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Structure-based design, synthesis, and biological evaluation of 1,1-dioxoisothiazole and benzo[*b*]thiophene-1,1-dioxide derivatives as novel inhibitors of hepatitis C virus NS5B polymerase

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ABSTRACT

A novel series of HCV NS5B polymerase inhibitors comprising 1,1-dioxoisothiazoles and benzo[*b*]thiophene-1,1-dioxides were designed, synthesized, and evaluated. SAR studies guided by structure-based design led to the identification of a number of potent NS5B inhibitors with nanomolar IC_{50} values. The most potent compound exhibited IC_{50} less than 10 nM against the genotype 1b HCV polymerase and EC_{50} of 70 nM against a genotype 1b replicon in cell culture. The DMPK properties of selected compounds were also evaluated.

Hepatitis C virus (HCV) is a major cause of acute hepatitis and chronic liver disease.[1] About 170 million people, 3% of the world's population, are infected with HCV and an estimated 3 million individuals become newly infected each year.[2] Currently there are no vaccines available to prevent hepatitis C. The existing combination treatment of pegylated interferon-α and ribavirin is costly, inconvenient, not well-tolerated, and has inadequate response rates, especially for genotype 1 patients.[3]

One promising avenue for treating HCV is to inhibit its replication by disabling the NS5B polymerase, a virally encoded protein essential for the HCV life cycle.

In 2006 GSK reported a series of benzothiadiazine analogs (**1**, Fig. 1) as potent HCV polymerase inhibitors.[4] These molecules bind to the "palm" region of NS5B in one of the three binding sites where non-nucleoside inhibitors typically interact with the protein.[5] Recently, we disclosed a related series of pyridazinone-containing NS5B inhibitors (**2**, Fig. 1), which also bind to the palm region of the enzyme.[6] The pyridazinone-containing compounds were found to display oral bioavailabilities in animals. In continuation, we sought to identify other structurally diverse, palm-binding NS5B inhibitors, which might exhibit improved pharmacokinetic (PK) properties.

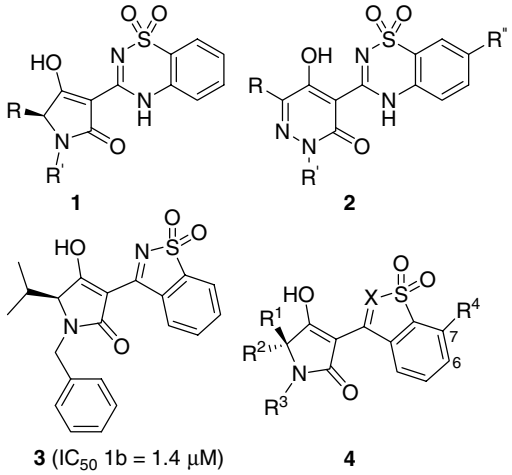

Figure 1. HCV NS5B polymerase inhibitors.

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Herein, we report a novel series of inhibitors of HCV polymerase in which the benzothiadiazine fragment present in **1** is replaced with a 1,1-dioxoisothiazole moiety. An early lead compound **3** (Fig. 1) exhibited reasonable potency (IC_{50} = 1.4 μM) against the genotype 1b NS5B enzyme and provided a good starting point for SAR development. In the present study, we focused on optimizing the lead structure via variation of R^1, R^2, R^3, R^4, and X (**4**, Fig. 1). As described below, the design of these target molecules was aided by several co-crystal structures we obtained of various pyridazinone-containing inhibitors complexed with the NS5B protein.[6]

The syntheses of these analogs began with the assembly of key synthetic precursors **8** and **13** (Scheme 3). Precursors **8** containing various R^1, R^2, and R^3 groups were prepared according to the general synthetic route outlined in Scheme 1. Reductive alkylation of amino acid esters **5** gave the corresponding secondary amines **6**, which were then subjected to an EDC-mediated coupling with mono-ethyl malonate to afford amides **7**.

