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Novel, Potent Phenethylamide Inhibitors of the Hepatitis C Virus (HCV) NS3 Protease: Probing the Role of P2 Aryloxyprolines with Hybrid Structures

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Abstract—Synthesis of hybrid HCV NS3 protease/NS4A inhibitors having the 4,4-difluoroaminobutyric acid (difluoroAbu) phenethylamides as P1–P1′ and quinolyloxyprolines as P2 fragments led to **7** (IC_{50} 54 nM). Molecular modelling suggests that this potent tripeptide inhibitor utilizes interactions in the S1′, S1, S2, S3 and S4 sites of the protease.

Despite its high global prevalence, the only current treatment for hepatitis C infection is α-interferon, either alone or in combination with the antiviral ribavirin.[1] These therapies are not of general efficacy and are characterised by severe side effects.

Several likely molecular targets for future drug therapy have been identified in recent years, among them the NS3 protease, a unique serine protease that acts in concert with a co-factor, the NS4A protein, to cleave the viral gene product at several defined positions, delivering mature viral peptides that are believed to be necessary for the life-cycle of the virus.[2] The NS3 protease is thus a primary focus for small-molecule drug discovery.

The NS3/NS4A protease/cofactor is an inherently daunting target for drug design, however, on account of its overall architecture and the molecular features of both the protease itself and its (minimal) polycarboxy decapeptide substrates. NS3 is subject to product inhibition: the thiol-containing, polycarboxy hexapeptide product inhibitors reported by us[3] and by others,[4] have been used as a starting point in the search for drug-like leads. We have described our progress in designing a P1 replacement for the ubiquitous cysteine and its subsequent utility in enabling the preparation of potent monocarboxy tripeptide ketoacid inhibitors.[5] The crystal structures of ternary ketoacid/protease/co-factor complexes[6] and solution NMR data[7] provided molecular-level understanding of the covalent and non-covalent interactions involved in the inhibition process.

We recently reported a new series of phenethyl amide NS3 protease inhibitors that feature a phenyl ring in P1′.[8] SAR showed that a carboxylic acid in the *para* position was beneficial for activity and that introduction of halogens *meta* to the carboxylate gave further improvements (Fig. 1). Molecular modelling, mutagenesis studies, and subsequent SAR strongly suggested that the phenyl ring found a unique binding site alongside Lys-136.[8] Additional SAR in this series showed that 3-substituted prolines were a preferred P2 group.[9]

Independently, workers at Boehringer produced an interesting and novel series of highly potent monocarboxy tripeptide product-like inhibitors that had unique aryl fragments (e.g., 4-quinolyloxy, (2-phenyl-7-methoxy)-4-quinolyloxy) attached at C3 of a P2 proline (**1**,**6**).[10]

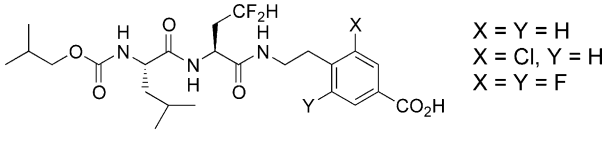

Figure 1. Tripeptide phenethylamides HCV protease inhibitors.[8]

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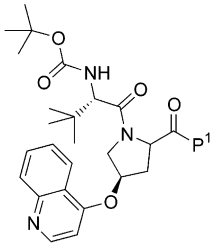
We were intrigued by the possibility that the aryl rings from our phenethyl amides and the Boehringer quinolines might occupy the same binding site on the protease. To probe this, we have made hybrid structures between the two families of inhibitors. We envisaged two positive outcomes:

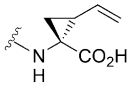
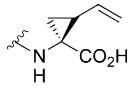
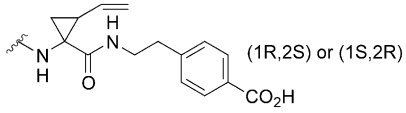
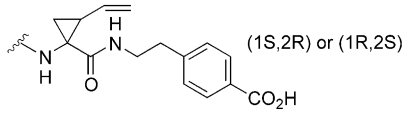
a. If the two series shared a common aryl binding site, novel hybrid series could be designed, for example where the phenethyl amide ring could be elaborated into substituted quinolines; or
b. If the aryl binding sites were discrete, then alternative novel hybrid series could result wherein both aryl groups could be present in the same structure.

Our unexpected findings are the subject of this communication.

