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Synthesis, Characterization and Biological Activity of Some Chalcone Derivatives of Cholic Acid

NABEEL A. ABDUL-REDA[1] and ABBAS S. ABD[2,*]

[1]Department of Chemistry, College of Science, University of Al-Qadisiyah, Al-Diwaniyah, Al-Diwaniyah Province, Iraq
[2]Department of Chemistry, College of Education, University of Al-Qadisiyah, Al-Diwaniyah, Al-Diwaniyah Province, Iraq

*Corresponding author: E-mail: nabeel.a.alradha@qu.edu.iq; abbassami94@yahoo.com

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N-(4-acetylphenyl)-3′,7′,12′-trihydroxy-5′-cholan-24-amide was synthesized from reaction of cholic acid with 4-amino acetophenone then the obtained product reacts with some substituted benzaldehyde derivatives to prepare chalcone derivatives. Finally, the products were allowed to react with thiourea to give thiazine derivatives. The reactions were monitored by thin-layer chromatography (TLC) technique. All the new compounds were characterized by melting points, elemental analysis, FT-IR, ^{1}H NMR, ^{13}C NMR spectroscopies. Antimicrobial activity of the synthesized derivatives was also determined.

Keywords: Cholic acid, Chalcone, Thiazine, Antimicrobial activity.

INTRODUCTION

Cholic acid is a major primary bile acid produced in liver and found in the bile of mammals and other vertebrates and usually conjugated with glycine or taurine. It facilitates cholesterol excretion and fat absorption [1]. A number of bile acid derivatives have many pharmacological activities, notably antimicrobial [2-4], antifungal [4-6], carbonic anhydrase inhibition [7,8] and potential antioxidant [9]. Several derived cholic acid facial amphiphiles have been reported to improve the permeability of membranes including bacterial cell wall [10].

Cholic acid and deoxycholic acid have attracted significant attention because of availability and the orientation of three hydroxy groups that may be exploited in podant-type receptors [11,12], linear dimeric hosts [13,14] or facial amphiphiles [15,16]. In addition, bile acids are natural ligands specifically recognized by hepatic cells and are amphiphilic molecules that undergo a biological recycling during enterohepatic circulation [10,17,18]. Cholic acid derivatives also displayed a remarkable activity against some viral infections. Salunke *et al.* [5] and Williams *et al.* [19] have reported the first examples of C-11 azido/amino functionalized cholic acid derivatives induce HIV replication and syncytia formation in T cells. Moreover, bile acids have been suggested as useful moieties to liver organotropic drugs, since cisplatin-bile acid derivatives have been used *in vitro* to determine the production of virions by HBV transfected hepatoblastoma cells (HepG2 2.2.15) [20] and effects of DNA-reactive bile acid derivatives on hepatitis B virus life cycle [21]. However, number of cholic acid analogues also revealed significant antimicrobial activity [22].

EXPERIMENTAL

All the compounds and reagents were of highest purity and supplied by Merck, Sigma Aldrich and Fluka Company. Melting points were measured on a Buchi melting point apparatus B-545 (Buchi Labortechnik AG, Switzerland). Microanalytical data were obtained with a Vario, Elementar apparatus (Shimadzu, Japan). The IR spectra were recorded on Schimadzu Fourier Transform Infrared spectrophotometer (Model 270) using KBr discs. NMR spectra were recorded on 600 MHz for ^{13}C and at 100 MHz for ^{1}H NMR (Bruker, Germany) using TMS as internal standard and on δ scale in ppm. Thin layer chromatography (TLC) was performed on silica gel for TLC and spots were visualized by iodine vapours. The reagents used were of analytical grade and purified before use.

Synthesis of N-(4-acetylphenyl)-3′,7′,12′-trihydroxy-5′-cholan-24-amide (2): To a cold solution of cholic acid (**1**) (409 mg, 1.0 mmol) in CH_2Cl_2/DMF (20 mL, 1:1 v/v) were added dimethyl amino pyridine (16 mg, 0.13 mmol) and 4-amino-acetophenone (1.0 mmol) with stirring for 10 min, followed

