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Antimicrobial Activities of some Synthesized Cyclo (N^{α}-dinicotinoyl) [L-phenylalanyl-L-leucine]Pentapeptide Candidates

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Some of cyclo (N^{α}-di-nicotinoyl)[L-phenylalanyl-L-leucine]pentapeptides**3-6** has been synthesized starting from bis-(1-carboxy-2-substituted)-3,5-diaminocarbonyl)pyridine **3** and N,N-bis-(1-hydrazonyl-2-substituted)-3,5 diaminocarbonyl) pyridine **2**. Treatment of **1** or **2** afforded the corresponding bis-ester derivative **3**, which was hydrolyzed with sodium hydroxideto give bis-acid **4**. Cyclization of bis-acid **4** with L-dibasic amino acid methyl esters, afforded the corresponding macrocyclic methyl esters **5a,b**, respectively. Finally, hydrazonolysis of **5a,b** with hydrazine hydrated in refluxing methanol afforded macrocyclic pentapeptide hydrazides **6a,b**, respectively. Some of these compounds exhibited antimicrobial activities comparable with Chloramphenicol and Fusidic acid as reference drugs.

Key words: Linear dipeptide pyridine, Macrocyclic pentaazapyridine, Antimicrobial agents

Introduction

Nicotinic acid and nicotinamide are similar in their vitamin activity. However, some of the new heterocyclic and peptide derivatives have been studied with respect to antiviral[1], anti-inflammatory[2], enzymatic peptide[3], antimicrobial activities[4,5] and there used as an antimicrobial for therapeutic applications[6]. On the other hand, some of reported macrocyclic peptides derivatives were synthesized from pyridinedicarboxylic acids with selected amino acids and evaluated of their biological and pharmacological activities[7-10]. The small molecules of peptide derivatives have been anticancer activity and effective against cancerous cells by either membranolytic mechanism or disruption of mitochondria[11-13]. Also, the synthesis of macrocyclic and complexing properties of azacrown compounds has been a subject of intensive exploration and existing antibacterial agents[14-18]. In view of these observations and as continuation of our previous works[7-10] in macrocyclic and heterocyclic chemistry, we have synthesized some new cyclo (Nα-di-nicotinoyl)[L-phenylalanyl-L-leucine]pentapeptides and they are tested as antimicrobial agents.

Materials and methods

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 (KBr) was recorded on a Nexus 670 FTIR Nicolet, Fourier Transform infrared spectrometer. Proton and carbon nuclear magnetic resonance (^{1}H- and ^{13}C NMR) spectra were run in (DMSO-d_6) on Jeol 500 MHz instruments. Mass spectra were run on a MAT Finnigan SSQ 7000 spectrometer (Shimadzu, Kyoto, Japan; Model: QP2010 ultra), using the electron impact technique (EI).

Synthesis of dimethyl 2,2'-((2,2'-((pyridine-3, 5-dicarbonyl) bis(azanediyl))bis(3-phenyl-propanoyl))bis(azanediyl))bis(4-methylpentanoate) **(3).** *Mixed anhydride[A]:* To a mixture of diacid**1** (1 mmol)

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and ethyl chloroformate (22g, 2 mmol) in dichloromethane (25 mL) at -20°C, triethylamine (0.2g, 2 mmol) was added, and stirred for 20 min., and then L-leucine methyl ester (2 mmol) was added at the same temperature. The reaction mixture was stirred at (-20°C) for 6 hrs and then overnight at room temperature. The obtained mixture was washed with H_2O, 1N HCl, 1N Na_2CO_3 and water then dried over anhydrous calcium chloride. The solvent was evaporated, and the crude product was purified by crystallization from ethanol/n-hexane to give the ester derivative**3** in 86% yield.

