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Identification and structure-based optimization of novel dihydropyrones as potent HCV RNA polymerase inhibitors

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Dedicated to Professor K. C. Nicolaou on the occasion of his 60th birthday.

Abstract—A novel class of non-nucleoside HCV NS5B polymerase inhibitors has been identified from screening. A co-crystal structure revealed an allosteric binding site in the protein that required a unique conformational change to accommodate inhibitor binding. Herein we report the structure–activity relationships (SARs) of this novel class of dihydropyrone-containing compounds that show potent inhibitory activities against the HCV RNA polymerase in biochemical assays.

Hepatitis C virus (HCV) infects approximately 3% of the global population and represents a major public health problem worldwide. In the US, it has been estimated that there are more than 4 million infections, mostly with genotype 1a and 1b. Of those individuals exposed to HCV, 80% become persistently infected, and about 20–30% of the chronically infected population eventually develops progressive liver diseases, including cirrhosis, hepatocellular carcinoma (HCC), and liver failure.[1] The current standard care of treatment for chronic hepatitis C is based on the combination of subcutaneous pegylated interferon-α and oral nucleoside drug ribavirin. However, serious side effects and poor response rates, particularly among patients with genotype 1, render the development of novel anti-HCV therapy an urgent need.[2]

HCV is a positive-sense, single-stranded-RNA virus. The viral genome encodes a single poly-peptide with about 3000 amino acid residues, which upon cleavage releases at least 10 structural and non-structural proteins. The HCV NS5B protein has been characterized as an RNA-dependent RNA polymerase (RdRp)[3] and is essential for viral replication. Because of the considerable differences as compared to human DNA and RNA polymerases, the HCV NS5B is an attractive target for selective anti-viral therapy.

Development of HCV NS5B polymerase inhibitors, particularly at allosteric binding sites, has gained increasing interests in recent years.[4] A number of compounds spanning multiple chemotypes have been reported in the literature (Fig. 1). Based on both mutational sites in the replicon system and protein crystallographic studies, several different allosteric binding sites of the HCV NS5B protein have been identified.[5] Benzimidazole inhibitor **1**[6] has been reported to bind to the non-catalytic GTP site within the thumb domain. An X-ray crystal structure with the indole analog **2**[7] also confirmed this allosteric binding site close to residue Pro-495. Benzothiadiazine analogs, such as **3**,[8] inhibit the initial step of RNA synthesis and induce a resistant mutation M414T close to the base of the thumb domain. Another allosteric binding site in the thumb domain was reported with both substituted phenylalanine **4**[9,10] and

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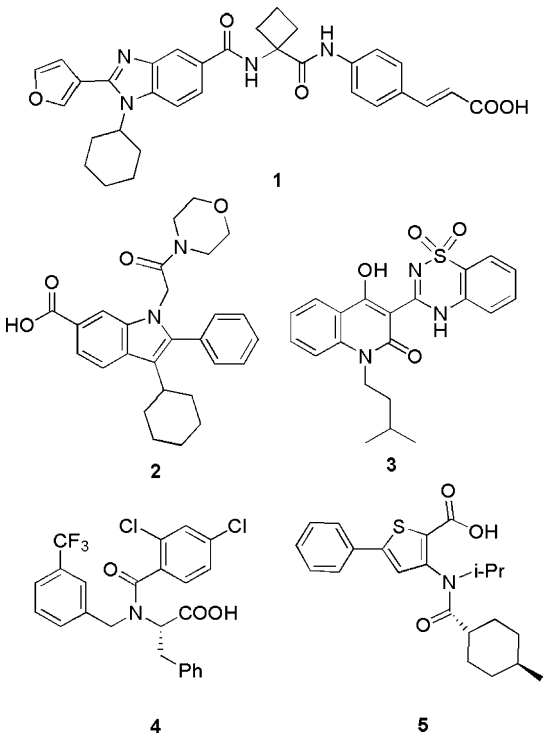


Figure 1. Selected HCV NS5B polymerase inhibitors.

thiophene carboxylic acid derivatives **5**.[11,12] Recently two new allosteric binding sites,[13,14] the anchor grip and the primer grip sites, were also reported. In this paper, we report our own effort in the synthesis, preliminary structure–activity relationship (SAR), and biological properties of a series of dihydropyrones as HCV NS5B polymerase inhibitors.

