ChemInform Abstract: Synthesis and Cytotoxic Evaluation of Substituted Sulfonyl-N-hydroxyguanidine Derivatives as Potential Antitumor Agents

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Synthesis and Cytotoxic Evaluation of Substituted Sulfonyl-*N*-hydroxyguanidine Derivatives as Potential Antitumor Agents

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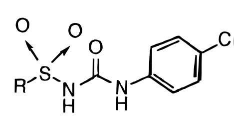
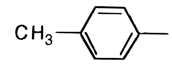
A series of sulfonyl-*N*-hydroxyguanidine derivatives was designed and synthesized for cytotoxic evaluation as potential anticancer agents on the basis of the lead compound LY-181984. Replacement of the ureido moiety of the lead compound with hydroxyguanidine provided a stable cytotoxic agent. The conformation of sulfonyl-*N*-hydroxyguanidine derivatives, such as *N*-(4-chlorophenyl)-*N*′-[(benzo[2,1,3]thiadiazol-4-yl)sulfonyl]-*N*″-hydroxyguanidine (**4g**), investigated utilizing HMBC NMR, theoretical calculations, and X-ray crystallography, indicated stacking of the two aromatic rings. The derivatives were evaluated for *in vitro* cytoxicity against five human tumor cell lines, including HepG2, TSGH 8302, COLO 205, KB, and MOLT-4. The cytotoxic activities of the derived compounds against the human tumor cell lines were equal to or greater than that of the lead compound. *N*-(4-Chlorophenyl)-*N*′-[[3,5-dichloro-4-(4-nitrophenoxy)phenyl]sulfonyl]-*N*″-hydroxyguanidine (**4n**) and *N*-(4-chlorophenyl)-*N*′-[[3,5-dichloro-4-(2-chloro-4-nitrophenoxy)phenyl]sulfonyl]-*N*″-hydroxyguanidine (**4o**) exhibited the greatest growth inhibition of solid tumor cell lines. Compound **4o** was found to possess antitumor activity against murine K1735/M2 melanoma xenografts.

Introduction

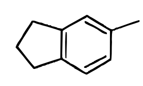
Sulfonylurea derivatives constitute an important class of therapeutical agents in medicinal chemistry.[1] More recently, a series of sulfonylurea derivatives, including LY 181984 (**1**) and LY186641 (**2**), was reported to possess a broad spectrum of activity in several solid tumor models,[2−5] and one of these compounds, LY 186641, is in extensive clinical trials based on its impressive preclinical activity and apparent lack of toxicity to proliferating normal tissues.[6−9] This series of compounds were initially discovered by directly utilizing *in vivo* tumor screening models to overcome the traditionally poor correlation between cytotoxity and antitumor activity.[10−12] However, it is of considerable interest that the mode of action of these compounds differ from traditional anticancer drugs which typically inhibit DNA, RNA, or protein synthesis.[10] Since sulfonylurea derivatives have been found to accumulate in the cell mitochondria, the mitochondria may be the target site for antitumor activity of these compounds.[13−15] Sulfonylurea derivatives, however, are susceptible to hydrolysis at physiological conditions.[16] In a previous communication of our synthetic studies of 1,2,4-benzothiadiazine 1,1-dioxides, 2,10-dihydro-10-hydroxy-3*H*-imidazo[1,2-*b*][1,2,4]benzothiadiazine 6,6-dioxide (**3**), which contains a built-in sulfonylhydroxyguanidine moiety, was found to exhibit activity against several tumor cell lines, including KB, COLO 205, TSGH 8302, and HepG2.[17,18] Nevertheless, hydroxyguanidine, which is considered to combine the imino group of guanidine with the hydroxylamino group of hydroxyurea, has been reported to exhibit potent antiviral and anticancer activities by inhibition of ribonucleotide reductase.[19−22] On the basis of these precedents, sulfonyl-*N*-hydroxyguanidine derivatives such as compounds **4a**−**r** can be regarded as bioisosters of sulfonylurea, which may possess increased stability. This paper herein describes the synthesis and biological evaluation of compounds **4a**−**r** as anticancer agents.



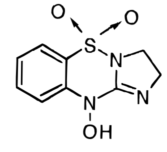
R=



(**1**, LY-181984)



(**2**, LY-186641)



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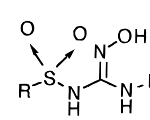

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Chemistry

The target compounds indicated in Table 4 were synthesized as outlined in Scheme 1. Starting with sulfonyl chloride **5**, amination and condensation of the resulting sulfonamide derivatives **6** (Table 1) with isothiocyanates were used for the preparation of the thiourea derivatives **7** (Table 2). Sulfonylthioureas are susceptible to nucleophilic attack. For example, these compounds decomposed if recrystallized from alcohol. Even when dissolved in DMSO-d_6 for NMR spectro-

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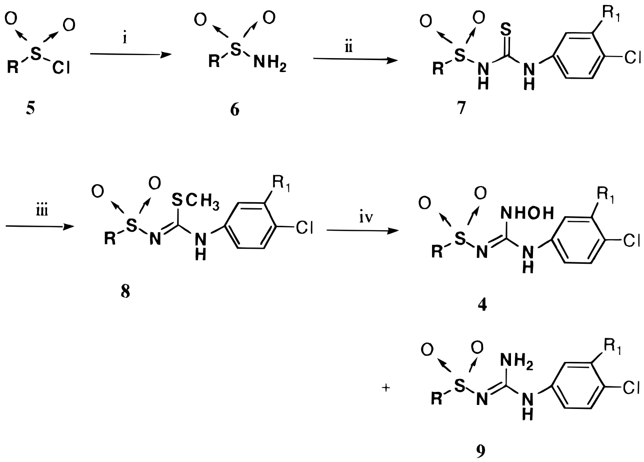
Scheme 1[a]



[a] (i) Liquid NH_3, CH_2Cl_2, −78 °C, 10 min; (ii) (a) 1 N NaOH, isothiocyanate, acetone, room temperature, 4−12 h; (b) 1 N HOAc; (iii) (a) 1 N NaOH, MeI, acetone, room temperature, 30 min; (b) 1 N HOAc; (iv) $NH_2OH{\cdot}HCl$, Et_3N, CH_3CN, 80−90 °C, 10−20 h or DMF, room temperature, 2−3 days.

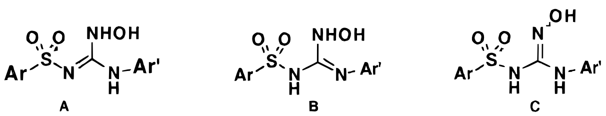


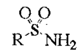
Figure 1. Three possible tautomeric forms of N,N′-disubstituted *N*″-hydroxyguanidine.

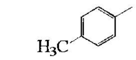
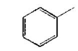
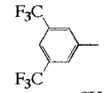
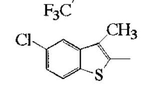
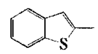
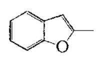
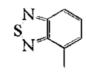
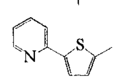
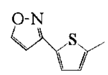
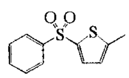
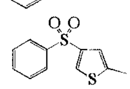
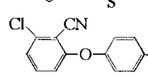
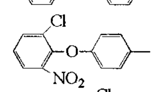
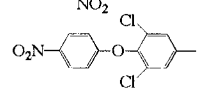
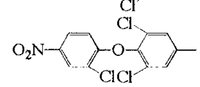
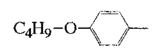
scopic analysis, the compounds gradually decomposed, in agreement with a previous report by J. E. Toth et al.[16] Therefore, compounds **7** was directly treated with methyl iodide without isolation to afford the methylpseudothiourea derivative **8** (Table 3), which was subsequently reacted with hydroxylamine hydrochloride to obtain the target compounds **4a**−**r** (Table 4). The guanidine derivatives **9** were also isolated as side products, probably due to ammonia salt contamination of the hydroxylamine hydrochloride. The low yield for compounds **4a**−**r** was due to the intensive column chromatography to separate the compounds **4a**−**r** and **9**, which were close to each other in the column.

The sulfonyl-*N*-hydroxyguanidine moiety of compound **4** can be illustrated in three tautomeric forms (Figure 1). The preferred tautomeric form is **A** and was elucidated from the following evidence. The ^{1}H NMR spectrum of **4g** exhibited two D_2O exchangeable proton peaks at δ 9.40 and 9.74. The former peak corresponded to one proton which was assigned to NH whereas the latter peak was assigned to NH and OH protons. To investigate the tautomeric state of this type of compound, *N*-(4-chlorophenyl)-*N*′-(benzo[2,1,3]thiadiazol-4-ylsulfonyl)-*N*″-hydroxyguanidine (**4g**) was chosed as a model compound. In the HMBC NMR spectrum of **4g**, the carbon signal (δ 125.0, C2 and C6) of the chlorophenyl group showed a ^{13}C−^{1}H long-range correlation with the N−H signal (δ 9.47). However, the absence of a long-range correlation between the N−H and the benzo moiety of the benzo[2,1,3]thiadiazole ring presented their unambiguous identification. Therefore, the HMBC NMR spectrum of **4g** is consistent with tautomers A and C, but not B.

The structure of **4g** was further investigated by X-ray crystallography (Figure 2). The three-dimensional structure of **4g** and lattice packing of **4g** surprisingly reveals base stacking (Figure 3). The bond distances and bond

Table 1. Chemical and Physical Properties of Sulfonamide Derivatives **5a**−**p**



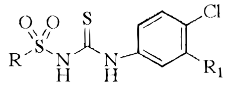
No.	R	mp, °C	yield, %	formula
6a		142-143 (H/EA)[a]	85.7	$C_7H_9NO_2S$
6b		155-158 (W)	90.2	$C_6H_7NO_2S$
6c		182 (W/E)	61.4	$C_8H_5F_6NO_2S$
6d		264-265 (M)	99.9	$C_9H_8ClNO_2S_2$
6e		210-211 (M/W)	7.8	$C_8H_7NO_2S_2$
6f		160-161 (M/W)	10.2	$C_8H_7NO_3S$
6g		134 (W/E)	100	$C_6H_5N_3O_2S_2.1/2H_2O$
6h		211-212 (M)	85.4	$C_9H_8N_2O_2S_2$
6i		171 (W/E)	96.5	$C_7H_6N_2O_3S_2$
6j		179 (M/W)	96	$C_{10}H_9NO_4S_3$
6k		157-158 (W)	89.9	$C_{10}H_9NO_4S_3$
6l		196-197 (W/E)	99.8	$C_{13}H_9ClN_2O_3S$
6m		181 (W/E)	94.8	$C_{12}H_9ClN_2O_5S$
6n		181 (W/E)	65.4	$C_{12}H_8Cl_2N_2O_5S$
6o		236 (W/E)	92.1	$C_{12}H_7Cl_3N_2O_5S$
6p		108 (W/E)	76.2	$C_{10}H_{15}NO_3S$

[a] Recrystallized from E (ethanol), EA (ethyl acetate), H (*n*-hexane), M (methanol), and W (water).

angles of **4g** are of interest to the issue of the tautomerism in **4g**. By comparison of these values with those of normal bases,[23] it was concluded that these molecules mostly adopt the **A** form in equilibrium with the other minor forms shown in Figure 1. The sulfonyl group together with the guanidine moiety adopts a planar conformation with the torsion angle of N3−C7−N5−C8 being nearly 0° (−7°) and the C2, S1, N3, C7, N5, and C8 atoms lying in a least-squares plane with mean deviation of 0.3 Å. The plane of the guanidine moiety is nearly perpendicular to the base plane whereas the hydrophobic ring systems cluster together into hydrophobic pockets in the crystal lattices.

There are three possible tautomers of **4g**. The bond lengths of **4g** in crystal, especially the bond distances of C7−N3, C7−N4, and C7−N5, do not unambigiously suggest that tautomer **A** is most favored. The differences in the total energy associated with these three

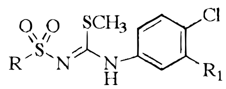
Table 2. Chemical, Physical Properties, and Cytotoxic Activities of N,N′-Disubstituted Thioureas **7a**,**b**,**d**,**g**,**j**,**k**,**q**,**r**



						IC50 (μg/mL)[a]				
no.	R	R_1	mp, °C	yield, %	formula	COLO 205	Hep-G2	KB	TSGH 8302	MOLT-4[b]
7a	(4-methylphenyl)sulfonyl	H	172–173 (E)[c]	22.6	$C_{14}H_{13}ClN_2O_2S_2$	87	232	80	125	59
7b	phenylsulfonyl	H	165–168 (T)	7.2	$C_{13}H_{11}ClN_2O_2S_2$	91	>300	66	>300	63
7d	(5-chloro-3-methylbenzo[*b*]thiophene-2-yl)sulfonyl	H	188–189 (AN)	91.0	$C_{16}H_{12}Cl_2N_2O_2S_3$	52	57	61	55	12
7g	benzo[2,1,3]thiadiazol-4-ylsulfonyl	H	192–193 (AN)	26.9	$C_{13}H_9ClN_4O_2S_3$	50	124	63	42	55
7j	5-(phenylsulfonyl)thiophene	H	156–157 (T/AC)	44.3	$C_{17}H_{12}ClN_2O_4S_4Na\cdot{}^3/_2H_2O$	65	216	66	79	59
7k	[4-(phenylsulfonyl)thiophene-2-yl]sulfonyl	H	257–258 (C/T)	12.8	$C_{17}H_{13}ClN_2O_4S_4\cdot{}^1/_3H_2O$	220	214	196	217	ND
7q	(4-methylphenyl)sulfonyl	Cl	252–253 (T/AC)	88.0	$C_{14}H_{11}Cl_2N_2O_2S_2Na\cdot 2H_2O$	45	120	41	55	39
7r	(5-chloro-3-methylbenzo[*b*]thiophene-2-yl)sulfonyl	Cl	256 (T/AC)	80.7	$C_{16}H_{10}Cl_3N_2O_2S_3Na\cdot{}^3/_2H_2O$	57	62	25	59	65
1						83	>300	72	106	33

[a] The cytotoxicity tests were replicated two times. Each treatment has three replications. The measurement of IC_{50} is described in Materials and Methods. [b] Percent inhibition in 20 μg/mL. [c] Recrystallized from AC (acetone), AN (acetonitrile), C (chloroform), E (ethyl acetate), and T (toluene).

