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In vitro anti-HIV and HCV activity of new thioureido and thiazole analogues of cholesterol conjugated α-amino acid residues and *in silico* molecular modeling study

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Abstract: New functional cholesteryl ester derivatives of thioureido-amino acid-3-carboxylic acids were synthesized from cholesteryl chloroformate and evaluated for their *in vitro* inhibitory activity against HIV and hepatitis virus C (HCV). Compound **6** was the most active analogue which exhibited a remarkable activity against HIV-2 with IC_{50} value of > 1.06 μM and CC_{50} = 54.03 μM, resulting with selectivity index (SI) = 51. In addition, compound **5** showed an activity against HCV genotype 1b in the Huh 5-2 replicon system with EC_{50} = 5.09 μM and SI = 7.65, meanwhile the analogues **5**, **7** and **8** exhibited inhibition of 76.5, 82.8 and 88.3%, respectively. Molecular modelling was used to rationalize the biochemical results.

Keywords: Anti-HCV activity / Anti-HIV activity, Cholesteryl chloroformate / Thioureido-amino acids / Molecular docking study

1. Introduction

In vitro, several synthesized cholesterol derivatives exhibited diverse pharmaceutical activities. Cosalane **1** (Figure 1), the cholesterol derivative, and its amide analogues have been reported as inhibitors of the cytopathic effect of HIV-1 and HIV-2 in cell culture [1-3]. Other cholesterol derivatives exhibited antiviral activity such as cholesteryl-3",4"-dimethoxycinnamate and *o*-coumaroyl ester of 3β-(2'-hydroxyethoxy)-cholest-5-en against poliovirus type 1 (Mahoney) [4]. Furthermore, piperazine derivatives of cholesterol were used to generate novel cationic lipids for formulation in non-viral nucleic acid vectors (DNA vectors in gene therapy) [5]. Letsinger *et al.* [6] have described the synthesis of cholesteryl-conjugated oligonucleotides as potential HIV inhibitors in cell culture, where cholesterol

was selected as the anchor since it is highly hydrophobic and membranes of HIV and HIV infected cells are rich in this steroid [7]. A series of sulfonated cholic acid derivatives have been reported to display selective AIDS antiviral activity [8].

Hepatitis C virus (HCV) continues to be a global problem as a major cause of liver failure, meanwhile the only drugs available for its treatment were pegylated interferon-alpha (PEG IFN-α) in combination with ribavirin with various adverse effects [9, 10]. Extensive efforts in the discovery of HCV *NS3* protease inhibitors have resulted in a number of drug candidates at various stages of clinical development [11]. The two most advanced compounds, telaprevir [12, 13] and boceprevir [14, 15], provided an early proof of concept in suppressing the virus and are currently undergoing Phase III clinical trials [16]. In addition, danoprevir [17], TMC-435 [18], BMS-791325 [19] and vaniprevir (MK-7009) [20] are in clinical development as promising HCV protease inhibitors. However, the virus cannot be eliminated from approximately half of the infected patients treated with these agents. In addition, the side effects of these agents are sometimes serious and unacceptable to patients. Therefore, alternative agents for the treatment and prevention of HCV infection are urgently needed. In 2014, Daclatasvir dihydrochloride **2** (Figure 1), developed by Bristol-Myers Squibb Company, is approved by FDA as effective drug for treatment of HCV [21, 22].

In the present work, we report the synthesis of new cholesteryl derivatives conjugated amino acids *via* the thioureido and thiazole moieties with evaluation of their *in vitro* activity on the replication of HIV-1, HIV-2 and HCV type 1b, as well as the *in silico* molecular modeling study.

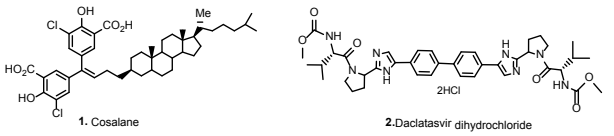


Figure 1. Some potentially active anti-HIV and anti-HCV agents

2. Experimental

2.1. *General methods*

Melting points were recorded on melting point apparatus (VEEGO), or a Büchi melting point apparatus B-545 (BÜCHI Labortechnik AG, Switzerland) and are uncorrected. ^{1}H and ^{13}C NMR spectra was recorded on a Bruker (Avance III, Germany) spectrospin-400 and 600 MHz (^{1}H) and 100 and 150.91 MHz (^{13}C) spectrometers using ($CDCl_3$) containing tetramethylsilane as internal standard (chemical shifts in δ, ppm), in order: multiplicity (bs, broad singlet; s, singlet; d, doublet; t, triplet; m, multiplet). Heteronuclear assignments were verified by ^{1}H,^{13}C HSQC, ^{1}H,^{13}C HMBC and ^{1}H,^{1}H NOESY NMR experiments. The IR spectra were measured as KBr disc by using F.T. IR-8400 (Shimadzu, Japan). Microanalytical data were obtained with a Vario elemental apparatus (Shimadzu, Japan). The purity of final products was confirmed by analytical high performance liquid chromatography (HPLC). HPLC/MS was performed on a MQS electrospray mass spectrometer (Thermo Fisher) or 70 eV EI and FAB MAT 8200 spectrometers (Finnigana MAT, USA). UV chamber was used for detection of spots in TLC. Eluent solvents of TLC were stated, while development reagent was iodine. Column chromatography was carried out with silica gel powder (230-400 mesh size) by using appropriate solvents.

2.2. *General procedure for the preparation of cholesterylthioureido amino acid derivatives* (**5-14**)

A solution of cholesteryl chloroformate (**3**) (174 mg, 0.40 mmol) and NH_4SCN (38

mg, 0.40 mmol) in acetone (10 ml) was heated under reflux for 1 h. After cooling, the solution was filtered and a suspension of amino acid (0.40 mmol) was added to the above filtrate. The mixture was heated under reflux for 6 h. After cooling, an excess of crashed ice was poured on the mixture with vigorous stirring. The result was collected, washed with acetone and purified on a SiO_2 column (5 g). Elution, in gradient, MeOH (0-10%) and $CHCl_3$ as eluent afforded the pure product.

2.2.1. *2-(3-(Cholesteroylcarbonyl)thioureido) acetic acid (**5**)*

From glycine (30 mg). Yield: 168 mg (79 %); MP: 158-160 °C; R_f = 0.48; IR (ν_{max}, cm^{-1}): 3136, 2840, 1722, 1500, 1404, 1330, 1253; ^{1}H NMR ($CDCl_3$): 10.36 (s, 1H, CO_2H, exchanged with D_2O), 9.23 (s, 1H, NH), 5.37 (t, 1H, *J* = 6.8 Hz, H-6), 4.77 (d, 2H, *J* = 5.2 Hz, CH_2-33), 4.46 (m, 1H, H-3), 2.34 (m, 2H, CH_2-4), 2.27 (m, 2H, CH_2-7), 1.95 (m, 3H, CH_2-20 + H-25), 1.78 (m, 2H, CH_2-15), 1.61-1.55 (m, 6H, CH_2-2+CH_2-12+CH_2-16), 1.52 (m, 2H, CH_2-11), 1.50 (m, 1H, H-17), 1.48 (m, 1H, H-14), 1.42 (m, 2H, CH_2-1), 1.32 (s, 3H, Me-19), 1.13 (m, 6H, CH_2-22+CH_2-23+CH_2-24), 1.03 (s, 3H, Me-18), 0.98 (bs, 9H, Me-21+Me-26+Me-27); ^{13}C NMR ($CDCl_3$): δ 180.2 (C=S), 172.3 (CO_2H), 151.9 (C=O), 138.6 (C-5), 124.1 (C-6), 72.3 (C-3), 56.4 (C-14), 55.9 (C-17), 49.7 (C-9+C-33), 42.1 (C-13), 39.2 (C-12+C-24), 38.1 (C-4), 36.4 (C-10), 36.3 (C-1), 36.0 (C-22), 35.5 (C-20), 31.7 (C-8), 31.6 (C-7), 28.1 (C-2), 27.7 (C-15+C-25), 24.1 (C-16), 23.5 (C-23), 22.9, 22.7 (C-26+C-27), 20.8 (C-11), 19.3 (Me-19), 18.9 (Me-21), 12.0 (Me-18); MS (FAB), *m/z* = 533 $[M+H]^+$; Elemental Analysis for $C_{30}H_{48}N_2O_4S$ (532.78); Cal: C, 67.63; H, 9.08; N, 5.26%; Found: C, 67.45; H, 9.01; N, 5.08%.

