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REVIEW

PCSK9 inhibitors: a patent review 2018-2023

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ABSTRACT

Introduction: Proprotein convertase subtilisin/kexin 9 (PCSK9) plays a crucial role in breaking down the hepatic low-density lipoprotein receptor (LDLR), thereby influencing the levels of circulating low-density lipoprotein cholesterol (LDL-C). Consequently, inhibiting PCSK9 through suitable ligands has been established as a validated therapeutic strategy for combating hypercholesterolemia and cardiovascular diseases.

Area covered: Patent literature claiming novel compounds inhibiting PCSK9 disclosed from 2018 to June 2023 available in the espacenet database, which contains more than 150 million patent documents from over 100 patent-granting authorities worldwide.

Expert opinion: The undisputable beneficial influence of PCSK9 as a pharmacological target has prompted numerous private and public institutions to patent chemical frameworks as inhibitors of PCSK9. While several compounds have advanced to clinical trials for treating hypercholesterolemia, they have not completed these trials yet. These compounds must contend in a complex market where new, costly, and advanced drugs, such as monoclonal antibodies and siRNA, are prescribed instead of inexpensive and less potent statins.

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KEYWORDS
PCSK9; hypercholesterolemia; CVD; LDL-C; patents

1. Introduction

Hypercholesterolemia is the most widespread form of dyslipidemias, causing, over the past years, a significant increase in the global mortality. Hypercholesterolemia is caused by high levels of low-density lipoprotein cholesterol (LDL-C) in the bloodstream, a major contributing factor to the development of atherosclerotic cardiovascular disease (CVD) [1]. It has been reported that, over 5 years of trial, a low circulating level of LDL-C leads to: i) a decrease in myocardial infarction or coronary death, ii) a reduction in coronary revascularization treatments, iii) the decrease in the number of strokes and the major vascular events [2]. These authors, together with others reaching the same conclusions, led the Endocrine Society Guidelines to recommend an LDL-C circulating level lower than 55 mg/dL (equivalent to 1.4 mmol/L), especially for patients affected by CVDs [3]. Nevertheless, when this level is not respected by dietary habits, or for the presence of genetic disorders (such as familial hypercholesterolemia), individuals are recommended to use statins as a drug preventing fatal or non-fatal CV events. Unfortunately, not all patients are susceptible to the treatments with 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoAR) inhibitors such as statins, due to intolerance or statin-resistant hypercholesterolemia [4,5]. Moreover, long-term use of them resulted in tachyphylaxis, the decreasing response to therapeutic agents [6]. Specifically, a study on 254 patients found that LDL-C levels initially decreased during statins treatment but subsequently increased over time, even after reaching maximum LDL-C reduction. This was particularly noticeable in patients treated with atorvastatin at exposure doses of 10 or 20 mg/day [6]. From a molecular point of view, through the direct ability to inhibit the endogenous cholesterol synthesis via HMG-CoAR inhibition, statins activate the SREBP-2 pathway leading to the level increase of both LDL receptor (LDLR) and HMG-CoAR [7]. In addition, it has been observed that statins also raise levels of proprotein convertase subtilisin/kexin type 9 (PCSK9), a protein modulating the LDLR degradation. Increases in PCSK9 levels also reduce the LDL-C response to statin therapy [8]. Therefore, there has been a rapid and substantial expansion in the range of lipid-lowering medications, acting by novel mechanisms of action. These mechanisms include monoclonal antibodies (mAbs) acting on angiopoietin-like 3 (evinacumab, Ulterius Pharmaceuticals), microsomal triglyceride transfer protein inhibitors (lomitapide, Aegerion), ATP-citrate lyase inhibitors (bempedoic acid), and last but not least, PCSK9 inhibitors [9–12]. As stated before, PCSK9 is an enzyme circulating in the bloodstream and is responsible for regulating the population of LDLRs on the surface of liver cells. The PCSK9/LDLR interaction results in the internalization and degradation of LDLR, reducing the liver cells' ability to capture LDL-C from the bloodstream [13]. Consequently, inhibiting the interaction between PCSK9 and LDLR leads to an increased number of LDLRs on the cell membrane, enhancing the liver cells' capacity to uptake LDL-C [14]. In addition to its crucial role in LDL-C metabolism, PCSK9 has been linked to various processes relevant to cardiovascular health. Particularly, PCSK9 levels can predict the occurrence of recurrent cardiovascular events in individuals with coronary artery disease, even when their LDL-C levels are

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Article highlights

- PCSK9 inhibitors may become a new generation of *drugs* for the treatment of hypercholesterolemia and the prevention of fatal or non-fatal cardiovascular events.
- Several chemical scaffolds as PCSK9 inhibitors have been reported to date.
- This review reports on the PCSK9 inhibitors patented from 2018 to June 2023.
- Nineteen patents have been deposited by several companies and universities.
- One PCSK9 inhibitor (MK-0616) entered phase III clinical trial.

well-controlled. This underscores the growing recognition of PCSK9 s involvement in platelet reactivity and the formation of blood clots, implying the clinical importance of inhibiting PCSK9 through pharmacological methods [15]. In the past few years, both academia and pharmaceutical corporations have invested significant resources in discovering compounds with the ability to block PCSK9, both the biosynthesis and the interaction with LDL-R [16]. Since PCSK9 inhibitors exert a mechanism of action that is completely different to the statins one, this makes them eligible for the treatment of patients who cannot be treated by statins, due to harsh side effects or to therapeutic (total/partial) ineffectiveness in some, severe, forms of hypercholesterolemia (e.g. familial hypercholesterolemia) [17]. As a consequence, this led to the development of effective therapeutic strategies for addressing hypercholesterolemia resistant to the statin treatment, as demonstrated by the introduction of two monoclonal antibodies, alirocumab (Sanofi) and evolocumab (Amgen), onto the market [18,19]. Novartis pioneered the development of the first siRNA drug, known as Inclisiran, which can disrupt the liver's PCSK9 transcription. This disruption results in long-lasting reductions in cholesterol levels among treated patients [20]. However, these medications are costly and face challenges in terms of patients' compliance due to their subcutaneous administration. Consequently, pharmaceutical companies and academic institutions have a strong interest in advancing the clinical development of orally bioavailable small molecules. This interest is evidenced by the substantial number of patent applications in this field in the last decades [21,22]. Herein, among the 810 patents resulted from the worldwide patent search conducted on espacenet database (https://worldwide.espacenet.com), we summarized the disclosures regarding only the compounds which directly interact and inhibit PCSK9, obtaining a total of nineteen patents, deposited from 2018 to June 2023. In particular, here we report on the patents containing the word 'PCSK9' in the 'Title or abstract' considering each year of the time period examined in this review, making use of the 'advanced search' webtool of espacenet.

2. Patent review

2.1. Year 2018

2.1.1. Cardio Therapeutics Pty Ltd

With this invention, Cardio Therapeutics Pty Ltd. patented a series of heterocyclic compounds as potential new PCSK9 small molecule inhibitors [23]. Based on the molecular modeling studies they have accomplished, it was concluded that these compounds can target PCSK9, thus preventing its interaction with the LDLR. This disclosure covers all the heterocyclic compounds with the general structure **1–4** (Figure 1). The embodiments of the patent cover any pharmaceutically acceptable salt, solvate, prodrug or polymorph of the compounds, in order to reduce LDL levels, through PCSK9 inhibition. The compounds of the invention may, moreover, be administered along with a pharmaceutical carrier, diluent, or excipient. Throughout the setting up and synthetic process of the molecules, the inventors selected some specific functional groups to put on the starting scaffold to enable these compounds to interact with some relevant amino acids in the protein sequence of PCSK9, such as: Ser221, Asp212, Lys223 and Lys258. The experimental approach used to establish these compounds' efficacy was, as a first thing, to measure their ability to disrupt the protein-protein interaction between LDLR and PCSK9 through a binding assay. Through this experiment, the inventors proved the mechanism of action of the compounds they proposed. The rate of inhibition was assessed as the absorbance of tested compounds at 450 nm and 540 nm, then plotted within IC_{50} curves. Among all the 31 compounds reported as examples, 5 of them showed an IC_{50} comprised between 0.1 and 1 μM (examples 5, 6, 7, 19, and 30), while only compound **1** (patented as example 27) exhibited an IC_{50} lower than 0.1 μM, representing the most potent PCSK9 inhibitor reported in this patent (Figure 2).

Furthermore, they performed a cell-based LDL uptake assay. The assay was carried out on human liver HepG2 cells, considering the examples 6, 25, 27, and 30, tested at the concentration of 0.1 and 1 μM. AMB-657286, a small molecule inhibitor of the PCSK9-LDLR interaction developed by Shifa Biomedical Corporation [24], was employed as a positive control. In this assay, the dynamic range of measuring LDL uptake was improved through the addition of a gain-of-function mutant of PCSK9, that furtherly impairs LDL uptake (increased binding and degradation of LDLR) and when inhibited represents a sign of functionality against the target. The quantification of the uptake was determined by fluorescence, on a reader set at excitation/emission wavelengths of 485 and 530 nm, respectively. Also in this case, compound **1** exhibited the best results (Figure 2). More details are available in their research article published on the Bioorganic and Medicinal Chemistry where the compound **1** (example 27) can be found with the name of 3d [25].

