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Pyrrolidine-5,5-*trans*-lactams. 5. Pharmacokinetic Optimization of Inhibitors of Hepatitis C Virus NS3/4A Protease

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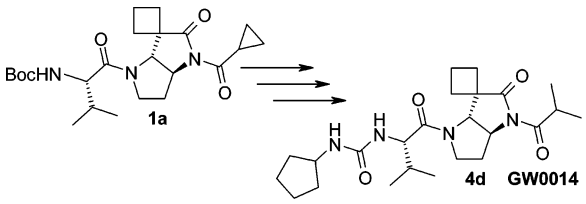
ABSTRACT



In this, the second of two Letters, the optimization of the pyrrolidine-5,5-*trans*-lactam template (exemplified by 1a) as a mechanism-based inhibitor of hepatitis C NS3/4A protease is described. "Right Box" analysis of cassette dosing screening pharmacokinetic data was used to rapidly categorize the compounds. GW0014 (compound 4d) emerged as the compound displaying an optimal balance of biochemical and replicon potency, along with low i.v. clearance in the dog.

Hepatitis C virus (HCV) infects chronically an estimated 3% of the global human population,[1] and the development of new therapies to treat HCV infection effectively is thus of considerable importance.[2] The viral genome encodes for a single polyprotein of 3010−3030 amino acids from which mature nonstructural proteins are released by the action of the viral proteases NS2 and NS3. It has been demonstrated that NS3 protease is an essential viral function and should prove to be an excellent target for the development of novel anti-HCV agents.[3]

Earlier communications have described the synthesis of disubstituted[4] core *trans*-lactam templates. In this, the second of two letters, we describe how an optimal combination of pyrrolidine and lactam ring substituents was achieved and how the critical parameter of in vivo pharmacokinetics, namely, clearance, was rapidly evaluated using cassette dosing in dog.

Although compounds **1a**−**e** (Table 1) are among the most potent HCV protease inhibitors hitherto described, they contain structural features that are regarded as potential liabilities. From a reactivity viewpoint, although the cyclopropylcarbonyl lactams **1a**−**c** offer excellent potency, the hydrolytic stability of this family is inferior to that of their isopropylcarbonyl counterparts **1d** and **1e**. Additionally, some

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

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

(4) (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.; Jones, P. S.; Mills, G.; Hind, S. L.; Slater, M. J.; Trivedi, N.; Wareing, K. J. *Bio. Med. Chem. Lett.* **2003**, *13*, 1657.

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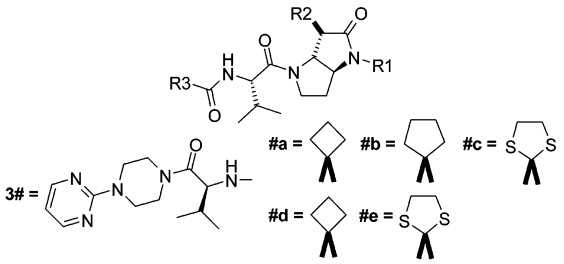
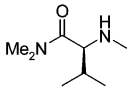
Table 1. *trans*-Lactam Array; Compounds Synthesized and K_{obs}/I Determinations (in italics, M^{-1} s^{-1})



R2 groups ⇨	R1 = cyclopropyl-carbonyl			R1 = isopropyl-carbonyl	
R3groups ⇩	**#a**	**#b**	**#c**	**#d**	**#e**
	**1a** *400*	**1b** *36*	**1c** *621*	**1d** *166*	**1e** *362*
	**2a** *2935*	**2b** *676*	**2c** *6112*	**2d** *1668*	**2e** *4865*
3#	**3a** *7761*	**3b** *989*	**3c** *8361*	**3d** *3099*	**3e** *5704*
	**4a** *912*	**4b** *122*	**4c** *1785*	**4d** *270*	**4e** *609*

of the previously described inhibitors, e.g., **3a**, have molecular weight over 600 Da. These concerns identified three main goals. First, improved biochemical potency was needed such that the compounds should reliably demonstrate activity in a replicon cell-based assay. Second, the molecular weight of the compounds should be reduced, preferably to below 500 (a threshold more consistent with membrane permeability and hence improved oral bioavailability). Third, clearance needed to be reduced, since initial studies had demonstrated that clearance (Clp) in the dog approximated to liver blood flow (38 mL/min/kg, too high to be useful for a systemically administered antiviral drug). Ultimately however, optimization of clearance (Clp), plasma half-life ($t_{1/2}$), and oral bioavailability (%F) is necessary to ensure that the required trough concentrations during dosing are obtained.

