European Journal of Chemistry

Journal webpage: www.eurjchem.com

Synthesis, characterization, physical and biological properties of some cholesterol derivatives

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ARTICLE INFORMATION

DOI: 10.5155/eurjchem.7.2.206-212.1410

Received: 10 February 2016
Received in revised form: 08 April 2016
Accepted: 10 April 2016
Published online: 30 June 2016
Printed: 30 June 2016

KEYWORDS

Steriods
Cholesterol
Liquid crystal
Thermal analysis
Mitsunobu reaction
Antimicrobial activity

ABSTRACT

A series of new cholesterol derivatives have been prepared via Mitsunobu reaction. The reaction was monitored by thin-layer chromatography (TLC) technique. All new compounds were characterized by melting points, elemental analysis, FT-IR, ^{1}H, ^{13}C and 2D-NMR spectroscopy. The antibacterial and antifungal activity of these derivatives was also determined. The phase transitions of the prepared derivatives were measured with the aid of differential scanning calorimetry. The textures of the mesophases have been determined with a hot stage equipped polarizing microscope, also the electrical conductivity of the solutions of these liquid crystals measured by electrical conductivity meter. The analysis showed that these prepared derivatives were liquid crystals.

Cite this: *Eur. J. Chem.* **2016**, *7(2)*, 206-212

1. Introduction

Steroids are derivative lipids [1], organic materials produced naturally from all the life forms that include microorganisms of plants and animals [2]. Cholesterol is one of the most important steroids [3], and is a very important substance found in the body of the organism enters in the composition of the animal cell wall [4].

Liquid crystals are mesophases between a crystalline phase (Solid phase) and isotropic phase (Liquid phase) [5,6]. Historically, the first liquid crystalline compound was discovered by the Austrian botanist, Friedrich Reinitzer in 1888 [7]. He had observed that when first heating of cholesteryl benzoate showed not a clear liquid state, but when increase the heating produced a clear liquid [8]. Liquid crystalline phases can be classified into two classes: Lyotropic LC and Thermotropic LC [9]. The phase of lyotropic LC characteristic ordering depends on solvent [10]. While the phase of thermotropic LC characteristic ordering depends only on temperature [11], thermotropic LC phases are: nematic, smectic, and cholesteric [12]. Cholesteric liquid crystals have many advanced technology applications [13] like liquid crystal displays [14], optical filters [15], imaging systems [16], chromatography techniques [17], optical storage systems [18], temperature sensors [19] and medical thermography [20].

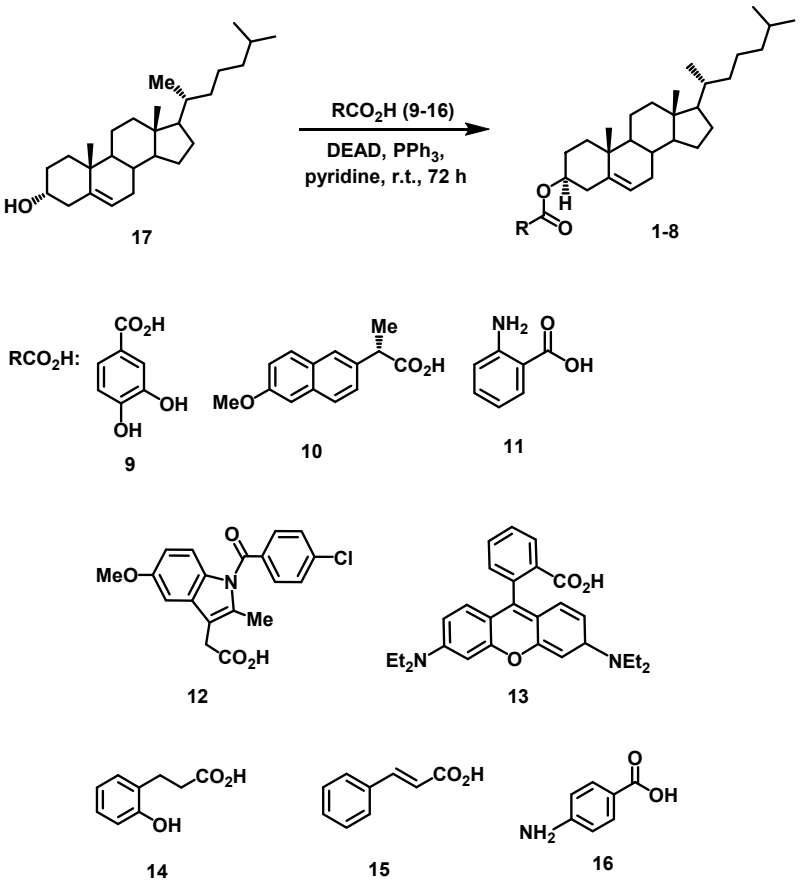
The aims of the study are synthesis of a new novel series of β-ester derivatives of cholesterol with inversion in configuration at C-3 by applying of Mitsunobu reaction and study the physical and biological properties.

2. Experimental

2.1. Instrumentation

The melting point was determined on a digital melting point instrument (SMP/Steuart). NMR were recorded on Avance III, Bruker spectrophotometer (400 MHz (^{1}H) and 100 MHz (^{13}C)) in DMSO-d_6 with TMS as internal standard and on the δ scale in ppm. Elemental analysis was performed by the Elemental Analyzer (Vario, Shimadzu, Japan). The FT-IR spectra were recorded in KBr pellets using Biotech, CO. Ltd. UK, FT-IR spectrometer. Polarized optical microscope analysis data were collected on a LEICA DM 2500P. Differential scanning calorimetry analysis data were collected on a LINSEIS STA PT-1000 DSC. Electrical conductivity analysis data were collected on a SensoDirect/Con200/Lovibond.

