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Tetrahydrobenzothiophene inhibitors of hepatitis C virus NS5B polymerase

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Abstract—A novel series of selective HCV NS5B RNA dependent RNA polymerase inhibitors has been disclosed. These compounds contain an appropriately substituted tetrahydrobenzothiophene scaffold. This communication will detail the SAR and activities of this series.

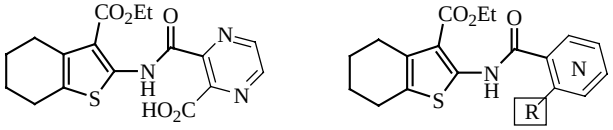
Hepatitis C virus (HCV) is a serious disease which often leads to chronic hepatitis, cirrhosis, and hepatocellular carcinoma.[1] These complications are responsible for about 10,000–12,000 deaths/year in the US.[1] The necessity for liver transplantation in the US is most often the result of chronic HCV infection.[1b] Recent worldwide estimates indicate that approximately 1–3% of the total population may be infected by HCV.[2]

Current therapies for HCV involve a combination of ribavirin and interferon-α.[3] Not only does this treatment regimen cause unfavorable side effects, but also only about 40% of patients achieve a sustained medical benefit. Recently, a pegylated interferon has been approved for treatment which appears to slightly improve the patient response rate.[3] Clearly there is a medical need for additional HCV antiviral agents.

Hepatitis C belongs to the *Flaviviridae* family of positive-single-stranded RNA viruses. The HCV genome encodes a 3000 amino acid polyprotein which is processed into structural and non-structural proteins.[4] One of the non-structural proteins that is essential for viral replication is the NS5B RNA dependent RNA polymerase.[5] This communication will center on a novel series of NS5B enzyme inhibitors discovered in our laboratories.[6,7]

During the course of our initial studies on HCV polymerase, we discovered a tetrahydrobenzothiophene (THBT) pyrazine carboxylate analog (**1**) from high throughput screening of compound libraries that exhibited low micromolar inhibition of HCV NS5B 4a full-length polymerase and was selective versus human DNA polymerase and calf thymus polymerase α.[8] A library of structurally related compounds was assembled which confirmed: (a) a preference for the bicyclic THBT scaffold over analogous non-cyclic 4,5-dialkyl- substituted thiophenes; and (b) the preference for an acidic functionality at the position proximal to the scaffold attachment point (**A**, Fig. 1).[9]

The core substituted thiophene scaffolds were assembled via chemistry first described by Gewald (Scheme 1).[10]



1

HCV 4a Pol 4 uM
CT Pol >100 uM

A

CO_2H favored
(vs. H, alkyl, etc.)

Figure 1. Tetrahydrobenzothiophene analogs and enzyme inhibition.

Keywords: HCV polymerase inhibitors; Tetrahydrobenzothiophenes.
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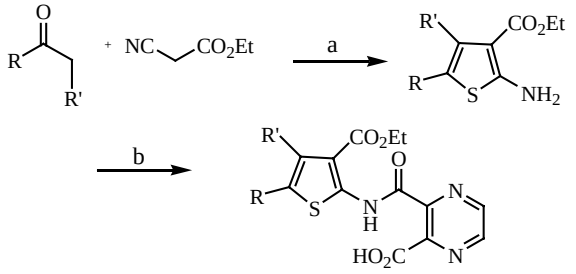
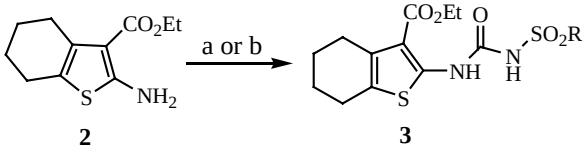


Scheme 1. Reagents and conditions: (a) S_8, morpholine, EtOH, Δ; (b) pyrazine carboxylate, DCC.



Scheme 2. Reagents: (a) $OCNSO_2R$; (b) triphosgene, then H_2NSO_2R, DIPEA.

