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Inhibitors of HCV NS5B polymerase: synthesis and structure–activity relationships of *N*-1-heteroalkyl-4-hydroxyquinolon-3-yl-benzothiadiazines

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Abstract—*N*-1-Alkylamino and *N*-1-alkyloxy-4-hydroxyquinolon-3-yl benzothiadiazines were synthesized and evaluated as inhibitors of genotype 1 HCV polymerase. The *N*-1-alkyloxy derivatives were not potent inhibitors, however *N*-1-alkylamino derivatives displayed comparable potency to carbon analogs. Analogs with aliphatic substituents were significantly more potent than those with benzylic substituents against genotype 1a polymerase. The most potent inhibitors contained small alkyl or carbocyclic substituents and exhibited IC_{50}'s of 50–100 and 200–400 nM against genotype 1b and 1a HCV polymerase, respectively.

Hepatitis C virus (HCV) infection is a leading cause of chronic liver disease and the leading cause of death from liver disease in the United States.[1] The Centers for Disease Control and Prevention estimate that more than 2.7 million individuals in the United States and 170 million people worldwide have ongoing HCV infection.[2] HCV has six major genotype classes,[3] with genotypes 1 and 2 being most prevalent in the United States, Europe, and Japan.

The goal in treating patients infected with HCV is eradication of the infection. The infection is considered eradicated when there is a sustained virologic response (SVR), defined as the absence of HCV RNA in serum at the end of treatment and six months post-treatment. The current standard of care for treating HCV is a combination therapy of pegylated interferon-α (PEG-IFN-α)/ribavirin. Response rates for HCV patients having genotypes 2 or 3 on a 24-week treatment regimen of PEG-IFN-α/ribavirin show a SVR approaching 80%. Patients infected with genotype 1 HCV do not respond as well to this combination therapy, demonstrating SVR rates of <50% even after treatment therapies of 48 weeks in duration.[4,5] In addition, nearly three out of four people treated with PEG-IFN-α/ribavirin experience systemic side effects, which can include neutropenia, depression, irritability, headaches, nausea, anemia, etc.[6] These factors result in a therapeutic need for new HCV therapies, particularly those directed at genotype 1 HCV.

Because of their demonstrated and vital roles in viral replication, the NS3 protease/helicase and NS5B RNA dependent RNA polymerase (RdRp) have been the most studied viral protein targets for small molecule HCV therapy. Numerous efforts directed at inhibiting HCV NS5B RdRp include nucleoside[7,8] and non-nucleoside inhibitors.[9–11] A series of *N*-1-alkyl-4-hydroxy-quinolon-3-yl benzothiadiazine non-nucleoside inhibitors **1** (Fig. 1) were identified from a high throughput screening assay[12] and were found to inhibit polymerase at the initiation step of RNA replication.[13] Most importantly, in the cell-based HCV replicon assay the compounds of class **1** potently inhibited the production of viral RNA.

Our preliminary SAR of the *N*-1-alkyl benzothiadiazines demonstrated equivalent polymerase activity for the quinolone core **1** and 1,8-naphthyridone core **2**.

Keywords: Hepatitis C; HCV; RNA dependent RNA polymerase; NS5B.
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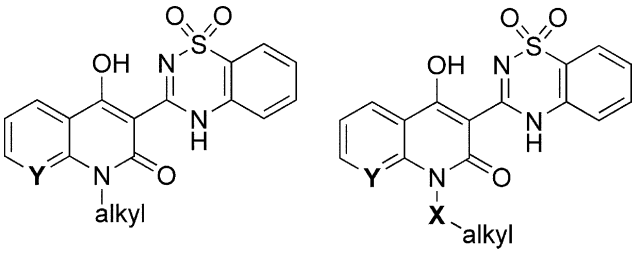


Figure 1.