ISSN 2153-2249 (Print) / ISSN 2153-2257 (Online)
http://dx.doi.org/10.5155/eurjchem.7.2.206-212.1410



Scheme 1

Analytical silica gel TLC plates $60F_{254}$ were purchased from Merck.

2.2. Synthesis

2.2.1. General procedure for the synthesis of 3β-substituted aryl ester derivatives of cholesterol by applying Mitsunobu reaction (1-8)

Carboxylic acids (**9**-**16**) (2.5 mmol), triphenylphosphine (Ph_3P) (2.5 mmol, 0.65 g) and diethylazodicarboxylate (DEAD) (2.5 mmol, 0.43 g) were added to a solution of cholesterol (2.5 mmol, 1 g) in dry pyridine (15 mL) and the mixture was stirred at 40 °C for 72 hours. The reaction was monitored by TLC (*n*-hexane:ethyl acetate, 2:3, *v*:*v*) [21]. The purity of products were checked by SiO_2 column chromatography (5 g) by mixture (MeOH:$CHCl_3$, 3:2, *v*:*v*) as elution afforded the pure desired products (Scheme 1).

(10R, 13R, 17R)-10, 13-dimethyl-17-((R)-6-methylheptan-2-yl)-2, 3, 4, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-yl 3, 4-dihydroxybenzoate (**1**): From 2,4-dihydroxy benzoic acid (9). Color: Yellow. Yield: 92%. M.p.:133-135 °C. FT-IR (KBr, ν, cm^{-1}): 1050-1250 (C-O, ester), 1400 (C-O, phenol), 1600 (C=C, aliphatic and aromatic), 1750 (C=O, ester), 3050 (=C-H, aliphatic), 3060 (C-H, aromatic), 3400 (O-H, phenol). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 0.69 (s, 3H, H18), 0.97 (d, 3H, H21), 1.04-1.05 (d, 6H, H26 + H27), 1.11 (s, 3H, H19), 1.131 (m, 1H, H9), 1.138 (m, 2H, H1B), 1.18-1.20 (m, 4H, H14 + H15B + H17), 1.208 (m, 2H, H24), 1.209 (m, 2H, H22), 1.21 (m, 2H, H23), 1.411 (m, 2H, H2B), 1.43 (m, 2H, H20 + H8), 1.44 (m, 2H, H12B), 1.45 (m, 2H, H16B), 1.52 (m, 2H, H11B), 1.53 (m, 2H, H7B), 1.57 (m, 1H, H25), 2.16 (m, 2H, H4), 2.19 (m, 1H, H3), 4.25 (s, 1H, OH), 5.24 (br, s, 1H, OH), 5.29 (t, 1H, H6), 6.58 (d, 1H, 5'Harom), 7.40 (s, 1H, 2'Harom), 7.43 (d, 1H, 6'Harom). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 14.8 (Me-18), 19.2 (Me-21), 19.5 (Me-19), 21.1 (C-11), 23.0 (C-23), 23.1 (C-26+ C-27), 23.8 (C-16), 24.4 (C-15), 27.9 (C-14), 28.0 (C-25), 31.99 (C-2), 32.0 (C-8), 32.1 (C-7), 36.8 (C-20), 37.5 (C-22), 37.7 (C-10), 39.55 (C-9), 39.8 (C-1), 40.1 (C-17), 40.21 (C-12), 40.29 (C-4), 40.68 (C-3), 40.75 (C-24), 40.80 (C-13), 116.3 (C-2' + C-5'), 123.1 (C-6'), 128.3 (C-1'), 142.1 (C-5), 144.7 (3'COH), 147.0 (4'COH), 164.8 (CO_2). Anal. calcd. for $C_{34}H_{50}O_4$: C, 78.12; H, 9.64. Found: C, 77.89; H, 9.56%.

(2S)-((10R, 13R, 17R)-10, 13-dimethyl-17-((R)-6-methyl heptan-2-yl)-2, 3, 4, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17-tetra decahydro-1H-cyclopenta[a]phenanthren-3-yl) 2-(6-methoxy naphthalen-2-yl)propanoate (2): From (S)-2-(6-methoxy naphthalen-2-yl)propanoic acid (10) (0.57 g). Color: Yellow. Yield: 89%. M.p.: 113-115 °C. FT-IR (KBr, ν, cm^{-1}): 1050-1200 (C-O, ester), 1356 (C-O, phenol), 1531 (C=C, aliphatic and aromatic), 1603 (C=O, ester), 3051 (=C-H, aliphatic), 3060 (C-H, aromatic). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 0.96 (s, 3H, H18), 1.05 (d, 3H, H21), 1.112-1.117 (d, 6H, H26 + H27), 1.13 (s, 3H, H19), 1.14 (m, 1H, H9), 1.15 (m, 2H, H1B), 1.190-1.209 (m, 4H, H14 + H15B + H17), 1.22 (m, 2H, H24), 1.23 (m, 2H, H22), 1.24 (m, 2H, H23), 1.42 (m, 2H, H2B), 1.44 (m, 2H, H20 + H8), 1.48 (m, 2H, H12B), 1.49 (m, 2H, H11B), 1.50 (d, 3H, 2"H), 1.51 (m, 2H, H7a), 1.52 (m, 2H, H16B), 1.55 (m, 1H, H25), 2.14 (m, 2H, H4), 3.30 (m, 1H, H3), 4.06 (s, 3H, OMe), 4.079 (d, 1H, 1"H), 5.279 (t, 1H, H6), 7.28 (d, 1H, 7' Harom), 7.30 (s, 1H, 5'Harom), 7.419 (d, 1H, 8' Harom), 7.76 (d, 2H, 2'Harom +

