IJPSR (2016), Vol. 7, Issue 4 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 07 October, 2015; received in revised form, 26 November, 2015; accepted, 20 February, 2016; published 01 April, 2016

SYNTHESIS AND CHARACTERIZATION OF NOVEL CHOLESTERYL LACTATE BASED FATTY ACID ANALOGS AND THEIR *IN VITRO* ANTIMICROBIAL ACTIVITY

Sathyam Reddy Yasa[1], Penumarthy Vijayalakshmi [1]* Poornachandra Yedla [2] and Ganesh Kumar Chityal [2]

Centre for Lipid Research [1], CSIR-Indian Institute of Chemical Technology (CSIR-IICT), Hyderabad, 500007, Telangana, India
Medicinal Chemistry and Pharmacology Division [2], CSIR-Indian Institute of Chemical Technology (CSIR-IICT), Hyderabad, 500007, Telangana, India.

Key words:

Cholesteryl lactate, Cholesteryl lactate-fatty acid conjugate, Antimicrobial activity, Unsaturated fatty acid

Correspondence to Author:
Dr. P. Vijayalakshmi

Chief Scientist,
Centre for Lipid Research,
CSIR-Indian Institute of Chemical Technology, Hyderabad 500007, Telangana, India.

Email: pvl@iict.res.in

ABSTRACT: A series of novel cholesteryl lactate-fatty acid conjugates (4a-i) were prepared by esterification of cholesterol with lactic acid, followed by coupling hydroxyl group of cholesteryl lactate with –COOH group of fatty acids including, saturated fatty acids of varying carbon chain of C_{10}-C_{18}, unsaturated fatty acids like 10-undecenoic, oleic ((9Z)-octadec-9-enoic), ricinoleic ((9Z,12R)-12-hydroxyoctadec-9-enoic) and 11-bromoundecanoic acid. Cholesteryl lactate-fatty acid conjugates were characterized by spectroscopic techniques like FT-IR, 1H-NMR, 13C-NMR, ESI-MS and HRMS. All the synthesized compounds were tested for their antimicrobial activity against a panel of seven bacterial strains like *Staphylococcus aureus* MTCC 96, *Bacillus subtilis* MTCC 121, *Micrococcus luteus* MTCC 2470, *Klebsiella planticola* MTCC 530, *Escherichia coli* MTCC 739, *Pseudomonas aeruginosa* MTCC 2453 and fungal strain like *Candida albicans*. Among them, the compounds with unsaturation (4f and 4h), and functional groups like bromine and hydroxyl group (4g and 4i) showed good antibacterial activity. But, promising activity was observed against *Staphylococcus aureus* MTCC 96 strain ranging from 7.8-15.6 µg/ml. These compounds (4f-i) are also exhibited good to moderate antifungal activity against different Candida strains ranging between 7.8-31.2 µg/ml.

INTRODUCTION: In recent years, antimicrobial resistance has gained a renewed attention in the clinical arena and has raised a serious public health concern due to the incidence of various drug-resistant microbial infections. Some of the infections are community acquired such as streptococcal infections, food poisoning, salmonellosis, pneumonia, etc., while some of them are of nosocomial origin caused by methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin resistant enterococci (VRE) or extended spectrum beta-lactamase (BSLE) enzyme producing Gram-negative bacteria and azole-resistant *Candida* species.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.7(4).1462-70

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.7 (4).1462-70

The primary reason for antimicrobial resistance is the wide usage or misuse of the currently available antimicrobial agents by the medical practitioners [1, 2]. In view of the increased threat from these drug-resistant Gram-positive and Gram-negative bacterial strains and also *Candida* strains, there is a continuous demand and perusal to identify new antimicrobial agents. Several reports claim that numerous potent biological activities such as, insect repellent [3], antibacterial [4-7], pesticidal [8], antifungal [9-11], anti-inflammation[12], antioxidant[10, 13], antifeedant [14], chemotherapeutic [5, 6, 15], as well as neuroprotective [16] properties are attributed to naturally occurring seed oils, fatty acids (FA) and their derivatives.

A host of FA analogs are reported to be promising candidates for treatment of cancer, hepatic, renal (anti-estrogenic) and cardiovascular diseases and dermatitis [17-20]. Some FA analogs are also reported for gene delivery applications [21, 22].

Cholesterol derivatives like steroidal 5-en-3-oxazolo and thiazoloquinoxalines [23], steroidal thiosemicarbazones [24], steroidal extract of *Pergularia extensa* N.E. Br plant [25] and steroid-based surfactants [26] are reported to exhibit antimicrobial activity, for treating asthma, diarrhea and for solubilization of poorly soluble drugs. In addition, a series of cholesteryl esters synthesized by using cholesterol and unsaturated fatty acids (10-undecenoic, oleic, linoleic, ricinoleic and arachidonic acid) and their derivatives also revealed that the product with bromine functionality on alkyl chain exhibited excellent antibacterial potency, while the fatty acids with unsaturation and hydroxyl functionality on alkyl chain showed considerable antibacterial and antifungal properties [27, 28].

In view of the above facts and the significance of long chain fatty acid esters of cholesterol as potent antimicrobial agents, the present study was undertaken to synthesize a series of cholesteryl lactate-fatty acid conjugates (**4a-i**) using fatty acids such as saturated fatty acids from C_{10}-C_{18} of varying carbon chain, unsaturated fatty acids (10-undecenoic, oleic and ricinoleic acids), and 11-bromo undecanoic acid, as well as cholesteryl lactate. The products were characterized by spectroscopic techniques like FT-IR, ^{1}H-NMR, ^{13}C-NMR, ESI-MS and HR-MS and further evaluated for their *in vitro* antimicrobial activity against a panel of Gram-positive, Gram-negative strains of bacteria and different fungal strains.

MATERIALS:

The raw materials needed for the synthesis of novel cholesteryl lactate-fatty acid conjugates (**4a-i**), such as cholesterol, lactic acid, different saturated fatty acids from C_{10}-C_{18} carbon chain and ricinoleic acid ((9Z,12R)-12-hydroxyoctadec-9-enoic acid) were purchased from S.D. Fine chemicals (Mumbai, India). 10-undecenoic, oleic (((9Z)-octadec-9-enoic) and 11-bromoundecanoic acids were purchased from Sigma-Aldrich Chemicals (St. Louis, MO, USA). All solvents and chemicals were of reagent grade and used directly without further purification. Silica gel (60-120 mesh) for column chromatography was purchased from Acme Synthetic Chemicals (Mumbai, India). Precoated TLC plates were purchased from Merck (Darmstadt, Germany). All microbial strains were obtained from Microbial Type Culture Collection and Gene Bank CSIR-Institute of Microbial Technology, Chandigarh, India.

Analytical Methods:

IR spectra were recorded on a Perkin Elmer (model: Spectrum BX) FT-IR spectrometer using $CHCl_3$ and KBr. All ^{1}H and ^{13}C-NMR spectra's were recorded on 500 MHz (Varian) and 125 (Bruker) spectrometer, respectively. ESI-MS spectra were recorded on Waters (Model Q STAR XL, Applied Biosystems, USA) mass spectrometer equipped with an electrospray ionization source. HRMS data were recorded on a Thermo Scientific Exactive Orbitrap mass spectrometer (Germany). Melting points were determined by using melting point apparatus MR-VIS (MR08190508).

