Risk of Late-Onset Alzheimer's Disease by Plasma Cholesterol: Rational *In Silico* Drug Investigation of Pyrrole-Based HMG-CoA Reductase Inhibitors

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ABSTRACT

Alzheimer's disease (AD), a worldwide renowned progressive neurodegenerative disorder, is the most common cause of dementia. There are several studies on the important role of cholesterol metabolism in AD pathogenesis, which indicated that the high concentrations of serum cholesterol increase the risk of AD. Biosynthesis of the plasma cholesterol and other isoprenoids is catalyzed by 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR) through the conversion of HMG-CoA to mevalonic acid in mevalonate pathway. Normally, the high level of plasma cholesterol is downregulated by HGMCR inhibition as the result of degradation of LDL, but in abnormal conditions, for example, *high blood glucose, the HMGCR over activated resulting in uncontrolled blood cholesterol. Selective HMGCR inhibitor drugs such as statins, which increase the catabolism of plasma LDL and reduce the plasma concentration of cholesterol, have been investigated as a possible treatment for AD. In the present study, we have identified the binding modes of 22 various derivatives of 3-sulfamoylpyrroles 16, prepared via a [3 + 2] cycloaddition of a münchnone with a sulfonamide-substituted alkyne, by using efficient biocomputational tools. Out of 22, 5 ligands, with code numbers 5b, 5c, 5d, 5i, and 5j, possessed most absorption, distribution, metabolism, and excretion (ADME) and toxicity profiles in acceptable ranges. Among ligands, 5j (sodium (3R,5R)-7-(3-(N,N-dimethylsulfamoyl)-5-(4-fluorophenyl)-2-isopropyl-4-phenyl-1H-pyrrol-1-yl)-3,5-dihydroxyheptanoate) could inhibit HMGCR enzyme in inhibitory binding site with affinity value −12.17 kcal/mol and binding energy −94.10 kcal/mol through 5 hydrogen bonds. It showed the best ADME and toxicity profiling and higher affinity values than other potent candidate and market drugs such as atorvastatin and rosuvastatin. Therefore, it is suggested for further* in vivo *investigation, the druggability of 5j and its cholesterol regulatory impact on AD.*

Keywords: Alzheimer's disease, plasma cholesterol, HMGCR inhibitor, 3-sulfamoylpyrroles 5, ADMET, docking score

INTRODUCTION

The brain is a lipid-rich organ. Since ∼50% of brain dry mass constituted lipids, specifically cholesterol, the dysregulation of brain cholesterol metabolism results in brain diseases, in particular Alzheimer's disease (AD).[1] AD, an age-related neurodegenerative disease, impairs cognitive functions, particularly memory. AD is known as the major form of dementia and diagnosed by age-dependent amyloid plaque deposition and neurofibrillary tangles.[2] After discovery of the isoform 4 (ε4) of the cholesterol transport protein (apolipoprotein E) synthesized in the liver and the central nervous system (CNS),[3] most attention focused on the role of cholesterol metabolism pathway on AD development.[4]

The role of cholesterol in many critical aspects of AD neuropathology has been proved by various evidence derived from genetic, epidemiological, and biochemical studies. It is discovered that a number of genes involved in cholesterol homeostasis caused late-onset AD.[5,6] There is evidence that cholesterol homeostasis dysregulation can significantly influence amyloid beta (Aβ) production, formation of amyloid plaques, Aβ toxicity, tau hyperphosphorylation, and other mechanisms leading to sporadic AD.[7,8]

One of the well-studied cholesterol homeostasis dysregulation is hypercholesterolemia. Hypercholesterolemia, known

as a risk factor for vascular dysfunction (VaD) and AD, can be controlled in every step of cellular cholesterol synthesis.[9] Cellular cholesterol is synthesized from acetyl-CoA in a multistep pre- and postsqualene mevalonate pathway. The presqualene mevalonate pathway starts by formation of acetoacetyl-CoA from two moles of acetyl Co-A in the presence of acetoacetyl-CoA thiolase, followed by synthesis of HMG-CoA from one mole of acetoacetyl-CoA and acetyl-CoA through 3-hydroxy-3-methylglutaryl (HMG) CoA synthase (HMGS) activity. Subsequently, 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR) converts HMG-CoA to mevalonic acid.[10]

HMGCR is the rate-limiting enzyme of the mevalonate pathway. The presence of two SRE motifs in the HMGCR promoter leads to a higher level sterol-dependent regulation.[11] HMGCR inhibition is a rapid (within 1 h) switch off of cholesterol synthesis. Thus, HMGCR is the main target for developing various cholesterol-synthesis inhibitory drugs such as statins.[11] Information on the status and regulation of HMGCR in AD is very limited. The crucial role of HMGCR in cholesterol regulation in both the brain and plasma has great impact on dementia and AD development directly via plaque formation in the brain or indirectly through hypercholesterolemia and VaD.[12]

The focus of the present study was to investigate the impact and mode of action of 22 various derivatives of 3-sulfamoylpyrroles 16 on HMGCR inhibition in hypercholesterolemia patients. Schrodinger Suite 2011 and TOPKAT approach of Accelrys technology, two most powerful and well-known tools for *in silico* drug investigation, have been used to elucidate the molecular mechanism, biological activity, and toxicity properties of druggable molecules.

MATERIALS AND METHODS

Selection of Proteins

The HMGCR structures (PDB Id: 2Q1L) were obtained from the RCSB Protein Data Bank (www.rcsb.org/pdb) with X-ray diffraction resolutions in 2.00 angstroms.