Cyclization of **7** under basic condition followed by the decarboxylation of the resulting ester intermediate provided the desired 2,4-pyrrolidiones **8**.

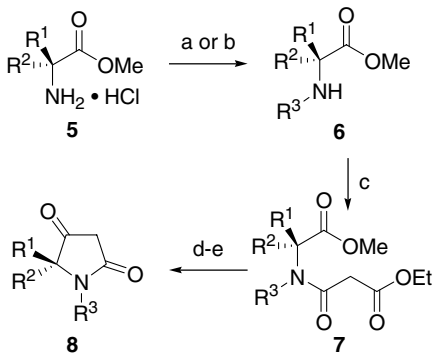
Different approaches were adopted for the construction of fragment **13** depending on the identity of R^4 and X.[7] Herein we discuss the synthesis of reactive fragment **12** as one example. Starting material **9** was prepared by using slightly modified literature procedures.[8] Benzylic bromination of **9** using one equivalent of NBS with benzoyl peroxide as an initiator gave predominantly the monobrominated product. Nucleophilic displacement of the bromine with Boc-protected methane sulfonamide resulted in the formation of **10**. Hot glacial acetic acid was then employed to simultaneously remove the *tert*-butyl and the Boc protecting groups to give rise to **11**. Subsequent chlorination with $POCl_3$ and catalytic amount of DMF afforded compound **12** (Scheme 2).

The key fragments **8** and **13** were successfully coupled by using NaH in THF as shown in Scheme 3. Nucleophilic displacement of the halogen atom of fragment **13** by the enolate of pyrrolidiones **8** provided the desired products **4** as yellow solids.[9]

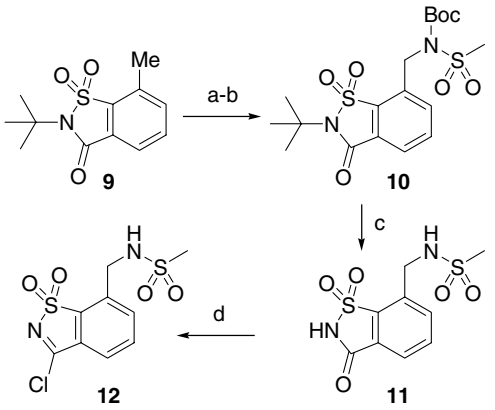
No attempts were made to rigorously determine the enantiomeric purity of final compounds **4**. It is therefore possible that racemization can occur during the synthesis of **8** (Scheme 1) or during the coupling reaction with **13** (Scheme 3).

The above-synthesized 1,1-dioxoisothiazole and benzo[*b*]thiophene-1,1-dioxide derivatives **4** were evaluated for in vitro activities against NS5B enzymes derived from HCV genotypes 1b[6a] and against an HCV genotype 1b replicon.[6a] The results of these assessments are shown in Table 1.

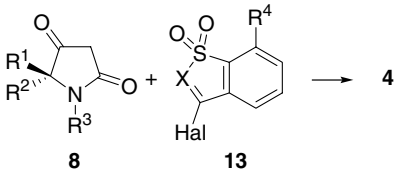
Our previous SAR studies, which were conducted with pyridazinone–benzothiadiazine NS5B inhibitors, demonstrated that polar



Scheme 1. Reagents and conditions: (a) for aromatic aldehydes: i—RCHO, TEA, $MgSO_4$, THF, 25 °C, 12 h; ii—$NaBH_4$, MeOH, 25 °C, 1 h; (b) for aliphatic aldehydes: RCHO, $NaBH_3CN$, MeOH, 25 °C, 12 h; (c) $HO_2CCH_2CO_2Et$, EDC·HCl, TEA, DCM, 25 °C, 12 h; (d) NaOEt, EtOH, 25 °C, 12 h; (e) 1 M H_2SO_4 (aq), reflux, 1 h.



