Bioorganic & Medicinal Chemistry Letters 22 (2012) 2629–2634

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Discovery of GS-9451: An acid inhibitor of the hepatitis C virus NS3/4A protease

X. Christopher Sheng *, Todd Appleby, Thomas Butler, Ruby Cai, Xiaowu Chen, Aesop Cho, Michael O. Clarke, Jeromy Cottell, William E. Delaney IV, Edward Doerffler, John Link, Mingzhe Ji, Rowchanak Pakdaman, Hyung-Jung Pyun, Qiaoyin Wu, Jie Xu, Choung U. Kim

Gilead Sciences, Inc., 333 Lakeside Dr, Foster City, CA 94404, USA

ARTICLE INFO

Article history:
Received 2 November 2011
Revised 4 January 2012
Accepted 9 January 2012
Available online 1 February 2012

Keywords:
HCV
GS-9451
NS3 Protease
Solubilizing group

ABSTRACT

The discovery of GS-9451 is reported. Modification of the P3 cap and P2 quinoline with a series of solubilizing groups led to the identification of potent HCV NS3 protease inhibitors with greatly improved pharmacokinetic properties in rats, dogs and monkeys.

Hepatitis C virus (HCV) has infected an estimated 3% of the world's population (170–200 million), making it a serious public health concern.[1] Since this disease was first reported in the 1980s,[2] extensive research has led to the first generation treatment of α-interferon (Peg-Intron® and Pegasys®) in combination with ribavirin. This therapy is only partially effective with ~50% of genotype 1 HCV patients demonstrating sustained viral responses (SVR). Recently, direct acting antivirals which inhibit the key enzymes in the viral replication process are emerging as new treatments with better overall response rates.[3] One of these viral targets is the serine protease NS3/4A. Efforts toward improved HCV therapy during the past several years have led to the recent FDA approval of telaprevir (VX-950)[4] and boceprevir (SCH 503034)[5], two α-keto-amide HCV NS3 protease inhibitors. However, some characteristics of these drugs can be improved, such as inconvenient dosing frequency (both are used three times a day).

The first carboxylic acid inhibitor of HCV NS3 protease, BILN-2061 (Fig. 1, compound **1**) was reported by Boehringer–Ingelheim with clinical proof of concept.[6] Through excellent design based on an inhibitor bound to the protease, cyclization of P1 to P3 led to a picomolar binding affinity and bioavailable NS3 protease inhibitor.[7]

It is well known that HCV protease has a featureless and relatively solvent exposed active site.[8] A potent inhibitor requires a series of weak lipophilic and electrostatic interactions distributed along its contact with the protease. As part of our ongoing antiviral drug discovery program, we decided to explore the potential for increased interactions using a fused bicyclic P3 cap and polar groups such as a weak amine attached to the P2 quinoline. In doing so, we envisioned we could (1) pick up additional interactions with the protease; (2) use polar groups to improve pharmaceutical properties such as aqueous solubility and PK. Herein we describe our efforts that culminated in the identification of a potent and highly bioavailable HCV NS3 inhibitor.

The chemistry used for our P3 cap modification is shown in Scheme 1. The bicyclic alcohol shown was converted to carbonate **2** in the presence of Et_3N. Activation of commercially available hydroxyl proline as the brosylate, followed by removal of the Boc group provided amine **3**. Coupling of **3** with (s)-*tert*-butyl glycine in the presence of HATU and subsequent saponification, provided the carboxylic acid dipeptide **4**. HATU mediated coupling provided tripeptide **5**. This tripeptide served as a key intermediate for the preparation of a number of analogs. For example, after the Boc protecting group removal, the free amine was reacted with *cis*-cyclopropylpentyl carbonate **2** to provide **6**. Displacement of the brosylate with the P2 quinoline and subsequent hydrolysis of the methyl ester provided compound **7**. Compounds **8** (*cis*-difluorocyclopropylpentyl) and **9** (*trans*-difluorocyclopropylpentyl) were synthesized in a similar fashion from the corresponding alcohols.

