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Pyrrolo[1,2-*b*]pyridazin-2-ones as potent inhibitors of HCV NS5B polymerase

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Abstract—Pyrrolo[1,2-*b*]pyridazin-2-one analogs were discovered as a novel class of inhibitors of genotype 1 HCV NS5B polymerase. Structure-based design led to the discovery of compound **3k**, which displayed potent inhibitory activities in biochemical and replicon assays (IC_{50} (1b) < 10 nM; EC_{50} (1b) = 12 nM) as well as good stability towards human liver microsomes (HLM $t_{1/2}$ > 60 min).

Hepatitis C virus (HCV) is a major cause of acute hepatitis and chronic liver disease, including cirrhosis and liver cancer. Globally, an estimated 170 million individuals, 3% of the world's population, are chronically infected with HCV and 3–4 million people are newly infected each year.[1] Currently, there is no vaccine available to prevent hepatitis C, nor a HCV-specific antiviral agent approved for treatment of chronic hepatitis C. The current standard of care is a combination of pegylated interferon (IFN) with ribavirin.[2] Low response rates, in particular for patients infected with genotype 1 HCV, along with significant side-effects of current HCV therapy result in a continuing medical need for improved treatments.[3]

Our research has been focused on identifying novel inhibitors of the HCV NS5B protein, a virally encoded RNA-dependent RNA polymerase (RdRp), the activity of which is critical for the replication of the virus.[4]

Most small molecule, non-nucleoside inhibitors of NS5B bind to one of three binding pockets, distinct from the active site.[5] Among these, we focused our attention on the palm binding site, which, based on our analysis, is highly conserved across various HCV genotype 1 sequences.

Several series of NS5B inhibitors have been reported to bind at the palm binding site.[6] More specifically, compound **1** (Fig. 1[7]), containing the benzo[1,2,4]thiadiazine-1,1-dioxide motif, has been reported to exhibit potent inhibitory activity against NS5B with an IC_{50} (1b) of 0.032–0.20 μM.[6c,8] As previously reported, we discovered that compounds containing 5-hydroxy-3(2*H*)-pyridazinones, as exemplified by compound **2**, can also function as potent NS5B inhibitors.[9] However, for many of these compounds we found it challenging to overcome their limited oral bioavailability. This was probably due to poor cell permeability likely caused by their high polar surface area (PSA), which is outside the normal range typically correlated with good absorption.[9c,d,10] Here we describe a related series of pyrrolo[1,2-*b*]pyridazin-2-one derivatives (**3**), which are derived from (**2**) by fusing C6 and N1 of the pyridazinone ring. We hypothesized that the resulting reduction in PSA combined with the increased lipophilicity might

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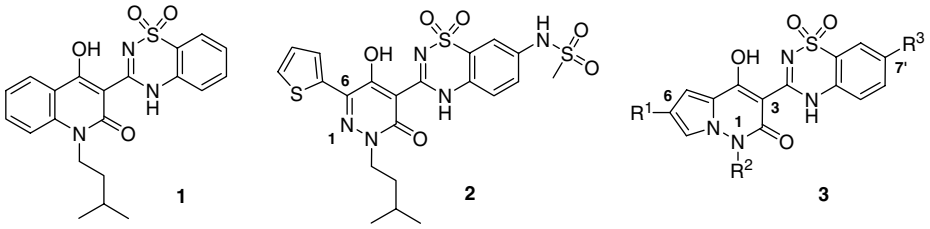


Figure 1. HCV NS5B polymerase inhibitors.

provide improved permeability properties and thereby afford a beneficial effect on the in vivo PK properties compared with analogs of compound **2**.

Table 1 details the structure–activity relationships (SAR) obtained for compounds **3**, focusing on their biochemical potencies against HCV genotype 1b, activities against the HCV genotype 1b subgenomic replicon in tissue culture, cytotoxicity, and stability against human liver microsomes (HLM).

