SAR and Mode of Action of Novel Non-Nucleoside Inhibitors of Hepatitis C NS5b RNA Polymerase

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Novel non-nucleoside inhibitors of the HCV RNA polymerase (NS5b) with sub-micromolar biochemical potency have been identified which are selective for the inhibition of HCV NS5b over other polymerases. The structures of the complexes formed between several of these inhibitors and HCV NS5b were determined by X-ray crystallography, and the inhibitors were found to bind in an allosteric binding site separate from the active site. Structure−activity relationships and structural studies have identified the mechanism of action for compounds in this series, several of which possess drug-like properties, as unique, reversible, covalent inhibitors of HCV NS5b.

Introduction

Hepatitis C virus (HCV) is a (+)-strand RNA virus of the *Flaviviridae* family that was first identified in 1989.[1,2] HCV infects ∼3% of the world population, and fatalities associated with HCV infection result from chronic liver disease (cirrhosis) and hepatocellular carcinoma.[3] HCV infection is responsible for the majority of liver transplants currently performed.[4,5] Current therapies include treatment with interferon-α (IFN-α), often in combination with Ribavirin,[6] or the recently approved poly(ethylene glycol)ated interferon-α (PEG-IFN-α),[7] which allows once-weekly administration. However, current combination therapy suffers from high cost and relapse rate and is successful in <50% of patients.[8] Thus, there remains an urgent need for efficacious therapies for the treatment of HCV. In this paper we present some of our work on the design of new inhibitors of HCV replication, and investigations into their mechanism of inhibition.

The HCV viral genome consists of a single-strand RNA of ∼9600 base pairs which encode a ∼3010 amino acid polypeptide. Protein products of the polypeptide include structural proteins and several functional proteins that are potential targets for the actions of small molecule drugs, including the NS3 serine protease and the associated NS4A ATP-ase dependent helicase (NS3·4A), the internal ribosomal entry site (IRES), and NS5b RNA dependent RNA polymerase (RdRp). Medicinal chemists have focused much attention on inhibitors of the NS3·4A protease, and efforts toward this end have been recently reviewed.[9,10] IRES inhibitors have been described,[11] and an IRES antisense oligonucleotide has been reported to be in clinical trials.[12] Reports on inhibitors of the NS5b polymerase have until recently been primarily restricted to patent applications, in which various groups, including ours,[13] have disclosed a variety of small molecule non-nucleoside inhibitors including diketobutanoic acids[14] and 2-aryldihydroxypyrimidine carboxylic acids[15] (IRBM), barbituric acid derivatives,[16] furanylidenylrhodanines,[17] 2-methylidenylbenzothiophenes[18] (ViroPharma), and 2-arylbenzimidazoles[19] (Japan Tobacco). ViroPharma has also reported a triazinoindole inhibitor of the NS5b polymerase from bovine viral diarrhea virus (BVDV), a member of the *Flaviviridae* family that is closely related to HCV.[20] Very recently, several groups, including our own,[21] have reported inhibitors of HCV NS5b in the scientific literature,[22−33] and the medicinal chemistry targeting hepatitis C has recently been reviewed.[34,35] In this paper we give a full report of our work on the discovery and structure−activity relationships of a novel class of small molecule inhibitors of the HCV NS5b polymerase.

The HCV NS5b RdRp is a 65 KDa polymerase that functions as a catalytic subunit of the viral replicase,[36−38] and the NS5b crystal structure has been independently solved by three groups.[39−41] The viral NS5b RNA polymerase is required for the transcription of genomic RNA, a process that is required for the replication of HCV. Viral polymerases are highly attractive targets for drug discovery, a position that has been amply demonstrated to date by the clinical success of both nucleoside and non-nucleoside inhibitors of HIV and hepatitis B virus (HBV). This success in other viral systems, combined with the possibility of three-dimensional target structural information, led us to investigate the possibility of small molecule inhibitors of HCV NS5b polymerase. High-throughput screening of a small-molecule compound library, using a radioactive filtration assay and truncated enzyme devoid of the C-terminal 21 hydrophobic amino acids (Δ21 NS5b),[42] led to the discovery of **1** (Figure 1), which is selective for HCV NS5b (IC_{50} = 1.5 μM) over BVDV NS5b and avian myeloblastosis virus (AMV) reverse transcriptase (IC_{50}'s >30 μM for both).

Chemistry. The syntheses of **1** and a variety of analogues are described in Schemes 1−3. Initial chemistry centered around the synthesis of truncated inhibitors **2** lacking the arylsulfonamide functionality (Scheme 1). Condensation of 3-aminorhodanine with a series of substituted benzaldehydes in methanol in the presence of sodium acetate, followed by filtration of the crude reaction mixtures, afforded pure 3-amino-5-benzylidenerhodanines **2a**−**h**. Similar condensations with rhodanine itself and 3-alkyl substituted rhodanines provided compounds **2i**−**n**, which were purified via flash chromatography. Compounds **2**

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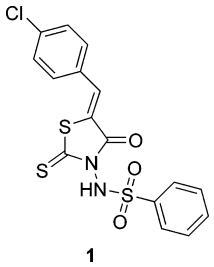
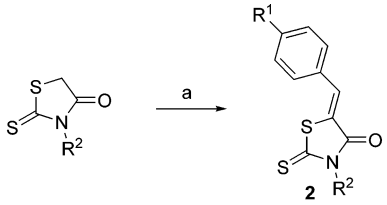


1

Figure 1. Structure of initial screening hit **1**.

Scheme 1. Synthesis of Truncated Analogues of **1**[a]

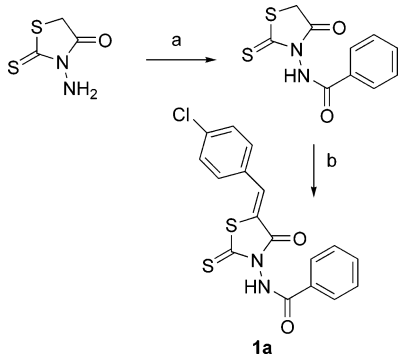


2

2	R^1	R^2	**2**	R^1	R^2
a	NMe_2	NH_2	**i**	Cl	C_2H_5
b	OMe	NH_2	**j**	Cl	CH_3
c	CH_3	NH_2	**k**	Cl	H
d	H	NH_2	**l**	Cl	CH_2Ph
e	Cl	NH_2	**m**	NO_2	CH_3
f	CN	NH_2	**n**	NO_2	H
g	F	NH_2			
h	NO_2	NH_2			

[a] Reagents: (a) Aldehyde, NaOAc, methanol, yield 30−90%.

Scheme 2. Synthesis of **1a**[a]



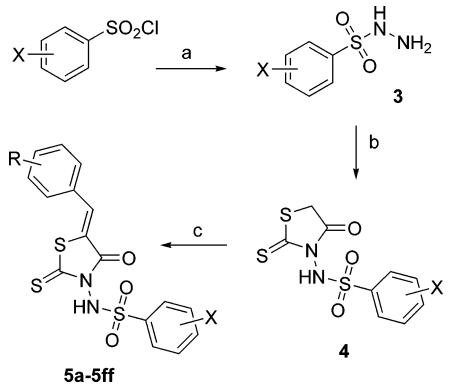
1a

[a] Reagents and conditions: (a) PhCOCl, 60 °C, THF, 82%. (b) 4-chlorobenzaldehyde, NaOAc/MeOH, rt, 58%.

were typically obtained as a single geometric olefin isomer, as determined via ^{1}H NMR spectral analysis, and were assigned as the *Z* isomer.

Treatment of 3-aminorhodanine with benzoyl chloride in THF followed by condensation with 4-chlorobenzaldehyde in MeOH/NaOAc gave the acyl 3-aminorhodanine derivative **1a** in fair yield (Scheme 2). However, in contrast to the reported acylation of 3-aminorhodanines with acyl chlorides,[43] attempts at direct sulfonamide formation of 3-aminorhodanines **2a**−**h** utilizing arylsulfonyl chlorides under a variety of conditions resulted only in the recovery of starting materials. Forcing conditions resulted only in combinations of unsubstituted and bis-sulfonylated products, so an alternative route was followed for the synthesis of arylsulfonamide compounds (Scheme 3). Formation of arylsulfonyl hydrazides **3** was accomplished by treatment of sulfonyl chlorides with hydrazine at 0 °C in THF, followed by

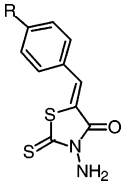
Scheme 3. General Route for the Synthesis of Analogues of **1**[a]



3 **4** **5a-5ff**

[a] Reagents and conditions: (a) hydrazine, 0 °C, THF. (b) bis(carboxymethyl) trithiocarbonate, H_2O, 95 °C. (c) aldehyde/NaOAc/MeOH, 65 °C.

Table 1. Effect of Benzylidene Substitution on HCV NS5b Polymerase Activity of 2-Amino Rhodanines



compound	R	IC_{50} (μM)[a]
2a	NMe_2	>30
2b	OMe	>30
2c	Me	>30
2d	H	>30
2e	Cl	16
2f	CN	7.0
2g	F	7.0
2h	NO_2	1.0

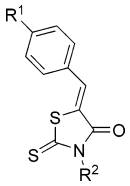
[a] IC_{50} values for inhibition of HCV NS5b calculated from two determinations in duplicate.

aqueous workup and crystallization from methanol. The resulting sulfonylhydrazides were treated with bis(carboxymethyl)trithiocarbonate[44] in water at 95 °C to give 3-(arylsulfonylamino)-rhodanines **4** following crystallization from ethanol. Condensation of rhodanines **4** with aldehydes in methanol with sodium acetate gave the desired compounds **5a**−**ff**. A similar procedure was followed to obtain 3-(alkylsulfonylamino)rhodanines **6a** and **6b** starting from methanesulfonyl chloride and butanesulfonyl chloride, respectively. As was the case for the truncated inhibitors **2**, compounds **5** were typically obtained as a single geometric olefin isomer, assigned as the *Z* isomer based on ^{1}H NMR spectral analysis.

Structure−**Activity Relationships.** Inhibition of HCV NS5b RNA polymerase was evaluated using recombinant Δ21 NS5b polymerase with a C-terminal 10-His tag, expressed from *E. coli*, and utilizing as a template the transcribed HCV 3′ UTR RNA (567nt) in a radioactive filtration format.

