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ORIGINAL ARTICLE

Evaluation of antimicrobial activity of glycinate and carbonate derivatives of cholesterol: Synthesis and characterization

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Abstract A series of glycinate and carbonate derivatives of cholesterol **(4a–t)** were synthesized, characterized and assessed for their *in vitro* antimicrobial activity. Our results revealed that the compounds exerted inhibitory activities against gram-negative bacteria and fungi.

1. Introduction

Steroids are an important class of multi-cyclic compounds that exhibit multiple pharmacological and physiological activities in living organisms. Interestingly many of them have shown promising biological activities such as antimicrobial (Lone et al., 2013; Salmi et al., 2008; Gogoi et al., 2012; Kakati et al., 2013; Krishnamurthy et al., 1998), antioxidant (Prokai-Tatrai et al., 2008; Mooradian, 1993), anti-inflammatory (Mohamed et al., 2012; Maitraie et al., 2009), anti-mitotic (Rao et al., 2002), cytotoxic (Mayer and Bracher, 2011; Shan et al., 2009) and anticancer (Fernandez-Herrera et al., 2012) activities. In recent years, many cholesterol heterocyclic derivatives have exhibited antibacterial and antifungal activities (Loncle et al., 2004; Brunel et al., 2005). Banday et al. reported that fatty acid analogues of cholesterol have shown better antimicrobial activities (Banday et al., 2010) (Fig. 1) and Bildziukevich et al. disclosed the cytotoxicity of cholesteryl ester derivatives (Bildziukevich et al., 2013) (Fig. 1). A number of the simple benzamides (Carpino et al., 1983; Chambhare, 2003; Moreno et al., 2010) (Fig. 2) and sulphonamides (Aslan et al., 2012; Kamal et al., 2013; Basanagouda et al., 2010) (Fig. 2) were revealed as potent antibacterial agents. With the knowledge of these previous reports available in the literature, we inspired to study *in vitro* anti-bacterial and anti-fungal activities of carboxamide, sulphonamide, carbamate, urea and thiourea derived from glycinate and carbonate derivatives of cholesterol and the results were presented here.

The hydroxyl group attached with ring A in cholesterol has been derived to glycinates and carbonates by the use of coupling agent (Paul and Anderson, 1960). The cholesteryl glycinates (Ha et al., 2011; Li et al., 2006) and carbonates derivatives were further built up to simple amides (Luo

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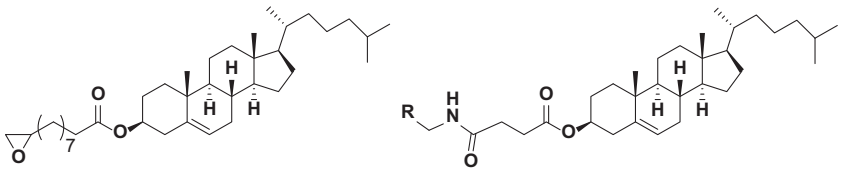


Figure 1 Examples for ester derivatives of cholesterol shown biological activities.

et al., 2001; De Logu et al., 2009), sulphonamide (Reddy et al., 2013; Ozbek et al., 2007; Keche et al., 2012), urea (Zhao et al., 2013; Faidallah et al., 2011; Vega-Perez et al., 2012) and thiourea (Hearn et al., 2006; Saeed et al., 2009; Abbas et al., 2013) by regular methodologies. The synthesized cholesterol derivatives were evaluated for their antimicrobial studies (Kakati et al., 2013). The pathogens have been chosen for antimicrobial screenings were *Bacillus subtilis*, *Staphylococcus epidermiditis*, *Proteus vulgaris* and *Escherichia coli* and for anti-fungal screening were *Candida albicans*.

2. Experimental

2.1. General considerations

Melting points were recorded on sigma melting apparatus SL111140. IR spectra were recorded in FT-IR Nicolet 6700 thermo scientific spectrometer using KBr pellet making method. ^{1}H NMR & ^{13}C NMR spectra were recorded on a Bruker 300 MHz instrument in $CDCl_3$ with TMS as an internal standard for proton and carbon spectra. Chemical shift values are mentioned in δ (ppm) and coupling constants are given in Hz. Mass spectra were recorded on Absciex 3000 LC-MS–MS. The progress of all reactions was monitored by TLC on 2 × 5 cm pre-coated silica gel 60 F254 plates of thickness of 0.25 mm (Merck). The chromatograms were visualized under UV 254–366 nm, iodine, and potassium permanganate strain solution. The elemental analyses were recorded in vario EL III CHNS element analyser.

2.2. General procedure for the synthesis of (3S,8S,9S,10R,13R,14S,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 (tert-butoxycarbonyl)glycinate (**2**)

The N-boc glycine (5 g, 28.5 mmol) was dissolved in chloroform (50 mL) and the solution was cooled to 0 °C. To that solution carbonyldiimidazole (CDI) (4.95 g, 30 mmol) was added under a nitrogen atmosphere and it was stirred for 15 min at room temperature. To that reaction mixture, cholesterol (**1**) (11 g, 28.5 mmol) was added and the reaction mixture was stirred for 24 h at ambient temperature. The completion of the reaction was monitored by thin layer chromatography. The reaction mixture was then diluted with chloroform (250 mL) and washed with water (2 × 200 mL) and brine solution (200 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated. The crude product obtained was subjected to column chromatography over silica gel (60–120 mesh) by using ethyl acetate/pet ether (5:95) mixture to obtain **2**.

White solid. Yield 73%. mp108–110 °C. IR (KBr) cm^{-1}: 3380, 2940, 1730, 1680, 1200, 1170. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.67 (s, 3H), 0.86 (d, J = 6.6 Hz, 3H), 0.87 (d, J = 6.6 Hz, 3H), 0.88 (d, J = 6.6 Hz, 3H), 0.90–2.0 (m, 38H), 2.33 (d, J = 7.8 Hz, 2H), 3.88 (d, J = 5.1 Hz, 2H), 4.62–4.75 (m, 1H), 5.03 (s, 1H carbamate NH), 5.36–5.38 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): 169.82, 155.85, 139.58, 123.06, 80.05, 75.34, 56.90, 56.40, 56.33, 50.27, 42.85, 42.52, 39.94, 39.70, 38.21, 37.12, 36.76, 36.38, 35.94, 32.07, 28.49, 28.36, 28.16, 27.90, 24.44, 24.02, 22.99, 22.93, 22.73, 22.69, 21.22, 19.42, 18.89, 12.02. ESI-LC/MS(M^+ + 1)calculated. m/z 544.8. Found 544.7. Anal.Calcd. for: $C_{34}H_{57}NO_4$: C, 75.09; H, 10.56; N, 2.58%. Found: C, 75.12; H, 10.53; N, 2.57%.

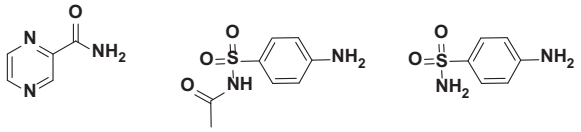


Figure 2 Examples for simple amide and sulphonamide based antibiotics.

2.3. General procedure for synthesis of compounds **3**, **4l**, **4r**

The compound **2** (5 g, 9.2 mmol) was dissolved in dichloromethane (DCM) (50 mL). To that solution trifluoroacetic acid (TFA) (7.0 mL, 0 92 mmol) was added and stirred for 30 min. Then the reaction mixture was concentrated, dried and washed with diethyl ether (25 ml) to obtain **3**.

2.3.1. 2-(((3S,8S,9S,10R,13R,14S,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)oxy)-2-oxoethan-1-aminium 2,2,2-trifluoroacetate (**3**)

White solid. Yield 92%. mp183–184 °C. IR (KBr) cm^{-1}: 3090, 2940, 1750, 1680, 1270, 1180. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.68 (s, 3H), 0.86 (d, J = 6.6 Hz, 3H), 0.87 (d, J = 6.6 Hz, 3H), 0.90 (d, J = 6.6 Hz, 3H), 0.99 (s, 3H), 1.00–2.03 (26H, cholesterol), 2.33 (d, J = 7.8 Hz, 2H), 3.74 (s, 2H), 4.62–4.73 (m, 1H), 5.36–5.38 (m, 1H). ^{13}C NMR (75 MHz, DMSO-D_6): δ 166.90, 139.09, 122.41, 75.14, 56.14, 55.66, 49.45, 41.85, 40.36, 39.53, 39.29, 37.42, 36.33, 36.04, 35.68, 35.16, 31.35, 27.71, 27.33, 27.12, 23.80, 23.22, 22.55, 22.30, 20.53, 18.85, 18.50, 11.60. ESI-LC/MS(M^+ + 1)calculated. m/z 444.7. Found 444.6. Anal.Calcd. for: $C_{31}H_{50}F_3NO_4$: C, 66.76; H, 9.04; N, 2.51%. Found: C, 66.73; H, 9.05; N, 2.53%.

