Journal of Medicinal Chemistry

Volume 50, Number 5 March 8, 2007

Letters

Optimization of Novel Acyl Pyrrolidine Inhibitors of Hepatitis C Virus RNA-Dependent RNA Polymerase Leading to a Development Candidate

Martin J. Slater,* Elizabeth M. Amphlett, David M. Andrews, Gianpaolo Bravi, George Burton, Anne G. Cheasty, John A. Corfield, Malcolm R. Ellis, Rebecca H. Fenwick, Stephanie Fernandes, Rossella Guidetti, David Haigh, C. David Hartley, Peter D. Howes, Deborah L. Jackson, Richard L. Jarvest, Victoria L. H. Lovegrove, Katrina J. Medhurst, Nigel R. Parry, Helen Price, Pritom Shah, Onkar M. P. Singh, Richard Stocker, Pia Thommes, Claire Wilkinson, and Alan Wonacott

Infectious Diseases CEDD and Medicinal Discovery Research, GlaxoSmithKline, GSK Medicines Research Centre, Gunnels Wood Road, Stevenage SG1 2NY, United Kingdom

Received October 16, 2006

Abstract: Optimization of a pyrrolidine-based template using structure-based design and physicochemical considerations has provided a development candidate **20b** (**3082**) with submicromolar potency in the HCV replicon and good pharmacokinetic properties.

An estimated 3% of the global human population is infected by hepatitis C virus (HCV),[1] an infection that often leads to cirrhosis, hepatocellular carcinoma, and liver failure in later life. It has been estimated that of those currently infected, 20% and 4% are likely to develop liver cirrhosis and liver cancer, respectively, in the next decade.[2] The current gold standard therapies are based upon the use of pegylated interferon-α in combination with ribavirin. These therapies provide a sustained virological response in around 50% of patients infected with genotype 1 and have the disadvantage of frequent and severe side effects.[3,4] The development of new therapies to treat HCV infection effectively is, therefore, of paramount importance and is currently an intensive area of research.[5]

HCV is a small, enveloped virus, the genome of which is a 9.5 kb single-stranded RNA that encodes for a single large polyprotein of 3010−3030 amino acids. This polyprotein is processed by cellular signal peptidases to produce the structural viral proteins, whereas viral proteases (NS2, NS3) are respon-

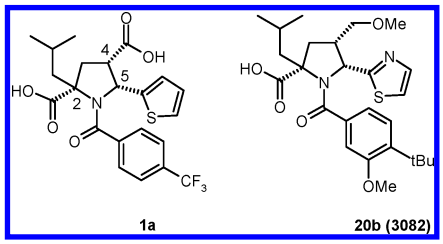
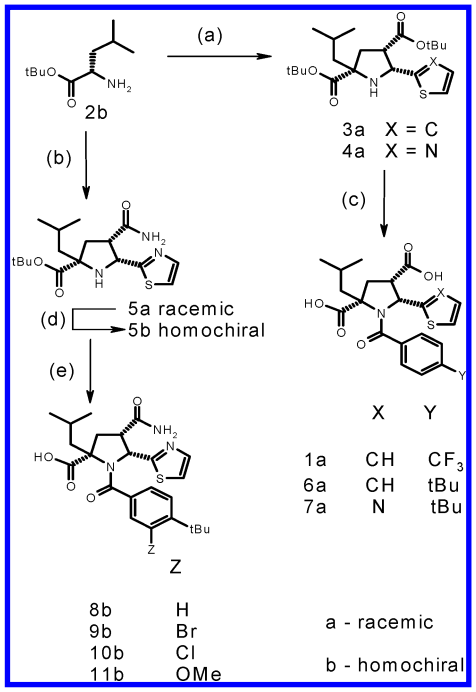


Figure 1.

Scheme 1. Synthesis of C4 Acids and C4 Primary Amides[a]



[a] Reagents: (a) (i) 2-thiophenecarboxaldehyde for **3a** or 2-thiazolecarboxaldehyde for **4a**, DCM; (ii) *tert*-butyl acrylate, LiBr, Et_3N, THF; (b) (i) 2-thiazolecarboxaldehyde, DCM; (ii) acrylamide, LiBr, Et_3N, THF; (c) (i) 4-CF_3 benzoyl chloride or 4-*tert*-butyl benzoyl chloride, Et_3N, DCM; (ii) TFA, DCM; (d) (i) (−)-di-*O*,*O*′-*p*-tolyl-L-tartaric acid, DCM; (ii) $NaHCO_3$; (e) (i) benzoyl chloride derivative, Et_3N, DCM; (ii) TFA, DCM.