Preparation of Ligands and Proteins

"LigPrep 2.5" module of Schrodinger Suite 2011 was utilized for ligand preparation using OPLS 2005 forcefield at biologically relevant pH. The preparation process included the assignment of the protonation states: disconnecting of group I metals in simple salts, protonating strong bases, and deprotonating strong acids, by adding explicit hydrogens and topological duplicates. The pathway of 4-sulfamoyl pyrrole synthesis[13] and the molecular properties of all compounds are presented in *Figure 1* and *Table 1*, respectively.

The preparation of retrieved target protein was performed using Protein Preparation Wizard of Schrodinger Suite 2011 (Schrödinger Suite; Epik version 2.2; Impact version 5.7; Prime version 2.3, Schrödinger, LLC, New York, NY, 2011). OPLS 2005 forcefield with RMSD as 0.30 was used for geometrical optimization and energy minimization of target protein. The receptor binding site was predicted based on the pose of presented ligands in crystalographic structure of protein extracted from PDB file.

Pharmacodynamic, Pharmacokinetic, and Toxicity Properties

The pharmacodynamic and pharmacokinetic study and quantitative prediction of absorption, distribution, metabolism, and excretion (ADME) have been carried out using

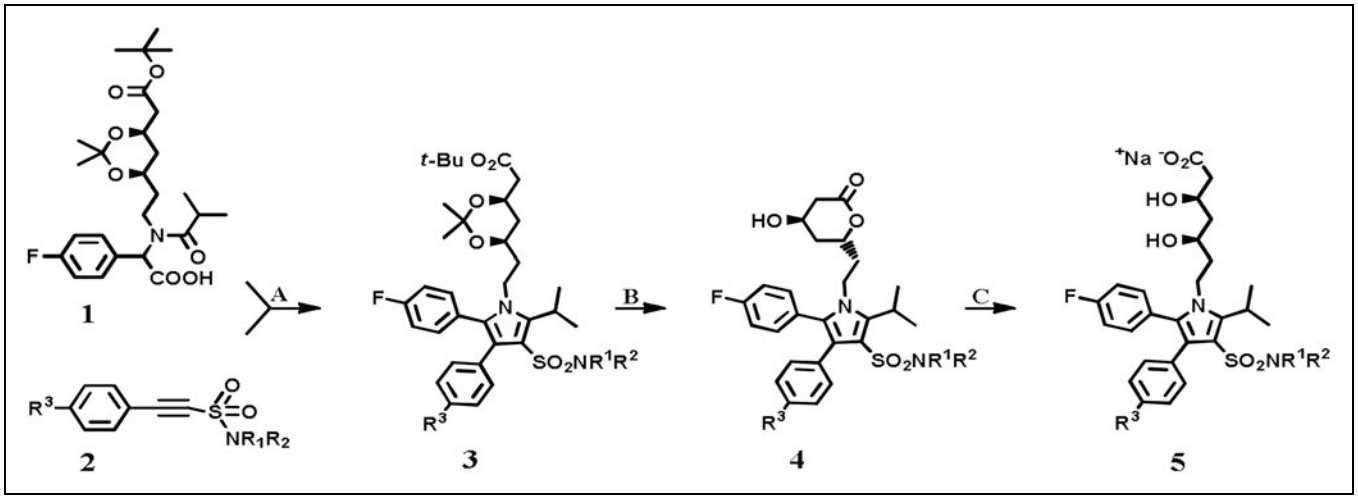
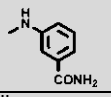
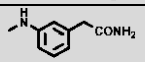
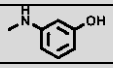
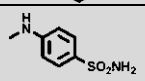
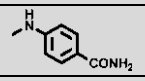
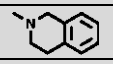
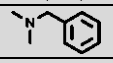
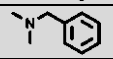
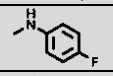


Fig. 1. The pathway of 4-sulfamoyl pyrrole synthesis: **(A)** acetic anhydride, toluene at 60°C, 5 h, 60–80%; **(B)** 30 (v/v) % TFA/DCM, room temperature, 2 h, quantitative; **(C)** one equivalent of 1 N NaOH/THF, room temperature, 4 h, quantitative (Ref. US Patent No. 7250444 B2).

Table 1. Two-Dimensional Structure and Molecular Properties of Ligands

Name	NR^1R^2	R^3	mol MW	donorHB	accptHB	QPlogPC16	QPlogPoct	QPlogPw	QPlogPo/w
5a		H	588.69	2	10.6	17.106	29.157	15.548	4.937
5b		H	586.717	2	8.9	17.779	28.796	14.082	5.973
5c		H	572.69	2	8.9	17.01	28.3	13.825	5.568
5d		H	586.717	2	8.9	17.778	28.796	14.081	5.973
5e		F	655.712	5	11.4	20.384	36.886	21.862	4.328
5f		F	669.739	5	11.4	20.563	37.182	24.629	4.19
5g		F	628.687	4	9.65	19.121	32.884	18.31	5.248
5h		F	626.714	3	8.9	19.373	31.887	16.02	6.395
5i		H	601.732	2	10.9	18.272	30.401	16.146	2.974
5j		H	546.653	2	8.9	16.528	27.264	13.949	5.035
5k		H	558.664	2	8.4	16.835	27.35	13.471	5.46
5l		F	564.643	2	8.9	16.105	27.51	13.724	5.264
5m		F	550.616	3	8.9	16.342	28.329	15.409	4.8
5n		F	622.741	2	9.4	17.174	29.275	14.233	5.841
5o		F	691.761	5	13.4	21.171	38.571	23.885	3.592
5p		F	655.712	5	11.4	20.458	36.886	21.896	4.288
5q		F	652.752	2	8.9	19.789	31.642	15.13	7.169
5r		F	640.741	2	8.9	19.884	30.833	14.846	7.006
5s		H	622.75	2	8.9	20.271	30.74	15.113	6.719
5t		F	630.678	3	8.9	18.393	31.258	16.095	6.169
5u		F	612.687	3	8.9	18.795	31.003	16.315	5.935
5v		H	594.697	3	8.9	19.197	30.748	16.534	5.702

