Ahmed M. Naglah, Abd El-Galil E. Amr*, Randa E. Abdel-Mageid, Elsayed A. Elsayed and Osama I. Abd El-Salam

Synthesis of chiral 3,5-bis(L-phenylalaninyl-L-leucinyl)pyridine Schiff base and their macrocyclic carboxamide derivatives using 3,5-bis(L-phenylalaninyl)-pyridine methyl ester

<https://doi.org/10.1515/znb-2019-0146>

Received September 04, 2019; accepted December 01, 2019

Abstract: A series of linear tetrapeptides **2–7** and cyclooctapeptedopyridine derivatives **8** and **9**, were synthesized using *N*^α-dinicotinoyl-bis[(L-phenylalaninyl-L-leucyl)hydrazide] **2** as starting material. Acid hydrazide **2** was reacted with aromatic or heterocyclic aldehydes to give Schiff base derivatives **3** and **4**, respectively. Additionally, compound **2** was reacted with dicarboxylic acid anhydrides or tetracarboxylic diacid anhydrides to give the corresponding linear diimide carboxamides **5–7**, and octapeptide tetraimides **8** and **9**, respectively. The synthesized products were elucidated by using spectroscopic evidences and they were evaluated for their antibacterial activity by using streptomycin as reference antibiotic drug.

Keywords: amino acids; antibacterial activity; octapeptide tetraimides; Schiff bases.

1 Introduction

Pyridines and their derivatives are of interest from both the chemical and biological aspects, such as hypotensive, anti-convulsant, anti-inflammatory, antimicrobial [1–5], anti-cancer [6] activities, and they have also been characterized with a broad range of biological activity [7–10]. In addition, some of hetero-nitrogen derivatives proved to be biologically active, such as antimicrobial [11], anti-inflammatory [12], antihistaminic [13], antihypertensive [14], hypnotic [15], and anticonvulsant [16] activity. From our previous work, Schiff bases were synthesized [17–19], and exhibit biological activities, such as antimicrobial [20–24], anti-inflammatory [25], and anticonvulsant [26] activities. In view of these observations and in continuation of our previous work in pyridine chemistry [17–26], we synthesized some new peptide compounds containing the pyridine ring substituted with a peptide linkage and other heterocyclic nucleus and tested their antimicrobial activities.

2 Results and discussion

2.1 Chemistry

In the present work, some of newly synthesized 3,5-pyridine tetrapeptides **1–4** were obtained by using *N*^α-dinicotinoyl-bis[L-phenylalaninyl acid] [27, 28] as starting materials. Treatment of **1** with L-leucine methyl ester to give *N*^α-dinicotinoyl-bis[L-phenylalaninyl-L-leucyl]methyl ester **1**, which was treated with hydrazine hydrated in refluxing methanol to give *N*^α-dipicolinoyl-bis[L-phenylalaninyl-L-leucine]hydrazide **2**. Treatment of hydrazide **2** with aromatic and heterocyclic aldehydes, namely, benzaldehyde, *p*-methylbenzaldehyde or pyridine-, 2-, 3- or 4-carbaldehydes in the presence of triethylamine (TEA)-diethylamine (DEA) afforded the corresponding Schiff base derivatives **3a**, **b**, and **4a–c**, respectively (Scheme 1).

Reaction of hydrazide **2** with mono acid anhydrides, namely, phthalic, 2,3,4,5-tetrachloro-phthalic anhydride, or 2,3-pyridinedicarboxylic acid anhydride afforded the

*Corresponding author: Abd El-Galil E. Amr, Pharmaceutical Chemistry Department, Drug Exploration and Development Chair (DEDC), College of Pharmacy, King Saud University, Riyadh 11451, Saudi Arabia; and Applied Organic Chemistry Department, Chemical Industries Research Division, National Research Center, Dokki 12622, Cairo, A.R. Egypt, e-mail: aamr@ksu.edu.sa

Ahmed M. Naglah: Pharmaceutical Chemistry Department, Drug Exploration and Development Chair (DEDC), College of Pharmacy, King Saud University, Riyadh 11451, Saudi Arabia; and Peptide Chemistry Department, Chemical Industries Research Division, National Research Centre, Dokki 12622, Cairo, A.R. Egypt

Randa E. Abdel-Mageid: Department of Photochemistry, Chemical Industries Research Division, National Research Center, Dokki 12622, Cairo, A.R. Egypt

Elsayed A. Elsayed: Bioproducts Research Chair, Zoology Department, College of Science, King Saud University, Riyadh 11451, Kingdom of Saudi Arabia; and Natural and Microbial Products Department, National Research Centre, Dokki 12622, Cairo, A.R. Egypt

Osama I. Abd El-Salam: Applied Organic Chemistry Department, Chemical Industries Research Division, National Research Center, Dokki 12622, Cairo, A.R. Egypt

Scheme 1: Synthetic routes and yields for compounds **2**, **3a, b**, and **4a–c**.

corresponding diimide derivatives **5a, b**, and **6**, respectively (Scheme 2).

Condensation of compound **2** with 1,2,4,5-benzene-tetracarboxylic acid dianhydride, or 1,4,5,8-naphthalene-tetracarboxylic acid dianhydride in refluxing acetic acid, afforded the corresponding 3,5-bis[L-phenylalaninyl-L-leucyl]benzene cyclic bridged octapeptide **8** and 3,5-bis-[L-phenylalaninyl-L-leucyl]naphthalene cyclic bridged octapeptide **9**, respectively (Scheme 3).

2.2 Antimicrobial testing

The newly synthesized compounds **2–9** were evaluated for their antibacterial activity against *Escherichia coli*,

Bacillus subtilis, and *Staphylococcus aureus* bacteria. The evaluated compounds have shown potent antibacterial activities at a concentration $50 \mu\text{g mL}^{-1}$ with a bioassay technique for antibiotics [29] specified in the US Pharmacopeia. Streptomycin was used as antibacterial reference drug. The results are summarized in Table 1. The obtained results revealed that all synthesized compounds showed varying degrees of antimicrobial activities against all tested three microbial pathogens. Furthermore, it can be seen that these activities were much higher when compared with the standard streptomycin antibiotic. Against the Gram negative *E. coli*, the most potent prepared compound (Compound **9**) showed higher antimicrobial activity by about 36.8% than the standard used antibiotic. Similarly, for Gram negative pathogens; i.e. *B. subtilis* and

Scheme 2: Synthetic routes and yields for compounds **5a**, **b**, **6**, and **7**.

