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Substituted benzothiadizine inhibitors of Hepatitis C virus polymerase

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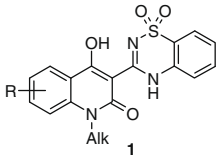
ABSTRACT

The synthesis and optimisation of HCV NS5B polymerase inhibitors with improved potency versus the existing compound **1** is described. Substitution in the benzothiadiazine portion of the molecule, furnishing improvement in potency in the high protein Replicon assay, is highlighted, culminating in the discovery of **12h**, a highly potent oxyacetamide derivative.

Hepatitis C virus (HCV) is responsible for a variety of clinical conditions ranging from acute viral hepatitis to chronic liver disease and cirrhosis. It is the major cause of liver cancer and about two thirds of all liver transplants are a result of HCV infection. There are an estimated 170 million people worldwide chronically infected and 3–4 million new infections annually.[2] In the United States, approximately 19,000 new infections occurred in 2006 and an estimated 3–4 million carry the disease, many of whom were infected through blood transfusions with contaminated blood prior to the start of screening for the virus in 1992.[3] There is currently no vaccine against the virus.

In a previous publication,[1] we describe the development of a series of 3-(benzothiadiazin-3-yl)quinolinone inhibitors **1** of the non-structural protein NS5B, an RNA dependent RNA polymerase encoded by the viral genome and known to be essential for viral replication.[4] Related benzothiadiazine inhibitors of NS5B have been described by our group[5] and others.[6]

This Letter describes the structure–activity relationships of substitution on the benzothiadizinyl ring as in compound **2** with the aim of improving the developability of the compounds and their cellular potency, particularly under high plasma-protein conditions more similar to those encountered physiologically. The preceding Letter describes an alternative approach to modulation of physical properties using related heterocyclic isosteres.[7]



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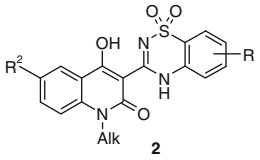

2

The compounds were synthesized according to the methods shown in the Schemes 1 and 2 (experimental details supplied in the Supplementary data).

Novel 2-Aminobenzenesulfonamides **4** were synthesized by reaction of anilines **3** with chlorosulfonyl isocyanate followed by cyclization with aluminium chloride and hydrolysis,[8] then acylated with ethyl malonyl chloride or diethyl malonate, and cyclised with phosphorus oxychloride or aqueous sodium carbonate to give the benzothiadiazine-3-acetic acid ester **5**. Ester **5** was condensed with N-alkyl isatoic anhydrides **6** and cyclised to the required compound **2** (Scheme 1).[1] In an alternative route, the isatoic anhydrides **6** were converted to quinoline-3-nitriles **7** by condensation

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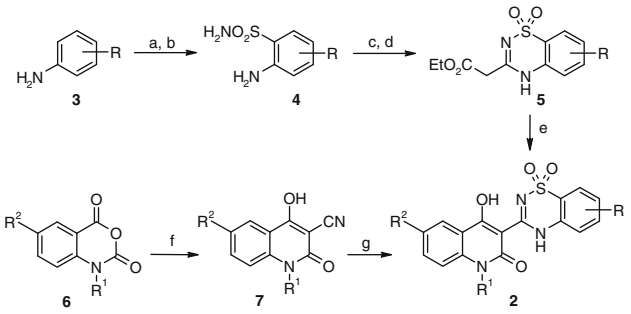
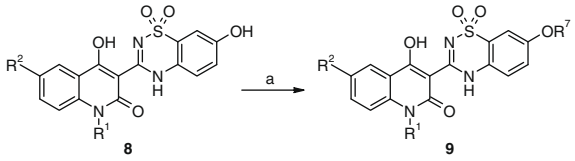


Scheme 1. Reagents and conditions: (a) $ClSO_2NCO$, $AlCl_3$, $EtNO_2$; (b) 50% H_2SO_4, reflux; (c) $EtO_2CCH_2CO_2Et$, DMF, Δ or $Cl(O)CCH_2CO_2Et$, Et_3N, DMAP, THF; (d) aq Na_2CO_3 or $POCl_3$, reflux; (e) **6**, NaH, then AcOH, THF, reflux or DBU, then AcOH, DMF; (f) EtO_2CCH_2CN, NaH, DMF, heat; (g) **4**, Me_3Al, 1,4-dioxane; then NaOH, reflux.



