



3-Hydroxyisoquinolines as inhibitors of HCV NS5b RNA-dependent RNA polymerase

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ABSTRACT

Isoquinoline-based non-nucleoside inhibitors of HCV NS5b RNA-dependent RNA-polymerase are described. The synthesis and structure–activity relationships are detailed, along with enzyme and cellular activity.

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Hepatitis C Virus (HCV) has become a major epidemic, infecting nearly 200 million people worldwide. Infection with HCV can lead to more serious liver diseases such as cirrhosis and hepatocellular carcinoma.¹ No vaccine is currently available for HCV and therapies are limited. The current best treatment option consists of a combination of pegylated interferon- α -2a and ribavirin, however, this therapy is only effective in about 50% of patients infected with the most prevalent genotype of the virus, genotype 1.¹

The HCV genome encodes for a polypeptide which is processed to produce both structural (C, E1, E2, and p7) and non-structural (NS2, NS3, NS4a, NS4b, NS5a, and NS5b) proteins. The non-structural proteins are considered excellent targets for new, much-needed HCV therapies. NS3 protease inhibitors have shown efficacy in human HCV patients,² and it is known that the NS5b RNA-dependent RNA-polymerase (RdRp) plays a central role in HCV virus replication.¹ For that reason, an investigation into developing small molecule NS5b inhibitors was undertaken in our labs.

Hydroxyquinolone-thiadiazines **1** (Fig. 1) have been reported as inhibitors of the NS5b polymerase and a crystal structure of **1a** bound to NS5b has been published.³ Docking comparisons made

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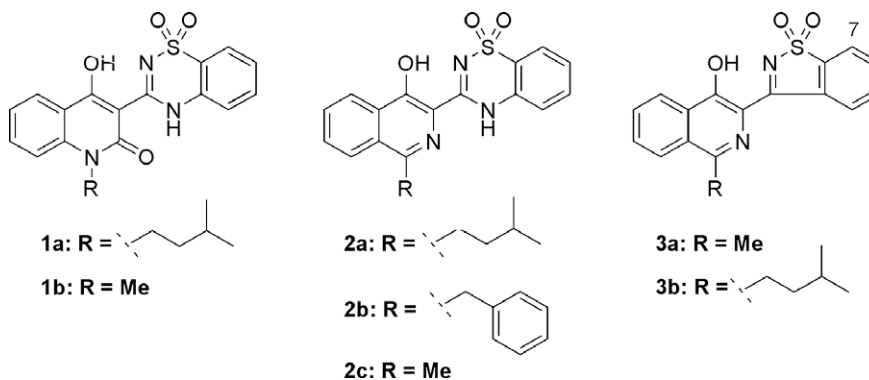


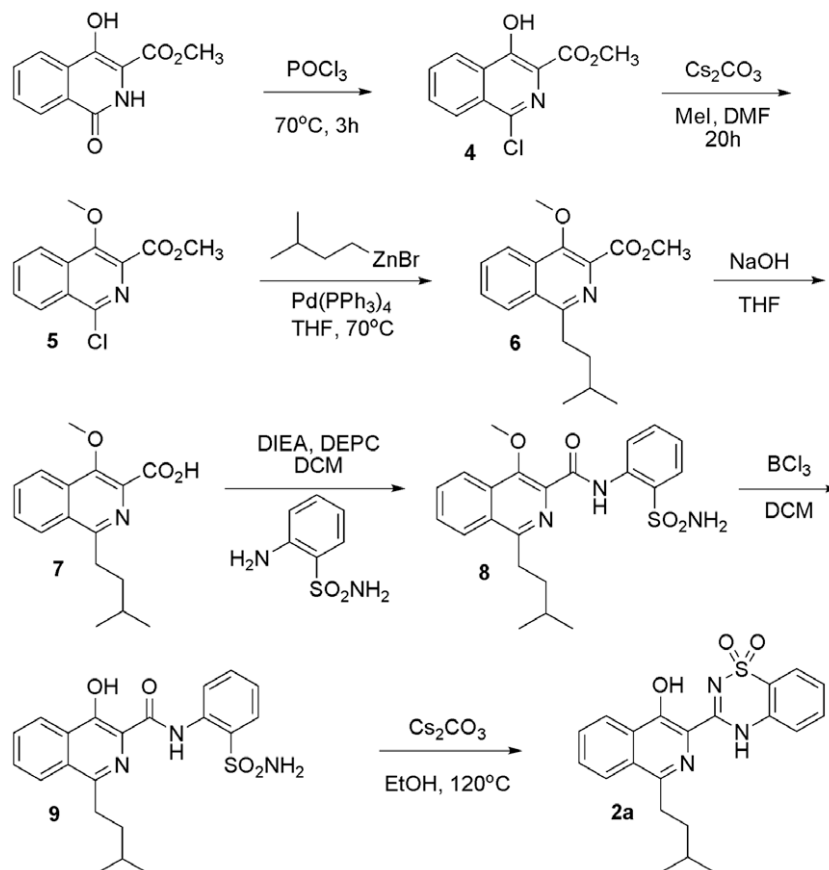
Figure 1. Hydroxyquinolone compounds **1** and the isoquinoline compounds **2** and **3**.

