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Original article

Synthesis and antimicrobial activity of cholic acid hydrazone analogues

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ABSTRACT

Synthesis and antimicrobial activity of cholic acid analogues **4a**–**t** are reported. The synthesis of **4a–t** was accomplished from ethylcholate **2**. The hydrazone moiety was introduced *via* coupling of the cholic acide hydrazide (**3**) with appropriately functionalized aldehyde utilizing acetic acid as a catalyst. Quiet of interest in relation to the synthesized hydrazones is the formation of two rotamers *s-cis.E* and *s-trans.E.* Most compounds showed stronger antimicrobial activity against Gram-positive bacteria than Cefaclor and Cefixime. Compounds **4d**, **4i** and **4j** indicated 15-fold stronger antimicrobial activities against *Enterobacter faecalis* compared to Cefaclor and Cefixime. Some of the synthesized compounds (e.g. **4a**, **4c**, **4d**, **4i**, and **4l**) reflected twofolds less activity against *Escherichia coli* relative to Cefixime.

1. Introduction

The widespread excessive use of antibacterial agents lead to development of more resistant bacteria to commonly used antibiotics. This has led to intense research for new types of antibiotics. A literature survey of antimicrobial steroids revealed that several amino cholesterol derivatives exhibit profound antimicrobial activity [1–4]. The preparation of various bile acid-based amino sterols was reported with a view to examine their activity as antimicrobial agents [5–7]. Several cholic acid derived facial amphiphiles have been reported to improve the permeability of membranes including bacterial cell wall [8]. Cholic acid and deoxycholic acid have attracted significant attention due to availability and the orientation of the hydroxy groups that may be exploited in podant-type receptors [9,10], linear dimeric hosts [11,12] or facial amphiphiles [13,14]. In addition, bile acids are natural ligands specifically recognised by hepatic cells and are amphiphilic molecules that undergo a biological recycling during enterohepatic circulation [8,15,16].

Novel steroid-based antimicrobials have been prepared as mimics of squalamine, a steroidal polyamine isolated from dogfish shark [17]. Very recently there are two reports of antimicrobial activity of cholic acid derivatives [3,4,18,19]. Willemen et al. reported that the antimicrobial activity of cholic acid against *Escherichia coli* and *Enterobacter faecalis* could be enhanced through introducing the cationic trimethylammonium groups [20]. Many cholic acid derived hydrazones are of special importance due to their activity against many cancer cell lines such as PC3, Bcap37 and BgC823 cells [21].

In continuation of our work toward finding lead compounds for certain disease states [22,23], we report herein the synthesis and biological evaluation of bile acid derived Schiff base conjugates **4a–t** in which amino functionality has been introduced at carboxylic acid appendage of steroid unit followed by coupling with different aldehydes. The rationale behind our investigation is twofolded: (1) the current upsurge in utilizing hydrazone derivatives on different scaffolds for finding antimicrobial lead compounds [24–27] and (2) the ongoing interests in the pharmacological importance of bile acid derivatives as antimicrobial agents [3,4,17–21].

2. Results and discussion

2.1. Chemistry

We have replaced the carboxylic acid moiety of cholic acid (**1**) with an amide functionality derived from hydrazine. This was carried out through esterification of the carboxylic acid moiety of cholic acid (**1**) with EtOH in the presence of H_2SO_4 to afford Cholic acid ethyl esters (**2**) in 99% yield. Formation of the amide **3** from the

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ester **2** is facile and takes place in refluxing EtOH with excess NH_2NH_2 in very good yield.

Thus, the synthesis of the target compounds (3α, 5β, 7α, 12α)-3, 7, 12-trihydroxy-*N*-[(1E)-phenylmethylene]cholan-24-ohydrazide **4a–t,** is straight forward through the condensation of carbonyl compounds (aldehydes or ketones) with cholylhydrazide **3** (Scheme 1). Subsequently, cholylhydrazide (**3**) was treated with appropriate aldehydes to produce the desired products **4a–t** (Scheme 2). The purity of all products was determined by thin layer chromatography using several solvent systems of different polarities. The synthesized compounds were characterized by ^1H, ^{13}C NMR and high resolution mass spectrometry.

Quiet of interest in relation to the synthesized hydrazones is the formation of two rotamers *s-cis.E* and *s-trans.E*, which resulted from the inversion of amide bonds. This was evident from their ^1H NMR and ^{13}C NMR spectra (see Section 4).

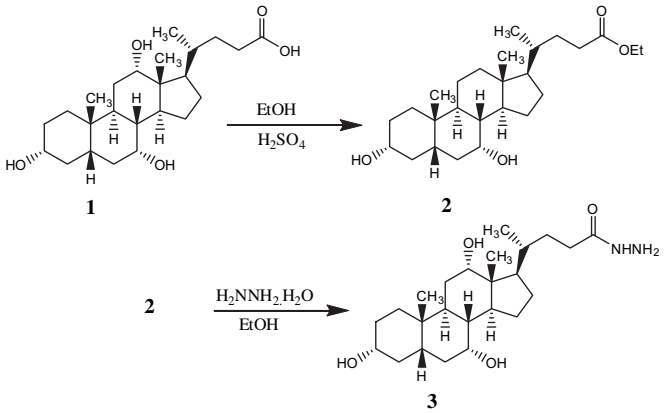
The N═CH protons of compounds **4(a–t)** appeared as expected as two separate signals in their 1H NMR spectra, in the region between $\delta = 7.8$ and 8.45 ppm due to nitrogen inversion. The nitrogen inversion also affected the (−CONH−) which appeared as two signals in the region between $\delta = 10.8$ and 11.6 ppm. The protons of the *s-trans.E* of both (N═CH) and (−CONH−) appeared at higher chemical shifts compared to those of *s-cis.E*. In the ^{13}C NMR spectra, the N═CH carbon atom resonated as two separate signals in the region between $\delta = 133$ and 148 ppm. On the other hand, the −CONH− carbons appeared as two signals at the expected chemical shift values in the range between $\delta = 169$ and 181 ppm.

2.2. Antimicrobial activity

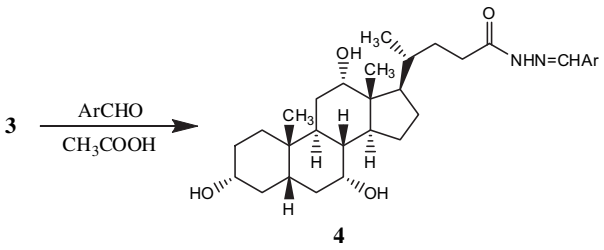
The antimicrobial activities of the compounds **4(a–t)** were tested against Gram-positive bacteria *Staphylococcus aureus*, *E. faecalis* and *Bacillus megaterium* and Gram-negative bacteria *E. coli*, *Pseudomonas aeruginosa* and *Enterobacter aerogens*. The results of *in vitro* antimicrobial activities of the hydrazones are listed in Table 1. Minimum inhibitory concentration (MIC) was measured as described in Section 4. All the compounds showed good antimicrobial activity against the tested bacteria except for *E. aerogens* and *P. aeruginosa*, in which the synthetic compounds were inactive.

As indicated from Table 1, strong activity for compounds **4c**, **4i** and **4j** against *E. faecalis* with MIC value of 1.96 μg/ml, which is 15-folds more potent than the positive controls cefaclor and cefixime.3H_2O, with MIC 31.25 μg/ml. Compounds **4d**, **4n** and **4o** also exhibited eightfolds activity with MIC 3.91 μg/ml against the same bacteria species compared to controls.

For *B. megaterium* bacteria all tested compounds showed the same MIC value as cefaclor except for compounds **4d**, **4o** and **4r**



Scheme 1. Synthesis of cholylhydrazide **3**.



Scheme 2. Synthesis of hydrazones **4a–t**.

which were more potent than cefaclor with MIC values of 7.82, 15.63 and 15.63 μg/ml, respectively.

For *S. aureus* many compounds showed the same or less activity than cefaclor and cefixime.3H_2O. Compounds **4a**, **4c**, **4d**, **4i** and **4l** were more active against *E. coli* (MIC 3.91 μg/ml) than cholic acid but twofolds less active than Cefixime.3H_2O.

