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Research paper

Design, synthesis and biological evaluation of pyrido[2,3-*d*]pyrimidin-7-(8*H*)-ones as HCV inhibitors

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ABSTRACT

The design and selection of a combinatorial library of pyrido[2,3-*d*]pyrimidin-7(8*H*)-ones (**4**) has allowed the synthesis of 121 compounds, using known and new synthetic methodologies, and the evaluation of the inhibitory activity against hepatitis C virus (HCV) genotype 1b replicon. Among these compounds, **21**{*4,10*} and **24**{*2,10*} presented very high activities [$EC_{50} = 0.027$ μM ($CC_{50} = 5.3$ μM) and $EC_{50} = 0.034$ μM ($CC_{50} = 13.5$ μM), respectively] and high selectivity indexes, 196 and 397. These values are similar to the EC_{50} reported for sofosbuvir (**2**) (0.048 μM) using a similar methodological approach and the same virus subtype. **21**{*4,10*} and **24**{*2,10*} are obtained through shorter synthetic itineraries than sofosbuvir and **24**{*2,10*} is achiral contrary to sofosbuvir which presents 4 stereogenic centers. *In silico* studies suggest that **21**{*4,10*} and **24**{*2,10*} inhibits NS5B polymerase through allosteric site binding.

1. Introduction

Hepatitis C is an infectious disease caused by the hepatitis C virus (HCV), which constitutes the leading cause of chronic liver diseases. HCV has infected an estimated 150–200 million people being an important health care problem worldwide [1]. The virus persists in the liver in about 85% of those infected.

HCV belongs to the genus *Hepacivirus*, a member of the family *Flaviviridae*. HCV is a positive-sense single-stranded RNA virus of approximately 9.6 Kb. Based on genetic differences between HCV isolates, the hepatitis C virus species is classified into seven genotypes (1–7) with several subtypes within each genotype [2], which differ by 30–35% of the nucleotide sites over the complete genome. For instance, subtypes 1a and 1b are found worldwide and cause 60% of hepatitis C cases.

HCV encodes structural (E1, E2, C and p7) and nonstructural proteins (NS2, NS3/4A, NS4B, NS5A, NS5B) [3]. Nonstructural proteins represent the main targets for intensive anti-HCV drug discovery programs.

Before the development of direct acting antivirals (DAAs), treatment for hepatitis C was based on a combination of (pegylated) interferon-alpha (PEG-IFN-α) plus ribavirin (**1**) for 24 or 48 weeks, depending on the HCV genotype [4]. This combination therapy yields a sustained virologic response (SVR) in more than 40% of patients infected by genotype 1 and 4 viruses and about 80% of those infected by genotypes 2 and 3. Besides the limited efficacy on HCV type 1, this combination therapy has significant side effects and is poorly tolerated in many patients. The most common side effects that may occur after treatment with PEG-IFN-α are: nausea, diarrhea, fever, chills, muscle and joint pain, difficulty concentrating, thyroid disorders, hair loss, insomnia, irritability, mild to severe depression, and rarely, suicidal thoughts. Other serious side effects that may occur are bone marrow toxicity, cardiovascular disorders, hypersensitivity disorders,

Abbreviations: BOP, (Benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate; *m*-CPBA, 3-Chloroperbenzoic acid; DBU, 1,8-Diazabicyclo [5.4.0]undec-7-ene; DDQ, 2,3-Dichloro-5,6-dicyano-p-benzoquinone; PSII, Palm site II of NS5B polymerase; PyBOP, (Benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate.

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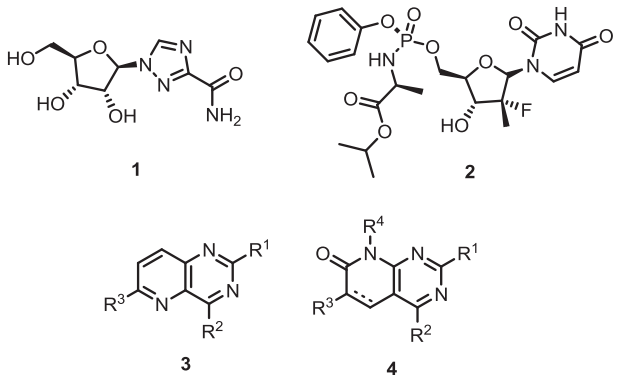


Fig. 1. Ribavirin (**1**), sofosbuvir (**2**), pyrido[3,2-*d*]pyrimidines active against HCV (**3**), and pyrido[2,3-*d*]pyrimidin-7-(8*H*)-ones (**4**).

endocrine disorders, pulmonary disorders, colitis, pancreatitis and ophthalmologic disorders. PEG-IFN-α can cause neuropsychiatric, autoimmune, ischemic and infectious fatal. With respect to ribavirin, anemia is often the most frequent side effect during the treatment of hepatitis C. Ribavirin can also cause decreased white blood cells and platelets [5].

In 2011, the first two DAAs (boceprevir and telaprevir) that targeted NS3/4A protease viral protein were introduced to the market. Added to PEG-IFN-α and ribavirin increased the SVR in genotype infected patients. However, several disadvantages were reported [6]. Thus, further pursuit of this strategy as a potential treatment was warranted. More recently, sofosbuvir (**2**, Sovaldi™) [7], a nucleotide analog, has been approved for the treatment of HCV infection. Treatment with this new drug without PEG-IFN-α provides higher cure rates, less side effects, and a reduction of the duration of the therapy. Sofosbuvir inhibits the NS5B polymerase, one of the most attractive targets of HCV because it presents a highly conserved region in the genome with respect to the different genotypes [8]. Sofosbuvir has changed the prognosis of this severe disease, however the price associated with the treatment (in the range 20,000–40,000 € per one year) has engendered considerable controversy including patent invalidity claims (Fig. 1).

