Bioorganic & Medicinal Chemistry Letters xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Design and synthesis of lactam–thiophene carboxylic acids as potent hepatitis C virus polymerase inhibitors

David Barnes-Seeman [a], Carri Boiselle [b], Christina Capacci-Daniel [b], Rajiv Chopra [a], Keith Hoffmaster [b], Christopher T. Jones [c,†], Mitsunori Kato [a], Kai Lin [c], Sue Ma [c,†], Guoyu Pan [b], Lei Shu [a], Jianling Wang [b], Leah Whiteman [c], Mei Xu [a], Rui Zheng [a], Jiping Fu [a,*,†]

[a] Global Discovery Chemistry, Novartis Institutes for BioMedical Research, 250 Massachusetts Avenue, Cambridge, MA 02139, United States
[b] Metabolism & Pharmacokinetics, Novartis Institutes for BioMedical Research, 250 Massachusetts Avenue, Cambridge, MA 02139, United States
[c] Department of Infectious Diseases, Novartis Institutes for BioMedical Research, 500 Technology Square, Cambridge, MA 02139, United States

ARTICLE INFO

Article history:
Received 23 April 2014
Revised 8 June 2014
Accepted 11 June 2014
Available online xxxx

Keywords:
HCV
NS5B polymerase
PXR
CYP induction
Lactam
Thiophene

ABSTRACT

Herein we report the successful incorporation of a lactam as an amide replacement in the design of hepatitis C virus NS5B Site II thiophene carboxylic acid inhibitors. Optimizing potency in a replicon assay and minimizing potential risk for CYP3A4 induction led to the discovery of inhibitor **22a**. This lead compound has a favorable pharmacokinetic profile in rats and dogs.

Hepatitis C virus (HCV), which chronically infects an estimated 3% of the world's population, is an important causative agent of liver fibrosis, cirrhosis and hepatocellular carcinoma.[1,2] In the last decade, the focus of anti-HCV drug discovery has been on developing interferon-free treatment regimens.[3,4] In order to effectively suppress viral resistance, it is necessary to use combination therapy with agents that target multiple viral proteins or host pathways. The HCV RNA-dependent RNA polymerase (NS5B) is an attractive drug target that is essential for the replication of the viral genome.[5,6] A number of nucleoside-based and non-nucleoside inhibitors of NS5B have demonstrated efficacy in clinical trials.[7–9] Sofosbuvir, a nucleotide-based HCV polymerase inhibitor and the first interferon-free treatment for HCV genotypes 2 and 3, was approved by the FDA in 2013.[10]

Thiophene carboxylic acids, with structures represented by compounds **1** and **2** (Fig. 1), are characteristic of a class of polymerase inhibitors that bind to the allosteric Thumb Site II of the NS5B protein.[11–15] This binding site is located near the base of the thumb domain and is approximately 35 Å away from the polymerase active site.[16] The interaction between this class of inhibitors and the NS5B enzyme is largely hydrophobic, with the only hydrogen bonding occurring between the carboxylic acid and the amide nitrogens of S476 and Y477 (Fig. 2).[17] The amide linkage and the isopropyl group are important structural elements that maintain an optimal dihedral angle between the amide and thiophene groups. These interactions ensure that the *trans*-methyl-cyclohexyl group is well positioned in the hydrophobic pocket.

Recently, a series of thumb Site II thiophene carboxylic acid inhibitors (exemplified by compound **3**) was reported that features aryl and heteroaryl groups as replacements for the amide linkage.[18] In these compounds, it is expected that the hetero aromatic group will maintain similar geometric, electronic and hydrogen bonding properties as the amide group. Although inhibitors such as **3** were potent inhibitors of the purified NS5B enzyme (IC_{50}, 0.1 μM), the compounds had weak potency in the replicon assay (EC_{50}, 15 μM). In this Letter, we describe the successful implementation of a lactam group as a replacement for the amide linkage, yielding thiophene–lactam carboxylic acids as potent NS5B Site II inhibitors.[19] These compounds were further optimized to reduce activation of the pregnane X receptor (PXR), the nuclear hormone receptor responsible for CYP3A4 induction.

Our initial design of Site II inhibitors, similar to designs described previously,[18] incorporated heterocycles as amide isoster-

* Corresponding author. Tel.: +1 510 923 2043.
E-mail address: jiping.fu@novartis.com (J. Fu).
† Current address: Novartis Institutes for BioMedical Research, 5300 Chiron way, Emeryville, CA 94608, United States.

http://dx.doi.org/10.1016/j.bmcl.2014.06.031
0960-894X/

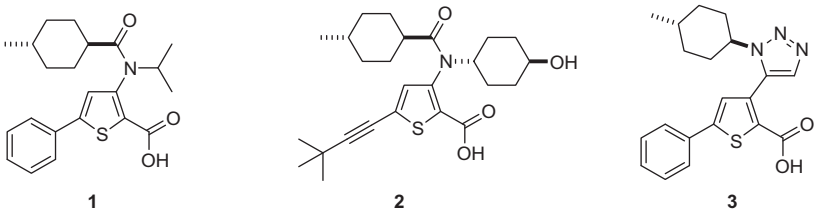


Figure 1. Thiophene carboxylic acid Site II inhibitors.

