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Bioorganic & Medicinal Chemistry Letters 13 (2003) 785–788

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Design and Synthesis of Bicyclic Pyrimidinone-Based HCV NS3 Protease Inhibitors

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Received 4 November 2002; revised 7 January 2003; accepted 10 January 2003

Abstract—A series of bicyclic pyrimidinone-based HCV NS3 protease inhibitors was synthesized via selective C8 position functionalization. Substituted phenylamides and phenylureas were preferred in the S2 binding pocket.

The hepatitis C virus (HCV) infects an estimated 1–3% of the world population. The clinical state of a proportion of those infected individuals eventually progresses to cirrhosis and hepatocellular carcinoma.[1] The prevalence of this disease and its adverse outcome make it the largest cause of liver transplantation. The current treatment of HCV, based upon pegylated interferon-α and the antiviral agent ribavirin, provides only limited sustained viral response and is accompanied by severe side effects.[2] New treatments for the disease exhibiting improved success rates and side effect profiles are needed.

Newer strategies for combating HCV infection target essential non-structural proteins of HCV. One such target is the HCV NS3 serine protease, which is responsible for proteolytic processing of non-structural proteins required for viral maturation. Functioning HCV NS3 has been shown to be required for infection in a chimpanzee model,[3] thus validating the protease as a therapeutic target and making it the focus of significant inhibitor design efforts.

Structural information and substrate requirements suggest the challenge that inhibiting HCV NS3 protease poses. X-ray crystal structures of the protease reveal a shallow, featureless binding pocket, with few opportunities for binding interactions.[4,5] Furthermore, substrates of ten or more amino acids are required for efficient enzymatic cleavage and sequence specificity is quite broad.[6] To overcome these challenges, we undertook a peptidomimetic approach with the goal of interacting with the enzyme binding site in ways unavailable to peptide-based inhibitors (see Fig. 1). Into a peptide boronic acid inhibitor,[7] we incorporated a bicyclic pyrimidinone-based P2–P3 dipeptide replacement[8,9] and focused on projecting substituents into the extended S2 binding pocket—a strategy shown to increase potency in peptidic inhibitors of HCV NS3 protease.[10] Towards this goal, we have developed regio- and stereoselective alkylation chemistry to functionalize the C8-position of the bicyclic pyrimidinone core.[11]

Synthesis of the bicyclic pyrimidinone core was accomplished in an eight-step sequence according to the method of Webber et al.,[9] beginning with pyroglutamic acid (**1**) (Scheme 1). *N*-Alkylation of Cbz-protected intermediate **2**, followed by amide coupling afforded anilide **3**. The 3-(trifluoromethyl)benzyl substituent was found to be an optimal P4 substituent in a related pyrazinone-based inhibitor[12] and was thus used in this series. Anilide **3** served as a key intermediate for the introduction of a variety of C8 substituents. Important

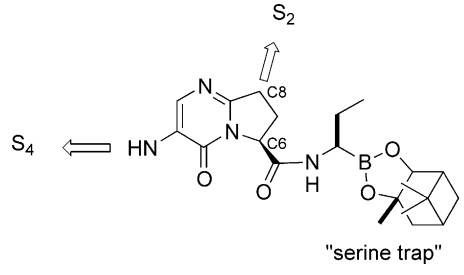

Figure 1. Bicyclic pyrimidinone-based inhibitor designed to reach into S2 via functionalization of C8 position.

