Identification of Anthranilic Acid Derivatives as a Novel Class of Allosteric Inhibitors of Hepatitis C NS5B Polymerase

Thomas Nittoli,* Kevin Curran, Shabana Insaf, Martin DiGrandi, Mark Orlowski, Rajiv Chopra, Atul Agarwal, Anita Y. M. Howe, Amar Prashad, M. Brawner Floyd, Bernard Johnson, Alan Sutherland, Karen Wheless, Boris Feld, John O'Connell, Tarek S. Mansour, and Jonathan Bloom

Chemical and Screening Sciences and Infectious Diseases Research, Wyeth Research, 401 N. Middletown Road, Pearl River, New York 10965

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A series of potent anthranilic acid-based inhibitors of the hepatitis C NS5B polymerase has been identified. The inhibitors bind to a site on NS5B between the thumb and palm regions adjacent to the active site as determined by X-ray crystallography of the enzyme−inhibitor complex. Guided by both molecular modeling and traditional SAR, the enzyme activity of the initial hit was improved by approximately 100-fold, yielding a series of potent and selective NS5B inhibitors with IC_{50} values as low as 10 nM. These compounds were also inhibitors of the HCV replicon in cultured HUH7 cells.

Introduction

Hepatitis C virus (HCV) is a blood-borne pathogen belonging to the *Flaviviridae*[1] family of viruses, which also includes the West Nile, Yellow Fever, and Dengue viruses. Although acute liver disease arising from HCV is uncommon,[2] as many as 85% of infected patients will progress to a chronic infection.[3] Over the course of 20−30 years, up to 20% of these patients will develop cirrhosis and hepatic carcinoma.[4] HCV infection is the leading cause of liver transplants in the United States and Europe[5] and affects four million people in the United States and 170 million worldwide;[6] it is about five times as prevalent as AIDS.[7]

Despite a proliferation of pharmaceutical and academic research in the past decade, no specific antiviral agents are available for the treatment of HCV, and there remains an enormous unmet medical need for adequate therapeutic options. Interferon[8] (IFN), and more recently pegylated IFN[9] (PEG-IFN), which acts by modification of immune function, are the only approved treatments for HCV infection; typically they are administered in combination with the viral mutagen ribavirin.[10,11] The sustained viral response to this therapy is about 50% for genotype 1 and up to 80% for genotypes 2 and 3,[12] and the regimen is poorly tolerated. Consequently, patient adherence is suboptimal.[13] Thus, the need for HCV-specific small molecule inhibitors is acute. The structures and domain targets of selected HCV clinical candidates are shown in Figure 1. By analogy to AIDS, most small molecule inhibitor approaches to HCV have focused on the inhibition of essential viral targets, particularly the NS3-4A protease (analogous to HIV protease) and the NS5B RNA-dependent RNA polymerase (analogous to HIV RT), although other targets are also being pursued.[14]

For the NS5B RNA-dependent RNA polymerase target, both active-site and allosteric inhibitors have appeared in the literature. For example, the nucleoside active-site inhibitor 2′-*C*-methyl-3′-valine ester cytidine (NM-283) and non-nucleoside active-site inhibitor isothiazoles target the growing RNA chain and have been effective at inhibiting HCV replication.[15] Recent success in the crystallization of inhibitors with various polymerase constructs has led to significant advances in the identification of allosteric inhibitors of HCV NS5B RNA-dependent RNA polymerase,[16] including several reports from our laboratories.[17] We have recently disclosed a novel class of proline sulfonamide allosteric binding inhibitors that bind approximately 10 Å away from the catalytic aspartic acids in the active site.[18] In addition, other groups recently reported inhibitors that bind approximately 8−9 Å away from the active site.[19]

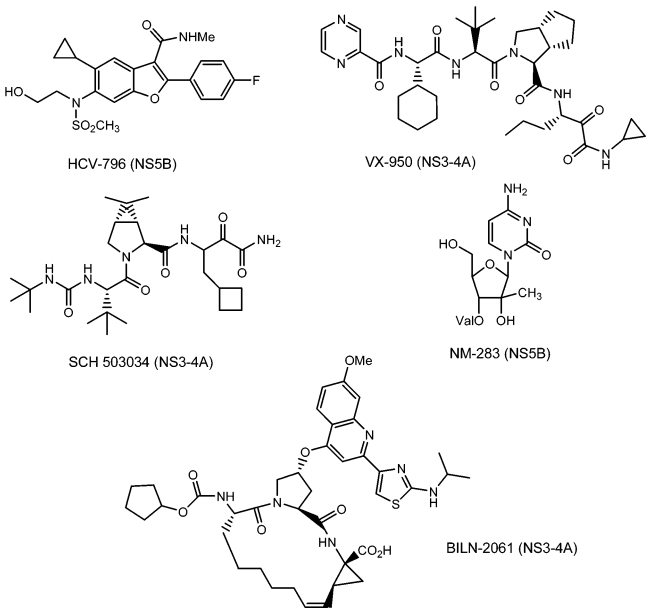

Figure 1. The structures and domain targets of selected HCV clinical candidates.

In our effort to identify allosteric inhibitors of NS5B polymerase, an HTS was developed to assay the Wyeth compound library collection. NS5B from the BK strain (1b subtype) was expressed in *E. coli* as a protein in which the 21 C-terminal amino acids were replaced with a short linker and a hexahistidine tag (GSHHHHHH). This screening expedition uncovered a lead anthranilate derivative with an IC_{50} of 1.6 μM. The lead was then soaked into the truncated NS5B polymerase to produce an X-ray crystal structure that revealed that these inhibitors bind approximately 7.5 Å away from the active site of NS5B in a similar fashion to the previous reports.[18,19] Optimization of this series of inhibitors resulted in analogues

* Corresponding author. Tel: (845) 602-5762, Fax: (845) 602-5561, E-mail: nittolt@wyeth.com.

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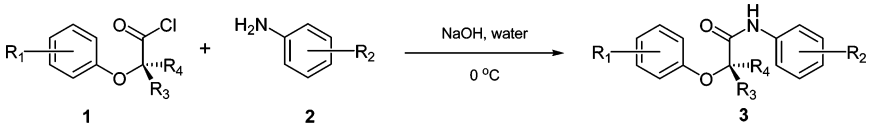
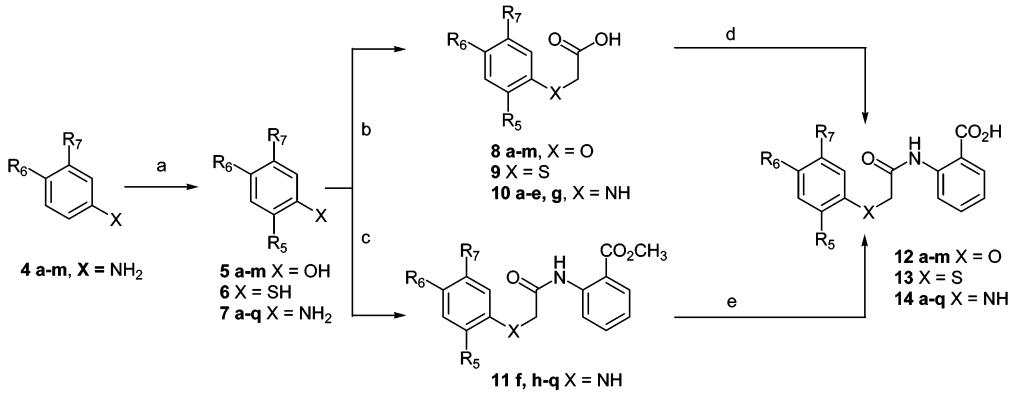
Scheme 1



#	R_1	R_2	R_3	R_4
3a	4-Cl	2-COOH	H	H
3b	4-Cl	3-COOH	H	H
3c	4-Cl	4-COOH	H	H
3d	4-Cl	2-COOMe	H	H
3e	4-Br	2-COOH	H	H
3f	3-Br	2-COOH	H	H
3g	H	2-COOH	H	H
3h	4-Cl	2-COOH	Me	Me
3i	4-CN	2-COOH	H	H
3j	4-F	2-COOH	H	H
3k	4-OMe	2-COOH	H	H

Scheme 2[a]