2.3.2. 2-((2-(((3S,8S,9S,10R,13R,14S,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)oxy)-2-oxoethyl)amino)-2-oxoethan-1-aminium 2,2,2-trifluoroacetate (**4l**)

White solid. Yield 91%. mp131–132 °C. IR (KBr) cm^{-1}: 3080, 2930, 1740, 1670, 1260, 1180. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.68 (s, 3H), 0.86 (d, J = 6.6 Hz, 3H), 0.87 (d, J = 6.6 Hz,

3H), 0.90 (d, J = 6.6 Hz, 3H), 0.99 (s, 3H), 1.00–1.99 (m, 26H, cholesterol), 2.31 (d, J = 7.8 Hz, 2H), 3.87–3.97 (m, 4H), 4.56–4.66 (m, 1H), 5.36–5.38 (m, 1H), 8.15 (s, 1H, amide NH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 169.27, 139.39, 122.83, 75.54, 56.78, 56.48, 50.05, 42.40, 41.56, 40.97, 39.87, 39.56, 37.88, 37.84, 36.94, 36.56, 36.33, 35.93, 31.93, 28.30, 28.01, 27.55, 24.35, 24.14, 22.78, 22.56, 21.13, 19.26, 18.79, 11.90. ESI-LC/MS(M^+ + 1)calculated. m/z 501.7. Found 501.5. Anal.Calcd. for: $C_{33}H_{53}F_3N_2O_5$: C, 64.47; H, 8.69; N, 4.56%. Found: C, 64.43; H, 8.72; N, 4.59%.

*2.3.3. 2-(((((3S,8S,9S,10R,13R,14S,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)oxy)carbonyl)oxy)ethan-1-aminium 2,2,2-trifluoroacetate (**4r**)*

White solid. Yield 90%. mp167–169 °C. IR (KBr) cm^{-1}: 3090, 2930, 1750, 1670, 1270, 1260. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.68 (s, 3H), 0.86 (d, J = 6.6 Hz, 6H), 0.87 (d, J = 6.6 Hz, 3H), 0.90 (d, J = 6.6 Hz, 3H), 0.99 (s, 3H), 1.00–2.04 (26H, cholesterol), 2.35 (d, J = 7.8 Hz, 2H), 3.24 (t, J = 9.9 Hz, 2H), 4.37 (t, J = 9.9 Hz, 2H), 4.42–4.52 (m, 1H), 5.38–5.40 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 153.77, 138.98, 122.83, 78.31, 77.59, 76.73, 63.51, 56.48, 55.98, 49.81, 42.11, 39.84, 37.73, 36.62, 36.30, 35.97, 35.52, 31.65, 27.95, 27.72, 27.40, 24.04, 23.60, 22.56, 22.32, 20.82, 19.01, 18.52, 11.64. ESI-LC/MS(M^+ + 1)calculated. m/z 474.7. Found 474.5. Anal.Calcd. for: $C_{32}H_{52}F_3NO_5$: C, 65.39; H, 8.92; N, 2.38%. Found: C, 65.36; H, 8.96; N, 2.32%.

*2.4. General procedure for synthesis of compounds **4a–k**, **4p** and **4s***

Pyrazine 2-carboxylic acid (0.11 g, 0.89 mmol) was dissolved in tetrahydrofuran (THF) (20 mL) (DMF is used as solvent for synthesizing compound **4p**). To that solution 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDCI.HCl) (0.2 g, 1.06 mmol), hydroxy benzotriazole (HOBT) (0.121 g, 0.89 mmol), triethylamine (TEA) (0.3 mL, 1.87 mmol) and compound **3** (0.5 g, 0.897 mmol) were added. The reaction mixture was stirred for 24 hours and it was poured into crushed ice. The white precipitate obtained was filtered which was subjected to column chromatography over silica gel (60–120 mesh) by using ethyl acetate/pet ether mixture.

*2.4.1. (3S,8S,9S,10R,13R,14S,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 (pyrazine-2-carbonyl)-glycinate (**4a**)*

White solid. Yield 70%. mp179–180 °C. IR (KBr) cm^{-1}: 3360, 1750, 1680, 1270, 1180. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.68 (s, 1H), 0.87 (d, J = 6.6 Hz, 3H), 0.88 (d, J = 6.6 Hz, 3H), 0.90 (d, J = 6.6 Hz, 3H), 0.99 (s, 3H), 1.01–2.04 (26H, cholesterol), 2.37 (d, J = 7,8 Hz, 2H), 4.25 (d, J = 5.4 Hz, 2H), 4.68–4.78 (m, 1H), 5.38–5.40 (m, 1H), 8.26 (s, 1H amide NH), 8.56–8.57 (m, 1H), 8.77 (d, J = 2.4 Hz, 1H), 9.40 (d, J = 1.2 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 168.97, 163.33, 147.58, 144.59, 144.28, 142.82, 139.48, 123.14, 75.81, 56.90, 56.39, 50.25, 42.52, 41.70, 39.93, 39.69, 38.20, 37.10, 36.76, 36.38, 35.93, 32.06, 28.35, 28.15, 27.90, 24.44, 24.01, 22.92, 22.68, 21.22, 19.43, 18.89, 12.02. ESI-LC/MS(M^+ + 1)calculated. m/z 550.8. Found 550.8. Anal.Calcd. for: $C_{34}H_{51}N_3O_3$: C, 74.28; H, 9.35; N, 7.64%. Found: C, 74.32; H, 9.30; N, 7.62%.

*2.4.2. (3S,8S,9S,10R,13R,14S,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 (thiophene-2-carbonyl) glycinate (**4b**)*

White solid. Yield 71%. mp158–159 °C. IR (KBr) cm^{-1}: 3360, 2950, 1740, 1670, 1210, 1180. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.68 (s, 3H), 0.86 (d, J = 6.6 Hz, 3H), 0.87 (d, J = 6.6 Hz, 3H), 0.90 (d, J = 6.6 Hz, 3H), 0.99 (s, 3H), 1.00–2.03 (26H, cholesterol), 2.36 (d, J = 7.8 Hz, 2H), 4.19 (d, J = 4.8 Hz, 2H), 4.65–4.75 (m, 1H), 5.39–5.41 (m, 1H), 6.57 (s, 1H, amide NH), 7.07–7.11 (m, 1H), 7.49 (d, J = 4.8 Hz, 1H), 7.56 (d, J = 3.3 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 169.51, 161.99, 139.47, 138.35, 130.37, 128.69, 127.76, 123.16, 75.82, 56.89, 56.39, 50.24, 42.50, 42.09, 39.92, 39.68, 38.18, 37.08, 36.74, 36.37, 35.93, 32.06, 28.35, 28.14, 27.88, 24.43, 24.01, 22.91, 22.68, 21.20, 19.41, 18.88, 12.01. ESI-MS(M^+ + 1)calculated. m/z 554.8. Found 554.8. Anal.Calcd. for: $C_{34}H_{51}NO_3S$: C, 73.73; H, 9.28; N, 2.53%. Found: C, 73.78; H, 9.30; N, 2.48%.

*2.4.3. (3S,8S,9S,10R,13R,14S,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 (4-aminobenzoyl)-glycinate (**4c**)*

White solid. Yield 76%. mp196–197 °C. IR (KBr) cm^{-1}: 3470, 3370, 2950, 1740, 1630, 1210, 1190. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.68 (s, 3H), 0.86 (d, J = 6.6 Hz, 3H), 0.87 (d, J = 6.6 Hz, 3H), 0.90 (d, J = 6.6 Hz, 3H), 0.99 (s, 3H), 1.00–2.04 (26H, cholesterol), 2.36 (d, J = 7.8 Hz, 2H), 4.00 (s, 2H, amine NH_2), 4.19 (d, J = 4.8 Hz, 2H), 4.66–4.76 (m, 1H), 5.38–5.41 (m, 1H), 6.54 (s, 1H, amide NH), 6.66 (d, J = 8.1 Hz, 2H), 7.64 (d, J = 8.1 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 169.99, 167.31, 150.05, 139.55, 129.02, 123.50, 123.06, 114.25, 75.59, 56.88, 56.37, 50.23, 42.49, 42.17, 39.92, 39.67, 38.19, 37.09, 36.74, 36.36, 35.91, 32.05, 28.34, 28.13, 27.88, 24.42, 24.00, 22.91, 22.67, 21.20, 19.41, 18.87, 12.00. ESI-LC/MS(M^+ + 1)calculated. m/z 563.8. Found 563.6. Anal.Calcd. for: $C_{36}H_{54}N_2O_3$: C, 76.82; H, 9.67; N, 4.98% Found: C, 76.79; H, 9.70; N, 2.49%.

