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Resveratrol A Potent Angiotensin Converting Enzyme Inhibitor: A Computational Study in Relevance to Cardioprotective Activity.

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ABSTRACT

Secondary metabolites obtained from different plants have been the starting material for designing different drugs. Resveratrol, a naturally occurring polyphenol found in grape skins, peanuts, and red wine shows myriad varieties of pharmacological activities. Different polyphenols have been found to be cardioprotective and paved the path towards development of cardioprotective formulations. In the present study we have analyzed the inhibitory potential of resveratrol, on the Angiotensin Converting Enzyme (ACE) - the enzyme responsible for various cardiovascular diseases. The study revealed that resveratrol have high ACE inhibiting potential and also low IC 50 values as compared to other known ACE inhibitors.

Keywords: Polyphenol, Resveratrol, ACE, Cardioprotective, IC50

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INTRODUCTION

Cardiovascular disease (CVD) refers to any disease that affects the cardiovascular system, principally cardiac disease, vascular diseases of the brain and kidney, and peripheral arterial disease. Diseases of the cardiovascular system include those that compromise the pumping ability of the heart, cause failure of the valves, or result in narrowing or hardening of the arteries. Injury or failure of the cardiovascular system, especially the heart, also affects the peripheral tissues that depend on the delivery of nutrients and the removal of wastes through the blood vascular system. CVD is a family of diseases that includes hypertension, atherosclerosis, coronary heart disease, and stroke [1] Angiotensin Converting Enzyme is the prime target for preventing CVD as the enzyme catalyses conversion of Angiotensin I into Angiotensin II [2] Angiotensin II is a vasoconstrictor that causes blood vessels to constrict thereby causing hypertension [3]. ACE is expressed in small pulmonary arteries normally [4]. However, during diabetes, obesity, hypertension the expression and activities of the enzyme increases in small pulmonary arteries [4]. This led to the development of ACE inhibitors which show significant cardioprotection through decreasing hypertension [5, 23 & 24]. This also relieves other hypertension linked ailments like kidney diseases, diabetes etc. [6].

Meanwhile, herbal based secondary metabolites are constantly being screened for drug discovery with respect to ACE inhibition. Resveratrol have been reported to be cardioprotective [7]. We, therefore, thought it prudent that resveratrol may inhibit ACE and thus provide cardioprotection. We validate the hypothesis using in silico tools.

MATERIALS AND METHODOLOGY

The Ligands

Resveratrol was selected for study using available literature [7], the structure of the ligand was drawn using Chemsketch [8], a chemically intelligent drawing interface freeware was used to construct the structure of the ligands. The three dimensional structure of the compound in PDB formats was generated and converted to SMILES using OpenBabel [9] and then converted to .sdf format again using OpenBabel. Known ACE inhibitors Benazepril, Captopril, Enalapril, Fosinopril, Imidapril, Lisinopril, Quinapril, Ramipril, Trandolapril and Zofenopril [25] were used as reference. The structures of these inhibitors were obtained from NCBI PubChem Compound (http://www.ncbi.nlm.nih.gov/pccompound).

ADME/Tox Screening

ADMET screening helps in detecting rug likeliness of compounds [10]. ADMET (Absorption, Distribution, Metabolism, Excretion and Toxicity) screening was done using Mobyle@rpbs server [11]. The compounds were loaded 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, No. of Carbons: >2, Hetero atoms: >2, 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

Table-1: ADMET Properties of resveratrol

Parameters	MW	Drs	Ars	FB	RB	#R	RL	C	Hetero atoms (H)	C/H	#Chrg	Chrg	LogP	PSA
Parameter standards	200-400	0-6	0-12	0-15	0-50	0-7	0-12	5-12	>2	0.1-1.0	0-3	(-2)-2	(-2)-6	0-150
Resveratrol	228.2	3	3	3	12	2	6	15	9	0.21	0	0	2.39	57.53

The receptor

The crystal structure of the drug target Angiotensin Converting Enzyme (PDB ID: 1O8A) was obtained from RCSB Protein Data Bank (http://www.rcsb.org). The protein has one chain (Chain A) of 589

residues determined by X-ray diffraction method at a resolution of 2.00 Å. It was deposited by: Natesh et al., in the year 2002.

