Bioorganic & Medicinal Chemistry Letters 22 (2012) 2866–2871

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Synthesis and SAR studies of novel heteroaryl fused tetracyclic indole-diamide compounds: Potent allosteric inhibitors of the hepatitis C virus NS5B polymerase

Min Ding [a,*], Feng He [a], Thomas W. Hudyma [a], Xiaofan Zheng [a], Michael A. Poss [a], John F. Kadow [c], Brett R. Beno [a], Karen L. Rigat [b], Ying-Kai Wang [b], Robert A. Fridell [b], Julie A. Lemm [b], Dike Qiu [b], Mengping Liu [b], Stacey Voss [b], Lenore A. Pelosi [b], Susan B. Roberts [b], Min Gao [b], Jay Knipe [a], Robert G. Gentles [a]

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

ARTICLE INFO

Article history:
Received 6 January 2012
Accepted 21 February 2012
Available online 1 March 2012

Keywords:
HCV NS5B polymerase inhibitors
Tetracyclic indoles

ABSTRACT

Presented here are initial structure–activity relationship (SAR) studies on a series of novel heteroaryl fused tetracyclic indole-based inhibitors of the hepatitis C viral polymerase, NS5B. The introduction of alternative heterocyclic moieties into the indolo-fused inhibitor class significantly expands the reported SAR and resulted in the identification of pyridino analogs, typified by compounds **44** and **45** that displayed excellent potency against the NS5B polymerase of both HCV 1a and HCV 1b genotypes.

The hepatitis C virus (HCV) currently infects about 180 million people globally,[1] and frequently results in serious and often fatal liver disease. The standard of care (SOC) for the treatment of HCV infection is expected to evolve significantly in coming years, from the current protocol of co-administration of pegylated interferon-α and ribavirin, (a regime that provides limited efficacy against HCV genotypes 1a and 1b, and is associated with significant adverse side-effects[2]), to protocols incorporating more selective agents, such the newly approved HCV specific protease inhibitors VICTRELIS™ and INCIVEK™.[3] In addition to the viral protease, the HCV non-structural protein 5B (NS5B) is an RNA-dependent RNA polymerase that is essential for viral replication. Several active-site and allosteric NS5B inhibitors have been reported, and recent clinical data[4] on both inhibitor classes have provided support for NS5B as a viable target for HCV therapy, with inhibitors of this enzyme expected to have utility in a number of potential combination therapies.

Described herein is our initial investigation into a series of indole based inhibitors related to the previously reported indolo analog **1**. Compounds structurally related to **1** have been demonstrated to bind in the 'thumb' domain of NS5B, as evidenced by a consistent P495L resistance profile, and more definitively, by a reported co-crystal structure.[5] A focus of our recent research[6] in this area has been the evaluation of indolo ring systems in which the fused aryl moiety in **1** has been replaced with a variety of heterocycles, as shown by **2** in Figure 1.

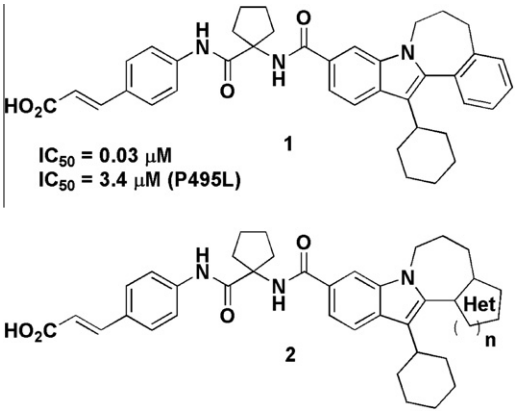


Figure 1. Aryl-fused tetracyclic indolo NS5B inhibitors **1**.[5] Generic structure of fused heterocyclic analogs, **2**. [IC_{50} values are the means of at least 2 experiments and were obtained using a previously reported enzymatic assay.[7]]

It can be anticipated that such substitutions would extend the range of physicochemical properties displayed by these ring systems and significantly expand the SAR reported in this area.

* Corresponding author.
E-mail address: min.ding@bms.com (M. Ding).

