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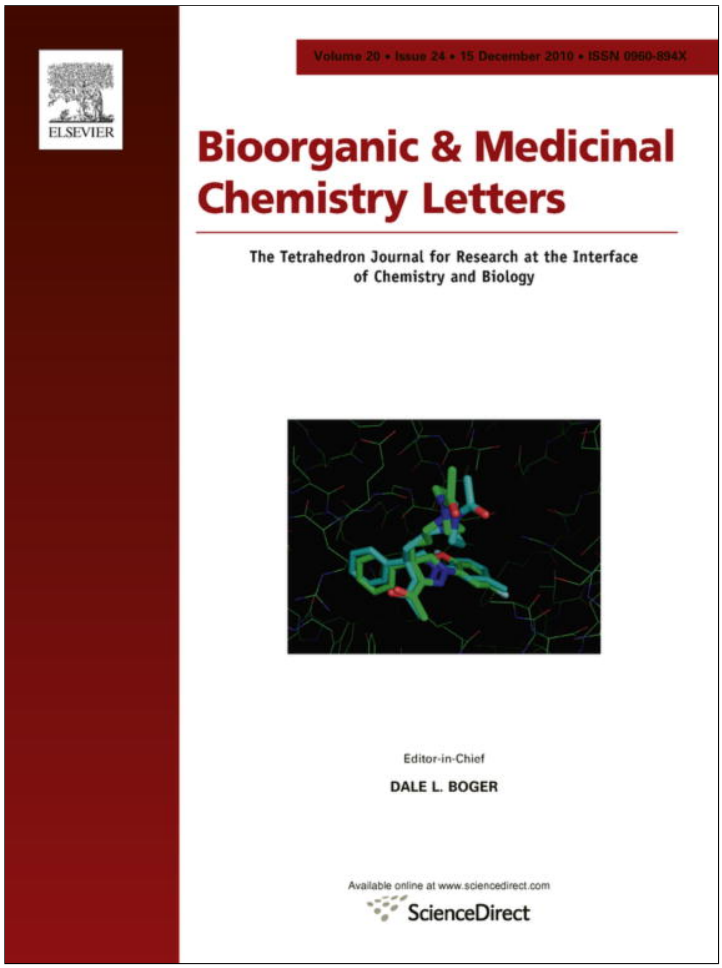

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Synthesis and biological evaluations of P4-benzoxaborole-substituted macrocyclic inhibitors of HCV NS3 protease

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ABSTRACT

We disclose here a series of P4-benzoxaborole-substituted macrocyclic HCV protease inhibitors. These inhibitors are potent against HCV NS3 protease, their anti-HCV replicon potencies are largely impacted by substitutions on benzoxaborole ring system and P2* groups. P2* 2-thiazole-isoquinoline provides best replicon potency. The in vitro SAR studies and in vivo PK evaluations of selected compounds are described herein.

Infection with Hepatitis C Virus (HCV) is a major cause of human liver disease throughout the world, affecting over 200 million individuals. In the US alone, an estimated 4.5 million Americans are chronically infected. HCV infection is responsible for 40–60% of all chronic liver disease cases and 30% of all liver transplants. The current standard of care for HCV infection is a combination of injectable pegylated interferon-α (PEG IFN-α) plus oral ribavirin, which is effective in only about 50% of genotype-1 patients achieving sustained viral response.[1] This protocol has been associated with side effects including neuropsychiatric events, flu-like symptoms and hematological toxicities.[2] Therefore, there has been tremendous interest in the development of more effective therapeutics in treating HCV infection. One of the validated targets is HCV NS3/4A serine protease.[3]

Extensive efforts in the discovery of HCV NS3 protease inhibitors have resulted in a number of drug candidates at various stages of clinical development.[4] The two most advanced compounds, VX-950 (telaprevir) and SCH-503034 (boceprevir), provided an early proof of concept in suppressing the virus and are currently undergoing Phase III clinical trials.[5] Newer protease inhibitors with improved potency, different mode of binding interaction with the protease enzyme and pharmacokinetic properties have emerged. These inhibitors, such as danoprevir (ITMN-191),[6] TMC-435,[7] BMS-791325[8] (Fig. 1), and vaniprevir (MK-7009)[9] are in clinical development representing structural diversity set of promising HCV protease inhibitors.

