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Isomannide-Based Peptidomimetics as Inhibitors for Human Tissue Kallikreins 5 and 7

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KEYWORDS. Serine Protease, Kallikrein, Peptidomimetics, Inhibitor, Molecular Dynamics.

ABSTRACT: Human kallikrein 5 (KLK5) and 7 (KLK7) are potential targets for the treatment of skin inflammation and cancer. Previously, we identified isomannide derivatives as potent and competitive KLK7 inhibitors. The introduction of N-protected amino acids into the isomannide-based scaffold was studied. Some KLK5 inhibitors with sub-micromolar affinity (K_i values of 0.3-0.7 μM) were identified and they were 6- to 13-fold more potent than our previous hits. Enzyme kinetics studies and the determination of the mechanism of inhibition confirmed that the new isomannide-based derivatives are competitive inhibitors of both KLK5 and KLK7. Molecular docking and MD simulations of selected inhibitors into the KLK5 binding site provide insight into the molecular mechanism by which these compounds interact with the enzyme. The promising results obtained in this study opens new prospects on the design and synthesis of highly specific KLK5 and KLK7 inhibitors.

Human tissue kallikreins are a family of serine peptidases present in almost every tissue of the human body (1). Among the members of the kallikrein family, Kallikrein 5 (KLK5) and Kallikrein 7 (KLK7) appear to be most abundantly expressed in human skin, where a possible role in the desquamation process has been reported (2). Also, there is now growing evidence that over-expression of these enzymes occurs in endocrine-related malignancies including ovarian, breast and testicular cancer (3). In addition, recently we have described the ability of KLK5 to hydrolyze plasminogen releasing active plasmin (4), which can represent a new way to understand its involvement in cancer and inflammation processes, in which KLK5 appear to be up regulated.

Despite the evidences of the importance of KLK5 and KLK7 for some pathological processes, so far there are few inhibitors described for both enzymes (5-7). Recently we have identified some natural and synthetic compounds that inhibited KLK5 and KLK7 in the low micromolar range (8-9). In our efforts to find new inhibitors for KLKs, herein we report a new series of potent KLK5 and KLK7 isomannide-based peptidomimetic inhibitors using enzyme inhibition kinetic studies and molecular modeling.

The synthesis of the isomannide-based peptidomimetic derivatives were previously described by Barros et al. (10).

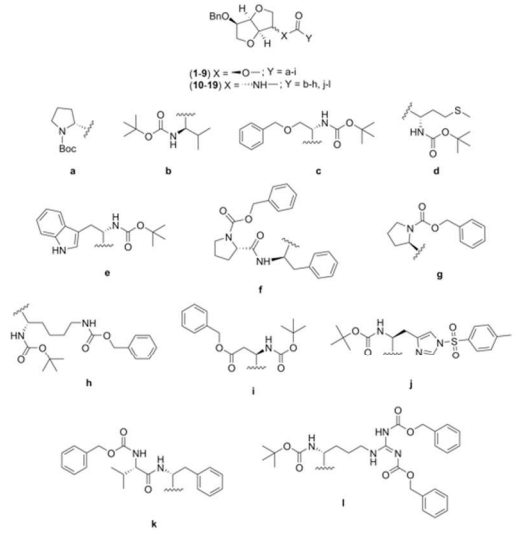


Figure 1. Structure of the isomannide-based amide and ester peptidomimetics inhibitors.

The use of the isomannide rigid scaffold was envisaged due to its structural analogy with cyclic rigid dipeptides. The

rigidity of this scaffold allows the compound to be fixed in its bioactive conformation. In our previous work (9), we identified two compounds that inhibited KLK5/KLK7 with K_i values in the micromolar range. In order to improve the inhibitory activity against KLK5 and KLK7, we designed a new series of peptidomimetics by the introduction of N-protected amino acid side chains into the isomannide-based scaffold. These new compounds are characterized by the presence of an N-protected amino acid coupled to the isomannide scaffold through an amide or ester bond (Figure 1). None of the compounds were able to inhibit the activity of KLK1 and KLK6 in an initial screen. On the other hand, seven compounds in these series showed significant to moderate activity against KLK5/KLK7 with IC_{50} ranging from 3.7 to 19.6 μM (Table 1).

