiva.

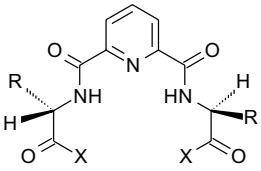
Potential bis-intercalating compounds *N*,*N*-dibenzylidene-N^2,$N^{2'}$-(pyridine-2,6-dicarbonyl)-di(aminoacidhydrazides) **4a**–**4j**, *N*,*N*′-substituted pyridine-2,6-bis(carbohydrazides) **5a**–**5d** and *N*,*N*′-substituted $N^2N^{2'}$-bis(pyridine-2,6-dicarbonyl)di(aminoacidhydrazides) **6a–6g**, both racemic and optically active, can be easily synthesized from pyridine-2,6-bis(carbohydrazide), natural amino acids and aromatic aldehydes or anhydrides of aromatic ortho-dicarboxylic acids.

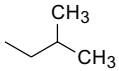
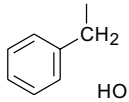
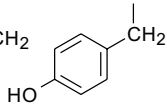
Key words: DNA intercalation; Chiral intercalators; Pyridine-2,6-dicarboxamides; Amino acids; Naphthalimides; Antimicrobial activity.

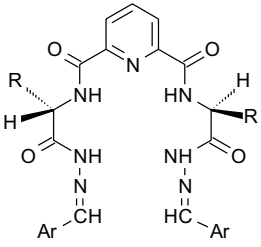
The concept of intercalation was first introduced by Lerman[1] to explain the reversible noncovalent binding of compounds with extended aromatic (most often heteroaromatic) moieties to double-helical DNA. The essence of intercalation is the insertion of such an almost planar moiety between adjacent base pairs of DNA, thereby extending and stabilizing the double helix; the base-pair separation at an intercalation site thus increases[2–8]. Whereas initial studies focused on molecules with a single intercalating moiety[9,10], their promising antitumor activity[11,12] and/or sequence specificity[13,14] has led researchers to investigate compounds that contain more than one intercalating group[15,16]. The family of intercalating antitumor drugs comprises also naphthalimides[17]. For some of them, the antitumor activity has been proved, several of them are now in phase I of clinical development[18–20]. It has been also known for a long time that combination of intrcalating unit with amino acid is promising[21]. Moreover there are peptide motifs occur-

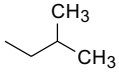
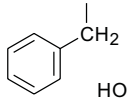
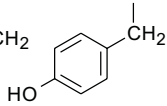
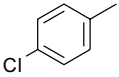


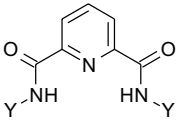
1a, X = OCH_3
1b, X = Cl
1c, X = $NHNH_2$

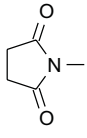


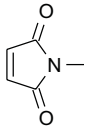
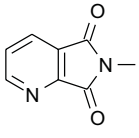
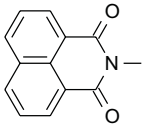
X \ R	H	iPr			
OCH_3	**2a**	**2b**	**2c**	**2d**	**2e**
$NHNH_2$	**3a**	**3b**	**3c**	**3d**	**3e**

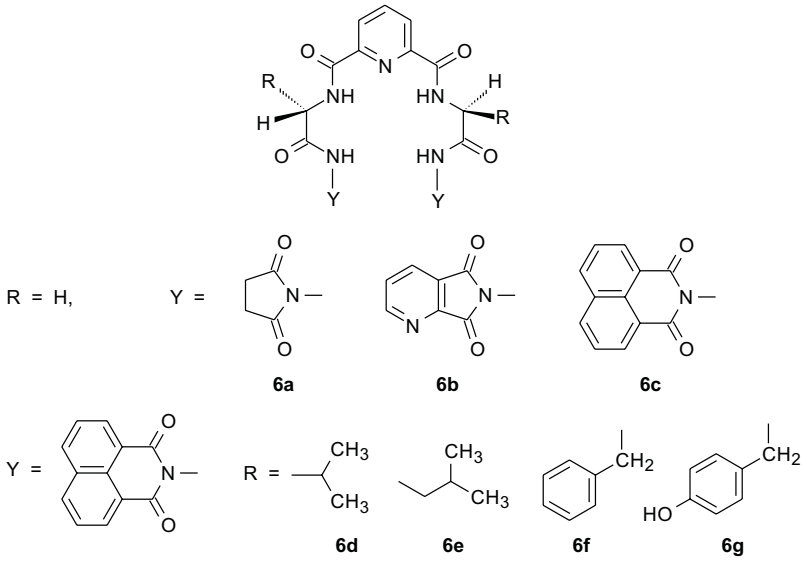


Ar \ R	H	iPr			
	**4a**	**4c**	**4e**	**4g**	**4i**
	**4b**	**4d**	**4f**	**4h**	**4j**



Y = 

5a  **5b**  **5c**  **5d**



ring repeateadly in carboxy-terminal domain of RNA polymerase II in mammals[22] which are believed to be responsible for the ability of this enzyme to bind to DNA by bis-intercalation[23,24]. The structure, conformation and binding ability of this peptide have been studied[25], its synthetic analogues were prepared and their ability to bind to DNA fragments were studied[26,27]. It was proved that the peptide adopt β-turn conformation enforced by several hydrogen bonds thus directing both flat units at the ends of the peptide to be perpendicular with distance about 700 pm which is in good correspondence with the spatial requirements for bis-intercalation. On the other hand, it is also known and very recently proved by X-ray crystallography, that peptides derived from pyridine-2,6-dicarboxylic acid and natural amino acids adopts spontaneously relatively rigid conformation reinforced by bi furcated hydrogen bonding between NH of carboxamides in position 2 and 6 of pyridine nucleus and its nitrogen[28]. This finding makes this structure very promising also from the viewpoint of bis-intercalation. Consequently, we have decided to study the possibility of using this conformationally restricted structure as a scaffold for attachment of aromatic moieties. In the first stage we have decided to use both acylhydrazones and imides as flat units.

