Original Research Article

Molecular docking studies of ephedrine, eugenol, and their derivatives as arginase inhibitors: Implications in asthma

Suhasini Donthi, Jayasree Ganugapati, Vijayalakshmi Valluri[1], Ramesh Macha[2], Krovvidi S. R. Sivasai

Department of Biotechnology, Sreenidhi Institute of Science and Technology, [1]Immunology Unit, Bhagwan Mahavir Hospital and Research Center, [2]Department of Chemistry, Osmania University, Hyderabad, Telangana, India

Abstract

Background: Arginine being a common substrate for arginase and nitric oxide synthase (NOS) an imbalance between enzymes could lead to a shift in airway responses. Reports suggest that increased arginase reduces the substrate availability to NOS and attributes to the airway hyperresponsiveness. Hence, inhibition of arginase might enhance the bioavailability of arginine to NOS and generates nitric oxide (NO) a bronchodilator. Molecules from Ephedra and Eugenia caryophyllus are documented for bronchodilator properties. However, the mechanism of action of these molecules for enhancing bronchodilation is not well characterized. The objective of the present study is to assess whether these molecules could inhibit the arginase by binding at its active site and helps in bronchodilation using *in silco* approach.

Methods: The crystal structures of the arginase and NOS enzymes were selected from the protein database. The molecules from Ephedra and Eugenia caryophyllus were obtained from Pubchem. Drug likeliness and bioactivity of the molecules were assessed by Molinspiration. The successful molecules were docked with active sites of enzymes using docking software, and the docked complexes were analyzed using Accelrys Discovery Studio.

Results: Molecules from Ephedra and Eugenia caryophyllus were able to interact to arginase at the active site whereas away from the active site in case of NOS. The molecules showed differential binding affinities, and some of them had higher binding affinity than substrate arginine.

Conclusion: *In silico* study suggests that molecules of Ephedra and Eugenia were capable of blocking the active site of arginase. We speculate that if these molecules are used as therapeutics, they could inhibit the arginase activity and this might increase arginine availability to NOS to produce NO which acts as bronchodilator. Our study suggests that molecules which bind to active site of arginine and do not affect the active site of NOS might be the potential molecules for arginase associated asthma.

Keywords: ArgusLab, Cambridge Crystallographic Data Center Genetic Optimization for Ligand Docking, discovery studio, genetic optimization, virtual screening

Address for correspondence: Dr. Krovvidi S. R. Sivasai, Department of Biotechnology, Sreenidhi Institute of Science and Technology, Yamnampet, Ghatkesar, Hyderabad - 501 301, Telangana, India.
E-mail: ksrsivasai@sreenidhi.edu.in

INTRODUCTION

Arginase is an important and final enzyme of urea cycle that utilizes arginine as substrate and converts to ornithine and urea. L-arginine also serves as a substrate for nitric oxide synthase (NOS) and thus both the enzymes compete for the substrate. Arginase is not only expressed in liver

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cells but also in other cells including lung and airways. Chronic asthma is characterized by airways constriction, inflammation, and airway hyperresponsiveness (AHR). High expression of arginase may attribute to airway remodeling in asthma by limiting the substrate arginine to NOS, thus preventing the generation of nitric oxide (NO), a bronchodilator.[1,2] Uptake of arginine by arginase not only reduces NO production but also enhances the synthesis of proline, putrescine, and polyamines that contribute to collagen deposition, cell proliferation, and smooth muscle contraction, respectively.[3] Reduced availability of L-arginine to NOS results in the accumulation of peroxynitrite that can induce inflammation, epithelial damage, and bronchoconstriction.[4]

On the other side, complete utilization of arginine by NOS and generation of high NO and its products also detrimental.[5] Hence, elevated levels of either enzyme have harmful effects on airways in asthma; therefore, two pathways must be under the regulation to maintain lung homeostasis.[6] In the past few years, high expression of arginase in asthma was established in animal models[7-9] and human[10-12] suggesting the enzyme role in pathogenesis. The novel drugs targeting the arginase inhibition may have favorable effects on asthma. The effectiveness of arginase inhibitors in different asthma models has been shown to reduce AHR and airway remodeling.[12] Based on these pieces of evidence, we designed our study to identify molecules from the plant sources that are known to have anti-asthmatic property and to assess whether their mode of the anti-asthmatic property is through inhibition of arginase.

Ephedra is a plant which has been used as herbal medicine for bronchial asthma, cold, flu, wheezing, and edema.[13,14] Ephedrine and pseudoephedrine are effective respiratory sedatives, cough remedies, and known to increase the blood pressure.[15] Drew *et al*. showed that D-(-)-ephedrine and L-(+)-pseudoephedrine isomers cause bronchodilation; however, the effect of ephedrine is double than pseudoephedrine.[16] Pseudoephedrine is the primary active component of many nasal decongestants, due to its effect on alpha-adrenoceptors in the nasal blood vessels.[17]

The second group of molecules we assessed is from Eugenia caryophyllus (commonly called clove) in which eugenol is the major compound. Research groups demonstrated antioxidant and bronchodilator activity of eugenol and its derivatives.[18] The anti-inflammatory effect of eugenol by modulating prostaglandin E2, NO, iNOS, and NF-kB was demonstrated by Maciele *et al*.[19] Raghavenra *et al*. showed the inhibitory activity of eugenol to 5-lipooxygenase and leukotriene-C4 in polymorphonuclear leukocytes cells of the human.[20] However, the effect of eugenol on arginase associated with asthma and the mechanism of bronchodilation is not well characterized.

