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Mini-review

Discovery of HCV NS5B thumb site I inhibitors: Core-refining from benzimidazole to indole scaffold

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ABSTRACT

Hepatitis C virus (HCV) NS5B RNA-depended-RNA-polymerase (RdRp) is an essential enzyme in HCV viral replication and has no functional equivalent in mammalian cells. Several classes of nucleoside and non-nucleoside inhibitors, targeting the different allosteric sites, have demonstrated efficacy in clinical trials. Compared to other allosteric sites, thumb site I is a more compelling allosteric target with a significant number of inhibitors in clinical trials. Among them, indole analogues are the most important series of NS5B thumb site I inhibitors with considerable antiviral activity. This review focuses on the discovery and development of indole inhibitors targeting on NS5B thumb site I. Five fundamental principles, the general structure–activity relationships (SARs) model of indole scaffold, were summarized, which could pave the way for further structural optimization of indole-based anti-HCV agents.

1. Introduction

HCV infection is a global health threat. Currently, about 180 million individuals have been chronically infected with HCV according to the database of World Health Organization (WHO) [1], and more than 350 000 people die every year from hepatitis related liver diseases caused by HCV infection, such as cirrhosis [2], liver failure, and hepatocellular carcinoma (HCC) [3].

Currently, vaccine against HCV is unavailable [4,5]. The traditional therapy named as standard of care (SOC), a combination regimen of regular pegalated α-interferon (PEG–IFN–α) injections with oral Ribavirin (RVB), is clinically used for treatment of HCV infection. However, it is associated with numerous side effects and yields at best a 50% sustained virological response (SVR) only for genotype 1 infected patients [6]. To address these deficiencies, extensive efforts have been made toward the identification of direct-acting antiviral agents (DAAs) that specifically target HCV [7,8]. The DAAs currently in research and development fall into three categories: (i) NS3/4A protease inhibitors, (ii) NS5A protein inhibitors, (iii) NS5B polymerase inhibitors. Among them, the most promising DAAs target at the NS3/4A serine protease and the NS5B polymerase. Two NS3/4A protease inhibitors, Telaprevir [9], and Boceprevir [10], were approved in Europe and the United States in 2011. Despite the availability of new therapies combining either of the two NS3/4A protease inhibitors, with PEG–IFN–α and RVB, there remains a high medical need for new DAAs with complementary modes of action.

The HCV NS5B RdRp is a central enzyme in the replication of the virus and has become a target of choice for the screening and design of small molecule inhibitors. The enzyme shares the common folds of other nucleic acid polymerases with characteristic thumb, finger, and palm domains (Fig. 1). Thumb site I, a pocket distal to the catalytic site at the interface of the finger and thumb domains of the polymerase, is a broad genotype coverage domain, endowing with an attractive and promising anti-HCV drug target. Over the past years, various thumb site I inhibitors have been reported including benzimidazoles, indoles, variations of indoles

Abbreviations used: HCV, hepatitis C virus; RdRp, RNA-depended-RNA-polymerase; WHO, World Health Organization; HCC, hepatocellular carcinoma; SOC, standard of care; PEG–IFN–α, pegalated α-interferon; RVB, Ribavirin; SVR, sustained virological response; DAAs, direct-acting antiviral agents; SARs, structure-activity relationships; HTS, high throughput screening; DOS, diversity-oriented synthesis.

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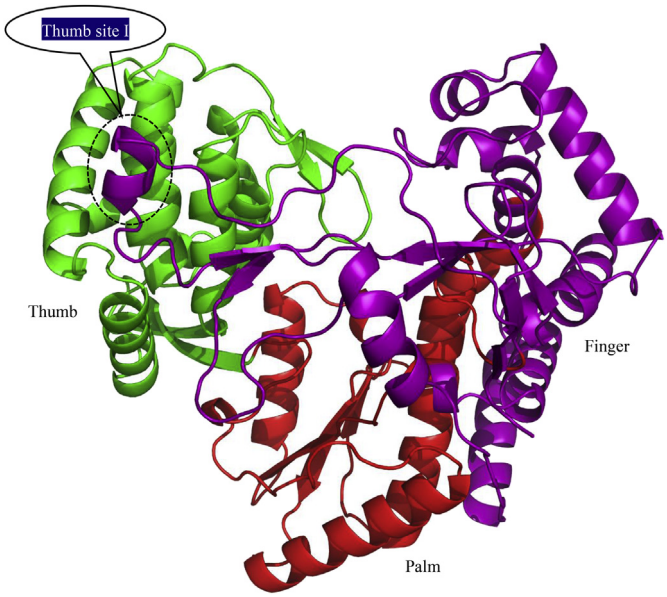

Fig. 1. Structure of NS5B (PDB code: 1CSJ [12]). Finger subdomain color coded purple, thumb subdomain color coded green, and palm subdomain color coded red. This figure was generated using PyMol (www.pymol.org). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

(thieno [3,2-b] pyrrole, quinoxalines, pyrazolylmethylacrylic acids), and aurones [11]. Among them, indoles are the most remarkable one, which have been deeply studied in SARs, specific action mode, and great potential for HCV therapy. In this article, we will review the discovery and development of indoles derivatives as potent thumb site I inhibitors.