* To whom correspondence should be addressed. Phone: +44 1438 763416. Fax: +44 1462 763620. E-mail: martin.j.slater@gsk.com.

10.1021/jm061207r CCC: $37.00
Published on Web 02/02/2007

Table 1. Enzyme, Replicon, and Selected Rat PK Data for 4-Carboxy and 4-Amido Acyl Pyrrolidine Derivatives

cmpd	chirality[a]	HCV polymerase[b] 1b FL (RB01) $IC_{50}/\mu M$	HCV replicon 1b ELISA $EC_{50}/\mu M$	Vero toxicity $CCID_{50}/\mu M$	rat[c] *F* %	rat[c] iv clearance mL/min/kg	ClogD[14]
1a	rac	20	>20	>500			−0.8
6a	rac	1.1	>20	>500			−0.1
7a	rac	3.8	>20	>500			−0.5
8b	h	0.97	>20	430	2	21	0.6
9b	h	0.014	0.57	>500	2	24	0.6
10b	h	0.020	1.2	>500	5	29	0.5
11b	h	0.005	0.61	>500	0.6	37	0.2

[a] rac = racemic and h = homochiral. [b] Filtermat assay using full length 1b enzyme and polyUrA homopolymer substrate.[15] [c] Dosed at 2 mg/kg oral and 1 mg/kg iv as a solution in 10% aqueous DMSO, $n = 3$ rats.

sible for the production of mature nonstructural proteins. These include the RNA-dependent RNA polymerase (RdRp, NS5B), which synthesizes the new viral RNA strands. It is a well characterized enzyme, essential for viral replication, with no known mammalian equivalent and thus represents an attractive target for the development of novel anti-HCV agents.[6]

A diverse range of non-nucleoside inhibitors of the HCV NS5B polymerase have been reported, including dihydroxypyrimidine carboxylic acids, benzimidazole derivatives, benzothiadiazines, thiophene carboxylic acids, phenylalanine derivatives, dihydropyrones, aminothiazoles, and pyranoindoles.[7] Recently, we disclosed the acyl pyrrolidine series identified through a high throughput screening program against the enzyme[8] (e.g., **1a**,[9] Figure 1). Coordinates for the structures of **1a** and **7a** bound to HCV polymerase BKd21 have been deposited in the Protein Data Bank under accession codes 2jc0 and 2jc1, respectively, and herein, we describe further optimization of the series leading to the identification of the development candidate **20b** (**3082**).

The previous report described a solid-phase approach to exploration of the structure−activity relationships (SAR) of acyl pyrrolidines.[8] These contained two carboxylic acid motifs, providing the lead molecule bearing a 4-trifluoromethyl benzoyl group at N1 and a 2-thiophene at C5 (**1a**, Figure 1). Separation of the racemic compound by chiral HPLC demonstrated that the enzyme activity resided with the (2*S*,4*S*,5*R*) enantiomer.[8,9] Although the dicarboxylic acids displayed good inhibitory activity in the polymerase biochemical assay, they were all inactive in the cellular HCV replicon assay.[10] The initial objective we set was to achieve significant inhibition in the HCV replicon, and our first approach was to replace the carboxylic acid at C4 by a primary amide. We exploited the solution-phase cycloaddition procedures described by Grigg and co-workers.[11] Imines derived from amino acids and aldehydes were reacted with *tert*-butyl acrylate or acrylamide as the dipolarophiles and with lithium bromide and triethylamine in THF. This procedure formed the racemic pyrrolidines in good yields, with excellent regiochemical and stereochemical control (Scheme 1). Standard acylation with a benzoyl chloride derivative and triethylamine followed by TFA deprotection of the *tert*-butyl esters afforded the racemic targets (**1a**, **6a**, and **7a**). Replacement of the 4-CF_3 group by a bulkier 4-*tert*-butyl in the benzamide gave a significant improvement in potency against the enzyme (compare **1a** and **6a**, Table 1). Another key SAR finding was that combining a 2-thiazole at C5 (in place of 2-thiophene) with a primary amide at C4 also provided good polymerase activity (**7a** vs **8b**). This indicated that we could dispense with the C4 carboxylic acid, although activity in the HCV replicon assay was still elusive.

