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Discovery of Novel Thiophene-Based, Thumb Pocket 2 Allosteric Inhibitors of the Hepatitis C NS5B Polymerase with Improved Potency and Physicochemical Profiles

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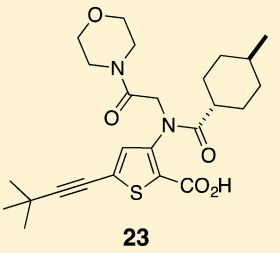
Supporting Information

ABSTRACT: The hepatitis C viral proteins NS3/4A protease, NS5B polymerase, and NS5A are clinically validated targets for direct-acting antiviral therapies. The NS5B polymerase may be inhibited directly through the action of nucleosides or nucleotide analogues or allosterically at a number of well-defined sites. Herein we describe the further development of a series of thiophene carboxylate allosteric inhibitors of NS5B polymerase that act at the thumb pocket 2 site. Lomibuvir (**1**) is an allosteric HCV NS5B inhibitor that has demonstrated excellent antiviral activity and potential clinical utility in combination with other direct acting antiviral agents. Efforts to further explore and develop this series led to compound **23**, a compound with comparable potency and improved physicochemical properties.



23

HCV replicon IC_{50}

Genotype 1a IC_{50} = 37 nM

Genotype 1b IC_{50} = 23 nM

INTRODUCTION

Hepatitis C virus (HCV) infection is a global public health issue affecting an estimated 80−115 million people worldwide, corresponding to regional infection rates of 1−4%.[1] Approximately 75−85% of individuals infected with HCV will develop chronic infection. An estimated 20−50% of these patients will develop chronic liver disease, liver fibrosis, cirrhosis, and hepatocellular carcinoma, the fourth leading cause of cancer deaths.[2] Hepatitis C is the leading cause of liver transplant in the United States, where an estimated 16,000 patients die each year as a consequence of HCV infection.[3] Thus, there remains a significant medical need to eradicate HCV and reduce the public healthcare burden.

The FDA approvals in May 2011 of two oral HCV protease inhibitors, telaprevir[4−6] and boceprevir,[7,8] initiated a revolution in the field of HCV treatment. These new treatment regimens, comprising a protease inhibitor in combination with the prior standard-of-care (SOC), pegylated interferon α (peg-IFN), and ribavirin (RBV), demonstrated significantly higher disease cure rates in clinical trials in comparison to the then SOC (peg-IFN/RBV) and have dramatically increased treatment success rates for patients infected with HCV genotype 1.[9] More recently, success rates for treatment have further improved, with several direct-acting antiviral agents having gained marketing authorization, some as part of combination therapy. These include the NS3/4A protease inhibitors simeprevir[10] and paritaprevir,[11] the nucleotide NS5B inhibitor sofosbuvir,[12] and the non-nucleotide NS5B allosteric inhibitor dasabuvir,[13] as well as the NS5A inhibitors ledipasvir,[14] daclatasvir,[15] and ombitasvir.[16] The FDA-approved combination drugs sofosbuvir with ledipasvir[17] and ombitasvir, paritaprevir, and dasabuvir combined with the CYP3A inhibitor ritonavir[18−20] represent the realization of the promise of all-oral, noninterferon-based therapy with shortened treatment times and high cure rates.

As part of our program to identify inhibitors of viral protein targets to add to the inventory of direct-acting antivirals with combination potential, we sought to further develop allosteric HCV NS5B inhibitors of the thiophene carboxylic acid class. Lomibuvir (**1**: also named VCH-222 and VX-222)[21] is a non-nucleoside, allosteric NS5B polymerase inhibitor that binds to the so-called thumb pocket 2, a distance of ∼35 Å from the active site.[22] Other reported inhibitors that act at the same allosteric site include related thiophene carboxylic acids, **2**−**5**,[23−26] as well as the structurally unrelated compounds **6** (filibuvir),[27] **7** (HCV-371),[28] and **8**[29] illustrated in Figure 1. Lomibuvir has

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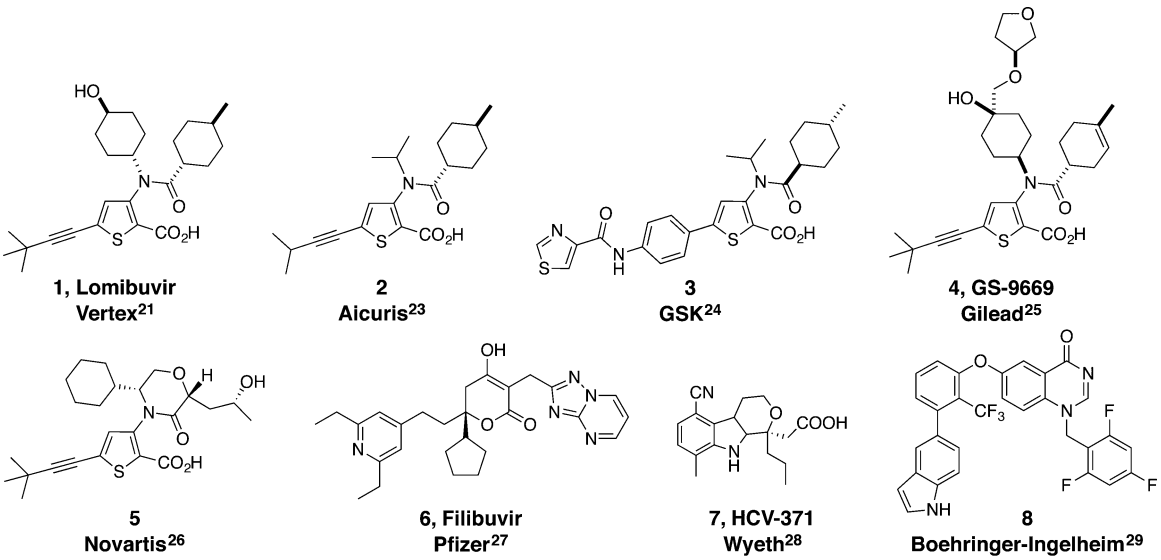


Figure 1. Thumb pocket 2 allosteric HCV NS5B polymerase inhibitors and originators.

demonstrated impressive antiviral effects in phase 1 trial, causing a 3.4 $\log_{10}$ reduction in hepatitis C viral load after 3 days of treatment.[30] Combination trials of lomibuvir plus telaprevir with and without peg-IFN/RBV have also been reported.[21]

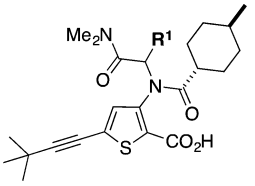
The structure of **1**, like that of other previously reported thiophene carboxylic acid NS5B inhibitors,[23,24,31,32] contains a single tertiary, anilinic nitrogen atom bearing two substituents, both of which possess a plane of symmetry. The *trans*-4-methylcyclohexyl group is known to be deeply buried in a hydrophobic pocket,[22] while the isopropyl or the *trans*-4-hydroxycyclohexyl substituent appears to extend into more open space. Our investigations sought to better understand this region within this class of molecules and to explore asymmetry and branching as a means of developing additional target interactions. To guard against discovering new molecular matter in undesirable chemical space, we also chose to apply specific design constraints, by keeping molecules "rule of 5" compliant, with molecular weight <500 and clogP < 5.[33]

CHEMISTRY

Compounds were synthesized by one of two general routes. Compounds in Table 1 were synthesized using the route shown in Scheme 1. Iodination of methyl 3-bromothiophene-2-carboxylate (**28**) was followed by Sonogashira reaction to introduce the *t*-butyl alkyne at the 5-position (**30**). A second Pd-catalyzed reaction, Buchwald–Hartwig amination at the 3-position, introduced the amino amide derivative, which was followed by acylation with *trans*-4-methylcyclohexyl carbonyl chloride and final ester saponification to give the compounds **9**–**16**. All compounds were purified by preparative HPLC and stereochemical purity confirmed by chiral SFC.

