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The introduction of P4 substituted 1-methylcyclohexyl groups into Boceprevir®: A change in direction in the search for a second generation HCV NS3 protease inhibitor

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ABSTRACT

In the search for a second generation HCV protease inhibitor, molecular modeling studies of the X-ray crystal structure of Boceprevir® **1** bound to the NS3 protein suggest that expansion into the S4 pocket could provide additional hydrophobic Van der Waals interactions. Effective replacement of the P4 *tert*-butyl with a cyclohexylmethyl ligand led to inhibitor **2** with improved enzyme and replicon activities. Subsequent modeling and SAR studies led to the pyridine **38** and sulfone analogues **52** and **53** with vastly improved PK parameters in monkeys, forming a new foundation for further exploration.

Hepatitis C virus (HCV) infection is a principle cause of chronic liver disease that leading to fibrosis, cirrhosis and/or hepatocellular carcinoma in humans.[1] HCV has infected more than 170 million people worldwide and it has emerged as a major global health problem. Currently available therapy is α-interferon, either alone or in combination with ribavirin. The latest combination therapy with peginterferon and ribavirin generates sustained virological response in only 40% of infected genotype 1 patients.[2] Sustained response rates of 80-90% are reported in genotype 2 and 3 individuals,[3] While results are achieved, existing therapies are associated with considerable side effects demonstrating a need to develop more effective antiviral agents. This has stimulated intensive research in finding potent and orally bioavailable small molecule drug candidates.[4]

The HCV viral RNA genome encodes a polyprotein which consists of structural and nonstructural (NS) proteins. The chymotrypsin-like serine protease is located in the *N*-terminal of NS3 nonstructural protein and is essential for viral replication.[5] It has been a valuable target for which a number of inhibitors have been reported in literature.[6] several drug candidates have or are being progressed into clinical trials in human beings. The earliest entry, BILN-2061,[7] an NS3 protease inhibitor from Boehringer–Ingelheim, was halted in phase I clinical trials due to toxicity. Currently, the most advanced candidates are Teleprivir (VX-950)[8] from Vertex® and Boceprevir® (SCH 503034)[9] from Schering-Plough. Both are currently in late stage (phase III) clinical trials.

Our first generation HCV NS3 protease inhibitor, compound **1** (Boceprevir®, Fig. 1), is a potent inhibitor of the NS3 protease with a enzyme assay (K_i^*) of 14 nM and a cell-based assay EC_{90} of 350 nM.[8] It had favorable pharmacokinetic (PK) profiles in rats and dogs (26% and 34% bioavailability, respectively) but low

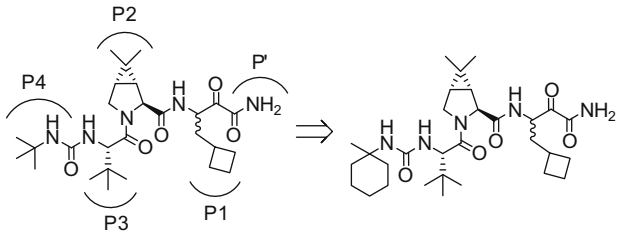

Figure 1. Boceprevir® **1**: the HCV protease inhibitor that is in phase III clinical trials. and its 1-methylcyclohexyl analogue **2**.

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bioavailability in monkeys (4–11%). In the clinic it was shown to be efficacious and well tolerated. Phase II trials have been completed and phase III studies have been initiated. Our goal for a next generation inhibitor was to develop more potent inhibitors with improved upon PK profile, particularly in monkeys, to target a once a day compound.

Boceprevir® **1**, and the compounds reported in this work were examined in a HCV protease continuous assay[10] using the NS4A-tethered single chain NS3 serine protease.[11] The K_i^* values determined in the experiments are a reflection of the equilibrium constant determined by the reversible covalent bond formed between the electrophilic ketone of the inhibitor and the active site serine residue and other interactions formed between the interacting species.[12] Active compounds were then evaluated in a replicon-based cell assay.[13] The EC_{90} was determined as the concentration of inhibitor required for 90% inhibition of viral replication. All compounds were isolated and tested as a mixture of diastereomers with respect to the epimerizable P1 center. The inhibitors were also tested against the serine protease, human neutrophil elastase (HNE), a closely related enzyme, and the ratio of activity HNE/HCV was taken as a measure of selectivity.

