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Bioorganic & Medicinal Chemistry Letters 17 (2007) 3406–3411
Bioorganic & Medicinal Chemistry Letters

Inhibitors of hepatitis C virus NS3·4A protease. Effect of P4 capping groups on inhibitory potency and pharmacokinetics

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Received 20 December 2006; revised 27 March 2007; accepted 29 March 2007
Available online 3 April 2007

Abstract—Reversible tetrapeptide-based compounds have been shown to effectively inhibit the hepatitis C virus NS3·4A protease. Inhibition of viral replicon RNA production in Huh-7 cells has also been demonstrated. We show herein that the inclusion of hydrogen bond donors on the P4 capping group of tetrapeptide-based inhibitors result in increased binding potency to the NS3·4A protease. The capping groups also impart significant effects on the pharmacokinetic profile of these inhibitors.

Hepatitis C virus (HCV) infection remains a significant public health problem throughout the world.[1] Current standard of care is interferon-based immunomodulatory therapy. It is suboptimal and morbidity is high.[2] The goal of direct antiviral therapy is potentially close at hand as a number of anti-HCV agents, including HCV NS3·4A protease inhibitors such as telaprevir (**1**, VX-950),[3–5] ciluprevir (BILN 2061),[6] and SCH 503034,[7] have recently entered clinical development.

The unusually shallow, solvent-exposed active site of the HCV NS3·4A protease has provided formidable hurdles for medicinal chemists to investigate ways to maximize inhibitor-protein interactions while trying to maintain drug-like properties in the resulting inhibitors. One common approach, based on the concept of product-based inhibition, has been to optimally fill the hydrophobic S2 pocket with large groups while truncating the inhibitor backbone that sits along the substrate-binding groove of NS3.[8–10] Hexapeptide inhibitors derived from the HCV NS5A-5B natural substrate which incorporate carboxylate residues at P5 and P6 that produce electrostatic interactions between inhibitor and protein, were among early inhibitors examined.[11,12] An alternative approach is to anchor a natural substrate-based inhibitor assembly with a serine-trap warhead at one end and a P4 capping group at the other end, which can form a hydrogen bond to a hydrogen-bond acceptor on the NS3 protease surface. P4 capping groups containing long-chained carboxylate functionality have also been described on tetrapeptide scaffolds.[13–15] These floppy, charged groups produce minimal, if any, specific interactions to the largely solvent-exposed S5 pocket of the NS3·4A enzyme. In this report, we sought to study the effect of introducing hydrogen-bond donors onto relatively rigid P4 capping groups that could anchor the inhibitor N-terminus to the enzyme surface in a tighter, more compact manner.[16]

Based on the hydrophobic bicyclic proline P2 of telaprevir (**1**),[3,17] a compound currently in Phase II clinical development,[18] the commercially available octahydroindole carboxylic acid fragment was used as the constant P2 moiety for this study, as for example, **2**.[19] This P2 replacement obviates the need to use the cyclopentaproline of **1**, which is not commercially available. The octahydroindole P2 was chosen because of the added potency it provides over the unsubstituted proline resulting in a larger dynamic range of usable SAR. The unsubstituted proline analogue of **2** has been previously described and displays $K_i = 1.15\ \mu M$ relative to $K_i = 0.133\ \mu M$ for **2**.[19] Herein, we describe the effect of various P4 capping groups on the binding of a series of tetrapeptide-based inhibitors to the NS3·4A protease

Keywords: Hepatitis C; Protease inhibitors; Tetrapeptides; Pharmacokinetics.
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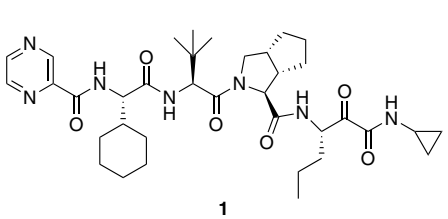
doi:10.1016/j.bmcl.2007.03.090



