Computational studies and cellular screening of new antitumor compounds for neuroblastoma therapy.

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Introduction

Neuroblastoma is the most common extracranial tumor in children and newborns. Many studies have shown that there is a link between the development of an aggressive form of neuroblastoma and the activation of receptor tyrosine kinase B (TrkB).[1] This finding triggered the research of Trk modulator with antitumoral activity. Nowadays, new molecules are at different stages of the drug discovery pipeline as Trk inhibitors. One of them, lestaurtinib, is under clinical investigation.

This work reports a bioactive compound, selected by means of cheminformatics tools that showed promising antitumoral activity against neuroblastoma cell lines.

Results and Discussion

The ligand-based virtual screening (LBVS) and structure-based virtual screening (SBVS) approaches were chosen to cherry pick new molecules with potential bioactivity in cell-based assays. To do so, known Trk inhibitors were gathered from the literature (Fig. 1) as well as the X-rays crystallographic structures available for the extracellular domain 5 of TrkA and TrkB (PDB codes: 2IFG and 1HCF, respectively).

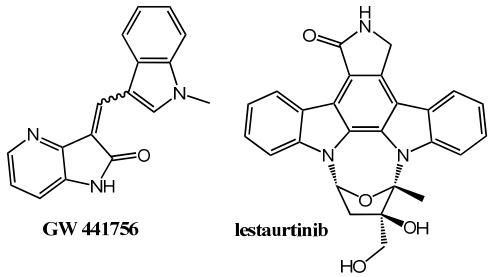

Figure 1. Chemical structures of some tyrosine kinase receptors inhibitors

Out of 5 million chemicals from eMolecules, 30 compounds were selected *in silico* to be tested *in vitro*. The computational process was composed by a set of 2D properties (FILTER, Openeye Inc.), 3D similarity (ROCS, Openeye Inc.) and docking into the extracellular domain for some compounds (FRED, Openeye Inc.).

Positive controls, like lestaurtinib, were tested *in vitro* together with the new molecules. The first one was the cell viability assay using the MTT method, followed by the dose-response study of the best molecule and the controls (Fig. 2).

Another screening was the cell cycle analyses with flow cytometry. Both compounds depicted cell arrest in sub-G1 phase at 10 μmol L^{-1} (Fig. 2) when compared with DMSO alone. Western Blotting for Trk phosphorylation and combined schemes of therapy with etoposide were also performed and they are going to be reported at the upcoming meeting.

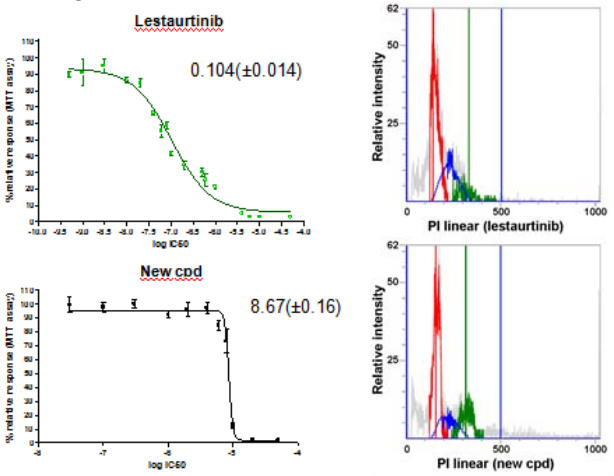

Figure 2. Comparative results of the new compound and lestaurtinib for the dose-response curves (μM) and cell cycle analyses.

Conclusions

The new compound is a hit that deserves further optimization in this drug discovery project. The next steps involve the identification of analogues and screening *in vitro* to determine a SAR; pharmacokinetics studies and *in vivo* tests using our mouse xenograft models.

Acknowledgements

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