Bioorganic & Medicinal Chemistry Letters 21 (2011) 3142–3147

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

SAR studies on a series of *N*-benzyl-4-heteroaryl-1-(phenylsulfonyl)piperazine-2-carboxamides: Potent inhibitors of the polymerase enzyme (NS5B) of the hepatitis C virus

Robert G. Gentles [a,*], Min Ding [a], Xiaofan Zheng [a], Louis Chupak [a], Michael A. Poss [a], Brett R. Beno [a], Lenore Pelosi [c], Mengping Liu [c], Julie Lemm [c], Ying-Kai Wang [c], Susan Roberts [c], Min Gao [c], John Kadow [b]

[a] Bristol Myers Squibb, Chemical and Protein Technologies, Research and Development, 5 Research Parkway, Wallingford, CT 06492, United States
[b] Bristol-Myers Squibb, Molecular Sciences and Candidate Optimization, Research and Development, 5 Research Parkway, Wallingford, CT 06492, United States
[c] Bristol Myers Squibb, Department of Virology, Research and Development, 5 Research Parkway, Wallingford, CT 06492, United States

ARTICLE INFO

Article history:
Received 7 January 2011
Revised 26 February 2011
Accepted 2 March 2011
Available online 6 March 2011

Keywords:
HCV
NS5B
Allosteric inhibitors
SAR

ABSTRACT

Described herein is the initial optimization of (+/−) *N*-benzyl-4-heteroaryl-1-(phenylsulfonyl)piperazine-2-carboxamide (**1**), a hit discovered in a high throughput screen run against the NS5B polymerase enzyme of the hepatitis C virus. This effort resulted in the identification of (*S*)-*N*-*sec*-butyl-6-((*R*)-3-(4-(trifluoromethoxy)benzylcarbamoyl)-4-(4-(trifluoromethoxy)phenylsulfonyl)piperazin-1-yl)pyridazine-3-carboxamide (**2**), that displayed potent replicon activities against HCV genotypes 1b and 1a (EC_{50} 1b/1a = 7/89 nM).

Hepatitis C virus (HCV) infection is a pressing medical problem: 4 million cases have been reported in the United States and approximately 180 million people are infected globally.[1] Patients with the disease frequently develop a persistent infection that results in variable hepatic necro-inflammation. Those patients presenting with high inflammatory activity are at significant risk of rapid disease progression leading to the development of fibrosis and cirrhosis, the sequelae of which include portal hypertension, liver failure and hepatocellular carcinoma.[2] The current optimal treatment involves extended administration (48 weeks) of pegylated interferon and ribavirin (Peg-IFN/RBV).[3] This regime is associated with a poor response rate with only 40–50% of patients infected with genotype 1 HCV able to achieve a sustained viral response (SVR).[4] In addition, concomitant severe flu-like side-effects and anemia associated with this treatment lead to significant patient discontinuation rates. This has stimulated a number of companies to attempt to identify direct acting antivirals (DAA's) that specifically target HCV.[5]

HCV non-structural protein 5B (NS5B) is an RNA-dependent RNA polymerase that is essential for viral replication.[6] Accordingly, it has long been considered an attractive target for drug development, and several active-site and allosteric NS5B inhibitors have been reported,[7] and a number of these are currently being clinically evaluated, see Figure 1.[8]

In this Letter, we report on our exploratory work on the optimization of compound **1**, a hit identified in an NS5B high throughput screen (HTS).[9] Preliminary structure–activity relationship (SAR) investigations quickly led to the identification of the potent piperazine based NS5B inhibitor **2,** as described below.[10]

The chemistry used in the preparation of analogs of **1** is shown in Scheme 1, and primarily employs standard protecting group manipulations and routine acylation and sulfonylation protocols.[11] However, in order to accommodate the greatest array of reagents in the N-arylation procedure (step **f**), a microwave mediated SN_{Ar} protocol was adopted that differed slightly from previously reported methodology.[12] For the syntheses of N4-heteroaryl carboxamide derivatives such as **2**, and **39–44**, the pendant heteroaryl groups were introduced onto the piperazine ring as the related heteroaryl esters that were subsequently hydrolyzed and condensed with amines to provide the desired amides. In the case of compound **2**, (*S*)-butan-2-amine was used to furnish the targeted pyridazine carboxamide.