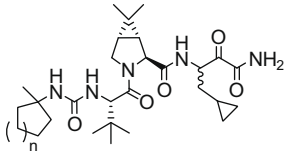
Modeling studies based on X-ray crystal structure of compound **1** revealed that the urea nitrogens donated two H-bonds with the carbonyl oxygen of alanine-157 and while the *tert*-butyl group occupied the S-4 pocket there was an opportunity for expansion in this cavity. To test this hypothesis we synthesized compounds **2** (Fig. 1) and the cyclopropyl analogues **3**–**8** (Table 1) and slight improvements in both enzyme and cell based potencies were observed.

As can be seen from Table 1, there was no significant change in potency with respect to P4 ring size, all four compounds (**3–6**) displaying enzyme and replicon potencies in line with the P1 cyclobutyl analogue **2**. The only noticeable advantage may lie with the elastase selectivity of the six-membered ring analogue **5**.

The X-ray structure of **2** bound to the enzyme revealed that in addition to the interactions observed with the clinical candidate **1**, the cyclohexyl group was nestled in the S4 pocket on one side and the other side was solvent exposed.[14] The methyl group of the P4 ligand clearly extended into the solvent in the direction of Cys159 of the protein backbone suggesting opportunities for additional interactions (Fig. 2).

Early attempts to further improve the physical parameters of our inhibitors involved introducing hydrophilic functionality into the methyl group of the P4 ligand. Using the P1 cyclopropylmethyl side chain, derived from commercially available racemic cyclopropylalanine, the alcohol analogue **7** and amine analogue **8** are prepared (Fig. 3). Unfortunately, the amine **8** proved to be relatively inactive and the alcohol **7**, while active in the enzyme assay, was not potent in the replicon assay, a possible indication of poor cell penetration.

Table 1
Attempted optimization of P4 cycloalkyl ring size the S4 pocket of HCV protease



Compound	*n*	K_i^* (nM)	HNE/ HCV	EC_{90} (nM)
3	0	24	110	200
4	1	10	300	200
5	2	16	640	250
6	3	15	155	250

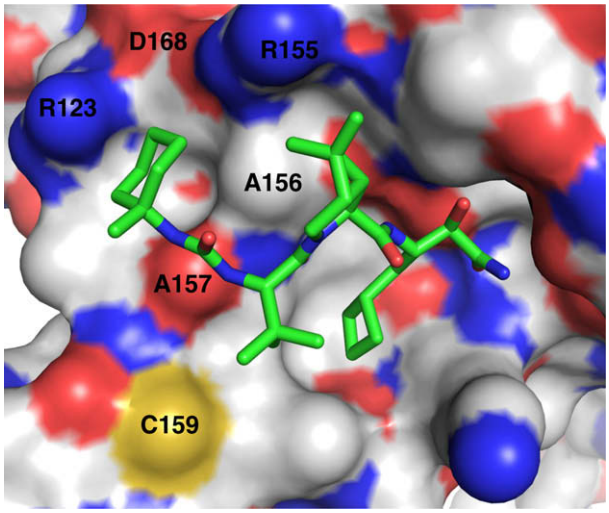
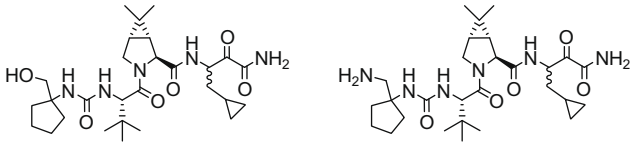


Figure 2. X-ray structure of compound **2** bound to the active site of HCV NS3/NS4A protease domain.