2. Discovery of benzimidazole 5-carboxylic acid scaffold

Benzimidazole 5-carboxylic acid scaffold is the precursor of indole class inhibitors. Compound **1** (Fig. 2), discovered by high-throughput screening (HTS) (47% inhibition at 10 μM), was the first reported compound to bind with thumb site I. The carboxylic acid analogue **2** ($IC_{50} = 3.2$ μM), derived from the amide analogue **1**, exhibited more potent antiviral potency, and was selected as the benchmark for seeking promising inhibitors. Initial optimization mainly focused on the cyclopentyl ring and substituents on the benzyl moiety of the lead **2**. Ring expansion of the cyclopentyl on the benzimidazole scaffold increased the potency, especially the cyclohexyl motif. The introduction of a substituent (such as Me, CF_3, *t*-Bu and Cl at the *para* position) on the benzyl moiety led to an unsatisfactory result. In contrast, a biphenylmethyl or diphenylmethyl motif on the benzyl part increased 2- to 4-fold activity. What's more, *ortho* fluorine on the benzyl ring of the diphenylmethyl subseries dramatically increased the potency. However, further introduction of substituents (Me, Cl and F *etc.*) on the diphenylmethyl part slightly decreased the potency. Although a slight increase was seen against 1a, compound **3** ($IC_{50} = 0.8$ μM towards genotype 1a, 0.096 μM towards 1b) achieved a 6-fold improvement in potency against the genotype 1b compared with the corresponding parent compound [13]. However, the cellular potency was undesirable, which suggested that the following work should center on the regulation of the lipophilic property of these molecules.

3. Modifications of benzimidazole-based NS5B thumb site I inhibitors

3.1. Modifications at the C-2 position of benzimidazole core

Initial SARs in the benzimidazole series had identified the cyclohexyl ring and the carbonyl group on the benzimidazole scaffold as the key contributors to potency. The *N*-1 position is very sensitive to small structural modifications, whereas the *C*-2 position was shown in early work to tolerate a variety of substituents.

Compound **4** ($IC_{50} = 0.3$ μM, Fig. 3), a new series of benzimidazole derivatives bearing substituted diarylmethyl groups as NS5B inhibitors, was found in the Hiromasa Hashimoto's earlier report and chosen as the new lead compound to address the challenge of improvement of the activity. Compared to the diarylmethyl series (**3**), the substituents of **4** on the phenyl rings showed more distinct SARs and generated prominent improvement in potency. Primary optimization mainly targeted on the A-ring, and small electron-withdrawing groups (chloro, carboxylic acid, carboxamide, or cyano, *etc.*) at the *C*-4 position tended to be preferred. Polar substituents at the *C*-4 position of B-ring, especially carbonyl/sulfonyl functional groups afforded potent biochemical and cellular activity. Generally, the activity was sensitive to the fluorine atom on the C-ring. Among the new compounds, compound **5** (JTK-109, $EC_{50} = 0.32$ μM), a new generation of HCV NS5B inhibitor, was further confirmed by oral absorption administration in rat, which demonstrated considerable antiviral activity ($IC_{50} = 0.062$ μM towards genotype 1a, 0.017 μM towards 1b, and 0.061 μM towards 3a). For its favorable pharmacokinetic profiles, high selectivity for NS5B, and good safety profiles, JTK-109 (**5**) has been carried into phase II clinical trials [14]. Unfortunately, the trial was terminated suddenly for undisclosed reasons. In addition, JTK-109 exhibited poor potency against genotype 2a ($IC_{50} = 6.4$ μM) and genotype 2b polymerases ($IC_{50} = 2.0$ μM), which was the common failing of this kind of inhibitors.

Although **5** possessed considerable potency, a relatively large 'shift in potency between biochemical assay and cellular assay' was observed. To address this problem, additional optimization efforts directed at investigating a non-biphenyl substructure got morpholine **6** ($IC_{50} = 0.042$ μM, $EC_{50} = 0.27$ μM). Regrettably, the EC_{50}/IC_{50} ratio was still very high, which signaled potential problems in membrane permeability, metabolism, and/or protein binding [15].

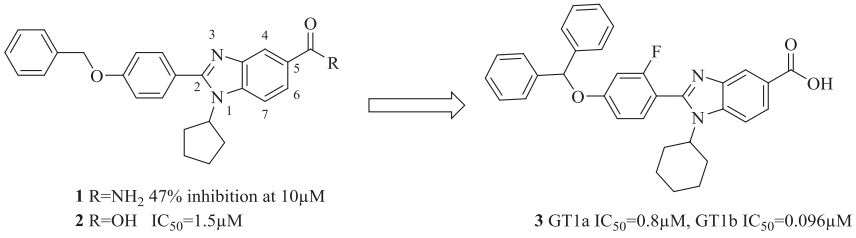

Fig. 2. Discovery of benzimidazole 5-carboxylic acid scaffold targeting NS5B thumb site I.