Bioorganic & Medicinal Chemistry Letters 19 (2009) 3642–3646

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Non-nucleoside inhibitors of HCV polymerase NS5B. Part 2: Synthesis and structure–activity relationships of benzothiazine-substituted quinolinediones

Javier de Vicente [a,*], Robert T. Hendricks [a], David B. Smith [a], Jay B. Fell [a], John Fischer [b], Stacey R. Spencer [b], Peter J. Stengel [b], Peter Mohr [b], John E. Robinson [b], James F. Blake [b], Ramona K. Hilgenkamp [a], Calvin Yee [a], George Adjabeng [a], Todd R. Elworthy [a], Jahari Tracy [a], Elbert Chin [a], Jim Li [a], Beihan Wang [a], Joe T. Bamberg [a], Rebecca Stephenson [a], Connie Oshiro [a], Seth F. Harris [a], Manjiri Ghate [a], Vincent Leveque [a], Isabel Najera [a], Sophie Le Pogam [a], Sonal Rajyaguru [a], Gloria Ao-Ieong [a], Ludmila Alexandrova [a], Susan Larrabee [a], Michael Brandl [a], Andrew Briggs [a], Sunil Sukhtankar [a], Robert Farrell [a], Brian Xu [a]

[a] Roche Palo Alto LLC, 3431 Hillview Avenue, Palo Alto, CA 94304, USA
[b] Array BioPharma Inc, 3200 Walnut Street, Boulder, CO 80301, USA

ARTICLE INFO

Article history:
Received 25 February 2009
Revised 30 April 2009
Accepted 4 May 2009
Available online 7 May 2009

Keywords:
Non-nucleoside inhibitors
HCV polymerase NS5B
Benzothiazine
Pharmacokinetic studies
DMPK properties
X-ray co-crystal structures

ABSTRACT

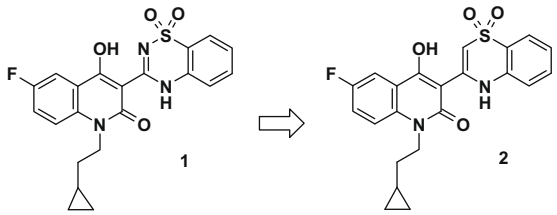
A new series of benzothiazine-substituted quinolinediones were evaluated as inhibitors of HCV polymerase NS5B. SAR studies on this series revealed a methyl sulfonamide group as a high affinity feature. Analogues with this group showed submicromolar potencies in the HCV cell based replicon assay. Pharmacokinetic and toxicology studies were also performed on a selected compound (**34**) to evaluate in vivo properties of this new class of inhibitors of HCV NS5B polymerase.

Hepatitis C virus (HCV) is a positive-stranded RNA virus of the Flaviridae family which was first characterized in 1989 as the major cause of non-A and non-B hepatitis infections.[1] About 170 million people, 3% of the global human population, are infected with HCV and an estimated 3 million individuals become newly infected each year.[2] HCV has been found to be a major cause of cirrhosis, hepatocellular carcinoma, as well as liver failure, and is the most common cause of the need for liver transplantation. HCV is most commonly transmitted by blood transfusions, hemodialysis, and intravenous drug use, and is quite insidious in that clinical manifestations can be mild or non-existent for years after initial infection. Currently there are no vaccines available to prevent hepatitis C. The current gold standard therapies are based upon the use of pegylated interferon-α-2a in combination with ribavirin. These therapies provide a sustained virological response in approximately 50% of patients infected with genotype 1, and have the disadvantage of frequent and severe side effects.[3] The cloning of HCV in 1989 eventually led to identification of several targets for small molecule intervention in the virus life cycle. In the literature, most reports relate to inhibitors of NS3/NS4A protease/helicase and the NS5B RNA-dependent RNA polymerase. With no known mammalian equivalent, HCV polymerase NS5B represents an attractive target for the development of novel anti-HCV agents.[4]

