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Benzimidazole Thumb Pocket I finger-loop inhibitors of HCV NS5B polymerase: Improved drug-like properties through C-2 SAR in three sub-series

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ABSTRACT

SAR at the C-2 position of benzimidazole-based Thumb Pocket I inhibitors of HCV NS5B polymerase revealed parallel activity for distinct sub-series that harbor 5-hydroxytryptophan amides, neutral thiazole isosteres or recently disclosed cinnamic acid diamides. The consistent SAR among the three sub-series suggest a common binding mode to the Thumb Pocket I allosteric site. New inhibitors with submicromolar cell-based replicon potency and improved 'drug-like' features are disclosed along with preliminary characterization of their ADME-PK profile.

Hepatitis C virus (HCV) infections, which are believed to afflict 1–3% of the world population, may lead to serious and often fatal liver diseases such as cirrhosis and hepatocellular carcinomas (HCC).[1] HCV is the leading cause of liver transplantation in the world and represents a major concern to public health in both industrialized and developing countries. The discovery of the virus 20 years ago and the development of diagnostic tools have greatly contributed to arresting the spread of new viral infections. Nevertheless, it is estimated that the number of newly diagnosed patients will continue to rise in the next 10 years before reaching a plateau and eventually decreasing as more effective and better tolerated antiviral therapies become available. The current standard of care, based on combinations of pegylated-interferon and ribavirin, is poorly tolerated and shows sub-optimal efficacy, particularly against genotype 1 (most abundant in the western world).[2]

NS5B, the RNA-dependent RNA polymerase of HCV is the enzyme that catalyzes replication of the virus's genome and has been the focus of extensive research efforts by academia and the pharmaceutical industry for the development of novel anti-HCV agents. NS5B polymerase is sensitive to inhibition by both substrate-based nucleoside analogs as well as non-nucleoside allosteric inhibitors. Several small molecule candidates from both classes of drugs have progressed through early stages of clinical development and provided proof of concept of antiviral efficacy in man.[3,4] Allosteric inhibitors of NS5B are divided into at least four different classes according to the location of their respective binding sites (referred to as Thumb Pocket I and II and Palm Sites I and II).[4]

Our group reported the discovery and subsequent optimization of benzimidazole-based allosteric inhibitors of HCV NS5B that bind to Thumb Pocket I of the enzyme (Fig. 1) and were shown by us and others to restrict conformational changes in the enzyme that are thought to be required at the initiation stage of RNA synthesis. We refer to this class of compounds as 'finger-loop inhibitors' since they prevent a key intra-molecular interaction of the λ1 loop that extends from the finger domain with the thumb domain of the enzyme. This interference with a protein conformational change prevents the formation of a productive enzyme complex for RNA synthesis.[5]

In a recent Letter, we reported optimization of the right-hand side of benzimidazole-based inhibitors and the discovery of novel diamide derivatives (e.g., compound **C1**) that displayed excellent inhibition in biochemical assays with micromolar potency in a cell-based 1b HCV sub-genomic replicon assay (Fig. 1).[6] We now describe C-2 SAR in this class of inhibitors and some comparison to previous series of compounds (5-hydroxytryptophans **A1** and thiazoles **B1**) that, despite significant structural changes on the right-hand side, provides evidence for parallel SAR and a common

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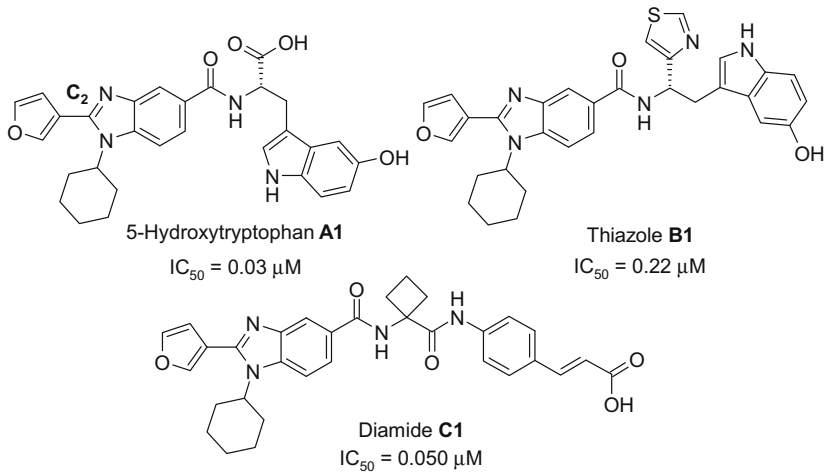


Figure 1. Representative members from three benzimidazole sub-series of finger-loop (Thumb Pocket I) inhibitors.

