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Syntheses and initial evaluation of a series of indolo-fused heterocyclic inhibitors of the polymerase enzyme (NS5B) of the hepatitis C virus

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ABSTRACT

Herein, we present initial SAR studies on a series of bridged 2-arylindole-based NS5B inhibitors. The introduction of bridging elements between the indole N1 and the *ortho*-position of the 2-aryl moiety resulted in conformationally constrained heterocycles that possess multiple additional vectors for further exploration. The binding mode and pharmacokinetic (PK) properties of select examples, including: *13-cyclohexyl-6-oxo-6,7-dihydro-5H-indolo[2,1-d][1,4]benzodiazepine-10-carboxylic acid* (**7**) (IC_{50} = 0.07 μM, *%F* = 18), are reported.

Hepatitis C is a liver disease caused by infection with the hepatitis C virus (HCV). It affects approximately 180 million of the world's population, with four million estimated to be infected in the United States alone.[1] Individuals exposed to the virus can contract a persistent infection that leads to variable hepatic necro-inflammation. Those patients that proceed to develop high inflammatory activity frequently deteriorate and ultimately present with fibrosis and cirrhosis, the consequences of which include liver failure, portal hypertension and hepatocellular carcinoma. The current optimal therapy: extended treatment (48 weeks) with pegylated interferon and ribavirin (Peg-IFN/RBV), is poorly effective in patients infected by genotype 1 HCV, with less than 50% achieving a sustained viral response (SVR).[2] This, coupled with poor compliance due to a severe side effect profile has prompted a number of pharmaceutical companies to attempt to develop direct acting antiviral agents (DAAs) for the treatment of HCV.[3]

HCV RNA-dependent RNA polymerase (NS5B) has long been considered an attractive target for therapeutic intervention. It is essential for viral replication and several companies have identified active-site or allosteric inhibitors of the NS5B enzyme. Many of these are being clinically evaluated.[4] An early focus of our research in this area was indole-based allosteric inhibitors[5] of the type shown in Figure 1.

In an attempt to further investigate these early indole leads we evaluated a number of related indolo-fused heterocycles of the types shown in Figure 2.[6] We designed these structures to retain known binding moieties, and to introduce bridging elements between the indole N1 and the 2-position of the pendant aryl group. We envisioned that such functionality could induce a degree of pre-organization through conformational constraint, as well as provide a scaffold from which additional pendant functionality could be evaluated. In the work presented here, we report on the impact of the introduction of such novel bridging elements in the context of both truncated **1** and extended **2** NS5B-inhibitor classes, as well as discuss the mode of binding and pharmacokinetic (PK) properties of select examples.

The novel core heterocycles evaluated in this study were prepared as outlined in Schemes 1–3. In the case of hydrocarbon-bridged analogs such as **3** and **4**, two complementary approaches were developed. The first utilized a key ring-closing metathesis (RCM) reaction[7] for construction of the indolo-fused heterocycle, as depicted in Scheme 1. Methyl 2-bromo-3-cyclohexylindole-6-carboxylate[8] (**11**) was coupled with 2-vinyl phenyl boronic acid using standard Suzuki conditions. Subsequent alkylation of the indole nitrogen with 3-bromoprop-1-ene afforded the cyclization

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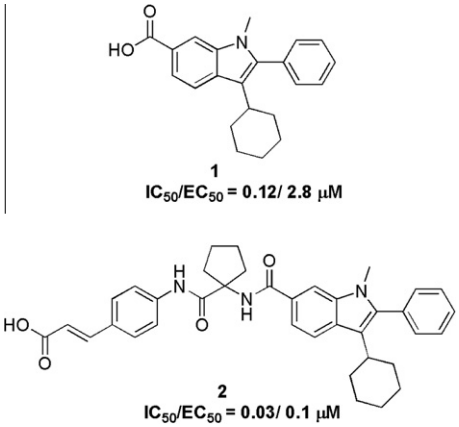


Figure 1. Early examples of truncated (1) and extended (2) indole-based allosteric HCV NS5B inhibitors.

