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Novel Peptidomimetic Hepatitis C Virus NS3/4A Protease Inhibitors Spanning the P2−P1′ Region

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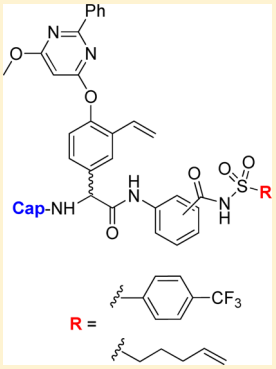
Supporting Information

ABSTRACT: Herein, novel hepatitis C virus NS3/4A protease inhibitors based on a P2 pyrimidinyloxyphenylglycine in combination with various regioisomers of an aryl acyl sulfonamide functionality in P1 are presented. The P1′ 4-(trifluoromethyl)phenyl side chain was shown to be particularly beneficial in terms of inhibitory potency. Several inhibitors with K_i-values in the nanomolar range were developed and included identification of promising P3-truncated inhibitors spanning from P2−P1′. Of several different P2 capping groups that were evaluated, a preference for the sterically congested Boc group was revealed. The inhibitors were found to retain inhibitory potencies for A156T, D168V, and R155K variants of the protease. Furthermore, in vitro pharmacokinetic profiling showed several beneficial effects on metabolic stability as well as on apparent intestinal permeability from both P3 truncation and the use of the P1′ 4-(trifluoromethyl)phenyl side chain.



KEYWORDS: *HCV, NS3, protease inhibitors, peptidomimetics, phenylglycine*

Hepatitis C is an inflammatory disease that affects around 2.2−3.0% of the world's population and is caused by the blood-borne hepatitis C virus (HCV).[1] The infection resides mainly in hepatocytes and becomes chronic in approximately 80% of infected individuals. Progression of the disease often develops into various stage of liver disease, such as cirrhosis or liver cancer, within a time period of 30 years.[2] There are several potential targets for anti-HCV therapy, of which the protease part of the bifunctional nonstructural protein 3 (NS3) is one of the most well-studied.[3] Proof-of-concept, that inhibition of the NS3/4A protease leads to reduction in plasma HCV RNA loads in infected patients, was established with ciluprevir (BILN 2061, Figure 1).[4] Thereafter, several NS3/4A protease inhibitors have been developed and entered into clinical trials, some of which are shown in Figure 1.[5] Two of the them, Incivek (Vertex) and Victrelis (Merck and Co.), have recently been launched on the market and are now used in combination with the standard therapy of pegylated interferon-α and ribavirin for treatment of HCV genotype 1.[6]

The above-mentioned inhibitors of HCV NS3/4A (as exemplified in Figure 1) share some structural similarities. They are peptide-based and share a proline or a proline mimic in the P2 position. Although they possess excellent antiviral capacities, the emergence of resistant strains of the protease is disquieting.[6,7] The mutations that have emerged in in vitro and

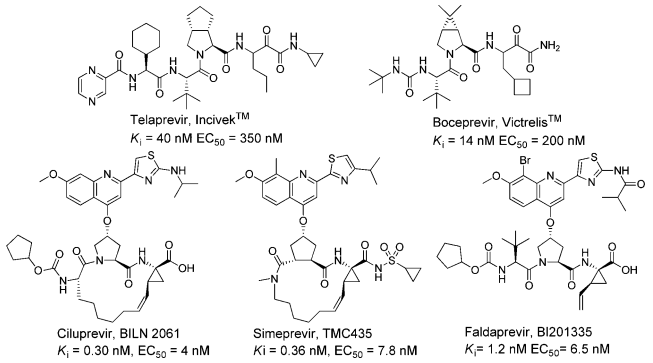


Figure 1. Selection of HCV NS3/4A protease inhibitors that have entered clinical trials.[5]

