Sultam Thioureas: Synthesis and Antiviral Activity Against West Nile Virus

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Abstract: The syntheses of eleven sultam thioureas, including nine new compounds, are described. These compounds were synthesized from thioureas and include the first sultam thioureas in which the two thiourea nitrogen groups are not identical. In addition, the first X-ray crystal structures of sultam thioureas and the antiviral activity of these compounds against West Nile virus (WNV) are reported.

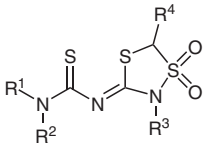
Key words: medicinal chemistry, antiviral agents, West Nile virus, heterocycles, sultam thioureas, thioureas

Sultam thioureas (Table 1) were identified as novel inhibitors of West Nile virus (WNV) replication from a screen of approximately 3,500 compounds.[1] Out of the four assayed sultam thioureas (**1**–**4**, Table 1), **1** was the most potent compound against WNV and appears to be non-toxic to cells. In addition, compounds **1** and **3** were found to provide some level of protection against Japanese encephalitis virus (JEV). With only four compounds, the obtained structure–activity relationship (SAR) data against WNV and JEV was limited. Further investigation of the antiviral activity of sultam thioureas is currently limited because very few have been synthesized.[2]

The reported syntheses of nine sultam thioureas were accomplished by cyclization between imino-1,2,4-dithiazoles and sulfenes.[2] Synthesis of imino-1,2,4-dithiazoles and the corresponding dithiazolium salt can be accomplished from either thiocarbamoyl chlorides[3,4] or thioureas.[3,5,6] Due to the antiviral activity of sultam thioureas and their limited availability, this paper reports initial work to synthesize and further characterize sultam thioureas. Specifically, the synthesis of eleven sultam thioureas (including nine new compounds) are reported and include the first compounds in which the R^1 and R^2 groups are not identical. In addition, the first X-ray crystal structures of sultam thioureas and their antiviral activities against WNV are reported.

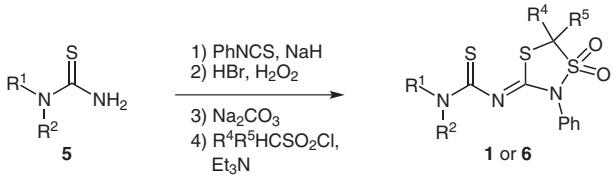
The method that was used to synthesize sultam thioureas (**6**) from thioureas (**5**) is shown in Table 2.[7] Initial work began with the synthesis of **1**. Commercially available

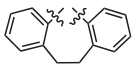
Table 1 WNV Activity for Four Sultam Thioureas[1]



Compd	$R^1 = R^2$	R^3	R^4	WNV EC_{50} (μM)	CC_{50} (μM)
1	Ph	Ph	H	0.7	>70
2	*i*-Pr	Me	Me	30	90
3	*i*-Pr	Ph	H	8	>80
4	Bn	Ph	H	65	65

Table 2 Synthesized Sultam Thioureas and WNV Activity



Compd	R^1/R^2	R^4/R^5	Overall yield (%)	WNV EC_{50} (μM)
1	Ph/Ph	H/H	31	1–3
6a	Ph/Ph	Me/H	32	>30
6b	Ph/Ph	Me/Me	26	>30
6c	*p*-tolyl/*p*-tolyl	H/H	32	>30
6d		H/H	31	>30
6e	Ph/Me	H/H	21	>30
6f	Ph/Et	H/H	45	>30
6g	Ph/Bn	H/H	35	>30
6h	Ph/Cy	H/H	20	1–3
6i	Et/Me	H/H	52	>30
6j	Me/Me	H/H	44	>30

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N,*N*-diphenylthiourea was reacted with sodium hydride and phenylisothiocyanate. Next, addition of hydrobromic acid followed by hydrogen peroxide resulted in the precipitation of the dithiazolium salt, which was isolated by filtration and used without further purification.[6] The dithiazolium salt was deprotonated and reacted with methanesulfonyl chloride under basic conditions[2] to provide **1** in 31% yield from *N*,*N*-diphenylthiourea (Table 2).[8] This procedure[7] was convenient because silica chromatography was only necessary for purification of the final product.

