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Structure-based design of a novel thiazolone scaffold as HCV NS5B polymerase allosteric inhibitors

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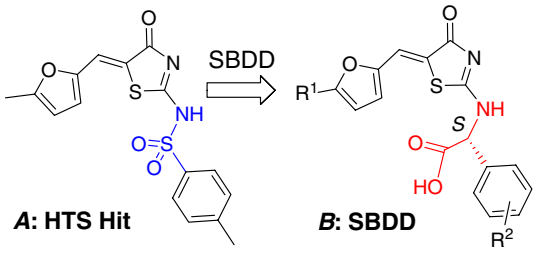
Abstract—A structure-based approach was performed to design a novel thiazolone scaffold as HCV NS5B inhibitors. A focused library was designed and docked by GOLD. One of the top-scored molecules was synthesized and shown to have similar potency to the initial hit. The X-ray complex structure was determined and validated our design rationale.

Identified in 1989, hepatitis C virus (HCV) has been recognized as the leading pathogen for the non-A, non-B virus hepatitis.[1] Chronic HCV infection is emerging as a worldwide health crisis. It is estimated that there are over 170 million individuals chronically infected with HCV globally and nearly 4 million are in the United States.[2] The current FDA-approved standard therapy provides a sustained viral response only for a fraction of patients.[3] Therefore, there is a growing unmet medical need to discover novel therapies for chronic hepatitis C.

HCV NS5B polymerase is one of the six non-structural proteins encoded in the approximately 9600 nucleotide HCV genome. It plays a pivotal role in the HCV viral replication and infection in a chimpanzee model,[4] and thus represents a key target for antiviral therapy against HCV.[5] Numerous classes of non-nucleoside inhibitors with different scaffolds have appeared lately in the literatures against HCV NS5B. Several scaffolds have been reported to bind several of the allosteric binding sites of NS5B, which are located on the thumb sub-domains distant from the polymerase active site.[6] Inhibitors bound to such allosteric sites of enzymes are generally considered to have more favorable on-target activities and less undesirable off-target side-effects.

One of our endeavors in HCV programs was aimed to discover novel molecules bound to the allosteric site of HCV NS5B. Our structure-based design strategies for new scaffolds were three folds: (1) analyze and retain key pharmacophores and potency of the initial hit; (2) establish reasonable size of explore-able chemistry space, (3) identify future opportunities for further diversification and optimization. Screening Valeant in-house compound collection identified **A** as a potential hit with an IC50 value of 2.0 μM against HCV-NS5B (Scheme 1) and subsequently X-ray complex structure from soaking experiment established its binding in the allosteric site (Fig. 1). Key interactions include three hydrogen-bonds and two hydrophobic contacts. Specifically, C=O and N of thiazolone-ring and sulfonamide moiety form hydrogen-bonds with backbone –NHs of Tyr 477, Ser476, and side-chain $-NH_3^+$ of Arg 501, respectively, while furan and phenyl make hydrophobic contacts with the protein. Efforts to explore more of the binding pocket by



Scheme 1. Structure-based designs of HCV NS5B allosteric-site inhibitor.

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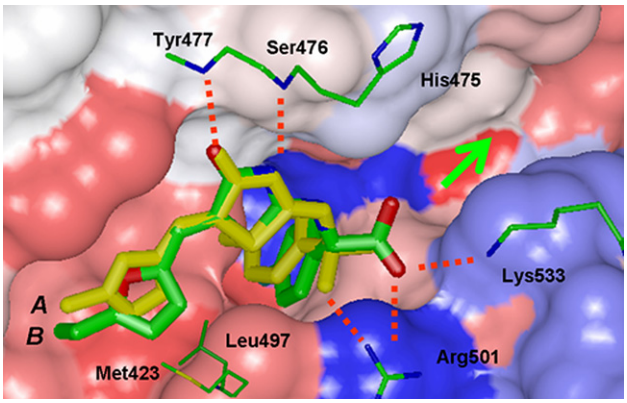


