Bioorganic & Medicinal Chemistry Letters xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis and biological evaluation of novel lipoamino acid derivatives

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ARTICLE INFO

Article history:
Received 9 September 2015
Revised 26 October 2015
Accepted 29 October 2015
Available online xxxx

Keywords:
Fatty acid
Lipoamino acid
Amino acid
Antimicrobial activity
Antibiofilm activity

ABSTRACT

Seven novel lipoamino acid conjugates were synthesized from methyl oleate and amino acids. Methyl oleate was grafted to different amino acids using thioglycolic acid as a spacer group. Seven derivatives (**3a**–**g**) were prepared and characterized by spectral data (NMR, IR and MS spectral studies). All the derivatives were studied for their antimicrobial, anti-biofilm and anticancer activities. Among all the derivatives, it was found that compound **3b** was the most potent antibacterial compound which showed good activity against four Gram positive bacterial strains and also exhibited excellent antifungal activity against a fungal strain. In the anti-biofilm assay, compound **3b** showed promising activity with IC_{50} value of 2.8 μM against *Bacillus subtilis* MTCC 121. All the compounds showed anticancer activities with **3c** showing promising anticancer activity (IC_{50} = 15.3–22.4 μM) against the four cell lines tested.

Emergence of antimicrobial resistance increases the number of diseases and poses a threat in prevention and treatment of infections caused by various bacteria, viruses, fungi and parasites.[1] This is a global concern and thus necessitated the interest and search for new and more promising potential antibiotics. Production of novel hybrid molecules by combining two biologically important and biocompatible molecules is a well known strategy for the synthesis of novel bioactives.[2,3] Fatty acids, one of the building blocks of biomolecules are reported to assist in host defense against pathogenic microorganisms. Fatty acids and their derivatives exhibit broad spectrum of antibacterial action with potencies comparable to some natural antimicrobial peptides.[4,5] The broad spectrum of bioactivity with non-specific mode of action makes them attractive substrates for antimicrobial agents for application in medicine, agriculture, cosmetics and nutraceutical areas.

Combination of fatty acids with natural amino acids produces amphiphilic lipoamino acids with combined properties of both lipids and amino acids.[6] Lipoamino acid derivatives are reported to be employed in the preparation of lipid core peptide systems for application in the delivery of genes, drugs and vaccines.[7] The lipoamino acid derivatives are expected to possess membrane like properties which could help in the passage of drugs through the biological membranes.[8] Studies of biological activities of *N*-palmitoylated amino acids and *N*-stearyl amino acids showed that these compounds function as potential biostatic additives.[9,10] Therefore, conjugation of amino acids with a fatty acid derivative changes the lipophilicity which influences the membrane permeability of the synthetic molecule. These types of lipoamino acid conjugates are used to enhance the oral absorption of drugs like peptides, β-lactam antibiotics and alkaloid-based molecules.[11] Moreover, products like lipoaminoacids and derivatives have been reported to be biodegradable and can find wide range of applications in food, pharmaceutical and cosmetic formulations.[12]

In the present study, we describe the functionalization of methyl oleate with thioglycolic acid group by thiol-ene coupling, followed by condensation of the carboxylic acid moiety with amine group of amino acid esters to produce novel lipoamino acid derivatives. Synthesis was carried out as shown in Scheme 1. Initially methyl oleate was functionalized with thioglycolic acid based on a reported method via thiol-ene coupling reaction using AIBN as initiator to give the corresponding product in 92.5% yield.[13] The second step was condensation of the acid group with amine group of different amino acid esters catalyzed by EDC.HCl (N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride) and HOBt (1-hydroxybenzotriazole hydrate) reagent which resulted in the final products (**3a**–**g**) in yields ranging from 78% to 82%.

Biological activities: All the prepared compounds were tested for biological activities such as antimicrobial, anti-biofilm and cytotoxic activities.

