Bioorganic & Medicinal Chemistry Letters 20 (2010) 7493–7497

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis of new acylsulfamoyl benzoxaboroles as potent inhibitors of HCV NS3 protease

Xianfeng Li [a,*], Yong-Kang Zhang [a], Yang Liu [a], Suoming Zhang [a], Charles Z. Ding [a], Yasheen Zhou [a], Jacob J. Plattner [a], Stephen J. Baker [a], Liang Liu [a], Wei Bu [a], Wieslaw M. Kazmierski [b,*], Lois L. Wright [b], Gary K. Smith [b], Richard L. Jarvest [c], Maosheng Duan [b], Jing-Jing Ji [b], Joel P. Cooper [b], Matthew D. Tallant [b], Renae M. Crosby [b], Katrina Creech [b], Zhi-Jie Ni [d], Wuxin Zou [e], Jon Wright [e]

[a] Anacor Pharmaceuticals, Inc., 1020 E. Meadow Circle, Palo Alto, CA 94303, USA
[b] GlaxoSmithKline, Five Moore Drive, Research Triangle Park, NC 27709, USA
[c] GlaxoSmithKline, Gunnels Wood Road, Stevenage, Herts SG1 2NY, UK
[d] Acme Bioscience, Inc., 3941 E. Bayshore Road, Palo Alto, CA 94303, USA
[e] BioDuro LLC, Building E, No. 29, Life Science Park Road, Beijing 102206, PR China

ARTICLE INFO

Article history:
Received 9 September 2010
Revised 28 September 2010
Accepted 1 October 2010
Available online 30 October 2010

Keywords:
Hepatitis C virus
HCV NS3
Protease inhibitor
Macrocyclic inhibitor
Benzoxaborole

ABSTRACT

HCV NS3/4A serine protease is essential for the replication of the HCV virus and has been a clinically validated target. A series of HCV NS3/4A protease inhibitors containing a novel acylsulfamoyl benzoxaborole moiety at the P1′ region was synthesized and evaluated. The resulting P1–P3 and P2–P4 macrocyclic inhibitors exhibited sub-nanomolar potency in the enzymatic assay and low nanomolar activity in the cell-based replicon assay. The in vivo PK evaluations of selected compounds are also described.

Hepatitis C virus (HCV) is a major cause of chronic liver disease that can lead to cirrhosis, carcinoma and liver failure. It is estimated that over 200 million people are chronically infected with this virus and it is the leading cause of liver transplants.[1] The current standard treatment for HCV infection is based on a combination therapy of injectable pegylated interferon-α (PEG IFN-α) and antiviral drug ribavirin. This treatment, indirectly targeting the virus, is associated with significant side effects often leading to treatment discontinuation in certain patient populations.[2] In addition, approximately 50% of genotype-1 (the predominant genotype in the US) patients do not respond to this treatment regimen. Giving the high prevalence of the disease infection worldwide, there is an enormous unmet medical need for new therapies against HCV infection.[3]

HCV NS3/4A protease inhibitors have emerged as a promising potential treatment for HCV infection.[4] Two major classes of NS3 protease inhibitors have been developed. The first class is comprised of serine-trap inhibitors. The most advanced drugs are VX-950 (telaprevir)[5] and SCH-503034 (boceprevir),[6] currently in Phase III clinical trials. The second class is represented by reversible noncovalent inhibitors such as BILN-2061 (ciluprevir, Fig. 1), the first compound in its class to achieve clinical proof of concept.[7] Although its development was halted due to cardiac issues in animals, BILN-2061 prompted extensive investigation on further optimization of the peptide framework and P1 carboxylic acid region. To replace the P1 carboxylic acid, many different groups (e.g., tetrazole, acylcyanamide, acysulfonamide, phosphonate) have been studied.[8] Among them, cyclopropyl acylsulfonamide appears to be the preferred replacement for the P1 carboxylic acid.[9] Further application of this strategy led to the discovery of a number of clinical candidates including ITMN-191 (danoprevir),[10] TMC-435350 (medivir)[11] and MK-7009 (vaniprevir),[12] currently in advanced clinical trials. However, rapid emergence of drug-resistance has recently been observed for HCV NS3 protease inhibitors.[13] Therefore, there remains a need for the discovery of new HCV NS3 protease inhibitors with novel binding properties.

