Target-hopping: a useful approach to identify novel Eph receptor antagonists

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INTRODUCTION:
Eph receptor tyrosine kinases and their ephrin ligands represent an important signaling system with widespread roles in cell physiology and disease, including tumor and vascular functions during carcinogenesis. In fact several evidence showed as deregulated expression and/or function of this system may promote tumorigenesis and the development of more aggressive and metastatic phenotypes in a large variety of human cancer.
In 2011, we identified lithocholic acid (LCA), a secondary bile acid, as a weak binder of Eph receptors inhibiting Eph kinases-ephrins interaction and Eph kinases activation. Recently, other classes of Eph antagonists have been discovered, with most of them directly derived from LCA, by chemical modulation of the 5-β cholanic scaffold or by conjugation with naturally occurring α-aminoacids. Due to the modest selectivity and the limited chemical flexibility of LCA-based derivatives, we focused our attention on the identification of alternative chemotypes able to bind the EphA2 receptor.

AIM:
In this work we explored the idea of 'target-hopping' in which a ligand for one target is used as a starting point to derive new leads for another target. In fact, lithocholic acid, participates in the control of bile acid homeostasis and glucose metabolism, mainly by binding and modulating the activity of the nuclear receptor FXR, and the G-protein coupled receptor TGR5. Consequently we hypothesized that the structural requirements for a small molecule to bind FXR or TGR5 might be similar to those for acting on the Eph receptors.

METHODS:
A set of commercially available FXR or TGR5 agonists, featured by a three-dimensional shape similar to that of the 5-β cholanic portion of LCA, were tested for their ability to prevent EphA2-ephrin-A1 association by an ELISA-binding assay, and in functional cellular assays on PC3 human prostate adenocarcinoma cells naturally expressing EphA2 receptor.

RESULTS:
TGR5 agonists ciprofloxacin, betulinic acid and oleanolic acid were not able to inhibit the interaction between ephrinA1 and EphA2 in the ELISA-binding assay. Conversely, among FXR agonists, GW4064 was able to significantly inhibit EphA2-ephrinA1 interaction at 50μM. Moreover GW4062 resulted able to dose-dependently disrupt EphA2-ephrin-A1 complex giving a IC_{50} value of 23 μM. In the same assay, LCA resulted less potent giving an IC_{50} value of 55 μM. As LCA, GW4062 showed a competitive e reversible binding to EphA2. According to binding study GW4062 resulted more potent than LCA to inhibit EphA2 phosphorylation in PC3 cells upon ephrin A1-Fc stimulation.
Finally starting from the stilbene carboxylic acid structure of GW4064, we synthetized a small series of analogues to be evaluated as a binders of the EphA2 receptor. One of derivatives was still able to inhibit EphA2-ephrinA1 interaction and to work like an antagonist on EphA2 receptor naturally expressed on PC3 cells.

CONCLUSION:
In the present work, by applying a 'target hopping' strategy, we discovered the stilbene carboxylic acid scaffold as a new template for obtaining novel antagonists of the Eph-ephrin system, avoiding the (5β)-cholan-24-oic acid scaffold of LCA.