Synthesis and Antiviral Activity of Novel 1,3,4-Thiadiazine Derivatives

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A series of novel 1,3,4-thiadiazine derivatives were synthesized *via* chemical optimization on phthiobuzone. Their anti-herpes simplex virus (HSV) activities *in vitro* were also tested. Several compounds exhibited more highly potent anti-HSV activity and much higher selectivity index (SI) values than those of phthiobuzone. The most potent anti-HSV compound was 4f, which showed marked inhibition against HSV-1 (IC_{50}=77.04 μg/ml) and HSV-2 (IC_{50}=30.00 μg/ml). Meanwhile it had low cytotoxicity (CC_{50}=1000.00 μg/ml), resulting in high ($SI_{HSV\text{-}1}$= 12.98, $SI_{HSV\text{-}2}$=33.33, respectively). Furthermore, a computational study for prediction of absorption, distribution, metabolism, excretion (ADME) properties of compound 4f was performed by determination of topological polar surface area, absorption and Lipinski parameters.

Key words 1,3,4-thiadiazine; phthiobuzone; antiviral activity; herpes simplex virus

Herpes viruses are responsible for a wide range of human diseases. Conventional antiviral chemotherapy using nucleoside analogues such as acyclovir is effective in most cases, but is also prone to toxicity and drug resistance. These limitations have fueled interest in developing novel antiviral agents.[1—3)]

Phthiobuzone (Ftibamzone, Tai Ding An, TDA, Fig. 1), 3-phthalimido-2-oxo-*n*-butyraldehyde bisthiosemicarbazone, was used in clinic for treatment of herpes and trachoma diseases in China and Egypt. *In vitro* anti-herpetic activity studies demonstrated that TDA possessed the effect of inhibiting virus plaque formation and reproduction against herpes simplex virus 1 (HSV-1) and herpes simplex virus 2 (HSV-2). Moreover, TDA has a unique mechanism of antiviral action, which might be associated with ribonucleotide reductase, different from those of the antiviral nucleotide analogues.[4,5)] But TDA has limited medicinal application due to its poor pharmaceutical profile, such as low activity and low solubility. As part of our further research of TDA, chemical optimization was performed on bisthiosemicarbazone, the core scaffold of TDA. In order to maintain degrees of similarity, we replaced bisthiosemicarbazone with different heterocycles containing sulfur and nitrogen atoms. This work resulted in the synthesis of a series of 1,3,4-thiadiazine derivatives without losing biological action. Herein, we reported the synthesis and antiviral activity of those novel 1,3,4-thiadiazine derivatives against HSV-1 and HSV-2.

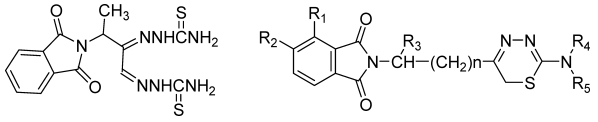
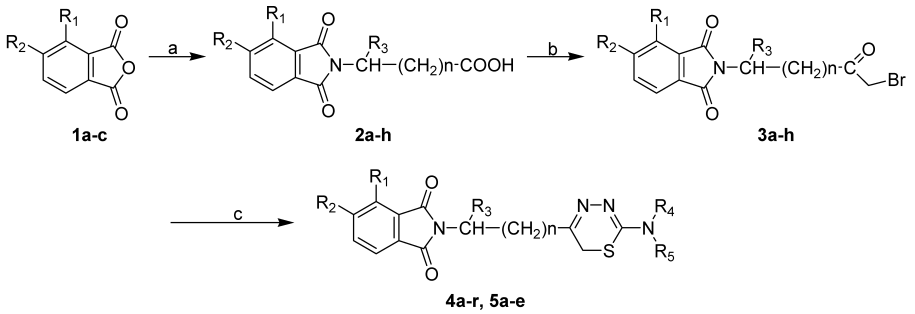


