Inhibitors of Hepatitis C Virus Polymerase: Synthesis and Biological Characterization of Unsymmetrical Dialkyl-Hydroxynaphthalenoyl-benzothiadiazines

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The hepatitis C virus (HCV) NS5B polymerase is essential for viral replication and has been a prime target for drug discovery research. Our efforts directed toward the discovery of HCV polymerase inhibitors resulted in the identification of unsymmetrical dialkyl-hydroxynaphthalenoyl-benzothiadiazines **2** and **3**. The most active compound displayed activity in genotypes 1a and 1b polymerase and replicon cell culture inhibition assays at subnanomolar and low nanomolar concentrations, respectively. It also displayed an excellent pharmacokinetic profile in rats, with a plasma elimination half-life after intravenous dosing of 4.5 h, oral bioavailability of 77%, and a peak liver concentration of 21.8 μg/mL.

Introduction

Hepatitis C virus (HCV) was unambiguously characterized in 1989 as the organism responsible for non-A and non-B hepatitis.[1] Because of an estimated 170 million people worldwide chronically infected with HCV, this virus represents a global public health problem.[2] After their initial acquisition of the virus, HCV-infected individuals pass through an acute phase, followed in most cases by development of chronic infection.[3] The majority of infected individuals remain asymptomatic for many years after the acute phase, with hepatic fibrosis progressing as the main complication of chronic HCV infection. While there is tremendous variation in the rate of fibrosis progression to cirrhosis, with no treatment, the median progression to cirrhosis is 30 years. Of this population, a substantial fraction will continue to experience progression of disease into hepatocellular carcinoma.[4] Complications due to chronic HCV infection have now become the predominant driver for liver transplantation.[5] Additionally, the leading cause of death upon kidney transplantation, which is the most commonly performed organ allograft, is due to chronic liver disease, most commonly precipitated by hepatitis C infection.[6] In 2004, 2.44 deaths per 100000 people could be attributed to complications due to HCV infection in the U.S.[7] According to one predictive model, this number is expected to rise to ~27700 deaths per year by 2010 and peak in 2030 at ~39800 deaths per year,[8] although a more conservative prediction places the peak mortality level in 2030 at 12900 deaths.[9]

The current standard of therapy for chronic HCV infection consists of combination treatment with pegylated interferon and ribavirin. While population genetics studies of HCV indicate an extremely diverse collection of genotypes (1−6) worldwide, HCV genotype 1 patients comprise ~75% of HCV infections in the U.S., Western Europe, and Japan and ~60% of HCV infection in Latin America.[10] Unfortunately, due to contraindications, only 40% of this population is eligible for therapy and 75% of this population refuse to undergo therapy, or drop out of treatment due to severe side effects associated with the drug regimen. Within those that remain on therapy, the infection cure rate is estimated to be only 50%.[11] For HCV genotype 1-infected individuals with additional risk factors, such as cirrhosis, HIV coinfection, dialysis treatment, or organ transplants, cure rates are substantially lower.[12] Clearly, there is a compelling need for new agents that will bolster the viral cure rate for this segment of the patient population.

The nonstructural protein 5B (NS5B) of HCV possesses RNA-dependent RNA polymerase activity, which is an activity that is not found in host liver cells.[13] The RNA polymerase plays an essential role in viral replication by directing the synthesis of both the replicative intermediate minus-strand RNA as well as the progeny plus-strand RNA molecules.[14] In general, viral polymerases are attractive drug targets, as shown by the success of viral polymerase inhibitors such as acyclovir for herpes virus infections, various nucleoside and non-nucleoside reverse transcriptase inhibitors for HIV, and lamivudine for HBV infections. More specifically, it has now been shown that inhibition of NS5B in HCV-infected patients leads to reduction in viral load. While nucleoside HCV polymerase inhibitors have garnered the most clinical attention in this mechanistic class, multiple non-nucleoside inhibitors have already entered into early trials and are beginning to show significant promise.[15]

Rationale. Our prior efforts resulted in the discovery of compound **1**.[16] Although, this compound demonstrated high in vitro potency and had a good pharmacokinetic profile, it and its sodium salt, **1a**, were plagued by poor aqueous solubility. As a response to the solubility limitation of compound **1**, a second-generation core was investigated in which the aza

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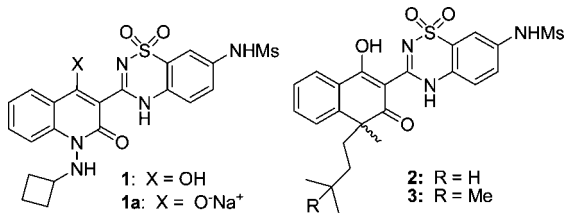
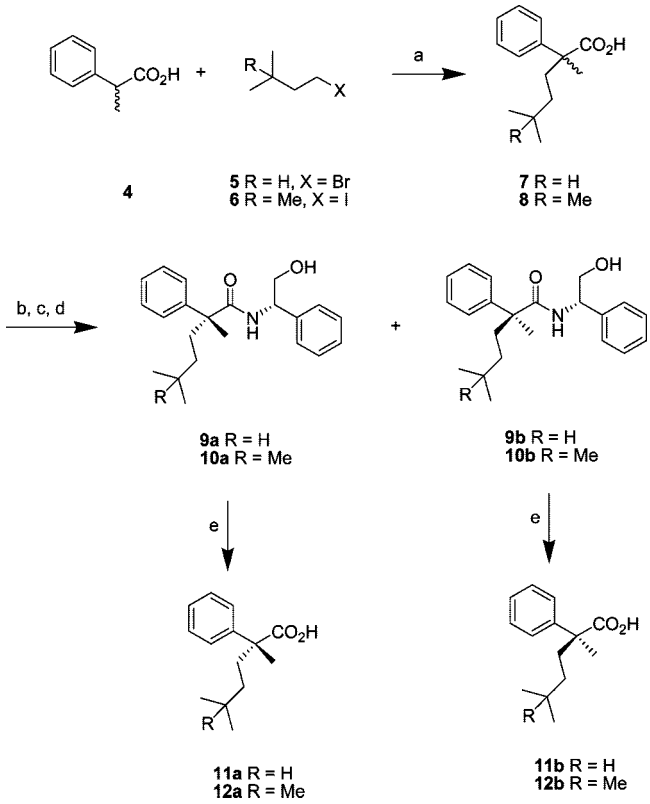


Figure 1. HCV polymerase inhibitor lead structures.

Scheme 1[a]



[a] Key: (a) LiHMDS, THF; (b) $(COCl)_2$, DMF, hexane; (c) (*S*)-phenylglycinol, Et_3N, CH_2Cl_2, DMAP; (d) chromatographic resolution; (e) H_2SO_4, dioxane.

functionality was replaced by a dialkyl motif. We speculated that the high degree of planarity of the tetracyclic ring system resulted in the poor solubility characteristics of compound **1**. The intention of the dialkyl replacement was to disturb the crystal packing forces of the flat tetracyclic system, thereby improving dissolution rates of the resultant analogues. This approach resulted in the identification of the racemates **2** and **3**.[17] The in vitro profiles of these new structures were comparable to compound **1**, and anecdotally, we observed these dialkylated analogues, **2** and **3**, to have better solubility characteristics than **1**. The logical path forward in this research program was to address the chiral aspect of the dialkyl compounds and develop a more complete biological profile of the more active enantiomers.

Chemistry. The primary requirements for the synthesis of the pure enantiomers of racemates **2** and **3** were 2-fold. Foremost, the materials obtained from the syntheses needed to have a high degree of enantiomeric purity. Additionally, we required a practical synthesis of multigram quantities of material. Given the unique challenges presented in an enantioselective synthesis of the quaternary stereocenter, we determined that an approach that allowed for the resolution of a racemic intermediate would be the most effective solution to this synthetic problem. We also determined that resolution of the stereoisomers at an early stage of the synthesis would be the most efficient approach.

The chiral synthesis was adapted from the synthesis developed for nonchiral dialkyl analogues. The synthesis of enantiomerically pure analogues was accomplished by a 13-step general procedure.

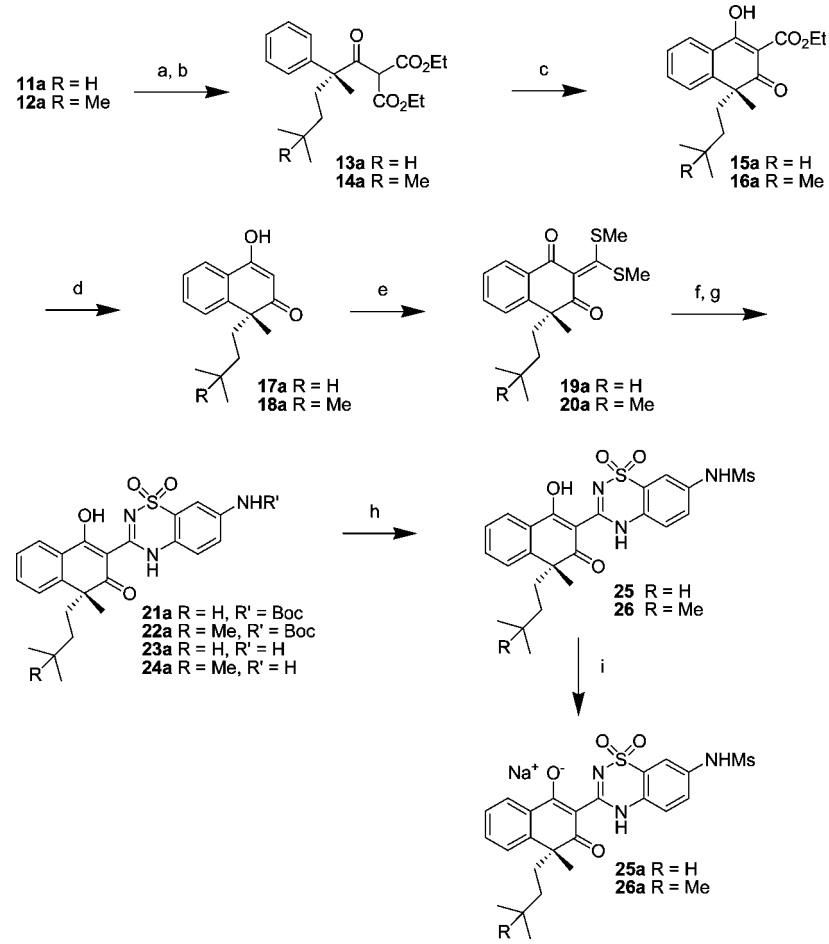
Shown in Scheme 1, the racemic dialkyl-carboxylic acids **7** and **8** were prepared by reaction of the dianion of carboxylic acid **4** with either of the appropriate alkyl halide **5** or **6**. The acid chlorides of racemic phenyl acetic acids **7** and **8** were allowed to react with (*S*)-phenylglycinol to give diastereomeric mixtures that were easily separated by flash column chromatography to give amides **9a**, **9b**, **10a**, and **10b**. At this point, the relative stereochemical configuration at the quaternary center of **10a** was determined by a single crystal X-ray experiment. With the configuration of the phenylglycinol fragment already established, the absolute stereochemistry could therefore be assigned. Each of the diastereomeric amides was then hydrolyzed to give the enantiomerically pure phenyl acetic acids **11a**, **11b**, **12a**, and **12b**.