Structure-based design played a key role in further potency enhancements. Soaking the acids **1a** and **7a** into polymerase crystals (1b BKΔ21) showed that these molecules bind in the palm region of the polymerase enzyme in an allosteric pocket close to the catalytic site (Figure 2). Moreover, **1a** and **7a** have different binding modes in which the bulkier *tert*-butyl substituent on the benzamide causes movement in the side chain of Leu384 and allows **7a** to bind deeper into the pocket (Figure 3). This is consistent with the improved potency of the *tert*-butyl containing compounds. A consequence of this binding mode is that a small lipophilic pocket is created, which is potentially accessible from the 3-position of the benzamide, which we sought to exploit.

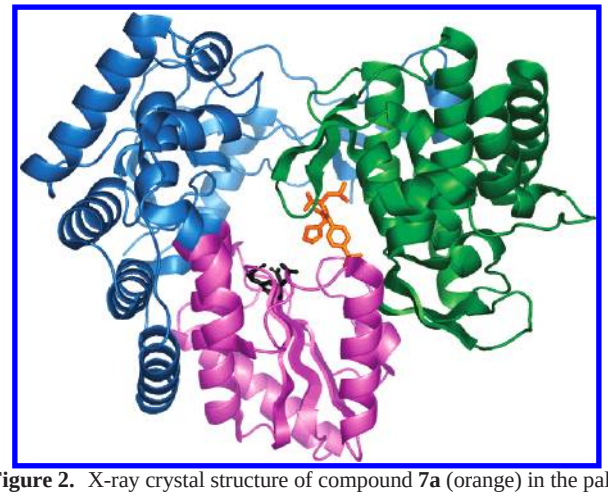


Figure 2. X-ray crystal structure of compound **7a** (orange) in the palm binding site of HCV polymerase; the catalytic aspartic acid residues are indicated in black.

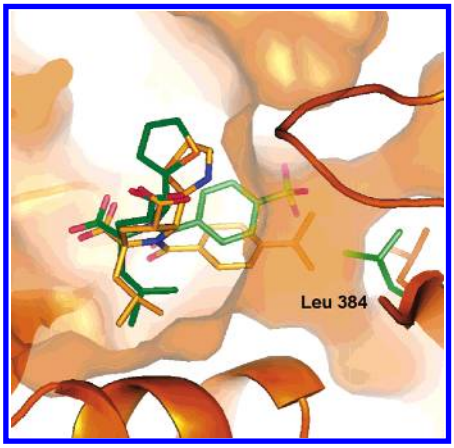
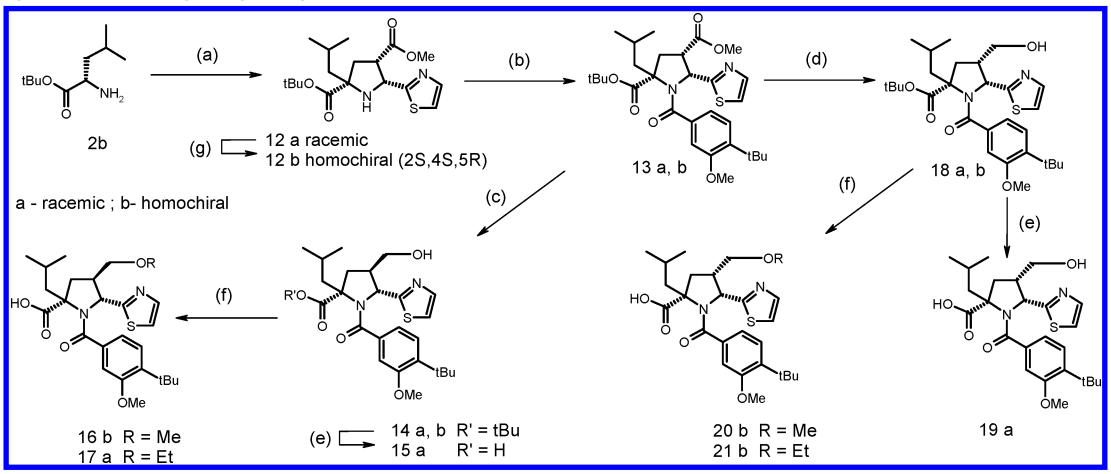


Figure 3. Comparison of the X-ray structures of compounds **1a** (green) and **7a** (orange), showing the different binding modes and movement of Leu384 to accommodate the *tert*-butyl group in **7a**. For clarity, the protein surface is shown only for **7a** (except for the side chain of Leu384).

