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Finger-loop inhibitors of the HCV NS5b polymerase. Part 1: Discovery and optimization of novel 1,6- and 2,6-macrocyclic indole series

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ABSTRACT

Novel conformationaly constrained 1,6- and 2,6-macrocyclic HCV NS5b polymerase inhibitors, in which either the nitrogen or the phenyl ring in the C2 position of the central indole core is tethered to an acylsulfamide acid bioisostere, have been designed and tested for their anti-HCV potency. This transformational route toward non-zwitterionic finger loop-directed inhibitors led to the discovery of derivatives with improved cell potency and pharmacokinetic profile.

One hundred and seventy million people worldwide are currently infected with hepatitis C virus (HCV).[1] Chronic infection is the leading cause of liver disease, the largest indication for transplantation in Europe and the United States, eventually leading to liver cirrhosis.[2] Hepatocellular carcinoma (HCC) related to HCV infection has become the fastest growing cause of cancer-related death in the US, and the incidence of HCC has tripled over the past 20 years.[3] Increased survival rates and improved clinical outcome may be associated with sustained virologic response (SVR).[4] A higher overall SVR could be achieved when direct anti-virals, recently approved by the FDA,[5,6] compliment the precedent standard of care treatment, that is, using ribavirin and pegylated interferon.[7] Novel medicaments like these are especially important for the difficult to treat population including those with genotype 1 and patients with liver cirrhosis.[5] HCV can develop resistance to anti-viral monotherapy within a matter of days. Future therapies will evaluate the combination of newly targeted agents, that could be used to enhance the anti-viral activity and potentially translate into higher cure rates in shorter time.[8] One of these potential targets is the RNA-dependant RNA-polymerase (NS5b), essential for viral replication.[9] HCV NS5b inhibitors can be divided into two classes; nucleoside[10] and non-nucleoside inhibitors,[11] targeting the active site or an allosteric site, respectively.[12] Allosteric finger loop inhibitors based on the 3-cyclohexyl indole structure, or analogs thereof, that target the Thumb Pocket 1 site of the HCV NS5b polymerase have been described (Fig. 1).[13] The first indole inhibitors suffered from poor aqueous solubility (e.g., amide analogs of compound **1**)[14] which hampered their development as drug candidates.

To address this drawback, scientists developed zwitterionic derivatives exemplified by **2**,[15] exhibiting improved water solubility and drug-like properties. However, this series of compounds has been reported to form glucoronide conjugates on the carboxylic acid, which might eventually be responsible for toxicity linked to their acylating potential.[15] Moreover, zwitterionic drugs are often absorbed in the GI tract at specific locations (pH driven), which may lead to higher patient variability. In this context, we envisioned a different strategy dealing with the introduction of an uncharged polar solubilizing group in a macrocycle, which might eventually counterbalance the very lipophilic nature of the 3-cyclohexyl-2-phenylindole moiety.

Previously reported X-ray structures with indoles bound to the HCV polymerase suggest that a solvent exposed tether from the C6 carbonyl to the indole nitrogen or to the C2 aromatic ring would not hinder the binding affinity of these macrocycles. Indeed, when the carboxylic acid in C6 is replaced by an acyl sulfamide bioisostere,[16] (e.g., **3**, Fig. 1) the key interaction with Arg-503[17] is maintained, resulting in NS5b inhibition. Furthermore, a tether to the

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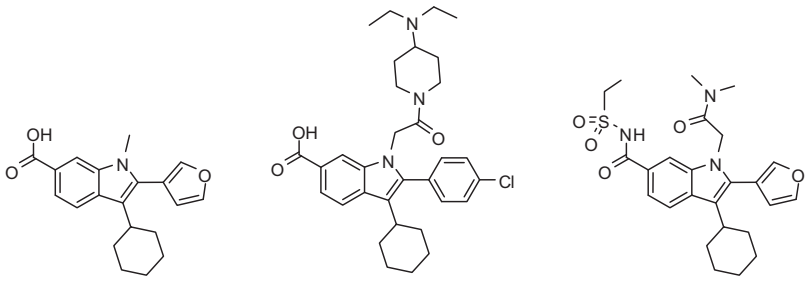


Figure. 1. Indole based finger loop inhibitors of NS5b polymerase.[14–16]

