ORGANIC LETTERS

2003
Vol. 5, No. 24
4627−4630

Pyrrolidine-5,5-*trans*-lactams. 4. Incorporation of a P3/P4 Urea Leads to Potent Intracellular Inhibitors of Hepatitis C Virus NS3/4A Protease

Martin J. Slater,* Elizabeth M. Amphlett, David M. Andrews, Paul Bamborough, Seb J. Carey, Martin R. Johnson, Paul S. Jones, Gail Mills, Nigel R. Parry, Donald O'N. Somers, Alan J. Stewart, and Tadeusz Skarzynski

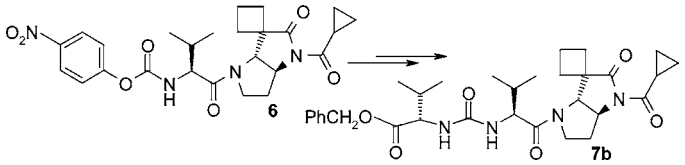
GlaxoSmithKline Medicines Research Center, Gunnels Wood Road, Stevenage, SG1 2NY, U.K.

martin.j.slater@gsk.com

Received September 22, 2003

Ⓦ **This paper contains enhanced objects available on the Internet at http://pubs.acs.org/orglett.**

ABSTRACT



In this, the first of two Letters, we describe how a P3/P4 urea linking unit was used to greatly enhance the biochemical and replicon potency of inhibitors based upon the pyrrolidine-5,5-*trans*-lactam template. Compound 7b demonstrated a 100 nM IC_{50} in the replicon cell-based surrogate HCV assay.

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.[2] 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.[3] Current therapies are based upon the use of interferon-α, alone or in combination with ribavirin. Although patients' sustained response rates are markedly improved using combination therapies, at least 50% of patients fail to show a sustained response. Additionally, these therapies have the disadvantage of frequent and severe side-effects.[4] The anticipated introduction of pegylated interferon offers the potential to improve sustained response rates against certain genotypes.[5] The development of new therapies to treat HCV infection effectively is therefore of paramount importance and is currently an intensive area of research.[6]

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

(1) World Health Organization *Weekly Epidemiological Record* **1997**, *72*, 65.

(2) (a) Gerber, M. A. *J. Hepatol*. **1993**, *17* (suppl 3), 108S. (b) Alter, M. J. *Hepatology* **1997**, *26*, 62S. (c) Hoofnagle, J. H. *Hepatology* **1997**, *26*, 15S. (d) Kwong, A. D. *Antiviral Res.* **1998**, *40*, 1. (e) Hoofnagle, J. H. *Digestion* **1998**, *59*, 563.

(3) (a) Di Bisceglie, A. M. *Lancet* **1998**, *351*, 351. (b) Brown, J. L. *Lancet* **1998**, *351*, 83.

(4) (a) Reichard, O.; Scvarcz, R.; Weiland, O. *Hepatology* **1997**, *26*, 108S. (b) Davis, G. L.; Esteban-Mur, R.; Rustgi, V.; Hoefs, J.; Gordon, S. C.; Trepo, C.; Schiffman, M. L.; Zeuzem, S.; Craxi, A.; Ling, M. H.; Albrecht, J. *N. Eng. J. Med*. **1998**, *339*, 1493. (c) McHutchinson, J. G.; Gordon, S. C.; Schiff, E. R.; Schiffman, M. L.; Lee, W. M.; Rustgi, V.; Goodman, Z. D.; Ling, M. H.; Cort, S.; Albrecht, J. *N. Eng. J. Med*. **1998**, *339*, 1485.

(5) (a) Luxon, B. A.; Grace, M.; Brassard, D.; Bordens, R. *Clin. Ther.* **2002**, *24*, 1363. (b) Youngster, S.; Wang, Y.-S.; Grace, M.; Bausch, J.; Bordens, R.; Wyss, D. F. *Curr. Pharm. Des.* **2002**, *8*, 2139.

(6) (a) Moradpour, D.; Brass, V.; Gosert, R.; Rainer, Wolk, B.; Blum, H. E. *Trends Mol. Med.* **2002**, *8*, 476. (b) Tan, S.-L, Pause, A.; Shi, Y.; Sonenberg, N. *Nat. Rev. Drug Discovery* **2002**, *11*, 867.

10.1021/ol035826v CCC: $25.00
Published on Web 11/06/2003

structural viral proteins (C, E1, E2, p7), whereas viral proteases (NS2, NS3) are responsible for the production of mature nonstructural replicative proteins. The multifunctional 70-kD NS3 protein has been extensively investigated.[7] The amino-terminal third of the protein is a trypsin-like serine protease that cleaves the NS3-4A, NS4A-NS4B, NS4B-NS5A, and NS5A-NS5B junctions.

