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Research paper

Molecular design, synthesis and biological evaluation of cage compound-based inhibitors of hepatitis C virus p7 ion channels

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ABSTRACT

The hepatitis C caused by the hepatitis C virus (HCV) is an acute and/or chronic liver disease ranging in severity from a mild brief ailment to a serious lifelong illness that affects up to 3% of the world population and imposes significant and increasing social, economic, and humanistic burden. Over the past decade, its treatment was revolutionized by the development and introduction into clinical practice of the direct acting antiviral (DAA) agents targeting the non-structural viral proteins NS3/4A, NS5A, and NS5B. However, the current treatment options still have important limitations, thus, the development of new classes of DAAs acting on different viral targets and having better pharmacological profile is highly desirable. The hepatitis C virus p7 viroporin is a relatively small hydrophobic oligomeric viral ion channel that plays a critical role during virus assembly and maturation, making it an attractive and validated target for the development of the cage compound-based inhibitors. Using the homology modeling, molecular dynamics, and molecular docking techniques, we have built a representative set of models of the hepatitis C virus p7 ion channels (Gt1a, Gt1b, Gt1b_L20F, Gt2a, and Gt2b), analyzed the inhibitor binding sites, and identified a number of potential broad-spectrum inhibitor structures targeting them. For one promising compound, the binding to these targets was additionally confirmed and the binding modes and probable mechanisms of action were clarified by the molecular dynamics simulations. A number of compounds were synthesized, and the tests of their antiviral activity (using the BVDV model) and cytotoxicity demonstrate their potential therapeutic usefulness and encourage further more detailed studies. The proposed approach is also suitable for the design of broad-spectrum ligands interacting with other multiple labile targets including various viroporins.

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1. Introduction

Viroporins are relatively small hydrophobic viral proteins that oligomerize to form aqueous pores or ion channels in the host and/or viral lipid bilayer membranes and can play a critical role in multiple stages of the viral life cycle by modifying the local environment to assist viral proliferation [1–4]. Thus, targeting these channels with small-molecule inhibitors may provide an attractive option in antiviral development. The members of the growing family of known viroporins have significant differences in their structure, characteristics, and functions. Two of them have been studied more thoroughly. The tetrameric M2 channel from the influenza A virus [5,6] is a highly selective proton channel that enables the acidification of the virion interior during virus uncoating in late endosomes (facilitating ribonucleoprotein release) as well as the deacidification of the Golgi network during virus maturation (preventing the premature conformational changes of hemagglutinin). On the other hand, the less selective p7

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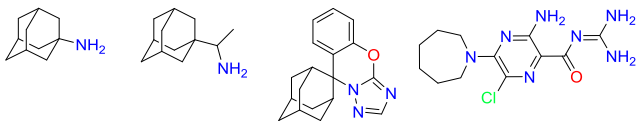
viroporin from the hepatitis C virus (HCV) [7,8] has more variable structure and a somewhat elusive function that also involves the deacidification of the Golgi network during virus assembly and maturation. (In addition, both the M2 and p7 proteins interact with other viral proteins during virion assembly [1,7]).

The hepatitis C caused by the hepatitis C virus (positive-sense single-strand RNA virus belonging to the Flaviviridae family [8]) is an acute and/or chronic liver disease ranging in severity from a mild brief ailment to a serious lifelong illness [9–11]. Chronic hepatitis C can lead to liver cirrhosis and fibrosis, hepatocellular carcinoma, degraded quality of life, liver failure and liver-related death, as well as a number of extrahepatic disorders. Globally, the hepatitis C infection is currently estimated to affect up to 185 million people, or about 3% of the world population, but the prevalence can be much higher in specific populations, regions, and age groups. Each year, up to 500 000 people die from hepatitis C-related liver diseases. The chronic hepatitis C also imposes significant and increasing social, economic, and humanistic burden, involving direct medical costs, loss of productivity, and decreased quality or loss of life [12].

The primary goal of the HCV therapy [10,13,14] is curing the infection, i.e., achieving a sustained virological response (SVR) defined as undetectable HCV RNA level in blood 3–6 months after treatment. Until recently, the historical standard of treatment had been based on the combination of PEGylated interferon α and ribavirin, characterized by inconvenient mode of administration, long treatment duration, high risk of severe side effects, and low resultant SVR rate (commonly less than 50%). Over the past decade, this field was revolutionized by the development and introduction into clinical practice of the direct acting antiviral (DAA) agents targeting the non-structural viral proteins NS3/4A, NS5A, and NS5B [15]. The current DAA-based 'interferon-free' treatment regimens are well tolerated, use convenient administration and relatively short duration, and usually yield SVR rates above 90%. Dozens of newer DAAs designed for even better properties are now in development or clinical trials. In 2016, the World Health Assembly has adopted the goal of eliminating viral hepatitis as a public health threat by 2030 [9]. However, the current treatment options still have important limitations, e.g., with respect to their genotype-dependent efficacy and potential virus resistance [13,16,17]. In addition, in some countries they may not be available and/or their prices could remain prohibitively high [10,11]. Thus, the development of new classes of DAAs acting on different viral targets and having better pharmacological profile is highly desirable.