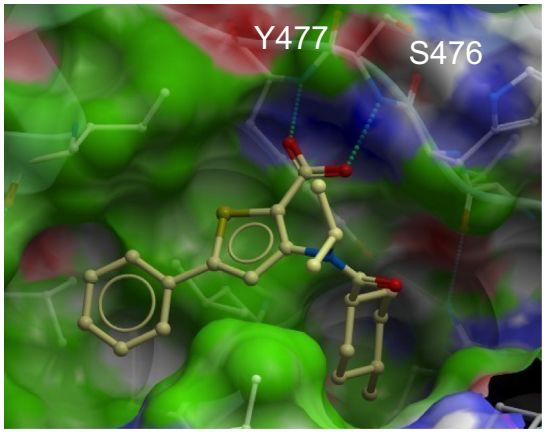
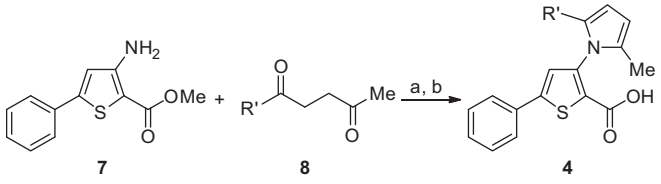


Figure 2. Co-crystal structure of **1** and HCV polymerase enzyme complex.[17]



Scheme 1. Synthesis of pyrrole analogs. Reagents and conditions: (a) R′ = *c*-hexyl, 150 °C; or R′ = 4-*trans*-Me-*c*-hexyl, I_2, THF; (b) NaOH or LiOH, THF, MeOH, water.

es (Fig. 3). In addition to heteroaryl groups, such as pyrrole, we also examined saturated lactams as amide replacements. These two structures are complementary in that they have different hybridization and therefore offer the possibility of tuning the trajectory of the hydrophobic group.

The pyrrole ring was constructed by a Paal–Knorr condensation reaction between 3-amino-thiophene **7** and diketone **8** either with heat or using I_2 as a promoter (Scheme 1).[20] Hydrolysis under basic conditions gave thiophene carboxylic acid **4**. The synthesis of lac-

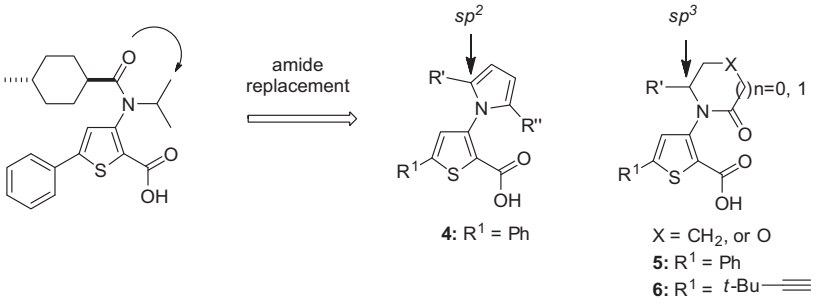
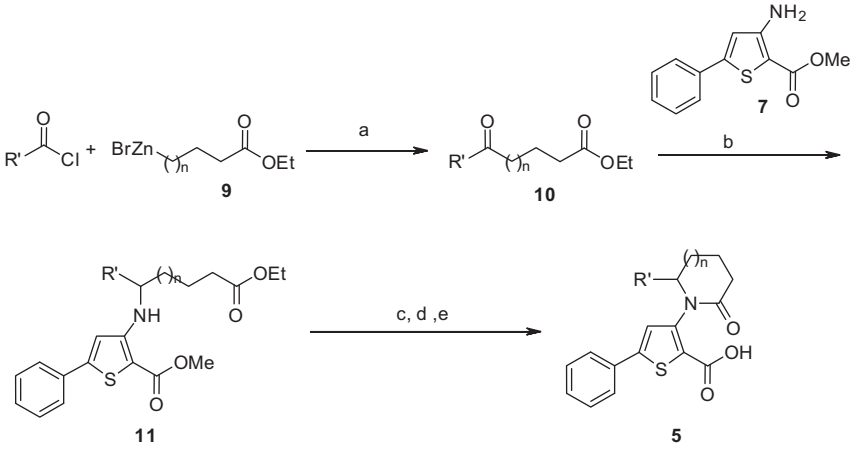
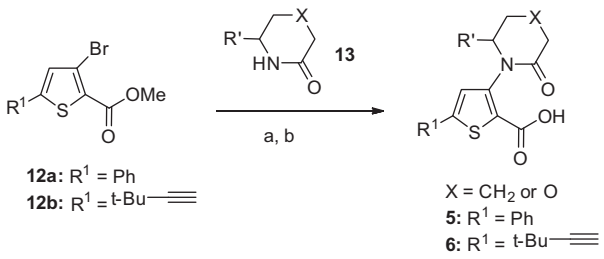


Figure 3. Replacement of amide with pyrrole and lactam.



Scheme 2. Synthesis of lactam analogs. Reagents and conditions: (a) $Pd(PPh_3)_4$, THF, rt; (b) Bu_2SnCl_2, Ph_3SiH, THF; (c) LiOH, THF, water, MeOH; (d) Py, $(Boc)_2O$, CH_2Cl_2; (e) LiOH, THF, MeOH, water.