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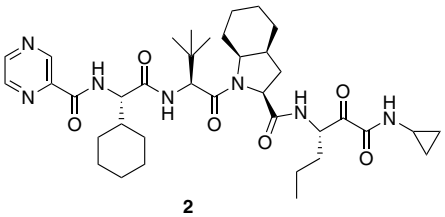


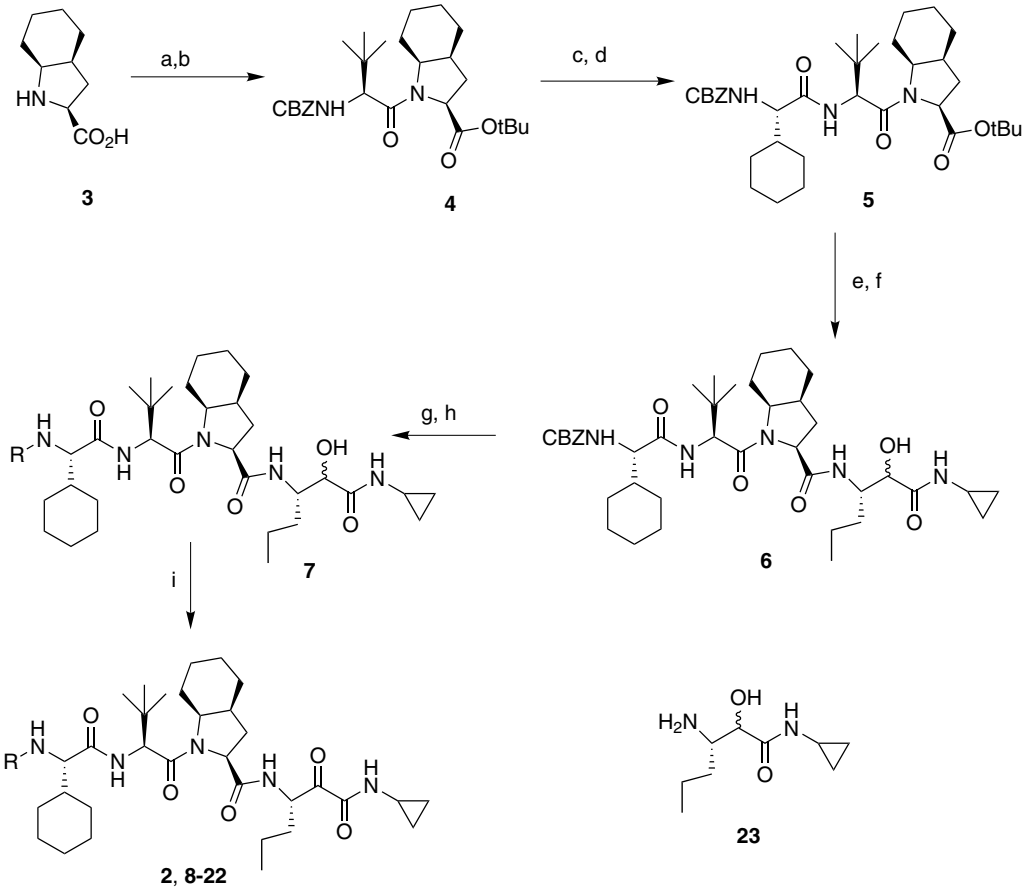
2

as well as the consequences of incorporating those moieties on the pharmacokinetic properties of this series. The structure–activity relationship of the resulting series of compounds reveals the contribution of key hydrogen bonds to inhibitor binding.