This initial excitement about the potential novel HCV treatments has been somewhat dampened by a quick emergence of enzyme resistance to these agents.[10] The opportunity for other chemical classes of HCV protease inhibitors with better resistance profile still exists. In our search for novel HCV protease inhibitors, we considered benzoxaborole as the P4 moiety, the fourth amino-acid residue from carboxyl terminus. Benzoxaboroles (core structure shown in compound **2** in Scheme 1) are a chemical class of organoboron compounds that have excellent physicochemical and biological properties.[11] They are metabolically stable, and exhibit good water solubility. Docking studies of compound **4** to the enzyme active site suggest that benzoxaborole moiety with suitable orientation and linkage can potentially interact with active site polar aminoacid residues: Ser122, Arg123, Arg155 and

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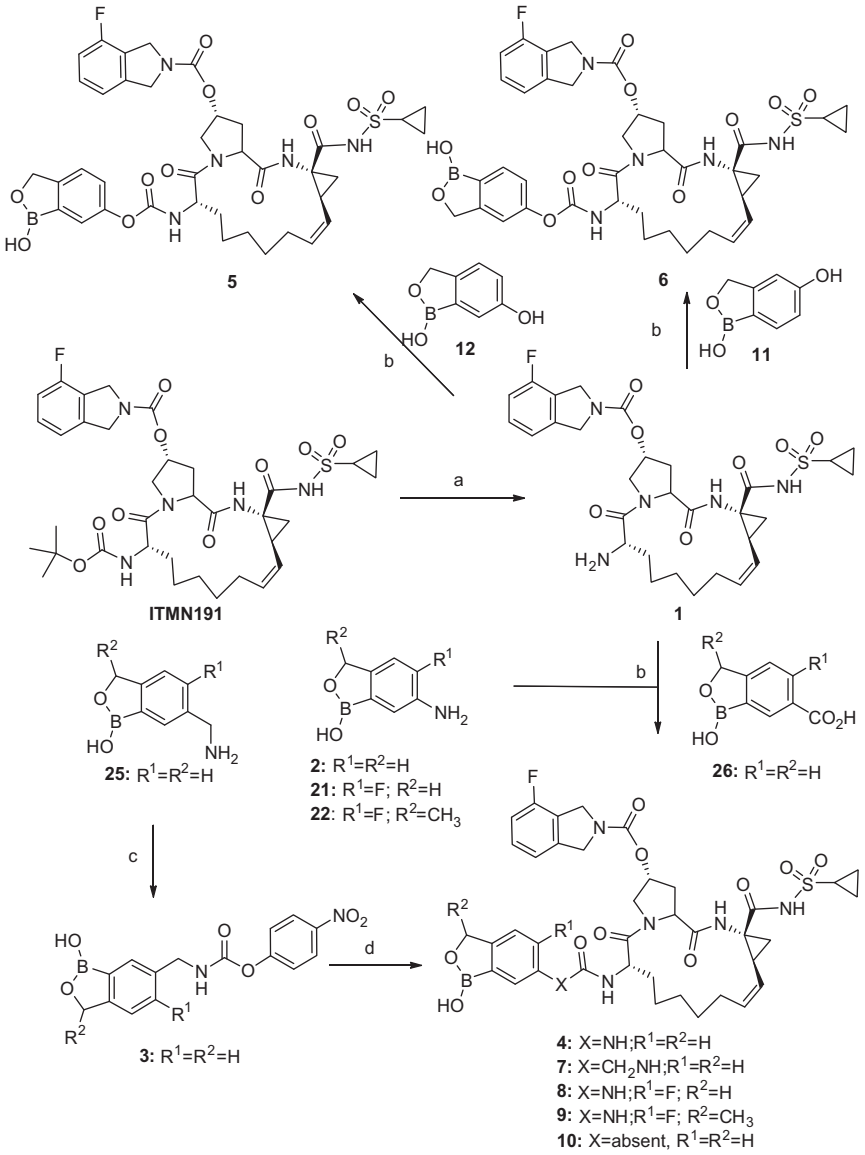
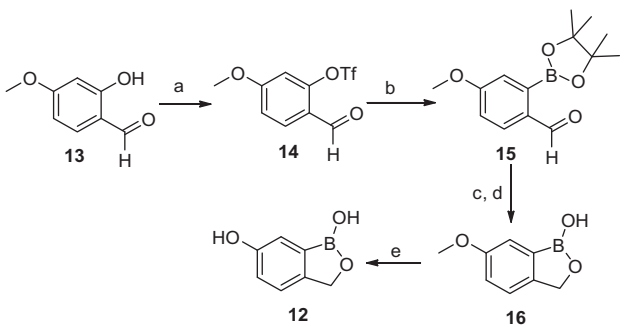


