Synthesis using microwave irradiation, characterisation and antibacterial activity of novel deoxycholic acid-triazole conjugates

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Novel deoxycholic acid 3α-triazole conjugates based on methyl 3α-chloroacetoxy-12α-hydroxy-cholanate have been synthesised. The synthesis is accelerated by microwave irradiation under solvent free conditions in the presence of K_2CO_3. Some of these compounds were tested for antibacterial activity against *B.subtilis*, *P.aeruginosa* and *S.aureus.* The preliminary results indicated that these deoxycholic acid-triazole conjugates have good inhibitory effect against *B.subtilis*. All of the compounds were characterised by ^{1}H NMR, IR, ESI-MS spectra and elemental analyses.

Keywords: deoxycholic acid, triazole, microwave irradiation, antibacterial activity

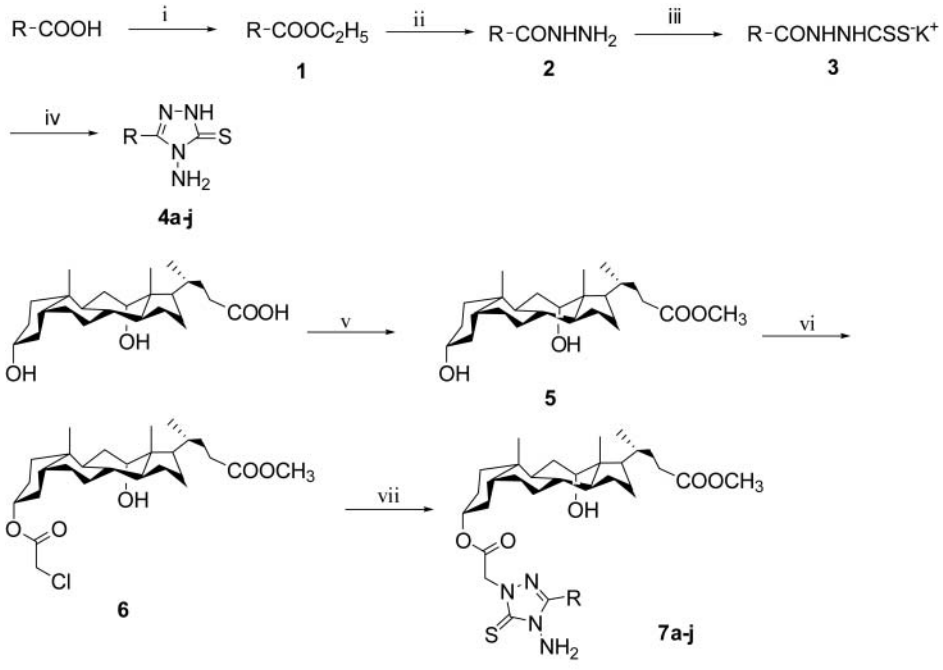
Bile acids are pharmacologically interesting as potential carriers of liver-specific drugs, absorption enhancers, and cholesterol lowering agents.[1] Moreover, owing to their own facially amphiphilic structures, it is possible to utilise bile acids as building blocks for other more complex amphiphilic molecules.[2] A variety of bile acids derivatives with anti-tumour, anti-microbial, anti-HCV, anti-HIV, carbonic anhydrase inhibitors and glucocorticoid receptor antagonists have been reported.[3–8] However, the common feature of these compounds are that the side chains of the bile acids are changed, whereas the hydroxyl groups are preserved. To the best of our knowledge, little work has been published on the synthesis and physicochemical properties of compounds in which the hydroxyl groups are replaced by heterocyclic compounds containing hydrophilic groups.

Azoles are the largest class of antibacterial agents in clinical use.[9] 1,2,4-Triazole moieties are attractive connecting units, and synthetic molecules containing 1,2,4-triazole units show diverse biological activities such as anti-bacterial, anti-proliferative, anti-cancer, analgesic and anti-oxidant agents.[10–14]

In recent years, microwave-assisted organic synthesis has been applied to a large and expanding range of chemical transformations, and this method of energy transfer to a reaction mixture can provide impressive enhancements in product yield, selectivity, and reaction rate.[15,16] In view of this, we now report a simple, efficient and rapid method for the synthesis of deoxycholic acid-triazole conjugates under microwave irradiation. The synthetic route is depicted in Scheme 1.