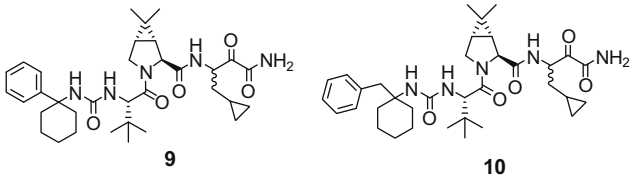


7
Ki* = 15 nM
EC_{90} = > 1,000 nM

8
Ki* = 140 nM

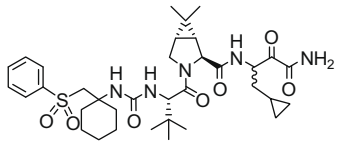
Figure 3. 1-Hydroxymethylcyclohexyl **7** 1-aminomethylcyclopentyl **8** analogues.

At this stage we realized that to achieve optimal interactions between substrate and protein we would have to replace the methyl group in the P4 ligands shown above with a more substantial template. To this end we prepared the phenyl **9**, benzyl **10** and sulfone **11** analogues (Fig. 4). Replacement of the terminal phenyl groups in **10** and **11** with alternative suitably substituted aromatic groups such as pyridyl, furyl and phenols which could become hydrogen bond donors or acceptors with the protein back-bone



9
Ki* = 47 nM
EC_{90} = 400 nM
HNE/HCV=320

10
Ki* = 12 nM
EC_{90} = 85 nM
HNE/HCV=395



11
Ki* = 3 nM
EC_{90} = 100 nM
HNE/HCV=390

Figure 4. 1-Phenylcyclopentyl **9**, 1-benylcyclohexyl **10** and phenylsulfone **11** analogues.

and/or impart physical properties conducive to improved PK parameters. Furthermore, functionality could also be introduced into the benzylic position of compound **10**, providing additional opportunities for interaction with the protein back-bone.

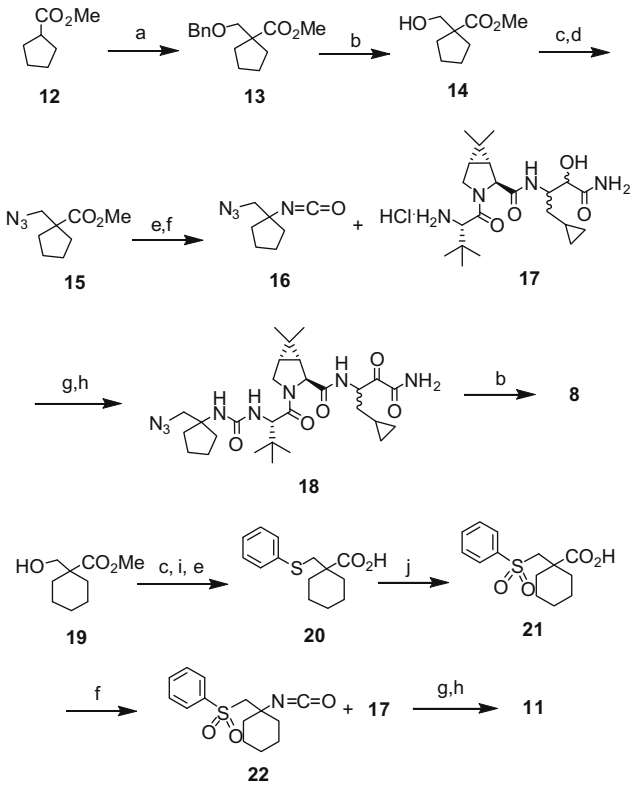
The syntheses of the compounds in this work, exemplified through the construction of the amine **8** and sulfone **11**, are outlined in Scheme 1, beginning with the appropriate cycloalkylcarboxylic methyl ester. Treatment of the anion generated from commercially available methyl cyclopentanecarboxylate methylester **12** with chloromethyl benzyl ether produced the benzyl ether **13** which is subsequently converted to the alcohol **14** under hydrogenation conditions. Mesylation and displacement with sodium azide gave the methyl ester **15** which was hydrolyzed to the corresponding carboxylic acid. Curtius rearrangement provided the isocyanate **16**, which is exposed to the previously described hydrochloride salt **17**[9] in the presence of triethylamine to the intermediate α-hydroxy amide was subsequently oxidized to the corresponding ketone **18**, under Pfitzner–Moffat conditions. Finally, reduction of the azide under hydrogenation conditions provided the target amine **8**. The synthesis of the sulfone **11** was obtained in a similar manner. Mesylation of the alcohol **19** followed by displacement with sodium thiophenoxide and hydrolysis of the methyl ester provides the sulfide-carboxylic acid **20**. Oxidation with oxone generates the sulfone **21**. Curtius rearrangement and exposure of the resulting isocyanate **22** with **17** in the presence of triethylamine provided the intermediate α-hydroxyamide which is finally oxidized to the target **11**.