The *N*-thiophenyl-*N*-acylglycine amide-derived compounds, summarized in Table 2, were synthesized using the route shown in Scheme 2. Trifluoroacetylation of 3-amino-2-carboxythiophene methyl ester (**33**), followed by LDA-mediated 5-iodination and subsequent deprotection, yielded the key starting material **36**. Acylation with *trans*-4-methylcyclohexanecarbonyl chloride gave the 5-iodothiophene amide **37** in 95% yield. *N*-Alkylation with *t*-butyl bromoacetate, followed by Sonogashira reaction and subsequent *t*-butyl ester deprotection gave the carboxylic acid **40**, which was coupled with various amines using HBTU as coupling agent. Methyl ester saponification gave products **17**–**27**.

Table 1. Structure–Activity Relationships for α-Carbon Substituents



compd	R^1	genotype 1b EC_{50} (nM)[a,b]	genotype 1a EC_{50} (nM)
1		7	53
9	(*S*)-Me	5	7
10	(*R*)-Me	109	ND[c]
11	(*S*)-Et	16	24
12	(*S*)-*n*-Pr	14	ND
13	(*S*)-*i*-Pr	51	ND
14	(*S*)-CH_2OH	60	ND
15	(*S*)-CH_2OMe	7	11
16	H	12	35

[a]All compounds were tested in duplicate. [b]CC_{50} > 20 μM for all compounds tested. [c]ND not determined

RESULTS AND DISCUSSION

To break the symmetrical nature of the N-substituent, the first analogues prepared were the (*S*)- and (*R*)-alanine dimethylamide derived compounds **9** and **10**. This pair immediately indicated a stereochemical preference, with the (*S*)-isomer being approximately 20-fold more active than the corresponding (*R*)-isomer (Table 1, entries 2 and 3) as measured in the antiviral replicon assays (described in the Supporting Information).[34] Further exploration of the analogous amino-acid-derived dimethylamides is summarized in Table 1. Small side chains with (*S*)-stereochemistry showed relatively small variations in activity, with (*S*)-methyl **9**, (*S*)-ethyl **11**, and (*S*)-*n*-propyl **12** all showing essentially the same level of activity in the range of 5–16 nM in the genotype 1b replicon assay. Branching of this side chain (**13**) showed a 10-fold loss in activity, as did the addition of

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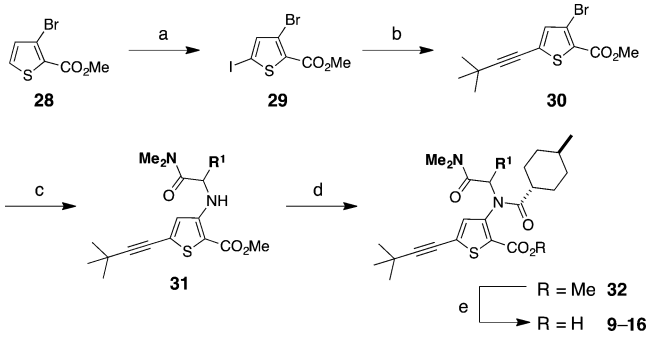
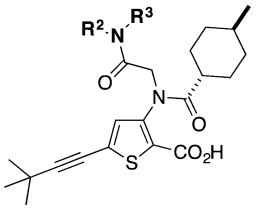
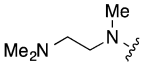
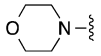
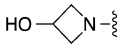
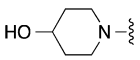
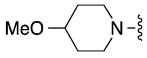
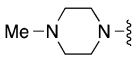
Scheme 1. Preparation of α-Branched Derivatives from α-Amino Amides[a]



[a](a) *i*-Pr_2NH, *n*-BuLi, I_2, THF, −78 °C, 45%; (b) *t*-BuC≡CH, CuI, Et_2NH, $Pd_2(dba)_3$, DMF, 0 °C to RT; (c) $H_2NCH(R^1)CONMe_2$, Cs_2CO_3, $Pd(OAc)_2$, BINAP, toluene, microwave, 15 min, 130 °C; (d) *trans*-4-Me-cyclohexyl-COCl, DMAP, DCE, pyridine, microwave, 24 h, 90 °C; (e) LiOH, THF, H_2O, 24 h.

Table 2. Glycine Amide Analogues[c]



Compd	NR^2R^3	Genotype 1b EC_{50} (nM)[a,b]	Genotype 1a EC_{50} (nM)[a,b]
16	Me_2N–⦚	12	35
17	MeNH–⦚	190	ND
18	*i*-PrNH–⦚	160	ND
19	Et_2N–⦚	23	51
20		21	32
21		67	ND
22		9	ND
23		23	37
24		66	ND
25		64	ND
26		17	ND
27		6	ND

[a]All compounds were tested in duplicate. [b]CC_{50} > 20 μM for all compounds tested [c]ND not determined

a polar, hydrogen bond-donating group (**14**). The analogous hydrogen-bond acceptor side chain, derived from (*S*)-*O*-methylserine **15**, was equipotent with the (*S*)-alanine derived compound **9**. Finally, we observed that the unsubstituted, glycine derived system, compound **16**, was also equipotent with the (*S*)-methyl derivative, suggesting that the (*S*)-substituent was providing no specific interactions with the polymerase system, while the (*R*)-methyl substituent may have created undesired steric interactions.

Subsequent investigations sought to better understand the nature of the amide substituents (Table 2). For these, we chose to focus on the glycine-derived system because the lack of C-substitution conferred no obvious disadvantage and the absence of chirality would make data interpretation and further development straightforward. Secondary amides **17** and the larger **18** exhibited significant loss of antiviral activity, indicating that tertiary amides would be preferred. Further exploration of the tertiary amides is summarized in Table 2. As observed with the α-carbon variations, changes in the tertiary amide N-substituents showed relatively little effect on antiviral activity. The pyrrolidine amide **20** was effectively equipotent with the dimethylamide, while larger ring amides varied from 6 nM for the 4-methylpiperazine amide (**27**) to 67 nM for the piperidine amide (**21**). The acyclic 2-dimethylaminoethyl(*N*-methyl) amide (**22**) showed potency comparable to **27**, while piperidine substitution either had no effect on activity (**25**) or restored activity to the level of the dimethylamide in the case of compound **26**. The morpholine amide **23** showed good potency, comparable to lomibuvir against both genotypes 1a and 1b. In general, potency was not significantly perturbed by the various tertiary amides. Unsurprisingly, zwitterionic compounds, such as **27**, although showing good antiviral activity, were poorly bioavailable in this series (data not shown). Compound **23**, possessing a desirable combination of lowered lipophilicity relative to lomibuvir (clogP 4.1 vs 5.6 for lomibuvir), good solubility, synthetic accessibility, structural simplicity, and a promising PK profile (vide infra) was selected for further profiling as a potential development candidate.