All compounds discussed below were tested as racemates, unless otherwise stated, and in those instances, specific enantiomers were isolated using chiral reverse-phase HPLC. In a limited number of cases (e.g., Table 1, entries **10** and **11**), specific enantiomers were prepared by beginning the synthesis with the *R*-enantiomer of the protected piperazine,[13] as shown in Scheme 1.

* Corresponding author.
E-mail address: robert.gentles@bms.com (R.G. Gentles).

0960-894X/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2011.03.008

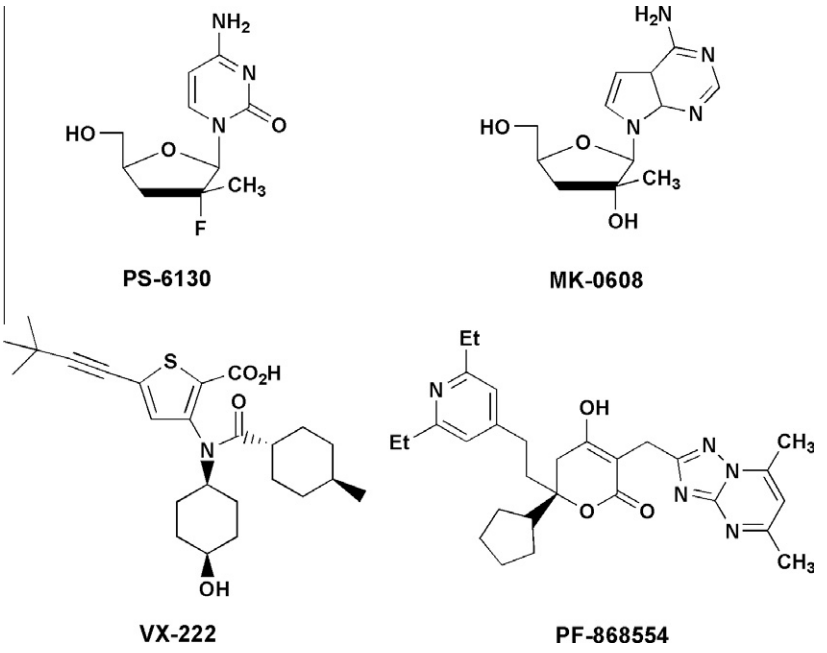
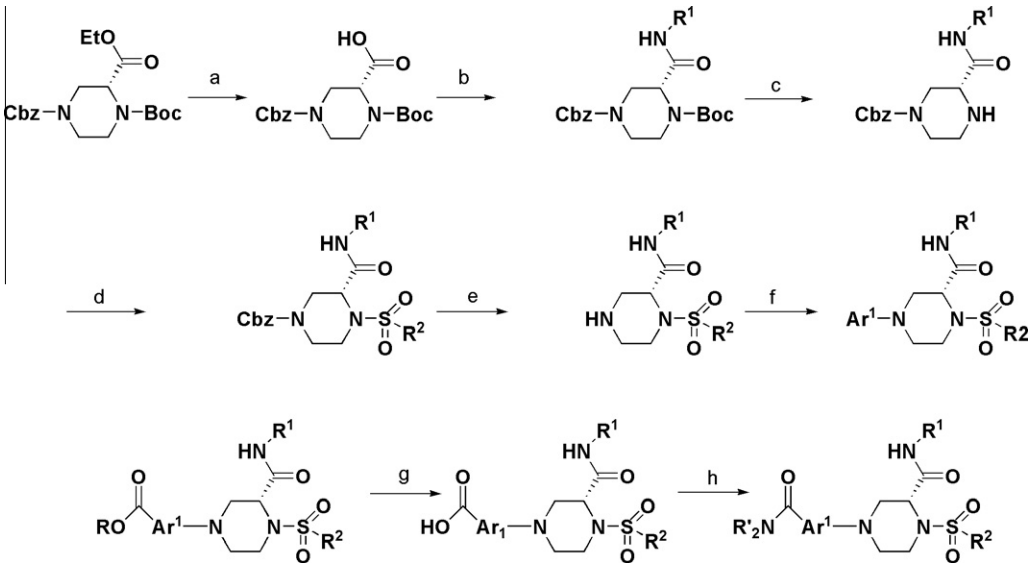


