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Structure-based drug design of 1,3,6-trisubstituted 1,4-diazepan-7-ones as selective human kallikrein 7 inhibitors

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ABSTRACT

A novel series of 1,3,6-trisubstituted 1,4-diazepan-7-ones were investigated as human kallikrein 7 (KLK7, stratum corneum chymotryptic enzyme) inhibitors. Based on the X-ray co-crystal structure of compound **1** bound to human KLK7, the derivatives of this scaffold were designed, synthesized, and evaluated. Through structure-activity relationship studies focused on the side chain located in the prime site region of the enzyme, representative compounds **15**, **33a**, and **35a** were identified as highly potent and selective inhibitors of human KLK7.

The skin functions as a barrier against environmental factors. The stratum corneum is responsible for this barrier function, and is composed of corneocytes, which are held together by corneodesmosomes.[1,2] A defective epidermal barrier increases moisture loss, and facilitates the penetration of irritants and allergens, thereby increasing the risk of inflammation.[1,2] Atopic dermatitis (AD) is a common and multifactorial chronic allergic skin disease. Skin barrier dysfunction has been implicated in the development of AD[1–3]; therefore, restoring this barrier function of the skin would promote AD therapy.[4–6] The desquamation of corneocytes is an essential process that occurs in the normal skin[7] upon the degradation of corneodesmosomes by skin-specific proteases.[1] However, increased activities of serine proteases in the skin are associated with skin barrier dysfunction.[8] Kallikrein 7 (KLK7, stratum corneum chymotryptic enzyme) is one such protease that promotes epidermal desquamation.[9–11] It has been reported that its level significantly increases in AD patients.[12,13] Therefore, we hypothesized that an inhibitor of KLK7 would aid AD therapy by restoring the barrier function of the skin.

Previously, we reported 1,3,6-trisubstituted 1,4-diazepan-7-one as a novel scaffold for human KLK7 inhibitors, and the X-ray co-crystal structure which reveals the binding mode of the compound **1** to the human KLK7.[14] Fig. 1 illustrates the binding of compound **1** within the active site of human KLK7. CH–O interactions were characteristically observed between the phenyl ring located in the S2′ site of the enzyme and the backbone carbonyl oxygens of Leu40 and His41 of KLK7, and between the carboxylic acid of **1** and the Phe151 of KLK7. Upon preliminary investigation, we found that the carboxyl group was not necessary, and *m*-substitution of the phenyl ring held more significance; accordingly, we found that a methanesulfonyl-substituted compound, **2**,[14] was a more potent human KLK7 inhibitor than **1**. Therefore, we hypothesized that substituting the carboxyl group with other electron withdrawing substituents would strengthen the CH–O interactions and improve the potency of **1**. Furthermore, because of the short distance between the carboxyl group of **1** and the Phe151 of KLK7, we expected an aryl group to interact better with the Phe151 side chain.[15] Additionally, our co-crystal structure revealed a space of the S1′ site adjacent to the methylene side chain (Fig. 1). We considered that the introduction of an appropriate substituent to occupy this space would increase the potency. In this report, we present the optimization of compound **1** to improve its human KLK7 inhibitory potency based on structure-based drug design.

Substituted anilide derivatives were synthesized as shown in Scheme 1. Compound **3**[14] was coupled with various aniline derivatives via acyl chloride to prepare **4**–**10** or using a condensation reagent to yield **11**–**15**. Branched side chain compounds were prepared from **16**[16] as depicted in Scheme 2. The nucleophilic

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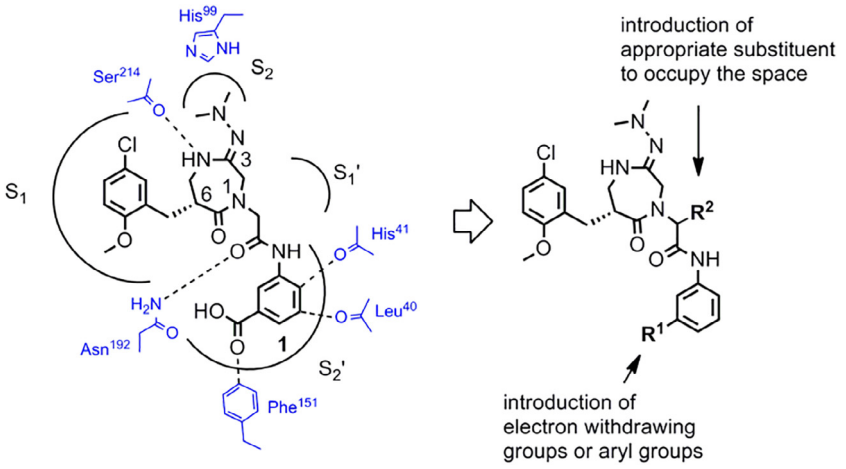
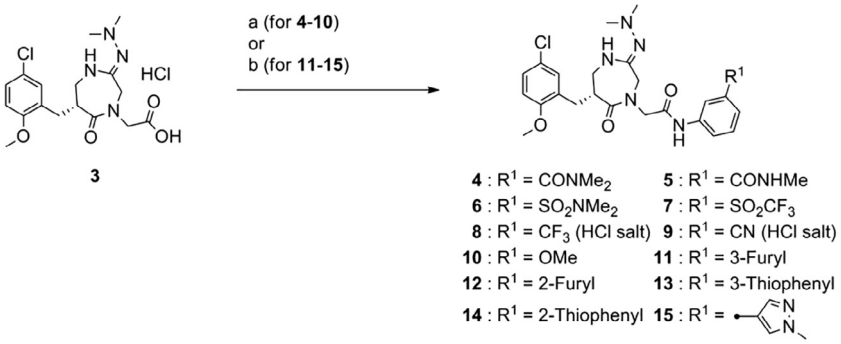


