Bioorganic & Medicinal Chemistry Letters 20 (2010) 1689–1692

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Towards the second generation of Boceprevir: Dithianes as an alternative P2 substituent for 2,2-dimethyl cycloproyl proline in HCV NS3 protease inhibitors

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ARTICLE INFO

Article history:
Received 2 November 2009
Revised 8 January 2010
Accepted 11 January 2010
Available online 20 January 2010

Keywords:
HCV protease
Dithianes
Glutarimides

ABSTRACT

Hepatitis C (HCV) infection is a global health crisis leading to chronic liver disease. In our efforts towards a second generation HCV NS3 serine protease inhibitor with improved profile, we have undertaken SAR studies in various regions of Boceprevir including P2. Herein, we report the synthesis and structure–activity relationship studies of inhibitors with (*S*)-1,4-dithia-7-azaspiro[4.4]nonane-8-carboxylic acid **2** as P2 substituent replacing the (1*R*,2*S*,5*S*)-6,6-dimethyl 3-azabicyclo[3.1.0]hexane-2-carboxylic acid. The systematic investigation led to the discovery of highly potent inhibitor **25** (K_i^* = 7 nM, EC_{90} = 30 nM) with improved rat exposure of 2.56 μM h.

The hepatitis C virus is a major health hazard affecting over 170 million individuals worldwide[1a] and its infection is a leading cause of chronic liver disease in the United States.[1b–d] The current standard of care treatment is a combination of subcutaneous pegylated interferon-α with oral nucleoside drug ribavirin.[2] Response rates for HCV patients having genotypes 2 or 3 on a 24 week treatment of this is 80%, whereas those with genotype 1 are treated for 48 weeks with response rates of less than 50%. With the opportunity to improve response rates, especially for genotype 1 patients, and given the side effects associated with the current therapy, it is necessary to search for novel potent and drug like inhibitors of NS3 enzyme of HCV with the intent of improving treatment outcomes and potentially shorten treatment duration.

The NS3 protease which is located at N-terminal portion of NS3 protein has a demonstrated vital role in the replication of the HCV virus.[3] Hence there are compelling evidences[4] to suggest that the inhibition of NS3 protease would be a viable strategy for the development of small molecules as antiviral agents. It has been determined that the HCV NS3 protease belongs to the trypsin or chymotrypsin super family of serine protease.[5] For efficient processing, the protease forms a complex with a small polypeptide co-factor NS4A.[6] The structure data of the protease have revealed a shallow and solvent exposed substrate binding region, where the binding energy is mainly derived from weak lipophilic and electrostatic interactions.[7] Despite tremendous difficulty encountered in the process, intensive efforts have been focused on NS3 serine protease and a number of novel inhibitors have been reported.[8] More recently, peptidomimetics like BILN-2061[9], VX-950[10] and MK-7009[11] are reported to show the antiviral activity by inhibiting the replication of the virus along with our clinical candidate SCH 503034 (Fig. 1).[12] Currently, the most advanced candidates are Telapravir (VX-950) from Vertex and Boceprevir (SCH 503034) from Schering-Plough which are in phase III clinical trials.

Synthesis of inhibitors with dithiane at P2 (Fig 2) starting from 4-oxo proline derivative **3**[13] was achieved according to the general Scheme 1. Refluxing **3** with 1,2-dithioethane and a catalytic amount of PTSA in glacial acetic acid resulted in the thioketal **4** in 56% isolated yield. The hydrobromide **4** was coupled with various N-Boc protected P3 amino acid under the HATU conditions

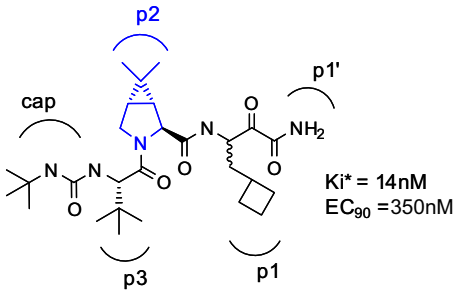

Figure 1. Boceprevir (SCH 503034).

