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Perspective

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Discovery and Development of Simeprevir (TMC435), a HCV NS3/4A Protease Inhibitor

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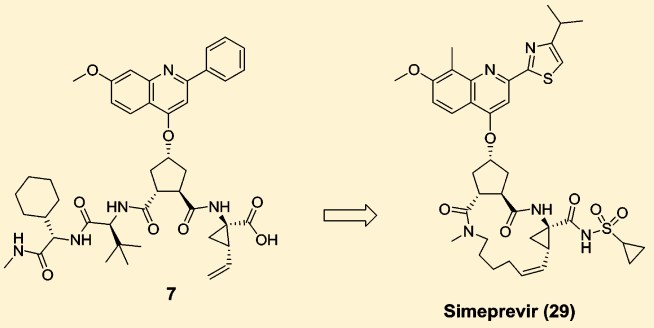
ABSTRACT: Hepatitis C virus is a blood-borne infection and the leading cause of chronic liver disease (including cirrhosis and cancer) and liver transplantation. Since the identification of HCV in 1989, there has been an extensive effort to identify and improve treatment options. An important milestone was reached in 2011 with the approval of the first-generation HCV NS3/4A protease inhibitors. However, new therapies are needed to improve cure rates, shorten treatment duration, and improve tolerability. Here we summarize the extensive medicinal chemistry effort to develop novel P2 cyclopentane macrocyclic inhibitors guided by HCV NS3 protease assays, the cellular replicon system, structure-based design, and a panel of DMPK assays. The selection of compound **29** (simeprevir, TMC435) as clinical candidate was based on its excellent biological, PK, and safety pharmacology profile. Compound **29** has recently been approved for treatment of chronic HCV infection in combination with pegylated interferon-α and ribavirin in Japan, Canada, and USA.



INTRODUCTION

In 1989, the hepatitis C virus (HCV) was identified as the agent causing non-A non-B hepatitis.[1] HCV is a positive-stranded RNA virus of the *Flaviviridae* family, replicating mainly in the liver and causing serious liver disease, beginning with fibrosis and subsequently slowly developing to cirrhosis, hepatocellular carcinoma, and liver failure. As a result, advanced liver disease due to HCV infection is currently the most common underlying cause of liver transplantation.[2,3] It has been estimated that 170−200 million people worldwide are chronically infected with HCV and that approximately 3−4 million people are newly infected each year,[4] with approximately 350 000 deaths annually due to HCV-related liver disease.

Since 1989, academic and pharmaceutical researchers have made tremendous progress in understanding the HCV virus and the complex nature of the diseases caused by HCV infection, establishing valuable research tools (e.g., the HCV replicon system[5]) and developing new treatment options for patients infected with HCV.[6] On the basis of the success of viral protease and polymerase inhibitors for the treatment of HIV infection, the HCV NS3/4A protease[7−9] and RNA-dependent RNA polymerase NS5B were the first HCV drug targets to be explored.[8,10,11] Subsequently, the viral NS5A RNA-binding protein emerged as an attractive additional target for drug development.[12,13] The molecular entities that directly modulate these viral proteins or protein complexes are termed direct-acting antiviral agents (DAAs).[14] In 2011, the massive effort to develop new HCV DAAs as rewarded by the approval of the first generation of HCV NS3/4A protease inhibitors, **1** (boceprevir)[15−17] and **2** (telaprevir),[18−23] which are used in

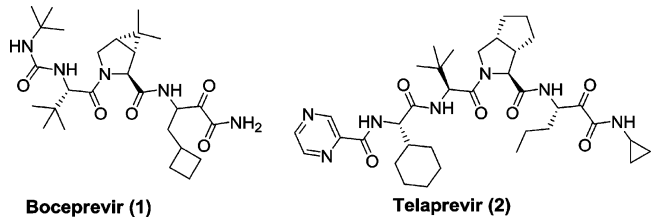


Figure 1. Structures of first-generation HCV NS3/4 protease inhibitors approved for clinical use.

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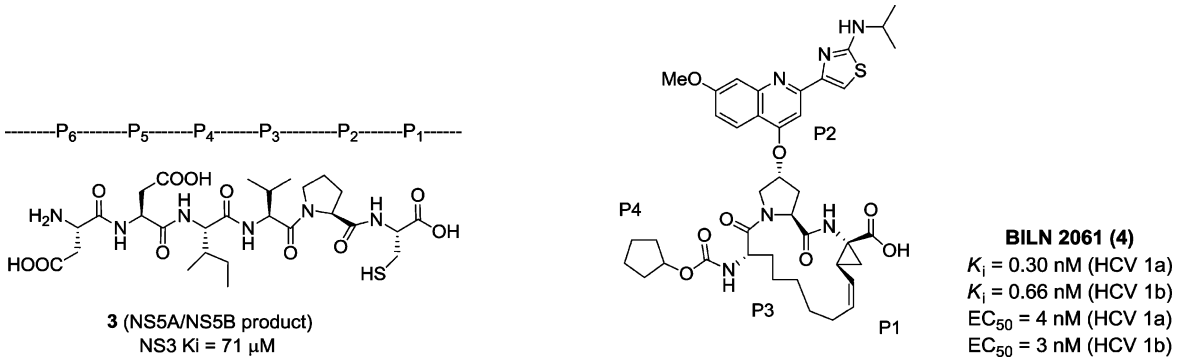

Figure 2. Structures of first product-based hexapeptide inhibitor (**3**) and compound **4**.[29−31]

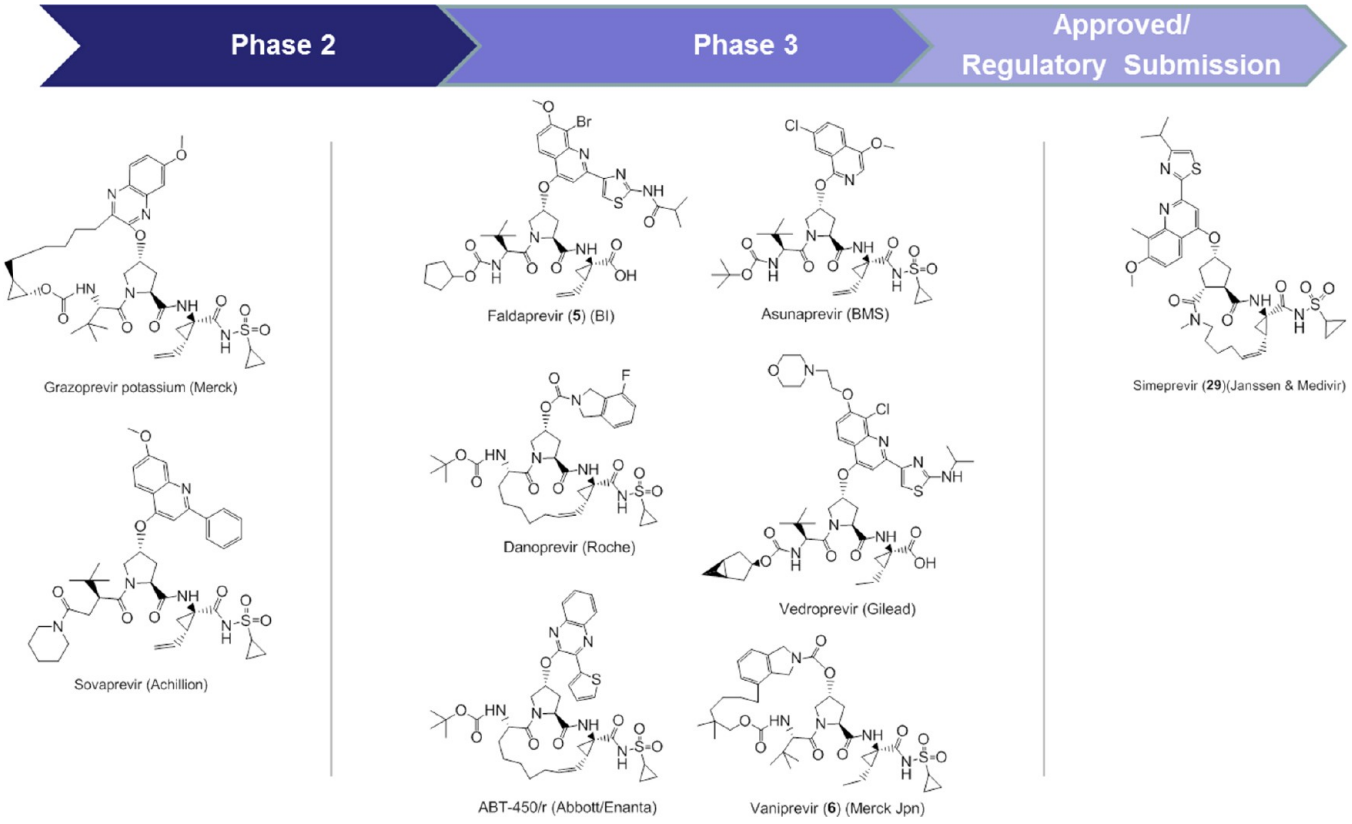

Figure 3. HCV NS3 protease inhibitors in late-stage clinical development.

combination with peg-IFN-α and ribavirin for the treatment of HCV genotype 1 infection in both treatment-naive and treatment-experienced patients (Figure 1). There is currently a large number of new DAAs and DAA combinations in clinical development, including second-generation NS3/4A protease inhibitors, nucleotide and non-nucleoside RNA-dependent RNA polymerase NS5B inhibitors, and NS5A replication complex inhibitors, making HCV one of the fastest expanding therapeutic areas in the pharmaceutical industry today.[6,24]

Treatment of HCV infection is highly complex, and outcome depends on multiple factors including viral genotype (G1−G6) and subtype, pretreatment viral load, patient population, prior treatment history (null responder, partial responder, relapser, or treatment-naive), patient IL28B polymorphisms (CC, CT, or TT genotype), and severity of patient liver disease (METAVIR score: F0−F4).[6,14,24,27]

The current standard of care for treatment-naive and treatment-experienced patients infected with HCV genotype 1 consists of compound **1** or **2** in combination with peg-IFN-α and ribavirin, with a treatment duration of 24−48 weeks depending on prior treatment response, liver disease status, and how well the patient responds to treatment. Compounds **1** and **2** are both given orally with food: compound **2** two or three times daily and compound **1** three times daily. Peg-IFN is administered as weekly injections, and ribavirin is given twice daily based on patient weight.

For compound **2**,[25] sustained virologic response (SVR) rates of 69−75% in treatment-naive patients and 83−88% in prior relapser patients have been reported. Partial responder and null responder patients show substantially lower SVR rates of 54−59% and 29−33%, respectively. The SVR rates for compound **1**[25] are 63−66%, 69−75%, and 40−52% for treatment-naive, relapser, and partial responder patients, respectively. For

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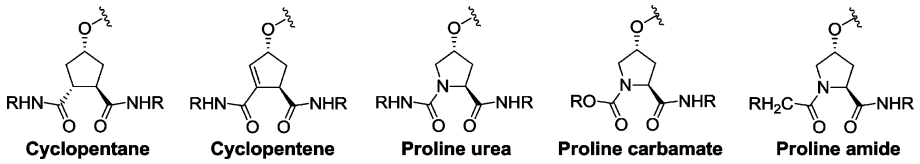

Figure 4. Bioisosteric replacement of the P2 *N*-acyl-(4*R*)-hydroxyproline with trisubstituted cyclopentane- and cyclopentenedicarbonyl moieties and proline urea, proline carbamate, and proline amide building blocks.

patients infected with HCV genotypes 2−6, current treatment consists of peg-IFN-α and ribavirin for 24−48 weeks. HCV G3 infection is less responsive to peg-IFN-α and ribavirin than G2, with SVR rates of 66−79% and 75−93%, respectively, after 24 weeks of therapy.[26]

The introduction of compounds **1** and **2** has resulted in increased cure rates in patients with HCV genotype 1 infection but has also added to the complexity of treatment and further increased the already severe side effects associated with HCV therapy.[27] Anemia, a known side effect of ribavirin, is more severe and frequent with **1**- or **2**-containing regimens, and treatment with **2** is associated with skin disorders, including rash and pruritis. Thus, there remains a major need for further improved treatments with higher cure rates (in particular for the difficult-to-treat genotype 1 patients), convenient dosing schedules, better tolerability and safety, and shorter treatment durations.

