Synthesis and Antiviral Activity of Phthiobuzone Analogues

Ya-Jun YANG,[a] Jing-Hua ZHAO,[b] Xian-Dao PAN,*[,a,b] and Pei-Cheng ZHANG*[,a]

[a] *Key Laboratory of Bioactive Substances and Resources Utilization of Chinese Herbal Medicine (Ministry of Education), Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College; Beijing 100050, People's Republic of China: and* [b] *Beijing Union Pharmaceutical Factory; Beijing 102600, People's Republic of China.*
Received September 14, 2009; accepted November 24, 2009; published online November 25, 2009

A series of phthiobuzone analogs, prepared from potassium phthalimide or phthalandione, have been evaluated for their antiviral activities. Among the candidates, compounds 5j and 5k, which contain the substituted 4-halogenated phenyl ring at *N*-4′,4″ position, show more potent antiviral activity than phthiobuzone against herpes simplex virus 1 (IC_{50}=8.56 and 2.85 μg/ml, respectively) and herpes simplex virus 2 (IC_{50}=1.75 and 4.11 μg/ml, respectively). Compounds 9c and 9d with a propylene linker between the phthalimide and bisthiosemicarbazone moieties display similar antiviral potency against herpes simplex virus 1 (IC_{50}=2.85 and 4.11 μg/ml, respectively).

Key words phthiobuzone; antiviral activity; herpes simplex virus; analogue

Herpes viruses are a large family of viruses containing eight known human viruses. Amongst them, herpes simplex virus 1 (HSV-1) and herpes simplex virus 2 (HSV-2) cause cold sores and genital infections, respectively.[1)] HSV infections are one of the most common contagious diseases in humans. Previous antiviral research on HSVs has primarily focused on the development of nucleoside analogs that target the viral polymerase.[2)] However, these antiviral agents cannot inhibit resistant viruses and can lead to hemotoxicity.[3)]

Phthiobuzone (Ftibamzone, Tai Ding An, TDA, Fig. 1), a derivative of thiosemicarzones, has been used in clinical treatment of herpes and trachoma diseases in China and in Egypt. TDA represents a type of molecule that combines 3-phthalimido-2-oxo-*n*-butyraldehyde and bisthiosemicarbazones in one structure. TDA has a unique mechanism of antiviral action, which is different from those of the antiviral nucleotide analogues.[4)] Moreover, recent research has demonstrated that phthiobuzone displays weak inhibitory activity against resistant HSV-1 strains.[5)] In view of this novel structural template, which differs from those of all reported anti-HSV agents, we are interested in a further study of the structure–activity relationship. In this article, we report the synthesis of a series of TDA analogues, particularly through the modification of the 4′,4″-*N*-substituted thiosemicarbazone group and the chain length between the phthalimide and bisthiosemicarbazone moieties.

Results and Discussion

The synthesis of compounds **5a**—**o** (Chart 1) began with potassium phthalimide (**1**). Treatment of **1** with 3-chloro-2-butanone afforded compound **2**,[6)] which was brominated by Br_2 in AcOH to give compound **3**. We prepared target compounds **5a**—**o** by oxidizing **3** using dimethyl sulfoxide (DMSO) to yield the α-keto aldehyde intermediate **4**, followed by direct condensation with 4-substituted-3-thiosemicarbazides.[7)] Some thiosemicarbazides were purchased from commercial suppliers, and others were prepared from the corresponding isothiocyanate with 85% hydrazine hydrate in ethanol. It should be pointed out that compounds **5a**—**o** were all racemates.

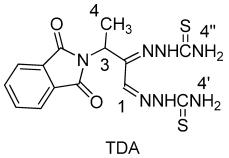
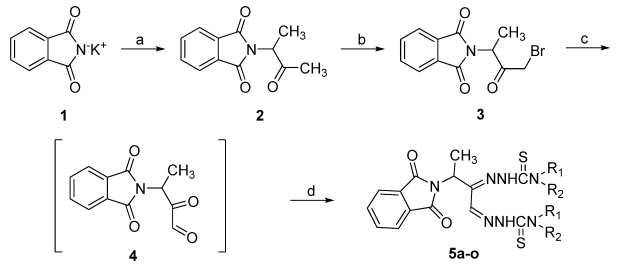