2.1.2. Merck sharp & Dohme corporation

In 2018, Merck Sharp & Dohme Corporation patented a series of novel PCSK9 allosteric modulators consisting of substituted 1-methyl-1,2,3,4-tetrahydroisoquinolines with general structure **5** (Figure 3), endowed with high affinity for a peculiar site of the protein, that is not involved in the PCSK9-LDLR interaction [26]. In particular, this unprecedented allosteric binding pocket was predicted *in silico* by using the Molecular Operating Environment Site Finder tool, and it is located between the catalytic and *C*-terminal domain of PCSK9 shaped

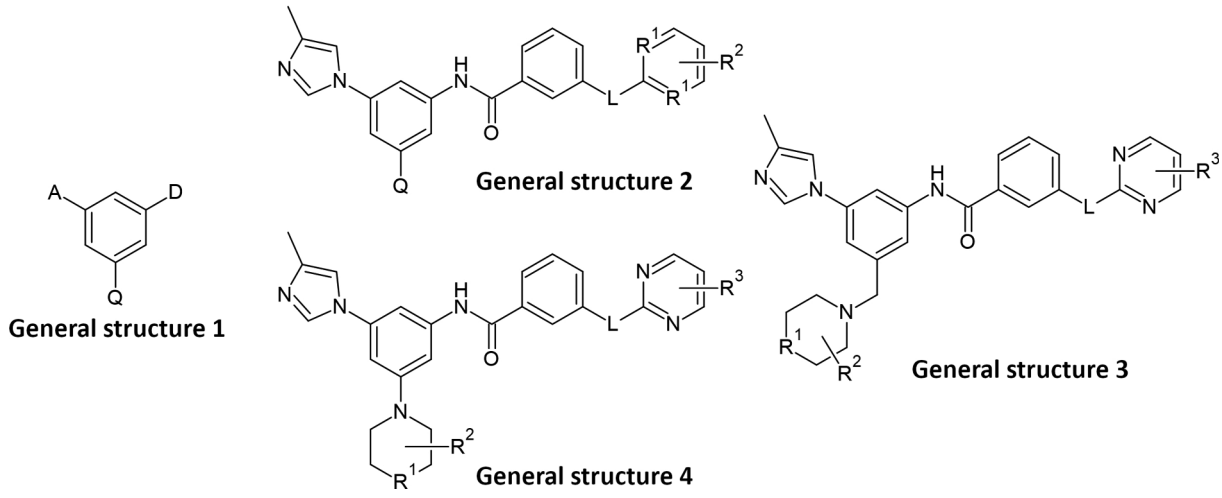


Figure 1. General structure 1–4 of the heterocyclic compounds patented by Cardio Therapeutics Pty Ltd as potential new PCSK9 small molecule inhibitors.

by Asp360, Arg357, Arg458 and Arg476 [27]. The authors also published the X-ray structures of several compounds, which are covered by the patent, in complex with the above-mentioned PCSK9 allosteric site (PDB accession codes: 6U26, 6U2N, 6U2P, 6U36, 6U38, 6U3X) [27]. Additionally, they seem to bind to such site independently from PCSK9's binding to LDLR. Besides inducing PCSK9 degradation through this unusual pathway, these compounds displayed the capacity of reducing intracellular levels of PCSK9 (both as pro-protein and mature form) and are potentially important in the treatment of clinical conditions affected by PCSK9 levels and functionality, such as atherosclerosis, hypercholesterolemia, coronary heart disease, metabolic syndrome, acute coronary syndrome, and related CV diseases. This invention covers any pharmaceutically acceptable salt or any pharmaceutical composition comprising one of said molecules or its salification product and any pharmaceutically accepted carrier. Talking about the combination, the other drug can be of various types, going from statins to Angiotensin – Converting Enzyme (ACE) inhibitors and sartans and many other classes. The embodiments cover all forms of the compounds presented, such as solvates, hydrates, stereoisomers, tautomers but also free acids and bases and alternative salts.

Several experiments were carried out to assess the compounds' capacity to bind the PCSK9 protein, such as Differential Scanning Calorimetry (DSC) and Fluorescence Polarization (FP), and their effectiveness in inhibiting its functionality performing a protein degradation assay. In the DSC assay, compounds designated as examples 1–92 and 109–112 were tested and most of them exhibited ΔTm values > 1°C. The higher the temperature shift, generally, the higher is the affinity. The stabilization of PCSK9 through these molecules' binding refers to an inactive form of the protein, that impairs its ability to bind to LDLR and, therefore, leads to an increased level of receptors on hepatocytes' surface and ameliorates the uptake of circulating LDL. The compound with the highest affinity in this test is compound **2** (example 85), exhibiting a ΔTm value of 9.623 (Figure 3). Also in the FP assay, the high affinity bond relates to the stabilization of an inactive conformation of PCSK9 and the consequent boost of

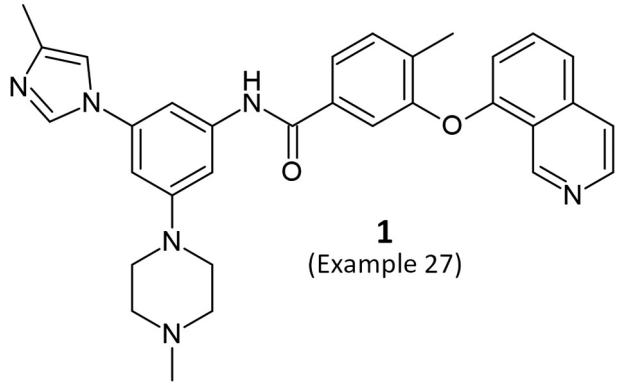


Figure 2. Chemical structure of the most potent PCSK9 inhibitor reported in the patent.

LDLR population. The lowest IC_{50} value was observed in the case of compound **3** (example 14, 2.37 nM), represented in Figure 3. Finally, in the protein degradation assay, the reported DC_{50} values represent the effective concentration of the tested molecule to lower PCSK9 protein levels, of both 'pro' and 'mature' forms, by 50%. The most effective compounds in lowering pro-PCSK9 are represented by compound **4** (example 94), examples 99 and 104, exhibiting DC_{50} values of 1.36, 1.37, and 1.53 μM, respectively, while the significantly most active against the mature form is compound **5** (example 96), showing a DC_{50} value of 1.05 μM (Figure 3).

2.1.3. Aarhus university

Liver heparan sulfate proteoglycans are PCSK9 receptors and essential for PCSK9-induced LDLR degradation. The heparan sulfate-binding site is located in the PCSK9 prodomain and formed by surface-exposed basic residues interacting with trisulfated heparan sulfate disaccharide repeats. Accordingly, heparan sulfate mimetics are potent PCSK9 inhibitors. It was proposed by Gustafsen et al., from Aarhus University of Denmark, that heparan sulfate proteoglycans lining the hepatocyte surface capture PCSK9 and facilitate subsequent PCSK9-LDLR complex formation [28].

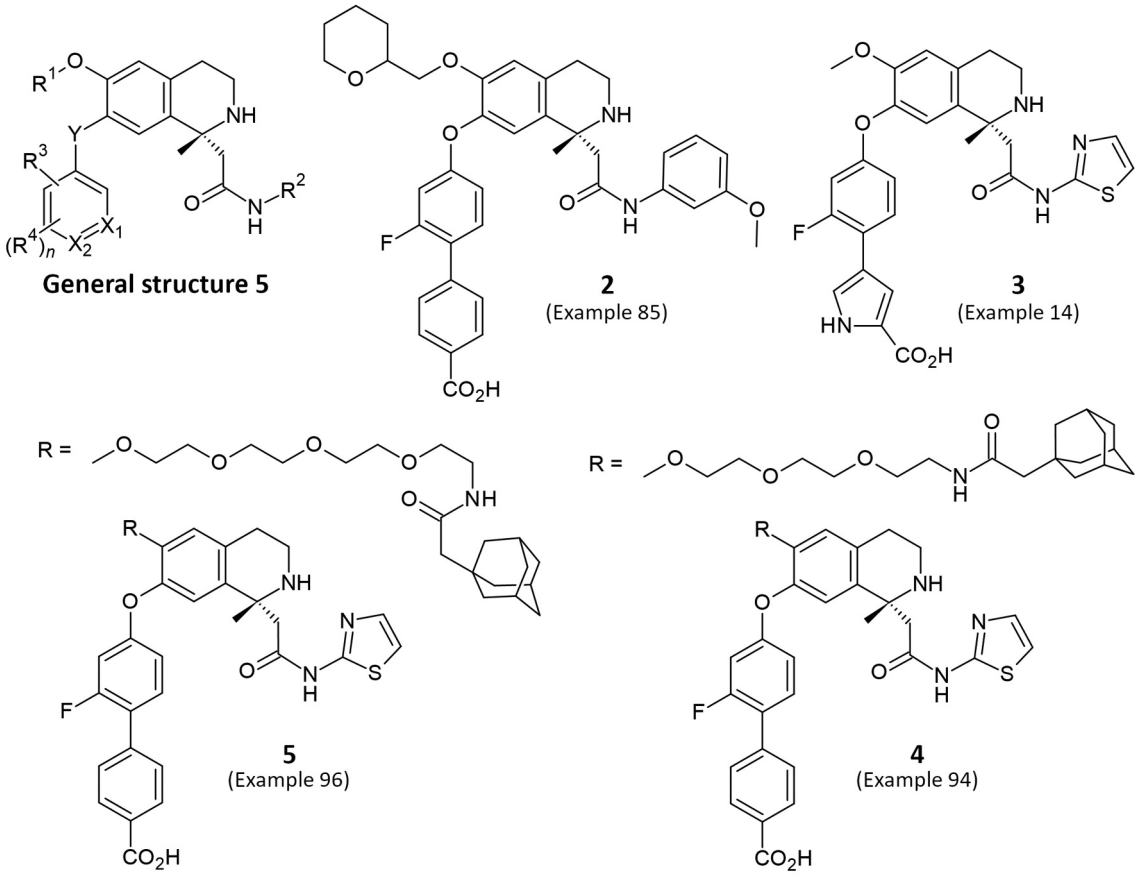


Figure 3. General structure and representative examples of PCSK9 allosteric binding tetrahydroisoquinolines patented by Merck Sharp & Dohme Corporation in 2018.

Notably, the patent entitled 'Compounds for treatment of lipoprotein metabolism disorders' by Gustafsen et al. relates to the use of heparin analogues as PCSK9 inhibitors for the treatment of lipid disorders [29]. In the first aspect, the invention relates to a composition comprising a compound with general structure **6**, or a pharmaceutically acceptable salt, solvate, polymorph or tautomer thereof, described in Figure 4.

