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Inhibitors of hepatitis C virus NS3·4A protease. Part 3: P_2 proline variants

Robert B. Perni,* Luc J. Farmer,* Kevin M. Cottrell, John J. Court, Lawrence F. Courtney, David D. Deininger, Cynthia A. Gates, Scott L. Harbeson, Joseph L. Kim, Chao Lin, Kai Lin, Yu-Ping Luong, John P. Maxwell, Mark A. Murcko, Janos Pitlik, B. Govinda Rao, Wayne C. Schairer, Roger D. Tung, John H. Van Drie, Keith Wilson and John A. Thomson

Vertex Pharmaceuticals Inc., 130 Waverly Street, Cambridge, MA 02139, USA

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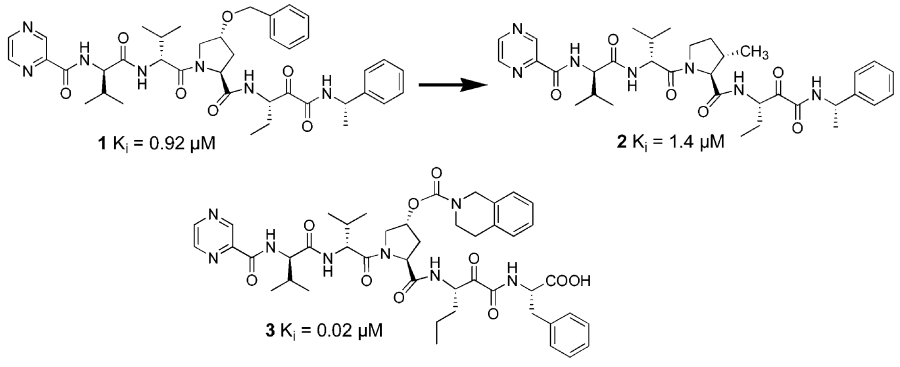
Abstract—We recently described the identification of an optimized α-ketoamide warhead for our series of HCV NS3·4A inhibitors. We report herein a series of HCV protease inhibitors incorporating 3-alkyl-substituted prolines in P_2. These compounds show exceptional enzymatic and cellular potency given their relatively small size. The marked enhancement of activity of these 3-substituted proline derivatives relative to previously reported 4-hydroxyproline derivatives constitutes additional evidence for the importance of the S_2 binding pocket as the defining pharmacophore for inhibition of the NS3·4A enzyme.

The hepatitis C NS3·4A protease is an important and attractive target for developing anti-viral therapies for HCV.[1] We recently reported on the design of a series of potent NS3·4A protease inhibitors based on a tetrapeptide scaffold.[2] Our design efforts have continued to focus on optimizing potency while improving the physicochemical properties of these molecules to enable convenient oral dosing of the inhibitor. Size is an important factor to address. Recently reported series of compounds displayed molecular weights ranging from 700–1000,[3] clearly above the optimal range for most orally delivered drugs. The enzyme S_2 pocket has been demonstrated to be important for inhibitor recognition, and bulky substituents have been identified as optimal at that position, contributing to the overall high molecular weight of the inhibitors.[3] We herein report on a



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* Corresponding author. Tel.: +1-617-444-6237; fax: +1-617-444-6766; e-mail: robert_perni@vrtx.com

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series of potent tetrapeptide inhibitors utilizing a 3-alkyl proline P_2 moiety that retains significant binding potency despite their relatively small molecular size.

Analogues derived from 4-hydroxyproline such as **1** and **3**[2] provide good binding to the S_2 pocket, but result in inhibitors possessing high cLogP values (e.g., 5.4 and 6.4 for **1** and **3**, respectively) and additional metabolically vulnerable functionality. Inhibitors such as **3**, which possess an obligatory charge have thus far displayed reduced cellular activity.[2b]