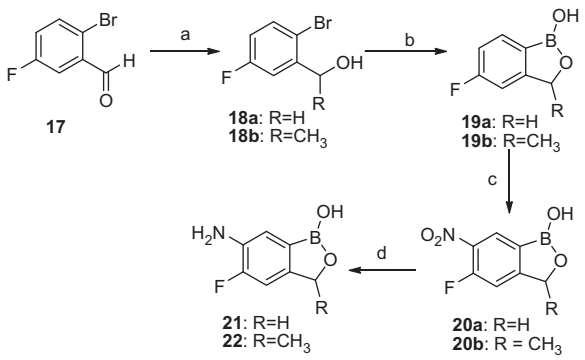
Scheme 1. General syntheses of benzoxaborole-substituted macrocyclic HCV inhibitors. Reagents and conditions: (a) TFA, DCM, rt basic work-up, 83%; (b) triphosgene, TEA, THF, −40 °C; then **2**, **12**, **21** and **22**, 15–30%; for **10**, HATU, DIEA, **26**, DMF, rt 50%; (c) *p*-O_2N-Ph-OCOCl, **25**, ACN, rt 90%; (d) amine **1**, ACN, 60 °C; 50%.

Asp168 of HCV NS3/4A serine protease. These additional interactions may provide better resistance profile than the known inhibitors that do not have. Syntheses of the target compound **4** and its analogs as well as their biological evaluations are reported herein.

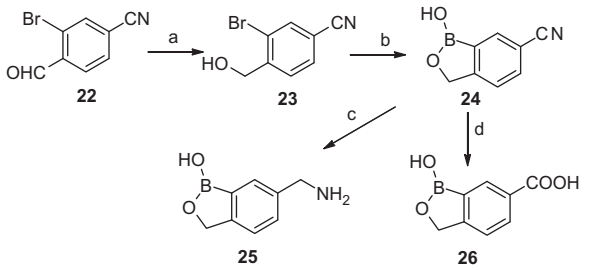


Scheme 2. Preparation of 6-hydroxybenzoxaborole. Reagents and conditions: (a) Tf_2O, pyridine, −78 °C to rt; (b) $(pinB)_2$, $PdCl_2(dppf)$, KOAc, dioxane, 80 °C; (c) $NaBH_4$, MeOH, rt; (d) HCl; (e) BBr_3, CH_2Cl_2, −78 °C to rt.

P4-Benzoxaborole-substituted macrocyclic compounds based on ITMN-191 scaffold were prepared using a general scheme as



Scheme 3. Preparation of substituted 6-aminobenzoxaboroles. Reagents and conditions: (a) for **18a**: $NaBH_4$, MeOH; for **18b**: MeMgBr, THF, 0 °C, ~93%; (b) $B(iPrO)_3$, *n*-BuLi, THF, −70 °C, 40–47%; (c) fuming HNO_3, −45 °C, 80–90%; (d) Raney Ni, hydrazine monohydrate, MeOH, rt, 70–80%.