In this communication, we report on facile synthesis of new type of potential bis-intercalating compounds **5** and **6**. The synthetic procedures are very simple, easily performed on multigram scale. Products are stable, crystalline compounds, any racemization has not been observed.

The synthesis starts from dimethyl pyridine-2,6-dicarboxylate (**1a**) or corresponding dichloride[29,30] (**1b**). Dihydrazide **1c** was prepared according to the literature[31]. Diesters **2a**–**2e** were prepared from acid chloride **1b** and ester of appropriate amino acid following the standard acylation procedure. They were used as starting materials for the synthesis of dihydrazides **3a**–**3e** using hydrazinolysis. Straightforward reaction of hydrazides **3a**–**3e** with selected commercial aldehydes has furnished **4a**–**4j**. The reaction of hydrazides **1c** and **3a**–**3e** with dicarboxanhydrides has furnished target imides, **5a**–**5d** and **6a**–**6g**, respectively. Preliminary biological activity screening of selected compounds has been performed against microorganisms representing Gram-positive, Gram-negative bacteria, yeast, and fungi, using the bioassay technique of antibiotics specified in US Pharmacopoeia at 50 γ/ml. Ampicillin and chloramphenicol were used as standards. The results obtained are summarized in Table I.

TABLE I
Results of antibiotics bioassay (US Pharmacopoeia, 50 γ/ml)

Compound	*Bacillus subtilis*	*Escherichia coli*	*Staphylococcus aureus*	*Candida albicans*	*Aspergillus niger*
Ampicillin	+	+	+	–	+
Chloramphenicol	+	+	+	–	+
2a	–	–	–	–	+
2b	–	–	–	–	+
3e	–	–	–	+	–
4a	–	–	–	–	+
4g	–	–	–	–	+
6b	–	–	–	–	+
6c	+	+	–	–	–
6d	–	–	–	–	+
6e	+	+	–	+	–
6f	+	–	–	+	+
6g	+	+	–	–	–

Selected Schiff bases **4** as well as imides **5** and **6** are now under study with respect to their ability to intercalate into DNA fragments.

EXPERIMENTAL

Melting points are uncorrected, the synthesized compounds were dried *in vacuo* for 8 h (or overnight), solvents were evaporated on rotary evaporator at 40 °C. NMR spectra (δ, ppm; *J*, Hz) with TMS as internal standard were recorded on a Varian Gemini 300 HC spectrometer in $CDCl_3$ at 25 °C. MS spectra (FAB) were measured on a ZAB-EQ (VG Analytical) using Xe atom bombardement and Finigan SPQ-7000, GC-MS spectrometer. Optical rotations were measured on an Atago Polax-D polarimeter at 30 °C and are given in deg cm^3 g^{-1} dm^{-1}. Antimicrobial activity screening have been performed by Prof. El-Diwany, Department of Natural and Microbial Product, National Research Center, Cairo, Egypt.

Diesters **2a**–**2e**. General Procedure

To a solution of amino acid methyl ester (2 mmol), obtained by addition of triethylamine (2 mmol) to amino acid (2 mmol) methyl ester hydrochloride in 50 ml of dry dichloromethane at –15 °C followed by 15 min stirring, pyridine-2,6-bis(carbonyl chloride) (**1b**) (1 mmol) in 15 ml of dry dichloromethane was added dropwise at –15 °C followed by triethylamine (2 mmol) in order to keep the reaction mixture slightly alkaline (pH 8). The reaction mixture was stirred at –15 °C for 3 h, at ambient tem perature for 12 h, then washed with water (2 × 50 ml), 1 M hydrochloric acid (2 × 50 ml), saturated sodium hydrogencarbonate (2 × 50 ml) and finely with water, dried over Na_2SO_4, evaporated and purified by crystallization.

Dimethyl N,N′-(pyridine-2,6-dicarbonyl)diglycinate (**2a**) was obtained in 45% yield. M.p. 135–140 °C (ethanol–petroleum ether). For $C_{13}H_{15}N_3O_6$ (309.3) calculated: 50.48% C, 4.88% H, 13.58% N; found: 50.36% C, 5.08% H, 13.48% N. ^{1}H NMR: 8.70–8.6 t, 2 H (NH); 8.2–8.1 d, 2 H (3 H-pyridine); 7.95–7.85 t, 1 H (4 H-pyridine); 4.2 d, 4 H (2 × CH_2); 3.75 s, 6 H (2 × CH_3). ^{13}C NMR: 41.49, 52.72, 125.35, 139.28, 148.23, 164.33, 171.23. Mass spectrum (M^+, *m/z* (rel. %)): 309 (72).

Dimethyl N,N′-(pyridine-2,6-dicarbonyl)di((S)-valinate) (**2b**) was obtained in 56% yield as oil. For $C_{19}H_{27}N_3O_6$ (393.4) calculated: 57.99% C, 6.92% H, 10.68% N; found: 57.85% C, 6.89% H, 10.63% N; $[\alpha]_D$ –20 (ethanol). ^{1}H NMR: 8.4–8.3 d, 2 H (NH); 8.3–8.2 d, 2 H (3 H-pyridine); 8.1–8.0 t, 1 H (4 H-pyridine); 4.8–4.65 t, 2 H (2 × CHN); 3.75 s, 6 H (2 × OCH_3), 2.4–2.2 m, 2 H (2 × CHC); 1.1–1.0 d, 12 H (4 × CH_3). ^{13}C NMR: 17.14, 18.53, 30.94, 51.67, 56.92, 124.72, 138.93, 147.95, 162.69, 171.32. Mass spectrum (M^+, *m/z* (rel. %)): 393 (51).