0960-894X/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2012.02.063

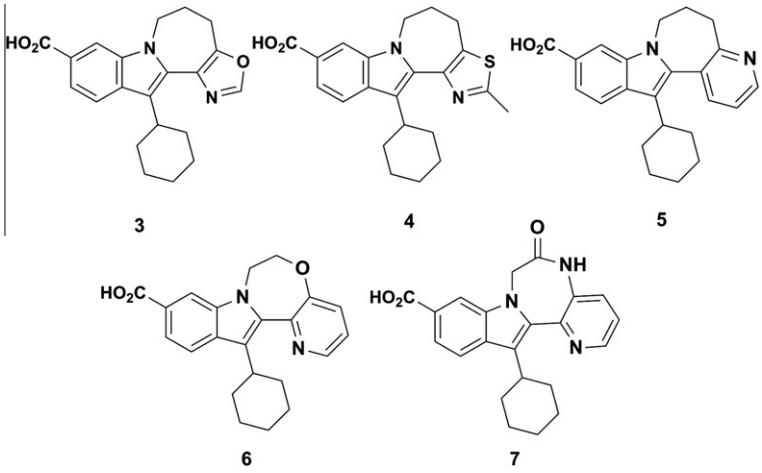


Figure 2. Novel heteroaryl-fused, indoloazepine, indolodiazepinone and indolooxazepine NS5B inhibitor classes.

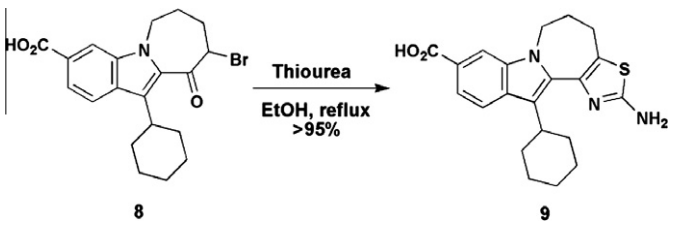
Several of the heterocyclic intermediates **3**–**7** required for these studies are shown in Figure 2.[8]

The fused thiazolo derivative **9** was prepared in a similar manner to that used to access compounds **3** and **4**, and utilized the previously reported[8] indoloazepinone intermediate **8** in a condensation reaction with thiourea to give the target thiazolo derivative **9** in essentially quantitative yield, as shown in Scheme 1.

Additional pyrazino- and imidazolo-fused indoloazepine intermediates **16** and **23** were prepared as shown in Scheme 2. In the case of the former, this involved an initial Suzuki coupling of 2,3-dichloropyrazine **11** with the pinacol boronate derivative **10**.[8] A subsequent Stille reaction with tributylvinyltin furnished the vinyl intermediate **13**, that was then alkylated using allylbromide to give the di-olefinic indole **14** in good overall yield. **14** was converted to the indolo-fused pyrazinoazepine **15** using the Grubb's metathesis reaction employing a second generation catalyst, and the desired target **16** was obtained by a final catalytic hydrogenation and basic hydrolysis. The related imidazo derivative **23** was prepared in an essentially analogous fashion using the SEM-protected 4,5-diiodo-imidazole intermediate (**18**), prepared as shown in Scheme 2.

A further furano-fused intermediate **30** was prepared as shown in Scheme 3 using a reaction sequence similar to that outlined above. 3-Bromofuran (**24**) was formylated using LDA and DMF to give intermediate **25**. This was then subjected to Suzuki coupling with the pinacol boronate **10** to afford the di-substituted furan **26**. This compound was used without additional protection in a Wittig reaction using an excess of base and triphenylphosphonium bromide to give **27**. Alkylation of **27** with allylbromide followed by olefin metathesis under conditions described above provided the indolo-fused compound **29**. This was converted to the desired saturated target **30** by catalytic hydrogenation and basic hydrolysis.

The synthesis of the final heterocyclic intermediate included in this study, the indolo-pyridooxazepine **37**, was prepared using the chemistry depicted in Scheme 4. Although **37** is a regioisomer of compound **6**, it cannot be accessed by the same intramolecular Heck strategy that we have discussed previously.[8] Correspondingly, we developed the alternative methodology depicted below that utilizes an intermolecular Mitsunobu reaction to construct the oxazepine ring. In this reaction sequence, 3-bromo-2-hydroxypyridine (**31**) is first derivatized as the *O*-benzyl ether **32**. This compound was coupled under Suzuki conditions with the pinacol boronate ester **10** to afford the pyridinyl derivative **33** in good yield. This intermediate was alkylated using benzyl 2-bromoethylether to give the di-ether **34**. Subsequent hydrogenolysis afforded the hydroxyl-pyridone **35** that under Mitsunobu conditions cyclized to give the target indolo-pyridooxazepine indole **36**. This was then hydrolyzed to **37** in moderate overall yield.