by addition of DCC (206 mg, 1.0 mmol). The reaction mixture was stirred at room temperature for 16-20 h and completion of reaction was monitored by TLC. A white precipitate was filtered and the solvent was allowed to evaporate to dryness and then the residue was dissolved in ethyl acetate (50 mL). The organic layer was washed with aqueous 4 % HCl and saturated aqueous solution of $NaHCO_3$ (3 × 30 mL) then dried (Na_2SO_4). The residue was purified on SiO_2 column chromatography using, in gradient, MeOH (0-10 %) and $CHCl_3$ as eluent to give compound **2**. Yield: 357 mg (68 %) as dark yellow solid; m.p. 128-130 ºC; (m.w. 525.73); R_f = 0.83; IR (KBr, ν_{max}, cm^{-1}): 3394 (OH), 2931, 2854 (CH_2), 1712 $(C=O)_{acetyl}$, 1697 $(C=O)_{amide}$, 1650 $(C=C)_{arom.}$; ^1H NMR (DMSO-d_6): δ 9.95 (s, 1H, NH_{amide}), 7.57 (d, 2H, *J* = 7.8 Hz, $H_{arom.}$- 3′ + $H_{arom.}$-5′), 7.44 (d, 2H, *J* = 7.8 Hz, $H_{arom.}$-2′ + $H_{arom.}$-6'), 6.61 (d, 1H, *J* = 7.9 Hz, OH), 5.58 (d, 1H, *J* = 7.6 Hz, OH), 4.30 (bs, 1H, OH), 4.06 (m, 1H, H-12), 3.78 (m, 1H, H-7), 3.18 (m, 1H, H-3), 2.58 (s, 3H, COMe), 2.24 (m, 1H, H-4a), 2.15 (m, 2H, H-9), 2.12 (m, 1H, H-23a), 2.06 (m, 1H, H- 23b), 1.94 (m, H, H-14a), 1.80 (m, 1H, H-6a), 1.78 (m, 1H, H-17), 1.75 (m, 1H, H-16a), 1.67 (m,2H, H-1a + H-22a), 1.64 (m, 2H, H-2a + H-15a), 1.48 (m, 1H, H-4b), 1.44 (m, 1H, H-11a), 1.39 (m, 1H, H-6b), 1.35 (m, 1H, H-11b), 1.33 (m, 2H, H-8 + H-20), 1.28 (m, 1H, H-2b), 1.25 (m, 1H, H-5), 1.23 (m, 1H, H-22b), 1.21 (m, 1H, H-5), 1.17 (m, 1H, H-16b), 0.93 (d, 3H, *J*Me21,H20 = 6.3 Hz, Me-21), 0.91 (m, 1H, H-15b), 0.83 (m, 1H, H-1b), 0.80 (s, 3H, Me-19), 0.57 (s, 3H, Me-18); ^{13}C NMR (DMSO-d_6): δ 191.2 (C=O), 171.5 (CONH), 139.7 (C-1′), 138.7 (C-4′), 131.3 (C-3′ + C-5′), 120.8 (C-2′ + C-6′), 73.1 (C-12), 70.9 (C-3), 66.2 (C-7), 47.1 (C-17), 45.6 (C-13), 41.5 (C-5), 41.3 (C-14), 40.1 (C-4 + C-8), 37.7 (C-20), 36.7 (C-1), 35.4 (C-6), 35.2 (C-10), 31.7 (C-2 + C-22), 30.9 (C-23), 28.4 (C-11), 27.2 (C-16), 26.1 (C-9), 24.5 (COMe), 18.4 (Me-21), 12.2 (Me-18). Anal. calcd. (found) (%) for $C_{32}H_{47}NO_5$: C, 73.11 (72.90); H, 9.01 (8.91); N, 2.66 (2.43).

General procedure for synthesis of chalcones (3-7): Compound **2** (26.28mg, 0.5 mmol) with different aromatic aldehydes (0.5 mmol) were dissolved in 10 mL of ethanol. Sodium hydroxide solution (3 mL, 10 %) was added slowly and the mixture stirred for 2 h until the entire mixture becomes very cloud then the mixture was poured slowly into 20 mL of water with constant stirring and kept in refrigerator for 24 h. The reaction was monitored by TLC (methanol:$CHCl_3$::1:9). The reaction was neutralized by HCl and the obtained precipitate was filtered, washed and recrystallized from ethanol.

Synthesis of (*R*)-N-(4-((*E*)-3-(4-bromophenyl)acryloyl)-phenyl)-4-(3′,7′,12′-triydroxy)-5′-cholan-24-amide (3): Using 4-bromobenzaldehyde (92.5 mg), compound **3** was obtained. Yield: 29.5 mg (85 %) as dark yellow solid; m.p. 110-112 ºC; m.w. 692.74; R_f = 0.62. IR (KBr, ν_{max}, cm^{-1}): 3394 (OH), 2931, 2854 (CH_2), 1681$(C=O)_{chalcone}$, 1665 $(C=O)_{amide}$, 1615 $(C=C)_{arom.}$; ^1H NMR (DMSO-d_6): δ 9.93 (s, 1H, NH_{amide}), 8.159 (d, 1H, H-b), 7.82 (d, 2H, $H_{arom.}$-3′ + $H_{arom.}$-5′), 7.77 (d, 2H, $H_{arom.}$-2′ + $H_{arom.}$-6′), 7.63 (d, 2H, $H_{arom.}$-3″ + $H_{arom.}$-5″ + $H_{chal.}$-a), 7.39 (d, 2H, $H_{arom.}$-2″+ $H_{arom.}$-6″), 4.74 (d, 1H, OH), 4.43 (brs, 1H, OH), 4.11 (m, 1H, H-12), 3.77 (m, 1H, H-7), 3.19 (m, 1H, H-3), 2.23 (m, 1H , H-4a), 2.18 (m, 2H, H-9), 2.13 (m, 1H, H-23a), 2.01 (m, 1H, H-23b), 1.95 (m, H, H-14a),1.80 (m, 1H, H-6a), 1.78 (m, 1H, H-17), 1.75 (m, 1H, H-16a), 1.66 (m, 2H, H-1a + H-22a), 1.61 (m, 2H, H-2a + H-15a), 1.46 (m, 1H, H-4b), 1.44 (m, 1H , H-11a), 1.38 (m, 1H, H-6b), 1.35 (m, 1H, H-11b), 1.33 (m, 2H, H-8 + H-20), 1.28 (m, 1H, H-2b), 1.25 (m, 1H, H-5), 1.23 (m, 1H, H-22b), 1.19 (m, 1H, H-5), 1.16 (m, 1H, H-16b), 0.92 (d, 3H, *J*Me21, H_2O = 6.3 Hz, Me-21),0.90 (m, 1H, H-15b), 0.82 (m, 1H, H-1b), 0.79 (s, 3H, Me-19), 0.55 (s, 3H, Me-18); ^{13}C NMR (DMSO-d_6): δ 183.15 $(C=O_{chal.})$, 173.42 (CONH), 159.38 (C-1′ + b), 150.62 (C-1″), 149.14 (C-4′), 130.82 (C-3″+ C-5″), 129.94 (C-3′ + C-5′), 126.09 (C-2″ + C-6″), 117.10 (C-2′ + C-6′), 113.30 (C-4″), 109.50 (C-a), 71.46 (C-12), 70.97 (C-3), 66.20 (C-7), 47.46 (C-17), 46.16 (C-13), 41.33 (C-5), 40.03 (C-14), 39.75 (C-4 + C-8), 38.64 (C-20), 35.27 (C-1), 35.11 (C-6), 34.54 (C-10), 32.51 (C-2 + C-22), 30.36 (C-23), 28.53 (C-11), 27.28 (C-16), 26.17 (C-9), 22.75 (Me-19), 17.18 (Me-21), 12.22 (Me-18); Anal. calcd. (found) (%) for $C_{39}H_{50}NO_5Br$: C 67.62 (67.55); H 7.28 (7.20); Br 11.53 (11.23); N 2.02 (1.95).