The virally encoded HCV NS3/4A serine protease is activated by the noncovalent association of NS3 with its cofactor NS4A. This chymotrypsin-like serine protease proteolytically cleaves four of the five sites of the viral polyprotein: the autocatalytic cis cleavage of the NS3/NS4A junction and the trans cleavage at the NS4A/NS4B, NS4A/NS5A, and NS5A/NS5B junctions in a process that is essential for viral replication.[8] The HCV NS3/4A protease also cleaves host cellular proteins, resulting in inhibition of the production of interferon and thereby impairing the host's innate immune response against viral infections.[28] Early studies showed that after processing of the viral polyprotein, the nonprime side cleavage product remained bound in the substrate-binding cleft and inhibited the protease itself.[29] This observation was the starting point of extensive exploratory structure−activity relationship (SAR) studies and refinements that led to the discovery of **4** (BILN 2061) (Figure 2) and, subsequently, compounds **1** and **2** (Figure 1), and other drug candidates currently in clinical development (Figure 3).[6,29−32]

Most of the HCV NS3/4A protease inhibitors fall into one of two mechanistic classes: reversibly covalent or noncovalent. The approved first-generation HCV protease inhibitors **1** and **2** are linear peptidomimetic structures incorporating an α-ketoamide that reacts reversibly with the catalytic Ser139. The noncovalent class of inhibitors[33−42] (Figure 3) includes either linear or macrocyclic structures, where the macrocyclic compounds are linked either via the P1 side chain to the P3 side chain or via the P2 side chain to the P4 side chain. All the inhibitors, with the exception of compound **29** which is based on a cyclopentyl equivalent, incorporate an *N*-acyl-(4*R*)-aryloxyproline moiety with extended P2 proline substituents such as quinoline, isoquinoline, quinoxaline, and indoline groups. Another common feature of all the noncovalent inhibitors is the vinylcyclopropylamino acid or ethylcyclopropylamino acid bioisostere in the P1 position.

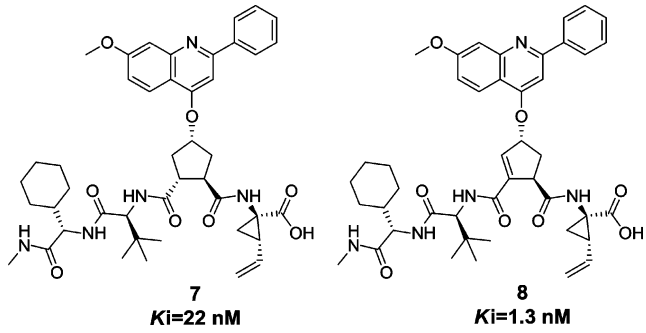

Figure 5. Structures of early Medivir peptidic inhibitors.

DISCOVERY OF SIMEPREVIR (COMPOUND 29)

During the early 2000s, scientists at Boehringer Ingelheim (BI) disclosed their groundbreaking work on the discovery of HCV NS3/4A protease inhibitors, detailing systematic peptide-based approaches in both patent applications and scientific journals. Over the same period, structural information relevant to NS3/4A (and NS5B) drug design started to emerge, with high-resolution crystal structures initially available in the late 1990s and inhibitor complexes emerging in the early 2000s. The BI group reported on work conducted to transform a linear hexapeptide inhibitor (**3**) into a potent, selective, and orally bioavailable macrocyclic tripeptide clinical candidate, compound **4** (Figure 3), which was the first HCV NS3 protease inhibitor to enter clinical trials.[29−31,43] Oral administration of compound **4** to patients with HCV genotype 1 resulted in a rapid and profound reduction of viral RNA levels (2−3 $\log_{10}$ after 2 days), thus establishing proof-of-concept for a HCV NS3/4A protease inhibitor as a therapeutic agent.[44] Subsequent clinical trials with compound **4** were halted because of cardiotoxicity at high doses in Rhesus monkeys.[45] The inhibitors from BI incorporate an *N*-acyl-(4*R*)-aryloxyproline moiety in the P2 position, a building block that subsequently has been adopted in a number of other noncovalent HCV protease inhibitors.

The design approach initially taken at Medivir, and subsequently in a research collaboration with Janssen, explored replacement of the central P2 *N*-acyl-(4*R*)-hydroxyproline with trisubstituted cyclopentane- and cyclopentenedicarbonyl "core moieties" or "building blocks" (Figure 4), an approach that even today remains unique for HCV protease inhibitors.[46,47] We envisaged that these novel core building blocks would be stable and able to mimic the P2 proline conformation of the HCV protease substrate despite reversal and shifting of the P2−P3 backbone amide. Thus, potent new HCV protease inhibitors incorporating these new cyclopentane and cyclopentene core P2 moieties were developed. The most potent inhibitors, **7** and **8**, displayed K_i values of 22 and 1.3 nM, respectively, against the full-length protease (G1a) (Figure 5).[46,48] The compounds were evaluated in an in vitro assay involving recombinant full length NS3 (3.5 nM) and NS4A in molar excess (14 μM). The

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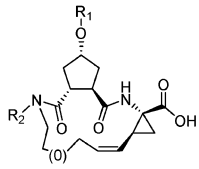
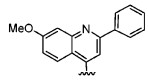
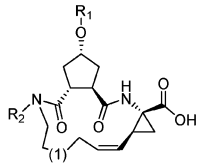
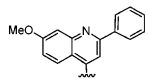
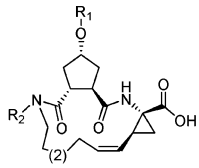
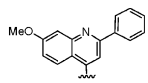
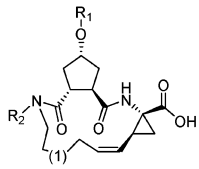
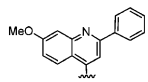
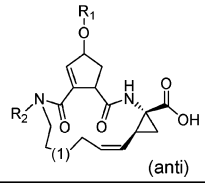
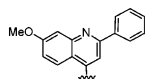
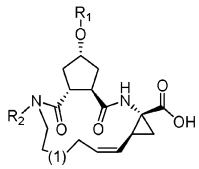
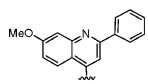
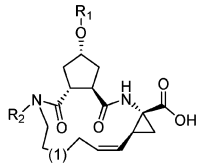
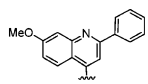
Table 1. Effect of P2 Scaffold, Ring Size, and P3 Capping Groups on Biological Activity for Macrocyclic P1 Carboxylic Acid Inhibitors

Cpd	Structure	Ring size	R_1	R_2	NS3/4A K_i (nM)[a]	HUH7-Rep EC_{50} (nM)[b]
9		13		Boc-NH	130	>10
10		14		Boc-NH	31	>10
11		15		Boc-NH	710	>10
12		14		H_2N	6	7.6
13		14		H_2N	15	5.4
14		14		H	260	>10
15		14		Me	44	2.2

[a]Inhibition of the full-length HCV NS3/4A protease 1a measured by the inhibition constants (K_i values). [b]Inhibition of HCV 1b replication in Huh-7-Rep cells (luciferase assay) measured by 50% effective concentration (EC_{50}).

enzyme activity was continuously measured over time (20 min), and K_i values were determined assuming competitive inhibition.[46,48]

In the work to characterize the SARs of these novel P2 ring structures, it was observed that an L configuration at both the P3 and P4 amino acids was preferred. It was also found that the P1 moiety (1*R*,2*S*)-1-amino-2-vinylcyclopropanecarboxylic acid and the P2 2-phenyl-7-methoxy-4-quinoline group further increased the potency of our novel inhibitors, thus making them attractive for further optimization. The initial focus was on improving enzyme and, more importantly, cell-based replicon activities (half maximal effective concentration, EC_{50}), the latter determined in the Huh7-Rep cell line containing the subgenomic bicistronic HCV 1b replicon clone ET with a luciferase readout.[5] This work included (i) macrocyclization, (ii) truncation of the peptide backbone, and (iii) replacement of the carboxylic acid with a bioisosteric acylsulfonamide group.[49] A series of 13- to 16-membered macrocycles incorporating the key P2 cyclopentane building block and with different P4 substituents (i.e., P3 capping

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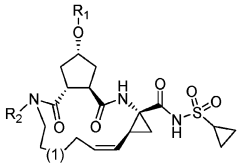
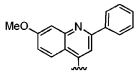
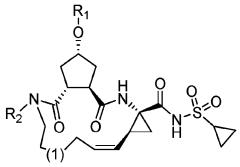
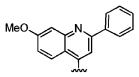
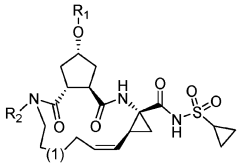
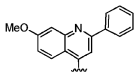
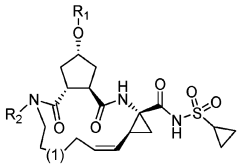
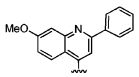
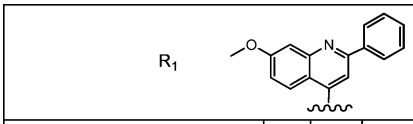
Table 2. Effect of Ring Size and P3 Capping Groups on Biological Activity for Macrocyclic P2 Cyclopentane P1 Cyclopropylacylsulfonamide Inhibitors

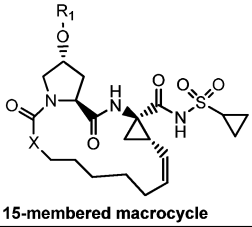
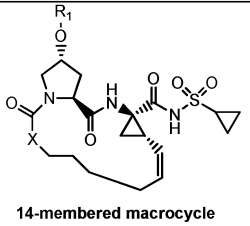
Cpd	Structure	Ring size	R_1	R_2	NS3/4A K_i (nM)[a]	HUH7-Rep EC_{50} (nM)[b]
16		14		Boc-NH	0.07	0.53
17		14		H_2N	0.19	0.033
18		14		H	2.2	4.4
19		14		Me	0.41	0.0091

[a]Inhibition of the full-length HCV NS3/4A protease 1a measured by the inhibition constants (K_i values). [b]Inhibition of HCV 1b replication in Huh-7-Rep cells (luciferase assay) measured by 50% effective concentration (EC_{50}).

groups) were synthesized and evaluated. Initial SAR work on these P2-cyclopentane-containing macrocyclic structures revealed potent inhibitors having either a P4 hydrazine or P4 NH-Boc functionalization (Table 1). Evaluation of 13- to 16-membered macrocycles showed a clear preference for 14-membered rings, exemplified by the P2 cyclopentane inhibitors **10** and **12** with K_i values of 31 and 6 nM, respectively. The corresponding 13- and 15-membered macrocyclic compounds **9** and **11** showed K_i values of 130 and 710 nM, respectively, whereas the 16-membered analogue was inactive (K_i > 10 μM). For the 14-membered macrocycle, a set of smaller P3 capping groups was investigated. Among these inhibitors, **14** (-H) and **15** (-Me) delivered K_i values of 260 and 44 nM, respectively (Table 1), and their corresponding P1 acylsulfonamides **18** and **19** furnished very promising K_i values of 2.2 and 0.41 nM, respectively (Table 2). More encouragingly, **19** delivered a replicon EC_{50} value of 9.1 nM. At the time of this work, this represented, to the best of our knowledge, the first reported HCV protease inhibitor lacking a P4 substituent, i.e., P3 capping group, while also maintaining high potency in the replicon assay. Since selectivity toward related human proteases is very important for the safety profile of protease inhibitors, our compounds were evaluated for selectivity against a range of human serine proteases, e.g., cathepsin B, chymotrypsin, and elastase. Compound **19** and the other compounds exhibited good selectivity against these proteases and did not exhibit any cytotoxicity in MT4-LTR-Luc and Huh7-Luc cell lines (CC_{50} > 20 μM).[49]

Table 3. Effect of Macrocycle Size and Proline *N*-Carbamate/Urea Linkage on Biological Activity



	Cpd	X	NS3/4A K_i (nM)[a]	HUH7-Rep EC_{50} (nM)[b]
 15-membered macrocycle	**20**	NH	0.24	15
	21	O	2.4	460
	22	NMe	10	4500
 14-membered macrocycle	**23**	NH	1.5	14
	24	O	0.35	62
	25	NMe	0.4	44

[a]Inhibition of the full-length HCV NS3/4A protease 1a measured by the inhibition constants (K_i values). [b]Inhibition of HCV 1b replication in Huh-7-Rep cells (luciferase assay) measured by 50% effective concentration (EC_{50}).