Synthesis:

Synthesis of cholesteryl lactate (or) cholesteryl 2-hydroxypropanoate (3):

Cholesterol (**1**) and lactic acid (**2**) were dissolved in dichloromethane (DCM) (1:4 equivalents) and stirred for 15 minutes, followed by the addition of PTSA catalyst (10% by weight of alcohol) and refluxed for 6 h. After 6 h, one drop of conc. H_2SO_4 was added for completion of the reaction by continuing further for 1 h. After evaporation of DCM, the crude product was worked up by washing with brine solution, and extracted with diethyl ether. Pure cholesteryl lactate was separated from worked up crude reaction product by silica gel (60-120 mesh) column chromatography using hexane and ethyl acetate (98:2, v/v) as eluent Column chromatography was monitored by TLC with hexane and ethyl acetate (9:1 v/v) solvent system and identified by iodine vapors. Isolated yield obtained was 95%.

Spectral data of cholesteryl lactate (or) cholesteryl 2-hydroxypropanoate (3):

White amorphous compound; Melting point 124.1-124.3°C; R_f = 0.61 (n-hexane/ethyl acetate 9:1 v/v), isolated yield 95%; IR (KBr, cm^{-1}): 3455, 2938, 1727, 1465, 1225; ^{1}H-NMR (500 MHz, $CDCl_3$): δ/ppm 5.37 (d, 1H, J = 3.97 Hz, C_6-H), 4.65-4.74 (br.m, 1H, $C_{3'}$-H), 4.20-4.26 (m, 1H, $C_3\beta$-H), 2.83 (d, 1H, J = 5.34 Hz, $C_{3'}$-H), 2.34 (2H, d, J = 7.78 Hz, C_4-H), 1.43-2.06 (br.m, 13H), 1.41(d, 3H, J =

6.87 Hz, $C_{3'}$-C<u>H</u>$_3$), 0.94-1.39 (br.m, 17H), 0.92 (d, 3H, *J* = 6.56 Hz, C_{21}-C<u>H</u>$_3$), 0.87 (d, 3H, *J* = 2.29 Hz, C_{27}-C<u>H</u>$_3$), 0.86 (d, 3H, *J* = 2.14 Hz, C_{26}-C<u>H</u>$_3$), 0.68 (s, 3H, C_{18}-C<u>H</u>$_3$); ^{13}C-NMR ($CDCl_3$, 125): δ/ppm 175.21, 139.17, 123.00, 75.45, 66.73, 56.63, 56.09, 49.95, 42.27, 39.67, 39.48, 37.95, 36.86, 36.53, 36.15, 35.76, 31.86, 31.80, 28.19, 27.99, 27.63, 24.24, 23.80, 22.80, 22.54, 21.00, 20.47, 19.27, 18.68, 11.82; M.W: 458.38. ESI-MS *m/z*: 481.49 ($M^+ + Na^+$).

General procedure for the synthesis of cholesteryl lactate-fatty acid conjugates:

Fatty acid (1 equivalent), DCC (1 equivalent) and cholesteryl lactate (**3**) (0.85 equivalent) in dry dichloromethane with catalytic amount of 4-dimethylaminopyridine (DMAP) were stirred mechanically at room temperature under N_2 atmosphere until esterification was complete [21]. The N, N-dicyclohexylurea was filtered off and the filtrate was washed with water (3 times), 5% acetic acid (3 times) and again with water (3 times) followed by drying over anhydrous sodium sulphate. The solvent was removed under vacuum and the crude product was purified by silica gel column chromatography using hexane: ethyl acetate (98:2 v/v) solvent system as eluent to afford the desired cholesteryl lactate-fatty acid conjugates from **4a-i** as shown in **Scheme** and all these novel compounds were further characterized from their spectral data.

Spectral data of cholesteryl lactate-fatty acid conjugates:

Cholesteryl lactate-decanoic acid conjugate (or) 1-((cholesteryl)-oxy)-1-oxopropane-2-yl decanoate (**4a**):

White solid compound; Melting point 45°C; R_f = 0.79 (n-hexane/ethyl acetate 9:1 v/v), isolated yield 89%; IR (KBr, cm^{-1}): 2931, 2856, 1746, 1465, 1377, 1206; ^{1}H-NMR (300 MHz, $CDCl_3$): δ/ppm 5.36 (d, 1H, *J* = 4.15 Hz, C_6-<u>H</u>), 5.01 (q, 1H, $C_{2'}$-<u>H</u>, *J* = 7.01 Hz), 4.71-4.58 (m, 1H, C_3β-<u>H</u>), 2.43 (t, 2H, *J* = 7.55 Hz, $C_{4'}$-<u>H</u>), 2.30 (d, 2H, *J* = 7.93 Hz, C_4-<u>H</u>), 2.05-1.49 (br.m, 15H), 1.47 (d, 3H, *J* = 7.01 Hz, $C_{2'}$-C<u>H</u>$_3$), 1.39-1.20 (br.m, 17H), 1.20-0.93 (br.m, 11H), 0.91 (d, 3H, *J* = 6.56 Hz, C_{21}-C<u>H</u>$_3$), 0.88 (t, 3H, *J* = 6.71 Hz, C_{19}-C<u>H</u>$_3$), 0.87 (d, 3H, *J* = 2.29 Hz, C_{27}-C<u>H</u>$_3$), 0.85 (d, 3H, *J* = 2.14 Hz, C_{26}-C<u>H</u>$_3$), 0.67 (s, 3H, C_{18}-C<u>H</u>$_3$); ^{13}C-NMR ($CDCl_3$, 125): δ/ppm 173.18, 170.34, 139.30, 122.84, 74.95, 68.57, 56.64, 56.10, 49.95, 42.28, 39.68, 39.49, 37.85, 36.86, 36.53, 36.15, 35.76, 33.96, 31.86, 31.84, 31.81, 29.38, 29.32, 29.24, 29.19, 29.16, 29.05, 28.84, 28.19, 27.98, 27.57, 24.83, 24.24, 24.20, 23.80, 22.79, 22.63, 22.62, 22.53, 21.00, 19.28, 18.69, 16.91, 14.08, 11.82; M.W: 612.51. ESI-MS *m/z*: 631 $(M + NH_4)^+$. HRMS (*m/z*) calculated for $C_{40}H_{72}O_4N$ is 630.5456 and found at 630.5457 $(M + NH_4)^+$.

