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In silico analysis of *Mentha pipertia* (phyto-constituents) as HMG coa reductase and squalene synthase inhibitors

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Abstract

Mentha piperita has been well known for its hypolipidemic activity. This prompted the present study to be carried out on a selected 12 phyto-constituents of *Mentha piperita* which are naringin, eriodictyol, eriodictyol 7-glucuronide, eriocitrin, hesperidin, isorohifolin, luteolin 7-glucoside, diosmin, rosmarinic acid, piperitoside, menthoside and caffeic acid. These phyto-constituents were evaluated on the docking behaviour of HMG CoA reductase (HMGR) and Squalene synthase (SQS) using Discovery Studio Version 3.1. In addition, molecular physicochemical, drug-likeness, ADMET (Absorption, Distribution, Metabolism, Excretion and Toxicity) and TOPKAT (Toxicity Prediction by Komputer Assisted Technology) analyses were done. The molecular physicochemical analysis revealed that eriodictyol, rosmarinic acid and caffeic acid (3 ligands) complied with Lipinski's rule of five. ADMET analysis showed that eriodictyol and caffeic acid exhibited good intestinal absorption property. Docking studies and binding free energy calculations revealed that menthoside (-70.0 kcal/mol) and piperitoside (-65.32 kcal/mol) exhibited the maximum interaction energy with HMGR and SQS respectively. Caffeic acid exhibited very least binding energy irrespective of its target protein. Caffeic acid showed interaction with Leu546 and Gln212 amino acid residue of HMGR and SQS. Hence, the results of this present study exhibited the potential of these twelve ligands as hypolipidemic agents.

Introduction

Cholesterol plays an important role in living cells and it has numerous crucial functions in human body (Pakpour, 2013). However, a very high level of cholesterol may lead to atherosclerosis (Lin *et al.*, 2015). Cholesterol biosynthesis in human is mainly regulated by the enzyme 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase (HMGR) (Goldstein and Brown, 1990). Thus, inhibition of this enzyme has proven to be the most efficient therapy for hyperlipidemia, since the enzymatic catalyses of HMG-CoA to mevalonate represents one of the rate-limiting steps of cholesterol biosynthesis (Jakobisiak and Golab, 2003). Until the present, statin has been the class of drugs prescribed for cholesterol biosynthesis inhibition via the HMG-CoA reductase activity (Tiwari and Pathak, 2011). However, the use of these drugs is known to have side or adverse-effects (Golomb *et al.*, 2008). Similarly, another important enzyme involves in cholesterol biosynthesis is Squalene synthase (SQS) which is also known as Farnesyl-diphosphate farnesyl transferase. It catalyzes the farnesyl pyrophosphate into squalene through dimerization. SQS inhibition leads to direct decrease in cholesterol biosynthesis resulted in reduction of plasma cholesterol level (Raeisi, 2013). Inhibition of SQS is considering as an approach of decreasing cholesterol levels in the prevention of cardiovascular disease (Griebenow *et al.*, 2011) and as well as in treatment of hypercholesterolemia and coronary heart disease (Ishihara *et al.*, 2003).

Mentha piperita L. (Lamiaceae family) is an ancient plant species known to Arab, Asian and Greek physicians (Abirami and Nirmala, 2014). It is commonly known as peppermint and used for culinary purposes. Peppermint tea is customarily used as a substitute for black tea. Peppermint tea and extracts are used orally for the treatment of dyspepsia, flatulence, intestinal colic and both for

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gall bladder and bile tract spasms (Fecka and Turek, 2007). *Mentha piperita* has been well known for its carminative, spasmolytic, analgesic, anti-oedema, anti-allergic, anti-nociceptive, anti-inflammatory, anti-microbial, anti-oxidant (Badal *et al*., 2011), anti-dermatophyte (Ibrahim and Abd El-Salam, 2015) and larvicidal (Chauhan *et al.*, 2016) activities. Recently, Radaelli *et al*. (2016) reported the anti-microbial activity against *Clostridium perfringens* which is one among the main causative agent of food-borne diseases. Barbalho *et al.* (2011) reported the hypolipidemic activity of *Mentha piperita.* Therefore, the present study aims to determine the twelve *Mentha piperita* phyto-constituents (namely naringin, eriodictyol, eriodictyol 7-glucuronide, eriocitrin, hesperidin, isorohifolin, luteolin 7-glucoside, diosmin, rosmarinic acid, piperitoside, menthoside and caffeic acid) as HMG CoA reductase (HMGR) and Squalene synthase (SQS) inhibitors using in silico analysis method. Outcome of the present study will provide useful information for the design of potent and selective hypercholesterolemia inhibitors from *Mentha piperita* phyto-constituents in the near future.