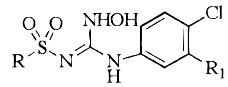
Table 3. Chemical and Physical Properties of N,N′-Disubstituted *S*-Methylpseudothioureas **8a**–**r**



no.	R	R_1	mp, °C	yield, %	formula
8a	(4-methylphenyl)sulfonyl	H	173–174 (M)[a]	86.5	$C_{15}H_{15}ClN_2O_2S_2$
8b	phenylsulfonyl	H	139–140 (M)	85	$C_{14}H_{13}ClN_2O_2S_2$
8c	[3,5-bis(trifluoromethyl)phenyl]sulfonyl	H	110 (M)	98.4	$C_{16}H_{11}ClF_6N_2O_2S_2$
8d	(5-chloro-3-methylbenzo[*b*]thiophene-2-yl)sulfonyl	H	189–190 (E/C)	72.8	$C_{17}H_{14}Cl_2N_2O_2S_3$
8e	benzo[*b*]thiophene-2-ylsulfonyl	H	189–190 (M)	91.0	$C_{16}H_{13}ClN_2O_2S_3$
8f	benzofuran-2-ylsulfonyl	H	163–164 (M)	48.7	$C_{16}H_{13}ClN_2O_3S_2$
8g	benzo[2,1,3]thiadiazol-4-ylsulfonyl	H	216–217 (E/AC)	83.7	$C_{14}H_{11}ClN_4O_2S_3$
8h	(2-pyrid-2-ylthiophene-5-yl)sulfonyl	H	206–207 (E/M)	94.9	$C_{17}H_{14}ClN_3O_2S_3$
8i	(5-isoxazol-3-ylthiophene-5-yl)sulfonyl	H	147 (M)	55.7	$C_{15}H_{12}ClN_3O_3S_3$
8j	5-(phenylsulfonyl)thiophene	H	167–168 (E/C)	75.9	$C_{18}H_{15}ClN_2O_4S_4$
8k	[4-(phenylsulfonyl)thiophene-2-yl]sulfonyl	H	98–99 (M)	43.1	$C_{18}H_{15}ClN_2O_4S_4\cdot{}^1/_2H_2O$
8l	[4-(3-chloro-2-cyanophenoxy)phenyl]sulfonyl	H	198 (E)	81.1	$C_{21}H_{15}Cl_2N_3O_3S_2$
8m	[4-(2-chloro-6-nitrophenoxy)phenyl]sulfonyl	H	196 (M)	95.3	$C_{20}H_{15}Cl_2N_3O_5S_2$
8n	[3,5-dichloro-4-(4-nitrophenoxy)phenyl]sulfonyl	H	165 (M)	99.6	$C_{20}H_{14}Cl_3N_3O_5S_2$
8o	[3,5-dichloro-4-(2-chloro-4-nitrophenoxy)phenyl]sulfonyl	H	184 (M)	89.7	$C_{20}H_{13}Cl_4N_3O_5S_2$
8p	(4-*n*-butoxyphenyl)sulfonyl	H	142 (M)	85.6	$C_{18}H_{21}ClN_2O_2S_2\cdot{}^1/_4H_2O$
8q	(4-methylphenyl)sulfonyl	Cl	133–134 (M)	96.9	$C_{15}H_{14}Cl_2N_2O_2S_2$
8r	(5-chloro-3-methylbenzo[*b*]thiophene-2-yl)sulfonyl	Cl	166–167 (E)	99	$C_{17}H_{13}Cl_3N_2O_2S_3$

[a] Recrystallized from AC (acetone), C (chloroform), E (ethanol), and M (methanol).

Table 4. Chemical, Physical Properties, and Cytotoxic Activities of N,N′-Disubstituted Sulfonyl-*N*-hydroxyquanidines **4a**−**r** against Human Tumor Cell Lines



no.	R	R_1	mp, °C	yield, %	formula	IC$_{50}$ (μg/mL) COLO 205[a]	Hep-G2	KB	TSGH 8302	MOLT-4
4a	(4-methylphenyl)sulfonyl	H	201−203 (M)[b]	12.7	$C_{14}H_{14}ClN_3O_3S$	58	49	46	46	6
4b	phenylsulfonyl	H	200−201 (M)	27.2	$C_{13}H_{12}ClN_3O_3S$	53	54	46	52	7
4c	[3,5-bis(trifluoromethyl)phenyl]sulfonyl	H	192 (M/W)	21.9	$C_{15}H_{10}ClF_6N_3O_3S$	58	233	57	66	44
4d	(5-chloro-3-methylbenzo[*b*]thiophene-2-yl)sulfonyl	H	245−247 (M)	40.9	$C_{16}H_{13}Cl_2N_3O_3S$	60	140	29	44	38
4e	benzo[*b*]thiophene-2-ylsulfonyl	H	264−266 (M)	27.6	$C_{15}H_{12}ClN_3O_3S_2$	80	176	55	76	57
4f	benzofuran-2-ylsulfonyl	H	207−209 (M)	63.9	$C_{15}H_{12}ClN_3O_4S$	>200	>200	67	ND	ND
4g	benzo[2,1,3]thiadiazol-4-ylsulfonyl	H	217−218 (M)	31.2	$C_{13}H_{10}ClN_5O_3S_2$	87	45	35	93	33
4h	(2-pyrid-2-ylthiophene-5-yl)sulfonyl	H	210(dec) (AN)	52.2	$C_{16}H_{13}ClN_4O_3S_2$	74	121	61	208	75
4i	(5-isoxazol-3-ylthiophene-5-yl)sulfonyl	H	184 (T)	37.5	$C_{14}H_{11}ClN_4O_4S_2$	52	>300	55	149	9
4j	5-(phenylsulfonyl)thiophene	H	195−196 (M)	11.6	$C_{17}H_{14}ClN_3O_5S_3$	59	92	29	41	ND
4k	[4-(phenylsulfonyl)thiophene-2-yl]sulfonyl	H	195(dec) (C)	19.8	$C_{17}H_{14}ClN_3O_5S_3$	45	175	10	37	22
4l	[4-(3-chloro-2-cyanophenoxy)phenyl]sulfonyl	H	199(dec) (M)	44.1	$C_{20}H_{14}Cl_2N_4O_4S$	178	>300	39	168	95
4m	[4-(2-chloro-6-nitrophenoxy)phenyl]sulfonyl	H	199 (AN)	35.2	$C_{19}H_{14}Cl_2N_4O_6S$	>100	>100	83	53	ND
4n	[3,5-dichloro-4-(4-nitrophenoxy)phenyl]-sulfonyl	H	212−214 (M)	23.9	$C_{19}H_{13}Cl_3N_4O_6S$	12	22	12	7	55
4o	[3,5-dichloro-4-(2-chloro-4-nitrophenoxy)-phenyl]sulfonyl	H	180 (C)	30.6	$C_{19}H_{12}Cl_4N_4O_6S{\cdot}H_2O$	6	49	7	7	56
4p	(4-*n*-butoxyphenyl)sulfonyl	H	175−176 (M)	34.3	$C_{17}H_{20}ClN_3O_3S$	49	63	50	37	18
4q	(4-methylphenyl)sulfonyl	Cl	195(dec) (M)	24.9	$C_{14}H_{13}Cl_2N_3O_3S$	10	72	8	67	7
4r	(5-chloro-3-methylbenzo[*b*]thiophene-2-yl)sulfonyl	Cl	232−233 (AN/E/EA)	24.8	$C_{16}H_{12}Cl_3N_3O_3S_2$	>300	>300	>300	>300	>300

[a] The cytotoxicity tests were replicated two times. Each treatment has three replications. The measurement of IC$_{50}$ is described in Materials and Methods. [b] Recrystallized from AC (acetone), AN (acetonitrile), C (chloroform), E (ethanol), EA (ethyl acetate), M (methanol), T (toluene), and W (water).

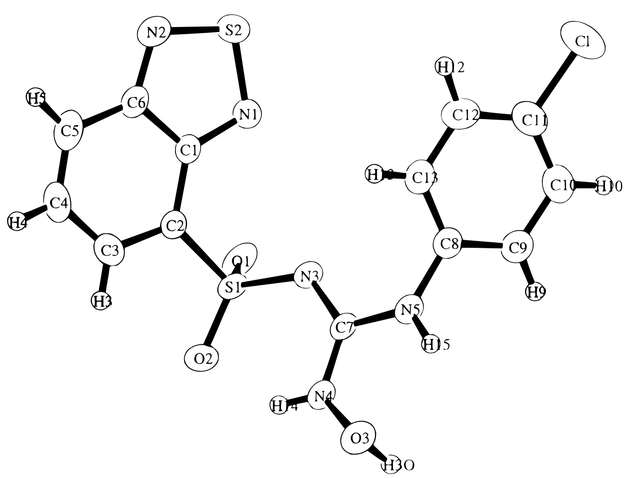


Figure 2. ORTEP drawing of *N*-(4-chlorophenyl)-*N*′-(benzo-[2,1,3]thiadiazol-4-ylsulfonyl)-*N*″-hydroxyguanidine (**4g**).

tautomers are 4.3 kcal/mol between tautomers **A** and **B** and 1.8 kcal/mol between tautomers **A** and **C**, based on the calculation using MOPAC[24] with PM3 force field parameters.[25] Although tautomer **A** is the most energetically favored, the bond distances of C7−N3, C7−N4, and C7−N5 are not equal to either pure C−N single or double bond lengths. This suggests some degree of coexistence of the three forms and/or some degree of delocalization among these bonds. However, the clear location of the proton density around N5 with correct geometry in the X-ray crystallography (Figure 2) indicates that tautomer **A** predominates in crystals. Hence, on the basis of the HMBC NMR spectrum and X-ray crystallograph, studies illustrated that tautomer **A** is overall most favored in this type of compound.

Results and Discussion

The sulfonylthioureas **7a**,**b**,**d**,**g**,**j**,**k**,**q**,**r** and sulfonyl-*N*-hydroxyguanidine derivatives **4a**−**r** were evaluated by the MTT assay for *in vitro* cytotoxicity against five human tumor cell lines, including human hepatocellular carcinoma (HepG2), human epidermoid cervical carcinoma (TSGH 8302), human colon adenocarcinoma (COLO 205), human epidermoid oral carcinoma (KB), and human acute lymphoblastic leukemia (MOLT-4). The human COLO 205, HepG2, KB, and TSGH 8302 cell lines were employed as a small panel for the

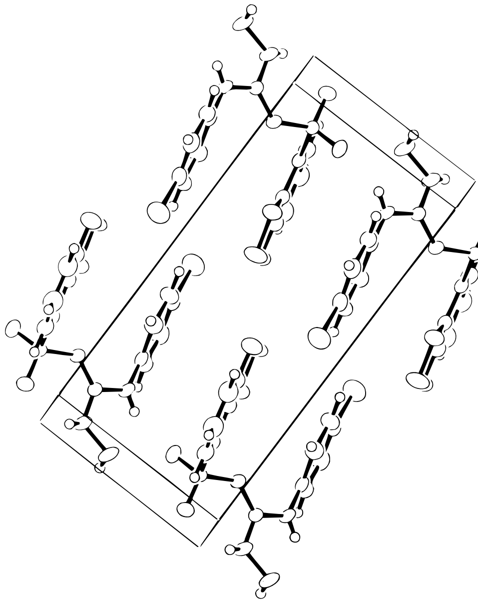


Figure 3. The lattice packing diagram of *N*-(4-chlorophenyl)-*N'*-[(benzo[2,1,3]thiadiazol-4-ylsulfonyl)-*N''*-hydroxyguanidine (**4g**).

screening of new antisolid tumor agents whereas MOLT-4 was used as a representative human blood cell. The criteria for selection of an agent for further *in vivo* investigation of drug efficacy was a demonstration of greater activity against COLO 205, HepG2, KB, and TSGH 8302 cells compared to MOLT-4 cells in the *in vitro* cytotoxicity assay.