*Azid method [B]:*A mixture of the dihydrazide**2** (1 mmol) in 5 N HCl (1.2 mL) with glacial acetic acid (2.4 mL) and water (10 mL) was stirred for 10 min at -5 ◦C, then $NaNO_2$ (0.138 g, 2 mmol in 6 mL of water) was added and the mixture was stirred for 30 min. The obtained azide was extracted with dichloromethane (120 mL), washed with cold water, 3% aqueous sodium bicarbonate followed by cold water, and dried over anhydrous calcium chloride. The obtained azide solution was added in one portion to a cold solution (-5 ◦C) of the L-lucine methyl ester (2 mmol) in dry dichloromethane (25 mL). The reaction mixture was stirred at the same temperature for 3 h, then overnight at r.t., washed with 1 N hydrochloric acid, 1N aqueous sodium bicarbonate, water and dried over anhydrous calcium chloride. The solvent was distilled off to give the corresponding 3,5-bis-ester derivative **3** in 65% yield, as identified by melting point and TLC in comparison with authentic sample prepared according to method A. m.p. 190-192 °C. $[\alpha]_D^{25}$ = - 95 (c = 0.5, MeOH). IR (film): ν = 3385 (NH), 1749 (C=O, ester), 1655, 1536, 1254 (3C=O, amides) cm^{-1}. ^1H NMR: δ = 0.85-1.02 (m, 12H, $4CH_3$), 1.62-1.68 (m, 4H, $2CH_2$), 2.35-2.39 (m, 2H, 2CH), 3.44 (d, 4H, $2CH_2$), 3.69 (s, 6H, $2OCH_3$), 4.12-4.25 (m, 2H, 2CH), 4.56-4.60 (m, 2H, 2CH), 6.98-7.46 (m, 10H, 2Ph-H), 8.40, 9.10 (2s, 3H, pyr-H) and 8.70, 8.88 (2s, 4H, 4NH, exchangeable with D_2O). ^{113}C NMR: δ = 20.32, 20.48, 22.98, 38.14, 40.62, 50.66, 52.75, 54.36, 125.25, 127.86, 129.00, 138.78, 132.00, 139.88, 152.04, 165.48, 168.76, 172.45 (39C).MS (EI, 70 eV): m/z (%) = 715 (95) $[M^+]$. $C_{39}H_{49}N_5O_8$ (715.84): Calcd: C 65.44, H 6.90, N 9.78; found: C 65.35, H 6.76, N 9.72.

Synthesis of 2,2'-((2,2'-((pyridine-3,5-dicarbonyl) bis(azanediyl))bis(3-phenylpropanoyl))bis-(azanediyl)) bis (4-methylpentanoic acid) **(4).** Sodium hydroxide (1N, 25 mL) was added to cold methanolic solution of the tetrapeptide ester **(3)** (1 mmol) with stirring at -5 °C. The reaction mixture was stirred for 2 h at the same temperature then for 3 h at room temperature. The solvent was distilled off, the aqueous solution was cooled and acidified with 1 N hydrochloric acid to pH ~ 3. The obtained solid was filtered off, washed with water, dried and crystallized from ethanol/water to give to give 3,5-bis acid derivative **4**. In 94% yield; m.p. 246-248 °C.$[\alpha]_D^{25}$ = **-** 105(c = 0.5, MeOH). IR (film): ν = 4568-3384 (OH, NH), 1723 (C=O, acid), 1658, 1535, 1255 (3C=O, amides) cm^{-1}. ^1H NMR: δ = 0.90-0.96 (m, 12H, $4CH_3$), 1.66-1.72 (m, 4H, $2CH_2$), 2.16-2.34 (m, 2H, 2CH), 3.48 (d, 4H, $2CH_2$), 4.28-4.32 (m, 2H, 2CH), 4.35-4.45 (m, 2H, 2CH), 7.08-7.46 (m, 10H, 2Ph-H), 8.51, 9.04 (m, 3H, pyr-H), 8.74, 8.90 (2s, 4H, 4NH, exchangeable with D_2O), 11.55 (s, 2H, 2OH, exchangeable with D_2O). ^{13}C NMR: δ = 21.05, 20.48, 22.88, 37.98, 40.50, 50.60, 52.70, 125.36, 127.84, 129.08, 138.86, 131.95, 139.92, 152.15, 166.50, 169.74, 174.32 (37C).MS (EI, 70 eV): m/z (%) = 688 (76) $[M^+]$. $C_{37}H_{45}N_5O_8$ (687.78): Calcd: C 64.61, H 6.59, N 10.18; found: C 64.45, H 6.52, N 10.14.