Active site identification

The PDB file was loaded into Q-Site Finder to identify the active site amino acids at default parameter setting [12].

Protein – Ligand interaction using FlexX

Docking is a term used for computational schemes that attempt to find the best matching between two molecules: a receptor and ligand [20]. The receptor Angiotensin Converting Enzyme (ACE) was docked with the known ACE inhibitors and resveratrol using a software FlexX [13, 14]. The active site amino acids were defined in the target molecule during the target preparation and residues within a radius of 10 Å were included within binding site. The SDF file of all the compounds was loaded in FlexX as docking library. The output file gave the energy values in Kcal/mol. For each docked molecule, this value was noted down.

Quantitative Structure Activity Relationship (QSAR) studies

The QSAR analysis [22] was performed by taking the known Angiotensin Converting Enzyme inhibitors *viz.* Benazepril, Captopril, Enalapril, Fosinopril, Imidapril, Lisinopril, Quinapril, Ramipril, Trandolapril and Zofenopril. The QSAR descriptors *viz.* Polarizability, Molar Refractivity, Molar volume, Molecular weight and LogP were generated for each of the molecule using ACD ChemSketch softwares [15]). The activities have been calculated by taking the inverse logarithm of IC50 values. The descriptors were tabulated in a MS Excel Sheet against their bioactivities (log IC_{50}^{-1}). The descriptors and activities were loaded in Easy QSAR software for multiple linear regression analysis. From the regression, the QSAR equation was generated and the activities of tea polyphenols were predicted [16].

RESULTS

ADMET screening revealed that resveratrol was non-toxic and obeyed Lipinski's rule.

The docked ligand-target complexes were analyzed carefully to identify the interactions. The docking score was noted down and docking poses were saved for reference (Figure 1).

The QSAR analysis of all the compounds showed significant correlation with R square value of 94.49% (The Rsq value should be definitely high for a good QSAR equation, Higher Rsq means higher fitting of the equation to the given data, Hence better predictions it will provide for new test data). The Adjusted Rsq is 85.31 % therefore the difference between Rsq and adjusted Rsq is less (High difference in Rsq and Adjusted Rsq indicates weaker overall prediction). The F statistics of the test is 10.29 and the critical F is 3.69 (The F statistics of the test should be greater than Critical F otherwise the generated equation is inefficient) [17].
The equation generated out of QSAR analysis is as follows:

Activity = 2.90 -0.168 Molar Volume) + 0.117 (Parachor) + 0.160 (Molar refractivity) + 1.68 (LogP) -0.179 (Molecular Weight).

From the above QSAR equation the IC 50 values of resveratrol was predicted and the value is **6.3 nM** (Figure 2).

Comparative analysis ACE inhibitory potential of Resveratrol and known inhibitors

From the docking score of resveratrol and the known inhibitors it was found that resveratrol have much more binding affinity compared to the known ACE inhibitors and the predicted activity (IC 50) of resveratrol is **6.3 nm** which is much less than that of known ACE inhibitors.

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Figure 1: Binding patterns of A -Resveratrol and B - known inhibitor Trandolapril with Angiotensin Converting Enzyme