The third group of molecules considered for the study is flavonoids, secondary metabolites of fruits, nuts, vegetables, flowers, and dark chocolates, which are found to have several biological activities such as antioxidant, anti-inflammatory, immune modulator, anti-carcinogenic, anti-diabetic, and anti-allergic properties. Studies show that flavonoids have an important role in controlling asthma through multiple mechanisms.[21,22] The scientific evidence regarding the effectiveness of these natural derivatives is limited and lacking mechanistic understanding prevented their incorporation into mainstream administration. In the present study, we assessed the probable mode of the anti-asthmatic mechanism of the selected molecule in the context of arginase inhibition that could provide therapeutic benefits in asthma.

MATERIALS AND METHODS

Selection of enzyme crystal structures

Crystal structure of human arginase I in complex with known inhibitor methionine 2(S)-amino-6-boronohexanoic acid (Me-ABH) and human NOS complexed with arginine were obtained from Protein database (PDB) with PDB IDs 3SJT[23] and 3NOS,[24] respectively.

Preparation of plant molecules

The selected molecules for the study were obtained as simple data format files from Pubchem National Center for Biotechnology Information. Energy minimization of the molecules was carried out using Swiss-PDB Viewer V.4.04.[25] Seventy molecules were selected for the study, of which 25 molecules belong to Ephedra, five from Eugenia caryophyllus, and 40 from flavonoids.

Molinspiration

To ensure the drug likeliness, bioactivity, and toxicity of the selected molecules, they were analyzed using Molinspiration tool.[26] This tool calculates molecular properties such as log *P* values, molecular weight, H bond donors, and acceptors. Lipinski's rule of five was applied to select the probable molecules.

Active site analysis

Active site analysis for arginase and NOS enzymes was performed using Accelrys Discovery Studio V 2.0. Me-ABH is a known arginase inhibitor, hence the amino acids of arginase interacting with Me-ABH were considered as active site region of arginase. NOS interacting amino

acids with arginine was considered as active site amino acids of NOS.

Docking studies

Docking studies of arginase and NOS with the selected molecules were carried out using ArgusLab 4.0.1,[27] Cambridge Crystallographic Data Centre Genetic Optimization For Ligand Docking (CCDC GOLD) 2.1.2[28] and then the docked complexes were visualized using Accelrys Discovery Studio version 2.0 (Accelrys Software Inc.,). ArgusLab 4.0.1 (ArgusLab – www.arguslab.com) is a molecular modeling and drug docking software. It is based on quantum mechanics, and it gives the result on the basis of pose energy. CCDC GOLD 2.1.2 is effective software for virtual screening, optimization, and identification of correct binding mode of molecules in the active site. GOLD utilizes genetic algorithm, and it is available through the Cambridge Crystallographic Data Center. Comprehensively validated and widely used, GOLD enables to make confident binding mode predictions, and achieve high database enrichments. GOLD reliably identifies the correct binding mode for a large range of test set cases and has been shown to perform favorably against other docking tools in numerous independent studies. With a wide range of available scoring functions and customizable docking protocols, GOLD provides consistently high performance across a diverse range of receptor types. Application of the GOLD software has been greatly enhanced to take into account water molecules in binding sites, metal centers, and flexible side chains.[29]

RESULTS

Arginase I has two chains A and B with resolution 1.60 A0. The length of the protein is 322 amino acids and was associated with a known inhibitor Me-ABH in its active site region. NOS associated with arginine substrate has two chains A and B with the length of 427 amino acids and resolution of 2.40 A0.

Molecules selected for docking studies and their PubMed compound identification numbers are listed in Supplementary Table 1. Of 70 molecules assessed, 31 possessed one and more violations, and hence, those molecules were eliminated from the study [Supplementary Table 1]. Twenty-three of 25 molecules from Ephedra, 5 of 5 from Eugenia caryophyllus and 11 of 40 flavonoids which satisfied Lipinski rule of five and hence were selected for docking studies.

HIS101, HIS141, GLU277, ASN130, GLY142, ASP232, ASP234, SER137, HIS126, ASP128, GLU186, ASP124, and ASP183 are found to interact with known inhibitor Me-ABH as shown in Figure 1a and b. ASN366, GLU361, TYR357, TRP356, ARG250, and GLN247 are found to interact with arginine at the active site of NOS [Figure 1c and d].

Docking studies of arginase and NOS with the selected molecules from Ephedra, Eugenia caryophyllus, and flavonoids were performed using ArgusLab and CCDC GOLD. The fitness scores and binding energies of the molecules are listed in Table 1. Flavonoids assessed in the study did not bind either to arginase or NOS at active site or any other location of the protein. Since no binding energies and fitness scores obtained, they are not considered for further analysis. GOLD fitness score is considered for further comparative analysis. However, the binding energies obtained from ArgusLab are depicted in Table 1.

