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A practical synthesis and spectroscopic study of new potentially biologically active *S*-lithocholic acid-substituted derivatives of 2-thiouracil

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ABSTRACT

Five new *S*-3α-acetoxy-5β-lithocholic acid methyl ester-substituted derivatives of 2-thiouracil and 6-methyl-2-thiouracil have been prepared. 5-Morpholino-methyl-2-thiouracil, 5-piperidinomethyl-2-thiouracil, and 5-(4-methylpiperidino)methyl-2-thiouracil have been obtained via the Mannich reaction between 6-methyl-2-thiouracil, paraformaldehyde, and the cyclic secondary amines morpholine, piperidine, or 4-methylpiperidine in ethanol. The structures of the compounds were confirmed by spectral (^{1}H NMR, ^{13}C NMR, and FT-IR) analyses and mass spectrometry. Estimation of the pharmacotherapeutic potential has been accomplished for the synthesized compounds on the basis of Prediction of Activity Spectra for Substances (PASS).

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Two very important classes of natural products, pyrimidine bases and steroids, play important roles in various biological systems. Thio derivatives of pyrimidine bases including 2-thiouracil, 6-methyl-2-thiouracil, and 2-thiocytosine are minor components of t-RNA, and furthermore, they have contributed remarkably to biological, pharmacological, and medicinal chemistry. Their S-, N-, or S,N-disubstituted analogs have shown therapeutic properties, especially antiviral, antithyroid, and antitumor activities.[1] They are also reported as biosensors and radioprotectors.[2] The prototropic tautomerism of thio derivatives of pyrimidine bases has attracted much attention. The tautomeric equilibrium of 2-thiouracil determines its chemoselectivity and regioselectivity, and is dependent on the temperature and on whether it is in the solution or in the solid state.[3]

In turn steroids such as cholesterol are very important components of cell membranes in eukaryotes and serve as substrates for the biosynthesis of bile acids (e.g., cholic acid, lithocholic acid, or deoxycholic acid), vitamin D, lipoproteins, and mammalian sex hormones. Moreover, synthetic receptors of steroids have been reported.[4] Steroids themselves have been used as building blocks for the design and construction of molecular receptors for the recognition of guest molecules of diverse chemical nature.[5]

However, to the best of our knowledge, no work has been published on the synthesis and physicochemical properties of *S*-3α-acetoxy-5β-lithocholic acid methyl ester-substituted derivatives of 2-thiouracil (**4a**) and 6-methyl-2-thiouracil (**4b**) and their 5-morpholinomethylene (**4c**), 5-piperidinomethylene (**4d**), and 5-(4-methylpiperidino)methylene (**4e**) analogs. Novel pharmacological actions of these compounds have been found on the basis of computer-aided drug discovery approaches with the program Prediction of Activity Spectra for Substances (PASS).[6] It is based on a robust analysis of structure–activity relationship in a heterogeneous training set currently including about sixty thousand biologically active compounds from different chemical series with about 4500 types of biological activity. Since only the structural formula of the chemical compound is necessary to obtain a PASS prediction, this approach can be used at the earliest stages of an investigation. There are many examples of the successful use of the PASS approach leading to new pharmacological agents.[6]

This Letter reports the synthesis and physicochemical properties of novel compounds **4a–e**. The structures of all the compounds obtained were determined by examining their ^{1}H and ^{13}C NMR, FT-IR, EI-MS, as well as ESI-MS spectra. Additionally, the analyses of the biological prediction activity spectra for compounds **4a–e** made herein are good examples of in silico studies of chemical compounds.

