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

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Negishi
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2-Aminobenzenesulfonamide
N-tert-Butyl-2-chlorobenzene-sulfonamide
3-Carbomethoxy-1,2,3,4-tetrahydroisoquinoline-1,4-dione
Computational
Model
Conformation

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.

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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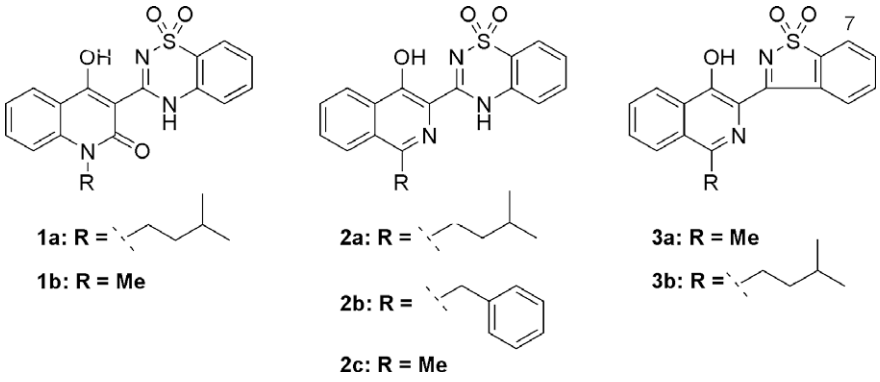
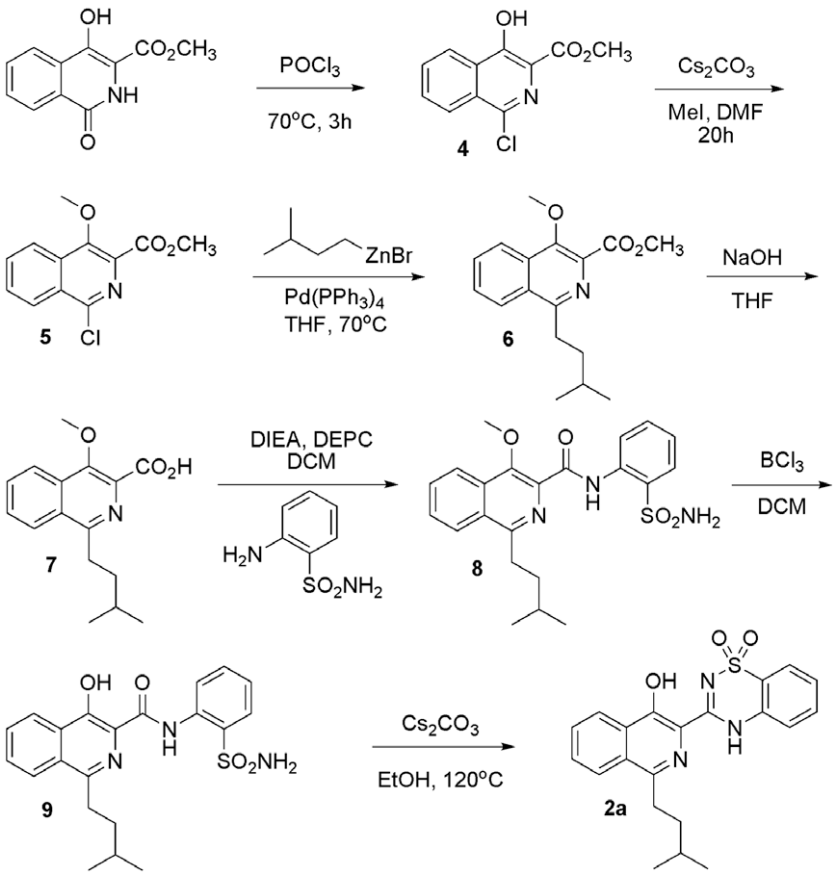


Figure 1. Hydroxyquinolone compounds **1** and the isoquinoline compounds **2** and **3**.

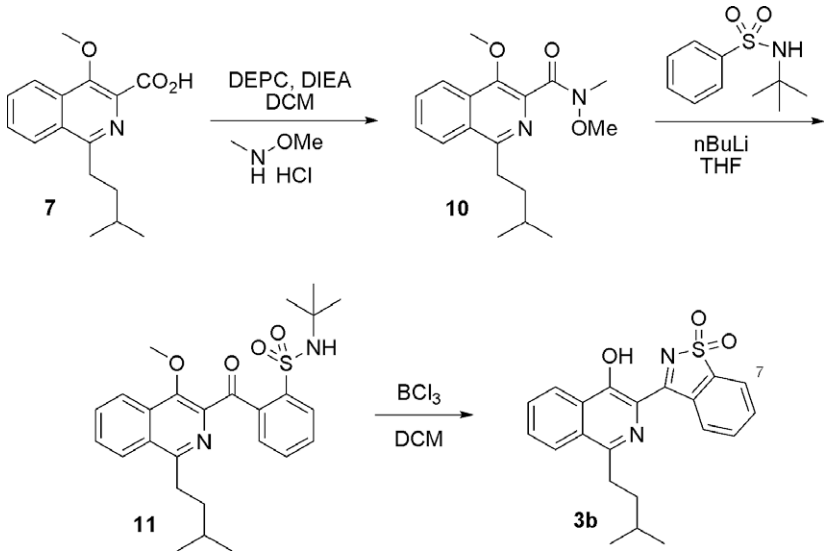
by placing the alternative 3-hydroxyisoquinoline scaffold **2** into the NS5b binding site of **1** suggest that the isoquinoline ring system should make similar favorable interactions in the binding site.

