3-(1,1-Dioxo-2*H*-(1,2,4)-benzothiadiazin-3-yl)-4-hydroxy-2(1*H*)-quinolinones, Potent Inhibitors of Hepatitis C Virus RNA-Dependent RNA Polymerase

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Recently, we disclosed a new class of HCV polymerase inhibitors discovered through high-throughput screening (HTS) of the GlaxoSmithKline proprietary compound collection. This interesting class of 3-(1,1-dioxo-2*H*-1,2,4-benzothiadiazin-3-yl)-4-hydroxy-2(1*H*)-quinolinones potently inhibits HCV polymerase enzymatic activity and inhibits the ability of the subgenomic HCV replicon to replicate in Huh-7 cells. This report will focus on the structure−activity relationships (SAR) of substituents on the quinolinone ring, culminating in the discovery of 1-(2-cyclopropylethyl)-3-(1,1-dioxo-2*H*-1,2,4-benzothiadiazin-3-yl)-6-fluoro-4-hydroxy-2(1*H*)-quinolinone (**130**), an inhibitor with excellent potency in biochemical and cellular assays possessing attractive molecular properties for advancement as a clinical candidate. The potential for development and safety assessment profile of compound **130** will also be discussed.

Introduction

Hepatitis C virus (HCV) was first characterized in 1989 as the major cause of non-A and non-B hepatitis infections.[1] Despite the routine screening of blood transfusion supplies since 1992, the number of new hepatitis C infections in 2001 was estimated at 25,000−35,000 cases in the United States. According to WHO figures, greater than 2% of the world population is chronically infected with HCV. The majority (approximately 80%) of these infections will develop into chronic hepatitis and a significant percentage (approximately 20%) will progress to cirrhosis, with many advancing to hepatocellular carcinoma.[2,3] The heterogeneity of the hepatitis C virus has hampered the development of a vaccine, and thus far, there is no universally effective therapy for all HCV genotypes. The standard of care for HCV infection is combination treatment using pegylated interferon-α and ribavirin, a nonspecific, antiviral nucleoside analogue. The rate of success obtained with this treatment is highly dependent upon the HCV genotype with which patients are infected, with a greater rate [70−80% sustained viral response (SVR) after 24 weeks of treatment] observed for those affected with genotypes 2 and 3. However for genotype 1, which accounts for approximately 70% of all HCV infections worldwide, the SVR is around 40−45% after 48 weeks of therapy.[4] In addition, the typical side effects associated with the use of interferons, which include headaches, nausea, fatigue, and other neuropsychological disorders, make patient compliance difficult, especially considering that people affected by the virus often proceed asymptomatically for several decades.

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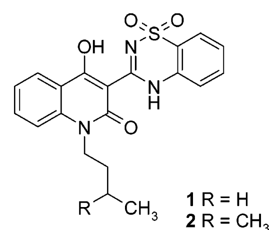
HCV is a single-stranded, positive-sense RNA virus of the *Flaviviridae* family. Its 9,600 nucleotide genome encodes for a single polyprotein of approximately 3,000 amino acids, which is processed by host cell and viral proteases into three structural proteins (C, E1 and E2) and six nonstructural proteins (NS2, NS3, NS4A, NS4B, NS5A, and NS5B).[4] Much research has been devoted to the discovery of inhibitors of NS3,[5] both protease and helicase components, and more recently, of NS5B, an RNA-dependent RNA polymerase (RdRp) and several classes of potent inhibitors have now been described.[6,7] The HCV polymerase is essential for viral replication and growth,[8,9] it has been structurally characterized,[10−12] and there are no known mammalian RdRps. Thus, it represents an excellent target for the development of anti-HCV agents.

High-Throughput Screening. In search of novel HCV polymerase inhibitors, a robust RdRp scintillation-proximity assay (SPA) using N-terminal-truncated Δ21-NS5B was developed as described previously.[13] HTS of the GlaxoSmithKline proprietary compound collection resulted in the identification of 1-butyl-3-(1,1-dioxo-2*H*-1,2,4-benzothiadiazin-3-yl)-4-hydroxy-2(1*H*)-quinolinone (**1**) as a potent HCV polymerase inhibitor. The biochemical characterization of this 4-hydroxy-2(1*H*)-quinolinone was previously described,[13] wherein it was shown that compound **1** did not interact with nucleic acid, it was noncompetitive with respect to GTP, and it reduced subgenomic viral-RNA replication in an Huh-7 cell-based HCV replicon system. In the same report, we also disclosed compound **2**, a more potent polymerase inhibitor. In this report we will



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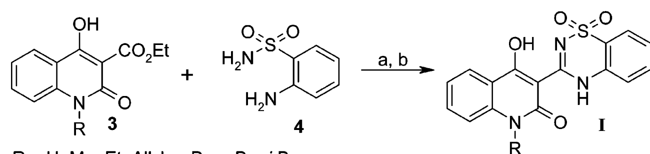
Scheme 1[a]



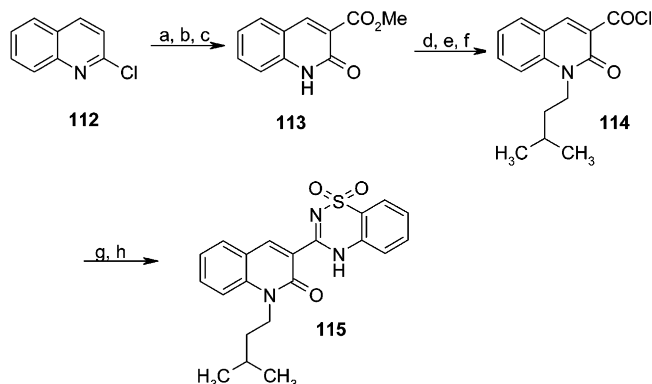
[a] Conditions: (a) Δ; (b) 10% aq KOH, reflux.

detail the structure–activity relationships (SAR) of the quinoline portion of compound **1** and how modifications of this screening hit led to the discovery of an inhibitor of viral replication with potency in the low nanomolar range.

Chemistry. The synthesis of 1-alkyl-3-(1,1-dioxo-2*H*-1,2,4-benzothiadiazin-3-yl)-4-hydroxy-2(1*H*)-quinolinones (**I**) was first reported by Ukrainets et al.[14] via the thermal condensation of ethyl 1-alkyl-4-hydroxy-2-oxoquinoline-3-carboxylates **3** with 2-aminobenzenesulfonamide **4**, followed by base-catalyzed heterocyclization (Scheme 1). For the synthesis of compounds **1**, **2**, **10–111**, **129**, and **130**, a more robust methodology was developed via the base-catalyzed condensation of an appropriately substituted 1-alkyl-2*H*-3,1-benzoxazine-2,4(1*H*)-dione **7** and ethyl (1,1-dioxo-2*H*-1,2,4-benzothiadiazin-3-yl)-acetate **9** (Scheme 2).[15] Ester **9** was obtained by reaction of 2-aminobenzensulfonamide **4** with ethyl malonyl chloride, followed by base-catalyzed cyclization, as previously described by Ukrainets et al.[16] In the absence of a commercial source of the required substituted isatoic anhydrides **6**, the latter were prepared from the corresponding anthranilic acids **5**, using phosgene or a phosgene equivalent. The N-1-substituent was installed by the reaction of **6** with an alkyl halide in the presence of sodium hydride or with the appropriate alcohol under Mitsunobu conditions.[17] Alternatively, in some cases the N-substituted 2*H*-3,1-benzoxazine-2,4(1*H*)-dione **7** was obtained by first coupling the 2-bromobenzoic acid **8** with the requisite alkylamine in the presence of Cu(II), followed by heterocyclization with phosgene (Scheme 2). Finally, elaboration of the 6-amino derivative **85** and 6-hydroxy derivative **86** via alkylation or acylation afforded compounds **90–102** (not illustrated).

To probe the structural requirements for inhibition by the heterocyclic 3-(1,1-dioxo-2*H*-1,2,4-benzothiadiazin-3-yl)-4-hydroxy-2(1*H*)-quinolinone template, a series of modified analogues (Figure 1) was prepared through a variety of synthetic approaches. Thus, the 4-deshydroxy analogue **115** was prepared (Scheme 3) from 2-chloroquinoline (**112**) via acyl chloride **114**, which was coupled with 2-aminobenzenesulfonamide **4**, followed by cyclization.

Scheme 3[a]

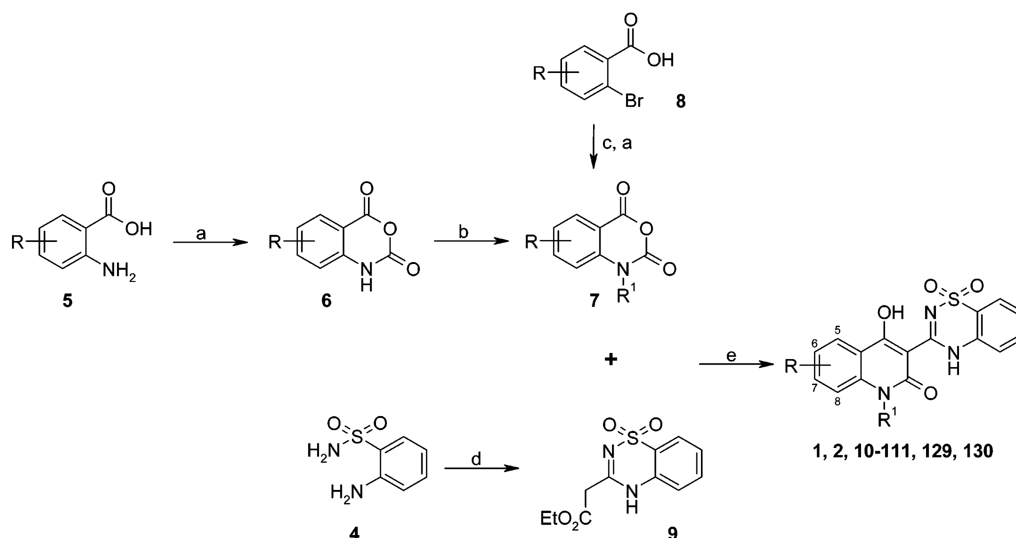


[a] Conditions: (a) (1) LDA, THF; (2) solid CO_2; (b) 6 N HCl, reflux; (c) concd H_2SO_4, MeOH; (d) NaH, *i*-AmBr, DMF; (e) NaOH, MeOH/H_2O; (f) $SOCl_2$, reflux; (g) **4**, Et_3N, DMAP, THF/DMF; (h) (1) 10% NaOH, 1,4-dioxane, reflux; (2) 3 N HCl.

The synthesis of the 2-desoxy compound **119** was accomplished starting from aniline (**116**), as shown in Scheme 4. Reductive amination with isovaleraldehyde, followed by condensation with diethyl ethoxymethylenemalonate and then PPA-mediated cyclization afforded intermediate ester **117**. Conversion of **117** to acyl chloride **118**, followed by coupling with 2-aminobenzenesulfonamide **4** and cyclization, gave the desired product.

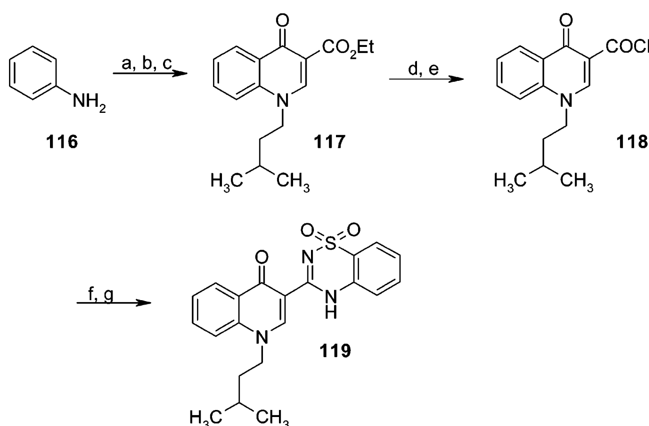
Thermal condensation of carbamate **121**[18] (Scheme 5) with the substituted isatoic anhydride **7** provided quinazolinedione **122**, in which the C-3 of inhibitor **2** was replaced by nitrogen.[19] The 4-*N*-methylbenzothiadiazine derivative **125** was synthesized from *N*-methylaniline (**123**, Scheme 6) by treatment with chlorosulfonyl isocyanate followed by acidic hydrolysis and then amide formation with ethyl malonyl chloride. Subsequent heterocyclization afforded ester **124**, which was condensed with 1-(3-methylbutyl)-2*H*-3,1-benzoxazine-2,4(1*H*)-dione to give

Scheme 2[a,b]



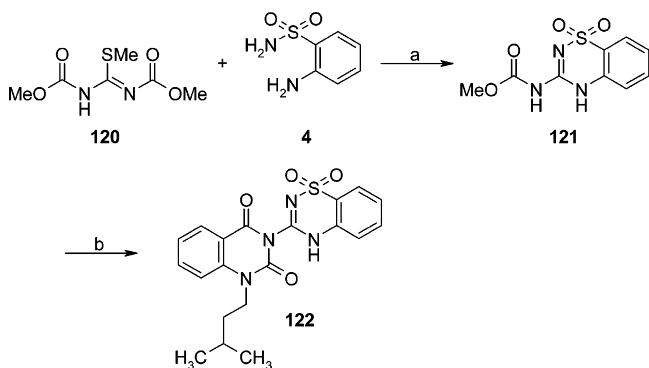
[a] Conditions: (a) $COCl_2$, Na_2CO_3, toluene or $(Cl_3CO)_2CO$, K_2CO_3, EtOAc; (b) NaH, R^1X, DMF or R^1OH, Ph_3P, DIAD; (c) R^1NH_2, $CuBr_2$, K_2CO_3, THF or DMF; (d) (1) $ClCOCH_2CO_2Et$, pyridine, CH_2Cl_2; (2) 10% aq Na_2CO_3; (e) NaH, THF, reflux, then AcOH, reflux or DBU, DMF. [b]For the definition of R^1, R^5, R^6, R^7, and R^8, see Tables 1–7.