Dimethyl N,N′-(pyridine-2,6-dicarbonyl)di((S)-leucinate) (**2c**) was obtained in 51% yield. M.p. 130–132 °C (ethanol–petroleum ether). For $C_{21}H_{31}N_3O_6$ (421.5) calculated: 59.84% C, 7.41% H, 9.96% N; found: 59.63% C, 7.70% H, 9.90% N; $[\alpha]_D$ –10 (ethanol). ^{1}H NMR: 8.4–8.3 d, 2 H (NH); 8.2 d, 2 H (3 H-pyridine); 8.1–8.0 t, 1 H (4 H-pyridine); 4.9–4.8 q, 2 H (2 × CHN); 3.75 s, 6 H (2 × OCH_3), 1.9–1.7 m, 2 H (2 × CH_2); 1.3–1.2 m, 2 H (2 × CHC); 1.1–0.9 d, 12 H (4 × CH_3). ^{13}C NMR: 22.52, 23.31, 25.53, 42.13, 51.57, 52.89, 125.77, 139.55, 148.89, 163.62, 173.71. Mass spectrum (M^+, *m/z* (rel. %)): 422 (100).

Dimethyl N,N′-(pyridine-2,6-dicarbonyl)di((S)-phenylalaninate) (**2d**) was obtained in 80% yield. M.p. 122–125 °C (ethanol–petroleum ether). For $C_{27}H_{27}N_3O_6$ (489.5) calculated:

66.24% C, 5.55% H, 8.58% N; found: 65.93% C, 5.61% H, 8.48% N; $[\alpha]_D$ –55 (ethanol). ^{1}H NMR: 8.4–8.3 d, 2 H (NH); 8.1–7.9 m, 3 H (pyridine); 7.4–7.1 m, 10 H (2 × Ph); 5.1–5.0 q, 2 H (2 × CHN); 3.8 s, 6 H (2 × OCH_3); 3.4–3.1 m, 4 H (2 × CH_2Ph). ^{13}C NMR: 38.30, 52.67, 53.93, 125.64, 127.48, 129.01, 129.53, 136.20, 139.29, 148.60, 163.32, 171.91. Mass spectrum (M^+, *m/z* (rel. %)): 489 (41).

Dimethyl N,N′-(pyridine-2,6-dicarbonyl)di((S)-tyrosinate) (**2e**) was obtained in 40% yield. M.p. 122–125 °C (ethanol–petroleum ether). For $C_{27}H_{27}N_3O_6$ (521.5) calculated: 62.18% C, 5.21% H, 8.05% N; found: 62.20% C, 5.35% H, 7.90% N; $[\alpha]_D$ –100 (DMF). ^{1}H NMR: 10.3 s, 2 H (2 × OH); 9.2–8.8 d, 2 H (NH); 8.3–8.1 m, 3 H (pyridine); 7.5–6.8 m, 8 H (2 × Ph); 4.6–4.5 t, 2 H (2 × CHN); 3.6–3.5 m, 4 H (2 × CH_2Ph); 3.2–3.0 s, 6 H (2 × OCH_3). ^{13}C NMR: 36.71, 52.98, 54.24, 122.28, 126.02, 130.84, 134.87, 139.54, 148.69, 156.19, 163.61, 172.55. Mass spectrum (M^+, *m/z* (rel. %)): 522 (20).

Dihydrazides **3a**–**3e**. General Procedure

Hydrazine hydrate (16 mmol, 80% aqueous solution) was added to a diester **2** (1 mmol) in methanol (50 ml), refluxed for 5 h, evaporated, triturated with light petroleum and purified by crystallization.

N^2,$N^{2'}$-(pyridine-2,6-dicarbonyl)di(glycinehydrazide) (**3a**) was obtained in 80% yield. M.p. 228–230 °C (DMF–water). For $C_{11}H_{15}N_7O_4$ (309.3) calculated: 42.71% C, 4.88% H, 31.70% N; found: 42.68% C, 4.74% H, 31.45% N. ^{1}H NMR: 9.70–9.55 t, 2 H (2 × NH_2-N**H**); 9.20–9.10 t, 2 H (2 × NH); 8.2–8.1 m, 3 H (pyridine); 4.3–4.2 s, 4 H (2 × CH_2); 4.0–3.90 s, 4 H (2 × NH_2). ^{13}C NMR: 41.02, 124.24, 139.34, 148.42, 163.58, 168.13. Mass spectrum (M^+, *m/z* (rel. %)): 309 (72).

N^2,$N^{2'}$-(pyridine-2,6-dicarbonyl)di((S)valinehydrazide) (**3b**) was obtained in 79% yield. M.p. 125–128 °C (dioxane–benzene). For $C_{17}H_{27}N_7O_4$ (393.45) calculated: 51.89% C, 6.91% H, 24.98% N; found: 51.97% C, 6.68% H, 24.89% N; $[\alpha]_D$ –145 (DMF). ^{1}H NMR: 9.50–9.30 d, 2 H (2 × NH_2-N**H**); 8.75–8.65 d, 2 H (2 × NH); 8.30–8.10 m, 3 H (pyridine); 4.35–4.20 t, 2 H (2 × N-CH); 4.20–3.50 bs, 4 H (2 × NH_2); 2.20–2.05 m, 2 H (2 × C-CH); 1.00–0.80 m, 12 H (4 × CH_3). ^{13}C NMR: 18.82, 19.62, 31.17, 57.57, 125.19, 140.09, 149.04, 163.37, 170.37. Mass spectrum (M^+, *m/z* (rel. %)): 394 (2).

N^2,$N^{2'}$-(pyridine-2,6-dicarbonyl)di((S)leucinehydrazide) (**3c**) was obtained in 85% yield. M.p. 110–113 °C (ethanol–water). For $C_{19}H_{31}N_7O_4$ (421.5) calculated: 54.14% C, 7.41% H, 23.26% N; found: 53.99% C, 7.25% H, 23.15% N; $[\alpha]_D$ +40 (DMF). ^{1}H NMR: 9.50–9.30 s, 2 H (2 × NH_2-N**H**); 9.10–9.00 d, 2 H (2 × NH); 8.30–8.10 m, 3 H (pyridine); 4.65–4.45 t, 2 H (2 × N-CH); 4.40–3.80 bs, 4 H (2 × NH_2); 1.90–1.70 m, 2 H (2 × C-CH); 1.70–1.50 t, 4 H (4 × CH_2); 1.00–0.80 m, 12 H (4 × CH_3). ^{13}C NMR: 22.22, 23.24, 24.90, 40.13, 51.04, 125.14, 139.67, 149.24, 163.58, 171.35. Mass spectrum (M^+, *m/z* (rel. %)): 422 (8).