Next, the final step of the reaction sequence was varied. The dithiazolium intermediate from the synthesis of **1** was reacted with either ethanesulfonyl chloride or isopropanesulfonyl chloride to produce **6a** and **6b** in 32 and 26% yield, respectively (Table 2).[8] Sultam thioureas with alkyl groups at both R^4 and R^5 have not previously been reported.

The previous SAR data indicated that the R^1/R^2 substituents are important for antiviral activity, therefore, a range of sultam thioureas were synthesized in which these groups were varied. Products **6e–j** required the synthesis of various thioureas.[9] Initially, two sultam thioureas, **6c** and **6d**, with identical R^1/R^2 aryl substituents were synthesized from the corresponding thioureas in 32 and 31% yield, respectively (Table 2).[10] Compound **6d** does not have a chiral center, but the ^{1}H NMR spectrum of **6d** indicated that the methylene protons of the five-membered heterocyclic ring are not equivalent (diastereotopic). To date, X-ray quality crystals of **6d** have not been obtained, however, crystals of the thiourea starting material **5d**[11] (synthesized according to literature procedure in 50% yield[12]) were obtained. The X-ray crystal structure of **5d**[11] was solved by direct methods with SHELXS-86 and refined by full-matrix least-squares with SHELXL-97;[13] the two hydrogen bonded enantiomeric forms of this compound are shown in Figure 1. Both **5d** and **6d** are new examples of chiral molecules without a chiral center and, prior to obtaining the X-ray structure of **5d**, preliminary computational data indicated that both compounds are chiral.[14]

All previously reported sultam thioureas had the same group at the R^1 and R^2 positions. Four sultam thioureas

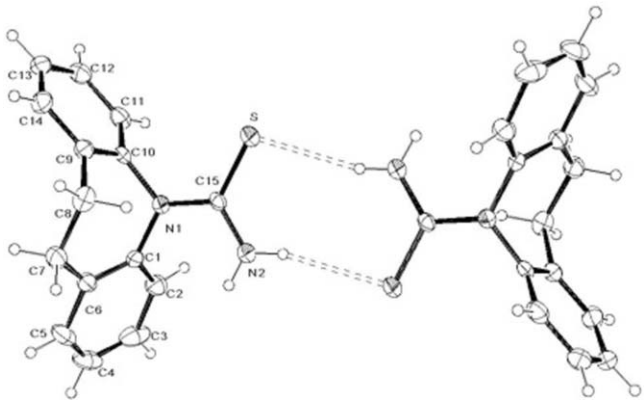

Figure 1 X-ray crystal structure of two enantiomeric forms of **5d**

(**6e–h**; Table 2) with a phenyl/alkyl group at R^1/R^2 were synthesized in 20–45% yield, and all of the compounds exhibited two sets of peaks for the alkyl groups in the ^{1}H NMR spectra.[15] The observed ratios for the two possible conformers were approximately 16:84, 31:69, and 38:62 for **6e**, **6f**, and **6g**, respectively, based on ^{1}H NMR integration values. These ratios are reasonably similar to the ratios predicted by B3 LYP/6-31G(d) calculations, which were 25:75, 45:55, and 47:53, respectively.[14] Variations between the experimental and computational ratios may be due to solvent effects during synthesis, because the calculations were carried out in a solvent and interaction-free environment. In addition, the interconversion barrier between the conformations has not yet been determined computationally, and kinetic versus thermodynamic control during synthesis may also lead to variations in the product ratios. The observation of two conformers by NMR analysis was reasonable considering the ^{1}H NMR data of the previously reported sultam thioureas and the NMR spectra of **1** and **6a–d**, in which the same groups at R^1 and R^2 are not equivalent. To further investigate these sultam thioureas, X-ray crystal structures of **6e** and **6f** were obtained and are shown in Figure 2.[16] For these two X-ray structures, the two phenyl rings are on the same side of the molecule. For **6f**, there are two molecules comprising the asymmetric unit, and one of the two has a disordered N-ethyl group, with the major conformer having an occupancy of 0.62(10). The observed conformers were the lower energy structures, as predicted by B3 LYP/6-31G(d) calculations.[14]