Synthesis of (*R*)-N-(4-((E)-3-(4-hydroxyphenyl)acryloyl)-phenyl)-4-(3α,7α,12α-triydroxy)-5β-cholan-24-amide (4): Using 4-hydroxybenzaldehyde (61.6 mg), compound **4** was obtained. Yield: 25.3 mg (80 %) as yellow solid; m.p. 123-125 ºC; m.w. 629.84; R_f = 0.52. IR (KBr, ν_{max}, cm^{-1}): 3394 (OH), 2931, 2854 (CH_2), 1681 (C=O), 1665 $(C=O)_{amide}$, 1650 $(C=C)_{arom.}$; ^1H NMR (DMSO-d_6): δ 9.87 (s, 1H, NH_{amide}), 8.04 (d, 2H, H-a + $H_{arom.}$-3′ + H′-5′), 7.88 (d, 2H, $H_{arom.}$-2′ + $H_{arom.}$-6′), 7.63 (d, 3H, H-b + $H_{arom.}$-2″ + $H_{arom.}$-6″), 7.04 (d, 2H, H-a + $H_{arom.}$-3″ + $H_{arom.}$-5″), 6.41 (d, 1H, OH), 5.41 (d, 1H, OH), 4.33 (brs, 1H, OH), 4.13 (m, 1H, H-12), 3.76 (m, 1H, H-7), 3.19 (m, 1H, H-3), 2.24 (m, 1H, H-4a), 2.16 (m, 2H, H-9), 2.12 (m, 1H, H-23a), 2.07 (m, 1H, H-23b), 1.91 (m, H, H-14a), 1.80 (m, 1H, H-6a), 1.75 (m, 1H, H-17), 1.71 (m, 1H , H-16a), 1.69 (m, 2H, H-1a + H-22a), 1.56 (m, 2H, H-2a + H-15a), 1.51 (m, 1H, H-4b), 1.46 (m, 1H, H-11a), 1.42 (m, 1H, H-6b), 1.35 (m, 1H, H-11b), 1.32 (m, 2H, H-8 + H-20), 1.28 (m, 1H, H-2b), 1.25 (m, 1H, H-5), 1.23 (m, 1H, H-22b), 1.21 (m, 1H, H-5), 1.17 (m, 1H, H-16b), 0.93 (d, 3H, *J*Me21, H_2O = 6.3 Hz, Me-21), 0.91 (m, 1H, H-15b), 0.83 (m, 1H, H-1b), 0.80 (s, 3H, Me-19), 0.57 (s, 3H, Me-18); ^{13}C NMR (DMSO-d_6): δ 198.59 $(C=O_{chal.})$, 190.66 (CONH), 157.92 (C-4′), 140.07 (C-1′), 132.03 (C-b), 130.34 (C-1″), 129.56 (C-3′ + C-5′ + C-2″ + C-6″), 121.23 (C-4′), 117.42 (C-2′ + C-6′), 115.86 (C-a), 113.61 (C-3″ + C-5″), 71.77 (C-12), 71.68 (C-3), 66.95 (C-7), 48.04 (C-17), 46.50 (C-13), 42.11 (C-5), 41.88 (C-14), 40.02 (C-4 + C-8), 37.7 (C-20), 36.7 (C-1), 35.65 (C-6), 35.26 (C-10), 30.55 (C-2 + C-22), 30.40 (C-23), 28.73 (C-11), 27.54 (C-16), 26.73 (C-9), 22.39 (C-19), 16.75 (Me-21), 12.00 (Me-18). Anal. calcd. (found) (%) for $C_{39}H_{51}NO_6$: C 74.3 (74.12); H 8.09 (7.95); N 2.22 (2.09).