Materials and Methods

Ligand preparation

Chemical structures of the ligands namely i) naringin[CID25244529]; ii) eriodictyol[CID440735]; iii) eriodictyol 7-glucuronide [CID124258]; iv) eriocitrin [CID83489]; v) hesperidin [CID3594]; vi) isorohifolin [CID5377847]; vii) luteolin 7-glucoside [CID5280637]; viii) diosmin [CID5281613]; ix) rosmarinic acid [CID5281792]; x) piperitoside [HMDB37348]; xi) menthoside [HMDB37350] and xii) caffeic acid [CID689043] were retrieved from PubMed *(www.pubmed.com)* compound and human metabolome database *(www.hmdb.ca/metabolites)* respectively.

Target protein identification and preparation

The three dimensional structure of the HMGR (PDB ID: 1DQ8 with resolution of 2.10 Å) and SQS (PDB ID: 3 ASX with resolution of 2.00 Å) were obtained from the Research Collaborator for Structural Bioinformatics (RCSB) Protein data bank (Anonymous,www.rcsb.org) respectively. A chain of HMGR was pre-processed separately by deleting other chains (B, C and D), ligand, as well as the crystallographically observed water molecules (water without hydrogen bonds). In case of SQS, a chain of protein was pre-processed separately by deleting the ligand and the crystallographically observed water molecules (water without hydrogen bonds).

Molecular descriptors calculation

Molinspiration online database was used for all selected ligands to calculate thirteen descriptors (www.molinspiration.com) which are logP, polar surface area, molecular weight, number of atoms, number of O or N, number of OH or NH, number of rotatable bond, volume, drug likeness including G protein coupled receptors (GPCR) ligand, ion channel modulator, kinase inhibitor, nuclear receptor ligand, and number of violations to Lipinski's rule..

ADMET and TOPKAT analysis

ADMET (Absorption, Distribution, Metabolism, Excretion and Toxicity) and TOPKAT (Toxicity Prediction by Komputer Assisted Technology) test was performed by using Discovery Studio® 3.1 (Accelrys, San Diego, USA). ADMET analysis was performed on human intestinal absorption (HIA), aqueous solubility (AS), blood brain barrier (BBB), cytochrome P450 2D6 (CYP2D6), plasma protein binding (PPB) and hepatotoxicity (HT) descriptors. As for the TOPKAT analysis, it was done on aerobic biodegradability (AB), ames mutagenicity (AM), ocular irritancy (OI), skin irritancy (SI), skin sensitization(SS) and oral toxicity in rat (LD_{50} in g/Kg of body weight) descriptors.

Docking studies

Docking studies were carried out on the crystal structure of HMGR and SQS which were retrieved from Protein Data Bank using the CDOCKER protocol under the protein-ligand interaction section in Discovery Studio® 3.1 (Accelrys, San Diego, USA). In general, CDOCKER is a grid-based molecular docking method that employs CHARMM force fields. This protein was firstly held rigid while the ligands were allowed to flex during the refinement. Two hundred random ligand conformations were then generated from the initial ligand structure through high temperature molecular dynamics followed by random rotations, refinement by grid-based (GRID I) simulated annealing, and a final grid-based or full force field minimisation (Wu *et al.*, 2003). In this experiment, the ligand was heated to the temperature of 700 K in 2000 steps. The cooling steps were set to 5000 steps to 300 K cooling temperature. The grid extension was set to 10 Å. Hydrogen atoms were added to the structure and all ionisable residues were set at their default protonation state at a neutral pH. For each ligand, top ten ligand binding poses were ranked according to their CDOCKER energies, and the predicted binding interactions were analysed. The