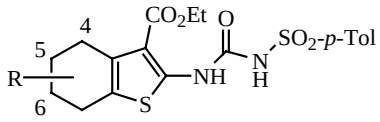
We found that when the 2-carboxyl-pyrazine group was replaced in its entirety by an alternative acidic moiety, such as an acyl sulfonamide,[11,12] the activity against HCV polymerase increased significantly while maintaining selectivity.[13] These compounds were assembled either by reaction of a preformed sulfonylisocyanate with the 2-amino-tetrahydrobenzothiophene or by conversion of this aminothiophene into the corresponding isocyanate followed by reaction with a sulfonamide (see, Scheme 2).[14,15] The structure–activity relationship within the sulfonamide subgroup was somewhat limited, although a trend emerged whereby aromatic R groups appeared to be preferred over alkyl substitution (Table 1). These compounds exhibited significantly higher IC_{50} values (>10 μM) when tested against the HCV polymerase Δ-21 BB7 isolate.[16,17]

During the course of exploring the impact of substitution on the THBT scaffold, we determined that alkyl substitution (particularly disubstitution) at the 6-position was favorable (Table 2). The 6-dimethyl-analog (**4d**) was determined to be among the most potent compounds in the series. In addition, compound **4d** showed submicromolar inhibition of the Δ-21 C-terminally truncated HCV polymerase BB7 isolate.

Table 1. SAR of sulfonamide analogs **3**

Compound	R	HCV 4a pol IC_{50} (μM)
3a	Phenyl	0.7
3b	4-Tolyl	1.0
3c	3-Tolyl	0.9
3d	2-Tolyl	0.9
3e	4-Fluorophenyl	1.7
3f	4-Chlorophenyl	1.0
3g	3-Methoxyphenyl	1.2
3h	4-Nitrophenyl	2.8
3i	4-Chlorothiophene	0.6
3j	1-Thiophene	0.6
3k	1-Naphthyl	0.7
3l	Me	3.8
3m	Bu	4.9

Table 2. Alkyl substituted derivatives of **4**



4

Compound	R	HCV 4a pol IC_{50} (μM)
4a	4-Me	2.0
4b	5-Me	0.5
4c	6-Me	0.2
4d[a]	6-Dimethyl	0.09

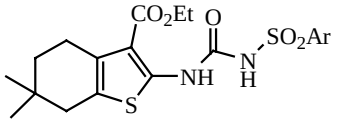
[a] IC_{50}, 0.4 μM against HCV Δ-21 BB7 isolate.

A subseries of analogs from the dimethyl-substituted THBT template was assembled (Table 3). All of these compounds inhibited the HCV 4a full-length and the BB7 Δ-21 polymerases at submicromolar levels.

Unfortunately, despite their potent HCV NS5B polymerase inhibitory activities and high permeabilities (>30 nM/s) in the Caco-2 assays,[18] these compounds were poorly active (EC_{50}s > 30 μM) in the subgenomic HCV replicon cell assay.[4a,19] Since sulfonyl ureas have historically exhibited high plasma protein binding, we speculated that this proclivity may be one of the contributing factors for the weak cellular activity.

In an attempt to modulate plasma protein binding by changing the physicochemical properties ($c\log P$, etc.) of the inhibitors, we focused our efforts on the SAR-tolerant SO_2R functionality. Specifically, we prepared a small sublibrary of more than 15 sulfamylureas in an attempt to decrease the protein binding.[20] These amin-

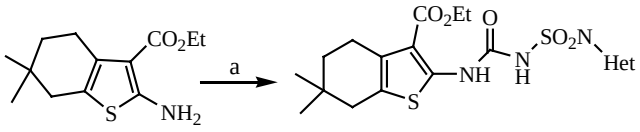
Table 3. Modification of the aryl group of **5**



5

Compound	Ar	HCV 4a pol IC_{50} (μM)	HCV BB7 pol IC_{50} (μM)
4d	4-Tolyl	0.09	0.4
5a	Phenyl	0.09	0.2
5b	4-Fluorophenyl	0.06	0.5
5c	2-Trifluoromethylphenyl	0.05	0.2
5d	2-Trifluoromethoxyphenyl	0.15	0.4
5e	4-Cyanophenyl	0.09	0.4
5f	2-Pyridyl	0.04	0.15
5g	5-Methyl-2-pyridyl	0.05	0.2
5h	2-Pyrimidine	0.05	0.3
5i	1-Methyl-5-pyrazole	0.05	0.26
5j	1-Methyl-2-imidazole	0.08	0.15

Table 4. Sulfamylurea derivatives **7** and enzyme inhibition



6 **7**

Compound	Heterocycle	HCV 4a pol IC_{50} (μM)	HCV BB7 pol IC_{50} (μM)
7a	Morpholine	0.3	2.5
7b	Piperidine	0.2	1
7c	Homopiperidine	0.5	1.1
7d	*N*-Methylpiperazine	0.4	2
7e	*N*-Acetylpiperazine	0.3	0.6
7f	Pyrrolidine	0.2	0.4
7g	Tetrahydroisoquinoline	0.1	0.6
7h	Indoline	0.2	0.26

