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Discovery of novel P3-oxo inhibitor of hepatitis C virus NS3/4A serine protease

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ABSTRACT

A novel series of P3 oxo-modified macrocyclic hepatitis C virus NS3/4A serine protease inhibitor was designed, synthesized and biologically evaluated. The hydroxy-substituted inhibitor **10** demonstrated high potency in genotype 1a and 1b replicon and in the panel of HCV protease mutants. Interestingly, the *t*-butyl carbonate analog **9c**, while not the most potent one in this series, exhibited a virtually flat potency profile in the panel of HCV protease mutants, thus providing opportunity for further optimization.

Hepatitis C virus (HCV) is the known causative pathogen for viral hepatitis. It has been estimated that 3% of the human population worldwide is infected with HCV.[1] If left untreated, HCV infection can lead to liver cirrhosis, hepatocellular carcinoma and subsequent liver failure.[2] Until the recent approval of boceprevir and telaprevir,[3] the standard of care (SOC) to treat HCV has involved a combination of pegylated interferon-alpha (IFN-α) and the nucleoside analog ribavirin. Effective clearance of HCV could be achieved in only less than 50% of the most prevalent genotype (gt) 1 infections, and the regimen often caused significant side-effects, such as flu-like symptoms and fatigue. Due to a heavy dosing regimen associated with the use of boceprevir and telaprevir, there is a continued need to develop more potent HCV Protease Inhibitors (PIs). Combined with improved pharmacokinetic properties, such compounds would enable lower pill burden compared to boceprevir and telaprevir, less frequent drug dosing, and as such would likely enhance patient compliance and thus improve treatment effectiveness. In addition, emergence of resistance to PIs also necessitates that the next generation PIs possess greater potency against clinically-relevant HCV protease mutants. To this end, new generation of protease inhibitors, such as ITMN-191[4] and TMC-435350[5] offer promise. Our own laboratories have also recently disclosed novel P4-urea based HCV protease inhibitor **1,** which was highly potent against the wt and mutants raised against HCV PIs.[6a,b] To further explore the SAR in the P4-P3 linker region, we substituted the P3-nitrogen with oxygen, resulting in until now unreported, novel P3-oxo series, Figure 1. Herein, we wish to report the synthesis, structure–activity relationship (SAR), mutant resistance profile and pharmacokinetic properties of key compounds discovered in our work.

The synthesis began with preparation of ethyl 2-hydroxy-8-nonenoate **2** (Scheme 1). Commercially available 7-bromo-1-heptene was treated with Mg in THF, and freshly generated Grignard reagent reacted with ethyl oxoacetate to give 2-hydroxyl ester **2**. Saponification of **2** followed by a HATU coupling of the resulting hydroxyl acid **3** with **4**[7] afforded **5**. Using *tert*-butylisocyanate, the free hydroxy group in **5** was derivatized to carbamate in **6a**, setting the stage to ring-closing metathesis. Macrocycle **7a** underwent hydrolysis with LiOH, and resulting acid **8a** was treated with CDI at elevated temperature, followed by addition of cyclopropanesulfonamide and DBU to furnish acylsulfonamide **9a**, Scheme 1. Compounds **9b–d** were prepared using the same synthetic sequence except for derivatization of the free OH in **5** with different functional groups, which yielded respective final compounds, Scheme 2. The free hydroxyl analog **10** was obtained by heating **9c** with NaOMe in MeOH. Finally, carbamate **11** was obtained by reacting **10** with CDI, Scheme 2.