Several heparin mimetic compounds were developed, and seven of them are currently being used in clinical settings for various purposes. These compounds are referred to as heparin/heparan sulfate mimetics and belong to different chemical classes, including oligosaccharides, oligonucleotides, and naphthalene derivatives [30]. Dedicated tests on a subset of these mimetics to evaluate their ability to bind PCSK9 and increase LDLR in HepG2 cells were carried out. The sulfated oligosaccharides dextran sulfate and pentosan sulfate both directly bound to PCSK9 with affinity constants of 180 and 381 μM, respectively, as estimated using Microscale Thermophoresis (MST). This binding resulted in a dose-dependent increase in cellular LDLR, reaching a plateau of around 400% compared to the control. It was observed that the interaction depended on the presence of sulfate groups, as non-sulfated dextran showed no affinity for PCSK9. Furthermore, the sulfated naphthalene derivative suramin, an antiparasitic drug used against African sleeping sickness, led to an up to 15-fold increase in LDLR and consequently ameliorated the HepG2 ability to uptake LDL. Phosphorothioate oligonucleotides are highly anionic and are known to interact with heparin-binding proteins [31,32]. Therefore, a modified 36–mer phosphorothioate oligodeoxycytidine (S-dC-36) was assessed demonstrating that it successfully binds to PCSK9 with a K_d of 4.8 μM, with inhibitory activity within this range of concentration. Based on this evidence, it appears clear that several negatively charged compounds exhibit inhibitory activity toward PCSK9. To obtain a better understanding of the structure – activity relationships, crystallographic data on a PCSK9-heparin mimetic complex was acquired, suggesting that a dextran sulfate disaccharide interacting with Arg93, Arg97, Arg104, and His139 of PCSK9. Overall, these findings suggest that the number and placement of negatively charged

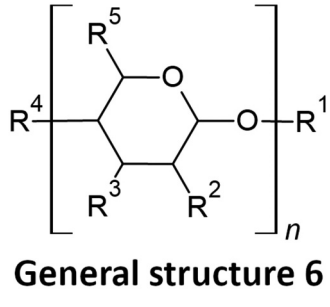


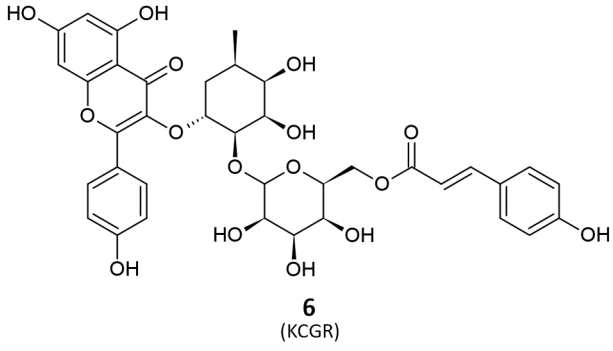
Figure 4. General structure of the heparin analogues patented by Aarhus University in 2018.

functional groups of sulfated sugars and mimetics are crucial determinants for their binding and activity toward PCSK9.

2.2. Year 2019

2.2.1. China pharmaceutical university

The 'China Pharmaceutical University' of Nanjing patented the use of Kaempferol 3-O-2'-(6''-*p*-coumaroyl)glucosyl rhamnoside (KCGR), a monomer component of a traditional Chinese medicine, for the treatment of diseases mediated by PCSK9 [33]. The chemical structure of KCGR (compound **6**) is made by a central rhamnose core which hemiacetal -OH group forms a glycosidic bond with a Kaempferol fragment while its hydroxymethyl functionality is esterified with a residue of *p*-coumaric acid (Figure 5). Study conducted by patent inventors demonstrated the efficacy of the inhibitor in providing a beneficial effect in the reduction of the level of lipids in blood. In particular, the authors discovered for the first time that KCGR possesses the specific activity of inhibiting both the combination of PCSK9 and LDLR. In fact, KCGR exhibited a reduction of the levels of TC (total cholesterol), TG (triglycerides) and LDL-C in the plasma of hyperlipidemic mice models, an increasement of the level of HDL-C (high-density lipoprotein cholesterol), and an improvement of the lipid metabolism. *In silico* studies showed the ability of KCGR to bind with high affinity to the active pocket region of PCSK9 crystal structure and establish stable H-bond interactions with multiple surrounding amino acid residues [34]. Moreover, a binding assay confirmed that KCGR and human recombinant PCSK9 protein had strong binding force ($K_d = 15$ nM) [34]. In particular, the bio-layer interferometry method was employed to ascertain the binding kinetics between the ligand and PCSK9, utilizing the Octet RED96 system at 25°C. Taken all together, these findings represent an advancement in the treatment of cardiovascular diseases and certain forms of hyperlipidemia.



6
(KCGR)

Figure 5. Chemical structure of Kaempferol 3-O-2''-(6'''-p-coumaroyl)glucosyl rhamnoside (KCGR).

2.2.2. Merck Sharp & Dohme corporation

In 2019, Merck Sharp & Dohme Corporation published two different patents covering novel macrocyclic inhibitors of PCSK9 with promising activity at nanomolar level. In the first patent, the chemical structures of the compound belong to a series of pseudopeptide macrocyclic derivatives bearing several modified aminoacidic residues, with general structure **7** (Figure 6) [35]. Selected compounds of the invention were examined by *in vitro* assays to determine their potency in blocking PCSK9 activity. Binding affinity was described as K_i and the values were obtained through biological assessments using Alexa FRET Plus (K_i-plus) and Alexa FRET Ultra (K_i-ultra) TR-FRET kits and they are reported in nanomolar (nM). The eight most promising compounds showed a K_i-plus lower than 0.00558 nM and a K_i-ultra comprised between 0.00074 (exhibited only by compound **7**, patented as EX-31) and 0.00597 nM (Figure 6).

The second patent highlights the advantages of using novel macrocyclic compounds as potent PCSK9 inhibitors [36]. Chemical structure of the inhibitors resembles a pseudopeptide backbone, embodying a functionalized tryptophan, specifically bearing a fluorinated indole moiety and methylated tyrosine and proline frameworks, with general structure **8** (Figure 7). Selected compounds of the invention were examined by two *in vitro* assays to determine their potency in blocking PCSK9 activity. K_i measures were obtained through biological assessments using Alexa FRET Standard (K_i standard) and Alexa FRET Plus (K_i plus) TR-FRET kits and the values are reported in nanomolar (nM). The most active compounds turned out to be compound **8** (patented as Ex-B03)

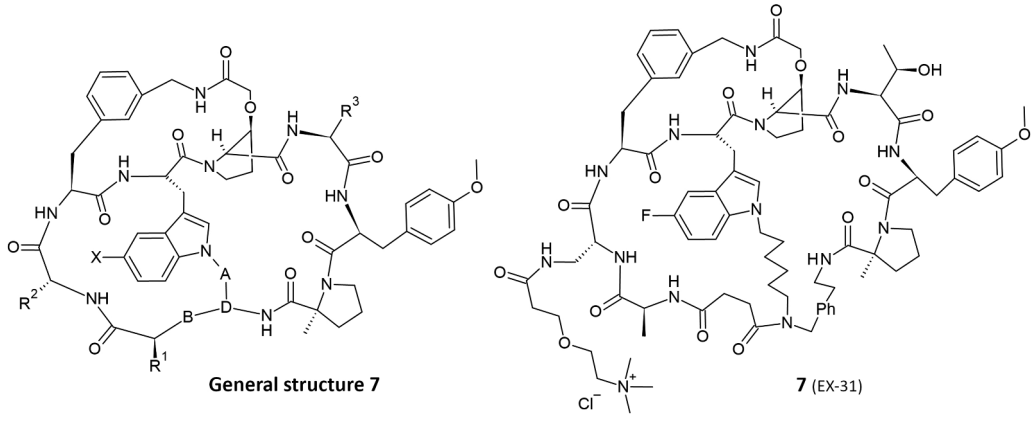


Figure 6. General structure of the compounds covered by Merck Sharp & Dohme Corporation, and the chemical structure of EX-31, which represent the most potent compound of the series.

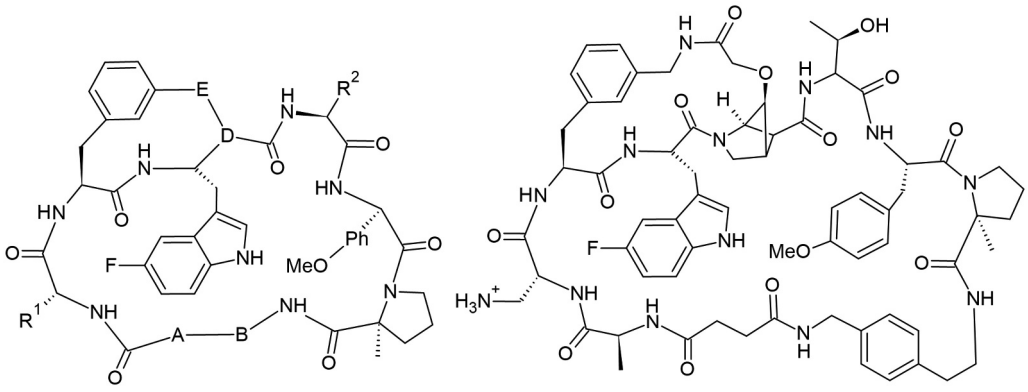


Figure 7. General structure of the compounds covered by the second patent of Merck Sharp & Dohme Corporation, and the chemical structure of Ex-B03, which represent the most potent compound of the series.

and Ex-B04, bearing an amide bridge between modified proline and phenylalanine residues, and derivatives EX-OT03–06, where the same amino acidic residues are connected via a triazole functionality to construct the tricyclic architecture. All these compounds showed a K_i standard lower than 1.26 nM and K_i plus comprised between 0.008 (exhibited only by compound **8**) and 0.32 nM (Figure 7).

Interestingly, this class of compounds was furtherly developed, finally attaining a promising drug candidate (see section 2.4.1 for details).

2.3. Year 2020

2.3.1. Novartis AG

In 2020, Novartis AG has filed two different patents regarding tetrameric and pentameric cyclic polypeptide PCSK9 inhibitors, respectively, which are helpful in the treatment of cholesterol lipid metabolism and other metabolic disorders in which PCSK9 protein is involved.

The first patent covers all the compounds with general structure **9** (Figure 8) and comprises their pharmaceutically acceptable salts, hydrates, solvates, prodrugs, stereoisomers, N-oxides, or tautomers [37]. The PCSK9 binding capacity of the compounds was measured using a TR-FRET assay which was performed on EnVision or PheraStar instruments. These experiments measured the ability of the compounds to interfere with the binding of human PCSK9 protein to human LDLR, providing the measures of both potency (IC_{50}) and efficacy. A total of 178 compounds were reported as examples, and, among them, the IC_{50} values ranged from 1.49 μM to 150 pM, however the IC_{50} values of the majority of the compounds fall in the picomolar range. In fact, a total of 106 polypeptides out of 178 showed IC_{50} values below 1 nM, and four of these showed values below 200 pM, as observed for compounds **9–12** (patented as example #10, #12, #138, and #139, respectively), representing the most potent PCSK9 inhibitors reported in the patent (Figure 8).