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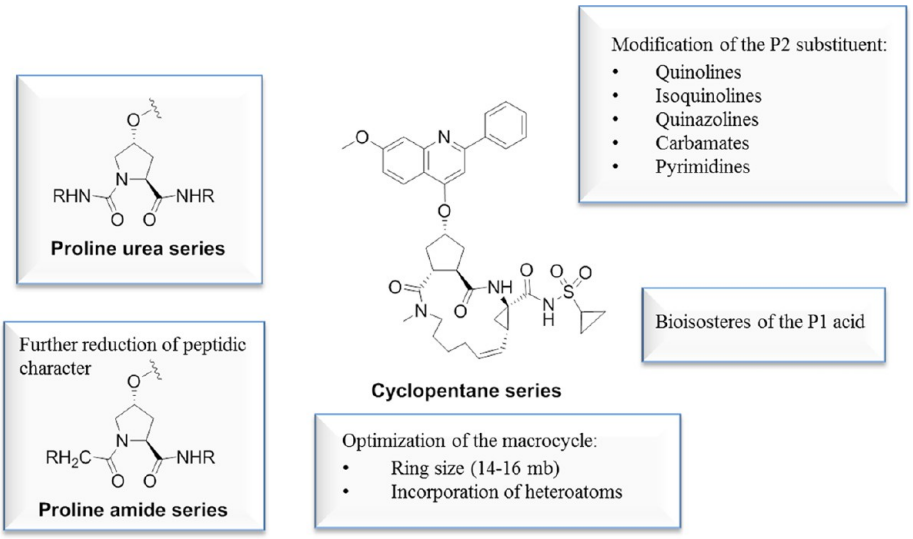

Figure 6. Lead optimization strategy.

Further work to expand and develop the concept of our novel P2 core moieties led to the development of inhibitors with proline *N*-urea or *N*-carbamate linkage to the P3 amino acid, again yielding inhibitors with excellent antiviral profiles (Figure 4).[50] From the initial results of these studies, it was thus evident that both the length and chemical nature of the linkage influenced inhibitor potency (Table 3). When 15-membered macrocycles were evaluated, the proline NH-urea chemotype proved to be the most active, for example, **20** with K_i = 0.24 nM and EC_{50} = 15 nM compared with the proline carbamate **21** and proline-NMe-urea **22** with EC_{50} values of 460 and 4500 nM, respectively. For the 14-membered ring compounds **23**, **24**, and **25**, the K_i values ranged from 0.4 to 1.5 nM. However, the proline-NH-urea-containing compounds **20** and **23** were the most active compounds in the cell-based replicon assay in both the 14- and 15-membered ring series, with EC_{50} values of 14 and 15 nM, respectively. The discrepancies observed between the enzymatic potency and replicon activity for the two carbamates, **21** and **24**, were larger than usually seen, likely attributable to lower cell membrane permeability and thereby making the proline carbamates less attractive for further development.

The most active macrocyclic P2 cyclopentane, **19**, showed promising in vitro Caco-2 permeability (P_{app} = 3.8 × 10^{-6} cm/s) and medium intrinsic clearance in both human and rat liver microsomes (HLM Cl_{int} = 46 μL min^{-1} mg^{-1}).[49] The rat pharmacokinetic (PK) profile for **19** was characterized by low oral bioavailability (F = 2.5%) due to a high plasma clearance (2.79 L h^{-1} kg^{-1}). Extensive bile excretion is a common feature of highly lipophilic compounds, and further characterization of **19** established that the poor PK profile was due to very high excretion of the parent drug into the bile. Indeed, 95% of the product was found to be unchanged in the bile 1 h after intravenous (iv) administration of 1 mg/kg **19** in rat.

In our efforts to improve and balance the biological and pharmacokinetic properties, we explored and optimized the substituent of the central P2 core. The P2 ether-linked quinoline was replaced with other heterocyclic groups such as isoquinolines, quinazolines, and pyrimidines. We also explored a carbamate linkage to connect the core P2 cyclopentane or pyrrolidine rings with the P2 heterocyclic group. Another aspect of our optimization work included further reduction of the peptidic nature of the inhibitors and the macrocyclic ring with respect to both the size of the macrocycle and the nature of the linkage between the P1 and P3 side chains. The substituents on the acylsulfonamide were also explored as another possibility to improve PK properties. Both the P2 cyclopentane and the P2 proline urea series were optimized in parallel, applying the strategy summarized above and in Figure 6. The P2 cyclopentene inhibitors were not included in the optimization work because of the challenges in the synthesis of the P2 cyclopentene inhibitors with difficult and time-consuming separation of diastereomeric mixtures.[47] Moreover, the risk for covalent addition through their Michael acceptor properties rendered this inhibitor class less attractive for further development.[47]

The macrocyclic lead compound in the P2 cyclopentane series, **19**, was optimized through replacement of the 2-phenyl group with five- and six-membered heterocycles and by adding a small substituent at the 8-position (Table 4).[51] The introduction of the 2-isopropylaminothiazol-4-yl moiety in the 2-position of the quinoline led to **26**, a potent (EC_{50} = 17 nM) but poorly permeable (P_{app} = 1.4 × 10^{-6} cm/s) compound. Low permeability was also observed for the thiazol-2-yl derivative **27** (P_{app} = 1.6 × 10^{-6} cm/s), while the 4-isopropylthiazol-2-yl derivative **28** showed significantly improved Caco-2 permeability with P_{app} = 13 × 10^{-6} cm/s and HLM stability with Cl_{int} = 16 μL min^{-1} mg^{-1}. Further improvement was achieved by the introduction of either a methyl or a chloro substituent in position 8 of the quinoline, leading to **29** or **32**, respectively. These two compounds showed improved activities (EC_{50} of 7.8 and 2.9 nM, respectively, for **29** and **32**) and improved stability in HLM (Cl_{int} of <6 μL min^{-1} mg^{-1} for **29** and 9.0 μL min^{-1} mg^{-1} for **32**). However, although the 8-methyl derivative **29** exhibits permeability similar to that of the parent **28** (P_{app} of 8.4 × 10^{-6} and 13 × 10^{-6} cm/s, respectively), the 8-chloro derivative **32** was found to be significantly less permeable (P_{app} = 5.8 × 10^{-6} cm/s). The 8-ethyl and 8-fluoro derivatives **30** and **31** were less active than the 8-hydrogen, 8-methyl, or 8-chloro derivatives. Finally, the bioisosteric replacement of the isopropylthiazole with an isopropylpyrazole and an isopropylpyridyl led to additional compounds **33** and **34** with excellent potencies and stability/permeability profiles.

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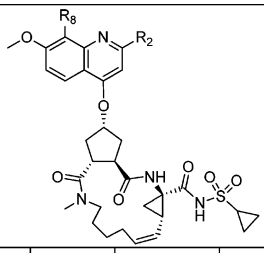
Table 4. Optimization in Cyclopentane Series



Cpd	R^2	R^8	NS3/4A K_i (nM)[a]	HUH7-Rep EC_{50} (nM)[b]	*Papp* A-B (cm/s x 10^{-6})[c]	Clint (μL/min/mg)[d]
19		H	0.41	9	3.8	46
26		H	1.4	17	1.4	15
27		H	0.20	11	1.6	38
28		H	0.84	17	13	16
29		Me	0.36	7.8	8.4	<6
30		Et	3.1	66	-	-
31		F	0.55	57	-	-
32		Cl	0.10	2.9	5.8	9.0
33		Me	0.16	9.7	15	<6
34		Me	0.30	6.8	12	<6

[a]Inhibition of the full-length HCV NS3/4A protease 1a measured by the inhibition constants (K_i values). [b]Inhibition of HCV 1b replication in Huh-7-Rep cells (luciferase assay) measured by 50% effective concentration (EC_{50}). [c]A−B apparent permeability coefficient (P_{app}) measured in Caco-2 cells. [d]Intrinsic clearance in human liver microsomes.

The optimization of the P2 proline urea series provided several highly attractive compounds with excellent antiviral and PK properties.[50,52] Both substituted quinolines and quinazolines could successfully be used as P2 substituents. Despite their relative similarities, the quinoline and quinazoline series displayed compound-specific SAR around the 2-position substituent. Table 5 provides a short summary of key compounds in the proline urea series. With respect to both potency and PK, thiazolyl substituents were preferred at the 2-position for quinoline derivatives whereas for the quinazolines unsubstituted or 4-substituted phenyl substituents were preferred in position 2 of the quinazoline. When quinolines were used as P2 substituents, a 15-membered proline-NH-urea type of macrocycle with a 4-isopropylthiazol-2-yl substituent in position 2 of the quinoline provided the best compound, **35**, with high potency at the enzyme level and activity in the replicon cell-based assay (K_i = 0.1 nM, EC_{50} = 4.5 nM) and with excellent permeability (P_{app} = 30 × 10^{-6} cm/s) but only moderate HLM stability (89% of the compound being metabolized after 15 min at 37 °C). On the other hand, with quinazolines as P2 substituents the more promising compounds were obtained with the 14-membered proline-NMe-urea type of macrocycle. These compounds, **36** and **37**, exhibited promising replicon activities of 11 and 3.0 nM, respectively, and excellent PK profiles with Caco-2 permeability values of P_{app} = 26 × 10^{-6} cm/s and P_{app} = 33 × 10^{-6} cm/s, respectively. **36** and **37** also showed good metabolic stability in HLM with Cl_{int} of 17 μL min^{-1} mg^{-1} for **36** and 8.0 μL min^{-1} mg^{-1} for **37**.

In an attempt to further improve the PK properties, the peptidic nature of the compounds was reduced by truncation of the P3 capping group, resulting in a new subseries of P2 proline dipeptide macrocyclic amide compounds (Table 6).[53] The rigid carbamate functionality of the P3 capping group has been reported to be responsible for positioning the N-terminal alkyl group in the shallow S4 binding pocket of the enzyme and thereby being important for the potency of the macrocyclic competitor compounds. As predicted, removal of the P3 capping group led to crucial decreases in potency and cell-based activity, as can be seen for **38** with a K_i value of 46 nM and an EC_{50} of 1980 nM compared to **4** with a K_i value of 0.3 nM and an EC_{50} of 1.6 nM. This loss of potency and biological activity could be countered by introducing a 4-isopropylthiazolyl moiety in position 2 of the quinazoline and by introducing a P1 acylsulfonamide group resulting in compound **39** with a K_i value of 0.65 nM and an EC_{50} of 18 nM. However, this lead P2 proline amide compound, **39**, exhibited low metabolic stability in human liver microsomes with 98% of the compound being metabolized after 15 min at 37 °C. A limited lead optimization effort based on our earlier SAR with a 14-membered macrocycle, introduction of an 8-methyl quinoline substituent, and introduction of a methyl group in the P1 cyclopropylsulfonamide moiety provided compound **40** where only 28.8% was metabolized after 15 min at 37 °C in the HLM assay. In addition, the biological activity was improved further by these structural changes (K_i = 0.2 nM, EC_{50} = 3.8 nM) and compound **40** exhibited good permeability in Caco-2 cells with a P_{app} value of 11.1 × 10^{-6} cm/s.

During the lead optimization work we also explored a carbamate linkage to the P2 substituent as well as quinolines with small substituents at the 2-position and pyrimidines as P2 substituents (Figure 7). When a carbamate linkage was used for the P2 substituent, the best compound, **41**, showed excellent biological activity (K_i = 0.2 nM, EC_{50} = 3.2 nM) and in vitro DMPK properties (Cl_{int} < 6 μL min^{-1} mg^{-1} and P_{app} = 15 × 10^{-6} cm/s) but also showed, at the time of this work, an unfavorable PK profile in rats with minimal plasma exposure in spite of high liver exposure. When quinolines with small substituents at the 2-position were used as in compound **42**, we could not obtain compounds with both good inhibitory activities and a favorable PK profile. When pyrimidines were examined as P2 substituents, only compounds with insufficient inhibitory activities were obtained, e.g., compound **43**. Changes in the linkage between the P1 and P3 substituents by introduction of oxygen resulted in decreased biological activity as exemplified by compound **44**, with a replicon EC_{50} of 320 nM (Figure 7).

