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Synthesis and biological evaluation of ricinoleic acid-based lipoamino acid derivatives

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ABSTRACT

A series of novel ricinoleic acid based lipoamino acid derivatives were synthesized from (*Z*)-methyl-12-aminooctadec-9-enoate and different L-amino acids (glycine, alanine, phenyl alanine, valine, leucine, isoleucine, proline and tryptophan). The structures of all the prepared compounds were characterized by ^{1}H NMR, ^{13}C NMR and mass spectral studies. The title compounds were evaluated for their antimicrobial and anti-biofilm activities. Among all the derivatives, compound **7a** (*Z*)-methyl-12-(2-aminoacetamido)octadec-9-enoate exhibited promising antibacterial activity (MIC, 3.9–7.8 μg/mL) and compounds **7b** (*Z*)-methyl 12-(2-aminopropanamido)octadec-9-enoate and **7g** (*Z*)-methyl-12-(pyrrolidine-2-carboxamido) octadec-9-enoate exhibited moderate activity (MIC, 7.8–31.2 μg/mL) selectively against four different Gram-positive bacterial strains such as *Staphylococcus aureus* MTCC 96, *Bacillus subtilis* MTCC 121, *S. aureus* MLS-16 MTCC 2940, *Micrococcus luteus* MTCC 2470. These compounds also exhibited excellent antifungal activity against studied fungal strains. Further, the compounds **7a**, **7b** and **7g** were also screened for anti-biofilm activity. Among these lipoamino acid derivatives, compound **7a** exhibited good anti-biofilm activity (IC_{50}, 1.9–4.1 μg/mL) against four Gram-positive bacterial strains.

Lipoamino acids (LAAs) are described as non-natural α-amino acid derivatives (having polar amino and carboxylic functionalities) which contain the structural features of lipids[1] (highly hydrophobic side chains). They are also called as fatty acid-amino acid conjugates which exists as endogenious substances with multiple biological activities.[2] The combination of complex (or) unusual fatty acids with natural α-amino acids produces amphiphilic LAAs as they have shown to exhibit great potential for a number of biologically active molecules. In fact, they have strong lipophilic nature due to the hydrophobic alkyl side chains when compared to polar characteristics of α-amino acids.[3,4] The bi-functional nature of LAAs is chemically conjugated to various drugs with a wide range of functional groups.[5] The functional nature of LAAs is not known; however, they have been found to exhibit anticancer activity against several cancer cell lines.[6]

LAAs are widely utilised in industry as lubricants[7] and have also been shown to possess therapeutic applications to inhibit both pancreatic lipase[8] and platelet phospholipase A_2 (PLA2)[9] and can function as anti-inflammatory agents. The presence of lipid and amino acid in LAAs makes them very attractive molecules to synthesize the prodrugs with increased bioavailability.[10,11] Today with increasing number of diseases and resistance to the existing antibiotics, there is an increasing demand to identify the drug candidates with novel modes of action. In this direction, the synthesis of novel bioactive molecules was designed by using a combination of two biologically active and biocompatible molecules (fatty acids and α-amino acids) which results in the production of novel hybrid bioactive lipoamino acid derivatives.

Literature reviews revealed that LAAs like *N*-palmitoylated amino acids and *N*-stearyl amino acids have been used as potential biostatic additives.[12] Moreover, the synthesis of non-natural lipoamino acid derivatives can find a wide range of applications in food, pharmaceutical and cosmetic formulations.[13] Ricinoleic acid is one of the naturally occurring unusual fatty acid with carboxyl and hydroxyl functionalities within the same molecule. It is a major fatty acid present in castor oil[14] and possesses various physical and chemical properties comparable to the normal edible oils because of the unusual nature of ricinoleic acid, it has been widely used for the preparation of several bioactive molecules.[15–17] L-Amino acids are also essential components in the living system. In view of the positive attributes of ricinoleic acid and L-amino acids, the present study was designed to synthesize a novel library of ricinoleic acid based bio-active LAA derivatives employing a five step procedure (Scheme 1).

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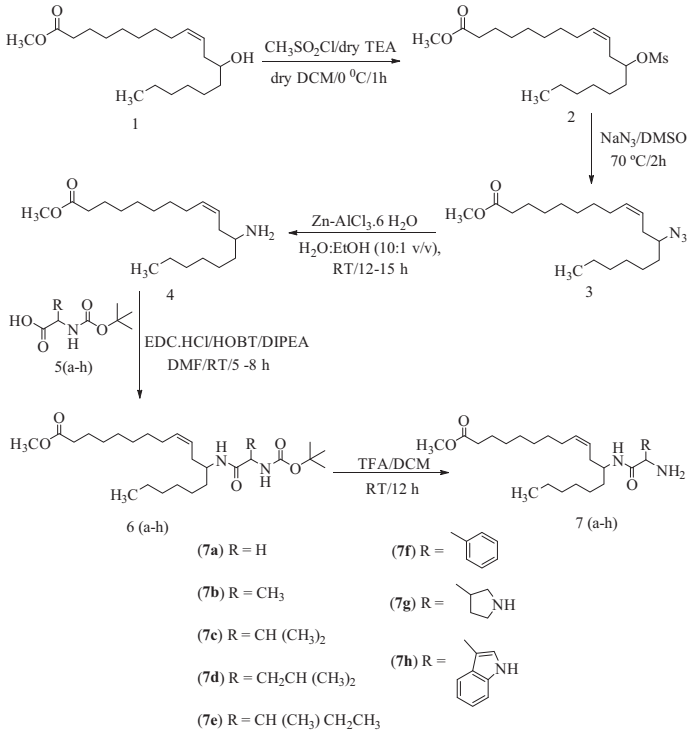

Scheme 1. Synthesis of ricinoleic acid based lipoamino acid derivatives.

