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Novel potent inhibitors of hepatitis C virus (HCV) NS3 protease with cyclic sulfonyl P3 cappings

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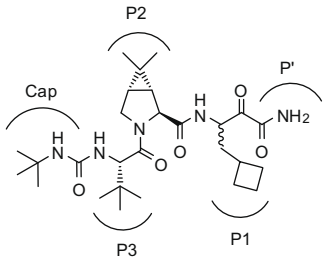
ABSTRACT

Extensive SAR studies of the P3 capping group led to the discovery of a series of potent inhibitors with sultam and cyclic sulfonyl urea moieties as the P3 capping. The bicyclic thiophene-sultam or phenyl-sultam cappings were selected for further SAR development. Modification at the P3 side chain determined that the *tert*-butyl group was the best choice at that position. Optimization of P1 residue significantly improved potency and selectivity. The combination of optimal moieties at all positions led to the discovery of compound **33**. This compound had the best overall profile in potency and PK profile: excellent K_i^* of 5.3 nM and activity in replicon (EC_{90}) of 80 nM, extremely high selectivity of 6100, and a good rat PO AUC of 1.43 μM h.

Hepatitis C virus (HCV) infection is a principle cause of chronic liver disease that leading to cirrhosis, hepatocellular carcinoma or liver failure 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 pegylated α-interferon and ribavirin generates sustained virological response in only 50% of infected patients.[2] These existing therapies are also associated with considerable side effects. The limited efficacy and adverse side effects of the current therapies has clearly demonstrated that there is a dire need to develop more effective antiviral agents, and has stimulated intensive research in finding potent and orally bioavailable small molecule drug candidates.[3]

The HCV viral RNA genome encodes a polyprotein which consists of structural and nonstructural (NS) proteins. The chymotrypsin-like serine protease located at N-terminal of NS3 nonstructural protein is essential for viral replication.[4] It has been a valuable target for which a number of inhibitors have been reported in literature.[5] Several drug candidates have or are being progressed into clinical trials in human beings. The earliest entry, BILN-2061,[6] an NS3 protease inhibitor from Boehringer–Ingelheim, failed in phase I clinical trials due to toxicity. Currently, the most advanced candidates are Telaprevir (VX-950)[7] from Vertex and Boceprevir (SCH 503034)[8] from Schering–Plough. Both are 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 activity in enzyme assay (K_i^*) of 14 nM and replicon assay (EC_{90}) of 350 nM.[8] It has a favorable pharmacokinetic (PK) profile in rats and dogs (26% and 34% bioavailability, respectively) but low bioavailability in monkeys (4–11%). Our goal for a next generation inhibitor was to improve the potency by at least fivefold while



1

Boceprevir (SCH503034)

Ki*= 14 nM
EC_{90} = 350 nM
Ratb ioavail. 26 %
Monkey bioavail. 4-11%
Dog bioavail. 30%

Figure 1. Boceprevir: the HCV protease inhibitor that is in phase III clinical trials.

