

Synthesis and Biological Activity of Novel Amino Acid-(N'-Benzoyl) Hydrazide and Amino Acid-(N'-Nicotinoyl) Hydrazide Derivatives.

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Abstract: The coupling reaction of benzoic acid and nicotinic acid hydrazides with N-protected L-amino acids including valine, leucine, phenylalanine, glutamic acid and tyrosine is reported. The target compounds, N-Boc-amino acid-(N'-benzoyl)- and N-Boc-amino acid-(N'-nicotinoyl) hydrazides **5a-5e** and **6a-6e** were prepared in very high yields and purity using N-[(dimethylamino)-1H-1,2,3-triazolo[4,5-b]pyridin-1-yl-methylene]-N-methyl-methanaminium hexafluorophosphate N-oxide (HATU) as coupling reagent. The antimicrobial activity of the Cu and Cd complexes of the designed compounds was tested. The products were deprotected affording the corresponding amino acid-(N'-benzoyl) hydrazide hydrochloride salts (**7a-7e**) and amino acid-(N'-nicotinoyl) hydrazide hydrochloride salts (**8a-8e**). These compounds and their Cu and Cd complexes were also tested for their antimicrobial activity. Several compounds showed comparable activity to that of ampicillin against *S. aureus* and *E. coli*.

Keywords: Benzoic acid hydrazide, nicotinic acid hydrazide, amino acid hydrazide derivatives, Copper and Cadmium complexes, antimicrobial agents.

Introduction:

Amino acids have proven to play a significant role in the synthesis of novel drug candidates with the use of non-proteinogenic and unnatural amino acids [1-8]. Recently a series of novel diflunilal hydrazide-hydrazone derivatives have demonstrated significant antimicrobial activity [9]. These synthesized compounds and additional hydrazide derivatives were screened for their wide range of biological activities, antimycobacterial activity against *Mycobacterium tuberculosis*, antimicrobial activities against various bacteria (*Escherichia coli* and *Bacillus subtilis*), fungi, and yeast species [10-13]. In addition, nicotinamide and nicotinic acid have been in use for 65 years due to their unusual antimicrobial spectrum. Many drugs possess modified pharmacological and toxicological properties when administered in form of metallic complexes [14-16]. In view of this it was interesting to synthesize several new compounds to evaluate the effect of amino acids on the bioactivity of both benzoic acid hydrazide and nicotinic acid hydrazide. It was also of interest to study the effect of metals such as Cu and Cd on the antibacterial activity of these compounds, thus both hydrazide ligands and complexes were tested for their antibacterial and antifungal activity.