Cholesteryl lactate-dodecanoic acid conjugate (or) 1-((cholesteryl) - oxy) - 1 - oxopropane - 2-yl dodecanoate **(4b)**:

White solid compound; Melting point 46°C ; R_f = 0.81 (n-hexane/ethyl acetate 9:1 v/v), isolated yield 95%; IR (KBr, cm^{-1}): 2927, 2854, 1746, 1466, 1376, 1203; ^{1}H-NMR (500 MHz, $CDCl_3$): δ/ppm 5.37 (d, 1H, *J* =3.66 Hz, C_6-<u>H</u>), 5.02 (q, 1H, $C_{2'}$-<u>H</u>, *J* =7.17 Hz), 4.61-4.70 (m, 1H, C_3β-<u>H</u>), 2.32-2.42 (m, 2H, $C_{4'}$-<u>H</u>), 2.30 (d, 2H, *J* = 7.63 Hz, C_4-<u>H</u>), 1.48-2.05 (br.m, 15H), 1.47 (d, 3H, *J* = 7.17 Hz, $C_{2'}$-C<u>H</u>$_3$), 1.22-1.44 (br.m, 17H), 0.93-1.20 (br.m, 11H), 0.90 (d, 3H, *J* = 6.56 Hz, C_{21}-C<u>H</u>$_3$), 0.87 (t, 3H, *J* = 6.71 Hz, C_{19}-C<u>H</u>$_3$), 0.86 (d, 3H, *J* = 2.29 Hz, C_{27}-C<u>H</u>$_3$), 0.85 (d, 3H, *J* = 2.14 Hz, C_{26}-C<u>H</u>$_3$), 0.67 (s, 3H, C_{18}-C<u>H</u>$_3$); ^{13}C-NMR ($CDCl_3$, 125): δ/ppm 173.23, 170.37, 139.30, 122.86, 74.97, 68.58, 56.63, 56.09, 49.94, 42.28, 39.68, 39.49, 37.85, 36.85, 36.54, 36.15, 35.77, 33.98, 31.90, 31.87, 31.81, 29.68, 29.60, 29.44, 29.33, 29.26, 29.07, 28.20, 27.99, 27.57, 24.84, 24.25, 23.80, 22.80, 22.67, 22.54, 20.99, 19.28, 18.68, 16.93, 14.11, 11.82; M.W: 640.54. ESI-MS *m/z*: 659 $(M + NH_4)^+$; HRMS (*m/z*) calculated for $C_{42}H_{72}O_4Na$ is 663.5328 and found at 663.4566 $(M + Na)^+$.

Cholesteryl lactate-tetradecanoic acid conjugate (or) 1 - ((cholesteryl)-oxy) – 1 - oxopropane-2-yl tetradecanoate **(4c)**:

White solid compound; Melting point 48°C; R_f = 0.83 (n-hexane/ethyl acetate 9:1 v/v), isolated yield 91%; IR (KBr, cm^{-1}): 2925, 2853, 1745, 1465, 1378, 1206; ^{1}H-NMR (300 MHz, $CDCl_3$): δ/ppm 5.36 (d, 1H, *J* = 3.77 Hz, C_6-<u>H</u>), 5.02 (q, 1H, $C_{2'}$-<u>H</u>, *J* = 6.79 Hz), 4.58-4.73 (m, 1H, C_3β-<u>H</u>), 2.37 (t, 2H, *J* = 7.55 Hz, $C_{4'}$-<u>H</u>), 2.30 (d, 2H, *J* = 7.55 Hz, C_4-<u>H</u>), 1.50-2.08 (br.m, 15H), 1.47 (d, 3H, *J* = 6.79 Hz, $C_{2'}$-C<u>H</u>$_3$), 1.20-1.44 (br.m, 25H), 0.96-1.99

(br.m, 11H), 0.82-0.95 (12H, m), 0.67 (s, 3H, C_{18}-C<u>H</u>$_3$); ^{13}C-NMR ($CDCl_3$, 125): δ/ppm 173.17, 170.33, 139.30, 122.84, 74.95, 68.57, 56.65, 56.11, 49.96, 42.28, 39.68, 39.49, 37.86, 36.87, 36.53, 36.16, 35.76, 33.97, 31.91, 31.86, 31.82, 29.67, 29.64, 29.60, 29.43, 29.34, 29.25, 29.06, 28.19, 27.99, 27.57, 24.84, 24.25, 23.80, 22.79, 22.67, 22.53, 21.00, 19.28, 18.68, 16.91, 14.10, 11.83; M.W: 668.57. ESI-MS *m/z*: 687 $(M + NH_4)^+$; HRMS (*m/z*) calculated for $C_{44}H_{80}O_4N$ is 686.6082 and found at 686.6077 $(M + NH_4)^+$.

Cholesteryl lactate-hexadecanoic acid conjugate (or) 1 - ((cholesteryl) - oxy) - 1-oxopropane-2-yl hexadecanoate **(4d)**:

White solid compound; Melting point 51°C; R_f = 0.84 (n-hexane/ethyl acetate 9:1 v/v), isolated yield 90%; IR (KBr, cm^{-1}): 2930, 2854, 1744, 1467, 1377, 1207; ^{1}H-NMR (500 MHz, $CDCl_3$): δ/ppm 5.37 (d, 1H, *J* =3.66 Hz, C_6-<u>H</u>), 5.02 (q, 1H, $C_{2'}$-<u>H</u>, *J* = 7.17 Hz), 4.61-4.70 (m, 1H, C_3β-<u>H</u>), 2.32-2.42 (m, 2H, $C_{4'}$-<u>H</u>), 2.30 (d, 2H, *J* = 7.63 Hz, C_4-<u>H</u>), 1.48-2.05 (br.m, 15H), 1.47 (d, 3H, *J* = 7.17 Hz, $C_{3'}$-C<u>H</u>$_3$), 1.20-1.39 (br.m, 29H), 0.93-1.20 (br.m, 11H), 0.91 (d, 3H, *J* = 6.56 Hz, C_{21}-C<u>H</u>$_3$), 0.88 (t, 3H, *J* = 6.71 Hz, C_{19}-C<u>H</u>$_3$), 0.87 (d, 3H, *J* = 2.29 Hz, C_{27}-C<u>H</u>$_3$), 0.85 (d, 3H, *J* = 2.14 Hz, C_{26}-C<u>H</u>$_3$), 0.67 (s, 3H, C_{18}-C<u>H</u>$_3$); ^{13}C-NMR ($CDCl_3$, 125): δ/ppm 173.19, 170.33, 139.26, 122.85, 74.91, 68.56, 56.60, 56.06, 49.79, 42.24, 39.65, 39.44, 37.81, 36.83, 36.13, 35.75, 33.95, 31.88, 31.84, 31.76, 29.67, 29.41, 29.33, 29.23, 29.03, 28.19, 27.96, 27.52, 24.81, 24.23, 23.76, 22.78, 22.66, 22.51, 20.97, 19.26, 18.64, 16.90, 14.09, 11.78; M.W: 696.61. ESI-MS *m/z*: 715 $(M + NH_4)^+$; HRMS (*m/z*) calculated for $C_{46}H_{84}O_4N$ is 714.6394 and found at 714.6386 $(M + NH_4)^+$.

