Research Article

Molecular Docking Studies of Berberine Derivatives as Novel Multitarget PCSK9 and HMGCR Inhibitors

Munchalika Jaitrong[1*], Pornthip Boonsri[2] and Siritron Samosorn[1]

Received: 11 May 2021
Revised: 26 May 2021
Accepted: 27 May 2021

ABSTRACT

Hypercholesterolemia is a high risk for cardiovascular diseases, stroke, and mortality. Multitarget directed ligands (MTDLs) with dual inhibition of Proprotein Convertase Subtilisin/Kexin type 9 (PCSK9) and 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGCR) are the potential targets for the treatment of hyperlipidemia. Berberine (BBR), an isoquinoline alkaloid, has been reported to reduce Low-Density Lipoprotein cholesterol (LDL-c) with no severe side effects by increasing Low-Density Lipoprotein cholesterol Receptor (LDLR) expression. In addition, BBR also suppressed the increased levels of PCSK9 mRNA. Therefore, BBR might be the potent inhibitor for both enzymes. In this work, a novel series of BBR derivatives was designed based on molecular docking to serve as MTDLs for PCSK9 and HMGCR. The binding energy of BBR derivatives was investigated, which confirmed that all the designed compounds showed better binding energy than the parent BBR for both enzymes. The effect of different benzenesulfonyl ring substituents was explored to improve the binding affinity. The obtained results indicated that there are significant differences in their interactions and mode of binding. All 24 designed compounds were identified as potent multitarget inhibitors

[1]Department of Chemistry and Center of Excellence for Innovation in Chemistry, Faculty of Science, Srinakharinwirot University, Thailand
[2]Department of Chemistry, Faculty of Science, Srinakharinwirot University, Thailand
[*]Corresponding author, email: munchalika.mild@g.swu.ac.th

because the increase of hydrogen bond hydrophobic and electrostatic interactions were observed. Among them, compound **12** could be selected as the best candidate for further study as potential multitarget PCSK9 and HMGCR inhibitors for anti-hypercholesterolemia. The obtained result suggested that the introduction of the nitro-group plays a vital role in the binding pose of the BBR derivative. Finally, *in silico* study confirmed that most of the compounds pass the drug likeliness properties.

Keywords: Berberine, HMG-CoA reductase (HMGCR), Molecular docking, Multitarget directed ligands (MTDLs), Proprotein Convertase Subtilisin/Kexin type 9 (PCSK9)

Introduction

Cardiovascular diseases (CVDs) have been the leading causes of morbidity and mortality in Western countries for decades. Elevated levels of plasma total cholesterol (TC) and low-density plasma lipoprotein (LDL) cholesterol are major risk factors for CVDs. It is widely acknowledged that inhibiting the cholesterol synthesis and accelerating the cholesterol breakdown are the critical targets for controlling the TC level in the body [1]. Usually, the most effective way to control cholesterol synthesis is by inhibiting the 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGCR). The most widely used lipid-lowering drugs are statins. These drugs act as competitive inhibitors of HMGCR, leading to a reduction of endogenous cholesterol synthesis, depletion of intracellular cholesterol levels, and increase of hepatic low-density lipoprotein receptor (LDLR) expression by up-regulating the transcription of LDLR gene through the sterol regulatory element-binding protein (SREBP) pathway [2].

Conversely, statins have also been shown to induce the proprotein convertase subtilisin/Kexin type 9 (PCSK9) gene identified as the third gene linked to the autosomal dominant hypercholesterolemia through the SREBP pathway [1], as shown in Figure 1. Drug therapy should dramatically decrease the risk of overall mortality. However, in clinical practice, more than half of the CVDs patients do not reach their LDL-c goal; many of them undergo cardiovascular events, even with statins as their first-line therapy [2]. Multitarget-directed ligands (MTDLs) strategy was a recent approach for the rational design of new drug candidates. It has been used to develop novel compounds capable of acting in diverse biological targets. Therefore, these innovative ligands could play a vital role in advancing a broader and more efficient therapy [3-4].

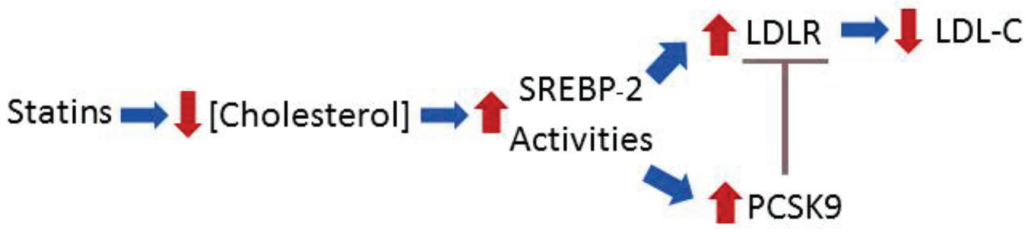


Figure 1 Regulation of the expression of LDLR and PCSK9 in the hepatic cell by statin adapted from mediating cholesterol homeostasis through SREBP2 LDLR PCSK9 signalling [5].

