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Design and synthesis of HCV agents with sequential triple inhibitory potentials ☆

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ABSTRACT

The union of HCV-796, a potent selective HCV NS5B polymerase inhibitor, and Ribavirin, a molecule with activities against a wide spectrum of viruses, resulted in a class of new anti-HCV agents with a sequential triple inhibitory mechanism.

Hepatitis C is a serious world wide health problem caused by RNA virus—hepatitis C virus infection that infects about 170 million people.[1] Failed treatment or under treatment, which is not uncommon due to its asymptotic nature, leads to chronic hepatitis C.[1] Chronic hepatitis C, defined as hepatitis C virus persisting for more than six months, can cause chronic liver inflammation that can develop into cirrhosis and cancer, and is the most common reason for liver transplantation. While some therapies exist, the result is far from ideal. The standard treatment for those who have contracted HCV, acute or chronic, is administration of the combination therapy of Ribavirin[2] (**1**) with pegylated interferon alpha (INF-α) for 24 weeks or 48 weeks depending on viral genotypes;[3] about 55% of patients obtain sustained viral response (SVR).[3,4] Quick mutation of the virus exacerbates the situation because it results in therapy resistance, meaning that only a portion of patients can achieve remission.[5] Another drawback is the severe adverse effect that is often observed with this therapy. Better medications are badly needed.

Unceasing efforts have been made in discovering and developing potent and specific anti-infectives for HCV. Many newer classes of selective antivirals, (like RNA polymerase inhibitors) have entered into clinical trials.[6,7] It is believed that a combination regimen involving agents of different mechanisms will one day cure HCV infection.[7] In the course of our development of a non-nucleoside polymerase inhibitor HCV-796 (**2**),[8] which has superior effect in clearing HCV when combined with standard treatment regimens—INF-α and ribavirin, we envisioned that combining **2** chemically with ribavirin (**1**) might produce a prodrug (**3**) that would release **2** and **1** in vivo. This offers benefits; for example, unlike the conventional combination therapy clinical trial, it can be tested as one entity, thus minimizing the cost and other undesired factors. Herein, we reported our finding that **3**, designed as a class of prodrug, demonstrates fairly potent anti-HCV activities, and might operate on a cascade of inhibitory mechanisms at different times during the course of action.

Ribavirin is a synthetic nucleoside analogue and has demonstrated activities against a wide spectrum of DNA and RNA viruses. For example, it shows activities against influenzas, flaviviruses and viral hemorrhagic fevers. More importantly, it also has synergic effect when combined with interferon, though it is not effective against HCV when given alone. The mechanism of action for Ribavirin's role in HCV treatment is not yet clear.[9] Conceivably, it is phosphorylated in vivo as other nucleoside anti-metabolites and interrupts the genetic replication of the virus. It was suggested that Ribavirin might exert its antiviral effect by inhibiting inosine monophosphate dehydrogenase (IMPDH),[10] but even more potent selective IMPDH inhibitor VX-497 did not show reducing HCV replication in the patient.[9] Thus far, it is generally agreed that Ribavirin gains non-specific effect on HCV both directly and indirectly.[9]

On the other hand, HCV-796 is selective non-nucleoside polymerase inhibitor by selective inhibitory binding to the Palm II region of HCV non-structural protein 5B polymerase (NS5B), thus operating on a specific mechanism. It is one of the most potent known anti-HCV agents with single digits to low double digits nM for enzyme

☆ Wyeth was acquired by Pfizer on October 16th, 2009.