The cytotoxic activities of these compounds are presented in Table 2 and 4. Although the thiourea derivatives **7a**,**b**,**d**,**g**,**j**,**k**,**q**,**r** were as active as LY 181984, they were as subject to nucleophilic attack as compound **1**. As shown in Table 4, replacement of the urea moiety of the lead compound LY 181984 with *N*-hydroxyguanidine produced compounds **4a**−**c** with similar cytotoxicity as LY-181984 in all cell lines. Compounds **4a**,**b** were more active against HepG2 and Molt-4 than LY181984 whereas compounds **4d**−**k** were as active as **4a**. This indicates that the aromatic ring attached to the sulfonyl moiety of **4a** can be substituted with different heterocycles such as benzothiophene, benzofuran, benzo[2,1,3]-thiadiazole, and thiophene without substantially affecting the general cytotoxicity of this class of compounds. Introduction of a butyloxy group (**4p**) at the para position of the phenylsulfonyl group of **4b** also retained the activity, indicating that the para position of the phenylsulfonyl moiety can tolerate a bulky substituent. However, replacement of the butyloxy moiety with a phenoxy, such as **4l** and **4m**, dramatically reduced activity. Activity, however, was significantly enhanced in compounds **4n** and **4o**, in which two chloro atoms were introduced at the 3'- and 5'-positions of the attached phenylsulfonyl moiety.

Compound **4o** exhibited enhanced activity against COLO 205 (IC_{50}, 6.32 μg/mL), KB (IC_{50}, 6.70 μg/mL), and TSGH 8302 (IC_{50}, 7.15 μg/mL) cells compared with that of MOLT-4 cells (IC_{50}, 55.88 μg/mL). Compound **4o** was therefore selected for further drug development. The activity of **4o** against solid tumors was examined in a murine K-1735/M2 melanoma xenograft model. LY-181984 or **4o** was given orally with a daily dose of 300 mg/kg for two 5-consecutive-day periods [(qid × 5)2]

Table 5. Antitumor Activity of LY181984 and **4o** against Murine K1735/M2 Melanoma Xenograft[a]

agent	dose (mg/kg)	administered route	treatment schedule (day)	body weight change (g)	TGI (%)
control	vehicle only	po	5−9, 12−16	3.0	
LY181984	300	po	5−9, 12−16	−1.2	64.7
4o	300	po	5−9, 12−16	1.3	70.7

[a] Tumor growth inhibition (TGI %) was determined at day 20 after tumor transplantation. Body weight change (gm/mice) was calculated from day 0 to day 20. The TGI % and tumor weight were estimated as described in Materials and Methods.

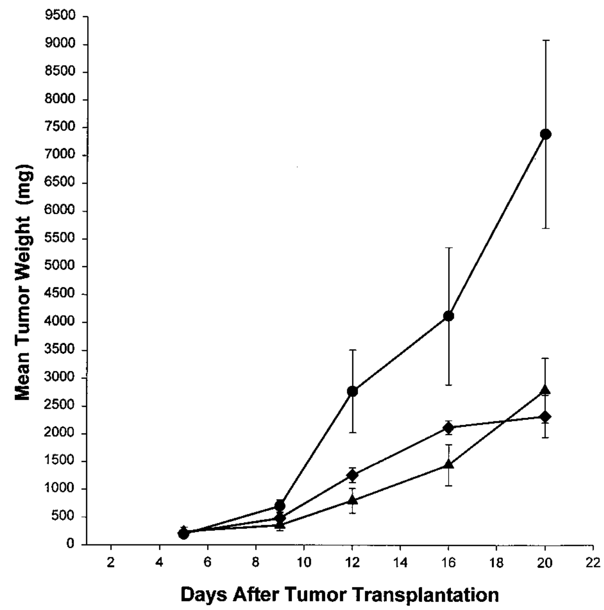


Figure 4. Tumor growth curves of murine K-1735/M2 melanoma xenograft treated with LY181984 and **4o**. Five days following tumor transplantation, mice were treated po with a daily dose of 300 mg/kg LY181984 or **4o** for two cycles of 5 consecutive days [(Q1DX5)2] at day 5 and day 12. Control mice were administered po with 2.5% cremophor. Each point, mean tumor weight (mg), were from five animals/group: (●) control, (▲) LY181984, (◆) **4o**.

starting on days 5 and 12. The tumor growth inhibition (TGI %) of LY-181984 and **4o** were 64.7% and 70.7%, respectively, 20 days after tumor transplantation (Table 5). Both LY-181984 and **4o** delayed the growth of solid melanoma tumor in the animal model (Figure 4). The mean body weight of mice increased 3 g in the control group and increased 1.3 g in the **4o** treatment group, but decreased 1.2 g in the LY-181984 treatment group, suggesting that **4o** was less toxic than LY-181984.

In summary, new *N*-hydroxyguanidine derivatives were synthesized *via* a bioisosteric displacement of the ureido moiety of LY 181984 with hydroxyguanidine. These molecules are chemically stable and displayed potent inhibitory properties against several solid tumor lines *in vitro*. No pharmacological mechanism has yet been determined to explain these effects.

Experimental Section

General Methods. Melting points were obtained on an Electrothermal apparatus and are uncorrected. ^{1}H and ^{13}C nuclear magnetic resonance spectra were recorded either on a JEOL JNM-EX400 spectrometer at the National Taiwan Normal University or on a Bruker Model AM 300 spectrometer at the National Taiwan University, Taipei, and are reported in parts per million with DMSO-d_6 as internal standard on a

δ scale. EI mass spectra were recorded on a JEOL JMS-D100 mass spectrometer at the National Taiwan University. Elemental analyses for C, H, and N were carried out either on a Heraeus elemental analyzer at the Cheng-Kong University, Tainan, or on a Perkin-Elmer 240 elemental analyzer in the National Taiwan University, Taipei, and were within ±0.4% of the theoretical values.

Preparation of Sulfonamides (6a–d,g–p): General Procedure. Liquid ammonia (20 mL) was added to a solution containing appropriate sulfonyl chloride (10 g, 52.45 mmol) in dichloromethane (100 mL) at −78 °C. After the mixture was stirred at −78 °C for 4 h, precipitates were removed by filtration and the filtrate was concentrated *in vacuo* to remove the solvent. The residue was then recrystallized from the appropriate solvent to give the desired compounds. The mp and yield data are summarized in Table 1. The analytical data are given below.

4-Toluenesulfonamide (6a): MS *m/z* 171 (M^+); ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.44 (s, 3H, CH_3), 4.89 (s, 2H, NH_2), 7.32 (d, J = 8.2 Hz, 2H, Ar*H*), 7.82 (d, J = 8.2 Hz, 2H, Ar*H*).

Benzenesulfonamide (6b): MS *m/z* 158 (M^+); ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.92 (s, 2H, NH_2), 7.60−7.51 (m, 3H, Ar*H*), 7.96−7.92 (m, 2H, Ar*H*).

3,5-Bis(trifluoromethyl)benzenesulfonamide (6c): MS *m/z* 293 (M^+); ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.76 (s, 2H, NH_2), 8.40 (s, 2H, Ar*H*), 8.44 (s, 1H, Ar*H*); ^{13}C NMR (75 MHz, DMSO-d_6) δ 122.6 (q, J = 270 Hz, CF_3), 125.8, 126.4, 131.2 (q, J = 34 Hz, CCF_3), 146.5. Anal. ($C_8H_5F_6NO_2S$) C, H, N.

5-Chloro-3-methylbenzo[*b*]thiophene-2-sulfonamide (6d): MS *m/z* 261 (M^+); ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.60 (s, 3H, CH_3), 7.53 (dd, J = 8.7 Hz, J = 2.0 Hz, 1H, Ar*H*), 7.88 (s, 2H, NH_2), 7.99 (d, J = 2.0 Hz, 1H, Ar*H*), 8.06 (d, J = 8.7 Hz, 1H, Ar*H*); ^{13}C NMR (75 MHz, DMSO-d_6) δ 12.3, 123.5, 125.1, 127.5, 130.7, 133.9, 136.7, 141.3, 141.9. Anal. ($C_9H_8ClO_2S_2$) C, H, N.

Benzo[*b*]thiophene-2-sulfonamide (6e): To a solution of benzothiophene (2.23 g, 16.6 mmol) in THF (40 mL) at room temperature was added 1.6 M *n*-butyllithium in hexane (10.4 mL, 16.6 mmol). The reaction mixture was refluxed for 4 h and then evaporated to dryness *in vacuo*. To the residue were added water (100 mL), sodium acetate (10.89 g, 0.13 mol), and hydroxylamine *O*-sulfonic acid (6.26 g, 0.05 mol). The mixture was allowed to stir at room temperature for 8 h and was then ether extracted (75 mL × 2). The organic layer was extracted with 1 N sodium hydroxide (50 mL × 3). The aqueous layer was collected and neutralized with 1 N hydrochloride solution followed by extraction with dichloromethane (50 mL × 3). The organic layer was collected and dried over anhydrous sodium sulfate. Concentration *in vacuo* yielded a yellow solid which was recrystallized from methanol and water (1:1) to give **6e** (0.28 g, 7.8%): mp 210−211 °C; MS *m/z* 213 (M^+); ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.46−7.54 (m, 2H, Ar*H*), 7.87 (s, 2H, NH_2), 7.92 (s, 1H, Ar*H*), 8.00−8.09 (m, 2H, Ar*H*); ^{13}C NMR (75 MHz, DMSO-d_6) δ 123.4, 125.8, 126.0, 127.1, 127.3, 138.0, 140.6, 146.2. Anal. ($C_8H_7NO_2S_2$) C, H, N.

Benzofuran-2-sulfonamide (6f) was prepared in 10.2% yield starting from benzofuran following the same procedures as for **6e**. An analytical sample was prepared by recrystallization from water and methanol (4:1): mp 160−161 °C; MS *m/z* 197 (M^+); ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.36−7.41 (m, 1H, Ar*H*), 7.45 (d, J = 1.1 Hz, 1H, furan-*H*), 7.48−7.54 (m, 1H, Ar*H*), 7.71 (d, J = 7.9 Hz, 1H, Ar*H*), 7.80 (d, J = 7.3 Hz, 1H, Ar*H*), 8.01 (s, 2H, NH_2); ^{13}C NMR (75 MHz, DMSO-d_6) δ 109.4, 112.3, 123.5, 124.6, 127.7, 154.9. Anal. ($C_8H_7NO_3S$) C, H, N.

Benzo[2,1,3]thiadiazole-4-sulfonamide (6g): MS *m/z* 215 (M^+); ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.65 (s, 2H, NH_2), 7.86 (dd, J = 8.7 Hz, J = 7.1 Hz, 1H, Ar*H*), 8.19 (d, J = 7.1 Hz, 1H, Ar*H*), 8.36 (d, J = 8.7 Hz, 1H, Ar*H*); ^{13}C NMR (75 MHz, DMSO-d_6) δ 125.4, 128.7, 128.9, 135.0, 148.8, 155.0. Anal. ($C_6H_5N_3O_2S_2 \cdot {}^1/_2H_2O$) C, H, N.

2-Pyrid-2-ylthiophene-5-sulfonamide (6h): MS *m/z* 240 (M^+); ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.37 (dd, J = 7.2 Hz, J = 5.0 Hz, 1H, Ar*H*), 7.56 (d, J = 3.8 Hz, 1H, thiophene-*H*), 7.73 (s, 2H, NH_2), 7.79 (d, J = 3.8 Hz, 1H, thiophene-*H*), 7.89 (td, J = 7.3 Hz, J = 1.7 Hz, 1H, Ar*H*), 8.01 (d, J = 7.4 Hz, 1H, Ar*H*), 8.57 (dd, J = 5.0 Hz, J = 1.7 Hz, 1H, Ar*H*); ^{13}C NMR (75 MHz, DMSO-d_6) δ 119.6, 124.0, 125.0, 131.2, 137.9, 146.9, 149.1, 150.0, 151.0. Anal. ($C_9H_8N_2O_2S_2$) C, H, N.

5-Isoxazol-3-ylthiophene-2-sulfonamide (6i): MS *m/z* 230 (M^+); ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.06 (s, 1H, isoxazole-*H*), 7.62 (d, J = 4.2 Hz, 1H, thiophene-*H*), 7.69 (d, J = 4.2 Hz, 1H, thiophene-*H*), 7.89 (s, 2H, NH_2), 8.70 (s, 1H, isoxzole-*H*); ^{13}C NMR (75 MHz, DMSO-d_6) δ 101.3, 127.5, 130.8, 131.7, 147.5, 152.0, 162.0. Anal. ($C_7H_6N_2O_3S_2$) C, H, N.