#	R_5	R_6	R_7
12a	Me	Cl	H
12b	OMe	Cl	H
12c	Cl	Cl	H
12d	COMe	Cl	H
12e	COMe	H	Cl
12f	COMe	Me	H
12g	COMe	F	H
12h	Cl	Cl	Cl
12i	F	F	F
12j	Br	Cl	Me
12k	COMe	Cl	F

#	R_5	R_6	R_7
12l	COMe	F	Cl
12m	COMe	Cl	Me
13	H	Cl	H
14a	COMe	Cl	H
14b	COEt	Cl	H
14c	COMe	Br	H
14d	COMe	Cl	Me
14e	COMe	Me	Cl
14f	COMe	Cl	Cl
14g	COMe	F	Me

#	R_5	R_6	R_7
14h	COMe	Cl	F
14i	COMe	F	Cl
14j	COMe	F	Br
14k	COMe	Br	Cl
14l	COMe	Cl	Et
14m	COMe	Me	Me
14n	H	H	Br
14o	H	Cl	Cl
14p*	H	Cl	Cl
14q	H	F	Cl

[a] Reagents and conditions: (a) (i) R−CN, BCl_3, Tol.; (ii) $AlCl_3$, reflux; (iii) 4 N HCl, reflux; (b) (i) K_2CO_3, DMF, 70 °C, $BrCH_2COOt$-Bu; (ii) TFA; or (i) K_2CO_3, DMF, 90 °C, ICH_2COOEt, (ii) NaOH, THF−MeOH; (c) DIPEA, Tol, $BrCH_2CONH$-2-C_6H_5COOMe. (d) $SOCl_2$, Pyr, anthranilic acid or BOP, DIPEA, anthranilic acid, DCM/DMF; (e) NaOH, THF or MeOH. * X = NMe.

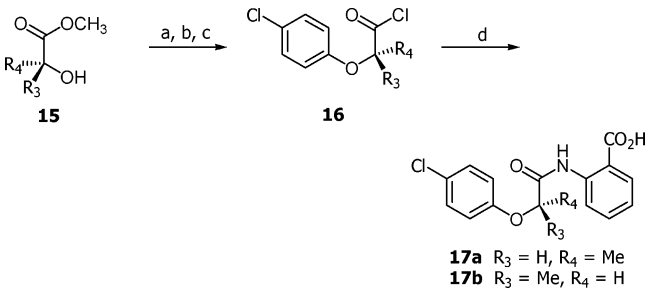
exhibiting nanomolar potency against the enzyme as well as inhibition of viral replication in the HCV replicon in cultured HUH7 cells.

Chemistry. Treatment of commercially available acid chlorides **1** with anilines **2** in the presence of aqueous NaOH gave amides **3** as shown in Scheme 1. Modifications of this procedure afforded the thioether **13** and anilino analogues **14** (Scheme 2). More elaborate analogues were prepared by the methods outlined in Scheme 2. Acylation of anilines **4** under Sugasawa[20] conditions afforded acetophenones **7**. A subset of four anilines were not acylated (**7n**−**q**) and carried forward. Subsequent alkylation with alkyl haloacetates gave the anthranilate esters, which were deprotected using standard methods to provide **8**−**10**. When possible, phenoxyacetic acid intermediates **8** were converted to the corresponding acid chlorides and then treated with anthranilic acid in pyridine to give amides **12**. In other cases, amide formation was accomplished by BOP activation and reaction with anthranilic acid to give amides **12** and **13**.

Alkylation of anilines **7** under basic conditions with the *N*-bromoacetyl anthranilates, derived from the reaction of methyl anthranilate with bromoacetyl bromide, gave esters **11**, which were then saponified to form acids **14**.

The preparation of *R* and *S* methylene-substituted anthranilic acids **17** (Scheme 3) commenced with the appropriate commercially available α-hydroxy methyl esters **15**, which were reacted with 4-chlorophenol under the conditions reported by Rao and co-workers,[21] hydrolyzed with 1 N NaOH, and then converted to acid chlorides **16**. Treatment of these compounds with anthranilic acid in 1 N NaOH afforded the desired products **17** in good yield.

Scheme 3[a]



[a] Reagents and conditions: (a) 4-ClPhOH, DEAD, PPh_3, THF; (b) 1 N NaOH; (c) oxalyl chloride; (d) 1 N NaOH, anthranilic acid.

Figure 2. Ribbon diagram of NS5B complex crystal structure indicating **3a** (space-filling atoms) between the thumb (colored green) and palm (colored purple) domains. The fingers domain is colored blue, and the β-loop is colored yellow.

Results and Discussion

The solved crystal structure of the initial lead compound **3a** at 2.6 Å resolution clearly shows the anthranilate inhibitor bound between the thumb and palm domains of NS5B (Figure 2), approximately 7.5 Å away from the NTP binding site. Key interactions between the enzyme and **3a** are detailed in Figure 3. The phenoxy ring and its substituents reside in a spherical hydrophobic pocket of 3.3−3.9 Å defined by Pro197, Leu384, Tyr448, Arg200, and Met414. Hydrogen bond interactions with the enzyme occur between the backbone NH of Tyr448 and the carboxylate oxygen of the anthranilate group (1.56 Å), as well as between the Tyr415 (2.48 Å) and Arg386 (3.09 Å) side chains and the amide carbonyl. The allosteric binding site we observed is in the region of the site occupied by our previously reported proline sulfonamides.[18] However, binding of the anthranilates differs in that the carboxyl oxygen of **3a** forms one hydrogen bond to the backbone NH of Tyr448 whereas the carboxyl oxygen of the proline sulfonamides forms hydrogen bonds to the backbone NH of Gly449 and the side chain of Gln446. In the proline sulfonamide series, one of the sulfonamide oxygens forms an H-bond to the Tyr448 NH and the other is bound to a water molecule.

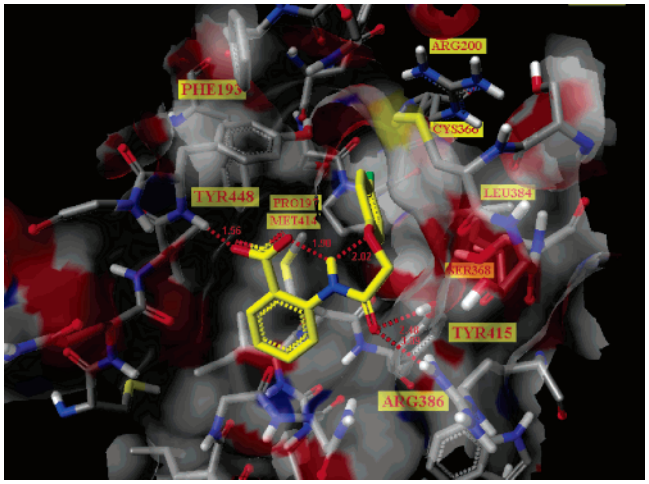
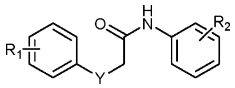


Figure 3. Compound **3a** in the active site of NS5B is depicted as capped-stick (yellow) and enzyme as capped-stick (gray) models. Interactions within the hydrogen-bonding distance are indicated as red dashed lines.

Table 1. Inhibition of NS5B BK with Anthranilates

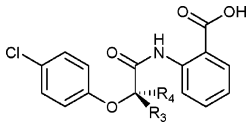


no.	Y	R_1	R_2	IC_{50} NS5B-Δ21 BK (μM)
3a	O	4-Cl	2-COOH	1.64
3b	O	4-Cl	3-COOH	>33
3c	O	4-Cl	4-COOH	>28
3d	O	4-Cl	2-COOMe	>31
3e	O	4-Br	2-COOH	3.23
3f	O	3-Br	2-COOH	1.14
3g	O	H	2-COOH	>37
3i	O	4-CN	2-COOH	5.1
3j	O	4-F	2-COOH	5.2
3k	O	4-OMe	2-COOH	35
13	S	4-Cl	2-COOH	4.4
14n	NH	3-Br	2-COOH	0.87

The inhibitor **3a** is fixed into a quasi-tricyclic conformation by a three-centered intramolecular hydrogen bond between the amide NH, the carboxylic acid oxygen, and the oxygen of the phenoxy group. The conformation contributes to the critical interactions with the enzyme. As would be predicted by this model, disruption of this system by relocation of the carboxylic acid to either the 3- (**3b**) or 4- (**3c**) positions, or esterification of the acid **(3d)**, rendered the compounds inactive. Furthermore, isosteric replacement of the phenoxy oxygen yields active inhibitors only when the resulting atom is capable of maintaining the intramolecular interaction, as shown by the comparison of **3a**, **3f**, **13**, and **14n**. Thus, the order of potency for the inhibitor linker atoms is N > O (**14n** vs **3f**) and O > S (**3a** vs **13**), as expected. The results of biological assays are compiled in Tables 1−4.