A series of benzothiadiazine-substituted quinolinedione compounds (i.e., **1**) were first reported in 2002 by GSK as HCV NS5B polymerase inhibitors (Fig. 1).[5,6] Our own analysis of **1** showed excellent activity against the NS5B polymerase, but in contrast to a recent report,[6b] we observed low in vivo exposure. We attributed the low in vivo exposure to poor solubility resulting from the extensive internal hydrogen bond network between the quinolinedione and benzothiadiazine rings. We recently reported on a new series of thiazine-based analogs,[7a] where one of the benzothiadiazine ring internal hydrogen bonds was disrupted by exchanging of one of the N atoms with a CH group. This new benzothiazine series (i.e., **2**) showed promising low micromolar potency and had a decreased melting point, slightly improved solubility, and significantly enhanced pharmacokinetic properties (Fig. 1). Herein, we report on our expanded lead optimization studies of the benzothiazine-substituted quinolinedione series as inhibitors of the HCV NS5B polymerase.[7b]

* Corresponding author. Tel.: +1 6508555632; fax: +1 6508556908.
E-mail address: javier.devicente@roche.com (J. de Vicente).

0960-894X/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2009.05.004



NS5B (IC50 uM):	0.03	1.1
Rep (EC50 uM):	0.25 / 10x	1.2
Solubility (ug/ml):	< 1	8
Melting point (°C):	263-265	188-189
AUC, po (ng·h/ml):	303	2321
%F (rat):	~10%	79%

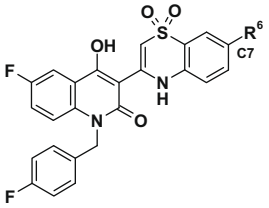
Figure 1. In vitro and in vivo (rat) comparison of compounds **1** and **2**.

Our general synthesis of variously substituted benzothiazine-substituted quinolinediones is presented in Scheme 1. The quinolinedione fragment was derived from isotoic anhydrides such as **3**. These were prepared by either direct alkylation of commercially available isotoic anhydrides (**4**) or by copper catalyzed amination of *ortho* bromo benzoic acids (**5**) followed by treatment with triphosgene. The synthesis of the benzothiazine core started by an alkylation of 2-aminobenzenethiols (**6**) with ethyl 4-chloroacetoacetate followed by an intramolecular condensation providing benzothiazine **7**. The benzothiazine nitrogen was protected with a BOC group and the sulfide oxidized to a sulfone group using *m*-chloroperbenzoic acid. Removal of the BOC protecting group under acidic conditions gave benzothiazine **8**. Deprotonation of benzothiazine ester **8** with potassium *tert*-butoxide followed by treatment with an isotoic anhydride **3** led to the desired benzothiazine-substituted quinolinediones (**9**) in one pot.[8]

A number of structurally diverse compounds synthesized in an analogous manner were evaluated in a biochemical assay against HCV polymerase NS5B.[9] In addition, the activities of these compounds in cell culture against HCV subgenomic replicon (GT-1b) were also determined.[10,11] These data are summarized in Table 1 below.

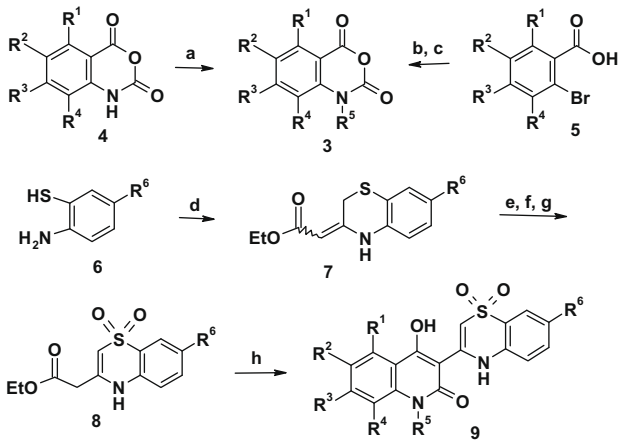
Our initial benzothiazine **2** showed improved in vivo properties but low microsomal stability and poor inhibitory activity (Fig. 1). During our initial explorations,[7a] we found that replacement of the cyclopropylethyl side chain by a *p*-fluorobenzyl group led to a slight improvement in inhibitory activity (compound **10** vs. compound **2**, Table 1). Thus, we commenced our structure activity relationship studies on benzothiazine-substituted quinolonediones with a *p*-fluorobenzyl group. Our initial work was focused on improving the inhibitory activity by addition of a substituent to the C-7 position of the benzothiazine ring. Incorporation of small groups such as fluorine or hydroxyl led to an improvement in enzyme potency with no improvement in our replicon assay (compounds **11** and **13**). In contrast, incorporation of a methyl sulfonamide group led to a significant improvement in both enzyme and replicon potency (compound **18**). Other polar groups such as *N*-acetyl (**17**) or methyl sulfone (**22**) did not provide required levels of potency. Extension of the methyl sulfonamide group or other polar groups did not improve inhibitory activity (**15**, **16** and **21**).

