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Unveiling Activity Determinants of ^{10}Panx1

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Introduction

Pannexin1 channels are heptameric conduits that allow the passage of ions and other molecules such as adenosine triphosphate (ATP) across the cell plasma membrane, thereby playing a role in multiple pathophysiological responses linked to inflammation [1,2]. Although pannexin1 channels are seen as highly interesting drug targets, the search for appropriate blockers is an arduous process [3,4]. As one of the known inhibitors' main problems is specificity, the pannexin1 field turned to peptides [5,6]. To this date, a peptide mimicking a ten amino-acid long sequence of the first extracellular loop of the pannexin1 protein, named ^{10}Panx1, remains one of the most commonly used pannexin1 channel inhibitor *in vitro*, yet is not fit for *in* vivo purposes because of poor stability [7,8]. The goal of this study was to identify important amino acid side chains of the lead sequence involved in the peptide protein interaction and to pinpoint scissile amide bonds in order to enable a more rational approach for the optimization of the sequence.

Results and Discussion

Important side chains were uncovered through the synthesis and *in vitro* testing of an Ala scan of the ^{10}Panx1 sequence (Figure 1). All analogs were obtained *via* Fmoc-solid phase peptide synthesis. Subsequently, the peptides were evaluated in an *in vitro* pannexin1 channel assay, in which their capacity to reduce pannexin1-mediated extracellular ATP release was compared with that of the lead sequence, after opening the channels through osmotic shock. Both ^{10}Panx1 and lanthanum, another well-known inhibitor of pannexin1 channel activity, were included in the assays as controls. *In vitro* testing of the Ala scan showed that replacing glutamine at position three (compound 9) and aspartic acid at position eight (compound 6) of the sequence abolished ^{10}Panx1's inhibitory potency significantly (Figure 1). Precipitation was observed in the case of the peptide in which glutamine was substituted by alanine. Hence, a more soluble analog was synthesized and compared against the respective control (Figure 1).

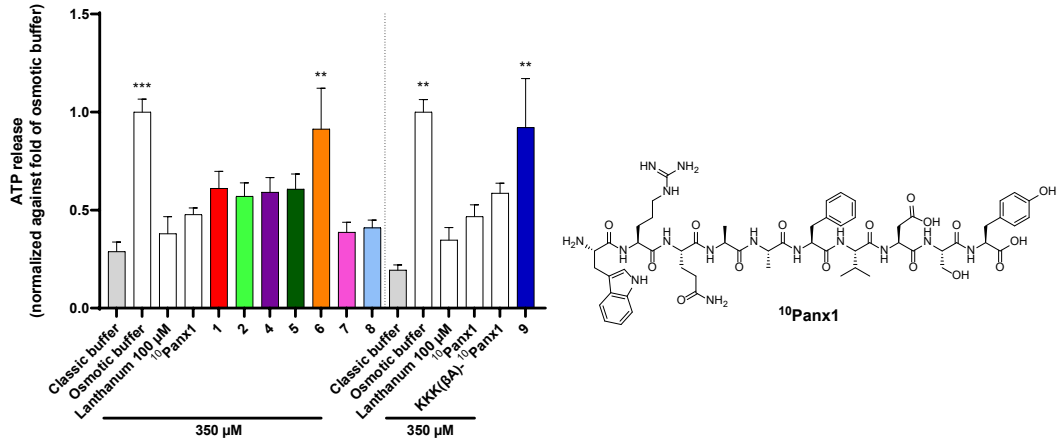

Fig. 1. Structure of the lead sequence ^{10}Panx1 and evaluation of ^{10}Panx1 and analogs.

