Multiple approaches for the discovery and development of small molecules targeting Eph-ephrin protein-protein interaction

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Eph receptor tyrosine kinases and their membrane-bound ephrin ligands are involved in many biological processes including 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 correlated with an aggressive tumor phenotype and poor prognosis[1] and it modulates the self-renewal of tumor propagating cells in human glioblastoma[2].
These evidences induced us to start a drug discovery program, some years ago, with the aim to discover and develop small molecules able to disrupt Eph-ephrin protein-protein interaction.
Due to the challenging task, we approached the problem through different complementary methodologies.

Screening
An hit compound (lithocholic acid, LCA) able to modulate Eph-ephrin activity, was discovered through an ELISA screening and it was used to synthesize and characterize a novel series of derivatives acting as EphA2 antagonists[3]. UniPR129 resulted as the best compound within this series with a submicromolar Ki and an IC_{50} in the low micromolar range in functional experiments, including in vitro angiogenesis[4]. Nevertheless, with the aim to improve the selectivity, the poor physico-chemical and pharmacokinetic properties of LCA derivatives, we turned our attention to the search for alternative chemotypes targeting Eph-ephrin system.
Target hopping
Given the ability of LCA to interact with farnesoid X receptors (FXR), G protein bile acid receptors (TGR5) and EphA2 receptors 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 microM, respectively. Interestingly, computational data obtained by docking compounds into the EphA2 receptor, ranked 6-EDCA and GW4064 as the best ones[5].
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. 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[6].
Medicinal chemistry
A class of Δ^5-cholenoyl-amino acids conjugates was finally synthesized identifying the *N*-L-tryptophan conjugate of 3β-hydroxy-Δ^5-cholenic acid (UniPR1331) as the first orally bioavailable small molecule antagonist of the Eph-ephrin system. UniPR1331 blocked in vitro angiogenesis (IC_{50}=1.3microM) in human umbilical vein endothelial cells and dramatically inhibited tumor growth in an in vivo xenograft model.

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