Fig. 1. The Structure of TDA

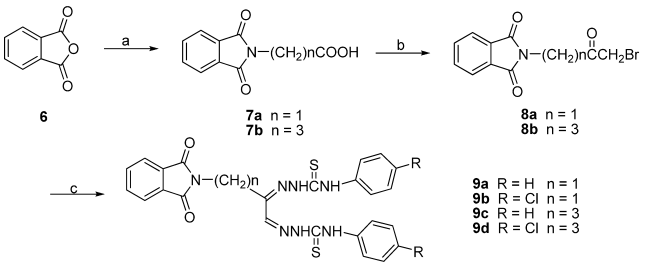
Compounds **7a** and **7b** were obtained by condensation in AcOH of **6** with aminoacetic acid and 4-aminobutyric acid, respectively.[8)] Using the method of Wang *et al.*,[9)] the α-bromoketones **8a** and **8b** were prepared in good yields. Oxidation and condensation of compounds **8a** and **8b** afforded compounds **9a**—**d** using the above method (Chart 2). Except **5g**, other TDA analogues were new compounds.

This series of TDA analogues were evaluated for their anti-HSV activity in Vero cells. The cytotoxic activity of the compounds was determined in parallel in the same cell line.



Reagents and Conditions: (a) 3-chloro-2-butanone, DMF; (b) Br_2, AcOH; (c) DMSO/H_2O(4:1); (d) 4-substituted-3-thiosemicarbazides.

Chart 1. The Synthetic Route of Compounds **5a**—**o**



Reagents and Conditions: (a) aminoacetic acid or 4-aminobutyric acid, AcOH; (b) i, $SOCl_2$; ii, new CH_2N_2, Et_2O; iii, HBr, THF; (c) i, DMSO/H_2O(4:1); ii, 4-substituted-3-thiosemicarbazides.

Chart 2. The Synthetic Route of Compounds **9a**—**d**

* To whom correspondence should be addressed. e-mail: xdp@imm.ac.cn; pczhang@imm.ac.cn

Table 1. Anti-HSV Activity (μg/ml), Cytotoxicity (μg/ml) and Selectivity Index of Compounds **5a**—**o** *in Vitro*

Compd.	R_1	R_2	CC_{50} [a]	HSV-1		HSV-2	
				IC_{50} [b]	SI [c]	IC_{50}	SI
5a	Me	Me	21.37	>4.11	—	NT [d]	
5b	H	*i*-Pr	74.07	>24.69	—	NT	
5c	H	Cyclohexyl	53.14	>12.34	—	NT	
5d	H	Allyl	77.04	>111.11	—	NT	
5e	H	Bn	21.38	>12.34	—	NT	
5f	H	4-Cl-Bn	21.38	>12.34	—	NT	
5g	H	Ph	21.38	4.11	5.20	NT	
5h	H	2-F-Ph	37.03	5.30	6.90	NT	
5i	H	4-Br-Ph	111.11	21.37	5.20	2.85	7.50
5j	H	4-F-Ph	64.15	8.56	7.49	1.75	36.80
5k	H	4-Cl-Ph	77.04	2.85	27.03	4.11	18.70
5l	H	3,4-Cl,Cl-Ph	333.33	25.68	13.00	>111.11	—
5m	H	3,5-CF_3,CF_3-Ph	53.41	12.34	—	NT	
5n	H	4-Me-Ph	21.38	>12.34	—	NT	
5o	H	4-MeO-Ph	21.38	12.34	1.73	NT	
TDA	H	H	384.90	95.44	4.03	74.07	5.19
ACV				1.00		8.98	

a) CC_{50}: 50% cytotoxic concentration; *b*) IC_{50}: 50% effective concentration; *c*) SI (selective index)=CC_{50}/IC_{50}; *d*) NT: not tested.