Compounds **7–9** were evaluated in a genotype 1b enzyme assay, and cellular activity was determined using the genotype 1b replicon. Table 1 summarizes the biochemical and replicon activities of these analogs. All three analogs are potent biochemical inhibitors of the HCV NS3 protease with IC_{50}'s in the single digit nanomolar range. However, in the replicon cells, *cis*-cyclopropylpentyl analogs

* Corresponding author. Tel.: +1 650 522 5902.
E-mail address: csheng@gilead.com (X.C. Sheng).

0960-894X/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2012.01.017

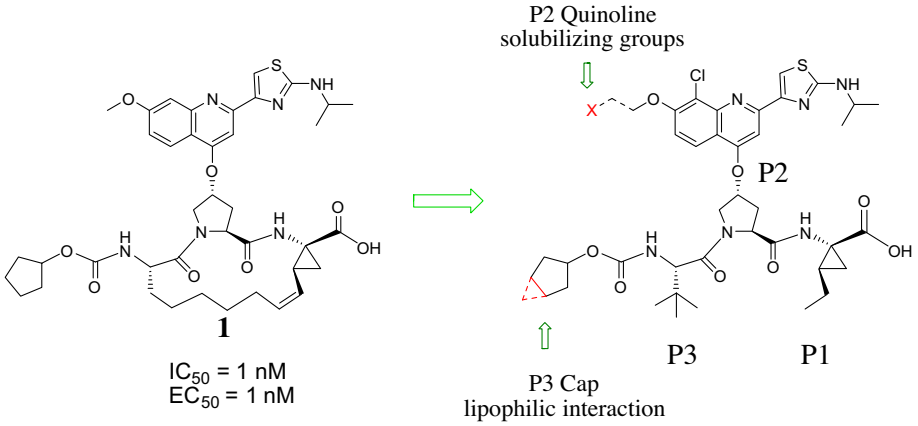
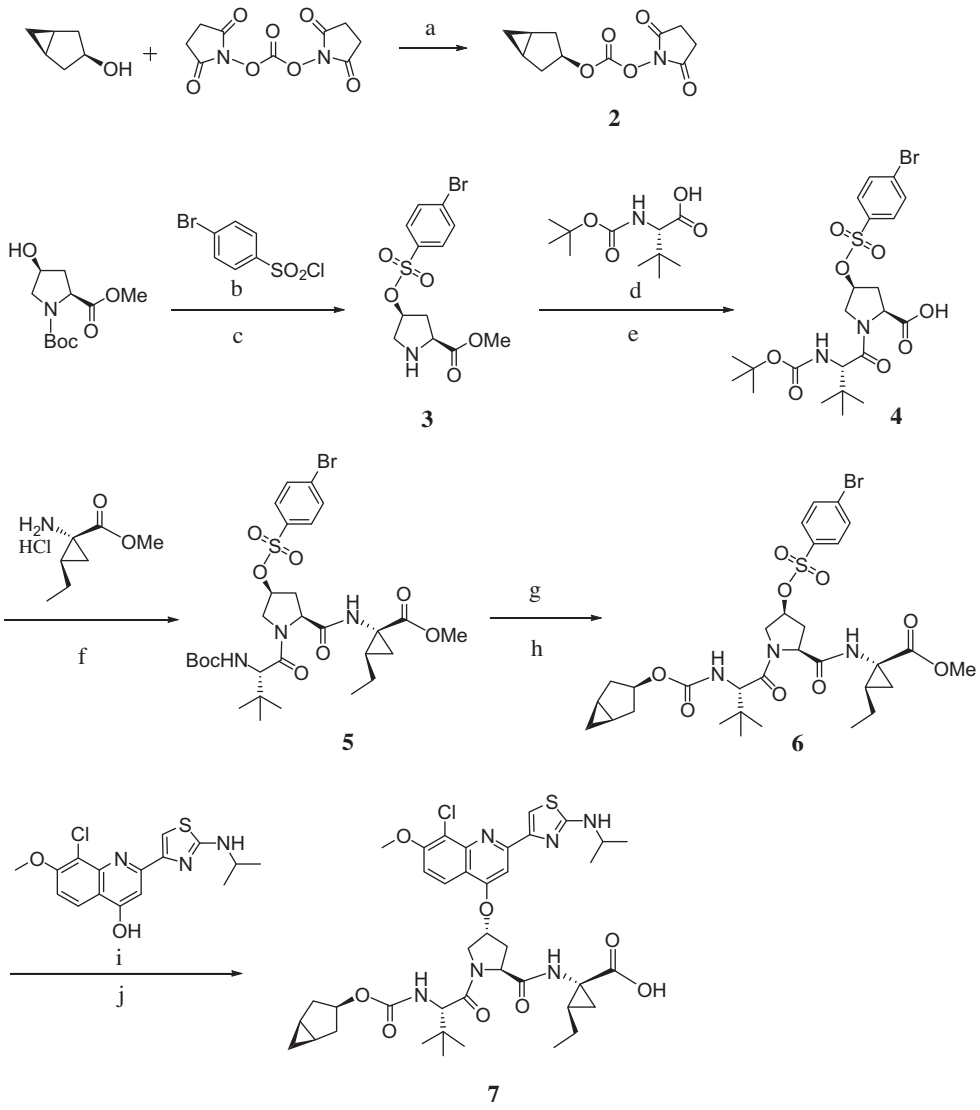