Among the synthesized compounds it was clear that those with bromine and chlorine atoms showed very good antimicrobial activity, while unsubstituted and cyano substituted hydrazones other than **4o** were inactive against bacteria under test. Generally, most of the synthesized compounds were active against Gram-positive strains.

3. Conclusion

In summary, we have explored the utilization of cholyl hydrazone derivatives as possible lead compounds against some Gram-positive and Gram-negative species. This in turn, opens up the possibility of elaborating on these derivatives for finding more effective broad spectrum antimicrobial derivatives of cholic acid.

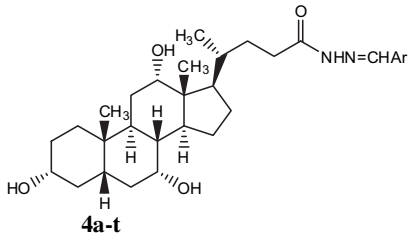
4. Experimental

4.1. General methods

The ^1H and ^{13}C NMR spectra were recorded on 300 MHz Brucker Advance. Chemical shifts were reported as δ values in ppm. Spectra were acquired in (DMSO-d_6 for hydrazones and $CDCl_3$ for cholic acid containing 1% TMS). The multiplicities of carbon atom were determined from Distortionless Enhancement by Polarization Transfer (DEPT) experiment. High resolution Mass spectra (HRMS) were recorded in positive ion mode by Electrospray Ionization (ESI) on a Bruker instrument. The samples were dissolved in acetonitrile, diluted in spray solution (methanol/water 1:1 v/v + 0.1% formic acid) and infused using a syringe pump with a flow rate of 2 μl/min. External calibration was conducted using Arginine cluster in a mass range m/z 175–871. For all HRMS data, mass error: 0.00–0.50 ppm.

Melting points (m.p.) were determined on an Electrothermal Melting point Apparatus and were reported uncorrected in °C. Angles rotations were determined on a Polarimeter Perkin–Elmer141 in degrees, with 10 mg/ml (DMSO) concentration in 10 cm cell length. Solvents used in this study were obtained from Scharlau, Fluka and Aldrich. Cholic acid obtained from Sigma. All reactions were monitored by thin layer chromatography (TLC) using Merck aluminum plates pre-coated with silica gel PF254; 20 × 20, 0.25 mm, and detected by visualization of the plate under UV lamp ($\lambda = 254$ or 365 nm). Spots were detected by spraying with anisaldehyde – sulpharic acid in ethanol, followed by heating to 140 °C. Compounds were purified through recrystallization or using column chromatography which was performed on Scharlau silica gel, packed by the slurry method.

Table 1
Minimum inhibitory concentration (MIC) in μg/ml of compounds **1**, **2**, **3** and hydrazones **4(a–t)**.



4a-t

Compound	Ar	Gram-negative			Gram-positive		
		E. coli	*P. aeruginosa*	*E. aerogens*	*S. aureus*	*E. faecalis*	*B. megaterium*
1		7.81	15.63	na	na	31.25	na
2		na	na	na	na	15.63	31.25
3		na	na	na	na	31.25	31.25
4a	C_6H_4–	3.91	na	na	62.5	15.63	31.25
4b	*p*-FC_6H_4–	na	na	na	31.25	62.5	31.25
4c	*p*-ClC_6H_4–	3.91	na	na	62.5	1.96	31.25
4d	*p*-BrC_6H_4–	3.91	na	na	62.5	3.91	7.82
4e	*p*-MeC_6H_4–	7.81	na	na	31.25	7.82	na
4f	*p*-$MeOC_6H_4$–	7.81	na	na	62.5	15.63	31.25
4g	*p*-NCC_6H_4–	7.81	na	na	31.25	15.63	31.25
4h	*p*-$NO_2C_6H_4$–	7.81	na	na	31.25	7.82	31.25
4i	*m*-ClC_6H_4–	3.91	na	na	31.25	1.96	31.25
4j	*o*-BrC_6H_4–	7.81	na	na	na	1.96	31.25
4k	*o*-HOC_6H_4–	na	na	na	31.25	7.82	31.25
4l	2,3-$(MeO)_2C_6H_4$–	3.91	na	na	na	15.63	31.25
4m	2,5-$(MeO)_2C_6H_4$–	na	na	na	na	15.63	31.25
4n	4-Cl-3-$NO_2C_6H_4$–	7.81	na	na	31.25	3.91	31.25
4o	2-indolyl	na	na	na	31.25	3.91	15.63
4p	5-MeO-3-indolyl	na	na	na	na	31.25	na
4q	2-Furanyl	na	na	na	na	na	31.25
4r	2-Thiophenyl	na	na	na	62.5	31.25	15.63
4s	2-Pyrrolyl	na	na	na	na	na	31.25
4t	2-Pyridinyl	7.81	na	na	na	na	31.25
Cefaclor		na	na	7.82	31.25	31.25	31.25
Cefixime		1.96	19.54	31.25	31.25	31.25	na

na: Not active.

4.2. Biology

Cefaclor and cefixime.$3H_2O$ were used as positive controls. Solution of different concentrations of cefaclor, cefixime.$3H_2O$ and compounds **4(a–t)** were prepared by dissolving them in DMSO. Eleven serial dilutions prepared by halving the concentration of the stock solution with initial concentration of (250 μg/ml). Microorganism's suspensions at 10^5 CFU (colony forming units)/ml were inoculated in the wells. The plates were incubated at 37 °C for 24 h. The minimum inhibitory concentration (MIC) values were determined according to turbidity test [28].

4.3. Synthesis

4.3.1. Preparation of ethylcholate (2)

Cholic acid (5 g) was dissolved in 20 ml of ethanol and 2 ml concentrated H_2SO_4. The mixture was stirred for 10 h then added to ice-water (100 ml). Characterized by melting point comparison with literature value [29]. The product was collected by suction filtration to give 99% yield; m.p. 160–162 °C, $[\alpha]_D = +26.3$.

4.3.2. Preparation of cholylhydrazide (3)

Ethylcholate (5 g) was refluxed for 10 h in 20 ml absolute alcohol and 2 ml hydrazine monohydrate. The reaction mixture was added to ice-water and left to stand for 10 h. The product was filtrated and washed with water and petroleum ether to yield 90%; m.p. 188–189 °C, $[\alpha]_D = +34.3$.

4.3.3. General procedure for hydrazones synthesis (4a–t)

Aromatic aldehyde (1.1 mmol) was added to a solution of 1 mmol of the cholylhydrazide **(3)** in absolute ethanol containing two drops of glacial acetic acid. The mixture was refluxed for 10 h, and the reaction progress was monitored by TLC. The mixture was then poured into cold water, and left standing for 10 h in order to complete precipitation. The precipitate formed was filtered and washed with petroleum ether and recrystalized from ethanol.

4.3.4. (3α, 5β, 7α, 12α)-3, 7, 12-Trihydroxy-N-[(1E)-phenylmethylene]cholan-24-ohydrazide. (4a)

This derivative was synthesized according to the general procedure. Yield 84.4%, as white solid, m.p. 189–190 °C, $[\alpha]_D = +20.5°$. ^{1}H NMR (300 MHz, DMSO-d_6, in ppm): 0.58 (s, 3H, 18-CH_3); 0.80 (s, 3H, 19-CH_3); 0.95 (d, 3H, $J = 6$, 21-CH_3); 0.92–2.20 (m, ca 22H, skeletal CH_2 and CH); 2.6 (m, 2H, 23-CH_2); 3.12 (bs, 1H, H-3β); 3.55 (bs, 1H, H-7β); 3.75 (bs, 1H, H-12β); 4.00 (d, 1H, $J = 3.17$, 3-OH); 4.10 (d, 1H, 7-OH); 4.30 (d, 1H, $J = 3.76$, 12-OH); 7.30–7.70 (m, skeletal CH-aromatic); 8.00 (8.15) (s, 1H, C=NH); 11.00 (11.20) (s, 1H, CONH). ^{13}C NMR (75 MHz, DMSO-d_6, in ppm): 12.80 (C-18); 17.70 (C-21); 23.10 (C-19); 23.30 (C-15); 26.70 (C-9); 27.70 (C-16); 29.00 (C-11); 30.90 (C-2); 31.40 (C-22); 31.70 (C-23); 34.99 (C-10); 35.40 (C-6); 35.60 (C-1); 35.80 (C-20); 40.60 (C-4); 40.90 (C-8); 41.86 (C-5); 41.98 (C-14); 46.50 (C-13); 46.60 (C-17); 66.71 (C-7); 70.90 (C-3); 71.46 (C-12); 134.87 (134.93) (C-1′); 129.21 (129.28) (C-2′ and 6′); 127(127.37) (C-3′ and 5′); 130.1 (130.26) (C-4′); 142.84

(146.12) (C═N); 169.64 (175.52) (C═O). ESIMS: m/z calculated for [M + Na^+]: 533.33553, found 533.33459, difference: 0.00094.