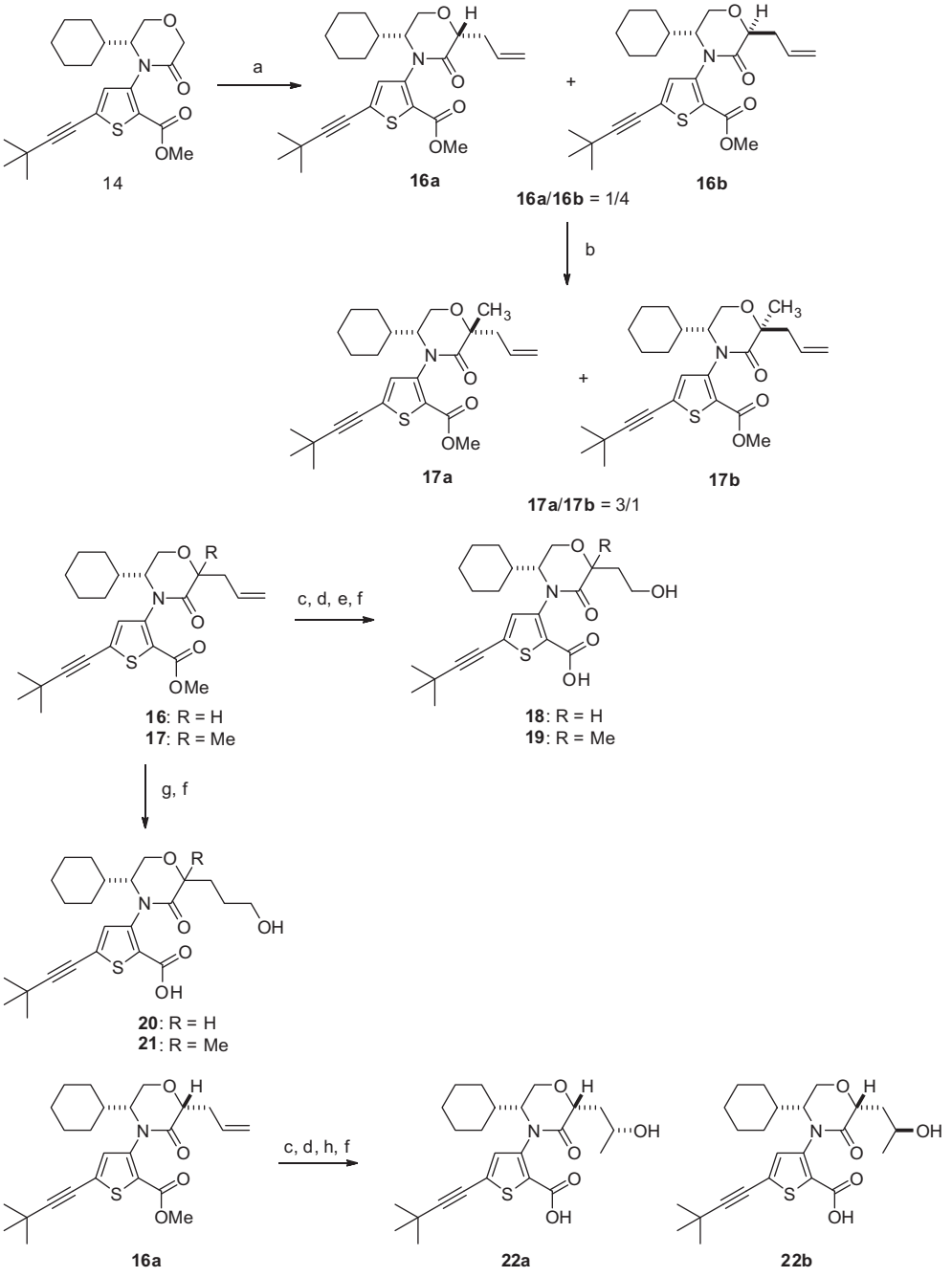
Please cite this article in press as: Barnes-Seeman, D.; et al. *Bioorg. Med. Chem. Lett.* (2014), http://dx.doi.org/10.1016/j.bmcl.2014.06.031



Scheme 3. Synthesis of lactam analogs by CuI catalyzed cross coupling. Reagents and conditions: (a) CuI, *trans*-cyclohexanediamine, K_2CO_3, dioxane, 110 °C; (b) LiOH or NaOH, THF, MeOH, water.

tam–thiophenes is illustrated in Scheme 2. Various acyl chlorides were coupled with zinc bromide **9** in the presence of catalytic $Pd(PPh_3)_4$.[21] A reductive amination between ketoester **10** and 3-aminothiophene **7** was accomplished using $PhSiH_3$ as the reducing agent and catalytic Bu_2SnCl_2.[22] The ethyl ester on the alkyl chain in **11** was then selectively hydrolyzed, and the resulting carboxylic acid cyclized to form the desired lactam with Boc_2O and pyridine. Finally, the thiophene carboxylic ester was hydrolyzed with LiOH to give **5**. All lactam analogs were prepared in racemic form using this synthetic route.

Thiophene lactams could also be synthesized by a Cu-catalyzed coupling reaction between thiophene bromide **12** and lactam **13** (Scheme 3).[23] In this route, enantiomerically pure products could be obtained by using enantiomerically pure lactams.