accptHB, estimated number of hydrogen bonds that would be accepted by the solute from water molecules in an aqueous solution: R.V. = 2.0–20.0; donorHB, estimated number of hydrogen bonds that would be donated by the solute to water molecules in an aqueous solution: R.V.:0.0–6.0); mol_MW, molecular weight: recommended value (R.V.):130–725; QPlogPC16, predicted hexadecane/gas partition coefficient: R.V. = 4.0–18.0; QPlogPoct‡, predicted octanol/gas partition coefficient: R.V. = 8.0–35.0; QPlogPw, predicted water/gas partition coefficient: R.V. = 4.0–45.0; QPlogPo/w, predicted octanol/water partition coefficient: R.V. = −2.0–6.5.

QikProp module of Schrodinger Suite 2011 (Schrödinger Press: QikProp 3.4 User Manual, LLC, 2011). These criteria for each compound were assessed by #start parameter, as the overall ADME acceptance score for drug likeness for 95% of known drugs.[14] #start includes the following: SASA/Smol (300–1,000), FISA (7–330), FOSA (0–750), PISA (0–450), total solvent- accessible volume (volume), Glob (0.75–0.95 for 95% of drugs), molecular weight (mol_MW, 130–725), donorHB (0–6), accptHB (2–20), partition coefficient, including QPlogPo/w (octanol/water, 2–6.5), QPlogPoct‡ (octanol/gas, 8–35), QPlogPw (water/gas, 4–45), and QPlogPC16 (hexadecane/gas, 4–18), number of likely metabolic reactions (Metab; 1–8 for 95% of drugs), QPlogKhsa (–1.5 to 1.5), QPlogHERG (concern, <–5), QPlogKp (–8 to –10), QPPMDCK (nm per sec; <25 poor, >500 great),[15] CNS activity (–2 to 2),[16] QPlogBB (–3 to 1.2),[17] QPPCaco (<25 poor, >500 great),[18] PM3 calculated ionization potential (IP(eV); 7.9–10.5), PM3 calculated electron affinity (EA(eV); –0.9 to 1.7), the human oral absorption level, the maximum transdermal transport rate (Jm; Kp XMWXS; $\mu gcm^{-2}h^{-1}$), and the number of violations of Lipinski's rule of five[19] of the various 3-sulfamoylpyrroles 16 derivatives.

The qualitative and quantitative toxicity properties of compounds were predicted using online TOPKAT approaches of Accelrys Environmental Chemistry and Toxicology Workbench, Accelrys Inc. (San Diego, CA; https://ect01.accelrysonline.com/webport/ECT/main.htm). TOPKAT features provide the accurate toxicity properties of compounds based on similarity with compounds registered in two largest chemical and drug data banks, FDA and NTP. Toxicity profiling includes prediction of rodent carcinogenicity from the FDA and NTP data sets for both female and male (v3.1), weight of evidence (WOE) (v5.1), developmental toxicity potential (DTP), mutagenicity (Ames test v3.1), ocular irritation (v5.1), skin sensitization (guinea pig maximization test) and irritancy (v6.1), aerobic biodegradability (v6.1), and EC50, LD50, and TD50.[20] The ADME and toxicity results of all compounds are listed in *Table 2* and *Supplementary Table S1* (Supplementary Data are available online at www.liebertpub.com/adt), respectively.

Receptor–Ligand Interactions

Dock scores and Molecular Mechanics/Generalized Born Surface Area (MMGBSA) values[21] for selected compounds in complex with receptor were computed using "Glide 5.7" module in Extra Precision (XP) mode[22,23] and Prime 3.0 application of Schrodinger Suite 2011 (Suite 2012: Prime, Version 3.1, Schrödinger, LLC, New York, NY, 2012), respectively. *Supplementary Table S2* represents docking scores and MMGBSA values.

Visualization of Interaction Between Top Score Candidate/s and Residues in Receptors

Candidates with the best ADMET (T, toxicity) profiles and top dock scores have been selected and their complexes with residues in binding site of receptor were visualized using XP visualizer approaches of Schrodinger 2011. The receptor surface was figured based on the electrostatic potential of residues in binding packet of protein and truncated in 5 Å from ligand with 20% transparency.

RESULTS AND DISCUSSION

The most common HMGCR inhibitor marketed drugs are within the statin family of drugs. However, there are various reports regarding the side effects of most statins, among which, myalgia is the main adverse effect, manifested by muscle stiffness, muscle weakness, fatigue, and cramps.[24] Myalgia is thought to be a consequence of inhibition of myocytic HMG-CoA reductase.[25] Also, it is reported that statins with greater hepatoselectivity may reduce the side effects due to less availability to muscle tissues.[26,27] Therefore, statins have been modified to increase the hepatoselectivity by utilizing organic anion transporting polypeptides, the hepatocyte-specific transporters.[28] Hydrophilic statins, by reducing the passive diffusion of nonselective ones into all cells, can increase selectivity for cells and internalize statins through active transport. *Figure 2* represents the role of statins or other HGMCR inhibitors in regulating the presqualene mevalonate pathway.