Initially, we prepared analog **3a** as a direct comparison with **1**. This compound displayed low micromolar NS5B inhibition properties and was also active in the replicon cell-based assay. Encouraged by these results, we introduced a sulfonamide R^3 substituent known from our previous studies to afford potent NS5B inhibitory properties.[9c] This modification quickly led to compound **3c**, which displayed excellent activity in both biochemical and replicon assays. In line with our previous findings, the R^3 substituent was critical for activity and very sensitive to structural changes.[9a,c] For example, N-methylation of the R^3 sulfonamide moiety present in **3c** led to a >11-fold loss in potency in the biochemical assay (**3f**), while replacing the R^3 sulfonamide with a methoxy group (**3b**) greatly diminished the biological activity.

As evident in the co-crystal structure of **3c** bound to NS5B[11] (Fig. 2) and as previously reported,[9c] the R^3 sulfonamide forms several H-bonds with the NS5B protein. These include an interaction between the sulfonamide NH and the sidechain of Asp318, as well as a H-bond between one sulfonamide oxygen and a key structural water molecule (Fig. 3). The other R^3 sulfonamide oxygen forms a H-bond with the sidechain of Asn291. These favorable interactions may explain the good activity of **3c** compared to **3a–b**, **3f**, **3i–j**, and **3m–n**, which presumably lack some of these H-bonds with the NS5B protein.

Somewhat surprisingly, introduction of a R^3 cyclopropylsulfonamide moiety into the pyrrolopyridazinone inhibitor design (compound **3g**) led to a considerable

Table 1. SAR of pyrrolo[1,2-*b*]pyridazin-2-one analogs **3**

Compound	Route	R^1	R^2	R^3	IC_{50} (1b)[a] (μM)	EC_{50} (1b)[a] (μM)	CC_{50} (GAPDH)[a] (μM)	HLM $t_{1/2}$[a] (min)
2	Ref. 9c and d	Figure 1	$CH_2CH_2CH(CH_3)_2$	$NHSO_2Me$	<0.01	0.005	>33	>60 (100%)[c]
3a	B,D	H	$CH_2CH_2CH(CH_3)_2$	H	0.98	5.3	>100	14
3b	B,D	H	$CH_2CH_2CH(CH_3)_2$	OMe	2.2	17	>100	ND[b]
3c	B,D	H	$CH_2CH_2CH(CH_3)_2$	$NHSO_2Me$	<0.01	0.0085	>1	42
3d	B,C	F	$CH_2CH_2CH(CH_3)_2$	$NHSO_2Me$	0.027	0.019	>1	>60 (78%)[c]
3e	B,C	CN	$CH_2CH_2CH(CH_3)_2$	$NHSO_2Me$	0.32	ND[b]	ND[b]	ND[b]
3f	A	H	$CH_2CH_2CH(CH_3)_2$	$NMeSO_2Me$	0.11	0.19	>33	>60 (55%)[c]
3g	B,D	H	$CH_2CH_2CH(CH_3)_2$	$NHSO_2cPr$	0.16	0.096	>33	59
3h	A	H	$CH_2CH_2C(CH_3)_3$	$NHSO_2Me$	<0.01	0.005	>1	10
3i	A	H	$CH_2CH_2C(CH_3)_3$	$NMeSO_2Me$	0.06	0.12	>33	49
3j	A	H	4-F-Bn	H	0.85	ND[b]	ND[b]	45
3k	A	H	4-F-Bn	$NHSO_2Me$	<0.01	0.012	>1	>60 (86%)[c]
3l	A	H	3-Cl,4-F-Bn	$NHSO_2Me$	0.025	0.022	>1	>60 (102%)[c]
3m	A	H	4-F-Bn	$NMeSO_2Me$	0.13	0.33	>10	>60 (90%)[c]
3n[d]	A	H	4-F-Bn		0.13	0.38	>33	>60 (100%)[c]

[a] See Ref. 9a for assay conditions.
[b] ND, not determined.
[c] For values >60 min, % remaining at 60 min is given in parentheses.
[d] Racemic.