The synthetic sequence to assemble the target compounds, **2**, **8**–**22**, is shown in Scheme 1.[16] The carboxylate of commercially available **3**, was protected as a *tert*-butyl ester with isobutylene and catalytic sulfuric acid and subsequently coupled to the CBZ protected *tert*-butyl glycine to give **4** via a standard EDC/HOBt protocol. Following deprotection of **4** via catalytic hydrogenation, the P4 cyclohexyl glycine was introduced to give **5**. Following the removal of the *tert*-butyl ester of **5** with TFA, the C-terminal warhead portion of the scaffold, **23**, was coupled (EDC/HOBt) to generate **6** as a mixture of hydroxyl diastereomers at the warhead position. Tetrapeptide **7** was produced, also as a mixture of diastereomers, after removal of the CBZ-protecting group of **6** by catalytic hydrogenation followed by condensation with the activated carboxylate of the commercially available[20] capping group carboxylic acids. Finally, **7** was oxidized to the target α-ketoamides (**2**, **8**–**22**) via Dess–Martin oxidation conditions.

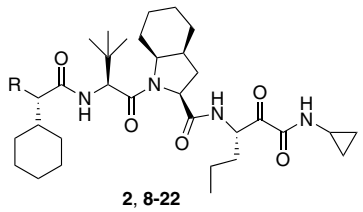
Target compounds were evaluated biochemically using a previously described enzymatic assay to provide K_i values.[21] Cellular data was obtained in Huh-7 replicon[22] cells in a two day assay as described.[3,21] Cytotoxicity results were generated from an MTS-based cell viability assay simultaneously with the corresponding replicon experiments. Both enzymatic and cellular data are shown in Table 1.

The SAR for this series is instructive. Pyrazine derivative, **2**, was more potent than the 2-pyridyl analogue, **8**, or 3-pyridyl derivative **9** for reasons that are unclear.

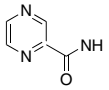
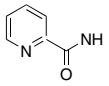
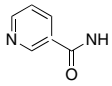
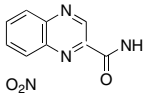
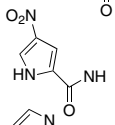
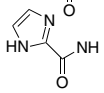
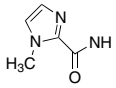
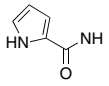
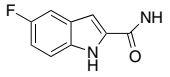
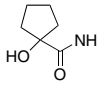
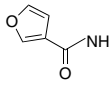
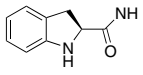
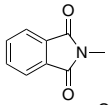
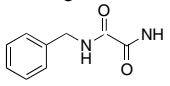
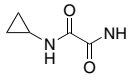
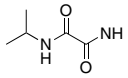


Scheme 1. Reagents: (a) *iso*-butylene, H_2SO_4; (b) CBZ-^{t}BuGly, EDC, HOBt, CH_2Cl_2; (c) 10% Pd/C, H_2, EtOH; (d) CBZ-cyclohexylglycine, EDC, HOBt, CH_2Cl_2; (e) TFA, CH_2Cl_2; (f) **23** (Ref. 23), EDC, HOBt, CH_2Cl_2; (g) 10% Pd/C, H_2, EtOH; (h) RCOOH, EDC, HOBt, CH_2Cl_2 (Ref. 20); (i) Dess–Martin periodinane, *t*-BuOH, CH_2Cl_2.

Table 1. Summary of enzymatic and cellular results for tetrapeptide inhibitors with varying P4 capping groups



2, 8-22

Compound	R	Inhibition K_i (μM)[a]	Replicon IC_{50} (μM)	Cytotoxicity CC_{50} (μM)[b]	CC_{50}/IC_{50}
1	Telaprevir[3]	0.04	0.35	82	230
2		0.15	1.1	47	43
8		0.52	1.9	>100	>52
9		0.24	1.7	>100	>58
10		0.44	1.2	>100	>81
11		0.05	0.37	>100	>270
12		0.12	0.23	>100	>442
13		1.2	>3	>100	—
14		0.09	0.50	>100	>201
15		0.12	0.49	>100	>203
16		0.64	1.6	>.100	>64
17		0.24	1.4	>100	>71
18		0.32	1.1	>100	>90
19		0.37	>3	>100	—
20		0.14	0.26	>100	>380
21		0.11	0.27	>100	>375
22		0.16	0.40	>100	>249

[a] Values are means of three experiments.
[b] Cytotoxicity data (CC_{50}) were determined in an MTS-based cell viability assay.