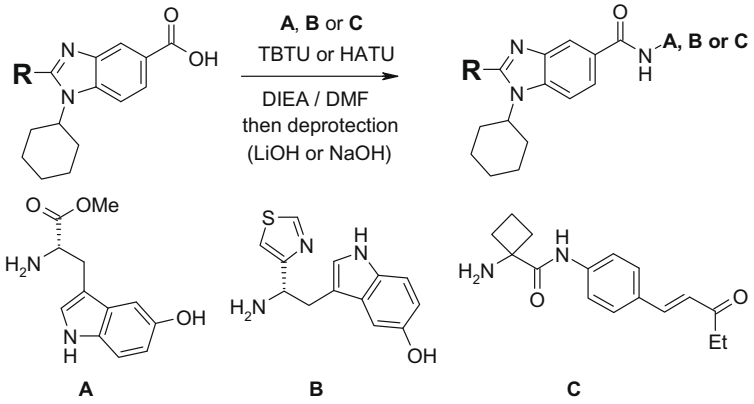
binding mode for the three sub-series. In addition, the furyl structural alert in lead inhibitors **A1, B1,** and **C1** was replaced and cellular potency improved to provide inhibitors with promising in vitro ADME and rat PK characteristics.

The three benzimidazole sub-series of NS5B inhibitors previously disclosed contain a 5-carboxybenzimidazole scaffold that features an optimal N^1-cyclohexyl moiety and a small heterocyclic C-2 substituent. While the N^1-position is very sensitive to small structural modifications, the C-2 position was shown in early work to tolerate a variety of substituents ranging from small heterocycles[7] to large poly-aromatic systems.[8] Recent disclosures from our group revealed how the electron-rich 5-hydroxytryptophan system may be replaced by a cinnamic acid moiety linked to the benzimidazole scaffold through an α,α-disubstituted cyclic α-amino acid linker.[6] We wished to re-investigate SAR at the C-2 position of the benzimidazole left-hand side to verify that SAR remained parallel in the three sub-series and identify a more drug-like replacement for the furyl heterocycle at C-2.[9]

The synthesis of inhibitors was performed in solution as described in Scheme 1. Benzimidazole carboxylic acids with variations at C-2 or N^1 were prepared according to previously published procedures[7,10] and coupled to amines **A**,[11] **B**,[12] or **C**[6] using standard reagents for amide bond formation (TBTU or HATU) followed by removal of protecting groups by saponification if necessary.[13]

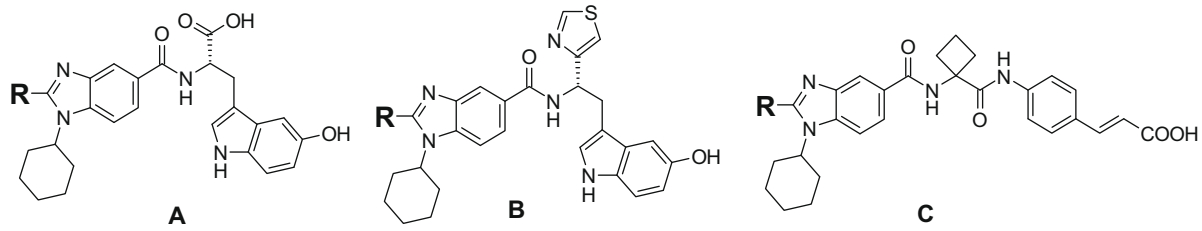
C-2 SAR was established early in truncated benzimidazole 5-carboxylic acid derivatives[7] where a relatively 'flat' SAR was observed at this position. Nonetheless, 2- and 3-furyl, 2-*N*-Me-pyrrole, 2- and 3-thiophenyl, and 2-pyridyl were identified as providing the best intrinsic potencies in a full-length NS5B biochemical assay.[14]

