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Antimicrobial activities of some synthesized macrocyclic pentaazapyridine and dipeptide pyridine derivatives.

Eman M. Flefel[1,2*], Mona A. Alsafi[1], Sana M. Alahmadi[1], Abd El-Galil E. Amr[3], Ahmed A. Fayed[2]

[1]Department of Chemistry, College of Science, Taibah University, Al-Madinah Al-Monawarah, Saudi Arabia

[2]Department of Photochemistry, Chemical Industries Research Division, National Research Centre, 33 EL-Bohouth St., Dokki, Giza, Egypt

[3]Applied Organic Chemistry Department, National Research Center, Cairo, Dokki, Egypt

Abstract

A series of tetracarboxamide Schiff base and macrocyclic pentaazapyridines has been prepared from 3,5-bis(N-(1-hydrazinyl-1-oxo-3-phenylpropan-2-yl))pyridine carboxamide 4 as starting material, which was synthesized from 3,5-dinicotinic acid 1. Treatment of 4 with 1,4-diaminobutane, 1,6-diaminohexane or cycloalkanone derivatives gave the corresponding macrocyclic tetracarboxamides 5a and 5b, and cycloalkyl Schiff bases 6a-6c, respectively. Treatment of 4 with acetophenone or acetylpyridine derivatives gave the corresponding Schiff bases 7a-7e and 8a-8c, respectively. Carboxylic acid hydrazide 4 was treated with acid anhydrides in glacial acetic acid to afford the corresponding diimide tetracarboxamides 9-11, respectively. The structures of newly synthesized compounds are established by physical and spectral data evidences. Some of the synthesized compounds were screened as antimicrobial agents.

Keywords: 3, 5-Bis (hydrazinyl) pyridine carboxamide, Bis-schiff bases, Macrocyclic pentaazapyridine, Antimicrobial agents.

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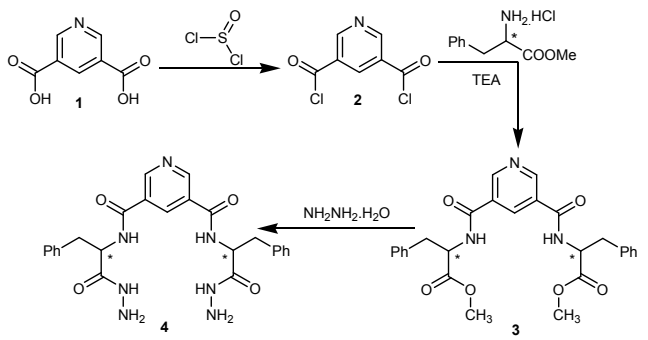
Introduction

The naturally occurring peptide derivatives were identified, and they were used in crucial roles in human physiology, ion channel ligands, including actions as hormones, growth factors, neurotransmitters, or anti-infectives [1-3]. A series of pyridine derivatives are correlated with several pharmacological properties, for example, antimycobacterial [4], anticancer [5], antiviral [6], anti-HlV [7], antifungal and antimicrobial [8], and anticonvulsant [9]. Also, some pyridinecarboxamide analogs were designed and used as PARP-1 inhibitors [10], mycobacterium tuberculosis agents [11] and as CB2 cannabinoid receptor partial agonists [12]. On the other hand, In addition, heterocyclic compounds containing an amino acid or a peptide structural moiety showed biological [13] and antibacterial activities [14]. A branched-chain amino acid (BCAA: Leu, Ile, and Val) mixture has been used for treatment of hypoalbuminemia in patients with decompensated liver cirrhosis in Japan [15]. In view of these observations and in continuation of our previous work [16-24] in heterocyclic and peptide chemistry, we have synthesized some new 3, 5-bis-(carboxamide Schiff base) pyridines and macrocyclic pentaazapyridines and we have evaluated some of them as antimicrobial agents.

Results and Discussion

Chemistry

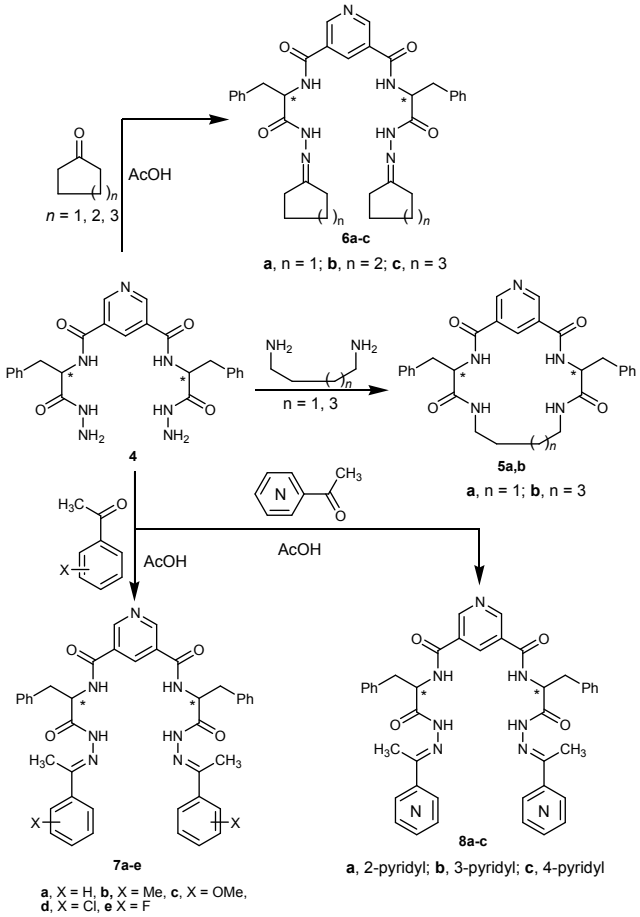
In this study, we report a series of linear dipeptide Schiff base derivatives by using N2, N2'-(pyridine-3, 5-dicarbonyl)-di-L-phenyalaninyl hydrazide **(4)** as starting material, which was synthesized from 3, 5-pyridinedicarboxylic acid according to a reported procedure [4] (Scheme 1).



