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Research Article

IN SILICO SCREENING OF CARDIOPROTECTIVE ACTIVITY OF SOME FLAVONOLS

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ABSTRACT

Objectives: Flavonoids constitute a large class of polyphenols found in plants. Among flavonoids, Flavonols are the most abundant and widely distributed in nature. Flavonols are present in considerable amounts in fruits and vegetables. They show a wide range of biological activities. In the present study, we wanted to access the cardioprotective activity of some selected flavonols using in silico tools.

Method: Binding affinity of flavonols against known cardioprotective drug target Malonyl Coenzyme A was calculated by performing the docking experiment using FlexX.

Results: The analysis showed that flavonols have high potential to inhibit the target. The docking studies also showed greater affinity of all selected flavonols towards the active site of Malonyl Coenzyme A with a docking score of -29.2358 Kcal/mol for Morin, -29.0486 Kcal/mol for Kaempferol, -28.3885 Kcal/mol for Fisetin, -27.5950 Kcal/mol for Quercetin, -27.5303 Kcal/mol for Isorhamnetin, -25.7692 Kcal/mol for Myricetin, -24.9174 Kcal/mol for 3-OH-flavone and -23.5732 Kcal/mol for Tamarixetin.

Conclusion: The study proposes that out of the eight selected flavonols, Morin is the suitable inhibitor of Malonyl Coenzyme A. Hence it is noted that flavonols – morin may act as cardioprotective agent by decreasing myocardial malonyl-CoA levels.

Keywords: Cardioprotective, Docking, Flavonols, FlexX, Malonyl Coenzyme A

INTRODUCTION

Flavonoids constitute a large class of polyphenols found in plants. This group includes several subclasses such as Flavonols, flavones, flavanones, anthocyanidines, Isoflavones, dihydro Flavonols and chalcones. Among flavonoids, Flavonols are the most abundant and widely distributed in nature. Flavonols are widely distributed in plants and are present in considerable amounts in fruits and vegetables, the richest sources include onions, apples, grapes, wine and tea. Flavonols lowered blood pressure in animal models of insulin resistance and metabolic syndrome. Flavonols has antihypertensive effects when given chronically in the most common rodent models of hypertension. Flavonols protect against atherosclerosis by preventing one or several processes involved in disease progression, such as oxidative stress, inflammation and endothelial dysfunction. Flavonols by preventing hypertension, atherosclerosis and endothelial dysfunction may protect the coronary vessels in the long term. Flavonols prevent endothelial dysfunction, atherosclerosis, hypertension and possibly thrombosis, all possible mechanism to prevent strokes [5,6,7]

Cardiovascular disease is the leading cause of death and disability for people living in western societies, with ischaemic heart disease accounting for the majority of this health burden [2]. The malonyl CoA is an exciting new target for the treatment of ischaemic heart disease. Malonyl CoA is a potent endogenous inhibitor of cardiac fatty acid oxidation, secondary to inhibiting carnitine palmitoyl transferase-I, the rate-limiting enzyme in the mitochondrial uptake of fatty acids [1]. Malonyl CoA is synthesized in the heart by acetyl CoA carboxylase, which in turn is phosphorylated and inhibited by 5' AMP-activated protein kinase. The degradation of myocardial malonyl CoA occurs via malonyl CoA decarboxylase (MCD) [3]. Inhibiting MCD will significantly increase cardiac malonyl CoA levels. This is associated with an increase in glucose oxidation, a decrease in acidosis, and an improvement in cardiac function and efficiency during and following ischemia[4].

Computers and computational methods are nowadays widely used in Biological Researches. *In silico* methods can help in identifying drug targets via bioinformatics tools. They can also be used to analyze the target structures for possible binding/ active sites, generate candidate molecules, check for their drug likeness, dock these molecules with the target, rank them according to their binding affinities, further optimize the molecules to improve binding characteristics. It has been observed that *in silico* approach to drug designing drastically minimizes the cost involved, as well as the time span required for a molecule to pass through the Drug Discovery pipeline. Virtual screening, the *in silico* analog of high throughput screening, also offers immense potential in identification of novel drug candidates [9, 14].

MATERIALS AND METHODS

Preparation of Ligand Structures

All the compounds used for docking study were selected from the lit erature. Chemsketch, chemically intelligent drawing interface freeware developed by Advanced Chemistry Development, Inc., (http://www.acdlabs.com) was used to construct the structure of the ligands. Using draw mode of Chemsketch, the ligands were drawn and the SMILE notations of the compounds were generated. The three dimensional structures of the compounds in PDB formats were generated using another online server called CORINA using the SMILE notation and again converted to SDF format using OpenBabel [11].

Preparation of Protein Structure

The crystal structure of the drug targets Malonyl Coenzyme A (PDB ID: 3NYQ has been obtained from RCSB Protein Data Bank (http://www.pdb.org).

Active site identification

The structure of the drug target was obtained from Protein Data Bank. The PDB file was loaded into Q-Site Finder [12, 13] to identify the active site amino acids.