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 oxychloride[4] and methylated to give **5** in good yield. A Negishi coupling[5] 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 potency[6] of the isoquinoline-thiadiazine analogs (**2a**: 51 μM, **2b**: 47 μM) indicated this particular scaffold was not ideal when compared with **1a** (1.1 μM). 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-benzoisothiazole **3b**.

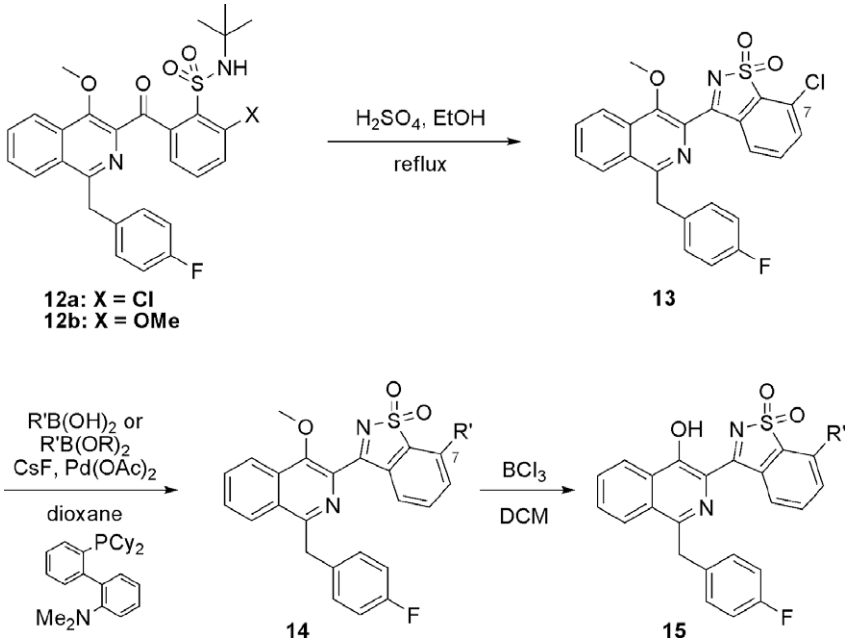
pair repulsion in the bound conformation. The observed low energy conformation for **2c** in the 'unbound' state results from a 180 degr rotation about the isoquinoline-thiadiazine linking bond. Based on these observations the 5-membered dioxo-benzoisothiazole ring was investigated in the isoquinoline 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-benzoisothiazole 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 dianion[8] of *N*-*tert*-butylbenzenesulfonamide. This gave ketone **11** which underwent a one-step deprotection/cyclization upon treatment with boron trichloride to generate the dioxo-benzoisothiazole ring system. Compounds **3c** and **3d** (Table 1) were prepared in an analogous fashion.

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

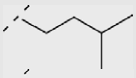
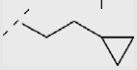
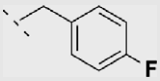
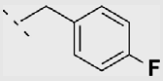
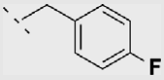
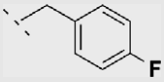
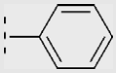
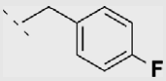
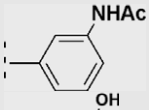
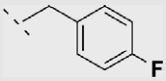
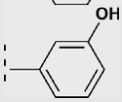
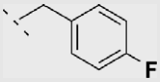
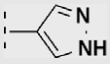
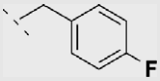
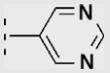
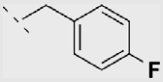
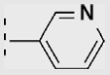
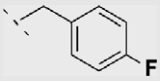
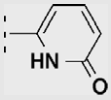
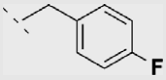
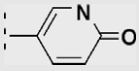
A model based on the X-ray structure[9] of the NS5b polymerase palm site region was used to further refine this series of inhibitors. Examination of **3d** in this model (Fig. 2) revealed a large pocket containing several water molecules directly off the 7-position of the dioxo-benzoisothiazole ring. 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-benzoisothiazoles **15a**–**j**.

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

Compound	R	R′	NS5B IC50, μM	Replicon IC50, μM	Solubility μg/mL
3b			4.5	nd	nd
3c			1.0	2.7	10
3d			2.2	0.44	16
15a			0.58	0.43	2
15b			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 **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-benzoisothiazole compound **13**. Aryl chloride **13** underwent Suzuki couplings[10] with a variety of boronic acids/esters. Deprotection of methyl ethers **14** gave analogs **15c–15j** (Table 1).

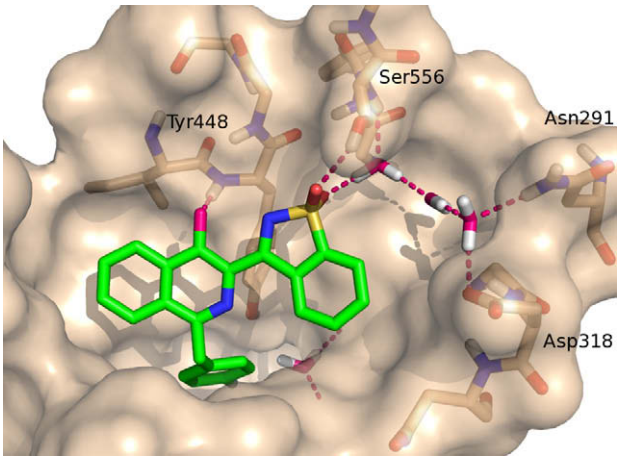


Figure 2. Model of isoquinoline **3d** in the NS5b active site binding region. Connolly surface is depicted.

Small substituents (**15a, 15b**) at the 7 position of the dioxo-benzoisothiazole 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-benzoisothiazole 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.

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