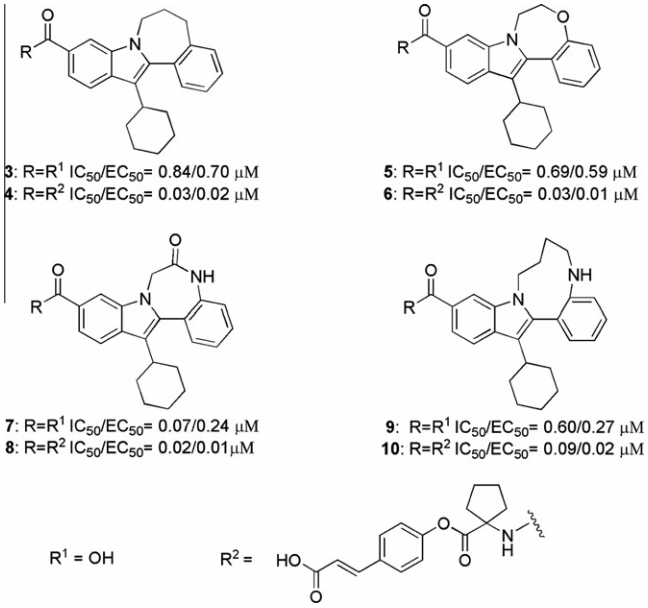


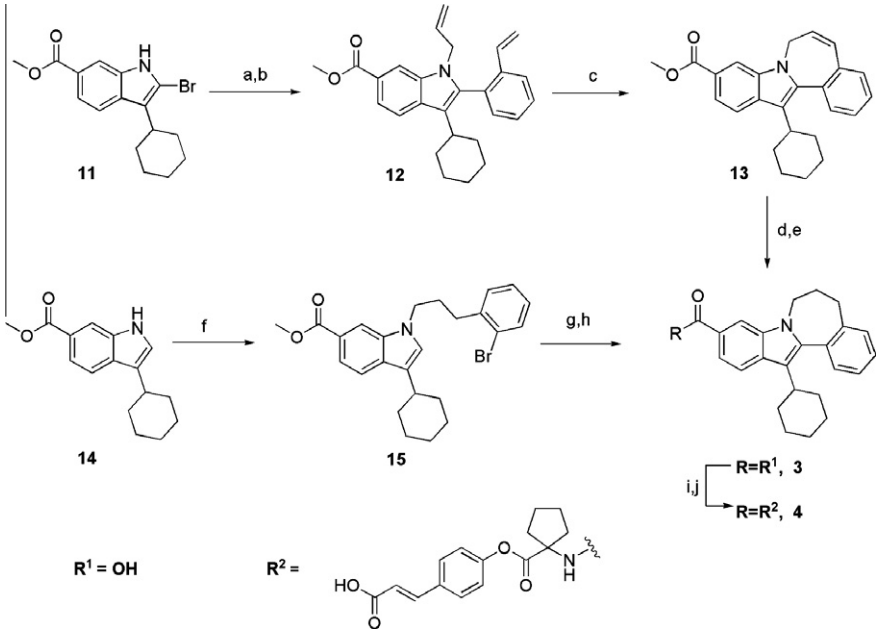
Figure 2. Novel truncated (1) and extended (2) indolo-fused heterocyclic NS5B inhibitors.

precursor **12**. RCM using the Grubb's 2nd generation catalyst[7] followed by catalytic hydrogenation and subsequent hydrolysis, provided the indolobenzazepine **3** in a concise fashion. This acid was coupled with the cinnamate side chain shown in Figure 2 and subjected to a further saponification to access the extended indolobenzazepine **4**. Alternatively, **3** could also be synthesized from indole **14** using a Heck reaction[9] as the key cyclization step (Scheme 1). Alkylation of the indole **14** with 1-bromo-2-(3-bromopropyl)benzene afforded the aryl bromide **15**. This was treated under standard Heck conditions to generate the desired indolobenzazepine **3**. A potential advantage that we anticipated for this route was that it would allow the introduction of more highly functionalized bridging elements that might be excluded when using the RCM approach.

