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Tetracyclic indole inhibitors of hepatitis C virus NS5B-polymerase

Ian Stansfield [a,*], Caterina Ercolani [a], Angela Mackay [a], Immacolata Conte [a], Marco Pompei [a], Uwe Koch [b], Nadia Gennari [c], Claudio Giuliano [d], Michael Rowley [a], Frank Narjes [a]

[a] Department of Medicinal Chemistry, Istituto di Ricerche di Biologia Molecolare "P. Angeletti" S.p.A., Merck Research Laboratories Rome, 00040 Pomezia, Italy
[b] Department of Computational Science, Istituto di Ricerche di Biologia Molecolare "P. Angeletti" S.p.A., Merck Research Laboratories Rome, 00040 Pomezia, Italy
[c] Department of Pharmacology, Istituto di Ricerche di Biologia Molecolare "P. Angeletti" S.p.A., Merck Research Laboratories Rome, 00040 Pomezia, Italy
[d] Department of Drug Metabolism Discovery and Development, Istituto di Ricerche di Biologia Molecolare "P. Angeletti" S.p.A., Merck Research Laboratories Rome, 00040 Pomezia, Italy

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ABSTRACT

We report the evolutionary path from an open-chain series to conformationally constrained tetracyclic indole inhibitors of HCV NS5B-polymerase, where the C2 aromatic is tethered to the indole nitrogen. SAR studies led to the discovery of zwitterionic compounds endowed with good intrinsic enzyme affinity and cell-based potency, as well as superior DMPK profiles to their acyclic counterparts, and ultimately to the identification of a pre-clinical candidate with an excellent predicted human pharmacokinetic profile.

HCV is a major human pathogen associated with chronic hepatitis and liver disease, cirrhosis, hepato-cellular carcinoma and liver failure,[1] with worldwide, an estimated 170 million chronic carriers.[2] Frontline therapies are based around interferon-α, commonly dosed in conjunction with ribavirin. Despite progress, including introduction of pegylated interferon,[3] sustained viral response (SVR) rates are still poor—particularly for genotype-1 infections that predominate in Europe, Japan, and the U.S.[4] In addition, therapy is often accompanied by significant adverse side effects[5]—consequently, there is a pressing need for new and broadly effective therapeutics to combat HCV.[3,6]

HCV is a small, enveloped, single stranded positive RNA virus in the *Flaviviridae* family. NS5B is the viral RNA-dependent RNA polymerase (RdRp) that is essential for viral replication.[7] With no functional equivalent in uninfected mammalian cells, it is an attractive target for drug discovery.[8] Inhibition of NS5B can be achieved through interaction at the active site, or at one of several allosteric inhibitor binding sites located distal to the catalytic centre.[9] Reports from our laboratories have documented the development of *N*-acetamidoindoles, such as **1** and **2**, as potent inhibitors interacting at allosteric site A lying close to a conserved amino acid, proline 495, on the surface of the thumb domain of the polymerase[10–12] (Fig. 1).

In this report we describe an alternative evolutionary path, from an open-chain series to tetracyclic indoles, **3**, where the C2 aromatic is tethered to the indole nitrogen, leading to the discovery of compounds endowed with good cell-based activity and superior DMPK profiles to their acyclic counterparts.

The compounds described herein were assessed for activity (IC_{50}) against the purified ΔC21 NS5B enzyme in the presence of heterogenic template RNA. Inhibition of replication of subgenomic HCV RNA was measured in HUH-7 cells using a modification of the procedure of Bartenschlager[13] (the so-called replicon assay). Unless otherwise stated, cell-based data (EC_{50}) were measured in the presence of 10% fetal calf serum.