Figure 1. Recently reported examples of active-site [PS-6130 and MK-0608] and allosteric [VX-759 and PF-868554] inhibitors of NS5B in clinical development.



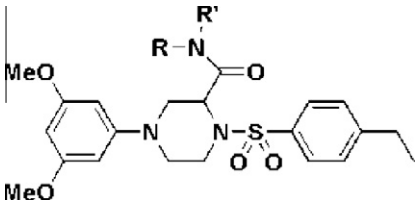
Scheme 1. Reagents and conditions: (a) NaOH, 1:1, MeOH, THF; (b) HOAt, DCM, R_1-NH_2; (c) 1:2 TFA/DCM rt 30 min; (d) R_2-SO_2Cl, pyridine, DCM, rt; (e) 10% Pd/C, MeOH, DCM; (f) *n*-butanol, 175 °C, Ar_1X; (g) NaOH, THF/MeOH/H_2O; (h) TBTU, DIPEA, R'_2NH_2.

The screening hit **1** can be viewed as a tri-functionalized piperazine with pendant moieties projecting along three vectors. Our choice of the order of exploration of these groups was influenced be the ease of chemistry. Correspondingly, we chose to sequentially optimize the carboxamide, sulfonamide are *N*-aryl moieties using the intermediates shown in Figure 3.

Compounds **3** and **5** can be accessed using variations of the reaction sequence shown in Scheme 1. Intermediate **4** was prepared using the chemistry depicted in Scheme 2.

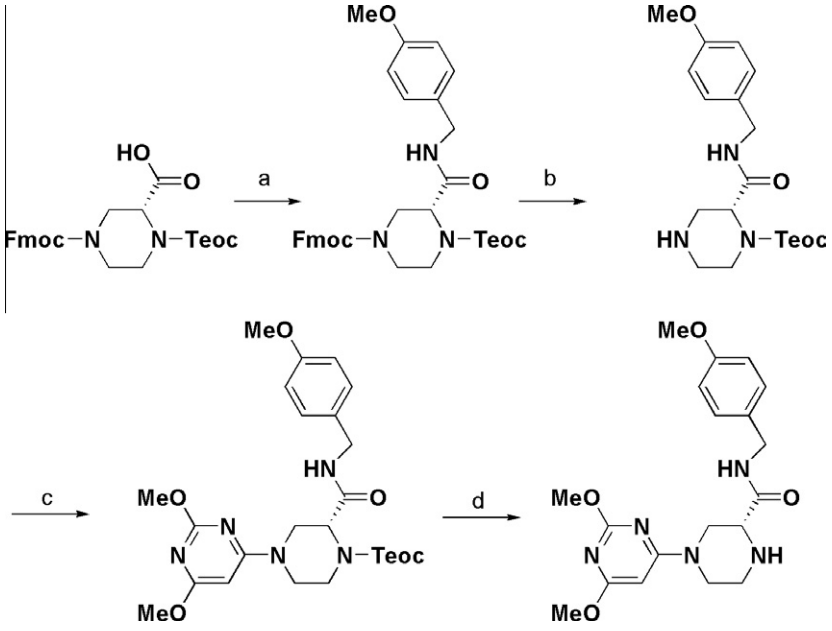
As stated above, our initial investigations involved the preparation of a diverse set of amides derived from intermediate **3** using a series of aliphatic, benzylic and heteroaromatic-methylamines. A representative set of the product carboxamides is shown in Table 1, together with their associated inhibition data generated from HCV 1b and 1a replicon assays.[14] From these results and additional data on related analogs (not reported), the following SAR could be discerned. Firstly, removal of the methoxy group from **1**, as shown in compound **6** is associated with significant loss of activity. Methylation at the benzylic position of the carboxamide, as exemplified in analog **7**, results in the generation of inactive compounds. This is also the case when the N–H moiety of the amide group is alkylated, as observed with derivative **8**. However, substitution at the four-position of the benzylic aromatic moiety is generally well tolerated. In relation to 1b activity, chloro **13**, methyl **9** and methoxy **1** derivatives are approximately equipotent, with the trifluoromethoxy analog **12** being slightly more active. A similar analysis of the 1a activities showed **13, 9** and **1** to have similar activity, but less than that of the trifluoromethoxy derivative **12,** suggesting