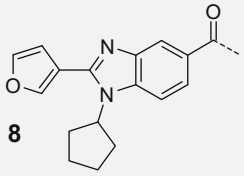
As the series progressed from the short benzimidazole carboxylic acid derivatives to more potent tryptophan-based amides (sub-series **A** and **B**), we had assumed similar inhibitor binding modes[15] and SAR at this position. In order to validate this hypothesis, a series of analogs that contained these privileged C-2 substituents was prepared and tested in both the full-length NS5B and the C-terminally deleted Δ21-NS5B format of the biochemical assays.[14] Both assays gave similar IC_{50} values and only data for the latter are reported in Table 1. As reported previously for the 3-furyl derivatives **A1** and **B1**, tryptophan analogs that contained a thiazole replacement for the α-carboxylic acid group were all less potent (3–12-fold), independent of the C-2 substituent. This observation may be rationalized by a key role for the carboxylic acid. It is believed to be solvent exposed and serves as a conformation orienting group,[12,15] and is favorably hydrated in sub-series **A** compared to the more hydrophobic thiazole group present in sub-series **B**. However, the charge and high polarity of carboxylic acids **A1–8** were incompatible with cellular permeability and all compounds in this sub-series were inactive in an HCV genotype 1b cellular replicon assay.[16] The more lipophilic and neutral thiazole analogs **B1–8**, on the other hand provided low-micromolar inhibition (EC_{50} = 2–4 μM) in the cell-based assay, regardless of the C-2 substituent present on the benzimidazole scaffold. As previously



Scheme 1. Synthesis of inhibitors.

Table 1
C-2 and N^1 SAR in three benzimidazole sub-series



R	IC_{50}, μM[a]	IC_{50}, μM[a]	EC_{50}, μM[a,b]	IC_{50}, μM[a]	EC_{50}, μM[a,b]	Calcd Log *P*[c]
1 	0.03 (±0.01)	0.22 (±0.04)	3.6 (±1.5)	0.05 (±0.02)	1.1 (±0.4)	4.4
2 	0.08 (±0.02)	0.27 (±0.1)	3.4 (±0.7)	0.11 (±0.01)	1.2 (±0.3)	4.7
3 	0.16 (±0.1)	0.52 (±0.17)	2.9 (±0.5)	0.12 (±0.05)	1.5 (±0.5)	4.9
4 	0.08 (±0.02)	0.45 (±0.06)	2.8 (±0.6)	0.11 (±0.05)	1.2 (±0.4)	4.9
5 	0.06 (±0.01)	0.73 (±0.36)	2.7 (±0.5)	0.09 (±0.02)	0.7 (±0.3)	4.3
6 	0.14 (±0.04)	0.69 (±0.4)	1.9 (±0.8)	0.10 (±0.04)	0.7 (±0.2)	4.7
7 	0.23 (±0.09)	1.16 (±0.28)	2.4 (±0.9)	0.20 (±0.08)	1.2 (±0.2)	5.1
8 	0.08 (±0.02)	0.38 (±0.06)	4.0 (±1.0)	0.10 (±0.02)	6.8 (±2)	4.0

[a] Values are means of at least three experiments, standard deviation is given in parentheses.
[b] EC_{50}s are corrected for actual compound concentration following the first dilution with assay buffer from 20 mM DMSO stock solutions. The concentration of compound was determined by HPLC after centrifugation of the sample.
[c] Log *P* predictions were calculated using the JChem 5.0.0 software from Chemaxon (see Ref. [17]).

observed for the truncated benzimidazole carboxylic acids, the 3-furyl analogs **A1** and **B1** provided the strongest intrinsic inhibition of the enzyme. Cyclopentyl N^1-analogs **A8** and **B8** were both 2–3-fold less potent than the corresponding cyclohexyl analogs **A1** and **B1** and suggest that addition of the amide substituent on the benzimidazole scaffold likewise does not affect binding at this sterically sensitive position compared to truncated analogs.[7]

