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Digest

# Small molecule modulators of PCSK9 – A literature and patent overview

Daniel Pettersen [a,*], Ola Fjellström [b]

[a] Medicinal Chemistry, Cardiovascular, Renal and Metabolism, IMED Biotech Unit, AstraZeneca, Gothenburg, Sweden
[b] Cardiovascular, Renal and Metabolism, IMED Biotech Unit, AstraZeneca, Gothenburg, Sweden

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ABSTRACT

Proprotein convertase subtilisin kexin like type 9 (PCSK9) has since its discovery been a key protein target for the modulation of LDL cholesterol. The interest in PCSK9 has grown even more with the positive clinical trial outcomes in cardiovascular disease recently reported for two PCSK9 antibodies. Currently, there are no PCSK9 small molecule programs active in clinical development. However, there has been a steady increase in publications and patent applications within the PCSK9 small molecule field. This digest will provide a summary of small molecule and peptide PCSK9 modulators reported both in scientific journals and in patent applications, most of them originating from the last 3–4 years. As such, this digest will serve as an introduction to the field and assist further identification and discovery of small molecule PCSK9 modulators.



Cardiovascular death is the leading cause of death in the world despite progress in the prevention and treatment.[1,2] Elevated LDL cholesterol (LDL-c) is an established risk factor in cardiovascular disease and causes dyslipidemia, and a key regulator of LDL-c is the proprotein convertase subtilisin kexin like type 9 (PCSK9) protein. PCSK9 was discovered in 2003[3] and its main biological role is to bind the LDL receptor (LDLR) and destine it for lysosomal degradation. An illustrative overview of the role of PCSK9 and the LDL receptor is shown in Fig. 1. Lower levels of LDLR on the cell surface of hepatocytes will cause decreased LDL-c liver uptake from the blood resulting in elevated circulating LDL-c levels. Genetic evidence support the PCSK9-LDL-c-disease link, where gain of function mutations in PCSK9 show increased levels of LDL-c and increased incidence of cardiovascular risk, whereas the opposite holds true for the PCSK9 loss of function mutations.[4,5] Studies in mice also showed that overexpression of PCSK9 reduced LDLR expression and increased circulating LDL-c, whereas in PCSK9 knockout mice the phenotype was reversed.[6,7] Based on the human genetic and preclinical animal data, PCSK9 became an attractive drug target within the pharmaceutical industry soon after its discovery.

Intense preclinical and clinical PCSK9 antibody programs have resulted in two marketed drugs, evolocumab[8] and alirocumab.[9] Positive clinical data were recently presented for evolocumab, showing a reduction in the composite of cardiovascular death, myocardial infarction, stroke, and hospitalization for unstable angina or coronary revascularization by 15% after a median of 2.2 years treatment.[8] These data provide evidence of a clinical benefit of PCSK9 inhibition as a drug target.

However, there is room for improved PCSK9 inhibitors considering e.g., potential intracellular effects, route of administration/-convenience for the patient and cost, since the marketed antibodies are only targeting extracellular PCSK9, are administered subcutaneously and are costly. This digest focus on small molecules, peptides, and peptide fragments acting as PCSK9 modulators demonstrating the efforts in identifying oral inhibitors of the PCSK9 biological activity, the Holy Grail in this area.

PCSK9 is not easily druggable by small molecules. Even though PCSK9 is a protease, it only cleaves itself, which is to ensure appropriate intracellular trafficking and secretion. To the best of our knowledge, no successful attempts to inhibit its autocatalytic activity has been reported, and the traditional and common approach is rather to inhibit the protein-protein interaction between PCSK9 and the LDLR. Even though crystal structures of PCSK9 reveal a relatively featureless and flat interaction surface making also this approach challenging,[10,11] this mode of action is actively pursued mainly through a stepwise peptide to small molecule tactic, and there are examples where other protein-protein interactions have been successfully targeted by small molecules.[12] However, the clinical success directly targeting the PCSK9-LDLR interaction has so far been limited to PCSK9 binding antibodies, e.g. evolocumab[8] and alirocumab.[9] Clearly, alternative small molecule therapeutic strategies to inhibit PCSK9 are welcomed. Thus, it is exciting that recent discoveries by Pfizer have identified a novel mode of action that halts translation of PCSK9 by small

* Corresponding author.
*E-mail address:* daniel.pettersen@astrazeneca.com (D. Pettersen).

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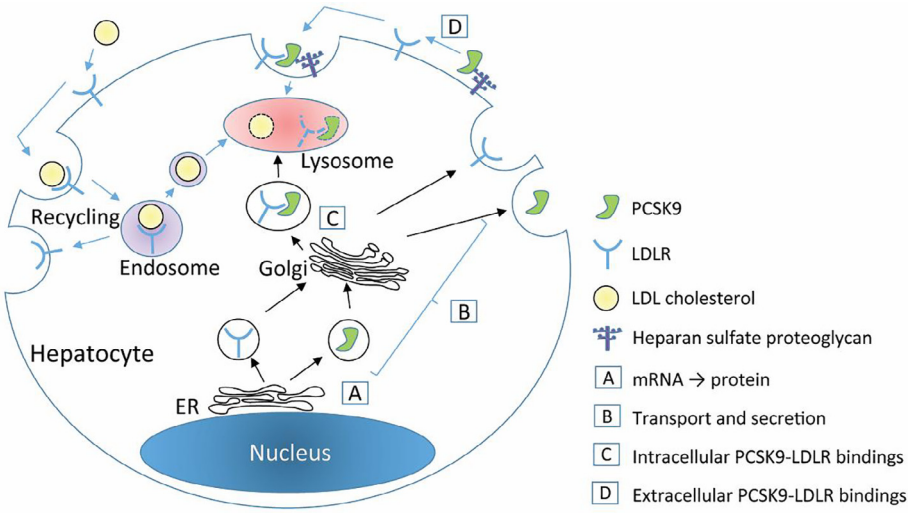