2.2.2. *2-(3-(Cholesteroylcarbonyl)thioureido) propanoic acid (**6**)*

From L-alanine (36 mg). Yield: 114 mg (52 %); MP: 196-198 °C; R_f = 0.63; IR (ν_{max}, cm^{-1}): 3091, 2935, 2823, 1616, 1411, 1361; ^{1}H NMR ($CDCl_3$): 10.38 (s, 1H, CO_2H, exchanged with D_2O), 9.23 (d, 1H, *J* = 3.5 Hz, NH), 5.40 (t, 1H, *J* = 6.5 Hz, H-6), 4.50 (m, 1H, H-3), 4.36 (dd, 2H, $J_{33,34}$ = 6.5 Hz, $J_{NH,33}$ = 3.5 Hz, CH_2-33), 2.33 (m, 2H, CH_2-4), 2.26 (m, 2H, CH_2-7), 1.93 (m, 3H, CH_2-20+ H-25), 1.80 (m, 2H, CH_2-15), 1.55 (m, 6H, CH_2-2+CH_2-12+CH_2-16), 1.49 (m, 2H, CH_2-11), 1.46 (m, 1H, H-17), 1.42 (m, 1H, H-14), 1.40 (m, 2H, CH_2-1), 1.30 (s, 3H, Me-19), 1.13 (m, 6H, CH_2-22+ CH_2-23+CH_2-24), 0.98 (s, 3H, Me-18), 0.89 (s, 3H, Me-21), 085 (s, 6H, Me-26+Me-27), 0.66 (d, 3H, $J_{33,34}$ = 6.5 Hz, Me-34); ^{13}C NMR ($CDCl_3$): 187.4 (C=S), 172.7 (CO_2H), 152.5 (C=O), 138.9 (C-5), 123.7 (C-6), 72.7 (C-3), 60.9 (C-33), 56.4 (C-14), 55.9 (C-17), 49.8 (C-9), 42.2 (C-13), 39.4 (C-24), 39.2 (C-12), 38.2 (C-4), 36.3 (C-10), 36.0 (C-22), 35.5 (C-20), 31.7 (C-7), 31.6 (C-8), 28.1 (C-2), 27.7 (C-25+C-15), 24.2 (C-16), 23.6 (C-23), 22.9, 22.7 (C-26+C-27), 20.9 (C-11), 19.3 (Me-19), 18.9 (Me-21), 17.5 (Me-34), 11.9 (Me-18); MS (FAB), *m/z* = 547 $[M+H]^+$; Elemental Analysisf or $C_{31}H_{50}N_{24}S$ (546.36); Cal: C, 68.09; H, 9.22; N, 5.12%; Found: C, 67.89; H, 9.14; N, 4.94%.

2.2.3. *2-(3-(Cholesteroylcarbonyl)thioureido)-4-(methylthio)butanoic acid (**7**)*

From L-methionine (60 mg). Yield: 208 mg (86 %); MP: 186-188 °C; R_f = 0.36; IR (ν_{max}, cm^{-1}): 3147, 2813, 1747, 1406; ^{1}H NMR ($CDCl_3$): 10.76 (s, 1H, CO_2H, exchanged with D_2O), 5.32 (t, 1H, *J* = 6.5 Hz, H-6), 4.47 (m, 1H, H-3), 4.29 (m, 2H, CH_2-33), 2.62 (m, 2H, CH_2-35), 2.30 (m, 4H, CH_2-4+CH_2-7), 2.14 (s, 3H, SMe), 1.97 (m, 5H, CH_2-20+H-25+CH_2-34), 1.82 (m, 2H, CH_2-15), 1.65 (m, 44 6H, CH_2-2+CH_2-12+CH_2-16), 1.49 (m, 2H, CH_2-11), 1.47 (m, 4H, CH_2-1+H-14+H-17), 1.30 (s, 3H, Me-19), 1.17 (m, 6H, CH_2-22+CH_2-23+CH_2-24), 0.98 (s, 3H, Me-18), 0.85 (m, 9H, Me-21+Me-26+Me-27); ^{13}C NMR ($CDCl_3$): 188.5 (C=S), 177.6 (CO_2H), 156.3 (C=O), 147.8 (C-5), 126.0 (C-6), 72.4 (C-3),

65.0 (C-33), 56.3 (C-14), 56.0 (C-17), 49.9 (C-9), 41.8 (C-13), 39.5 (C-24), 39.3 (C-12), 39.0 (C-4), 36.9 (C-1+C-10), 36.5 (C-20+C-22), 32.2 (C-34+C-35), 31.2 (C-7+C-8), 27.4 (C-2), 26.1 (C-15+C-25), 23.8 (C-16+C-23), 22.6, 22.2 (C-26+C-27), 20.1 (C-11), 18.9 (Me-19+Me-21), 14.5 (S-Me), 11.5 (Me-18); MS (FAB), *m/z* = 629 $[M+Na]^+$; Elemental Analysis for $C_{33}H_{54}N_2O_6S_2$ (606.92); Cal: C, 65.31; H, 8.97; N, 4.62%; Found: C, 65.17; H, 8.89; N, 4.44%.

2.2.4. *2-(3-(Cholesteroylcarbonyl)thioureido)-3-hydroxypropanoic acid (**8**)*

From L-serine (42 mg). Yield: 140 mg (62 %); MP: 158-160 °C; R_f = 0.52; IR (ν_{max}, cm^{-1}): 3151, 2952, 2844, 1596, 1409, 1120; ^1H NMR ($CDCl_3$): 10.40 (s, 1H, CO_2H, exchanged with D_2O), 9.30 (s, 1H, NH), 6.25 (m, 1H, OH), 5.41 (m, 1H, H-6), 4.80 (m, 1H, CH_2-OH), 4.78 (m, 1H, H-3), 4.45 (m, 1H, CH_2-33), 2.31 (m, 2H, CH_2-4), 2.25 (m, 2H, CH_2-7), 1.96 (m, 3H, CH_2-20+H-25), 1.82 (m, 2H, CH_2-15), 1.52 (m, 6H, CH_2-2+CH_2-12+CH_2-16), 1.49 (m, 2H, CH_2-11), 1.43 (m, 1H, H-17), 1.40 (m, 1H, H-14), 1.38 (m, 2H, CH_2-1), 1.31 (s, 3H, Me-19), 1.12 (m, 6H, CH_2-22+CH_2-23+CH_2-24), 0.98 (s, 3H, Me-18), 0.90 (m, 9H, Me-21+Me-26+Me-27); ^{13}C NMR ($CDCl_3$): 188.0 (C=S), 173.3 (CO_2H), 152.8 (C=O), 139.1 (C-5), 124.5 (C-6),71.9 (C-3), 68.5 (C-33), 61.3 (CH_2OH), 56.0 (C-14), 55.5 (C-17), 41.7 (C-13), 40.0 (C-24), 39.9 (C-4), 39.6 (C-12), 39.2 (C-24), 38.8 (C-1), 37.4 (C-10), 35.9 (C-22), 35.6 (C-20), 31.3 (C-7), 31.1 (C-8), 27.7 (C-2), 27.3 (C-25+C-15), 23.8 (C-16), 23.1 (C-23), 22.6 (Me-26), 22.3 (Me-27), 20.5 (C-11), 18.9 (Me-21), 18.5 (Me-19), 11.6 (Me-18); MS (FAB), *m/z* = 563 $[M+H]^+$; Elemental Analysis for $C_{31}H_{50}N_2O_5S$ (562.80); Cal: C, 66.16; H, 8.95; N, 4.98%; Found: C, 65.93; H, 8.86; N, 4.76%.