Scheme 4. Synthesis of α-substituted morpholinone analogs. Reagents and conditions: (a) LDA, allyl iodide, THF; (b) LDA, MeI, THF; (c) OsO_4, NMO, acetone, water; (d) $NaIO_4$, THF, water; (e) $NaBH_4$, MeOH; (f) LiOH, THF, MeOH, water; (g) 9-BBN, THF; H_2O_2, $NaHCO_3$, water; (h) MeMgBr, THF.

Please cite this article in press as: Barnes-Seeman, D.; et al. *Bioorg. Med. Chem. Lett.* (2014), http://dx.doi.org/10.1016/j.bmcl.2014.06.031

The α-position of morpholinone could be further substituted to generate an additional series of analogs **18**–**21** (Scheme 4). Morpholinone **14** was alkylated with an allyl group to give **16a** and **16b** in a 1:4 ratio. The two diastereomers were separated by silica gel chromatography and converted to the thiophene carboxylic acids separately. The stereochemical assignment of the alkylation product **16a** was assigned based on a cocrystal structure of its derivative **22a** and the NS5B enzyme.[24] The major product **16a** derived from the allyl group reacting on the face of enolate opposite to the cyclohexane group. This observation is consistent with the diastereoselectivity previously reported on morpholinone alkylations.[25] Morpholinone **17** with a quaternary carbon center at the α-position was prepared by methylation of **16** with LDA as the base. The methylation gave a 3:1 mixture of diastereomers. The major diastereomer was putatively assigned to be **17a**, with the methyl group adding from the face opposite to cyclohexane group.

The diastereomers in both **16** and **17** were further converted to the desired thiophene carboxylic acids with tethered hydroxyl groups. The allyl group was converted to ethyl alcohols **18** and **19** or propyl alcohols **20** and **21** through standard olefin cleavage/reduction or hydroboration sequences followed by basic hydrolysis. Secondary alcohols **22a** and **22b** were synthesized by addition of methyl Grignard to the olefin cleavage product of **16a** followed by hydrolysis.

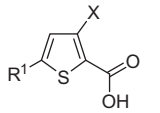
The potencies of pyrroles **4** and lactams **5** and **6** against purified NS5B enzyme and in the cell-based GT-1b replicon system are summarized in Table 1. Replacing the amide in **1** with pyrroles, as in compound **4a** and **4b**, resulted in significant reductions in potency against the enzyme. Neither **4a** nor **4b** had measurable inhibition in the replicon assay. In this scaffold, similar to the thiophene amide series, a *trans* methyl group on the cyclohexyl ring was found to improve potency ten-fold in the enzyme assay.

In the lactam series, our initial attempt was to replace the amide in **1** with a piperidinone. Unfortunately, compound **5a**, which contains a 4-*trans*-methyl-cyclohexyl group, showed low potency in both enzymatic and replicon assays. We hypothesized that the positioning and trajectory of the cyclohexyl group might be different in **1** and **5a**, and therefore, the fit of the *trans*-methyl-cyclohexyl in the pocket might not be optimal. Thus, we also prepared compound **5b** with a cyclohexyl group. Remarkably, this analog showed promising enzyme inhibition and potency in the replicon assay. Having observed how subtle changes in this lipophilic R group can result in dramatic effects on inhibitory activity, we went on to survey other substituents, including cyclopentyl, THP and phenyl groups (Table 1, compounds **5c**, **5d**, **5e**). Unfortunately, these modifications uniformly resulted in a significant loss in potency. Finally, the pyrrolidinone **5f** was slightly less potent compared to the piperidinone analog **5b**.

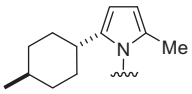
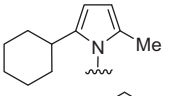
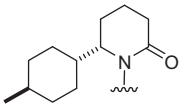
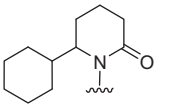
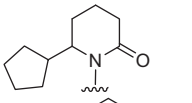
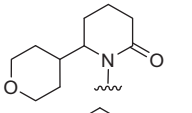
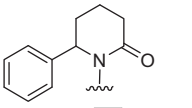
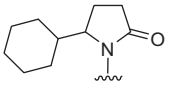
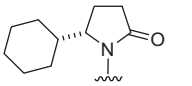
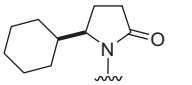
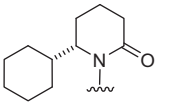
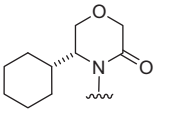
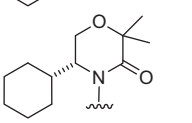
Compounds **5a**–**f** were all synthesized in racemic form. Compound **5f** was further separated into two enantiomers **5g** and **5h**. Only one enantiomer (**5g**) was responsible for the observed inhibition. The absolute configuration of **5g** was established based on a co-crystal structure with NS5B enzyme (Fig. 4).[26] This structure showed that the cyclohexyl group overlaps very well with the methyl-cyclohexyl group of **1** and fully occupies the hydrophobic pocket. This observation explains why the addition of a methyl group to the cyclohexyl ring of **5g** would be detrimental to the potency of the compound against the enzyme. Incidentally, in another class of thumb Site II inhibitor, namely dihydropyran-2-ones, discovered by Pfizer, the cyclopentane group also occupies this hydrophobic pocket and is the optimal substituent for enzymatic potency.[27–29]