Table 1
Enzyme and replicon data for benzothiazine derivatives **10**–**22**



Compds	R^6	NS5B IC_{50}[a] (μM)	Replicon EC_{50}[a,b] (μM)
10	H	0.705	0.726
11	F	0.148	1.284
12	CN	0.694	4.638
13	OH	0.069	1.743
14	NH_2	0.734	0.827
15	OCH_2CONH_2	0.01	1.188
16	$OCH_2CONHCH_3$	0.025	0.869
17	$NHCOCH_3$	0.869	10.87
18	$NHSO_2CH_3$	0.005	0.014
19	$NHSO_2NH_2$	0.007	0.015
20	$NMeSO_2CH_3$	0.106	1.471
21	$CH_2NHSO_2CH_3$	0.068	6.928
22	SO_2CH_3	3.7	11.89

[a] IC_{50} and EC_{50} values for inhibition calculated from two determinations in duplicate.
[b] CC50 > 50 M for all analogues.



Scheme 1. Reagents and conditions: (a) NaH, DMA, rt, then R_5Br, 70 °C; (b) R_5NH_2, K_2CO_3, $CuBr_2$, THF; (c) triphosgene, K_2CO_3, EtOAc; (d) $EtO_2CCH_2COCH_2Cl$, AcOH, AcOEt, 70 °C; (e) Boc_2O, DMAP, THF; (f) $KMnO_4$, HCOOH, acetone, THF, 0 °C; (g) TFA, DCM, rt; (e) *t*-BuOK, THF, rt, 16 h.

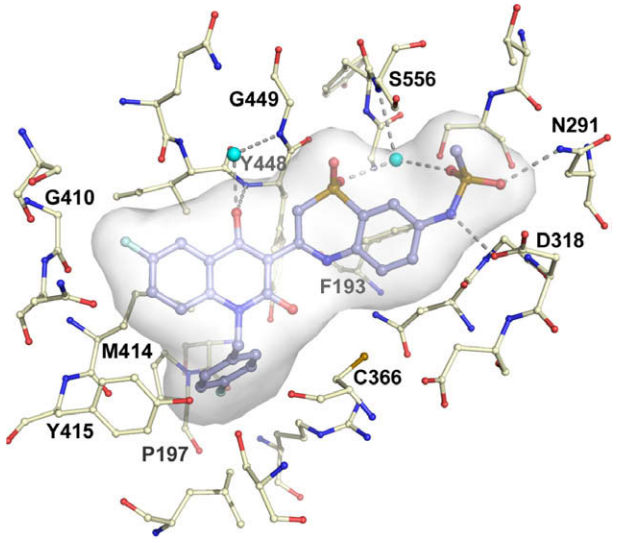


Figure 2. X-ray co-crystal structure of compound **18** bound to the palm site of CΔ21 NS5B polymerase.

A crystal structure of compound **18** complexed with the enzyme was obtained (Figs. 2 and 3)[12] and confirmed our earlier hypothesis that the thiazine inhibitors do indeed bind in the palm site of the NS5B polymerase, near the catalytic site. The quinolinedione and benzothiazine rings adopted a nearly co-planar geometry, while the benzothiazine ring displays a shallow U-shaped bend nearly identical with that predicted by our previous modeling study.[7a] The phenyl group of the quinolinedione is positioned in a hydrophobic site organized by Met414 and Gly410. The quinolinedione also serves as an anchor point for the fluorobenzyl lipophilic tail that fills a hydrophobic pocket shaped by Tyr448, Cys366 and Tyr415.