Cholesteryl lactate-octadecanoic acid conjugate (or) 1 - ((cholesteryl) - oxy) - 1-oxopropane-2-yl octadecanoate **(4e)**:

White solid compound; Melting point 55°C; R_f = 0.85 (n-hexane/ethyl acetate 9:1 v/v), isolated yield 92%; IR (KBr, cm^{-1}): 2928, 2854,1745, 1466, 1376, 1207; ^{1}H-NMR (500 MHz, $CDCl_3$): δ/ppm 5.37 (d, 1H, *J* = 4.27 Hz, C_6-<u>H</u>), 5.02 (q, 1H, *J* = 7.17 Hz, $C_{2'}$-<u>H</u>), 4.60-4.70 (m, 1H, C_3β-<u>H</u>), 2.33-2.41 (m, 2H, $C_{4'}$-<u>H</u>), 2.31 (d, 2H, *J* = 7.78 Hz, C_4-<u>H</u>), 1.49-2.04 (br.m, 15H), 1.47 (d, 3H, *J* = 7.17 Hz, $C_{2'}$-C<u>H</u>$_3$), 1.21-1.39 (br.m, 33H), 0.95-1.18 (br.m, 11H), 0.91 (d, 3H, *J* = 6.04 Hz, C_{21}-C<u>H</u>$_3$), 0.88 (t, 3H, *J* = 6.71 Hz, C_{19}-C<u>H</u>$_3$), 0.87 (d, 3H, *J* = 2.89 Hz, C_{27}-C<u>H</u>$_3$), 0.85 (d, 3H, *J* = 2.89 Hz, C_{26}-C<u>H</u>$_3$), 0.67 (s, 3H, C_{18}-C<u>H</u>$_3$); ^{13}C-NMR ($CDCl_3$, 125): δ/ppm 173.21, 170.36, 139.26, 122.85, 74.94, 68.56, 56.61, 56.06, 49.89, 42.24, 39.65, 39.44, 37.81, 36.83, 36.13, 35.75, 33.95, 31.88, 31.82, 31.76, 29.67, 29.41, 29.32, 29.23, 29.03, 28.19, 27.95, 27.52, 24.81, 24.23, 23.76, 22.77, 22.66, 22.51, 20.97, 19.25, 18.64, 16.90, 14.08, 11.78; M.W: 724.64. ESI-MS *m/z*: 743 $(M + NH_4)^+$; HRMS (*m/z*) calculated for $C_{48}H_{88}O_4N$ is 742.6707 and found at 742.6703 $(M + NH_4)^+$.

Cholesteryl lactate-undec-10-enoic acid conjugate (or) 1 - ((cholesteryl)-oxy) – 1 - oxopropane-2-yl undec-10-enoate (**4f**):

White solid compound; Melting point 42°C; R_f = 0.80 (n-hexane/ethyl acetate 9:1 v/v), isolated yield 81%; IR (KBr, cm^{-1}): 3072, 2930, 2855, 1744, 1466, 1379, 1207; ^{1}H-NMR (300 MHz, $CDCl_3$): δ/ppm 5.81 (m, 1H, $J_{H\text{-}11'CH2}$ = 6.79 Hz, $J_{H\text{-}HZ}$ = 10.57 Hz, $J_{H\text{-}HE}$ = 17.37 Hz, H_EH_ZC=C<u>H</u>-CH_2), 5.37 (d, 1H, *J* = 3.78 Hz, C_6-<u>H</u>), 5.08-4.89 (m, 3H, $C_{2'}$-<u>H</u>, H_ZC=CH- and H_EC=CH-), 4.72-4.59 (m, 1H, C_3β-<u>H</u>), 2.37 (t, 2H, *J* = 7.55 Hz, $C_{4'}$-<u>H</u> (-OC-C<u>H</u>$_2$-CH_2)), 2.31 (d, 2H, *J* = 7.55 Hz, C_4-<u>H</u>), 2.09-1.49 (br.m, 17H), 1.47 (d, 3H, *J* = 7.55 Hz, $C_{2'}$-C<u>H</u>$_3$), 1.39-0.93 (br.m, 26H), 0.91 (d, 3H, *J* = 6.80 Hz, C_{21}-C<u>H</u>$_3$), 0.88 (d, 6H, *J* = 6.80 Hz, C_{19}-C<u>H</u>$_3$, C_{26}-C<u>H</u>$_3$), 0.67 (s, 3H, C_{18}-C<u>H</u>$_3$); ^{13}C-NMR ($CDCl_3$, 125): δ/ppm 173.20, 170.36, 139.28, 139.12, 122.86, 114.11, 74.96, 68.58, 56.62, 56.07, 49.92, 42.26, 39.66, 39.48, 37.84, 36.84, 36.52, 36.14, 35.76, 33.94, 33.77, 31.85, 31.80, 29.25, 29.18, 29.03, 28.87, 28.20, 27.98, 27.55, 24.81, 24.24, 23.80, 22.80, 22.54, 20.99, 19.28, 18.68, 16.92, 11.85, 11.79; M.W: 624.51. ESI-MS *m/z*: 643 $(M + NH_4)^+$. HRMS (*m/z*) calculated for $C_{41}H_{72}O_4N$ is 642.5456 and found at 642.5457 $(M + NH_4)^+$.

Cholesteryl lactate-11-bromoundecanoic acid conjugate (or) 1-((cholesteryl)-oxy)-1-oxopropane-2-yl-11-bromo undecanoate (**4g**):

Colorless viscous liquid; R_f = 0.85 (n-hexane/ethyl acetate 9:1 v/v), isolated yield 90%; IR (KBr, cm^{-1}): 2935, 2856, 1744, 1463, 1378, 1206; ^{1}H-NMR (500 MHz, $CDCl_3$): δ/ppm 5.37 (d, 1H, *J* = 4.53 Hz, C_6-<u>H</u>), 5.02 (q, 1H, *J* = 7.02 Hz, $C_{2'}$-<u>H</u>), 4.72-4.58 (m, 1H, C_3β-<u>H</u>), 3.40 (t, 2H, *J* = 6.80 Hz, $C_{13'}$-

H), 2.37 (t, 2H, *J* = 7.55 Hz, $C_{2'}$-H), 2.30 (d, 2H, *J* = 7.55 Hz, C_4-H), 2.07-1.41 (br.m, 11H), 1.47 (d, 3H, *J* = 7.02 Hz, $C_{2'}$-CH_3), 1.38-0.96 (br.m, 24H), 0.91 (d, 3H, *J* = 6.80 Hz, C_{21}-CH_3), 0.86 (d, 6H, *J* = 6.80 Hz, C_{27}-CH_3, C_{26}-CH_3), 0.67 (s, 3H, C_{18}-CH_3); ^{13}C-NMR ($CDCl_3$, 125): δ/ppm 173.18, 170.34, 139.29, 122.85, 74.96, 68.58, 56.62, 56.08, 49.92, 42.27, 39.66, 39.47, 37.84, 36.85, 36.52, 36.14, 35.76, 34.00, 34.00, 33.94, 32.79, 31.86, 31.81, 29.33, 29.27, 29.17, 29.00, 28.70, 28.19, 28.13, 27.99, 27.56, 24.79, 24.24, 23.79, 22.79, 22.53, 20.99, 19.28, 18.68, 16.92, 11.82; M.W: 704.44. ESI-MS *m/z*: 722.75 $(M + NH_4)^+$, 725 $(M+ 2 + NH_4)^+$. HRMS (*m/z*) calculated for $C_{41}H_{73}O_4NBr$ is 722.4717 and found at 722.4687 $(M + NH_4)^+$, 724.4661 $(M+ 2 + NH_4)^+$.