Derivatives have been developed from sodium (3S,5S)-7-(2-(4-fluorophenyl)-5-isopropyl-3-phenyl-4-sulfamoyl-1H-pyrrol-1-yl)-3,5-dihydroxyheptanoate, yielding modifying atorvastatin in multiple steps. The 5-member heterocyclic core in atorvastatin structure has been retained as a key scaffold for interacting within the active site of the enzyme. For synthesizing the different 4-sulfamoyl pyrrole analogs, the 2-(N-(2-((4S,6S)-6-(2-(tert-butoxy)-2-oxoethyl)-2,2-dimethyl-1,3-dioxan-4-yl)ethyl)isobutyramido)-2-(4-fluorophenyl)acetic acid was prepared stepwise from 2-(4-fluorophenyl)acetic acid and heated in acetic anhydride/toluene to form münchnone *in situ*; subsequently, münchnone interacted with alkynyl sulfonamides to afford the pyrroles 3 *in situ*.[13] By treating the pyrroles 3 with trifluoroacetic acid, the trimethoxybenzyl group has been eliminated from the sulfonamide nitrogen and cleaved the tert-butyl esters and acetonide to form the lactones 4. By treating the lactone with one equivalent of 1 N NaOH and lyophilizing, the pyrrole sodium salts 5 has been yielded. Finally, various 4-sulfamoyl pyrrole analogs have been prepared by interacting different residues with the sulfonamide moiety of pyrrole sodium salts 5. The various residues and their physiochemical properties are presented in *Table 1* and the pyrrole sodium salts 5 synthesizing pathway is depicted in *Figure 1*.

Table 2. Absorption, Distribution, Metabolism, and Excretion Properties

Name	QPlogS	CIQPlogS	QPlog HERG	QPP Caco	QPlog BB	QPP MDCK	QPlog Kp	QPlog Khsa	#metab	HOA	RF	RT	Jm
5a	−6.08	−7.99	−4.11	32.47	−2.11	28.02	−3.17	0.41	5	2	1	1	0.000333
5b	−7.32	−8.57	−4.52	36.13	−2.16	31.45	−3.00	0.85	4	1	2	1	0.000028
5c	−6.64	−8.29	−4.05	29.91	−2.14	25.64	−3.27	0.73	4	1	2	1	0.00007
5d	−7.32	−8.57	−4.52	36.14	−2.16	31.46	−3.00	0.85	4	1	2	1	0.000028
5e	−6.86	−9.01	−4.90	1.71	−3.79	2.10	−5.23	0.39	6	1	2	2	0.000001
5f	−5.40	−8.65	−3.40	1.36	−3.49	3.79	−4.66	0.16	7	1	2	2	0.000059
5g	−6.90	−9.26	−4.89	7.87	−2.93	10.96	−3.84	0.57	6	1	2	2	0.000011
5h	−7.77	−9.62	−5.03	16.76	−2.59	24.82	−3.16	0.94	5	1	2	2	0.000007
5i	−6.44	−7.08	−5.36	8.05	−1.85	6.87	−5.16	0.56	5	1	1	2	0.000002
5j	−6.18	−7.73	−4.13	24.47	−2.24	21.03	−3.38	0.52	4	2	2	1	0.000151
5k	−6.72	−7.98	−4.27	26.30	−2.25	22.32	−3.32	0.69	4	1	2	1	0.000051
5l	−6.53	−8.09	−4.00	24.52	−2.14	38.14	−3.51	0.55	4	1	2	1	0.000051
5m	−6.45	−7.73	−4.23	16.63	−2.38	24.90	−3.79	0.42	4	1	1	2	0.000032
5n	−7.18	−9.17	−4.04	27.73	−1.99	76.55	−3.43	0.70	4	1	2	1	0.000015
5o	−6.66	−8.96	−5.09	0.81	−4.37	0.96	−5.79	0.11	4	1	3	2	0
5p	−6.95	−9.01	−4.96	1.53	−3.88	1.87	−5.33	0.38	5	1	2	2	0
5q	−8.74	−10.29	−5.40	34.27	−2.17	53.77	−2.61	1.22	6	1	2	1	0.000003
5r	−8.48	−10.00	−5.59	28.38	−2.43	44.30	−2.53	1.08	5	1	2	1	0.000006
5s	−8.01	−9.63	−5.67	24.86	−2.57	21.21	−2.49	1.03	5	1	2	1	0.00002
5t	−7.76	−9.70	−4.96	17.53	−2.39	47.15	−3.27	0.81	4	1	2	2	0.000006
5u	−7.40	−9.33	−5.09	17.51	−2.49	26.02	−3.14	0.77	5	1	2	2	0.000018
5v	−7.04	−8.97	−5.23	17.50	−2.58	14.37	−3.00	0.73	5	1	2	2	0.000054

CIQPlogS, conformation-independent predicted aqueous solubility; $-6.5 < x < 0.5$; CNS, central nervous system activity −2, −1, 0, 1, 2: −2 = completely inactive, −1 = very low activity, 0 = low activity, 1 = medium activity, 2 = completely active, 3 = high; HOA, human oral absorption level; 1,2, 3; 1 = low, 2 = medium; Jm, maximum transdermal transport rate; QPlogBB, predicted brain/blood partition coefficient; −3.0 to 1.2; QPlogHERG, predicted IC50 value for blockage of hERG K+ channels; <−5 = concern; QPlogKp, predicted skin permeability; range = $-8 < x < -1$; QPlogKhsa, prediction of binding to human serum albumin; −1.5 to 1.5; QPlogS, prediction of aqueous solubility level; recommended range $-6.5 < x < 0.5$; QPPCaco, predicted apparent gut-blood barrier permeability; <25 = poor, >500 = great; QPPMDCK, predicted apparent Madin-Darby canine kidney cell permeability; <25 = poor, >500 = great; #Metab, number of likely metabolic reactions; 1–8; RF, the number of violations of Lipinski's rule of five; RT, the number of violations of Jorgensen's rule of three.

Protein and Ligand Preparation

The structures of HMGCR, PDB Ids: 2Q1L, have been downloaded from the Protein Data Bank website (www.rcsb.org/pdb) and prepared by using ProPrep and Grid Generation Wizard of Schrödinger 2011 by optimizing the tertiary structure and predicting the binding sites for the ligands. The ligands, also, have been prepared using LigPrep 2.5 of Schrödinger 2011 and their molecular properties have been computed via QikProp 3.4 (*Table 1*).