Scheme 1. *Synthetic routes to starting compound* ***4****.*

Treatment of 3, 5-bis-hydrazide **4** with 1, 4-diaminobutane or 1, 6-diaminohexane afforded the corresponding macrocyclic tetracarboxamide derivatives **5a** and **5b**. Condensation of **4**

with cycloalkanones in refluxing glacial acetic acid gave the corresponding cycloalkanyl Schiff base derivatives **6a-6c**. Additionally, reaction of **4** with acetophenone or acetylpyridines gave the corresponding Schiff base derivatives **7a-7e** and **8a-8c**, respectively (Scheme 2).

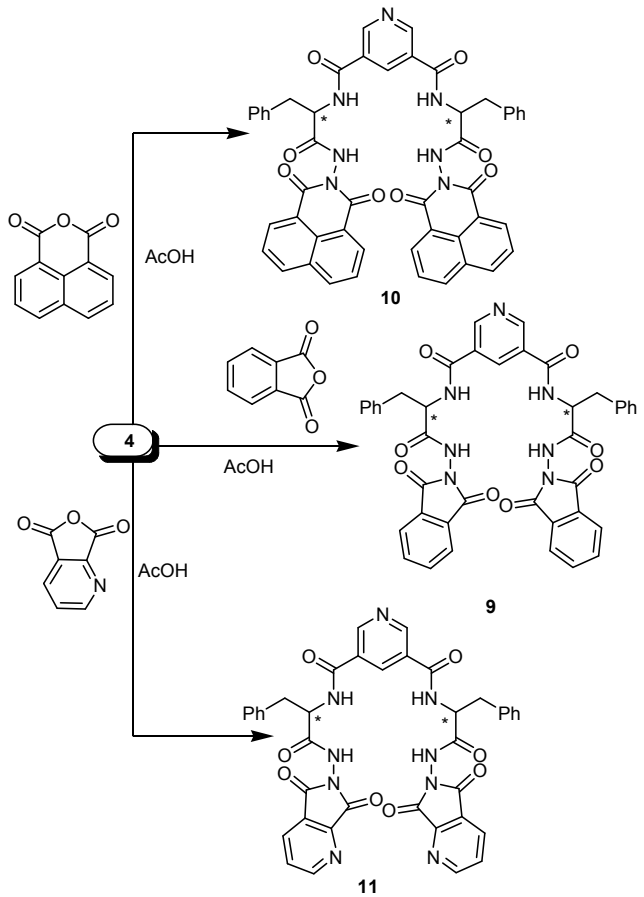


Scheme 2. *Synthetic routes to compounds* ***5a*** *and* ***5b, 6a-6c, 7a-7e*** *and* ***8a-8c****.*

Finally, carboxylic acid hydrazide **4** was treated with acid anhydrides, namely, phthalic, tetrachloro-phthalic, 1, 8-naphthaline or 2, 3-pyridinedicarboxylic acid anhydride in glacial acetic acid to afford the corresponding diimide tetracarboxamide derivatives **9-11**, respectively (Scheme 3).

Antimicrobial activity

The newly synthesized compounds **5-11** were tested for their preliminary antimicrobial activity against different microorganisms representing gram-positive (*Staphylococcus aureus*, *Bacillus aureus* and *Bacillus subtilis*), gram-negative bacteria (*Escherichia coli*), fungi (*Aspergillus niger*) and yeast (*Candida albican*). The obtained results (Table 1) showed that all synthesized compounds exhibited both antibacterial and antifungal activities on all tested microbial strains, except for compounds **5a, 5b, 7a, 7e, 8a, 8c, 9** and **11**, which did not showed antifungal activity against *Candida albicans*. In terms of antifungal activities, compounds **5a, 7c, 7d** and **10** were the most active and their activities was higher than that of the positive control (fusidic acid) by about 2.6, 2.6, 5.0 and 5.0%, respectively. Regarding antibacterial activities, it can be clearly observed that compounds **6a, 6b, 7d, 8b, 10** and **11** were the highly active compounds.



