Editorial

Optimisation of a potent series of HCV helicase drug candidates

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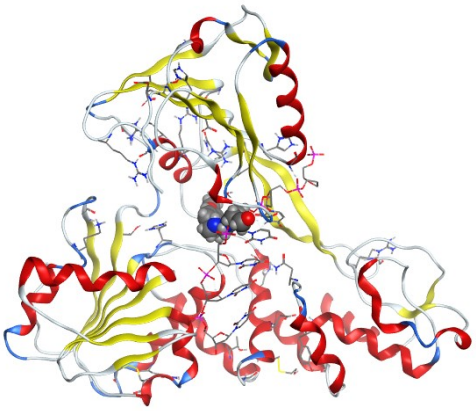
Introduction

Infection with the Hepatitis C virus (HCV) has recently become the second commonest chronic viral disease in the world. It is believed that its global prevalence exceeds 180 million people worldwide. That is over 3% of the world population, who are chronically infected with the virus (Brass *et al.* 2006). Progression of HCV infection usually results in liver cirrhosis and/or hepatocellular carcinoma (Craxì *et al.* 2008). To date, there is neither vaccination available nor any prophylactic approach against HCV infection and such vaccine is not expected anytime soon (Hayashi & Takehara 2006). Current treatment of HCV infected patients involves the co-administration of pegylated interferon with ribavirin, which is associated with serious clinical adverse effects. There is a range of recent drug candidates that are currently in preclinical or clinical development stages. However, most of those drugs aim either the protease or the polymerase enzymes of the Hepatitis C virus (Lam & Frick 2006). Provided that years of extensive research concerning the latter enzymes have failed to come up with an answer to the HCV infection, new targets must be identified. One of the most important novel targets today is the HCV helicase enzyme. There has been no extensive research on the HCV helicase, even though it is one of the most important enzymes for the survival and proliferation of the virus (Bailey *et al.* 1997, Janetka *et al.* 2000, Maga *et al.* 2005, Soriano *et al.* 2009, Zhang *et al.* 2003). The role of the HCV helicase is to unwind double stranded RNA (dsRNA) into single stranded RNA (ssRNA) during the viral replication cycle. Consequently, inhibition of the HCV helicase would interrupt the viral replication life cycle.

Current Research

Only recently we introduced and published a novel and successful de novo structure-based drug design approach on the HCV helicase (Kandil *et al.* 2009). Computer-aided drug design followed by extensive molecular dynamics simulations, helped establish a series of novel compounds, capable of interacting with the putative binding site on the HCV helicase ssRNA channel. Our best-ranking compounds were chemically synthesised and biologically evaluated against the purified enzyme. They exhibited inhibition of the HCV helicase enzyme in submicromolar concentrations. Our rational drug design approach was based on the structure of the HCV helicase, obtained by X-ray crystallography (Kim *et al.* 1998). In one of these crystals, the HCV helicase was co-crystallised with a single strand of a DNA fragment, providing invaluable information about the interaction pattern of the oligonucleotide with the enzyme. Conserved "key" residues were soon isolated and selected as suitable amino acid targets for the structure-based drug design approach. Notably the most important one was an Arginine amino acid (Arg393), which was clearly involved in interactions with the DNA fragment. Molecular biology studies on the HCV helicase confirmed that upon mutation of the Arg393 to an Ala amino acid (R393A) the enzyme loses its unwinding properties (Lam *et al.* 2003). It was evident that the Arg393 amino acid was responsible for the processing of the viral genetic material. By further observing the HCV helicase crystal structure we noticed that a Cysteine amino acid (Cys431) managed to establish a disulphide bond with a mercaptoethanol molecule that was present in the crystallisation solution. Cys431 was located around ~10Å away from Arg393 and constituted an ideal target for drug design. Since it was able to interact with the mercaptoethanol molecule, it was definitely exposed and reactive enough to form a disulphide bond. A compound capable of covalently bonding with Cys431 would be impossible to be displaced by the incoming oligonucleotide, water or any other solvent ions, in contrast to competitive drugs that establish bonds of electrostatic or hydrophobic nature.