Next, **6i** was synthesized with an ethyl and methyl group at the R^1/R^2 positions in 52% yield and an X-ray crystal structure was obtained (Figure 2).[17] In the crystal, both conformers were observed (N-methyl *syn* to S; N-ethyl *syn* to S; ratio 76:24). Two conformers were also observed in solution by ^{1}H NMR analysis (ratio 1:1.5). In all three structures, weak thiourea ring S1···S2 interactions are present with distances of 2.94(1) Å for **6e**, 2.998(5) and 2.989(5) Å for **6f**, and 3.061(6) Å for **6i**. These distances are much longer than typical disulfide bonds (about 2.05 Å), but are shorter than the sum of the van der Walls radii (about 3.6 Å).[18] Lastly, compound **6j**,[19] which has previously been reported,[2] was synthesized in 44% yield to provide another example of a compound with alkyl/alkyl groups at the R^1/R^2 positions.

All of the synthesized sultam thioureas were assayed for cytotoxicity and were found to have a 50% cytotoxicity concentration (CC_{50}) value of >100 μM.[20] The ability of each of the compounds to inhibit WNV replication is shown in Table 2.[21] Newly synthesized **1** (Table 2) was found to reproduce the WNV antiviral activity of the originally discovered compound (**1**; Table 1). The fifty percent effective concentration (EC_{50}) values for **6a** and **6b** clearly indicate that replacement of one or both of the hydrogen atoms at R^4 and R^5 with a methyl group causes a significant increase in the EC_{50} values. Out of the eight compounds with various R^1/R^2 groups (**6c–j**), only **6h** was found to have similar activity to **1**.