High-throughput screening (HTS) of a proprietary collection of in-house compounds identified compound **6** (Fig. 2) as an HCV RNA polymerase inhibitor with modest potency (IC_{50} = 0.93 μM). Subsequent biochemical experiments demonstrated that compound **6** was a specific, reversible inhibitor of the HCV NS5B polymerase that bound non-competitively with respect to GTP. An X-ray co-crystal structure[15] of this compound with NS5B (genotype 1b, BK strain, CΔ21) triple mutant (Lys114Asn/Leu47Gln/Phe101Tyr) identified an allosteric binding site close to the junction of thumb and finger domain, approximately 30 Å away from the active site. Upon inhibitor binding the protein structure underwent

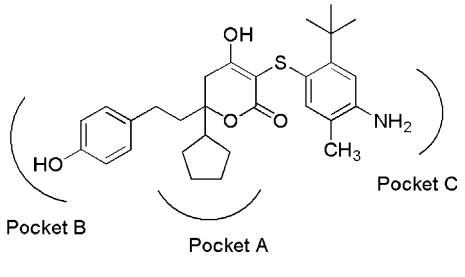


Figure 2. Dihydropyrone lead (**6**) from high-throughput screening.

a conformational rearrangement. During this process Met-423 and Leu-497 moved away from their original positions, opening up a new hydrophobic pocket (pocket A, Fig. 2). Examination of the X-ray co-crystal structure revealed some key interactions between compound **6** and the HCV polymerase protein. The enol/ketone oxygen of the dihydropyrone forms a direct hydrogen bond to the backbone amide NH of Ser-476 and a water-mediated hydrogen bond to the amide NH of Tyr-477 (the donor–donor motif). The lactone carbonyl of the dihydropyrone is involved in a water-mediated hydrogen bond to Arg-501. The phenol functional group forms another hydrogen bond with Leu-497 through a water molecule, while the phenyl ring occupies an otherwise hydrophobic pocket (pocket B). The extension of an additional aromatic ring from the dihydropyrone, through a sulfur linker, appears to make limited interactions with the protein (pocket C, Fig. 3).

The preparation of dihydropyrone analogs is outlined in Scheme 1. Two efficient synthetic approaches toward key intermediate ketone **11** were utilized. The first started from substituted phenylpropanoic acids, which upon reaction with dipyridyl disulfide and triphenylphosphine at ambient temperature provided pyridyl thioester **8** in good yields. Further reaction of intermediate **8** with cyclopentyl magnesium bromide afforded the desired ketone. The second method utilized a unique palladium catalyzed Heck reaction protocol between 1-cyclopentyl-2-propen-1-ol **10** and various aryl bromides (or triflates) **9**. After carbon–carbon bond formation and the subsequent enol/ketone isomerization, this route provided the desired ketone **11** in one single step compatible with broad R1 variation. Formation of the dihydropyrone ring from ketone **11** was straightforward. The dianion formed from methyl acetoacetate (NaH followed by *n*-BuLi) was treated with the ketone to afford the open chain product **12**, which upon reaction with NaOH in aqueous THF provided the ring-closed product **13**. Chlorination of intermediate **13** at the 3-position was accomplished by reaction of SO_2Cl_2 in CH_2Cl_2 with high regio-selectivity. The chloro-dihydropyrone product **14** served as a versatile template and reacted readily with various thiophenols under basic conditions in DMF to provide sulfur-linked analogs of general structure **15** in good yields.

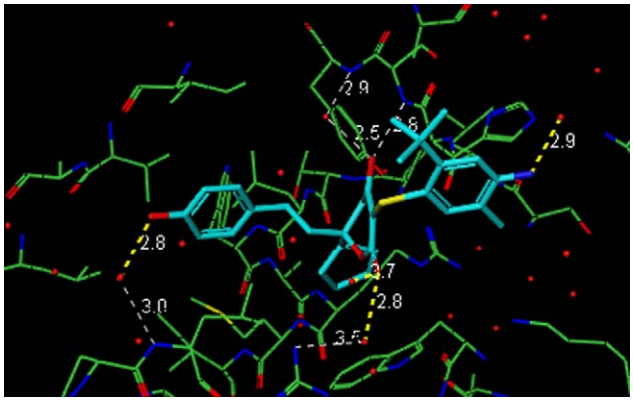
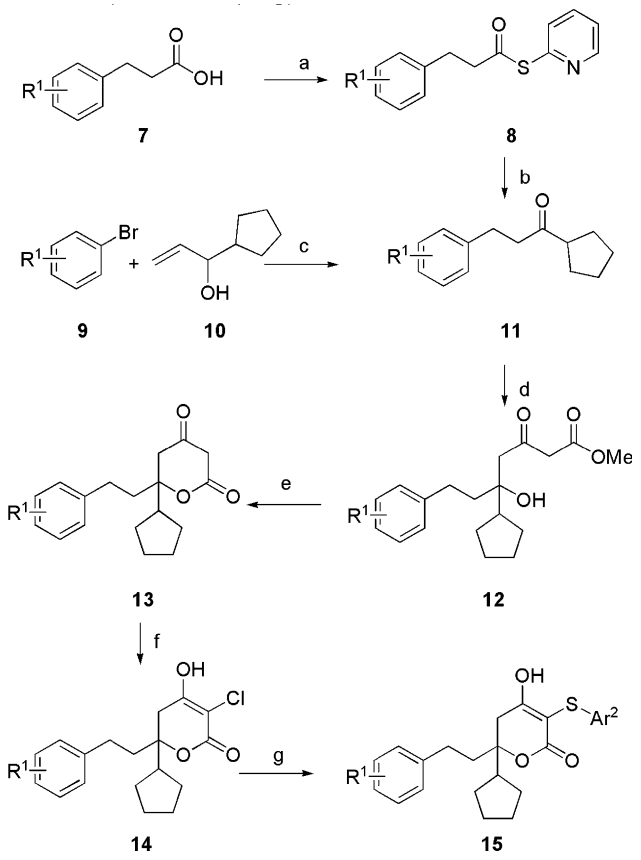