Quinolinedione **18** binds to the palm I site as an anion due to its ionization at physiological pH (experimental pK_a = 3.8). The anionic charge is conveniently delocalized on this cyclic 1,3-dicarbonyl functionality which interacts with two conserved water molecules and the backbone N–H of Tyr448. The benzothiazine ring system makes an edge to face interaction with Phe193. One of the sulfone oxygens interacts with a structural water molecule bridging to Ser556 and the methyl sulfonamide group. The sulfonamide group also interacts with the NS5B residue Asn291. The acidic N–H of the sulfonamide group makes a strong hydrogen bond with the carboxylate group of Asp318. This unique three point interaction of the sulfonamide group helps explain the 50-fold boost in potency observed upon incorporation this polar group.

Once the methyl sulfonamide was discovered as a high affinity polar group, we focused our efforts on optimizing lipophilic interactions on the quinolinedione core (Table 2). We evaluated the in vitro potency in our NS5B enzyme assay and HCV subgenomic replicon cell based assay in the presence of 5% Fetal Bovine Serum (FBS). The effect of protein binding on the efficiency of the inhibitors was quantified by a plasma shifted replicon assay, which uses 40% Human Serum Albumin (HSA). Addition of small substituents, such as fluorine or chlorine, were tolerated at any position of the quinoline ring. Since addition of a fluorine atom at the C-6 position of the quinoline ring provided a boost in potency (compound **18**) and blocks this site from further metabolism via *para*-hydroxylation, we choose this substitution pattern for further optimization.

We next turned our attention to the optimization of the lipophilic benzyl substituent (Table 3). The benzyl group is often metabolized at the *para* position or on the benzylic carbon. In order to block any metabolism, halogens were incorporated on the *meta* and *para* positions. With this aim, pyridyl groups were also evaluated. Among this set of diverse aromatic groups the *p*-fluorobenzyl group provided adequate levels of potency in our enzyme and cell based assays. Incorporation of a methyl group at the *meta* position in the *p*-fluorobenzyl series provided an added boost in potency in our PS replicon assay (**34**). Several alkyl groups were also evaluated. Surprisingly, small alkyl groups (**41**) were as potent as the benzyl groups, but were not metabolically stable. Thus, compound **34** became our key lead structure.

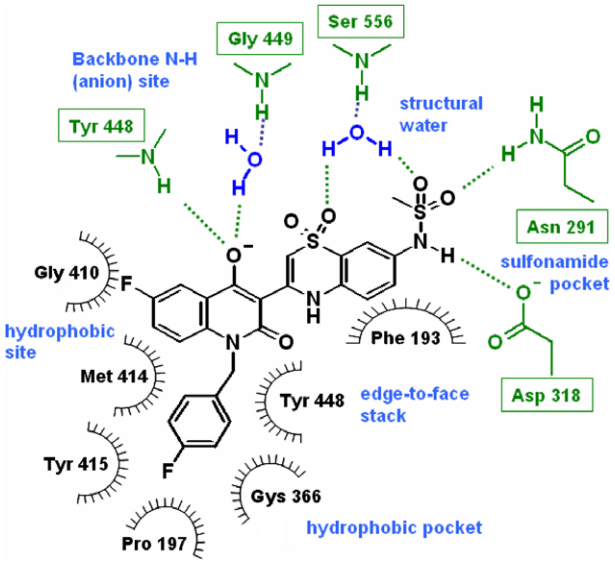
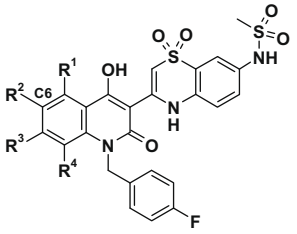


Figure 3. Schematic representation of the interactions between compound **18** and the NS5B protein. Hydrogen bonds that are shorter than 3.0 Å are represented as dashed lines and the residues which make up the enzyme-binding subsites are shown.

