Development of small molecule antagonists of TrkA

Neurotrophins, including nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3) and neurotrophin-4 (NT-4) are a family of secreted growth factors that bind to their cell surface receptors as homodimers and lead to receptor dimerisation and intracellular signal transduction. Neurotrophins promote the survival, differentiation and maintenance of specific neuronal populations. Recent studies have also shown that neurotrophins have other important functions, including regulation of activity-dependent synaptic plasticity, stimulation of neurite outgrowth, and protection and repair of neurons during tissue injury. All neurotrophins bind to $p75^{NGFR}$ receptor but selectively interact with their individual high-affinity protein tyrosine kinase receptors of the Trk family (TrkA, TrkB and TrkC). NGF mediates majority of its biological effects via TrkA receptor.

Re-arranged constitutively active TrkA receptors are functioning as proto-oncogenes in several cancers, including colon carcinoma and thyroid papillary carcinomas. Overexpression of TrkA is also associated with the development of several cancers, including neuroblastoma, breast cancer and others. The expression of NGF is high in injured and inflamed tissues, and activation of its trkA receptor on nociceptive neurons triggers and potentiates pain signalling. Inhibition of NGF function and signalling blocks pain sensation both in neuropathic and inflammatory pain. In agreement with this, mutations in NGF and TrkA have been demonstrated in humans with insensitivity to pain. Since NGF has a crucial role in the development of cancer and generation of pain and hyperalgesia in several acute and chronic pain states, developing small molecule antagonists of TrkA receptor is a therapeutic strategy for the treatment of cancer and pain. BTD, LTd. Has aimed to develop novel efficient low molecular weight antagonistic compounds for TrkA receptors.

We have identified novel compounds CA1, CA2, CA3 and CA4 as NGF antagonists.

(1) These compounds inhibit the phosphorylation of TrkA by NGF (Fig. 1).

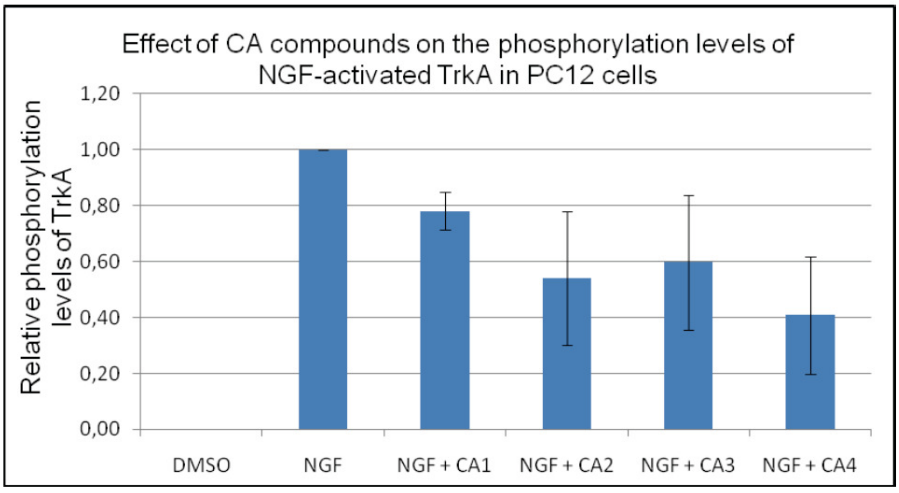

Figure 1. Effect of CA1, CA2, CA3 and CA4 on TrkA phosphorylation by NGF in PC12 cells. After the cells were treated with NGF (20 ng/ml) and different CA compounds (30 µM), the cells were lysed and the lysates immuno-precipitated with anti-phospho-tyrosine antibody. Precipitated proteins were analysed with Western blotting using anti-TrkA antibody. Shown is average of TrkA signal of three independent experiments.

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(2) These compounds inhibit the effect of NGF on the neuronal differentiation of PC12 cells (Fig. 2).

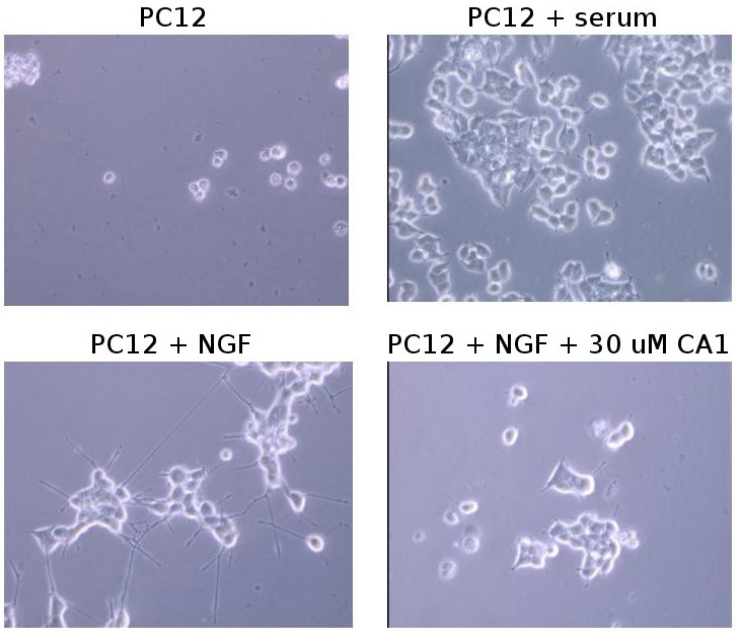

Figure 2. Effect of CA1 (30 μM) on the ability of NGF (20 ng/ml) to induce growth of neurite-like extensions of PC12 cells. Serum-starved PC12 cells (up, left) normally die, while addition of serum to the medium allows the cells to thrive and multiply. Serum-starved and NGF-activated PC12 cells stay alive and grow neurites, but addition of CA1 to the growth medium reduces NGF effect.

(3) Using computer simulations (blind docking), we predicted the interaction sites of CA1 with TrkA. We found a promising binding site of CA1 between TrkA and NGF (figure 3).

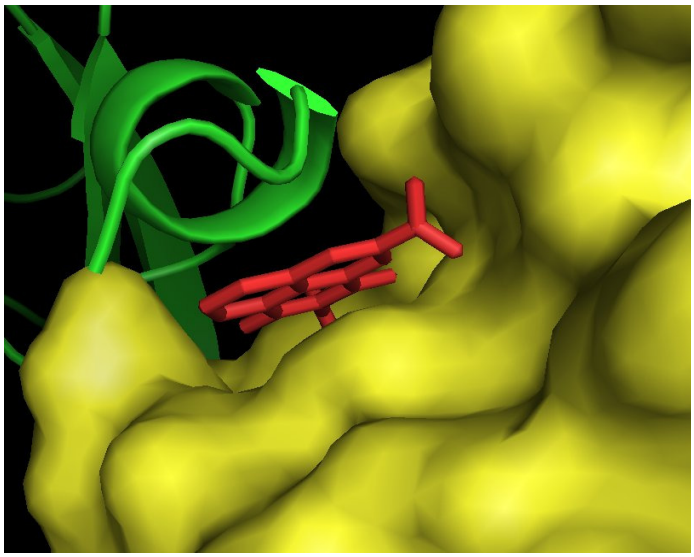

Figure 3. The predicted location of CA1 molecule (red) on TrkA receptor (yellow) – on the interface of TrkA and NGF (green) binding site.

Conclusion: CA1, CA2, CA3 and CA4 are good candidates as lead compounds for the development of TrkA antagonists.