



Design, Synthesis and SAR of α -Amino Cyclic Boronate-containing Macrocyclic Inhibitors of HCV NS3/4A Serine Protease

Xianfeng Li¹, Y.-K. Zhang¹, Y. Liu¹, C. Z. Ding¹, Q. Li¹, Y. Zhou¹, J. J. Plattner¹, S. J. Baker¹, E. Kraynack¹, S. Zhang¹, L. Feng¹, L. Liu¹, X. Qian², D. Fan², L. Liao², D. Chen², Z.-J. Ni², M. J. Slater³, R. L. Jarvest³, P. Thommes³, M. Ellis³, C. M. Edge³, J. A. Hubbard³, P. Nassau³, B. McDowell³, T. J. Skarzynski³, W. M. Kazmierski⁴, L. L. Wright⁴, G. K. Smith⁴, R. M. Crosby⁴, A. A. Wang⁴, R. M. Grimes⁴, M. Wu⁴, X. Dong⁵, P. Liang⁵, J. Xiao⁵, G. Li⁵, S. Wang⁵, C. Li⁵, G. Lü⁵, B. Zhao⁵, X. Zhou⁵, W. Zou⁵, J. Wright⁵, L. E. Pennicott⁶, I.-M. Pietsch⁶

¹Anacor Pharmaceuticals, Inc., 1020 E. Meadow Circle, Palo Alto, CA; ²Acme Bioscience, Inc., 3941 E. Bayshore Road, Palo Alto, CA; ³GlaxoSmithKline, Gunnels Wood Road, Stevenage, Herts, UK; ⁴GlaxoSmithKline, Five Moore Drive, Research Triangle Park, NC; ⁵BioDuro LLC, Life Science Park Road, Changping District, Beijing, China; ⁶SussChem Ltd., Scitech Stores, Falmer, Brighton, East Sussex, BN1 9QJ, UK.

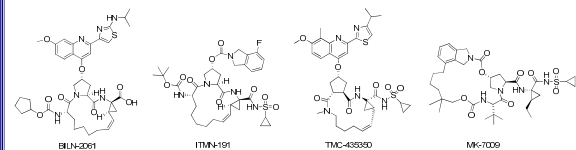
Abstract

A novel series of macrocyclic HCV NS3/4A serine protease inhibitors with α -amino cyclic boronates as warheads at the P1 site was designed and synthesized. When compared to their linear analogs, the P2-P4 macrocyclic inhibitors exhibited remarkable improvement in replicon activity. The SARs around α -amino cyclic boronates which include the influence of ring size, chirality, and substitution patterns are discussed. Furthermore, X-ray co-crystal structure of boronate inhibitor **14a** with HCV NS3 protease reveals that the enzyme is inhibited via the warhead boron trapping Ser139 at the enzyme active site.

Introduction

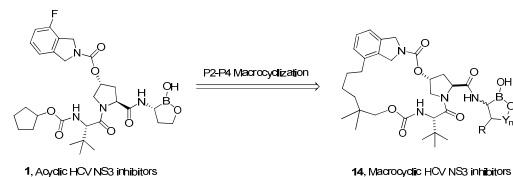
Hepatitis C Virus (HCV) infection affects more than 200 million people worldwide, leading to chronic liver disease such as cirrhosis, carcinoma and liver failure. The current standard of care for HCV infection is a combination therapy of injectable pegylated interferon- α (PEG IFN- α) plus oral ribavirin. Unfortunately, approximately 50% of genotype-1 (the predominant genotype in the U.S.) patients do not respond to this treatment regimen. Recently, HCV NS3/4A protease inhibitors have emerged as a promising potential treatment for HCV infection. Unlike interferons, which work by stimulating the immune system's response to viral infection, NS3 protease inhibitors target the virus directly by inhibiting NS3/4A protease, a key mechanism implicated in HCV replication.

The first clinical proof of concept was achieved with BILN-2061, a macrocyclic P1-P3 inhibitor. Subsequently, the search for HCV NS3 protease inhibitors incorporating new structure motifs led to the discovery of several other P1-P3 macrocyclic inhibitors including ITMN-191 and TMC-435350 in clinical trials. Recently, Merck identified a novel class of P2-P4 macrocyclic inhibitors of HCV NS3 protease including MK-7009, currently being evaluated in clinical trials. Therefore, macrocyclic HCV inhibitors have proved to be a viable structural class with improved binding and pharmacokinetic properties.