10'Harom), 7.77 (d, 1H, 3' Harom). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 12.1 (Me-18), 14.8 (C-2"), 19.2 (Me-21), 19.5 (Me-19), 21.3 (C-11), 23.1 (C-23), 23.2 (C-26+C-27), 23.9 (C-16), 24.2 (C-15), 27.9 (C-14), 28.0 (C-25), 32.0 (C-2), 32.1 (C-8), 32.2 (C-7), 39.4 (C-20+C-22), 39.6 (C-10), 40.01 (C-1), 40.02 (C-9), 40.20 (OMe), 40.28 (C-14), 40.29 (C-17), 40.30 (C-12), 40.35 (C-4), 40.41 (C-1"), 40.71 (C-24), 40.75 (C-13), 40.79 (C-3), 107.0 (C-5'), 126.9 (C-7'), 127.1 (C-6), 128.5 (C-3+C-10'), 128.9 (C-8'+C-9'), 129.2 (C-4'+C-2'), 129.30 (C-1'), 134.0 (C-5), 156.8 (C-6'), 164.8 (CO_2). Anal. calcd. for $C_{41}H_{58}O_3$: C, 82.22; H, 9.76. Found: C, 81.87; H, 9.44%.

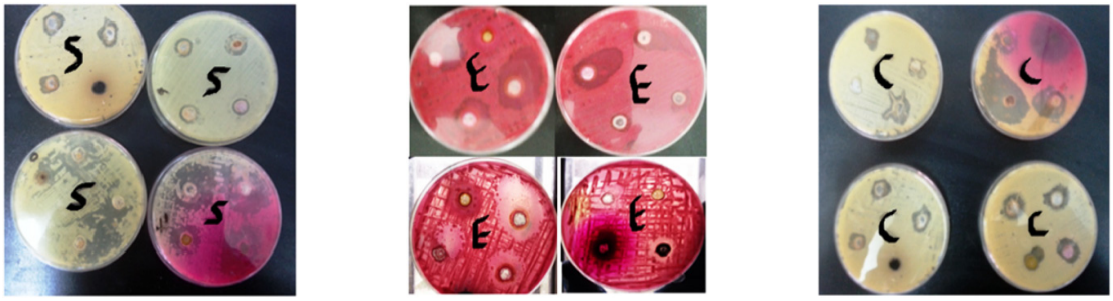
(10R, 13R)-10, 13-dimethyl-17-((R)-6-methylheptan-2-yl)-2, 3, 4, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-yl 2-aminobenzoate (**3**): From 2-aminobenzoicacid (11) (0.34 mg). Color: White. Yield: 89%. M.p.: 108-110 °C. FT-IR (KBr, ν, cm^{-1}): 1019-1209 (C-O, ester), 1354 (C-N, aromatic), 1516 (N-H, b, aromatic amine), 1609 (C=C, aliphatic and aromatic), 1754 (C=O, ester), 3035 (=C-H, aliphatic), 3074 (C-H, aromatic), 331 -3375 (N-H, s, aromatic amine). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 0.73 (s, 3H, H18), 1.04 (d, 3H, H21), 1.10 (d, 6H, H26+H27), 1.12 (s, 3H, H19), 1.135 (m, 1H, H9), 1.139 (m, 2H, H1B), 1.18-1.19 (m, 4H, H14+H15B+H17), 1.201 (m, 2H, H24), 1.205 (m, 2H, H22), 1.210 (m, 2H, H23), 1.425 (m, 2H, H2B), 1.448 (m, 2H, H20+H8), 1.50 (m, 2H, H12B), 1.529 (m, 2H, H11B), 1.535 (m, 2H, H7B), 1.54 (m, 2H, H16B), 1.57 (m, 1H, H25), 2.16 (m, 2H, H4), 3.31 (m, 1H, H3), 5.29 (t, 1H, H6), 6.53 (m, 1H, 5'Harom), 6.735 (d, 1H, 3' Harom), 6.77 (m, 1H, 4' Harom), 7.22 (d, 1H, 6'Harom), 8.62, (br. s, 2H, NH_2). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 12.2 (Me-18), 19.2 (Me-21), 19.6 (Me-19), 21.3 (C-11), 23.0 (C-23), 23.2 (C-27+C-26), 23.9 (C-16), 24.4 (C-15), 28.0 (C-14), 28.1 (C-25), 32.1 (C-2), 32.11 (C-8), 32.12 (C-7), 37.5 (C-20), 38.0 (C-22), 39.5 (C-10), 39.6 (C-9), 40.1 (C-1), 40.19 (C-17), 40.25 (C-12), 40.30 (C-4), 40.60 (C-3), 40.80 (C-24), 40.89 (C-13), 110.2 (C-1'), 116.0 (C-5'), 118.0 (3'-CMe), 120.4 (C-6), 130.6 (C-6'), 132.0 (C-4'), 141.9 (C-5), 151.6(C-2'), 165.0 (CO_2). Anal. calcd. for $C_{34}H_{51}NO_2$: C, 80.74; H, 10.16; N, 2.77. Found: C, 80.46; H, 9.95; N, 2.49%.

