Microwave Assisted Solvent-Free Synthesis of Novel Chenodeoxycholic Acid Thiosemicarbazone Derivatives and Studies on Antibacterial Activities

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Received December 28, 2011: Revised June 04, 2012: Accepted June 24, 2012

Abstract: A rapid and efficient method for the synthesis of novel chenodeoxycholicacid acid thiosemicarbzones under solvent-free conditions using microwave has been developed. Their structures were elucidated by ESI-MS, IR, ^{1}H NMR and elemental analysis. Nine novel compounds have been synthesized in good yields. Compared to the conventional method, microwave irradiation method, main advantages are short reaction times, good conversions and the environmentally friendly nature of the process. Preliminary results showed that some of these compounds possess inhibitory effects against *S. typhimurium* and *E. coli*.

Keywords: Antibacterial activity, chenodeoxycholic acid, microwave irradiation, solvent-free, thiosemicarbazone.

1. INTRODUCTION

Thiosemicarbazones and their metal complexes have received considerable attention in view of their various biological activities, variables bonding modes, and structural diversity [1-3]. The biological activity of thiosemicarbazones was exploited in the 1960s using 1-methylisatin-3-thiosemicarbazone, which was effective as prophylactic against smallpox [4] and vaccinia [5]. 3-aminopyridine-2-carboxaldehyde thiosemicarbazones are now being evaluated in clinical trials against several malignancies [6]. Compounds with thiosemicarbazones structures have numerous applications like anti-tubercular, anti-tumor, anti-viral, anti-fungal, anti HIV, anticancer and other biological activities [7-15].

Recent development in "green chemistry" can minimize the environment harmfulness of classical reactions. One of the most popular approaches is the application of microwave techniques for organic synthesis. This technology can enhance the selectivity and improve product yields [16, 17]. For this reason, the application of microwave irradiation under solvent-free conditions has gained popularity over the usual homogeneous and heterogeneous reactions.

As is evident from the literature, our research group has been working on the microwave solvent-free synthesis of thiosemicarbazone derivatives [18-20]. As a continuation of this work, we report good yields in the synthesis of novel chenodexoycholic acid thiosemicarbazone under solvent-free conditions using neutral aluminum oxide as a mineral support. Some compounds were tested *in vitro* against bacteria such as *Salmonella typhimurium*, *Staphylococcus pyogenes* and *Escherichia coli*. The synthesis route is depicted in Scheme **1**.

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2. RESULTS AND DISCUSSION

The structures of all the compounds **4a-i** were confirmed by Mass, IR, ^{1}H NMR, elemental analysis. Their mass spectra showed the expected molecular ions at high intensity. The IR spectra of these compounds exhibited a characteristic strong absorption at 3287-3003 cm^{-1} due to NH stretching vibration; the strong bands in the region 1731-1736 cm^{-1} indicated the adsorption of C=O. Strong absorption bands falling within the range of 1519-1543cm^{-1} and the range of 1034-1065 cm^{-1} were assigned to the C=N and C=S respectively. In the ^{1}H NMR spectra, between δ 9.08-9.45 ppm and δ 8.59-8.94 ppm were assigned to the protons of the NH. In addition, the singlet peaks at 1.16-1.19 ppm, 0.68-0.74 ppm and the doublet at 0.95-0.97 ppm were the characteristics of steroidal structure. The singlet at 3.66-3.68 ppm was assigned to the protons of $COOCH_3$.

In searching for the best conditions, we also carried out a series of experiments, varying the microwave irradiation (MWI) power, time and different supporters. We used the synthesis of **4a** for example and we found that the highest yield was obtained when the time was 5.0 min at 450 W by using neutral aluminum oxide as the solid support.

As shown in Table **1**, we carried out the synthetic comparison of **4a-i** between MWI in the solvent-free conditions and conventional heating. It was easy to see that MWI greatly decreased the reaction time form 360-500 min to 4.5-6.5 min. However, the yields also increased from 45-52% to 84-93%. Consequently, the use of microwave technology in conjunction with the use of solvent-free conditions allows expeditious and efficient procedures in this organic synthesis.