N^2,$N^{2'}$-(pyridine-2,6-dicarbonyl)di((S)phenylalaninehydrazide) (**3d**) was obtained in 83% yield. M.p. 197–200 °C (ethanol–diethyl ether). For $C_{25}H_{27}N_7O_4$ (489.5) calculated: 61.33% C, 5.55% H, 20.02% N; found: 61.26% C, 5.53% H, 20.18% N; $[\alpha]_D$ –100 (DMF). ^{1}H NMR: 9.50 s, 2 H (2 × NH_2-N**H**); 9.20–9.10 d, 2 H (2 × NH); 8.20–8.00 m, 3 H (pyridine); 7.50–7.10 m, 10 H (2 × Ph); 4.70–4.60 s, 2 H (2 × N-CH); 4.50–3.80 bs, 4 H (2 × NH_2); 2.50 d, 4 H (2 × CH_2). ^{13}C NMR: 37.72, 53.66, 124.63, 126.33, 128.26, 137.81, 139.23, 148.61, 163.05, 169.87. Mass spectrum (M^+, *m/z* (rel. %)): 490 (10).

N^2,$N^{2'}$-(pyridine-2,6-dicarbonyl)di((S)tyrosinehydrazide) (**3e**) was obtained in 71% yield. M.p. 225–228 °C (methanol–water). For $C_{25}H_{27}N_7O_6$ (521.5) calculated: 57.57% C, 5.21% H,

18.80% N; found: 57.49% C, 5.23% H, 18.74% N; $[\alpha]_D$ –110 (DMF). ^{1}H NMR: 10.65–10.50 s, 2 H (2 × OH); 9.40–9.30 s, 2 H (2 × NH_2-N**H**); 9.20–9.00 d, 2 H (2 × NH); 8.20–8.00 m, 3 H (pyridine); 7.30–7.10 s, 4 H (Ph); 6.80–6.60 s, 4 H (Ph); 4.70–4.50 s, 2 H (2 × N-CH); 4.30–4.10 s, 4 H (2 × NH_2); 3.10–2.95 d, 4 H (2 × CH_2). ^{13}C NMR: 37.38, 54.22, 115.45, 124.04, 124.98, 128.13, 139.61, 148.98, 156.09, 163.32, 170.35.

Dihydrazones **4a**–**4j**. General Procedure

A solution of a dihydrazide **3a**–**3e** (1 mmol) and an aromatic aldehyde (2 mmol) in 100 ml of absolute methanol was refluxed for 6 h. The reaction mixture was cooled and most of the solvent was evaporated. The crystalline crude product was collected by filtration, washed with ether and purified by crystallization.

N,N′-Dibenzylidene-N^2,$N^{2'}$-(pyridine-2,6-dicarbonyl)di(glycinehydrazide) (**4a**) was obtained in 42% yield. M.p. 297–300 °C (DMF–water). For $C_{25}H_{23}N_7O_4$ (485.5) calculated: 61.84% C, 4.77% H, 20.19% N; found: 61.79% C, 4.88% H, 20.14% N. ^{1}H NMR: 9.90–9.80 t, 2 H (2 × N-NH); 9.80–9.60 s, 2 H (2 × NH); 8.35–8.05 m, 3 H (pyridine); 7.75–7.45 m, 10 H (2 × Ph); 4.60 s, 2 H (2 × N=CH); 4.10 s, 4 H (2 × CH_2). ^{13}C NMR: 41.79, 124.47, 127.10, 128.85, 130.07, 134.24, 139.58, 143.72, 147.52, 163.84, 165.48, 170.18. Mass spectrum (M^+, *m/z* (rel. %)): 486 (35).

N,N′-Bis(4-chlorobenzylidene)-N^2,$N^{2'}$-(pyridine-2,6-dicarbonyl)di(glycinehydrazide) (**4b**) was obtained in 86% yield. M.p. 328–330 °C (DMF–water). For $C_{25}H_{21}Cl_2N_7O_4$ (554.4) calculated: 54.16% C, 3.81% H, 17.68% N; found: 54.10% C, 3.82% H, 17.54% N. ^{1}H NMR: 9.90–9.60 s, 4 H (4 × NH); 8.25–8.05 m, 3 H (pyridine); 7.80–7.55 m, 8 H (2 × Ph); 4.80–4.60 s, 2 H (2 × N=CH); 4.10 s, 4 H (2 × CH_2). ^{13}C NMR: 41.13, 124.40, 128.45, 128.85, 132.97, 134.30, 139.52, 143.62, 145.62, 148.42, 163.64, 165.40, 170.16. Mass spectrum (M^+, *m/z* (rel. %)): 554 (10).

N,N′-Dibenzylidene-N^2,$N^{2'}$-(pyridine-2,6-dicarbonyl)di((S)valinehydrazide) (**4c**) was obtained in 61% yield. M.p. 120–122 °C (ethanol–water). For $C_{31}H_{35}N_7O_4$ (596.7) calculated: 65.36% C, 6.19% H, 17.21% N; found: 65.28% C, 6.14% H, 17.12% N; $[\alpha]_D$ +10 (DMF). ^{1}H NMR: 9.35 s, 2 H (2 × N-NH); 8.65 d, 2 H (2 × C-NH); 8.40–8.05 m, 3 H (pyridine); 7.80–7.30 m, 10 H (2 × Ph); 5.40 s, 2 H (2 × N=CH); 4.40–4.20 t, 2 H (2 × CH-N); 2.25–2.05 m, 2 H (2 × C**H**$(CH_3)_2$); 1.00 t, 6 H (2 × CH_3); 0.80 t, 6 H (2 × CH_3). ^{13}C NMR: 18.35, 19.09, 30.54, 57.05, 124.39, 127.27, 128.67, 130.26, 133.89, 139.52, 147.46, 148.51, 163.24, 165.52, 172.24.