ADME/Tox Screening

The ADME/Tox parameters of the compounds were studied using online server Mobyle@RPBS maintained by the University of Paris. The compounds were input in the server in SMILES format using the following parameters:

Molecular weight: min 200.0 max 600.0

Hydrogen donors: min 0.0 max 6.0

Hydrogen acceptors: min 0.0 max 12.0

Flexible bonds: min 0.0 max 15.0

Rigid bonds : min 0.0 max 50.0

Ring number : min 0.0 max 7.0

Ring size : min 0.0 max 12.0

Atom number : min #carbons: 5.0 min #non carbons 2.0

Ratio carbon/hetero : min 0.1 max 1.0

Charge number : min 0.0 max 3.0

Total charge : min -2.0 max 2.0

logP : min -2.0 max 6.0

Polar Surface Area : min 0.0 max 150.0

Protein – Ligand interaction using FlexX

The PDB file of the target was loaded in the BioSolveIT FlexX [15]. The active site amino acids were defined in the target molecule during the target preparation step of FlexX. A sphere of 9Å radius was defined as active site. The SDF file of all the compounds was loaded in FlexX as docking library. The Protein Ligand clash was set to 2.9 Å and Intra Ligand clash was set to 0.6 in the docking. The docking was performed to study the binding efficacy of the compounds and the drug target. The docked ligand-target complexes were analyzed carefully to identify the interactions [8, 10]. The docking score was noted down and docking poses were saved for reference.

RESULTS

Malonyl Coenzyme A, which is a target for cardiovascular disease was selected based on the literature survey. The structure of Malonyl Coenzyme A was obtained in PDB format. The active site characterisation of the enzyme using Q-site finder showed that THR-143, LYS 390, GLY-285, ARG-283, LYS-395, SER-261, THR-287, TYR 142 and LEU 388 are the key amino acids forming active site.

For any molecule to become a drug it should not have any toxic or allergenic effects and it should possess all the ADME/Tox properties. The ADME/Tox screening of the compounds has not shown any negative results, which indicates the potentiality of these molecules to become drugs. The results of the ADME/Tox screening is described in table 1.

Table 1*:* Molecular weight, Hydrogen donors, Hydrogen acceptors, Flexible bonds, Rigid bonds, Ring number, Ring size, Carbon number, Non carbon number, Ratio carbon/non carbon, Charge number, Total charge, logP and Polar Surface Area of the compounds obeying to Lipinski's rule of drug likeness

Parameters	MW	Drs	Ars	FB	RB	#R	RL	C	nC	C/nC	#Chrg	Chrg	LogP	PSA
Parameter standards	**200-400**	**0-6**	**0-12**	**0-15**	**0-50**	**0-7**	**0-12**	**5-2**	**>2**	**0.1-1.0**	**0-3**	**(-2)-2**	**(-2)-6**	**0-150**
3-OH-Flavone	238.2	1	3	1	18	3	6	15	9	0.600000	0	0	4.010000	46.530000
Quercetin	302.2	5	7	1	18	3	6	15	9	0.600000	0	0	3.250000	127.450000
Kaempferol	286.2	4	6	1	18	3	6	15	9	0.600000	0	0	3.230000	107.220000
Myricetin	318.2	6	8	1	18	3	6	15	9	0.600000	0	0	3.060000	147.680000
Isorhamnetin	316.2	4	7	2	18	3	6	16	9	0.562500	0	0	3.140000	116.450000
Tamarixetin	316.2	4	7	2	18	3	6	16	9	0.562500	0	0	3.140000	116.450000
Morin	302.2	5	7	1	18	3	6	15	9	0.600000	0	0	2.820000	127.450000
Fisetin	286.2	4	6	1	18	3	6	15	9	0.600000	0	0	3.230000	107.220000

Interaction energies between ligand and receptor play the most crucial role in drug designing. In this work, Malonyl Coenzyme A (PDB ID: 3NYQ) was selected as drug target and the interactions of the compounds were studied using FlexX.

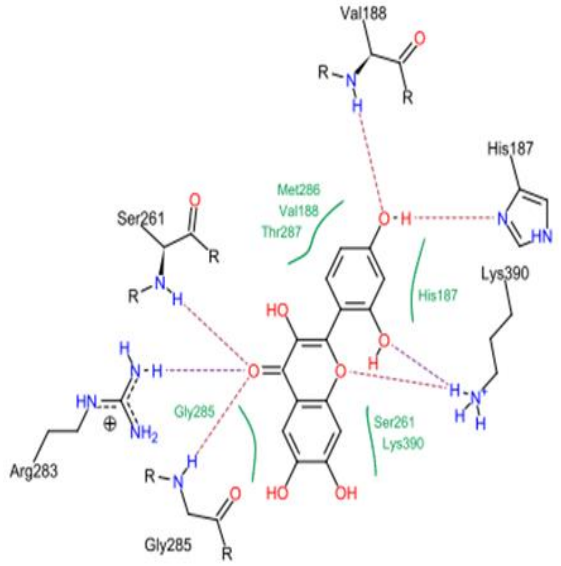

Fig.1: Docking of Morin with Malonyl Coenzyme A.

