Supporting information

Towards the first class of suicide inhibitors of key kallikreins involved in skin diseases

Xiao Tan, Feryel Soualmia, Laetitia. Furio, Jean-François Renard, Isabelle Kempen, Lixian Qin, Bernard Pirotte, Maurice Pagano, Chahrazade El Amri, Alain Hovnanian, Michèle Reboud-Ravaux

Table of contents

Figure legends

Figure 1S. Inhibition of kallikreins by selected coumarin derivatives after 15 min incubation at pH 8.0 and 37°C. Inhibition of KLK5 (A) and KLK7 (B) by compound **4d** and KLK5 by compound **56** (C). $[KLK5]_0$ = 0.6 nM; $[Boc\text{-}VPR\text{-}AMC]_0$ = 100 μM; $[KLK7]_0$ = 7.6 nM; $[Succ\text{-}LLVY\text{-}AMC]_0$ = 40 μM. Experimental points were fitted to equation 2.

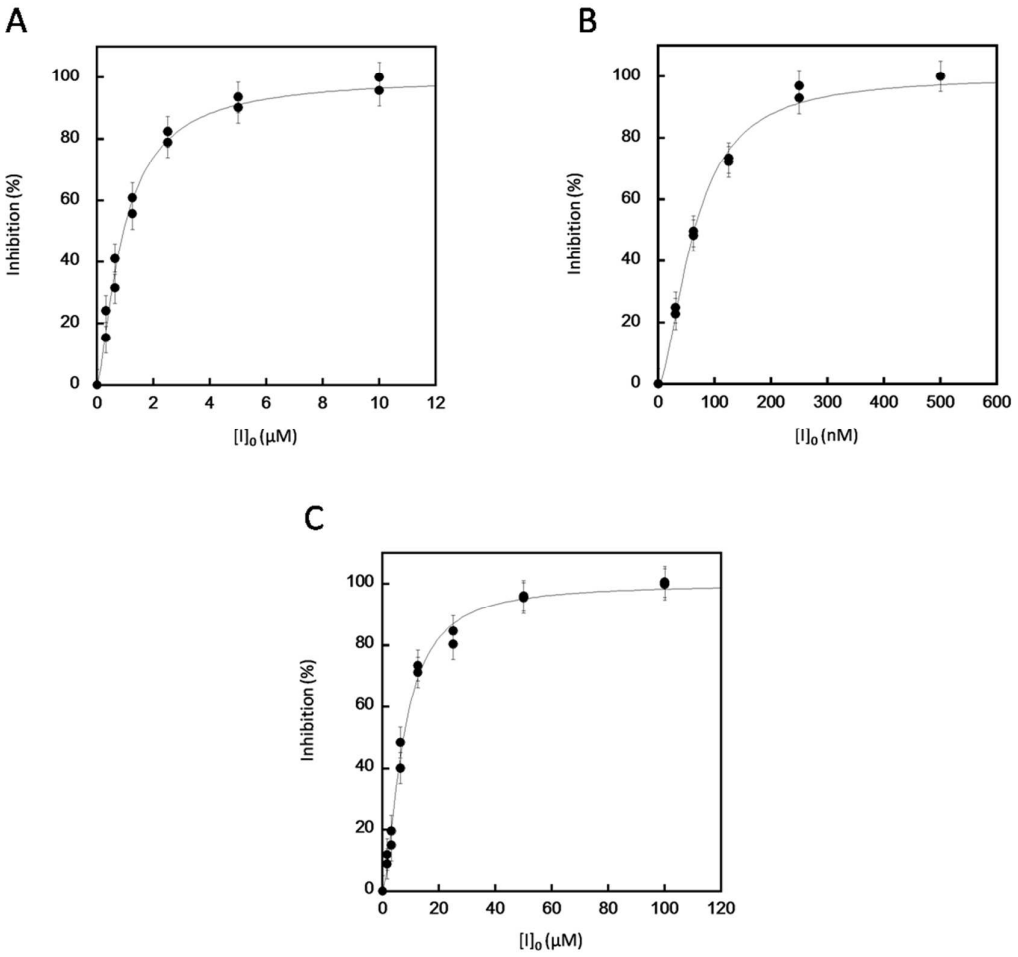
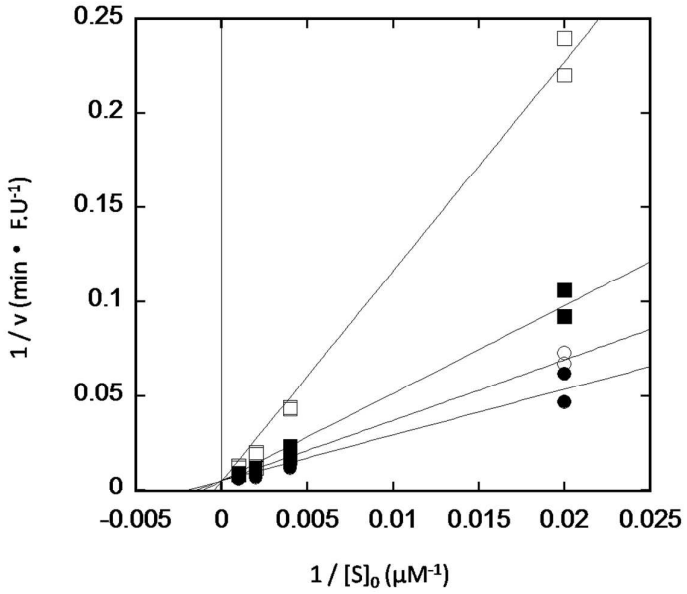