A similar study was conducted in the diamide-cinnamic acid sub-series **C**, where we suspected that introduction of a carboxylic acid moiety at the far right-hand side of the molecules could conceivably affect overall inhibitor binding interactions. As seen in Table 1 however, binding was consistent throughout the series of C-2 substituents investigated, with IC_{50} values ranging from 50 to 200 nM and the trends reflected those of tryptophan sub-series **A**. Again, a twofold decrease in potency was observed between the optimized cyclohexyl analog **C1** and its cyclopentyl counterpart **C8**. The C-2 SAR detailed in Table 1 are represented graphically in Figure 2 for the three sub-series. Despite the differences in absolute potency, the trends at C-2 and N^1 of the benzimidazole scaffold were consistent as the inhibitor series progressed from small, less potent truncated benzimidazole 5-carboxylic acids[7] to more elaborated amide derivatives (sub-series **A**–**C**).

Representative compounds from sub-series **C** were tested for specificity against another RNA-dependent RNA polymerase from

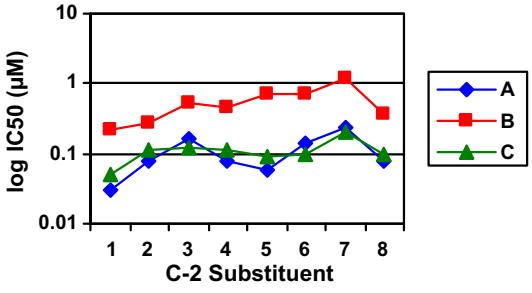


Figure 2. Comparison of C-2 SAR in three benzimidazole sub-series. Refer to Table 1 for structure of C-2 substituents (compounds **1–8**) and series **A–C**.

polio virus and a mammalian DNA-dependent RNA polymerase II isolated from calf-thymus.[7]

In all cases, no significant inhibition was observed at concentrations up to 250 μM (IC_{50} >250 μM). Cinnamic acid derivatives from sub-series **C** also provided improved potency in the cell-based gt1b replicon assay with EC_{50} = 0.7–6.8 μM. In particular, the 2-pyridyl analog **C6**, with an EC_{50} = 700 nM, provided improved potency and a more 'drug-like' alternative to 3-furyl analogs such as **C1**. Expansion of the cyclobutyl linker ring of **C6** to a cyclopentyl ring

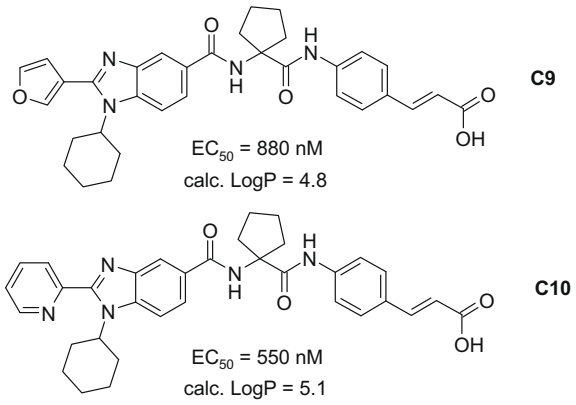


Figure 3. Cyclopentyl diamide derivatives.

provided compounds **C9** and **C10** with slight increases in lipophilicity[17] and sub-micromolar cell culture activity (Fig. 3).

Following the discovery of benzimidazole-cinnamic acid inhibitors with sub-micromolar cell-based potency in the replicon assay and drug-like structural features, the in vitro ADME profiles of analogs such as **C9** and **C10** were investigated and compared to thiazole-based inhibitor **B1**. Human and rat liver microsome stabilities, Caco-2 permeability, protein binding and CYP inhibition data are presented in Tables 2 and 3.

Neutral thiazole analog **B1** was insoluble at pH 7.2 and exhibited no detectable apical to basolateral permeability in the Caco-2 cell model. Furthermore, the compound was rapidly metabolized in the presence of human liver microsomes and displayed strong inhibition of CYP3A4 in the sub-micromolar range (see Table 3). In contrast, cinnamic acid analogs **C9** and **C10** had moderate solubility at pH 7.2 due to the presence of the weakly ionized carboxylic acid function. Both inhibitors showed good permeability of Caco-2 monolayers, and suggest that the weakly basic benzimidazole scaffold and the deactivated carboxylic acid function do not promote formation of zwitterionic species to a significant extent. Phase 1 metabolic stability and CYP450 inhibition profiles were significantly improved with neither compound inhibiting CYP3A4 at 30 μM. Despite the relatively high lipophilicity (calcd Log *P* 4.8–5.4)[17] and presence of a free carboxylic acid function, both compounds exhibited a significant unbound fraction in the presence of human plasma protein.