Further antiviral activity profiling for compound **23** in comparison with lomibuvir is summarized in Table 3. Both compounds show similar potency against genotypes 1a and 1b, with no significant activity against genotype 2a. Lack of activity against this genotype is due to the difference of NS5B amino acid residues at positions 419, 482, and 494 in non-1 genotypes that are known resistance sites for lomibuvir. Measures of antiviral activity in the presence of human serum show approximately 10–20-fold shift, consistent with other molecules of this class. Both molecules possess a similar profile of activity against resistant viral strains clinically relevant to lomibuvir. Resistance selection studies of **23** showed common single amino acid substitutions with those observed for lomibuvir, consistent with similar binding interactions of these two compounds to the NS5B protein. Modeling, on the basis of the X-ray crystal structure of related thiophenes bound to NS5B,[22,35] indicates that both molecules interact with the protein in an identical manner (Figure 2), while the regions of each inhibitor that are different are located away from the enzyme surface. Thus, the resistance profiles are similar. Compound **23** maintains strong antiviral activity against clinically observed resistance mutations to telaprevir (Supporting Information, Table S1) and the NS5A inhibitor BMS-790052[36] (Supporting Information, Table S2). Compound **23** also showed no activity toward human polymer-

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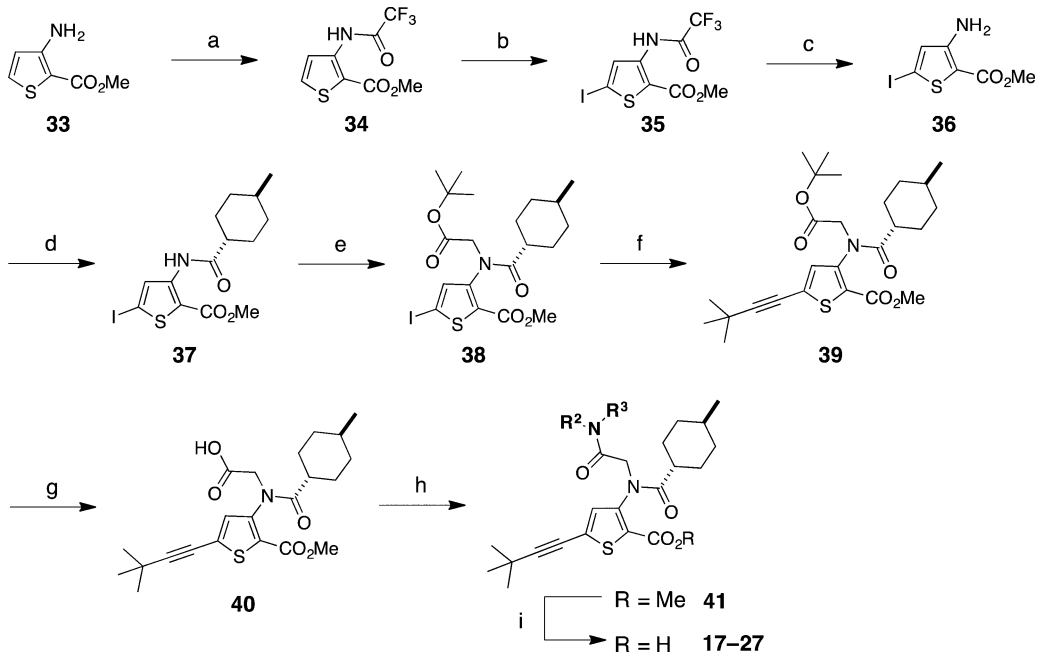
Scheme 2. General Synthesis of *N*-Thiophenyl-*N*-acylglycine Amide Analogues[a]



[a](a) TFAA, Et_2O, 0 °C, 98%; (b) I_2, LDA, 2-MeTHF, −78 °C, 80%; (c) K_2CO_3, H_2O, MeOH, 96%; (d) *trans*-4-Me-cyclohexane-COCl, DMAP, DCM, pyridine, 0 °C to RT, 99%; (e) LiHMDS, NaI, *t*-BuOCOCH$_2$Br, THF, 0−60 °C, 64%; (f) *t*-BuC≡CH, CuI, *i*-Pr_2NH, $PdCl_2(PPh_3)_2$, 0 °C to RT, 83%; (g) TFA, CH_2Cl_2, RT; (h) R^2R^3NH, HBTU, DIEA, DMF, RT; (i) NaOH, MeOH, H_2O, RT.

Table 3. Comparison of Antiviral Activity (Replicon EC_{50}) of 23 and Lomibuvir (All Values in nM)

assay	23	lomibuvir
genotype 1b	24	7
genotype 1b + 40% human serum	340	66
genotype 1a	37	53
genotype 2a	4800	8100
genotype 1a mutants		
L419M	240	320
L419I	400	320
M423I	89	30
M423T	280	310
M423V	120	65

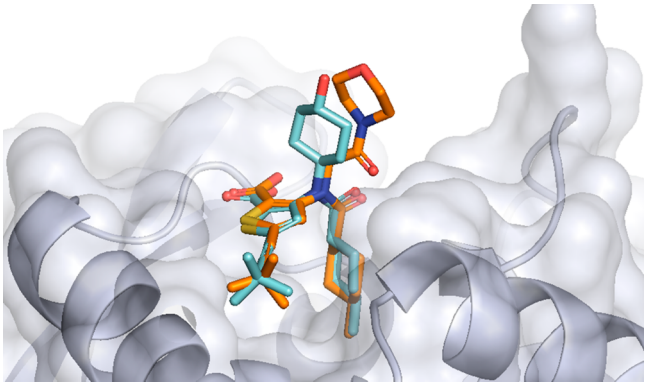


Figure 2. Three dimensional model of compound **23** (gold) overlaid with lomibuvir (light-blue) bound to HCV NS5B (gray) based on PDB ID 2GIR.[35]

ase α, β, and γ, nor toward other viral targets (see Supporting Information, Tables S3, S4, and S5).

Combination antiviral studies of **23** with human interferon α2a and ribavirin, or **23** with telaprevir, demonstrated synergistic activities in vitro (Supporting Information, Figure S1). Further in vitro studies demonstrated synergy with NS5A inhibitors (Supporting Information, Figure S1), suggesting the potential of **23** as a valuable molecule with combination potential for further development.

The in vitro pharmacokinetic ADME profile of **23** across four species is summarized in Table 4. Overall, **23** shows excellent in vitro stability and a low propensity toward metabolism. Further studies showed that CYP 3A4 is the cytochrome enzyme primarily responsible for oxidative metabolism of **23**, however no single metabolite of >5% of total material was seen in incubation with human S9 liver fraction after 1 h. The in vitro studies foretold in vivo observations in rat, where intact excretion of **23** (in plasma, bile, and feces) was observed as the major route of elimination (data not shown). Compound **23** showed no significant CYP P450 inhibition across tested isozymes, with only CYP 2E1 showing any measurable inhibition (IC_{50} = 14 μM; all others >40 μM). In addition, **23** showed no induction of CYP 1A2 or 3A4 in hepatocytes, no change in mRNA levels, and low potential for induction in a PXR-gene mediated activation assay.

As expected for a carboxylic acid, plasma protein binding of **23** is relatively high across species, ranging from 97.5% bound in dog plasma to 99.7% bound in rat plasma. Partitioning into red blood cells was not observed across species and was less than unity. Liver protein binding is in the range of 83−91% across species.

