Bioorganic & Medicinal Chemistry Letters 19 (2009) 451–458

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

5,6-Dihydro-1*H*-pyridin-2-ones as potent inhibitors of HCV NS5B polymerase

Frank Ruebsam *, Chinh V. Tran, Lian-Sheng Li, Sun Hee Kim, Alan X. Xiang, Yuefen Zhou, Julie K. Blazel, Zhongxiang Sun, Peter S. Dragovich, Jingjing Zhao, Helen M. McGuire, Douglas E. Murphy, Martin T. Tran, Nebojsa Stankovic, David A. Ellis, Alberto Gobbi, Richard E. Showalter, Stephen E. Webber, Amit M. Shah, Mei Tsan, Rupal A. Patel, Laurie A. LeBrun, Huiying J. Hou, Ruhi Kamran, Maria V. Sergeeva, Darian M. Bartkowski, Thomas G. Nolan, Daniel A. Norris, Leo Kirkovsky

Department of Medicinal Chemistry, Anadys Pharmaceuticals, Inc., 3115 Merryfield Row, San Diego, CA 92121, USA

ARTICLE INFO

Article history:
Received 26 September 2008
Revised 12 November 2008
Accepted 13 November 2008
Available online 18 November 2008

Keywords:
Hepatitis C virus (HCV)
NS5B polymerase
Small molecule
Non-nucleoside NS5B inhibitor
5,6-Dihydro-1*H*-pyridin-2-ones

ABSTRACT

5,6-Dihydro-1*H*-pyridin-2-one analogs were discovered as a novel class of inhibitors of genotype 1 HCV NS5B polymerase. Among these, compound **4ad** displayed potent inhibitory activities in biochemical and replicon assays (IC_{50} (1b) < 10 nM; IC_{50} (1a) < 25 nM, EC_{50} (1b) = 16 nM), good in vitro DMPK properties, as well as moderate oral bioavailability in monkeys (F = 24%).

Hepatitis C virus (HCV) is a small RNA virus, a member of the genus *Hepacivirus* in the *Flaviviridae* family of viruses,[1] that is recognized as a major cause of acute hepatitis and chronic liver disease, including cirrhosis and liver cancer.[2] An estimated 180 million people are chronically infected with HCV worldwide with approximately 4.1 million individuals affected in the United States.[3] The current standard of care, a combination of pegylated interferon (Peg-IFN) and ribavirin,[4] achieves sustained virologic response (SVR) rates of ~80% in patients infected with genotypes 2 or 3 HCV. However, individuals infected with the genotypes most common in the US (1a and 1b, approx. 75% of the patients) do not respond as well to this treatment with SVR rates typically <50%. This treatment regimen also suffers from frequent adverse effects such as flu-like symptoms, anemia, and depression. These significant shortcomings in current HCV therapy, combined with the fact that there is no vaccine available to prevent hepatitis C, result in an urgent need for improved treatments, in particular those specifically directed at genotype 1 HCV.[5]

Due to its critical role in viral replication, we have focused our research efforts on identifying novel non-nucleoside inhibitors of the HCV NS5B RNA-dependent RNA polymerase (RdRp).[6] Among several inhibitor binding pockets distinct from the active site,[7] we chose to pursue the structure-based design of inhibitors targeting the palm region of the enzyme. Other groups have also reported on their series of NS5B inhibitors that have been shown to bind to NS5B at the same location.[8]

In our earlier efforts to optimize benzothiadiazine-containing inhibitors as exemplified by compound **1**[9c,d] (Fig. 1),[10] we concluded that their poor oral bioavailability was likely due to poor intestinal permeability. We therefore explored strategies to reduce the compounds' high polar surface areas (PSA) that we believed were responsible for the undesirable PK properties. We recently reported that molecules containing the fused pyrrolo[1,2-*b*]pyridazinone motif, as exemplified by compound **2**, yield potent inhibitors of the NS5B polymerase with IC_{50} (1b) values of <0.01 μM.[11] Unfortunately, these molecules exhibited poor oral bioavailability in animals (F_{po} = 1% for **2**). Formal saturation of the fused ring system present in **2** led to hexahydro-pyrrolo[1,2-*b*]-pyridazinone compounds such as **3**. While these molecules also displayed potent biochemical and antiviral activity, their in vitro DMPK properties still remained unsatisfactory, resulting in poor oral bioavailability (best compound (**3**) exhibited F_{po} = 7%).[12] Here we describe our continued efforts to improve both intestinal permeability and oral bioavailability of the benzothiadiazine-containing compounds by removing a ring nitrogen atom from the hexahydro-pyrrolo[1,2-*b*]-pyridazinone inhibitor design (**3**). We envisioned that the lower PSA values of the resulting 5,6-dihydro-1*H*-pyridin-2-ones (exemplified by general structure **4**) might favorably impact permeability relative to compounds such as **2** and **3**.

* Corresponding author. Tel.: +1 858 530 3708; fax: +1 858 530 3644.
E-mail address: fruebsam@anadyspharma.com (F. Ruebsam).