Initial SAR investigation focused on truncated systems without the arylsulfonyl group present, and a batch mode Topliss tree[45] was generated with para-substituted compounds **2a**−**h** (Table 1). Substitution with electron-donating groups such as dimethylamino (**2a**) or methoxy (**2b**) gave compounds that were inactive at the highest concentration tested (30 μM), as did substitution with methyl (**2c**) and hydrogen (**2d**). Conversely, substitution with electron-withdrawing groups on the benzylidene aryl ring produced compounds with increased activity, the nitro-substituted compound **2h** being the most potent in the series, with an IC_{50} of 1.0 μM, about 30-fold more potent than

Table 2. Effect of Imide N-Substitution on Benzylidene-Substituted Rhodanine HCV NS5b Polymerase Inhibition



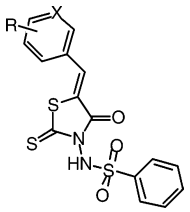
compound	R_1	R_2	IC_{50} (μM)[a]
2i	Cl	CH_3CH_2	>30
2j	Cl	CH_3	>30
2k	Cl	H	>30
2e	Cl	NH_2	16
1	Cl	NH_2SO_2Ph	1.5
1a	Cl	NHC(O)Ph	>30
2l	Cl	CH_2Ph	>30
2m	NO_2	CH_3	30
2n	NO_2	H	22
2h	NO_2	NH_2	1.0
5k	NO_2	NH_2SO_2Ph	2.0

[a] IC_{50} values for inhibition of HCV NS5b calculated from two determinations in duplicate.

the unsubstituted compound **2d**. Next, a series of simple 3-amino replacements were investigated (Table 2) in order to ascertain the importance of the 3-amino group. When the para benzylidene substituent R^1 is chlorine, removal of the 3-amino group, as in **2k**, eliminated activity, and the activity could not be regained with simple alkyl substitution of the imide nitrogen by methyl or ethyl (compounds **2i** and **2j**), while the 3-(phenylsulfonyl)-amino substituted lead compound **1** remains 10-fold more potent than the 3-amino compound **2e**. Changing R^1 from chloro to nitro resulted in a similar trend. Interestingly, in the case of the nitro series, elaboration of the 3-amino group (**2h**) to 3-(phenylsulfonyl)amino (**5k**) did not result in the same increase in potency observed in the chloro series, indicating that when the 3-(phenylsulfonyl)amino group is present the importance of the electron-withdrawing group at R^1 is reduced. Replacement of the 3-(phenylsulfonyl)amino group with acylamino moieties such as 3-(benzoyl)amino resulted in inactive compounds (e.g., **1a**), again suggesting specific interactions associated with the 3-(phenylsulfonyl)amino group.

The SAR was further investigated in the 3-(phenylsulfonyl)-aminorhodanine core via a series of analogues with varied benzylidene aryl substitution (Table 3). The unsubstituted benzylidene analogue **5a** (IC_{50} = 30 μM) was 15-fold less potent than the initial lead **1** (R = 4-Cl), while substitution with 4-methyl (**5c**) improved potency to 10 μM. Moving the methyl group to the 3 position (**5d**) reduced potency to 25 μM, although activity remained better than the unsubstituted analogue **5a**. Contrary to the SAR observed in the truncated inhibitors **2a**−**h**, incorporation of electron donating groups such as methoxy at the 3 or 4 positions (**5f** and **5e**, respectively) improved potency relative to the electronically neutral methyl substitution, consistent with the results observed with nitro-substituted compounds **2h** and **5k** and suggestive of the importance of lipophilic interactions. Similarly, replacement of the electron-withdrawing chloro group of **1** with the electronically weaker but more lipophilic bromide **5h** resulted in an increase in potency. Following the above SAR, addition of a second Cl substituent to **1** (**5o**) improved potency to 200 nM. Trifluoro-substituted compounds (**5s**−**u**) again highlight the importance of specific interactions, as a 10-fold difference in potency was noted between the 2,3,4-trifluoro compound **5s** (IC_{50} = 1.9 μM) and the 2,4,5-trifluoro compound **5u** (IC_{50} = 200 nM), even though both compounds possess very similar electronic character. The effect of lipophilicity and size in the benzylidene substitution was further investigated as shown in Table 4. The trends observed in Tables 2 and 3 were confirmed, and potency increased with increasing lipophilicity of the benzylidene group.

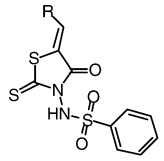
Table 3. Effect of Benzylidene Substitution on 2-(Phenylsulfonyl)amino-Substituted Rhodanine HCV NS5b Polymerase Inhibition

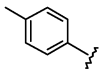
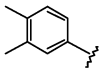
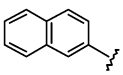
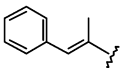


compound	R	X	IC_{50} (μM)[a]
5a	H	C	30
5b	H	N	15
5c	4-CH_3	C	10
5d	3-CH_3	C	25
5e	4-OCH_3	C	5
5f	3-OCH_3	C	5
5g	3-OCH_2O-4	C	15
5h	4-Br	C	0.7
1	4-Cl	C	1.5
5i	4-F	C	1.0
5j	2-F	C	6.5
5k	4-NO_2	C	2.0
5l	4-CN	C	0.9
5m	4-CF_3	C	1.0
5n	3-F, 4-Cl	C	1.0
5o	3,4-di-Cl	C	0.2
5p	3,4-di-Br	C	0.2
5q	2,4-di-F	C	3
5r	2,5-di-F	C	1.7
5s	2,3,4-tri-F	C	1.9
5t	3,4,5-tri-F	C	1.0
5u	2,4,5-tri-F	C	0.2

[a] IC_{50} values for inhibition of HCV NS5b calculated from two determinations in duplicate.

Table 4. Effect of 4-Benzylidene Extension on the Inhibition of HCV NS5b Polymerase



Compound	R	IC_{50} (μM)[a]
5a		30
5c		10
5v		8
5w		3
5x		0.2

[a] IC_{50} values for inhibition of HCV NS5b calculated from two determinations in duplicate.

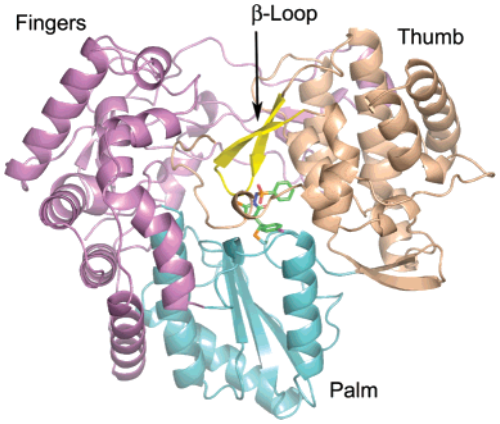


Figure 2. Overall structure of the HCV NS5b polymerase in the standard orientation with the fingers domain in the top left (violet), the thumb domain in the top right (light brown), and the palm domain at the bottom (light blue). The inhibitor **5h** is shown bound to the palm domain below the β-loop (yellow). This and the following crystallography figures were made using PyMOL.[46]

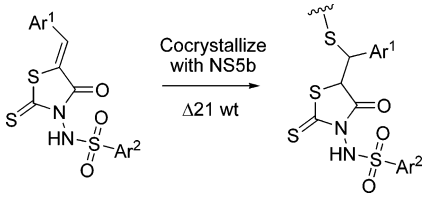


Figure 3. Cocrystallization of compounds **5h**, **5o**, **5u**, **5x**, and **5ee** with Δ21 wt NS5b revealed covalent attachment to Cys366 (resolution 2.0−2.15 Å).

This trend resulted in the synthesis of the potent inhibitor **5x** (IC_{50} = 200 nM).

Structure of an HCV NS5b−Inhibitor Complex. The overall structure for unliganded HCV NS5b has been extensively described elsewhere.[39−41] HCV NS5b retains the general structural features common to other polymerases and can be described as a hand with fingers, thumb, and palm domains (Figure 2). The palm domain contains the conserved Asp residues that bind Mg^{2+} ions and are responsible for the polymerase activity. Polymerase fingers and thumb domains channel the single-stranded nucleic acid template and double-stranded product through the active site and have been crystallized in two conformations: closed, in the presence of nucleotide,[47] and open, in the absence of nucleotide.[48,49] In the case of NS5b (and other RNA-dependent RNA polymerases)[50] the fingers domain contains an extra region that bridges the fingers and thumb domains. The extensive contacts that result between the fingers and thumb domains of these polymerases reduce their flexibility, though different conformations have been observed for the NS5b polymerase that may correspond to active and inactive forms of the protein.[51]

In all of the X-ray crystal structures of inhibitor−NS5b complexes obtained by us, the inhibitors bound covalently to Cys366 in the palm domain (Figure 3), approximately 8 Å from the Gly-Asp-Asp sequence of the active site. The walls of the binding pocket include residues from both the palm and the thumb domains (Figures 4a−c). The compounds form van der Waals interactions with Phe193, Pro197, and Arg200 from the palm domain, and Leu384, Met414, Tyr415, and Tyr448 from the thumb domain. In addition, one of the sulfonamide oxygen atoms forms a direct hydrogen bond with the backbone amide of Tyr448 and a water-mediated hydrogen bond with the backbone amide of Gly449. Finally, the hydroxyl of Tyr415 interacts with the inhibitor's carbonyl group through a water molecule.

SAR, Chemical, and Structure-Based Mechanistic Considerations. Cocrystallization of the benzylidene compounds as described above showed that the inhibitors are covalently linked to the enzyme via Cys366 (Figure 3) at the site of the growing polynucleotide. However, the compounds were found to be *reversible* inhibitors of HCV NS5b (via preincubation/dilution experiments) in vitro.

Based on the chemical structures of the inhibitors, the correlation observed between potency and inductive effects in the truncated inhibitors **2a**−**h** (but not the fully elaborated inhibitors **5**), and the X-ray evidence, concerns were raised about the potential reactivity of the double bond as a nonselective Michael acceptor. However, the compounds described were not found to be nonspecific alkylators or broad-spectrum inhibitors of proteins with biochemically active cysteine residues. Compound **5o** (HCV NS5b IC_{50} = 200 nM) showed no inhibition of any tested cysteine proteases, including cathepsin B, calpain, human caspases 1, 3, 6, 7, and 8, or aldose reductase.[43] Additionally, there was no effect on tubulin polymerization in vitro.[52] Thus, it was concluded that **5o** is not a particularly "hot" thiol acceptor, in accordance with the SAR of the 3-(phenylsulfonyl)amino compounds **5**, where no inductive electronic substituent effects were observed on potency. General affinity labels (*N*-ethylmaleimide, *N*-propylmaleimide, ethyl iodoacetate, dipyridyl disulfides, diphenydisulfide, glutathione disulfide, and assorted acrylates) showed no inhibition of HCV NS5b under our standard assay conditions, indicating that Cys366 is not a particularly "hot" thiol. Additionally, the compounds were found to have excellent separation of in vitro potency against HCV NS5b and cytotoxicity in E−304 and HFF cells (IC_{50} >50 and >100 μM, respectively).