Result and discussion

In the ^{1}H NMR (DMSO-d_6, 400 MHz) spectrum of compounds **7a–j** the protons of the ArH appeared at 8.12–7.06 ppm. A single peak in the region 6.61–5.72 ppm attributed to the NH_2 group. The 3β-H and 12β-H appeared in the region 4.70–4.64 ppm (multiplet) and 4.28–4.24 ppm, respectively. The 12α-OH appeared at 3.78 ppm (singlet). The resonance



R = C_6H_5 (**a**), 3-$CH_3C_6H_4$ (**b**), 4-$CH_3C_6H_4$ (**c**), 2-$CH_3OC_6H_4$ (**d**), 4-$CH_3OC_6H_4$ (**e**),

2-$C_2H_5OC_6H_4$ (**f**), 3-ClC_6H_4 (**g**), 4-ClC_6H_4 (**h**), 4-BrC_6H_4 (**i**), 1-naphthalene (**j**)

Scheme 1 Reagents and conditions: (i) $SOCl_2$, C_2H_5OH, MWI; (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) **4a–j**, K_2CO_3, DMF, MWI.

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corresponding to –$OCOCH_2N$– protons was identified at the region 4.14–4.04 ppm (singlet). Moreover, the three hydrogen singlets in the range 0.92–0.90, 0.87–0.85 and 0.60–0.59 ppm were assigned to the 21-CH_3, 19-CH_3 and 18-CH_3, respectively. The IR spectra of all compounds **7a–j** revealed strong characteristic bands in the region 3361–3310 cm^{-1}, 3286–3170 cm^{-1} and 1732–1729 cm^{-1} which were assigned to the 12α-OH NH_2 and –$COOCH_3$, respectively. The strong bands at 1631–1572 cm^{-1} corresponded with the absorption of C=N. The most characteristic peaks were the bands at 1384–1379 cm^{-1} and 1094–1040 cm^{-1} assigned to the C=S. In addition, they gave satisfactory elemental analyses and mass spectra. Compounds **7a–j** showed molecular ion peak at $[2M+H]^+$ (or $[2M+Na]^+$).

In conclusion, all of the spectroscopy and elemental analysis data were consistent with the structures of the target compounds.

Comparison of microwave irradiation and conventional heating: In order to identify the best reaction conditions, we examined the synthesis of **7a** with different microwave irradiation power and reaction time.

As shown in Table 1, the reaction was irradiated with different power for the same reaction time (20 min). As a result, 150 W was the optimum power and the use of decreased the yield.

As shown in Table 2, the reaction was irradiated for a different time with the same power (150 W). It was found that 20 min was the best reaction time.

As shown in Table 3, compared to conventional method, microwave method decreased the reaction time from 1560–2880 min to 20–50 min. It was obvious that the yields increased from 45–55% to 93–98%. From these data, we conclude that microwave irradiation method is a simple, efficient and rapid synthetic method.

In vitro *antibacterial activity:* Compounds **7c**, **7d**, **7g**, **7j** were evaluated for their antibacterial activities by the method of plate culture count. The Gram-positive and Gram-negative bacteria which utilised in this study consisted of *B.subtilis*, *P.aeruginosa* and *S.aureus.* In the method of plate culture count, compounds were dissolved in DMSO. In each case, 0.1 mL of test compound and 0.1 mL test bacterial suspension were pipette to provide a uniform coating on the tablet. The bacteria tablets were incubated at 37 °C for 24 h. The results are presented in Table 4, Figs 1,2 and 3. The preliminary results indicated that these deoxycholic acid-triazole conjugates have good inhibitory effects against *B.subtilis*. The details of compounds **7a–j** for their antibacterial activity are under further studies.

