Microwave-assisted synthesis and *in vitro* antibacterial activity of novel steroidal 1,2,4-triazole Schiff base derivatives

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A series of novel Schiff bases containing both a 1,2,4-triazole and deoxycholic acid skeleton have been synthesised in excellent yields and in high purity by means of microwave irradiation. The structures of the target compounds were characterised by MS, IR and NMR as well as elemental analysis. All the products were evaluated for their *in vitro* antibacterial activities against Gram positive (*Staphylococcus aureus* and *Bacillus subtilis*) and Gram negative bacteria (*Pseudomonas seruginosa* and *Escherichia coli*). Four of the compounds displayed varying levels of antibacterial activity against *P. aeruginosa* and *S. aureus*, but none of the compounds showed any activity against *E. coli* and *B. subtilis*.

Keywords: 1,2,4-triazole, Schiff base, deoxycholic acid, microwave irradiation, antibacterial activity

1,2,4-Triazoles have a wide range of biological activities including antibacterial activity.[1] In previous work bile acids such as deoxycholic acid have been used as conjugates with triazoles and have been shown to have anti-bacterial activity.[2] Schiff bases derived from triazoles have also been shown to have anti-bacterial activity.[3,4] Moreover, in recent years, microwave irradiation has gained popularity as powerful tool in green synthetic chemistry.[5] This method of synthesis is not only green, economic and easy to handle, but it also gives a significant improvement in product yield, selectivity and reaction time.[6,7]

In this work, three components, deoxycholic acid, a 1,2,4-triazole and a Schiff base have been combined. The anti-bacterial activity has been evaluated against *Pseudomonas seruginosa*, *Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtilis*. The value of microwave irradiation has been demonstrated in the preparation of these compounds. The synthetic route is shown in Scheme 1.

Results and discussion

The structures of all the compounds **7** and **8a–j** were confirmed by IR, mass spectroscopy, NMR and elemental analysis. Their mass spectra showed the expected molecular ion peaks in high intensity. The IR spectra of compound **7** exhibited a characteristic strong absorption at 3684–3149 cm^{-1} due to N–H stretching vibration, which was absent in the IR spectra of **8a–j**. The appearance of a medium to strong absorption peak at 1615–1602 cm^{-1} is due to the stretching vibration of C=N bond formation in the synthetic products. The carbonyl absorption band observed at 1734–1732 cm^{-1} and C=S stretching band was seen in the range of 1297–1280 cm^{-1}. In the ^1H NMR spectra, compound **7** showed a singlet peak at δ 6.17 ppm attributed to the NH_2 group which was not present in the spectra of compounds **8a–j**. The singlet peaks at δ 9.10–8.76 ppm were assigned to the N=CH proton. The protons of ArH appeared at δ 8.69–6.91 ppm. In the ^{13}C NMR spectra, the triazole C3 and C5 of compounds **7** and **8a–j** were observed at δ 151.16–150.31 ppm and δ 174.26–174.21 ppm respectively. The carbons of C=N were found in the region at δ 146.59–146.38 ppm.

In vitro *antibacterial activity*

The *in vitro* antibacterial activities of compounds **8a–j** were examined using cultures of the gram-negative bacteria (*P. seruginosa* and *E. coli*) and gram-positive bacteria (*S. aureus* and *B. subtilis*). Amoxicillin and ciprofloxacin were used as the standards. The MIC was evaluated by double dilution method using standard inocula of 10^5 CFU mL^{-1}. Serial dilutions of the test compounds, previously dissolved in DMSO were prepared to give concentrations of 256, 128, 64, 32, 16, 8, 4, 2, 1, 0.5 and 0.25 μg mL^{-1}. Each tube contained 1 mL bacterial fluid of 0.5 McFarland standards. The minimum inhibitory concentration (MIC) was determined visually after incubation for 16 h, at 37°C. The lowest concentration, which showed no visible growth, was taken as an end point for minimum MIC.

A standard inoculum of 10^5 CFU mL^{-1} was introduced onto the surface of sterile agar plates. The filter papers measuring 6 mm in diameter previously soaked in a required concentration (640, 320, 160, 80, 40, 20, 10 and 5 μg mL^{-1}) of the test compounds were placed in nutrient agar medium. Solvent and growth controls were kept. The plates were incubated for 16 h at 37 °C. The inhibition zones were measured and compared with the controls, which helped to provide the IC_{50} values. The results of MIC and IC_{50} are presented in Table 1.