Results and Discussion

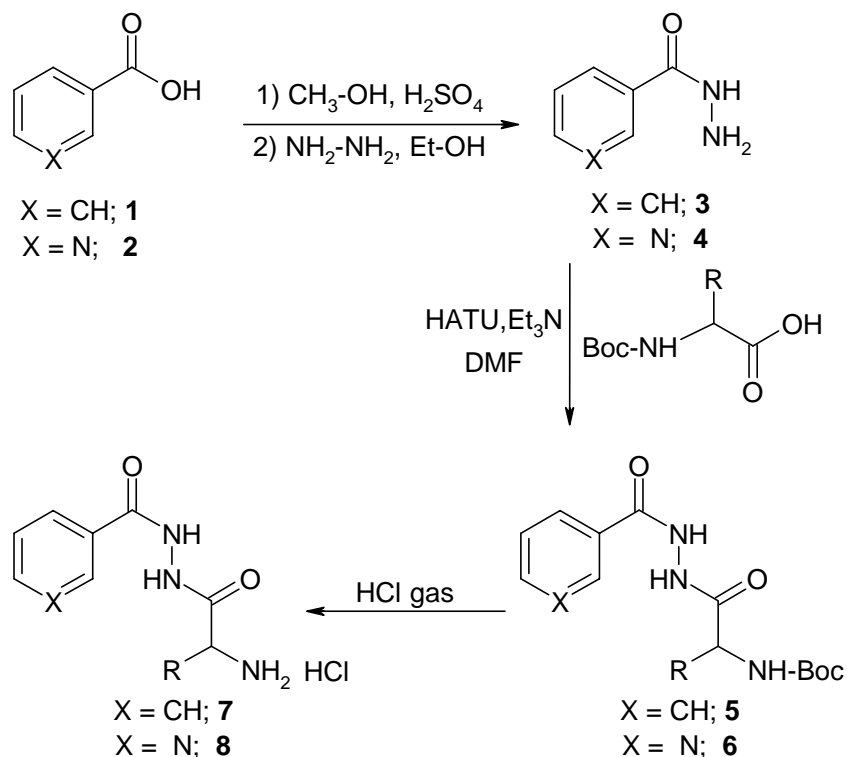
Chemistry

For the synthesis of the desired compounds, the sequence of reactions shown in Scheme 1 was followed. The reaction of benzoic acid hydrazide (**3**) with different N-Boc-L-amino acids in dimethylformamide in the presence of triethylamine (Et₃N) as a base and N-[(dimethylamino)-1H-1,2,3-triazolo[4,5-b]pyridin-1-yl-methyl-ene]-N-methylmethanaminium hexafluorophosphate N-oxide (HATU) as coupling reagent at 0°C gave the desired compounds **5a-5e**. The structures of compounds **5a-5e** were established by means of IR, ¹H-NMR, ¹³C-NMR, and elemental analyses. The infrared spectra of these compounds showed the absorption peaks of the urethane and the amide carbonyls in the 1715-1727 cm⁻¹ and 1663-1693 cm⁻¹ ranges, respectively, and the absorption peak of the N-H in the 3226-3336 cm⁻¹ range. The ¹H-NMR showed the presence of three exchangeable signals for the NH and the ¹³C-NMR detected the presence of one urethane carbonyl and two amide carbonyls in the 153-173 ppm range. Similarly, nicotinic acid hydrazide (**4**) was allowed to react under the same conditions with the same N-Boc-L-amino acids to give the desired compounds **6a-6e**. The structures of compounds **6a-6e** were also established by means of IR, ¹H-NMR, ¹³C-NMR, and elemental analyses. Compounds **5** and **6** were subjected to N-deprotection by passing HCl gas into a solution of these compounds in methylene chloride/ether. The latter series upon deprotection of Boc group afforded the new series of compounds **7** and **8** (Table 1, Table 2).

Compounds **5**, **6**, **7** and **8** were allowed to undergo complexation with Cu and Cd. All complexes were obtained by reacting one equivalent of the ligands **5**, **6**, **7** or **8** once with 2 equivalents of Cu(NO₃)₂ and once with 2 equivalents of Cd(CH₃COO)₂ in methanol.

The Cu and Cd complexes were later decomposed and their Cu and Cd contents were analyzed by atomic absorption to determine the ratio of complex formation of Cu and Cd to ligand. The atomic absorption analysis showed the formation of complexes Cu:L and Cd:L in the ratio (1:1).

Scheme 1



a, R = CH(CH₃)₂; **b**, R = CH₂-CH₂-COOBn; **c**, R = CH₂-CH(CH₃)₂;
d, R = CH₂-Ph; **e**, R = CH₂-[4-(OBn)C₆H₄].

Table 1. Yields, Color, Mps., and Elemental Analyses of Compounds **7** and **8**.

Cmpd	Yield (%)	Color	mp (°C)	Elemental Analysis (Found)		
				C	H	N
7a	75	White	244	53.04(52.89)	6.68 (6.83)	15.46(15.62)
7b	84	White	155	58.24(58.39)	5.66 (5.89)	10.72(10.96)
7c	93	White	190	54.64(54.91)	7.05 (7.32)	14.70(14.95)
7d	83	White	238	60.09(59.84)	5.67 (5.92)	13.14(13.32)
7e	89	White	233	64.86(64.58)	5.68 (5.89)	9.87 (9.59)
8a *	77	White	290	-----	-----	-----
8b *	81	White	267	-----	-----	-----
8c *	78	White	253	-----	-----	-----
8d *	89	White	215	-----	-----	-----
8e *	96	White	197	-----	-----	-----

* = Elemental analysis was unreliable due to their hygroscopic nature.

Table 2. $^1\text{H-NMR}$ Data of Compounds **7** and **8**.

Compound	$^1\text{H-NMR}$ (DMSO- d_6): (δ) ppm
7a	0.89, 0.97 (2d, 6H, 2 CH ₃), 1.92 (m, 1H, CH), 4.89 (d, 1H, CH), 7.40-7.88 (m, 5H, aromatic), 8.38 (br.s, 2H, 2 NH), 10.64 (br.s, 2H, 2 NH).
7b	1.99 (m, 2H, CH ₂), 2.63, 2.75 (2m, 2H, CH ₂ -CO), 4.55 (m, 1H, CH), 5.14 m, 2H, CH ₂ -O), 7.21-7.90 (m, 10H, aromatic), 8.36 (br.s, 2H, 2 NH), 10.66 (br.s, 2H, 2 NH).
7c	0.91 (dd, 6H, 2 CH ₃), 1.63 (m, 2H, CH ₂), 1.81 (m, 1H, CH), 3.83 (m, 1H, CH), 7.46-7.87 (m, 5H, aromatic), 8.42 (br.s, 2H, 2 NH), 10.61 (br.s, 2H, 2 NH).
7d	3.07 (2m, 2H, CH ₂), 4.16 (m, 1H, CH), 7.22-7.89 (m, 10H, aromatic), 8.34 (br.s, 2H, 2 NH), 10.65 (br.s, 2H, 2 NH).
7e	3.08 (m, 2H, CH ₂), 4.29 (m, 1H, CH), 5.11 (br.s, 2H, CH ₂), 7.24-7.96 (m, 14H, aromatic), 8.45 (br.s, 2H, 2 NH), 10.59 (br.s, 2H, 2 NH).
8a	δ 0.87, 0.96 (2d, 6H, 2 CH ₃), 1.96 (m, 1H, CH), 4.01 (m, 1H, CH), 7.79 (d, 2H, aromatic), 8.40 (br.s, 2H, 2 NH), 8.78 (d, 2H, aromatic), 10.62 (br.s, 2H, 2 NH).
8b	2.03 (m, 2H, CH ₂), 2.35 (m, 2H, CH ₂ -CO), 4.09 (m, 1H, CH), 5.14 m, 2H, CH ₂ -O), 7.33-7.82 (m, 7H, aromatic), 8.44 (br.s, 2H, 2 NH), 8.77 (d, 2H, aromatic), 10.66 (br.s, 2H, 2 NH).
8c	δ 0.92 (d, 6H, 2 CH ₃), 1.59 (m, 2H, CH ₂), 1.79 (m, 1H, CH), 4.07 (m, 1H, CH), 7.79 (d, 2H, aromatic), 8.39 (br.s, 2H, 2 NH), 8.75 (d, 2H, aromatic), 10.71 (br.s, 2H, 2 NH).
8d	3.09 (m, 2H, CH ₂), 4.33 (m, 1H, CH), 7.25-7.39 (m, 5H, aromatic), 7.85 (d, 2H, aromatic), 8.43 (br.s, 2H, 2 NH), 8.79 (d, 2H, aromatic), 10.79 (br.s, 2H, 2 NH).
8e	3.04 (m, 2H, CH ₂), 4.27 (m, 1H, CH), 5.14 (br.s, 2H, CH ₂), 7.24-7.47 (m, 9H, aromatic), 7.82 (d, 2H, aromatic), 8.45 (br.s, 2H, 2 NH), 8.79 (d, 2H, aromatic), 10.62 (m, 2H, 2 NH);

