Microwave-assisted synthesis of new steroidal thiosemicarbazones derived from methyl 3-oxocholanate under solvent-free conditions

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A series of novel steroidal thiosemicarbazones derived from methyl 3-oxocholanate were synthesised in good yields via microwave irradiation under solvent-free conditions. The structures of the compounds were confirmed by spectroscopic data. Compared to the conventional method, microwave irradiation was a fast and simple method. These compounds were tested for antibacterial activity against *S. aureus*, *S. pyogenes*, and *E. coli* bacteria.

Keywords: steroidal thiosemicarbazones, microwave irradiation, solvent-free conditions

Thiosemicarbazones are important classes of compounds which have attracted attention, owing to their remarkable biological and pharmacological properties.[1–3] The antibacterial properties of thiosemicarbazones have been studied since 1946, when Domagk et al. reported their activity against *M. tuberculosis*.[4] The biological activity of thiosemicarbazones were dependent on the substituents. Minor modifications in the thiosemicarbazones can lead to significant change in biological activity.[5] Therefore, thiosemicarbazones are considered to be important scaffolds and are embedded in many biologically active compounds.[6]

Microwave irradiation, a non-conventional energy source, has emerged as a powerful technique for promoting a variety of chemical reactions and has become a useful technology in organic chemistry.[7,8] The combination of solvent-free conditions and microwave irradiation considerably reduces reaction time, enhances conversions as well as selectivity and has environmental advantages. These constitute an eco-friendly green approach.[9,10]

In 2007, Salman *et al.*[11] synthesised a series steroidal thiosemicarbazones from the reaction of cholest-5-en-7-one with thiosemicarbazides, and evaluated them for their antibacterial activity against two Gram-positive and two Gram-negative bacteria. The results showed that those compounds inhibit growth of both types of the bacteria. Steroidal thiosemicarbazones have a considerable research potential.[12–14]

Recently, we reported the preparation of indole-based thiosemicarbazones under solvent-free conditions using microwave methods.[15] As a continuation of this work, we have synthesised a series of new steroidal thiosemicarbazones based on methyl 3-oxocholanate by the condensation of the steroidal ketones with the thiosemicarbazides under these green conditions. The synthetic route is shown in Scheme 1.

Results and discussion

Spectroscopic studies

The structures of **7a–j** were confirmed by IR, mass, ^{1}H NMR and elemental analysis. Their mass spectra showed the expected molecular ion peaks with a high intensity. The IR spectra of these compounds exhibited characteristic strong absorption at 3178–3456 cm^{-1} due to N–H stretching vibration; The strong bands in the region 1731and 1747 cm^{-1} indicated the absorption of C=O; The strong absorption bands falling within the range of 1511–1648 cm^{-1} and the range of 1229–1286 cm^{-1} were assigned to the C=N and C=S respectively. In the ^{1}H NMR spectra, the singlets between δ 9.11 and 9.78 ppm were assigned to the protons of the ArNH. A singlet due to the other NH proton was observed at 8.55–8.77 ppm. In addition, the singlet peaks at 0.63–0.68 ppm, 1.00–1.09 ppm and the double peaks at 0.92–0.93 ppm were the characteristic of the steroidal structure. The singlet peaks between 3.67 and 3.69 ppm was assigned to the protons in the $COOCH_3$.

The effect of different oxidants

When we synthesised the intermediate **6** (Scheme 1), we found that PCC was the best oxidant for this reaction. PCC is a mild oxidant and this was a selective oxidation compared to other stronger oxidants such as $KMnO_4$, $K_2Cr_2O_7/H_2SO_4$. In addition, the oxidation reaction was carried out at room temperature.

The comparison of microwave irradiation and conventional heating

In searching for the best conditions, we also carried out a series of experiments, to study microwave irradiation power, time and different supporters. We took the synthesis of **7a** for example and found that the highest yield would be obtained when the time was 4 min at 460W using neutral aluminum oxide as the solid support.