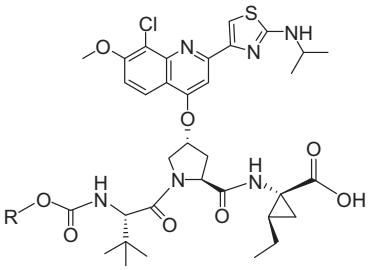
Figure 1. Carboxylic acid HCV NS3 protease inhibitors.

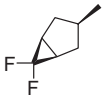
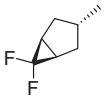


Scheme 1. Preparation of compound **7**. (a) alcohol, CH_2Cl_2, Et_3N, 75%; (b) DABCO, PhMe; (c) 4 N HCl, CH_2Cl_2, 94% for two steps (d) HATU, Huniq's base, DMF; (e) LiOH, THF–MeOH–H_2O, 82% for two steps; (f) HATU, Huniq's base, DMF, 90%; (g) 4 N HCl, CH_2Cl_2, 90%; (h) **2**, satd $NaHCO_3$, EtOAc, 92%; (i) Cs_2CO_3, NMP, 60 °C, 75%; (j) LiOH, THF–MeOH–H_2O, 85%.

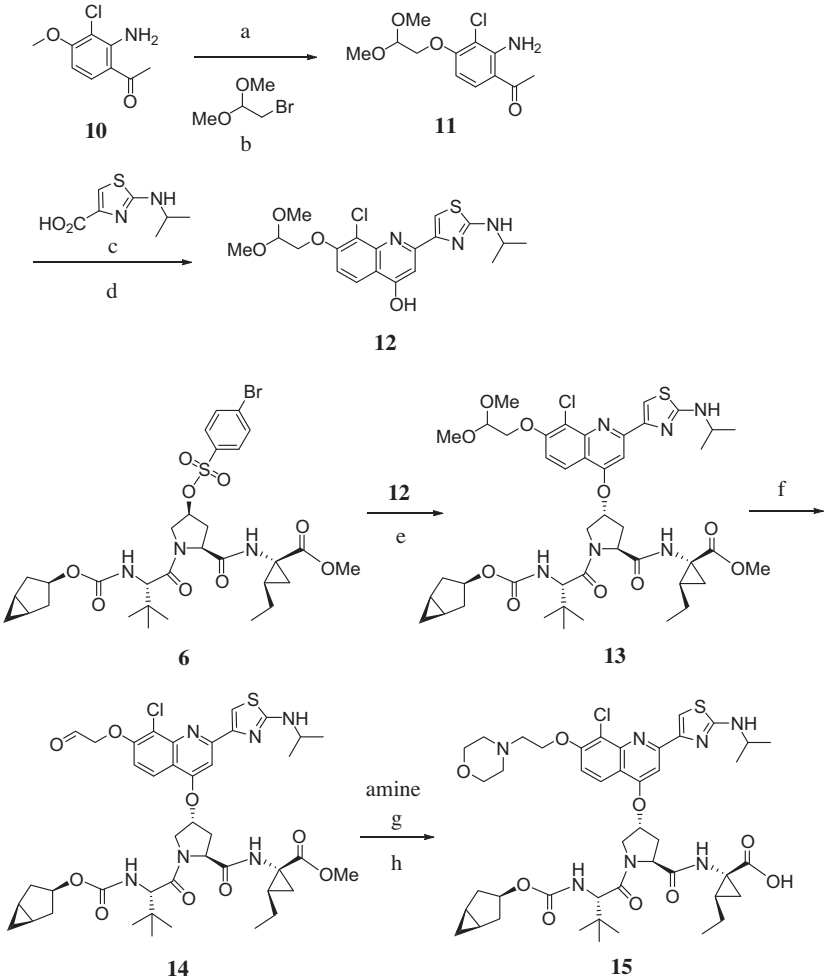
(compound **7** and **8**) were much more active than the *trans* analog (compound **9**). To further differentiate the two *cis* analogs, we tested their thermodynamic solubility and Caco-2 permeability. Compound **7** showed very promising solubility, especially at neutral pH as well as good forward permeability. Given the excellent potency and favorable aqueous solubility profile, the