To further investigate the significance of the covalent attachment to Cys366 observed in the X-ray structures, the importance of the olefin moiety was investigated via additional SAR studies. Condensation of 3′,4′-dichloroacetophenone with **4** under Dean−Stark conditions in refluxing benzene/acetic acid/piperidine gave the tetrasubstituted olefin **7** as a 4.9:1 mixture of olefin isomers (Scheme 4). Reduction of the olefin was accomplished via a modified conjugate addition protocol[53] by treatment of **5o** with a mixture of $CoCl_2$/NaOH/$NaBH_4$ in THF/DMF/H_2O to give the reduced product **8** (5%) and the byproduct **9** (22%) resulting from overreduction (Scheme 5). The investigation into the necessity of the olefin (Table 5) gave results inconsistent with the absolute requirement for Michael addition. Addition of a methyl group as in tetrasubstituted olefin **7** should significantly decrease the reactivity of the double bond to Michael addition; however, only a 3-fold decrease in potency was observed. Additionally, removal of the double bond altogether, as in **8**, decreases potency 50-fold but does not completely abrogate activity, demonstrating further that specific interactions are important for binding, and indicating again that the compounds are not simple nonselective Michael acceptors. Further deconstruction of the pharmacophore by reduction of the carbonyl to the alcohol, as in **9**, again reduces potency but does not abolish it altogether. The structure of **5o** was determined via single-crystal X-ray crystallography, and the olefin geometry was determined to be *Z*. The geometric isomer **10** was independently synthesized by treatment of **5o** with thiophenol in refluxing benzene, followed by aprotic workup and isolation of the isomers via flash chromatography to give both **10** and **5o** (Scheme 6). The *E* isomer **10** was found to be

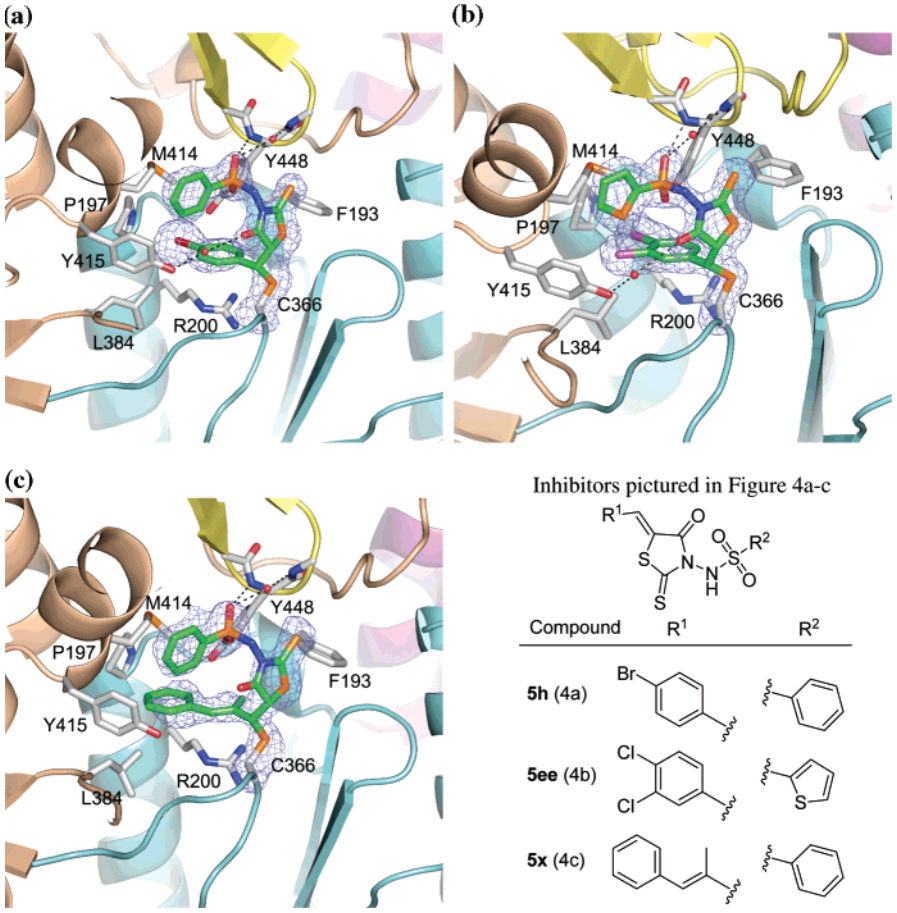
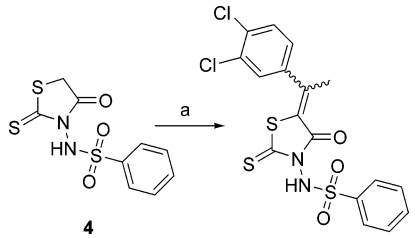


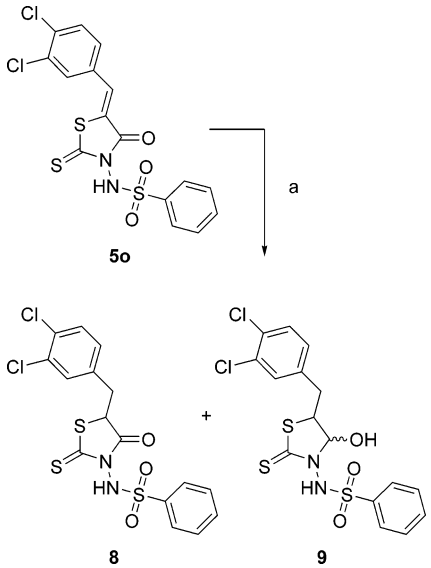
Figure 4. Complexes formed between inhibitors and NS5b (coloring scheme as in Figure 2). The covalent bond between Cys366 and each inhibitor is shown along with other amino acids involved in the interaction. Hydrogen bonds are indicated as dotted lines. The Fo-Fc electron density maps were calculated excluding the ligand and Cys366, and are contoured at 2.5 σ. Inhibitors shown are **5h** (a), **5ee** (b), and **5x** (c). The longer benzylidene substituent of compound **5x** increases the distance to Tyr415 and prevents formation of the water mediated hydrogen bond observed in parts a and b.

Scheme 4. Synthesis of Tetrasubstituted Olefin **7**[a]



[a] Reagents and conditions: (a) 3′,4′-dichloroacetophenone, AcOH/benzene, reflux, 6 h.

Scheme 5. Synthesis of **8** and **9**[a]



[a] Reagents and conditions: (a) $CoCl_2/NaBH_4$/2,2′-dipyridyl, H_2O/DMF/THF, 0 °C to room temperature, yield 5% (**8**), 22% (**9**).

equipotent in the biochemical assay and was found to isomerize back to **5o** under the assay conditions or in the presence of buffer, methanol, or other protic solvents, explaining the potency equivalence of **10** and **5o**, and indicating an equilibrium strongly shifted toward the *Z* form. This very facile isomerization is consistent with ab initio calculations (geometry optimizations, B3LYP/6-31G**) which indicate a large difference in calculated energies between the *Z* (**5o**, 0.0 kcal/mol) and *E* (**10**, +3.7 kcal/mol) forms, again suggesting predominance of the *Z* form to the extent that there is an equilibrium. The combination of the above results with the benzylidene SAR of the 3-(phenylsulfonyl)aminorhodanines **5**, which does not show a substituent electronic component, and where increasing electrophilicity of the double bond does not increase potency, suggests that the binding to Cys366 is perhaps less important than the other interactions and the geometry of the inhibitors, and that specific recognition elements are clearly important for activity.

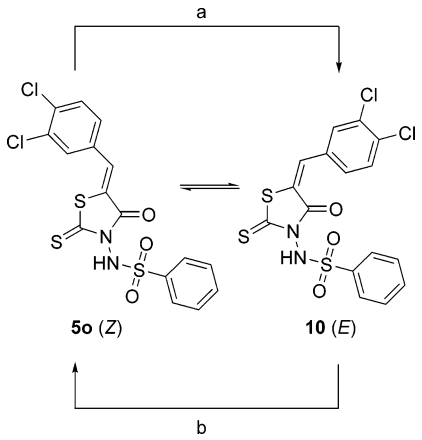
Mechanism of Inhibition of HCV NS5b. The crystal structure of NS5b shows two regions of the protein, a β-loop from the thumb domain (residues 443−454) and the C-terminus

Table 5. Effect of Benzylidene Alkene Modification on the Inhibition of HCV NS5b Polymerase

compound	**5o**	**7**[a]	**8**	**9**[b]
IC_{50} (μM)[c]	0.2	0.6	11	27

[a] Compound **7** tested as a 4.9:1 mixture of olefin isomers. [b] Compound **9** tested as a mixture of isomers. [c] IC_{50} values for inhibition of HCV NS5b calculated from two determinations in duplicate.

Scheme 6. Synthesis of **10**[a]



[a] Reagents and conditions: (a) PhSH, benzene, 80 °C. (b) Presence of MeOH, H_2O, or buffer.

(residues 545−562), that fold in toward the active site.[39−41] Modeling an initiation complex into the NS5b structure using structurally similar amino acid residues from the palm domain of the bacteriophage ϕ6 polymerase structure as a guide[54] shows that, in their crystallized conformations, both the β-loop and the C-terminus block the channel required for the double-stranded RNA product to exit the polymerase. As is the case for the bacteriophage ϕ6 polymerase, these regions may be involved in template binding or act as a scaffold for the formation of the initiation complex.[54] Once RNA synthesis begins, these areas would have to undergo a conformational change to allow the double-stranded RNA product to exit the polymerase.[39−41]

From the X-ray structure information, a compound binding to Cys366 is unlikely to interfere with the active site or compete with nucleotide binding,[55] consistent with the observed reaction kinetics, Lineweaver−Burke plots of which suggest that inhibition by these compounds is noncompetitive with respect to nucleotide substrates.[56] Instead, binding to this site likely inhibits the polymerase activity in one of two ways. The β-loop described above makes up one wall of the binding pocket for our compounds. Stabilizing the position of this β-loop through interaction with the compound could inhibit a conformational change in the thumb domain likely required to bind template or form the platform for assembly of the initiation complex. Furthermore, even if the initiation complex were able to form, strand elongation would not be able to continue. Binding to Cys366 places the compounds in the channel that the RNA daughter strand would use to exit the polymerase and would block the advancement of the RNA daughter strand (Figure 5).

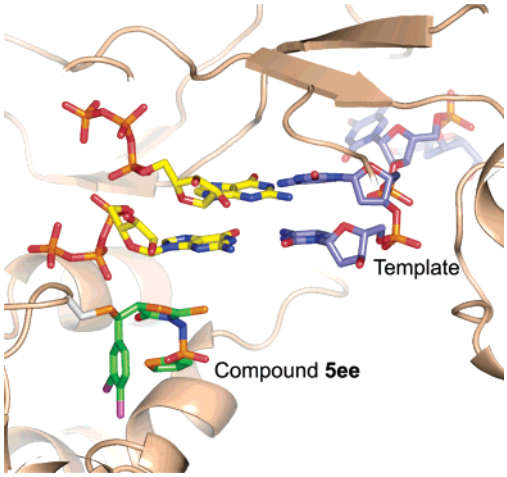


Figure 5. Model of the HCV NS5b initiation complex based on the initiation complex observed in bacteriophage ϕ6.[54] The oligonucleotide template (violet) and the complimentary GTPs (yellow) were modeled into the NS5b polymerase after aligning structurally equivalent residues from the palm domains of the two proteins. Compound **5ee** is shown bound to the NS5b protein, where it would block the advancement of the RNA daughter strand. The β-loop (residues 443−454) and C-terminus (residues 545−562) have been removed for clarity.

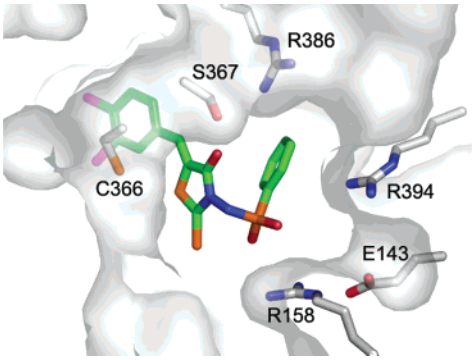


Figure 6. Model of the pre-reacted inhibitor **5o** prior to attack by Cys366.