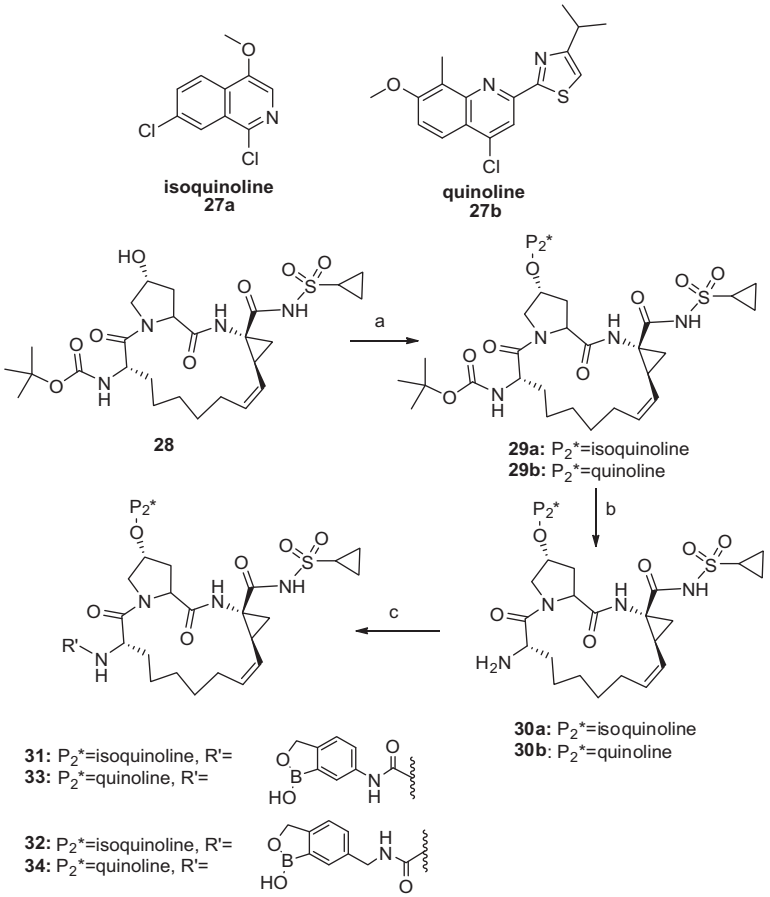


Scheme 4. Preparation of amine 25 and acid 26. Reagents and conditions: (a) $NaBH_4$, MeOH, 0 °C, 99%; (b) $B(iPrO)_3$, BuLi, THF, −78 °C, 50%; (c) LAH, THF, rt, 46%; (d) concn HCl, reflux, 85%

shown in Scheme 1. ITMN-191 was prepared by following a patent procedure.[12] The P4 BOC group was removed by treatment with TFA in dichloromethane to give amine **1**. The coupling of the amine to 6-aminobenzoxaboroles (**2**, **21** and **22**) providing urea compounds (**4**, **8** and **9**) was accomplished via an isocyanide intermediate, which was prepared by treatment of compound **1** with triphosgene in the presence of triethylamine at low temperature. The intermediate was not isolated, 6-aminobenzoxaboroles were added in situ. This reaction sequence provided urea compounds in modest 30% isolated yield.[13] The carbamate-linked P4-benzoxaborole-substituted macrocyclic compounds (**5** and **6**) were generated going through the same isocyanate intermediate reacting with hydroxybenzoxaboroles instead (**11** and **12**). The synthesis of urea compound **7** involves conversion of 6-aminomethylbenzoxaborole **25** to its *p*-nitrophenyl carbamate **3**, followed by reaction of it with macrocyclic amine **1** in somewhat higher (50%) yield. The amide **10** was prepared in 50% by coupling of 6-benzoxaborole carboxylic acid **26** with macrocyclic amine **1** in the presence of HATU, DIEA in DMF at room temperature.