2-(Phenylsulfonyl)thiophene-5-sulfonamide (6j): MS *m/z* 303 (M^+); ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.60 (d, J = 3.8 Hz, 1H, thiophene-*H*), 7.68 (t, J = 7.3 Hz, 2H, Ar*H*), 7.77 (t, J = 7.3 Hz, 1H, Ar*H*), 7.88 (d, J = 3.8 Hz, 1H, thiophene-*H*), 8.02 (s, 2H, NH_2), 8.02 (d, J = 7.3 Hz, 2H, Ar*H*); ^{13}C NMR (75 MHz, DMSO-d_6) δ 127.7, 130.5, 130.8, 134.9, 140.8, 145.7, 153.4. Anal. ($C_{10}H_9NO_4S_3$) C, H, N.

4-(Phenylsulfonyl)thiophene-2-sulfonamide (6k): MS *m/z* 303 (M^+); ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.65−7.77 (m, 4H, Ar*H*), 7.88 (s, 2H, NH_2), 8.02 (d, J = 7.1 Hz, 2H, Ar*H*), 8.70 (d, J = 1.6 Hz, 1H, thiophene-*H*); ^{13}C NMR (75 MHz, DMSO-d_6) δ 127.4, 127.7, 127.7, 130.4, 134.6, 137.0, 140.8, 149.5. Anal. ($C_{10}H_9NO_4S_3$) C, H, N.

4-(3-Chloro-2-cyanophenoxy)benzenesulfonamide (6l): MS *m/z* 308 (M^+ − 1); ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.11 (d, J = 8.3 Hz, 1H, Ar*H*), 7.36 (d, J = 8.7 Hz, 2H, Ar*H*), 7.41 (s, 2H, NH_2), 7.57 (d, J = 8.2 Hz, 1H, Ar*H*), 7.73 (t, J = 8.3 Hz, 1H, Ar*H*), 7.90 (d, J = 8.7 Hz, 2H, Ar*H*); ^{13}C NMR (75 MHz, DMSO-d_6) δ 106.2, 113.4, 118.0, 119.6, 125.7, 128.7, 136.5, 137.1, 141.0, 157.7, 159.4. Anal. ($C_{13}H_9ClN_2O_3S$) C, H, N.

4-(2-Chloro-6-nitrophenoxy)benzenesulfonamide (6m): MS *m/z* 229 (M^+); ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.07 (d, J = 8.7 Hz, 2H, Ar*H*), 7.32 (s, 2H, NH_2), 7.63 (t, J = 8.2 Hz, 1H, nitrophenoxy-*H*), 7.81 (d, J = 8.7 Hz, 2H, Ar*H*), 8.07 (dd, J = 8.0 Hz, J = 1.0 Hz, 1H, nitrophenoxy-*H*), 8.19−8.15 (m, 1H, nitrophenoxy-*H*); ^{13}C NMR (75 MHz, DMSO-d_6) δ 115.2, 125.0, 127.9, 128.1, 129.2, 136.0, 138.8, 142.2, 144.3, 158.6. Anal. ($C_{12}H_9ClN_2O_5S$) C, H, N.

3,5-Dichloro-4-(4-nitrophenoxy)benzenesulfonamide (6n): MS *m/z* 362 (M^+); ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.15 (d, J = 9.1 Hz, 2H, Ar*H*), 7.71 (s, 2H, NH_2), 8.05 (s, 2H, Ar*H*), 8.26 (d, J = 9.1 Hz, 2H, Ar*H*); ^{13}C NMR (75 MHz, DMSO-d_6) δ 115.7, 126.4, 127.1, 129.1, 143.1, 147.6, 160.2. Anal. ($C_{12}H_8Cl_2N_2O_5S$) C, H, N.

4-(2-Chloro-4-nitrophenoxy)-3,5-dichlorobenzenesulfonamide (6o): MS *m/z* 229 (M^+); ^{1}H NMR (300 MHz, DMSO-d_6) δ 6.95 (d, J = 9.2 Hz, 1H, Ar*H*), 7.72 (s, 2H, NH_2), 8.13 (dd, J = 9.0 Hz, J = 3.1 Hz, 2H, Ar*H*), 8.52 (d, J = 3.2 Hz, 1H, Ar*H*); ^{13}C NMR (75 MHz, DMSO-d_6) δ 114.8, 122.0, 124.6, 126.5, 127.1, 128.8, 143.2, 144.0, 147.4, 155.5. Anal. ($C_{12}H_7Cl_3N_2O_5S$) C, H, N.

4-*n*-Butoxybenzenesulfonamide (6p): MS *m/z* 362 (M^+); ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.92 (t, J = 7.3 Hz, 3H, CH_3), 1.49−1.36 (m, 2H, CH_2), 1.75−1.64 (m, 2H, CH_2), 4.03 (t, J = 6.4 Hz, 2H, OCH_2), 7.06 (d, J = 8.6 Hz, 2H, Ar*H*), 7.16 (s, 2H, NH_2), 7.72 (d, J = 8.7 Hz, 2H, Ar*H*); ^{13}C NMR (75 MHz, DMSO-d_6) δ 13.6, 18.6, 30.5, 67.6, 114.4, 127.6, 136.0, 161.0. Anal. ($C_{10}H_{15}NO_3S$) C, H, N.

Preparation of N,N′-Disubstituted Thioureas 7a,b,d,-g,j,k,q,r: General Procedure. Sodium hydroxide solution (1 N, 1 mL, 1 mmol) was added to a solution containing the appropriate sulfonamide (1.0 mmol) in acetone (25 mL). After the mixture was stirred at room temperature for 30 min, the appropriate isothiocyanate (1.0 mmol) was added. After 4 h of reflux, the mixture was cooled to room temperature and neutralized with 1 N acetic acid solution to pH 5. The mixture was allowed to stir at room temperature for 30 min before water (45 mL) was added to produce a white precipitate which was collected by filtration. After the solid was dried in the oven, it was recrystallized from the appropriate solvent to give the desired compound. The mp and yield data are summarized in Table 2. The analytical data are given below.

***N*-[(4-Methylphenyl)sulfonyl]-*N′*-(4-chlorophenyl)thiourea (7a):** MS *m/z* 341 (M^+ + 1); ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.40 (s, 3H, CH_3), 7.37−7.48 (m, 6H, Ar*H*), 7.82 (d, J =

7.8 Hz, 2H, Ar*H*), 10.22 (brs, 1H, N*H*); ^{13}C NMR (75 MHz, DMSO-d_6) δ 21.5, 126.1, 126.5, 128.1, 128.9, 129.2, 129.8, 137.7, 144.3, 178.3. Anal. ($C_{14}H_{13}ClN_2O_2S_2$) C, H, N.

***N*-(Phenylsulfonyl)-*N′*-(4-chlorophenyl)thiourea (7b):** MS *m/z* 295 ($M^+ - 32$); ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.39 (d, *J* = 8.1 Hz, 2H, Ar*H*), 7.47 (d, *J* = 8.4 Hz, 2H, Ar*H*), 8.17−7.62 (m, 3H, Ar*H*), 7.94 (d, *J* = 8.1 Hz, 2H, Ar*H*), 10.30 (s, 1H, N*H*). Anal. ($C_{13}H_{11}ClN_2O_2S_2$) C, H, N.

***N*-(4-Chlorophenyl)-*N′*-[(5-chloro-3-methylbenzo[*b*]thiophene-2-yl)sulfonyl]thiourea (7d):** MS *m/z* 398 ($M^+ -$ 33); ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.63 (s, 3H, C*H*$_3$), 7.36 (d, *J* = 8.8 Hz, 2H, Ar*H*), 7.56 (d, *J* = 8.8 Hz, 3H, Ar*H*), 8.03 (d, *J* = 1.9 Hz, 1H, Ar*H*), 8.08 (d, *J* = 8.7 Hz, 1H, Ar*H*), 10.15 (brs, 1H, N*H*); ^{13}C NMR (75 MHz, DMSO-d_6) δ 12.6, 121.2, 123.7, 124.1, 125.0, 125.1, 127.4, 127.6, 128.7, 129.1, 130.6, 138.0, 140.7. Anal. ($C_{16}H_{12}Cl_2N_2O_2S_3$) C, H, N.

***N*-(4-Chlorophenyl)-*N′*-(benzo[2,1,3]thiadiazol-4-yl)sulfonyl)thiourea (7g):** MS *m/z* 350 ($M^+ - 35$); ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.36 (d, *J* = 8.8 Hz, 2H, Ar*H*), 7.51 (d, *J* = 8.8 Hz, 2H, Ar*H*), 7.91 (dd, *J* = 8.8 Hz, 7.1 Hz, Ar*H*), 8.36 (d, *J* = 7.1 Hz, 1H, Ar*H*), 8.43 (d, *J* = 8.8 Hz, 1H, Ar*H*), 10.18 (brs, 1H, N*H*); ^{13}C NMR (75 MHz, DMSO-d_6) δ 120.9, 125.0, 125.8, 127.4, 128.7, 129.0, 129.2, 129.4, 133.1, 155.2. Anal. ($C_{13}H_9ClN_4O_2S_3$) C, H, N.

***N*-(4-Chlorophenyl)-*N′*-[[2-(phenylsulfonyl)thiophene-5-yl]sulfonyl]thiourea (7j):** MS *m/z* 473 (M^+); ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.21 (d, *J* = 9.0 Hz, 2H, Ar*H*), 7.46 (d, *J* = 7.2 Hz, 1H, Ar*H*), 7.63−7.73 (m, 7H, Ar*H*), 7.99 (d, *J* = 7.2 Hz, 2H, Ar*H*), 9.51 (brs, 1H, N*H*); ^{13}C NMR (75 MHz, DMSO-d_6) δ 117.1, 122.3, 125.5, 127.3, 127.4, 128.2, 130.0, 130.3, 132.7, 134.4, 140.2, 143.4. Anal. ($C_{17}H_{13}ClN_2O_4S_4\cdot{}^3/_2H_2O$) C, H, N.

***N*-(4-Chlorophenyl)-*N′*-[[2-(phenylsulfonyl)thiophene-4-yl]sulfonyl]thiourea (7k):** MS *m/z* 351 ($M^+ - 125$); ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.14 (d, *J* = 8.9 Hz, 2H, Ar*H*), 7.46 (d, *J* = 8.9 Hz, 2H, Ar*H*), 7.54 (d, *J* = 1.6 Hz, 1 H, thiophene-*H*), 7.61−7.74 (m, 3H, Ar*H*), 7.94−7.97 (m, 2H, Ar*H*), 8.40 (d, *J* = 1.6 Hz, 1H, thiophene-*H*), 8.67 (brs, 2H, N*H*). Anal. ($C_{17}H_{13}ClN_2O_4S_4\cdot{}^1/_3H_2O$) C, H, N.

***N*-(3,4-Dichlorophenyl)-*N′*-(4-tolylsulfonyl)thiourea (7q):** MS *m/z* 375 (M^+); ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.38 (s, 3H, CH_3), 7.37 (d, *J* = 8.0 Hz, 2H, Ar-H), 7.45 (dd, *J* = 8.8 Hz, *J* = 2.3 Hz, 1H, Ar-H), 7.54 (d, *J* = 8.8 Hz, 1H, Ar-H), 7.79 (d, *J* = 8.2 Hz, 2H, Ar-H), 7.90 (s, 1H, Ar-H), 10.10 (brs, 1H, Ar-H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 21.4, 123.5, 124.6, 126.0, 128.1, 128.2, 129.5, 129.7, 130.6, 130.9, 139.6, 179.4. Anal. ($C_{14}H_{11}Cl_2N_2O_2S_2Na\cdot 2H_2O$) C, H, N.

***N*-(3,4-Dichlorophenyl)-*N′*-[(5-chloro-3-methylbenzo[*b*]thiophen-2-yl)sulfonyl]thiourea (7r):** MS *m/z* 46 (M^+); ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.48 (s, 3H, CH_3), 7.40−7.45 (m, 2H, Ar-H), 7.66 (dd, *J* = 9.0 Hz, *J* = 2.3 Hz, 1H, Ar-H), 7.84 (d, *J* = 2.0 Hz, 1H, Ar-H), 7.95 (d, *J* = 8.6 Hz, 1H, Ar-H), 8.22 (d, *J* = 2.3 Hz, 1H, Ar-H), 9.52 (br s, 1H, Ar-H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 12.3, 120.0, 121.2, 122.6, 122.7, 124.5, 125.9, 129.7, 130.2, 130.6, 131.3, 137.4, 141.4, 141.6, 183.3. Anal. ($C_{16}H_{10}Cl_3N_2O_2S_3Na\cdot H_2O$) C, H, N.

Preparation of N,N′-Disubstituted *S*-methylpseudothioureas 8a−r: General Procedure. Sodium hydroxide solution (1 N, 5.0 mL) was added to a solution containing the appropriate sulfonamide **6a**−**p** (5.0 mmol) in acetone (100 mL). A solution of the appropriate isothiocyanates (5.0 mmol) in acetone (25 mL) was added. After the mixture was stirred at room temperature for 4 h, methyl iodide (5.6 mmol) was added to the filtrate. The reaction mixture was stirred for 30 min before being neutralized with 1 N acetic acid (5.5 mL). The solid was collected and recrystallized from the appropriate solvent. The mp and yield data of the compounds are summarized in Table 3. The analytical data are given below.