Fig. 1. Structures of TDA and 1,3,4-Thiadiazine Derivatives

Results and Discussion

The synthesis of 1,3,4-thiadiazine derivatives began with phthalandiones **1a**—**c** (Chart 1). Condensation of compounds **1a**—**c** with amino acids gave **2a**—**h**,[6)] which were converted into a-bromo ketones **3a**—**h** according to the previous method.[5,7)] Using $NaHCO_3$ as base, cyclization of compounds **3a**—**h** with 4-substituted thiosemicarbazides in refluxing EtOH gave compounds **4a**—**n**, **4s**, **4t** and **5a**—**e** in moderate to good yields. The synthesis of compounds **4o**—**r** and **7** were illustrated in Chart 2. Acylation and alkylation of **4f** afforded compounds **4o**, **4p** and **4q**, **4r**, respectively. Compound **4f** was reduced by $NaBH_4$ to yield **6**, which was further methylated to give the corresponding compound **7**.

The potential anti-HSV activity and cytotoxicity of the



Reagents and Conditions: (a) amino acids, AcOH; (b) i, $SOCl_2$; ii, new CH_2N_2, Et_2O; iii, HBr, THF; (c) 4-substituted-thiosemicarbazides, $NaHCO_3$, EtOH.

Chart 1. The Synthesis Route of Compounds **4a**—**n**, **4s**, **4t** and **5a**—**e**

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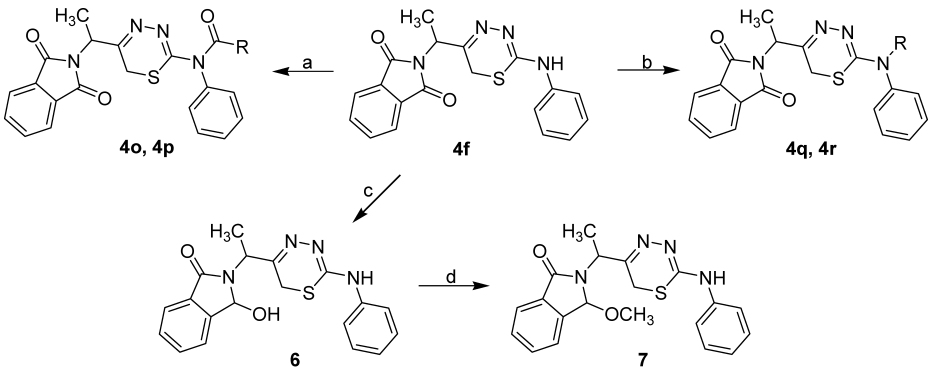

Chart 2. The Synthesis Route of Compounds **4o**—**r** and **7**

Table 1. Anti-HSV-1 Activity (μg/ml), Cytotoxicity (μg/ml) and Selectivity Index of Compounds **4a**—**t**, **5a**—**e** and **7** *in Vitro*

Comp.	R_1	R_2	R_3	R_4	R_5	*n*	CC_{50} [a)]	IC_{50} [b)]	SI [c)]
4a	H	H	Me	Me	Me	0	333.33	>111.11	
4b	H	H	Me	H	*c*-Hexyl	0	192.45	>111.11	
4c	H	H	Me	H	4-Cl-Bn	0	192.45	>111.11	
4d	H	H	Me	H	Allyl	0	557.35	77.04	7.23
4e	H	H	Me	H	Bn	0	192.45	77.04	2.50
4f	H	H	Me	H	Ph	0	1000.00	77.04	12.98
4g	H	H	Me	H	4-MeO-Ph	0	192.45	64.15	3.00
4h	H	H	Me	H	4-Me-Ph	0	192.45	>111.11	
4i	H	H	Me	H	4-NO_2-Ph	0	192.45	>111.11	
4j	H	H	Me	H	2-F-Ph	0	21.38	>12.34	
4k	H	H	Me	H	4-F-Ph	0	64.15	>37.03	
4l	H	H	Me	H	4-Cl-Ph	0	64.15	>37.03	
4m	H	H	Me	H	3-Pyridyl	0	557.35	>333.33	
4n	H	H	Me	H	2-Pyridyl	0	333.33	333.33	1.00
4o	H	H	Me	Bz	Ph	0	192.45	111.11	1.73
4p	H	H	Me	Ac	Ph	0	111.11	>37.04	
4q	H	H	Me	Me	Ph	0	192.45	>111.11	
4r	H	H	Me	Bn	Ph	0	192.45	47.72	4.03
4s	H	Br	Me	H	Ph	0	192.45	>111.11	
4t	NO_2	H	Me	H	Ph	0	480.75	111.11	4.32
5a	H	H	H	H	Ph	0	192.45	>111.11	
5b	H	H	H	H	Ph	1	192.45	>111.11	
5c	H	H	H	H	Ph	2	192.45	25.68	7.49
5d	H	H	Bn	H	Ph	0	53.41	12.34	4.32
5e	H	H	*i*-Bu	H	Ph	0	192.45	>111.11	
7							160.25	37.03	4.32
TDA							353.55	106.52	3.32