(10R, 13R)-10, 13-dimethyl-17-((R)-6-methylheptan-2-yl)-2, 3, 4, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-yl 2-(1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1H-indol-3-yl)acetate (**4**): From 2-(1-(4-chloro benzoyl)-5-methoxy-2-methyl-1H-indol-3-yl)acetic acid (12) (0.8 g). Color: Brown. Yield: 94%. M.p.: 102-104 °C. FT-IR (KBr, ν, cm^{-1}): 1026-1063 (C-O, ester), 1370 (C-O, phenol), 1410 (C-N, hetero), 1562 (C=C, aliphatic and aromatic), 1650 (C=O, amid), 1718 (C=O, ester), 3043 (=CH, aliphatic), 3059 (C-H, aromatic). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 0.87 (s, 3H, H18), 1.10 (d, 3H, H21), 1.11-1.12 (d, 6H, H26 H27), 1.14 (s, 3H, H19), 1.17 (m, 1H, H9), 1.24-1.26 (m, 6H, H1B + H14 + H15B + H17), 1.34 (m, 6H, H22+H24+H23), 1.37 (m, 4H, H20 + H8 + H12B), 1.46 (m, 2H, H11B), 1.47 (m, 2H, H7A), 1.49 (m, 2H, H16A), 1.53 (m, 1H, H25), 1.86 (m, 2H, H2B), 2.28 (m, 2H, H4), 2.29 (s, 2H, 1"H), 3.27 (m, 1H, H3), 3.315 (s, 3H, C2'Me), 3.57 (s, 3H, 5'OMe), 5.29 (t, 1H, H6), 7.61-7.625 (m, 7H, Harom). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 12.18 (Me-18), 14.89 (C2'-Me), 19.13 (Me-21), 19.57 (Me-19) 21.21 (C-11), 22.95 (C-23), 22.96 (C-27 +C-26), 23.80 (C-16), 23.85 (C-15), 27.80 (C-14), 27.82 (C-25), 32.15 (C-2), 32.16 (C-8), 32.17 (C-7), 32.18 (C-1"), 35.60 (C-20), 37.0 (C-22), 39.50 (C-10), 39.92 (C-1), 40.35 (C-9), 40.40 (C-12), 40.45 (C-4), 40.50 (5'OMe), 40.58 (C-17), 42.60 (C-24), 42.70 (C-13), 50.42 (C-3), 113.29 (C-3'), 120.70 (C-4'), 128.0 (C-7'), 129.04 (C-6'), 130.0 (C-6), 131.97-131.99 (C-arom), 132.31 (C-3a'), 132.32 (C-5 + C-Cl), 156.93 (C-5'), 167.78 (CO2+ NCOAr). Anal. calcd. for $C_{46}H_{60}ClNO_4$: C, 76.06; H, 8.33; N, 1.93. Found: C, 75.80; H, 8.18; N, 1.71%.

(10R, 13R)-10, 13-dimethyl-17-((R)-6-methylheptan-2-yl)-2, 3, 4, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-yl 2-(3, 6-bis(diethylamino)-9H-xanthen-9-yl)benzoate (**5**): From 2-(3,6-*bis*(diethyl amino)-9*H*-xanthen-9-yl)benzoic acid (13) (1.1 g). Color: Pink. Yield: 90%. M.p.: 198-200 °C. FT-IR (KBr, ν, cm^{-1}): 1037-1242 (C-O, ester), 1100 (C-O, hetero), 1254 (C-N, aliphatic), 1300 (C-N, aromatic), 1592 (C=C, aliphatic and aromatic), 1746 (C=O, ester), 3018 (=C-H, aliphatic), 3040 (C-H, aromatic). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 0.87 (s, 3H, H18), 1.01 (d, 3H, H21), 1.03-1.04 (d, 6H, H26+H27), 1.18 (s, 3H, H19), 1.189 (m, 1H, H9), 1.193 (m, 2H, H1B), 1.21-1.255 (m, 4H, H14 + H15B + H17), 1.26 (m, 2H, H24), 1.27 (m, 2H, H22), 1.28 (m, 2H, H23), 1.29 (t, 12H, 16'H+19'H+23'H+20'H), 1.461 (m, 2H, H20+H8), 1.481 (m, 2H, H12B), 1.489 (m, 2H, H11B), 1.49 (m, 2H, H7B), 1.531 (m, 2H, H16B), 1.55 (m, 1H, H25),1.60 (m,2H,H2), 2.16 (m, 2H, H4), 4.06 (m, 1H, H3), 5.26 (m, 8H, 17'H + 18'H + 22'H + 21'H), 7.15 (t, 1H, H6), 7.16 (s, 1H, 9'H), 7.162-7.168 (d, 2H, 2'Harom + 7'Harom), 7.27 (s, 2H, 5'Harom + 4'Harom), 7.41-7.419 (d, 2H, 8' Harom + 1'Harom), 7.56-7.58 (m, 3H, 13'H + 14'H + 15'Harom), 7.76-7.78 (d, 1H, 12'Harom). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 12.2 (Me-18), 14.9 (C-16'+C-19'+C-23'+C-20'), 19.2 (Me-21), 19.6 (Me-19), 21.3 (C-11), 23.1 (C-23), 23.2 (C-26+C-27), 24.1 (C-16), 24.3 (C-15), 27.9 (C-14), 28.0 (C-25), 32.09 (C-2), 32.15 (C-8), 32.19 (C-7), 37.7 (C-20), 39.5 (C-22), 39.8 (C-10), 40.0 (C-1), 40.31 (C-12), 40.38 (C-4), 40.8 (C-24), 40.81 (C-13), 40.9 (C-9), 42.6 (C-9'), 42.8 (C-17'+C-18 '+C-22'+C-21'), 44.0 (C-17), 54.0 (C-3), 106.71 (C-5'), 106.72 (C-4'), 119.1 (C-7'), 119.2 (C-2'), 128.9 (C-6), 129.0 (8-a' + 9-a'), 129.4 (C-13'), 130.3 (C-1'+C-8'+C-15'), 130.7 (C-11'+C-12'), 130.8 (C-14'), 141.0 (C-5), 142.1 (C-10'), 144.4 (C-3'), 148.0 (5-a'), 148.1 (4-a'), 164.8 (CO_2). Anal. calcd. for $C_{55}H_{76}N_2O_3$: C, 81.23 ; H, 9.42; N, 3.44. Found: C, 80.95; H, 9.30; N, 3.21%.