Compound	Y	KLK5		KLK7	
		IC_{50} (μM)	K_i (μM)	IC_{50} (μM)	K_i (μM)
1	a	520.7 ± 6.6	ND	24.4 ± 2.5	15.6 ± 1.2
2	b	222.9 ± 21.1	ND	239.0 ± 9.8	ND
3	c	15.5 ± 2.9	2.4 ± 0.3	16.0 ± 1.5	2.3 ± 0.2
4	d	356.6 ± 13.4	ND	28.7 ±1.9	18.4 ± 1.0
5	e	376.6 ± 32.4	ND	59.2 ± 5.3	ND
6	f	3.9 ± 0.1	0.4 ± 0.05	19.6 ± 1.3	2.8 ± 0.9
7	g	3.7 ± 0.1	0.3 ± 0.02	16.6 ± 2.4	1.9 ± 0.6
8	h	9.0 ± 0.8	0.7 ± 0.06	13.7 ± 0.7	1.4 ± 0.1
9	i	4.7 ± 0.1	0.6 ± 0.07	10.2 ± 0.8	1.3 ± 0.2
10	b	479.8 ± 20.4	ND	> 1000	ND
11	c	53.9 ± 0.5	ND	84.5 ± 3.1	ND
12	d	37.0 ± 1.3	26.6 ± 2.2	456.8 ± 31.8	ND
13	e	39.2 ± 3.7	28.2 ± 1.8	418.3 ± 32.6	ND
14	f	157.4 ± 5.4	ND	249.3 ± 12.7	ND
15	g	320.6 ± 17.7	ND	23.6 ± 0.5	15.1 ± 0.6
16	h	13.3 ± 0.3	2.4 ± 0.5	23.6 ± 0.6	2.9 ± 0.3
17	j	156.9 ± 4.1	ND	27.8 ± 3.8	4.4 ± 0.5
18	k	21.7 ± 1.5	3.4 ± 0.4	56.6 ± 1.6	3.8 ± 0.4
19	l	11.8 ± 1.1	1.7 ± 0.2	16.9 ± 0.9	2.4 ± 0.5

Table 1. Inhibitory potency (IC_{50}) and inhibition constant (K_i) values for the isomannide-based ester (X = O) and amide (X = NH) derivatives. ND, not determined.

A pairwise analysis between the isomannide-based ester **2**-**8** and amide **10**-**16** derivatives shows that in general the former were better KLK5 inhibitors than the later ones. The isomannide-based ester derivatives with the Boc-protecting group and containing a hydrophobic amino acid side chain (Compounds **1**, **2**, **4** and **5**) were poor KLK5 inhibitors, but their potencies against KLK7 were between 6 and 21-fold higher. The only exception was compound **2**, which is the weakest inhibitor of both enzymes. On the other hand, the compounds bearing a protected charged or polar amino acid side chain (Compounds **3**, **8** and **9**) exhibited potency parameters comparable against both enzymes. A progressive enhancement in the potency was observed in the case of compounds **6** and **7** with IC_{50} against KLK5 of 3.9 μM and 3.7 μM, respectively. They also inhibit KLK7, but with an IC_{50} between 4-5 times higher. Compound **6** has a phenylalanine residue between the isomannide ring and a Cbz-protected proline, while in compound **7** the proline is directly attached do isomannide ring. The comparison of these two compounds shows that phenylalanine residue can be eliminated without compromising the potency. Also, it appears that the stereochemistry configuration of the pyrrolidine ring in proline has a drastic effect on the potency of the compounds against KLK5. In compound **1** an *S*-configuration in the pyrrolidine ring shows an IC_{50} of 521 ± 7 μM and was 140-fold less potent than the corresponding *R*-enantiomer (compound **7**; KLK5 IC_{50} = 3.7 ± 0.1 μM). In contrast, KLK7 seems to be less restrictive since the potency against this enzyme for these two compounds are nearly identical.

For the isomannide-based amide derivatives the SAR indicates that the Boc-protected compounds with hydrophobic amino acid side chains (Compounds **10**, **12** and **13**) are more potent against KLK5 than KLK7, which is in opposite to the observed inhibition pattern for the ester derivatives. Moreover, compounds with a protected charged or polar amino acid side chain (Compounds **11**, **16** and **19**) inhibit both enzymes with almost the same potencies. Compounds with a Cbz-protecting group (Compounds **14** and **15**) inhibit the enzymes in different levels. While compound **14** is a weak inhibitor for both KLK5 and KLK7 enzymes, compound **15** is a poor inhibitor of KLK5 but inhibits KLK7 with an IC_{50} of 23.6 μM. Compound **17** with a protected histidine side chain inhibits KLK7 with an IC_{50} six times lower than KLK5. Finally, compound **18** with a phenylalanine residue between the isomannide ring and a Cbz-protected valine is a slightly better inhibitor of KLK5 than KLK7.