Scheme 4[a]



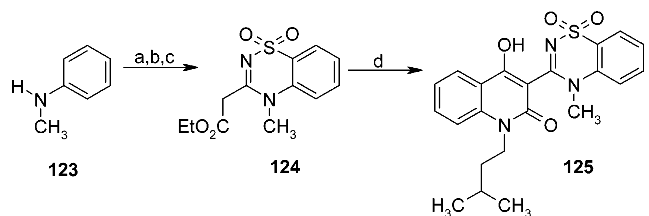
[a] Conditions: (a) isovaleraldehyde, $NaBH(OAc)_3$, CH_2Cl_2; (b) $(CO_2Et)_2$-C=CHOEt; (c) PPA, Δ; (d) NaOH, EtOH/H_2O; (e) $SOCl_2$, reflux; (f) **4**, Et_3N, DMAP, THF/DMF; (g) (1) 10% NaOH, 1,4-dioxane, reflux; (2) 3 N HCl.

Scheme 5[a]



[a] Conditions: (a) toluene, reflux; (b) **7** ($R^1 = -(CH_2)_2CH(CH_3)_2$), sealed tube, 250 °C.

Scheme 6[a]



[a] Conditions: (a) $ClSO_2NCO$, $AlCl_3$, $EtNO_2$; (b) 50% H_2SO_4, Δ; (c) (1) $ClCOCH_2CO_2Et$, pyridine, $CHCl_3$; (2) $POCl_3$, reflux; (d) **7** ($R^1 = -(CH_2)_2CH(CH_3)_2$), NaH, 1,4-dioxane, reflux, then AcOH, reflux.

125. Derivatization of compound **2** via treatment with tosyl chloride, followed by aqueous ammonia, provided 4-aminoquinoline **126**. 2,4(1*H*,3*H*)-Quinazolinone **128**, wherein the sulfonyl group of the benzothiadiazine is replaced with a carbonyl group, was synthesized in a straightforward manner following the procedure described in Scheme 2, substituting 2-aminobenzensulfonamide **4** with 2-aminobenzamide.

Results and Discussion

Inhibition of HCV NS5B Polymerase Activity. The primary testing of compounds was performed using a scintillation proximity assay to measure the incorporation of radio-labeled ^{33}P-GTP into a biotinylated oligo-G_{13} primer in the presence of a poly-C template catalyzed by 10 nM of HCV NS5B (genotype 1b, J4 strain, Δ21 construct). After immobilization of the primer to streptavidin-coated Flash-plates, quantification

Table 1. Inhibition of HCV NS5B Polymerase Activity by N-1 Alkyl and Cycloalkyl-alkyl Derivatives of 3-(1,1-Dioxo-2*H*-(1,2,4)-benzothiadiazin-3-yl)-4-hydroxy-2(1*H*)-quinolinones

Entry	R^1	RdRp IC_{50} (nM)	Entry	R^1	RdRp IC_{50} (nM)
1	CH_3	200	**19**	CH_3	150
2	CH_3, CH_3	32	**20**	CH_3, CH_3	289
10	H	>10,000	**21**	CH_2	90
11	CH_3	5,592	**22**	CH	118
12	CH_3	2,220	**23**	CH	179
13	CH_3	1,550	**24**		78
14	CH_3, CH_3	108	**25**	CH_3	41
15	CH_3, CH_3, CH_3	21	**26**		13
16	CH_3	80	**27**		49
17	CH_3, CH_3	65	**28**		132
18	CH_2	>10,000	**29**		367

of radioactivity at varying inhibitor concentrations generated inhibition curves.[13]

The ability of NS5B to polymerize GTP under these assay conditions proves to be very sensitive to the nature of the quinoline N-1 substituent of the 3-(1,1-dioxo-2*H*-1,2,4-benzothiadiazin-3-yl)-4-hydroxy-2-(1*H*)-quinolinones (Table 1). Compounds containing no substitution or only a short alkyl chain (1−3 carbon atoms, Table 1, compounds **10**−**13**) are weak inhibitors of NS5B. However, increasing the length of the linear alkyl chain to four or five carbon atoms results in a significant increase in inhibitory potency (Table 1, compounds **1** and **16**). Most promisingly, incorporation of a branched alkyl chain containing between five and six carbons results in a further increase in activity, as observed when comparing *n*-butyl analogue **1** (IC_{50} = 200 nM) with 3-methylbutyl analogue **2** (IC_{50} = 32 nM) and 3,3-dimethylbutyl compound **15** (IC_{50} = 21 nM). Incorporation of unsaturation into the N-1 chain provides several alkenes and alkynes (compounds **18**−**23**), which are generally weaker inhibitors of NS5B, whereas cycloalkyl-substituted variants are fairly well tolerated. In particular, within the homologous series of C_3 to C_6 cycloalkylmethyl derivatives (compounds **24** and **27**−**29**), the cyclobutylmethyl derivative **27** is the most potent (IC_{50} = 49 nM), with activity dropping off as the ring size increases. Further branching by substitution on the cyclopropyl ring of compound **24** (IC_{50} = 78 nM) with a methyl group (compound **25**, mixture of isomers) results in a modest 2-fold increase in potency. However, an even greater increase in potency (6-fold) is seen

Table 2. Inhibition of HCV NS5B Polymerase Activity by Compounds Containing Polar Functionalities in the N-1 Alkyl Substituent of 3-(1,1-Dioxo-2*H*-(1,2,4)-benzothiadiazin-3-yl)-4-hydroxy-2(1*H*)-quinolinones

Entry	R[1]	RdRp IC_{50} (nM)	Entry	R[1]	RdRp IC_{50} (nM)
30	CF_3	106	**42**	OH	>10,000
31	CF_3, CH_3	44	**43**	OH	2,815
32	Br	290	**44**	OH	1,301
33	N	172	**45**	CH_3, N, CH_3	>10,000
34	N	750	**46**	N	>10,000
35	O, CH_3	819	**47**	NH_2	>10,000
36	O, CH_3, CH_3	82	**48**	CO_2Et	2,036
37	O	484	**49**	CO_2Et	>10,000
38	O	528	**50**	$CONH_2$	>10,000
39	S, CH_3	53	**51**	CO_2H	>10,000
40	O^-, S^+, CH_3	2,537	**52** (**C-6 = F**)	CH_3, N, CH_3, O	>10,000
41	O, O, S, CH_3	2,322	**53** (**C-6 = F**)	CH_3, CH_3, CH_3, O	696

Table 3. Inhibition of HCV NS5B Polymerase Activity by N-1 Aromatic, Alkyl-aromatic, and Alkyl-heteroaromatic Derivatives of 3-(1,1-Dioxo-2*H*-(1,2,4)-benzothiadiazin-3-yl)-4-hydroxy-2(1*H*)-quinolinones

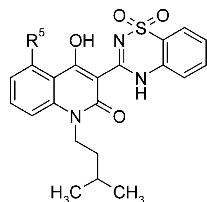
Entry	R[1]	RdRp IC_{50} (nM)	Entry	R[1]	RdRp IC_{50} (nM)
54		>10,000	**63**	Br	95
55		82	**64**	Br	36
56		>10,000	**65**	N	2,694
57		>10,000	**66**	N	316
58	NC	780	**67**	N	61
59	O_2N	>10,000	**68**	O	27
60	NO_2	81	**69**	S	>10,000
61	NO_2	2,442	**70**	S	>10,000
62	CH_3, CH_3, CH_3	>10,000	**71**	N, S, CH_3	304

upon homologation to the cyclopropylethyl derivative **26** (IC_{50} = 13 nM), which is among the most active compounds within this series.

Substitution of the N-1 alkyl substituent with halogens or polar functionality generally proves to be deleterious to the inhibitory activity of the 3-(1,1-dioxo-2*H*-1,2,4-benzothiadiazin-3-yl)-4-hydroxy-2(1*H*)-quinolinones, even with simple isosteric modifications (Table 2, compounds **30**−**53**). Relative to *n*-butyl analogue **1**, the 1-methoxyethyl derivative **35** is much less active, while the more lipophilic (methylthio)ethyl compound **39** is slightly more potent. Similarly, incorporation of a basic nitrogen into the side chain of *i*-amyl derivative **2** (IC_{50} = 32 nM) provides 1-(*N,N*-dimethylamino)ethyl derivative **45**, a compound devoid of activity. Interestingly, while the activity of methoxyethyl compound **35** is reduced 4-fold relative to the *n*-butyl analogue **1**, branched methoxypropyl compound **36** (IC_{50} = 82 nM) is nearly equipotent to its isosteric all-carbon analogue **17** (IC_{50} = 65 nM). Replacement of protons by fluorine atoms is tolerated; for example, compounds **30** and **31** have similar activities to their parent, nonfluorinated alkyl derivatives. Interestingly, addition of a relatively large bromine atom near the terminus of the butyl chain (compound **32**) results in an inhibitor of equivalent potency to the unsubstituted butyl compound **1**. Cyanopropyl derivative **33** (IC_{50} = 172 nM) is equipotent with its isosteric alkyne **23** (IC_{50} = 179 nM); however, the slightly larger, homologated cyanobutyl compound **34** is somewhat less potent (IC_{50} = 750 nM). The two isomeric (tetrahydrofuranyl)methyl derivatives **37** and **38** are less active than the corresponding cyclopentane derivative **28**. As previously alluded, incorporation of polar functionality, such as in the homologous series of hydroxylalkyl analogues (compounds **42**−**44**) or aminoalkyl derivative **47**, is poorly tolerated. Therefore, despite the presence of amino acid side chains containing potential hydrogen-bonding functionality in the N-1 binding pocket (vide infra), all attempts to increase potency by engaging hydrophilic interactions with such residues or the peptide backbone amide functionality have been unfruitful to date.

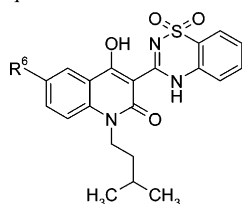
Aromatic and heteroaromatic substituents at N-1 are generally tolerated, provided that they are separated from the heterocyclic template by a one-carbon atom spacer (Table 3). For example, while the *N*-phenyl (**54**), *N*-phenethyl (**56**), and the *N*-phenylpropyl (**57**) compounds are essentially inactive at the concentrations tested, the *N*-benzyl compound **55** displays excellent inhibitory activity. In general, *m*-substituted benzyl derivatives are more effective inhibitors than the corresponding *p*- and *o*-derivatives. An approximate 30-fold increase in activity is observed when a nitro group is moved from the 4-position on an N-1 benzyl substituent (compound **61**, IC_{50} = 2442 nM) to the 3-position (compound **60**, IC_{50} = 81 nM). The 4-bromobenzyl derivative **63** (IC_{50} = 95 nM) is about 2.5-fold less active than 3-bromobenzyl derivative **64** (IC_{50} = 36 nM), and overall, bromo substitution is better tolerated than nitro substitution. Although a relatively weak inhibitor, the 2-cyanobenzyl derivative **58** (IC_{50} = 780 nM) is considerably more active than the 2-nitrobenzyl derivative **59** (IC_{50} > 10,000 nM). Among the isomeric series of (pyridinyl)methyl analogues (compounds **65**−**67**), the pyridine-4-yl compound **67** is the most active (IC_{50} = 61 nM). The smaller 3-(furanyl)methyl substituent (derivative **68**) also appears to be well-accommodated in the N-1 binding pocket (IC_{50} = 27 nM). As in the case of the *N*-phenethyl derivative **56**, the two isomeric (thienyl)ethyl derivatives **69** and **70** are poor inhibitors of NS5B activity.