S. aureus, the most potent derivatives, **4b** and **2**, respectively, showed increased activities by about 20% and 21%, respectively. Generally, the order of descending activities against Gram negative pathogen can be summarized as Compound **9** > **4b** > **3b** > **7** > **2**, **3a**, **5a**, **5b** > **6**, **8** > **4c** > **4a**. On the other hand the obtained descending order of activities against Gram positive pathogens differed depending on the tested pathogen. For *B. subtilis*, the order was **4b** > **8** > **5a** > **9** > **5b** > **6** > **2** > **3a**, **7** > **4c** > **3b** > **4a**, while for *S. aureus*, the order was **2** > **7**, **9** > **8** > **4b**, **5a** > **3b** > **4c** > **5b**, **6** > **3a** > **4a**. A comparison of our results with previous results reported for antimicrobial activities of oligopeptides shows that our synthesized derivatives have potential antimicrobial activities against tested pathogens. Abd El Rahman et al. [30] obtained their highest antimicrobial activities (13.1 mm against *B. subtilis*) for their synthesized peptide (PEG-Gly-Tyr-Tyr-Ala-NH₂) at their used concentration of 10 mg mL⁻¹, while this peptide was not active at all against *S. aureus* and *E. coli*. It is noteworthy to mention that our results were obtained at the 50 µg mL⁻¹ level. The difference in the obtained antimicrobial activity is generally explained due to the difference in the structure of cell membranes of each microbial cell type [31, 32]. The higher obtained activities of these compounds can be attributed to the presence of macrocyclic octapeptide ring, the Schiff base of 3-pyridine carbaldehyde and the hydrazide bond in compounds **9**, **4b** and **2**, respectively. Generally, the antimicrobial activity of the synthesized peptides can be due to the interaction of the peptides with microbial cell

membranes, and thus affecting the integrity of the cell walls [33, 34]. Furthermore, peptides have been reported to effect microbial cells intracellularly by interacting with cellular DNA or enzyme machinery [35].

3 Experimental section

3.1 Reagents

3,5-Pyridinedicarbonylchloride was prepared and elucidated according to literature procedures [36]. L-Leucine methyl ester, ethanol, methanol, dichloromethane, hydrazine hydrate, ethyl chloroformate, benzaldehyde, *p*-methylbenzaldehyde, and sodium hydroxide were all purchased from Sigma-Aldrich (Buchs, Switzerland). Melting points were determined in open glass capillary tubes with an Electro Thermal Digital melting point apparatus (Shimadzu, Tokyo, Japan), (model: IA9100) and are uncorrected. Elemental microanalysis for carbon, hydrogen and nitrogen (Microanalytical Unit, NRC) was found within the acceptable limits of the calculated values. Infrared spectra (KBr) were recorded using a Nexus 670 FTIR Nicolet, Fourier Transform infrared spectrometer (Perkin Elmer, Hopkinton, MA, USA). ¹H and ¹³C NMR spectra were run in (DMSO-*d*₆) using Jeol 500 MHz instruments (Tokyo, Japan). Mass spectra were run using a MAT Finnigan SSQ 7000 spectrometer (Shimadzu, Kyoto, Japan; Model:

Scheme 3: Synthetic routes for compounds 8 and 9.

Table 1: Antibacterial activities of compounds 2–9.

Comp. No.	Zones of inhibition (mm)		
	<i>E. coli</i>	<i>B. subtilis</i>	<i>S. aureus</i>
2	0.75	1.83	1.58
3a	0.75	1.85	1.92
3b	0.68	1.87	1.77
4a	0.90	1.96	1.95
4b	0.65	1.62	1.75
4c	0.84	1.86	1.78
5a	0.76	1.74	1.75
5b	0.75	1.78	1.86
6	0.78	1.80	1.85
7	0.72	1.85	1.65
8	0.78	1.68	1.67
9	0.60	1.75	1.65
Streptomycin	0.95	2.00	2.00

E. coli, *Escherichia coli*; *B. subtilis*, *Bacillus subtilis*; *S. aureus*, *Staphylococcus aureus*.

QP2010 ultra), using the electron impact technique (EI). Analytical thin layer chromatography was performed on silica gel aluminum sheets, 60 F₂₅₄ (E. Merck).

3.2 Syntheses

3.2.1 Synthesis of *N*^α-dinicotinoyl-bis[L-phenylalanine-L-leucyl]methyl ester (1)

Compound 1 was prepared according to the mixed anhydride method [37] by using *N*^α-dinicotinoyl-bis[L-phenylalaninyl acid] [27, 28] and L-leucine methyl ester as starting materials. Yield 90%, m.p. 134°C–136°C. – $[\alpha]_D^{25} = -109$ ($c = 0.5$, DMF). – IR (film): $\nu = 3540$ – 3380 (br, NH), 3086 (CH–Ar), 2992 (CH–aliph.), 1754 (C=O, estr), 1653, 1534, 1256 (C=O, amide I, II and III) cm^{-1} . – ^1H

NMR (500 MHz, DMSO- d_6): δ = 0.92 (d, 12H, J = 6.5 Hz, 4CH₃), 1.70 (t, 4H, J = 7.88 Hz, 2CH₂), 2.22–2.26 (m, 2H, 2CH), 3.42 (d, 4H, J = 5.2 Hz, 2CH₂), 3.70 (s, 6H, 2OCH₃), 4.15 (t, 2H, J = 5.4 Hz, 2CH), 4.42 (t, 2H, J = 4.6 Hz, 2CH), 7.12–7.64 (m, 10H, 2Ph-H), 8.35, 9.02 (2s, 3H, pyr-H), 8.72, 8.80 (2s, 4H, 4NH, exchangeable with D₂O). – ¹³C NMR (125 MHz, DMSO- d_6): δ = 17.40, 18.55 (4C, 4 CH₃), 23.74 (2C, 2CH), 40.15 (2C, 2CH₂), 42.54 (2C, 2CH₂), 51.36 (2C, 2OCH₃), 52.64, 53.13 (4C, 4 CH), 124.24, 128.30, 129.42, 138.65 (12C, 2Ph-C), 131.62, 140.18, 152.16 (5C, pyr-C), 163.75, 169.15 (4C, 4 CO-amide), 171.40 (2C, 2CO-ester). – MS (EI, 70 eV): m/z (%) = 716 (42) [M]⁺. – C₃₉H₄₉N₅O₈ (715.85): calcd. C 65.44, H 6.90, N 9.78; found C 65.32, H 6.86, N 9.70.