The functionalized benzoxaborole compounds necessary for the preparation of benzoxaborole-substituted macrocyclic HCV inhibitors were prepared as follows. The 6-aminobenzoxaborole **2** (R^1=NH_2, R=R^2=H) was prepared according to a published procedure.[14] Both 5- and 6-hydroxybenzoxaboroles (**11** and **12**) were prepared in the same fashion starting from separate starting material 5 or 4-methoxysalicylaldehyde, as illustrated for 6-hydroxybenzoxaborole in Scheme 2. 4-Methoxysalicylaldehyde was converted to its triflate **14** by treatment with triflic anhydride in the presence of pyridine at low temperature. Boronation was accomplished by pinacol diborate in the presence of a palladium catalyst to provide boronobenzaldehyde **15**. Reduction with sodium borohydride and acidic work up gave 6-methoxybenzoxaborole **16**. Cleavage of the methoxy group by BBr_3 provided 6-hydroxybenzoxaborole **12**.

6-Amino-5-fluorobenzoxaboroles **21** and **22** were prepared as shown in Scheme 3 from 2-bromo-5-fluorobenzaldehyde **17**. Reduction with sodium borohydride or addition of methyl magnesium bromide to **17** produced benzyl alcohol **18a** or **18b** in high yield. Halogen-metal exchange reaction and deprotonation were accomplished using two equivalents of *n*-butyllithium, the resulting dianion was reacted with triisopropyl borate and acidic work-up providing benzoxaboroles **19a** and **19b**. Nitration of both compounds with fuming nitric acid produced 6-nitro derivatives



Scheme 5. Preparation of isoquinoline- and quinoline-based HCV inhibitors. Reagents and conditions: (a) KOtBu, DMF, rt; then, **27a** or **27b**, 60–70%; (b) HCl(g), dioxane, rt, ~85%; (e) triphosgene, Et_3N, CH_2Cl_2, −40 °C, then, compound **2** or **25**, 20–30%.

20a and **20b** with high regio-selectivity and yield. Raney nickel reduction of the two nitro compounds provided 6-amino-7-fluorobenzoxaboroles **21** and **22**.

6-Aminomethylbenzoxaboroles **25** and benzoxaborole-6-carboxylic acid **26**[15] were prepared as shown in Scheme 4. 2-Bromo-4-cyanobenzaldehyde was converted to 6-cyanobenzoxaborole **24** by following the same sequence of reactions as shown in Scheme 3 for preparation of benzoxoborole **19a** and **19b**. The aminomethylbenzoxaborole **25** was obtained from reduction of compound **24** using lithium aluminium hydride, while compound **26** was obtained from hydrolysis of compound **24** with concn HCl.

Inhibitors with P2* groups other than isoindoline (ITMN-191 scaffold) were prepared as described in Scheme 5 through heteroaryl displacement reactions. Prerequisite chloro-isoquinoline **27a** and chloro-quinoline **27b** were prepared by following the published procedures.[16] The other coupling partner hydroxymacrocycle **28** was also prepared according to a published reference procedure.[17] Heteroaryl displacement reaction of compound **28** with either **27a** or **27b** was achieved in good yield by treatment of **28** with slightly more than two equivalents of potassium *tert*-butoxide in DMF at room temperature. Both products **29a** and **29b** were treated with HCl in dioxane to produce aminomacrocycles **30a** and **30b**. Linking of either **30a** or **30b** with both 6-aminobenzoxaborole **2** and 6-aminomethylbenzoxaborole **25** was accomplished by the same reaction sequence as described in Scheme 1. Compounds **31** and **32** are products of amine **30a** coupled to 6-aminobenzoxaborole **2** and aminomethylbezoxaborole **25**; while **33** and **34** are products of amines **30b** coupled with the same benzoxaboroles.