Synthesis of 3,5-pyridine-cyclic [L-phenylalanyl-L-leucine]pentapeptide methyl esters **(5a,b).** The same procedure which was used in synthesized compound **3**, by using compound **2** instead of compound **1**.

Methyl 4,19-dibenzyl-7,16-diisobutyl-2,5,8,15,18,21-hexaoxo-3,6,9,14,17,20-hexaaza-1(3,5)-pyridinacyclo-henicosaphane-10-carboxylate **(5a)**. Yield 72%; m.p. 202-204 °C. $[\alpha]_D^{25}$ = - 112 (c = 0.5, MeOH). IR (film): ν = 3388 (NH), 1752 (C=O, ester), 1655, 1538, 1254 (3C=O, amides) cm^{-1}. ^1H NMR: δ = 0.86-0.92 (m, 12H, $4CH_3$), 1.25-1.46 (m, 4H, $2CH_2$), 1.58-1.76 (m, 4H, 2 CH_2), 2.18-2.34 (m, 2H, 2CH), 3.02-3.22 (m, 2H, CH_2), 3.34 (d, 4H, $2CH_2$), 3.53 (s, 3H, OCH_3), 3.86-4.08 (m, 4H, 4CH), 4.36-4.46 (m, 1H, CH), 7.10-7.34 (m, 10H, 2Ph-H),8.45, 9.00 (2s, 3H, pyr-H), 8.84, 8.96, 9.20 (3s, 6H, 6NH, exchangeable with D_2O). ^{13}C NMR: δ = 20.85, 21.24, 23.05, 28.56, 30.48, 37.95, 37.80, 41.10, 50.98, 52.69, 53.66, 60.50, 125.42, 127.54, 128.00, 139.12, 132.10, 140.05, 152.10, 166.74, 170.14, 172.85 (43C).MS (EI, 70 eV): m/z (%) = 798 (58) $[M^+]$. $C_{43}H_{55}N_7O_8$ (797.94): Calcd: C 64.72, H 6.95, N 12.29; Found: C 64.58, H 6.80, N 12.22.

Methyl 4,20-dibenzyl-7,17-diisobutyl-2,5,8,16,19,22-hexaoxo-3,6,9,15,18,21-hexaaza-1(3,5)-pyridinacyclo-docosaphane-10-carboxylate **(5b).** Yield 65%; m.p. 190-192 °C. $[\alpha]_D^{25}$ = - 98 (c = 0.5, MeOH). IR (film):

ν = 3362 (NH), 1746 (C=O, ester), 1656, 1534, 1252 (3C=O, amides) cm^{-1}. ^1H NMR: δ = 0.88-0.94 (m, 12H, $4CH_3$), 1.23-1.46 (m, 4H, $2CH_2$), 1.60-1.75 (m, 4H, 2 CH_2), 2.28-2.35 (m, 2H, 2CH), 3.00-3.26 (m, 4H, 2 CH_2), 3.38 (d, 4H, $2CH_2$), 3.56 (s, 3H, OCH_3), 3.96-4.04 (m, 4H, 4CH), 4.40-4.45 (m, 1H, CH), 7.10-7.48 (m, 10H, 2Ph-H), 8.45, 9.00 (2s, 3H, pyr-H), 8.86, 8.96, 9.20 (3s, 6H, 6NH, exchangeable with D_2O). ^{13}C NMR: δ = 20.75, 21.18, 23.02, 22.60, 28.72, 30.52, 43.90, 37.92, 41.15, 51.02, 52.74, 53.70, 60.52, 125.40, 127.50, 128.04, 139.16, 132.12, 140.00, 152.14, 167.00, 170.02, 173.12 (44C). MS (EI, 70 eV): *m/z* (%) = 812 (62) [M^+]. $C_{44}H_{57}N_7O_8$ (811.97): Calcd: C 65.09, H 7.08, N 12.08; Found: C 64.97, H 7.01, N 11.98.