Antiviral activities of the compounds were evaluated by FRET enzyme assay with NS3/4A 1a protease domain[8] and 1a and 1b HCV Huh7 replicon assays.[9] Results are summarized in Table 1.[10] Although the hydroxy **10** and imidazolyl carbamate **11** compounds were somewhat less potent than ITMN-191 in the protease enzyme assay, they were essentially equipotent to ITMN-191 in both the gt1a and gt1b replicon assays, potentially reflecting improved

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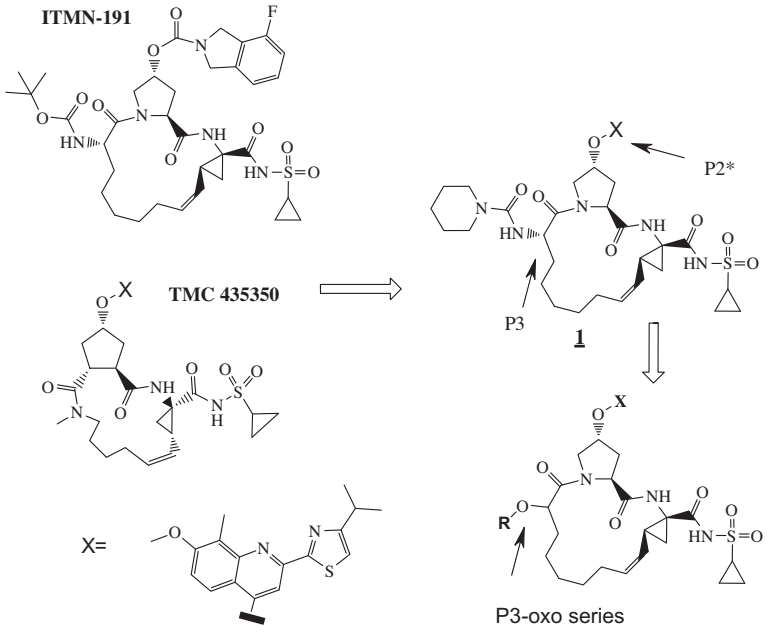
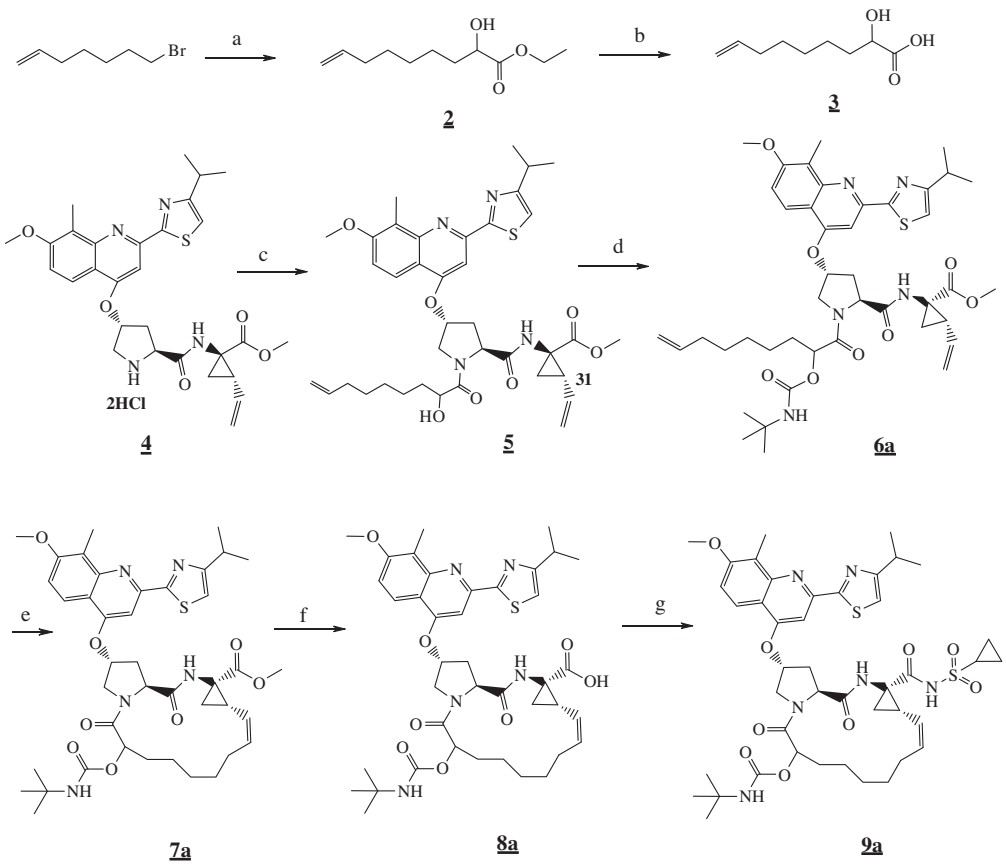
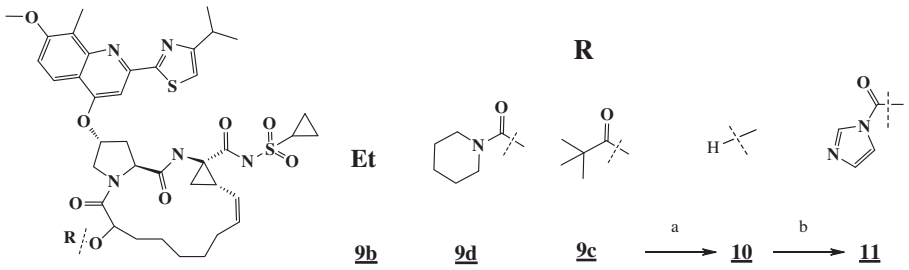