The antimetabolite of thymine, 5-morpholinomethyl-2-thiouracil was synthesized previously via Mannich reaction of 2-thiouracil, formalin, or formaldehyde and morpholine, in ethanol as the solvent.[7] We used this method to prepare 5-morpholinomethyl-6-methyl-2-thiouracil, 5-piperidinomethyl-6-methyl-2-thiouracil, and 5-(4-methylpiperidino)methyl-6-methyl-2-thiouracil (Scheme 1). Methyl 3α-hydroxy-5β-cholan-24-oate (**2**) and methyl 3α-chloroacetoxy-5β-cholan-24-oate (**3**) were prepared according to the literature procedures.[8,9] Compounds **4a–e** were synthesized regio- and chemoselectively via reaction of 2-thiouracil, 6-methyl-2-thiouracil, 5-morpholinomethyl-6-methyl-2-thiouracil, 5-piperidinomethyl-6-methyl-2-thiouracil, or 5-(4-methylpiperidino)methyl- 6-methyl-2-thiouracil with methyl 3α-chloroacetoxy-5β-cholan-24-oate (**3**) in dry DMF in the presence of anhydrous K_2CO_3 at room temperature for 24 h. The reaction of 2-thiouracil

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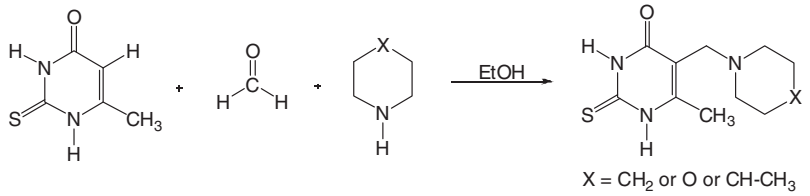
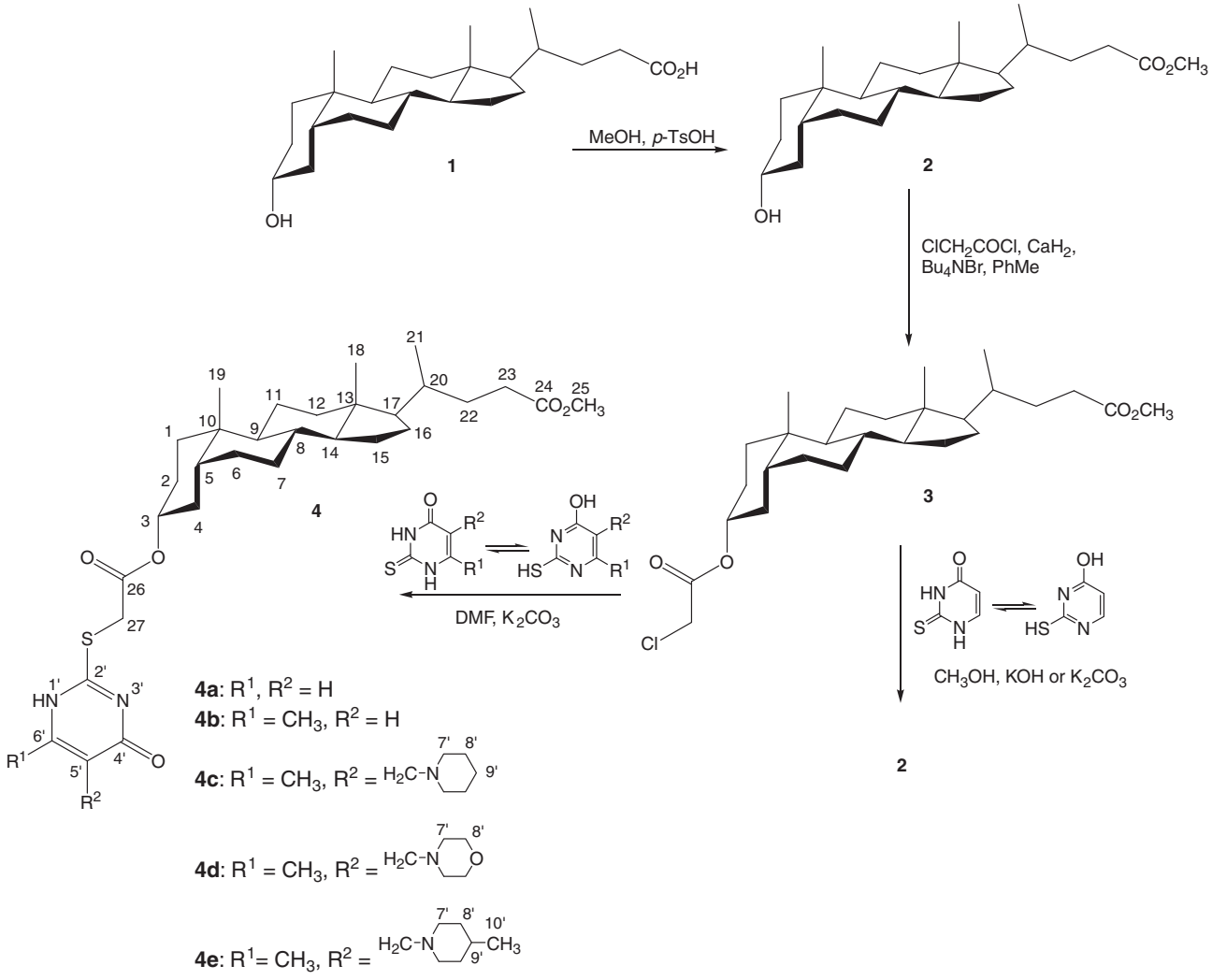