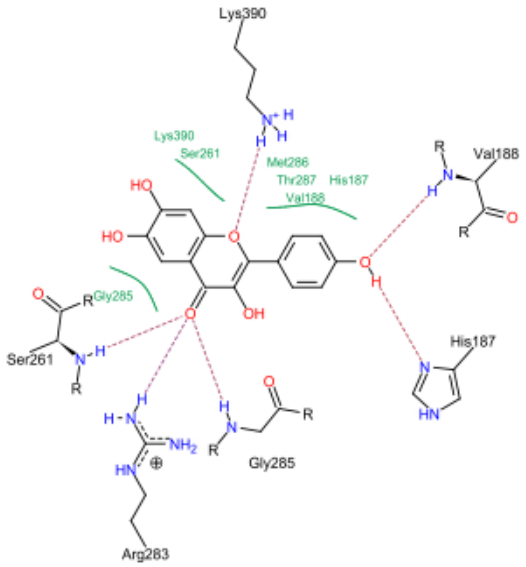

Fig. 2: Docking of Kaempferol with Malonyl Coenzyme A.

Table 2: Docking results of Flavonols with Malonyl Coenzyme A

Compounds	Total	Bond Property

	Score (Kcal/mol)	Bonds	Energy (Kcal/mol)	Length(Å)
3-OH-flavone	-24.9174	LYS-390-O28	-4.68	2.61
		GLY-285-O27	-4.70	2.25
		ARG-283-O27	-4.27	1.74
		ARG-283-O27	-2.18	2.23
Quercetin	-27.5950	ARG-283-H16	-4.70	2.18
		THR-143-H18	-4.35	1.57
		LYS-395-O28	-3.08	1.84
		ARG-283-O29	-4.70	1.61
Kaempferol	-29.0486	LYS-390-O29	-3.80	2.11
		GLY-285-O31	-4.06	2.22
		ARG-283-O31	-2.55	2.00
		SER-261-O31	-3.68	2.20
		VAL-188-O27	4.43	1.58
		HIS-187-H25	-4.70	1.95
Myricetin	-25.7692	ARG-283-H16	-4.70	2.18
		ARG-283-O30	-4.34	1.60
		LYS-395-O29	-3.10	1.84
		THR-143-H18	-4.70	1.57
Isorhamnetin	-27.5303	HIS-187-H20	-4.70	1.83
		LYS-390-O33	-4.13	2.04
		GLY-285-O30	-4.21	2.19
		ARG-283-O30	-2.94	2.07
		SER-239-O30	-2.44	2.31
Tamarixetin	-23.5732	HIS-189-O32	-4.70	2.08
		VAL-188-O31	-4.10	1.86
		HIS-189-H17	-4.70	2.08
		THR-287-O30	-4.02	1.58
Morin	-29.2358	VAL-188-O27	-4.64	1.53
		HIS-187-H25	-4.70	2.03
		LYS-390-O31	-3.34	2.21
		GLY-285-O29	-4.38	2.18
		SER-261-O29	-3.56	2.22
		ARG-283-O29	-2.44	2.06
Fisetin	-28.3885	ARG-283-O29	-4.40	1.99
		HIS-189-O27	-4.70	1.88
		VAL-188-O26	-3.64	1.81
		THR-287-O17	-2.27	2.32

DISCUSSION

Cardiovascular disease is the leading cause of death and disability for people living in western societies, with ischaemic heart disease accounting for the majority of this health burden. So, there is always a high demand of effective drug molecules to combat the disease. And plant derived drugs are always of top preference in any diseases. Application of modern tools to validate the ethnobotanical information in designing newer effective drugs may lead to the development of promising drugs with lesser side effects. Flavonols in this way may be potent drug for cardiovascular diseases. Toxicity analysis has been performed using FAF Drugs ADME/Tox filtering server. All the compounds successfully passed through the ADME/Tox filter. The ADME/Tox screening of the compounds has not shown any negative results, which indicates the potentiality of these molecules to become drugs. Docking studies yielded crucial information concerning the orientati on of the inhibitors in the binding pocket of the target protein. All the compounds bind successfully to the active site of the target proteins. The docking scores were the highest for Morin with Malonyl CoA with -29.2358 Kcal/mol followed by Kaempferol with -29.0486 Kcal/mol, Fisetin with -28.3885 Kcal/mol, Quercetin with -27.5950 Kcal/mol, Isorhamnetin with -27.5303 Kcal/mol, Myricetin with -25.7692 Kcal/mol, 3-OH-flavone with -24.9174 Kcal/mol and Tamarixetin with -23.5732 Kcal/mol. Thus from the docking scores it can be inferred that the compounds binds successfully with the drug targets and the compounds may be used as cardiovascular drugs. Further *in vivo* experiments in this regard will surely help these molecules in reaching the market as commercial drug.

CONCLUSION

Based on in silico docking result it has been observed that out of the eight selected flavonols, Morin has the greater affinity to bind with Malonyl Coenzyme A. Present work therefore, proposes that Morin may be a suitable Cardioprotective agent by decreasing myocardial malonyl-CoA levels.

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CONFLICT OF INTEREST STATEMENT: The authors declare no conflict of interests.

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