As shown in Table 1, compounds **8b**, **8d**, **8f** and **8h** exhibited moderate to poor antibacterial activity against *P. aeruginosa* and *S. aureus*. They exhibited better antibacterial activity against *P. aeruginosa* than *S. aureus*. None of the compounds showed any activity against *E. coli* and *B. subtilis*. The relationship between the structures and biological activity indicated that monosubstituted halogen compounds showed greater antibacterial activity, whereas disubstituted halogen compounds had weaker antibacterial activity. For example, the antibacterial activity of the compound **8b** was better than that of the compound **8d**. Otherwise, compounds with an electron-withdrawing group generally showed stronger activity than

Table 1 Bacterial inhibition results for compounds **8a–j** and two positive controls (ciprofloxacin and amoxicillin)

Compd	*E. coli*		*B. subtilis*		*P. aeruginosa*		*S. aureus*	
	MIC	IC_{50}	MIC	IC_{50}	MIC	IC_{50}	MIC	IC_{50}
Ciprofloxacin	0.25	0.18	0.125	0.1	0.5	0.41	3.12	1.82
Amoxicillin	16	7.3	2	1	4	2.5	16	6.4
8a	–	–	–	–	–	–	–	–
8b	–	–	–	–	32	17.34	32	18.72
8c	–	–	–	–	–	–	–	–
8d	–	–	–	–	64	32.45	–	–
8e	–	–	–	–	–	–	–	–
8f	–	–	–	–	64	32.26	64	32.46
8g	–	–	–	–	–	–	–	–
8h	–	–	–	–	64	33.45	128	63.43
8i	–	–	–	–	–	–	–	–
8j	–	–	–	–				

– Inactive to inhibit bacteria.

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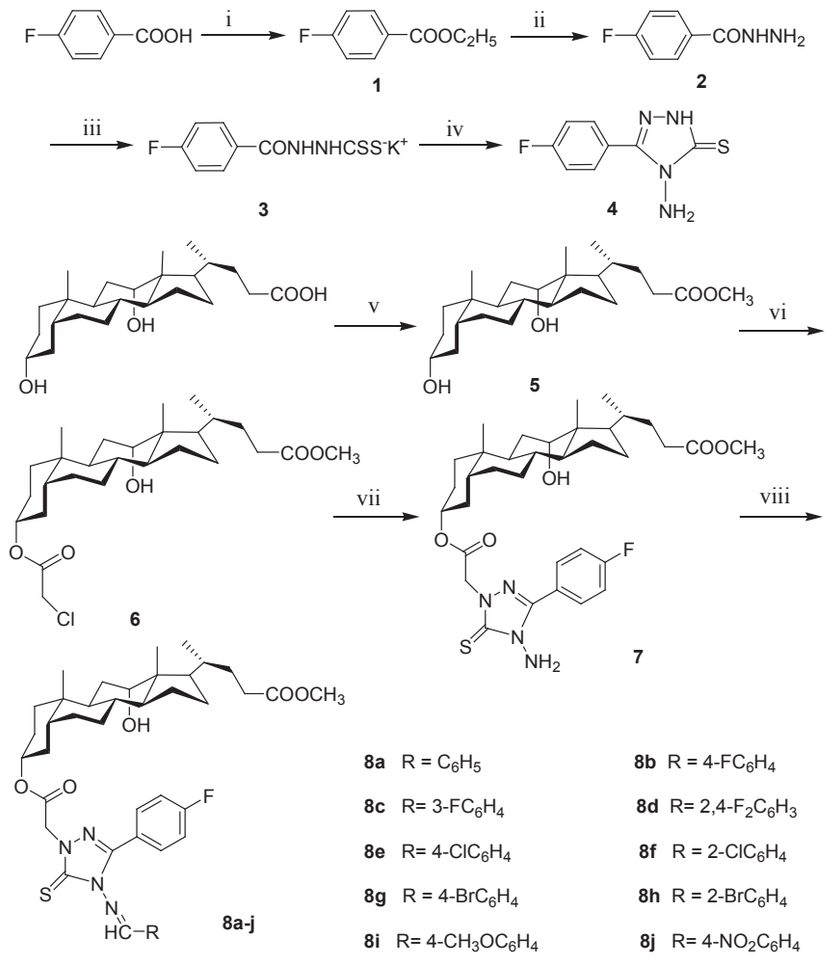

Scheme 1 Reagents and conditions: (i) $SOCl_2$, C_2H_5OH, MWI (microwave irradiation); (ii) $NH_2NH_2{\cdot}H_2O$, MWI; (iii) CS_2, KOH, C_2H_5OH; (iv) $NH_2NH_2{\cdot}H_2O$, MWI; (v) CH_3COCl, CH_3OH; (vi) pyridine, $ClCH_2COCl$, $CHCl_3$, MWI; (vii) 4, K_2CO_3, DMF, MWI; (viii) RCHO, AcOH, MWI.

those with an electron-donating group. The potency order was F (fluorine) > Cl (chlorine) > Br (bromine). Further antibacterial activity studies are in progress.

