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Microwave-assisted synthesis and in vitro antibacterial activity of novel steroidal thiosemicarbazone derivatives

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ABSTRACT

Herein, we reported the synthesis of 16 novel steroidal thiosemicarbazone derivatives via the condensation of steroidal ketones and substituted thiosemicarbazides under solvent-free conditions using microwave irradiation. The yields obtained are in the range of 84–96% using microwave method and 46–62% using conventional method. All the synthesized compounds (**7a–p**) have been characterized by ^{1}H NMR, ESI-MS, IR and elemental analyses. All the series compounds (**7a–p**) were evaluated for their antibacterial activity against and the results were compared with the standard drug Amoxicillin. Some of the compounds from the series like **7c**, **7o** and **7p** were equipotent with Amoxicillin against *Pseudomonas aeruginosa*. Also compound **7h** was better than Amoxicillin against *Staphylococcus aureus* and *Bacillus subtilis*.

The diverse parasitic bacteria such as *Staphylococcus aureus* and *Pseudomonas aeruginosa* have significant impact on the mucosal health of humans. *P. aeruginosa* is one of the leading causes of hospital-acquired pneumonia and urinary tract infections and is the major cause of death in Cystic Fibrosis patients.[1] Hence, the present strategy for new drug development is directed towards identifying the essential enzyme systems in the bacterial and developing molecules to inhibit them. The present work is aimed towards developing novel molecules with improved potential for treating bacterial infections and with decreased probability for developing drug resistance.

The biological activity of Schiff base thiosemicarbazones were exploited in the 1960s using *N*-methylisatin-β-thiosemicarbazone, which was effective as prophylactic agent against smallpox and vaccinia.[2,3] 3-Aminpyridine-2-carboxaldehyde thiosemicarbazone is now being evaluated in clinical trials against several malignancies.[4] Currently, compounds with a thiosemicarbazone structure are known to possess antibacterial, antitumous, antiviral and antimalarial properties.[5–7]

The application of microwave techniques for organic synthesis has attracted considerable interest in recent years. Microwave-assisted organic synthesis has proven to be a valuable technique for reducing reaction times, giving cleaner reactions, improving yields, simplifying work-up and designing energy-saving protocols.[8] Moreover, with the development of 'green chemistry', the focus has now shifted to less cumbersome solvent-free methods, undergoing facile reactions to provide high yields of pure products, thus eliminating or minimizing the use of organic solvents.[9–13]

Recently, steroidal building blocks have attracted the interest of research groups in many branches of science and technology, such as the medical and pharmacological fields, supramolecular chemistry and nanotechnology.[14–17] To the best of our knowledge, the research about steroidal (cholesterol) thiosemicarbazone derivatives screening on bacteria has been rarely reported.[18,19] However, above reported synthetic methods suffer from one or more drawbacks like prolonged reactions times, the use of environmentally unfavorable solvents and frequently with low yields. Our research group has been reported with solid inorganic support (such as: Al_2O_3) in a special microwave oven to synthesize the thiosemicarbazone compounds.[20,21] Besides, earlier reports on N(4)-substituted thiosemicarbazones have concluded that the presence of bulky group at the N(4) position of the thiosemicarbazone moiety greatly enhances biological activity.[22] Inspired by these earlier work, herein, we report the environmentally friendly, highly efficient and selective method for the synthesis of novel steroidal thiosemicarbazone derivatives in the solvent-free conditions under microwave irradiation. The synthetic route is depicted in Scheme 1.

The substituted thiosemicarbazides were synthesized by using the literature procedure.[21] All the thiosemicarbazone derivatives were prepared through the condensation of steroidal ketones and thiosemicarbazides which mixture were placed in a porcelain mortar in the presence of a few drops of HCl using neutral aluminum oxide as supporter under microwave. In the experiment, ketones of compounds **4**, **6** used 2 equiv of substituted thiosemicarbazides for the preparation of disubstituted compounds (**7a–h**). Reaction of ketones **5** or **6** with one equivalent of substituted

