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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.

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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; nucleoside¹⁰ 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-503¹⁷ 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 $\mathbf{4}^{14,18}$ 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 5¹⁴ 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** (IC₅₀ = 9.5 μ M), and **12b** were reached by catalytic hydrogenation of **11a** (IC_{50} = 2.2 μ M) 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 (IC₅₀) was measured against purified HCV NS5b Δ 21C isolate.²⁰ The open amide intermediates 10a and 10b (Table 1) displayed, relatively low activity in the HCV replicon (EC₅₀ = 14 μ M and 10 μ M, respectively). Subsequent ring-closed products 11a and 11b, displayed activities that increased by fourfold (EC₅₀ = $3.4 \,\mu$ M and $2.6 \,\mu$ M, 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 ($P_{app} < 1 \times 10^{-6}$ cm/s), and a high efflux ratio >25.²¹ In contrast, a marked increase in cell potency was observed with the acy-Isulfamide derivative 11d, which was found to be 10 times more potent than **11c** (EC₅₀ = 0.72 μ M, and 7.5 μ M, 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(PPh₃)₄, Na₂CO₃, 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 H₂, rt, 6 h.

Table 1

1,6-macrocylic indoles via ring closing metathesis





Scheme 2. Synthesis of 1,6-macrocyclic indoles. Reagents and conditions: (i) 1 M aq Na₂CO₃, R₁-B(OH)₂, LiCl, EtOH/toluene, Pd(PPh₃)₄, 80 °C, 12 h; (ii) NaH, t-butyl bromoacetate, DMF, rt; (iii) (1) TFA, DCM, rt, (2) HATU, **15a–d**, DCM, DIPEA, rt; (iv) (1) TFA, DCM, 15 h, rt, (2) Sulfamide, dioxane 100 °C, 40 min; (v) (1) aq NaOH, methanol, THF, (2) CDI, CH₃CN, rt, then DBU, CH₃CN, rt.

TFA in dichloromethane, and subsequent reaction with mono-boc protected diamines (**15a–d**) under standard aminoacid coupling conditions afforded **16a–e**. Acid mediated deprotection of the

boc-protected linkers in TFA/DCM, formation of the free base, then subsequent heating with excess sulfamide in dioxane at 100 °C affords **17a–e** in yields above 50%. Methyl ester hydrolysis proceeded

Table 2

HCV inhibition of the 1,6-macrocyclic indole series

 Compd	R ₁	Linker	$IC_{50}(\mu M)$	REP Huh-7 EC_{50} (μM)
18a 18b	3-Furan 4- CH ₃ OPh	, . N N H	0.11 0.18	1.58 1.76
18c	4- CH₃OPh	, N N N N N	0.44	3.47
18d	4- CH₃OPh	, N, N, N,	NA	3.5
18e	4- CH₃OPh	H N	0.19	0.35
18f	4-ClPh		0.19	0.59
18g	Ph		NA	0.43
18h	4-ClPh		0.44	0.53

NA = not available.

in good yield (>90%) to afford the corresponding acid intermediates, which were then treated with carbonyl diimidazole (CDI) to form an acyl imidazole intermediate purified by flash chromatography. Following isolation, the compound was reconstituted in acetonitrile, then DBU was added to effect macrocyclization at room temperature giving rise to products **18a–e** (Table 2). Compounds **18f–h** (Table 2) have been synthesized from 2-(4-chlorophenyl)-3-cyclopentyl-6-carboxylic acid methyl ester (**18f** and **18h**) following a similar pathway as reported for the synthesis of **12a,b** (Scheme 1). The dehalogenated **18g** was obtained as a side product of **18f** during the final hydrogenation over 10% Pd/C in methanol at room temperature.

The 16-membered ring compound **18e** displayed more favorable replicon inhibition over the 15-membered ring analog **18b**. Introduction of a basic nitrogen in the linker (**18c**, **18d**) decreased potency by 10-fold versus the carbon chain linker of the same length (**18e**) (EC₅₀ = 3.47 μ M, 3.5 μ M, 0.35 μ M, respectively). Moreover, the potency is maintained when the phenyl ring is substituted with a chlorine (**18f**, **18h**) or unsubstituted (**18g**).



Scheme 3. Synthesis of 2,6-macrocyclic indoles. Reagents and conditions: (i) iodoalkane (I-R₁), NaH, THF, rt; (ii) 2-hydroxyphenyl-(R₂,R₃)-boronic acid, Pd(PPh₃)₄, K₂CO₃, DME/water, 100 °C, 12 h; (iii) (1) methyl bromoacetate, K₂CO₃, CH₃CN, rt, (2) LiOH(aq), THF/methanol, (3) amine, HATU, DIPEA, THF. For **22d–e**; Ns deprotection via Cs₂CO₃, thiophenol, DMF, rt; (iv) sulfamide, dioxane 100 °C, 45 min.; (v) (1) TFA, DCM, rt, (2) CDI, CH₃CN then DBU.