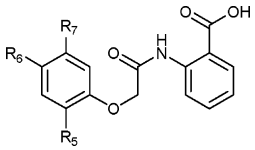
The effect of substitution of the methylene linker was also examined (Table 2). The *S* methyl enantiomer (**17a**), with an IC_{50} of 0.76 μM, was twice as potent as the corresponding analogue having no substitution on the methylene linker (**3a**, IC_{50} = 1.6 μM). The weak activity of the *R* methyl isomer **17b** (IC_{50} = 50 μM) may be due to unfavorable interactions between the methyl group of the inhibitor and the backbone carbonyl of Cys366 and the Tyr415 hydroxyl (1.17 Å and 1.76 Å, respectively). In contrast, the *S* methyl enantiomer's (**17a**) nearest interactions are with the sidechains of Arg386 (2.44 Å) and Ser368 (3.00 Å). The geminal dimethyl analogue **3h** was not active.

Table 2. Effect of Linker Substitution on Inhibition of NS5B BK



no.	R_3	R_4	IC_{50} NS5B-Δ21 BK (μM)
3a	H	H	1.64
17a	H	Me	0.76
17b	Me	H	50
3h	Me	Me	>30

Table 3. Inhibition of NS5B BK by Substituted Phenoxy Anthranilates[a]



no.	R_5	R_6	R_7	IC_{50} NS5B-Δ21 BK (μM)
12a	Me	Cl	H	0.57
12b	OMe	Cl	H	0.57
12c	Cl	Cl	H	0.45
12d	Ac	Cl	H	0.53
12e	Ac	H	Cl	0.86
12f	Ac	Me	H	2.8
12g	Ac	F	H	1.5
12h	Cl	Cl	Cl	0.03
12i	F	F	F	1.27
12j	Br	Cl	Me	0.11
12k	Ac	Cl	F	0.11
12l	Ac	F	Cl	0.23
12m	Ac	Cl	Me	0.14

[a] Compounds tested in the replicon and MTS assays had IC_{50} > 11 μM and >115 μM, respectively.

Tables 1 and 3 define some of the size and electronic parameters for phenoxy ring substitution. For example, for 4-monosubstituted analogues, a medium-sized, electron-withdrawing group is required for activity. Of this group, the 4-chloro analogue **3a** is the best inhibitor (1.6 μM), while the 4-bromo **3e** (3.2 μM), 4-cyano **3i**, (5.1 μM), and the 4-fluoro **3j** (5.2 μM) analogues are less potent. Substitution of a 4-methoxy group (**3k)** for the chlorine of **3a** also resulted in the loss of activity. Addition of substituents ortho to the ether group of analogues **3** improved activity, regardless of their electron-withdrawing or donating capacity. These results are attributed to an additional interaction between the ortho group and a hydrophobic pocket in the vicinity of Phe193. For example, addition of a methyl, methoxy, chloro, or acetyl group (compounds **12a**−**d**) all improved activity by 2−3 fold over the corresponding unsubstituted analogue (**3a**). The most potent disubstituted compound was the 2,4-dichloro analogue (**12c**) with an IC_{50} value of 0.45 μM.

In general, trisubstitution on the phenoxy ring further increased the potency of the inhibitors (**12h**−**m**, Table 3). The 2,4,5-trichlorophenoxy analogue **12h** was the most potent trisubsubstituted compound with an IC_{50} of 0.03 μM. The trichlorophenoxy group appears to form favorable interactions with the spherical pocket of Pro197, Leu384, Tyr448, Arg200, and Met414. As was the case with mono- and disubstitution, fluorine was not an acceptable replacement for chlorine, with the 2,4,5-trifluorophenoxy analogue **12i** being >40-fold less potent than **12h**. The nature of the ortho substituent was again consistent with trends observed with disubstituted analogues (Table 3). Electron-donating or -withdrawing groups all made positive contributions to activity. For example, the 2-bromo-4-chloro-5-methylphenoxy **12j** and the 2-acetyl-4-chloro-5-fluoro **12k** analogues were both very potent with IC_{50} of approximately 100 nM.

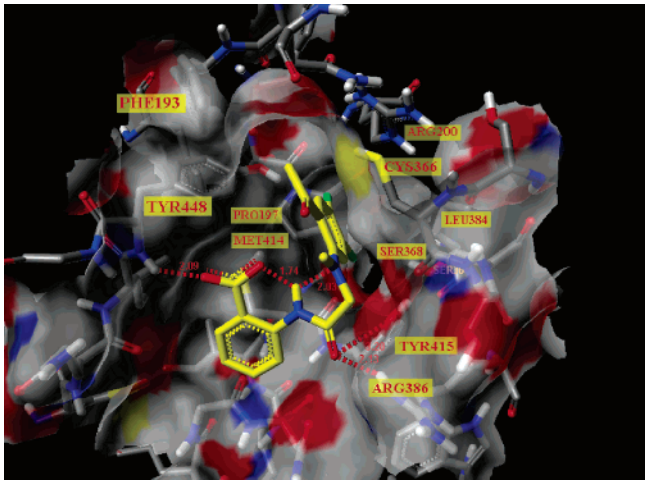


Figure 4. Compound **14i** in the active site of NS5B is depicted as capped-stick (yellow) and enzyme as capped-stick (gray) models. Interactions within the hydrogen-bonding distance are indicated as dashed lines.

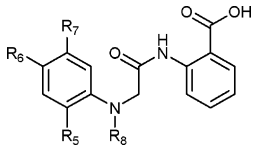
Introduction of an ortho acetyl group in the anilino series generally resulted in potent inhibitors. For example, compound **14a** (Table 4), having a 4-chloro-2-acetyl aniline group (IC_{50} = 230 nM), is comparable to the dihalogenated series **12k**−**m** (IC_{50} = 230−110 nM). Interestingly, the corresponding propionyl **14b** was approximately 14-fold less potent, which is contrary to our expectation based on crystallographic data since there would be space available (2.64 Å) between the side chain of Cys366 and the propionyl group.

In general, for the anilino analogues, the same electronic trends were seen as with the phenoxy series. The simple dihalogenated analogues **14o**−**q** all inhibit in the 200 nM range. These compounds have cellular activity, but **14o** and **14p** have MTS IC_{50} values in the toxic range. It is interesting to note that the *N*-methylanilino analogue **14p** has similar activity to the desmethyl compound **14o**. This is consistent with the model proposed in Figure 3 in that the anilino NH does not participate in fixing the inhibitor into the required quasi-tricyclic conformation and therefore tolerates the *N*-methyl group.

The trisubstituted anilino analogues were significantly more potent than the corresponding trisubstituted phenoxy analogues. Once again, electron-withdrawing groups on the anilino ring improved the potency of these inhibitors by approximately 10-fold. Analogues with good enzymatic potency (<100 nM) **14d**, **14f**, **14i**−**l** varied in activity for the replicon and MTS assays. The ortho acetyl analogues with R_6 = Cl and R_7 = alkyl (**14d** and **14l**) were more potent in the replicon assay, with only **14l** having toxicity. For the ortho acetyl hetero-dihalogenated analogues, the more potent derivatives in the replicon assay had bromo or chloro at R_7 (**14i**−**k**), as compared to **14h** (R_6 = Cl). Two analogues (**14i** and **14j**) were the most active with IC_{50} values of 17 and 10 nM, respectively, in the polymerase assay and had cellular activity of 2 μM. In addition, analogues **14i** and **14j** were selective over calf thymus A, HIV-RT, RNA II, and human α,β,γ-polymerases (Table 5).