All other compounds described in this Letter were prepared from variations of the chemistry described in Scheme 1. For example, the benzyl analogue **10** was obtained from the benzylation of the anion generated from cyclohexanecarboxylate methylester, transformation to the isocyanate and incorporation into the target compound via addition to hydrochloride salt **17** followed by oxidation. The P4 ligand found in inhibitor **9** was obtained directly from commercially available 1-phenyl-1carboxylic acid. However, as the ring size study with the methyl analogues (**3**–**6**; Table 1) provided a somewhat flat SAR, we decided to reinvestigate these structural changes with respect to the benzyl analogue **10** before conducting further modifications. The results derived from the respective cyclopentyl **23** and cycloheptyl **24** analogues are shown in Figure 5. Both five- and seven-membered ring (**23**, **24**, respectively) analogues were clearly less active than the cyclohexyl derivative **10** in both the enzyme and cell based assays.

Based on the results in Figure 5, we decided to keep the six-membered ring a constant and a series of analogues based on compound **10** is shown in Table 2.

Using the benzyl analogue **10** as a bench-mark in this study we observed that when functionality was introduced at the benzylic position in the P4 residue that although enzyme activity was retained and selectivity against HNE was vastly improved, it was detrimental to the relatively more important replicon assay. The methyl **25**, ethyl (**26**) and hydroxy **(27**) substituted derivatives were less active than the parent **10** by more than a factor of four in the cell based analysis while the ketone **28** by a factor of more than **20**. The naphthyl derivatives **29** and **30** were clearly less active in both enzyme and cell based studies and as a consequence were no further interest. Of more interest, the phenol analogues (**31–33**) displaying an interesting SAR pattern. While, the 4-hydroxy **33** analogue proved to be obviously less active, the *ortho*-**31** and *meta*-substituted **32** derivatives displayed excellent potency comparable to the parent compound **10**. Similarly, the thiophenes (**34** and **35**), furan **36**, thiazole **37** and 2-pyridyl derivative **38** all displayed similar activities and were in line with the benzyl derivative **10**. The isomeric pyridyls **39** and **40** were observed to be less potent.

Inhibitors that displayed interesting biological activities were subsequently analyzed for their PK properties by the oral route in rats while exceptionally important analogues were also studied in monkeys. For example, the methyl analogues **4** (AUC = 0.21 μM h; 1% bioavailability) and **5** (AUC = 0.11 μM h; 1% bioavailability) when administered at 10 mpk by the oral route in rats. Similarly the potent phenol analogue **32** (AUC = 0.09 μM h) and the thiophene **35** (AUC = 0.23 μM h; 4% bioavailability) provided equally disappointing results. Interestingly, the more biologically active pyridine **38** generated more encouraging results (AUC = 0.56 μM h; 7% bioavailability) possibly providing evidence that by changing the physical properties of these inhibitors as a result of modifying the terminal aromatic group in the P4 ligands could improve serum concentrations following oral administration in this class of compound. This phenomenon became more evident when these molecules were administered to monkeys. For example, the compound **38** that provided the most encouraging results in rats delivered excellent serum concentrations in monkeys (AUC = 16.4 μM h) after oral administration at 3 mpk, while other potent derivatives such as



Scheme 1. Reagents and conditions: (a) KHMDS, BOMCl, THF; (b) H_2, Pd–C, EtOH; (c) MsCl, Et_3N, CH_2Cl_2; (d) NaN_2, DMF, reflux; (e) KOH, MeOH; (f) $Ph_2P(O)N_3$, Et_3N, toluene, 110 °C; (g) Et_3N, CH_2Cl_2; (h) Cl_2CCO_2H, EDCI, DMSO, toluene; (i) NaSPh, EtOH; (j) oxone, H_2O/MeOH.