0960-894X/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2008.11.048

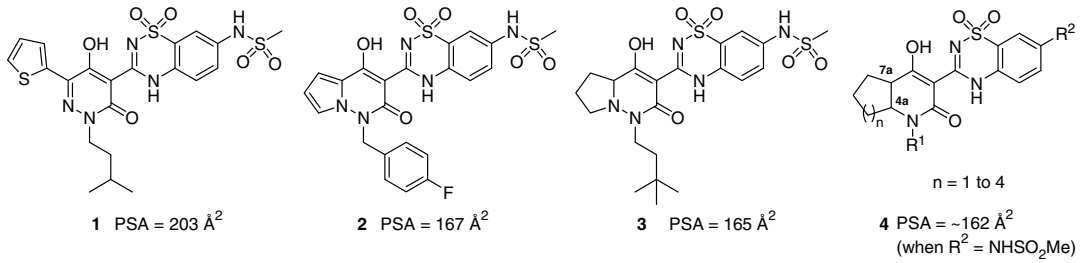


Figure 1. HCV NS5B polymerase inhibitors.

We began our synthesis of the described inhibitors (**4**) from commercially available racemic β-amino acids (or their hydrochlorides) **5** or the corresponding esters **6** (Scheme 1). N-Alkylated β-amino esters **7** were obtained either via reductive alkylation of the β-amino esters **6** with aldehydes or ketones or by reductive amination of commercially available racemic β-keto esters **8** with primary amines.[13] Coupling of intermediates **7** with acids **9**[14] using standard methods for amide formation afforded the corresponding amide intermediates **10**, which upon treatment with NaOEt cyclized to yield the desired final products **4a–ap**.[15] Optically enriched inhibitors were synthesized following the same route described in Scheme 1 starting from commercially available chiral β-amino acids or *N*-Boc-protected amino acid derivatives.[16] Alternatively, optically active intermediate **6** ($n = 2$) was prepared via enantioselective desymmetrization of *cis*-1,2-cyclohexanedicarboxylic anhydride followed by Curtius rearrangement and Cbz deprotection.[17]

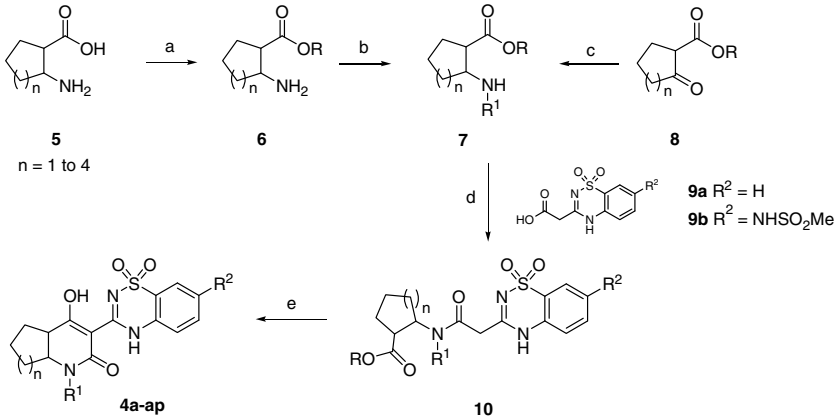
We initiated our first inhibitor synthesis from commercially available racemic *cis*-2-aminocyclohexancarboxylic acid ethyl ester hydrochloride following the chemistry described in Scheme 1. Exposure of **10a**-*cis* (Scheme 2) to an excess (4 equiv) of warm NaOEt for 23 h afforded one major cyclized product (>90% peak area) as determined by LC–MS and HPLC analysis of the crude reaction mixture. Somewhat surprisingly based on the well-known thermodynamics of simple decalin hydrocarbons,[18] the purified major product thus obtained was shown to be the *cis*-isomer (**4a**-*cis*) by a 2D NOE experiment rather than the expected *trans*-isomer.[19]

To further explore this unexpected phenomenon, we prepared the *trans*-isomer intermediate **10a**-*trans* from commercially available racemic *trans*-2-aminocyclohexanecarboxylic acid ethyl ester hydrochloride using a reaction sequence identical to that employed for the synthesis of **10a**-*cis*. Cyclization of **10a**-*trans* under basic conditions for 28 h again yielded **4a**-*cis* as the major cyclized product (>90% HPLC peak area). Similar preparation of the 5- and 7-membered fused ring analogs corresponding to **4a** from the appropriate racemic *cis*-β-amino acid esters also afforded *cis*-cyclization products (**4b**-*cis* and **4c**-*cis*, respectively; each >90% HPLC peak area).[19] While it has not been experimentally verified, we believe that the *cis*-isomer is the more thermodynamically stable entity.

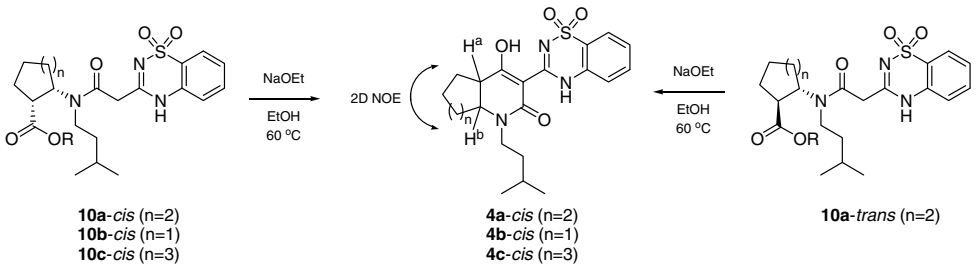
Next, ab initio calculations were performed to better understand the thermodynamic properties of the new heterocyclic system under study using the deprotonated model structures shown in Figure 2.[20]

As shown in Figure 2, these calculations correctly assessed the experimental difference in formation energy between *trans* and *cis*-decalin isomers (3.1 kcal/mol)[20] with the former proving to be thermodynamically favored. In contrast, calculations performed on model system **12** indicated the thermodynamic preference for the *cis*-isomer which was consistent with the isolation of the *cis*-product (**4a**-*cis*) observed in the experiments described above. The conjugated enol-amide in **4a** and **12** allows for a distribution of the negative charge. This conjugation requires a close to planar conformation of the dihydro-pyridinone ring. The *cis*-products can adopt this planar conformation with a significantly smaller strain than the *trans*-products thus explaining the energy difference. Consistent with this hypothesis, ab initio calculations on model compound **13** containing a more strained fused 5-membered ring predicted a higher energy difference between *cis*- and *trans*-isomers relative to **12**.