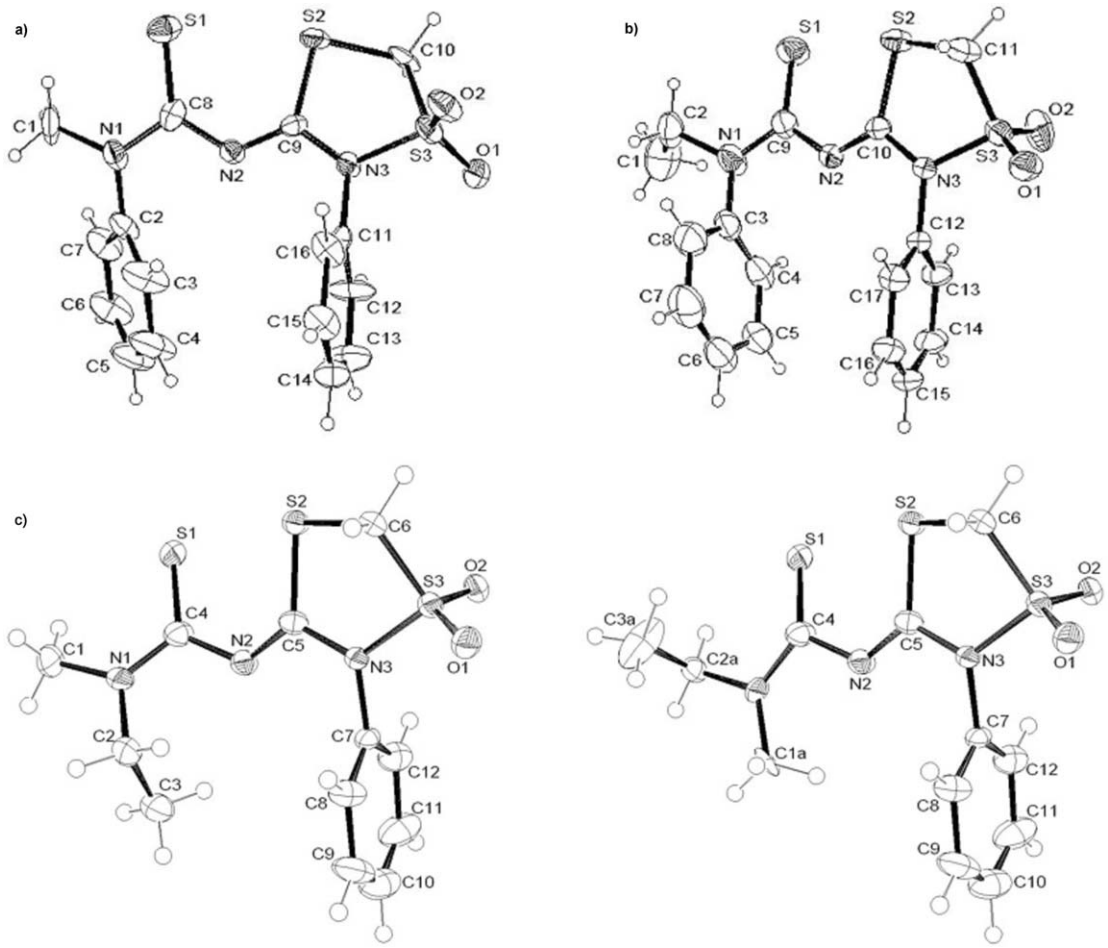

Figure 2 X-ray crystal structures of (a) **6e**, (b) **6f**, and (c) two conformers of **6i**

In summary, this paper reports the syntheses of eleven sultam thioureas and further characterization of these compounds, including X-ray structures. Using the synthetic procedure described, sultam thioureas can be synthesized with alkyl/alkyl, aryl/alkyl, or aryl/aryl groups at the R^1/R^2 positions and with alkyl groups at the R^4 and/or R^5 positions. The results of the assay against WNV indicate that hydrogen atoms at R^4 and R^5 are critical for activity and further demonstrate that the R^1 and R^2 groups are significant for the inhibition of WNV replication. Work is underway to synthesize more sultam thioureas, especially compounds with diverse groups at the R^3 position.

Supporting Information for this article is available online at http://www.thieme-connect.com/ejournals/toc/synlett. It includes general experimental information and the procedures and characterization of the thioureas used to synthesize the sultam thioureas.

Acknowledgment

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(7) **Typical procedure for the synthesis of sultam thioureas.** To a flame-dried flask under nitrogen, were added thiourea (1 equiv) and THF to make a 0.4 M solution. Next NaH

(60% in paraffin, 1.5 equiv) was added in portions. Phenylisothiocyanate (1 equiv) was added, the reaction mixture was heated in a 45 °C oil bath, and the reaction was monitored by TLC. The reaction mixture was cooled to room temperature and then cooled in an ice–water bath. To this reaction mixture was added a 0.5 M solution of HBr (5 equiv) in THF (made using 5.1 M HBr in acetic acid), followed by the addition of hydrogen peroxide (3.5 equiv, 30% H_2O_2 solution). The reaction was stirred overnight and filtered, after which, the solid was dissolved in CH_2Cl_2 (10 mL) and washed with sat. Na_2CO_3 (10 mL). The organic layer was removed and the aqueous layer was washed with a second portion of CH_2Cl_2 (10 mL). The combined CH_2Cl_2 solution was dried with anhydrous $MgSO_4$, filtered and concentrated in vacuo. The solid was dissolved in CH_2Cl_2 to make a 0.05 M solution, which was cooled in an ice bath under nitrogen. To this solution was added triethylamine (7.2 equiv based on the crude weight of the filtered solid), followed by dropwise addition of a 0.75 M solution of methanesulfonyl chloride (1.5 equiv based on the crude weight of the filtered solid) in CH_2Cl_2. After stirring the reaction for 1 h, the reaction was diluted with CH_2Cl_2 and washed twice with H_2O. The organic layer was dried with anhydrous $MgSO_4$, filtered and concentrated in vacuo. The residue was purified by silica chromatography (ethyl acetate–*n*-hexane, 95% or CH_2Cl_2–*n*-hexane, 95%). The purity of the product was confirmed by HPLC analysis using two different conditions with a C18, 5 μm (250 × 4.6 mm) column at 40 °C, and monitored at 220 nm.