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

synthesized 1,3,4-thiadiazine derivatives, together with those of the reference drug TDA, were evaluated in Vero cells. The result was summarized in Table 1. The subclass of compounds **4a**—**r** had a wide variety of substituents on the 2-position of 1,3,4-thiadiazine ring. Compounds **4d**—**g** and **4r** showed potent activity against HSV-1 with IC_{50} values ranging from 47.72 to 77.04 μg/ml. On the other hand, the introduction of other substituents in the same position decreased the inhibitory potency (compounds **4a**—**c**, **4h**, **4i**, **4m**—**o**, **4q**) or led to an increase in toxicity (compounds **4j**—**l**, **4p**). Therefore, it could be concluded that suitable electron-donating group was in favor of antiviral activity. Most importantly, compound **4f** had higher selectivity index (SI) than that of TDA. To study the effect of the chain between the phthalimide and 1,3,4-thiadiazine ring, different linkers were introduced, producing compounds **4f** and **5a**—**e**. Of these, compounds **5c** and **5d** were 4—6 folds more potent than TDA. This result suggests that the alkyl linker was important for antiviral activity. The introduction of substituents on the phthalimide (compounds **4s**, **4t**) generally led to loss of activity. In addition, compound **7** exhibited similar antiviral activity to compound **4r**.

Compounds that showed good activity against HSV-1 and good separation between anti-HSV activity and toxicity were tested for activity against HSV-2 (Table 2). However, compounds **4d** and **5c** lost suppressant property against HSV-2. The most potent anti-HSV compound was **4f**, which showed marked inhibition of HSV-1 (IC_{50}=77.04 μg/ml) and HSV-2 (IC_{50}=30.00 μg/ml). Meanwhile it had low cytotoxicity (CC_{50}=1000.00 μg/ml), resulting in high selectivity index

Table 2. Anti-HSV-2 Activity (μg/ml), Predicted ADME and Lipinski Parameters of Compounds **4d**, **4f** and **5c**

Comp.	HSV-2			%ABS	TPSA[d]	Lipinski's rule[d]			
	CC_{50}[a]	IC_{50}[b]	SI[c]			*n*-ON	*n*-OHNH	log *P*	MW
4d	557.35	>333.33		82.8	75.831	6	1	2.562	328.40
4f	1000.00	30.00	33.33	82.8	75.831	6	1	3.579	364.43
5c	192.45	>111.11		82.8	75.831	6	1	5.007	440.53
TDA	353.55	73.05	4.84	60.7	139.904	9	6	0.843	377.46

a) CC_{50}: 50% cytotoxic concentration; *b*) IC_{50}: 50% effective concentration; *c*) SI (selective index)=CC_{50}/IC_{50}; *d*) www.molinspiration.com/cgi-bin/properties.

($SI_{HSV\text{-}1}$=12.98, $SI_{HSV\text{-}2}$=33.33, respectively). In addition, compound **4f** was evaluated for its solubility profile *in vitro* in differet solutions (2 mg/2 ml), including CH_2Cl_2, EtOAc, acetone, and MeOH. Compound **4f** showed improved solubility (>1 mg/ml) when compared to TDA (only soluble in dimethyl sulfoxide). A computational study of compound **4f** was performed by determination of Lipinski's rule and topological polar surface area (TPSA).[8] The percent absorption (%ABS) was estimated using equation: %ABS=109−0.345×TPSA, according to Zhao *et al.*[9] Our results revealed that the lipophilicity (LogP), molecular weight (MW), hydrogen bond acceptors (*n*-ON) and donors (*n*-OHNH) of compound **4f** fulfilled Lipinski's rule. Furthermore, **4f** demonstrated low TPSA and high %ABS values, suggesting its good oral bioavailability and absorption.