Table 4. Inhibition of HCV NS5B Polymerase Activity by C-5 Substituted Derivatives of 3-(1,1-Dioxo-2*H*-(1,2,4)-benzothiadiazin-3-yl)-4-hydroxy-2(1*H*)-quinolinones



entry	R^5	RdRp IC_{50} (nM)	compd	R^5	RdRp IC_{50} (nM)
2	H	32	**75**	CH_3	206
72	F	340	**76**	Ph	144
73	Cl	33	**77**	OH	47
74	Br	105	**78**	NO_2	338

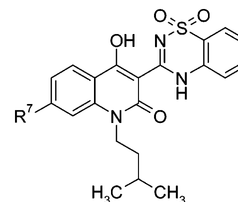
Table 5. Inhibition of HCV NS5B Polymerase Activity by C-6 Substituted Derivatives of 3-(1,1-Dioxo-2*H*-(1,2,4)-benzothiadiazin-3-yl)-4-hydroxy-2(1*H*)-quinolinones



entry	R^6	RdRp IC_{50} (nM)	entry	R^6	RdRp IC_{50} (nM)
2	H	32	**91**	OCH_2CN	34
79	F	20	**92**	$O(CH_2)_3CN$	4,328
80	Cl	43	**93**	$O(CH_2)_2OCH_3$	1,741
81	Br	205	**94**	OCH_2CONH_2	24
82	I	>10,000	**95**	$NHCH_2CO_2H$	21
83	CH_3	47	**96**	$NHCH_2CO_2Et$	115
84	NO_2	455	**97**[a]	$NH(CH_2)_2OH$	48
85	NH_2	62	**98**	$NHCOCH_3$	>10,000
86	OH	198	**99**	$NHCOCH_2CH(CH_3)_2$	6,503
87	OCH_3	23	**100**	$NHCOCH_2N(CH_3)_2$	5,805
88	CO_2Et	>10,000	**101**	NHCO-cyclopentyl	973
89	CO_2H	1,035	**102**	NHCOPh	4,235
90	OCH_2Ph	3,950			

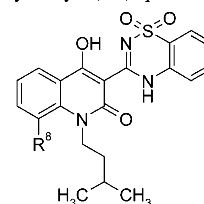
[a] (R^1 = 3,3-diMe-Bu).

Table 6. Inhibition of HCV NS5B Polymerase Activity by C-7 Substituted Derivatives of 3-(1,1-Dioxo-2*H*-(1,2,4)-benzothiadiazin-3-yl)-4-hydroxy-2(1*H*)-quinolinones



entry	R^7	RdRp IC_{50} (nM)	entry	R^7	RdRp IC_{50} (nM)
2	H	32	**106**	NO_2	>10,000
103	F	554	**107**	CO_2Et	>10,000
104	Cl	>10,000	**108**	CO_2H	>10,000
105	Br	>10,000	**109**	$CH{=}CHCONH_2$	>10,000

Table 7. Inhibition of HCV NS5B Polymerase Activity by C-8 Substituted Derivatives of 3-(1,1-Dioxo-2*H*-(1,2,4)-benzothiadiazin-3-yl)-4-hydroxy-2(1*H*)-quinolinones



entry	R^8	RdRp IC_{50} (nM)
2	H	32
110[a]	F	90
111	NO_2	>10,000

[a] (R^1 = 2-cyclopropylethyl).

The structure−activity relationships for varying the substituents on the quinolinone ring are also in good agreement with those predicted by analysis of an inhibitor-bound crystal structure (vide infra), wherein the tight fit of the quinolinone portion of the molecule into the binding pocket allows for substitution to be tolerated only at positions C-5 and C-6 (Figure 2b). Small- to medium-sized groups are tolerated at C-5, although little improvement in NS5B inhibition is observed with these derivatives (compounds **72**−**78**, Table 4). A wider range of substituents is tolerated at C-6, with most of the C-6 derivatized compounds demonstrating potent NS5B inhibitory activity (Table 5). Short linear chains bearing polar substituents are particularly active (i.e., entries **91**, **94**, **95**, and **97**, IC_{50} = 34, 24, 21, and 48 nM, respectively). Smaller groups (polar or nonpolar) are also quite potent. In addition to their outstanding enzymatic activities, the 6-fluoro (**79**, IC_{50} = 20 nM), 6-methyl (**83**, IC_{50} = 47 nM), and 6-amino (**85**, IC_{50} = 62 nM) analogues are also among the most active compounds with respect to their ability to inhibit cellular replication of subgenomic HCV RNA in Huh-7 cells (vide infra). 6-Amido substituents are poorly tolerated, as can be seen from the relatively poor activity of *N*-acyl analogues (**98**−**102**) when compared with the *N*-alkyl-substituted compounds **95**−**97**.

Substitution at C-7 is very poorly tolerated, as illustrated by the small set of analogues in Table 6 (compounds **103**−**109**). With the exception of fluorine derivative **103** (IC_{50} = 554 nM), all the other derivatives have an IC_{50} > 10,000 nM. Due to unfavorable steric interactions between C-8 and N-1, substitution at the C-8 position proved to be synthetically challenging; however, the two synthetically accessible derivatives **110** and **111** do not show any improvement in activity (Table 7).

Speculation over the importance of the hydrogen-bonding atoms in the pyridinone and thiadiazinedioxide portions of these heterocyclic inhibitors led to the investigation of a series of derivatives aimed at probing these interactions (Figure 1). Removal of either the 4-hydroxy group (compound **115**) or 2-oxo atom (compound **119**) or replacement of the 4-hydroxy group with a 4-amino group (compound **126**) affords compounds with very little inhibitory activity at the concentrations tested (IC_{50}s > 10,000 nM). Changing the 4-hydroxyquinolin-2-one heterocycle to a quinazolin-2,3-dione (compound **122**), which replaces the quinoline C-3 with nitrogen in order to lock the C-4 functionality into the keto tautomer, also affords a derivative with lower potency against NS5B (IC_{50} = 9,940 nM). Modification of the benzothiadiazine portion of the molecule, including excision of the sulfone completely to give a benzimidazole **127** or replacement with a carbonyl group to give a quinazolinone **128**, also results in poorly active NS5B inhibitors. Similarly, analogue **125**, possessing a methyl substitution on the benzothiadiazine 4-nitrogen atom, is approximately 75-fold less active (IC_{50} = 2,430 nM) than parent compound **2** (IC_{50} = 32 nM). It is therefore apparent that each of these transformations remove or change some critical binding element with the NS5B enzyme binding pocket, either by losing the capacity to bind to

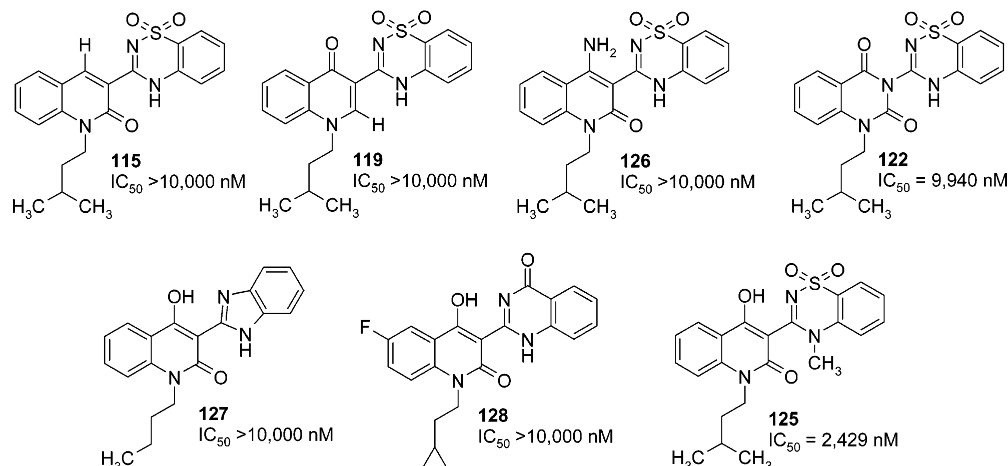


Figure 1. Core modifications.

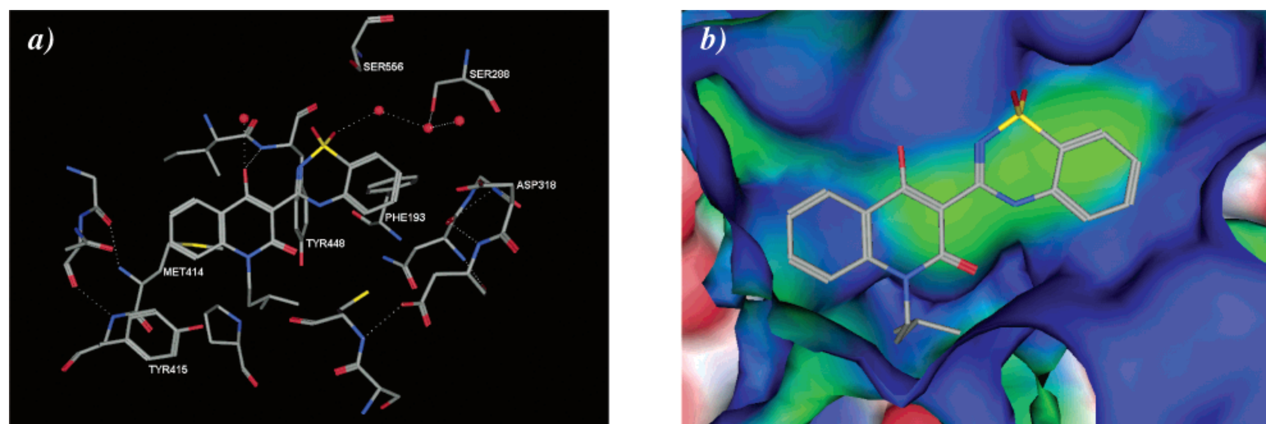


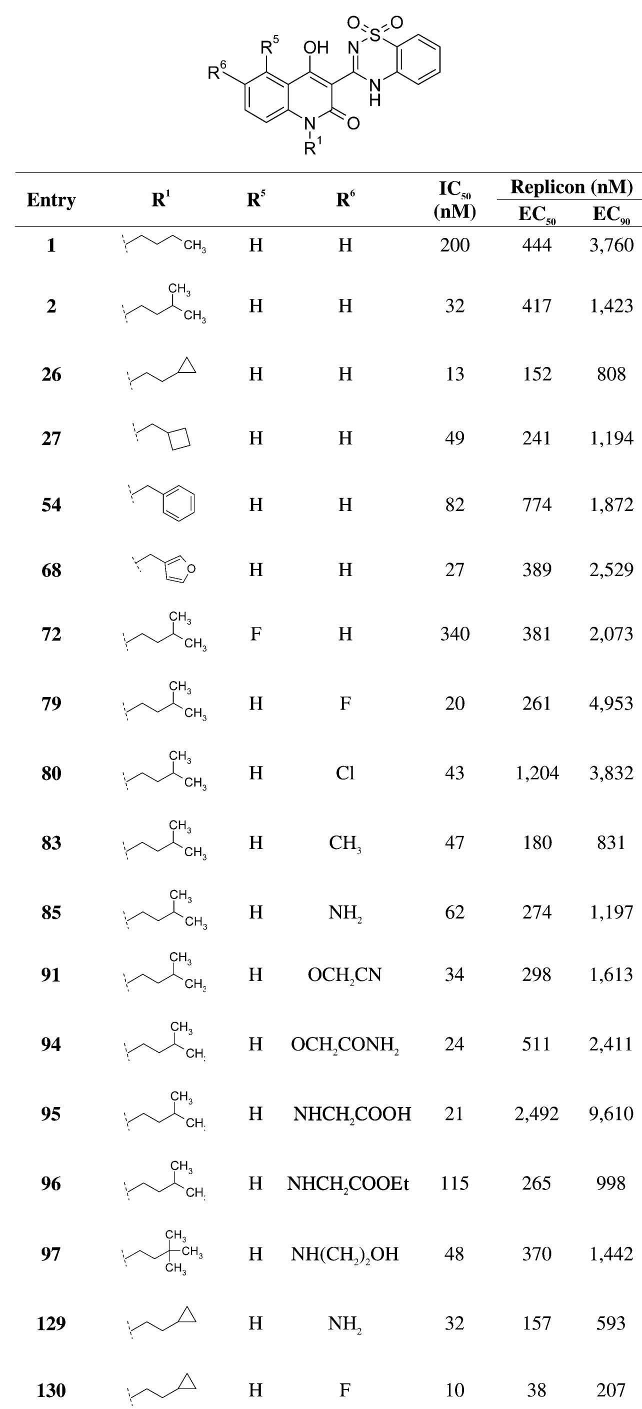
Figure 2. (a) Close up of the NS5B−compound **2** complex binding site. (b) Gaussian−Connolly surface for the protein binding site (red, exposed surface; green, hydrophobic; blue, hydrophilic).

water molecules present in the binding complex (vide infra) or by losing the degree of planarity (through intramolecular hydrogen bonding) necessary to stabilize the requisite binding conformation.