Scheme 2. Reagents and conditions: (a) NBS, $(BzO)_2$, CCl_4, reflux, 1 d, 49%; (b) NHBocMs, Cs_2CO_3, DMF, 35–70 °C, 6 h, 81%; (c) AcOH, reflux, 1–2 d, 94%; (d) $POCl_3$, DMF, reflux, 10 min, 70%.



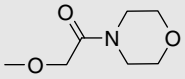
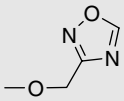
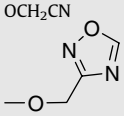
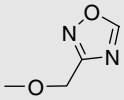
Scheme 3. Reagent and condition: (a) NaH, THF, 25 °C, 1 h. Hal = Cl or Br; X = N or CH.

substituents properly appended to the benzothiadiazine ring could dramatically improve inhibitor potency.[6] Accordingly, we sought to introduce similar polar fragments into our lead inhibitor **3** to determine whether analogous potency improvements could be realized. Due to the different NS5B-binding geometries adopted by the benzothiadiazine and 1,1-dioxoisothiazole-containing inhibitors, the 7-position of the latter ring system (R^4) appeared to be the ideal location to introduce such modifications. Adding an OEt fragment in this manner to **3** resulted in a slight decrease of NS5B enzyme inhibition (**14**, Table 1).

However, the corresponding hydroxy analog (**15**) displayed improved anti-NS5B characteristics and incorporating other polar R^4 moieties (**16**–**21**) afforded several compounds with improved NS5B inhibition properties relative to **3** (e.g., **16** and **21**). Although the 7-OEt substituent was not the optimal R^4 fragment identified by this initial SAR effort, it was synthetically convenient and therefore was employed in our exploration of R^1 and R^3 modifications.

Accordingly, we replaced the R^3 benzyl substituent present in **14** with a 3,3-dimethylbutyl fragment that had imparted good NS5B inhibition to the previously studied pyridazinone–benzothiadiazines.[6] The resulting compound (**22**) displayed slightly weaker NS5B inhibition levels relative to **14**. However, replacement of the R^1 *iso*-propyl group of compound **22** with a *tert*-butyl moiety (**23**) resulted in an increase in activity in the enzymatic assay. Additional analogs of compound **23** were therefore synthesized. Incorporation of larger aliphatic or aromatic groups at the R^1 position impaired inhibitor potency (**24**–**26**). However, combination of an R^1 *tert*-butyl entity with substituted R^3 benzyl moieties further improved potency (**27** and **28**) with the 3-Cl, 4-F-benzyl group proving to be the optimal R^3 fragment. Inclusion of a less bulky aliphatic moiety at the R^3 position was also explored but was found to be detrimental to inhibitor potency (compare**29** to **23** and **28**). As expected from our initial SAR studies, removal of the OEt group present in **23** resulted in a slight improvement in NS5B inhibition properties (compound **30**). But compound **30** is clearly much more

Table 1
1,1-Dioxoisothiazole analogs (**4**)[a]