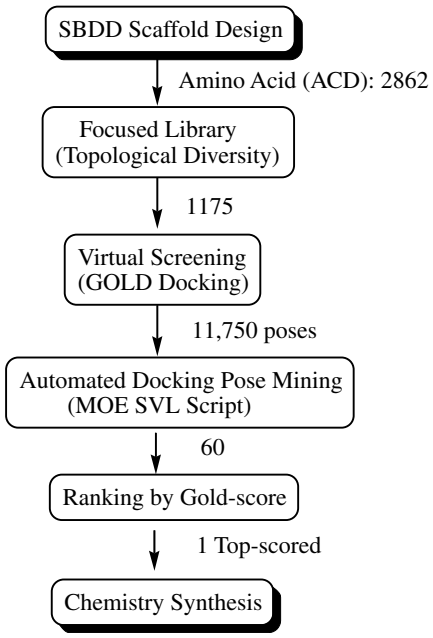
Figure 1. Overlay of binding modes of A and B in the allosteric site of HCV NS5B. A (in yellow), X-ray soaking complex structure; B (colored by atom-type), GOLD docking pose.

diversifying aryl-sulfonamide were unsuccessful. Based on the X-ray complex structure of **A** with NS5B, we envisioned that a partial pharmacophore equivalent, in particular, a suitable (*S*)-amino acid, replacement of aryl-sulfonamide moiety in **A** would satisfy our underlying design approaches (Scheme 1).

The predicted binding mode of **B** by GOLD docking program closely mimicked that of **A** in X-ray structure.[7] Moreover, not only does carboxylic acid of **B** engage in the same hydrogen-bond with Arg 501 as sulfonamide group does in **A**, but it also picks up additional hydrogen-bond with Lys 533. In particularly, carboxylic acid moiety of **B** could serve as an ideal starting point to be further functionalized to explore the right-side channel of the binding pocket (Fig. 1).

With the new scaffold **B** in place, we set out to focus on finding a favorable amino acid replacement. Analysis of GOLD docking results for both *S* and *R* chirals showed that the binding site prefers a (*S*)-amino acid. It seemed to us from previous SAR knowledge of **A** series that a small substituted-furan group for R1 would be optimal, and thus we kept it with minimal variations in the first round of design (Scheme 2). A substructure search of ACD database[8] afforded 2862 amino acids and the number was brought down to 1175 by applying a topological diversity analysis implemented in MOE software package.[9] The subsequently enumerated virtual library was subjected to GOLD virtual screening using the standard parameters. The bound structure of **A** from X-ray was employed as shape template similarity constraint with a constraint weight of 10. For each molecule in the virtual library, 10 docking poses were saved, and the combined 11,750 poses were filtered based on the identity to the preferred binding-modes using an in-house developed MOE SVL script.[10] As expected, only 60 molecules survived this filter and they were then ranked by Gold-score. One of the 10 top-scored molecules was proposed for synthesis to prove this design principle.

The designed molecule **6** was prepared according to Scheme 3. Rhodinine, **1**, was reacted with aldehyde **2** in a condensation reaction to furnish **3**.[11]



Scheme 2. The design flow-chart.

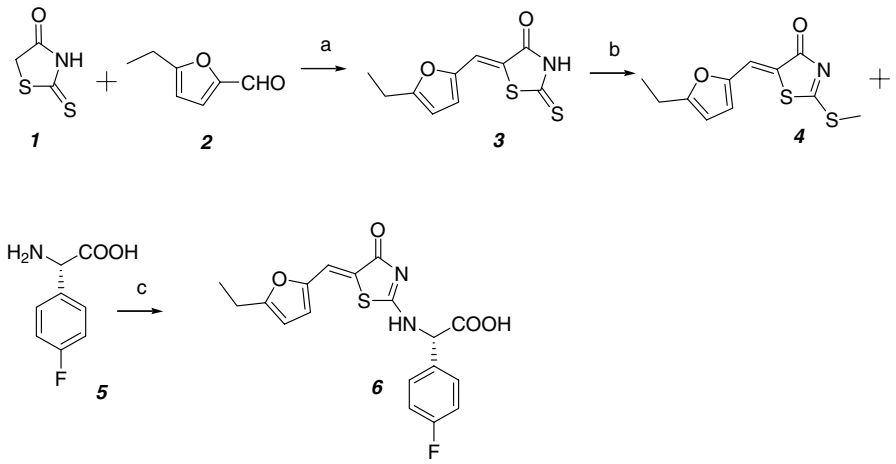
Subsequently, **3** was coupled with MeI to give an intermediate **4**, which was allowed to react with (*S*)-2-amino-2-(4-fluorophenyl)acetic acid, **5**, to afford (*S*,*Z*)-2-(5-((55-ethylfuran-2-yl)methylene)-4-oxo-4,5-dihydrothiazol-2-ylamino)-2-(4-fluorophenyl)acetic acid as the desired product **6** in 90% overall yield.