2.2.5. *2-(3-(Cholesteroylcarbonyl)thioureido)-3-hydroxybutanoic acid (**9**)*

From L-threonine (48 mg). Yield: 143 mg (62 %); MP: 210-212 °C;R_f = 0.69; IR (ν_{max}, cm^{-1}): 3026, 2960, 2856, 1728, 1623, 1487, 1404, 1294; ^1H NMR ($CDCl_3$): 10.36 (s, 1H, CO_2H, exchanged with D_2O), 9.23 (s, 1H, NH), 5.37 (t, 1H, *J* = 6.5 Hz, H-6), 4.43 (m, 1H, H-3), 4.37 (m, 1H, H-34), 3.89 (d, 1H, *J* = 5.1 Hz, H-33), 2.43 (m, 2H, CH_2-4), 2.27 (m, 2H, CH_2-7), 2.28 (m, 2H, CH_2-7), 1.93 (m, 3H, CH_2-20+H-25), 1.81 (m, 2H, CH_2-15), 1.62-1.53 (m, 6H, CH_2-2+CH_2-12+CH_2-16), 1.50 (m, 2H, CH_2-11), 1.49 (m, 1H, H-17), 1.46 (m, 3H, CH_2-1+H-14), 1.31 (s, 3H, Me-19), 1.18 (bs, 2H, Me-35), 1.13 (m, 6H, CH_2-22+CH_2-23+CH_2-24), 0.98 (s, 3H, Me-18), 0.88 (m, 9H, Me-21+Me-26+Me-27); ^{13}C NMR ($CDCl_3$): 186.4 (C=S), 172.0 (CO_2H), 152.6 (C=O), 140.9 (C-5), 123.5 (C-6), 69.3 (C-3+C-33), 66.9 (C-34), 55.5 (C-14+C-17), 49.5 (C-9), 41.8 (C-13), 39.4 (C-24), 39.1 (C-12), 38.8 (C-4), 37.5 (C-10), 36.0 (C-1), 35.6 (C-22), 35.2 (C-20), 31.3 (C-7+C-8), 27.4 (C-2), 27.4 (C-15+C-25), 23.8 (C-16), 23.2 (C-23), 22.7, 22.4 (C-26+C-27), 20.6 (Me-35), 20.1 (C-11), 18.9 (C-21), 18.5 (Me-19), 11.7 (C-18); MS (FAB), *m/z* = 599 $[M+Na]^+$; Elemental Analysis for $C_{32}H_{52}N_2O_5S$ (576.83); Cal: C, 66.63; H, 9.09; N, 4.86%; Found: C, 66.46; H, 9.00; N, 4.65%.

2.2.6. *2-(3-(Cholesteroylcarbonyl)thioureido)-3-(1H-imidazol-4-yl)propanoic acid (**10**)*

From L-histidine (62 mg). Yield: 169 mg (69 %); MP: 182-184 °C; R_f = 0.54; IR (ν_{max}, cm^{-1}): 2943, 2862, 1737, 1623, 1473, 1382, 1230, 1029; ^1H NMR ($CDCl_3$): 10.78 (s, 1H, CO_2H, exchanged with D_2O), 9.24 (s, 1H, NH), 7.07-7.00 (m, 2H, H-2'$_{imidazol}$+H-5'$_{imidazol}$), 5.36 (t, 1H, *J* = 6.2 Hz, H-6), 4.37 (d, 1H, *J* = 4.9 Hz, H-3), 3.89 (m, 1H, H-33), 2.94 (m, 2H, CH_2-34), 2.29 (m, 2H, CH_2-4), 2.15 (m, 2H, CH_2-7), 1.97 (m, 3H, CH_2-20 + H-25), 1.83 (m, (m, 2H, CH_2-15), 1.60 (m, 6H, CH_2-2+CH_2-12+CH_2-16), 1.48 (m, 2H, CH_2-11), 1.43 (m, 2H, H-14+H-17), 1.35 (m, 2H, CH_2-1), 1.32 (s, 3H, Me-19), 1.13 (m, 6H, CH_2-22+CH_2-23+CH_2-24), 0.98 (s, 3H, Me-18), 0.84 (m, 9H, Me-21+Me-26+Me-27);

^{13}C NMR ($CDCl_3$): 188.4 (C=S), 174.0 (CO_2H), 155.6 (C=O), 140.4 (C_{indol}-1), 135.1 (C_{indol}-3), 121.7 (C-6), 118.9 (C_{indol}-4),71.2 (C-3+C-33), 55.5 (C-14), 51.6 (C-17), 49.3 (C-9), 41.8 (C-13), 39.6 (C-24), 39.2 (C-12), 38.8 (C-4), 36.8 (C-10), 36.5 (C-1), 37.5 (C-10), 36.0 (C-1), 36.0 (C-22), 35.9 (C-20), 31.3 (C-7+C-8), 27.7 (C-2), 27.3 (C-15+C-25), 26.3 (C-34), 24.3 (C-16), 23.1 (C-23), 22.6, 22.3 (C-26+C-27), 20.1 (C-11), 18.9 (C-21), 18.5 (Me-19), 11.6 (C-18); MS (FAB), *m/z* = 635 $[M+Na]^+$; Elemental Analysis for $C_{34}H_{52}N_4O_4S$ (612.87); Cal: C, 66.63; H, 8.55; N, 9.14%; Found: C, 66.45; H, 8.46; N, 8.93%.

2.2.7. *2-(3-(Cholesteroylcarbonyl)thioureido) hexanoic acid (**11**)*

From L-lysine (58 mg). Yield: 126 mg (52 %); MP: 198-200 ^oC; R_f = 0.31; IR (ν_{max}, cm^{-1}): 3157, 2933, 2896, 1625, 1504, 1411; ^{1}H NMR ($CDCl_3$): 10.81 (s, 1H, CO_2H, exchanged with D_2O), 7.24 (bs, 1H, NH_2), 5.42 (m, 1H, H-6), 4.44 (m, 1H, H-3), 4.32 (m, 1H, H-33), 2.78 (m, 2H, CH_2-37), 2.31 (m, 2H, CH_2-4), 2.27 (m, 2H, CH_2-7), 1.97 (m, 3H, CH_2-20+H-25), 1.77 (m, 2H, CH_2-15+CH_2-34), 1.55 (m, 8H, CH_2-2+CH_2-12 + CH_2-16 + CH_2-36), 1.47 (m, 2H, CH_2-11), 1.39 (m, 1H, H-17), 1.36 (m, 1H, H-14), 1.30 (m, 4H, CH_2-1+CH_2-35), 1.28 (s, 3H, Me-19), 1.13 (m, 6H, CH_2-22+CH_2-23+CH_2-24), 1.01 (s, 3H, Me-18), 0.87 (m, 9H, Me-21+Me-26+Me-27); ^{13}C NMR ($CDCl_3$): 188.0 (C=S), 173.1 (CO_2H), 151.3 (C=O), 137.7 (C-5), 124.1 (C-6), 70.6 (C-3), 56.0 (C-33), 55.5 (C-14+C-17), 49.1 (C-9), 41.7 (C-13+C-38), 39.4 (C-12+C-24), 38.9 (C-4), 36.3 (C-10), 36.0 (C-1), 35.9 (C-22), 35.5 (C-20), 32.6 (C-34+C-37), 31.3 (C-7+C-8), 27.7 (C-2+C-36), 27.3 (C-15+C-25), 23.8 (C-16+C-37), 23.1 (C-23), 22.6, 22.3 (C-26+C-27), 20.5 (C-11), 18.8 (C-19), 18.5 (C-21), 11.5 (C-18); MS (FAB), *m/z* = 604 $[M+H]^+$; Elemental Analysis for $C_{34}H_{57}N_3O_4S$ (603.90); Cal: C, 67.62; H, 9.51; N, 6.96%; Found: C, 67.46; H, 9.43; N, 6.80%.

