Discovery of MK-1220: A Macrocyclic Inhibitor of Hepatitis C Virus NS3/4A Protease with Improved Preclinical Plasma Exposure

Michael T. Rudd,[*,†] John A. McCauley,[†] John W. Butcher,[†] Joseph J. Romano,[†] Charles J. McIntyre,[†] Kevin T. Nguyen,[†] Kevin F. Gilbert,[†] Kimberly J. Bush,[†] M. Katharine Holloway,[‡] John Swestock,[†] Bang-Lin Wan,[†] Steven S. Carroll,[§] Jillian M. DiMuzio,[§] Donald J. Graham,[§] Steven W. Ludmerer,[§] Mark W. Stahlhut,[§] Christine M. Fandozzi,[∥] Nicole Trainor,[∥] David B. Olsen,[§] Joseph P. Vacca,[†] and Nigel J. Liverton[†]

[†]Departments of Medicinal Chemistry, [‡]Molecular Systems, [§]Antiviral Research, and [∥]Drug Metabolism, Merck Research Laboratories, West Point, Pennsylvania 19486, United States

Ⓢ Supporting Information

ABSTRACT: The discovery of MK-1220 is reported along with the development of a series of HCV NS3/4A protease inhibitors containing a P2 to P4 macrocyclic constraint with improved preclinical pharmacokinetics. Optimization of the P2 heterocycle substitution pattern as well as the P3 amino acid led to compounds with greatly improved plasma exposure following oral dosing in both rats and dogs while maintaining excellent enzyme potency and cellular activity. These studies led to the identification of MK-1220.

KEYWORDS: Hepatitis C, NS3/4A, HCV, macrocycle, ring-closing metathesis
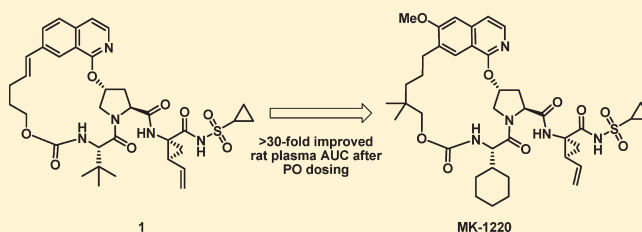
Hepatitis C virus (HCV) is a chronic infection that affects an estimated 170−200 million people worldwide.[1] The positive RNA strand *flaviviridae* family virus replicates primarily in the liver, and while the disease progresses over of a number of years, a significant fraction of those infected develop serious liver disease, including cirrhosis and hepatocellular carcinoma.[2] HCV is also the most common indication for liver transplantation.[3] HCV displays a high degree of genetic heterogeneity, with genotypes 1, 2, and 3 accounting for more than 90% of the infections in the developed world and with genotype 1 being predominant (∼70%) in the U.S., Europe, and Japan. The current standard of care treatment for chronic HCV infection is combination therapy with pegylated Interferon-α and Ribavirin[4−6] for up to 48 weeks. Sustained viral response (SVR) rates vary by genotype, with ∼45% of HCV genotype 1-infected patients and ∼80% of genotype 2- and 3-infected patients achieving SVR. The limited efficacy, coupled with a number of serious side-effects, limits the number of patients who complete treatment.[7,8]

Efforts toward improved HCV treatment include the development of direct antivirals which inhibit key steps in the viral replication process.[9] One of the more promising targets is the serine protease NS3/4A,[10−14] with clinical proof of concept established by Boehringer-Ingelheim with BILN-2061 (Figure 1).[15] Subsequently, both Vertex (VX-950, telaprevir)[16] and Schering-Plough (SCH-503034, boceprevir)[17] have moved α-keto-amide HCV NS3/4A inhibitors into phase 3 clinical trials, while a number of noncovalent inhibitors (danoprevir (ITMN-191),[18,19] TMC435350,[20−22] BI201335,[23] and vaniprevir[24] (Figure 1)) have also advanced into late stage trials.

