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Capped dipeptide phenethylamide inhibitors of the HCV NS3 protease

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Abstract—The N-terminal aminoacid of phenethylamide tripeptide inhibitors of the hepatitis C virus NS3 protease can be replaced with an α-hydroxy acid to obtain more 'drug like' inhibitors with low micromolar activity. The preferred *S*-configuration of the capping residue can be explained by molecular modeling studies.

1. Introduction

The hepatitis C virus (HCV) represents the chief etiologic agent in non-A and non-B hepatitis, a disease that causes liver cirrhosis and hepatocellular carcinoma[1,2] and affects more than 170 million people worldwide.[3] Although the introduction of a combination therapy, pegylated interferon-α and ribavirin, has markedly improved clinical outcomes, less than 50% of infected individuals can be expected to have a favourable response to this treatment.[4] The HCV NS3/4A protease is an important target to treat this disease and has been intensively studied because of its central role in the viral replication. A recent important study has shown that NS3 protease is necessary for HCV replication in chimpanzee.[5]

The identification of clinically relevant compounds—low molecular weight, orally bioavailable, cell-penetrant entities—is exceptionally challenging and the reason can be found in the unique structural characteristics of the target itself, the NS3/NS4 protease/co-factor complex: the featureless and highly polar substrate binding region causes the need for unusually large, polyanionic decapeptide substrate molecules.[2,6]

Inhibitors described in the literature are predominantly based on the substrates of NS3[2] and due to their peptidic nature they will likely suffer from poor oral bioavailability. Nevertheless, enormous progress has been made in the peptidomimetic area, where macrocyclic inhibitors showed a discrete oral absorption and an antiviral effect in clinical trials.[7]

Only a few other small molecule inhibitors whose structure is not based on the peptide substrates of the NS3 protease have been reported:[2] their mechanism of action is not clear and none of these are competitive inhibitors of NS3.

Recently we reported[8] the evolution of potent capped dipeptide α-ketoacid inhibitors of HCV NS3 protease based on the primary discovery that the α-ketoacid moiety is a particularly effective serine trap for NS3 protease[9] and that 4,4-difluoro-2-aminobutyric acid (difluoroAbu)[10] is an excellent and chemically inert replacement for the canonical P1 cysteine found in most of the cleavage sequences of NS3 protease.

Capped dipeptides showed that the entire P3 residue of the N-protected tripeptides can be replaced by a small hydroxylated residue (e.g., **1**, IC_{50} 3 μM) whose chirality is critical for binding recognition (Fig. 1). Contrary to the *S*-configuration found in the P3 aminoacids, α-hydroxy acids with *R*-configuration were more potent than those with *S*-configuration. We explained this with a conformational preference for a coplanar arrangement of the C–O and N–H bonds, obtained by an intramolecular hydrogen bond between these groups, as shown in Figure 1.

Keywords: HCV NS3 protease; Phenethylamide dipeptide.
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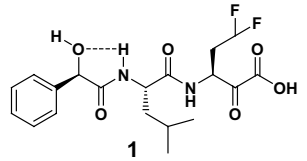


Figure 1. Capped dipeptide α-ketoacid lead **1** (IC_{50} 3.00 μM).

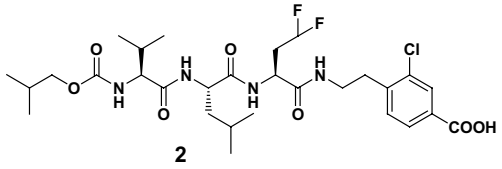


Figure 2. Tripeptide phenethylamide lead (IC_{50} 2.4 μM).

The more recent discovery[11] that the α-ketoacid moiety of potent tripeptides can be replaced by an amide residue (e.g., **2**, IC_{50} 2.4 μM, Fig. 2), led us to develop a new series of reversible, competitive inhibitors of HCV NS3 protease, which are characterized by the presence of a phenethylamide group in the P1′ position. These inhibitors, even if they are slightly less potent than the corresponding tripeptide ketoacids, have the clear advantage of lacking the electrophilic carbonyl group. They also explore novel interactions in the prime side of the NS3/4A protease, which renders them highly specific against related proteases as for example elastase.

