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Synthesis and evaluation of NS5A inhibitors containing diverse heteroaromatic cores

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Abstract

Inhibitors of the HCV NS5A nonstructural protein are showing promising clinical potential in the treatment of hepatitis C when used in combination with other direct-acting antiviral agents. Current NS5A clinical candidates such as daclatasvir, ledipasvir, and ombitasvir share a common pharmacophore that features a pair of (*S*)-methoxycarbonylvaline capped pyrrolidines linked to various cores by amides, imidazoles and/or benzimidazoles. In this letter, we describe the evaluation of NS5A inhibitors which contain alternative heteroaromatic replacements for these amide mimetics. The SAR knowledge gleaned in the optimization of scaffolds containing benzoxazoles was parlayed toward the identification of potent NS5A inhibitors containing other heteroaromatic replacements such as indoles and imidazopyridines.

Letter

Hepatitis C virus (HCV) is currently one of the most significant world health concerns. It is estimated that 130-170 million individuals (~3%) worldwide and more than 5 million Americans (1.8%) are infected with HCV.[1] In the majority of cases (80%), the viral infection leads to a chronic form of hepatitis and without therapeutic intervention can lead to morbidity or mortality through either cirrhosis and liver failure or hepatocellular carcinoma.[2] An extended, moderately efficacious and poorly tolerated

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regimen of pegylated interferon-α (PEG-IFN-α) and ribavirin (RBV) had served as the HCV standard of care until recently. Improvements in cure rates and treatment durations of the most dominant form of the virus, genotype 1, were realized through the addition of the direct-acting antiviral (DAA) agents telaprevir, boceprevir, simeprevir (NS3•4a protease) and/or sofosbuvir (NS5B polymerase) to the standard of care.[3,4] Although further optimization in the areas of resistance, tolerability, and pan-genotypic efficacy is a necessity, ongoing clinical trials employing all oral interferon-free combinations of DAAs are yielding promising results.[5]

The HCV NS5A protein has evolved into an attractive drug target. NS5A is a large phosphoprotein (49 kDa) with no apparent enzymatic activity or homologues in prokaryotes and eukaryotes. Although the mechanistic role of NS5A in the HCV replication cycle currently is unknown, it is thought to play important roles in viral replication, virion assembly and secretion from infected cells.[6] The elucidation of NS5A's mechanistic role remains an active area of investigation.[7] Proof of concept for this target was initially demonstrated by daclatasvir (BMS-790052, Figure 1), which displayed an impressive $3\log_{10}$ reduction of HCV RNA in 24 h with one 10 mg oral dose (genotypes 1a and 1b); a viral load drop that was considerably faster than had been observed with HCV inhibitors acting by other mechanisms.[8] These results stimulated considerable interest in the design and development of NS5A replication complex inhibitors (RCIs) and several related clinical candidates are currently being evaluated.[9,10] The pharmacophore of the NS5A RCIs in Figure 1 is comprised of a pair of (*S*)-methoxycarbonylvaline capped pyrrolidines attached to various cores by anilides, imidazoles and/or benzimidazoles, suggesting the importance of a group with H-bond donating and accepting properties (Figure 1). In this manuscript, we expand upon previously reported NS5A RCIs containing amides, imidazoles and benzimidazoles to describe inhibitors with alternate heteroaromatic replacements.

C_2-symmetric *bis*-benzimidazole analogs with aryl linkers, such as **1** (Figure 1), have been shown to be potent inhibitors of NS5A genotype 1a and 1b replicons.[11] Subsequent efforts to desymmetrize these scaffolds led to the identification of the thienoimidazole isostere as an attractive surrogate for benzimidazole.[12] We therefore set out to design nonsymmetrical inhibitors with diverse heteroaromatic cores, with an initial

focus on analogs containing 2,5- and 2,6-substituted benzoxazoles.