The second Novartis AG disclosure relates to PCSK9 inhibitors with general structure **10** (Figure 9) [38]. The company, already in the market with Inclisiran, in this patent covered cyclic pentameric *N*-substituted peptides in which X is C or N atoms and R_n substituents can be often H, C1-C6 alkyl or haloalkyl groups (Figure 9). More than 300 examples of compounds were reported in the patent. For all of them the PCSK9 inhibiting activity was measured by TR-FRET assays using unlabeled human PCSK9 as positive control and buffer/DMSO as negative control. The highest inhibitory activity, associated with the lowest IC_{50} value (0.9 nM), was displayed by compound **13** (patented as example 85) reported in Figure 9. Interestingly, 13 other compounds showed IC_{50} values lower than 1 nM and a protein inhibition activity higher than 88%.

As usual in the patent descriptions, no structural or mechanistic information is provided and, to the best of our knowledge, the inventors did not publish any data on these compounds, only one of the inventors (Yang L.), together with other Novartis researchers, published an interesting paper in which macrocyclic natural peptides were discovered [39]. In this effort, the Novartis researchers, through an affinity-based screening process involving over a trillion in vitro-translated macrocyclic peptides, successfully pinpointed compounds that exhibit a strong binding affinity to PCSK9 at the low-nanomolar level. These compounds not only disrupted the function of PCSK9 but also made use of a distinctive, induced-fit pocket of PCSK9 [39]. It is possible that the compounds covered by this patent could share with the macrocyclic peptides reported in the published article the discovery process and the action mechanism.

2.3.2. Dogma Therapeutics, Inc

The patent of Dogma Therapeutics, Inc. describes novel heteroaryl compounds of general structure **11** differently substituted (Figure 10), or their pharmaceutically acceptable salts, which are able to interact with a new discovered binding pocket defined by amino acid residues Val589 and Ser636 of human PCSK9 protein [40]. In September 2020, AstraZeneca AB acquired the oral PCSK9 inhibitor programme from Dogma Therapeutics, Inc. These compounds can inhibit the activity of PCSK9, thus resulting in an increase in LDLR levels on the liver surface. The affinity (K_D) of the compounds on human recombinant PCSK9 protein was measured through Surface Plasmon Resonance (SPR) experiments, in particular, the biophysical data were collected on a Biacore™ system (GE Healthcare).

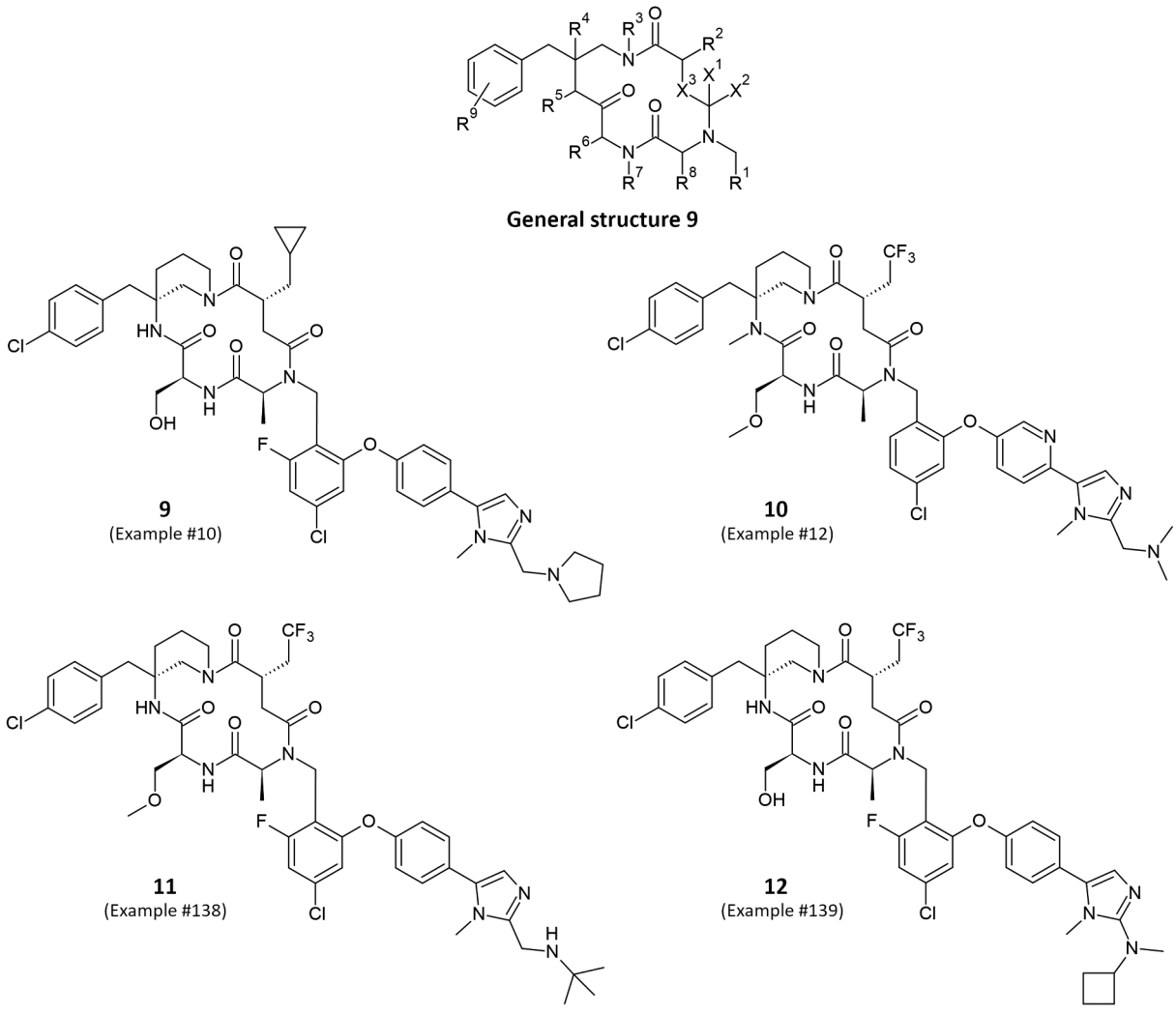


Figure 8. Chemical structure of the cyclic polypeptides which inhibit the PCSK9 protein, patented by Novartis AG, and chemical structures of the compounds 9–12, capable of inhibiting the PCSK9/LDLR interaction with an IC_{50} of 180 pM, 152 pM, 152 pM, and 150 pM, respectively.

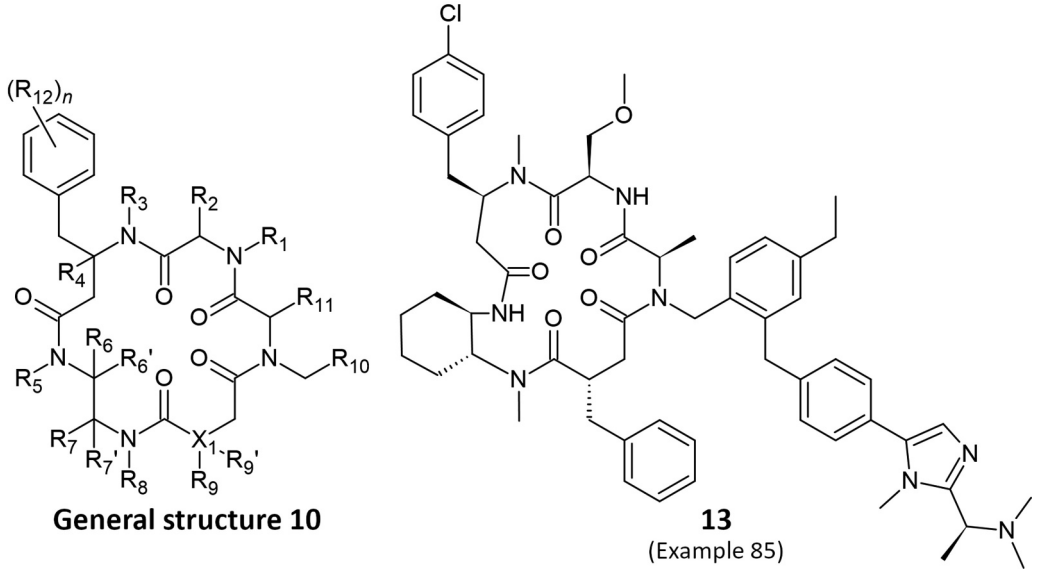


Figure 9. General structure of the compounds covered by Novartis AG patent, and chemical structure of compound 13 (example 85), which showed the highest inhibitory activity of the series.

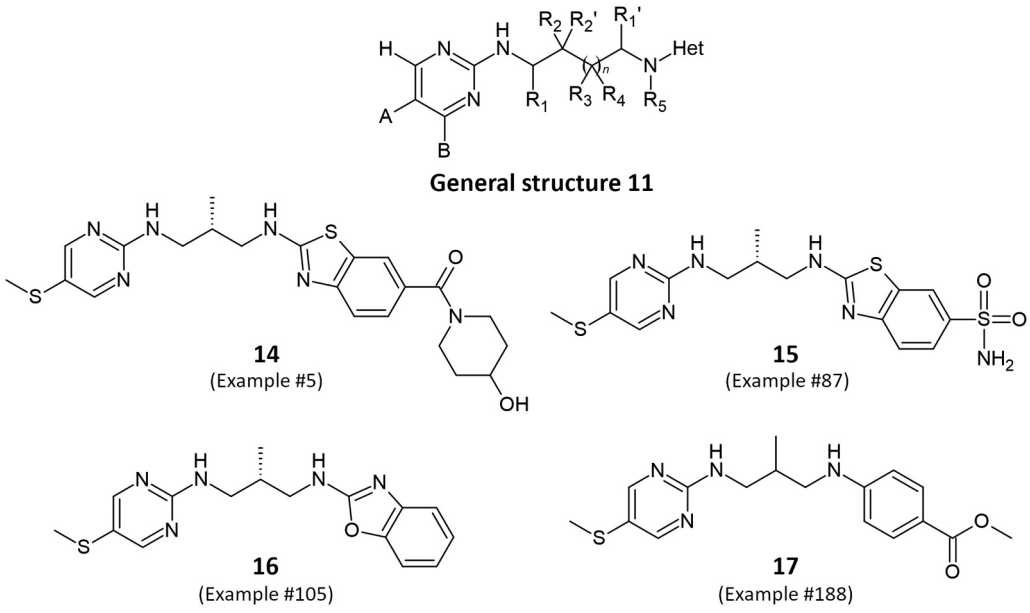


Figure 10. General structure of the novel heteroaryl allosteric inhibitors of PCSK9 protein, patented by Dogma Therapeutics Inc., and chemical structure of the representative compounds 14–17.