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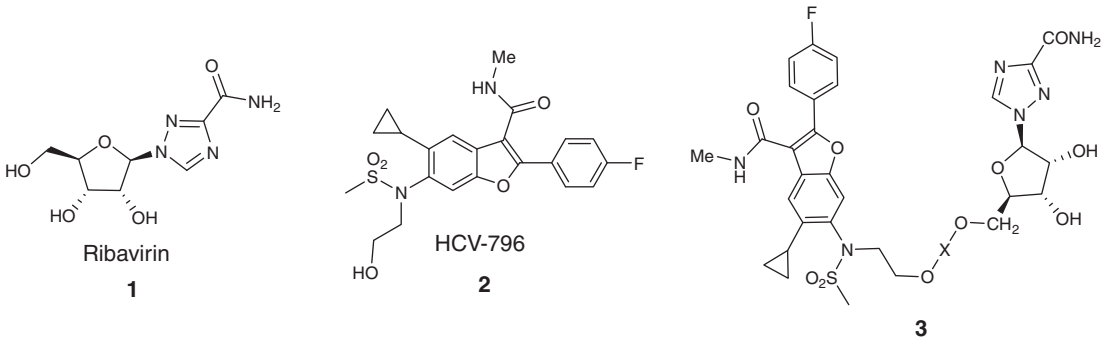
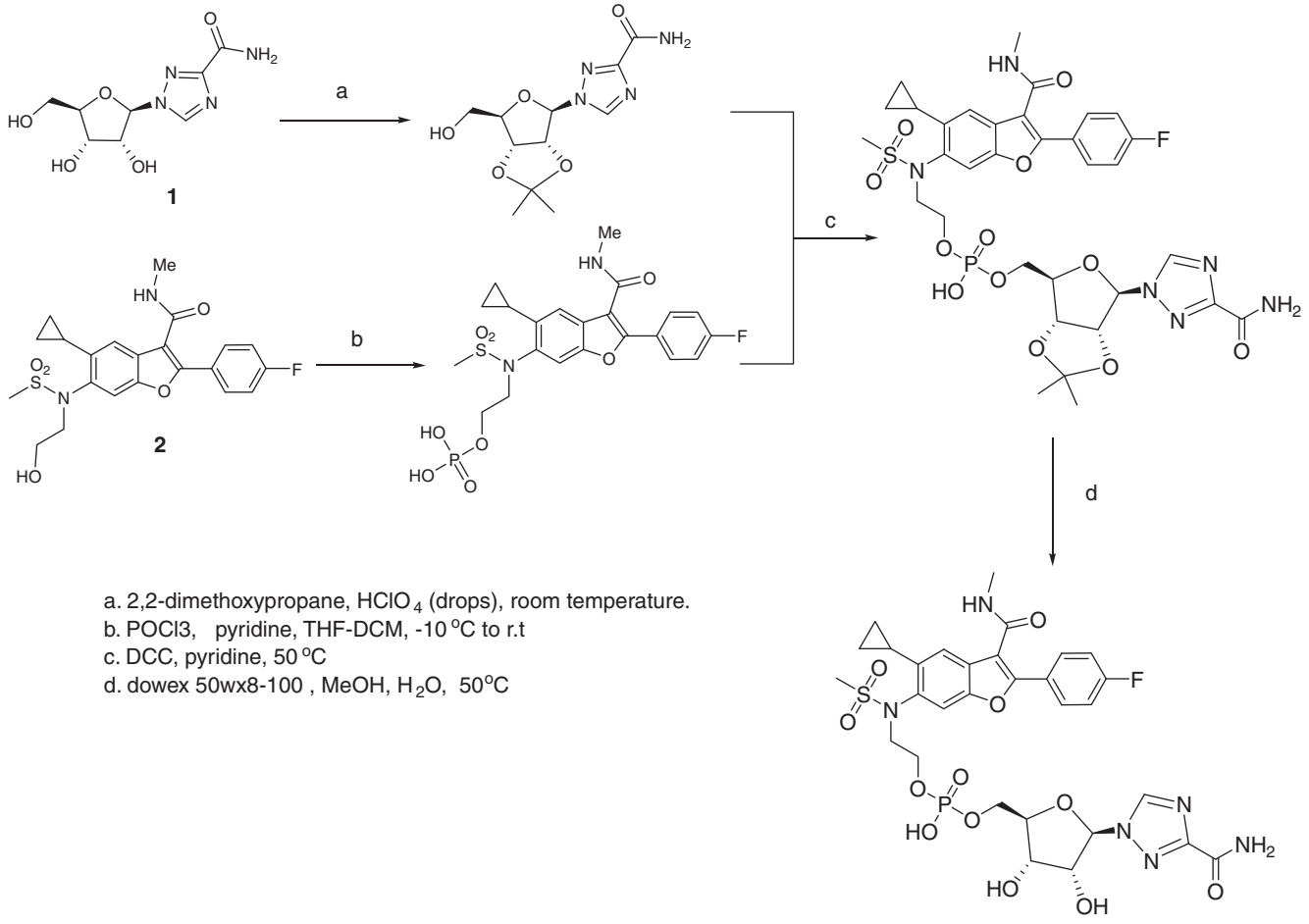


inhibitory activity. It is efficacious as a mono-therapeutic agent for HCV in animal study and early phase clinical trials, but the effect is transient. When it is given in addition to the standard regimen, a significant portion of HCV patients achieved notable reduction of virus load to an undetectable level.