Additionally, in vitro experiments demonstrated that the amount of full length RNA hybrid product was reduced with increasing inhibitor concentration, while no additional shorter products were obtained under the same conditions (again suggestive of an inhibition of initiation mechanism).[56]

Discussion

On the basis of our findings, we have combined the information described above into a cohesive picture of a possible mechanism for the inhibition of NS5b. Our hypothesis places the prereacted inhibitor in the proper relationship to residues allowing for prerecognition from both lipophilic (Pro197, Leu384, Met414, Arg200, Phe193, Tyr448, and Tyr415) and H-bonding (Ser367, Arg158, Arg386) interactions (Figure 6), as well as reaction (H-transfer relay and stabilization in the attack of Cys366) (Figure 7). Placement of the benzylidene alkene in the *Z* geometry, known to have the lowest energy and the highest population, results in attack on the pro-*R* face (Figure 8), consistent with the absolute configuration of the site of cysteine attack obtained in all ($n = 5$) of our X-ray structures of covalent inhibitor−NS5b complexes. The proposed mechanism is consistent with the requirement for a relatively small lipophilic substituent on the arylsulfonyl group. The hydrophobic interaction between the prebound inhibitor arylsulfonyl group and the protein side chains Ser407 and Arg394 is balanced by

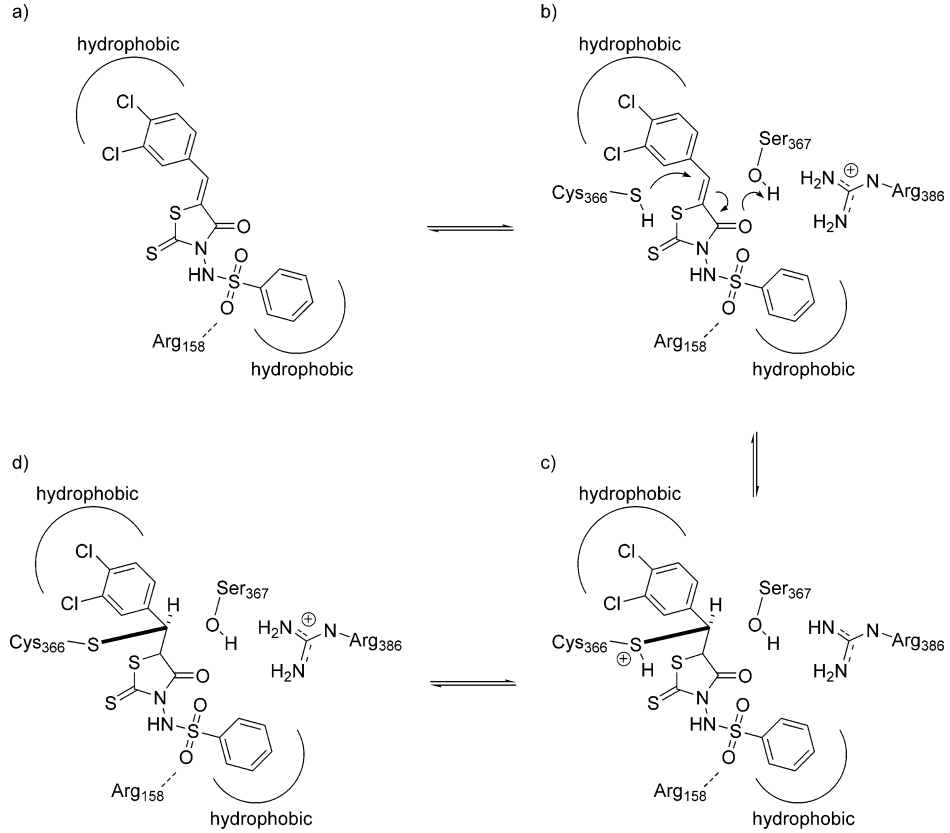


Figure 7. Model of the enzyme−inhibitor complex prior to the putative attack by Cys366 and the proposed mechanism of reversible covalent binding: (a) Pre-recognition of **5o** in the NS5b binding pocket (lipophilic and H-bonding); (b) Attack by Cys366 mediated by a H-transfer relay resulting in c, followed by proton transfer to give the product (d).

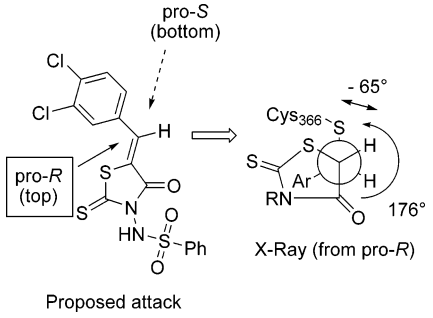
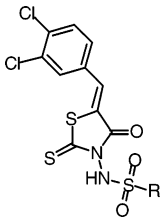


Figure 8. Proposed stereochemistry in the attack by Cys366 on compound **5o**, and the resulting torsional angles for the staggered conformation observed in the X-ray structure of the **5o**−NS5b complex.

the requirement for this group to rotate during the transition between the pre-recognition complex and the covalent complex. This is consistent with the arylsulfonyl replacement SAR, in which substitution of the sulfonylhydrazide aromatic ring consistently reduces potency, presumably by hindering rotation which must occur during the reaction. For example, the methanesulfonyl substituent of compound **6a** (IC_{50} = 2.0 μM) should rotate freely, but lacks the aromatic ring which provides enhanced lipophilic interaction, while the butylsulfonyl substituent of compound **6b** (IC_{50} = 0.7 μM) should maintain freedom of rotation while increasing lipophilic interaction (Table 6). In the covalently bound state, the increased size of para and meta-substituted arylsulfonyl groups reduces freedom of rotation in the mechanism proposed due to steric clashes with the protein at Tyr415 and Asn411, which results in the lower potency observed for these compounds. This analysis led to the potent arylsulfonyl modifications **5z** (2-fluorophenyl) and **5ee** (2-thiophene) which maintain or improve lipophilic interaction

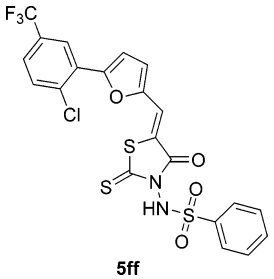
Table 6. Effect of Sulfonyl Substitution on HCV NS5b Polymerase Activity of 2-Amino Rhodanines



compound	R	IC_{50} (μM)[a]
6a	CH_3	2
6b	$CH_3CH_2CH_2CH_2$	0.7
5o	Ph	0.2
5y	4-F-Ph	0.7
5z	2-F-Ph	0.2
5aa	4-CH_3-Ph	0.6
5bb	3-OCH_3-Ph	0.8
5cc	4-OCH_3-Ph	6
5dd	4-Cl-Ph	0.5
5ee	2-thiophene	0.2

[a] IC_{50} values for inhibition of HCV NS5b calculated from two determinations in duplicate.

without hindering freedom of rotation during the course of the mechanism proposed. Torsional angles for the covalently bound Cys366−inhibitor complexes show a staggered low energy conformation (Figure 8). Rotation of either 60 or 120 degrees to the syn or anti-periplanar conformation is required for elimination in the proposed reversible mechanism, which should be allowed without high energy "bumping" of the rhodanine thiocarbonyl into residues Asp318 and Asp319 at the lip of the pocket. However, if the benzylidene group is made to be too large to fit into the Pro197 lipophilic pocket, this should shift



5ff

Figure 9. A potent, time-dependent nonreversible inhibitor of HCV NS5b.

Table 7. Rat Pharmacokinetic Parameters for Compounds **5o** and **5se**

parameter	compound **5o**[a]	compound **5ee**[a]
intravenous dose (mg/kg)[b]	0.16	0.86
CL (L/h/kg)	0.0075 ± 0.0013	0.17 ± 0.022
V_{dss} (L/kg)	0.090 ± 0.017	0.70 ± 0.14
$T_{1/2}$ (h)	9.2 ± 0.30	3.3 ± 1.0
oral dose (mg/kg)[c]	4.1	4.3
C_{max} (ng/mL)	3740 ± 228	3970 ± 438
T_{max} (h)	3.3 ± 2.3	1.7 ± 1.2
$AUC_{(0-8h)}$ (μg·h/L)	25500 ± 1810	25600 ± 3520

[a] Mean ± standard deviation of three male Sprague-Dawely rats. [b] iv bolus. [c] Dosed as a suspension 1% Tween 80 in 1% aqueous methylcellulose.

the rhodanine thiocarbonyl closer to the Asp318 and Asp319 residues in the covalently bound complex, preventing rotation due to steric clashes and thus preventing elimination. Using the model above, we have developed the ability to control whether the compounds are reversible or not by controlling the size of the benzylidene substituent. Increasing the size of the benzylidene substituent beyond that capable of fitting into the benzylidene pocket should therefore make the inhibitors irreversible, which was demonstrated by the synthesis of **5ff** (Figure 9), a potent time-dependent (IC_{50} = 2.0 to <0.1 μM, decreasing with increasing incubation time) irreversible inhibitor of HCV NS5b, although no structure of this NS5b−inhibitor complex was obtained in this case and it is possible that **5ff** may inhibit the enzyme through an alternative mechanism.

Conclusions

We have identified a new class of HCV NS5b polymerase inhibitors following screening of an extensive compound library. SAR studies gave access to inhibitors with improved potency against HCV NS5b, and the compounds displayed good pharmacokinetic properties, characterized by low in vivo clearance, moderate half-life, and high exposure following oral administration (Table 7). These compounds are reversible inhibitors of HCV NS5b, and the X-ray crystal structures of the inhibitor−NS5b complexes show that the compounds bind covalently to Cys366, approximately 8 Å from the Gly-Asp-Asp sequence of the active site. Data from the modification of the inhibitor double bond, X-ray structure analysis, modeling, and structure activity relationships have resulted in a predictive model for the mechanism of interaction with NS5b polymerase, resulting in potent inhibitors of HCV NS5b polymerase. The binding site observed is distinct from those described for other non-nucleoside allosteric inhibitors of HCV NS5b reported to bind at the base of the thumb domain,[21,51,57] or adjacent to Pro495 on the surface of the thumb domain in the case of the benzimidazole[58] and indole-*N*-acetamide[33] inhibitors. Interestingly, very recently and after our initial report,[21] Pfefferkorn et al. reported a series of non-nucleoside inhibitors for HCV NS5b which also bind in the hydrophobic pocket around the β-hairpin where C366 is located,[59,60] but in a noncovalent mode. The binding site of these inhibitors with NS5b partially overlaps that of our inhibitors, especially in the deep hydrophobic pockets, although the sulfonamide in our inhibitors picks up unique interactions with the β-loop not observed in the NS5b−inhibitor complexes of Pfefferkorn et al.

Experimental Section

Reagents and solvents used below were obtained from commercial sources and when required were purified in the accepted fashion. ^{1}H NMR spectra were obtained on a Varian Gemini 400 MHz NMR spectrometer. Electron ionization (EI) mass spectra were recorded on a Hewlett-Packard 5989A mass spectrometer. Electrospray ionization (ESI) mass spectrometry analysis was performed using a Hewlett-Packard 1100 MSD electrospray mass spectrometer using the HP1 100 HPLC for sample delivery. Combustion analyses were performed by Atlantic Microlab Inc. (Norcross, GA). Air and/or moisture sensitive reactions were carried out under N_2 using flame dried glassware and standard syringe/septa techniques. Ab initio calculations were performed with the Jaguar computational package: Jaguar 5.5, Schrödinger, L.L.C., Portland, OR, 1991−2003.