Synthesis of (*R*)-*N*-(4-((*E*)-3-(4-hydroxy-3-methoxyphenyl)-acryloyl)phenyl-4-(3α,7α,12α-triydroxy)-5β-cholan-24-amide (5): Using vanillin (76.07 mg), compound **5** was obtained. Yield: 24.2 mg (73.3 %) as white solid; m.p. 113-115 ºC; m.w. 659.86; R_f = 0.29. IR (KBr, ν_{max}, cm^{-1}): 3394 (OH), 3080 $(CH)_{arom.}$, 2931, 2854 (CH_2), 1691 $(C=O)_{amide}$, 1660 $(C=O)_{chal.}$, 1650 $(C=C)_{arom.}$; ^1H NMR (DMSO-d_6): δ 9.96 (s, 1H, NH_{amide}), 9.52 (s, 1H, Ar-OH), 7.99 (d, 1H, H-b), 7.96 (d, 2H, $H_{arom.}$-3′ + $H_{arom.}$-5′), 7.85 (d, 2H, $H_{arom.}$-2′ + $H_{arom.}$-6′), 7.62 (d, 1H, H-a), 7.42 (s, 1H, H-1″), 7.27 (d, 1H, H-5″), 6.85 (d, 1H, H-6″), 6.41 (d, 1H, OH), 5.18 (d, 1H, OH), 4.73 (brs, 1H, OH), 4.28 (m, 1H, H-12), 3.80 (s, 3H, OMe), 3.61 (m, 1H, H-7), 3.18 (m, 1H, H-3), 2.23 (m, 1H, H-4a), 2.19 (m, 2H,

H-9), 2.15 (m, 1H, H-23a), 2.09 (m, 1H, H-23b),1.95 (m, H, H-14a), 1.92 (m, 1H, H-6a), 1.67 (m, 1H, H-17), 1.52 (m, 1H, H-16a), 1.67 (m, 2H, H-1a + H-22a), 1.56 (m, 2H, H-2a + H-15a), 1.52 (m, 1H, H-4b), 1.46 (m, 1H, H-11a), 1.39 (m,1H, H-6b), 1.34 (m, 1H, H-11b), 1.32 (m, 2H, H-8 + H-20), 1.28 (m, 1H, H-2b), 1.25 (m, 1H, H-5), 1.23 (m, 1H, H-22b), 1.19 (m, 1H, H-5), 1.12 (m, 1H, H-16b), 0.93 (d, 3H, *J*Me21, H_2O = 6.3 Hz, Me-21), 0.91 (m, 1H, H-15b), 0.83 (m, 1H, H-1b), 0.80 (s, 3H, Me-19), 0.57 (s, 3H, Me-18); ^{13}C NMR (DMSO-d_6): δ 171.56 (C=O $_{chal.}$), 167.09 (CONH), 158.48 (C-4″), 151.01 (C-1′), 147.14 (C-b), 133.74 (C-4′), 129.57 (C-3′ + C-5′), 123.40 (C-1″), 121.52 (C-2′+ C-6′), 116.96 (C-a), 114.93 (C-5″), 112.68 (C-2″), 70.92 (C-12), 70.36 (C-3), 66.16 (C-7), 55.67 (Ar-OMe), 47.46 (C-17), 46.12 (C-13), 41.48 (C-5), 41.33 (C-14), 40.27 (C-4 + C-8), 37.7 (C-20), 35.26 (C-1), 35.12 (C-6), 35.00 (C-10), 30.75 (C-2 + C-22), 30.32 (C-23), 28.49 (C-11), 27.25 (C-16), 26.18 (C-9), 22.74 (C-19), 22.55 (Me-19), 17.09 (Me-21), 12.10 (Me-18). Anal. calcd. (found) (%) for $C_{40}H_{53}NO_7$: C 72.81 (72.71); H, 8.10 (7.95); N, 2.12 (2.05).

Synthesis of (*R*)-*N*-(4-((*E*)-3-(*p*-tolyl)acryloyl)phenyl)-4-(3α,7α,12α-triydroxy)-5β-cholan-24-amide (6): Using 4-methyl benzaldehyde (60.07 mg), compound **6** was obtained. Yield: 22.7 mg (72.3 %) as yellow solid; m.p. 95-97 ºC; m.w. 627.87; R_f = 0.41. IR (KBr, ν_{max}, cm^{-1}): 3394 (OH), 2931, 2854 (CH_2), 1697(C=O)$_{amide}$, 1670 (C=O)$_{chal.}$, 1650 (C=C)$_{arom.}$; ^{1}H NMR (DMSO-d_6): δ 9.88 (s,1H,NH$_{amide}$), 8.06 (d, 2H, a +H$_{arom.}$-3″ + H$_{arom.}$-5″ + H-b), 7.68 (d, 2H, H$_{arom.}$-2′ + H$_{arom.}$-6′), 7.21 (d, 3H, H-a + H$_{arom.}$-2″ + H$_{arom.}$-6″), 6.69 (d, 2H, H$_{arom.}$-3′ + H$_{arom.}$-5′), 6.41 (d, 1H, OH), 5.41 (d, 1H, OH), 4.33 (brs, 1H, OH), 4.13 (m, 1H, H-12), 3.76 (m, 1H, H-7), 3.19 (m, 1H, H-3), 2.30 (s, 3H, A-Me), 2.24 (m, 1H, H-4a), 2.16 (m, 2H, H-9), 2.12 (m, 1H, H-23a), 2.07 (m, 1H, H-23b), 1.91 (m, H, H-14a), 1.80 (m, 1H, H-6a), 1.75 (m, 1H, H-17), 1.71 (m, 1H, H-16a), 1.69 (m, 2H, H-1a + H-22a), 1.56 (m, 2H, H-2a + H-15a), 1.51 (m, 1H, H-4b), 1.46 (m, 1H, H-11a), 1.42 (m, 1H, H-6b), 1.35 (m, 1H, H-11b), 1.32 (m, 2H, H-8 + H-20),1.28(m, 1H, H-2b), 1.25 (m,1H, H-5), 1.23 (m, 1H, H-22b), 1.21 (m, 1H, H-5), 1.17 (m, 1H, H-16b), 0.93 (d, 3H, *J*Me21, H_2O = 6.3 Hz, Me-21), 0.91 (m, 1H, H-15b), 0.83 (m, 1H , H-1b), 0.80 (s, 3H, Me-19), 0.57 (s, 3H, Me-18); ^{13}C NMR (DMSO-d_6): δ 181.0 (C=O$_{chal.}$), 177.54 (CONH), 146.56 (C-b), 143.92(C-1′), 143.49 (C-4″), 138.63 (C-4′), 133.74 (C-1″), 129.24 (C-3′ + C-5′), 128.48 (C-3″ + C-5″ + C-2″ + C-6″), 121.52 (C-a), 120.46 (C-2′ + C-6′), 70.97 (C-12), 70.36 (C-3), 65.99 (C-7), 48.00 (C-17), 46.00 (C-13), 42.11(C-5), 41.32 (C-14), 40.02 (C-4 + C-8), 37.93 (C-20), 35.06 (C-1), 34.64 (C-6), 34.30 (C-10), 34.16 (C-2 + C-22), 31.79 (C-23), 28.73 (C-11), 27.05 (C-16), 2493 (Ar-Me), 24.30 (C-9), 22.08 (C-19), 22.08 (Me-19), 16.83 (Me-21), 11.75 (Me-18). Anal. calcd. (found) (%) for $C_{40}H_{53}NO_5$: C, 76.52 (76.45); H, 8.51 (8.40); N, 2.23 (2.17).