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in vivo studies of the viral genome, under a selective pressure of HCV NS3/4A protease inhibitors, are often related to amino acids close to the S2 position of the protease, thus accounting for cross-resistance issues.[6−9] We have therefore searched for alternatives to the commonly employed P2 proline residue in HCV NS3/4A protease inhibitors. A promising alternative is the use of a phenylglycine as a P2 moiety.[10,11] In addition, P2 phenylglycine-based inhibitors have been shown to retain activity against mutated strains of the protease.[11−13] Further development and optimization are still necessary in order to improve antiviral activities. During our initial studies, we discovered that a 2-phenyl-6-methoxypyrimidine based P2 substituent was beneficial in combination with a vinylated phenylglycine, an aromatic P1 moiety, and alkenylic P1′ substituents (**1**, Figure 2). We also discovered that a P3 truncated inhibitor (**2**) showed reasonable inhibitory potency (K_i = 190 nM, Figure 2)

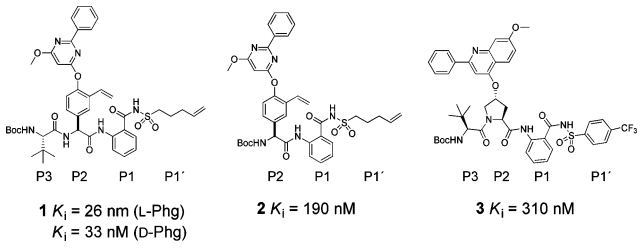


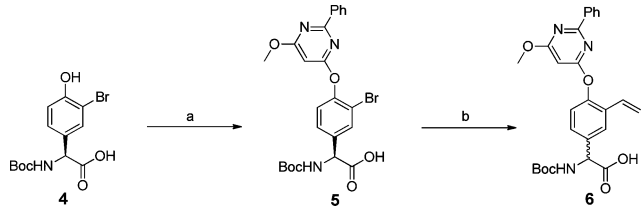
Figure 2. P3−P1′ spanning HCV NS3/4A protease inhibitor (**1**), its P3 truncated analogue (**2**), and a proline-based HCV NS3/4A protease inhibitor (**3**).[14]

We have investigated the use of an aromatic P1 moiety in proline-based inhibitors previously (e.g., **3** in Figure 2).[14] Because the aromatic P1 enables synthesis of regioisomers, it was appealing to us to utilize this property to examine how acyl sulfonamides at different positions around the aromatic P1 would influence inhibitory potency for phenylglycine-based inhibitors. Furthermore, we felt prompted to investigate the possibility to develop smaller, less peptide-like but more drug-like inhibitors with only one α-amino acid and therefore designed P3 truncated inhibitors (exemplified by **2**). Thus, we herein present the synthesis and biochemical evaluation of a series of P3−P1′ and P2−P1′ spanning HCV NS3/4A protease inhibitors comprising a vinylated hydroxy phenylglycine substituted with a 2-phenyl-6-methoxy-pyrimidine, combined with an ortho (*o*-), meta (*m*-), or para (*p*-)-regioisomer of a P1 aryl acyl sulfonamide with either the pent-4-enyl side chain or the 4-(trifluoromethyl)phenyl side chain in P1′-position. For the P3 truncated inhibitors, a scan of various P2 *N*-capping groups was also performed to establish any important inhibitor-protease interactions at the S3−S2 border. Since we are aiming for inhibitors with a unique binding mode, the ability to inhibit known drug resistant enzyme variants was evaluated, as well as their in vitro pharmacokinetic properties.

The synthesis of the P2 building block **6** is described in Scheme 1. Compound **6** was synthesized from a 3-bromo, 4-hydroxy-phenylglycine derivative via a substitution reaction followed by a Suzuki reaction to give the partially racemic compound **6**. The racemization is a known side-effect during Suzuki couplings with phenylglycine.[15]

In a previous study, we found that a 4-(trifluoromethyl)-benzene sulfonamide was beneficial as a P1′ substituent in combination with an aromatic P1.[14] For the synthesis of the