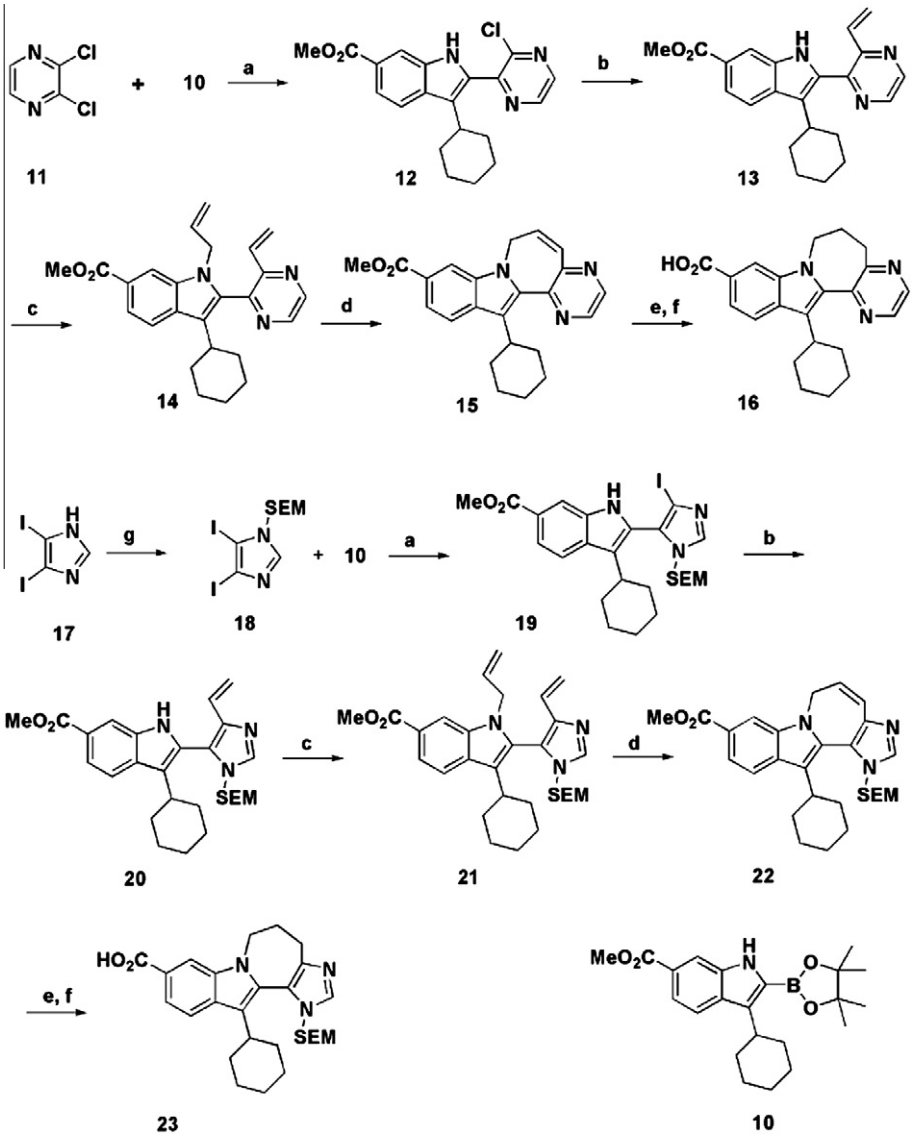
Scheme 1. Synthesis of compound **9**.

With all of the key intermediates in hand, each was subsequently converted to the desired (*E*)-3-(4-(1-aminocyclopentanecarboxamido)phenyl)acrylic acid carboxamide[9] using the methodology shown in Scheme 5. Each of the core heterocyclic acids was first condensed with (*E*)-methyl 3-(4-(1-aminocyclopentanecarboxamido)phenyl)acrylate using TBTU as coupling agent with the final target acids obtained by basic hydrolysis with sodium hydroxide, except in the case of **45**. Compound **45** was obtained by the use of LiI in pyridine as the lactam moiety in the molecule was sensitive to base-catalyzed hydrolysis conditions. Compound **40** was obtained by treating the SEM-protected imidazole precursor with TBAF in THF. The structures of the final products are shown in Scheme 6.

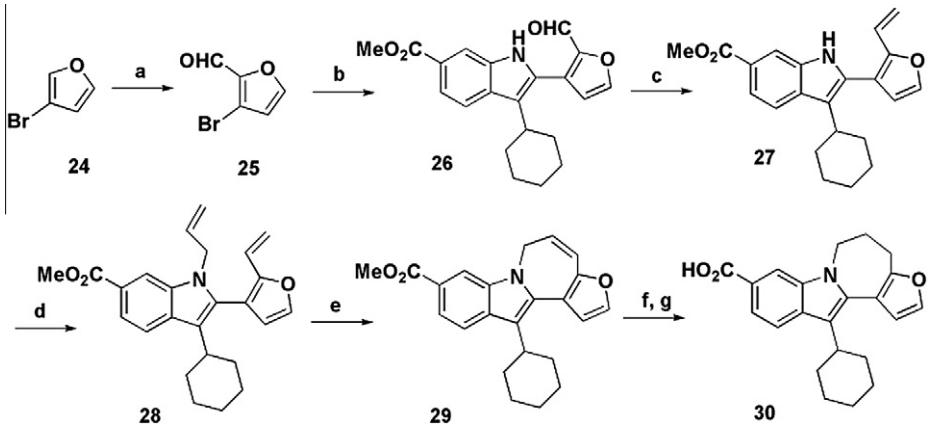
Table 1 lists the activities of these compounds against both wild-type and P495L mutant genotype 1b NS5B enzymes, as well as their activities in HCV genotype 1a and 1b replicon systems.[10]

