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SAR and pharmacokinetic studies on phenethylamide inhibitors of the hepatitis C virus NS3/NS4A serine protease

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Abstract—SAR on the phenethylamide **1** (K_i 1.2 μM) in the P2- and the P′-position led to potent inhibitors, one of which showed good exposure and low clearance when administered intramuscularly to rat.

Hepatitis C virus (HCV) infections constitute a global health problem, which affects more than 170 million individuals. Most of these individuals, if untreated, will develop chronic liver disease, which can develop into liver cirrhosis and hepatocellular carcinoma.[1] In contrast to hepatitis B virus infections, no vaccine or general therapy is available. The limited efficacy of the current therapy, α-interferon in combination with ribavirin,[2] has stimulated intense research programs to find a broadly effective antiviral therapy.[3]

The nonstructural (NS) protein 3 of the viral genome harbors a serine protease, which forms a heterodimer with the NS4A protein. This chymotrypsin-like NS3/4A protease is essential for viral replication,[4] and has been studied intensively in the search for new effective antiviral agents. Several classes of inhibitors of NS3 have been described in the literature, the majority derived from the substrates of NS3.[5] The classical serine trap approach yielded small peptidic or peptidomimetic inhibitors with good potency,[5,6] but it is doubtful if such electrophilic molecules would ever become drugs. Another approach, based on product inhibition led to noncovalent macrocyclic inhibitors,[7] one of which showed a substantial antiviral effect in clinical trials.[8]

We recently disclosed a novel class of noncovalent inhibitors of the NS3/4A protease, which contain a C-terminal phenethylamide moiety.[9] The initial polyanionic hexapeptide lead could be truncated to tripeptides like **1** (Fig. 1), which contain only one carboxylic acid. Amide **1** was shown to be a competitive, reversible inhibitor of the NS3/4A protease. The compound has comparable activity on the protease domain and on the full-length NS3 protein/NS4A complex, comprising protease-helicase/NTPase (Fig. 1).

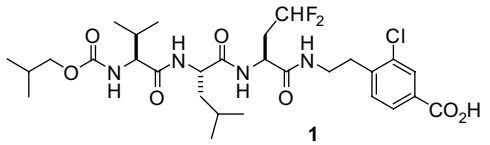

Figure 1.

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From SAR data, molecular modeling, and inhibition data generated on mutagenized enzymes, it was concluded that the phenethyl group is binding in the prime site of the enzyme, with the strictly required phenyl group occupying a unique position, where it stacks against the aliphatic part of the side-chain of Lys136.[9]

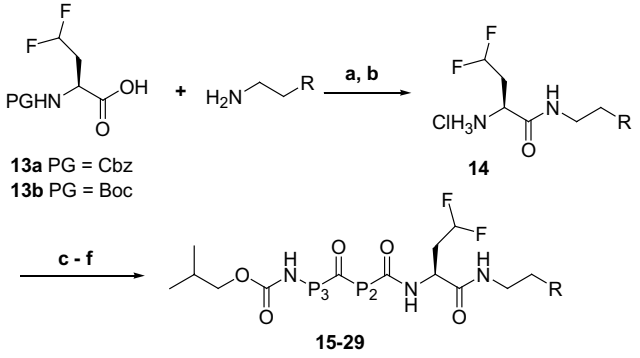
In this letter we report the further optimization of **1** towards more potent inhibitors and disclose first encouraging PK data.

The synthesis of representative phenethylamines **4a**,**b**, **8** and **12** is shown in Scheme 1. For the installation of the ethylamine moiety we relied on the *ortho*-directing effect of the fluorine group.[10] For example, *tert*-butyl-3,5-difluorobenzoate **2a** was treated with LDA and the resulting anion quenched with DMF. The formyl group was then converted into the nitroethyl group via the nitrostyrene to give **3a**, whose reduction lead to the phenethylamine **4a**.

Tripeptides **15**–**29** were assembled as previously reported (Scheme 2).[9] *trans*-4-Cyclohexyl proline was obtained by hydrogenation of the commercially available 4-phenylproline.[11] Acylsulfonamide **22** was obtained from **18** and methyl sulfonamide using EDCI.