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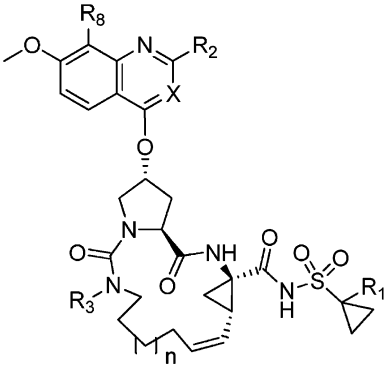
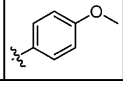
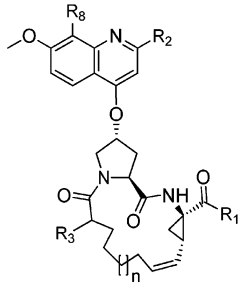
Table 5. Lead Optimization in Proline Urea Series

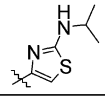
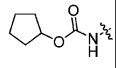


Cpd	X	n	R^1	R^2	R^3	R^8	NS3/4A K_i (nM)[a]	HUH7-Rep EC_{50} (nM)[b]	*Papp* A-B ($cm/s \times 10^{-6}$)[c]	Clint ($\mu L/min/mg$)[d]
35	C	2	Me		H	H	0.1	4.5	30	89[e]
36	N	1	H		Me	Me	0.2	11	26	17
37	N	1	H		Me	Me	0.6	3	33	8

[a]Inhibition of the full-length HCV NS3/4A protease 1a measured by the inhibition constants (K_i values). [b]Inhibition of HCV 1b replication in Huh-7-Rep cells (luciferase assay) measured by 50% effective concentration (EC_{50}). [c]A−B apparent permeability coefficient (P_{app}) measured in Caco-2 cells. [d]Intrinsic clearance in human liver microsomes. [e]Human liver microsomes stability measured by the % of metabolized product after 15 min at 37 °C in presence of 5 μM tested compound.

Table 6. Summary of Optimization of the Proline Amide Series



Cpd	n	R^1	R^2	R^3	R^8	NS3/4A K_i (nM)[a]	HUH7-Rep EC_{50} (nM)[b]	HLM stab (% metab)[c]
4	2	OH			H	0.3	1.63	2.5
38	2	OH		H	H	46	1980	-
39	2			H	H	0.65	18	98
40	1			H	CH_3	0.40	14	28.8

[a]Inhibition of the full-length HCV NS3/4A protease 1a measured by the inhibition constants (K_i values). [b]Inhibition of HCV 1b replication in Huh-7-Rep cells (luciferase assay) measured by 50% effective concentration (EC_{50}). [c]Human liver microsomes stability measured by the % of metabolized product after 15 min at 37 °C in presence of 5 μM tested compound.

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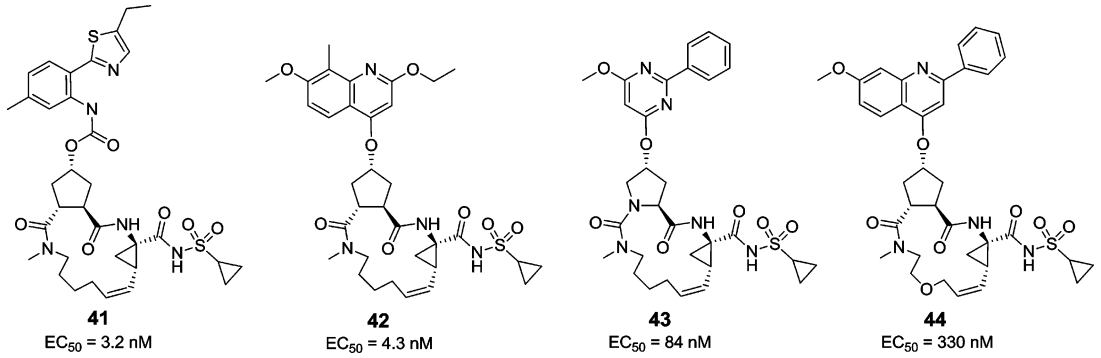

Figure 7. Example compounds from lead optimization.

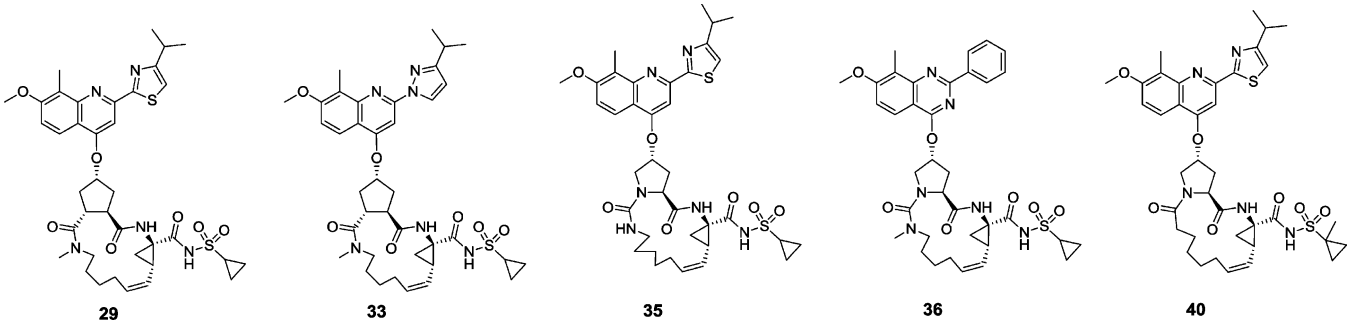

Figure 8. Promising compounds obtained from the extensive lead optimization effort.

The extensive lead optimization effort summarized above provided several promising inhibitor compounds in all of the series explored. A number of these compounds were assessed as candidates for further development, e.g., the P2 cyclopentanes (**29** and **33**), a 14-membered proline-NMe-urea (**36**), a 15-membered proline-NH-urea (**35**), and also a prolineamide (**40**) (Figure 8). All compounds possessed excellent profiles with respect to potency and activity as well as in vitro and in in vivo PK. The in vivo rat PK data for the top compounds in each of the selected series are summarized in Table 7. As can be seen from Table 7, compound **29** showed a favorable PK profile after a single iv administration of 2 mg/kg and after oral administration of 10 mg/kg in the male Sprague–Dawley rats with mean time to maximum plasma concentration of 3.0 h following oral dosing, indicating a medium rate of absorption. Low clearance (Cl = 0.505 L h^{-1} kg^{-1}) associated with a low Vd_{ss} (0.490 L/kg), high C_{max} (0.73 μM), and high AUC (2.79 μM·h) was seen with iv administration.[51] Given that viral replication of HCV occurs almost exclusively in hepatocytes, achieving high drug concentrations in the liver is considered to be critical for HCV DAAs. Data from oral administration in rats showed that compound **29** was well distributed with a high concentration observed in the liver and with a liver/plasma ratio of 32. In dogs, compound **29** had a superior PK profile compared to the other compounds evaluated, characterized by complete absorption (F = 100%) after oral administration of 6.5 mg/kg, a high C_{max} (4.72 μM) and AUC (14 986 ng·h/mL), and a long half-life ($T_{1/2}$ = 5.1 h).[54]

Table 7. Mean Plasma and Tissue Levels and Basic Pharmacokinetic Parameters after a Single Intravenous Administration of 2 mg/kg in 20% Hydroxypropyl-β-cyclodextrine or Oral Administration of 10 mg/kg in 50% PEG400 Containing 2.5% of Vitamin E-TPGS in the Male Sprague–Dawley Rat[a]

	compound				
	29	**33**	**35**	**36**	**40**
Intravenous (2 mg/kg), n = 2					
Cl (L h^{-1} kg^{-1})	0.51	2.5	0.59	0.69	0.94
Vd_{ss} (L/kg)	0.49	4.6	1.0	1.1	0.56
AUC (μM·h)	5.21	1.05	nd	8.4	nd
liver/plasma ratio (6 h)		23.5	550	38	nd
Oral (10 mg/kg), n = 2					
AUC (μM·h)	2.79	1.30	6	15	nd
C_{max} (μM)	0.73	0.31	1.7	3.4	0.93
T_{max} (h)	3.0	1.5	0.75	5	0.75
$T_{1/2}$ (h)	2.8	2.2	3	nd	2.7
F (%)	11	25	54	73	31
liver/plasma ratio (6 h)	32	44	100	32	nd

[a]AUC, area under the plasma concentration–time curve; Cl, clearance; C_{max}, maximum plasma concentration; F, percentage of dose reaching systemic circulation; T_{max}, time to maximum plasma concentration; $T_{1/2}$, plasma half-life; Vd_{ss}, volume of distribution at steady state.

■ ANALYSIS OF 3D STRUCTURES

Contemporaneous with our discovery work, an emerging body of publicly available NS3/4A 3D structural information was growing rapidly, and this information was exploited through analysis and modeling that guided aspects of our molecular design efforts. However, we did not have an internal crystallography program providing us with iterative structural guidance as we progressed through our drug discovery program. The peptidic features common to many of the emerging inhibitors of interest meant that much of the available 3D information was applicable, or at least relevant, to many or all of the ongoing drug discovery programs across the NS3/4A field, facilitating relatively reliable modeling to guide many

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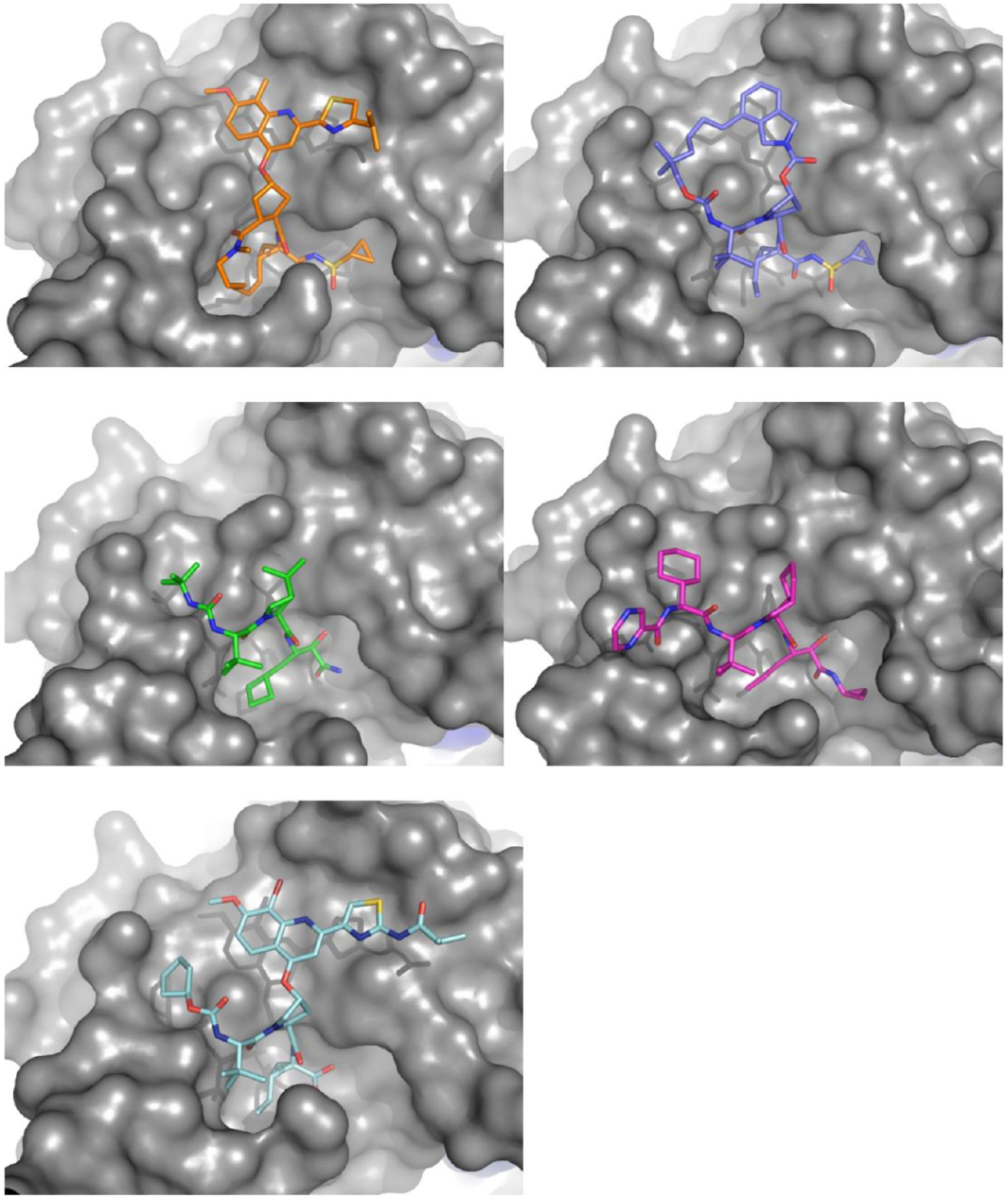

Figure 9. Inhibitor binding modes: global views of the binding modes of **29** (top left), **6** (top right), **1** (middle left), **2** (middle right), and **5** (bottom left).

aspects of inhibitor design; this statement is even more applicable today, with the PDB containing a rich body of NS3/4A data relevant to structure-based drug design.