Table 1
SAR of P3 pharmacophore



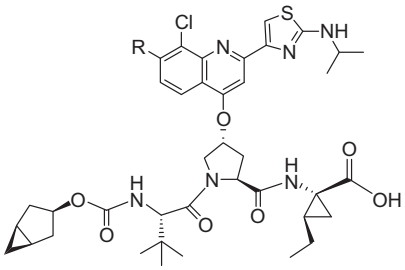
Compound	R	IC_{50}[a]/EC_{50}[b] (nM)	Sol (μM) pH7.3/2.2	Caco-2 A − B/B − A ($\times 10^{-6}$ cm/s)
7		1.4/9.0	72/BLQ	7.3/25
8		2.6/5.0	57/20	4.5/18
9		5.0/115	9/<6	6.8/49

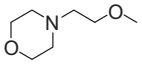
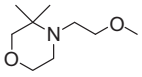
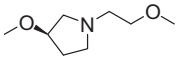
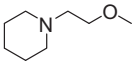
[a] IC_{50} determined by enzymatic assay using an HCV genotype 1b NS3/4A protein.
[b] EC_{50} determined by cell based assay using Huh-luc cells harboring subgenomic HCV genotype 1b replicon.



Scheme 2. Preparation of modified quinoline derivatives. (a) 48% HBr, reflux, 61%; (b) Cs_2CO_3, DMF, 65 °C, 67%; (c) $POCl_3$, Py, −40 to 0 °C, 85%; (d) NaH, PhMe, reflux, 86%; (e) Cs_2CO_3, NMP, 65 °C, 60%; (f) 1.4 N HCl, HOAc, 60°; (g) amine, CH_2Cl_2, $NaBH(OAc)_3$; (h) LiOH, THF–MeOH–H_2O, 71% over three steps.

Table 2
IC_{50}/EC_{50} measurements of modified quinoline analogs



Compound	R	IC_{50}/EC_{50} (nM)	Sol (μM) pH7.3/2.2	Caco-2 A − B/B − A ($\times 10^{-6}$ cm/s)
15		3.2/2.0	38/790	0.8/8.2
16		4.2/3.2	22/1900	6.6/10
17		2.9/3.8	59/>100	6.1/30
18		25/7.5	80/>100	6.7/20

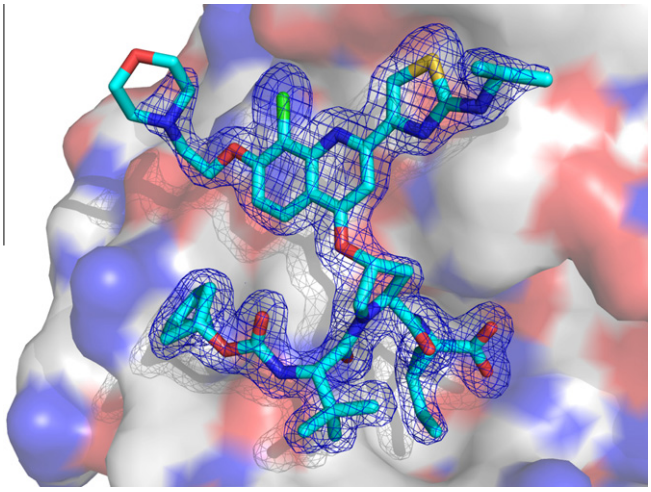


Figure 2. The refined 1.85 Å resolution 2Fo–Fc electron density map superimposed on the X-ray crystal structure of compound **15** bound to HCV protease.