Plasma exposure of compounds **C9** and **C10** were evaluated in rat following oral administration of a 5 mg/kg dose.[18] While no **C9** plasma exposure was observed, compound **C10** was readily detected in plasma with a C_{max} = 0.58 μM (T_{max} = 0.5 h) and AUC = 0.51 μM h. Consistent with the modest AUC, an oral MRT (mean residence time) of 0.74 h was measured, indicative of rapid clearance from the plasma compartment.

In conclusion, we established that SAR at the C-2 and N^1-position on the scaffold of benzimidazole-based Thumb Pocket I inhibitors of HCV NS5B polymerase is not affected by the nature of the right-hand side of the molecule (truncated carboxylic acids or three distinct amide sub-series) which suggests a common binding mode of these molecules to the enzyme allosteric site. We also identified a more 'drug-like' replacement for the initial 3-furyl group that provides slightly improved potency and ADME profile. A prototype from this series provided modest plasma exposure and a short MRT after oral administration to rats. Further optimization of this class of inhibitors will be reported in the near future.

Table 2
In vitro ADME profile for compounds **B1**, **C9** and **C10**

Compd	Calcd Log *P*[a]	Solubility[b] (μg/mL)	Caco-2 (cm/s)	HLM/RLM[c] $T_{1/2}$ (min)	%PB[d]
B1	5.4	<0.1	N.A.	26/N.A.	N.A.
C9	4.8	125	3.9×10^{-6}	77/54	96.2
C10	5.1	100	9.5×10^{-6}	67/17	87.6

N.A.: not available.
[a] Log *P* predictions were calculated using the JChem 5.0.0 software from Chemaxon (see Ref. 17).
[b] 24 h shaking flask method (pH 7.2 phosphate buffer, amorphous TFA salt).
[c] $T_{1/2}$ at 10 μM in human or rat liver microsomes.
[d] Protein binding was determined by the equilibrium dialysis method.

Table 3
CYP inhibition profile for compounds **B1**, **C9** and **C10**

Compd	IC_{50} CYP450 inhibition (μM)				
	1A2	2C9	2C19	2D6	3A4
B1	>30	2.3	2.4	4.0	0.44
C9	>30	11.5	7.7	>30	>30
C10	>30	4.0	7.4	>30	>30

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16. EC_{50} determinations were performed in duplicates (or more) using Huh-7 cells with a stable sub-genomic HCV 1b replicon that encodes a modified luciferase reporter gene as previously described in: Tsantrizos, Y. S.; Chabot, C.; Beaulieu, P. L.; Brochu, C.; Poirier, M.; Stammers, T.; Thavonekham, B.; Rancourt, J. WO 05/080388, 2005. Compounds were devoid of cytotoxicity (TC_{50} >10 μM) at inhibitory concentrations. A sub-set of inhibitors were also tested in a 72 h sub-genomic replicon assay where HCV RNA levels were normalized to total cellular RNA. Similar EC_{50} values were determined in both assays and the quantification of total RNA recovery allowed for an alternative assessment of cellular homeostasis to eliminate the possibility of antiviral activity due to

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17. JChem 5.0.0 (http://www.chemaxon.com) was used for Log *P* predictions. The prediction method is based on the algorithm described in Viswanadhan, V. N.; Ghose, A. K.; Revankar, G. R.; Robins, R. K. *J. Chem. Inf. Comput. Sci.* **1989**, *29*, 163.
18. Male Sprague-Dawley rats were starved overnight and dosed by oral gavage at 5 mg/kg using 0.5% methocel and 0.3% Tween-80 as vehicle. Plasma samples from three animals were pooled at each time points (0–8 h) for analysis. Compound detection in plasma samples was performed following liquid–liquid extraction and HPLC analysis using UV detection.