General Procedures for the Synthesis of Truncated Inhibitors 2a−h. 5-(4-Dimethylaminobenzylidene)-3-amino-4-oxo-2-thionothiazolidine (2a). A flask containing 20 mL methanol was charged with 1.0 g 3-aminorhodanine (6.75 mmol, 1.0 equiv), 1.2 g 4-dimethylaminobenzaldehyde (8.05 mmol, 1.2 equiv), and 1.66 g NaOAc (20.2 mmol, 3.0 equiv). The solution was placed in a 65 °C bath and allowed to stir for 25 min. The flask was then removed from the heating bath, and the bright red solid was collected via vacuum filtration. The solid was washed 4 × MeOH and dried under vacuum to give the product as a red solid (912 mg, 3.27 mmol, 48%). ^{1}H NMR (DMSO, 400 MHz) δ 7.73 (s, 1 H), 7.50 (d, J = 8.6 Hz, 2 H), 6.85 (d, J = 9.6 Hz, 2 H), 5.95 (s, 2 H), 3.06 (s, 6 H). Anal. ($C_{12}H_{13}N_3OS_2$): C, H, N, S.

5-(4-Methoxybenzylidene)-3-amino-4-oxo-2-thionothiazolidine (2b). As described for the synthesis of **2a** starting with 3-aminorhodanine and 4-methoxybenzaldehyde. Yield 57%. ^{1}H NMR (DMSO, 400 MHz) δ 7.83 (s, 1 H), 7.63 (d, J = 8.6 Hz, 2 H), 7.13 (d, J = 8.3 Hz, 2 H), 5.95 (s, 2 H), 3.82 (s, 3 H). Anal. ($C_{11}H_{10}N_2O_2S_2$): C, H, N, S.

5-(4-Methylbenzylidene)-3-amino-4-oxo-2-thionothiazolidine (2c). As described for the synthesis of **2a** starting with 3-aminorhodanine and 4-methylbenzaldehyde. Yield 60%. ^{1}H NMR (DMSO, 400 MHz) δ 7.83 (s, 1 H), 7.56 (d, J = 8.1 Hz, 2 H), 7.38 (d, J = 7.6 Hz, 5.95 (s, 2 H), 2.36 (s, 3 H). Anal. ($C_{11}H_{10}N_2OS_2$): C, H, N, S.

5-(Benzylidene)-3-amino-4-oxo-2-thionothiazolidine (2d). As described for the synthesis of **2a** starting with 3-aminorhodanine and benzaldehyde. Yield 60%. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.88 (s, 1 H), 7.60−7.45 (m, 5 H). Anal.($C_{10}H_8N_2OS_2$): C, H, N, S.

5-(4-Chlorobenzylidene)-3-amino-4-oxo-2-thionothiazolidine (2e). As described for the synthesis of **2a** starting with 3-aminorhodanine and 4-chlorobenzaldehyde. Yield 33%. ^{1}H NMR (DMSO, 400 MHz) δ 7.87 (s, 1 H), 7.70 (d, J = 8.6 Hz, 2 H), 7.64 (d, J = 8.7 Hz, 2 H), 5.95 (s, 2 H). Anal. ($C_{10}H_7ClN_2OS_2$): C, H, N, S.

5-(4-Cyanobenzylidene)-3-amino-4-oxo-2-thionothiazolidine (2f). As described for the synthesis of **2a** starting with 3-aminorhodanine and 4-cyanobenzaldehyde. Yield 60%. ^{1}H NMR (DMSO, 400 MHz) δ 8.0 (d, J = 8.1 Hz, 2 H), 7.91 (s, 1 H), 7.83 (d, J = 8.8 Hz, 2 H), 5.95 (s, 2 H). Anal. ($C_{11}H_7N_3OS_2$): C, H, N, S.

5-(4-Fluorobenzylidene)-3-amino-4-oxo-2-thionothiazolidine (2g). As described for the synthesis of **2a** starting with 3-aminorhodanine and 4-fluorobenzaldehyde. Yield 62%. ^{1}H NMR (DMSO, 400 MHz) δ 7.92 (s, 1 H), 7.72 (m, 2 H), 7.40 (d, J = 7.4 Hz, 2 H), 5.95 (s, 2 H). Anal. ($C_{10}H_7FN_2OS_2$): C, H, N, S.

5-(4-Nitrobenzylidene)-3-amino-4-oxo-2-thionothiazolidine (2h). As described for the synthesis of **2a** starting with 3-aminorhodanine and 4-nitrobenzaldehyde. Yield 55%. ^{1}H NMR (DMSO, 400 MHz)

δ 8.39 (d, J = 8.6 Hz, 2 H), 7.96 (s, 1 H), 7.91 (d, J = 8.8 Hz, 2 H), 5.97 (s, 2 H). Anal. ($C_{10}H_7N_3O_3S_2$): C, H, N.

5-(4-Chlorobenzylidene)-3-ethyl-4-oxo-2-thionothiazolidine (2i). As described for the synthesis of **2a** starting with 3-ethylrhodanine and 4-chlorobenzaldehyde. Yield 87%. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.71 (s, 1 H), 7.49 (d, J = 8.6 Hz, 2 H), 7.45 (d, J = 8.6 Hz, 2 H), 4.22 (dd, J = 5.2, 14.2 Hz, 2H), 1.29 (t, J = 7.3 Hz, 3 H). Anal. ($C_{12}H_{10}ClNOS_2$): C, H, N, S.

5-(4-Chlorobenzylidene)-3-methyl-4-oxo-2-thionothiazolidine (2j). As described for the synthesis of **2a** starting with 3-methylrhodanine and 4-chlorobenzaldehyde. Yield 100%. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.70 (s, 1 H), 7.48 (d, J = 8.6 Hz, 2 H), 7.45 (d, J = 8.6 Hz, 2 H), 3.50 (s, 3 H). Anal. ($C_{11}H_8ClNOS_2$): C, H, N, S.

5-(4-Chlorobenzylidene)-4-oxo-2-thionothiazolidine (2k). As described for the synthesis of **2a** starting with rhodanine and 4-chlorobenzaldehyde. Yield 33%. ^{1}H NMR (DMSO, 400 MHz) δ 7.66 (s, 1 H), 7.62 (m, 4 H); ESI-MS m/z 254.0, 255.9 ($M+H^+$). Anal. ($C_{10}H_6ClNOS_2$): C, H, N, S.

5-(4-Chlorobenzylidene)-3-benzyl-4-oxo-2-thionothiazolidine (2l). As described for the synthesis of **2a** starting with 3-benzylrhodanine and 4-chlorobenzaldehyde. Yield 81%. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.75 (s, 1 H), 7.50 (m, 5 H), 7.30 (m, 4 H), 5.32 (s, 2 H). Anal. ($C_{17}H_{12}ClNOS_2$): C, H, N, S.

5-(4-Nitrobenzylidene)-3-methyl-4-oxo-2-thionothiazolidine (2m). As described for the synthesis of **2a** starting with 3-methylrhodanine and 4-nitrobenzaldehyde. Yield quantitative. ^{1}H NMR (DMSO, 400 MHz) δ 8.32 (m, 2 H), 7.88 (m, 3 H), 3.41 (s, 3 H). Anal. ($C_{11}H_8N_2O_3S_2$): C, H, N, S.

5-(4-Chlorobenzylidene)-3-(benzoylamino)-4-oxo-2-thionothiazolidine (1a). A dry flask was charged with 1.00 g 3-aminorhodanine (6.75 mmol, 1.0 equiv) and 10 mL THF followed by the addition of 784 μL benzoyl chloride (6.75 mmol, 1.0 equiv), and the resulting solution was heated to 53 °C. After 20 h the crude solution was concentrated under reduced pressure. The resulting solid was recrystallized from hot toluene, filtered, and washed (3 × toluene) to give 3-(benzoylamino)rhodanine as a white solid (1.399 g, 5.55 mmol, 82%).

The 3-(benzoylamino)rhodanine prepared above (100 mg, 0.397 mmol, 1.0 equiv) was dissolved in 2 mL MeOH followed by the addition of 100 mg of 4-chlorobenzaldehyde (0.711 mmol, 1.8 equiv) and 3.0 mg NaOAc (0.037 mmol, 0.092 equiv). The solution was allowed to stir for 6 h, and the resulting yellow suspension was then concentrated under reduced pressure. The resulting semisolid was taken up in toluene and concentrated under reduced pressure to give a yellow solid. The solid was taken up in MeOH, filtered, washed 3 × MeOH, and dried under vacuum to give 87 mg of the product as a yellow solid (0.232 mmol, 58%). ^{1}H NMR (DMSO, 400 MHz) δ 8.00 (m, 3 H), 7.78 (m, 2 H), 7.68 (m, 3 H), 7.60 (m, 2 H). Anal. ($C_{17}H_{11}ClN_2O_2S_2$): C, H, N, S.

3-(Benzenesulfonylamino)-4-oxo-2-thionothiazolidine (4). Benzenesulfonyl hydrazide (25.0 g, 145 mmol, 1.0 equiv) was suspended in 300 mL H_2O and heated to 95 °C. After 2 h the hydrazide was fully dissolved and 32.85 g of bis(carboxymethyltrithiocarbonate (145 mmol, 1.0 equiv) was added. The resulting solution was allowed to stir for 20 h and was then removed from the heat bath and allowed to cool to room temperature. The resulting yellow suspension was filtered and the solid washed (3 × H_2O). The solid was then recrystallized from hot EtOH to give 16.39 g of the product as light yellow crystals (56.91 mmol, 39%). ^{1}H NMR (DMSO, 400 MHz) δ 7.82 (m, 2 H), 7.65 (m, 1 H), 7.58 (m, 2 H), 4.37 (broad s, 2 H). Anal. ($C_9H_8N_2O_3S_2$): C, H, N, S.

General Procedures for the Synthesis of 3-(Bennzenesulfonylamino) Inhibitors 1 and 5a−x. 5-(4-Chlorobenzylidene)-3-(benzenesulfonylamino)-4-oxo-2-thionothiazolidine (1). A dry flask was charged with 544 mg 3-(benzenesulfonylamino)-4-oxo-2-thionothiazolidine **4** (1.89 mmol, 1.0 equiv) and 8 mL MeOH followed by the addition of 266 mg 4-chlorobenzaldehyde (1.89 mmol, 1.0 equiv) and 16 mg NaOAc (0.189 mmol. 0.1 equiv). The solution was allowed to stir at room temperature for 5 h and then concentrated under reduced pressure. The resulting semisolid was concentrated from toluene and was then taken up in MeOH, filtered, and washed (3 × MeOH) to give the product as a yellow solid (370 mg, 0.903 mmol, 48%). ^{1}H NMR (DMSO, 400 MHz) δ 7.88 (m, 3 H), 7.73−7.58 (m, 7 H); ESI-MS m/z 410.9 ($M + H^+$). Anal. ($C_{16}H_{11}ClN_2O_3S_3$): C, H, N, S.

5-(Benzylidene)-3-(benzenesulfonylamino)-4-oxo-2-thionothiazolidine (5a). As described for the synthesis of **1** starting with 3-(benzenesulfonylamino)rhodanine **4** and benzaldehyde. Yield 27%. ^{1}H NMR (DMSO, 400 MHz) δ 7.87 (m, 3 H), 7.74−7.64 (m, 3 H), 7.63−7.54 (m, 5 H). Anal. ($C_{16}H_{12}N_2O_3S_3$): C, H, N, S.