Scheme 3. *Synthetic routes to compounds* ***9-11****.*

Table 1. *Antimicrobial activities of some newly synthesized compounds.*

Compound	Inhibition zome (cm)					
	Gram +ve		Gram -ve		Fungi	Yeast
	Staphylococcus aureus	*Bacillus subtilis*	*Bacillus aureus*	*Escherichia coli*	*Aspergillus niger*	*Candida albicans*
5a	1.65	1.46	1.80	0.66	1.95	-
5b	1.68	1.75	1.55	0.60	1.70	-

6a	1.80	1.65	1.96	0.78	1.48	0.94
6b	1.85	1.85	1.92	0.80	1.56	0.92
6c	1.76	1.72	1.58	0.74	1.80	0.95
7a	1.56	1.56	1.50	0.66	1.68	-
7b	1.75	1.65	1.74	0.75	1.85	1.05
7c	1.64	1.76	1.75	0.80	1.95	1.05
7d	1.95	1.85	2.00	0.90	2.00	0.96
7e	1.78	1.45	1.65	0.62	1.75	-
8a	1.55	1.85	1.50	0.65	1.75	-
8b	1.85	1.80	1.95	0.78	1.55	1.00
8c	1.66	1.72	1.22	0.60	1.75	-
9	1.65	1.65	1.83	0.64	1.58	-
10	1.77	1.84	1.88	0.92	2.00	1.10
11	1.80	1.70	1.65	0.64	1.75	-
Chloramphenicol	2.00	2.00	2.10	0.95	-	-
Fusidic acid	-	-	-	-	1.9	1.9

Experimental section

Chemistry: Melting points were determined in open glass capillary tubes with an Electro Thermal Digital melting point apparatus (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. IR was recorded on a Nexus 670 FTIR Nicolet, Fourier Transform infrared spectrometer. ^{1}H N MR and ^{13}C NMR spectra were run in DMSO-d6 on Jeol 500 MHz (^{1}H) and 125 MHz (^{13}C) instruments. Mass spectra were run on a MAT Finnigan SSQ 7000 spectrometer, using the Electron Impact technique (EI). Analytical Thin Layer Chromatography (TLC) was performed on silica gel aluminum sheets, 60 F254 (E. Merck). Antimicrobial activities were evaluated in National Research Center, Dokki, Cairo, Egypt.

Synthesis of cyclo-(Nα-dinicotinoyl)-bis (L-phenylalaninyl)-1, 4- or 1, 6-alkanediamine 5a and 5b: To a cold (-5°C) and stirred solution of the dihydrazide 4 (1 mmol) in 5 N aq. HCl (3 ml) and acetic acid (3 ml), sodium nitrite solution (10%, 0.13 g, 2 mmol) was added at the same temperature. Stirring was continued for 30 min, the reaction mixture was extracted with ether, washed with water, $NaHCO_3$, and water, dried over anhydrous sodium sulfate. The cold ethereal solution (-5°C) was then added to a cold (-5°C) dichloromethane solution of 1, 4-butanediamine, or 1, 6-hexanediamine (1 mmol, 10 ml of CH_2Cl_2). Stirring was continued for 5 h at -5°C and at room temperature for 2 h. The reaction mixture was washed with 1 N hydrochloric acid, water and then dried over anhydrous calcium chloride. The solvent was evaporated under reduced pressure and crystallized from ethanol/ether to afford the corresponding title compounds **5a** and **5b**, respectively.

*Cyclo-(Nα-dinicotinoyl)-bis [L-phenylalaninyl]-1, 4-butanediamine (***5a***):* Yield, 58%; M. p. 204-206°C; $[\alpha]^{25}_{D}$=-110 (c=0.5, DMF); IR (KBr) ν_{max} in cm^{-1}: 3365 (NH), 3076 (CH-Ar), 2984 (CH-aliph.), 1662, 1535, 1234 (C=O, amides I-III) cm^{-1}; ^{1}H MR (DMSO-d6, δ ppm): 1.40-1.48 (m, 4 H, 2 CH_2), 2.62-2.74 (m, 4 H, 2 CH_2), 3.42 (d, 4 H, 2 CH_2), 4.05-4.10 (m, 2 H, 2 CH), 6.95-7.52 (m, 10 H, 2 Ph-H), 8.58, 9.05 (2 s, 3 H, pyr-H), 8.72, 8.95 (2 s, 4 H, 4 NH, exchangeable with D_2O); MS, m/z (%): 514 (12) $(M)^{+}$; Anal. Calcd. for $C_{29}H_{31}N_5O_4$ (513.58): C 67.82, H 6.08, N 13.64; found C 67.70, H 6.00, N 13.57.

*Cyclo-(Nα-dinicotinoyl)-bis[L-phenylalaninyl]-1, 6-hexanediamine (***5b***):* Yield, 60%; M. p. 194-196°C; $[\alpha]^{25}_{D}$=-96 (c=0.5, DMF); IR (KBr) ν_{max} in cm^{-1}: 3358-3312 (NH), 3080 (CH-Ar), 2975 (CH-aliph.), 1660, 1530, 1240 (C=O, amides I-III) cm^{-1}; ^{1}H MR (DMSO-d6, δ ppm): 1.24-1.28 (m, 4 H, 2 CH_2), 1.38-1.44 (m, 4 H, 2 CH_2), 2.60-1.68 (m, 4 H, 2 CH_2), 3.42 (d, 4 H, 2 CH_2), 4.08-4.20 (m, 2 H, 2 CH), 6.96-7.55 (m, 10 H, 2 Ph-H), 8.62, 9.00 (2 s, 3 H, pyridyl-H), 8.74, 8.95 (42 s, H, 4 NH, exchangeable with D_2O); MS, m/z (%): 542 (22) $(M)^{+}$; Anal. Calcd. for $C_{31}H_{35}N_5O_4$ (541.64): C 68.74, H 6.51, N 12.93; found C 68.62, H 6.44, N 12.85.