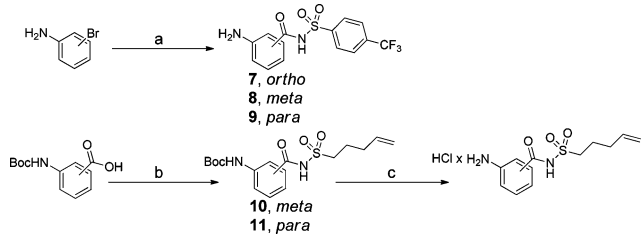
Scheme 1. Synthetic Route[a]



[a]Reagents: (a) 4-chloro-6-methoxy-2-phenylpyrimidine, KO*t*Bu, DMSO, 64 °C (87%); (b) 2,4,6-trivinylcycloboroxane pyridine complex, $Pd(OAc)_2$, $[(tBu)_3PH]BF_4$, K_2CO_3, DME, H_2O, microwave irradiation at 100 °C for 15 min (82%).

P1−P1′ building blocks, a microwave-assisted carbonylative approach including bromoaniline, *trans*-bis(acetato)bis[*o*-(di-*o*-tolylphosphino)benzyl]-dipalladium(II) (Herrmann's palladacycle), [(*t*Bu)3PH]BF4, 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), and $Mo(CO)_6$ as a solid carbon monoxide source was applied (Scheme 2).[16] Using this method, the P1−P1′

Scheme 2. Synthetic Route to P1−P1′ Building Blocks[a]



[a]Reagents: (a) Herrmann's palladacycle, [(tBu)3PH]BF4, $Mo(CO)_6$, DBU, 1,4-dioxane, microwave irradiation at 140 °C for 25 min (**7**, 50%; **8**, 64%; **9**, 62%); (b) CDI, DBU, THF, 60 °C → rt (**10**, 71%; **11**, 69%); (c) 4.0 M HCl in 1,4-dioxane (quantitative).

building blocks as *o*-, *m*- and *p*-isomers **7**−**9** could be synthesized in only 25 min, without need for amine protection. The P1−P1′ building blocks containing a pent-4-enyl sulfonamide substituent were synthesized in the standard way by coupling of Boc-protected *m*- and *p*-aminobenzoic acids with pent-4-ene sulfonamide using carbonyldiimidazole (CDI) in dry THF with DBU as base (Scheme 2).

Amide bond formation between the P2 building block **6** and the poorly nucleophilic P1−P1′ moieties **7**−**11** was employed using HATU and *N*,*N*-diisopropylethylamine (DIEA) in dry CH_2Cl_2 at 45 °C and gave P2−P1′spanning compounds **12**−**16** in reasonable yields. The P3−P1′ spanning analogues **17L**−**21D** were obtained after standard HATU promoted couplings of P3 Boc-L-*t*Leu and RP-HPLC purification. Only the L-Phg isomers could be isolated for compounds **17** and **18** (Scheme 3).

To evaluate the effect of the N-capping group, compounds **13**−**15** were *N*-deprotected and reacted with *t*-butylisocyanate, acetyl chloride, 4-morpholinecarbonyl-chloride, nicotinoyl-chloride hydrochloride, or cyclopentyl chloroformate, giving compounds **23**−**29** (Scheme 4).

For compounds **1**, **2**, and **12**−**29**, K_i values were determined in a biochemical inhibition assay using full length NS3 protein of genotype 1a (see Supporting Information) and a 16 amino acid peptide corresponding to the activating region of NS4a (Tables 1 and 2).

