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Thiazolone-acylsulfonamides as novel HCV NS5B polymerase allosteric inhibitors: Convergence of structure-based drug design and X-ray crystallographic study

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Abstract—A novel series of thiazolone-acylsulfonamides were designed as HCV NS5B polymerase allosteric inhibitors. The structure based drug designs (SBDD) were guided by docking results that revealed the potential to explore an additional pocket in the allosteric site. In particular, the designed molecules contain moieties of previously described thiazolone and a newly designed acyl-sulfonamide linker that is in turn connected with a substituted aromatic ring. The selected compounds were synthesized and demonstrated low μ M activity. The X-ray complex structure was determined at a 2.2 Å resolution and converged with the SBDD principle.

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Hepatitis C virus (HCV) is a small positive-stranded RNA virus whose viral genome encodes a single polyprotein.¹ NS5B polymerase—one of the non-structural proteins released by the proteolytic processing of this polyprotein—is responsible for the replication and transcription of the viral genome and is critical for viral infectivity.^{2,3} Thus, a small molecule that inhibits NS5B polymerase may prevent HCV from replicating and consequently stop viral infection. Novel anti-HCV therapies utilizing a small molecule inhibitor with such a mechanism of action are not yet available, but would be ultimately desired for the treatment of the growing HCV patient population worldwide.

Our structure-based drug design (SBDD) effort focusing on an allosteric site of NS5B had resulted in the discovery of thiazolone-carboxylic acid (A) and its isosteric tetrazole derivatives as inhibitors of this enzyme.^{4,5} X-ray complex structures established the predicted binding mode of these inhibitors, that is, they bind to the site on the thumb subdomain distant from the active site. Inhibitors of different scaffolds interacting with this same binding pocket have also been reported by others.^{6–8} All of these disclosed scaffolds, though structurally diverse, hydrogen-bond with Ser476, Tyr477, and Arg501, either directly or via a water molecule, and in the same region, make similar hydrophobic contacts with the side-chains of Leu419, Met423, Ile482, Val485, Leu489, Leu497, and Trp528 (Fig. 1a). Close examination of the inhibitor-protein interactions revealed that in the vicinity of these inhibitor-binding interactions there seems to be an additional pocket that has yet to be fully explored by the reported inhibitors.⁹ Notably, this added pocket is flanked by two basic residues, His475 and Lys533, near its entrance (Fig. 1a). An inhibitor with a suitable moiety to establish interactions specifically with these two residues will represent a new molecular platform for the exploration of this extra pocket. More importantly, a corresponding X-ray complex structure of such a novel inhibitor with NS5B will provide further structural insights for the future design of novel and specific HCV NS5B inhibitors.

Therefore as a continued SBDD support for our inhouse anti-HCV program targeting the above-mentioned NS5B allosteric site, we envisioned that a design of such a new scaffold would constitute three iterative

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Figure 1. (a) Surface representation of X-ray structure of an analog of A; (b) Predicted binding mode of a new design molecule from docking.



Scheme 1. SBDD of novel acylsulfonamides as NS5B inhibitors.

steps (Scheme 1). First, a fragment template, that is, part **B** in Scheme 1, was selected as an anchor to the binding site for the purpose of establishing the same interactions with the protein as previously described for **A**.⁴ Second, an acylsulfonamide (1) or its reverse form (2) that has a comparable pK_a with –COOH was identified as a candidate linker with **B** in order to hydrogen-bond with the basic side-chain of Lys533. The design was completed by connecting an aromatic moiety to the other side of the linker in order to pick up a seemingly aromatic π - π stacking with His475.

The predicted binding mode by GOLD docking for an exemplary molecule agreed with the initial design principle (Fig. 1b).¹⁰ Namely, the thiazolone moiety interacts with NS5B in the same way, as anticipated, as A.⁴ The acylsulfonamide moiety hydrogen-bonds extensively with the basic side-chains of Arg501 and more importantly Lys533 as it was designed to do. Additionally, the added phenyl ring connected with the linker on the model inhibitor appears to engage in an anticipated π stacking with His475. It is worthy noting that GOLD docking seems to favor the face-edge stacking (Fig. 1b), but cursory assessment of Protein Data Bank (PDB) revealed that the other possibility, the face-face stacking, is equally plausible.¹¹

To test the validity of the design principle, a small focused set of thiazolone-acylsulfonamides, as well as

one exemplary compound with linker **2**, was synthesized. Previous SAR on scaffold **A** showed that the optimal groups for $\mathbb{R}^{11}/\mathbb{R}^{12}$ are F/H or Cl/Cl^{4,5} and the same $\mathbb{R}^{11}/\mathbb{R}^{12}$ groups were therefore adopted. General procedures for the synthesis of the desired compounds are shown in Scheme 2 and Scheme 3.