Docking studies with 23 molecules of Ephedra with arginase revealed that the GOLD fitness scores ranged from 52.15 for D-Ephedrine phosphate (Ester) to 29.2 for N-methyl ephedrine [Table 1]. Molecules such as D-Ephedrine phosphate (Ester), L-Ephedrine

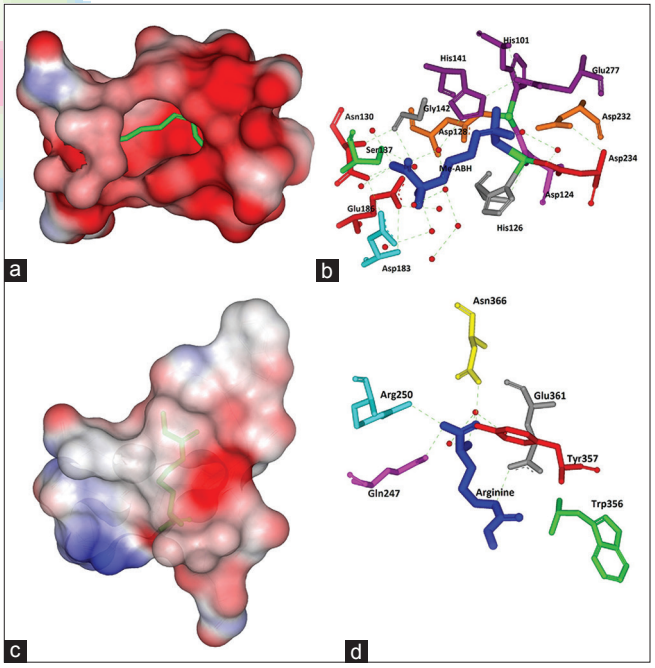

Figure 1: Active site analysis of arginase enzyme and nitric oxide synthase using methionine 2(s)-amino-6-boronohexanoic acid and arginine as ligands, respectively. (a and b) Are the active site of arginase enzyme. (a) Represents an electrostatic surface of arginase active site. Red represents negative potential, blue represents positive potential, and gray represents neutral potential. (b) Is the visualization of interacting residues (labeled) of arginase. (c and d) Are nitric oxide synthase active site visualizations. (c) Is electrostatic representation in which blue represents positive potential, gray represents neutral potential, and red represents negative potentials. (d) Is stick model representation of interacting residues of nitric oxide synthase (labeled)

Table 1: List of binding energies and fitness values of arginase and nitric oxide synthase with plant molecules by Argus lab and Cambridge Crystallographic Data Centre genetic optimization for ligand docking

Compound name	CID code	Arginase			Nitric oxide synthase		
		Argus lab	GOLD fitness	GOLD binding energy	Argus lab	GOLD	GOLD binding energy
Ephedra plant molecules							
D-Ephedrine phosphate (ester)	CID71292	−5.78291	52.15	−3.22	−6.91108	48.58	−3.98
L-Ephedrine phosphate (ester)	CID71293	−5.75937	47.52	−6.68	−7.29048	46.29	−5.85
Ephedrine, N-TFA-O-TMS	CID528872	Nil interactions	46.84	−2.79	Nil interactions	38.58	−3.9
Phospharyl Ephedrine	CID71287	−6.16019	45.78	−7.63	−7.49935	47.99	−3.93
L-Ephedrine Levulinate	CID44135556	−6.22777	43.28	−11.19	−7.70729	44.31	−6.78
Racephedrine	CID5032	−5.89678	41.53	−6.64	−7.5046	30.44	−6.02
Ephedrine, N-propyloxycarbonyl	CID6420918	−5.74051	40.89	−8.12	−7.09996	37.45	−7.09
L-Ephedrine	CID9294	−5.91348	40.85	−5.47	−7.49997	Nil interactions	Nil interactions
Phenylpropanolamine	CID 162265	−5.83999	40.44	−5.38	−7.43605	34.05	−3.13
dl-Desoxyephedrine	CID 1206	−5.38014	39.73	−4.11	−7.12249	34.07	−4.12
N-Methacryloyl-L-ephedrine	CID38091	−5.75191	39.15	−13.26	−7.55571	23.71	−10.97
Ephedrine, O-trimethylsilyl	CID547244	−5.21962	38.64	−1.87	−7.44809	34.12	−9.73
Ephedrine acetate	CID547373	−6.21474	38.53	−9.31	−7.46503	34.48	−7.59
D-Ephedrine	CID9457	−5.95452	37.73	−10.92	−7.22884	Nil interactions	Nil interactions
D-(-)-Pseudoephedrine	CID62946 D	−5.96462	37.67	−7.17	−7.14381	31.95	−7.11
(1R,2S)-(-)-ephedrine	CID6922965	−5.9855	37.35	−6.8	−7.15289	34.5	−7.54
L(-)-Pseudoephedrine	CID62946 L	−5.96462	35.62	−4.93	−7.14381	31.95	−7.11
O-Acetylephedrine	CID71291	−5.32433	35.52	−5.14	−7.60631	37	−8.26
AC1OCLO0	CID6922967	−5.95408	35.36	−9.34	−6.95629	38.38	−7.39
Norephedrine	CID26934	−5.80233	34.84	−2.49	−7.65086	37.58	−3.34
Pseudoephedrine	CID7028	−5.65701	31.84	−6.46	−7.26654	32.82	−6.79
N-methylephedrine	CID64782	−5.11913	29.88	−12.95	−7.0851	27.21	−14.42
N-methylephedrine	CID4374	−5.24258	29.2	−9.98	−7.84594	27.48	−12.67
Eugenia caryophyllus molecules							
Eugenol benzyl ether	CID93649	−5.30129	52.01	−5.21	−9.49727	Nil interactions	Nil interactions
Eugenol benzoate	CID62362	−5.76781	51.79	−8.59	Nil interactions	44.71	−7.5
Acetyleugenol	CID7136	−5.93757	46.08	−4.61	−7.62072	Nil interactions	Nil interactions
Methyleugenol	CID7127	−5.37646	43.81	−4.66	−7.40723	36.33	−5.5
Eugenol	CID3314	−5.79385	40.47	−4.61	−7.4065	32.76	−4.55

