SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF SOME NEW *L*-LYSINE GLYCOSIDE DERIVATIVES

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Novel glycosides 2-(1,5-diaminopentyl)-*S*-fructosyl-1,3,4-oxadiazole-5-thione , 3-(1,5-diaminopentyl)-5-*S*-glucosyl-1,2,4-triazole-5-thiole and 3-(1,5-diaminopentyl)-4-amino-5-*S*- or 4-*N*-glucosyl-1,2,4-triazole-5-thiole are obtained by reacting the appropriate azoles with *D*-fructose and *D*-glucose. All intermediates and final products are characterized by IR, ^{1}H-NMR and ^{13}C-NMR. The antimicrobial activities are assessed using the paper disk diffusion and broth dilution methods against *Acinetobacter*, *Pseudomonas aeruginosa*, *Bacillus cereus, Staphylococcus aureus* and *Spongospora subterranean*. Some of the synthesized compounds showed promising activity against microorganisms under test in comparison with commercially available antibiotics Polymixine and Oxytetracycline.

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Introduction

L-Lysine is a necessary building block for all proteins in the body. It also plays a major role in calcium absorption, building muscle protein, recovering from surgery or sports injuries and body's production of hormones, enzymes, and antibodies.[1] Some studies have found that lysine may be beneficial for those with herpes simplex infections.[2] *L*-lysine has a anxiolytic action through its effects on serotonin receptors in the intestinal tract, and is also hypothesized to reduce anxiety through serotonin regulation in the amygdala.[3,4] There are lysine conjugates that show promise in the treatment of cancer, by causing cancerous cells to destroy themselves when the drug is combined with the use of phototherapy, while leaving non-cancerous cells unharmed.[5] *L*-lysine is an important additive to animal feed because it is a limiting amino acid when optimizing the growth of certain animals such as chickens for the meat production.[6]

Several modifications to *L*-lysine concerning the two amino groups, substitution of hydrogen of five carbon chain and OH group of carboxylic acid residue have been reported.[7, 8]

Lysine can be modified through (acetyllysine) methylation (methyllysine), ubiquitination, sumoylation, neddylation, biotinylation, pupylation, and carboxylation, which tends to modify the function of the protein of which the modified lysine residue(s) are a part.[9] To best of our knowledge, no modification of carboxylic group to azole rings with thione or thiol residue such as 1,3,4-oxadiazylthione, 1,2,4-triazylthiol and 4-amino-1,2,4-triazylthiol are reported.

In continuation of our investigations in this field to synthesize derivatives of 1,3,4-oxadiazylthione, 1,2,4-triazylthiol,[7] and 4-amino-1,2,4-triazylthiol,[8] and their respective glycosides,[9, 10,11] the synthesis of three azole-thiol derived from *L*-lysine and their antimicrobial effect is reported here.

Experimental

The IR-spectra, reported in wavenumber (ν, cm^{-1}), were recorded using KBr discs using a Jasco V-530 spectrophotometer. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker Avance AQS 300 MHz spectrometer at 300 and 75.5 respectively. Chemical shifts were measured in DMSO-d_6 as solvent to TMS as the internal standard.

The bacterial strains used for the experiment were collected as pure culture from Nekache El Sghir Hospital (Hopital El Mohgoun), Arzew, Oran. The Mueller Hinton medium was supplied by Difco.

All reactions were monitored by thin layer chromatographic analysis (silica gel supplied by Merck), and iodine used for visualization. The melting points were measured with a Büchi 540 melting point apparatus and were uncorrected.

Synthesis

Methyl *L*-lysinate (2).

Method (1): 5 ml H_2SO_4 was added dropwise with continuous stirring to *L*-lysine **1** (8.3 g, 0.06 mol) dissolved in methanol (200 mL) and the mixture was refluxed at 110 °C for 19 h. Excess of methanol was removed under vacuum at room temperature and aq. $NaHCO_3$ was added to neutralise the acid. The resulting solution was extracted with CH_2Cl_2, and dried over anhydrous $MgSO_4$, filtered and evaporated to dryness to give a solid substance which was recrystallized from ethanol to yield colorless crystalline methyl L-lysinate (**2**) (7.6 g, 83.4 %), m.p. 162 °C. IR, 3464 (broad, NH_2), 1725 (C=O).

Method (2): *L*-lysine **1** (2.0 gr, 0.014 mol), dissolved in methanol (75 ml), was treated with 5 ml chlorotrimethylsilane added dropwise with continuous stirring under N_2 atmosphere. The mixture was stirred further for 24 h. Volatiles were removed under vacuum to give a white solid which was recrystallized from ethanol to give colourless crystalline methyl L-lysinate (**2**) (1.95 g, 88.6 %). IR, 3469 (broad, NH_2), 1731 (C=O).

