Biological Science

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Crystal Structure of HCV NS5B RNA Polymerase Complexed with a Tetracyclic Inhibitor

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Introduction

Hepatitis C virus (HCV) NS5B RNA polymerase is the key enzyme involved in the replication of the HCV gene and has been one of the main targets for drug development. We synthesized a series of 2-phenyl-benzimidazole and 2-phenyl-indole derivatives as inhibitors of NS5B. Among them, conformationally constrained tetracyclic derivatives by fixing the 6,5-bicyclic and phenyl rings with a bridging group increased inhibitory potency against NS5B. We analyzed the crystal structure of NS5B complexed with the tetracyclic inhibitor (Figure 1), one of the compounds with high inhibitory potency [1].

Materials and Methods

The expression, purification and crystallization of NS5B were performed as described elsewhere [2]. The NS5B/inhibitor complex was prepared by soaking the NS5B crystal in the solution of the inhibitor. Diffraction data for the crystal were collected at PF-AR NW12. The data collection and refinement statistics are summarized in Table 1. Coordinates and structure factors for the structure were released in the Protein Data Bank (PDB ID 2DXS).

Results and Discussion

Binding of the inhibitor was identified from the difference Fourier maps. The inhibitor is located at the known allosteric pocket of the thumb domain of NS5B (Figure 2), where the tip of the peptide loop is substitutionally located in case of the apo structures.

In the structure-activity relationship (SAR) studies of tetracyclic compounds, inhibitory potency roughly correlates with the dihedral angle of the indole and phenyl planes based on the calculation of the most stable conformation. The optimal angle for inhibitory potency seems to be approximately 46°. The crystal structure of the complex reveals that the dihedral angle in the complex structure is 47°, which is in good agreement with the angle predicted by the above SAR and the conformational calculation. We conclude that conformational constraint of benzimidazole and indole inhibitors afforded a tetracyclic scaffold favorable for binding to the allosteric pocket of NS5B and resulted in enhanced enzyme inhibition potencies.

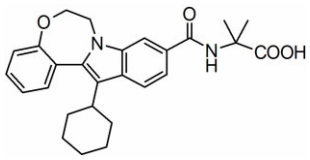

Figure 1: Tetracyclic inhibitor for analysis

Table 1: Data processing and refinement statistics

Data processing	
Space group	$P2_1$
Cell parameters	a = 96.4 Å, b = 67.2 Å, c = 297.9 Å, β = 93.8°
Resolution	30 – 2.2 Å
Completeness	0.993 (0.983)
Observed reflections	224,858
Unique reflections	63,257
$\langle I/\sigma(I)\rangle$	10.4 (3.3)
R_{merge}	0.122 (0.340)
Refinement	
Resolution	30 – 2.2 Å
R factor	0.217
R_{free}	0.259

Numbers in parentheses refer to data in the highest resolution shell (2.32 - 2.20 Å).

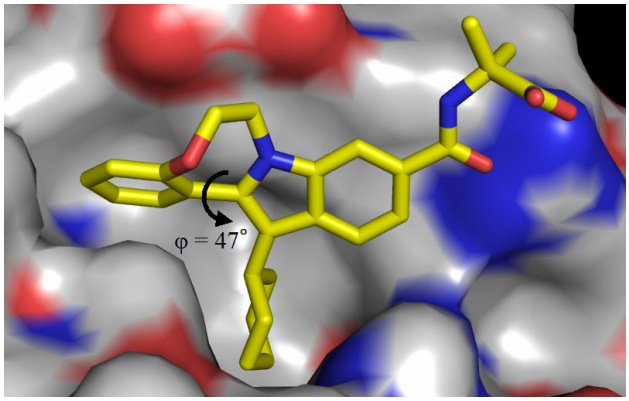

Figure 2: Inhibitor bound to the thumb domain of NS5B

References

[1] K. Ikegashira et al., J. Med. Chem. 49, 6950 (2006).
[2] T. Adachi et al., Biochim. Biophys. Acta 1601, 38 (2002).

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