The tetracyclic indoles reported herein were prepared as outlined in Schemes 1 and 2. Thus, for the most part the synthetic strategy used standard chemistry,[14] starting from methyl 2-bromo-3-cyclohexylindole-6-carboxylate substrates **4** and **15**,[10] to afford the cyclization pre-cursors **5**, **17** and **21**: alkylation to install the appropriate side chain on the indole nitrogen, Suzuki cross-coupling and, where necessary, manipulation of the *ortho*-functionality. In the case of **5**, following acetal deprotection, the resultant reactive intermediate was not isolated. Dilution with MeOH, adjusting the pH and reduction afforded the cyclic amine. Conversion to the desired product **6** was by simple ester hydrolysis. Alternatively, isolation of the intermediate following deprotection of **5** allowed a Strecker reaction to give the cyclic α-amino nitrile. Nitrile reduction to the primary amine, reductive amination and ester hydrolysis yielded **7**. Of particular note is the chemistry

* Corresponding author. Tel.: +39 06 91093286; fax: +39 06 91093654.
E-mail address: ian_stansfield@merck.com (I. Stansfield).

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to assemble the tetracyclic cores **10** and **13**. Following Suzuki coupling and deprotection to yield *ortho*-aniline **9**, reaction with α-chloro methyl acrylate afforded solely indolobenzo-diazepinedicarboxylate regio-isomer **10**—presumably via a selective conjugate addition–cyclization cascade. Instead, triphenylphosphine catalyzed α-addition[15] of indole **8** to methyl propiolate yielded α-acrylate **12**, which, after Boc deprotection, could be induced to participate in a conjugate addition to afford tetracyclic regio-isomer **13** under the same conditions employed in the cascade reaction above. In both cases, reductive amination to methylate the anilinic nitrogen, side chain manipulation to install the basic amine, and ester hydrolysis gave the final compounds **11** and **14**.

For the benzylic cyclic amides, following deprotection of **17**, cyclization under standard amide bond forming conditions (or base mediated for R^2 = Me) and methyl ester cleavage with BBr_3 afforded the endocyclic amides **19**. Reduction of **18** was an alternative to give the corresponding cyclic amine, and for R = H afforded the secondary amine suitable for accessing exocyclic amides **20.** The anilinic tetracycles were prepared in similar fashion from **21**. Interestingly, deprotection of **21** led to spontaneous ring closure affording **22** in the case of the 7-membered ring, whilst peptide coupling conditions were required for the 8-membered homolog. N-Alkylation and ester cleavage gave cyclic amides **23**. Facile reduction of the cyclic amide (presumably aided by conformational constraints in the 7-membered system) prior to ester hydrolysis led to cyclic amines **24**.

The binding mode at allosteric site A of the indole (and related benzimidazole) class has been well documented[11,16] and is illustrated in Figure 2. Salient features include the C3 cyclohexyl ring buried deep in a lipophilic pocket, the C2 phenyl rotated out of the indole plane and occupying a lipophilic channel and a key electrostatic interaction between the carboxylic acid and arginine 503. The substituent on the indole nitrogen is oriented towards solvent and as such offers a suitable handle for modulating physicochemical properties. Similarly, whilst one *ortho* position on the C2 phenyl moiety in (**1**) faces the lipophilic surface of the polymerase, the other projects to solvent.

Thus, we and others[17,18] have reasoned that it ought to be possible to improve intrinsic affinity for the binding site on the enzyme by constraining the C2 aromatic in the bound conformation via tethering from the *ortho* position to the indole nitrogen. Our analysis suggested that tethering to form either an 8- or a 7-membered ring would be suitable, and pleasingly (as illustrated by **25**) the entropic gain from tethering gave a 6-fold boost in biochemical potency (Table 1). Activity in the cell-based assay, however, deteriorated by an order of magnitude. In this instance the reduced cellular potency could likely be ascribed to raised affinity of **25** for serum protein (human plasma protein binding (hPPB) 99.7%) with