4.3.5. (3α, 5β, 7α, 12α)-3, 7, 12-Trihydroxy-N-[(1E)-4-fluorophenylmethylene]cholan-24-ohydrazide. (**4b**)

This derivative was synthesized according to the above general procedure. Yield 40%, as white solid, m.p. 153–154 °C, $[\alpha]_D = +20.0°$. ^{1}H NMR (300 MHz, DMSO-d_6, in ppm): 0.58 (s, 3H, 18-CH_3); 0.80 (s, 3H, 19-CH_3); 0.95 (d, 3H, $J = 6$, 21-CH_3); 0.92–2.20 (m, ca 22H, skeletal CH_2 and CH); 2.60 (m, 2H, 23-CH_2); 3.12 (bs, 1H, H-3β); 3.55 (bs, 1H, H-7β); 3.75 (bs, 1H, H-12β); 4.00 (d, 1H, $J = 2.83$, 3-OH); 4.10 (d, 1H, $J = 3.65$, 7-OH); 4.30 (d, 1H, $J = 4.19$, 12-OH); 7.10–7.70 (m, 4H, skeletal CH-aromatic); 7.95 (8.10) (s, 1H, C═NH); 11.10 (11.20) (s, 1H, CONH). ^{13}C NMR (75 MHz, DMSO-d_6, in ppm): 12.84 (C-18); 17.66 (17.59) (C-21); 23.12 (C-19); 23.3 (C-15); 26.72 (C-9); 27.87 (C-16); 29.06 (29.58) (C-11); 30.9(C-2); 31.33 (C-22); 31.7 (C-23); 34.88 (C-10); 35.38 (C-6); 35.65 (C-1); 35.82 (C-20); 40.53 (C-4); 40.8 (C-8); 41.9 (C-5); 42 (C-14); 46.4 (C-13); 46.51 (C-17); 66.7 (C-7); 70.88 (C-3); 71.43 (C-12); 131.45 (131.49) (C-1′); 129.1–129.6 (C-2′ and 6′); 116.17–116.54 (C-3′ and 5′); 161.67 (C-4′); 141.66 (144.92) (C═N); 169.65 (175.5) (C═O). ESIMS: m/z calculated for [M + Na^+]: 551.32611, found 551.328607, difference: 0.00249.

4.3.6. (3α, 5β, 7α, 12α)-3, 7, 12-Trihydroxy-N-[(1E)-4-chlorophenylmethylene]cholan-24-ohydrazide (**4c**)

This derivative was synthesized according to the above general procedure. Yield 89%, as white solid, m.p. 260–261 °C, $[\alpha]_D = +19.3°$. ^{1}H NMR (300 MHz, DMSO-d_6, in ppm): 0.58 (s, 3H, 18-CH_3); 0.80 (s, 3H, 19-CH_3); 0.95 (d, 3H, $J = 6$, 21-CH_3); 0.92–2.20 (m, ca 22H, skeletal CH_2 and CH); 2.60 (m, 2H, 23-CH_2); 3.12 (bs, 1H, H-3β); 3.50 (bs, 1H, H-7β); 3.75 (bs, 1H, H-12β); 4.00 (d, 1H, $J = 3.17$, 3-OH); 4.10 (d, 1H, 7-OH); 4.30 (d, 1H, $J = 3.76$, 12-OH); 7.40–7.70 (m, 4H, skeletal CH-aromatic); 7.90 (8.10) (s, 1H, C═NH); 11.20 (11.40) (s, ^{1}H, CONH). ^{13}C NMR (75 MHz, DMSO-d_6, in ppm): 12.84(C-18); 17.66(17.59) (C-21); 23.12(C-19); 23.3(C-15); 26.72 (C-9); 27.87 (C-16); 29.06 (29.58) (C-11); 30.9(C-2); 31.33 (C-22); 31.7 (C-23); 34.88 (C-10); 35.38 (C-6); 35.65 (C-1); 35.82 (C-20); 40.53 (C-4); 40.8 (C-8); 41.9 (C-5); 42 (C-14); 46.4 (C-13); 46.51 (C-17); 66.7 (C-7); 70.88 (C-3); 71.43 (C-12); 133.87 (C-1′); 129.3, 129.36 (C-2′ and 6′); 128.61, 129 (C-3′ and 5′); 134.71 (C-4′); 141.57 (144.76) (C═N); 169.73 (175.52) (C═O). ESIMS: m/z calculated for [M + Na^+]: 567.29656, found 567.302527, difference: 0.00597.

4.3.7. (3α, 5β, 7α, 12α)-3, 7, 12-Trihydroxy-N-[(1E)-4-bromophenylmethylene]cholan-24-ohydrazide (**4d**)

This derivative was synthesized according to the above general procedure. Yield 80%, as white solid, m.p. 269–270 °C, $[\alpha]_D = +18.6°$. ^{1}H NMR (300 MHz, DMSO-d_6, in ppm): 0.58 (s, 3H, 18-CH_3); 0.80 (s, 3H, 19-CH_3); 0.95 (d, 3H, $J = 6$, 21-CH_3); 0.92–2.20 (m, ca 22H, skeletal CH_2 and CH); 2.60 (m, 2H, 23-CH_2); 3.12 (bs, 1H, H-3β); 3.55 (bs, 1H, H-7β); 3.75 (bs, 1H, H-12β); 4.00 (d, 1H, 3-OH); 4.10 (d, 1H, 7-OH); 4.30 (d, 1H, $J = 3.85$, 12-OH); 7.50–7.65 (m, 4H, skeletal CH-aromatic); 7.90 (8.10) (s, 1H, C═NH); 11.10 (11.25) (s, 1H, CONH). ^{13}C NMR (75 MHz, DMSO-d_6, in ppm): 12.85 (C-18); 17.66 (17.59) (C-21); 23.08 (C-19); 23.26 (C-15); 26.66 (C-9); 27.8 (C-16); 29.64 (29) (C-11); 30.9 (C-2); 31.3 (C-22); 31.7 (C-23); 34.88 (C-10); 35.36 (C-6); 35.64 (C-1); 35.8 (C-20); 40.53 (C-4); 40.81 (C-8); 41.88 (C-5); 42 (C-14); 46.24 (C-13); 46.59 (C-17); 66.74 (C-7); 70.92 (C-3); 71.47 (C-12); 134.14 (134.22) (C-1′); 128.9 (129.28) (C-2′ and 6′); 132.25 (132.3) (C-3′ and 5′); 123.26 (123.49) (C-4′); 141.63 (144.8) (C═N); 169.72 (175.51) (C═O). ESIMS: m/z calculated for [M + Na^+]: 611.24604, found 611.245942, difference: 0.00010.