2.2.8. *2-(3-(Cholesteroylcarbonyl)thioureido)-3-(4-hydroxyphenyl)propanoic acid (**12**)*

From L-tyrosine (72 mg). Yield: 192 mg (75 %); MP: 230-232 ^oC; R_f = 0.61; IR (ν_{max}, cm^{-1}): 3207, 3014, 2956, 2887, 1595, 1415, 1326, 1244; ^{1}H NMR ($CDCl_3$): 10.82 (s, 1H, CO_2H, exchanged with D_2O), 9.22 (s, 1H, NH), 7.45-7.20 (m, 4H, $H_{arom.}$), 5.37 (t, 1H, *J* = 6.6 Hz, H-6), 4.53 (m, 1H, H-3), 4.39 (m 1H, H-33), 3.10 (m, 2H, CH_2-34), 2.39 (m, 2H, CH_2-4), 2.32 (2H, CH_2-7), 2.03 (m, 3H, CH_2-20+H-25), 1.81 (m, 2H, CH_2-15), 1.62-1.55 (m, 6H, CH_2-2+CH_2-12+CH_2-16), 1.50 (m, 2H, CH_2-11), 1.48 (m, 1H, H-17), 1.41 (m, 3H, CH_2-1+H-14), 1.30 (s, 3H, Me-19), 1.16 (m, 6H, CH_2-22+CH_2-23+CH_2-24), 0.98 (s, 3H, Me-18), 0.92 (s, 9H, Me-21+Me-26+Me-27); ^{13}C NMR ($CDCl_3$): 188.5 (C=S), 177.7 (CO_2H), 155.7 ($C_{arom.}$-OH), 154.0 (C=O), 135.2 (C-5), 132.1 ($C_{arom.}$-2+$C_{arom.}$-6), 124.0 (C-6), 116.7 ($C_{arom.}$-3+$C_{arom.}$-5), 70.1 (C-3), 65.5 (C-33), 56.1 (C-14), 53.1 (C-17), 48.7 (C-9), 41.8 (C-13), 39.6 (C-12+C-24), 38.4 (C-4), 36.2 (C-10), 36.0 (C-1+C-34), 35.6 (C-22), 35.2 (C-20), 31.3 (C-7+C-8), 27.4 (C-2), 27.3 (C-15+C-25), 23.2 (C-16+C-23), 22.7, 22.4 (C-26+C-27), 20.5 (C-11), 18.9 (C-19), 18.5 (C-21), 11.7 (C-18); MS (FAB), *m/z* = 625 $[M+Na]^+$; Elemental Analysisfor $C_{34}H_{57}N_3O_4S$ (603.90); Cal: C, 67.62; H, 9.51; N, 6.96%; Found: C, 67.46; H, 9.43; N, 6.80%.

2.2.9. *2-(3-(Cholesteroylcarbonyl)thioureido)-3-phenylpropanoic acid (**13**)*

From L-phenylalanine (66 mg). Yield: 137 mg (55 %); MP: 198-200 ^oC.; R_f = 0.74; IR (ν_{max}, cm^{-1}): 3031, 2918, 1689, 1481, 1404; ^{1}H NMR ($CDCl_3$): 10.79 (s, 1H, CO_2H, exchanged with D_2O), 9.23 (bs, 1H, NH), 7.10-7.00 (m, 5H, $H_{arom.}$), 5.35 (t, 1H, *J* = 6.4 Hz, H-6), 4.47 (m, 1H, H-3), 4.31 (d, 1H, *J* = 5.0 Hz, H-33), 3.10 (m, 2H, CH_2-34), 2.41 (m, 2H, CH_2-4), 2.26 (m, 2H, CH_2-7), 1.95 (m, 3H, CH_2-20+H-25), 1.80 (m, 2H, CH_2-15), 1.64-1.53 (m, 6H, CH_2-2+CH_2-12+CH_2-16), 1.50 (m, 2H, CH_2-11), 1.47 (m, 1H, H-17), 1.40 (m, 3H, CH_2-1+H-14),

1.27 (s, 3H, Me-19), 1.11 (m, 6H, CH_2-22+CH_2-23+CH_2-24), 0.89 (s, 3H, Me-18), 0.88 (m, 9H, Me-21+Me-26+Me-27); ^{13}C NMR ($CDCl_3$): 186.1 (C=S), 177.8 (CO_2H), 153.7 (C=O), 134.5 (C-5+$C_{arom.}$), 128.7, 126.7 ($C_{arom.}$), 125.6 (C-6), 69.9 (C-3), 66.9 (C-3), 56.2 (C-14+C-17), 48.7 (C-9), 41.9 (C-13), 39.4 (C-12+C-24), 38.1 (C-4), 36.4 (C-10), 36.1 (C-1+C-34), 35.7 (C-22), 35.2 (C-20), 31.4 (C-7+C-8), 27.4 (C-2+C-15+C-25), 23.2 (C-16+C-23), 22.7, 22.5 (C-26+C-27), 19.0 (C-11), 18.8 (C-19), 18.5 (C-21), 11.7 (C-18); MS (FAB), *m/z* = 623 $[M+H]^+$; Elemental Analysis for $C_{37}H_{54}N_2O_4S$ (622.90); Cal: C, 71.34; H, 8.74; N, 4.50%; Found: C, 71.09; H, 8.66; N, 4.32%.

2.2.10. *2-(3-(Cholesteroylcarbonyl)thioureido)-3-(1H-indol-3-yl)propanoic acid (**14**)*

From L-tryptophane (82 mg). Yield: 236 mg (89%); MP: 146-148 ^{o}C; R_f = 0.62; IR (ν_{max}, cm^{-1}): 3143, 2812, 1757, 1633, 1406, 1232; ^{1}H NMR ($CDCl_3$): 11.12 (s, 1H, CO_2H, exchangable with D_2O), 10.10 (s., 1H, NH_{Indol}), 9.24 (s, 1H, NH), 7.81-7.22 (m, 4H, $H_{arom.}$), 6.04 (d, 1H, *J* = 4.9 Hz, H_{indol}-2), 5.37 (t, 1H, *J* = 6.5 Hz, H-6), 4.47 (m, 1H, H-3), 4.22 (m, 1H, H-33), 3.32 (m, 2H, CH_2-34), 2.42 (m, 2H, CH_2-4), 2.20 (m, 2H, CH_2-7), 1.97 (m, 3H, CH_2-20+H-25), 1.81 (m, 2H, CH_2-15), 1.64-1.56 (m, 6H, CH_2-2+CH_2-12+CH_2-16), 1.50 (m, 2H, CH_2-11), 1.44 (m, 1H, H-17), 1.41 (m, 3H, CH_2-1+H-14), 1.28 (s, 3H, Me-19), 1.04 (m, 6H, CH_2-22+CH_2-23+CH_2-24), 0.89 (s, 3H, Me-18), 0.91 (m, 9H, Me-21+Me-26+Me-27); ^{13}C NMR ($CDCl_3$): 187.3 (C=S), 174.6 (CO_2H), 153.5 (C=O), 135.0 (C-5+C_{indol}-7) 127.1 (C_{indol}-3a+C-3), 122.2 (C_{indol}-2), 120.9 (C_{indol}-6), 118.0 (C_{indol}-4+C_{indol}-5), 111.8 (C_{indol}-7), 109.9 (C_{indol}-1), 70.0 (C-3), 67.9 (C-33), 55.6 (C-14), 55.1 (C-17), 48.2 (C-9), 41.3 (C-13), 39.4 (C-12+C-24), 38.2 (C-4), 36.0 (C-10), 35.6 (C-1), 35.1 (C-22), 34.1 (C-20), 30.8 (C-7+C-8), 27.3 (C-2), 26.9 (C-15 +C-25+C-34), 23.3 (C-16), 22.7 (C-23), 22.2, 21.9 (C-26+C-27), 20.0 (C-11), 18.5 (C-19), 18.0 (C-21), 11.2 (C-18); MS (FAB), *m/z* = 684 $[M+Na]^+$; Elemental Analysis for $C_{39}H_{55}N_3O_4$; (661.94); Cal: C, 70.76; H, 8.37; N, 6.35%. Found: C, 71.09; H, 8.66; N, 4.32%.

2.3. *General procedure for the preparation of 2-((Z)-2-((((cholest-5-en-3β-yl)oxy)carbonyl) imino)-4-arylthiazol-3(2H)-yl)propanoic acids ((**16-18**)*.A mixture of **6** (280 mg, 0.50 mmol) and *α*-bromocarbonyl compound (0.50 mmol) was stirred in 1-butyl-3-methylimidazolium bromide ([bmim]Br) (5 ml) for 1 h at r.t. Then water (5 ml) was added and the mixture was extracted with ethyl acetate (3× 10 ml). The solvent was evaporated to dryness and the residue was purified by column chromatography (SiO_2; hexane/EtOAc 3:2) to afford the desired pure products **18-20**.