*2.4.4. (3S,8S,9S,10R,13R,14S,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 (2-(1H-indol-3-yl)-acetyl)glycinate (**4d**)*

White solid. Yield 74%. mp192–193 °C. IR (KBr) cm^{-1}: 3380, 3260, 2940, 1730, 1650, 1290, 1230. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.67 (s, 3H), 0.87 (d, J = 6.6 Hz, 3H), 0.88 (d, J = 6.6 Hz, 3H), 0.90 (d, J = 6.6 Hz, 3H), 0.99 (s, 3H), 1.00–2.00 (26H, cholesterol), 2.24 (d, J = 7.8 Hz, 2H), 3.79 (s, 2H), 3.94 (d, J = 5.4 Hz, 2H), 4.53–4.64 (m, 1H), 5.32–5.34 (m, 1H), 6.19 (s, 1H, amide NH), 7.13–7.23 (m, 3H), 7.39 (d, J = 8.1 Hz, 1H), 7.60 (d, J = 7.8 Hz, 1H), 8.44 (s, 1H, indole NH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 171.93, 169.27, 139.49, 136.65, 127.24, 123.99, 123.02, 122.67, 120.14, 118.84, 111.58, 108.76, 75.46, 56.87, 56.39, 50.22, 42.49, 41.77, 39.91, 39.67, 38.09, 37.05, 36.70, 36.37, 35.91, 33.28, 32.03, 28.33, 28.13, 27.78, 24.41, 24.00, 22.90, 22.67, 21.18,

19.39, 18.87, 11.99. ESI-LC/MS(M^+ + 1)calculated. *m/z* 601.8. Found 601.6. Anal.Calcd. for: $C_{39}H_{56}N_2O_3$: C, 77.96; H, 9.39; N, 4.66%. Found: C, 77.99; H, 9.43; N, 4.62%.

*2.4.5. (3S,8S,9S,10R,13R,14S,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 nicotinoylglycinate (**4e**)*

White solid. Yield 70%. mp139–140 °C. IR (KBr) cm^{-1}: 3370, 2940, 1750, 1650, 1260, 1210. ^1H NMR (300 MHz, $CDCl_3$): δ 0.68 (s, 3H), 0.86 (d, *J* = 6.6 Hz, 3H),0.87 (d, *J* = 6.6 Hz, 3H), 0.90 (d, *J* = 6.6 Hz, 3H), 0.99 (s, 3H), 1.00–2.04 (26H, cholesterol), 2.36 (d, *J* = 7.8 Hz, 2H), 4.21 (d, *J* = 4.8 Hz, 2H), 4.67–4.77 (m, 1H), 5.37–5.39 (m, 1H), 7.22 (s, 1H, amide NH), 7.36–7.40 (m, 1H), 8.14 (d, *J* = 7.8 Hz, 1H), 8.71 (d, *J* = 2.7 Hz, 1H), 9.04 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 169.36, 165.73, 152.53, 148.29, 139.40, 135.26, 129.76, 123.57, 123.18, 75.91, 56.87, 56.38, 50.23, 42.49, 42.18, 39.90, 39.66, 38.16, 37.06, 36.73, 36.35, 35.91, 32.04, 28.33, 28.12, 27.86, 24.41, 24.00, 22.89, 22.66, 21.19, 19.40, 18.87, 11.99. ESI-LC/MS(M^+ + 1)calculated. *m/z* 549.8. Found 549.7. Anal.Calcd. for: $C_{35}H_{52}N_2O_3$: C, 76.60; H, 9.55; N, 5.10%. Found: C, 76.66; H, 9.51; N, 5.11%.

*2.4.6. (3S,8S,9S,10R,13R,14S,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 ((E)-3-(3,4,5-trimethoxyphenyl)acryloyl)glycinate (**4f**)*

White solid. Yield 71%. mp108–109 °C. IR (KBr) cm^{-1}: 3400, 2940, 1750, 1670, 1240, 1200. ^1H NMR (300 MHz, $CDCl_3$): δ 0.68 (s, 3H), 0.87 (d, *J* = 6.6 Hz, 3H), 0.88 (d, *J* = 6.6 Hz, 3H), 0.90 (d, *J* = 6.6 Hz, 3H), 0.99 (s, 3H), 1.00–2.06 (26H, cholesterol), 2.35 (d, *J* = 7.8 Hz, 2H), 3.87 (s, 9H), 4.16 (d, *J* = 4.5 Hz, 2H), 4.65–4.75 (m, 1H), 5.34–5.36 (m, 1H), 6.37 (s, 1H, amide NH), 6.45 (d, *J* = 8.1 Hz, 1H), 6.71 (s, 2H), 7.51 (d, *J* = 15.6 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): 169.72, 166.02, 153.58, 141.69, 140.13, 139.46, 130.37, 123.11, 119.53, 105.48, 75.69, 61.00, 56.88, 56.32, 50.24, 42.48, 41.96, 39.90, 39.66, 38.18, 37.07, 36.72, 36.35, 35.91, 32.03, 28.31, 28.11, 27.87, 24.40, 23.98, 22.88, 22.65, 21.19, 19.40, 18.87, 11.98. ESI-LC/MS(M^+ + 1)calculated. *m/z* 664.9. Found 664.6. Anal.Calcd. for: $C_{41}H_{61}NO_6$: C, 74.17; H, 9.26; N, 2.11%. Found: C, 74.21; H, 9.25; N, 2.08%.

*2.4.7. (3S,8S,9S,10R,13R,14S,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 (4-hydroxybenzoyl)-glycinate (**4g**)*

White solid. Yield 72%. mp197–199 °C. IR (KBr) cm^{-1}: 3570, 3400, 2950, 1790, 1660, 1200, 1180. ^1H NMR (300 MHz, $CDCl_3$): δ 0.68 (s, 3H), 0.86 (d, *J* = 6.6 Hz, 3H), 0.87 (d, *J* = 6.6 Hz, 3H) 0.90 (d, *J* = 6.6 Hz, 3H), 0.99 (s, 3H), 1.00–2.04 (26H, cholesterol), 2.35 (d, *J* = 7.8 Hz, 2H), 4.13 (d, *J* = 5.1 Hz, 2H), 4.64–4.74 (m, 1H), 5.35–5.37 (m, 1H), 6.86 (d, *J* = 8.1 Hz, 2H), 7.39 (s, 1H, amide NH), 7.72 (d, *J* = 8.4 Hz, 2H), 9.47 (s, 1H, OH). ^{13}C NMR (75 MHz, $CDCl_3$):δ 169.54, 167.30, 160.53, 139.29, 128.96, 124.73, 122.61, 115.22, 74.99, 56.53, 56.02, 49.89, 42.49, 42.18, 39.90, 39.66 , 37.88, 36.76, 36.40, 36.01, 35.55, 31.70, 27.99, 27.76, 27.55, 24.08, 23.63, 22.59, 22.36, 20.85, 19.08, 18.55, 11.68. ESI-LC/MS(M^+ + 1)calculated. *m/z* 564.4. Found 565.5. Anal.Calcd. for: $C_{36}H_{53}NO_4$: C, 76.69; H, 9.47; N, 2.48%. Found: C, 77.71; H, 9.45; N, 2.48%.