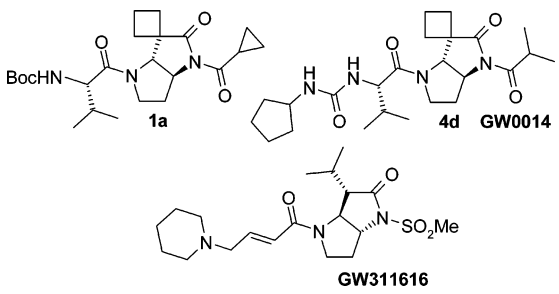
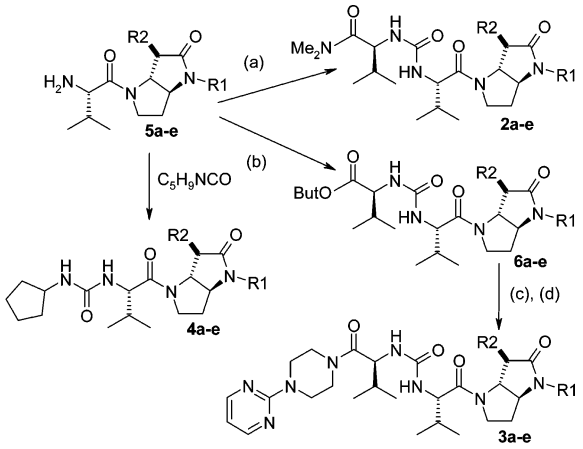


Figure 1. Urethane- and urea-substituted pyrrolidine-5,5-*trans*-lactams **1a** and **4d**; cf. cassette standard GW311616.

Capitalizing on the earlier observation that a urea linker appended to the amino acid moiety (Figure 1) bestows improved potency upon the series,[5] an array of 15 compounds was synthesized (Scheme 1) for comparison with the reference compounds **1a**−**e**.

Scheme 1. Synthesis of **2a**−**4e**[a]



[a] Reagents and conditions: (a) *p*-nitrophenyl chloroformate, DCM, Et_3N, L-valine dimethylamide; (b) *p*-nitrophenyl chloroformate, DCM, Et_3N, L-valine *tert*-butyl ester; (c) trifluoroacetic acid, DCM; (d) HATU, DIPEA, MeCN or DMF, 2-(1-piperazinyl)-pyrimidine.

The previously reported amines **5a**−**e**[4] were reacted with *p*-nitrophenyl chloroformate to form activated carbamate species that were either quenched with L-valine dimethylamide (a) or L-valine *tert*-butyl ester (b) to give the valyl ureas **2a**−**e** and **6a**−**e**. Further deprotection and coupling of **6a**−**e** generated the piperidino analogues **3a**−**e**. The ureas **4a**−**e** were prepared straightforwardly in a single step by reaction of cyclopentyl isocyanate with **5a**−**e**. Biochemical potency data, as well as the structure of all the compounds evaluated, is summarized in Table 1.

Compounds were scheduled for parallel evaluation in multiple assays to facilitate rapid identification of compounds exemplifying both good potency and pharmacokinetics. Screening relatively large numbers of compounds in the biochemical proteinase and cellular replicon assays was anticipated to be straightforward, but evaluating clearance using traditional pharmacokinetics assays would clearly not offer the throughput required. We therefore chose to extend previously developed in-house dog cassette methodology[6] to assist with the rapid evaluation of analogues with superior pharmacokinetics to **1a**.

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Table 2. In Vivo Pharmacokinetic and in Vitro Potency Data for Pyrrolidine-5,5-*trans*-lactams

compound	molecular weight	compound Clp (mL/min/kg)	standard Clp (mL/min/kg)	Clp as ratio to Std (Std = 1)	$t_{1/2}$ (h)	Vd (L/kg)	K_{obs}/I (M^{-1} s^{-1})	IC_{50} (μM)
1a	433	65	no[a]		0.3	0.3	400	4.0
1b	447	18	no		1.4	2.2	36	nt[b]
1c	483	nt					621	86
1d	435	36	no				166	3.1
1e	487	63	no		0.2	1.3	362	17
2a	503	54	12	4.5	0.4	0.6	2935	<0.78[c]
2b	517	54	11	4.9	0.1	0.5	676	nt[d]
2c	553	86	28	3.1	0.1	0.8	6112	nt[d]
2d	505	44	34	1.3	0.5	2.0	1668	nt[d]
2e	555	45	34	1.3	0.2	0.9	4865	nt[d]
3a	622	45	34	1.3	0.8	3.0	7761	0.45
3b	636	25	28	0.9	0.6	1.2	989	2.2[e]
3c	672	77	28	2.8	0.3	2.1	8361	nt
3d	624	48	11	4.4	0.2	0.6	3099	1.8[e]
3e	674	60	12	5.0	0.1	0.6	5704	nt[d]
4a	444	20	43	0.5	0.5	0.9	912	2.6
4b	458	39	43	0.9	0.7	2.4	122	1.6[e]
4c	494	85	43	2.0	0.9	6.6	1785	80
4d	446	33	43	0.77	0.5	1.4	270	1.0
4e	496	72	43	1.7	0.2	1.0	609	6.6

[a] No reference run (compound run as single discrete entity). [b] Not tested; biochemical activity too low to translate to cellular activity. [c] First dose response performed at too high a concentration. Compound shows very high clearance; therefore IC_{50} determination was not repeated. [d] Not tested; clearance higher than standard. [e] In-assay toxicity observed at concentrations <10-fold greater than the quoted IC_{50}.