Synthesis of Schiff bases 6a-6c: To a solution of acid hydrazide 4 (1 mmol) in glacial acetic acid (30 ml), cyclopentanone, cyclohexanone or cycloheptanone (2 mmol) was added. The reaction mixture was refluxed for 6 h, poured onto ice water, the obtained solid was filtered off, washed with water, dried, and crystallized from the proper solvents to give the corresponding Schiff bases derivatives **6a-6c**, respectively.

*N,N'-Bis[1-(cyclopentanyl-1-methylhydrazonyl)-2-L-phenylalaninyl]-3,5-(diaminocarbonyl) pyridine (**6a**):* Yield, 82%; M. p. 178-180°C (EtOH/H_2O); $[\alpha]^{25}_D$=-124 (c=0.5, DMF); IR (KBr) ν_{max} in cm^{-1}: 3446-3367 (NH), 3085 (CH-Ar), 2982 (CH-aliph.), 1661, 1521, 1314 (C=O, amides I-III) cm^{-1}; ^{1}H MR (DMSO-d6, δ ppm): 1.28-1.34 (m, 16 H, 2 cyclopentyl-H), 3.36 (d, 4 H, 2 CH_2), 4.62-4.73 (m, 2 H, 2 CH), 6.98-7.52 (m, 10 H, 2 Ph-H), 7.90, 8.64 (2 s, 4 H, 4 NH, exchangeable with D_2O), 8.68, 9.12 (2 s, 3 H, pyr-H); ^{13}C-NMR (DMSO-d6): 41.35 (2 C, 2 CH_2), 53.14 (2 C, 2 CH), 125.66, 127.70, 128.64, 139.44 (12 C, 2 Ph-C), 131.46, 141.23, 152.36 (5 C, Pyr-C), 167.18 (2 C, 2 C=O), 176.64 (2 C, 2 C=O), 26.04, 36.86, 186.74 (10 C, cyclopentyl-C); MS, m/z (%): 622 (6) $(M)^+$; Anal. Calcd. for $C_{35}H_{39}N_7O_4$ (621.72): C 67.61, H 6.32, N 15.77; found C 67.52, H 6.22, N 15.69.

*N,N'-Bis[1-(cyclohexanyl-1-methylhydrazonyl)-2-L-phenylalaninyl]-3,5-(diaminocarbonyl)pyridine (**6b**):* Yield, 66 %; M. p. 206-208°C (AcOH/H_2O); $[\alpha]^{25}_D$ =-116 (c=0.5, DMF); IR (KBr) ν_{max} in cm^{-1}: 3565-3432 (NH), 3084 (CH-Ar), 2974 (CH-aliph.), 1664, 1525, 1318 (C=O, amides I-III) cm^{-1}; ^{1}H MR (DMSO-d6, δ ppm): 1.25-1.36 (m, 20 H, 2 cyclohexyl-H), 3.42 (d, 4 H, 2 CH_2), 4.65-4.70 (m, 2 H, 2 CH), 6.96-7.54 (m, 10 H, 2 Ph-H), 7.96, 8.68 (2 s, 4 H, 4 NH, exchangeable with D_2O), 8.76, 9.10 (2 s, 3 H, Pyr-H); ^{13}C-NMR (DMSO-d6): 41.36 (2 C, 2 CH_2), 53.32 (2 C, 2 CH), 125.65, 127.74, 128.67, 139.48 (12 C, 2 Ph-C), 131.42, 141.28, 152.33 (5 C, Pyr-C), 167.24 (2 C, 2 C=O), 176.44 (2 C, 2 C=O), 23.22, 26.86, 28.14, 161.33 (12 C, cyclohexyl-C); MS, m/z (%): 650 (22) $(M)^+$; Anal. Calcd. for $C_{37}H_{43}N_7O_4$ (649.78): C 68.39, H 6.67, N, 15.09; found C 68.30, H 6.60, N 15.00.

*N,N'-Bis[1-(cycloheptanyl-1-methylhydrazonyl)-2-L-phenylalaninyl]-3,5-(diaminocarbonyl)pyridine (**6c**):* Yield, 72 %; M. p. 232-234°C (EtOH); $[\alpha]^{25}_D$=-112 (c=0.5, DMF); IR (KBr) νmax in cm^{-1}: 3554-3428 (NH), 3090 (CH-Ar), 2978 (CH-aliph.), 1661, 1526, 1318 (C=O, amides I-III) cm^{-1}; ^{1}H MR (DMSO-d6, δ ppm): 1.20-1.36 (m, 24 H, 2 cycloheptyl-H), 3.31 (d, 4 H, 2 CH_2), 4.62-4.71 (m, 2 H, 2 CH), 6.99-7.56 (m, 10 H, 2 Ph-H), 7.94, 8.66 (2 s, 4 H, 4 NH, exchangeable with D_2O), 8.79, 9.18 (2 s, 3 H, Pyr-H); ^{13}C-NMR (DMSO-d6): 41.48 (2 C, 2 CH_2), 53.15 (2 C, 2 CH), 125.76, 127.54, 128.62, 139.50 (12 C, 2 Ph-C), 132.13, 141.18, 152.42 (5 C, Pyr-C), 167.24 (2 C, 2 C=O), 175.98 (2 C, 2 C=O), 23.56, 25.42, 29.70, 183.84 (14 C, cycloheptyl-C); MS, m/z (%): 678 (15) $(M)^+$; Anal. Calcd. for $C_{39}H_{47}N_7O_4$ (677.83): C 69.10, H 6.99, N 14.46; found C 69.00, H 6.90, N 14.40.