Cholesteryl lactate-oleic acid conjugate (or) 1-((cholesteryl)-oxy)-1-oxopropane-2-yl octadec-9-(Z)-enoate (**4h**):

Colorless viscous liquid; R_f = 0.81 (n-hexane/ethyl acetate 9:1 v/v), isolated yield 85%; IR (KBr, cm^{-1}): 2936, 2856, 1743, 1463, 1376, 1207, 758; ^{1}H-NMR (500 MHz, $CDCl_3$): δ/ppm 5.37 (d, 1H, *J* = 5.49 Hz, C_6-H), 5.36-5.32 (m, 2H, -HC=CH-), 5.02 (q, 1H, *J* = 7.17 Hz, $C_{2'}$-H), 4.70-4.62 (m, 1H, C_3β-H), 2.43-2.32 (m, 2H, $C_{4'}$-H), 2.30 (d, 2H, *J* = 7.78 Hz, C_4-H), 2.05-1.49 (br.m, 19H), 1.47 (d, 3H, *J* = 7.17 Hz, -$C_{2'}$-CH_3), 1.39-0.94 (br.m, 36H), 0.91 (d, 3H, *J* = 6.56 Hz, C_{21}-CH_3), 0.88 (t, 3H, *J* = 6.71 Hz, C_{19}-CH_3), 0.87 (d, 3H, *J* = 2.89 Hz, C_{27}-CH_3), 0.85 (d, 3H, *J* = 2.89 Hz, C_{26}-CH_3), 0.67 (s, 3H, C_{18}-CH_3). ^{13}C-NMR ($CDCl_3$, 125): δ/ppm 173.18, 170.35, 139.30, 129.94, 129.72, 122.85, 74.98, 68.58, 56.64, 56.10, 49.95, 42.28, 39.68, 39.48, 37.85, 36.86, 36.54, 36.15, 35.76, 33.96, 31.82, 31.88, 29.74, 29.67, 29.50, 29.29, 29.14, 29.07, 29.03, 28.19, 27.99, 27.57, 27.19, 27.15, 24.81, 24.25, 23.80, 22.79, 22.66, 22.53, 21.00, 19.28, 18.68, 16.91, 14.08, 11.82; M.W: 722.62. ESI-MS *m/z*: 741 $(M + NH_4)^+$. HRMS (*m/z*) calculated for $C_{48}H_{86}O_4N$ is 740.6551 and found at 740.6547 $(M + NH_4)^+$.

Cholesteryl lactate-ricinoleic acid conjugate (or) 1-((cholesteryl)-oxy)-1-oxopropane-2-yl -12'-(R)-hydroxyoctadec-9'(Z)-enoate (**4i**):

Colorless viscous liquid; R_f = 0.73 (n-hexane/ethyl acetate 9:1 v/v), isolated yield 87%; IR (KBr, cm^{-1}): 3688, 2936, 2855, 1742, 1458, 1374, 1207; ^{1}H-NMR (500 MHz, $CDCl_3$): δ/ppm 5.59-5.52 (m, 1H, $C_{12'}$-H), 5.44-5.39 (m, 1H, $C_{11'}$-H), 5.37 (d, 1H, *J* = 5.12 Hz, C_6-H), 5.02 (q, 1H, *J* = 7.17 Hz, $C_{2'}$-H), 4.70-4.61 (m, 1H, C_3β-H), 3.65-3.57 (m, 1H, $C_{14'}$-H (-C-OH)), 2.43-2.33 (m, 2H, $C_{13'}$-H), 2.30 (d, 2H, *J* =7.63 Hz, C_4-H), 2.21 (t, 2H, *J* =6.56 Hz, $C_{4'}$-H), 2.05-1.49 (brm, 16H), 1.47 (d, 3H, *J* = 7.17 Hz, $C_{2'}$-CH_3), 1.39-0.94 (br.m, 36H), 0.91 (d, 3H, *J* = 6.56 Hz, C_{21}-CH_3), 0.88 (t, 3H, *J* = 6.71 Hz, C_{19}-CH_3), 0.87 (d, 3H, *J* = 2.89 Hz, C_{27}-CH_3), 0.85 (d, 3H, *J* = 2.89 Hz, C_{26}-CH_3), 0.67 (s, 3H, C_{18}-CH_3); ^{13}C-NMR ($CDCl_3$, 125): δ/ppm 173.18, 170.35, 139.30, 133.34, 125.15, 122.85, 74.98, 71.50, 68.58, 56.64, 56.10, 49.95, 42.28, 39.68, 39.48, 37.85, 36.85, 36.82, 36.54, 36.15, 35.76, 35.31, 33.94, 31.86, 31.81, 29.55, 29.32, 29.10, 29.05, 28.98, 28.19, 27.98, 27.57, 27.35, 25.69, 24.79, 24.24, 23.80, 22.79, 22.59, 22.53, 20.99, 19.28, 18.68, 16.91, 14.06, 11.82; M.W: 738.62. ESI-MS *m/z*: 757 $(M + NH_4)^+$. HRMS (*m/z*) calculated for $C_{48}H_{86}O_5N$ is 756.6501 and found at756.6496 $(M + NH_4)^+$.

Antimicrobial activity:

Antimicrobial activity of the cholesteryl lactate-fatty acid conjugates was screened using well diffusion method against a panel of pathogenic bacterial strains, including *Bacillus subtilis* MTCC 121, *Staphylococcus aureus* MTCC 96, *Staphylococcus aureus* MLS16 MTCC 2940, *Micrococcus luteus* MTCC 2470, *Escherichia coli* MTCC 739, *Klebsiella planticola* MTCC 530, *Pseudomonas aeruginosa* MTCC 2453 and different *Candida* strains such as *Candida albicans* MTCC 183, *C. albicans* MTCC 227, *C. albicans* MTCC 854, *C. albicans* MTCC 1637, *C. albicans* MTCC 3017, *C. albicans* MTCC 3018, *C. albicans* MTCC 3958, *C. albicans* MTCC 4748, *C. albicans* MTCC 7315, *Candida parapsilosis* MTCC 1744, *Candida aaseri* MTCC 1962, *Candida glabrata* MTCC 3019, *Candida krusei* MTCC 3020 and *Issatchenkia hanoiensis* MTCC 4755 which were procured from the Microbial Type Culture Collection (MTCC), CSIR-Institute of Microbial Technology, Chandigarh, India [29, 30].

The pathogenic reference strains were seeded on the surface of Muller-Hinton agar Petri plates with 0.1 ml of previously prepared microbial suspensions individually containing 1.5×10^8 cfu/mL (equal to 0.5 McFarland standard). Wells of