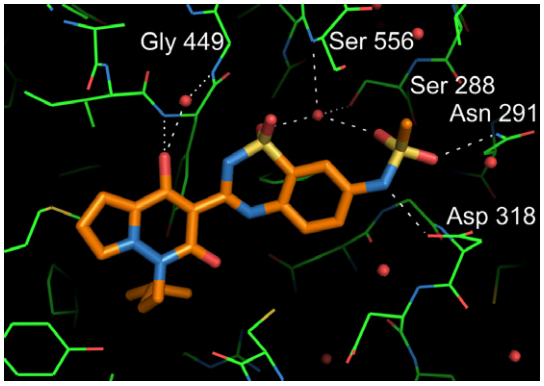


Figure 2. Co-crystal structure of compound **3c** bound to the NS5B protein (2.1 Å).

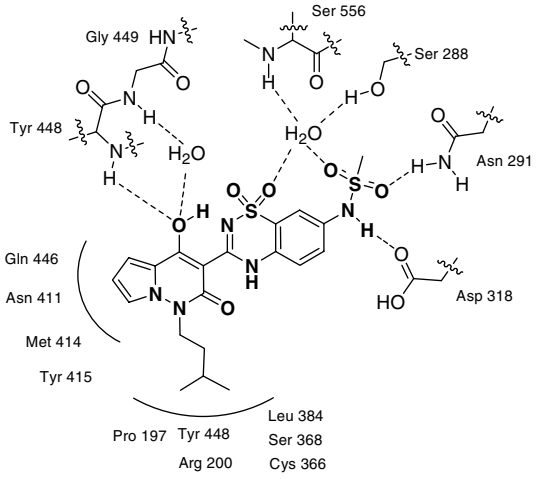


Figure 3. Schematic representation of the interactions between compound **3c** and the NS5B protein. Hydrogen bonds are represented as dashed lines, and the residues which make up the enzyme binding subsites are shown.

loss in activity in the enzymatic assay. Similar introduction of this fragment into the substituted pyridazinone inhibitors we previously studied (e.g., **2**) led only to a 3- to 4-fold reduction in NS5B inhibitory potencies.[9c] While the former result is not completely understood, it appears likely that this modification may cause a change in the overall geometry of the pyrrolopyridazinone structure in the binding pocket, resulting in the observed loss in activity.

Changes in the R^2 moiety were generally well tolerated, with the *tert*-butyl ethyl analog **3h** showing comparable activities to **3c**. Also, the 4-fluorobenzyl analog **3k** retained good enzymatic and antiviral potencies, while introduction of an additional chlorine atom on the R^2 benzyl ring led to a slight loss of activity (**3l** vs **3k**). This result may indicate a sterically and/or electronically unfavorable interaction. Again, N-methylation of the R^3 sulfonamide for the analog containing a *tert*-butyl ethyl R^2 moiety also resulted in loss of activities (**3i** vs **3h**). However, this change was not as pronounced as that observed for compounds containing isoamyl and 4-fluorobenzyl fragments in the R^2 position (**3f** vs **3c** and **3m** vs **3k**). We went on to explore changes in the R^1 substituents by installing a nitrile group at the 6-position of the pyrrolopyridazinone ring. This modification resulted in a substantial loss in activity (**3e** compared to **3c**), suggesting that the nitrile group may be either sterically too encumbered or too polar in nature to fit well in the shallow hydrophobic R^1 binding pocket. Installation of the smaller and more lipophilic fluoro moiety (**3d**) was better tolerated but still led to a $\geqslant$3-fold loss in potency in the biochemical assay (**3d** vs **3c**).