N,N′-Bis(4-chlorobenzylidene)-N^2,$N^{2'}$-(pyridine-2,6-dicarbonyl)di((S)valinehydrazide) (**4d**) was obtained in 54% yield. M.p. 208–210 °C (DMF–water). For $C_{31}H_{33}Cl_2N_7O_4$ (638.6) calculated: 58.30% C, 5.20% H, 15.35% N; found: 58.41% C, 5.18% H, 15.29% N; $[\alpha]_D$ +150 (DMF). ^{1}H NMR: 9.23 s, 2 H (2 × N-NH); 8.45 d, 2 H (2 × C-NH); 8.40–8.10 m, 3 H (pyridine); 7.80–7.30 m, 8 H (2 × Ph); 5.20 s, 2 H (2 × N=CH); 4.45–4.24 t, 2 H (2 × CH-N); 2.30–2.15 m, 2 H (2 × C**H**$(CH_3)_2$); 1.05 t, 6 H (2 × CH_3); 0.85 t, 6 H (2 × CH_3). ^{13}C NMR: 18.92, 19.45, 30.75, 58.36, 125.08, 128.61, 129.01, 134.60, 134.77, 139.92, 146.23, 148.86, 163.21, 167.64, 172.54.

N,N′-Dibenzylidene-N^2,$N^{2'}$-(pyridine-2,6-dicarbonyl)di((S)leucinehydrazide) (**4e**) was obtained in 60% yield. M.p. 176–179 °C (ethanol–petroleum ether). For $C_{33}H_{39}N_7O_4$ (597.7) calculated: 66.30% C, 6.58% H, 16.41% N; found: 66.18% C, 6.46% H, 16.36% N. ^{1}H NMR: 9.65 s, 2 H (2 × N-NH); 8.76 d, 2 H (2 × C-NH); 8.45–8.15 m, 3 H (pyridine); 7.90–7.35 m, 10 H (2 × Ph); 4.80 s, 2 H (2 × N=CH); 4.38–4.18 m, 2 H (2 × CH-N); 3.50 t, 4 H (2 × CH_2); 2.27–2.15 m, 2 H (2 × C**H**$(CH_3)_2$); 1.10 t, 6 H (2 × CH_3); 0.90 t, 6 H (2 × CH_3). ^{13}C NMR:

21.57, 22.72, 24.66, 41.52, 51.33, 124.65, 127.01, 129.14, 133.49, 134.37, 139.19, 145.70, 148.69, 163.21, 168.31, 173.26.

N,N′-Bis(4-chlorobenzylidene)-N^2,$N^{2'}$-(pyridine-2,6-dicarbonyl)di((S)leucinehydrazide) (**4f**) was obtained in 67% yield. M.p. 123–125 °C (ethanol). For $C_{33}H_{37}Cl_2N_7O_4$ (666.6) calculated: 59.45% C, 5.59% H, 14.70% N; found: 59.23% C, 5.68% H, 14.59% N; $[\alpha]_D$ +80 (DMF). ^{1}H NMR: 9.40 s, 2 H (2 × N-NH); 9.20–9.10 t, 2 H (2 × C-NH); 8.30–8.10 m, 3 H (pyridine); 7.70–7.40 m, 8 H (2 × Ph); 5.50 s, 2 H (2 × N=CH); 4.70 t, 2 H (2 × CH-C); 3.40 d, 4 H (2 × CH_2); 2.00–1.70 m, 2 H (2 × C**H**$(CH_3)_2$); 1.10 t, 6 H (2 × CH_3); 0.90 t, 6 H (2 × CH_3). Mass spectrum (FAB, glycerol–thioglycerol–DMSO) (M^+, *m/z* (rel. %)): 666 (M + H, 100).

N,N′-Dibenzylidene-N^2,$N^{2'}$-(pyridine-2,6-dicarbonyl)di((S)phenylalaninehydrazide) (**4g**) was obtained in 60% yield. M.p. 145–147 °C (methanol–water). For $C_{39}H_{35}N_7O_4$ (665.8) calculated: 70.36% C, 5.29% H, 14.72% N; found: 70.58% C, 5.33% H, 14.42% N; $[\alpha]_D$ +150 (DMF). ^{1}H NMR: 9.40 s, 2 H (2 × N-NH); 8.85 s, 2 H (2 × C-NH); 8.40–8.10 m, 3 H (pyridine); 7.90–7.15 m, 20 H (2 × Ph); 4.90 s, 2 H (2 × N=CH); 4.50 t, 2 H (2 × CH-N); 3.50 t, 4 H (2 × CH_2). ^{13}C NMR: 40.25, 51.33, 124.45, 126.54, 128.09, 128.37, 128.52, 128.75, 133.03, 134.27, 134.37, 139.19, 145.70, 148.69, 163.21, 168.31, 173.26.

N,N′-Bis(4-chlorobenzylidene)-N^2,$N^{2'}$-(pyridine-2,6-dicarbonyl)di((S)phenylalaninehydrazide) (**4h**) was obtained in 55% yield. M.p. 215–220 °C (methanol–water). For $C_{39}H_{33}Cl_2N_7O_4$ (734.6) calculated: 63.76% C, 4.52% H, 13.34% N; found: 63.86% C, 4.47% H, 13.55% N; $[\alpha]_D$ +70 (DMF). ^{13}C NMR: 41.25, 51.44, 125.20, 127.27, 127.52, 128.78, 129.82, 130.52, 131.75, 134.24, 134.56, 139.81, 147.85, 148.98, 163.85, 167.73, 172.59. Mass spectrum (M^+, *m/z* (rel. %)): 734 (80).

N,N′-Dibenzylidene-N^2,$N^{2'}$-(pyridine-2,6-dicarbonyl)di((S)tyrosinehydrazide) (**4i**) was obtained in 65% yield. M.p. 210–212 °C (methanol–diethyl ether). For $C_{39}H_{35}N_7O_6$ (697.8) calculated: 67.13% C, 5.06% H, 14.05% N; found: 66.95% C, 5.12% H, 14.12% N; $[\alpha]_D$ +70 (DMF). ^{13}C NMR: 41.52, 53.23, 123.98, 127.65, 127.96, 128.25, 130.25, 134.75, 139.62, 146.21, 148.65, 153.95, 163.75, 165.39, 172.54. Mass spectrum (M^+, *m/z* (rel. %)): 698 (3).