Scheme 1.

with **3** in anhydrous methanol in the presence of a strong base such as KOH or the weak base K_2CO_3 gave methyl 3α-hydroxy-5β-cholan-24-oate (**2**) (Scheme 2). Chattopadhyay et al. described the reaction between methyl 3α-bromoacetoxy-5β-cholan-24-oate or methyl 3α-bromoacetoxy-12α-hydroxy-5β-cholan-24-oate and uracil in DMF in the presence of dry K_2CO_3 at room temperature for 12 h.[9a] In this reaction they observed N1-alkylation of the uracil. In 2-thiouracil and 6-methyl-2-thiouracil, the sulfur atom is more nucleophilic than the nitrogen atoms (N1 or N3), and hence we observed the S-substituted derivatives of 2-thiouracil, 6-methyl-2-thiouracil, 5-morpholinomethyl-6-methyl-2-thiouracil, 5-piperidinomethyl-6-methyl-2-thiouracil, and 5-(4-methylpiperidino)methyl-6-methyl- 2-thiouracil.

The biological activity spectra were predicted for all five synthesized compounds **4a–e** using PASS. We also selected the types of activities that were predicted for a potential compound with the highest probability (focal activities), (Table 1 in Supplementary data). According to these data the most frequently predicted types of biological activity are cholesterol antagonist, acylcarnitine hydrolase inhibition, galactosylgalactosylglucosylceramide β-D-acetylgalactosaminyltransferase inhibition, and alkylacetylglycerophosphatase inhibition.

The ^{1}H NMR spectra of compounds **4a–e** in $CDCl_3$ showed characteristic multiplets in the range 4.79–4.81 ppm assigned to the Cβ3–H protons of the steroid skeleton and three hydrogen singlets in the range 0.64, 0.92, and 0.90–0.91 ppm assigned to CH_3-18,



Scheme 2.

CH_3-19, and CH_3-21, respectively. The protons of the CO_2CH_3 group gave signals in the range 3.66 ppm. Moreover, the ^{1}H NMR spectra of compounds **4a–e** showed characteristic singlets in the range 3.88–3.92 ppm assigned to the S–CH_2–CO protons. Two doublets at 6.22 and 7.80 ppm for C5′-H and C6′-H of the 2-thiouracil ring of compound **4a** and a singlet at 6.00 ppm due to C5′-H of the 6-methyl-2-thiouracil ring of compound **4b** were present. Diagnostic singlets for compounds **4b–e** in the range 2.16–2.23 ppm were assigned to the C6′-CH_3 protons of the 6-methyl-2-thiouracil ring.