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0960-894X/$ - see front matter © 2010 Published by Elsevier Ltd.
doi:10.1016/j.bmcl.2010.01.037

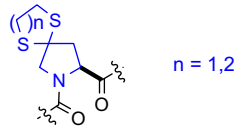
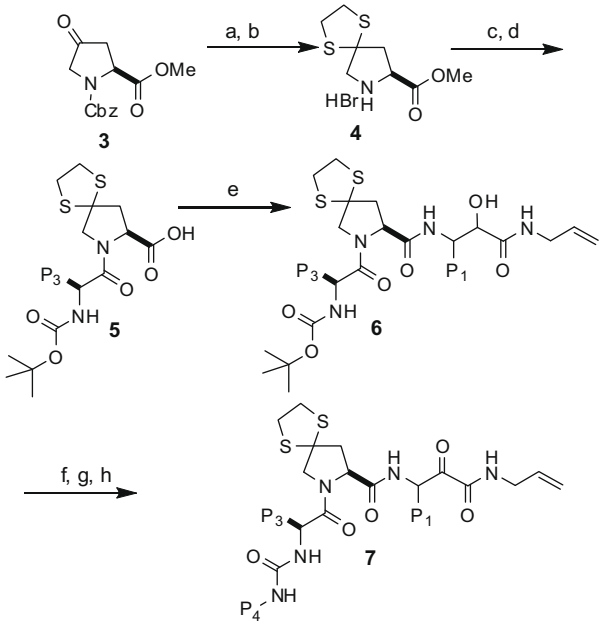


Figure 2. Dithiane P2.



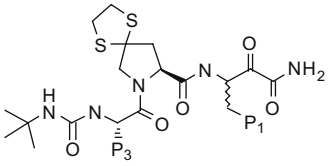
Scheme 1. Synthesis of the dithiane inhibitors. Reagents and conditions: (a) 1,2-dithioethane, PTSA, glacial acetic acid, reflux; (b) 20% HBr, acetic acid; (c) HATU, DIPEA, P3 Boc amino acid; (d) 1 N LiOH (aq); (e) HATU, DIPEA, P1 hydroxy allylamide hydrochloride; (f) 4 M HCl/dioxane; (g) P4 isocyanate, triethylamine; (h) DMSO, dichloroacetic acid, EDAC·HCl.

to get a dipeptide which up on hydrolysis with LiOH afforded the acids of type **5**. Acid **5** were reacted with P1 hydroxy allyl amides under the standard coupling conditions to afford the hydroxy allyl amide intermediates **6**. *tert*-Butyl carbamates **6** were progressed to the P4 extended ureas **7** by Boc deprotection and reaction with isocyanates[14]followed by Moffat oxidation (Scheme 1).[15]

All inhibitors were tested in the HCV continuous enzymatic assay[16] using the NS4A-tethered single chain NS3 serine protease.[17] The K_i^* values 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.[18] The concentration required for inhibition of 90% of virus replication, EC_{90}, was obtained as a measure of replicon cellular potency.[19] Inhibitors were tested for the activity against one of the structurally close related serine protease, human neutrophil elastase (HNE) to determine the selectivity between HCV and HNE.

Results from our previous structural studies showed that introduction of a hydrophobic sulfide moiety at P2 provided some improvement in binding potency.[20]The initial set of compounds for the current study were primary amides **8–11** (Table 1). The exact dithiane analog of Boceprevir (**8**, K_i^* = 600 nM) was less active. It was known from our previous SAR studies that cyclohexyl at P3 improved the potency.[21] Although tertiary butyl P3 replacement with cyclohexyl enhanced the potency (**9**, K_i^* = 400 nM), it was not potent enough to pursue further. We had observed earlier that cyclopropyl alanine at P1 improved potency.[20] Thus, a twofold improvement in the potency was observed with inhibitor **10** (K_i^* = 300 nM). Further enhancement was achieved with cyclohexyl

Table 1
SAR of dithianes and primary amides

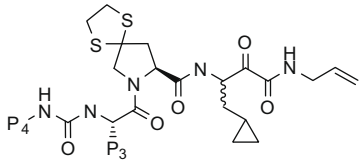


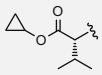
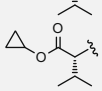
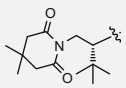
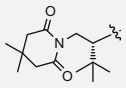
Entry	P3	P1	K_i^* (μM)
8			0.600
9			0.400
10			0.300
11			0.200

P3 replacement (**11**, K_i^* = 200 nM) to achieve a threefold improvement in potency compared to **8**. Assessment of resistance mutation selected by Boceprevir suggested that larger flexible P2 could provide better results for V170A mutant profile by retaining more interaction between Arg 155 and the inhibitor.[22] Structural modelling predicted that the side chain of Val 170 was in Van der Waals contact with the side chain of Arg 155.[22] Although the initial results from analogs **8–11** were disappointing, we decided to continue the SAR studies and quickly identified compounds with desired potency by incorporating P3 caps (Table 2).