Comparison of microwave irradiation and conventional heating

As shown in Table 2, microwave irradiation significantly reduced the reaction time from 420–780 min to 3–7 min and the yields increased from 62–70% to 86–94%. We can conclude that microwave irradiation is a simple, efficient and rapid synthetic method.

Table 2 Comparison of microwave irradiation with conventional heating for the efficiency of synthesis of compounds **8a–j**

Compd	RCHO (R)	Conventional method		Microwave method		t_C/t_{MW}
		t/min	Yield/%	t/min	Yield/%	
8a	C_6H_5	480	69	3.5	93	137
8b	4-F–C_6H_4	600	66	5.0	91	120
8c	3-F–C_6H_4	660	64	4.5	89	147
8d	2,4-F_2–C_6H_3	450	70	4.0	94	113
8e	4-Cl–C_6H_4	660	64	6.0	90	110
8f	2-Cl–C_6H_4	720	67	7.0	93	103
8g	4-Br–C_6H_4	540	63	5.0	87	108
8h	2-Br–C_6H_4	600	65	4.5	90	133
8i	4-OCH_3–C_6H_4	780	62	7.0	86	111
8j	4-NO_2–C_6H_4	420	69	3.0	92	140

t_C, Conventional method time; t_{MW}, microwave method time.

In summary, we have developed a simple method for the synthesis of novel Schiff bases containing both 1,2,4-triazole and deoxycholic acid skeleton under microwave irradiation giving excellent yields of the products in shorter reaction time. All the synthesised compounds have been investigated for their *in vitro* antibacterial activities. From the activity studies, compounds **8b**, **8d**, **8f** and **8h** were shown to possess some antibacterial activity against *P. aeruginosa* and *S. aureus*. This study may provide valuable information for further designing and developing more potent antibacterial agents for the biological and pharmacological use.

Experimental

Melting points were determined on a micro-melting point apparatus and the thermometer was uncorrected. IR spectra were obtained on 1700 PerkinElmer FTIR using KBr disks. ^1H NMR and ^{13}C NMR spectra were recorded on a Bruker 400 MHz spectrometer using DMSO-d_6 as solvent and TMS as internal standard. Mass spectra were determined on FinniganLCQ^{DECA} instrument. Elemental analysis (C, H, and N) was performed on a VarioMICRO auto analyser. All reactions were performed in a commercial microwave reaction (XH-100A, 100–1000 W, Beijing XiangHu Science and Technology Development Co. Ltd, Beijing, P.R. China). The disinfection of apparatus and reagents used *in vitro* antimicrobial activity test was conducted in a portable stainless steel pressure steam steriliser (YX280A, Shanghai Sanshen Medical Instrument Co., Ltd, Shanghai, P.R. China). A sterile operation was carried out on a super clean bench

(DL-CJ-1N, Donglian Electronic & Technology Development Co. Ltd, Beijing, P.R. China). Bacterial culture was operated in a biologically constant temperature incubator (ECA-9272, Beijing ECOA Science & Development Co., Ltd, Beijing, P.R. China). All the chemicals and solvents were dried and purified before use. Intermediates **3** and **4** were prepared following the literature methods.[8]

Synthesis of the intermediate methyl 3α-(2-(4-amino-3-(4-fluorophenyl)-5-thioxo-4,5-dihydro-1,2,4-triazol-1-yl)-acetoxy)-12α-hydroxy-cholanate **7**

Methyl 3α-(2-(4-amino-3-(4-fluorophenyl)-5-thioxo-4,5-dihydro-1,2,4-triazol-1-yl)-acetoxy)-12α-hydroxy-cholanate **7** was synthesised using a literature method.[2] The solid product obtained was purified by flash chromatography (elution with ethyl acetate : petroleum ether = 4 : 1–8 : 1). White solid; yield 96%; m.p. 121–122 °C; $[\alpha]_D^{20}$–45.8 (*c* 0.17, CH_2Cl_2); IR (KBr)(cm^{-1}): 3324, 3195, 2938, 2867, 1732, 1606, 1458, 1382, 1298, 1184, 1041, 982, 897, 843. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.01–7.98 (m, 2H, ArH), 7.34 (t, *J* = 8.0 Hz 2H, ArH), 6.17 (s, 2H, NH_2), 4.63 (s, 1H, 3*β*-H), 4.21 (s, 1H, 12*β*-H), 4.03 (s, 2H, –OCOCH$_2$N), 3.75 (s, 1H, 12*α*-OH), 3.54 (s, 3H, –CO_2CH_3), 0.88 (d, *J* = 6.4 Hz, 3H, 21-CH_3), 0.83 (s, 3H, 19-CH_3), 0.56 (s, 3H, 18-CH_3); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 174.22, 168.33, 164.53, 153.27, 130.55, 130.47, 123.78, 123.75, 116.14, 115.92, 75.61, 71.44, 51.62, 47.82, 46.54, 46.42, 41.60, 35.93, 35.36, 34.82, 34.15, 33.79, 33.23, 32.06, 31.10, 30.87, 28.94, 27.57, 27.02, 26.43, 26.29, 23.88, 23.16, 17.29, 12.81; ESI-MS *m/z* (%): 1335 ([2M + Na]$^+$, 100). Anal. calcd for $C_{35}H_{49}FN_4O_5S$: C, 64.00; H, 7.52; N, 8.53; found: C, 64.13; H, 7.50; N, 8.50%.