Fitness values and binding energies are depicted for 28 molecules. Molecules which did not show any interactions with enzymes of corresponding software are highlighted as "Nil interactions" against to the molecule. Molecules were aligned as per descending order of GOLD fitness values with arginase enzyme. CID: PubMed compound identification, GOLD: Genetic optimization for ligand docking

phosphate (Ester), Ephedrine N-TFA-O-TMS, Phosphoryl Ephedrine, and L-Ephedrine levulinate have best fitness scores toward arginase compared to other molecules such as N-methylephedrine (CID4374), N-methylephedrine (CID64782), pseudoephedrine, norephedrine, and AC1OCLO0 [Table 1]. Among Eugenia caryophyllus molecules, the fitness scores ranged from 52.01 for Eugenol benzyl ether to 40.47 for Eugenol [Table 1]. Eugenol benzyl ether and Eugenol benzoate obtained best fitness scores compared to Acetyleugenol, Methyleugenol, and Eugenol as shown in Table 1. To compare the fitness scores of plant molecules with the original substrate arginine, we performed the docking studies with arginine and obtained fitness score 38.09 [Table 2]. Interestingly, 13 molecules of Ephedra and all 5 molecules of Eugenia caryophyllus have more fitness scores compared to original substrate arginine toward arginase, suggesting the possibility of these plant molecules as inhibitors which has the ability to compete with arginine. Docking studies of NOS with 23 Ephedrine molecules revealed that the GOLD fitness scores ranged from 48.58 for D-Ephedrine phosphate (Ester) to 23.71 for N-methacryloyl-L-ephedrine [Table 1]. The fitness scores for five eugenol molecules ranged from 44.71 for Eugenol benzoate to 32.76 for Eugenol as shown in Table 1. The molecules such as D-Ephedrine phosphate (Ester), Phospharyl Ephedrine, and L(-)-Pseudoephedrine are found to have the best fitness scores compared to L-Ephedrine Levulinate and Ephedrine, N-TFA-O-TMS. Molecules such as L-Ephedrine, D-Ephedrine, Acetyleugenol, and Eugenol benzyl ether have not shown any affinity toward NOS, and hence, no fitness scores were obtained from CCDC GOLD. To compare the molecules fitness scores with original substrate arginine, NOS was docked with arginine obtained fitness score of 30.79. Eighteen molecules of Ephedra and three molecules of Eugenia caryophyllus have high fitness scores to NOS compared to arginine.

Arginine being the common substrate for both enzymes one would expect that the arginine and plant molecules assessed in the study might obtain similar binding affinity or fitness scores toward both the enzymes. Noteworthy observation of the study is arginine has a differential binding affinity to

Table 2: Percentage of difference in fitness scores of ephedrine and Eugenol derivatives in comparison to Arginase and nitric oxide synthase

Molecules	GOLD Arginase fitness	GOLD NOS fitness	Percentage of difference#
Me-ABH	61.08	NA	NA
Arginine	38.09	30.79	NA
L-Ephedrine Levulinate	43.28	44.31	−2.35
L-Ephedrine phosphate (ester)	47.52	46.29	2.62
Pseudoephedrine	31.84	32.82	−3.03
O-Acetylephedrine	35.52	37	−4.08
Phospharyl Ephedrine	45.78	47.99	−4.71
N-methylephedrine	29.2	27.48	6.06
D-Ephedrine phosphate (ester)	52.15	48.58	7.08
(1R,2S)-(-)-ephedrine	37.35	34.5	7.93
Norephedrine	34.84	37.58	−7.56
AC1OCLO0	35.36	38.38	−8.19
Ephedrine, N-propyloxycarbonyl	40.89	37.45	8.78
N-methylephedrine	29.88	27.21	9.35
L(-)-Pseudoephedrine	35.62	31.95	10.86
Ephedrine acetate	38.53	34.48	11.09
Ephedrine, O-trimethylsilyl	38.64	34.12	12.42
Eugenol benzoate	51.79	44.71	14.67
dl-Desoxyephedrine	39.73	34.07	15.33
Phenylpropanolamine	40.44	34.05	17.15
D-(-)-Pseudoephedrine	31.95	37.67	−16.43
Methyleugenol	43.81	36.33	18.66
Eugenol	40.47	32.76	21.05
Ephedrine, N-TFA-O-TMS	46.84	38.58	19.33
Racephedrine	41.53	30.44	30.81
N-Methacryloyl-L-ephedrine	39.15	23.71	49.12
D-Ephedrine	37.73	Nil*	37.73
L-Ephedrine	40.85	Nil	40.85
Acetyleugenol	46.08	Nil	46.08
Eugenol benzyl ether	52.01	Nil	52.01

CCDC GOLD fitness values are compared between two enzymes. Percentage differences in fitness are depicted. *Nil interactions: In CCDC GOLD docking these ligands was not shown any interactions with NOS, hence the fitness values are not obtained. Serial number 1 and 2 are the known inhibitor and original substrate respectively and their GOLD fitness scores, #Percentage difference in fitness scores was obtained by dividing difference if fitness scores of arginase and NOS by fitness scores average of arginase and NOS×100. NI: Nil interaction, GOLD: Genetic optimization for ligand docking, CCDC: Cambridge Crystallographic Data Centre, NOS: Nitric oxide synthase