Prior to inclusion of dog cassette dosing in the screening sequence, the technique was validated to ensure that within this series no drug−drug interactions occurred. Five spirocyclobutyl-substituted *trans*-lactams (previously examined in the dog as singletons) were coadministered with the well-characterized elastase inhibitor GW311616 (Figure 1).[7] The data confirmed that the rank order of clearance did not alter and that, in general, the absolute values for clearance did not vary much between the discrete and cassette doses. With one exception, the values were within 25%. In light of the generally self-consistent data, this variability was ascribed to interanimal variation rather than a compound or technique issue (see Supporting Information).

The *tert*-butyloxy reference compounds **1a**−**e** had been subject to prior examination by discrete dosing, but because none combined biochemical potency with low clearance, they were not subject to reevaluation in cassette. The standard compound, GW311616 has a mean clearance of 25 mL/min/kg, around two-thirds liver blood flow (about 38 mL/min/kg), but as seen in Table 2, the actual value can vary between 11 and 43 mL/min/kg. This indicated a higher degree of interanimal variability than we had observed in our initial validation experiments. In choosing which compounds to progress for further study, we therefore paid less attention to the absolute clearance values, preferring to interpret clearance data as a ratio to the standard (i.e., a "Right Box" approach). Compounds that were cleared more rapidly than the standard were not progressed further down the testing cascade, and in some cases, if replicon data had not already been obtained, cellular potency data was not collected. Independently, White and Manitpisitkul have shown by reanalysis of literature reports that Right Box analysis is one of the most useful screening cassette pharmacokinetics approaches.[8]

It can be appreciated readily that the valine dimethylamide substituted compounds **2a**−**e**, although showing good biochemical potency, were all cleared rapidly and therefore not progressed to the replicon assay in most cases. It is likely that the high clearance could arise (in part) as a result of rapid amide cleavage, demethylation, or N-oxidation.

The (piperazinyl)pyrimidine-substituted valineamides **3a**−**e** demonstrated marginally improved potency compared to that of **2a**−**e**, and one example (**3a**) combined moderate clearance with sub-micromolar replicon potency, but the majority of compounds (**3**) showed high clearance. In view of this, and since **3a**−**e** all had molecular weights above 600, the latter compounds were not progressed further.

The cyclopentyl urea series **4a**−**e** was of particular interest, since molecular weight was in a lower range and predictive of better membrane permeability. The dithiolanes **4c** and **4e**, although biochemically highly potent, failed to show good replicon potency and were also cleared more rapidly than the standard. Indeed, all of the diothiolane members of the other series were also rapidly cleared, leading to the conclusion that this moiety is too unstable to confer druglike properties. The poor translation of biochemical potency to

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(8) (a) Christ, D. D. *Drug Metab. Dispos* **2001**, *29*, 935. (b) White, R. E.; Minitpisitkul, P. *Drug Metab Dispos* **2001**, *29*, 957.

cellular activity is in agreement with our earlier findings and suggests that hydrolytic instability is an inherent liability with dithiolanes. The spirocyclopentyl lactam **4b** showed reasonably low clearance but poor biochemical activity, and the measured replicon potency could not be distinguished from in-assay toxicity. The spirocyclobutyl lactams **4a** and **4d** demonstrated an optimal combination of low clearance (i.e., less than the standard and around one-third dog liver blood flow), good biochemical potency, and micromolar replicon potency.

Interestingly, the isopropylcarbonyl-substituted lactam **4d**, although less potent in the biochemical assay than its cyclopropyl-substituted counterpart **4a**, demonstrated better cellular potency. This mirrors the situation with the analogues **1a** and **1d**. This trend has been previously explained;[4c] **1d** shows considerably greater stability in aqueous media than **1a**.

The favorable pharmacokinetic behavior of **4d** extends to other species: clearance was within an acceptable range in marmoset, rat, and hamster (data not shown). Lactam **4d** (GW0014) has been progressed to pharmacodynamic evaluation in GBV-B infected marmosets and shows good efficacy (three logs lower virus titer compared to animals given placebo) following subcutaneous administration.[9]

In conclusion, described here is the strategy for the successful discovery of potent, low molecular weight, druglike inhibitors of hepatitis C NS3/4A proteinase. Through the use of rapid parallel synthesis and purification, a substantial number of compounds were prepared for evaluation, a subset of which is described herein. The use of the dog cassette protocol permitted a thorough evaluation of the limiting property of the series, namely, clearance, and allowed the rapid identification of a candidate for in vivo efficacy testing.

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Supporting Information Available: Experimental procedures for synthesis of representative compounds, abridged characterization data for test compounds, and experimental procedures and validation data for dog cassette dosing. This material is available free of charge via the Internet at http://pubs.acs.org.

OL035827N

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