by placing the alternative 3-hydroxyisoquinoline scaffold **2** into the NS5b binding site of **1** suggest that the isoquinoline ring system should make similar favorable interactions in the binding site.

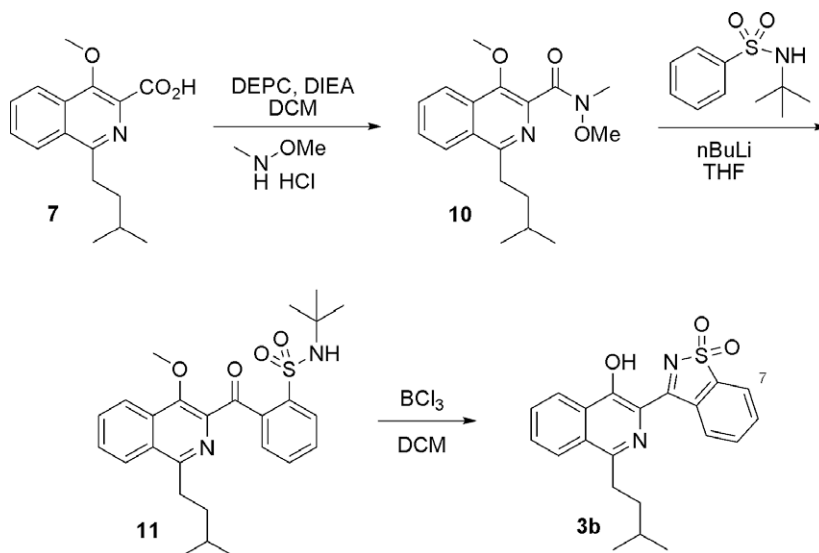
The synthesis of isoquinoline-thiadiazine **2a** began with commercially available 3-carbomethoxy-1,2,3,4-tetrahydroisoquinoline-1,4-dione (Scheme 1). The dione was selectively mono-chlorinated using phosphorous oxychloride⁴ and methylated to give **5** in good yield. A Negishi coupling⁵ with isoamyl zinc bromide, followed by hydrolysis, afforded acid **7**. Coupling with 2-aminobenzenesulfonamide, removal of the methyl protecting group, and cyclization under basic conditions (sealed tube) provided the desired compound **2a**. The corresponding benzyl analog **2b** was prepared similarly.