2.3.1. *2-((Z)-2-((((chloest-5-en-3β-yl)oxy) carbonyl)imino)-4-phenylthiazol-3(2H)-yl) propanoic acid (**18**)*. From phenacyl bromide (100 mg). Yield: 261 mg (79 %); MP: 176-178 ^{o}C; ^{1}H NMR ($CDCl_3$): 10.80 (s, 1H, CO_2H, exchanged with D_2O), 8.09-7.50 (m, 5H, $H_{arom.}$), 6.65 (s, 1H, $H_{thiazole}$), 5.41 (t, 1H, *J* = 6.6 Hz, H-6), 4.66 (d, 1H, $J_{H,Me}$ = 6.6 Hz, N*CH*Me), 4.53 (m, 1H, H-3), 2.35 (m, 2H, CH_2-4), 2.28 (m, 2H, CH_2-7), 1.94 (m, 3H, CH_2-20+ H-25), 1.82 (d, 3H, $J_{H,Me}$ = 6.6 Hz, NCH*Me*), 1.79 (m, 2H, CH_2-15), 161-155 (m, 4H, CH_2-2+CH_2-12+CH_2-16), 1.51 (m, 2H, CH_2-11), 1.48 (m, 1H, H-17), 1.45 (m, 1H, H-14), 1.41 (m, 2H, CH_2-1), 1.31 (s, 3H, Me-19), 1.15 (m, 6H, CH_2-22+ CH_2-23+CH_2-24), 0.99 (s, 3H, Me-18), 0.90 (s, 3H, Me-21), 087 (s, 6H, Me-26+Me-27); ^{13}C NMR ($CDCl_3$): 173.2 (CO_2H), 166.2 (C=N), 157.2 (C=O), 140.5 ($C_{thiazole}$-4'), 139.1 (C-5), 131.0, 129.8, 128.3, 124.6 ($_{Carom.}$), 123.8 (C-6), 107.8 ($C_{thiazole}$-5'), 72.4 (C-3), 56.1 (C-14), 55.5 (C-17), 49.7 (C-9), 42.0 (C-13), 39.6 (C-24), 39.2 (C-12), 38.0 (C-4), 36.6 (C-10), 36.1 (C-22), 35.4 (C-20), 31.6 (C-7), 31.8 (C-8), 28.2 (C-2), 27.8 (C-25+C-15), 24.4 (C-16), 23.5 (C-23), 23.1, 22.8 (C-26+C-27), 21.0 (C-11), 19.2 (Me-19), 19.0 (Me-21), 15.9 (CH*Me*),

11.8 (Me-18); MS (FAB), *m/z* = 684 $[M+H]^+$; Elemental Analysis for $C_{40}H_{56}N_2O_4S$ (660.96); Cal: C, 72.69; H, 8.54; N, 4.24%. Found: C, 72.43; H, 8.44; N, 4.09%.

2.3.2. *2-((Z)-2-((((chloest-5-en-3β-yl)oxy) carbonyl)imino)-4-(4-methoxyphenyl) thiazol-3(2H)-yl)propanoic acid(**19**)*. From 4-methoxyphenacyl bromide (115 mg). Yield: 283 mg (82 %); MP: 183-185 °C; ^{1}H NMR ($CDCl_3$): 11.20 (s, 1H, CO_2H, exchanged with D_2O), 7.60 (d, 2H, *J* = 8.0 Hz, $H_{arom.}$-3'+$H_{arom.}$-5'), 7.30 (d, 2H, *J* = 8.0 Hz, $H_{arom.}$-2'+$H_{arom.}$-6'), 6.69 (s, 1H, $H_{thiazole}$), 4.65 (d, 1H, $J_{H,Me}$ = 6.5 Hz, N*CH*Me), 4.54 (m, 1H, H-3), 3.89 (s, 3H, OMe), 2.36 (m, 2H, CH_2-4), 2.29 (m, 2H, CH_2-7), 1.98 (m, 3H, CH_2-20+H-25), 1.80 (d, 3H, $J_{H,Me}$ = 6.5 Hz, NCH*Me*), 1.80 (m, 2H, CH_2-15), 165-155 (m, 6H, CH_2-2+CH_2-12+CH_2-16), 1.53 (m, 2H, CH_2-11), 1.50 (m, 1H, H-17), 1.44 (m, 1H, H-14), 1.42 (m, 2H, CH_2-1), 1.33 (s, 3H, Me-19), 1.17 (m, 4H, CH_2-22+ CH_2-23+CH_2-24), 0.98 (s, 3H, Me-18), 0.92 (s, 3H, Me-21), 088 (s, 6H, Me-26+Me-27); ^{13}C NMR ($CDCl_3$): 172.6 (CO_2H), 167.0 (C=N), 168.1 (*C*-OMe), 157.6 (C=O), 140.2 ($C_{thiazole}$-4'), 139.4 (C-5), 129.5 ($C_{arom.}$), 123.5 (C-6), 122.1, 121.9, 121.0 ($C_{arom.}$), 108.1 ($C_{thiazole}$-5'), 72.2 (C-3), 56.0 (C-14), 55.8 (OMe), 55.3 (C-17), 49.5 (C-9), 42.1 (C-13), 39.5 (C-24), 39.4 (C-12), 38.2 (C-4), 36.5 (C-10), 36.0 (C-22), 35.3 (C-20), 31.6 (C-7), 31.4 (C-8), 28.1 (C-2), 27.7 (C-25+C-15), 24.3 (C-16), 23.3 (C-23), 23.0, 22.6 (C-26+C-27), 20.9 (C-11), 19.1 (Me-19), 18.9 (Me-21), 15.6 (*CHMe)*, 11.8 (Me-18); MS (FAB), *m/z* = 714 $[M+Na]^+$; Elemental Analysis for $C_{41}H_{58}N_2O_5S$ (690.98); Cal: C, 71.27; H, 8.46; N, 4.05%. Found: C, 70.08; H, 8.37; N, 3.97%.

2.3.3.*2-((Z)-4-(4-Bromophenyl)-2-((((chloest-5-en-3β-yl)oxy)carbonyl)imino)thiazol-3(2H)-yl)propanoic acid (**20**)*. From *p*-bromophenacyl bromide (139 mg). Yield: 263 mg (71 %); MP: 183-185 °C; ^{1}H NMR ($CDCl_3$): 10.86 (s, 1H, CO_2H, exchangable with D_2O), 7.36 (d, 2H, *J* = 8.1 Hz, $H_{arom.}$-2'+$H_{arom.}$-6'), 7.01 (d, 2H, *J*= 8.1 Hz, $H_{arom.}$-3'+$H_{arom.}$-5'), 6.55 (s, 1H, $H_{thiazole}$), 4.69 (d, 1H, $J_{H,Me}$ = 6.7 Hz, N*CH*Me), 4.56 (m, 1H, H-3), 2.38 (m, 2H, CH_2-4), 2.30 (m, 2H, CH_2-7), 1.97 (m, 3H, CH_2-20+ H-25), 1.82 (d, 3H, $J_{H,Me}$ = 6.7 Hz, NCH*Me*), 1.79 (m, 2H, CH_2-15), 166-155 (m, 6H, CH_2-2+CH_2-12+CH_2-16), 1.54 (m, 2H, CH_2-11), 1.50 (m, 1H, H-17), 1.43 (m, 1H, H-14), 1.44 (m, 2H, CH_2-1), 1.35 (s, 3H, Me-19), 1.19 (m, 4H, CH_2-22+ CH_2-23+CH_2-24), 0.99 (s, 3H, Me-18), 0.93 (s, 3H, Me-21), 088 (s, 6H, Me-26+Me-27); ^{13}C NMR ($CDCl_3$): 172.4 (CO_2H), 166.7 (C=N), 158.0 (C=O), 140.5 ($C_{thiazole}$-4'), 139.5 (C-5), 132.1 ($C_{arom.}$), 131.2, 128.9, 123.6 (C-6), 121.8 ($C_{arom.}$), 107.8 ($C_{thiazole}$-5'), 72.0 (C-3), 55.8 (C-14), 55.0 (C-17), 49.3 (C-9), 41.7 (C-13), 39.4 (C-24), 39.4 (C-12), 38.0 (C-4), 36.7 (C-10), 36.1 (C-22), 35.2 (C-20), 31.4 (C-7), 31.3 (C-8), 28.0 (C-2), 27.5 (C-25+C-15), 24.5 (C-16), 23.2 (C-23), 23.1, 22.7 (C-26+C-27), 21.0 (C-11), 19.2 (Me-19), 18.9 (Me-21), 15.8 (*CHMe)*, 11.9 (Me-18); MS (FAB), *m/z* = 761/763 $[M+Na]^+$; Elemental Analysis for $C_{40}H_{55}BrN_2O_4S$ (739.85); Cal: C, 64.94; H, 7.49; N, 3.79%. Found: C, 64.77; H, 7.40; N, 3.57%.