Synthesis of compounds **8a–j***; general procedure*

Conventional method: Compound **7** (0.164 g, 0.25 mmol), aldehyde (0.26 mmol), and glacial acetic acid (10 mL) were placed in a round-bottomed flask and the reaction mixture was heated to reflux for 420–780 min. When the reaction was complete (monitored by TLC), the flask was cooled to room temperature and evaporated to remove the glacial acetic acid. The solid residue was purified by flash chromatography (elution with ethyl acetate : petroleum ether = 1 : 1–2 : 1) to give pure **8a–j** (62–70%).

Microwave irradiation method: Compound **7** (0.164 g, 0.25 mmol), aldehyde (0.26 mmol), and glacial acetic acid (5 mL) were mixed thoroughly in a sealed vessel. The vessel was then placed in a microwave oven and the mixture was irradiated at 300 W for 3–7 min. The reaction was monitored by TLC until it was complete. The solvent was removed and the solid residue was subject to flash chromatography (elution with ethyl acetate : petroleum ether = 1 : 1–2 : 1) for purification to give pure **8a–j** (86–94%). The physical and spectra data of compounds **8a–j** are as follows:

Methyl 3α-(2-(4-(benzylideneamino)-3-(4-fluorophenyl)-5-thioxo-4,5-dihydro-1,2,4-triazol-1-yl)acetoxy)-12α-hydroxy-cholanate (**8a**): White solid; yield 93%; m.p. 86–88 °C; $[\alpha]_D^{20}$ –101.3 (*c* 0.17, CH_2Cl_2); IR (KBr)(cm^{-1}): 3440, 2937, 2866, 1733, 1608, 1532, 1441, 1290, 1165, 1092, 840; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.88 (s, 1H, =CH), 7.96–7.90 (m, 4H, ArH), 7.68 (t, *J* = 7.2 Hz, 1H, ArH), 7.60 (t, *J* = 7.4 Hz, 2H, ArH), 7.37 (t, *J* = 8.8 Hz, 2H, ArH), 4.59 (s, 1H, 3*β*-H), 4.22 (s, 1H, 12*β*-H), 4.05 (s, 2H, –OCOCH$_2$N), 3.78 (s, 1H, 12*α*-OH), 3.57 (s, 3H, –CO_2CH_3), 0.91 (d, *J* = 6.0 Hz, 3H, 21-CH_3), 0.84 (s, 3H, 19-CH_3), 0.58 (s, 3H, 18-CH_3); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 174.23, 168.81, 167.83, 164.62, 150.80, 146.49, 133.86, 131.85, 130.63, 130.54, 129.81, 129.63, 123.29, 116.51, 116.29, 75.60, 71.39, 51.63, 47.79, 46.52, 46.41, 41.55, 35.90, 35.84, 35.36, 34.77, 34.11, 33.21, 31.96, 31.10, 30.84, 28.94, 27.57, 27.00, 26.36, 26.18, 23.85, 27.16, 17.30, 12.81; ESI-MS *m/z* (%): 745 ([M + 1]$^+$, 100). Anal. calcd for $C_{42}H_{53}FN_4O_5S$: C, 67.72; H, 7.17; N, 7.52; found: C, 67.84; H, 7.15; N, 7.54%.