As compound **29** progressed through early clinical development, we succeeded in determining a 2.4 Å resolution structure of the complex with the NS3/4A protease. At the time of publication in 2010, our disclosure became the first publicly available example of a noncovalent NS3/4A inhibitor complex, and also represented the first available inhibitor complex structure showing occupation of the extended S2 subsite, with Arg155 in an induced (or selected) conformation allowing face-to-face stacking between the side chain guanidine and the substituted quinoline of compound **29**.[55] This latter feature was potentially of broad interest, since this type of interaction was fundamental to many ongoing NS3/4A-based drug discovery efforts at the time. While it had been analyzed briefly in an earlier publication from the BI group,[56] the relevant complex structure had not been made available.

When bound to NS3/4A, compound **29** occupies the S3−S1′ region of the active site, forming two substrate backbone-like intermolecular hydrogen bonds with Arg155:O and Ala157:N, respectively, and one water-mediated and four direct intermolecular hydrogen bonds between the key acylsulfonamide group and the enzyme catalytic region and oxyanion hole. The P1- and P3-mimetic side chains of compound **29** are linked, forming a hydrophobic section of the 14-membered macrocycle that makes multiple hydrophobic contacts with several active site residues. The P2 cyclopentyl ring serves as a trisubstituted "core" or "scaffold", similar to the pyrrolidine ring common to the field, with the oversized substituted quinoline occupying an extended S2 subsite that is obscured in structures not involving an inhibitor with an extended P2 substituent.

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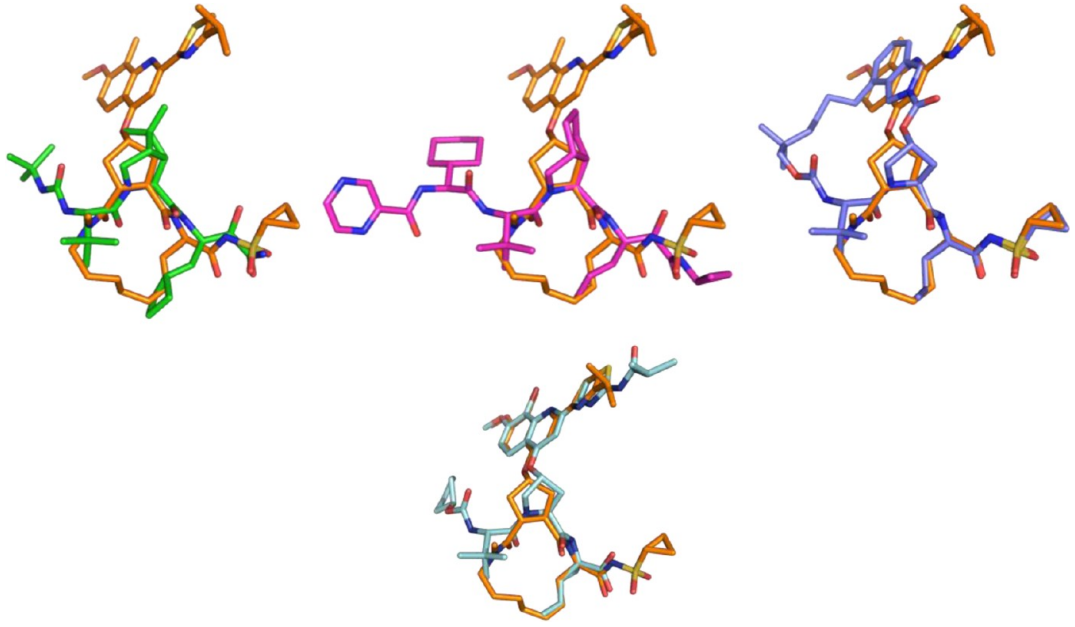

Figure 10. Comparison of bound inhibitors. Protein-based overlay of compound **29** (orange) with **1** (green), **2** (magenta), **6** (purple), and **5** (turquoise).

Since our initial description of the **29**–NS3/4A complex,[55] in which we compared the compound **29** and **1**[57] complexes, numerous additional inhibitor complex structures have become available, including those of compounds **2** and **6**[58] and compound **5**.[59] Figure 9 compares a global perspective on the binding modes for these five inhibitors, making it clear that while much is common to the complexes, each has unique features. Both compound **29** and compound **6** are macrocycles with extended P2 substituents, although the P1–P3 cyclization scheme of compound **29** leads to a markedly different molecular shape than the P2–P4 scheme of **6**. Compound **5**, while not a macrocycle, does employ an extended P2 group; this compound also has a carboxylate in place of the acylsulfonamide that is most common in current late-stage development compounds or the ketoamide present in compound **1** and compound **2** (Figures 1 and 3). The P1 and P3 side chains of bound compound **5** are identical to those of compound **6** (Figures 3, 9, and 10). Interestingly, while the cyclopropylacylsulfonamide groups of compounds **28** and **6** are positioned identically, the cyclopropyl moiety of the compound **2** ketoamide occupies a different region of the S1′ subsite (Figures 9 and 10; the cyclopropyl moiety is not present in the ketoamide group of compound **1**). As noted above, the cyclopentane ring of compound **29**, similar to the pyrrolidine rings of the other inhibitors, serves as a central trisubstituted core that in all cases is positioned in the same region of the binding site. However, the cyclopentane ring of compound **29** is able to pack closer to the enzyme surface, while the carbonyl of the adjacent *N*-methylamide group maintains the canonical substrate-like hydrogen bond with Ala157:N that is seen for all five of these inhibitors (Figure 11; see also Figures 9 and 10). This difference can be ascribed to a combination of the increased flexibility of the cyclopentane ring as well as the added conformational freedom of the fully exocyclic amide, with both of these structural features unique to compound **29**.

The new complex structures of compounds **2**, **5**, and **6** are consistent with our original analysis of the induced fit required for binding of the extended P2 group of compound **29**,[55] as

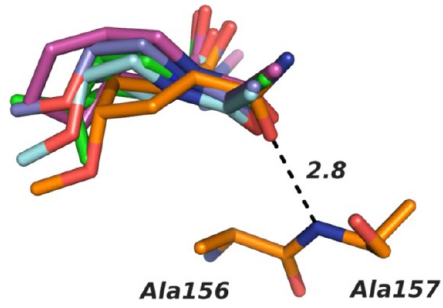

Figure 11. Core cyclopentane and pyrrolidine rings. Close-up of the core five-membered rings, with the canonical substrate-like hydrogen bond detailed for compound **29**. Protein-based overlay of compound **29** (orange) with **1** (green), **2** (magenta), **6** (purple), and **5** (turquoise).

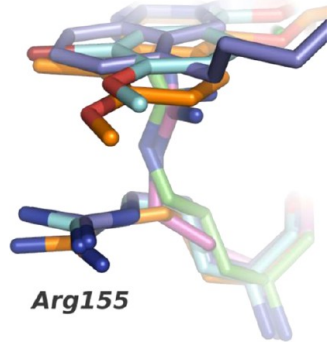

Figure 12. Conformation of Arg155 with large and small P2 inhibitors. Large P2 inhibitors such as compounds **29** and **6** bind with the guanidine of Arg155 "down", whereas apo (not shown) and small P2 inhibitors such as **2** and **1** bind with Arg155 "up". Shown is the protein-based overlay of compound **29** (orange) with **1** (green), **2** (magenta), **6** (purple), and **5** (turquoise). Arg155 from the respective complexes is colored according to the inhibitor scheme.

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well as with the earlier analysis of an analogue of compound **4**.[56] Similar to the compound **29** complex, the structures of compounds **5** and **6** show Arg155 in the "down" conformation, opening up the extended S2 subsite and stacking against the bicyclic moieties of the extended P2 groups of the bound inhibitors (Figures 9 and 12). In the compound **2** complex, as in the earlier compound **1** complex, Arg155 is seen in the "up" conformation obscuring the extended S2 pocket (Figure 12). The extended S2 pocket occupied by large P2 groups forms part of the helicase protease protein−protein interface in full-length NS3.[60] Previous work aimed specifically at understanding the role of helicase residues in protease inhibitor binding showed relatively minor (less than 10-fold difference in K_i values) effects of various helicase mutations.[61,62] Our original analysis of the **29**−NS3/4A complex was consistent with earlier structural results[55] and further confirmed the occupancy of the induced extended S2 subsite. These results and the structural work of many others in this field validate the use of the truncated NS3 protease for early stage HCV drug discovery work. A recent analysis of a complex involving a P1′−P3 macrocyclic inhibitor with an extended P2 group bound to full-length NS3 established that the bound inhibitor formed only weak contacts with the helicase domain but concluded that inhibitor modifications could lead to improved inhibitor potency through enhanced interactions with helicase residues.[63] To the best of our knowledge, a systematic application of such a strategy has not (yet) been reported.

PRECLINICAL CHARACTERIZATION

On the basis of the promising biology and PK data described above, compound **29** was selected as a candidate for clinical development and further characterized with respect to its biological, PK, and early safety profile.

One important part of the biological profiling was to evaluate the effect of compound **29** on different viral genotypes and subtypes. The effect on HCV NS3/4A proteolytic activity was determined for both genotypes 1a and 1b HCV NS3/4A proteases, with median K_i values of 0.5 and 1.4 nM, respectively.[54]

In the subgenomic genotype 1b (G1b) replicon assay, EC_{50} and EC_{90} values were 9.4 and 19 nM, respectively. To exclude a replicon clone-specific effect and to determine the potency on a genotype 1a replicon, Huh7-derived replicon cells engineered with either genotype 1a (H77) or genotype 1b (con1b) sequences were incubated with compound **29** and HCV replicon RNA levels were measured, showing EC_{50} values of 28 and 25 nM, respectively.[54] Compound **29** was also evaluated against HCV genotypes 2−6. Median FC values against genotype 2, genotype 3, and genotype 4 baseline isolates compared with genotype 1 were 25 (N = 4), 1014 (N = 2), and 0.3 (N = 8), respectively.[64]

Components of human serum have been shown to bind to, and reduce the activity of, a number of drugs. Therefore, since compound **29** binds extensively to plasma proteins (>99%), the antiviral replicon activity was tested in the presence of human plasma proteins (α-1 acid glycoprotein and human serum albumin alone and for combination). Only a minor 2.4-fold shift of the replicon EC_{50} value was observed with the addition of 50% human serum albumin.[54]

Selectivity against a panel of 20 human cellular proteases, including human leukocyte elastase, trypsin, chymotrypsin, thrombin, factor VIIa, and factor X, was also evaluated.[54] To summarize, compound **29** was highly selective, with >1000-fold selectivity for NS3/4A versus most of the evaluated human proteases. Compound **29** only exhibited submicromolar activity against cathepsin S in an enzyme assay. In a relevant cellular assay for cathepsin S activity (p10 fragment accumulation assay), compound **29** was found to be inactive up to the highest concentrations tested (10 μM), suggesting that the observed in vitro inhibition would not translate to in vivo activity.