While compound **4a** displayed only weak biochemical activity (IC_{50} (1b) = 9.0 μM), the corresponding analog bearing a methylsulfonamide R^2 substituent (**4ab**, Table 2) exhibited ~225-fold



Scheme 1. Reagents and conditions: R = Me, Et; (a) $TMSCHN_2$, PhH, MeOH (94–99%); (b) RCHO or R_2CO, $NaCNBH_3$, NaOAc, 4 Å MS, MeOH, 25 °C, 16 h (18–93%) or i—RCHO, NEt_3, $MgSO_4$, THF, 25 °C, 16 h; ii—$NaBH_4$, MeOH, 25 °C, 1 h (79–87%); (c) R^1-NH_2, $NaCNBH_3$, AcOH, EtOH, 50 °C, 16 h (15–87%) or i—R^1-NH_2, AcOH, toluene, 70–145 °C, 3 h; ii—$NaBH(OAc)_3$, CH_3CN, AcOH, 0–25 °C, 19 h (77% over two steps); (d) **9**, DCC, DCM/DMF, 25 °C, 2–16 h or EDC, NMM, DMF, 25 °C, 2–16 h; (e) NaOEt, EtOH, 60 °C, 2–16 h (22–71% over two steps).



Scheme 2. Equilibrium after ring closure favors *cis*-isomer as confirmed by 2D NOE experiment. All compounds shown are racemic.

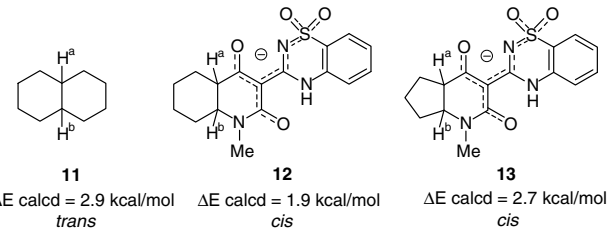


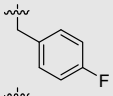
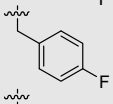
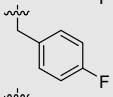
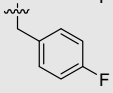
Figure 2. Ab initio calculations performed for decalin (**11**) and model systems **12** and **13**. For each structure, ΔE values refer to the calculated energy difference between *cis*- and *trans*-isomers. The more thermodynamically stable isomer is listed below each ΔE value.

improved potency in the enzymatic assay. This result was consistent with the previously noted importance of an R^2 sulfonamide moiety in obtaining potent benzothiadiazine-containing inhibitors.[9,11] In addition, substitution of the R^1 isoamyl moiety present in **4ab** with a 4-fluorobenzyl group provided a further increase in anti-NS5B activity combined with improved HLM stability (**4e**, Table 1). We therefore chose to first explore the effects of varying the aliphatic ring size using molecules containing 4-fluorobenzyl R^1 and methylsulfonamide R^2 substituents as shown in Table 1.

While the 5-membered compound **4d** was the most potent inhibitor in this subset, the activities of the 6- and 7-membered analogs (**4e** and **4f**) were only slightly weaker. However, expanding the ring size further (**4g**) led to a significant loss in potency, indicating that the larger ring size cannot easily be accommodated in the binding pocket. To verify that this SAR was not unique to compounds containing the 4-fluorobenzyl R^1 group, we also evaluated the corresponding analogs that contained a *tert*-butylethyl R^1 moiety (**4h–k**) and confirmed the previously observed trend.

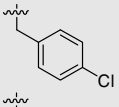
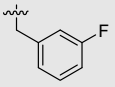
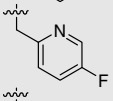
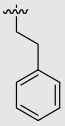
Table 2 shows additional structure–activity relationships (SAR) observed for variations of the R^1 substituents in racemic compounds **4** containing fused 5- and 6-membered ring systems (R^2 = $NHSO_2Me$). In cases where the R^1 moiety was either short (**4l–4n**), α-branched (**4n**, **4r**, **4s**), or fairly long (**4z**), the activity in the enzymatic assay (1b) was very weak. Introduction of a cyclobutylmethyl group (**4o**) led to improved biochemical potency, but the replicon activity remained weak. The optimal chain length

Table 1
Exploration of various aliphatic ring sizes (see Fig. 1) with R^2 = $NHSO_2Me$

Compound[a]	*n*	R^1	IC_{50} (1b)[b] [μM]	IC_{50} (1a)[c] [μM]	EC_{50} (1b)[b] [μM]	CC_{50} (GAPDH)[b] [μM]	HLM $t_{1/2}$[b,d] [min]
4d	1		<0.01	<0.025	0.023	>1	>60 (98%)
4e	2		0.022	ND[e]	0.15	>33	>60 (81%)
4f	3		0.057	0.042	0.074	>33	47
4g	4		0.13	1.0	7.3	>33	>60 (66%)
4h	1		<0.01	<0.025	0.024	>1	7
4i	2		0.043	0.066	0.033	>33	16
4j	3		0.039	0.073	0.022	>10	27
4k	4		6.5	ND	ND	ND	ND

[a] All compounds are racemic. Major isomer is *cis* with the amount of *trans*-isomer possibly present assumed to be <10%. See Ref. 19 for additional details.
[b] See Ref. 9a for assay conditions and error.
[c] See Ref. 9b for assay conditions and error.
[d] For values >60 min,% remaining at 60 min is given in parentheses. All compounds were tested at 1 μM.
[e] ND, not determined.