Although isolated NS3 is enzymatically active, it forms a heterodimer with the NS4A cofactor constituting an integral structural component of the enzyme.[8] It has been reported that when appropriate mutations were made in the NS3 protease region of the HCV genome, the infectivity of these RNAs in chimpanzees was abolished.[9] NS3 protease is thus an essential viral function and an excellent target for the development of novel anti-HCV agents.

Our initial report described the design of α or β ethyl-substituted pyrrolidine *trans*-lactams such as **1**.[10] Subsequently, we published the further elaboration of the template to provide compound **2**, in which the symmetrical spirocyclobutyl group has replaced the ethyl P1 substituent and cyclopropylcarbonyl has replaced the methanesulfonyl substituent on the lactam. (Figure 1).[11] Herein we describe the

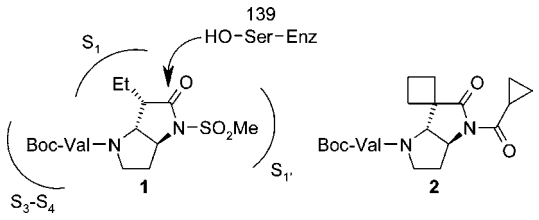


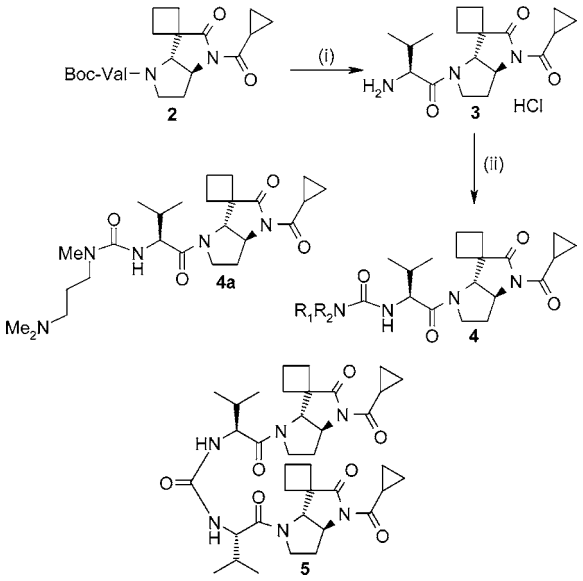
Figure 1. Pyrrolidine-5,5-*trans*-lactams as inhibitors of HCV NS3/4A protease.

optimization of the interactions in the S3 and S4 protein subsites leading to sub-micromolar inhibitors in the HCV replicon assay.

In the course of the optimization of **2**, a diverse range of analogues was prepared wherein the pyrrolidine nitrogen of the homochiral template is amide- and sulfonamide-substituted. Even the most promising leads from this approach were reduced in potency by approximately 3-fold compared with the standard Boc-valine derivative (**2**) and could not be improved upon, either with further Boc replacements or cyclic substituents (data not shown). This is consistent with earlier amino acid scanning using Boc and CBZ-protected amino acids and confirms that valine is the optimal choice for P3.[11] Our attention then focused on the linker from the valine nitrogen. Carbamate derivatives of this type have been previously disclosed, and indeed there is a strong preference for small hydrophobic groups such as *tert*-butyl.[11]

Commercially available isocyanates were employed to synthesize an initial small set of ureas. To gain access to a larger and more diverse set of ureas such as **4**, we prepared a *p*-nitrophenyl carbamate derivative of the amine **3** in situ and reacted this with a set of amines (R_1R_2NH) (Scheme 1).

Scheme 1. Initial Array Synthesis of P3-P4 Ureas[a]



[a] Reagents and conditions: (i) HCl, dioxan; (ii) *p*-nitrophenyl chloroformate (1.5 equiv), $EtiPr_2N$ (2 equiv), CH_2Cl_2, THF; then R_1R_2NH (3 equiv), MeCN in situ then aqueous sodium carbonate workup.