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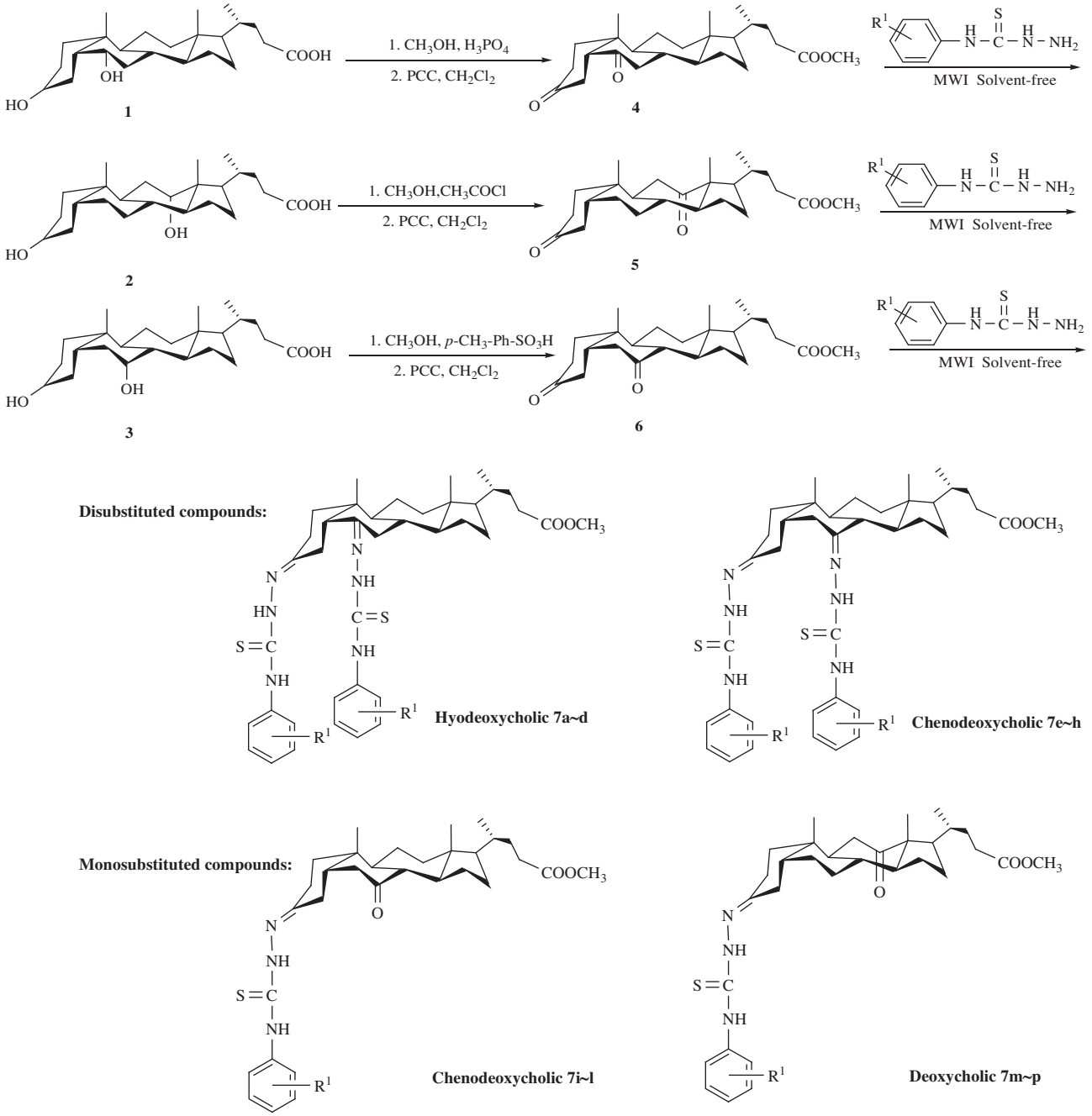

Scheme 1. Synthetic protocol of titled compounds **7a–p**.

thiosemicarbazides only gave the monosubstituted compounds (**7i–p**). It has been known carbonyl group at C-3 position is more reactive to the nucleophilic addition reaction than that of C-7 position of steroidal scaffold.[23]