Table 2
SAR around R^1 substituent (R^2 = $NHSO_2Me$)

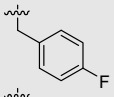
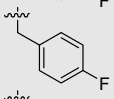
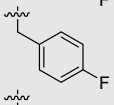
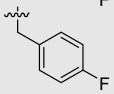
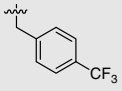
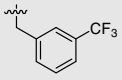
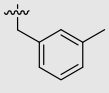
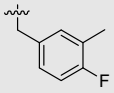
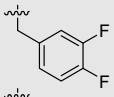
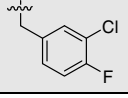
Compound[a]	*n*	R^1	IC_{50} (1b)[b] [μM]	IC_{50} (1a)[c] [μM]	EC_{50} (1b)[b] [μM]	CC_{50} (GAPDH)[b] [μM]	HLM $t_{1/2}$[b,d] [min]
2		Figure 1	<0.01	<0.025	0.012	>1	>60 (86%)
3		Figure 1	<0.01	<0.025	0.034	>1	59
4l	1		9.1	ND[e]	ND	ND	ND
4m	1		0.95	ND	2.1	>33	ND
4n	1		0.78	ND	13.4	>33	ND
4o	1		0.051	ND	0.7	>33	4
4p	1		<0.01	<0.025	0.06	>1	10
4q	1		0.035	0.051	0.12	>33	25
4r	1		3.4	ND	ND	ND	ND
4s	1		0.73	ND	4.3	>10	ND
4t	1		3.5	ND	ND	ND	ND
4u	1		0.046	0.099	0.1	>33	50
4v	1		0.028	ND	0.88	>10	>60 (90%)
4w	1		0.02	0.13	0.17	>33	51
4x	1		0.43	0.47	8.1	>33	>60 (76%)
4y	1		0.12	0.19	3.1	>33	>60 (100%)
4z	1		0.57	ND	1.5	>33	25
4aa	1		6.8	ND	ND	ND	>60 (97%)
4ab	2		0.04	0.047	0.039	>33	28
4ac	2		0.91	ND	3.2	>33	ND

[a] All compounds are racemic. Major isomer is *cis* with the amount of *trans*-isomer possibly present assumed to be <10%. See Ref. 19 for additional details.
[b] See Ref. 9a for assay conditions and error.
[c] See Ref. 9b for assay conditions and error.
[d] For values >60 min, % remaining at 60 min is given in parentheses. All compounds were tested at 1 μM.
[e] ND, not determined.

and/or volume of the aliphatic R^1 moiety with respect to the activities in the enzymatic and replicon assays was reached with the isoamyl (**4p**) and the *tert*-butylethyl (**4h**, Table 1) substituents. However, these compounds displayed fairly short half-lives when exposed to human liver microsomes (HLM) and prompted us to also explore aromatic R^1 groups. A R^1 phenyl group directly attached to the ring nitrogen showed poor activity (**4t**), while introduction of a benzyl group (**4u**) led to improved biochemical activity and microsomal stability. The SAR for the substitution pattern around the benzyl moiety was tighter and more subtle compared to that observed for the aliphatic R^1 fragments described above. Addition of a fluorine atom in the 4-position yielded one of the most active and stable compounds (**4d**, Table 1) while larger substituents in the 4-position, such as a chlorine atom (**4v**), or *meta*-substitution (e.g., **4w**) resulted in a significant loss of replicon activity.

We also investigated the impact of including heteroatoms in the R^1 substituents and introduced a 2-pyridyl-methyl group (**4x**) into our design. This led to a substantial loss in enzymatic activity suggesting unfavorable polar interactions in the hydrophobic binding sub-pocket. In addition, **4x** exhibited weak activity in the replicon assay, possibly due to poor cell permeability as a result of its increased polar nature. A similar effect was observed when a nitrogen atom was introduced into the aliphatic R^1 side chain (**4aa** vs **4p**). While introduction of a fluorine atom in the 4-position of **4x** led to a ~2- to 4-fold improvement in biochemical and antiviral potencies (compare to **4y**), the absolute activities of the fluorinated analog **4y** remained poor. As observed in the SAR described in

Table 3
SAR around R^1 substituent in the chiral 5- and 6-membered ring systems (R^2 = $NHSO_2Me$)