Figure 3. X-ray co-crystal structure (2.2 Å resolution) of compound **6** bound to the HCV ΔC21 NS5B protein.



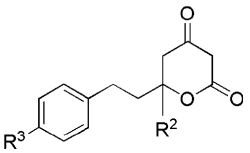
Scheme 1. Synthesis of dihydropyrone derivatives. Reagents and conditions: (a) PPh_3, 2,2′-dipyridyl disulfide, CH_2Cl_2; (b) cyclopentyl magnesium bromide, THF, −78 °C; (c) $Pd(PPh_3)_2Cl_2$, $NaHCO_3$, NMP, 140 °C; (d) NaH, methyl acetoacetate, followed by *n*-BuLi, −78 °C; (e) NaOH, THF/H_2O; (f) SO_2Cl_2, CH_2Cl_2; (g) Ar^2SH, DMF, 50 °C.

All compounds synthesized were assayed against HCV genotype 1b (BK strain) NS5B ΔC21 enzyme to assess their inhibitory activity (IC_{50}) as previously described.[15]

Early exploration was focused on evaluation of binding features of compound **6** in pockets A and B. Truncation of the sulfur-linked aniline of compound **6** (pocket C) resulted in an 8 fold loss in potency in the enzymatic assay (Table 1, **19**). However, this unsubstituted dihydropyrone simplified chemical synthesis and served as a good starting point for the further optimization of pockets A and B. Comparison of the co-crystal structure of compound **6** and the apo structure of the HCV NS5B protein revealed that the cyclopentyl ring forced a reorganization of residues Met-423 and Leu-497. This resulted in the placement of the cyclopentyl group, with a high degree of complementarity, into the newly formed pocket A. As summarized in Table 1, substitutions at this position with either larger or smaller groups proved to be detrimental for activity against HCV NS5B enzyme (compounds **16**–**18** and **20**).

A close analysis of the pocket B binding site revealed two smaller hydrophobic pockets, almost perpendicular to each other (Fig. 4).[16] Methylation of the hydroxyl

Table 1. Enzymatic potencies of dihydropyrones: pocket A optimization



Compound	R2	R3	IC_{50} (μM)
16	Me	OH	>100
17	*n*-Pr	OMe	>50
18	Cyclobutyl	OH	93
19	Cyclopentyl	OH	8.2
20	Cyclohexyl	OH	52

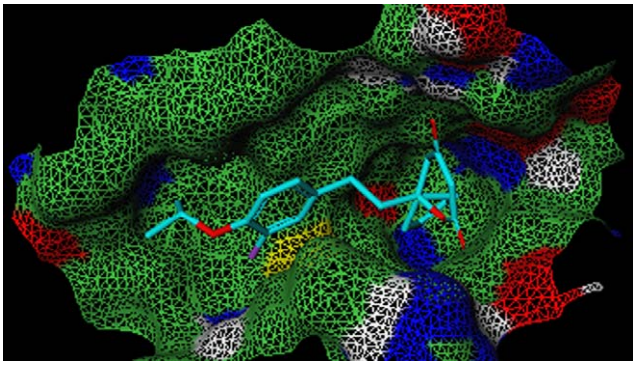
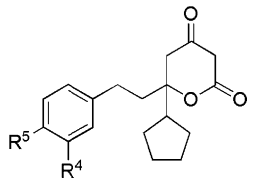


Figure 4. X-ray co-crystal structure of compound **33** (1.58 Å resolution) bound to HCV NS5B ΔC21 protein.