Further potency improvements were achieved by replacing the phenyl group in the thiophene ring with a *t*-butyl alkynyl group.[13] With the knowledge that an *S*-configuration is required for potency, the lactam analogs were prepared in enantiomerically pure form (**6a**–**6c**). Compound **6a** has an EC_{50} of 12 nM in the replicon assay, which is significantly more potent than the corresponding phenyl analog **5b**. The piperidinone could be replaced by morpholinone (**6b**) without losing potency. Dimethyl substitution in the α position

Table 1
Amide replacement SAR



4, 5: R^1 = Ph
6: R^1 = *t*-Bu—≡

Compound	Heterocycle X	NS5B IC_{50} (μM)	Replicon 1b EC_{50} (μM)
1		0.060	0.27
4a		1.5	>33
4b		10.5	>33
5a		0.74	11.6
5b		0.073	0.33
5c		0.72	3.7
5d		>10	>33
5e		0.87	5.8
5f		0.18	0.64
5g		0.12	0.39
5h		>10	>33
6a		0.010	0.012
6b		0.003	0.017
6c		0.002	0.005

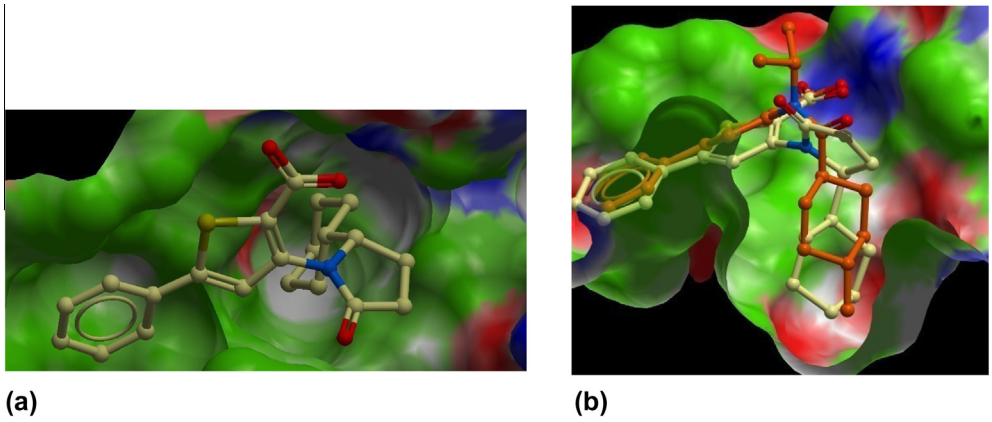
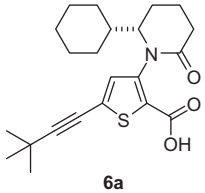
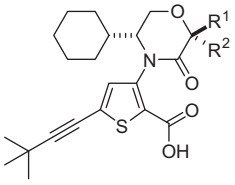


Figure 4. (a) Co-crystal structure of **5g** and HCV polymerase enzyme; (b) Overlay co-crystal structure of **1** and **5g** complexed with the HCV polymerase enzyme.

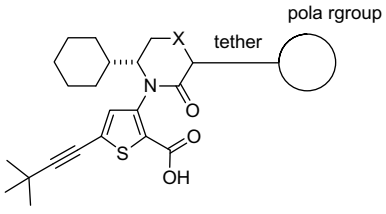
Table 2
Anti-HCV activity and PXR activation profiles of piperidinone and morpholinones



6a



Cpd	R^1	R^2	NS5B IC_{50} (μM)	Replicon 1b EC_{50} (μM)	PXR activation at 10 μM
6a			0.01	0.012	141%
6b	H	H	0.003	0.017	87%
6c	Me	Me	0.002	0.005	145%
18a	H	CH_2CH_2OH	0.002	0.007	3%
18b	CH_2CH_2OH	H	0.012	0.066	NA
19a	Me	CH_2CH_2OH	0.002	0.003	77%
19b	CH_2CH_2OH	Me	0.006	0.020	29%
20a	H	$CH_2CH_2CH_2OH$	0.002	0.014	5%
20b	$CH_2CH_2CH_2OH$	H	0.035	0.083	NA
21a	Me	$CH_2CH_2CH_2OH$	0.002	0.011	34%
21b	$CH_2CH_2CH_2OH$	Me	0.004	0.025	8%
22a	H		0.003	0.008	9%
22b	H		0.002	0.030	4%



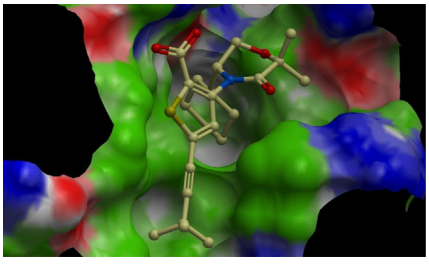


Figure 5. (a) Strategy to reduce PXR induction. (b) Model of morpholinone **6c** binding to the NS5B enzyme.

of morpholinone further improved replicon EC_{50} to 5 nM (**6c**). Since the polymerase enzymatic assay has a lower limit of detection of 2 nM, the most potent compounds could not be differentiated based on this method.