These properties led us to investigate, replacing the N-terminal aminoacid of the phenethylamide, inhibitors with an α-hydroxy acid in a manner analogous to that in the serine trap ketoacid series.[8]

In the investigation of the SAR in the tripeptide series, we achieved the highest potencies with the 4-(2-aminoethyl)-3,5-difluorobenzoic acid as phenethylamine[12] and the (*S*,*S*)-4-phenylproline in P2,[13] and a corresponding optimized tripeptide is shown in Figure 3.

The combination of an α-hydroxyamide in P3 with the phenylproline in P2 removes the amide N–H of this residue, which favoured a coplanar arrangement with the P3 C–O and thus the *R*-configuration of the α-hydroxyamide (Fig. 1). Accordingly, we expected in this case the *S*-configuration to be favoured as for the aminoacids.

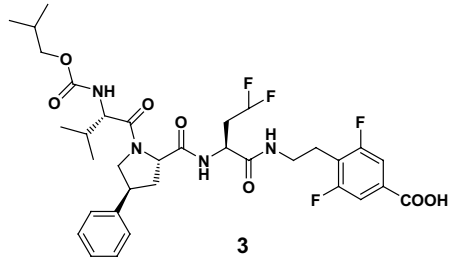


Figure 3. Optimized phenethylamide tripeptide (IC_{50} 0.9 μM).

Combining these informations, we made a series of compounds aimed towards potent reversible capped dipeptide phenethylamides as HCV NS3/4A protease inhibitors.

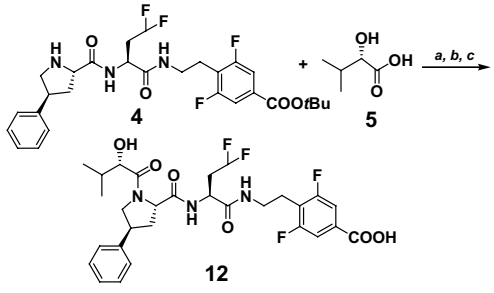
2. Synthesis

The synthesis of the capped dipeptide **12** from the intermediate **4**[12] is shown in Scheme 1 and is representative for other analogues. Condensation of intermediate **4** with unprotected (*S*)-isovaleric acid **5**, followed by acid promoted removal of the *tert*-butyl ester, gave the desired capped dipeptide phenethylamide **12**.

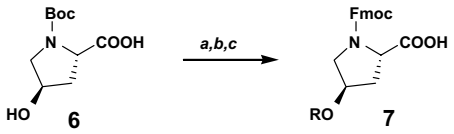
All crude phenethylamides were purified by HPLC and when necessary the two diastereoisomers, resulting from the use of racemic α-hydroxy acids, were separated at this stage as well. The more active diastereoisomer always eluted first and was later shown by use of chiral fragments, such as (*R*)- or (*S*)-isovaleric acids and (*S*)-hexahydromandelic acid, to have the *S*-configuration at the capping residue. The *l*-configuration at P1, and the 2*S*,4*S*-configuration at P2 was in accord with the results from tripeptide work.[11]

As far as the P2 residues reported in Table 2, they have been synthesized as described in Scheme 2. Following a procedure described in literature,[14] direct alkylation of the sodium dianion of **6**, in the presence of NaI, followed by exchange of the protecting group gave the desired intermediate in moderate to good yields.

Compounds were then evaluated as inhibitors using the full length HCV NS3/NS4A protease complex as described in Ref. 15.



Scheme 1. Reagents and conditions: (a) HATU, 2,6-lutidine, DCM–DMF (55%); (b) TFA–DCM–H_2O (99%); (c) RP-HPLC.



Scheme 2. Reagents and conditions: (a) NaH, THF, RBr, NaI (25–76%); (b) HCl, dioxane (67–98%); (c) Fmoc-OSuc, $NaHCO_3$, H_2O/acetone (78–95%).

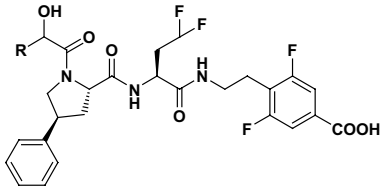
3. Results and discussion

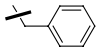
A selection of capped dipeptide analogues was prepared to investigate the hypothesis of replacing an amide or a carbamate by an alcohol, wherein the lipophilic substituents R and the stereochemistry of the alcohol were varied (Table 1).

Of these, certain alkyl or cycloalkyl analogues were active at the micromolar level.

