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Des-A-ring benzothiadiazines: Inhibitors of HCV genotype 1 NS5B RNA-dependent RNA polymerase

Pamela L. Donner,* Qinghua Xie, John K. Pratt, Clarence J. Maring, Warren Kati, Wen Jiang, Yaya Liu, Gennadiy Koev, Sherie Masse, Debra Montgomery, Akhter Molla and Dale J. Kempf

Antiviral Research, Global Pharmaceutical Research, and Development, Abbott Laboratories, Abbott Park, IL 60064, USA

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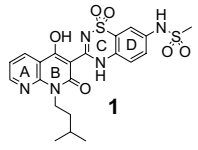
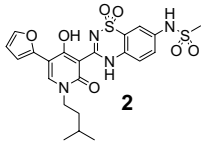
Abstract—In our program to discover non-nucleoside, small molecule inhibitors of genotype 1 HCV polymerase, we investigated a series of promising analogs based on a benzothiadiazine screening hit that contains an ABCD ring system. After demonstrating that a methylsulfonylamino D-ring substituent increased the enzyme potency into the low nanomolar range, we explored a minimum core required for activity by truncating to a three-ring system. Described herein are the syntheses and structure–activity relationship of a set of inhibitors lacking the A-ring of an ABCD ring system. We observed that small aromatic rings and alkenyl groups appended to the 5-position of the B-ring were optimal, resulting in inhibitors with low nanomolar potencies.

Hepatitis C virus infects over 3% of the world population.[1] HCV infection is a leading cause of chronic liver disease and the leading cause of death from liver disease in the United States.[2] Despite the fact that new infections are decreasing due to better blood screening and education in the United States and Europe, the death rate from HCV is expected to continue to rise through 2015.[3] Hepatitis C virus (HCV) is a (+)-strand RNA virus of the *Flaviviridae* family and was first identified in 1989.[4] HCV has six major genotype classes,[5] with genotypes 1 and 2 being the most prevalent in the US, Europe and Japan. The goal in treating patients infected with HCV is eradication of the infection. The current standard of care for treating HCV is a combination therapy of pegylated interferon-α (PEG-IFN-)/ribavirin. Sustained virologic response (SVR) rates for HCV patients having genotypes 2 or 3 approach 80%. However, patients infected with genotype 1 HCV do not respond well to this combination therapy, demonstrating SVR rates of <50% even after prolonged treatment.

Additionally, nearly 3 out of 4 people treated with PEG-IFN-/ribavirin experience systemic side effects including neutropenia, depression, irritability, headaches, nausea and anemia.[6] These factors result in a therapeutic need for new HCV therapies, particularly those directed at genotype 1 HCV.

Due to its necessary role in viral replication, NS5B RNA-dependent RNA polymerase (RdRp) has been one of the most studied viral protein targets for small molecule HCV therapy.





	1	2
1a IC_{50}	2 nM	8 nM
1b IC_{50}	7 nM	23 nM
1a Replicon EC_{50}	16 nM	14 nM
1b Replicon EC_{50}	2 nM	3 nM

Figure 1. A comparison of enzyme potencies of the 4-ring benzothiadiazine system and the truncated 3-ring core.

Keywords: HCV polymerase; Benzothiadiazines; HCV genotype 1; Antivirals.
* Corresponding author. Tel.: +1 847 937 3245; fax: +1 847 938 2756; e-mail: Pamela.l.donner@abbott.com

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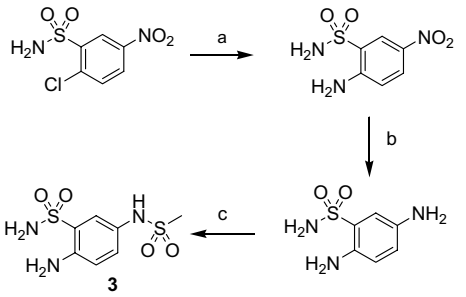
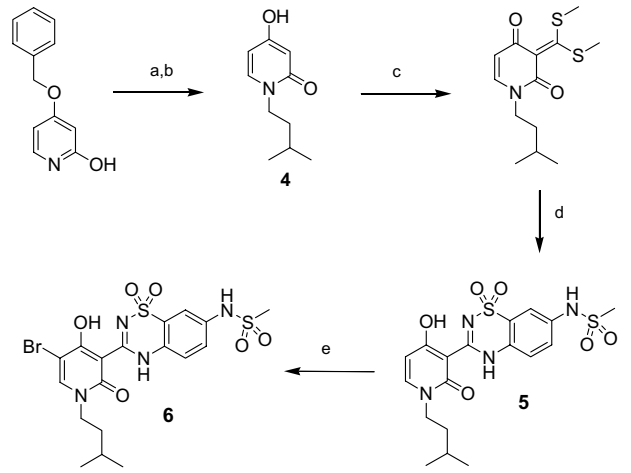
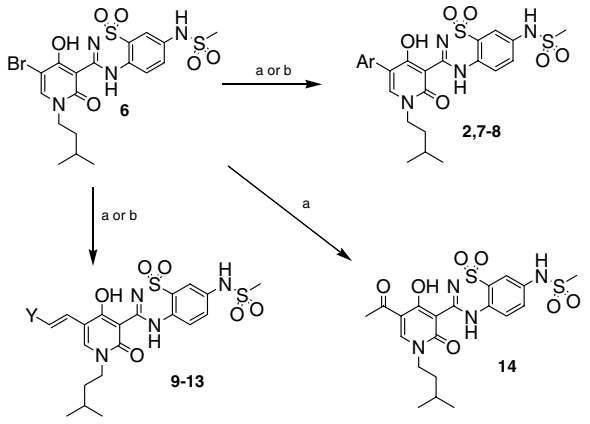