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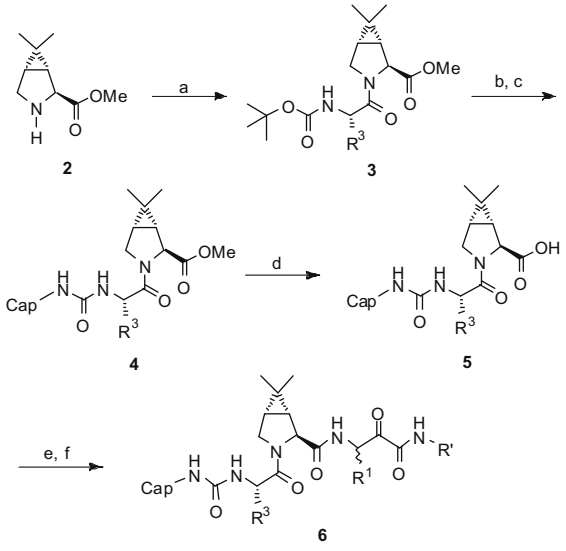
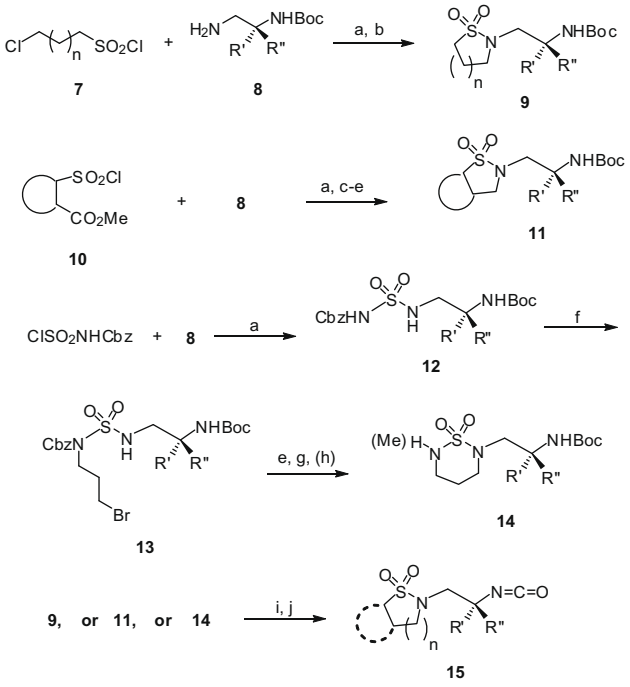
maintain or improve upon PK profile. Modeling studies based on X-ray crystal structure of compound **1** revealed that there was ample room for the P3 cap to be extended beyond current *tert*-butyl group. The Cys159 amino acid residue of the protein backbone in the S4 region provided an opportunity for additional hydrogen bonding interaction. We envisioned that an extended P3 cap having sulfonyl or carbonyl groups at right position could engage in a hydrogen bonding with Cys159. In the course of our extensive search for the optimal functionality to establish the hydrogen bond, we discovered that sultams and cyclic sulfonyl ureas derived from *tert*-leucinol provided potent P3 capped inhibitors. Thus, a number of mono-cyclic and bicyclic sultams and sulfonyl ureas were studied. We also systematically investigated SAR at other sites of the inhibitor, namely P3, P1 and P′ residues. The combination of the best moieties at all positions gave rise to a potent inhibitor with favorable PK profile. Herein, we report our SAR development and the identification of a novel potent sultam-based HCV NS3 protease inhibitor.

The general synthesis of the inhibitors is outlined in Scheme 1. It has been described in details in our previous publications.[8,9] Starting from our unique *gem*-dimethylcyclopropylproline P2 core **2** and a Boc-protected P3 amino acid, dipeptide **3** was obtained through a standard EDC mediated coupling. Removal of the Boc protecting group and subsequent reaction with a capping isocyanate afforded compound **4**. Hydrolysis of the methyl ester to a carboxylic acid **5** and its coupling to a P1 α-hydroxy-β-aminoamide gave rise to a tripeptide. Dess–Martin periodinane[10] oxidation of the hydroxyamide furnished the desired α-ketoamide **6**. Since our early research indicated the difficulty in obtaining acceptable PK from primary ketoamides (R′ = H), we decided to perform all SAR studies on secondary ketoamides. Among various R′ groups investigated, allyl group was one of the best moieties for potency and PK. Thus, R′ was fixed as allyl group for current study.

Synthesis of the capping group, the focus of the current SAR development, is shown in Scheme 2. Thus, the amino acid derived α,β-di-amine (**8**), which could be obtained from commercial protected amino acid through a reduction to amino alcohol, a Mitsunobu reaction[11] with phthalimide and subsequent removal of the phthaloyl group with hydrazine, was treated with chloroalkyl sulfonyl chloride (**7**) in the presence of triethylamine. Sulfonylation of



Scheme 1. Reagents and conditions: (a) Boc-*tert*-leucine, EDC, HOOBt, DMF/CH_2Cl_2; (b) 4 M HCl; *p*-dioxane; (c) appropriate isocyanate, *i*-Pr_2NEt, CH_2Cl_2; (d) LiOH, THF/MeOH/H_2O; (e) P1-α-hydroxy-β-aminoamide, EDC, HOOBt, DMF/CH_2Cl_2; (f) Dess–Martin periodinane, CH_2Cl_2.



Scheme 2. Reagents and conditions: (a) Et_3N, CH_2Cl_2; (b) NaH, DMF; (c) DIBAL-H, toluene; (d) MsCl, Et_3N, CH_2Cl_2; (e) Cs_2CO_3, DMF; (f) 3-bromopropanol, DIAD, PPh_3, CH_2Cl_2; (g) H_2, Pd–C, EtOH; (h) Cs_2CO_3, MeI, DMF; (i) 4 M HCl, *p*-dioxane; (j) phosgene, $NaHCO_3$, H_2O/CH_2Cl_2.