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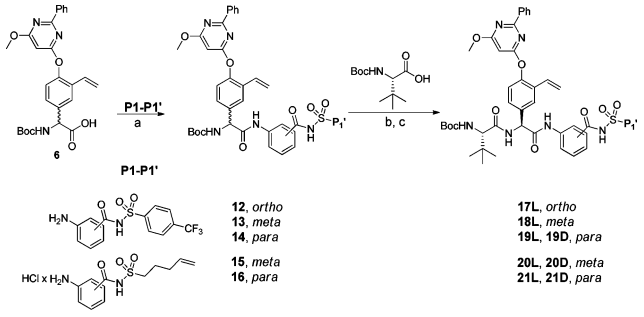
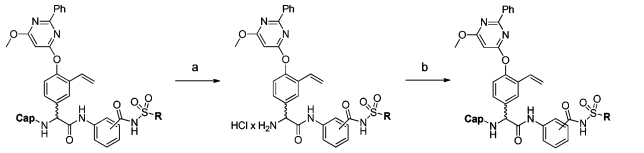
Scheme 3. Synthetic Route[a]



[a]Reagents: (a) HATU, DIEA, CH_2Cl_2, 45 °C (**12**, 83%; **13**, 50%; **14**, 56%; **15**, 74%; **16**, 73%); (b) 4.0 M HCl in 1,4-dioxane; (c) HATU, DIEA, DMF, rt (**17L**, 31%; **18L**, 29%; **19L**, 16%; **19D**, 5%; **20L**, 34%; **20D**, 10%; **21L**, 22%; **21D**, 12%).

Scheme 4. Synthetic Route[a]



13, Cap=Boc, *m*, R=4-CF_3Ph
14, Cap=Boc, *p*, R=4-CF_3Ph
15, Cap=Boc, *m*, R=pent-4-enyl

22, *m*, R=4-CF_3Ph

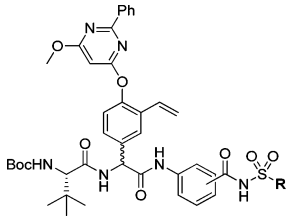
23, Cap=*t*-butyl carbamoyl, *m*, R=4-CF_3Ph
24, Cap=acetyl *m*, R=4-CF_3Ph
25, Cap=4-morpholinecarbonyl *m*, R=4-CF_3Ph
26, Cap=nicotinoyl *m*, R=4-CF_3Ph
27, Cap=cyclopentyloxycarbonyl *m*, R=4-CF_3Ph
28, Cap=cyclopentyloxycarbonyl *p*, R=4-CF_3Ph
29, Cap=cyclopentyloxycarbonyl *m*, R=pent-4-enyl

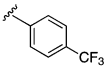
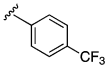
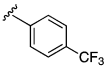
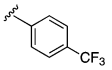
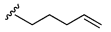
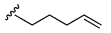
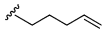
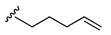
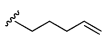
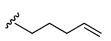
[a]Reagents: (a) 4.0 M HCl in dioxane (100%); (b) CH_2Cl_2 or THF, Et_3N/DMF, DIEA, *t*-butylisocyanate (**23**, 55%), acetyl chloride (**24**, 34%), 4-morpholinecarbonylchloride (**25**, 17%), nicotinoylchloride × HCl (**26**, 39%), or cyclopentyl chloro-formate (**27**, 48%; **28**, 56%; **29**, 51%).

Gratifyingly, the use of a P1′ 4-(trifluoromethyl)phenyl side chain was shown to be beneficial in terms of inhibitory potency giving several inhibitors in the nanomolar range (**13**, **14**, **17**−**19L**, **19D**, **27**, and **28**, $K_i \approx 20-70$ nM, Tables 1 and 2). The aromatic P1′ side chain was especially advantageous for the truncated inhibitors **13** and **14** (K_i = 58 and 46 nM) as compared to their P1′ pent-4-enylic counterparts **15** and **16** ($K_i \approx 200$ nM). Biochemical evaluation of truncated inhibitors comprising P1 regioisomers **12** (*o*-, K_i = 240 nM), **13** (*m*-, K_i = 58 nM), and **14** (*p*-, K_i = 46 nM), with a 4-(trifluoromethyl)-phenyl P1′ substituent, revealed a preference for the *m*- and *p*-isomers.

