Negative Allosteric Modulators of TrkA for the Treatment of Pain

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Background

Chronic pain is currently treated by conventional therapies e.g., NSAIDs, opioids and selective COX2 inhibitors. Unfortunately, these therapies are associated with gastrointestinal and cardiovascular side effects as well as a risk for addiction and development of tolerance even with short-term use of opioids. A potential novel analgesic mechanism involves blocking the activity of the pain mediator nerve growth factor (NGF) or its receptor TrkA. NGF plays a key role in pathophysiological processes in several human neuropathies and its role in pain sensation in patients with osteoarthritis has been extensively investigated [1]. Anti-NGF antibody therapies in clinical development have demonstrated pharmacological validation of the NGF/TrkA pathway in pain sensation [2]. However, a small number of these patients developed rapidly progressing osteoarthritis.

Targeting the TrkA receptor with a small molecule negative allosteric modulator (NAM) with low blood-brain permeability might offer a more selective way of disrupting the NGF signalling than anti-NGF antibodies

Methods

Active compounds were identified using a previously described cell-based assay from DiscoveRx [3]. In vitro metabolic stability was addressed using mouse, rat or human liver microsomes. Pharmacokinetic properties were investigated by i.v. and p.o. administration of compounds to rats. In vivo analgesic effects 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

A series of potent and selective TrkA-NAMs were identified during a lead optimization program. During the optimization, a structure-activity relationship (SAR) was established. The lead optimization led to very potent and selective TrkA-NAM's with the most potent compound demonstrating an IC50 value of 2.5 pM and >700,000 fold selective for TrkA over TrkB (figure 1 and table 1).

Figure 1. Effects of AC-0027628 on TrkA or TrkB in cell-based assays

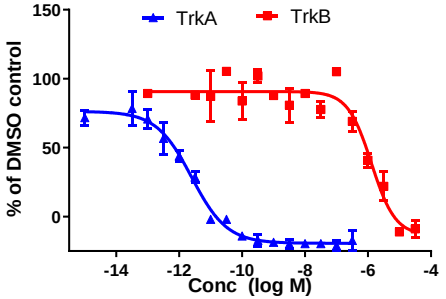

Figure 1. U2OS-TrkA/SHC1-p75 or U2OS-TrkB/SHC1-p75 cells were plated in a 384-well plate and incubated for 24 hours at 37°C/5% CO2. Cells were stimulated with NGF or BDNF (10 ng/ml) and incubated for 180 min with AC-0027628 at the indicated concentrations. Chemiluminescence was detected using the PathHunter® detection reagent provided in the kit.

Table 1. Effects of TrkA-NAM's in U2OS cell-based assays

Compound	TrkA IC50 (nM)	TrkB IC50 (nM)	Ratio
AC-0027628	0.0025	1800	720,000
Array #10 [4]	0.037	211	5,700
Pfizer #23 [5]	0.640	145	226

The selectivity towards TrkB was further evaluated in mouse cortical neurons. The TrkA-NAM compound AC-0026669 did not inhibit BDNF-induced phosphorylation of ERK, whereas a non-selective Trk-inhibitor showed similar potency in inhibition of phosphorylation of ERK as the inhibition observed with U2OS-cells overexpressing rat TrkA (fig 2). This clearly demonstrate that TrkA-NAM are selective also in more complex cellular models.

Figure 2. BDNF-induced phosphorylation of ERK in primary cortical neurons is not affected by a TrkA-NAM