Table 1. Molecular physicochemical descriptors analysis of twelve ligands using

Ligand	Log A[a]	TPSA[b]	Natoms[c]	MW[d]	noN[e]	nOHNH[f]	Nviolations[g]	Nrotb[h]	Volume[i]
Naringin	-0.37	225.1	41	580.5	14	8	3	6	486.2
Eriodictyol	1.63	107.2	21	288.3	6	4	0	1	238.3
Eriodictyol 7-glucuronide	-0.28	203.4	33	464.4	12	7	2	4	372.6
Eriocitrin	-0.86	245.3	42	596.5	15	9	3	6	494.3
Hesperidin	-0.55	234.3	43	610.6	15	8	3	7	511.8
Isorohifolin	-0.23	229	41	578.5	14	8	3	6	480.0
Luteolin 7-glucoside	0.19	190.3	32	448.4	11	7	2	4	364.2
Diosmin	-0.21	238.2	43	608.5	15	8	3	7	505.6
Rosmarinic acid	1.63	144.5	26	360.3	8	5	0	7	303.5
Piperitoside	1.88	216.6	43	594.5	13	7	3	8	491
Menthoside	1.18	275.5	53	740.7	17	9	3	10	614.8
Caffeic acid	0.94	77.8	13	180.2	4	3	0	2	154.5

[a] Octanol-Water partition coefficient, [b] Polar surface area, [c] Number of non hydrogen atoms, [d] Molecular weight, [e] Number of hydrogen bond acceptors [O and N atoms], [f] Number of hydrogen bond donors [OH and NH groups], [g] Number of Rule of 5 violations, [h] Number of rotatable bonds, [i] Molecular volume

best among the ten ligand binding poses was chosen and carried out in situ ligand minimization using standard protocol.

Results

Molecular descriptors analysis

Violation of zero will be the main target compound which was observed for eriodictyol, rosmarinic acid and caffeic acid suggesting that these compounds complied very well with the Lipinski's rule of five. However, eriodictyol 7-glucuronide and luteolin 7-glucoside showed two violations and naringin, eriocitrin, hesperidin, isorohifolin, diosmin, piperitoside and menthoside showed three violations as shown in Table 1. All of the ligands except menthoside and caffeic acid showed better drug-likeness score towards enzyme inhibitor descriptors. However, these compounds exhibited active to moderate active score for other descriptors as shown in the Table 2.

ADMET and TOPKAT analysis

Table 3 shows the ADMET profile of the twelve ligands, wherein eriodictyol and caffeic acid showed good intestinal absorption property compared to that of other ligands. All of the compounds except eriodictyol were predicted to have Cytochrome P450 2D6 (CYP2D6) induction effect. The toxicity profile of twelve ligands as depicted in Table 4 exhibits eriodictyol and rosmarinic acid as non-degradable towards aerobic biodegradability nature compared to the other ligands. All of the ligands were predicted to have ocular/eye irritancy effect in human.

Docking analysis with HMG CoA reductase (HMGR)

Table 5A shows the docking studies and binding free energy calculations in which menthoside exhibited the highest interaction energy (-70.0 kcal/mol)and caffeic acid in contrast showed the least interaction energy (-27.6 kcal/mol). In the present study all of the ligands except eriodictyol and caffeic acid showed interaction with Lys549 amino acid residue of HMGR as shown in the Table 5A. Most of the ligands beside eriodictyol and diosmin showed interaction with Arg582 amino acid residue. On the other hand, hesperidin and caffeic acid exhibited interaction with Cys545 and Leu546 amino acid residue of HMGR respectively as shown in the Table 5A.

Docking analysis with Squalene synthase (SQS)

Piperitoside exhibited the maximum interaction energy (-65.32 kcal/mol) while caffeic acid showed the least interaction energy (-27.91 kcal/mol) in the docking studies and binding free energy calculations (Table 5B). Four ligands which are: eriodictyol 7-glucuronide, luteolin 7-glucoside, piperitoside and

Table 2. Drug-likeness property analysis of 12 ligands using Molinspiration online software tool