Beginning in 2005,[24−27] our program focused on a series of macrocyclic inhibitors of HCV NS3/4A protease which contain a linker from P2 to P4, in contrast to the P1 to P3 linker present in BILN-2061 and several other known inhibitors (Figure 1). In 2008, we disclosed the general strategy for the discovery of this series and the subsequent optimization of genotype 1b potency and liver exposure, which led to compound **1**[26] and vaniprevir (MK-7009) (Figure 1).[24] Compound **1** has picomolar binding affinity for HCV NS3/4A protease (1b K_i = 0.04 nM), good cellular potency (1b replicon IC_{50} = 4.5 nM), and excellent liver exposure in rat following a 5 mg/kg oral dose ([liver]$_{4h}$ = 18,600 nM).[26] Obtaining high liver exposure may be critical to the success of any anti-HCV compound because the primary HCV replication site is in hepatocytes.[28] However, high systemic exposure would also be desirable given the evidence of extrahepatic replication sites,[28] and unfortunately, compound **1** does not achieve significant plasma levels following oral dosing in preclinical species (rat plasma AUC = 0.36 μM·h; 5 mg/kg, PO).[26] With a goal of finding compounds in this series, with high liver exposure and improved plasma exposure following oral dosing in preclinical species while maintaining excellent potency,
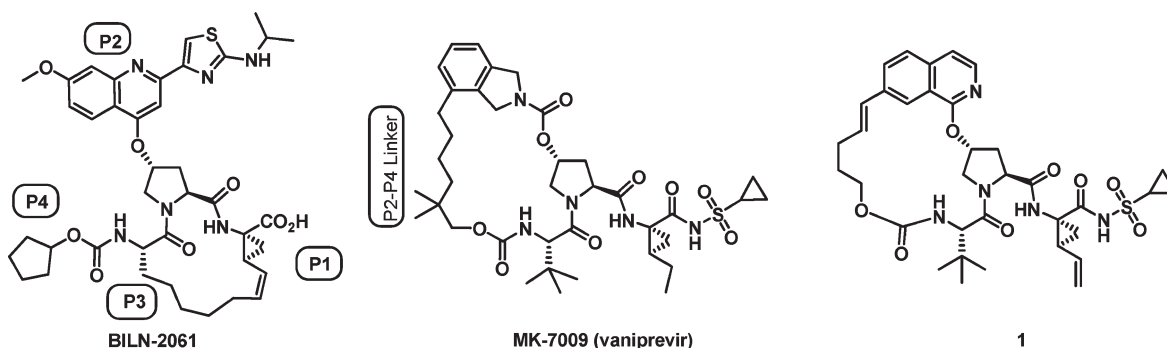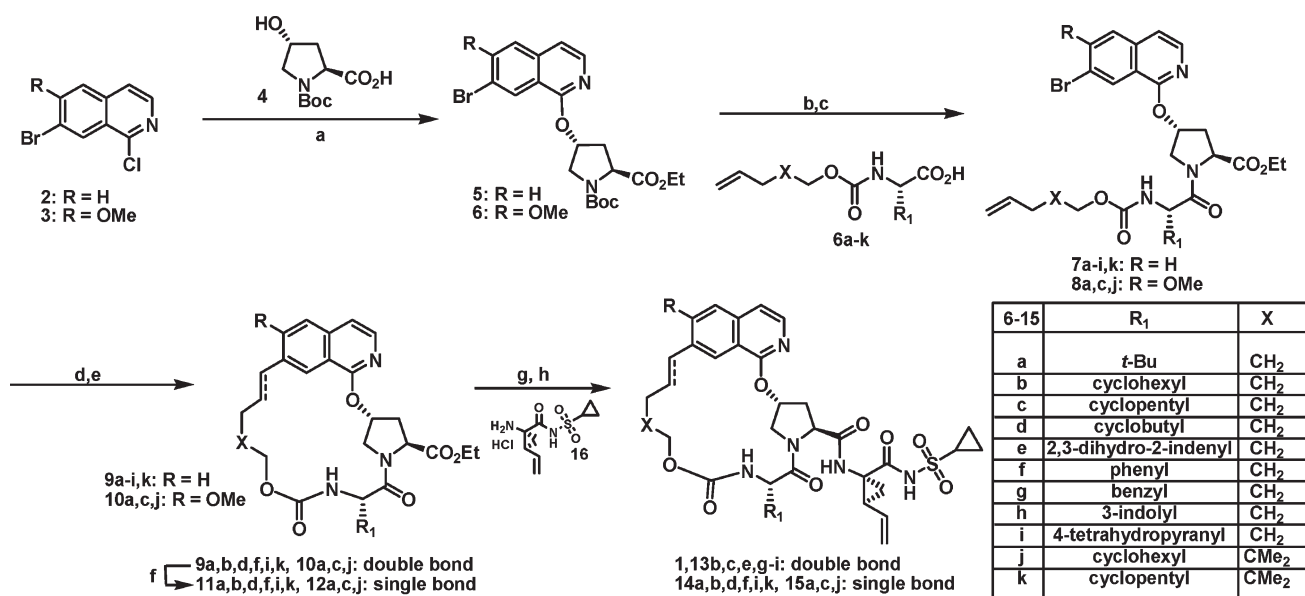
Figure 1. Macrocycle HCV NS3/4A protease inhibitors.