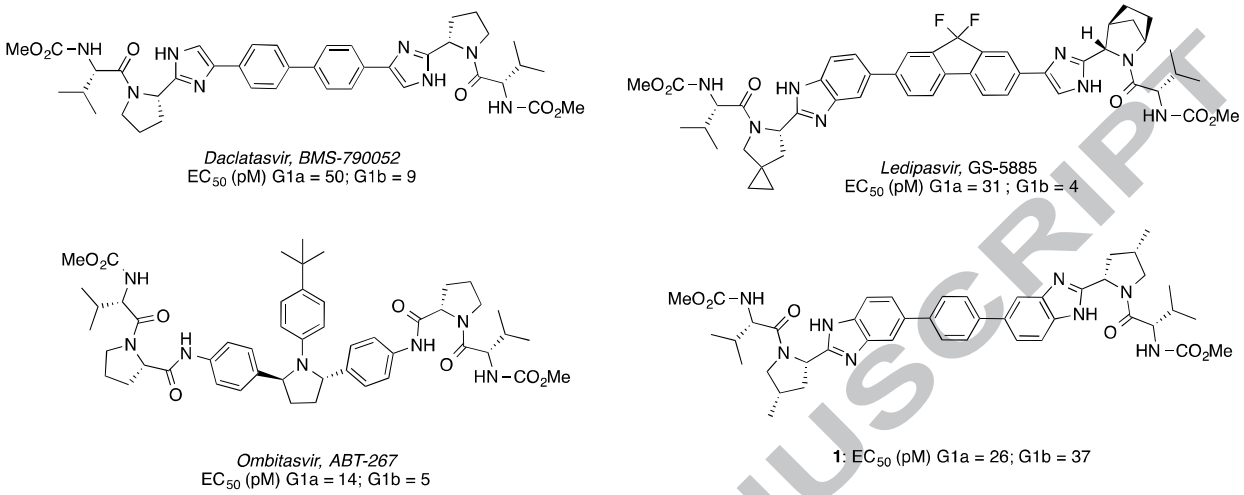
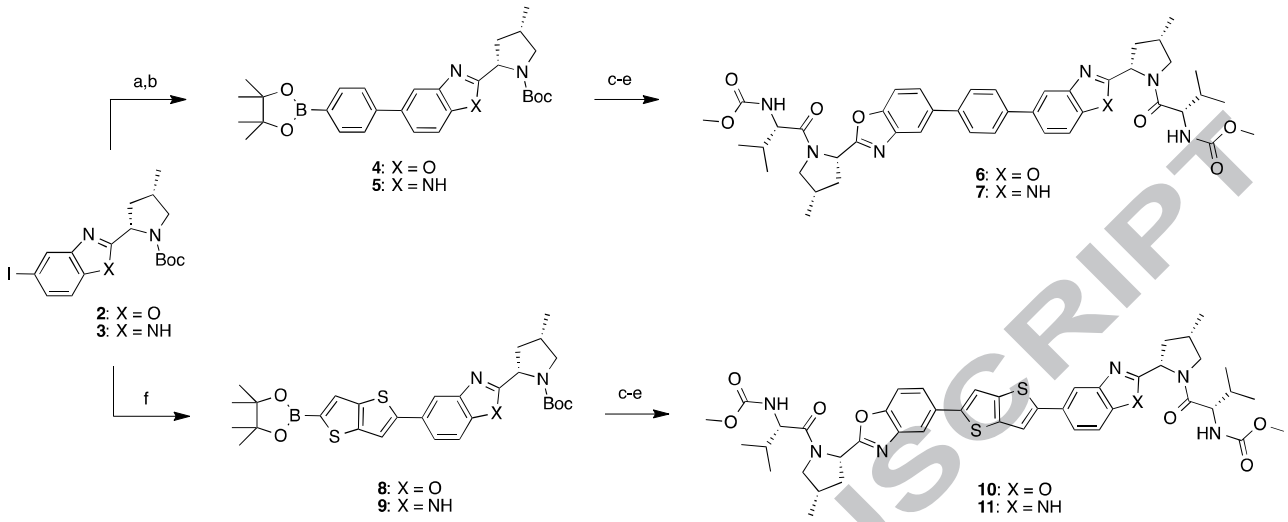