Natural products have received much attention as new therapeutic agents due to their pharmacological features, less toxicity, and cost-effectiveness. Studies of developing natural products as lipid-lowering agents have been reported in recent years. Berberine (BBR, Figure 1) is a quaternary ammonium salt derived from the protoberberine class of isoquinoline alkaloids and quite popular in the online market as a supplementary food. At present, increasing evidence has been reported as BBR can reduce LDL-c in animals, and several clinical studies have also tested in hypercholesterolemic patients with almost none of the side effects. Clinical studies showed that the administration of BBR reduced LDL-c level by 20% [2], mediated by increasing LDLR expression at the post-transcriptional level, the mechanism distinct from statins [6]. Because of its unique action and safety record, BBR is ideal for combination therapy with statins to treat hypercholesterolemia. In addition, the combination of BBR and Simvastatin showed an improved lipid-lowering effect with a 31.8% reduction of serum LDL-c [2]. Moreover, there are few reports on BBR's effects on levels of PCSK9 mRNA. It has been reported that BBR reduces the level of PCSK9 protein secreted into the media. In addition, the combination of BBR and mevastatin increases LDLR protein levels while suppressing the increased levels of PCSK9 mRNA caused by mevastatin alone [7]. Besides, BBR decreased PCSK9 mRNA and protein levels in a time- and dose-dependent manner. It was not due to the increased PCSK9 mRNA degradation but most likely to reduce transcription of PCSK9 [1]. Then, BBR might be the potent therapeutic agent for HMGCR that reduced LDL-c level by increased expression of LDLR like statin or/and decreased PCSK9 expression.

Nevertheless, a detailed molecular hyperlipidemia study on multifunctional PCSK9 and HMGCR inhibitors by BBR was still not deciphered. Due to the synergistic effect of BBR and Simvastatin on reducing serum LDL-c, naphthalene moiety, which is a part of Simvastatin and mevastatin structures (Figure 2), was designed to substitute at the C-13 of BBR. In addition, polar substituents such as the benzenesulfonyl group were also substituted for the C-9 methyl side chain of BBR to provide stronger binding to both targets. In this study, based on the core structure of BBR, a novel series of naphthalene-containing BBR as multitarget PCSK9 and HMGCR inhibitors were designed by computer-aided drug design that may represent a possible way to solve the time-consuming as shown in Figure 2. The interaction between BBR and both target enzymes will be allowed to characterize and explain compounds' behavior and the detailed structure-activity relationship (SAR) analysis via molecular docking to restrict the number of experimental models worth testing for biochemical processes.

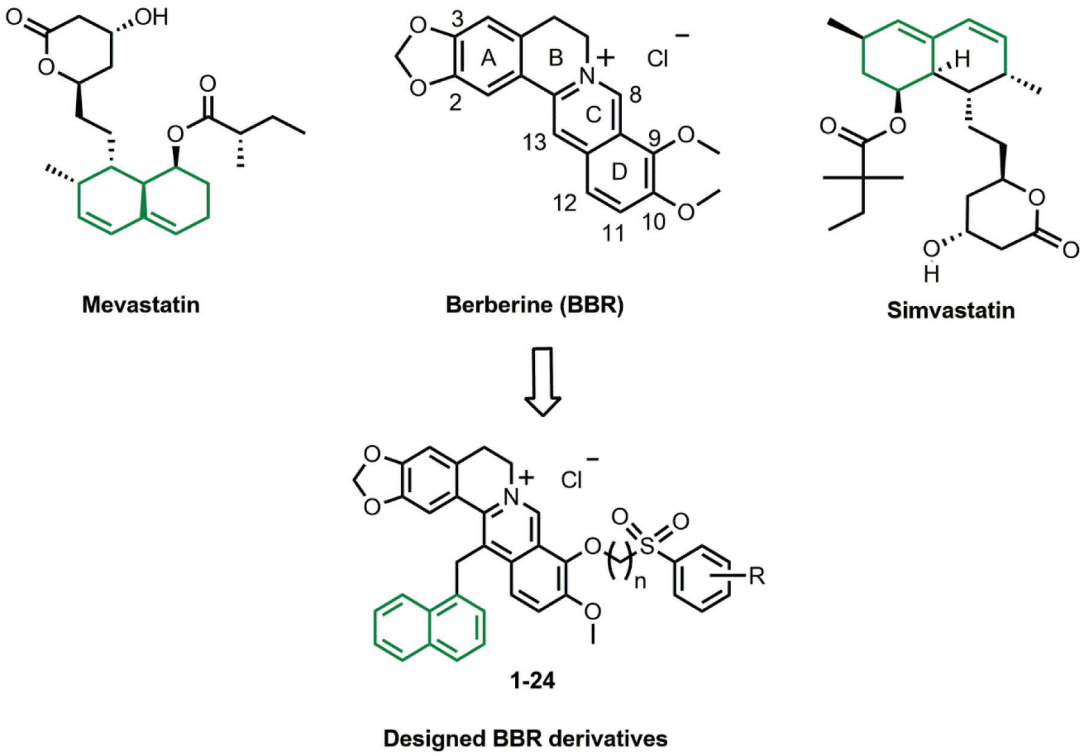


Figure 2 Design of BBR derivatives based on the structures of BBR and statins (Mevastatin and Simvastatin).

Materials and Methods

1. Molecular Docking studies

1.1 Preparation of protein

The X-ray crystal structure of the human PCSK9 and HMGCR were downloaded from the RCSB protein data bank (http://www.rcsb.org). PCSK9 (PDB code 3GCX) with a resolution of 2.70, constructed in the presence of the EGF-A domain of the LDLR at neutral pH [8]. In contrast, the structure of HMGCR (PDB 3CDB) was a tetramer resolved at 2.3. Chains A and B were reserved because the binding site was composed of A and B chains [9]. Both protein structures were firstly checked for missing atoms, residues, bonds, and contacts. Discovery studio 2020 was used to remodel both target enzymes structure by removing water molecules and co-factors. Then, the hydrogen atoms were added to the protein using AutoDock Tools (ADT) 1.5.6 [10].

1.2 Preparation of ligands

Structures of Berberine (BBR) were downloaded from the PubChem Compound Database (National Center for Biotechnology Information; https://pubchem.ncbi.nlm.nih.gov/). The structures of BBR and its derivatives called ligands were energy minimized at the B3LYP/

6-31G (d,p) using the G09 program [11]. The stable 3D ligands were prepared in combinations with nonpolar hydrogens, gasteiger charges, rotatable bonds based on the ADT program.