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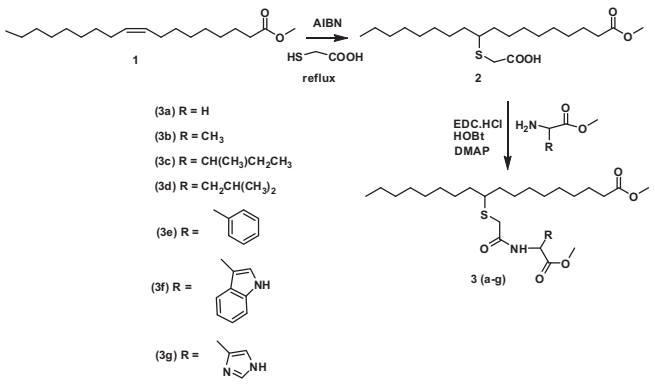
http://dx.doi.org/10.1016/j.bmcl.2015.10.086
0960-894X/



Scheme 1. Synthetic route for lipoamino acid conjugates.

Antimicrobial activity: The antimicrobial activities of the novel lipoamino acid esters were determined using well diffusion method[14] against different pathogenic strains. The synthesized compounds were evaluated for antimicrobial activity against seven bacterial organisms, namely *Micrococcus luteus* (*M. luteus*) MTCC 2470, *Staphylococcus aureus* (*S. aureus*) MTCC 96, *S. aureus* MLS-16 MTCC 2940, *Bacillus subtilis* (*B. subtilis*) MTCC 121, *Escherichia coli* (*E. coli*) MTCC 739, *Pseudomonas aeroginosa* (*P. aeroginosa*) MTCC 2453 and *Klebsiella planticola (K. planticola)* MTCC 530 and also against a fungal strain, *Candida albicans* (*C. albicans*) MTCC 3017. The MIC (Minimum inhibitory concentration) was determined by comparing the standard Ciprofloxacin and Miconazole as reference drugs for evaluating the antibacterial and antifungal activities, respectively. Out of the compounds tested, two compounds showed both antibacterial and antifungal activity and two compounds showed only antibacterial activity. The results of antimicrobial evaluation are presented in Table 1. The compounds whose MIC was less than 125 μg/mL were considered as active antimicrobial compounds and were taken up for further evaluation.

From the antimicrobial activity data, it was observed that four compounds showed activity out of the total seven compounds screened. Among the four active compounds, compounds **3a** and **3b** exhibited both antibacterial and antifungal activity with compound **3b** being more potent than **3a**. Compound **3b** exhibited good antimicrobial activity with MIC value of 7.8, 15.6, 7.8 and 3.9 μg/mL against *M. luteus* MTCC 2470, *S. aureus* MTCC 96, *S. aureus* MLS-16 MTCC 2940 and *B. subtilis* MTCC 121, respectively. Compound **3c** showed moderate antibacterial activity (MIC value of 31.2 μg/mL) against both *M. luteus* MTCC 2470 and *S. aureus* MTCC 96. Compound **3g** showed promising antibacterial activity (MIC value of 3.9 μg/mL) against both *M. luteus* MTCC 2470 and *B. subtilis* MTCC 121.

Based on the preliminary antibacterial activity data screening, further studies on minimum bactericidal concentration (MBC) and biofilm inhibition assay was conducted on four bacterial strains.[15] The compounds which showed antibacterial activity were tested for determining the MBC and it was found that compound **3b** was the most potent compound showing very good activity against all the four Gram positive microbes, namely *M. luteus* MTCC 2470, *S. aureus* MTCC 96, *S. aureus* MLS-16 MTCC 2940 and *B. subtilis* MTCC 121.

The results of the MBC data are presented in Table 2. All the compounds showed activity against *M. luteus* MTCC 2470 with compounds **3b** and **3g** being the most potent compounds, both showing MIC value of 7.8 μg/mL. Compounds **3a** and **3g** showed promising bactericidal activity against *B. subtilis* MTCC 121 with MIC value of 3.9 μg/mL. However, the values were than the standard reference drug, Ciprofloxacin which had MIC values ranging between 0.9 and 1.9 μg/mL.