***N*-[(4-Methylphenyl)sulfonyl]-*N′*-(4-chlorophenyl)-*S*-methylpseudothiourea (8a):** MS *m/z* 354 (M^+); ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.38 (s, 3H, CH_3), 2.47 (s, 3H, SC*H*$_3$), 7.35−7.38 (m, 4H, Ar*H*), 7.45 (d, *J* = 8.4 Hz, 2H, Ar*H*), 7.74 (d, *J* = 8.0 Hz, 2H, Ar*H*), 9.66 (s, 1H, N*H*); ^{13}C NMR (75 MHz, DMSO-d_6) δ 15.2, 21.3, 126.3, 127.6, 129.1, 129.7, 131.3, 136.7, 139.8, 142.8, 166.4. Anal. ($C_{15}H_{15}ClN_2O_2S_2$) C, H, N.

***N*-(Phenylsulfonyl)-*N′*-(4-chlorophenyl)-*S*-methylpseudothiourea (8b):** MS *m/z* 343 ($M^+ + 2$); ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.47 (s, 3H, SC*H*$_3$), 7.36 (d, *J* = 8.7 Hz, 2H, Ar*H*), 7.45 (d, *J* = 8.7 Hz, 2H, Ar*H*), 7.53−7.66 (m, 3H, Ar*H*), 7.86 (d, *J* = 8.4 Hz, 2H, Ar*H*), 9.71 (s, 1H, N*H*); ^{13}C NMR (100 MHz, DMSO-d_6) δ 14.8, 126.2, 127.2, 128.7, 128.9, 131.3, 132.2, 136.3, 142.3, 166.3. Anal. ($C_{14}H_{13}ClN_2O_2S_2$) C, H, N.

***N*-(4-Chlorophenyl)-*N′*-[[3,5-bis(trifluoromethyl)phenyl]sulfonyl]-*S*-methylpseudothiourea (8c):** MS *m/z* 477 (M^+); ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.56 (s, 3H, SC*H*$_3$), 7.35 (d, *J* = 8.7 Hz, 2H, Ar*H*), 7.45 (d, *J* = 8.7 Hz, 2H, Ar*H*), 8.38 (s, 2H, Ar*H*), 8.45 (s, 1H, Ar*H*), 10.04 (s, 1H, N*H*); ^{13}C NMR (75 MHz, DMSO-d_6) δ 15.4, 123.0 (q, *J* = 271 Hz, C*F*$_3$), 126.5, 126.7, 127.2, 128.2, 129.2, 131.5 (q, *J* = 33.9 Hz, CC*F*$_3$), 136.6, 145.5, 167.7. Anal. ($C_{16}H_{11}F_6ClN_2O_2S_2$) C, H, N.

***N*-(4-Chlorophenyl)-*N′*-[(5-chloro-3-methylbenzo[*b*]thiophene-2-yl]sulfonyl]-*S*-methylpseudothiourea (8d):** MS *m/z* 446 ($M^+ + 1$); ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.56 (s, 3H, C*H*$_3$), 2.57 (s, 3H, SC*H*$_3$) 7.40−7.50 (AB q, *J* = 8.9 Hz, 4H, Ar*H*), 7.55 (dd, *J* = 8.6 Hz, 2.0 Hz, 1H, Ar*H*), 8.00 (d, *J* = 2.0 Hz, 1H, Ar*H*), 8.07 (d, *J* = 8.6 Hz, 1H, Ar*H*); ^{13}C NMR (75 MHz, DMSO-d_6) δ 12.4, 15.4, 123.6, 125.0, 127.5, 127.6, 129.2, 130.7, 131.5, 134.7, 136.6, 137.1, 141.1, 167.3, 167.4. Anal. ($C_{17}H_{14}Cl_2N_2O_2S_3$) C, H, N.

***N*-(4-Chlorophenyl)-*N′*-(benzo[*b*]thiophene-2-ylsulfonyl)-*S*-methylpseudothiourea (8e):** MS *m/z* 398 (M^+); ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.55 (s, 3H, SC*H*$_3$), 7.40−7.55 (m, 6H, Ar*H*), 8.00−8.08 (m, 3H, Ar*H*), 9.86 (s, 1H, N*H*); ^{13}C NMR (75 MHz, DMSO-d_6) δ 35.2, 106.6, 123.3, 123.4, 125.80, 126.2, 127.4, 127.7, 128.2, 129.2, 136.6, 137.7, 141.1, 143.9. Anal. ($C_{16}H_{13}ClN_2O_2S_3$) C, H, N.

***N*-(4-Chlorophenyl)-*N′*-(benzofuran-2-ylsulfonyl)-*S*-methylpseudothiourea (8f):** MS *m/z* 383 (M^+); ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.56 (s, 3H, SC*H*$_3$), 7.36−7.57 (m, 7H, Ar*H*), 7.73−7.81 (m, 2H, Ar*H*), 9.95 (s, 1H, N*H*); ^{13}C NMR (75 MHz, DMSO-d_6) δ 15.0, 110.8, 112.5, 123.6, 124.6, 126.2, 127.7, 127.9, 129.2, 131.7, 136.5, 152.7, 155.2. Anal. ($C_{16}H_{13}ClJN_2O_3S_2$) C, H, N.

***N*-(4-Chlorophenyl)-*N′*-(benzo[2,1,3]thiadiazol-4-ylsulfonyl)-*S*-methylpseudothiourea (8g):** MS *m/z* 339 ($M^+ +$ 1); ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.42 (s, 3H, SC*H*$_3$), 7.38−7.45 (m, 4H, Ar*H*), 7.86 (dd, *J* = 8.8 Hz, 7.1 Hz, 1H, Ar*H*), 8.26 (d, *J* = 7.1 Hz, 1H, Ar*H*), 8.37 (d, *J* = 8.8 Hz, 1H, Ar*H*), 9.85 (s, 1H, N*H*); ^{13}C NMR (75 MHz, DMSO-d_6) δ 14.9, 125.8, 127.0, 128.7, 129.6, 130.9, 133.4, 136.1, 148.8, 155.0, 166.8. Anal. ($C_{14}H_{11}ClN_4O_2S_3$) C, H, N.

***N*-(4-Chlorophenyl)-*N′*-[(2-pyrid-2-ylthiophene-5-yl)sulfonyl]-*S*-methylpseudothiourea (8h):** MS *m/z* 424 (M^+); ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.54 (s, 3H, SC*H*$_3$), 7.36−7.49 (m, 5H, Ar*H*), 7.68 (d, *J* = 4.0 Hz, 1H, thiophene-*H*), 7.81 (d, *J* = 4.0 Hz, 1H, thiophene-*H*), 7.87−7.92 (m, 1H, Ar*H*), 8.04 (d, *J* = 7.8 Hz, 1H, Ar*H*), 8.58 (d, *J* = 4.8 Hz, 1H, Ar*H*), 9.80 (s, 1H, N*H*); ^{13}C NMR (75 MHz, DMSO-d_6) δ 15.3, 119.7, 124.1, 124.9, 127.7, 129.2, 131.5, 132.4, 136.6, 137.9, 144.5, 150.0, 150.9. Anal. ($C_{17}H_{14}ClN_3O_2S_3$) C, H, N.

***N*-(4-Chlorophenyl)-*N′*-[(5-isoxazol-3-ylthiophene-2-yl)sulfonyl]-*S*-methylpseudothiourea (8i):** ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.56 (s, 3H, SC*H*$_3$), 7.09 (d, *J* = 2.0 Hz, 1H, Ar*H*), 7.40 (d, *J* = 8.8 Hz, 2H, Ar*H*), 7.49 (d, *J* = 8.8 Hz, 2H, Ar*H*), 7.71 (d, *J* = 4.0 Hz, 1H, Ar*H*), 7.75 (d, *J* = 4.0 Hz, 1H, Ar*H*), 8.74 (d, *J* = 2.0 Hz, 1H, Ar*H*), 9.89 (s, 1H, N*H*); ^{13}C NMR (75 MHz, DMSO-d_6) δ 15.0, 101.4, 127.3, 127.4, 128.8, 131.7, 132.5, 136.0, 145.3, 151.9, 162.0, 167.3; HREIMS (exact mass HREMS) calcd for $C_{15}H_{12}ClN_3O_3S_3$ *m/e* 412.9729, found 412.9729.

***N*-(4-Chlorophenyl)-*N′*-[[5-(phenylsulfonyl)thiophene-2-yl]sulfonyl]-*S*-methylpseudothiourea (8j):** MS *m/z* 440 ($M^+ - 47$); ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.56 (s, 3H, SC*H*$_3$), 7.56−7.49 (m, 4H, Ar*H*), 7.64−7.87 (m, 5H, Ar*H*), 8.02−8.07 (m, 2H, Ar*H*), 9.99 (brs, 1H, N*H*); ^{13}C NMR (100 MHz, DMSO-d_6) δ 15.4, 106.2, 118.9, 127.7, 127.8, 127.8, 129.2, 130.5, 131.2, 131.3, 134.1, 134.6, 134.9, 205.4. Anal. ($C_{18}H_{15}ClN_2O_4S_4$) C, H, N.

***N*-(4-Chlorophenyl)-*N′*-[[4-(phenylsulfonyl)thiophene-2-yl]sulfonyl]-*S*-methylpseudothiourea (8k):** MS *m/z* 438 ($M^+ - 49$); ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.17 (s, 3H, SC*H*$_3$), 7.29 (d, $J = 8.7$ Hz, 2H, Ar*H*), 7.45 (d, $J = 8.7$ Hz, 2H, Ar*H*), 7.64−7.78 (m, 3H, Ar*H*), 7.93 (s, 1H, Ar*H*), 8.03 (d, $J = 7.4$ Hz, 2H, Ar*H*), 8.72 (d, $J = 1.6$ Hz, 1H, Ar*H*), 9.92 (s, 1H, N*H*); ^{13}C NMR (75 MHz, DMSO-d_6) δ 15.4, 127.8, 127.9, 128.3, 129.2, 130.3, 131.8, 134.5, 136.3, 137.8, 140.8, 141.1, 147.3, 167.9. Anal. ($C_{18}H_{15}ClN_2O_4S_4 \cdot {}^1/_2H_2O$) C, H, N.

***N*-(4-Chlorophenyl)-*N*′-[[4-(2-chloro-2-cyanophenoxy)-phenyl]sulfonyl]-*S*-methylpseudothiourea (8l):** MS *m/z* 491 (M$^+$ − 1); ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.50 (s, 3H, SC*H*$_3$), 7.16 (d, $J = 9.2$ Hz, 1H, Ar*H*), 7.32−7.47 (m, 6H, Ar*H*), 7.57 (d, $J = 8.4$ Hz, 1H, Ar*H*), 7.73 (t, $J = 8.4$ Hz, 1H, Ar*H*), 7.94 (d, $J = 8.8$ Hz, 2H, Ar*H*), 9.74 (s, 1H, N*H*); ^{13}C NMR (75 MHz, DMSO-d_6) δ 15.0, 104.9, 113.0, 117.8, 119.1, 125.4, 127.3, 128.8, 129.0, 131.0, 136.1, 136.3, 136.7, 138.6, 157.7, 158.8, 166.3. Anal. ($C_{21}H_{15}Cl_2N_3O_3S_2$) C, H, N.

***N*-(4-Chlorophenyl)-*N*′-[[4-(2-chloro-6-nitrophenoxy)-phenyl]sulfonyl]-*S*-methylpseudothiourea (8m):** MS *m/z* 512 (M$^+$); ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.49 (s, 3H, SC*H*$_3$), 8.07 (d, $J = 8.9$ Hz, 2H, Ar*H*), 7.35 (d, $J = 8.9$ Hz, 2H, Ar*H*), 7.44 (d, $J = 8.9$ Hz, 2H, Ar*H*), 7.64 (t, $J = 8.2$ Hz, 1H, Ar*H*), 7.86 (d, $J = 8.9$ Hz, 2H, Ar*H*), 8.09 (dd, $J = 8.2$ Hz, $J = 1.5$ Hz, 1H, Ar*H*), 8.19 (dd, $J = 8.2$ Hz, $J = 1.6$ Hz, 1H, Ar*H*), 9.71 (s, 1H, N*H*); ^{13}C NMR (75 MHz, DMSO-d_6) δ 15.2, 115.7, 125.5, 127.6, 128.3, 129.1, 129.2, 129.5, 131.3, 136.4, 136.7, 137.3, 142.6, 144.6, 159.3, 166.6. Anal. ($C_{20}H_{15}Cl_2N_3O_5S_2$) C, H, N.

