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Combining pharmacology, target hopping and computational medicinal chemistry to discover novel scaffold targeting EPH-ephrin interaction

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EPH receptor tyrosine kinases and their membrane-bound ephrin ligands are involved in many biological processes as cell migration and morphology, synaptic plasticity and angiogenesis both during embryogenesis and in adult tissues. Alterations of this system have been found in human cancers and in particular, EPHA2 overexpression is often correlated with aggressive tumor phenotypes and poor prognosis. Therefore, EPH-ephrin targeting could represent a promising approach in targeted chemotherapy.

Pharmacology

Through an ELISA screening, we recently identified lithocholic acid (LCA) as a hit compound able to modulate EPH-ephrin activity and we synthetize and characterize a novel series of LCA derivatives acting as EPHA2 antagonists. UniPR129, the homotryptophan conjugator of lithocholic acid, resulted as the best compound within this series with a submicromolar Ki and an IC50 in the low micromolar range in functional experiments, including in vitro angiogenesis. Nevertheless, with the aim to improve both the selectivity and the poor physico-chemical properties of LCA derivatives, we turned out our attention to the search for alternative chemotypes targeting EPH system.

Target hopping

Given the ability of LCA to interact with farnesoid X receptor (FXR), G protein-coupled bile acid receptor (TGR5) and EPHA2 receptor we hypothesized that structural requirements for a small molecule to bind each of these receptors might be similar. The stilbene carboxylic acid GW4064 and the bile acid 6-ECDCA, were identified as new EPHA2 binders within a set of FXR and TGR5 ligands. In particular, GW4064 was slightly more potent than LCA both in binding and functional assays showing IC_{50}s of 23 and 46 micromolar, respectively. Interestingly, computational data obtained by docking compounds into the EPHA2 receptor, ranked 6-EDCA and GW4064 as the best ones.

Computational approach

The ability of our computational approach to identify novel EPHA2 receptor antagonists, suggested us to conduct a virtual screening campaign, combining shape-similarity and docking calculations, on a set of commercially available compounds. A combined score, taking into account both ligand- and structure-based results, was then used to identify the most promising candidates. Two compounds, selected among the best-ranked ones, were identified as EphA2 receptor antagonists with micromolar affinity. The 3-hydroxy-cholenic acid emerged as the best ligand inhibiting both EPHA2-ephrin-A1 protein-protein interaction in an ELISA assay and ligand-induced

EPHA2 phosphorylation in PC3 cells (IC_{50}=19microM). Although this compound directly derives from a class of potent LXR agonists (i.e. the oxysterols), it does not affect cellular responses mediated by LXR functioning and fails to activate physiological targets of LCA including FXR and PXR receptors, suggesting that it is possible to achieve EPHA2 selectivity working around the cholenic acid nucleus.