In our quest to search an optimal reaction condition, we examined the condensation of steroidal ketones with substituted thiosemicarbazide, employing conventional and microwave solvent-free thermal procedures. The reaction was monitored with or without additives catalyst such as HCl, acetic acid or H_2SO_4 under refluxing in ethanol, DMF or DMSO. The result showed that the yields **7a** was in the 18–50% range. But it required a longer reaction time. Among the results obtained, the use of HCl in EtOH received the yield (50%) for the synthesis of compound **7a**. So catalytic property of HCl has been studied considering synthesis of compound **7a** in the solvent-free conditions under microwave. Then effect of various supports like silica gel H, neutral Al_2O_3 and K_2CO_3 have also been studied under the catalyst of HCl. Among the results obtained, the use of HCl in neutral Al_2O_3 received the best yield (94%) for the synthesis of compound **7a** (Table 1). Finally, it showed a dramatic improvement that is in the yield the reaction time (2 min), when we employed a microwave-assisted method. Thus this protocol effectively worked for the affording new steroidal thiosemicarbazones **7a–p**: mixture of steroidal ketones (1 mmol), substituted thiosemicarbazide (1 mmol or 2 mmol) and netural aluminium oxide (0.8 g) in the presense of a catalytic amount of HCl was placed in a microwave oven for 1.5–6.0 min at 240–500 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 crude product was recrystallized by ethanol to give pure compounds **7a–p**.

Table 1
Optimization of the reaction conditions synthesizing compound **7a**

Entry	Solvent	Catalyst	Supporter	Time (min)	Yield (%)
1	EtOH	—	—	380	18[a]
2	EtOH	HCl	—	380	50[a]
3	EtOH	acetic acid	—	380	42[a]
4	EtOH	H_2SO_4	—	380	37[a]
5	DMF	—	—	380	31[a]
6	DMSO	—	—	380	33[a]
7	—	—	Silica gel H	2.0	37[b]
8	—	HCl	Silica gel H	2.0	48[b]
9	—	—	Neutral Al_2O_3	2.0	23[b]
10	—	HCl	Neutral Al_2O_3	2.0	94[b]
11	—	—	K_2CO_3	2.0	14[b]
12	—	HCl	K_2CO_3	2.0	39[b]

[a] Conventional method.
[b] Microwave method.

We compared the synthesis of compounds **7a–p** between microwave in the solvent-free conditions and conventional heating. Compared to conventional thermal heating, microwave irradiation technique decreased the reaction time from 300–480 min to 1.5–6.0 min. It was obvious that yields were increased from 46–62% to 84–96%. Consequently, the use of microwave technology in conjunction with solvent-free conditions allows expeditious, environmentally friendly and efficient procedures in organic methodology. The comparison data are given in Table 2.

The purity of the compounds was checked by the TLC and elemental analyses, and the structures of compounds were identified by spectral data. Compounds **7a–p** analytical data (IR, ESI-MS, ^{1}H NMR, Elemental analysis) are in good agreement with the composition of thiosemicarbazones. The elemental analysis results were within ±0.4% of the theoretical values.[24] Their mass spectra showed the expected molecular peaks at high intensity. Selected diagnostic bands of the IR spectra of the steroidal thiosemicarbazone derivatives (**7a–p**) showed useful information about the structure of the compounds. All the compounds exhibited a characteristic strong absorption at 3270–3370 cm^{-1} due to N–H stretching vibration. Intense absorption bands in the regions 1166–1200 cm^{-1} and 1505–1546 cm^{-1} were attributed to the ν (C=S) and ν (C=N) stretching vibration which also confirms the formation of desired thiosemicarbazones in all compounds. The sharp peaks at 1717–1739 cm^{-1} attributable to ester carbonyl. But compounds **7i–p** showed another sharp peak at 1697–1710 cm^{-1} attributable steroidal ketones carbonyl (7-C or 12-C). In the ^{1}H NMR spectra, the peaks between δ 8.59 and 9.27 ppm can be assigned to the protons of the NHN. Peaks due to the other NH proton were observed at δ 8.56–9.45 ppm. In addition, the singlet peaks at 0.69 (**7a–d**), 0.68 (**7e–l**), 1.05 (**7m–p**), 0.94 (**7a–d**), 1.16–1.27 (**7e–l**), 1.09 (**7m–p**) ppm and the doublet at 0.94–0.96 (**7a–d**), 0.93–0.96 (**7e–l**), 0.86 (**7m–p**) ppm were the characteristics of steroidal structure. The singlet at 3.66–3.68 ppm was assigned to the protons of $COOCH_3$.