Table 1. IC_{50} values against genotype 1b polymerase: effects of branching at the α-carbon for analogs of **2**

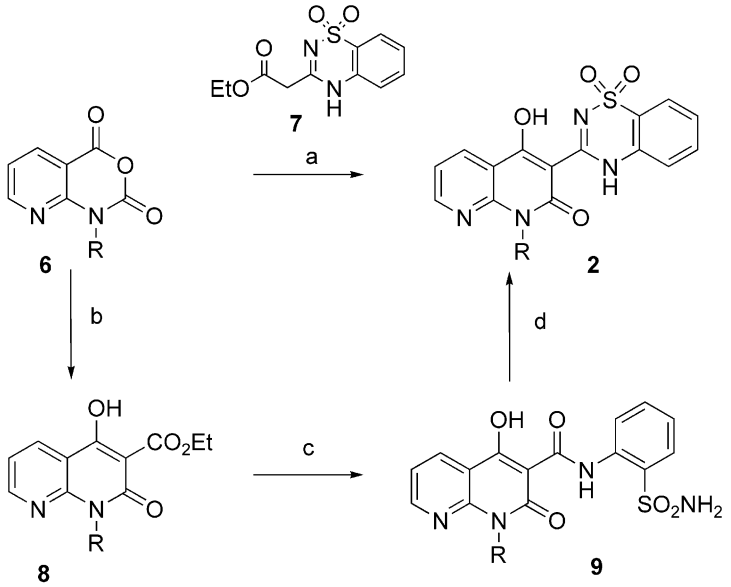
Compd	R	1b IC_{50}, μM[a]
2c	$-CH_2Ph$	0.083
2d	(*S*)–CHMePh	4.63
2e	$-CH_2CH(Et)_2$	0.058
2f	$-CH(Et)_2$	13.2
2g		0.093
2h		50.0

[a] IC_{50} values in all tables are means of at least two independent determinations, standard deviation ± 10%. Detailed protocols can be found in the Supplementary data.

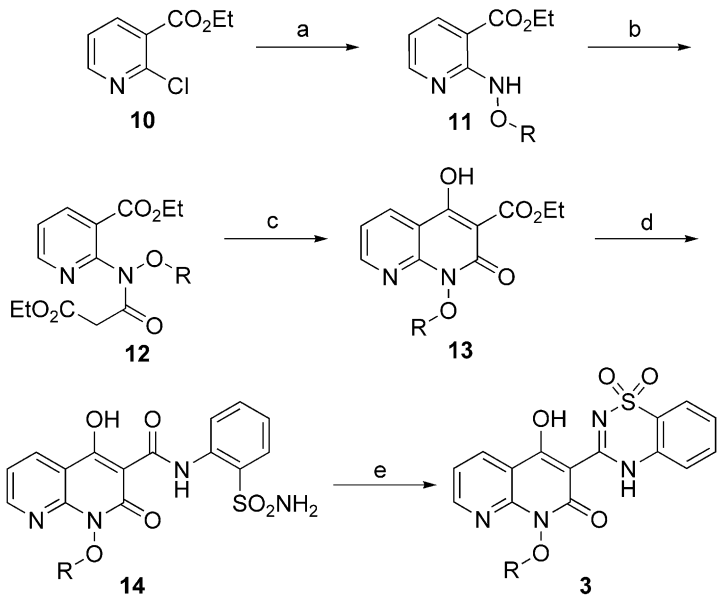
We also observed that the nature of the hydrophobic functionality at the N-1 position of the quinolone ring substantially impacted biochemical potency. In particular, branching at the α-carbon of the alkyl group dramatically decreased inhibitory activity (Table 1) suggesting either that this position was sterically restricted or that the branched analogs could not adopt a low energy conformation suitable for binding. This paper describes the synthesis and structure–activity relationships of quinolone and naphthyridione benzothiadiazines in which the α-carbon of the N-1 side chain is replaced with either an oxygen (compound **3**) or a nitrogen (compounds **4** and **5**). We rationalized that these replacements could allow the attached alkyl groups access to hydrophobic binding via slightly different bond angles/conformations. However, the effect of changing a lipophilic carbon atom to a more polar nitrogen or oxygen atom on the interaction of the inhibitor with the protein was unknown.