These observations led us to inspect other possibilities for P_2 proline substitution that would not possess the liabilities afforded by 4-hydroxyproline derivatives. During our analysis of a crystallographic co-complex of **1** with the NS3·4A protein we observed poorly defined electron density in the region where we expected to find the catalytic histidine. We initially interpreted the electron density map of this area close to the aspartate (Asp 81) and serine (Ser 139), as representing a structural water molecule. Additional refinement of the structure is shown in Figure 1, with a putative bound water molecule (green sphere). Molecular modeling studies based on the X-ray structures of 4-hydroxyproline-substituted inhibitors indicated that a vector from the 3-position of the P_2 proline ring might allow access to the active site via a relatively small substituent (also shown in Fig. 1). We hypothesized that the putative crystallographically-defined water molecule could then be displaced with a 1–4 carbon group extending from the α-face of the proline 3-position, potentially boosting the binding affinity by ~2 kcal/mol from the entropic gain from displacing that bound water[4] thereby compensating for affinity lost upon removal of the 4-substituent. To test this hypothesis, we prepared **2** from commercially available 3-methyl proline as shown in Scheme 4.[5] The affinity of **2** was determined to be 1.4 μM, consistent with expectations. The yellow structure in Figure 2 depicts the modeled orientation of the bound inhibitor if the histidine were displaced from its normal orientation. The actual X-ray structure of the co-complex showed that the ligand bound similarly to the modeled conformation with the catalytic histidine in the normal conformation (green structure in Fig. 2). In this structure we found the 3-position methyl group to be subtly tilted away from the active site due to the position of the histidine. The initial interpretation of density near catalytic Asp 81 and His 139 as a structural water appears to be incorrect in view of this result. The similar enzymatic activity of **2** relative to **1** (1.4 μM versus 0.92 μM respectively, cLogP = 4.4) provided the impetus to explore this series of 3-substituted proline analogues in detail.

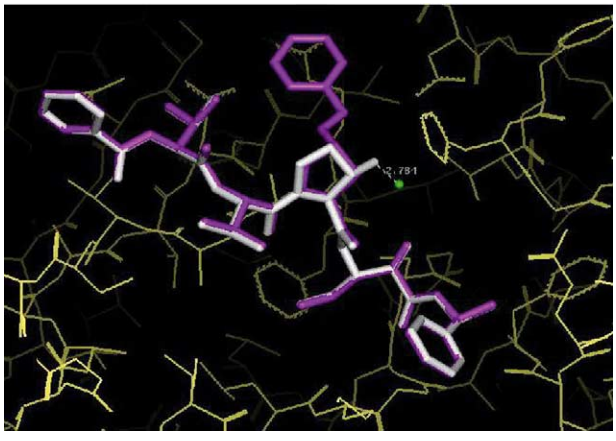


Figure 1. Predicted structure of **2** (white) bound to the tNS3·4A protein relative to X-ray structure of **1** (purple).

The syntheses of 3-functionalized prolines **9a–g** are described in Schemes 1 and 2. Cuprate addition,[6] of

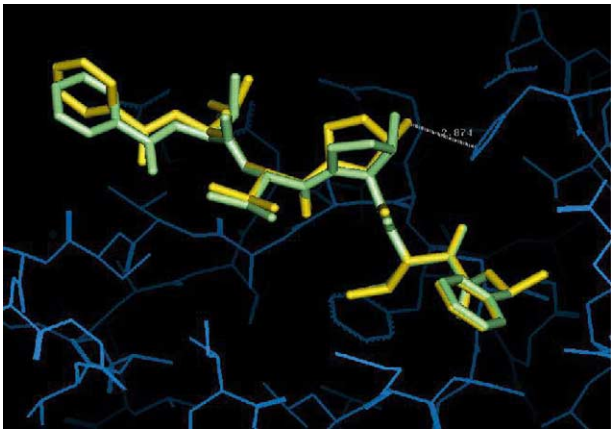
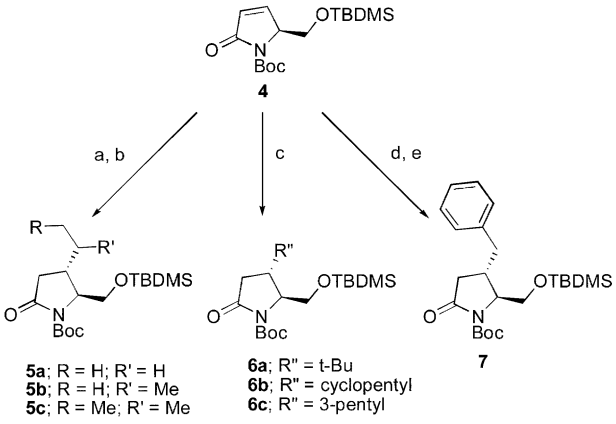
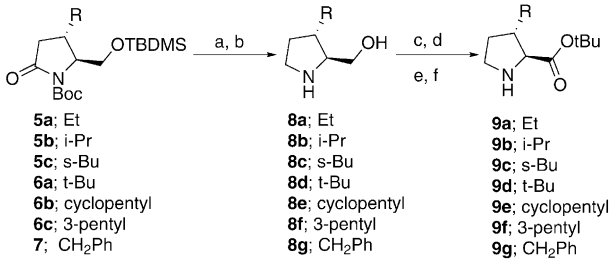