Synthesis of (*R*)-*N*-(4-((*Z*)-3-(2,4-dinitrophenyl)acryloyl)-phenyl-4-(3α,7α,12α)-triydroxy-5β-cholan-24-amide (7): Using 2,4-dinitrobenzaldehyde (98.05 mg), compound **7** was obtained. Yield: 25.1 mg (71.3 %) as dark yellow solid; m.p. 108-110 ºC; m.w. 703.83; R_f = 0.56. IR (KBr, ν_{max}, cm^{-1}): 3394 (OH), 2931, 2854 (CH_2), 1681 (C=O)$_{amide}$, 1660 (C=O)$_{chal.}$, 1650 (C=C)$_{arom.}$; ^{1}H NMR (DMSO-d_6): δ 9.84 (s, 1H, NH$_{amide}$), 8.14 (s, 1H, H-3″), 7.91 (s, 1H, H-5″), 7.79 (s, 1H, H-b), 7.57 (d, 2H, H$_{arom.}$-3′ + H$_{arom.}$-5′), 7.39 (s, 1H, H-6″), 7.19 (d, 2H , H$_{arom.}$-2′ + H$_{arom.}$-6′), 7.03 (s, 1H, H-a), 7.04 (d, 2H, H-a), 6.34 (d, 1H, OH), 5.60 (d, 1H, OH), 4.44 (brs, 1H, OH), 4.18 (m, 1H, H-12), 3.87 (m, 1H, H-7), 3.35 (m, 1H, H-3), 2.37 (m, 1H, H-4a), 2.19 (m, 2H, H-9), 2.10 (m, 1H, H-23a), 2.05 (m, 1H, H-23b), 1.95 (m, H, H-14a), 1.80 (m, 1H, H-6a), 1.75 (m, 1H, H-17), 1.71 (m, 1H, H-16a), 1.67 (m, 2H, H-1a + H-22a), 1.52 (m, 2H, H-2a + H-15a), 1.51 (m, 1H, H-4b), 1.46 (m, 1H, H-11a), 1.42 (m, 1H, H-6b), 1.39 (m, 1H, H-11b), 1.33 (m, 2H, H-8 + H-20), 1.25 (m, 1H, H-2b), 1.23 (m, 1H ,H-5), 1.21 (m, 1H, H-22b), 1.18 (m, 1H, H-5), 1.16 (m, 1H, H-16b), 0.93 (d, 3H, Me-21), 0.91 (m, 1H, H-15b), 0.83 (m, 1H, H-1b), 0.80 (s, 3H, Me-19), 0.57 (s, 3H, Me-18); ^{13}C NMR (DMSO-d_6): δ 191.35 (C=O$_{chal.}$), 171.48 (CONH), 156.57 (C-1′ + C-2″ + C-b), 150.62 (C-4″), 149.14 (C-1″ + C-4′), 130.51 (C-3′ + C-5′), 129.94 (C-5″), 129.60 (C-6″), 126.09 (C-2′ + C-6′), 111.61 (C-a), 109.37 (C-3″), 70.97 (C-12), 70.39 (C-3), 66.20 (C-7), 47.46 (C-17), 46.16 (C-13), 41.33 (C-5), 40.03 (C-14), 39.75 (C-4 + C-8), 38.64 (C-20), 35.27 (C-1), 35.11 (C-6), 34.54 (C-10), 32.51 (C-2 + C-22), 30.36 (C-23), 28.53 (C-11), 27.28 (C-16), 26.17 (C-9), 22.75 (Me-19), 17.10 (Me-21), 12.25 (Me-18); Anal. calcd. (found) (%) for $C_{39}H_{49}N_3O_9$: C, 66.55 (66.49); H, 7.02 (6.95); N, 5.97 (5.85).