The in vitro antibacterial activities of compounds **7a–p** were carried out using the culture of gram-negative bacteria (*P. seruginosa*) and gram-positive bacterium (*S. aureus* and *B. subtilis*). Amoxicillin (30 μg) was used as the standard drug, where MIC was evaluated by the macro-dilution test using standard inoculums of 10^{-5} CFU mL^{-1}. Serial dilutions of the test compounds, previously dissolved in DMSO were prepared to final concentrations of 128, 64, 32, 16, 8, 4 and 2 μg/mL. To each tube was added 100 μL of 24 h old inoculums. The MIC which inhibits the visible growth after 18 h, was determined visually after incubation for 18 h, at 37 °C. The lowest concentration, which showed no visible growth, was taken as an end point for minimum inhibitory concentration (MIC). The MIC level of the tested compounds against these organisms is given in Table 2.

The preliminary results of antibacterial activities indicated that some of the compounds exhibited a moderate to good activity against gram-negative bacteria (*P. seruginosa*) and gram-positive bacterium (*S. aureus* and *B. subtilis*). Notably, compounds **7a**, **7b**, **7c**, **7f**, **7n**, **7o** and **7p** found active against Gram-negative bacteria *P. seruginosa* with MIC values lying in the 2–4 μg/mL range. Furthermore, compounds **7c**, **7o** and **7p** were found resembled with a standard Amoxicillin drug against *P. seruginosa*, whilst three compounds **7d**, **7i**, **7j** were found weak activity. Rapid glance at the Table 2, compounds **7a**, **7d**, **7g** and **7h** found activity against Gram-positive bacteria *S. aureus* with MIC values lying in the 8–32 μg/mL range. Among compounds **7a**, **7d** and **7g** were found resembled with a standard Amoxicillin drug against *S. aureus*. Additionally compounds **7g** (IC_{50} = 2.85 ± 0.10 μg/mL) and **7h** (IC_{50} = 1.02 ± 0.05 μg/mL) also showed as potent as a standard reference drug Amoxicillin (IC_{50} = 2.84 ± 0.05 μg/mL) against *S. aureus* (Table 3). More exceptional result was due to **7h** which showed activity against Gram-positive *B. subtilis* bacteria with a MIC value of 8 μg/mL, a more potency then all other reference drugs used in the study. But majority of compounds showed poor activity against

Table 2
Synthesis comparison of between microwave irradiation and conventional, in vitro antibacterial activity of compounds **7a–p**

Compd	R^1	Conventional		Microwave		MIC (μg/mL)		
		T (min)	Yield (%)	*T* (min)	Yield (%)	*S. aureus*	*B. subtilis*	*P. seruginosa*
7a	4-OCH_3	380	50	2.0	94	32	128	4
7b	4-CH_3	300	61	2.0	90	128	128	4
7c	4-Br	420	60	1.5	96	64	128	2
7d	3-F	450	58	1.5	95	32	128	128
7e	H	360	52	5.0	91	>128	128	8
7f	4-OCH_3	450	46	5.5	84	64	>128	4
7g	4-Br	400	52	4.5	93	32	>128	16
7h	3-F	450	49	5.0	89	8	8	32
7i	4-OCH_3	360	53	4.0	94	128	32	128
7j	4-F	420	62	5.5	86	128	128	128
7k	4-CH_3	360	52	4.0	90	>128	32	16
7l	NA[a]	480	49	6.0	87	>128	32	16
7m	H	360	58	2.0	91	>128	64	32
7n	4-F	380	57	2.0	84	>128	>128	4
7o	4-Br	420	60	1.5	94	>128	>128	2
7p	4-OCH_3	450	59	2.0	89	>128	>128	2
Standard[b]	—	—	—	—	—	32	16	2

[a] Naphthalene.
[b] Amoxicillin.

Table 3
Antibacterial activity of compounds **7f–h** against *S. aureus* (IC_{50} μg/mL)

	7f	7g	7h	Amoxicillin
S. aureus	3.25 ± 0.12	2.85 ± 0.10	1.02 ± 0.05	2.84 ± 0.05

B. subtilis. Among the thiosemicarbazones, substituents with deactivating electron withdrawing groups like fluoric group in the phenyl ring showed excellent antibacterial activity. It was also observed that the Chenodeoxycholic acid thiosemicarbazone disubstituted derivatives of biological activity better than monosubstituted Deoxycholic acid and Hyodeoxycholic. The importance of such work lies in the possibility that the new compounds might be more effective against bacteria for which a thorough investigation regarding the structure–activity relationship, toxicity and the biological effects which would be helpful in designing more potent antibacterial agents for therapeutic use is required.