5-(3-Pyridylmethylene)-3-(benzenesulfonylamino)-4-oxo-2-thionothiazolidine (5b). As described for the synthesis of **1** starting with 3-(benzenesulfonylamino)rhodanine **4** and 3-pyridylcarboxaldehyde. Yield 25%. ^{1}H NMR (DMSO, 400 MHz) δ 11.66 (s, 1 H), 8.89 (s, 1 H), 8.68 (s, 1 H), 8.01 (m, 1 H), 7.87 (m, 3 H), 7.71 (m, 1 H), 7.62 (m, 3 H). Anal. ($C_{15}H_{11}N_3O_3S_3$): C, H, N, S.

5-(4-Methylbenzylidene)-3-(benzenesulfonylamino)-4-oxo-2-thionothiazolidine (5c). As described for the synthesis of **1** starting with 3-(benzenesulfonylamino)rhodanine **4** and 4-methylbenzaldehyde. Yield 33%. ^{1}H NMR (DMSO, 400 MHz) δ 7.83 (m, 3 H), 7.70 (m, 1 H), 7.50 (m, 4 H), 7.32 (m, 2 H), 2.38 (s, 3 H). Anal. ($C_{17}H_{14}N_2O_3S_3$): C, H, N, S.

5-(3-Methylbenzylidene)-3-(benzenesulfonylamino)-4-oxo-2-thionothiazolidine (5d). As described for the synthesis of **1** starting with 3-(benzenesulfonylamino)rhodanine **4** and 3-methylbenzaldehyde. Yield 49%. ^{1}H NMR (DMSO, 400 MHz) δ 7.88 (m, 2 H), 7.84 (s, 1 H), 7.70 (m, 1 H), 7.60 (m, 2 H), 7.45 (m, 3 H), 7.38 (m, 1 H), 2.35 (s, 3 H). Anal. ($C_{17}H_{14}N_2O_3S_3$): C, H, N, S.

5-(4-Methoxybenzylidene)-3-(benzenesulfonylamino)-4-oxo-2-thionothiazolidine (5e). As described for the synthesis of **1** starting with 3-(benzenesulfonylamino)rhodanine **4** and 4-methoxybenzaldehyde. Yield 5%. ^{1}H NMR (DMSO, 400 MHz) δ 11.55 (broad s, 1 H), 7.86 (m, 3 H), 7.72−7.58 (m, 5 H), 7.12 (m, 2 H), 3.85 (s, 3 H). Anal. ($C_{17}H_{14}N_2O_4S_3$): C, H, N, S.

5-(3-Methoxybenzylidene)-3-(benzenesulfonylamino)-4-oxo-2-thionothiazolidine (5f). As described for the synthesis of **1** starting with 3-(benzenesulfonylamino)rhodanine **4** and 3-methoxybenzaldehyde. Yield 30%. Anal. ($C_{17}H_{14}N_2O_4S_3 \cdot 1/2\ H_2O$) calcd: C, 49.14; H, 3.64; N, 6.74; S, 23.15. Found: C, 48.61; H, 3.45; N, 6.66; S, 22.96.

5-(5-Benzo[1,3]dioxolemethylene)-3-(benzenesulfonylamino)-4-oxo-2-thionothiazolidine (5g). As described for the synthesis of **1** starting with 3-(benzenesulfonylamino)rhodanine **4** and benzo-[1,3]dioxole-5-carboxaldehyde. Yield 15%. ^{1}H NMR (DMSO, 400 MHz) δ 7.88 (d, J = 7.5 Hz, 2 H), 7.80 (s, 1 H), 7.71 (t, J = 7.2 Hz, 1 H), 7.60 (m, 2 H), 7.25 (dd, J = 1.5, 8.2 Hz, 1 H), 7.20 (d, J = 1.6 Hz, 1 H), 7.14 (d, J = 8.2 Hz, 1 H), 6.17 (s, 2 H). Anal. ($C_{17}H_{12}N_2O_5S_3$): C, H, N, S.

5-(4-Bromobenzylidene)-3-(benzenesulfonylamino)-4-oxo-2-thionothiazolidine (5h). As described for the synthesis of **1** starting with 3-(benzenesulfonylamino)rhodanine **4** and 4-bromobenzaldehyde. Yield 61%. ^{1}H NMR (DMSO, 400 MHz) δ 11.51 (s, 1 H), 7.80 (m, 3 H), 7.78 (m, 2 H), 7.65 (m, 1 H), 7.55 (m, 4 H). Anal. ($C_{16}H_{11}BrN_2O_3S_3$): C, H, N, S.

5-(4-Fluorobenzylidene)-3-(benzenesulfonylamino)-4-oxo-2-thionothiazolidine (5i). As described for the synthesis of **1** starting with 3-(benzenesulfonylamino)rhodanine **4** and 4-fluorobenzaldehyde. Yield 10%. ^{1}H NMR (DMSO, 400 MHz) δ 7.90−7.86 (m, 3 H), 7.77−7.70 (m, 3 H), 7.61 (t, J = 8.8 Hz, 2 H), 7.42 (t, J = 8.8 Hz, 2 H). Anal. ($C_{16}H_{11}FN_2O_3S_3$); C, H, N, S.

5-(2-Fluorobenzylidene)-3-(benzenesulfonylamino)-4-oxo-2-thionothiazolidine (5j). As described for the synthesis of **1** starting with 3-(benzenesulfonylamino)rhodanine **4** and 2-fluorobenzaldehyde. Yield 48%. ^{1}H NMR (DMSO, 400 MHz) δ 7.89 (m, 2 H), 7.79 (s, 1 H), 7.71 (m, 1 H), 7.62 (m, 4 H), 7.42 (m, 2 H). Anal. ($C_{16}H_{11}FN_2O_3S_3$): C, H, N, S.

5-(4-Nitrobenzylidene)-3-(benzenesulfonylamino)-4-oxo-2-thionothiazolidine (5k). As described for the synthesis of **1** starting with 3-(benzenesulfonylamino)rhodanine **4** and 4-nitrobenzaldehyde. Yield 16%. ^{1}H NMR (DMSO, 400 MHz) δ 8.34 (d, J = 8.8 Hz, 2 H), 7.87 (d, J = 8.8 Hz, 2 H), 7.75 (s, 1 H), 7.68 (m, 2

H), 7.42−7.32 (m, 3 H); ESI-MS *m*/*z* 420.2 (M−H). Anal. ($C_{16}H_{11}N_3O_5S_3$): C, H, N, S.

5-(4-Cyanobenzylidene)-3-(benzenesulfonylamino)-4-oxo-2-thionothiazolidine (5l). As described for the synthesis of **1** starting with 3-(benzenesulfonylamino)rhodanine **4** and 4-cyanobenzaldehyde. Yield 29%. ^{1}H NMR (DMSO, 400 MHz) δ 11.69 (broad s, 1 H), 8.00 (m, 2 H), 7.98−7.79 (m, 5 H), 7.58 (m, 1 H), 7.50 (m, 2 H). Anal. ($C_{17}H_{11}N_3O_3S_3$): C, H, N, S.

5-(4-Trifluoromethylbenzylidene)-3-(benzenesulfonylamino)-4-oxo-2-thionothiazolidine (5m). As described for the synthesis of **1** starting with 3-(benzenesulfonylamino)rhodanine **4** and 4-trifluoromethylbenzaldehyde. Yield 35%. ^{1}H NMR (DMSO, 400 MHz) δ 7.88 (s, 1 H), 7.86−7.83 (m, 6 H), 7.66 (m, 1 H), 7.60 (m, 2 H). Anal. ($C_{17}H_{11}F_3N_2O_3S_3$): C, H, N, S.

5-(4-Chloro-3-fluorobenzylidene)-3-(benzenesulfonylamino)-4-oxo-2-thionothiazolidine (5n). As described for the synthesis of **1** starting with 3-(benzenesulfonylamino)rhodanine **4** and 4-chloro-3-fluorobenzaldehyde. Yield 30%. ^{1}H NMR (DMSO, 400 MHz) δ 7.87 (m, 3 H), 7.80 (m, 1 H), 7.76 (m, 1 H), 7.69 (m, 1 H), 7.61 (m, 2 H), 7.50 (m, 1 H). Anal. ($C_{16}H_{10}ClFN_2O_3S_3$): C, H, N, S.

***Z*-5-(3,4-Dichlorobenzylidene)-3-(benzenesulfonylamino)-4-oxo-2-thionothiazolidine (5o).** As described for the synthesis of **1** starting with 3-(benzenesulfonylamino)rhodanine **4** and 3,4-dichlorobenzaldehyde. Yield 71%. ^{1}H NMR (DMSO, 400 MHz) δ 7.99 (d, J = 2.1 Hz, 1 H), 7.88 (m, 3 H), 7.84 (d, J = 8.4 Hz, 1 H), 7.73 (ddd, J = 1.2, 7.4, 8.6 Hz, 1 H), 7.63 (m, 3 H); IR 3211.1, 1734.4, 1604.0, 1580.6, 1469.9, 1428.6, 1363.4, 1232.7, 1215.2, 1174.2,1113.2, 1027.7, 724.7, 683.9, 581.0, 540.8 cm^{-1}; ESI-MS *m*/*z* 442.9 (M−H). Anal. ($C_{16}H_{10}Cl_2N_2O_3S_3$): C, H, N, S.

5-(3,4-Dibromobenzylidene)-3-(benzenesulfonylamino)-4-oxo-2-thionothiazolidine (5p). As described for the synthesis of **1** starting with 3-(benzenesulfonylamino)rhodanine **4** and 3,4-dibromobenzaldehyde. Yield 36%. ^{1}H NMR (DMSO, 400 MHz) δ 8.08 (d, J = 2.5 Hz, 1 H), 7.94 (d, J = 8.5 Hz, 1 H), 7.87 (m, 3 H), 7.73 (t, J = 7.0 Hz, 1 H), 7.61 (m, 2 H), 7.52 (dd, J = 1.9, 8.3 Hz, 1 H). Anal. ($C_{16}H_{10}Br_2N_2O_3S_3$): C, H, N, S.

5-(2,4-Difluorobenzylidene)-3-(benzenesulfonylamino)-4-oxo-2-thionothiazolidine (5q). As described for the synthesis of **1** starting with 3-(benzenesulfonylamino)rhodanine **4** and 2,4-difluorobenzaldehyde. Yield 40%. ^{1}H NMR (DMSO, 400 MHz) δ 7.87 (m, 2 H), 7.75−7.65 (m, 3 H), 7.60 (m, 2 H), 7.53 (m, 1 H), 7.30 (m, 1 H). Anal. ($C_{16}H_{10}F_2N_2O_3S_3$): C, H, N, S.

5-(2,5-Difluorobenzylidene)-3-(benzenesulfonylamino)-4-oxo-2-thionothiazolidine (5r). As described for the synthesis of **1** starting with 3-(benzenesulfonylamino)rhodanine **4** and 2,5-difluorobenzaldehyde. Yield 12%. ^{1}H NMR (DMSO, 400 MHz) δ 7.87 (m, 2 H), 7.71 (m, 2 H), 7.60 (m, 2 H), 7.50 (m, 2 H), 7.43 (m, 1 H). Anal. ($C_{16}H_{10}F_2N_2O_3S_3$): C, H, N, S

5-(2,3,4-Trifluorobenzylidene)-3-(benzenesulfonylamino)-4-oxo-2-thionothiazolidine (5s). As described for the synthesis of **1** starting with 3-(benzenesulfonylamino)rhodanine **4** and 2,3,4-trifluorobenzaldehyde. Yield 34%. ^{1}H NMR (DMSO, 400 MHz) δ 7.87 (m, 2 H), 7.71 (m, 2 H), 7.60 (m, 2 H), 7.49 (m, 2 H). Anal. ($C_{16}H_9F_3N_2O_3S_3$): C, H, N, S.