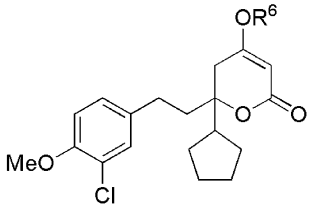
group of compound **19** at the *para* position of the phenyl ring afforded analog **22** with almost equal potency, indicating that a hydrogen bond acceptor is sufficient at this position to provide the interaction with Leu-497 through a water-mediated hydrogen bond. Extension from the oxygen with bulkier groups, such as Et or *i*-Pr (Table 2, **23** and **24**), further increased compound potency, probably by providing more hydrophobic interactions. Elimination of the oxygen atom from compound **24** in pocket B resulted in a 3-fold loss of potency (**25**, IC_{50} = 4.3 μM), which can be attributed to the loss of hydrogen bonding and decrease in hydrophobic interaction (Fig. 5).

Consistent with the X-ray co-crystal structure, substitutions at the *meta* position of the phenyl ring in pocket B were well tolerated and provided a second hydrophobic interaction (Table 2, **26**–**29**). A simple chlorine substitution (**27**, IC_{50} = 5 μM) afforded 10-fold potency improvement over unsubstituted derivative **21** (IC_{50} = 48 μM) and slightly larger groups, such as Et and *i*-Pr, further enhanced compound potency to 3.6 and 1.8 μM, respectively. In an attempt to evaluate the combined substitution effects at both *meta* and *para* positions, compounds **30**–**33** were prepared. It was found that a smaller group at the *meta* position, such as fluorine, in combination with *para*-alkyloxy substitution, afforded the most potent compound **33** with an IC_{50} of 0.53 μM.

Table 2. Enzymatic potencies of dihydropyrones: pocket B optimization



Compound	R4	R5	IC_{50} (μM)
21	H	H	48
22	H	MeO	9.7
23	H	EtO	2.9
24	H	*i*-PrO	1.7
25	H	*i*-Pr	4.3
26	Me	H	23
27	Cl	H	5.0
28	Et	H	3.6
29	*i*-Pr	H	1.8
30	Cl	MeO	1.0
31	F	MeO	0.89
32	F	EtO	0.79
33	F	*i*-PrO	0.53



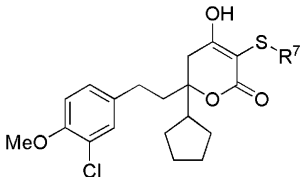
Compound 30, R^6 = H, IC_{50} = 1.0 uM
Compound 34, R^6 = Me, IC_{50} = 29 uM

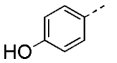
Figure 5. The importance of the enol hydrogen-bond donor.

The dihydropyrone core was essential for the potency of this series of compounds. Selective O-methylation of compound **30** afforded compound **34**, which was almost 30-fold less potent when compared with the free OH analog. It is reasonable to assume that a substantial contribution to this loss in potency of the racemate is accounted for by that enantiomer which places the extra methyl in the very constrained steric environment to interfere with the water-mediated hydrogen-bonding network between the dihydropyrone enol/ketone oxygen and the donor–donor motif (Ser-476 and Tyr-477) in the HCV NS5B protein.

With a 15-fold potency improvement from the pocket B optimization, pocket C fragments were re-introduced to further explore potency enhancement against HCV NS5B. Examination of the X-ray structure revealed that this portion of the molecule resided in a shallow canyon on the protein surface, with a possible π–π stacking interaction with His-475. A survey of various arylthio moieties demonstrated that a simple phenyl ring (Table 3, **35**) provided a moderate 2-fold advantage over the corresponding unsubstituted dihydropyrone **30**. An additional hydroxyl group, at the *para* position to the sulfur linker, afforded a 4-fold further

Table 3. Enzymatic potencies of dihydropyrones: pocket C optimization



Compound	R^7	IC_{50} (μM)
35		0.56
36		0.13
37		0.28
38		0.14
39		0.038

improvement (IC_{50} = 0.13 μM, Table 3, **36**). Five-membered heterocyclic ring systems generally provided better inhibitory potency against the HCV NS5B enzyme and the N-methyl triazole **39** exhibited the best potency within the series (IC_{50} = 0.038 μM).

All compounds were tested in replicon assays in the Huh-7 hepatoma cell line harboring a self-replicating HCV subgenomic replicon of genotype 1b.[17] However, none of them exhibited any significant antiviral activity up to 10 μM.

In conclusion, we have described the identification of a novel series of dihydropyrones as potent allosteric inhibitors of the HCV NS5B polymerase. Initial optimization of the series has resulted in more than a 30-fold increase in compound potency and led to analogs with nanomolar IC_{50} against the enzyme. Further efforts to improve compound enzymatic potency and physical/chemical properties to achieve antiviral activity in the cell-based replicon system will be reported in future communications.

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