In conclusion, we have demonstrated a rapid, efficient and eco-friendly method for synthesis of steroidal thiosemicarbazones under solvent-free conditions using microwave irradiation. The reaction was conducted in the presence of neutral aluminum oxide, without using solvent. The present method has some distinct advantages comparing to the conventional method, such as shorter reaction times, good product yields and finally agreement with the green chemistry protocols. All the synthesized compounds were tested for in vitro antibacterial activity. Based on the activity data, many new synthesized compounds are good antibacterial activity. Among compounds **7a**, **7b**, **7c**, **7f**, **7n**, **7o** and **7p** exhibited potency as close as a standard drug Amoxicillin against Gram-negative bacteria. Compound **7h** even received more than reference drugs against Gram-positive bacterium *S. aureus* and *B. subtilis*. Compound **7h** can serve as an important pharmacophore for the design of new antibacterial agent. Studies to establish their in vitro efficacy and safety are being planned for their further development.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.09.083.

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24. Compound **7a**: white solid, mp 208–209 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.09 (d, 2H, *J* = 8.8 Hz, NH), 8.72–8.68 (m, 2H, NH), 7.53–7.43 (m, 4H, ArH), 6.90 (t, 4H, *J* = 6.8 Hz, ArH), 3.81 (s, 6H, Ar-OCH_3), 3.67 (s, 3H, OCH_3), 0.96 (d, 3H, *J* = 6.4 Hz, 21-CH_3), 0.92 (s, 3H, 19-CH_3), 0.69 (s, 3H, 18-CH_3). IR (KBr, cm^{-1}): 3301, 2941, 1739, 1594, 1525, 1480, 1178, 1034, 827. ESI-MS *m*/*z* (%): 761 ($[M+1]^+$, 100). Ana1. Calcd for $C_{41}H_{56}N_6O_4S_2$: C, 64.71; H, 7.42; N, 11.04; found: C, 64.59; H, 7.40; N, 11.01. Compound **7b**: white solid, mp 176–177 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.17 (d, 2H, *J* = 9.6 Hz, NH), 8.74–8.65 (m, 2H, NH), 7.54–7.44 (m, 4H, ArH), 7.17 (d, 4H, *J* = 4.4 Hz, ArH), 3.67 (s, 3H, OCH_3), 2.34 (s, 6H, Ar-CH_3), 0.96 (d, 3H, *J* = 6.4 Hz, 21-CH_3), 0.93 (s, 3H, 19-CH_3), 0.69 (s, 3H, 18-CH_3). IR (KBr, cm^{-1}): 3295, 2943, 1737, 1591, 1529, 1478, 1175, 1051, 814. ESI-MS *m*/*z* (%): 729 ($[M+1]^+$, 100). Ana1. Calcd for $C_{41}H_{56}N_6O_2S_2$: C, 67.55; H, 7.74; N, 11.53; found: C, 67.63; H, 7.69; N, 11.56. Compound **7c**: white solid, mp 170–171 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.22 (t, 2H, *J* = 6.4 Hz, NH), 8.71 (d, 2H, *J* = 10.8 Hz, NH), 7.63–7.46 (m, 8H, ArH), 3.68 (s, 3H, OCH_3), 0.95 (d, 3H, *J* = 6.0 Hz, 21-CH_3), 0.93 (s, 3H, 19-CH_3), 0.69 (s, 3H, 18-CH_3). IR (KBr, cm^{-1}): 3280, 2942, 1735, 1587, 1534, 1488, 1179, 1066, 1008, 