As shown in Table 1, we compared the synthesis of **7a–j** using microwave irradiation under the solvent-free conditions and conventional heating. \this showed that that microwave irradiation greatly decreased the reaction time from 180–300 min to 2–4 min. However, the yields were increased from 60–75% to 88–95%. The use of microwave technology in conjunction with the use of solvent-free conditions allows expeditious and efficient procedures for this synthesis.

In vitro *antibacterial activity*

Compounds **7b, 7e, 7g, 7h** were evaluated for their *in vitro* antibacterial activity against *S. aureus*, *S. pyogenes* and *E. coli* using agar dilution method. The preliminary results seemed promising and these thiosemicarbazones had a good inhibitory effect. Furtherresearch work is underway.

In conclusion, we have synthesised 10 novel streroidal thiosemicarbazones derived from methyl 3-oxocholanate under solvent-free conditions using microwave irradiation. This method was a convenient and rapid procedure compared to the conventional synthesis. In addition, these compounds may have good antibacterial activity and this requires more research. The importance of this work lies in the possibility that these new compounds may be more helpful in designing more potent antibacterial agents for therapeutic use.

Experimental

Melting points were determined on a micro-melting point apparatus and the thermometer was uncorrected. Infrared spectra were obtained on 1700 PerkinElmer FTIR using KBr disks. ^{1}H NMR spectra were recorded on a Varian INOVA 400 MHz spectrometer using $CDCl_3$ as solvent and TMS as internal standard. Mass spectra were determined on FinniganLCQ^{DECA} instrument. Elemental analysis was performed on a Carlo-Erba-1106 auto analyzer. Microwave irradiation was carried out with a MCL-3 microwave oven (very safe) at full power (700 W), which was modified from domestic microwave oven and

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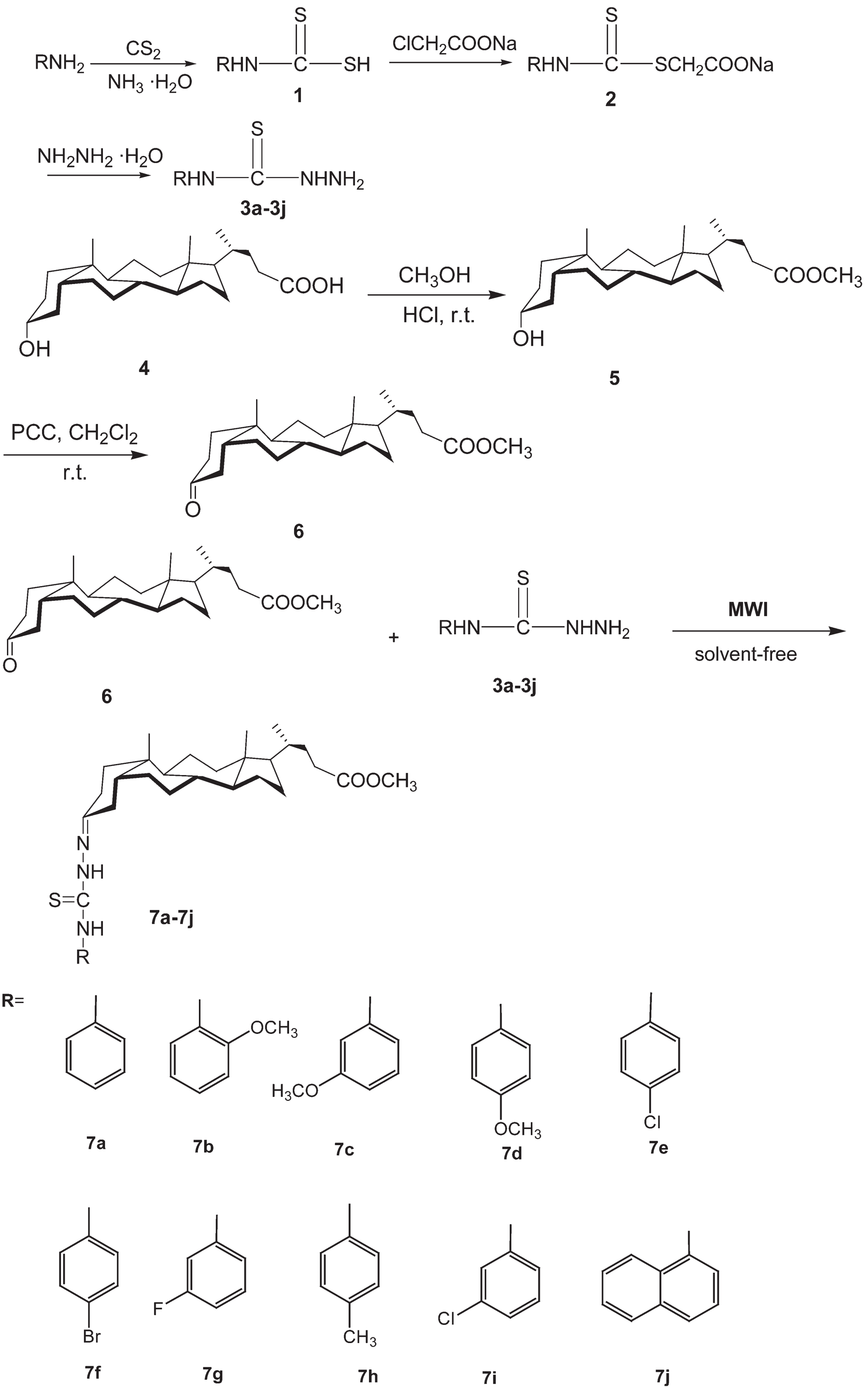