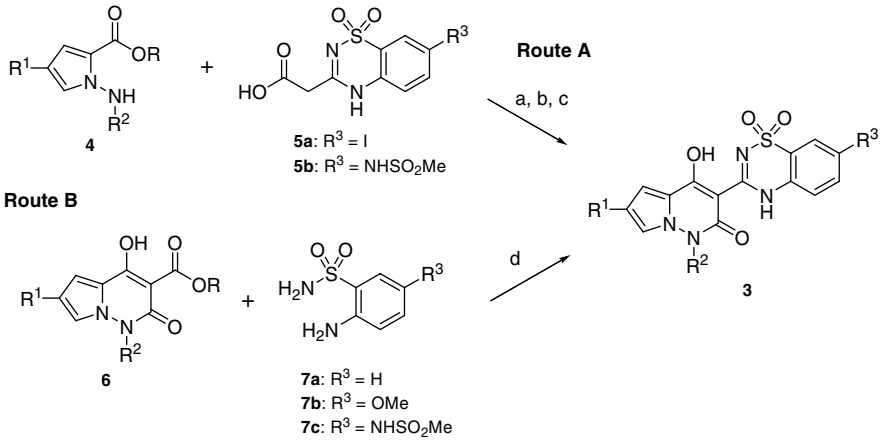
We tested the stability of the above compounds toward human liver microsomes, indicated as their HLM $t_{1/2}$ in Table 1. The majority of compounds exhibited moderate (>30 min) to long half-lives (>60 min). Compound **3a** lacking the R^3 substituent was among the least stable compounds tested. While the most potent compound **3c** had a reasonable half-life (42 min), introduction of a R^1 fluorine atom (**3d**) led to an improvement in stability ($t_{1/2}$ > 60 min, 78% remaining at 60 min). This result suggested that the fluorine may reduce potential metabolism occurring on the pyrrole ring. When comparing **3h** with **3c**, a significant loss in stability was observed, presumably because the *tert*-butyl ethyl moiety at R^2 represents a better substrate for interactions with CYPs as compared with the isoamyl group. Interestingly, the stability could be restored by N-methylation of the R^3 sulfonamide but unfortunately at the cost of potency as shown in compound **3i**. Compound **3k** proved to be the optimal compound exhibiting a favorable combination of long HLM half-life and potent activities in biochemical and replicon assays. Collectively, our results suggest that modifications in the R^2 and R^3 regions can be utilized to successfully overcome potential metabolic liabilities in this series of NS5B inhibitors.

Table 2 details the results obtained from our in vivo PK assessment of a selected number of compounds. To our disappointment, compounds **3c** and **3k**, which were initially identified to be the most promising compounds in this series, showed only low exposure levels after oral dosing in cynomolgus monkeys. These compounds displayed good stability towards monkey liver microsomes as well as reasonable solubility in our biochemical assays. However, as observed previously, MLM stability did not correlate well with the corresponding clearance data suggesting this process may not be primarily mediated via biotransformation.[9d] We therefore assumed that their poor permeability, as indicated by their low P_{app} values in the Caco-2 assay, was responsible for the unsatisfactory PK properties. Accordingly, we reasoned that lowering the PSA and/or increasing the lipophilicity of the compounds under study might improve their intestinal permeability and oral bioavailability. N-Alkylation of the sulfonamides led to an increase in lipophilicity as predicted by their *c*log *P* values (compare **3f** and **3m** with **3c**). Importantly, these changes also resulted in lower PSA values compared to the unsubstituted sulfonamide **3c**, which we believe to be beneficial for achieving good cell permeability. Indeed, in these cases Caco-2 permeability was increased but only translated into similar or slightly improved exposure levels for