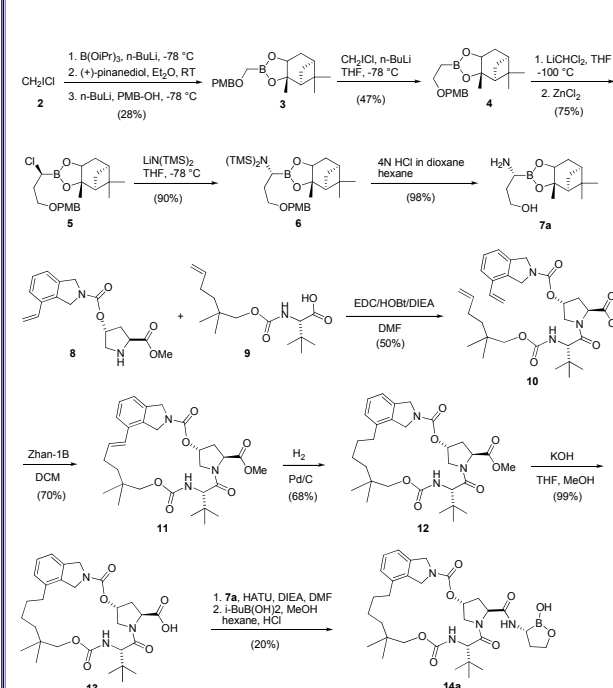


Design of α -Amino Cyclic Boronate Based Macrocyclic Inhibitors

Boron-containing compounds are emerging as potential therapeutics.¹ Recently we discovered an acyclic series of HCV NS3 protease inhibitors derived from α -amino cyclic boronates (**1**).² To improve their replicon activities, we focused our discovery efforts on P2-P4 macrocyclic series.

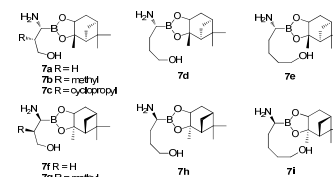


Representative Synthesis of Macrocyclic Inhibitor **14a**



Key Boronate Intermediates **7a-i** Prepared

The key α -amino boronates **7a-i** were prepared based on similar synthetic routes using (+) or (-) pinanediol as a chiral auxiliary and were subsequently used to incorporate α -amino cyclic boronates in the macrocyclic inhibitors.

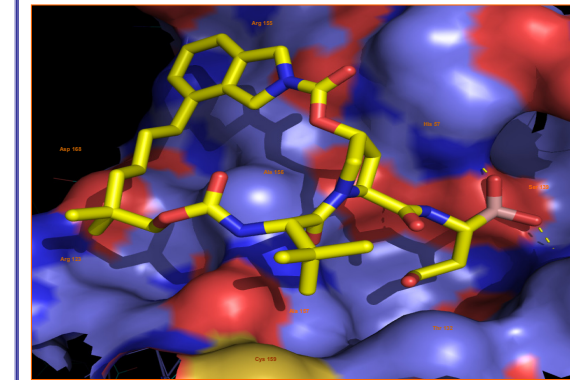


SAR

Cpds	Boronate 7 used	Cyclic boronate R	NS3/4A 1a IC ₅₀ (uM)*	Replicon EC ₅₀ (uM) 1a	1b
14a	7a		0.15	0.676	0.376
14b	7f		1.39	11.35	>50.0
14c	7b		0.62	1.90	2.12
14d	7c		0.11	2.64	4.17
14e	7g		>10.0	33.1	29.5
14f	7d		0.061	0.70	2.82
14g	7h		1.29	13.2	>25.0
14h	7e		0.13	0.426	0.278
14i	7i		0.62	3.67	2.37

*FRET assay (QXL520) with HCV NS3/4A 1a protease domain in the buffer containing 20% sucrose

X-ray Structure of **14a** Complexed with NS3 Protease



Conclusion

- The P2-P4 macrocyclic series had improved replicon activities by over 10 fold when compared to linear analogs
- (R) α -Amino cyclic boronates inhibitors were significantly more potent than the corresponding (S) isomers, which validates the specificity of these inhibitors for inhibiting HCV NS3 protease
- The 5-, 6- and 7-membered cyclic boronates appear to have similar enzymatic and replicon activities, suggesting that the ring size of α -amino cyclic boronates has minimum influence
- The substitution at the β -position of 5-membered cyclic boronates resulted in several fold loss in potency, indicating that the small substituents are not well-tolerated
- X-ray co-crystal structure of **14a** with NS3 protease showed that the boron is covalently linked to the Ser-139 in the enzyme active site and is locked in the negatively-charged tetrahedral form mimicking the transition state. The oxygen-boron bond inside the 5-membered oxaborole ring was forced open, resulting in the hydroxyethyl side chain in the S1 pocket

References

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- YK Zhang, X Li, et al., *BIT's 7th Annual Congress of International Drug Discovery Science and Technology*, Shanghai, China, October 25, **2009**.