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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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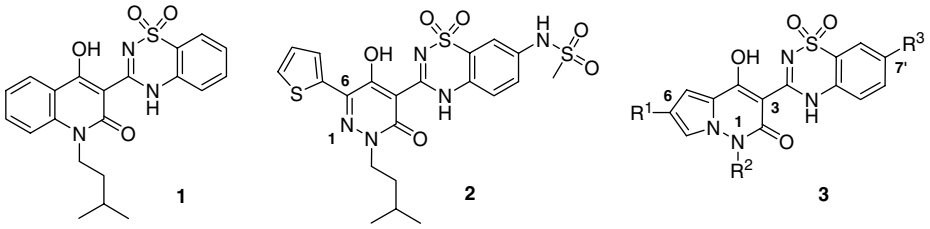

Figure 1. HCV NS5B polymerase inhibitors.

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.