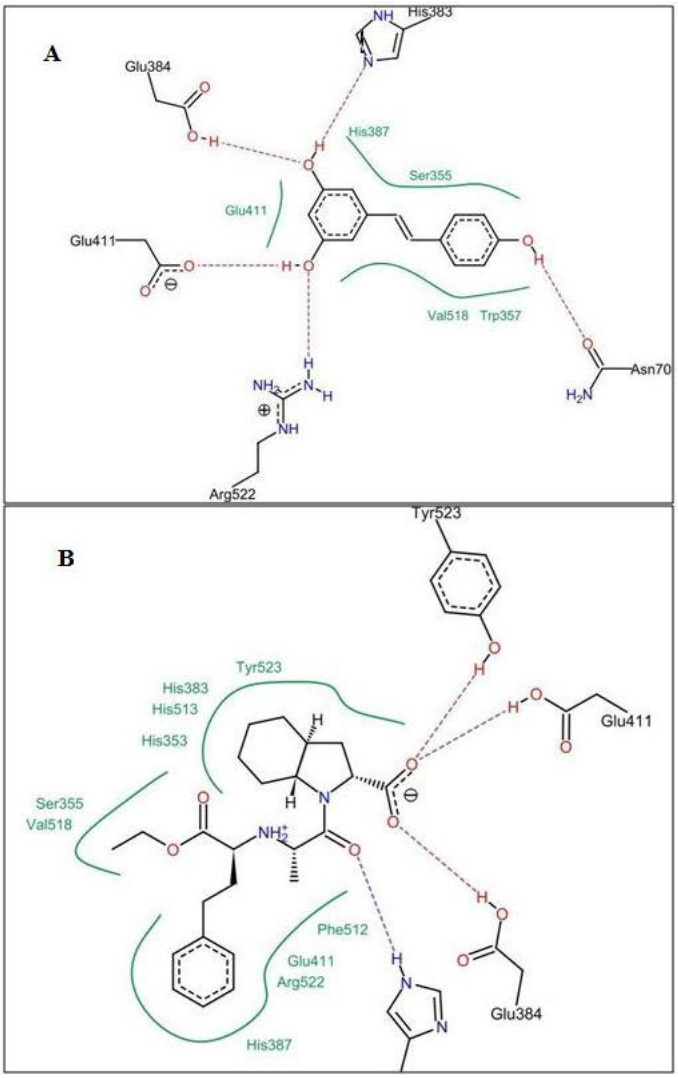

Figure 2: The multiple regression plots (linear) for ten ACE inhibitors.

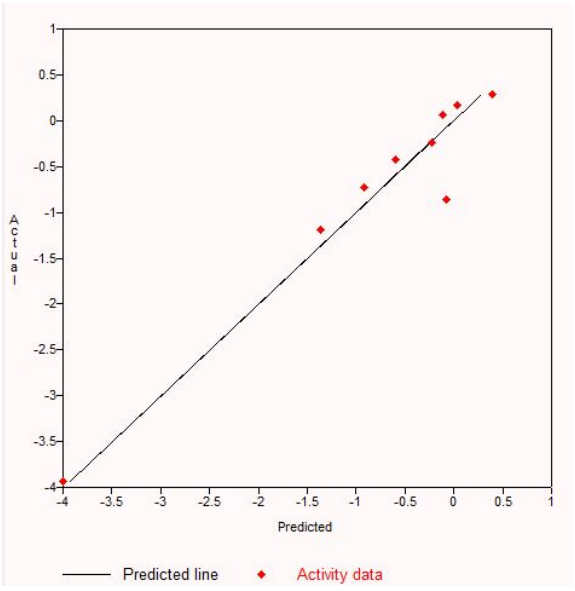

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Table 2: Docking of Resveratrol with Angiotensin Converting Enzyme

Compound	Total Score (Kcal/mol)	Bond Properties		
		Bonds	Bond Energy (Kcal/mol)	Bond Length (Å)
Resveratrol	-29.2392	HIS 383 – H28	-4.50	2.16
		GLU 384 – O2	-4.70	1.62
		GLU 411 – H27	-3.60	2.23
		ARG 522 – O1	-4.70	2.07
		ASN 70 - H29	-4.70	2.19