Synthesis of 3,5-pyridine cyclic [L-phenylalanyl-L-leucine]pentapeptide hydrazides **(6a,b).** A mixture of pentapeptide esters**5a,b**(1 mmol) and hydrazine hydrate (0.2 mL, 4 mmol) in absolute methanol (25 mL) was refluxed for 6 h. The solvent was evaporated to dryness, the obtained residue was washed with n-hexane. The obtained solid was crystallized from methanol to give cyclic [L-phenylalanyl-L-leucine]pentapeptide hydrazides**6a,b**, respectively.

4,19-Dibenzyl-7,16-diisobutyl-2,5,8,15,18,21-hexaoxo-3,6,9,14,17,20-hexaaza-1(3,5)-pyridinacyclo henicosaphane-10-carbohydrazide **(6a).** Yield 72%; m.p. 248-250 °C. $[\alpha]^{25}_{D}$ = - 102 (*c* = 0.5, MeOH). IR (film): ν = 3390-3370 (NH, NH_2), 1654, 1536, 1255 (3C=O, amides) cm^{-1}. ^1H NMR: δ = 0.84-0.92 (m, 12H, $4CH_3$), 1.24-1.45 (m, 4H, $2CH_2$), 1.62-1.74 (m, 4H, 2 CH_2), 2.26-2.34 (m, 2H, 2CH), 3.10-3.18 (m, 2H, CH_2), 3.35 (d, 4H, $2CH_2$), 3.86-4.00 (m, 4H, 4CH), 4.23 (s, 2H, NH_2), 4.38-4.45 (m, 1H, CH), 7.10-7.34 (m, 10H, 2Ph-H),8.40, 9.08 (2s, 3H, pyr-H), 9.10 (s, 1H, CONH, D_2O exchangeable), 8.82, 8.95, 9.18 (3s, 6H, 6NH, exchangeable with D_2O). ^{13}C NMR: δ = 20.68, 21.42, 23.22, 25.56, 28.48, 32.84, 37.62, 40.65, 50.70, 52.85, 54.90, 125.70, 126.96, 128.08, 139.00, 131.87, 139.65, 151.90, 169.78, 170.75, 170.12 (42C). MS (EI, 70 eV): *m/z* (%) = 798 (65) [M^+]. $C_{42}H_{55}N_9O_7$ (797.94): Calcd: C, 63.22; H, 6.95; N, 15.80; Found: C, 63.00, H, 6.85, N, 15.72.

4,20-Dibenzyl-7,17-diisobutyl-2,5,8,16,19,22-hexaoxo-3,6,9,15,18,21-hexaaza-1(3,5)-pyridinacyclo docosaphane-10-carbohydrazide **(6b).** Yield 65%; m.p. 265-267 °C. $[\alpha]^{25}_{D}$ = - 115 (*c* = 0.5, MeOH). IR (film): ν = 3420-3394 (NH, NH_2), 1650, 1534, 1252 (3C=O, amides) cm^{-1}. ^1H NMR: δ = 0.86-0.92 (m, 12H, $4CH_3$), 1.25-1.46 (m, 4H, $2CH_2$), 1.58-1.74 (m, 4H, 2 CH_2), 2.28-2.36 (m, 2H, 2CH), 3.00-3.24 (m, 4H, $2CH_2$), 3.35 (d, 4H, $2CH_2$), 3.90-4.05 (m, 4H, 4CH), 4.30 (s, 2H, NH_2), 4.36-4.46 (m, 1H, CH), 7.15-7.32 (m, 10H, 2Ph-H),8.45, 9.00 (2s, 3H, pyr-H), 9.10 (s, 1H, CONH, D_2O exchangeable, Hydrazide), 8.85, 8.95, 9.18 (3s, 6H, 6NH, exchangeable with D_2O). ^{13}C NMR: δ = 20.55, 21.65, 22.99, 22.15, 28.55, 31.95, 44.65, 37.60, 40.82, 52.15, 53.45, 56.84, 125.36, 126.72, 128.12, 139.05, 131.84, 139.68, 151.92, 169.66, 170.58, 170.05 (43C). MS (EI, 70 eV): *m/z* (%) = 812 (55) [M^+]. $C_{43}H_{57}N_9O_7$ (811.97): Calcd: C 63.61; H 7.08; N 15.53; Found: C 63.55, H 6.95, N 15.45.