We noted that compounds such as the dimethylaminopropyl urea **4a** were more active than the standard (**2**) when tested initially, whereas the highly purified sample of **4a** was consistently less active than the standard (see Table 1). On further investigation by LC-MS, we noted that the "active sample" contained a higher molecular weight impurity consistent with the *trans*-lactam dimer **5**. We reasoned that formation of the carbamate of **3** had been incomplete and that remaining **3** was available to react with the *p*-nitrophenyl carbamate and form the dimer **5**. Indeed, on repeating the synthesis with 2 equiv of *trans*-lactam **3** and in the absence of an additional amine, **5** became the major product. The activity of **5** was striking ($K_{obs}/I = 1530\ M^{-1}\ s^{-1}$) and the most potent inhibitor of NS3/4A we had encountered, which

(7) (a) Clarke, B. E. *J. Gen. Virol.* **1997**, *78*, 2397. (b) Major, M. E.; Feinstone, S. H. *Hepatology* **1997**, *25*, 1527.

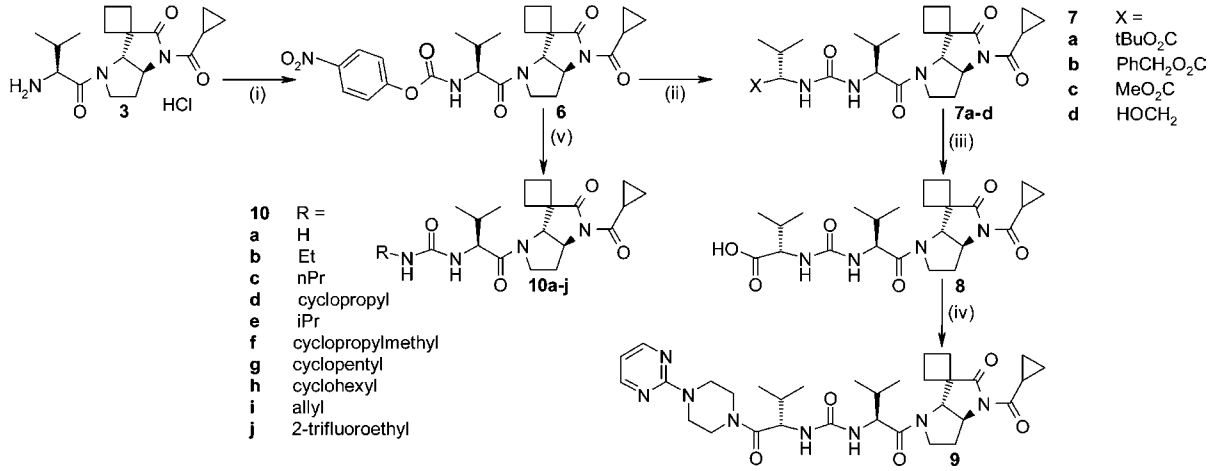
(8) Kim, J. L.; Morgenstern, K. A.; Lin, C.; Fox, T.; Dwyer, M. D.; Landro, J. A.; Chambers, S. P.; Markland, W.; Lepre, C. A.; O'Malley, E. T.; Harbeson, S. L.; Rice, C. M.; Murcko, M. A.; Caron, P. R.; Thomson, J. A. *Cell* **1996**, *8*, 344.

(9) Kolykhalov, A. A.; Mihalik, K.; Feinstone, S. M.; Rice, C. M. *J. Virol.* **2000**, *74*, 2046.

(10) Slater, M. J.; Andrews, D. M.; Baker, G.; Bethell, S.; Carey, S.; Chaignot, H.; Clarke, BE.; Coomber, B.; Ellis, M.; Good, A.; Gray, N.; Hardy, G.; Jones, P.; Mills G.; Robinson, E. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 3359.

(11) (a) Andrews, D. M.; Carey, S. J.; Chaignot, H.; Coomber, B. A.; Gray, N. M.; Hind, S. L.; Jones, P. S.; Mills, G.; Robinson, J. E.; Slater, M. J. *Org. Lett.* **2002**, *4*, 4475. (b) Andrews, D. M.; Chaignot, H.; Coomber, B. A.; Good, A. C.; Hind, S. L.; Johnson, M. R.; Jones, P. S.; Mills, G.; Robinson, J. E.; Skarzynski, T.; Slater, M. J.; Somers, D. O'N. *Org. Lett.* **2002**, *4*, 4479. (c) Andrews, D. M.; Chaignot, H. M.; Coomber, B. A.; Dowle, M. D.; Hind, S. L.; Johnson, M. R.; Jones, P. S.; Mills, G.; Patikis, A.; Pateman, T. J.; Robinson, E.; Slater, M. J.; Trivedi, N. *Eur. J. Med. Chem.* **2003**, *38*, 339.