Scheme 2. Reagents and Conditions: (a) NaH or K_2CO_3, R^7Br, R^7Cl or R^7OTs, DMF or aq DME, heat.

with ethyl cyanoacetate and cyclization. The nitriles **7** were coupled with 2-aminobenzenesulfonamides **4** to give, after cyclization with sodium hydroxide, the required compound **2**.

Benzothiadiazines (**10f, m–q**) were made after the heterocycle had been constructed, either by palladium-catalyzed substitution of a halide,[9,10] or by nitrile hydrolysis/methanolysis. 7-Amino compounds **10i** and **10j** were made by nitration of the parent heterocycle followed by hydrogenation, then reductive alkylkation. Hydroxy compound **10l** was made by demethylation of the methoxy compound **10k**, while **12b** was made as in Scheme 1 with the appropriate benzothiadiazine-3-acetic acid ester **5**. Carbon-linked compounds **12i** and **12j** were made by palladium-catalysed coupling of the corresponding 7-iodo compound with acrylamide,[11] followed by hydrogenation. Hydroxymethyl compound **12d** was made from the corresponding ester by reduction with lithium aluminum hydride and converted to the carbamate derivative **12k** by reaction with trichloroacetyl isocyanate followed by hydrolysis.[12]

More elaborate alkoxy compounds **9** were made by alkylation of the hydroxy derivatives **8** using the appropriate alkyl bromide, chloride or tosylate as in Scheme 2, followed, if necessary, by functional group manipulation. Compounds **12n** and **12o** were made by alkylation with the appropriate homochiral 2-tosyloxypropionamide without loss of chiral integrity.[13]

An X-ray structure of compound **10a** bound to the NS5B polymerase in the palm/thumb domain region showed a tight fit of the compound in the binding site (Fig. 1).[1] It was apparent from the structure that, while most substitutable positions around the quinolinone ring were close to the protein surface, there was space available around the benzothiadiazine ring for further substitution. An examination of the protein structure showed a number of hydrophilic residues in close proximity to the benzothiadiazine 7-position and prompted us to explore substitution in the thiadiazine with an emphasis on polar substituents.

The goal of this strategy was not only to improve potency by making additional H-bonding interactions with the protein, but also to reduce the highly lipophilic nature of our earlier compounds[1] in an attempt to lower protein binding and improve activity in cell-based assays and in vivo settings. Substitution at other positions 5, 6 and 8 was also explored, but bulky polar

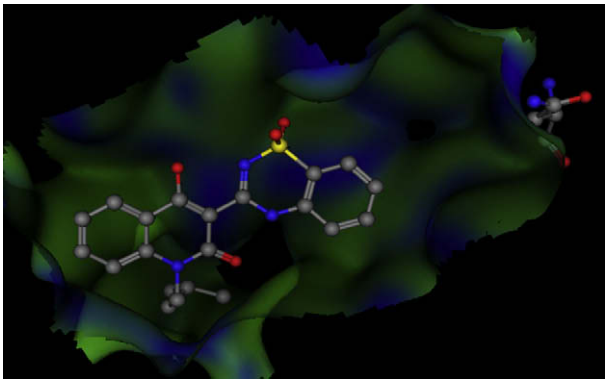


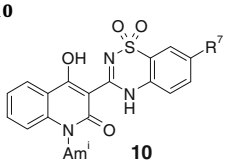
Figure 1. X-ray of **10a** bound to NS5B (genotype 1b, BK strain, Δ21 construct, PDB deposition code 2FVC).

functionality at these positions was poorly tolerated or synthetically less accessible (data not shown).