Synthesis of compounds 7a-7e and 8a-8c: A mixture of **4** (1 mmol) and a substituted acetophenone (acetophenone, 4-methyl-, 4-methoxy-, 4-chloro-, 4-fluoroacetophenone) or an acetylpyridine (2-acetyl-, 3-acetyl-, 4-acetylpyridine, 2 mmol) in glacial acetic acid (30 ml) was refluxed for 4-7 h. The reaction mixture was poured into ice-water, and then neutralized with 1 N aq. sodium carbonate. The obtained solid was filtered off, washed with water, dried, and crystallized from the proper solvent to give the corresponding Schiff bases **7a-7e** and **8a-8c**, respectively.

*N,N'-Bis[(1-phenyl-1-methyl-hydrazonyl-2-L-phenylalaninyl]-3,5-(diaminocarbonyl)pyridine (**7a**):* Yield, 75 %; M. p. 242-244°C (DMF/H_2O); $[\alpha]^{25}_D$=-98 (c=0.5, DMF); IR (KBr) νmax in cm^{-1}: 3367-3342 (NH), 3078 (CH-Ar), 2990 (CH-aliph.), 1652, 1537, 1254 (CO, amides I-III) cm^{-1}; ^{1}H MR (DMSO-d6, δ ppm): 1.04 (s, 6 H, 2 CH_3), 3.24 (d, 4 H, 2 CH_2), 4.68-4.75 (m, 2 H, 2 CH), 7.05-7.68 (m, 20 H, 4 Ph-H), 8.54, 8.65 (2 s, 4 H, 4 NH, D_2O exchangeable), 8.75, 9.08 (2 s, 3 H, pyr-H); ^{13}C-NMR (DMSO-d6): 14.43 (2 C, 2 CH_3), 41.22 (2 C, 2 CH_2), 53.98 (2 C, 2 CH), 125.56, 127.72, 128.55, 139.35 (12 C, 2 Ph-C), 127.82, 128.55, 130.70, 133.36 (12 C, 2 Ph-C), 131.72, 140.34, 151.86 (5 C, pyr-C), 167.42 (2 C, 2 CO), 168.35 (2 C, 2 C=N), 176.98 (2 C, 2 CO); MS, m/z (%): 694 (16) $(M)^+$. Anal. Calcd. for $C_{41}H_{39}N_7O_4$ (693.79): C 70.98, H 5.67, N 14.13; found C 70.90, H 5.60, N 14.05.

*N,N'-Bis[1-(4-methylphenyl-1-methylhydrazonyl)-2-L-phenylalaninyl]-3,5-(diaminocarbonyl)pyridine (**7b**):* Yield, 68 %; M. p. 226-228°C (AcOH/H_2O); $[\alpha]^{25}_D$=-105 (c=0.5, DMF); IR (KBr) νmax in cm^{-1}: 3388-3335 (NH), 3082 (CH-Ar), 2985 (CH-aliph.), 1653, 1534, 1252 (CO, amides I-III) cm^{-1}; ^{1}H MR (DMSO-d6, δ ppm): 1.00 (s, 6 H, 2 CH_3), 2.23 (s, 6 H, 2 CH_3), 3.26 (d, 4 H, 2 CH_2), 4.65-4.72 (m, 2 H, 2 CH), 7.12-7.72 (m, 18 H, 4 Ph-H), 8.65, 8.78 (2 s, 4 H, 4 NH, D_2O exchangeable), 8.72, 9.12 (2 s, 3 H, pyr-H); ^{13}C-NMR (DMSO-d6): 14.22, 23.45 (4 C, 4 CH_3), 41.25 (2 C, 2 CH_2), 54.10 (2 C, 2 CH), 125.62, 127.70, 128.58, 139.45 (12 C, 2 Ph-C), 128.74, 129.08, 130.86, 140.35 (12 C, 2 Ph-C), 131.84, 140.38, 151.85 (5 C, pyr-C), 167.36 (2 C, 2 CO), 168.45 (2 C, 2 C=N), 176.95 (2 C, 2 CO); MS, m/z (%): 722 (8) $(M)^+$; Anal. Calcd. for $C_{43}H_{43}N_7O_4$ (721.84): C 71.55, H 6.00, N 13.58; found C 71.48, H 5.92, N 13.50.

*N,N'-Bis[1-(4-methoxylphenyl-1-methylhydrazonyl)-2-L-phenylalaninyl]-3,5-(diaminocarbonyl)pyridine (**7c**).* Yield, 78 %; M. p. 268-270°C (dioxane); $[\alpha]^{25}_D$=-78 (c=0.5, DMF); IR (KBr) νmax in cm^{-1}: 3374-3328 (NH), 3080 (CH-Ar), 2988 (CH-aliph.), 1654, 1535, 1255 (CO, amides I-III) cm^{-1}; ^{1}H MR (DMSO-d6, δ ppm): 1.12 (s, 6 H, 2 CH_3), 3.32 (d, 4 H, 2 CH_2), 2.68 (s, 6 H, 2 OCH_3), 4.62-4.68 (m, 2 H, 2 CH), 6.95-7.65 (m, 18 H, 4 Ph-H), 8.68, 8.82 (2 s, 4 H, 4 NH, D_2O exchangeable), 8.74, 9.10 (2 s, 3 H, pyr-H); ^{13}C-NMR (DMSO-d6): 14.36 (2 C, 2 CH_3), 41.34 (2 C, 2 CH_2), 54.18 (2 C, 2 CH), 55.32 (2 C, 2 OCH_3), 125.65, 127.73, 128.54, 139.42 (12 C, 2 Ph-C), 113.95, 125.78, 129.94, 162.48 (12 C, 2 Ph-C), 131.76, 140.42, 151.80 (5 C, pyr-C), 167.43 (2 C, 2 CO), 168.35 (2 C, 2 C=N), 176.88 (2 C, 2 CO); MS, m/z (%): 754 (12) $(M)^+$; Anal. Calcd. for $C_{43}H_{43}N_7O_6$ (753.84): C 68.51, H 5.75, N 13.01; found C 68.40, H 5.70, N 12.90.