***N*-(4-Chlorophenyl)-*N*′-[[3,5-dichloro-4-(4-nitrophenoxy)phenyl]sulfonyl]-*S*-methylpseudothiourea (8n):** MS *m/z* 547 (M$^+$); ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.56 (s, 3H, SC*H*$_3$), 7.18 (d, $J = 9.2$ Hz, 2H, Ar*H*), 7.41 (d, $J = 8.8$ Hz, 2H, Ar*H*), 7.49 (d, $J = 8.8$ Hz, 2H, Ar*H*), 8.10 (s, 2H, Ar*H*), 8.26 (d, $J = 9.2$ Hz, 2H, Ar*H*), 9.96 (s, 1H, N*H*); ^{13}C NMR (75 MHz, DMSO-d_6) δ 15.4, 116.2, 126.8, 128.1, 129.2, 129.5, 130.3, 131.8, 136.5, 142.3, 143.4, 148.2, 160.6, 167.8. Anal. ($C_{20}H_{14}Cl_3N_3S_2$) C, H, N.

***N*-(4-Chlorophenyl)-*N*′-[[4-(2-chloro-4-nitrophenoxy)-3,5-dichlorophenyl]sulfonyl]-*S*-methylpseudothiourea (8o):** MS *m/z* 533 (M$^+$ − 48); ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.56 (s, 3H, SC*H*$_3$), 7.01 (d, $J = 9.1$ Hz, 1H, Ar*H*), 7.41 (d, $J = 8.8$ Hz, 2H, Ar*H*), 7.49 (d, $J = 8.8$ Hz, 2H, Ar*H*), 8.10−8.14 (m, 3H, Ar*H*), 8.54 (d, $J = 2.7$ Hz, 1H, Ar*H*), 9.96 (s, 1H, N*H*); ^{13}C NMR (75 MHz, DMSO-d_6) δ 15.4, 115.4, 122.4, 125.0, 126.9, 128.1, 128.2, 129.2, 131.7, 136.7, 142.7, 143.6, 148.0, 156.0. Anal. ($C_{20}H_{13}Cl_4N_3O_5S_2$) C, H, N.

***N*-(4-Chlorophenyl)-*N*′-[(4-*n*-butoxyphenyl)sulfonyl]-*S*-methylpseudothiourea (8p):** MS *m/z* 412 (M$^+$ − 1); ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.93 (t, $J = 7.3$ Hz, 3H, C*H*$_3$), 1.32−1.43 (m, 2H, C*H*$_2$), 1.67−1.76 (m, 2H, C*H*$_2$), 2.46 (s, 3H, SC*H*$_3$), 4.05 (t, $J = 6.4$ Hz, 2H, OC*H*$_2$), 7.07 (d, $J = 8.9$ Hz, 2H, Ar*H*), 7.36 (d, $J = 8.8$ Hz, 2H, Ar*H*), 7.45 (d, $J = 8.8$ Hz, 2H, Ar*H*), 7.78 (d, $J = 8.9$ Hz, 2H, Ar*H*), 9.63 (s, 1H, N*H*); ^{13}C NMR (75 MHz, DMSO-d_6) δ 14.0, 15.2, 19.1, 30.9, 68.0, 114.8, 127.6, 128.8, 129.1, 131.2, 134.2, 136.8, 161.8, 166.1. Anal. ($C_{18}H_{21}ClN_2O_3S_2 \cdot {}^1/_4H_2O$) C, H, N.

***N*-(3,4-Dichlorophenyl)-*N*′-(4-tolylsulfonyl)-*S*-methylpseudothiourea (8q):** MS *m/z* 388 (M$^+$); ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.38 (s, 3H, SC*H*$_3$), 2.54 (s, 3H, C*H*$_3$), 7.36−7.41 (m, 3H, Ar*H*), 7.64 (d, $J = 8.6$ Hz, 1H, Ar*H*), 7.68 (d, $J = 2.3$ Hz, 1H, Ar*H*), 7.73 (d, $J = 8.3$ Hz, 2H, Ar*H*), 9.66 (s, 1H, N*H*); ^{13}C NMR (75 MHz, DMSO-d_6) δ 15.3, 21.4, 125.2, 126.6, 126.7, 129.3, 129.8, 130.9, 131.2, 138.0, 139.7. Anal. ($C_{15}H_{14}Cl_2N_2O_2S_2$) C, H, N.

***N*-(3,4-Dichlorophenyl)-*N*′-[(5-chloro-3-methylbenzo[*b*]thiophene-2-yl)sulfonyl]-*S*-methylpseudothiourea (8r):** MS *m/z* 478 (M$^+$ − 1); ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.58 (s, 3H, SC*H*$_3$), 2.61 (s, 3H, SC*H*$_3$), 7.43 (dd, $J = 8.7$ Hz, $J = 2.4$ Hz, 1H, Ar*H*), 7.56 (dd, $J = 8.6$ Hz, $J = 2.0$ Hz, 1H, Ar*H*), 7.68 (d, $J = 8.7$ Hz, 1H, Ar*H*), 8.07 (d, $J = 8.6$ Hz, 1H, Ar*H*), 9.86 (s, 1H, N*H*); ^{13}C NMR (75 MHz, DMSO-d_6) δ 12.4, 15.5, 96.6, 123.6, 125.0, 125.4, 127.0, 127.6, 127.7, 130.7, 131.0, 131.3, 134.9, 137.7, 141.1, 201.5, 209.8. Anal. ($C_{17}H_{13}Cl_3N_2O_2S_3$) C, H, N.

Preparation of N,N′-Disubstituted *N*″-Hydroxyguanidines 4a−r: General Procedure. The appropriate S-methyl pseudourea **8a−r** (2.8 mmol) was added to a stirred solution of hydroxylamine hydrochloride (7.4 mmol) and triethylamine (1.2 mL, 8.4 mmol) in chloroform (50 mL). The solution was refluxed for 48 h, and the solvent was removed by evaporation to produce a solid residue. Ether (20 mL) was added to the residue, and the white precipitate was collected by filtration. The solid was then heated with toluene, and the undissolved solid was removed by filtration. The filtrate was again evaporated to obtain a solid which was subsequently recrystallized from the appropriate solvent to yield the desired compound.

***N*-[(4-Methylphenyl)sulfonyl]-*N*′-(4-chlorophenyl)-*N*″-hydroxyguanidine (4a):** MS *m/z* 294 (M$^+$ − 32); ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.36 (s, 3H, C*H*$_3$), 7.31−7.36 (m, 4H, Ar*H*), 7.45 (d, $J = 8.8$ Hz, 2H, Ar*H*), 7.71 (d, $J = 8.1$ Hz, 2H, Ar*H*), 9.40 (s, 1H, N*H*), 9.69 (brs, 2H, N*H* and O*H*); ^{13}C NMR (100 MHz, DMSO-d_6) δ 20.9, 124.7, 125.9, 128.2, 128.3, 129.1, 136.2, 140.6, 141.6, 154.0. Anal. ($C_{14}H_{14}ClN_3O_3S$) C, H, N.

***N*-(Phenylsulfonyl)-*N*′-(4-chlorophenyl)-*N*″-hydroxyguanidine (4b):** MS *m/z* 328 (M$^+$ + 2); ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.34 (d, $J = 8.9$ Hz, 2H, Ar*H*), 7.44 (d, $J = 8.9$ Hz, 2H, Ar*H*), 7.49−7.61 (m, 3H, Ar*H*), 7.81−7.84 (dd, $J = 7.8$ Hz, $J = 1.8$ Hz, 2H, Ar*H*), 9.42 (s, 1H, N*H*), 9.81 (brs, 2H, N*HOH*); ^{13}C NMR (100 MHz, DMSO-d_6) δ 124.8, 125.8, 128.2, 128.4, 128.7, 131.6, 136.2, 143.4, 154.1. Anal. ($C_{13}H_{12}ClN_3O_3S$) C, H, N.

***N*-(4-Chlorophenyl)-*N*′-[[3,5-bis(trifluoromethyl)phenyl]-sulfonyl]-*N*″-hydroxyguanidine (4c).** After the reaction was complete, the solvent was removed *in vacuo*, and the residue was purified by column chromatography (silica gel, solvent system: *n*-hexane/ethyl acetate = 1:1). The $R_f = 0.44$ fraction was collected to obtain **4c** (318 mg, 21.93%): MS *m/z* 461.4 (M$^+$); ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.30−7.40 (m, 4H, Ar*H*), 8.32 (s, 2H, Ar*H*), 8.37 (s, 1H, Ar*H*), 9.61 (s, 1H, N*H*), 10.01 (s, 1H, N*H*), 10.27 (s, 1H, O*H*); ^{13}C NMR (75 MHz, DMSO-d_6) δ 123.1 (q, $J = 272$ Hz, C*F*$_3$), 125.7, 126.0, 126.9, 128.6, 129.4, 131.3 (q, $J = 33$ Hz, C*CF*$_3$), 136.3, 146.8, 154.2. Anal. ($C_{15}H_{10}F_6ClN_3O_3S$) C, H, N.

***N*-(4-Chlorophenyl)-*N*′-[(5-chloro-3-methylbenzo[*b*]-thiophene-2-yl)sulfonyl]-*N*″-hydroxyguanidine (4d).** After the reaction was complete, the precipitate was removed, and the filtrate, after concentration *in vacuo* to dryness, was purified by column chromatography (silica gel, solvent system: chloroform) to obtain two products. The $R_f = 0.28$ fraction was collected to give **4d** (0.62 g, 40.9%): mp 245−247 °C dec; MS *m/z* 413 (M$^+$ − 17); ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.52 (s, 3H, C*H*$_3$), 7.35 (d, $J = 8.9$ Hz, 2H, Ar*H*), 7.45 (d, $J = 8.9$ Hz, 2H, Ar*H*), 7.52 (dd, $J = 8.7$ Hz, $J = 1.9$ Hz, 1H, Ar*H*), 7.96 (d, $J = 1.9$ Hz, 1H, Ar*H*), 8.05 (d, $J = 8.7$ Hz, 1H, Ar*H*), 9.60 (s, 1H, N*H*), 9.97 (s, 2H, N*H* and O*H*); ^{13}C NMR (75 MHz, DMSO-d_6) δ 12.3, 123.3, 124.9, 125.6, 127.1, 128.6, 129.2, 130.5, 133.4, 136.7, 141.4, 154.5; HREIMS (exact mass HREMS) calcd for $C_{16}H_{13}O_3S_2N_3Cl_2$ *m/z* 428.9775, found 428.9774. The $R_f = 0.37$ fraction was collected and recrystallized from ethanol and acetonitrile (v/v = 1:1) to furnish **9d** (203 mg, 15.5%): mp 256−257 °C dec; MS *m/z* 413 (M$^+$ − 1); ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.58 (s, 3H, C*H*$_3$), 7.12 (s, 2H, N*H*$_2$), 7.38 (s, 4H, Ar*H*), 7.53 (dd, $J = 8.7$ Hz, $J = 2.1$ Hz, 1H, Ar*H*), 7.97 (d, $J = 2.1$ Hz, 1H, Ar*H*), 8.05 (d, $J = 8.7$ Hz, 1H, Ar*H*), 9.34 (s, 1H, N*H*); ^{13}C NMR (75 MHz, DMSO-d_6) δ 12.3, 123.4, 123.9, 125.0, 127.2, 128.6, 129.1, 130.6, 133.6, 136.7, 136.8, 141.4, 141.8, 155.0. Anal. ($C_{16}H_{13}O_2S_2N_3Cl_2$) C, H, N.

***N*-(4-Chlorophenyl)-*N*′-(benzo[*b*]thiophene-2-ylsulfonyl)-*N*″-hydroxyguanidine (4e).** After the reaction was complete, the filtrate was concentrated *in vacuo* to dryness and purified by column chromatography (silica gel, solvent system: chloroform/methanol = 98:2) to obtain two products. The $R_f = 0.16$ fraction was collected to give **4e** (0.28 g, 27%): mp 264−266 °C; MS *m/z* 381 (M$^+$); ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.36−7.39 (m, 2H, Ar*H*), 7.45−7.50 (m, 4H, Ar*H*), 7.96−8.05 (m, 2H, Ar*H*), 9.57 (s, 1H, N*H*), 9.99 (s, 2H, OH and N*H*); ^{13}C NMR (75 MHz, DMSO-d_6) δ 123.3, 123.8, 125.6, 125.7, 125.8, 127.1, 128.6, 129.1, 136.4, 137.9, 140.8, 145.2, 154.4. Anal. ($C_{15}H_{12}ClN_3O_3S_2$) C, H, N. The $R_f = 0.27$ fraction was collected and recrystallized from methanol to furnish **9e** (25 mg, 2.6%): mp 205−206 °C; MS *m/z* 367 (M$^+$); ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.15 (s, 2H, N*H*$_2$), 7.37 (s, 4H, Ar*H*), 7.46−

7.50 (m, 2H, Ar*H*), 7.95 (s, 1H, Ar*H*), 7.97−8.06 (m, 2H, Ar*H*), 9.33 (s, 1H, N*H*). Anal. ($C_{15}H_{12}ClN_3O_2S_2$) C, H, N.