Compound[a]	*n*	Stereo-chemistry	R^1	IC_{50} (1b)[b] [μM]	IC_{50} (1a)[c] [μM]	EC_{50} (1b)[b] [μM]	CC_{50} (GAPDH)[b] [μM]	HLM $t_{1/2}$[b,d] [min]
4ad	1	(4a*R*,7a*S*)		<0.01	<0.025	0.016	>100	>60 (85%)
4ae	1	(4a*S*,7a*R*)		0.031	0.026	0.22	>33	>60 (84%)
4af	2	(4a*R*,8a*S*)		0.027	0.063	0.12	>1	>60 (99%)
4ag	2	(4a*S*,8a*R*)		0.047	0.043	0.35	>33	>60 (80%)
4ah	1	(4a*R*,7a*S*)		0.21	ND[e]	0.94	>33	>60 (99%)
4ai	1	(4a*R*,7a*S*)		<0.01	0.051	0.032	>1	23
4aj	1	(4a*R*,7a*S*)		<0.01	<0.025	0.009	>1	16
4ak	1	(4a*R*,7a*S*)		0.62	ND	4.8	>33	>60 (85%)
4al	1	(4a*R*,7a*S*)		0.57	ND	15	>33	>60 (70%)
4am	1	(4a*R*,7a*S*)		<0.01	0.074	0.16	>1	>60 (67%)
4an	1	(4a*R*,7a*S*)		<0.01	0.028	0.034	>1	>60 (66%)
4ao	1	(4a*R*,7a*S*)		<0.01	0.029	0.045	>1	>60 (88%)
4ap	1	(4a*R*,7a*S*)		<0.01	0.028	0.055	>1	>60 (81%)

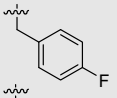
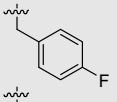
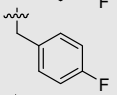
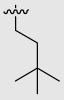
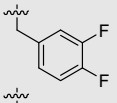
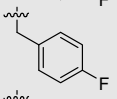
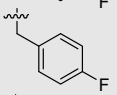
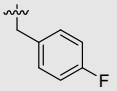
[a] Major isomer is *cis* with the amount of *trans*-isomer possibly present assumed to be <10%. See Ref. 19 for additional details.
[b] See Ref. 9a for assay conditions and error.
[c] See Ref. 9b for assay conditions and error.
[d] For values >60 min, % remaining at 60 min is given in parentheses. All compounds were tested at 1 μM.
[e] ND, not determined.

Table 1, the inhibitory activities in the enzymatic assay for compounds containing a fused 6-membered ring ($n = 2$) were generally weaker than those observed for the corresponding 5-membered ring systems (**4e**, **4i**, **4ab**, and **4ac**).

The shift between the IC_{50} (1b) and EC_{50} (1b) values typically observed was ~5- to 30-fold, which can likely be attributed to protein binding under the assay conditions. However, it is interesting to note that this shift was much smaller for some of the aliphatic R^1 moieties, in particular in compounds bearing 6- and 7-membered fused aliphatic rings. Specifically, compounds containing an isoamyl moiety (**4ab**) or a *tert*-butylethyl group (**4i** and **4j**, vide supra) displayed minimal shifts between the enzymatic and replicon activities. Possible reasons for these observations include reduced protein binding and/or better cell permeability associated with these structural motifs (see Table 4 for Caco-2 data of selected compounds).

Having thus far only explored racemic compounds, we next focused our attention on investigating the SAR of enantiopure inhibitors (Table 3). Comparing the 5-membered enantiomers **4ad** and **4ae**, we found the (4a*R*,7a*S*)-isomer **4ad** to have significantly greater (~10-fold) antiviral potency. The same trend was also observed when comparing the corresponding 6-membered enantiomeric pair (**4af** vs **4ag**), but the difference was not as pronounced (~3-fold). Similar to what was observed for the racemic inhibitors, the corresponding enantiopure 5-membered compound **4ad** was significantly (~8-fold) more potent than its 6-membered analog **4af**. Based on these results, we continued our SAR exploration around the R^1 moiety only for the (4a*R*,7a*S*)-isomers in the 5-membered series of inhibitors. The enantiopure analogs **4ai** and **4aj** displayed slightly improved potencies compared to the corresponding racemic compounds (**4h** and **4p**). Introducing a larger trifluoromethyl-substituent in the para- (**4ak**) or

Table 4
In vitro and in vivo DMPK parameters for selected compounds

Compound	n	R^1	MLM $t_{1/2}$[a,b] [min]	P_{app}[a,c] [(cm/s) × 10^{-6}]	F_{po}[d] [%]	AUC_{inf} [ng/h/mL] po/iv[d]	CL (iv)[d] [mL/min/kg]	C_{12h} (po)/EC_{50}[e]
2	1		>60 (90%)	0.1	1	6/539	31	NC[f]
3	1		7.5	0.44	7	344/5178	3.3	0.29
4ad	1		>60 (~100%)	1.6	21	6041/29086	0.63	10.49
4ae	1		>60 (~100%)	0.88	12	573/4839	3.45	1.54
4p	1		13	3.3	10	273/2786	6.2	NC
4h	1		5.4	4.15	16	636/3924	4.3	0.19
4ao	1		>60 (~100%)	0.83	12	1845/15950	1.2	1.33
4af	2		>60 (~100%)	0.67	12	2913/25311	0.7	0.90
4ag	2		>60 (83%)	1	6	610/10023	1.7	0.03
4ab	2		44	3.1	7	67/992	18.2	NC
4i	2		8	3.1	7	239/3515	4.9	0.11
4f	3		>60 (81%)	0.95	14	655/4825	4.1	0.37

[a] See Ref. 9c for assay conditions and error.
[b] For values >60 min, % remaining at 60 min is given in parentheses. All compounds were tested at 1 μM.
[c] Controls: P_{app} atenolol (low) = (0.4–0.7) × 10^{-6} (cm/s), P_{app} propranolol (high) = (10–16) × 10^{-6} (cm/s).
[d] 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.
[e] C_{12h} (po)/EC_{50} = plasma concentration 12 h after oral administration divided by EC_{50} (1b) value.
[f] NC, not calculated.