	R^1	R^2	R^3	R^4	X	IC_{50} 1b (μM)	EC_{50} 1b (μM)	HLM $T_{1/2}$ (min)
3	$CH(CH_3)_2$	H	CH_2Ph	H	N	1.4	24	>60
14	$CH(CH_3)_2$	H	CH_2Ph	OEt	N	3.6	18	>60
15	$CH(CH_3)_2$	H	CH_2Ph	OH	N	0.47	47	>60
16	$CH(CH_3)_2$	H	CH_2Ph	OCH_2CONH_2	N	0.52	88	>60
17	$CH(CH_3)_2$	H	CH_2Ph	$OCH_2CH_2CONH_2$	N	1.2	100	–[c]
18	$CH(CH_3)_2$	H	CH_2Ph	$OCH_2COC(CH_3)_3$	N	21	>33	–
19	$CH(CH_3)_2$	H	CH_2Ph	OCH_2CN	N	1.6	12	>60
20	$CH(CH_3)_2$	H	CH_2Ph		N	3.2	>33	>60
21	$CH(CH_3)_2$	H	CH_2Ph		N	0.35	15	>60
22	$CH(CH_3)_2$	H	$CH_2CH_2C(CH_3)_3$	OEt	N	9.0	–	–
23	$C(CH_3)_3$	H	$CH_2CH_2C(CH_3)_3$	OEt	N	1.5	–	>60
24	$CH_2CH(CH_3)_2$	H	$CH_2CH_2C(CH_3)_3$	OEt	N	5.1	–	–
25	C_6H_{11}	H	$CH_2CH_2C(CH_3)_3$	OEt	N	4.0	–	–
26[b]	2-Thiophene	H	$CH_2CH_2C(CH_3)_3$	OEt	N	2.9	–	–
27	$C(CH_3)_3$	H	CH_2(3-Cl-Ph)	OEt	N	1.6	–	>60
28	$C(CH_3)_3$	H	CH_2(3-Cl-4-F-Ph)	OEt	N	0.76	–	>60
29	$C(CH_3)_3$	H	$CH_2CH_2CH(CH_3)_2$	OEt	N	5.1	–	–
30	$C(CH_3)_3$	H	$CH_2CH_2C(CH_3)_3$	H	N	0.42	4.3	10
31	$C(CH_3)_3$	H	CH_2(3-Cl-4-F-Ph)	OCH_2CONH_2	N	0.033	7.9	>60
32	$C(CH_3)_3$	H	CH_2(3-Cl-4-F-Ph)	OCH_2CN	N	0.07	0.7	>60
33	$C(CH_3)_3$	H	CH_2(3-Cl-4-F-Ph)		N	0.18	1.8	>60
34	$C(CH_3)_3$	H	CH_2(3-Cl-4-F-Ph)	$CH_2NHSO_2CH_3$	N	<0.010	0.07	>60
35	$C(CH_3)_3$	H	CH_2(3-Cl-4-F-Ph)	$CH_2N(CH_3)SO_2CH_3$	N	0.023	0.09	>60
36	$C(CH_3)_3$	H	CH_2(3-Cl-4-F-Ph)	$NHSO_2CH_3$[d]	N	0.094	13	>60
37	$C(CH_3)_3$	H	$CH_2CH_2C(CH_3)_3$	OCH_2CONH_2	N	0.2	11	48
38	$C(CH_3)_3$	H	$CH_2CH_2C(CH_3)_3$		N	0.65	2	44
39	$C(CH_3)_3$	H	$CH_2CH_2C(CH_3)_3$	$CH_2NHSO_2CH_3$	N	0.018	0.35	>60
40	$C(CH_3)_3$	H	$CH_2CH_2C(CH_3)_3$	$CH_2CH_2SO_2CH_3$	N	0.045	2.6	>60
41	$-CH_2CH_2-$		CH_2(3-Cl-4-F-Ph)	$CH_2NHSO_2CH_3$	N	0.13	>33	>60
42	$-CH_2CH_2-$		CH_2(3-Cl-4-F-Ph)	$CH_2N(CH_3)SO_2CH_3$	N	0.62	–	>60
43	CH_2CH_3	CH_3	CH_2(3-Cl-4-F-Ph)	$CH_2N(CH_3)SO_2CH_3$	N	0.41	–	>60
44	CH_3	CH_3	CH_2(3-Cl-4-F-Ph)	$CH_2N(CH_3)SO_2CH_3$	N	0.46	–	>60
45	$CH(CH_3)_2$	CH_3	$CH_2CH_2C(CH_3)_3$	$CH_2N(CH_3)SO_2CH_3$	CH	13	–	>60
46	$C(CH_3)_3$	H	$CH_2CH_2C(CH_3)_3$	$CH_2N(CH_3)SO_2CH_3$	CH	0.92	5.8	>60
47	$C(CH_3)_3$	H	$CH_2CH_2C(CH_3)_3$	$CH_2NHSO_2CH_3$	CH	0.24	6.4	>60

[a] CC_{50} (GAPDH) > 33 μM for all analogs.
[b] Racemic amino acid ester **5** was employed in synthesis.
[c] Not determined.
[d] This substitution is at the 6-position of the isothiazole ring.

labile in the HLM assay compared to the other compounds in the series.