3.2.2 Synthesis of *N*^α-dinicotinoyl-bis[L-phenylalanine-L-leucyl]hydrazide (2)

A mixture of *N*^α-dinicotinoyl-bis[L-phenylalanine-L-leucyl]methyl ester (1) (0.715 g, 1 mmol) and hydrazine hydrate (0.35 mL, 10 mmol) in methanol (20 mL) was refluxed for 3 h, solvent was evaporated under reduced pressure. The formed solid was washed with ether, filtered off, and crystallized from ethanol-ether to afford the corresponding title compound (2). Yield 85%, m.p. 245°C–247°C. – $[a]_D^{25}$ = –115 (c = 0.5, DMF). – IR (film): ν = 3510–3375 (br, NH₂, NH), 3085 (CH–Ar), 2990 (CH–aliph.), 1652, 1536, 1257 (C=O, amide I, II and III)cm^{–1}. – ¹H NMR (500 MHz, DMSO- d_6): δ = 0.90 (d, 12H, J = 6.4 Hz, 4CH₃), 1.72 (t, 4H, J = 7.9 Hz, 2CH₂), 2.23–2.28 (m, 2H, 2CH), 3.44 (d, 4H, J = 5.2 Hz, 2CH₂), 4.10 (bs, 4H, 2NH₂, exchangeable with D₂O), 4.12 (t, 2H, J = 5.4 Hz, 2CH), 4.40 (t, 2H, J = 4.6 Hz, 2CH), 7.10–7.65 (m, 10H, 2Ph-H), 8.36, 9.00 (s, 3H, pyr-H), 8.70, 8.82, 9.22 (3s, 6H, 6NH, exchangeable with D₂O). – ¹³C NMR (125 MHz, DMSO- d_6): δ = 17.42, 18.60 (4C, 4 CH₃), 23.78 (2C, 2CH), 40.12 (2C, 2CH₂), 42.56 (2C, 2CH₂), 52.65, 53.10 (4C, 4 CH), 124.23, 128.32, 129.45, 138.68 (12C, 2Ph-C), 131.60, 140.15, 152.18 (5C, pyr-C), 163.78, 169.12 (4C, 4 CO-amide), 171.45 (2C, 2CO-hydrazide). – MS (EI, 70 eV): m/z (%) = 716 (32) [M]⁺. – C₃₇H₄₉N₉O₆ (715.86): calcd. C 62.08, H 6.90, N 17.61; found C 61.95, H 6.73, N 17.50.

3.2.3 Synthesis of *N*^α-dinicotinoyl-bis-[L-phenylalaninyl-L-leucyl]-*p*-substituted phenyl hydrazones (3a, b)

A mixture of hydrazide derivative 2 (1 mmol) and aromatic aldehydes, namely benzaldehyde, or *p*-methylbenzaldehyde (2 mmol) in absolute ethanol (25 mL) in

the presence of a mixture of TEA-DEA (4 mL, 2:2) was refluxed for 3–5 h. The solvent was evaporated to dryness, the obtained residue was triturated with icewater with stirring. The precipitated solid was filtered off, washed with water, and crystallized from the proper solvents to give the corresponding *N*^α-dinicotinoyl-bis[L-phenylalaninyl-L-leucyl] substituted phenypyridines (3a, b), respectively.

3.2.3.1 *N*^α-Dinicotinoyl-bis-[L-phenylalaninyl-L-leucyl]phenylhydrazone (3a)

Yield 65%, m.p. 198°C–200°C (dioxane-ether). – $[a]_D^{25}$ = –98 (c = 0.5, DMF). – IR (film): ν = 3490–3355 (NH), 3092 (CH–Ar), 2978 (CH–aliph.), 1656, 1535, 1252 (C=O, amide I, II and III)cm^{–1}. – ¹H NMR (500 MHz, DMSO- d_6): δ = 0.96 (d, 12H, J = 6.5 Hz, 4CH₃), 1.75 (t, 4H, J = 7.4 Hz, 2CH₂), 2.25–2.32 (m, 2H, 2CH), 3.42 (d, 4H, J = 7.9 Hz, 2CH₂), 4.16 (t, 2H, J = 5.5 Hz, 2CH), 4.45 (t, 2H, J = 4.5 Hz, 2CH), 7.00–7.68 (m, 22H, 20Ar-H + 2CH=N), 8.36, 9.14 (2s, 3H, pyr-H), 8.70, 8.82, 9.26 (3s, 6H, 6NH, exchangeable with D₂O). – ¹³C NMR (125 MHz, DMSO- d_6): δ = 18.68, 19.25 (4C, 4 CH₃), 22.47 (2C, 2CH), 40.16 (2C, 2CH₂), 42.46 (2C, CH₂), 52.40, 53.30 (4C, 4 CH), 125.87, 127.46, 128.34, 128.50, 129.08, 131.08, 133.25, 138.66 (24C, 4Ph-C), 131.65, 140.35, 152.46 (5C, pyr-C), 143.16 (2C, 2C=N), 163.92, 169.42 (4C, 4 CO-amide), 178.42 (2C, 2CO-hydrazone). – MS (EI, 70 eV): m/z (%) = 892 (25) [M]⁺. – C₅₁H₅₇N₉O₆ (892.07): calcd. C 68.67, H 6.44, N 14.13; found C 68.54, H 6.35, N 14.08.