*2.4.8. (3S,8S,9S,10R,13R,14S,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 ((1R,3R)-adamantane-1-carbonyl)glycinate (**4h**)*

White solid. Yield 72%. mp177–178 °C. IR (KBr) cm^{-1}: 3350, 2930, 1750, 1670, 1250, 1200. ^1H NMR (300 MHz, $CDCl_3$): δ 0.68 (s, 3H), 0.86 (d, *J* = 6.6, 3H), 0.87 (d, *J* = 6.6 Hz, 3H), 0.90 (d, *J* = 6.6 Hz, 3H), 0.99 (s, 3H), 1.00–2.05 (42H), 2.34 (d, *J* = 7.8 Hz, 2H), 3.99 (d, *J* = 4.8 Hz, 2H), 4.62–4.72 (m, 1H), 5.37–5.39 (m, 1H), 6.18 (s, 1H, amide NH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 178.19, 169.90, 139.45, 123.07, 75.52, 60.50, 56.81, 56.27, 50.13, 42.43, 41.63, 40.73, 39.84, 39.64, 39.26, 38.13, 37.02, 36.69, 36.62, 36.31, 35.90, 32.02, 31.96, 28.34, 28.21, 28.13, 27.82, 24.40, 23.95, 22.93, 22.68, 21.15, 19.40, 18.84, 14.31, 11.97. ESI-LC/MS(M^+ + 1)calculated. *m/z* 606.9. Found 606.8. Anal.Calcd. for: $C_{40}H_{63}NO_3$: C, 79.29; H, 10.48; N, 2.31%. Found: C, 79.31; H, 10.48; N, 2.28%.

*2.4.9. (3S,8S,9S,10R,13R,14S,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 (2,4-dihydroxybenzoyl)glycinate (**4i**)*

White solid. Yield 71%. mp232–233 °C. IR (KBr) cm^{-1}: 3400, 3350, 2940, 1730, 1640, 1220, 1170. ^1H NMR (300 MHz, $CDCl_3$): δ 0.68 (s, 3H), 0.86 (d, *J* = 6.6 Hz, 3H), 0.87 (d, *J* = 6.6 Hz, 3H), 0.90 (d, *J* = 6.6 Hz, 3H), 0.99 (s, 3H), 1.00–2.03 (26H, cholesterol), 2.35 (d, *J* = 7.8 Hz, 2H), 4.10 (d, *J* = 5.4 Hz, 2H), 4.62–4.73 (m, 1H), 5.36–5.38 (m, 1H), 6.37 (d, *J* = 2.4 Hz, 2H), 7.51 (d, *J* = 8.7 Hz, 1H), 7.80 (s, 1H, amide NH), 9.58 (bs, 1H, OH), 12.32 (bs, 1H, OH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.00, 169.25, 163.07, 162.64, 139.28, 128.21, 122.68, 107.53, 106.53, 103.51, 75.15, 56.56, 56.05, 49.91, 42.18, 41.36, 39.90, 39.66, 37.90, 36.79, 36.44, 36.04, 35.58, 31.73, 28.03, 27.80, 27.58, 24.11, 23.67, 22.63, 22.39, 20.89, 19.12, 18.59, 11.71. ESI-LC/MS(M^+ + 1)calculated. *m/z* 580.8. Found 580.7. Anal.Calcd. for: $C_{36}H_{53}NO_5$: C, 74.57; H, 9.21; N, 2.42%. Found: C, 74.55; H, 9.18; N, 2.45%.

*2.4.10. (3S,8S,9S,10R,13R,14S,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 (2-(4-hydroxyphenyl)acetyl)glycinate (**4j**)*

White solid. Yield 70%. mp146–147 °C. IR (KBr) cm^{-1}: 3400, 3300, 2950, 1750, 1650, 1220, 1190. ^1H NMR (300 MHz, $CDCl_3$): δ 0.67 (s, 3H), 0.86 (d, *J* = 6.6 Hz, 3H), 0.87 (d, *J* = 6.6 Hz, 3H), 0.90 (d, *J* = 6.6 Hz, 3H), 0.99 (s, 3H), 1.00–2.03 (26H, cholesterol), 2.30 (d, *J* = 7.8 Hz, 2H), 3.55 (s, 2H), 3.98 (d, *J* = 5.1 Hz, 2H), 4.58–4.68 (m, 1H), 5.34–5.36 (m, 1H), 6.15 (s, 1H, amide NH), 6.75 (d, *J* = 8.4 Hz, 2H), 7.09 (d, *J* = 8.4 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 172.73, 169.40, 155.87, 139.33, 130.86, 125.58, 123.19, 116.19, 75.84, 56.81, 56.29, 50.12, 42.56, 42.45, 41.88, 39.85, 39.65, 38.07, 37.00, 36.68, 36.33, 35.93, 32.02, 31.96, 28.36, 28.15, 27.78, 24.41, 23.99, 22.95, 22.70, 21.16, 19.40, 18.85, 11.99. ESI-LC/MS(M^+ + 1)calculated. *m/z* 578.8. Found 578.7. Anal.Calcd. for: $C_{37}H_{55}NO_4$: C, 76.91; H, 9.59; N, 2.42%. Found: C, 76.90; H, 9.60; N, 2.41%.

*2.4.11. (3S,8S,9S,10R,13R,14S,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 (tert-butoxycarbonyl)glycylglycinate (**4k**)*

White solid. Yield 71%. mp120–121 °C. IR (KBr) cm^{-1}: 3330, 2940, 1750, 1680, 1250, 1200. ^1H NMR (300 MHz, $CDCl_3$): δ 0.68 (s, 3H), 0.86 (d, J = 6.6 Hz, 3H), 0.87 (d, J = 6.6 Hz, 3H), 0.90 (d, J = 6.6 Hz, 3H), 0.99 (s, 3H), 1.00–2.06 (35H), 2.33 (d, J = 7.8 Hz, 2H), 3.86 (d, J = 5.4 Hz, 2H), 4.03 (d, J = 5.4 Hz, 2H), 4.61–4.72 (m, 1H), 5.36–5.38 (m, 1H), 6.15 (s, 1H, carbamate NH), 6.85 (s, 1H, amide NH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 169.94, 169.30, 156.17, 139.39, 123.10, 80.41, 75.60, 56.80, 56.27, 50.12, 42.42, 41.55, 39.83, 39.62, 38.09, 37.00, 36.66, 36.30, 35.90, 32.00, 31.94, 28.42, 28.33, 28.11, 27.78, 24.38, 23.96, 22.92, 22.67, 21.14, 19.39, 18.83, 11.96. ESI-LC/MS(M^+ + OAc) calculated. m/z 659.8. Found 659.8. Anal.Calcd. for: $C_{36}H_{60}N_2O_5$: C, 71.96; H, 10.06; N, 4.66%. Found: C, 71.94; H, 10.05; N, 4.67%.

*2.4.12. (3S,8S,9S,10R,13R,14S,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 2-(1H-tetrazol-1-yl)-acetate (**4p**)*

White solid. Yield 42%. mp195–196 °C. IR (KBr) cm^{-1}: 2930, 1750, 1240, 1170. ^1H NMR (300 MHz, $CDCl_3$): δ 0.68 (s, 3H), 0.86 (d, J = 6.6 Hz, 3H), 0.87 (d, J = 6.6 Hz, 3H), 0.90 (d, J = 6.6 Hz, 3H), 0.99 (s, 3H), 1.00–2.04 (26H, cholesterol), 2.35 (d, J = 7.8 Hz, 2H), 4.70–4.80 (m, 1H), 5.23 (s, 2H), 5.38–5.40 (m, 1H), 8.81 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 169.82, 155.85, 139.58, 123.06, 75.34, 56.90, 56.40, 50.27, 42.85, 42.52, 39.94, 39.70, 38.21, 37.12, 36.76, 36.38, 35.94, 32.07, 28.49, 28.36, 28.16, 27.90, 24.44, 24.02, 22.93, 22.69, 21.22, 19.42, 18.89, 12.02. ESI-LC/MS(M^+ + 1)calculated. m/z 497.7. Found 497.7. Anal.Calcd. for: $C_{30}H_{48}N_4O_2$: C, 72.54; H, 9.74; N, 11.28%. Found: C, 72.54; H, 9.75; N, 11.27%.