Table 1
Replicon EC_{50} data on 4-(2,6-dimethoxypyrimidin-4-yl)-1-(4-ethylphenylsulfonyl)piperazine-2-carboxamides



Compound	Carboxamide N-substituent(s) R,R′	1b Replicon EC_{50}[a] (μM)	1a Replicon EC_{50} (μM)
6	Phenylmethyl	8.8	na
7	2-Phenethyl (diastereomeric mixture)	na	na
8	*N*-Methyl, *N*-benzyl	>50	>50
9	4-Methylphenylmethyl	1.7	20.5
1	4-Methyoxyphenylmethyl	2.2	23
10	4-Methyoxyphenylmethyl *R* isomer	0.9	6.2
11	4-Methyoxyphenylmethyl *S* isomer	10.1	14.1
12	4-Trifluoromethoxyphenylmethyl	0.5	9.2
13	4-Chlorophenylmethyl	2.1	30.8
14	2-Chlorophenylmethyl	3.7	>50
15	2,4-Dichlorophenylmethyl	2.4	>50
16	2,4-Dimethoxyphenylmethyl	1.2	12.4
17	3,4-Dimethoxyphenylmethyl	0.9	14.6
18	4-(Thiophen2-yl)phenylmethyl	1.2	47.0
19	*N*-Cyclopropylmethyl	na	na
20	(3,5-Dimethyl-1-phenyl-1*H*-pyrazol-4-yl)methyl	2.1	20.8

[a] Values are means of three experiments, and standard deviations are <40% of mean (na = not active, or therapeutic index <10).



Scheme 2. Reagents and conditions: (a) DIPEA, HATU, *p*-trifluoromethoxybenzylamine, DMF, rt; (b) piperidine/DMF (1:5), rt; (c) 6-chloro-2,4-dimethoxypyrimidine, DIPEA, *n* BuOH, 180 °C, microwave; (d) TFA, DCM, rt.

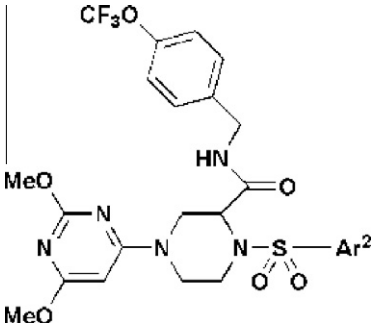
the latter to be a preferred substituent. Analogs containing functionality at the 2-position of the aryl group as in **14**, or the disubstituted analogs **15** and **16**, generally did not lose 1b activity, but were less active against the 1a genotype than the original lead structure **1**. Disubstitution at positions 3- and 4- also did not lead to any significant enhancement of activity as exemplified by compound **17**. In a related exercise, all attempts to replace the aryl moiety of the benzylic group with an aliphatic substituent resulted in inactive compounds, as typified by analog **19**. More productive, was the replacement of the phenyl group in **1** with a variety of heterocycles, which although they did not enhance the activity of the series, could introduce significant structural diversity into the chemotype at little expense to potency, as observed with derivative **20**.