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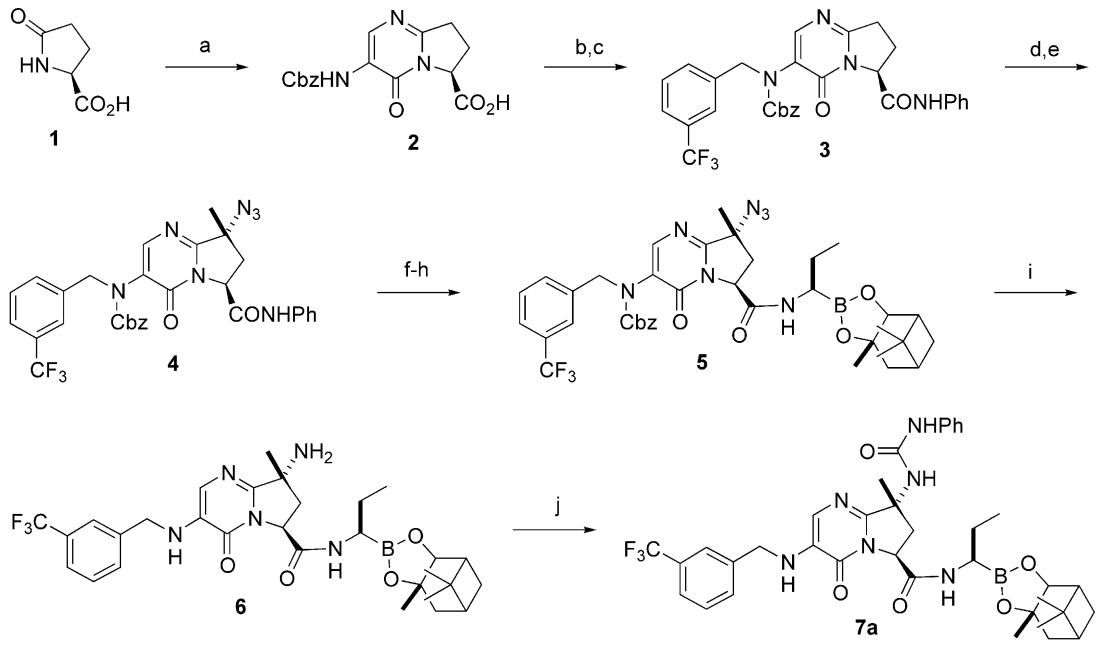
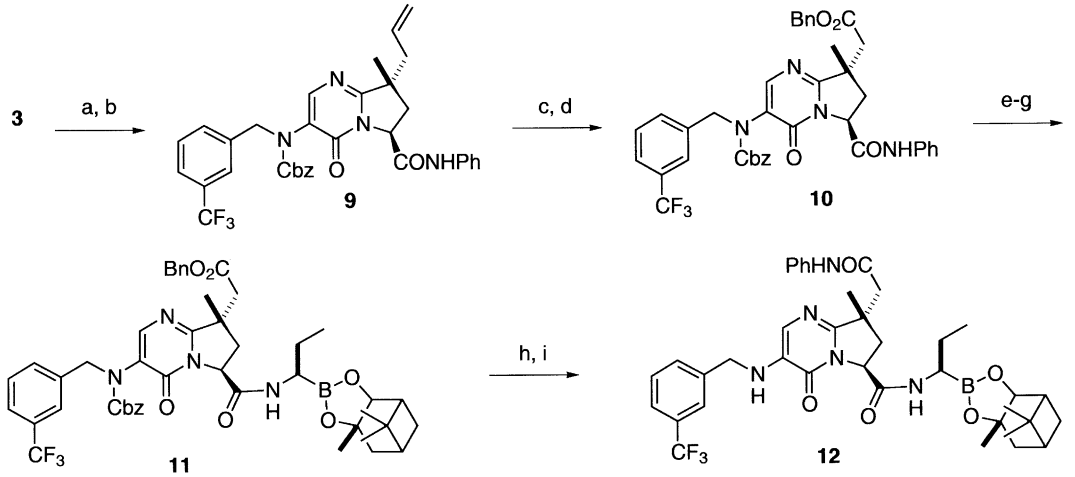
doi:10.1016/S0960-894X(03)00022-2



Scheme 1. Reagents and conditions: (a) eight steps, ref 9; (b) NaH, *m*CF_3–BnBr, TBAI; (c) aniline, EDCI, HOAt, $NaHCO_3$ (74%, two steps); (d) LiHMDS, MeI; (e) LiHMDS, trisyl azide (83%, two steps); (f) Boc_2O, DMAP, CH_3CN (100%); (g) LiOH, H_2O_2; (h) EDCI, HOAt, (1*R*)-1-aminopropyl boronic acid pinanediol ester (75%, two steps); (i) H_2, Pd–C, MeOH (100%); (j) PhNCO, CH_2Cl_2 (40%).

features of this intermediate are the anilide, which serves as a synthetically convenient, protected carboxylic acid and prevents Cα deprotonation/alkylation,[13] and the tertiary substitution of the C3 amine, which facilitates deprotonation of the C8 position.

An initial series of inhibitors, based upon a C8 amino group designed to project a substituent into the extended S2 pocket, was synthesized beginning with phenylamide **3**. Deprotonation of **3** (LiHMDS, 2.0 equiv) followed by quenching of the resultant dianion with MeI gives a *trans*/*cis* mixture (~2:1) of C8 mono-alkylation products. A second deprotonation followed by treatment with trisyl azide installed the quaternary center of intermediate **4** in which the azide is oriented *trans* to the C6 carboxamide in 83% overall yield for the two-step sequence. The stereochemical outcome, as determined by nOe studies, is consistent with the *trans* selectivity observed in alkylations of *N*-protected pyroglutamic acid esters.[14,15] The carboxylic acid functionality was revealed by conversion of the amide to an imide, followed by hydrolysis of the imide to the acid. The acid was coupled with (1*R*)-1-aminopropyl boronic acid pinanediol ester, prepared according to the method of Matteson et al.[16,17] The azide functionality was reduced to the amine via hydrogenolysis with concomitant Cbz removal. Treatment of amine **6** with phenyl isocyanate afforded phenyl urea-based inhibitor **7a**. In addition, a series of amides and a carbamate were prepared by treatment of amine **6** with various acid chlorides or