(10R, 13R)-10, 13-dimethyl-17-((R)-6-methylheptan-2-yl)-2, 3, 4, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-yl 3-(2-hydroxyphenyl)propanoate (**6**): From 2-hydroxy benzyl acetic acid (14), (0.4 g). Color: Brown. Yield: 92%. M.p: 125-127 °C. FT-IR (KBr, ν, cm^{-1}): 1037-1239 (C-O, ester), 1350 (C-O, phenol), 1539 (C=C, aliphatic and aromatic), 1740 (C=O, ester), 3029 (=C-H, aliphatic)), 3070 (C-H, aromatic), 3360 (O-H, phenol). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 0.67 (s, 3H, H18), 0.88 (d, 3H, H21), 0.910-0.911 (d, 6H, H26 + H27), 0.93 (s, 3H, H19), 0.97 (m, 1H, H9), 0.99 (t, 2H, H1B), 1.189-1.220 (m, 4H, H14 + H15B + H17), 1.355 (m, 4H, H22 + H24), 1.357 (m, 2H, H23), 1.409 (m, 2H, H2B), 1.410 (m, 2H, H20+H8), 1.415 (m, 2H, H12B), 1.50 (m, 2H, H11B), 1.51 (m, 2H, H7B) 1.52 (m, 2H, H16B), 1.54 (m, 1H, H25), 2.15 (m, 2H, H4), 4.059 (m, 1H, H3), 4.35 (m, 2H, 3"H), 4.37 (m, 2H, 4"H), 5.28 (t, 1H, H6), 7.631-7.639 (m, 4H, Harom), 8.00 (s, 1H, OH). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 12.1 (Me-18), 19.5 (Me-21), 19.6 (Me-19), 21.3 (C-11), 23.0 (C-4"), 23.1 (C-23+C-26+C-27), 24.0 (C-16), 24.4 (C-15), 27.9 (C-14), 28.1 (C-25), 32.18 (C-2), 32.19 (C-8), 32.2 (C-7), 37.7 (C-20), 37.8 (C-3"), 39.6 (C-22), 39.9 (C-10), 40.0 (C-1), 40.39 (C-12), 40.4 (C-4), 40.85 (C-24), 40.89 (C-13), 41.1 (C-9), 42.7 (C-17), 56.5 (C-3), 131.0 (C-6), 133.0-133.9 (C_{arom}), 141.0 (C-5), 155.0 (C2'-OH), 166.0 (CO_2). Anal. calcd. for $C_{36}H_{54}O_3$: C, 80.85; H, 10.18. Found: C, 80.66; H, 10.10%.

(10R, 13R)-10, 13-dimethyl-17-((R)-6-methylheptan-2-yl)-2, 3, 4, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-yl cinnamate (**7**): From cinammic acid (15), (0.37 g). Color: Yellow. Yield: 72%. M.p.: 113-115 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 1.02 (s, 3H, H-18), 1.15 (d, 3H, H-21), 1.23-1.24 (d, 6H, H-26+H-27), 1.255 (s, 3H, H-19), 1.27 (m, 1H, H-9), 1.28 (m, 2H, H-1B), 1.36-1.40 (m, 4H, H-14+H-15B+H-17), 1.41 (m, 4H, H-22+H-24), 1.419 (m, 2H, H-23), 1.50 (m, 2H, H-2B), 1.52 (m, 2H, H-20+H-8), 1.53 (m, 2H, H-12B), 1.545 (m, 2H, H-11B), 1.555 (m, 2H, H-7B), 1.557 (m, 2H, H-16A), 1.57 (m, 1H, H-25), 2.11 (m, 2H, H-4), 3.29 (m, 1H, H-3), 5.28 (t, 1H, H-6), 7.45 (d, 1H, 3'H Olefin), 7.56 (d, 1H, 4'H Olefin), 7.58-7.60 (m, 5H, 2"Harom + 6"Harom + 3"Harom + 4"Harom + 5"Harom). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 12.17 (Me-18), 19.12 (Me-21), 19.57 (Me-19), 21.20 (C-11), 22.97 (C-23), 22.98 (C-26+C-27), 23.90 (C-16), 24.35 (C-15),



(a) (b) (c)

Figure 1. Zone of inhibition of compounds against (a) *Staphylococcus aureues*, (b) *Escherichia coli*, (c) *Candida albicans.*

28.10 (C-14), 28.13 (C-25), 32.00 (C-2), 32.10 (C-8), 32.13 (C-7), 36.28 (C-20+C-22), 36.68 (C-10), 37.50 (C-1), 39.50 (C-12 + C-4), 40.90 (C-9), 42.70 (C-24), 42.75 (C-17), 42.79 (C-13), 56.39 (C-3), 129.1 (C-3'), 129.2 (C-6), 129.5-130.0 (C-arom), 133.18 (C-1'), 140.0 (C-5), 141.98 (C-4'), 167.0 (CO_2H). Anal. calcd. for $C_{36}H_{52}O_2$: C, 83.67; H, 10.14. Found: C, 83.45; H, 9.98%.