Scheme 1. Preparation of 6-Substituted and Unsubstituted Isoquinoline Derivatives[a]

6-15	R_1	X
a	t-Bu	CH_2
b	cyclohexyl	CH_2
c	cyclopentyl	CH_2
d	cyclobutyl	CH_2
e	2,3-dihydro-2-indenyl	CH_2
f	phenyl	CH_2
g	benzyl	CH_2
h	3-indolyl	CH_2
i	4-tetrahydropyranyl	CH_2
j	cyclohexyl	CMe_2
k	cyclopentyl	CMe_2

[a] Reagents and conditions: (a) t-BuOK, DMSO, then HCl, EtOH. (b) HCl, dioxane. (c) HATU, DIEA, DMF. (d) vinylSnBu$_3$, Pd(PPh$_3$)$_4$. (e) Zhan 1b, DCM. (f) Pd/C, H$_2$. (g) LiOH, THF, EtOH. (h) **16**, HATU, DIEA, DMF.

we decided to further explore the effect of substituents on the P2 isoquinoline group as well as various P3 and P4 groups. These efforts, which ultimately led to the discovery of MK-1220, are described herein.
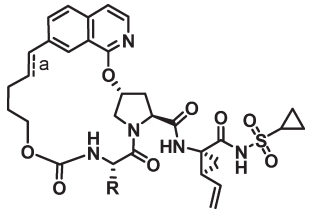
The synthesis of the various P2-isoquinoline analogues was carried out using the modular approach detailed in Scheme 1. N-Boc hydroxyproline **4** was reacted with either 6-substituted or unsubstituted bromo-chloroisoquinolines **2** and **3**, which, following esterification, gave **5** and **6**. Boc-deprotection, followed by coupling with acids **6a−k**, gave **7a−i**, **7k** and **8a**, **8c**, **8j**, which could be vinylated under Stille conditions and then cyclized using the Zhan 1b metathesis catalyst[29] to give **9a−i**, **9k** and **10a**, **10c**, and **10j**. The resulting double bond could alternatively be left intact or saturated under standard conditions to give **11a** and **b**, **11d**, **11f**, **11i**, **11k**, and **12a**, **12c**, and **12j**. The targeted compounds **1**, **13b** and **c**, **13e**, **13g−i**, **14a**, **14b**, **14d**, **14f**, **14i**, **14k**, and **15a**, **15c**, and **15j** were then prepared via hydrolysis of the ester and coupling with amine **16**.[30] 5-Substituted analogues (see Supporting Information Scheme 2 for general scheme and experimental details) were prepared by a regioselective halogenation/cross-coupling sequence from **11a**, which provided the

desired acyl-sulfonamides **18a−h** after hydrolysis and coupling with **16**. 5-Trifluoromethoxy analogue **18i** was prepared analogously to the compounds in Scheme 1 using the corresponding trifluoromethoxy isoquinoline, and P3-cyclohexyl analogue **19** was prepared analogously to **18c** using **6b**.

Compounds were evaluated in a genotype 1b enzyme binding assay,[31] and cellular activity was determined using the genotype 1b replicon system in the presence of 10% fetal bovine serum (FBS) and 50% normal human serum (NHS).[32,33] The plasma and liver pharmacokinetics of lead HCV protease compounds have been characterized and shown to result from the complex interplay of absorption, uptake transport, efflux transport, protein binding, and metabolism. Since fully characterizing each of these individual components in vitro may or may not translate into accurate in vivo predictions, the team took an empirical approach and differentiated potential lead compounds using initial pharmacokinetic analysis focused on plasma AUC and liver concentration at 4 h after a 5 mg/kg oral dose in rats.

As Table 1 illustrates, the effect on rat exposure in both liver and plasma of various P3 groups can be dramatic. Simple substitution the t-Bu of compound **1**[26] for a cyclohexyl group
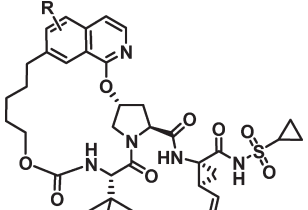
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Table 1.