Figure 1. ITMN-191, TMC 435350, urea-based PI **1** and the new P3-oxo-series.



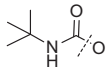
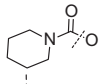
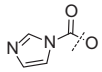
Scheme 1. Reagents and conditions: (a) Mg, I_2, THF, rt, then ethyl oxoacetate, −78 °C-rt, 4 h, 13%; (b) LiOH, THF/H_2O, rt, overnight, then 1 N HCl, quant.; (c) **3**, HATU, *i*Pr_2NEt, DMF, rt, 45%; (d) *tert*-butylisocyanate, CH_2Cl_2, 40 °C, overnight, 36%; (e) Zhan-1b, $ClCH_2CH_2Cl$, 70 °C, overnight, 50%; (f) LiOH, THF/*t*BuOH/H_2O, rt, overnight, then, 1 N HCl, 80%; (g) CDI, THF, 40 °C, 1.5 h, then cyclopropanesulfonamide, DBU, rt, overnight, 15%.

membrane transport of these compounds. Carbamate **9a** exhibited somewhat less attractive potency in the enzyme and gt 1a assays, but was equipotent to ITMN-191 in the gt1b replicon assay. Interestingly, compounds with no H-bond donor in the P4 region, **9b–9c**, were substantially less potent in both assays, Table1. It may be worth noting that the imidazole in **11** can be protonated



Scheme 2. Reagents and conditions: (a) NaOMe, MeOH, 50 °C, 2 h, 40%; (b) CDI, THF, 60 °C, 1.5 h, 11%.

Table 1
Anti-HCV activities of analogs and ITMN-191

Compound	R	NS3/4A 1a IC_{50} (nM)	Replicon EC_{50} (nM)	
			1a	1b
9a		31.0	5.0	0.8
9b		107.2	251.2	79.4
9c		195.0	50.1	39.8
9d		56.2	10.0	3.2
10		6.8	1.0	0.8
11		3.2	0.8	0.5
ITMN-191		0.4	1.0	1.1

ITMN-191 lit.[4a] NS3/4A 1a IC_{50} = 0.2 nM, replicon 1b EC_{50} = 1.8 nM.

Table 2
Genotype 1b transient replicon activity of **9a–d**, **10**, **11** and ITMN-191[a]

Compound	1b transient replicon EC_{50} (nM)						
	wt	A156S	A156T	A156V	D168A	D168V	R155K
9a	1.2	2.5	84.6	322.5	462.2	1071.5	323.6
9b	63.1	29.4	397.2	434.4	514.0	693.5	3981.0
9c	63.1	118.6	70.8	73.7	88.1	131.8	170.5
9d	7.1	9.7	90.2	657.7	2152.8	3981.1	1484.5
10	1.3	0.1	2.6	3.2	62.8	45.7	60.3
11	0.5	0.1	2.8	3.9	70.0	151.0	47.9
ITMN-191	0.1	1.6	6.8	8.3	48.5	24.5	80.8

[a] Literature data for ITMN-191 EC_{50} (nM): wt = 1.5, A156T = 4, D168V = 12, R155K = 180 (Seiwert et al., poster #938, EASL 2009, Copenhagen, April 22–26, 2009).

under physiological condition, resulting in potential H-bond donating capability of this moiety.