2.3. *Biological evaluations*

2.3.1. *In vitro HIV assay*

Evaluation of the antiviral activity of compounds **5-14** and **17-19** against the HIV-1 strain (III_B) and the HIV-2 strain (ROD) in MT-4 cells was performed using an MTT assay as described previously [23, 24]. In brief, stock solutions (10 times final concentration) of test compounds were added in 25-µl volumes to two series of triplicate wells to allow simultaneous evaluation of their effects on mock and HIV-infected cells at the beginning of each experiment. Serial 5-fold dilutions of test compounds were made directly in flat-bottomed 96-well microtiter trays using a Biomek 3000 robot (Beckman

instruments). Untreated control, HIV and mock-infected cell samples, were included for each sample. HIV-1 (III_B) [25] or HIV-2 (ROD) [26] stock (50 µl) at 100-300 $CCID_{50}$ (50 % cell culture infectious dose) or culture medium was added to either of the infected or mock-infected wells of the microtiter tray. Mock-infected cells were used to evaluate the effect of test compound on uninfected cells in order to assess the cytotoxicity of the test compounds. Exponentially growing MT-4 cells [27] were centrifuged for 5 min at 1000 rpm, and the supernatant was discarded. The MT-4 cells were resuspended at 6×105 cells per ml, and 50-µl volumes were transferred to the microtiter tray wells. Five days after infection, the viability of the mock- and HIV-infected cells was examined spectrophotometrically.

2.3.2. In vitro HCV assay

Huh-5-2 cells, containing the hepatitis C virus genotype 1b I389luc-ubi-neo/NS3-3'/5.1 replicon [28] were sub-cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% Fetal calf serum (FCS), 1% non-essential amino acids, 1% penicillin/streptomycin and 2% Geneticin at a ratio of 1:3 to 1:4, and grown for 3-4 days in 75 cm² tissue culture flasks. One day before the addition of the compound, cells were harvested and seeded in assay medium (DMEM, 10% FCS, 1% non-essential amino acids, 1% penicillin/streptomycin) at a density of 6500 cells/well (100 µl/well) in 96-well tissue culture microtiter plates for the evaluation of anti-metabolic effect and culture plate (Perkin Elmer) for the evaluation of the antiviral effect. The microtiter plates were incubated overnight (37 °C, 5% CO_2, 95-99 % relative humidity), yielding a non-confluent cell monolayer.

The evaluation of the anti-metabolic as well as antiviral effect of each compound was performed in parallel. Four-step, 1-to-5 compound dilution series were prepared for the first screen, to collect data for a more detailed dose-response curve, an eight-step, 1-to-2 dilution series was used. Following assay setup, the microtiter plates were incubated for 72 h. (37 °C, 5% CO_2, 95-99 % relative humidity). For the evaluation of anti-metabolic effects, the assay medium was aspirated, replaced with 75 µl of a 5% MTS solution in phenol red-free medium and incubated 45 for 1.5 h. (37 °C, 5% CO_2, 95-99% relative humidity). Absorbance was measured at a wavelength of 498 nm (Safire², Tecan), and optical densities (OD values) were converted to percentage of untreated controls. For the evaluation of antiviral effects, the assay medium was aspirated and the cell monolayers were washed with phosphate buffered saline (PBS). The wash buffer was aspirated, and 25 µl of Glo Lysis Buffer (Promega) was added allowing for cell lysis to proceed for 5 min at room temperature. Subsequently, 50 µl of Luciferase Assay System (Promega) was added, and the luciferase luminescence signal was quantified immediately (1,000 ms integration time/well, Safire², Tecan). Relative luminescence units were converted into percentage of untreated controls. The EC_{50} (values calculated from the dose-response curve) represent the concentrations at which 50% inhibition, respectively, of viral replication is achieved. The CC_{50} (value calculated from the dose-response curve) represents the concentration at which the metabolic activity of the cells is reduced by 50% as compared to untreated cells. A concentration of a compound is considered to elicit a genuine antiviral effect in the HCV replicon system when the anti-replicon effect is well above the 70% threshold at concentrations where no significant anti-metabolic activity is observed [28].

2.3.3. *Cells and HCV*

The Huh-5-2 and Huh-9-13 HCV subgenomic replicon-containing cells were provided by Prof

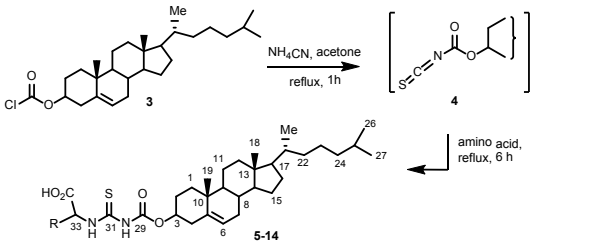
R. Bartenschlager (University of Heidelberg, Heidelberg, Germany).

3. Results and discussion

3.1. *Chemistry*

Treatment of the cholesteryl chloroformate (**3**) with NH_4SCN following Kabbani approach [29], afforded the intermediate **4**, which was directly treated with the desired amino acids to give, after chromatographic purification, the cholesteryl thioureido-amino acid derivatives **5-15** in 52-86% yield. The synthetic reactions are summarized in Scheme 1.

The structures of **5-14** were determined by their ^{1}H, ^{13}C NMR and by mass spectra, where the cholesteryl protons showed almost a similar pattern. In the ^{1}H NMR spectra of **5-14**, the two singlets resonated at the regions δ 1.12-10.32 and 9.30-9.22 ppm were assigned to the CO_2H) exchanged with D_2O) and NH protons, respectively. H-3 and CH_2-4 were appeared as multiplets at the regions δ 4.53-4.37 and 2.43-2.29 ppm, respectively, while H-6 resonated as triplets ormultiplets at δ 5.41-5.24 ppm (J ~ 6.5 Hz). The other aliphatic protons of the cholesterol scaffold were fully analysed (*c.f.* Experimental section). The three methyl groups at C-21, C-26 and C-27

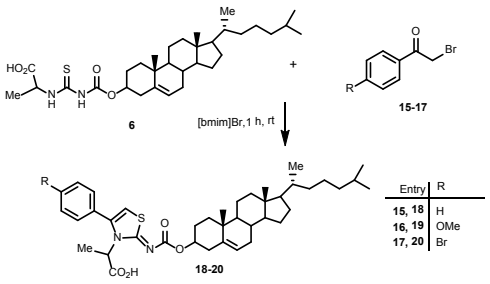


Entry	R	Amino acids
5	H	glycine
6	Me	L-alanine
7	$(CH_2)_2Me$	L-methionine
8	CH_2OH	L-serine
9	CHOHMe	L-threonine
10	CH_2-4-imidazole	L-histidine
11	$(CH_2)_4NH_2$	L-lysine
12	CH_2-(4-OH-C_6H_4)	L-tyrosine
13	$CH_2C_6H_4$	L-phenylalanine
14	CH_2-3-indole	L-tryprophane

