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Original article

Therapeutic potential of nitric oxide synthase inhibitor from natural sources for the treatment of ischemic stroke

Bader Alshehri [a,1], Rajendran Vijayakumar [b], Subramanian Senthilkumar [c,*], Ahmed Ismail [d,e], Ahmed Abdel-Hadi [a,f], Ranjay K. Choudhary [a], Kamal S. Albenasy [a], Saeed Banawas [a,g], Mohammed A Alaidarous [a], Palanisamy Manikandan [a,h,1]

[a] Department of Medical Laboratory Sciences, College of Applied Medical Sciences, Majmaah University, Majmaah 11952, Saudi Arabia, Kingdom of Saudi Arabia
[b] Department of Biology, College of Science, Majmaah University, Majmaah 11952, Saudi Arabia, Kingdom of Saudi Arabia
[c] Department of Medicine, College of Medicine and Health Science, Jigjiga University, Ethiopia
[d] Department of Public Health, College of Applied Medical Sciences, Majmaah University, Majmaah 11952, Saudi Arabia, Kingdom of Saudi Arabia
[e] Department of Biotechnology, Faculty of Agriculture, Al-Azhar University, Cairo, Egypt
[f] Botany and Microbiology Department, Faculty of Science, Al-Azhar University, Assiut Branch, Egypt
[g] Department of Biomedical Sciences, Oregon State University, Corvallis, OR 97331, USA
[h] Greenlink Analytical and Research Laboratory India Private Limited, Coimbatore 641 014, India

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ABSTRACT

Nitric oxide (NO) is one of the major signalling molecules in the mammalian body playing critical role in regulation of blood pressure, cardiovascular disease including stroke, immune activation, neuronal and cell communication. Moreover, hyper production of NO by the activity of nitric oxide synthase (NOS) involved in neuropathic pain, neurodegenerative disorders and stroke. Hence, the search on small molecules from the natural sources for the inhibition of NOS is desirable in therapeutic point of view. The elevated level of NO caused by NOS enzyme become a novel target in finding new inhibitors from natural sources as antistroke agents. The present study focuses on the molecular docking of quercetin and its analogues against NOS. The active site of the enzyme was docked with the ligand and pharmacological properties were analysed. From this result, we suggest the therapeutic property of quercetin and its analogues against NOS.

1. Introduction

Nitric oxide (NO) is synthesized by the enzymatic activity of nitric acid synthase (EC. 1.14.13.39) exerts significant impact through free radicals and involves a potent role in various regulation process in cellular functions (Rubbo et al., 1996). It is one of the signalling molecules involved in signal transduction pathway and this bioactive signalling molecule was initially investigated from the mammals. In mammals, it involves in various physiological processes such as, apoptosis, immune regulation, neuronal communication, and relaxation of smooth muscle (Schmidt and Walter, 1994). In plants, it was considered as one of the important signalling molecules, involved in various physiological functions such as senescence of organs, ripening of fruits, flowering, germination, plant growth and development (Arasimowicz et al., 2007). NO also involved in some toxic effect effects to the animals based on the concentration. It also mediates tissue protective function under stress conditions (Ferreira et al., 2010). It has been reported that the exposure of sub-lethal and lethal doses of NO impel adaptive mechanisms that allows mammalian cells highly resistance to lethal concentrations of peroxidises and NO. It has been previously reported that NO produced by inducible nitric oxide synthase (iNOS) effectively inhibits the proliferation of T- lymphocytes. The role of iNOS in rheumatoid arthritis, cardiovascular diseases, diabetes, and cancer has been reported previously (Laspina et al., 2005). In mammals, three different forms of iNOS are reported, two isoforms are expressed in neurons and

* Corresponding author at: Department of Medicine, College of Medicine and Health Science, Jigjiga University, Ethiopia.
E-mail addresses: senthilkumarsubramanian@jju.edu.et (S. Senthilkumar), m.palanisamy@mu.edu.sa (P. Manikandan).
[1] Contributed equally to the work.

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endothelium and third isoform is induced by cytokines (Ghosh et al., 1999).

