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The discovery and structure–activity relationships of pyrano[3,4-*b*]indole-based inhibitors of hepatitis C virus NS5B polymerase

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ABSTRACT

We describe the structure–activity relationship of the C7-position of pyrano[3,4-*b*]indole-based inhibitors of HCV NS5B polymerase. Further exploration of the allosteric binding site led to the discovery of the significantly more potent compounds **13** and **14**.

Hepatitis C virus (HCV) infection continues to be a major global health issue with recent total population infection estimates of 1-3%.[1] HCV is the leading cause of advanced liver disease and transplantation.[2] Complications from chronic infection are responsible for about 10,000–12,000 deaths/year in the U.S.[2]

Current therapies for HCV involve a combination of the nucleoside analog ribavirin and interferon-α.[3] This treatment regimen causes unfavorable side effects which often results in poor patient compliance. Furthermore, only about 40% of patients infected with HCV achieve a sustained virologic response.[4] Recently, pegylated forms of interferon-α (Pegasys™ and PEG-INTRON™) have improved patient tolerance and response rates of over 50% have been achieved. Sustained virologic responses have also been reported with the combination of long-acting interferons with several NS3/4a protease inhibitors of HCV.[5] Some promising NS5B HCV polymerase inhibitors are currently in various stages of development. Similar to the successful anti-HIV 'cocktail' treatments, future HCV therapies will most likely involve the combination of agents that inhibit different viral targets. Clearly there is a medical need for additional HCV antiviral agents.

Hepatitis C belongs to the *Flaviviridae* family of positive-single stranded RNA viruses.[6] The HCV genome encodes a 3000 amino acid polyprotein which is processed into structural and non-structural proteins. One of the non-structural proteins that is essential for viral replication is the NS5B RNA-dependent RNA polymerase.[7,8] The HCV NS5B is an attractive target owing to the success of other antiviral agents that inhibit viral polymerases such as the HIV non-nucleoside reverse transcriptase inhibitors (NNRTI's).[5] We have recently reported on a pyranoindole series of allosteric HCV NS5B inhibitors that resulted in a potent analog demonstrating anti-viral activity in the chimeric mouse model of HCV infection.[9] This communication will describe our optimization efforts and structure-activity relationships at the C7-position of this novel pyranoindole series of allosteric HCV NS5B enzyme inhibitors that ultimately led to significantly more potent analogs.

Several thumb domain directed inhibitors of HCV NS5B have been identified (Fig. 1).[8] Compounds **1–4** occupy the same allosteric binding site (Thumb Pocket II) of HCV NS5B with their acidic functionalities participating in hydrogen bonding to the Ser^{476} and Tyr^{477} residues.

Another critical feature of these ligands is the ability to occupy a hydrophobic region defined by residues Leu^{419}, Trp^{528}, Tyr^{477}, and Arg^{422}. Co-crystallographic analysis has determined that this hydrophobic 'dimple' on the NS5B protein is optimally occupied by the C1 *n*-propyl group of **1**.[9] Further examination of the protein

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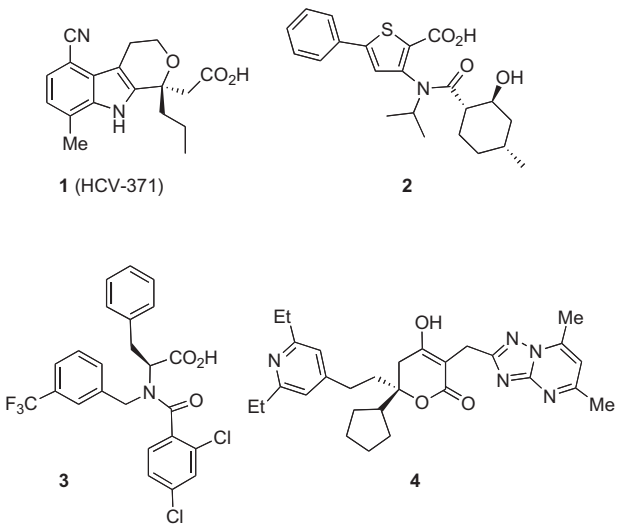


Figure 1. Reported allosteric thumb pocket II HCV NS5B inhibitors.