In summary, we synthesized a series of novel 1,3,4-thiadiazine derivatives *via* chemical optimization on TDA. Their anti-HSV activities *in vitro* were also tested. It was worth noting several derivatives exhibited more highly potent anti-HSV activity and much higher SI values than those of TDA. This founding refined our present understanding of the TDA pharmacophore and established certain structure–activity relationships. To our knowledge, this is the first report on the anti-HSV activity of 1,3,4-thiadiazine derivatives. A computational study revealed the potential of compound **4f** as a new antiviral agent. Further studies are required to investigate the antiviral mechanism of this compound.

Experimental

Biology. Antiviral Assay The antiviral activity of compounds **4a**—**t**, **5a**—**e** and **7** 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 $CCID_{50}$ (the 50% cell culture infective dose) of virus. 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.[10]

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 time-of-flight (TOF) spectrometer, using electrospray ionization (ESI) as ionization mode. IR spectra were measured on a Nicolet 5700 spectrometer (microscope transmission). Flash column chromatography was performed with 200—300 mesh silica gel.

General Procedure for the Preparation of Compounds 4a—n, 4s, t and 5a—e Compounds **3a**—**h** (10 mmol), $NaHCO_3$ (168 mg, 20 mmol) and 4-substituted-3-thiosemicarbazides (1.2 eq) were suspended in EtOH (20 ml). The suspension was refluxed for 1 h and poured into water (20 ml). The mixture were extracted with EtOAc (30 ml×3). The organic extracts were successively washed with water and brine, dried over absolute $MgSO_4$, and then filtered. The filtrate was evaporated to give a residue, which was subjected to silica gel column chromatography to afford target compounds.

4a: Yellow oil, yield: 57.7%. ^{1}H-NMR ($CDCl_3$) δ: 1.78 (3H, d, *J*=7.2 Hz), 2.15 (3H, s), 3.12—3.17 (5H, m), 5.28 (1H, q, *J*=7.2 Hz), 7.69—7.83 (4H, m). HR-ESI-MS (positive) *m/z*: 317.1077 $[M+H]^+$ (Calcd for $C_{15}H_{17}N_4O_2S$: 317.1072). IR (cm^{-1}): 3224, 1775, 1710, 1385, 879, 720, 531.

4b: Yellow solid, yield: 51.8%, mp: 175.1—176.2 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.11—1.42 (6H, m), 1.59—1.73 (3H, m), 1.80 (3H, d, *J*=7.2 Hz), 2.04—2.06 (2H, m), 3.15 (2H, dd, *J*=16.8 Hz), 5.29 (1H, q, *J*=7.2 Hz), 7.71—7.86 (4H, m). HR-ESI-MS (positive) *m/z*: 371.1523 $[M+H]^+$ (Calcd for $C_{19}H_{23}N_4O_2S$: 371.1542). IR (cm^{-1}): 3160, 1778, 1710, 1606, 1384, 1348, 883, 718.

4c: Yellow solid, yield: 35.1%, mp: 165.4—166.8 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.67 (3H, d, *J*=6.9 Hz), 3.28 (2H, s), 3.43 (2H, dd), 5.12 (1H, q, *J*=6.9 Hz), 7.29—7.37 (4H, m), 7.82—7.86 (4H, m), 10.87 (1H, s). HR-ESI-MS (positive) *m/z*: 413.0834 $[M+H]^+$ (Calcd for $C_{20}H_{18}ClN_4O_2S$: 413.0839). IR (cm^{-1}): 3184, 1775, 1711, 1605, 1382, 882, 848, 716, 535.

4d: Yellow solid, yield: 80.4%, mp: 99.9—102.2 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.76 (3H, d, *J*=6.9 Hz), 3.13 (2H, s), 4.04 (2H, m), 5.09—5.29 (3H, m), 5.85—5.94 (1H, m), 7.67—7.82 (4H, m). HR-ESI-MS (positive) *m/z*: 329.1067 $[M+H]^+$ (Calcd for $C_{16}H_{17}N_4O_2S$: 329.1072). IR (cm^{-1}): 3301, 1774, 1702, 1529, 1382, 1332, 1263, 1041, 879, 720, 529.