Methyl 3α-(2-(4-(4-fluorobenzylideneamino)-3-(4-fluorophenyl)-5-thioxo-4,5-dihydro-1,2,4-triazol-1-yl)acetoxy)-12α-hydroxy-cholanate (**8b**): White solid; yield 91%; m.p. 77–78 °C; $[\alpha]_D^{20}$ –48.1 (*c* 0.20, CH_2Cl_2); IR (KBr)(cm^{-1}): 3442, 2939, 2868, 1733, 1602, 1583, 1510, 1479, 1297, 1158, 1092, 840; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 8.88 (s, 1H, =CH), 8.05–8.02 (m, 2H, ArH), 7.93–7.90 (m, 2H, ArH), 7.45 (t, *J* = 8.2 Hz, 2H, ArH), 7.36 (t, *J* = 8.6 Hz, 2H, ArH), 4.57 (s, 1H, 3*β*-H), 4.24 (s, 1H, 12*β*-H), 4.01 (s, 2H, –OCOCH$_2$N), 3.78 (s, 1H, 12*α*-OH), 3.57 (s, 3H, –CO_2CH_3), 0.91 (d, *J* = 6.4 Hz, 3H, 21-CH_3), 0.83 (s, 3H, 19-CH_3), 0.58 (s, 3H, 18-CH_3); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 174.22, 167.78, 167.55, 164.63, 164.29, 150.79, 146.49, 132.48, 132.36, 130.61, 130.53, 128.56, 128.54, 123.29, 123.26, 117.06, 117.00, 116.57, 116.49, 75.57, 71.38, 51.62, 47.82, 46.53, 46.40, 41.55, 35.94, 35.88, 35.34, 34.75, 34.09, 33.23, 31.94, 31.09, 30.85, 28.95, 27.56, 26.99, 26.36, 26.14, 23.82, 23.16, 17.31, 12.79; ESI-MS *m/z* (%): 1547 ([2M + Na]$^+$, 100). Anal. calcd for $C_{42}H_{52}F_2N_4O_5S$: C, 66.12; H, 6.87; N, 7.34; found: C, 66.01; H, 6.85; N, 7.31%.

Methyl 3α-(2-(4-(3-fluorobenzylideneamino)-3-(4-fluorophenyl)-5-thioxo-4,5-dihydro-1,2,4-triazol-1-yl)acetoxy)-12α-hydroxy-cholanate (**8c**): White solid; yield 89%; m.p. 81–82 °C; $[\alpha]_D^{20}$ –151.5 (*c* 0.15, CH_2Cl_2); IR (KBr)(cm^{-1}): 3442, 2940, 2867, 1733, 1608, 1582, 1476, 1448, 1294, 1159, 1092, 842; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.90 (s, 1H, =CH), 7.94–7.90 (m, 2H, ArH), 7.80–7.76 (m, 2H, ArH), 7.68–7.63 (m, 1H, ArH), 7.54 (t, *J* = 7.6 Hz, 1H, ArH), 7.38 (t, *J* = 8.8 Hz, 2H, ArH), 4.58 (s, 1H, 3*β*-H), 4.22 (s, 1H, 12*β*-H), 4.02 (s, 2H, –OCOCH$_2$N), 3.78 (s, 1H, 12*α*-OH), 3.57 (s, 3H, –CO_2CH_3), 0.91 (d, *J* = 6.4 Hz, 3H, 21-CH_3), 0.84 (s, 3H, 19-CH_3), 0.58 (s, 3H, 18-CH_3); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 174.26, 167.84, 167.13, 164.67, 163.96, 150.91, 146.48, 134.13, 134.06, 132.10, 132.01, 130.72, 130.63, 126.13, 123.15, 123.12, 120.81, 120.60, 116.52, 116.30, 115.56, 115.34, 75.63, 71.41, 51.62, 47.75, 46.48, 46.39, 41.54, 35.96, 35.87, 35.35, 34.74, 34.08, 33.19, 31.93, 31.07, 30.81, 28.88, 27.57, 26.97, 26.34, 26.18, 23.82, 23.12, 17.26, 12.76; ESI-MS *m/z* (%): 763 ([M + 1]$^+$, 100). Anal. calcd for $C_{42}H_{52}F_2N_4O_5S$: C, 66.12; H, 6.87; N, 7.34; found: C, 66.23; H, 6.89; N, 7.37%.