The isoform iNOS is homodimer in nature and iNOS gene has been located on chromosome 17 (Michal, 1999; Xu et al., 1994). NO production was regulated by iNOS during translation and transcription process, the active iNOS produces unrestricted biosynthesis of NO until the available substrate completely depleted (Hickey et al., 2001). The important role of NO significantly affects the activity and expression of ongogenes which are very significant to the apoptosis and cell cycle (Sandau et al. 1997). In recent years, computer aided design has been used to identify antistroke agent targeting nitric oxide synthase from various natural sources. Either hemorrhagic stroke or ischemic stroke affected oxygen flow and brain cells severely affected. Generally, the dead brain cells cannot replaced, so the effect is leading to death to the brain cells (Sims and Muyderman, 2009). NO synthase calalyzes NO is one of the important pathological chains. In a study, Cacha et al. (2002) reported the role of excel NO in hypoxia condition and its reaction with superoxide free radicals to form peroxynitrite free radicals which allows the nitration of DNA, proteins and lipids culminating into severe damaging the neurons. Nitric oxide synthase was inhibited 2-imino biotin and showed protective role on neurons (Cacha et al., 2002). Continuous production of NO by the activity of nitric oxide synthase caused septic shock and stroke. Nitric oxide synthase inhibitors targeted arginine binding site of nitric oxide synthase. The present investigation was aimed to perform docking studies on NOS enzyme against quercetin and its analogues which is predominant in grains, leaves, vegetables, and fruits. Quercetin and its analogues plays significant role in cancer prevention, reduce the risk of cancer among human population who consume more vegetables and fruits (Verschoyle et al., 2007). García-Mediavilla et al. (2007) reported anticancer property of quercetin by inhibiting iNOS enzyme. Moreover, the clinical application of quercetin is very less because of low bioavailability, and therefore molecular modelling studies are needed to study its pharmacological potentials.

2. Materials and methods

2.1. Homology modeling and validation

In this study, human inducible NO synthase (NOS) was searched using the accession number P35228 from the UniProtKB database (http://www.uniprot.org/) and was retrieved from NCBI database with BLASTp. Further, the homology model of NOS was designed using the obtained sequences with the 100% similarity as template using the SWISS-MODEL server (https://swissmodel.expasy.org). The generated model was validated using ProSA and SAVES server (https://prosa.services.came.sbg.ac.at/prosa.php and https://saves.mbi.ucla.edu).

2.2. Molecular docking studies

2.2.1. Ligands

In this study, the structure of quercetin was obtained from PubChem database from NCBI and the structure of the chemical analogues were performed using PubChem database. The related compounds with 97% tanimoto value were selected for this study and 10 compounds showed maximum similarity were selected for molecular docking. The ligands were selected for docking studies with the help of LigPrep tool using the Schrodinger software suite. The selected ligands were optimized as suggested by Harder et al. (2016) in OPLS3e forcefield and the optimized pH value was pH 7.0 ± 2.0 using Epik. The generated ligands were the combinations of stereoisomers and tautomer. The determined chirality was used to make at most 32 per ligand and further minimization process using OPLS3 force field in H_2O using Powell - Reeves conjugate gradient method (PRCG). This experimental procedure consists of 2500 steps and the convergence threshold limit was 0.05.

2.2.2. Protein preparation

The homology model of the protein was analyzed as described previously by Sastry et al. (2013) using protein preparation wizard in Schrodinger software. Further, pre-processing was performed using CCD database to assign bond orders. During this process hydrogen bonds were added with zero-order bonds to metals. While preparing protein, addition of disulfide bonds to the water and protein molecules beyond Å from the hetero were generally removed. The hydrogen bond (H) was applied using PROPKA at pH 7.0 using water orientations. During protein preparation the water molecules with less than three or less hydrogen bonding distance were simply deleted from the protein. OPLS3 restrained minimization was carried out and further heavy atoms were converged to RMSD 0.3 Å.

2.2.3. Glide generation and docking

Glide was used for the generation of receptor grid as described by Friesner et al. (2004) and Halgren et al. (2004) for the prepared protein based on the active sites predicted using the CASTp server. In this study, no constrains were applied to the selected protein. The generated conformers for the earlier prepared ligands were carefully docked in the grid. In this study, standard precision (SP) docking analysis was carried out using flexible ligand sampling and Epik state penalties and docking scores were analyzed. Further, extra precision (XP) docking was performed with flexible ligand and Epik state penalties and docking scores were analyzed. Planarity of conjugated pi groups and intramolecular hydrogen bonds were enhanced.