Table 1 Effect of microwave powers on yields

Power/W	100	125	150	175	200
Yields/%	–	60	94	85	0

Table 2 Effect of microwave irradiation time yields

Time/min	10	20	30	40	50
Yields/%	80	95	90	88	79

Table 3 Comparison of the synthesis of **7a–j** using microwave and conventional methods

Compd	Conventional method		Microwave method		t_c/t_w[a]
	Time/min	Yields/%	Time/min	Yield%	
7a	1440	50	20	95	72
7b	2040	45	30	93	68
7c	2220	49	25	97	88
7d	2520	55	30	95	84
7e	2640	50	45	93	58
7f	2880	51	50	95	57
7g	2520	46	25	98	100
7h	2760	49	40	95	69
7i	2520	45	45	92	56
7j	1800	55	35	96	51

t_c, conventional method time; t_w, microwave method time

Experimental

Melting points were determined on a micro-melting point apparatus and the thermometer was uncorrected. IR spectra were obtained on 1700 Perkin-Elmer FTIR using KBr disks. ^{1}H NMR spectra were recorded on a Varian INOVA 400 MHz spectrometer using TMS as internal standard. Mass spectra were determined on Finnigan LCQ^{DECA} instrument. Elemental analysis was performed on a Carlo-Erba-1106 autoanalyser. All reactions were performed in a commercial microwave reactor (XH-100A, 100–1000 W, Beijing Xianghu Science and Technology Development Co. Ltd, Beijing, P.R. China). Optical rotations were measured on a Wzz-2B polarimeter. All the solvents were purified before use.

Synthesis of compounds **4a–j**;[17] *typical procedure*

Substituted acid was esterified with ethanol in the presence of $SOCl_2$. Then the ester refluxed with hydrazine hydrate to obtain carbohydrazide. The carbohydrazide on reaction with carbon disulphide in ethanolic potassium hydroxide yielded the corresponding dithiocarbazinate in good yield then used directly for the next step without further purification. Triazole was synthesised by refluxing dithiocarbazinate with hydrazine hydrate. The crude product was then recrystallised from ethanol to give a pure sample. (Table 5)

Synthesis of compound **5**;[18] *general procedure*

Deoxycholic acid (5 mmol) and anhydrous methanol (50 mL) were added to a round-bottomed flask and the mixture was stirred in ice bath. Then pure acetyl chloride was added slowly. The reaction was monitored by TLC. The reaction solution was then poured into ice water, and the required solid was precipitated out. It was filtered, dried under vacuum. The crude product was purified by column chromatography on silica gel using $V(CHCl_3)$: $V(CH_3COCH_3)$: $V(CH_3OH)$ = 70:20:1 as elution agent to give 1.82 g. White solid, yield 90%, m.p. 79–80 ºC (lit.[18] m.p. 74–76 ºC).

Synthesis of compound **6**;[19] *general procedure*

Compound **5** (1 mmol) and chloroacetyl chloride (1.2 mmol) were dissolved in dry chloroform (20 mL). A solution of anhydrous pyridine (1mmol) in dry chloroform (10 mL) was added slowly. Then, the mixture was refluxed in the microwave reactor (TLC). The mixture was evaporated to dryness under vacuum, and the residue was dissolved in $CH_3COOC_2H_5$, The solution was washed with $NaHCO_3$, brine, dried over Na_2SO_4 and evaporated to dryness under vacuum. The impure product was then purified by flash chromatography (elution with $CH_3COOC_2H_5$: petroleum ether = 1:5) to give 0.44g. White solid, yield 92%; m.p. 130–132 ºC (lit.[19] 134–135 ºC).

Synthesis of compounds **7a–j**; *microwave method*

Triazole (0.5 mmol), methyl 3α-chloroacetoxy-12α-hydroxy-cholanoate (0.5 mmol) and anhydrous K_2CO_3 (1.0 g) were placed in a porcelain mortar. After grinding, dry DMF (the amount of catalyst) was added and the mixture was placed in the microwave reactor. Then it was irradiated for 20–50 min at 150 W (TLC). The mixture was cooled to room temperature and dissolved in $CH_3COOC_2H_5$. It was filtered and evaporated to dryness under vacuum. The impure product was then purified by flash chromatography (elution with $CH_3COOC_2H_5$: petroleum ether = 4:1–8:1) to give **7a–j** (92–98%).