Fig. 1. Schematic illustration of the X-ray co-crystal structure of compound **1** in the active site of human KLK7, and the design of a KLK7 inhibitors.



Scheme 1. Reagents and conditions: (a) (i) oxalylchloride, DMF, CH_2Cl_2, 0 °C, (ii) aniline derivatives, Et_3N or $(i\text{-}Pr)_2NEt$, CH_2Cl_2, 0 °C–rt, (then 4 M HCl-AcOEt, rt, for **8**, **9**); (b) aniline derivatives, EDCI·HCl, DMAP, CH_2Cl_2, 0 °C.

substitutions of **16** were accomplished using corresponding α-bromo *tert*-butyl esters. The stereoisomers of the methyl (**17a** and **17b**) and ethyl (**18a** and **18b**) branched compounds were successfully separated by column chromatography; however, the *n*-propyl (**19**) and *n*-butyl (**20**) branched compounds were obtained as mixtures of diastereomers. The alkylated compounds were treated with Belleau's reagent to prepare the corresponding thioamides, **21**–**24**; diastereoisomers of **23** and **24** were isolated by column chromatography at this point. Thioamides **21**–**24** were converted to amidorazones **25**–**28** by reacting with *N*,*N*-dimethylhydrazine in the presence of silver acetate. Compounds **29**–**32** were obtained after simultaneously removing the *tert*-butyl and the 2,4,6-trimethoxybenzyl (TMB) group under an acidic condition. Further, they were condensed with 3-(1-methyl-1*H*-pyrazol-4-yl) aniline to yield **33**–**36**, respectively. To determine the stereochemistry of the diastereomers, an alternative synthesis was conducted as shown in Scheme 3. The substitution reaction of **16** with a chiral D-lactic acid derivative **37** yielded an *S*-isomer, whose ^1H NMR spectrum was consistent with that of **17a**. Therefore, compound **33a**, which was derived from **17a**, was determined to have an *S* configuration. The stereochemistry of the R^2 group of compounds **34**–**36** was determined by comparing their ^1H NMR spectra with that of **33a** or **33b**.

Previously, we identified that compound **2** showed improved human KLK7 inhibition (Table 1) and hypothesized that the electron withdrawing effect of its methanesulfonyl group enhances CH–O interaction and increases its inhibition potency.[14] Further, we evaluated the human KLK7 inhibitory activities of compounds substituted with other electron withdrawing substituents (Table 1).

Although the methyl amide derivative, **5**, exhibited an increased potency, the inhibitory activity of the dimethyl amide derivative, **4**, was decreased compared with that of **1**. The sulfonamide derivative, **6**, also showed lesser potency than **2**. We presumed the poor electron withdrawing abilities of the amide and sulfonamide groups as well as steric hindrance to be responsible for the reduced potency. Therefore, we investigated the effects of compounds substituted with smaller electron withdrawing groups, such as trifluoromethansulfonyl (**7**), trifluoromethyl (**8**), and nitrile (**9**) groups. Contrary to our expectations, these compounds exhibited decreased inhibitory activities. In addition, compound **10** with a methoxy group, exhibited a similar inhibitory activity as compounds **7**, **8**, and **9**. These results indicated that the electronic effects of substituents contributed limitedly to strengthening the CH–O interactions and improving the inhibitory activity.

X-ray co-crystal structure revealed that replacing the carboxyl group of **1** with an aromatic substituent could strengthen its interaction with the Phe151 of KLK7, thereby increasing its potency. To confirm this hypothesis, we introduced heteroaryl groups, which are likely to interact with Phe151, and evaluated the human KLK7 inhibitory activities of the resultant compounds (Table 2).

As shown in Table 2, all heteroaryl substituents were tolerated. Compounds substituted with furan (**11** and **12**) and pyrazole rings (**15**) showed more potent inhibitory activities than **1**; however, those substituted with a thiophene group (**13** and **14**) were slightly less potent than other heteroaryl-substituted compounds. The