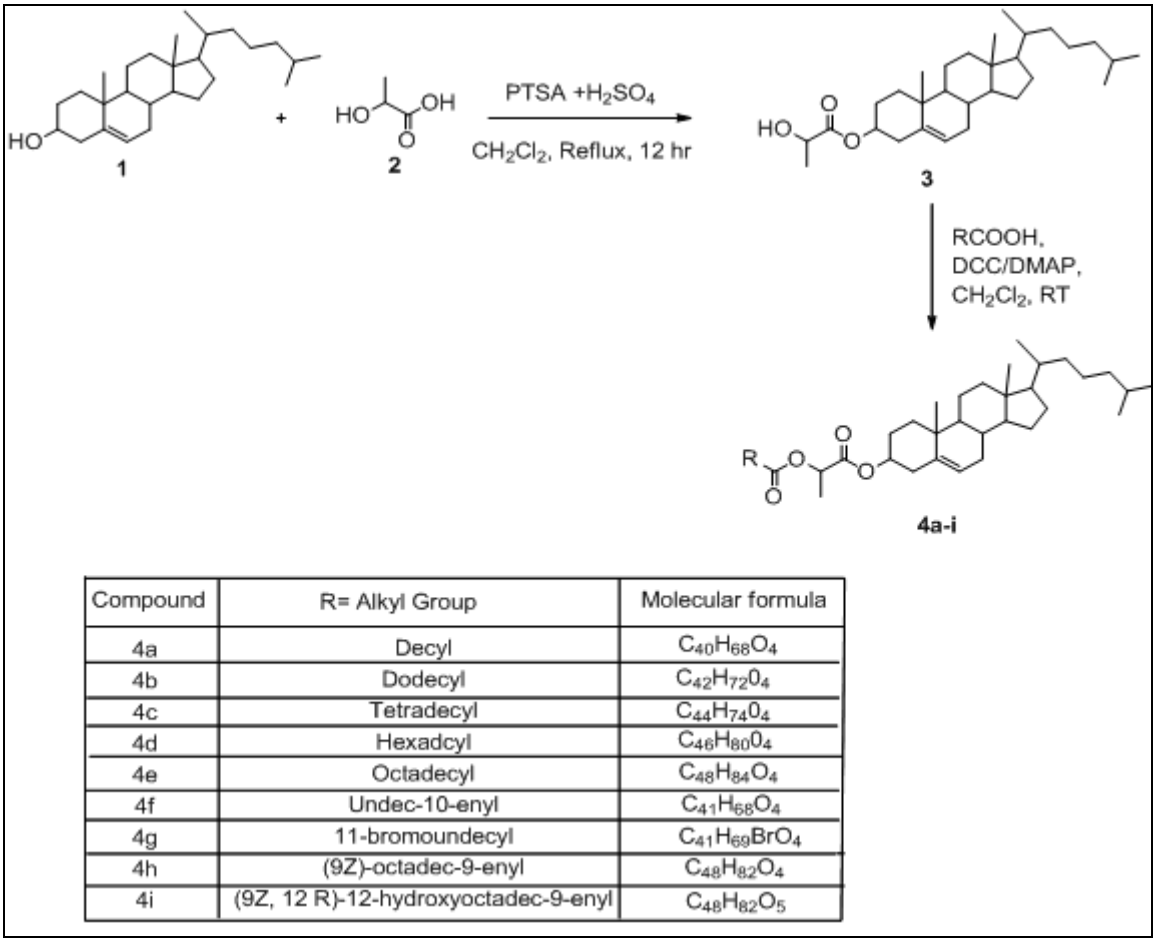
6.0 mm diameter were prepared in the media plates using a cork borer and the prepared cholesteryl lactate-fatty acid conjugates at a dose range of 125 - 1.95 µg was added to each well under sterile conditions in a laminar air flow chamber. Standard antibiotic solution of Ciprofloxacin (bacterial strains) and Miconazole (*Candida* strains) at a dose range of 125-1.95 µg/well and the well containing methanol served as positive and negative controls, respectively.

The plates were incubated for 24 h at 37 °C for bacterial and 30 °C for different *Candida* strains and the well containing the least concentration showing the inhibition zone was considered as the minimum inhibitory concentration. All experiments were carried out in duplicates and mean values are represented.

RESULTS AND DISCUSSION:

Synthesis: Cholesteryl lactate-fatty acid conjugates (**4a-i**) were synthesized in two steps as shown in **Scheme**. Initially, cholesterol (**1**) was esterified with lactic acid (**2**) to form cholesteryl lactate (**3**). Further, the obtained cholesteryl lactate (**3**) having the hydroxyl functionality was reacted with different fatty acids like decanoic, dodecanoic, tetradecanoic, hexadacanoic, octadacanoic, 10-undecenoic (undec-10-enoic), 11-bromo undecanoic, oleic ((9*Z*)-octadec-9-enoic) and ricinoleic ((9*Z*,12*R*)-12-hydroxyoctadec-9-enoic) acids to obtain the desired cholesteryl lactate-fatty acid conjugates (**4a-i**).

The synthesized esters were characterized by FT-IR, ^{1}H-NMR, ^{13}C-NMR, ESI-MS and HR-MS spectroscopic methods.



Compound	R= Alkyl Group	Molecular formula
4a	Decyl	$C_{40}H_{68}O_4$
4b	Dodecyl	$C_{42}H_{72}0_4$
4c	Tetradecyl	$C_{44}H_{74}0_4$
4d	Hexadcyl	$C_{46}H_{80}0_4$
4e	Octadecyl	$C_{48}H_{84}O_4$
4f	Undec-10-enyl	$C_{41}H_{68}O_4$
4g	11-bromoundecyl	$C_{41}H_{69}BrO_4$
4h	(9Z)-octadec-9-enyl	$C_{48}H_{82}O_4$
4i	(9Z, 12 R)-12-hydroxyoctadec-9-enyl	$C_{48}H_{82}O_5$

SCHEME: SYNTHESIS OF CHOLESTERYL LACTATE-FATTY ACID CONJUGATES (4a-i)

Antimicrobial Activity:

Antimicrobial activities of the cholesteryl lactate-fatty acid conjugates were screened using well diffusion method [29, 30]. From a structure-activity relationship perspective, nine cholesteryl lactate-fatty acid conjugates were synthesized by keeping cholesteryl lactate as an unchanged component, and the alkyl chain was altered at the other end i.e.,

conjugated with different alkyl chain fatty acids including saturated, unsaturated, bromoalkyl and hydroxy fatty acids. These synthesized compounds were further tested for antimicrobial activity against various pathogenic microorganisms comprising of Gram-positive bacteria like S*taphylococcus aureus* MTCC 96, *Bacillus subtilis* MTCC 121, *Staphylococcus aureus* MLS-16 MTCC 2940, *Micrococcus luteus* MTCC 2470, Gram-negative bacteria such as *Escherichia coli* MTCC 739, *Pseudomonas aeruginosa* MTCC 2453 and *Klebsiella planticola* MTCC 530 as well as a fungal strain, *Candida albicans* MTCC 3017. Miconazole and Ciprofloxacin were used as standard references and the results showed in **Table 1**.

TABLE 1: ANTIMICROBIAL ACTIVITY OF CHOLESTERYL LACTATE-FATTY ACID CONJUGATES (4a-i)

Compound	Minimum inhibitory concentration (µg/ml)							
	[a]S. a.	[a]B. s.	[a]S. m.	[a]M. l.	[b]K. p.	[b]E. c.	[b]P. a.	[c]C. a.
4a	62.5	>125	31.2	62.5	>125	31.2	62.5	>125
4b	>125	>125	62.5	>125	>125	>125	>125	>125
4c	>125	>125	>125	>125	>125	>125	>125	>125
4d	>125	>125	>125	>125	>125	>125	>125	>125
4e	>125	>125	>125	>125	>125	>125	>125	>125
4f	15.6	15.6	7.8	7.8	7.8	15.6	62.5	31.2
4g	15.6	15.6	15.6	7.8	15.6	>125	>125	31.2
4h	7.8	15.6	7.8	31.2	31.2	>125	>62.5	15.6
4i	7.8	31.2	31.2	15.6	31.2	31.2	>31.2	15.6
Miconazole	-	-	-	-	-	-	-	7.8
Ciprofloxacin	0.9	0.9	0.9	0.9	0.9	0.9	0.9	-

No activity

MIC-Minimum Inhibition Concentration and the values are mean of three determinations.