Out of 511 compounds tested, 261 showed a K_D value lower than 200 nM.

The representative compounds with general structure **11**, for a total of eight compounds, were then selected for the *in vitro* cellular assay to measure the secreted PCSK9 levels, cellular LDLR levels and cell viability. An inhibitor of PCSK9 translation (SI – 1) was used as positive control and was synthesized according to the patent deposited by Pfizer in 2014 [41]. At the tested concentration of 75 µM, the compounds were able to increase LDLR levels up to 136% (in the case of example #89) and did not induce significant changes regarding cellular viability (values range between −14% and 12%). Surprisingly, the compounds inhibited also the PCSK9 secretion level up to 72% (in the case of example #175), but the mechanism of action was not highlighted in the patent description. Finally, the pharmacophore characterization was assessed by crystallographic experiments performed on compounds **14–17** (patented as examples #5, #87, #105, and #188, respectively), which are endowed with a K_D value lower than 200 nM (Figure 10). Crystals were cryo-cooled for synchrotron data collection at ESRF beamline ID30A −1 or DLS beamline I04 on a Pilatus3 2 M or 6 M −F detector, respectively.

2.4. Year 2021

2.4.1. Merck Sharp & Dohme Corporation

In 2021, Merck Sharp & Dohme Corporation patented macrocycles representing the final stage of the development of PCSK9 inhibitors obtained by mRNA display technology [42–44]. This technique is a robust *in vitro* affinity selection system, enabling the examination of extensive peptide libraries genetically encoded. This platform controls the transcription/translation apparatus and post-translation cyclization, all within cell-free settings, to assemble these libraries. A noteworthy aspect of this technique is the covalent linkage of mRNA to the peptides it encodes, allowing for the amplification of desired peptides exhibiting interactions with the target of interest. The use of this technique, together with structure-based studies, led to the development of highly potent macrocyclic peptides with general structure **12**, considering the patent (Figure 11) [45]. As can be noted, the chemical structure of macrocyclic peptides bears a tricyclic amino acid portion with naturally occurring and non-naturally occurring amino acids variously linked via side chains.

The patented structures resulted from studies on linear peptides, progressively cyclized creating bridges between different points of the loops, giving the structure considerable conformational rigidity, protecting the cleavage sites susceptible to gut enzymes like trypsin, chymotrypsin [44]. In the first patent examples, it is interesting to note that the R^2 substituents are long chains containing various PEG, ethanolamine, glutamic acid, glycolic acid, glutamate, and long-chain dicarboxylic acids (C16-C18). These groups, variably linked by ester, ether, or amide bonds, impart zwitterionic characteristics to the structures, maybe enabling the fine tuning of the chemical-physical properties of the resulting compounds. The published papers [43,44] do not report any of these long chains, neither clarify the importance of them. Conversely, in both papers the authors stated that this portion is responsible for the PK properties of the compounds, influencing also the OATP inhibition and the mast cell degranulation, particularly when a LYS residue composes the R^2 substituent of the macrocycles. Nevertheless, the use of a PEG linker, equipped with a trimethylammonium group substituting the side chain of LYS, solved this PK issue maintaining, at the same time, the low Ki value displayed by the compounds.

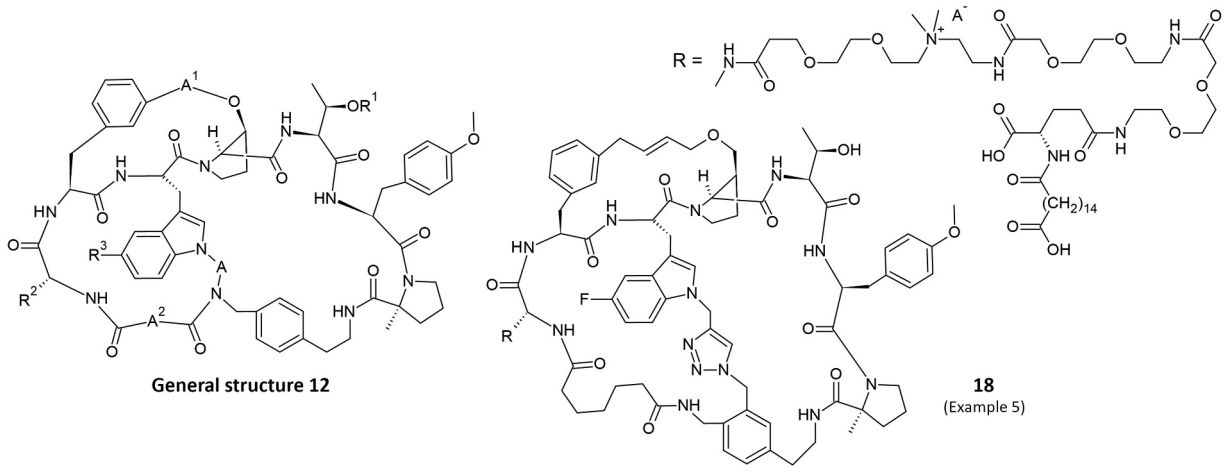


Figure 11. General structure of Merck Sharp & Dohme Corporation compounds, and chemical structure of compound 18 (Example 5), that represents the most potent compound. A^- is any pharmaceutical acceptable counter ion.

Structural information is known about the PCSK9/peptide complex and the binding mode of the compounds on PCSK9. In fact, from the deposited X-ray structures (PDB accession codes 6XIB to -F [43], 7S5G and 7S5H [42]), the macrocycles interact with PCSK9, preventing PPI with LDL-R, in the area shaped by the S153 and the β-hairpin in which the disulfide bridge C375-C378 is located.

The biological activity of the peptides was measured by Alexa TR-FRET experiments. Particularly, biotinylated PCSK9 was used while the peptides were tagged with AlexaFluor647, in the presence of Lance streptavidin europium. Among the 13 compounds reported as examples (described in the Tables 1 and 2 of the patent original document), the most active was compound **18** (patented as example 5), reported in Figure 11, displaying a K_i value of 0.13 nM.

In the same year, Merck Sharp & Dohme Corporation deposited another patent [46]. Comparing the structures covered by both patents, the general structure **13** (Figure 12) seems essentially the same but, in this patent, the A^1 and A^2 portions of the first patent (Figure 11) are better denoted. In particular, A^1 is -CH_2-NHCO-CH_2- while A^2 is -CHR^1-NHCO$(CH_2)_2$-. The previous patent already covered carbonyl derivatives as substituents at positions A^1 and A^2, but in this patent, 68 examples are given, displaying new compounds bearing new substituents A, R^1, and R^2 for which K_i values spanned from 0.131 to 0.001 nM, as resulted by K_i measurements accomplished by using the same TR-FRET method. The compound showing the lowest K_i value (0.001 nM) is compound **19** (patented as example 18), reported in Figure 12.

As can be seen, compound **19** has a -$(CH_2)_6$- alkyl chain as A substituent, a methyl group as R^1, and an R^2 chain bearing the dicarboxylic acid of compound 5 of the first patent. The gain in activity could be due to the optimal A^1 and A^2 substituents of the generic formula covered by the first patent. Furthermore, in the 68 compounds shown as examples (Table 1 of the original patent), the A-spacer of the general formula is always an alkyl chain bearing 5 or 6 carbon atoms, sometimes also unsaturated (i.e. examples 25, 44, 46). Compounds exhibiting these variations continued to show K_i <0.010 nM, an indication that these variations had little influence on the biological activity of the compounds tested. On the other hand, Merck Sharp & Dohme Corporation reported 68 examples bearing huge R^2 substituents, do not clearly displaying the structure of MK–0616, a very promising drug candidate entering the clinical phase III [47]. In the structure of MK-0616, the PEG linker as R^2 substituent, was replaced by a simpler -$(CH_2)_5$-$N^+(CH_3)_3$, do not present in any of the 68 reported example.

Despite the simplicity of the chain, MK-0616 retains a K_i value of 5 pM, indicating that the R^2 is not really critical for the PCSK9 inhibiting activity of the compounds. MK–0616, not only exhibited a strong affinity for PCSK9 in laboratory settings, but it demonstrated poor renal and hepatic clearance, adequate safety, and oral bioavailability during preclinical

Table 1. Structure and inhibition rate values of the most potent compounds reported in the patent.

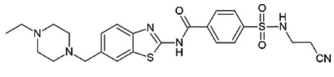
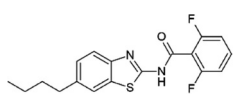
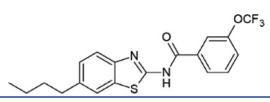
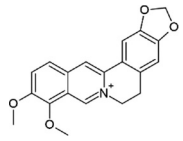
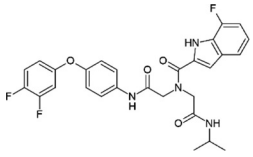
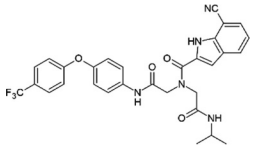
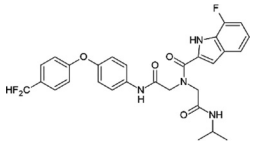
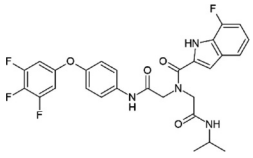
Compound	Inhibition rate (%)	Structure	IUPAC
24 (J14)	90.2		4-[N(2-ethyl)sulfonyl]-N-[6(4–2-ethylpiperazinylmethyl)benzothiazol-2-yl]benzamide
25 (J20)	86.8		*N*-(6-butylbenzothiazol-2-yl)-2–6-difluorobenzamide
26 (J22)	86.0		*N*-(6-butylbenzothiazol-2-yl)-3-(trifluoromethoxy)benzamide

Table 2. Biological activity and cytotoxicity of the positive control (Berberine) and compounds 28–31, ordered by PCSK9 inhibition. Notes: all values reported in the table below are intended to be percentage of activity at 10 μM of concentration. [1] the value refers to HepG2 ELISA test; [2] the value refers to a HepG2 RT-Q-PCR analysis PCSK9 mRNA analysis; [3] the value refers to cell viability measured in HepG2 cells.