Racemic pyrrolidine **5a** was efficiently resolved using (−)-di-*O*,*O*′-*p*-tolyl-L-tartaric acid in dichloromethane. The chiral pyrrolidine **5b** was acylated under standard conditions with the

Scheme 2. Synthesis of C4 Hydroxymethyl Derivatives[a]



[a] Reagents: (a) (i) 2-thiazolecarboxaldehyde, DCM; (ii) methyl acrylate, LiBr, Et_3N, THF; (b) 3-methoxy-4-*tert*-butyl benzoyl chloride, Et_3N, DCM; (c) $LiBH_4$, THF; (d) $LiAlH_4$, −40 °C, THF; (e) TFA, DCM; for **16b** and **20b**, (f) MeI, NaH, DMF then TFA, DCM; for **17a** and **21b**, (f) EtI, NaH, DMF then TFA, DCM; (g) chiral resolution **12a** into **12b**, (*R*)-(−)-1,1′-binaphthyl-2,2′-diyl-hydrogen phosphate, *i*-PrOH, 90 °C, then Et_3N, Et_2O to form the free base.

Table 2. Enzyme, Replicon, and Selected Rat PK Data for 4-Alkoxymethyl and 4-Hydroxymethyl Acyl Pyrrolidine Derivatives

cmpd	chirality[a]	HCV polymerase[b] 1b FL (RB01) $IC_{50}/\mu M$	HCV replicon 1b ELISA $EC_{50}/\mu M$	Vero toxicity $CCID_{50}/\mu M$	rat[c] *F* %	rat[c] iv clearance mL/min/kg	ClogD[14]
15a	rac	1.0	1.3	482	11	39	0.6
16b	h	2.3	5.7	265	44	12	1.2
17a	rac	3.2	12.8	214			1.7
19a	rac	0.73	0.21	358	12	36	0.6
20b	h	0.44	0.39	240	59	5	1.2 (2.2)[d]
21b	h	0.43	0.47	142	120	5	1.7 (2.7)[d]

[a] rac = racemic and h = homochiral. [b] SPA assay using full length 1b enzyme and rCrG homopolymer substrate.[15] [c] Dosed at 2 mg/kg oral and 1 mg/kg iv as a solution in aqueous 10% DMSO, $n = 3$ rats. [d] Measured logD.

3-bromo, 3-chloro, and 3-methoxy derivatives of 4-*tert*-butyl benzoyl chloride, and the esters deprotected to form **9b**, **10b**, and **11b** (Scheme 1). These compounds were very potent in the enzyme assay, with IC_{50} values 50−100-fold better than the unsubstituted compound **8b** and additionally delivered very good potency in the HCV replicon assay, with the 3-bromo and 3-methoxy containing analogues (**9b** and **11b**) now achieving submicromolar EC_{50} values (Table 1).

All of the amides **8b**−**11b** had poor oral bioavailability in the rat ($F \leq 5\%$, Table 1). Because clearance from the plasma compartment was moderate (24−42% of liver blood flow), we considered that poor membrane permeability may be an issue. Although formally these molecules comply with Lipinski's rule of 5,[12] calculated logD (ClogD) values are in the range 0.2−0.6. We reasoned that replacing the C4-amide with a more lipophilic moiety while maintaining molecular weight below 500 may improve permeability and deliver improved PK properties. C4-Methyl ether derivatives were selected as appropriate targets that met these criteria.