Scheme 1 The synthetic route of the steroidal thiosemicarbazones **7a–j.**

tested to conform to the performance index before use. All the solvents were purified before use.

Preparation of substituted thiosemicarbazides **3a–j***; general procedure*[16]

A solution of substituted amine (0.01 mol) in ethanol (10 mL) and concentrated aqueous ammonia (2 mL) was treated with carbon disulfide dropwise (0.8 mL), and the mixture was stirred at 15–20 °C for 1–2 h to form a solid. Sodium chloroacetate (1.2 g) was added to the stirred mixture and then 85% hydrazine hydrate (1.2 mL) was added and the stirring continued at 60 °C for 4 h to give a solid. The crude product was then recrystallised from ethanol to obtain each thiosemicarbazide in 62–80% yields. The melting points of thiosemicarbazides **3a–j** are shown in Table 2.

Table 1 Synthetic comparison of thiosemicarbazones **7a–j** between solvent-free conditions under microwave irradiation and conventional heating

Comp.	Conventional method		Microwave method		t_C/t_{MW} ^{a}
	t/min	Yield/%	t/min	Yield/%	
7a	300	60	4.0	88	75
7b	180	75	2.0	95	90
7c	240	65	3.0	88	80
7d	240	65	3.0	88	80
7e	240	70	4.0	90	60
7f	240	70	4.0	90	60
7g	300	70	4.0	90	75
7h	240	65	3.0	89	80
7i	300	70	3.0	90	100
7j	300	60	4.0	88	75

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

Table 2 The melting points of thiosemicarbazide **3a–j**

Product	Formula	M.p./°C	Lit M.p./°C
3a	$C_7H_{10}N_3OS$	137–139	136–138[17]
3b	$C_8H_{11}N_3OS$	155–157	159[18]
3c	$C_8H_{11}N_3OS$	157–158	161[19]
3d	$C_8H_{11}N_3OS$	147–149	152[20]
3e	$C_7H_8N_3ClS$	178–180	178[21]
3f	$C_7H_8N_3BrS$	165–168	163–165[21]
3g	$C_7H_8N_3FS$	137–138	138[22]
3h	$C_8H_{11}N_3S$	135–136	137[23]
3i	$C_7H_8N_3ClS$	126–127	125[24]
3j	$C_{11}H_{11}N_3S$	135–137	138–139[18]

