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Identification of novel plasmin inhibitors possessing nitrile moiety as warhead

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ABSTRACT

Lysine-nitrile derivatives having a trisubstituted benzene, which belongs to a new chemical class, were prepared and tested for inhibitory activities against plasmin and the highly homologous plasma kallikrein and urokinase. The use of the novel chemotype in the development of plasmin inhibitors has been demonstrated by derivatives of compound **9**.

Plasmin is best known as the key molecule in the fibrinolytic system and is critical for clot lysis,[1] which directly degrades extracellular matrix components including the glycoproteins fibronectin, laminin, and elastin, as well as proteoglycans.[2] Otherwise, plasmin can initiate matrix metalloproteinase (MMP) activation cascade. MMPs, themselves, are implicated in a complex cascade of activation.[3] Along with MMP, plasmin is suggested to be involved in physiological processes including angiogenesis, tissue repair, tumor invasion and metastasis.[4–6] It seems likely that the aging process is influenced by the activity of serum proteolytic enzymes, MMP-9 and plasmin.[7] Thus, the research area on plasmin has dynamically shifted from historical molecule in the fibrinolytic system to a newly confirmed drug target. Although plasmin inhibitors should be now perceived as a promising new principle in the treatment of diseases triggered by plasmin, few plasmin inhibitors are available in recognized compounds, such as tranexamic acid pharmaceuticals, or YO-2 and peptidic inhibitors possessing cyclic ketone group as experimental tools.[8–10]

Up to the mid of 1980's, only a few studies identified the substrates for plasmin; however, they were not selective against other serine proteases.[11–13] Subsequently, some research focused on increasing the selectivity against other members of this enzyme family. From these studies it was discovered that plasmin exhibited a strong preference for hydrophobic D-amino acids and a free amine group at the P3 position, such that D-Ile-Phe-Lys-*p*NA was identified as a specific substrate for plasmin .[14,15]

A selective peptidic substrate for a targeted protease provides many potential approaches for developing a selective inhibitor against the protease. Many chemotypes have been described as peptidic and non-peptidic inhibitors with warhead of cysteine and serine proteases.[16,17] A number of cathepsin K inhibitors with a nitrile group as warhead have been identified that covalently bind to active site Cys25 of cathepsin K.[18,19] Likewise, the dipeptidyl peptidase (DPP-IV) inhibitor Vildagliptin (LAF237) is clinically used as an anti-diabetes drug also has a nitrile group that covalently interacts with Ser630 in the active site of DPP-IV.[20] Merck recently reported that the electrophilicity and reactivity of nitrile-containing inhibitors have an impact on the reversibility of the enzyme–inhibitor complex.[21] In addition, a survey of nitrile-containing pharmaceuticals and clinical candidates identified several roles for the nitrile group, including an effect on the ADME profile.[22] Identification of the nitrile group as a warhead had a significant impact on the development of cysteine and serine protease inhibitors. This accounts for our attempts to design new plasmin inhibitors with nitrile group.

As the first step in tackling plasmin inhibitors possessing a nitrile group, compound **1** was designed by replacing the scissile amide bond of substrate (D-Ile-Phe-Lys-*p*NA) with the warhead (electron deficient center), which was capable of reacting in a reversible manner (Table 1). Compound **2** was also designed to elucidate whether the nitrile group had an impact on the reactivity of the enzyme–inhibitor complex formation. Both the peptide–nitrile

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Table 1
Inhibition of plasmin, plasma kallikrein and urokinase by compounds **1**and **2**

Compd nos.	Structures	IC_{50} (μM)		
		Plasmin	Plasma kallikrein	Urokinase
1	D-Ile-Phe-Lys-CN	78	38% at 1000	15% at 1000
2	D-Ile-Phe-Lys-NH_2	290	0% at 1000	0% at 1000

derivatives were prepared and tested for inhibitory activities against plasmin and the highly homologous plasma kallikrein (PK) and urokinase (UK).[23] Compound **1** displayed potent plasmin inhibition combined with significant selectivity for plasmin over PK and UK. Compound **2** showed about a 3-fold decrease in the inhibitory activity for the target enzyme and proved to be less active for other enzymes. These results suggest that the nitrile group is covalently bound to the Ser active site. Additionally, molecular modeling of D-Ile-Phe-Lys substrate complexed with plasmin supported experimental data that the each amino acid in the inhibitor sequence occupied well-recognized S1 subsite in plasmin and a wider subsite, comprising S2 and S3,[24] was also occupied by the hydrophobic residues of D-Ile and Phe (Fig. 1). The initial investigation led to the development of the first nitrile-containing peptide-derived plasmin inhibitor, which was the basis for the subsequent development of nitrile-containing non-peptide-related plasmin inhibitors.