Reacting methyl acrylate with the imine derived from leucine *tert*-butyl ester (**2b**) and 2-thiazole carboxaldehyde gave racemic pyrrolidine **12a** in excellent yield in our standard cycloaddition conditions (Scheme 2). Initially this was processed in racemic form to give the benzamide derivative **13a**. We selected the 3-methoxy substituent over the equipotent 3-bromo substituent as the 3-methoxy series had superior solubility properties and lower molecular weights. To resolve the racemic pyrrolidine **12a**, in this case, (*R*)-(−)-1,1′-binaphthyl-2,2′-diyl-hydrogen phosphate in hot isopropanol was effective, providing the required salt in good yield with high ee (98.8%).[13] As the phosphate salt had poor solubility in dichloromethane and many other solvents, prior to the acylation step it was necessary to liberate the pyrrolidine base **12b** using triethylamine in ether. Standard reaction conditions with 3-methoxy-4-*tert*-butyl benzoyl chloride then afforded **13b**. A number of reducing reagents were evaluated to convert the methyl ester to the desired alcohol. Lithium borohydride in THF gave a mixture of alpha and beta methyl alcohols, in which the beta methyl alcohol **14a** was the major product (70% yield) and could be separated from the minor alpha epimer using silica chromatography. Lithium aluminum hydride reduction of **13a** at −40 °C gave a good yield of the alpha methyl alcohol **18a**. It was crucial to maintain the temperature around −40 °C for this reaction, as at higher temperatures, a significant quantity of the beta epimer **14a** was formed due to epimerisation of the C4-ester prior to reduction. Additionally, partial reduction of the benzamide (giving 3-methoxy-4-*tert*-butyl benzoic acid after work up) was observed at 0 °C. The racemic alcohols **14a** and **18a** were deprotected to give the C4-methyl alcohols **15a** (beta) and **19a** (alpha), respectively. The methyl ether derivatives were prepared by alkylation of the alcohols with methyl or ethyl iodide using sodium hydride in DMF and subsequently deprotected in the usual manner (Scheme 2).[16]

Potency and rat pharmacokinetic properties for these compounds are summarized in Table 2. In all cases, the alpha epimers are more potent than the corresponding beta epimers.[15] The racemic alcohols (**15a** and **19a**) have good activity in the replicon assay, especially the alpha methyl alcohol (**19a**) with an EC_{50} of 0.21 uM. However, both alcohols possess PK profiles in the rat similar to the C4-amides (poor absorption and moderate clearance). The beta methyl and ethyl ethers (**16b** and **17a**) have moderate activities in the replicon assay. Gratifyingly, the alpha methyl (**20b**) and ethyl ethers (**21b**) have very good potencies in this cellular assay, with EC_{50} values of 0.39 and

0.47 uM, respectively. Moreover, both **20b** and **21b** displayed excellent pharmacokinetic profiles in the rat, with good oral bioavailabilities and low iv clearance values. The stereochemistry of **21b** was confirmed as (2*S*,4*S*,5*R*) by single molecule X-ray crystallography. It is noteworthy that the beta methyl ether **16b** also demonstrated a good PK profile in the rat.

Compound **20b** thus achieved our objective of combining very good potency in the HCV replicon with a good PK profile in the rat and was selected for further development. In the marmoset, **20b** had a similarly good PK profile ($F = 50\%$, iv clearance = 10 mL/min/kg, 17% of liver blood flow). In further studies in the replicon system, negligible attenuation (~2-fold) of the potency was observed in the presence of human serum albumin (30 mg/mL); additionally, **20b** and interferon α-2a demonstrated a synergistic inhibitory effect in combination assays. A detailed biological profile of **20b** will be published in due course.[17]

In summary, we have described the optimization of the acyl pyrrolidine series leading to submicromolar inhibitors in the replicon. Replacing the polar amide at the C4-position with a lipophilic methoxymethyl moiety resulted in **20b**, a molecule combining very good replicon potency and good pharmacokinetics suitable for further development as an anti-HCV agent.

Acknowledgment. We thank Stephen Richards for NMR spectroscopic work, Bill Leavens for accurate mass determinations, Eric Hortense for chiral analysis and separations, Steve Jackson for chiral separations, and Tadeusz Skarzynski and Maire Convery for assistance with structural aspects.

Supporting Information Available: Experimental procedures for the preparation of compounds, spectral and analytical characterizing data, procedures for the biochemical assay, replicon assay, and Vero cytotoxicity assay, and rat pharmacokinetic studies. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Poordad, F. F.; Tran, T.; Martin, P. F. Developments in hepatitis C therapy during 2000−2002. *Expert Opin. Emerging Drugs* **2003**, *8* (1), 9−25.

(2) (a) Gerber, M. A. Relation of hepatitis C to hepatocellular carcinoma. *J. Hepatol*. **1993**, *17* (suppl 3), S108−111. (b) Alter, M. J. Epidemiology of hepatitis C. *Hepatology* **1997**, *26*, 62S−65S. (c) Hoofnagle, J. H. Hepatiitis C: The clinical spectrum of disease. *Hepatology* **1997**, *26*, 15S−20S. (d) Di Bisceglie, A. M. Hepatitis C. *Lancet* **1998**, *351*, 351−355. (e) Hoofnagle, J. H. Therapy of viral hepatitis. *Digestion* **1998**, *59*, 563−578.