Figure 1. Structures of NS5A inhibitors



Scheme 1: General preparation of benzoxazole-containing analogs, exemplified by **6,7,10, and 11**. Reagents and conditions: (a) (4-bromophenyl)boronic acid (1.0 eq), $Pd(PPh_3)_4$, K_3PO_4, 5:1 PhMe:MeOH, 70 °C; (b) bis(pinacolato)diboron, $PdCl_2$dppf, KOAc, DMF, 85 °C; (c) **2** (1.0 eq), $Pd(OAc)_2$, ^{S}SPhos, $NaHCO_3$(aq), *i*-PrOH, 100 °C; (d) TFA, DCM, rt; (e) (*S*)-2-((methoxycarbonyl)-amino)-3-methylbutanoic acid, HATU, DIPEA, DMF, rt; (f) 4,4,5,5-tetramethyl-2-[5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)thieno[3,2-b]thiophen-2-yl]-1,3,2-dioxaborolane (1.5 eq), $Pd(OAc)_2$, ^{S}SPhos, K_2CO_3, 3:1 2-MeTHF:H_2O, 90 °C

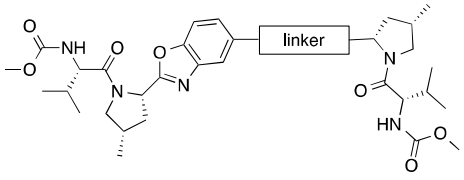
Symmetrical and nonsymmetrical benzoxazole-containing compounds were prepared by the general approach shown in Scheme 1. Coupling of iodo-benzoxazole **2** and iodo-benzimidazole **3** with (4-bromophenyl)boronic acid and reaction of the resulting aryl bromide with bis(pinacolato)diboron under Suzuki conditions provided phenyl-boronate intermediates **4** and **5**, respectively. The boronates **4** and **5** were coupled with iodo-oxazole **2,** Boc-deprotected under acidic conditions, and coupled with *N*-(methoxycarbonyl)-L-valine in the presence of HATU to give analogs **6** and **7**. Compounds containing the thienothiophene (e.g., **10** and **11**) or biphenyl-imidazole linker (**12**) were prepared by analogous sequences.[13]

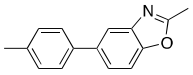
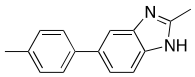
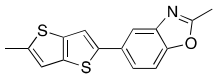
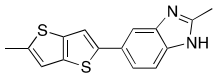
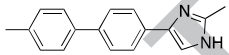
In comparison to **1**, *bis*-benzoxazole analogs **6** and **10** exhibited a marked reduction in activity against both genotype 1a (G1a) and genotype 1b (G1b) replicons

(Table 1).[12] The inclusion of benzimidazole in the nonsymmetrical analogs **7, 11,** and **12** resulted in the return of potent G1b activity (EC_{50} = 17 pM). Notably, replacement of the phenyl linker in compound **7** with thienothiophene **(11)** resulted in a 1800-fold increase in G1a activity (EC_{50} = 32 pM). Further lengthening of the scaffold with a biphenyl-imidazole linker, as in **12**, resulted in diminished G1a potency (11 nM).

Table 1

Activity of analogs containing 2,5-substituted benzoxazole

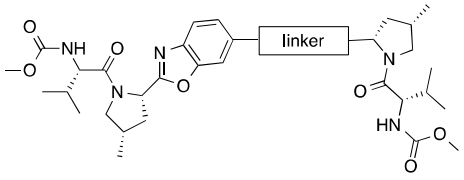


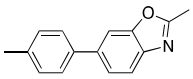
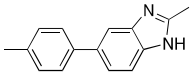
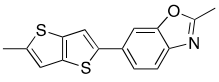
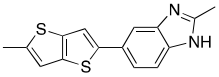
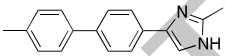
Compd	Linker	G1a EC_{50} (nM)	G1b EC_{50} (nM)	CC_{50} (μM)
6		>2000	223	7
7		58	0.017	>20
10		>2000	23	>20
11		0.032	0.003	>20
12		11	0.023	>20

The investigation of inhibitors containing 2,6-substituted benzoxazoles is shown in Table 2. In contrast to the 2,5-substituted benzoxazole inhibitors (Table 1), both symmetric and nonsymmetric analogs in the 2,6-substituted benzoxazole series exhibited picomolar G1b activity. Increased G1a activity was also observed in this series and is highlighted by a 30-fold potency difference between analogs **7** and **14** (G1a EC_{50} = 58 and 1.8 nm, respectively) and a 60-fold potency boost when the diphenyl-imidazole linker was employed (compare **12** and **17**). Gratifyingly, the thienothiophene-benzimidazole linker also stood out in the 2,6-substituted benzoxazole series and furnished inhibitor **16**, which exhibited excellent potency against both G1b and 1a replicons (EC_{50} = 4 and 29 pM, respectively).