The compounds with an IC_{50} lower than 50 μM were chosen for further studies to determine their mechanism of inhibition. The inhibition of KLK5/KLK7 was measured at three different inhibitor concentrations and Lineweaver–Burk plots were prepared. The inhibition constants (K_i) were obtained through the secondary plot of K_m/V_{max} vs. [I]. Representative plots for the best two inhibitors, compounds **6** and **7**, are shown in Figure 2.

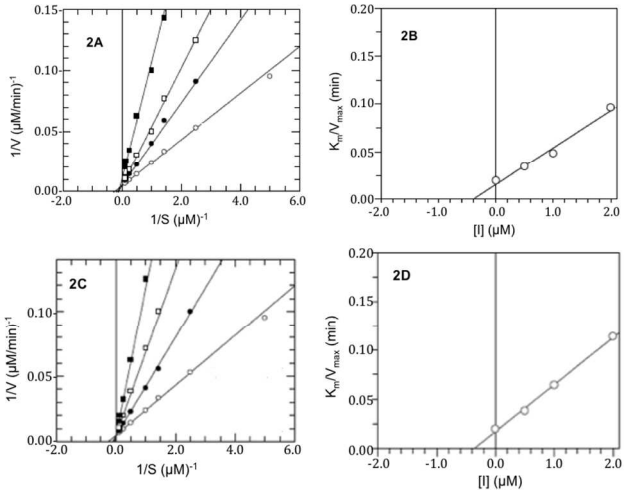


Figure 2. Representative graphs for the kinetic constants determination. The mechanism of inhibition was determined by the Lineweaver-Burk plots for the hydrolysis of FRET substrate Abz-KLRSSKQ-EDDnp by KLK5, in the presence of inhibitor **6** (2A) and **7** (2C). The K_i values were determined by the graph K_m/V_{max}vs [I] for the compounds **6** (2B) and **7** (2D). In 2A and 2C the solid lines represent the linear regression in fits obtained by software GraFit 7.0 in absence of inhibitor (○) and three different inhibitor concentrations (0.5 μM (●), 1.0 μM (□), and 2.0 μM (■).

All of the inhibitions followed a competitive mechanism, and the Lineweaver-Burk plot (Figure 2A and 2C) reveals a common intersection point on the y-axis ($1/V_{max}$) at different inhibitor concentrations, but with different slopes

(K_m/V_{max}) and x-intercepts ($-1/K_m$). The K_i value was obtained from the replot of the slopes (K_m/V_{max}) vs. [I] (Figure 2B and 2D). This plot generates a straight line, which intersects the [I] axis at a value equal to $-K_i$. The measured K_i values are in reasonable agreement with IC_{50} values, with the compounds **6**-**9** being the best inhibitors of KLK5 showing a K_i in the range of 0.3 – 0.7 μM. Compounds **7**-**9** were all similarly potent inhibitors of KLK7 with K_i values ranging from 1.3 to 1.9 μM. A similar rank order was also observed in the K_i values for the amide derivatives with compounds **16** and **19** inhibiting both enzymes in the low micromolar range.

To investigate the binding mode of these new inhibitors, we performed molecular docking studies with compound **6** and **14** into the KLK5 binding site using the structure of KLK5 in complex with the inhibitor leupeptin (11). Binding modes of the newly found KLK5 inhibitors were addressed with Autodock 4.2 (12). The docking pose of the two complexes are remarkably similar as well as their docking scores: -10.5 and -9.5 kcal/mol for compound **6** and **14**, respectively. Thus, from molecular docking results, it was very uncertain to rationalize the difference in the potency of these two compounds. We then applied molecular dynamics simulation combined with Molecular Mechanics/Generalized Born Surface Area (MM/GBSA) as a more accurate method to investigate the protein-ligand interactions (13). The binding mode of the complexes identified with Autodock was used as starting structures for 10ns of MD simulations using AMBER (14) (for details see Supporting Information).