With the necessary enantiomerically pure building blocks in hand, the remaining syntheses of the optically active thiadiazines were completed in tandem. Although syntheses of the biologically active enantiomers are depicted in Scheme 2, the inactive enantiomers were obtained by the same methodology, and ultimately the absolute configuration of virologically active **25a** was assigned by analogy to **26a**. The corresponding acid chlorides of carboxylic acids **11a** and **12a** were allowed to react with the magnesium enolate of diethyl malonate, and the resulting ketodiesters **13a** and **14a** were then cyclized to the dihydronapthalene esters **15a** and **16a** by treatment with methanesulfonic acid. Esters **15a** and **16a** were decarboxylated and converted to dithioketene acetals **19a** and **20a**, which were treated with (4-amino-3-sulfamoyl-phenyl)-carbamic acid *tert*-butyl ester. The syntheses were completed by deprotection of the aniline group of the tetracyclic compounds **21a** and **22a**, followed by reaction with methanesulfonyl chloride provided **25** and **26**. Final treatment with aqueous sodium hydroxide gave the enantiomerically pure target compounds **25a** and **26a**.

Results and Discussion

The primary in vitro tools used to screen analogues was a polymerase enzyme inhibition assay as well as a subgenomic HCV cell culture system. Although HCV has a very robust replication rate in vivo, efforts to propagate the virus in cell culture systems have been largely unsuccessful for genotype 1 despite the success of a genotype 2a infectious system.[18] This difficulty has led to the development of subgenomic HCV replicons that can be propagated efficiently in the human hepatoma cell line Huh-7.[19] These replicons contain 5′ and 3′ nontranslated regions as well as all of the HCV nonstructural genes. Similar to conventional tissue culture and in vivo viral replication, HCV replicon RNA efficiently replicates by undergoing a cycle consisting of translation, polyprotein cleavage (by the NS3 protease), RNA synthesis (by the NS5B polymerase), unwinding of the double-stranded RNA (by the NS3 helicase), and assembly of replication complexes containing the HCV nonstructural proteins within Huh-7 cells. This system produces 1000−10000 copies of RNA per cell, which is substantially greater than that estimated for in vivo infection (50−100 particles/cell). However, unlike conventional tissue culture and in vivo viral infection, this system is limited by the lack of

Scheme 2[a]



[a] Key: (a) $(COCl)_2$, DMF, hexane; (b) diethyl malonate, $MgCl_2$, Et_3N, CH_3CN; (c) CH_3SO_3H; (d) 1 N HCl, dioxane; (e) (bis-methylsulfanyl-methylene)-methyl-sulfonium tetrafluoroborate, pyridine, dioxane; (f) *tert*-butyl 4-amino-3-sulfamoylphenylcarbamate, toluene; (g) HCl/dioxane; (h) CH_3SO_2Cl, pyridine, acetone; (i) NaOH, water.

Table 1. Biochemical and Antiviral Activity[a]

	HCV polymerase inhibition IC_{50} (nM)		HCV replicon EC_{50} (nM)		cytotoxicity TD_{50}(nM)	HCV replicon + 40% human	
	genotype 1a	genotype 1b	genotype 1a	genotype 1b	Huh-7 MTT	serum EC_{50} (nM) 1b	serum effect
1	2.0 $n = 2$ (1.5 to 2.7)	1.6 $n = 1$	3 $n = 1$	0.7 $n = 2$(0.63−0.86)	>10000 $n = 2$(>10000, >30000)	46 $n = 2$(41.4−50.0)	66×
25a	1.3 $n = 4$ (0.7 to 2.3)	0.9 $n = 4$ (0.5 to 1.4)	11 $n = 10$ (8.5−13.6)	3 $n = 10$ (0.8−4.2)	35300 $n = 1$	42 $n = 7$ (19.5−58.7)	14×
25b	624 $n = 3$ (370−1000)	2300 $n = 3$ (830−6400)	ND	ND	ND	ND	ND
26a	0.7 $n = 3$ (0.5 to 0.9)	0.6 $n = 3$ (0.5 to 0.6)	4 $n = 10$ (2.9−5.8)	2 $n = 5$ (1.1−3.0)	21700 $n = 5$ (14000−33000)	28 $n = 5$ (22.0−33.2)	17×
26b	444 $n = 3$ (405−468)	518 $n = 3$ (395−632)	ND	ND	ND	ND	ND

[a] ND: not determined.

assembly and release of virus particles. Therefore, the replicon system is incapable of producing infectious virus, and viral infection of new cells (entry) does not occur. Nevertheless, the use of HCV replicons to evaluate HCV inhibitors has been validated with the NS3 protease inhibitor BILN-2061. This compound has an EC_{50} of 3−4 nM against 1a and 1b subgenomic replicons in cell culture. When this compound was dosed for 48 h in HCV genotype 1-infected patients, it produced a 2−3 $\log_{10}$ drop in viral titer.[20] The potency of these current compounds are reported as EC_{50} values against the subgenomic genotype 1a and 1b replicons measuring the reduction of HCV RNA by real time PCR. To establish the effect of nonspecific protein binding on the antiviral activity, we tested these compounds in the presence of 5% fetal calf serum (FCS) with or without 40% human serum.

Preparation and in vitro profiling of the corresponding optically active isomers of compounds **2** and **3** revealed the (*R*)-configured isomers **25a** and **26a** to be the more active stereoisomers. Both compounds **25a** and **26a** inhibited genotypes 1a and 1b polymerase activity and HCV replicon replication at low nanomolar concentrations. These data compare similarly to the corresponding data obtained for lead compound **1**. (Table 1.)

Table 2. Physical Property Comparison of **1a** and **26a**

	solubility at 25 °C (mg/mL)				melting point (°C)	
compd	water (pH 4.0)[a]	water (pH 7.4)[b]	PEG400	logD (pH 7.4)	non-salt form	sodium salt
1a	not available	<0.00007	>191.8	1.45 ± 0.02	295−297 decomp.	264−272
26a	<0.0002	0.11 ± 0.004	150	2.26 ± 0.0045	129[c]	>375

[a] 50 mM citrate buffer. [b] 50 mM phosphate buffer. [c] amorphous glass transition temperature.

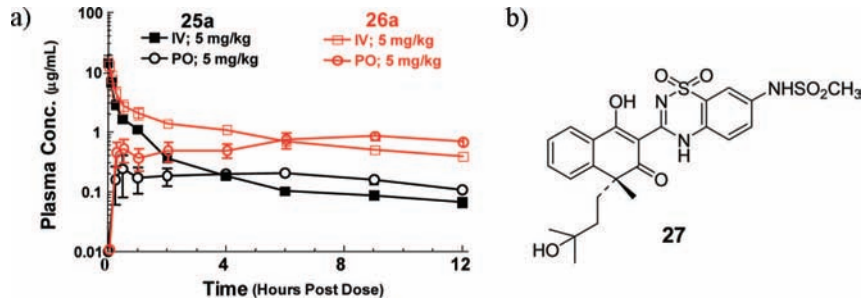


Figure 2. (a) Rat IV and Oral Pharmacokinetics upon 5 mg/kg dosing of **25a** and **26a**, **2b**. (b) Major metabolite of **25a** isolated from rat bile.

Table 3. Rat Pharmacokinetic Parameters of 25a and 26a, iv/Oral Dosing (5 mg/kg)

	iv dose (5 mg/kg)					oral dose (5 mg/kg)					liver concentrations after oral dosing (5 mg/kg)	
	$t_{1/2}$ (h)	V_{ss} (L/kg)	$V\beta$ (L/kg)	AUC (µg·h/mL)	CLp (L/h·kg)	$t_{1/2}$ (h)	C_{max} (µg/mL)	T_{max} (h)	AUC (µg·h/mL)	F (%)	6 h (µg/g)	12 h (µg/g)
25a	1.2	1.72	1.97	5.2	0.96	7.6	0.31	4.2	3.3	62.7	3.8	1.43
26a	4.5	2.22	2.39	14.9	0.34	unable to calculate	0.89	9	11.5	77.3	21.8	4.19

Table 4. Comparison of Dog and Monkey PK Parameters for Compounds **25a** and **26a**

		iv dose					oral dose				
	species	$t_{1/2}$ (hr)	V_{ss} (L/kg)	$V\beta$ (L/kg)	AUC (µg·h/mL)	CLp (L/h·kg)	$t_{1/2}$ (hr)	C_{max} (µg/mL)	T_{max} (hr)	AUC (µg·h/mL)	F (%)
25a	dog (5 mg/kg)	2.7	1.10	1.74	11.8	0.44	10.7	1.94	0.4	10.5	89.3
	monkey (5 mg/kg)	0.7	0.19	0.50	10.2	0.51	1.9	0.52	2.7	1.5	15.2
26a	dog (2.5 mg/kg)	12.3	0.38	0.46	115.6	0.02	14.8	5.7	2.2	121	~100
	monkey (5 mg/kg)	2.1	0.77	1.77	10.2	0.56	2	0.48	2.7	1.9	19

Table 5. Multispecies in Vitro Microsomal and Hepatocyte Metabolism of **25a** and **26a**

		intrinsic clearance (µL/min/mg)			
compd	assay system	rat	dog	monkey	human
25a	microsomes	36.3	3.5	62.0	29.6
	hepatocytes	9.0	2.2	29.2	6.8
26a	microsomes	8.7	2.4	14.4	23.6
	hepatocytes	1.6	0.5	3.5	2.7

However, the potencies of compounds **25a** and **26a** were attenuated by the presence of 40% human serum in the replicon assay by 14- and 17-fold, respectively, which compared favorably to the 66-fold attenuation observed with compound **1**.

