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N-Acetamideindolecarboxylic acid allosteric 'finger-loop' inhibitors of the hepatitis C virus NS5B polymerase: discovery and initial optimization studies

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ABSTRACT

SAR studies at the N^1-position of allosteric indole-based HCV NS5B inhibitors has led to the discovery of acetamide derivatives with good cellular potency in subgenomic replicons (EC_{50} <200 nM). This class of inhibitors displayed improved physicochemical properties and favorable ADME-PK profiles over previously described analogs in this class.

The Hepatitis C Virus (HCV) infects millions of people worldwide leading in some cases to severe and often fatal liver damage (e.g., cirrhosis or hepatocellular carcinoma). The best current treatment options consist of pegylated interferon and ribavirin combinations and provide limited efficacy against the most prominent HCV genotype (1a/1b) with significant side effects. In industrialized nations, HCV infection has become the major reason for orthotopic liver transplants.[1] The NS5B RNA-dependent RNA polymerase of the hepatitis C virus is a promising target for the development of novel anti-HCV therapeutics.[2] Recently, nucleoside analogs[3] and non-nucleoside allosteric inhibitors[2,4] of the enzyme have demonstrated efficacy in the clinic, particularly in combination with interferon-based regimens aimed at retarding emergence of resistant strains of the virus. Our own efforts in the field have centered on benzimidazole-based non-nucleoside allosteric inhibitors of the enzyme (e.g., compound **1**) which were discovered through screening of our corporate sample collection.[5,6] We and others recently described the progression of this class of inhibitors towards more potent and cell-permeable indole-based derivatives that inhibited HCV subgenomic RNA replication in cell culture (replicon assay) at low μM concentrations (e.g., compounds **2** and **3**, Fig. 1).[7]

Two strategies were explored to further improve the cell-based potency of these inhibitors. Incorporation of the indole modification into previously described amide analogs[8] led to non-ionized

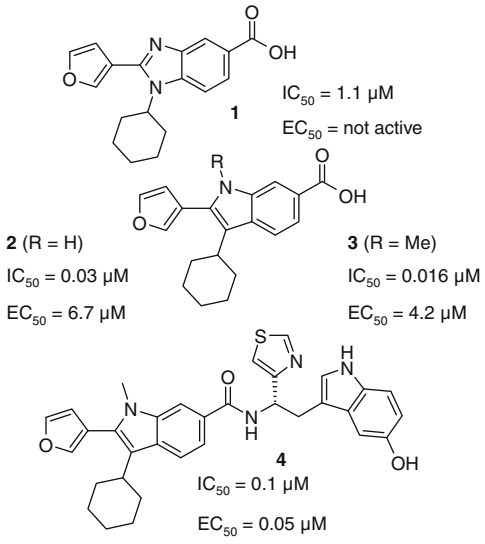

Figure 1. Initial indole-based NS5B inhibitors.

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compounds such as **4**, a very potent inhibitor of HCV RNA replication (EC_{50} = 0.05 μM).[7a] Unfortunately, amide **4** had inadequate physicochemical properties (high lipophilicity and very poor aqueous solubility) that compromised further progression of such derivatives into development for oral therapy. In contrast, indole carboxylic acid derivatives **2** and **3** had desirable physicochemical properties (low MW, good solubility) as well as remarkable intrinsic potency against the polymerase enzyme, albeit with modest replicon potency.

The strategy to improve the cell-based potency of compounds **2** and **3** was based on optimization of the N^1-indolic substituent while preserving the ionizable carboxylic acid function in order to maintain adequate physicochemical properties. We now report the results of studies directed toward exploring SAR at this newly accessible position extending from the nitrogen atom of the indole scaffold of these derivatives (R group in Fig. 2).

Initial data from compounds **2** and **3**[7a] suggested that substitution of the indole nitrogen was tolerated. The indole NH did not contribute to the improved potency that accompanied the replacement of the benzimidazole scaffold by indoles. The possibility of exploiting new interactions with the enzyme was thus explored by incorporating highly diverse NH substituents. Table 1 summarizes the highlights from an extensive alkylation study performed with the 2-(3-furyl) analog **2**.[9]

N-Alkylation of **2** with alkyl groups larger than Me (compound **3**), including aliphatics, allylics, benzylic aryls and benzylic heterocyclics (compounds **5**–**15**, Table 1) did not improve enzymatic and cell-based potencies. Some notable steric effects were observed in the case of branched alkyls (results not shown) and *ortho*-disubstituted benzylic groups (cf. compound **11**). On the other hand, SAR appeared to be somewhat insensitive to electronic/polarity effects as both basic and acidic substituents were well tolerated (e.g., **12**–**15**). Initial results exposed a rather unproductive SAR at this position and little improvement was achieved relative to compound **3** that had a simple methyl.