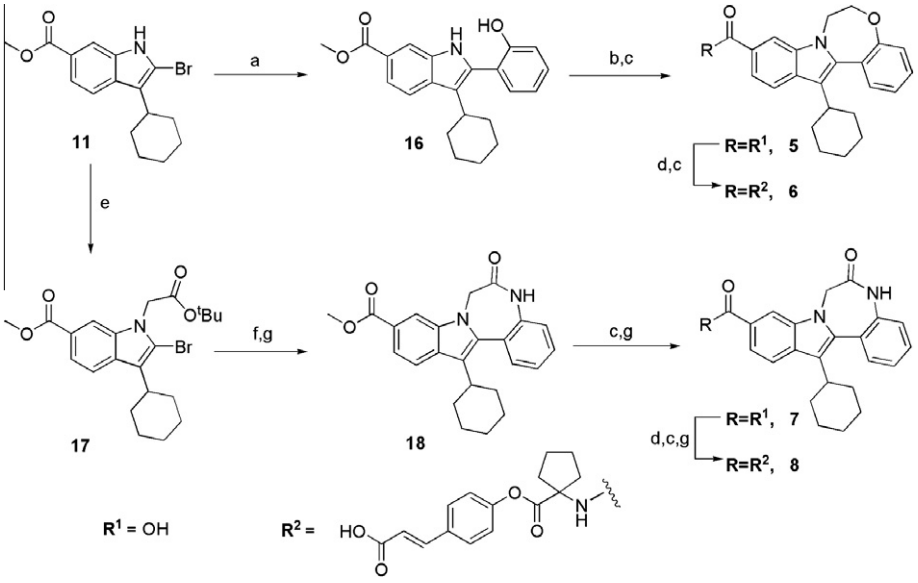
The indolobenzoxazepine derivatives **5** and **6** were also prepared from methyl 2-bromo-3-cyclohexylindole-6-carboxylate (**11**), as shown in Scheme 2.

Suzuki coupling of **11** with the pinacol ester of 2-hydroxyphenyl boronic acid provided the hydroxyphenyl indole derivative **16** in good yield (92%). Alkylation of this intermediate with 1,2-dibromoethane provided access to the indolobenzoxazepine ring system in a single step. Subsequent base hydrolysis afforded the target analog **5**. This, in turn, could be coupled with (*E*)-4-(3-methoxy-3-oxoprop-1-enyl)phenyl-1-aminocyclopentane carboxylate using standard coupling conditions, and the resultant product saponified to provide the extended indolobenzoxazepine target **6**.

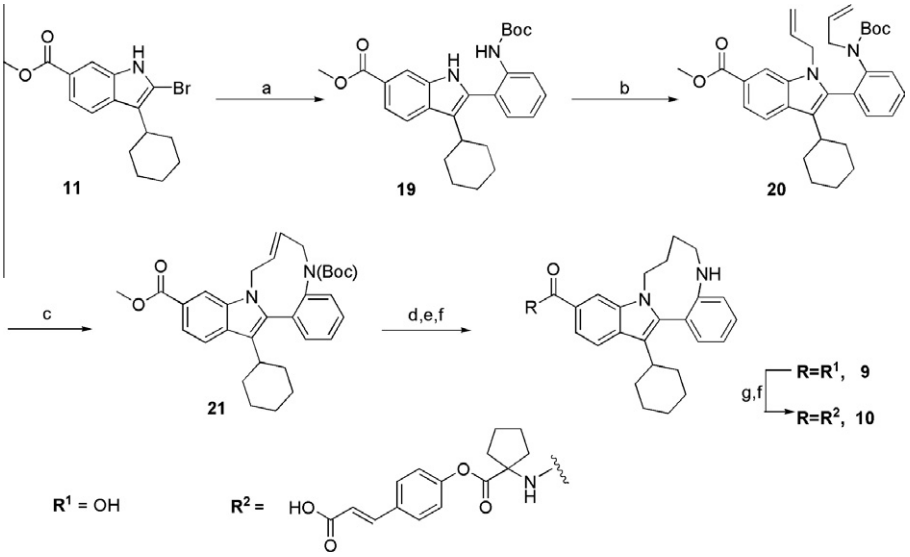
For the synthesis of indolobenzodiazepine analogs **7** and **8**, a related methodology was utilized, as described in the bottom of