On conclusion of this exercise we next used the intermediate **4** that contains one of the preferred carboxamide functionalities identified above to examine the SAR of the pendant sulfonamide moiety. Initial attempts to delete or replace the sulfonamide

linkage with a number of alternative moieties (alkyl, carboxamide, carbamate, and urea) proved unsuccessful, as all activity at both genotypes was lost. Subsequently, the sulfonamide derivatives shown in Table 2 were prepared and the most salient aspects of the SAR observed are discussed below.

Deletion of the ethyl group in **1** as shown in analog **21**, resulted in a significant loss of both 1b and 1a activities, as did substitution at the *ortho*-position of the arylsulfonamide, as observed in derivative **23**. Replacement of the phenyl group with an aliphatic moiety, as shown in analog **22**, was also unsuccessful. Substitution at

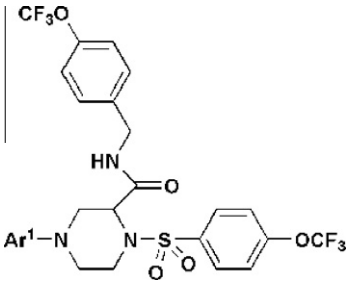
Table 2
Replicon EC_{50} data on 4-(2,6-dimethoxypyrimidin-4-yl)-*N*-(4-methoxy)benzyl)piperazine-1-sulfonamides



Compound	Sulfonyl substituent Ar^2	1b Replicon EC_{50}[a] (μM)	1a Replicon EC_{50} (μM)
21	Phenyl	20.3	na
22	Cyclohexyl	na	na
23	*o*-Fluorophenyl	na	na
24	*m*-Methylphenyl	1.4	41
25	*m*-Trifluoromethoxyphenyl	5.3	58
26	*m*-Trifluoromethylphenyl	6.2	53
27	*m*-(4-Fluorophenyl)phenyl	na	na
28	*p*-Trifluoromethoxyphenyl	0.7	8.1
29	*p*-Trifluoromethylphenyl	1.2	13
30	*p*-Phenylphenyl	2.4	56
31	*p*-Methoxyphenyl	5.9	64
32	*p*-Fluorophenyl	8.1	na
33	3,4-Dimethoxyphenyl	1.1	13

[a] Values are means of three experiments, and standard deviations are <40% of mean (na = not active, or therapeutic index <10).

Table 3
Replicon EC_{50} data on 4-heteroaryll-*N*-(4-(trifluoromethoxy) benzyl)-1-(4-(trifluoromethoxy)phenylsulfonyl)piperazine-2-carboxamides



Compound	Ar^1 substituent	1b Replicon EC_{50}[a] (μM)	1a Replicon EC_{50}[a] (μM)
34	2,4-Dimethoxypyrimidin-4-yl	0.4	2.0
35	6-Carboxylic acid-pyridazin-3-yl	na	na
36	5-Carboxylic acid-pyrazin-2-yl	na	na
37	Methyl-6-carboxylatepyridazin-3-yl	0.38	3.9
38	Methyl-5-carboxylatepyrazin-2-yl	0.14	1.6
39	*N*-Methyl-6-carboxamidepyridazin-3-yl	0.02	0.33
40	*N*-Methyl-5-carboxyamidepyrazin-2-yl	0.22	2.23
41*	*N*-Methyl-6-carboxamidepyridazin-3-yl	0.007	0.22
42*	*N*-Cyclopropyl-6-carboxamidepyridazin-3-yl	0.009	0.169
43*	*N*-Isopropyl-6-carboxamidepyridazin-3-yl	0.008	0.139
2*	(*R*)-*N*-*sec*-butyl-6-carboxamidepyridazin-3-yl	0.007	0.087
44	*N*-*tert*-pentyl-6-carboxamidepyridazin-3-yl	0.043	0.46