Castor oil was chosen as the raw material for the isolation of ricinoleic acid methyl ester[18] (methyl ricinoleate) used in the present study, which deals with the synthesis of a series of novel lipoamino acid (LAA) derivatives by the functionalization of methyl ricinoleate (**1**). Methyl ricinoleate was prepared by the transesterification of castor oil containing 87–90% ricinoleic acid. The obtained mixture of castor fatty acid methyl esters was purified by column chromatography to obtain ricinoleic acid of 99% purity (GC). The synthesized ricinoleic acid based lipoamino acid derivatives were further evaluated for antimicrobial and anti-biofilm activities to identify the lead molecules.

Initially, methyl ricinoleate (**1**) was converted to (*Z*)-methyl-12-aminooctadec-9-enoate (**4**) according to the literature procedure.[15] The obtained compound **4** was reacted with *N*-BOC-amino acids in the presence of EDCI/HOBt/DIPEA which afforded the corresponding *N*-BOC-LAA derivatives with 60–75% yields. The structure of the BOC-protected LAA derivatives **6** (**a**–**h**) were confirmed by ^{1}H NMR and mass spectroscopy. The ^{1}H NMR spectra of compound **6a** showed the presence of BOC protons at δ 1.46 as singlet, two protons (–C*H*$_2$–NH–) at 3.74 as doublet and one proton (–CH–N*H*–CO–) at δ 5.83 as singlet. The structure of the compound **6a** was also confirmed by ESI-MS which showed *m/z* 469 [M+H]$^+$ and 491 [M+Na]$^+$. Finally, the deprotection of *N*-BOC-LAA derivatives **6** (**a**–**h**) using trifluoroacetic acid (TFA) afforded the corresponding LAA derivatives **7** (**a**–**h**) with yields ranging between 55% and 68%. The structures of all LAA derivatives were characterized by ^{1}H NMR, ^{13}C NMR and mass spectral studies. The ^{1}H NMR spectra of a compound **7a** indicated the presence of two protons (–C*H*$_2$–NH$_2$) at δ 3.34 as singlet and one hydrogen (–CH–N*H*–CO–) as δ 7.03 as doublet. The structure of compound **7a** was also confirmed by ESI-MS showed *m/z* 369 [M+H]$^+$. Similarly, the structures of the remaining compounds **7** (**b**–**h**) were also confirmed by its ^{1}H NMR and mass spectral studies which are represented in the spectral data. The details of the synthetic procedure and the spectral data of the synthesized compounds are given in the Supplementary data. The synthesized compounds were evaluated for their antimicrobial and anti-biofilm activities.

Antimicrobial activity: The antimicrobial activity of the LAA derivatives were determined using well-diffusion method[19] against several pathogenic bacterial strains namely *Staphylococcus aureus* MTCC 96, *Bacillus subtilis* MTCC 121, *S. aureus* MLS16 MTCC 2940, *Micrococcus luteus* MTCC 2470, *Micrococcus luteus* MTCC 2470, *Klebsiella planticola* MTCC 530, *Escherichia coli* MTCC 739, *Pseudomonas aeruginosa* MTCC M 2453 and several fungal pathogenic *Candida* strains namely *C. albicans* MTCC 183, *C. albicans* MTCC 227, *C. albicans* MTCC 854, *C. albicans* MTCC 1637, *Candida albicans* MTCC 3017, *C. albicans* MTCC 3018, *C. albicans* MTCC 3958, *C. albicans* MTCC 4748, *C. albicans* MTCC 7315, *C. parapsilosis* MTCC 1744, *C. aaseri* MTCC 1962, *C. glabrata* MTCC 3019, *C. krusei* MTCC 3020 and *Issatchinikia hanoiensis* MTCC 4755, which were procured from the Microbial Type Culture Collection (MTCC), CSIR-Institute of Microbial Technology, Chandigarh, India.

Out of the eight tested compounds, only three compounds (**7a**, **7b** and **7g**) exhibited both antibacterial and antifungal activities against the tested strains. While, the other compounds showed MIC values of >125 μg/mL for all the tested microbial strains. The antimicrobial activity results of LAA derivatives are shown in Table 1.

The obtained data revealed that only three compounds exhibited antimicrobial activity; compound **7a** exhibited promising antibacterial activity with MIC values of 3.9–7.8 μg/mL against all the Gram-positive bacterial strains and compounds **7b** and **7g** exhibited good to moderate antibacterial activity with MIC values of 7.8–31.2 μg/mL selectively against *S. aureus* MTCC 96, *B. subtilis* MTCC 121, *S. aureus* MLS16 MTCC 2940 and *M. luteus* MTCC 2470.

Based on the observed antibacterial activity, further studies of minimum bactericidal concentration (MBC)[20] and anti-biofilm activity[21] was screened against four different Gram-positive bacterial strains. It was observed that compound **7a** exhibited promising bactericidal activity with MBC values of 7.8 μg/mL against *S. aureus* MTCC 96, *B. subtilis* MTCC 121, *S. aureus* MLS16 MTCC 2940 and