Okada et al. discovered a non-peptidic plasmin inhibitor, YO-2.[9] The inhibitor had tranexamic acid instead of Lys and occupied the S1 subsite. Modeling studies suggested that the *O*-picolyl-Tyr moiety in YO-2 assumes an extended conformation along with the wall of S2 site.[24] Molecular modeling of the substrate and YO-2 complexed with plasmin suggested that a rigid spacer extended from the N-terminus of the P1 residue, Lys, would be directed toward the broad and hydrophobic space comprised by the S2 and S3 subsites; for example, in the case of R1 (P3 moiety as R1 in Fig. 2a) which is a benzyl moiety. Incorporation of picolyl group into R1 resulted in plasmin inhibitor **3** with no substantial change in the selectivity against PK and UK (Table 2). Replacement of the nitrile in **3** with an amide exhibited a reduced potency against plasmin (IC_{50} >1000 μM). This substantial difference of the activity is another supportive evidence that the nitrile group works as the warhead for plasmin. Molecular modeling supported that if the nitrile group was covalently bound to the active site serine, the picolyl group would occupy the S3 subsite while the S2 subsite would remain unoccupied (Fig. 3). In addition, modeling suggested that the substitutent at the *meta*-position (P2 moiety as R2 in Fig. 2b) of the hydrophobic group would point toward the S2 subsite. Hence, derivatives of compound **3** were prepared to explore the role of R2 for inhibitory activity against plasmin. As indicated in Table 2, introduction of an aromatic ring into R2 lost their inhibitory activity against plasmin (**3** vs **4**). Compound **5**, which differs from **4** by the replacement of *p*-fluorobenzene with 2-naphthyl, showed increased potency against plasmin, whereas compound **6**, the regioisomer of **5**, lost its inhibitory activity. Compounds **5** and **6** increased the affinity for PK as well, whereas the indole derivatives **7**–**9** completely lost the inhibitory activity against the untargeted enzyme, PK. Inhibitors **7** and **8** were found to be potent and selective to a similar level to that of compounds **3** and **5**. Replacement of *t*-butoxycarbonyl with a *p*-fluorobenzyl group exhibited increased inhibitory activity for plasmin without substantial change in the selectivity against PK and UK (**7** vs **9**). As expected above from molecular modeling, these results suggested that the P2 moiety could have an interaction with the S2 pocket in plasmin. The structures of **9** and **10** imply that the picolyl group in P3 moiety contributes to the increase in inhibitory activity against plasmin while retaining selectivity against PK and UK. Especially, since compound **3** possesses considerable inhibitory activity against plasmin, the picolyl group as the P3 moiety seems to be an essential asset for the scaffold of plasmin inhibitors reported here. Molecular modeling of **9** reported here will be covered in future publications.

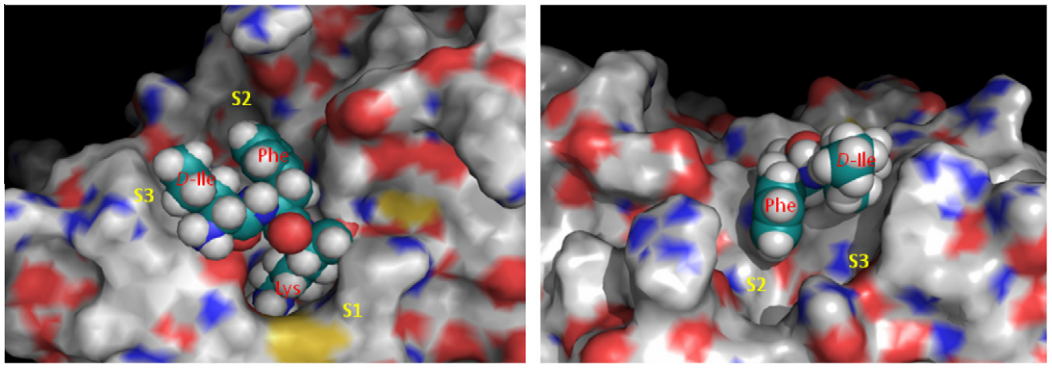

Figure 1. D-Ile-Phe-Lys substrate docked into the active site of plasmin.[24]

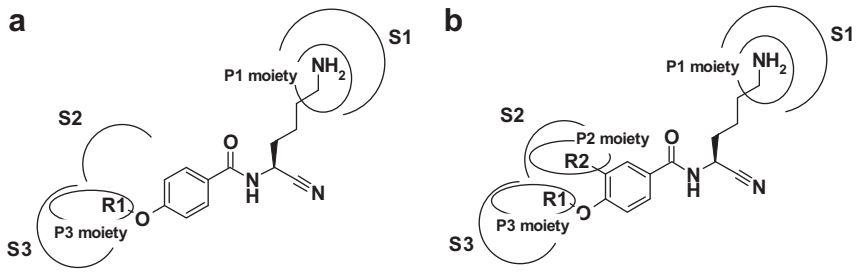

Figure 2. Design of new chemotypes for plasmin inhibitors.