arginase with a fitness score of 38.09 and NOS with fitness scores 30.79 [Table 2]. Similarly, plant molecules assessed also showed different fitness scores to arginase and NOS. The percentage difference between the same plant molecules for arginase and NOS is calculated by dividing difference if fitness scores of arginase and NOS by fitness scores average of arginase and NOS × 100, which is depicted in Table 2. The percentage difference which is represented by negative score [Table 2] is an indication of NOS having better fitness scores than arginase, and positive score indication is high fitness scores of arginase compared to NOS. As shown in Table 2, the molecules D-Ephedrine, L-Ephedrine, Acetyleugenol, and Eugenol benzyl ether would be strong inhibitors for arginase as these molecules did not bind to NOS. Plant molecules which have the positive score in percentage difference between 15% and 49% would be ideal compounds for arginase inhibition with partial effect on NOS, whereas rest of the molecules which are less positive scored could be ideal compounds where both arginase and NOS might have similar inhibition effect. Molecules which are negatively scored in percentage difference would be ideal compounds for NOS inhibition compared to arginase.

The docked complexes of arginase and NOS enzymes with molecules were further assessed for interacting residues between them. The molecules interactions were compared with interactions of Me-ABH-arginase and NOS-arginine.

We observed that the majority of interacting residues of arginase to 23 Ephedra molecules and five Eugenia caryophyllus molecules are similar to Me-ABH-binding residues of arginase. A representative table with ten ephedrine and five eugenol molecules is shown in Table 3. The predominant residues of arginase binding to molecules are depicted in bold in Table 3, and they are HIS-126, ASP-128, ASN-130, SER-137, HIS-141, ASP-183, ASP-234 GLU-277, and ASP-232. Negative potential amino acids found to be high in active site region of arginase [Figure 2a and c]. Even though the plant molecules bound to arginase differ in their fitness scores, the interacting residues turn out to be common. This suggests that the molecules assessed could be used as ideal inhibitors since they are binding to the active site of the enzyme. Two best molecules from both groups are visualized in Figure 2. Hydrogen bond interactions of molecules with active site

Table 3: Interacting residues (amino acid) of Arginase active site with Ephedrine, Eugenol and their derivatives by Accelrys DS Visualizer 2.0

Serial number	Molecule	Interacting amino acids of arginase with molecules
1	Arginase complexed with Me-ABH by SPDBV	**HIS-126, ASP-128, ASN-130, SER-137, HIS-141, ASP-183, ASP-234 GLU-277, ASP-232, HIS-101, GLY-142, GLU-186 and ASP-124**
		Ephedrine and its derivatives
1	D-Ephedrine phosphate (Ester)	**HIS-126, ASP-128, ASN-130, SER-137, ASP-234, GLU-277, HIS-101, HIS-141, GLY-142, ASP-232, GLU-186, ASP-183,** ASP-181, THR-246, ASN-139, THR-127, VAL-182
2	L-Ephedrine phosphate (Ester)	**HIS-126, ASP-128, ASN-130, SER-137, ASP-232, ASP-234, GLU-277,** HIS-101, ASN-139, THR-146
3	Phospharyl Ephedrine	**HIS-126, ASP-128, ASN-130, SER-137, ASP-232, ASP-234, GLU-277,** THR-246, ASP-100, SER-102, IS-101, ASN-139, GLY-142, ASP-124
4	L-Ephedrine Levulinate	**HIS-126, ASP-128, ASN-130, SER-137, HIS-141, ASP-232, ASP-234, GLU-277,** HIS-101, THR-246, ASP-122, ASP-124
10	Ephedrine	**HIS-126, ASP-128, ASN-130, ASP-232, ASP-234, GLU-277,** GLU-142, THR-246, HIS-101
4	Ephedrine acetate	**HIS-126, ASP-128, ASN-130, SER-137, HIS-141, ASP-232, ASP-234, GLU-277,** ASN-139, HIS-101, ASP-122, ASP-124, THR-246
9	O-Acetylephedrine	**HIS-126, ASP-128, HIS-141, SER-137, ASP-232, ASP-234, GLU-277,** ASP-124, HIS-101, GLY-142, THR-246
8	AC1OCLO0	**HIS-126, ASP-128, SER-137, HIS-141, ASP-232, GLU-277,** THR-246, ASP-234, ASP-124, HIS-101
9	Pseudoephedrine	**HIS-126, ASP-128, ASN-130, ASP-234, GLU-277,** HIS-101, GLY-142, THR-246
10	N-methylephedrine	**ASP-128, HIS-141, ASP-232, GLU-277,** SER-102, HIS-101, PRO-144, THR-246
		Eugenol and its derivatives
1	Eugenol benzyl ether	**HIS-126, ASP-128, ASN-130, ASP-234, GLU-277, HIS-141, ASP-232, HIS-101, GLY-142, GLU-186, ASP-124, SER-137, ASP-183,** THR-246, VAL-182, PRO-184, ASP-181
2	Eugenol benzoate	**HIS-126, ASP-128, ASN-130, SER-137, ASP-232, ASP-234, GLU-277,** ARG-21, PRO-20, HIS-101, ASN-139, THR-246, GLU-236, ASP-130
3	Acetyleugenol	**HIS-126, ASP-128, ASN-130, SER-137, ASP-232,** Arg-21, HIS-101, ASN-139, ASP-124, THR-246
4	Methyleugenol	**HIS-126, ASP-128, ASN-130, SER-137, ASP-232, ASP-234, GLU-277,** HIS-101, ASN-139, GLY-142, ASP-124, THR-246
5	Eugenol	**HIS-126, ASP-128, SER-137, HIS-141, ASP-232, ASP-234, GLU-277,** ASN-139, HIS-101, THR-246, HIS-124