(10R, 13R)-10, 13-dimethyl-17-((R)-6-methylheptan-2-yl)-2, 3, 4, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-yl 4-aminobenzoate (**8**): From 4-Amino benzoic acid (16), (0.34 g). Color: Brown. Yield: 89%. M.p.: 138-140 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 0.69 (s, 3H, H-18), 0.929 (d, 3H, H-21), 0.97-0.98 (d, 6H, H-26+H-27), 1.02 (s, 3H, H-19), 1.03 (m, 1H, H-9), 1.12 (m, 2H, H1B), 1.15-1.18 (m, 4H, H-14+H-15B+H-17), 1.19 (m, 4H, H-22+H-24), 1.35 (m, 2H, H-23), 1.42 (m, 2H, H-2B), 1.43 (m, 2H, H-20+H-8), 1.44 (2H, H-12B), 1.51 (m, 2H, H-11B), 1.52 (m, 2H, H-7A), 1.535 (m, 2H, H-16B), 1.55 (m, 1H, H-25), 2.17 (m, 2H, H-4), 3.30 (m, 1H, H-3), 5.29 (t, 1H, H-6), 7.05 (s, 2H, NH_2), 7.64-7.67 (m, 4H, 2'H arom+3'H arom+5'H arom+6'H arom). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 11.9 (Me-18), 19.35 (Me-21), 19.41 (Me-19), 21.9 (C-11), 22.8 (C-27), 22.9 (C-26), 24.1 (C-16), 24.3 (C-15), 28.1 (C-14), 28.2(C-25), 31.16 (C-2), 31.18 (C-8+C-7), 36.55 (C-20), 36.59 (C-22), 37.0 (C-10), 37.2 (C-1), 39.3 (C-12), 39.4 (C-4), 42.1 (C-24), 42.2 (C-13), 55.0 (C-9), 56.5 (C-17), 70.5 (C-3), 114.6 (C-6), 114.8 (C-3'), 114.9 (C-5'), 120.7 (C-1'), 130.7 (C-2'+C-6'), 142.0 (C-5), 154.0 (C-4'), 164.0 (CO_2). Anal. calcd. for $C_{34}H_{51}NO_2$: C, 80.74; H, 10.16; N, 2.77. Found: C, 80.51; H, 10.01; N, 2.52%.

2.2.2. Biological activity

The screening was performed according to Agar diffusion method by dissolve (0.02 g) from each prepared derivative in 10 mL ethanol, then put 0.1 mL from every prepared sample in holes Agar dishes that cultivated by microorganisms, the dishes incubated at 37 °C for 24 hr for bacteria while 72 hr for fungal, zone of inhibition measured by ruler [22].

2.2.3. Physical properties study

2.2.3.1. Polarized optical microscopy (POM)

The idea of polarized optical microscopy can be down by taking a small amount of the sample (1-2 mg) and placed between two sheets of glass and tablets placed on the hot stage [23].

2.2.3.2. Differential scanning calorimetry (DSC)

Liquid crystal phases were characterized by using a differential scanning calorimetery (DSC) by taking about 2-3 mg of dry matter and heated inside the apparatus under inert gas atmosphere of nitrogen [24].

2.2.3.3. The electrical conductivity

The electrical conductivity of solutions of prepared derivatives can be determined by taking about 0.1 g from derivatives (**1**-**5**) and dissolved in 100 mL ethanol, then put the electrode inside a sample solution with 0.002 M concentration in the experiment [25].

3. Results and discussion

3.1. Chemistry

Ester compounds (**1**-**8**) have been synthesis by reaction of cholesterol with compounds **9**-**16**, respectively, in the presence of pyridine, triphenylphosphine and diethylazodicarboxylate as catalysts (Scheme 1).

The reaction proceeds via nucleophileic attack of the Ph_3P on azo group in DEAD and nucleophilic attack of carboxylate anion on triphenylphosphonium according to S_N2 mechanism [26].

The structures of these compounds have been characterized by disappearance of OH band of cholesterol and appearance of new bands in the prepared compounds **1**-**8** via R_f value, melting points, C.H.N. analysis, FT-IR, ^{1}H NMR and ^{13}C NMR.

3.2. Biological activity

The newly synthesized compounds were screened for their antimicrobial activity invitro against bacteria (*Staphylococcus aureues*, *Escherichia coli)* and fungal (*Candida albicans)* as shown in Figure 1. From the results given in Table 1, compounds **1** and **2** showed slightly activity against *Escherichia coli* and *Staphylococcus aureues* while showed moderated activity against *Candidia albicans.* Compound **3** showed no activity against *Escherichia coli* while showed moderated activity against *Staphylococcus aureus and Candidia albicans.* Compound **4** and **5** showed moderated activity against *Escherichia coli and Candidia albicans* while compound **4** showed very activity against *Staphylococcus aureus.* Compound **5** showed slightly activity against *Staphylococcus aureus.* Compound **6** showed moderated activity against *Escherichia coli* while showed high activity against *Staphylococcus aureus* and *Candidia albicans.* Compound **7** showed no activity against *Escherichia coli* while showed very activity against *Staphylococcus aureus* and *Candidia albicans.* Compound **8** showed moderated activity against *Escherichia Coli* and *Staphylococcus aureus* while showed no activity against *Candidia albicans.*

Table 1. Antimicrobial activity *.

Derivatives	*Escherichia coli*	*Staphylococcus aureues*	*Candida albicans*
1	+	+	++
2	+	+	++
3	-	++	++
4	++	+++	++
5	++	+	++
6	++	++++	++++
7	-	+++	+++
8	++	++	-

* +: 0-3 mm slightly active, ++: 6-9 mm moderated active, +++: 10-17 mm very active, ++++: 15-18 high active, and -: no active.