Prodrug[11] is a certain derivative of a pharmaceutical agent that is either not active or significantly less active until it is converted back to the agent in vivo after it is administered. This particular derivatization technique is often used to improve many properties of an active; indeed many marketed drugs are in the forms of prodrugs. Quite a few ribavirin derivatives have been synthesized to minimize adverse effects of ribavirin,[12] but there is no report of chemically linking Ribavirin with another anti-HCV agent, for example HCV-796; such compounds, if designed properly, could release ribavirin and HCV-796 simultaneously on site in a seemingly prodrug manner.

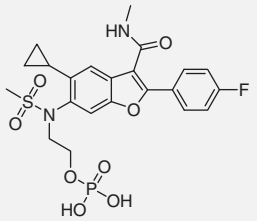
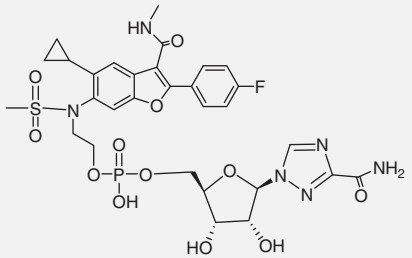
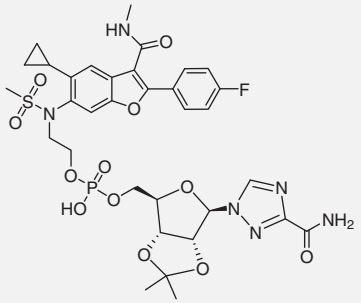
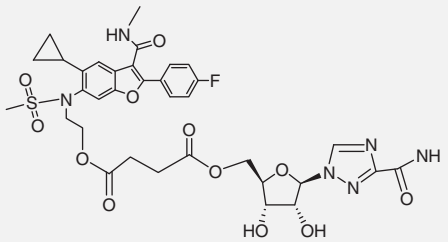
The two primary hydroxyl groups of Ribavirin and HCV-796 are an apparent choice of linking in our study. They were attached together by a linker to yield **3**, molecules hopeful to deliver two anti-HCV agents in vivo. The linkers employed are phosphate and succinic. Both are common and acceptable in pharmaceutical development for analoguing and salting active pharmaceutical agents; many phosphates and succinates are prodrugs.

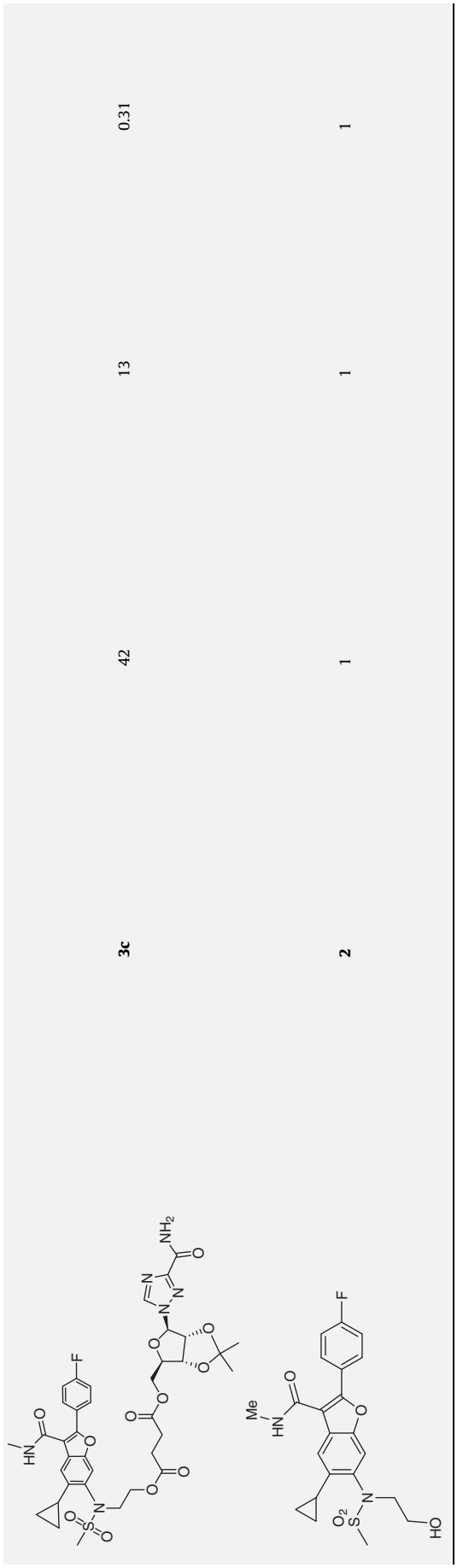
We set out to synthesize the molecules with a short convergent synthetic sequence (Scheme 1). The 3- and 4-hydroxyl groups of Ribavirin were first protected according to known procedure.[13]