[a] Gram-positive bacteria; [b] Gram-negative bacteria; [c] fungus; *S. a.* (*Staphylococcus aureus* MTCC 96); *B. s.* (*Bacillus subtilis* MTCC 121); *S. m.* (*Staphylococcus aureus* MTCC 2940); *M. l.* (*Micrococcus luteus* MTCC 2470); *K. p.* (*Klebsiella planticola* MTCC 530); *E. c.* (*Escherichia coli* MTCC 739); *P. a.* (*Pseudomonas aeruginosa* MTCC 2453); *C. a.* (*Candida albicans* MTCC 3017).

Based on the obtained results, it was observed that the compounds **4e-i** showed good to moderate activity against Gram-positive microorganisms as compared to Gram-negative microbial strains. Among them, the compounds **4h** and **4i** containing oleic and ricinoleic acid conjugated to cholesteryl lactate (**3**) showed significant activity on S*taphylococcus aureus* MTCC 96 with MIC value of 7.8µg/ml. In addition, **4f** and **4g** with 10-undecenoic and bromoundecanoic acid were conjugated to cholesteryl lactate (**3**) as a functional unit inhibited the growth of the same strain with MIC value of 15.6µg/ml.

Some of the compounds like **4f**, **4g, 4h** and **4i** showed good antimicrobial activity against *Staphylococcus aureus* MTCC 2940 with MIC values of 7.8, 15.6, 7.8 and 31.2µg/ml respectively, and showed better growth inhibition against *Micrococcus luteus* MTCC 2470 with MIC values of 7.8, 7.8, 31.2 and 15.6µg/ml, respectively. Among all the cholesteryl lactate-fatty acid conjugates, compound **4f** with terminal unsaturation (10-undecenoic acid) on the carbon chain exhibited good antimicrobial activity against all the tested strains such as *Staphylococcus aureus* MTCC 96, *Bacillus subtilis* MTCC121, *Staphylococcus aureus* MLS-16 MTCC2940 and *Micrococcus luteus* MTCC 2470, *Klebsiella planticola* MTCC53, *Escherichia coli* MTCC739 and *Pseudomonas aeruginosa* MTCC2453 with 15.6, 15.6, 7.8, 7.8, 15.6, 7.8 and 62.5 µg/ml respectively.

Moreover, these compounds **4b-d** lacked both unsaturation and functional groups on the carbon chain and were not active even up to the maximum tested concentration of >125 µg/ml against all the tested strains. Whereas, the compound **4a** with decyl chain, a small chain saturated fatty acid conjugated to cholesteryl lactate showed moderate activity used in this study on *Staphylococcus aureus* MTCC 96, *Staphylococcus aureus* MTCC 2940, *Micrococcus luteus* MTCC 2470, *Escherichia coli* MTCC 739 and *Pseudomonas aeruginosa* MTCC 2453 strains except *Bacillus*

subtilis MTCC 121, *Klebsiella planticola* MTCC 530 and *Candida albicans* MTCC 3017 strains.

TABLE 2: ANTIFUNGAL ACTIVITY OF CHOLESTERYL LACTATE-FATTY ACID CONJUGATES (4f-i)

Fungal strain	Minimum inhibitory concentration (µg/ml)				
	4f	4g	4h	4i	Miconazole
C.a. 183	7.8	62.5	31.2	15.6	7.8
C.a. 227	15.6	31.2	62.5	31.2	7.8
C.a. 854	15.6	31.2	62.5	31.2	7.8
C.a. 1637	7.8	>125	62.5	15.6	7.8
C.a. 3018	7.8	>125	31.2	15.6	7.8
C.a. 3958	7.8	>125	>125	15.6	7.8
C.a. 4748	15.6	62.5	>125	15.6	7.8
C.a. 7315	31.2	31.2	62.5	31.2	7.8
C. p. 1744	15.6	31.2	31.2	31.2	7.8
C.as. 1962	7.8	15.6	15.6	31.2	7.8
C. g. 3019	31.2	31.2	31.2	15.6	7.8
C. k. 3020	7.8	31.2	31.2	31.2	7.8
I. s. 4755	7.8	31.2	62.5	15.6	7.8

MIC-Minimum Inhibition Concentration and the values are mean of three determinations. *C. a.* 183 *(Candida albicans* MTCC 183); *C. a.* 227 *(C. albicans* MTCC 227); *C. a.* 854 *(C. albicans* MTCC 854); *C. a.* 1637 *(C. albicans* MTCC 1637); *C. a.* 3017 *(C. albicans* MTCC 3017); *C. a.* 3018 *(C. albicans* MTCC 3018); *C. a.* 3958 *(C. albicans* MTCC 3958); *C. a.* 4748 *(C. albicans* MTCC 4748); *C. a.* 7315 *(C. albicans* MTCC 7315); *C. p.* 1744 *(Candida parapsilosis* MTCC 1744); *C. as.* 1962 *(Candida aaseri* MTCC 1962); *C. g.* 3019 *(Candida glabrata* MTCC 3019); *C. k.* 3020 *(Candida krusei* MTCC 3020) and *I. s.* 4755 *(Issatchenika hanoiensis* MTCC 4755).

The synthesized compounds were also tested against a panel different *Candida* strains and the results to this regard are presented in **Table 2**. The compounds **4f**, **4g**, **4h** and **4i** showed good to moderate antifungal activity. Among them, the compound **4f** showed promising activity on different *Candida* strains like *Candida albicans* MTCC 1637, *Candida albicans* MTCC 3018, *Candida albicans* MTCC 3958 and *Candida aaseri* MTCC 1962 with MIC value of 7.8µg/ml. From a structure-function relationship perspective, it was observed that the presence of unsaturation and functional unit on carbon chain proved important to exhibit the antifungal activity. This observation also corroborates the observations made in the earlier reports that compounds containing alkyl chain with unsaturation and functional group conjugated to cholesterol as a core moiety which exhibited promising antibacterial and antifungal activities [21, 22].

CONCLUSIONS: In the present work, nine cholesteryl lactate-fatty acid conjugates were synthesized that would function as antimicrobial agents. The antimicrobial studies revealed that some of the compounds exhibit promising activity against the Gram-positive microorganisms and moderate activity against the Gram-negative strains. Interestingly the compounds with unsaturation and functional groups (bromine and hydroxyl) on alkyl chain **4f-i**, exhibited pronounced growth inhibitory activity MIC values ranging from 7.8-31.2 µg/ml. On the other hand, these compounds also showed significant activity against most of the fungal strains. Based on the results, it can be summarized that cholesteryl lactate-fatty acid conjugates with unsaturation and functional groups on alkyl chain could be used to generate antimicrobial agents.

ACKNOWLEDGEMENTS: One of the authors YSR gratefully acknowledges the Department of Biotechnology, New Delhi for the financial assistance under sponsored project and Director, CSIR-IICT for providing the facilities.

REFERENCES

1. Kapil A: The challenge of antibiotic resistance: Need to contemplate. Indian Journal of Medical Research 2005; 121, 83–91.
2. Balsalobre LC, Dropa M and Matte HM: An overview of antimicrobial resistance and its public health significance. Brazilian Journal of Microbiology 2014; 45, 1–5.
3. Khan MYW, Ahmad F, Ahmad I and Osman SM: Nonedible seed oils as insect repellent. Journal of American Oil Chemical Society 1983; 60, 949–950.
4. Rauf A and Praveen H: Preparation, characterization and antimicrobial activity of fatty alkanoates. Indian Journal of Chemistry 2005; 44B, 1273–1276.