*2.4.13. (3S,8S,9S,10R,13R,14S,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 (2-(nicotinamido)-ethyl) carbonate (**4s**)*

White solid. Yield 70%. mp146–147 °C. IR (KBr) cm^{-1}: 3380, 2950, 1740, 1650, 1260, 1240. ^1H NMR (300 MHz, $CDCl_3$): δ 0.68 (s, 3H), 0.86 (d, J = 6.6 Hz, 3H), 0.87 (d, J = 6.6 Hz, 3H), 0.90 (d, J = 6.6 Hz, 3H), 0.99 (s, 3H), 1.00–2.04 (26H, cholesterol), 2.38 (d, J = 7.8 Hz, 2H), 3.79 (q, 2H), 4.35 (t, J = 9.6 Hz, 2H), 4.43–4.54 (m, 1H), 5.37–5.39 (m, 1H), 7.00 (s, 1H, amide NH), 7.40 (m, 1H), 8.14 (d, J = 8.1 Hz, 1H), 8.73 (d, J = 4.8 Hz, 1H), 9.01 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): **δ** 165.84, 154.78, 152.43, 148.11, 139.19, 135.36, 130.00, 123.64, 123.31, 78.59, 66.42, 56.79, 56.25, 50.10, 42.43, 39.82, 39.68, 39.63, 38.10, 36.92, 36.64, 36.30, 35.90, 31.94, 28.33, 28.12, 27.78, 24.39, 23.94, 22.93, 22.68, 21.16, 19.36, 18.83, 11.97. ESI-LC/MS(M^+ + 1)calculated. m/z 579.8. Found 579.7. Anal.Calcd. for: $C_{36}H_{54}N_2O_4C$, 74.70; H, 9.40; N, 4.84%. Found: C, 74.71; H, 9.41; N, 4.85%.

*2.5. General procedure for synthesis of compounds **4n** and **4o***

The compound **3** (0.5 g, 0.897 mmol) was dissolved in dichloromethane (DCM) (20 mL). To that solution triethylamine (TEA) (0.31 mL, 2.24 mmol) and isocyanate (0.1 g, 0.897 mmol) were added at 0 °C. The reaction mixture was stirred for 30 min at room temperature. The product was extracted with dichloromethane (100 mL) and washed with water (2 × 75 mL) and brine (75 mL) solution. The organic layer was separated, dried over anhydrous Na_2SO_4, filtered and concentrated. The crude product obtained was purified by column chromatography over silica gel using ethyl acetate/pet ether mixture.

*2.5.1. (3S,8S,9S,10R,13R,14S,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 (phenylcarbamoyl)-glycinate (**4n**)*

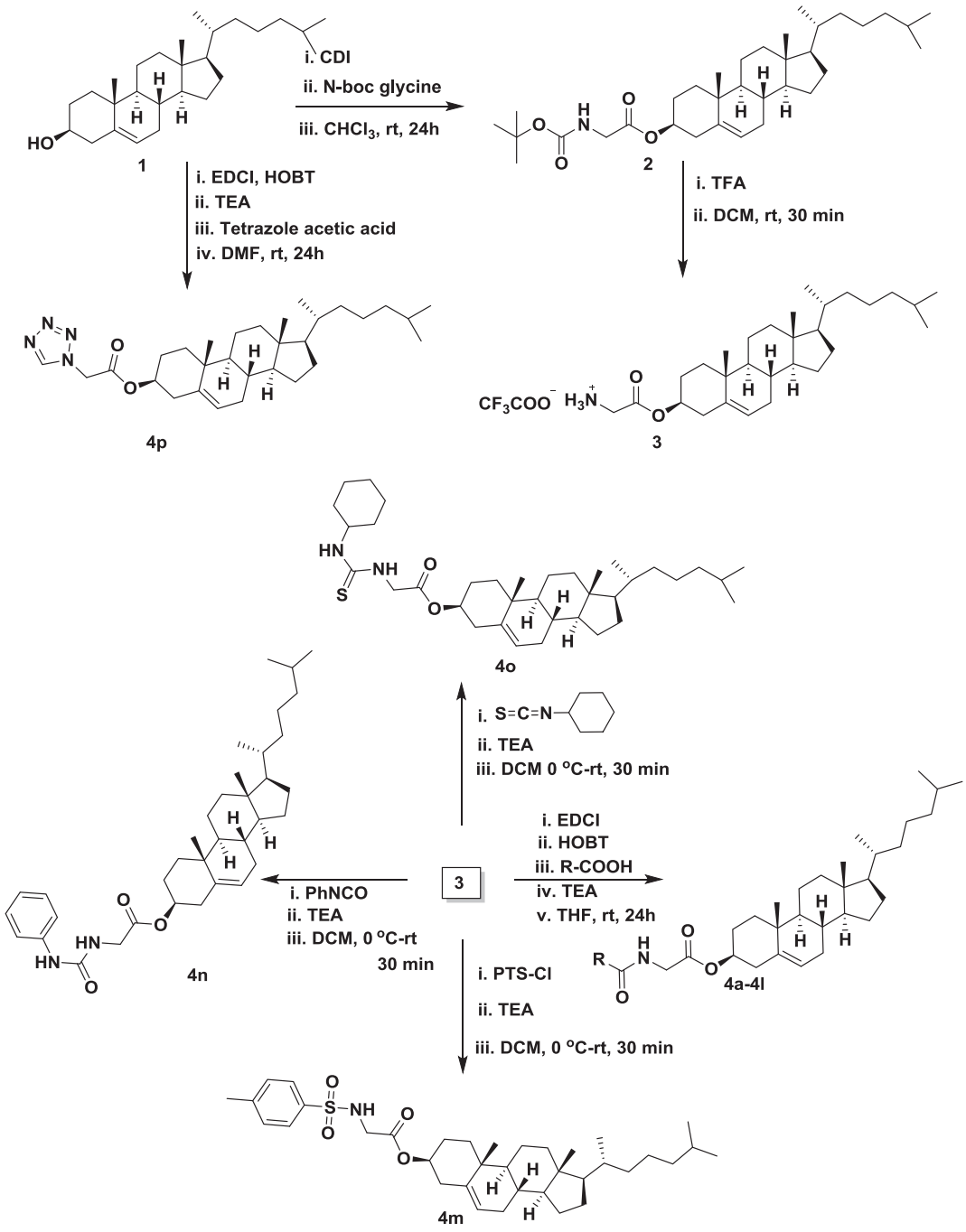
White solid. Yield 70%. mp177–178 °C. IR (KBr) cm^{-1}: 3330, 2940, 1750, 1680, 1250, 1200. ^1H NMR (300 MHz, $CDCl_3$): δ 0.67 (s, 3H), 0.86 (d, J = 6.6 Hz, 3H), 0.87 (d, J = 6.6 Hz, 3H), 0.90 (d, J = 6.6 Hz, 3H), 0.99 (s, 3H), 1.00–2.03 (26H, cholesterol), 2.30 (d, J = 7.8 Hz, 2H), 3.99 (d, J = 4.8 Hz, 2H), 4.58–4.68 (m, 1H), 5.32-5.34 (m, 1H), 5.99 (s, 1H, urea NH), 6.98–7.03 (m, 1H), 7.19–7.33 (m, 4H), 7.55 (s, 1H, urea NH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.88, 156.24, 139.45, 138.65, 129.25, 129.17, 123.72, 123.07, 120.91, 120.58, 75.57, 56.85, 56.34, 50.14, 42.46, 39.88, 39.65, 38.12, 37.03, 36.68, 36.34, 35.95, 32.03, 31.96, 28.37, 28.14, 27.81, 24.41, 24.03, 22.95, 22.70, 21.16, 19.41, 18.86, 11.99. ESI-LC/MS(M^+ + 1)calculated. m/z 563.8. Found 563.5. Anal.Calcd. for: $C_{36}H_{54}N_2O_3$:C, 76.82; H, 9.67; N, 4.98%. Found: C, 76.81; H, 9.67; N, 4.99%.

*2.5.2. (3S,8S,9S,10R,13R,14S,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 (cyclohexylcarbamothioyl)glycinate (**4o**)*

White solid. Yield.71%. mp141–142 °C. IR (KBr) cm^{-1}: 3330, 2930, 1740, 1550, 1260, 1210. ^1H NMR (300 MHz, $CDCl_3$): δ 0.68 (s, 3H), 0.86 (d, J = 6.6 Hz, 3H), 0.87 (d, J = 6.6 Hz, 3H), 0.90 (d, J = 6.6 Hz, 3H), 0.99 (s, 3H), 1.00–2.04 (36H, cholesterol), 2.35 (d, J = 7.8 Hz, 2H), 3.63–3.73 (m, 1H), 4.38 (d, J = 4.5 Hz, 1H), 4.63–4.73 (m, 1H), 5.37–5.39 (m, 1H), 6.33–6.39 (m, 2H, thiourea NH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 181.03, 170.06, 139.43, 123.17, 75.94, 56.88, 56.37, 52.91, 50.23, 46.93, 42.49, 39.90, 39.66, 38.15, 37.08, 36.74, 36.35, 35.92, 32.79, 32.05, 28.34, 28.13, 27.85, 25.53, 24.78, 24.41, 23.99, 22.92, 22.67, 21.19, 19.41, 18.86, 12.00. ESI-LC/MS(M^+ + 1)calculated. m/z 585.9. Found 585.6. Anal.Calcd. for: $C_{36}H_{60}N_2O_2S$: C, 73.92; H, 10.34; N, 4.79%. Found: C, 73.91; H, 10.33; N, 4.79%.