As part of our continued efforts to discover novel HCV NS3 protease inhibitors,[14] we envisioned that acylsulfamoyl benzoxaborole could be used to replace the cyclopropyl acylsulfonamide moiety (Fig. 2). Benzoxaboroles[15,16] are organoboron compounds that have emerged as a new class of potential therapeutic agents.

* Corresponding authors. Tel.: +1 650 543 7587; fax: +1 650 543 7660 (X.L.); tel.: +1 919 483 9462; fax: +1 919 315 6923 (W.M.K.).
E-mail addresses: xli@anacor.com (X. Li), wieslaw.m.kazmierski@gsk.com (W.M. Kazmierski).

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doi:10.1016/j.bmcl.2010.10.007

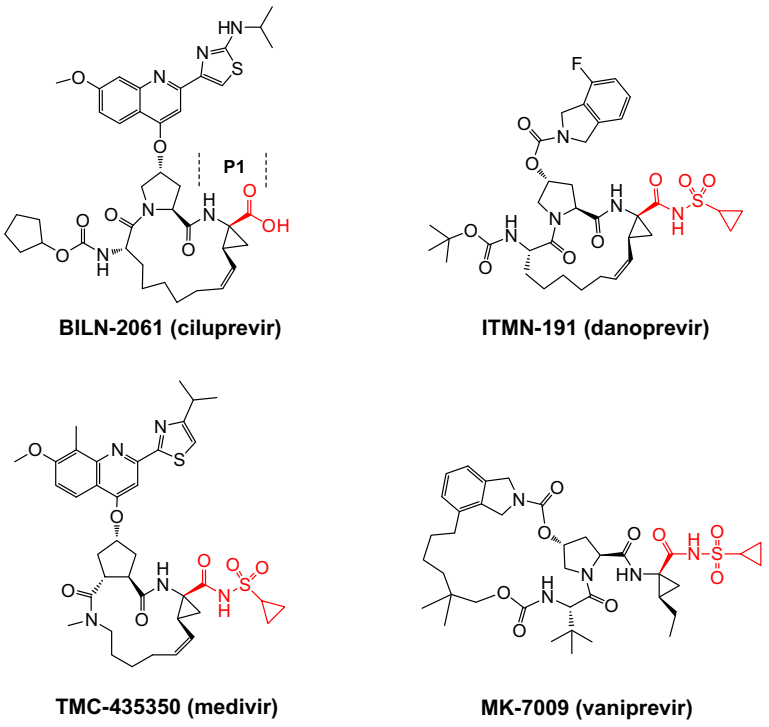


Figure 1. Selected reversible noncovalent inhibitors of HCV NS3 protease in advanced clinical trials.

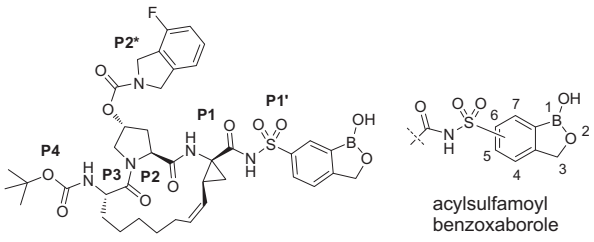


Figure 2. Hypothetical P1–P3 macrocyclic inhibitor in which an acylsulfamoyl benzoxaborole is used to replace the cyclopropyl acylsulfonamide in danoprevir.

Compounds containing a benzoxaborole moiety have been shown to interact with a variety of biological targets and also exhibit good drug properties.[15] Studies of a hypothetical benzoxaborole inhibitor derived from danoprevir (ITMN-191) docked into HCV NS3 protease suggest the benzoxaborole moiety can potentially form polar interactions with Thr 42, and positively charged Lys 136 (Fig. 3).[17] Our strategy was to scan for new potential benzoxaborole interactions with the protease by exploring the impact of two distinct macrocyclic series and different regioisomers of acylsulfamoyl benzoxaboroles on the inhibitory potency.