2.2.4. Binding free energy analysis

The binding free energies (ΔG°) for docking studies were analyzed for human inducible NOX complex and selected ligands. The binding free energy (ΔG_{bind}) between inhibitors and protein which form a protein-inhibitor complex are determined using the following equations:

$$\Delta G_{bind} = \Delta H - T\Delta S = \Delta E_{MM} + \Delta G_{sol} - T\Delta S$$

$$\Delta E_{MM} = \Delta E_{internal} + \Delta E_{electrostatic} + \Delta E_{vdw}$$

$$\Delta G_{sol} = \Delta G_{PB/GB} + \Delta G_{SA}$$

where, ΔEMM is the gas phase molecular mechanics energy; -TΔS is the conformational entropy; ΔG_{sol} is the solvation free energy; $\Delta E_{internal}$ is the one which includes bond, angle, and dihedral angles.

To determine the protein/ligand binding free energies, Molecular Mechanics - Generalized-Born Surface Area (MM-GBSA) was used, which is generally accurately determine the binding free energies for a congeneric series of ligands. The ligand – protein complexes resulted from docking (XP) were carried out using MM/GBSA determination. MM-GBSA was used to analyze the free energy characters of the conformers. VSGB and OPLS3e forcefield model was used in this study.

2.2.5. ADME toxicity analysis

ADME toxicity was predicted using the top docking hits using QikProp tool from Schrodinger suites and the tool ADMETlab 2.0 webserver (https://admetmesh.scbdd.com/service/evaluation/index) was used to predict ADME, physicochemical properties and toxicity properties. The solubility of the analyzing compounds, Log D, availability of the compound, absorption, distribution,

LD50 value, health effect for the maximum docked compounds were tested and probability of health effects also analyzed.

3. Results

3.1. Homology modelling of nitric oxide synthase

Homology modelling was performed using templates generated by the accession number, P35228 (Fig. S1). The homodimer template 3e7g.1 with 100% similarity was subjected for modelling studies. Local quality estimate was used to predict local similarity to target (Fig. S2). Normalized QMEAN4 score was used to compare non-redundant set of PDB structures and the result was described in Fig. S3. The 3D structure of NOS was generated and described in Fig. 1. The generated model was validated with the use of MolProbity tool with the SWISS-MODEL server and the obtained score was 1.07. The Ramachandran plot was generated and the protein model was described in Fig. 2 with a clash score of 0.65. The generated model was validated and SAVES tool was used and the obtained ERRAT score was 92.8398. The generated model was passed VERIFY3D with 87.41% of residues had 3D-1D score below 0.2. The overall Z-score of the designed model residues was plotted with ProSA server against X-ray diffraction and NMR structures and described in Fig. 3.

3.2. Molecular docking

The 10 most relevant structural analogues retrieved from the PubChem database similar to quercetin with > 97% similarity were selected for docking studies. The selected analogues were, 7,8-dihydroxyflavone, quercetin, luteolin, chrysoeriol, baicalein, diosmetin, fisetin, isorhamnetin, myricetin, rhamnetin, and tricetin. The selected 10 ligands including quercetin showed 25 different conformers after the completion of minimization steps, which were further applied in docking studies. Molecular docking was carried out to analyze the potential of the selected compounds as inhibitors for human iNOS. The compounds were ranked after docking with SP and XP analysis and based on XP GScore, glide emodel, glide gscore, docking score and Boltzmann population. Glide SP and XP results of generated conformers of 10 ligands for docking against iNOS were tabulated (Table 1; Table 2). Quercetin (CID: 5280343) showed high docking scores than the compounds such as, Luteolin (CID: 5280445), Isorharmnetin (CID: 5281654) and fisetin (CID: 5281614) and these analogues showed interactions with the active site of iNOS (Table 3). The compounds such as quercetin, fisetin, isorhamnetin and myricetin have interacted with Glu 296 receptor and HEM 5 in the active site by hydrogen bond interactions. Luteolin interacted with Trp 291 and HEM 5 by hydrogen bond. Rhamnetin interacted with Ala 270, Pro 269, Tyr 266 and Asp 301 residues of the protein in the active site by hydrogen bond. Pi-Pi stacking interactions were observed with HEM 5 in quercetin, fisetin, isorhamnetin, myricetin, luteolin and rhamnetin (Fig. 4). Free energy between the receptor ligand complexes for 25 conformers showed varying energy. Free energy was low (-0.119) for rhamnetin and it was maximum (3.104) and quercetin had 1.772 free energy.