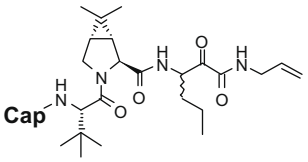
the amine was followed by an in-situ intra-molecular cyclization to form the desired sultam **9**. Similarly, *o*-aromatic sulfonyl chloride carboxylic esters were reacted with **8** to give sulfonamides. The esters were reduced to alcohols which were then converted to mesylates or chlorides. Finally, intra-molecular cyclization afforded bicyclic aromatic sultams **11**. To gain access to the cyclic sulfonyl urea of type **14**, amine **8** was first treated with Cbz-amino sulfonyl chloride to give sulfonyl urea **12**, which was then alkylated with 3-bromopropanol under Mitsunobu reaction conditions to give **13**, cyclized, and finally hydrogenated to remove Cbz-protecting group. The intermediate sultams **9**, **11** and cyclic sulfonyl urea **14** were treated with 4 M HCl to remove the Boc group and then reacted with phosgene to provide key isocyanate intermediates of type **15**, which were used to make final targets **6** (Scheme 1). All compounds were isolated as mixture of two diastereomers at P1 α-center. The ratio of two isomers varied between 2:1 and 1:2.

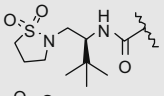
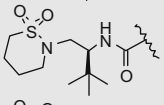
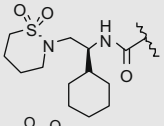
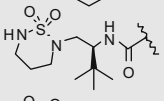
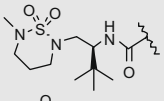
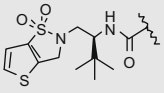
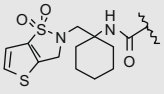
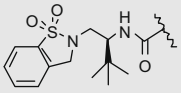
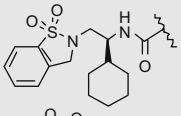
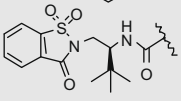
All inhibitors synthesized were tested in HCV continuous assay[12] using the NS4A-tethered single chain NS3 serine protease.[13] The K_i^* values in the assay reflected the equilibrium constant determined by the reversible covalent bond formed between the ketone and serine and other interactions between the inhibitors and the enzyme.[14] Compounds with good potency were then evaluated in a replicon assay.[15] EC_{90}, the concentration required for inhibition of 90% of replicon replication, was obtained as a measure of activity in replicon. To address the issue of selectivity among serine proteases, all inhibitors were assayed against human neutrophil elastase (HNE), a serine protease with most structural similarity to HCV NS3 serine protease. The ratio of activity in these two proteases (HNE/HCV) was used as a measure of the selectivity. Previously, a multiplex Taqman RT reaction was done to measure both HCV and endogenous GAPDH RNA levels for analogous compounds. GAPDH RNA level did not change when measured up to a maximum concentration of 5 μM. Thus, cytotoxicity was never an issue for this class of inhibitors, and GAPDH was not measured for most recent compounds.

P3 capping SAR: Discovery of sultam and sulfonyl urea as potent capping moiety. As demonstrated by X-ray structure of compound **1** bound to the protein, the S4 pocket and Cys159 were potential targets for additional interactions. After extensive exploration with the moieties that could both reach into the S4 pocket and form hydrogen bonding with Cys159, compounds with sulfonyl functionality were proved to be promising. Among them, cyclic sulfonamides (sultams) and sulfonyl ureas were found to be more interesting. Thus, a set of inhibitors with these types of P3 capping were prepared and evaluated (Table 1). The P1 side chain was fixed as *n*-propyl for this study.

A number of structure variations were incorporated into the P3 capping group as shown in Table 1. The caps in compounds **16**–**20** were mono-cyclic alkyl sultams or sulfonyl ureas, while those in compounds **21–25** were bicyclic fused aromatic sultams. Most of the caps were derived from *tert*-leucine amine (**8**, R′ = H, R″ = *tert*-butyl), except those in compounds **18**/**24** and **22**, where amine **8** was prepared from cyclohexyl glycinol and 1-amino-1-cyclohexylmethanol, respectively. We started with compound **16** which had a *tert*-leucine derived 5-membered sultam P3 cap. It had good potency in both enzyme assay (K_i^* = 11 nM) and replicon assay (EC_{90} = 200 nM), but had a moderate selectivity against human neutrophil elastase (HNE/HCV = 230). This compound had poor rat PK with oral area-under-curve (PO AUC) of only 0.09 μM h. Expanding the sultam ring size to six led to compound **17**. Enzyme potency, selectivity and oral PK AUC were improved significantly over that of **16**, while activity in replicon remained the same at 200 nM. Replacement of the *tert*-butyl side chain of the P3 cap with a cyclohexyl gave group compound **18** which resulted in a loss in both enzyme and activity in replicon (20 and 250 nM, respectively), lower selectivity (50) and rat PO AUC (0.18 μM h). It was hoped that addition of more heteroatoms to the P3 cap rings might increase the opportunity of additional hydrogen bonding, thus improving potency. Initial result from a