Boronate **4** or **5**, an important intermediate towards the 6-acylsulfamoyl benzoxaboroles, was prepared according to Scheme 1. Reaction of bromide **1** with pinacol diborane in the presence of palladium catalyst afforded boronate **2**. Bromination of **2** with NBS and AIBN gave benzyl bromide **3**. Subsequently, treatment of **3** with sodium acetate in glacial acetic acid gave the corresponding benzyl acetate **4**. Reaction of **4** with sodium hydroxide followed by acid treatment resulted in 6-sulfamoyl benzoxaborole **5**. We found that either boronate **4** or **5** could be coupled to HCV NS3 inhibitor scaffolds to form the targeted acylsulfamoyl benzoxaborole inhibitors.[18]

Similarly, towards the 5-acylsulfamoyl benzoxaboroles, the boronate intermediate **10** was prepared according to Scheme 2, while

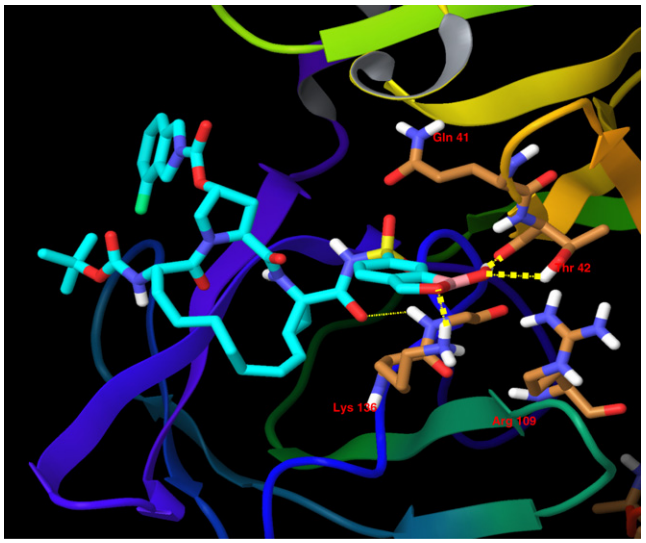
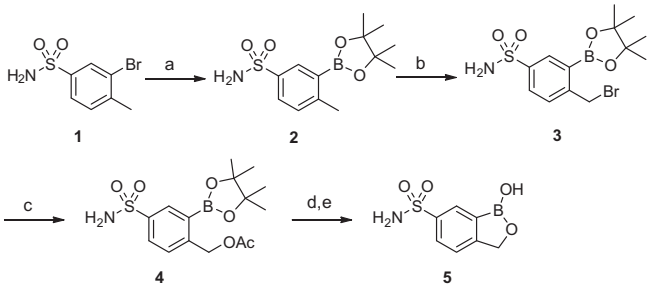


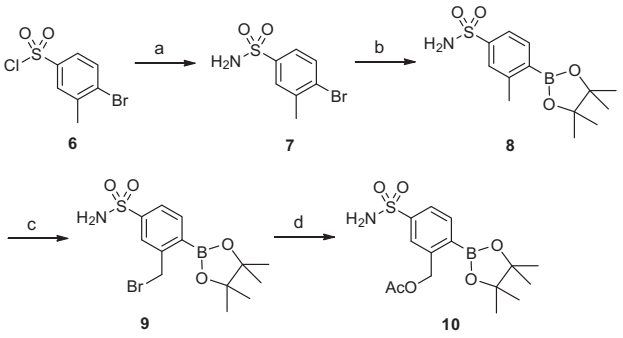
Figure 3. Modeling of a hypothetical benzoxaborole inhibitor derived from danoprevir with HCV NS3 protease. Potential polar interactions for P1′-benzoxaborole include main chain and side chain of Thr 42, and positively charged Lys 136. The Boc group in danoprevir was replaced with a chemically more-stable cyclopentyl carbamate in compound **17**.

the boronate **15**, an intermediate towards 4-acylsulfamoyl benzoxaboroles, was prepared according to Scheme 3. The sulfonamides **7** and **12** used in the synthesis were made from the starting sulfonyl chlorides **6** and **11**, respectively.

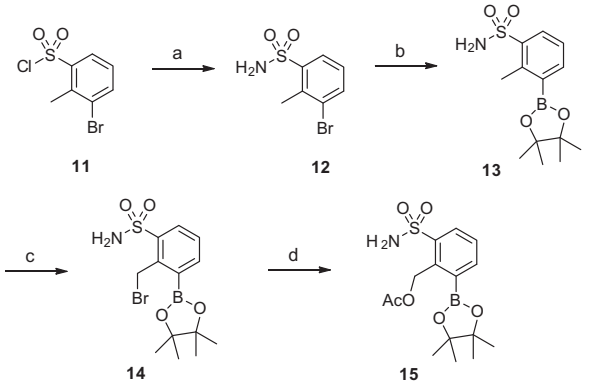
With these key intermediates in hand, we set out to investigate the P1–P3/P2–P4 macrocyclic series and also explore the impact of regioisomers of acylsulfamoyl benzoxaboroles. The P1–P3 macrocyclic inhibitors **17**–**18** were synthesized according to Scheme 4. Initially one of our targeted compounds was the hypothetical P1–P3 macrocyclic inhibitor in which 6-acylsulfamoyl benzoxaborole is