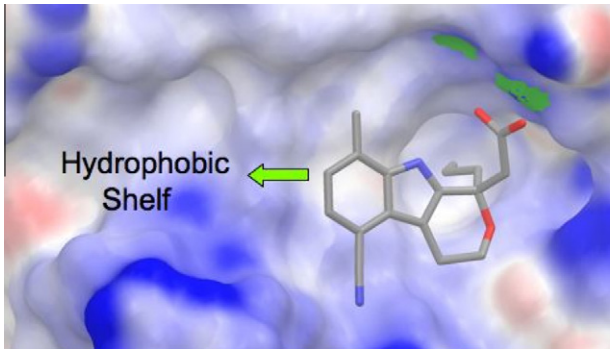


Figure 2. Crystal structure of NS5B with tetrahydro[3,4-*b*]indole inhibitor. Electrostatic potential representation: Red-negative charge; Blue-positive charge; White-non-polar; Ser^{476} & Tyr^{477} highlighted in Green.

reveals a hydrophobic shelf near the aromatic core of the pyranoindole (Fig. 2). We hypothesized that this shelf may provide an opportunity for additional protein-ligand interactions affording compounds with enhanced binding affinity. Structural analysis suggested that substitution at the C7 position of the pyranoindole nucleus would provide the required trajectory for accessing this hydrophobic region (See Fig 2).

Our strategy relied on a flexible synthetic route that allows for introduction of a variety of substitutions with the initial focus on carboxamides as shown in Scheme 1.

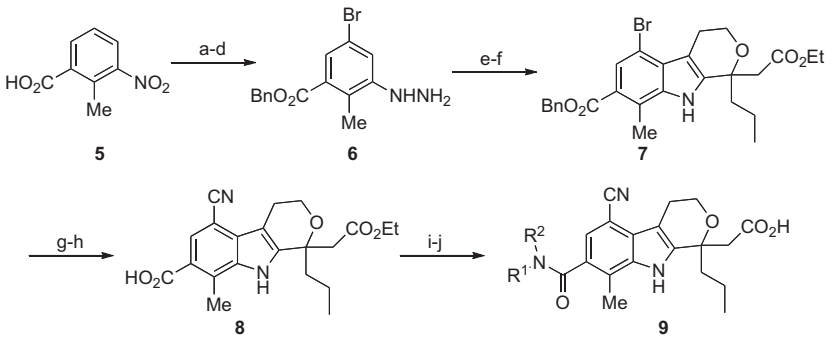
Representative assay results for the carboxamide series are shown in Table 1. All of these analogs were prepared as racemates. These amide analogs are generally less active against the HCV NS5B enzymes than the unsubstituted derivative (**1**), although **9e** and **9g** appear to be equipotent to **1**.[10] Disubstituted amides such as **9b** are not well tolerated.

We were encouraged by these results because they demonstrated that the C7-substituted pyranoindoles were tolerated in the thumb domain binding site. However, the C7-containing benzamides had poor activity in the cell-based HCV replicon assay.

We reasoned that the decrease in cellular activity was related to the higher polar surface areas (PSA) of the amides relative to **1**,[11] and that replacement of the amide linker with a less polar functionality may restore the cellular activity. Therefore, we turned our attention to preparing a series of C7-substituted ethers (Scheme 2). The benzoic acid functionality in **8** was converted into the aldehyde derivative (**10**) which then underwent a Baeyer–Villiger oxidation to provide phenol **11**. The racemic ethers (**12**) were formed by either standard alkylation or Mitsunobu reactions.

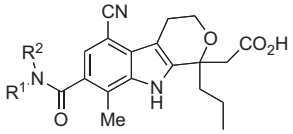
Representative C7-ether analogs were significantly more potent against the HCV polymerase enzymes compared to **1** (Table 2). Phenol **12a** was approximately twice as potent as **1** against BK and BB7 enzymes but possessed no appreciable cellular activity. The C7 methyl ether (**12b)** was similarly active against the polymerase and equipotent to **1** in the cell-based replicon assay. Optimal tether lengths were observed through preparation of the ethyl, *n*-propyl and the methoxyethyl analogs (**12c**, **12d**, and **12f**, respectively). These analogs all demonstrated similar cellular activities as well as improved affinities against the isolated NS5B enzymes suggesting that these compounds were accessing the hydrophobic shelf. Alkyl branching (**12e**) or incorporation of a basic amine (**12g**) were not well tolerated.