**Figure 1.** Schematic illustration of PCSK9, LDLR and LDL-c biology in hepatocytes. A. mRNA to protein translation of PCSK9 and LDLR. PCSK9 autocatalytic cleavage occurs in ER. B. Transport and secretion path C. Intracellular and D. extracellular PCSK9-LDLR binding, the latter facilitated by heparan sulfate proteoglycan, resulting in lysosomal degradation. ER: endoplasmic reticulum.

molecules.[13,14] This mechanism may be compared to a small interfering RNA (siRNA) approach to block PCSK9 translation, and the siRNA inclisiran was recently reported to robustly lower PCSK9 and LDL-c levels in high cardiovascular risk patients with elevated LDL-c.[15] Furthermore, it has recently been reported that PCSK9 binding to proteoglycan is important for PCSK9-LDLR binding, and thus blocking the PCSK9-proteoglycan interaction may provide an additional opportunity for small molecules PCSK9 inhibitors.[16]

The multitude of innovative strategies to target PCSK9 is a tribute to the central role of PCSK9 in the lipid biology and in its link to disease. Even though there are vast challenges for small molecules to inhibit PCSK9 activity, as evident from the quite slow progress in the field, there are numerous actors pursuing this area. There are reports in the PCSK9 field focusing on other modalities than small molecules and peptides.[17,18] However, to our understanding there are no reports summarizing the area of small molecules, including peptides, known to modulate PCSK9. This digest will provide a selective summary of the small molecule endeavors, most of them originating from the last 3–4 years, as reported both in the scientific journals and in patent applications. However, it is difficult to get a comprehensive view of the quality of the reported small molecule area since many critical questions are not addressed in the patent applications (e.g. lack of information regarding mode of action, pharmacokinetics and PCSK9 selectivity). As authors, we have listed some of these limitations and we direct the readers to the original publications for further information.

*Small molecule modulators of PCSK9:* Abdel-Meguid et al. reported a class of e.g. hydroxy pyrrolidone and thia-triazolo derivatives that modulate the physiological action of PCSK9 resulting in lowering of LDL-c levels in blood.[19] The authors do not specifically claim by which mode of action the molecules modulate PCSK9 activity but the compounds are reported to dose dependently increase the uptake of fluorescent Dil-LDL (low density lipoprotein coupled with 1,1′dioctadecyl-3.3.3′,3′-tetramethylindocarbocyanine perchlorate) in HepG2 cells and increase the LDLR expression levels in both HepG2 and HEK293 cells in the presence of PCSK9 protein (Fig. 2).

Compound **1** was subcutaneously dosed up to 2 weeks (4 mg/kg, once daily dosing) to mice fed high fat diet. There was a 32% mean reduction ($p < 0.01$) of total cholesterol levels compared to non-compound treated mice. A second study was performed dosing atorvastatin (40 mg/kg, for 3 weeks) resulting in 27% reduction of total cholesterol levels showing that compound **1** could match the cholesterol reducing effect of a statin at the doses studied.

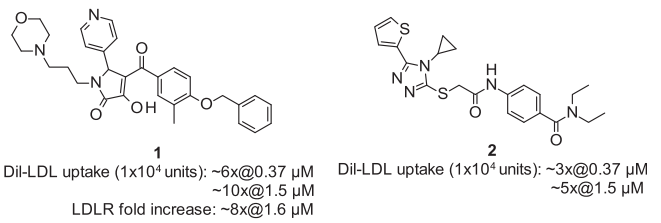


**Figure 2.** Hydroxy pyrrolidone and thia-triazolo derivatives as PCSK9 modulators.

In a series of several patent applications from Portola Pharmaceuticals, compounds able to increase LDL uptake and to increase LDLR expression in HepG2 cells have been reported.[20–23] The compounds were shown to be PCSK9 protein dependent since they did not have any effect on LDL uptake in control experiments without PCSK9 protein present. However, no details are provided about what mode of actions is behind the compound capacity to modulate the biological activity of PCSK9 and they are generally

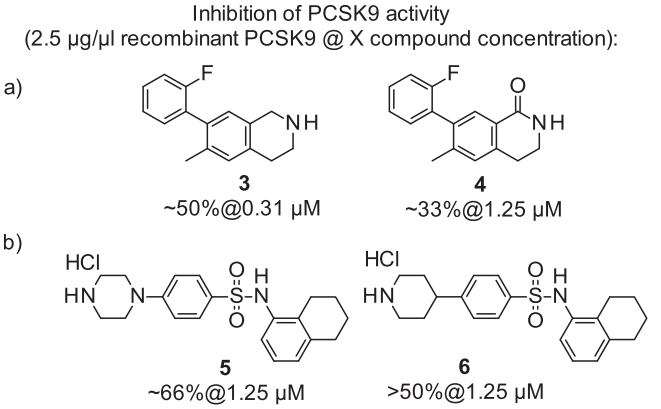


**Figure 3.** PCSK9 modulators reported by Portola Pharmaceuticals. (a) Tetrahydroisoquinolines and tetrahydroisoquinolinones and (b) phenyl piperazines and phenyl piperidines as PCSK9 modulators.