To afford an initial comparison, the diastereomeric phenethyl amides **3** and **4** were prepared from the parent acids **1**, **2**[10] (Table 1). In contrast to **1** (IC_{50} 330 nM) which was, as reported,[10] a potent inhibitor of the protease, the phenethyl amides **3**, **4** were completely inactive up to a concentration of 100 μM. Inhibition data were obtained on the full-length NS3/NS4A complex.[11]

Table 1. P1 vinylcyclopropyl acids and corresponding phenethylamides



Compd	P1	IC_{50} (μM)
1	 (1R,2S)	0.33[a,b]
2	 (1S,2R)	na[a,c]
3	 (1R,2S) or (1S,2R)	na[c]
4	 (1S,2R) or (1R,2S)	na[c]

[a]Ref 10.
[b]IC_{50} <500 nM,[10] confirmed by synthesis and testing of the compounds.
[c]na, not active.

Given that the P1 difluoroethyl substituent is compatible with either a terminal carboxy group or the *p*-carboxyphenethyl amide,[8] the data in Table 1 suggest an incompatibility between the P1 vinylcyclopropyl substituent and/or the P2 quinolyl group with the phenethyl amide group in **3**, **4**.

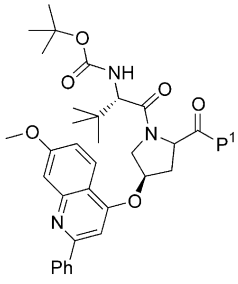
To discern whether the incompatibility was due to P1 or P2, analogues were synthesized that had 4,4-difluoro-2-aminobutyric acid (difluoroAbu) in P1.[12] In addition, we chose to use the potent Boehringer P2 (2-phenyl-7-methoxyquinolyl proline[10]), together with the most potent Merck phenethyl amide (difluoro-carboxyphenyl).[13] The acid **5** was prepared as a mixture of P1 diastereomers (Table 2).

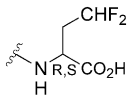
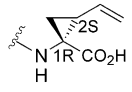
Allowing for the presence of one (largely) inactive diastereomer,[12] (*S*)-**5** (IC_{50} ~90 nM) had an activity somewhat less, but in the same range as that of **6** (IC_{50} ~25 nM).[10] This result demonstrated that the vinylcyclopropyl group could be successfully replaced by the difluoroethyl group. However, by contrast with the results of Table 1, making the carboxyphenethyl amide of (*S*)-**5** gave the potent hybrid **7** (IC_{50} 54 nM) (Table 3).

With difluoroAbu in P1, we can therefore conclude that the P1′ phenethyl amide ring and the P2 quinoline (or substituents) are binding at separate sites.

The comparison of **3**, **4** with **7** suggests interference between the phenethyl amide and the vinylcyclopropyl group in the enzyme-bound conformation of **3**/**4**. According to our modelling, the vinylcyclopropyl group causes a distinct change in the binding conformation of the phenethyl amide, reducing favorable interactions in the oxoanion cavity and between the phenyl ring and Lys136 (Fig. 2).[8] Consequently, the P1′ SAR for the P1 vinylcyclopropyl series is different from the difluoroAbu

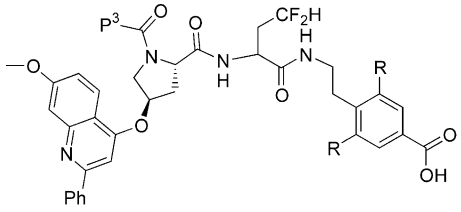
Table 2. Comparison of P1 vinylcyclopropyl and difluoroethyl acids

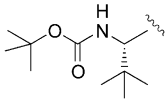
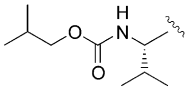


Compd	P1	IC_{50} (μM)
5		0.180
6		0.025[a]

[a]Ref 10.

Table 3. P1-Difluoroabu-phenethylamides



Compd	P3	R	IC_{50} (μM)
			[a]
7		F	0.054
8		H	0.4
9		H	11
10		H	na[b]
11	Me	F	38,150[c]

[a]Single diastereomer, *S* configuration at P1.
[b]na, not active.
[c]Values for the two diastereomers.