4e: Yellow oil, yield: 63.4%. ^{1}H-NMR (DMSO-d_6) δ: 1.67 (3H, d, *J*=6.9 Hz), 3.27 (2H, s), 4.46 (2H, s), 5.12 (1H, q, *J*=6.9 Hz), 6.79—7.33 (5H, m), 7.82—7.89 (4H, m), 10.75 (1H, s). HR-ESI-MS (positive) *m/z*: 379.1229 $[M+H]^+$ (Calcd for $C_{20}H_{19}N_4O_2S$: 379.1229). IR (cm^{-1}): 3133, 1774, 1713, 1562, 1382, 881, 715.

4f: White solid, yield: 48.1%, mp: 178.9—180.7 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.66 (3H, d, *J*=6.9 Hz), 3.43 (2H, s), 5.14 (1H, q, *J*=6.9 Hz), 6.96—7.01 (3H, m), 7.22—7.27 (2H, m), 7.83—7.90 (4H, m), 10.98 (1H, s); ESI-MS: $[M+H]^+$ 365.1089, $[M+Na]^+$ 387.0906. HR-ESI-MS (positive) *m/z*: 365.1078 $[M+H]^+$ (Calcd for $C_{19}H_{17}N_4O_2S$: 365.1072). IR (cm^{-1}): 3083, 1777, 1708, 1385, 1125, 883, 721.

4g: Yellow solid, yield: 33.0%, mp: 171.7—173.1 °C. ^{1}H-NMR (CD_3COCD_3) δ: 1.75 (3H, d, *J*=7.2 Hz), 3.48 (2H, dd, *J*=21 Hz), 3.74 (2H, s), 5.23 (1H, q, *J*=7.2 Hz), 6.81—6.89 (4H, m), 7.83—7.86 (4H, m). HR-ESI-MS (positive) *m/z*: 395.1172 $[M+H]^+$ (Calcd for $C_{20}H_{19}N_4O_3S$: 395.1178). IR (cm^{-1}): 3169, 1777, 1703, 1592, 1503, 1239, 1153, 1040, 879, 721, 531.

4h: White solid, yield: 52.8%, mp: 179.6—180.9 °C. ^{1}H-NMR (CD_3COCD_3) δ: 1.75 (3H, d, *J*=6.9 Hz), 2.25 (3H, s), 3.48 (2H, dd, *J*=20.7 Hz), 5.23 (1H, q, *J*=6.9 Hz), 6.88 (2H, m), 7.05 (2H, m), 7.83—7.86 (4H, m). HR-ESI-MS (positive) *m/z*: 379.1223 $[M+H]^+$ (Calcd for $C_{20}H_{19}N_4O_2S$: 379.1229). IR (cm^{-1}): 3179, 1776, 1703, 1592, 1384, 1162, 1044, 878, 722.

4i: White solid, yield: 58.6%, mp: 178.1—178.8 °C. ^{1}H-NMR (CD_3COCD_3) δ: 1.77 (3H, d, *J*=7.2 Hz), 3.59 (2H, s), 5.27 (1H, q, *J*=7.2 Hz), 7.09 (2H, s), 7.87 (4H, s), 8.17 (2H, m). HR-ESI-MS (positive) *m/z*: 410.0918 $[M+H]^+$ (Calcd for $C_{19}H_{16}N_5O_4S$: 410.0923). IR (cm^{-1}): 3145, 1777, 1703, 1320, 1107, 839, 718.

4j: Yellow solid, yield: 62.8%, mp: 129.1—131.5 °C. ^{1}H-NMR (CD_3COCD_3) δ: 1.74 (3H, d, *J*=7.2 Hz), 3.56 (2H, dd, *J*=18.6 Hz), 5.27 (1H, q, *J*=7.2 Hz), 6.95—6.7.11 (4H, m), 7.83—7.86 (4H, m). HR-ESI-MS (positive) *m/z*: 383.0973 $[M+H]^+$ (Calcd for $C_{19}H_{16}FN_4O_2S$: 383.0978). IR (cm^{-1}): 3248, 1777, 1710, 1607, 1385, 1218, 762, 719.

