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I. Novel HCV NS5B polymerase inhibitors: Discovery of indole 2-carboxylic acids with C3-heterocycles

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ABSTRACT

SAR development of indole-based palm site inhibitors of HCV NS5B polymerase exemplified by initial indole lead **1** (NS5B IC_{50} = 0.9 μM, replicon EC_{50} >100 μM) is described. Structure-based drug design led to the incorporation of novel heterocyclic moieties at the indole C3-position which formed a bidentate interaction with the protein backbone. SAR development resulted in leads **7q** (NS5B IC_{50} = 0.032 μM, replicon EC_{50} = 1.4 μM) and **7r** (NS5B IC_{50} = 0.017 μM, replicon EC_{50} = 0.3 μM) with improved enzyme and replicon activity.

Hepatitis C virus (HCV) is a major health hazard affecting over 170 million individuals worldwide[1a] and its infection is a leading cause of chronic liver cirrhosis and death from liver disease in the United States.[1b,c] The current standard of care is treatment with a combination of subcutaneous pegylated interferon administration with oral dosing of the cytotoxic nucleoside drug ribavirin.[2] The response rate is >75% for HCV patients with genotypes 2 and 3 after a 24 week treatment regimen while genotype 1 patients have a response rate of less than 50% after 48 weeks of treatment.[3] With a clear opportunity to improve clinical outcomes, and given the side effects associated with the current standard of care, it is valuable to discover potent inhibitors of HCV replication that will improve outcomes and shorten treatment duration.

The HCV NS5B protein is an RNA-dependent RNA polymerase critical for the synthesis of progeny viral genomes. The crystal structure of HCV NS5B displayed an overall subdomain architecture similar to other members of the PolI family,[4] with a deep active site cavity, located at the top of the 'palm' subdomain, and sealed at its base by a unique β-loop. Furthermore, there was an unexpected interaction identified between the tip of the 'fingers' subdomain and the 'thumb' subdomain to encircle the presumed nucleoside triphosphate substrate entry trajectory (Fig. 1).

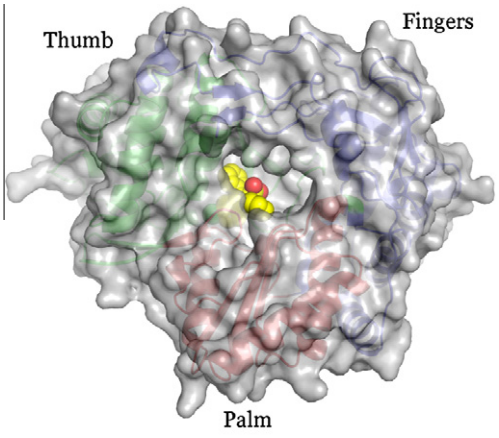

Figure 1. Overall protein molecular surface of NS5B with compound **1** (yellow spheres) bound within the active site cavity at the palm site. The 'fingers', 'palm', and 'thumb' domains are labeled and colored in blue, red, and green, respectively, beneath the molecular surface.

Sequence variation analysis suggests that residues lining the active site cavity ('palm site') more conserved than in other regions, such as the 'thumb-site'. This makes the palm site an attractive target for inhibition of the viral polymerase, though

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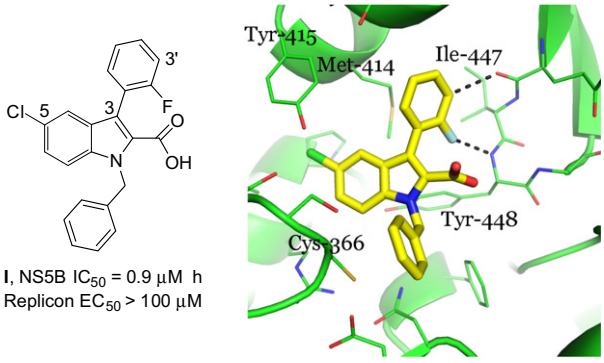


Figure 2. X-ray structure of lead indole acid analog **1** bound within the active site cavity of NS5B at the palm site. Residues presenting sidechain atoms within 5 Å of the compound are shown, along with backbone atoms of Ile-447 and Tyr-448. An unusual F···HN interaction and a close CH···O=C approach are illustrated by dotted lines.

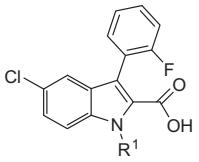
not all residues lining this site are absolutely conserved. Clinical efficacy has been demonstrated with non-nucleoside inhibitors binding at the palm, thumb, and finger-loop subdomains.[5]

High throughput screening of HCV NS5B polymerase using a novel trinucleotide-primed assay,[6] designed to respond to both initiation and elongation inhibitors, revealed a structurally-similar cluster of weak indole inhibitors exemplified by initial lead **1** with ~0.9 μM binding affinity. The X-ray structure of NS5B with initial lead **1** (Fig. 2), confirms that compound **1** binds within the active site cavity of the apoprotein at the 'palm site'. The *N*-benzyl group of inhibitor **1** stacks upon Cys-366. The C2 carboxylate does not contact the protein directly. Hydrophobic interactions from the indole core and the N1 phenyl ring dominated the contacts, and make no specific polar interactions. The indole core and the C3 phenyl ring are in contact with the side chain of Met-414. Of particular note is the identification of an unusual F···HN interaction with a 2.6 Å distance between the C3 aromatic fluorine and the protein backbone of Tyr-448. The phenyl 3′-carbon adjacent to the fluorine is only 2.9 Å from the backbone carbonyl oxygen of Ile-447, well within the van der Waals contact distance. The indole 5-chlorine atom protrudes into a constricted tunnel formed primarily by hydrophobic and aromatic residues such as Leu-384 and Tyr-415.