Permeability of **23** was assessed in Caco2 cells; transport from the basolateral-to-apical side (permeability = 9.8 × 10^{-6} cm/s) was much greater than that in the apical-to-basolateral direction (permeability = 0.7 × 10^{-6} cm/s): the efflux ratio of 14 indicates that **23** is actively transported. Similar results were observed in MDCK cells that overexpress MDR1, which showed an efflux ratio of 9.8. These data indicate that **23** has low permeability and

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Table 4. In Vitro Stability and Protein Binding of 23 in Four Species

assay		rat	dog	monkey	human
hepatocyte stability (% remaining t = 2 h; 10 μM)		100	70	30	77
microsomal stability (% remaining t = 2 h; 1 μM)	S9 + NADPH	99	82	19	76
	S9 + NADPH + UDPGA	79	82	14	73
liver protein binding (% at 1 μM)		83.8	86.0	90.7	84.7
plasma protein binding (% at 1 μM)		99.7	97.5	97.7	98.6

Table 5. Pharmacokinetic Profile of 23 across Three Species

	rat			
	Sprague–Dawley[a]	Fischer 344[a]	dog[b]	monkey[c]
IV Dose				
AUC_{0-inf} (μg·h/mL)	4.27 ± 4.14	1.58 ± 0.96	1.36 ± 0.44	1.73 ± 0.32
CL (mL/min/kg)	14.9 ± 14.0	13.8 ± 6.9	10.1 ± 3.2	9.9 ± 2.0
$T_{1/2}$ (h)	2.35 ± 0.52	1.67 ± 0.83	9.8 ± 9.5	2.2 ± 0.3
V_{ss} (L/kg)	1.55 ± 1.63	0.86 ± 0.46	1.57 ± 2.08	0.23 ± 0.09
PO Dose[d]				
AUC_{0-inf} (μg·h/mL)	8.86 ± 9.07	0.31 ± 0.18	2.34 ± 0.96	
C_{max} (μg/mL)	1.73 ± 1.57	0.24 ± 0.24	1.55 ± 0.91	
%F	69	6.5	57.3	
$T_{1/2}$ (h)	3.04 ± 0.71	2.10 ± 0.68	6.6 ± 4.1	

[a]IV bolus dose of 1 mg/kg in dimethyl isosorbide (DMI; 10%), EtOH (15%), PEG400 (35%), 5% aqueous dextrose (D5W; 40%). n = 6 animals. [b]IV bolus doses of 1 mg/kg in 0.9% saline, 1% NaOH solution pH 7. n = 6 animals. [c]IV bolus doses of 1 mg/kg in 100 mM sodium phosphate buffer (pH 7.4). n = 3 animals. [d]Oral doses of 3 mg/kg in 0.5% methylcellulose, 0.5% Tween 80 in H_2O. n = 6 animals.

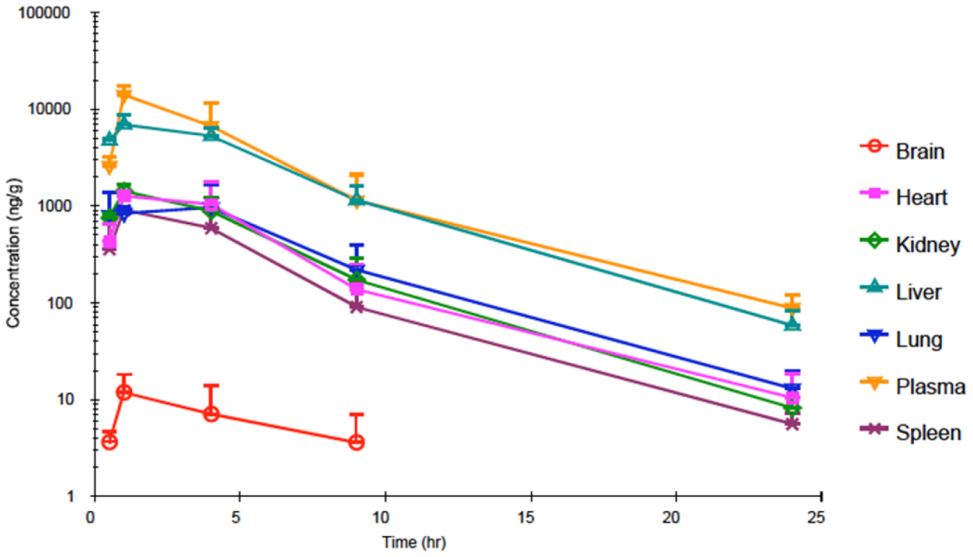

Figure 3. Mean plasma and tissue concentration–time profile of **23** in male Sprague–Dawley rats administered a single nominal oral dose of 10 mg/kg.

is a P-glycoprotein (Pgp) substrate. Conversely, **23** showed uptake into rat and human hepatocytes via an active transport process and was actively taken up into MDCK cells expressing the organic anion transporter OATP1B1 3-fold higher than wild-type MDCK.

The in vivo pharmacokinetic profile of **23** is summarized in Table 5. Following IV dosing, **23** showed a low clearance across three species and a low volume of distribution. Interanimal variability was high in Sprague–Dawley rats, lower in Fischer 344 rats and in dogs, and minimal in monkeys. Dogs appeared to exhibit enterohepatic recirculation, as evidenced by multiphase elimination causing an extended half-life (see Supporting Information, Figure S2). In PO-dosed Sprague–Dawley rats, bile duct cannulation studies showed that 14–50% of the dosed **23** was excreted intact, also indicating the potential for enterohepatic recirculation. Following administration of nominal oral doses of 3 mg/kg in male Sprague–Dawley rats and dogs, **23** had moderate oral bioavailability in both species. Again, interanimal variability was high in Sprague–Dawley rats and moderate in dogs. Bioavailability in Fischer rats was low and interanimal variablility was also low, consistent with Fischer 344 rats being a more inbred strain than Sprague–Dawley rats.

Tissue distribution studies of **23** showed that exposure was highest in the liver and plasma and significantly lower, by a factor of at least 10, in other major organs (Figure 3), consistent with an active hepatic uptake mechanism. This profile was considered ideal for an orally delivered drug targeting a disease of the liver.

The overall activity and pharmacokinetic profile of **23** is highly promising for an antiviral development candidate designed for use in combination with other antiviral agents. Compound **23**

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demonstrated low potential for causing drug−drug interaction as a result of the lack of CYP inhibition, low levels of metabolism, and low CYP induction potential. Further studies focused on profiling the physicochemical properties of **23** to better understand its potential for pharmaceutical development. Compound **23** is a crystalline solid that demonstrates excellent solubility at neutral pH. Solubility of **23** in pH 7 potassium phosphate buffer was measured at 1.8 mg/mL, while solubility in fasted and fed simulated intestinal fluids is similarly high: FaSSIF (1.7 mg/mL at pH 6.3) and FeSSIF (0.80 mg/mL at pH 5.2). In addition, the compound shows excellent stability on long-term storage in solution. (Figure 3)

CONCLUSIONS

Beginning with the structure of lomibuvir and related thiophene analogues, we sought to explore the effect of changes in the N-substituent, particularly with respect to α-branching and asymmetry. Replacement of the *trans*-4-hydroxycyclohexyl with chiral and unbranched, achiral α-amino amide derived systems revealed molecules possessing a high degree of antiviral activity and excellent physical chemical properties. Compound **23**, an achiral, non-α-substituted, and synthetically accessible structure, showed an antiviral profile comparable to lomibuvir. Antiviral activity and a clean ADME profile suggest strong potential for application in combination therapies. Compound **23** possesses excellent solubility and physicochemical properties and presents no significant concerns regarding drug−drug interaction potential.