Alkylation of the indole scaffold with an acetic acid side chain (compound **16**) was also well tolerated and was seen as an opportunity to introduce further diversity in high-throughput fashion through the synthesis of amide libraries. The carboxylic acid function did not contribute specifically to potency as reduction to the alcohol (**17**) or esterification (**18**) maintained activity. The 1C methylene linker between the indole scaffold and the carboxyl function appeared to be optimal as extension to the propionic analog decreased potency threefold (cf. **16** vs **19**). Consistent with previously observed steric restrictions, substitution at the α-position of the indole scaffold (**20**) was not tolerated. Encouragingly however, primary amide analog **21** seemed to provide a slight improvement in cellular potency compared to **3** despite a small decrease in NS5B enzymatic inhibition. Compound **21** was selected as starting point for further studies.[10]

As mentioned previously, the presence of the carboxylic acid function in **16** provided an opportunity for rapidly expanding diversity through the formation of amide derivatives as depicted in Scheme 1.

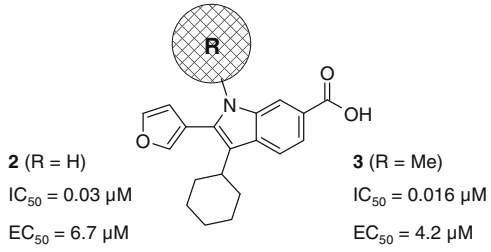
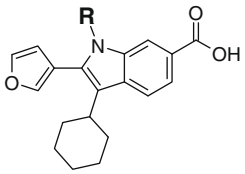


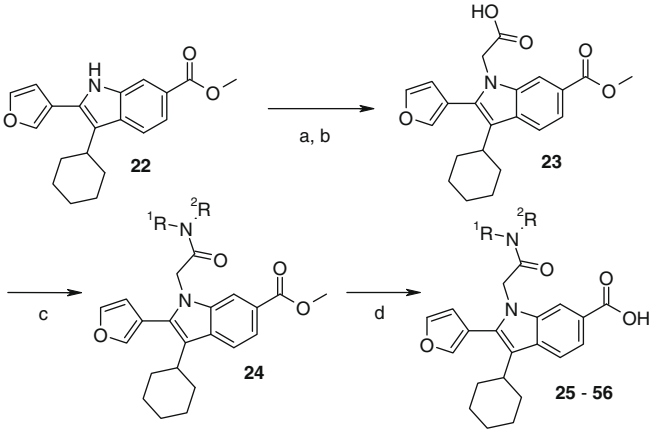
Figure 2. General structure of indole-based NS5B inhibitors.

Table 1
N-Alkylation studies on indole carboxylic acid **2**



Compds	R	HT-NS5B-Δ21 IC_{50}[a] (μM)	1b Replicon EC_{50}[a] (μM)
5	Ethyl	0.07	>10
6	Allyl	0.08	>10
7	Isopropenyl	0.36	>10
8	Benzyl	0.25	N.T.
9	2-Picoly	0.13	N.T.
10	2-Me–$C_6H_4CH_2$	0.29	N.T.
11	2,6-Me_2–$C_6H_3CH_2$	>138	N.T.
12	2-(NH_2)–$C_6H_4CH_2$	0.09	2.9
13	3-(NH_2)–$C_6H_4CH_2$	0.13	>8
14	3-(COOH)–$C_6H_4CH_2$	0.07	N.T.
15	4-(COOH)–$C_6H_4CH_2$	0.04	N.T.
16	CH_2COOH	0.06	N.T.
17	CH_2CH_2OH	0.10	>10
18	CH_2COOEt	0.09	8.5
19	CH_2CH_2COOH	0.21	N.T.
20	(±)-$CH(CH_3)COOH$	2.8	N.T.
21	CH_2CONH_2	0.04	2.4

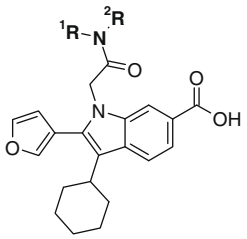
N.T. (not tested).
[a] Values are means of duplicate experiments on two separate weightings.