Name	Structure	PCSK9 Inhibition[1]	Reduction of the expression of PCSK9[2]	Cytotoxicity[3]
Berberine (positive control)		52	35	n/a
28 (201 – 277)		≃ 100	86	90
31 (201 – 284)		94	90	≃ 100
30 (201 – 327)		90	88	98
29 (201 – 331)		87	92	≃ 100

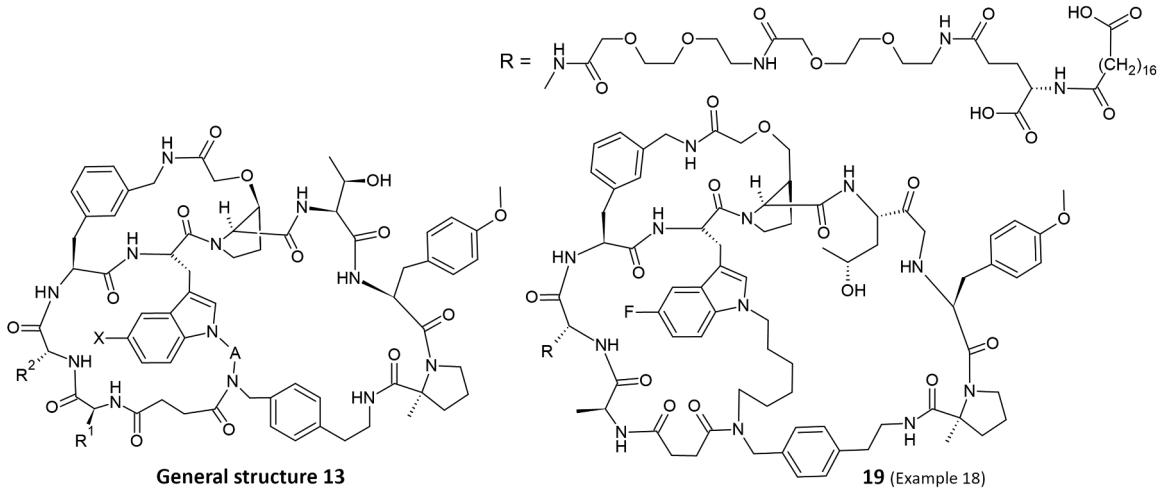


Figure 12. General structure of the second patent of Merck Sharp & Dohme Corporation compounds, deposited in 2021, and chemical structure of compound 19 (Example 18), that represents most potent compound described in the patent.

evaluations, facilitating its progression into clinical trials. MK-0616 suggests that, sometimes, the Lipinsky rule cannot be respected to attain drug candidates. In fact, it represents an example of high molecular weight drug deriving from the optimization of active peptides produced by mRNA display technologies. In Phase 1 clinical trials involving healthy adults, the administration of single oral doses of MK–0616 resulted in a substantial reduction of more than 93% in mean levels of free, unbound plasma PCSK9 (95% CI, 84–103). Among participants concurrently receiving statin therapy, the use of multiple oral doses of 20 mg MK-0616 once daily for 14 days led to a maximum reduction of 61% in mean levels of low-density

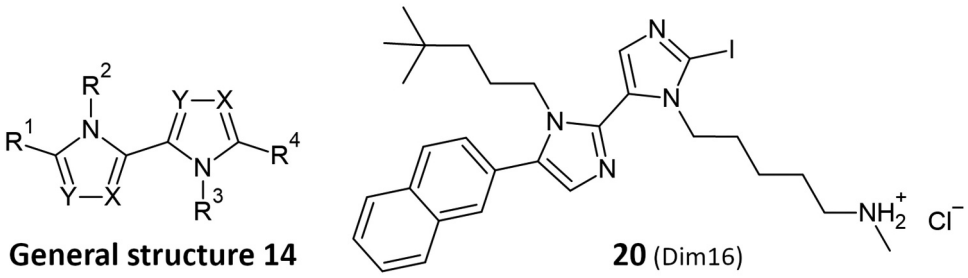


Figure 13. General structure of the diimidazole analogues and the most potent example of the Dim series (Dim16) patented by the University of Milan in 2021.

lipoprotein cholesterol from baseline (95% CI, 43–85) [48]. This work validates the approach of using peptide libraries from mRNA display technologies for the identification of novel oral therapeutic agents, exemplified by the invention of MK-0616.

2.4.2. University of milan

In 2021, the University of Milan patented a disclosure relying on diimidazole analogues with general structure **14**, endowed with high PCSK9 inhibiting activity (Figure 13) [49]. As stated by the authors in the papers preceding the application for the patent [50–54], the source of inspiration of these compounds derived from the presence of a β-sheet shaping the interaction between the EGF-A domain of LDL-R and PCSK9, as can be clearly seen in the X-ray structure deposited by McNutt et al.. (PDB accession code 3GCX) [55]. Stucchi et al. demonstrated that a poli-imidazole chain well resembles the β-strand conformation of a poli-Ala chain [50]. Then, the synthesis and the preliminary biological investigation of a simple N-Methyltetraimidazole (MeIm) validated this hypothesis, since it displayed an IC_{50} value in the micromolar range. The further optimization of the chemical structure of MeIm chain led to the Rim compounds [54] and, finally, to the synthesis of compound **20** (patented as Dim16) reported in Figure 13, displaying an IC_{50} value lower than 1 nM [56]. Further biological investigations of the compounds demonstrated that they can decrease the HepG2 LDL-C uptake and increase the LDLR population on the cell surface. Remarkably, similarly to other natural peptides derived from lupin seeds hydrolysis, Dim series displayed an inhibitory activity on the LDL-C metabolic pathway. In fact, some of the compounds covered by the patent can inhibit HMG-CoAR, the enzyme targeted by the statins. Additionally, compound **20** exerted platelet antiaggregating activity, opening the way to the development of a new therapeutical application of PCSK9 inhibitors [56].

2.5. Year 2022

2.5.1. Cardio Therapeutics Pty Ltd

Researchers from Cardio Therapeutics Pty Ltd. described the synthesis and biological evaluation of a series of small molecules, with general structure **15** (Figure 14) [57].

They are constituted by a central aromatic or heteroaromatic six-membered ring, which is decorated with a variety of substitution patterns. One hundred and fifty-four different compounds have been prepared by straightforward synthetic protocols and fully characterized by means of LC/MS(ESI) and ^{1}H NMR experiments. All compounds were assayed for their ability to inhibit the binding between PCSK9 and the LDLR using a CircuLex PCSK9-LDLR *in vitro* binding assay kit, displaying IC_{50} values in the high nanomolar range. All compounds were assessed for their oral suitability in Caco-2 assay. Caco-2 cells are a human colon epithelial cancer cell line used as a model of human intestinal absorption of drugs. The permeability coefficient (Papp) denotes the permeability of the drug through a monolayer of cells. *Ex vivo* LDLR surface expression on primary human lymphocytes was evaluated using most promising derivatives, namely example 3f (IC_{50} 939 nM), **compound 21** (patented as example 29, IC_{50} 320 nM, Figure 14), example 122 (IC_{50} 279 nM) and example 131 (IC_{50} 547 nM), in comparison with the positive control alirocumab, a fully humanized monoclonal antibody. These compounds, endowed with high bioavailability in the *in vitro* Caco2 study, were further assessed in a dedicated *in vivo* pharmacokinetic study. Within these tests, various additional

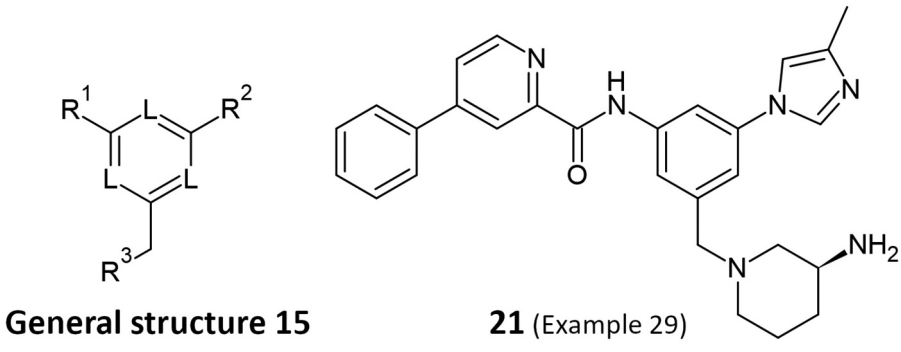


Figure 14. Representation of the general structure of the small molecules patented by Cardio Therapeutics Pty Ltd. and the most promising one, compound 21 (Example 29), showing potent PCSK9-LDLR inhibiting activity and tested in vivo.

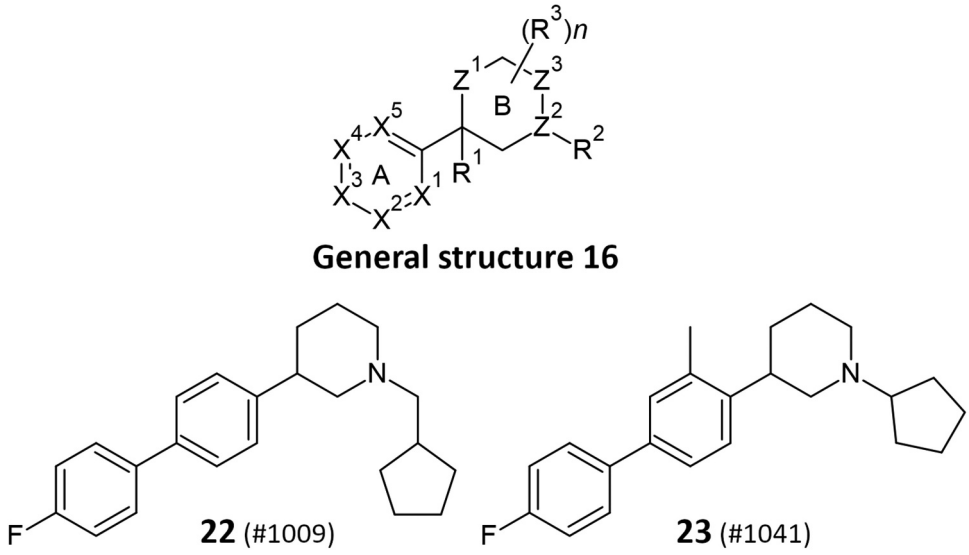


Figure 15. (Top) General structure of the small molecules patented by SRX Cardio LLC in 2022. (Bottom) Structure of the most promising compound 22 (#1009) from biological assays, and structure of the representative compound 23 (#1041) selected to evaluate the oral bioavailability and efficacy in mice.

