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Inhibitors of HCV NS5B polymerase: Synthesis and structure–activity relationships of *N*-alkyl-4-hydroxyquinolon-3-yl-benzothiadiazine sulfamides

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Abstract—Substituted *N*-alkyl-4-hydroxyquinolon-3-yl-benzothiadiazine sulfamides were investigated as inhibitors of genotype 1 HCV polymerase. Structure–activity relationship patterns for this class of compounds are discussed.

Hepatitis C virus (HCV) is a common pathogen that can lead to cirrhosis, hepatocellular carcinoma (HCC), and liver failure. It is estimated that 170 million people were infected worldwide by the year 2000, and that the virus is responsible for at least 10,000 deaths annually in the United States alone.[1] HCV has six major genotype classes, with genotypes 1 and 2 being most prevalent in the United States, Europe, and Japan.[2] Currently combination drug treatment of genotype 2 or 3 is more successful than treatment of genotype 1 infection.[3,4] Moreover, existing therapies are hampered by drug-related toxicities. Therefore, there is a particular need for new therapies directed toward genotype 1 HCV infection. Our group has been pursuing inhibition of the HCV NS5B RNA-dependent RNA polymerase (RdRp) enzyme by hydroxyquinolon-3-yl-benzothiadiazines.[5] Other groups have reported nucleoside as well as other non-nucleoside inhibitors of this viral enzyme.[6]

We have recently shown that the addition of a methyl sulfonamide group to the 7-position of the D-ring of the benzothiadiazine core (compound **1**, IC_{50} = 2.4 nM) dramatically improved the potency of the series.[7] In an effort to further probe the thiadiazine binding site and possibly obtain even greater potency against the RdRp, we sought to extend more functionality away from the methyl sulfonamide. An attractive strategy for accomplishing this endeavor was to synthesize a number of diverse thiadiazine sulfamide derivatives (Fig. 1).

A variety of sulfamide carbamates were synthesized according to the protocol shown in Scheme 1. Chloro-

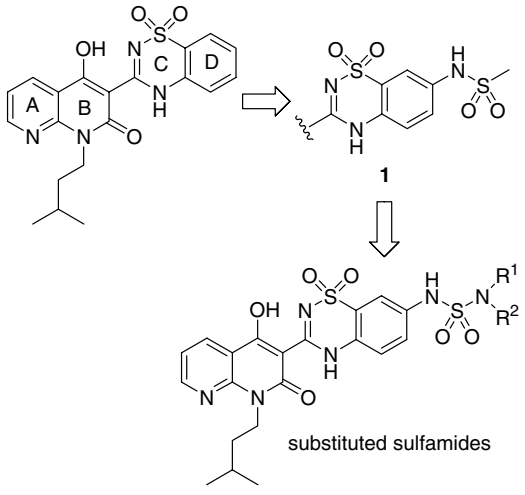

Figure 1. Substituted sulfamide analog strategy.

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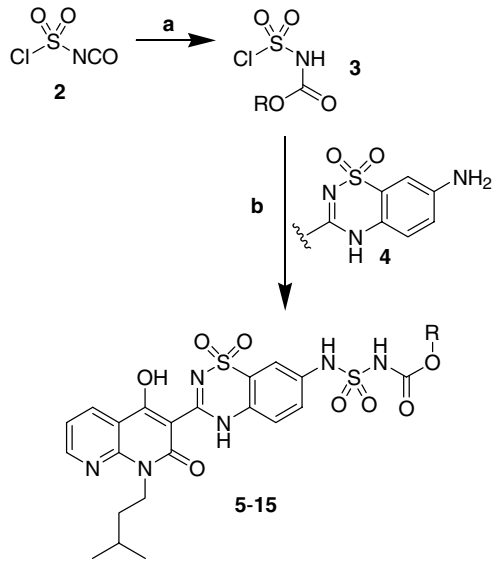


Scheme 1. Reagents and conditions: (a) primary alcohol, CH_2Cl_2, 1 h, 100%; (b) compound **4**, Et_3N, CH_2Cl_2, 22 h, rt, 40–80%.