Table 3

HCV inhibition and metabolic stability of compounds in the 2,6-macrocyclic indole series

Compd	R ₁ , R ₂ , R ₃	Linker	IC_{50} (μM)	REP Huh-7 EC50 µM	RLM ²²	HLM ²²
25a	CH ₃ , H, H		1.9	7.56	_	_
25b	CH ₃ , H, H	, N N N	0.097	0.31	60	92
25c	CH ₃ , H, OCH ₃		0.054	0.17	64	96
25d	CH ₃ , H, H	H	0.033	0.18	44	71
25e	CH ₃ , F, H	N	0.088	0.68	56	59
25f	CH ₃ , H, F	N	0.110	0.46	58	81
25g	CH ₃ , H, OCH ₃		0.056	0.15	52	69
25h	CH ₃ , H, H		0.11	0.19	50	65
25i	<i>i</i> Pr, H, H		1.66	11.9	35	64
25j	Cyclopentyl _. H, H		1.49	15.7	40	74
25k	CH ₃ , H, H	N N N	0.14	0.24	35	56

Table 4	
Pharmacokinetic profile of compounds 12b , 25b and 25h in rat ^a	

Compd	CL ^b (L/h/kg)	$V_{\rm dss}^{\rm c}$ (L/kg)	$T_{1/2}^{d}(h)$	F ^e (%)
12b	7.4	2.4	0.4	40
25b	7.7	2.6	0.3	9
25h	6.9	7.0	1	14

^a Administration: iv 2 mg/kg in PEG400/saline (70:30); po 10 mg/kg PEG400/2% Vitamin E TPGS.

^b Clearance from plasma.

^c Volume of distribution.

^d Half-life.

^e Oral bioavailability.

Attention then turned to installing the linker between the C2 aromatic, holding the aryl group in its preferred conformation, and the acid bioisostere, described in Scheme 3. Basic hydrolysis of methyl ester 4, followed by reaction with dimethylformamide di-t-butylacetal (DMF-DBA) afforded the indole t-butylester 19. Alkylation of 19 over sodium hydride in the presence of alkyl iodide in DMF gave rise to **20a–c** (R_1 = methyl, isopropyl, cyclopentyl in yields of 90%, 60%, and 20%, respectively). Typical Suzuki-Miyaura conditions were used in the coupling of 2-bromoindoles 20a-c with boronic acids to afford intermediates 21a-f. The phenol oxygen was alkylated with methyl bromoacetate in DMF over potassium carbonate. Subsequent regioselective deprotection of the intermediate methyl esters was carried out in basic media, followed by standard aminoacid coupling conditions in DMF, employing linkers **22a-e**, (the nitrogen of the newly formed bond is shown in bold, Scheme 3, Table 3), allowed the formation of the corresponding amide intermediates 23a-k in 70-90% yield. Formation of the sulfamides **24a-k**, and subsequent ring closure was effected as in Scheme 2 to give the 2,6-indole macrocycles 25a-k (3).

Alkylation of the indole nitrogen proved detrimental to activity (e.g., **25i**, EC₅₀ = 11.9 μ M, IC₅₀ = 1.66 μ M), the augmented alkyl size potentially displaces the linker to clash with the protein, or alternatively, alters the dihedral angle of the 2-aryl group. Contrary to the 1,6-macrocyclic indoles, this series tolerated a tether containing a basic amine (**25k**, EC₅₀ = 0.24 μ M, IC₅₀ = 0.14 μ M).

The potent macrocyclic indoles 12b, 25b and 25h were studied for their pharmacokinetic properties in Sprague-Dawley rats, and the data is summarized in Table 4. Plasma kinetics were determined after a single iv administration of 2 mg/kg compound in PEG400/saline (70:30) as a vehicle. These data were compared to the oral dose at 10 mg/kg in PEG400 containing 2% Vitamin E TPGS. Systemic exposure was attainable, albeit with low to moderate bioavailability ranging from 9% to 40%. High clearance from plasma and half-lives of 1 h or less were observed. HCV replication is known to occur in the liver,²⁴ thus the high drug concentrations observed in the target organ 7 h post dosing (12b. 25b. 25h = 3743, 1524, 1717 ng/g, respectively), corresponding to favorable liver to plasma ratios (150, 99, and 64 for 12b, 25b and 25h, respectively), were encouraging results. No formation of glutathione conjugates was observed after incubation of 25h with human liver microsomes, fortified with glutathione (GSH),²⁵ bolstering the development potential of these macrocyclic compounds.

In summary, we have described two series of macrocyclic indoles where a tether connects a carboxylic acid bioisostere in the 6-position to either the indole nitrogen or 2-aryl position. Optimization afforded potent allosteric inhibitors of the HCV NS5b enzyme, reduction of subgenomic HCV RNA replication in Huh-7 cells, and bioavailability in rats. These findings contribute to further modifications that will be described in Part 2.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012. 03.097. These data include MOL files and InChiKeys of the most important compounds described in this article.

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