1.3 Molecular docking

Molecular docking was performed using the AutoDock 4.2 [12] package that explores the conformational space of the ligand using the Lamarkian genetic algorithm (LGA), which is a hybrid of a genetic algorithm (G.A.) with an adaptive local search (L.S.) method. Ligands were docked individually to the protein with grid coordinates (grid center) and grid boxes of specific sizes for each receptor. For PCSK9, the grid size was set at 60 x 60 x 60 (x, y, and z) points, and the grid center was designated at x, y, and z dimensions of 13.805, -36.325, 0.195, respectively, with a resolution of 0.375. While docking study of HMGCR, the grid size was set at 60 x 60 x 52 (x, y, and z) points, and the grid center was designated at x, y, and z dimensions of 13.915, 6.736, 47.782, respectively. Then, the initial population was set to 150 individuals; the maximum number of energy evaluations was 2.5 x 10^5, and a root mean square deviation (RMSD) tolerance set to 2.0 angstroms, the maximum number of generations was 27,000. Finally, the docking results were analyzed to find the best-clustered compounds with the lowest free energy of binding. Post-docking analyses were visualized using the Discovery Studio program, which showed the mode of binding, the intermolecular protein-ligand interaction, and bonding distances as interaction radii of <5 Å position the docked ligand.

2. Analysis of drug likeliness

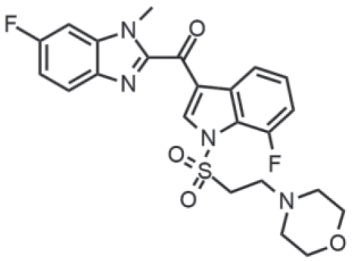
Bioavailability plays an essential role in the progress of bioactive compounds as healing agents [13]. The drug-likeness properties were analyzed to select the compounds that comply with Lipinski's rule of five. Accordingly, the potential orally drug is active when the criteria of this rule were determined. Compounds are more likely permeable and active such as molinspiration Log partition coefficient (miLogP), molecular weight, number of hydrogen bond acceptors and donors, molecular polar surface area (TPSA), and number of rotatable bonds were calculated by using free software (http://www.molinspiration.com/) [14].

Results and Discussion

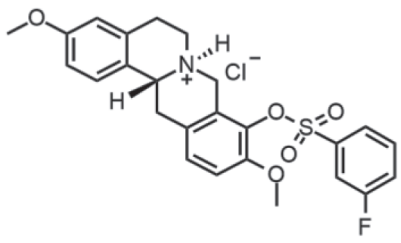
AutoDock approach was applied to study the binding affinities of BBR derivatives to both PCSK9 and HMGCR proteins to obtain the novel multitarget inhibitors. The docking simulations provide insights into the possible binding modes and orientations between ligand and protein. In the present work, the 24 designed compounds were docked into the target proteins.

Designing a new series of Berberine

Statins, as competitive inhibitors of HMGCR, are the most widely used lipid-lowering drugs. These drugs improved the clearance of intracellular cholesterol levels by increased hepatic LDLR expression. *Cameron et al.* studied the effect of BBR and mevastatin on the amount of PCSK9 and LDLR mRNA. The results suggested that mevastatin has also been shown to induce the expression of PCSK9, whereas BBR decreased PCSK9 mRNA and increased LDLR mRNA. Besides, combining BBR and mevastatin could be helpful due to suppression of mevastatin's upregulation of PCSK9 mRNA [1]. To improve the therapeutic potential and pharmacokinetic limitations of BBR, several structures were modified at the C-2, C-3, C-8, C-9, C-10, C-12, and C-13 positions of the BBR scaffold (Figure 2) [15]. The structure-activity relationship (SAR) analysis of BBR indicated modifications on C-9 could essentially enhance cholesterol-lowering activity; however, there was a limited report on applying this strategy to structure optimization at the C-9 position. Most of these studies focused on lipophilic modifications by introducing long-chain alkyl groups [16]. Compounds **1-7** were designed by introducing a long alkyl chain branched (n = 0-6) with the benzenesulfonyl group at the 9-position. The substituted group shared a similar structure with the previous study, compound **33** as PCSK9 modulators (Figure 3), when introduced with a sulfonamide alkyl linker, showed very promising activity with EC_{50} values less than 1 nM [17]. In addition, **CVI-LM001** (Figure 3), the fluorobenzenesulfonate derivative of corydaline, demonstrated increased hepatic LDLR expression, inhibition of PCSK9 expression, and activation of AMP-activated protein kinase (AMPK) [18].



33

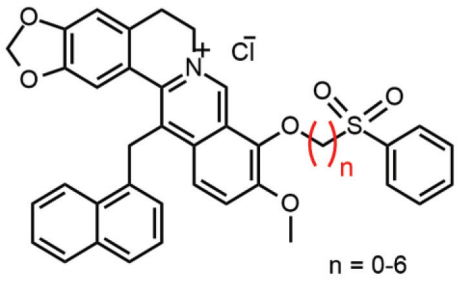


CVI-LM001

Figure 3 Structures of small molecules as inhibitors of PCSK9.