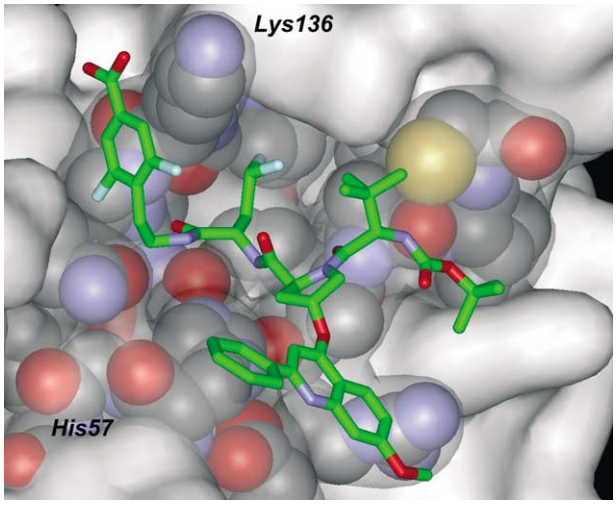


Figure 2. Modeling of **7** in the NS3/NS4A complex.

series. Interestingly, whereas addition of the phenethylamide to tripeptides with a P2 Leu caused a significant increase in activity with respect to the corresponding carboxylic acid,[8] in our case of tripeptides with a P2 quinolinoxy-proline such a significant improvement was not observed. The SAR trends are therefore not parallel, perhaps not surprisingly given the structural differences at P2 between the two series.

With potent phenethyl amides in hand, P3 SAR was briefly studied in an effort to further reduce peptide character and molecular weight in these non-covalent inhibitors (Table 3). Replacing the terminal urethane in **8** by an *iso*-butyl alcohol[14] gave **9** that was some 25–30-fold less active. Capping the proline as a *t*-butyl amide abolished activity (**10**: $IC_{50} >> 100\,\mu M$). Simplification of **7** gave **11**, which was essentially inactive.

Simplifications at P3 resulted in significant drop in activity. The mode of binding and SAR of the P3 side chain and the capping group, which essentially binds to the S4 site, is similar to that observed for other phenethylamides (Fig. 2).[8] We observe close contacts between the P1 and P3 and the P2 and capping group side chains indicating a direction for further optimization.