The synthesis of thiazolone-acylsulfonamides **8–13**, symbolized by **C**, was started with commercially available amino-acids (**3**) (Scheme 2). Protection of amino acid **3** gave **4**, a compound which underwent coupling reaction with sulfonamides upon treatment with EDC to afford acylsulfonamide intermediate **5**. De-protection of **5** was carried out under acidic conditions to provide amino acylsulfonamide **6**, which was coupled with **7** to afford desired products using previously described procedures.⁴

Compound **21** was synthesized in nine steps from amino acid **14** with reasonable overall yield (Scheme 3). Amino acid **14** was first Boc-protected and its COOH was then reduced to produce amino alcohol **15**.¹² Bromination of **15** with CBr₄ in the presence of PPh₃ readily formed **16**.¹³ Treatment of **16** with KSAc, followed by chlorination and amination, provided sulfonamide intermediate **19**.^{14–16} Coupling reaction of **19** with PhCOCl under DIEA/DMAP, followed by de-protection under acidic condition, gave **20**, which was allowed to react with **7** to furnish the final compound **21**.⁴



Scheme 2. General procedures for the synthesis of compounds 8–13. Reagents and conditions: (a) NaOH(1N, aq), dioxane, 0°C \rightarrow rt, 14 h, 73%; (b) R₂–Ph–SO₂NH₂, EDC, DMAP, DCM, 0 °C \rightarrow rt, 48 h, 76%; (c) HCl in dioxane (4N), rt, 14 h, 100%; (d) 7, DIEA, EtOH, Microwave, 10min, 80 °C, 67%.



Scheme 3. General procedures for the synthesis of reverse acylsulfonamide 21. Reagents and conditions: (a) (Boc)₂O, NaOH, dioxane, 14 h 90%; (b) BH₃ • THF, THF, 0 °C \rightarrow rt, 3.5 h, 61%; (c) CBr₄, Ph₃P, DCM, 0 °C, 10 min \rightarrow rt, 2.5 h, 26%; (d) potassium thioacetate, DMF, 4 Å mol sieves, rt, 18 h, 100%; (e) Cl₂(gas), H₂O, 0 °C, 40 min, then extraction with DCM; (f) NH₃(gas), THF, 0 °C, 30 min \rightarrow rt, 16 h, 98%; (g) Ph-COCl, DMAP, DIEA, DCM, rt, 16 h, 89%; (h) TFA, DCM, 1.5 h, 100%; (i) 7, DIEA, EtOH, 80 °C, 2 h, 70%.

The resultant compounds 8–13 and 21 were evaluated for the inhibition against BK strain of HCV NS5B polymerase using a previously described protocol.¹⁷ As shown in Table 1, these compounds inhibited NS5B with potencies in the low μ M range. It seems, from the limited SAR available, that para-substituted R₂ (8, 9, and 13) resulted in better potency than the meta-substituted ones (10, 11, and 12). Interestingly, compound 21, with a reverse acylsulfonamide linker 2, had an IC₅₀ of 16.0 μ M. This inhibitory activity is comparable to those of the most potent compounds (8, 9, and 13) with acylsulfonamide linker 1, suggesting that both linkers might be interchangeable, in general, for future designs of novel inhibitors against other disease targets.

Encouraged by the moderate potency and more importantly, aiming to substantiate the SBDD principle for this new series of inhibitors, we turned our attention to understanding the mechanism of action structurally. As such, an X-ray complex structure of NS5B with compound **13** was obtained at a resolution of 2.2 Å from soaked crystals.¹⁸ The resulting structure revealed that the compound was bound to an allosteric site at the thumb domain as anticipated (Fig. 2a and b). This

Compound	R ¹¹ /R ¹²	\mathbb{R}^2	$IC_{50}{}^a \left(\mu M \right)$
8	F/H	4-Me	9.3
9	Cl/Cl	4-NO ₂	6.6
10	F/H	3-NO ₂	15
11	F/H	3-COOMe	14
12	F/H	3-COOH	12
13	F/H	$4-NO_2$	7.0
21			16

Table 1. IC₅₀ values against HCV NS5B polymerase

^a IC_{50} values are means of three experiments, standard deviation is less than 10% of the IC_{50} values.

binding site is about 30 Å away from the active site that includes a conserved catalytic GDD motif located in the palm domain. The electron density was evident for inhibitor 13 and the surrounding amino acids, and the inhibitor structure fits adequately into the electron density map (Fig. 2c). In particular, the electron density map of the side-chain of Lys533 is unambiguous, and it was the first time we saw such well-defined density for its long linear side-chain, indicating a favorable stabilizing force in that area between the linker atoms of the inhibitor 13 and NS5B.