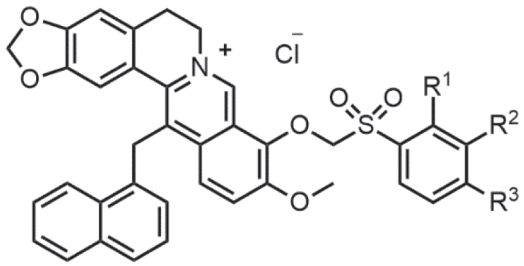
Also, it has been reported that the long hydrophobic alkyl chains at 13-position were suggested to increase the bioavailability [15]. The naphthalene moiety, an HMG-CoA-like moiety hydrophobic ring structure important in binding to the HMGR, was attached to compound **2-8** at 13-position. The results showed that all designed compounds demonstrated better binding energy (BE) than parent BBR, as shown in Table 1. Among them, compound **3** shows the lowest BE. The results confirmed that introducing an alkyl group at the C-9 position to BBR could decrease hypercholesterolemia, supporting the previous study [16]. The naphthalene moiety at C-13 positions of the BBR scaffold could improve the binding affinity and might be the right candidate multitarget directed ligands (MTDLs) with dual inhibition of PCSK9 and HMGCR.

Table 1 Binding energy (BE) of BBR and BBR derivatives to PCSK9 and HMGCR were obtained from molecular docking studies compared to the standard drug, Mevastatin.



Compound	$[CH_2]_n$	BE (kcal/mol)	
		PCSK9	HMGCR
1	0	-7.56	-8.58
2	1	-8.00	-9.75
3	2	-7.35	-8.45
4	3	-7.52	-9.04
5	4	-7.07	-9.36
6	5	-7.40	-9.05
7	6	-6.68	-7.85
BBR	-	-5.50	-5.69
Mevastatin	-	-5.14	-7.42

Table 2 Binding energy (BE) studied the effect of substituent group at the benzenesulfonyl ring of BBR derivatives on PCSK9 and HMGCRs



Compound	R^1	R^2	R^3	BE (kcal/mol)	
				PCSK9	HMGCR
8	F	H	H	-7.64	-8.53
9	H	F	H	-8.14	-9.56
10	H	H	F	-8.09	-9.95
11	NO_2	H	H	-7.92	-11.04
12	H	NO_2	H	-7.80	-11.56
13	H	H	NO_2	-7.80	-11.11
14	CH_3	H	H	-7.59	-10.20
15	H	CH_3	H	-8.35	-10.20
16	H	H	CH_3	-8.41	-8.85
17	OCH_3	H	H	-7.87	-9.24
18	H	OCH_3	H	-7.73	-9.18
19	H	H	OCH_3	-8.18	-8.64
20	H	CN	H	-7.47	-9.78
21	H	OH	H	-8.03	-9.33
22	H	$C(CH_3)_3$	H	-8.06	-8.93
23	H	Cl	H	-7.88	-9.46
24	H	CF_3	H	-7.73	-8.49
BBR	-	-	-	-5.50	-5.69
Mevastatin	-	-	-	-	-7.42

Effect of substituent group at the benzenesulfonyl ring

Structure-activity relationships were investigated in order to explore the potent PCSK9 and HMGCR inhibitors. Therefore, the effect of substituent position at the benzenesulfonyl ring was firstly focused. Compounds **8-13** contained F and NO_2 represent an electron-withdrawing group at *ortho*-, *meta*-, *para*-position. The obtained results indicated that there are no significant differences in their interactions and mode of binding. An electron-donating group was CH_3 and OCH_3 to give **14-19**, which give similar results to an electron-withdrawing group as shown in Table 2. The *meta*-position was identified as the most potent inhibitor due to strong interactions for both enzymes. Furthermore, the different substituents on *meta*-position were introduced to improve the binding affinity. The binding energy between BBR derivatives and each target protein was shown in Table 2, and the molecular interaction was considered insight into the binding modes.

3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase

The enzyme HMG-CoA reductase, an important molecular target of statins, catalyzes the mevalonate pathway's reaction to convert HMG-CoA to mevalonate. Statins effectively reduce cholesterol serum levels, attenuating cholesterol synthesis in the liver by competitive inhibition regarding the substrate HMG-CoA. In contrast, the HMGCR binding pocket is located between the L- and S-domains [19]. The previous study found that twenty-one residues from neighboring monomers contribute to the binding, namely: Arg590, Ser661, Val683, Ser684, Asn686, Asp690, Lys691, and Lys692 residues of chain A and Glu559, Gly560, Cys561, Leu562, Ser565, Arg568, Lys735, Ala751, His752, Asn755, Ser852, Leu853, Ala856, and Leu857 residues of chain B [20-21]. In addition, an analysis of binding energies between compounds **9, 12, 15, 18, 20-24** and HMGCR protein showed excellent binding energies at -8.49 to -11.56 kcal/mol (Table 2), which also better than parent BBR (-5.69 kcal/mol) and mevastatin (-7.42 kcal/mol). Then, the docking poses of designed compounds were considered in comparison to the standard drug. Mevastatin explored hydrogen bonds to the key residues, including Glu559, Lys691, Lys692, and Asn755, located at the HMGCR binding site. Remarkably, compounds **9** and **12** showed hydrogen bonding interactions similar to mevastatin. Whereas compounds **18, 20, 21** did not involve those key residues. Interestingly, all compounds occurred hydrophobic interactions with the amino acid comparable to the standard drug shown in Table 3. Besides, Arg590 and His861 which found in BBR might be the crucial residues for the electrostatic interaction. Even though the different substituent groups at the *meta*-benzenesulfonyl ring significantly influenced their conformation change, those compounds are still bound to the key residues of HMGCR. Considering the interaction, compound **12** showed the strongest

binding affinity with HMGCR. The 2D and 3D binding posed between compound **12** and HMGCR were shown in Figure 4(A, B). To summarize, the results indicated that introducing the naphthalene and the benzenesulfonyl moiety increased the hydrogen bond and hydrophobic interaction to the HMGCR binding site in comparison with BBR.