Compound **6** was then evaluated for inhibition of HCV NS5B polymerase, and we were pleased to see **6** had an IC50 value of 3.0 μM, which was essentially the same as that of the initial hit **A**.[12]

To further validate our design rationales, **6** was successfully soaked into crystalline *C*Δ21 NS5B protein and the X-ray crystal structure of HCV NS5B complexed with **6** was established at 2.0 Å resolution (Fig. 2).[13] Gratifyingly, we found inhibitor **6** bound in a binding mode just as predicted in the 'thumb' sub-domain, which is on the protein's surface about 30 Å away from the enzyme's catalytic center (Fig. 2).[6]

A superimposition of early GOLD docking pose with the bound structure of **6** later determined by X-ray soaking experiment afforded a rmsd value of only 0.15. Such a remarkably low rmsd revealed an excellent predictability of GOLD docking program for this scaffold in the allosteric-site of HCV NS5B polymerase (Fig. 3).[7]

Taken together (Figs. 1–3), the complex structure of bound **6** revealed several critical hydrogen-bonding interactions and hydrophobic contacts. Namely, the 4-F-phenyl is buried into a small deep hydrophobic pocket determined by the side chains of Leu 419, Met 423, Tyr 477, and Trp 528. The furan and thiazolone rings are nearly co-planar with a dihedral angle of ~7.6°. Ethyl-furan moiety of the inhibitors is bound to the surface of another hydrophobic pocket defined by Leu 419, Met 423, Ile 482, Val 485, Leu 489, and Leu 497.



Scheme 3. General procedures for the synthesis of **6**. Reagents and conditions: (a) EtONa, HOAc, 95 °C, 12 h; (b) MeI, EtOH, DIEA, rt, 10–16 h; (c) i—EtOH, DIEA, 80 °C, 21 h; ii—NaOH (aq, 1 N), 100 °C, 30 min; iii—HCl (aq, 1 N).

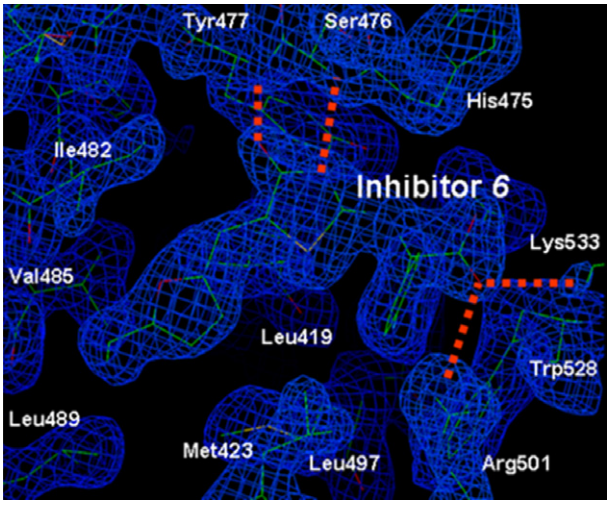


Figure 2. X-ray electron density map of inhibitor **6** complexed with NS5B in the allosteric site with a 2.0 Å resolution.

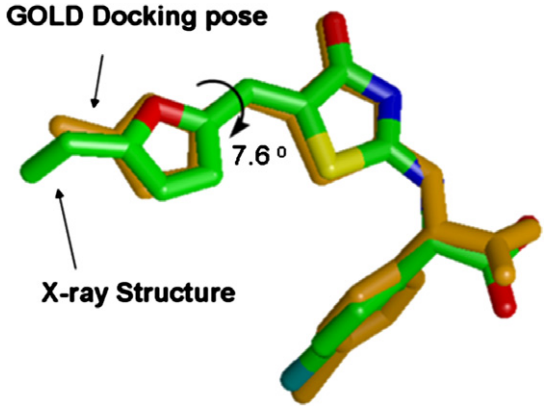


Figure 3. Superimposition of GOLD docking pose and X-ray structure.