These four compounds were also tested for biofilm inhibition assay according to a reported protocol.[16] The bacteria which caused biofilm formation were reported to tolerate antibiotics which posed a major threat in treatment of bacterial infections.[17] The compounds prepared in the present study are derived from amino acids and fatty acid and therefore can be projected as new molecules which can prevent the formation of biofilm. Accordingly, the compounds which showed activity in the preliminary antimicrobial assay were screened for biofilm inhibition assay and the results to this regard are presented in Table 3. It was observed that compound **3g** showed excellent anti-biofilm activity against *B. subtilis* MTCC 121 and *M. luteus* MTCC 2470 with IC_{50} values of 1.9 and 2.1 μM, respectively. Compound **3b** showed anti-biofilm activity against all the strains with IC_{50} values ranging from 2.8 to 9.3 μM. Compound **3a** showed moderate activity against *M. luteus* MTCC 2470 and *B. subtilis* MTCC 121, whereas compound **3c** showed lowest activity among all the compounds tested for biofilm inhibition assay.

From a structure–activity relationship perspective, the prepared lipoamino acid ester derivatives (**3a–g**) indicated that compound **3b** derived from alanine showed promising activity with respect to the evaluation of antimicrobial and anti-biofilm activities. The lipoamino acid from glycine (**3a**) and alanine (**3b**) were found to

Table 1
Antimicrobial activity of the lipoamino acid conjugates **3** (**a–g**)

S. No	Test compounds	Minimum inhibitory concentration (μg/ml)							
		M i[a]	*S a*[b]	*S a*[c]	*B s*[d]	*E c*[e]	*P a*[f]	*K p*[g]	*C a*[h]
1	**3a**	15.6	>125	>125	7.8	>125	>125	>125	31.2
2	**3b**	7.8	15.6	7.8	3.9	>125	>125	>125	7.8
3	**3c**	31.2	31.2	>125	>125	>125	>125	>125	>125
4	**3d**	>125	>125	>125	>125	>125	>125	>125	>125
5	**3e**	>125	>125	>125	>125	>125	>125	>125	>125
6	**3f**	>125	>125	>125	>125	>125	>125	>125	>125
7	**3g**	3.9	>125	>125	3.9	>125	>125	>125	>125
Ciprofloxacin (Standard)		0.9	0.9	0.9	0.9	0.9	0.9	0.9	>125
Miconazole (Standard)		>125	>125	>125	>125	>125	>125	>125	7.8

[a] *M. luteus* MTCC 2470.
[b] *S. aureus* MTCC 96.
[c] *S. aureus* MLS-16 MTCC 2940.
[d] *B. subtilis* MTCC 121.
[e] *E. coli* MTCC 739.
[f] *P. aeruginosa* MTCC 2453.
[g] *K.planticola* MTCC 530.
[h] *C. albicans* MTCC 3017.

Table 2
Minimum bactericidal concentration data

Test compounds	Minimum bactericidal concentration (μg/mL)			
	M l[a]	*S a*[b]	*S a*[c]	*B s*[d]
3a	31.2	—	—	3.9
3b	7.8	31.2	15.6	7.8
3c	31.2	31.2	—	—
3g	7.8	—	—	3.9
Ciprofloxacin (Standard)	0.9	1.9	1.9	1.9

[a] *M. luteus* MTCC 2470.
[b] *S. aureus* MTCC 96.
[c] *S. aureus* MLS-16 MTCC 2940.
[d] *B. subtilis* MTCC 121.

exhibit antifungal activities too. It was interesting to note that compound **3e** bearing phenylalanine did not show antimicrobial activity which suggests that the phenyl moiety could be affecting the bioactivity. However, compound **3g** derived from histidine showed excellent activity against *M. luteus* MTCC 2470 and *B. subtilis* MTCC 121 with MIC value of 3.9 μg/mL. In the anti-biofilm activity, compound **3g** showed promising activity against *B. subtilis* MTCC 121 and *M. luteus* MTCC 2470 with IC_{50} values of 1.9 and 2.1 μM, respectively. However, compound **3b** was observed to exhibit biofilm inhibition against all the four bacterial strains tested. It can be observed that all the compounds showed lower activity as compared with the reference drug, Ciprofloxacin in the antibacterial and biofilm inhibition assays studied. Based on the structural characteristics, it is interesting to note that compounds **3b** and **3e** derived from alanine and phenylalanine, respectively, showed considerable differences in the activities studied in the present investigation.