*Synthesis of methyl 3α-hydroxy-5β-cholan-24-oate (***5***)*[25]

Lithocholic acid (1g, 2.7mmol), anhydrous methanol (20 mL) and *p*-toluenesulfonic acid (0.1g, 0.52mmol) were added to a round bottom flask and this was stirred at room temperature for 24 h. Then the solvent was evaporated and the residue dissolved with ethyl acetate (30 mL). The solution was washed with $NaHCO_3$ (3 × 20 mL) and saturated brine (3 × 20 mL). After driying over anhydrous sodium sulfate, the solvent was removed under reduced pressure, and the crude product was purified by chromatography on silica gel using (CH_2Cl_2):$(CH_3COOC_2H_5)$ V/V 10:1 to give a white solid, yield 94%, m.p. 126–127 °C, $[\alpha]_D^{20}$ = +34.7 (*c* 0.9, CH_2Cl_2); IR (KBr) (cm^{-1}): 3520, 2981, 2959, 1719, 1101; ^1H NMR (400 MHz, $CDCl_3$) *δ*: 3.63 (s, 3H, $COOCH_3$), 3.63–3.59 (m, 1H, 3β-H), 0.94 (s, 3H, 19-CH_3), 0.90 (d, *J* = 6.4 Hz, 3H, 21-CH_3), 0.64 (s, 3H, 18-CH_3); ESI-MS *m/z* (%): 803 ($[2M+Na]^+$, 100).

*Synthesis of methyl 3α-oxo-5β-cholano-24-oate (***6***)*[26]

Pyridinium chlorochromate (PCC) (0.74g, 0.34 mmol) was added to a solution of compound **5** (0.78g, 0.2 mmol) in dried CH_2Cl_2 (30 mL) at room temperature. The reaction was completed in 12h. The solvent was removed under reduced pressure, and the crude product was purified by chromatography on silica gel using ethyl acetate to give white solid, yield 87%; m.p. 115–117 °C $[\alpha]_D^{20}$ = +190.0 (*c* 0.1, CH_2Cl_2); IR (KBr) (cm^{-1}): 2940, 2860, 1731, 1536, 1180; ^1H NMR (400 MHz, $CDCl_3$) *δ*: 3.67 (s, 3H, $COOCH_3$), 1.02 (s, 3H, 19-CH_3), 0.93 (d, *J* = 6.4 Hz, 3H, 21-CH_3), 0.68 (s, 3H, 18-CH_3); ESI-MS *m/z* (%): 799 ($[2M+Na]^+$, 100).

General procedure for the preparation of thiosemicarbazones ***7a–j***

Conventional method

Steroidal ketones (**6**) (0.1g, 0.258mmol) and substituted thiosemicarbazide (**3**) (0.258 mmol) were dissolved in ethanol (10 mL). After completely dissolving, two drops of concentrated sulfuric acid were added. The mixture was stirred for 3–5 h at room temperature. A solid was separated, which was recrystallised from DMSO and H_2O in 60–75% yields.

Microwave irradiation method

Steroidal ketones (**6**) (0.1g, 0.258mmol), substituted thiosemicarbazide (0.258 mmol) (**3**) and neutral aluminium oxide (0.3 g) were placed in a porcelain mortar, then concentrated sulfuric acid (two drops) was added to it. After grinding, the mixture was put in a round-bottom flask (25 mL) and was kept under microwave oven. Then it was irradiated for 2–4 min at 200–460 W. The reaction mixture was cooled to room temperature and was dissolved in DMSO and filtered. The filtrate was added water and the product was formed. The product was recrystallised from DMSO and H_2O in 88–95% yields. The physical and spectra data of the compounds **7a–j** are as follows.