4.3.8. (3α, 5β, 7α, 12α)-3, 7, 12-Trihydroxy-N-[(1E)-4-methylphenylmethylene]cholan-24-ohydrazide (**4e**)

This derivative was synthesized according to the above general procedure. Yield 48.3%, as white solid, m.p. 151–152 °C, $[\alpha]_D = +24.6$. ^{1}H NMR (300 MHz, DMSO-d_6, in ppm): 0.58 (s, 3H, 18-CH_3); 0.80 (s, 3H, 19-CH_3); 0.95 (d, 3H, $J = 6$, 21-CH_3); 0.92–2.20 (m, ca 22H, skeletal CH_2 and CH); 2.25 (s, 3H, CH_3-aromatic) 2.60 (m, 2H, 23-CH_2); 3.12 (bs, 1H, H-3β); 3.55 (bs, 1H, H-7β); 3.75 (bs, 1H H-12β); 4.00 (d, 1H, $J = 3.06$, 3-OH); 4.10 (d, 1H, $J = 3.66$, 7-OH); 4.30 (d, 1H, $J = 4.18$, 12-OH); 7.30–7.70 (m, 4H, skeletal CH-aromatic); 7.90 (8.10) (s, 1H, C═NH); 11.00 (11.15) (s, 1H, CONH). ^{13}C NMR (75 MHz, DMSO-d_6, in ppm): 12.85 (C-18); 17.67 (17.6) (C-21); 21.51 (CH_3-aromatic); 23.13 (C-19); 23.31 (C-15); 26.71 (C-9); 27.86 (C-16); 29.06 (29.71) (C-11); 30.9 (C-2); 31.47 (C-22); 31.71 (C-23); 34.89 (C-10); 35.37 (C-6); 35.65 (C-1); 35.84 (C-20); 40.53 (C-4); 40.81 (C-8); 41.89 (C-5); 42 (C-14); 46.24 (C-13); 46.56 (C-17); 66.74 (C-7); 70.92 (C-3); 71.47 (C-12); 132.15 (132.18) (C-1′); 129.87 (129.93) (C-2′ and 6′); 127.03 (127.4) (C-3′ and 5′); 139.86 (140.08) (C-4′); 142.86 (146.1) (C═N); 169.51 (175.4) (C═O). ESIMS: m/z calculated for [M + Na^+]: 547.35118, found 547.35097, difference: 0.00021.

4.3.9. (3α, 5β, 7α, 12α)-3, 7, 12-Trihydroxy-N-[(1E)-4-methoxyphenylmethylene]cholan-24-ohydrazide (**4f**)

This derivative was synthesized according to the above general procedure. Yield 93.8%, as white solid, m.p. 155–156 °C, $[\alpha]_D = +22.7°$. ^{1}H NMR (300 MHz, DMSO-d_6, in ppm): 0.58 (s, 3H, 18-CH_3); 0.80 (s, 3H, 19-CH_3); 0.95 (d, 3H, $J = 6$, 21-CH_3); 0.92–2.20 (m, ca 22H, skeletal CH_2 and CH); 2.60 (m, 2H, 23-CH_2); 3.12 (bs, 1H, H-3β); 3.55 (bs, 1H, H-7β); 3.75 (bs, 1H, H-12β); 3.75 (s, 3H, OCH_3); 4.00 (d, 1H, $J = 1.54$, 3-OH); 4.10 (d, 1H, $J = 3.69$, 7-OH); 4.30 (d, 1H, $J = 4.19$, 12-OH); 6.90–7.60 (m, 4H, skeletal CH-aromatic); 7.90 (8.05) (s, 1H, C═NH); 10.90 (11.10) (s, 1H, CONH). ^{13}C NMR (75 MHz, DMSO-d_6, in ppm): 12.85 (C-18); 17.6 (17.7) (C-21); 23.13 (C-19); 23.3 (C-15); 26.7 (C-9); 27.8 (C-16); 29 (C-11); 30.85 (C-2); 31.34 (C-22); 31.65 (C-23); 34.88 (C-10); 35.37 (C-6); 35.66 (C-1); 35.83 (C-20); 40.53 (C-4); 40.8 (C-8); 41.89 (C-5); 42(C-14); 46.24 (C-13); 46.5 (C-17); 55.76 (OCH_3); 66.75 (C-7); 70.92 (C-3); 71.48 (C-12); 127.45 (C-1′); 128.57 (128.98) (C-2′ and 6′); 114.75 (114.8) (C-3′ and 5′); 160.93 (161.1) (C-4′); 142.7 (142.96) (C═N); 169.38 (175.26) (C═O). ESIMS: m/z calculated for [M + Na^+]: 563.34609, found 563.350418, difference: 0.00433.

4.3.10. (3α, 5β, 7α, 12α)-3, 7, 12-Trihydroxy-N-[(1E)-4-cyanophenylmethylene]cholan-24-ohydrazide (**4g**)

This derivative was synthesized according to the above general procedure. Yield 78.9%, as white solid, m.p. 278–279 °C, $[\alpha]_D = +20.7°$. ^{1}H NMR (300 MHz, DMSO-d_6, in ppm): 0.58 (s, 3H, 18-CH_3); 0.80 (s, 3H, 19-CH_3); 0.95 (d, 3H, $J = 6$, 21-CH_3); 0.92–2.20 (m, ca 22H, skeletal CH_2 and CH); 2.60 (m, 2H, 23-CH_2); 3.12 (bs, 1H, H-3β); 3.55 (bs, 1H, H-7β); 3.75 (bs, 1H, H-12β); 4.00 (d, 1H, 3-OH); 4.10 (d, 1H, $J = 3.5$, 7-OH); 4.30 (d, 1H, $J = 4.13$, 12-OH); 7.70–7.90 (m, 4H, skeletal CH-aromatic); 8.00 (8.25) (s, 1H, C═NH); 11.30 (11.45) (s, 1H, CONH). ^{13}C NMR (75 MHz, DMSO-d_6, in ppm): 12.84 (C-18); 17.66 (17.58) (C-21); 23.12 (C-19); 23.3 (C-15); 26.67 (C-9); 27.82 (C-16); 29.63 (29) (C-11); 30.89 (C-2); 31.71 (C-22); 31.81 (C-23); 34.88 (C-10); 35.37 (C-6); 35.64 (C-1); 35.81 (C-20); 40.53 (C-4); 40.81 (C-8); 41.89 (C-5); 42 (C-14); 46.24 (C-13); 46.54 (C-17); 66.73 (C-7); 70.92 (C-3); 71.48 (C-12); 139.32 (139.46) (C-1′); 127.61 (127.97) (C-2′ and 6′); 133.18 (133.25) (C-3′ and 5′); 111.95 (112.13) (C-4′); 119.21 (CN); 140.92 (144.1) (C═N); 170 (175.78) (C═O). ESIMS: m/z calculated for [M + Na^+]: 558.33078, found 558.334985, difference: 0.00421.

4.3.11. (3α, 5β, 7α, 12α)-3, 7, 12-Trihydroxy-N-[(1E)-4-nitrophenylmethylene]cholan-24-ohydrazide (**4h**)

This derivative was synthesized according to the above general procedure. Yield 90%, as yellow solid, m.p 271–272 °C, $[\alpha]_D = +27.2$.

^{1}H NMR (300 MHz, DMSO-d_6, in ppm): 0.55 (s, 3H, 18-CH_3); 0.75 (s, 3H, 19-CH_3); 0.95 (d, 3H, $J = 3.98$, 21-CH_3); 0.92–2.20 (m, ca 22H, skeletal CH_2 and CH); 2.60 (m, 2H, 23-CH_2); 3.15 (bs, 1H, H-3β); 3.55 (bs, 1H, H-7β); 3.75 (bs, 1H, H-12β); 3.95 (bs, 1H, 3-OH); 4.10 (d, 1H, $J = 3.06$, 7-OH); 4.30 (d, 1H, $J = 3.42$, 12-OH); 7.80–8.30 (m, 4H, skeletal CH-aromatic); covered by aromatic (1H, C═NH); 11.35 (11.6) (s, 1H, CONH). ^{13}C NMR (75 MHz, DMSO-d_6, in ppm): 12.81 (C-18); (17.57) (C-21); 23.07 (C-19); 23.26 (C-15); 26.67 (C-9); 27.83 (C-16); 29.67 (29) (C-11); 30.86 (C-2); 31.19 (C-22); 31.68 (C-23); 34.87 (C-10); 35.36 (C-6); 35.64 (C-1); 35.87 (35.8) (C-20); 40.53 (C-4); 40.8 (C-8); 41.88 (C-5); 42 (C-14); 46.23 (C-13); 46.26 (C-17); 71.44 (C-7); 75.63 (C-3); 76.18 (C-12); 145.94 (146.08) (C-1′); 129.2 (129.26) (C-2′ and 6′); 132.61 (133.02) (C-3′ and 5′); 152.73 (152.88) (C-4′); 145.22 (148.31) (C═N); 174.85 (180.56) (C═O). ESIMS: m/z calculated for [M + Na^+]: 578.32061, found 578.320200, difference: 0.00041.