The mostly hydrophobic nature of viroporins (and many other ion channels) makes them suitable targets for the cage compound derivatives, in particular, substituted adamantanes [18,19]. In addition to lipophilicity, these scaffolds also have other unique properties such as spheroid shape and controlled conformation. Indeed, the aminoadamantane inhibitors of the influenza A virus M2 proton channel (amantadine **1** and rimantadine **2**) were the first adamantane derivatives that have found an application in medicinal chemistry more than five decades ago. Some preliminary studies also suggested the possible clinical efficacy of amantadine against hepatitis C [20] and identified the hepatitis C virus p7 ion channel as its probable target [21] involved in the deacidification of intracellular compartments that is required for productive HCV infection [22].



1 amantadine **2** rimantadine **3** OVA09 **4** hexamethylene amiloride

However, the utility of classical aminoadamantane antivirals turned out to be rather limited and transient, which is perhaps not surprising taking into account their relatively simple structures as well as highly flexible nature of their targets. For the hepatitis C virus, after the first promising results, a lack of significant benefits of amantadine regarding the mortality, morbidity or SVR outcomes was shown in more broad and thorough clinical trials [23], leading to a call of "enough is enough" of unfulfilled hopes [24]. The *in vitro* studies also indicate substantial differences (up to several orders of magnitude) in the amantadine activity towards the p7 channel of different HCV genotypes; on the other hand, the rimantadine activity was more uniformly high [25,26]. In addition, the mutant p7 variants resistant to aminoadamantanes were found both in clinical [27,28] and *in vitro* [29,30] studies.

In spite of these issues, the viroporins present validated (and potentially clinically relevant) therapeutic targets as well as an area of active ongoing inhibitor research [1,3,31,32]. For the influenza A virus M2 channel, significant progress has been made in the recent years, and a number of promising inhibitor compounds were discovered [6]. In most of them, the bulky hydrophobic adamantane fragment is retained (although other cage systems with similar properties may also be used). A positively ionizable group as well as other additional functions are usually present. For some of the lead compounds, *in vivo* activity against the currently circulating viruses and a higher barrier to drug resistance has been demonstrated [33,34].

Aiming to design broad-spectrum inhibitors targeting the wild-type and resistant variants of the influenza A virus M2 proton channel, the molecular dynamics and molecular docking techniques were recently used to refine their dynamic structures, analyze the inhibitor binding sites, identify a number of potential inhibitors, and clarify the binding modes and probable mechanisms of action of a promising compound OVA09 (**3**) [35]. The proposed approach is also suitable for the design of broad-spectrum ligands of other multiple labile targets. Taking this into account, we have attempted to design potential broad-spectrum inhibitors of the hepatitis C virus p7 channel based on the cage compounds.

2. Results and discussion

2.1. Design approach

Similar to the approach used earlier for the M2 channel inhibitors [35], our approach to the design of p7 inhibitors involves (1) the analysis of the behavior of the selected target channel variants during the thermal motion by means of molecular dynamics; (2) the determination of the representative binding site structures; (3) the identification of potential inhibitor structures by means of the virtual screening; (4) the verification of the selected inhibitor structures by the molecular dynamics simulations of their complexes with target p7 channels in order to confirm the stability of binding and to analyze their binding modes.

The HCV p7 protein is relatively short, consisting of 63 amino acid residues. In a monomeric form, it adopts a flexible hairpin-like conformation with two (trans)membrane regions formed by three helical segments (H1 and H2 in the N-terminal and H3 in the C-terminal part of the sequence) [1,8,36]. In a membrane, the protein oligomerizes into cation-selective channels. The predominant form is a flower-shaped hexameric assembly, although hepta- and pentameric forms may also be present in the equilibrium [1,37,38]. Two basic models of the hexamer architecture have been proposed [8,39]. In one approach, the monomeric hairpin p7 units (represented by various NMR-based or predicted structures) are assembled into a hexameric or heptameric bundle with the N-terminal transmembrane domains forming the channel pore. A fundamentally different model of the hexamer structure was derived directly

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