The compounds **4a-e** were tested for their antibacterial activities by disc-diffusion method using nutrient broth medium. The Gram-positive and Gram-negative bacteria utilized in this study consisted of *S. typhimurium*, *S. pyogenes*,

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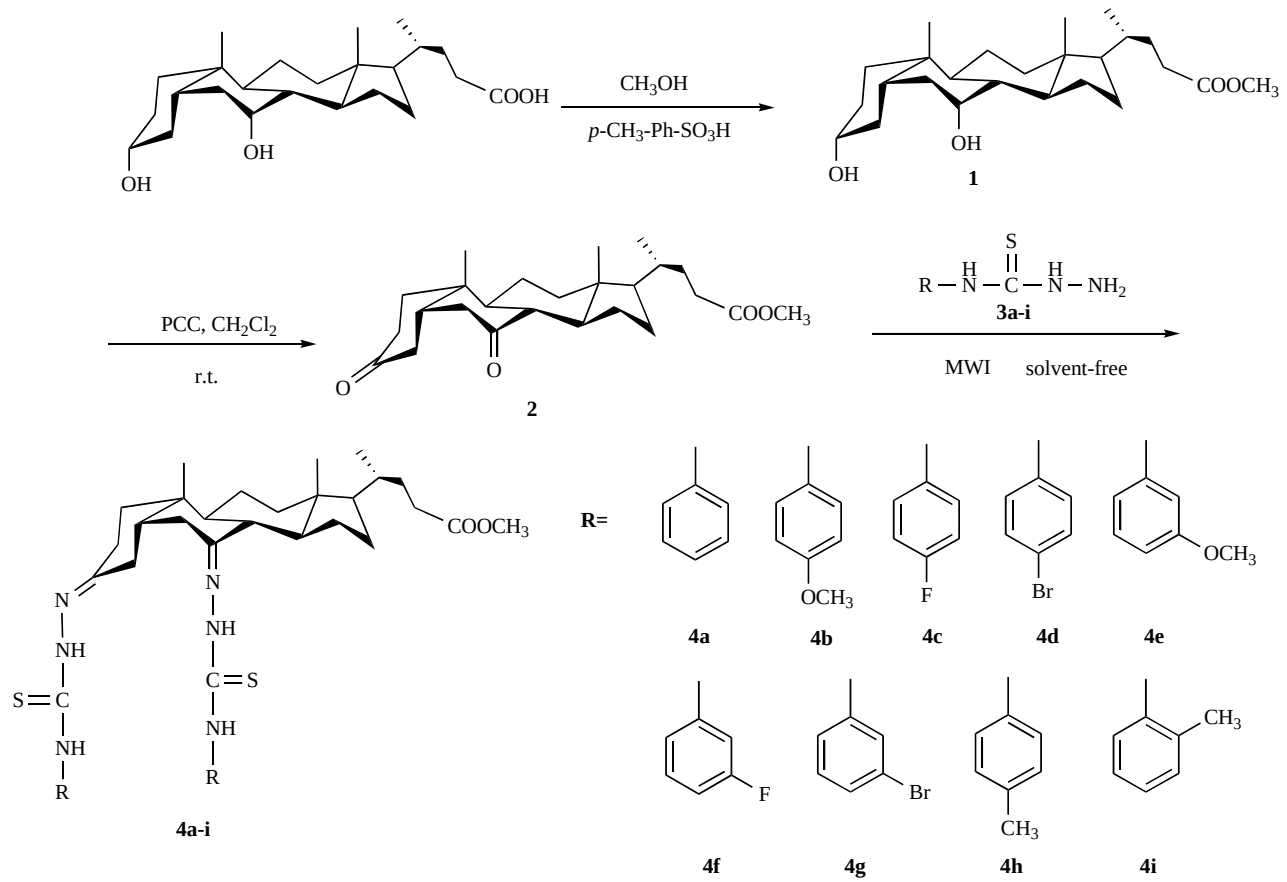

Scheme 1. Synthesis of the chenodeoxycholic acid thiosemicarbazone derivatives **4a-i**.

Table 1. Synthetic Comparison of the Chenodeoxycholic Acid Thiosemicarbazone Derivatives 4a-i Between MWI and Conventional Heating.