Table 3
Dog PK parameters

Compound	iv $t_{1/2}$ (h)	Oral AUC (nM h L^{-1})	Cl (L/h/kg)	Vdss (L/kg)	*F* (%)
15	4.2	21760	0.26	1.15	142
16	5.8	38287	0.03	0.21	24
17	2.1	8331	0.22	0.66	45
18	3.0	19740	0.13	0.39	32

Dose, iv 1.0 mg/kg; po 4.0 mg/kg.

cis-cyclopropylpentyl carbamate functionality (P3 cap for compound **7**) was selected in our next round of SAR study which explores modified P2 qinoline analogs.

The synthesis of modified quinoline analogs is depicted in Scheme 2. Commercially available aniline **10** was treated with aqueous HBr to remove the methyl group. Alkylation with 2-bromo-1,1-dimethoxyethan provided **11**, which was converted to quinoline **12** following literature reported procedures.[7] Displacement of the brosylate **6** with quinoline **12** went smoothly to provide **13**. The conversion of the acetal group to aldehyde was accomplished with aqueous HCl in acetic acid to yield the key aldehyde intermediate **14**. Our synthesis of different analogs relies on reductive amination with a variety of amines using this aldehyde. As shown in Scheme 2, reductive amination with morpholine proceeded smoothly, and hydrolysis of the methyl ester afforded compound **15**. In a similar manner dimethylmorpholine, methoxypyrrolidine and pyperidine, provided compounds **16**, **17** and **18** in high yield.

Table 4
PK parameters of compound **15** (GS-9451)

	Rat	Dog	Cyno Monkey
Cl (L/h/kg)	4.4	0.26	0.29
Vdss (L/kg)	2.3	1.15	0.76
iv $t_{1/2}$ (h)	0.62	4.2	3.9
F (%)	62	143	49
Oral AUC (nM h L^{-1})	1409	21760	13121
Oral C_{max} (nM)	576	3937	3467
Unbound free fraction	1.1%	2.6%	1.6%
MS stability $t_{½}$ (min)	>395	>395	79
Human free fraction	2.6%		
Human MS stability $t_{½}$ (min)	>395		

Dose, iv 1.0 mg/kg; po 4.0 mg/kg.

All compounds of this series showed excellent antiviral activity in the cell-based HCV replicon assay (Table 2). In particular, the simple morpholine analog, compound **15**, was four-fold more active than the methoxy analog **7**. Compared to **7**, compound **15** showed dramatically increased solubility at low pH (790 μM vs BLQ) obviously a result of the charged morpholine under acidic conditions.

In order to confirm the binding mode for this class of inhibitors, we crystallized the HCV NS3/4A protease in the presence of compound **15** (Fig. 2). The X-ray crystal structure reveals that the *cis*-cyclopropyl bicycle stabilizes the required axial orientation of the cyclopentyl group. Addition of the bicyclic P3 cap also results in increased van der Waals contacts with the enzyme. As predicted from earlier modeling studies, the morpholine group is solvent