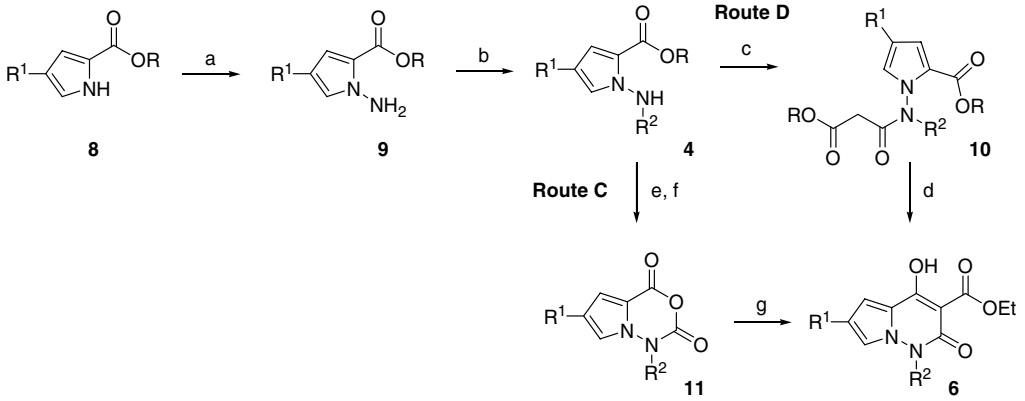
Table 2. Correlation of calculated physicochemical parameters, in vitro DMPK data and oral bioavailabilities of selected pyrrolo[1,2-*b*]pyridazin-2-one analogs **3**

Compound	PSA[a]	*c*log *P*[a]	MLM $t_{1/2}$[b] (min)	Solubility limit[d] (μM)	P_{app}[b,e] [(cm/s) × 10^{-6}]	*F* (%)[f]	AUC_{inf}[f] (ng/h/mL) po/iv	CL (iv)[f] (mL/min/kg)
2	203	−0.1	>60 (82%)[c]	>100	0.03	2	30/1334	14
3a	112	1.99	11	>200	11	63	12,887/20,478	49
3c	167	0.48	>60 (66%)[c]	>100	0.2	ND[g]	83/ND[g]	ND[g]
3f	158	1.23	60	>100	4.3	7	79/1063	16
3g	167	0.85	>60 (87%)[c]	>100	0.8	4	141/3774	5
3h	167	0.83	14	>100	0.7	2	25/1209	14
3k	167	0.66	>60 (90%)[c]	>100	0.1	1	6/539	31
3m	158	0.73	>60 (90%)[c]	>60	0.9	6	107/1776	9

[a] Calculated using ACD/Labs, version 10.0, Advanced Chemistry Development, Inc., Toronto ON, Canada. Available from www.acdlabs.com, 2006.
[b] See Ref. 9c for assay conditions.
[c] For values >60 min, % remaining at 60 min is given in parentheses. Compound **2** was tested at 5 μM, all other compounds were tested at 1 μM.
[d] Determined by UV absorption (2% DMSO in 20 mM Tris–HCl, pH 7.5, 20 mM NaCl, 5 mM $MgCl_2$, 5 mM dithiothreitol, 0.1 g/L bovine serum albumin, and 100 U/mL RNAse inhibitor).
[e] Controls: P_{app} atenolol (low) = 0.4×10^{-6} (cm/s), P_{app} propranolol (high) = 10×10^{-6} (cm/s).
[f] Cynomolgus monkeys; dose: 1 mg/kg; formulation (for both po and iv administration): 1% DMSO, 9.9% Cremophor EL in 50 mM PBS, pH 7.4.
[g] ND, not determined.



Scheme 1. Reagents and conditions: (a) DCC, DCM or EDC, DMAP, DMF, rt, 12 h; (b) NaOEt, EtOH, 60–80 °C, 8–12 h (39–82% over two steps); (c) when R^3 = I: $NHRSO_2R'$, CuI, sarcosine, K_3PO_4, DMF, 100 °C, 4–16 h, (15–65%); (d) i—pyridine, 120 °C, 3 h; ii—DBU (2 equiv), 120 °C, 16 h (7–10% over two steps); or i—neat, 180 °C, 20 min; ii—aq KOH, 110 °C, 20 h (47–52% over two steps).