The NS5b (GT-1b) inhibitory potency⁶ of the isoquinoline-thiadiazine analogs (**2a**: 51 μ M, **2b**: 47 μ M) indicated this particular

scaffold was not ideal when compared with **1a** (1.1 μ M). However, we were encouraged that our first attempts at least showed measurable activity. We hypothesized that the reduced NS5b potency was likely due to a mismatch between the required binding conformation and the ground-state conformation of the isoquinoline-thiadiazine combination. This was verified by computing the 'bound' and 'unbound' (i.e., lowest energy) structures of methyl-prototypes **1b** and **2c** via ab initio molecular orbital calculations at the 6-311++G(d,p)//6-311++G(d,p) level of theory with the GAMESS program.⁷ The enolic anion form of each prototype was examined. Compound **1b** requires ca 1.56 kcal/mol to adopt the proper bound conformation, while compound **2c** requires ca 9.49 kcal/mol. The favored thiadiazine ring tautomer is as shown in Figure 1. Delocalization of the anion in **2c** is less favored, leading to significant lone



Scheme 1. Synthesis of isoquinoline thiadiazine **2a**.

Scheme 2. Synthesis of isoquinoline-dioxo-benzisothiazole **3b**.

pair repulsion in the bound conformation. The observed low energy conformation for **2c** in the 'unbound' state results from a 180 deg rotation about the isoquinoline-thiadiazine linking bond. Based on these observations the 5-membered dioxo-benzisothiazole ring was investigated in the isoquinoline series (**3**; Fig. 1). The ab initio calculations for the prototype **3a** found that the predicted bound conformation was only ca. 0.57 kcal/mol higher in energy relative to the lowest energy conformation.

Synthesis of dioxo-benzisothiazole isoquinoline **3b** is shown in Scheme 2. Using the same acid intermediate **7** from Scheme 1, Weinreb amide **10** was prepared and treated with the dianion⁸ of *N*-*tert*-butylbenzenesulfonamide. This gave ketone **11** which underwent a one-step deprotection/cyclization upon treatment with boron trichloride to generate the dioxo-benzisothiazole ring system. Compounds **3c** and **3d** (Table 1) were prepared in an analogous fashion.

The initial dioxo-benzisothiazole analogue **3b** showed an NS5b IC50 of 4.5 μ M, only 4-fold less potent than **1a** (1.1 μ M). The cyclopropyl-ethyl side chain provided a modest improvement in the enzyme potency (**3c**: 1.0 μ M) and was active in a cellular HCV GT-1b replicon assay (2.7 μ M).⁶ The 4-fluorobenzyl substituent showed similar enzyme potency (**3d**: 2.2 μ M), but more importantly showed improved cellular activity (0.44 μ M).

A model based on the X-ray structure⁹ of the NS5b polymerase palm site region was used to further refine this series of inhibitors. Examination of **3d** in this model (Fig. 2) revealed a large pocket containing several water molecules directly off the 7-position of the dioxo-benzisothiazole ring. Based on this observation a number of analogues substituted at C-7 were designed to probe this area with the goal of either interacting with or displacing one or more of the bound water molecules.

The synthesis of the C-7 substituted compounds is outlined in Scheme 3. This route utilized the ortho-substituted analogs **12a**

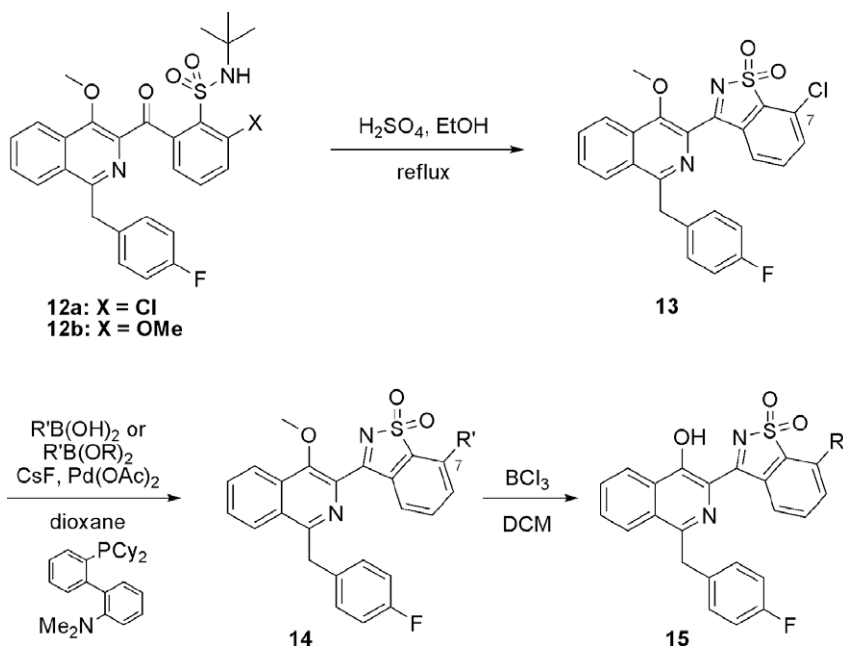
Scheme 3. Synthesis of 7-substituted dioxo-benzisothiazoles **15a-j**.