During 10 ns of molecular dynamics simulations, the complex between compound **6** and KLK5 (Figure 3a) showed a stable binding mode, which does not substantially diverge from the one previously obtained by docking, being observed only fluctuations in the O-benzyl moieties (Figure S1). The binding of compound **6** is further stabilized by three hydrogen bonds with the backbone of Gly193, Ser214, and Gly216 (Figure 3a and Figure S2).

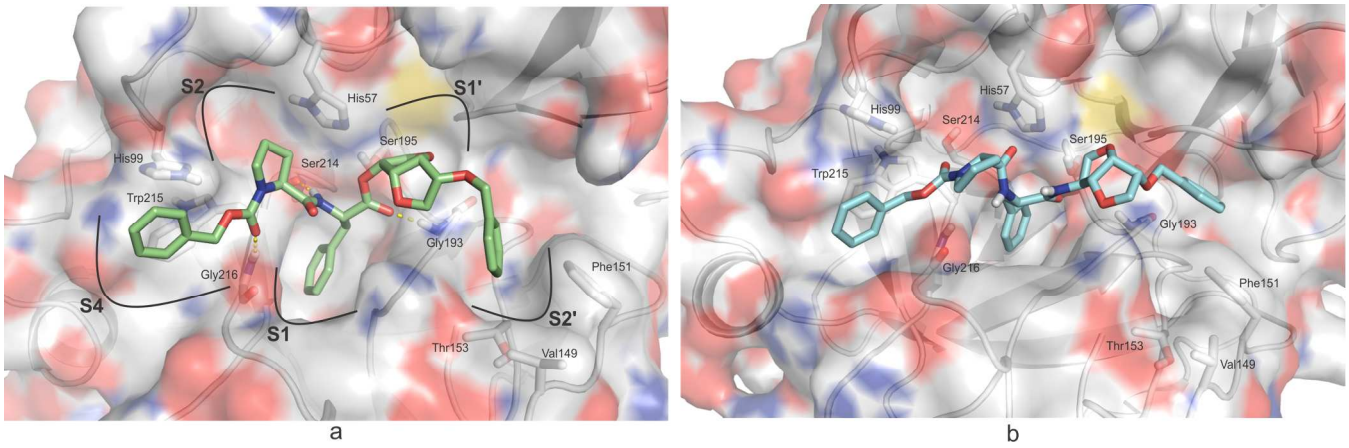
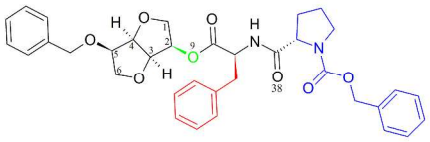


Figure 3. Predicted binding mode of inhibitor **6** (a) and **14** (b) in the active site of KLK5, illustrating the essential residues, the hydrogen bonds (yellow dash line) and the surface of the enzyme (colored by element).

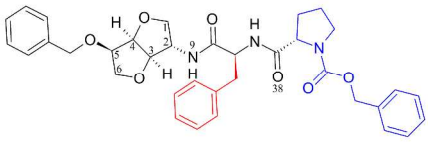
For compound **14**, significant differences in its position and conformation were observed throughout the MD simulation. The ligand gradually left the starting conformation and moves away from the binding pocket losing its contact with the enzyme (Figure 3b). As a consequence, the hydrogen bonds with Ser195 and Gly193 are lost (Figure S2). According to MM-GBSA calculations, these structural changes are reflected in the free energy of binding between compound **14** and KLK5 (ΔG_{GBSA} = –30.1 kcal/mol), which is around 17 kcal/mol lower than that obtained in the simulation of compound **6** (ΔG_{GBSA} = –46.9 kcal/mol), which corroborates our experimental results.