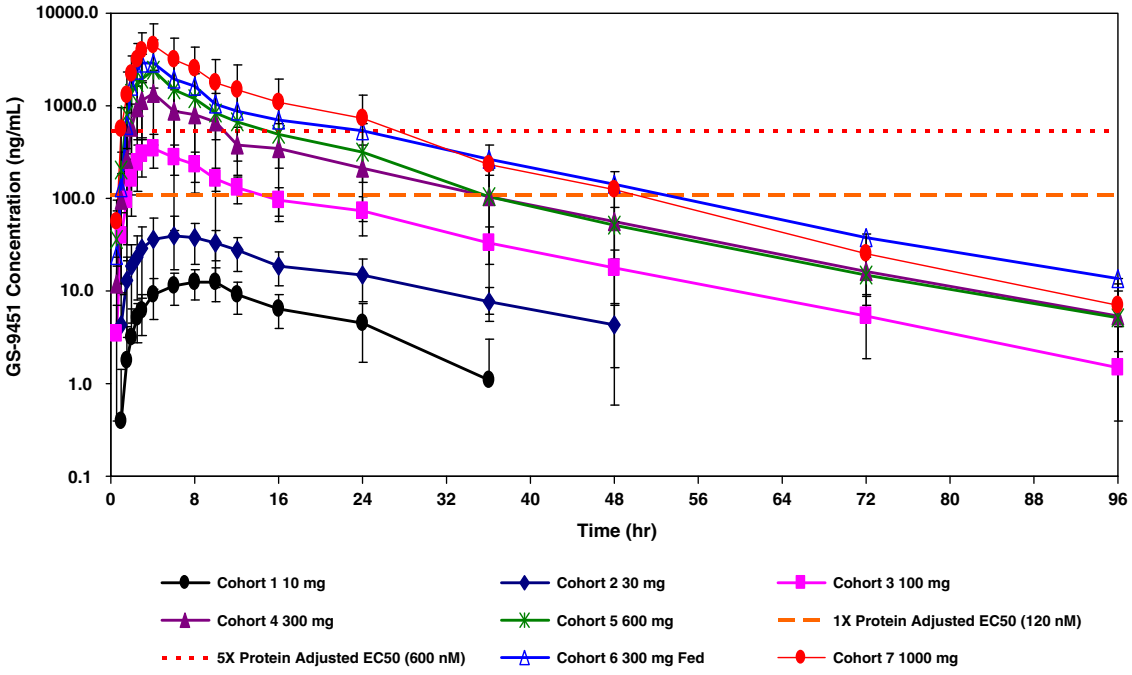
exposed and is disordered in the structure as indicated by weak electron density. The structure of compound **15** bound to NS3/4A suggests that this class of inhibitors is capable of forming extensive interactions with protein atoms in the active site and shallow substrate binding groove of the protease. In addition, the structure confirms our initial hypothesis that we can introduce functional groups which confer enhanced pharmaceutical and pharmacokinetic properties to the inhibitors without interfering with binding to the protease.

We next evaluated the pharmacokinetic profile of compounds **15**–**18**. In general, the compounds were characterized by low clearance and good oral bioavailability (Table 3). Compound **15** exhibited the highest oral bioavailability (142%) and volume of distribution (1.15 L/kg).

Because of its excellent potency and bioavailability, compound **15** was selected as a clinical development candidate. Table 4 summarizes pharmacokinetic parameters of **15** in rat, dog and cynomolgus monkey. Our aim was to determine if sufficient drug exposure would permit once daily dosing. Excellent plasma levels were observed after oral administration to dog and monkey. In rat, **15** was rapidly cleared (Cl 4.4 L/h/kg). The biliary excretion was examined in bile-duct cannulated rats following intravenous administration. Approximately 45 to 61% of the total dose was recovered as the parent compound; with glucuronide conjugates accounted for the remainder of total dose.

Compound **15** (GS-9451) was further profiled against a panel of mammalian proteases such as cathepsin D/L, human proteinase 3 and leukocyte elastase. GS-9451 showed selectivity of more than 50,000-fold selectivity over the human proteases assayed. In safety pharmacology screens, GS-9451 was not an inhibitor of CYP-450 enzymes and did not block the hERG channel at concentrations up to 25 μM. GS-9451 showed moderate dose-dependent inhibition of P-gp, MRP1 and MRP2 with IC_{50} values of 34, 14.9 and 12 μM, respectively. It was a relatively potent inhibitor of BCRP (breast cancer resistant protein), with an IC_{50} value of 1.4 μM. GS-9451 could potentially affect the disposition of drugs that are substrates of BCRP.

In a phase 1 single-dose escalation study, GS-9451 demonstrated excellent exposure over the range of 10–1000 mg. The mean elimination half-life was around 12–14 h for all dose groups, suitable for once daily dosing. For the 300 mg dose group, the trough plasma concentration (C_{24} ~400 nM) is 3 times the protein adjusted EC_{50} (120 nM).



When administered orally to patients infected with HCV genotype 1a, median HCV RNA declines were −0.88, −3.2 and −3.6 log 10 IU/mL for three days at doses of 60, 200, and 400 mg, respectively.[9]

In summary, a new class of carboxylic acid HCV NS3 protease inhibitors with excellent antiviral activity and bioavailability was identified. Compound **15** (GS-9451) exhibited low toxicity as determined by cell culture CC_{50} and high selectivity over mammalian proteases. In a phase 1b study, GS-9451 showed a rapid onset of viral load reduction, comparable to known inhibitors reported so far. GS-9451 is currently in phase 2b clinical trial for the treatment of hepatitis C infection.