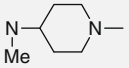


Scheme 1. Synthesis of *N*-acetamidoindole NS5B inhibitors. Reagents and conditions: (a) NaH (1.25 equiv), DMF, 0 °C, 1 h then *tert*-butylbromoacetate (1.24 equiv), rt, 18 h (86% yield); (b) TFA, 4 h, rt (99% yield); (c) TBTU or HATU (1.2 equiv), Et*i*Pr_2N (5 equiv), DMF or DMSO, rt, 10 min then $^1R^2RNH$, rt, 1–18 h; (d) 10 N NaOH (5–10 equiv), 5:1 DMSO-water, 1–2 h, then purification by reversed-phase HPLC (>95% homogeneity).

Toward this end, alkylation of indole ester **22**[7] with NaH/*tert*-butylbromoacetate followed by cleavage of the *tert*-butyl group under acidic conditions afforded key intermediate **23** that was coupled to various amines under standard peptide coupling conditions to provide *N*-acetamidoindole esters **24** in good yields. Saponification under mild basic conditions gave inhibitors **25**–**56** that were purified to >97% homogeneity by reversed-phase HPLC. Approxi-

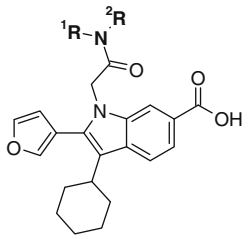
Table 2
N-Acetamidoindole carboxylic acid inhibitors

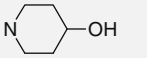
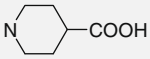
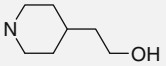
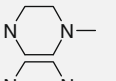
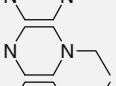
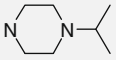
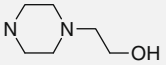
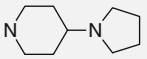
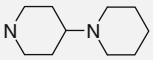
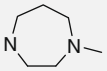


Compds	N $^1R^2R$	HT-NS5B-Δ21 IC_{50}[a] (μM)	1b Replicon EC_{50}[a] (μM)
25	NHMe	0.05	2.0
26	NH*i*Pr	0.05	N.T.
27	$NHCH_2(c\text{-}C_6H_{11})$	0.12	N.T.
28	NHPh	0.08	N.T.
29	$NHCH_2Ph$	0.06	N.T.
30	$NH(CH_2)_2OMe$	0.05	N.T.
31	NMe_2	0.02	0.8
32	NMe(*n*Pr)	0.05	1.7
33	NMe(*i*Pr)	0.03	0.8
34	NMe(*c* C_6H_{11})	0.11	N.T.
35	N $(iPr)_2$	0.07	2.3
36	$NMe(CH_2CH_2OH)$	0.02	1.5
37	$NMe(CH_2CH_2OMe)$	0.05	1.3
38	$NMe(CH_2CH_2NMe_2)$	0.02	0.44
39	$NMe(CH_2CH_2NEt_2)$	0.03	0.46
40	$NMe(CH_2CH_2CH_2NMe_2)$	0.02	0.58
41	NMe[CH_2CH_2-(4-pyridyl)]	0.03	0.63
42	$NEtCH_2$-(4-pyridyl)	0.04	N.T.
43		0.02	0.13

N.T. (not tested).
[a] Values are means of duplicate experiments on two separate weightings.

Table 3
Cyclic *N*-acetamidoindole carboxylic acid inhibitors



Compds	N $^1R^2R$	HT-NS5B-Δ21 IC_{50}[a] (μM)	1b Replicon EC_{50}[a] (μM)
44		0.04	N.T.
45		0.04	0.82
46		0.03	0.60
47		0.02	2.0
48		0.03	>94
49		0.07	1.3
50		0.04	0.45
51		0.03	0.41
52		0.04	0.83
53		0.02	1.3
54		0.02	0.38
55		0.03	0.78
56		0.02	0.33

N.T. (not tested).
[a] Values are means of duplicate experiments on two separate weightings.

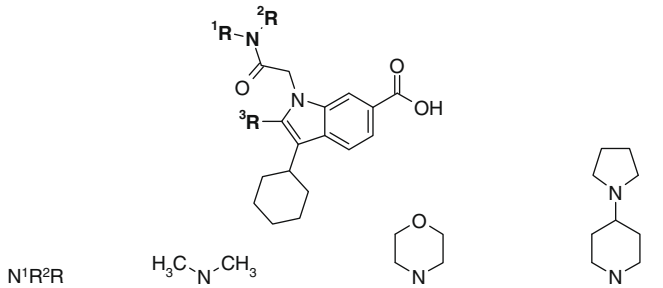
mately 125 amide analogs were prepared in this fashion and the results for a representative set are shown in Tables 2 and 3.[11]

