Abstract

Elevated LDL cholesterol (LDL-C) is an established risk factor in cardiovascular disease, the leading cause of death in the world. To date three main therapeutic strategies have been evaluated to reduce circulating LDL-C including: a) inhibition of critical steps of cholesterol synthesis, b) regulation of hepatic expression of the LDL-receptor (LDLR), and c) inhibition of Proprotein Convertase Subtilisin/Kexin type 9 (PCSK9)-mediated LDLR degradation.

Berberine (BBR) is a well known hypolipidemic agent able to upregulate LDLR expression. This alkaloid is also endowed with hypoglycemic, antitumor, antiviral, antioxidant and neuroprotective properties. However, BBR has poor oral bioavailability, is a substrate of P-glycoprotein (P-gp) and can affect the CYP450 system. Therefore, the development of new BBR analogues is encouraged in order to obtain new hypolipidemic compounds with improved pharmacokinetic/pharmacodynamic profile.

PCSK9 is a protein that binds LDLR and promotes its lysosomal degradation after internalization. Thus, LDLR cannot be recycled to the hepatic cell surface; on the contrary, the inhibition on PCSK9/LDLR increases hepatic cell-surface LDLR and improves the uptake of the LDL particles by the liver. To date, only two mAbs (alirocumab and evolocumab) directed against PCSK9 have been approved by FDA. However, these drugs need to be administered by injection, are expensive, and these aspects can greatly affect adherence to therapeutic regimen.

Therefore, an orally available small molecule inhibitor of PCSK9/LDLR interaction would be a desirable alternative in the management of hyperlipidemia, based on its ease of administration and lower cost.

This research project aimed to develop new lipid-lowering agents through two different approaches targeting: a) modulation of LDLR expression, and b) inhibition of PCSK9/LDLR interaction.

In this regard, for the first approach a small library of BBR analogues was designed and synthesized with the aim to improve the bioavailability compared to our lead compound, and shed light on the minimal structural requirements for its hypolipidemic action. The cytotoxicity of a small set of synthetized BBR analogues was evaluate by MTT assay on HepG2 and HaCaT cell lines, using BBR as reference compound. The results obtained were used to select three promising BBR analogues **18l**, **31** and **25a** which were tested to assess their ability to reduce total cholesterol (TCHO) and triglycerides (TG) levels in HepG2 cells after 48h of treatment at 5, 10 and 30μM. Compound **25a** proved to be the most active one showing at 30μM an inhibition rate of about 60% and 88% against TCHO and TG respectively. Finally, increased LDLR expression *vs* control was observed for **18l** and **31** by qRT-PCR analysis.

For the second approach, two sets of pyrrole derivatives were rationally designed through molecular docking studies, in order to mimic the critical residues of the LDLR implicated in the interaction with its protein partner PCSK9. Computational studies were performed using the crystallographic structure of the known PCSK9/LDLR inhibitor peptide Pep2-8 bound to PCSK9 (PBD code: 4NMX). For some synthesized putative PCSK9/LDLR inhibitors an *in vitro* binding assay (SPR) was performed, and two derivatives showed K_D lower than that of Pep2-8, used as reference compound.