Table 2.

Activity of analogs containing 2,6-substituted benzoxazole

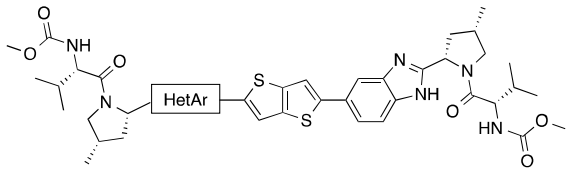


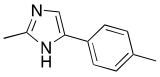
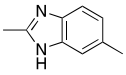
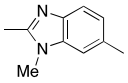
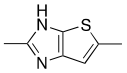
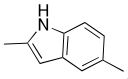
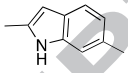
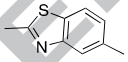
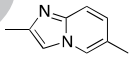
Compd	Linker	G1a EC_{50} (nM)	G1b EC_{50} (nM)	CC_{50} (μM)
13		630	0.006	7
14		1.8	0.008	>20
15		43	0.019	>20
16		0.029	0.004	>20
17		0.164	0.005	>20

In order to evaluate if the potent activity profiles observed with **11** and **16** were inherent to analogs containing benzoxazoles, additional heteroaromatic groups were evaluated in the thienothiophene-benzimidazole scaffold (Table 3). This survey included heteroaromatics with both H-bond donating and accepting capabilities, such as benzimidazole **20** and thienoimidazole **22**, as well as rings with exclusively H-bond donating or H-bond accepting capacities. Remarkably, the thienothiophene-benzimidazole scaffold proved to be tolerant of heteroaromatics from each of these donor/acceptor classes, and with the exception of indole **23** and benzthiazole **25,** all compounds exhibited potent and balanced G1a and 1b activity. Compounds in Tables 1-3 were generally noncytotoxic, with most having CC_{50} values greater than 20 μM.[14]

Table 3.

Activity of thienothiophene-benzimidazole scaffold analogs



Compd	HetAr	G1a EC_{50} (nM)	G1b EC_{50} (nM)	CC_{50} (μM)
18		0.021	0.016	>20
19		0.008	0.006	>20
20		0.004	0.009	>20
21		0.024	0.004	>20
22		0.011	0.004	>20
23		1.3	0.006	>20
24		0.020	0.006	>20
25		6.8	0.025	>20
26		0.006	0.004	8

The thienothiophene-benzimidazole scaffold analogs discussed above generally displayed low IV clearance and long $T_{1/2}$ in rat PK experiments. Unfortunately, the low rat oral bioavailablity observed in this series remains an area for improvement. Rat PK details for representative compounds **16**, **19**, and **24** are given in Table 4.

Table 4.
Rat pharmacokinetics of selected compounds.

Compound	***16***	***19***	***24***
IV Rat PK			
Cl (mL/min/kg)	*6.2*	*8.1*	*4.7*
$T_{1/2}$(h)	*5.6*	*3.5*	*4.6*
V_{ss} (L/kg)	*2.8*	*1.6*	*1.6*
PO Rat PK			
$AUC_{0\text{-}8h}$(μg h/mL)	*0.376*	*0.281*	*0.659*
$T_{1/2}$ (h)	*14.8*	*17.2*	*16.6*
C_{max} (ng/mL)	*0.01*	*0.01*	*0.02*
% F	*7.3*	*8.4*	*8.4*

In summary, the evaluation of two series of benzoxazole-containing HCV NS5A inhibitors led to the identification of a thienothiophene-benzimidazole scaffold, which delivered analogs exhibiting potent and balanced G1a and 1b activity. Furthermore, it was shown that the excellent potency profiles observed with the thienothiophene scaffold analogs could be maintained when the benzoxazoles were replaced with a range of other heteroaromatic rings.