Methyl 3α-(2-(4-amino-3-phenyl-5-thioxo-4,5-dihydro-1,2,4-triazol-1-yl)acetoxy)-12α-hydroxy-cholanate (**7a**): White solid, yield 95%, m.p. 91–93 ºC; $[\alpha]_D^{20}$–71.7 (*c* 0.19, CH_2Cl_2); IR (cm^{-1}): 3339, 3191, 2939, 2867,1730, 1631, 1456, 1379, 1296, 1172, 1042, 772, 695; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.96 (d, *J* = 2.4 Hz, 2H, ArH), 7.51 (s, 3H, ArH), 6.21 (s, 2H, NH_2), 4.67 (bs, 1H, 3β-H), 4.24 (s, 1H, 12β-H), 4.07 (s, 2H, –$OCOCH_2N$), 3.78 (s, 1H, 12α-OH), 3.57 (s, 3H, –$COOCH_3$), 0.91 (d, *J* = 6.4 Hz, 3H, 21-CH_3), 0.87 (s, 3H, 19-CH_3),

Table 4 The antibacterial rate of compounds **7c**, **7d**, **7g** and **7j** against *B. subtilis*, *P. aeruginosa* and *S. aureus* (%)

		512 μg mL^{-1}	256 μg mL^{-1}	128 μg mL^{-1}	64 μg mL^{-1}	32 μg mL^{-1}
7c	*B.subtilis*	89.6	88.3	59.7	49.3	44.1
	P.aeruginosa	21.5	26.7	6.1	28.6	23.9
	S.aureus	50.8	4.8	–	–	–
7d	*B.subtilis*	83.1	77.9	72.7	53.2	36.3
	P.aeruginosa	34.7	16.9	31.9	18.3	31.4
	S.aureus	22.6	16.1	–	–	–
7g	*B.subtilis*	66.2	61.0	32.4	6.4	–
	P.aeruginosa	7.9	31.4	23.4	20.1	2.3
	S.aureus	–	–	–	–	–
7j	*B.subtilis*	74.0	70.1	64.9	44.1	–
	P.aeruginosa	35.6	–	22.0	19.2	22.5
	S.aureus	–	–	–	–	–
Amoxicillin	*B.subtilis*	100.0	100.0	100.0	100.0	100.0
	P.aeruginosa	100.0	100.0	100.0	100.0	100.0
	S.aureus	100.0	95.5	88.8	75.5	37.7

–No antibacterial activity.

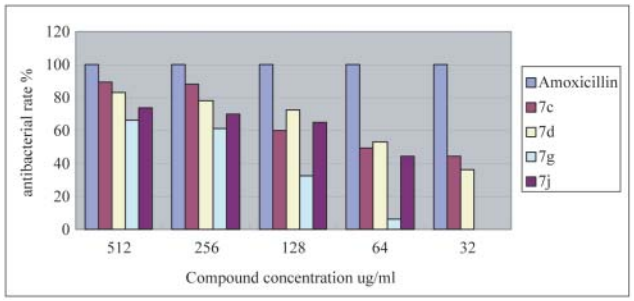

Fig. 1 Comparison of compounds **7c**, **7d**, **7g**, and **7j** against *B.subtilis* antibacterial rate.

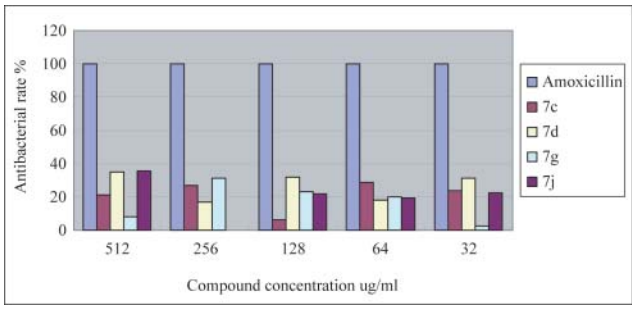

Fig. 2 Comparison of compounds **7c**, **7d**, **7g** and **7j** against *P.aeruginosa* antibacterial rate.

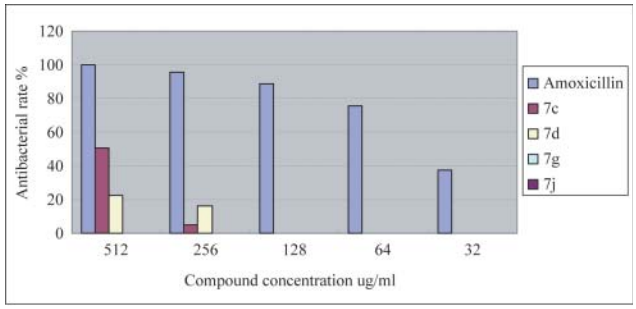

Fig. 3 Comparison of compounds **7c**, **7d**, **7g** and **7j** against *S.aureus* antibacterial rate.