Scheme 1. Reagents and conditions: (a) $(NH_4)_2CO_3$, $CuSO_4$, NH_4OH, 120 °C, 4 h, sealed tube; (b) $Na_2S_2O_4$, 1 N NaOH; (c) CH_3SO_2Cl, pyridine, rt, 24 h.



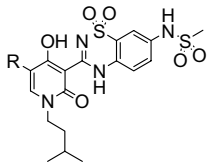
Scheme 2. Reagents and conditions: (a) NaH, benzyl bromide, DMF, rt, 20 h; (b) HCO_2NH_4, PdOH/C, MeOH/THF, 70 °C 2–8 h; (c) $(MeS)_3C^+MeSO_4^-$, pyridine, dioxane, 90 °C 1 h; (d) **13**, dioxane, 90 °C 1 h; (e) *N*-bromohydantoin, THF, rt.

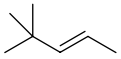
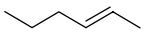
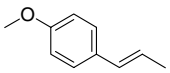
We have investigated *N*-1-alkyl-4-hydroxyquinolon-3-yl benzothiadiazines as inhibitors of HCV NS5B polymerase.[7] This class of compounds binds near the active site of the enzyme in the palm domain.[8] Optimization of this series led to an analog, **1**, containing a methylsulfonylamino D-ring substituent that increased enzyme potency into the low nanomolar range.[9]



Scheme 3. Reagents and conditions: (a) cat. $Pd(PPh_3)_2Cl_2$, appropriate tributyltin reagent, THF, 75 °C, 20 h; (b) cat. $Pd(PPh_3)_2Cl_2$, appropriate boronic acid reagent, 4:1.5:1 DME/H_2O/EtOH, potassium carbonate, 110 °C, 25 min, microwave.

Table 1. Biochemical potencies



Compound	R	1a IC_{50} (nM)	1b IC_{50} (nM)
5	H	274	231
6	Br	55	150
7	Phenyl	425	334
2	2-Furyl	8	23
8	2-Thiophene	11	36
9		24	95
10		6	35
11		406	483
12		67	105
13	 acetyl	480	12622
14	Acetyl	280	799
1	See Figure 1	2	7

We then investigated the possibility of truncating this four-ring system to the minimum core required for activity. Our models and published X-ray crystal structures[11] indicated that the A-ring of benzothiadiazine compounds fit snugly into a small hydrophobic surface of the binding site. When the A-ring was eliminated, the potency fell off considerably. Substitutions from the

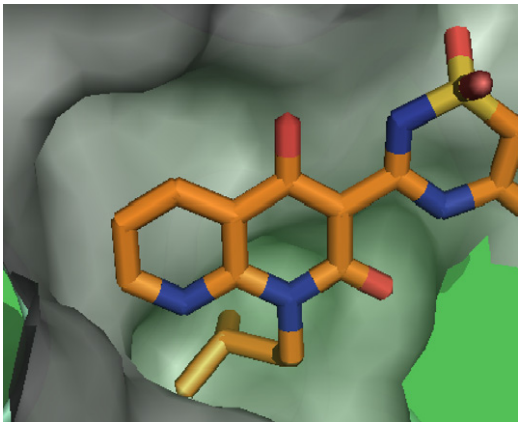


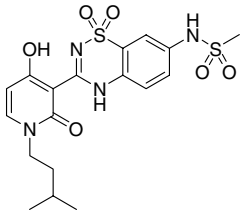
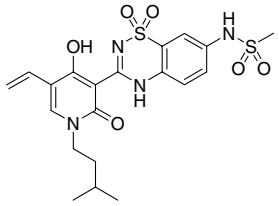
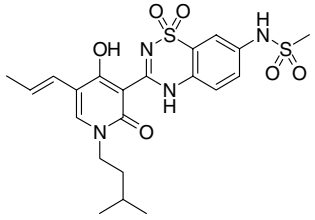
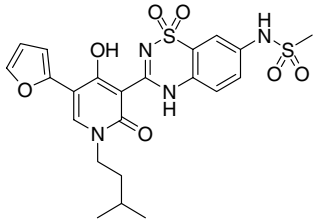
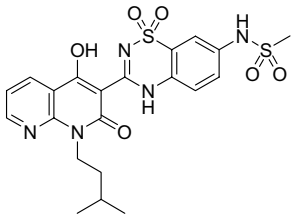
Figure 2. A/B ring potion of benzothiadiazine 1 modeled into the binding site of the enzyme based on reported binding orientation of analog.[11]