Biological Test Results

The synthesized compounds **5**, **6**, **7** and **8** and their **Cu** and **Cd** complexes have been evaluated for their antimicrobial activity. The microdilution susceptibility test in Müller-Hinton Broth (Oxoid) and Sabouraud Liquid Medium (Oxoid) were used for the determination of antibacterial and antifungal activity [17]. The minimal inhibitory concentration (MIC) values listed in Table 3 show that all the test compounds have lower antifungal activity than clotrimazole (Canesten[®], Bayer).

Table 3. Minimal inhibitory concentration (MIC) of test compounds in µg/mL.

Test compound	E. coli	S. aureus	C. albicans	Test compound	E. coli	S. aureus	C. albicans
ampicillin	25	12.5	-----	(Cu:L),(1:1) of 6e	100	12.5	200
clotrimazole	-----	-----	12.5	(Cd:L),(1:1) of 5a	100	>200	>200
5a	>200	>200	>200	(Cd:L),(1:1) of 5b	>200	25	>200
5b	>200	>200	>200	(Cd:L),(1:1) of 5c	>200	>200	>200
5c	100	100	>200	(Cd:L),(1:1) of 5d	>200	25	>200
5d	50	>200	>200	(Cd:L),(1:1) of 5e	>200	>200	>200
5e	>200	>200	>200	(Cd:L),(1:1) of 6a	>200	>200	>200
6a	>200	>200	>200	(Cd:L),(1:1) of 6b	>200	100	>200
6b	100	>200	>200	(Cd:L),(1:1) of 6c	100	>200	>200
6c	50	>200	>200	(Cd:L),(1:1) of 6d	>200	>200	>200
6d	100	>200	>200	(Cd:L),(1:1) of 6e	100	>200	>200
6e	50	>200	>200	(Cu:L),(1:1) of 7a	>200	>200	>200
7a	100	100	>200	(Cu:L),(1:1) of 7b	>200	>200	>200
7b	25	>200	>200	(Cu:L),(1:1) of 7c	>200	100	>200
7c	100	50	>200	(Cu:L),(1:1) of 7d	>200	50	>200
7d	100	>200	>200	(Cu:L),(1:1) of 7e	>200	50	>200
7e	200	50	>200	(Cu:L),(1:1) of 8a	>200	>200	>200
8a	25	>200	>200	(Cu:L),(1:1) of 8b	>200	>200	>200
8b	100	100	>200	(Cu:L),(1:1) of 8c	>200	>200	>200
8c	50	>200	>200	(Cu:L),(1:1) of 8d	100	>200	>200
8d	100	100	>200	(Cu:L),(1:1) of 8e	>200	100	>200
8e	50	100	>200	(Cd:L),(1:1) of 7a	100	>200	>200
(Cu:L),(1:1) of 5a	200	100	>200	(Cd:L),(1:1) of 7b	>200	>200	>200
(Cu:L),(1:1) of 5b	>200	>200	100	(Cd:L),(1:1) of 7c	100	12.5	>200
(Cu:L),(1:1) of 5c	100	50	>200	(Cd:L),(1:1) of 7d	50	>200	>200
(Cu:L),(1:1) of 5d	>200	50	>200	(Cd:L),(1:1) of 7e	>200	>200	>200
(Cu:L),(1:1) of 5e	200	25	>200	(Cd:L),(1:1) of 8a	>200	>200	>200
(Cu:L),(1:1) of 6a	>200	100	>200	(Cd:L),(1:1) of 8b	100	>200	>200
(Cu:L),(1:1) of 6b	>200	>200	>200	(Cd:L),(1:1) of 8c	>200	>200	>200
(Cu:L),(1:1) of 6c	>200	>200	>200	(Cd:L),(1:1) of 8d	100	>200	>200
(Cu:L),(1:1) of 6d	200	100	>200	(Cd:L),(1:1) of 8e	>200	>200	>200