N,N′-Bis(4-chlorobenzylidene)-N^2,$N^{2'}$-(pyridine-2,6-dicarbonyl)di((S)tyrosinehydrazide) (**4j**) was obtained in 58% yield. M.p. 178–180 °C (methanol–water). For $C_{39}H_{33}Cl_2N_7O_6$ (766.6) calculated: 61.10% C, 4.34% H, 12.79% N; found: 60.85% C, 4.45% H, 12.82% N; $[\alpha]_D$ –25 (DMF). ^{1}H NMR: 11.50 s, 2 H (2 × OH); 9.40–9.30 d, 2 H (2 × N-NH); 8.70 s, 2 H (2 × C-NH); 8.90–8.30 m, 3 H (pyridine); 7.70–7.60 d, 8 H (2 × Ph); 7.20–7.10 m, 8 H (2 × Ph); 5.75 s, 2 H (2 × N=CH); 4.75 t, 2 H (2 × CH-N); 3.40 d, 4 H (2 × CH_2). ^{13}C NMR: 42.05, 54.62, 124.32, 127.26, 127.89, 128.55, 129.65, 133.24, 134.39, 134.81, 139.26, 145.23, 147.96, 154.26, 163.65, 165.43, 171.95.

Bis-Diimides **5a**–**5d** and **6a**–**6g**. General Procedure

A mixture of pyridine-2,6-bis(carbohydrazide) (**1c**, **3a**–**3e**) (1 mmol) and an appropriate dicarboxylic acid anhydride (2 mmol) was refluxed in 50 ml of glacial acetic acid for 6 h. The reaction mixture was evaporated, cooled and the crystalline product was collected by filtration. The crude product was washed with cold acetic acid, dried and purified by crystallization to yield compounds **5a**–**5d** and **6a**–**6g**.

N,N′-Disuccinylpyridine-2,6-bis(carbohydrazide) (**5a**) was prepared from **1c** and succinic anhydride in 68% yield. M.p. >350 °C (acetic acid–diethyl ether). For $C_{15}H_{13}N_5O_6$ (359.3) calculated: 50.13% C, 3.65% H, 19.50% N; found: 50.32% C, 3.58% H, 19.41% N. ^{1}H NMR: (DMSO-d_6): 11.50 bs, 2 H (NH); 8.34 s, 3 H (arom.); 2.93 bs, 8 H ($COCH_2$). ^{13}C NMR

(DMSO-d_6): 26.44, 126.26, 140.58, 146.53, 161.24, 174.22. Mass spectrum (FAB, glycerol–thioglycerol–DMSO), *m/z* (rel. %): 360 (M + H, 70).

N,N′-Dimaleoylpyridine-2,6-bis(carbohydrazide) (**5b**) was prepared from **1c** and maleic anhydride in 90% yield. M.p. 305 °C (DMF–water). For $C_{15}H_9N_5O_6$ (355.3) calculated: 50.71% C, 2.55% H, 19.71% N; found: 50.68% C, 2.60% H, 19.85% N. ^{1}H NMR (DMSO-d_6): 11.24 bs, 2 H (NH); 8.27 s, 3 H (arom.); 6.47, 6.43, 6.37, 6.33 AB system, 4 H (4 × CH=). ^{13}C NMR (DMSO-d_6): 125.41, 126.80, 133.18, 140.07, 147.66, 161.93, 163.47, 167.15. Mass spectrum, *m/z* (rel. %): 355 (18).

N,N′-Bis(pyridine-2,3-dicarbonyl)pyridine-2,6-bis(carbohydrazide) (**5c**) was prepared from **1c** and pyridine-2,3-dicarboxylic anhydride in 82% yield. M.p. >300 °C (DMF–water). For $C_{21}H_{11}N_7O_6$ (457.4) calculated: 55.15% C, 2.42% H, 21.44% N; found: 55.25% C, 2.35% H, 21.17% N. ^{1}H NMR (DMSO-d_6): 11.99 bs, 2 H (NH); 9.14 d, 2 H, J = 4.4 (H-2 H-pyridine); 8.52 d, 2 H, J = 7.7 (H-4 H-pyridine); 8.39 s, 3 H (Ar-pyridine); 7.96 dd, 2 H, J = 4.4, J = 7.7 (3 H-pyridine). ^{13}C NMR (DMSO-d_6): 126.36, 127.17, 129.44, 132.84, 141.17, 146.89, 149.72, 156.52, 162.52, 164.15, 164.25.

N,N′-Bis(naphthalene-1,8-dicarbonyl)pyridine-2,6-bis(carbohydrazide) (**5d**) was prepared from **1c** and naphthalene-1,8-dicarboxylic anhydride in 72% yield. M.p. >300 °C (DMF). For $C_{31}H_{17}N_5O_6$ (555.5) calculated: 67.03% C, 3.08% H, 12.60% N; found: 66.70% C, 3.35% H, 12.50% N. ^{1}H NMR (DMSO-d_6): 11.99 bs, 2 H (NH); 8.62 m, 8 H (βH-naphthalene); 8.42 m, 3 H (pyridine); 7.97 m, 4 H (αH-naphthalene). Mass spectrum (FAB, glycerol–thioglycerol–DMSO), *m/z* (rel. %): 556.1 (M + H, 100).

N,N′-Dimaleolyl-N^2,$N^{2'}$-(pyridine-2,6-dicarbonyl)di(glycinehydrazide) (**6a**) was prepared from **3a** and maleic anhydride in 70% yield. M.p. >235–240 °C. For $C_{19}H_{15}N_7O_8$ (469.4) calculated: 48.62% C, 3.22% H, 20.89% N; found: 48.55% C, 3.14% H, 20.84% N. ^{1}H NMR (DMSO-d_6): 11.37 bs, 2 H (NH); 9.71 bt, 2 H, J = 6 (N**H**-CH); 8.21 bs, 3 H (pyridine); 6.33 m, 4 H (CH=); 4.09 bd, 4 H, J = 6 (C**H**$_2$NH). ^{13}C NMR (DMSO-d_6): 41.16, 125.24, 128.50, 133.34, 140.35, 149.22, 163.55, 164.52, 167.58, 168.18. Mass spectrum, *m/z* (rel. %): 469 (2).