Acknowledgments

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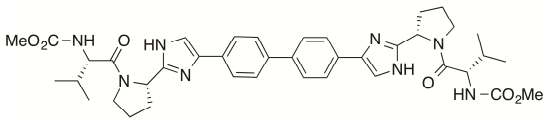
References and notes

1. For recent reviews, see: (a) Thomas, D. L. *Nat. Med.* **2013**, *19*, 850-858, (b) Scheel, T. K. H.; Rice, C. M. *Nat. Med.* **2013**, *19*, 837-849, (c) Lavanchy, D. *Microbiol. Infect.* **2011**, *17*, 107-115.
2. Tong, M. J.; El-Farra, N. S.; Reikes, A. R.; Co, R. L. *N. Engl. J. Med*. **1995**, *332*, 1463-1466.
3. Lemon, S. M.; McKeating, J. A.; Pietschmann, T.; Frick, D. N.; Glenn, J. S.; Tellinghuisen, T. L.; Symons, J.; Furman, P. A. *Antiviral Res*. **2010**, *86*, 79-92.
4. Aghemo, A.; De Francesco, R. *Hepatology* **2013**, *58*, 428-438.
5. Asselah, T.; Marcellin, P. *Liver Int.* **2013**, *33* (Suppl. 1), 93-104.

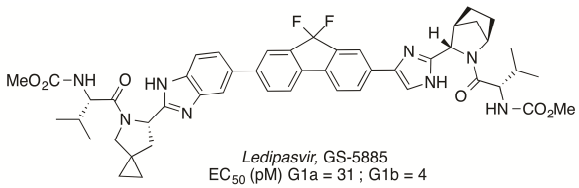
6. Guedj, J.; Dahari, H.; Rong, L.; Sansone, N. D.; Nettles, R. E.; Cotler, S. J.; Layden, T. J.; Uprichard, S. L.; Perelson, A. S. *Proc. Natl. Acad. Sci. U.S.A.* **2013**, *110*, 3991-3996.
7. (a) Reghellin, V.; Donnici, L.; Fenu, S.; Berno, V.; Calabrese, V.; Pagani, M.; Abrignani, S.; Peri, F.; De Francesco, R.; Neddermann, P. *Antimicrob. Agents Chemother.* **2014**, doi:10.1128/AAC.03293-14; (b) Mani, N.; Yuzhakov, A.; Yuzhakov, O.; Coll, J. T.; Black, J.; Saxena, K.; Fulghum, J. R.; Lippke, J. A.; Govinda Rao, B.; Rijnbrand, R.; Kwong, A. D. *J. Virol.* **2014**, *88*, *In Press*
8. Gao, M.; Nettles, R. E.; Belema, M.; Snyder, L. B.; Nguyen, V. N.; Fridell, R. A.; Serrano-Wu, M. H.; Langley, D. R.; Sun, J.-H; O'Boyle, D.R., II; Lemm, J. A.; Wang, C.; Knipe, J. O.; Chien, C.; Colonno, R. J.; Grasela, D. M.; Meanwell, N. A.; Hamman, L.G. *Nature* **2010**, *465*, 96-100.
9. Belema, M.; Lopez, O. D.; Bender, J. A.; Romine, J. L.; St. Laurent, D. R.; Langley, D. R.; Lemm, J. A.; O'Boyle, D. R., II; Sun, J.-H.; Wang, C.; Fridell, R. A.; Meanwell, N. A. *J. Med. Chem.* **2014**, *57*, 1643-1672.
10. On October 10th, 2014, the FDA approved Gilead Sciences' Harvoni® (sofosbuvir and ledipasvir) for the treatment of chronic HCV genotype 1 infection.
http://www.fda.gov/newsevents/newsroom/pressannouncements/ucm418365.htm
11. Henderson, J. A.; Maxwell, J.; Vaillancourt, L.; Morris, M.; Grey, R., Jr.; Giroux, S.; Kong, L. C. C.; Das, S. K.; Liu, B.; Poisson, C.; Cadilhac, C.; Bubenik, M.; Reddy, T. J.; Falardeau, G.; Yannopoulos, C.; Wang, J.; Pereira, O. Z.; Bennani, Y. L.; Pierce, A. C.; Bhisetti, G. R.; Cottrell, K. M.; Morone, V. Patent US 8,765,731 B2
12. For details of the NS5A replicon assays used in this letter, see: Giroux, S.; Xu, J.; Reddy, T. J.; Morris, M.; Cottrell, K. M.; Cadilhac, C.; Henderson, J. A.; Nicolas, O.; Bilimora, D.; Denis, F.; Mani, N.; Ewing, N.; Shawgo, R.; L'Heureux, L.; Selliah, S.; Chan, L.; Chauret, N.; Berlioz-Seux, F.; Namchuk, M. N.; Grillot, A-L.; Bennani, Y. L.; Das, S. K.; Maxwell, J. P. *ACS Med. Chem Lett*. **2014**, *5*, 240-243.