7a: White crystal, yield 88%, m.p. 126–128 °C, $[\alpha]_D^{20}$ = +141.7 (*c* 0.1, CH_2Cl_2); IR (KBr)(cm^{-1}): 3278, 3178, 2946, 2863, 1731, 1536, 1428, 1262, 1188; ^1H NMR (400 MHz, $CDCl_3$) δ: 9.30 (s, 1H, ArNH), 8.59 (s, 1H, NH), 7.67 (d, 2H, *J* = 8.0 Hz, ArH), 7.40 (t, 2H, *J* = 7.6 Hz, ArH), 7.24–7.20 (m, 1H, ArH), 3.67 (s, 3H, $COOCH_3$), 1.00 (s, 3H, 19-CH_3), 0.93 (d, 3H, *J* = 6.4 Hz, 21-CH_3), 0.68 (s, 3H, 18-CH_3); ESI-MS *m/z* (%): 538 ($[M+1]^+$,100). Ana1.Calcd for $C_{32}H_{47}N_3O_2S$: C, 71.47; H, 8.81; N, 7.81. Found: C, 71.40; H, 8.79; N, 7.82%.

7b: White crystal, yield 95%, m.p. 120–123 °C. $[\alpha]_D^{20}$ = +33.3 (*c* 0.1, CH_2Cl_2); IR (KBr) (cm^{-1}): 3270, 3191, 2929, 1739, 1602, 1544, 1233, 1163, 740; ^1H NMR (400 MHz, $CDCl_3$) δ: 9.78 (s, 1H, ArNH), 8.71 (s, 1H, NH), 8.55 (d, 1H, *J* = 5.2 Hz, ArH), 7.15 (t, 1H, *J* = 7.2 Hz, ArH), 7.02 (t, 1H, *J* = 7.2Hz, ArH), 6.93 (d, 1H, *J* = 8.0 Hz, ArH), 3.89 (s, 3H, OCH_3), 3.67 (s, 3H, $COOCH_3$), 1.00 (s, 3H, 19-CH_3), 0.93 (d, 3H, *J* = 6.4 Hz, 21-CH_3), 0.68 (s, 3H, 18-CH_3); ESI-MS *m/z* (%): 568 ($[M+1]^+$,100). Ana1.Calcd for $C_{33}H_{49}N_3O_3S$: C, 69.80; H, 8.70; N, 7.40. Found: C, 69.73; H, 8.68; N, 7.43%.

7c: White crystal, yield 88%, m.p. 150–153 °C, $[\alpha]_D^{20}$ = +130.0 (*c* 0.1, CH_2Cl_2); IR (KBr) (cm^{-1}): 3286, 3211, 2941, 2863, 1743, 1593, 1539, 1286, 1158, 780; ^1H NMR (400 MHz, $CDCl_3$) δ: 9.30 (s, 1H, ArNH), 8.55 (s, 1H, NH), 7.49 (s, 1H, ArH), 7.28 (t, 1H, *J* = 7.6 Hz, ArH), 7.15 (d, 1H, *J* = 7.6 Hz, ArH), 6.77 (d, 1H, *J* = 8.0 Hz, ArH), 3.83 (s, 3H, OCH_3), 3.67 (s, 3H, $COOCH_3$), 1.00 (s, 3H, 19-CH_3), 0.93 (d, 3H, *J* = 6.4 Hz, 21-CH_3), 0.68 (s, 3H, 18-CH_3); ESI-MS *m/z* (%): 568 ($[M+1]^+$,100). Ana1.Calcd for $C_{33}H_{49}N_3O_3S$: C, 69.80; H, 8.70; N, 7.40. Found: C, 69.72; H, 8.72; N, 7.42%.