Compd.	Conventional method			Microwave method			Tc[a]/Tmw[b]
	Solvent(ml)	T/min	Yield/%	Solvent(ml)	T/min	Yield/%	
4a	20	360	52	/	5.0	91	72
4b	20	450	46	/	5.5	84	82
4c	20	360	50	/	4.5	91	80
4d	20	400	52	/	4.5	93	89
4e	20	420	47	/	5.0	87	84
4f	20	450	49	/	5.0	89	90
4g	20	400	48	/	6.0	86	67
4h	20	500	45	/	6.5	84	77
4i	20	420	49	/	6.0	87	70

[a]Time of conventional heating
[b]Time of MWI

E.coli. In the disc-diffusion method, sterile paper discs (5 mm) were impregnated with compound dissolved in DMSO at the concentration of 0.01% (mass fraction). The plates were incubated at 35°C for 24 h. The result is presented in Table **2**. The compounds investigated have good biological activity against *S. typhimurium* and *E. coli*. Further antibacterial activities are under study.

3. CONCLUSIONS

In summary, we have developed a highly efficient and eco-friendly method for the preparation of chenodeoxycholic acid thiosemicarbazones. The reaction was conducted in the presence of neutral aluminum oxide, without using solvent, and assisted by microwave irradiation. The present method has many advantages compared to the conventional method,

Table 2. Antibacterial Activities of Compounds 4a-e.

Compounds	*S. typhimurium*	*S. pyogenes*	*E. coli*
4a	++	-	++
4b	+	-	++
4c	++	+	++
4d	++	+	++
4e	+	-	++
DMSO	-	-	-

++: strong; +: moderate; -: weak or no.

including shorter reaction times, good product yields and it fulfills green chemistry protocols. The importance of such work lies in the possibility that these new compounds might be more helpful in designing more potent antibacterial agents for biological and therapeutic use.

4. EXPERIMENTAL

4.1. General

Melting points were determined on a micro-melting point apparatus and are uncorrected. IR spectra were obtained on 1700 PerkinElmer FTIR using KBr disks. ^{1}H NMR spectra recorded on a Varian INOVA 400 MHz spectrometer spectra were determined on FinniganLCQ^{DECA} instrument. Elemental analysis was performed on a Carlo-Erba-1106 auto analyzer. Optical rotation was measured on a Wzz-2B polarimeter. All reactions were performed in a commercial microwave apparatus (XH-100A, 100-1000W, Beijing Xianghu Science and Technology Development Co. Ltd, Beijing, China). All the solvents were purified before use. Methyl (3α, 5β, 7α)-3,7-dihydroxycholan-24-oate [21] and Methyl (5β)-3,7-dioxocholan-24-oate [22] were prepared by known procedures. Thiosemicarbazides **3a-i** were also prepared by known procedures [22].

4.2. Methyl (5β)-3,7-dioxocholan-24-oate (2)

Pyridinium chlorochromate [23] (PCC) (3.03 mmol) was added to a solution of methyl (3α, 5β, 7α)-3,7-dihydroxycholan-24-oate (0.25 g, 0.615 mmol) in dried CH_2Cl_2 (20 ml) at room temperature. The reaction was completed in 24 h. The solvent was removed under reduced pressure, and the crude product was purified by column chromatography on silica gel using ethyl acetate to give 2 as white solid; yield 86%; Mp: 155-156 °C (lit. Mp: 154-156 °C [24]). $[\alpha]_D^{20}$ = -21.5 (*c* 0.12, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.69 (s, 3H, 18-CH_3), 0.93 (d, 3H, *J* = 6.4 Hz, 21-CH_3), 1.31 (s, 3H, 19-CH_3), 3.67 (s, 3H, $COOCH_3$); IR (KBr, cm^{-1}): 2953, 2873, 1709, 1403, 1375, 1214, 1174, 1010, 966; ESI—MS *m/z* (%): 827 [$(2M+23)^+$, 100]; Elemental analysis: Found (%):C, 74.53; H, 9.52 Calcd. For $C_{25}H_{38}O_4$: C, 74.59; H, 9.51.

4.3. Microwave Procedure for the Preparation of Steroidal Thiosemicarbazones 4a-i

The steroidal diketone **2** (1 mmol), the thiosemicarbazides **3a-i** (2 mmol) and neutral aluminium oxide (1.0 g) were placed in a porcelain mortar, then two drops of concentrated acetic acid were added. After grinding, the mixture was put in round-bottom flask (25 ml) in a microwave oven. It was irradiated for 4.5~6.5 min at 400~600 W. The reaction mixture was cooled to room temperature, dissolved in DMSO and filtered. The filtrate was added to water and the product precipitated. It was recrystallized from ethanol and obtained in 84~93% yields. The physical and spectra data of the compounds **4a-i** are as follows.

