Designed EVH1 inhibitors derived from synthetic diproline mimetics (ProMs) rigidified in a PPII helix conformation

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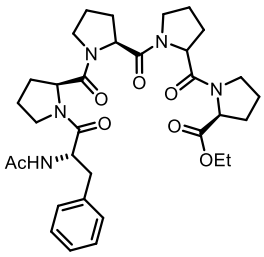
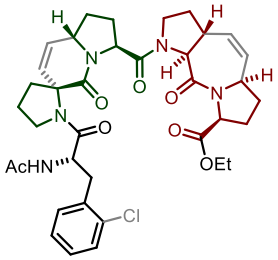
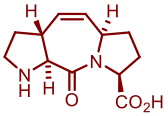
We combined computational modeling, X-ray crystallography, organic synthesis and biological investigations to develop small-molecule inhibitors for the Ena/VASP EVH1 domain, which is involved in the migration and chemotaxis (metastasis) of invasive cancer cells.[1] Using synthetic diproline mimetics such as **ProM**-1,[2a] **ProM-2**,[2b] **ProM-13**[2c] and **ProM-15**[2d] we succeeded to reduce the wild-type oligopeptide sequence **WT1** to an equivalent of the pentapeptidic proline rich core sequence **WT2**, which itself only shows weak binding. By freezing pairs of prolines in a PPII helix conformation (by formal introduction of a vinylidene bridge) in combination with the introduction of a chlorine atom to the phenylalanine residue, the first nanomolar EVH1 inhibitors (**L1**-**L3**) were obtained.[1]



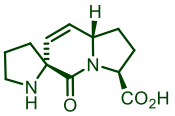
Ac-**FPPPP**-OEt



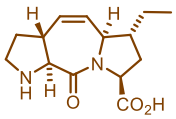
Ac-**[2-Cl-F][ProM-2][ProM-1]**-OEt



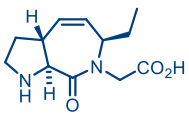
H**[ProM-1]**OH



H**[ProM-2]**OH



H**[ProM-13]**OH



H**[ProM-15]**OH

Ligand	Ligand composition	K_d [µM]	ΔG [KJ mol^{-1}]
WT1	Ac-SFE-**FPPPP**-TEDEL-NH_2	13.0 ± 0.6	-27.9 ± 0.1
WT2	Ac-**FPPPP**-OEt	153 ± 8	-21.9 ± 0.1
L1	Ac-**[2-Cl-F][ProM-2][ProM-1]**-OEt	4.1 ± 0.3	-30.8 ± 0.2
L2	Ac-**[2-Cl-F][ProM-2][ProM-15]**-OMe	0.47 ± 0.03	-36.1 ± 0.1
L3	Ac-**[2-Cl-F][ProM-2][ProM-13]**-OEt	0.12 ± 0.01	-39.5 ± 0.3

Besides exhibiting strong affinity, the optimized inhibitors were shown to bind to the EVH1 domain in the canonical fashion. While *in vitro* cell migration experiments proved the inhibition of chemotaxis, the extravasation of MDA-MB-231 cancer cells was demonstrated *in vivo* using transgenic zebrafish embryos.[1b] Notably, the compounds showed high selectivity towards EVH1 in comparison to other proline-rich motif-recognizing (off-target) protein domains (Fyn SH3, Profilin, Yap1 WW1) as revealed by $^{1}H^{15}N$ HSQC NMR experiments.

References:

[1] (a) R. Opitz, M. Müller, C. Reuter *et al*., *Proc. Natl. Acad. Sci. U.S.A*. **2015**, *112*, 5011-5016; (b) M. Barone, M. Müller, S. Chiha *et al*., *Proc. Natl. Acad. Sci. U.S.A.* **2020**, *117*, 29684–29690.

[2] (a) J. Zaminer, C. Brockmann, P. Huy *et al*., *Angew. Chem. Int. Ed.* **2010**, *49*, 7111–7115; (b) C. Reuter, R. Opitz, A. Soicke *et al*., *Chem. Eur. J.* **2015**, *21*, 8464–8470; (c) S. Chiha, A. Soicke, M. Barone *et al*., *Eur. J. Org. Chem.* **2018**, *2018*, 455–460; (d) S. Dohmen, M. Reiher, D. Albat *et al*., *Chem. Eur. J.* **2020**, *26*, 3049–3053.