An X-ray crystal structure (2.9 Å resolution) was obtained for **14i** with NS5B-21 (Figure 4). The structure confirms that this compound binds at the same site and with similar conformation and hydrogen bonding interactions as was observed for **3a**. The increase in potency for molecules bearing an ortho acetyl group could arise from interactions with the

Table 4. Inhibition of NS5B BK by Substituted Anilino Anthranilates



no.	R_5	R_6	R_7	R_8	IC_{50} NS5B-Δ21 BK (μM)	IC_{50} cell (μM)	IC_{50} MTS (μM)
14a	COMe	Cl	H	H	0.23	12	>144
14b	COEt	Cl	H	H	3.36	-	-
14c	COMe	Br	H	H	0.11	43	>128
14d	COMe	Cl	Me	H	0.08	3.1	>139
14e	COMe	Me	Cl	H	1.05	30	>139
14f	COMe	Cl	Cl	H	0.03	10	123
14g	COMe	F	Me	H	7.47	-	-
14h	COMe	Cl	F	H	0.18	17	>137
14i	COMe	F	Cl	H	0.017	2.0	>137
14j	COMe	F	Br	H	0.010	2.0	>122
14k	COMe	Br	Cl	H	0.022	30	>117
14l	COMe	Cl	Et	H	0.032	5.0	44
14m	COMe	Me	Me	H	0.21	32	>147
14n	H	H	Br	H	0.87	-	-
14o	H	Cl	Cl	H	0.20	13	47
14p	H	Cl	Cl	Me	0.17	14	47
14q	H	F	Cl	H	0.21	40	155

Table 5. Selectivity for Compounds **14i** and **14j** among Polymerases

no.	NS5B-Δ2 1 BK (μM)	CT-A pol (μM)	HIV-RT pol (μM)	RNA pol II (μM)	h α pol (μM)	h β pol (μM)	h γ pol (μM)
14i	0.017	>137	>137	>137	>137	>137	>27
14j	0.010	>122	>122	>122	>122	>122	>24

hydrophobic pocket in the vicinity of Phe193 or the additional intramolecular hydrogen bond between the acetyl and the anilino NH thereby yielding a rigid quasi-pentacyclic structure.

Conclusion

This report details our efforts to identify potent hepatitis C NS5B polyermase inhibitors. These allosteric inhibitors bind to NS5B adjacent to the active site, between the thumb and palm regions, as determined by X-ray crystallography. The enzyme activity of the initial lead compound was improved approximately 100-fold producing two inhibitors, **14i** and **14j**, with $IC_{50} \leq 17$ nM. These compounds had cellular activity with selectivity indexes over 7100 for the MTS assay. In addition, compounds **14i** and **14j** had selectivity indexes of over 1400 relative to calf thymus A, HIV-RT, RNA II, and human α,β,γ-polymerases.

Experimental Section

Expression and Purification of Recombinant NS5B. Cloning, expression, and purification of the recombinant NS5B enzyme was the same as described.[22] Briefly, the plasmid encoding the His-tagged C-terminal 21-amino acid deleted NS5B (genotype 1b, BK isolate) was transformed in *Escherichia coli* cells. The recombinant protein was expressed upon isopropyl β-D-galactoside induction. NS5B was purified using nickel affinity resin (Nickel-NTA, Qiagen), followed by cation exchange (Poros HS, Perseptive Biosystem) and size exclusion (Superdex 200, Amersham Pharmacia Biotech) chromatographies. The protein was concentrated up to 5 mg/mL and exchanged into buffer containing 50% glycerol, 25 mM HEPES, pH 7.5, 10 mM DTT, 600 mM NaCl, and stored at −20 °C.

NS5B RNA-Dependent RNA Polymerase Assay. The RNA-dependent RNA polymerase (RdRP) assay was performed in 24 nM of NS5B, 20 mM HEPES, pH 7.5), 5 mM $MgCl_2$, 1 mM dithiothreitol (DTT), 0.05 mg/mL bovine serum albumin (BSA), 0.5 μM UTP, 1 μM ATP, 0.08 μM CTP, 0.025 μM GTP spiked with 0.125 μCi of [α-^{33}P]GTP, 3 nM of RNA substrate, 0.4 U/μL of RNasin in the presence or absence of escalating concentrations of compounds (average of three or more determinations). The reaction was incubated at room temperature for 2 h. Product RNA containing the incorporated radioactive nucleotides was collected after thorough washing with 0.5 M sodium phosphate buffer (pH 7.0) using a Millipore Manifold. The filters containing the reaction products were allowed to dry at room temperature, and radioactivity was quantified using a Wallac MicroBeta counter.

Cellular Assay. Intracellular activities of the compounds were evaluated in Huh7 cells harboring the genotype 1b, BB7 replicon and was conducted as previously described.[22] Briefly, cells were incubated with the compound at increasing concentrations in the presence of 2% FCS without G418 in Dulbecco's minimal essential medium (DMEM) for 3 days (average of three or more determinations). The levels of HCV, ribosomal, and glyceraldehyde-3-phosphate mRNAs were determined using quantitative TaqMan RT-PCR.

Polymerase Selectivity. The selectivity over calf thymus polymerase A, human α, β, γ, and HIV-RT polymerases was conducted as previously described.[22] Briefly, the enzyme was incubated with the compound in a buffered reaction containing four dNTPs spiked with [α-33P]TTP, and respective DNA templates (poly(dA)-oligo-$(dT)_{12}$ for HIV RT, DNase I activated calf thymus DNA for CT Pol α, hPol α and β, and singly primed M13 DNA for hPol γ (average of three or more determinations). All NTPs and DNA templates were adjusted to the respective K_m levels. The reaction was carried out at 37 °C for 1−2 h. Product DNA containing incorporated radioactive nucleotides was collected by filtration through Millipore Multiscreen plates, washed, and quantified using a Wallac MicroBeta counter.

X-ray Crystallography. A C-terminally truncated version of HCV:BK NS5B (NS5B-21) was purified (as described previously)[23] and was buffer exchanged into 600 mM NaCl, 10% glycerol, 25 mM Tris pH 7.5, and 20 mM DTT and concentrated to 70 mg/mL over the course of 12 h. Crystals were grown using the vapor diffusion hanging-drop method. The drop contained 2 μL of protein and 2 μL of well solution: 26% PEG 4K, 50 mM sodium acetate

Table 6. Statistics from Crystallographic Analysis of HCV NS5B with Inhibitors

	3a	**14i**
space group	$P2_12_12_1$	$P2_12_12_1$
unit cell (Å)	101.34 101.47 251.21	70.84 70.88 251.65
resolution (Å) (last shell)	20−2.6 (2.7−2.6)	20−2.4 (2.5−2.4)
total observations	1269229	720284
unique observations	70074	50296
completeness (%)	87.8 (89.2)	99.1 (92.2)
R_{sym}[a]	8.5 (28.0)	5.9 (38.7)
$I/\sigma I$	7.0 (3.4)	26.3 (2.9)
refinement		
resolution (Å)	20-2.6	20-2.9
R_{work}/R_{free}[b] (%)	23.4/27.7	25.6/27.9
RMSd[c] bonds (Å)	0.011	0.008
RMSd[c] angles (deg)	1.1	1.6

[a] $R_{sym} = \Sigma |I - \langle I \rangle|/I$, where I is the observed intensity, $\langle I \rangle$ is the average intensity of multiple observations of symmetry- related reflections. [b] $R_{work} = \Sigma ||F_{obs}| - |F_{calc}|| \Sigma |F_{obs}|$, R_{free} is equivalent to R_{work}, but calculated for a randomly chosen 5% of reflections omitted from the refinement process. [c] RMSd is the root-mean-square deviation from ideal geometry.

pH 4.6, 10% glycerol. Protein crystals with plate-like morphology grew to dimensions of 200 μm × 200 μm × 50 μm overnight. Inhibitors were stored as a 100 mM stock solution in 100% deuterated DMSO and diluted to a final concentration of 1 mM in a buffer containing 26% PEG 4K, 50 mM sodium acetate pH 4.8, 10% glycerol, and 100 mM NaCl. Crystals were transferred to this solution for 12 h prior to rapid cooling in liquid nitrogen in a buffer containing 26% PEG 4K, 50 mM sodium acetate pH 4.8, 100 mM NaCl, and 30% glycerol. The structure was phased using the technique of molecular replacement with the CCP4 suite of software (CCP4 1994).[24] The search model used was based on the published crystal structure of NS5B.[25] Refinement and model building was performed in iterative cycles using the programs CNX and QUANTA (Accelrys Inc.). See Table 6 for refinement statistics.