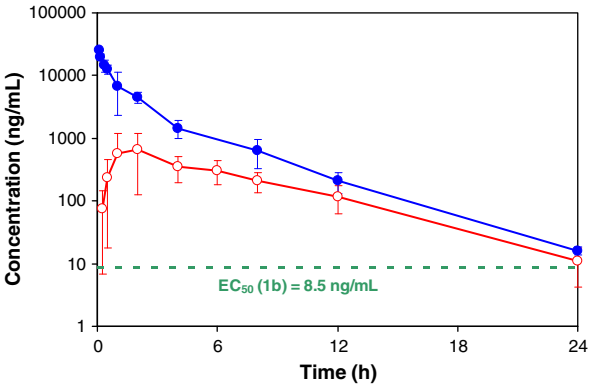


Figure 3. Plasma concentrations of compound **4ad** in cynomolgus monkeys at various times after iv (solid symbols) and po (open symbols) administration of a single 1 mg/kg dose.

meta-positions (**4al**) of the R^1 benzyl group resulted in significant loss of activity. Compound **4am**, bearing a smaller 3-methylbenzyl R^1 substituent, displayed improved activities compared to **4al**. Potencies in the enzymatic and replicon assays were also restored by introducing 3,4-disubstituted benzyl groups containing a fluorine atom in the 4-position as illustrated by compounds **4an**–**4ap**.

Table 4 details the in vitro and in vivo DMPK parameters for selected 5-membered (**4ad**, **4ae**, **4p**, **4h**, **and 4ao**), 6-membered (**4af**, **4ag**, **4ab**, **and 4i**), and 7-membered (**4f**) compounds.[21] All pyridinones in Table 4 exhibited good solubility in the biochemical assay (>100 μM) and generally displayed low to moderate in vivo clearance. Most compounds bearing aliphatic R^1 moieties (**4p**, **4h**, **4ab**, and **4i**) displayed low stability toward monkey liver microsomes (MLM), while all inhibitors containing benzylic R^1 substituents (**4ad**, **4ae**, **4ao**, **4af**, **4ag**, **and 4f**) were stable in such assessments. Generally, MLM stability correlated loosely with the corresponding in vivo clearance data suggesting that the clearance of less stable compounds bearing aliphatic R^1 substituents is likely to be mediated via oxidative biotransformation. Encouragingly, Caco-2 data indicated improved permeability for the new inhibitors described in Table 4 relative to the previously studied compounds **2** and **3**. The bioavailabilities and AUCs of compounds **4** observed following oral dosing were also improved relative to those exhibited by compounds **2** and **3**, suggesting that the permeability gains, when combined with good microsomal stability (MLM $t_{1/2} > 60$ min), translated into increased in vivo exposures. Interestingly, comparison of enantiomers **4af** and **4ag** revealed that these compounds showed distinct in vivo DMPK parameters, with the (4a*R*,7a*S*)-isomer **4af** being superior. This difference was even more pronounced in the 5-membered enantiomeric pair **4ad** and **4ae**, again with the (4a*R*,7a*S*)-isomer (**4ad**) favorably standing out. However, these differences in the in vivo parameters were not easily predicted from the corresponding in vitro DMPK data (MLM, Caco-2), possibly suggesting differential recognition of each enantiomer pair by biological systems.[22] Thus, while we originally envisioned that the lower PSA values associated with molecules **4** containing the 5,6-dihydro-1*H*-pyridin-2-one motif would afford improved permeabilities relative to inhibitors such as **2** and **3**, we currently suspect that the superior in vivo performance of the former compounds may partially result from more favorable interactions with one or more biological systems.[23] We hypothesize that the incorporation of two sp^3-hybridized centers into the design of **4** significantly altered the shape of the resulting molecules relative to those we previously studied and thereby contributed to the in vivo DMPK improvements. Additional experiments are underway to better characterize the pharmacokinetic properties of the dihydropyridinone-containing molecules, and results from these assessments will be reported in the future. Importantly, the enantiopure inhibitor **4ad** showed a good combination of replicon potency and in vitro/in vivo DMPK properties and exhibited plasma levels in monkeys that significantly exceeded its replicon (1b) EC_{50} value for at least 12 h following oral dosing (Fig. 3). The other compounds described in Table 4 did not exhibit similarly high C_{12h}/EC_{50} values, either due to poorer PK properties or weaker antiviral activity (or both).

In summary, we describe a novel series of non-nucleoside inhibitors of genotype 1b HCV NS5B polymerase (**4**) that incorporate an aliphatic, fused 5,6-dihydro-1*H*-pyridin-2-one moiety. Extensive SAR studies identified a number of very potent compounds in both biochemical and replicon assays. This work led to the discovery of the promising inhibitor **4ad** which exhibited potent antiviral activity and significantly improved oral bioavailability compared to our previously reported NS5B inhibitors. Our ongoing efforts to further improve the PK properties of the benzothiadiazine-containing NS5B inhibitors will be reported in a future communication.

Acknowledgement

The authors thank Drs. Devron Averett and Steve Worland for their support and helpful discussions during the course of this work.