The preparation of the 1,8-naphthyridone benzothiadiazines **2**, shown in Scheme 1, is similar to the synthesis of compound **1**.[14] Treatment of 8-azaisatoic anhydrides **6** with NaH and (1,1-dioxo-1,4-dihydro-1λ⁶-benzo-[1,2,4]- thiadiazin-3-yl)-acetic acid ethyl ester[15] (compound **7**) followed by acetic acid provided **2** directly. Alternatively, the 8-azaisatoic anhydrides were allowed to react with diethyl malonate and NaH to give compound **8**, which were in turn treated with 2-aminobenzenesulfonamide in refluxing toluene to give the carboxanilides **9**. The thiadiazine ring was then formed by an intramolecular dehydration using 15% aqueous KOH at reflux to give **2**.

The synthesis of *N*-1-alkyloxy derivatives **3**, shown in Scheme 2, entailed treating ethyl 2-chloronicotinate **10** with an alkoxyamine in the presence of Hunig's base at high temperature to give **11** in poor yields. Attempts to convert **11** or the corresponding nicotinic acid to an isatoic anhydride with phosgene or phosgene equivalents were unsuccessful. However, **11** could be acylated with ethyl 3-chloro-3-oxopropionate followed by cyclization with sodium ethoxide to give the naphthyridone **13**. Treatment of **13** with 2-aminobenzenesulfonamide



Scheme 1. Reagents and conditions: (a) compound **7**, NaH, THF, reflux, 3 h then AcOH, reflux 1 h, 50–88%; (b) diethylmalonate, NaH, DMA, 120 °C, 2 h, 74–95%; (c) 2-aminobenzenesulfonamide, toluene, reflux, 5 h, 50–60%; (d) 15% aqueous KOH, reflux, 12 h, 95%.



Scheme 2. Reagents and conditions: (a) alkoxyamine hydrochloride, *N*,*N*-diisopropylethylamine, dioxane, 140 °C, 24 h, 30–35%; (b) ethyl 3-chloro-3-oxopropionate, triethylamine, dichloromethane, 2 h, 42–65%; (c) NaOEt, EtOH, 82–98%; (d) 2-aminobenzenesulfonamide, toluene, reflux, 12 h, 86–89%; (e) 10% aqueous KOH, reflux, 12 h, 90–93%.