The same comparison of the P1′ pent-4-enyl regioisomers **15** (*m*-, K_i = 210 nM) and **16** (*p*-, K_i = 220 nM) reveals that these are equipotent to *o*-compound **2** (K_i = 190 nM), although with a slightly decreased inhibitory potency compared to the P1′ 4-(trifluoromethyl)-phenyl containing inhibitors. Coupling of **12**−**14** with the P3 Boc-L-*t*Leu gave P3−P1′ spanning inhibitors **17L** (*o*-, K_i = 45 nM), **18L** (*m*-, K_i = 24 nM), **19L** (*p*-, K_i = 30 nM), and **19D** (*p*-, K_i = 30 nM). Again, a slight preference was shown for the *m*- and *p*-isomers, although the contribution to inhibitory potency for the *m*- and *p*-isomers from the P3 substituent was only 1.5- and 2.5-fold, respectively, as compared with 5-fold for the *o*-isomer. The same increase in potency was noted for the pent-4-enyl containing inhibitors after coupling with the P3 Boc-L-*t*Leu. Here, it was revealed that the *m*-compounds (**20L**, K_i = 88 nM; **20D**, K_i = 100 nM) and *p*-compounds (**21L**, K_i = 96 nM; **21D**, K_i = 110 nM) were less potent than the *o*-compounds (**1L**, K_i = 26 nM; **1D**, K_i = 33 nM). Moreover, no apparent difference was seen between the stereoisomers of these inhibitors. Noteworthy, a comparison with the proline-based aromatic P1 inhibitor **3** (Figure 2) reveals that the aromatic P1 moiety is better suited for combination with phenylglycine than with proline. Both the 4-(trifluoromethyl)phenyl and the pent-4-enyl P1′side chain combined with a P2 2-(4-((6-methoxy-2-phenylpyrimidin-4-yl)oxy)-3-vinylphenylglycine (compounds **17L**, **1L**, and **1D**) gives inhibitors with ca. 10-fold better inhibitory potency than to the corresponding proline inhibitor **3** (Figure 2).

Table 1. Inhibition Constants for NS3/4A Protease[a]



Compound	R	Position	K_i ± SD (nM)
17L		*ortho*	45±5.0
18L		*meta*	24±3.1
19L		*para*	30±3.8
19D		*para*	30±4.0
1L		*ortho*	26±2.5
1D		*ortho*	33± 3.3
20L		*meta*	88±10
20D		*meta*	100±10
21L		*para*	96±20
21D		*para*	110±20

[a]K_i values of P3−P1′ spanning inhibitors were measured with full-length NS3/4A protein from genotype 1a.

A further evaluation of the *N*-capping group of the P3 truncated *m*-isomer **13** showed that removal of the Boc-protecting group (compound **22**, K_i = 1200 nM) caused a 20-fold impairment in the inhibitory activity. The replacement of the Boc-group with an *N*-acetyl capping group reduced the inhibitory potency 14-fold (**24**, K_i = 700 nM) possibly as a result of the reduced bulk. The larger 4-morpholinecarbonyl group (compound **25**, K_i = 590 nM) was slightly better than the *N*-acetyl capping group but was still 12 times less potent compared to inhibitors bearing the Boc-group. The introduction of an *N*-nicotinoyl capping group (compound **26**, K_i = 240 nM) gave a 2-fold improvement compared to the 4-morpholinecarbonyl capping group but was still 4 times less favorable than the Boc-group. The *N*-*t*-butyl carbamoyl (**23** K_i = 140 nM) and the *N*-cyclopentyloxy carbonyl (**27**, K_i = 72 nM) capping groups proved to be the best alternatives to the Boc-group. Thus, the steric bulk of the capping group is clearly of importance. Indeed, exchange of the Boc group on the P3 truncated *p*-compound **14** (K_i = 46 nM) by a cyclopentyl carbamate gave the equipotent inhibitor **28** (K_i = 55 nM).