Scheme 2. Synthesis of Valyl Urea Pyrrolidine-5,5-*trans*-lactams[a]



[a] Reagents and conditions: (i) (a) sodium hydrogen carbonate/CH_2Cl_2 extraction to generate free base; (b) *p*-nitrophenyl chloroformate, pyridine, CH_2Cl_2, (86%); (ii) valine derivative, $EtiPr_2N$, MeCN or Et_3N, CH_2Cl_2; (iii) H_2, 5% Pd/C, EtOH (92%) when X = $PhCH_2O_2C$; (iv) 4-(2-pyrimidinyl)piperazine, HATU, $EtiPr_2N$, MeCN; (v) RNH_2, Et_3N, CH_2Cl_2.

also translated into potent inhibition in the surrogate cellular assay, the HCV replicon.[12]

Table 1. NS3/4A Protease and HCV Replicon Activities

compound	NS3/4A protease IC_{50} (μM)	NS3/4A protease K_{obs}/I (M^{-1} s^{-1})	HCV replicon[12] IC_{50} (μM)
2	0.5	400	4.0
4a[a]	0.66	nt[b]	14
5	nt	1530	0.39

[a] Purified sample of **4a**. [b] Not tested.

Further SAR to exploit this observation was carried out, and our initial objective was to assess the requirement for two *trans*-lactam units. To prevent the formation of **5**, the carbamate **6** was purified by silica column chromatography and then subjected to a separate reaction step with amine nucleophiles (Scheme 2). Three simple esters of valine and valinol were selected and provided the bisvalyl ureas **7a**−**d**. Biochemical potency data (Table 2) clearly illustrated that the second *trans*-lactam unit could be dispensed with, and the critical role of the second valine unit was evident, since these molecules possessed similar or better potency than **5**.

Moreover, activity in the HCV replicon assay was maintained, with **7b**, the benzyl ester, providing excellent cellular activity (IC_{50} = 100 nM). Additionally, the valinol derivative **7d** demonstrated good activity in both assays, indicating that the ester could also be dispensed with.

(12) (a) Lohmann, V.; Korner, F.; Koch, J.-O.; Herian, U.; Theilmann, L.; Bartenschlager, R. *Science* **1999**, *285* (5424), 110. (b) Blight, K. J.; Kolykhalov, A. A.; Rice, C. M. *Science* **2000**, *290* (5498), 1972. (c) Bartenschlager, R.; Lohmann, V. *Antiviral Res.* **2001**, *52*, 1 (d) Parry, N. R.; Chung, V.; Viner, K. C.; Carroll, A. R. *Antiviral Res.* **2002**, *53* (3) A73.

A small program of work was undertaken based upon the bisvalyl urea amides, particularly with a view to improving aqueous solubility. Thus, the benzyl ester in **7b** was removed by standard hydrogenolysis, and the resulting acid **8** was coupled to a range of amines. The 4-(2-pyrimidinyl)-piperazinyl amide **9** illustrates the results of this approach and is one of the most potent enzyme inactivators of this series, with sub-micromolar potency in the HCV replicon (Table 2).

We then investigated replacing the carbonyl moiety in the urea by a sulfonyl group. Treatment of the amine **3** with sulfuryl chloride followed by the *tert*-butyl ester of valine

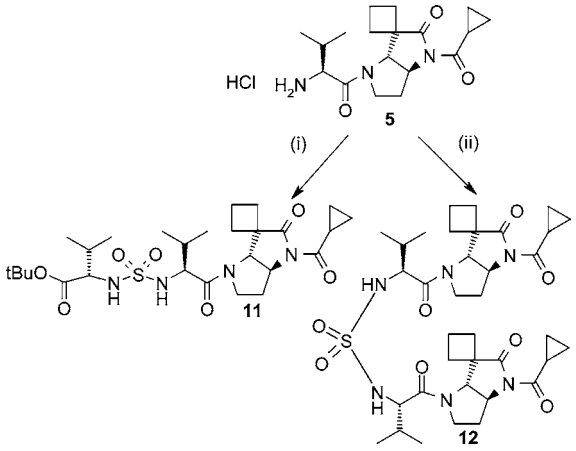
Table 2. NS3/4A Protease and HCV Replicon Activities of Urea Pyrrolidine-5,5-*trans*-lactams

compound	NS3/4A protease K_{obs}/I (M^{-1} s^{-1})	HCV replicon IC_{50} (μM)
5	1530	0.39
7a	1750	0.48
7b	1335	0.10
7c	2290	0.38
7d	1072	0.50
9	7761	0.30
10a	395	2.2
10b	376	4.5
10c	324	1.6
10d	167	8.5
10e	390	6.3
10f	853	2.8
10g	912	2.6
10h	922	1.2
10i	543	1.6
10j	458	3.5
11	89	nt[a]
12	340	nt[a]