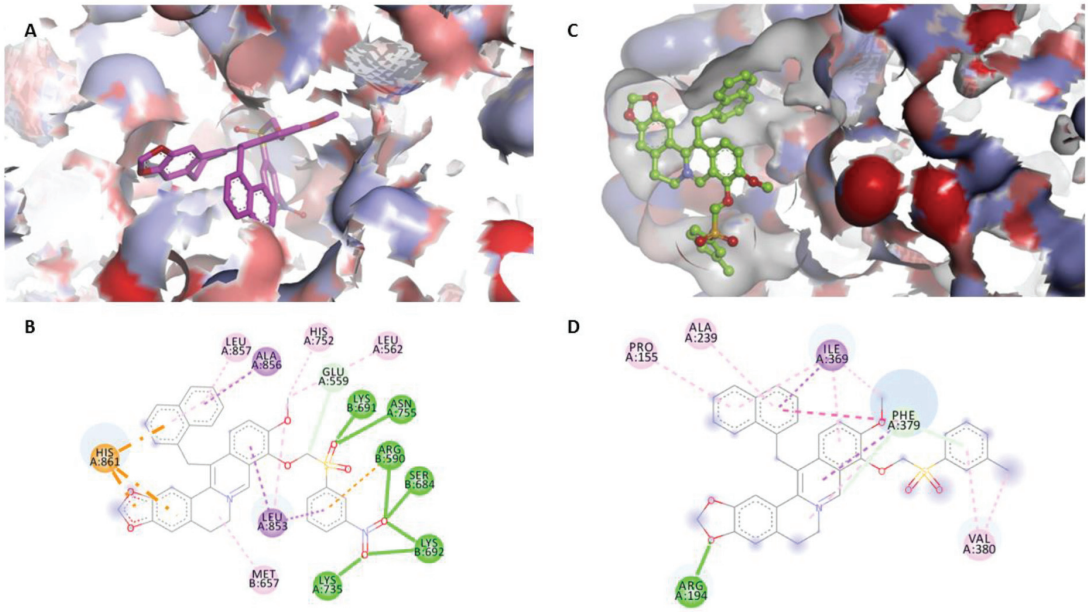


Figure 4 (A) Illustrate the 3D interaction of the best docking pose of compound **12** in the binding sites of HMGCR (B) 2D molecular docking model of compound **12** with HMGCR. (C) Illustrate the 3D interaction of best docking pose of compound **15** on the surface of PCSK9 (D) 2D diagram of docking structure of compound **15** with PCSK9. The hydrogen bonds are represented as solid green lines, hydrophobic interactions as dashed pink and purple lines, electrostatic interactions as dash-dotted orange lines.

Table 3 Interactions of HMGCR amino acid residues with the different substituent groups at *meta*-position of benzenesulfonyl BBR derivatives.

Compound	Amino acids involved		
	Hydrogen bond	Hydrophobic interactions	Electrostatic interactions
9	Glu559, Arg590, Ser684, Lys691, His752, Asn755, His861	Leu562, Ala564, Ser565, His752, Leu853, Ala856	Arg590
12	Glu559, Arg590, Ser684, Lys691, Lys692, Lys735, Asn755	Leu562, Met657, His752, Leu853, Ala856, Leu857, His861	Arg590, His861
15	Arg590, Lys691, His752, Asn755, His861	Cys561, Leu562, Ala564, His752, Leu853, Ala856, Leu857	Arg590
18	Thr558, Gly560, His861	Cys561, Leu562, Lys662, Leu853, Ala856, Leu857, His861	Glu559, Lys691
20	Ala525, Thr558, Gly560, Asn658, Gly808	Cys561, Leu562, Leu853, Ala856, Leu857, His861	Glu559, His861
21	Ala525, Thr558, Gly560, Asn658, Gly808, Ala856	Cys561, Leu853, Ala856, Leu857, His861	Asp767, His861
22	Lys691, Asn755	Leu562, Met657, Lys662, Leu853, Leu857, His861	Arg590, Glu559, His861
23	Ala525, Glu559, Gly560, Asn658, Ala856	Cys561, Ala654, Met655, Met657, Leu853, Ala856, Leu857, His861	His861
24	Lys691, His752, His861	Cys561, Leu562, Ala564, Met657, His752, Leu853, Ala856	Arg590, Asp690, His861
BBR	Ser684, Lys735, Ala751	Met657, Leu853, Ala856, Leu857	Arg590, His861
Mevastatin	Glu559, Lys691, Lys692, Asn755	Leu562, Met657, Val683, His752, Leu853, Leu857, His861	-

Proprotein Convertase Subtilisin/Kexin type 9 (PCSK9)

Multiple clinical studies have shown that injectable antibody therapeutics that impede the PCSK9-LDLR protein-protein interaction (PPI) significantly decrease circulating LDL levels. Subsequently, two antibody drugs that disrupt PCSK9-LDLR were FDA approved; they appear to be tolerated well, with no severe side effects and are efficacious. Small molecule drugs have been the preferred modality for disruption of PCSK9-LDLR; however, based on the mode of administration, cost, shelf life, and immunogenic response issues [8]. PCSK9 binds to LDLRs on the cell surface, leading to their degradation. The binding site of PCSK9 localizes the epidermal growth factor A (EGF-A) domain of the LDLR. The critical interactions of PCSK9 with EGF-A

are between residues 377-379 of PCSK9. Also, Arg194, Asp238, Ile369 of PCSK9 are essential for the binding [18]. BBR was reported to down-regulate PCSK9 [1]. In the molecular study, BBR interacted with three key residues; hydrophobic interaction formed with Ile369, Phe379, and strong hydrogen bond with Arg194. While compounds **9, 12, 15, 18** and **20-24** were investigated, focusing on the different substituent groups on *meta*-position at the benzenesulfonyl ring. The results showed significant differences in their interactions and binding mode. All compounds interacted well with PCSK9 showing binding energies of -7.47 to -8.35 kcal/mol (Table 2), which better than BBR (-5.50 kcal/mol). Most designed compounds correlated with PCSK9 protein by forming two hydrogen bonds with Arg194, Phe379. In addition, all compounds involved with two amino acids in hydrophobic formation interactions; Ile369, Phe379, except compound **22.** For an electrostatic interaction, compounds **12, 18, 21,** and **24** were formed with Arg194. Interestingly, the result indicated that hydrophobic interaction was the main contribution for BBR derivatives to bind with PCSK9 shown in Table 4. From this set of compounds, the *meta*-NO_2 substituent at the benzenesulfonyl moiety **(15)** showed the lowest BE. The docking posed of compound **15** located on the surface of PCSK9 which shown in Figure 4(C). The 2D interactions were clearly explained in Figures 4(D). Finally, the novel BBR derivatives established similar binding modes to the PCSK9 binding site as compared to EGF-A.