Despite the many advantages of peptides as therapeutics, one of the major drawbacks in the field is proteolytic instability [9]. ^{10}Panx1, as an unmodified linear peptide, is highly prone to be cleaved by different proteases, as reflected by a half-life of merely a few minutes in human plasma. Well-established techniques such as terminal modification and chiral switches were used to increase proteolytic stability. Both acetylation of the *N*-terminus (compound 12) and *C*-terminal amidation (compound 11) on their own as well as the combination of these two modifications (compound 13) did not alter the inhibitory activity (Figure 2). The latter did however increase the half-life by almost a ten-fold (Figure 2). Chiral switches of the tryptophan created a loss in activity (compound 14), while the opposite holds true for the tyrosine at the *C*-terminus (compound 16). Combining the terminal modifications with the integration of a D-tyrosine (compound 16) increased the plasma half-life up to more than a 20-fold, in comparison to the $t_{1/2}$ of the lead sequence (Figure 2). Additionally, the all D-amino acid containing sequences with (compound 17) and without (compound 10) terminal modifications did not show any loss of activity in the pannexin1 channel assay (Figure 2). In conclusion, we have obtained fully stable ^{10}Panx1 analogs with a similar inhibitory activity as the lead sequence.

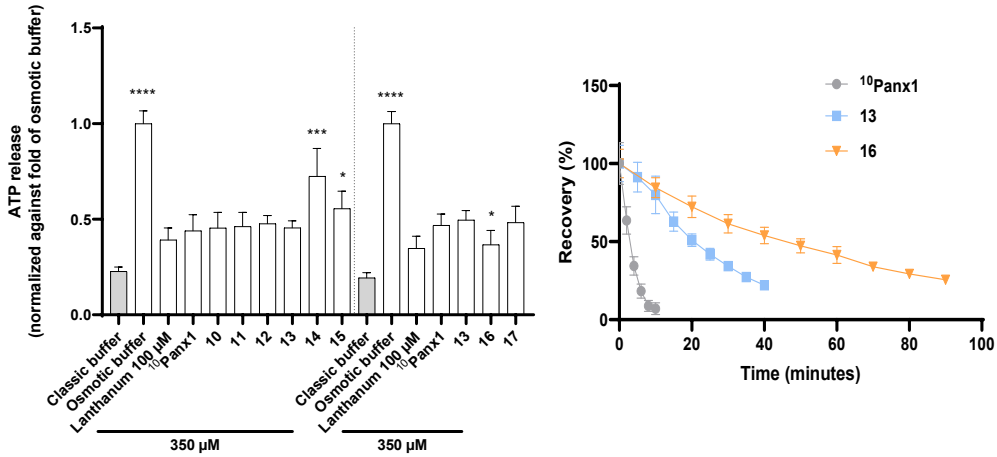

Fig. 2. Inhibitory activity and plasma stability testing of stabilized ^{10}Panx1 analogs.

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References

1. Jin, Q., et al. *Cell Research* **30**, 449-451 (2020), https://doi.org/10.1038/s41422-020-0310-0
2. Koval, M., et al. *Purinergic Signalling* **17,** 521-531 (2021), https://doi.org/10.1007/s11302-021-09804-8
3. Dahl, G., et al. *Neuropharmacology* **75**, 583-593 (2013), https://doi.org/10.1016/j.neuropharm.2013.02.019
4. Willebrords, J., et al. *Pharmacology & Therapeutics* **180**, 144-160 (2017), https://doi.org/10.1016/j.pharmthera.2017.07.001
5. Pelegrin, P., and Surprenant, A. *EMBO journal* **25**, 5071-5082 (2006), https://doi.org/10.1038/sj.emboj.7601378
6. Caufriez, A., et al. *Expert Opinion on Drug Discovery* **15**, 1213-1222 (2020), https://doi.org/10.1080/17460441.2020.1773787
7. Pelegrin, P., and Surprenant, A. *J.Biol.Chem.* **282**, 2386-2394 (2007), https://doi.org/10.1074/jbc.M610351200
8. Maes, M., et al. *Archives of Toxicology* **91**, 2245-2261 (2017), https://doi.org/10.1007/s00204-016-1885-6
9. Muttenthaler, M., et al. *Nature Reviews Drug Discovery* **20**, 309-325 (2021), https://doi.org/10.1038/s41573-020-00135-8