Chemistry. ^{1}H NMR spectra were determined with a Bruker DRX400 spectrometer at 400 MHz. Chemical shifts, δ, are in parts per million relative to the internal standard tetramethylsilane. Electrospray (ES) mass spectra were recorded in positive and negative mode on a Micromass Platform spectrometer. Electron impact (EI) and high-resolution mass spectra (HRMS) were obtained on a Finnigan MAT-90 spectrometer. Some high-resolution electrospray mass spectra with higher precision were obtained on a Bruker 9.4T FTMS spectrometer. Elemental analyses were obtained from Robertson Microlit. Chromatographic purifications were by flash chromatography using EMD Chemicals, Inc., 60 μm silica gel. Analytical HPLC was conducted on an HP 1100 liquid chromatography system over a 4.6 mm × 150 mm Prodigy ODS3 column (5 um) or a 4.6 mm × 150 mm X-Bridge BEH C18 column (3.5 um). For method 1, a gradient elution of increasing concentrations of CH_3CN in H_2O containing 0.02% TFA (10−95% over 20 min, wavelength 215 nm) and a flow rate of 1 mL/min was employed. For method 2, a gradient elution of increasing concentrations of CH_3CN in H_2O containing 10 mM ammonium acetate (10−95% over 20 min, wavelength 215 nm) or MeOH in H_2O containing 0.02% TFA (10−95% over 20 min, wavelength 215 nm) and a flow rate of 1 mL/min were employed.

Syntheses and Spectral Data of Select Noncommercial Compounds.

2-[2-(4-Chlorophenoxy)acetylamino]benzoic Acid (3a). To a stirred solution of 496 mg (3.62 mmol) of anthranilic acid in 50 mL of H_2O was added 290 mg of NaOH. The solution was cooled to 0 °C, and 0.57 mL (3.62 mmol) of 4-chlorophenoxyacetyl chloride was added dropwise then stirred for 1 h. The mixture was diluted with 40 mL of 1 N HCl and extracted with 2 × 100 mL of EtOAc. The combined organic layers were dried over Na_2SO_4 and concentrated *in vacuo*. Crystals were obtained from EtOH. 226 mg (20%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.81 (bs, 1H), 12.25 (s, 1H), 8.68 (d, 1H, J = 4.2 Hz), 8.02 (d, 1H, J = 3.6 Hz), 7.62 (t, 1H, J = 7.8), 7.41 (d, 2H, J = 4.7), 7.19 (t, 1H, J = 7.8 Hz), 7.11 (d, 2H, J = 4.4 Hz), 4.75 (s, 2H); Anal. Calcd for $C_{15}H_{12}ClNO_4$: C, H, N.

(4-Chloro-2-methoxyphenoxy)acetic Acid (8b). (4-Chloro-2-methoxyphenoxy)acetic Acid *tert*-Butyl Ester. To a stirred solution of 951 mg (6 mmol) of 2-methoxy-4-chlorophenol and 1170 mg (6 mmol) of *tert*-butyl bromoacetate in 8 mL of dry DMF was added 850 mg (6.15 mmol) of K_2CO_3. The reaction was heated at 65 °C for 48 h. The reaction was then concentrated *in vacuo* and dissolved in hexanes. The organic layer was washed with 1 N NaOH and then passed through a pad of silica gel. The solution was concentrated *in vacuo* to yield 1063 mg (65%) of the desired compound. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.04 (d, J = 2.27 Hz, 1 H) 6.83−6.93 (m, 2 H) 4.63 (s, 2 H) 3.79 (s, 3 H) 1.41 (s, 9 H); MS (ESI) m/z 273.1; Anal. Calcd for $C_{13}H_{17}ClO_4$: C, H, N.

A 1016 mg (3.72 mmol) amount of the above ester was dissolved in 4 mL of TFA. The solution was stirred for 1 h and then concentrated *in vacuo* to yield 796 mg (98%) of the desired compound. ^{1}H NMR (400 MHz, DMSO-d$_6$) δ 7.03 (d, J = 2.27 Hz, 1 H) 6.84−6.94 (m, 2 H) 4.66 (s, 2 H) 3.79 (s, 3 H); MS (ESI) m/z 215.

(2-Acetyl-4-chloro-5-fluorophenoxy)acetic Acid (8k). 1-(5-Chloro-4-fluoro-2-hydroxyphenyl)ethanone. A stirred solution of 3712 mg (25.36 mmol) of 4-chloro-3-fluorophenol and 2389 mg (30.43 mmol) of acetyl chloride in 100 mL of toluene was heated to 40 °C for 1 h. $AlCl_3$ (6.76 g, 50.7 mmol) was added portionwise and heated to 80 °C for 3 h. The reaction was cooled to room temperature and 1 N HCl added dropwise. The mixture was extracted with DCM, dried over Na_2SO_4, filtered, and allowed to crystallize to yield 2917 mg (61%); ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.70 (s, 1H), 7.85 (d, 1H, J = 9.8 Hz), 7.21 (d, 1H, J = 6.2 Hz), 2.62 (s, 3H); Anal. Calcd for $C_8H_6ClFO_2$: C, H.

(2-Acetyl-4-chloro-5-fluoro-phenoxy)acetic Acid Ethyl Ester. To a stirred solution of 933 mg (4.95 mmol) of 1-(5-chloro-4-fluoro-2-hydroxyphenyl)ethanone and 1059 mg (4.95 mmol) of ethyl iodoacetate in 100 mL of dry DMF was added 750 mg (5.43 mmol) of K_2CO_3. The mixture was heated to 90 °C for 18 h. The mixture was poured into water, and the solids collected by filtration and crystallized from EtOAc−hexanes to yield 1350 mg 95% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.55 (d, 1H, J = 9.6 Hz), 7.49 (d, 1H, J = 6.0 Hz), 5.00 (s, 2H), 4.19 (q, 2H, J = 7.2 Hz), 2.62 (s, 3H), 1.22 (t, 3H, J = 7.4 Hz); Anal. Calcd for $C_{12}H_{12}ClFO_4$: C, H.

To a stirred solution of 2110 mg (7.68 mmol) of the above ester in 5 mL of THF was added 3 mL of 5 N NaOH. The reaction was stirred for 18 h and then poured into water. The mixture was made acidic with 1 N HCl, and the solids were filtered, washed with water, and dried to yield 1810 mg (96%); ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.54 (d, 1H, J = 9.6 Hz), 7.45 (d, 1H, J = 5.9 Hz), 4.91 (s, 2H), 2.62 (s, 3H); Anal. Calcd for $C_{10}H_8ClFO_4$: C, H

2-{[(4-Chloro-2-methylphenoxy)acetyl]amino}benzoic Acid (12a). Stirred 247 mg (0.77 mmol) of **8a** in 1 mL of oxalyl chloride and 1 mL of DCM with 1 drop of DMF for 30 min. The reaction mixture was concentrated in vacuo. Pyridine (1 mL) and 137 mg (1 mmol) of anthranilic acid was then added and stirred for 1 h. The reaction was poured into water and made acidic with 1 N HCl, and the solid was filtered, washed with water, and then dried. The desired compound was crystallized from acetonitrile to yield 145 mg (40%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.70 (bs, 1 H) 11.91 (s, 1 H) 8.70 (dd, J = 8.56, 1.01 Hz, 1 H) 8.02 (dd, J = 7.93, 1.64 Hz, 1 H) 7.61 − 7.66 (m, 1 H) 7.28 (d, J = 2.01 Hz, 1 H) 7.20 (td, J = 7.99, 1.89 Hz, 2 H) 6.99 (d, J = 8.56 Hz, 1 H) 4.77 (s, 2 H) 2.38 (s, 3 H); Anal. Calcd for $C_{16}H_{14}ClNO_4 \cdot 0.2H_2O$: C, H, N; MS (ESI, M − H) m/z 318.01.