We have reported in the 2,2-dimethyl proline P2 series that extension of the P3 unit resulted in additional interaction with Cys159 and hence improved the potency.[23] Since it was known from our previous SAR studies which led to the discovery of **1** that allyl amide P1′ provided inhibitors with good rat PK profile[12], we kept allyl amides at P1′ for our P4 and P3 optimization studies. As observed before, extending the P3 unit to P4 with cyclopropyl ester resulted in inhibitor **13** (K_i^* = 84 nM, EC_{90} = 300 nM) with ninefold improvement in potency compared to their urea analog (**11**, K_i^* = 700 nM). Changing the P3 to cyclohexyl glycine did not

Table 2
SAR of P3 cap



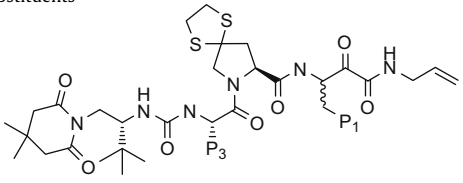
Entry	P3	P4	K_i^* (μM)	HNE/HCV	EC_{90} (μM)
12			0.700	NA	NA
13			0.084	40	0.300
14			0.072	15	0.300
15			0.021	290	0.070
16			0.018	200	0.050

improve the activity (**14**, K_i^* = 72 nM, EC_{90} = 300 nM) albeit showed low selectivity. Replacing the ester P3 cap of **13** with the glutarimide demonstrated a profound effect in potency (**15**, K_i^* = 21 nM, EC_{90} = 70 nM) and eightfold improvement in selectivity. In order to benefit from potency enhancement (Table 1, in primary amide series) of the cyclohexyl and the selectivity advantage of the tertiary butyl group we used β-methyl cyclohexyl group, a combination of these two, at P3. We found the imide **16** (K_i^* = 18 nM, EC_{90} = 50 nM) showed a slight improvement in potency but suffered from loss of selectivity compared to **15**.

Our next focus was to optimize the P1 unit keeping 4,4-dimethyl glutarimide as P4. From our previous structure–activity studies,[12] it was demonstrated that combination of norvaline at P1 with allyl amide at P1′ provided better rat exposure. Thus, changing the P1 from cyclopropyl (**16**) to norvaline afforded **17** (K_i^* = 6 nM, EC_{90} = 40 nM) with similar potency in cellular assays and improvement in enzymatic potency and HNE selectivity. Replacement of P3 with tertiary butyl retained the potency with two to threefold enhancement in selectivity (**18**, K_i^* = 5 nM, EC_{90} = 40 nM). From our previous structure–activity studies,[12] it was demonstrated that norleucine at P1 improved the HNE selectivity. Indeed changing the P1 from norvaline (**18**) to norleucine boosted the selectivity by twofold. But this replacement was detrimental to the potency (**19**, K_i^* = 13 nM, EC_{90} = 100 nM) with more than twofold loss in both enzymatic and cellular potencies. Since it was known that longer P1 residues were optimal for HNE selectivity[12] we synthesized the homocyclopropyl as a combination of cyclopropyl and norleucine at P1. However, inhibitor **20** (K_i^* = 13 nM, EC_{90} = 70 nM) was similar to their norleucine counterpart **19** in potency and selectivity (Table 3). We could improve both the enzymatic and cellular potency by incorporating the P4 imide cap and P1′ allyl amide. However, none of those inhibitors showed better rat PK profile compared to **1** (Table 5).

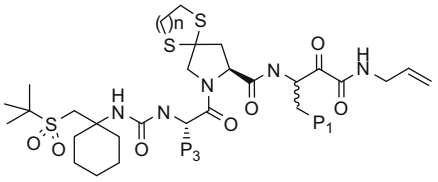
Encouraged by the results obtained from P3 cap exploration we further studied different P3 cap units, identified in 2,2-dimethyl proline P2 series[23] to improve PK. Sulfones[24] emerged out as one of the best class of capping group. The sulfone analog of **17** (**21**, K_i^* = 19 nM, EC_{90} = 100 nM) showed 2–3-fold loss in potency and poor selectivity. However, there was a profound effect on the oral rat exposure for this compound with 33-fold improvement (**21**, Table 5). As observed before change of P3 to tertiary butyl further improved the selectivity (fivefold) and potency (**22**, K_i^* = 11 nM, EC_{90} = 70 nM), but exhibited lower exposure. The cyclopropyl P1 congener **23** (K_i^* = 33 nM, EC_{90} = 100 nM) was less active in the enzymatic assay and cellular assay compared to **22.** Even though we could identify compounds with desired potency and acceptable PK profile, the selectivity for these compounds had to be further improved.