Table 1
P3 capping SAR development



Compound	P3 cap	K_i^* (nM)	HNE/HCV	EC_{90} (nM)	Rat PO AUC[a] (μM h)
16		11	230	200	0.09
17		6	650	200	0.67
18		30	50	250	0.18
19		59	80	350	—
20		14	490	150	0.20
21		10	150	150	0.43
22		41	130	400	—
23		3	650	150	0.37
24		18	55	NA	—
25		15	310	230	—

[a] 3 mg/kg, 0.4% MC.

6-membered sulfonyl urea ring (compound **19**) was not encouraging (K_i^* = 59 nM, EC_{90} = 350 nM).

However, methylation of the nitrogen gave compound **20** with enhanced potency and selectivity. It had one of the best activity in replicon among all mono-cyclic alkyl sulfonyl capped inhibitors in Table 1.

The fused bicyclic aromatic sulfonyl moieties were of great interest because of their potential to improve PK properties. Thus, bicyclic thiophene-sultam analog of **16** was synthesized. Compound **21** maintained enzyme activity (K_i^* = 10 nM) but lost some selectivity (HNE/HCV = 150). However, it had better activity in replicon (EC_{90} = 150 nM) than **16**. The same bicyclic thiophene sultam did not work well with the 1,1-disubstituted-cyclohexyl moiety in compound **22**, as evidenced by the drop in potency and selectivity. Changing from 5,5- to 6,5-ring system resulted in the fused bicyclic phenyl-sultam analog **23**. This compound had the best overall profile among all the inhibitors discussed so far. It was the most potent in both enzyme and replicon assays (3 and 150 nM, respectively) and it had the highest selectivity of 650. It also had moderate rat PK AUC (0.34 μM h). When this phenyl-sultam moiety was combined with cyclohexyl glycine amine as in compound **24**, significant loss of potency and selectivity were observed. We also investigated inhibitors with bicyclic phenyl acyl sulfonamide capping (**25**). Compared to **23**, this compound was inferior in both potency and selectivity.

Based on these results, we decided that *tert*-leucine amine-derived thiophene- and phenyl-sultam P3 capping groups (in compounds **21** and **23**, respectively) were most interesting for further SAR development. Optimization at other positions was thus performed with these two capping moieties.

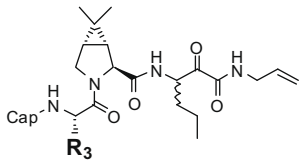
P3 side chain modifications. Both X-ray structures[16] and our earlier SAR studies indicated that the S3 pocket of HCV NS3 protease was highly lipophilic and the P3 moiety bound to the enzyme mainly through hydrophobic interactions. As a result, possible modifications to the P3 side chain was very limited. Branched alkyl or carbocyclic groups were among the best options. In the current study, three P3 residues were incorporated: *tert*-leucine, cyclohexyl glycine and β-methyl cyclohexyl glycine. They were combined with either thiophene or phenyl-sultam P3 cappings. The results are summarized in Table 2.

In the series with thiophene-sultam P3 cap, compounds having cyclohexyl and β-methylcyclohexyl P3 side chains (**26** and **27**) achieved better activity in replicon (EC_{90} = 100 nM). Compound **27** had better enzyme potency as well (K_i^* = 3 nM). However, both of these compounds were less selective against elastase with HNE/HCV of 10 and 68, respectively. When phenyl-sultam P3 cap was incorporated, all three compounds (**23**, **28**, **29**) had good activity in replicon (EC_{90} = 150 nM) and excellent K_i^* (3–7 nM). However, the selectivity against elastase decreased significantly for compounds **28** and **29** (HNE/HCV = 19 and 50, respectively). Based on the results from these two series, cyclohexyl and β-methyl cyclohexyl P3 side chains did not provide much improvement in activity in both enzyme and replicon assays, but resulted substantial loss in elastase selectivity. Judging from the overall profile of these compounds, inhibitor **23** appeared to the most interesting. A decision was tentatively made to perform further SAR studies using compound **23** as a template, with P3 cap as phenyl-sultam and P3 side chain as *tert*-butyl group.