Table 2
Enzyme and replicon data for benzothiazine derivatives **8**, **23**–**30**



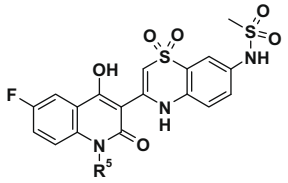
Compds	R^1	R^2	R^3	R^4	NS5B IC_{50}[a] (μM)	Replicon EC_{50}[a,b] (μM)	
						5% FBS	40% HSA (PS)[c]
23	H	H	H	H	0.002	0.042	0.542 (13)
24	Cl	H	H	H	0.012	0.048	0.593 (12.3)
18	H	F	H	H	0.005	0.014	0.406 (29)
25	H	Cl	H	H	0.01	0.166	nd
26	H	Me	H	H	0.017	0.025	nd
27	H	OMe	H	H	0.005	0.143	2.053 (14.3)
28	H	H	F	H	0.01	0.074	0.898 (12.1)
29	H	H	Cl	H	0.384	2.278	nd
30	H	H	H	F	0.008	0.103	2.777 (26.9)

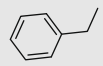
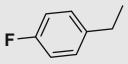
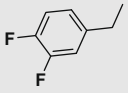
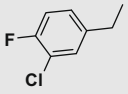
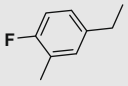
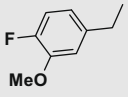
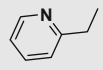
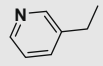
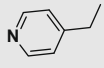
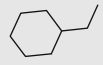
[a] IC_{50} and EC_{50} values for inhibition calculated from two determinations in duplicate.
[b] CC50 > 50 M for all analogues.
[c] Plasma IC_{50} fold shift values shown in parenthesis.

In addition to having excellent in vitro potency, **34** also demonstrated adequate physical chemical properties for this series and was therefore selected for in vivo pharmacokinetics studies. The requirement of multigram quantities led us to optimize the original synthesis (Scheme 2). The large scale synthesis of benzothiazine commenced from 6-nitrobenzothiazole (**46**). The thiazole ring of **46** was opened under basic conditions and then doubly alkylated in situ upon treatment with ethyl 4-chloroacetoacetate, providing benzothiazine core **47** as a 1:1 mixture of exocyclic olefin isomers. The benzothiazine sulfur was oxidized selectively by treatment with potassium permanganate at low temperature to prevent over oxidation. Ester **48** was deprotonated and then treated with isotoic anhydride **45**, which was readily obtained by reductive alkylation of anthranilic acid **43** followed by anhydride formation. Selective reduction of the nitro group with Raney-nickel, followed by treatment with methanesulfonyl chloride in pyridine, led to a mixture of mono and disulfonylated benzothianes. This mixture was converted to the desired monosulfonylated benzothiazine **34** following treatment with aqueous sodium hydroxide.

In vivo pharmacokinetic data was obtained for compound **34** following administration of single intravenous and oral doses to rats or dogs (Table 4). The limited intravenous plasma concentration of **34** in rat was attributed to high first pass clearance (186 mL/kg/min). The rat plasma clearance was ca. threefold higher than rat liver blood flow, but was inconsistent with the observed high rat hepatocyte stability in vitro (1.6 μL/min/10^6cells). In contrast to the rat data, **34** displayed moderate clearance and good