Eventually, the *in silico* drug design part was finished and our most promising compounds were chemically synthesised and biologically evaluated by a strand-displacement enzymatic assay against the HCV helicase enzyme. Compound no. 4 produced an IC_{50} of 0.26μM, while at the same time its regioisomer Com-



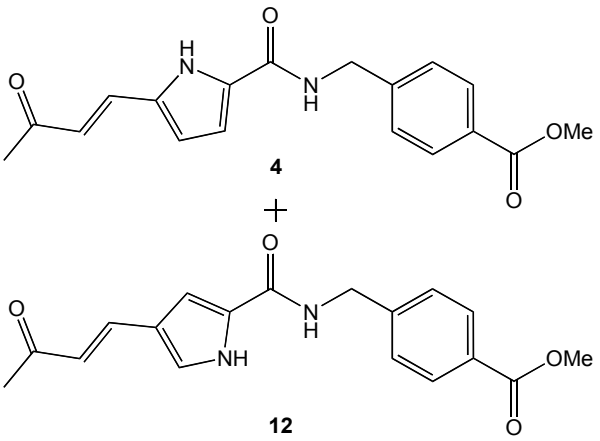


Figure 1. Left: The docked complex of the HCV helicase. The inhibitor covalently bonds with Cys431, while bridging the ssRNA channel via interactions with Arg393. Right: The chemical structures of Compounds 4 and 12.

pound no. 12 did not show any activity at all (Figure 1). That can be explained by our molecular modelling analysis, where the 2-4 substituted pyrrole did not interact with the Michael acceptor moiety positioned in proximity to the Cys431 amino acid. The potency and inhibitory properties of Compound no. 4 were then confirmed by biological evaluation on HCV subgenomic replicon replication assay. Compound no. 4 turned out to be quite cytostatic to the hepatoma cells with an EC_{50} value of 3μg/ml and a IC_{50} value of 10μg/ml. (Kandil *et al.* 2009). That could possibly be explained by the fact that the vinyl ketone moiety is a known potent toxicophore. The ester analogues were also synthesized and tested, although our in silico model suggests that the free acid version of that compound should be capable of improved interaction with Arg393 and Cys431 amino acids. However it must be noted that the increased polarity and charges of the above compounds could affect the cellular permeability of the drugs.

What the future holds

We have reached a critical point in our research where, we need to further exploit our existing work on the helicase inhibitors in order to achieve a better understanding of the mechanism of action of those molecules and proceed with the design and development of an improved series of compounds as drug candidates against the Hepatitis C Virus (Figure 2). The first step will be the lead optimization process of our most potent compounds using computer-aided *in silico* techniques, starting from Compound no. 4. The drug design process based on our previous compounds is very promising, since the sample that will be used here is from a pool of highly active compounds (Balatsos *et al*. 2012, Dalkas *et al.* 2012, Loukatou *et al.* 2014, Papangelopoulos *et al.* 2013). Further *in silico* studies must be incorporated in an attempt to minimize the potential adverse effects of our drug candidates. Using large databases and bioinformatics tools we aim to generate an improved series of drug candidates against the HCV helicase that will still have the same inhibitory potency, but far less toxicity and improved bioavailability (Papageorgiou *et al.* 2013, Sellis *et al.* 2009, Vlachakis *et al.* 2013a).

The new line of improved drug candidates will be initially evaluated in silico for their predicted efficacy against the HCV enzymes. That involves molecular docking, energy minimizations, molecular dynamics and Monte Carlo simulations (Vlachakis *et al.* 2013c). As soon as the computer-aided simulations are over the best ranking compounds will be chemically synthesized. Since all drug candidates descent from our current lead compounds, the major part of the synthetic pathway is known to us and already improved in such a way that we may obtain large quantities of highly pure product within a very limited time-course (Vlachakis *et al.* 2013d, Vlachakis & Kossida 2013). Finally, an enzymatic assay will help determine the *in vitro* activity of the newly synthesized drug candidates. The enzymatic assay has been customly developed for rapid testing of small molecule drugs against the helicase protein of HCV (unpublished data). Our assay can determine the potency of a certain compound within a few hours and speed up the screening process that is usually the bottleneck of biological evaluations (Vlachakis *et al.* 2013e). All compounds that exhibit at least some activity in our enzymatic assay will be further tested in a replicon cellular assay that will provide us with invaluable information about the bioavailability and toxicity levels of each drug candidate. Eventually it is our aim to obtain a crystal structure of the complex to validate our approach. This in turn may allow to further rationally optimize these compounds and to obtain a more specific and mechanism driven class of potent anti-HCV inhibitors (Vlachakis *et al.* 2012, 2013b,f, 2014).