P1 residue optimization. The X-ray structure of the HCV NS3 protease revealed that the S1 pocket was small and narrow.[16] The preferred P1 side chain is either a short linear alkyl chain or a small ring attached to a short alkyl chain. Compound **1** had a cyclobutyl methyl P1 side chain, which was one of the largest group the S1 pocket could accommodate. Compound **23** had a smaller *n*-propyl side chain. It could serve as the starting point for further modifications. The results of this study are listed in Table 3. The first two compounds had small ring side chains. Compound **30** had the same cyclobutylalanine P1 residue as compound **1**. Unfortunately, in the enzyme assay it was substantially less potent (K_i^* = 30 nM) than similar analogs in Table 2, even though its selectivity (HNE/HCV = 1500) was much higher than that of compound **23**. The smaller ring analog, compound **31**, however, had very good enzyme potency of 7 nM, along with good activity in replicon (150 nM). The profile of **31** was comparable to that of **23**, except that selectivity was more than twofold higher. We then investigated terminal alkyne P1 side chain as shown in compound **32**. It definitely improved enzymatic activity by several fold, but selectivity and activity in replicon decreased. Finally, inhibitor with norleucine as P1 residue was prepared (**33**). The side chain was one carbon longer than that of **23**. We were delighted that compound had excellent potency in enzyme assay (K_i^* = 5 nM) and extremely high selectivity (HNE/HCV = 6100). More importantly, it achieved the best activity in replicon (EC_{90} = 80 nM). This compound (**33**) clearly had the best overall profile in potency and selectivity.

Besides a compound's biological activity, equally important is its pharmacokinetic properties. PK profile determines whether a chemical entity has the desired characteristics of a drug. Along our SAR development, selected promising compounds were evaluated in PK studies. The oral AUC in rats for three most interesting compounds is listed in Table 3. Both compounds **31** and **23** had rat PO AUC below 1.0 μM h. Fortunately, compound **33,** the most potent of the series, demonstrated good rat PO AUC of 1.43 μM h. Thus, through the combination of the novel phenyl-sultam P3 cap, *tert*-butyl P3 side chain and norleucine P1 residue, a potent HCV NS3 protease inhibitor with good PK was discovered. It had best overall profile: K_i^* of 5 nM, EC_{90} of 80 nM, excellent selectivity HNE/HCV of 6100, and a favorable rat PK of 1.43 μM h PO AUC. This

Table 2
P3 side chain modifications



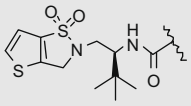
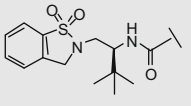
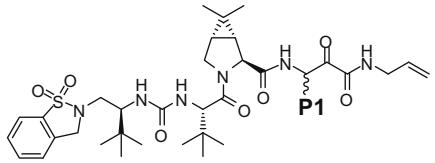
Compound	P3	P3	K_i^* (nM)	HNE/HCV	EC_{90} (nM)
21			10	150	150
26			14	10	100
27			3	68	100
23			**3**	**650**	**150**
28			7	19	150
29			5	50	150

Table 3
Modifications on P1 side chains



Compound	P1	K_i^* (nM)	HNE/HCV	EC_{90} (nM)	Rat PO AUC[a] (μM h)
30		30	1500	NA	NA
31		7	1600	150	0.705
23		3	650	150	0.373
32		0.9	380	250	NA
33		**5**	**6100**	**80**	**1.43**

[a] 3 mg/kg, 0.4% MC.

compound is under further evaluation and will serve as the lead compound for future SAR development.

In summary, a number of novel and potent HCV protease inhibitors with various sultam or sulfonyl urea P3 capping groups were synthesized and investigated. The *tert*-leucine amine-derived thiophene-sultam or phenyl-sultam cappings were the most interesting. The capping group SAR study identified compound **23** as a starting point for further optimization. Modification on the P3 side chain established that the *tert*-butyl group was the best moiety for that position. With the optimum combination of the phenyl-sultam P3 cap and *tert*-butyl P3 side chain, an SAR investigation on the P1 residues significantly improved potency and selectivity further. The combination of optimal moieties at all positions led to the discovery of compound **33**, which had the best overall profile in potency and PK properties: excellent K_i^* (5 nM) and activity in replicon (EC_{90} = 80 nM), extremely high selectivity against elastase (HNE/HCV = 6100), and a good rat oral PK AUC of 1.43 μM h.

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