The ^{13}C NMR spectra of compounds **4a–e** in $CDCl_3$ showed characteristic signals in the ranges 12.00–12.02 ppm, 23.26–23.28 ppm, and 18.24–18.26 ppm assigned to CH_3-18, CH_3-19, and CH_3-21, respectively. The carbons of the CO_2CH_3 group gave signals in the ranges 174.78–174.81 ppm and 51.48–51.49 assigned to CO_2 and CH_3, respectively. Two diagnostic signals for C26=O and S-CH_2 were present at 167.60–168.66 ppm and 33.13–33.38 ppm, respectively. The thiouracil ring exhibited signals in the range 107.95–111.39 ppm and 154.64–161.07 ppm assigned to C5′=C6′, respectively. The ^{13}C NMR spectra of compounds **4b–e** showed the presence of a methyl group from the 6-methyl-2-thiouracil ring at 23.85–24.13 ppm.

The FT-IR spectra of all the compounds (KBr discs) revealed two strong characteristic bands in the region 1666–1639 cm^{-1} and 1587–1546 cm^{-1}, assigned to ν(C4′=O) and ν(C5′=C6′), respectively. The most characteristic peaks in the FT-IR spectra of water-free **4a–e** in KBr were the bands at 1746–1730 cm^{-1} and 1272-1263 cm^{-1}, 2870–2865 cm^{-1}, and 1445–1432 cm^{-1}, assigned to ν(C24=O) and ν(C24O_2), ν(S–CH_2), and δ(S–CH_2), respectively.

In conclusion, five new compounds **4a–e** were prepared from methyl 3α-chloroacetoxy-5β-cholan-24-oate (**3**) in dry DMF in the presence of K_2CO_3 at room temperature for 24 h by reaction with 2-thiouracil, 6-methyl-2-thiouracil, 5-morpholinomethyl-6-methyl-2-thiouracil, 5-piperidinomethyl-6-methyl-2-thiouracil, or 5-(4-methylpiperidino)methyl-6-methyl-2-thiouracil.[10]

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2010.05.094.