*N,N'-Bis[1-(4-chlorophenyl-1-methylhydrazonyl)-2-L-phenylalaninyl]-3,5-(diaminocarbonyl)pyridine (**7d**):* Yield, 64 %; M. p. 234-236°C (AcOH); $[\alpha]^{25}_D$=-86 (c=0.5, DMF); IR (KBr) νmax in cm^{-1}: 3398-3354 (NH), 3085 (CH-Ar), 2978 (CH-aliph.), 1654, 1535, 1252 (CO, amides I-III) cm^{-1}; ^{1}H MR (DMSO-d6, δ ppm): 1.10 (s, 6 H, 2 CH_3), 3.26 (d, 4 H, 2 CH_2), 4.65-4.70 (m, 2 H, 2 CH), 7.15-7.72 (m, 18 H, 4 Ph-H), 8.52, 8.68 (2 s, 4 H, 4 NH, D_2O exchangeable), 8.76, 9.14 (2 s, 3 H, pyr-H). ^{13}C-NMR (DMSO-d6): 14.52 (2 C, 2 CH_3), 41.28 (2

C, 2 CH_2), 53.85 (2 C, 2 CH), 125.64, 127.70, 128.62, 139.38 (12 C, 2 Ph-C), 128.82, 129.55, 131.65, 136.35 (12 C, 2 Ph-C), 131.80, 140.40, 151.82 (5 C, pyr-C), 167.45 (2 C, 2 CO), 168.48 (2 C, 2 C=N), 176.86 (2 C, 2 CO); MS, m/z (%): 763 (32) $(M)^+$; Anal. Calcd. for $C_{41}H_{37}Cl_2N_7O_4$ (762.68): C 64.57, H 4.89, Cl 9.30, N 12.86; found C 64.50, H 4.80, Cl 9.22, N 12.80.

*N,N'-Bis[1-(4-fluorophenyl-1-methylhydrazonyl)-2-L-phenylalaninyl]-3,5-(diaminocarbonyl)pyridine (***7e***):* Yield, 70 %; M. p. 216-218°C (AcOH); $[\alpha]^{25}_D$=-116 (c=0.5, DMF); IR (KBr) *ν*max in cm^{-1}: 3378-3342 (NH), 3080 (CH-Ar), 2972 (CH-aliph.), 1652, 1533, 1254 (CO, amides I-III) cm^{-1}; ^1H MR (DMSO-d6, δ ppm): 1.15 (s, 6 H, 2 CH_3), 3.24 (d, 4 H, 2 CH_2), 4.68-4.72 (m, 2 H, 2 CH), 7.10-7.75 (m, 18 H, 4 Ph-H), 8.68, 8.70 (2 s, 4 H, 4 NH, D_2O exchangeable), 8.75, 9.10 (2 s, 3 H, pyr-H); ^{13}C-NMR (DMSO-d6): 14.56 (2 C, 2 CH_3), 41.32 (2 C, 2 CH_2), 53.88 (2 C, 2 CH), 125.58, 127.64, 128.60, 139.44 (12 C, 2 Ph-C), 114.65, 128.62, 129.68, 164.78 (12 C, 2 Ph-C), 131.76, 140.38, 151.80 (5 C, pyr-C), 167.41 (2 C, 2 CO), 168.52 (2 C, 2 C=N), 176.82 (2 C, 2CO); MS, m/z (%): 730 (25) $(M)^+$; Anal. Calcd. for $C_{41}H_{37}F_2N_7O_4$ (729.77): C 67.48, H 5.11, N 13.44; found C 67.40, H 5.05, N 13.40.

*N,N'-Bis[1-(2-pyridyl-1-methylhydrazonyl)-2-L-phenylalaninyl]-3,5-(diaminocarbonyl)pyridine (***8a***):* Yield, 65%; M. p. 217-219°C (AcOH/H_2O); $[\alpha]^{25}_D$=- 92 (c=0.5, DMF); IR (KBr) *ν*max in cm^{-1}: 3454-3324 (NH), 3088 (CH-Ar), 2974 (CH-aliph.), 1655, 1534, 1253 (C=O, amides I-III) cm^{-1}; ^1H MR (DMSO-d6, δ ppm): 0.99 (s, 6 H, 2 CH_3), 3.62 (d, 4 H, 2 CH_2), 4.64-4.70 (m, 2 H, 2 CH), 7.02-7.60 (m, 10 H, 2 Ph-H), 7.75-8.56 (m, 6 H, 2-pyridyl-H), 8.65, 8.92 (2 s, 4 H, 4 NH, D_2O exchangeable), 8.80 (s, 2 H, pyridyl-H), 8.75, 9.08 (2 s, 3 H, 3,5-pyridyl-H); ^{13}C-NMR (DMSO-d6): 13.65 (2 C, 2 CH_3), 41.82 (2 C, 2 CH_2), 53.74 (2 C, 2 CH), 125.35, 127.62, 128.55, 139.58 (12 C, 2 Ph-C), 122.88, 126.12, 131.68, 135.84, 140.32, 148.65, 152.28, 154.48 (15C, 3 pyr-C), 144.98 (2 C, 2 C=N), 167.72 (2 C, 2 CO), 176.85 (2 C, 2 CO); MS, m/z (%): 696 (10) $(M)^+$; Anal. Calcd. for $C_{39}H_{37}N_9O_4$ (695.76): C 67.32, H 5.36, N 18.12; found C 67.21, H 5.30, N 18.05.