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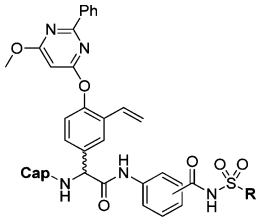
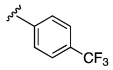
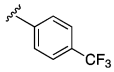
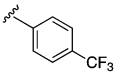
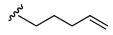
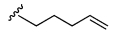
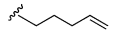
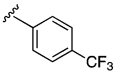
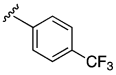
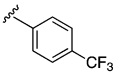
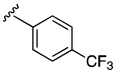
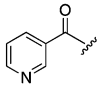
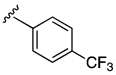
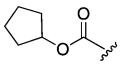
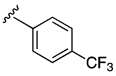
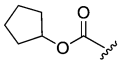
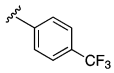
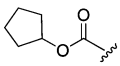
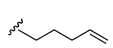
Table 2. Inhibition Constants for NS3/4A Protease[a]



Compound	Cap	R	Position	$K_i \pm$ SD (nM)
12	Boc		*ortho*	240±50
13	Boc		*meta*	58±5.2
14	Boc		*para*	46±5.3
2	Boc		*ortho*	190±20
15	Boc		*meta*	210±40
16	Boc		*para*	220±30
22	H		*meta*	1200±220
23			*meta*	140±20
24			*meta*	700±170
25			*meta*	590±130
26			*meta*	240±50
27			*meta*	72±10
28			*para*	55±7.3
29			*meta*	130±30

[a] K_i values of truncated inhibitors were measured with full-length NS3/4A protein from genotype 1a.

Substitution of Boc by a cyclopentyl carbamate on *m*-compound **15** (K_i = 210 nM) gave compound **29** (K_i = 130 nM) with a slightly improved potency.

Additionally, compounds **13** and **18L** were evaluated toward the drug resistant A156T, D168V, and R155K variants of the protease (Table 3). Vitality values were calculated to normalize the inhibitory effects of the inhibitors with respect to the effects of amino acid substitutions on catalytic efficiency (k_{cat}/K_m) of the enzyme variants. A vitality value less than 1 indicates a more efficient inhibitor against the mutated variant compared to the wild-type enzyme, whereas a value larger than 1 shows that the inhibitor is less efficient against the mutated virus.[12] The possibility to endure antiviral activity on mutated strains of the protease differs among compounds that have entered clinical trials. For example, Incivek (Figure 1) is favored by the D168V substitution[17] (V = 0.4) but highly affected by the A156T substitution (V = 480).[12] Ciluprevir is also highly affected by both the A156T (V = 1600) and D168V (V = 3200) substitutions.[12] Herein, the vitality values for compounds **13** and **18L** shows that the inhibitors have the capacity to retain most of their inhibitory activity on the A156T, D168V, and R155K variants of the protease (vitality values around 1). Compound **18L** is equally affected by the A156T, D168V, and R155K substitutions (V = 1.9, 1.9, and 1.6), while compound **13**, lacking a P3 substituent, is slightly less affected by the D168V substitution (V = 1.3) than the A156T and R155K (V = 1.6 and 1.8) substitutions.

Table 3. Inhibition Constants and Vitality Values Evaluated with A156T, D168V, and R155K Variants of NS3

	A156T		D168V		R155K	
compd	$K_i \pm$ SD (nM)	V^a	$K_i \pm$ SD (nM)	V^a	$K_i \pm$ SD (nM)	V^a
13	110	1.6	180	1.3	580	1.8
18L	53	1.9	110	1.9	210	1.6

[a] Vitality values (V) were calculated using the equation $V = [K_i \times (k_{cat}/K_m)]_{variant}/[K_i \times (k_{cat}/K_m)]_{wild\text{-}type}$.[12]

Compounds **1** (racemate), **13**, and **18L** were also tested for EC_{50}-values in a replicon Huh-7 cell line containing subgenomic HCV RNA genotype 1b replicon with firefly luciferase.[18] Compound **1** and the P2−P1′ inhibitor **13** showed EC_{50} values of 12.5 and 27.5 μM ($CC_{50} \geq 50$ μM; in Huh-7 cells), respectively, whereas the P3-comprising inhibitor **18L** had a EC_{50} value of >50 μM ($CC_{50} \geq 50$ μM). In analogy with acyl sulfonamide based NS3/4A protease inhibitors of submicromolar potency in general there is a significant discrimination between cellular and enzymatic potencies.[19] Clearly, there is room for optimization of the overall potencies, down to low or subnanomolar, for these type of inhibitors.