Biological evaluations of the compounds were done both using HCV protease and replicon assays. Results are shown in Table 1. The protease inhibitory IC_{50}'s were determined using a FRET assay with HCV NS3/4A 1a protease domain.[18] The replicon EC_{50}'s were determined using a replicon luciferase cell-based assay.[19] The initially designed compound **4** proved equipotent than danoprevir (ITMN-191) with enzymatic activity IC_{50} of 0.4 nM. While **4** was potent in the genotype 1b assay (EC_{50} 3.7 nM), the P4 structural

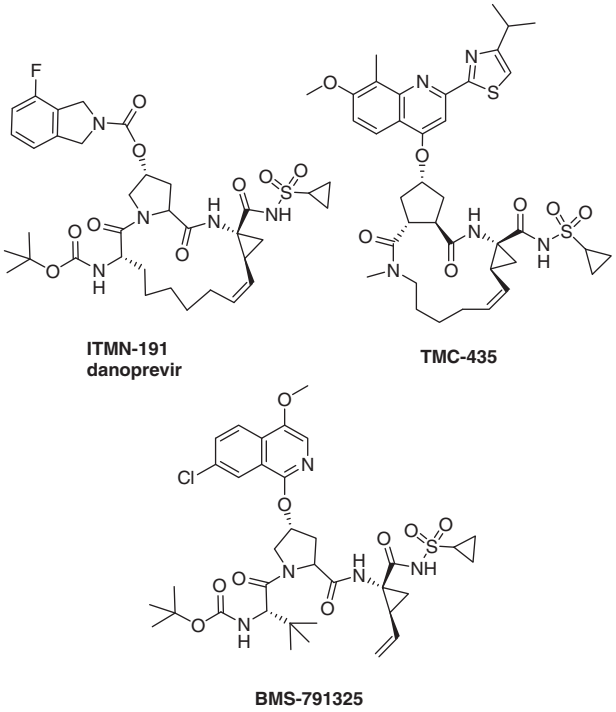
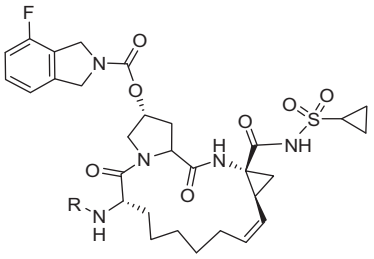
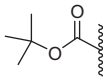
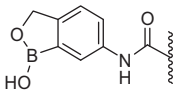
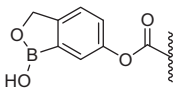
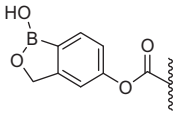
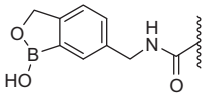
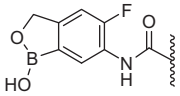
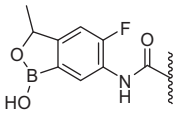
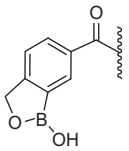


Figure 1. Representative HCV protease inhibitors in clinical development.

Table 1
HCV protease NS3/4 1a IC_{50} and replicon EC_{50} values for ITMN191-based P4-benzoxaborole-substituted macrocyclic inhibitors as compared to ITMN191

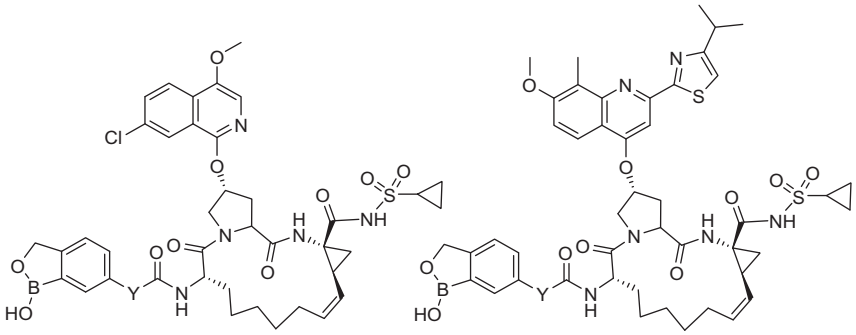


Compds	R	NS3/4 IC_{50} (nM)[a]	HCV replicon EC_{50} (nM)	
			1a	1b
ITMN191		0.4	1.0	1.1
4		0.4	159	3.7
5		1.6	661	15.0
6		2.4	3548	172
7		0.4	160	0.9
8		<0.2	35	1.5
9		0.4	20	1.2
10		1.0	201	3.2