The subjective observation that the dialkyl-series compounds had physical characteristics that were superior to the aza-series compounds was borne out by solubility studies of compounds **1a** and **26a** (Table 2). In aqueous 50 mM phosphate buffer adjusted to pH 7.4 at 25 °C, the solubility of compound **26a** exceeded that of compound **1a** by at least 3 orders of magnitude. In acidic aqueous buffer, however, **26** suffered from significant insolubility and was not detected in the sample indicating a solubility less than 0.2 µg/mL. In the more organic medium of PEG400, the solubility differences, between **1a** and **26a** were less evident, with some advantage being yielded to **1a**. HPLC was used to determine the LogD at pH 7.4 and provided a partition coefficient for compound **26a** higher than **1a**. Interestingly, Table 2 shows significantly different phase transition points of nonsalts **1** and **26**. We interpret this observation, in part, as indicating tighter self-association of **1** vs **26**, perhaps due to the flatter shape of the molecule. In comparison, the sodium salt form of amorphous **1a** shows little change in melting point, however, the sodium salt of **26a** is much higher due to a combination of its crystallinity and greater ionic character of the deprotonated diketone sodium salt than the deprotonated 3-ketoamide of **1**.

Compound **25a**, our initial in vivo study compound, demonstrated an encouraging pharmacokinetic profile in rats (Figures 1 and 2a, Table 3). Following intravenous dosing, the plasma elimination half-life was 1.2 h and the plasma clearance value was 0.96 L/h·kg. Oral dosing resulted in bioavailability of 63%. A study performed on bile duct cannulated rats indicated that biliary secretion was the major excretory route in rats. Microsomal metabolism studies indicated that the major metabolite showed an increase in molecular weight of 16 amu. Mass spectral fragment analysis of this metabolite indicated that it was formed by oxidation of tertiary carbon of the terminal alkyl chain, yielding carbinol **27** (Figure 2b). This structure was confirmed by independent synthesis.[21]

Given the site of metabolism of compound **25a**, we rationalized that compound **26a** might have a superior pharmacokinetic profile due to the blocking effect of an extra methyl group on its alkyl side chain.

Table 6. Concentrations of 25 and 26 in Rat Liver after Oral dosing

	rat plasma concentrations after oral dosing (5 mg/kg)		rat liver concentrations after oral dosing (5 mg/kg)		fold over 1b EC_{50} in presence of 40% human serum	
compd	6 h (nM)	12 h (nM)	6 h (nM)	12 h (nM)	6 h	12 h
25	445	135	7340	2860	175	68
26	2240	320	39300	7550	1404	270

A pharmacokinetic study of compound **26a** in rat confirmed a superior profile to compound **25a**. The plasma elimination half-life after intravenous dosing improved by nearly 4-fold to 4.5 h, and the plasma clearance dropped by 3-fold to 0.34 L/h·kg. While oral bioavailability improved only slightly, it should be noted that the oral exposure more than tripled to 11.5 μg·h/mL when compared to analogue **25a**. One peculiar aspect to the oral pharmacokinetic behavior of compound **26a** relates to its prolonged oral half-life, precluding our ability to determine its value in this experiment. Although the solubility characteristics were improved in comparison to compound **1**, we believe that the relatively flat dose/plasma concentration response curve is due to dissolution rate limited absorption resultant from protonation of **26a** in the acidic stomach environment. Indeed, Table 2 reports the significant insolubility of **26a** in acidic aqueous media.

Further characterization of the pharmacokinetics in dog and monkey underscored the differences of **26a** in comparison to **25a**. In both species, the iv half-life of **26a** was significantly improved relative to **25a**. The iv data for **26a** are consistent with metabolic stability and profiling of **26a** in rat, dog, and monkey where the dog liver microsomes did not produce a key metabolite that was found in the other species. While this metabolite was isolated from rat bile and characterized by mass spectral fragmentation, tentatively assigning the structure as that shown in Figure 3, lack of production of **28** in the dog was also reflected in reduced hepatocyte clearance (Table 4 and 5). Unfortunately, attempts to synthesize metabolite **28** were met with failure and therefore thwarted our efforts to provide for an independent verification of this structure.

While the IV PK parameters show a significant improvement for **26a** relative to **25a** across all species examined, the advantage of **26a** upon oral administration is less obvious. Nevertheless, the improved pan-species microsomal/hepatocellular stability of **26a** in comparison to **25a** (Table 5) cause it to be the preferred compound to test in human subjects. Demonstration of antiviral activity in patients is a key goal of this program, however, it is impossible to predict with certainty the pharmacokinetic behavior of **26a** in HCV infected human subjects.

In an attempt to understand liver uptake, hepatic distribution studies of **25a** and **26a** were conducted in rats. While reasonable plasma exposures were achieved upon dosing compounds **25a** and **26a**, we were interested in determining the concentrations of the free acids, **25** and **26**, in the target organ. Four groups of rats were dosed orally at 5 mg/kg with each of **25a** and **26a** and sacrificed at the 6 and 12 h time points.

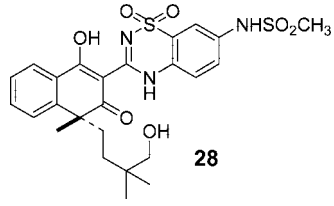


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Figure 3. Primary rat metabolite of **26a**.

As shown in Table 6, the results from this experiment indicate that both drugs are found at high concentrations in rat liver tissue. The liver to plasma ratios at the 6 h time point were ca. 17-fold for both compounds and increased to slightly better than 20-fold for both compounds at the 12 h time point. After 6 and 12 h, concentrations representing large multiples of the EC_{50} values are achievable after a single oral dose, with compound **26** achieving a concentration 270-fold of the serum adjusted replicon EC_{50} 12 h after oral dosing. The improved rat liver levels of **26** over **25** at all time points is again consistent with the in vitro metabolism data.

Conclusions

While the in vitro potency of our aza-series provided NS5B inhibitors of exceptional antiviral potency, the strategy of exploring the unsymmetrical dialkyl class of inhibitors has proven to extend the promising properties of the antecedent series by successfully optimizing their physical characteristics. We have identified the (*R*)-configured isoamyl and *neo*-hexyl substituted compounds **25a** and **26a** to be promising drug candidates for the treatment of HCV infection. These compounds are potent inhibitors of HCV genotype 1 replicons in the presence of human serum. By achieving high liver concentrations, a high liver/plasma ratio, good bioavailability, and a long half-life after oral dosing, compounds **25a** and **26a** also demonstrate a desirable pharmacological profile. An important milestone for either compound would be the demonstration of antiviral activity in human patients. Unfortunately, human PK parameters bear on this critical question and will remain unanswered without further clinical investigation. As an interim milestone, we were pleased to find that compound **25a** provided the first in vivo chimpanzee validation of the antiviral efficacy of our dialkyl-hydroxynaphthalenoyl-benzothiadiazine series.[22] Further in vivo characterization of compound **26a**, including chimpanzee efficacy studies, will be reported in due course.

Experimental Section

Synthetic Materials and Methods. Unless otherwise specified, reactions were performed under an inert atmosphere of nitrogen and monitored by thin-layer chromatography (TLC). All reagents were purchased from commercial suppliers and used as provided. Flash column chromatography was carried out on silica gel. All ^{1}H NMR and ^{13}C NMR spectra were recorded on 300 or 500 MHz instruments, are given ppm (δ), and are referenced to an internal standard of tetramethylsilane (δ 0.00). ^{1}H−^{1}H couplings are assumed to be first-order, and peak multiplicities are reported in the usual manner. Elemental analyses were performed by Robertson Microlit Laboratories (Madison, NJ) or Qualitative Technologies Inc. (Whitehouse, NJ).

Solvents used for solubility determinations included PEG 400 (The Dow Chemical Company, Midland MI), water, 50 mM citrate buffer adjusted to pH 4, and 50 mM phosphate buffer adjusted to pH 7.4. Buffers were adjusted to an ionic strength of 0.155 M with NaCl. Solvents used for HPLC analysis and sample preparation were HPLC grade. Acetonitrile and TFA were used for mobile phase preparation. Distilled deionized water was obtained from a Millipore Milli-Q water purification system equipped with a 0.22 μm filter.

2,5-Dimethyl-2-phenylhexanoic acid (7). A solution of lithium bis(trimethylsilyl)amide (13.94 g, 83.28 mmol) in 40 mL of THF was cooled in an ice bath. A solution of 2-phenylpropanoic acid (4.56 mL, 33.3 mmol) in 5 mL of THF was added, followed by addition of 1-bromo-3-methylbutane (6.66 mL, 53.3 mL.) The cooling bath was removed, and the reaction solution was stirred for 30 min. The reaction solution was then heated in a 50 °C oil bath and stirred for 16 h. After cooling, the reaction solution was concentrated and the resulting residue was dissolved in 250 mL of 1 N NaOH. The aqueous solution was washed with 2:1 hexane/*i*-PrOAc, acidified to pH 1 with 6 N HCl, and extracted 3× with EtOAc. The combined organic layers were dried over Na_2SO_4 and concentrated to give 7.29 g (99%) of a clear, colorless oil. ^{1}H NMR ($CDCl_3$) δ 7.31 (m, 5H), 1.99 (m, 2H), 1.56 (s, 3H), 1.50 (m, 1H), 1.09 (m, 2H), 0.88 (d, 3H, $J = 6.62$), 0.85 (d, 3H, $J = 6.25$). ^{13}C NMR ($CDCl_3$) δ 182.6, 143.0, 128.4, 126.8, 126.2, 49.9, 36.8, 33.5, 28.5, 22.5, 22.5, 22.3. MS (DCI/NH_3) *m/z* 238 $(M + NH_4)^+$.

2,5,5-Trimethyl-2-phenylhexanoic acid (8). Following the procedure for compound **7**, except substituting 1-iodo-3,3-dimethylbutane for 1-bromo-3-methylbutane, the title compound **8** (7.66 g, 98%) was obtained as a clear, yellow oil. ^{1}H NMR ($CDCl_3$) δ 7.31 (m, 5H), 1.97 (m, 2H), 1.56 (s, 3H), 1.08 (m, 2H), 0.86 (s, 9H). ^{13}C NMR ($CDCl_3$) δ 182.8, 143.0, 128.4, 126.8, 126.2, 49.8, 38.1, 33.8, 30.2, 29.3, 22.1. MS (DCI/NH_3) *m/z* 252 $(M + NH_4)^+$.