(8) **Compound 1**: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 4.38 (s, 2 H), 6.90–7.44 (m, 15 H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 46.46, 126.7, 126.8, 127.7, 127.9, 128.8, 128.9, 129.3, 129.4, 129.5, 131.9, 144.0, 144.8, 161.8, 188.4. HRMS (ESI+): *m*/*z* $[M + H]^+$ calcd for $C_{21}H_{18}N_3O_2S_3$: 440.0556; found: 440.0545. HPLC conditions A: MeCN/H_2O with 0.1% AcOH and the following gradient: 5% MeCN for 2 min, 5–95% MeCN over 16 min, 95% MeCN for 2 min at 0.5 mL/min. HPLC conditions B: MeOH/H_2O with 0.1% AcOH and the following gradient: 5% MeOH for 2 min, 5–95% MeOH over 16 min, 95% MeOH for 2 min at 1.0 mL/min. The retention time of the product under conditions A was 16.9 min (>99% purity) and under conditions B it was 17.9 min (>99% purity).

Compound 6a: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.83 (d, *J* = 6.8 Hz, 3 H), 4.51 (q, *J* = 6.8 Hz, 1 H), 6.85–7.50 (m, 15 H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 14.7, 55.1, 126.7, 126.8, 127.6, 127.9, 128.6, 128.6, 129.2, 129.4, 129.4, 132.2, 144.1, 144.8, 160.7, 188.4. HRMS (ESI+): *m*/*z* $[M + H]^+$ calcd for $C_{22}H_{20}N_3O_2S_3$: 454.0712; found: 454.0701. HPLC conditions A: MeCN/H_2O with 0.1% AcOH and the following gradient: 45% MeCN for 3 min, 45–95% MeCN over 15 min, 95% MeCN for 2 min at 1.0 mL/min. HPLC conditions B: MeOH/H_2O with 0.1% AcOH and the following gradient: 55% MeOH for 3 min, 55–95% MeOH over 15 min, 95% MeOH for 2 min at 1.0 mL/min. The retention time of the product under conditions A was 17.4 min (>99% purity) and under conditions B was 18.0 min (>99% purity).

Compound 6b: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.91 (s, 6 H), 6.90–7.02 (m, 4 H), 7.02–7.14 (m, 3 H), 7.14–7.22 (m, 2 H), 7.22–7.42 (m, 6 H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 23.4, 63.7, 126.7, 126.8, 127.6, 127.9, 128.4, 128.7, 129.2, 129.3, 129.3, 132.5, 144.1, 144.9, 160.1, 188.5. HRMS (ESI+): *m*/*z* $[M + H]^+$ calcd for $C_{23}H_{22}N_3O_2S_3$: 468.0869; found: 468.0860. HPLC conditions A: MeCN/H_2O with 0.1% AcOH and conditions B: MeOH/H_2O with 0.1% AcOH. The following gradient was used: 40% organic solvent for 3 min, 40–95% organic solvent over 15 min, 95% organic solvent for 2 min at 1.0 mL/min. The retention time of the product under conditions A was 13.6 min (>99% purity) and under conditions B it was 15.8 min (>99% purity).

(9) For procedures see the Supporting Information.

(10) **Compound 6c**: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.27 (s, 3 H), 2.32 (s, 3 H), 6.77–7.32 (m, 13 H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 21.0, 21.2, 46.4, 126.3, 127.4, 128.9, 129.1, 129.1, 129.3, 130.0, 132.0, 136.3, 137.5, 141.7, 142.3, 161.5, 188.2. HRMS (ESI+): *m*/*z* $[M + H]^+$ calcd for $C_{23}H_{22}N_3O_2S_3$: 468.0859; found: 468.0857. The purity of the product was confirmed by HPLC using the same two conditions used for **6b**. The retention time of the product under conditions A was 14.1 min (98% purity) and under conditions B was 16.5 min (>99% purity).