1-(2-Amino-5-chlorophenyl)ethanone (7a). To a stirred solution of 75 mL of BCl_3 in heptane (75 mmol) at 0 °C was added 9.2 g (72.2 mmol) of 4-chloroaniline in 90 mL of acetonitrile dropwise over 20 min. To this suspension was added 10.6 g (79.5 mmol) of $AlCl_3$ in three portions. The mixture was then heated to reflux overnight. The mixture was cooled to 0 °C, and 120 mL of 4 N HCl was added and then refluxed for 2 h. The reaction was cooled

and extracted with 3 × 150 mL of DCM. The combined organic layers were washed with 1 × 100 mL of 2 N HCl, dried over Na_2SO_4, and concentrated *in vacuo*. The crude material was purified by silica gel chromatography using 25% EtOAc/Hex to give 2684 mg (22%); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.66 (d, 1H, J = 2.3 Hz), 7.21 (dd, 1H, J = 8.8, 2.3 Hz), 6.60 (d, 1H, J = 8.8 Hz), 6.26 (s, 2H), 2.56 (s, 3H); Anal. Calcd for C_8H_8ClNO: C, H, N.

[(4-Chlorophenyl)thio]acetic Acid (9). To a stirred solution of 930 mg (7.29 mmol) of 4-chlorobenzenethiol and 1217 mg (7.29 mmol) of ethyl iodoacetate in 100 mL of dry DMF was added 1509 mg (10.94 mmol) of K_2CO_3. The mixture was heated to 90 °C for 18 h. The mixture was poured into water, and the solids were collected by filteration and crystallized from EtOAc−hexanes to yield 1203 mg, 72% yield.

To a stirred solution of 1016 mg (4.40 mmol) of the above ester in 5 mL of THF was added 3 mL of 5 N NaOH. The reaction was stirred for 18 h and then poured into water. The mixture was made acidic with 1 N HCl, and the solids were filtered, washed with water, and dried to yield 810 mg (91%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.81 (s, 1 H) 7.36 (m, 4 H) 3.83 (s, 2 H); Anal. Calcd for $C_8H_7ClO_2S$: C, H, N.

***N*-(2-Acetyl-4-chlorophenyl)glycine (10a).** (**2-Acetyl-4-chlorophenylamino)acetic Acid Ethyl Ester.** To a stirred solution of 1026 mg (6.05 mmol) of 1-(2-amino-5-chlorophenyl)ethanone (**7a**) in 10 mL of dry DMF were added 1.98 mL (16.8 mmol) of ethyl iodoacetate and 4.3 g (30.8 mmol) of K_2CO_3. The vessel was sealed then heated to 90 °C for 2 h. The mixture was cooled and then poured into 200 mL of H_2O, followed by extracting into 2 × 150 mL of Et_2O. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated *in vacuo*. The crude material was purified by silica gel chromatography using 5% EtOAc/hexanes to yield 850 mg (56%); ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.20 (s, 1H), 7.73 (d, 1H, J = 2.3 Hz), 7.31 (dd, 1H, J = 9.1, 2.5 Hz), 6.50 (d, 1H, J = 9.1 Hz), 4.25 (q, 2H, J = 7.1 Hz), 3.99 (d, 2H, J = 5.5 Hz), 2.58 (s, 3H), 1.30 (t, 3H, J = 7.2 Hz); Anal. Calcd for $C_{12}H_{14}ClNO_3$: C, H, N.

To a stirred solution of 649 mg (2.54 mmol) of (2-acetyl-4-chlorophenylamino)acetic acid ethyl ester in 25 mL of MeOH was added 25 mL of 1 N NaOH. The mixture was stirred 1 h and then poured into 150 mL of 1 N HCl. The solids were filtered off and crystallized from MeOH. 373 mg (51%); ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.05 (s, 1H), 7.74 (d, 1H, J = 2.8 Hz), 7.31 (dd, 1H, J = 9.1, 2.5 Hz), 6.58 (d, 1H, J = 9.1 Hz), 3.33 (s, 2H, obscured), 2.51 (s, 3H, obscured); Anal. Calcd for $C_{10}H_{10}ClNO_3$·Na·2H_2O: C, H, N.

2-[2-(2-Acetyl-4,5-dichlorophenylamino)acetylamino]benzoic Acid Methyl Ester (11f): To a solution of 610 mg (3.00 mmol) of **7f** and 816 mg (3.00 mmol) of 2-(2-bromoacetylamino)benzoic acid methyl ester in 50 mL of dry toluene were added 0.78 mL (4.50 mmol) of DIPEA and 75 mg of tetrabutylammonium iodide. The solution was heated to 90 °C for 18 h. The solution was concentrated *in vacuo*, dissolved in DCM, and filtered through a pad of magnesol. The solution was concentrated *in vacuo* and crystallized from EtOH. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.96 (s, 1H), 9.18 (t, 1H, J = 5.7 Hz), 8.40 (d, 1H, J = 8.6 Hz), 8.09 (s, 1H), 7.91 (dd, 1H, J = 7.9, 1.5 Hz), 7.63 (td, 1H, J = 8.0, 1.6 Hz), 7.20 (td, 1H, J = 7.6, 1.0 Hz), 6.96 (s, 1H), 4.24 (d, 2H, J = 5.9 Hz), 3.78 (s, 3H), 2.63 (s, 3H); Anal. Calcd for $C_{18}H_{16}Cl_2N_2O_4$: C, H, N.

2-({[(4-Chlorophenyl)sulfanyl]acetyl}amino)benzoic Acid (13). To a stirred solution of 300 mg (1.48 mmol) of **9** in 10 mL of dry DCM and 10 mL of dry DMF were added 796 mg (1.80 mmol) of BOP and 0.31 mL (1.80 mmol) of DIPEA. To this solution was added 202 mg (1.48 mmol) of anthranilic acid. The mixture was stirred 48 h and then poured into 50 mL of 1 N HCl. The mixture was extracted with 2 × 100 mL of Et_2O, and the organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. The crude material was purified by silica gel chromatography using 5% MeOH/DCM to yield 125 mg (39%) of the desired compound. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.70 (s, 1H), 8.50 (d, 1H, J = 12.0 Hz), 7.95, (d, 1H, J = 12.0 Hz), 7.55 (t, 1H, J = 8.8 Hz), 7.35−7.45 (m, 5H), 4.05 (s, 2H); Anal. Calcd for $C_{15}H_{12}ClNO_3S$·0.25H_2O: C, H. N; MS (ESI, (M − H$^-$)) *m*/*z* 319.96.

2-({*N*-(2-Acetyl-4-chlorophenyl)glycyl]amino}benzoic Acid (14a). To a stirred solution of 317 mg (1.39 mmol) of **10a** in 10 mL of dry DCM and 10 mL of dry DMF were added 753 mg (1.70 mmol) of BOP and 0.30 mL (1.70 mmol) of DIPEA. To this solution was added 210 mg (1.39 mmol) of anthranilic acid. The mixture was stirred 48 h and then poured into 50 mL of 1 N HCl. The mixture was extracted with 2 × 100 mL of Et_2O, and the organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. The crude material was purified by silica gel chromatography using 5% MeOH/DCM to yield 130 mg (27%) of the desired compound. ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.51 (bs, 1H), 11.67 (s, 1H), 9.18 (s, 1H), 8.63 (d, 1H, J = 8.3 Hz), 7.93 (dd, 1H, J = 7.8, 1.3 Hz), 7.90 (d, 1H, J = 7.9 Hz), 7.59 (td, 1H, J = 7.9, 1.5 Hz), 7.42 (dd, 1H, J = 8.9, 2.4 Hz), 7.14 (t, 1H, J = 7.4 Hz), 6.67 (d, 1H, J = 9.1 Hz) 4.15 (d, 2H, J = 5.8 Hz), 2.61 (s, 3H); Anal. Calcd for $C_{17}H_{15}ClN_2O_4$·2H_2O: C, H, N; MS (ESI, M − H) *m*/*z* 345.1.