Previously, we have identified compound **20** (named 9g in that publication) as a selective KLK7 inhibitor over KLK5 (13). In order to rationalize the observed structure–activity relationship trend it would be helpful to compare the structure of compound **6** and **14** with that of compound **20**. First, the comparison between compounds **14** and **20** shows that they are highly similar (Figure 4). The only differences are the replacement of the 2-methylthiophene (red) and phenyl moieties (blue) in **20** by a benzyl and N-Cbz-L-proline moieties, respectively, in compound **14**. As a result of these changes, the potency against KLK7 is abolished along with the worsening of the inhibitory effects against KLK5. In compound **6**, the same substituents, as in **14**, replace the 2-methylthiophene and phenyl moieties in **20**. Moreover, two other additional changes are observed. First the stereochemistry of the carbon atoms C-2 and C-4 in the isomannide ring which is 2*S* and 4*S*, respectively, in compounds **14** and **20** is converted to 2*R*, and 4*R*, respectively, in compound **6**. This fact explains, at first glance, the reduced potency of compound **14**. The second difference between compounds **14** (and **20**) and **6** is the bioisosteric substitution of NH by O (green in Figure 4) group converting an amide into an ester. The amide-to-ester substitution reduces the electronegativity of the carbonyl oxygen, which weakens its hydrogen bonding acceptor strength and eliminates a hydrogen bond donor (15, 16). In theory these factors would contribute negatively to the affinity of the compound since the amide-to-ester substitution will reduce the number and the strength of potential hydrogen bonds the compound can make. Also there is a reduction in the rotational barrier of the ester bond (11 kcal/mol) compared with that of amide bond (20 kcal/mol), increasing the conformational flexibility of the molecule. Locking the compound in its binding conformation or increasing its flexibility are well known strategies used in drug design (17, 18). For the

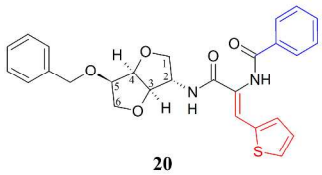
compounds evaluated in the present work the higher conformational flexibility is crucial for the biological activity of compound **6**. In fact, along with being more rigid than **6** because of the higher rotational barrier of the amide bond, analysis of the MD simulations for compound **14** showed the presence of a stable intramolecular hydrogen bond between the amide nitrogen N-9 and the carbonyl oxygen O-30 producing a



6
KLK5
IC_{50} = 3.9 μM
K_i = 0.4 μM
ΔG_{GBSA} = -46.9 kcal/mol
KLK7
IC_{50} = 19.6 μM
K_i = 2.8 μM



14
KLK5
IC_{50} = 157.4 μM
ΔG_{GBSA} = -30.1 kcal/mol
KLK7
IC_{50} = 249.3 μM



20
KLK5
IC_{50} = 99.2 μM
K_i = 70.7 μM
KLK7
IC_{50} = 13.3 μM
K_i = 1.8 μM

Figure 4. Chemical structures of compounds **6**, **14** and **20**. The common substructure shared by the KLK5/KLK7 inhibitors is colored in black. The substituents 2-methylthiophene (red) and phenyl (blue) in **20** are replaced by a benzyl (red) and N-Cbz-L-proline (blue) moieties, respectively, in compounds **6** and **14**. The green oxygen highlights the amide-to-ester substitution between **6** and **14**.

seven-member heterocyclic ring. This imposes an additional conformational constraint to **14** reducing its ability to fit in the KLK5 binding site with the correct conformation. In **6** the amide-to-ester substitution prevents the formation of this intramolecular hydrogen bond. Taken together, the stereochemistry of the isomannide ring and the lower conformational flexibility provide insight to explain the reduced potency of the amide derivative **14** against KLK5.

The binding of compounds **6** and **14** to KLK7 were also investigated. In the MD simulation followed by the MM/GBSA calculations, the predicted free energy of binding for compound **6** (ΔG_{GBSA} = -32.9 kcal/mol) was lower than that of **14** (ΔG_{GBSA} = -38.9 kcal/mol), which contradicts the experimental data. Looking back to the docking results we noticed that the 2nd ranked pose had almost the same energy than the 1st one, with the phenylalanine side chain still occupying the S1 pocket, but the isomannide and pyrrolidine rings are placed in an opposite orientation. After the MD simulation of this pose the ΔG_{GBSA} calculated using the MM/GBSA method was -40.0 and -35.6 kcal/mol for the compound **6** and **14**, respectively. The 2nd ranked pose of **6** is the ligand binding pose most likely to be correct since it is approximately 7 kcal/mol more stable than the one best ranked. The complex between compound **6** and KLK7 (Figure S4) exhibited a stable binding mode, stabilized by two hydrogen bonds with Asn192 Gly193 amino acids (Figure S5). A similar binding pose was observed for the KLK7-**14** complex, and the interaction is stabilized by two hydrogen bonds with Asn192 and Ser195 (Figure S5).