The specificity of compound **29** was further supported by the absence of any antiviral effect toward a panel of DNA and RNA viruses, including closely related *Flaviviridae* viruses such as bovine viral diarrhea virus and yellow fever virus, up to the highest concentrations tested (10 or 100 μM).[54]

Resistance to compound **29** was characterized in HCV genotypes 1a and 1b replicon-containing cells. Ninety-six percent of **29**-selected genotype 1 replicons carried one or more amino acid substitutions at NS3 protease positions 43, 80, 155, 156, and/or 168, with substitutions at NS3 position 168 being most frequently observed (78%) (Figure 9).[65,66] The relevance of these positions for compound **29** inhibitory activity was confirmed in a transient replicon assay using replicons carrying single or multiple mutations. For replicons with mutations at position 168, the change in the EC_{50} values compared to the EC_{50} for the wild type ranged from <10-fold

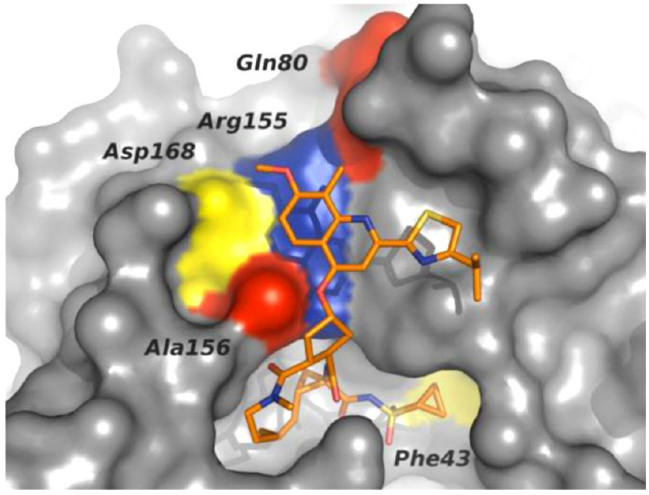
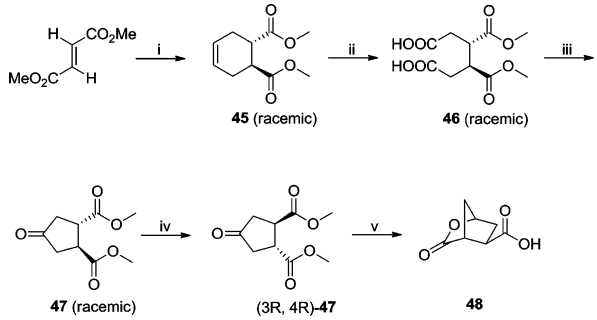


Figure 13. Resistance mutations and the compound **29** binding mode. Selected residues are highlighted on the NS3/4A binding surface.

Scheme 1[a]



[a]Reagents and conditions: (i) 3-sulfolene, hydroquinone, EtOH, 105−110 °C; (ii) $KMnO_4$, H_2O, 0 °C to room temperature; (iii) Ac_2O, NaOAc, reflux; (iv) PLE, NaOH, phosphate buffer (pH 7), room temperature; (v) (1) $NaBH_4$, MeOH, 0 °C, (2) NaOH, MeOH, room temperature, (3) Ac_2O, pyridine, room temperature.

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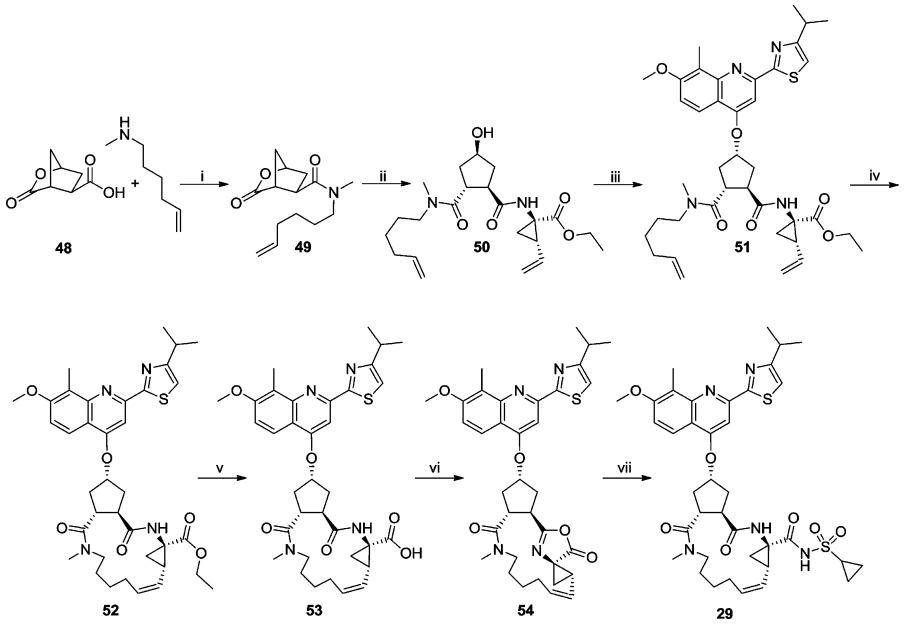
Scheme 2[a]



[a]Reagents and conditions: (i) HATU, diisopropylethylamine, DMF, 0 °C to room temperature; (ii) (a) LiOH, THF/methanol/water, 0 °C, (b) (1*R*,2*S*)-1-amino-2-vinylcyclopropanecarboxylic acid ethyl ester hydrochloride, HATU, diisopropylethylamine, DMF, 0 °C to room temperature; (iii) 4-hydroxy-2-(4-isopropylthiazol-2-yl)-7-methoxy-8-methylquinoline (**60**), PPh_3, DIAD, THF, −15 °C to room temperature; (iv) Hoveyda−Grubbs first-generation catalyst, dichloroethane, 70 °C; (v) LiOH, THF/methanol/water, room temperature; (vi) CDI, THF, reflux; (vii) cyclopropylsulfonamide, DBU, THF, 50 °C.

for those with the Asp168Gly or Asp168Asn mutation to ∼2000-fold for those with the Asp168Val or Asp168Ile mutation. Mutations at residue Gln80 had the least impact on the activity of compound **29** (<10-fold change in EC_{50} values), while greater effects were observed for replicons with some mutations at positions 43, 155, and 156. These in vitro results are consistent with the observed binding mode for compound **29** (Figure 13). Phe43, Arg155, and Ala156 make direct contact with compound **29**, and Asp168 plays a central role in positioning Arg155 in the conformation that opens up the extended S2 pocket. Gln80, while also involved in positioning Arg155 through both a backbone and side chain contact, is more peripheral to the inhibitor binding site. Compound **29** remained active against replicons with some mutations observed after in vitro or in vivo exposure to **1** or **2**, including most replicons with changes at positions 36, 54, and 55 (<2-fold change in EC_{50} values).

Importantly, replicons carrying mutations affecting the activity of compound **29** remained fully susceptible to NS5A and NS5B inhibitors and peg-IFN-α. SVR rates are greatly improved by combining a DAA drug with peg-IFN-α and ribavirin or from combinations of DAAs with different modes of actions. In vitro combination studies of compound **29** with interferon, ribavirin, NS5A or NS5B inhibitors resulted in additive or synergistic effects.[54] Additionally, combinations of compound **29** with peg-IFN, HCV NS5B polymerase, or HCV NS5A inhibitors prevented the formation of drug-resistant replicon colonies. These data indicate that the use of compound **29** in combination with other DAAs with complementary mechanisms of action could reduce or prevent the accumulation of drug-resistance-conferring mutations in vivo and thereby have a positive effect on SVR rates.

Evaluation of cytotoxic and cytostatic concentrations of compound **29** in a panel of human cell lines derived from different tissues and primary PBMC showed CC_{50} and CsC_{50} values of ≥10 μM, resulting in a selectivity index of >1000. Compound **29** was also found to be inactive in a panel of genotoxicity assays and showed an in vivo safety pharmacology (central nervous system, cardiovascular, and pulmonary functions) profile that provided a strong basis for continued development.

In summary, an extensive medicinal chemistry effort to explore novel P2 cyclopentane macrocyclic inhibitors, guided by HCV NS3 protease assays, the cellular replicon system, and structure-based design, led to the discovery of potent lead compounds. In addition, a panel of in vitro DMPK assays in conjunction with PK analysis in rats was used to identify potent inhibitors showing good oral bioavailability, typically the biggest challenge during lead optimization. Over the course of our discovery effort, approximately 1000 compounds were synthesized and evaluated by the Medivir and Janssen teams. The selection of compound **29** as a clinical candidate was based on its excellent in vitro and in vivo biological, antiviral, PK, and safety pharmacology profile.

SYNTHESIS OF COMPOUND 29

Lead Optimization Route. For the synthesis of target compounds with the P2 cyclopentane building block, a bicyclic lactone acid, **48**, was used as key starting material.[67] The synthesis started with a Diels−Alder reaction of dimethyl fumarate and 3-sulfolene, affording compound **45** in 98% yield. Oxidative cleavage of the double bond followed by cyclization and decarboxylation gave compound **47** in a total yield of 48% over two steps. Resolution of the racemic material **47** using pig liver esterase (PLE) in phosphate buffer, pH 7, containing 5% acetone provided the enatiomerically pure material *trans*-(3*R*,4*R*)-bis(methoxycarbonyl)cyclopentanone ((3*R*,4*R*)-**47**) in 36% yield.

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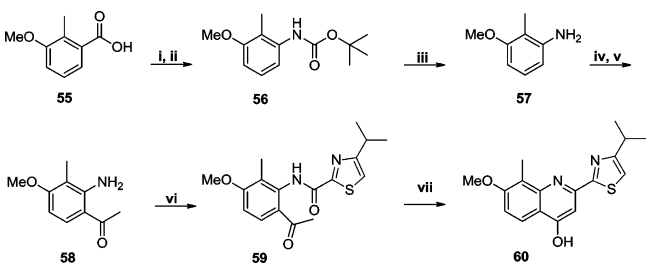
The enantiomerically pure cyclopentanone compound ((3*R*,4*R*)-**47**) was treated with sodium borohydride in methanol to reduce the ketone. Subsequent hydrolysis with sodium hydroxide in methanol followed by lactonization with acetic anhydride in pyridine provided the bicyclic lactone **48** in 42% yield over two steps (Scheme 1). The overall yield of the key building block **48** was 7% from dimethyl fumarate.

The macrocyclic target compounds were effectively prepared in a seven-step procedure from the bicyclic lactone acid **48** (Scheme 2).[51,68] The synthesis is exemplified with the structure of compound **29** in Scheme 2. In the first steps the lactone acid **48** was coupled with the P3 side chain, *N*-methylhex-5-enamine, using HATU and DIPEA in DMF providing compound **49** in 68% yield. Opening of the lactone with LiOH in THF and water followed by subsequent coupling with the P1 building block (1*R*,2*S*)-1-amino-2-vinylcyclopropane ethyl ester, using HATU and DIPEA in DMF, gave the open compound **50** in 60% yield. The P2 substituent was then introduced with inversion of configuration using Mitsunobu-like conditions using 4-hydroxy-2-(4-isopropylthiazol-2-yl)-7-methoxy-8-methylquinolinol (**60**), triphenylphosphine, and DIAD in THF, giving the open diene **51** in 56−72% yield depending on the P2 substituent. Ring closing olefin metathesis using the Hoveyda−Grubbs first-generation catalyst in refluxing dichloroethane provided the cis-macrocyclic ester **52** in satisfactory yields (60−83%). In this reaction the cis-derivatives were obtained as major products with traces of the trans isomers. For the metathesis reactions we also used the Hoveyda−Grubbs second-generation catalyst in refluxing dichloroethane or under microwave irradation, furnishing the cyclized compounds in good yields (30−80%). Subsequent hydrolysis of the ethyl ester with LiOH in THF/methanol/water at room temperature followed by the activation of the resulting acid **53** with CDI afforded the corresponding oxazolidinone, **54**, which was readily opened with cyclopropylsulfonamide in the presence of DBU to afford the final target compounds exemplified with compound **29** in 40−78% yield. NMR analysis confirmed that the stereochemistry of the different chiral centers was retained throughout the synthesis.

The 4-hydroxy-2-(4-isopropylthiazol-2-yl)-7-methoxy-8-methylquinolinol **60** and the other quinolinoles used in our work were synthesized from the corresponding 2-substituted 3-methoxybenzoic acid **55** as described in Scheme 3. Curtius rearrangement and electrophilic aromatic substitution of 3-methoxyanilines **56** with boron trichloride followed by the addition of acetonitrile and aluminum chloride afforded the corresponding ketones **58** in 40−73% yield. Finally, the desired quinolin-4-ols **60** were obtained in 58−88% yield via acylation of the anilines **58** followed by a subsequent treatment of intermediates **59** with potassium *tert*-butoxide via a tandem of ring closure and aromatization reactions.

Scheme 3[a]



[a]Reagents and conditions: (i) TEA, diphenylphosphorylazide (dppa), toluene, 100 °C; (ii) *t*-BuOH, toluene, 100 °C; (iii) TFA, CH_2Cl_2, 20 °C; (iv) BCl_3, xylene, 0 °C; (v) CH_3CN, $AlCl_3$, CH_2Cl_2, 0−70 °C; (vi) R^3COOH, $POCl_3$, pyridine, −20 to 0 °C; (vii) *t*-BuOK, *t*-BuOH, 80 °C.