Having identified the *tert*-butyl and 3-Cl, 4-F-benzyl groups as optimal R^1 and R^3 moieties, respectively, we then examined appending highly polar substituents to the 1,1-dioxoisothiazole ring system. As shown in Table 1, inclusion of such fragments into the inhibitor design afforded several compounds with excellent NS5B inhibitory properties (**31**–**36**). Many of these molecules also exhibited good activity in the replicon assay that was clearly distinct from cytotoxic effects. In particular, a compound containing a $CH_2NHSO_2CH_3$ R^4 substituent (**34**) displayed NS5B inhibition activity that was more potent than the quantitation limit of the enzymatic assay (<10 nM). As described in the X-ray analysis section below, the methylene (CH_2) moiety represents an optimal spacer between the polar $NHSO_2CH_3$ fragment and the 1,1-dioxoisothiazole ring system. Additional SAR studies demonstrated that the sulfonamide NH present in **34** could be methylated with minimal loss of in vitro enzymatic potencies (compare **35** with **34**). This result was consistent with the absence of an H-bond observed between the polymerase and the sulfonamide NH in the NS5B-**34** co-crystal structure. As predicted by X-ray analysis, directly attaching the sulfonamide to the 6-position of the 1,1-dioxoisothiazole ring system, in analogy to the optimal functionalization of the pyridazinone–benzothiadiazines we previously described,[6] was highly detrimental to anti-NS5B potencies (compare **36** to **34**). The activity loss was further magnified in the replicon assay, probably due to the poor cell permeability of **36** (comparing with **34**).

An additional set of R^4 variations was also investigated using a combination of *tert*-butyl and 3,3-dimethylbutyl moieties as R^1 and R^3 substituents, respectively (**37**–**40**). As observed previously, the most potent of these analogs incorporated a $CH_2NHSO_2CH_3$ moiety at the R^4 position (**39**). The minimal activity loss, when

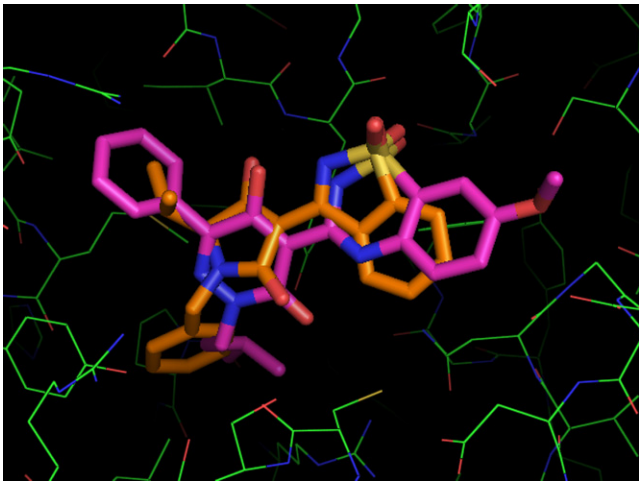


Figure 2. X-ray co-crystal structure of compound **3** (orange) bound to NS5B polymerase (2.3 Å). A pyridazinone–benzothiadiazine compound (cf, structure **2**, R = Ph, R′ = $CH_2CH_2CH(CH_3)_2$, R″ = OMe) as observed in an independently determined co-crystal structure[6] is superimposed in purple.

the R^4 sulfonamide NH was methylated (compare **35** with **34**) in the previous set of analogs, led us to explore whether the NH group of the R^4 sulfonamide present in **39** could be replaced with a methylene linker. Interestingly, this modification reduced the potency minimally despite the significant structural change.