Inhibition of Subgenomic Replication in Huh-7 Cells. Since no direct antiviral cell culture system for HCV has been developed to date,[20] the ability of 3-(1,1-dioxo-2*H*-1,2,4-benzothiadiazin-3-yl)-4-hydroxy-2(1*H*)-quinolinones to inhibit HCV RNA replication intracellularly was evaluated in the subgenomic replicon system.[13] In this assay, carried out in Huh-7 cells, several compounds show low micromolar to low nanomolar inhibition of viral RNA replication. In general, biochemical potency correlates well with activity in the replicon assay, provided poor cell membrane permeability and/or decreased solubility under the assay conditions does not preclude the determination of an accurate cellular EC_{50}.

A list of selected compounds and their cellular EC_{50}s and EC_{90}s is shown in Table 8. Comparing EC_{50} values, the 1-(3-methyl)butyl analogue **2** and the 1-butyl derivative **1** possess very similar levels of inhibitory activity in the replicon assay, despite the 6-fold difference in their enzyme inhibition. The 1-(2-cyclopropyl)ethyl analogue **26** is approximately 3-fold more active than compounds **1** and **2** in the Replicon system. A small increase in potency of less than 2-fold over compounds **1** and **2** was observed with 1-(cyclobutyl)methyl substitution (Table 8; compound **27**). Substitution at the N-1 position with (aryl)-methyl groups (compounds **54** and **68**) was not as advantageous, resulting in poor inhibitors of cellular replication. The 5-fluoro substituted analogue **72** possesses cellular activity similar to that of the unsubstituted parent compound **2**, while addition of small substituents at the C-6 position generally results in equivalent or modestly improved potency in the Replicon assay; 6-fluoro (compound **79**), 6-methyl (compound **83**), and 6-amino (compound **85**) substitutions all demonstrate improved activity over unsubstituted compound **2**. Interestingly, substitution at the C-6 position with a variety of polar functionalities provides a series of generally potent cellular inhibitors, seemingly irrespective of the functionality present (compounds **91**, **94**, **95**, and **97**). The relatively poor correlation between enzyme inhibition and inhibition of cellular replication for such analogues could potentially be attributed to the relative abilities of the inhibitors to penetrate the cellular membrane of Huh-7 cells. For example, carboxylic acid **95** shows excellent levels of enzyme inhibition but an unexpectedly poor cellular effect (NS5B IC_{50} = 21 nM, cellular EC_{50} = 2,492 nM). This result is in contrast with that for the corresponding ethyl ester (compound **96**), which shows a more robust inhibition of cellular replication despite possessing a decreased ability to inhibit enzymatic activity (NS5B IC_{50} = 115 nM, cellular EC_{50} = 265 nM). It is most important to ascertain whether the improved potency effects of independently optimized substituents are additive or synergistic when incorporated into the same inhibitor. Indeed, for the series of 3-(1,1-dioxo-2*H*-1,2,4-benzothiadiazin-3-yl)-4-hydroxy-2(1*H*)-quinolinones under investigation, this is the case, as can be seen from Table 8; compound **129** and, more remarkably, compound **130**, in which the combination of a preferred C-6 substituent (fluoro) with the N-1 (2-cyclopropyl)ethyl moiety results in an inhibitor

Table 8. Huh-7 Replicon Cell Data for Selected Compounds



Entry	R^1	R^5	R^6	IC_{50} (nM)	Replicon (nM) EC_{50}	Replicon (nM) EC_{90}
1		H	H	200	444	3,760
2		H	H	32	417	1,423
26		H	H	13	152	808
27		H	H	49	241	1,194
54		H	H	82	774	1,872
68		H	H	27	389	2,529
72		F	H	340	381	2,073
79		H	F	20	261	4,953
80		H	Cl	43	1,204	3,832
83		H	CH_3	47	180	831
85		H	NH_2	62	274	1,197
91		H	OCH_2CN	34	298	1,613
94		H	OCH_2CONH_2	24	511	2,411
95		H	$NHCH_2COOH$	21	2,492	9,610
96		H	$NHCH_2COOEt$	115	265	998
97		H	$NH(CH_2)_2OH$	48	370	1,442
129		H	NH_2	32	157	593
130		H	F	10	38	207

with excellent enzymatic and cellular potencies (NS5B IC_{50} = 10 nM, cellular EC_{50} = 38 nM).

Crystal Structure of 2 Bound to NS5B. The cocrystal structures of NS5B (BK strain, Δ21 construct) with a number of inhibitors from the 3-(1,1-dioxo-2*H*-1,2,4-benzothiadiazin-3-yl)-4-hydroxy-2(1*H*)-quinolinone class have been determined. In general, these inhibitors fit tightly into a single, high occupancy site in the palm/thumb domain interface.[10] A close up of the NS5B/compound **2** complex is shown in Figure 2a,b, from which it can be appreciated how, due to the tight fit, only certain positions on the inhibitor are open for further substitution. Specifically, the C-5 and C-6 positions of the quinolinone ring, consistent with the observed SAR (vide supra), along with the C-7 and C-8 positions of the benzothiadiazine ring (the full exploration of these latter positions will be described in a future publication) are amenable to substitution. This inhibitor binding site is outlined by residues from the palm, finger, and thumb domains and resides in a region distinct from other reported non-nucleoside allosteric inhibitors that bind mainly in the thumb domain.[7] Also shown in Figure 2a are some highly conserved, tightly bound water molecules, mediating the hydrogen-bond interactions of the inhibitor with the protein, consistently present in all the structures determined. Another key feature observed in all of the structures determined is an apparent edge-to-face π-interaction between Phe193 and the benzo portion of the benzothiadiazine ring system. Although a small molecule X-ray crystal structure of compound **2** had shown coplanarity between the quinolone and benzothiadiazine rings (data not shown), due primarily to internal hydrogen bonds, experimental electron density indicates that the inhibitor molecule is distorted away from planarity when bound to NS5B.

Table 9. Selectivity Data for 130

biochemical assay	IC_{50} (nM)
NS5B type 1a	49
NS5B type 1b	10
NS5B type 2a	13
NS5B type 3a	60% inhibn @10 μM
GBV-B	>50,000
DNA pol-α	>50,000
DNA pol-β	>50,000

Activity against Other HCV Genotypes and Other Polymerases. Hepatitis C virus is a rapidly mutating virus and has been classified into six major genotypes (1−6) with numerous subtypes. Testing of one of our most potent inhibitors, 1-(2-cyclopropylethyl)-3-(1,1-dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-3-yl)-6-fluoro-4-hydroxy-1-quinolin-2-one (**130**), against a selection of NS5B genotypes demonstrates that compound **130** is a potent inhibitor of genotypes 1a, 1b, and 2a (Table 9), while inhibition of NS5B from genotype 3a is greatly attenuated. The reasons for the attenuation of activity against genotype 3a are unclear at this time. Analysis of the sequences of several type 3a polymerases reveals no obvious mutations in close proximity to the observed binding site for our benzothiadiazine inhibitors. Compound **130** is also highly selective for HCV NS5B versus other viral polymerases (e.g., the closely related GBV-B, IC_{50} > 50,000 nM) and against human DNA polymerases (IC_{50} > 50,000 nM) (Table 9).

Animal Pharmacokinetics and Safety Assessment. To establish the suitability of our benzothiadiazine inhibitors as potential clinical candidates, the pharmacokinetic profiles of selected compounds were determined in Sprague−Dawley rats, beagle dogs, and cynomolgous monkeys. The results of these experiments are summarized in Table 10. All four benzothiadiazine inhibitors have moderate plasma half-lives, low plasma clearances, and very good oral bioavailability (≥35%) in all three species. All of the benzothiadiazines tested have relatively low volumes of distribution along with high binding to human plasma proteins (generally >99%). These factors raise concerns over how well the compounds will distribute into the liver tissues, the site of action for the targeted disease. Analyses of liver tissue drug concentrations following oral administration in rats indicate that the compounds distribute well into hepatic tissue, with ≥2−5-fold liver tissue to plasma concentration ratios for the four compounds tested.

Due to the favorable combination of high oral bioavailability and low intrinsic clearance, compound **130** shows high exposure upon oral suspension dosing in rats, dogs, and monkeys [oral DNAUC (μg·h/mL/mg/kg) = 5.3, 20, and 23.5, respectively].

Table 10. Mean Pharmacokinetic Parameter Values for Compound **2**, **26**, **83**, and **130** Following Single Dose Administration to Rats, Dogs, and Monkeys

entry	species	dose iv/po (mg/kg)	C_{max} (ng/mL)	$T_{1/2}$ (min)	CL (mL/min/kg)	Vdss (L/kg)	oral *F* (%)
2	rat	0.8/5.8	1,096 ± 484	36.4 ± 7.9	10.1 ± 3.2	0.44 ± 0.01	70 ± 7
	dog	0.8/1.8	2,230	859	0.50	0.53	89
26	rat	1.3/7.7	6,460 ± 1231	112.4 ± 9.9	1.7 ± 0.3	0.25 ± 0.05	78 ± 19
	dog	0.9/1.1	5,398	289	0.47	0.15	∼100
	monkey	1.8/1.6	7,917	81	1.73	0.19	>40
83	rat	1.2/3.7	1,217 ± 251	317.5 ± 69.7	1.65 ± 0.50	0.81 ± 0.11	∼100
	dog	0.9/3.6	1,958 ± 261	543.5 ± 290.1	1.09 ± 0.30	0.72 ± 0.28	>50
	monkey	1.0/3.6	2,136 ± 471	49.7 ± 10.8	5.32 ± 1.60	0.34 ± 0.09	>53
130	rat	1.3/3.4	4,141 ± 980	182.2 ± 7.5	1.4 ± 0.4	0.36 ± 0.10	44 ± 11
	dog	0.8/4.0	4,455 ± 565	103.5 ± 7.2	0.86 ± 0.05	0.15 ± 0.01	>35
	monkey	0.8/4.0	5,437 ± 1568	96.9 ± 0.7	0.76 ± 0.2	0.11 ± 0.03	>53

Table 11. Protein Binding Studies in Huh-7 Replicon Cell Assay for Compound 130

cellular assay	EC_{50} (nM)
replicon (type 1b)	38
replicon + 45 mg/mL HSA	13,000
replicon + 1 mg/mL AAG	180
replicon + 45 mg/mL HSA, 1 mg/mL AAG	>20,000

Further profiling of compound **130** reveals that it has no major CYP450 liabilities with the exception of modest potency at inhibiting the 2C9 isozyme ($IC_{50} \sim 1\ \mu M$) and a modest potential for 3A4 induction (PXR $EC_{50} = 5.3\ \mu M$).[21] The clinical relevance of these findings is not clear at this point, but such activities do not preclude advancing compound **130** toward clinical studies. Furthermore, no macroscopic adverse events manifested themselves in a 4-day rat toxicology study at doses up to 300 mg/kg/day in both male and female rats. Importantly, dose proportional increases in AUC are observed, confirming high levels of exposure.