In general, all of the compounds showed good concordance between their activities against the isolated wild-type enzyme and in both replicon systems. The analogs also consistently displayed a reduced activity toward the P495L mutant enzyme. Given this, and their close structural relationship to compounds known to bind in the well-studied[5,6,11] NS5B inhibitor binding pocket proximal to P495, it would appear reasonable to assume that these compounds bind in a similar fashion, vide infra.

In terms of discernable SAR's, the two fused thiazoles **41** and **42** and the imidazole analog **40** show a significant reduction in potency relative to the phenyl analog **2**. In relation to **41** and **42**, each has a pendant functionality that can encounter a steric clash with the enzyme with an obvious negative impact on potency, vide infra. This is in contrast to the two other oxygen containing five-membered rings **38** and **39** that display similar potency to **2**. With respect to imidazole **40**, reported[12] experimental pK_a values of the conjugate acids of dialkylsubstituted imidazoles are between 7.2 and 8.7. Correspondingly, this compound may be significantly



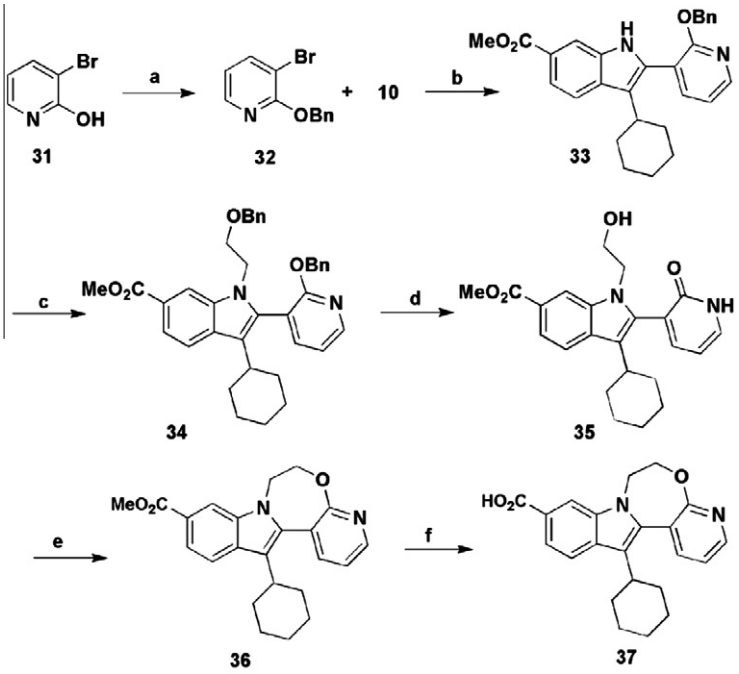
Scheme 2. Reagents and conditions: (a) $Pd(PPh_3)_4$, LiCl, Na_2CO_3, EtOH/toluene, 80 °C, 41–43%; (b) tributylvinyltin, $PdCl_2(PPh_3)_2$, LiCl, DMF, 100 °C, 29–88%; (c) KH, allyl bromide, 75%; (d) Grubbs catalyst 2nd generation, CH_2Cl_2, reflux, 64–100%; (e) H_2/Pd-C, 84–91%; (f) NaOH, MeOH/THF, 87–100%; (g) SEMCl, K_2CO_3, DMF, 92%.