Scheme 1. Reagents and conditions: (a) pinacol diborane, $PdCl_2$(dppf), KOAc, dioxane, reflux, N_2, 86%; (b) NBS, AIBN, CCl_4, reflux; (c) NaOAc, glacial acetic acid, reflux, N_2, 23% in two steps; (d) 4 N NaOH, rt, 16 h; (e) 6 N HCl, 40 °C, THF, 16 h, 100% in two steps.

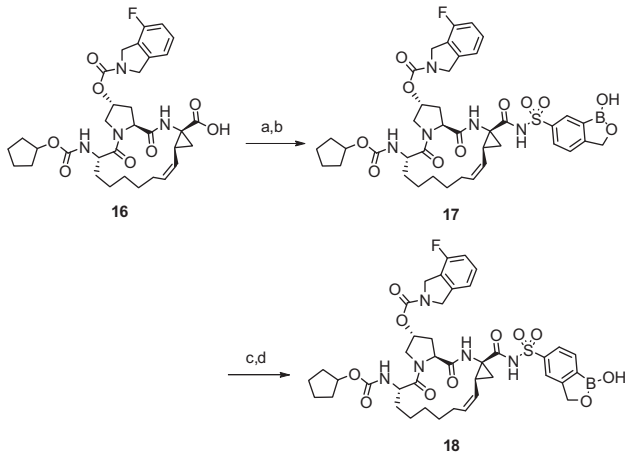


Scheme 2. Reagents and conditions: (a) NH_4OH, dioxane, 86%; (b) pinacol diborane, $PdCl_2$(dppf), KOAc, dioxane, reflux, N_2, 67%; (c) NBS, AIBN, CCl_4, reflux; (d) NaOAc, glacial acetic acid, reflux, N_2, 26% in two steps.



Scheme 3. Reagents and conditions: (a) NH_4OH, dioxane, 100%; (b) pinacol diborane, $PdCl_2$(dppf), KOAc, dioxane, reflux, N_2, 73%; (c) NBS, AIBN, CCl_4, reflux; (d) NaOAc, glacial acetic acid, reflux, N_2, 21% in two steps.

used to replace the cyclopropyl acylsulfonamide in danoprevir (structure shown in Fig. 2). During the synthesis, we found P4-Boc group did not survive the acid treatment in the final step and thus it was replaced with a chemically more-stable cyclopentyl carbamate. The P1–P3 macrocyclic acid **16**, derived from danoprevir, was made according to a published procedure.[10] Acid **16** was converted to the corresponding acylsulfonamides using boronate **4** or **10** in the presence of HATU and DIEA. The removal of pinacol/acetate groups and spontaneous formation of benzoxaborole ring was accomplished by acid treatment in the presence of isobutyl boronic acid and 1 N HCl to afford the desired product **17** and **18**, respectively.



Scheme 4. Reagents and conditions: (a) **4**, HATU, DIEA, DMAP, DBU, anhydrous DMF; (b) *i*-BuB$(OH)_2$, 1 N HCl, hexane–MeOH (1:1), 28% in two steps; (c) **10**, HATU, DIEA, DMAP, DBU, anhydrous DMF; (d) *i*-BuB$(OH)_2$, 1 N HCl, hexane–MeOH (1:1), 7% in two steps.