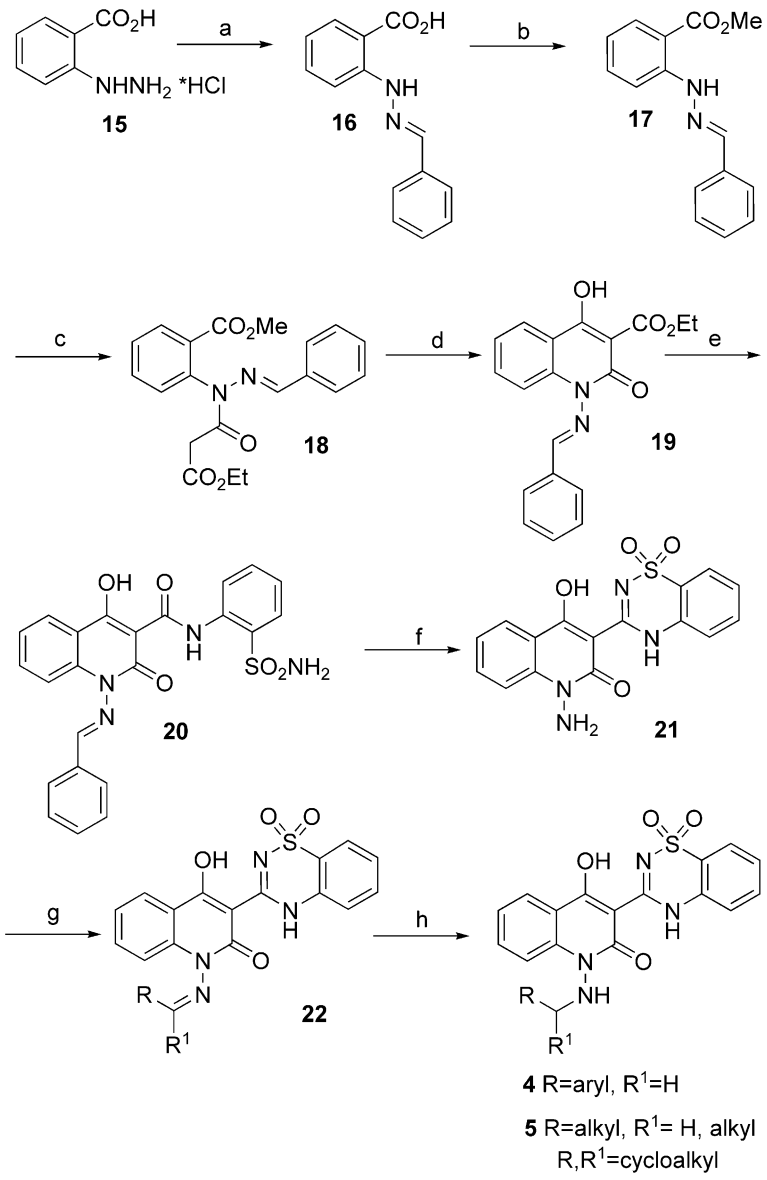
followed by heating in aqueous KOH gave the *N*-1-alkyloxy benzothiadiazine derivatives **3**.

Preparation of the *N*-1-alkylamino benzothiadiazines[16] with the general structures of **4** and **5** could be accomplished using a similar approach, but it required the development of a protecting group strategy for the terminal hydrazine nitrogen, as shown in Scheme 3. Commercially available 2-hydrazinobenzoic acid hydrochloride **15** was treated with benzaldehyde to give the hydrazone **16**[17] and the carboxylic acid was converted to the methyl ester **17** with trimethylsilyldiazomethane. Treatment of **17** with ethyl 3-chloro-3-oxopropionate in refluxing toluene afforded **18**, followed by cyclization to the 4-hydroxyquinolone **19** with sodium ethoxide in ethanol. Compound **19** was allowed to react with 2-aminobenzenesulfonamide in refluxing toluene to give the carboxamide **20** in moderate yield. Treatment of **20** with refluxing 20% aqueous potassium hydroxide resulted in cyclization of the sulfonamide to the benzothiadiazine accompanied by concomitant hydrolysis of the benzaldehyde hydrazone to give **21**. This intermediate was treated with excess aldehyde or ketone (typically 5–10 mol equiv) and heated in a microwave reactor to produce the hydrazones of choice. Similar transformations using a sealed tube in an oil bath proved unsatisfactory due to long reaction times and low conversion of the hydrazine to the hydrazone, especially with hindered ketones. The hydrazone derivatives **22** were then reduced to the final products **4** or **5** with lithium borohydride in THF.

Table 1 illustrates the effect of branching at the N-1 α-carbon on biochemical potency.[16] The IC_{50} of the benzyl derivative **2c** against 1b HCV polymerase was <0.1 μM, whereas the α-methyl benzyl amine **2d** was much less active (IC_{50} 4.6 μM). A more pronounced potency difference was observed between the alkyl derivatives **2e**–**f** and the cycloalkyl analogs **2g**–**h**, with the cyclohexyl analog **2h** displaying >500-fold lower potency than the cyclohexane methyl analog **2g**.