The observed activities were compared with those of TDA and acyclovir (ACV). The subclass of compounds **5a**—**o** had a wide variety of substituents on the 4′,4″-*N* residue. As can be seen in Table 1, alkyl groups in 4′,4″-positions did not improve potency (**5a**—**f**). On the other hand, the introduction of aryl substituents in the same positions generally led to an increase in inhibitory potency (**5g**—**o**). Especially, a notable anti-HSV-1 activity was seen when a halogen atom was present at the 4-position on the phenyl ring (**5i**—**k**). Substituting the halogen atom at the 4-position for a methyl (**5n**) or a methoxy (**5o**) decreasesd the activity of the molecules. Compounds that showed good activity against HSV-1 and good separation between anti-HSV activity and toxicity were tested for activity against HSV-2. Several of the compounds showed activity similar to ACV against HSV-1 and HSV-2. However, compound **5l** lost suppressant property against HSV-2. The most potent anti-HSV compounds were **5j** and **5k**, which showed marked inhibition of HSV-1 (IC_{50}=8.56 and 2.85 μg/ml, respectively) and HSV-2 (IC_{50}=1.75 and 4.11 μg/ml, respectively). Meanwhile they had low cytotoxicity (CC_{50}>64.15 μg/ml), resulting in high selectivity index ($SI_{HSV\text{-}1}$=7.49 and 27.03, $SI_{HSV\text{-}2}$=36.8 and 18.7, respectively).

To study the effect of the chain between the phthalimide and bisthiosemicarbazone moieties, two alkyl linkers were introduced, producing the four analogous compounds **9a**—**d**. The propylene analogues were 3—4 fold more potent than the corresponding methylene analogues (Table 2). Like compounds **5j** and **5k**, compounds **9c** and **9d** demonstrated similar anti-HSV-1 potency with IC_{50}s of less than 4.11 μg/ml. More importantly, compound **9d** also exhibited a high selectivity index (SI=12.9). This result suggests that the alkyl linker is important for antiviral activity.

On the basis of the above-described results, the following conclusions could be drawn: (a) alkyl groups in the 4′,4″-positions are found to decrease activity; (b) aryl groups in the same positions generally increase activity; (c) a 4-halogenated aromatic ring results in good selectivity; and (d) the alkyl linker between the phthalimide and bisthiosemicarbazone moieties is important for antiviral activity. These results support further development of more specific and potent analogues for antiviral drug therapy. Additional efforts will be devoted to investigate the mechanism of antiviral action of these compounds.

Table 2. Anti-HSV-1 Activity (μg/ml), Cytotoxicity (μg/ml) and Selectivity Index of Compounds **9a**—**d** *in Vitro*

Compd.	*n*	R_1	CC_{50} [a]	HSV-1	
				IC_{50} [b]	SI [c]
9a	1	H	37.30	12.34	3.00
9b	1	Cl	21.08	12.34	1.73
9c	3	H	8.56	2.85	3.00
9d	3	Cl	53.41	4.11	12.90
TDA			384.90	95.44	4.03
ACV				1.00	

a) CC_{50}: 50% cytotoxic concentration; *b*) IC_{50}: 50% effective concentration; *c*) SI (selective index)=CC_{50}/IC_{50}.

Experimental

Biology. Antiviral Assay The antiviral activity of compounds **5a**—**o** and **9a**—**d** was determined using a cytopathic effect (CPE) reduction assay against HSV-1 (VR733) and HSV-2 (SAV) in Vero cell cultures. Cells grown to confluency in 96-well plates were infected with 100 $CCID_{50}$ of virus, one $CCID_{50}$ being the 50% cell culture infective dose. After an adsorption period of 2 h at 37 °C, virus was removed and serial dilutions of the compounds were added. Virus-infected wells without compounds were used as cytopathogenicity controls. Viral cytopathogenicity was completed 1—2 d after viral infection. Antiviral activity is expressed as the IC_{50} (50% inhibitory concentration).

Cytotoxic Assay Cytotoxicity of the compounds for the host cells was evaluated in parallel with their antiviral effects, based on the inhibition of cell growth.

Chemistry. General ^{1}H-NMR spectra were recorded on a Varian MERCURY-300 (300 MHz) system. Chemical shift values (δ) are given in ppm relative to TMS as internal standard. HR-MS spectra were obtained on a LC/MSD TOF spectrometer, using ESI as ionization mode. Flash column chromatography was performed with 200—300 mesh silica gel.

3-Phthalimido-2-butanone (2) To a solution of 3-chloro-2-butanone (3.21 g, 20 mmol) in DMF (20 ml) was added potassium phthalimide (6.12 g, 22 mmol) with stirring. The reaction mixture was stirred at room temperature for 3 h and then poured into water (180 ml). The desired product can be collected *via* filtration without further purification. ^{1}H-NMR ($CDCl_3$) δ: 1.66 (3H, d, *J*=6.9 Hz), 2.20 (3H, s), 4.80 (1H, q, *J*=6.9 Hz), 7.73—7.88

(4H, m). ESI-MS (positive) *m/z*: 218.0802 $[M+H]^+$.