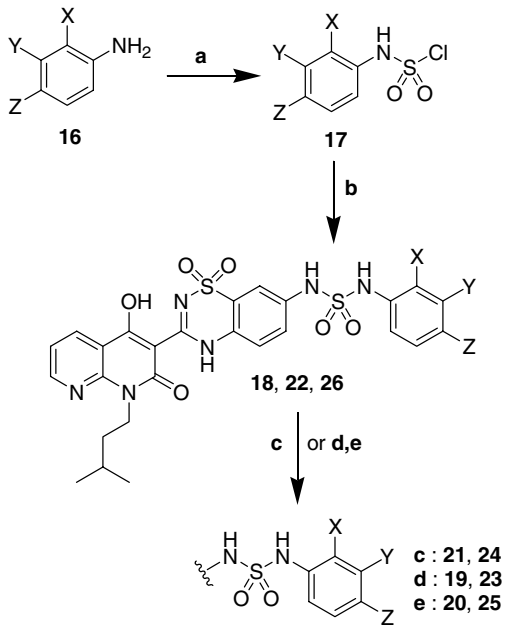
sulfonyl isocyanate was treated with a wide variety of primary alcohols to provide the requisite intermediate sulfonyl chlorides (**3**), which were allowed to react with thiadiazine aniline **4**[7] to provide the desired sulfamide carbamate analogs **5**–**15**. The biochemical activities of these compounds measured against the genotype 1a HCV polymerase are shown in Table 1.[8] For the most part the analogs displayed similar potencies regardless of the hydrophobic or hydrophilic functionality appended to the sulfamide group. The primary amine **11** was the most potent analog in this initial series (IC_{50} = 21 nM).

A series of phenyl-substituted sulfamides were also constructed according to the conditions shown in Scheme 2. Substituted anilines (**16**) were treated sequentially with chlorosulfonic acid and phosphorus pentachloride to provide phenyl-substituted chlorosulfonamides (**17**). Subsequent reaction with thiadiazine aniline **4** provided the desired ester analogs **18**, **22**, and **26**. These

Table 1. Biochemical potency of sulfamide carbamate derivatives **5**–**15**

Compound	R	1a IC_{50}[a] (nM)
5	CH_3	63
6	CH_2CH_2Cl	189
7	CH_2CHCH_2	88
8	CH_2CCH	72
9	CH_2CH_2CN	35
10	CH_2Ph	58
11[b]	$CH_2CH_2NH_2$	21
12	$CH_2CO_2CH_2CH_3$	61
13	$CH_2CH_2OCH_3$	87
14	$CH_2CH_2OCH_2Ph$	96
15[c]	CH_2CO_2H	50

[a] IC_{50} values in all tables are means of at least two independent determinations, standard deviation ± 10%. Detailed protocols can be found in Supplementary data.
[b] Prepared from the cleavage of the Boc-protected primary amine.
[c] Prepared from the hydrolysis of compound **12**.



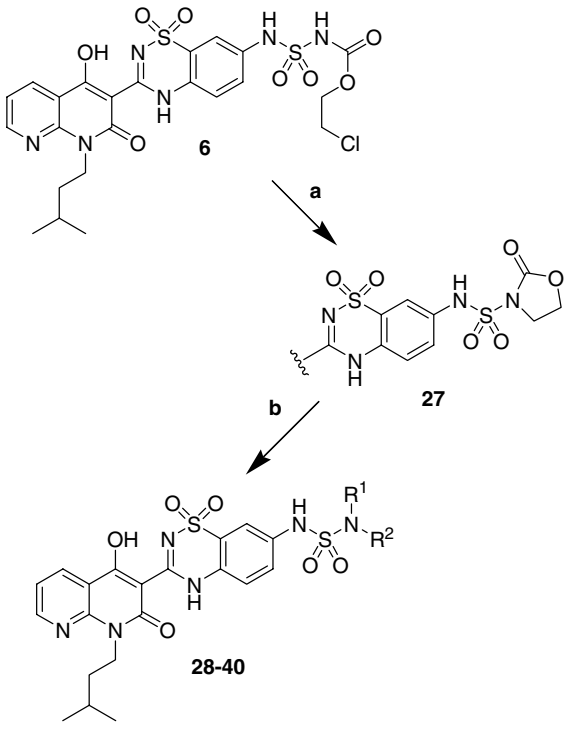
Scheme 2. Reagents and conditions: (a) chlorosulfonic acid, CH_2Cl_2, 0 °C, then rt, 1 h, then PCl_5, reflux, 3.5 h; (b) compound **4**, Et_3N, rt, 3 h, 5–48%; (c) NH_4OH, rt, 72 h, 100%; (d) LiOH, THF, H_2O, rt, 20 h, 90–95%; (e) HOBT, EDAC, DMF, rt, 18 h, 64–97%.