***N*-(4-Chlorophenyl)-*N*′-(benzofuran-2-ylsulfonyl)-*N*″-hydroxyguanidine (4f):** MS *m/z* 366 (M^+); ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.33−7.50 (m, 7H, Ar*H*), 7.69 (d, $J = 8.2$ Hz, 1H, Ar*H*), 7.77 (d, $J = 7.7$ Hz, 1H, Ar*H*), 9.62 (s, 1H, N*H*), 10.09 (s, 2H, N*H* and O*H*); ^{13}C NMR (75 MHz, DMSO-d_6) δ 106.6, 109.4, 112.3, 123.3, 124.4, 125.6, 126.5, 127.4, 128.6, 129.1, 136.4, 154.0, 154.2. Anal. ($C_{15}H_{12}ClN_3O_4S$) C, H, N.

***N*-(4-Chlorophenyl)-*N*′-(benzo[2,1,3]thiadiazol-4-ylsulfonyl)-*N*″-hydroxyguanidine (4g).** Compound **4g** (R_f = 0.04) was separated in 31.2% yield by column chromatography (silica gel, solvent system: chloroform/methanol = 99:1): MS *m/z* 366.5 ($M^+ - 17$); ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.24 (d, $J = 8.9$ Hz, 2H, Ar*H*), 7.43 (d, $J = 8.9$ Hz, 2H, Ar*H*), 7.83 (dd, $J = 8.8$ Hz, 7.1 Hz, 1H, Ar*H*), 8.22 (dd, $J = 7.1$ Hz, 1.1 Hz, 1H, Ar*H*), 8.31 (dd, $J = 8.8$ Hz, 1.1 Hz, 1H, Ar*H*), 9.47 (s, 1H, N*H*), 9.95 (s, 2H, N*H* and O*H*); ^{13}C NMR (75 MHz, DMSO-d_6) δ 125.0, 125.5, 128.4, 128.6, 129.2, 129.4, 135.1, 136.5, 154.6, 155.5, 158.3. Anal. ($C_{13}H_{10}ClN_5O_3S_2$) C, H, N. The R_f = 0.05 fraction was collected and recrystallized from acetonitrile to furnish **9g** (104 mg, 8.9%): mp 260 °C dec; MS *m/z* 368.5 ($M^+ - 19$); ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.17 (s, 2H, NH_2), 7.28−7.37 (AB q, $J = 9.0$ Hz, 4H, Ar*H*), 7.84 (dd, $J = 8.8$ Hz, 7.1 Hz, 1H, Ar*H*), 8.22 (dd, $J = 7.1$ Hz, 1.1 Hz, 1H, Ar*H*), 8.33 (dd, $J = 8.8$ Hz, 1.1 Hz, 1H, Ar*H*), 9.24 (s, 1H, N*H*); ^{13}C NMR (75 MHz, DMSO-d_6) δ 116.3, 123.3, 125.6, 128.0, 129.0, 129.2, 135.1, 137.1, 155.1, 155.5, 170.5. Anal. ($C_{13}H_{10}ClN_5O_2S_2$) C, H, N.

***N*-(4-Chlorophenyl)-*N*′-[(2-pyrid-2-ylthiophene-5-yl)sulfonyl]-*N*″-hydroxyguanidine (4h).** Compound **4h** (R_f = 0.29) was separated in 52.2% yield by column chromatography (silica gel, solvent system: chloroform/methanol = 98:2): MS *m/z* 411 ($M^+ + 1$); ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.33−7.39 (m, 3H, Ar*H*), 7.47 (d, $J = 8.9$ Hz, 2H, Ar*H*), 7.61 (d, $J = 4.0$ Hz, 1H, thiophene-*H*), 7.77 (d, $J = 4.0$ Hz, 1H, thiophene-*H*), 7.88 (td, $J = 7.8$ Hz, $J = 1.6$ Hz, 1H, Ar*H*), 8.00 (d, $J = 7.8$ Hz, 1H, Ar*H*), 9.55 (s, 1H, N*H*), 9.97 (s, 2H, N*H* and O*H*); ^{13}C NMR (75 MHz, DMSO-d_6) δ 119.6, 123.9, 124.7, 125.4, 128.6, 129.0, 131.3, 136.5, 137.8, 146.1, 148.9, 150.0, 151.1, 154.3. Anal. ($C_{16}H_{13}ClN_4O_3S_2$) C, H, N. The R_f = 0.35 fraction was collected and recrystallized from methanol to furnish **9h** (100 mg, 6.5%): mp 229−230 °C; MS *m/z* 394 (M^+); ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.13 (s, 2H, NH_2), 7.33−7.41 (m, 5H, Ar*H*), 7.60 (d, $J = 4.0$ Hz, 1H, thiophene-*H*), 8.77 (d, $J = 4.0$ Hz, 1H, thiophene-*H*), 8.88 (td, $J = 7.6$ Hz, $J = 1.7$ Hz, 1H, Ar*H*), 8.00 (d, $J = 7.6$ Hz, 1H, Ar*H*), 8.55 (dd, $J = 5.0$ Hz, $J = 1.1$ Hz, 1H, Ar*H*), 9.30 (s, 1H, N*H*); ^{13}C NMR (75 MHz, DMSO-d_6) δ 119.2, 123.2, 123.4, 124.4, 127.0, 128.7, 130.6, 136.5, 137.4, 145.6, 148.6, 149.5, 150.6, 154.6. Anal. ($C_{16}H_{13}ClN_4O_2S_2$) C, H, N.

***N*-(4-Chlorophenyl)-*N*′-[(5-isoxazol-3-ylthiophene-5-yl)sulfonyl]-*N*″-hydroxyguanidine (4i).** Compound **4i** (R_f = 0.19) was separated in 37.5% yield by column chromatography (silica gel, solvent system: chloroform/methanol = 98:2): MS *m/z* 396 ($M^+ - 3$); ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.05 (d, $J = 1.8$ Hz, 1H, thiophene-*H*), 7.41 (q, $J = 9.0$ Hz, 4H, Ar*H*), 7.68 (s, 2H, Ar*H*), 7.72 (d, $J = 1.8$ Hz, thiophene-*H*), 9.60 (s, 1H, N*H*), 10.05 (s, 2H, N*H* and O*H*). Anal. ($C_{14}H_{11}ClN_4O_4S_2$) C, H, N.

***N*-(4-Chlorophenyl)-*N*′-[[5-(phenylsulfonyl)thiophene-2-yl]sulfonyl]-*N*″-hydroxyguanidine (4j).** Compound **4j** (R_f = 0.35) was separated in 11.6% yield by column chromatography (silica gel, solvent system: chloroform): MS *m/z* 413 ($M^+ - 59$); ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.32 (s, 4H, Ar*H*), 7.59 (d, $J = 4.0$ Hz, 1H, thiophene-*H*), 7.65−7.78 (m, 3H, Ar*H*), 8.03 (d, $J = 7.1$ Hz, 2H, Ar*H*), 9.65 (s, 1H, N*H*), 10.05 (s, 1H, O*H*), 10.20 (s, 1H, N*H*); ^{13}C NMR (75 MHz, DMSO-d_6) δ 125.6, 125.9, 127.6, 128.6, 129.4, 130.2, 130.4, 134.0, 134.8, 134.8, 136.1, 141.0, 145.2, 153.4, 154.2. Anal. ($C_{17}H_{14}ClN_3O_5S_3$) C, H, N. The R_f = 0.44 fraction was collected and recrystallized from toluene to give **9j** (37 mg, 2.7%): mp 233−234 °C; MS *m/z* 349 ($M^+ - 107$); ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.20 (s, 2H, NH_2), 7.28 (d, $J = 8.8$ Hz, 2H, Ar*H*), 7.37 (d, $J = 8.8$ Hz, 2H, Ar*H*), 7.61−7.83 (m, 4H, Ar*H*), 7.84 (d, $J = 3.7$ Hz, 1H, thiophene-*H*), 8.03 (d, $J = 7.5$ Hz, 2H, Ar*H*), 9.36 (s, 1H, N*H*); ^{13}C NMR (75 MHz, DMSO-d_6) δ 106.2, 106.6, 124.3, 127.6, 129.2, 130.3, 130.5, 134.9, 136.5, 140.9, 145.4, 155.2. Anal. ($C_{17}H_{14}ClN_3O_4S_3$) C, H, N.

***N*-(4-Chlorophenyl)-*N*′-[[4-(phenylsulfonyl)thiophene-2-yl]sulfonyl]-*N*″-hydroxyguanidine (4k).** Compound **4k** (R_f = 0.4) was separated in 19.8% yield by column chromatography (silica gel, solvent system: chloroform): MS *m/z* 413 ($M^+ - 59$); ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.34 (s, 4H, Ar*H*), 7.64−7.75 (m, 3H, Ar*H*), 7.81 (d, $J = 1.6$ Hz, 1H, thiophene-*H*), 8.01 (d, $J = 7.4$ Hz, 2H, Ar*H*), 8.63 (d, $J = 1.6$ Hz, 1H, thiophene-*H*), 9.59 (s, 1H, N*H*), 10.04 (s, 1H, O*H*), 10.15 (s, 1H, N*H*); ^{13}C NMR (75 MHz, DMSO-d_6) δ 106.2, 125.8, 127.0, 127.7, 128.6, 129.3, 130.3, 134.5, 136.2, 136.8, 140.8, 140.9, 154.1. Anal. ($C_{17}H_{14}ClN_3O_5S_3$) C, H, N. The R_f = 0.47 fraction was collected and recrystallized from chloroform to give **9k** (65 mg, 0.06%): MS *m/z* 438 ($M^+ - 18$); ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.19 (s, 2H, NH_2), 7.27 (d, $J = 8.9$ Hz, 2H, Ar*H*), 7.36 (d, $J = 8.9$ Hz, 2H, Ar*H*), 7.66 (t, $J = 7.4$ Hz, 2H, Ar*H*), 7.75 (t, $J = 7.4$ Hz, 1H, Ar*H*), 7.86 (d, $J = 1.6$ Hz, 1H, thiophene-*H*), 8.02 (d, $J = 7.4$ Hz, 2H, Ar*H*), 8.65 (d, $J = 1.6$ Hz, 1H, thiophene-*H*), 9.30 (s, 1H, N*H*); ^{13}C NMR (75 MHz, DMSO-d_6) δ 124.0, 124.2, 127.2, 127.8, 128.8, 129.1, 129.2, 130.3, 134.5, 136.6, 137.0, 155.1. Anal. ($C_{17}H_{14}ClN_3O_4S_3$) C, H, N.

***N*-(4-Chlorophenyl)-*N*′-[[4-(3-chloro-2-cyanophenoxy)phenyl]sulfonyl]-*N*″-hydroxyguanidine (4l).** Compound **4l** (R_f = 0.23) was separated in 44.1% yield by column chromatography (silica gel, solvent system: chloroform:methanol = 98:2): MS *m/z* 418 ($M^+ - 59$); ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.11 (d, $J = 8.3$ Hz, 1H, Ar*H*), 7.30−7.37 (m, 4H, Ar*H*), 7.45 (d, $J = 8.9$ Hz, 2H, Ar*H*), 7.56 (d, $J = 8.2$ Hz, 1H, Ar*H*), 7.72 (t, $J = 8.3$ Hz, 1H, Ar*H*), 7.90 (d, $J = 8.7$ Hz, 2H, Ar*H*), 9.47 (s, 1H, N*H*), 9.88 (s, 2H, N*H* and O*H*); ^{13}C NMR (75 MHz, DMSO-d_6) δ 104.8, 113.0, 117.5, 119.0, 124.8, 125.2, 128.2, 128.4, 128.5, 136.0, 136.2, 136.5, 140.0, 154.0, 157.1, 159.0. Anal. ($C_{20}H_{14}Cl_2N_4O_4S$) C, H, N.

***N*-(4-Chlorophenyl)-*N*′-[[4-(2-chloro-6-nitrophenoxy)phenyl]sulfonyl]-*N*″-hydroxyguanidine (4m).** Compound **4m** (R_f = 0.36) was separated in 35.2% yield by column chromatography (silica gel, solvent system: chloroform:methanol = 98:2): MS *m/z* 364.5 ($M^+ - 132$); ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.03 (d, $J = 8.8$ Hz, 2H, Ar*H*), 7.33 (d, $J = 8.9$ Hz, 2H, Ar*H*), 7.43 (d, $J = 8.9$ Hz, 2H, Ar*H*), 7.63 (t, $J = 8.2$ Hz, 1H, Ar*H*), 7.81 (d, $J = 8.8$ Hz, 2H, Ar*H*), 8.08 (dd, $J = 8.2$ Hz, $J = 1.5$ Hz, 1H, Ar*H*), 8.18 (dd, $J = 8.2$ Hz, $J = 1.5$ Hz, 1H, Ar*H*), 9.46 (s, 1H, N*H*), 9.85 (brs, 2H, N*H* and O*H*); ^{13}C NMR (75 MHz, DMSO-d_6) δ 115.5, 125.2, 125.4, 128.3, 128.6, 128.7, 128.8, 129.6, 136.4, 136.6, 138.3, 142.7, 144.7, 154.3, 158.9. Anal. ($C_{19}H_{14}Cl_2N_4O_6S$) C, H, N.