824. ESI-MS *m*/*z* (%): 859 ($[M+1]^+$, 100). Ana1. Calcd for $C_{39}H_{50}Br_2N_6O_2S_2$: C, 54.54; H, 5.87; N, 9.79; found: C, 54.67; H, 5.89; N, 9.81. Compound **7d**: white solid, mp 189–190 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.31 (d, 2H, *J* = 5.6 Hz, NH), 8.76 (s, 2H, NH), 7.75–7.63 (m, 2H, ArH), 7.41–7.15 (m, 4H, ArH), 7.06–6.83 (m, 2H, ArH), 3.68 (s, 3H, OCH_3), 0.94 (d, 3H, *J* = 6.8 Hz, 21-CH_3), 0.92 (s, 3H, 19-CH_3), 0.69 (s, 3H, 18-CH_3). IR (KBr, cm^{-1}): 3290, 2945, 1736, 1602, 1539, 1487, 1442, 1198, 1048, 775. ESI-MS *m*/*z* (%): 737 ($[M+1]^+$, 100). Anal. Calcd for $C_{39}H_{50}F_2N_6O_2S_2$: C, 63.56; H, 6.84; N, 11.40; found: C, 63.77; H, 6.82; N, 11.45. Compound **7e**: white solid, mp 188–189 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.36–9.25 (m, 2H, NH), 8.66 (s, 1H, NH), 8.63 (s, 1H, NH), 7.71–7.64 (m, 4H, ArH), 7.43–7.34 (m, 4H, ArH), 7.22 (dd, 2H, *J* = 9.6, 7.6 Hz, ArH), 3.67 (s, 3H, $COOCH_3$), 1.19 (s, 3H, 19-CH_3), 0.96 (d, 3H, *J* = 6.4 Hz, 21-CH_3), 0.73 (s, 3H, 18-CH_3). IR (KBr, cm^{-1}): 3303, 2942, 1734, 1595, 1536, 1441, 1327, 1266, 1182, 1064, 751. ESI-MS *m*/*z* (%): 701 [(M+1)$^+$, 100]. Anal. Calcd for $C_{39}H_{52}N_6O_2S_2$: C, 66.82; H, 7.48; N, 11.99; found: C, 66.85; H, 7.47; N, 12.01. Compound **7f**: white solid, mp 197–198 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.15–9.08 (m, 2H, NH), 8.61 (s, 1H, NH), 8.59 (s, 1H, NH), 7.53–7.45 (m, 4H, ArH), 6.96–6.89 (m, 4H, ArH), 3.83 (s, 3H, Ar-OCH_3), 3.81 (s, 3H, Ar-OCH_3), 3.66 (s, 3H, $COOCH_3$), 1.16 (s, 3H, 19-CH_3), 0.96 (d, 3H, *J* = 6.4 Hz, 21-CH_3), 0.74 (s, 3H, 18-CH_3). IR (KBr, cm^{-1}): 3301, 2943, 1733, 1595, 1520, 1469, 1244, 1177, 1034, 829. ESI-MS *m*/*z* (%): 761 [(M+1)$^+$, 100]. Anal. Calcd for $C_{41}H_{56}N_6O_4S_2$: C, 64.71; H, 7.42; N, 11.04; found: C, 64.76; H, 7.40; N, 11.03. Compound **7g**: white solid, mp 164–165 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.32–9.21 (m, 2H, NH), 8.77 (s, 1H, NH), 8.70 (s, 1H, NH), 7.62–7.52 (m, 4H, ArH), 7.50–7.44 (m, 4H, ArH), 3.68 (s, 3H, $COOCH_3$), 1.19 (s, 3H, 19-CH_3), 0.96 (d, 3H, *J* = 6.0 Hz, 21-CH_3), 0.68 (s, 3H, 18-CH_3). IR (KBr, cm^{-1}): 3291, 2944, 1732, 1585, 1530, 1260, 1179, 1065, 1010, 823. ESI-MS *m*/*z* (%): 859 [(M+1)$^+$, 100]. Anal. Calcd for $C_{39}H_{50}Br_2N_6O_2S_2$: C, 54.54; H, 5.87; N, 9.79; found: C, 54.59; H, 5.85; N, 9.81. Compound **7h**: white solid, mp 188–187 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.45–9.29 (m, 2H, NH), 8.74 (s, 1H, NH), 8.69 (s, 1H, NH), 7.79–7.65 (m, 2H, ArH), 7.39–7.19 (m, 4H, ArH), 6.85–6.94 (m, 2H, ArH), 3.68 (s, 3H, $COOCH_3$), 1.17 (s, 3H, 19-CH_3), 0.97 (d, 3H, *J* = 6.0 Hz, 21-CH_3), 0.73 (s, 