Antimicrobial evaluation

The experimental method which was used in the antimicrobial evaluation has been adopted from Nehad *et al*[19].

Result and Discussion

Chemistry

In the present work, a series of several linear tetrapeptide and macrocyclic pentapeptide derivatives were synthesized based on compounds **1** and **2** which was synthesized from 3,5-pyridinedicarbonyl chloride, according to the previous reported procedures[1,20,21] (Figure 1). The synthesis of N^{α}-dinicotenoyl-bis [L-phenylalanyl-L-leucine]methyl ester] derivative **(3)** was based on the acid **1** and the hydrazide **2.** Treatment of L-phenylalanine methyl ester hydrochloride with dipeptide acid **3** (*Mixed anhydride method*) or dipeptide acid hydrazide **2** (*Azide method*) afforded the corresponding N^{α}-dinicotinoyl-bis[L-phenylalanyl-L-leucine]methyl ester] derivative **(3)**. The hydrolysis of bis-ester **3** with sodium hydroxide (2% methanolic solution) afforded the N^{α}-dinicotinoyl-bis[L-

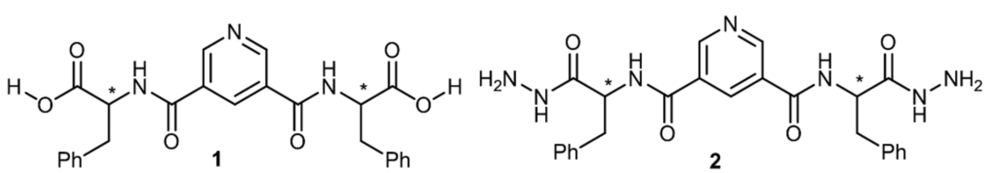
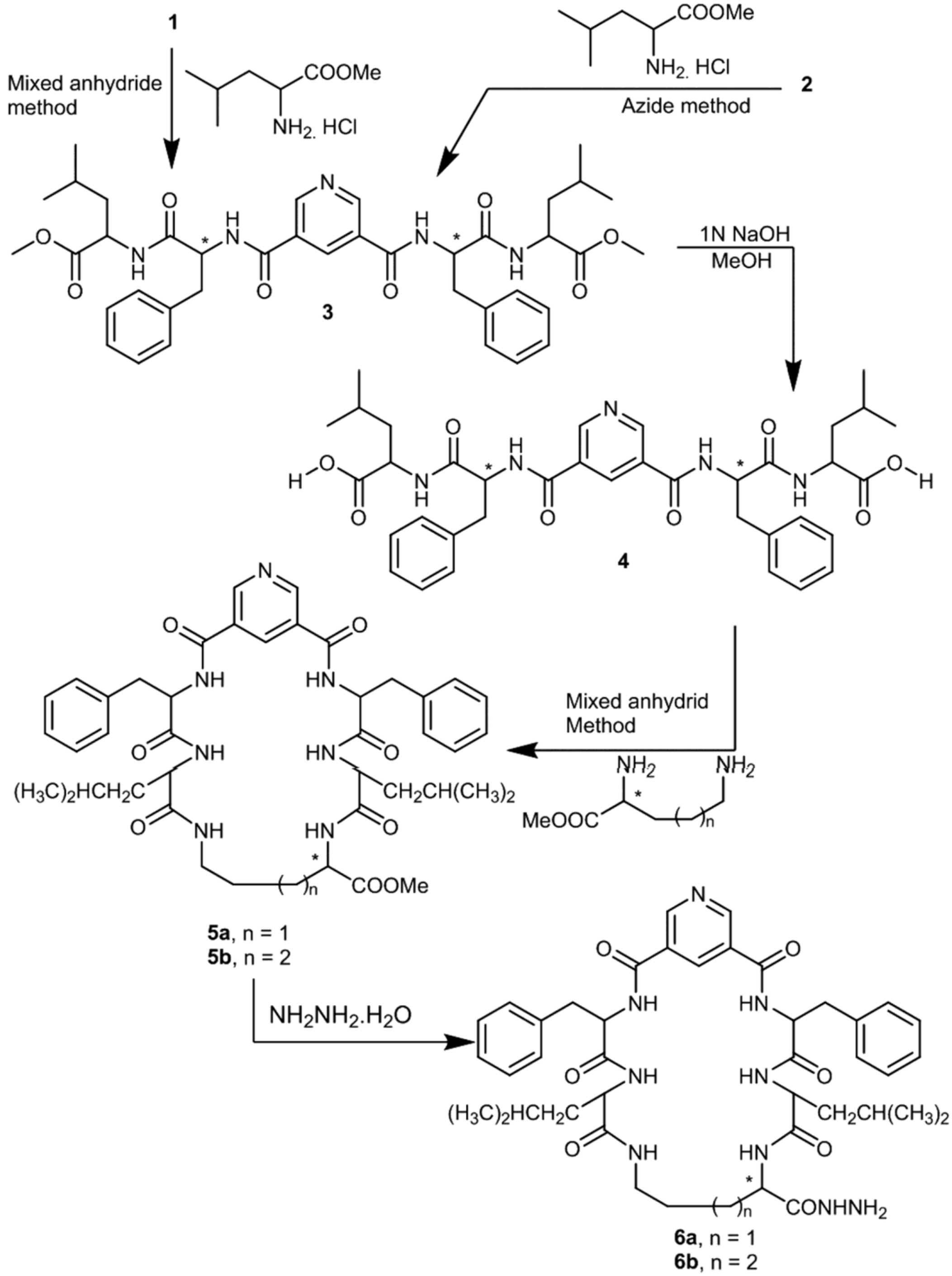