Ligand	GPCR* ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor
Naringin	0.11	-0.40	-0.24	0.04	0.09	0.24
Eriodictyol	0.07	-0.20	-0.22	0.46	-0.09	0.21
Eriodictyol 7-glucuronide	0.17	-0.10	-0.28	0.45	0.09	0.39
Eriocitrin	0.06	-0.47	-0.28	-0.08	0.05	0.16
Hesperidin	-0.01	-0.59	-0.36	-0.20	-0.00	0.06
Isorohifolin	0.05	-0.32	-0.01	-0.03	0.01	0.24
Luteolin 7-glucoside	0.09	-0.02	0.15	0.27	-0.01	0.42
Diosmin	0.05	-0.53	-0.13	-0.23	-0.06	0.09
Rosmarinic acid	0.17	-0.08	-0.18	0.57	0.15	0.24
Piperitoside	-0.09	-0.55	-0.21	-0.02	-0.06	0.07
Menthoside	-1.07	-2.10	-1.57	-1.59	-0.73	-1.18
Caffeic acid	-0.48	-0.23	-0.81	-0.10	-0.79	-0.09

*GPCR- G Protein coupled receptors

Table 3. ADMET analysis of twelve ligands

Ligand	HIA			AS		BBB		PPB	CYP 2D6	HT
	PSA	ALog P8	L*	Log(SW)	L**	Log BB	L***	Prediction		
Naringin	228.48	-0.42	3	-4.18	2	0	4	F	F	F
Eriodictyol	109.49	2.13	0	-2.82	3	0	4	F	T	T
Eriodictyol 7-glucuronide	207.1	0.33	3	-4.02	2	0	4	F	F	T
Eriocitrin	249.29	-0.66	3	-5.07	2	0	4	F	F	T
Hesperidin	237.41	-0.43	3	-4.49	2	0	4	F	F	T
Isorohifolin	228.48	-0.38	3	-4.21	2	0	4	F	F	T
Luteolin 7-glucoside	189.80	0.24	3	-3.33	3	0	4	F	F	T
Diosmin	237.41	-0.40	3	-4.53	2	0	4	F	F	T
Rosmarinic acid	147.61	2.71	2	-3.21	3	0	4	F	F	F
Piperitoside	216.03	2.14	3	-4.64	2	0	4	F	F	F
Menthoside	275.52	1.28	3	-5.5	2	0	4	F	F	T
Caffeic acid	79.75	1.44	0	-1.06	4	0	3	F	F	F

HIA-Human intestinal absorption, AS- Aqueous solubility, BBB-Blood brain barrier, PPB-Plasma protein binding, CYP2D6- cytochrome P450 2D6, HT-hepatotoxicity, L-Level, F-False and T-True.] *(0-good; 1-moderate; 2-poor and 3-very poor) ;**(0-extremely low; 1-very low; 2-low; 3-good; 4-optimal; 5-too soluble and 6-warning);***(0-very high penetrate; 1-high; 2-medium; 3-low and 4-undefined)

Table 4. Toxicity prediction analysis of twelve ligands

Ligand	AB*	AM**	OI#	SI##	SS♦	Oral toxicity▲
Naringin	Degradable	Non-mutagen	Irritant	Non-irritant	Non-sensitizer	5.52
Eriodictyol	Non-degradable	Non-mutagen	Irritant	Non-irritant	Sensitizer	1.82
Eriodictyol 7-glucuronide	Degradable	Non-mutagen	Irritant	Non-irritant	Non-sensitizer	4.67
Eriocitrin	Degradable	Non-mutagen	Irritant	Non-irritant	Non-sensitizer	6.75
Hesperidin	Degradable	Non-mutagen	Irritant	Non-irritant	Non-sensitizer	3.48
Isorohifolin	Degradable	Non-mutagen	Irritant	Non-irritant	Non-sensitizer	2.96
Luteolin 7-glucoside	Degradable	Non-mutagen	Irritant	Non-irritant	Non-sensitizer	1.47
Diosmin	Degradable	Non-mutagen	Irritant	Non-irritant	Non-sensitizer	1.87
Rosmarinic acid	Non-degradable	Non-mutagen	Irritant	Non-irritant	Sensitizer	2.44
Piperitoside	Degradable	Non-mutagen	Irritant	Non-irritant	Non-sensitizer	1.01
Menthoside	Degradable	Non-mutagen	Irritant	Non-irritant	Non-sensitizer	2.33
Caffeic acid	Degradable	Non-mutagen	Irritant	Non-irritant	Sensitizer	1.48

AB*- Aerobic biodegradability; AM**- Ames mutagenicity; OI#- Ocular irritancy; SI##- Skin irritancy; SS♦- Skin sensitization and Oral toxicity▲- Oral toxicity in rat (LD_{50} in g/Kg of body weight-rat)

caffeic acid showed interaction with Gln212 amino acid residue. Eriodictyol, on the other hand, showed interaction with Arg218 amino acid residue of SQS, as shown in the Table 5B.