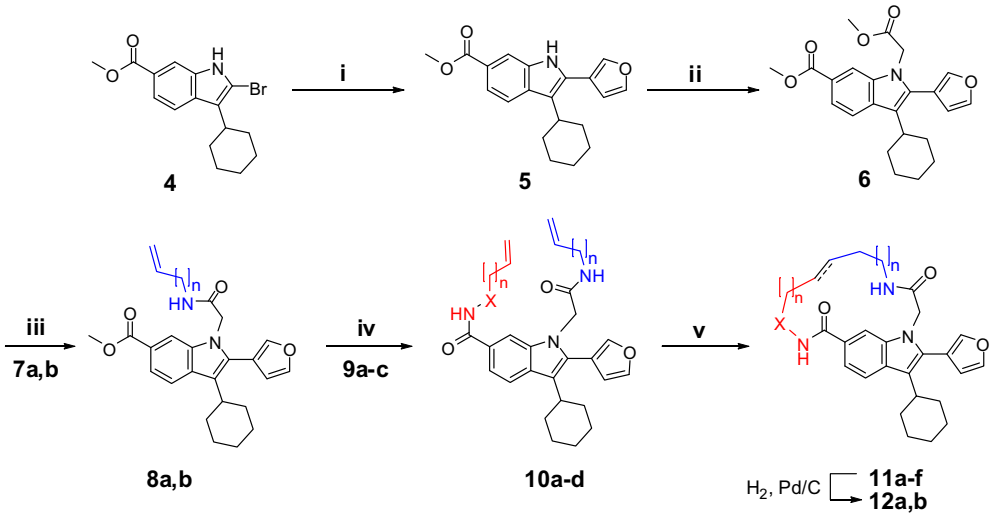
C2 aromatic ring would increase the affinity by holding the 46° dihedral angle between the phenyl and indole moiety as previously reported.[13c]

The synthesis of the macrocyclic indole derivatives **11a–d**, via ring closing metathesis (RCM), started with the 2-bromoindole derivative **4**[14,18] following the five step procedure outlined in Scheme 1. Aryl bromide **4** reacted with 3-furanboronic acid under standard Suzuki–Miyaura conditions in ethanol/toluene to give intermediate **5** in 90% yield. Subsequent alkylation of **5**[14] with bromomethylacetate with NaH in DMF led to acetate **6** in 90% yield. Regioselective ester cleavage of intermediate **6** at 0 °C in THF/methanol and aqueous LiOH, was followed by standard aminoacid coupling with the alkenylamines **7a,b** (Table 1, in blue) using HATU in DMF afforded amides **8a,b**. Basic hydrolysis of the second ester group and subsequent coupling of the alkenes **9a–c** (Table 1, in red) using standard aminoacid coupling conditions in DMF provided dialkenes **10a–d** in good yields (>80%). Final ring closing metathesis using Hoveyda–Grubbs 1st generation catalyst (5 mol %) in dichloroethane at 80 °C for 15 h, afforded macrocyclic products **11a–d** in 20–30% yield. The saturated macrocycle targets **12a** (IC_{50} = 9.5 μM), and **12b** were reached by catalytic hydrogenation of **11a** (IC_{50} = 2.2 μM) and **11d**, respectively (Table 1).

The cell-based activity was measured as the inhibition of HCV RNA replication in Huh-7 cells, based on a bicistronic expression construct.[19] Inhibition was calculated as the concentration of compound that caused a 50% reduction in signal as compared to the control. Enzymatic activity (IC_{50}) was measured against purified HCV NS5b Δ21C isolate.[20] The open amide intermediates **10a** and **10b** (Table 1) displayed, relatively low activity in the HCV replicon (EC_{50} = 14 μM and 10 μM, respectively). Subsequent ring-closed products **11a** and **11b**, displayed activities that increased by fourfold (EC_{50} = 3.4 μM and 2.6 μM, respectively) potentially as a result of the entropic gain in macrocyclization. Acyl sulfonamide **11c** exhibits decreased cell potency for a comparable ring size, attributed to its poor permeability, measured in CACO-2 cells (P_{app} <1 × 10^{-6} cm/s), and a high efflux ratio >25.[21] In contrast, a marked increase in cell potency was observed with the acylsulfamide derivative **11d**, which was found to be 10 times more potent than **11c** (EC_{50} = 0.72 μM, and 7.5 μM, respectively). **11c,d** and **12b** displayed moderate metabolism in liver microsomes (e.g., **12b**: 63% rat, 67% human),[22] in contrast to the rapid metabolism seen for the macrocyclic diamides **11a–b**, and **12a** (>95% metabolized in both rat and human liver microsomes).[22]

With these encouraging results in hand, we embarked on the synthesis of additional macrocyclic indole derivatives incorporating the acyl sulfamide via the five step protocol outlined in Scheme 2. The indole nitrogen of **5**[14] and **13**[23] were then alkylated with

t-butyl bromoacetate using sodium hydride in DMF at room temperature. Regioselective unmasking of the *t*-butylesters **14a,b** via



Scheme 1. Synthesis of macrocyclic indoles. Reagents and conditions: (i) 3-furanylboronic acid, $Pd(PPh_3)_4$, Na_2CO_3, LiCl, ethanol/toluene, 80 °C, 3.5 h; (ii) NaH, methyl bromoacetate, DMF, 0 °C; (iii) (a) LiOH, water/THF/methanol, 0 °C, 15 h, (b) **7a,b**, HATU, DMF, rt, 6 h; (iv) (a) NaOH, water/THF/methanol, rt, 24 h, (b) for **9a,b**: HATU, DIPEA, DMF; for **9c**: EDC, DMAP, DMF, rt, 24 h; (v) Hoveyda–Grubbs 1st generation catalyst (5 mol %), DCE, 80 °C, 15 h. Subsequent hydrogenation of the double bond: 10% Pd/C, methanol, 1 atm H_2, rt, 6 h.