Acknowledgments

The authors thank Huiling Yang, Margaret Robinson and Scott Hluhanich for determining biological activities.

References and notes

1. a De Francesco, R.; Migliaccio, G. *Nature* **2005**, *436*, 953; b Sheldon, J.; Barreiro, P.; Soriano, V. *Expert Opin. Investig. Drugs* **2007**, *16*, 1171.
2. Choo, Q.; Kuo, G.; Weiner, A.; Overby, L.; Bradley, D.; Houghton, M. *Science* **1989**, *244*, 359.
3. (a) Ni, Zhi-Jie.; Wagman, Allan S. *Curr. Opin. Drug Disc. Devel.* **2004**, *7*, 4; (b) Kwong, Ann D.; McNair, Lindsay.; Jacoboson, Ira.; George, Shelley. *Curr. Opin. Pharm.* **2008**, *8*, 1; (c) Jensen, Donald M.; Ascione, Antonio. *Antivir. Ther.* **2008**, 31–36; d Irena, Melnikova. *Nat. Rev. Drug Disc.* **2011**, *10*, 94.
4. (a) Reesink, H. W.; Zeuzem, S.; Weegink, C. J.; Forestier, N.; van Vliet, A.; van de Wetering de Rooij, J.; McNair, L.; Purdy, S.; Kauffman, R.; Alam, J.; Jansen, P. L. M. *Gastroenterology* **2006**, *131*, 997; (b) Jacobson, Ira M.; McHutchison, John G.; Dusheiko, Geoffrey.; Di Bisceglie, Adrian M.; Reddy, K. Rajender.; Bzowej, Natalie H.; Marcellin, Patrick.; Muir, Andrew J.; Ferenci, Peter.; Flisiak, Robert.; George, Jacob.; Rizzetto, Mario.; Shouval, Daniel.; Sola, Richard.; Terg, Ruben A.; Yoshida, Eric M.; Adda, Nathaile.; Bengtsson, Leif.; Sankoh, Abdul J.; Kieffer, Tara L.; George, Shelley.; Kauffman, Robert S.; Zeuzem, Stefen. *N. Engl. J. Med* **2011**, *364*, 2405.

5. a Malcolm, B. A.; Liu, R.; Agrawal, S.; Belanger, B.; Butkiewicz, N.; Chase, R.; Gheyas, F.; Hart, A.; Hesk, D.; Ingravallo, P.; Jiang, C.; Kong, R.; Lu, J.; Pichardo, J.; Prongay, A.; Skelton, A.; Tong, X.; Venkatraman, S.; Xia, E.; Girijavallabhan, V.; Njoroge, F. G. *Antimicrob. Agents Chemother.* **2006**, 1013; (b) Njoroge, F. George.; Chen, Kevin X.; Shin, Neng-Yang.; Piwinski, John J. *Acc. Chem. Res.* **2008**, *41*, 1.
6. Lamarra, D. A.; Bailey, M., et al *Nature* **2003**, *426*, 186.
7. Goudreau, N.; Brochu, C.; Cameron, D.; Duceppe, J.; Faucher, A.; Ferland, J.; Grand-Maitre, C.; Poirier, M.; Simoneau, B.; Tsantrizos, Y. *J. Org. Chem.* **2004**, 6185.
8. a Tsantrizos, Y. S.; Bolger, G.; Bonneau, P.; Cameron, D. R.; Goudreau, N.; Kukolj, G.; LaPlante, S. R.; Llinas-Brunet, M.; Nar, H.; Lamarre, D. *Angew. Chem., Int. Ed.* **2003**, *42*, 1355; b LaPlante, S. R.; Llinas-Brunet, M. *Curr. Med. Chem.-Anti-Infective Agents* **2005**, *4*, 111.
9. Lawitz, E.; Marbury, T.; Vince, B.; Grunenberg, N.; Rodriguez-Torres, M.; DeMicco, M.; Tarro, J.; Shelton, M.; West, S.; Zong, J.; Bae, A.; Wong, K.; Mo, H.; Oldach, D.; Delaney, W.; Rousseau, F. EASL, 2010.