Scheme 1. Synthesis of **3**.

Table 1
Summary relative activities of **3** in cell and cell-free assay

Compound	Compound number	Enzymatic (BB7)	BB7 cells	Enzymatic to cellular activity attenuation
	**4**	0.4	8.9	22.2
	**3b**	6.2	7.3	1.18
	**3a**	11.8	18.6	1.57
	**3d**	22	6.2	0.28



3c	42	1		0.31
2	1	1		1

Meanwhile the HCV-796 was added a linker to become its phosphate **4**, by a one-pot two-steps conversion. The union of these two motifs **4** and **1** by coupling rendered **3a**. A mild de-protection to free the 3- and 4-hydroxyls on the sugar of **3a** afforded **3b**. No effort was made to optimize the reaction and/or separation. Relevant reaction products were purified if necessary either by HPLC or by preparative TLC. Molecules with a succinic acid as the linker, **3c** and **3d**, were synthesized in a similar way.

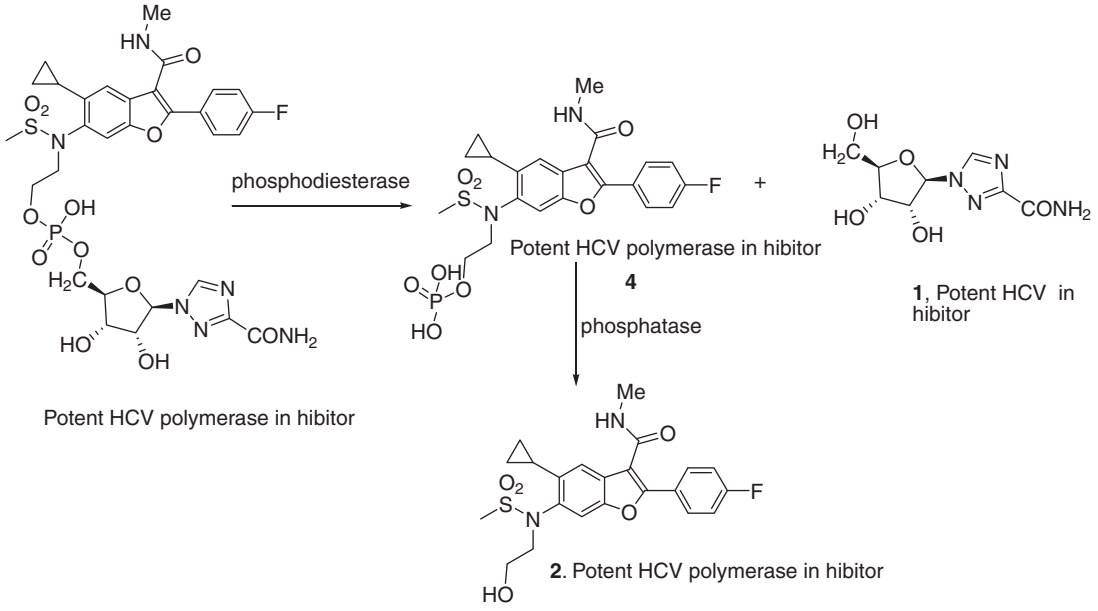
The availability of **3** enabled us to test their enzymatic activities, which were repeated several times, whose averages are then normalized against those of HCV-796 which is set to 1.[14] As a comparison, **4** was also tested. Results of relative activities are summarized in Table 1. Unlike typical prodrugs that usually have no intrinsic activities, these compounds showed various degrees of activities in enzymatic assay. From the known relationship of structure and activity of this benzofuran class compound, the hydroxyl-ethyl tail of HCV-796 is tolerated for some small modification. It is somewhat surprising that with a large group or highly polar group added, it still retains most activity. In fact, phosphate of HCV-796, **4**, showed higher enzymatic activity than HCV-796 itself.