3.2.3.2 *N*^α-Dinicotinoyl-bis-[L-phenylalaninyl-L-leucyl]-*p*-methylphenylhydrazone (3b)

Yield 78%, m.p. 132°C–234°C (DMF-H₂O). – $[a]_D^{25}$ = –125 (c = 0.5, DMF). – IR (film): ν = 3460–3348 (NH), 3090 (CH–Ar), 2982 (CH–aliph.), 1654, 1536, 1250 (C=O, amide I, II and III)cm^{–1}. – ¹H NMR (500 MHz, DMSO- d_6): δ = 0.98 (d, 12H, J = 6.6 Hz, 4CH₃), 1.72 (t, 4H, J = 7.3 Hz, 2CH₂), 2.21–2.34 (m, 8H, 2CH + 2 CH₃), 3.46 (d, 4H, J = 7.9 Hz, 2CH₂), 4.18 (t, 2H, J = 5.4 Hz, 2CH), 4.40 (t, 2H, J = 4.6 Hz, 2CH), 6.98–7.56 (m, 20H, 18Ar-H + 2CH=N), 8.45, 9.18 (s, 3H, pyr-H), 8.65, 8.85, 9.24 (3s, 6H, 6NH, exchangeable with D₂O). – ¹³C NMR (125 MHz, DMSO- d_6): δ = 18.65, 19.30, 24.18 (6C, 6 CH₃), 23.45 (2C, 2CH), 40.22 (2C, 2CH₂), 42.48 (2C, CH₂), 52.45, 53.45 (4C, 4CH), 125.85, 127.66, 128.54, 128.95, 129.18, 130.48, 139.45, 140.64 (24C, 4Ph-C), 131.62, 140.50, 152.56 (5C, pyr-C), 142.90 (2C, 2C=N), 164.14, 169.55 (4C, 4 CO-amide), 178.68 (2C, 2CO-hydrazone). – MS (EI, 70 eV): m/z (%) = 920 (15) [M]⁺. – C₅₃H₆₁N₉O₆ (920.13): calcd. C 69.18, H 6.68, N 13.70; found C 69.05, H 6.58, N 13.64.

3.2.4 Synthesis of *N*^α-dinicotinoyl-bis[(L-phenylalaninyl-L-leucyl)]-pyridyl hydrazones] (4a–c)

A mixture of **2** (0.715 g, 1 mmol) and pyridinecarbaldehyde, namely, pyridine 2-, 3-, or 4-carbaldehydes (2 mmol) in glacial acetic acid (30 mL) was refluxed for 3–5 h. The solvent was concentrated to dryness, the obtained residue was triturated with water. The formed precipitate was filtered off, washed with water, dried, and crystallized from the proper solvents to give 3,5-bisSchiff bases **4a–c**, respectively.

3.2.4.1 *N*^α-Dinicotinoyl-bis[(L-phenylalaninyl-L-leucyl)]-2-pyridylpyridine] (4a)

Yield 60%, m.p. 224°C–226°C (DMF-H₂O). – $[α]_D^{25} = -112$ (*c* = 0.5, DMF). – IR (film): $\nu = 3450$ – 3354 (NH), 3092 (CH–Ar), 2986 (CH–aliph.), 1654, 1534, 1252 (C=O, amide I, II and III) cm^{–1}. – ¹H NMR (500 MHz, DMSO-*d*₆): $\delta = 0.90$ (d, 12H, *J* = 6.3 Hz, 4CH₃), 1.78 (t, 4H, *J* = 7.3 Hz, 2CH₂), 2.26–2.32 (m, 2H, 2CH), 3.52 (d, 4H, *J* = 8.10 Hz, 2CH₂), 4.25 (t, 2H, *J* = 5.3 Hz, 2CH), 4.54 (t, 2H, *J* = 4.5 Hz, 2CH), 6.98–7.72 (m, 12H, 2Ph-H + 2CH=N), 7.80–8.50 (m, 10H, pyr-H), 9.12 (s, 1H, pyr-H), 8.72, 8.86, 9.22 (3s, 6H, 6NH, exchangeable with D₂O). – ¹³C NMR (125 MHz, DMSO-*d*₆): $\delta = 17.98$, 18.60 (4C, 4 CH₃), 23.76 (2C, 2CH), 40.26 (2C, 2CH₂), 42.42 (2C, CH₂), 52.57, 53.38 (4C, 4CH), 124.24, 128.30, 129.32, 138.64 (12C, 2Ph-C), 139.80 (2C, 2C=N), 122.90, 126.00, 131.66, 135.85, 140.24, 148.66, 152.25, 154.48 (15C, 3pyr-C), 163.86, 169.40 (4C, 4 CO-amide), 178.64 (2C, 2CO-hydrazone). – MS (EI, 70 eV): *m/z* (%) = 895 (6) [M + 1]⁺. – C₄₉H₅₅N₁₁O₆ (894.05): calcd. C 65.83, H 6.20, N 17.23; found C 65.72, H 6.12, N 17.18.

3.2.4.2 *N*^α-Dinicotinoyl-bis[(L-phenylalaninyl-L-leucyl)]-3-pyridylpyridine] (4b)

Yield 58%, m.p. 208°C–210°C (AcOH-H₂O). – $[α]_D^{25} = -132$ (*c* = 0.5, DMF). – IR (film): $\nu = 3467$ – 3350 (NH), 3075 (CH–Ar), 2990 (CH–aliph.), 1652, 1536, 1252 (C=O, amide I, II and III) cm^{–1}. – ¹H NMR (500 MHz, DMSO-*d*₆): $\delta = 0.96$ (d, 12H, *J* = 6.4 Hz, 4CH₃), 1.75 (t, 4H, *J* = 7.4 Hz, 2CH₂), 2.18–2.36 (m, 2H, 2CH), 3.48 (d, 4H, *J* = 8.0 Hz, 2CH₂), 4.18 (t, 2H, *J* = 5.6 Hz, 2CH), 4.52 (t, 2H, *J* = 4.6 Hz, 2CH), 6.96–7.68 (m, 12H, 2Ph-H + 2CH=N), 7.76–8.62 (m, 8H, pyr-H), 9.05, 9.20 (2s, 3H, pyr-H), 8.76, 8.85, 9.28 (3s, 6H, 6NH, exchangeable with D₂O). – ¹³C NMR (125 MHz, DMSO-*d*₆): $\delta = 17.98$, 18.88 (4C, 4 CH₃), 23.98 (2C, 2CH), 40.32 (2C, 2CH₂), 42.65 (2C, CH₂), 52.45, 53.65 (4C, 4 CH), 124.32, 128.45, 129.52, 138.65 (12C, 2Ph-C), 123.44, 126.00, 131.65, 136.46, 140.34, 150.80, 151.20, 152.20 (15C, 3 pyr-C), 142.95 (2C, 2C=N), 164.08, 169.48 (4C, 4 CO-amide), 178.74 (2C, 2CO-hydrazone). – MS (EI, 70 eV): *m/z* (%) = 894 (18) [M]⁺. – C₄₉H₅₅N₁₁O₆ (894.05): calcd. C 65.83, H 6.20, N 17.23; found C 65.74, H 6.15, N 17.16.