5-(3,4,5-Trifluorobenzylidene)-3-(benzenesulfonylamino)-4-oxo-2-thionothiazolidine (5t). As described for the synthesis of **1** starting with 3-(benzenesulfonylamino)rhodanine **4** and 3,4,5-trifluorobenzaldehyde. Yield 13%. ^{1}H NMR (DMSO, 400 MHz) δ 7.85 (m, 3 H), 7.71 (m, 1 H), 7.65 (m, 4 H). Anal. ($C_{16}H_9F_3N_2O_3S_3$): C, H, N, S.

5-(2,4,5-Trifluorobenzylidene)-3-(benzenesulfonylamino)-4-oxo-2-thionothiazolidine (5u). As described for the synthesis of **1** starting with 3-(benzenesulfonylamino)rhodanine **4** and 2,4,5-trifluorobenzaldehyde. Yield 16%. ^{1}H NMR (DMSO, 400 MHz) δ 7.78 (ddd, J = 6.7, 10.4, 10.8 Hz, 1 H), 7.65 (m, 2 H), 7.59 (m, 1 H), 7.46 (s, 1 H), 7.32 (m, 3 H). Anal. ($C_{16}H_9F_3N_2O_3S_3$): C, H, N, S.

5-(3,4-Dimethylbenzylidene)-3-(benzenesulfonylamino)-4-oxo-2-thionothiazolidine (5v). As described for the synthesis of **1** starting with 3-(benzenesulfonylamino)rhodanine **4** and 3,4-dimethylbenzaldehyde. Yield 12%. ^{1}H NMR (DMSO, 400 MHz) δ 7.90 (m, 2 H), 7.82 (s, 1 H), 7.72 (m, 1 H), 7.60 (m, 2 H), 7.42 (m, 2 H), 7.38 (m, 1 H), 2.25 (s, 6 H). Anal. ($C_{18}H_{16}N_2O_3S_3$): C, H, N, S.

5-(2-Naphthylmethylene)-3-(benzenesulfonylamino)-4-oxo-2-thionothiazolidine (5w). As described for the synthesis of **1** starting with 3-(benzenesulfonylamino)rhodanine **4** and 2-naphthylaldehyde. Yield 71%. ^{1}H NMR (DMSO, 400 MHz) δ 8.28 (s, 1 H), 8.09 (m, 2 H), 7.99 (m, 2 H), 7.90 (m, 2 H), 7.73 (m, 2 H), 7.62 (m, 4 H). Anal. ($C_{20}H_{14}N_2O_3S_3$): C, H, N, S.

5-(2-Methyl-3-phenyl-allylidene)-3-(benzenesulfonylamino)-4-oxo-2-thionothiazolidine (5x). As described for the synthesis of **1** starting with 3-(benzenesulfonylamino)rhodanine **4** and 2-methyl-3-phenylpropenal. Yield 46%. ^{1}H NMR (DMSO, 400 MHz) δ 7.88 (d, J = 7.4 Hz, 1 H), 7.73 (t, J = 7.4 Hz, 1 H), 7.62 (m, 3 H), 7.47 (m, 4 H), 7.41 (m, 2 H), 2.20 (s, 3 H); IR (KBr pellet) 3227.6, 1726.5, 1712.9, 1571.7, 1561.1, 1449.6, 1362.6, 1240.9, 1221.5, 1172.6, 1115.9, 1089.2, 753.8, 723.2, 684.1, 589.5, 537.0 cm^{-1}. Anal. ($C_{19}H_{16}N_2O_3S_3$): C, H, N, S.

5-(3,4-Dichlorobenzylidene)-3-(4-fluorobenzenesulfonylamino)-4-oxo-2-thionothiazolidine (5y). As described for the synthesis of **1** starting with 3-(4-fluorobenzenesulfonylamino)rhodanine (prepared from 4-fluorobenzenesulfonylhydrazide as in example **4** in 34% yield) and 3,4-dichlorobenzaldehyde. Yield 67%. ^{1}H NMR (DMSO, 400 MHz) δ 7.96 (d, J = 2.2 Hz, 1 H), 7.92 (d, J = 5.6 Hz, 1 H), 7.90 (d, J = 5.1 Hz, 1 H), 7.83 (s, 1 H), 7.81 (d, J = 9.1 Hz, 1 H), 7.58 (dd, J = 2.3, 8.5 Hz, 1 H), 7.40 (t, J = 9.1 Hz, 2 H). Anal. ($C_{16}H_9Cl_2FN_2O_3S_3$): C, H, N, S.

5-(3,4-Dichlorobenzylidene)-3-(2-fluorobenzenesulfonylamino)-4-oxo-2-thionothiazolidine (5z). As described for the synthesis of **1** starting with 3-(2-fluorobenzenesulfonylamino)rhodanine (prepared from 2-fluorobenzenesulfonylhydrazide as in example **4** in 28% yield) and 3,4-dichlorobenzaldehyde. Yield 27%. ^{1}H NMR (DMSO, 400 MHz) δ 7.97 (d, J = 2.0 Hz, 1 H), 7.87 (s, 1 H), 7.80 (m, 2 H), 7.59 (dd, J = 2.2, 8.4 Hz, 1 H), 7.47 (t, J = 9.6 Hz, 1 H), 7.35 (t, J = 7.6 Hz, 1 H). Anal. ($C_{16}H_9Cl_2FN_2O_3S_3$): C, H, N, S.

5-(3,4-Dichlorobenzylidene)-3-(4-methylbenzenesulfonylamino)-4-oxo-2-thionothiazolidine (5aa). As described for the synthesis of **1** starting with 3-(4-methylbenzenesulfonylamino)rhodanine (prepared from 4-methylbenzenesulfonylhydrazide as in example **4** in 31% yield) and 3,4-dichlorobenzaldehyde. Yield 9%. ^{1}H NMR (DMSO, 400 MHz) δ 7.98 (d, J = 2.1 Hz, 1 H), 7.98 (s, 1 H), 7.83 (d, J = 8.3 Hz, 1 H), 7.76 (d, J = 8.8 Hz, 2 H), 7.61 (dd, J = 2.1, 8.3 Hz, 1 H), 7.41 (d, J = 7.9 Hz, 2 H), 2.41 (s, 3 H). Anal. ($C_{17}H_{12}Cl_2N_2O_3S_3$): C, H, N, S.

5-(3,4-Dichlorobenzylidene)-3-(3-methoxybenzenesulfonylamino)-4-oxo-2-thionothiazolidine (5bb). As described for the synthesis of **1** starting with 3-(3-methoxybenzenesulfonylamino)-rhodanine (prepared from 3-methoxybenzenesulfonylhydrazide as in example **4** in 20% yield) and 3,4-dichlorobenzaldehyde. Yield 43%. ^{1}H NMR (DMSO, 400 MHz) δ 7.97 (m, 1 H), 7.86 (d, J = 9.5 Hz, 1 H), 7.81 (d, J = 8.3 Hz, 1 H), 7.59 (m, 1 H), 7.52 (m, 1 H), 7.43 (m, 1 H), 7.73 (m, 1 H), 7.27 (m, 1 H), 3.81 (s, 3 H); ESI-MS *m*/*z* 472.9, 474.9 (M−H). Anal. ($C_{17}H_{12}Cl_2N_2O_4S_3$): C, H, N, S.

5-(3,4-Dichlorobenzylidene)-3-(4-methoxybenzenesulfonylamino)-4-oxo-2-thionothiazolidine (5cc). As described for the synthesis of **1** starting with 3-(4-methoxybenzenesulfonylamino)-rhodanine (prepared from 4-methoxybenzenesulfonylhydrazide as in example **4** in 14% yield) and 3,4-dichlorobenzaldehyde. Yield 19%. ^{1}H NMR (DMSO, 400 MHz) δ 7. 98 (d, J = 2.3 Hz, 1 H), 7.87 (d, J = 5.4 Hz, 1 H), 7.84 (d, J = 8.9 Hz, 1 H), 7.78 (m, 2 H), 7.59 (dd, J = 2.5, 8.3 Hz, 1 H), 7.12 (m, 2 H). Anal. ($C_{17}H_{12}Cl_2N_2O_4S_3$): C, H, N, S.

5-(3,4-Dichlorobenzylidene)-3-(4-chlorobenzenesulfonylamino)-4-oxo-2-thionothiazolidine (5dd). As described for the synthesis of **1** starting with 3-(4-chlorobenzenesulfonylamino)rhodanine (prepared from 4-chlorobenzenesulfonylhydrazide as in example **4** in 10% yield) and 3,4-dichlorobenzaldehyde. Yield 19%. ^{1}H NMR

(DMSO, 400 MHz) δ 11.80 (broad s, 1 H), 7.97 (m, 1 H), 7.86 (m, 4 H), 7.69 (m, 2 H), 7.58 (m, 1 H). Anal. ($C_{16}H_9Cl_3N_2O_3S_3$): C, H, N, S.

5-(3,4-Dichlorobenzylidene)-3-(2-thiophenesulfonylamino)-4-oxo-2-thionothiazolidine (5ee). As described for the synthesis of **1** starting with 3-(2-thiophenesulfonylamino)rhodanine (prepared from 2-thiophenesulfonylhydrazide as in example **4** in 5% yield) and 3,4-dichlorobenzaldehyde. Yield 19%. ^{1}H NMR (DMSO, 400 MHz) δ 8.06 (d, J = 5.0 Hz, 1 H), 8.00 (d, J = 2.0 Hz, 1 H), 7.90 (s, 1 H), 7.83 (d, J = 8.4 Hz, 1 H), 7.72 (dd, J = 1.2, 3.8 Hz, 1 H), 7.61 (dd, J = 2.0, 8.4 Hz, 1 H), 7.20 (m, 1 H); IR (KBr) 3224.3, 3142.9, 1742.9, 1705.2, 1605.1, 1469.0, 1639.9, 1243.5, 1215.9, 1166.8, 1117.4, 1024.0, 737.0, 718.0, 619.6 cm^{-1}. Anal. ($C_{14}H_8Cl_2N_2O_3S_4$): C, H, N, S.

5-(3,4-Dichlorobenzylidene)-3-(methanesulfonylamino)-4-oxo-2-thionothiazolidine (6a). As described for the synthesis of **1** starting with 3-(methanesulfonylamino)rhodanine (prepared from methanesulfonylhydrazide as in example **4** in 57% yield) and 3,4-dichlorobenzaldehyde. Yield 55%. ^{1}H NMR (DMSO, 400 MHz) δ 8.01 (d, J = 1.0 Hz, 1 H), 7.95 (s, 1 H), 7.84 (d, J = 8.4 Hz, 1 H), 7.63 (dd, J = 2.1, 8.4 Hz, 1 H), 3.28 (s, 3 H). Anal. ($C_{11}H_8Cl_2N_2O_3S_3$): C, H, N, S.