In the absence of a readily accessible animal model, the effects of the high affinity for human plasma proteins on the cellular activity of compound **130** can be tested in the replicon assay by the addition of α-acid glycoprotein (AAG), human serum albumin (HSA), or the combination of both. In the presence of 1 mg/mL AAG, the EC_{50} for compound **130** increases by only 4−5-fold, but the presence of 45 mg/mL HSA reduces the activity of **130** dramatically (Table 11). The relevance of this HSA-mediated attenuation of potency in the replicon system and the impact of protein binding on the in vivo efficacy of compound **130** are, as yet, unknown. It is well-known that high affinity for plasma proteins can dramatically reduce the effectiveness of, for example, HIV antiretroviral agents;[22] however a similar correlation has not yet been established for HCV. It is encouraging, however, as discussed above, that compound **130** preferentially distributes into liver tissues following repeated oral dosing in the rat, despite its high protein binding. Nonetheless, addressing the effects of protein binding on compound potency may be critical to the successful discovery of clinically effective benzothiadiazine non-nucleoside HCV NS5B inhibitors, and work toward this goal will be the subject of future publications.

Conclusions

A potent class of HCV NS5B polymerase inhibitors has been discovered through high throughput screening of the GSK compound collection. Preliminary SAR studies around this scaffold culminated in the discovery of compound **130**, which was ultimately progressed into preclinical development. This class of compounds represents an example of non-nucleoside inhibitors in which excellent RdRp inhibition has translated in low-nanomolar replicon activity. The lack of a practical animal model for HCV infection, however, does not allow speculation about the relevance of the replicon system as a means to discover potent clinical HCV polymerase inhibitors, and only clinical data will provide insight on the matter.

Experimental Section

Chemistry. General Methods. Starting materials were either commercially available or prepared as reported previously in the literature, unless otherwise noted. Solvents and reagents were used without further purification, except THF, which was distilled from sodium/benzophenone. Reactions were monitored by TLC, performed on silica gel glass plates containing F-254 indicator (Kieselgel 60 F_{254}, Merck or Uniplate Silica Gel GF, Analtech). Visualization on TLC was achieved by UV light, potassium permanganate, or iodine indicator. Column chromatography was performed with Merck 40−63 mesh silica gel. Reaction temperatures refer to the oil bath or cold bath temperature. ^1H NMR spectra were recorded on a Bruker ARX-300 or Avance-400 instrument. Chemical shifts (δ) are reported in ppm downfield from internal TMS standard or from solvent references. Mass spectra were recorded on a ScieX API 150EX electrospray LC−mass spectrometer. Melting points were determined on a Melt-Temp apparatus and are uncorrected. Elemental analyses were performed by QTI Technologies, Inc., Whitehouse, NJ. Compound **127** was purchased from AsInEx.

General Method for the Alkylation of Isatoic Anhydride. Method A: 1-(3-Methylbutyl)-2*H*-3,1-benzoxazine-2,4(1*H*)-dione [7, R = $(CH_2)_2CH(CH_3)_2$]. Sodium hydride (6.4 g of a 60% suspension in mineral oil, 159.4 mmol) was washed with hexanes, dried under a N_2 stream, and suspended in dimethylacetamide (240 mL). Isatoic anhydride (20.0 g, 122.6 mmol) was then added portionwise, followed by *i*-amyl iodide (24.0 mL, 183.9 mmol). The reaction mixture was then stirred for 20 h at room temperature, poured into ice/water, and stirred for 40 min. The precipitate which formed was collected, washed with water, and dried in a vacuum oven to give the title compound as a tan powder (25.0 g; 58%): ^1H NMR ($CDCl_3$) δ 8.17 (dd, *J* = 7.9, 1.6 Hz, 1H), 7.76 (ddd, *J* = 8.4, 7.4, 1.6 Hz, 1H), 7.29 (t, *J* = 7.5 Hz, 1H), 7.15 (d, *J* = 8.4 Hz, 1H), 4.09−4.02 (m, 2H), 1.76 (sept, *J* = 6.6 Hz, 1H), 1.67−1.61 (m, 2H), 1.03 (d, *J* = 6.6 Hz, 6H).

Method B: 1-(3,3-Dimethylbutyl)-2*H*-3,1-benzoxazine-2,4-(1*H*)-dione [7, R = $(CH_2)_2C(CH_3)_3$]. Diisopropyl azodicarboxylate (0.663 mL, 3.37 mmol) was added dropwise to a solution of isatoic anhydride (500 mg, 3.06 mmol), 3,3-dimethylbutan-1-ol (0.408 mL, 3.37 mmol), and Ph_3P (883 mg, 3.37 mmol) in CH_2Cl_2 (10.0 mL). The resulting mixture was stirred overnight at room temperature, concentrated under reduced pressure, and purified by flash chromatography (hexanes/ethyl acetate 75:25) to give the title compound (336 mg, 44%) as a colorless powder: ^1H NMR ($CDCl_3$) δ 8.17 (dd, *J* = 7.8, 1.6 Hz, 1H), 7.77 (ddd, *J* = 8.54, 7.4, 1.6 Hz, 1H), 7.32 (t, *J* = 7.4 Hz, 1H), 7.15 (d, *J* = 8.4 Hz, 1H), 4.13−4.05 (m, 2H), 1.68−1.621 (m, 2H), 1.07 (s, 9H).

Ethyl (1,1-Dioxido-2*H*-1,2,4-benzothiadiazin-3-yl)acetate (9). Ester **9** was prepared according to the literature procedure reported

by Kovalenko et al.[16] Ethyl 3-chloro-3-oxopropanoate (22.5 mL, 175.4 mmol) was added dropwise to a solution of 2-aminobenzenesulfonamide (30.2 g, 175.4 mmol) and pyridine (14.2 mL, 175.4 mmol) in CH_2Cl_2 (200 mL) at 0 °C. The reaction mixture was stirred overnight at room temperature, the solvent evaporated under reduced pressure, and the residue partitioned between 1 M aqueous hydrochloric acid and EtOAc. The separated organic layer was washed with water and then brine and dried over Na_2SO_4 and the solvent evaporated under reduced pressure. The residue was triturated in hexane/EtOAc 1:1 to give ethyl 3-{[2-(aminosulfonyl)-phenyl]amino}-3-oxopropanoate (31.0 g; 61%) as colorless needles.

Ethyl 3-{[2-(aminosulfonyl)phenyl]amino}-3-oxopropanoate (30.0 g, 104.8 mmol) was suspended in 10% aqueous sodium carbonate (900 mL) and the suspension warmed to 45 °C until all solid dissolved (**Caution:** *maintain internal temperature below 40 °C*) and then further stirred at room temperature for 40 min. The reaction mixture was then cooled in an ice bath, and 6 N HCl was slowly added until pH ~7. After cooling the suspension for 2 h the precipitate which formed was collected by filtration, washed with water, and dried under vacuum to give compound **9** (13.3 g; 47%) as a colorless powder: ^{1}H NMR (DMSO-d_6) δ 12.27 (br s, 1H), 7.82 (dd, J = 8.0, 1.3 Hz, 1H), 7.69 (td, J = 8.1, 1.3 Hz, 1H), 7.47 (td, J = 8.1, 1.0 Hz, 1H), 7.33 (d, J = 7.9 Hz, 1H), 4.17 (q, J = 7.1 Hz, 2H), 3.71 (s, 2H), 1.22 (t, J = 7.1 Hz, 3H); MS (ES+) *m/e* 269 $[M + H]^+$.

General Method for the Condensation of Isatoic Anhydride with Ester 9. Method C: 3-(1,1-Dioxo-1,4-dihydrobenzo[1,2,4]-thiadiazin-3-yl)-4-hydroxy-1-(3-methylbutyl)-1*H*-quinolin-2-one (2). Sodium hydride (687 mg of a 60% suspension, 17.2 mmol) was added to a suspension of 1-(3-methylbutyl)-2*H*-3,1-benzoxazine-2,4(1*H*)-dione (1.0 g, 4.29 mmol) and ester **9** (1.15 g, 4.29 mmol) in anhydrous THF (50.0 mL). The reaction mixture was then heated under reflux for 1.5 h, cooled, treated with glacial AcOH (1.5 mL), heated under reflux for 1 h, cooled, and then poured into 1 M aqueous hydrochloric acid. The precipitate that formed was collected by filtration and recrystallized from ethyl acetate. The solid was collected, washed with Et_2O, and dried in a vacum oven to give 455 mg (26%) of **2** as pale yellow crystals: ^{1}H NMR ($CDCl_3$) δ 15.21 (br s, 1H), 14.58 (br s, 1H), 8.29 (dd, J = 8.1, 1.5 Hz, 1H), 7.99 (dd, J = 8.0, 0.7 Hz, 1H), 7.74 (ddd, J = 8.6, 7.2, 1.5 Hz, 1H), 7.63 (td, J = 8.2, 1.5 Hz, 1H), 7.48−7.27 (m, 4H), 4.35−4.30 (m, 2H), 1.83 (sept, J = 6.6 Hz, 1H), 1.67−1.61 (m, 2H), 1.08 (d, J = 6.5 Hz, 6H); MS (ES+) *m/e* 412 $[M + H]^+$. Anal. ($C_{21}H_{21}N_3O_4S$·0.25 EtOAc) C, H, N.

3-(1,1-Dioxo-1,4-dihydro-benzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-1-methyl-1*H*-quinolin-2-one (11). Following the procedures from methods A and C, compound **11** (319 mg, 23%) was obtained as a colorless solid as the monosodium salt after recrystallization from ethanol/1M aqueous sodium hydroxide: ^{1}H NMR (DMSO-d_6) δ 16.3 (s, 1H), 8.12 (dd, J = 7.8, 1.5 Hz, 1H), 7.66 (dd, J = 7.8, 1.3 Hz, 1H), 7.58−7.52 (m, 2H), 7.31−7.26 (m, 3H), 7.12 (td, J = 7.0, 1.0 Hz, 1H), 3.49 (s, 3H).

3-(1,1-Dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-3-yl)-1-ethyl-4-hydroxy-1*H*-quinolin-2-one (12). Following the procedures from methods A and C, except substituting iodoethane for *i*-amyl iodide, compound **12** (323 mg, 67%) was obtained as a colorless solid after recrystallization from AcOH: ^{1}H NMR ($CDCl_3$) δ 15.23 (br s, 1H), 14.58 (br s, 1H), 8.31 (dd, J = 8.1, 1.5 Hz, 1H), 8.00 (d, J = 8.0 Hz, 1H), 7.76 (ddd, J = 8.6, 7.1, 1.6 Hz, 1H), 7.63 (ddd, J = 8.1, 7.5, 1.4 Hz, 1H), 7.47−7.43 (m, 2H), 7.37 (td, J = 8.0, 0.8 Hz, 1H), 7.30 (dd, J = 8.2, 0.5 Hz, 1H), 4.40 (q, J = 7.1 Hz, 2H), 1.41 (t, J = 7.1 Hz, 3H); MS (ES+) *m/e* 370 $[M + H]^+$. Anal. ($C_{18}H_{15}N_3O_4S$·0.25AcOH) C, H, N.

3-(1,1-Dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-1-propyl-1*H*-quinolin-2-one (13). Following the procedures from methods A and C, except substituting *n*-propyl iodide for *i*-amyl iodide, compound **13** (225 mg, 60%) was obtained as a pale yellow powder after washing with water, diethyl ether, and hexanes, sequentially: ^{1}H NMR ($CDCl_3$) δ 15.23 (br s, 1H), 14.58 (br s, 1H), 8.30 (dd, J = 8.1, 1.5 Hz, 1H), 7.99 (dd, J = 7.8, 1.3 Hz, 1H), 7.74 (ddd, J = 8.5, 7.2, 1.5 Hz, 1H), 7.63 (ddd, J = 8.1, 7.5, 1.5 Hz, 1H), 7.45(td, J = 8.1, 0.9 Hz, 1H), 7.41 (d, J = 8.6 Hz, 1H), 7.36 (td, J = 8.1, 0.8 Hz, 1H), 7.31 (d, J = 8.1 Hz, 1H), 4.28 (t, J = 7.9 Hz, 2H), 1.81 (sext, J = 7.6 Hz, 2H), 1.09 (t, J = 7.4 Hz, 3H); MS (ES+) *m/e* 384 $[M + H]^+$. Anal. Calcd for $C_{19}H_{17}N_3O_4S$: C, 59.52; H, 4.47; N, 10.96. Found: C, 60.01; H, 4.09; N, 10.88.

3-(1,1-Dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-1-(2-methylpropyl)-1*H*-quinolin-2-one (14). Following the procedures from methods A and C, except substituting 1-bromo-2-methylpropane for *i*-amyl iodide, the title compound **14** (100 mg, 28%) was obtained as tan crystals after washing with water and then hexanes and trituration with ethyl acetate: ^{1}H NMR ($CDCl_3$) δ 15.28 (br s, 1H), 14.62 (br s, 1H), 8.31 (dd, J = 8.1, 1.6 Hz, 1H), 7.99 (dd, J = 8.0, 1.3 Hz, 1H), 7.74 (ddd, J = 8.7, 7.1, 1.6 Hz, 1H), 7.63 (td, J = 8.1, 1.5 Hz, 1H), 7.48−7.34 (m, 4H), 4.23−4.20 (m, 2H), 2.26 (m, J = 6.9 Hz, 1H), 1.02 (d, J = 6.8 Hz, 6H); MS (ES+) *m/e* 398 $[M + H]^+$. Anal. ($C_{20}H_{19}N_3O_4S$) C, H, N.

