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Discovery of Novel Allosteric HCV NS5B Inhibitors. 2. Lactam-Containing Thiophene Carboxylates

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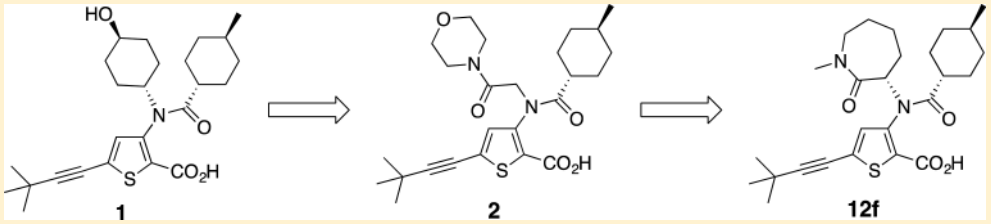
Supporting Information



ABSTRACT: Lomibuvir (**1**) is a non-nucleoside, allosteric inhibitor of the hepatitis C virus NS5B polymerase with demonstrated clinical efficacy. Further development efforts within this class of inhibitor focused on improving the antiviral activity and physicochemical and pharmacokinetic properties. Recently, we reported the development of this series, leading to compound **2**, a molecule with comparable potency and an improved physicochemical profile relative to **1**. Further exploration of the amino amide-derived side chain led to a series of lactam derivatives, inspired by the X-ray crystal structure of related thiophene carboxylate inhibitors. This series, exemplified by **12f**, provided 3−5-fold improvement in potency against HCV replication, as measured by replicon assays. The synthesis, structure−activity relationships, *in vitro* ADME characterization, and *in vivo* evaluation of this novel series are discussed.

KEYWORDS: *Lomibuvir, non-nucleoside, hepatitis C, NS5B, polymerase, antiviral activity*

Hepatitis C virus (HCV) infects an estimated 80−115 million people worldwide.[1] Chronic infection is associated with increased risk of liver disease, fibrosis and cirrhosis, and hepatocellular carcinoma.[2] In 2011, the HCV NS3 protease inhibitors boceprevir[3] and telaprevir[4] were approved as the first direct-acting antiviral agents, administered in combination with pegylated interferon α and ribavirin.[5] In the following years, more efficacious agents and combinations have been introduced, and these two protease inhibitor drugs have been superseded.[6,7] In our quest for new chemical agents needed to decrease the duration of treatment and reduce treatment side effects, we focused on developing inhibitors of the NS5B polymerase, an approach anticipated to deliver an all-oral, interferon-free treatment regimen when applied in combination with telaprevir. The viral NS5B polymerase plays an essential role in the life cycle of HCV, being responsible for viral genomic replication.[8] Inhibitors of this enzyme in clinical development and practice fall into two classes: nucleoside (or nucleotide) analogues and non-nucleoside, allosteric inhibitors.[9] Lomibuvir (**1**: also named VX-222 and VCH-222) is a selective, non-nucleoside polymerase inhibitor that binds to thumb pocket 2 of the HCV NS5B polymerase.[10] A 3-day, monotherapy viral kinetic study in which treatment-naïve patients with genotype 1 HCV infection were treated with 250, 500, or 750 mg lomibuvir twice daily, or 1500 mg once daily, showed it to be well tolerated, and patients achieved a mean HCV RNA reduction ranging from 3.1 to 3.4 $\log_{10}$.[11] To continue to develop this series, our program focused on improving physicochemical and pharmacokinetic properties. As we recently reported, replacement of the *trans*-4-hydroxycyclohexane ring in **1** with a glycine-derived amide (**2**) resulted in comparable potency and improved physicochemical properties.[12] During the course of this work, we established that although compounds such as **3b** showed higher activity than the corresponding *R*-enantiomer **3a**, the unsubstituted compound **2** was sufficiently active, and we elected to advance the unsubstituted (glycine) series. However, the question remained whether the side chain was simply disadvantageous in the wrong configuration, or could this region of the molecule be developed to show a specific advantage, perhaps through engagement of target interactions in the correct configuration. To further develop this class of

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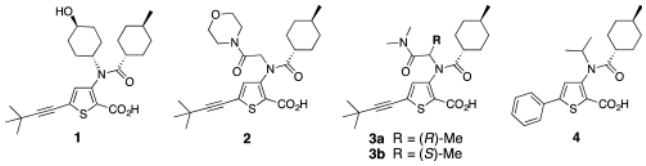
molecules, we applied a structure-guided design approach, based on the X-ray crystal structures of the NS5B polymerase with bound ligands of this class.