Process Route. Development of the large-scale synthesis route was done along well-established principles, focusing on process safety, robustness, quality, environmental impact, and cost of goods.[69−71] *trans*-Cyclopentanone-3,4-dicarboxylic acid **61**[72] was hydrogenated in water over Raney Ni as its triethylamine salt. The resulting aqueous solution of the hydroxydiacid was subjected to cyclization to the corresponding lactone using 2-chloro-4,6-dimethoxy-1,3,5-triazine (CDMT) and NMM in water−acetone and then treated directly with cinchonidine to obtain the highly crystalline cinchonidine salt **62** with 97% enantiomeric purity in 26% yield from **61**. This procedure gave a stable (**62** can be stored for at least 3 years at room temperature without decomposition) intermediate in a high-quality robust and controlled process with a minimal number of operations and eliminated the need to use PLE for resolution (problematic for large-scale pharma manufacturing). Amide coupling of **62** with *N*-methylhexenylamine using EEDQ (rather than the hazardous HATU) in refluxing THF, with coupled recovery of cinchonidine, gave the lactone amide, which without isolation was subjected to ring-opening of the lactone function by acid-catalyzed methanolysis. The resulting secondary alcohol was isolated as a toluene solution and coupled with 4-hydroxy-2-(4-isopropylthiazol-2-yl)-7-methoxy-8-methylquinolinol (**60**) by a Mitsunobu reaction with DIAD/TPP to give the crystalline intermediate **63** in 65% yield from **62**. This new intermediate afforded an advanced purification point by crystallization for this route, which was essential to ensure process robustness of the following metathesis reaction. Hydrolysis of the ester function of **63** with LiOH in water−THF followed by direct amidation in the same reaction mixture with the P1 building block (1*R*,2*S*)-1-amino-2-vinylcyclopropane ethyl ester using EEDQ gave the key diene intermediate **51**, isolated in toluene solution (**51** and **64** are oils). Cyclization of **51** was for the early development batches performed in refluxing 1,2-dichloroethane using 2.5 mol % GH1 catalyst to the macrocycle **52**, at 0.01 M concentration. A significant improvement of the volume yield (82% yield at 0.05 M substrate concentration) of the early method was obtained by the alternative cyclization method of **64** in refluxing toluene 0.3 mol % M2 catalyst using SHD techniques. Boc protection and deprotection were done using standard methods, and compound **52** could be obtained by simple crystallization, thereby avoiding the resource- and time-consuming chromatography needed for the cleanup of the product after the early development RCM method. Hydrolysis with NaOH in refluxing water−EtOH gave **53**. Activation of the carboxylic function in **53** with EDCI at room temperature and coupling with cyclopropylsulfonamide followed by controlled crystallization gave compound **29** with a high degree of control over impurities in the desired (most stable) polymorphic form (Scheme 4).[73]

CLINICAL DEVELOPMENT

Phase I Clinical Studies. The safety, tolerability, and pharmacokinetics of compound **29** was first tested in the first-in-human study TMC435350-C101, a phase I, randomized, double-blind, placebo-controlled trial in 49 healthy volunteers, followed by an open-label, nonplacebo-controlled panel in six

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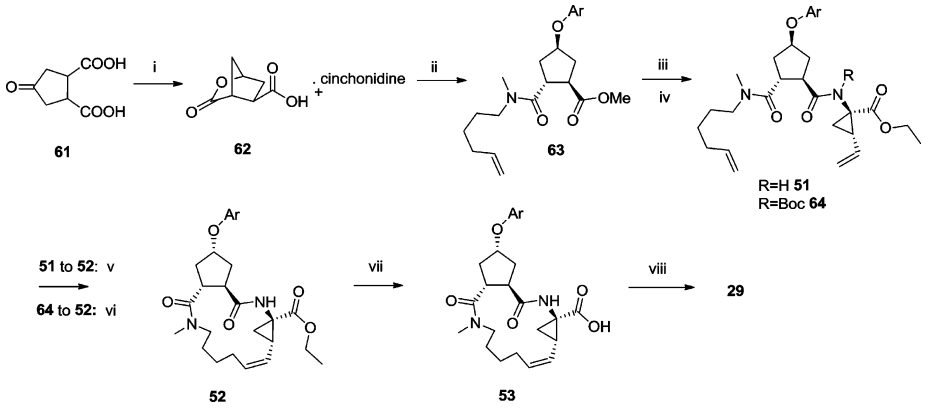
Scheme 4[a]



[a]Reagents and conditions: (i) 1) H_2/Raney Ni, H_2O, Et_3N, 20 bar, (2) CDMT, NMM, H_2O, acetone, 25 °C, (3) cinchonidine, 40 °C to room temperature, 26%; (ii) (1) *N*-Me hexenylamine, EEDQ, THF reflux, (2) MeOH, $MeSO_3H$, reflux, (3) **60**, PPh_3, DIAD, toluene, 0 °C, 65%; (iii) (1) LiOH, H_2O, THF, 25 °C, (2) (1*R*,2*S*)-1-amino-2-vinylcyclopropanecarboxylic acid ethyl ester $^1/_2H_2SO_4$, EEDQ 50 °C; (iv) Boc_2O, DMAP, THF, 20 °C, 95%; (v) GH1, DCE, reflux, 0.01 M, chromatography, 47%; (vi) (1) M2, toluene, reflux, 0.05 M, 82%, (2) H_2SO_4, toluene/EtOH, reflux; 85%; (vii) NaOH, ethanol/water, reflux, 90%; (viii) (1) ECDI, DCM, 20 °C, (2) cyclopropylsulfonamide, DBU, DCM, 20 °C, 89%.

Table 8. Primary and Key Secondary Efficacy Data for Compound 29 in the Phase IIb PILLAR Study[75,a]

rate, % (n/N)	SVR, 75 mg + PR, 12 weeks	SVR, 75 mg + PR, 24 weeks	SVR, 150 mg + PR, 12 weeks	SVR, 150 mg + PR, 24 weeks	placebo + PR
SVR W72	80.8 (63/78)	70.7 (53/75)	77.9 (60/77)*	84.8 (67/79)*	64.9 (50/77)
SVR24	82.1 (64/78)*	74.7 (56/75)	80.5 (62/77)*	86.1 (68/79)*	64.9 (50/77)

[a]PR, peginterferon + ribavirin; SVR, sustained viral response; SVR W72, SVR at w 72 after planned end of treatment; SVR24, SVR at week 24 after planned end of treatment. The asterisk (*) indicates statistically significant difference versus placebo + PR ($p < 0.05$).

genotype 1 hepatitis C patients. The study established that once daily oral compound **29** was generally safe and well tolerated and that it showed potent antiviral activity when dosed over 5 days. Plasma HCV-RNA levels dropped rapidly in all patients, with a median maximal reduction of 3.9 $\log_{10}$ IU/mL and a median of 6 days to maximal reduction.[74]

Phase IIa Clinical Studies. Phase IIa studies TMC435-C202 (NCT00812331) and OPERA-1 (NCT00561353) evaluated compound **29**, given as monotherapy for 7 days, followed by combination with peg-IFN-α-2a and ribavirin therapy (PR) for 21 or 28 days. PR was then continued for up to 48 weeks. These studies demonstrated that compound **29** was generally well tolerated, with antiviral activity against HCV genotypes 1,[75] 2, 4, 5, and 6.[64,76]

TMC435-C202 and OPERA-1 identified mild and transient increases in bilirubin in patients treated with compound **29**.[75,76] Related in vitro investigations found that compound **29** is an inhibitor of the OATP1B1 and MRP2 transport proteins, both of which are involved in the metabolism of bilirubin.[77]

Subsequent phase IIb and phase III clinical studies have evaluated the efficacy and safety of compound **29** in adult patients with HCV genotypes 1 and 4. In all of these studies, compound **29** was administered once daily (q.d.) as a single pill in combination with PR.

Phase IIb Clinical Studies. Two randomized, double-blind, placebo-controlled phase IIb dose-finding studies (PILLAR (NCT00882908[78]) and ASPIRE (NCT00980330)) assessed the efficacy and safety of compound **29** with PR.

In the PILLAR study, 386 treatment-naive patients received triple therapy with compound **29** (75 mg or 150 mg q.d.; n = 309) or placebo (n = 77) and PR for 12 weeks or 24 weeks, with PR continuing up to 24 or 48 weeks according to response guided therapy (RGT) criteria.[78] The primary end point for PILLAR was the proportion of patients with SVR (HCV RNA of <25 IU/mL undetectable) at the end of treatment (EOT) and at 72 weeks after EOT (SVR W72). The proportion of patients achieving SVR at 24 weeks after the planned EOT (SVR24) was evaluated as a secondary end point.[78] In the compound **29** treatment group, 70.7−84.8% of patients achieved SVR W72 vs 64.9% for placebo ($p < 0.05$ for simeprevir 150 mg, Table 8). SVR24 rates were also significantly higher in patients receiving compound **29** (74.7−86.1%) vs placebo (64.9%, $p < 0.05$, Table 8). Furthermore, 79.2−86.1% of **29**-treated patients were eligible to stop all treatment after 24 weeks, 85.2−95.6% of whom subsequently achieved SVR24.[78]

In the ASPIRE study, patients who had previously failed at least one prior course of PR received 48 weeks of PR with compound **29** (100 mg or 150 mg q.d.) or concurrent placebo for the first 12, 24, or 48 weeks according to randomization.[79] The primary objective of the trial was to evaluate the efficacy of compound **29** (as measured using SVR24) versus placebo in combination with PR. Secondary evaluations included efficacy according to prior treatment response, PK, safety, and tolerability of **29**-based therapy.

Phase III Studies. Three pivotal phase III, randomized, placebo-controlled studies, QUEST-1 (NCT01289782), QUEST-2 (NCT01290679), and PROMISE (NCT01281839), have evaluated the efficacy and safety of compound **29** (150 mg q.d.) in treatment-naive patients (QUEST-1 and -2) and in prior relapsers (PROMISE). In all three studies, the duration of compound **29** treatment was 12 weeks, with PR therapy continuing to week 24 or week 48 according to RGT criteria: patients with HCV RNA of <25 IU/mL (detectable or undetectable) at week 4 and undetectable HCV RNA (<25 IU/mL undetectable) at week 12 were eligible to stop all treatment at week 24. The primary efficacy end point for all three studies was SVR at week 12 (SVR12), defined as

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HCV RNA undetectable at EOT and (<25 IU/mL or HCV RNA undetectable) at 12 weeks after the planned EOT. The studies varied in the composition of PR: whereas patients in QUEST-1 and PROMISE received peg-IFN-α-2a, patients in QUEST-2 received either peg-IFN-α-2a or peg-IFN-α-2b.

The primary objective of the QUEST-1, QUEST-2, and PROMISE studies was to demonstrate superiority of compound **29** versus placebo as part of triple therapy with PR. Secondary evaluations included rates of SVR24, treatment failure and relapse, proportion of patients eligible to stop treatment at week 24 (according to RGT criteria), assessment of fatigue, safety, and tolerability.

Ongoing evaluations of compound **29** are investigating drug−drug interactions, efficacy according to baseline fibrosis stage (especially in patients with METAVIR fibrosis stage F4), efficacy in patients co-infected with HIV, and the use of compound **29** in interferon-free treatment regimens.

FUTURE DEVELOPMENTS AND PERSPECTVES

New drug applications have been submitted for compound **29** in Japan, the United States, and Europe based on four phase III Japanese studies and three phase III studies in Europe and the U.S., complemented by data from several phase II studies. The application covers compound **29** in combination with peg-IFN-α and ribavirin for the treatment of adult patients with chronic HCV genotype 1.

Compound **29** was approved in September 2013 in Japan under the trade name SOVRIAD and in November 2013 in Canada under the trade name GALEXOS and in November 2013 in the U.S. under the trade name OLYSIO for the treatment of chronic hepatitis C infection as part of an antiviral treatment regimen in combination with pegylated interferon and ribavirin in genotype 1 infected adults with compensated liver disease, including cirrhosis. To date, more than 3700 patients have been treated with compound **29** in clinical trials.

During the past few years, the HCV field has continued to develop, and the next step to further improve the treatment options for patients with HCV is the new wave of all-oral therapies, interferon-free and ribavirin-free treatment regimens, currently in phase II/III studies. Results so far indicate that high HCV cure rates can be achieved by combining two or three DAAs, with or without ribavirin. The most advanced of these therapies, targeted for the treatment of HCV genotype 1, is likely to become available in early 2015.