Table 1Enzyme and replicon (GT-1b) inhibitory potency, and aqueous solubility for compounds **3b–d** and **15a–j**

Compound	R	R'	NS5B IC ₅₀ , μ M	Replicon IC ₅₀ , μ M	Solubility μ g/mL
3b		—H	4.5	nd	nd
3c		—H	1.0	2.7	10
3d		—H	2.2	0.44	16
15a		—Cl	0.58	0.43	2
15b		—OMe	0.61	0.76	40
15c			3.9	0.8	41
15d			0.53	0.94	17
15e			0.35	1.1	3
15f			0.72	0.78	nd
15g			1.8	0.38	9
15h			0.55	0.15	3
15i			0.73	0.40	6
15j			0.41	0.075	7

and **12b** prepared by coupling *N*-*tert*-butyl-2-chlorobenzene-sulfonamide and *N*-*tert*-butyl-2-methoxybenzene-sulfonamide with

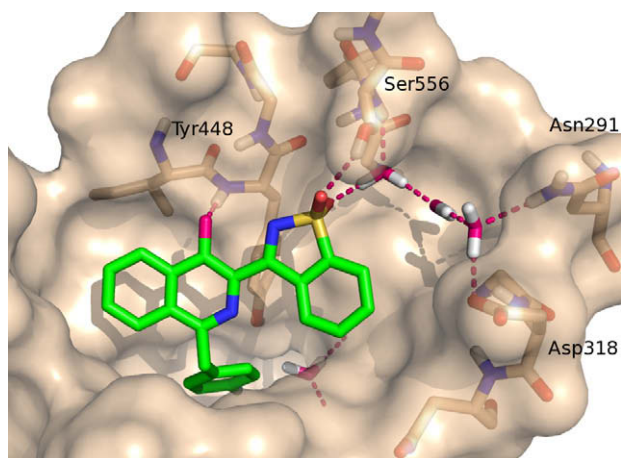


Figure 2. Model of isoquinoline **3d** in the NS5b active site binding region. Connolly surface is depicted.

10 in a similar manner as described previously for **11**. Treatment of **12a** and **12b** directly with BCl_3 gave **15a** and **15b**. Cyclization of **12a** using a modified procedure employing sulfuric acid in EtOH in place of BCl_3 gave the O-protected 7-chloro-dioxo-benzisothiazole compound **13**. Aryl chloride **13** underwent Suzuki couplings¹⁰ with a variety of boronic acids/esters. Deprotection of methyl ethers **14** gave analogs **15c–15j** (Table 1).

Small substituents (**15a**, **15b**) at the 7 position of the dioxo-benzisothiazole ring were well-tolerated, but did not show any improvement in cellular potency over the 7-H compound **3d**. Phenyl (**15c**), substituted phenyl (**15d**, **15e**) and heterocycles (**15f–j**) were all tolerated, but only the 6-membered heterocycles **15h** and **15j** showed any noticeable improvement in cellular activity. The smaller 7-H and 7-OMe substituents, along with the unsubstituted 7-phenyl analog showed the best aqueous solubilities within the series examined.

In summary, a series of new isoquinoline-based HCV NS5b polymerase inhibitors have been efficiently synthesized in 7–9 steps from 3-carbomethoxy-1,2,3,4-tetrahydroisoquinoline-1,4-dione. A structure–activity relationship was generated around the 7-position of the dioxo-benzisothiazole series, and the pyridone analogue **15j** was identified as the most potent HCV replicon

inhibitor in this investigation (0.075 μM).^{11,12} These isoquinolines represent a promising new series of HCV NS5b inhibitors and efforts to further enhance their properties are ongoing.