Some limited efforts were then expended to determine whether the sp^3 carbon present in the 4-hydroxy-1,5-dihydropyrrol-2-one moiety of the above inhibitors could tolerate geminal substitution. Accordingly, several spirocyclic or quaternary analogs of compounds **34** and **35** were prepared (**41**–**44**), but all of them exhibited considerably decreased anti-NS5B activities relative to the parent compounds. We also explored replacing the 1,1-dioxoisothiazole present in the current study with a benzo[*b*]thiophene-1,1-dioxide moiety. Unfortunately, the three examples that were synthesized (**45**–**47**) displayed relatively weak NS5B inhibition properties. Therefore additional benzo[*b*]thiophene-1,1-dioxide-containing molecules were not prepared (for the most direct assessment of this change, compare **47** with **39**).

X-ray analysis. Prior to conducting the analog synthesis described above, we utilized the analysis of several NS5B inhibitor co-crystal structures to identify the optimal location to append R^4 substituents to the 1,1-dioxoisothiazole ring system. As shown in Figure 2, we superimposed[10] the co-crystal structure of our early 1,1-dioxoisothiazole lead (**3**[11]) with that of a pyridazinone-containing compound (**2**,[6a] R = Ph, R′ = $CH_2CH_2CH(CH_3)_2$, R″ = OMe)

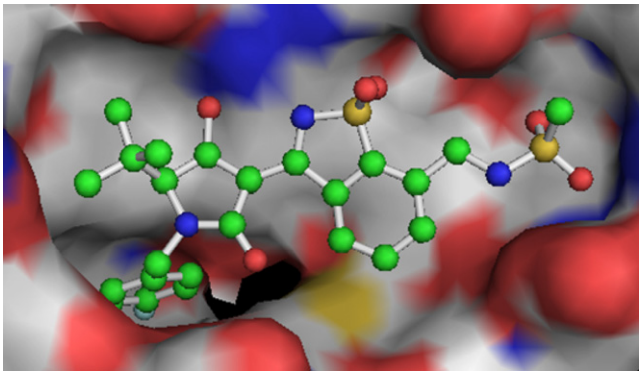


Figure 3. X-ray co-crystal structure of compound **34** bound to the NS5B polymerase (2.2 Å).

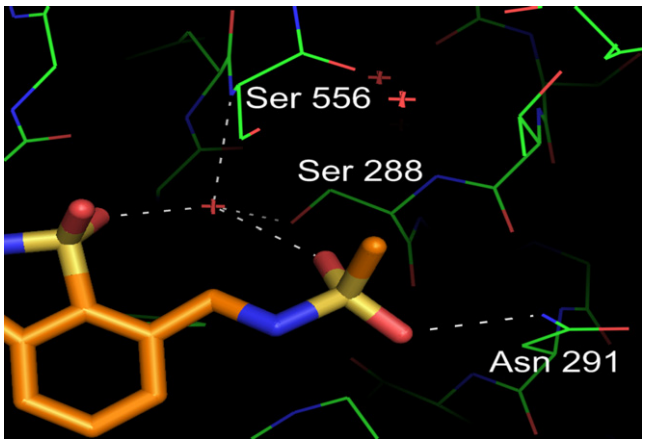


Figure 4. X-ray co-crystal structure showing hydrogen-bonding interaction of compound **34** with Asn 291 (2.2 Å).