1-Bromo-3-phthalimido-2-butanone (3) To a stirred solution of **2** (2.17 g, 10 mmol) in AcOH (20 ml), bromine water (1.76 g, 11 mmol) was added drop-wise. After complete addition, stirring was continued at room temperature for 1 h. The reaction mixture was then poured onto ice cold water, and the solid product was filtered, washed with H_2O, dried, and crystallized from 95% ethanol. ^{1}H-NMR ($CDCl_3$) δ: 1.67 (3H, d, J=5.4 Hz), 3.99 (2H, dd, J=11.4 Hz), 5.22 (1H, q, J=5.4 Hz), 7.73—7.88 (4H, m). ESI-MS (positive) *m/z*: 295.9926 $[M+H]^+$.

General Procedure for the Preparation of 3-Phthalimido-2-oxo-*n*-butyraldehyde-bis-(4-alkyl or aryl)thiosemicarbazone (5a—o) A solution of **3** (296 mg, 1 mmol) in DMSO/H_2O (4 : 1, 2 ml) was heated at 80 °C for 3 h. The appropriate thiosemicarbazide (2 mmol) in hot 95% ethanol was added and stirring under reflux was continued for 1—3 h, during which period a precipitate was formed. The precipitate was filtered, washed with ethanol, dried, and purified by flash chromatography on silica gel to afford **5a**—**o**.

5a: ^{1}H-NMR ($CDCl_3$) δ: 1.67 (3H, d, J=7.2 Hz), 3.44—3.48 (12H, m), 5.21 (1H, q, J=7.2 Hz), 7.72 (1H, s), 7.72—7.84 (4H, m), 9.36 (1H, s), 12.23 (1H, s). HR-ESI-MS (positive) *m/z*: 434.1389 $[M+H]^+$ (Calcd for $C_{18}H_{24}N_7O_2S_2$: 434.1433).

5b: ^{1}H-NMR (DMSO-d_6) δ: 1.14—1.64 (12H, m), 1.62 (3H, d, J=6.9 Hz), 4.39—4.41 (2H, m), 5.46 (1H, q), 7.58 (1H, d, J=8.1 Hz), 7.68 (1H, d), 7.82 (1H, s), 7.86—7.88 (4H, m), 11.56 (2H, s). HR-ESI-MS (positive) *m/z*: 484.1545 $[M+Na]^+$ (Calcd for $C_{20}H_{27}N_7O_2NaS_2$: 484.1565).

5c: ^{1}H-NMR (DMSO-d_6) δ: 0.74—1.98 (23H, m), 4.10 (2H, m), 5.46 (1H, q), 7.58 (1H, d), 7.70 (1H, d), 7.82 (4H, m), 7.89 (1H, s), 11.54 (1H, s), 11.56 (1H, s). HR-ESI-MS (positive) *m/z*: 542.2388 $[M+H]^+$ (Calcd for $C_{26}H_{36}N_7O_2S_2$: 542.2372).

5d: ^{1}H-NMR ($CDCl_3$) δ: 1.73 (3H, d), 4.37 (m, 4H), 5.34 (m, 5H), 5.92 (m, 1H), 7.39 (s, 1H), 7.79 (m, 4H), 9.75 (s, 1H), 11.71 (s, 1H). HR-ESI-MS (positive) *m/z*: 458.1406 $[M+H]^+$ (Calcd for $C_{20}H_{24}N_7O_2S_2$: 458.1433).

5e: ^{1}H1NMR (DMSO-d_6) δ: 1.62 (3H , d, J=6.9 Hz), 4.73—4.88 (4H, m), 5.49 (1H, q, J=6.9 Hz), 7.22—7.34 (10H, m), 7.68—7.81 (4H, m), 7.94 (1H, s), 8.50 (1H, t, J=6.3 Hz), 8.57 (1H, s, J=6.3 Hz), 11.66 (1H, s), 11.71 (1H, s, NH). HR-ESI-MS (positive) *m/z*: 558.1744 $[M+H]^+$ (Calcd for $C_{28}H_{28}N_7O_2S_2$: 558.1746).