Table 3
SAR of P1 substituents



Entry	P3	P1	K_i^* (μM)	EC_{90} (μM)	HNE/HCV
17			0.006	0.040	240
18			0.005	0.040	630
19			0.013	0.100	1500
20			0.013	0.070	1700

Table 4
SAR of inhibitors with sulfone as P4



Entry	P3	P1	*n*	K_i^* (μM)	EC_{90} (μM)	HNE/HCV
21			1	0.019	0.100	10
22			1	0.011	0.070	50
23			1	0.033	0.100	40
24			2	0.018	0.050	130
25			2	0.007	0.030	128

The SAR above clearly showed that a spirocyclohexyl with an appended *tert*-butyl sulfone at P4 improved DMPK properties. Then we decided to change the ring size of the dithiane and selected analogs were synthesized to evaluate their effect on selectivity. Thus, the six membered dithiane analog **24** (K_i^* = 18 nM, EC_{90} = 50 nM) with cyclopropyl P1 improved the potency twofold both in enzymatic and cellular assays with a threefold augmentation in selectivity and a much improved rat exposure of 1.69 μM h. Changing the P1 unit to norvaline (**25**, K_i^* = 7 nM, EC_{90} = 30 nM) further improved the potency threefold maintaining the selectivity and improved rat exposure (2.56 μM h) (Table 4). Thus, by systematic investigations on different regions of the molecule we identified a potent dithiane inhibitor with enhanced potency and DMPK profile.

From the model of compound **25** bound to the active site of HCV NS3/4A protease, it was evident that the dithiane P2 fitted into the S2 pocket very well (Fig. 3). The inhibitor is represented as stick model, and the protein as surface. The S1–S4 subsites and the amino acid residues forming the S2 pocket are labeled. The larger size flexible P2 of inhibitor **25** reaches more towards R155 compared to smaller P2 residue. The resistance profiles of these inhibitors are yet to be studied.

In conclusion, we identified a new class of compounds as potent HCV NS3 serine protease inhibitors. Replacement of the 2,2-dimethyl cyclopropyl proline with the spiro dithiane proline P2 in compound **1** resulted in loss of activity. However, by the systematic SAR studies of the different regions of the molecule we could achieve an improvement in replicon activity by 12-folds (**25**,

Table 5
Rapid rat PO AUC of selected analogs

Compound	K_i^* (nM)	EC_{90} (μM)	PO AUC μM h (10 mpk)
16	18	0.070	0.20
17	6	0.040	0.04
18	5	0.040	0.06
19	13	0.100	0.2
21	19	0.100	1.34
22	11	0.100	0.57
23	33	0.100	0.77
24	18	0.050	1.69
25	7	0.030	2.56
SCH503034 (1)	14	0.350	0.14

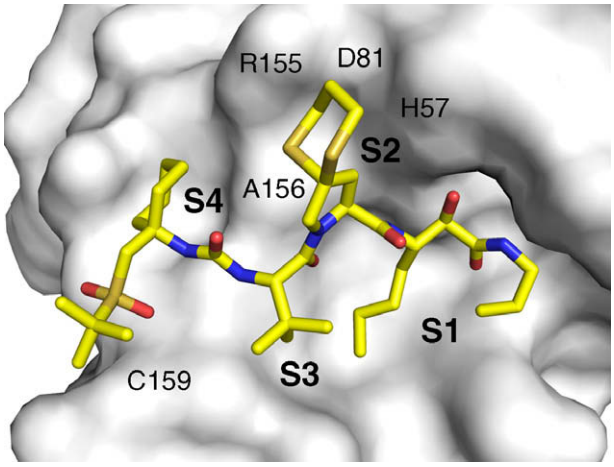


Figure 3. Model of compound **25** bound to the active site of HCV NS3/4A protease.

K_i^* = 7 nM, EC_{90} = 30 nM). All of the inhibitors with sulfone as P3 cap showed improved pharmacokinetic properties compared to **1** in rat model. Our SAR studies with (*S*)-6, 10-dithia-2-azaspiro[4.5]decane-3-carboxylic acid (**2,** n = 2) as the new P2 culminated potent inhibitor, **25,** with excellent potency (K_i^* = 7 nM, EC_{90} = 30 nM) and good rat exposure with acceptable selectivity against elastase.

Acknowledgment

The authors wish to thank virology, DMPK and Structural Chemistry group of Schering-Plough Research Institute for their help in in vitro, in vivo and X-ray crystal studies, respectively.

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