[a] Values are means of three experiments, and standard deviations are <40% of mean (na = not active, or therapeutic index <10).
* Compounds that have an asterisk have the *R* absolute configuration at the 2-position of the piperazine ring in addition to the designated stereochemistry indicated in table.

other vectors off the aromatic group is generally tolerated within certain size constraints (see **27**). However, again the preferred substituents identified were the *p*-substituted fluoroalkyl **29** and trifluoromethoxy **28** groups. These observations are not likely attributable to electronic effects, as **33**, an electron rich system, is equipotent with **28** and **29,** both of which are moderately electron deficient.

Following these initial optimization exercises, we next used intermediate **5** to explore the SAR at the four-position of the piperazine. Several compound arrays were prepared using a variety of linking chemistries. However, direct heteroarylation of N4 of the pirperazine was clearly preferred, and from an extensive set of heterocycles evaluated, carboxyl- and carboxamide-substituted pyridazines and pyrazines clearly displayed the greatest potency, as shown in Table 3.

Although the two acids **35** and **36** displayed no significant activity in the replicon assay, we attributed this to poor cellular penetration, as both compounds displayed excellent activity in the enzyme assay (data not shown). Correspondingly, we prepared the esters **37** and **38**, and were gratified to see significant activity with both compounds. More interesting, was the methyl carboxamide **39** that displayed more than a 10-fold improvement in potency against both genotypes relative to carboxylate **37**. Unfortunately, the related pyrazine carboxamide **40** did not show a similar enhancement in activity, being approximately equipotent to the related ester **38**. We attributed this to the relative disposition of a nitrogen atom (∗) in the preferred conformation of the pyridazine and pyrazine carboxamides, as shown in Figure 4. This will be discussed this in greater detail in a future manuscript.

Compound **39** was subsequently resolved, and the more active (*R*)-enantiomer **41** displayed the expected enhancement in activity, with EC_{50}'s of 7 nM and 220 nM against genotypes 1b and 1a, respectively. A subsequent homologation exercise focused on the introduction of small alkyl substituents onto the nitrogen atom of the secondary carboxamide. The final products from these syntheses were fractionated using chiral reverse-phase HPLC, resulting in the isolation of specific enantiomers **42** and **43,** which displayed similar activities to **41.** Derivative **2,** prepared by condensing (*S*)-butan-2-amine with the appropriate racemic pyridazine carboxamide, and isolation by preparative HPLC, displayed a moderate enhancement of activity against genotype 1a, and was the only analog within the series to display an EC_{50} of <100 nM against this genotype. Initial attempts to extend this homologation further resulted in a moderate reduction in activity, as seen with **44**. However, it is apparent that further work around the aryl carboxamide moiety of the chemotype is clearly merited.

All of the compounds discussed above were screened against NS5B genotype 1b and 1a enzymes, with activities that were generally consistant with those observed in the replicon system, as typified by the data presented in Table 4 and Figure 2. In addition, the compounds were assayed in a replicon system possessing a Y452H mutation in the NS5B protein that is located within the palm domain of the enzyme. All of the examples discussed above displayed significantly less activity in the mutant replicon system,

Table 4
NS5B enzymatic activity of compounds **1** and **2** against genotypes 1b and 1a and a mutant Y452H replicon[9]

Compound	Wild type 1b enzyme IC_{50} (μM)	Wild type 1a enzyme IC_{50} (μM)	Mutant Y452H replicon EC_{50} (μM)
1	0.3	12.5	19
2	0.013		0.4

Values are means of three experiments, and standard deviations are <40% of mean.