EXPERIMENTAL SECTION

Reagents and anhydrous solvents were obtained from commercial sources and were used without further purification. Reactions heated by microwave were performed in sealed tubes on a Biotage Initiator microwave synthesis reactor (Biotage, Uppsala, Sweden). Preparative chromatography was performed on ISCO Combiflash systems using prepacked silica gel cartridges. High resolution mass spectra were acquired on a Thermo QExactive Orbitrap mass spectrometer following chromatography on a Waters Acquity UPLC system. High-resolution mass spectra (HRMS) were collected by direct infusion on a Thermo QExactive. The samples were dissolved in MeOH at a concentration of approximately 0.2 mg/mL and infused with a flow rate of 5 μL/min. Electrospray ionization in positive ion mode was employed with a spray voltage of 4.0 kV. The mass resolution was set to 35000. ^{1}H NMR spectra (δ, ppm) were recorded using a Bruker Avance 300 (300 MHz) or Bruker Avance-III (400 MHz) instrument. Proton resonances are recorded in parts per million (ppm) downfield from tetramethylsilane (TMS). ^{13}C and ^{19}F NMR spectra were recorded on a Bruker Avance 300 operating at 101 and 282 MHz, respectively. Purity of compounds was assessed by reversed-phase LCMS. The purity of compounds was determined by LC absorbance at two wavelengths, typically 220 and 254 nm, and all tested compounds were >95% pure by peak area, unless otherwise noted. Enantiomeric purity was determined by chiral HPLC on an Agilent 1100 system equipped with a Phenomenex Cellulose 4.6 mm × 250 mm column. Elution conditions were 15% MeOH, 15% EtOH, and 70% hexanes (containing 0.2% Et_2NH) eluted at 1 mL/min, and product was detected at 280 nm absorbance. Preparative HPLC was performed on a Gilson HPLC system using a suitable gradient of MeCN (0.1% TFA) in H_2O (0.1% TFA) at a flow rate of 15 mL/min over 10 min on either YMC ODS-AQ C18 or Kromasil C8 columns (10 μm particle size; 21 mm × 150 mm). Antiviral activity of the compounds described was determined by the previously described replicon methods for hepatitis C genotypes 1a and 1b.[37] A full description of the assay method can be found in the Supporting Information.

Methyl 3-Bromo-5-iodothiophene-2-carboxylate (29). *n*-BuLi (109 mL of 2.5 M, 272 mmol) was added dropwise to a solution of diisopropylamine (41 mL, 294 mmol) in 2-MeTHF (400 mL) at 0 °C and stirred for 30 min. The reaction mixture was cooled to −70 °C, and methyl 3-bromothiophene-2-carboxylate (**27**) (50 g, 226 mmol) in 2-MeTHF (200 mL) was added dropwise over 30 min. The reaction mixture was stirred for 30 min then warmed to −60 °C. Iodine (63.1 g, 250 mmol) in 2-MeTHF (100 mL) was added dropwise over 30 min, maintaining the internal temperature at −60 °C, and stirred at this temperature for 45 min, then allowed to warm to 0 °C. The reaction mixture was quenched with aqueous NH_4Cl (300 mL) and diluted with MTBE (1 L). The organic layer was separated, washed with 1 M $Na_2S_2O_3$ (200 mL), H_2O (200 mL), and brine (200 mL), and dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude product was purified over a silica gel plug using 5−10% EtOAc in hexanes as eluent to afford **29** (85% purity by HPLC). This material was recrystallized from methanol to afford desired product **29** (35.4 g, 45% yield) as a light-yellow colored solid. HRMS: *m*/*z* calcd for $C_6H_5BrIO_2S^+$ $[M + H]^+$, 346.8233; found, 346.8229. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.25 (s, lH), 3.88 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.82, 141.62, 132.96, 116.86, 82.10, 52.38.

Methyl 3-Bromo-5-(3,3-dimethylbutyn-l-yl)thiophene-2-carboxylate (30). A dry 2 L flask under N_2 atmosphere was charged with $PdCl_2(PPh_3)_2$ (4.93 g, 7.0 mmol), copper iodide (1.33 g, 7.0 mmol), and dioxane (400 mL) and the mixture stirred for 5 min. Diisopropylamine (49 mL, 350 mmol) and **29** (70 g, 233 mmol) were added and the mixture stirred for 5 min. 3,3-Dimethylbutyne (31 mL, 250 mmol) in dioxane (500 mL) was added at ambient temperature and the mixture stirred for 2 h. The reaction was diluted with MTBE (1 L), stirred for 20 min, then filtered through Celite. The filtrate was concentrated under reduced pressure and the residue purified on a short silica gel column eluted with 5% EtOAc in hexanes. Evaporation of solvent yielded **30** as an off-white solid (68 g, 96%). LCMS *m*/*z* 301.0, 303.0 $[M + H^+]$. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.37 (s, 1H), 3.82 (s, 3H), 1.29 (s, 9H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 159.6, 136.1, 128.9, 126.0, 116.3, 107.0, 70.7, 52.5, 30.1, 28.0.

(*S*)-Methyl 3-((1-(Dimethylamino)-1-oxobutan-2-yl)amino)-5-(3,3-dimethylbut-1-ynyl)-thiophene-2-carboxylate 31 (R^1 = (*S*)-Me). To a solution of **30** (604 mg, 2.0 mmol) in dioxane (10 mL) at RT was added Cs_2CO_3 (1.0 g, 7.52 mmol), BINAP (250 mg, 0.40 mmol), $Pd(OAc)_2$ (185 mg, 0.11 mmol), and (*S*)-2-amino-*N*,*N*-dimethyl-propanamide (400 mg, 2.62 mmol). The mixture was degassed by passing nitrogen through the mixture for 10 min and then heated with stirring at 90 °C for 24 h. The reaction was checked for completion by LCMS, then diluted with EtOAc (20 mL) and filtered through a pad of silica gel washing with EtOAc (3 × 30 mL). The combined eluent was evaporated and purified by silica gel chromatography (ISCO 40 g column, eluted with a gradient of 5−40% EtOAc in hexanes 5 CV, held at 40% for 2 CV, then eluted to 90% EtOAc in hexanes over 7 CV) to afford product **31** (250 mg, 37%). LCMS *m*/*z* 337.6 $[M + H^+]$. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.32 (d, *J* = 8.1 Hz, 1H), 7.03 (s, 1H), 4.65 (dd, *J* = 8.0, 6.5 Hz, 1H), 3.64 (s, 3H), 3.00 (s, 3H), 2.79 (s, 3H), 1.21 (s, 9H), 1.13 (d, *J* = 6.5 Hz, 3H).

(*R*)-Methyl 3-((1-(Dimethylamino)-1-oxobutan-2-yl)amino)-5-(3,3-dimethylbut-1-ynyl)thiophene-2-carboxylate 31 (R^1 = (*R*)-Me). The (*R*) enantiomer (279 mg) was prepared in identical fashion as described above in 41% isolated yield.

Methyl 3-(*N*-((*S*)-1-(Dimethylamino)-1-oxopropan-2-yl)-(*trans*-4-methylcyclohexane)-1-carboxamido)-5-(3,3-dimethyl-but-1-ynyl)thiophene-2-carboxylate 32 (R^1 = (*S*)-Me). A mixture containing **31** (R^1 = (*S*)-Me) (100 mg, 0.29 mmol), *trans*-4-methylcyclohexanecarbonyl chloride (95 mg, 0.56 mmol), pyridine (50 μL, 0.5 mmol), and a catalytic amount of DMAP (4 mg, 0.032 mmol) in 1,2-dichloroethane (3 mL) was heated in a sealed vial at 90 °C for 24 h. A 1:1 mixture of water and brine was added, and the solution was extracted with CH_2Cl_2 (2 × 10 mL), then dried over Na_2SO_4, filtered, and the solvent removed to give crude product which was purified by reversed-phase chromatography (ISCO C18-AQ eluted with 0−90% MeCN in H_2O (0.1% TFA)) to give **32** (R^1 = (*S*)-Me) (94 mg, 69%). LCMS *m*/*z* 461.2 $[M + H^+]$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.29 (s, 1H), 5.34 (q, *J* = 7.2 Hz, 1H), 3.79 (s, 3H), 3.06 (br s, 3H), 2.88 (br s, 3H), 1.94 (t, *J* = 11.4 Hz, 1H), 1.70−1.42 (m, 4H), 1.32 (s, 9H),

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J. Med. Chem. XXXX, XXX, XXX−XXX

1.27−1.09 (m, 3H), 0.91 (d, J = 7.2 Hz, 3H), 0.78 (d, J = 6.5 Hz, 3H), 0.76−0.51 (m, 2H).