ADMET properties of compounds of the invention were assessed, such as HepG2 cytotoxicity (HepG2 is a human hepatoma commonly used in drug metabolism and hepatotoxicity studies), hERG assay (the hERG channel inhibition assay is a highly sensitive measurement which identifies compounds exhibiting cardiotoxicity), and CYP assay (the CYP enzyme inhibition assay predicts a drug candidate's predisposition to inhibit cytochrome P450 enzymes). Among them, compound **21** was selected for *in vivo* efficacy study, as monotherapy and in combination with atorvastatin. As monotherapy, oral dosing of compound **21** at 30 mg/kg and 50 mg/kg were able to reduce total plasma cholesterol levels by more than 30% and 50%, compared to the vehicle control, respectively, over a period of four weeks. The results also show an increase in plasma PCSK9 levels over the same time period. The increase in PCSK9 plasma levels indicates that compound **21** is inhibiting the interaction of the protein with LDLR *in vivo* and, as such, PCSK9 cannot be taken up by the liver cells from the blood. Finally, the results from experiments in combination with atorvastatin demonstrate at least an additive effect. All these results confirm the efficacy of compound **21** to modulate PCSK9 levels and to reduce the circulating LDL-C following the oral dosing of the compound.

More information is also available in the research article published on the Journal of Lipid Research,in which the compound **21** (example 29) is reported as NYX-PCSK9i [58].

2.5.2. SRX cardio LLC

The patent of SRX Cardio LLC describes a series of small molecules with general structure **16** (Figure 15). They are constituted by a six-membered aromatic or heteroaromatic ring A and a six-membered non-aromatic ring B, linked together and decorated with various substituents. They present a stereocenter and are described as single stereoisomers or mixtures of stereoisomers. Seventy-five molecules are listed in the examples section, but the synthesis and structural characterization, as racemic mixtures, are briefly reported only for the most promising molecules [59].

Biological assays data are reported for selected compounds. Cells, such as HepG2, HuH7, FL83B, or a cell line transfected with a short-hairpin PCSK9 knockdown sequence have been cultured and analyzed for both cell viability marker (dead cells) and LDLR levels in live cells, using a flow cytometer. Cells incubated with small molecule compounds that are inhibitors of PCSK9 are expected to show increased amounts of LDLR, relative to control (no compound) specimens. The percentage recovery in the LDLR assay at 10 µM concentration showed that only molecules #1003, #1006, compound **22** (patented as #1009), and molecule #1035, have demonstrated a LDLR percentage recovery higher than 80%. Fluorescent-LDL uptake analysis by flow cytometric analysis was also carried out. The LDL-uptake assay showed that only compound **22** (Figure 15), and molecules #1010 and #1011, have demonstrated an EC_{50} at concentrations lower than 0.5 µM, representing the most promising compounds.

Finally, a representative compound **23** (patented as **#1041**) was tested for oral bioavailability and efficacy in mice (Figure 15). Following repeated treatment with the compound, a 72.1% reduction in AST (aspartate aminotransferase) levels and a 77.7% reduction in ALT (alanine aminotransferase) levels provide direct experimental evidence that the compound was reversing liver damage induced by a high fat diet and could be useful as a treatment for liver disease or liver dysfunction.

2.5.3. Hebei normal university & institute of medical biotechnology CAMS

Hebei Normal University and the Institute of Medical Biotechnology Chinese Academy of Medical Sciences patented a series of benzothiazole compounds with general structures **17 – 19**, or a pharmaceutically acceptable salt thereof, described in Figure 16 [60].

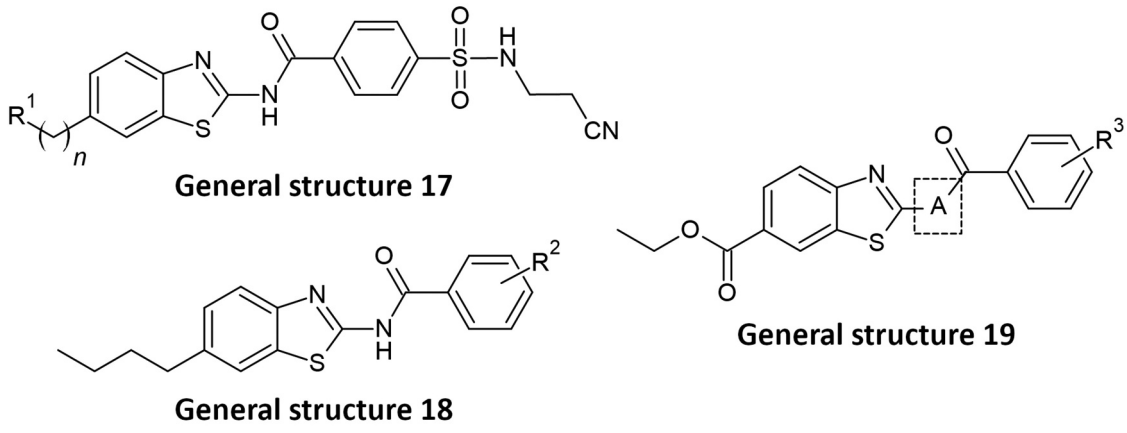


Figure 16. General structure of the benzothiazole patented by Hebei Normal University and Institute of Medical Biotechnology Chinese Academy of Medical Sciences in 2022.

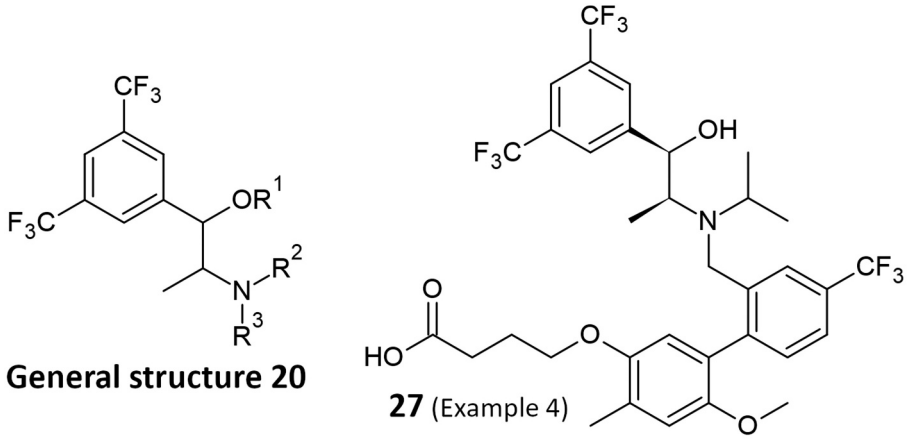


Figure 17. General structure of the new discovered amino alcohol derivatives and the most potent PCSK9-LDLR inhibitor reported in the patent of Daegu Gyeongbuk Medical Innovation Foundation and Kyungpook National University.

All the benzothiazole compounds mentioned above exhibit a significant inhibitory effect on PCSK9, making them suitable for the development of PCSK9 inhibitor drugs. In this experiment, HepG2 cells served as the experimental model and the inhibitory effect of the test compounds on PCSK9 activity was determined by chemiluminescent experiments.

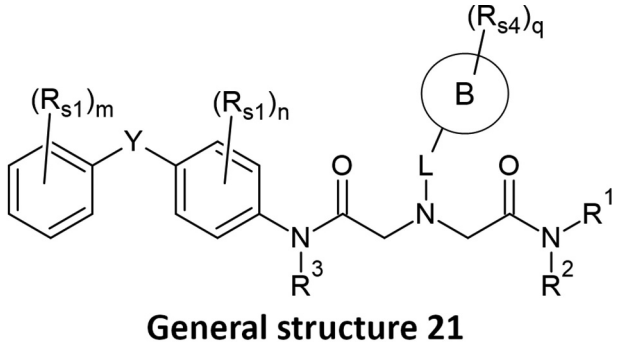
In this patent, the inhibitory activity of 49 compounds against PCSK9 protein was tested. Only the examples J13–23, J32–36, J43–44, and J49, exhibited an inhibition rate equal to or greater than 50%. Among all, the compounds **24–26** (patented as J14, J20, and J22, respectively) demonstrated the most significant inhibitory effect (Table 1).

2.5.4. Daegu gyeongbuk medical innovation foundation & kyungpook national university

The patent deposited by Daegu Gyeongbuk Medical Innovation Foundation and Kyungpook National University relates to the potential use of newly discovered amino alcohol derivatives with general structure **20** (Figure 17) [61]. This patent also comprises the pharmaceutically acceptable salts, hydrates, solvates, tautomeric forms, and stereoisomers of the compounds displayed. From the point of view of the formulation, the embodiments of the invention cover several administration methods: oral (sublingual, buccal), intrathoracic, subcutaneous, intramuscular, and parenteral.

A PCSK9-LDLR binding assay experiment was conducted on a culture of HepG2 cells to assess the inhibitory activity of the amino alcohol derivatives. As a positive control, the well-established PCSK9 inhibitor SBC – 115076 compound was used as described in the patent published by Shifa Biomedical Corporation in 2014 [24]. In particular, the binding inhibition rate of PCSK9 with LDLR was assessed by measuring the relative binding rate. The inhibitor's effect was evaluated by comparing the PCSK9-LDLR binding rate of the treated group to the control group (not treated with the inhibitor), set at 100. Results demonstrated that the reference compound SBC-115076, when treated at a single concentration of 10 μM, effectively inhibited the PCSK9-LDLR binding, showing a 47.39% inhibition rate.