References and notes

- (a) Tan, S.-L.; Pause, A.; Shi, Y.; Sonenberg, N. *Nat. Rev. Drug Discov.* **2002**, *1*, 867; (b) Brass, V.; Blum, H. E.; Moradpour, D. *Expert Opin. Ther. Targets* **2004**, *8*, 295; (c) Beaulieu, P. L.; Tsantrizos, Y. S. *Curr. Opin. Invest. Drugs* **2004**, *5*, 838.
- Lamarre, D.; Anderson, P. C.; Bailey, M., et al *Nature* **2003**, *426*, 186.
- (a) Dhanak, D.; Duffy, K. J.; Johnston, V. K., et al *J. Biol. Chem.* **2002**, *277*, 38322; (b) Tedesco, R.; Shaw, A. N.; Bambal, R., et al *J. Med. Chem.* **2006**, *49*, 971.
- Weidmann, K.; Baringhaus, K. -H.; Tschank, G.; Werner, U. U.S. Patent 6,093,730, 2000.
- Shiota, T.; Yamamori, T. *J. Org. Chem.* **1999**, *64*, 453.
- Klumpp, K.; Leveque, V.; Le Pogam, S.; Ma, H.; Jiang, W.-R.; Kang, H.; Granycome, C.; Singer, M.; Laxton, C.; Hang, J. Q.; Sarma, K.; Smith, D. B.; Heindl, D.; Hobbs, C. J.; Merrett, J. H.; Symons, J.; Cammack, N.; Martin, J. A.; Devos, R.; Najera, I. *J. Biol. Chem.* **2006**, *281*, 3793.
- GAMESS Version 27 June 2005 (R2). Schmidt, M. W.; Baldridge, K. K.; Boatz, J. A.; Elbert, S. T.; Gordon, M. S.; Jensen, J. J.; Koseki, S.; Matsunaga, N.; Nguyen, K. A.; Su, S.; Windus, T. L.; Dupuis, M.; Montgomery, J. A. *J. Comput. Chem.* **1993**, *14*, 1347–1363.
- (a) Lombardino, J. G. *J. Org. Chem.* **1971**, *36*, 1843; (b) Dauban, P.; Dodd, R. H. *Org. Lett.* **2000**, *2*, 2327.
- X-ray crystal structure (1C2P) from Lesburg, C. A.; Cable, M. B.; Ferrari, E.; Hong, Z.; Mannarino, A. F.; Weber, P. C. *Nat. Struct. Biol.* **1999**, *6*, 937.
- Old, D. W.; Wolfe, J. P.; Buchwald, S. L. *J. Am. Chem. Soc.* **1998**, *120*, 9722.
- Portions of this work have previously been described See: Blake, J. F.; Fell, J. B.; Fischer, J. P.; Hendricks, R. T.; Spencer, S. R.; Stengel, P. J. U.S. Patent Application US 20060252785, 2006.; Blake, J. F.; Fell, J. B.; Fischer, J. P.; Hendricks, R. T.; Robinson, J. E.; Spencer, S. R.; Stengel, P. U.S. Patent Application US 2006040927, 2006.
- A related series of 7-substituted dioxo benzo-isothiazole containing NS5b inhibitors has recently been described. See: Kim, S. H.; Tran, M. T.; Ruebsam, F.; Xiang, A. X.; Ayida, B.; McGuire, H.; Ellis, D.; Blazel, J.; Tran, C. V.; Murphy, D. E.; Webber, S. E.; Zhou, Y.; Shah, A. M.; Tsan, M.; Showalter, R. E.; Patel, R.; Gobbi, A.; LeBrun, L. A.; Bartkowski, D. M.; Nolan, T. G.; Norris, D. A.; Sergeeva, M. V.; Kirkovsky, L.; Zhao, Q.; Han, Q.; Kissinger, C. R. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 4181.