Methyl 3-(*N*-((*R*)-1-(Dimethylamino)-1-oxopropan-2-yl)-(*trans*-4-methylcyclohexane)-1-carboxamido)-5-(3,3-dimethylbut-1-ynyl)thiophene-2-carboxylate 32 (R^1 = (*R*)-Me; R^2, R^3 = Me). Compound **32** (*R*) enantiomer (91 mg) was prepared in identical fashion as the *S*-enantiomer in 66% isolated yield. LCMS m/z 461.2 $[M + H^+]$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.27 (s, 1H), 5.32 (q, J = 7.2 Hz, 1H), 3.77 (s, 3H), 3.04 (s, 3H), 2.83 (s, 3H), 1.95−1.89 (m, 1H), 1.70−1.30 (m, 4H), 1.31 (s, 9H), 1.29−1.05 (m, 3H), 0.87 (d, J =J = 7.2 Hz, 3H), 0.76 (d, J = 6.4 Hz, 3H), 0.74−0.51 (m, 2H).

3-(*N*-((*S*)-1-(Dimethylamino)-1-oxopropan-2-yl)-(*trans*-4-methylcyclohexane)-1-carboxamido)-5-(3,3-dimethylbut-1-ynyl)thiophene-2-carboxylic Acid 9. To methyl ester **32** (100 mg, 0.22 mmol) dissolved in THF (1 mL) and H_2O (1 mL) was added LiOH (50 mg, 0.22 mmol). The mixture was stirred at RT for 24 h. The reaction mixture was quenched with 1 N HCl (3 mL), and the mixture was extracted with EtOAc (2 × 10 mL). The organic extracts were combined and washed with brine and dried over Na_2SO_4. After filtration and removal of the solvent, the residue was purified by HPLC to give the title compound **9** (75 mg, 89%). Chiral HPLC R_t = 7.98 min, 97.4% ee. LCMS m/z 447.1 $[M + H^+]$. HRMS: m/z calcd for $C_{24}H_{35}N_2O_4S^+$ $[M + H]^+$, 447.2312; found, 447.2317. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.25 (s, 1H), 5.66−5.28 (m, 1H), 2.96 (s, 6H), 1.98 (s, 1H), 1.67−1.40 (m, 4H), 1.33 (s, 9H), 1.30−1.11 (m, 2H), 0.95 (d, J = 7.1 Hz, 3H), 0.79 (d, J = 6.5 Hz, 3H), 0.73−0.60 (m, 3H).

3-(*N*-((*R*)-1-(Dimethylamino)-1-oxopropan-2-yl)-(*trans*-4-methylcyclohexane)-1-carboxamido)-5-(3,3-dimethylbut-1-ynyl)thiophene-2-carboxylic Acid 10. Compound **10** was prepared as described for **9** and isolated in 87% yield. Chiral HPLC R_t = 6.41 min, 99.2% ee. LCMS m/z 447.1 $[M + H^+]$. HRMS: m/z calcd for $C_{24}H_{35}N_2O_4S^+$ $[M + H]^+$, 447.2312; found, 447.2316. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.23 (s, 1H), 5.31 (d, J = 7.3 Hz, 1H), 2.94 (bs, 6H), 1.96 (s, 1H), 1.68−1.38 (m, 4H), 1.30 (s, 9H), 1.27−1.10 (m, 2H), 0.93 (d, J = 7.2 Hz, 3H), 0.77 (dd, J = 6.6, 2.1 Hz, 3H), 0.73−0.49 (m, 3H).

Compounds **11**−**16** were prepared by the methods described above for the preparation of **9** and as illustrated in Scheme 1. Analytical data is summarized in the Supporting Information.

Methyl 3-(2,2,2-Trifluoroacetamido)thiophene-2-carboxylate 34. To a stirred, cold (0 °C) solution of **33** (501 g, 3.19 mol) in Et_2O (5 L) was added trifluoroacetic anhydride (465 mL, 3.35 mol) dropwise. The resulting reaction mixture was slowly allowed to warm to RT and stirred for 12 h, at which point HPLC and LCMS-analysis revealed consumption of the starting material. The reaction mixture was partitioned between ethyl acetate (2 L) and 1 N HCl (500 mL), the organic layer was separated and washed with 1 N HCl (500 mL), satd aqueous $NaHCO_3$ solution (2 × 500 mL), and brine (500 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure to afford **34** (792 g, 98% yield) as a white solid. HRMS: m/z calcd for $C_8H_7F_3NO_3S^+$ $[M + H]^+$, 254.0093; found, 254.0091. ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.17 (s, 1H), 8.07 (d, J = 5.4 Hz, 1H), 7.56 (d, J = 5.5 Hz, 1H), 3.94 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 164.53, 154.66, 154.29, 153.91, 153.53 (C═O, quart, J_{C-F} = 38.1 Hz), 141.41, 132.16, 121.89, 119.90, 117.04, 114.17, 111.31 (CF3, quart, J_{C-F} = 288.1 Hz), 113.37, 52.41. ^{19}F NMR (282 MHz, $CDCl_3$) δ −75.94.

Methyl 5-Iodo-3-[(2,2,2-trifluoroacetyl)amino]thiophene-2-carboxylate 35. A stirred solution of diisopropylamine (72 mL, 514 mmol) in THF (400 mL) was cooled to 0 °C and *n*-BuLi (175 mL of 2.5 M, 438 mmol) was added dropwise over 25 min and the reaction mixture was stirred for a further 30 min, then cooled to −78 °C. Compound **34** (50 g, 198 mmol) in THF (200 mL) was added dropwise using addition funnel over 30 min, and the reaction mixture was stirred for 30 min. Iodine (60 g, 237 mmol) in 2-MeTHF (150 mL) was added dropwise. The reaction mixture was stirred at −70 to −40 °C over 2 h, at which point HPLC-analysis revealed 5−10% starting material. The reaction mixture was allowed to warm to RT, quenched with satd aqueous NH_4Cl solution (300 mL), and diluted with MTBE (1 L), and the organic layer was separated, washed with 1 M aqueous $Na_2S_2O_3$ (2 × 100 mL), H_2O (200 mL), and brine (200 mL), dried over Na_2SO_4, filtered through silica gel (50 g) plug, and the filtrate concentrated under reduced pressure. The crude product was triturated with methanol (2 × 100 mL) to afford **35** (40 g, 54% yield) as a light-yellow color solid. Additional product (20 g, 26% yield) was isolated from the filtrate after silica gel chromatography (5−10% EtOAc in hexanes eluent). HRMS: m/z calcd for $C_8H_6F_3INO_3S^+$ $[M + H]^+$, 379.9060; found, 379.9060. ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.12 (s, 1H), 8.28 (s, 1H), 3.92 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.13, 154.72, 154.33, 153.95, 153.57 (C═O, quart, J_{C-F} = 38.5 Hz), 141.38, 131.35, 119.73, 116.87, 114.01, 111.14 (CF_3, quart, J_{C-F} = 288.2 Hz), 118.70, 84.15, 52.62. ^{19}F NMR (282 MHz, $CDCl_3$) δ −75.89.

