CASE STUDY: **SELECTIVE INHIBITORS FOR ONE OF SIX ION CHANNEL ISOFORMS**

RECEPTOR.AI

BACKGROUND:

Challenging target: The family of highly similar ion channel isoforms, only one of which have to be targeted to achieve the tissue-specific therapeutic effect. The exact location of the most favourable binding pocket for achieving selectivity is unknown.

Goal: Design of highly selective inhibitors against 6 ion channel isoforms.

METHODOLOGY:

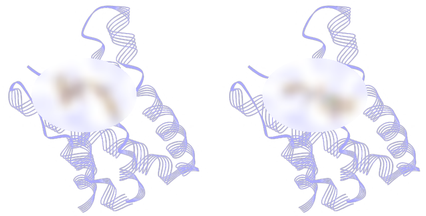
- Three tentative binding sites were identified for each protein isoform by proprietary Receptor.AI **pocket detection workflow:** in the outer channel pore, inside the channel cavity and between the functionally important transmembrane helices.
- Selectivity assessment based on differential pocket pharmacophore's representation combined with generative AI binding pose prediction was used.
- Pre-filtered stock chemical space of 662K compounds was used as well as custom focused diversity database of 50K compounds.
- 291 compounds were selected for experimental validation.

662K compounds screened	**291** hit candidates selected	**>40%** hit rate	**5** selective compounds (>x2 selectivity)	**3 compounds active *in vivo*** (including 1 highly potent)

RESULTS:

Three metrics were used:

1. **Fold increase** of effect on target isoform in comparison to off-target isoforms.
2. **UFD effect:** the preference of the compound to block the active channel state relative to the resting state.
3. **Peak blocking** of the target channel at 120 μM of compound relative to vehicle.



Binding mode of hit compounds

Hit compounds:

- The best compound have shown **x3.16 selectivity** against the target isoform.
- Top 5 compounds demonstrate **>x2 selectivity** and specificity to active state of the target channel, which demonstrates correct mechanism of action against the active channel conformation.
- Top 10 compounds are attributed to all **three** binding pockets.

IN VIVO VALIDATION:

- The top 3 compounds was **tested for activity *in vivo*** (murine model) and are proven to be active (including a **highly potent one**) and non-toxic. (*in vivo* tests of other compounds are ongoing).

Compound	Fold Increase (isoform selectivity)	UFD effect (conformation selectivity)	Peak blocking (relative to vehicle)	Max similarity to training set (all isoforms)	Max similarity to ChEMBL (v.33)	Max similarity to Murcko assemblies of training set (all isoforms)	Max similarity to Murcko assemblies of ChEMBL (v.33)	Comments
#1	3.6	4.69	0.08	0.12	0.34	0.13	0.43	Completely new class
#2	3.08	5.98	0.07	0.23	0.67	0.39	1	New class for target of interest
#3	2.65	2.22	0.24	0.21	0.48	0.34	1	New class for target of interest
#4	2.57	3.43	0.17	0.18	0.41	0.3	0.56	New class for target of interest
#5	2.32	3.53	0.15	0.2	0.71	0.27	0.82	Completely new class
#6	1.84	8.07	0.04	0.22	0.5	0.26	1	New class for target of interest
Competitor #1	1.42	1.99	0.35					
Competitor #2	1.23	1.4	0.05					

*Similarity was calculated as Tanimoto similarity between Morgan fingerprints, 3 radius.