Compound	Single (S) or double bond (D)[a]	R	1b K_i (nM)[b]	1b Replicon IC$_{50}$ (nM)[c] 10% FBS	1b Replicon IC$_{50}$ (nM)[c] 50% NHS	Rat PO Plasma AUC (μM·h)[d]	Rat [Liver] @4h (μM)[e]
1	D	*t*-butyl	0.07	4.5	14	0.36	18.6
13b	D	Cyclohexyl	0.063	2.5	78	0.67	46.9
14b	S	**Cyclohexyl**	**0.15**	4.5	181	3.1	29.8
13c	**D**	**Cyclopentyl**	**0.1**	**2.0**	**40**	**4.2**	**9.2**
14d	S	Cyclobutyl	0.18	7.0	130	--	--
13e	D	indane	0.07	5.0	20	0.15	4.5
14f	S	Phenyl	1.0	--	--	--	--
13g	D	Benzyl	0.65	3	--	0.43	10.0
13h	D	3-indolyl	2.0	20	--	0.07	0.4
13i	D	tetrahydropyran	0.07	9.0	20	0.5	0.2
14i	S		0.07	15.0	34	0.8	0.09

[a] Single or double bond at styryl position. [b] NS3/4A protease time-resolved fluorescence assay. [c] Cell-based replicon assay. [d] 5 mg/kg dosed orally in PEG400 (*n* = 2); AUC calculated based on 4 h experiment. [e] 5 mg/kg dosed orally in PEG400 (*n* = 2); liver levels after 4 h.

(**13b**) led to a 2-fold improvement in rat plasma AUC and liver concentration, while reduction of the styryl-olefin (**14b**) led to a nearly 10-fold improvement in rat AUC (3.1 μM·h). Unfortunately, while **14b** had improved rat plasma exposure, the genotype 1b replicon IC$_{50}$ in the presence of 50% NHS was shifted by greater than 10-fold, which may be due to the increased lipophilicity of the cyclohexyl group. Introduction of a cyclopentyl P3 group (**13c**) led to even greater plasma exposure (AUC = 4.2 μM·h) with a reduced loss in 50% NHS genotype 1b replicon potency. Given these dramatic effects, a number of other macrocycles with various cyclic P3 groups were prepared. Smaller groups, such as cyclobutyl (**14d**), led to much reduced potency, while larger groups, such as indane (**13e**) or benzyl (**13g**), resulted in compounds with reduced potency and/or moderate rat plasma and liver exposure. Aromatic P3 groups (**14f**, **13h**) resulted in compounds with poor potency. In a striking example of the unpredictable nature of the pharmacokinetics seen in this series, simple substitution of oxygen for carbon in the cyclohexyl ring (compare **13b/14b** to **13i/14i**) resulted in potent compounds which had almost no rat liver exposure (<0.2 μM @ 4 h) and moderate plasma levels. This type of result emphasizes the potential benefit of screening a large number of compounds for rat oral plasma and liver exposure due to the unpredictable pharmacokinetics of this series of compounds.[26]

Next, we explored the effect of 5- or 6-substitution on the P2-isoquinoline ring, which in general led to increased plasma exposure with variable effects on potency (Table 2). Interestingly, while the 5-iodo derivative (**18b**) possessed a moderate 20-fold shift in the replicon assay from 10% FBS to 50% NHS and good plasma exposure (AUC = 1.4 μM·h), the 5-bromo analogue (**18a**) had a much larger shift in replicon IC$_{50}$ and only moderate plasma and liver exposure, thus demonstrating that lipophilicity is not necessarily directly coupled to plasma/liver exposure. All

Table 2.

compd	R	1b K_i (nM)[a]	1b Replicon IC$_{50}$ (nM)[b] 10% FBS	1b Replicon IC$_{50}$ (nM)[b] 50% NHS	rat PO plasma AUC (μM·h)[c]	rat [liver] @ 4 h (μM)[d]
14a	H	0.18	8.7	46	0.27	13.4
18a	5-Br	0.04	2	170	0.28	5.8
18b	5-I	0.03	2.3	56	1.4	24.5
18c	**5-OMe**	**0.04**	**2.7**	**35**	**17.7**	**33.2**
18d	5-OEt	0.37	6	500	6.9	15.2
18e	5-Et	0.14	2.5	90	2.1	12.5
18f	5-SMe	0.03	2	34	1.8	12.6
18g	5-CF$_3$	0.25	21	410		
18h	5-SO$_2$Me	0.11	280	>1000		
18i	5-OCF$_3$	0.75	50	4400	1.6	8.8
15a	6-OMe	0.04	3	113	9.6	3.0