5-(3,4-Dichlorobenzylidene)-3-(butylsulfonylamino)-4-oxo-2-thionothiazolidine (6b). As described for the synthesis of **1** starting with 3-(butylsulfonylamino)rhodanine (prepared from butylsulfonylhydrazide as in example **4** in 12% yield) and 3,4-dichlorobenzaldehyde. Yield 42%. ^{1}H NMR (DMSO, 400 MHz) δ 11.05 (s, 1 H), 8.00 (d, J = 2.1 Hz, 1 H), 7.94 (s, 1 H), 7.83 (d, J = 8.4 Hz, 1 H), 7.62 (m, 1 H), 3.31 (m, 2 H), 1.81 (m, 2 H), 1.41 (m, 2 H), 0.90 (t, J = 7.3 Hz, 3 H). Anal. ($C_{14}H_{14}Cl_2N_2O_3S_3$): C, H, N, S.

5-(3,4-Dichlorophenyl-1-ethylene)-3-(benzenesulfonylamino)-4-oxo-2-thionothiazolidine (7). A 50 mL flask equipped with a Dean−Stark trap and condenser was charged with 486 mg 3-(benzenesulfonylamino)rhodanine **4** (1.69 mmol, 1.0 equiv), 319 mg 3′,4′-dichloroacetophenone (1.69 mmol, 1.0 equiv), 1.84 mL piperidine (13.07 mmol, 11.0 equiv), 3.84 mL acetic acid (67.5 mmol, 40.0 equiv), and 25 mL benzene. The solution was heated to reflux for 6 h, followed by removal of the volatile components under reduced pressure. Purification by flash chromatography (SiO_2, 2% MeOH/hexanes) gave the product as a yellow solid (22 mg, 0.0483 mmol, 3%). ^{1}H NMR (DMSO, 400 MHz, mixture of olefin isomers) δ (major isomer) 7.83 (m, 2 H), 7.80 (m, 1 H), 7.69 (m, 1 H), 7.65−7.55 (m, 3 H), 7.51 (m, 1 H), 2.7 (s, 3 H); ESI-MS m/z 458.1 (M + H$^+$). Anal. ($C_{17}H_{12}Cl_2N_2O_3S_3$): C, H, N, S.

5-(3,4-Dichlorobenzyl)-3-(benzenesulfonylamino)-4-oxo-2-thionothiazolidine (8) and 5-(3,4-Dichlorobenzyl)-3-(benzenesulfonylamino)-4-hydroxy-2-thionothiazolidine (9). A flask containing 8 mL H_2O was charged with 12 mg cobalt chloride hexahydrate (0.051 mmol, 0.06 equiv) and 29 mg 2,2′-dipyridyl (0.189 mmol, 0.22 equiv) followed by the addition of two drops of 1 M NaOH and 16 mg (0.429 mmol, 0.5 equiv) sodium borohydride. The solution was cooled to 0 °C followed by the dropwise addition of 6 mL of a 2:1 THF/DMF solution of 3,4-dichlorobenzylidene **5o** (381 mg, 0.858 mmol, 1.0 equiv). When the addition of **5o** was completed the ice bath was removed and the solution was allowed to warm to room temperature. After 60 min 5% AcOH was added until a pH of ∼6 was reached. The solution was then diluted with 20 mL H_2O and extracted with CH_2Cl_2, washed with H_2O, dried (Na_2SO_4), and concentrated under reduced pressure. Purification by flash chromatography (SiO_2, 0−2% MeOH/CH_2Cl_2) gave the product **8** as a white solid (18.6 mg, 0.042 mmol, 5%) followed by **9** (83 mg, 0.185 mmol, 22%) as a white solid. **(8)**: ^{1}H NMR (DMSO, 400 MHz) δ 7.84 (m, 2 H), 7.69 (ddd, J = 1.2, 7.5, 8.7 Hz, 1 H), 7.61 (s, 1 H), 7.55 (m, 2 H), 7.47 (d, J = 8.2 Hz, 1 H), 7.38 (d, J = 2.1 Hz, 1 H), 7.13 (dd, J = 2.1, 8.2 Hz, 1 H), 4.48 (dd, J = 4.4, 9.1 Hz, 1 H), 3.52 (dd, J = 4.4, 14.3 Hz, 1 H), 3.20 (dd, J = 9.1, 14.3 Hz, 1 H); ESI-MS m/z 444.9, 446.9 (M − H). Anal. ($C_{16}H_{12}Cl_2N_2O_3S_3$): C, H, N, S. **(9)**: ^{1}H NMR (DMSO, 400 MHz mixture of two diastereomers) δ 7.89 (d, J = 7.3 Hz, 1.4 H), 7.82 (m, 1.4 H), 7.68−7.52 (m, 7 H), 7.39 (dd, J = 1.9, 8.2 Hz, 0.7 H), 7.32 (dd, J = 1.9, 8.2 Hz, 0.4 H), 7.26 (m, 0.4 H), 7.16 (m, 0.4 H), 5.55 (t, J = 9.6 Hz, 0.4 H), 5.41 (d, J = 4.7 Hz, 0.4 H), 4.50 (ddd, J = 4.8, 6.7, 8.9 Hz, 0.4 H), 3.09 (dd, J = 6.7, 13.7 Hz, 0.7 H), 3.20 (dd, J = 9.5, 14.1 Hz, 0.7 H), 2.90 (dd, J = 9.1, 14.1 Hz, 0.4 H); ESI-MS m/z 446.9, 448.9 (M−H). Anal. ($C_{16}H_{14}Cl_2N_2O_3S_3$): C, H, N, S.

***E*-5-(3,4-Dichlorobenzylidene)-3-(benzenesulfonylamino)-4-oxo-2-thionothiazolidine (10).** A flask was charged with 208 mg *Z*-5-(3,4-dichlorophenylmethylene)-3-(benzenesulfonylamino)-4-oxo-2-thionothiazolidine (**5o**) (0.469 mmol, 1.0 equiv), 10 mL benzene, 241 mg thiophenol (2.19 mmol, 4.7 equiv), and 20 μL diisopropylethylamine (0.115 mmol. 0.25 equiv), and the resulting solution was heated to 80 °C for 18 h. The solution was then allowed to cool to room temperature, and the benzene solution was applied directly to a SiO_2 column which was preequilibrated with 30% EtOAc/hexanes. Elution with 30−100% EtOAc/hexanes gave a mixture of *Z* starting material **5o** and the more polar *E* product **10**. The mixture was taken up in CH_2Cl_2 at which point crystals formed. The solid was collected via vacuum filtration and washed (5 × CH_2Cl_2) to give 12 mg of the desired *E*-isomer **10** (0.027 mmol, 6%) as a yellow solid. ^{1}H NMR (DMSO, 400 MHz) δ 7.92 (d, J = 1.9 Hz, 1 H), 7.81 (d, J = 8.4 Hz, 1 H), 7.68 (m, 2 H), 7.61 (s, 1 H), 7.56 (dd, J = 1.9, 8.4 Hz, 1 H), 7.33 (m, 3 H); ESI-MS m/z 442.9, 444.9 (M − H). Anal. ($C_{16}H_{10}Cl_2N_2O_3S_3$): C, H, N, S.

5-(5-[2-Chloro-5-(trifluoromethyl)phenyl]-furan-2-methylene)-3-(benzenesulfonylamino)-4-oxo-2-thionothiazolidine (5ff). As described for the synthesis of **1** starting with 3-(benzenesulfonylamino)rhodanine **4** and 5-[2-chloro-5-(trifluoromethyl)phenyl]-2-furaldehyde. Yield 64%. ^{1}H NMR (DMSO, 400 MHz) δ 8.23 (d, J = 1.6 Hz, 1 H), 7.88 (m, 4 H), 7.82 (s, 1 H), 7.72 (t, J = 7.9 Hz, 1 H), 7.62 (m, 3 H), 7.46 (d, J = 3.8 Hz, 1 H); IR (KBr) 3206.7, 3049.7, 1728.2, 1606.2, 1547.4, 1360.5, 1328.6, 1227.6, 1171.7, 1123.4, 1095.4, 1036.3, 805.7, 723.1, 684.7, 581.9 cm^{-1}. Anal. ($C_{21}H_{12}ClF_3N_2O_4S_3$): C, H, N, S.

Evaluation of HCV NS5b Polymerase Inhibitory Activity. Materials: Recombinant HCV Δ21 NS5b polymerase with a C-terminal 10-His tag was expressed from *E. coli*. Template is transcribed HCV 3′ UTR RNA (567nt) as described in ref 13. Signal detection was by ^{32}P or ^{33}P CTP incorporation. Compounds were serially diluted in DMSO in a 96-well plate. Controls were enzyme without compound (100% activity) and no enzyme (0% activity background).

Assay Protocol: 40 μL Template label mixture (5 mM $MgCl_2$, 2 mM DTT, 5 μM ATP, 1 μM UTP, 0.05 μM CTP, 0.05 μM 3′ UTR RNA template, 0.001 to 0.1 μCi ^{32}P CTP, water to 40 μL) was added to the assay plates followed by 2.5 μL of each compound dilution. 10 μL Enzyme dilution mixture (20 mM Tris 7.5, 30 mM KCl, 2 mM DTT, 10% glycerol, 100 nM HCV Δ21 NS5b polymerase, water to 10 μL) was then added, the assay plates were covered and incubated at 41 °C for 60 min. Reactions were then filtered through 96-well DEAE-81 filter plates via vacuum. The plates which contained the captured labeled reaction products were then washed under vacuum with multiple volumes of 0.5 M $NaHPO_4$ or 2X PBS to remove unincorporated label. Plates were then counted on a Fuji Bio-imager to assess the level of product synthesis over background controls.

NS5b Purification, Crystallization and Structure Determination. Δ21 NS5b (amino acids 1−570) with a C-terminal 10-His tag was expressed in *E. coli*. The protein was purified first using a Ni-NTA column, followed by purification using a heparin column. The heparin column eluate was further purified by size exclusion chromatography using a Superdex 200 column equilibrated in 20 mM Tris pH 7.5, 300 mM NaCl, 10 mM $MgCl_2$, 5 mM DTT, and 10% glycerol. Fractions were assayed by SDS−PAGE, and the purified NS5b protein was pooled and concentrated to 4−6 mg/mL for crystallization. Crystals were grown by the hanging drop method using a well solution of 200 mM ammonium sulfate, 27−30% (vol/vol) PEG-5000 MME, and 100 mM Na-acetate pH 5. Protein−inhibitor complexes were formed prior to crystallization by adding 3× the molar equivalent of compound to the protein solution, and cocrystals were grown using similar conditions.

Diffraction data were collected at 90 Kelvin at ALS Beamline 5.0.2, and processed using Denzo and Scalepack.[61] The NS5b structure was solved by molecular replacement with EPMR[62] using NS5b (1QUV)[39,63] as the search model. NS5b−inhibitor complex structures were determined by rigid-body refinement using the unliganded NS5b structure as the starting model. Structure improvement proceeded with multiple rounds of manual model building in Quanta, followed by refinement using CNX.[64] Ligands were fitted into clear Fo-Fc electron density in the later stages of refinement. Data and refinement statistics are given in the Supporting Information.

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Supporting Information Available: C, H, N, S analysis for all compounds and X-ray data and refinement statistics for NS5b+**5h**, NS5b+**5ee**, and NS5b+**5x**. This material is available free of charge via the Internet at http://pubs.acs.org.

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