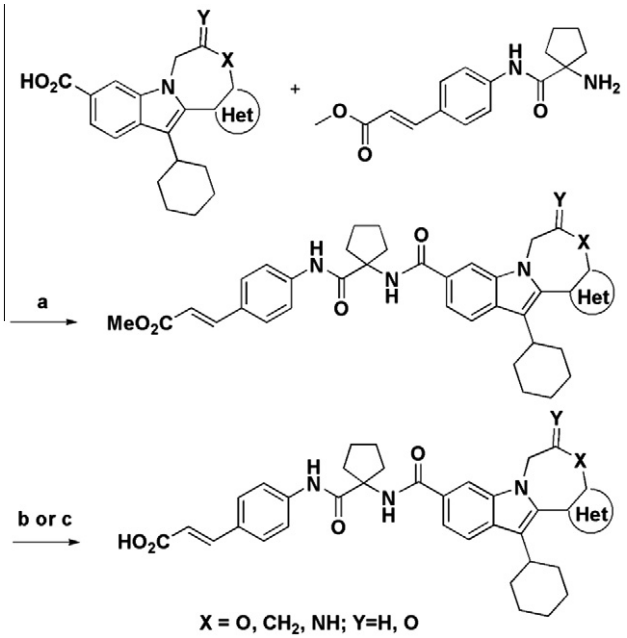
Scheme 3. Reagents and conditions: (a) LDA, DMF, −78 °C to rt, 60%; (b) **10**, $Pd(PPh_3)_4$, LiCl, Na_2CO_3, EtOH/toluene, 80 °C, 50%; (c) *n*-BuLi, methyl triphenylphosphonium bromide, 65 °C, 54%; (d) KH, allyl bromide, 67%; (e) Grubbs catalyst 2nd generation, CH_2Cl_2, reflux, 85%; (f) H_2/Pd-C, 80%; (g) NaOH, MeOH/THF, 90%.

ionized under our assay conditions (pH 7.2–7.5), and this may account for the observed reduction in potency.

The six-membered isosteric fused heterocyclic systems **43**, **44**, **46** and **47** are essentially equipotent with the phenyl analog **2**, as



Scheme 4. Reagents and conditions: (a) NaH, benzyl bromide, DMF, 52%; (b) $Pd(PPh_3)_4$, LiCl, Na_2CO_3, EtOH/toluene, 80 °C, 70%; (c) NaH, benzyl 2-bromoethyl ether, DMF, 49%; (d) H_2/Pd-C, 90%; (e) PPh_3, DBAD, THF, 85 °C, 58%; (f) NaOH, MeOH/THF, 65%.

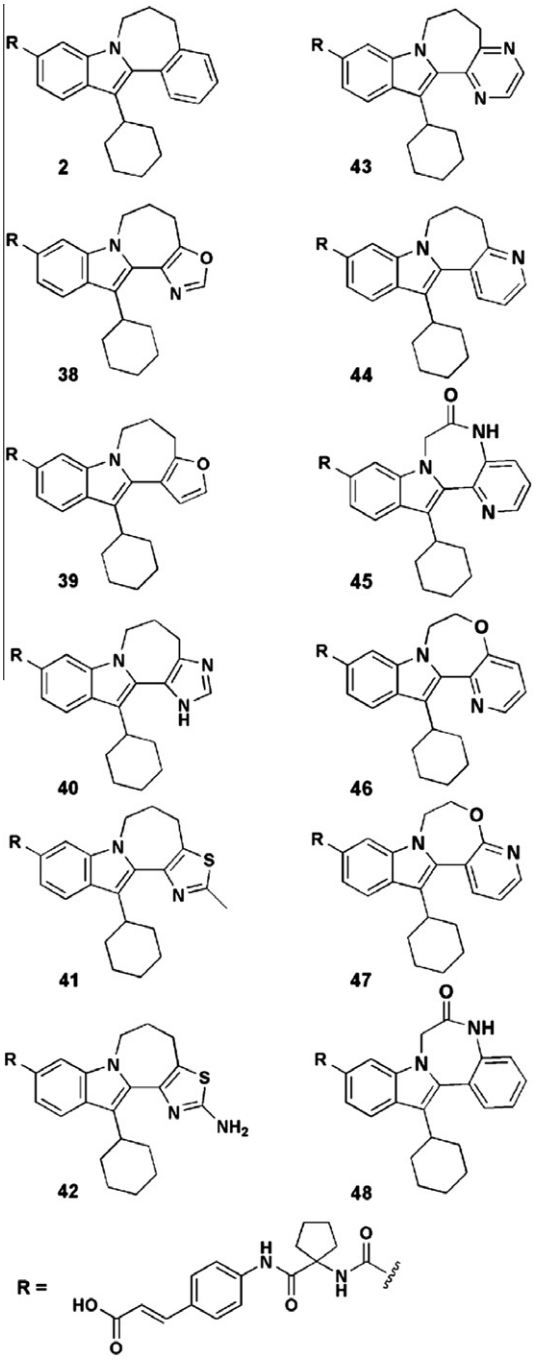


Scheme 5. Reagents and conditions: (a) TBTU, diisopropylethyl amine, DMF, 30–87%; (b) NaOH, MeOH, THF, H_2O, 90 °C, microwave, 26–86%; (c) LiI, pyridine, 150 °C, 69%.

is analog **45** which possesses a more conformationally constrained bridging element. Collectively these molecules demonstrate that polarity can be tolerated both in the bridge as well as the fused heterocyclic moieties. Interestingly, analogs **46** and **47** are isomeric, differing only in the placement of a nitrogen atom. **47** displays similar activity to the other six-membered heterocycles, but its positional isomer **46** shows a significant reduction in potency against genotype 1a. To date, we have not been able to obtain a co-crystal of any of the analogs discussed above with NS5B genotype 1a (and correspondingly, can only speculate on the origin of the observed potency differences).