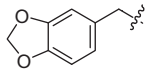
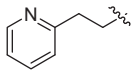
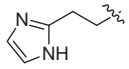
Further SAR development of the C7 ethers showed that heteroaromatic substrates tethered via an alkyl chain were consistently among the most potent compounds (Table 3). The 2-pyridylmethyl compound (**12i**) was significantly more active than **1** in the replicon assay. The 4-pyridylmethyl analog was slightly less active, and the addition of an extra methylene unit into the tether resulted in a 10-fold drop in enzyme inhibition. Alkyl-substitution on the pyridine ring was generally well- tolerated but did not provide significant advantages (not shown), nor did addition of a second nitrogen (**12k**). Five-membered heteroaromatic substituents were also highly potent inhibitors of the HCV polymerase. The 5-methylisoxazole (**12l**) was among the most potent with sub-micromolar activity in the cell-based replicon assay. Co-crystallographic analy-



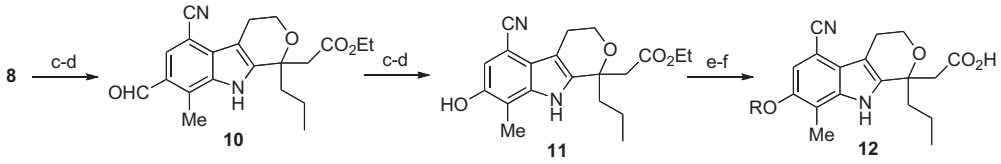
Scheme 1. Reagents: (a) *N,N*-dibromodimethyl-hydantoin, H_2SO_4, 99%; (b) BnBr, DIPEA, THF, 84%; (c) Fe, NH_4Cl, H_2O, 100 °C, 92%; (d) $NaNO_2$, HCl, then $SnCl_2$, 93%; (e) Dihydrofuran, $ZnCl_2$, H_2O, ethylene glycol, 100 °C, 27%; (f) Ethyl-3-oxapentanoate, $BF_3{\cdot}OEt_2$, DCM, 84%; (g) CuCN, NMP, 190 °C, 82%; (h) H_2, $Pd(OH)_2$, MeOH/EtOAc, 96%; (i) R^2R^1NH, HATU, NMM, DCM; (j) NaOH, H_2O, EtOH.

Table 1
C7-Pyranoindole carboxamides



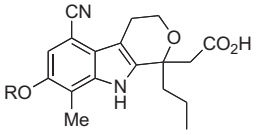
Compd	R^1	R^2	HCV pol (BK) IC_{50}, μM	HCV pol (BB7) IC_{50}, μM	HCV NS5A EC_{50}, μM	PSA
1	—	—	0.3	0.4	13.1	86.1
9a	H	Me	1.3	0.97	>100	115.2
9b	Me	Me	16	20	>100	106.4
9c	H	*n*Pr	3	2	>100	115.2
9d	H	Bn	0.98	0.67	>100	115.2
9e	H		0.54	0.3	56	133.6
9f	H		1.2	0.64	>100	128.1
9g	H		0.86	0.56	>100	143.9

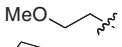
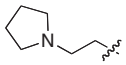
*A description of the assay conditions used to determine IC_{50}'s and EC_{50}'s can be found in Ref. 9d.



Scheme 2. Reagents and conditions: (a) BH_3·THF, THF, 77%; (b) MnO_2, C_6H_6, 1,2-DCE, reflux, 96%; (c) SeO_2 (0.1 equiv), H_2O_2, *t*-BuOH, H_2O, TFA; (d) K_2CO_3, MeOH, 73%-2 steps; (e) R-X, K_2CO_3, DMF or ROH, DIAD, PPh_3, DCM; (f) NaOH, H_2O, EtOH.

Table 2
Initial C7-alkoxy-pyranoindoles



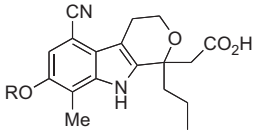
Compound	R	HCV pol (BK) IC_{50}, μM	HCV pol (BB7) IC_{50}, μM	HCV NS5A EC_{50}, μM	PSA
1	—	0.3	0.4	13.1	86.1
12a	H	0.26	0.2	47	106.3
12b	CH_3	0.36	0.13	20	95.3
12c	Et	0.11	0.07	3.5	95.3
12d	*n*Pr	0.07	0.07	2.9	95.3
12e	*i*Pr	0.48	0.22	19.7	95.3
12f		0.04	0.09	4.9	104.6
12g		1.1	0.95	26	98.6

sis has determined that the pendant isoxazole group of **12l** occupies the proximal hydrophobic shelf (See Fig 3). Dimethylpyrazole **12m** had an EC_{50} of 0.76 μM in the replicon assay. The 2-pyrazolylethyl analog (**12n**) was among the most active analogs prepared in this series. The incorporation of a (*S*)-methyl group adjacent to the pyrazole ring of **12n** increased potency even further, generating a compound (**12o**) with a replicon EC_{50} of 0.12 μM while diastereomer **12p** was less active (EC_{50} = 0.34 μM).