[a] NS3/4A protease time-resolved fluorescence assay. [b] Cell-based replicon assay. [c] 5 mg/kg dosed orally in PEG400 (*n* = 2); AUC calculated based on 4 h experiment. [d] 5 mg/kg dosed orally in PEG400 (*n* = 2); liver levels after 4 h.

the other 5-substituted derivatives tested in the standard rat PK experiment showed good to excellent plasma exposure, with most of the analogues losing significant replicon potency in the presence of 50% NHS (**18d** and **e**, **18 g–i**). In contrast, thiomethyl analogue **18f** displayed excellent in vitro and replicon potency (gt 1b K_i = 0.03 nM; 1b replicon IC$_{50}$ = 2 nM) and a more modest shift in the presence of 50% NHS (IC$_{50}$ = 34 nM); however, the rat plasma AUC following a 5 mg/kg PO dose was only moderate (1.8 μM·h). The compound with the most balanced profile was the 5-methoxyisoquinoline **18c**, with good gt 1b replicon IC$_{50}$ (2.7 nM (10% FBS); 35 nM (50% NHS)) and 17.7 μM·h plasma AUC following an oral 5 mg/kg dose in rat, which is a nearly 100-fold improvement in plasma AUC compared to the unsubstituted analogue **14a**. A similar plasma exposure enhancing effect was also seen with the 6-methoxyisoquinoline **15a** (9.6 μM·h), although this compound did have a larger 50% NHS replicon shift and lower liver exposure.

While a number of the most promising analogues (**14b**, **13c**, **18c**, **15a**) possessed the improved rat plasma exposure we desired, all lost replicon potency in the presence of 50% NHS to a degree that they were less potent than the targeted potency of parent compound **1** (gt 1b replicon IC$_{50}$ = 14 nM (50% NHS)). We then investigated whether the plasma enhancing effects of P3 cycloalkyl groups and P2-isoquinoline substituents could be combined to produce a compound which maintained high rat plasma while improving replicon potency (Table 3). 5-Methoxyisoquinoline analogue **19** showed reduced rat plasma exposure compared to **18c**, while 6-methoxy analogue (**15c**) showed an additive effect of the

Table 3.
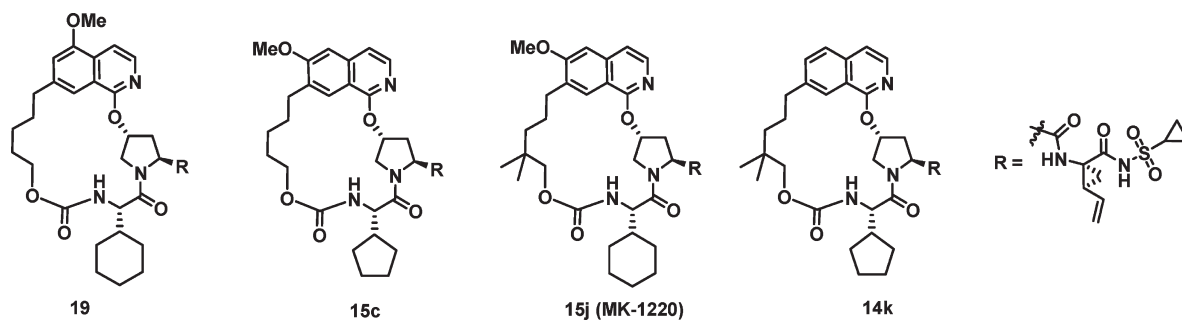
compd	1b K_i (nM)[a]	Replicon IC$_{50}$ (nM)[b]			rat PO plasma AUC (μM·h)[c]	rat [liver] @ 4 h (μM)[d]
		1b 10% FBS	1b 50% NHS	2a 10% FBS		
19	0.05	3.0	60.0	6.0	2.5	13.2
15c	0.03	2.0	80.0	-	18.1	8.0
15j (MK-1220)	**0.02**	**4.0**	**11.0**	**5.0**	**11.8**	**23.0**
14k	0.02	2.0	10.0	11.0	0.18	16.0

[a] NS3/4A protease time-resolved fluorescence assay. [b] Cell-based replicon assay. [c] 5 mg/kg dosed orally in PEG400 ($n = 2$); AUC calculated based on 4 h experiment. [d] 5 mg/kg dosed orally in PEG400 ($n = 2$); liver levels after 4 h.