1-(3,3-Dimethylbutyl)-3-(1,1-dioxo-1,4-dihydrobenzo[1,2,4]-thiadiazin-3-yl)-4-hydroxy-1*H*-quinolin-2-one (15). Following the procedures of methods B and C, the title compound **15** (211 mg, 61%) was obtained as a colorless solid after trituration with diethyl ether: ^{1}H NMR (DMSO-d_6) δ 15.18 (br s, 1H), 14.29 (s, 1H), 8.19 (dd, J = 8.1, 1.3 Hz, 1H), 7.94 (d, J = 7.8 Hz, 1H), 792−7.68 (m, 4H), 7.59−7.54 (m, 1H), 7.44 (t, J = 7.6 Hz, 1H), 4.36−4.31 (m, 2H), 1.59−1.53 (m, 2H), 1.07 (s, 9H); MS (ES+) *m/e* 426 $[M + H]^+$. Anal. ($C_{22}H_{23}N_3O_4S$) C, H, N.

1-(2-Cyclopropylethyl)-3-(1,1-dioxo-1,4-dihydro-1-benzo[1,2,4]-thiadiazin-3-yl)-4-hydroxy-1*H*-quinolin-2-one (26). Following the procedures of methods B and C, except substituting 2-cyclopropylethanol for 3,3-dimethylbutan-1-ol, the title compound **26** (206 mg, 58%)was obtained as a colorless solid: ^{1}H NMR ($CDCl_3$) δ 15.24 (s, 1H), 14.60 (s, 1H), 8.31 (dd, J = 8.1, 1.4 Hz, 1H), 8.01 (dd, J = 8.0, 0.6 Hz, 1H), 7.75 (ddd, J = 8.6, 7.2, 1.6 Hz, 1H), 766−7.61 (m, 1H), 7.48−7.31 (m, 4H), 4.43 (t, J = 7.7 Hz, 2H), 1.68 (q, J = 7.6 Hz, 2H), 0.84−0.79 (m, 1H), 0.55−0.49 (m, 2H), 0.17−0.12 (m, 2H); MS (ES+) *m/e* 410 $[M + H]^+$. Anal. ($C_{21}H_{19}N_3O_4S$) C, H, N.

5-Chloro-3-(1,1-dioxo-1,2-dihydrobenzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-1-(3-methylbutyl)-1*H*-quinolin-2-one (73). A solution of 6-chloroanthranilic acid (1.15 g, 6.73 mmol) in tetrahydrofuran (20.0 mL) was treated with triphosgene (1.0 g, 3.3 mmol) and stirred at 50 °C overnight. Saturated sodium hydrogen carbonate solution was added and the mixture extracted with ethyl acetate. Evaporation of the organic solution gave 5-chlorobenzo[*d*][1,3]oxazine-2,4-dione (1.25 g, 94%): ^{1}H NMR (DMSO-d_6) δ 11.85 (s, 1H), 7.65 (t, J = 8.0 Hz, 1H), 7.30 (d, J = 8.0 Hz, 1H), 7.10 (d, J = 9.0 Hz, 1H).

5-Chlorobenzo[*d*][1,3]oxazine-2,4-dione (517 mg, 2.6 mmol), triphenylphosphine (688 mg, 2.6 mmol), and 3-methylbutanol (0.3 mL, 2.75 mmol) were stirred together in dichloromethane and treated with diethyl azodicarboxylate (0.42 mL, 2.6 mmol). The reaction was stirred under a nitrogen atmosphere overnight, evaporated onto silica, and purified by chromatography (silica gel, ethyl acetate/hexanes) to give 1-(3-methylbutyl)-5-chlorobenzo[*d*]-[1,3]oxazine-2,4-dione (440 mg, 63%): ^{1}H NMR ($CDCl_3$) δ 7.62 (t, J = 8.0 Hz, 1H), 7.33 (d, J = 8.0 Hz, 1H), 7.08 (d, J = 9.0 Hz, 1H), 4.06 (m, 2H), 1.78 (m, 1H), 1.61 (m, 2H) 1.02 (d, J = 6.5 Hz, 6H).

Sodium hydride (263 mg of a 60% suspension in mineral oil, 6.56 mmol) was added to a mixture of 1-(3-methylbutyl)-5-chlorobenzo[*d*][1,3]oxazine-2,4-dione (440 mg, 1.64 mmol) and ester **9** (440 mg, 1.64 mmol) in tetrahydrofuran (20.0 mL). The mixture was heated under reflux for 1.5 h, cooled, and acidified with acetic acid. The mixture was then heated under reflux for an additional 1.5 h and cooled and water was added. The product was collected and washed with water, diethyl ether, and hexanes to give the title compound **73** (520 mg, 71%): ^{1}H NMR (DMSO-d_6) δ 16.10 (br s, 1H), 14.43 (s, 1H), 7.94 (d, J = 8.0 Hz, 1H), 7.57−7.82 (m, 5H), 7.48 (d, J = 8.0 Hz, 1H), 4.33 (m, 2H), 1.80 (m, 1H), 1.53 (m, 2H) 1.00 (d, J = 7 Hz, 6H). Anal. ($C_{21}H_{20}ClN_3O_4S$) C, H, N.

5-Bromo-3-(1,1-dioxo-1,2-dihydrobenzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-1-(3-methylbutyl)-1*H*-quinolin-2-one (74). A solution of a 1:1 mixture of 4-bromoindole-2,3-dione and 6-bromoindole-2,3-dione (prepared by the method of Katsifis and McPhee[24]) (3.7 g; 0.016 mol.) in glacial acetic acid (25.0 mL) was treated with 30% aqueous hydrogen peroxide solution (5.0 mL). The suspension was then stirred at 50 °C for 5 h. The mixture was concentrated and the residue was washed with diethyl ether to give a mixture of 7-bromo-2*H*-3,1-benzoxazine-2,4(1*H*)-dione (~17% measured by ^{1}H NMR) and 5-bromo-2*H*-3,1-benzoxazine-2,4(1*H*)-dione (~83% measured by ^{1}H NMR) (4.0 g, yield 100%) as a yellow solid: MS (ES+) *m*/*e* 244, 242 $[M + H]^+$.

A solution of triphenylphosphine (1.24 g, 4.7 mmol) and 3-methylbutanol (0.47 mL, 4.3 mmol) was treated with the mixture obtained in the previous step (1.1 g; 4.5 mmol) and then treated dropwise with diisopropyl azodicarboxylate (1.0 mL, 4.8 mmol). The solution was stirred at room temperature and then concentrated to give a gum. The gum was purified by chromatography (silica gel, 10% ethyl acetate/hexanes) to give 5-bromo-2*H*-3,1-benzoxazine-1-(3-methylbutyl)-2,4-dione (0.57 g; 41%) {^{1}H NMR ($CDCl_3$) δ 7.6 (dd, 1H), 7.5 (t, 1H), 7.1 (dd, 1H), 4.1 (m, 2H), 1.8 (m, 1H), 1.7 (m, 2H), 1.0 (d, 6H); MS (ES+) *m*/*e* 314, 312 $[M + H]^+$} followed by 7-bromo-2*H*-3,1-benzoxazine-1-(3-methylbutyl)-2,4-dione (0.10 g; 7%) {^{1}H NMR (300 MHz, $CDCl_3$) δ 8.0 (d, 1H), 7.4 (dd, 1H), 7.3 (d, 1H), 4.6 (m, 2H), 1.8 (m, 3H), 1.0 (d, 6H); MS (ES+) *m*/*e* 314, 312 $[M + H]^+$}.

A solution of 1-(3-methylbutyl)-5-bromobenzo[*d*][1,3] oxazine-2,4-dione (200 mg, 0.6 mmol) and ethyl (1,1-dioxo-1,2-dihydrobenzo-[1,2,4]thiadiazin-3-yl)acetate (172 mg, 0.6 mmol) in tetrahydrofuran (15.0 mL) was treated with sodium hydride (60% dispersion in mineral oil) (100 mg; 2.6 mmol) and heated under reflux for 1.5 h. The mixture was then cooled to room temperature and acidified with excess glacial acetic acid. The mixture was then heated under reflux for an additional 1.5 h, cooled, and quenched with water. The product was collected and washed with water, ether, and then hexanes to give **74** (180 mg; 60%) as colorless powder: ^{1}H NMR (DMSO-d_6) δ 8.0 (d, $J = 7.5$ Hz, 1H), 7.8 (t, $J = 8.0$ Hz, 1H), 7.7 (m, 4H), 7.6 (t, $J = 8.0$ Hz, 1H), 4.4 (m, 2H), 1.8 (m, 1H), 1.5 (m, 2H), 1.0 (d, $J = 7.0$ Hz, 6H).

3-(1,1-Dioxo-1,2-dihydrobenzo[1,2,4]thiadiazin-3-yl)-6-fluoro-4-hydroxy-1-(3-methylbutyl)-1*H*-quinolin-2-one (79). A solution of 5-fluoroanthranilic acid (1.0 g, 6.44 mmol) in tetrahydrofuran (20.0 mL) was treated with a 20% solution of phosgene in toluene (4.0 mL) and stirred overnight. Saturated sodium hydrogen carbonate solution was added and the mixture extracted with ethyl acetate. The organic layer was evaporated and the residue crystallized from diethyl ether to afford 6-fluorobenzo[*d*][1,3]oxazine-2,4-dione (960 mg, 82%): ^{1}H NMR ($CDCl_3$) δ 11.81 (s, *N*H), 7.70 (m, 2H), 7.67 (m, 1H).

6-Fluorobenzo[*d*][1,3]oxazine-2,4-dione (940 mg, 5.2 mmol) was added portionwise to a suspension of sodium hydride (60% suspension in mineral oil) (240 mg, 6.0 mmol) in anhydrous *N,N*-dimethylformamide. After 30 min, 1-bromo-3-methylbutane (0.9 mL, 7.1 mmol) was added and the mixture was stirred at 80 °C for 16 h. The mixture was poured onto ice, acidified with glacial acetic acid, and extracted with ethyl acetate. The organic layer was evaporated and the residue crystallized from hexanes to afford 1-(3-methylbutyl)-6-fluorobenzo[*d*][1,3]oxazine-2,4-dione (380 mg, 23%): ^{1}H NMR (DMSO-d_6) δ 7.72−7.81 (m, 2H), 7.47−7.51 (m, 1H), 4.02 (m, 2H) 1.73 (m, 1H), 1.54 (m, 2H) 0.96 (d, $J = 6.5$ Hz, 6H).

Sodium hydride (130 mg of a 60% suspension in mineral oil, 3.25 mmol) was added to a mixture of 1-(3-methylbutyl)-6-fluorobenzo[*d*][1,3]oxazine-2,4-dione (250 mg, 0.8 mmol) and ethyl 1,1-dioxo-2*H*-benzo-1,2,4-thiadiazinyl-3-acetate (215 mg, 0.8 mmol) in tetrahydrofuran (15.0 mL). The mixture was heated under reflux for 1.5 h, cooled, and acidified with glacial acetic acid. The mixture was then heated under reflux for an additional 1.5 h and cooled and water was added. The product was collected and washed with water, diethyl ether, and hexanes to give compound **79** (220 mg, 64%): ^{1}H NMR (DMSO-d_6) δ 15.10 (br s, 1H), 14.21 (s, 1H), 7.93 (d, $J = 7$ Hz, 1H), 7.59−7.54 (m, 5H) 7.55 (dd, 1H), 4.29 (m, 2H),1.78 (m, 1H), 1.52 (m, 2H), 0.99 (d, $J = 6.5$ Hz, 6H). Anal. ($C_{21}H_{20}FN_3O_4S$) C, H, N.