However, we have previously reported the co-crystal structure of compound **48** with a genotype 1b NS5B construct,[13] the model of which is shown in Figure 3.

In an effort to elucidate the origin of the reduced potency of **41** and **42** relative to the most potent analog **47**, the A-chain from the NS5B 1b/**48** co-crystal structure (PDB: 3Q0Z) was used to model the binding of these three compounds to NS5B. The initial NS5B 1b/**48** model was prepared by assigning bond orders and adding hydrogen atoms to the atoms associated with the A-chain in the co-crystal structure followed by optimization of the hydrogen bonding network. These operations were performed with the Protein Preparation Wizard[16] in Maestro™ v. 9.2.[17] The resultant model was then subjected to 500 steps of PRCG[18] minimization using the OPLS2005 force-field[19] and implicit water solvation[20] with the positions of all non-hydrogen atoms fixed. Two additional rounds of 100 steps of steepest descent energy minimization were then performed. In the first, the positions of the protein backbone atoms were fixed, and in the second round, no positional restraints were imposed. All energy minimization calculations utilized MacroModel® v. 9.9.[21] Models of compounds **41**, **42**, and **47** in their putative bioactive conformations were then created by manual editing of the structure of **48** from the refined complex model in Maestro™.[17] Models of the complexes of 1b NS5B with compounds **41**, **42**, and **47** were then each energy minimized (OPLS2005, implicit water solvation, 500 steps PRCG) with the positions of all atoms except those of the ligands held fixed. Figure 4 shows the three compounds in the NS5B 1b P495 binding site after energy minimization. Both of the less potent compounds **41** and **42** are displaced slightly upward and sit less deeply in the binding pocket compared to potent analog **47**. Furthermore, the methyl and amino substituents of **41** and **42**, respectively, make close contacts with Ala393 and/or Leu492. This suggests that the relatively poor potency of the two thiazole analogs may be a result of steric interactions between the substituents and the protein which prevent the polycyclic cores from achieving optimal contact with the enzyme. In



Scheme 6. Final product acids that were examined in enzymatic and replicon assay systems.

the case of **42**, the presence of the polar amino moiety in a relatively hydrophobic protein environment may also contribute to the observed sub-optimal potency.

A number of compounds from this series were investigated in pharmacokinetic (PK) studies in the rat, with the data on two analogs, **44** and **45** compiled in Table 2. Compound **44** displayed low oral bioavailability (4%) with an AUC of 1.7 μM h, but had relatively low clearance characteristics (CL = 7.2 mL/min/kg; $T_{1/2}$ = 1 h), suggesting the molecule suffered from poor absorption. Compound **45** had essentially no oral bioavailability and displayed similar clearance characteristics to **44** (CL = 8.7 mL/min/kg). We attributed this lack of oral exposure to a combination of poor solubility and membrane permeability. [In a Caco-2 assay designed to determine if these analogs were substrates for P-gp, their poor solubility prevented the generation of inter-

Table 1
Inhibitory activities of indolo-fused heterocycles as determined in biochemical assays with NS5B wild-type and P495L mutant enzymes. Inhibitory activities in HCV replicon 1b and 1a systems are also provided

Final product acid	Enzyme WT IC_{50}[a] (μM)	Enzyme P495L IC_{50}[a] (μM)	Replicon 1b/1a EC_{50}[a] (μM)
2	0.034	3.39	0.014/0.071
38	0.044	5.85	0.078/0.380
39	0.034	3.72	<0.069/ND
40	0.228	ND	0.738/2.51
41	0.108	5.1	0.683*/4.19*
42	0.250	4.84	1.81/3.78
43	0.024	2.28	0.024/0.089
44	0.030	1.731	0.009/0.035
45	0.024	3.1	0.012/0.039
46	0.015	>0.6	0.022/0.157
47	0.014	2.80	0.021/0.028

[a] Values are means of at least three experiments, unless otherwise noted. Compounds **2**, **44** and **45** had CC_{50}'s of 30, 63 and 45 μM, respectively in the replicon system.
* Data from a single test occasion.