4.3.12. (3α, 5β, 7α, 12α)-3, 7, 12-Trihydroxy-N-[(1E)-3-chlorophenylmethylene]cholan-24-ohydrazide (**4i**)

This derivative was synthesized according to the above general procedure. Yield 89.13%, as white solid, m.p. 150–151 °C, $[\alpha]_D = +18.5°$. ^{1}H NMR (300 MHz, DMSO-d_6, in ppm): 0.58 (s, 3H, 18-CH_3); 0.80 (s, 3H, 19-CH_3); 0.95 (d, 3H, $J = 6$, 21-CH_3); 0.92–2.20 (m, ca 22H, skeletal CH_2 and CH); 2.60 (m, 2H, 23-CH_2); 3.12 (bs, 1H, H-3β); 3.55 (bs, 1H, H-7β); 3.75 (bs, 1H, H-12β); 4.00 (d, 1H, 3-OH); 4.10 (d, 1H, 7-OH); 4.30 (d, 1H, $J = 4.11$, 12-OH); 7.40–7.70 (m, 4H, skeletal CH-aromatic); 7.90 (8.10) (s, 1H, C═NH); 11.10 (11.30) (s, 1H, CONH). ^{13}C NMR (75 MHz, DMSO-d_6, in ppm): 12.86 (C-18); 17.71 (17.61) (C-21); 23.13 (C-19); 23.31 (C-15); 26.73 (C-9); 27.85 (C-16); 29 (C-11); 30.87 (C-2); 31.41 (C-22); 31.68 (C-23); 34.9 (C-10); 35.38 (C-6); 35.63 (C-1); 35.82 (35.91) (C-20); 40.6 (C-4); 40.87 (C-8); 41.89 (C-5); 42 (C-14); 46.28 (C-13); 46.59 (C-17); 66.76 (C-7); 70.94 (C-3); 71.5 (C-12); 137.1(137.18) (C-1′); 126.27 (126.7) (C-2′); 134.07(134.14) (C-3′); 131.15 (131.22) (C-4′); 129.73 (129.92) (C-5′); 125.82 (126.07) (C-6′); 141.19 (144.38) (C═N); 169.85 (175.66) (C═O). ESIMS: m/z calculated for [M + Na^+]: 567.29656, found 567.299726, difference: 0.00317.

4.3.13. (3α, 5β, 7α, 12α)-3, 7, 12-Trihydroxy-N-[(1E)-2-bromophenylmethylene]cholan-24-ohydrazid (**4j**)

This derivative was synthesized according to the above general procedure. Yield 30%, as white solid, m.p. 193–194 °C, $[\alpha]_D = +16.4°$. ^{1}H NMR (300 MHz, DMSO-d_6, in ppm): 0.58 (s, 3H, 18-CH_3); 0.80 (s, 3H, 19-CH_3); 0.95 (d, 3H, $J = 6$, 21-CH_3); 0.92–2.20 (m, ca 22H, skeletal CH_2 and CH); 2.60 (m, 2H, 23-CH_2); 3.12 (bs, 1H, H-3β); 3.55 (bs, 1H, H-7β); 3.75 (bs, 1H, H-12β); 4.00 (d, 1H, $J = 2.7$, 3-OH); 4.10 (d, 1H, $J = 2.04$, 7-OH); 4.30 (d, 1H, $J = 3.71$, 12-OH); 7.20–7.90 (m, 4H, skeletal CH-aromatic); 8.30 (8.45) (s, 1H, C═NH); 11.35 (11.60) (s, 1H, CONH). ^{13}C NMR (75 MHz, DMSO-d_6, in ppm): 12.85 (C-18); 17.67 (C-21); 23.13 (C-19); 23.3 (C-15); 26.74 (C-9); 27.85 (C-16); 29.07 (29.67) (C-11); 30.92 (C-2); 31.31 (C-22); 31.7 (C-23); 34.9 (C-10); 35.39 (C-6); 35.64 (C-1); 35.81 (C-20); 40.59 (C-4); 40.87 (C-8); 41.91 (C-5); 42 (C-14); 46.26 (C-13); 46.51 (C-17); 66.75 (C-7); 70.94 (C-3); 71.5 (C-12); 133.66 (C-1′); 123.61 (C-2′); 131.8 (C-3′); 133.59 (C-4′); 127.28 (127.63) (C-5′); 132 (C-6′); 141.19 (144.32) (C═N); 169.82 (175.64) (C═O). ESIMS: m/z calculated for [M + Na^+]: 611.24604, found 611.254085, difference: 0.00805.

4.3.14. (3α, 5β, 7α, 12α)-3, 7, 12-Trihydroxy-N-[(1E)-2-hydroxyphenylmethylene]cholan-24-ohydrazide (**4k**)

This derivative was synthesized according to the above general procedure. Yield 30%, as white solid, m.p. 273–274 °C, $[\alpha]_D = +21.1$. ^{1}H NMR (300 MHz, DMSO-d_6, in ppm): 0.58 (s, 3H, 18-CH_3); 0.80 (s, 3H, 19-CH_3); 0.95 (d, 3H, J = 6, 21-CH_3); 0.92–2.20 (m, ca 22H, skeletal CH_2 and CH); 2.60 (m, 2H, 23-CH_2); 3.12 (bs, 1H, H-3β); 3.55 (s, 1H, H-7β); 3.75 (s, 1H, H-12β); 4.00 (d, 1H, 3-OH); 4.10 (d, 1H, $J = 2.97$, 7-OH); 4.30 (d, 1H, $J = 3.79$, 12-OH); 6.70–7.60 (m, 4H, skeletal CH-aromatic); 8.20 (8.30) (s, 1H, C═NH); 11.10 (11.20) (s, 1H, CONH); 10.10 (11.55) (s, 1H, OH-aromatic). ^{13}C NMR (75 MHz, DMSO-d_6, in ppm): 12.85 (C-18); 17.67 (C-21); 23.13 (C-19); 23.3 (C-15); 26.74 (C-9); 27.85 (C-16); 29.07 (29.67) (C-11); 30.92 (C-2); 31.31 (C-22); 31.7 (C-23); 34.9 (C-10); 35.39 (C-6); 35.64 (C-1); 35.81 (C-20); 40.59 (C-4); 40.87 (C-8); 41.91 (C-5); 42 (C-14); 46.26 (C-13); 46.51 (C-17); 66.75 (C-7); 70.94 (C-3); 71.5 (C-12); 119.1 (120.56) (C-1′); 156.82 (157.79) (C-2′); 116.56 (116.76) (C-3′); 131.29 (131.56) (C-4′); 119.7 (119.9) (C-5′); 131.29 (131.56) (C-6′); 141.08 (146.8) (C═N); 169.44 (175) (C═O). ESIMS: m/z calculated for [M + Na^+]: 549.33044, found 549.33050, difference: 0.00006.