The test compounds are more active against *S. aureus* and *E. coli*. Compounds **(Cu:L),(1:1) of 6e** and **(Cd:L),(1:1) of 7c** have antimicrobial activity against *S. aureus* comparable to that of ampicillin, while the activity of compounds **(Cu:L),(1:1) of 5e**, **(Cd:L),(1:1) of 5b** and **(Cd:L),(1:1) of 5d** is about 50% of that of ampicillin. Compounds **7b** and **8a** have antimicrobial activity against *E. coli*

comparable to that of ampicillin, while the activity of compounds **5d**, **6c**, **6e**, **8c**, **8e**, (Cd:L),(1:1) of **7d** is about 50% of that of ampicillin.

Conclusions

The present work describes the synthesis of amino acid derivatives and its metal complexes which showed interesting results comparable to ampicillin. The uncomplexed compounds showed higher antimicrobial activity against *E. coli* compared to their respective complexes. While the complexes showed higher antimicrobial activity against *S. aureus* compared to their respective ligands. These results prompted us to further pursue in SAR as our future plane.

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Experimental

General

Melting points were determined on a Mel-Temp apparatus and are uncorrected. ¹H-NMR and ¹³C-NMR spectra were recorded on a Bruker 300 MHz spectrometer with chemical shift values reported in δ units (ppm) relative to an internal standard (tetramethylsilane). Infrared data were obtained on a Perkin-Elmer 1600 series Fourier transform instrument as KBr pellets. Amino acids are abbreviated and designated following the rules of the IUPAC-IUB Commission of Biochemical *Nomenclature* (*J. Biol. Chem.*, **1972**, *247*, 977). The abbreviations: HATU (N-[(dimethylamino)-1H-1,2,3-triazolo[4,5-b]pyridin-1-ylmethylene]-N-methyl-methanaminium hexafluorophosphate N-oxide); Boc (*t*-butyloxy-carbonyl); Et₃N (triethylamine) and DMF (N,N-dimethylformamide) were used throughout the manuscript. Follow up of the reactions and checking the homogeneity of the compounds were made by TLC on silica gel-protected aluminum sheets (Type 60 F254, Merck) and the spots were detected by exposure to UV-lamp at λ 254 nm for few seconds. Elemental analyses were performed in the Chemistry Department, Faculty of Science, Cairo, University.

Chemistry

General Procedure for the Reaction of N-Boc-L-Amino Acids with Benzoic Acid Hydrazide.

A mixture of *N*-Boc-*L*-amino acid (1 mmol), HATU (0.38 g, 1 mmol) and Et₃N (0.28 mL, 2 mmol) was stirred at 0°C for 3 minutes in DMF (2 mL). Then benzoic acid hydrazide (**3**, 0.122 g, 1 mmol)

was added. The reaction mixture was stirred at 0°C for 1 hour and left overnight at room temperature. The reaction mixture was diluted with ethyl acetate (80 mL) and the mixture was washed successively with 5% aqueous citric acid solution (2×10 mL), saturated sodium bicarbonate solution (2×10 mL) and saturated sodium chloride solution (2×10 mL). The organic layer was dried over anhydrous sodium sulphate, filtered and the solvent was removed *in vacuo*. The crude product was crystallized from methylene chloride-hexane. In the case of *N*-Boc L-(*O*-benzyl)-tyrosine (**5e**) and *N*-Boc-L-phenylalanine (**5d**) the reaction mixture was poured into ice water, filtered, washed with 5% aqueous citric acid solution, saturated sodium bicarbonate solution and finally with water, dried and recrystallized from methylene chloride-hexane.

Boc-valyl(N-benzoyl)-hydrazide (**5a**): White crystals, 0.32 g (95.4 % yield); mp 156-157°C; IR: 3308, 3226 (NH), 1715 (C=O, urethane), 1689 (C=O, amide), 1668 (C=O, amide) cm⁻¹; ¹H-NMR (CDCl₃): δ 0.87, 0.95 (2d, 6H, 2 CH₃), 1.35 (s, 9H, 3 CH₃), 1.88 (m, 1H, CH), 4.93 (d, 1H, CH), 7.20-7.77 (m, 6H, 1 NH, aromatic), 9.17, 9.34 (2 br.s, 2H, 2 NH); ¹³C-NMR (CDCl₃): δ 19.61, 19.70, 26.40, 28.62, 50.49, 80.69, 127.40, 129.00, 129.71, 131.79, 132.70, 136.58, 156.00, 164.62, 169.01; *Anal.* Calcd for C₁₇H₂₅N₃O₄: C, 60.88; H, 7.51; N, 12.53. Found: C, 60.72; H, 7.68; N, 12.81.