Discussion

Today, worldwide pharmaceutical industries are investing torrential in virtual screening technologies as an alternative drug development strategy for many challenging areas including cancer drug development. Lipinski rule of five is a rule applied to evaluate molecular physicochemical and drug-likeness properties of compounds which when further determined a lead compound having a certain pharmacological or biological activity could be made into an orally active drug for human (Lipinski *et al*., 2001). Violation of the Lipinski's rule of five is when logP>5; molecular weight (MW) >500; number of N, O (hydrogen bond acceptor) >10; number of OH and NH (hydrogen bond donor) >5 and number of rotatable bond (rotb) >15. With regard to drug-likeness score, if the score is >0 is active, -5.0 to 0.0 is moderate active, and <-5.0 is inactive (Sadhana Singh *et al.*, 2013).

The elevated serum cholesterol, especially low-density lipoprotein (LDL) cholesterol is one of the major risk factors for coronary heart disease (CHD). The most effective therapeutic approach to reduce the level of plasma LDL cholesterol is now achieved by inhibition of cholesterol biosynthesis (Choi *et al*., 2007). HMG-CoA reductase (HMGR) is composed of four regions they are i) the N-terminal region, ii) the conserved membrane domain, iii) the linker region and iv) the catalytic (or cytosolic) domain. HMGR is one of the effective approaches for treating hypercholesterolemia including cardiovascular disease (Pakpour, 2013). Squalene synthase (SQS) inhibition may also decrease circulating LDL cholesterol levels by inducing LDL receptors (Menys and Durrington, 2003). In the present study, eriodictyol failed to dock with HMGR, whereas naringin, eriocitrin, hesperidin, isorohifolin, diosmin and menthoside failed to dock with SQS which might be generally due to the poor binding phenomenon (Akdogan *et al.*, 2011). Previously three natural compounds such as curcumin *(Curcuma longa),* docosanol *(Saccharum arundinaceum)* and salvianolic acid C *(Salvia miltiorrhiza)* have been reported to inhibit the HMG-CoA reductase (HMGR) activity (Lin *et al.*, 2015). Similarly, chlorogenic acid *(Prunus mume)* has been reported to inhibit the Squalene synthase (SQS) activity (Choi *et al*., 2007).

Although *Mentha piperita* crude extract has been reported to exhibit hypolipidemic activity (Badal *et al.*, 2011; Barbalho *et al*., 2011), no constituents have been reported to this properties. Some of the constituents identified in *M. piperita* were reported to have anti-hyperlipidemia. Sharma (1980) found that caffeic acid did not exhibit hypolipidemic activity, however, Chao *et al*. (2009), reported that hypolipidemic activity of ellagic acid was significantly better than caffeic acid. Miyake *et al*., (2006); Yang *et al.*, (2012) and Lim *et al.*, (2013)

Table 5 A. The interaction energy analysis of twelve ligands with that of HMGR using Discovery Studio® 3.1