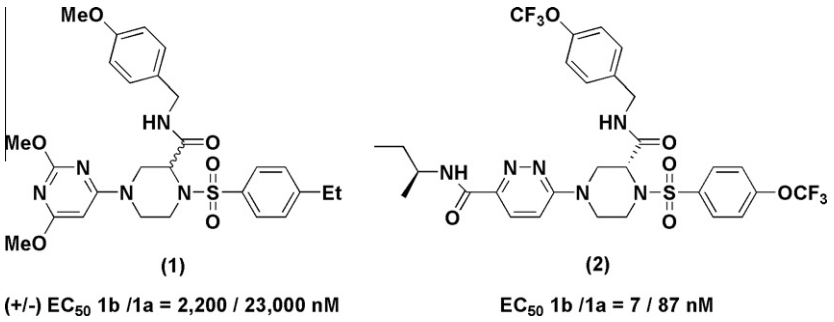


Figure 2. Screening hit **1** and optimized NS5B inhibitor, (*S*)-*N*-*sec*-butyl-6-((*R*)-3-(4-(trifluoromethoxy)benzylcarbamoyl)-4-(4-(trifluoromethoxy) phenylsulfonyl)piperazin-1-yl)pyridazine-3-carboxamide (**2**).

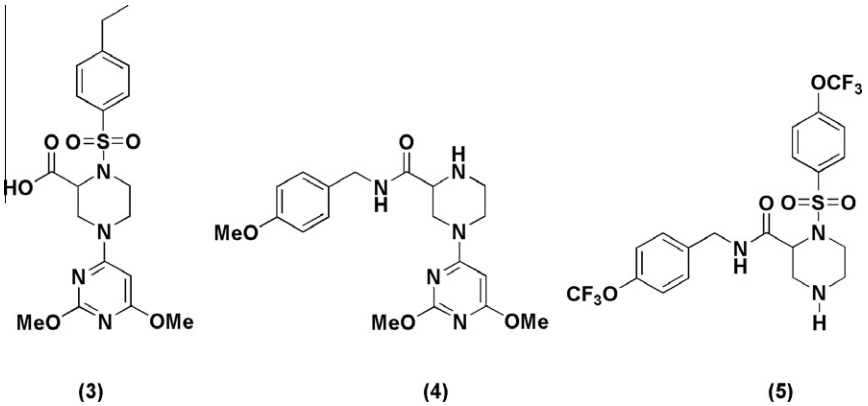


Figure 3. The intermediates **3** and **5** can all be accessed using the chemical procedures shown in Scheme 1. For intermediate **4**, see Scheme 2.

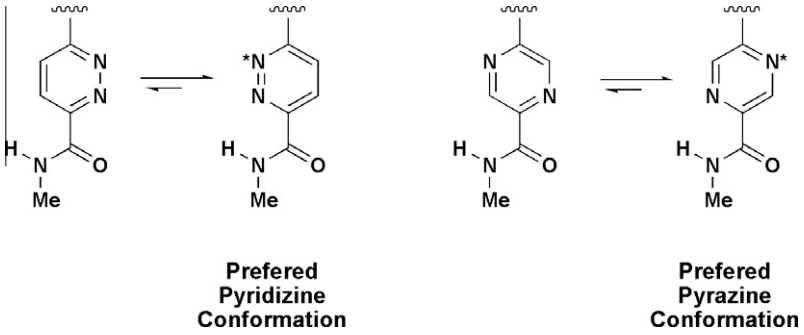


Figure 4. Preferred conformation of structurally related carboxamide substituted pyrazines and pyridazines.

suggesting that these compounds bind in, or proximal to the palm domain. This is discussed in detail in a related manuscript.[15]

In conclusion, we have identified a novel class of piperazine NS5B inhibitors using high throughput screening. Subsequent exploratory lead optimization led to a rapid improvement in potency against HCV genotypes 1b (>300-fold) and 1a (>200-fold). Key aspects of the pharmacophore have been clearly identified, as have potential paths for the further advancement of this series.

