Discovery and development of Eph-ephrin antagonists endowed with antiangiogenic properties

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Eph-ephrin system is involved in many biological processes including cell migration and morphology, axon guidance, synaptic plasticity and angiogenesis both during embryogenesis and in adult tissues. Many reports correlate a deregulation of Eph-ephrin system to aggressive tumor phenotypes in a number of human cancers and, in particular EphA2 and EphB4 overexpression, is associate with a poor prognosis. This evidence indicates Eph-ephrin system as a new promising target in cancer field.

By means of an ELISA-based binding screening, we recently identified lithocholic acid (LCA), a secondary bile acid able to modulate Eph-ephrin activity showing a Ki value of 49 μM. Further investigations brought out LCA as a novel specific reversible antagonist of Eph receptors, able to dose-dependently interfere with functional effects of Eph activation (i.e. Eph phosphorylation, cell rounding).

Using LCA scaffold as a track, first of all we identified the structural elements essential for the binding to Eph receptors, allowing the extrapolation of the pharmacophoric group and, on the other hand, we could design and synthesize a first new series of derivatives analyzing the structure-activity relationship. This analysis indicated the necessity of the large hydrophobic region (cyclopenta[*a*]perhydrophenantrene scaffold) and the anionic hydrogen bond acceptor group (carboxylate function), whereas modifications on the hydroxy group in -3 can be allowed. During this phase of the work, cholanic acid emerged as the most potent compound showing a Ki value of 5.1 μM. Furthermore, the conjugation of LCA with a glycine generated an interesting active compound (glycolithocholic acid, Ki=38 μM) that we used for the synthesis of a new series of derivatives whose structure was functionalized with amino acids.

Among this new series, the LCA conjugate with a L-Trp (PCM126) emerged as specific antagonist, able to reversibly disrupt EphA2–ephrin-A1 binding in the ELISA assay, showing a Ki value of 1.2 μM. The improved activity observed in PCM126 could be due to the facilitated interaction between the carboxilyc function of the amino acid conjugate and the essential amino acid residue Arg 103 of the EphA2 ligand binding domain (LBD). Starting from this observation, we tried to even further improve the binding affinity to Eph receptors reinforcing this crucial interaction and synthetisizing the superior homolog of PCM126 (the homo-L-Trp-conjugate of LCA), namely PCM129.

PCM129 resulted the best compound of the series, both in binding assays, showing a ki value of 370 nM towards the EphA2-ephrin-A1 interaction and also in functional assays, in particular, it vaunted high potency in blocking *in vitro* angiogenesis on HUVE cells, suggesting promising applications as antiangiogenic compound. Moreover, PCM129 is endowed not only with better potency in Eph-ephrin antagonism but also with an improvement of the cytotoxicity profile, pointing out this compound as a good candidate for further development.