6-Chloro-3-(1,1-dioxo-1,2-dihydrobenzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-1-(3-methylbutyl)-1*H*-quinolin-2-one (80). 6-Chloro-1*H*-benzo[*d*][1,3]oxazine-2,4-dione (1.27 g, 6.43 mmol) was added portionwise to a suspension of sodium hydride (60% suspension in mineral oil) (284 mg, 7.1 mmol) in anhydrous *N,N*-dimethylformamide. After 15 min, 1-bromo-3-methylbutane (0.9 mL, 7.1 mmol) was added and the mixture was stirred at 80 °C for 16 h. The mixture was poured onto ice, acidified with glacial acetic acid, and extracted with ethyl acetate. Purification using flash chromatography (20% ethyl acetate in hexanes) gave 6-chloro-1-(3-methylbutyl)benzo[*d*][1,3]oxazine-2,4-dione (1.0 g, 58%): ^{1}H NMR ($CDCl_3$) δ 8.35 (d, 1H), 7.85 (dd, 1H), 7.04 (d, 1H), 4.04 (m, 2H), 1.72 (m, 1H), 1.60 (m, 2H), 1.02 (d, $J = 6.5$ Hz, 6H).

Sodium hydride (165 mg of a 60% suspension in mineral oil, 4.0 mmol) was added to a mixture of 6-chloro-1-(3-methylbutyl)-benzo[*d*][1,3]oxazine-2,4-dione (267 mg, 1.0 mmol) and ethyl 1,1-dioxo-2*H*-benzo-1,2,4-thiadiazinyl-3-acetate (270 mg, 1.0 mmol) in tetrahydrofuran (15.0 mL). The mixture was heated under reflux for 1.5 h, cooled, and acidified with glacial acetic acid. The mixture was then heated under reflux for an additional 1.5 h and cooled and water was added. The product was collected and washed with water, diethyl ether, and hexanes to give compound **80** (180 mg, 41%): ^{1}H NMR (DMSO-d_6) δ 15.94 (s, 1H), 8.06 (d, $J = 3.0$ Hz, 1H), 7.68 (dd, $J = 8.0, 1.0$ Hz, 1H), 7.54−7.59 (m, 2H), 7.26−7.32 (m, 3H), 4.01 (m, 2H), 1.7 (m, 1H), 1.45 (m, 2H), 0.98 (d, $J = 6.5$ Hz, 6H). Anal. ($C_{21}H_{20}ClN_3O_4S$) C, H, N.

6-Bromo-3-(1,1-dioxo-1,2-dihydrobenzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-1-(3-methylbutyl)-1*H*-quinolin-2-one (81). 6-Bromo-1*H*-benzo[*d*][1,3]oxazine-2,4-dione (1.62 g, 6.69 mmol) was added portionwise to a suspension of sodium hydride (60% suspension in mineral oil) (296 mg, 7.4 mmol) in anhydrous *N,N*-dimethylformamide. After 15 min, 1-bromo-3-methylbutane (0.9 mL, 7.1 mmol) was added and the mixture was stirred at 80 °C for 16 h. The mixture was poured onto ice, acidified with glacial acetic acid, and extracted into ethyl acetate. Purification using flash chromatography (20% ethyl acetate in hexanes) gave 6-bromo-1-(3-methylbutyl)-benzo[*d*][1,3] oxazine-2,4-dione (1.0 g, 48%): ^{1}H NMR ($CDCl_3$) δ 8.11 (d, $J = 3.0$ Hz, 1H), 7.70 (dd, $J = 9.0, 3.0$ Hz, 1H), 7.10 (d, $J = 9.0$ Hz, 1H), 4.04 (m, 2H), 1.62 (m, 1H) 1.60 (m, 2H), 1.02 (d, $J = 6.5$ Hz, 6H).

Sodium hydride (160 mg of a 60% suspension in mineral oil, 4.0 mmol) was added to a mixture of 6-bromo-1-(3-methylbutyl)-benzo[*d*][1,3]oxazine-2,4-dione (312 mg, 1.0 mmol) and ethyl 1,1-dioxo-2*H*-benzo-1,2,4-thiadiazinyl-3-acetate (270 mg, 1.0 mmol) in tetrahydrofuran (15.0 mL). The mixture was heated under reflux for 1.5 h, cooled, and acidified with glacial acetic acid. The mixture was then heated under reflux for an additional 1.5 h and cooled and water was added. The product was collected and washed with water, diethyl ether, and hexanes to give compound **81** (400 mg, 81%): ^{1}H NMR (DMSO-d_6) δ 15.93 (s, 1H), 8.20 (d, $J = 2.5$ Hz, 1H), 7.67 (m, 2H), 7.59 (m, 1H), 7.30 (m, 3H), 4.10 (m, 2H), 1.75 (m, 1H), 1.45 (m, 2H), 1.0 (d, $J = 6.5$ Hz, 6H). Anal. ($C_{21}H_{20}BrN_3O_4S$) C, H, Br, N.

3-(1,1-Dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-6-iodo-1-(3-methylbutyl)-1*H*-quinolin-2-one (82). Triphosgene (5.64 g, 19 mmol) was added portionwise to a stirred solution of 2-amino-5-iodobenzoic acid (10.0 g, 38 mmol) in tetrahydrofuran (60.0 mL). The mixture was stirred for 1 h and then a mixture of water/ice/sodium hydrogen carbonate solution was added in portions to the mixture. The solid was collected, washed with water then diethyl ether, and dried to give 6-iodo-1*H*-benzo[*d*][1,3]oxazine-2,4-dione (9.68 g, 88%): ^{1}H NMR (DMSO-d_6) δ 11.83 (s, 1H), 8.12 (d, 1H), 8.00 (dd, 1H), 6.95 (d, 1H).

A solution of diethyl azodicarboxylate (3.76 mL, 23.88 mmol) in chloroform (50.0 mL) was added dropwise to a stirred suspension of 6-iodo-1*H*-benzo[*d*][1,3]oxazine-2,4-dione (6.90 g, 23.88 mmol), triphenylphosphine (6.26 g, 23.88 mmol), and isoamyl alcohol (2.5 mL, 23.88 mmol) in chloroform (150 mL). The mixture was stirred

overnight and evaporated onto silica gel. Purification using flash chromatography (silica gel, gradient, hexanes/ethyl acetate 0−15%) gave 6-iodo-1-(3-methylbutyl)-1*H*-benzo[*d*][1,3]oxazine-2,4-dione as a colorless solid (5.45 g, 53%): ^{1}H NMR ($CDCl_3$) δ 8.42 (d, 1H), 8.00 (dd, 1H), 6.92 (d, 1H), 4.02 (m, 2H), 1.60 (m, 1H), 1.57 (m, 2H), 1.01 (d, 6H).

Sodium hydride (2.4 g of a 60% suspension in mineral oil, 60 mmol) was added to a mixture of 6-iodo-1-(3-methylbutyl)-1*H*-benzo[*d*][1,3]oxazine-2,4-dione (5.39 g, 15 mmol) and ester **9** (4.02 g, 15 mmol) in tetrahydrofuran (100 mL). The mixture was heated under reflux for 1.5 h, cooled, and acidified with glacial acetic acid. The mixture was then heated under reflux for an additional 1.5 h and cooled and water was added. The precipitate was collected and washed with water, diethyl ether, and hexanes to give compound **82** (5.5 g, 68%): ^{1}H NMR (pyridine-d_5) δ 15.24 (br s, 1H), 14.50 (br s, 1H), 8.59 (d, 1H), 8.25 (m, 1H), 8.05 (m, 1H), 7.89 (m, 1H), 7.79 (m, 1H), 7.69 (m, 1H), 7.59 (m, 1H), 4.44 (m, 2H), 1.94 (m, 1H), 1.70 (m, 2H), 1.16 (d, 6H); MS (ES+) *m/e* 438 $[M + H]^+$. Anal. Calcd for $C_{21}H_{20}IN_3O_4S$: C, 46.94; H, 3.75; N, 7.82. Found: C, 46.42; H, 3.41; N, 7.62.

6-Amino-3-(1,1-dioxo-1,2-dihydrobenzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-1-(3-methylbutyl)-1*H*-quinolin-2-one (85). A solution of compound **84** (78 mg, 0.17 mmol) in *N,N*-dimethylformamide (20.0 mL) and glacial acetic acid (2.0 mL) was hydrogenated over 10% palladium-on-charcoal at 50 psi for 2 h. The mixture was filtered through Celite, washed through with methanol, and evaporated to a solid. The residual compound was crystallized from ethanol/4 M hydrogen chloride in dioxane to give the title compound **85** (35 mg, 48% as the hydrochloride salt): ^{1}H NMR (DMSO-d_6) δ 14.49 (s, 1H), 7.94 (d, *J* = 8.0 Hz, 1H), 7.54−7.80 (m, 6H), 4.29 (m, 2H), 1.77 (m, 1H), 1.53 (m, 2H) 1.01 (d, *J* = 6.5 Hz, 6H). Anal. Calcd for $C_{21}H_{22}N_4O_4S{\cdot}1.4HCl$: C, 52.82; H, 4.94; N, 11.73. Found: C, 53.04; H, 4.89; N, 11.45.

3-(1,1-Dioxo-1,4-dihydrobenzo[1,2,4]thiadiazin-3-yl)-4,6-dihydroxy-1-(3-methylbutyl)-1*H*-quinolin-2-one (86). *tert*-Butyldimethylsilyl chloride (5.05 g, 33.5 mmol) was added to a solution of 6-hydroxy-1*H*-benzo[*d*]oxazine-2,4-dione (6.0 g, 33.5 mmol) and imidazole (2.28 g, 33.5 mmol) in chloroform. The mixture was stirred overnight and then evaporated onto silica gel. Purification using flash chromatography (silica gel, 0−40% ethyl acetate/hexanes) gave 6-(*tert*-butyldimethylsilanyloxy)-1*H*-benzo[*d*]oxazine-2,4-dione (5.5 g, 56%) as a colorless solid: ^{1}H NMR ($CDCl_3$) δ 9.29 (s, *N*H), 7.27 (d, *J* = 2.0 Hz, 1H), 6.99 (dd, *J* = 2.0, 8.0 Hz, 1H), 6.80 (d, *J* = 8.0 Hz, 1H), 0.77 (s, 9H), 0.00 (s, 6H).

6-(*tert*-Butyldimethylsilanyloxy)-1*H*-benzo[*d*]oxazine-2,4-dione (324 mg, 1.1 mmol), triphenylphosphine (289 mg, 1.1 mmol), and isoamyl alcohol (0.18 mL, 1.1 mmol) were stirred together in chloroform and treated with diethyl azodicarboxylate (0.12 mL, 1.1 mmol). The reaction was stirred under a nitrogen atmosphere overnight, evaporated onto silica, and purified by flash chromatography (silica gel, ethyl acetate−hexanes) to give 6-(*tert*-butyldimethylsilanyloxy)-1-(3-methylbutyl)-1*H*-benzo[*d*]oxazine-2,4-dione (175 mg, 44%): ^{1}H NMR ($CDCl_3$) δ 7.53 (d, *J* = 3.0 Hz, 1H), 7.23 (dd, *J* = 3.0, 8.0 Hz, 1H), 7.03 (d, *J* = 8.0 Hz, 1H), 4.01 (m, 2H), 1.63 (m, 1H), 1.60 (m, 2H), 1.00 (d, *J* = 6.0 Hz, 6H), 0.97 (s, 9H), 0.20 (s, 6H), 0.50 (m, 2H), 0.22 (s, 6H).

Sodium hydride (75.0 mg of a 60% suspension in mineral oil, 1.88 mmol) was added to a mixture of 6-(*tert*-butyl-dimethylsilanyloxy)-1-(3-methylbutyl)-1*H*-benzo[*d*]oxazine-2,4-dione (170 mg, 0.46 mmol) and ester **9** (125 mg, 0.46 mmol) in tetrahydrofuran (20.0 mL). The mixture was heated under reflux for 1.5 h, cooled, and acidified with glacial acetic acid. The mixture was then heated under reflux for an additional 1.5 h and cooled and water was added. The product was collected; washed with water, diethyl ether, and hexanes; dried; and suspended in tetrahydrofuran (5.0 mL). Tetrabutylammonium fluoride (1.0 M solution in tetrahydrofuran; 0.50 mL) was then added and the mixture was stirred until a clear yellow solution was obtained. After 10 min, 3 M aqueous hydrochloric acid (10.0 mL) was added, followed by water until a precipitate was obtained. The solid was collected and washed with water, diethyl ether, and hexanes to give compound **86** (53 mg, 96%) as a yellow solid: ^{1}H NMR (DMSO-d_6) δ 15.12 (br s, 1H), 14.63 (br s, 1H), 10.00 (br s, *O*H), 7.93 (d, *J* = 8.0 Hz, 1H), 7.77 (dd, *J* = 8.0 Hz, 1H), 7.66 (d, *J* = 8.0 Hz, 1H), 7.54 (m, 3H), 7.36 (dd, *J* = 3.0, 8.0 Hz, 1H), 4.29 (m, 2H), 1.80 (m, 1H), 1.54 (m, 2H), 1.00 (d, *J* = 3.0 Hz, 12H). Anal. ($C_{21}H_{21}N_3O_5S$ $0.2H_2O$) C, H, N.