Figure 2S. Mechanism of inhibition of KLK5 by compound **56**. Lineweaver and Burk plot obtained at pH 8.0 and 37 °C. $[E]_0$ = 0.6 nM; $[I]_0$ = 50 μM (□); 20 μM (■); 10 μM (○); 0 (●). F.U. = arbitrary fluorescence unit.



Human samples

For experiments using human samples, we obtained written informed consent from healthy donors in the context of a protocol approved by the local CPP Ile de France VI committee (N° 2013-A00275-40).

Molecular docking specification

The binding of selected bioactive molecules within the enzyme active sites were carried out using the docking engine CLC Drug Discovery Workbench version 1.0.2. The enzyme structures were retrieved from PDB (file 2PSX for KLK5[1], file 2QXH for KLK7[2] and file 1EAX for matriptase[3]). In all cases, ligands structures (leupeptin for KLK5, succinyl-Ala-Ala-Pro-Phe-chloromethylketone for KLK7 and benzamidine for matriptase), and heteroatoms were removed from the crystal. Hydrogen atoms were added. The binding pocket around the catalytic triad residues was calculated by 'Find Binding Pockets' tool (CLC Drug Discovery Workbench 1.0.2). The scoring function $PLANTS_{PLP}$ search algorithm was used.[4] The coumarin three-dimensional structures were generated by Balloon program[5] and flexible docking calculations were performed (100 iterations for each ligand). Up to 50 poses were subsequently analyzed. Figures were prepared with Pymol (DeLano Scientific LLC, San Carlos, CA, USA).

References of supporting information

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(2) Debela, M.; Hess, P.; Magdolen, V.; Schechter, N. M.; Steiner, T.; Huber, R.; Bode, W.; Goettig, P. Chymotryptic specificity determinants in the 1.0 A structure of the zinc-inhibited human tissue kallikrein 7. *Proc. Natl. Acad. Sci. U. S. A.* **2007***, 104*, 16086-16091.

(3) Friedrich, R.; Fuentes-Prior, P.; Ong, E.; Coombs, G.; Hunter, M.; Oehler, R.; Pierson, D.; Gonzalez, R.; Huber, R.; Bode, W.; Madison, E. L. Catalytic domain structures of MT-SP1/matriptase, a matrix-degrading transmembrane serine proteinase. *J. Biol. Chem.* **2002***, 277*, 2160-2168.

(4) Korb, O.; Stutzle, T.; Exner, T. E. Empirical scoring functions for advanced protein-ligand docking with PLANTS. *J. Chem. Inf. Model.* **2009***, 49*, 84-96.

(5) Vainio, M. J., Johnson, M.S. Generating Conformer Ensembles Using a Multiobjective Genetic Algorithm *J. Chem. Inf. Model.* **2007***, 47*, 2462-2474.