All inhibitors were then evaluated for inhibitory activity on the NS3/NS4A full-length complex.[12]

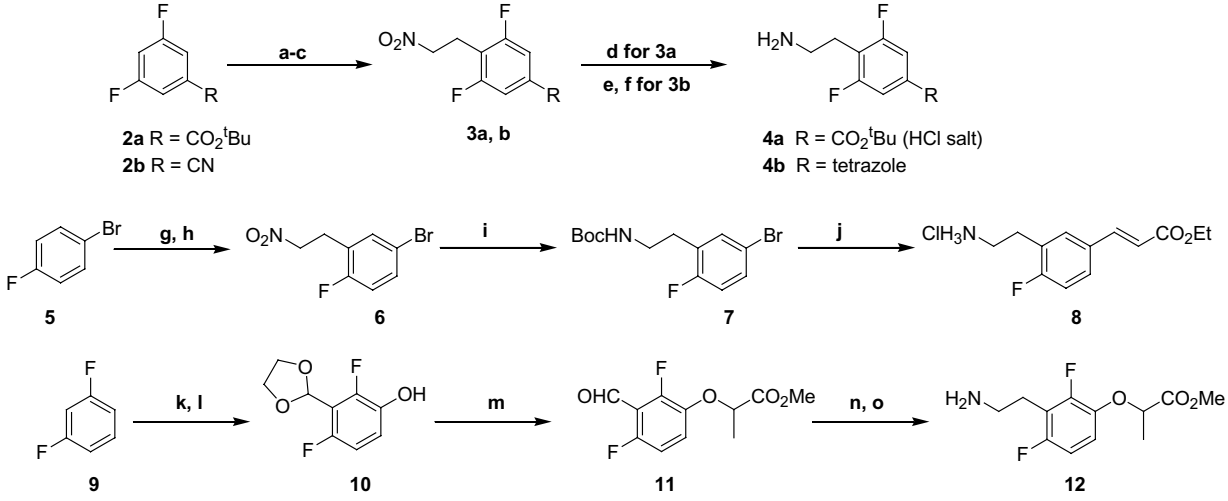
Extensive SAR in the previous Glu-Leu-Cys series had established the requirement of the 4-benzoic acid moiety as the optimal residue. Improvement of potency in this series could be achieved by incorporation of a chlorine substituent.[9] To evaluate if another halogen would further enhance the activity, we synthesized tripeptide **15**, containing the 2,6-difluorobenzoic acid, which was indeed threefold more potent than **1** (Table 1).



Scheme 2. (a) EDCI, HOBt, DCM, *i*-Pr_2NEt; (b) **13a**: H_2, Pd/C MeOH; HCl/Et_2O; **13b**: HCl/EtOAc; (c) P2-residue, EDCI, HOBt, *i*-Pr_2NEt; HCl/EtOAc (Boc) or 4-AMP, DCM; phosphate buffer, pH 5.5 (Fmoc); (d) *i*-Boc-P3-residue, HATU, 2,6-lutidine, DMF; (e) deprotection: TFA/DCM/H_2O (1:1:0.1) or NaOH, MeOH; (f) RP-HPLC.

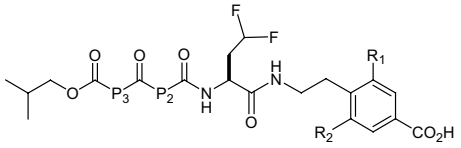
Several groups had reported that substituted proline derivatives in the P2-position can significantly enhance potency in product inhibitor and serine-trap based series.[7,13] We tried this approach in our phenethylamide series[14a] and the results are presented in Table 1.

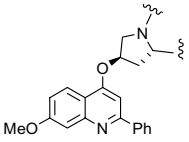
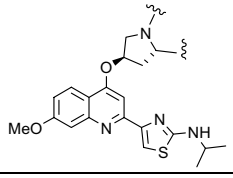
Exchanging leucine in **15** with *trans*-4-benzyloxyproline gave equipotent **16a**. Inverting the stereochemistry of the 4-substituent to *cis* as in **16b**, resulted in a 10-fold loss of potency. Due to the potential metabolic instability of the benzyloxy residue, we investigated carbon-linked 4-substituents and found that a 4-phenyl- or a 4-cyclohexyl residue are equally well tolerated. The resulting inhibitors **17** and **18** are not significantly more potent compared to the leucine containing compound **15**, but the cyclic P2-residue renders these structures less peptidic and therefore should increase their hydrolytic stability. As we have recently reported,[14b] a major gain