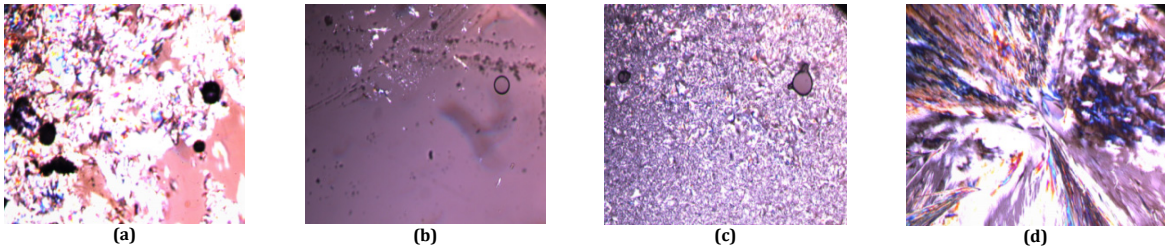


Figure 2. Images of polarized optical microscope for compound **1**, (a) Crystal phase (30 °C heating) (b) Sematic phase (72 °C heating) (c) Liquid phase (130 °C heating) (d) Blue phase (117 °C cooling).

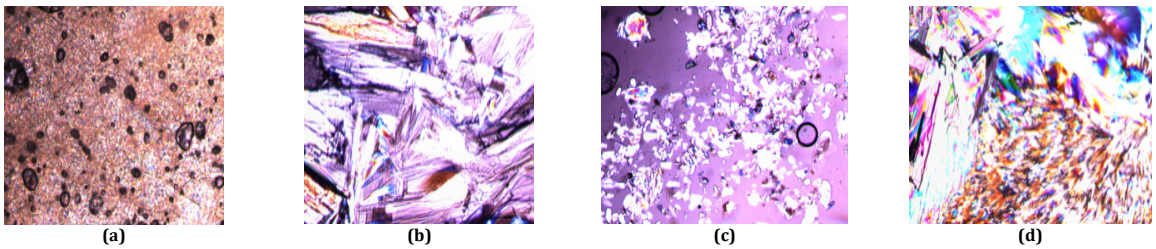


Figure 3. Images of polarized optical microscope for compound **2**, (a) Crystal phase (30 °C heating) (b) Smectic phase (98 °C heating) (c) Liquid phase (112 °C heating) (d) Blue phase (95 °C cooling).

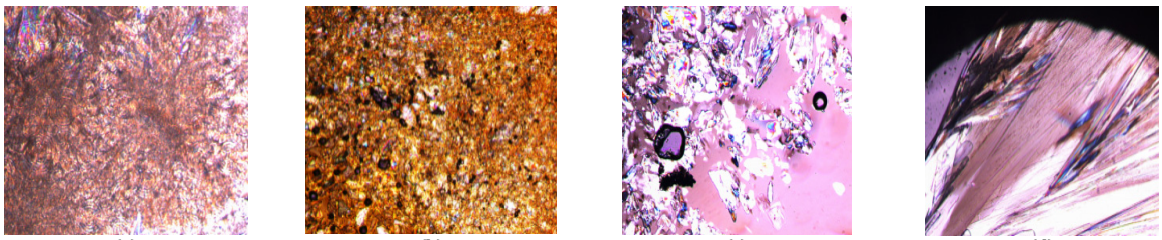


Figure 4. Images of polarized optical microscope for compound **3**, (a) Crystal phase (30 °C heating) (b) Smectic phase (80 °C heating) (c) Liquid phase (106 °C heating) (d) Blue phase (76 °C cooling).

3.3. Physical properties study

3.3.1. Polarized optical microscopy (POM) study

When melting the solid phase moves to the liquid crystalline phase. First, the shape will appear histological shape most systematic (Smectic phase), the continues of increase temperature gets quick change refers to move to another phase transitions less regularly (Nematic phase) [27], then moves down to the isotropic liquid phase, when cooling the fluid isotropic will appear blue phase (Liquid crystal phase) and then cooling down phase blue turn into a solid phase [28]. The results obtained by POM are shown in Figures 2-6.

3.3.2. Differential scanning calorimetry (DSC) study

The derivatives **1**-**4** showed four peaks, two peaks at heating and two peaks at cooling. At heating the first peak represent thermal transition from solid phase to liquid crystal phase; the second peak represents thermal transition from liquid crystal phase to isotropic phase [29]. At cooling the first peak represent thermal transition from isotropic phase to liquid crystal phase, the second peak represent phase thermal transition from liquid crystal to solid phase, While derivative **5** showed only two peaks, one peak at heating represent thermal transition from solid phase to liquid phase, the other peak at cooling represent thermal transition from liquid phase to solid phase [30].

Table 2. Data of thermal transition temperatures at heating *.

Derivatives	T /°$C_{C\rightarrow M}$	T /°$C_{M\rightarrow I}$	T / °$C_{C\rightarrow I}$	ΔT / °C
1	72	130	-	58
2	98	112	-	14
3	80	106	-	56
4	88	102	-	71
5	-	-	200	-

* C: Crystal phase, M: Meso phase, I: Iso phase.

Table 3. Data of thermal transition temperatures at cooling.

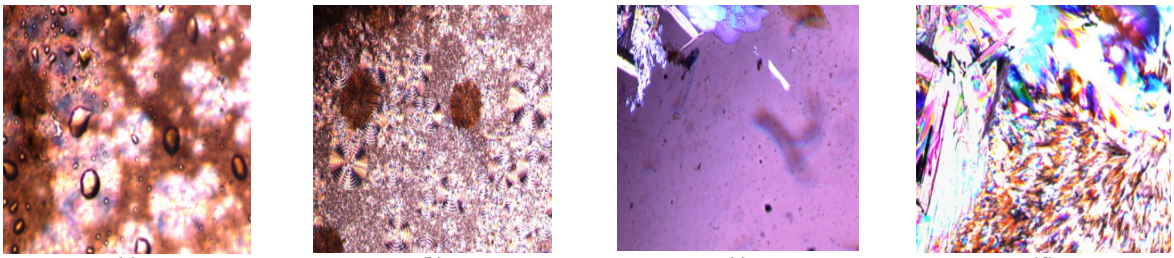
Derivatives	T / °$C_{I\rightarrow M}$	T / °$C_{M\rightarrow C}$	T / °$C_{I\rightarrow C}$	ΔT / °C
1	117	79	-	-38
2	95	75	-	-20
3	96	76	-	-20
4	95	75	-	-72
5	-	-	185	-

Table 4. Values of enthalpy and entropy of derivatives at heating.