0.59 (s, 3H, 18-CH_3); ESI-MS *m/z* (%): 1277 ([2M+H]$^+$, 100). Anal. Calcd for $C_{35}H_{50}N_4O_5S$: C, 65.80; H, 7.89; N, 8.77. Found: C, 66.06; H, 7.86; N, 8.80%.

Methyl 3α-(2-(4-amino-3-(3-methylphenyl)-5-thioxo-4,5-dihydro-1,2,4-triazol-1-yl)-acetoxy)-12α-hydroxy-cholanate (**7b**): White solid, yield 93%, m.p. 171–173 ºC; $[\alpha]_D^{20}$ –129.8 (*c* 0.14, CH_2Cl_2); IR (cm^{-1}):

Table 5 The melting point of triazole **4a–j**

Compd	Formula	M.p./ °C	Lit. m.p./ °C
4a	$C_8H_8N_4S$	202–204	203–204[20]
4b	$C_9H_{10}N_4S$	203–205	206–208[21]
4c	$C_9H_{10}N_4S$	201–203	201[21]
4d	$C_9H_{10}N_4OS$	211–212	216–219[21]
4e	$C_9H_{10}N_4OS$	206–207	208[21]
4f	$C_{10}H_{12}N_4OS$	149–150	153–154[22]
4g	$C_8H_7N_4ClS$	213–214	215–217[21]
4h	$C_8H_7N_4ClS$	214–216	210–212[21]
4i	$C_8H_7N_4BrS$	208–210	205–206[21]
4j	$C_{12}H_{10}N_4S$	204–205	206[23]

3340, 3187, 2937, 2867, 1731, 1613, 1452, 1382, 1296, 1185, 1094, 978, 793, 714; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.77 (t, J = 6.4 Hz, 2H, ArH), 7.40 (t, J = 7.2 Hz, 1H, ArH), 7.31 (d, J = 7.6 Hz, 1H, ArH), 6.19 (s, 2H, NH_2), 4.67–4.64 (m, 1H, 3β-H), 4.27 (s, 1H, 12β-H), 4.06 (s, 2H, –OCOCH_2N), 3.78 (s, 1H, 12α-OH), 3.57 (s, 3H, –COOCH_3), 2.38 (s, 3H, Ar-CH_3), 0.91 (d, J = 6.0 Hz, 3H, 21-CH_3), 0.87 (s, 3H, 19-CH_3), 0.59 (s, 3H, 18-CH_3). ESI-MS *m/z* (%): 1305 ([2M+H]$^+$, 100). Anal. Calcd for $C_{36}H_{52}N_4O_5S$: C, 66.23; H, 8.03; N, 8.58. Found: C, 65.93; H, 8.07; N, 8.55%.

Methyl 3α-(2-(4-amino-3-(4-methylphenyl)-5-thioxo-4,5-dihydro-1,2,4-triazol-1-yl)-acetoxy)-12α-hydroxy-cholanate (**7c**): White solid, yield 97%, m.p. 104–106 ºC; $[\alpha]_D^{20}$–80.2 (*c* 0.17, CH_2Cl_2); IR (cm^{-1}): 3343, 3195, 2939, 2868, 1730, 1623, 1452, 1380, 1297, 1173, 1045, 983, 823, 726; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.86 (d, J = 8.0 Hz, 2H, ArH), 7.32 (d, J = 8.0 Hz, 2H, ArH), 6.17 (s, 2H, NH_2), 4.66–4.64 (m, 1H, 3β-H), 4.26 (s, 1H, 12β-H), 4.05 (s, 2H, –OCOCH_2N), 3.77 (s, 1H, 12α-OH), 3.57 (s, 3H, –COOCH_3), 2.37 (s, 3H, Ar-CH_3), 0.90 (d, J = 6.0 Hz, 3H, 21-CH_3), 0.86 (s, 3H, 19-CH_3), 0.59 (s, 3H, 18-CH_3); ESI-MS *m/z* (%): 1305 ([2M+H]$^+$, 100). Anal. Calcd for $C_{36}H_{52}N_4O_5S$: C, 66.23; H, 8.03; N, 8.58. Found: C, 66.36; H, 8.06; N, 8.61%.