Compound 6d: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.65–2.80 (m, 2 H), 3.12–3.33 (m, 2 H), 4.30 (d, *J* = 12.0 Hz, 1 H), 4.34 (d, *J* = 12.0 Hz, 1 H), 6.70–6.82 (m, 2 H), 6.96–7.03 (m, 4 H), 7.17–7.37 (m, 7 H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 30.1, 30.2, 46.4, 126.4, 126.8, 127.3, 127.4, 128.0, 128.4, 128.8, 129.2, 129.4, 130.4, 131.9, 134.1, 135.3, 142.2, 143.1, 161.5, 188.5. HRMS (ESI+): *m*/*z* $[M + H]^+$ calcd for $C_{23}H_{20}N_3O_2S_3$: 466.0712; found: 466.0700. The purity of the product was confirmed by HPLC using the same two conditions used for **6b**. The retention time of the product under conditions A was 12.7 min (96% purity) and under conditions B was 15.1 min (98% purity).

(11) **Compound 5d**: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.76–2.92 (m, 2 H), 3.34–3.49 (m, 2 H), 6.05 (br s, 2 H), 7.18–7.52 (m, 8 H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 30.0, 30.5, 126.7, 127.8, 128.3, 129.1, 129.3, 130.0, 131.7, 135.7, 136.9, 140.1, 143.7, 183.3. HRMS (ESI+): *m*/*z* $[M + H]^+$ calcd for $C_{15}H_{15}N_2S$: 255.0951; found: 255.0951. HPLC conditions A: MeCN/H_2O with 0.1% AcOH and conditions B: MeOH/H_2O with 0.1% AcOH. The following gradient was used: 35% organic solvent for 3 min, 35–95% organic solvent over 15 min, 95% organic solvent for 2 min at 1.0 mL/min. The retention time of the product under conditions A was 6.6 min (>99% purity) and under conditions B was 10.5 min (>99% purity).

Crystal Data: CCDC 838607.[22] $C_{15}H_{14}N_3S$; MW = 254.34; monoclinic; *a* = 7.3785 (10) Å, *b* = 15.847 (3) Å, *c* = 11.1495 (16) Å, *β* = 101.204 (14)°; *U* = 1278.8 (3) Å^3; *T* = 298 (2) K; space group P 2_1/n (#14); λ = 0.71073 Å; Z = 4; D_c = 1.321 Mg/m^3; *F*(000) = 536; colorless; dimensions 0.20 × 0.20 × 0.50 mm; *μ* = 0.235 mm^{-1}; 3.32 < Θ < 32.23°, 7898 reflection measured, 4179 unique reflections, R_{int} = 0.0773. *R*1 = 0.2121, 0.3045, *wR*2 = 0.2746, 0.3530, 1.772 goodness of fit on F^2. Crystals are invariably twinned. The structure was solved by direct methods with SHELXS-86 and refined by full-matrix least-squares with SHELXL-97. In each case, non-hydrogen atoms were found in the initial E-maps. All non-hydrogen atom positions and anisotropic vibrational parameters were refined in the developed models, which included contributions from the hydrogen atoms placed in calculated positions and assigned U_{iso} values equal to 120% of the U_{eq} of the adjacent atom.

