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4-(1,1-Dioxo-1,4-dihydro-1λ^6-benzo[1,4]thiazin-3-yl)-5-hydroxy-2*H*-pyridazin-3-ones as potent inhibitors of HCV NS5B polymerase

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ABSTRACT

4-(1,1-Dioxo-1,4-dihydro-1λ^6-benzo[1,4]thiazin-3-yl)-5-hydroxy-2*H*-pyridazin-3-one analogs were discovered as a novel class of inhibitors of HCV NS5B polymerase. Structure-based design led to the identification of compound **3a** that displayed potent inhibitory activities in biochemical and replicon assays (1b IC_{50} < 10 nM; 1b EC_{50} = 1.1 nM) as well as good stability toward 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 to 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] Inadequate response rates in patients infected with genotype 1 HCV along with adverse side-effects result in a continuing medical need for improved therapy.[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 is well conserved across various HCV genotype 1 sequences.

Several series of NS5B inhibitors have been reported to bind at the palm binding site.[6] In particular, compounds **1a** and **1b** (Fig. 1),[7] containing the pyridazinone motif, were reported to exhibit potent inhibitory activity against genotype 1b NS5B.[8a] However, these compounds possess high polar surface areas (PSA), which are outside the range expected to impart favorable gut permeability properties.[9] Accordingly, they displayed poor permeabilities in Caco-2 assays and, as a likely result, exhibited poor bioavailabilities following oral administration to cynomolgus monkeys.[8a]

We hypothesized that by lowering the PSA and/or increasing the *clog P* of the pyridazinone-containing compounds under study, we would improve their Caco-2 permeabilities and thereby increase the corresponding bioavailability values.[8b] One strategy we pursued to accomplish this objective involved the synthesis of compounds containing a fused pyrrolo-pyridazinone motif (**2**) in lieu of the pyridazinone moiety present in **1a** and **1b**.[10] In this report, we describe an alternative approach to reducing the PSA of the NS5B inhibitors under study which entails replacing the benzothiadiazine fragment contained in them with a benzothiazine moiety (e.g., structures **3** and **4**).[11]

We began our exploration of the new NS5B inhibitor series by combining the benzothiazine moiety with other fragments that had been previously determined to interact favorably with NS5B during our exploration of the benzothiadiazine-containing

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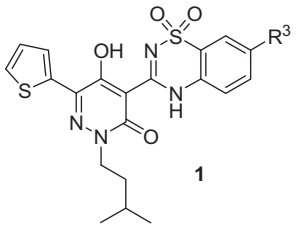
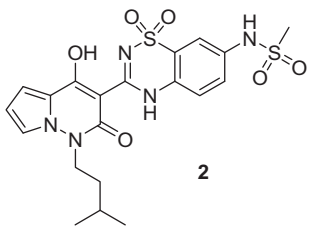


1

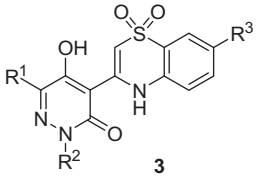
1a R^3 = $NHSO_2Me$
IC_{50} 1b <10 nM
EC_{50} 1b = 5 nM
PSA = 203 Å^2
P_{app} = 0.02 x 10^{-6}(cm/sec)
F = 2 %

1b R^3 = $NMeSO_2Me$
IC_{50} 1b = 23 nM
EC_{50} 1b = 130 nM
PSA = 194 Å^2
P_{app} = 0.20 x 10^{-6}(cm/sec)
F = 12 %



2

IC_{50} 1b = <10 nM
EC_{50} 1b = 8.5 nM
PSA = 167 Å^2
P_{app} = 0.2 x 10^{-6}(cm/sec)
F = 0 %



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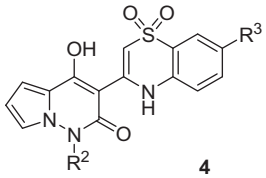

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Figure 1. HCV NS5B polymerase inhibitors.