Methyl 3α-(2-(4-(2,4-difluorobenzylideneamino)-3-(4-fluorophenyl)-5-thioxo-4,5-dihydro-1,2,4-triazol-1-yl)acetoxy)-12α-hydroxy-cholanate (**8d**): White solid; yield 94%; m.p. 77–79 °C; $[\alpha]_D^{20}$–175.6 (*c* 0.20, CH_2Cl_2); IR (KBr)(cm^{-1}): 3450, 2940, 2867, 1734, 1615, 1583, 1476, 1437, 1280, 1159, 1092, 968, 843; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.98 (s, 1H, =CH), 8.17–8.11 (m, 1H, ArH), 7.93–7.90 (m, 2H, ArH), 7.58–7.52 (m, 1H, ArH), 7.40–7.32 (m, 3H, ArH), 4.58–4.54 (m, 1H, 3*β*-H), 4.23 (s, 1H, 12*β*-H), 4.02 (s, 2H, –OCOCH$_2$N), 3.77 (s, 1H, 12*α*-OH), 3.57 (s, 3H, –CO_2CH_3), 0.92 (d, *J* = 6.4 Hz, 3H, 21-CH_3), 0.83 (s, 3H, 19-CH_3), 0.58 (s, 3H, 18-CH_3); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 174.21, 167.80, 167.27, 164.74, 164.69, 164.61, 164.24, 164.11, 161.67, 161.54, 150.95, 146.38, 130.79, 130.71, 123.28, 123.25, 116.67, 116.60, 116.57, 116.46, 116.24, 113.99, 113.77, 105.96, 105.70, 105.45, 75.62, 71.40, 51.60, 47.78, 46.52, 46.40, 41.56, 36.02, 35.89, 35.35, 34.76, 34.09, 33.21, 31.94, 31.09, 30.81, 28.93, 27.56, 26.99, 26.35, 26.15, 23.82, 23.13, 17.29, 12.78; ESI-MS *m/z* (%): 803 ([M + Na]$^+$, 100). Anal. calcd for $C_{42}H_{51}F_3N_4O_5S$: C, 64.60; H, 6.58; N, 7.30; found: C, 64.71; H, 6.55; N, 7.32%.

Methyl 3α-(2-(4-(4-chlorobenzylideneamino)-3-(4-fluorophenyl)-5-thioxo-4,5-dihydro-1,2,4-triazol-1-yl)acetoxy)-12α-hydroxy-cholanate (**8e**): White solid; yield 90%; m.p. 76–78 °C; $[\alpha]_D^{20}$–85.2 (*c* 0.13, CH_2Cl_2); IR (KBr)(cm^{-1}): 3436, 2934, 2866, 1732, 1603, 1532, 1446, 1289, 1165, 1090, 835; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 8.89 (s, 1H, =CH), 7.97 (d, *J* = 8.4 Hz, 2H, ArH), 7.73–7.89 (m, 2H, ArH), 7.69 (d, *J* = 8.4 Hz, 2H, ArH), 7.36 (t, *J* = 8.6 Hz, 2H, ArH), 4.56–4.53 (m, 1H, 3*β*-H), 4.24 (s, 1H, 12*β*-H), 4.01 (s, 2H, –OCOCH$_2$N), 3.78 (s, 1H, 12*α*-OH), 3.57 (s, 3H, –CO_2CH_3), 0.91 (d, *J* = 6.4 Hz, 3H, 21-CH_3), 0.83 (s, 3H, 19-CH_3), 0.58 (s, 3H, 18-CH_3); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 174.22, 167.74, 167.35, 164.65, 150.82, 146.50, 138.58, 131.26, 130.76, 130.65, 130.56, 130.04, 123.26, 123.23, 116.49, 116.27, 75.56, 71.37, 51.62, 47.85, 46.52, 46.40, 41.53, 36.09, 35.87, 35.32, 34.75, 32.14, 33.23, 31.93, 31.10, 30.86, 28.95, 27.55, 26.98, 26.36, 26.12, 23.82, 23.16, 17.32, 12.80; ESI-MS *m/z* (%): 801 ([M + Na]$^+$, 100). Anal. calcd for $C_{42}H_{52}ClFN_4O_5S$: C, 64.72; H, 6.72; N, 7.19; found: C, 64.60; H, 6.75; N, 7.16%.

Methyl 3α-(2-(4-(2-chlorobenzylideneamino)-3-(4-fluorophenyl)-5-thioxo-4,5-dihydro-1,2,4-triazol-1-yl)acetoxy)-12α-hydroxy-cholanate (**8f**): White solid; yield 93%; m.p. 85–86 °C; $[\alpha]_D^{20}$ –160.3 (*c* 0.13, CH_2Cl_2); IR (KBr)(cm^{-1}): 3448, 2938, 2867, 1734, 1607, 1585, 1478, 1438, 1295, 1159, 1091, 841, 755; ^{1}H NMR (DMSO-d_6, 400 MHz)