Compound **29** is also being evaluated in phase II interferon-free trials with and without ribavirin, in combination with the Janssen non-nucleoside inhibitor 2,19-methano-3,7:4,1-dimetheno-1*H*,11*H*-14,10,2,9,11,17-benzoxathiatetraazacyclodocosine-8,18(9*H*,15*H*)-dione 27-cyclohexyl-12,13,16,17-tetrahydro-22-methoxy-11,17-dimethyl-10,10-dioxide (TMC647055),[80] with the Gilead nucleotide inhibitor sofosbuvir,[81] with the Bristol-Myers Squibb NS5A replication complex inhibitor daclatasvir,[82] with the Idenix NS5A replication complex inhibitor samatasvir,[83] and with the Vertex nucleotide analog inhibitor VX-135 (structure not disclosed).

To summarize, patients chronically infected with HCV can now look to higher cure rates than previously achieved, which will continue to rise even for the previously most difficult-to-treat patients, offering bright prospects for the HCV patient population as a whole. There will, however, remain issues and obstacles on the road ahead, some of these being the need for diagnosis of patients with HCV (in particular those with advanced liver disease who would benefit most from treatment), reimbursement policies, and not least, the delivery of affordable treatments for use in the developing world.

We at Janssen and Medivir are proud of being part of this exciting and highly rewarding work, and we are looking forward to seeing simeprevir become one of the key components to help patients with HCV to overcome their disease.

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The authors declare the following competing financial interest(s): Åsa Rosenquist, employee of the Medivir AB; Bertil Samuelsson, employee of the Medivir AB; Per-Ola Johansson, employee of the Medivir AB; Maxwell D. Cummings, employee of the Janssen Research & Development, LLC; Oliver Lenz; employee of the Janssen Infectious Diseases BVBA; Pierre Roboisson, employee of the Janssen Infectious Diseases BVBA; Kenny Simmen, employee of the Janssen Infectious Diseases BVBA; Sandrine Vendeville; employee of the Janssen Infectious Diseases BVBA; Herman deKockMagnus Nilsson, employee ot the AstraZeneca R&D; Andras Horvath, employee of the Janssen Infectious Diseases BVBA; Ronald Kalmeijer, employee of the Janssen Infectious Diseases BVBA; Guy de la Rosa, employee of the Janssen Global Services, LLC; Maria Beumont-Mauviel, employee of the Janssen Infectious Diseases BVBA.

Biographies

Åsa Rosenquist received her Ph.D. degree in Chemistry working with nucleoside analogues for treatment of HIV in 1996 at Linköping University, Sweden. She then continued as a Postdoctoral Research Fellow with Professor J. Ellman at University of California, Berkeley, USA. When returning to Sweden, Åsa joined Astra-Zeneca in Mölndal and later Medivir AB in Huddinge. At Medivir she has been project leader for several protease and polymerase projects including the described HCV protease project leading to the development of simeprevir. Since 2008 she has held the position of Director of Medicinal and Analytical Chemistry at Medivir. During the period 1999−2010 she also held a part time position as Assistant and Associate Professor at Linköping University.

Bertil Samuelsson obtained his Ph.D. at Stockholm University, Sweden, in 1980 and then went on as a Postdoctoral Research Fellow with Professor E. J. Corey at Harvard University, Cambridge, MA, USA. After returning to Sweden, he joined Astra (later AstraZeneca) where he held the position as Executive Director, Medicinal Chemistry. In 1999 he joined Medivir as Vice President, Discovery Research and later as CSO, Head of R&D. He is currently active in Medivir as Chief Scientific Advisor. During the period 1985−2010 he also held a position as Professor (adjunct, 20% of full time) at Stockholm University and has published over 170 research articles in scientific journals.

Per-Ola Johansson graduated with a M.Sc. degree in Chemistry at Linköping University, Sweden, in 1999 and subsequently received a Ph.D. degree in 2005 at Linköping University under the guidance of Professor Ingemar Kvarnström and Professor Bertil Samuelsson. Following graduation, he was hired by Medivir AB as a Research Scientist and subsequently as a Senior Research Scientist in the Medicinal Chemistry Department, a position which he still holds. His main research interests are design and synthesis of protease and polymerase inhibitors targeting enzymes such as thrombin, HCV NS3,

HCV NS5B, cathepsins K and S, and BACE in order to discover new chemotherapies for various severe diseases.

Max Cummings is a computational chemist at Janssen R&D, working on drug discovery in various therapeutic areas. He earned his Ph.D. in Biochemistry under the supervision of Randy J. Read at the University of Alberta, Canada. Dr. Cummings worked in medicinal chemistry and enzymology at SynPhar (now NAEJA), Canada, from 1988 to 1996 and in the computational chemistry group at SmithKline Beecham, USA, from 1997 to 2000. In 2000 he joined the computational chemistry group at 3-Dimensional Pharmaceuticals, Exton, USA, which later become part of J&J PRD. In 2007 he moved to Tibotec BVBA, Belgium, and in 2011 returned to the Janssen R&D site in Spring House, PA, USA. Dr. Cummings has contributed to the discovery of several clinical candidates, including the HCV inhibitors simeprevir and TMC647055.

Oliver Lenz obtained his Ph.D. in Human Biology at the University of Marburg, Germany, working on hemorrhagic fever viruses. After his postdoctoral training at the European Molecular Biology Laboratories in Grenoble, France, doing research in HIV structural biology, he joined the HCV Discovery Group at Tibotec (now Janssen Infectious Diseases) in Belgium. Dr. Lenz has been has been involved in the discovery of simeprevir and has continued to support the clinical development of this drug. He is currently Scientific Director within Global Clinical Virology at Janssen Infectious Diseases.

Pierre Raboisson obtained his Pharmacist Degree and Master Diploma in Strasbourg, France. He did his Ph.D. thesis with Prof. Wermuth in Strasbourg before joining ArQule in 2001. He joined Janssen Pharmaceuticals at Exton in 2002 and then moved to the Belgium facility in 2004 as Head of HCV Medicinal Chemistry. He was the medicinal chemistry project leader of different HCV projects including the HCV protease project leading to simeprevir. His work in HCV was recognized twice by the Philip B. Hofmann Research Scientist Award in 2009 for the discovery of simeprevir and in 2013 for the discovery of TMC647055, a HCV polymerase inhibitor being developed in combination with simeprevir in Ph2. Pierre is currently leading the Medicinal Chemistry of Infectious Diseases and Vaccines at Janssen Pharmaceuticals.

Kenny Simmen is the Vice President, Research & Early Development & Scientific Partnership Strategy, Janssen Infectious Diseases & Vaccines. He has overseen the drug discovery and progression of compounds from the bench up to the clinical "proof-of-concept", particularly for HCV, including simeprevir (TMC435), an FDA approved protease inhibitor. He has worked on discovery and development programs in HIV, influenza, and other respiratory infections. With almost 20 years in the pharma industry, Dr. Simmen holds an B.Sc.(Hons) degree in Molecular Biology from the University of Edinburgh, U.K., and obtained his Ph.D. at the European Molecular Biology Laboratory (EMBL), Heidelberg.

Sandrine Vendeville is a Principal Scientist and medicinal chemistry project leader at Janssen Infectious Diseases in Beerse, Belgium. Over 14 years she has contributed to multiple lead optimization projects in the antiviral field, including HIV protease inhibitors active on multidrug resistant mutants, HCV protease and non-nucleoside inhibitors, and RSV fusion inhibitors. Prior to joining Janssen, she obtained a Ph.D. in Medicinal Chemistry from the University of Pharmacy of Lille (France) in 1999 and a Master in Pharmaceutical Sciences from the School of Pharmacy of UCL (Belgium, 2010). She also carried out a postdoc at University College London, U.K., with Prof. Karl Hale on the total synthesis of the natural compound bryostatin 1.

Herman de Kock studied Chemistry at Ghent University, Belgium, and received his doctoral degree from the University of Antwerp, Belgium, studying the antiviral activity of synthetic flavonoids against picornaviruses. He started his professional career at Tibotec, where he took up positions with increasing responsibility overseeing research and development activities in HIV and HCV. After moving to SEPS Pharma, where he managed the Internal Development Department, he took up a position at Galapagos where he currently heads different development programs in the field of immunoinflammatory diseases, osteoarthritis, cystic fibrosis, and antibacterial development.

Magnus Nilsson studied Chemistry at Stockholm University, Sweden, and received his doctoral degree from the Swedish University of Agricultural Science, Uppsala under the supervision of Professor Thomas Norberg. His professional career in Medicinal Chemistry at Medivir AB, Huddinge, Sweden, includes discovery of antiviral compounds in the field of HCV such as simeprevir and TMC649128 as well as cathepsin K inhibitors, such as MIV-711, for treatment of osteoarthritis. He is currently holding a position at AstraZeneca R&D, Mölndal, Sweden, in the Medicinal Chemistry Department of the Respiratory, Inflammation, and Autoimmunity (RIA) iMed.

András Horváth holds an M.S. in Pharmaceutical Chemistry from the Technical University of Budapest, Hungary, and a Ph.D. in Organic Chemistry from the University of Debrecen, Hungary. He is currently Scientific Director in the API Small Molecule Development—Process Research Department at the Janssen Pharmaceutical Companies of Johnson & Johnson (Beerse, Belgium) and is in charge of the development of several drug projects including synthesis method selection, optimization, scale-up, and transfer to manufacturing. He is actively involved in new technology initiatives in the J&J Pharma division and is member of the editorial advisory board for *Organic Process Research & Development*. Dr. Horváth has been the chemistry project leader for simeprevir (TMC435), J&J's HCV protease inhibitor launched in 2013.

Ronald Kalmeijer received his M.D. degree from the University of Leuven, Belgium, and a MBA from the Wharton Business School in Philadelphia, PA. Ronald works for Janssen as Compound Development Team Leader, leading a cross-functional team for the HCV protease inhibitor simeprevir.

Guy de la Rosa earned his medical degree from the University of Panama School of Medicine. He completed his internship and residency in Internal Medicine in Boston, MA. Subsequently, Dr. de la Rosa joined The University of Texas Medical School at Houston where he completed his fellowship in Infectious Diseases, followed by a fellowship in HIV/AIDS. In 2001, he attended the London School of Hygiene and Tropical Medicine earning his postdoctoral degree in tropical infectious diseases. Upon completion of his training, Dr. de la Rosa was appointed Clinical Assistant Professor at the University of Iowa College of Medicine. Dr. Guy de la Rosa is currently Global Medical Affairs Leader at Janssen. Dr. de la Rosa is board certified in both Internal Medicine and Infectious Diseases.

Maria Beumont-Mauviel is a Senior Medical Director who has overseen simeprevir clinical development activities, an FDA approved protease inhibitor. She has worked as medical lead of HCV projects since 2006 and was previously involved in HIV development programs. With 14 years in the pharma industry, Dr. Beumont-Mauviel is an infectious diseases trained physician with a degree from the University of Pennsylvania.

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■ ABBREVIATIONS USED

AUC, area under the plasma concentration−time curve; BI, Boehringer Ingelheim; CC_{50}, cytotoxic inhibitor concentration reducing the cell viability by 50%; CsC_{50}, cytostatic inhibitor concentration reducing the cell proliferation by 50%; C_{max}, maximum plasma concentration; DAA, direct-acting antiviral agent; DMPK, drug metabolism and pharmacokinetics; E, enzyme; EC_{50}, inhibitor concentration causing 50% inhibition of replication in a cell culture system; EC_{90}, inhibitor concentration causing 90% inhibition of replication in a cell culture system; FC, fold change; G, genotype (e.g., G1a); HCV, hepatitis C virus; I, inhibitor; iv, intravenous; K_i, dissociation constant of an enzyme−inhibitor (E−I) complex, $K_i = [E][I]/[EI]$; peg-IFN-α, pegylated interferon-α; PBMC, peripheral blood mononuclear cell; PDB, Protein Data Bank; PK, pharmacokinetic; PLE, pig liver esterase; RGT, response-guided therapy; SAR, structure−activity relationship; SHD, simulated high dilution, also called Ziegler's infinite dilution method; SVR, sustained viral response; Vd_{ss}, volume of distribution at a steady state; PR, peg-IFN-α-2a and ribavirin therapy; rt, room temperature

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