*N,N'-Bis[1-(3-pyridyl-1-methylhydrazonyl)-2-L-phenylalaninyl]-3,5-(diaminocarbonyl)pyridine (***8b***):* Yield, 55 %; M. p. 241-243°C (EtOH); $[\alpha]^{25}_D$=-75 (c=0.5, DMF); IR (KBr) *ν*max in cm^{-1}: 3415-3332 (NH), 3075 (CH-Ar), 2982 (CH-aliph.), 1652, 1534, 1255 (CO, amides I-III) cm^{-1}. ^1H MR (DMSO-d6, δ ppm): 1.05 (s, 6 H, 2 CH_3), 3.75 (d, 4 H, 2 CH_2), 4.60-4.66 (m, 2 H, 2 CH), 7.00-7.58 (m, 10 H, 2 Ph-H), 7.70-8.50 (m, 6 H, pyridyl-H), 8.65, 8.86 (2 s, 4 H, 4 NH, D_2O exchangeable), 8.75, 9.10 (2 s, 3 H, 3,5-pyridyl-H), 9.18 (s, 2 H, pyridyl-H-2); ^{13}C-NMR (DMSO-d6): 12.98 (2 C, 2 CH_3), 41.68 (2 C, 2 CH_2), 53.84 (2 C, 2 CH), 125.64, 127.58, 128.72, 139.67 (12 C, 2 Ph-C), 122.56, 125.52, 136.92, 150.75, 151.24 (10 C, 3-Pyridyl-C), 131.48, 140.65, 152.32 (5 C, 3,5-pyridyl-C), 167.05 (2 C, 2 CO), 168.42 (2 C, 2 C=N), 176.78 (2 C, 2 CO); MS, m/z (%): 696 (35) $(M)^+$; Anal. Calcd. for $C_{39}H_{37}N_9O_4$ (695.76): C 67.32, H 5.36, N 18.12; found C 67.20, H 5.28, N 18.05.

*N,N'-Bis[1-(4-pyridyl-1-methylhydrazonyl)-2-L-phenylalaninyl]-3,5-(diaminocarbonyl)pyridine (***8c***):* Yield, 62 %; M. p. 255-257°C (MeOH); $[\alpha]^{25}_D$=-102 (c=0.5, DMF); IR (KBr) *ν*max n cm^{-1}: 3410-3330 (NH), 3078 (CH-Ar), 2986 (CH-aliph.), 1653, 1532, 1251 (CO, amides I-III) cm^{-1}; ^1H MR (DMSO-d6, δ ppm): 0.98 (s, 6 H, 2 CH_3), 3.78 (d, 4 H, 2 CH_2), 4.62-4.65 (m, 2 H, 2 CH), 6.98-7.46 (m, 10 H, 2 Ph-H), 7.78, 8.70 (d, 8 H, pyridyl-H), 8.68, 8.88 (2 s, 4 H, 4 NH, D_2O exchangeable), 8.78, 9.12 (2 s, 3 H, 3,5-pyridyl-H); ^{13}C-NMR (DMSO-d6): 13.12 (2 C, 2 CH_3), 41.60 (2 C, 2 CH_2), 53.80 (2 C, 2 CH), 125.60, 127.50, 128.65, 139.60 (12 C, 2 Ph-C), 123.65, 136.52, 148.65 (10 C, pyridyl-C), 131.55, 140.32, 152.44 (5 C, 3,5-pyridyl-C), 167.00 (2 C, 2 CO), 168.30 (2 C, 2 C=N), 176.84 (2 C, 2 CO); MS, m/z (%): 696 (16) $(M)^+$; Anal. Calcd. for $C_{39}H_{37}N_9O_4$ (695.76): calcd. C 67.32, H 5.36, N 18.12; found C 67.18, H 5.24, N 18.08.

Synthesis of compounds 9-11: A mixture of **4** (1 mmol) and dicarboxylic acid anhydride derivatives (phthalic anhydride, tetrachlorophthalic anhydride, 1, 8-naphthalenedicarboxylic acid anhydride or 2, 3-pyridinedicarboxylic acid anhydride, 2 mmol) was refluxed in glacial acetic acid (50 ml) for 6 h. The reaction mixture was poured into ice-water, the obtained precipitate was collected by filtration, washed with water, dried, and crystallized from DMF/H_2O to give the corresponding bisimide hexacarboxamide derivatives **9-11**, respectively.