Me-ABH is a know inhibitor for arginase and the interactive residues (amino acids) between arginase and Me-ABH were depicted for comparison. Amino acids in the active site of arginase interacting with selected ephedrine and Eugenol derivatives are listed. Bold residues, are similar to Me-ABH interactive amino acids between plant molecules and arginase

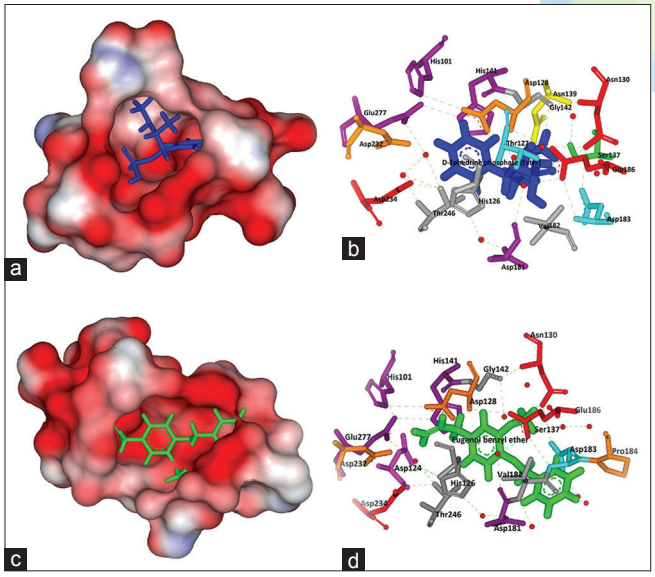

Figure 2: Arginase complexed with D-Ephedrine phosphate and Eugenol benzyl ether. (a) Electrostatic surface representation (red is negative potential, blue is positive potential, and gray is neutral potential) and (b) Stick representation (active site residues labeled) of arginase with D-Ephedrine phosphate (deep blue). Green-dashed lines are hydrogen bonds. (c) Electrostatic surface representation (red is negative potential, blue is positive potential, and gray is neutral potential) and (d) stick representation (active site residues labeled) of arginase with Eugenol benzyl ether (Green). Green-dashed lines are hydrogen bonds

residues of arginase are visualized in Figure 2b and d, and the electrostatic surface of arginase with the molecule in its active cleft is represented in Figure 2a and c.

Similarly, the binding interactions of molecules with NOS were assessed and compared with the active site amino acids of NOS. We observed that TYR475, PHE473, ARG474, PHE105, ALA472, ARG107, PRO106, VAL104, ALA181, PHE468, ARG183, PRO182, and ASP444 are predominant interacting amino acids of NOS with all the plant molecules [Figure 3]. The striking observation is that they are completely different from NOS-arginine interactions. Figure 3b and d indicates that these plant molecules have not interacted with the active site residues and hence did not fit into the active cleft but interacted with residues that are outside the active site region.

DISCUSSION

Increased arginase activity may involve in the pathogenesis of asthma through reducing the NO production and by promoting cell proliferation and collagen deposition in the airways.[10] Therefore, arginase inhibition may offer therapeutic benefits in the treatment of asthma.[11] The molecules from Ephedra and eugenol are known to have

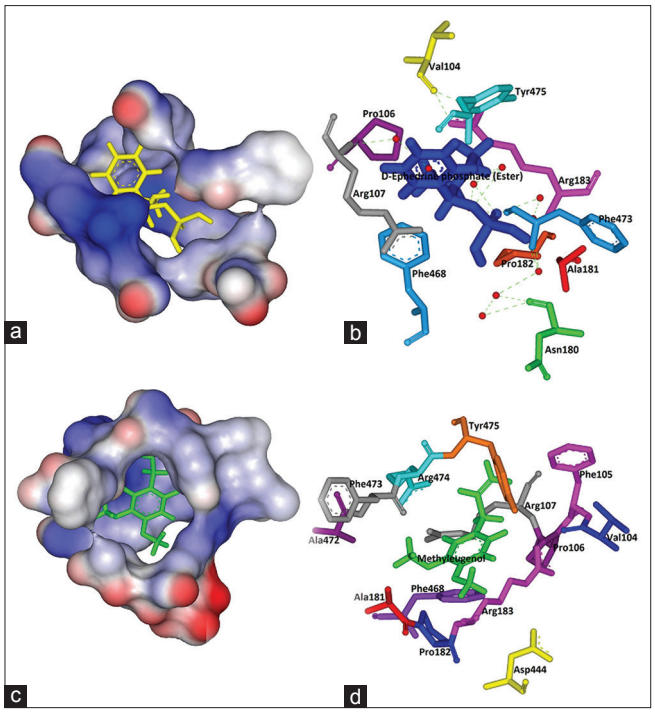

Figure 3: Nitric oxide synthase complexed with D-Ephedrine phosphate and methyl eugenol. (a) Electrostatic surface representation (blue is positive potential, red is negative potential, gray is neutral potential, and yellow is ligand) and (b) Stick representation (residues labeled) of nitric oxide synthase with D-Ephedrine phosphate (deep blue). Green-dashed lines are hydrogen bonds. (c) Electrostatic surface representation (blue is positive potential, red is negative potential, and gray is neutral potential) and (d) stick representation (residues labeled) of nitric oxide synthase with methyl eugenol (green). Green-dashed lines are hydrogen bonds

bronchodilator property and used for respiratory diseases since 3000 BC.[18] However, the actual mechanism of action is not illustrated. Our *in silico* study suggests that these molecules could bind to active site of arginase enzyme and thus block/inhibit the arginase enzyme which might help in the availability of arginine to NOS eventually generate NO that helps in bronchodilation.