Scheme 2. Reagents and conditions: (a) i—NaH, DMF, rt, 1 h; ii—NH_2Cl, Et_2O; or NH_2Cl, MTBE, Aliquat® 336, rt, 1.5 h (62–75%); (b) RCHO, $NaCNBH_3$, MeOH, 16 h (37–71%); (c) $EtOOCCH_2COCl$, 1,4-dioxane, 100 °C, 1 h, (quant.); (d) NaOEt, EtOH, 40–60 °C (25–82%); (e) $Pd(PPh_3)_4$, $NH_2OBn{\cdot}HCl$, DCM, 40 °C, 16 h (82%); (f) $COCl_2$, toluene, K_2CO_3, H_2O, 0 °C to rt, 16 h (57%); (g) $CH_2(CO_2Et)_2$, NaH, DMA, 120 °C, 16 h (55%).

compounds **3f** and **3m**. Although introduction of a terminal cyclopropyl group into the sulfonamide (**3g**) led to an increase in lipophilicity, the polar surface area remained unchanged. Consistent with our hypothesis, only modest improvements in cell permeability and exposure after oral dosing were observed compared to **3c**. While some improvements were achieved, the calculated log *P* and polar surface area values for the majority of the compounds in Table 2 are still outside the range of most known orally bioavailable drugs (<140 Å^2).[10] Consistent with this hypothesis, when the polar R^3 sulfonamide group was removed (**3a**), a significantly lower PSA combined with increased lipophilicity was achieved, resulting in good Caco-2 permeability and oral bioavailability. While the compounds described in this work, with the exception of compound **3a**, suffer from high polarity that prevents them from being absorbed effectively, these results provided us with additional direction to further improve the PK properties of the benzothiadiazine-containing NS5B inhibitors while retaining potent biological activity.

Compounds **3** were synthesized following Routes **A** or **B** as shown in Scheme 1. In Route **A**, aminoesters **4** were coupled with acids **5**[12] in the presence of DCC or EDC to form the corresponding amide intermediates. Treatment with NaOEt afforded the desired compounds **3**. Alternatively, in Route **B**, the esters **6** were condensed with 2-aminobenzensulfonamides **7**[13] by heating in pyridine to furnish the corresponding amide intermediates, which were then cyclized in the presence of DBU to yield the desired compounds **3**. When intermediate **5a**, bearing an iodo group at the 7-position, was employed, the corresponding sulfonamides **3** could be accessed via Cu-mediated displacement of the iodo moiety.[14]

The synthesis of key intermediates **4** and **6** is illustrated in Scheme 2. Pyrrole 2-allyl esters **8** (with the exception of **8e**, where the methyl ester was used) were prepared according to literature procedures.[15] N-Amination, using freshly or in situ prepared monochloramine,[16] followed by reductive alkylation in the presence of $NaCNBH_3$, provided key intermediates **4**. These could be further elaborated into **6** via different routes as shown in Scheme 2. Initially, we investigated accessing **6** via formation of cyclic anhydrides **11**, which upon treatment with diethylmalonate in the presence of NaH afforded the desired products **6** (Route **C**). However, this route required hydrolysis of the esters prior to treatment with phosgene, which initially led to decomposition of the starting materials **4** when simple alkyl esters (e.g., methyl esters) were employed. While employing an allyl ester, which was later removed in the presence of $Pd(PPh_3)_4$, effectively solved that issue, we later focused on the reaction sequence shown as Route **D** in Scheme 2. In this route, compounds **4** were treated with ethyl malonyl chloride to form the intermediates **10**, which were then converted to **6** in the presence of NaOEt.

In summary, we have synthesized a novel class of pyrrolo[1,2-*b*]pyridazin-2-ones as potent inhibitors of genotype 1 HCV NS5B polymerase. Our optimization efforts led to the discovery of compound **3k**, which exhibited low nanomolar activity in both biochemical and replicon assays as well as good stability toward HLM. However, PK studies indicated that the introduction of the polar R^3 sulfonamide moiety contained in **3k** significantly reduced oral bioavailability, presumably due to an increase in PSA that resulted in poor absorption. Results from additional modifications to this inhibitor series will be reported in the future.

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