7d: White crystal, yield 88%, m.p. 175–177 °C, $[\alpha]_D^{20}$ = +193.0 (*c* 0.1, CH_2Cl_2); IR (KBr)(cm^{-1}): 3434, 3286, 2917, 2842, 1731, 1631, 1528, 1229, 1163, 823; ^1H NMR (400 MHz, $CDCl_3$) δ: 9.11 (s, 1H, ArNH), 8.55 (s, 1H, NH), 7.49 (d, 2H, *J* = 8.4 Hz, ArH), 6.92 (d, 2H, *J* = 8.4 Hz, ArH), 3.82 (s, 3H, OCH_3), 3.67 (s, 3H, $COOCH_3$), 1.00 (s, 3H, 19-CH_3), 0.93 (d, 3H, *J* = 6.4 Hz, 21-CH_3), 0.67 (s, 3H, 18-CH_3); ESI-MS *m/z* (%): 568 ($[M+1]^+$,100). Ana1.Calcd for $C_{33}H_{49}N_3O_3S$: C, 69.80; H, 8.70; N, 7.40. Found: C, 69.76; H, 8.68; N, 7.39%.

7e: White crystal, yield 90%, m.p. 176–179 °C, $[\alpha]_D^{20}$ = +176.7 (*c* 0.1, CH_2Cl_2); IR (KBr)(cm^{-1}): 3444, 3278, 2925, 2855, 1731, 1648, 1511, 1270, 1175, 827; ^1H NMR (400 MHz, $CDCl_3$) δ: 9.27 (s, 1H, ArNH), 8.60 (s, 1H, NH), 7.60 (d, 2H, *J* = 8.4 Hz, ArH), 7.50 (d, 2H, *J* = 8.4 Hz, ArH), 3.67 (s, 3H, $COOCH_3$), 1.09 (s, 3H, 19-CH_3), 0.93 (d, 3H, *J* = 6.4 Hz, 21-CH_3), 0.68 (s, 3H, 18-CH_3); ESI-MS *m/z* (%): 572 ($[M+1]^+$,100). Ana1.Calcd for $C_{32}H_{46}ClN_3O_2S$: C, 67.16; H, 8.10; N, 7.34. Found: C, 67.10; H, 8.05; N, 7.30%.

7f: White crystal, yield 90%, m.p. 185–187 °C, $[\alpha]_D^{20}$ = +111.7 (*c* 0.1, CH_2Cl_2); IR (KBr)(cm^{-1}): 3444, 3278, 2925, 2855, 1731, 1648, 1511, 1270, 1175, 827; ^1H NMR (400 MHz, $CDCl_3$) δ: 9.27 (s, 1H, ArNH), 8.58 (s, 1H, NH), 7.60 (d, 2H, *J* = 8.4 Hz, ArH), 7.50 (d, 2H, *J* = 8.4 Hz, ArH), 3.69 (s, 3H, $COOCH_3$), 1.00 (s, 3H, 19-CH_3), 0.93 (d, 3H, *J* = 6.4 Hz, 21-CH_3), 0.68 (s, 3H, 18-CH_3); ESI-MS *m/z* (%): 616 ($[M+1]^+$,100). Ana1.Calcd for $C_{32}H_{46}BrN_3O_2S$: C, 62.32; H, 7.52; N, 6.81. Found: C, 62.30; H, 7.49; N, 6.79%.

7g: White crystal, yield 90%, m.p. 130–131 °C. $[\alpha]_D^{20}$ = +170.0 (*c* 0.1, CH_2Cl_2); IR (KBr)(cm^{-1}): 3286, 3195, 2938, 2863, 1743, 1606, 1548, 1266, 1163, 781; ^1H NMR (400 MHz, $CDCl_3$) δ: 9.36 (s, 1H, ArNH), 8.58 (s, 1H, NH), 7.70 (d, 1H, *J* = 10.4 Hz, ArH), 7.39 (d, 1H, *J* = 7.6 Hz, ArH), 7.34–7.28 (m, 1H, ArH), 6.92 (t, 1H, *J* = 6.8 Hz, ArH), 3.67 (s, 3H, $COOCH_3$), 1.00 (s, 3H, 19-CH_3), 0.93 (d, 3H, *J* = 6.4 Hz, 21-CH_3), 0.68 (s, 3H, 18-CH_3); ESI-MS *m/z* (%): 556 ($[M+1]^+$,100). Ana1.Calcd for $C_{32}H_{46}FN_3O_2S$: C, 69.15; H, 8.34; N, 7.56. Found: C, 69.10; H, 8.30; N, 7.60%.