3H, 18-CH_3). IR (KBr, cm^{-1}): 3300, 2944, 1735, 1604, 1539, 1486, 1436, 1272, 1200, 1064, 858, 775. ESI-MS *m*/*z* (%): 737 [(M+1)$^+$, 100]. Anal. Calcd for $C_{39}H_{50}F_2N_6O_2S_2$: C, 63.56; H, 6.84; N, 11.40; found: C, 63.54; H, 6.86; N, 11.39. Compound **7i**: white solid, mp 164–165 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.07 (s, 1H, NH), 8.58 (d, 1H, *J* = 7.2 Hz, NH), 7.46 (d, 2H, *J* = 8.8 Hz, ArH), 6.91 (d, 2H, *J* = 7.6 Hz, ArH), 3.81 (s, 3H, Ar-OCH_3), 3.67 (s, 3H, $COOCH_3$), 1.27 (s, 3H, 19-CH_3), 0.93 (d, 3H, *J* = 6.4 Hz, 21-CH_3), 0.69 (s, 3H, 18-CH_3). IR (KBr, cm^{-1}): 3286, 3174, 2951, 2875, 1721, 1594, 1547, 1516, 1244, 1177, 1033, 833. ESI-MS *m*/*z* (%): 604 ($[M+23]^+$, 100). Anal. Calcd for $C_{33}H_{47}N_3O_4S$: C, 68.12; H, 8.14; N, 7.22; found: C, 68.04; H, 8.12; N, 7.20. Compound **7j**: white solid, mp 115–116 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.15 (s, 1H, NH), 8.75 (d, 1H, *J* = 7.2 Hz, NH), 7.56 (t, 2H, *J* = 8.0 Hz, ArH), 7.07 (t, 2H, *J* = 8.4 Hz, ArH), 3.67 (s, 3H, $COOCH_3$), 1.27 (s, 3H, 19-CH_3), 0.93 (d, 3H, *J* = 6.4 Hz, 21-CH_3), 0.69 (s, 3H, 18-CH_3); IR (KBr, cm^{-1}): 3293, 2950, 2871, 1738, 1710, 1609, 1521, 1258, 1186, 1053, 835. ESI-MS *m*/*z* (%): 570 ($[M+1]^+$, 100). Anal. Calcd for $C_{32}H_{44}FN_3O_3S$: C, 67.46; H, 7.78; N, 7.37; found: C, 67.43; H, 7.75; N, 7.35. Compound **7k**: white solid, mp 151–152 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.15 (s, 1H, NH), 8.59 (d, 1H, *J* = 7.2 Hz, NH), 7.48 (d, 2H, *J* = 8.0 Hz, ArH), 7.18 (t, 2H, *J* = 8.0 Hz, ArH), 3.67 (s, 3H, $COOCH_3$), 2.35 (s, 3H, Ar-CH_3), 1.27 (s, 3H, 19-CH_3), 0.93 (d, 3H, *J* = 6.4 Hz, 21-CH_3), 0.69 (s, 3H, 18-CH_3). IR (KBr, cm^{-1}): 3282, 3220, 2951, 2870, 1737, 1708, 1591, 1546, 1481, 1439, 1269, 1176, 1045, 819. ESI-MS *m*/*z* (%): 1131 ($[2\ M+1]^+$, 100). Anal. Calcd for $C_{33}H_{47}N_3O_3S$: C, 70.05; H, 8.37; N, 7.43; found: C, 69.99; H, 8.35; N, 7.40. Compound **7l**: white solid, yield 87%, mp 118–119 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.42 (s, 1H, NH), 8.75 (d, 1H, *J* = 9.2 Hz, NH), 7.93–7.85 (m, 3H, ArH), 7.82 (d, 1H, *J* = 8.0 Hz, ArH), 7.55–7.51 (m, 3H, ArH), 3.67 (s, 3H, $COOCH_3$), 1.29 (s, 3H, 19-CH_3), 0.94 (d, 3H, *J* = 6.4 Hz, 21-CH_3), 0.70 (s, 3H, 18-CH_3). IR (KBr, cm^{-1}): 3307, 2946, 2871, 1735, 1708, 1596, 1505, 1470, 1380, 1268, 1199, 1166, 1093, 1019, 775. ESI-MS *m*/*z* (%): 1225 ($[2M+23]^+$, 100). Anal. Calcd for $C_{36}H_{47}N_3O_3S$: C, 71.84; H, 7.87; N, 6.98; found: C, 71.82; H, 7.85; N, 6.97. Compound **7m**: white solid, mp 175–176 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.27 (s, 1H, NH), 8.57 (d, 1H, *J* = 10.8 Hz, NH), 7.66