4.3.1. Methyl (5β)-3,7-bis[2-[[(phenylamino)thioxomethyl]hydrazinylidene]-12-oxocholan-24-oate (4a)

Obtained according to the MWI method as a white solid; yield 91%; Mp: 188-189 °C. $[\alpha]_D^{20}$ = -73.50 (*c* 0.10, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.73 (s, 3H, 18-CH_3), 0.96 (d, 3H, *J* = 6.4 Hz, 21-CH_3), 1.19 (s, 3H, 19-CH_3), 3.67 (s, 3H, $COOCH_3$), 7.22 (dd, 2H, *J* = 9.6, 7.6 Hz, ArH), 7.34-7.43 (m, 4H, ArH), 7.64-7.71 (m, 4H, ArH), 8.63 (s, 1H, NH), 8.66 (s, 1H, NH), 9.25-9.36 (m, 2H, NH); IR (KBr, cm^{-1}): 3303, 2942, 1734, 1595, 1536, 1441, 1327, 1266, 1182, 1064, 751; ESI—MS *m/z* (%): 701 [$(M+1)^+$, 100]; Elemental analysis: Found (%): C, 66.85; H, 7.47; N, 12.01 Calcd. For $C_{39}H_{52}N_6O_2S_2$: C, 66.82; H, 7.48; N, 11.99.

4.3.2. Methyl (5β)-3,7-bis[2-[[(4-methoxyphenyl)amino]thioxomethyl]hydrazinylidene]-12-oxochola n-24-oate (4b)

Obtained according to the MWI method as a white solid; yield 84%; Mp: 197-198 °C. $[\alpha]_D^{20}$ = -108.40 (*c* 0.10, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.74 (s, 3H, 18-CH_3), 0.96 (d, 3H, *J* = 6.4 Hz, 21-CH_3), 1.16 (s, 3H, 19-CH_3), 3.66 (s, 3H, $COOCH_3$), 3.81 (s, 3H, Ar-OCH_3), 3.83 (s, 3H, Ar-OCH_3), 6.89-6.96 (m, 4H, ArH), 7.45-7.53 (m, 4H, ArH), 8.59 (s, 1H, NH), 8.61 (s, 1H, NH), 9.08-9.15 (m, 2H, NH); IR (KBr, cm^{-1}): 3301, 2943, 1733, 1595, 1520, 1469, 1244, 1177, 1034, 829; ESI—MS *m/z* (%): 761 [$(M+1)^+$, 100]; Elemental analysis: Found (%): C, 64.76; H, 7.40; N, 11.03 Calcd. For $C_{41}H_{56}N_6O_4S_2$: C, 64.71; H, 7.42; N, 11.04.

4.3.3. Methyl (5β)-3,7-bis[2-[[(4-fluorophenyl)amino]thioxomethyl]hydrazinylidene]-12-oxocholan- 24-oate (4c)

Obtained according to the MWI method as a white solid; yield 91%; Mp: 156-157 °C. $[\alpha]_D^{20}$ = -80.37 (*c* 0.10, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.74 (s, 3H, 18-

CH_3), 0.96 (d, 3H, *J* = 6.0 Hz, 21-CH_3), 1.19 (s, 3H, 19-CH_3), 3.67 (s, 3H, $COOCH_3$), 7.02-7.12 (m, 4H, ArH), 7.53-7.62 (m, 4H, ArH), 8.71 (s, 1H, NH), 8.79 (s, 1H, NH), 9.15-9.31 (m, 2H, NH); IR (KBr, cm^{-1}): 3297, 2944, 1733, 1608, 1519, 1373, 1263, 1219, 1181, 1062, 830; ESI—MS *m/z* (%): 737 [$(M+1)^+$, 100]; Elemental analysis: Found (%): C, 63.50; H, 6.86; N, 11.43 Calcd. For $C_{39}H_{50}F_2N_6O_2S_2$: C, 63.56; H, 6.84; N, 11.40.