4.3.15. (3α, 5β, 7α, 12α)-3, 7, 12-Trihydroxy-N-[(1E)-2, 3-dimethoxyphenylmethylene] cholan-24-ohydrazide (**4l**)

This derivative was synthesized according to the above general procedure. Yield 47.3%, as white solid, m.p. 159–160 °C, $[\alpha]_D = +10.5°$. ^{1}H NMR (300 MHz, DMSO-d_6, in ppm): 0.58 (s, 3H, 18-CH_3); 0.80 (s, 3H, 19-CH_3); 0.95 (d, 3H, $J = 6$, 21-CH_3); 0.92–2.20 (m, ca 22H, skeletal CH_2 and CH); 2.60 (m, 2H, 23-CH_2); 3.12 (bs, 1H, H-3β); 3.55 (bs, 1H, H-7β); 3.70 (s, 3H, OCH_3); 3.80 (s, 3H, OCH_3); 3.75 (bs, 1H, H-12β); 4.00 (d, 1H, $J = 3.22$, 3-OH); 4.10 (d, 1H, $J = 3.46$, 7-OH); 4.30 (d, 1H, $J = 4.21$, 12-OH); 7.00–7.40 (m, 3H, skeletal CH-aromatic); 8.20 (8.40) (s, 1H, C═NH); 11.00 (11.25) (s, 1H, CONH). ^{13}C NMR (75 MHz, DMSO-d_6, in ppm): 12.8 (C-18); 17.62 (17.55) (C-21); 23.09 (C-19); 23.27 (C-15); 26.67 (C-9); 27.8 (C-16); 29.02 (29.64) (C-11); 30.85 (C-2); 31.32 (C-22); 31.71 (C-23); 34.89 (C-10); 35.38 (C-6); 35.66 (C-1); 35.82 (C-20); 40.26 (C-4); 40.8 (C-8); 41.89 (C-5); 42 (C-14); 46.23 (C-13); 46.47 (C-17); 56.17 (61.58) (OCH_3 on position 2′ and 3′); 66.73 (C-7); 70.92 (C-3); 71.47 (C-12); 128.25 (128.31) (C-1′); 153.12 (153.18) (C-2′); 148.17 (148.25) (C-3′); 117 (117.36) (C-4′); 114.16 (C-5′); 114.41 (C-6′); 138.56 (141.52) (C═N); 169.52 (175.42) (C═O). ESIMS: m/z calculated for [M + Na^+]: 593.35666, found 593.364688, difference: 0.00803.

4.3.16. (3α, 5β, 7α, 12α)-3, 7, 12-Trihydroxy-N-[(1E)-2, 5-dimethoxyphenylmethylene] cholan-24-ohydrazide (**4m**)

This derivative was synthesized according to the above general procedure. Yield 51.7%, as yellow solid, m.p. 177–178 °C, $[\alpha]_D = +8.0$. ^{1}H NMR (300 MHz, DMSO-d_6, in ppm): 0.58 (s, 3H, 18-CH_3); 0.80 (s, 3H, 19-CH_3); 0.95 (d, 3H, $J = 6$, 21-CH_3); 0.92–2.20 (m, ca 22H, skeletal CH_2 and CH); 2.60 (m, 2H, 23-CH_2); 3.12 (bs, 1H, H-3β); 3.55 (bs, 1H, H-7β); 3.75 (bs, 1H, H-12β); 4.00 (d, 1H, $J = 3.15$, 3-OH); 4.10 (d, 1H, $J = 3.17$, 7-OH); 3.68 (3.70) (s, 3H, OCH_3); 3.74 (3.76) (s, 3H, OCH_3); 4.30 (d, 1H, $J = 1.33$, 12-OH); 6.80–7.00 (m, 3H, skeletal CH-aromatic); 8.20 (8.40) (s, 1H, C═NH); 11.00 (11.20) (s, 1H, CONH). ^{13}C NMR (75 MHz, DMSO-d_6, in ppm): 12.83 (C-18); 17.69 (17.56) (C-21); 23.12 (C-19); 23.31 (C-15); 26.69 (C-9); 27.85 (C-16); 29.06 (30) (C-11); 30.89 (C-2); 31.64 (C-22); 31.75 (C-23); 34.88 (C-10); 35.36 (C-6); 35.64 (C-1); 35.79 (C-20); 40.52 (C-4); 40.8 (C-8); 41.85 (C-5); 42(C-14); 46.24 (C-13); 46.53 (C-7); 55.82 (55.91) and 56.63 (56.68) (OCH_3 on position 2′ and 5′); 66.73 (C-7); 70.93 (C-3); 71.46 (C-12); 123.36 (123.47) (C-1′); 152.43 (152.57) (C-2′); 109. 4 (C-3′); 117.27 (117.82) (C-4′); 153.68 (C-5′); 113.72 (113.77) (C-6′); 138.22 (141.41) (C═N); 169.48 (175.48) (C═O). ESIMS: m/z calculated for [M + Na^+]: 593.35666, found 593.355674, difference: 0.00099.

4.3.17. (3α, 5β, 7α, 12α)-3, 7, 12-Trihydroxy-N-[(1E)-4-chloro-3-nitrophenylmethylene] cholan-24-ohydrazide (**4n**)

This derivative was synthesized according to the above general procedure. Yield 93.1%, as orange solid, m.p. 205–206 °C, $[\alpha]_D = +10.3°$. ^{1}H NMR (300 MHz, DMSO-d_6, in ppm): 0.58 (s, 3H, 18-CH_3); 0.80 (s, 3H, 19-CH_3); 0.95 (d, 3H, $J = 6$, 21-CH_3); 0.92–2.20

(m, ca 22H, skeletal CH_2 and CH); 2.60 (m, 2H, 23-CH_2); 3.12 (bs, 1H, H-3β); 3.55 (bs, 1H, H-7β); 3.75 (bs, 1H, H-12β); 3.90 (d, 1H, $J = 3.35$, 3-OH); 4.10 (d, 1H, $J = 3.34$, 7-OH); 4.30 (d, 1H, $J = 4.05$, 12-OH); 7.70–8.30 (m, 3H, skeletal CH-aromatic); covered by aromatic (1H, C═NH); 11.40 (11.60) (s, 1H, CONH). ^{13}C NMR (75 MHz, DMSO-d_6, in ppm): 12.84 (C-18); 17.66 (17.59) (C-21); 23.12 (C-19); 23.3 (C-15); 26.72 (C-9); 27.87 (C-16); 29.06 (29.58) (C-11); 30.9 (C-2); 31.33 (C-22); 31.7 (C-23); 34.88 (C-10); 35.38 (C-6); 35.65 (C-1); 35.82 (C-20); 40.53 (C-4); 40.8 (C-8); 41.9 (C-5); 42 (C-14); 46.4 (C-13); 46.51 (C-17); 66.73 (C-7); 70.92 (C-3); 71.48 (C-12); 125.73 (126) (C-1′); 123.5 (123.83) (C-2′); 148.28 (148.39) (C-3′); 135.58 (135.69) (C-4′); 131.31 (131.81) (C-5′); 132.55 (C-6′); 139.41 (142.61) (C═N); 170.07 (175.74) (C═O). ESIMS: m/z calculated for $[M + Na^+]$: 612.28163, found 612.289643, difference: 0.00801.

4.3.18. (3α, 5β, 7α, 12α)-3, 7, 12-Trihydroxy-N-[(1E)-1H-indole-2-ylmethylene]cholan-24-ohydrazide (**4o**)

This derivative was synthesized according to the above general procedure. Yield 90%, as yellow solid, m.p. 223–224 °C, $[\alpha]_D = +19.0$. ^{1}H NMR (300 MHz, DMSO-d_6, in ppm): 0.58 (s, 3H, 18-CH_3); 0.80 (s, 3H, 19-CH_3); 0.95 (d, 3H, $J = 5.39$, 21-CH_3); 0.92–2.20 (m, ca 22H, skeletal CH_2 and CH); 2.60 (m, 2H, 23-CH_2); 3.15 (bs, 1H, H-3β); 3.60 (bs, 1H, H-7β); 3.80 (bs, 1H, H-12β); 4.00 (bs, 1H, 3-OH); 4.10 (bs, 1H, 7-OH); 4.30 (bs, 1H, 12-OH); 7.00–8.30 (m, 5H, skeletal CH-aromatic); covered by aromatic (1H, C═NH); 10.80 (11.00) (s, 1H, CONH); 11.50 (bs, NH). ^{13}C NMR (75 MHz, DMSO-d_6, in ppm): 12.88 (C-18); 17.63 (C-21); 23.11 (C-19); 23.33 (C-15); 26.69 (C-9); 27.88 (C-16); 29 (29.93) (C-11); 30.9 (C-2); 31 (C-22); 31.05 (C-23); 34.89 (C-10); 35.37 (C-6); 35.71 (C-1); 35.82 (C-20); 40.55 (C-4); 40.83 (C-8); 41.84 (C-5); 42(C-14); 46.27 (C-13); 46.84 (C-17); 66.76 (C-7); 70.96 (C-3); 71.54 (C-12); 124.81 (C-2′); 112.11 (C-3′); 120.71 (120.84) (C-4′); 124.57 (C-5′); 123 (C-6′); 130.28 (7′); 137.46 (137.52) (C-8′); 112.18 (112.33) (C-9′); 140.29 (143.28) (C═N); 168.83 (174.75) (C═O). ESIMS: m/z calculated for $[M + Na^+]$: 573.35425, found 573.35700, difference: 0.00275.