3-(1,1-Dioxo-1,2-dihydrobenzo[1,2,4]thiadiazin-3-yl)-7-fluoro-4-hydroxy-1-(3-methylbutyl)-1*H*-quinolin-2-one (103). A solution of 4-fluoroanthranilic acid (1.2 g, 7.7 mmol) in tetrahydrofuran (20.0 mL) was treated with triphosgene (2.3 g, 7.7 mmol) and stirred at 50 °C overnight. Saturated sodium hydrogen carbonate solution was added and the mixture extracted with ethyl acetate. Evaporation of the organic solution gave 7-fluorobenzo[*d*][1,3]oxazine-2,4-dione (1.09 g, 78%): ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.88 (s, 1H), 8.00 (m, 1H), 7.11 (m, 1H), 6.88 (m, 1H).

7-Fluorobenzo[*d*][1,3]oxazine-2,4-dione (500 mg, 2.75 mmol), triphenylphosphine (721 mg, 2.75 mmol), and 3-methylbutanol (0.3 mL, 2.75 mmol) were stirred together in dichloromethane and treated with diisopropyl azodicarboxylate (0.54 mL, 2.75 mmol). The reaction was stirred under a nitrogen atmosphere overnight, evaporated onto silica, and purified by chromatography (silica gel, ethyl acetate/hexanes) to give 1-(3-methylbutyl)-7-fluorobenzo[*d*]-[1,3]oxazine-2,4-dione (448 mg, 65%): ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.09 (m, 1H), 7.37 (m, 1H), 7.20 (m, 1H), 4.00 (m, 2H), 1.71 (m, 1H), 1.52 (m, 2H) 0.96 (d, *J* = 6.5 Hz, 6H).

Sodium hydride (130 mg of a 60% suspension in mineral oil, 3.25 mmol) was added to a mixture of 1-(3-methylbutyl)-7-fluorobenzo[*d*][1,3]oxazine-2,4-dione (200 mg, 0.8 mmol) and ester **9** (215 mg, 0.8 mmol) in tetrahydrofuran (15.0 mL). The mixture was heated under reflux for 1.5 h, cooled, and acidified with glacial acetic acid. The mixture was then heated under reflux for an additional 1.5 h and cooled and water was added. The product was collected and washed with water, diethyl ether, and hexanes to give compound **103** (46 mg, 15%): ^{1}H NMR (DMSO-d_6) δ 15.22 (br s, 1H), 14.11 (s, 1H), 8.13 (dd, *J* = 6.5 and 9 Hz, 1H), 7.78 (m, 1H), 7.69 (d, *J* = 8 Hz, 1H), 7.56 (m, 2H), 7.30 (m, 1H), 4.29 (m, 2H), 1.80 (m, 1H), 1.54 (m, 2H), 1.00 (d, *J* = 6.5 Hz, 6H). Anal. ($C_{21}H_{20}FN_3O_4S$) C, H, N.

7-Chloro-3-(1,1-dioxo-1,2-dihydrobenzo[1,2,4]thiadiazin-3-yl)-4-hydroxy-1-(3-methylbutyl)-1*H*-quinolin-2-one (104). Compound **104** was obtained by the alkylation of commercially available 4-chloroisatoic anhydride with 1-bromo-3-methylbutane under the conditions of method A, followed by the coupling of the resulting 7-chloro-1-(3-methylbutyl)-2*H*-3,1-benzoxazine-2,4(1*H*)-dione with ester **9** under the conditions of method C: ^{1}H NMR (DMSO-d_6) δ 15.0 (br s, 1H), 14.07 (br s, 1H), 8.15 (d, *J* = 8.5 Hz, 1H), 7.93 (d, *J* = 7.4 Hz, 1H), 7.53−7.80 (m, 3H), 7.47 (t, *J* = 1.3 Hz, 1H), 7.44 (d, *J* = 1.4 Hz, 1H), 4.31 (m, 2H), 1.76 (m, 1H), 1.54 (m, 2H), 0.99 (d, *J* = 6.6 Hz, 6H); MS (ES+) *m/e* 446 $[M + H]^+$. Anal. ($C_{21}H_{20}ClN_3O_4S$) C, H, N.

1-(2-Cyclopropylethyl)-3-(1,1-dioxo-1,4-dihydrobenzo[1,2,4]-thiadiazin-3-yl) -6-fluoro-4-hydroxy-1-quinolin-2-one (130). A solution of 6-fluorobenzo[*d*][1,3]oxazine-2,4-dione (1.0 g, 5.58 mmol) (obtained as described for compound **79**), triphenylphosphine (1.44 g, 5.58 mmol), and 2-cyclopropylethanol (1.0 g, 11.6 mmol) in chloroform was treated with diethyl azodicarboxylate (0.875 mL, 5.58 mmol). The reaction was stirred under a nitrogen atmosphere overnight, evaporated onto silica, and purified by chromatography (silica gel, ethyl acetate−hexanes) to give 1-(2-cyclopropylethyl)-6-fluorobenzo[*d*][1,3]oxazine-2,4-dione (722 mg, 51%): ^{1}H NMR ($CDCl_3$) δ 7.80 (dd, *J* = 3 and 7 Hz, 1H), 7.48 (m, 1H), 7.08 (dd, *J* = 4 and 9 Hz, 1H), 4.15 (m, 2H), 1.65 (m, 2H) 0.74 (m, 1H), 0.51 (m, 2H), 0.09 (m, 2H).

Sodium hydride (463 mg of a 60% suspension in mineral oil, 6.56 mmol) was added to a mixture of 1-(2-cyclopropylethyl)-6-fluorobenzo[*d*][1,3]oxazine-2,4-dione (722 mg, 2.90 mmol) and ester **9** (777 mg, 2.90 mmol) in tetrahydrofuran (30.0 mL). The mixture was heated under reflux for 1.5 h, cooled, and acidified with glacial acetic acid. The mixture was then heated under reflux for an additional 1.5 h and cooled and water was added. The product was collected and washed with water, diethyl ether, and hexanes to give compound **130** (800 mg, 65%): ^{1}H NMR (DMSO-d_6) δ

15.19 (br. s, 1H), 14.31 (s, 1H), 7.57−7.96 (m, 7H), 4.43 (m, 2H), 1.60 (m, 2H), 0.86 (m, 1H), 0.43 (m, 2H), 0.08 (m, 2H). A portion was converted to the sodium salt: Anal. ($C_{21}H_{17}FNaN_3O_4S{\cdot}0.5H_2O$) C, H, N, Na.

Biology. Inhibition of HCV NS5B Polymerization Activity.[13] The high-throughput polymerization assay was carried out in 384-well plates using 50 or 10 nM NS5B, [α-^{33}P]GTP, GTP, 5′-biotinylated oligo(rG_{13})/poly(rC) in 20 mM Tris-Cl, pH7.5, $MgCl_2$, KCl, DTT, and 0.05% bovine serum albumine as previously described. After 2 h at 25 °C, the reaction was terminated upon addition of an equal volume of 100 mM EDTA and transferred to a streptavidin-coated FlashPlate. After incubation for 30 min, the plate was washed and counted using a Packard TopCount microplate reader ($n = 4$).

Method for Positive Strand Replicon HCV-RNA Detection in Replicon Cells.[13] Replicon cells were plated at 3×10^3 cells per well in a 96-well plate plates at 37° and 5% CO_2 in DMEM (Dulbecco's minimal essential medium) containing 10% FCS (fetal calf serum), 1% NEAA (nonessential amino acids), and 1 mg/mL Geneticin (G418 neomycin). After allowing 4 h for cell attachment, 1 μL of a solution of candidate antiviral agent was added to the medium ($n = 8$ wells per dilution). Briefly, 11 2.5-fold dilutions of 1 mM stock test compound in dimethyl sulfoxide were prepared with final concentration ranging from 10,000 to 1.0 nM. Plates were incubated for 40 h, until reaching 80% confluence. After removal of medium, 150 μL of buffer RLT (Qiagen, Valencia, CA) was added to each well, and RNA purified according to manufacturer's recommendations (Qiagen RNAeasy) was eluted twice in 45 μL of distilled H_2O prior to RT-PCR. Approximately 40 μL of TaqMan EZ RT-PCR (Applied Biosystems, Foster City, CA) master mix (1× TaqMan EZ Buffer, 3 mM $Mn(OAc)_2$, 0.3 mM dATP, 0.3 mM dCTP, 0.3 mM dGTP, 0.6 mM dUTP, 0.2 mM neo-forward, 0.2 mM neo-reverse, 0.1 mM neo-probe, 1× cyclophilin mix, 0.1 unit/μL r*Tth* DNA polymerase, 0.01 unit/μL AmpErase UNG, and H_2O to 40 μL) was added to each tube of 96-tube optical plate along with 10 μL of RNA elution. Primers and probes specific for the positive strand RNA detection of the neomycin gene were as follows: neo-forward, 5′CCGGCTACCTGCCCATTC3′ (SEQ ID NO 1); neo-reverse, 5′CCAGATCATCCTGATCGACAAG3′ (SEQ ID NO 2); neo-probe, 5′FAM-ACATCGCATCGAGCGAGCACG-TAC-TAMRA3′ (SEQ ID NO 3). For negative strand RNA detection, the cDNA primer used was 5′ACA TGC GCG GCA TCT AGA CCG GCT ACC TGC CCA TTC3′ (SEQ ID NO 4), wherein the first 18 bases represent SEQ ID NO 5 linked to neo sequences: neo-forward tag, 5′ACA TGC GCG GCA TCT AGA3′ (SEQ ID NO 5); neo reverse, 5′CCAGATCATCCTGATCGACAAG3′ (SEQ ID NO 6); neo probe, 5′FAM-ACA TCG CAT CGA GCG AGC ACG TAC-TAMRA3′ (SEQ ID NO 3). Additionally, the PDAR control reagent human cyclophilin was used for normalization. Samples were mixed briefly and placed in an ABI7700 (Applied Biosystems) at 50 °C for 2 min; 60 °C for 30 min; and 95 °C for 5 min, with cycling parameters set to 94 °C and 20 s, 55 °C for 1 min for 40 cycles. The relative cDNA levels for neo and cyclophilin were determined compared to DMSO-only treated controls and the ratio of neo:cyclophilin was used for IC_{50} calculation ($n = 8$).

Method for Negative Strand Replicon HCV-RNA Detection in Replicon Cells.[13] To achieve strand-specific detection, a primer containing HCV RNA (or replicon RNA sequences such as neomycin gene) and an 18 base tag of nonrelated sequence at the 5′ end was for the reverse transcription (RT) reaction: 5′ACAT-GCGCGGCATCTAGACCGGCTACCTGCCCATTC3′ (SEQ ID NO 4). A Thermoscript-RT-PCR system (Invitrogen) was used for the room-temperature reaction according to the manufacturer's protocol, with approximately 9 μL of the cell-harvested RNA and 1 μL of primer (10 μM) incubated with room temperature at 60 °C for 1 h. Following that incubation, 2 μL of cDNA product containing the 5′ tag was amplified for TaqMan quantification using 48 μL of TaqMan Universal Master Mix (Applied Biosystems) as well as the following primers: neo-forward tag, 5′ACA TGC GCG GCA TCT AGA3′ (SEQ ID NO 5); neo reverse, 5′CCAGAT-CATCCTGATCGACAAG3′ (SEQ ID NO 6); and neo probe, 5′FAM-ACA TCG CAT CGA GCG AGC ACG TAC-TAMRA3′ (SEQ ID NO 3). Samples were mixed briefly and placed in an ABI7700 (Applied Biosystems) at 50 °C for 2 min, 95 °C for 10 min, with cycling parameters set to 94 °C for 15 s, 55 °C for 1 min for 40 cycles. The negative strand copy number in each reaction was determined using linear regression analysis based on the slope and intercept generated with a negative strand copy standard curve. The negative strand copies per cell were determined by dividing the total negative strand copies per reaction by the total cells per reaction.

Supporting Information Available: Experimental procedures and spectral data for compounds **16**−**25**, **27**−**72**, **75**−**78**, **83**, **84**, **87**−**102**, and **105**−**129**. This material is available free of charge via the Internet at http://pubs.acs.org.

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