Supporting Information

Identification of new specific snake venom metalloproteinase inhibitors using compound screening and rational peptide design

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Experimental section

Materials

BaP1 was isolated from *B. asper* venom as previously described.[1] Solvents and reagents were purchased from commercial suppliers and were used without further purification. Batimastat was purchased from Tocris Bioscience. Analytical RP-HPLC separations were performed on Discovery BIO Wide Pore C8 column (25 cm x 10.0 mm), using an Agilent Serie 1200 HPLC system equipped with a UV detector. 0.1% TFA/water and 0.1% TFA/acetonitrile were used as the mobile phase. The fluorogenic substrate Abz-Ala-Gly-Leu-Ala-Nba was purchased from Bachem.

Inhibition of proteolytic activity

Inhibition of the proteolytic activity of BaP1 was assessed using a fluorogenic substrate Abz-Ala-Gly-Leu-Ala-Nba; λex/em= 340/460 nm.[2] The assays were performed in black flat-bottom polypropylene 96-well plates (PerkinElmer) using 200 µL total reaction volumes. The samples were buffered in 0.02 M Tris, pH 7.5, supplemented with 0.02 M $CaCl_2$ and 0.15 M NaCl. BaP1 (1.7 µM) was incubated for 30 min at 37 ºC in the presence of inhibitor compounds. Batimastat (final concentration in the assay: 2.3 µM) was used as a positive control of inhibition. It was dissolved in 20% DMSO and 0.01% Tween-20 as stock solution (46 µM). Negative controls consisted of BaP1 incubated without inhibitors. Then, the fluorogenic substrate was added at a final concentration of 85 µM. The reaction was monitored in a Perkin Elmer Victor 2 microplate reader station. Assays were performed at least in triplicate. Statistical analysis and non-linear regression were performed using Prism 5.0 (GraphPad Software, Inc.).

Screening of the Prestwick Chemical Library

We used the Prestwick Chemical Library composed of 880 compounds. The compounds were assayed at a concentration of 100 µM in a buffer containing 0.02 M Tris pH 7.5, 0.02 M $CaCl_2$, 0.15 M NaCl, 2% DMSO and 1.7 µM BaP1. The library compounds and the enzyme were preincubated in 96-well microplates for 30 min at 37 °C under stirring. Thereafter, the fluorogenic substrate was added to the incubation mixture at 85 µM and measurements were performed in a Perkin Elmer Victor 2 microplate reader station, as

described above. For molecules exerting inhibition, a ten-point dose-response analysis was performed in order to estimate the Median Inhibitory Concentration (IC_{50}), i.e. the concentration at which the activity of BaP1 was reduced by 50%. Assays were performed at least in triplicate. Statistical analysis and non-linear regression were performed using Prism 5.0 (GraphPad Software, Inc.)

Peptide synthesis

N-Fmoc-hydroxylamine 2-chlrotrityl resin (Glycopep) and Fmoc-protected Rink amide linker AM RAM (IRIS Biotech GmbH) were used for the synthesis of hydroxamate peptides and non-hydroxamate peptides, respectively. The synthesis was carried out manually in ‘‘tea-bags’’, using Fmoc chemistry. Peptides were purified from the crude reaction mixture by semi-preparative HPLC using Discovery BIO Wide Pore C8 column (25 cm x 10.0 mm), using an Agilent Serie 1200 HPLC system equipped with a UV detector. CH_3CN-H_2O mixtures containing 0.1% TFA as mobile phases and a flow rate of at 5 mL/min were used.

Peptide affinity towards zinc

The zinc-binding assay was carried out using PCA-1 as described earlier[3] with some modifications. PAC-1 was weighed and exact concentration in buffer A (50 mM Hepes, 100 mM KNO_3, pH 7.4) was spectrophotometrically determined at 405 nm.[3] Functionalized tripeptides (see text) at 300 μM in buffer A were incubated for 30 min in the presence of 1.1 mM $ZnSO_4$ solution. Then, 25 μL of 88 μM of PAC-1, in buffer A, were added, followed by mixing and further incubation for 30 min at room temperature. The amount of PAC-1 bound to zinc was determined by measuring the absorbance at 405 nm.

Docking with BaP1

Protein-docking was performed with GOLD (Cambridge Crystallographic Data Centre, version 3.0.1). The high-resolution crystal structure of BaP1 complexed with a peptidomimetic was used (2W12 deposited in the RCSB Protein Data Bank; the ligand and waters were removed). The ligands were drawn in Hyperchem V6.0. The active site of the protein was defined using the co-crystallized ligand. The docking calculation was stopped

if the best 10 results had a R.M.S.D of 1.5 Å and the ratio of the active site was 10 Å. The "GOLDScore" was activated with a hydrogen bond of 4 Å and van der Waals 2.5. In the panel "Genetic Algorithm Parameters" the Population Size was 100, the Selection Pressure 1.1, the No. of Operations 100,000, the No. of Islands 5, the Nich size 2, the Migrate 10, the Mutate 95, and the Crossover 95.

References

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