Scheme 1. Reagent and conditions: (a) 2-vinylphenylboronic acid, $Pd(Ph_3P)_4$, Na_2CO_3(1 N), LiCl, toluene, EtOH; (b) NaH, allyl bromide, DMF, rt; (c) RCM, Grubb's 2nd generation catalyst; (d) H_2, Pd/C; (e) NaOH, MeOH, THF, H_2O, 90 °C, microwave, 10 min; (f) 1-bromo-2-(3-bromopropyl)benzene, NaH, THF, rt; (g) $Pd(Ph_3P)_4$, KOAc, DMA, 150 °C, microwave, 30 min; (h) NaOH, MeOH, THF, H_2O, 90 °C, microwave, 10 min; (i) (*E*)-4-(3-methoxy-3-oxoprop-1-enyl)phenyl 1-aminocyclopentanecarboxylate, TBTU, diisopropylethyl amine, DMF, rt; (j) NaOH, MeOH, THF, H_2O, 90 °C, microwave, 10 min.



Scheme 2. Reagents and conditions: (a) 2-(4, 4, 5, 5-tetramethyl-1, 3, 2-dioxaborolan-2-yl)phenol, $Pd(Ph_3P)_4$, Na_2CO_3(1 N), LiCl, toluene, EtOH; (b) NaH, THF, dibromoethane; (c) NaOH, MeOH, THF, H_2O, 90 °C, microwave, 10 min. (d) (*E*)-4-(3-methoxy-3-oxoprop-1-enyl)phenyl 1-amino-cyclopentanecarboxylate, TBTU, DIPEA, DMF, rt; (e) *tert*-butylbromo-acetate, NaH, THF; (f) 2-(*tert*-butoxycarbonyl-amino)phenylboronic acid, $Pd(Ph_3P)_4$, Na_2CO_3(1 N), LiCl, toluene, EtOH; (g) TFA, CH_2Cl_2.



Scheme 3. Reagents and conditions: (a) 2-(*tert*-butoxycarbonyl-amino)phenylboronic acid, $Pd(Ph_3P)_4$, Na_2CO_3 (1 N), LiCl, toluene, EtOH; (b) NaH, allyl bromide, DMF, rt; (c) RCM, Grubb's 2nd generation catalyst; (d) TFA, CH_2Cl_2; (e) H_2, Pd/C; (f) NaOH, MeOH, THF, H_2O, 90 °C, microwave, 10 min; (g) (*E*)-4-(3-methoxy-3-oxoprop-1-enyl)phenyl 1-aminocyclopentanecarboxylate, TBTU, diisopropylethyl amine, DMF, rt.

Scheme 2. Intermediate **11** was first alkylated using *t*-butyl 2-bromoacetate to provide the bromoindole derivative **17**. A subsequent Suzuki reaction allowed the introduction of a pendant N-protected 2-aminoaryl group. Removal of the Boc protecting moiety led to spontaneous ring closure and the generation of the indolobenzodiazepine **18**. Hydrolysis of the ester functionality in this derivative resulted in the concomitant cleavage of the diazepinone ring system. However, this was easily reversed under acidic conditions and target **7** could be isolated in reasonable yield. Coupling of intermediate **7** with the aforementioned cinnamate and final saponification also resulted in the opening of the lactam ring, which fortunately, once again spontaneously closed on acidification to provide the targeted extended indolobenzodiazepine **8**.