2-[2-(2-Acetyl-4,5-dichlorophenylamino)acetylamino]benzoic Acid (14f). To a solution of **11f** (200 mg, 0.51 mmol) in EtOH (5 mL) and tetrahydrofuran (5 mL) was added sodium hydroxide solution (1 N, 5 mL), and the reaction mixture was stirred for 2 h at room temperature. The reaction mixture was partitioned between water and methylene chloride, and the aqueous layer was acidified with 1 N HCl solution and extracted with methylene chloride. The latter was dried over sodium sulfate and concentrated to give 155 mg (41%) of the desired product. The compound can be crystallized from EtOH. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.54 (bs, 1H), 11.56 (s, 1H), 9.23 (t, 1H, J = 5.9 Hz), 8.61 (d, 1H, J = 8.4 Hz), 8.09 (s, 1H), 7.96 (dd, 1H, J = 8.1, 1.3 Hz), 7.61 (td, 1H, J = 7.9, 1.7 Hz), 7.16 (td, 1H, J = 7.8, 1.0 Hz), 6.94 (s, 1H), 4.23 (d, 2H, J = 5.9 Hz), 2.62 (s, 3H); Anal. Calcd for $C_{17}H_{14}Cl_2N_2O_4$*0.14 H_2O: C, H, N; MS (ESI, M − H) *m*/*z* 379.1.

2-({[(2*S*)-2-(4-Chlorophenoxy)propanoyl]amino}benzoic Acid (17a). (*S*)-2-(4-Chlorophenoxy)propionic Acid Methyl Ester. To a solution of 4-chlorophenol (1.36 g; 10.6 mmol), (*R*)-methyl 2-hydroxypropanoate (1.00 g; 9.6 mmol), and triphenylphosphine (3.04 g; 11.6 mmol) in 25 mL of dry DCM was added neat diethyl azodicarboxylate (1.8 mL; 1.99 g; 11.4 mmol) dropwise over 0.25 h and was stirred at room temperature for an additional 1 h. Water was added, and the layers were separated. After the aqueous layer was washed with a second portion of DCM, the combined organic layers were washed brine, dried over $MgSO_4$, and filtered through silica gel. Concentration *in vacuo* gave an oil that was loaded onto silica gel and eluted with 10% to 20% EtOAc/hexanes to give the desired product as an oil (1.55 g; 75%) ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.22 (d, 2H, J = 9 Hz), 6.80 (d, 2H, J = 9 Hz), 4.72 (q, 1H, J = 7 Hz), 3.75 (s, 3H), 1.61 (d, 3 H, J = 7 Hz); Anal. Calcd for $C_{10}H_{11}ClO_3$: C, H.

(***S*)-2-(4-Chlorophenoxy)propionic Acid**. (*S*)-2-(4-Chlorophenoxy)propionic acid methyl ester (1.01 g; 4.7 mmol) was dissolved in MeOH (20 mL), and 1 N NaOH (7.0 mL; 7.0 mmol) was added. The reaction was stirred overnight at room temperature, and the volatiles were removed in vacuo. The resulting residue was then partitioned between water and ether. The aqueous layer was drawn off and acidified with concentrated HCl to pH 2, which was then extracted with EtOAc. The organic layers were combined, washed with brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo* to give the desired product as a white crystalline solid (0.85 g; 90%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.35 (bs, 1H), 7.24 (d, 2H, J = 9 Hz), 6.82 (d, 2H, J = 9 Hz), 4.75 (q, 1H, J = 7 Hz), 1.66 (d, 3 H, J = 7 Hz); Anal. Calcd for $C_9H_9ClO_3$: C, H.

To a solution of (*S*)-2-(4-chlorophenoxy)propanoic acid (0.53 g = 2.6 mmol) in DCM (10 mL) was added oxalyl chloride (2 mL of a 2.0 M DCM solution; 4.0 mmol) and 2 drops of DMF. The reaction was stirred at room temperature for 5 h and concentrated to give the crude acid chloride as a yellow syrup. This syrup was treated with anthranilic acid (0.36 g = 2.6 mmol), and 1 N NaOH (5.0 mL; 5.0 mmol) was added. After being stirred at room

temperature for 0.5 h, during which time an oil separated, the mixture was acidified with concentrated aqueous HCl to pH 3 and extracted with ether twice. The organic layer was dried over $MgSO_4$, filtered, and concentrated *in vacuo* to give the desired product as a light brown solid 630 mg (75%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.8 (s, 1H), 11.3 (bs, 1H), 8.82 (d, 1H, $J = 9$ Hz), 8.13 (dd, 1H, $J = 1.6$, 8 Hz), 7.63 (dt, 1H, $J = 1.6$, 8 Hz), 7.22 (m, 2H), 6.94 (d, 2H, $J = 9$ Hz), 6.86 (d, 1H, $J = 9$ Hz), 4.83 (q, 1H, $J = 6.8$ Hz), 1.69 (s, 3H, $J = 6.8$ Hz); HRMS: calcd for $C_{16}H_{14}ClNO_4 + H^+$, 320.06841; found (ESI-FTMS, $[M + H]^+$), 320.06847.