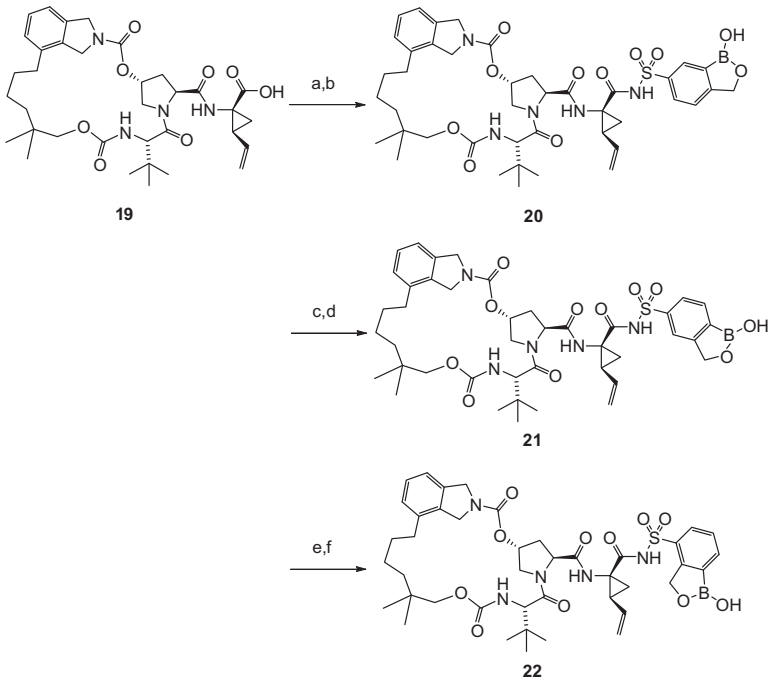
The P2–P4 macrocyclic inhibitors **20**–**22** were synthesized according to Scheme 5. The starting macrocyclic acid **19**, derived from MK-7009, was made according to a reported procedure.[12] Acid **19** was converted to the corresponding acylsulfonamides by reaction with boronate **4**, **10** or **15** as described previously, followed by acid treatment to give isomeric benzoxaboroles **20**–**22**.[19]

These compounds were evaluated for enzymatic potency in FRET assay with NS3/4A 1a protease domain.[20] The cellular activity was determined using 1a and 1b HCV replicon assays.[21] As shown in Table 1, P1–P3 macrocyclic compounds **17**–**18** and P2–P4 macrocyclic compounds **20**–**22** inhibited NS3 1a enzyme with IC_{50} values in the sub-nanomolar range (IC_{50} = 0.3–0.8 nM). They were equipotent against NS3 1a enzyme, compared to danoprevir. These benzoxaboroles exhibited low nanomolar potency against replicon 1b (EC_{50} = 8.0–20 nM), approaching that of danoprevir (EC_{50} = 1.1 nM). However, a higher shift between the enzyme potency IC_{50} and replicon activity EC_{50} (especially for replicon 1a) was observed for these benzoxaborole inhibitors than that observed for danoprevir, which could be attributed to their poor cell membrane permeability.

Interestingly, the regioisomers of acylsulfamoyl benzoxaboroles appear to have a minimal influence on the inhibitor activity. For the P1–P3 macrocyclic series, 6-acylsulfamoyl benzoxaborole **17** is equipotent in the enzyme and replicon 1a and 1b assay, compared to 5-acylsulfamoyl analog **18**. For the P2–P4 macrocyclic series, very little difference in potency was observed with 6-, 5- or 4-acylsulfamoyl benzoxaboroles (**20**–**22**) in the enzyme and replicon assay. These results demonstrate that regioisomers of acylsulfamoyl benzoxaboroles are well tolerated, consistent with the shallow enzyme binding pocket that is able to incorporate a variety of diverse inhibitors.

Our results in acylsulfamoyl benzoxaboroles show that the P1–P3 macrocyclic inhibitors are equipotent in the enzyme and replicon assay compared to the P2–P4 macrocyclic inhibitors, suggesting that these two macrocyclization strategies are equally effective in enhancing inhibitor activity. Further in vivo PK evaluation was undertaken to prioritize these two potent sub-series, which is shown in Table 2.

Despite increased solubility relative to danoprevir, both the P1–P3 macrocyclic inhibitor **17** and the P2–P4 macrocyclic inhibitor **22** displayed undetectable to low oral exposures in rats. Portal vein sampling evidenced very low absorption of **17** and **22**. By contrast, danoprevir exhibited calculated 17.4% absorption and



Scheme 5. Reagents and conditions: (a) **4**, HATU, DIEA, DMAP, DBU, anhydrous DMF; (b) *i*-BuB(OH)$_2$, 1 N HCl, hexane–MeOH (1:1), 40% in two steps; (c) **10**, HATU, DIEA, DMAP, DBU, anhydrous DMF; (d) *i*-BuB(OH)$_2$, 1 N HCl, hexane–MeOH (1:1), 25% in two steps; (e) **15**, HATU, DIEA, DMAP, DBU, anhydrous DMF; (f) *i*-BuB(OH)$_2$, 1 N HCl, hexane–MeOH (1:1), 19% in two steps.