Compounds were also evaluated in the wild type (wt) and mutant genotype 1b transient replicon assays,[11] Table 2. Compounds **10** and **11** were essentially equipotent to danoprevir (ITMN-191). Carbamates **9a**, **9b**, **9d** and to a lesser degree **9c** were less potent, particularly against mutants A156V, D168A and D169V.

The ratio of EC_{50}(mutant)/EC_{50}(wt) may be relevant in compound dose consideration to provide sufficient coverage for all relevant mutants. Danoprevir (ITMN-191), **9a**, **9b**, **9d, 10** and **11** exhibited relatively high mutant/wt ratios for some mutants, especially for D168A, D168V and R155K, Table 2. On the other hand, the *t*-butyl-carbonate compound **9c** had a near-flat profile in this assay. Compound **9c** had only modest potency in the wt enzyme and stable gt1a and gt1b replicon assays, consistent with its moderate potency in the wt transient replicon.

Compounds in this series had low permeability in the parallel artificial membrane permeability assay (PAMPA), as exemplified by **11** (Papp = 12 nM/s at pH 7.05), and perhaps for that reason exhibited only low to moderate bioavailability in the rat PK model (F = 12%). Compound **11** was also characterized by high clearance in rat PK model (Cl = 53.7 mL/min/kg).

In summary, we described novel P3-oxo inhibitors of HCV NS3/4A serine protease. Novel hydroxy **10** and carbonyl-imidazole **11** compounds were found to be essentially equipotent to danoprevir (ITMN-191) in the wt gt1a and gt 1b replicon assays (Table 1) and had a noticeably improved viral mutant profiles EC_{50}(mutant)/EC_{50}(wt), Table 2. The *tert*-butyl-carbonate **9c**, which was less potent in both stable replicon assays (Table 1), has a near-flat potency profile in the transient mutant assay (Table 2), and as such is an attractive lead molecule for further potency optimization towards HCV protease inhibitors with high potency across all HCV protease mutants. Further PK improvement will also be necessary for this class to be further developable. This can potentially be accomplished by reducing the polar surface area PSA in this class (PSA of **11** is 201), which should improve compound permeability and bioavailability. The biological properties of compounds in this class, especially of **9c**, **10** and **11** warrant further optimization. These efforts will be described in due course.