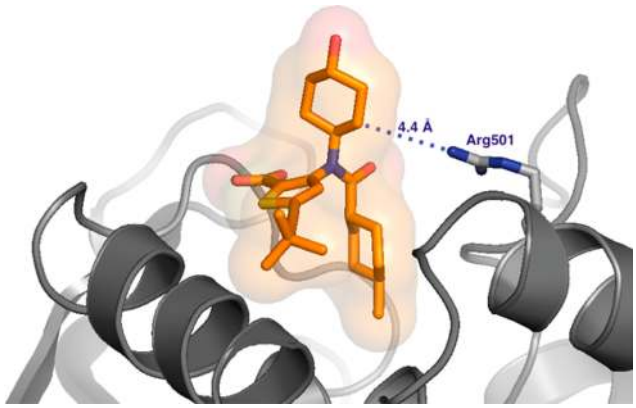
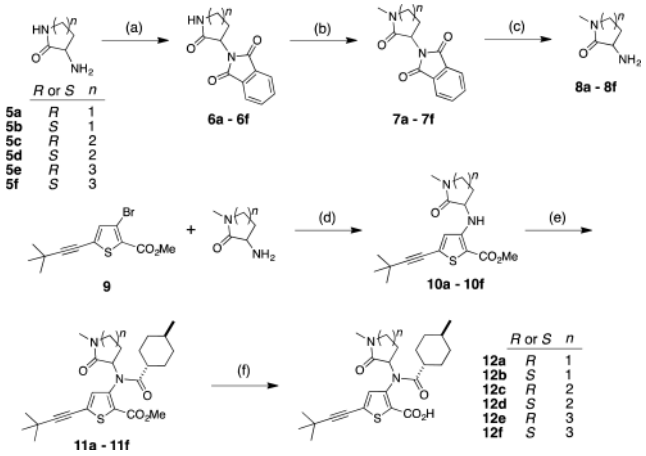


Figure 1. Model of **1** bound to HCV genotype 1b NS5B showing the proximity of the *trans*-4-hydroxycyclohexyl ring to Arg501.

Scheme 1. General Synthesis of Compounds 12a−12f[a]

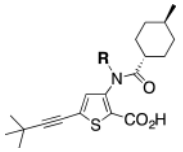


[a]Reaction conditions: (a) ethyl 1,3-dioxoisoindoline-2-carboxylate, Na_2CO_3, H_2O, RT; (b) NaH, MeI, DMF, 0 °C; (c) NH_2NH_2, MeOH, RT; (d) $Pd(OAc)_2$, BINAP, Cs_2CO_3, toluene, 90 °C; (e) *trans*-4-methylcyclohexylcarbonyl chloride, pyridine, DCE, reflux; (f) LiOH, THF, H_2O, RT

Based on the X-ray crystal structure of compound **4** bound to the thumb pocket 2 allosteric site of NS5B (PDB: 2GIR),[13] compound **1** was docked, maintaining the same key contacts. The model illustrated in Figure 1 shows the *trans*-4-hydroxycyclohexyl group protruding from the binding site and in proximity to the surface residue Arg501 of the long helix forming the side of the binding pocket. Our objective was to capitalize on this proximity and derive additional binding interactions.

Compounds of this series were prepared from commercially available (*R*)- and (*S*)-5-, 6-, and 7-membered lactams derived from α-amino acids as illustrated in Scheme 1. The lactams

Table 1. Structure−Activity Relationships of the Lactam Series in the Anti-Viral Replicon Assay[14]



Compd	R	HCV Genotype 1b inhibition (nM)[a,b] IC$_{50}$	IC$_{90}$	IC$_{50}$ +40% HS[c]
1		25 ± 13	97 ± 62	203 ± 88
2		23 ± 13	100 ± 38	340 ± 114
3a		110 ± 29	610 ± 83	1320
3b		5 ± 3	24 ± 20	49 ± 24
12a		16 ± 1	96 ± 28	140
12b		4 ± 1	13 ± 2	40
12c		10 ± 1	44 ± 2	105 ± 17
12d		3 ± 1	8 ± 2	24 ± 4
12e		29 ± 10	63 ± 16	156 ± 17
12f		4 ± 2	12 ± 6	23 ± 6

[a]All compounds were tested in duplicate. Values are means of at least two determinations ± standard deviation. [b]CC_{50} > 20 μM for all compounds tested. [c]IC_{50} in the presence of human serum at a final concentration of 40%.

(**5a−f**) were phthaloyl-protected by treatment with ethyl 1,3-dioxoisoindoline-2-carboxylate to give products **6a−f**. *N*-Methylation of the lactam was accomplished by treatment with NaH and MeI in DMF. Hydrazine deprotection of the phthaloyl group gave the desired lactams (**8a−f**), which were coupled to the thiophene **9**[12] under Buchwald−Hartwig conditions to provide ester products **10a−f**, which were converted to the corresponding amides **11a−f** using *trans*-4-methylcyclohexylcarbonyl chloride. Hydrolysis of the methyl esters gave desired products **12a−12f**. Experimental details are provided in the Supporting Information.