The first compounds **10** with an *N*-isoamyl quinolinone ring (Table 1) showed that substitution is well tolerated at the 7 position with a wide variety of substituents. Generally, smaller, hydrophilic groups are preferred. Fluorine maintains the activity in the NS5B enzymatic assay,[14] while activity drops as the halogen gets larger. Both electron donating (e.g., OMe, compound **10k**) and electron-withdrawing substituents (e.g., CN, compound **10f**) are tolerated. Hydrophilic OH (compound **10l**), NH_2 (compound **10i**) substituents gave compounds with activity similar to the parent. Introduction of a carbonyl function is well tolerated. Ester **10n** and acid **10o** have similarly potencies, while the 7-carboxamide derivative **10p** is about fivefold more potent than the unsubstituted parent **10a**. The more bulky tertiary amide group (compound **10q**) resulted in significantly lower enzymatic activity.

In general, activity in the Replicon assay[15] tracks fairly well with enzymatic activity. The hydroxy compound **10l**, however, is

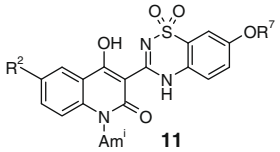
Table 1
Polymerase inhibition,[14] type 1b Replicon assay data[15] under high and low protein conditions for compounds **10**



Compd	R^7	NS5B[a] IC_{50} (nM)	Replicon IC_{50} (nM)	PA-Replicon[b] IC_{50} (nM)
10a	H	32	417	NT[c]
10b	F	26	334	NT
10c	Cl	76	2321	>50,000
10d	Br	173	5141	NT
10e	I	>10,000	NT	NT
10f	CN	57	486	NT
10g	Me	50	1784	NT
10h	NO_2	238	>20,000	NT
10i	NH_2	13	218	45,000
10j	NMe_2	142	1247	NT
10k	OMe	55	1394	NT
10l	OH	30	>20,000	NT
10m	C(O)Me	127	5195	NT
10n	CO_2Me	65	2625	NT
10o	CO_2H	45	>20,000	NT
10p	$CONH_2$	6	383	NT
10q	$CONMe_2$	102	>20,000	NT

[a] Δ21-truncated NS5B used in the assay.
[b] PA-Replicon = protein-attenuated Replicon assay, run in the presence of 45 mg/mL HSA and 1 mg/mL AAG.
[c] NT = not tested.

Table 2
Polymerase inhibition, type 1b Replicon assay data under high and low protein conditions for compounds **11**



11

Compd	R^2	R^7	NS5B IC_{50} (nM)	Replicon IC_{50} (nM)	PA-Replicon IC_{50} (nM)
11a	H	CH_2CN	7	81	NT
11b	H	$CH_2CO_2CH_3$	23	815	NT
11c	H	CH_2CO_2H	8	1604	NT
11d	H	CH_2CONH_2	6	15	2646
11e	H	$(CH_2)_2NH_2$	142	568	NT
11f	H	$(CH_2)_2N(CH_3)_2$	685	NT	NT
11g	F	CH_2CN	5	29	8000
11h	F	CH_2CONH_2	18	13	3097

very weak in the cellular assay and the biochemically potent 7-carboxamide derivative **10p** is equipotent with the parent **10a**, despite its higher biochemical activity.

The 7-hydroxyl group was a useful handle for introducing further functionality through alkylation, and allowed us to reach out further into the binding pocket. Table 2 shows such alkylated derivatives, **11**.