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10. (a) General procedure for the synthesis of 5-morpholinomethyl-6-methyl-2-thiouracil, 5-piperidinomethyl-6-methyl-2-thiouracil, and 5-(4-methylpiperidino)methyl-6-methyl-2-thiouracil: a mixture of 6-methyl-2-thiouracil (5.0 g, 35.21 mmol), paraformaldehyde (3.0 g, 35.21 mmol), and morpholine (3.07 mL, 35.21 mmol), or piperidine (3.46 mL, 35.21 mmol), or 4-methylpiperidine (4.15 mL, 35.21 mmol) was suspended in 300 mL of EtOH (99.8%) and heated at reflux for 48 h. The obtained homogeneous solution was filtered and concentrated on a rotary evaporator to 150 mL. The reaction mixture was kept at room temperature for 24 h. The precipitated solid was isolated by filtration, dried at room temperature, and recrystallized from methanol. (b) General procedure for the synthesis of **4a** and **4b**: a mixture of 2-thiouracil (54.81 mg, 0.42 mmol) or 6-methyl-2-thiouracil (60.81 g, 0.42 mmol) and K_2CO_3 (59.10 mg, 0.42 mmol) in dry DMF (5 mL) was stirred at room temperature for 2 h. Next, methyl 3α-chloroacetoxy-5β-cholan-24-oate (**3**) (200 mg, 0.42 mmol) was added and the mixture was stirred for 24 h at room temperature (TLC). It was then poured onto crushed ice and extracted with benzene/Et_2O (1:1, 3 × 15 mL). The extract was washed with H_2O (3 × 15 mL) and brine (20 mL) and then dried over $MgSO_4$. The solvent was evaporated under reduced pressure to afford the crude product. (c) General procedure for the synthesis of **4c–e**: A mixture of 5-piperidinomethyl-6-methyl-2-thiouracil (145.18 mg, 0.42 mmol) or 5-morpholinomethyl-6-methyl-2-thiouracil (103.21 mg, 0.42 mmol) or 5-(4-methylpiperidino) methyl-6-methyl-2-thiouracil (108.35 mg, 0.42 mmol) and K_2CO_3 (59.10 mg, 0.42 mmol) in dry DMF (10 mL) was stirred at room temperature for 2 h. Next, methyl 3α-chloroacetoxy-5β-cholan-24-oate (**3**) (200 mg, 0.42 mmol) was added and the mixture was stirred for 24 h at room temperature (TLC). It was then poured onto crushed ice and extracted with benzene/Et_2O (1:1, 3 × 20 mL). The extract was washed with H_2O (3 × 20 mL), brine (30 mL), and dried over $MgSO_4$. The solvent was evaporated under reduced pressure to afford the crude product. The spectral and ES-HRMS data of compounds **4a–e** are given below.
Compound **4a** (isolated yield 79%, mp 175–176 °C): ^{1}H NMR (300 MHz, $CDCl_3$, TMS, ppm): δ 12.10 (1H, br s, N1-H), 7.80 (1H, d, *J* = 7.8 Hz, C6′-H), 6.22 (1H, d, *J* = 7.8 Hz, C5′-H), 4.81 (1H, m, Cβ3–H), 3.92 (2H, s, 27-CH_2), 3.66 (3H, s, –CO_2CH_3), 2.40–0.98 (27H, m, steroid skeleton), 0.92 (3H, s, 19-CH_3), 0.90 (3H, d, *J* = 6.4 Hz, 21-CH_3), 0.64 (3H, s, 18-CH_3). ^{13}C NMR (75 MHz, $CDCl_3$, TMS, ppm): 174.78 (C24), 167.60 (C26), 164.28 (C2′), 160.45 (C4′), 154.64 (C6′), 111.39 (C5′), 76.48 (C3), 56.40 (C14), 55.94 (C17), 51.49 (C25), 42.70 (C13), 41.85 (C5), 40.36 (C9), 40.06 (C12), 35.75 (C8), 35.34 (C20), 34.91 (C1), 34.55 (C10), 33.13 (C27), 32.01 (C4), 31.04 (C23), 30.97 (C22), 28.16 (C2), 26.97 (C16), 26.44 (C6), 26.38 (C7), 24.25 (C15), 23.26 (C19), 20.80 (C11), 18.24 (C21), 12.01 (C18). FT-IR (KBr, cm^{-1}): ν(C4′=O) 1664, ν(C5′=C6′) 1552, ν(C24=O) 1733, ν(C24O_2) 1265, ν(S–CH_2) 2870, δ(S–CH_2) 1445, ν(C26=O) 1749, ν(C26O_2) 1271. EI MS (*m*/*z*, % int.): 558 ($M^{+\cdot}$, 8), 372 (100), 215 (72). ES-HRMS $[C_{31}H_{46}N_2O_5S+H]^+$: calcd 559.3203, found 559.3208.
Compound **4b** (isolated yield 84%, yellow oil): ^{1}H NMR (300 MHz, $CDCl_3$, TMS, ppm): δ 12.15 (1H, br s, N1-H), 6.00 (1H, s, C5′-H), 4.79 (1H, m, Cβ3–H), 3.88 (2H, s, 27-CH_2), 3.66 (3H, s, –CO_2CH_3), 2.41–0.98 (28H, m, steroid skeleton), 2.16 (3H, s, C6′-CH_3), 0.92 (3H, s, 19-CH_3), 0.91 (3H, d, *J* = 6.4 Hz, 21-CH_3), 0.64 (3H, s, 18-CH_3). ^{13}C NMR (75 MHz, $CDCl_3$, TMS, ppm): 174.78 (C24), 168.66 (C26), 165.30 (C2′), 163.43 (C4′), 161.07 (C6′), 107.95 (C5′), 76.32 (C3), 56.36 (C14), 55.92 (C17), 51.48 (C25), 42.69 (C13), 41.86 (C5), 40.35 (C9), 40.04 (C12), 35.76 (C8), 35.34 (C20), 34.93 (C1), 34.56 (C10), 33.38 (C27), 32.06 (C4), 31.04 (C23), 30.97 (C22), 28.16 (C2), 27.01 (C16), 26.44 (C6), 26.27 (C7), 24.17 (C15), 23.85 (C6′-CH_3), 23.27 (C19), 20.79 (C11), 18.24 (C21), 12.00 (C18). FT-IR (KBr, cm^{-1}): ν(C4′=O) 1659, ν(C5′=C6′) 1549, ν(C24=O) 1738, ν(C24O_2) 1272, ν(S–CH_2) 2869, δ(S–CH_2) 1443, ν(C26=O) 1748, ν(C26O_2) 1269. EI MS (*m*/*z*, % int.): 572 ($M^{+\cdot}$, 10), 372 (100), 215 (45). ES-HRMS $[C_{32}H_{48}N_2O_5S+H]^+$: calcd 573.8068, found 573.8063.
Compound **4c** (isolated yield 87%, mp 157–157 °C): ^{1}H NMR (300 MHz, $CDCl_3$, TMS, ppm): δ 12.15 (1H, br s, N1-H), 4.81 (1H, m, Cβ3–H), 3.88 (2H, s, 27-CH_2),