References and notes

1. White, P. W.; Llinas-Brunet, M.; Bos, M. *Prog. Med. Chem.* **2006**, *44*, 65.
2. (a) Cohen, J. *Science* **1999**, *285*, 26; (b) Alter, M. J.; Kruszon-Mran, D.; Nainan, O. V.; McQuillan, G. M.; Gao, F.; Moyer, L. A.; Kaslow, R. A.; Margolis, H. S. *N. Engl. J. Med.* **1999**, *341*, 566.
3. (a) Neumann, A. U.; Lam, N. P.; Dahari, H.; Gretch, D. R.; Wiley, T. E.; Layden, T. J.; Perelson, A. S. *Science* **1998**, *282*, 103; (b) Rosen, H. R.; Gretch, D. R. *Mol. Med. Today* **1999**, *5*, 393; (c) Di Bisceglie, A. M.; Mchutchison, J.; Rice, C. M. *Hepatology* **2002**, *35*, 224.
4. (a) Seiwert, S. D.; Andrews, S. W.; Jiang, Y.; Serebryany, V.; Tan, H.; Kossen, K.; Rajagopalan, P. T.; Misialek, S.; Stevens, S. K.; Stoycheva, A.; Hong, J.; Lim, S. R.; Qin, X.; Rieger, R.; Condroski, K. R.; Zhang, H.; Do, M. G.; Lemieux, C.; Hingorani, G. P.; Hartley, D. P.; Josey, J. A.; Pan, L.; Beigelman, L.; Blatt, L. M. *Antimicrob. Agents Chemother.* **2008**, *52*, 4432; (b) WO 2007015824.
5. Raboisson, P.; de Kock, H.; Rosenquist, A.; Nilsson, M.; Salvador-Oden, L.; Lin, T. I.; Roue, N.; Ivanov, V.; Wähling, H.; Wickström, K.; Hamelink, E.; Edlund, M; Vrang, L.; Vendeville, S.; Van de Vreken, W.; McGowan, D.; Tahri, A.; Hu, L.; Boutton, C.; Lenz, O.; Delouvroy, F.; Pille, G.; Surleraux, D., Wigerinck, P.; Samuelsson, B.; Simmen, K. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 4853.
6. (a) Cooper, J. P.; Duan, M.; Grimes, R. M.; Kazmierski, W. M.; Tallant, M. D. WO 2010088394.; (b) Kazmierski, W. M.; Hamatake, R.; Duan, M.; Wright, L. L.; Smith, G. K.; Jarvest, R. L.; Ji, J.-J.; Cooper, J. P.; Tallant, M. D.; Crosby, R. M.; Creech, K.; Wang, A.; Li, X.; Zhang, S.; Liu, Y.; Zhang, Y.-K.; Liu, L.; Ding, C. Z.; Zhou, Y.; Plattner, J. J.; Baker, S. J.; Bu, W.; Liu, L. J. Med. Chem., accepted for publication. doi:10.1021/jm201278q.
7. See patent for preparation: Cooper, J. P.; Duan, M.; Grimes, R. M.; Kazmierski, W. M.; Tallant, M. D. WO 2010088394.
8. (a) *Compounds were assayed in the fluorescence enzymatic assay using HCV NS3/4A 1a protease domain*: Conditions: 0.75 nM enzyme (1a domain), 2 μM NS4A, 0.5 μM peptide substrate (Ac-DE-Dap(QXL520)-EE-Abu-ψ-[COO]-AS-C(5-FAMsp)-NH_2 is the FRET substrate purchased from Anaspec Inc., San Jose, CA) in 50 mM HEPES, 20% sucrose, 5 mM DTT, and 0.05% NP-40. Wavelengths of 490 ex and 520 em were used on a Molecular Devices plate reader to measure

initial rates.; (b) *Compounds were assayed in the fluorescence enzymatic assay using HCV NS3/4A 1a protease domain*: Conditions: 1 nM enzyme (1a domain), 2 μM NS4A, 5 μM peptide substrate (Ac-DE-D(EDANS)-EE-Abu-ψ-[COO]-AS-K(DABCYL)-NH_2 is the FRET substrate purchased from Anaspec Inc., San Jose, CA) in 50 mM HEPES, 20% sucrose, 5 mM DTT, and 0.05% NP-40. Wavelengths of 355 ex and 495 em were used on a Molecular Devices plate reader to measure initial rates.

9. Lohmann, V.; Korner, F.; Koch, J.-O.; Herian, U.; Theilman, L.; Batenschlager, R. *Science* **1999**, *285*, 110.
10. The P3-oxo stereochemistry results in 1:1 ratio of diastereomers.
11. Add wild type 1b RNA or mutation RNA into 400uL of ET cured cells, then transfer into a cuvette with a gap width of 0.4 cm and electroporate immediately using a Gene Pulser system (Bio-Rad), settings: 270 V, 950 μF with a capacitance extender. Transfer the electroporated cells from the cuvette into a 50 mL Corning tube containing appropriate amount of medium; then, transfer 190 μL into 96-well assay plates from column 1 to column 11. To column 12 add medium only. Finally, add 10 μL compounds to each well from column 1 to 10. Place the plates in a 37 °C humidified incubator at atmosphere of 5% CO_2 in air. After 72 h, aspirate out the media and add 25 μL of Bright-Glo Luciferase substrate (Promega) to each well. Read luminescence on Perkin Elmer Envision Multilabel Reader. Generate IC_{50} values, by plotting % of inhibition against the compound concentrations.