(*R*)-*N*-((*S*)-2-Hydroxy-1-phenylethyl)-2,5-dimethyl-2-phenylhexanamide (9a) and (*S*)-*N*-((*S*)-2-Hydroxy-1-phenylethyl)-2,5-dimethyl-2-phenylhexanamide (9b). To a solution of compound **7** (7.92 g, 35.9 mmol) in 800 mL of hexane was added DMF (2.78 mL, 35.9 mmol) and $(COCl)_2$ (9.40 mL, 107 mmol). After stirring for 2 h, the reaction mixture was filtered through a plug of celite and concentrated to an oily residue.

A solution of the residue, (*S*)-phenylglycinol (7.39 g, 53.9 mmol), and Et_3N (10.5 mL, 75.5 mmol) in 500 mL of CH_2Cl_2 was stirred overnight. The reaction mixture was washed with 1 N HCl and saturated $NaHCO_3$. The organic layer was dried over Na_2SO_4, concentrated, and purified by chromatography on silica gel with 10−30% EtOAc in toluene as eluent to give 4.52 g (37%) of a more polar compound **9a** and 3.90 g (32%) of a less polar compound **9b**. More polar compound **9a**: white solid. ^{1}H NMR (DMSO-d_6) δ 7.41 (d, 1H, $J = 7.7$), 7.23 (m, 10H), 4.86 (m, 1H), 4.72 (m, 1H), 3.51 (m, 2H), 1.98 (m, 1H), 1.81 (m, 1H), 1.42 (m, 4H), 0.92 (m, 2H), 0.79 (d, 6H, $J = 6.6$). ^{13}C NMR (DMSO-d_6) δ 174.7, 145.7, 141.5, 128.0, 127.7, 126.9, 126.5, 126.2, 126.1, 64.2, 55.3, 49.6, 36.5, 33.1, 28.1, 23.6, 22.5, 22.4. MS (ESI) *m/z* 338 $(M - H)^-$. Anal. ($C_{22}H_{29}NO_2$) C, H, N. Less polar compound **9b**: white solid. ^{1}H NMR (DMSO-d_6) δ 7.28 (m, 11H), 4.85 (m, 1H), 4.72 (m, 1H), 3.51 (m, 2H), 1.98 (m, 1H), 1.83 (m, 1H), 1.47 (m, 1H), 1.40 (s, 3H), 1.01 (m, 2H), 0.83 (d, 6H, $J = 6.6$). ^{13}C NMR (DMSO-d_6) δ 174.7, 145.8, 141.6, 128.0, 127.8, 126.8, 126.5, 126.3, 126.1, 64.3, 55.3, 49.7, 36.3, 33.3, 28.1, 23.9, 22.6. MS (ESI) *m/z* 338 $(M - H)^-$. Anal. ($C_{22}H_{29}NO_2$) C, H, N.

(*R*)-*N*-((*S*)-2-Hydroxy-1-phenylethyl)-2,5,5-trimethyl-2-phenylhexanamide (10a) and (*S*)-*N*-((*S*)-2-Hydroxy-1-phenylethyl)-2,5,5-trimethyl-2-phenylhexanamide (10b). Following the procedure for **9a** and **9b**, the title compounds were prepared from compound **8** and were obtained as 4.95 g (39%) of a more polar compound **10a** and 5.08 g (40%) of a less polar compound **10b**. More polar compound **10a**: white solid. ^{1}H NMR (DMSO-d_6) δ 7.27 (m, 11H), 4.86 (m, 1H), 4.72 (t, 1H), 3.51 (m, 2H), 1.96 (m, 1H), 1.77 (m, 1H), 1.41 (s, 3H), 0.92 (m, 2H), 0.78 (s, 9H). ^{13}C NMR (DMSO-d_6) δ 174.7, 145.7, 141.4, 127.9, 127.7, 126.9, 126.5, 126.3, 126.1, 64.2, 55.2, 49.4, 37.6, 33.3, 29.8, 29.1, 23.5. MS (ESI) *m/z* 352 $(M-H)^-$. Anal. ($C_{23}H_{31}NO_2$) C, H, N. Less polar compound **10b**: white solid. ^{1}H NMR (DMSO-d_6) δ 7.27 (m, 11H), 4.85 (m, 1H), 4.72 (m, 1H), 3.52 (m, 2H), 1.97 (m, 1H), 1.81 (m, 1H), 1.39 (s, 3H), 1.02 (m, 2H), 0.82 (s, 9H). ^{13}C NMR (DMSO-d_6) δ 174.6, 145.8, 141.5, 127.9, 127.7, 126.8, 126.4, 126.3, 126.1, 64.2, 55.2, 49.5, 37.8, 33.1, 29.8, 29.2, 23.8. MS (ESI) *m/z* 352 $(M - H)^-$. Anal. ($C_{23}H_{31}NO_2$) C, H, N.

(*R*)-2,5-Dimethyl-2-phenylhexanoic Acid (11a). A solution of compound **9a** (3.55 g, 9.87 mmol) and 75 mL of 6 N H_2SO_4 in 75 mL of dioxane was heated at reflux for 2 d. The reaction solution was cooled and concentrated. The resulting residue was suspended in 100 mL of H_2O and extracted 3× with Et_2O. The combined organic layers were dried over $MgSO_4$, concentrated, and purified by chromatography on silica gel with 20% EtOAc in hexane as eluent to give 1.91 g (88%) of a clear, colorless oil. ^{1}H NMR ($CDCl_3$) δ 7.31 (m, 5H), 1.99 (m, 2H), 1.56 (s, 3H), 1.50 m, 1H), 1.08 (m, 2H), 0.88 (d, 3H, $J = 6.6$), 0.85 (d, 3H, $J = 6.3$). ^{13}C NMR ($CDCl_3$) δ 182.4, 143.1, 128.4, 126.8, 126.2, 49.9, 36.8, 33.5, 28.5, 22.6, 22.5, 22.3. MS (DCI/NH_3) *m/z* 238 $(M + NH_4)^+$. Anal. ($C_{14}H_{20}O_2$) C, H.

(*S*)-2,5-Dimethyl-2-phenylhexanoic Acid (11b). Following the procedure for compound **11a**, except substituting compound **9b** for compound **9a**, the title compound **11b** (2.01 g, 93%) was obtained as a clear, colorless oil. H NMR ($CDCl_3$) δ 7.31 (m, 5H), 1.99 (m, 2H), 1.56 (s, 3H), 1.50 (m, 1H), 1.09 (m, 2H), 0.88 (d, 3H, $J = 6.6$), 0.85 (d, 3H, $J = 6.3$). ^{13}C NMR ($CDCl_3$) δ 182.6, 143.0, 128.4, 126.8, 126.2, 49.9, 36.8, 33.5, 28.5, 22.5, 22.5, 22.3. MS (DCI/NH_3) *m/z* 238 $(M + NH_4)^+$. Anal. ($C_{14}H_{20}O_2$) C, H.

(*R*)-2,5,5-Trimethyl-2-phenylhexanoic acid (12a). Following the procedure for compound **11a**, except substituting compound **10a** for compound **9a**, the title compound **12a** (2.55 g, 93%) was obtained as a clear, colorless oil. ^{1}H NMR ($CDCl_3$) δ 7.31 (m, 5H), 1.97 (m, 2H), 1.55 (s, 3H), 1.08 (m, 2H), 0.85 (s, 9H). ^{13}C NMR ($CDCl_3$) δ 182.9, 143.0, 128.4, 126.8, 126.2, 49.8, 38.1, 33.8, 30.2, 29.3, 22.1. MS (DCI/NH_3) *m/z* 252 $(M + NH_4)^+$. Anal. ($C_{15}H_{22}O_2$) C, H.

(*S*)-2,5,5-Trimethyl-2-phenylhexanoic Acid (12b). Following the procedure for compound **11a**, except substituting compound **10b** for compound **9a**, the title compound **12b** (2.70 g, 96%) was obtained as a clear, colorless oil. ^{1}H NMR ($CDCl_3$) δ 7.31 (m, 5H), 1.97 (m, 2H), 1.56 (s, 3H), 1.08 (m, 2H), 0.86 (s, 9H). ^{13}C NMR ($CDCl_3$) δ 182.8, 143.0, 128.4, 126.8, 126.2, 49.8, 38.1, 33.8, 30.2, 29.3, 22.1. MS (DCI/NH_3) *m/z* 252 $(M + NH_4)^+$. Anal. ($C_{15}H_{22}O_2$) C, H.

(*R*)-Diethyl 2-(2,5-Dimethyl-2-phenylhexanoyl)malonate (13a). A solution of diethyl malonate (1.33 g, 8.21 mmol) in 15 mL of CH_3CN was cooled in an ice bath. Magnesium chloride (0.798 g, 8.21 mmol) and Et_3N (2.40 mL, 17.2 mmol) were added to the reaction solution. After stirring for 15 min, the reaction mixture was removed from the cooling bath and stirred for an additional 2 h.

In a separate flask, to a solution of compound **11a** (1.81 g, 8.21 mmol) and DMF (0.70 mL, 9.03 mmol) in 275 mL of hexane was added $(COCl)_2$ (2.15 mL, 24.6 mmol). After stirring for 2 h, the reaction mixture was filtered through a plug of celite and concentrated to an oily residue. The residue was dissolved in 10 mL of CH_3CN and added to the magnesium malonate solution. The resulting reaction mixture was removed from the cooling bath and heated in a 50 °C oil bath for 18 h. The reaction mixture was cooled and concentrated. The oily residue was suspended in 100 mL of EtOAc and 30 mL of 1 N HCl and stirred vigorously until all solids dissolved. The layers were separated, and the aqueous layer was extracted 3× with EtOAc. The combined organic layers were dried over Na_2SO_4, concentrated, and purified by chromatography on silica gel with 20% EtOAc in hexane as eluent to give 2.41 g (81%) of a clear, colorless oil. ^{1}H NMR ($CDCl_3$) δ 7.31 (m, 5H), 4.51 (s, 1H), 4.11 (m, 2H), 4.05 (m, 2H), 1.96 (m, 2H), 1.53 (s, 3H), 1.46 (m, 1H), 1.21 (t, 3H, $J = 7.0$), 1.16 (t, 3H, $J = 7.4$), 1.07 (m, 1H), 0.84 (m, 7H). ^{13}C NMR ($CDCl_3$) δ 200.1, 164.3, 164.3, 139.8, 128.7, 127.5, 127.4, 62.1, 61.9, 60.5, 56.9, 35.0, 32.9, 28.5, 22.5, 22.4, 19.9, 13.8, 13.8. MS (ESI) *m/z* 361 $(M - H)^-$. Anal. ($C_{21}H_{30}O_5$) C, H.