The selected plant molecules although showed high-affinity binding to NOS, they did not interact with the active site region of NOS enzyme where arginine substrate binds. This raises two possibilities. One as they are not binding to active site of NOS, they may not affect the arginine interaction and further steps. The second possibility is that these molecules on interaction with NOS might alter the function of NOS by changing the conformational form of the NOS leading to either loss or enhancement of function. Wet laboratory studies are being carried out presently for understanding the interaction of molecules and its impact on function of NOS.

We extrapolate from our findings that Ephedrine acetate to N-methacryloyl-L-ephedrine [Table 2] which have a percentage difference of 10%–49% would be ideal compounds for arginase inhibition with partial effect on NOS. Remaining molecules which are positive and negative difference fitness score within 10% range such as L-ephedrine levulinate to N-methylephedrine could be ideal compounds where both arginase and NOS might play a role in disease scenario. Some of the molecules such as D-Ephedrine, L-Ephedrine, Acetyleugenol, and Eugenol benzyl ether have not shown any interactions with NOS but interacted with arginase, and thus, these molecules could be considered as specific inhibitors of arginase.

The anti-asthmatic properties of flavonoids in the prevention and management of asthma are documented. Quercetin, Epicatechin, and Kampferol are found have inhibitory activity against IL-4-mediated allergic asthma.[22] However, our *in silico* analysis with these flavonoid molecules has not yielded any binding energies, confirming their inability to block the arginase and NOS enzymes. Their mode of anti-asthmatic action may not be through arginase pathway. In contrast to our observations, methanol extract of *Caesalpinia pulcherrima* (L.) Sw. stem bark that contains flavonoids shown to have the significant inhibitory activity of arginase.[30] We speculate that the flavonoid molecules present in *C. pulcherrima* (L.) Sw. stem bark might be different from the molecules we used for our docking studies.

CONCLUSION

Docking studies indicated that molecules derived of Ephedra and Eugenia caryophyllus interact/bind with active site of arginase enzyme with fitness score higher than the arginine itself. In case of NOS, the molecules did not bind in active site but outside the active site. The interactive residues of arginase active site with different plant molecules predominantly remained same when compared to arginine substrate. Hence, these molecules could be used as inhibitors in arginase associated asthma and arginase-related diseases. Our molecular docking study suggests that anti-asthmatic properties of Ephedra and Eugenia caryophyllus may be by inhibiting the arginase activity and thus helps in enhancing the recovery of airways.

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Conflicts of interest
There are no conflicts of interest.

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Supplementary Table 1: List of the molecules from ephedrine, Eugenol and flavonoids