compounds were converted to the corresponding primary amides and substituted amide analogs. The *para*-substituted analogs (**18**–**21**) as a class were generally more potent than the corresponding *ortho*- (**26**) and *meta*- (**22**–**25**) substituted analogs, as shown in Table 2. However, there were few discernable structure–activity relationships among the *para*-substituted analogs **18**–**21**, and all displayed roughly equivalent potencies.

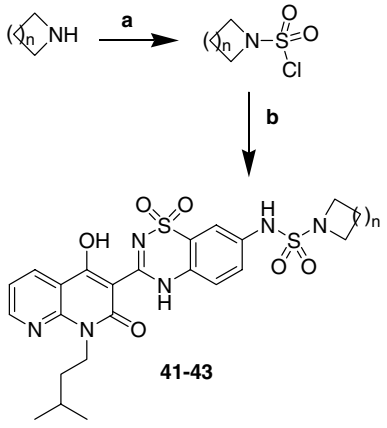
Several other substituted benzothiadiazine sulfamides (compounds **28**–**40**) were prepared by primary amine displacement of the oxazolidinone ring from compound **2** as shown in Scheme 3.[9] This method was less efficient for cyclic secondary amines, thus sulfamides **41**–**43** were prepared by reaction of the corresponding sulfamyl chlorides with thiadiazine aniline **4** (Scheme 4). Most of these sulfamide analogs (Table 3) demonstrated similar potencies when compared to the sulfamide carbamates (Table 1) and phenyl sulfamides (Table 2). The exception was benzyl derivative **29**, with an IC_{50} of 5.5 nM.

Table 2. Biochemical potency of phenyl sulfamide derivatives **18**–**26**

Compound	X	Y	Z	1a IC_{50} (nM)
18	H	H	CO_2Me	48
19	H	H	CO_2H	20
20	H	H	CONHMe	43
21	H	H	$CONH_2$	18
22	H	CO_2Et	H	132
23	H	CO_2H	H	115
24	H	$CONH_2$	H	43
25	H	$CONHCH_2CONH_2$	H	43
26	CO_2Me	H	H	129



Scheme 3. Reagents and conditions: (a) excess Et_3N, CH_2Cl_2, rt, 6 h, 82%; (b) primary amines, CH_2Cl_2, CH_3CN, 70 °C, 24 h, 25–50%.



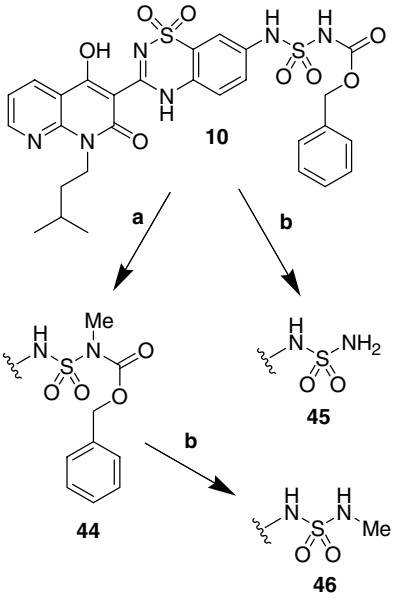
Scheme 4. Reagents and conditions: (a) sulfuryl chloride, CH_2Cl_2, −20 °C, then 0 °C for 2 h, 20–35%; (b) compound **4**, Et_3N, CH_2Cl_2, rt, 18 h, 25–45%.

Simple unencumbered sulfamides **45** and **46** were prepared from the benzyl carbamate **10** (Scheme 5). These two analogs were nearly as potent as the methyl sulfonamide (**1**) against genotype 1a HCV polymerase (Table 4). In addition, two unencumbered sulfamides from a different thiadiazine series[5] were prepared and tested (Scheme 6).