Ligand name	-cDocker interaction energy (kcal/mol)	Interaction amino acid residue	Bond distance (Å)
Naringin	49.10	Glu548	2.2
		Lys549	1.7
		Arg582	1.7
		Ser580	1.0 and 2.6
Eriodictyol	F*	Nil	Nil
Eriodictyol 7-glucuronide	53.20	Asp547	2.8 and 3.4
		Glu548	2.9
		Lys549	2.1 and 2.9
		Arg582	2.2
		Ala579	2.3
		Arg840	2.7
Eriocitrin	61.0	Asp547	2.9
		Lys549	1.9 and 2.1
		Ala579	1.7
		Arg582	2.0
		Arg840	2.6
Hesperidin	51.50	Cys545	2.2 and 2.4
		Asp547▲	2.3
		Lys549	1.8
		Glu550	0.67
		Gly577	1.8
		Gly578	1.1
		Arg582■	6.0
		Arg840	1.5
Isorohifolin	65.22	Glu548	2.3
		Lys549	1.0
		Glu550	2.4
		Gly577	0.9
		Ser580	1.9
		Arg582	1.3 and 2.1
Luteolin 7-glucoside	61.6	Lys549	0.87 and 2.2
		Gly577	2.5
		Ser580	2.2
		Arg582	0.59 and 2.9
		Arg840	2.2
Diosmin	47.1	Asp547▲	1.9
		Glu548	1.1 and 2.0
		Lys549■	3.2 and 3.4
		Ala579	2.1
		Arg840	1.4 and 1.5
Rosmarinic acid	42.4	Glu548	2.4
		Lys549	0.62 and 1.1
		Ser580	2.0
		Arg582	2.4 and 3.5
Piperitoside	63.83	Asp547	3.8
		Lys549	1.6 and 1.8
		Gly574	2.0
		Ser580	1.3
		Arg582	2.2
		Arg840	1.6
Menthoside	70.0	Lys549	1.6 and 2.0
		Glu548	1.9
		Glu550	2.5
		Ser580	1.2
		Arg582	2.1
		Arg840	1.3
Caffeic acid	27.6	Leu546	2.5
		Asp547	1.8
		Ser580	2.5
		Arg582	2.5
		Arg840	3.8

F*-Failed to dock; ▲-π-sigma interaction; ■-π-+ interaction

have reported the hypolipidemic activity of eriocitrin, luteolin 7-glucoside and eriodictyol respectively. Rosmarinic acid was shown to be having anti-lipid peroxidative activity (Lin *et al*., 2002). Bok *et al.* (1999), showed that the mixture of naringin and hesperidin fed rats exhibited reduction in HMGR activity when compared to that untreated rats. Apart from these reports on the compounds, until the present there is no study available with regard to their docking studies.

Table 5 B. The interaction energy analysis of twelve ligands with that of SQS using Discovery Studio® 3.1

Ligand name	-cDocker interaction energy (kcal/mol)	Interaction amino acid residue	Bond distance (Å)
Naringin	F*	Nil	Nil
Eriodictyol	40.1	Arg218	1.8
Eriodictyol 7-glucuronide	46.9	Arg77	1.6
		Gln212	2.4
		Asn215	2.1
		Asp219	2.2
		Asp223	1.2
Eriocitrin	F*	Nil	Nil
Hesperidin	F*	Nil	Nil
Isorohifolin	F*	Nil	Nil
Luteolin 7-glucoside	45.32	Arg77	1.2
		Arg77	3.8
		Asp80	1.7
		Asn215	2.2
		Gln212	1.5 and 1.9
		Gln293	1.9
Diosmin	F*	Nil	Nil
Rosmarinic acid	46.80	Asp80	and 1.1
		Ala176	2.0
		Gly180	2.3
		Asn215	2.4
Piperitoside	65.32	Gln212	1.4
		Arg218	2.2
		Asp219	0.54
		Glu222	1.7
		Phe230♦	6.0
Menthoside	F*	Nil	Nil
Caffeic acid	26.91	Gln212	0.93 and 1.6

F*-Failed to dock; ♦-π-π interaction

Conclusion

In the present study, all of the tested ligands except for eriodictyol have shown to dock and bind with HMGR. Interestingly six ligands which are naringin, eriocitrin, hesperidin, isorohifolin, diosmin and menthoside exhibited docking selectivity towards HMGR. On the other hand, six ligands which are naringin, eriocitrin, hesperidin, isorohifolin, diosmin and menthoside failed to dock with SQS. Only eriodictyol exhibited docking selectivity towards SQS. Among the ligands, eriodictyol 7-glucuronide, luteolin 7-glucoside, piperitoside, rosmarinic acid and caffeic acid were shown to dock and bind with both HMGR and SQS. However, caffeic acid exhibited the very least binding energy irrespective of the target protein. .The results from the present study provide new insight in understanding these twelve ligands as potential hypolipidemic agents in which the molecular docking studies could contribute for further development and understanding of the HMGR and SQS inhibitors for the prevention of hypercholesterolemia associate disorders.

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