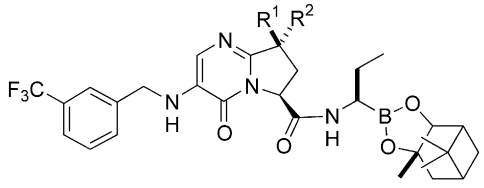


Scheme 2. Reagents and conditions: (a) LiHMDS, MeI; (b) LiHMDS, allyl bromide (48%, two steps); (c) $RuCl_3$, $NaIO_4$; (d) Cs_2CO_3, BnBr (42%, two steps); (e) Boc_2O, DMAP, CH_3CN (93%); (f) LiOH, H_2O_2 (100%); (g) EDCI, HOAt, (1*R*)-1-aminopropyl boronic acid pinanediol ester (52%); (h) H_2, Pd–C, MeOH (100%); (i) $PhNH_2$, HATU, DIEA (54%).

phenyl chloroformate (see Table 1). A series of substituted phenyl urea-based inhibitors (**7f–z**) was prepared by treatment of amine **6** with substituted phenyl isocyanates (Table 2).

A second series of bicyclic pyrimidinone-based inhibitors was prepared that incorporates an acetate group at the C8 position, designed to project a substituent into the S2 pocket (Scheme 2). To obtain the *trans*-disposed acetate moiety, it was necessary to proceed through the intermediacy of an allyl substituent (intermediate **9**), which was readily installed with the desired *trans* stereochemistry via alkylation of bicyclic pyrimidinone **3** with allyl bromide. Attempts to directly install the acetate group via alkylation with *tert*-butyl bromoacetate afforded predominantly *cis* substitution relative to the C6 carboxamide as determined by nOe. Subsequent oxidation of the allyl group to the acid ($RuCl_3$, $NaIO_4$), followed by benzylation afforded the acetate functionality with the desired *trans* stereochemistry, protected as the benzyl ester (**10**). As in the previous example, the C-terminal acid was revealed and coupled with (1*R*)-1-aminopropyl boronic acid pinanediol ester to provide protected intermediate **11**. Hydrogenation unmasked the carboxylate group, which was coupled with a series of amines to provide *trans* substituted amides **12a–j** (Table 3).

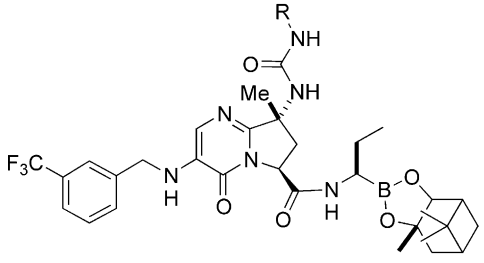
Table 1. Inhibition of HCV NS3 protease by C8-nitrogen linked bicyclic pyrimidinones[a]



Compd	R^1	R^2	IC_{50}, μM
8	H	H	7.0
7a	Me	–NH(CO)NHPh	0.79
7b	Me	–NH(CO)NHCH$_2$Ph	1.2
7c	Me	–NHCOPh	3.4
7d	Me	–NHCOCH$_2$Ph	2.4
7e	Me	–NHCO$_2$Ph	1.4

[a]Each IC_{50} is the average of two determinations.

Table 2. Inhibition of HCV NS3 protease by substituted phenyl ureas[a]



Compd	R	IC_{50} (μM)		R	IC_{50} (μM)
7a	Ph	0.70	**7p**	*o*-(CO$_2$Et)Ph	0.10
7f	*o*-NO$_2$Ph	0.18	**7q**	*m*-(CO$_2$Et)Ph	1.55
7g	*o*-ClPh	0.37	**7r**	*p*-(CO$_2$Et)Ph	1.10
7h	*p*-ClPh	1.10	**7s**	*o*-MeSPh	0.71
7i	*o*-MeOPh	0.36	**7t**	*m*-MeSPh	0.13
7j	*o*-EtOPh	0.16	**7u**	*p*-MeSPh	2.36
7k	*o*-PhOPh	0.30	**7v**	*o*-PhPh	30
7l	*p*-PhOPh	4.74	**7w**	*m*-FPh	0.50
7m	*o*-(CO$_2$Me)Ph	0.08	**7x**	*p*-FPh	1.03
7n	*m*-(CO$_2$Me)Ph	1.77	**7y**	*o*-MePh	0.78
7o	*o*-(CO$_2$H)Ph	6.40	**7z**	1-Naph	0.27