B-ring into the space previously occupied by the A-ring produced analogs that maintained nanomolar enzyme inhibitory potency in both isolated enzyme and cell culture systems (HCV subgenomic replicon). Figure 1 shows one of the most potent des-A-ring analogs **2** with an IC_{50} value of less than 10 nM against genotype 1a.

Chemistry used to prepare the D-ring is shown in Scheme 1. 2-Chloro-5-nitrobenzenesulfonamide was converted to the amino benzenesulfonamide. Reduction of the nitro moiety followed by a chemoselective sulfonylation provided benzothiadiazine ring precursor **3**. The B-ring portion of the molecule was constructed, as shown in Scheme 2. 4-Benzyloxy-pyridin-2-ol was alkylated with 1-bromo-3-methyl butane, followed by deprotection to give compound **4**. Construction of the benzothiadiazine ring system was achieved by converting **4** to the dithioketene acetal[12] followed by reaction with **3** to provide **5**. Bromination of **5** produced **6**, which could be coupled with a variety of boronic acids and tin reagents using Suzuki or Stille conditions as shown in Scheme 3 to provide analogs **2**, **7**–**14**.

Table 1 shows the activities of these inhibitors in the HCV polymerase enzymatic assays. The unsubstituted analog, where R = H, is 100-fold less active compared

Table 2. Replicon potencies in 40% human serum

Compound		EC_{50} 1b replicon 5% FCS (nM)	EC_{50} 1b replicon 40% HS (nM)	Fold Incr.
5		163	911	5.6
9		8.8	62.5	7.1
10		5.6	38.2	6.8
2		2.5	59.9	24
1		2	736	368

IC_{50} and EC_{50} values are means of at least two independent determinations, standard deviation ±10%. HCV 1b replicon EC_{50} values are measurements of RNA production by real-time RT-PCR. HCV 1a replicon EC_{50} values are measurements using a SEAP reporter gene construct.[7,10]

to the original four-ring system, **1**. Presumably, this is due to the loss of a hydrophobic contact in the binding site of the enzyme that the A-ring provides in the 4-ring system. Figure 2 shows this model of the A/B ring region of the benzothiadiazine, **1**, demonstating a tight hydrophobic fit with the surface of the enzyme in this portion of the molecule. As hydrophobicity in that region is added back to the molecule, potency increases. Analogs with vinyl, methylvinyl, thiophene, and furyl substituents all have IC_{50} values less than 25 nM against genotype 1a. The remaining examples, with larger substituents, show poorer activity. The size of the hydrophobic binding site appears to be limited, and is demonstrated by the fact that the thiophene and furyl 5-membered ring aromatics are in the 10 nM range, but the analog with a phenyl substituent is 40-fold less active. Although all of these inhibitors are slightly less active against the genotype 1b enzyme, the trend is similar (see Table 2).

We also determined the potency in the replicon assays for these inhibitors. One of the major drawbacks of the tetracyclic benzothiadiazine system is a high degree of protein binding. This can be illustrated by comparing the fold difference in activity in 0% versus 40% human serum. In Table 2, the difference between the replicon potency in the presence of 5% FCS with no human serum and the replicon potency in the presence of 40% human serum is shown for a representative set of inhibitors in this class. We observed that the truncated des-A-ring series exhibited a lower protein-binding effect. Thus, the potencies of these Des-A analogs are typically attenuated by 6- to 24-fold compared to 60- to 360-fold for the benzothiadiazine four-ring system. Notably, analogs **2**, **9**, and **10** are 10- to 20-fold more active in 1b replicon than the tetracyclic analog, **1**, in the presence of human serum.

In our investigation of a set of HCV polymerase inhibitors with a benzothiadiazine core, we have discovered that a three-ring system with small substituents attached to the 5-position of the B-ring can, indeed, retain much of the antiviral potency of the original 4-ring system, in both the biochemical and replicon assays, as our models predicted. This new class of inhibitors can be readily synthesized from a commercially available 4-benzyloxy-pyridin-2-ol using known chemistry via a dithioketene acetal intermediate followed by coupling with tin or boronic acid reagents to provide the desired products. Importantly, these inhibitors are less protein with relatively high potencies in the HCV replicon in the presence of human serum, and therefore address one of the key liabilities associated with the benzothiadiazine class with a 4-ring system of HCV polymerase inhibitors.

Acknowledgment

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