δ 9.10 (s, 1H, =CH), 8.13 (d, *J*=7.2 Hz, 1H, ArH), 7.93–7.89 (m, 2H, ArH), 7.67 (d, *J*=3.6 Hz, 2H, ArH), 7.58–7.54 (m, 1H, ArH), 7.40 (t, *J*=8.8 Hz, 2H, ArH), 4.61–4.56 (m, 1H, 3*β*-H), 4.22 (s, 1H, 12*β*-H), 4.07 (s, 2H, $-OCOCH_2N$), 3.77 (s, 1H, 12*α*-OH), 3.57 (s, 3H, $-CO_2CH_3$), 0.91 (d, *J*=6.4 Hz, 3H, 21-CH_3), 0.84 (s, 3H, 19-CH_3), 0.58 (s, 3H, 18-CH_3); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 174.23, 167.82, 164.71, 151.12, 146.44, 135.79, 135.06, 131.00, 130.92, 130.90, 129.38, 128.74, 128.65, 123.33, 123.30, 116.52, 116.30, 75.66, 71.40, 51.63, 47.76, 46.51, 46.41, 41.56, 35.90, 35.85, 35.36, 34.76, 34.11, 33.21, 31.97, 31.09, 30.84, 28.92, 27.57, 27.00, 26.37, 26.19, 23.85, 23.16, 17.30, 12.80; ESI-MS *m/z* (%): 1579 ([2M+Na]$^+$, 100). Anal. calcd for $C_{42}H_{52}ClFN_4O_5S$: C, 64.72; H, 6.72; N, 7.19; found: C, 64.81; H, 6.69; N, 7.17%.

Methyl 3α-(2-(4-(4-bromobenzylideneamino)-3-(4-fluorophenyl)-5-thioxo-4,5-dihydro-1,2,4-triazol-1-yl)acetoxy)-12α-hydroxy-cholanate (**8g**): White solid; yield 87%; m.p. 90–91 °C; $[\alpha]_D^{20}$ –189.2 (*c* 0.15, CH_2Cl_2); IR (KBr)(cm^{-1}): 3441, 2938, 2867, 1733, 1608, 1589, 1475, 1438, 1295, 1159, 1097, 841; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.88 (s, 1H, =CH), 7.93–7.89 (m, 4H, ArH), 7.83 (d, *J*=8.0 Hz, 2H, ArH), 7.36 (t, *J*=8.8 Hz, 2H, ArH), 4.58–4.53 (m, 1H, 3*β*-H), 4.24 (s, 1H, 12*β*-H), 4.02 (s, 2H, $-OCOCH_2N$), 3.78 (s, 1H, 12*α*-OH), 3.57 (s, 3H, $-CO_2CH_3$), 0.93 (d, *J*=6.0 Hz, 3H, 21-CH_3), 0.83 (s, 3H, 19-CH_3), 0.58 (s, 3H, 18-CH_3); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 174.22, 167.71, 167.44, 164.66, 150.82, 146.48, 132.97, 131.35, 131.09, 130.64, 130.56, 127.70, 123.24, 116.47, 116.25, 75.56, 71.39, 51.62, 47.85, 46.52, 46.40, 41.54, 35.87, 35.88, 35.32, 34.75, 34.06, 33.23, 31.93, 31.09, 30.81, 28.95, 27.56, 26.97, 26.35, 26.11, 23.82, 23.15, 17.31, 12.79; ESI-MS *m/z* (%): 847 ([M+Na]$^+$, 100). Anal. calcd for $C_{42}H_{52}BrFN_4O_5S$: C, 61.23; H, 6.36; N, 6.80; found: C, 61.10; H, 6.38; N, 6.78%.

Methyl 3α-(2-(4-(2-bromobenzylideneamino)-3-(4-fluorophenyl)-5-thioxo-4,5-dihydro-1,2,4-triazol-1-yl)acetoxy)-12α-hydroxy-cholanate (**8h**): White solid; yield 90%; m.p. 82–83 °C; $[\alpha]_D^{20}$ –169.5 (*c* 0.18, CH_2Cl_2); IR (KBr)(cm^{-1}): 3450, 2937, 2866, 1734, 1608, 1560, 1476, 1437, 1295, 1159, 1092, 841, 756; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 9.02 (s, 1H, =CH), 8.12–8.10 (m, 1H, ArH), 7.92–7.88 (m, 2H, ArH), 7.84–7.82 (m, 1H, ArH), 7.60–7.58 (m, 2H, ArH), 7.40 (t, *J*=8.8 Hz, 2H, ArH) 4.61–4.59 (m, 1H, 3*β*-H), 4.22 (s, 1H, 12*β*-H), 4.08 (s, 2H, $-OCOCH_2N$), 3.77 (s, 1H, 12*α*-OH), 3.57 (s, 3H, $-CO_2CH_3$), 0.93 (d, *J*=6.4 Hz, 3H, 21-CH_3), 0.83 (s, 3H, 19-CH_3), 0.58 (s, 3H, 18-CH_3); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 174.22, 167.80, 164.75, 151.16, 146.41, 135.18, 134.17, 131.05, 130.96, 130.89, 129.12, 129.05, 126.05, 123.33, 123.30, 116.43, 116.31, 75.67, 71.40, 51.62, 47.76, 46.51, 46.41, 41.57, 35.90, 35.81, 35.36, 34.77, 34.11, 33.21, 31.99, 31.09, 30.84, 28.92, 27.57, 27.01, 26.37, 26.20, 23.85, 23.15, 17.29, 12.79; ESI-MS *m/z* (%): 823 ([M+1]$^+$, 100). Anal. calcd for $C_{42}H_{52}BrFN_4O_5S$: C, 61.23; H, 6.36; N, 6.80; found: C, 61.31; H, 6.33; N, 6.82%.