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Supporting Information Available: Additional synthetic procedures and characterization data. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Matsuura, Y. Expression and characterization of hepatitis C virus (HCV) proteins. *Uirushu* **1995**, *45*, 105−115.
(2) Farci, P.; Alter. J. H.; Shimoda, A.; Govindarajan, S.; Cheung, L. C.; Melpolder, J. C.; Sacher, R. A.; Shih, J. W.; Purcell, R. H. Hepatitis C virus−associated fulminant hepatic failure. *N. Engl. J. Med*. **1996**, *335*, 631−634.
(3) Hoofnagle, J. H. Hepatitis C−the clinical spectrum of disease. *Hepatology* **1997**, *26*, S15−S20.
(4) McHutchison. J. G. Understanding hepatitis C. *Am. J. Manag. Care* **2004**, *10*, S21−29.
(5) National Institutes of Health Consensus Development Conference Panel. National Institutes of Health Consensus Development Conference Panel statement: management of hepatitis C. *Hepatology* **1997**, *26(suppl 1*), 2S−10S.
(6) Alter, M. J.; Kruszon-Moran, D.; Nainan, O. V.; McQuillan, G. M.; Gao, F.; Moyer, L. A.; Kaslow, R. A.; Margolis, H. S. The prevalence of hepatitis C virus infection in the United States, 1988 through 1994. *N. Engl. J. Med*. **1999**, *341*, 556−562.
(7) Tan, S.; Pause, A.; Shi, Y.; Sonenberg, N. Hepatitis C therapeutics: Current status and emerging strategies. *Nat. Rev. Drug Discovery* **2002**, *11*, 867−881.
(8) McHutchison, J. G.;. Gordon, S. C; Schiff, E.R.; Shiffman, M. L.; Lee, W. M.; Rustgi, V. K.; Goodman, Z. D.; Ling, M.; Cort, S.; Albrecht, J. K. Interferon alfa-2b alone or in combination with ribavirin as initial treatment for chronic hepatitis C. *N. Engl. J. Med*. **1998**, *339*, 1485−1492.
(9) Manns, M. P.; McHutchison, J. G.; Gordon, S. C.; Rustgi, V. K.; Shiffman, M.; Reindollar, R.; Goodman, Z. D.; Koury, K.; Ling, M.; Albrecht, J. K. Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. *Lancet* **2001**, *358*, 958−965.
(10) Asahina, Y.; Izumi, N.; Enomoto, N.; Kurosaki, M.; Onuki, Y.; Nishimura, Y.; Ueda, K.; Tsuchiya, K.; Nakanishi, H.; Kitamura, T.; Miyake, S. Mutagenic effects of ribavirin and response to interferon/ ribavirin combination therapy in chronic hepatitis C. *J. Hepatol.* **2005**, *43*, 623−629.
(11) Dixit, N. M.; Perelson. The metabolism, pharmacokinetics and mechanisms of antiviral activity of ribavirin against hepatitis C virus. *Cell Mol. Life Sci*. **2006**, *63*, 832−842.
(12) (a) Pawlotsky, J.; McHutchison, J. G. Hepatitis C. Development of new drugs and clinical trials: promises and pitfalls. Summary of an AASLD Hepatitis Single Topic Conference, Chicago, IL, February 27-March 1, 2003. (b) Nakagawa, S.; Umehara, T.; Matsuda, C.; Kuge, S.; Sudoh, M.; Kohara. M. Hsp90 inhibitors suppress HCV replication in replicon cells and humanized liver mice. *Biochem. Biophys. Res. Commun.* **2007**. 353. 882−888.
(13) McHutchison, J. G.; Manns, M.; Keyur, P.; Poynard, T.; Lindsay, K. L.; Trepo, C.; Dienstag, J.; Lee, W. M.; Mak, C.; Garaud, J.; Albrect, J. K. Adherence to combination therapy enhances sustained response in genotype-1-infected patients with chronic hepatitis C. *Gastroenterology* **2002**, *123*, 1061−1069.
(14) (a) Gordon, C. P.; Keller, P. A. Control of hepatitis C: A medicinal chemistry perspective. *J. Med. Chem.* **2005**, *48*, 1−20. (b) De Francesco, R.; Migliaccio, G. Challenges and successes in developing new therapies for hepatitis C. *Nature* **2005**, *436*, 953−960.
(15) (a) Carroll, S. S.; Olsen, D. B. Nucleoside analog inhibitors of hepatitis C virus replication. *Infectious Disorders: Drug Targets* **2006**, *6*, 17−29. (b) Yan, S.; Appleby, T.; Gunic, E.; Shim, J. H.; Tasu, T.; Kim, H.; Rong, F.; Chen, H.; Hamatake, R.; Wu, J. Z.; Hong, Z.; Yao, N. Isothiazoles as active-site inhibitors of HCV NS5B polymerase. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 28−33.
(16) (a) Koch, U.; Narjes, F. Allosteric inhibition of the hepatitis C virus NS5B RNA dependent RNA polymerase. *Infect. Disord.: Drug Targets* **2006**, *6*, 31−41. (b) Beaulieu, P. L. The discovery of finger loop inhibitors of the hepatitis C virus NS5B polymerase: status and prospects for novel HCV therapeutics. *IDrugs* **2006**, *9*, 39−43.
(17) (a) Gopalsamy, A.; Shi, M.; Ciszewski, G.; Park, K.; Ellingboe, J. W.; Orlowski, M.; Feld, B.; Howe, A. Y. M. Design and synthesis of 2,3,4,9-tetrahydro-1H-carbazole and 1,2,3,4-tetrahydro-cyclopenta-[b]indole derivatives as non-nucleoside inhibitors of hepatitis C virus NS5B RNA-dependent RNA polymerase. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 2532−2534. (b) Gopalsamy, A; Aplasca, A.; Ciszewski, G.; Park, K.; Ellingboe, J. W.; Orlowski, M.; Feld, B; Howe, A. Y. M. Design and synthesis of 3,4-dihydro-1H-[1]-benzothieno[2,3-c]-pyran and 3,4-dihydro-1H-pyrano[3,4-b]benzofuran derivatives as non-nucleoside inhibitors of HCV NS5B RNA dependent RNA polymerase. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 457−460. (c) Gopalsamy, A.; Lim, K.; Ciszewski, G.; Park, K.; Ellingboe, J. W.; Bloom, J.; Insaf, S.; Upeslacis, J.; Mansour, T. S.; Krishnamurthy, G.; Damarla, M.; Pyatski, Y.; Ho, D.; Howe, A. Y. M.; Orlowski, M.; Feld, B.; O'Connell, J. Discovery of Pyrano[3,4-*b*]indoles as Potent and Selective HCV NS5B Polymerase Inhibitors. *J. Med. Chem.* **2004**, *47*, 6603−6608. (d) Gopalsamy, A.; Lim, K.; Ellingboe, J. W.; Krishnamurthy, G.; Orlowski, M.; Feld, B.; van Zeijl, M.; Howe, A. Y. M. Identification of [(naphthalene-1-carbonyl)-amino]-acetic acid derivatives as nonnucleoside inhibitors of HCV NS5B RNA dependent RNA polymerase. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 4221−4224.
(18) Gopalsamy, A.; Chopra, R.; Lim, K.; Ciszewski, G.; Shi, M.; Curran, K. J.; Sukits, S. F.; Svenson, K.; Bard, J.; Ellingboe, J. W.; Agarwal, A.; Krishnamurthy, G.; Howe, A. Y. M.; Orlowski, M.; Feld, B.; O'Connell, J.; Mansour, T. S. Discovery of proline sulfonamides as potent and selective hepatitis C virus NS5b polymerase inhibitors. evidence for a new NS5b polymerase binding site. *J. Med. Chem.* **2006**, *49*, 3052−3055.
(19) (a) Powers, J. P.; Piper, D. E.; Li, Y.; Mayorga, V.; Anzola, J.; Chen, J. M.; Jaen, J. C.; Lee, G.; Liu, J.; Peterson, M. G.; Tonn, G. R.; Ye, Q.; Walker, N. P. C.; Wang, Z. SAR and mode of action of novel non-nucleoside inhibitors of hepatitis C NS5b RNA polymerase. *J. Med. Chem.* **2006**, *49*, 1034−1046. (b) Pfefferkorn, J. A.; Greene, M. L.; Nugent, R. A.; Gross, R. J.; Mitchell, M. A.; Finzel, B. C.; Harris, M. S.; Wells, P. A.; Shelly, J. A.; Anstadt, R. A.; Kilkuskie, R. E.; Kopta, L. A.; Schwende, F. J. Inhibitors of HCV NS5B polymerase. Part 1: Evaluation of the southern region of (2Z)-2-(benzoylamino)-3-(5-phenyl-2-furyl)acrylic acid. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 2481−2486. (c) Pfefferkorn, J. A.; Nugent, R.; Gross, R. J.; Greene, M.; Mitchell, M. A.; Reding, M. T.; Funk, L. A.; Anderson, R.; Wells, P. A.; Shelly, J. A.; Anstadt, R.; Finzel, B. C.; Harris, M. S.; Kilkuskie, R. E.; Kopta, L. A.; Schwende, F. J. Inhibitors of HCV NS5B polymerase. Part 2: Evaluation of the northern region of (2Z)-2-benzoylamino-3-(4-phenoxy-phenyl)-acrylic acid. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 2812−2818.
(20) Sugasawa, T.; Toyoda, T.; Adachi, M.; Sasakura, K. Aminohaloborane in organic synthesis. 1. Specific ortho substitution reaction of anilines. *J. Am. Chem. Soc*. **1978**, *100*, 4842−4852.
(21) Rao, A. V. R.; Gaitonde, A. S.; Prakash, K. R. C.; Rao, S. P. A concise synthesis of chiral 2-methyl chroman-4-ones: Stereo selective build-up of the chromanol moiety of anti-HIV agent, calanolide A. *Tetrahedron Lett.* **1994**, *35*, 6347−6350.
(22) Howe, A. Y.; Bloom, J.; Baldick, C.; Christophers, J.; Cheng, H.-M.; Christensen, J. S.; Chunduru, S. K.; Coburn, G. A.; Feld, B.; Gopalsamy, A.; Gorczyca, W. P.; Herrman, S.; Johann, S.; Jiang, X.; Kimberland, M. L.; Krishnamurthy, G.; Olson, M. W.; Orlowski, M.; Swanberg, S.; Thompson, I.; Thorn, M.; Del Vecchio, A. M.; Young, D. C.; van Zeijl, M.; Ellingboe, J. W.; Upeslacis, J.; Collett, M. S.; Mansour, T. S.; O'Connell, J. Indentification of a novel non-nucleoside inhibitor of hepatitis C virus RNA-dependent RNA polymerase. *Antimicrob. Agents Chemother.* **2004**, *48*, 4813−4821.

(23) Liu, C.; Chopra, R.; Swanberg, S.; Olland, S.; O'Connell, J.; Herrmann, S. Elongation of synthetic RNA templates by hepatitis C virus NS5B polymerase. *J. Biol. Chem.* **2004**, *279*, 10738−10746.
(24) CCP4. 1994. The CCP4 suite: programs for protein crystallography. *Acta Crystallogr. D50*, 760−763.
(25) Lesburg, Charles A.; Cable, Michael B.; Ferrari, Eric; Hong, Zhi; Mannarino, Anthony F.; Weber, Patricia C. Crystal structure of the RNA-dependent RNA polymerase from hepatitis C virus reveals a fully encircled active site. *Nat. Struct. Biol.* **1999**, *6*, 937−943.

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