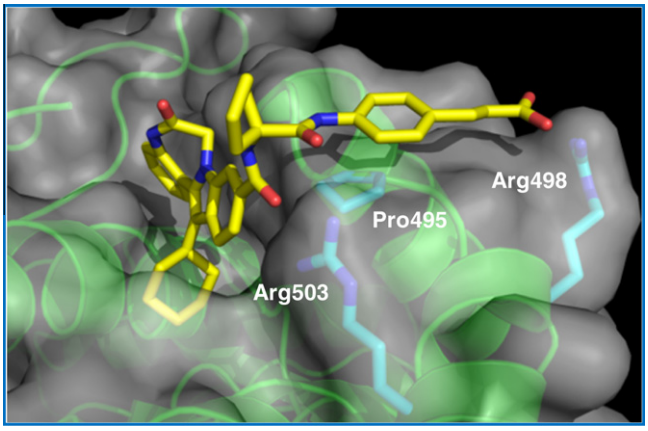


Figure 3. The co-crystal structure of compound **48** bound to the thumb domain of HCV genotype 1b NS5B (Bartenschlager).[14] The proximity of the binding site to P495 is clearly shown. The binding site is represented by a surface (gray) and cartoon (green). The side chains of Pro495, Arg498, and Arg503 and compound **48** are represented by sticks (cyan and yellow carbon atoms, respectively). Image created with PyMOL.[15]

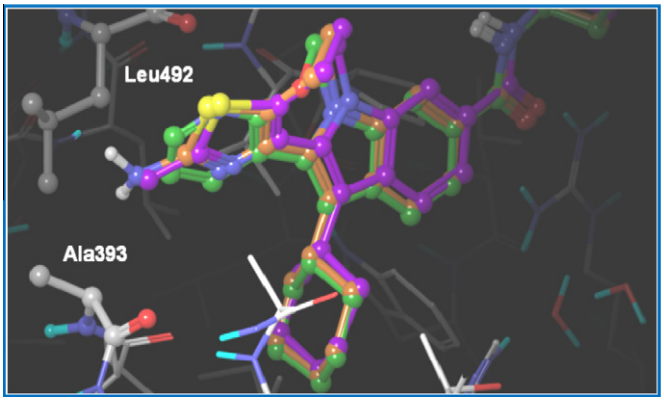


Figure 4. Compounds **41**, **42**, and **47** (shown with purple, orange, and green carbon atoms, respectively) are shown modeled into the P495 binding site in NS5B 1b. Potent analog **47** sits more deeply in the binding pocket than either of the less potent analogs which also have unfavorable steric interactions with Ala393 and/or Leu492. Figure created with Maestro™.[17]

Table 2
Rat PK results observed with select examples of the indolo-fused tetracyclic NS5B inhibitors shown in Scheme 6

Compound	F^a (%)	AUC (μM h)	Clearance[b] (mL/min/kg)
44	4	1.7	7.2
45	<1	Not detected	8.7

The above results are the average values calculated from $n = 3$. Vehicle iv and po PEG400.
[a] Oral bioavailability.
[b] Iv (dose 2 mg/kg).

pretable data.] Addressing this issue will be the subject of a future manuscript.

In summary, we have described the syntheses of a series of novel heteroaryl fused tetracyclic indole-diamide compounds and have provided data on their inhibition of NS5B. Pyridino-fused analogs with different bridging elements displayed excellent potency against both HCV 1a and HCV 1b genotypes, with compounds **44** and **45** demonstrating EC_{50}'s of 9 and 12 nM, respectively against 1b.