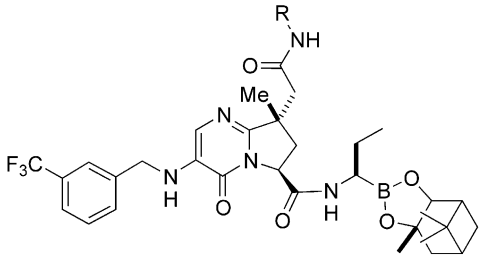
[a]Each IC_{50} is the average of two determinations.

IC_{50} values[18] determined for the initial series of bicyclic pyrimidinone inhibitors (Table 1) show a preference for a phenyl urea substituent (**7a**) in the S2 pocket over benzyl urea **7b**, amides **7c** and **7d**, or phenyl carbamate **7e**. The phenyl urea has approximately a 10-fold effect on inhibition relative to C8-unsubstitued analogue **8** (prepared by coupling of 2 with (1*R*)-1-aminopropyl boronic acid pinanediol ester, hydrogenation, and reductive amination with 3-(trifluoromethyl) benzaldehyde).

IC_{50} determinations for the substituted phenyl ureas are listed in Table 2. The most potent HCV NS3 protease inhibitors in this series are *ortho* alkyl esters **7m** and **7p**, which are approximately 10-fold more potent than the unsubstituted phenyl urea (**7a**). Moving this substitutent to either the *meta* (**7n** and **7q**) or *para* (**7r**) positions results in a significant loss in inhibition, as does removing the alkyl group to give *ortho* carboxylate **7o**. Potent inhibitors are also obtained with alkoxy substitution at the *ortho* position (**7i**, **7j** and **7k**) or a *meta* methylsulfanyl group (**7t**). Substitution at the *para* position diminishes inhibitory activity relative to the unsubstituted phenyl urea.

Inhibition determinations for the *trans* substituted amides, listed in Table 3, follow trends observed in the phenyl urea series. The unsubstituted phenylamide (**12a**) is 4-fold more potent than the corresponding phenyl urea **7a**. Preferences are seen for the methyl ester and alkoxy substituents at the *ortho* position (compounds

Table 3. Inhibition of HCV NS3 protease by *trans* substituted C8 amides[a]



Compd	R	IC_{50} (μM)		R	IC_{50} (μM)
12a	Ph	0.17	**12f**	1-Naphthyl	0.17
12b	*o*-(CO$_2$Me)Ph	0.10	**12g**	1-Isoquinolinyl	0.44
12c	*o*-MeOPh	0.20	**12h**	*o*-(CONH$_2$)Ph	0.15
12d	*o*-NO$_2$Ph	2.53	**12i**	*o*-(CONHMe)Ph	0.18
12e	*m*-MeSPh	0.09	**12j**	*o*-(CONMe$_2$)Ph	1.46

[a]Each IC_{50} is the average of two determinations.

12b and **12c**, respectively) as well as a methylsulfanyl group at the *meta* position (**12e**). Primary and secondary carboxamides at the *ortho* position (**12h** and **12i**) are tolerated, whereas a tertiary amide (**12j**) is not. In contrast to the trends observed in the urea series, an *ortho* nitro substituent (**12d**) results in a 15-fold loss in activity relative to the unsubstituted phenylamide. Inhibitors prepared based upon the *cis* substitution pattern (prepared via alkylation of intermediate **3** with *tert*-butyl bromoacetate) displayed flat SAR, which is consistent with molecular modeling that predicts the C8 substituent to be solvent exposed in the *cis* system.

In summary, we have developed potent, non-peptide HCV NS3 protease inhibitors based upon a bicyclic pyrimidinone scaffold. Interaction with the S2 binding pocket by these inhibitors via urea- and amide-based linkers contributed significantly to inhibition of the enzyme. To access the extended S2 binding pocket of the enzyme and make these binding interactions, we have developed alkylation chemistry to regio- and stereoselectively functionalize the bicyclic pyrimidinone ring system at the C8 position. This chemistry allows late-stage modification of the C8 position of the scaffold without necessitating resynthesis of the bicyclic pyrimidinone core for each analogue (i.e., pyrrolidinone alkylation, followed by elaboration to the bicyclic pyrimidinone).

Acknowledgements

We thank Lorraine Gorey-Feret and James L. Meek for IC_{50} determinations, Laurine G. Galya and Thomas H. Scholz for NMR structural determinations, and Professor David Evans for helpful discussions.

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