[a] FRET assay with HCV NS3 1a protease domain. Values are means of duplicate or triplicate experiments; errors are usually within ±10%.

change turned out to be detrimental to compound's potency against genotype 1a replicon activity (EC_{50} 159 nM). Encouraged by this result, we set out to further explore the linker SAR. Changing the urea linker to its corresponding carbamate (compound **5**), the potency dropped off in both enzymatic or replicon assays. Compound **6** is a regioisomer of compound **5**, its potency was even worse. However, homologation of the urea linker (in compound **7**) restored enzyme activity and, compared to lead compound **4**, improved replicon EC_{50} by 4 fold for genotype 1b, although similar for genotype 1a. The amide linker in compound **10** provided similar potency or slightly inferior to compound **4**. Next, we explored substitutions on benzoxaborole ring system by improving lipophilicity, reducing polar surface area (PSA) of the ring while maintaining the oxaborole functionality, aimed to improve replicon potency for genotype 1a. We kept the urea linker for this exploration because it showed best potency and ease of synthesis. Compound **8**

Table 2
HCV protease NS3/4 1a IC_{50} and replicon EC_{50} values for isoquinoline and quinoline P2* inhibitors as compared to TMC435



31: Y=NH
32: Y=CH_2NH

33: Y=NH
34: Y=CH_2NH

Compds	NS3/4 IC_{50} (nM)	HCV replicon EC_{50} (nM)	
		1a	1b
TMC435	10.0	2.3	1.4
31	0.6	60.3	3.9
32	0.4	50.1	3.9
33	5.6	12.5	1.2
34	4.1	4.2	0.5

is a fluoro derivative of compound **4** with excellent enzyme potency and improvement in both genotype 1a and 1b replicon activity by 2 to 3-fold. The compound **9** with both methyl and fluoro substitutions on benzoxaborole ring improved further cellular potency in genotype 1a. Preliminary SAR in this series strongly suggests that it should be possible to further optimize cellular potency by further modifications in the benzoxaborole ring.

We decided to explore the impact of P2* groups on the anti-HCV potency of P4-benzoxoborole substituted macrocyclic compounds. We picked representative isoquinoline and quinolone groups from other classes of HCV protease inhibitors, namely BMS-791325 and TMC-435. The in vitro profiles of these compounds are summarized in Table 2. Interestingly, compounds **31** and **32** with isoquinoline P2* have similar enzyme and replicon potency profile to isoindoline P2*. The more bulky, elaborated quinoline P2*-containing compounds **33** and **34** showed better replicon potency, although enzymatic potency fell off. We believe that some of the differences between the enzyme and replicon potency may originate from factors that differentiate both assays, such as different transcellular transport.

Selected compounds were evaluated in rats for their pharmacokinetic parameters, blood samples from both jugular and portal vein were drawn and drug concentrations were measured. The results are shown in Table 3. The oral absorption was calculated from the portal vein drug concentrations and oral bioavailability was calculated from jugular vein drug concentrations as compared to the drug concentrations after IV administration. ITMN-191 exhibits calculated 17.4% absorption and 20% oral bioavailability in rats. However, benzoxaborole-substituted macrocylic inhibitors **4**, **7** and **34** displayed minimal to undetectable level of absorption and oral bioavailability. We noticed good water solubility of these inhibitors compared to ITMN191 when the PK samples were made, however, their permeability and absorption are almost certainly limited by their high molecular weight and high polar surface area (PSA).

Table 3
Physicochemical properties and oral PK parameters of selected benzoxaborole-macrocyclic inhibitors

Compds	MW	*c* log *P*	PSA	Oral PK in rats[a]	
				% Absorption	% BA
ITMN191	732	5.6	181	17.4	20
4	807	4.5	213	0.9	0.5
7	821	4.7	213	0.6	0.7
34	939	7.7	226	<LOD	<LOD

[a] % Absorption was calculated from portal vein drug concentration, while % BA was from jugular vein.