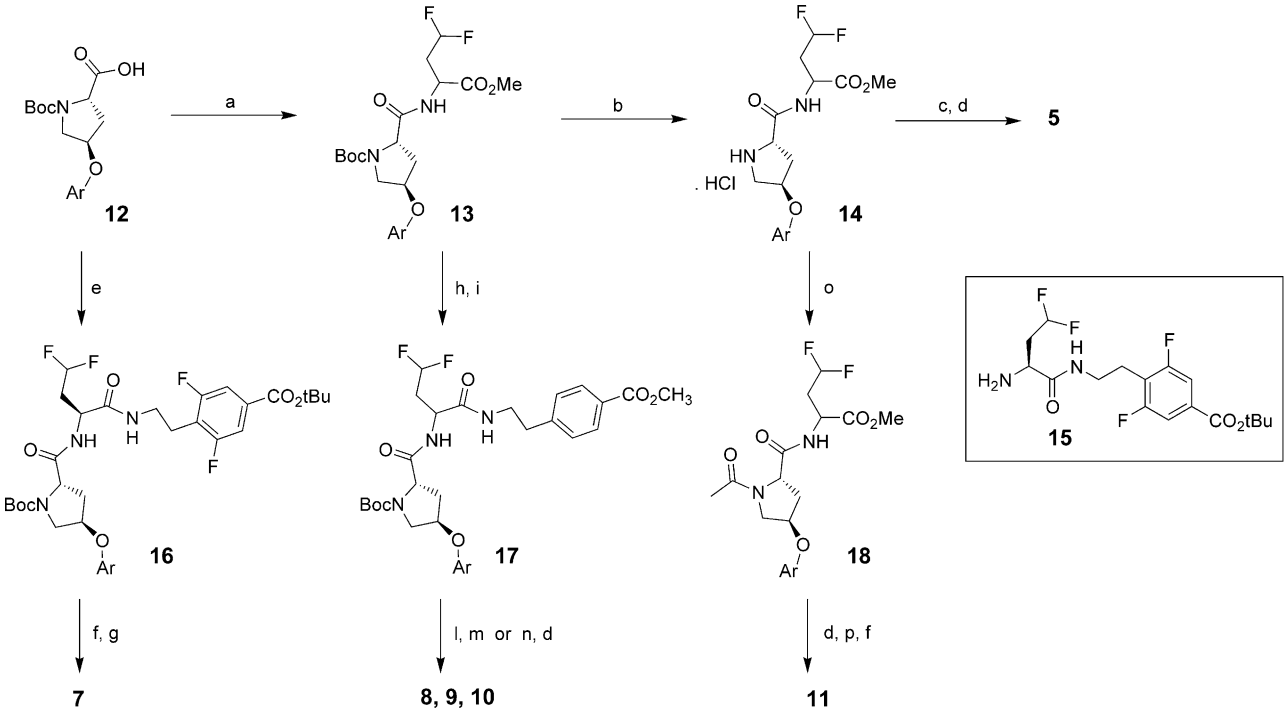
Synthesis

All the final compounds were isolated by preparative RP-HPLC.[15] **1**, **2** were synthesized according to the literature.[10] **3**, **4** were prepared from the diastereomeric mixture at P1 of acids **1**, **2** by coupling with 4-methoxycarbonylphenethylamine (HATU, DIPEA, DCM), separation of the two diastereomers by flash chromatography, followed by hydrolysis (NaOH, MeOH/THF). The synthesis of **5**, **7**–**11** is summarized in Scheme 1. Boc-protected proline **12**[10] was coupled with methyl 4,4-difluoro-2-aminobutyrate, readily obtained from 4,4-difluoro-2-aminobutyric acid,[12] to give **13** that, after Boc removal (**14**), was reacted with *N*-Boc-(L)-*t*-Bu-GlyCOF, obtained by fluorination with trifluorotriazine[16] of *N*-Boc-(L)-*t*-Bu-Gly, and the crude methyl ester was hydrolyzed to **5**. Coupling of **12** with the preformed phenethylamide **15** (2,5-difluoro-4-*t*-butoxycarbonylphenethylamide of (*S*)-4,4-difluoro-2-aminobutyric acid),[13] produced **16**, that underwent simultaneous Boc and *t*-Bu ester deprotection, followed by coupling of the resulting aminoacid with an excess of *N*-Boc-(L)-*t*-Bu-GlyOH, to give **7**.

Phenethyl amides **8**–**10** were prepared from **13**, which was hydrolyzed to the corresponding carboxylic acid and coupled with 4-methoxycarbonylphenethylamine to give **17**. **17** was Boc-deprotected and coupled with the required carboxylic acid (**8**, **9**) or acylated with pivaloyl chloride (**10**) and finally hydrolyzed to the final compounds. The two diastereomers of **11** were synthesized from **14** by acetylation followed by separation of the two diastereomers at P1 (**18**) by flash chromatography. Each of the two diastereomers was then separately hydrolyzed, coupled with 2,5-difluoro-4-*t*-butoxycarbonylphenethylamine, and finally deprotected.

In conclusion, through synthesizing hybrid inhibitors that incorporated diverse P1, P2 and P1′ groups we generated potent tripeptide inhibitors that utilize interactions in the S1′, S1, S2, S3 and S4 sites of the protease. We established that the phenyl rings of the P1′ phenethyl amide and 2-phenyl-7-methoxyquinoline substituent of P2 proline bind at different sites on the protease.

With 4,4-difluoro-2-aminobutyric acid in P1, the phenethyl amide group is compatible with the presence of



Scheme 1. (a) Methyl 4,4-difluoro-2-aminobutyrate (1 equiv), HATU (1.2 equiv), DIPEA (3 equiv), DCM, 82%; (b) 4 N HCl in dioxane; (c) Boc-(L)-*t*BuGlyCOF (1.2 equiv), TEA (3 equiv), DCM; (d) 1 N NaOH, MeOH, THF; (e) (*S*)-**15** (1 equiv), HATU (1.2 equiv), DIPEA (2.5 equiv), DCM, 96%; (f) TFA–H_2O; (g) Boc-(L)-*t*BuGlyOH (4 equiv), HATU (3.5 equiv), DIPEA (7 equiv), DCM; (h) 1 N NaOH, MeOH; (i) 4-methoxycarbonylphenethylamine (1 equiv), HOBt (2 equiv), DIPEA (3 equiv), EDC (1.5 equiv), DCM; (l) TFA; (m) RCO_2H (1 equiv), HATU (1.2 equiv), DIPEA (5 equiv), DCM; (n) *t*BuCOCl (2 equiv), DIPEA (5 equiv), DCM; (o) AcCl (1.5 equiv), Et_3N (5 equiv), DCM; flash chromatography; (p) 2,5-difluoro-4-*t*-butoxycarbonylphenethylamine (1.1 equiv), HATU (1.3 equiv), DIPEA (12 equiv), DCM.

the phenyl-methoxyquinoline at P2. However, the P1 vinylcyclopropyl group is not compatible with the phenethyl amide.

Acknowledgements

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References and Notes

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