4k: White solid, yield: 44.5%, mp: 155.2—156.8 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.65 (3H, d, *J*=6.0 Hz), 3.45 (2H, s), 5.13 (1H, q, *J*=6.0 Hz), 6.79 (2H, s), 7.07—7.09 (2H, m), 7.83—7.90 (4H, m), 10.98 (1H, s). HR-ESI-MS (positive) *m/z*: 383.0961 $[M+H]^+$ (Calcd for $C_{19}H_{16}FN_4O_2S$: 383.0978). IR (cm^{-1}): 3248, 1779, 1709, 1612, 1503, 1384, 1199, 880, 717.

4l: White solid, yield: 65.3%, mp: 163.1—164.3 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.66 (3H, d, J=6.9 Hz), 3.47 (2H, s), 5.14 (1H, q, J=6.9 Hz), 6.79 (2H, s), 7.27—7.30 (2H, m), 7.83—7.90 (4H, m), 11.05 (1H, s). HR-ESI-MS (positive) m/z: 399.0678 $[M+H]^+$ (Calcd for $C_{19}H_{16}ClN_4O_2S$: 399.0683). IR (cm^{-1}): 3089, 1775, 1707, 1609, 1386, 879, 720.

4m: Yellow solid, yield: 49.3%, mp: 180.5—181.5 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.65 (3H, d, J=7.2 Hz), 3.50 (2H, s), 5.13 (1H, q, J=7.2 Hz), 7.20 (1H, s), 7.26—7.30 (1H, m), 7.83—7.90 (4H, m), 8.07 (1H, s), 8.20 (1H, s), 11.19 (1H, s). HR-ESI-MS (positive) m/z: 366.0998 $[M+H]^+$ (Calcd for $C_{18}H_{16}N_5O_2S$: 366.1025). IR (cm^{-1}): 3086, 1776, 1709, 1591, 1569, 1389, 882, 715.

4n: Yellow solid, yield: 41.1%, mp: 170.3—171.9 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.67 (3H, d, J=6.9 Hz), 3.28 (2H, s), 5.16 (1H, q, J=6.9 Hz), 6.94—6.98 (1H, m), 7.03—7.06 (1H, m), 7.63—7.69 (1H, m), 7.84—7.91 (4H, m), 8.29—8.30 (1H, m), 11.27 (1H, s). HR-ESI-MS (positive) m/z: 366.0998 $[M+H]^+$ (Calcd for $C_{18}H_{16}N_5O_2S$: 366.1025). IR (cm^{-1}): 3059, 1775, 1715, 1570, 1546, 1466, 1382, 1155, 795, 720.

4s: White solid, yield: 25.0%, mp: 189.2—190.8 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.65 (3H, d, J=6.3 Hz), 3.42 (2H, s), 5.12 (1H, q, J=6.3 Hz), 6.79 (1H, s), 6.96—7.01 (1H, m), 7.22—7.27 (3H, m), 7.81 (1H, m), 8.04—8.09 (2H, m), 10.97 (1H, s). HR-ESI-MS (positive) m/z: 443.0154 $[M+H]^+$ (Calcd for $C_{19}H_{16}BrN_4O_2S$: 443.0177). IR (cm^{-1}): 3083, 1773, 1708, 1605, 1377, 775, 743.

4t: White solid, yield: 40.2%, mp: 94.7—96.1 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.76 (3H, d, J=6.9 Hz), 3.33 (2H, s), 5.24 (1H, q, J=6.9 Hz), 6.91—6.93 (1H, m), 6.99—7.08 (1H, m), 7.22—7.27 (2H, m), 7.89—7.94 (2H, m), 8.04—8.12 (2H, m). HR-ESI-MS (positive) m/z: 410.0910 $[M+H]^+$ (Calcd for $C_{19}H_{16}N_5O_4S$: 410.0923). IR (cm^{-1}): 3086, 1783, 1719, 1583, 1543, 1382, 1358, 723, 699.