Methyl 3-Amino-5-iodo-thiophene-2-carboxylate 36. A stirred solution of **35** (96 g, 253.2 mmol) in 2-MeTHF (500 mL), methanol (300 mL), and H_2O (250 mL) was cooled to 0 °C, and K_2CO_3 (87 g, 630 mmol) was added portionwise. The resulting reaction mixture was slowly allowed to ambient temperature and stirred for 12 h, at which point HPLC and LCMS analysis revealed consumption of the starting material. The reaction mixture was concentrated under reduced pressure to remove methanol and 2-MeTHF. The residue was partitioned between EtOAc (2 L) and H_2O (500 mL), and the organic layer was separated, washed with brine (2 × 100 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure to afford methyl 3-amino-5-iodo-thiophene-2-carboxylate **36** (69.0 g, 96% yield) as a yellow solid. LCMS m/z = 283.9 $[M + H^+]$. HRMS: m/z calcd for $C_6H_7FNO_2S^+$ $[M + H]^+$, 283.9237; found, 283.9233. ^{1}H NMR (300 MHz, DMSO-d_6) δ 6.85 (s, 1H), 6.59 (br s, 2H), 3.69 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.47, 153.91, 129.51, 106.57, 83.54, 51.32.

Methyl 3-(*trans*-4-Methylcyclohexylcarbonylamino)-5-iodo-thiophene-2-carboxylate 37. *trans*-4-Methylcyclohexane-1-carboxylic acid (55.2 g, 389 mmol) was suspended in toluene (400 mL) and $SOCl_2$ (31 mL, 424 mmol) and DMF (0.5 mL) added and the mixture heated to 40 °C for 2 h. The solvent was evaporated and the residue taken up in toluene (400 mL) and re-evaporated. In a separate flask, **36** (100 g, 353 mmol) was dissolved in DCM (600 mL) and pyridine (35 mL, 425 mmol) and DMAP (2.16 g, 17.7 mmol) added and cooled to 0 °C. The previously prepared *trans*-4-tethylcyclohexane-1-carbonyl chloride was dissolved in DCM (400 mL) and slowly added to the mixture, maintaining an internal temperature below 20 °C. After addition was complete, the reaction was stirred for 1 h, allowing the reaction mixture to warm to RT. The reaction was quenched by addition of H_2O (500 mL) and 2 N HCl (500 mL). The organic phase was separated and washed with saturated aqueous $NaHCO_3$, H_2O, and brine. The solution was dried (Na_2SO_4), filtered, and rotary evaporated. Product **37** was isolated as a golden crystalline solid (144 g, 99%). LCMS m/z = 408.1 $[M + H^+]$. ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.22 (s, 1H), 8.41 (s, 1H), 3.89 (s, 3H), 2.26 (m, 1H), 2.03 (d, 2H), 1.83 (d, 2H), 1.65−1.28 (m, 2H), 1.43−1.28 (m, 3H), 1.11−0.95 (m, 2H), 0.90 (d, 3H).

Methyl 3-(*N*-(2-(*tert*-Butoxy)-2-oxoethyl)-(*trans*-4-methylcyclohexylcarbonyl)-amino)-5-iodo-thiophene-2-carboxylate 38. Methyl 3-(*trans*-4-methylcyclohexylcarbonylamino)-5-iodo-thiophene-2-carboxylate **37** (2.8 g, 6.88 mmol) was dissolved in THF (50 mL) and cooled to 0 °C under an argon atmosphere. A 1.0 M solution of LiHMDS (8.6 mL) in THF was added dropwise. The reaction was stirred at 0 °C for 30 min followed by the addition of *t*-butyl 2-bromoacetate (1.61 g, 8.25 mmol) in THF (5 mL). The reaction was warmed to RT then heated to 60 °C for 24 h. The reaction was cooled to RT then diluted with EtOAc (100 mL) and washed with water and brine, dried over anhydrous Na_2SO_4, filtered, and evaporated to afford a yellow oil, which was purified by silica gel column chromatograhy eluting with a gradient of 0−100% EtOAc in hexanes. The desired fractions were combined and evaporated in vacuo to afford product **38** as a light-yellow foam (2.29 g, 64%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.38 (s, 1H), 4.87 (s, 1H), 3.83 (s, 3H), 3.45 (s, 1H), 2.07 (m, 1H), 1.82−1.49 (m, 6H), 1.46 (s, 9H), 1.39−1.23 (m, 1H), 0.82 (d, 3H), 0.78−0.59 (m, 2H) (spectrum shows amide cis/trans isomers).

Methyl 3-(*N*-(2-(*tert*-Butoxy)-2-oxoethyl)-(*trans*-4-methylcyclohexylcarbonyl)-amino)-5-(3,3-dimethylbut-1-ynyl)-thiophene-2-carboxylate 39. Compound **38** and 3,3-dimethylbut-1-yne (1.55 mL, 13 mmol) were taken into diethylamine (51 mL) and placed under an argon atmosphere. The reaction was cooled to 0 °C. CuI (246 mg, 1.29 mmol) was added to the mixture and stirred for 5 min, followed

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J. Med. Chem. XXXX, XXX, XXX−XXX

by the addition of $Pd(PPh_3)_2Cl_2$ (304 mg, 0.43 mmol). The reaction was stirred for 30 min then warmed to RT and stirred overnight. The reaction was evaporated in vacuo and the residue purified by silica gel column chromatography eluting with a gradient of 0−50% EtOAc in hexanes. The desired fractions were combined and evaporated in vacuo to afford a yellow solid, **39** (1.7 g, 83%). LCMS m/z 476.4 $[M + H^+]$. ^1H NMR (300 MHz, $CDCl_3$) δ 7.16 (s, 1H), 4.87 (d, J = 17.2 Hz, 1H), 3.85 (s, 3H), 3.46 (d, J = 17.2 Hz, 1H), 2.23−1.99 (m, 1H), 1.86−1.50 (m, 7H), 1.47 (s, 9H), 1.34 (s, 9H), 0.83 (d, J = 6.5 Hz, 3H), 0.69 (m, 2H).

Methyl 3-(*N*-(2-(*tert*-Butoxy)-2-oxoethyl)-(*trans*-4-methylcyclohexylcarbonyl)-amino)-5-(3,3-dimethylbut-1-ynyl)-thiophene-2-carboxylate 40. Methyl 3-(*N*-(2-(*tert*-butoxy)-2-oxoethyl)-(*trans*-4-methylcyclohexylcarbonyl)-amino)-5-(3,3-dimethylbut-1-ynyl)-thiophene-2-carboxylate **39** (1.9 g, 4.0 mmol) was dissolved in DCM (10 mL). TFA (1.5 mL, 20 mmol) was added to the solution and the mixture stirred at RT for 5 h. The solution was evaporated to dryness then redissolved in DCM and the solvent evaporated (2×) to remove residual TFA. The product **40** was obtained as a foam (1.7 g), which was used without further purification. LCMS m/z 420.2 $[M + H^+]$.