The oxyacetonitrile substituted compound **11a** is very potent in the enzymatic assay and also shows higher potency in the Replicon assay than all previous compounds **10a–q**. The corresponding oxyacetic acid **11c** and its methyl ester **11b** are also potent, but weaker in the cellular assay. The oxyacetamide **11d** stands out as it has very high potency in both the enzymatic and the cellular assay, being about 25-fold more potent in the latter assay than the parent compound **10a**. The 7-aminoethoxy (compound **11e**) or 7-dimethylaminoethoxy (compound **11f**) derivatives are weaker NS5B inhibitors. Incorporation of a fluoro substituent at the 6 position of the quinolinone ring has previously been shown to improve cellular activity[1] and exhibits a similar effect here, as exemplified by the 7-oxyacetonitrile (compound **11g**) and 7-oxyacetamide (compound **11h**) derivatives. Indeed, the 7-oxyacetamide analog **11h** was, at the time, the most potent compound tested in the cellular Replicon system. Importantly, compounds **11d**, **11g**, **11h** have low micromolar inhibitory activity in the type 1b Replicon assay when run in the presence of 45 mg/mL HSA and 1 mg/mL alpha acid glycoprotein (AAG), representing a significant improvement over earlier compounds with smaller, lipophilic C-7 substituents (e.g., compound **10c**, Table 1). An X-ray structure of **11d** bound to the type 1b NS5B was solved and shows the oxyacetamide group filling out the binding pocket and making a favorable hydrogen bond to the side-chain amide of Asn_{291} (Fig. 2).

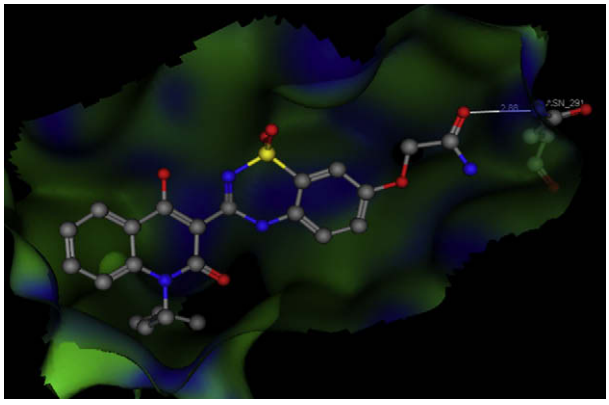
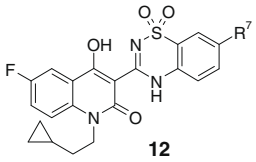


Figure 2. X-ray of **11d** bound to NS5B (genotype 1b, BK strain, Δ21 construct, PDB deposition code 3HHK).

The optimal 6-fluoro and 1-2-(cyclopropyl)ethyl substituents on the quinolinone ring,[1] were next combined with a panel of the best substituents from the benzothiadiazine SAR studies described above to maximize the activity of the substituted thiadiazines both enzymatically and in the cellular assay. The activity of these compounds **12** is shown in Table 3.

The unsubstituted thiadiazine compound **12a**, our first development candidate,[1] is potent against the isolated enzyme and in the standard Replicon assay, but much weaker in the protein-attenuated Replicon assay in the presence of physiologically relevant concentrations of HSA and AAG. The 7-hydroxy and 7-methoxy compounds **12b** and **12c** are similar to the parent, but the longer side-chain analogs again showed significant improvements in potency. The oxyacetic acid **12e** is potent but weak in cells, probably due to high polarity and therefore poor membrane permeability. The corresponding tetrazole **12g** offers no improvement in cells, nor does the 1,1-dimethyloxyacetic acid **12p**. The oxyacetonitrile **12f** shows high potency and low micromolar activity in the protein-attenuated Replicon assay, but the oxyacetamide **12h** is more potent still, mirroring the SAR trends seen in the previously described isoamyl series. Interestingly, an all carbon-linked side-chain, either saturated or unsaturated, (compounds **12j** and **12i**) abolishes the gain in the high-protein Replicon assay, and the isomeric carbamate **12k** of amide **12h** is weaker in both primary assays. Confident that the oxyacetamide imparted the best activity of the compounds made at that time, we looked at simple substitution on this substituent for further improvement. The N-methyl secondary amide **12l** is slightly less potent in both Replicon assays and the N-dimethyl tertiary amide **12m** significantly weaker, presumably due to the steric perturbation of the interaction with Asn_{291}. Introduction of a methyl group into the oxyacetamide side-chain gives one enantiomer ((*R*) **12n**) with activity comparable to **12h** in enzymatic and high protein cellular assays. Interestingly, the (*S*)-enantiomer **12o** is weaker biochemically, as was