(*S*)-Diethyl 2-(2,5-Dimethyl-2-phenylhexanoyl)malonate (13b). Following the procedure for compound **13a**, except substituting compound **11b** for compound **11a**, the title compound **13b** (2.83 g, 95%) was obtained as a clear, colorless oil. ^{1}H NMR ($CDCl_3$) δ 7.31 (m, 5H), 4.51 (s, 1H), 4.11 (m, 2H), 4.05 (m, 2H), 1.96 (m, 2H), 1.53 (s, 3H), 1.46 (m, 1H), 1.21 (t, 3H, $J = 7.0$), 1.16 (t, 3H, $J = 7.4$), 1.07 (m, 1H), 0.84 (m, 7H). ^{13}C NMR ($CDCl_3$) δ 200.1, 164.3, 164.3, 139.8, 128.7, 127.5, 127.4, 62.1, 61.9, 60.5, 56.9,

35.0, 32.9, 28.5, 22.5, 22.4, 19.9, 13.8, 13.8. MS (ESI) *m*/*z* 361 $(M - H)^-$. Anal. ($C_{21}H_{30}O_5$) C, H.

(*R*)-Diethyl 2-(2,5,5-Trimethyl-2-phenylhexanoyl)malonate (14a). Following the procedure for compound **13a**, except substituting compound **12a** for compound **11a**, the title compound **14a** (2.78 g, 90%) was obtained as a clear, colorless oil. ^{1}H NMR ($CDCl_3$) δ 7.31 (m, 5H), 4.52 (s, 1H), 4.12 (m, 2H), 4.04 (m, 2H), 1.95 (m, 2H), 1.53 (s, 3H), 1.21 (m, 3H), 1.15 (m, 3H), 1.08 (m, 1H), 0.82 (m, 10H). ^{13}C NMR ($CDCl_3$) δ 200.2, 164.3, 164.3, 139.7, 128.7, 127.5, 127.4, 62.1, 61.9, 60.6, 56.8, 37.5, 32.1, 30.1, 29.2, 19.8, 13.9, 13.8. MS (ESI) *m*/*z* 375 $(M - H)^-$.

(*S*)-Diethyl 2-(2,5,5-Trimethyl-2-phenylhexanoyl)malonate (14b). Following the procedure for compound **13a**, except substituting compound **12b** for compound **11a**, the title compound **14b** (2.75 g, 89%) was obtained as a clear, colorless oil. ^{1}H NMR ($CDCl_3$) δ 7.31 (m, 5H), 4.52 (s, 1H), 4.12 (m, 2H), 4.04 (m, 2H), 1.95 (m, 2H), 1.53 (s, 3H), 1.21 (m, 3H), 1.15 (m, 3H), 1.08 (m, 1H), 0.82 (m, 10H). ^{13}C NMR ($CDCl_3$) δ 200.2, 164.3, 164.3, 139.7, 128.7, 127.5, 127.4, 62.1, 61.9, 60.6, 56.8, 37.5, 32.1, 30.1, 29.2, 19.8, 13.9, 13.8. MS (ESI) *m*/*z* 375 $(M - H)^-$.

(*R*)-Ethyl 1-Hydroxy-4-isopentyl-4-methyl-3-*oxo*-3,4-dihydronaphthalene-2-carboxylate (15a). A solution of compound **13a** (2.63 g, 7.25 mmol) in 15 mL of methanesulfonic acid was stirred for 18 h. The reaction solution was poured into 100 mL of H_2O, and the resulting aqueous mixture was extracted 3× with EtOAc. The combined organic layers were dried over Na_2SO_4, concentrated, and purified by chromatography on silica gel with 20% EtOAc in hexane as eluent to give 1.47 g (64%) of a clear, colorless oil. ^{1}H NMR ($CDCl_3$) δ 15.15 (s, 0.5H), 15.06 (s, 0.5H), 8.23 (m, 0.5H), 8.16 (m, 0.5H), 7.59 (m, 1H), 7.42 (m, 2H), 4.47 (m, 2H), 2.28 (m, 1H), 1.94 (m, 0.5H), 1.78 (m, 0.5H), 1.64 (s, 1.5H), 1.57 (s, 1.5H), 1.47 (m, 3H), 1.36 (m, 1H), 0.87 (m, 1H), 0.73 (m, 6H), 0.52 (m, 1H). ^{13}C NMR ($CDCl_3$) δ 172.9, 133.0, 126.7, 126.5, 125.4, 62.1, 61.4, 41.7, 39.9, 33.6, 28.8, 28.1, 22.4, 22.1, 14.2, 14.0. MS (ESI) *m*/*z* 315 $(M - H)^-$. Anal. ($C_{19}H_{24}O_4$) C, H.

(*S*)-Ethyl 1-Hydroxy-4-isopentyl-4-methyl-3-*oxo*-3,4-dihydronaphthalene-2-carboxylate (15b). Following the procedure for compound **15a**, except substituting compound **13b** for compound **13a**, the title compound **15b** (1.70 g, 74%) was obtained as a clear, colorless oil. ^{1}H NMR ($CDCl_3$) δ 15.15 (s, 0.5H), 15.06 (s, 0.5H), 8.23 (m, 0.5H), 8.16 (m, 0.5H), 7.59 (m, 1H), 7.42 (m, 2H), 4.47 (m, 2H), 2.28 (m, 1H), 1.94 (m, 0.5H), 1.78 (m, 0.5H), 1.64 (s, 1.5H), 1.57 (s, 1.5H), 1.47 (m, 3H), 1.36 (m, 1H), 0.87 (m, 1H), 0.73 (m, 6H), 0.52 (m, 1H). ^{13}C NMR ($CDCl_3$) δ 172.9, 133.0, 126.7, 126.5, 125.4, 62.1, 61.4, 41.7, 39.9, 33.6, 28.8, 28.1, 22.4, 22.1, 14.2, 14.0. MS (ESI) *m*/*z* 315 $(M - H)^-$. Anal. ($C_{19}H_{24}O_4$) C, H.

(*R*)-Ethyl 4-(3,3-Dimethylbutyl)-1-hydroxy-4-methyl-3-*oxo*-3,4-dihydronaphthalene-2-carboxylate (16a). Following the procedure for compound **15a**, except substituting compound **14a** for compound **13a**, the title compound **16a** (2.06 g, 86%) was obtained as a clear, colorless oil. ^{1}H NMR ($CDCl_3$) δ 15.15 (s, 0.5H), 15.03 (s, 0.5H), 8.24 (m, 0.5H), 8.16 (m, 0.5H), 7.59 (m, 1H), 7.41 (m, 2H), 4.47 (m, 2H), 2.28 (m, 1H), 1.94 (m, 0.5H), 1.76 (m, 0.5H), 1.65 (s, 1.5H), 1.57 (s, 1.5H), 1.47 (m, 3H), 0.86 (m, 1H), 0.75 (m, 4.5H), 0.73 (m, 4.5H), 0.51 (m, 1H). ^{13}C NMR ($CDCl_3$) δ 196.5, 192.7, 180.4, 177.8, 173.1, 172.8, 147.8, 143.6, 133.3, 132.6, 131.6, 126.9, 126.6, 126.2, 126.0, 125.9, 124.8, 104.6, 101.4, 62.2, 62.1, 51.6, 45.2, 38.4, 28.1, 36.9, 36.6, 30.0, 29.8, 29.5, 29.1, 29.0, 28.8, 14.2, 14.2. MS (ESI) *m*/*z* 329 $(M - H)^-$.

(*S*)-Ethyl 4-(3,3-Dimethylbutyl)-1-hydroxy-4-methyl-3-*oxo*-3,4-dihydronaphthalene-2-carboxylate (16b). Following the procedure for compound **15a**, except substituting compound **14b** for compound **13a**, the title compound **16b** (2.04 g, 85%) was obtained as a clear, colorless oil. ^{1}H NMR ($CDCl_3$) δ 15.15 (s, 0.5H), 15.03 (s, 0.5H), 8.24 (m, 0.5H), 8.16 (m, 0.5H), 7.59 (m, 1H), 7.41 (m, 2H), 4.47 (m, 2H), 2.28 (m, 1H), 1.94 (m, 0.5H), 1.76 (m, 0.5H), 1.65 (s, 1.5H), 1.57 (s, 1.5H), 1.47 (m, 3H), 0.86 (m, 1H), 0.75 (m, 4.5H), 0.73 (m, 4.5H), 0.51 (m, 1H). ^{13}C NMR ($CDCl_3$) δ 196.5, 192.7, 180.4, 177.8, 173.1, 172.8, 147.8, 143.6, 133.3, 132.6, 131.6, 126.9, 126.6, 126.2, 126.0, 125.9, 124.8, 104.6, 101.4, 62.2, 62.1, 51.6, 45.2, 38.4, 28.1, 36.9, 36.6, 30.0, 29.8, 29.5, 29.1, 29.0, 28.8, 14.2, 14.2. MS (ESI) *m*/*z* 329 $(M - H)^-$.