Table 4. Pharmacokinetic Parameters for 15j (MK-1220)
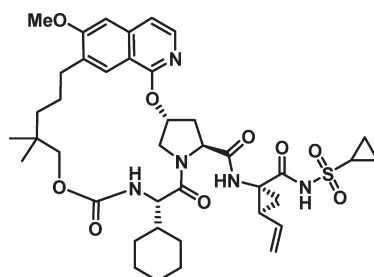
species	Cl[a] (mL/(min kg))	$T_{1/2}$[a] (h)	%F	PO[b] C_{max} (μM)	PO[b] AUC (μM·h)	PO[b] [liver] (μM)	PO[b] [liver] (μM)
rat	3.4	5.7	37%	4.4	12.1	23 @ 4 h	9.9 @ 24 h
dog	3.6	2.5	41%	4.0	12.4	54 @ 2 h	10.3 @ 26 h
monkey	8.3	1.3	9%	0.3	1.2		

[a] IV: Rat ($n = 4$) and dog ($n = 2$) at 2 mg/kg and monkey ($n = 3$) at 1 mg/kg; DMSO. [b] PO (5 mg/kg, $n = 3$ in rat and $n = 2$ in dog and monkey; 10% Tween).

P2-methoxy and the P3-cycloalkyl groups with a rat plasma AUC of 18.1 μM·h. This compound still possessed a 40-fold shift in replicon IC$_{50}$ in the presence of 50% NHS; however, as we demonstrated in the development of MK-7009 (vaniprevir),[24] incorporation of longer and/or substituted P2−P4 linkers can both improve potency and decrease the 50% NHS replicon shift. In this case, addition of the *gem*-dimethyl group into the P2−P4 linker led to a compound (**15j**, MK-1220)[34] with excellent genotype 1 and 2 biochemical and replicon potency and excellent rat plasma (plasma AUC = 11.8 μM·h) and liver exposure (23 μM @ 4 h). The dramatic effect of the 6-methoxy group can be seen with the contrastingly low plasma exposure seen with related P2-unsubstituted analogue **14k** (plasma AUC = 0.18 μM·h).

Given the excellent potency and initial high plasma and liver exposure following oral dosing in rat, **15j** was profiled further (Table 4). Compound **15j** demonstrates low IV clearance in rat, dog, and rhesus monkey, moderate half-life

and bioavailability, and excellent exposure in rat liver even at 24 h (9.9 μM; 5 mg/kg). Plasma and liver exposure in dog are similar to those in rat, with good plasma AUC (12.4 μM·h) and liver levels at both 2 h and 26 h (54 μM, 10.3 μM) following a 5 mg/kg oral dose. In contrast, plasma exposure in rhesus monkey is more limited (Table 4). Of particular note is that the 24 h liver concentration following a 5 mg/kg oral dose in both rats and dogs is ~1000-fold greater than the 50% NHS-shifted replicon IC$_{50}$ (11 nM). Following an IV dose to bile-duct cannulated animals, **15j** is primarily excreted into the bile as a mixture of parent and oxidative metabolites in rat and dog. Compound **15j** also has no significant activity versus other serine proteases (>50,000-fold versus trypsin and chymotrypsin) or in a broad Panlabs screen (>15,000-fold selectivity). Given the excellent potency, favorable preclinical pharmacokinetic properties, and clean off-target profile, **15j** was determined to be the optimal compound in this series and was selected for clinical development.

In summary, optimization for potency and rapid screening for rat plasma exposure (following oral dosing) in the P2-isoquinoline series of the P2–P4 macrocyclic HCV NS3/4A protease inhibitors has led to the discovery of several key structural features: notably the plasma exposure enhancing effects of P2-methoxy isoquinolines and P3-cycloalkyl groups. Within this optimized series, MK-1220 (**15j**) emerged as a candidate suitable for development.

■ ASSOCIATED CONTENT

■ AUTHOR INFORMATION

Corresponding Author
*Phone: 215-652-9727. Fax: 215-652-3971. E-mail: michael_rudd@merck.com.

■ ACKNOWLEDGMENT

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