3.2.4.3 *N*^α-Dinicotinoyl-bis[(L-phenylalaninyl-L-leucyl)]-4-pyridylpyridine] (4c)

Yield 75%, m.p. 196°C–198°C (EtOH-H₂O). – $[α]_D^{25} = -140$ (*c* = 0.5, DMF). – IR (film): $\nu = 3466$ – 3348 (NH), 3080 (CH–Ar), 2986 (CH–aliph.), 1655, 1535, 1255 (C=O, amide I, II and III) cm^{–1}. – ¹H NMR (500 MHz, DMSO-*d*₆): $\delta = 0.98$ (d, 12H, *J* = 6.3 Hz, 4CH₃), 1.77 (t, 4H, *J* = 7.5 Hz, 2CH₂), 2.20–2.35 (m, 2H, 2CH), 3.46 (d, 4H, *J* = 8.0 Hz, 2CH₂), 4.22 (t, 2H, *J* = 5.6 Hz, 2CH), 4.54 (t, 2H, *J* = 4.6 Hz, 2CH), 6.97–7.51 (m, 12H, 2Ph-H + 2CH=N), 8.10 (d, 4H, *J* = 6.25 Hz, pyr-H), 8.78 (d, 4H, *J* = 6.25 Hz, Pyr-H), 8.60, 9.00 (2s, 3H, pyr-H-4), 8.72, 8.89, 9.26 (3s, 6H, 6NH, exchangeable with D₂O). – ¹³C NMR (125 MHz, DMSO-*d*₆): $\delta = 17.90$, 18.98 (4C, 4 CH₃), 23.82 (2C, 2CH), 41.04 (2C, 2CH₂), 42.60 (2C, CH₂), 52.55, 53.65 (4C, 4 CH), 124.22, 128.40, 129.46, 138.60 (12C, 2Ph-C), 143.12 (2C, 2C=N), 123.95, 132.90, 139.02, 144.20, 149.18, 150.94 (15C, 3 pyr-C), 163.86, 169.12 (4C, 4 CO-amide), 177.82 (2C, 2CO-hydrazone). – MS (EI, 70 eV): *m/z* (%) = 892 (15) [M – 2]⁺. – C₄₉H₅₅N₁₁O₆ (894.06): calcd. C 65.83, H 6.20, N 17.23; found C 65.75, H 6.16, N 17.15.

3.2.5 Synthesis of *N*^α-dinicotinoyl-bis[(L-phenylalaninyl-L-leucyl)] substituted aromatic or heterocyclic bisimide derivatives 5–7

A mixture of **2** (0.715 g, 1 mmol) and mono acid hydride derivatives, namely, phthalic, tetrachlorophthalic, 2,3-pyridinedicarboxylic, or 1,8-naphthalene dicarboxylic acid anhydrides (2 mmol) was refluxed in glacial acetic acid (50 mL) for 4–6 h. The reaction mixture was poured into ice-water, the obtained precipitate was collected by filtration, washed with water, dried, and crystallized from the proper solvents to give bisimidehexacarboxamide derivatives **5a**, **b**, **6**, and **7**, respectively.

3.2.5.1 *N*^α-dinicotinoyl-bis[(L-phenylalaninyl-L-leucyl)]-*N*-(1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl)-imide] (5a)

Yield 72%, m.p. 236°C–238°C. – $[α]_D^{25} = -110$ (*c* = 0.5, DMF). – IR (film): $\nu = 3455$ – 3345 (NH), 3082 (C–H_{arom}), 2986 (C–H_{aliph}), 1652, 1534, 1254 (C=O, amide I, II and III) cm^{–1}. – ¹H NMR (500 MHz, DMSO-*d*₆): $\delta = 0.96$ (d, 12H, *J* = 6.4 Hz, 4CH₃), 1.74 (t, 4H, *J* = 7.9 Hz, 2CH₂), 2.24–2.30 (m, 2H, 2CH), 3.40 (d, 4H, *J* = 5.2 Hz, 2CH₂), 4.24 (t, 2H, *J* = 5.4 Hz, 2CH), 4.54 (t, 2H, *J* = 4.6 Hz, 2CH), 7.00–7.98 (m, 18H, 4Ph-H), 8.40, 9.08 (2s, 3H, pyr-H), 8.65, 8.82, 9.22 (3s, 6H, 6NH, exchangeable with D₂O). – ¹³C NMR (125 MHz, DMSO-*d*₆): $\delta = 17.36$, 18.52 (4C, 4 CH₃), 23.78 (2C, 2CH), 40.10 (2C, 2CH₂), 42.27 (2C, CH₂), 52.45, 53.06

(4C, 4 CH), 124.15, 127.23, 128.24, 129.35, 131.86, 132.18, 138.72 (24C, 4Ph-C), 131.60, 140.12, 152.08 (5C, pyr-C), 164.64 (4C, 4 CO-imide), 163.82, 169.42, 170.10 (6C, 6 CO-amide). – MS (EI, 70 eV): m/z (%) = 976 (24) $[M]^+$. – $C_{53}H_{53}N_9O_{10}$ (976.06): calcd. C 65.22, H 5.47, N 12.92; found C 65.05, H 5.38, N 12.86.