The overall binding mode of **13** with NS5B established by the X-ray crystallographic study concurs with previously determined binding conformation and that predicted by GOLD docking. The determined complex structure showed, as expected, that the thiazolone moiety of **13** binds to the pocket of NS5B similarly as the compound described before.⁴ Namely, the C= O and lone-pair N on the thiazolone ring hydrogen-bond with -NHs of backbone Tyr477 and Ser476, respectively (Fig. 2c and d), whereas the ethyl-furan extends to a hydrophobic channel defined by residues of Met423, Ile482, Val485, Leu489, and Leu497. In the same region, the 4-*F*-phenyl of **13** is positioned nearly parallel with the indole-ring of Trp528 in a π - π stacking, as indicated clearly by the electron-density map, and further contacted by hydrophobic side-chains of Leu419, Met423, and Tyr477.

We were pleased to find that the newly incorporated moieties were bound in the predicted mode. In particular, the designed linker (1) makes extensive hydrogenbonding with the protein. The deprotonated N and one of the O atoms on the sulfonamide engage in hydrogen-bonding with the basic side-chain of Arg501, and both O atoms on -SO₂NH⁻ form hydrogen-bonds with $-NH_3^+$ of Lys533 (Fig. 2d). Interestingly, the 4-NO₂-Ph involves a possible face-face π stacking with His 475, which is in contrast with edge-face stacking predicted initially by GOLD docking (Fig. 1b). Because both stacking patterns are well represented in the PDB database and in the absence of a higher resolution X-ray complex structure, a definitive assignment of a stacking pattern would be elusive. Furthermore, a superimposition of the bound structure of 13 and the top-scored docking pose of a model structure afforded a RMSD of less than 1.0 Å (Fig. 3). Such a small RMSD value reveals a remarkable convergence of the initial structurebased drug design and the subsequently established complex structure by X-ray cryptography.²²

In conclusion, a novel series of thiazolone-acylsulfonamides were designed as HCV NS5B polymerase



Figure 2. X-ray complex structure of HCV NS5B with **13** at a resolution of 2.2 Å. (a) Molecular surface representation of NS5B with bound inhibitor **13**, electron density of the inhibitor in solid-shape with green color; (b) ribbon representation; GDD motif represents the activated site of NS5B. (c) electron-density map contoured at a 2σ level; (d) binding mode of **13** with removal of electron-density map for clarity. The pictures were generated by VMD1.8 and rendered by POV-Ray3.5.¹⁹⁻²¹



Figure 3. Superimposition of X-ray structure of bound inhibitor 13 and the top-scored GOLD docking pose of the model structure; atom-type coloring scheme is for 13, and yellow color is for the model.

allosteric inhibitors. The structure-based drug designs were guided by docking results that revealed the potential to explore an additional pocket in the allosteric site. In particular, such designed molecules contain moieties of previously described thiazolone, and a newly designed acylsulfonamide linker that is in turn connected with a substituted aromatic ring. The selected compounds were synthesized and demonstrated to be active with low μ M potency. The X-ray complex structure was established at a 2.2 Å resolution and converged with the initial SBDD principle.

References and notes

- Choo, Q. L.; Kuo, G.; Weiner, A. J.; Overby, L. R.; Bradley, D. W.; Houghton, M. Science 1989, 244, 359.
- Ali, N.; Tardif, K. D.; Siddiqui, A. J. Virol. 2002, 76, 12001.
- Kolykhalov, A. A.; Mihalik, K.; Feinstone, S. M.; Rice, C. M. J. Virol. 2000, 74, 2046.
- Yan, S.; Appleby, T.; Larson, G.; Wu, J. Z.; Hamatake, R.; Hong, Z.; Yao, N. *Bioorg. Med. Chem. Lett.* 2006, 16, 5888.
- 5. Yan, S.; Larson, G.; Wu, J. Z.; Appleby, T.; Ding, Y.; Hamatake, R.; Hong, Z.; Yao, N. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 63.
- Love, R. A.; Parge, H. E.; Yu, X.; Hickey, M. J.; Diehl, W.; Gao, J.; Wriggers, H.; Ekker, A.; Wang, L.; Thomson, J. A.; Dragovich, P. S.; Fuhrman, S. A. J. Virol. 2003, 77, 7575.
- Chan, L.; Reddy, T. J.; Proulx, M.; Das, S. K.; Pereira, O.; Wang, W.; Siddiqui, A.; Yannopoulos, C. G.; Poisson, C.; Turcotte, N.; Drouin, A.; Alaoui-Ismaili, M. H.; Bethell, R.; Hamel, M.; L'Heureux, L.; Bilimoria, D.; Nguyen-Ba, N. J. Med. Chem. 2003, 46, 1283.