Derivatives	$\Delta H_{K.J/mol\ C\rightarrow M}$	$\Delta H_{K.J/mol\ M\rightarrow I}$	$\Delta H_{K.J/mol\ C\rightarrow I}$	$\Delta S_{K.J/mol.K\ C\rightarrow M}$	$\Delta S_{K.J/mol.K\ M\rightarrow I}$	$\Delta S_{K.J/mol.K\ C\rightarrow I}$
1	-8×10^{-5}	-4×10^{-5}	-	-6×10^{-7}	-5×10^{-7}	-
2	-3×10^{-7}	-2×10^{-7}	-	-3×10^{-9}	-1×10^{-9}	-
3	-7×10^{-5}	-2×10^{-5}	-	-8×10^{-7}	-1×10^{-7}	-
4	-3×10^{-6}	-2×10^{-6}	-	-3×10^{-8}	-1×10^{-9}	-
5	-	-	3×10^{-5}	-	-	-3×10^{-7}

Table 5. Values of enthalpy and entropy of derivatives at cooling.

Derivatives	$\Delta H_{K.J/mol\ I\rightarrow M}$	$\Delta H_{K.J/mol\ M\rightarrow C}$	$\Delta H_{K.J/mol\ I\rightarrow C}$	$\Delta S_{K.J/mol.K\ I\rightarrow M}$	$\Delta S_{K.J/mol.K\ M\rightarrow C}$	$\Delta S_{K.J/mol.K\ I\rightarrow C}$
1	7×10^{-5}	3×10^{-5}	-	5×10^{-7}	3×10^{-7}	-
2	3×10^{-5}	2×10^{-7}	-	4×10^{-7}	2×10^{-9}	-
3	4×10^{-5}	3×10^{-5}	-	5×10^{-7}	3×10^{-7}	-
4	2×10^{-5}	2×10^{-7}	-	2×10^{-5}	2×10^{-7}	-
5	-	-	-2×10^{-5}	-	-	-2×10^{-7}



(a) (b) (c) (d)

Figure 5. Images of polarized optical microscope for compound **4**, (a) Crystal phase (30 °C heating) (b) Nematic phase (88 °C heating) (c) Liquid phase (102 °C heating) (d) Blue phase (95 °C cooling).

(a) (b)

Figure 6. Images of polarized optical microscope for compound **5**, (a) Crystal phase (30 °C heating) (b) Liquid phase (100 °C heating).

The cause of not appearance liquid crystal phase in derivative **5** is the middle group (CO) that makes the molecules not linear, this make the liquid crystal phase lower thermal stability and cause losing the liquid crystal phase. The results obtained by DSC analysis are given in Table 2-5.

The values of entropy (ΔS) were calculated by free Gipps relationship (ΔG) [31] at every degree of thermal transition in crystal phase, mesophase and isotropic phase as in Equations (1-6).

At heating

$$\Delta G_{C\text{-}M} = \Delta H_{C\text{-}M} - T_M.\ \Delta S_{C\text{-}M} \quad (1)$$

$$\Delta G_{M\text{-}I} = \Delta H_{M\text{-}I} - T_I.\ \Delta S_{M\text{-}I} \quad (2)$$

$$\Delta G_{C\text{-}I} = \Delta H_{C\text{-}I} - T_I.\ \Delta S_{C\text{-}I} \quad (3)$$

At cooling

$$\Delta G_{C\text{-}M} = \Delta H_{C\text{-}M} - T_M.\ \Delta S_{C\text{-}M} \quad (4)$$

$$\Delta G_{M\text{-}I} = \Delta H_{M\text{-}I} - T_I.\ \Delta S_{M\text{-}I} \quad (5)$$

$$\Delta G_{C\text{-}I} = \Delta H_{C\text{-}I} - T_I.\ \Delta S_{C\text{-}I} \quad (6)$$

Table 6. The electrical conductivity for starting materials and derivatives.

Starting materials	Electrical Conductivity (μs)	Derivatives	Electrical Conductivity (μs)
Cholesterol	900	-	-
9	919	1	911
10	100	2	95
11	918	3	906
12	97	4	80
13	850	5	700

3.3.3. The electrical conductivity study

The behavior of electrical conductivity of prepared derivatives solutions were measured by using (0.002M) as a concentration in the experiment and the data were illustrated in Table 6.

The results showed that the electrical conductivity of derivatives (**1**-**5**) Less than electrical conductivity for starting materials (**9**-**13**), respectively, the reason was decreasing the polar property in these derivatives due to converting a high polar (COOH) group in the starting materials to the less polar ester group [32] in the derivatives **1**-**5**.

4. Conclusion

A series of new ester derivatives of cholesterol has been described by applying Mitsunobu reaction. All the compounds were achieved according to the data shown by the physical and chemical analysis including (TLC, melting point, elemental analysis, FT-IR spectroscopy, NMR spectroscopy, POM analysis, DSC analysis and electrical conductivity measurements. All the derivatives showed inversion in configuration at C-3 during the formation of β-ester analogues. Most of these compounds showed high microbial activity against some bacteria and fungal. Most of these compounds are liquid crystals.

Acknowledgement

We thank Prof Dr. Najim Al-Masoudi and Miss Friemel, Chemistry Department, Konstanz University, Germany, for making and analyser NMR spectra.

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