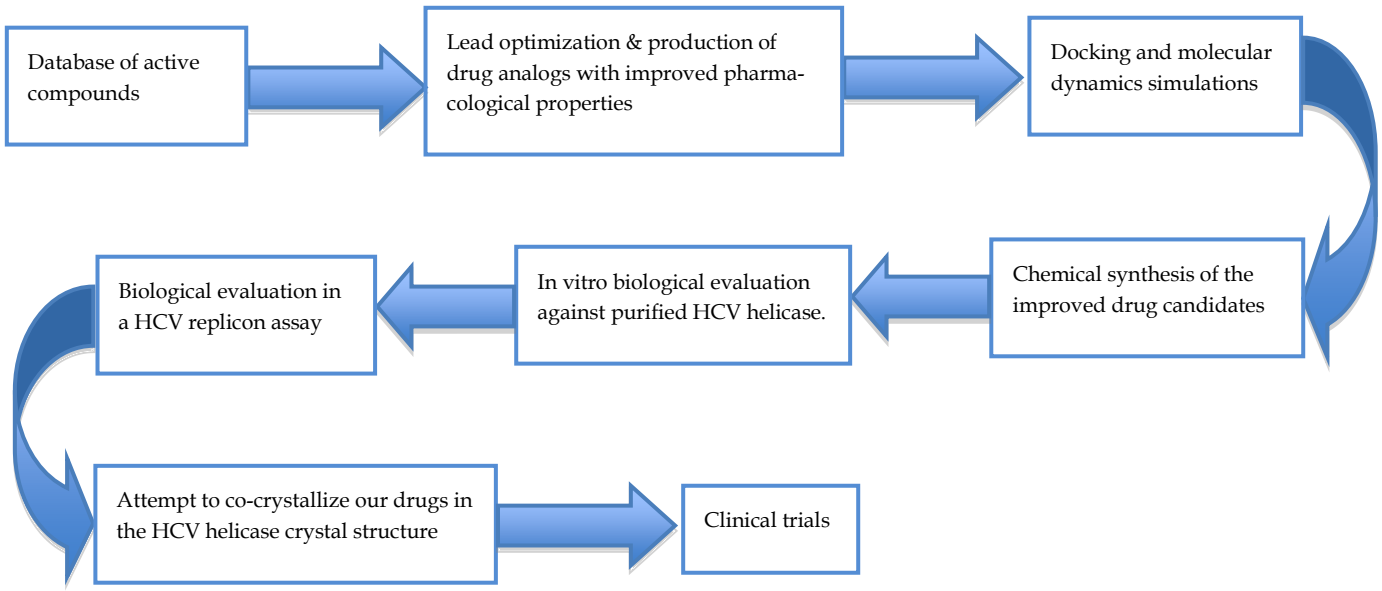


Figure 2. Representation of the proposed research, highlighting the different developmental stages in the design of HCV helicase drugs.

Conclusion

In conclusion, we have used a *de novo* approach to design a novel HCV helicase inhibitor that could target a newly identified pocket on the enzyme. Our compounds propose a novel and unique approach to the battle against HCV. They are the only compounds known to literature capable of covalently interacting with the ssRNA channel of the helicase enzyme of HCV, while at the same time being very easy and cheap to synthesize, in compliance with the huge pharmacoeconomic issues that run though most of the countries that suffer from HCV epidemics. Furthermore, they have demonstrated activity within submicromolar concentrations, when evaluated in biological *in vitro* and replicon cellular assays. Funding and continuation of our work would enable the exploitation of our drug candidates in order to further improve them and subject them in clinical trials, aiming for a significant contribution to the fight against the lethal HCV epidemic.

Conflicts of interest

The authors have no conflicts of interest.

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