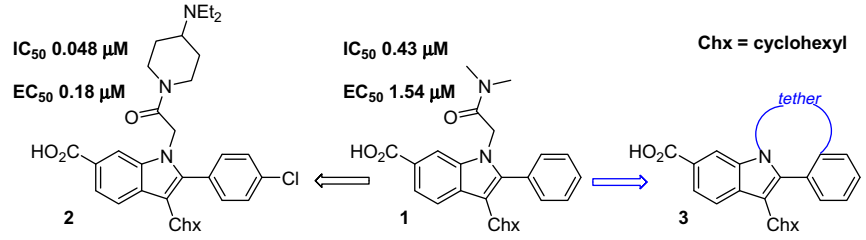
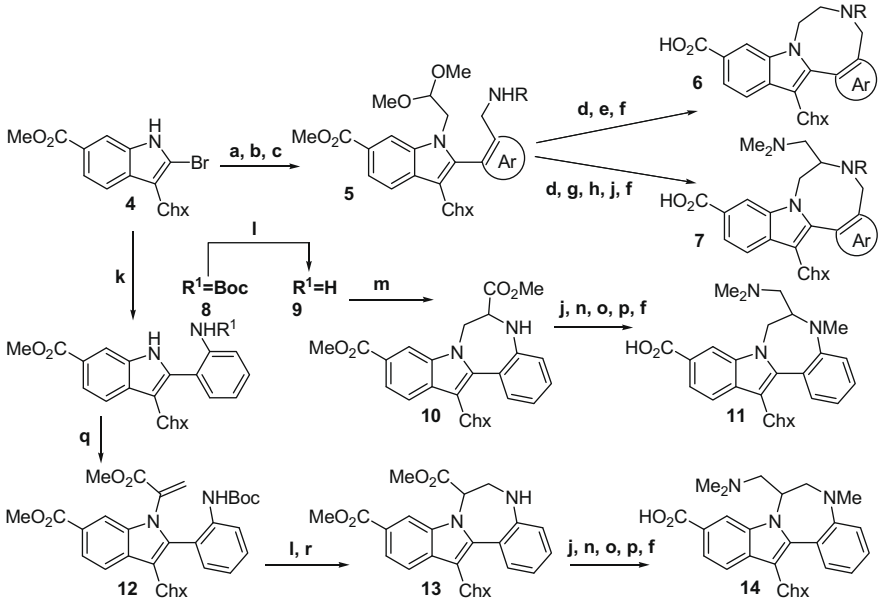
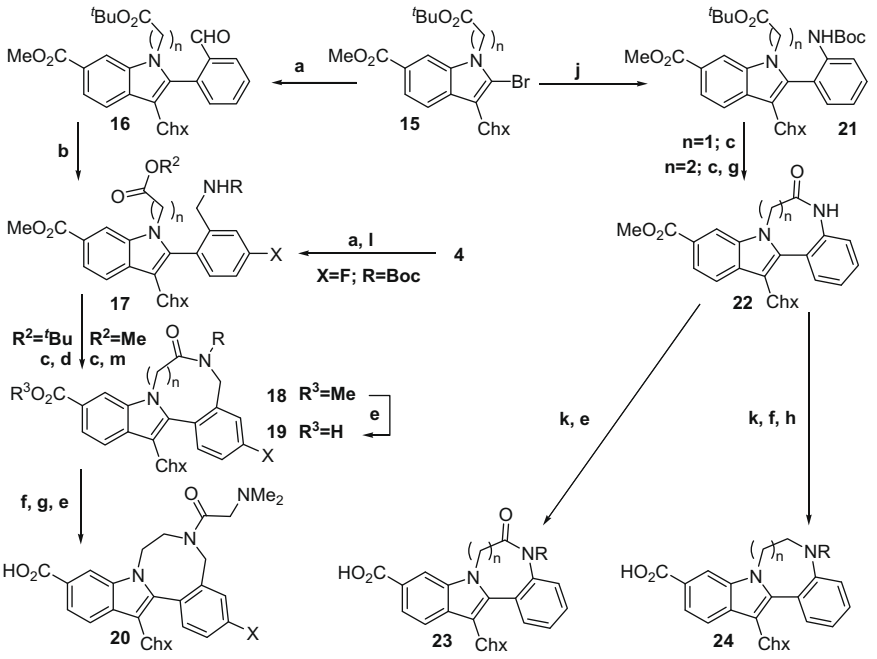


Figure 1. Acyclic *N*-acetamide and tethered indole inhibitors of HCV NS5B-polymerase.