5f: ^{1}H-NMR (DMSO-d_6) δ: 1.62 (3H, d, J=6.9 Hz), 4.69—4.86 (4H, m), 5.48 (1H, q, J=6.9 Hz), 7.29—7.39 (8H, m), 7.68—7.80 (4H, m), 7.92 (1H, s), 8.52 (1H, t, J=6.3 Hz), 8.63 (1H, s, J=6.3 Hz), 11.65 (1H, s), 11.74 (1H, s). HR-ESI-MS (negative) *m/z*: 624.0812 $[M-H]^-$ (Calcd for $C_{28}H_{24}N_7O_2Cl_2S_2$: 624.0805).

5g: ^{1}H-NMR (DMSO-d_6) δ: 1.73 (3H, d, J=6.9 Hz), 5.68 (1H, q, J=6.9 Hz), 7.21—7.43 (6H, m), 7.60—7.71 (4H, m), 7.84 (4H, m), 8.04 (1H, s), 9.78 (1H, s), 9.80 (1H, s), 11.96 (1H, s), 11.98 (1H, s). HR-ESI-MS (negative) *m/z*: 528.1238 $[M-H]^-$ (Calcd for $C_{26}H_{22}N_7O_2S_2$: 528.1271).

5h: ^{1}H-NMR (DMSO-d_6) δ: 1.70 (3H, d, J=7.2 Hz), 5.64 (1H, q, J=7.2 Hz), 7.20—7.46 (8H, m), 7.48—7.88 (4H, m), 8.06 (1H, s), 9.41 (1H, s), 9.65 (1H, s), 12.03 (1H, s), 12.14 (1H, s). HR-ESI-MS (negative) *m/z*: 564.1132 $[M-H]^-$ (Calcd for $C_{26}H_{20}N_7O_2F_2S_2$: 564.1083).

5i: ^{1}H-NMR (DMSO-d_6) δ: 1.71 (3H, d, J=7.2 Hz), 5.66 (1H, q, J=7.2 Hz), 7.56—7.71 (8H, m), 7.84 (4H, m), 8.03 (1H, s), 9.84 (2H, s), 12.04 (1H, s), 12.05 (1H, s). HR-ESI-MS (negative) *m/z*: 683.9463 $[M-H]^-$ (Calcd for $C_{26}H_{20}N_7O_2Br_2S_2$: 683.9481).

5j: ^{1}H-NMR (DMSO-d_6) δ: 1.71 (3H, d, J=6.9 Hz), 5.65 (1H, q, J=6.9 Hz), 7.21—7.58 (8H, m), 7.84 (4H, m), 8.03 (1H, s), 9.77 (1H, s), 9.81 (1H, s), 11.99 (2H, s). HR-ESI-MS (negative) *m/z*: 564.1083 $[M-H]^-$ (Calcd for $C_{26}H_{20}N_7O_2F_2S_2$: 564.1131).

5k: ^{1}H-NMR (DMSO-d_6) δ: 1.72 (d, 3H), 5.66 (dd, 1H), 7.45—7.74 (m, 8H), 7.76 (m, 4H), 8.03 (s, 1H), 9.85 (d, 2H), 12.03 (d, 2H). HR-ESI-MS (negative) *m/z*: 596.0497 $[M-H]^-$ (Calcd for $C_{26}H_{20}N_7O_2Cl_2S_2$: 596.0497).

5l: ^{1}H-NMR (DMSO-d_6) δ: 1.71(3H, d, J=6.8 Hz), 5.66 (1H, q, J=6.8 Hz), 7.79—7.81 (6H, m), 7.83 (4H, m), 8.04 (1H, s), 9.94 (1H, s), 9.99 (1H, s), 12.15 (2H, s). HR-ESI-MS (negative) *m/z*: 663.9700 $[M-H]^-$ (Calcd for $C_{26}H_{18}N_7O_2Cl_4S_2$: 663.9712).

5m: ^{1}H-NMR (DMSO-d_6) δ: 1.73 (3H, d, J=7.2 Hz), 5.66 (1H, q, J=7.2 Hz), 7.77—7.87 (4H, m), 8.03—8.47 (6H, m), 8.61 (1H, s), 9.75 (1H, s), 10.05 (1H, s), 10.34 (1H, s), 12.32 (1H, s). HR-ESI-MS (negative) *m/z*: 800.0756 $[M-H]^-$ (Calcd for $C_{30}H_{18}N_7O_2F_{12}S_2$: 800.0753).