Table 3
Polymerase inhibition, type 1b Replicon assay data under high and low protein conditions for compounds **12**



12

Compd	R^7	NS5B IC_{50} (nM)	Replicon IC_{50} (nM)	PA-Replicon IC_{50} (nM)
12a[1]	H	10	38	24,461
12b	OH	<5	57	50,000
12c	OMe	24	342	>50,000
12d	CH_2OH	17	233	NT
12e	OCH_2CO_2H	<5	2,295	NT
12f	OCH_2CN	<5	8	2388
12g	OCH_2(tetrazole)	6	2000	NT
12h	OCH_2CONH_2	<5	2	1,484
12i	(E)-CH=CH$CONH_2$	202	39	50,000
12j	$(CH_2)_2CONH_2$	5	208	>50,000
12k	CH_2OCONH_2	36	48	NT
12l	$OCH_2CONHCH_3$	<5	9	4,737
12m	$OCH_2CON(CH_3)_2$	12	211	NT
12n	(*R*)-$OCH(CH_3)CONH_2$	<5	3	1017
12o	(*S*)-$OCH(CH_3)CONH_2$	12	4	50,000
12p	$OC(CH_3)_2CO_2H$	22	3,742	NT
12q	$OC(CH_3)_2CONH_2$	9	272	5358

Table 4
Comparison of selected properties of HCV NS5B inhibitors

Property	**12a**	**12h**	**12n**
Type 1b NS5B IC_{50} (nM)	10	<5	<5
Type 1b Replicon IC_{50} (nM)	38	2	3
Protein-attenuated 1b Replicon IC_{50} (nM)	24,461	1484	1017
Equilibrium dialysis% in human plasma	99.9	99.9	99.8
Rat PK[a]: $T_{1/2}$ (h), F(%)	3, 44	1, 33	0.7, 35
CYP2C9 IC_{50} (μM)	0.84	5.4	1.7

[a] Low dose (~4 mg/kg) oral and iv pharmacokinetics in rat. $T_{1/2}$ = terminal half-life, F = bioavailability.

predicted by prior modeling studies showing a small pocket for a group in the (*R*)-configuration but not for the one in the (*S*)-configuration (data not shown). Consistently, dimethylation of the oxyacetamide side-chain also gives a compound (**12q**) with lower biochemical potency than **12n**, although the activity in the protein-attenuated Replicon assay, surprisingly, is only about fivefold worse.

Most of the compounds described in this Letter were tested in a large panel of developability assays, including pharmacokinetics, human serum albumin (HSA) column retention and cytochrome P450 assays. The data for the most promising compounds **12h** and **12n** are compared to those of the first development candidate **12a** in Table 5‘. CYP2C9 inhibition was routinely measured, since, historically, the benzothiadiazine inhibitors show the most potent inhibition against this isoform. Inhibitory potencies against other P450 enzymes (data not shown) are significantly lower than for 2C9 for these compounds.

While the human serum protein binding, measured by equilibrium dialysis, ultimately was not significantly reduced for **12h** and **12n**, their potencies in the key assay, the protein-attenuated Replicon assay, were significantly improved over that of **12a**. Protein binding, as measured by HSA column retention, was not in fact significantly reduced for any of the compounds described here (data not shown).

Inhibitor **12h** showed the best overall profile of the compounds examined and was selected as an improved follow-up development candidate for the treatment of HCV infection.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.05.091.

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