Methyl 3α-(2-(4-amino-3-(2-methoxyphenyl)-5-thioxo-4,5-dihydro-1,2,4-triazol-1-yl)-acetoxy)-12α-hydroxy-cholanate (**7d**): White solid, yield 95%, m.p. 102–104 ºC; $[\alpha]_D^{20}$ –139.8 (*c* 0.13, CH_2Cl_2); IR (cm^{-1}): 3361, 3286, 2939, 2867, 1732, 1610, 1529, 1471, 1379, 1251, 1169, 1044, 985, 758; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.54 (t, J = 8.0 Hz, 1H, ArH), 7.41 (d, J = 7.2 Hz, 1H, ArH), 7.19 (d, J = 8.4 Hz, 1H, ArH), 7.08 (t, J = 7.2 Hz, 1H, ArH), 5.72 (s, 2H, NH_2), 4.70–4.64 (m, 1H, 3β-H), 4.28 (s, 1H, 12β-H), 4.06 (s, 2H, –OCOCH_2N), 3.82 (s, 3H, Ar–OCH3), 3.77 (s, 1H, 12α-OH), 3.57 (s, 3H, –COOCH_3), 0.91 (d, J = 6.0 Hz, 3H, 21-CH_3), 0.87 (s, 3H, 19-CH_3), 0.59 (s, 3H, 18-CH_3); ESI-MS *m/z* (%): 1337 ([2M+H]$^+$, 100). Anal. Calcd for $C_{36}H_{52}N_4O_6S$: C, 64.64; H, 7.84; N, 8.38. Found: C, 64.49; H, 7.88; N, 8.35%.

Methyl 3α-(2-(4-amino-3-(4-methoxyphenyl)-5-thioxo-4,5-dihydro-1,2,4-triazol-1-yl)-acetoxy)-12α-hydroxy-cholanate (**7e**): White solid, yield 93%, m.p. 175–177 ºC; $[\alpha]_D^{20}$ –53.4 (*c* 0.17, CH_2Cl_2); IR (cm^{-1}): 3310, 3170, 2940, 2868, 1732, 1611, 1464, 1381, 1299, 1177, 1033, 986, 832; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.91 (d, J = 8.4 Hz, 2H, ArH), 7.06 (d, J = 8.8 Hz, 2H, ArH), 6.61 (s, 2H, NH_2), 4.69–4.63 (m,

1H, 3*β*-H), 4.26 (s, 1H, 12*β*-H), 4.04 (s, 2H, –OCOCH$_2$N), 3.81 (s, 3H, Ar–OCH3), 3.78 (s, 1H, 12*α*-OH), 3.57 (s, 3H, –COOCH$_3$), 0.90 (d, *J* = 6.0 Hz, 3H, 21-CH$_3$), 0.86 (s, 3H, 19-CH$_3$), 0.59 (s, 3H, 18-CH$_3$); ESI-MS *m/z* (%): 1337 ([2M+H]$^+$, 100). Anal. Calcd for $C_{36}H_{52}N_4O_6S$: C, 64.64; H, 7.84; N, 8.38. Found: C, 64.85; H, 7.80; N, 8.40%.