5a: White solid, yield: 65.7%, mp: 171.4—173.3 °C. ^{1}H-NMR (DMSO-d_6) δ: 3.46 (2H, s), 4.59 (2H, s), 6.79 (1H, s), 6.94—6.98 (2H, m), 7.21—7.26 (2H, m), 7.85—7.94 (4H, m), 10.85 (1H, s). HR-ESI-MS (positive) m/z: 351.0896 $[M+H]^+$ (Calcd for $C_{18}H_{15}N_4O_2S$: 351.0916). IR (cm^{-1}): 3092, 1777, 1734, 1608, 1585, 1420, 1391, 947, 700.

5b: White solid, yield: 41.9%, mp: 201.1—202.0 °C. ^{1}H-NMR (DMSO-d_6) δ: 2.72 (2H, s), 3.41 (2H, s), 3.82 (2H, s), 6.74 (1H, s), 6.95—6.98 (2H, m), 7.22—7.27 (2H, m), 7.82—7.89 (4H, m), 10.77 (1H, s). HR-ESI-MS (positive) m/z: 365.1053 $[M+H]^+$ (Calcd for $C_{19}H_{17}N_4O_2S$: 365.1072). IR (cm^{-1}): 3082, 1769, 1695, 1612, 1594, 993, 776, 716, 695.

5c: White solid, yield: 40.1%, mp: 150.6—151.8 °C. ^{1}H-NMR (DMSO-d_6) δ: 2.90—2.95 (2H, m), 2.49—2.54 (4H, m), 3.65 (2H, s), 6.74 (1H, s), 6.93—6.98 (2H, m), 7.22—7.31 (2H, m), 7.82—7.94 (4H, m), 10.75 (1H, s). HR-ESI-MS (positive) m/z: 379.1212 $[M+H]^+$ (Calcd for $C_{20}H_{19}N_4O_2S$: 379.1229). IR (cm^{-1}): 3172, 1769, 1710, 1603, 1398, 1024, 773, 720, 701.

5d: Yellow solid, yield: 68.2%, mp: 136.2—137.0 °C. ^{1}H-NMR (DMSO-d_6) δ: 3.50—3.54 (4H, m), 5.33 (1H, m), 6.79 (1H, s), 6.95—7.32 (9H, m), 7.80 (4H, s), 10.05 (1H, s). HR-ESI-MS (positive) m/z: 441.1373 $[M+H]^+$ (Calcd for $C_{25}H_{21}N_4O_2S$: 441.1385). IR (cm^{-1}): 3077, 1771, 1761, 1574, 1383, 769, 715.

5e: White solid, yield: 33.3%, mp: 161.1—162.3 °C. ^{1}H-NMR (DMSO-d_6) δ: 0.87—0.91 (6H, m), 1.48—1.52 (1H, m), 1.86—1.96 (1H, m), 2.24—2.34 (1H, m), 3.47 (2H, s), 5.07—5.12 (1H, m), 6.96—7.01 (3H, m), 7.22—7.27 (2H, m), 7.85—7.92 (4H, m), 10.66 (1H, s). HR-ESI-MS (positive) m/z: 407.1532 $[M+H]^+$ (Calcd for $C_{22}H_{23}N_4O_2S$: 407.1542). IR (cm^{-1}): 3060, 1776, 1712, 1586, 1383, 719.

General Procedure for the Preparation of Compounds 4o—p A solution of compound **4f** (364 mg, 10 mmol), Et_3N (202 mg, 20 mmol), acetic anhydride or Benzoyl chloride (1.2 eq) in CH_2Cl_2 (10 ml) were stirred at room temperature for 12 h. The reaction mixture was poured into water (20 ml) and extracted with CH_2Cl_2 (30 ml×3). The organic extracts were successively washed with water and brine, dried over absolute $MgSO_4$, and then filtered. The filtrate was evaporated to give a residue, which was subjected to silica gel column chromatography to afford target compounds.

4o: White oil, yield: 58.3%. ^{1}H-NMR ($CDCl_3$) δ: 1.79 (3H, d, J=6.9 Hz), 2.88 (1H, d, J=14.1 Hz), 2.30 (1H, d, J=14.1 Hz), 5.31 (1H, q, J=6.9 Hz), 7.01—7.49 (10H, m), 7.71—7.87 (4H, m). HR-ESI-MS (positive) m/z: 469.1352 $[M+H]^+$ (Calcd for $C_{26}H_{21}N_4O_3S$: 469.1334). IR (cm^{-1}): 1776, 1710, 1384, 1334, 719, 696.