Ribavirin has not been reported to be an HCV polymerase inhibitor;[15] activities of **3** are reasonably believed from the contribution of HCV-796 moiety-those compounds bind to the same site of HCV-796 in a similar manner as the benzofuran motif fitting into the hydrophobic pocket and tail being oriented toward aqueous phase. The influence of the linker is not fully explored; but it is evident that it plays roles in possessing activity from this limited data set. This opens the possibility of further exploration and tuning.

Next, cellular activities of **3** and **4** were investigated. All compounds demonstrated activities and were reported as relative cellular activity for a fair comparison as well (Table 1).[14] All showed weaker cellular activities than HCV-796 for different reasons. In most cases, the readout of a compound's cellular activity would be weaker than its enzymatic readout, being attenuated with a factor. HCV-796's cellular activity was recorded here to be 7.5-fold weaker than its enzymatic activity in nM absolute readings in current study. Most compounds here have weaker enzymatic activities to start with. While HCV-796 Phosphate, **4**, has more potent cell free inhibitory activity than HCV-796, it has far less permissibility than HCV-796, for it is predictive that this phosphate is in ionic forms under physiological PH and will not penetrate into the cells.

However, when relative attenuations of cellular activity compared to enzymatic activities are taken into a closer look in terms of the ratio of its cellular activities to that of enzymatic activity (Table 1, 5th column), something interesting was revealed. Other compounds (e.g., **3a**–**3d**) showed enhanced relative cellular activity. Neutral compounds (e.g., **3c** and **3d**) showed enhanced relative cellular activity as they have smaller attenuations than that of HCV-796. Those neutral molecules are expected to have the same permissibility as HCV-796. Acidic compounds (**3a** and **3b**) showed comparable attenuation to HCV-796 even though they are expected to have less penetration in cells.

That implies the involvement of more than the molecule being tested. This can be explained to be a combination effect of all species. After a compound enters a cell, it would release HCV-796 and Ribavirin to some degree by esterases; the cellular activity reflects the sum of all the chemical species present inside the cell. For instance **3b** is surmised to be hydrolyzed inside the cell by appropriate enzymes. Though only a portion of **3b** was expected to enter the cell, the hydrolyzed product **2** is more potent. Together with the synergy from Ribivarin, it makes **3b** potent in the cellular setting. This notion is supported by the enzymatic degradation study of **3b**, which is a phosphate diester that is expected to be a good



Scheme 2. Enzymatic hydrolysis of **3b** to release **4**, **1** and **2** successively.

substrate for phosphodiesterase and phosphatase. Indeed, phospholipase D was found to catalyze the hydrolysis of **3b** into to **1** and **4**, which was further slowly hydrolyzed to **2** under this condition. Another experiment showed that **4** was quickly converted to **2** catalyzed by alkaline phosphatase. In short, **3b** gains cellular activity by itself, from its hydrolyzed intermediates (**4** and **1**) and final hydrolyzed products (**2** and **1**) during the course of **3b** inside the cell (Scheme 2).

It is known that HCV replicates with high frequency and with very high error rate so that HCV readily develops resistance to therapies, especially selective inhibitors with specific mechanism. Mutants resistant to HCV-796 and other newer class of anti-virals have already been discovered.[5,7,16] It is this very reason that most monotherapies provide transient effect at best and a combination is needed for a sustained HCV eradication. A triple combination of INF, Ribavirin and an orthogonally selective HCV inhibitor is believed to be needed for a sustained HCV eradication.[7,17] The combination of HCV-796 with Ribavirin and INF-2a is a good example. Another example is the observation of synergic effect of VX-950, a selective protease inhibitor when used with Ribavirin and INF. The therapy duration and dosage are the other two main factors in achieving therapy efficacy and mitigating adverse effects.

In this context, current approach can not only deliver two or three agents in a single entity fashion, but can also provide additional benefits. It could operate on different mechanisms at different times during the course of action and with longer duration of overall inhibition. This strategy might be applicable to other inhibitors and in particular, but not limited to, other benzofuran classes of the inhibitors that occupy the same binding pocket of NS5B.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.06.156.

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