3.66 (3H, s, $-CO_2CH_3$), 2.96 (2H, s, C5′-CH_2), 2.49 (4H, t, J = 12.2 Hz, C7′-H), 2.41–1.08 (28H, m, steroid skeleton), 2.22 (3H, s, C6′-CH_3), 1.59 (4H, m, C8′-H), 1.43 (2H, m, C9′-H), 0.92 (3H, s, 19-CH_3), 0.91 (3H, d, J = 6.4 Hz, 21-CH_3), 0.64 (3H, s, 18-CH_3). ^{13}C NMR (75 MHz, $CDCl_3$, TMS, ppm): 174.79 (C24), 167.79 (C26), 165.49 (C2′), 164.16 (C4′), 158.72 (C6′), 108.71 (C5′), 76.57 (C3), 56.42 (C14), 55.95 (C17), 55.56 (C5′-CH_2), 52.11 (C7′), 51.49 (C25), 42.71 (C13), 41.85 (C5), 40.39 (C9), 40.07 (C12), 35.75 (C8), 35.35 (C20), 34.92 (C1), 34.56 (C10), 33.21 (C27), 32.09 (C4), 31.05 (C23), 30.98 (C22), 29.69 (C8′), 28.17 (C2), 26.98 (C16), 26.48 (C6), 26.28 (C7), 24.16 (C15), 24.01 (C6′-CH_3), 23.27 (C19), 22.68 (C9′), 20.81 (C11), 18.26 (C21), 12.02 (C18). FT-IR (KBr, cm^{-1}): ν(C4′=O) 1639, ν(C5′=C6′) 1546, ν(C24=O) 1730, ν(C24O_2) 1269, ν(S–CH_2) 2867, δ(S–CH_2) 1432, ν(C26=O) 1743, ν(C26O_2) 1268. EI MS (m/z, % int.): 669 ($M^{+\cdot}$, 11), 372 (84), 215 (100). ES-HRMS $[C_{38}H_{59}N_3O_5S+H]^+$: calcd 670.9651, found 670.9656.