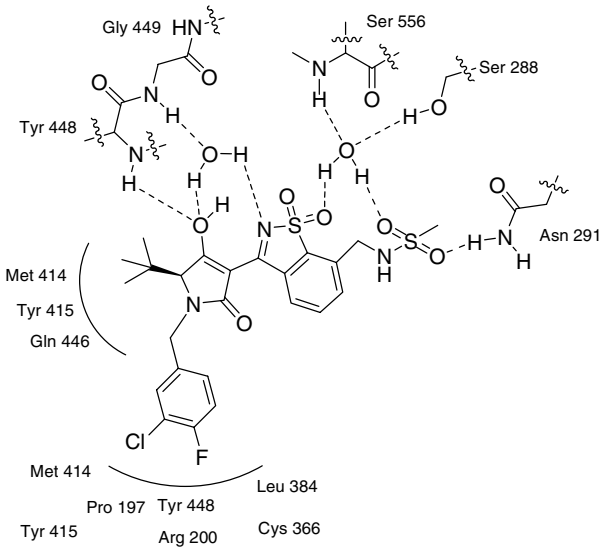


Figure 5. Schematic representation of the interactions between compound **34** and the NS5B protein. Hydrogen bonds that are shorter than 3.2 Å are represented as dashed lines, and the residues which make up the enzyme-binding subsites are shown.

whose anti-NS5B potency had been significantly improved by increasing the polarity of the R′ OCH_3 group.[6a] This exercise suggested that derivatizing the 1,1-dioxoisothiazole 7-position would better orient any appended fragments for interaction with the NS5B protein relative to performing similar modifications to the corresponding 6-location. Based on this observation, we synthesized most of the inhibitors described in this work with the R^4 substitution appended to the 1,1-dioxoisothiazole 7-position.

Figure 3 shows the X-ray co-crystal structure of compound **34** complexed with the NS5B protein. It clearly illustrates how the inclusion of a CH_2 spacer between the 1,1-dioxoisothiazole ring system and the sulfonamide moiety allows the functionality to appropriately access the far right side of the palm-binding pocket.[12] As depicted in Figures 4 and 5, the sulfonamide oxygen atoms form direct and water-mediated hydrogen bonds with NS5B residues Asn291 and Ser288, respectively. These interactions are likely responsible for the significant potency improvements exhibited by compounds **34** and **39**. Similar H-bonds were also noted in X-ray co-crystal structures of optimized pyridazinone–benzothiadiazine inhibitors complexed with the

Table 2
PK data for selected analogs (**30**, **34**, **35**)

Compound	P_{app} [(cm/s) × 10^{-6}][a]		(B-to-A)/(A-to-B) ratio	AUC_{24} (h ng/mL)[b]	C_{12} (ng/mL)[b]	F(%)[b]
	A-to-B	B-to-A				
30	9.9	19	2	4685	5	ND[c]
34	0.1	15	150	38	0.5	1
35	0.2	29	132	193	3.1	4

[a] See Ref.6c for assay conditions. Controls: P_{app} Atenolol (low) = 0.4–0.8 (cm/s) × 10^{-6}, P_{app} Propanolol (high) = 10–16 (cm/s) × 10^{-6}.
[b] Cynomolgus monkeys; 1 mg/kg single oral dose; 1% DMSO, 9.9% Cremophor EL in 50 mM PBS, pH 7.4.
[c] Not determined.[13]

NS5B protein.[6c] In contrast with the pyridazinone–benzothiadiazine co-crystal structures previously examined, the sulfonamide NH moiety present in **34** did not form a hydrogen bond with the NS5B protein, suggesting that it could be modified without significantly sacrificing inhibitor potency.

Pharmacokinetic studies. As part of our inhibitor optimization effort, in vivo PK data were obtained for selected compounds in the 1,1-dioxoisothiazole series following administration of a single oral dose to cynomolgus monkeys (**30**, **34**, and **35**, Table 2). The results demonstrated that as the polarity of the molecules increased, the corresponding oral exposures drastically deteriorated, as measured by AUC_{24} and concentrations of the compounds in monkey plasma 12 h after administration (C12). The PK data are consistent with the reduction of intestinal permeability with increasing compound polarity and this trend was observed in Caco-2 assessments of the three compounds (Table 2). Similar relationships between compound polarity, Caco-2 Papp values and oral exposures were also noted during our study of pyridazinone–benzothiadiazine NS5B inhibitors.[6d] Furthermore, Caco-2 results indicate that compounds **34** and **35** may act as efficient efflux transporter substrates (see (B-to-A)/(A-to-B) ratio, Table 2) which resulted in low exposures after oral dosing. Finally, our most potent NS5B inhibitors of interest (**34** and **35**) showed poor oral bioavailability values (1% and 4%, respectively) and inadequate plasma concentrations.

