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ORIGINAL ARTICLE

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

Rajendran Sribalan, Vediappen Padmini *, Andiappan Lavanya, Kandasamy Ponnuvel

Department of Organic Chemistry, Madurai Kamaraj University, Madurai 625 021, Tamil Nadu, India

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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

* Corresponding author. Mobile: +91 9095169125.
E-mail addresses: padimini_tamilenthi@yahoo.co.in, padmini.chem@mku.org (V. Padmini).
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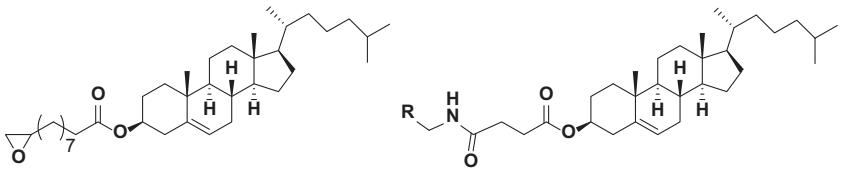

Figure 1 Examples for ester derivatives of cholesterol shown biological activities.

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%.

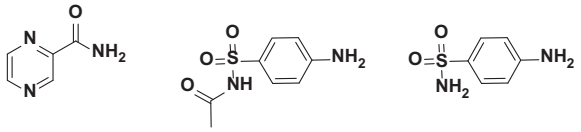

Figure 2 Examples for simple amide and sulphonamide based antibiotics.

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,