In vitro and *in vivo* efficacy of an orally bioavailable antagonist of Eph-ephrin system in tumor angiogenesis and growth

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The Eph receptors represent the largest family of receptor tyrosine kinases (RTK) in humans. They are divided in 2 classes, EphA and EphB, based on sequence homology of extracellular domain and their affinity for ephrin ligands, proteins tethered to the cell membrane by either glycosylphosphatidyl-inositol linkage (ephrin-As) or a transmembrane domain and cytoplasmic tail (ephrin-Bs). Eph-ephrin signaling plays a key role during embryogenesis, where regulates the morphogenetic processes of organs and tissues, whilst in the adult several evidence showed as a deregulated expression and/or function of these proteins may promote tumorigenesis and the development of more aggressive and metastatic phenotypes in a large variety of solid tumors. Moreover this system has a prominent role in tumor angiogenesis and for these reasons is an emerging target for the development of novel antiangiogenic therapies. Research programs aimed at developing small molecules antagonists of the Eph receptors are still in their initial stage as available compounds suffer for chemical or pharmacological drawbacks, limiting their application both *in vitro* and *in vivo*. Here, we report the pharmacological characterization of a class of delta^5-cholenoyl-amino acids conjugates as Eph antagonists, and the *in vitro* and *in vivo* efficacy of the most promising one as antiangiogenic agent. In particular our research led us to discover a new potent and selective aminoacid conjugate of 3beta-hydroxy-delta^5-cholenic acid (UniPR1331) that was able to inhibit EphA2-ephrin-A1 binding with a pIC_{50} value of 5.45 and a Ki value of 1.4μM. The functional study on human prostate adenocarcinoma cells (PC3 cells), naturally expressing EphA2 receptor, showed the ability of the compound to inhibit EphA2 phosporylation upon ephrin-A1 stimulation with an IC_{50} value of 9.3 μM. Notably, UniPR1331 did not affect PC3 cells viability highlighting as the observed reduction in the phosphorylation levels was not due to a condition of cellular stress. Moreover, UniPR1331 was inactive when tested as enzymatic inhibitor of the EphA2 kinase domain confirming to be a protein-protein interaction inhibitor (i-PPI). When tested in *in vitro* angiogenesis UniPR1331 was able to inhibit human umbilical vein endothelial cells (HUVEC) tubes formation in the low micromolar range. Finally, UniPR1331 showed a promising pharmacokinetic profile in mice that allowed us to test its efficacy in *in vivo* xenografts after oral administration.