General procedure for synthesis thiazine compounds derivatives: Synthesized chalcone ((0.001mol) was dissolved in 10 mL of ethanolic NaOH and 0.001mol thiourea was was added slowly. The mixture were stirred about 3-4 h with a magnetic stirrer and then the mixture was poured into 5 mL of cold water with continuous stirring for an hour and then kept in refrigerator for 24 h, the reaction was monitored by TLC (methanol:$CHCl_3$::1:9). The obtained precipitate was filtered, washed and recrystallized with ethanol.

Synthesis of (*R*)-*N*-(4-((6*S*)-2-amino-6-(4-bromophenyl)-5,6-dihydro-4*H*-1,3-thiazin-4-yl)phenyl-4-(3α,7α,12α-trihydroxy)-5β-cholan-24-amide (8): Using compound **3** (292.7 mg), compound **8** was obtained. Yield: 520 mg (69 %) as yellow solid; m.p: 123-125 ºC; m.w. 752; R_f = 0.45. IR (KBr, ν_{max}, cm^{-1}): 3394 (OH), 2931, 2854 (CH_2), 1680 (C=O)$_{amide}$, 1630 (C=C) $_{arom.}$; ^{1}H NMR (DMSO-d_6): δ 10.48 (s, 1H, NH$_{amide}$), 8.09 (s, 2H, NH_2), 7.95-6.73 (m, 4H, H$_{arom.}$), 4.63 (d, 1H, OH), 4.46 (brs, 1H, OH), 4.08 (m, 1H, H-12), 3.77 (m, 1H, H-7+t, 2H, H-b), 3.60 (m, 1H, H-3), 2.42 (d, 2H, H-a + H-c), 2.23 (m, 1H, H-4a), 2.18 (m, 2H, H-9), 2.13 (m, 1H, H-23a), 2.01 (m, 1H, H-23b), 1.95 (m, 1H, H-14a), 1.91 (m, 1H , H-6a), 1.78 (m, 1H, H-17), 1.70 (m, 1H, H-16a), 1.65 (m, 2H, H-1a + H-22a), 1.61 (m, 2H, H-2a + H-15a), 1.46 (m, 1H, H-4b), 1.40 (m, 1H, H-11a), 1.38 (m, 1H, H-6b), 1.35 (m, 1H, H-11b), 1.33 (m, 2H, H-8 + H-20), 1.28 (m, 1H, H-2b), 1.23 (m, 1H, H-5), 1.21 (m, 1H, H-22b), 1.16 (m, 1H, H-5), 1.13 (m, 1H, H-16b), 0.92 (d, 3H, *J*Me21, H_2O = 6.3 Hz, Me-21), 0.91 (m, 1H, H-15b), 0.82 (m, 1H, H-1b), 0.79 (s, 3H, Me-19), 0.56 (s, 3H, Me-18); ^{13}C NMR (DMSO-d_6): δ 173.49 (CONH), 163.23 (C=N), 139.25 (C-1″), 136.65 (C-1′), 133.74 (C-3″ + C-5″), 128.48 (C-2″ + C-6″), 126.86 (C-2′ + C-6′ + 4′), 121.22 (C-3′ + C-5′), 120.46 (C-4″), 71.43 (C-12), 70.83 (C-3), 66.65 (C-7), 60.21 (C-a), 47.76 (C-17), 46.55 (C-b), 46.10 (C-13), 41.79 (C-5), 40.35 (C-14), 39.79 (C-4 + C-8), 38.67 (C-20), 35.58 (C-1 + C-c), 35.17 (C-6), 34.73 (C-10), 32.76 (C-2 + C-22), 30.61 (C-23), 28.87 (C-11), 27.67 (C-16), 26.62 (C-9), 22.75 (Me-19), 17.40 (Me-21), 12.45 (Me-18). Anal. calcd. (found) (%) for $C_{40}H_{54}N_3O_4S$: C, 63.82 (63.75); H, 7.23 (7.15); N, 5.58 (5.50).