Boc-(o-benzyl)-glutamyl(N-benzoyl)-hydrazide (**5b**): White powder, 0.38 g (80 % yield); mp 126°C; IR: 3336, 3253 (NH), 1753 (C=O, ester), 1727 (C=O, urethane), 1686 (C=O, amide), 1663 (C=O, amide) cm⁻¹; ¹H-NMR (CDCl₃): δ 1.37 (s, 9H, 3 CH₃), 1.90, 2.05 (2m, 2H, CH₂), 2.60, 2.71 (2m, 2H, CH₂-CO), 4.50 (m, 1H, CH), 5.15 m, 2H, CH₂-O), 7.20-7.81 (m, 11H, 1 NH, aromatic), 9.17, 9.34 (2 br.s, 2H, 2 NH); ¹³C-NMR (CDCl₃): δ 24.96, 28.16, 28.67, 49.70, 66.94, 80.72, 127.79, 128.65, 128.94, 128.95, 129.02, 131.87, 132.69, 136.10, 136.16, 153.64, 165.20, 169.82, 173.20; *Anal.* Calcd for C₂₄H₂₉N₃O₆: C, 63.28; H, 6.42; N, 9.22. Found: C, 63.02; H, 6.21; N, 8.96.

Boc-leucyl(N-benzoyl)-hydrazide (**5c**): White crystals, 0.26 g (74 % yield); mp 147°C; IR: 3318, 3234 (NH), 1726 (C=O, urethane), 1689 (C=O, amide), 1672 (C=O, amide) cm⁻¹; ¹H-NMR (DMSO-d₆): δ 0.91 (d, 6H, 2 CH₃), 1.38 (s, 9H, 3 CH₃), 1.51 (m, 2H, CH₂), 1.72 (m, 1H, CH), 4.12 (m, 1H, CH), 6.85 (d, 1H, 1 NH), 7.2-7.77 (m, 5H, aromatic), 10.10, 10.40 (2 br.s, 2H, 2 NH); ¹³C-NMR (DMSO-d₆): δ 21.88, 23.00, 23.29, 24.47, 28.54, 51.64, 79.20, 126.00, 127.50, 131.65, 153.65, 156.11, 171.15; *Anal.* Calcd for C₁₈H₂₇N₃O₄: C, 61.87; H, 7.79; N, 12.03. Found: C, 62.05; H, 7.92; N, 12.26.

Boc-phenylalanyl(N-benzoyl)-hydrazide (**5d**): White powder, 0.31 g (81 % yield); mp 168°C; IR: 3314, 3233 (NH), 1725 (C=O, urethane), 1692 (C=O, amide), 1673 (C=O, amide) cm⁻¹; ¹H-NMR (DMSO-d₆): δ 1.30 (s, 9H, 3 CH₃), 2.82, 3.08 (2m, 2H, CH₂), 4.30 (m, 1H, CH), 7.04 (d, 1H, 1 NH), 7.18-7.92 (m, 10H, aromatic), 10.18, 10.29 (2 br.s, 2H, 2 NH); ¹³C-NMR (DMSO-d₆): δ 28.50, 37.68, 54.85, 126.58, 127.80, 128.40, 128.81, 129.59, 132.18, 153.62, 164.9, 173.10; *Anal.* Calcd for C₂₁H₂₅N₃O₄: C, 65.78; H, 6.57; N, 10.96. Found: C, 65.98; H, 6.78; N, 10.71.

Boc-(o-benzyl)-tyrosyl(N-benzoyl)-hydrazide (**5e**): White crystals, 0.47 g (96 % yield); mp 177°C; IR: 3322, 3245 (NH), 1726 (C=O, urethane), 1693 (C=O, amide), 1673 (C=O, amide) cm⁻¹; ¹H-NMR (DMSO-d₆): δ 1.31 (s, 9H, 3 CH₃), 2.85, 3.05 (2m, 2H, CH₂), 4.25 (m, 1H, CH), 5.05 (br.s, 2H, CH₂), 6.92-7.93 (m, 15H, aromatic, 1NH), 10.15, 10.46 (2s, 2H, 2 NH); ¹³C-NMR (DMSO-d₆): δ 26.52,

32.01, 53.48, 68.50, 77.52, 112.81, 125.90, 126.02, 126.81, 128.69, 130.21, 135.6, 153.62, 155.04, 163.81, 170.00; *Anal.* Calcd for C₂₈H₃₁N₃O₅: C, 68.69; H, 6.38; N, 8.58. Found: C, 68.51; H, 6.15; N, 8.73.

General Procedure for the Reaction of N-Boc-L-Amino Acids with Nicotinic Acid Hydrazide.