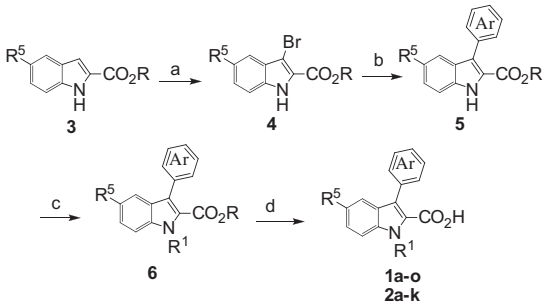
The initial efforts were focused on variation of the indole N1 substituent with selected SAR is presented in Table 1. Synthesis of the analogs in Table 1 generally followed a route as outlined in Scheme 1. Commercially available indole 2-carboxylic acid esters (ethyl or methyl) were selectively brominated at the C3 position by treatment with *N*-bromosuccinimide. Bromide **4** was subjected to Suzuki coupling with aryl boronic acids using $Pd(dppf)Cl_2$ as the catalyst. N-alkylation of the indole was achieved by treatment with an appropriate halide and Cs_2CO_3 as the base. It should be noted that other bases such as sodium hydride also gave satisfactory yields. In some examples the arylation and alkylation steps were done in a reverse order. In the final step, the indole C2 acids were prepared by hydrolysis of the corresponding ester using aqueous 1 N LiOH. In cases were there was no substitution at N1 (**1a**), the corresponding functionalization step was omitted.

The 5-chloro indole analog (**1a**) with an unsubstituted N1 was approximately 10-fold less potent than **1**, while introducing small alkyl substitutions like methyl at the N1 position were tolerated (**1b**). Both acetyl and benzoyl substitutions at the N1 position showed eightfold reduced enzyme activity (**1c,d**). Bulky lipophilic substitution such as naphthalene-2-ylmethyl was not tolerated (**1e**). The observed N1 SAR trend, coupled with the fact that the screening hits were part of a larger library with diverse N1 substitutions (not described), suggested the critical nature of the N1

Table 1
Indole-N1 substitutions



Compds	R^1	IC_{50} (μM)[7]
1	Benzyl (lead)	0.9
1a	H	7.3
1b	Methyl	1.3
1c	Acetyl	8.1
1d	Benzoyl	7.8
1e	Naphthalene-2-ylmethyl	4.4
1f	3-Me-benzyl	2.5
1g	3-CF_3-benzyl	>44
1h	3-CF_3O-benzyl	1.8
1i	2,5-F-benzyl	0.3
1j	2-NH_2-benzyl	0.6
1k	3-NH_2-benzyl	0.2
1l	3-NH_2, 4-Me-benzyl	1.3
1m	3-AcNH-benzyl	0.6
1n	3-BnNH-benzyl	0.7
1o	Pyridin-3-ylmethyl	1.3
1p	Pyridin-4-ylmethyl	1.2



Scheme 1. General synthesis of analogs. Reagents and conditions: (a) NBS, THF, rt; (b) Ar-$(B(OH)_2$, $Pd(dppf)Cl_2$, K_2CO_3, H_2O, DME, reflux; (c) R^1-Br, Cs_2CO_3, DMF, rt; (d) aq 1 N LiOH, TFH, reflux.

benzyl scaffold despite the lack of any obvious interaction with the protein. The focus was shifted to substitutions around the phenyl portion of the N1-benzyl group. The X-ray structure with **1**, showed that the benzyl group was close enough to the protein backbone that substitutions offered a chance to identify new interactions. Lipophilic benzyl substituents such as 3-methyl (**1f**), 3-trifluoromethyl (**1g**) and the 3-trifluoromethoxy analog (**1h**) were deleterious relative to the initial lead. On the other hand, 2,5-difluoro substitution of the benzyl group had a beneficial effect on the enzyme activity (**1i**, IC_{50} = 0.3 μM). Both 2- and 3- amino substitutions also showed improved activity, with a regiochemical preference (**1j**,**k**, IC_{50} = 0.6–0.2 μM) for 3-amino substituted benzyl. A follow up focused library of ~300 compounds with functionalized 3-amino substitutions to probe additional interactions with protein backbone did not identify any compounds with improved activity. The analogs which showed comparable activity to **1k** were the 3-acetyl and 3-amino benzyl analogs, **1m** and **1n**. The aniline liability of **1k** prompted preparation of pyridine–methyl analogs **1o** and **1p** which were equipotent with initial lead **1**.

The X-ray structure of the lead **1** revealed that the C3-fluorophenyl group is close to the protein backbone. Efforts were made to understand the potential interactions in this region as