KLK5 is a trypsin-like serino protease with a negatively charged Asp189 and the polar Ser190 at the bottom of the S1 pocket. On the other hand, KLK7 is a chymotrypsin-like protease, with a polar Asn189 and a hydrophobic Ala190 at the S1 pocket. Also, the side chains of Tyr218 and Gln192, which frames the entrance to the KLK5 S1 pocket, are replaced by Phe218 and Asn192 in KLK7. These differences make the S1 pocket in KLK7 more open and hydrophobic. These observations provide support for the design of more potent and selective inhibitors for KLK5 and KLK7. Although the phenylalanine side chain of compound **6** binds inside the S1 pocket in both enzymes, this moiety does not fully occupy the pocket or make direct contacts with the amino acids 189 and 190. So, for example, replacing the phenyl ring in the phenylalanine by the benzamidine group, that mimics the arginine side chain, would extend further the inhibitor into the S1 pocket where additional interactions could be formed with the Asp189 and Ser190.

In conclusion, we reported a new class of small isomannide-based peptidomimetic compounds that inhibit the activity of KLK5 and KLK7. Enzyme kinetics studies and the determination of the mechanism of inhibition confirmed that all of the compounds are competitive inhibitors of KLK5 and KLK7. Compared to our previous hit, the introduction of new structural moieties and changes in the stereochemistry of the isomannide ring, as well as an amide-to-ester substitution resulted in a new series of isomannide-based ester derivatives with K_i values in the sub-micromolar range and rank among the best inhibitors of KLK5 reported so far. Docking and molecular dynamics studies allowed us to rationalize the difference in activity between the new series of compounds. Thus, the novel KLK5 inhibitors reported here represent potential leads for future optimization studies.

ASSOCIATED CONTENT

Supporting Information

Detailed materials and methods for the experimental and theoretical procedures. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript. ‡These authors contributed equally.

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REFERENCES

(1) Emami, N.; Diamandis, E.P. Human tissue kallikreins: a road under construction. Clin. Chim. Acta. 2007, 381, 78-84.

(2) Borgoño, C.A.; Michael, I.P.; Komatsu, N.; Jayakumar, A.; Kapadia, R.; Clayman, G. L.; Sotiropoulou, G.; Diamandis, E.P. A potential role for multiple tissue kallikrein serine proteases in epidermal desquamation J. Biol. Chem. 2007, 282, 3640-3652.

(3) Oikonomopoulou, K.; Diamandis, E.P.; Hollenberg, M.D., Kallikrein-related peptidases: proteolysis and signaling in cancer, the new frontier. Biol Chem. 2010, 391, 299-310.

(4) de Souza, L.R.; Melo, P.M.; Paschoalin, T.; Carmona, A K.; Kondo, M.; Hirata, I.Y.; Blaber, M.; Tersariol, I.; Takatsuka, J.; Juliano, M.A.; Juliano, L.; Gomes, R.A.; Puzer, L. Human tissue kallikreins 3 and 5 can act as plasminogen activator releasing active plasmin Biochem. Biophys. Res. Commun. 2013, 433, 333-337.

(5) Tan, X.; Furio, L.; Reboud-Ravaux, M.; Villoutreix, B.O.; Hovnanian, A.; El Amri, C. 1,2,4-Triazole derivatives as transient inactivators of kallikreins involved in skin diseases Bioorg Med. Chem.Lett. 2013, 23, 4547-4551.

(6) Liang, G.; Chen, X.; Aldous, S.; Pu, S.F.; Mehdi, S.; Powers, E.; Xia, T.; Wang, R. Human kallikrein 6 inhibitors with a para-amidobenzylanmine P1 group identified through virtual screening. Bioorg. Med. Chem. Lett. 2012, 22, 2450-2455.

(7) Liang, G.; Chen, X.; Aldous, S.; Pu, S.; Mehdi, S.; Powers, E.; Giovanni, A.; Kongsamut, S.; Xia, T.; Zhang, Y.; Wang, R.; Gao, Z.; Merriman, G.; McLean, L.R.; Morize, I. Virtual Screening and X-ray Crystallography for Human Kallikrein 6 Inhibitors with an Amidinothiophene P1 Group ACS Med. Chem. Lett. 2012, 3, 159-164.

(8) Teixeira, T.S.P.; Freitas, R.F.; Abrahão, O. Jr.; Devienne, K.F.; de Souza, L.R.; Blaber, S.I.; Blaber, M.; Kondo, M.Y.; Juliano, M.A.; Juliano, L.; Puzer, L. Biological evaluation and docking studies of natural isocoumarins as inhibitors for human kallikrein 5 and 7. Bioorg. Med. Chem. Lett. 2011, 21, 6112–6115.