(*R*)-4-Hydroxy-1-isopentyl-1-methylnaphthalen-2(1*H*)-one (17a). A solution of compound **15a** (1.50 g, 4.75 mmol) and 15 mL of 1 N HCl in 15 mL of dioxane was heated at reflux for 2 h. The reaction mixture was cooled and partitioned between 100 mL of H_2O and 150 mL of EtOAc. The layers were separated and the organic layer was washed with H_2O and brine, dried over Na_2SO_4, and concentrated. The resulting solid was triturated with warm hexane and filtered to give 801 mg (69%) of a white solid. ^{1}H NMR ($CDCl_3$) δ 8.10 (m, 0.5H), 7.66 (m, 0.5H), 7.46 (m, 3H), 6.01 (s, 0.5H), 3.74 (m, 1H), 2.24 (m, 0.5H), 2.03 (m, 0.5H), 1.84 (m, 1H), 1.56 (s, 1.5H), 1.51 (s, 1.5H), 1.42 (m, 0.5H), 1.31 (m, 0.5H), 1.05 (m, 0.5H), 0.80 (m, 4H), 0.72 (d, 1.5H, $J = 6.6$), 0.68 (d, 1.5H, $J = 6.6$), 0.44 (m, 0.5H). ^{13}C NMR ($CDCl_3$) δ 192.1, 181.5, 147.5, 135.1, 132.0, 131.4, 129.5, 127.3, 127.2, 126.5, 126.3, 125.5, 125.4, 103.8, 53.2, 53.0, 46.9, 40.5, 39.4, 34.1, 33.3, 28.8, 28.2, 28.0, 24.0, 22.4, 22.2. MS (ESI) *m*/*z* 243 $(M - H)^-$. Anal. ($C_{16}H_{20}O_2$) C, H.

(*S*)-4-Hydroxy-1-isopentyl-1-methylnaphthalen-2(1*H*)-one (17b). Following the procedure for compound **17a**, except substituting compound **15b** for compound **15a**, the title compound **17b** (1.06 g, 91%) was obtained as a white solid. ^{1}H NMR ($CDCl_3$) δ 8.10 (m, 0.5H), 7.66 (m, 0.5H), 7.46 (m, 3H), 6.01 (s, 0.5H), 3.74 (m, 1H), 2.24 (m, 0.5H), 2.03 (m, 0.5H), 1.84 (m, 1H), 1.56 (s, 1.5H), 1.51 (s, 1.5H), 1.42 (m, 0.5H), 1.31 (m, 0.5H), 1.05 (m, 0.5H), 0.80 (m, 4H), 0.72 (d, 1.5H, $J = 6.6$), 0.68 (d, 1.5H, $J = 6.6$), 0.44 (m, 0.5H). ^{13}C NMR ($CDCl_3$) δ 192.1, 181.5, 147.5, 135.1, 132.0, 131.4, 129.5, 127.3, 127.2, 126.5, 126.3, 125.5, 125.4, 103.8, 53.2, 53.0, 46.9, 40.5, 39.4, 34.1, 33.3, 28.8, 28.2, 28.0, 24.0, 22.4, 22.2. MS (ESI) *m*/*z* 243 $(M - H)^-$. Anal. ($C_{16}H_{20}O_2$) C, H.

(*R*)-1-(3,3-Dimethylbutyl)-4-hydroxy-1-methylnaphthalen-2(1*H*)-one (18a). Following the procedure for compound **17a**, except substituting compound **16a** for compound **15a**, the title compound **18a** (1.07 g, 87%) was obtained as a white solid. ^{1}H NMR ($CDCl_3$) δ 8.09 (m, 1H), 7.50 (m, 3H), 6.06 (s, 0.67H), 3.73 (s, 0.67H), 2.25 (m, 0.67H), 2.01 (m, 0.33H), 1.83 (m, 1H), 1.56 (s, 1H), 1.48 (s, 2H), 1.06 (m, 0.33H), 0.82 (m, 4H), 0.70 (s, 6H), 0.41 (m, 0.67H). ^{13}C NMR ($CDCl_3$) δ 192.1, 181.5, 147.5, 132.0, 129.5, 126.3, 125.5, 125.4, 103.9, 46.9, 37.9, 36.3, 29.8, 29.1. MS (ESI) *m*/*z* 257 $(M - H)^-$.

(*S*)-1-(3,3-Dimethylbutyl)-4-hydroxy-1-methylnaphthalen-2(1*H*)-one (18b). Following the procedure for compound **17a**, except substituting compound **16b** for compound **15a**, the title compound **18b** (1.09 g, 89%) was obtained as a white solid. ^{1}H NMR ($CDCl_3$) δ 8.09 (m, 1H), 7.50 (m, 3H), 6.06 (s, 0.67H), 3.73 (s, 0.67H), 2.25 (m, 0.67H), 2.01 (m, 0.33H), 1.83 (m, 1H), 1.56 (s, 1H), 1.48 (s, 2H), 1.06 (m, 0.33H), 0.82 (m, 4H), 0.70 (s, 6H), 0.41 (m, 0.67H). ^{13}C NMR ($CDCl_3$) δ 192.1, 181.5, 147.5, 132.0, 129.5, 126.3, 125.5, 125.4, 103.9, 46.9, 37.9, 36.3, 29.8, 29.1. MS (ESI) *m*/*z* 257 $(M - H)^-$. Anal. ($C_{17}H_{22}O_2$) C, H.

(*R*)-2-(Bis(methylthio)methylene)-4-isopentyl-4-methylnaphthalene-1,3(2*H*,4*H*)-dione (19a). A solution of compound **17a** (953 mg, 3.90 mmol) and pyridine (2.52 mL, 31.2 mmol) in 20 mL of dioxane was treated with (bis-methylsulfanyl-methylene)-methyl-sulfonium tetrafluoroborate (4.14 g, 15.6 mmol).[23] The reaction solution was heated in a 100 °C oil bath for 1 h. The reaction mixture was cooled and partitioned between 100 mL of H_2O and 150 mL of EtOAc. The layers were separated and the organic layer was washed with H_2O and brine, dried over Na_2SO_4, and concentrated. The resulting solid was purified by chromatography on silica gel with 15% EtOAc in hexane as eluent to give 1.20 g of a yellow solid. The product was immediately used in the following step without further purification.

(*S*)-2-(Bis(methylthio)methylene)-4-isopentyl-4-methylnaphthalene-1,3(2*H*,4*H*)-dione (19b). Following the procedure for compound **19a**, except substituting compound **17b** for compound **17a**, the title compound **19b** (1.33 g) was obtained as a yellow solid. The product was immediately used in the following step without further purification.

(*R*)-2-(Bis(methylthio)methylene)-4-(3,3-dimethylbutyl)-4-methylnaphthalene-1,3(2*H*,4*H*)-dione (20a). Following the procedure for compound **19a**, except substituting compound **18a** for compound **17a**, the title compound **20a** (1.40 g) was obtained as a yellow solid. The product was immediately used in the following step without further purification.

(*S*)-2-(Bis(methylthio)methylene)-4-(3,3-dimethylbutyl)-4-methylnaphthalene-1,3(2*H*,4*H*)-dione (20b). Following the procedure for compound **19a**, except substituting compound **18b** for compound **17a**, the title compound **20b** (1.40 g) was obtained as a yellow solid. The product was immediately used in the following step without further purification.

{3-[(*R*)-1-Hydroxy-4-methyl-4-(3-methyl-butyl)-3-*oxo*-3,4-dihydro-naphthalen-2-yl]-1,1-dioxo-1,4-dihydro-1λ^6-benzo[1,2,4]thiadiazin-7-yl}-carbamic Acid *tert*-Butyl Ester (21a). A solution of compound **19a** (1.32 g, 3.78 mmol) and *tert*-butyl 4-amino-3-sulfamoylphenylcarbamate (1.27 g, 3.97 mmol) in 25 mL of toluene was heated at reflux for 6 h.[24] The reaction solution was cooled, concentrated, and purified by chromatography on silica gel with 30% EtOAc in hexane as eluent to give 1.24 g (61%) of a white solid. ^{1}H NMR (DMSO-d_6) δ 13.78 (s, 1H), 9.89 (s, 1H), 8.15 (m, 2H), 7.76 (m, 2H), 7.66 (m, 2H), 7.54 (m, 1H), 2.23 (m, 1H), 2.06 (m, 1H), 1.57 (s, 3H), 1.50 (s, 9H), 1.31 (m, 1H), 0.80 (m, 1H), 0.72 (d, 3H, J = 6.6), 0.69 (d, 3H, J = 6.6), 0.42 (m, 1H). ^{13}C NMR (DMSO-d_6) δ 155.2, 152.7, 138.3, 133.9, 128.3, 127.2, 126.2, 125.9, 123.4, 123.2, 120.3, 109.9, 80.0, 33.2, 29.2, 28.0, 27.6, 22.3, 22.1. MS (ESI) m/z 538 (M − H)$^-$.

{3-[(*S*)-1-Hydroxy-4-methyl-4-(3-methyl-butyl)-3-*oxo*-3,4-dihydro-naphthalen-2-yl]-1,1-dioxo-1,4-dihydro-1λ^6-benzo[1,2,4]thiadiazin-7-yl}-carbamic Acid *tert*-Butyl Ester (21b). Following the procedure for compound **21a**, except substituting compound **19b** for compound **19a**, the title compound **21b** (1.71 g, 84%) was obtained as a white solid. ^{1}H NMR (DMSO-d_6) δ 13.78 (s, 1H), 9.89 (s, 1H), 8.15 (m, 2H), 7.76 (m, 2H), 7.66 (m, 2H), 7.54 (m, 1H), 2.23 (m, 1H), 2.06 (m, 1H), 1.57 (s, 3H), 1.50 (s, 9H), 1.31 (m, 1H), 0.80 (m, 1H), 0.72 (d, 3H, J = 6.6), 0.69 (d, 3H, J = 6.6), 0.42 (m, 1H). ^{13}C NMR (DMSO-d_6) δ 155.2, 152.7, 138.3, 133.9, 128.3, 127.2, 126.2, 125.9, 123.4, 123.2, 120.3, 109.9, 80.0, 33.2, 29.2, 28.0, 27.6, 22.3, 22.1. MS (ESI) m/z 538 (M − H)$^-$.

{3-[(*R*)-4-(3,3-Dimethyl-butyl)-1-hydroxy-4-methyl-3-*oxo*-3,4-dihydro-naphthalen-2-yl]-1,1-dioxo-1,4-dihydro-1λ^6-benzo[1,2,4]thiadiazin-7-yl}-carbamic Acid *tert*-Butyl Ester (22a). Following the procedure for compound **21a**, except substituting compound **20a** for compound **19a**, the title compound **22a** (1.80 g, 86%) was obtained as a white solid. ^{1}H NMR (DMSO-d_6) δ 13.76 (s, 1H), 9.90 (s, 1H), 8.15 (m, 2H), 7.76 (m, 2H), 7.66 (m, 2H), 7.54 (m, 1H), 2.23 (m, 1H), 2.06 (m, 1H), 1.58 (s, 3H), 1.50 (s, 9H), 0.80 (m, 1H), 0.70 (s, 9H), 0.39 (m, 1H). ^{13}C NMR (DMSO-d_6) δ 155.1, 152.6, 138.4, 134.1, 128.1, 127.3, 126.2, 125.9, 123.5, 123.3, 120.4, 109.8, 80.0, 37.8, 36.4, 29.7, 29.5, 28.9, 28.0. MS (ESI) m/z 552 (M − H)$^-$.