_L_-Lysine hydrazide (3).

20 mL hydrazine hydrate 64 % is added to methyl *L*-lysinate **2**(8.63 g, 0.05 mol) dissolved in ethanol (60 ml). The mixture is refluxed at 110 °C for 18 h, a white precipitate is formed during this period. The precipitate was filtered off and the filtrate is evaporated almost to dryness under vacuum at room temperature to give more solid which is combined with the previous one and recrystallized from water/ethanol to yield colorless crystalline *L*-lysine hydrazide **3** (6.25 g, 72 %), m.p. 187 °C. IR, 3446 (broad, NH_2), 1689 (N-C =O).

2-(1,5-Diaminopentyl)-1,3,4-oxadiazole-5-thione hydrochloride (4).

KOH (0.16 g, 0.004 mol) dissolved in ethanol (30 ml), was added to *L*-lysine hydrazide(3) (5.00 g, 0.03 mol) dissolved in ethanol (60 ml). Next, carbon disulphide (15 ml) is added and the mixture was refluxed at 110 °C for 13 h. The reaction mixture is then acidified to pH 5 by HCl (10 %). Excess ethanol was removed under vacuum, water was added and the aqueous mixture was then extracted by ethyl acetate, dried over anhydrous $MgSO_4$ and filtered. Volatiles were then removed under vacuum to give fine solid which was recrystallized from toluene/ethanol to colorless crystals (**4**) (4.18 g, 55.7 %), m.p. 173 °C. IR, 3426.3 (NH), 2655(S-H), 1644.93 (C=N), 1415.49(C=S), 1106.08(C-O-C). ^{1}H NMR (400 MHz) at 8.8 (HN-C=S), 4.3 (N=C-S-H) ppm of oxadiazole ring, 2.7-1.1 ppm for C-H side chain and ^{13}C NMR 206.37(C=S), 172.00(N=C=SH), 154.00, 54.70, 31.10, 29.90, 26.60 and 16.20.

1,5-Diaminopentylthiosemicarbazide (5).

Ammonium thiocyanate (0.85 g, 0.2 mol) was added to *L*-lysine hydrazide (**3**) (1.00 g, 0.06 mol), dissolved in ethanol (80 ml). Next, HCl (20 ml) was added dropwise and the mixture was refluxed at 80 °C for 15 h. Excess of ethanol was then removed under vacuum to give white solid which was recrystallized from toluene/petroleum ether to yield 1,5-diaminopentylthiosemicarbazide (**5**) as white crystals (0.82 g, 84 %), m.p. 197 °C. IR, 3456, 3075(N-H?, 1686.3 (C=O), 1211.08 (C=S).

Potassium-1,5-diaminopentylthiosemicarbazinate (6).

KOH (1.86 g, 0.03 mol) was added to *L*-lysine hydrazide (3) (1.00 g, 0.06 mol) dissolved in ethanol (50 ml) at room temperature. CS_2 (5 ml) was next added drop by drop with stirring. Reaction mixture was allowed to stand for 18 h. Excess of volatiles were evaborated under vacuum at room temperature. Diethylether (30 ml) was then added and the mixture was stirred for 1 h, filtered to give the solid (6) (1.32 g, 88 %), m.p. 193 °C. IR, 3380 (strong-NH_2), 2650(S-H), 1625(N-C=O) and 1590(N-C=S).

2-(1,5-Diaminopentyl)-1H-1,2,4-triazole-5-thiol (7).

1,5-Diaminopentylthiosemicarbazide (**5**) (2.0 g 0.01 mol) is dissolved in ethanol (200 ml), and an ethanolic KOH solution (KOH, 0.842 g in 20 ml of ethanol) was added and the mixture was refluxed for 13 h. Progress of the reaction was monitored by TLC. Careful neutralization was carried out with HCl. Volatiles were removed under vacuum, the reaction mixture was then extracted three times with ethyl acetate (50 ml), dried over $MgSO_4$, filtered and evaporated to dryness to give white solid (7) (1.10 g, 55 %), m.p. 203 °C. IR, 3342.5(N-H_2), 1526(C=N) and 1385.6 (S-H).

2-(1,5-Diaminopentyl)-1-amino-1H-1,2,4-triazole-5-thiol (8).