Methyl 3α-(2-(4-amino-3-(2-ethoxyphenyl)-5-thioxo-4,5-dihydro-1,2,4-triazol-1-yl)-acetoxy)-12α-hydroxy-cholanate (**7f**): White solid, yield 95%, m.p. 101–103 ºC; $[\alpha]_D^{20}$ –85.2 (*c* 0.16, CH_2Cl_2); IR (cm^{-1}): 3358, 3286, 2938, 2867, 1733, 1609, 1466, 1380, 1247, 1167, 1040, 986, 756; ^{1}H NMR (DMSO-*d*$_6$, 400 MHz): *δ* 7.52 (t, *J* = 7.4 Hz, 1H, ArH), 7.43 (d, *J* = 7.2 Hz, 1H, ArH), 7.18 (d, *J* = 8.4 Hz, 1H, ArH), 7.08 (t, *J* = 7.4 Hz, 1H, ArH), 5.72 (s, 2H, NH$_2$), 4.69–4.64 (m, 1H, 3*β*-H), 4.28 (s, 1H, 12*β*-H), 4.19–4.10 (m, 2H, Ar–OCH$_2$CH$_3$), 4.06 (s, 2H, –OCOCH$_2$N), 3.78 (s, 1H, 12*α*-OH), 3.57 (s, 3H, –COOCH$_3$), 1.28 (t, *J* = 7.0 Hz, 3H, Ar–OCH$_2$CH$_3$), 0.91 (d, *J* = 6.0 Hz, 3H, 21-CH$_3$), 0.87 (s, 3H, 19-CH$_3$), 0.59 (s, 3H, 18-CH$_3$); ESI-MS *m/z* (%): 1365 ([2M+H]$^+$, 100). Anal. Calcd for $C_{37}H_{54}N_4O_6S$: C, 65.07; H, 7.97; N, 8.20. Found: C, 64.74; H, 8.01; N, 8.23%.

Methyl 3α-(2-(4-amino-3-(3-chlorophenyl)-5-thioxo-4,5-dihydro-1,2,4-triazol-1-yl-)acetoxy)-12α-hydroxy-cholanate (**7g**): White solid, yield 98%, m.p. 165–167 ºC; $[\alpha]_D^{20}$–107.1 (*c* 0.14, CH_2Cl_2); IR (cm^1): 3341, 3260, 2937, 2867, 1731, 1572, 1450, 1384, 1299, 1189, 1094, 977, 889; ^{1}H NMR (DMSO-*d*$_6$, 400 MHz): *δ* 8.07 (s, 1H, ArH), 7.94 (dd, *J* = 1.6 Hz and *J* =1.6 Hz, 1H, ArH), 7.59–7.53 (m, 2H, ArH), 6.25 (s, 2H, NH$_2$), 4.69–4.64 (m, 1H, 3*β*-H), 4.25 (s, 1H, 12*β*-H), 4.07 (s, 2H, –OCOCH$_2$N), 3.78 (s, 1H, 12*α*-OH), 3.57 (s, 3H, –COOCH$_3$), 0.90 (d, *J* = 6.4 Hz, 3H, 21-CH$_3$), 0.86 (s, 3H, 19-CH$_3$), 0.59 (s, 3H, 18-CH$_3$); ESI-MS *m/z* (%): 1367 ([2M+H]$^+$, 100). Anal. Calcd for $C_{35}H_{49}ClN_4O_5S$: C, 62.43; H, 7.34; N, 8.32. Found: C, 62.72; H, 7.38; N, 8.29%.

Methyl 3α-(2-(4-amino-3-(4-chlorophenyl)-5-thioxo-4,5-dihydro-1,2,4-triazol-1-yl)-acetoxy)-12α-hydroxy-cholanate (**7h**): White solid, yield 95%, m.p. 160–162 ºC; $[\alpha]_D^{20}$–64.9 (*c* 0.13, CH_2Cl_2); IR (cm^{-1}): 3324, 3196, 2936, 2869, 1732, 1632, 1462, 1383, 1296, 1186, 1093, 998, 885, 836; ^{1}H NMR (DMSO-*d*$_6$, 400 MHz): *δ* 8.01 (d, J = 8.4 Hz, 2H, ArH), 7.60 (d, *J* = 8.4 Hz 2H, ArH), 6.23 (s, 2H, NH$_2$), 4.66–4.64 (m, 1H, 3*β*-H), 4.26 (s, 1H, 12*β*-H), 4.07 (s, 2H, –OCOCH$_2$N), 3.87 (s, 1H, 12*α*-OH), 3.57 (s, 3H, –COOCH$_3$), 0.90 (d, *J* = 6.0 Hz, 3H, 21-CH$_3$), 0.86 (s, 3H, 19-CH$_3$), 0.59 (s, 3H, 18-CH$_3$); ESI-MS *m/z* (%): 1367 ([2M+H]$^+$, 100). Anal. Calcd for $C_{35}H_{49}ClN_4O_5S$: C, 62.43; H, 7.34; N, 8.32. Found: C, 62.61; H, 7.30; N, 8.35%.