*Nα-Dinicotinoyl-bis(L-phenylalaninyl-isoindoline-1,3-dione-imide) (***9***):* Yield, 72%; M. p. 256-258°C; $[\alpha]^{25}_D$=- 117 (c=0.5, DMF); IR (KBr) *ν*max in cm^{-1}: 3398-3343 (NH), 3078 (CH-Ar), 2986 (CH-aliph.), 1653, 1533, 1254 (C=O, amides I-III) cm^{-1}; ^1H MR (DMSO-d6, δ ppm): 3.40 (d, 4 H, 2 CH_2), 4.56-4.63 (m, 2 H, 2 CH), 7.08-8.05 (m, 18 H, 4 Ph-H), 8.45, 9.00 (2 s, 3 H, pyr-H), 8.64, 8.75 (2 s, 4 H, 4 NH, D_2O exchangeable); ^{13}C-NMR (DMSO-d6): 40.84 (2 C, 2 CH_2), 53.03 (2 C, 2 CH), 124.34, 127.42, 128.22, 138.86 (12 C, 2 Ph-C), 127.24, 131.22, 131.86, (12 C, 2 Phth-C), 131.65, 140.14, 152.13 (5 C, pyr-C), 164.56 (4 C, 4 CO-imide), 167.12, 170.15 (4 C, 4 CO-amide); MS, m/z (%): 750 (14) $(M)^+$; Anal. Calcd. for $C_{41}H_{31}N_7O_8$ (749.72): C 65.68, H 4.17, N 13.08; found C 65.60, H 4.10, N 13.00.

*Nα-Dinicotinoyl-bis(L-phenylalaninylbenzo[de]isoquinoline-1,3(2H)-dione-imide) (***10***):* Yield, 65%; M. p. 264-266°C; $[\alpha]^{25}_D$=-68 (c=0.5, DMF); IR (KBr) *ν*max in cm^{-1}: 3416-3332 (NH), 3073 (CH-Ar), 2987 (CH-aliph.), 1655, 1535, 1255 (C=O, amides I-III) cm^{-1}; ^1H MR (DMSO-d6, δ ppm): 3.40 (d, 4 H, 2 CH_2), 4.45-4.52 (m, 2 H, 2 CH), 7.12-7.86 (m, 22 H, Ar-H), 8.57, 9.02 (2 s, 3 H, pyr-H), 8.60, 8.78 (2 s, 4 H, 4 NH, exchangeable with D_2O); ^{13}C-NMR (DMSO-d6): 41.45 (2 C, 2 CH_2), 53.12 (2 C, 2 CH), 122.12, 124.65, 127.95, 130.10, 136.86, 137.35 (20 C, naphthyl-C), 125.60, 127.54, 128.54, 139.68 (12 C, 2 Ph-C), 131.62, 140.18, 152.17 (5 C, pyr-C), 158.55 (4 C, 4 CO-imide), 167.24, 170.26 (4 C, 4 CO-amide); MS, m/z (%): 850 (8) $(M)^+$; Anal. Calcd. for $C_{49}H_{35}N_7O_8$ (849.84): C 69.25, H 4.15, N 11.54; found C 69.12, H 4.05, N 11.43.

*Nα-Dinicotinoyl-bis[L-phenylalaninylpyrrolo[3,4-b]pyridine-5,7-dione-imide] (**11**):* Yield, 68%; M. p. 234-236°C; $[\alpha]^{25}_{D}$=-96 (c=0.5, DMF); IR (KBr) *ν*max in cm^{-1}: 3445-3313 (NH), 3083 (CH-Ar), 2992 (CH-aliph.), 1653, 1535, 1252 (C=O, amides I-III) cm^{-1}; ^{1}H MR (DMSO-d6, δ ppm): 3.45 (d, 4 H, 2 CH_2), 4.50-4.61 (m, 2 H, 2 CH), 6.94-7.48 (m, 10 H, 2 Ph-H), 8.62, 9.05 (2 s, 3 H, pyr-H), 7.96-8.32 (m, 4 H, pyr-H), 8.95 (t, 2 H, pyr-H), 8.68, 8.75 (2 s, 4 H, 4 NH, exchangeable with D_2O); ^{13}C-NMR (DMSO-d6): 42.28 (2 C, CH_2), 52.87 (2 C, 2 CH), 124.32, 128.36, 129.43, 138.76 (12 C, 2 Ph-C), 127.21, 128.05, 131.66, 137.94, 140.12, 145.12, 152.10, 152.56 (15 C, pyr-C), 164.63, 164.95 (4 C, 4 CO-Imide), 169.48, 170.34 (4 C, 4 CO-amide); MS, m/z (%): 752 (20) $(M)^{+}$; Anal. Calcd. for $C_{39}H_{29}N_9O_8$ (751.70): C 62.31, H 3.89, N 16.77; found C 62.20, H 3.80, N 16.70.

Antimicrobial activity

The antimicrobial activities of the synthesized compounds were determined by the agar diffusion method as recommended by the National Committee for Clinical Laboratory Standards (NCCLS) [25]. The concentrations of the tested compounds (10 µg/ml) were used according to modified Kirby-Bauer's disk diffusion method [26]. The degree of inhibition is measured in comparison with that of Chloramphenicol® and fusidic acid taken as standards.

Conclusion

In the present work, a series of tetracarboxamide and macrocyclic tripeptides has been prepared starting from 3,5-bis[N-(1-hydrazinyl-1-oxo-3-phenylpropan-2-yl)]pyridine-carboxamide as starting material. Some of the synthesized compounds were screened as antimicrobial agents.

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*Correspondence to

Eman M. Flefel

Department of Chemistry

College of Science

Taibah University

Saudi Arabia