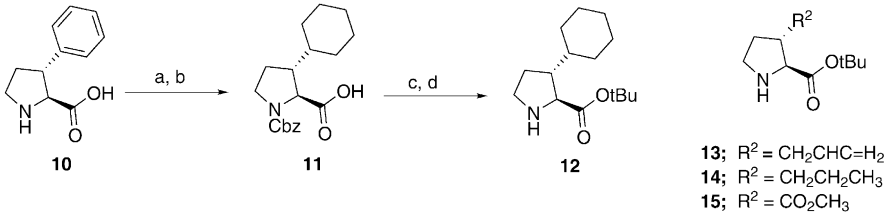
Figure 2. X-ray structure of **2** (green) bound to the tNS3·4A protein relative to the predicted model structure of **2** from Figure 1 (yellow).



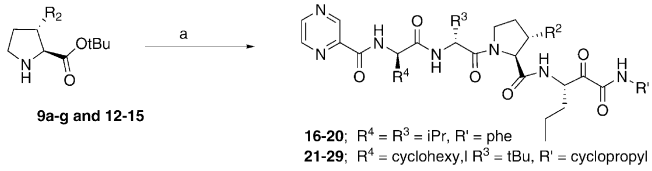
Scheme 1. (a) 2-vinyl, 2-propenyl or 2-butenyl-MgBr, CuBr·DMS, ether, −20 °C then TMSCl, −78 °C (65%, 73%, 84%); (b) 10% Pd–C, H_2, 1 atm, EtOH (90%, 92%, 89%); (c) R″ZnBr, THF, −30 °C, BF_3OEt_2 then TMSCl (64%, 40%, 37%); (d) PhCH(Li)SPh, BuLi, TMEDA, −78 °C (45%); (e) Ra-Ni, acetone/water (1:1), reflux, 12 h (83%).



Scheme 2. (a) HCl gas, EtOAc, −20 °C (80–90%); (b) LAH, THF, reflux (85–90%); (c) CbzCl, K_2CO_3, THF/H_2O (1:1) (60–85%); (d) Jones, acetone, (70–80%); (e) isobutylene, H_2SO_4 cat., DCM (67–85%); (f) 10% Pd–C, H_2, 1 atm, EtOAc (90–95%).



Scheme 3. (a) PtO_2, EtOH/AcOH/H_2O (7:2:1), H_2, 50 psi; (b) CbzCl, Na_2CO_3, acetone/H_2O (1:1) (90%, two steps); (c) isobutylene, H_2SO_4 cat., CH_2Cl_2; (d) 10% Pd–C, H_2, 1 atm, EtOH (84%, two steps).

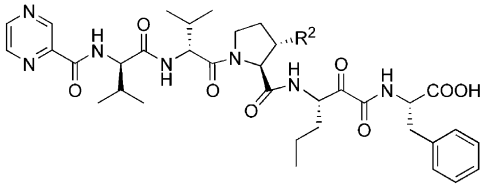


Scheme 4. (a) See ref 5.

vinyl, 2-propenyl and 2-butenylmagnesium bromide in the presence of copper bromide dimethylsulfide complex and trimethylsilyl chloride on pyroglutaminol[7] **4** provided, after hydrogenation, compounds **5a**, **b** and **c**. The 1,4-addition of organozinc to **4** afforded intermediates **6a**, **b**, and **c**.[8] Finally, the 3-benzyl pyroglutaminol **7** was prepared via the addition of 2-lithio benzylphenyl sulfide[9] to compound **4** followed by Raney-nickel reduction.

The 3-substituted pyroglutaminols **5a–c**, **6a–c** and **7** were converted to proline derivatives **9a–g** in six steps (Scheme 2). Tandem removal of the silyl group and *t*-butyl carbamate in the presence of anhydrous hydrochloric acid in ethyl acetate generated pyroglutaminol intermediates, which, upon LAH reduction in refluxing THF, provided hydroxymethyl pyrrolidine **8a–g**. Protection of the 3-alkyl hydroxymethyl pyrrolidines as the benzylic carbamate[10,11] with benzyloxycarbonyl chloride in the presence of K_2CO_3 followed by Jones oxidation gave the corresponding Cbz-protected prolines that were esterified with isobutylene in dichloromethane with a catalytic amount of sulfuric acid. The resulting carbamate esters were hydrogenated to give the pivotal proline ester scaffolds **9a–g**.