References and notes

1. Choo, Q. L.; Kuo, G.; Weiner, A. J.; Overby, L. R.; Bradley, D. W.; Houghton, M. *Science* **1989**, *244*, 359.
2. Shepard, C. W.; Finelle, L.; Alter, M. J. *Lancet Infect. Dis.* **2005**, *5*, 558.
3. CDC Hepatitis Fact Sheet, March 6, 2008, www.cdc.gov/hepatitis.
4. (a) Sidwell, R. W.; Huffman, J. H.; Khare, G. P.; Allen, L. B.; Witkowski, J. T.; Robins, R. K. *Science* **1972**, *177*, 705; (b) Smith, R. A.; Kirkpatrick, W.. In *Ribavirin a Broad Spectrum Antiviral Agent*; Academic Press: New York, 1980; Vol. 3, p 237; (c) De Clercq, E. *Adv. Virus Res.* **1994**, *42*, 1.
5. (a) Hayashi, N.; Takehara, T. *J. Gastroenterol.* **2006**, *41*, 17; (b) Hoofnagle, J. H.; Seeff, L. B. *N. Engl. J. Med.* **2007**, *355*, 2444.
6. Kolykhalov, A. A.; Agapov, E. V.; Blight, K. J.; Mihalik, K.; Feinstone, S. M.; Rice, C. M. *Science* **1997**, *277*, 570.
7. Koch, U.; Narjes, F. *Curr. Top. Med. Chem.* **2007**, *7*, 1302.
8. (a) Slater, M. J.; Amphlett, E. M.; Andrews, D. M.; Bravi, G.; Burton, G.; Cheasty, A. G.; Corfield, J. A.; Ellis, M. R.; Fenwick, R. H.; Fernandes, S.; Guidetti, R.; Haigh, D.; Hartley, C. D.; Howes, P. D.; Jackson, D. L.; Jarvest, R. L.; Lovegrove, V. L. H.; Medhurst, K. J.; Parry, N. R.; Price, H.; Shah, P.; Singh, O. M. P.; Stocker, R.; Thommes, P.; Wilkinson, C.; Wonacott, A. *J. Med. Chem.* **2007**, *50*, 897; (b) Dhanak, D.; Duffy, K. J.; Johnston, V. K.; Lin-Goerke, J.; Darcy, M.; Shaw, A. N.; Gu, B.; Silverman, C.; Gates, A. T.; Nonnemacher, M. R.; Earnshaw, D. L.; Casper, D. J.; Kaura, A.; Baker, A.; Greenwood, C.; Gutshall, L. L.; Maley, D.; DelVecchio, A.; Macarron, R.; Hofmann, G. A.; Alnoah, Z.; Cheng, H.-Y.; Chan, G.; Khandekar, S.; Keenan, R. M.; Sarisky, R. T. *J. Biol. Chem.* **2002**, *277*, 38322; (c) Evans, K. A.; Chai, D.; Graybill, T. L.; Burton, G.; Sarisky, R. T.; Lin-Goerke, J.; Johnston, V. K.; Rivero, R. A. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 2205; (d) Blake, J. F.; Fell, J. B.; Fischer, J. P.; Hendricks, R. T.; Spencer, S. R.; Stengel, P. J. WO2006117306, 2006.; (e) Pratt, J. K.; Donner, P.; McDaniel, K. F.; Maring, C. J.; Kati, W. M.; Mo, H.; Middleton, T.; Liu, Y.; Ng, T.; Xie, Q.; Zhang, R.; Montgomery, D.; Molla, A.; Kempf, D. J.; Kohlbrenner, W. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 1577; (f) Hutchinson, D. K., et al. U.S. Patent US2005107364, 2005.; (g) Bosse, T. D.; Larson, D. P.; Wagner, R.; Hutchinson, D. K.; Rockway, T. W.; Kati, W. M.; Liu, Y.; Masse, S.; Middleton, T.; Mo, H.; Montgomery, D.; Jiang, W.; Koev, G.; Kempf, D. J.; Molla, A. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 568.
9. (a) Zhou, Y.; Webber, S. E.; Murphy, D. E.; Li, L.-S.; Dragovich, P. S.; Tran, C. V.; Sun, Z.; Ruebsam, F.; Shah, A.; Tsan, M.; Showalter, R.; Patel, R.; Li, B.; Zhao, Q.; Han, Q.; Hermann, T.; Kissinger, C.; LeBrun, L.; Sergeeva, M. V.; Kirkovsky, L. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 1413; (b) Zhou, Y.; Li, L.-S.; Dragovich, P. S.; Murphy, D. E.; Tran, C. V.; Ruebsam, F.; Webber, S. E.; Shah, A.; Tsan, M.; Averill, A.; Showalter, R.; Patel, R.; Han, Q.; Zhao, Q.; Hermann, T.; Kissinger, C.; LeBrun, L.; Sergeeva, M. V. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 1419; (c) Li, L.-S.; Zhou, Y.; Murphy, D. E.; Stankovic, N.; Zhao, J.; Dragovich, P. S.; Bertolini, T.; Sun, Z.; Ayida, B.; Tran, C. V.; Ruebsam, F.; Webber, S. E.; Shah, A. M.; Tsan, M.; Showalter, R. E.; Patel, R.; LeBrun, L. A.; Bartkowski, D. M.; Nolan, T. G.; Norris, D. A.; Kamran, R.; Brooks, J.; Sergeeva, M. V.; Kirkovsky, L.; Zhao, Q.; Kissinger, C. R. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 3446; (d) Sergeeva, M. V.; Zhou, Y.; Bartkowski, D. M.; Nolan, T. G.; Norris, D. A.; Okamoto, E.; Kirkovsky, L.; Kamran, R.; LeBrun, L. A.; Tsan, M.; Patel, R.; Shah, A. M.; Lardy, M.; Gobbi, A.; Li, L.-S.; Zhao, J.; Bertolini, T.; Stankovic, N.; Sun, Z.; Murphy, D. E.; Webber, S. E.; Dragovich, P. S. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 3421.