3.2.5.2 *N*^α-dinicotinoyl-bis[(L-phenylalaninyl-L-leucyl)-*N*-(4,5,6,7-tetrachloro-1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl)imide] (5b)

Yield 78%, m.p. 256°C–258°C. – $[α]_D^{25} = -125$ ($c = 0.5$, DMF). – IR (film): $\nu = 3460$ –3318 (NH), 3085 (C–H_{arom}), 2992 (C–H_{aliph}), 1650, 1532, 1254 (C=O, amide I, II and III) cm^{-1} . – 1H NMR (500 MHz, DMSO- d_6): $\delta = 0.95$ (d, 12H, $J = 6.4$ Hz, 4CH₃), 1.73 (t, 4H, $J = 7.8$ Hz, 2CH₂), 2.25–2.35 (m, 2H, 2CH), 3.42 (d, 4H, $J = 5.3$ Hz, 2CH₂), 4.30 (t, 2H, $J = 5.4$ Hz, 2CH), 4.48 (t, 2H, $J = 4.5$ Hz, 2CH), 6.95–7.56 (m, 10H, 2Ph-H), 8.35, 9.14 (2s, 3H, pyr-H), 8.70, 8.78, 9.18 (3s, 6H, 6NH, exchangeable with D₂O). – ^{13}C NMR (125 MHz, DMSO- d_6): $\delta = 17.45$, 18.56 (4C, 4 CH₃), 23.82 (2C, 2CH), 40.16 (2C, 2CH₂), 42.32 (2C, CH₂), 52.50, 53.17 (4C, 4 CH), 124.24, 128.02, 128.44, 129.46, 132.74, 138.12, 138.68 (24C, 4Ph-C), 131.53, 140.20, 152.32 (5C, pyr-C), 164.76 (4C, 4 CO-imide), 163.84, 169.45, 170.15 (6C, 6 CO-amide). – MS (EI, 70 eV): m/z (%) = 1251 (6) $[M - 1]^+$. – $C_{53}H_{45}Cl_8N_9O_{10}$ (1251.07): calcd. C 50.86, H 3.62, Cl 22.66, N 10.07; found C 50.70, H 3.56, Cl 22.58, N 10.01.

3.2.5.3 *N*^α-dinicotinoyl-bis[(L-phenylalaninyl-L-leucyl)-*N*-(5,7-dioxo-5,7-dihydro-6*H*-pyrrolo[3,4-*b*]pyridine-6-yl)imide] (6)

Yield 75%, m.p. 218°C–220°C. – $[α]_D^{25} = -98$ ($c = 0.5$, DMF). – IR (film): $\nu = 3450$ –3345 (NH), 3090 (C–H_{arom}), 2988 (C–H_{aliph}), 1652, 1540, 1255 (C=O, amide I, II and III) cm^{-1} . – 1H NMR (500 MHz, DMSO- d_6): $\delta = 0.94$ (d, 12H, $J = 6.2$ Hz, 4CH₃), 1.72 (t, 4H, $J = 7.7$ Hz, 2CH₂), 2.20–2.32 (m, 2H, 2CH), 3.44 (d, 4H, $J = 5.4$ Hz, 2CH₂), 4.26 (t, 2H, $J = 5.6$ Hz, 2CH), 4.50 (t, 2H, $J = 4.5$ Hz, 2CH), 6.96–7.48 (m, 10H, 2Ph-H), 8.48, 9.05 (2s, 3H, pyr-H), 7.98 (t, 2H, $J = 7.6$ Hz, pyr-H), 8.54 (d, 2H, $J = 7.9$ Hz, pyr-H), 8.95 (d, 2H, $J = 7.8$ Hz, pyr-H), 8.65, 8.75, 9.14 (3s, 6H, 6NH, exchangeable with D₂O). – ^{13}C NMR (125 MHz, DMSO- d_6): $\delta = 18.00$, 18.85 (4C, 4 CH₃), 24.00 (2C, 2CH), 39.84 (2C, 2CH₂), 42.30 (2C, CH₂), 52.48, 53.00 (4C, 4 CH), 124.25, 128.24, 129.32, 138.62 (12C, 2Ph-C), 127.16, 128.04, 131.64, 138.00, 140.22, 145.00, 152.10, 152.50 (15C, pyr-C), 164.54, 165.10 (4C, 4 CO-imide), 163.80, 169.46, 170.15 (6C, 6 CO-amide). – MS (EI, 70 eV): m/z (%) = 978 (10) $[M]^+$. – $C_{51}H_{51}N_{11}O_{10}$ (978.04): calcd. C 62.63, H 5.26, N 15.75; found C 62.50, H 5.18, N 15.66.

3.2.5.4 *N*^α-dinicotinoyl-bis[(L-phenylalaninyl-L-leucyl)-*N*-(1,3-dioxo-1*H*-benzo[*de*]iso-quinolin-2(3*H*)-yl)imide] (7)

Yield 74%, m.p. 254°C–256°C. – IR (film): $\nu = 3468$ –3350 (NH), 3090 (C–H_{arom}), 2988 (C–H_{aliph}), 1652, 1532, 1252 (C=O, amide I, II and III) cm^{-1} . – 1H NMR (500 MHz, DMSO- d_6): $\delta = 0.86$ (d, 12H, $J = 6.5$ Hz, 4CH₃), 1.73 (t, 4H, $J = 7.4$ Hz, 2CH₂), 2.18–2.26 (m, 2H, 2CH), 3.45 (d, 4H, $J = 5.6$ Hz, 2CH₂), 4.32 (t, 2H, $J = 5.7$ Hz, 2CH), 4.50 (t, 2H, $J = 4.6$ Hz, 2CH), 7.00–7.95 (m, 22H, Ar-H), 8.40, 9.05 (2s, 3H, pyr-H), 8.60, 8.72, 9.12 (3s, 6H, 6NH, exchangeable with D₂O). – ^{13}C NMR (125 MHz, DMSO- d_6): $\delta = 18.00$, 18.72 (4C, 4 CH₃), 23.70 (2C, 2CH), 40.12 (2C, 2CH₂), 41.20 (2C, 2CH₂), 52.72, 53.15 (4C, 4 CH), 123.55, 125.19, 128.35, 130.38, 137.14, 137.80 (20C, naphtha-C), 124.20, 128.15, 129.32, 138.75 (12C, 2Ph-C), 131.48, 140.18, 152.00 (5C, pyr-C), 164.70, 169.84, 171.30 (6C, 6 CO-amide), 168.85 (4C, 4CO-imide). – MS (EI, 70 eV): m/z (%) = 1075 (8) $[M - 1]^+$. – $C_{61}H_{57}N_9O_{10}$ (1075.42): calcd. C 68.08, H 5.34, N 11.71; found C 67.96, H 5.23, N 11.60.

