The use of PathHunter cell lines to discover modulators of Trk-receptors. From HTS to phase 1.

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Introduction

The neurotrophins, including nerve growth factor (NGF) and brain derived neurotrophic factor (BDNF), regulate neuronal functions such as survival, differentiation and plasticity. Decreased NGF-signalling in the central nervous system (CNS) contributes to the dysfunction of basal forebrain cholinergic neurons during Alzheimer's disease (AD) whereas increased BDNF signalling has been demonstrated to mediate improved synaptic plasticity.

In the peripheral nervous system (PNS), NGF is well known to mediate pain sensation. The role of NGF/TrkA in pain signalling is well validated by human genetics and anti-NGF antibody therapies have demonstrated pharmacological validation of the NGF/TrkA pathway.

The large unmet medical need in AD strongly supports the development of positive modulators of neurotrophin signalling as cognitive enhancers for the treatment of AD.

Additionally, targeting the TrkA receptor with a selective negative allosteric modulator (NAM) with low blood-brain permeability might offer a more selective and safer way of disrupting the NGF signalling than anti-NGF antibodies.

Methods

Active compounds were identified by screening a compound library using the U2OS-hTrkA/SHC1-p75 cell line as described (fig. 1). After completion of the screen, a total of 756 compounds were re-screened at two different concentrations of NGF (1 and 10 ng/mL) and at three different concentrations of compound (3, 10 and 30 µM).

Lead optimization of different chemical series led to the discovery of novel TrK-PAM or TrkA-NAM molecules (fig. 1).

The effects of Trk-PAM molecules were investigated in different in vivo behavioural models including old animals with a reduced cognitive capability.

In vivo analgesic effects of TrkA-NAM molecules were assessed in two different rat models, the oxaliplatin-induced painful neuropathy model and in the Complete Freund's adjuvant (CFA)-induced arthritis model.

Results

We identified hits with three different modes of action (fig. 2). The Trk-PAM's act by binding to the intracellular domain of Trk's, thereby increasing the catalytic efficiency (fig. 2, lower panel). The optimization of lead series E led to the identification of potent and selective TrkA-NAM molecules (fig. 3).

Interestingly, the modulation of the Trk-receptors was for both Trk-PAM's and TrkA-NAM's dependent on the full-length receptor (fig 4) since correct modulation was not observed when using the intracellular kinase domain.

Results, cont.

After identifying the mechanism of action of both Trk-PAM's and TrkA-NAM's, we performed in vivo efficacy experiments for both classes of compounds.

Pan-Trk-PAM compounds, here exemplified by ACD856, were able to improve long-term memory in old animals (20 months of age). As shown in figure 5, there was a trend to improved memory already 24 hours after administration of ACD856 and the training session. The improvement of memory in ACD856 treated old animals was statistically significant after the second measurement, which occurred 11 days after administration of ACD856.

The pharmacokinetic data of ACD856 from clinical phase 1 single and multiple ascending dose studies in healthy volunteers have shown a rapid absorption, high bioavailability (~100% in comparison with i.v. microdose data), a terminal half-life of approx. 20 hours and a linear dose-dependent exposure.

The TrkA-NAM molecule AC-0027496 has been tested in the CFA-induced arthritis model (fig. 6, left graph) and in a model of chemotherapy-induced neuropathic pain, i.e the rat oxaliplatin-induced neuropathy model (fig. 6, right graph). Analgesic efficacy was observed already at the lowest dose tested, 3 mg/kg. The highest dose tested (40 mg/kg) demonstrated similar efficacy as 100 mg/kg of Duloxetine. No acute CNS-related side effects were observed at any dose tested as judged by the Irwin test