Methyl 3α-(2-(4-amino-3-(4-bromophenyl)-5-thioxo-4,5-dihydro-1,2,4-triazol-1-yl)-acetoxy)-12α-hydroxy-cholanate (**7i**): White solid, yield 92%, m.p. 99–101 ºC; $[\alpha]_D^{20}$–129.8 (*c* 0.14, CH_2Cl_2); IR (cm^{-1}): 3336, 3195, 2927, 2865, 1729, 1630, 1453, 1380, 1297, 1171, 980, 831, 724; ^{1}H NMR (DMSO-*d*$_6$, 400 MHz): *δ* 7.96 (d, J = 8.4 Hz, 2H, ArH), 7.74 (d, *J* = 8.4 Hz, 2H, ArH), 6.23 (s, 2H, NH$_2$), 4.70–4.65 (m, 1H, 3*β*-H), 4.26 (s, 1H, 12*β*-H), 4.07 (s, 2H, –OCOCH$_2$N), 3.78 (s, 1H, 12*α*-OH), 3.58 (s, 3H, –COOCH$_3$), 0.92 (d, *J* = 6.4 Hz, 3H, 21-CH$_3$), 0.87 (s, 3H, 19-CH$_3$), 0.60 (s, 3H, 18-CH$_3$); ESI-MS *m/z* (%): 1459 ([2M+Na]$^+$, 100). Anal. Calcd for $C_{35}H_{49}BrN_4O_5S$: C, 58.57; H, 6.88; N, 7.81. Found: C, 58.83; H, 6.85; N, 7.85%.

Methyl 3α-(2-(4-amino-(3-naphthalen-1-yl)-5-thioxo-4,5-dihydro-1,2,4-triazol-1-yl)-acetoxy)-12α-hydroxy-cholanate (**7j**): White solid, yield 96%, m.p. 129–114 ºC; $[\alpha]_D^{20}$–75.7 (*c* 0.18, CH_2Cl_2); IR (cm^{-1}): 3348, 3187, 2939, 2867, 1731, 1630, 1448, 13790, 1295, 1093, 984, 805, 777; ^{1}H NMR (DMSO-*d*$_6$, 400 MHz): *δ* 8.12 (d, *J* = 8.4 Hz, 1H, ArH), 8.03 (t, *J* = 8.4 Hz, 2H, ArH), 7.84 (d, *J* = 7.2 Hz, 1H, ArH), 7.66 (t, *J* = 7.6 Hz, 1H, ArH), 7.62–7.55 (m, 2H, ArH), 6.03 (s, 2H, NH$_2$), 4.75–4.69 (m, 1H, 3*β*-H), 4.28 (s, 1H, 12*β*-H), 4.14 (s, 2H, –OCOCH$_2$N), 3.79 (s, 1H, 12*α*-OH), 3.58 (s, 3H, –COOCH$_3$), 0.92 (d, *J* = 6.0 Hz, 3H, 21-CH$_3$), 0.85 (s, 3H, 19-CH$_3$), 0.60 (s, 3H, 18-CH$_3$); ESI-MS *m/z* (%): 1377 ([2M+H]$^+$, 100). Anal. Calcd for $C_{39}H_{52}N_4O_5S$: C, 67.99; H, 7.61; N, 8.13. Found: C, 67.65; H, 7.63; N, 8.17%.

Synthesis of compounds **7a–j**; *general procedure*

In a typical procedure, the triazole (0.5 mmol) and anhydrous K_2CO_3 (0.5 mmol) in dry DMF (5 mL) was stirred at room temperature for 2 h. Next, methyl 3*α*-chloroacetoxy-12α-hydroxy-cholanoate (0.5 mmol) was added and the mixture was stirred for 24–48 h at room temperature (TLC). Then, it was poured into crushed ice and extracted with $CH_3COOC_2H_5$. The extract was washed with H_2O, brine, and dried over Na_2SO_4. The solvent was evaporated under reduce pressure to afford the impure product. The impure product was then purified by flash chromatography (elution with $CH_3COOC_2H_5$: petroleum ether = 4:1–8:1) to give **7a–j** (45–55%).

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