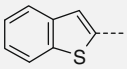
Some general conclusions that can be drawn from the data are presented in Tables 2 and 3. Secondary amides (**25**–**30**) exhibited comparable enzymatic and cellular potencies as unsubstituted analog **21**. IC_{50}s were not significantly affected by the size/nature of the amide substituent, with <2-fold variation observed between small or large aliphatic, aromatic or benzylic groups. The first improvement was noted with the tertiary amide analogs (**31**–**43**). Dimethylation of the acetamide nitrogen (compound **31**) provided an inhibitor with comparable enzymatic potency to *N*-methylindole **3** but with a fivefold improvement in cell culture and represented the first compound with EC_{50} <1 μM in this series. The enhanced replicon potency of **31** relative to compound **3** may, in part, be attributable to improved cellular permeability (Caco-2 = 12.8×10^{-6} cm/s) rather than increased lipophilicity (Log *D* at pH 7.4 = 1.45 and 3.26 for **31** and **3**, respectively). Based on this finding, the 3° amide chemotype was expanded but subsequent evaluation revealed a relatively flat SAR with IC_{50} values ranging from 20 to 110 nM. Trends were consistent with the hypothesis that this part of the molecule is solvent-exposed since polar side chains were generally preferred to large lipophilic groups. Of greater significance however, were the patterns that developed in the replicon assay. Several analogs, in particular those carrying basic side chains (e.g., **38**, **39** and **43**), had EC_{50} <500 nM. *N*-Methylpiperidine **43** was the most potent inhibitor (EC_{50} = 130 nM) generated in this class, and approached the biological potency of compound **4** but with improved physicochemical properties (solubility at pH 7.2 = 290 μg/mL).[12]

Investigations on 3° acetamide derivatives were extended to derivatives in which the nitrogen atom was incorporated within a cyclic system (compounds **44**–**56**, Table 3). Derivatives showed excellent inhibition of NS5B (all IC_{50}s <40 nM except for **49**) but no improvement was achieved over unsymmetrical 3° amides (e.g., compound **43**, Table 2). Yet again, diverse functionality was tolerated on the ring systems including neutral, basic and acidic groups. As described in Table 2, basic derivatives generally improved potency in the cellular replicon assay, with several compounds featuring EC_{50} values <500 nM (e.g., **50**, **51**, **54** and **56**).

Earlier SAR studies at the N^1-position of indole **2** led to the identification of novel acetamideindole NS5B inhibitors[10] and the compounds from this study suggest that substituents extending into this space are likely solvent-exposed and contribute little to inhibitor potency relative to a simple methyl group since most compounds had similar IC_{50} values relative to compound **3**. However, the inclusion of basic, lipophilic functionality in this position provided zwitterionic species with up to 30-fold increase in cellular activity. These results justified further expansion on this class and combinations of representative acetamide side chains with various C-2 aromatic/heterocyclic substituents were evaluated as

Table 4
Acetamide–C2 combinations



	^{3}R	IC_{50}[a] (μM)	EC_{50}[a] (μM)	IC_{50}[a] (μM)	EC_{50}[a] (μM)	IC_{50}[a] (μM)	EC_{50}[a] (μM)
57		0.04	1.5	0.04	1.0	0.03	1.2
58		0.04	0.64	0.04	0.33	0.03	1.0
59		0.02	0.60	0.03	0.60	0.02	0.46
60		0.04	1.4	0.07	0.70	0.04	0.38
61		0.24	3.4		—		
62		0.16	N.T.	0.10	N.T.	0.06	10
63		0.21	2.5	0.16	1.1	0.16	1.0

N.T. (not tested).
[a] Values are means of duplicate experiments on two separate weightings.

described in Table 4. Analogs of compound **22** that bear alternate C-2 heterocyclic substituents were elaborated to compounds **57**–**59** in a similar manner to those described in Scheme 1.[7]

Though removal of the C-2 substituent (i.e., ^{3}R = H) or the introduction of small C-2 alkyl groups at that position (e.g., Et) led to substantially decreased intrinsic potency (IC_{50} >1 μM; results not shown), several combinations with enzymatic potency comparable to 3-furyl analogs (IC_{50} <40 nM) were identified. In most cases, zwitterions bearing the lipophilic-basic side chain **C** were more potent than neutral amides **A** and **B**. In cell culture assays, 2-furyl derivatives (**57A**–**C**) were significantly less cell-permeable and had reduced replicon potency relative to the 3-furyl isomers. On the other hand, more lipophilic thiophene analogs (**58A**–**C**, **59A**–**C**) had EC_{50} values similar to the corresponding 3-furyl containing molecules, providing optional C-2 alternatives. In the case of 2-phenylindole derivatives, only the combination with a lipophilic and basic acetamide side chain had desirable cell culture potency (**60C**). Surprisingly, incorporation of a basic nitrogen atom in the phenyl ring at C-2 (**61A**) resulted in a sixfold drop in enzymatic potency. It is possible that intramolecular interactions between the basic pyridyl nitrogen and the acetamide carbonyl group may modify the side chain orientation of the latter, resulting in a less favorable binding conformation. Indeed, the presence of less basic pyrazine nitrogens at C-2 (e.g., **62**) was better tolerated (compare **60C** and **62C**) but the modification was highly detrimental to cell culture activity. Finally, the presence of more bulky C-2 groups (e.g., benzothiophene **63**) also negatively impacted on potency.