Compounds **12** (*o*), **13** (*m*), and **14** (*p*) were modeled into the binding site of the full-length NS3/4A protein after removal of the cocrystallized macrocyclic inhibitor.[20] The modeling showed that compound **13** (*m*) finds key interactions with amino acid backbone of the protease (R155 and A157) and that the P1 carbonyl interacts with the oxyanion hole (G137 and S139) (Figure 3). A possible π stacking interaction is seen between the 4-(trifluoromethyl)phenyl substituent and Q41. This type of interaction is seen for the original cocrystallized macrocyclic inhibitor, as well as for a prime side extended α-keto amide inhibitor.[20,21] Additionally, hydrogen bond interaction with the helicase (Q526) is captured by compound **13** (*m*), in analogy with the cocrystallized structure used in the molecular modeling. The *p*-regioisomer **14** showed a similar binding mode in the protein active site. However, the *o*-regioisomer **12** did not fit well in the protein active site. In the docking suggested conformations, several of the significant interactions with the protein were missing, supporting the preference of *m*- and *p*-isomers observed for this type of inhibitors.

To further address the potential of this series of inhibitors and the P3 truncated inhibitors in particular, drug-like properties were evaluated for the various regioisomers of the most potent P1′ 4-(trifluoromethyl)phenyl containing inhibitors with the Boc capping group (**12**−**14**), using in vitro pharmacokinetic profiling (Table 4). One inhibitor each of the

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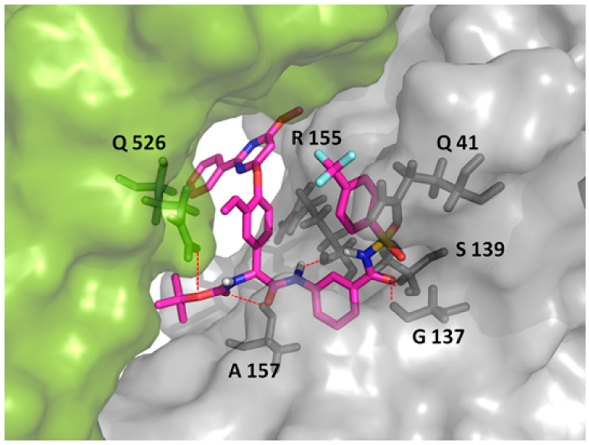

Figure 3. Compound **13** (*m*) docked in the binding site of the full-length NS3/4A protein (PDB code: 4A92).[20] The protease domain is shown in gray; the helicase domain is shown in green. Hydrogen bonds are highlighted as red dashed lines.

corresponding urea- (**23**), P3 *t*-leucine- (**18L**), and the P1′ pent-4-enyl (**2**) analogues were included for comparison. Although the predicted log *D* values were in the same range, the compounds showed relatively large variation in solubility. Whereas the inhibitor comprising the P1′ pent-4-enyl side chain (**2**) showed excellent solubility (>100 μM), the corresponding P1′ 4-(trifluoromethyl)phenyl based inhibitors exhibited lower solubility. The ortho analogue (**12**) displayed a poor solubility of 5 μM, while the *p*-, P3-comprising-, and the urea analogue showed acceptable (>20 μM) solubilities in the range of 30−40 μM.