In summary, we have designed and synthesized a series of P4-benzoxaborole-substituted macrocyclic HCV protease inhibitors. We suggest that the benzoxaborole moiety can be a useful moiety towards developing compounds retaining potency against resistant enzymes.[20a] These compounds exhibited potent inhibitory activity against HCV NS3/4 protease. Their cellular replicon potencies were impacted by substitutions on the benzoxaborole ring system and P2* groups, but even a limited exploration with compounds **8** and **9** suggest that further potency optimization should be possible. These compounds had high polar surface area (PSA), which may initially limit their oral absorption and bioavailability. However, our results with a related series suggest that relatively simple structural changes can bring these molecules back into more drug-like parameters.[20b]

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13. *Description of synthesis of a representative compound* **4**: A solution of compound **1** (65 mg, 0.103 mmol) and Et_3N (26 mg, 0.258 mmol) in 6 mL of THF was cooled to −40 to −50 °C. Triphosgene (11.3 mg, 0.038 mmol) was added. The reaction mixture was stirred at −40 °C for 1 h and 6-aminobenzo[*c*]-[1,2]oxaborol-1(3*H*)-ol (11.3 mg, 0.103 mmol) was added. Then the reaction mixture was stirred at −40 °C for another 1 h and allowed to warm to room temperature for 24 h. The reaction mixture was added 1 N HCl (20 mL) and extracted with CH_2Cl_2 (15 mL × 3). The combined organic layers was washed with brine (20 mL), dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by prep-HPLC to give the product as a white solid (25 mg, 30%). ^{1}H NMR (300 MHz, CD_3OD): δ 8.20 (s, 1H), 7.40 (d, 1H), 7.15 (m, 1H), 7.18 (m, 1H), 7.05 (m, 1H), 6.75–7.00 (m, 2H), 6.71 (m, 1H), 5.72 (m, 1H), 5.55 (m, 1H), 5.03 (m, 1H), 4.93–5.03 (m, 1H), 4.45–4.77 (m, 6H), 4.36 (m, 1H), 3.90 (d, 1H), 3.60 (m, 1 H), 2.89–2.91 (m, 1H), 2.65 (m, 1H), 2.41–2.56 (m, 2H), 1.72–2.00 (m, 3H), 0.95–1.58 (m, 16H). LC-MS: 807 $(M+H)^+$. Purity on HPLC: 96.6% (214 nm), 98.1% (254 nm).
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18. 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)-NH2 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. Compounds were also assayed for activity in the HCV genotype 1a and 1b subgenomic (NS3-NS5B) replicon model systems. The genotype 1b ET cell line is stably transfected with RNA transcripts harboring a I389luc-ubi-neo/NS3-3'/ET replicon with firefly luciferase-ubiquitin-neomycin phosphotransferase fusion protein and EMCV-IRES driven NS3-NS5B polyprotein containing cell culture adaptive mutations. The genotype 1a replicon is a stable cell line licensed from Apath LLC, modified to contain the firefly luciferase gene. The cells were grown in DMEM supplemented with 10% fetal calf serum (SAFC Biosciences, Lenexa, KS), 1× GlutaMax-1, penicillin (100 IU/mL)/streptomycin (100 μg/mL), 1× nonessential amino acids, and 500 μg/mL geneticin (all from Life Technologies, Bethesda, MD). The cells were plated at 5 × 103 cells/well in 384-well plates containing compounds. The final concentration of compounds ranged from 170 pM to 10 μM, with a final DMSO concentration of 1%. Luciferase activity was measured after 48 h by adding Steady-Glo (Promega, Madison, WI). Percent inhibition at each compound dose was calculated relative to the no compound control. EC50s were determined from an 11-point dose response curve and 3-fold serial dilutions for each compound, using a standard four parameter logistic fit equation.
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20. (a) Preliminary results of co-crystal structure of compound **4** with HCV protease enzyme did not show specific ligand–protein interactions. Co-crystal structures with other ligands were unknown; (b) Publication of the series is pending.