ADME Prediction

The main parameter of druggability is the ability of a compound to cross the oral and intestinal barriers, enter the blood circulation for delivery to its target wherever in the body, be metabolized by the target, and excrete from the body during a period of time. The Lipinski's rule of 5 (ro5) was utilized to predict the drug likeness of orally administered compounds.[29] ro5 includes standard ranges for molecular weight (MW <500), number of hydrogen bond donor (<5),

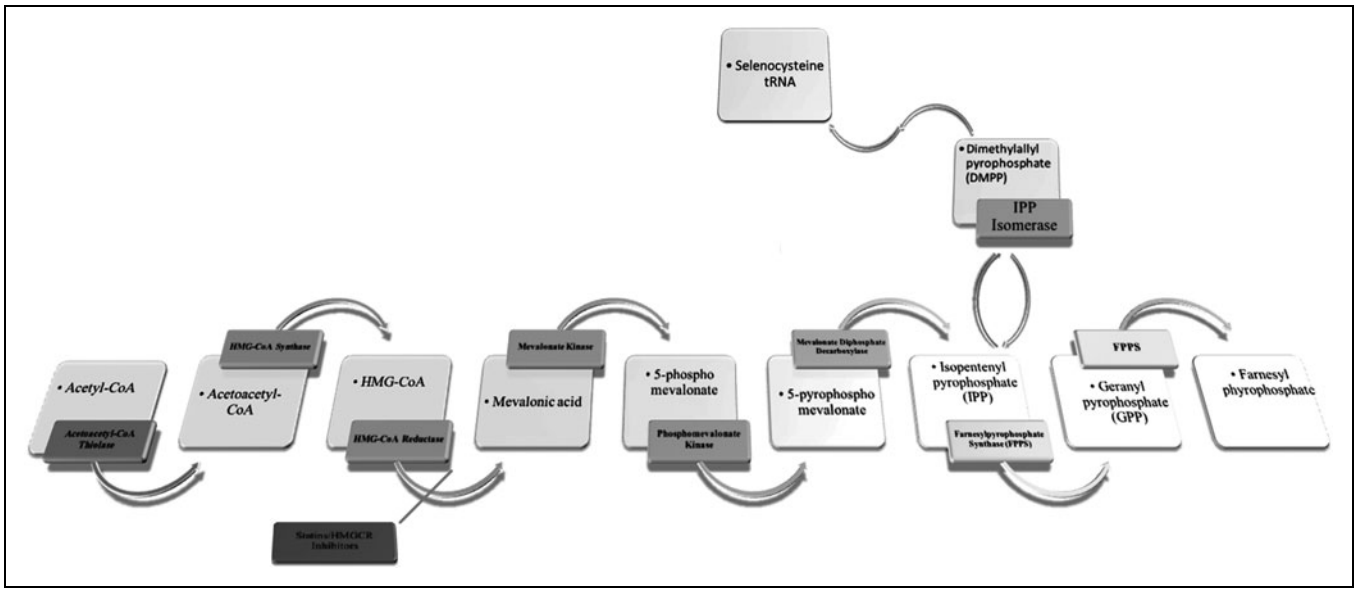


Fig. 2. Presqualene mevalonate pathway and the role of statins or other HGMCR inhibitors.

number of hydrogen bond acceptor (≤10), and predicted octanol/water partition coefficient (log$P$ < 5). Lipinski's criteria are presented in the scatter plots of correlation between MW and rest of parameters for all compounds, depicted in *Figure 3*. According to the result, none of the compounds had molecular weight in the recommended range by ro5 and could not satisfy drug likeness criteria. Approximately, 31.82%, 72.73%, and 100% of all compounds are in the recommended range for LogP, accptHB, and donorHB of drug likeness, respectively.[15] However, all MW values are in the recommended range for 95% of known drugs indicated by #star.[20] The ADME properties of all compounds are presented in *Table 2*.

Bioavailability

Absorption and the first-pass metabolism of the liver are two processes by which the bioavailability of each compound can be predicted. The absorption influenced by effective factors, including the solubility of compounds, the gut wall permeability to the compounds, and the ability of compound to interact with shuttles in the gut wall such as transporters and metabolizing enzymes, depended on the functional groups in the compound structure.

The computational methodology for oral absorption prediction has been offered by Jorgensen, known as "Rule of Three" (ro3). The parameters of oral availability likelihood include log S>−5.7, QPPCaco >22 nm/s, and primary metabolites, #metab <7. However, there are other important parameters that directly affect the bioavailability of compounds such as the prediction of the qualitative human oral absorption, the percentage of human oral absorption, and the conformation-independent aqueous solubility, CIlog S. CIlog S is computed based on the similarity of compounds with their close analogs. The adjusted formula is given in Equation (1) for similarity >0.9.

$$\text{Ppred} = \text{SPexp} + (1 - \text{S})\text{PQP}, \quad (1)$$

where S is the similarity, Pexp and PQP are the respective experimental and QikProp predictions for the most similar molecule within the training set. There are several parameters that have to be considered for prediction of bioavailability of a compound such as Log S, predict the aqueous solubility levels, #metab, number of likely metabolic reactions, and Caco-2, the gut/blood barrier permeability that is a nonactive transport in nm/s, respectively.[30] The results indicate that 36.36% of compounds possessed QPPCaco in recommended range 25–500 nm/s and rest showed low gut permeability. All compounds showed metabolic behaviors in recommended range and the aqueous solubility levels of all compounds were out of recommended range −6.5 to 0.5. Increasing the hydrophilicity could dramatically decrease the gut permeability of compounds and in turn reduce the bioavailability of orally administered drugs, but can increase the specificity and selectivity of statins.[13]