Methyl 3α-(2-(4-(4-methoxybenzylideneamino)-3-(4-fluorophenyl)-5-thioxo-4,5-dihydro-1,2,4-triazol-1-yl)acetoxy)-12α-hydroxy-cholanate (**8i**): White solid; yield 86%; m.p. 91–92 °C; $[\alpha]_D^{20}$ –140.7 (*c* 0.10, CH_2Cl_2); IR (KBr)(cm^{-1}): 3442, 2937, 2867, 1734, 1602, 1568, 1513, 1438, 1296, 1169, 1093, 839; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.76 (s, 1H, =CH), 7.93–7.90 (m, 4H, ArH), 7.36 (t, *J*=7.8 Hz, 2H, ArH), 7.15 (d, *J*=9.2 Hz, 2H, ArH), 4.57 (s, 1H, 3*β*-H), 4.24 (s, 1H, 12*β*-H), 4.00 (s, 2H, $-OCOCH_2N$), 3.86 (s, 3H, $-OCH_3$), 3.78 (s, 1H, 12*α*-OH), 3.57 (s, 3H, $-CO_2CH_3$), 0.92 (d, *J*=6.4 Hz, 3H, 21-CH_3), 0.83 (s, 3H, 19-CH_3), 0.58 (s, 3H, 18-CH_3); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 174.22, 168.59, 167.81, 164.56, 163.91, 150.65, 146.51, 131.74, 130.48, 130.39, 124.35, 123.39, 116.44, 116.22, 115.32, 75.59, 71.40, 56.04, 51.61, 47.80, 46.54, 46.41, 41.56, 35.90, 34.71, 35.36, 34.77, 34.10, 33.23, 31.94, 31.11, 30.84, 28.95, 27.57, 27.00, 26.37, 26.17, 23.83, 23.15, 17.29, 12.78; ESI-MS *m/z* (%): 1571 ([2M+Na]$^+$, 100). Anal. calcd for $C_{43}H_{55}FN_4O_6S$: C, 66.64; H, 7.15; N, 7.23; found: C, 66.52; H, 7.12; N, 7.25%.

Methyl 3α-(2-(4-(4-nitrobenzylideneamino)-3-(4-fluorophenyl)-5-thioxo-4,5-dihydro-1,2,4-triazol-1-yl)acetoxy)-12α-hydroxy-cholanate (**8j**): Yellow solid; yield 92%; m.p. 101–102 °C; $[\alpha]_D^{20}$ –133.8 (*c* 0.16, CH_2Cl_2); IR (KBr)(cm^{-1}): 3436, 2939, 2867, 1733, 1608, 1596, 1526, 1438, 1346, 1295, 1159, 1095, 845; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 9.05 (s, 1H, =CH), 8.45 (d, *J*=8.4 Hz, 2H, ArH), 8.22 (d, *J*=8.4 Hz, 2H, ArH), 7.95–7.90 (m, 2H, ArH), 7.37 (t, *J*=8.8 Hz, 2H, ArH), 4.59–4.51 (m, 1H, 3*β*-H), 4.26 (s, 1H, 12*β*-H), 4.04 (s, 2H, $-OCOCH_2N$), 3.79 (s, 1H, 12*α*-OH), 3.57 (s, 3H, $-CO_2CH_3$), 0.93 (d, *J*=6.4 Hz, 3H, 21-CH_3), 0.82 (s, 3H, 19-CH_3), 0.58 (s, 3H, 18-CH_3); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 174.22, 167.65, 165.73, 164.75, 151.06, 150.31, 146.59, 137.57, 130.82, 130.75, 130.73, 124.91, 123.14, 123.11, 116.52, 116.31, 75.53, 71.38, 51.62, 47.90, 46.59, 46.40, 41.52, 36.38, 35.86, 35.32, 34.72, 34.06, 33.23, 31.94, 31.08, 30.81, 28.96, 27.53, 26.97, 26.36, 26.09, 23.79, 23.16, 17.32, 12.81; ESI-MS *m/z* (%): 812 ([M+Na]$^+$, 100). Anal. calcd for $C_{42}H_{52}FN_5O_7S$: C, 63.86; H, 6.63; N, 8.87; found: C, 63.75; H, 6.65; N, 8.89%.

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