Further profiling of **6a**–**6c** in off-target safety assays indicated that these compounds were strong activators of PXR, a key transcription factor responsible for upregulation of drug metabolizing enzymes, notably CYP3A4.[30–34] In a PXR reporter gene assay, at a 10 μM

concentration, compounds **6a**–**6c** activated PXR by 87–145% relative to rifampicin (Table 2). Morpholinone **6b** had slightly lower PXR activation than piperidinone **6a**. Adding two methyl groups at the α-position regained PXR activation. The potential for **6b** to induce CYP3A4 was further confirmed by measuring testosterone metabolism (a CYP3A4 substrate) and CYP3A4 mRNA expression in human hepatocytes. Pretreatment of hepatocytes with 10 μM of **6b** increased CYP3A4-mediated testosterone metabolism by 61% and increased CYP3A4 mRNA expression level by 66% as compared to treatment with 10 μM rifampicin.

From a regulatory perspective, a negative in vitro result for CYP3A4 induction in the PXR assay eliminates the need for additional in vitro or in vivo induction studies for CYP3A4.[32] In early drug discovery, PXR data, as expressed in the percentage of activation compared with a positive control such as rifampicin at a particular concentration (e.g., 10 μM), have been widely used to rank order of the CYP3A4 induction potential of test compounds. Mitigating the risk of CYP3A4 induction early in drug discovery can help streamline the development process, and maximize the ability to combine our NS5B inhibitors with other important HCV therapies. Thus, we sought to reduce PXR activation in the reporter gene assay to less than 25% of that induced by 10 μM rifampicin for this series. For promising compounds that met this criteria, follow up studies in human hepatocytes were used to confirm a lack of CYP3A4 induction.

Several crystal structures of the human PXR ligand-binding domain (hPXR LBD) complexed with xenobiotic ligands have been reported.[35–38] The human PXR ligand binding site is unusually large and flexible. A pharmacophore containing one H-bond acceptor and three hydrophobic groups for binding to the PXR protein has been described.[39,40] Compounds **6a**–**6c** contain a carboxylic acid and several hydrophobic groups and therefore contain functionality consistent with this pharmacophore model. One strategy to reduce PXR activation is to introduce polar groups to the activator in order to destabilize interactions in the hydrophobic areas of the PXR ligand-binding pocket (Fig. 5a).[40] In compound **6**, the *t*-butyl group and cyclohexyl group form hydrophobic interactions with NS5B, and are required for binding. The position α to the carbonyl is located near the solvent opening of the binding pocket and appears to be the ideal position to append polar groups (Fig. 5b). Based on this hypothesis, morpholinones **18**–**21** with alcohols attached to the α position were prepared (Table 2).

Initial profiling revealed that the two diastereomers had markedly different potencies in both the enzymatic and replicon assays (Table 2). Compounds **18a**, **19a**, **20a**, and **21a**, which have tethered hydroxyl groups *cis* to the cyclohexyl group, were more potent than the diastereomers **18b**, **19b**, **20b**, and **21b**. Compounds **18a** and **20a**, which are monosubstituted at the α-position, had slightly improved replicon potency compared to **6b**. Meanwhile, the isomers **18b** and **20b** had much reduced potency in the NS5B enzyme and replicon assays. The two potent diastereomers **18a**, **20a** had much reduced PXR activation in the reporter gene assay, with 3% and 5% of the maximum activation by rifampicin at 10 μM, respectively. Compared to compounds **18a**, **18b**, **20a**, and **20b**, the addition of a methyl group afforded compounds **19a**, **19b**, **21a** and **21b**, which had slightly improved replicon potency. However, this change increased PXR activation especially for **19a** and **19b**. Secondary alcohols **22a** and **22b** also had much reduced PXR activation. Among these two, the more potent diastereomer **22a** had an EC_{50} 8 nM in the replicon assay, which was comparable to the EC_{50} of primary alcohol **18a**.

Compounds with the most promising profile based on potency in the replicon assay and low CYP3A4 induction risk were further tested in a human hepatocyte CYP3A4 activity assay. Whereas compound **6b** increased CYP3A4-mediated testosterone metabolism by 61%, compounds **18a**, **20a**, and **22a** only caused 5%, 2%, and 2% increases, respectively, as compared to treatment with 10 μM rifampicin. These compounds are therefore anticipated to have a low risk of CYP3A4 induction.

Table 3
Comparison of pharmacokinetic parameters of **18a**, **20a** and **22a** in rats and dogs

Compound		18a	20a	22a
Rat IV (1 mg/kg)	AUC [nmol h/L]	1420	984	2758
	Cl [mL/min/kg]	27	38	14
	V_{ss} [L/kg]	2.1	1.2	3.0
	$T_{1/2}$ (h)	2	1.1	5.2
Rat PO (5 mg/kg)	AUC [nmol h/L]	3374	2108	10580
	F%	48	43	77
Dog IV (0.3 mg/kg)	AUC [nmol h/L]	2077	2343	1452
	Cl [ml/min/kg]	5.6	5.4	7.7
	V_{ss} [L/kg]	0.3	0.1	0.7
	$T_{1/2}$ (h)	3.8	2.4	5
Dog PO (3.0 mg/kg)	AUC [nmol h/L]	5684	2924	6302
	F%	27	12	43