For the syntheses of the indolobenzodiazonines **9** and **10**, an RCM reaction was again employed, as shown in Scheme 3. Starting from intermediate **11**, Suzuki coupling with an N-protected 2-aminophenyl boronic acid provided intermediate **19** in satisfactory yield.

Treatment with an excess of sodium hydride and allyl bromide resulted in a double alkylation, and the intermediate **20** could be obtained in good yield (77%). RCM of **20** subsequently yielded the target heterocycle **21**. After removal of the Boc moiety, similar procedures to those outlined above for the preparation of **3**, allowed access to the desired indolobenzodiazonines, **9** and **10**.

With these compounds in hand, we evaluated the initial proposition that such bridged structures might demonstrate improved potency with respect to their non-bridged congeners due to appropriate pre-organization and conformational constraint.[9]

The acyclic analogue **1** was used as a basis for comparison. The minimized structure of **1** (Fig. 3) displays a dihedral of 65.6°

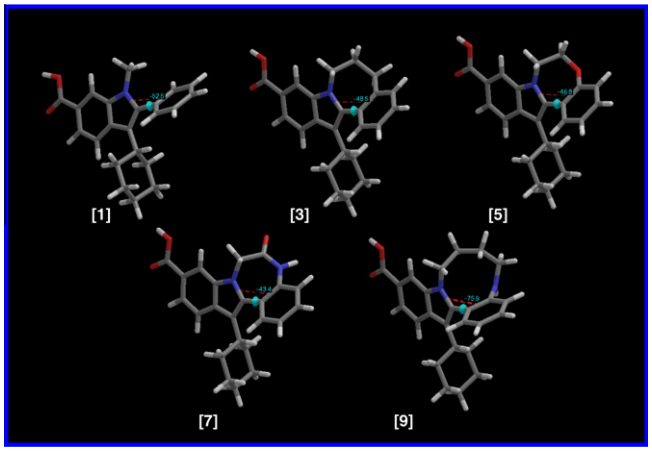


Figure 3. Molecular mechanics (OPLS)[10] minimized structures of the indolo-fused heterocycles depicted in Figure 2. The dihedral angle between the phenyl and indole ring systems for each compound is listed in Table 1.

Table 1
Comparison of inhibitor potency with dihedral angle, as indicated in Figure 3

Compound	Dihedral angle (°)	IC_{50}[a] (μM)
7	43	0.07
3	45	0.84
5	49	0.69
1	66	0.12
9	71	0.60

[a] Values are means of at lease two test occatsions run in duplicate, standard deviations are <40% of mean.

between the pendant phenyl and the core indole ring, and has an IC_{50} of 0.12 μM.[11] When constrained with a seven-membered lactam bridge, as in **7**, the dihedral angle is 43° and a modest increase in potency is observed to 0.07 μM. However, locking in similar dihedral angles, as in **3** and **5**, causes a loss in potency (Table 1). The cyclic analogs **3**, **5**, and **9** are approximately equipotent with IC_{50}s of 0.84, 0.69, and 0.60 μM, respectively. Compound **9** contains a flexible nine-membered ring that adopts a preferred dihedral angle of ~71°; closer to the dihedral angle observed with compound