4.3.4. Methyl (5β)-3,7-bis[2-[[(4-bromophenyl)amino]thioxomethyl]hydrazinylidene]-12-oxocholan- 24-oate (4d)

Obtained according to the MWI method as a white solid; yield 93%; Mp: 164-165 °C. $[\alpha]_D^{20}$ = -109.50 (*c* 0.10, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.68 (s, 3H, 18-CH_3), 0.96 (d, 3H, *J* = 6.0 Hz, 21-CH_3), 1.19 (s, 3H, 19-CH_3), 3.68 (s, 3H, $COOCH_3$), 7.44-7.50 (m, 4H, ArH), 7.52-7.62 (m, 4H, ArH), 8.70 (s, 1H, NH), 8.77 (s, 1H, NH), 9.21-9.32 (m, 2H, NH); IR (KBr, cm^{-1}): 3291, 2944, 1732, 1585, 1530, 1260, 1179, 1065,1010, 823; ESI—MS *m/z* (%): 859 [$(M+1)^+$, 100]; Elemental analysis: Found (%): C, 54.59; H, 5.85; N, 9.81 Calcd. For $C_{39}H_{50}Br_2N_6O_2S_2$: C, 54.54; H, 5.87; N, 9.79.

4.3.5. Methyl (5β)-3,7-bis[2-[[(3-methoxyphenyl)amino]thioxomethyl]hydrazinylidene]-12-oxochola n-24-oate (4e)

Obtained according to the MWI method as a white solid; yield 87%; Mp: 191-192 °C. $[\alpha]_D^{20}$ = -139.50 (*c* 0.10, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.74 (s, 3H, 18-CH_3), 0.96 (d, 3H, *J* = 6.0 Hz, 21-CH_3), 1.19 (s, 3H, 19-CH_3), 3.67 (s, 3H, $COOCH_3$), 3.81-3.87 (m, 6H, Ar-OCH_3), 6.77 (dd, 2H, *J* = 6.0, 6.8 Hz, ArH), 7.06 (d, 1H, *J* = 8.0 Hz, ArH), 7.11-7.20 (m, 1H, ArH), 7.23-7.32 (m, 2H, ArH), 7.47-7.55 (m, 2H, ArH), 8.61 (brs, 2H, NH), 9.26-9.37 (m, 2H, NH); IR (KBr, cm^{-1}): 3287, 2943, 1734, 1601, 1540, 1488, 1460, 1286, 1216, 1160, 1047, 851, 693; ESI—MS *m/z* (%): 761 [$(M+1)^+$, 100]; Elemental analysis: Found (%): C, 64.74; H, 7.41; N, 11.06 Calcd. For $C_{41}H_{56}N_6O_4S_2$: C, 64.71; H, 7.42; N, 11.04.

4.3.6. Methyl (5β)-3,7-bis[2-[[(3-fluorophenyl)amino]thioxomethyl]hydrazinylidene]-12-oxocholan- 24-oate (4f)

Obtained according to the MWI method as a white solid; yield 89%; Mp: 188-187 °C. $[\alpha]_D^{20}$ = -100.89 (*c* 0.10, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.73 (s, 3H, 18-CH_3), 0.97 (d, 3H, *J* = 6.0 Hz, 21-CH_3), 1.17 (s, 3H, 19-CH_3), 3.68 (s, 3H, $COOCH_3$), 6.85-6.94 (m, 2H, ArH), 7.19-7.39 (m, 4H, ArH), 7.65-7.79 (m, 2H, ArH), 8.69 (s, 1H, NH), 8.74 (s, 1H, NH), 9.29-9.45 (m, 2H, NH); IR (KBr, cm^{-1}): 3300, 2944, 1735, 1604, 1539, 1486, 1436, 1272, 1200, 1064, 858, 775; ESI—MS *m/z* (%): 737 [$(M+1)^+$, 100]; Elemental analysis: Found (%): C, 63.54; H, 6.86; N, 11.37 Calcd. For $C_{39}H_{50}F_2N_6O_2S_2$: C, 63.56; H, 6.84; N, 11.40.

4.3.7. Methyl (5β)-3,7-bis[2-[[(3-bromophenyl)amino]thioxomethyl]hydrazinylidene]-12-oxocholan- 24 -oate (4g)

Obtained according to the MWI method as a white solid; yield 86%; Mp: 142-143 °C. $[\alpha]_D^{20}$ = -131.80 (*c* 0.10, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.74 (s, 3H, 18-CH_3), 0.96 (d, 3H, *J* = 6.4 Hz, 21-CH_3), 1.17 (s, 3H, 19-CH_3), 3.67 (s, 3H, $COOCH_3$), 7.18-7.24 (m, 2H, ArH), 7.27-7.36 (m, 2H, ArH), 7.46-7.51 (m, 1H, ArH), 7.57-7.67 (m, 1H, ArH), 7.90-7.99 (m, 2H, ArH), 8.79 (brs, 2H, NH), 9.24-9.34 (m, 2H, NH); IR (KBr, cm^{-1}): 3288, 2944, 1731, 1584, 1530, 1474, 1424, 1258, 1181, 1063, 773, 671; ESI—MS *m/z* (%): 859 [$(M+1)^+$, 100]; Elemental analysis: Found (%): C, 54.57; H, 5.86; N, 9.81 Calcd. For $C_{39}H_{50}Br_2N_6O_2S_2$: C, 54.54; H, 5.87; N, 9.79.