4.3.19. (3α, 5β, 7α, 12α)-3, 7, 12-Trihydroxy-N-[(1E)-5-methoxy-1H-indole-3-ylmethylene] cholan-24-ohydrazide (**4p**)

This derivative was synthesized according to the above general procedure. Yield 95%, as orange solid, m.p. 224–225 °C, $[\alpha]_D = +16.9$. ^{1}H NMR (300 MHz, DMSO-d_6, in ppm): 0.58 (s, 3H, 18-CH_3); 0.80 (s, 3H, 19-CH_3); 0.95 (d, 3H, $J = 6$, 21-CH_3); 0.92–2.20 (m, ca 22H, skeletal CH_2 and CH); 2.60 (m, 2H, 23-CH_2); 3.12 (bs, 1H, H-3β); 3.55 (bs, 1H, H-7β); 3.73 (s, 3H, OCH_3); 3.75 (bs, 1H, H-12β); 4.00 (bs, 1H, 3-OH); 4.10 (bs, 1H, 7-OH); 4.30 (bs, 1H, 12-OH); 6.70–8.30 (m, 4H, skeletal CH-aromatic); covered by aromatic (1H, C═NH); 10.80 (10.90) (s, 1H, CONH); 11.50 (bs, NH). ^{13}C NMR (75 MHz, DMSO-d_6, in ppm): 12.82 (C-18); 17.63 (C-21); 23.13 (C-19); 23.32 (C-15); 26.69 (C-9); 27.88 (C-16); 29 (29.93) (C-11); 30.9 (C-2); 31 (C-22); 31.05 (C-23); 34.89 (C-10); 35.37 (C-6); 35.71 (C-1); 35.82 (C-20); 40.55 (C-4); 40.83 (C-8); 41.84 (C-5); 42(C-14); 46.27 (C-13); 47 (C-17); 55.47 (55.81) (OCH_3); 66.73 (C-7); 70.94 (C-3); 71.5 (C-12); 103.43 (C-2′); 132.41 (132.49) (C-3′); 104.64 (C-4′); 154.79 (C-5′); 113.04 (C-6′); 130.67 (7′); 132.49 (132.73) (C-8′); 111.82 (112.58) (C-9′); 140.34 (143.5) (C═N); 168.71 (174.35) (C═O). ESIMS: m/z calculated for $[M + Na^+]$: 602.35699, found 602.35649, difference: 0.00050.

4.3.20. (3α, 5β, 7α, 12α)-3, 7, 12-Trihydroxy-N-[(1E)-furan-2-ylmethylene]cholan-24-ohydrazide. (**4q**)

This derivative was synthesized according to the above general procedure. Yield 57.4%, as yellow solid, m.p. 146–147 °C, $[\alpha]_D = +24.0°$. ^{1}H NMR (300 MHz, DMSO-d_6, in ppm): 0.58 (s, 3H, 18-CH_3); 0.80 (s, 3H, 19-CH_3); 0.95 (d, 3H, $J = 6$, 21-CH_3); 0.92–2.20 (m, ca 22H, skeletal CH_2 and CH); 2.60 (m, 2H, 23-CH_2); 3.12 (bs, 1H, H-3β); 3.55 (bs, 1H, H-7β); 3.75 (bs, 1H, H-12β); 4.00 (d, 1H, $J = 3.17$, 3-OH); 4.10 (d, 1H, 7-OH); 4.30 (d, 1H, $J = 3.76$, 12-OH); 6.50–6.90 (m, 3H, skeletal CH-aromatic); 8.00 (8.15) (s, 1H, C═NH); 11.00 (11.20) (s, 1H, CONH). ^{13}C NMR (75 MHz, DMSO-d_6, in ppm): 12.85 (C-18); 17.65 (17.59) (C-21); 23.13 (C-19); 23.3 (C-15); 26.71 (C-9); 27.75 (C-16); 29.06 (29.21) (C-11); 30.9 (C-2); 31.1 (C-22); 31.74 (C-23); 34.89 (C-10); 35.36 (C-6); 35.63 (C-1); 35.8 (C-20); 40.55 (C-4); 40.82 (C-8); 41.88 (C-5); 42 (C-14); 46.23 (C-13); 46.43 (C-17); 66.74 (C-7); 70.92 (C-3); 71.49 (C-12); 149.86 (149.98) (C-2′); 112.46 (112.54) (C-3′); 113.12 (113.42) (C-4′); 145.2 (145.4) (C-5′); 133.03 (136.1) (C═N); 169.58 (175.31) (C═O). ESIMS: m/z calculated for $[M + Na^+]$: 523.31479, found 523.320816, difference: 0.00600.

4.3.21. (3α, 5β, 7α, 12α)-3, 7, 12-Trihydroxy-N-[(1E)-thiophen-2-ylmethylene]cholan-24-ohydrazide (**4r**)

This derivative was synthesized according to the above general procedure. Yield 70%, as white solid, m.p. 170–171 °C, $[\alpha]_D = +22.8°$. ^{1}H NMR (300 MHz, DMSO-d_6, in ppm): 0.58 (s, 3H, 18-CH_3); 0.80 (s, 3H, 19-CH_3); 0.95 (d, 3H, $J = 6$, 21-CH_3); 0.92–2.20 (m, ca 22H, skeletal CH_2 and CH); 2.60 (m, 2H, 23-CH_2); 3.12 (bs, 1H, H-3β); 3.55 (bs, 1H, H-7β); 3.75 (bs, 1H, H-12β); 4.00 (d, 1H, 3-OH); 4.10 (d, 1H, $J = 2.69$, 7-OH); 4.30 (d, 1H, $J = 4.07$, 12-OH); 7.10–7.60 (m, 3H, skeletal CH-aromatic); 8.10 (8.30) (s, 1H, C═NH); 11.00 (11.10) (s, 1H, CONH). ^{13}C NMR (75 MHz, DMSO-d_6, in ppm): 12.86 (C-18); 17.66 (17.59) (C-21); 23.12 (C-19); 23.31 (C-15); 26.7 (C-9); 27.79 (C-16); 29.05 (29.69) (C-11); 30.9 (C-2); 31 (C-22); 31.9 (C-23); 34.88 (C-10); 35.37 (C-6); 35.37 (C-1); 35.82 (C-20); 40.54 (C-4); 40.82 (C-8); 41.86 (C-5); 42 (C-14); 46.23 (C-13); 46.5 (C-17); 66.73 (C-7); 70.92 (C-3); 71.48 (C-12); 139.72 (C-2′); 130.3 (130.95) (C-3′); 128.98 (C-4′); 128.23 (128.46) (C-5′); 137.89 (141.31) (C═N); 169.49 (175.25) (C═O). ESIMS: m/z calculated for $[M + Na^+]$: 539.29195, found 539.294343, difference: 0.00239.