Table 1
In vitro activity of acylsulfamoyl benzoxaborole inhibitors against HCV NS3/4A 1a, replicon 1a and 1b

Compounds	NS3/4A 1a IC_{50}[a] (nM)	HCV replicon 1a EC_{50}[b] (nM)	HCV replicon 1b EC_{50}[b] (nM)
Danoprevir	0.4	1.0	1.1
17	0.8	66	8.0
18	0.8	70	12
20	0.4	78	20
21	0.6	66	15
22	0.3	52	12

[a] FRET assay with HCV NS3 1a protease domain in the buffer containing 20% sucrose, as described in Ref. 20.
[b] Replicon assay performed as described in Ref. 21.

Table 2
Physicochemical properties and PK parameters of selected inhibitors in male Sprague-Dawley rats[a]

Parameter	Compound		
	Danoprevir	**17**	**22**
MW	732	836	848
c Log *P*	5.6	5.4	6.3
PSA	181	210	210
CL (mL/h/kg), iv	643	6640	4922
$AUC_{0\text{-inf}}$ (h μg/mL), po	1.75	[b]	0.014
% Absorption[c]	17.4	[b]	1.6
%*F*[d]	20.0	[b]	1.2

[a] Compounds were dosed orally at a dose of 5 mg/kg ($n = 3$) and intravenously at a dose of 1 mg/kg ($n = 3$).
[b] Below the limit of detection (0.5 ng/mL) and no PK data generated.
[c] Calculated from portal vein drug concentrations after oral administration as compared to that after IV administration.
[d] Calculated from jugular vein drug concentrations after oral administration as compared to that after IV administration.

20% oral bioavailability in rats. These results suggest that the poor oral bioavailability of the benzoxaborole-containing compounds is caused by their limited absorption. We believe that the introduction of an unsubstituted benzoxaborole moiety results in unbalanced physicochemical properties, such as high molecular weight (MW) and high polar surface area (PSA), of the final molecule (Table 2). Further rebalancing of MW and PSA with carefully redesigned benzoxaborole moieties could improve the permeability of the final inhibitors, contributing to simultaneous increase of potency in the replicon assay as well as improvement of bioavailability in the in vivo PK assays. In fact, we applied this strategy to another benzoxaborole-based series, resulting in more drug-like properties in these molecules (manuscript in preparation).

In summary, we have designed and explored synthetic routes towards novel acylsulfamoyl benzoxaborole-based HCV NS3 protease inhibitors. Interestingly, the resulting, unoptimized P1–P3 and P2–P4 macrocyclic inhibitors were equipotent in an enzyme assay and somewhat less potent in replicon assays, compared to danoprevir. Further optimization of the benzoxaborole moiety may allow to rebalance the physicochemical properties (e.g., MW, PSA) of the resulting compounds and improve their membrane absorption, potency and bioavailability. In addition, it will be interesting to examine the HCV protease inhibitor resistance profiles of these compounds due to the anticipated additional interactions of P1′ benzoxaborole with the enzyme active site as shown in Figure 3.

Acknowledgments

The authors acknowledge Dazhong Fan, Xuelei Qian, Liang Liao, Dianjun Chen, Xiantao Dong, Peng Liang, Jianbin Xiao, Guanghui Li, Shuanghui Wang, Chong Li, Gang Lü, Baojuan Zhao and Xinming Zhou for technical assistance and timely supply of key intermediates.