Table 3: Docking result of known Angiotensin Converting Enzyme inhibitors

Compounds	Total Score (Kcal/mol)	Hydrogen Bond Properties	
		Hydrogen Bonds	Bond Energy (Kcal/mol)
Benazepril	-13.1522	GLU 411 - H 38	-4.5
		GLU 411 - O 1	-4.3
		HIS 383 - O 1	-4.7
		HIS 513 - O 4	-4.6
		HIS 387 - H 59	-2.2
Captopril	-21.8193	HIS 383 -O 2	-4.7
		GLU 411 - O2	-4.7
		TYR 523 - O2	-3.5
		HIS 513 - O3	-2.6
		HIS 353 - O3	-4.7
Enalapril	-23.6967	GLU 384 - O4	-3.6
		HIS 383 -O 5	-4.7
		GLU 411 - O5	-4.7
		TYR 523 - O5	-4.0
		GLU 411 - H55	-3.2
		ARG 522 - O3	-4.0
Imidapril	-13.0579	HIS 383 -O 5	-2.5
		GLU 411 - O5	-4.7
		GLU 384 - O3	-4.7
		TYR 523 - O5	-2.4
Lisinopril	-22.7837	HIS 383 -O 5	-4.7
		GLU 411 - O5	-4.7
		TYR 523 - O5	-2.9
		HIS 387 - O4	-4.7
		GLU 411 - H59	-4.3
		ARG 522 - O3	-3.0
		ALA 356 - H52	-4.1
Perindopril	-15.3663	HIS 383 - O2	-4.7
		GLU 411 - O2	-4.7
		GLU 384 - O3	-4.7
		HIS 513 - O5	-4.5
		ALA 354 - H58	-4.4
Quinapril	-17.6071	HIS 383 - O3	-4.7
		GLU 411 - O3	-3.8
		TYR 523 - O3	-2.8
		GLU 384 - O2	-4.2
		ALA 356 - O5	-2.5
Ramipril	-14.2360	HIS 383 - O5	-4.7
		GLU 411 - O5	-4.7
		TYR 523 - O5	-3.8
		GLU 384 - O4	-3.5
		HIS 357 - H43	-2.7
Trandolapril	-2.9183	TYR 523 - O3	-4.6
		GLU 384 - O2	-4.7
Zofenopril	-14.6572	HIS 383 - S1	-2.9
		GLU 411 - S1	-2.7
		TYR 523 - S1	-3.1
		ALA 356 - O4	-4.0
		ARG 522 - O6	-3.2

DISCUSSION

While considering better ligands, the least score in docking was preferred as it indicates more stability in binding [13]. The interaction of resveratrol was screened based on hydrogen bonding based prediction [18] which shows they binds to the active site residues i.e., ASN70, SER355, TRP357, HIS 383, GLU384, HIS387, GLU411, ARG522, VAL518 etc. which was confirmed by the bonded residues in Flex-X. Activity of compound in question has been predicted from QSAR model [19] as inverse logarithm of IC50. It showed that the IC50 of resveratrol was better than the known inhibitors.

After choosing resveratrol as better option on the basis of docking score, IC50 and bonding pattern, cross validation was done by target fishing using Pharm mapper software and found that the target comes in suitable range. This analysis indicates suitability of the chosen ligand for the target in one hand and validate the docking result obtained from Flex X.

Angiotensin Converting Enzyme (ACE) produces Angiotensin II - a very potent chemical that causes hypertension [21]. By decreasing the production of angiotensin II through inhibiting the activity of the enzyme ACE, the function of a failing heart can be improve and thus the chances of hypertension and other CVDs can be reduce. Since, resveratrol binds to the active sites of the enzyme ACE and forms stable bonds therefore; resveratrol may be used as Angiotensin Converting Enzyme inhibitor. Resveratrol shows stable bonding pattern in compare to known inhibitors as they shows least score in docking, forms maximum number of hydrogen bonds with the active residues of the enzyme, therefore resveratrol have more ACE inhibitory potentials.

CONCLUSION

Based on present observation of docking score of both resveratrol and known inhibitors, IC50 value of known inhibitors and predicted IC50 of resveratrol we suggests that resveratrol may be Angiotensin Converting Enzyme targeted potent new drug for treating Cardiovascular diseases. However, further studies are required to validate the same in vivo or in vitro.

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