{3-[(*S*)-4-(3,3-Dimethyl-butyl)-1-hydroxy-4-methyl-3-*oxo*-3,4-dihydro-naphthalen-2-yl]-1,1-dioxo-1,4-dihydro-1λ^6-benzo[1,2,4]thiadiazin-7-yl}-carbamic Acid *tert*-Butyl Ester (22b). Following the procedure for compound **21a**, except substituting compound **20b** for compound **19a**, the title compound **22b** (1.80 g, 86%) was obtained as a white solid. ^{1}H NMR (DMSO-d_6) δ 13.76 (s, 1H), 9.90 (s, 1H), 8.15 (m, 2H), 7.76 (m, 2H), 7.66 (m, 2H), 7.54 (m, 1H), 2.23 (m, 1H), 2.06 (m, 1H), 1.58 (s, 3H), 1.50 (s, 9H), 0.80 (m, 1H), 0.70 (s, 9H), 0.39 (m, 1H). ^{13}C NMR (DMSO-d_6) δ 155.1, 152.6, 138.4, 134.1, 128.1, 127.3, 126.2, 125.9, 123.5, 123.3, 120.4, 109.8, 80.1, 37.8, 36.4, 29.7, 29.5, 28.9, 28.0. MS (ESI) m/z 552 (M − H)$^-$.

(R)-3-(7-Amino-1,1-dioxo-1,4-dihydro-1λ^6-benzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-1-methyl-1-(3-methyl-butyl)-1*H*-naphthalen-2-one Hydrochloride (23a). A solution of compound **21a** (1.60 g, 2.97 mmol) in 2 mL of CH_2Cl_2 was treated with 15 mL of 4.0 M HCl in dioxane. The reaction solution was stirred for 1 h and then concentrated. The resulting white solid was triturated with ether to give 1.29 g (91%) of a white solid. ^{1}H NMR (DMSO-d_6) δ 13.58 (s, 1H), 8.16 (m, 1H), 7.77 (m, 2H), 7.55 (m, 1H), 7.49 (m, 2H), 7.07 (m, 2H), 2.23 (m, 1H), 2.08 (m, 1H), 1.57 (s, 3H), 1.31 (m, 1H), 0.79 (m, 1H), 0.72 (d, 3H, J = 6.6), 0.68 (d, 3H, J = 6.6), 0.41 (m, 1H). ^{13}C NMR (DMSO-d_6) δ 154.5, 145.7, 141.1, 134.2, 127.3, 127.1, 126.3, 125.9, 123.7, 123.6, 121.0, 109.5, 100.6, 48.2, 33.2, 29.1, 27.6, 22.3, 22.1. MS (ESI) m/z 438 (M − H)$^-$. Anal. ($C_{23}H_{26}ClN_3O_4S$) C, H, N.

(*S*)-3-(7-Amino-1,1-dioxo-1,4-dihydro-1λ^6-benzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-1-methyl-1-(3-methyl-butyl)-1*H*-naphthalen-2-one Hydrochloride (23b). Following the procedure for compound **23a**, except substituting compound **21b** for compound **21a**, the title compound **23b** (1.27 g, 89%) was obtained as a white solid. ^{1}H NMR (DMSO-d_6) δ 13.58 (s, 1H), 8.16 (m, 1H), 7.77 (m, 2H), 7.55 (m, 1H), 7.49 (m, 2H), 7.07 (m, 2H), 2.23 (m, 1H), 2.08 (m, 1H), 1.57 (s, 3H), 1.31 (m, 1H), 0.79 (m, 1H), 0.72 (d, 3H, J = 6.6), 0.68 (d, 3H, J = 6.6), 0.41 (m, 1H). ^{13}C NMR (DMSO-d_6) δ 154.6, 145.7, 140.4, 134.2, 127.5, 127.3, 126.3, 125.9, 123.9, 123.6, 121.0, 110.0, 100.7, 48.2, 33.2, 29.1, 27.6, 22.3, 22.1. MS (ESI) m/z 438 (M − H)$^-$. Anal. ($C_{23}H_{26}ClN_3O_4S$) C, H, N.

(*R*)-3-(7-Amino-1,1-dioxo-1,4-dihydro-1λ^6-benzo[1,2,4]thiadiazin-3-yl)-1-(3,3-dimethyl-butyl)-4-hydroxy-1-methyl-1*H*-naphthalen-2-one Hydrochloride (24a). Following the procedure for compound **23a**, except substituting compound **22a** for compound **21a**, the title compound **24a** (1.38 g, 95%) was obtained as a white solid. ^{1}H NMR (DMSO-d_6) δ 13.58 (s, 1H), 8.16 (m, 1H), 7.77 (m, 2H), 7.55 (m, 1H), 7.49 (m, 2H), 7.07 (m, 2H), 2.23 (m, 1H), 2.08 (m, 1H), 1.57 (s, 3H), 1.31 (m, 1H), 0.79 (m, 1H), 0.70 (m, 9H), 0.41 (m, 1H). ^{13}C NMR (DMSO-d_6) δ 154.7, 145.7, 140.3, 134.1, 127.7, 127.4, 126.3, 125.9, 124.0, 123.6, 121.1, 110.2, 37.8, 36.3, 29.7, 29.4, 28.9. MS (ESI) m/z 454 (M − H)$^-$.

(*S*)-3-(7-Amino-1,1-dioxo-1,4-dihydro-1λ^6-benzo[1,2,4]thiadiazin-3-yl)-1-(3,3-dimethyl-butyl)-4-hydroxy-1-methyl-1*H*-naphthalen-2-one Hydrochloride (24b). Following the procedure for compound **23a**, except substituting compound **22b** for compound **21a**, the title compound **24b** (1.37 g, 94%) was obtained as a white solid. ^{1}H NMR (DMSO-d_6) δ 13.58 (s, 1H), 8.17 (m, 1H), 7.77 (m, 2H), 7.55 (m, 1H), 7.48 (m, 1H), 7.04 (m, 2H), 2.23 (m, 1H), 2.08 (m, 1H), 1.59 (s, 3H), 0.79 (m, 1H), 0.71 (s, 9H), 0.39 (m, 1H). ^{13}C NMR (DMSO-d_6) δ 154.7, 145.7, 140.3, 134.1, 127.7, 127.4, 126.3, 125.9, 124.0, 123.6, 121.1, 110.2, 37.8, 36.3, 29.7, 29.4, 28.9. MS (ESI) m/z 454 (M − H)$^-$.

Sodium (*R*)-2-(7-Methanesulfonylamino-1,1-dioxo-1,4-dihydro-1λ^6-benzo[1,2,4]thiadiazin-3-yl)-4-methyl-4-(3-methyl-butyl)-3-*oxo*-3,4-dihydro-naphthalen-1-olate (25a). A solution of compound **23a** (1.14 g, 2.40 mmol), MsCl (0.744 mL, 9.61 mmol), and pyridine (1.56 mL, 19.2 mmol) in 30 mL of acetone was stirred for 18 h. The reaction mixture was diluted with 150 mL of EtOAc and washed with 0.1 M citric acid, H_2O, and brine. The organic layer was dried over Na_2SO_4, concentrated, and purified by chromatography on silica gel with 2.5% MeOH in CH_2Cl_2 as eluent. The resulting solid was suspended in 7 mL of EtOAc, and the mixture was heated at reflux until the solid completely dissolved. Heptane (7 mL) was added to the solution at a rate that maintained reflux. The heat source was removed and the solution was stirred vigorously overnight. The solid was filtered, washed with a 1 mL of heptane, and dried under vacuum to give 1.02 g of a pale-yellow crystalline solid.

To a suspension of the solid in 15 mL of H_2O was added 1 N NaOH (1.97 mL, 1.97 mmol). The suspension was stirred for 2 h and then lyophilized to give 1.06 g (82%) of a yellow solid. ^{1}H NMR (DMSO-d_6) δ 15.50 (s, 1H), 9.88 (s, 1H), 8.05 (m, 1H), 7.47 (m, 3H), 7.33 (m, 3H), 2.96 (s, 3H), 2.14 (m, 1H), 1.73 (m, 1H), 1.38 (s, 3H), 1.28 (m, 1H), 0.79 (m, 1H), 0.69 (m, 6H), 0.45 (m, 1H). ^{13}C NMR (DMSO-d_6) δ 195.5, 180.4, 156.7, 145.0, 135.2, 133.0, 132.4, 130.7, 125.6, 125.6, 125.2, 124.8, 122.9, 118.9, 113.3, 101.6, 48.3, 39.7, 39.1, 33.7, 29.5, 27.7, 22.5, 22.2. MS (ESI) m/z 516 (M − H)$^-$. HRMS (ESI) m/z calcd ($C_{24}H_{27}N_3O_6S_2$) 518.1414, found 518.1411 (M + H)$^+$, −0.6 ppm error.

Sodium (*S*)-2-(7-Methanesulfonylamino-1,1-dioxo-1,4-dihydro-1λ^6-benzo[1,2,4]thiadiazin-3-yl)-4-methyl-4-(3-methyl-butyl)-3-*oxo*-3,4-dihydro-naphthalen-1-olate (25b). Following the procedure for compound **25a**, except substituting compound **23b** for compound

23a, the title compound **25b** (1.11 g, 86%) was obtained as a yellow solid. ^{1}H NMR (DMSO-d_6) δ 15.47 (s, 1H), 9.88 (s, 1H), 8.05 (m, 1H), 7.45 (m, 3H), 7.31 (m, 3H), 2.94 (s, 3H), 2.15 (m, 1H), 1.73 (m, 1H), 1.38 (s, 3H), 1.28 (m, 1H), 0.80 (m, 1H), 0.69 (m, 6H), 0.45 (m, 1H). ^{13}C NMR (DMSO-d_6) δ 195.5, 180.4, 156.7, 145.0, 135.2, 133.0, 132.4, 130.7, 125.6, 125.6, 125.2, 124.8, 122.9, 118.9, 113.3, 101.6, 48.3, 39.7, 39.1, 33.7, 29.5, 27.7, 22.5, 22.2. MS (ESI) *m*/*z* 516 $(M - H)^-$. HRMS (ESI) *m*/*z* calcd ($C_{24}H_{27}N_3O_6S_2$) 518.1414, found 518.1412 $(M + H)^+$, −0.3 ppm error.