4.3.8. Methyl (5β)-3,7-[2-[[(4-methylphenyl)amino]thioxomethyl]hydrazinylidene]-12-oxocholan-24 -oate (4h)

Obtained according to the MWI method as a white solid; yield 84%; Mp: 190-191 °C. $[\alpha]_D^{20}$ = -90.50 (*c* 0.10, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.72 (s, 3H, 18-CH_3), 0.95 (d, 3H, *J* = 6.0 Hz, 21-CH_3), 1.17 (s, 3H, 19-CH_3), 2.33 (s, 3H, Ar-CH_3), 2.34 (s, 3H, Ar-CH_3), 3.67 (s, 3H, $COOCH_3$), 7.14-7.22 (m, 4H, ArH), 7.46-7.54 (m, 4H, ArH), 8.75 (d, 1H, *J* = 4.0 Hz, NH), 8.81 (d, 1H, *J* = 6.8 Hz, NH), 9.16-9.28 (m, 2H, NH); IR (KBr, cm^{-1}): 3295, 2941, 2868, 1734, 1590, 1532, 1371, 1263, 1177, 1062, 812, 731; ESI—MS *m/z* (%): 729 [$(M+1)^+$, 100]; Elemental analysis: Found (%): C, 67.57; H, 7.72; N, 11.57 Calcd. For $C_{41}H_{56}N_6O_2S_2$: C, 67.55; H, 7.74; N, 11.53.

4.3.9. Methyl (5β)-3,7-bis[2-[[(2-methylphenyl)amino]thioxomethyl]hydrazinylidene]-12-oxocholan -24-oate (4i)

Obtained according to the MWI method as a white solid; yield 87%; Mp: 196-197 °C. $[\alpha]_D^{20}$ = -60.78 (*c* 0.10, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.74 (s, 3H, 18-CH_3), 0.96 (d, 3H, *J* = 6.4 Hz, 21-CH_3), 1.18 (s, 3H, 19-CH_3), 2.38 (s, 3H, Ar-CH_3), 2.39 (s, 3H, Ar-CH_3), 3.67 (s, 3H, $COOCH_3$), 7.02-7.07 (m, 2H, ArH), 7.23-7.32 (m, 2H, ArH), 7.40-7.44 (m, 1H, ArH), 7.48 (d, 2H, *J* = 6.0 Hz, ArH), 7.56 (d, 1H, *J* = 8.0 Hz, ArH), 8.61 (s, 1H, NH), 8.94 (s, 1H, NH), 9.20-9.28 (m, 2H, NH); IR (KBr, cm^{-1}): 3294, 2942, 1736, 1602, 1543, 1487, 1326, 1271, 1197, 1164, 1063, 780, 698; ESI—MS *m/z* (%): 729 [$(M+1)^+$, 100]; Elemental analysis: Found (%): C, 67.51; H, 7.77; N, 11.48 Calcd. For $C_{41}H_{56}N_6O_2S_2$: C, 67.55; H, 7.74; N, 11.53.

4.4. Conventional Procedure for the Preparation of Steroidal Thiosemicarbazones 3a-i

The steroidal diketone **2** (1 mmol) and the thiosemicarbazides **3a-i** (2 mmol) were dissolved in ethanol (20 ml). After completely dissolving, two drops of acetic acid were added. The mixture was stirred for 360-500 min at 80 °C. After cooling, the products were filtered, recrystallized from ethanol and obtained 45-52% yields.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflicts of interest.

ACKNOWLEDGEMENTS

We greatly thank the Science and Technology Department of Si Chuan Province (No. 2011JY0035) and the Fundamental Research Funds of Central Universities, Southwest University for Nationalities (No. 11NZYTH05) for the financial support.

SUPPLEMENTARY MATERIAL

Supplementary material is available on the publishers Web site along with the published article.

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