compounds (Table 1).[8a–c] Thus, pyridazinone-benzothiazine hybrids bearing an R^1 thiophene, a branched or cyclic C_4–C_7 alkyl group at the R^2 position, and a $–NHSO_2CH_3$ R^3 moiety displayed potent NS5B inhibition properties along with excellent anti-HCV activity in cell culture (**3a**–**3d**). As was observed in our earlier studies,[8a–c] introduction of an R^2 benzyl group or a R^1 phenyl moiety into the inhibitor design reduced the NS5B inhibitory properties of the corresponding molecules (compare **3e** and **3f**, respectively, with **3a**). Similarly, substitution of the R^1 thiophene present in **3e** with a methyl group in the 5-position resulted in further loss of NS5B inhibitory potency (compound **3g**). Somewhat surprisingly, N-methylation of the R^3 sulfonamide moiety contained in **3a** drastically reduced NS5B inhibition activity (compound **3h**) in a manner that was not suggested by our previous study of benzothiadiazine-containing compounds (e.g., compare **1a** with **1b**). This unexpected result could be explained by analysis of the co-crystal structure of **3a** complexed with the NS5B protein (see below). Incorporation of optimal R^2 fragments into the fused pyrrolopyridazinone benzothiazine hybrids containing R^3 $–NHSO_2CH_3$ groups also afforded potent NS5B inhibitors which displayed excellent antiviral properties in cell culture (**4a** and **4b**). However, as was noted for the benzothiazine-containing compounds **3**, methylation of the R^3 sulfonamide moiety present in **4b** resulted in significant loss of NS5B inhibition properties (**4c**).

Collectively, the benzothiazine-containing compounds described in this work displayed NS5B inhibition properties and replicon activities that were similar to those exhibited by the corresponding benzothiadiazines (e.g., compare **1a** with **3a** and **2** with **4b**).[8a,10] These observations suggested that the N-2 atom present in the benzothiadiazine moiety was not critical for effective recognition by the NS5B protein. As was previously noted for benzothiadiazine inhibitors containing a R^3 sulfonamide moiety,[8a,10] the ratios of EC_{50} and IC_{50} values of the benzothiazines under study were typically small (<5-fold). The stability of the benzothiazines toward human liver microsomes was also assessed, and all compounds tested displayed moderate to long half-lives (30 to >60 min, Table 1).

To better understand the interactions of the benzothiazine inhibitors with the NS5B protein, a crystal structure of compound **3a** complexed with the enzyme was obtained.[12] As shown in Figure 2, the benzothiazine-containing compound occupied the NS5B palm binding site in a manner that was similar to that observed previously for the corresponding benzothiadiazine (**1a**).[8a] However, the benzothiazine ring system of **3a** adopted a more planar conformation than that noted for the benzothiadiazine moiety present in **1a**. This alteration resulted in a significantly different binding orientation for the R^3 $–NHSO_2CH_3$ groups contained in the two molecules. In particular, the sulfonamide NH vector of **1a** pointed out of the binding cavity toward solvent while the corresponding NH vector of **3a** was oriented toward the protein. This subtle but important difference is likely responsible for the significantly different reductions in NS5B inhibition activity observed when a methyl group is appended to the N-atom of the R^3 sulfonamide moiety of benzothiadiazine and benzothiazine inhibitors. As can be seen in Figure 2, the binding geometry of the former compounds appears to accommodate the additional methyl group more easily than the orientation of the latter inhibitors.

Table 2 details results from in vitro and in vivo DMPK assessments of a selected number of benzothiazine-containing compounds.

Compound **3a** displayed poor Caco-2 permeability properties that were very similar to those exhibited by the corresponding benzothiadiazine-containing molecule (**1a**). Not surprisingly, **3a** displayed low bioavailability after oral administration to cynomolgus monkeys. Most of the other benzothiazines examined exhibited similarly poor Caco-2 and bioavailability properties. One exception was compound **4a** which displayed the highest Caco-2 fluxes of all tested benzothiazines containing a R^3 sulfonamide moiety. Unfortunately, this compound was quite unstable toward monkey liver microsomes and did not show improved oral bioavailability relative to the other compounds in Table 2. While some trend between lower PSA values and improved Caco-2 fluxes was noted for the compounds in Table 2, the absolute permeabilities of all molecules tested were still low (compared to the control