Scheme 1. Reagents and conditions: (a) LDA, THF, −78 °C, 1 h; DMF, **2a** 77%; **2b** 60%; (b) $MeNO_2$, KF, NMM, *i*-PrOH, 0 °C; Ac_2O, NaOAc, 60 °C; (c) $NaBH_4$, SiO_2, *i*-PrOH, $CHCl_3$, **3a** 45% (two steps), **3b** 40% (two steps); (d) H_2, Pd/C; Et_2O/HCl, 98%; (e) NaN_3, NH_4Cl, DMF, 42%; (f) H_2, Pd/C, 67%; (g) as in (a), 83%; (h) as in (b) and (c), 65%; (i) $NiCl_2$, $NaBH_4$, MeOH; Boc_2O, NEt_3, DCM, 57%; (j) $Pd(OAc)_2$, P(*o*-tol)$_3$, NaOAc, CH_2=$CHCO_2Et$, DMF; EtOAc, HCl, 75%; (k) LDA, THF, DMF; ethylenglycol, PPTs, 85%; (l) BuLi, TMEDA; $B(OMe)_3$; oxone, 63%; (m) $Me(Br)CHCO_2Me$, K_2CO_3, acetone; PPTs, H_2O, acetone, reflux, 76%; (n) as in (b) and (c), 55%; (o) as in (f), 85%.

Table 1. SAR on inhibitor **1**



No.	P2	P3	R_1, R_2	K_i (μM)
1	Leu	Val	Cl, H	1.2
15	Leu	Val	F, F	0.40
16a	*trans*-4-BnO-Pro	Val	F, F	0.55
16b	*cis*-4-BnO-Pro	Val	F, F	4.5
17	*trans*-4-Phe-Pro	Val	F, F	0.45
18	*trans*-4-Cy-Pro	Val	F, F	0.35
19		*t*-Bu-gly	F, F	0.03
20		Val	F, F	0.02

in potency can be achieved by incorporation of a large (2-aryl-7-methoxy)-4-quinolyl fragment, discovered by the Boehringer group.[7] Compounds **19**[14b] and **20** are about 20-fold more active than **15**–**18**, but their molecular weight is now well above 850. For this reason we chose **17** and **18** for the investigation of the SAR in the P′-site.

An acid is required for activity, as inactive methyl ester **21** shows (Table 2), but it can be successfully converted to known isosteres like an acylsulfonamide (**22**, K_i 0.45 μM) or a tetrazole (**23**, K_i 0.1 μM). Interestingly, this compound is threefold more active than the parent acid **18**.

Previous SAR in the series related to **1** had shown that elongation of the acid led to an increase in potency only in the *meta*-position.[15] In the P2-proline series, the *meta*-cinnamic acid **24** (K_i 1.3 μM) is fourfold less active than **18**, but also here incorporation of a fluorine increases potency (**25**, K_i 0.35 μM). The position of the halogen is not relevant for the activity, as equipotent **26** shows (Table 2).

Since numerous attempts to increase activity by increasing the size of the aromatic ring were not successful in the Glu-Leu-Cys series,[9] we investigated the possibility of appending hydrophobic residues in the context of a structure similar to **24**. Indeed, we had seen earlier that substituted phenyl acetic acid derivatives give compounds with good affinity for NS3 (e.g. **27a** and **27b**). Further investigation of this series showed that the phenyl ring does not contribute significantly to potency, as the ethyl derivative **28**, (K_i 0.5 μM) illustrates. The major gain in potency is thus due to the presence of the two fluorines, and this is demonstrated with tripeptides **29a**,**b**, both of which achieve potencies similar to tetrazole **23**.

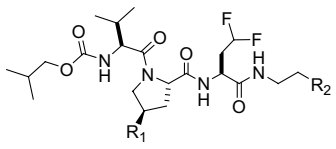
Having increased potency about 10-fold, we investigated the pharmacokinetic behavior of our molecules.