[a] Not tested.

yielded **11**, the sulfonyl urea analogue of **7a**. Employing a molar excess of the translactam amine **3** provided a sample of the sulfonyl urea dimer **12** after column chromatography (Scheme 3). Biochemical potencies of **11** and **12** were

Scheme 3. Synthesis of Bisvalyl Sulfonyl Urea Pyrrolidine-5,5-*trans*-lactams[a]



[a] Reagents and conditions: (i) 2-amino-3-methyl-butyric acid *tert*-butyl ester, SO_2Cl_2, $EtiPr_2N$ (5 equiv), CH_2Cl_2 (18%); (ii) SO_2Cl_2, $EtiPr_2N$ (3 equiv), CH_2Cl_2, (9%).

considerably reduced, indicating a strong preference for the planar urea linker (Table 2).

Because our goal is the development of anti-HCV drug molecules suitable for delivery by oral administration, we were concerned that the bisvalyl urea amides have molecular weights in the region 600 Da. We thus directed our attention to simpler ureas more likely to achieve a combination of good potency in the cellular assay with an acceptable pharmacokinetic profile. Table 2 illustrates the SAR for some of these simple ureas, again prepared from the carbamate **6** (Scheme 2). Smaller groups such as ethyl and *n*-propyl demonstrated activities similar to that of the *tert*-butyl carbamate standard. Many of these lipophilic P4 substituents conferred similar levels of biochemical and replicon potency, with cellular activities in the 1−5 μM range. Cyclohexyl and cyclopentyl urea substituents (**10g**, **10h**) were identified as having the best overall profile of biochemical and cellular potency.

An X-ray structure of the cyclopentyl compound **10g** was obtained by soaking into preformed crystals of the NS3 protease with domain:NS4A cofactor.[13] Features of the *trans*-lactam core were consistent with those previously described following acylation of the active site serine.[11] Both of the urea NH moieties are clearly shown to be capable of forming a hydrogen bond with the main chain carbonyl of Ala 157, facilitating a good fit of the valine isopropyl onto the S3 surface and the cyclopentyl moiety into the S4 subsite (Figure 2). The urea-carbonyl bidentate interaction can facilitate the initial binding interaction and accommodate the movement of the *trans*-lactam upon acylation.

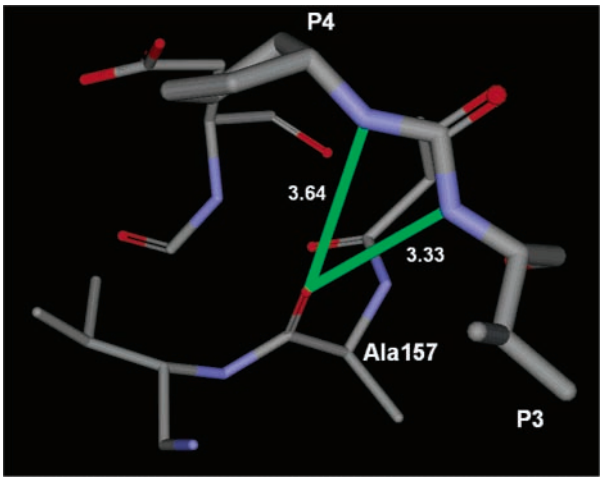


Figure 2. Pyrrolidine-5,5-*trans*-lactam crystal structure. Numbers indicate distances between heteroatoms in angstroms.

Ⓦ A 3D rotatable image of the structure in PDB format is available.

In summary, we have optimized the P3 and P4 interactions of the pyrrolidine *trans*-lactam template utilizing a urea linker. Bisvalyl ureas afford sub-micromolar inhibitors in the HCV replicon assay, and simple lipophilic ureas provide low micromolar inhibitors with excellent developability properties. The pharmacokinetic optimization of these potent molecules is described in the following paper.

Acknowledgment. We thank Drs. Berwyn Clarke and George Hardy for support and encouragement; Graham Baker, Sue Bethell, and Malcolm Ellis for provision of NS3 protease protein and initial assay systems; Michael Barnes for the synthesis of intermediates; and Gianpaolo Bravi for assistance with molecular modeling.

Supporting Information Available: Detailed experimental procedures for synthesis of representative compounds, characterization data for test compounds, biochemical and replicon assay methodology, and X-ray crystallographic methodology. This material is available free of charge via the Internet at http://pubs.acs.org.

OL035826V

(13) Deposition of the atomic coordinates into the Protein Data Bank is in progress.