(a) Chlorosulfonylisocyanate, Et_2O then HNRR′, THF, TEA.

osulfonylurea compounds were prepared from the pivotal 2-amino-tetrahydrobenzothiophene with chlorosulfonylisocyanate followed by treatment with a variety of amines.[21] As indicated for a representative subset in Table 4, these compounds were submicromolar inhibitors of HCV 4a polymerase but were less active against the BB7 Δ-21 polymerase isolate. Unfortunately, these compounds still exhibited high plasma protein binding and poor activities in the replicon cell assay.[22]

In conclusion, we have identified a novel series of tetrahydrobenzothiophene compounds that are selective HCV polymerase inhibitors. In vitro activity for the 6,6-disubstituted analogs was particularly strong. Attempts to access corresponding cellular activity in both the sulfonyl- and aminosulfonyl-urea subseries were unsuccessful.

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15. General procedure A: a solution of *N*-sulfonyl ethylcarbamate (0.7–7 mmol) in dry toluene (6–20 mL) was treated with TEA (1–2 equiv) followed by chlorodimethylsilane (1–2 equiv) at room temperature. After 16 h, a solution of the 2-amino-tetrahydrobenzothiophene (1 equiv) in toluene (10 mL) was added dropwise. After 1–4 h, the solution was diluted with 1 M HCl and extracted with EtOAc. The combined organics were dried with Na_2SO_4, filtered and concentrated. The products were purified by either crystallization from a suitable solvent or by flash chromatography. General procedure B: a solution of amine (3–6 mmol) in dry dichloroethane (20 mL) was treated with triphosgene (1.2 equiv) in dichloroethane (20 mL). After 2–3 h, DMAP (0.5 equiv) was added followed by dropwise addition of triethylamine (5–6 equiv). The mixture was concentrated and diluted with diethyl ether. The mixture was filtered through Celite and the filtrate was concentrated. The isocyanate was used without further purification. To a solution of isocyanate (3–6 mmol) in dry THF (20 mL) was added sulfonamide (1 equiv) in THF (20 mL) with DIPEA (1 equiv) at room temperature. After 16 h, the solution was diluted with brine and extracted with EtOAc. The combined organics were dried with Na_2SO_4, filtered and concentrated. The crude products were purified by either crystallization from suitable solvents or by flash chromatography to give the sulfonylureas (20–60%).
16. The IC_{50}'s are an average of multiple experiments with the standard deviations generally within 10% of this value.
17. RNA dependent RNA polymerase assay. Plasmid containing full-length BB7 NS5B gene was licensed from APATH, LLC (St. Louis, MO). The HCV NS5B region was amplified by PCR from BB7 plasmid DNA containing HCV genotype 1b (BB7), cloned expressed, and purified from *Escherichia coli*. The RNA dependent RNA polymerase (RdRp) assay was performed in a final volume of 50 μl per reaction. Twenty microliters of the NS5B enzyme mix containing 24 nM NS5BdCT21-His, 20 mM HEPES (pH 7.5), 5 mM $MgCl_2$, 1 mM DTT, 0.05 mg/ml BSA, 0.5 μM UTP, 1 μM ATP, 0.08 μM CTP, and 0.025 μM GTP (all in final concentrations) was incubated in the presence of test compounds at varying concentrations

(3 nM–30 μM) or EDTA dissolved in DMSO (10 μl) for 15 min at room temperature. Concentrations of RNA and NTPs were kept at apparent K_m levels. The final concentration of DMSO present in the reaction was 3%. The reaction was initiated by adding 3 nM pOF transcribed RNA substrate, 0.4 U/μl RNasin, and 0.125 μCi [α-^{33}P]GTP (indicated are final concentrations in the 50 μl reaction mix). After 120 min at room temperature, the amount of RNA synthesized was quantified by collecting the radiolabeled product RNA on Millipore multiscreen membrane filter plates. The filters containing the reaction products were allowed to dry at room temperature and counted in a Wallac MicroBeta after an addition of 50 μl of Optiphase™ scintillant. Inhibition data were analyzed using the sigmoidal dose–response (variable slope) equation in GraphPad Prism (GraphPad Software Inc., San Diego CA). The 50% inhibitory concentration (IC_{50}) was the drug concentration that decreased the enzyme activity by 50% relative to control samples incubated without compound.

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22. In general, these compounds displayed high cellular penetrations as determined by the Caco-2 assay.