Serial number	Compound	CID number	milogP	TPSA	natoms	MW	nO N	nOHNH	nviolations	Nrotb
			Ephedrine plant molecules							
1	L-Ephedrine	9294	1.241	32.255	12.0	165.23	2	2	0	3
2	D-Ephedrine	9457	1.241	32.255	12.0	165.23	2	2	0	3
3	Pseudoephedrine	7028	1.241	32.255	12.0	165.23	2	2	0	3
4	Racephedrine	5032	1.241	32.255	12.0	165.23	2	2	0	3
5	(-)-Pseudoephedrine	62946	1.241	32.255	12.0	165.23	2	2	0	3
6	L-Ephedrine phosphate (ester)	71293	0.711	78.788	16.0	245.21	5	3	0	5
7*	(1R2R)-Ephedrine, N-(2-phenylbutanoyl)-O-TMS	530349	6.795	29.543	27.0	383.60	3	0	1	8
8	D-Ephedrine phosphate (ester)	71292	0.711	78.788	16.0	245.21	5	3	0	5
9	(1R,2S)-(-)- ephedrine	6922965	-1.741	36.833	12.0	166.24	2	3	0	3
10	Methamphetamine	1206	2.232	12.027	11.0	149.23	1	1	0	3
11	Ephedrine acetate	547373	2.082	38.332	15.0	207.27	3	1	0	5
12	N-Methacryloyl-L-ephedrine	38091	1.999	40.537	17.0	233.31	3	1	0	4
13*	Ephedrine di-TMS	582495	6.816	12.472	20.0	309.60	2	0	1	6
14	Ephedrine, N-TFA-O-TMS	528872	4.774	29.543	22.0	333.42	3	0	0	6
15	Phenylpropanolamine	162265	0.332	46.251	11.0	151.20	2	3	0	2
16	Phenylpropanolamine	26934	0.332	46.251	11.0	151.20	2	3	0	2
17	O-Acetylephedrine	71291	2.082	38.332	15.0	207.27	3	1	0	5
18	Amphetamine	3007	1.323	26.023	10.0	135.21	1	2	0	2
19	Ephedrine, O-trimethylsilyl	547244	4.283	21.261	16.0	237.41	2	1	0	5
20	Ephedrine, N-propyloxycarbonyl	6420918	2.438	49.771	18.0	251.32	4	1	0	6
21	ephedrine phosphate	71287	0.711	78.788	16.0	245.21	5	3	0	5
22	L-EPHEDRINE LEVULINATE	44135556	1.65	55.403	19.0	263.33	4	1	0	8
23	N-methylephedrine	64782	1.486	23.466	13.0	179.26	2	1	0	3
24	AC1OCLO0	6922967	-1.741	36.833	12.0	166.24	2	3	0	3
25	N-methylephedrine	4374	1.486	23.466	13.0	179.26	2	1	0	3
			Eugenol plant molecules							
1	Eugenol	3314	2.1	29.462	12.0	164.20	2	1	0	3
2	Methyleugenol	7127	2.408	18.468	13.0	178.23	2	0	0	4
3	Acetyleugenol	7136	1.903	35.539	15.0	206.24	3	0	0	5
4	Eugenol benzoate	62362	4.219	35.539	20.0	268.31	3	0	0	6
5	Eugenol benzyl ether	93649	4.002	18.468	19.0	254.32	2	0	0	6
			Flavonoid molecules							
1*	(-) Epicatechin-3-gallate	107905	2.537	177.135	32	442.37	10	7	1	4
2*	(-) Epigallocatechin-3-gallate	65064	2.245	197.363	33	458.37	11	8	3	4
3	Apigenin	5280443	2.463	90.895	20	270.24	5	3	0	1
4	Kampferol	5280863	1.683	131.351	22	302.23	7	5	0	1
5	Quercetin	5280343	1.683	131.351	22	302.23	7	5	0	1
6	Caffeine	2519	0.063	61.836	14	194.19	6	0	0	0
7	Luteolin	5280445	1.974	111.123	21	286.23	6	4	0	1
8*	(-)-Epigallocatechin	72277	1.077	130.602	22	306.27	7	6	1	1
9*	Theaflavin-3-gallate	22833650	3.278	284.352	53	730.63	16	11	4	5
10	(-)-Epicatechin	72276	1.365	110.374	21.0	290.27	6	5	0	1
11	CATECHIN; Cianidanol; (+)-catechin	9064	1.369	110.374	21.0	290.27	6	5	0	1
12*	(-)-Epicatechin-3-gallate; (-)-Epicatechin-3-O-gallate; L-Epicatechin gallate	65056	2.375	177.135	32.0	442.37	10	7	1	4
13	(+)-Epicatechin; 35323-91-2; ent-Epicatechin	182232	1.365	110.364	21.0	290.27	6	5	0	1
14*	Proanthocyanidin A2	124025	2.568	209.754	42.0	576.51	12	9	3	2
15*	Procyanidin; epicatechin-4alpha	107876	2.108	229.982	43.0	594.52	13	10	3	4
16	Epicatechin-2-sulfonate sodium salt	23712880	-2.545	167.573	25.0	392.32	9	5	0	2
17	DL-Catechin; NSC81746; L-Epicatechin	1203	1.369	110.374	21.0	290.27	6	5	0	1
18*	Davallin	16131425	5.078	421.268	83.0	1139.03	23	19	4	7
19*	(-)-epicatechingallate; (-)-Epicatechin gallate	367141	2.537	177.135	32.0	442.37	10	7	1	4
20*	Procyanidin B1; Procyanidin B2; Epicatechin-(4beta->8)-ent-epicatechin	11250133	2.581	220.748	42.0	578.52	12	10	3	3
21*	Procyanidin B2	122738	2.581	220.748	42.0	578.52	12	10	3	3
22*	Procyanidin C1	169853	3.792	331.122	63.0	866.77	18	15	3	5
23*	Epicatechin, TMS	6428957	10.085	55.404	41.0	651.18	6	0	2	11
24*	Procyanidin B4	147299	2.581	220.748	42.0	578.52	12	10	3	3
25*	Procyanidin B5	124017	2.373	220.748	42.0	578.52	12	10	3	3
26*	Cinnamtannin A4	16129623	7.428	662.244	126.0	1731.54	36	30	4	11
27*	4-beta-Carboxymethyl-(-)-epicatechin;	148001	0.955	147.673	25.0	348.30	8	6	1	3
28	AC1Q1VCF	23677926	-2.545	167.573	25.0	392.32	9	5	0	2

Contd...

Supplementary Table 1: Contd...

Serial number	Compound	CID number	milogP	TPSA	natoms	MW	nO N	nOHNH	nviolations	Nrotb
				Flavonoid molecules						
29*	Gallocatechin-(4alpha–>8) epicatechin	11527214	2.289	240.976	43.0	594.52	13	11	3	3
30*	Gallocatechin-(4alpha–>8) epicatechin	5317458	2.289	240.976	43.0	594.52	13	11	3	3
31*	Epicatechin-8-C-beta-D-galactopyranoside	9911680	-0.577	200.52	32.0	452.41	11	9	2	3
32*	Epicatechin-8-C-beta-D-galactopyranoside; AC1NSV2Y;	5317057	-0.577	200.52	32.0	452.41	11	9	2	3
33*	ECG-trimer; (-)-Epicatechin gallate trimer;	16170076	7.297	531.405	96.0	1323.09	30	21	4	14
34*	ECG-tetramer; (-)-Epicatechin gallate tetramer; Benzoic acid, Cis-trimer	16197484	8.985	708.54	128.0	1763.45	40	28	4	19
35*	Procyanidin B7	474541	2.373	220.748	42.0	578.52	12	10	3	3
36*	AC1LCTJV	637122	2.277	229.982	43.0	592.50	13	10	3	2
37*	AC1L9VM1	476783	2.289	240.976	43.0	594.52	13	11	3	3
38*	Proanthocyanidin A1	474542	2.277	229.982	43.0	592.50	13	10	3	2
39*	AC1L9D7K	442678	3.457	307.737	54.0	746.63	17	13	3	6
40*	AC1L9VM4	476784	2.565	229.982	44.0	608.55	13	10	3	4

*Molecules possessing violation 1 or more are excluded from the study. CID: PubMed compound identification