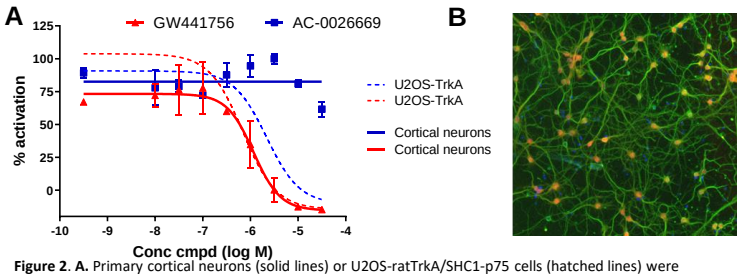

Figure 2. **A.** Primary cortical neurons (solid lines) or U2OS-ratTrkA/SHC1-p75 cells (hatched lines) were incubated with GW441756 or AC-0026669. Cortical neurons were stimulated with BDNF (10 ng/ml) for 15 minutes. Phosphorylated ERK was detected using anti-phospho ERK1/2 antibody (CS4370) in an automated microscopy. Results are expressed as total fluorescence intensity. **B.** Representative image of cortical neurons stained for nuclei (blue), tubulin (green) and phosphorylated ERK (red).

The lead optimization work included improving pharmacological potency and simultaneously improving the physicochemical and pharmacokinetic properties of the series. The efforts led to the discovery of AC-0027496 as a potent, selective, and orally bioavailable TrkA-NAM. The half-life in rats after oral administration is approx. 3 hours with a bioavailability of 30%.

AC-0027496 has been tested in the CFA-induced arthritis model (fig. 3) and in a model of chemotherapy-induced neuropathic pain, i.e the rat oxaliplatin-induced neuropathy model (fig. 4). 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.

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Figure 3. Effects of AC-0027496 or Celexocib on CFA-induced arthritic pain

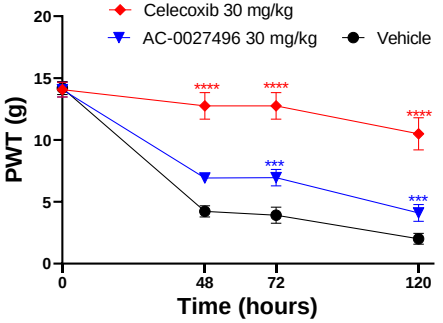

Figure 3. Complete Freund's adjuvant was administered in the intraplantar region of the left hind limb. Animals were thereafter dosed orally twice daily with vehicle (black line), AC-0027496 (30 mg/kg)(blue line). A third group of animals were dosed on day 2, 3 and 5 with Celecoxib on day 2, 3 or 5 (30 mg/kg) (red line). Paw withdrawal threshold (PWT) was evaluated by the use of Von Frey monofilaments.

Figure 4. Effect of a single oral administration of AC-0027496 in a rat model of oxaliplatin-induced cold allodynia in rats

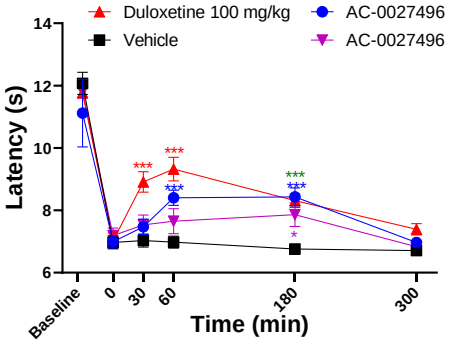

Figure 4. Intraperitoneal injection of Oxaliplatin 10 mg/kg was performed on day 0 (after baseline recording). Three days after induction, latency of hindpaw withdrawal was measured (pre-treatment baseline). Then, rats was treated with vehicle (blac), AC-0027496 (30 mg/kg (blue) or 3 mg/kg (magenta) or Duloxetine 100 mg/kg (red) and the paw withdrawal latency was measured after immersion of the hindpaw in a cryothermostat with a temperature fixed at 10°C (± 0.5°C) 30 min, 60 min, 180 min and 300 min after treatment.

Conclusion

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. Identification of selective and potent TrkA-NAM's could potentially avoid some of the side effects observed for anti-NGF antibodies or for non-selective Trk-inhibitors, while retaining the analgesic efficacy. We have in our lead optimization program identified very potent and selective TrkA-NAM's demonstrating analgesic efficacy in vivo. These compounds are suitable candidates for further development for the treatment of both nociceptive and neuropathic pain.

References

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