However, a properly situated hydrogen-bond donor placed on the capping group, may allow a hydrogen bonding interaction with the NS3 binding groove, as suggested by the increased potency for **11** and **14** relative to **2**. The X-ray structure of **11** bound to the NS3·4A protease is shown in Figure 1 (PDB code: 2P59). Indeed, the structure clearly shows the hydrogen bond from the pyrrole NH of **11** to the protein. Although imidazole **12** was not significantly more potent than **2**, blocking of the NH of the imidazole with a methyl group, as in the case of **13** (K_i = 1.2 μM), resulted in a 10-fold loss of potency relative to **12** (K_i = 0.12 μM). With the exception of **2** and **13**, groups that are incapable of donating hydrogen bonds to the protein surface (e.g., **8**–**10**, **17**, and **19**) displayed a relatively flat SAR, though they were generally less potent than **2**. Although **16** possess a donor hydroxyl group, the potency was relatively poor, possibly due to improper geometry of the quaternary hydroxyl center relative to the NS3 surface. Oxamate derivatives **20**–**22** displayed good enzymatic potency as would be expected from compounds incorporating an NH moiety. They did not differ significantly in potency from each other (K_i = 140, 110 and 160 nM, respectively, for **20**–**22**). As a whole in this series, the cellular potency in a HCV replicon assay[3,22] tracked well with enzymatic potency. In the HCV replicon assay, compounds containing a hydrogen-bond donating capping group were generally more potent than those without. Again an exception is the alcohol **16**, which showed reduced enzymatic binding affinity as well as lower cellular potency. Cytotoxicity for this series was relatively low, consistent with previous results from tetrapeptide ketoamide inhibitors.[21,23]

Hydrogen bond donors on the P4 capping position are shown to increase enzymatic potency relative to moieties that cannot form hydrogen bonds. Pharmacokinetic data in mice following the oral administration of inhibitors (Table 2) showed that capping groups lacking acidic protons resulted in better oral absorption than those containing acidic hydrogen donor functionality. There is some evidence that more acidic protons on the capping group may lower the liver uptake (e.g., **11** relative to **14**), although the data is limited. Unfortunately, while stronger hydrogen bonds provide for tighter binding, the resultant increased acidity of the cap hydrogen appears to reduce oral bioavailability in mice (Table 2). While **13** displayed good oral exposure