N,N′-Bis(pyridine-2,3-dicarbonyl)-N^2,$N^{2'}$-(pyridine-2,6-dicarbonyl)di(glycinehydrazide) (**6b**) was prepared from **3a** and pyridine-2,3-dicarboxylic anhydride in 60% yield. M.p. >300 °C (DMF–water). For $C_{25}H_{17}N_9O_8$ (571.5) calculated: 52.543% C, 3.00% H, 22.06% N; found: 52.67% C, 3.05% H, 21.96% N. ^{1}H NMR (DMSO-d_6): 11.05 bs, 2 H (NH); 9.83 bt, 2 H, J = 6.1 (N**H**-CH); 9.07 d, 2 H, J = 4.9 (2 H-pyridine); 8.41 d, 2 H, J = 7.7 (4 H-pyridine); 8.25 m, 3 H (pyridine); 7.88 dd, 2 H, J = 4.9, J = 7.7 (3 H-pyridine); 4.32 bd, 4 H (C**H**$_2$NH). Mass spectrum (FAB, glycerol–thioglycerol–DMSO), *m/z* (rel. %): 572.0 (M + H, 55).

N,N′-Bis(naphthalene-1,8-dicarbonyl)-N^2,$N^{2'}$-(pyridine-2,6-dicarbonyl)di(glycinehydrazide) (**6c**) was prepared from **3a** and naphthalene-1,8-dicarboxylic anhydride in 65% yield. M.p. 235–240 °C (dioxane–benzene). For $C_{35}H_{23}N_7O_8$ (669.6) calculated: 62.78% C, 3.46% H, 14.64% N; found: 62.75% C, 3.42% H, 14.60% N. ^{1}H NMR (DMSO-d_6): 10.80 s, 2 H (2 × NH); 8.90–8.80 m, 2 H (2 × NH); 8.52–8.50 m, 8 H (βH-naphthalene); 8.10 m, 3 H (pyridine); 7.90–7.80 m, 4 H (αH-naphthalene); 3.58 d, 4 H (C**H**$_2$NH). Mass spectrum, *m/z* (rel. %): 669 (M^+, 15).

N,N′-Bis(naphthalene-1,8-dicarbonyl)-N^2,$N^{2'}$-(pyridine-2,6-dicarbonyl)di((S)-valinehydrazide) (**6d**) was prepared from **3b** and naphthalene-1,8-dicarboxylic anhydride in 65% yield. M.p. >280 °C (DMF–water); $[\alpha]_D$ +20 (acetic acid). For $C_{41}H_{35}N_7O_8$ (753.8) calculated: 65.32% C, 4.68% H, 13.01% N; found: 65.20% C, 4.52% H, 12.89% N. ^{1}H NMR (DMSO-d_6): 11.10 bs, 2 H (NH); 8.68 m, 2 H (N**H**-CH); 8.47 m, 8 H (βH-naphthalene); 8.24 m, 3 H (pyridine); 7.86 m, 4 H (αH-naphalene); 4.76 m, 2 H (C**H**NH); 2.33 m, 2 H (C**H**$(CH_3)_2$); 1.08 d and 1.02 d, 2 ×

6 H, *J* = 7.1 (CH_3). ^{13}C NMR (DMSO-d_6): 17.77, 19.13, 31.36, 56.38, 121.53, 125.04, 127.26, 131.46, 132.49, 135.10, 135.40, 140.00, 148.61, 161.51, 162.98, 169.44. Mass spectrum, *m/z* (rel. %): 753 (M^+, 10).

N,N′-Bis(naphthalene-1,8-dicarbonyl)-N^2,$N^{2'}$-bis(pyridine-2,6-dicarbonyl)di((S)-leucinehydrazide) (**6e**) was prepared from **3c** and naphthalene-1,8-dicarboxylic anhydride in 75% yield. M.p. 230–235 °C (dioxane–benzene); $[\alpha]_D$ –30 (acetic acid). For $C_{43}H_{39}N_7O_8$ (781.5) calculated: 66.06% C, 5.03% H, 12.54% N; found: 65.95% C, 4.89% H, 12.50% N. ^1H NMR (DMSO-d_6): 11.15 s, 2 H (2 × NH); 9.20–9.10 m, 2 H (2 × N**H**-CH); 8.60–8.52 m, 8 H (βH-naphthalene); 8.16–8.13 m, 3 H (pyridine); 8.02–7.90 m, 4 H (αH-naphthalene); 4.80 m, 2 H (2 × C**H**NH); 3.75 t, 4 H (2 × CH_2); 2.25 m, 2 H (2 × C**H**$(CH_3)_2$); 1.80 d, 12 H (4 × CH_3). Mass spectrum, *m/z* (rel. %): 781 (M^+, 12).

N,N′-Bis(naphthalene-1,8-dicarbonyl)-N^2,$N^{2'}$-bis(pyridine-2,6-dicarbonyl)di((S)-phenylalaninehydrazide) (**6f**) was prepared from **3d** and naphthalene-1,8-dicarboxylic anhydride in 78% yield. M.p. 186–188 °C (dioxane–benzene); $[\alpha]_D$ +40 (acetic acid). For $C_{49}H_{35}N_7O_8$ (849.7) calculated: 69.25% C, 4.15% H, 11.54% N; found: 69.20% C, 4.10% H, 11.51% N. ^1H NMR (DMSO-d_6): 11.28 s, 2 H (2 × NH); 9.31–9.21 m, 2 H (2 × N**H**-CH); 8.58–8.50 m, 8 H (βH-naphthalene); 8.15–8.13 m, 3 H (pyridine); 7.95–7.87 m, 4 H (αH-naphthalene); 7.49–7.19 m, 10 H (2 × Ph); 5.19 t, 2 H (2 × C**H**NH); 3.56 d, 4 H (2 × CH_2). Mass spectrum, *m/z* (rel. %): 849 (M^+, 2).