Scheme 1. Reagents and conditions: (a) NaH, $BrCH_2CH(OMe)_2$, KI (cat), DMF, 60 °C, 79%; (b) $Pd(PPh_3)_2Cl_2$, Na_2CO_3 (aq), (2-formylaryl)boronic acid, 1,4-dioxane, reflux, 85–95%; (c) i—H_2NR, AcOH (cat), THF; ii—$NaBH_4$, MeOH, 85–100%; (d) 3 N HCl (aq), THF, 60 °C; (e) pH 5–6; $NaBH_4$, MeOH; (f) i—NaOH (aq), MeOH, 60 °C; ii—RP-HPLC, 20–50%; (g) KCN, H_2O, MeCN, pH 5, 65%; (h) PtO_2, H_2, 50 psi, AcOH (1 equiv), EtOH, 75%; (j) CH_2O, $NaBH(OAc)_3$, DCE, 72–80%; (k) $Pd(PPh_3)_2Cl_2$, Na_2CO_3 (aq), *tert*-butyl [2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl]-carbamate, 1,4-dioxane, reflux, 95%; (l) CF_3CO_2H, CH_2Cl_2, H_2O, 57–70%; (m) methyl α-chloro acrylate, K_2CO_3, $BnEt_3NCl$, MeCN, 60 °C, 75%; (n) LiOH (aq), THF, MeOH, 81%; (o) TBTU, iPr_2NEt, $HNMe_2$, CH_2Cl_2, 70–80%; (p) BH_3·DMS, THF, 70–80%; (q) PPh_3, methyl propiolate, CH_2Cl_2, 80%; (r) K_2CO_3, $BnEt_3NCl$, MeCN, 60 °C, 53%.



Scheme 2. Reagents and conditions: (a) $Pd(PPh_3)_2Cl_2$, Na_2CO_3 (aq), (aryl)boronic acid, 1,4-dioxane, reflux, 53–84%; (b) i—H_2NR, AcOH (cat), THF; ii—$NaBH_3CN$, MeOH, 88–95%; (c) CF_3CO_2H, CH_2Cl_2, 53–100%; (d) HATU, iPr_2NEt, CH_2Cl_2, 98%; (e) i—BBr_3, CH_2Cl_2; ii—RP-HPLC, 20–50%; (f) BH_3·DMS, THF, 80–83%; (g) TBTU, iPr_2NEt, RCO_2H, CH_2Cl_2, 70–80%; (h) i—NaOH (aq), MeOH, 60 °C; ii—RP-HPLC, 20–50%; (j) $Pd(PPh_3)_2Cl_2$, Na_2CO_3 (aq), *tert*-butyl [2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl]-carbamate, 1,4-dioxane, reflux, 60–95%; (k) NaH, RX, DMF, 73–90%; (l) $BrCH_2CO_2Me$; K_2CO_3, DMSO, 60 °C, 70%; (m) NaOMe, MeOH, 100%.

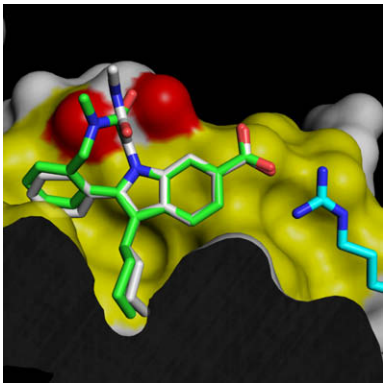


Figure 2. Superimposition of tetracyclic **25** and acyclic indole *N*-acetamide **1**, docked in the allosteric inhibitor binding site.

respect to **1** (hPPB 97.1%). Previously in the indole class, zwitterionic species have been found to exhibit reduced PPB and improved cell permeability over related non-zwitterionic systems—enhancing cell-based activity. Accordingly, introduction in **26** of a distal basic centre improved activity in the replicon assay by close on 2 orders of magnitude. With in vitro and cell-based activity in hand, the 7- and 9-membered cyclic analogs were prepared for comparison. Modelling predicted the dihedral angle between the plane of the indole and the C2 phenyl for the 7- and 8-membered rings (Φ_{calc} 48° and 56°, respectively) to be in line with the enzyme bound conformation[11] (Φ 51°), whilst the 9-membered ring diverges (Φ_{calc} 73°). In agreement with the prediction, **28** shows significantly reduced affinity on the isolated enzyme and **26**, **27** both show good (and equivalent) levels of activity. The observation that these 8- and 7-membered tetracyclic amides are equipotent is in contrast to what has been reported to date for tetracyclic indoles—where a clear preference for 7- over 8-membered systems has been noted.[17] Cell-based activity proved better for **26** and we report here data primarily from the 8-membered ring class.