Figure 1. PathHunter assay and chemical series derived from HTS

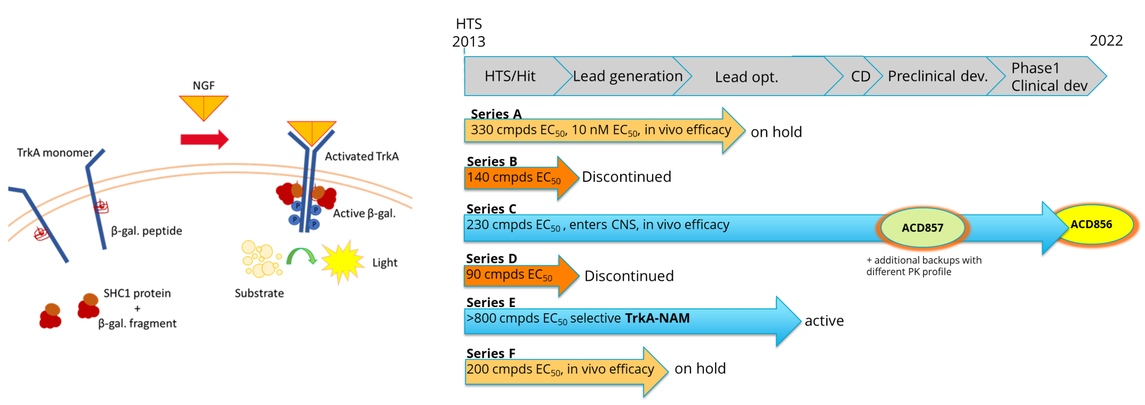


Figure 2. Mechanism of action of HTS-hits and ACD856

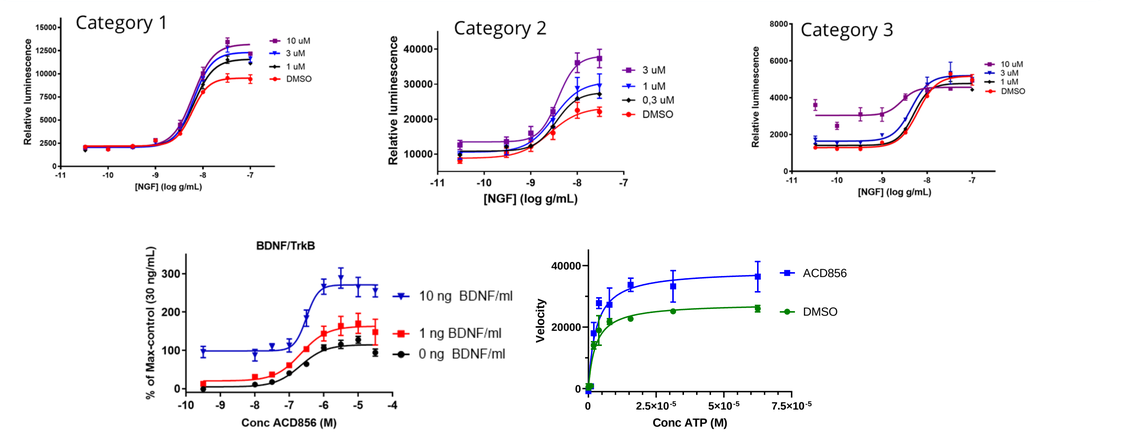
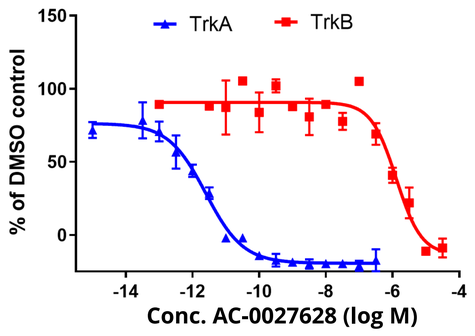


Figure 3. Effects of a TrkA-NAM on TrkA or TrkB and a comparison to known TrkA-NAM molecules



Compound	TrkA IC50 (nM)	TrkB IC50 (nM)	Ratio TrkA vs TrkB
AC-0027628	0.0025	1800	720,000
Array #10	0.037	211	5,700
Pfizer #23	0.640	145	226

Figure 4. Effects of Trk-modulators in cell-based assay or in a biochemical kinase assay