5. Mohini Y, Prasad RBN, Karuna MSL, Ganesh Kumar C, Poornima M, Sujitha P: Synthesis of fatty acid Schiff base esters as potential antimicrobial and chemotherapeutic agents. Journal of Medicinal Chemistry Research 2013; 22, 4360–4366.
6. Satyavani T, Mohini Y, Karuna MSL, Prasad RBN, Ganesh Kumar C, Poornima M, Sujitha P: Synthesis and biological evaluation of fatty imidazolines. Journal of Medicinal Chemistry Research 2014; 23, 3617–3623.
7. Ma DC, Yoon AJ, Faull KF, Desharnais R, Zemanick ET, Porter E: Cholesteryl Esters are Elevated in the Lipid Fraction of Bronchoalveolar Lavage Fluid Collected from Pediatric Cystic Fibrosis Patients. PLoS ONE 2015; 10, e0125326.
8. Mujeebur RVP, Mukhtar S, Ansari WH and Lemiere G: Synthesis, stereochemistry and biological activity of some novel long alkyl chain substituted thiazolidin-4-ones and thiazan-4 one from 10-undecenoic acid hydrazide. European Journal of Medicinal Chemistry 2005; 40, 173–184.
9. Ahmed SM, Ahmad F and Osman SM: Preparation and characterization of derivatives of isoricinoleic acid and their antimicrobial activity. Journal of American Oil Chemical Society 1985; 62, 1578–1580.
10. Orhan I, Ozcelik B, Sener B: Evaluation of antibacterial, antifungal, antiviral, and antioxidant potentials of some edible oils and their fatty acid profiles. Turkish Journal of Biology 2011; 35, 251–258.
11. Chuealee R, Wiedmann TS, Srichana T: Physicochemical properties and antifungal activity of amphotericin B incorporated in cholesteryl carbonate esters. Journal of Pharmaceutical Sciences 2011; 100, 1727–1735.
12. Pugliese PT and Pugliese PM: zwitterionic-fatty acid compounds having anti-inflammatory properties. U S Patent, U.S. 6,448,251. Chemical Abstracts 2002; 137, 216818t.
13. Viklunda F, Alanderb J and Hulta K: Antioxidative Properties and Enzymatic Synthesis of Ascorbyl FA Esters. Journal of American Oil Chemical Society 2003; 80, 795–799.
14. Mallavadhani UV, Mahapatra A, Raja SS and Manjula C: Antifeedant Activity of Some Pentacyclic Triterpene Acids and Their Fatty Acid Ester Analogues. Journal of Agricultural and Food Chemistry 2003; 51, 1952–1955.
15. Grunberg E and Titsworth EH: Chemotherapeutic Properties of Heterocyclic Compounds: Monocyclic Compounds with Five-Membered Rings. Annual Review of Microbiology 1973; 27, 317–346.
16. Takahashi H, Kosaka M, Watanabe Y, Nakade K and Fukuyama Y: Synthesis and neuroprotective activity of bergenin derivatives with antioxidant activity. Bioorganic Medicinal Chemistry 2003; 11, 1781–1788.
17. Gupta R, Pathak D and Jindal DP: Synthesis and biological activity of azasteroidal [3,2-c]- and [17,16-c] pyrazoles. European Journal of Medicinal Chemistry 1996; 31, 241–247.
18. Manson AJ, Stonner FW and Neumann HC: Steroidal Heterocycles. VII. Androstano[2,3-d]isoxazoles and Related Compounds. Journal of Medicinal Chemistry 1963; 6, 1–9.
19. Hirschmann R, Steinberg NG, Buchschacher P, Fried GH, Kent GJ and Tishler M: Synthesis and Structure of Steroidal 4-Pregneno [3,2-c] Pyrazoles. A Novel Class of Potent Anti-Inflammatory Steroids. Journal of American Chemical Society 1963; 85, 120–122.
20. Swaminathan K and Mason KV: PCT Int Appl WO, 03, 86,348. Chemical Abstracts 2003; 139, 341744h.
21. Kedika B, Patri SV: Synthesis and gene transfer activities of novel serum compatible reducible tocopherol-based cationic lipids. Molecular Pharmaceutics 2012; 9, 1146-1162.
22. Medvedeva DA, Maslov MA, Serikov RN, Morozova NG, Serebrenikova GA, Sheglov DV, Latyshev AV, Vlassov VV and Zenkova MA: Novel Cholesterol-Based Cationic Lipids for Gene Delivery. Journal of Medicinal Chemistry 2009; 52, 6558–6568.
23. Khan SA: Synthesis, characterization and in vitro antibacterial activity of new steroidal 5-en-3-oxazolo and thiazoloquinoxaline. European Journal of Medicinal Chemistry 2008; 43, 2040–2044.
24. Khan SA, Kumar P, Joshi R, Iqbal PF and Saleem K: Synthesis and in vitro antibacterial activity of new steroidal thiosemicarbazone derivatives. European Journal of Medicinal Chemistry 2008; 43, 2029–2034.
25. Anjaneyulu ASR, Raju DVSN and Rao SS: Chemical Examination of Pergularia-Extensa NE-BR. Indian Journal of Chemistry 1998; 37B, 318–320.
26. Menard N, Tsapis N, Poirier C, Arnauld T, Moine L, Lefoulon F, Pean JM, Fattal E: Physicochemical characterization and toxicity evaluation of steroid-based surfactants designed for solubilization of poorly soluble drugs. European Journal of Pharmaceutical Sciences 2011; 44, 595-601.
27. Banday MR, Farshori NN, Ahmad A, Khan AU and Rauf A: Synthesis and characterization of novel fatty acid analogs of cholesterol: *In vitro* antimicrobial activity. European Journal of Medicinal Chemistry 2010; 45, 1459–1464.
28. Thai QD, Safiehkhatoon M, Patricia C, Suda A, Adelina P, Annie C, Beth M, Kym FF, William E, Su MC, Gary F, Catherine FC and Krishna F. Edith, P: Lipids Including Cholesteryl Linoleate and Cholesteryl Arachidonate Contribute to the Inherent Antibacterial Activity of Human Nasal Fluid. Journal of Immunology 2008; 181, 4177–4187.
29. Amsterdam D: Susceptibility testing of antimicrobials in liquid media. In: V. Loman (Ed.) Antibiotics in Laboratory Medicine, 4th Edition, Williams and Wilkins, Baltimore, MD. 1996; 52–111.
30. Kamal A, Hussaini SM, Sucharitha ML, Poornachandra Y, Sultana F, Ganesh Kumar C: Synthesis and antimicrobial potential of nitrofuran-triazole congeners. Organic Biomolecular Chemistry 2015; 13, 9388-9397.

How to cite this article:
Yasa SR, Vijayalakshmi P, Yedla P and Chityal GK: Synthesis and Characterization of Novel Cholesteryl Lactate Based Fatty Acid Analogs and there *in vitro* Antimicrobial Activity. Int J Pharm Sci Res 2016; 7(4): 1462-70.doi: 10.13040/IJPSR.0975-8232.7(4).1462-70.