(3) Cornberg, M.; Wedemeyer, H.; Manns, M. P. Treatment of chronic hepatitis C with PEGylated interferon and ribavirin. *Curr. Gastroenterol. Rep.* **2002**, *4*, 23−30.

(4) Hugle, T.; Cerny, A. Current therapy and new molecular approaches to antiviral treatment and prevention of hepatitis C. *Rev. Med. Virol.* **2003**, *13*, 361−371.

(5) (a) Pawlotsky, J.-M.; McHutchinson, J. G. Hepatitis C. Development of new drugs and clinical trials: promises and pitfalls. *Hepatology* **2004**, *39*, 554−567. (b) Penin, F.; Dubuisson, J.; Rey, F. A.; Moradpour, D.; Pawlotsky, J.-M. Structural biology of hepatitis C virus. *Hepatology* **2004**, *39*, 5−19.

(6) (a) Behrens, S. E.; Tomei, L.; De Francisco, R. Identification and properties of the RNA-dependent RNA polymerase of hepatitis C virus. *EMBO J.* **1996**, *15*, 12−22. (b) Kolykhalov, A. A.; Mihalik, K.; Feinstone, S. M.; Rice, C. M. Hepatitis C virus-encoded enzymatic activities and conserved RNA elements in the 3′-nontranslated region are essential for virus replication in vivo. *J. Virol.* **2000**, *74*, 2046−2051. (c) Ago, H.; Adachi, T.; Yoshida, A.; Yamamoto, M.; Habuka, N.; Yatsunami, K.; Miyano, M. Crystal structure of the RNA-dependent RNA polymerase of hepatitis C virus. *Structure* **1999**, *7*, 1417−1426. (d) Lesburg, C. A.; Cable, M. B.; Ferrari, E.; Hong, Z.; Mannarino, A. F.; Weber, P. 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. (e) Bressanelli, S.; Tomei, L.; Roussel, A.; Incitti, I.; Vitale, R. L.; Mathieu, M.; De Francesco, R.; Rey, F. A. Crystal structure of the RNA-dependent RNA polymerase of hepatitis C virus. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 13034−13039.

(7) (a) Beaulieu, L. P.; Tsantrizos, Y. S. Inhibitors of the HCV NS5B polymerase: New hope for the treatment of hepatitis C infections. *Curr. Opin. Invest. Drugs* **2004**, *5*, 838−850. (b) Gordon, C. P.; Keller, P. A. Control of hepatitis C: A medicinal chemistry perspective. *J. Med. Chem.* **2005**, *48*, 1−20. (c) Gopalsamy, A.; Aplaska, A.; Ciszewski, G.; Park, K.; Ellingboe, J. W.; Orlowski, M.; Feld, B.; Howe, A. Y. M. Design, and synthesis of 3,4-dihydro-1*H*-[1]-benzothieno[2,3-*c*]pyran and 3,4-dihydro-1*H*-[1]-benzofuran derivatives as non-nucleoside inhibitors of HCV NS5B RNA dependent RNA polymerase. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 457−460. (d) Aminothiazole, inhibitors of HCV RNA polymerase. *Bioorg. Med. Chem. Letters* **2005**, *15*, 115−119. (e) Chan, L.; Pereira, O.; Reddy, T. J.; Das, S. K.; Poisson, C.; Courchesne, M.; Proulx, M.; Siddiqui, A.; Yannopoulos, C. G.; Nguyen-Ba, N.; Roy, C.; Nasturica, D.; Moinet, C.; Bethell, R.; Hamel, M.; L'Heureux, L.; Maud, D.; Nicolas, O.; Cortemanche-Asselin, P.; Brunette, S.; Bilimoria, D.; Bedard, J. Discovery of thiophene-2-carboxylic acids as potent inhibitors of HCV NS5B polymerase and HCV subgenomic RNA replication. Part 2: Tertiary amides. *Bioorg. Med. Chem. Letters* **2004**, *14*, 797−800. (f) Li, H.; Tatlock, J.; Linton, A.; Gonzalez, J.; Borchardt, A.; Dragovich, P.; Jewell, T.; Prins, T.; Zhou, R.; Blazel, J.; Parge, H.; Love, R.; Hickey, M.; Doan, C.; Shi, S.; Duggal, R.; Lewis, C.; Fuhrman, S. Identification and structure-based optimization of novel dihydropyranones as potent HCV RNA polymerase inhibitors. *Bioorg. Med. Chem. Letters* **2006**, *16*, 4834−4838. (g) Tedesco, R.; Shaw, A. N.; Bambel, R.; Chai, D.; Concha, N. O.; Darcy, M. G.; Dhanak, D.; Fitch, D. M.; Gates, A.; Gerhardt, W. G.; Halegoua, D. L.; Han, C.; Hofmann, G. A.; Johnston, V. K.; Kaura, A. C.; Liu, N.; Keenan, R. M.; Lin-Goerke, J.; Sarisky, R. T.; Wigall, K. J.; Zimmerman, M. N.; Duffy, K. J. 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. *J. Med. Chem.* **2006**, *49* (3), 971−983.