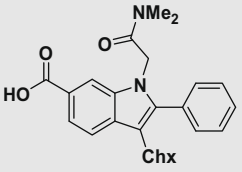
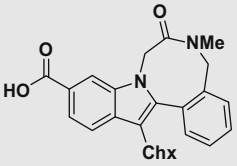
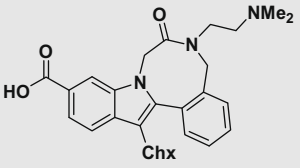
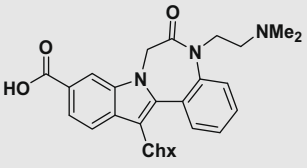
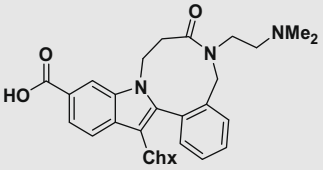
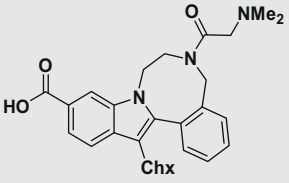
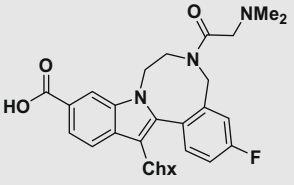
Interestingly, exocyclic amide analog **29** is essentially equipotent to **26**. Considering calculated pK_a values,[19] this suggests that a strongly basic amine is not required for cell-based activity. Rather, a less basic centre will suffice so long as a significant percentage of the protonated species exists at physiological pH. Substitution of the C2 aromatic afforded incremental improvements in intrinsic enzyme affinity and cell-based activity (e.g., **30**).

Given the projection of the tether moiety itself into solvent, we believed it ought to be possible to generate a zwitterionic species and improve cell-based activity by moving from an amide (**25**) to an amine linker (**31**). Pleasingly, combining the basic centre and the conformational constraint in a single structural motif (form and function) was not only tolerated with regard to enzyme affinity, but afforded sub-μM cell-based activity (Table 2). *Para*-methoxy substitution of the aryl at C2 (**32**), afforded a further 3-fold boost in both intrinsic and replicon potency—providing, for the first time, simplified structures with activity to rival **2** and **26**.

Introduction of a distal amine to the aminotetracycle core (**33**, **36**, **11**, and **37**) gave a further 6- to 7-fold potency boost and consolidated cell-based activity in the 100 nM range—with **33** offering the advantage of not introducing a chiral branch point to the tether. Substitution on the C2 aromatic in this class provided at best incremental gains in replicon activity (e.g., **34**). Consistent with our earlier predictions and results, **33** and its counterpart in the 7-membered ring series **36** are essentially equipotent. Interestingly, data for hybrid anilinic 8-membered tetracycle **35** is more in keeping with what has been reported elsewhere in the literature[17]—the compound losing potency with regard to **33** and **36**. In addition, **14** would suggest that branching alpha to the indole nitrogen is not tolerated.