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(15) **Compound 6e**: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 3.11 (s, 0.5 H), 3.60 (s, 2.5 H), 4.33 (s, 1.7 H), 4.35 (s, 0.3 H), 6.79–6.83 (m, 2 H), 6.89–6.94 (m, 2 H), 7.05–7.56 (m, 6 H). HRMS (ESI+): *m*/*z* $[M + H]^+$ calcd for $C_{16}H_{16}N_3O_2S_3$: 378.0399; found: 378.0391. The purity of the product was confirmed by HPLC using the same two conditions used for **5d**. The retention time of the product under conditions A was 11.4 min (95% purity) and under conditions B was 13.3 min (98% purity).
Compound 6f: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.80 (t, *J* = 7.1 Hz, 0.93 H), 1.19 (t, *J* = 7.1 Hz, 2.07 H), 3.52 (q, *J* = 7.1 Hz, 0.62 H), 4.13 (q, *J* = 7.1 Hz, 1.38 H), 4.32 (s, 1.35 H), 4.36 (s, 0.65 H), 6.76–6.82 (m, 1 H), 6.83–6.88 (m, 1 H), 7.08–7.18 (m, 4 H), 7.22–7.58 (m, 4 H). HRMS (ESI+): *m*/*z* $[M + H]^+$ calcd for $C_{17}H_{18}N_3O_2S_3$: 392.0556; found: 392.0546. The purity of the product was confirmed by HPLC using the same two conditions used for **5d**. The retention time of the product under conditions A was 16.5 min (98% purity) and under conditions B was 14.2 min (98% purity).
Compound 6g: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 4.31 (s, 1.25 H), 4.33 (s, 0.75 H), 4.74 (s, 0.76 H), 5.36 (s, 1.24 H), 6.61–6.66 (m, 1 H), 6.68–6.72 (m, 1 H), 6.82–6.88 (m, 1 H), 6.97–7.41 (m, 12 H). HRMS (ESI+): *m*/*z* $[M + H]^+$ calcd for $C_{22}H_{20}N_3O_2S_3$: 454.0712; found: 454.0703. The purity of the product was confirmed by HPLC using the same two conditions used for **5d**. The retention time of the product under conditions A was 17.9 min (99% purity) and under conditions B was 15.7 min (95% purity).
Compound 6h: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.64–0.81 (m, 1 H), 0.81–1.15 (m, 3 H), 1.27–1.46 (m, 2 H), 1.46–1.63 (m, 2 H), 1.64–1.78 (m, 1 H), 1.87–2.02 (m, 1 H), 4.22 (tt, *J* = 3.5, 11.9 Hz, 0.5 H), 4.29 (s, 1.3 H), 4.33 (s, 0.7 H), 5.07 (tt, *J* = 3.5, 11.9 Hz, 0.5 H), 6.64–6.78 (m, 2 H), 6.98–7.05 (m, 1 H), 7.05–7.28 (m, 4 H), 7.28–7.59 (m, 3 H). HRMS (ESI+): *m*/*z* $[M + H]^+$ calcd for $C_{21}H_{24}N_3O_2S_3$: 466.1025; found: 446.1015. The purity of the product was confirmed by HPLC using the same two conditions used for **6b**. The retention time of the product under conditions A was 15.0 min (>99% purity) and under conditions B was 16.9 min (97% purity).

(16) **Crystal Data for 6e**: CCDC 838606.[22] $C_{16}H_{15}N_3O_2S_3$; MW = 377.49; orthorhombic; *a* = 16.708 (4) Å, *b* = 18.397 (3) Å, *c* = 5.6729 (8) Å, *β* = 90°; *U* = 1743.7 (6) Å^3; *T* = 300(2) K; space group P n a 21 (#33); λ = 0.71073 Å; *Z* = 4; D_c = 1.438 Mg/m^3; *F*(000) = 784; yellow; dimensions 0.69 × 0.06 × 0.03 mm; *μ* = 0.439 mm^{-1}; 3.54 < Θ < 29.19°, 9073 reflection measured, 4001 unique reflections, R_{int} = 1163. *R*1 = 0.0625, 0.1818, *wR*2 = 0.0656, 0.0781, 0.877 goodness of fit on F^2. The structure was solved by direct methods and refined as described above.
Compound 6f: CCDC 838608.[22] $C_{17}H_{17}N_3O_2S_3$; MW = 391.54; triclinic; *a* = 10.2747 (5) Å, *b* = 11.2638 (5) Å, *c* = 18.1559 (8) Å, *β* = 80.766 (4)°; *U* = 1866.99 (15) Å^3; *T* = 299 (2) K; space group P-1; λ = 0.71073 Å; Z = 4; D_c = 1.393 Mg/m^3; *F*(000) = 816; yellow; dimensions 0.46 × 0.32 × 0.05 mm; *μ* = 0.413 mm^{-1}; 3.35 < Θ < 30.59°, 21500 reflection measured, 11234 unique reflections, R_{int} = 0.0342. *R*1 = 0.0407, 0.1264, *wR*2 = 0.0482, 0.0515, 1.005 goodness of fit on F^2. The structure was solved by direct methods and refined as described above.