Based on the preliminary antimicrobial evaluation data, it was found that compounds **3a** and **3b** exhibited antifungal activity against *C. albicans* MTCC 3017 with MIC values of 31.2 and 7.8 μg/mL, respectively. From the observed preliminary antimicrobial activity evaluation, compound **3b** was further screened for antifungal activity against several fungal strains and the results to this regard are presented in Table 4. Based on the MIC values, it was observed that compound **3b** was equipotent to Miconazole showing excellent activity against *C. albicans* MTCC 183, *C. albicans* MTCC 3958 and *C. parapsilosis* MTCC 1744 with MIC value of 7.8 μg/mL similar to Miconazole. It showed good activity against other fungal strains with MIC values of 15.6 and 31.2 μg/mL. To further asses the antifungal activity, minimum fungicidal concentration (MFC) was determined against the fungal strains. It was interesting to observe that compound **3b** showed excellent antifungal activity against most of the *Candida* strains with MFC values ranging from 7.8 to 62.5 μg/mL. In particular, compound **3b** showed antifungal activity against *C. parapsilosis* MTCC 1744 with MFC value of 7.8 μg/mL which was on par with Miconazole.

Table 3
Anti-biofilm activity of the selected compounds

Test compounds	IC_{50} values in (μM)			
	M l[a]	*S a*[b]	*S a*[c]	*B s*[d]
3a	8.2 ± 0.22	—	—	4.4 ± 0.21
3b	4.1 ± 0.31	9.3 ± 0.11	3.2 ± 0.26	2.8 ± 0.31
3c	16.8 ± 0.44	21.5 ± 0.42	—	—
3g	2.1 ± 0.28	—	—	1.9 ± 0.12
Ciprofloxacin (Standard)	0.5 ± 0.08	0.3 ± 0.11	0.4 ± 0.09	0.5 ± 0.10

[a] *M. luteus* MTCC 2470.
[b] *S. aureus* MTCC 96.
[c] *S. aureus* MLS-16 MTCC 2940.
[d] *B. subtilis* MTCC 121.

Table 4
Antifungal activity of compound **3b**

S No	Test organism	MIC (μg/mL)		MFC (μg/mL)	
		3b	Miconazole	**3b**	Miconazole
1	*C. albicans* MTCC 183	7.8	7.8	15.6	7.8
2	*C. albicans* MTCC 227	15.6	7.8	31.2	15.6
3	*C. albicans* MTCC 854	31.2	7.8	62.5	7.8
4	*C. albicans* MTCC 1637	31.2	7.8	31.2	15.6
5	*C. albicans* MTCC 3018	15.6	7.8	31.2	7.8
6	*C. albicans* MTCC 3958	7.8	7.8	15.6	7.8
7	*C. albicans* MTCC 4748	15.6	7.8	15.6	7.8
8	*C. albicans* MTCC 7315	15.6	7.8	31.2	15.6
9	*C. parapsilosis* MTCC 1744	7.8	7.8	7.8	7.8
10	*C. aaseri* MTCC 1962	15.6	7.8	31.2	15.6
11	C. glabrata MTCC 3019	31.2	7.8	31.2	7.8
12	*C. krusei* MTCC 3020	15.6	7.8	15.6	7.8
13	Issatchenika hanoiensis MTCC 4755	15.6	7.8	31.2	7.8

It is know that many antifungal drugs currently available for treatment of Candida infections target the ergosterol biosynthetic pathway or its end product, ergosterol.[18] Considering this fact, we examined the compound **3b** in comparison with the standard miconazole drug to delineate the mode of action in the ergosterol biosynthetic pathway against one of the highly susceptible strain of *C. parapsilosis* MTCC 1744 and the results to this regard are shown in Table 5.