Based on their potency in the NS5B enzymatic and replicon assays, low PXR and CYP3A4 induction liability, and limited off-target liabilities, the pharmacokinetic profiles of **18a**, **20a** and **22a** were characterized. The in vivo pharmacokinetic parameters in Sprague–Dawley rats and beagle dogs are summarized in Table 3. In both rats and dogs, all three compounds showed low to moderate plasma clearance relative to hepatic blood. Following oral dose studies, plasma exposures of **22a** were found to be higher than **18a** and **20a** in both rats and dogs. The oral bioavailability of **22a** was adequate to support further pharmacokinetic and toxicology studies in both species. Finally, in a further antiviral activity profiling, **22a** had a modest shift in replicon potency (EC_{50}, 44 nM) in the presence of 40% human serum and was also active against HCV G1a replicon (EC_{50}, 11 nM).

In summary, we have successfully incorporated lactams as amide replacements in the design of HCV NS5B Site II thiophene carboxylic acid inhibitors. In contrast to the amide scaffold, in which a 4-*trans*-cyclohexyl group was important for replicon potency, a cyclohexyl group on the lactam was an optimal substituent. Appending a tethered hydroxyl group off the α position of the morpholinone lactam ring mitigated the risk of CYP3A4 induction with the lead compounds while maintaining cellular potency. In addition, compound **22a** had a favorable pharmacokinetic profile in rats and dogs and is a promising candidate for the clinical treatment of HCV infection.

Acknowledgment

We thank Dr. Zachary Sweeney and Catherine Jones for help with the manuscript preparation.

References and notes

1. Negro, F.; Alberti, A. *Liver Int.* **2011**, *31*, 1.
2. Thomas, D. L. *Nat. Med.* **2013**, *19*, 850.
3. Hellard, M. E.; Doyle, J. S. *Lancet* **2014**, *383*, 491.
4. Lawitz, E.; Poordad, F. F.; Pang, P. S.; Hyland, R. H.; Ding, X.; Mo, H.; Symonds, W. T.; McHutchison, J. G.; Membreno, F. E. *Lancet* **2014**, *383*, 515.
5. Behrens, S. E.; Tomei, L.; De Francesco, R. *EMBO J.* **1996**, *15*, 12.
6. Joyce, C. M.; Steitz, T. A. *J. Bacteriol.* **1995**, *177*, 6321.
7. Mayhoub, A. S. *Bioorg. Med. Chem.* **2012**, *20*, 3150.
8. Patil, V. M.; Gupta, S. P.; Samanta, S.; Masand, N. *Curr. Med. Chem.* **2011**, *18*, 5564.
9. Sofia, M. J.; Chang, W.; Furman, P. A.; Mosley, R. T.; Ross, B. S. *J. Med. Chem.* **2012**, *55*, 2481.
10. http://www.fda.gov/forconsumers/byaudience/forpatientadvocates/ucm377920.htm.
11. Chan, L.; Das, S. K.; Reddy, T. J.; Poisson, C.; Proulx, M.; Pereira, O.; Courchesne, M.; Roy, C.; Wang, W.; Siddiqui, A.; Yannopoulos, C. G.; Nguyen-Ba, N.; Labrecque, D.; Bethell, R.; Hamel, M.; Courtemanche-Asselin, P.; L'Heureux, L.; David, M.; Nicolas, O.; Brunette, S.; Bilimoria, D.; Bedard, J. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 793.