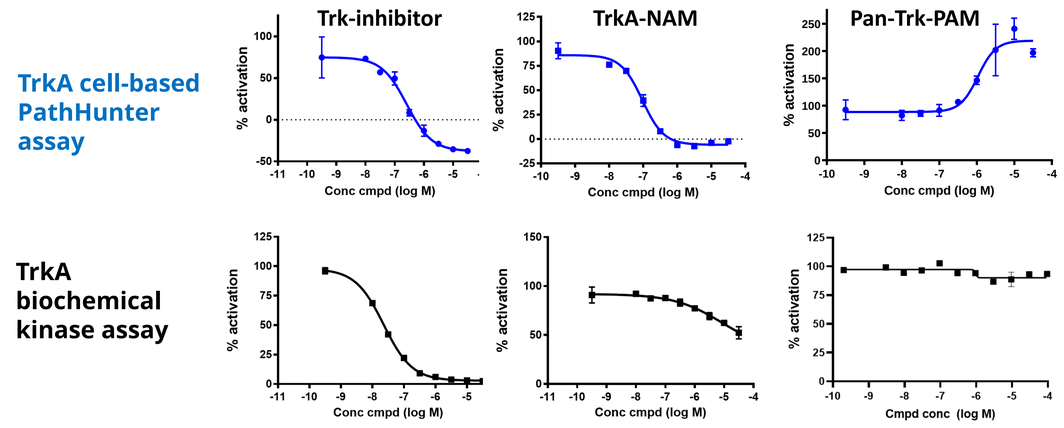


Figure 5. Effects of a pan-Trk-PAM (ACD856) on long-term memory in 20-months old mice

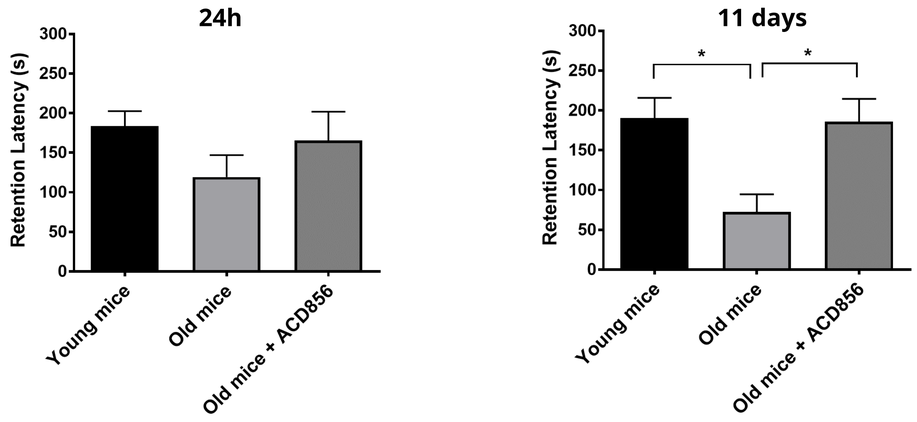
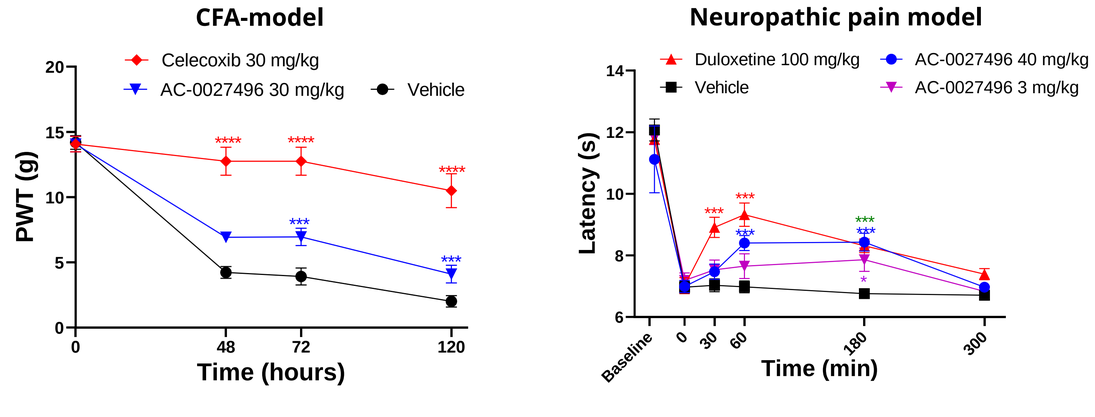


Figure 6. Effect of a TrkA-NAM molecule in a model of neuropathic or nociceptive pain in rats



Conclusion

We have demonstrated that the PathHunter cell-based assay is a suitable assay for identifying compounds that act as allosteric modulators on Trk-receptors. The use of biochemical kinase assay using the intracellular domain could not replicate the findings from the cell-based assay.

We have identified ACD856 as a novel pan-Trk-PAM enhancing cognitive function, suggesting its use for treating cognitive dysfunction in AD, and currently in clinical studies.

The NGF/TrkA pathway is a well validated and a promising alternative for new analgesics without the side effects and dependency issues observed with opioids or the side-effects observed for anti-NGF antibodies, and we have identified compounds showing analgesic effects in both preclinical neuropathic and nociceptive pain models.

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