Scheme 1. Synthesis of cholestryl thioureido-amino acid derivatives

were resonated together as singlets at δ 0.98-0.84 ppm, whereas the doublets at the regions δ 4.77-3.89 ppm were assigned to CH_2-33 (doublet for analogue **5**, J = 5.2 Hz, and doublet of doublets for analogue **7**, J = 6.5 and 3.5 Hz). In addition, the three protons H-25 and CH_2-15 were appeared as multiplets at δ 2.03-1.95 ppm, meanwhile the methylene protons at C_{22}-C_{24} were resonated together as singlets at the regions δ 1.17-1.04 ppm. The methyl protons (Me-34) of **6** appeared as a doublet at δ 0.66 ppm ($J_{33,34}$ = 6.5 Hz), while the methylene protons (CH_2-34) of **7** appeared together with CH_2-20 and CH_2-25 as multiplet at δ 1.97 ppm. The same protons of **8-14** were resonated as multiplets at δ 4.80, 4.37, 2.94, 3.10 (2), and 3.32 ppm, respectively, except **11** where CH_2-34 was resonated together with CH_2-15 as multiplet at δ 1.77 ppm. CH_2-35 of **7** and **9** appeared as multiplet, and broad singlet at δ 2.62 and 1.18 ppm, respectively, whereas the same protons of **11** resonated together with CH_2-2, and CH_2-16 at δ 1.30 ppm. CH_2-37 of **11** appeared as a multiplet at δ 2.78 ppm. The aromatic protons of **10**, **12** and **13** were resonated as multiplets at the regions δ 7.45-7.00 ppm, whereas the spectrum of **14** was characterized by the presence of four aromatic protons as multiplet at δ 7.81-7.22 ppm, in addition to a doublet at δ 6.04 ppm (J = 4.9 Hz) assigned for H_{indol}-2. In the ^{13}C NMR spectra of **5-14**, the lower field resonances at δ 188.5-180.2 ppm and δ 177.8-171.0 ppm were assigned for C=S and CO_2H groups, respectively. Resonances at the regions δ 156.3-151.3 and 147.8-134.5 ppm were attributed to C=O and C-5, respectively. Furthermore, C-33 resonated at δ 70.0-56.0 ppm, except for the analogues **8** and **9** which appeared together with C-3 at δ 69.3 and 71.2 ppm, respectively. The aromatic, other cholesterol aliphatic carbon atoms and the amino acid substituents have been fully identified (*c.f.* Experimental section). All the compounds have been further identified by their ^{1}H,^{13}C HSQC [30], HMBC [31], and ^{1}H,^{1}H NOESY NMR [32] NMR spectra.

Next, other models of cholesterol derivatives bearing a thiazole moieties were prepared via a simple and efficient method [33], aiming to evaluate their anti-HIV activity in comparison for those of the cholesteryl-thioureido analogues **4-15**. Thus, compound **6** was selected for preparation of the new thiazole analogues **18-20**, in 79, 82 and 71 % respectively, by treatment with substituted phenacyl bromides **15-17** in ionic liquid [bmim]Br (Scheme 2).



Scheme 2. Synthesis of 2-((*Z*)-2-(((((cholest-5-en-3β-yl)oxy)carbonyl)imino)-4-arylthiazol-3(*2H*)-yl)propanoic acids (**18-20**)

The assignment of protons and carbons of the cholesterol backbone was deduced in comparison to compounds **5-14**. The protons and carbon atoms of the substituted thiazole residues were fully analysed (*c.f.* Experimental section). Compound 1**9** was selected for further NMR experiments. The gradient HMBC [31] NMR spectrum of 1**9** revealed four $^{3}J_{C,H}$ couplings: CO_2 of the ester group at δ 157.6 ppm with H-3 of the cholesterol backbone at δ 4.54 ppm, C=N at δ 167.0 ppm with H-5' of the thiazole ring at δ 6.69 ppm in addition to coupling between NC*H*Me of the amino acid residue at δ 4.65 ppm and C=N at δ 167.0 ppm. The fourth $^{3}J_{C,H}$ coupling was observed between C-4' of the thiazole moiety at δ 140.2 ppm and H-2'' together with H-6'' of the aromatic residue at δ 7.30 ppm (Figure 2).

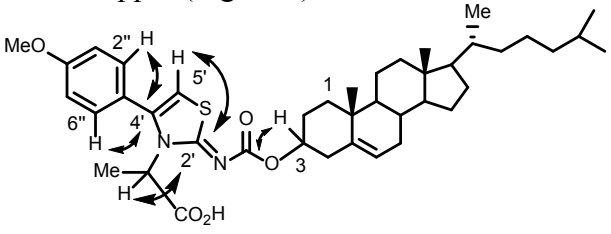


Figure 2. $J_{C,H}$ correlations in the HMBC NMR spectrum of compound **19**.

3.2. *In vitro anti-HIV activity*

Compounds **5-14** and **17-19** were tested for their *in vitro* anti-HIV-1 (strain III_B) and HIV-2 (strain ROD) activity in human T-lymphocyte (MT-4) cells based on a Microculture Tetrazolium (MTT) assay [27, 28]. The results are summarized in Table 1, in which nevirapine (BOE/BIRG587) [34] was used as a reference drug. The cytotoxicity of the compounds was determined in parallel. It is interesting to report that compounds **6** was effected a 50% reduction of the cytopathogenicity induced by HIV-2 (III_B strain) at a concentration of IC_{50}> 1.06 μM and CC_{50} of 54.03 μM, resulting with selectivity index (SI) of 51 at non-toxic concentration, meanwhile no antiretroviral activity was observed (SI < 1) against HIV-1. On the other hand, compound **19** exhibited moderate inhibition activity against HIV-1 at a concentration of IC_{50}> 2.90 μM and CC_{50} of 23.20 μM resulting with selectivity index (SI) of 8 at non-toxic concentration. The remaining compounds exhibited an average to poor activity with SI <1. However, the activity of the compound is much lower than those of the corresponding reference compound nevirapine. The synthesis of new analogues of these thioureido and thiazole derivatives of cholesterol could lead to the discovery of more potent and selective analogues that will subject for further structural development.

Table 1. *In-vitro* anti-HIV-1[a] and HIV-2[b] of new cholesteryl derivatives **4-15** and **17-19**

Compd.	HIV-1 ($IIII_B$) IC_{50} [μM]	HIV-2 (ROD) IC_{50} [μM]	CC_{50} (μM)[b]	SI (III_B)	SI (ROD)
5	> 14	> 14	14	< 1	< 1
6	> 1.06	> 54.03	54.03	< 1	51
7	> 60.55	> 60.55	60.55	< 1	< 1
8	> 67.75	> 67.75	67.75	< 1	< 1
9	> 47.55	> 47.55	47.55	< 1	< 1
10	> 98.0	> 98.0	98.0	< 1	< 1
11	> 69.35	> 69.35	69.35	< 1	< 1
12	> 81.4	> 81.4	81.4	< 1	< 1
13	> 68.15	> 68.15	68.15	< 1	< 1
14	> 70.1	> 70.1	70.1	< 1	< 1

17	>57.55	>57.55	57.55	< 1	< 1
18	>21.22.	>21.22	21.22	< 1	< 1
19	>2.90	>23.20	23.20	<8	<1
Nevirapine	0.027	> 4.0	> 4.0	> 147	X1

[a] Anti-HIV-1 activity measured with strain III_B; [b] anti-HIV-2 activity measured with strain ROD; [c]compound concentration required to achieve 50 % protection of MT-4 cells from the HIV-1 and 2-induced cytopathogenic effect; [d] compound concentration that reduces the viability of mock-infected MT-4 cells by 50 %; [e] SI: selectivity index (CC_{50}/EC_{50}).

3.3. *In vitro anti-HCV activity*

The establishment of stable HCV subgenomic replicon systems [35, 36] in the human hepatoblastoma cell line Huh-7 has provided a useful system for the development of new antiviral approaches against HCV [37-39]. The overriding aims of the new therapeutic strategies are higher efficacy associated with shortened duration of treatment, favourable mode of administration, and thus improved tolerability and adherence.