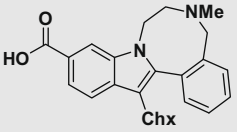
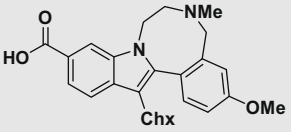
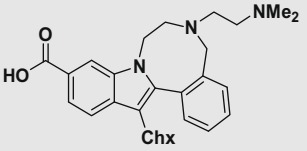
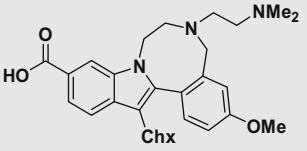
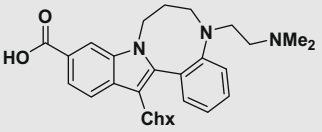
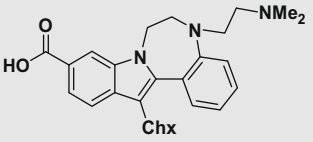
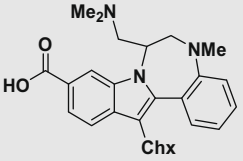
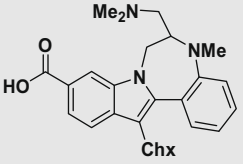
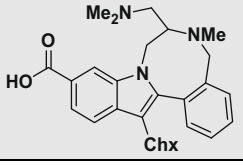
The PK properties for potent tetracyclic indoles (**30**, **32**, and **33**) are summarized in Table 3, alongside acyclic *N*-acetamide **2** for comparison. As mentioned above, the structural simplicity of **32** made it particularly attractive—indeed this simplicity may well contribute to its excellent PK properties in pre-clinical species also. Ultimately, however, cell-based potency focused attention on compounds **30** and **33**.

Table 1
Tetracyclic indole amides versus acyclic indole **1**

Compound	Structure	IC_{50}/EC_{50}[a] (μM)	Amine $pK_{a\,calc}$[19]
1		0.43/1.54	—
25		0.08/18.9	—
26		0.11/0.26	8.9
27		0.13/0.41	8.9
28		0.86/1.44	8.9
29		0.13/0.22	7.8
30		0.06/0.17	7.8

[a] IC_{50}/EC_{50} values are the mean from at least two experiments, standard deviation ± 20%.

Table 2
Aminotetracycle indole inhibitors of HCV polymerase

Compound	Structure	IC_{50}/EC_{50}[a] (μM)	Amine $pK_{a\,calc}$[19]
31		0.17/0.89	8.0
32		0.055/0.29	8.1
33		0.027/0.14	9.2/9.5[c]
34		0.035/0.10	9.2
35		0.077/0.54	8.8
36		0.028/0.13	8.8
14[b]		1.92/13.5	9.0
11[b]		0.043/0.16	9.0
37[b]		0.035/0.14	8.9

[a] IC_{50}/EC_{50} values are the mean from at least two experiments, standard deviation ± 20%.
[b] Racemic mixture.
[c] Measured value.

In general, compounds in this class have been found to be cleared mainly by metabolism, with only minimal biliary and renal excretion of intact drug. Thus, it was judged reasonable to extrapolate human PK predictions based on data from pre-clinical species and relative intrinsic metabolic stability in vitro. The relative rank order across species of intrinsic clearance (Cl_{int}) was seen as a more reliable means of comparing compounds than absolute Cl_{int} values, as it is known that compounds from this class are highly bound to microsomal protein.[20] This carries a number of provisos, not least that for a given compound there is a similar significant percentage unbound to plasma protein across species. In this regard, ca. 2% **30** is unbound in PPB (rat, dog, human), whilst values for **33** are 3–4% (dog, human) and 7% in rat.

Table 3
In vivo and in vitro pharmacokinetic properties for indole inhibitors of the HCV NS5B-polymerase

Compound	Species[a]	F[b] (%)	$AUC_{dose(mpk)}$ (μM h)	Clp[c] (ml/min/kg)	Clint[d] (ml/min/kg) r, d, rh, h
2	Rat	10	0.2_3	44	
	Rat	83	2.9_3	32	
32	Dog	67	18.7_2	2.9	43, 6, 16, 7
	Rat	48	4.0_3	12	
30	Dog	83	17.6_2	3.0	16, 8, 42, 13
	Rat	31	4.2_{15}	61	
33	Dog	74	23_2	2.5	100, 26, 51, 5
	Rhesus	57	2.0_2	22	

[a] Compounds were dosed as HCl or trifluoroacetate salts; $n = 3$. Vehicle iv 20% DMSO/60% PEG400/20% H_2O; po PEG400.
[b] Oral bioavailability.
[c] Plasma clearance.
[d] Intrinsic clearance in liver microsomes from rat (r), dog (d), rhesus (rh), human (h), incubated in the presence of NADPH.