A mixture of N-Boc-L-amino acid (1 mmol), HATU (0.38 g, 1 mmol) and Et₃N (0.15 mL, 1 mmol) was stirred at 0°C for 3 minutes in DMF (2 mL). Then nicotinic acid hydrazide (**4**, 0.123 g, 1 mmol) was added. The reaction mixture was stirred at 0°C for 1 hour and left overnight at room temperature. The reaction mixture was diluted with methylene chloride (80 mL) and the mixture was washed with 5% aqueous citric acid solution (2×10 mL), saturated sodium bicarbonate solution (2×10 mL) and saturated sodium chloride solution (2×10 mL). The organic layer was dried over anhydrous sodium sulphate, filtered and the solvent was removed *in vacuo*. The crude product was crystallized from methylene chloride-hexane.

Boc-valyl(N'-nicotinoyl)-hydrazide (6a): White crystals, 0.28 g (83.3 % yield); mp 209°C; IR: 3344, 3242 (NH), 1726 (C=O, urethane), 1708 (C=O, amide), 1661 (C=O, amide) cm⁻¹; ¹H-NMR (DMSO-d₆): δ 0.86, 0.94 (2d, 6H, 2 CH₃), 1.39 (s, 9H, 3 CH₃), 1.96 (m, 1H, CH), 3.93 (m, 1H, CH), 6.81 (d, 1H, 1 NH), 7.77 (d, 2H, aromatic), 8.75 (d, 2H, aromatic), 10.12, 10.68 (2 br.s, 2H, 2 NH); ¹³C-NMR (DMSO-d₆): δ 18.7, 19.5, 28.6, 30.7, 58.7, 78.4, 121.7, 139.8, 150.7, 155.7, 164.1, 171.1; *Anal.* Calcd for C₁₆H₂₄N₄O₄: C, 57.13; H, 7.19; N, 16.66. Found: C, 57.34; H, 7.35; N, 16.41.

Boc-(o-benzyl)-glutamyl(N'-nicotinoyl)-hydrazide (6b): White powder, 0.42 g (92 % yield); mp 120°C; IR: 3334, 3243 (NH), 1740 (C=O, ester), 1727 (C=O, urethane), 1681 (C=O, amide) cm⁻¹; ¹H-NMR (DMSO-d₆): δ 1.38 (s, 9H, 3 CH₃), 1.84, 2.04 (2m, 2H, CH₂), 2.32 (m, 2H, CH₂-CO), 4.07 (m, 1H, CH), 5.14 m, 2H, CH₂-O), 7.02 (m, 1H, 1 NH), 7.34 (br.s, 5H, aromatic), 7.77 (d, 2H, aromatic), 8.75 (d, 2H, aromatic), 10.13, 10.66 (2 br.s, 2H, 2 NH); ¹³C-NMR (DMSO-d₆): δ 26.6, 28.3, 30.0, 53.6, 66.2, 78.7, 121.6, 128.1, 128.4, 128.5, 128.7, 136.3, 139.7, 150.8, 156.0, 164.3, 171.1, 172.6, 175.8; *Anal.* Calcd for C₂₃H₂₈N₄O₆: C, 60.52; H, 6.18; N, 12.27. Found: C, 60.37; H, 6.32; N, 12.45.

Boc-leucyl(N'-nicotinoyl)-hydrazide (6c): White crystals, 0.25 g (71.3 % yield); mp 210°C; IR: 3301, 3222 (NH), 1710 (C=O, urethane), 1662 (C=O, amide) cm⁻¹; ¹H-NMR (DMSO-d₆): δ 0.91 (d, 6H, 2 CH₃), 1.38 (s, 9H, 3 CH₃), 1.53 (m, 2H, CH₂), 1.72 (m, 1H, CH), 4.13 (m, 1H, CH), 6.85 (d, 1H, 1 NH), 7.77 (d, 2H, aromatic), 8.71 (d, 2H, aromatic), 10.10, 10.70 (2 br.s, 2H, 2 NH); ¹³C-NMR (DMSO-d₆): δ 21.88, 23.00, 23.29, 24.47, 28.54, 51.64, 78.75, 121.64, 139.77, 150.48, 155.55, 163.97, 172.38; *Anal.* Calcd for C₁₇H₂₆N₄O₄: C, 58.27; H, 7.48; N, 15.99. Found: C, 58.02; H, 7.21; N, 16.26.

Boc-phenylalanyl(N'-nicotinoyl)-hydrazide (6d): White powder, 0.29 g (75.4 %) yield, mp 145°C; IR: 3309, 3234 (NH), 1726 (C=O, urethane), 1695 (C=O, amide), 1677 (C=O, amide) cm⁻¹; ¹H-NMR (DMSO-d₆): δ 1.29 (s, 9H, 3 CH₃), 2.82, 3.07 (2m, 2H, CH₂), 4.31 (m, 1H, CH), 7.05-7.37 (m, 6H, aromatic, 1 NH), 7.81 (d, 2H, aromatic), 8.76 (d, 2H, aromatic), 10.33, 10.82 (2 br.s, 2H, 2 NH); ¹³C-NMR (DMSO-d₆): δ 28.12, 28.48, 37.82, 54.87, 78.4, 121.68, 126.62, 128.42, 129.57, 138.30, 139.70,

150.77, 155.67, 164.18, 171.70; *Anal.* Calcd for C₂₀H₂₄N₄O₄: C, 62.49; H, 6.29; N, 14.57. Found: C, 62.65; H, 6.50; N, 14.83.