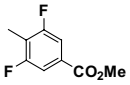
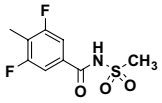
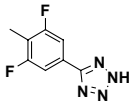
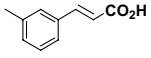
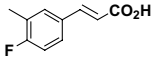
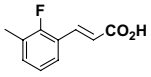
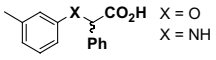
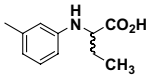
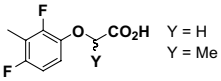
Some selected compounds were dosed intravenously to rats to examine their pharmacokinetic parameters (Table 3). Their behaviour was mostly characterized by high clearance and a short half-life, with compound **16a** exhibiting the highest plasma clearance. Exchanging the benzyloxy substituent on proline to phenyl (**17**) or cyclohexyl (**18**) improved the clearance, although half-life for the latter compound is short. Exchanging the acid in **18** for acid mimetics was beneficial in the case of tetrazole **23**, which exhibited low clearance and reasonable half-life and AUC, whereas acylsulfonamide **22** was comparable to parent acid **18**.

Acylsulfonamide **22** was also dosed intramuscularly to rat and showed a remarkable profile (Fig. 2). Very good exposure with an estimated bioavailability of 85% was observed over an extended period of time.

In summary we have optimized the potency of the noncovalent tripeptide phenethylamide series of NS3/4A inhibitors. Although a substantial gain in potency can be achieved with large lipophilic substituents in P2, as compound **20** (K_i 0.02 μM) demonstrates, this results in compounds with high molecular weight (933 Daltons), which is above the optimal range for most orally active drugs. Small changes in the phenethylamide moiety by incorporation of two fluorines and modifications on the acidic group led to tripeptides **23** and **29b** with K_i's of

Table 2.



No.	R_1	R_2	K_i (μM)
21	Cy		Na[b]
22	Cy		0.45
23	Cy		0.10
24	Ph		1.30
25	Ph		0.35
26	Ph		0.40
27a	Ph	 X = O	1.0[a]
27b	Ph	X = NH	0.65[a]
28	Ph		0.50[a]
29a	Cy	 Y = H	0.19[a]
29b	Cy	Y = Me	0.12[a]

[a] Mixture of diastereomers.
[b] Na: not active at 50 μM.

Table 3. PK parameters of selected phenethylamides[a]

No.	$t_{1/2}$ (min)	Cl_p (mL/min/kg)	V_{dss} (L/kg)	AUC (μM × h)
16a	48	222	4.5	0.4
17	72	36	2.4	1.3
18	15	44	0.45	2.7
22[b]	19	46	0.8	0.9
23	74	9	0.15	12.4

[a] Compound dosed (iv) to male Sprague–Dawley rats as a suspension in DMSO/EtOH/water (20:20:60) at 5 mg/kg.
[b] Dosed at 2 mg/kg.

about 100 nM, less active than **20**, but with lower molecular weight. Despite its relatively peptidic nature tetrazole **23** showed an improved behavior compared to acid **18** upon iv dosage to rat. The acylsulfonamide **22** exhibited good exposure upon intramuscular dosing.

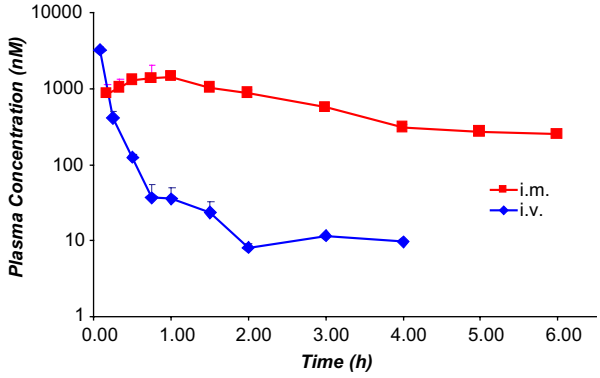


Figure 2. Profile of compound **22** after iv (2 mg/kg) and im dosage (10 mg/kg, solution in 80% DMSO and 20% water) to rat.

Acknowledgements

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15. For example: *i*-BocGluLeuCysNH$(CH_2)_2$ Ph(4-CHCH-CO_2H), K_i 5 μM; *i*-BocGluLeuCysNH$(CH_2)_2$ Ph(3-CH=CH–CO_2H), K_i 1.5 μM; *i*-BocValLeu-difluoroAbuNH$(CH_2)_2$Ph(2-CH=CHCO_2H), K_i >30 μM.