4.3.22. (3α, 5β, 7α, 12α)-3, 7, 12-Trihydroxy-N-[(1E)-1H-pyrrol-2-ylmethylene]cholan-24-ohydrazide (**4s**)

This derivative was synthesized according to the above general procedure. Yield 38%, as white solid, m.p. 267–268 °C. ^{1}H NMR (300 MHz, DMSO-d_6, in ppm): 0.58 (s, 3H, 18-CH_3); 0.80 (s, 3H, 19-CH_3); 0.95 (d, 3H, $J = 6$, 21-CH_3); 0.92–2.20 (m, ca 22H, skeletal CH_2 and CH); 2.60 (m, 2H, 23-CH_2); 3.12 (bs, 1H, H-3β); 3.55 (bs, 1H, H-7β); 3.75 (bs, 1H, H-12β); 4.00 (d, 1H, 3-OH); 4.10 (d, 1H, $J = 2.69$, 7-OH); 4.30 (d, 1H, $J = 4.07$, 12-OH); 6.00–6.90 (m, 3H, skeletal CH-aromatic); 7.80(8.00) (s, 1H, C═NH); 10.80 (11.00) (s, 1H, NH); 11.15 (11.40) (s, 1H, CONH). ^{13}C NMR (75 MHz, DMSO-d_6, in ppm): 12.86 (C-18); 17.66 (17.59) (C-21); 23.12 (C-19); 23.31 (C-15); 26.7 (C-9); 27.79 (C-16); 29.05 (29.69) (C-11); 30.9 (C-2); 31 (C-22); 31.9 (C-23); 34.88 (C-10); 35.37 (C-6); 35.37 (C-1); 35.82 (C-20); 40.54 (C-4); 40.82 (C-8); 41.86 (C-5); 42 (C-14); 46.23 (C-13); 46.5 (C-17); 66.73 (C-7); 70.92 (C-3); 71.48 (C-12); 127.79 (127.58) (C-2′); 111.94 (113.23) (C-3′); 109.51 (C-4′); 121.92 (122.54) (C-5′); 135.89 (139.34) (C═N); 169.07 (174.87) (C═O). ESIMS: m/z calculated for $[M + Na^+]$: 522.33078, found 522.33023, difference: 0.00055.

4.3.23. (3α, 5β, 7α, 12α)-3, 7, 12-Trihydroxy-N-[(1E)-pyridin-2-ylmethylene]cholan-24-ohydrazide (**4t**)

This derivative was synthesized according to the above general procedure. Yield 67%, as white solid, m.p. 184–186 °C, $[\alpha]_D = +6.0°$. ^{1}H NMR (300 MHz, DMSO-d_6, in ppm): 0.58 (s, 3H, 18-CH_3); 0.80 (s, 3H, 19-CH_3); 0.95 (d, 3H, $J = 6$, 21-CH_3); 0.92–2.20 (m, ca 22H, skeletal CH_2 and CH); 2.60 (m, 2H, 23-CH_2); 3.12 (bs, 1H, H-3β); 3.55 (bs, 1H, H-7β); 3.75 (bs, 1H, H-12β); 4.00 (s, 1H, 3-OH); 4.10 (s, 1H, 7-OH); 4.30 (d, 1H, $J = 4.01$, 12-OH); 7.20–8.60 (m, 4H, skeletal CH-aromatic); 8.00 (8.15) (s, 1H, C═NH); 11.35 (11.50) (s, 1H, CONH). ^{13}C NMR (75 MHz, DMSO-d_6, in ppm): 12.86 (C-18); 17.66 (17.59) (C-21); 23.12 (C-19); 23.31 (C-15); 26.7 (C-9); 27.79 (C-16); 29.05

(29.69) (C-11); 30.9 (C-2); 31 (C-22); 31.9 (C-23); 34.88 (C-10); 35.37 (C-6); 35.37 (C-1); 35.82 (C-20); 40.54 (C-4); 40.82 (C-8); 41.86 (C-5); 42 (C-14); 46.23 (C-13); 46.5 (C-17); 66.73 (C-7); 70.92 (C-3); 71.47 (C-12); 153.69 (C-2′); 119.66 (120.21) (C-3′); 137.35 (C-4′); 124.57 (C-5′); 149.92 (C-6′); 143.35 (146.34) (C═N); 169.9 (175.73) (C═O). ESIMS: m/z calculated for $[M + Na^+]$: 534.33078, found 534.332224, difference: 0.00144.

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References

[1] J. Barnett, B.E. Ryman, F. Smith, J. Chem. Soc. (1946) 528.
[2] C. Loncle, J.M. Brunel, N. Vidal, M. Dherbomez, Y. Letourneux, Eur. J. Med. Chem. 39 (2004) 1067.
[3] H.M. Willemen, L.C.P.M. de Smet, A. Koudijs, M.C.A. Stuart, I.G.A.M. Heikamp-de Jong, A.T.M. Marcelis, E.J.R. Sudhlter, Angew. Chem. Int. Ed. 41 (2002) 4275.
[4] P.B. Savage, Eur. J. Org. Chem. (2002) 759.
[5] S.P. James, F. Smith, M. Stacy, M. Webb, J. Chem. Soc. (1946) 665.
[6] M.L. Hilton, A.S. Jones, J.R.B. Westwood, J. Chem. Soc. (1955) 3449.
[7] A. Fini, G. Fazio, A. Roda, A.M. Bellini, E. Mencini, M. Guarneri, J. Pharm. Sci. 81 (1992) 726.
[8] C. Li, A.S. Peters, E.L. Meridith, G.W. Allman, P.B. Savage, J. Am. Chem. Soc. 120 (1998) 2961.
[9] R. Boyce, G. Li, H.P. Nestler, T. Suenaga, W.C. Still, J. Am. Chem. Soc. 116 (1994) 7955.
[10] A.P. Davis, J.J. Perry, R.P. Williams, J. Am. Chem. Soc. 119 (1997) 1793.
[11] J. McKenna, D.W. Thornthwaite, J. Chem. Soc. Chem. Commun. (1977) 809.
[12] V. Janout, M. Lanier, S.L. Regen, J. Am. Chem. Soc. 118 (1996) 1573.
[13] Y. Cheng, D.M. Ho, C.R. Gottlieb, D. Kahne, M.A. Bruck, J. Am. Chem. Soc. 114 (1992) 7319.
[14] P. Venkatasan, Y. Cheng, D. Kahne, J. Am. Chem. Soc. 116 (1994) 6955.
[15] W. Kramer, G. Wess, Eur. J. Clin. Invest. 26 (1996) 715.
[16] T. Lehmann, J. Engels, Bioorg. Med. Chem. 9 (2001) 1827.
[17] K.S. Moore, S. Wehrli, H. Roder, M. Rogers, J.N. Forrest Jr., D. McCrimmon, M. Zasloff, Proc. Natl. Acad. Sci. U.S.A. 90 (1993) 1354.
[18] X. Yuan, H. Li, X. Zhu, H. Woo, J. Chem. Technol. Biotechnol. 81 (2006) 746.
[19] P. Savage, C. Li, U. Taotafa, B. Ding, Q. Guan, FEMS Microbiol. Lett. 217 (2002) 1.
[20] H. Willemen, L. Smet, I. Jong, A. Marcelis, E. Sudholter, Angew. Chem. Int. Ed. 41 (2002) 4275–4277.
[21] L. Jin, J. Chen, B. Song, Z. Chen, S. Yang, Q. Li, D. Hu, R. Xu, Bioorg. Med. Chem. Lett. 16 (2006) 5036.
[22] T.H. Al-Tel, R.A. Al-Qawasmeh, M.F. Schmidt, A. Al-Aboudi, S.N. Rao, S.S. Sabri, W. Voelter, J. Med. Chem. 52 (2009) 6484.
[23] T.H. Al-Tel, R.A. Al-Qawasmeh, S.S. Sabri, W. Voelter, J. Org. Chem. 74 (2009) 4690.
[24] K.-K. Bedia, O. Elçin, U. Seda, K. Fatma, S. Nathaly, R. Sevim, A. Dimoglo, Eur. J. Med. Chem. 41 (2006) 1253.
[25] G. Küçükgüzel, S. Rollas, I. Küçükgüzel, M. Kiraz, Eur. J. Med. Chem. 34 (1999) 1093.
[26] V.S. Palekar, A.J. Damle, S.R. Shukla, Eur. J. Med. Chem. 44 (2009) 5112.
[27] K. Sztanke, J. Rzymowska, M. Niemczyk, I. Dybała, A.E. Kozio, Eur. J. Med. Chem. 44 (2006) 539.
[28] J. Andrews, Antimicrob. Agents Chemother. 48 (2001) 5.
[29] F. Cortese, J. Am. Chem. Soc. 59 (1937) 2532.