Potassium-1,5-diaminopentylthiosemicarbazinate (**6**) (1.23 g, 0.47 mmol) and hydrazine hydrate (64 %, 12 ml) were refluxed for 10 h. The progress of the reaction was monitored by TLC. Reaction mixture was cooled to 5 °C and acidified with HCl to pH 1 to yield a yellowish solid of **8**.HCl (2.05 g, 85 %), m.p. 190 °C. IR: 3456 (NH_2), 2640 (SH), 1456.9 (C=N).^{1}H NMR, 9.71(d, 2H, NH_2), 3.38 (m, 4H, 2NH_2), 2.53 (s, 1H, SH), 1.86, 1.78, 1.50 (m, 9H, $CH(CH_2)_4$.

Synthesis of 2-(1,5-diaminopentyl)-5-S-*D*-fructosyl-1,3,4-oxadiazole (9), 2-(1,5-diaminopentyl)-5-S-*D*-fructosyl-1h-1,2,4-triazole (10), 2-(1,5-diaminopentyl)-1-amino-N-*D*-glucosyl-1H-1,2,4-triazole-5-thiol and 2-(1,5-diaminopentyl)-1-amino-5-S-*D*-glucosyl-1H-1,2,4-triazole (11).

Thiols (**4**), (**7**) and (**8**) were dissolved in DMF and few drops of HCl and an appropriate amount of *D*-fructose or *D*-glucose was added and the mixture was refluxed (see Table 2). The progress of the reactions was monitored with TLC The mixtures were neutralized with solid $NaHCO_3$, and excess of solvent was removed by vacuum under moderate temperature. Residues were extracted with CH_2Cl_2 (50 ml), dried over $MgSO_4$, filtered and evaporated to dryness to give **9-11**. The yields were summarized in Table 1.

Antimicrobial test

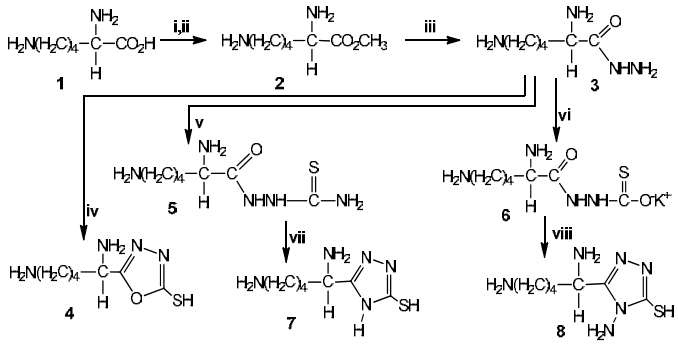
The test sample solutions containing the compounds were made by dissolving in calculated volumes of solvents separately and applied to sterile discs (6 mm diameter) at a concentration of 200 µg/disc and carefully dried to evaporate the residual solvents. Discs containing the test compound were placed on nutrient agar medium uniformly seeded with the test microorganisms. Standard antibiotics polymixine and oxytetracycline (30 µg/disc) discs and blank discs (impregnated with solvents) were used as positive and negative controls, respectively. The antimicrobial activity of the test agent was determined by measuring the diameter of zone of inhibition expressed in millimeter. The experiments carried out in duplicates.

Table 1. Reaction conditions of the thiols **4**, **7** and **8** with sugars

Compound no.	Thiols (g mol^{-1})	*D*-Fructose (g mol^{-1})	*D*-Glucose (g mol^{-1})	Reflux time (h)	Result no.	Yield (%)	M.p. (°C)
4	0.35/0.002	1.8/ 0.01		12	**9**	68	231
7	02.3/0.010	1.8/ 0.01		08	**10**	40	253
8	0.105/0.0004		1.2/ 0.007	24	**11**	46	110

Results and discussion

The three nucleobases 1,3,4-oxadiazole-5-thione (4), 1,2,4-triazole-5-thiole (7) and 4-amino-1,2,4-triazole-5-thiole (8) derived from *L*-lysine via *L*-lysine hydrazide (3) (see Scheme 1).



i) CH_3OH, H_2SO_4; ii) CH_3OH, $Si(CH_3)_3Cl$; iii) $NH_2NH_2.H_2O$; iv) NaOH, CH_3OH; CS_2, NaOH; vi) NH_4SCN; vii) NaOH; viii) NH_2NH_2.