4p: White solid, yield: 66.3%, mp: 160.0—161.4 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.68 (3H, d, J=7.2 Hz), 1.91 (3H, s), 3.15 (2H, dd, J=23.7 Hz), 5.19 (1H, q, J=7.2 Hz), 7.35—7.49 (5H, m), 7.85 (4H, s). HR-ESI-MS (positive) m/z: 407.1165 $[M+H]^+$ (Calcd for $C_{21}H_{19}N_4O_3S$: 407.1178). IR (cm^{-1}): 1772, 1709, 1672, 1388, 1275, 730, 697.

General Procedure for the Preparation of Compounds 4q—r A solution of compound **4f** (364 mg, 10 mmol), K_2CO_3 (276 mg, 20 mmol), iodomethane or benzyl bromide (1.2 eq) in *N*,*N*-dimethylformamide (DMF) (10 ml) were stirred at room temperature for 12 h. The reaction mixture was poured into water (20 ml) and extracted with EtOAc (30 ml×3). The organic extracts were successively washed with water and brine, dried over absolute $MgSO_4$, and then filtered. The filtrate was evaporated to give a residue, which was subjected to silica gel column chromatography to afford target compounds.

4q: White oil, yield: 60.2%. ^{1}H-NMR ($CDCl_3$) δ: 1.74 (3H, d, J=7.2 Hz), 3.29 (2H, s), 3.55 (3H, s), 5.21 (1H, q, J=7.2 Hz), 6.83—6.85 (2H, m), 7.03—7.08 (1H, m), 7.25—7.30 (2H, m), 7.73—7.77 (2H, m), 7.85—7.88 (2H, m). HR-ESI-MS (positive) m/z: 379.1230 $[M+H]^+$ (Calcd for $C_{20}H_{19}N_4O_2S$: 379.1229). IR (cm^{-1}): 1777, 1749, 1710, 1581, 1385, 1197, 723.

4r: White solid, yield: 82.5%, mp: 136.1—137.7 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.71 (3H, d, J=7.2 Hz), 3.25 (2H, s), 5.16 (1H, q, J=7.2 Hz), 5.22 (2H, s), 6.77—6.79 (2H, m), 7.01—7.06 (1H, m), 7.21—7.28 (4H, m), 7.33—7.37 (3H, m), 7.70—7.73 (2H, m), 7.81—7.84 (2H, m). HR-ESI-MS (positive) m/z: 455.1549 $[M+H]^+$ (Calcd for $C_{26}H_{23}N_4O_2S$: 455.1542). IR (cm^{-1}): 1778, 1711, 1780, 1382, 736, 722.

Preparation of Compounds 7 A solution of compound **4f** (364 mg, 10 mmol) and $NaBH_4$ (76 mg, 20 ml) in tetrahydrofuran (THF) and MeOH (10 ml, 1 : 1) were stirred at 0 °C for 2 h. The reaction mixture was poured into water (20 ml) and filtered. The solid was dried to give compound **6**, which was added to MeOH (10 ml) containing *p*-toluenesulfonic acid (10 eq). The solution were refluxed for 8 h, poured into water (20 ml) and extracted with EtOAc (30 ml×3). The organic extracts were successively washed with water and brine, dried over absolute $MgSO_4$, and then filtered. The filtrate was evaporated to give a residue, which was subjected to silica gel column chromatography to afford compound **7**.

7: White oil, yield: 53.9%. ^{1}H-NMR (DMSO-d_6) δ: 1.67 (3H, d, J=7.2 Hz), 3.26 (2H, s), 3.48 (3H, s), 5.11 (1H, q, J=7.2 Hz), 6.95—7.09 (3H, m), 7.24—7.30 (2H, m), 7.51—7.64 (3H,m), 7.82—7.84 (2H, m), 10.95 (1H, s). HR-ESI-MS (positive) m/z: 381.1387 $[M+H]^+$ (Calcd for $C_{20}H_{21}N_4O_2S$: 381.1385). IR (cm^{-1}): 3031, 1778, 1710, 1580, 1383, 1070, 736, 722.

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