Table 3
Enzyme and replicon data for benzothiazine derivatives **31**–**42**



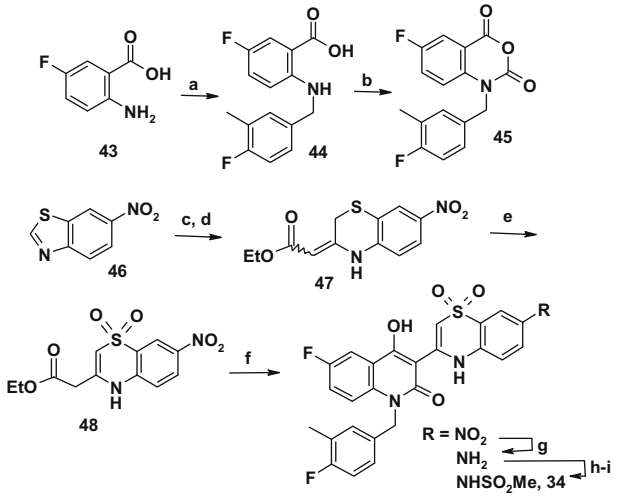
Compds	R^5	NS5B IC_{50}[a] (μM)	Replicon EC_{50}[a,b] (μM)	
			5% FBS	40% HSA (PS)[c]
31		0.007	0.044	0.406 (9.2)
18		0.005	0.014	0.46 (32.8)
32		0.014	0.015	nd
33		0.016	0.018	nd
34		0.013	0.023	0.088 (3.8)
35		0.01	0.1	0.528 (5.3)
36		0.103	1.2	nd
37		0.022	0.589	nd
38		0.011	0.064	0.688 (10.7)
39		0.018	0.1	nd
40		0.041	0.009	nd
41		0.012	nd	0.241
42		0.01	0.047	1.196 (25.4)

[a] IC_{50} and EC_{50} values for inhibition calculated from two determinations in duplicate.
[b] CC50 > 50 M for all analogues.
[c] Plasma IC_{50} fold shift values shown in parenthesis.

exposure in dogs at lower dose. In addition to better microsomal stability in dog versus rat, **34** is highly protein bound (99.8% HSA) and it is possible that interspecies protein binding variations may partly explain the reduced clearance in dog. Although the low-dose oral exposure of **34** in both species was quite low, rat liver levels at 2 and 6 h confirmed that higher exposure in the target organ was possible.

In order to better assess the effects of increasing po doses of **34**, a seven day rat toxicology study was performed using both female and male rats (Table 5). There were no toxicological findings observed in this study: NOAEL > 500 mkd (AUC ca. 1700 ng h/mL). However, the ability of higher doses of **34** to overwhelm clearance mechanisms was less than expected and exposures were generally low. Regardless, compound **34** showed good dose proportionality and a liver-to-plasma ratio of ca. 10 after 24 h. The liver accumulation on day eight was also in line with a ca. 2.6-fold plasma accumulation.

The poor levels of exposure of compound **34** were attributed to low solubility (6.7 μg/mL) and high rat clearance. To address the effects of solubility versus permeability and evaluate exposure levels in a different species, **34** was dosed in dog using three different dosage formulations (Fig. 4). Oral administration of 2 mg/kg to female dogs using either normal or nanosized particles, or via cannulated delivery of a fully solubilized intraduodenal dose all showed low levels of exposure with C_{max} below 15 ng/mL. These results



Scheme 2. Reagents and conditions: (a) 4-fluoro-3-methyl benzaldehyde, $NaBH(OAc)_3$, MTBE, AcOH, 0–25 °C, 16 h; (b) EtOCOCl, DME, 85 °C, 3 h, then $(COCl)_2$, DME, 85 °C, 1.5 h, 85%; (c) $EtO_2CCH_2COCH_2Cl$, AcOH, AcOEt, 70 °C, 16 h, 80% (2 steps); (d) $KMnO_4$, HCOOH, acetone, THF, 0 °C, 1 h, 90%; (e) *t*-BuOK, THF, rt, 16 h; (f) H_2, Raney-Ni, rt, 2 h; (g) MsCl, pyridine, 0 °C, 30 min; (h) 1 N NaOH, THF, rt, 30 min.