5n: ^{1}H-NMR (DMSO-d_6) δ: 1.65 (3H, d, J=6.9 Hz), 2.24 (3H, s), 2.33 (3H, s), 5.63 (1H, q, J=6.9 Hz), 7.10 (2H, d, J=8.7 Hz), 7.22 (2H, d, J=8.4 Hz), 7.32 (2H, d, J=8.4 Hz), 7.55 (2H, d, J=8.4 Hz), 7.35—7.82 (4H, m), 8.33 (1H, s), 9.45 (1H, s), 9.66 (1H, s), 11.94 (1H, s), 12.14 (1H, s). HR-ESI-MS (positive) *m/z*: 558.1744 $[M+H]^+$ (Calcd for $C_{28}H_{28}N_7O_2S_2$: 558.1746).

5o: ^{1}H-NMR (DMSO-d_6) δ: 1.82 (d, 3H, J=6.9 Hz), 3.80 (3H, s), 3.81 (3H, s), 5.61 (1H, q, J=6.9 Hz), 6.92—6.98 (4H, m), 7.60 (2H, d, J=9.0 Hz), 7.7 (2H, d, J=9.0 Hz), 7.84 (4H, m), 8.16 (1H, s), 9.76 (1H, s), 10.81 (1H, s), 11.20 (1H, s), 11.57 (1H, s). HR-ESI-MS (positive) *m/z*: 4590.1621 $[M+H]^+$ (Calcd for $C_{28}H_{28}N_7O_2S_2$: 590.1644).

General Procedure for the Preparation of 2-*N*-Phthalimide Acetic Acid (7a) and 4-*N*-Phthalimide Butyric Acid (7b) Phthalandione (2.96 g, 20 mmol) and the appropriate amino acid (1.2 eq) were suspended in AcOH (20 ml). The suspension was refluxed for 2 h and then cooled and poured into water (20 ml). The resulting precipitate was filtered, washed with water, and dried to give the desired product.

7a: ^{1}H-NMR ($CDCl_3$) δ: 4.50 (2H, s), 7.74—7.76 (2H, m), 7.89—7.91 (2H, m). ESI-MS (positive) *m/z*: 206.0511 $[M+H]^+$.

7b: ^{1}H-NMR (DMSO-d_6) δ: 1.79—1.85 (2H, m), 2.56 (2H, t), 3.59 (2H, t), 7.80—7.87 (4H, m), 12.05 (1H, s). ESI-MS (positive) *m/z*: 297.9875 $[M+H]^+$.

General Procedure for the Preparationof 1-Bromo-3-phthalimido-2-aceton (8a) and 1-Bromo-3-phthalimido-2-pentanone (8b) A solution of **7a** or **7b** (10 mmol) in $SOCl_2$ (5 ml) was refluxed for 2 h. After that, all volatiles were removed under reduced pressure and the crude acid chloride thus obtained was dissolved in toluene (1 ml) and added dropwise to an ice-cold solution of new CH_2N_2 [prepared from nitrosomethyl urea (6 g) and KOH (5 g) in water (20 ml) in ether (45 ml)] over 20 min. The mixture was allowed to reach room temperature and excess diazomethane was destroyed with a few drops of AcOH. After addition of satd $NaHCO_3$ solution (10 ml), the ether layer was separated and the aqueous portion extracted with ether (2×20 ml). The combined ether fractions were dried, evaporated under reduced pressure. Hydrobromic acid (47%, 1 ml) was added dropwise to a solution of *N*-Pht α-diazoketones in THF (10 ml) at 0 °C. After stirring at room temperature for 1 h, the reaction was neutralized with satd $NaHCO_3$ solution and extracted with ethyl acetate (3×30 ml). The combined ethyl acetate layer was washed with brine, dried and evaporated under reduced pressure to give the corresponding **8a** or **8b** which were purified by recrystallization.

8a: ^{1}H-NMR ($CDCl_3$) δ: 4.01 (2H, s), 4.78 (2H, s), 7.75—7.77 (2H, m), 7.88—7.90 (2H, m). ESI-MS (positive) *m/z*: 281.9760 $[M+H]^+$.

8b: ^{1}H-NMR ($CDCl_3$) δ: 2.00—2.04 (2H, m), 2.72 (2H, t), 3.73 (2H, t), 3.92 (2H, s), 7.71—7.74 (2H, m), 7.83—7.86 (2H, m). ESI-MS (positive) *m/z*: 310.0077 $[M+H]^+$.