(d, 2H, J = 8.0 Hz, ArH), 7.38 (dd, 2H, J = 8.0, 7.6 Hz, ArH), 7.22 (dd, 1H, J = 7.2, 7.6 Hz, ArH), 3.67 (s, 3H, $COOCH_3$), 1.09 (s, 3H, 19-CH_3), 1.05 (s, 3H, 18-CH_3), 0.86 (d, 3H, J = 6.4 Hz, 21-CH_3). IR (KBr, cm^{-1}): 3286, 2937, 2872, 1717, 1697, 1594, 1528, 1490, 1439, 1352, 1265, 1178, 1040, 750. ESI-MS m/z (%): 1103 [$(2M+1)^+$, 100]. Anal. Calcd for $C_{32}H_{45}N_3O_3S$: C, 69.65; H, 8.22; N, 7.62; found: C, 69.57; H, 8.19; N, 7.64. Compound **7n**: white solid, mp 198–199 °C. ^1H NMR (400 MHz, $CDCl_3$): δ 9.17 (s, 1H, NH), 8.57 (d, 1H, J = 10.4 Hz, NH), 7.59–7.55 (m, 2H, ArH), 7.07 (dd, 2H, J = 8.4, 8.4 Hz, ArH), 3.67 (s, 3H, $COOCH_3$), 1.09 (s, 3H, 19-CH_3), 1.05 (s, 3H, 18-CH_3), 0.86 (d, 3H, J = 6.4 Hz, 21-CH_3). IR (KBr, cm^{-1}): 3271, 2931, 2867, 1738, 1699, 1519, 1380, 1330, 1268, 1197, 1053, 847. ESI-MS m/z (%): 570 [$(M+1)^+$, 100]. Anal. Calcd for $C_{32}H_{44}FN_3O_3S$: C, 67.46; H, 7.78; N, 7.37; found: C, 67.47; H, 7.77; N, 7.39. Compound **7o**: white solid, mp 196–197 °C. ^1H NMR (400 MHz, $CDCl_3$): δ 9.24 (s, 1H, NH), 8.64 (d, 1H, J = 10.0 Hz, NH), 7.59–7.56 (m, 2H, ArH), 7.48 (dd, 2H, J = 7.2, 1.6 Hz, ArH), 3.67 (s, 3H, $COOCH_3$), 1.09 (s, 3H, 19-CH_3), 1.05 (s, 3H, 18-CH_3), 0.86 (d, 3H, J = 6.4 Hz, 21-CH_3). IR (KBr, cm^{-1}): 3270, 2931, 2867, 1735, 1699, 1582, 1519, 1488, 1383, 1328, 1267, 1175, 1061, 1009, 76. ESI-MS m/z (%): 1261 [$(2M+3)^+$, 100]. Anal. Calcd for $C_{32}H_{44}BrN_3O_3S$: C, 60.94; H, 7.03; N, 6.66; found: C, 60.78; H, 7.01; N, 6.68. Compound **7p**: white solid, mp 198–199 °C. ^1H NMR (400 MHz, $CDCl_3$): δ 9.09 (s, 1H, NH), 8.56 (d, 1H, J = 11.2 Hz, NH), 7.49–7.46 (m, 2H, ArH), 6.91 (d, 2H, J = 8.4 Hz, ArH), 3.82 (s, 3H, Ar-OCH_3), 3.67 (s, 3H, $COOCH_3$), 1.09 (s, 3H, 19-CH_3), 1.05 (s, 3H, 18-CH_3), 0.86 (d, 3H, J = 6.4 Hz, 21-CH_3). IR (KBr, cm^{-1}): 3285, 2931, 2866, 1734, 1699, 1589, 1522, 1379, 1329, 1238, 1174, 1036, 840. ESI-MS m/z (%): 604 [$(M+23)^+$, 100]. Anal. Calcd for $C_{33}H_{47}N_3O_4S$: C, 68.12; H, 8.14; N, 7.22; found: C, 68.01; H, 8.13; N, 7.24.