Figure 1. General scheme for synthesis of azole-thiols (**4**), (**7**), and (**8**) from *L*-lysine

The oxadiazole thione (4) was obtained by refluxing hydrazide (3) with CS_2 in ethanol followed by acidification with HCl when the desired oxadiazole thion (4) was formed in 72 % yield, as a mixture of (thiol 4a⇄ thion 4b) tautomers.[11]

4a **4b**

Figure 2. Enol-keto [**4a** ⇄ **4b**] tautomerism of oxadiazolethione (**4b**)

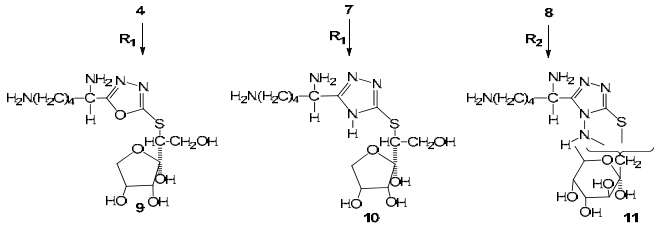
It was confirmed by IR spectroscopic measurements which showed bands at cm-1 2655 (SH) 1480 (oxadiazole ring) and 1415(C=S). 1H NMR exhibited signals at 8.8 (HN-C=S), 4.3 (N=C-SH) ppm of oxadiazole ring, 2.7-1.1 ppm for C-H side chain and 206.37(C=S), 172(N=C-SH), 154,54.7, 31.1, 29.9,26.6 and 16.2 for ^{13}C NMR.

1,5-Diaminopentylthiosemicarbazide (5) was obtained in good yield (84 %) by treating hydrazide (3) with ammonium thiocyanate as confirmed by TLC, IR and melting point.[14]

Refluxing (5) with ethanolic KOH solution yields 2-(1,5-diaminopentyl)-1H-1,2,4-triazole-5-thiol (7) which exhibits the characteristic IR bands of the 1,2,4-triazole ring at 1533 cm-1.13

Hydrazide (3) was treated with CS_2 in alkaline medium to yield potassium 1,5-diaminopentylthiosemicarbazinate (6) which was detected by TLC and IR and showed the following characteristic bands: 3380 (strong-NH_2), 2650(SH), 1625(N-C=O and 1590(N-C=S). The salt (6), without further purification was treated with hydrazine to give 2-(1,5-diaminopentyl)-1-amino-1H-1,2,4-triazole-5-thiol (8) which was confirmed with disappearance of IR bands at 1625 and 1590 and development of triazole bands at 1691, 1618, 1509 and 1402 attributed for C=N and C=S bonds. ^{1}H-NMR exhibited signals at 9.72 (1H, SH aromatic), 3.38(6H, 3NH_2), 2.54(1H, adjacent to NH_2 and triazole ring), and 1.86-1.5 (8H, 4CH_2).[14]

Oxadiazole (4) reacts as its thiol tautomer (4a) and triazole (7) are condensed with *D*-fructose while *N*-amino triazole (8) is condensed with *D*-glucose to give the corresponding S-glycosides (9), (10) and (11) (see Figure 3). Sugar carbonyl favors condensation with SH group than N-H group.



R_1 = *D*-Fructose R_2 = *D*-Glucose

Figure 3. General synthesis scheme for glyosides (**9**), (**10**) and (**11**) from *L*-lysine

The acyclic thioglycoside supposed to be formed undergoes cyclization to give **9**, **10** and **11** *via* the proposed mechanism illustrated in Figure 4. The compound **11** shows acyclic-cyclic (furano- ⇄ pyrano-) equilibrium and quick trans S- to N-glycosidation.

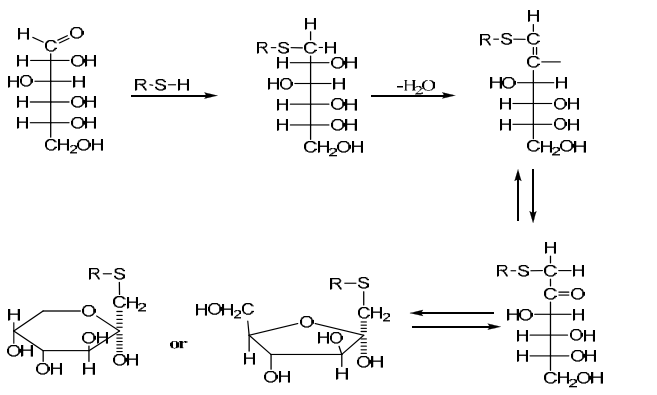


Figure 4. The proposed mechanism of acyclic-cyclic sugar S-glycoside tautomerism.

Antimicrobial screening

The antimicrobial activities of the tested compounds are evaluated in vitro using the paper disk diffusion method,[15] against two gram-negative bacteria: *acinetobacter*, *Pseudomonas aeruginosa*, gram-positive bacteria: *Bacillus cereus*, *Staphylococcus aureus* and one fungus *Spora subterranea*. DMSO known as bacterial static in the concentration of 2 µg ml^{-1} is used as negative control and two standard disks (Mast Diagnostics, UK), saturated with known antibiotics polymixine and oxytetracycline (30 µg/disc) as positive control are applied.

