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The discovery and optimization of naphthalene-linked P2-P4 Macrocycles as inhibitors of HCV NS3 protease

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ABSTRACT

Naphthalene-linked P2-P4 macrocycles within a tri-peptide-based acyl sulfonamide chemotype have been synthesized and found to inhibit HCV NS3 proteases representing genotypes 1a and 1b with single digit nanomolar potency. The pharmacokinetic profile of compounds in this series was optimized through structural modifications along the macrocycle tether as well as the P1 subsite. Ultimately a compound with oral bioavailability of 100% in rat, and a long half-life in plasma was obtained. However, compounds in this macrocyclic series exhibited cardiac effects in an isolated rabbit heart model and for this reason further optimization efforts were discontinued.

Hepatitis C virus (HCV) infection is one of the leading causes of chronic liver disease. It is estimated that approximately four million people are infected with HCV in the United States while 177 million people are infected worldwide.[1] HCV is an insidious disease as early symptoms are somewhat benign and disease progression is relatively slow. However, over time, infected individuals progress through various stages of cirrhosis which can lead to hepatocellular carcinoma.[2,3] There is a significant mortality rate associated with HCV, as this disease is responsible for approximately 399,000 deaths per year.[2,3] The genetic diversity of HCV is broad, consisting of seven genotypes (GTs) with GTs 1–4 contributing to the largest percentage of infections worldwide.[4] The HCV genome comprises of a single strand of RNA that encodes a polyprotein that is ~3000 amino acids in length. The polyprotein is divided into structural and non-structural (NS) proteins. The non-structural proteins NS3/4A, NS4B, NS5A, and NS5B are involved in the virus replication process.[5] A complex of the NS3 protease and co-factor NS4A is responsible for cleaving the polyprotein at four sites to release functional non-structural proteins that are essential for replication of the HCV genome.[6] Due to its critical role in virus replication, the NS3 protease has been an attractive target for drug discovery and to date efforts targeting this protein have led to six approved NS3 protease inhibitors that are used in combination therapies to treat HCV infection.[7,8]

We have previously reported on the discovery of BMS-605339 (**1**, Fig. 1) which demonstrated a robust antiviral effect following a single oral dose to HCV GT 1-infected patients.[9] However, clinical studies with **1** were terminated due to the observation of cardiac effects which were unanticipated based on pre-clinical toxicology studies.[9,10] These drug-related effects included mild bradycardia, PR interval prolongation, and junctional escape rhythms.[10] This cardiovascular observation in humans prompted the employment of the isolated heart model (Langendorff model) as a pre-clinical tool to more effectively assess potential cardiovascular (CV) liabilities.[11] As previously described, perfusing isolated hearts with **1**, at concentrations similar to those which precipitated CV events in humans, triggered a similar response in this *ex vivo* model.[10] The isolated heart model was integral to the program screening tier and enabled the discovery of asunaprevir (**2**) which demonstrates low nM antiviral activity against replicons representing HCV GT 1a (H77) and 1b (Con1) (Table 1). In clinical studies with **2**, a robust antiviral response was observed in HCV GT 1-infected patients after a single dose.[10] However, the pharmacokinetic-pharmacodynamic relationship observed in humans was not supportive of once-daily (QD) dosing but was instead suggestive of a twice-daily (BID) dosing profile.[12] Follow-up efforts targeted the identification of a back-up compound with an antiviral profile similar to **2**, but with the potential for QD dosing in humans. While this approach