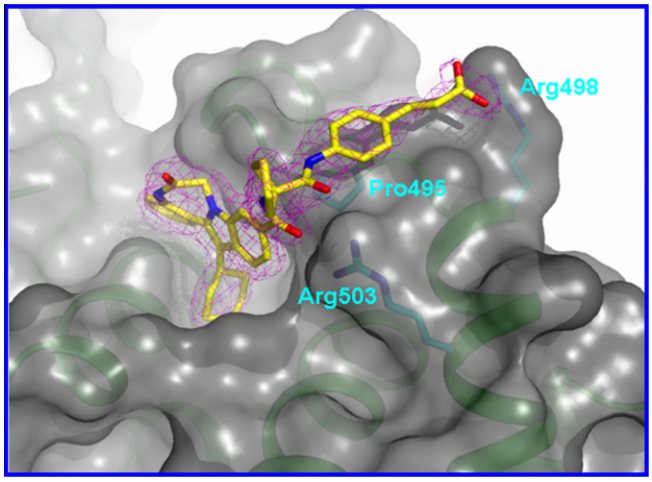


Figure 4. Compound **8** bound to HCV genotype1b NS5B (Bartenschlagger).[14] The protein in the vicinity of the P495 site is represented by a surface (gray) and cartoon (green). The side chains of Pro495, Arg498, and Arg503 and compound **8** are represented by sticks (cyan and yellow carbon atoms, respectively). The initial (prior to placing compound **8** in the model) 2Fo-Fc electron density is contoured at 1σ (magenta). Figure drawn with PyMola.[15]

Table 2
Rat PK[a] results observed with select examples of the indolo-fused tetracyclic NS5B inhibitors shown in Figure 2

Compound	F^b (%)	AUC (μM*h)	Clearance[c] (mL/min/kg)
3	6	1.3	53
4	8	0.28	250
6	5	0.79	17
7	17.6	2.2	35
8	3	0.66	13
10	12.3	3.61	8.7

[a] $n = 3$. Vehicle iv and po PEG400.
[b] Oral bioavailability.
[c] iv (dose 2 mg/kg).

1 (65°). Despite this, **9** displays potency similar to compounds with more rigid seven-membered rings and smaller dihedral angles. In these compounds, the IC_{50}s depend more on the content of the bridge than on the enforced dihedral angle. Taken collectively, one interpretation of these results is that the ring systems evaluated here do not lock the pendant aryl group in an optimal position. Instead, the observed differences in potencies may be attributed to direct interactions between the bridging elements and the enzyme. This idea will be elaborated upon in future publications.

Previous reports have noted that the introduction of an (*E*)-3-(4-(1-aminocyclopentanecarboxamido)phenyl) acrylic acid side chain can significantly increase the potency of indole-6-carboxylate NS5B inhibitors.[12] As described above, this was explored with analogs **4**, **6**, **8**, and **10**, as shown in Figure 2. In all cases, significant increases in potency of 10- to 20-fold were observed relative to the 6-carboxylates. In the case of compound **8**, the EC_{50} of the extended indolobenzodiazepinone was 10 nM, which represented the optimal activity achieved with these early examples. Subsequently, a co-crystal of this analog complexed to NS5B [1b Bartenschlager construct][13] was obtained, and is shown in Figure 4.

From this structure it can be seen that compound **8** binds in an area in the NS5B thumb domain.[14] The key-cyclohexyl ring binds in a small hydrophobic pocket that is known to interact with a residue in the finger domain of the enzyme when NS5B adopts a transcriptionally active conformation. The amide N–H moiety in the pendant side chain forms a hydrogen-bond with ARG 503, and the terminal carboxylate interacts with ARG 498. The pendant phenyl group appears to lie in a hydrophobic cleft and adopts a dihedral angle relative to the indole ring of ~46°, suggesting the bioactive conformation to be similar to that observed in the minimized structures reported above. Notable by their apparent absence are specific interactions between the bridging element and the enzyme. However, in the structures that we have obtained to date, the Δ1 fingertip region of the enzyme, which normally binds at this site, appears disordered, and no such contacts can be assigned.

Finally, the PK profiles obtained in the rat for select examples of the tetracyclic indoles described above are summarized in Table 2. PK data were obtained for the truncated analogs **3** and **7**, and for the extended inhibitors **4**, **6**, **8**, and **10**. In the case of the former, the more polar lactam **7** showed significantly increased exposure and improved bioavailability relative to the propano-bridged derivative **3**. The extended inhibitors **4**, **6**, **8**, and **10** all exhibited poor PK profiles in rat. Of the compounds examined, compound **7** appeared to be the most promising from a PK perspective. Replacement of the acid functionality in this molecule might be expected to improve the exposure achievable with this series, and as we have established that this vector can be exploited to improve the potency, an obvious path for the further optimization of this class of inhibitor is apparent. This will be commented upon in future manuscripts.

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