(9) Freitas, R.; Teixeira, T.S.; Barros, T.G.; Santos, J.A.; Kondo, M.Y.; Juliano, M.A.; Juliano, L.; Blaber, M.; Antunes, O.A.; Abrahão, O. Jr.; Pinheiro, S.; Muri, E.M.; Puzer, L. Isomannide derivatives as new class of inhibitors for human kallikrein 7. Bioorg Med Chem Lett. 2012, 22, 6072-6075.

(10) Barros, T.G.; Zorzanelli, B.C.; Pinheiro, S.; de Brito, M.A.; Tanuri, A.; da Costa, E.C.B.; Mohana-Borges, R.S.; Rodrigues, C.R.; de Souza, A.M.T.; Ferreira, V.F.; Muri, E.M.F. Novel Peptide Mimetics Based on N-protected Amino Acids Derived from Isomannide as Potential Inhibitors of NS3 Serine Protease of Hepatitis C Virus. Let. Org.Chem. 2012, 9, 239-249.

(11) Debela, M.; Goettig, P.; Magdolen, V.; Huber, R.; Schechter, N.M.; Bode, W. Structural basis of the zinc inhibition of human tissue kallikrein 5. J. Mol. Biol. 2007, 373, 1017-1031.

(12) Morris, G. M.; Huey, R.; Lindstrom, W.; Sanner, M.F.; Belew, R.K.; Goodsell, D.S.; Olson, A. J. AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. J. Comput. Chem. 2009, 16, 2785- 2791.

(13) Hou, T.; Wang J.; Li, Y.; Wang, W. Assessing the Performance of the MM/PBSA and MM/GBSA Methods. 1. The Accuracy of Binding Free Energy Calculations Based on Molecular Dynamics Simulations. J. Chem. Inf. Model. 2011, 51, 69-82.

(14) Case, D.A.; Darden, T.A.; Cheatham, T.E.; Simmerling, C.L.; Wang, J.; Duke, R.E.; Luo, R.; Walker, R.C.; Zhang, W.; Merz, K.M.; Robertson, B.; Wang, B.; Hayik, S.; Roitberg, A.; Seabra, G.; Kolossvary, I.; Wong, K.F.; Paesani, F.; Vanicek, J.; Liu, J.; Wu, X.; Brozell, S.; Steinbrecher, T.; Gohlke, H.; Cai, Q.; Ye, X.; Wang, J.; Hsieh, M.J.; Cui, G.; Roe, D.R.; Mathews, D.H.; Seetin, M.G.; Sangui, C.; Babin, V.; Luchko, T.; Gusarov, S.; Kovalenko, A.; Kollman, P. A. AMBER 11; University of California: San Francisco, 2010.

(15) Valiyaveetil, F.I.; Sekedat, M.; MacKinnon, R.; Muir, T.W. Structural and functional consequences of an amide-to-ester substitution in the selectivity filter of a potassium channel. J. Am. Chem. Soc. 2006, 128, 11591-11599.

(16) Choudhary, A.; Raines, R.T. An Evaluation of Peptide-Bond Isosteres. Chembiochem 2011, 12, 1801-1807.

(17) Velazquez-Campoy, A., Todd, M. J.; Freire, E. HIV-1 protease inhibitors: enthalpic versus entropic optimization of the binding affinity. Biochemistry, 2000, 39, 2201-2207.

(18) Parish, C.A.; Yarger, M.; Sinclair, K.; Dure, M.; Goldberg, A. Comparing the conformational behavior of a series of diastereomeric cyclic urea HIV-1 inhibitors using the low mode:monte carlo conformational search method. J. Med. Chem. 2004, 47,4838-4850.

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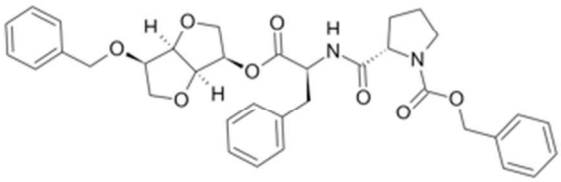

6
KLK5
$K_i = 0.4\ \mu M$
KLK7
$K_i = 2.8\ \mu M$