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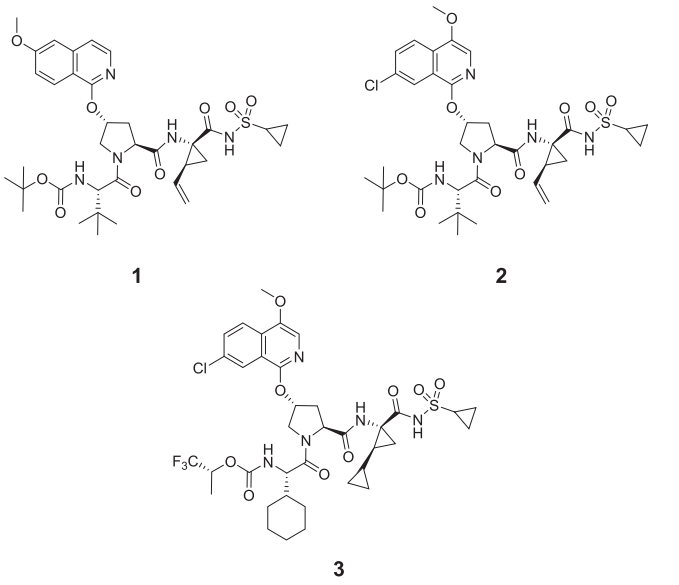
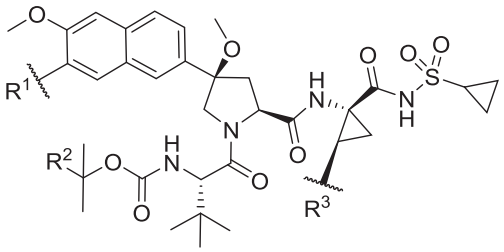


Fig. 1. The structures of BMS-605339 (**1**), asunaprevir (**2**), and pre-clinical back-up compound BMS-890068 (**3**).

provided **3** as a back-up molecule,[13] a parallel workstream pursued a structurally distinct chemical series based on a P2-P4 macrocycle chemotype.[14,15] This manuscript describes the results of that study and the properties of these P2-P4 macrocycles with respect to antiviral activity, pharmacokinetic properties and cardiac effects.

We have previously reported on the effects of replacing the isoquinoline P2* moiety with a naphthalene ring and modifying the connectivity for this element to the P2 proline ring, as demonstrated by compounds **4**–**7** (Table 2).[16] Therein, it was hypothesized that the reduced potency observed with **4**–**7** when compared to **2** and **3** was caused by a conformational bias between the naphthalene and proline rings which resulted in a suboptimal overlay of the P2* region with the NS3 protease.[10] Macrocyclization is an established strategy to enhance biological activity by limiting conformational ensembles and thereby favoring active conformers.[14,15,17] This has been an effective strategy to gain inhibitory activity against NS3 protease.[18,19] Against this backdrop, tethering of the P2* naphthalene ring in **4** to P4 was proposed as a potential approach to constrain the aromatic moiety in an active conformation. Models of the binding mode of acyclic compound **4**, 6-carbon tether macrocyclic naphthalene **10**, and **2** superimposed with **10** on the HCV NS3 protease provided support for these hypotheses (Fig. 2). Hence, the initial goal of this work was to synthesize P2-P4 macrocycles and determine if potency could be secured in this series.

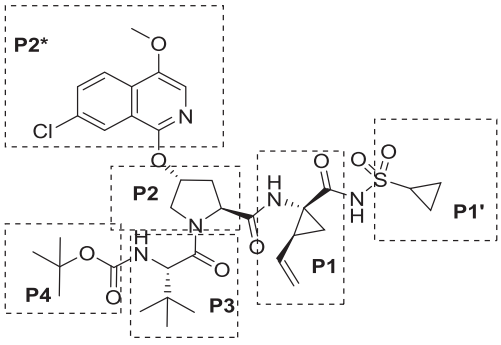
Table 2
Antiviral activity of acyclic compounds with naphthalene directly bound to C-4 of the proline P2 moiety.



Compound	Structure			EC_{50} (nM)[a]		HLM[b] $t_{1/2}$ (min)
	R^1	R^2	R^3	1a	1b	
4	H	CH_3		55	34	21
5	H	CH_3		244	253	–
6	H	CF_3		320	302	–
7		CH_3		–	50	36

[a] EC_{50} values obtained in GT 1a (H77) and GT 1b (Con1) replicons.
[b] Half-lives determined using liver microsomes in the presence of NADPH.

Table 1
Activity in GT 1a (H77) and 1b (Con1) replicons and rat PK profiles of asunaprevir (**2**) and the back-up compound **3**.



Compound	EC_{50}(nM)[a]		Rat PK (5mg/15 mg IV/Oral)[b]		
	1a	1b	Cl(mL/min/kg)	%F	PO 24 h liver (μM)
asunaprevir (**2**)	4	1.2	38	12	15.2
3	1	4.5	12.5	86.4	51

[a] EC_{50} values obtained in GT 1a-H77 and GT 1b-Con1 replicons.
[b] Dose Formulations: PEG 400/Ethanol (90:10).