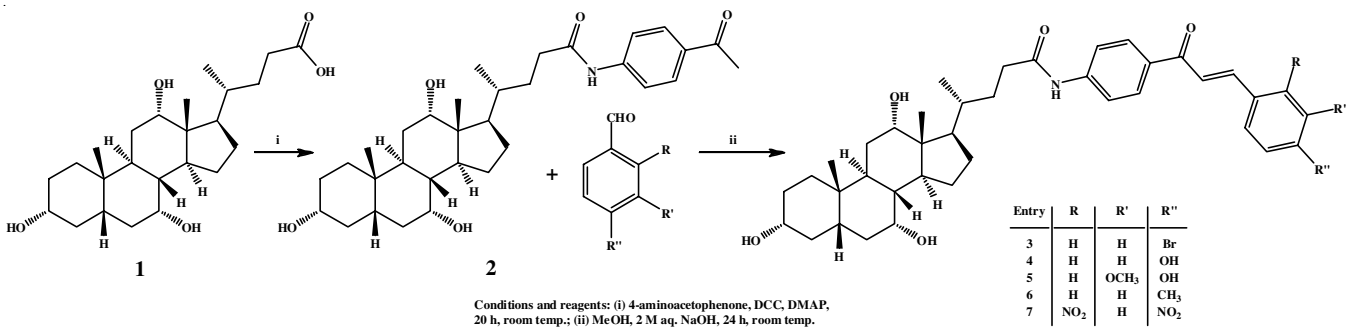
Synthesis of (*R*)-*N*-(4-((6*S*)-2-amino-6-(*p*-tolyl)-5,6-dihydro-4*H*-1,3-thiazin-4-yl)phenyl)-4-(3α,7α,12α)-trihydroxy-5β-cholan-24-amide (9): Using compound **6** (627.84 mg), compound **9** was obtained. Yield: 510 mg (74 %) as white solid; m.p. 133-135 ºC; m.w. 687.98; R_f = 0.44. IR (KBr, ν_{max}, cm^{-1}): 3394 (OH), 2931, 2854 (CH_2), 1681 $(C=O)_{amide}$, 1650 $(C=C)_{arom.}$; ^1H NMR (DMSO-d_6): δ 9.90 (s, 1H, NH_{amide}), 8.85 (s, 2H, NH_2), 8.06 (d, 2H, $H_{arom.}$-2′ + $H_{arom.}$-6′), 7.82 (d, 2H, $H_{arom.}$-3′ + $H_{arom.}$-5′), 7.47 (d, 2H, $H_{arom.}$-2″ + $H_{arom.}$-6″), 7.04 (d, 2H, $H_{arom.}$-3″ + $H_{arom.}$-5″), 4.98 (d, 1H, OH), 4.48 (d, 1H, OH), 4.21 (brs, 1H, OH), 4.18 (m,1H, H-12), 3.77 (m, 1H, H-7+t, 2H, H-b), 3.17 (m, 1H, H-3), 2.72 (d, 2H, H-a + H-c), 2.30 (m, 1H, H-4a), 2.19 (m, 2H, H-9), 2.14 (m, 1H, H-23a), 2.07 (m, 1H, H-23b), 1.95 (m, H, H-14a), 1.85 (m, 1H, H-6a), 1.75 (m, 1H, H-17), 1.66 (m, 1H, H-16a), 1.63 (m, 2H, H-1a + H-22a), 1.52 (m, 2H, H-2a + H-15a), 1.46 (m, 1H, H-4b), 1.45 (m, 1H, H-11a), 1.42 (m, 1H, H-6b), 1.39 (m, 1H, H-11b), 1.34 (m, 2H, H-8 + H-20), 1.28 (m, 1H, H-2b), 1.25 (m, 1H, H-5), 1.23 (m, 1H, H-22b), 1.21 (m, 1H, H-5), 1.19 (m, 1H, H-16b), 0.93 (d, 3H, Me-21), 0.92 (m, 1H, H-15b), 0.86 (m, 1H, H-1b), 0.80 (s, 3H, Me-19), 0.56 (s, 3H, Me-18); ^{13}C NMR (DMSO-d_6): δ 173.49 (CONH), 157.33 (C=N), 143.92 (C-1″), 141.52 (C-1′ + C-4″), 138.63 (C-3″ + C-5″), 136.65 (C-4′), 133.74 (C-3′ + C-5′) , 128.48 (C-2″ + C-6″), 121.52 (C-2′ + C-6′), 70.97 (C-12), 70.36 (C-3), 67.96 (C-7), 55.48 (C-a), 48.00 (C-17 + C-b), 46.00 (C-13), 45.59 (C-5), 41.32 (C-14), 40.02 (C-4 + C-8), 37.93 (C-20), 35.06 (C-1 + C-c), 34.64 (C-6), 34.30 (C-10), 34.16 (C-2 + C-22), 31.79 (C-23), 28.73 (C-11), 27.05 (C-16), 24.93 (C-9), 24.30 (Ar-Me), 22.08 (C-19), 22.08 (Me-19), 16.83 (Me-21), 11.81 (Me-18). Anal. calcd. (found) (%) for $C_{41}H_{57}N_3O_4S$: C, 71.58 (71.51); H, 8.35 (8.30); N, 6.11 (6.01).

Antimicrobial activity: The newly synthesized compounds were selected for their antimicrobial activities against bacteria and fungi. The microorganisms used were *Staphylococcus aureus* (Gram +ve), *Candida albicans* by using the agar diffusion method to select the most potent compounds. Each compound (5 mg) was dissolved in 1 mL of dimethyl sulfoxide (DMSO) then complete up to 10 mL with distilled water to give a concentration of 500 μg/mL. The bacteria were maintained on Muller hentone agar media, the dishes incubated at 37 ºC for 24 h for bacteria while 72 h for fungal. The efficiency of the tested compounds was compared to water and DMSO.