Table 4 Interactions of PCSK9 amino acid residues with the different substituent groups at *meta*-position of benzenesulfonyl BBR derivatives.

Compound	Amino acids involved		
	Hydrogen bond	Hydrophobic interactions	Electrostatic interactions
9	Arg194, Phe379	Pro155, Ala239, Ile369, Phe379, Val380	-
12	Arg194, Phe379, Ser381	Pro155, Ala239, Ile369, Phe379, Val380	Arg194
15	Arg194, Phe379	Pro155, Ala239, Ile369, Phe379, Val380	-
18	Arg194, Thr377, Phe379	Pro155, Ala239, Ile369, Phe379, Val380	Arg194
20	Phe379, Ser381	Ile369, Phe379	-
21	Arg194, Ser372, Phe379	Pro155, Ala239, Ile369, Phe379, Val380	Arg194
22	Arg194, Thr377, Phe379	Ala239, Cys375, Cys378, Phe379, Val380	-
23	Arg194, Phe379, Ser381	Pro155, Ala239, Ile369, Phe379, Val380	-
24	Arg194, Thr377, Phe379	Pro155, Ala239, Ile369, Phe379, Val380	Arg194
BBR	Arg194	Ile369, Phe379	-

Drug likeness

The observation of the most biologically active drugs was evaluated by Lipinski's rule of five. This simple rule is based on a molecular weight (MW) of approximately 500, log P values not exceeding 5, hydrogen donor bond (HBD) not more than 5, and hydrogen bond acceptor bond (HBA) not greater than 10. The bioavailability of drugs links to the polar topological surface (TPSA) and the number of rotatable bonds (nRB) [22]. The molecular properties of ten designed compounds were calculated using molinspiration software compared to standard drugs' values: mevastatin and the natural product (BBR). Considering these recommendations, the results presented in Table 5 indicate that all the compounds analyzed have MW > 500. So, they have poor drug distribution that depends on drug size and lipophilicity, confirmed by miLogP. The value of miLogP must be estimated 0.4 to 5; the obtained results showed that most of the compounds agree with miLogP, except **22** miLogP value > 5. Highly lipophilic value was indicated that drug only be absorbed after dissolving in the digestive tract. In addition, TPSA and nRB are a perfect descriptor characterizing drug absorption, including intestinal absorption, bioavailability, and penetration of the blood-brain barrier. The designed compounds showed TPSA < 140 $Å^2$ and nRB < 10. Then, they should exhibit good intestinal absorption. In addition, low TPSA and total hydrogen counts (HBD and HBA) are significant predictors of good oral bioavailability. All compounds passed the evaluation (HBD and HBA counts) and qualified to be used as drugs. Furthermore, the designed BBR derivatives fulfill the Lipinski's rule, which implies potent oral bioavailability multitarget inhibitors, except **22**.

Dual binding inhibitor of novel Berberine derivatives

A recent approach for the rational design of the MTDLs approach has gained increasing attention, which has developed various hybrid compounds acting simultaneously on diverse biological targets such as Alzheimer's disease [23]. Hypercholesterolemia is the risk factor of mortality; however, more than 50% of patients do not reach their goal for prevention and treatment. In the present work, the novel series of BBR derivatives were designed based on *in silico* study as the multitarget inhibitor for anti-hypercholesterolemia. To summarize, the obtained docking studies of all compounds to both target enzymes clearly explained the molecular rationality design. Most of them showed satisfying results in the mode of binding, binding affinity, and drug-likeness properties, except compound **22;** MW > 500, miLogP > 5. In considering, **12** is the *meta*-NO_2 substituent at the benzenesulfonyl moiety, chosen as the excellent candidate for further development to be the lead compound. It showed the binding affinity to PCSK9 and HMGCR at -7.38 and -11.56 kcal/mol, respectively. More importantly, **12** showed a similar mode of binding to the previous works concerning PCSK9 [18] and

Table 5 Physicochemical properties of *meta*-benzenesulfonyl *ring*-containing BBR derivatives.

Compound	MW	miLogP	HBA	HBD	TPSA	nRB
2	616.72	3.73	7	0	74.96	7
9	634.71	3.87	7	0	74.96	7
12	661.71	3.66	10	0	120.79	8
15	630.74	4.15	7	0	74.96	7
18	646.74	3.76	8	0	84.20	8
20	641.73	3.46	8	0	98.75	7
21	632.71	3.22	8	1	95.19	7
22	672.82	5.41	7	0	74.96	8
23	651.16	4.38	7	0	74.96	7
24	684.71	4.60	7	0	74.96	8
BBR	336.37	0.20	5	0	40.82	2
Mevastatin	404.55	4.35	5	1	72.84	7

MW: Molecular Weight; miLogP: molinspiration Log partition coefficient; HBA: Hydrogen bond acceptor; HBD: Hydrogen bond donor; TPSA: topological polar surface area; nRB: Number of rotatable bonds.