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17. The crystal structure of HCV NS3-4A complexed with a ketoamide inhibitor SCH446211 (PDB code 2FM2) was selected for the docking analysis. The bound inhibitor was deleted and hydrogen atoms added to the protein structure. The hypothetical benzoxaborole macrocyclic compound was first built and minimized in extended conformation. Monte Carlo search method was then used to dock the macrocyclic molecule into the active site allowing side chain flexibility of surrounding residues. GB/SA water solvation model combined with OPLS_2001 forcefield was used for the docking procedure. All modeling work was done using Schrodinger Maestro package.
18. Experimental procedure for the preparation of compound **4**: To a mixture of 3-bromo-4-methylbenzenesulfonamide **1** (5.0 g, 20 mmol), 2,4,4,5,5-pentamethyl-1,3,2-dioxaborolane (7.62 g, 30 mmol), and KOAc (7.85 g, 80 mmol) in 80 mL of dioxane was added $PdCl_2$(dppf) (740 mg, 0.91 mmol). After degassed three times with nitrogen, the reaction mixture was heated up to reflux for 16 h under nitrogen. Subsequently the mixture was cooled to room temperature and filtered through Celite. The filtrate was concentrated and the residue was purified by ISCO CombiFlash silica chromatography eluted with 0–40% ethyl acetate in hexane to give 5.1 g of compound **2** as a white solid (yield 86%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.26 (s, 1H), 7.81 (d, 1H),7.25 (d, 1H), 5.15 (s, 2H), 2.57 (s, 3H), 1.32 (s, 12H). To a solution of boronate **2** (5.1 g, 17.2 mmol) in 170 mL of CCl_4 were added NBS (6.09 g, 34.2 mmol) and AIBN (68 mg). The mixture was heated to reflux for 16 h under N_2 atmosphere. Subsequently, the mixture was cooled to room temperature and diluted with ethyl acetate and washed with brine. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated under vacuum to give a yellow syrup **3**. To a solution of this crude syrup in 80 mL of HOAc was added NaOAc (5.46 g, 66.6 mmol). The mixture was heated to reflux under nitrogen for 16 h. Then the mixture was evaporated to dry and diluted in ethyl acetate. The organic layer was washed with saturated $NaHCO_3$, brine and dried over anhydrous Na_2SO_4, filtered and concentrated under vacuum. The residue was purified by ISCO CombiFlash silica chromatography eluted with 0–50% ethyl acetate in hexane to give 1.4 g of compound **4** as a white solid (yield 23% in two steps). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.37 (s, 1H), 7.97 (d, 1H), 7.52 (d, 1H), 5.42 (s, 2H), 4.82 (s, 2H), 2.12 (s, 3H), 1.34 (s, 12H).
19. Experimental procedure for the synthesis of compound **20**: To a stirred solution of acid **19** (78 mg, 0.12 mmol), HATU (52 mg, 0.14 mmol) in 1 mL of anhydrous DMF was added DIEA (90 μL, 0.50 mmol). The reaction mixture was stirred at room temperature for 1 h. Subsequently a solution of **4** (178 mg, 0.50 mmol), DMAP (61 mg, 0.5 mmol), and DBU (76 mg, 0.5 mmol) in 2 mL of anhydrous DMF was added. The reaction mixture was stirred at room temperature for three days. The mixture was diluted with ethyl acetate, and washed with aqueous NaOAc buffer, 5% $NaHCO_3$ and brine. The organic layer was dried over Na_2SO_4, filtered and concentrated to give the coupling product as a yellow oil. MS *m/z* 990.4 $[M+1]^+$, 988.5 $[M-1]^-$ (calcd MS 989.5). To a stirred solution of this coupling product in 5 mL of hexane and 5 mL of MeOH were added isobutyl boronic acid (40 mg, 0.39 mmol) and HCl (1 mL, 6 N), respectively. The reaction was stirred at room temperature for 16 h. The mixture was concentrated in vacuo, diluted with ethyl acetate and washed with brine. The organic layer was dried over Na_2SO_4, filtered and concentrated in vacuo. The crude residue was purified on a reversed-phase column eluted with ACN and H_2O. The pure fractions were collected and ACN was removed in vacuo. The aqueous solution was extracted with ethyl acetate three times. The organic layer was dried over Na_2SO_4, filtered and concentrated to give compound **20** as a white solid (36 mg, yield 40%). MS *m/z* 848.4 $[M+1]^+$, 846.4 $[M-1]^-$ (calcd MS 847.3). ^{1}H NMR (300 MHz, $CDCl_3$): δ 11.0 (1H, s), 9.60 (1H, s), 8.46 (1H, s), 7.82 (1H, m), 7.76 (1H, b), 7.42 (1H, d), 7.07 (1H, d), 7.15 (1H, t), 7.07 (1H, d), 5.76 (1H, d), 5.00–5.50 (5H, m), 4.93 (1H, d), 4.70 (2H, m), 4.36–4.64 (4H, m), 4.21 (1H, m), 3.88 (1H, d), 3.07 (1H, d), 2.81 (1H, m), 1.60–2.40 (7H, m), 1.10–1.50 (6H, m), 1.06 (9H, m), 0.78 (3H, s), 0.60 (3H, s).
20. 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.
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