We consider that this scenario shows that there is still a need for clinically effective agents for the treatment of infections caused the hepatitis C virus (HCV), which may improve current therapeutic strategies, in particular, which may reduce unwanted side effects of current drugs.

We decided to focus on the NS5B polymerase in order to develop a non-nucleotide inhibitor. As starting point we consider a series of patents of Gilead Sciences on the use of pyrido[3,2-*d*]pyrimidines (**3**) as HCV inhibitors (see for instance [9]) due to the structural similarity between such compounds and the 7-oxopyrido[2,3-*d*] pyrimidines (**4**) developed by our group.

In this context, our group has a broad experience in the synthesis of 4-amino-5,6-dihydropyrido[2,3-*d*]pyrimidin-7-(8*H*)-ones (**9**) (Scheme 1) from α,β-unsaturated esters (**5**). Thus, 2-methoxy-6-oxo-1,4,5,6-tetrahydropyridin-3-carbonitriles (**7**) are obtained by reaction of an α,β-unsaturated ester (**5**) and malononitrile (**6**) in NaOMe/MeOH [10]. Treatment of pyridones **7** with guanidine systems (**8**, R^4 = H, alkyl, aryl) affords 4-amino-pyrido[2,3-*d*]pyrimidines (**9**, R^4 = H, alkyl, aryl) [11].

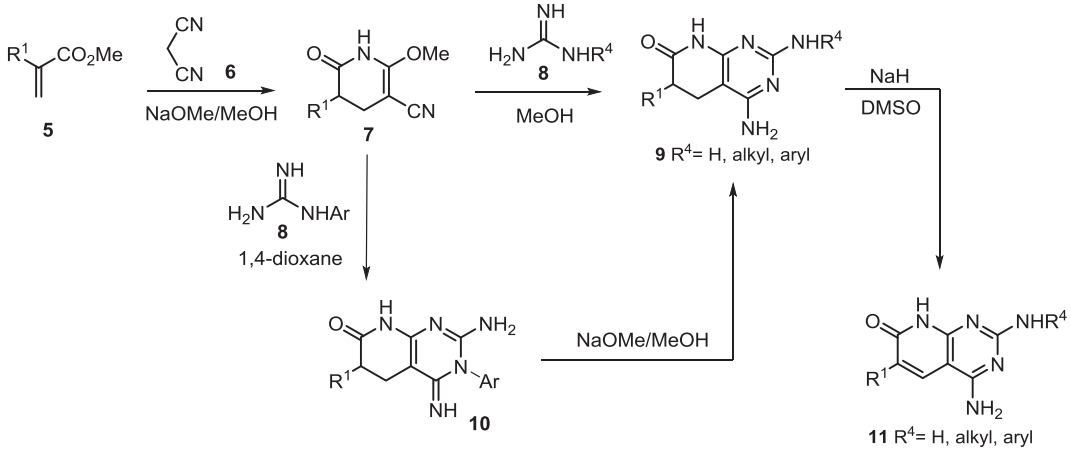
More recently, we have developed protocols for the dehydrogenation of 5,6-dihydropyrido[2,3-*d*]pyrimidin-7(8*H*)-ones **12** to pyrido[2,3-*d*]pyrimidin-7(8*H*)-ones **11** [12] and a methodology for the synthesis of 2-arylamino substituted 4-amino-5,6-dihydropyrido[2,3-*d*]pyrimidin-7(8*H*)-ones (**9**, R^4 = aryl) via the corresponding 3-aryl substituted pyridopyrimidines **10**, formed upon treatment of pyridones **7** with an arylsubstituted guanidine **8** in dioxane, which underwent the Dimroth rearrangement to the desired 4-aminopyridopyrimidines (**9**, R^4 = aryl) with NaOMe/MeOH. The overall yields of such two-step protocol are in general higher than the reaction between pyridones **7** and an arylsubstituted guanidine **8** (Scheme 1) [13].

The present paper deals with the use of such methodology for the synthesis of pyrido[2,3-*d*]pyrimidin-7-(8*H*)-ones as a potential treatment of HCV.

2. Results and discussion

2.1. Virtual combinatorial library of pyrido[2,3-d]pyrimidin-7-(8H)-ones

Inhibitory activity of several privileged scaffolds has been intensively researched as potential anti-HCV candidates. Among them, Gilead Sciences reported a large series of pyrido[3,2-*d*]pyrimidines with promising activities but the main HCV target of these molecules remains unclear [9]. A chemical and structural similarity search of the crystallized targets, using a subset of pyrido [3,2-*d*]pyrimidines as query, yielded similarity scores higher than 0.7 for molecules targeting NS5B polymerase (see Methods for details), thus we hypothesize that this scaffold could potentially target this HCV enzyme. Nevertheless, in view of the potency of pyrido[3,2-*d*]pyrimidines and own expertise complemented by computer-assisted rational design, we chose to evaluate the activity



Scheme 1. Synthesis of 7-oxopyrido[2,3-*d*]pyrimidines **9**, **10**, and **11** from α,β-unsaturated esters **5** via pyridones **7**.