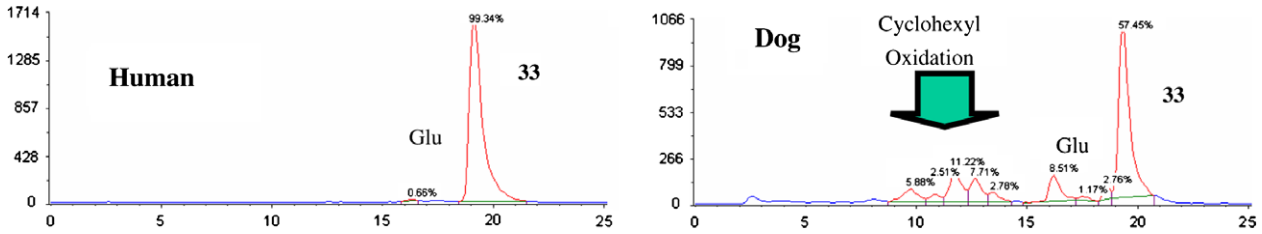


Figure 3. HPLC-radiochromatograms of [^{3}H]-**33** after 4 h incubation with human and dog hepatocytes.

Compounds **30** and **33** have moderate to good oral bioavailability in the rat, with good systemic exposure attainable. Although clearance of **33** in rat was relatively high, the compound was found to be dose proportional. Furthermore, elevated compound concentrations (liver/plasma ratio = 16 for **33**; 8 for **30**) were found in rat liver 6 h after oral administration (encouraging when considering liver is the target organ for an anti-HCV agent). In dog, both **30** and **33** showed excellent PK profiles with low clearance, high oral bioavailability and systemic exposure, and a plasma half-life of over 5 h. Discriminating on the basis of relative metabolic stability singled out **33** for further profiling. Human PK for **33** would be expected to be even better than that seen in dog, whilst **30** would be predicted to be worse. Stability in hepatocytes supported relative Cl_{int} data, with **33** showing the same rank order of stability across species and proving appreciably more stable in human preparations (Fig. 3).

Significantly, following oral dosing, **33** was cleared mainly by metabolism, with only minimal excretion of intact drug (rat urine 1%, bile 4%; dog urine <1%). The main oxidative metabolic pathways for **33** were found to be cyclohexyl hydroxylation (CYP3A4 mediated), N-demethylation (CYP3A4, 2D6 mediated). Taken as a whole, these data lead to an excellent predicted human PK profile for **33** that is similar or, more probably, superior to that determined in dog.

Compound **33** was characterized further and showed no activity (at 10 μM) in human PXR-dependent reporter gene assays. Furthermore, there was no significant inhibition of CYP3A4, 2D6 and 2C9 up to 100 μM and no evidence of time dependent inhibition of CYP3A4 in human liver microsomes. Overall, this suggests **33** has little potential to cause drug–drug interactions or autoinduction of metabolism in humans. The compound also showed no inhibition of human DNA polymerases and no significant off-target activities in extensive counterscreening efforts.[21] Lastly, **33** had low potential for covalent binding to rat and human microsomal protein in the presence of NADPH and no propensity to prolong the QT interval in anesthetized dogs (escalating dose up to 10 mpk).

In summary, we have described the development of tetracyclic indoles, where the C2 aromatic is tethered to the indole nitrogen as part of an 8-membered ring, that are potent allosteric inhibitors of the HCV NS5B enzyme. Optimization furnished zwitterionic inhibitors that show potency in the blockade of subgenomic HCV RNA replication in HUH-7 cells. PK profiling of potent analogs from three diverse classes (**30**, **32** and **33**) showed good to excellent PK properties in pre-clinical species. Compound **33** was advanced on the basis of a superior predicted human PK profile. Further characterization showed **33** to be clean in an extensive panel of counterscreening assays. On the basis of these data, **33** progressed as a pre-clinical candidate.

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