*2.6. General procedure for synthesis of compound **4m** and **4t***

The compound **3** (0.5 g, 0.897 mmol) was dissolved in dichloromethane (DCM) (5 mL). To that solution triethylamine (TEA) (0.32 mL, 2.25 mmol) and p-toluene sulphonyl chloride (PTS-Cl) (0.17 g, 0.897 mmol) were added at 0 °C. The reaction mixture was stirred for 30 min at room temperature. Then the reaction mixture was diluted with dichloromethane, (100 mL) and washed with water (2 × 75 mL) and brine (75 mL) solution. The organic layer was separated, dried over anhydrous Na_2SO_4, filtered and concentrated. The crude product obtained was purified by column chromatography over silica gel using ethyl acetate/pet ether mixture.



Scheme 1 Synthetic route for glycinate derivatives of cholesterol (**4a–o**).

*2.6.1. (3S,8S,9S,10R,13R,14S,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 tosylglycinate (**4m**)*

White solid. Yield: 80%. mp130–131 °C. IR (KBr) cm^{-1}: 3320, 2940, 1740, 1710, 1240, 1210. ^1H NMR (300 MHz, $CDCl_3$): δ 0.67 (s, 3H), 0.86 (d, J = 6.6 Hz, 3H), 0.87 (d, J = 6.6 Hz, 3H), 0.90 (d, J = 6.6 Hz, 3H), 0.99 (s, 3H), 1.00–2.02 (26H, cholesterol), 2.16 (d, J = 7.8 Hz, 2H), 2.32 (s, 3H), 3.75 (d, J = 4.8 Hz, 2H), 4.46–4.56 (m, 1H), 5.17 (s, 1H), 5.31–5.33 (m, 1H), 7.30 (d, J = 8.1 Hz, 2H), 7.75 (d, J = 8.1 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 168.31, 143.87, 139.19, 136.37, 129.87, 127.44, 123.20, 75.96, 56.78, 56.26, 50.09, 44.51, 42.42, 39.81, 39.63, 37.93, 36.93, 36.62, 36.30, 35.90, 31.93, 28.33, 28.13, 27.62, 24.39, 23.96, 22.94, 22.69, 21.67, 21.13, 19.36, 18.84, 11.97. ESI-LC/MS($M^+ - 1$)calculated. m/z 596.8. Found 596.5. Anal.Calcd. for: $C_{36}H_{55}NO_4S$: C, 72.32; H, 9.27; N, 2.34%. Found: C, 72.31; H, 9.25; N, 2.33%.

*2.6.2. (3S,8S,9S,10R,13R,14S,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 (2-((4-methylphenyl)-sulfonamido)ethyl) carbonate (**4t**)*

White solid. Yield: 78%. mp148–149 °C. IR (KBr) cm^{-1}: 3270, 2940, 1740, 1650, 1450, 1270, 1200. ^1H NMR (300 MHz, $CDCl_3$): δ 0.68 (s, 3H), 0.87 (d, J = 6.6 Hz, 3H), 0.88 (d, J = 6.6 Hz, 3H), 0.90 (d, J = 6.5 Hz, 3H), 0.99 (s, 3H),

1.00–2.00 (26H, cholesterol), 2.36 (d, J = 7.8 Hz, 2H), 2.43 (s, 3H), 3.22–3.26 (q, 2H), 4.14 (t, J = 10.2 Hz, 2H), 4.39–4.49 (m, 1H), 5.01 (s, 1H), 5.38–5.40 (m, 1H), 7.31 (d, J = 8.4 Hz, 2H), 7.75 (d, J = 8.4 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$):δ 154.22, 143.75, 139.23, 136.97, 129.93, 127.19, 123.26, 78.50, 77.58, 77.16, 76.74, 66.17, 56.79, 56.25, 50.10, 42.43, 42.24, 39.83, 39.63, 38.06, 36.92, 36.63, 36.30, 35.90, 31.95, 28.34, 28.12, 27.74, 24.39, 23.95, 22.94, 22.68, 21.65, 21.15, 19.37, 18.84, 11.97. ESI-LC/MS($M^+ - 1$)calculated. m/z 626.9. Found 626.6. Anal.Calcd. for: $C_{37}H_{57}NO_5S$: C, 70.77; H, 9.15; N, 2.23%. Found: C, 70.76; H, 9.18; N, 2.20%.

2.7. General procedure for preparation of compound **4q**

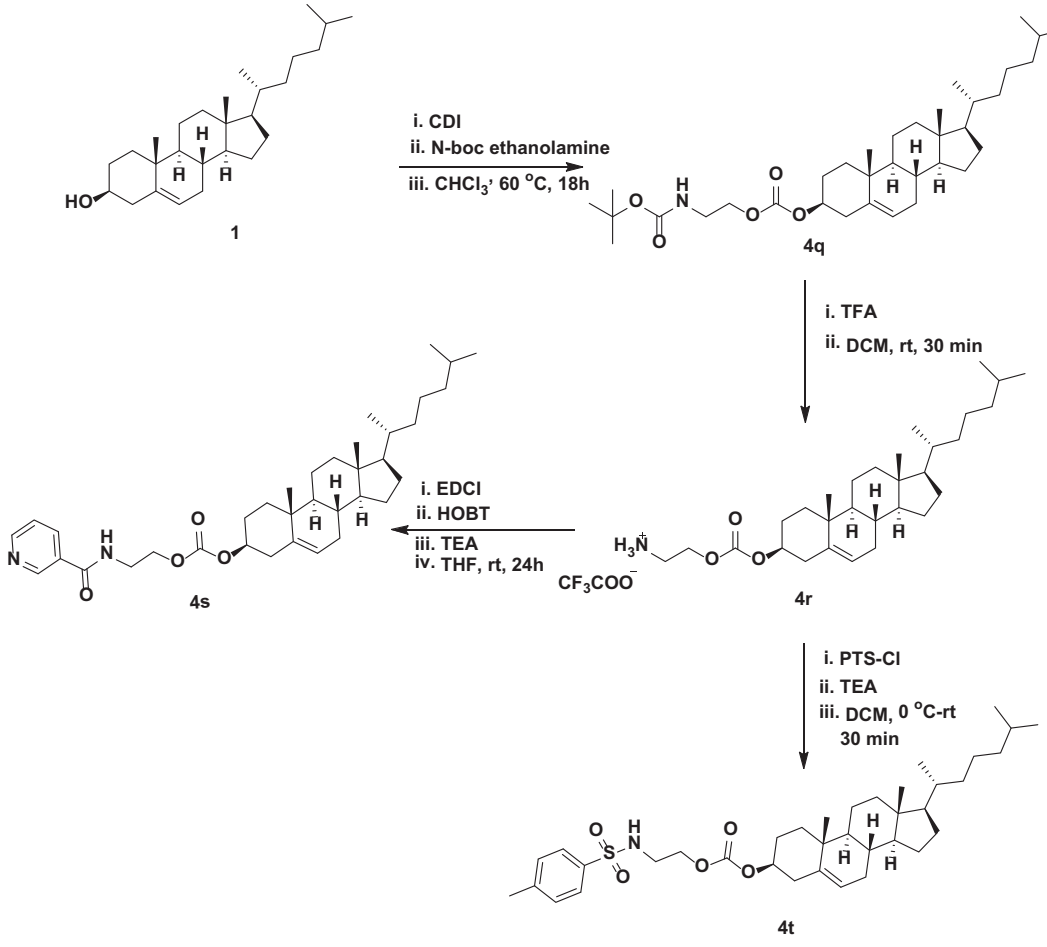
The cholesterol **1** (2 g, 5.1 mmol) was dissolved in chloroform ($CHCl_3$) (20 mL). To that solution carbonyldiimidazole (CDI) (0.839 g, 5.1 mmol) was added and refluxed for an hour. Then N-boc ethanolamine (0.834 g, 5.1 mmol) was added and it was further refluxed for 3 h. The completion of the reaction was monitored by thin layer chromatography. The product was extracted with chloroform (200 mL) and washed with water (2 × 150 mL) and brine (150 mL) solution. The organic layer was separated and dried over anhydrous Na_2SO_4, filtered and concentrated. The product was purified by column chromatography over silica gel using ethyl acetate/pet ether (1:9) mixture to obtain **4q**.