Sodium (*R*)-4-(3,3-Dimethyl-butyl)-2-(7-methanesulfonylamino-1,1-dioxo-1,4-dihydro-1λ^6-benzo[1,2,4]thiadiazin-3-yl)-4-methyl-3-*oxo*-3,4-dihydro-naphthalen-1-olate (26a). A suspension of **26** (223 mg, 0.42 mmol) in ethanol (1.7 mL) was heated to 75 °C and mixed to dissolve the solids. A solution of sodium ethoxide (31 mg, 0.46 mmol) in ethanol (0.55 mL) was added to the solution via syringe over 50 min while maintaining the temperature at 70−72 °C. The solution was cooled to room temperature and mixed for 15 h. The product was isolated by filtration and dried to give the sodium salt **26a** as a white solid (223 mg, 96%). ^{1}H NMR (DMSO-d_6) δ 15.50 (s, 1H), 8.05 (m, 1H), 7.46 (m, 3H), 7.31 (m, 3H), 2.94 (s, 3H), 2.16 (m, 1H), 1.71 (m, 1H), 1.39 (s, 3H), 0.80 (m, 1H), 0.70 (s, 9H), 0.44 (m, 1H). ^{13}C NMR (DMSO-d_6) δ 196.3, 181.2, 157.4, 145.7, 136.7, 133.8, 132.7, 131.3, 126.4, 126.3, 125.9, 125.6, 123.6, 119.5, 113.8, 102.3, 49.0, 39.7, 39.0, 37.2, 30.6, 30.3, 29.8. MS (ESI) *m*/*z* 530 $(M - H)^-$. HRMS (ESI) *m*/*z* calcd ($C_{25}H_{29}N_3NaO_6S_2$) 554.13900, found 554.13946 $(M + Na)^+$, −0.8 ppm error.

Sodium (*S*)-4-(3,3-Dimethyl-butyl)-2-(7-methanesulfonylamino-1,1-dioxo-1,4-dihydro-1λ^6-benzo[1,2,4]thiadiazin-3-yl)-4-methyl-3-*oxo*-3,4-dihydro-naphthalen-1-olate (26b). Following the procedure for compound **25a**, except substituting compound **24b** for compound **23a**, the title compound **26b** (1.24 g, 93%) was obtained as a yellow solid. ^{1}H NMR (DMSO-d_6) δ 15.48 (s, 1H), 8.05 (m, 1H), 7.46 (m, 3H), 7.30 (m, 3H), 2.93 (s, 3H), 2.16 (m, 1H), 1.71 (m, 1H), 1.39 (s, 3H), 0.81 (m, 1H), 0.70 (s, 9H), 0.44 (m, 1H). ^{13}C NMR (DMSO-d_6) δ 195.5, 180.4, 156.6, 145.1, 136.6, 133.1, 131.7, 130.7, 125.7, 125.7, 125.2, 124.9, 122.9, 118.8, 112.9, 101.7, 48.3, 39.0, 38.3, 36.5, 30.0, 29.7, 29.1. MS (ESI) *m*/*z* 530 $(M - H)^-$. HRMS (ESI) *m*/*z* calcd ($C_{25}H_{29}N_3O_6S_2$) 532.1571, found 532.1570 $(M + H)^+$, <0.1 ppm error.

HCV Polymerase Inhibition Assays. Dilutions of the inhibitors were incubated with 20 mM Tris-Cl pH 7.4, 2 mM $MnCl_2$, 80 mM potassium glutamate, 1 mM dithiothreitol, 1 mM ethylene diamine tetraacetic acid (EDTA), 0.1 mg/ml BSA, 125 uM GTP and 20 nM NS5B, HCV strain 1a (H77, Genbank accession number AF011751) or 62.5 uM GTP and 50 nM NS5B, HCV strain 1b (BK) for 15 min at room temperature. The reaction was initiated by the addition of 20 μM CTP, 20 μM ATP, 0.284 μM (0.5 μCi) of 5,6-[^{3}H] UTP, 5 nM template RNA, and 0.1 U/μL RNase inhibitor (RNasin, Promega), and allowed to proceed for 3 h at 30 °C. Reaction volume was 50 μL. The reaction was terminated by the addition of 1 volume of 4 mM spermine in 10 mM Tris-Cl pH 8.0, 1 mM EDTA. After incubation for at least 15 min at room temperature, the precipitated RNA was captured by filtering through a GF/B filter (Millipore) in a 96-well format. The filter plate was washed five times with 200 μL each of 2 mM spermine, 10 mM Tris-Cl pH 8.0, and 1 mM EDTA and 2 times with ethanol. After air drying, 30 μL of Microscint 20 scintillation cocktail (Packard) was added to each well and the retained cpm were determined by scintillation counting. The initial rates of 24 inhibitor concentrations were fit to the tight binding equation 1 to obtain IC_{50}.

$$V = k_{cat}[S]/2(K_m + [S])\left(\sqrt{(IC_{50} + I - E)^2 + (4IC_{50}E)} - (IC_{50} + I - E)\right) \quad (1)$$

Replicon Assays with Laboratory Strains. A genotype 1b-con1 strain subgenomic replicon[18] and a chimeric genotype 1a-H77/1b-con1 subgenomic replicon were used in these studies.[25] For the chimeric replicon, the nonstructural genes NS3 (except for the N-terminal 73 amino acids), NS4A, NS5B, and the 3′ nontranslated region were derived from the 1a strain H77 and the first 73 amino acids of NS3 along with all of NS4B and NS5A were from the 1b-con1 strain. The inhibitory potencies of A-848837 against these HCV subgenomic replicons were measured based on the reduction of HCV RNA copy number in the presence of inhibitor over a four day period as described previously.[25] Cytotoxicity was determined by the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay.[26] The protein binding effect on inhibitor potency was assessed by including 40% human serum in culture media containing 5% FBS during the four day incubation period.

Solubility Determinations. Approximately 0.5−10 mg of the lyophilized amorphous powder samples of **1a**, **26**, and crystalline **26a** were weighed using a microbalance (model M5P, Sartorius Corp., Bohemia, NY) and transferred into 2 mL glass HPLC vials. Approximately 0.5−1 mL of medium was added into the vials. The vials were wrapped with aluminum foil and equilibrated with rotation in a water bath maintained at 25 °C (Vankel Industries) for 4 days. The final pH of the aqueous solutions was measured before HPLC assay and crystalline form of residual solid for **26a** was confirmed by powder X-ray diffraction (Rigaku Ultima).

HPLC Analysis. Sample analysis was conducted using an isocratic reverse phase HPLC method with a PhenomenexLuna 5 μ CN, 250 mm × 4.60 mm or PhenomenexLuna 5 μm C18 (2), 150 mm × 4.60 mm column with detection wavelength was 262 nm. The aqueous mobile phase component was either 0.1% TFA or 25 mM potassium phosphate, pH 2.5 with acetonitrile used as the organic component. Beckman Peak Pro software CALS version 8.4a was used to acquire and process the chromatograms. Sample concentrations were determined against a linear standard curve.

LogD Determinations. Distribution coefficient was measured between *n*-octanol and 50 mM phosphate buffer, made isotonic with NaCl and adjusted to pH 7.4. The compound was first dissolved in the *n*-octanol phase and then mixed with the buffer phase. The mixture was tumbled in a 25 °C water bath at 25 rpm for 2−3 days. The sample was then removed from the water bath and centrifuged at 3000 rpm for 10 min (Jouan GT 422 centrifuge) to separate the two phases. The separated phases were transferred into separate vials by discarding the interface. The buffer phase was injected into HPLC column without dilution, while the octanol phase was diluted with mobile phase to achieve a final concentration within the range of the standard curve and assayed by HPLC. The distribution coefficient D is expressed as the ratio of concentration in the octonal phase over that in the buffer phase.

Pharmacokinetic Profiles. 25a and **26a** were formulated in DMSO:PEG400 (10:90; v/v) for single oral dosing of 5 mg/kg in rat, dog (2.5 mg/kg only for **26a**), and monkey. Heparinized plasma samples were withdrawn at 0.1 (IV only), 0.25, 0.5, 1, 2, 3 (dog only), 4, 6, 9, 12, 15 (dog only), and 24 h postdosing. Plasma or rat liver homogenate drug concentrations were determined by a liquid chromatography−mass spectrometry (LC-MS) assay as follows. Compounds **25a** and **26a** were separated from plasma or liver homogenate samples using protein precipitation with acetonitrile. A 250 μL aliquot of each sample or spiked standard was combined with 25 μL of internal standard and 500 μL of acetonitrile. The samples were vortexed vigorously for 1 min, followed by centrifugation for 10 min at 4 °C. Each supernatant was transferred to a well of a 96-well plate and evaporated to dryness with a gentle stream of nitrogen. The samples were reconstituted with sequential aliquots of acetonitrile and 20 mM ammonium acetate. Compounds **25a** or **26a** and the internal standard were separated from each other and coprecipitated contaminants on a 50 mm × 3 mm Clipeus 5 μm column (Higgins Analytical, Inc.) with a 1:1 acetonitrile:20 mM ammonium acetate mobile phase at a flow rate of 0.3 mL/min. Analysis was performed on a Sciex API2000 biomolecular mass analyzer with a turbo-ionspray interface, in the negative ion mode. Detection was in the multiple reaction monitoring (MRM) mode at *m*/*z* 516.2→437.0. Compounds **25a** or **26a** and internal standard peak areas were determined using Sciex TurboQuan software. The drug concentration of each sample was calculated by least-squares linear regression analysis (non-weighted) of the peak area ratio (parent/internal standard) of the spiked standards versus concentration. The method, generally

evaluated over the concentration range 0−6.4 μg/mL, was linear (correlation coefficient >0.999), with mean accuracy values from 96−107% of theoretical for the analysis of triplicate standards at seven separate concentrations. The limit of quantitation was estimated to be ∼20 ng/mL from a 0.25 mL plasma sample.

Supporting Information Available: ORTEP plot of single crystal X-ray structure determination of intermediate **10a**. This material is available free of charge via the Internet at http://pubs.acs.org.

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