HMGCR [20], making it a highly potent inhibitor than other compounds. Through analysis, the NO_2-substituent group enhanced the binding affinity of BBR derivatives to both target enzymes, as shown in Figure 5. Considering PCSK9, BBR scaffold, and the naphthalene moiety occurred much hydrophobic interaction through the residues Pro155, Ala239, Ile369, and Phe379 via Pi-alkyl, alkyl. In addition, the core structure of BBR formed electrostatic interaction with Arg194 residue. The benzenesulfonyl moiety generated Pi-sulfur to key residue Cys378, hydrophobic interaction with Val380, and hydrogen bond via SER381. Interestingly, the key residue Phe379 generated a conventional hydrogen bond and Pi-lone pair with the nitro- group, as shown in Figure 5(A). For HMGCR binding, ring A of BBR structure occurred electrostatic interaction with His861. Five hydrophobic interactions formed to ring B (Met657), ring D (Leu853), and methyl at C-10 position (Leu562 and His752). The naphthalene moiety in the BBR scaffold was established hydrophobic with Ala856, Leu857, and electrostatic interactions through His861. The benzenesulfonyl moiety showed hydrogen bonds with Lys691, Asn755, generated electrostatic interactions of the benzyl group through Arg590 and formed hydrophobic

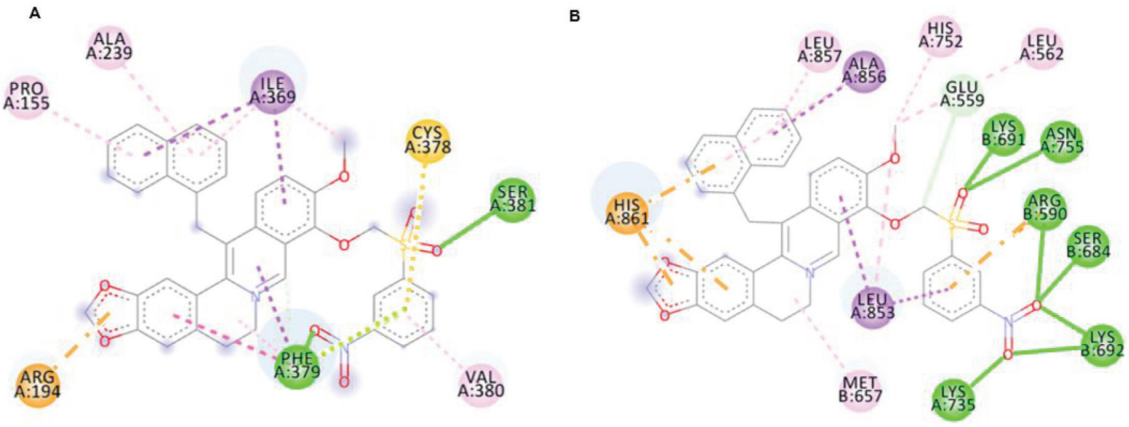


Figure 5 2D interactions between **12** and target proteins (A) PCSK9 (B) HMGCR. The hydrogen bonds are represented as solid green lines, hydrophobic interactions as dashed pink and purple lines, electrostatic interactions as dash-dotted orange lines.

interaction with Leu853. Surprisingly, the nitro-group formed five conventional hydrogen bonds to Arg590, Ser684, Lys692, and Lys735, which play an essential role in HMGCR binding, as shown in Figure 5(B).

Conclusion and Discussion

A novel series of berberine derivatives as multifunctional inhibitors has been designed and evaluated the binding affinities for PCSK9 and HMGCR. The obtained results showed that most of the designed compounds exhibited strong binding interactions to both target proteins than the parent BBR and the standard drugs (Mevastatin). Hence, the introducing aromaticity at the C-13 position and benzenesulfonyl moiety at the C-9 position increases the binding affinity of BBR derivatives. The different benzenesulfonyl substituents improved the binding mode and orientation, enhancing the possibility of binding with key residues. Additionally, *in silico* study confirmed that most of the compounds fulfilled drug-likeness properties. Finally, further experimental approaches can be applied to prove that the novel berberine derivatives can be further developed as potential multitarget PCSK9 and HMGCR inhibitors for hypercholesterolemia treatment.

Acknowledgements

This work has been provided computing resources by the grant numbers 483/2563 and 485/2563 from the Faculty of Science, Srinakharinwirot University. Department of Chemistry, Faculty of Science, Srinakharinwirot University is gratefully acknowledged for providing research facilities. Finally, the Center of Excellence for Innovation in Chemistry (PERCH-CIC) is thankfully acknowledged for scholarship for MJ.