2.7.1. Tert-butyl (2-(((((3S,8S,9S,10R,13R,14S,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)oxy)carbonyl)oxy)ethyl)carbamate (**4q**)

White solid. Yield. 64%. mp79–80 °C. IR (KBr) cm^{-1}: 3370, 2960, 1750, 1690, 1260, 1170. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.68 (s, 3H), 0.86 (d, J = 6.6 Hz, 3H), 0.87 (d, J = 6.6 Hz, 3H), 0.90 (d, J = 6.6 Hz, 3H), 0.99 (s, 3H), 1.00–2.03 (35H), 2.40 (d, J = 7.8 Hz, 2H), 3.40–3.43 (q, 2H), 4.18 (t, J = 9.9 Hz, 2H), 4.43–4.53 (m, 1H), 4.89 (s, 1H, carbamate NH), 5.49–5.51 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 155.84, 154.52, 139.42, 123.21, 79.76, 78.27, 77.58, 77.16, 76.74, 66.99, 56.86, 56.29, 50.15, 42.48, 39.87, 39.66, 38.16, 37.00, 36.69, 36.34, 35.93, 32.04, 28.50, 28.36, 28.15, 27.84, 24.42, 23.97, 22.96, 22.70, 21.19, 19.40, 18.86, 12.00. ESI-LC/MS($M^+ + 1$)calculated. m/z 591.8. Found 591.7. Anal.Calcd. for: $C_{35}H_{59}NO_5$: C, 73.26; H, 10.36; N, 2.44%. Found: C, 73.29; H, 10.35; N, 2.42%.

2.8. Antimicrobial activity

The bacterial strains used for the examinations were *B. subtilis (ATCC 10876)*, *S. epidermiditis (ATCC 25923)*, *P. vulgaris (ATCC 27836)* and *E. coli (ATCC 25922)*, and fungal strain is *C. albicans (ATCC 66027)* obtained from either American type culture collection or purchased from Himedia, Mumbai.



Scheme 2 Synthetic route for carbonate derivatives of cholesterol (**4q–t**).

Table 1 List of synthesised cholesterol derivatives **4a–t**.

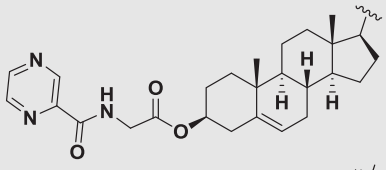
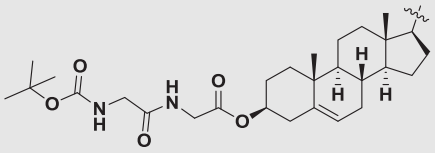
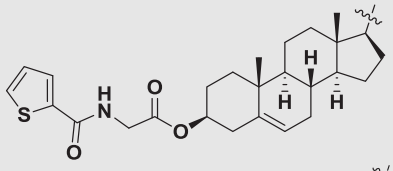
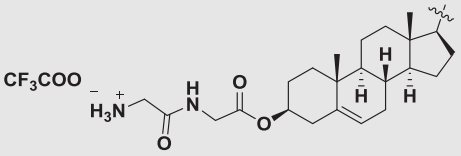
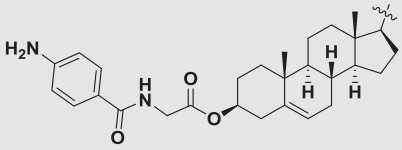
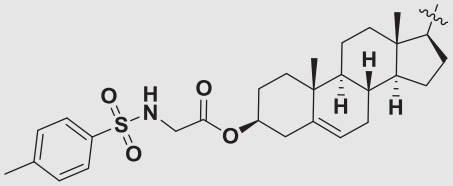
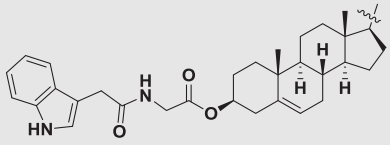
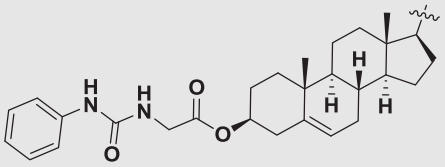
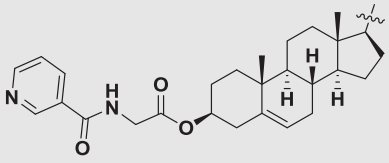
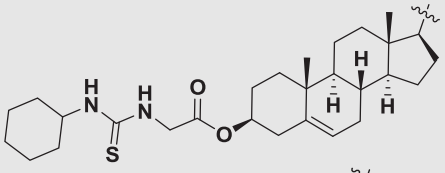
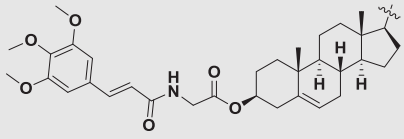
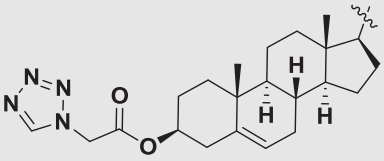
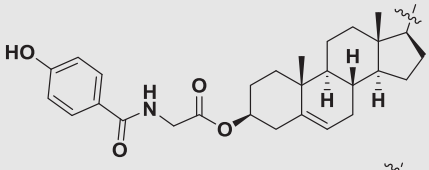
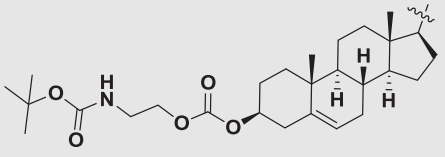
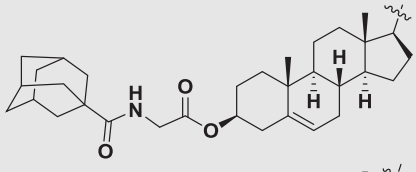
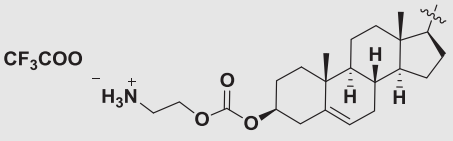
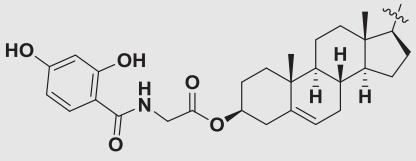
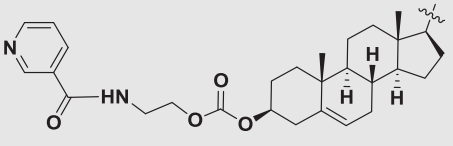
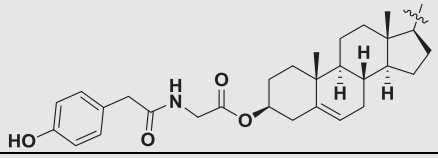
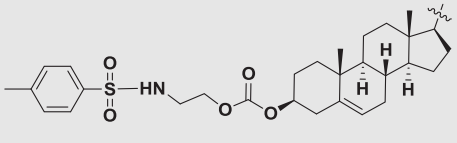
Entry	Structure of the compound	Entry	Structure of the compound
4a		4k	
4b		4l	
4c		4m	
4d		4n	
4e		4o	
4f		4p	
4g		4q	
4h		4r	
4i		4s	
4j		4t	

Table 2 Antimicrobial activity of cholesterol derivatives (**4a–t**).