General Procedure for the Preparation of Compounds 9a—d Compounds **9a**—**d** were prepared from **8a** or **8b** using the general procedure for the preparation of Compounds **5a**—**o**.

9a: ^{1}H-NMR (DMSO-d_6) δ: 4.91 (2H, s), 7.20—7.28 (2H, m), 7.36—7.46 (4H, m), 7.47—7.63 (4H, m), 7.78—8.16 (5H, m), 9.77 (1H, s), 10.47 (1H, s), 11.31 (1H, s), 12.23 (1H, s). HR-ESI-MS (negative) *m/z*: 514.1118 $[M-H]^-$ (Calcd for $C_{25}H_{20}N_7O_2S_2$: 514.1120).

9b: ^{1}H-NMR (DMSO-d_6) δ: 4.91 (2H, s), 7.42—7.62 (8H, m), 7.81—7.90 (5H, m), 9.82 (1H, s), 10.53 (1H, s), 11.38 (1H, s), 12.32 (1H, s). HR-ESI-MS (negative) *m/z*: 582.0321 $[M-H]^-$ (Calcd for $C_{25}H_{19}Cl_2N_7O_2S_2$: 582.0340).

9c: ^{1}H-NMR (DMSO-d_6) δ: 1.78—1.82 (2H, m), 3.00 (2H, t), 3.72 (2H, t), 7.18—7.23 (2H, m), 7.34—7.38 (4H, m), 7.50—7.56 (4H, m), 7.56—7.81 (4H, m), 7.80 (1H, s), 9.83 (1H, s), 10.26 (1H, s), 11.27 (1H, s), 12.10 (1H, s). HR-ESI-MS (negative) *m/z*: 542.1434 $[M-H]^-$ (Calcd for $C_{27}H_{25}N_7O_2S_2$: 542.1433).

9d: ^{1}H-NMR (DMSO-d_6) δ: 1.77—1.81 (2H, m), 3.00 (2H, t), 3.71 (2H, t), 7.39—7.42 (4H, dd), 7.52—7.60 (4H, dd), 7.61—7.91 (4H, m), 7.80 (1H, s), 9.87 (1H, s), 10.31 (1H, s), 11.35 (1H, s), 12.19 (1H, s). HR-ESI-MS (negative) *m/z*: 610.0643 $[M-H]^-$ (Calcd for $C_{27}H_{23}Cl_2N_7O_2S_2$: 610.0653).

Acknowledgement Financial support of Beijing Municipal Commission of Commerce is acknowledged. Technical assistance of professor Wan Lin is greatly appreciated.

References

1) Gudmundsson K. S., Johns B. A., Allen S. H., *Bioorg. Med. Chem. Lett.*, **18**, 1157—1161 (2008).
2) Gudmundsson K. S., Johns B. A., *Bioorg. Med. Chem. Lett.*, **17**, 2735—2739 (2007).
3) Tomé J. P. C., Neves M. G. P. M. S., Tomé A. C., Cavaleiro J. A. S., Mendonça A. F., Pegado I. N., Duarteb R., Valdeirab M., *Bioorg. Med. Chem.*, **13**, 3878—3888 (2005).
4) Yin M. B., Cheng M., Ye Q. R., Yang H., M., Gao Y. J., Wang L., Liu

Y. Y., *Acta Academiae Medicinae Sinicae*, **9**, 79—83 (1987).
5) Hu N., Guan H. J., Sang A. M., Yang L., *Jiangsu Med. J.*, **31**, 688—700 (2005).
6) Zhao Z. Z., Wang L., Jiang X. J., Wang Z. H., Wei Z., *Acta Chimi. Sin.*, **38**, 67—77 (1980).
7) Wang L., Yang H. M., Zhao Z. Z., *Acta Pharmaceut. Sin.*, **29**, 427—432 (1994).
8) Medjahed W., Tabet Zatla A., Kajima Mulengi J., Baba Ahmed F. Z., Merzouk H., *Tetrahedron Lett.*, **45**, 1211—1213 (2004).
9) Wang G. X., Wang L., Zhao Z. Z., Tao P. Z., Wang S. Q., *Acta Pharmaceut. Sin.*, **31**, 831—836 (1996).