(17) **Compound 6i**: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.78 (t, *J* = 7.2 Hz, 1.8 H), 1.17 (t, *J* = 7.1 Hz, 1.2 H), 2.78 (s, 1 H), 3.21–2.28 (m, 3.3 H), 3.81 (q, *J* = 7.2 Hz, 0.7 H), 4.35 (s, 0.8 H), 4.36 (s, 1.2 H), 7.36–7.42 (m, 2 H), 7.45–7.53 (m, 3 H). HRMS (ESI+): *m*/*z* $[M + H]^+$ calcd for $C_{12}H_{16}N_3O_2S_3$: 330.0399; found: 330.0399. The purity of the product was confirmed by HPLC using the same two conditions used for **6b**. The retention time of the product under conditions A was 8.6 min (97% purity) and under conditions B was 10.3 min (96% purity).
Crystal Data: CCDC 838605.[22] $C_{12}H_{15}N_3O_2S_3$; MW = 329.47; monoclinic; *a* = 5.2548 (4) Å, *b* = 15.8241 (13) Å, *c* = 17.885 (2) Å, *β* = 97.681 (9)°; *U* = 1473.8 (2) Å^3; *T* = 150 (2) K; space group P 21/n (#14); *λ* = 0.71073 Å; *Z* = 4; D_c = 1.485 Mg/m^3; F(000) = 688; colorless; dimensions 0.62 × 0.04 × 0.01 mm; *μ* = 0.507 mm^{-1}; 3.45 < Θ < 27.59°, 10662 reflection measured, 3285 unique reflections, R_{int} = 0.0831. *R*1 = 0.0637, 0.1217, *wR*2 = 0.1185, 0.1248, 1.008 goodness of fit on F^2. The structure was solved by direct methods and refined as described above.

(18) *Structure Correlation*, Vol. 2; Burgi, H.-B.; Dunitz, J. D., Eds.; VCH Publishers: Weinheim, **1994**.

(19) **Compound 6j**: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.87 (s, 3 H), 3.31 (s, 3 H), 4.37 (s, 2 H), 7.37–7.43 (m, 2 H), 7.47–7.54 (m, 3 H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 39.6, 42.0, 46.5, 129.5, 129.6, 130.1, 132.7, 161.3, 185.2. HRMS (ESI+): *m*/*z* $[M + H]^+$ calcd for $C_{11}H_{14}N_3O_2S_3$: 316.0243; found: 316.0243. The purity of the product was confirmed by HPLC using the same two conditions used for **5d**. The retention time of the product under conditions A was 8.7 min (>99% purity) and under conditions B was 10.1 min (99% purity).

(20) Cytotoxicity analyses were performed by using the assay developed for determining dehydrogenase activities in metabolically active cells as described in ref. 1.

(21) Fifty percent effective (EC_{50}) concentrations of compounds against WNV were determined by evaluation of cytopathic effects (CPE) in Vero cells by limiting dilution, as described in ref. 1.

(22) Crystallographic data for structures **5d, 6e**, **6f**, and **6i** have been deposited with the Cambridge Crystallographic Data Centre (CCDC 838607, 838606, 838608, and 838605, respectively). Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, United Kingdom [Fax: +44 (1223)336033 or e-mail: deposit@ccdc.cam.ac.uk].