Table 1. 3-Substituted proline structure–activity summary



	R^2	K_i (μM)[a]
16	CH_2CH_3	0.22
17	$CH_2CH=CH_2$	0.21
18	$CH_2CH_2CH_3$	1.2
19	$CH(CH_3)CH_2CH_3$	0.12
20	CO_2CH_3	0.18

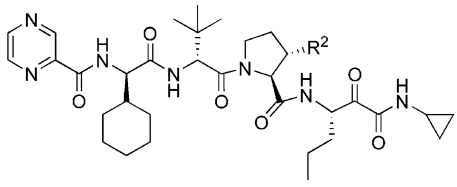
[a] HPLC based enzymatic assay performed as described in ref 2b.

The 3-cyclohexylproline derivative, **12**, was prepared according to Scheme 3 in four steps from commercially available 3-phenylproline. Compounds **13–15** were prepared according to literature procedures.[11,12]

The final tetrapeptides **16–28** (Tables 1 and 2) were then elaborated using the same methodology as previously reported[5] (Scheme 4).

We produced our initial set of inhibitors (**16–20**) on a scaffold containing a phenylalanine $P_{1'}$ terminus in order to maximize binding potency at the possible expense of cellular activity. With the exception of **18** these closely related inhibitors possess essentially identical activity versus the NS3·4A dimer (Table 1). It is unclear why the *n*-propyl derivative, **18**, is 5- to 10-fold less potent than the other compounds. This result, obviously, does not appear related to steric demand given

Table 2. Enzymatic and cellular results from optimized 3-substituted proline derivatives



	R^2	K_i (μM)[a]	IC_{50} (μM)[b]	CC_{50} (μM)[c]
21	CH_2CH_3	0.15	0.45	30
22	$CH(CH_3)_2$	0.09	0.91	59
23	cyclohexyl	0.10	0.76	28
24	$CH(CH_3)CH_2CH_3$	0.10	—	—
25	$CH(CH_2CH_3)_2$	0.33	>3	74
26	cyclopentyl	0.25	2.9	43
27	$C(CH_3)_3$	0.46	>3	74
28	$CH_2C_6H_5$	0.31	1.1	22
29	H	1.2	—	—

[a] HPLC based enzymatic assay performed as described in ref 2b.
[b] Cellular HCV replicon[13] assay performed as described in ref 2b.
[c] Cytotoxicity of the compounds (CC_{50}) was determined by an MTS-based cell viability assay.

that other compounds containing small substituents on the 3-position of the P_2 proline were nearly as potent at many similar compounds with large 4-hydroxyproline groups (e.g., **19** versus **3**). Nevertheless, these initial results were extremely encouraging.

Having developed smaller P_2 containing inhibitors that possess significant enzymatic potency we embarked on a broader survey of potential non-polar 3-position analogues on an optimized scaffold that bears no charge and would presumably be better able to penetrate cells. Those results are presented in Table 2.

This series of proline variants, **21**–**29**, was based on a scaffold comprised of optimized $P_{1'}$[2b] and P_3, P_4[14] substituents. The resulting inhibitors demonstrated class-wide binding potency relative to carboxy-terminal inhibitors, **16**–**20**. Again, there is little spread in the data with inhibitory constants ranging from 0.09 μM to 0.46 μM with no visible steric trends in this, albeit limited, series. As hoped significant cellular activity[13] is evident in this series. Several compounds exhibit IC_{50} values of <1 μM in an HCV replicon assay. While **21** incorporates the smallest proline substituent of the series shown in Table 2, an ethyl group, it is among the most potent compounds, along with **22** and **23**, in the replicon cellular assay ($IC_{50} = 0.45$ μM, cLogP = 5.5) indicating that size alone is not the major determinant of activity. The unsubstituted proline P_2 derivative, **29** ($K_i = 1.2$ μM), provides a baseline comparator that clearly demonstrates the advantage of incorporating a substituent at the 3-position relative to no substitution. The combination of a small proline moiety and optimized P_3–P_4 substituents results in a molecule with good cellular activity probably due to an improved balance between enzymatic binding affinity and overall physicochemical properties.

Although the original hypothesis of targeting a substituent from the P_2 proline into the NS3 catalytic site was not possible as initially formulated, exploiting the subtleties of the enzyme inhibitor interactions at this site led to the discovery of a series potent inhibitors with compact P_2 substituents and improved physicochemical parameters relative to the corresponding 4-hydroxyproline-derived analogues. Further investigation of this series is in progress and will be reported in due course.[15]

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