The Prediction of Blood/Brain Penetration (QPlogBB)

The prediction of the blood/brain barrier (BBB) permeability using QPlogBB was used to check the accessibility of compounds for CNS based on the polarity of compounds.[31] For the blood/brain penetration (logB/B) as well as CNS activity,

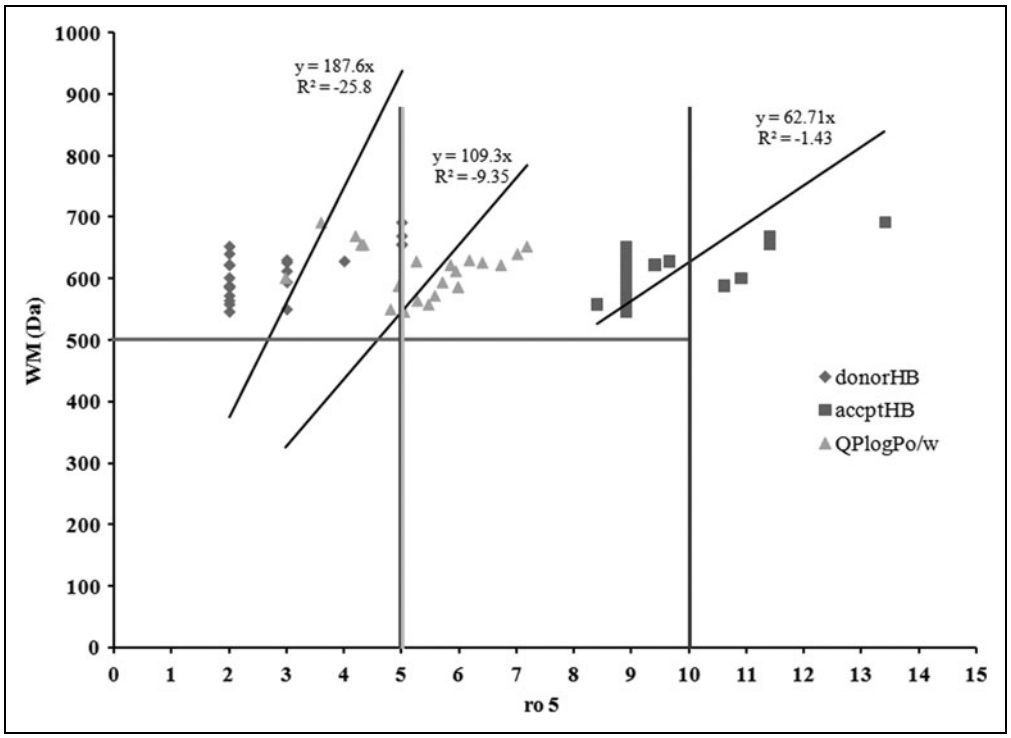


Fig. 3. Scatter plots of correlation between MW and the rest of the parameters for all compounds. MW, molecular weight.

Madin-Darby canine kidney (MDCK) was used to compute the level of BBB penetration to druggable molecules.[31] Results indicated that none of compounds was active in CNS (predicted value −2), except 5e, 5f, 5o, and 5p, almost all compounds were in the recommended range for the logB/B prediction (−3.0 to 1.2) and ~54.54% of all compounds were in the recommended range for the prediction of nonactive transportation through the MDCK (25–500).

The Prediction of Plasma Protein Binding

The plasma proteins such as glycoproteins, human serum albumin, lipoproteins, and globulins (a, b, and c) are targets for the prediction of pharmacodynamics of a druggable molecule by investigating the ability of ligands to bind to blood proteins, which can directly affect the efficacy of a drug.[32] On the contrary, the availability of drug for target is in direct relation to the rate of plasma protein binding. The high plasma protein binding results in reducing the rate of distribution of drug through general blood circulation.[33] Therefore, for designing a drug, a less degree of plasma protein binding is desirable. QPlogKhsa was used as a unique parameter for the estimation of tendency level of a compound to bind to plasma proteins. The results showed that all compounds were in the recommended range (−1.5 to 1.5) for plasma protein binding.

The Prediction of Metabolism

The accessibility level of compounds for their target after entering into the blood stream, as the number of likely metabolic reactions, can be computed by QikProp. The #metadata were used to predict the average number of possible metabolic reactions of each compound. Based on #metadata, all compounds possessed #metavalues within the recommended range of metabolic reaction 1–8.

The Prediction of Blockage of Human *Ether-à-go-go*-Related Gene Potassium Channel

One of the most important targets for testing the cardiac toxicity of druggable molecules is the human *Ether-à-go-go*-related gene (hERG) because of its role in the electrical activity during systolic and diastolic periods of the heart by encoding the potassium ion (K+) channel.[34] This channel has modulatory function for the nervous system as well[35] and involves neurocardiac disorders such as long QT syndrome (Torsade de pointes).[36] Therefore, blockage of hERG K+ channel can be considered as the potential toxicity of a compound for the nervous and cardiac system, which is indicated by IC50 in drug designing.[37] The QPlogHERG indicator was used by QikProp module of Schrodinger Suite 2011 to simulate the IC50 values of hERG channel toxicity of a compound. The values showed that 63.63% of compounds had no toxicity potential for

neurocardiac systems and their values fell in the recommended range of IC50 (>—5) for blockage of hERG K+ channels.