Finally, representative *N*-acetamideindoles were tested for specificity against another RNA-dependent RNA polymerase from polio virus and a mammalian DNA-dependent RNA polymerase II isolated from calf-thymus.[5] In all cases, no significant inhibition was observed at concentrations up to 250 μM (IC_{50} >250 μM).

The identification of a series of compounds with promising antiviral efficacy in the replicon assay system merited ADME-PK profiling of a representative set of compounds, in order to evaluate their potential for further development. The data are summarized in Table 5.[13]

In general, most of the compounds had good metabolic stability in the presence of human liver microsomes with $T_{1/2}$ >200 min. In addition, there was low potential for inhibition of major CYP450 isozymes ($IC_{50} \geqslant 8$ μM). Permeability across Caco-2 cell monolayers was assessed in the apical to basal direction and the values ranged from non-permeable (zwitterions) to highly permeable (neutral side chains) depending on structural features. The equilibrium solubilities in pH 7.2 buffer[12] of these inhibitors also spanned a large range.

Several compounds were screened for oral absorption in rat using cassette dosing (four compounds per cassettes, 4 mg/kg each). The most potent compound (**43**) had no plasma exposure (consistent with its low Caco-2 permeability). The inhibitor that exhibited the best overall ADME-PK profile in rat was compound **46** and contained a neutral morpholine acetamide. The compound was moderately soluble and had reasonable permeability, excel-

Table 5
Summary of ADME-PK properties of selected acetamidoindole inhibitors of NS5B polymerase

	Solubility[a] (μg/mL)	Caco-2[b] ($\times 10^{-6}$ cm/s)	CYP450[c] IC_{50} (μM)	HLM $T_{1/2}$[d] (min)	Rat PK[e] C_{1h} (μM)
31	240	12.8	⩾26 (3A4)	250	n.d.
36	1300	BLD	n.d.	275	n.d.
43	290	0.2	n.d.	>300	0.2
46	105	5.2	⩾24 (2C9)	>300	1.0
50	7	1.7	>30	70	0.6
51	12	6.5	⩾16 (3A4)	>300	0.3
52	18	7.0	⩾8 (3A4)	>300	0.4
54	79	1.4	n.d.	>300	0.0
59C	60	0.2	⩾11 (2C19)	>300	n.d.
60C	47	<0.1	n.d.	111	0.0[f]

n.d.: not determined.
[a] Determined with amorphous solids.[12]
[b] Apical to Basal permeability at pH 7.4.
[c] IC_{50} values were determined for 1A2, 2C9, 2C19, 2D6 and 3A4 isozymes. The values represent the lowest IC_{50} against the most sensitive enzyme (in brackets).
[d] Human liver microsome stability data at 2 μM compound concentration.
[e] Compounds were administered po in suspension (0.3% tween-80/0.5% methocel) as mixtures of 4 compounds (each compound at 4 mg/kg). Plasma concentrations are reported for the 1 h time point.
[f] Experiment performed at 2 mg/kg po BLD: below limit of detection.

lent metabolic stability (RLM $T_{1/2}$ = 288 min) and low potential for CYP-mediated drug–drug interactions. At an oral dose of 4 mg/kg, plasma concentration at the 1 h time point reached 1 μM. Several piperazine analogs (**50**–**52**, **54**) were also identified that provided promising plasma exposures in the 0.3–0.6 μM range, 1 h post-dosing.

In conclusion, we described how alkylation of the nitrogen atom of indole-based allosteric inhibitors of HCV NS5B polymerase with a variety of acetamide side chains led to the discovery of compounds with generally good cell culture potency in subgenomic HCV RNA replicon assays. The SAR patterns suggested that the acetamide substituent is solvent-exposed and allows for the incorporation of chemical diversity and the modulation of the molecule's physicochemical properties. Many analogs showed improved aqueous solubility and promising ADME-PK profiles in rats and in human in vitro assays over previously reported series. Further optimization of this class of compounds will be the focus of future studies.

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