Table 4
Mean pharmacokinetic parameter values for compound **34** following single dose administration to rats and dog

Caco-2 A to B (ER)[a]	Species	LM[b] (μL/min/mg)	C_{max} (ng/mL)	AUC (ng h/mL)	Cl (mL/kg/min)	$T_{1/2}$ (h)
0.97 (3.78)	Rat iv[c]	126	130	158	186	1.1
	Rat po[c]		2[e]	nd	—	nd
	Dog iv[d]	24	4350	954	19	1.6
	Dog po[d]		10.6	34.8	—	1.2

[a] Caco-2 assay run for 21 days. ER = (B-to-A)/(A-to-B).
[b] LM: in vitro liver microsomal stability data.
[c] Rat PK study: dose iv = 2 mg/kg; po = 5 mg/kg.
[d] Dog PK study: dose iv = 1 mg/kg; po = 1 mg/kg.
[e] BQL in 2 out of 3 rats after 2 h; 2 h and 6 h C_{liver} = 151 and 22 ng/gm.

Table 5
Pharmacokinetic data for benzothiazine **34** following oral administration on a seven days rat toxicology study

Po dose (mg/kg)	Gender	C_{max} (ng/mL)		AUC (0–24 h) (ng h/mL)		C_{min} (24 h) (ng/mL)	
		Day 1	Day 7	Day 1	Day 7	Day 1	Day 7
10	M	2.06	2.76	25.4	34.4	0	0
10	F	3.26	2.80	25.6	21.2	0	0
100	M	20.4	21.1	223	322	2.8	19.6
100	F	13.0	15.8	116	299	2.1	12.1
500	M	93.2	152	821	1910	11.3	41.7
500	F	48.2	89.3	701	1466	1.3	39.5

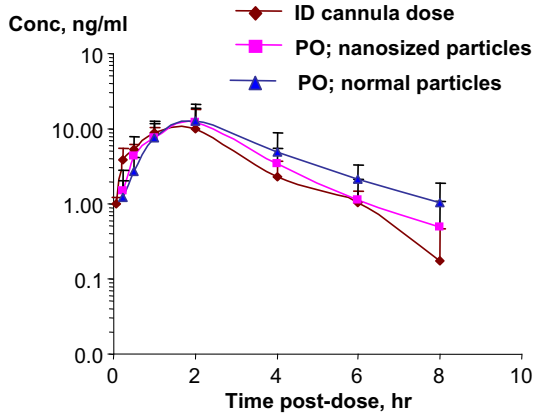


Figure 4. Graphical representation of plasma concentrations dependence of time after oral administration of benzothiazine **34** in dog using different dosage formulations.

confirm limited absorption as the key factor resulting in poor compound exposure in dog.

In conclusion, we have disclosed our structure activity studies and in vivo evaluation of a series of benzothiazine-substituted quinolinediones as a new class of non-nucleoside inhibitors of HCV polymerase NS5B. The incorporation of the methyl sulfonamide group at C-7 led to potent inhibitors in vitro, and in both standard and plasma shifted cell based assays. We have developed a short and convergent synthesis of these compounds that allowed for rapid evaluation and scale up. In vivo pharmacokinetic evaluation of compound **34** demonstrated good dose proportionality and a favorable toxicological profile. Pharmacokinetic studies also revealed poor exposure due to low absorption. Template modifications focused on improving the physicochemical properties of this scaffold will be reported in subsequent publications.

Acknowledgments

The authors thank Drs. Eric Sjogren, Hans Maag, and John Josey for their support and helpful discussion during the course of this work.