12. Chan, L.; Pereira, O.; Reddy, T. J.; Das, S. K.; Poisson, C.; Courchesne, M.; Proulx, M.; Siddiqui, A.; Yannopoulos, C. G.; Nguyen-Ba, N.; Roay, C.; Nasturica, D.; Moinet, C.; Bethell, R.; Hamel, M.; L'Heureux, L.; David, M.; Nicolas, O.; Courtemanche-Asselin, P.; Brunette, S.; Bilimoria, D.; Bedard, J. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 797.
13. Chan, L.; Das, S. K.; Poisson, C.; Yannopoulos, C. G.; Falardeau, G.; Vaillancourt, L.; Denis, R. Preparation of alkynylthiophenecarboxylates for the treatment or prevention of flavivirus infections. WO 2008058393 A1 20080522.
14. Canales, E.; Carlson, J. S.; Appleby, T.; Fenaux, M.; Lee, J.; Tian, Y.; Tirunagari, N.; Wong, M.; Watkins, W. J. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 4288.
15. Lazerwith, S. E.; Lew, W.; Zhang, J.; Morganelli, P.; Liu, Q.; Canales, E.; Clarke, M. O.; Doerffler, E.; Byun, D.; Mertzman, M.; Ye, H.; Chong, L.; Xu, L.; Appleby, T.; Chen, X.; Fenaux, M.; Hashash, A.; Leavitt, S. A.; Mabery, E.; Matles, M.; Mwangi, J. W.; Tian, Y.; Lee, Y.-J.; Zhang, J.; Zhu, C.; Murray, B. P.; Watkins, W. J. *J. Med. Chem.* **2014**, *57*, 1893.
16. Love, R. A.; Parge, H. E.; Yu, X.; Hickey, M. J.; Diehl, W.; Gao, J.; Wriggers, H.; Ekker, A.; Wang, L.; Thomson, J. A.; Dragovich, P. S.; Fuhrman, S. A. *J. Virol.* **2003**, *77*, 7575.
17. Biswal, B. K.; Cherney, M. M.; Wang, M.; Chan, L.; Yannopoulos, C. G.; Bilimoria, D.; Nicolas, O.; Bedard, J.; James, M. N. G. *J. Biol. Chem.* **2005**, *280*, 18202.
18. Yang, H.; Hendricks, R. T.; Arora, N.; Nitzan, D.; Yee, C.; Lucas, M. C.; Yang, Y.; Fung, A.; Rajyaguru, S.; Harris, S. F.; Leveque, V. J. P.; Hang, J. Q.; Pogam, S. L.; Reuter, D.; Tavares, G. A. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 4614.
19. Barnes, D.; Cohen, S. L.; Fu, J.; Shu, L.; Zheng, R. Preparation of 2, 3, 5-trisubstituted thiophene compounds useful in treating diseases caused by or associated with HCV infection. WO 2012010663 A1 20120126.
20. Banik, B. K.; Samajdar, S.; Banik, I. *J. Org. Chem.* **2004**, *69*, 213.
21. Amat, M.; Griera, R.; Fabregat, R.; Molins, E.; Bosch, J. *Angew. Chem., Int. Ed.* **2008**, *47*, 3348.
22. Apodaca, R.; Xiao, W. *Org. Lett.* **2001**, *3*, 1745.
23. Klapars, A.; Huang, X.; Buchwald, S. L. *J. Am. Chem. Soc.* **2002**, *124*, 7421.
24. The co-crystal structure coordinates have been deposited with Protein Data Bank with access code 4TLR.
25. Norman, B. H.; Kroin, J. S. *J. Org. Chem.* **1996**, *61*, 4990.
26. The co-crystal structure coordinates have been deposited with Protein Data Bank with access code 4TN2.
27. Li, H.; Tatlock, J.; Linton, A.; Gonzalez, J.; Borchardt, A.; Dragovich, P.; Jewell, T.; Prins, T.; Zhou, R.; Blazel, J.; Parge, H.; Love, R.; Hickey, M.; Doan, C.; Shi, S.; Duggal, R.; Lewis, C.; Fuhrman, S. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 4834.
28. Li, H.; Linton, A.; Tatlock, J.; Gonzalez, J.; Borchardt, A.; Abreo, M.; Jewell, T.; Patel, L.; Drowns, M.; Ludlum, S.; Goble, M.; Yang, M.; Blazel, J.; Rahavendran, R.; Skor, H.; Shi, S.; Lewis, C.; Fuhrman, S. *J. Med. Chem.* **2007**, *50*, 3969.
29. Li, H.; Tatlock, J.; Linton, A.; Gonzalez, J.; Jewell, T.; Patel, L.; Ludlum, S.; Drowns, M.; Rahavendran, S. V.; Skor, H.; Hunter, R.; Shi, S. T.; Herlihy, K. J.; Parge, H.; Hickey, M.; Yu, X.; Chau, F.; Nonomiya, J.; Lewis, C. *J. Med. Chem.* **2009**, *52*, 1255.
30. Chai, X.; Zeng, S.; Xie, W. *Expert Opin. Drug Metab. Toxicol.* **2013**, *93*, 253.
31. Dickins, M. *Curr. Top. Med. Chem.* **2004**, *4*, 1745.
32. Guidance for Industry: Drug Interaction Studies—Study Design, Data Analysis, Implications for Dosing, and Labeling Recommendations. http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM292362.pdf.
33. Sinz, M.; Kim, S.; Zhu, Z.; Chen, T.; Anthony, M.; Dickinson, K.; Rodrigues, A. D. *Curr. Drug Metab.* **2006**, *7*, 375.
34. Wang, J. *Curr. Pharm. Des.* **2009**, *15*, 2195.
35. Watkins, R. E.; Wisely, G. B.; Moore, L. B.; Collins, J. L.; Lambert, M. H.; Williams, S. P.; Willson, T. M.; Kliewer, S. A.; Redinbo, M. R. *Science* **2001**, *292*, 2329.
36. Watkins, R. E.; Davis-Searles, P. R.; Lambert, M. H.; Redinbo, M. R. *J. Mol. Biol.* **2003**, *331*, 815.
37. Watkins, R. E.; Maglich, J. M.; Moore, L. B.; Wisely, G. B.; Noble, S. M.; Davis-Searles, P. R.; Lambert, M. H.; Kliewer, S. A.; Redinbo, M. R. *Biochemistry* **2003**, *42*, 1430.
38. Chrencik, J. E.; Orans, J.; Moore, L. B.; Xue, Y.; Peng, L.; Collins, J. L.; Wisely, G. B.; Lambert, M. H.; Kliewer, S. A.; Redinbo, M. R. *Mol. Endocrinol.* **2005**, *19*, 1125.
39. Ekins, S.; Erickson, J. A. *Drug Metab. Dispos.* **2002**, *30*, 96.
40. Gao, Y.-D.; Olson, S. H.; Balkovec, J. M.; Zhu, Y.; Royo, I.; Yabut, J.; Evers, R.; Tan, E. Y.; Tang, W.; Hartley, D. P.; Mosley, R. T. *Xenobiotica* **2007**, *37*, 124.