Parallel efforts of optimizing the C1 position of the pyranoindole demonstrated that replacement of the *n*-propyl group with (*S*)-*sec*-butyl afforded susbstrates with improved potencies.[9b] Towards this end we combined the C7-ethyl-linked pyrazole group with the (*S*)-*sec*-butyl substitution (**13** and **14**, Table 4) that produced significant increases in cellular activities.[9a] The pyrazole containing analogs are the most potent pyranoindole-based HCV inhibitors prepared to date, and **13** has been shown to be efficacious in a chimeric mouse model of hepatitis C infection.[9a]

We have described the evolution and SAR of a C7-substituted pyranoindole-based series of HCV NS5B polymerase inhibitors. Through structure-based design, we have identified and detailed

Table 3
C7-Heteroaryl-containing pyranoindoles



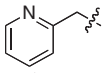
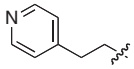
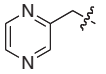
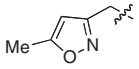
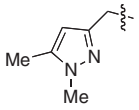
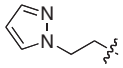
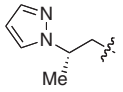
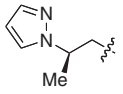
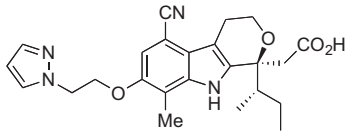
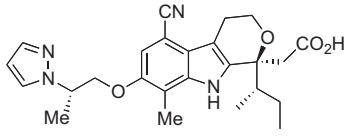
Compound	R	BK IC_{50} (μM)	BB7 IC_{50} (μM)	HCV NS5A EC_{50} (μM)	PSA
1	–	0.3	0.4	13.1	86.1
12i		0.02	0.02	0.39	108.2
12j		0.31	0.23	8.3	108.2
12k		0.019	0.02	1.6	121.1
(**1R**)-**12l**		0.006	0.008	0.48	121.4
(**1R**)-**12m**		0.03	0.02	0.76	113.2
(**1R**)-**12n**		0.005	0.005	0.88	113.2
(**1R**)-**12o**		0.003	0.003	0.12	113.2
(**1R**)-**12p**		0.007	0.007	0.34	113.2

Table 4
C1-*sec*-Butyl analogs

Compound	Structure	BK IC_{50} (μM)	BB7 IC_{50} (μM)	HCV RNA EC_{50} (μM)
13		0.012	0.003	0.02
14		0.002	0.002	0.02

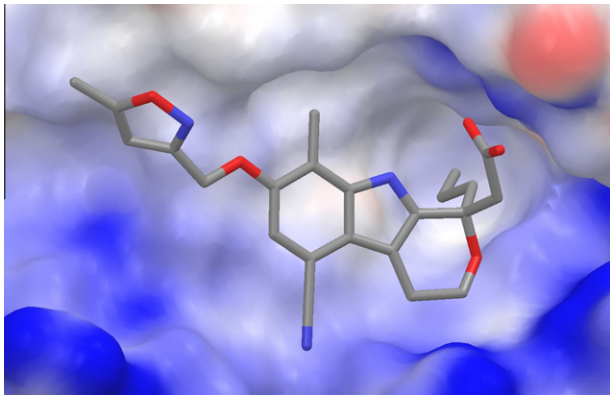


Figure 3. Crystal structure of NS5B with tetrahydro[3,4-*b*]indole inhibitor **12l**. Electrostatic potential representation: Red-negative charge; Blue-positive charge; White-non-polar.

the preparation and biological activities of these potent inhibitors. Significant improvements in enzyme and cellular potencies were realized by incorporating ether substitutions at the C7-position of the pyranoindole nucleus. Ultimately, we were able to identify ethoxy- pyrazole analogs **13** and **14** that were significantly more potent than **1**.

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10. The IC_{50}'s are an average of multiple experiments with the standard deviations generally within 10% of this value.
11. Physicochemical properties calculated using Schrödinger 2010 Suite, Maestro 9.1-QikProp 3.3; Schrödinger, LLC: New York.