7h: White crystal, yield 89%, m.p. 186–188 °C, $[\alpha]_D^{20}$ = +190.0 (*c* 0.1, CH_2Cl_2); IR (KBr)(cm^{-1}): 3419, 3274, 2925, 2863, 1747, 1615, 1536, 1279, 1179, 739; ^1H NMR (400 MHz, $CDCl_3$) δ: 9.20 (s, 1H, ArNH), 8.55 (s, 1H, NH), 7.51 (d, 2H, *J* = 8.0 Hz, ArH), 7.19 (d, 2H, *J* = 8.0 Hz, ArH), 3.67 (s, 3H, $COOCH_3$), 2.69 (s, 3H, Ar-CH_3), 1.00 (s, 3H, 19-CH_3), 0.93 (d, 3H, *J* = 6.4 Hz, 21-CH_3), 0.68 (s, 3H, 18-CH_3); ESI-MS *m/z* (%): 552 ($[M+1]^+$,100). Ana1.Calcd for $C_{33}H_{49}N_3O_2S$: C, 71.83; H, 8.95; N, 7.61. Found: C, 71.77; H, 8.90; N, 7.59%.

7i: White crystal, yield 90%, m.p. 144–146 °C, $[\alpha]_D^{20}$ = +106.7 (*c* 0.1, CH_2Cl_2); IR (KBr)(cm^{-1}): 3293, 3207, 2938, 2851, 1739, 1594, 1266, 1188, 698; ^1H NMR (400 MHz, $CDCl_3$) δ: 9.30 (s, 1H, ArNH), 8.59 (s, 1H, NH), 7.77 (s, 1H, ArH), 7.62 (d, 1H, *J* = 7.6 Hz, ArH), 7.32 (t, 1H, *J* = 8.0 Hz, ArH), 7.19 (d, 1H, *J* = 8.0 Hz, ArH), 3.67 (s, 3H, $COOCH_3$), 1.00 (s, 3H, 19-CH_3), 0.93 (d, 3H, *J* = 6.4 Hz, 21-CH_3), 0.68 (s, 3H, 18-CH_3); ESI-MS *m/z* (%): 572 ($[M+1]^+$,100). Ana1.Calcd for $C_{32}H_{46}ClN_3O_2S$: C, 67.16; H, 8.10; N, 7.34. Found: C, 67.10; H, 8.09; N, 7.35%.

7j: White crystal, yield 88%, m.p. 163–165 °C, $[\alpha]_D^{20}$ = +150.0 (*c* 0.1, CH_2Cl_2); IR (KBr)(cm^{-1}): 3435, 3290, 2929, 2863, 1731, 1631, 1548, 1233, 1167; ^1H NMR (400 MHz, $CDCl_3$) δ: 9.48 (s, 1H, ArNH), 8.77 (s, 1H, NH), 7.96 (d, 1H, *J* = 8.0 Hz, ArH), 7.91 (t, 2H, *J* = 7.2 Hz, ArH), 7.83 (d, 1H, *J* = 8.4 Hz, ArH), 7.55–7.51 (m, 3H, ArH), 3.67 (s, 3H, $COOCH_3$), 1.02 (s, 3H, 19-CH_3), 0.92 (d, 3H, *J* = 6.4 Hz, 21-CH_3), 0.63 (s, 3H, 18-CH_3); ESI-MS *m/z* (%): 588 ($[M+1]^+$,100). Ana1.Calcd for $C_{36}H_{49}N_3O_2S$: C, 73.55; H, 8.40; N, 7.15. Found: C, 73.50; H, 8.42; N, 7.10%.

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