Table 2. Antibacterial activity of the synthesized compounds

Compounds	Inhibition zone (mm)				Relative inhibition, %
	a	b	c	d	
	Acinitobaccter				
1	5	5	0	0	71.41
2	12	8	5	0	>100
3	7	0	0	0	100
4	10	10	5	0	>100
5	0	0	0	0	-
6	0	0	0	0	-
7	0	0	0	0	-
8	13	12	5	5	>100
9	7	7	6	6	58.30
10	11	11	9	4	91.66
11	10	8	0	0	71.80
DMSO	-	-	-	-	-
Polymixine	12				100
Oxytetracycline	7				100
	P. aeruginosa				
1	12	7	5	0	60
2	0	0	0	0	-
3	7	7	5	5	35
4	12	12	7	0	60
5	8	8	8	0	40
6	8	0	0	0	33.33
7	10	7	4	0	41.66
8	8	0	0	0	60
9	9	9	8	0	35
10	8	6	0	0	40
11	8	5	3	0	60
DMSO	-	-	-	-	-
Polymixine	24				100
Oxytetracycline	20				100
	S. aureus				
1	10	7	0		83.33
2	9	9	5	5	75
3	12	9	6		>100
4	11	9	6	0	91.66
5	12	8	0	0	>100
6	0	0	0	0	-
7	7	6	5	0	43.70
8	9	9	9	9	56
9	8	8	6	6	50
10	10	7	5	3	62.50
11	9	7	6	3	56
DMSO	-	-	-	-	-
Polymixine	12				100
Oxytetracycline					100
	B. cereus				
1	16	10	8	6	100
2	17	13	7	7	>100
3	15	15	12	12	93.75
4	9	7	4	4	56.25
5	15	11	9	9	93.75
6	6	0	0	0	30
7	9	7	7	7	35
8	13	10	8	6	65
9	16	15	12	12	>100
10	21	11	9	9	>100
11	11	10	10	10	56.20
DMSO	-	-	-	-	-
Polymixine	20				100
Oxytetracycline	16				100
	S. subterranea				
1	3	7	7	5	86.66
2	12	10	7	7	80
3	20	15	6	6	>100
4	12	10	10	9	80
5	13	13	13	13	86.66
6	8	5	0	0	53.33
7	10	10	9	9	66.66
8	8	8	8	7	53.33
9	12	10	9	7	80
10	4	0	0	0	26.66
11	7	7	0	0	46
DMSO	-	-	-	-	-
Polymixine	15				100
Oxytetracycline	15				100

Highly active = (inhibition zone > 20 mm), moderately active = (inhibition zone 11- 19 mm), slightly active = (inhibition zone 6 – 10 mm), Inactive = (inhibition zone < 6 mm); MIC a, b, c, d = (1, 1/2 , 1/4, 1/8) 2 µg mL-1 ,*10 %, v/v= 1/1,

After incubation at 37 °C for 24 h, the zone of inhibition of growth around each disk is measured in mm and zone diameters are interpreted in accordance with clsi and nccls.[16-18] The experiments are performed in duplicates and the average results are summarized in Table 2. Appreciable effects of compounds (2), (4), (7) and (10) were observed on *Acinetobacter at* concentration of 400 µg mL^{-1} and certain extent at 200 µg mL^{-1}but there was no any response in cases of compounds 5-7. *P. Aeruginosa* was affected by all compounds except ester (2) In c ases of both G-strains slight effect were observed G+bactrria, *B. cereus* and S. aureus were affected by great extent by the studied compounds even at 50 µg mL^{-1} concentration. The studied compounds also exhibited antifungal activity resembled by *S. subterrane*

Conclusion

We have presented synthesis and characterization of three new diazole derivatives (**4**), (**7**) and (**8**) and related S- and N-glycosides (**9**), (**10**) and (**11**). The structure of new compounds and their synthetic intermediates are confirmed by spectral data IR, ^{1}H and ^{13}C-NMR. All compounds have been investigated for their antimicrobial activity against two gram-negative bacteria: *Acinetobacter*, *Pseudomonas aeruginosa*, and two gram-positive bacteria: *Bacillus cereus*, *Staphylococcus aureus* and one fungus: *Spora subterranea*.

The investigation of antibacterial and antifungal data showed that heterocyclic derivatives and their synthetic intermediates had variable effect, while all the glycosides (**9**)-(**11**) exhibited appreciable antimicrobial effect.

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