Comparing the two isopropyl α-hydroxy acetic acid derivatives **11** and **12** it was found that one isomer was active, whereas the other one did not show any inhibition even up to 100 μM. In the case of the two cyclopentyl α-hydroxy acetic acid derivatives **13** and **14** one isomer was almost 5-fold more potent than the other one. The most active diastereoisomer has the (*S*)-configuration at the hydroxy alcohol residue, in agreement with our expectations.

Table 1. SAR of capped dipeptide phenethylamides



Compds	R	P3 conf.	IC_{50}, μM[a]
8		(*R*)	na
9		(*S*)	na
10		2 diaster.	na
11		(*R*)	na
12		(*S*)	18
13		(*R*)	62
14		(*S*)	13
15		(*S*)	18
16		2 diaster.	50
17		(*S*)	14
18		2 diaster.	na

[a] na means $IC_{50} > 100$ μM.

Modeling of the compounds in the active site of NS3[16] show that the α-hydroxy group superimposes with the P3 amide NH participating in the same P3–S3 hydrogen bonds to Ala157 as the tripeptide **2** (Fig. 4). In this conformation the lipophilic cyclopentyl side chain is orientated towards the S3 site, which it is capable of occupying completely.

Compounds **19** and **20** lacking the hydroxy group, have been prepared as well (Fig. 5). The 3-fold lower activity (IC_{50} 34 and 58 μM, respectively) confirm the importance of the hydroxy group for the activity of the capped dipeptides.

Having discovered that the commercially available (*S*)-hydroxy-isovaleric residue in P3 was able to replace the N-protected aminoacid we tried to optimize the P2 residue and the compounds reported in Table 2 were made. Only compounds **22** and **24**, containing, respectively, the *p*-Br-benzyloxy and the 3-phenylpropyloxy moieties instead of the phenyl group in the 4-position of the pyrrolidine ring gave a small increase in potency.

Previously we had used the α-hydroxy acid replacement in the design of phenethylamide-Boehringer hybrid structures to explore the role of bigger P2 aryloxyprolines.[17]

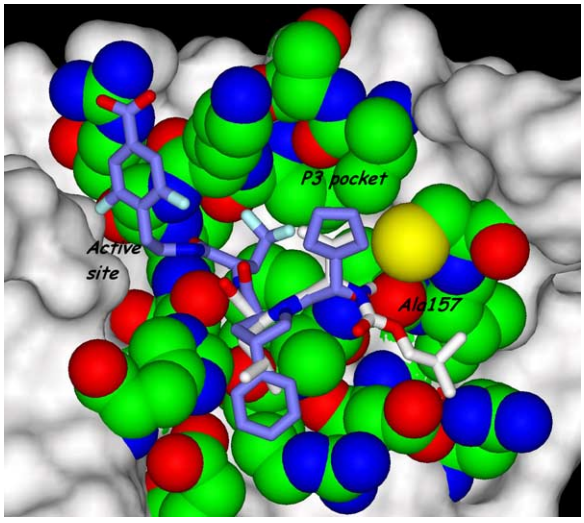


Figure 4. Superposition of **14** (blue) and **2** (only P2, P3 and capping group shown, white).

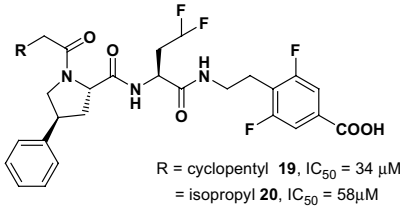
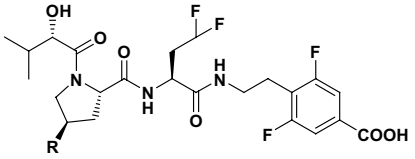
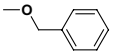
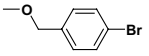
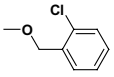
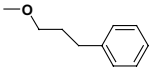


Figure 5. Capped dipeptide phenethylamides without OH in the capping group.

Table 2. P2 SAR of capped dipeptide phenethylamides



Compds	R	IC_{50}, μM[a]
21		27
22		12
23		na
24		11
25		32

[a] na means $IC_{50} > 100\,\mu M$.

In summary, we have shown that also in the non-covalent series of tripeptide phenethylamide inhibitors, an α-hydroxy acid is a viable replacement for the P3-aminoacid, obtaining compounds with lower peptidic character. The preferred (*S*)-configuration was correctly inferred from molecular modeling. Compounds like **14**, **17**, **22** and **24** are active at the low micromolar level and no longer contain the serine trap present in **1**.

Acknowledgements

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