References and notes

1. Choo, Q. L.; Weiner, A. J.; Overby, L. R.; Bradley, D. W.; Houghton, M. *Science* **1989**, *244*, 359.
2. For a review of HCV epidemiology, pathogenesis, and clinical treatment, see: (a) Lauer, G. M.; Walker, B. D. *N. Eng. J. Med.* **2001**, *345*, 41; (b) Di Bisceglie, A. M. *Lancet* **1998**, 351; (c) Alter, M. J.; Kruszon-Moran, D.; Nainan, O. V.; McQuillan, G. M.; Gao, F.; Moyer, L. A.; Kaslow, R. A.; Margolis, H. S. *N. Eng. J. Med.* **1999**, *341*, 556.
3. Manns, M. P.; McHutchison, J. G.; Gordon, S. C.; Rustgi, V. K.; Shiffman, M.; Reindollar, R.; Goodman, Z. D.; Koury, K.; Ling, M.-H.; Albrecht, J. K. *Lancet* **2002**, *347*, 975.
4. Koch, U.; Narjes, F. *Curr. Top. Med. Chem.* **2007**, *7*, 1302; Rönn, R.; Sandström, A. *Curr. Top. Med. Chem.* **2008**, *8*, 533; Zapf, C. W.; Bloom, J. D.; Levin, J. I. *Ann. Rep. Med. Chem.* **2007**, 281. See also references therein.
5. 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.
6. (a) Gu, B.; Johnston, V. K.; Gutshall, L. L.; Nguyen, T. T.; Gontarek, R. R.; Darcy, M. G.; Tedesco, R.; Dhanak, D.; Duffy, K. J.; Kao, C. C.; Sarisky, R. T. *J. Biol. Chem.* **2003**, *278*, 16602; (b) Tedesco, R.; Shaw, A. N.; Bambal, R.; Chai, D.; Concha, N. O.; Darcy, M. G.; Danak, D.; Fitch, D. M.; Gates, A.; Gerhardt, W. G.; Halegoua, D. L.; Han, C.; Hofmann, G. A.; Johnston, V. K.; Kaura, A. C.; Liu, N.; Keenan, R. M.; Lin-Goerke, J.; Sarisky, R. T.; Wiggall, K. J.; Zimmerman, M. N.; Duffy, K. J. *J. Med. Chem.* **2006**, *49*, 971.
7. (a) Hendricks, R. T.; Fell, J. B.; Blake, J. F.; Fischer, J. P.; Robinson, J. E.; Spencer, S. R.; Stengel, P. J.; Bernaki, A. L.; Leveque, V. J. P.; LePogam, S.; Rajyaguru, S.; Najera, I.; Josey, J. A.; Harris, J. R.; Swallow, S. *Biog. Med. Chem. Lett.* preceding paper.; (b) Blake, J. F.; Fell, J. B.; Fischer, J. P.; Fischer, J. P.; Hendricks, R. T.; Robinson, J. E.; Spencer, S. R.; Stengel, P. J. U.S. Patent Application 2006/040927, 2006.
8. Hardtmann, G. E.; Koletar, G.; Pfister, O. R. *J. Heterocycl. Chem.* **1975**, *12*, 565.
9. For a detailed description of the enzyme assay see: Ma, H.; Jiang, W.-R.; Robledo, N.; Leveque, V.; Ali, S.; Lara-Jaime, T.; Masjedizadeh, M.; Smith, D. B.; Cammack, N.; Klumpp, K.; Symons, J. *J. Biol. Chem.* **2007**, *282*, 29812.
10. For a detailed description of the replicon assay see: Klumpp, K.; Leveque, V.; Le Pogam, S.; Ma, H.; Jiang, W.-R.; Kang, H.; Granycome, C.; Singer, M.; Laxton, C.; Hang, J. Q.; Sarma, K.; Smith, D. B.; Heindl, D.; Hobbs, C. J.; Merrett, J. H.; Symons, J.; Cammack, N.; Martin, J. A.; Devos, R.; Najera, I. *J. Biol. Chem.* **2006**, *281*, 3793.
11. A representative number of benzothiazine-substituted quinolinediones were also evaluated in a GT-1a replicon assay. This class of compounds had a minimal loss in inhibitory activity of 2–10 fold relative to our standard GT-1b replicon assay.
12. Atomic coordinates of the structure **18** bound to CΔ21 NS5B polymerase were deposited with the RCSB Protein Data Bank (PDB) under the accession code **3G86**. The crystal structure was determined following previously described procedures: Le Pogam, S.; Kang, H.; Harris, S. F.; Leveque, V.; Giannetti, A. M.; Ali, S.; Jiang, W.; Rajyaguru, S.; Tavares, G.; Oshiro, C.; Hendricks, T.; Klumpp, K.; Symons, J.; Browner, M. F.; Cammack, N.; Najera, I. *J. Virol.* **2006**, *80*, 6146.