It is also noticeable from electron density map that ethyl group on furan moiety tilts into a small hydrophobic pocket surrounded by side chains of Met 423, Val 485, and Leu 489. The C=O moiety in the thiazolone ring of **6** accepts a hydrogen-bond from the backbone –NH of Tyr 477, while its lone-pair N makes another hydrogen-bond to the backbone –NH of Ser 476. Moreover, one of the carboxylic oxygen atoms of **6** forms an ionic hydrogen-bond to the side-chain guanidium moiety of Arg 501, and meanwhile captures another weaker hydrogen-bond with side-chain $-NH_3^+$ of Lys 533. Interestingly, the other oxygen atom of carboxylic moiety of **6** in the bound X-ray complex structure with an anticipated position provides structural foundation to probe the right-side channel of this pocket in HCV NS5B polymerase (Figs. 1 and 2).

In conclusion, a novel thiazolone scaffold has been designed based on the complex structure of initial hit. To validate the design rationale, one of the top-scored molecules, **6**, from virtual screening was synthesized and had an IC50 value similar to the initial hit. Consequently, **6** was soaked into crystalline of HCV NS5B and the solved X-ray complex structure revealed nearly identical binding mode as the one predicted originally by GOLD docking. Further designs by functionalizing carboxylic group and more detail analysis of SAR for this scaffold will be reported in due course.

Acknowledgments

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12. In vitro HCV RNA transcript assay was performed as following. A HCV mini-genome was constructed from an internal deletion between two *Kpn*I sites in the HCV replicon plasmid, pFK389/NS3-#'/wt (Lohmann, V.; Korner, F.; Koch, J.; Herian, U.; Theilmann, L.; Bartenschlager, R. *Science* **1999**, *285*, 110.), to yield a 2.1 kb region that contains the entire 5′-UTR (untranslated region), part of the NS5B sequence, and the entire 3′-UTR. To generate the HCV mini-genome RNA, the plasma DNA was linearized with *Ase*I and *Sca*I, and transcribed *in vitro* using the Megascript kit (Ambion, Austin, TX). After phenol–chloroform extraction and isopropanol precipitation, the RNA was resuspended in RNase-free water and stored at −80 °C prior to use. This HCV CON1 strain-based RNA template was used for the standard NS5B RdRp-catalyzed elongation assay. NS5B RdRp enzyme (100 nM) was incubated with the RNA template (75 ng) in a reaction buffer that contained 50 mM Tris, pH 7.0, 10 mM $MgCl_2$, 50 mM NaCl, 5 mM DTT (add fresh), and 0.1 mg/ml BSA prior to the initiation of the reaction by adding the mixture containing [α-^{33}P]-CTP (1 μCi) and 80 μM of ATP, GTP, and UTP. The reaction mixture was incubated for 1 h at 23 °C in a total volume of 25 μL. The assay was terminated by adding 75 μL of 1% trichloroacetic acid (TCA) and 0.5% pyrophosphate. The quenched solution was incubated for 10 min at room temperature to allow the RNA to precipitate, and then subsequently transferred to a 96-well white GF/B filter plate (Perkin-Elmer, Wellesley, MA) using a Perkin-Elmer Filtermate Universal Harvester. The filter plate was washed ten times with water and once with ethanol before completely drying. 40 μL of Microscint™ (Perkin-Elmer, Wellesley, MA) was added to each well and the incorporated radioactivity was counted using a Perkin-Elmer TopCount. Compounds were titrated in this assay and IC_{50} values were determined. The reported IC_{50} values are the average of at least two sets of data.
13. BK strain of ***C***Δ21 HCV NS5B protein was used. The soaking experiment was carried out by taking 20 μM DMSO stock compound solution, diluted in 1:100 soaking buffer, and undergoing overnight soaking. Coordinates for structures have been deposited at Protein Data Bank (www.rcsb.org) under PDB codes: 2HWH and 2HWI.