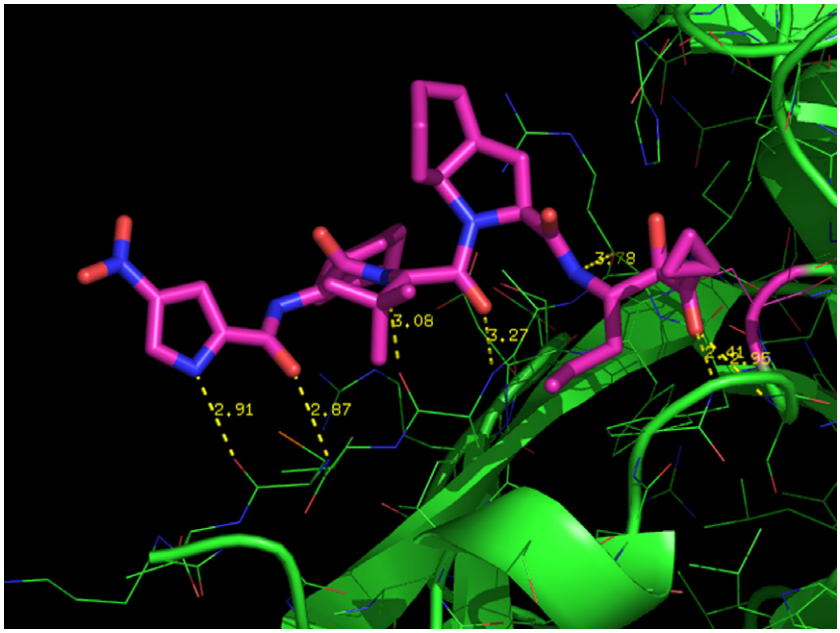


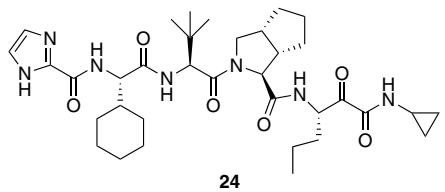
Figure 1. X-Ray structure of inhibitor **11** in a co-complex with HCV NS3·4A protease showing the hydrogen-bonding network from the P2 carbonyl to the P4 cap. The pyrrole NH forms a hydrogen bond with the Cys 159 of the NS3 protein.

Table 2. Pharmacokinetic data for selected compounds in CD-1 mice

	K_i (μM)	IC_{50} (μM)	AUC_{liver}(μM h)	AUC_{plasma}(μM h)	$C_{liver\ ave}$ (μM)	$C_{plasma\ ave}$ (μM)	$C_{liver\ ave}/IC_{50}$
1	0.04	0.35	19.0	3.0	2.4	0.37	54
2	0.15	1.1	2.9	1.5	0.36	0.18	0.33
11	0.05	0.37	0.06	BQL	0.01	BQL	0.03
12	0.12	0.23	0.47	0.05	0.06	0.01	0.26
13	1.2	>3	2.1	1.3	0.26	0.17	—
14	0.09	0.50	1.3	0.36	0.17	0.04	0.34
24	0.03	0.20	10.5	0.36	1.3	0.04	52

Mice were dosed orally with compounds as a solution in propylene glycol at a concentration of 50 mg/kg. AUC values are 0–8 h. For liver concentrations, it is assumed that liver tissue has a density of 1 g/mL. BQL, below quantitation limit.

in mice, it was a relatively weak inhibitor of the protease (K_i = 1.2 μM). Conversely, **11**, a potent inhibitor in enzymatic assay (K_i = 50 nM) as well as in the replicon system (IC_{50} = 0.37 μM), showed relatively poor oral bioavailability in mice. For orally bioavailable compounds, the high lipophilicity resulted in higher liver concentrations relative to plasma drug levels, a potentially advantageous property given that the vast majority, if not all, of the replicating HCV virions reside in the liver.[24] For example, **2** had an average liver concentration that was approximately two-fold higher than the average plasma concentration over 8 h. Similar results were observed for **13** and **14** (approximately 1.5- and four-fold, respectively). To observe the effect of incorporating a hydrogen bond-donating P4 cap onto the telaprevir scaffold, compound **24**,[25] the P2 analogue of **12**, was prepared. Liver exposure for **24** was increased 20-fold over that of **12**. However, the plasma exposure for **24** increased to a lesser degree than did the liver concentration. Relative to telaprevir, liver exposure was modestly reduced (2×) but plasma exposure was reduced approximately nine-fold.



24

The SAR of a series of inhibitors containing P4 capping groups with varying abilities to donate hydrogen bonds to the NS3 binding groove has been described in this report. Some of these compounds were potent inhibitors of the HCV NS3·4A protease. The results of enzymatic assays showed that incorporation of groups that form strong hydrogen bonds with the NS3·4A protein derive additional affinity. In general, cellular activity tracked reasonably well with enzyme binding affinity. The pharmacokinetic properties of a subset of the inhibitor series demonstrate that the inclusion of hydrogen bond donors adversely affected oral bioavailability for this series. Careful balancing of these competing properties (binding affinity vs. oral bioavailability) is required to identify compounds with the requisite drug-like properties.

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