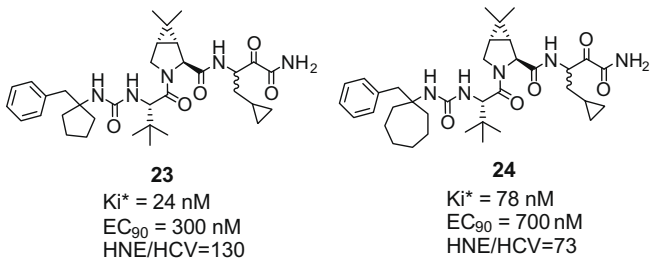
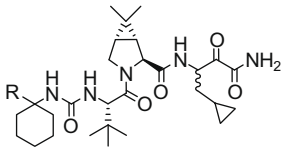


Figure 5. Cyclopentyl 23, and cycloheptyl 24 analogues of 10

Table 2
P4 1-Benzylcyclohexyl analogues of Boceprevir®



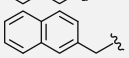
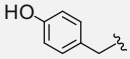
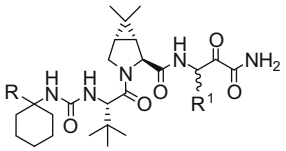
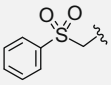
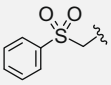
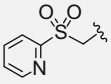
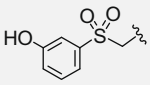
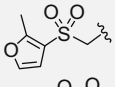
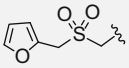
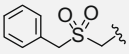
Compound	R	K_i^* (nM)	HNE/HCV	EC_{90} (nM)
10		12	395	85
25		11	2600	400
26		20	1500	400
27		11	1171	400
28		100	230	2000
29		44	470	400
30		300		
31		19	440	150
32		9	455	70
33		30	260	400
34		9	470	200
35		10	280	100
36		11	440	100
37		11	620	200
38		7	310	180
39		25	200	400
40		39	250	300

Table 3
Sulfone analogues of Boceprevir®



Compound	P3 Cap	R^1	K_i^* (nM)	HNE HCV	EC_{90} (nM)
11			3	570	100
41			9	1100	200
42			10	1566	300
43			6	200	1000
44			10	260	400
45			4	200	500
46			5	90	400
47			2	460	300
48			4	2400	500
49			11	290	400
50			5	1200	90
51			12	110	100
52			3	730	80

the thiophene (**35**), the furan (**36**) and the phenol (**32**) analogues provided extremely disappointing PK results (AUC <0.2 μM h).

Concurrent with the aforegoing study, we investigated the influence of compounds containing P4 ligands with similar aromatic groups linked through a sulfone (e.g., compound **11**; Fig. 4) would have on potency and subsequently PK properties. In addition to the variables presented to the previous study, we investigated the effects of substituting of cyclopropylalanine at P1 in the inhibitors with the analogous cyclobutyl ligand, the residue present in our clinical candidate **1**. The results from this study are displayed in Table 3.

Using the phenyl sulfone **11** as the standard, we can see that by introducing cyclobutylalanine at P1, resulting in compound **41**, a loss in both enzyme and cell based potencies were observed. Not surprisingly, as in previous studies,[10] the introduction of this relatively larger ligand provided an improvement in elastase selectivity. The pyridyl derivative **42** although equally potent in the enzyme assay with its phenyl counterpart **41**, provided a further drop in cell based activity. Reverting back to cyclopropylalanine at P1, a number heteroaromatic groups were introduced into the sulfone P4 ligand. The phenol **43**, the furans **44** and **45** and benzyl derivatives **46**, although potent in the enzyme assay provided disappointing cell based potency (EC_{90} = 400–1000 nM), Partly based on these results and for the sake of completeness we decided to examine the effect of replacing these aromatic moieties with simple alkyl groups (entries **47–52**). As with the aromatic derivatives the major distinguishing feature became the cell based replicon activity. Although initial results from analogues **48–50** were disappointing, we quickly observed that by introducing cyclobutylalanine in the P1 position improvements in cell based potency could be achieved by introducing minor modifications to the P4 alkyl group. For example, ethyl analogue **50** displayed excellent enzyme (IC_{50} = 5 nM) and cell based (EC_{90} = 90 nM) activities, as well as excellent enzyme selectivity (HNE/HCV = 1200). This encouraging

