SMALL CHEMICAL ENTITIES TARGETING THE PROPROTEIN CONVERTASE SUBTILISIN/KEXIN TYPE 9: SYNERGISTIC EFFECT WITH SIMVASTATIN ON LOW-DENSITITY LIPOPROTEIN UPTAKE BY HEPG2CELLS

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Introduction: The discovery of proprotein convertase subtilisin/kexin type 9(PCSK9) as a key modulator in cholesterol homeostasis led to the development of several strategies to inhibit its activity in hypercholesterolemic patients. To this aim, monoclonal antibodies (mAbs), small interfering RNA (siRNA) and vaccines have been developed for targeting PCSK9. Objective of this study was the in vitro pharmacological study of new potential PCSK9inhibitors, as a starting point for the development of a new class of hypocholesterolemic agents.

Methods: Four structurally related chemical entities were derived from a compound (MR-39) previously identified as potential PCSK9inhibitor by a high throughput screening analysis. From MR-39, three chemically-related compounds were synthesized. Thus, the effect of all four small chemical entities on PCSK9expression were assessed by using HepG2cell line by the means of RT-qPCR, ELISA, Western Blot, Luciferase Promoter Activity and low-density lipoprotein (LDL) uptake assays.

Results: MR-39, strongly inhibited PCSK9mRNA levels in a concentration-dependent manner (-46% at 6.25μM, the lowest concentration tested) and the protein expression at both intracellular (-70%) and extracellular (-30%) levels. MR-39increased both sterol regulatory element-binding protein 2(SREBP2) and hepatocyte nuclear factor 1α (HNF1α) protein, two transcriptional factors involved in the regulation of PCSK9. However, no effect was observed by MR-39on PCSK9promoter activity and it did not significantly improve the LDL uptake by HepG2cells. Interestingly, MR-39counteracted the transcriptional induction of PCSK9by simvastatin and significantly improved the LDL uptake of simvastatin (+30% and +75% at 6.25μM and 12.5μM of MR39, respectively). Similar results were observed for all four small chemical entities.

Discussion and conclusions: The inhibition of PCSK9is challenging, with the monoclonal antibodies therapy the most promising approach. Nevertheless, treating patients with mAbs is economically onerous. The development of small chemical entities specifically targeting PCSK9could be a valid alternative to the current therapies. MR small molecules can be considered as starting point for developing new innovative inhibitors of PCSK9to be utilized in combination with simvastatin.