N,N′-Bis(naphthalene-1,8-dicarbonyl)-N^2,$N^{2'}$-bis(pyridine-2,6-dicarbonyl)di((S)-tyrosinehydrazide) (**6g**) was prepared from **3e** and naphthalene-1,8-dicarboxylic anhydride in 75% yield. M.p. 208–210 °C (dioxane–benzene); $[\alpha]_D$ –50 (acetic acid). For $C_{49}H_{35}N_7O_{10}$ (881.7) calculated: 66.74% C, 4.00% H, 11.12% N; found: 66.70% C, 3.95% H, 11.10% N. ^1H NMR (DMSO-d_6): 12.00 s, 2 H (2 × OH); 11.20 s, 2 H (2 × NH); 9.18–9.10 m, 2 H (2 × N**H**-CH); 8.67–8.50 m, 8 H (βH-naphthalene); 8.14–8.12 m, 3 H (pyridine); 8.01–7.85 m, 4 H (αH-naphthalene); 7.30–7.15 m, 8 H (2 × Ph); 5.20 m, 2 H (2 × C**H**NH); 3.60 d, 4 H (2 × CH_2). Mass spectrum, *m/z* (rel. %): 881 (M^+, 9).

This work was supported by the Grant Agency of the Czech Republic (grant No. 203/94/0933).

REFERENCES

1. Lerman L.: *J. Mol. Biol.* **1961**, *3*, 18.
2. Waring M. J.: *Annu. Rev. Biochem.* **1981**, *50*, 159.
3. Wakelin L. P. G.: *Med. Chem. Rev.* **1986**, *6*, 275.
4. Schneider E., Hsiang Y., Liu. L. F.: *Adv. Pharmacol.* **1990**, *21*, 149.
5. Wakelin L. P. G., Waring M. J. in: *Comprehensive Medicinal Chemistry* (P. G. Sammes, Ed.), Vol. 2, p. 720. Pergamon, Oxford 1990.
6. Johnson D. S., Boger D. L. in: *Comprehensive Supramolecular Chemistry* (J.-M. Lehn, Ed.), Vol. 4, p. 73. Pergamon, Oxford 1995.
7. Hurley L. H.: *J. Med. Chem.* **1989**, *32*, 2027.
8. Waring M. J., Fox K. R. in: *Molecular Aspects of Anti-Cancer Drug Action* (S. Neidle and M. J. Waring, Eds), p. 127. Verlag Chemie, Weinheim 1984.
9. Wilson W. D., Jones R. L.: *Adv. Pharmacol. Chemother.* **1981**, *18*, 177.
10. Wakelin L. P. G.: *Med. Res. Rev.* **1986**, *6*, 275.

11. Chaires J. B., Leng F., Przewloka T., Fokt I., Ling Y. H., Perez-Soler R., Proiebe W.: *J. Med. Chem.* **1997**, *40*, 261.
12. Bousquet P. F., Braňa M. F., Conlon D., Fitgerald K. M., Perron D., Cocchiaro C., Miller R., Moran M., George J., Qian X. D., Keilhauer G., Romerdahl C. A.: *Cancer Res.* **1995**, *55*, 1176.
13. Bailly C., Braňa M. F., Waring M. J.: *Eur. J. Biochem.* **1996**, *240*, 195.
14. Delepine M., Milhe C., Namane A., Dinh T. H., Roques B. P.: *Biopolymers* **1991**, *31*, 331.
15. Denny W. A. in: *Cancer. Chemotherapeutic Agents* (W. O. Foye, Ed.), p. 218. ACS, Washington DC 1995.
16. Lokey R. S., Kwok Y., Guelev V., Pusell C. J., Hurley L. H., Iverson B. L.: *J. Am. Chem. Soc.* **1997**, *119*, 7202.
17. Waring M. J., Gonzáles A., Jiménez A., Vázquez D.: *Nucleic Acids Res.* **1979**, *7*, 217.
18. Braňa M. F., Castellano J. M., Moran M., Perez de Vega M. J., Romerdahl C. A., Qian X. D., Bousquet P. F., Emling F., Schlick E., Keilhauer G.: *Anti-Cancer Drug Des.* **1993**, *8*, 257.
19. Braňa M. F., Castellano J. M., Moran M., Perez de Vega M. J., Qian X. D., Romerdahl C. A., Keilhauer G.: *Eur. J. Med. Chem.* **1995**, *30*, 235.
20. Braňa M. F., Castellano J. M., Perron D., Maher C., Conlon D., Bousquet P. F., George J., Qian X. D., Robinson S. P.: *J. Med. Chem.* **1997**, *40*, 449.
21. Kelly D. P., Mack P. O.-L., Martin R. F., Wakelin L. P. G.: *Int. J. Pept. Protein Res.* **1985**, *26*, 400.
22. Corden J. L., Cadena D. L., Ahearu J. M., Jr., Dahmus M. E.: *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 7934.
23. Young R. A.: *Annu. Rev. Biochem.* **1991**, *60*, 689.
24. Suzuki M.: *Nature* **1990**, *344*, 562.
25. Harding M. M.: *J. Med. Chem.* **1992**, *35*, 4658.
26. Huang X., Long E. C.: *Bioorg. Med. Chem. Lett.* **1995**, *5*, 1937.
27. Harding M. M., Krippner G. Y., Shelton C. J., Rodger A., Sanders K. J., Mackay J. P., Prakash A. P.: *Biopolymers* **1997**, *42*, 387.
28. Ranganathan D., Haridas V., Gilari R., Karle I. L.: *J. Am. Chem. Soc.* **1998**, *120*, 10793.
29. Roderick A. B., Fales H. M.: *J. Am. Chem. Soc.* **1953**, *75*, 975.
30. Graf R., Zettl F.: *J. Prakt. Chem.* **1936**, *147*, 188.
31. Supniewski J., Bany T., Krupinska J.: *Bull. Acad. Pol. Sci.* **1955**, *3*, 55; *Chem. Abstr.* **1956**, *50*, 7800.