Boc-(o-benzyl)-tyrosyl(N'-nicotinoyl)-hydrazide (6e): White crystals, 0.38 g (77.5 %) yield, mp 141°C; IR: 3311, 3237 (NH), 1727 (C=O, urethane), 1695 (C=O, amide), 1677 (C=O, amide) cm⁻¹; ¹H-NMR (DMSO-d₆): δ 1.33 (s, 9H, 3 CH₃), 2.83, 3.05 (2m, 2H, CH₂), 4.26 (m, 1H, CH), 5.10 (br.s, 2H, CH₂), 6.96-7.47 (m, 10H, aromatic, 1NH), 7.83 (d, 2H, aromatic), 8.78 (d, 2H, aromatic), 10.36, 10.63 (2m, 2H, 2 NH); ¹³C-NMR (DMSO-d₆): δ 28.20, 28.54, 37.03, 55.19, 69.50, 78.38, 114.77, 121.70, 127.99, 128.77, 130.63, 137.60, 140.06, 150.72, 155.68, 157.32, 164.08, 171.64; *Anal.* Calcd for C₂₇H₃₀N₄O₅: C, 66.11; H, 6.16; N, 11.42. Found: C, 65.82; H, 5.95; N, 11.59.

General Procedure for the Preparation of the Hydrochloride Salts 7 and 8.

5 or **6** (1 mmol) were dissolved in a mixture of methylene chloride (4 mL) and ether (4 mL). HCl gas was passed through the solution for 2 hours. A white precipitate that formed was filtered and washed with anhydrous ether. The crude product was recrystallized from methyl alcohol-anhydrous ether to give white crystals. The amino acid-(*N*-nicotinoyl) hydrazide hydrochloride salts **8a-8e** were obtained as hygroscopic salts.

General Procedure for the Preparation of the Cu Complexes.

A solution of Cu(NO₃)₂ (0.48 gm, 2 mmol) in methyl alcohol (2 mL) was added to a solution of **5**, **6**, **7** or **8** (1 mmol) in methyl alcohol (4 mL). A change was observed in the color of the solution. The Cu complexes were obtained as green crystals. The Cu complexes were later decomposed and their Cu content was analyzed by atomic absorption to determine the ratio of complex formation of Cu to ligand. The atomic absorption analysis showed the formation of complexes Cu:L in the ratio (1:1).

General Procedure for the Preparation of the Cd Complexes

A solution of Cd(CH₃COO)₂ (0.46 gm, 2 mmol) in methyl alcohol (2 mL) was added to a solution of **5**, **6**, **7** or **8** (1 mmol) in methyl alcohol (4 mL). The Cd complexes were obtained as white crystals. The Cd complexes were later decomposed and their Cd content was analyzed by atomic absorption to determine the ratio of complex formation of Cd to ligand. The atomic absorption analysis showed the formation of complexes Cd:L in the ratio (1:1).

In vitro antimicrobial activity

The microdilution susceptibility test in Müller-Hinton Broth (Oxoid) and Sabouraud Liquid Medium (Oxoid) were used for the determination of antibacterial and antifungal activity [17, 18]. The utilized test organisms were: *Escherichia coli* (*E. coli*) ATCC 25922 as an example of Gram-negative bacteria, *Staphylococcus aureus* (*S. aureus*) ATCC 19433 as an example of Gram-positive bacteria and *Candida albicans* (*C. albicans*) as yeast-like fungi. Ampicillin trihydrate and clotrimazole were used as standard antibacterial and antifungal agents, respectively. Solutions of the test compounds,

ampicillin trihydrate and clotrimazole were prepared in DMSO to a concentration of 1600 µg/mL. Twofold dilutions of the compounds were prepared (800, 400, ... 6.25 µg/mL). Microorganism suspensions at 10⁶ CFU/mL (Colony Forming Unit/mL) concentrations were inoculated to the corresponding wells. Plates were incubated at 36°C for 24 h to 48 h. The incubation chamber was kept sufficiently humid. At the end of the incubation period, the minimal inhibitory concentrations (MIC) were determined.