3-(*N*-(-1-(Pyrrolidino)-1-oxoethan-2-yl)-(*trans*-4-methylcyclohexane)-1-carboxamido)-5-(3,3-dimethylbut-1-ynyl)-thiophene-2-carboxylic Acid Methyl Ester 41 (NR^2R^3 = Pyrrolidine). Carboxylic acid **40** (192 mg, 0.41 mmol) and a 0.5 M solution of HBTU (1.66 mL, 0.83 mmol) in DMF were placed in a reaction vial. DIEA (180 μL, 1.0 mmol) and pyrrolidine (72 μL, 0.86 mmol) were added and the reaction stirred at overnight at RT. Brine (60 mL) was added and the mixture extracted with iPrOAc (2 × 60 mL). The combined extracts were dried over Na_2SO_4, filtered, and evaporated. The product was purified by silica gel flash chromotography (ISCO 12 g column), eluted with 0−50% EtOAc in hexanes to afford the desired amide product **41** (NR^2R^3 = pyrrolidine) (130 mg, 68%). LCMS m/z 473.5 $[M + H^+]$. ^1H NMR (400 MHz, DMSO-d_6) δ 7.27 (s, 1H), 4.79 (d, J = 16.6 Hz, 1H), 3.78 (s, 3H), 3.77−3.69 (m, 2H), 3.37 (m, 2H), 3.29−3.18 (m, 2H), 2.16−2.01 (m, 1H), 1.86−1.80 (m, 2H), 1.79−1.69 (m, 2H), 1.68−1.55 (m, 2H), 1.53−1.31 (m, 2H), 1.30 (s, 9H), 1.29−1.11 (m, 2H), 0.77 (d, J = 6.5 Hz, 3H), 0.74−0.59 (m, 2H).

3-(*N*-(-1-(Pyrrolidino)-1-oxoethan-2-yl)-(*trans*-4-methylcyclohexane)-1-carboxamido)-5-(3,3-dimethylbut-1-ynyl)-thiophene-2-carboxylic Acid 20. Methyl ester **41** (NR^2R^3 = pyrrolidine) (130 mg, 0.28 mmol) was dissolved in MeOH (5 mL), NaOH (2N, 5 mL, 10 mmol) was added, and the reaction was stirred at RT overnight. The volatile solvent was evaporated and 1 N HCl (15 mL) and brine (15 mL) added. The mixture was extracted with 1:1 Et_2O:EtOAc (2 × 30 mL), and the combined extracts dried over Na_2SO_4, filtered, and evaporated. The crude product was purified by HPLC (40−95% CH_3CN in H_2O; 0.1% TFA) to give desired compound **20** (55 mg, 42%). LCMS m/z 459.5 $[M + H^+]$. ^1H NMR (400 MHz, DMSO-d_6) δ 7.25 (s, 1H), 4.77 (d, J = 16.7 Hz, 1H), 3.78 (d, J = 16.7 Hz, 1H), 3.62−3.09 (m, 5H), 2.18−2.03 (m, 1H), 1.89−1.83 (m, 2H), 1.80−1.68 (m, 2H), 1.66−1.49 (m, 4H), 1.35 (m, 1H), 1.29 (s, 9H), 1.28 (s, 1H), 0.78 (d, J = 6.5 Hz, 3H), 0.66 (m, 2H).

Compounds **17**−**27** were prepared by the methods described above for the preparation of **9** and as illustrated in Scheme 2. Analytical data is summarized in the Supporting Information.

Compound **23** was prepared at a larger scale by direct alkylation of **37** with chloroacetylmorpholine

Methyl 3-(*N*-(-1-(Morpholino)-1-oxoethan-2-yl)-(*trans*-4-methylcyclohexane)-1-carboxamido)-5-(3,3-dimethylbut-1-ynyl)thiophene-2-carboxylate, 41 (NR^2R^3 = morpholine). Compound **37** was dissolved in THF (2.2 L) and DMA (120 mL) and the solution cooled to 0 °C. A 1 M solution of LiHDMS in THF (840 mL) was added while keeping the reaction mixture below 15 °C, then allowing the temperature to rise to RT. NaI (120 g, 800 mmol) and 2-chloroacetylmorpholine (131 g, 800 mmol) were added and the mixture brought to 60 °C and stirred overnight. The reaction was cooled, aqueous NH_4Cl (10 L) was added, the pH was adjusted to ca. 5 with 2 N HCl, and the mixture was extracted with EtOAc (2×). The combined extracts were washed with water and brine and dried (Na_2SO_4). Solvent was evaporated and the residue filtered over silica gel (800 g), eluting with DCM and subsequently 25% EtOAc in hexane to elute product. Evaporation of solvent of solvent afforded **41** (NR^2R^3 = morpholine) which was isolated as a foam, 215 g, 66%. LCMS m/z 408.1 $[M + H^+]$. ^1H NMR (300 MHz, $CDCl_3$) δ 7.25 (s, 1H), 5.15 (d, J = 8 Hz, 1H), 3.8 (s, 3H), 3.75−3.25 (m, 9H), 2.1−2.0 (m, 1H), 1.8−1.7 (m, 1H), 1.7−1.3 (m, 6H), 1.25 (s, 9H), 0.75 (d, J = 4 Hz, 3H), 0.7−0.55 (m, 2H).

3-(*N*-(-1-(Morpholino)-1-oxoethan-2-yl)-(*trans*-4-methylcyclohexane)-1-carboxamido)-5-(3,3-dimethylbut-1-ynyl)-thiophene-2-carboxylic Acid 23. Compound **41** (NR^2R^3 = morpholine) (215 g, 440 mmol) was dissolved in THF (1 L) and H_2O (1 L). LiOH (31.6 g, 1.32 mol) was added and the reaction stirred at RT overnight. The mixture was acidified to pH 2 by addition of c·HCl and the mixture extracted with EtOAc (3×). The combined extracts were washed with water and brine, dried (Na_2SO_4), and evaporated to yield an oil that gradually crystallized. MTBE was added and the product **23** (180 g, 86%) isolated by filtration. LCMS m/z 475.6 $[M + H^+]$, 473.5 $[M - H^-]$. ^1H NMR (300 MHz, $CDCl_3$) δ 6.90 (s, 1H), 4.94 (d, J = 16.3 Hz, 1H), 4.02 (d, J = 16.3 Hz, 1H), 3.79−3.35 (m, 8H), 2.28 (m, 1H), 1.81 (d, J = 13.3 Hz, 1H), 1.74−1.44 (m, 4H), 1.30 (s, 9H), 0.88−0.58 (m, 5H). ^{13}C NMR (101 MHz, $CDCl_3$) 177.0, 167.7, 161.7, 142.1, 130.8, 130.0, 128.2, 107.2, 71.4, 66.5, 66.2, 50.1, 45.3, 42.6, 40.8, 34.2, 33.8, 31.8, 30.4, 29.8, 28.5, 28.4, 22.4

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jmedchem.6b00541.

Enzyme inhibition data, full replicon profiling, antiviral combination studies, dog PK data, compound analytical data, compound ^1H NMR spectra (PDF)

Molecular formula strings (CSV)

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Notes

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

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■ ABBREVIATIONS USED

CV, column volumes; ee, enantiomeric excess; ELISA, enzyme-linked immunosorbent assay; FaSSIF, fasted-state simulated intestinal fluid; FeSSIF, fed-state simulated intestinal fluid; HCV, hepatitis C virus; HRMS, high resolution mass spectrometry; IFN, interferon; LCMS, liquid chromatography−mass spectrometry; NS5B, nonstructural protein 5B; RBV, ribavirin; SFC, supercritical fluid chromatography; SOC, standard of care

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J. Med. Chem. XXXX, XXX, XXX−XXX

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