In analogy, several of the inhibitors with the higher aromatic ring count penetrated the intestinal epithelial cells very well (P_{app} = (4−9) × 10^{-6} cm/s, compounds **12**, **14**, and **18L**). The *m*-isomers showed moderate permeability, and when an additional hydrogen bond donor is introduced as in the urea group, this appears to have a negative impact on the transport over cell membranes as shown from the decreased P_{app} value (0.4 × 10^{-6} cm/s) of compounds **23** as compared to the corresponding carbamate **13** (P_{app} value = 1.0 × 10^{-6} cm/s), although the difference is not statistically significant. Gratifyingly, all the truncated inhibitors with a P1′ 4-(trifluoromethyl)-phenyl side chain (**12**−**14** and **23**) showed a very low risk for high oxidative metabolism in microsomes (Cl_{int} = 7−25 μL/min/mg). These compounds clearly benefitted from the removal of the P3 amino acids since the P3−P1′ spanning inhibitor **18L** showed a significantly higher intrinsic clearance value (80 μL/min/mg). Additionally, the inhibitor with a P1′ pent-4-enyl side chain (**2**, Cl_{int} = 66 μL/min/mg) was more susceptible for oxidative metabolism in microsomes compared to its aromatic counterparts.

In summary, truncated phenylglycine based HCV NS3/4A protease inhibitors of nanomolar potency have been discovered. The aryl acyl sulfonamide moiety in P1 position was preferably combined with a 4-(trifluoromethyl)phenyl P1′ side chain in *m*- and *p*-positions, and the sterically congested Boc group turned out to be the preferred P2 capping group. In general, the P2−P1′spanning inhibitors comprising the Boc and 4-(trifluoromethyl)phenyl groups exhibited very low risk for high first pass metabolism, good apparent intestinal permeability, and moderate solubility under in vitro settings. Further optimization should be devoted to improving the cell-based inhibitory potency. This could probably be achieved by further optimization of the inhibitory potency against the protease and by improving solubility, while maintaining good cell-permeability and metabolic stability. Special attention should be dedicated to drug resistance variants in the lead optimization process since the inhibitors displayed promising inhibition of the A156T, D168V, and R155K variants of the protease.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

All experimental details such as synthetic procedures and characterization of compounds **4**−**29**, enzymatic inhibition assays, molecular modeling procedures, in silico prediction, and in vitro pharmacokinetic assays. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

The manuscript was written through contributions of all authors.

Notes

The authors declare no competing financial interest.

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Table 4. In Vitro and in Silico Determined Properties

	in vitro				in silico	
compd	solubility (μM) pH 7.4	Caco-2 permeability P_{app} (10^{-6} cm/s)[a] a−b[b]	Cl_{int} (μL/min/mg)[c]	$t_{1/2}$[d] (min)	pK_a[e]	log $D_{7.4}$
12	5	4.0	16	88	4.8	4.4
13	n.d.[f]	1.0 ± 0.1	12	117	4.1	3.8
14	37	5.7 ± 0.5	25	55	4.4	3.9
18L	43	9.7 ± 0.9	80	17	4.3	4.5
23	31	0.36 ± 0.3	7	194	4.2	4.1
2	101	1.0 ± 0.7	66	21	5.8	4.0

[a]P_{app} = apparent permeability coefficient. [b]a−b = apical to basolateral. [c]Cl_{int} = in vitro intrinsic clearance. [d]$t_{1/2}$ = in vitro half-life. [e]Acidic pK_a (acyl sulfonamide). [f]n.d. = not determined.

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■ ABBREVIATIONS

HCV, hepatitis C virus; NS, nonstructural; Phg, phenylglycine; Herrmann's palladacycle, *trans*-bis(acetato)bis[*o*-(di-*o*-tolylphosphino)-benzyl]-dipalladium(II); DBU, 1,8-diazabicyclo-[5.4.0]undec-7-ene; HATU, *N*-[(dimethylamino)-1*H*-1,2,3-triazolo-[4,5-*b*]pyridin-1-ylmethylene]-*N*-methylmethanaminium hexa-fluorophosphate *N*-oxide; CDI, carbonyldiimidazole; DIEA, *N*,*N*-diisopropylethylamine; Cl_{int}, intrinsic clearance; P_{app}, apparent permeability coefficient

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