From the ergosterol biosynthesis inhibition assay, it was observed that the ergosterol content decreased significantly with an increase in the concentration of test compound **3b**. Similarly, dose-dependent decrease in ergosterol content was observed when the *C. parapsilosis* MTCC 1744 was cultured in presence of miconazole. Our findings suggest that the lipoamino acid conjugate (**3b**) alters the sterol profile and thus exerts its antifungal activity through inhibition of ergosterol biosynthesis. The selective cytotoxic behavior of this compound suggests that it has affinity to the specific target site in the ergosterol biosynthetic pathway. The fungicidal activity of the compound **3b** may also be due to the cell membrane permeability and subsequent cell death. It was also earlier reported that lipid derivatives disorganized the cytoplasm by disintegrating the plasma membrane which could be the plausible reason for effective antifungal activity.[19] Also the strong lipophilic character of the derivative could have helped it to affect the cellular membrane and the peptide linkage would have exhibited the selective antimicrobial activity.

All the prepared compounds were further evaluated for *in vitro* cytotoxicity by MTT assay[20] against four different cell lines and the data of this regard is presented in Table 6. It can be observed that compounds **3a** and **3c** exhibited good cytotoxic activities as compared to other compounds. According to structure–activity relationship between these two derivatives, compound **3c** derived from isoleucine showed excellent activity against HepG2 and SKOV3 cell lines with IC_{50} values of 15.3 and 16.4 μM, respectively. Compounds **3f** and **3g** bearing heterocyclic amino acid moiety did not show any activity against the tested cell lines. It was interesting to note that among compounds **3b** and **3e**, the compound **3e** derived from phenylalanine showed better activity as compared to **3b**. In general, the synthesized compounds showed good to moderate cytotoxicity as compared to the reference drug, Doxorubicin.

Novel lipoamino conjugates involving amino acids and fatty acid were synthesized employing reported protocols. All the product structures were characterized by ^{1}H NMR, ^{13}C NMR, FT-IR and MS spectral data. Among the prepared derivatives, compounds

Table 5
Ergosterol biosynthesis inhibition of the lipoamino acid conjugate (**3b**)

Test compound	Mean ergosterol content of cells grown with compounds at a concentration (μg/ml)				
	0	2	4	8	16
3b	1.62 ± 0.08	1.41 ± 0.11	0.63 ± 0.11	0.38 ± 0.08	0.11 ± 0.04
Miconazole	1.62 ± 0.08	1.28 ± 0.09	0.54 ± 0.07	0.24 ± 0.05	0.09 ± 0.02

Table 6
Anticancer activity of lipoamino acid conjugates (**3a–g**)

Test compound	IC_{50} values in (μM)			
	DU145	HepG2	SKOV3	MDA-MB 231
3a	30.8 ± 0.23	29.6 ± 0.29	25.2 ± 0.52	31.4 ± 0.13
3b	154.4 ± 0.44	102.5 ± 0.25	113.6 ± 0.36	98.7 ± 045
3c	19.0 ± 0.22	15.3 ± 0.18	16.4 ± 0.24	22.4 ± 0.11
3d	214.9 ± 0.52	—	—	152.1 ± 0.48
3e	49.1 ± 0.39	33.6 ± 0.32	42.5 ± 0.16	38.6 ± 0.29
3f	—	—	—	—
3g	—	—	—	—
Doxorubicin (Control)	0.6 ± 0.11	0.8 ± 0.09	0.8 ± 0.12	0.7 ± 0.08

3b derived from alanine was found to be potent for all the activities tested. In addition, it also exhibited excellent antifungal activity which was on par with the standard drug. Among the heterocyclic amino acid based derivatives, compound **3g** prepared from histidine showed excellent antibacterial activity against two strains along with anti-biofilm activity. The prepared derivatives were found to exhibit moderate cytotoxicity with isoleucine based lipoamino acid conjugate **3c** showing better cytotoxicity as compared to others. Based on this study, these types of molecules can be projected as novel amphiphilic antimicrobials which are derived from natural compounds as substrates.

Acknowledgements

One of the authors A. Sammaiah is thankful to UGC – India for the support in the form of SRF.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2015.10.086.

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