Among all the compounds tested, 27 (patented as example 4), reported in Figure 17, demonstrated a superior PCSK9-LDLR binding inhibition (40.38% inhibition rate) compared to the positive control, indicating its potential as a PCSK9 inhibitor. These findings suggest that the compounds of the present invention have the potential to prevent, improve, or treat hypercholesterolemia by inhibiting the binding of PCSK9-LDLR.



General structure 21

Figure 18. General structure of the novel compounds patented by Shengke Pharmaceuticals Jiangsu LTD in 2023.

2.6. Year 2023

2.6.1. Shengke Pharmaceuticals Jiangsu LTD

The patent of Shengke Pharmaceuticals Jiangsu LTD describes the synthesis of 28 novel compounds based on a central glycine core, modified with a para–amino biaryl structure and an indole moiety with general structure **21** (Figure 18) [62]. The invention showed promising results for most of the compounds and the most interesting examples are reported in Table 2. Fluorinated biaryl structures are endowed in the most active molecules, where the halogen directly functionalizes the phenyl ring (compounds **28** and **29**, patented as 201 – 277 and 201 – 331, respectively) or through difluoro- (compound **30**, patented as 201 – 327) or trifluoro- (compound **31**, patented as 201 – 284) groups.

The compounds turned out to be potent down-regulators of the activity of PCSK9, producing a blood cholesterol-reducing action. In particular, the biological activity of the compounds was measured through the treatment of HepG2 cells with the respective drug and a reduction of the level of PCSK9 was observed and described as percentage of inhibition (Table 2). Moreover, a RT-Q-PCR analysis was conducted to clarify the mechanism of action of the molecules, strengthening their role in the depletion of the expression of PCSK9 (Table 2). The compounds showed a good level of safety during conventional cell viability assays. In addition, the authors performed a metabolic stability test incubating the compounds with human or rat liver microsomes, indicating that compound **31** (201 – 284), bearing a -CN group on the indole moiety and a para-CF3 substituted phenyl ring, represented the most stable candidate. The isoquinoline alkaloid berberine was used as a positive control in the biological experiments performed (Table 2).

2.6.2. China pharmaceutical university

In March 2023, the public China Pharmaceutical University provided a class of structurally novel compounds endowed with PCSK9 inhibitory activity to be used alone or in combination for the prevention and/or treatment of hyperlipidemia and related metabolic diseases [63]. Compounds **32–35** (Figure 19) were discovered *via* virtual screening and their biological activity was verified through *in vitro* protein-protein interaction inhibition experiments, protein binding force test experiments and *in cell* test experiments.

Moreover, the compounds could also be subjected to pharmaceutical formulation such as capsules, powders, tablets, granules, pills, injections, syrups, oral liquids, inhalants, ointments, suppositories or patches and other conventional pharmaceutical preparation forms. Compounds **32–35** demonstrated to inhibit the binding of PCSK9 protein to LDLR on the surface of liver cells by directly binding to PCSK9 protein, thereby inhibiting the degradation of LDLR, increasing the level of LDLR on the surface of liver cells, promoting the uptake and processing of LDL from the blood, and reducing cholesterol levels.

A lipid–lowering effect was observed for compounds **32–35** and can be further used in the development of hypolipidemic drugs also in association with already marketed pharmaceuticals. The experimental results show that compounds **32** (AG-205/07687040, CAS: 339023-59-5), **33** (AN-153/13396332, CAS: 797808-17-4), **34** (AH-487/41802246, CAS: 664316-79-4) and **35** (AN-919/15529007, CAS: 2099167-44-7) can directly inhibit the protein–protein interaction between PCSK9 and LDLR, as shown PCSK9-LDLR binding inhibition in vitro. During the assay, the inhibitory rates of compounds with formula **32–35** at

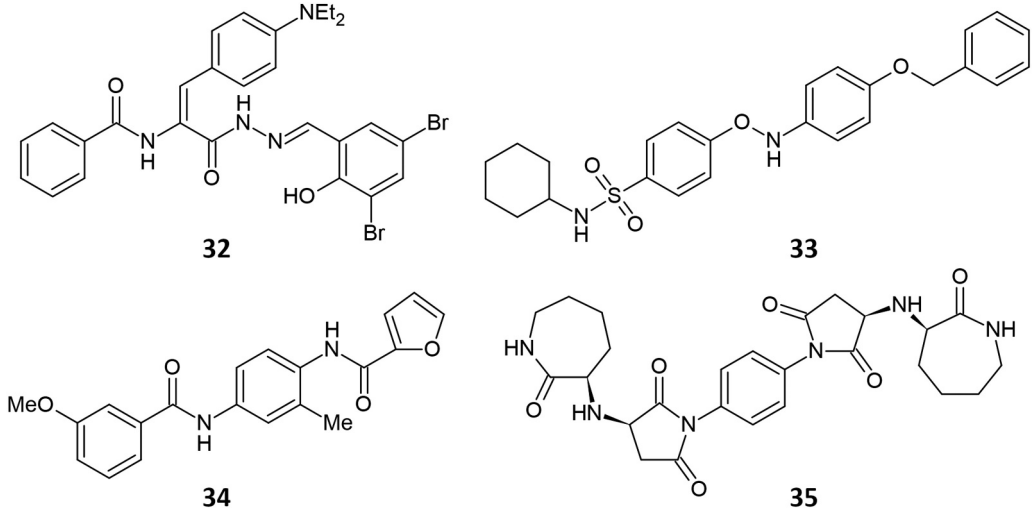


Figure 19. Chemical structures of compounds 32–35 patented by the public China Pharmaceutical University in 2023.

a concentration of 25 μM on PCSK9-LDLR protein-protein interaction were 42.0 ± 6.8%, 82.8 ± 1.7%, 45.9 ± 1.6% and 64.2 ± 0.7%, respectively. Further dilution experiments showed that the inhibitory activity of **35** on PCSK9-LDLR protein-protein interaction was dose–dependent (IC_{50} = 7.57 ± 1.40 μM). A strong binding to PCSK9 was observed for compound **35** using a SPR method, and the stability of the binding was also confirmed by computational studies. Compound **35** was tested for its ability to restore LDL uptake showing optimal results and the treatment of cells with high concentration of compound **35** showed a good inhibition of the expression of LDLR. Such features, combined with low cytotoxicity, indicated that compound **35** possesses a potential cholesterol-lowering activity and lipid-lowering effect, and can be further used to prepare blood-lipid-lowering drugs.

2.7. Expert opinion

Genetic studies have revealed that variations in the PCSK9 gene, specifically the R46L polymorphism, present in 2–3% of the population, are linked to lower levels of LDL-C and a protective effect against cardiovascular issues. This finding prompted the development of strategies aimed at inhibiting or eliminating PCSK9 activity. The rationale behind this approach stems from the observation that PCSK9 appears to primarily regulate the population of LDL receptors on liver cells, thereby influencing circulating LDL-C, with no apparent physiological roles beyond this function. The significant therapeutic potential of the PCSK9 inhibition has led numerous entities worldwide, including companies and academic institutions, to patent diverse compounds such as small molecules, natural and non-natural peptides, siRNA, and monoclonal antibodies. Now, only monoclonal antibodies such as alirocumab (Sanofi) or evolocumab (Amgen) and siRNA (Inclisiran, Alnylam and Novartis) reached the market for the treatment of CVD. Nevertheless, the high cost makes these remedies unusable in the underdeveloped countries and for this reason orally bioavailable small molecules never lose interest. For this reason, to date, many companies and universities have continued to search for PCSK9 modulators, as evidenced by the 810 patents filed in the last 5 years.

It was interesting to note that some patented compounds do not act only inhibiting the PCSK9/LDLR interaction. In fact, the compounds patented by us (diimidazoles) are also active in inhibiting HMG-CoAR (as statins), improving their hypocholesterolemic effect. Remarkably, some of the patented structures have started the processes of clinical development, in particular MK-0616 (demonstrating PCSK9 affinity in the pico molar range) has successfully progressed to clinical application and has shown to be effective in reducing cholesterol levels. However, the intense competition in this therapeutic field is causing pharmaceutical companies, such as AstraZeneca or Novo Nordisk, to reconsider projects that seem competitive in cholesterol reduction. This is because these projects may face challenges when integrating into a market that already has a longer-acting alternative, like Novartis' inclisiran. For this reason, other targets implicated in dyslipidemia are being contemplated as potential alternatives. Among them, seems promising decreasing the level of lipoprotein(a) [Lp(a)], an LDL cholesterol variant that contains an apolipoprotein(a) [apo(a)]. Novartis is leading Phase III program for pelacarsen, an antisense oligonucleotide (ASO), interacting with hepatocyte apo(a) mRNA, forming an ASO/mRNA complex. This complex hinders the translation of apo(a), resulting in reduced apo(a) production and consequently, diminished levels of circulating Lp(a) [64]. In the same category of RNA-based drugs belong volanesorsen (Akcea Therapeutics Ireland Limited) and olezarsen (Ionis Pharmaceuticals), ASO directed to apoC-III mRNA, disrupting apoC-III translation. This disruption results in decreased apoC-III levels, leading to reduced chylomicron and triglyceride levels. Phase III clinical trial findings for volanesorsen, published in 2021, revealed a 71.8% reduction in triglyceride levels compared to the placebo group over a three-month period [65]. Unfortunately, volanesorsen creates reactions in the injection site, and thrombocytopenia has been observed. Fortunately, lower side effects were observed by the subcutaneous administration of olezarsen [65].

In conclusion, the identification of PCSK9's involvement in cholesterol homeostasis has illuminated a wonderful player in cholesterol metabolism and in the prevention of coronary heart disease. PCSK9 represents a promising target for reducing LDL-C levels, amplifying the efficacy of other lipid-lowering drugs, particularly statins, and advancing the prevention and treatment of hypercholesterolemia. However, a plethora of academia and pharmaceutical companies are still involved in the development of PCSK9 inhibitors. It is hoped that other compounds can proceed into clinical development soon, in order to obtain powerful and effective drugs, as siRNA and monoclonal antibodies are, but accessible to all patients at low prices.

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Author contribution statement

All authors contributed to gather the patent-related information and to the preparation of the original draft. EMA Fassi created the figures. EMA Fassi and G Grazioso contributed to the manuscript revisions. All authors read and approved the final version of the manuscript.

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