Fig.1 — Chemical structure for starting materials 1 and 2

phenylalanyl-L-leucine] acid] derivative **(4)**. Cyclization of tetrapeptidebis-acid **(4)** with L-dibasic amino acid methyl esters, namely, L-ornithine methyl ester or L-lysine methyl ester afforded the corresponding cyclo (N^{α}-di-nicotinoyl)[L-phenylalanyl-L-leucine] pentapeptide methyl esters**5a** and **5b**, respectively. Finally, hydrazonolysis of the last compounds **5a,b** by treating with hydrazine hydrated in refluxing methanol afforded macrocyclic pentapeptide hydrazides **6a,b**, respectively (Scheme 1).

Antimicrobial activity

The newly synthesized compounds **2-6** were tested for their preliminary antimicrobial activity against the



Scheme 1 — Synthetic routes for compounds 3-6

Table 1 — Antimicrobial activities of some newly synthesized compounds 2, 3, 4, 5a,band 6a,b.

Comp. No.	Inhibition zone (mm)						
	Gram-negative		Gram-positive		Fungi		Yeast
	E. coli	*P. putide*	*B. subtilis*	*Staph. Lactis*	*A. niger*	*Penicillium Sp.*	*C. albican*
2	1.66	1.72	1.22	0.60	1.75	2.00	-
3	1.65	1.65	1.83	0.64	1.58	1.65	-
4	1.66	1.72	1.22	0.60	1.75	1.50	-
5a	1.78	1.45	1.65	0.62	1.75	1.95	0
5b	1.85	1.80	1.95	0.78	1.55	1.22	1.85
6b	1.85	1.85	1.92	0.80	1.56	1.83	1.5
6a	1.80	1.70	1.65	0.64	1.75	1.20	1.40
Chlora-mphenicol	2.00	2.00	2.10	0.95	-	-	-
Fusidic acid	-	-	-	-	1.9	1.9	1.8

following microorganisms: *Escherichia coli* (*E. coli*), *Pseudomonas putide* (*P. putide*), *Bacillus subtilis* (*B. subtilis*), *Streptococcuslactis* (*Staph. Lactis*), *Aspergillus niger* (*A. niger*), *Penicillium sp*. and *Candida albicans* (*C. albican*). From the results in Table 1 showed that all synthesized compounds exhibited both antibacterial and antifungal activities on all tested microbial strains, except for compounds **2, 3**, and **4**, which did not showed activity against *Candida albicans*.

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