Toxicity

Accelrys Environmental Chemistry and Toxicology Workbench invented TOPKAT online approaches for *in silico* investigation of the most important pharmacological toxicity parameters of compounds. TOPKAT toxicity profiling includes carcinogenicity, mutagenicity, skin and ocular sensitizing and irritancy, lethal dose for oral administration, effective concentration, tolerable dose, lethal concentration for inhalation, and toxicity potential based on structural similarity of compounds with structures available in both the FDA (U.S. Food and Drug Administration) and NTP (National Toxicology Program) databases. The carcinogenicity of all compounds for male mouse (MM), male rat (MR), female mouse (FM), and female rat (FR) was predicted. Based on the NTP database, only 13.64% all compounds were carcinogenic for FM with a probability of 0.58–0.64 and just 10% carcinogenic for MR 0.61–0.64 and none of compounds showed carcinogenicity for MR and FM. According to the FDA database, around 63.64% of all compounds were carcinogenic for MR with a probability of 0.31–0.42 and no carcinogenicity for FM, MM, and FR. The predicted data indicated that all compounds were nonmutagenic and noncarcinogenic based on the WOE for rodent carcinogenicity. Around 68.18% of all compound DTP with a probability of 0.49–0.58. All compounds were nonirritant for skin, however, around 90% of all showed weak to strong skin sensitizing with a probability of 0.75–0.84 and approximately 81.82% of all compounds irritate oculus with a probability of 0.97–1.00. None of compounds was degradable through an aerobic biodegradability process with a probability of 0.19–0.48. The levels of maximum tolerated dose by feeding, oral LD50, inhalation and fathead minnow LC50 for rat, Daphnia EC50, TD50 for mouse and rat were evaluated and are presented in *Supplementary Table S1*.

In a glance, all compounds with big molecular sizes and more hydrophilic modification were not recommended for oral administration and could not satisfy Lipinski's and Jorgensen's criteria, but by having good distribution and metabolism in the body can be used in suggested doses as interveinal injections. Among them, 5b (sodium (3R,5R)-7-(2-(4-fluorophenyl)-5-isopropyl-3-phenyl-4-(piperidin-1-ylsulfonyl)-1H-pyrrol-1-yl)-3,5-dihydroxyheptanoate), 5c (sodium (3R,5R)-7-(2-(4-fluorophenyl)-5-isopropyl-3-phenyl-4-(pyrrolidin-1-ylsulfonyl)-1H-pyrrol-1-yl)-3,5-dihydroxyheptanoate), 5d (sodium (3R,5R)-7-(2-(4-fluorophenyl)-5-isopropyl-3-phenyl-4-(piperidin-1-ylsulfonyl)-1H-pyrrol-1-yl)-3,5-dihydroxyheptanoate), 5i (sodium (3R,5R)-7-(2-(4-fluorophenyl) –5- isopropyl-4- ((4-methylpiperazin-1-yl)sulfonyl) –3-phenyl-1H-pyrrol-1-yl) –3,5-dihydroxyheptanoate), and 5j (sodium (3R,5R)-7-(3-(N,N-dimethylsulfamoyl)-5-(4-fluorophenyl)-2-isopropyl-4-phenyl-1H-pyrrol-1-yl)-3,5-dihydroxyheptanoate) showed better ADME and toxicity profiling and can be selected for further study.

Docking Calculations Using Schrodinger 2011

The five selected compounds have been docked to the binding site of HMGCR enzyme via Glide v.5.7 feature of Schrodinger Suite 2011. The result indicated that among all

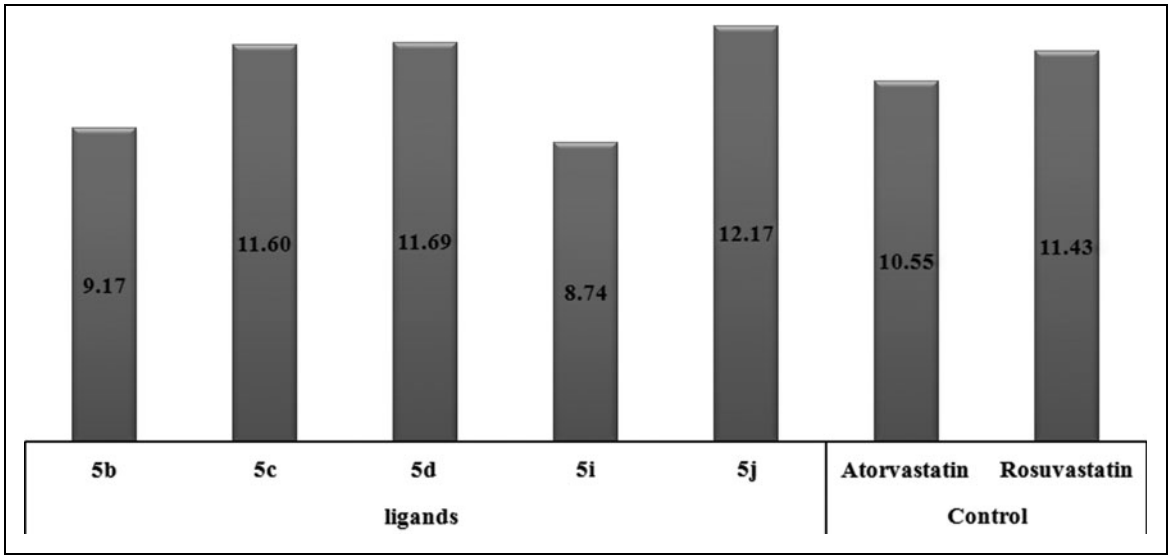


Fig. 4. Dock scores of five selected compounds in comparison with two marketed drugs, atorvastatin and rosuvastatin.

five compounds, 5j with affinity value −12.17 kcal/mol and binding energy −94.10 kcal/mol has a significantly better dock score than both the marketed drugs atorvastatin and rosuvastatin with docking scores −10.55 and −11.43 kcal/mol, respectively. The dock scores of five selected compounds in comparison with two marketed drugs, atorvastatin and rosuvastatin, have been depicted in *Figure 4*.