result prompted us to prepare the *tert*-butyl derivatives **51** and **52** and we were particularly pleased to see that in addition to having acceptable enzyme potency and selectivity, both analogues displayed excellent cell based activity (EC_{90} = 100 and 80 nM, respectively).

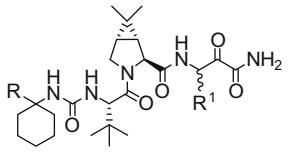
As in the previous study, inhibitors that displayed interesting biological activity were examined for their PK properties in rats and monkeys. Unfortunately, the phenyl sulfone analogue **11** and the *tert*-butyl sulfone derivatives **51** and **52** provided disappointing results in rat PK (AUC = 0.01, 0.05 and 0.07 μM h at 10 mpk, respectively). Although the monkey PK for phenyl sulfone **11** was also poor (AUC = 0 μM h at 3 mpk), the *tert*-butyl sulfone inhibitor **51** displayed surprisingly good exposure (AUC = 4.0 μM h at 3 mpk). The P1 cyclobutylalanine analogue **52** provided equally impressive monkey PK (AUC = 3.2 μM h at 3 mpk).

In summary, in the search for a second generation protease inhibitor with improved PK parameters, guided by X-ray structure of the inhibitor bound to the NS3 protease, we explored the extension of the P3 capping group into the S4 pocket. Expansion of the *tert*-butyl group led to a series of cycloalkyl analogues (**2–6**) with improved cell based replicon activities. Subsequent X-ray structure of one of these derivatives with the protein suggested further modifications which prompted the development of two series of inhibitors (Tables 2 and 3). The three leading compounds (**38**, **51** and **52**) from this study are listed in Table 4. They had improved potency in replicon assay (2–4-fold). Further, PK in monkeys was improved compared to the first generation protease inhibitors.

Although the sulfone capped analogues **51** and **52** clearly lacked acceptable PK in rats, based on previous observations that oral exposure in rat could be vastly improved through the introduction of a P1′ ligand such as an allyl group,[9] (e.g., **53** vs **54** in Fig. 6), we believed that secondary α-ketoamides with the sulfone capping group could provide us with compounds with acceptable rat PK. The improvement of monkey PK provides an opportunity for assessment of additional compounds.

The results of the investigation of the introduction of P1′ ligands into compounds such as **38**, **51** and **52** will be reported in a separate publication.

Table 4
Primary α-ketoamides with improved replicon activity and monkey PK



Compound	R	R^1	K_i^* (nM)	EC_{90} (nM)	Rat PO AUC, 10 mpk (μM h)	Monkey AUC 3 mpk (μM h)
Boceprevir©			14	350	1.52	0.12
38			7	180	0.56	16.4
51			12	100	0.05	4.0
52			3	80	0.07	3.2

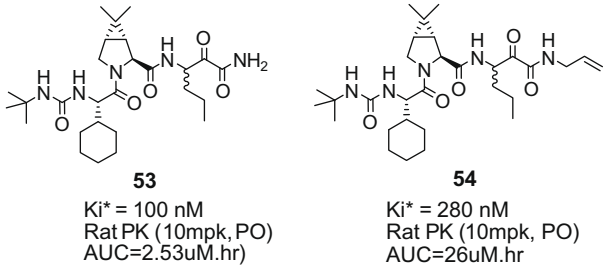


Figure 6. Improvement of PK parameters in rats by introduction of a P1′ ligand (allyl amide; **53** vs **54**).

References and notes

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14. Crystallographic data for compound **2** bound to HCV NS3 protease have been deposited with the RCSB Protein Data Bank and has been assigned the RCSB ID code rcsb057547 and PDB ID code 3LOX.