References

1. Barrett, D.; Tanaka, A.; Harada, K.; Ohki, H.; Watabe, E.; Maki, K.; Ikeda, F. Synthesis and biological activity of novel macrocyclic antifungals: acylated conjugates of the ornithine moiety of the lipopeptidolactone FR901469. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 479-482.
2. Kovalainen, J. T.; Christians, J. A. M.; Kotisaari, S.; Laitinen, J. T.; Mannisto P.T. Synthesis and in vitro pharmacology of a series of new chiral histamine H3-receptor ligands: 2-(R and S)-amino-3-(1H-imidazol-4(5)-yl)propyl ether derivatives. *J. Med. Chem.* **1999**, *42*, 1193-202.
3. El-Faham, A.; El Massry, A. M.; Amer, A.; Gohar, Y. M., A versatile synthetic route to chiral quinoxaline derivatives from amino acids precursors. *Lett. Pept. Sci.* **2002**, *9*, 49-54.
4. a) Polyak, F.; Lubell, W. D. Rigid dipeptide mimics: Synthesis of enantiopure 5- and 7-benzyl, and 5,7-dibenzyl indolizidinone amino acids via enolization and alkylation of δ-oxo α,ω-di-[N-(9-(9-phenylfluorenyl))amino]azelaate esters. *J. Org. Chem.* **1998**, *63*, 5937-5949; b) Roy, S.; Lombart, H.-G.; Lubell, W. D.; Hancock, R. E. W.; Farmer, S. W. Exploring relationships between mimic configuration, peptide conformation and biological activity in indolizidin-2-one amino acid analogs of gramicidin S. *J. Peptide Res.* **2002**, *60*, 198-214.
5. Okada, Y.; Tsukatani, M.; Taguchi, H.; Yokoi, T.; Bryant, S. D.; Lazarus, L. H. Amino acids and peptides. Design and synthesis of opioidmimetics containing pyrazinone ring and examination of their opioid receptor binding activity. *Chem. Pharm. Bull.* **1998**, *46*, 1374-82.
6. Sun, G.; Uretsky, N. J.; Wallace, L. J.; Shams, G.; Weinstein, D. M.; Miller, D. D. Synthesis of chiral 1-(2'-amino-2'-carboxyethyl)-1,4-dihydro-6,7-quinoxaline-2,3-diones: α-amino-3-hydroxy-5-methyl-4-isoxazole propionate receptor agonists and antagonists. *J. Med. Chem.* **1996**, *39*, 4430-38.
7. Marsham, P. R.; Wardleworth, J. M.; Boyle, F. T., Hennequin, L. F.; Kimbell, R.; Brown, M.; Jackman, A. L. Design and synthesis of potent nonpolyglutamatable quinoxaline antifolate thymidylate synthase inhibitors. *Med. Chem.*, **1999**, *42*, 3809-20.
8. Xia, Y.; Yang, Z.-Y.; Xia, P.; Bastow, K. F.; Nakanishi, Y.; Lee, K.-H. Antitumor agents. Part 202: Novel 2'-amino chalcones: design, synthesis and biological evaluation. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 699-701.
9. Kucukguzel, S. G.; Mazi, A.; Sahin, F.; Ozturk, S.; Stables J. Synthesis and biological activities of diflunisal hydrazide-hydrazones. *Eur. J. Med. Chem.* **2003**, *38*, 1005-1013.
10. Hassan, H. M.; Shedid, S. A. M. Synthesis and antimicrobial activity of some new Mannich bases, N-(2-hydroxy-1-naphthyl) amino acid, methyl ester and hydrazide derivatives. *J. Serb. Chem. Soc.* **1998**, *63*, 125-130.
11. Dogan, H. N.; Rollas, S.; Erdeniz, H., Synthesis, structure elucidation and antimicrobial activity of some 3-hydroxy-2-naphthoic acid hydrazide derivatives. *Farmaco* **1998**, *53*, 462-7.

12. Cesur, Z.; Büyüktimkin, S.; Büyüktimkin, N.; Derbentli, T. Synthesis and antimicrobial evaluation of some arylhydrazones of 4-[(2-methylimidazo[1,2-a]pyridine-3-yl)azo]benzoic acid hydrazine. *Arch. Pharm. (Weinheim)* **1990**, *323*, 141-4.
13. Cocco, M. T.; Congiu, C.; Onnis, V.; Pusceddu, M. C.; Schivo, M. L.; De Logu, A. Synthesis and antimycobacterial activity of some isonicotinoylhydrazones. *Eur. J. Med. Chem.* **1999**, *34*, 1071–6.
14. Uğur, A.; Mercimek, B.; Özler, M. A.; Şahin, N. Antimicrobial effects of bis(Δ^2 -2-imidazoliny)-5,5'-dioxime and its mono- and tri-nuclear complexes. *Trans. Met. Chem.* **2000**, *25*, 421-25.
15. Chohan, Z. H.; Farooq, M. A.; Scozzafava, A.; Supuran, C. T. Antibacterial Schiff bases of oxalyl-hydrazine/ diamide incorporating pyrrolyl and salicylyl moieties and their zinc(II) complexes. *J. Enz. Inhib. Med. Chem.* **2002**, *17*, 1-7.
16. Wu, G.; Wang, G.; Fu, X.; Zhu, L. Synthesis, crystal structure, stacking effect and antimicrobial studies of novel quaternary copper(II) complex with quinolone. *Molecules*, **2003**, *8*, 287-296.
17. Henderson B. In *Textbook of Immunopharmacology*, 3rd ed; Dale M. M.; Foreman J. C.; Fan T-P. D. (eds), Blackwell Scientific Publications: London, **1994**; pp. 16, 193.
18. Murray, P. R.; Baron, E. J.; Pfaller, M. A.; F. C. Tenover; Tenover, R. H. Manual of clinical microbiology. In: *Antimicrobial Agents and Susceptibility Testing*; Woods, G. L.; Washington J. A. (Eds.); American Society for Microbiology: Washington, DC, **1995**.

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