From modeling, we anticipated that the polymerase surface residue Arg501 might provide hydrogen bonding opportunities if a suitably oriented H-bond acceptor was engineered into the molecule. The side chain of this residue is located approximately 4 Å from the cyclohexyl group of **1** (Figure 1). Thus, introduction of strategically placed polar residues into the

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Table 2. Rat IV and PO Pharmacokinetic Profiles of Lactams 12a−12f

	12a	12b	12c	12d	12e	12f
Rat ppb[a]	99.8	98.1	99.96	98.9	99.8	99.2
			Rat IV PK Parameters			
n[b]	3	2	3	3	3	3
dose[c]	1	1	0.5	0.5	1	1
$AUC_{0\text{-}INF}$[d]	1.74 ± 1.86	0.229 ± 0.038	25.4 ± 20.7	8.77 ± 7.31	1.13 ± 0.12	1.48 ± 0.82
Unbound $AUC_{0\text{-}INF}$[e]	3.48 ± 3.72	4.35 ± 0.71	10.2 ± 8.3	96.5 ± 80	2.26 ± 0.23	11.8 ± 6.6
CL[f]	22.5 ± 24.1	73.7 ± 12.1	1.19 ± 1.67	8.30 ± 13.3	15.9 ± 1.59	16.1 ± 6.8
Unbound CL[f]	11,300 ± 12000	3880 ± 640	2980 ± 4180	755 ± 1210	7950 ± 800	2010 ± 850
$T_{1/2}$[g]	2.73 ± 0.56	3.07 ± 2.54	5.57 ± 3.08	6.68 ± 3.75	2.79 ± 0.82	2.12
Vss[h]	1.93 ± 1.97	6.04 ± 3.52	0.221 ± 0.167	1.17 ± 1.20	1.05	1.95 ± 0.81
			Rat PO PK Parameters			
n[b]	2	2	3	3	3	3
dose[c]	3	3	1.5	1.5	3	3
$AUC_{0\text{-}INF}$[d]	3.32 ± 3.98	0.176 ± 0.060	24.4 ± 29.1	6.39 ± 9.45	3.53 ± 2.55	3.82 ± 2.56
Unbound $AUC_{0\text{-}INF}$[e]	6.64 ± 7.96	3.34 ± 1.14	9.76 ± 11.6	70.3 ± 104	7.06 ± 5.10	30.6 ± 20.5
C_{max}[i]	1.31 ± 1.5	0.119 ± 0.006	2.54 ± 2.15	0.751 ± 0.728	0.589 ± 0.21	1.04 ± 0.452
%F	64	26	32	24	122	97
$T_{1/2}$[g]	6.49 ± 4.77	4.66 ± 1.89	6.13 ± 3.85	4.80 ± 4.36	4.31 ± 2.68	2.91 ± 1.94

[a]Plasma protein binding, %. [b]Number of animals. [c]mg/kg. [d]μg·h/mL. [e]ng·h/mL. [f]mL/min/kg. [g]hours. [h]L/kg. [i]μg/mL.

Table 3. PXR Data

Compound	PXR EC_{50} (μM)	PXR max. activation vs control[a]	PXR max. activation[b]
12c	20.4	7.3-fold	28%
12d	17.3	15.1-fold	63%
12e	>30	ND[c]	16%
12f	>30	ND[c]	10%

[a]Control contains 0.1% DMSO. [b]Relative to 20 μM rifampicin. [c]Not detectable.

Table 4. Pharmacokinetic Parameters for Compound 12f Following a Single IV Bolus Dose of 1 mg/kg in Dogs and Monkeys

Species	Dog	Monkey
$AUC_{0\text{-}INF}$[a]	1.45 ± 0.815	0.443 ± 0.094
Unbound $AUC_{0\text{-}INF}$[b]	10.1 ± 5.7	4.43 ± 0.94
CL[c]	15.0 ± 6.4	33.5 ± 7.17
Unbound CL[c]	2140 ± 910	3350 ± 717
$T_{1/2}$[d]	1.39 ± 0.21	5.44 ± 0.55
V_{SS}[e]	1.59 ± 0.58	5.27 ± 3.39

[a]μg·h/mL. [b]ng·h/mL. [c]mL/min/kg. [d]hours. [e]L/kg.