S no.	Compound	Zone of inhibition (mm)				
		Escherchia coli	*Proteus vulgaris*	*Staphylococcus epidermiditis*	*Bacillus subtilis*	*Candida albicans*
1	4a	6.2 ± 0.2	5.0 ± 0.2	–	–	12.2 ± 0.3
2	4b	5.1 ± 0.3	4.2 ± 0.3	–	–	9.1 ± 0.2
3	4c	–	–	–	–	–
4	4d	4.9 ± 0.2	15.1 ± 0.3	–	–	5.8 ± 0.5
5	4e	8.3 ± 0.1	14.4 ± 0.2	–	–	6.8 ± 0.4
6	4f	–	14.5 ± 0.2	–	–	–
7	4g	–	–	–	–	–
8	4h	–	11.2 ± 0.1	–	–	8.0 ± 0.4
9	4i	–	–	–	–	–
10	4j	–	–	–	–	–
11	4k	–	8.9 ± 0.5	–	–	9.1 ± 0.5
12	4l	9.4 ± 0.4	14.1 ± 0.2	–	–	8.0 ± 0.3
13	4m	6.8 ± 0.4	13.8 ± 0.4	–	–	11.2 ± 0.2
14	4n	14.1 ± 0.3	16.2 ± 0.2	–	–	–
15	4o	7.3 ± 0.2	–	–	–	7.9 ± 0.3
16	4p	–	–	–	–	–
17	4q	–	9.3 ± 0.5	–	–	11.2 ± 0.2
18	4r	9.3 ± 0.3	18.1 ± 0.2	–	–	9.4 ± 0.3
19	4s	8.1 ± 0.2	11.2 ± 0.3	–	–	14.1 ± 0.2
20	4t	–	–	–	–	–
21	Control	–	–	–	–	–
22	Amikacin	17.0 ± 0.1	20.1 ± 0.2	18.2 ± 0.1	19.8 ± 0.3	–
23	Ketoconazole	–	–	–	–	17.0 ± 0.2

1. (–) no inhibition.
2. Control: DMSO.

Amikacin and ketoconazole are used as standard for antibacterial and antifungal substances respectively. The analogous conditions, dimethyl sulfoxide (DMSO) was used as negative control. The biological studies were carried out in the Bose clinical laboratory, Madurai, India.

The test organisms were overnight cultured on agar slants and incubated at (37 ± 0.5 °C and 24–48 h) for bacteria and fungi (37 ± 0.5 °C and 36 h) respectively to get the freshly prepared cultures. The cholesterol derivatives were evaluated for antimicrobial activity by the well diffusion method. Muller Hinton Agar (MHA) media were used for bacteria and Potato dextrose agar (PDA) media were used for fungal strains respectively. After sterilization, the medium was inoculated with freshly cultured bacterial strains under sterile condition that is under Laminar Flow. The inoculation was carried out when the temperature of the medium reached until 40–50 °C. The medium inoculated with test microorganisms was transferred into the plates of 90 mm size under sterile conditions. The medium was allowed to solidify and the wells (4/plate) of 6 mm diameter and 50 μL volume were bored on it by using sterile cork borer. The solution of test compounds 1000 μg/mL was prepared in DMSO and the wells bored on the medium were each filled (50 μg) with test compound using micropipette (20–200 μL). Four wells were bored on the plates and each filled with same compound and two plates for each test compound were taken and the experiments were repeated twice. The discs of amikacin and ketoconazole were also incorporated into the medium for comparison. The plates containing test organism and test material in contact were incubated at 37 ± 0.5 °C for 24 h. Identical procedure was employed for antifungal activity, however the culture strains of fungi and the plates were incubated at 37 ± 0.2 °C for 36–72 h. Inhibition of growth of test organisms (bacterial and fungal) in the presence of the test material and the standard was measured with the help of standard scale and the mean values of inhibition zones were reported in Table 2.

3. Results and discussions

3.1. Chemistry

In the present work, we synthesized a new series of glycinate derivatives of cholesterol **4a–o**, tetrazolyl acetate of cholesterol **4p** and carbonate derivatives of cholesterol **4q–r**. The first step in Scheme 1 was the coupling of commercially available cholesterol with N-boc glycine in the presence of carbonyldiimidazole (yield 73%). The deprotection of N-boc cholesteryl glycinate afforded the compound **3** in 92% yield. Further the compound **3** was converted into carboxamide (**4a–l**) by the reaction of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide, hydroxy benzotriazole and corresponding acids (yield 70–76%). Again, the compound **3** was converted into sulphonamide (**4m**) derivative by the reaction of p-toluene sulphonyl chloride in the presence of triethylamine (yield 80%). The phenyl urea and cyclohexyl thiourea derivatives of cholesterol (**4n** and **4o**) were synthesized with excellent yield by using phenyl isocyanate and cyclohexyl isothiocyanate in the presence of triethylamine. The compound **4p** built by the coupling of tetrazole acetic acid with cholesterol was followed by the same procedure which was used for compound **(4a–l)** (yield 65%).

Scheme 2 was the outline for synthesis of carbonate **(4q–r)** derivatives of cholesterol. The compound **4q** was synthesized by the carbonylation reaction between cholesterol and N-boc

ethanolamine (yield 64%). The deprotection of compound **4q** gave the product **4r** in 92% yield. The compound **4r** was further subjected to amide and sulphonamide by following the same protocol as described for **4s** and **4t**. All the synthesized compounds were characterized by ^{1}H NMR, ^{13}C NMR, Mass and IR spectroscopies. The appearance of NH protons in ^{1}H NMR, the appearance of carbonyl peak in ^{13}C NMR and the observed stretching frequency of $\sim$1700 cm^{-1} in IR spectra indicate the formation of either amides or urea derivatives. In addition 2D NMR spectra (^{1}H–^{1}H, ^{13}C–^{1}H and HMBC correlation spectrum) were recorded for the compounds **4d** and **4t**. For the compound **4d**, the correlation of 7.39 & 7.20, 8.44 & 7.14 and 6.12 & 3.93 in ^{1}H–^{1}H Cosy, and the correlation of 7.59 & 118.83, 7.39 & 111.57, 7.20 & 122.64, 3.94 & 42.49 in ^{13}C–^{1}H cosy indicate the presence of indolyl acetate and glycinyl unit. Similarly the correlation of 3.94 & 171.93, 6.19 & 171.93, 3.78 & 169.72, 8.44 & 123.99, 8.44 & 136.65, 7.59 & 108.75 in HMBC also indicates the presence of indolyl and glycinyl unit. In the compound **4t**, the correlation of 7.73 & 7.30 in ^{1}H–^{1}H cosy and the correlation of 7.74 & 127.19, 7.74 & 129.92, 2.42 & 50.09 in ^{13}C–^{1}H cosy indicate the presence of tosyl ring. The correlation of 4.43 & 154.22, 5.00 & 154.22 in HMBC indicates that the carbonate was attached with cholesterol. The correlation of 2.42 & 136.96, 2.42 & 127.19 indicates the presence of tosyl group. The list of synthesized compounds was summarized in Table 1.

3.2. Biology

3.2.1. Antimicrobial activity

A series of 20 compounds were tested against microorganism such as *B. subtilis*, *S. epidermiditis*, *P. vulgaris* and *E. coli* and fungi *C. albicans*. Among the synthesized compounds, **4a**, **4b**, **4d**, **4e**, **4l**, **4m**, **4n**, **4o**, **4r** and **4s** were exhibited activity against *E. coli* and **4a**, **4b**, **4d**, **4e**, **4f**, **4h**, **4k**, **4l**, **4m**, **4n**, **4q**, **4r** and **4s** were showed moderate activity against *P. vulgaris*. The compounds **4a**, **4b**, **4d**, **4e**, **4h**, **4k**, **4l**, **4m**, **4o**, **4q**, **4r** and **4s** were exerted activity against fungi. None of these compounds were active against gram-positive bacteria such as *B. subtilis* and *S. epidermiditis*. Furthermore, the compounds **4a**, **4b**, **4d**, **4e**, **4l**, **4m**, **4r**, **4s** and **4t** were active against both gram-negative bacteria and fungi. From the data, it was clear that the compounds **4a**, **4b**, **4d**, **4e** and **4s** possess heterocyclic unit is responsible for antimicrobial activities. On the other hand, compounds **4m** and **4t** have sulphonyl group might provoke antimicrobial activity, because it is a well known fact that many drugs for antibiotics are of sulpha drugs. The zones of inhibition (in mm) of synthesized compounds were summarized in Table 2.

4. Conclusion

In summary, a new series of 20 glycinates and carbonates derivatives of cholesterol were synthesized and evaluated their antimicrobial activity. Among all the compounds, **4a**, **4b**, **4d**, **4e**, **4l**, **4m**, **4r**, **4s** and **4t** were active against both gram-negative bacteria and fungi. With these achievements, we are trying to improve the biological activities further with the cholesterol derivatives.

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