(8) (a) Burton, G.; Ku, T. W.; Carr, T. J.; Kiesow, T.; Sarisky, R. T.; Lin-Goerke, J.; Baker, A.; Earnshaw, D. L.; Hofmann, G. A.; Keenan, R. M.; Dhanak, D. Identification of small molecule inhibitors of the hepatitis C RNA-dependent RNA polymerase from a pyrrolidine combinatorial mixture. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 1553−1556. (b) Burton, G.; Ku, T. W.; Carr, T. J.; Kiesow, T.; Sarisky, R. T.; Lin-Goerke, J.; Hofmann, G. A.; Slater, M. J.; Haigh, D.; Dhanak, D.; Johnson, V. K.; Parry, N. R.; Thommes, P. Studies on acyl pyrrolidine inhibitors of HCV RNA-dependent RNA polymerase to identify a molecule with replicon antiviral activity. *Bioorg. Med. Chem. Lett.* **2007**, accepted for publication.

(9) The stereochemistry of the (+) enantiomer was shown to be (2*S*,4*S*,5*R*) by small molecule x-ray crystallography. Compounds labeled as **a** (e.g., **1a**) are racemic and those labeled as **b** are homochiral.

(10) Lohmann, V.; Korner, F.; Koch, J.-O.; Herian, U.; Theilmann, L.; Bartenschlager, R. Replication of subgenomic heptatits C virus RNAs in a hepatoma cell line. *Science* **1999**, *285*, 110−113.

(11) Barr, D. A.; Dorrity, M. J.; Grigg, R.; Hargreaves, S.; Malone, J. F.; Montgomery, J.; Redpath, J.; Stevenson, P.; Thornton-Pett, M. X = Y−ZH compounds as potential 1,3-Dipoles. Part 43. Metal ion asymmetric 1,3-dipolar cycloaddition reactions of imines and menthyl acrylate. *Tetrahedron* **1995**, *51*, 273−294.

(12) Lipinski, C. A. Lead and drug-like compounds: The rule-of-five revolution. *Drug Discovery Today* **2004**, *1*, 337−341.

(13) The opposite pyrrolidine enantiomer was obtained from the mother liquors and processed through to the inactive enantiomer of **20b** using identical chemistry, confirming that (*R*)-BINAP phosphate gave the desired enantiomer.

(14) *ACD/CLogD* (at pH 7.4), version 8.0, Advanced Chemistry Development, Inc., Toronto ON, Canada, www.acdlabs.com, 2003.

(15) Note that the IC_{50} values obtained from the filtermat assay are generally 4−10-fold lower than the ones from the SPA assay. For example, **9b** has $IC_{50} = 0.99$ uM in the SPA assay (0.014 uM in filtermat assay) and **11b** has $IC_{50} = 0.053$ uM in the SPA assay (0.005 uM in filtermat assay), while **21b** has $IC_{50} = 0.43$ uM in the SPA assay (0.16 uM in the filtermat assay). Both assays are described in the Supporting Information. All enzyme and replicon determinations are the average of at least two assays.

(16) Chemistry was initially conducted in the racemic series (a) and then repeated in the homochiral series (b).

(17) Biological properties of the acyl pyrrolidine 3082, a novel NS5B HCV polymerase inhibitor, unpublished results (manuscript in preparation, Thommes, P. et al.).

JM061207R