Visualization of Interaction Between Compounds and Residues in Receptors

In a glance, 5j (sodium (3R,5R)-7-(3-(N,N-dimethylsulfamoyl)-5-(4-fluorophenyl)-2-isopropyl-4-phenyl-1H-pyrrol-1-yl)-3,5-dihydroxyheptanoate) among five eligible compounds with the best dock score, ADME, and toxicity profiling has been selected for visualizing the interaction with the residues in binding packet of HMGCR with PDB id 2Q1L.

All compounds have been docketed in same binding pocket of HMGCR enzyme with PDB ids: 2Q1L, in which 5j interacted with residue inhibitory binding pocket, including GLU559, CYS561, LEU562, SER565, ARG568, LYS735, ALA751, HIS752, ASN755, LEU853, ALA856, HIS861, ARG590, MET657, SER661, VAL683, SER684, ASP690, LYS691, and LYS692 through five hydrogen bonds with Arg590, Asn755, Asp690, Glu559, and Lys735with bond distances 1.872, 1.912, 1.880, 1.726, and 1.601 Å, respectively. The cocrystal of 5j with HMG-CoA reductase shown in *Figure 5*, salient features of the binding of (sodium (3R,5R)-7-(3-(N,N-dimethylsulfamoyl)-5-(4-fluorophenyl)-2-isopropyl-4-phenyl-1H-pyrrol-1-yl)-3,5-dihydroxyheptanoate) to the enzyme include strong hydrogen bonding interactions provided by the 3,5,7-trihydroxy heptanoic acid fragment of 5j; the isopropyl group fitting into a small lipophilic pocket. The number of hydrogen bonds and related residues for all compounds are presented in *Supplementary Table S2*.

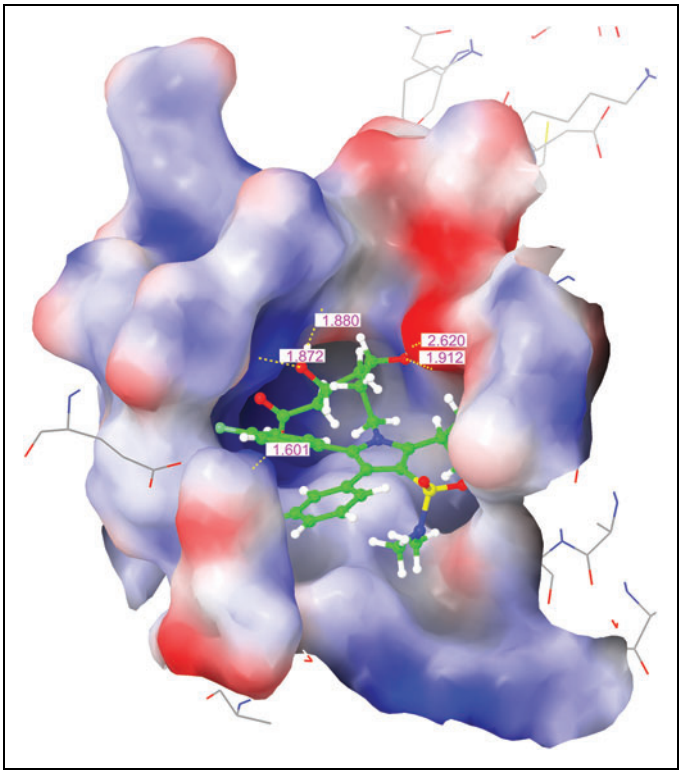


Fig. 5. Cocrystal of 5j with HMG-CoA reductase. Color images available online at www.liebertpub.com/adt

CONCLUSION

The important role of cholesterol metabolism in AD pathogenesis has been reported that the high concentrations of serum cholesterol can increase the risk of AD. Since the biosynthesis of plasma cholesterol and other isoprenoids is initiated by catalytic function of 3-hydroxy-3-methyl-glutaryl-CoA reductase (HMGCR) through the conversion of HMG-CoA to mevalonic acid in the mevalonate pathway, HMGCR is now considered as an interesting target for AD drug development and treatment. In this article, we have focused on modified statin derivatives, which act selectively on hepatocyte to reduce the risk of myalgia, the well-known side effect of statins. There have been designed 22 varieties of 4-sulfamoyl pyrroles, the statin derivatives and the biological activities and pharmacological properties of such compounds have been predicted. Among all compounds, 5j (sodium (3R,5R)-7-(3-(N,N-dimethylsulfamoyl)-5-(4-fluorophenyl)-2-isopropyl-4-phenyl-1H-pyrrol-1-yl)-3,5-dihydroxyheptanoate) showed the best ADME and toxicity profiling and higher affinity values than other potent candidates. It could inhibit the HMGCR enzyme in inhibitory binding site with affinity value −12.17 kcal/mol and binding energy −94.10 kcal/mol through five hydrogen bonds, much better than atorvastatin and rosuvastatin. Most of ADMET properties were in recommended ranges and can be considered as potent hepatoselective pyrrole drugs for cholesterol and AD treatment.

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DISCLOSURE STATEMENT

No competing financial interests exist.

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Abbreviations Used

AD = Alzheimer's disease
ADME = absorption, distribution, metabolism, and excretion
Aβ = amyloid beta
BBB = blood/brain barrier
CNS = central nervous system
DTP = developmental toxicity potential
FM = female mouse
FR = female rat
hERG = human *Ether-à-go-go*-related gene
HMGCR = 3-hydroxy-3-methylglutaryl-CoA reductase
MDCK = Madin-Darby canine kidney
MM = male mouse
MMGBSA = Molecular Mechanics/Generalized Born Surface Area
MR = male rat
MW = molecular weight
VaD = vascular dysfunction
WOE = weight of evidence
XP = extra precision