In general, the SAR in Tables 1–4 suggest that larger sulfamide derivatives do not offer any extra binding interactions with the polymerase binding site over the unsubstituted sulfamide **45**. Rather, it is likely that the sulfamide substituents in many cases either project into solvent or undergo hydrophobic collapse to produce flat SAR trends.

Table 3. Biochemical potency of sulfamide derivatives **28–43**

Compound	R^1	R^2	1a IC_{50} (nM)
28	H	CH_2CH_2Ph	18
29	H	CH_2Ph	5.5
30	H	Ph	41
31	H	CH_2CH_2OH	107
32	H	Cyclohexane	67
33	H	Cyclopentane	39
34	H	$CH_2CH_2NH_2$	10
35	H	4-Piperidine	32
36	H	$CH_2CH_2CONH_2$	20
37	H	CH_2(4-OMe–Ph)	10
38	H	CH_2(3-OMe–Ph)	15
39	H	CH_2(2-OMe–Ph)	57
40	H	CH_2(3-NO_2–Ph)	13
41		Piperidine	51
42		Pyrrolidine	27
43		Azetidine	24

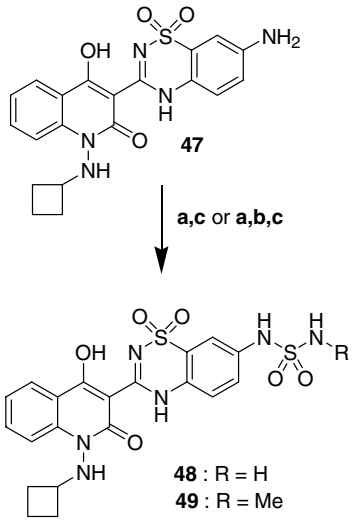


Scheme 5. Reagents and conditions: (a) TMS–diazomethane, THF, rt, 16 h, 15%; (b) MeOH, 10% Pd/C, H_2 gas, 1 atm, rt, 3 h, 70–80%.

Table 4. Biochemical and cell culture potency of selected compounds

Compound	IC_{50} (nM)		1b EC_{50}[a,b] (nM)	1b EC_{50}[a,c] (nM) (40% serum)
	1a	1b		
1	2.4	6.0	3.0	1310
9	35	6.7	98	—
28	18	37	1266	—
29	5.5	44	398	—
41	51	45	940	—
44	121	—	—	—
45	9.0	2.8	17	—
46	14	—	—	—
48	5.2	0.4	3.0	81
49	8.7	—	11	420

[a] EC_{50} values in this table are means of at least two independent determinations, standard deviation ± 10%. Detailed protocols can be found in Supplementary data.
[b] Assay run with 5% fetal calf serum.
[c] Assay run with 40% human serum.



Scheme 6. Reagents and conditions: (a) chlorosulfonylisocyanate, benzyl alcohol, CH_2Cl_2, 1 h, then compound **47**, CH_2Cl_2, Et_3N, 2 h, 66%; (b) TMS–diazomethane, THF, MeOH, 70 °C, 16 h, 98%; (c) MeOH, THF, 10% Pd/C, H_2 gas, 1 atm, rt, 24 h, 17–83%.

The biochemical activities of several analogs were measured against the genotype 1b HCV polymerase as shown in Table 4. The analogs displayed similar activities against the 1a and 1b virus strains. Many of these analogs were also tested in a replicon cell culture assay based on the 1b virus genotype (Table 4).[10] Consistent with the biochemical results, larger substituted sulfamide analogs displayed weaker activities. The primary sulfamide (**48**) and methyl sulfamide (**49**) derivatives potently inhibited HCV replication, with EC_{50} values in the single digit nanomolar range. In addition, the activity of compound **48** was attenuated to a lesser extent than that of compound **1** (81 vs 1310 nm) when the cell culture assay was run using 40% human serum versus 5% fetal calf serum.

In summary, we synthesized a number of substituted benzothiadiazine sulfamide analogs and assessed their inhibitory potency against genotype 1 HCV polymerase. Overall we observed that the simple methyl and unsubstituted benzothiadiazine sulfamides displayed the most potent biochemical and cell culture activities.

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

Supplementary data

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.bmcl.2006.04.015.

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