In summary, a novel series of NS5B inhibitors derived from 1,1-dioxoisothiazoles and benzo[*b*]thiophene-1,1-dioxides were synthesized and evaluated. The compounds demonstrated potent inhibitory activities against both genotype 1a and 1b HCV polymerases. Structure-based design and molecular modeling were employed to guide the optimization of the associated SAR. The most potent compound (**34**) in this series contained a R^1 *tert*-butyl group, a R^3 3-chloro-4-fluorobenzyl group, and a R^4 methyl methanesulfonamide group. However, PK results obtained from selected compounds in this series demonstrated that although the incorporation of polar R^4 substituents was important for enhancing the biological potencies of these molecules, such modification also compromised their oral PK properties.[14]

Acknowledgements

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Supplementary data

Supplementary material describing additional syntheses of individual 1,1-dioxoisothiazoles and benzo[*b*]thiophene-1,1-dioxides **4** can be found, in the online version, at doi:10.1016/j.bmcl.2008.05.083.

References and notes

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11. Crystals of HCV NS5B polymerase (genotype 1b, strain BK, Δ21) were grown by the hanging drop method at room temperature using a well buffer of 20% PEG 4K, 50 mM ammonium sulfate, 100 mM sodium acetate, pH 4.7 with 5 mM DTT. The crystals formed in space group $P2_12_12_1$ with approximate cell dimensions, a = 85 Å, b = 106 Å, c = 127 Å, containing two protein molecules in the asymmetric unit. Protein-inhibitor complexes were prepared by soaking these NS5B crystals for 4 h in solutions containing 15–20% DMSO, 20% glycerol, 20% PEG 4K, 0.1 M Hepes, 10 mM $MgCl_2$ at pH 7.6, and inhibitors at concentrations of 2 mM. Diffraction data were collected to a resolution of 2.3 Å for compound **3**. Diffraction data for **3** were collected on beamline 14IDB at the Advanced Photon Source at Argonne National Laboratory. Crystal structures discussed in this paper have been deposited in the Protein Databank (www.rcsb.org) with entry codes: 3D28 and 3BSC. Full details of structure determination are given in the respective PDB entries.
12. Crystals of HCV NS5B polymerase (genotype 1b, strain BK, Δ21) were grown by the hanging drop method at room temperature using a well buffer of 20% PEG 4K, 50 mM ammonium sulfate, 100 mM sodium acetate, pH 4.7 with 5 mM DTT. The crystals formed in space group $P2_12_12_1$ with approximate cell dimensions, a = 85 Å, b = 106 Å, c = 127 Å, containing two protein molecules in the asymmetric unit. Protein-inhibitor complexes were prepared by soaking these NS5B crystals for 20 h in solutions containing 15–20% DMSO, 20% glycerol, 20% PEG 4K, 0.1 M Hepes, 10 mM $MgCl_2$ at pH 7.6, and inhibitors at concentrations of 5 mM. Diffraction data were collected to a resolution of 2.2 Å for compound **34**. Diffraction data for **34** were collected on beamline 14IDB at the Advanced Photon Source at Argonne National Laboratory. Crystal structures discussed in this paper have been deposited in the Protein Databank (www.rcsb.org) with entry codes: 3D5M. Full details of structure determination are given in the respective PDB entries.
13. As part of a strategy to rapidly evaluate the oral PK properties of the NS5B inhibitors under study, the corresponding PK data following IV administration of the described compounds were not obtained.
14. During the course of this study, similar HCV NS5B inhibitors were described in the patent literature. See: Blake, J. F.; Fell, J. B.; Fischer, J. P.; Hendricks, R. T.; Spencer, S. R.; Stengel, P. J. U.S. Patent Application 2006/0252785, **2006**.