References

1. Cameron, J., Ranheim, T., Kulseth, M. A., Leren, T. P., & Berge, K. E. (2008). Berberine decreases PCSK9 expression in HepG2 cells. *Atherosclerosis, 201*(2), 266-273.
2. Kong, W.-J., Wei, J., Zuo, Z.-Y., Wang, Y.-M., Song, D.-Q., You, X.-F., Zhao, L.-X., Pan, X.-N., & Jiang, J.-D. (2008). Combination of Simvastatin with berberine improves the lipid-lowering efficacy. *Metabolism Clinical and Experimenta, 57,* 1029-1037.
3. Bremner, J. (2021). *Multiple Action-Based Design Approaches to Antibacterials.* Springer Nature Singapore Pte Ltd.
4. de Freitas, S. M., Dias , K. S. T., Gontijo, V.S., Ortiz, C.J.C., & Viega Jr, C. (2018). Multi-target directed drugs as a modern approach for drug design towards Alzheimer's disease: An update. *Current Medicinal Chemistry, 25*(29), 3491-3525.
5. May, O. (2008). Mediating cholesterol homeostasis through SREBP2 LDLR PCSK9 signalling. Retrieved March 1, 2021, from https://www.caymanchem.com/news/mediating-cholesterol-homeostasis-through-srebp2-ldlr-pcsk9-signalling.
6. Kong, W., Wei, J., Abidi, P., Lin, M., Inaba, S., Li, C., Wang, Y., Wang, Z., Si, S., Pan, H., Wang, S., Wu, J., Wang, Y., Li, Z., Liu, J., & Jiang, J.-D. (2004). Berberine is a novel cholesterol-lowering drug working through a unique mechanism distinct from statins. *Nature Medicine, 10*(12), 1344-51.
7. Ye, X., He, K., Zhu, X., Zhang, B., Chen, X., Yi, J., & Li, X. (2012). Synthesis and antihyperlipidemic efficiency of berberine-based HMG-CoA reductase inhibitor. *Medicinal Chemistry Research, 21,* 1353-1362.
8. Taechalertpaisarn, J., Zhao, B., Liang, X., & Burgess, K. (2019). Small molecule Inhibitors of the PCSK9·LDLR interaction. *Journal of the American Chemical Society, 140*(9), 3242-3249.
9. Gai, W., Zhang, Y.-L., Ai, L., & Qiao, Y.-J. (2010). Screening of HMG-CoA reductase inhibitors from composite Salvia miltiorrhiza using AutoDock. *Chinese Journal of Natural Medicines, 8*(1), 51-56.

10. Sanner, M. F. (1999). A programming language for software integration and development. *Journal of Molecular Graphics and Modelling, 17*(1), 57-61.
11. Frisch, M. J., Trucks, G. W., Schlegel, H. B., Scuseria, G. E., Robb, M. A., Cheeseman, J. R., Ścalmani, G., Barone, V., Mennucci, B., Petersson, G. A., Nakatsuji, H., Caricato, M., Li, X., Hratchian, H. P., Izmaylov, A. F., Bloino, J., Zheng, G., Sonnenberg, J. L., Hada, M., . . . Fox D. J. (2009). Gaussian 09 Revision B.01. Gaussian. Wallingford CT, Inc.
12. Morris, G. M., Huey, R., Lindstrom, W., Sanner, M. F., Belew, R. K., Goodsell, D. S., & Olson, A. J. (2009). AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. *Journal of Computational Chemistry, 30*(16), 2785-2791.
13. Chidambaram, S., El-Sheikh, M. A., Alfarhan, A. H., Radhakrishnan, S., & Akbar, I. (2020). Synthesis of novel coumarin analogues: Investigation of molecular docking interaction of SARS-CoV-2 proteins with natural and synthetic coumarin analogues and their pharmacokinetics studies. *Saudi Journal of Biological Sciences, 28*(1), 1100-1108.
14. Alwan, S. M. (2016). Computational calculations of molecular properties and molecular docking of new and reference cephalosporins on penicillin binding proteins and various β-Lactamases. *Journal of Pharmacy and Pharmacology, 4*(5), 212-225.
15. Gaba, S., Saini, A., Singh, G., Monga, V. (2021), An insight into the medicinal attributes of berberine derivatives: A review. *Bioorganic & Medicinal Chemistry, 38,* 116143.
16. Li, D.-D, Yu, P., Xu, H., Wang, Z.-Z., Xiao, W., & Zhao, L.-G. (2021), Discovery of C-9 modified berberine derivatives as novel lipid-lowering agents. *Chemical and Pharmaceutical Bulletin, 69*(1), 59-66.
17. Xie, H., Yang, K., Winston-McPherson, G. N., Stapleton, D. S., Keller, M. P., Attie, A. D., Smith, K. A., & Tang W. (2020), From methylene bridged diindole to carbonyl linked benzimidazoleindole: Development of potent and metabolically stable PCSK9 modulators. *European Journal of Medicinal Chemistry, 206,* 112678.
18. Xu, S., Luo, S., Zhu , Z., & Xu, J. (2019). Small molecules as inhibitors of PCSK9: Current status and future challenges. *European Journal of Medicinal Chemistry, 162,* 212-233.
19. Christopher Murphy, C., Deplazes, E., Cranfield, C. G., & Garcia, A. (2020). The role of structure and biophysical properties in the pleiotropic effects of statins. *International Journal of Molecular Sciences, 21*(22), 8745.
20. da Costa, R. F., Freire, V. N., Bezerra, E. M., Cavada, B. S., Caetano, E. W. S., de Lima Filho, J. L., & Albuquerque, E. L. (2012), Explaining statin inhibition effectiveness of HMG-CoA reductase by quantum biochemistry computations. *Physical Chemistry Chemical Physics, 14*(4), 1389-1398.

21. Ercan, S., Çinar, E., Özaydn, C., Ertrk, N. E., & Çakmak, R. (2020), Inhibitor design for 3-hydroxy-3-methyl-glutaryl CoA reductase enzyme; molecular docking and determination of molecular and electronic properties of ligands by density functional theory method. *The Journal of Heterocyclic Chemistry, 57*(7), 2875-2888.
22. Benguerba, Y., & Bououden, W. (2020). Designing, Cytotoxic Evaluation, Molecular Docking and in Silico Pharmacokinetic Prediction of New Hydrocortisone Derivatives as Anti-Asthmatics Drugs. *Journal of Drug Delivery and Therapeutics, 10*(4), 8-16.
23. Gontijo, V. S., Viegas, F. P. D., Ortiz, C. J. C., de Freitas, S. M., Damasio, C. M., Rosa, M. C., Campos, T. G., Couto, D. S., Dias, K. S. T., & Viegas Jr, C. (2020). Molecular hybridization as a tool in the design of multi-target directed drug candidates for neurodegenerative diseases. *Current Neuropharmacology, 18*(5), 348-407.