Compounds **5-14** were selected for the evaluation of their *in vitro* selective anti-hepatitis C virus (HCV) activity in the Huh-5-2 replicon system (type 1b, Con1 strain) [28]. The analogues activity ranged between EC_{50} values of 5.09 and 45.1 μM with CC_{50} between 39 and >50 μM. Compound **5** was the most active inhibitor of this series since it exhibited EC_{50} of 5.09 μM with SI of 7.65. Furthermore, the analogues **5**, **7** and **8** exhibited inhibition of 76.5, 82.8 and 88.3%, respectively. The results are summarized in Table 2. However, none of these compounds matched the selection criteria of a selective inhibitor of virus replication in this assay (i.e. >70% inhibition at concentrations that do not elicit an antimetabolic effect on the host cells).

Table 2.*In vitro* anti-HCV type 1b, activity, cytotoxicity and inhibition% of cholesteryl ester derivatives **5-14**

Compd.	CC_{50} [μM]	EC_{50}[μM] [a]	SI	Inhibition [%]	Conc. [μM][a]
5	39.0	5.09	7.65	76.5	19.8
6	> 50	10.24	> 4.88	33.7	2.0
7	> 50	18.8	> 2.66	82.8	47.6
8	> 50	17.1	> 2.93	88.3	32.6
9	> 50	28.5	> 1.75	73.1	50.0
10	> 50	38.4	> 1.30	58.5	50.0
11	> 50	22.1	> 2.26	77.1	50.0
12	> 50	26.2	> 1.91	76.9	49.4
13	> 50	45.1	> 1.11	52.4	50.0
14	> 50	17.7	> 2.82	75.3	45.8

CC_{50}: 50% Cytostatic/Cytotoxicity concentration (concentration at which 50% adverse effect is observed on the host cell); EC_{50}: 50% Effective concentration (concentration at which 50% inhibition of virus replication is observed; SI: Selectivity Index (CC_{50}/EC_{50}).

3.4. *Molecular docking study*

The docking studies of RNA-dependent RNA polymerase were carried out to define the binding pockets, inhibitors interactions, and their specificity and energy requirement. Our *in silico* docking study using the Graphical User Interface program AutoDock Tools and AutoDockTools4 [40]. The X-ray structure of the HCV RNA-dependent RNA polymerase was obtained from the Protein Data Bank (PDB 2brl) [41] as the inhibitor receptor.

Compound **5** was selected for docking study as a best dock pose with the following energy data (kcal/mol): binding energy = -9.50, competitive inhibition (*K*i) = 107.89 nM, intermolecular energy = -11.8, internal energy = -2.47, torsional energy = 3.58 and unbound extended energy = -1.19, where the model was visualized in Figure 2. The number of conformations generated by compound **5** which indicated that flexibility is an important parameter for the ligand to docked

deeply within the binding pocket of hepatitis C virus RNA-dependent RNA polymerase enzyme. The energy of conformation for compound **5** was -9.50 which indicate compound is active at lowest energy of conformation. The study revealed two hydrogen bonds between NH_2 protons of guanidine residue of Arg158 with *O* atom of the carboxylic acid group as well as *O* atom of the ester group at C-3. The third hydrogen bond is shown between *O* atom of the ester group of cholesterol scaffold and NH_2 proton of Lys141 of RNA-dependent RNA polymerase of HCV residues. The amino acids Ser282, Ph193, Asn316, Arg200 and Tyr448 are non bonded residues which are involved in flexible alignment as well orientation of ligand into the receptor activity. Apparently, the residue Arg 158 may contribute to the binding and stabilization of compound **5** in the cavity space of RNA polymerase.

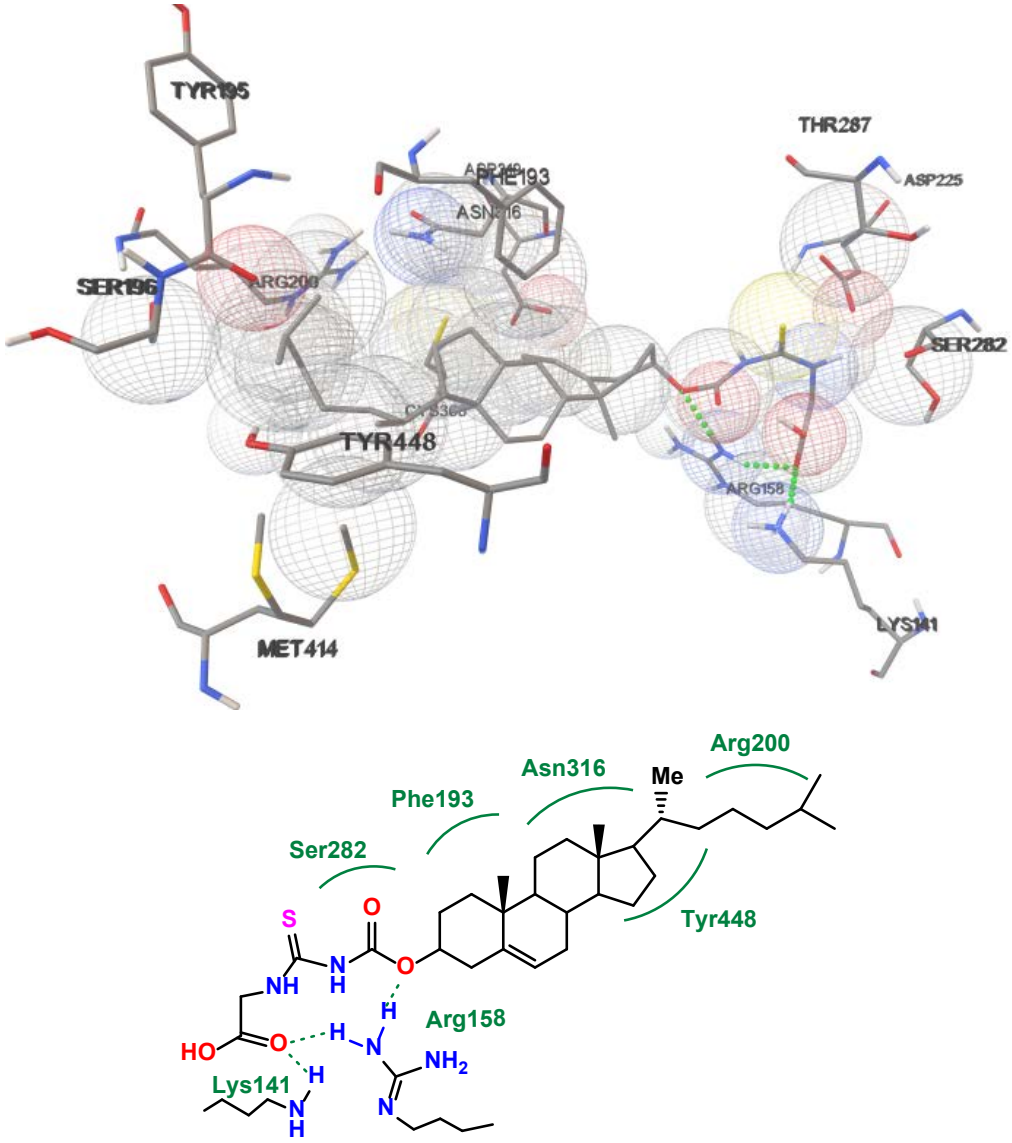


Fig. **2**. Docked conformation of **5** showing three hydrogen bonds: Arg298 with *O* atom of the carboxylic acid group as well as with *O* atom of the ester group at C-3, while the third hydrogen bond is shown between *O* atom of the ester group and NH_2 proton of Lys141 of RNA-dependent RNA polymerase of HCV residues.

4. Conclusion

In summary, we synthesized and evaluated anti-HIV activity of a new series of cholesteryl ester derivatives of thioureido-amino acid-3-carboxylic acids **5-14** and the substituted thiazole analogues **17-19**. Compound **6** having thioureido-L-alanine residueis the most active analogue from both series against HIV-2, meanwhile **19** exhibited moderate activity against HIV-1 and both analogues being promising agents for further structural modification and pharmacological evaluation. Compounds **5-14** were evaluated for their activity against HCV genotype 1b, where the cholesteryl conjugated thioureido-glycine group (compound **5**) exhibited anti-HCV activity with a 50% effective concentration (EC_{50}) value of 5.09 μM and a selective index (SI) value of 7.65. These result suggest that **5** might act as new lead candidate for inhibition of HCV.

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