Table 2 highlights the effect of substituting a heteroatom for the methylene at the N-1 α-position. Compounds **2a** and **2b**, with a methylene link at N-1, displayed IC_{50} values of 1.49 and 0.069 μM, respectively. The oxygen-linked homologs **3a** and **3b** were much less potent, with IC_{50}'s of 58 and 20.5 μM, respectively. In contrast, the nitrogen-linked analogs **4a** and **5e** inhibited HCV 1b polymerase at <0.2 μM, indicating that nitrogen replacement for the methylene is tolerated and prompts further investigation of this series. The large discrepancy in potency between the oxygen- and nitrogen-linked analogs is of interest. Potentially, the latter might preferentially interact with the protein via hydrogen bond donation. Alternately, an unfavorable interaction between the lone pairs of the nitrogen at the 8-position of the napthyridine ring and the oxygen linker could disfavor the conformation required for productive binding of the alkyl group. Oxygen-linked analogs lacking the 8-heteroatom and nitrogen-linked napthyridines were not readily synthetically accessible to test this hypothesis.

With the available synthesis for the *N*-1-alkylamine series **4** and **5**, the scope of the SAR was determined. The inhibitory potencies for these derivatives are shown in Tables 3 and 4. The compounds were tested against polymerases representing both HCV 1a and 1b genotypes. Table 3 summarizes benzylic and heteroaromatic derivatives **4**, all of which have a methylene spacer between the α-nitrogen and the aromatic ring. Compound **4a**, the unsubstituted benzyl analog, inhibited genotype 1b polymerase with an IC_{50} of 0.129 μM, but was significantly less potent against 1a (5.1 μM). Substitution at the *ortho* position of the phenyl ring with either a bromo or methyl substituent produced a 4-fold decrease in



Scheme 3. Reagents and conditions: (a) benzaldehyde, EtOH/H_2O, 98%; (b) (trimethylsilyl)diazomethane, THF/MeOH, 16 h, 90%; (c) ethyl 3-chloro-3-oxopropionate, toluene, reflux, 4 h, 95%; (d) NaOEt, EtOH, 95%; (e) 2-aminobenzenesulfonamide, toluene, reflux, 5 h, 60%; (f) 20% aqueous KOH, reflux, 16 h, 95%; (g) aldehyde/ketone, 135 °C microwave, 30 min, 60–80%; (h) $LiBH_4$, cat. MeOH, THF, 0 °C to rt, 1 h, 50–80%.

Table 2. Comparison of N-1 linking atoms (C vs N vs O)

Compd	R	R^1	1b IC_{50}, μM
2a	$–CH_2CH_2Ph$	—	1.49
2b	$–CH_2CH_2CH(Me)_2$	—	0.069
3a	$–CH_2Ph$	—	58.0
3b	$–CH_2CH(Me)_2$	—	20.5
4a	Ph	H	0.129
5e	$–CH(Me)_2$	H	0.101

potency against 1b polymerase but no change in activity against 1a. The 3-bromo derivative **4c** was 6-fold more active than the 2-bromo analog **4b**. In comparison, the 3-methyl analog **4f** was less potent against both 1a and 1b polymerases. A dramatic decrease in activity against 1b polymerase was observed with derivatives substituted at the para position, **4d** and **4g**, suggesting a size limitation to the binding pocket. Compounds **4h–n** represent heteroaromatic replacements for the phenyl ring. In general, none of these derivatives displayed significantly improved activity over the phenyl analog.