***N*-(4-Chlorophenyl)-*N*′-[[3,5-dichloro-4-(4-nitrophenoxy)phenyl]sulfonyl]-*N*″-hydroxyguanidine (4n).** Compound **4n** (R_f = 0.59) was separated in 23.9% yield by column chromatography (silica gel, solvent system: ethyl acetate:hexane = 1:1): MS *m/z* 473.8 ($M^+ - 58$); ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.16 (d, $J = 9.2$ Hz, 2H, Ar*H*), 7.38 (d, $J = 8.9$ Hz, 2H, Ar*H*), 7.46 (d, $J = 8.9$ Hz, 2H, Ar*H*), 8.06 (s, 2H, Ar*H*), 8.26 (d, $J = 9.2$ Hz, 2H, Ar*H*), 9.61 (s, 1H, N*H*), 10.06 (s, 1H, O*H*), 10.16 (s, 1H, N*H*); ^{13}C NMR (75 MHz, DMSO-d_6) δ 115.7, 123.6, 125.4, 126.4, 127.4, 128.3, 128.8, 136.0, 143.0, 143.3, 147.3, 153.8, 160.3. Anal. ($C_{19}H_{13}Cl_3N_4O_6S$) C, H, N.

***N*-(4-Chlorophenyl)-*N*′-[[3,5-dichloro-4-(2-chloro-4-nitrophenoxy)phenyl]sulfonyl]-*N*″-hydroxyguanidine (4o).** Compound **4o** (R_f = 0.53) was separated in 30.6% (266 mg) yield by column chromatography (silica gel, solvent system: ethyl acetate:hexane = 1:1): MS *m/z* 552 ($M^+ + 4$); ^{1}H NMR (300 MHz, DMSO-d_6) δ 6.96 (d, $J = 9.2$ Hz, 2H, Ar*H*), 7.37 (d, $J = 8.9$ Hz, 2H, Ar*H*), 7.46 (d, $J = 8.9$ Hz, 2H, Ar*H*), 8.08 (s, 2H, Ar*H*), 8.11 (dd, $J = 9.2$ Hz, $J = 2.7$ Hz, 1H, Ar*H*), 8.53 (d, $J = 2.7$ Hz, 1H, Ar*H*), 9.61 (s, 1H, N*H*), 10.08 (brs, 2H, N*H* and O*H*); ^{13}C NMR (75 MHz, DMSO-d_6) δ 115.3, 122.4, 125.0, 125.8, 126.9, 127.9, 128.7, 128.9, 129.2, 136.4, 143.6, 144.0, 147.5, 154.1, 156.1. Anal. ($C_{19}H_{12}Cl_4O_6S \cdot H_2O$) C, H, N. The R_f = 0.56 fraction was collected and recrystallized from acetonitrile to give **9o** (100 mg, 12.1%): mp 193 °C; MS *m/z* 550 (M^+); ^{1}H NMR (300 MHz, DMSO-d_6) δ 6.99 (d, $J = 9.1$ Hz, 1H, Ar*H*), 7.23 (s, 2H, NH_2), 7.34−7.41 (m, 4H, Ar*H*), 8.08−8.12 (m, 3H, Ar*H*), 8.53 (d, $J = 2.8$ Hz, 1H, Ar*H*), 9.31

(s, 1H, N*H*); ^{13}C NMR (75 MHz, DMSO-d_6) δ 115.4, 122.4, 124.1, 125.0, 16.9, 127.7, 128.6, 129.1, 129.2, 136.8, 143.6,-144.0, 147.6, 155.0, 156.0. Anal. ($C_{19}H_{12}Cl_4N_4O_5S$) C, H, N.

***N*-(4-Chlorophenyl)-*N*′-[(4-*n*-butoxyphenyl)sulfonyl]-*N*′′-hydroxyguanidine (4p).** Compound **4p** (R_f = 0.51) was separated in 34.3% (397 mg) yield by column chromatography (silica gel, solvent system: ethyl acetate:hexane = 3:7): MS *m*/*z* 381 (M^+ − 16); ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.93 (t, J = 7.3 Hz, 3H, C*H*$_3$), 1.37−1.49 (m, 2H, C*H*$_2$), 1.65−1.75 (m, 2H, C*H*$_2$), 4.02 (t, J = 6.4 Hz, 2H, OC*H*$_2$), 7.03 (d, J = 8.9 Hz, 2H, Ar*H*), 7.35 (d, J = 8.9 Hz, 2H, Ar*H*), 7.47 (d, J = 8.9 Hz, 2H, Ar*H*), 7.74 (d, J = 8.9 Hz, 2H, Ar*H*), 9.38 (s, 1H, N*H*), 9.77 (s, 2H, N*H* and O*H*); ^{13}C NMR (75 MHz, DMSO-d_6) δ 14.0, 19.0, 31.0, 67.9, 114.6, 125.0, 128.4, 128.6, 135.6, 136.7, 154.3, 161.4, 162.5. Anal. ($C_{17}H_{20}ClN_3O_4S$) C, H, N.

***N*-(3,4-Dichlorophenyl)-*N*′-(4-tolylsulfonyl)-*N*′′-hydroxyguanidine (4q).** Compound **4q** (R_f = 0.36) was separated in 24.9% (256 mg) yield by column chromatography (silica gel, solvent system: methanol:chloroform = 2:98): MS *m*/*z* 375 (M^+); ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.36 (s, 3H, C*H*$_3$), 7.34 (d, J = 8.1 Hz, 2H, Ar*H*), 7.46−7.56 (m, 2H, Ar*H*), 7.71−7.75 (m, 3H, Ar*H*), 9.51 (s, 1H, N*H*), 9.92 (s, 2H, N*H* and O*H*); ^{13}C NMR (75 MHz, DMSO-d_6) δ 20.9, 122.7, 124.0, 125.8, 129.2, 130.1, 130.4, 137.6, 140.5, 141.7, 153.4. Anal. ($C_{14}H_{13}Cl_2N_3O_3S$) C, H, N. The R_f = 0.42 fraction was collected to give **9q** (230 mg, 23.9%): mp 190 °C; MS *m*/*z* 359 (M^+ + 1); ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.36 (s, 3H, C*H*$_3$), 7.09 (s, 2H, N*H*$_2$), 7.24 (dd, J = 8.8 Hz, J = 2.6 Hz, 1H, Ar*H*), 7.35 (d, J = 8.2 Hz, 2H, Ar*H*), 7.54 (d, J = 8.8 Hz, 2H, Ar*H*), 9.26 (s, 1H, N*H*); ^{13}C NMR (75 MHz, DMSO-d_6) δ 21.3, 106.6, 121.5, 122.8, 126.1, 129.7, 130.9, 138.5, 139.3, 142.3, 154.4, 160.9. Anal. ($C_{14}H_{13}Cl_2N_3O_2S$) C, H, N.

***N*-(3,4-Dichlorophenyl)-*N*′-[(5-chloro-3-methylbenzo[*b*]thiophene-2-yl)sulfonyl]-*N*′′-hydroxyguanidine (4r):** MS *m*/*z* 466 (M^+ + 1); ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.56 (s, 3H, C*H*$_3$), 7.45 (dd, J = 8.8 Hz, J = 2.4 Hz, 1H, Ar*H*), 7.51−7.57 (m, 2H, Ar*H*), 7.73 (d, J = 2.4 Hz, 1H, Ar*H*), 7.97 (d, J = 2.0 Hz, 1H, Ar*H*), 8.05 (d, J = 8.6 Hz, 1H, Ar*H*), 9.69 (s, 1H, N*H*), 10.10 (brs, 2H, N*H* and O*H*); ^{13}C NMR (75 MHz, DMSO-d_6) δ 12.3, 100.7, 106.6, 123.4, 123.5, 124.8, 124.9, 127.2, 130.5, 130.6, 133.4, 136.7, 137.7, 141.4, 153.8. Anal. ($C_{16}H_{12}Cl_3N_3O_3S_2$) C, H, N.

X-ray Crystallography. Crystals of **4g** were grown from a warm solution of the compound from methanol by slow cooling. The X-ray diffraction data were collected for each compound (to 2θ = 120°) on a Rigaku AFC-5R (RU-300) rotating anode X-ray diffractometer at 22 °C using the $\omega-2\theta$ scan mode with graphite-monochromated Cu Kα radiation (λ = 1.5418 Å). The power of the X-ray generator was set at 50 kV and 40 mA with 0.2 × 2 mm^2 fine-focus anode cup. Unit cell dimensions were determined using Cu Kα1 radiation (λ = 1.5406 Å). The crystallographic parameters are as follows: space group $P\bar{1}$, a = 10.573(1) Å, b = 21.955(2) Å, c = 14.360(1) Å, β = 110.65(1)°, no. of 3σ F_o's = 4585, R = 0.047. There is one molecule per asymmetric unit. The structure was solved by the direct method using the program SHELXS-86.[26] They were refined by the full-matrix least-squares refinement procedure using the SHELXL package.[27] Hydrogen atoms were also included in the refinement with variable positions and isotropic temperature factors.

***In Vitro* Cytotoxicity Assays.** MOLT-4, COLO 205, and HepG2 were obtained from the American Tissue Culture Collection (ATCC). KB and TSGH 8302 cells were obtained from the Chinese patients of the Tri-Service General Hospital, Taipei, Taiwan. COLO 205, KB, and TSGH 8302 cells were grown as a monolayer in RPMI 1640 (Gibco, BRL) supplemented with 5% fetal bovine serum (FBS) (Hyclone). HepG2 cells were grown as a monolayer in IMDM medium (Gibco, BRL) supplemented with 10% FBS. MOLT-4 was grown as a suspension culture in RPMI 1640 medium supplemented with 10% FBS and 1% penicillin/streptomycin (Gibco, BRL). Exponentially growing cell cultures were maintained in a humidified incubator with an atmosphere of 5% CO_2−95% air (NAPCO, Model 5410) at 37 °C. In principle, the assay is dependent on the cellular reduction of MTT (Sigma Chemical Co.) to a blue formazan product by the mitochondrial dehydrogenase of viable cells.[29−31] The viable cell number/well is directly proportional to the production of formazan, which following solubilization, can be measured spectrophotometrically. Single-cell suspensions were obtained by mechanical disaggregation of the floating cell line (MOLT-4) and by trypsinization of the monolayer cultures (COLO 205, HepG2, KB, and TSGH 8302) and counted by trypan blue exclusion. The cells were then seeded into 96-well plates (Nunc 67008) in a 180 μL volume using a multichannel pipet (Gilson) and incubated for 24 h. The drug was dissolved in 10% DMSO (Sigma, D-8779) and 90% DPBS solution. Drug solution (20 μL) was dispensed within appropriate wells (each treatment group and control, N = 3) at final drug concentrations from 100 μg/mL to 0.01 μg/mL by a 10-time dilution. Peripheral wells for each plate (lacking cells) were utilized for drug blank and medium/tetrazolium reagent blanks “background determinations”. The cells were then incubated for another 72 h. MTT (20 μL, 5 mg/mL) was added to each well and incubated for a further 4 h. Culture plates containing MOLT-4 cells were centrifuged at 1000 rpm for 5 min. Culture medium supernatant (170 μL) was removed from each well and replaced with 200 μL/well DMSO using a multichannel pipet. Following formazans solubilization, the absorbance of each well was measured using an ELISA reader (Molecular Devices Emax) at (545−690 nm) interfaced with IBM computer Softmax software. Cell growth inhibition was calculated according to (1 − (OD of drug treatment/OD of control)) × 100%. The IC_{50} values were obtained by determining the drug concentration producing 50% growth inhibition by drawing the drug concentration vs growth inhibition percentage.

***In Vivo* Antitumor Tests.** C3H/HeN mice (16−20 g, 5 weeks old) were obtained from the animal center of the Cheng-Kung University and allowed to acclimate to their new environment for one week. Murine K-1735/M2 melanoma cells were obtained from Dr. Mien-Chie Hung (M.D. Anderson Cancer Center, Houston, TX) and subcutaneously inoculated in C3H/HeN mice. The tumors were maintained by serial transplantation in C3H/HeN mice. Tumors were grown until a size of approximately 15 × 20 mm. Tumors were then resected, minced, and used for subcutaneous inoculation in mice for the antitumor agent test. The tumors were grown until approximately 150 mg (range 100−200 mg). The mice were randomly divided into six mice per group. The compounds (**4o** and LY181984) were resuspended in 2.5% cremophor/saline. The desired dosage of compound was orally administered for two cycles of 5 consecutive days [(qid × 5)2] at day 5 and day 12. Tumor weight was estimated by two-dimensional caliper measurements and calculated with the formula for an ellipsoid. Tumor weight = $LW^2/2$, where L is the major axis and W is the width of the tumor.[2,32,33] The percentage of tumor growth inhibition (TGI %) was calculated as (1 mean tumor weight of treated group/mean tumor weight of control group) × 100%. Moderate activity and significant activity were defined as TGI of 58−89% and ≥90%, respectively.[32,34]

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Supporting Information Available: HMBC spectrum and X-ray diffraction data for **4g**, including crystal data and structure refinement, atomic coordinates, bond distance, parameters, and bond angles (7 pages). Ordering information is given on any current masthead page.

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