- Pfefferkorn, J. A.; Nugent, R.; Gross, R. J.; Greene, M.; Mitchell, M. A.; Reding, M. T.; Funk, L. A.; Anderson, R.; Wells, P. A.; Shelly, J. A.; Anstadt, R.; Finzel, B. C.; Harris, M. S.; Kilkuskie, R. E.; Kopta, L. A.; Schwende, F. J. *Bioorg. Med. Chem. Lett.* 2005, 15, 2812.
- X-ray studies of NS5B inhibitors having a binding to this additional pocket have been reported by Love et al. in Ref
 But at the time when this work was initiated, to our best knowledge, no such inhibitors were publicly known.
- 10. GOLD v2.1 docking software from: www.cdc.cam.ac.uk, Cambridge, UK.
- Berman, H. M.; Westbrook, J.; Feng, Z.; Gilliland, G.; Bhat, T. N.; Weissig, H.; Shindyalov, I. N.; Bourne, P. E. Nucleic Acids Res. 2000, 28, 235.
- Tucci, F. C.; Zhu, Y. F.; Guo, Z.; Gross, T. D.; Connors, P. J., Jr.; Gao, Y.; Rowbottom, M. W.; Struthers, R. S.; Reinhart, G. J.; Xie, Q.; Chen, T. K.; Bozigian, H.; Killam Bonneville, A. L.; Fisher, A.; Jin, L.; Saunders, J.; Chen, C. J. Med. Chem. 2004, 47, 3483.
- Mewshaw, R. E.; Zhou, D.; Zhou, P.; Shi, X.; Hornby, G.; Spangler, T.; Scerni, R.; Smith, D.; Schechter, L. E.; Andree, T. H. J. Med. Chem. 2004, 47, 3823.
- 14. Bhattacharya, S.; Srivastava, A. Proc. Indian Acad. Sci. (Chem. Sci.) 2003, 115, 613.
- Tomlinson, I. D.; Grey, J. L.; Rosenthal, S. J. *Molecules* 2002, 7.
- Cherney, R. J.; Mo, R.; Meyer, D. T.; Hardman, K. D.; Liu, R. Q.; Covington, M. B.; Qian, M.; Wasserman, Z. R.; Christ, D. D.; Trzaskos, J. M.; Newton, R. C.; Decicco, C. P. J. Med. Chem. 2004, 47, 2981.
- Shim, J.; Larson, G.; Lai, V.; Naim, S.; Wu, J. Z. Antiviral Res. 2003, 58, 243.
- Coordinates for the complex structure have bee deposited at Protein Data Bank (www.rcsb.org) under the Code#: 2O5D.
- Humphrey, W.; Dalke, A.; Schulten, K. J. Mol. Graph. 1996, 14, 27.
- VMD 1.8 is available from: http://www.ks.uiuc.edu/Research/vmd/.
- 21. Pov-Ray is available from: http://www.povray.org.
- 22. It is worth noting that the described molecules are very rigid, and may possess entropic disadvantage for a surface binding site like this which normally requires subtle structural adjustment of both ligand and engaging residues of protein for a favorable binding. Furthermore, the newly designed moiety targets hydrophilic residues of Lys, Arg, and His on the surface which are presumably solvated in apo state of the enzyme. Any direct interaction from a ligand to these residues would have to overcome desolvation first. The desolvation process is energetically unfavorable and could offset the additional interaction observed by X-ray structure. This may account for the flatness of the SAR for this scaffold in this region of the binding site despite the predicted/observed additional protein-ligand interactions. Future direction should be directed toward the relaxation of the rigidity of the inhibitors, taking into consideration the desolvation, and at the same time retaining the similar protein-ligand interaction network.