Table 4 shows the SAR of three classes of alkyl and cycloalkyl derivatives: aliphatic groups linked by a methylene to the nitrogen (compounds **5a–g**), branched aliphatics (compounds **5h–m**) and carbocyclic derivatives (compounds **5n–r**). In general, these aliphatic analogs were equipotent to the aromatic analogs against 1b polymerase, but several compounds **5d**, **5f**, **5n**, and **5p** were significantly more active against the 1a genotype. This suggests that the 1a enzyme is more discriminating than 1b polymerase toward binding quinolone benzothiadiazines in this pocket. The branched aliphatic analogs were considerably less active against both 1b and 1a, compared to the methylene-linked alkyls. The carbo-

Table 3. Biochemical potency of benzylic and heterocyclic derivatives **4**

Compd	R	R^1	1b IC_{50}, μM	1a IC_{50}, μM
4a	Ph	H	0.129	5.09
4b	2-BrPh	H	0.469	6.72
4c	3-BrPh	H	0.075	1.04
4d	4-BrPh	H	4.69	10.36
4e	2-MePh	H	0.481	6.12
4f	3-MePh	H	0.212	8.21
4g	4-MePh	H	>5	>5
4h	3-Pyridine	H	0.221	>5
4i	2-Thiophene	H	0.125	2.33
4j	2-Thiazole	H	0.674	35.8
4k	2-Furan	H	0.305	3.26
4l	3-Furan	H	0.133	1.55
4m	2-Thiophene-3-Me	H	1.19	15.0
4n	2-Thiophene-5-Cl	H	1.45	4.78

Table 4. Biochemical potency of aliphatic derivatives **5**

Compd	R	R^1	1b IC_{50}, μM	1a IC_{50}, μM
5a	Pr	H	0.161	0.951
5b	Bu	H	0.100	0.928
5c	*i*-Bu	H	0.134	0.629
5d	Neopentyl	H	0.135	0.411
5e	*i*Pr	H	0.101	0.941
5f	Cyclopropyl	H	0.145	0.285
5g	Cyclohexyl	H	0.150	2.528
5h	Me	Me	0.640	6.35
5i	Et	Et	0.120	2.46
5j	Et	Pr	0.386	1.89
5k	Pr	Pr	1.80	2.24
5l	Pr	*i*-Pr	1.13	6.40
5m	Me	Ph	1.49	16.5
5n	Cyclobutyl		0.108	0.278
5o	Cyclopentyl		0.150	0.747
5p	Cyclohexyl		0.060	0.356
5q	Cycloheptyl		0.217	1.04
5r	4-Pyranyl		0.439	4.63

cyclic derivatives **5n–r** displayed good potency against both 1a and 1b polymerases. However, the introduction of an oxygen heteroatom into the carbocyclic ring **5r** produced a marked decrease in activity, further illustrating the hydrophobic nature of this portion of the binding pocket.

Several compounds were evaluated in a cell-based HCV replicon system[18,19] against genotype 1b (Table 5). Alkyl derivatives **5a**, **5e**, **5f**, and **5p** exhibited EC_{50} values in the 1–4 μM range, a 10–20-fold decrease from their respective biochemical potencies. The benzyl derivative **4a** lost over a hundred fold in potency having an EC_{50} of 15.7 μM. The cyclobutyl analog **5n** was equipotent in the biochemical and replicon assay suggesting this compound has superior membrane permeability than the other analogs that were tested.

In conclusion, we have determined that a nitrogen-for-carbon replacement at the N-1 position of 4-hydroxyquinolin-3-yl benzothiadiazines yields potent inhibitors of genotype 1 HCV polymerase. The optimal functional groups at N-1 are small, branched aliphatics and carbocyclics. The structure–activity relationship data imply that preferred N-1 substituents extend in a linear fashion away from the quinolone core (e.g., through a CH_2 or NH link) and bend into a sterically restrictive, highly lipophilic environment that is less discriminating in strain 1b than in strain 1a.

Table 5. 1b replicon potency of selected *N*-1-alkylamino derivatives

Compd	R	R^1	1b EC_{50}, μM
4a	Ph	H	15.7
5a	Pr	H	3.48
5e	*i*Pr	H	1.35
5f	Cyclopropyl	H	1.05
5n	Cyclobutyl		0.103
5p	Cyclohexyl		1.27

Detailed biological protocols for biochemical IC_{50} determinations and replicon assay EC_{50} determinations are available in the Supplementary data.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2005.01.071.

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