3.2.6 Synthesis of cyclo(*N*^α-dinicotinoyl)-bis(L-phenylalaninyl-L-leucyl)benzene or naphthalene tetraimide] derivatives (8) and (9)

The same procedure for synthesis of compounds 5–7 by using 1,4,5,8-naphthylenetetracarboxylic dianhydride or 1,2,4,5-benzenetetracarboxylic dianhydride (as anhydride derivatives) (1 mmol) and compound 2 (1 mmol) in refluxing glacial acetic acid.

3.2.6.1 Cyclo(*N*^α-dinicotinoyl)-bis[(L-phenylalaninyl-L-leucyl)benzene tetraimide] derivatives (8)

Yield 65%, m.p. 242°C–244°C. – $[α]_D^{25} = -115$ ($c = 0.5$, DMF). – IR (film): $\nu = 3460$ –3340 (NH), 3087 (C–H_{arom}), 2985 (C–H_{aliph}), 1653, 1534, 1252 (C=O, amide I, II and III) cm^{-1} . – 1H NMR (500 MHz, DMSO- d_6): $\delta = 0.96$ (d, 24H, $J = 6.4$ Hz, 8CH₃), 1.72 (t, 8H, $J = 7.5$ Hz, 4CH₂), 2.10–2.18 (m, 4H, 4CH), 3.46 (d, 8H, $J = 5.6$ Hz, 4CH₂), 4.26 (t, 4H, $J = 5.6$ Hz, 4CH), 4.54 (t, 4H, $J = 4.7$ Hz, 4CH), 7.00–7.78 (m, 20H, 4Ph-H), 8.72 (s, 4H, Ar-H), 8.42, 9.02 (s, 6H, 2pyr-H), 8.64, 8.80, 9.10 (3s, 12H, 12NH, exchangeable with D₂O). – ^{13}C NMR (125 MHz, DMSO- d_6): $\delta = 17.95$, 18.66 (8C, 8 CH₃), 23.72 (4C, 4CH), 40.30 (4C, 4CH₂), 41.48 (4C, 4CH₂), 53.55, 57.25 (8C, 8 CH), 124.95, 135.25 (12C, benzene-C), 124.36, 128.46, 129.54, 138.70 (24C, 4Ph-C), 131.48, 140.24, 152.38 (10C, 2pyr-C), 164.75, 173.35, 174.15 (12C, 12 CO-amide), 164.66 (8C, 8 CO-imide). – MS (EI, 70 eV): m/z (%) = 1795 (14) $[M - 1]^+$. – $C_{94}H_{94}N_{18}O_{20}$ (1795.67): calcd. C 62.87, H 5.28, N 14.04; found C 62.75, H 5.18, N 13.94.

3.2.6.2 Cyclo(*N*^ε-dinicotinoyl)-bis[(L-phenylalaninyl-L-leucyl)naphthalene tetraimide] derivatives (9)

Yield 60%, m.p. 278°C–280°C. – $[α]_D^{25} = -132$ ($c = 0.5$, DMF). – IR (film): $\nu = 3460$ – 3312 (NH), 3090 (C–H_{arom}), 2984 (C–H_{aliph}), 1652, 1534, 1250 (C=O, amide I, II and III) cm^{-1} . – ^1H NMR (500 MHz, DMSO- d_6): $\delta = 0.89$ (d, 24H, $J = 6.6$ Hz, 8CH₃), 1.70 (t, 8H, $J = 7.6$ Hz, 4CH₂), 2.12–2.16 (m, 4H, 4CH), 3.45 (d, 8H, $J = 5.5$ Hz, 4CH₂), 4.32 (t, 4H, $J = 5.4$ Hz, 4CH), 4.52 (t, 4H, $J = 4.6$ Hz, 4CH), 6.95–7.64 (m, 20H, 4Ph-H), 8.10–8.15 (m, 8H, naphtha-H), 8.48, 9.10 (2s, 6H, 2pyr-H), 8.62, 8.80, 9.15 (3s, 12H, 12NH, exchangeable with D₂O). – ^{13}C NMR (125 MHz, DMSO- d_6): $\delta = 17.98$, 18.40 (8C, 8 CH₃), 23.70 (4C, 4CH), 40.15 (4C, 4CH₂), 41.36 (4C, 4CH₂), 52.75, 53.25 (8C, 8 CH), 119.95, 135.18, 139.72 (20C, naphtha-C), 124.25, 128.22, 129.35, 138.72 (24C, 4Ph-C), 131.30, 140.12, 152.15 (10C, 2pyr-C), 164.65, 169.75, 171.30 (12C, 12 CO-amide), 158.80 (8C, 8CO-imide). – MS (EI, 70 eV): m/z (%) = 1896(12) [M]⁺. – C₁₀₂H₉₈N₁₈O₂₀ (1896.01): calcd. C 64.62, H 5.21, N 13.30; found C 64.48, H 5.16, N 13.26.

Acknowledgments: The authors are grateful to King Saud University for funding the work through Researchers Supporting Project (Project No. RSP-2019-066).