References and notes

1. (a) Choo, Q.-L.; Kuo, G.; Weiner, A. J.; Overby, L. R.; Bradley, D. W.; Houghton, M. *Science* **1989**, *244*, 359; (b) Lavanchy, D. *Liver Int.* **2009**, *29*(Suppl. 1), 74.
2. Farnik, H.; Mihm, U. A.; Zeuzem, S. *Liver Int.* **2009**, *29*, 23.
3. Ghany, M. G.; Nelson, D. R.; Strader, D. B.; Thomas, D. L.; Seeff, L. B. *Hepatology* **2011**, *54*, 1433.
4. (a) Brown, N. A. *Expert Opin. Investig. Drugs* **2009**, *18*, 709; (b) Beaulieu, P. L. *Expert Opin. Ther. Patents* **2009**, *19*, 145; (c) Lin, K.; Hazuda, D. J.; Otto, M. J. *Expert Opin. Investig. Drugs* **2010**, *19*, 963.
5. Zheng, X.; Hudyma, T. W.; Martin, S. W.; Bergstrom, C. P.; Ding, M.; He, F.; Romine, J.; Poss, M. A.; Kadow, J. F.; Chang, C.-H.; Wan, J.; Witmer, M. R.; Morin, P.; Camac, D. M.; Sheriff, S.; Beno, B. R.; Rigat, K. L.; Wang, Y.-K.; Fridell, R.; Lemm, J.; Qiu, D.; Liu, M.; Voss, S.; Pelosi, L.; Roberts, S. B.; Gao, M.; Knipe, J.; Gentles, R. G. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 2925.
6. Hudyma, T. W.; Zheng, X.; He, F.; Ding, M.; Bergstrom, C. P.; Hewawasam, P.; Martin, S. W.; Gentles, R. G. U.S. Patent 7,153,848, 2006.
7. (a) Wang, Y.-K.; Rigat, K. L.; Roberts, S. R.; Gao, M. *Anal. Biochem.* **2006**, 106; (b) Wang, Y.-K.; Rigat, K. L.; Sun, J.-H.; Roberts, S. R.; Gao, M. *Arch. Biochem. Biophys.* **2008**, *470*, 146.
8. Ding, M.; He, F.; Poss, M. A.; Rigat, K. L.; Wang, Y.-K.; Roberts, S. B.; Qiu, D.; Fridell, R. A.; Gao, M.; Gentles, R. G. *Org. Biomol. Chem.* **2011**, *9*, 6654.
9. Beaulieu, P. L.; Dansereau, N.; Duan, J.; Garneau, M.; Gillard, J.; McKercher, G.; LaPlante, S.; Lagacee, L.; Thauvette, L.; Kukolj, G. *Bioorg. Med. Chem. Lett.* **1825**, *2010*, 20.
10. (a) Fridell, R. A.; Qiu, D.; Wang, C.; Valera, L.; Gao, M. *Antimicrob. Agents Chemother.* **2010**, *54*, 3641; (b) O'Boyle, D. R. I. I.; Nower, P. T.; Lemm, J. A.; Valera, L.; Sun, J.-H.; Rigat, K.; Colonno, R.; Gao, M. *Antimicrob. Agents Chemother.* **2005**, *49*, 1346–1353.
11. (a) Beaulieu, P. L.; Fazal, G.; Kukolj, G.; Jolicoeur, E.; Gillard, J.; Poupart, M.-A.; Rancourt, J. Int. Patent Appl. 2003, WO 03010140.; (b) Ikegashira, K.; Oka, T.; Hirashima, S.; Noji, S.; Yamanaka, H.; Hara, Y.; Adachi, T.; Tsuruha, J.-I.; Doi, S.; Hase, Y.; Noguchi, T.; Ando, I.; Ogura, N.; Ikeda, S.; Hashimoto, H. *J. Med. Chem.* **2006**, *49*, 6950; (c) Beaulieu, P. L.; Gillard, J.; Bykowski, D.; Brochu, C.; Dansereau, N.; Duceppe, J.-S.; Hache, B.; Jakalian, A.; Lagace, L.; LaPlante, S.; McKercher, G.; Moreau, E.; Perreault, S.; Stammers, T.; Thauvette, L.; Warrington, J.; Kukolj, G. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 4987.
12. Lenarcik, B.; Ojczenasz, P. *J. Heterocycl. Chem.* **2002**, *39*, 287–290.
13. Lohmann, V.; Körner, F.; Koch, J. O.; Herian, U.; Theilmann, L.; Bartenschlager, R. *Science* **1999**, *285*, 110.
14. PDB: 3Q0Z.
15. The PyMOL Molecular Graphics System, version 1.3, Schrödinger, LLC: New York, NY, 2011.
16. Schrödinger Suite 2011 Protein Preparation Wizard; Epik version 2.2, Schrödinger, LLC: New York, NY, 2011; Impact version 5.7, Schrödinger, LLC: New York, NY, 2011; Prime version 3.0, Schrödinger, LLC: New York, NY, 2011.
17. Maestro, version 9.2, Schrödinger, LLC: New York, NY, 2011.
18. Polak, E.; Ribiere, G. *Rev. Fr. Inf. Rech. Oper., Serie Rouge* **1969**, *16*, 35.
19. Jorgensen, W. L.; Maxwell, D. S.; Tirado-Rives, J. *J. Am. Chem. Soc.* **1996**, *118*, 11225.
20. Still, W. C.; Tempczyk, A.; Hawley, R. C.; Hendrickson, T. A. *J. Am. Chem. Soc.* **1990**, *112*, 6127.
21. MacroModel, version 9.9, Schrödinger, LLC: New York, NY, 2011.