Acknowledgments

The authors would like to thank Dr. Carl Bergstrom for his help in reviewing this manuscript.

References and notes

1. (a) Shepard, C. W.; Finelli, L.; Alter, M. J. *Lancet Infect. Dis.* **2005**, *5*, 558; (b) http://www.who.int/accine_research/diseases/viral_cancers/en/index2.html.
2. (a) Lauer, G. M.; Martin, K. A. *N. Eng. J. Med.* **2001**, *345*, 41; (b) Shackel, N. A.; Patel, K.; McHutchison, J. *Genomic Pers. Med.* **2009**, *2*, 1375.
3. Zeuzem, S. *Nat. Clin. Pract. Gastroenterol. Hepatol.* **2008**, *5*, 610.
4. Field, J. J.; Hoofnagle, J. H. *Nature* **2005**, *436*, 967.
5. Li, H.; Shi, S. T. *Future Med. Chem.* **2010**, *2*, 121.
6. Vo, N. V.; Tuler, J. R.; Lai, M. M. C. *Biochemistry* **2004**, *43*, 10579.
7. Beaulieu, P. L. *Expert Opin. Ther. Patents* **2009**, *19*, 145.
8. (a) Legrand-Abravanel, F.; Nicot, F.; Izopet, J. *Expert Opin. Invest. Drugs* **2010**, *19*, 963; (b) Enomoto, M.; Tamori, A.; Kawada, N. *Rev. Recent Clin. Trials* **2009**, *4*, 179.
9. An NS5B enzyme-based screen of the BMS Compound Library was conducted. Inhibition of a genotype 1b enzyme (Con-1 with a C-terminal deletion of 18 amino acids) was detected as a decrease in ^{3}H-UTP incorporation using a SPA format (polyA template/biotin-labeled dT primer). Confirmed hits were tested for activity and cytotoxicity in an HCV replicon assay.[14]
10. During the course of our work the following patent reported this compound class to be active against HCV: Abe, H.; Tanaka, M.; Sugimoto, K.; Suma, A.; Yokota, M.; Shiozaki, M.; Iio, K.; Ueyama, K.; Motoda, D.; Noguchi, T.; Adachi, T.; Tsuruha, J.; Doi, S. PCTInt. Appl. WO 2007119889; *Chem. Abstr.* **2007**, *120*, 4767.
11. Breitenbucher, J. G.; Johnson, C. R.; Haight, M.; Phelan, J. C. *Tetrahedron Lett.* **1998**, 1295.
12. Xiang, J.; Wan, Z. K.; Li, H. Q.; Ipek, M.; Binnun, E.; Nunez, J.; Chen, L.; McKew, J. C.; Mansour, T. S.; Xu, X.; Suri, V.; Tam, M.; Xing, Y.; Li, X.; Hahm, S.; Tobin, J.; Saiah, E. *J. Med. Chem.* **2008**, *51*, 4068.
13. Neya, M.; Yamazaki, H.; Kayakiri, N.; Sato, K.; Oku, T. PCT Int. Appl. WO 9827069, 1998.
14. Hudyma, T. W.; Zheng, X.; He, F.; Ding, M.; Bergstrom, C. P.; Hewawasam, P.; Martin, S. W.; Gentles, R. G. PCT Int. Appl. WO 2006020082, 2006.
15. Gentles, R. G.; Sheriff, S.; Beno, B. R.; Wan, C.; Kish, K.; Ding, M.; Zheng, X.; Chupak, L.; Poss, M. A.; Witmer, M. R.; Morin, M.; Wang, Y. K.; Rigat, R.; Lemm, J.; Voss, S.; Liu, M.; Pelosi, P.; Roberts, S. B.; Gao, M.; Kadow, J. F. *Bioorg. Med. Chem. Lett.*, in press.