References

- [1] A. Samatov, S. T. Akramov, S. Yu. Yunusov, *Chem. Nat. Compd* **1967**, 3, 150.
- [2] T. U. Rakhmatullaev, S. T. Akramov, S. Yu. Yunusov, *Chem. Nat. Compd* **1969**, 5, 531.
- [3] S. K. Bhattacharya, S. Ghosal, R. K. Chaudhuri, P. V. Sharma, *J. Pharm. Sci.* **1974**, 63, 1341.
- [4] R. E. Hancock, E. F. Haney, E. E. Gill, *Nat. Rev. Immunol.* **2016**, 16, 321.
- [5] T. J. Opperman, S. M. Kwasny, H. S. Kim, S. T. Nguyen, C. Houseweart, S. D'Souza, G. C. Alker, N. P. Peet, H. Nikaido, T. L. Bowlin, *Antimicrob. Agents Chemother.* **2014**, 58, 722.
- [6] S. Dennison, M. Whittaker, F. Harris, D. Phoenix, *Curr. Protein Pept. Sci.* **2006**, 7, 487.
- [7] A. Galán, L. Moreno, J. Párraga, A. Serrano, M. J. Sanz, D. Cortes, N. Cabed, *Bioorg. Med. Chem.* **2013**, 21, 3221.
- [8] R. E. Martin, J. D. Aebi, B. Hornsperger, H. J. Krebs, B. Kuhn, A. Kuglstatler, A. M. Alker, H. P. Märki, S. Muller, D. Burger, G. Ottaviani, W. Riboulet, P. Verry, X. Tan, K. Amrein, A. V. Mayweg, *J. Med. Chem.* **2015**, 58, 8054.
- [9] H. M. Faidallah, Sh. A. F. Rostom, A. M. Asiri, K. A. Khan, M. F. Radwan, H. Z. Asfour, *J. Enzyme Inhib. Med. Chem.* **2013**, 28, 123.
- [10] T. Kaiho, R. Sannohe, S. Kajiya, T. Suzuki, K. Otsuka, T. Ito, J. Kamiya, M. Maruyama, *J. Med. Chem.* **1989**, 32, 351.
- [11] S. P. Singh, S. S. Parmar, K. Raman, V. I. Stenherb, *Chem. Pharm. Bull.* **1981**, 81, 197.
- [12] P. B. Patel, J. T. Trivedi, *Ind. Chem. Soc.* **1987**, 54, 765.
- [13] D. M. Vittoria, O. Mazzoni, E. Piscopo, A. Caligmano, A. Bolognese, *J. Med. Chem.* **1992**, 35, 2910.
- [14] A. M. Omar, N. H. Eshba, *J. Pharm. Sci.* **1984**, 73, 1166.
- [15] M. Chaudhary, S. S. Parmar, S. K. Chaudhari, B. V. Ramasastry, *J. Pharm. Sci.* **1976**, 65, 443.
- [16] F. A. Ragab, M. M. Hussain, M. M. Hanna, G. S. Hassan, *J. Pharm. Sci.* **1993**, 34, 387.
- [17] A. A. Ibrahim, A. M. Mohamed, A. E. Amr, M. A. Al-Omar, *Russ. J. Gen. Chem.* **2015**, 85, 1506.
- [18] A. M. Naglah, A. E. Amr, N. M. Khalifa, M. A. Al-Omar, *Russ. J. Gen. Chem.* **2015**, 85, 2838.
- [19] A. E. Amr, O. I. Abd El-Salam, A. M. Mohamed, *Russ. J. Gen. Chem.* **2015**, 85, 1161.
- [20] A. E. Amr, A. M. Mohamed, *Molecules* **2010**, 15, 4711.
- [21] O. A. Ismail, M. A. Al-Omar, A. E. Amr, *Curr. Org. Synth.* **2012**, 9, 406.
- [22] M. E. Azab, A. E. Amr, *Russ. J. Gen. Chem.* **2015**, 85, 1513.
- [23] M. E. Azab, S. A. Rizk, A. E. Amr, *Molecules* **2015**, 20, 18201.
- [24] M. E. Azab, A. E. Amr, M. A. Al-Omar, *Russ. J. Gen. Chem.* **2015**, 85, 1952.
- [25] N. H. Ouf, M. I. Sakran, A. E. Amr, *Res. Chem. Intermed.* **2015**, 41, 2521.
- [26] S. S. Al Thagfan, A. A. Fayed, S. A. Bahshwan, A. E. Amr, N. Aljuhani, M. A. Al-Omar, M. E. Azab, M. M. Abdalla, *Biomed. Res.* **2018**, 29, 3605.
- [27] M. G. Mamolo, D. Zampieri, V. Falagiani, L. Vio, M. Fermeglia, M. Ferrone, S. Pricl, E. Banfi, G. Scialino, *ARKIVOC*, **2004**, 5, 231.
- [28] M. E. Azab, E. M. Felfel, N. M. Sabry, A. E. Amr, *Z. Naturforsch.* **2016**, 71b, 803.
- [29] A. A. Abou-Zeid, Y. M. Shehata, *Indian J. Pharm.* **1969**, 31, 72.
- [30] S. E. Abd El Rahman, A. M. F. Eissa, A. M. Naglah, *Int. J. Pharmacol.* **2015**, 11, 726.
- [31] A. E. Amr, M. H. Abo-Ghaila, G. O. Moustafa, M. A. Al-Omar, E. Nossir, E. A. Elsayed, *Molecules* **2018**, 23, 2416.
- [32] A. E. Amr, M. El-Naggar, M. A. Al-Omar, E. A. Elsayed, M. M. Abdalla, *Molecules* **2018**, 23, 1572.
- [33] G. Bhattacharya, R. P. Giri, A. Dubey, S. Mitra, R. Priyadarshini, A. Gupta, M. K. Mukhopadhyay, S. K. Ghosh, *Chem. Phys. Lipids* **2018**, 215, 1.
- [34] Y. Patil-Sen, S. R. Dennison, T. J. Snape, *Bioorg. Med. Chem.* **2016**, 24, 4241.
- [35] T. Li, Q. Liu, D. Wang, J. Li, *Fish Shellfish Immunol.* **2019**, 92, 881.
- [36] A. G. Talma, J. Patrick, J. G. De Vries, C. B. Troostwijk, G. H. W. Buning, J. K. Waninge, J. Visscher, R. M. Kellogg, *J. Am. Chem. Soc.* **1985**, 107, 3981.
- [37] A. E. Amr, A. M. Naglah, N. M. Sabry, A. A. Ibrahim, E. A. Elsayed, A. Attar, *Z. Naturforsch.* **2019**, 74b, 473.