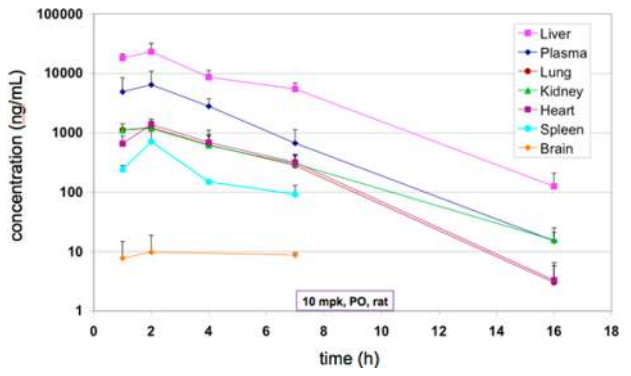

Figure 2. Mean plasma and tissue concentration−time profile of compound **12f** in male Sprague−Dawley rats.

pendant ring might create this desired interaction. Consideration of the chiral derivatives **3a** and **3b** suggested that constraining the dimethylamide back onto the amino acid side chain would create lactams with potential to interact with NS5B surface residues. Thus, a series of lactam derivatives were synthesized that provided 3- to 5-fold improvements in potency against NS5B polymerase (genotype 1a and 1b), as measured by replicon assays,[14] relative to **1** and **2**. The genotype 1b data for these compounds is summarized in Table 1. Each of the lactams, whether 5-, 6-, or 7-membered, or of *R*- or *S*-configuration, demonstrate strong antiviral activity in the genotype 1b replicon assay, as measured by IC_{50} or IC_{90}, and good inhibition in the presence of 40% human serum. In general, *S*-isomers were more potent than the corresponding *R*-isomers, but ring size was not a significant factor. The (*S*)-lactams were all comparable in activity to the acyclic *S*-Ala *N,N*-dimethylamide derivative **3b**.

Since each compound in the series **12a−12f** was an effective antiviral agent, all were advanced to *in vivo* pharmacokinetic studies. Table 2 summarizes the IV and PO PK studies in rats. For the 5-membered lactams **12a** and **12b**, the *R*-enantiomer **12a** shows a superior IV profile in terms of clearance (CL) and exposure (AUC); however, when accounting for protein binding, the unbound profiles are essentially the same. For the 6-membered lactams **12c** and **12d**, again the clearance and AUC of the *R*-enantiomer appears superior, but the unbound exposure of *S*-enantiomer **12d** is notably better. Finally, for the 7-membered lactams **12e** and **12f**, the profiles are almost identical, though again the *S*-enantiomer **12f** shows superior unbound exposure. Following oral dosing in rats, a similar outcome is observed: both *S*-enantiomers **12d** and **12f** show higher unbound exposure than their corresponding *R*-enantiomers **12c** and **12e**. For the 5-membered lactams, **12a** and **12b** show similar unbound exposure following PO dosing. Based on the strong antiviral activity and encouraging PK profiles, both **12d** and **12f** were further evaluated as potential development compounds.

A comparison of the *in vitro* ADME profiles of **12d** and **12f** revealed little distinction. Both compounds show high plasma protein binding (~99%) and acceptable stability in rat and

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human liver microsomes (Supporting Information Table S2). While both **12d** and **12f** show no propensity for CYP inhibition (3A4, 2D6, 2C9 all >30 μM), **12d** did exhibit CYP induction potential, as evaluated in human PXR-gene mediated activation, whereas **12f** showed no induction potential (Table 3). A similar propensity for PXR gene activation has been reported with related thiophene carboxylate NS5B inhibitors.[15] Based on this difference, **12f** was selected for further profiling.

Further *in vivo* profiling is summarized in Table 4. IV pharmacokinetic profiles in dog and monkey showed good exposure and moderate clearance.

An *in vitro* hepatocyte uptake study showed that **12f** was actively transported, with 5-fold higher concentration in hepatocytes at 37 °C than at 4 °C after 15 min. Correspondingly, a rat bile duct cannulation study showed elimination primarily in bile; 35% of the dose was excreted intact in bile, suggesting both active uptake and efflux mechanisms are involved. Finally, tissue distribution studies of **12f** in rats showed that exposure was highest in the liver and the second highest in plasma; it was significantly lower, by a factor of at least 10, in other major organs (Figure 2). This profile was considered ideal for an orally delivered drug targeting a disease of the liver.

To develop the thiophene carboxylate class of allosteric HCV NS5B inhibitors, we applied both structure-guided design and conformational constraint to identify compounds with enhanced antiviral activity, possibly resulting from additional binding interactions between protein and ligand. We successfully developed a new series of compounds containing a lactam motif that provided further potency, possibly as a result of the desired designed interactions between the compounds and NS5B residue Arg501. Compound **12f** is 3–5-fold more potent against NS5B polymerase relative to **1**, as measured by replicon assays, and it possesses pharmaceutical properties and an *in vivo* profile consistent with parameters desirable for further development.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmedchemlett.6b00479.

General experimental details and synthetic procedures, analytical data, and microsomal stability data (PDF)

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Notes

The authors declare the following competing financial interest(s): All of the authors of this manuscript are current or former employees of Vertex Pharmaceuticals.

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■ ABBREVIATIONS

HCV, hepatitis C virus; NS5B, nonstructural protein 5B

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