10. All structures are arbitrarily drawn as one of several possible tautomers.
11. Ruebsam, F.; Webber, S. E.; Tran, M. T.; Tran, C. V.; Murphy, D. E.; Zhao, J.; Dragovich, P. S.; Kim, S. H.; Li, L.-S.; Zhou, Y.; Han, Q.; Kissinger, C. R.; Showalter, R. E.; Lardy, M.; Shah, A. M.; Tsan, M.; Patel, R.; LeBrun, L. A.; Kamran, R.; Sergeeva, M. V.; Bartkowski, D. M.; Nolan, T. G.; Norris, D. A.; Kirkovsky, L. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 3616.
12. Ruebsam, F.; Sun, Z.; Ayida, B. K.; Webber, S. E.; Zhou, Y.; Zhao, Q.; Kissinger, C. R.; Showalter, R. E.; Shah, A. M.; Tsan, M.; Patel, R.; LeBrun, L. A.; Kamran, R.; Sergeeva, M. V.; Bartkowski, D. M.; Nolan, T. G.; Norris, D. A.; Kirkovsky, L. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 5002.
13. Bartoli, G.; Cimarelli, C.; Marcantoni, E.; Palmieri, G.; Petrini, M. *J. Org. Chem.* **1994**, *59*, 5328.
14. Ruebsam, F.; Tran, M. T.; Webber, S. E.; Dragovich, P. S.; Li, L.-S.; Murphy, D. E.; Kucera, D. J.; Sun, Z.; Tran, C. V. PCT International Patent Application, 2007, WO2007150001 A1.
15. For additional experimental details, see: Tran, C. V.; Ruebsam, F.; Zhou, Y.; Dragovich, P. S.; Xiang, A. X. PCT International Patent Application, 2008, WO2008073982 A2.
16. The optical purity (ee) of compound **4ad** prepared from commercially available (1*R*,2*S*)-2-amino-cyclopentanecarboxylic acid hydrochloride (>98% ee) was rigorously determined by chiral HPLC to be >98% (using enantiomer **4ae** and racemic analog **4d** as HPLC references). This result established that the chemistries depicted in Scheme 1 which transform β-amino acids **5** to inhibitors **4** do not result in significant racemization. The optical purities of other chiral inhibitors **4** described in this work were therefore assumed to be similar to those of the corresponding stating materials (typically >98% ee).
17. (a) Bolm, C.; Schiffers, I.; Dinter, C. L.; Gerlach, A. *J. Org. Chem.* **2000**, *65*, 6984; (b) Bolm, C.; Schiffers, I.; Dinter, C. L.; Defrère, L.; Gerlach, A.; Raabe, G. *Synthesis* **2001**, 1719.
18. van Duin, A. C. T.; Dasgupta, S.; Lorant, F.; Goddard, W. A., III *J. Phys. Chem. A* **2001**, *105*, 9396.
19. The 500 MHz ^{1}H and 125 MHz ^{13}C NMR spectra of **4a**-*cis* obtained in $CDCl_3$ at 25 °C were primarily comprised of signals from two compound tautomers (1:1 ratio; precise structure unknown). The H^a and H^b resonances for both tautomers were unambiguously identified via a HMQC experiment and exhibited cross-peaks in a 2D NOESY spectra. A 3rd set of signals (ca. 10%) was also apparent in the ^{1}H and ^{13}C NMR spectra, but it is currently unknown whether these resonances arise from another **4a**-*cis* tautomer or the **4a**-*trans*-isomer. The ^{1}H and ^{13}C NMR spectra of **4b** (C_6D_6) and **4c** ($CDCl_3$) were similar in appearance to those associated with **4a** and were similarly analyzed and annotated (1:1 mixture of tautomers, major resonances = *cis*-isomers). The purified R^2 sulfonamide-containing benzothiadiazines described in this work exhibited a single major LC–MS peak (>90% area) that displayed the correct molecular weight. However, they were typically only soluble in polar organic solvents (DMSO, DMF, pyridine) and exhibited ^{1}H NMR spectra that were often broad and difficult to rigorously characterize. Based on the LC–MS analysis of these molecules and the HPLC, LC–MS, and NMR data associated with compounds **4a**–**4c**, we believe that the more polar sulfonamide-containing inhibitors were prepared predominantly as the *cis*-isomers (which exist in multiple tautomeric forms in polar organic solvents) and that they contain no more than 10% of the corresponding *trans*-isomers (if any).
20. (a) The calculations were performed using PC Gamess (Version 6) employing the HF/6-31g*//MP2/6-31+g* level of theory. Nemukhin A. V.; Grigorenko B. L.; Granovsky A. A. *Moscow University Chemistry Bulletin* **2004**, *45*, 75.; (b) The enol moiety present in structures **12** and **13** (or **4a**) is presumed to be completely deprotonated under the basic cyclization conditions.; (c) The location of the benzothiadiazine NH proton in structures **12** and **13** reflects the most stable tautomer predicted by calculation at the same level of theory.
21. The analysis of in vitro DMPK properties and oral bioavailability data can be complicated by comparison of racemic compounds with optically pure molecules.
22. The exact nature of the biological systems is currently unknown, but possibilities include transporters, conjugation enzymes or enzymes involved in metabolism not included in MLM.
23. We cannot exclude the possibility that the improved in vivo properties of compounds **4** result from increased aqueous solubility that was not detected in our high-throughput (biochemical) solubility assessments.