Compound **4d** (isolated yield 80%, mp 123–125 °C): ^{1}H NMR (300 MHz, $CDCl_3$, TMS, ppm): δ 12.28 (1H, br s, N1-H), 4.79 (1H, m, Cβ3–H), 3.88 (2H, s, 27-CH_2), 3.66 (3H, s, $-CO_2CH_3$), 3.60 (4H, t, J = 12.2 Hz, C8′-H), 2.62 (2H, s, C5′-CH_2), 2.39–1.08 (28H, m, steroid skeleton), 2.23 (4H, t, J = 12.2 Hz, C7′-H), 2.21 (3H, s, C6′-CH_3), 0.92 (3H, s, 19-CH_3), 0.90 (3H, d, J = 6.4 Hz, 21-CH_3), 0.64 (3H, s, 18-CH_3). ^{13}C NMR (75 MHz, $CDCl_3$, TMS, ppm): 174.81 (C24), 168.62 (C26), 164.22 (C2′), 163.78 (C4′), 157.88 (C6′), 108.67 (C5′), 76.58 (C3), 66.43 (C8′), 56.41 (C14), 55.95 (C17), 54.53 (C5′-CH_2), 52.92 (C7′), 51.49 (C25), 42.71 (C13), 41.86 (C5), 40.34 (C9), 40.07 (C12), 35.75 (C8), 35.35 (C20), 34.98 (C1), 34.57 (C10), 33.23 (C27), 32.09 (C4), 31.04 (C23), 30.98 (C22), 28.17 (C2), 27.01 (C16), 26.48 (C6), 26.29 (C7), 24.17 (C15), 24.13 (C6′-CH_3), 23.28 (C19), 20.81 (C11), 18.25 (C21), 12.01 (C18). FT-IR (KBr, cm^{-1}): ν(C4′=O) 1656, ν(C5′=C6′) 1578, ν(C24=O) 1746, ν(C24O_2) 1263, ν(S–CH_2) 2865, δ(S–CH_2) 1436, ν(C26=O) 1741, ν(C26O_2) 1269. EI MS (m/z, % int.): 671 ($M^{+\cdot}$, 15), 372 (87), 215 (100). ES-HRMS $[C_{37}H_{57}N_3O_6S+H]^+$: calcd 672.9055, found 672.9060.

Compound **4e** (isolated yield 78%, mp 183–184 °C): ^{1}H NMR (300 MHz, $CDCl_3$, TMS, ppm): δ 12.31 (1H, br s, N1-H), 4.79 (1H, m, Cβ3–H), 3.91 (2H, s, 27-CH_2), 3.66 (3H, s, $-CO_2CH_3$), 2.96 (2H, s, C5′-CH_2), 2.44 (4H, t, J = 12.2 Hz, C7′-H), 2.39–1.06 (28H, m, steroid skeleton), 2.23 (3H, s, C6′-CH_3), 1.79 (4H, q, J = 12.2 Hz, C8′-H), 1.39 (1H, m, C9′-H), 0.92 (3H, s, 19-CH_3), 0.90 (3H, d, J = 6.4 Hz, 21-CH_3), 0.89 (3H, d, C10′-CH_3), 0.64 (3H, s, 18-CH_3). ^{13}C NMR (75 MHz, $CDCl_3$, TMS, ppm): 174.78 (C24), 167.73 (C26), 165.57 (C2′), 164.58 (C4′), 158.82 (C6′), 108.68 (C5′), 76.36 (C3), 56.40 (C14), 55.94 (C17), 55.18 (C5′-CH_2), 51.79 (C7′), 51.49 (C25), 42.71 (C13), 41.85 (C5), 40.37 (C9), 40.06 (C12), 35.75 (C8), 35.35 (C20), 34.92 (C1), 34.56 (C10), 34.28 (C8′), 33.17 (C27), 32.08 (C4), 31.05 (C9′), 31.03 (C23), 30.98 (C22), 28.17 (C2), 26.98 (C16), 26.48 (C6), 26.28 (C7), 24.15 (C15), 24.03 (C6′-CH_3), 23.27 (C19), 22.97 (C10′), 20.81 (C11), 18.25 (C21), 12.01 (C18). FT-IR (KBr, cm^{-1}): ν(C4′=O) 1666, ν(C5′=C6′) 1587, ν(C24=O) 1745, ν(C24O_2) 1269, ν(S–CH_2) 2868, δ(S–CH_2) 1438, ν(C26=O) 1738, ν(C26O_2) 1265. EI MS (m/z, % int.): 683 ($M^{+\cdot}$, 22), 372 (100), 215 (75). ES-HRMS $[C_{39}H_{61}N_3O_5S+H]^+$: calcd 684.9916, found 684.9911.