13. For synthetic procedures and characterization of intermediates and biologically evaluated compounds see: Pereira, O.; Maxwell, J. P.; Bennani, Y. B. Patent WO 2011/119858A1.

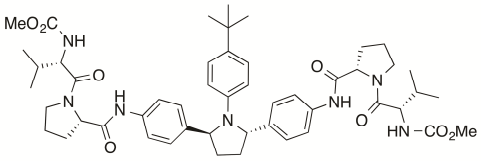
14. CC_{50} in Huh-7 ET cells were determined using a [^{3}H]-thymidine incorporation assay from 12 concentrations of the test compound in duplicate, and data were considered accurate when the CC50 of the positive control was within 40% of the mean value.



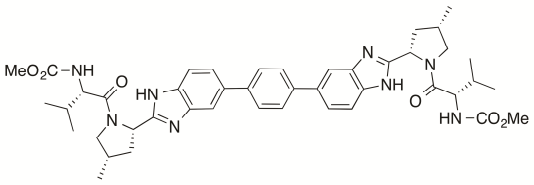
Daclatasvir, BMS-790052
EC_{50} (pM) G1a = 50; G1b = 9



Ledipasvir, GS-5885
EC_{50} (pM) G1a = 31 ; G1b = 4

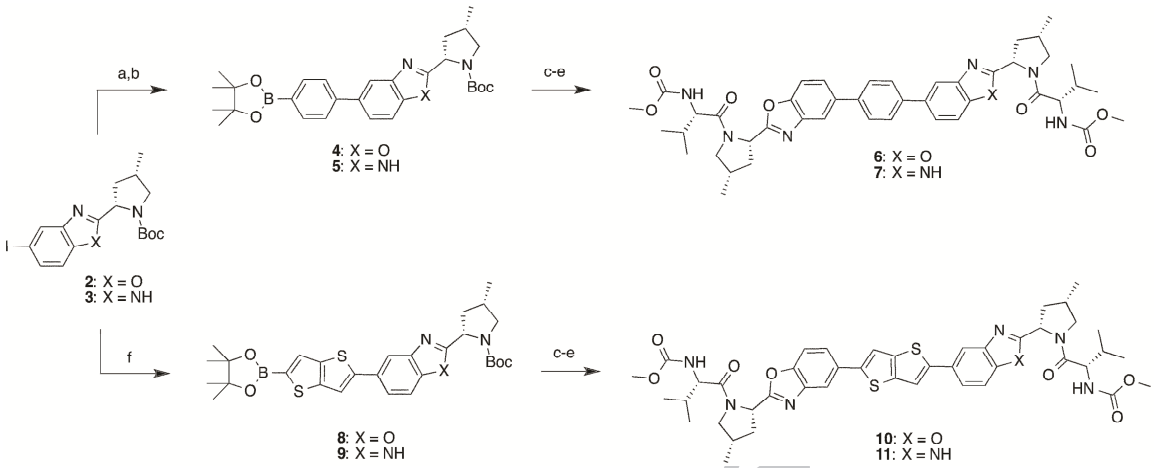


Ombitasvir, ABT-267
EC_{50} (pM) G1a = 14; G1b = 5



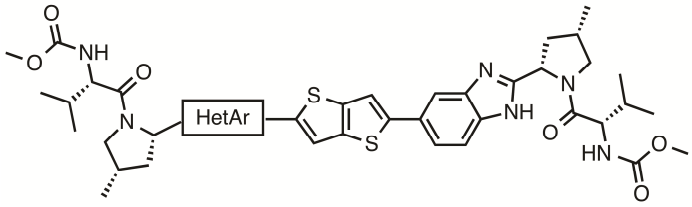
1: EC_{50} (pM) G1a = 26; G1b = 37

Figure 1



Scheme 1.

Graphical abstract



HetAr = benzoxazole, indole, imidazopyridine

EC_{50} G1a = 4 - 32 pM