Design of new cyclic plasmin inhibitors

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Plasmin (Plm), a trypsin like protease, is involved in the fibrinolysis pathway. Additionally, the localized activation of plasminogen (Plg) and action of Plm at the specific regions of the extracellular milieu lead to the digestion of many proteins at the localized cell surface that results in inducing cell invasion and metastasis and alternating the expression of cytokines. A growing body of data suggests that a Plm inhibitor is a potential candidate as an anti-inflammatory and anti-cancer agent as well as for hemostatics. The development of selective Plm inhibitors is required to study the precise role of Plm and its relationship in treating several diseases.

We previously revealed the binding mode of the YO-2 (Plm inhibitor)[1]- ≠ Plm complex with the crystal structure analysis [2]. In the complex, the aminomethylcyclohexyl moiety of YO-2 extends into the S1 subsite. At the S1' subsite, the Tyr moiety forms a hydrogen bond with K607, and the pyridine moiety forms an imperfect π - π stacking. In contrast, the hydrophobic octyl moiety does not have extensive interaction with the S2' subsite; it points away from the protease surface. In this work, we deal with the structure-based design of Plm inhibitors by focusing on the P' residues; namely, we paid attention to the enhanced interaction at the S' pocket. Incorporation of a ring segment could create extensive interactions to make the inhibitor fit better in the S' subsites. We cyclized YO-2 *via* ring closing metathesis (RCM), or through lactam formation between the side chains of P2' and P3' residues.

Figure 1: The structure of YO-2 and design of new cyclic derivatives.

Scheme 1 summarizes the synthetic route for the preparation of cyclic derivatives 3-5. The synthesis started from H-Tyr-OMe, which was derived to the dialkene intermediates, which were treated with TFA giving the linear derivatives. On the other hand, the intermediates were cyclized with RCM using Grubbs catalyst, followed by treatment of TFA, giving the cyclic derivatives 3-5. Final products were identified by analytical HPLC and MALDI (ESI)-TOF mass spectrometry. The Plm inhibitory activity of newly synthesized compounds is summarized in Table 1. An addition of benzene ring into the hydroxy group of a Tyr residue dramatically enhanced the inhibitory activity (1, 2 vs 3, 4, 5), which proved that the π - π interaction at the S3' subsite played a critical role. Among the compounds, the cyclic compound possessing an olefin with 20-membered ring had the strongest Plm inhibition (4: $IC_{50} = 3.68 \pm M$), which, however, was seven-times weaker than YO-2, the parent compound.

In conclusion, the cyclization performed between the side chains of P2' and P3' residues and effectively enhanced the interaction at S' subsites; however, the other elements, such as hydrophobic interaction or their combination are necessary to optimize the interaction at the contact surface. Further efforts to improve Plm inhibition are continuing.

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Scheme 1: Preparation of cyclic derivatives 3-5

Reagents and conditions: (a) TEA, Boc-Tra-OH, HOBt-H₂O, EDC·HCl (1.2 equiv); (b) K₂CO₃ (4.0 equiv), Methyl 2-(chloromethyl)benzoate (2.0 equiv)/DMF, MeOH; (c) 1M NaOH/DMF; (d) Unsaturated amines (2.5 equiv), HOBt-H₂O, EDC·HCl (2.2 equiv); (e) Grubbs catalyst (0.25 equiv)/DCE at 60°C under N₂; (f) TFA-anisole for 1h.

Table 1: Plm inhibition of newly synthesized derivatives

compd.	cyclic			linear		Index: IC ₅₀ (linear)/IC ₅₀ (cyclic)
	structure	ring size	IC ₅₀ (μM)	structure	IC ₅₀ (μM)	
YO-2					0.53	/
PSI-325					0.20	/
1		16	25.0% inhibition at 100 μM		20.9	<0.21
2		17	42.6% inhibition at 100 μM		39.9% inhibition at 100 μM	1.06
3		18	5.88		27.6	4.69
4		20	3.68		16.8	4.56
5		22	7.14		8.97	1.25

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