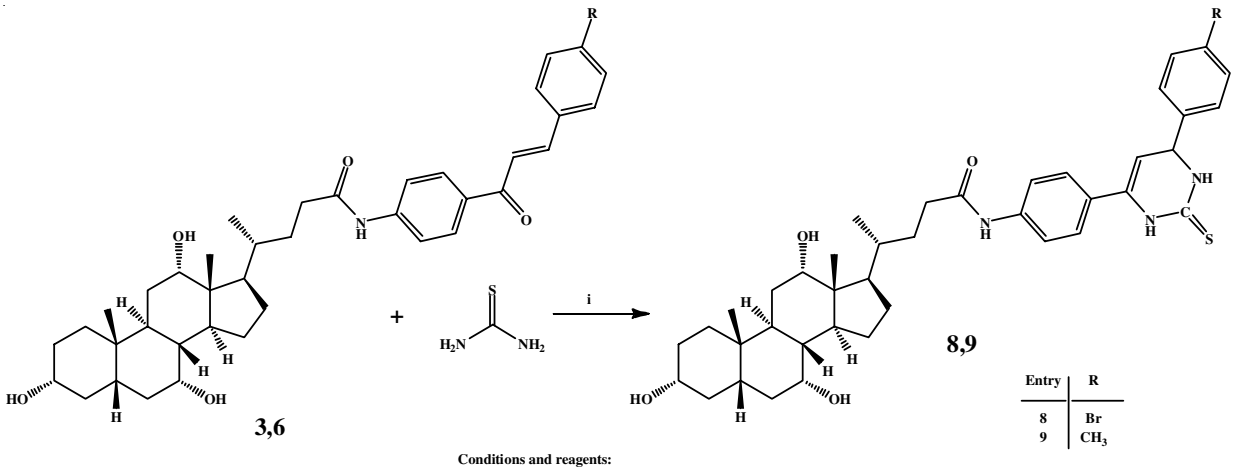
RESULTS AND DISCUSSION

Treatment of cholic acid (**3**) with 4-aminoacetophenone in the precence of DCC as coupling reagent and DMAP as catalyst in CH_2Cl_2-DMF gave *N*-(4-acetylphenyl)-3α,7α,12α-trihydroxy-5β-cholan-24-amide (**2**) in 68 % yield (**Scheme-I**). The synthesis of new chalcone derivatives aiming to examine their antimicrobial activities by treatment of compound **2** with adefferent aromatic aldehydes gives cholic acid chalcones (**3-7**) in 71.3-85 % yield **Scheme-I**.

Chalcone (**3** and **6**) reacted with thiourea in the presence ethanolic NaOH to give corresponding (**8** and **9**) in the presence absolute ethanol to give thiazine derivatives in (69 and 74 %) yield (**Scheme-II**).



Scheme-I



Scheme-II

The structures of compounds **3-7** were assigned on the basis of their IR and NMR (^{1}H, ^{13}C) spectra, which showed rather similar patterns of the aliphatic proton and carbon atoms. The ^{1}H NMR spectrum of compound **2** characterized by the presence of aromatic protons and carbon atoms, indicative for amidation of cholic acid backbone. The singlet at δ 9.95 ppm attributed to proton of NH amide. The aromatic protons H-3′ and H-5′ of compound **2**, appeared as doublets at δ ppm (J = 7.8 Hz), while H-2′ and H-6′ were resonated at δ ppm (J = 7.8Hz). The aromatic protons H-3′ and H-5′ of all analogues (**3-7**), appeared as doublets at the regions δ 6.69-8.04 ppm, while H-2′ and H-6′ were resonated at the regions δ 7.88-7.19 ppm. The aromatic protons H-3′′ and H-5′′ of compounds **1**, **2**, **3** and **5**, appeared as doublets at the regions δ 7.04-8.06 ppm, while H-2′′ and H-6′′ of compounds **1**, **2** and **4** were resonated at the regions δ 7.39, 7.63 and 7.21 ppm, respectively.The proton H-a and H-b were resonated at the regions δ 6.84-8.04 and δ 7.63.84-8.21 ppm, respectively. The proton H-a, H-c of compound **8** appears as triplet signal at δ 2.42 ppm and H-b appears as triplet signal at δ 3.77 ppm. While the proton H-a, H-c of compound **9** appears as atrpilet sginal at δ 2.72 ppm and the H-b appears as triplet signal at δ 3.77 ppm. In ^{13}C NMR, the resonances at the regions δ 171.56-198.18 ppm were assigned for carbonyl carbon atoms of amide group (CONH), whereas for carbonyl carbon atoms of chalcone group appears at δ 167.09-190.66 ppm. The signals at δ 129.24-132.30 ppm were attributed to C-2′ and C-5′ of compounds **1**, **2**, **3**, **4** and **5**, whereas the resonances at δ 117.10 and 1126.69 ppm were attributed to C- 2′ and C-5′ of compounds **1**, **2**, **3**, **4** and **5** too.The carbon atoms C-a,C-b and H-c of compound **8** were resonated at δ ppm, 46.55 ppm and 35.58 ppm, respectively while the carbon atoms C-a,C-b and H-c of compound **9** were resonated at δ 55.48, 48.00 and 35.06 ppm, respectively.

Biological activities: The antimicrobial activities of compounds (**3-9**) were tested against Gram +ve bacteria *S. aureus* and fungal (*Candida albicans*). The results of *in vitro* antimicrobial activities of hydrazones are listed in Table-1.

TABLE-1

Compd.	Gram-positive *S. aureus*	Fungal *Candida albicanas*
3	0.8	1.0
4	1.0	1.9
5	1.2	1.5
6	1.2	5.5
7	1.3	3.7
8	1.1	3.8
9	0.9	4.5
Control DMSO	0.0	0.0
Distilled water	0.0	0.0

Conclusion

In this study, we reported the synthesis of several chalcone and thiazine derivatives from cholic acid amide (**2**) with aromatic aldehydes in the presence of ethanolic NaOH as catalysts. Thiazine derivatives (**8**,**9**) synthesized by reaction of compounds **3** and **6** with thiourea. All the chalcone derivatives have weak to moderate activity against *S. aureus* but all derivatives show a moderate to strong activity aganist *Candida albicans*.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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