3.3. ADME toxicity of quercetin and structural analogues

The ADME of the docking hits ranged between 84.687% and 28.177% for oral absorption. Two conformers of myricetin violate one rule of Lipinski where in the Jorgensen's rule of three one violation was observed for both conformers of myricetin, quercetin and tricetin structures, the other compounds not violated any rules

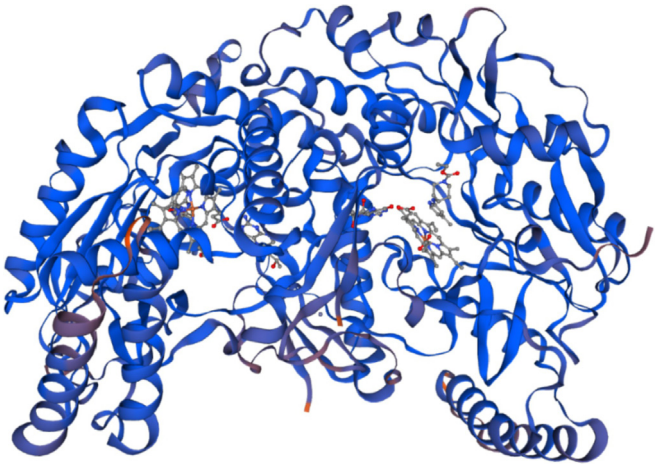

Fig. 1. 3D. Structure of the NO synthase retrieved from the UniProtKB database.

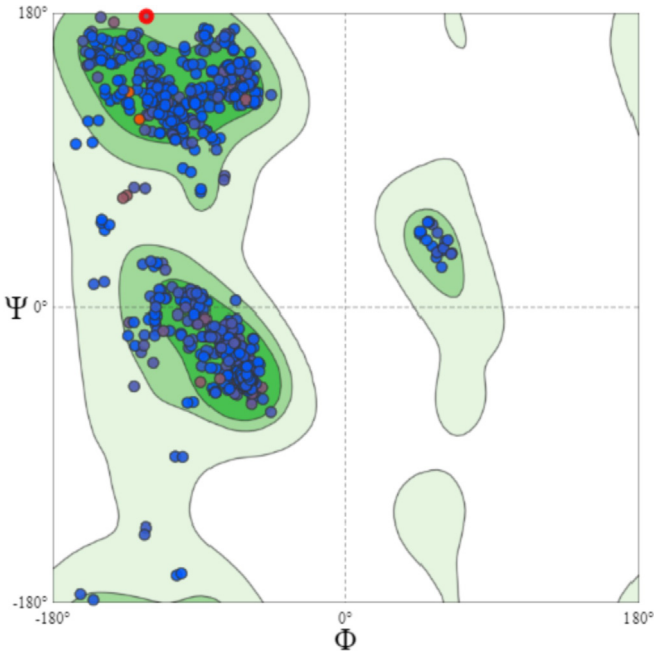

Fig. 2. Ramachandran plot for the homology model of the receptor protein.

(Table 4). ADMETlab 2.0 analysis of 10 structural analogues of quercetin revealed that all structures pass Lipinski's, pfizer's, GSK's and Golden triangle rules except for 7,8-Dihydroxyflavone failing in Pfizer's rules (Table 4). The physicomeical properties of legends used for docking studies was predicted using ADMETlab 2.0 server and the result was described in Fig. S4. The LogD at 7.0 pH for the selected ligands were less than 3 and for quercetin the predicted value was 2.248 which wsa optimal for absorption. The QED score for drug likeliness shows luteolin was above 0.67 which was the most desired value. Quercetin has good absorption values but has poor bioavailability. It has high protein plasma binding of 98.9% which implied that it could have low therapeutic index, but has a very good blood–brain barrier capability. Of all the slected ten ligands, quercetin has one of the highest possibility of drug induced brain injury. Luteolin and chrysoeriol have the higher probability of being the substrate for the enzyme ensurung higher metabolism than all other ligands.

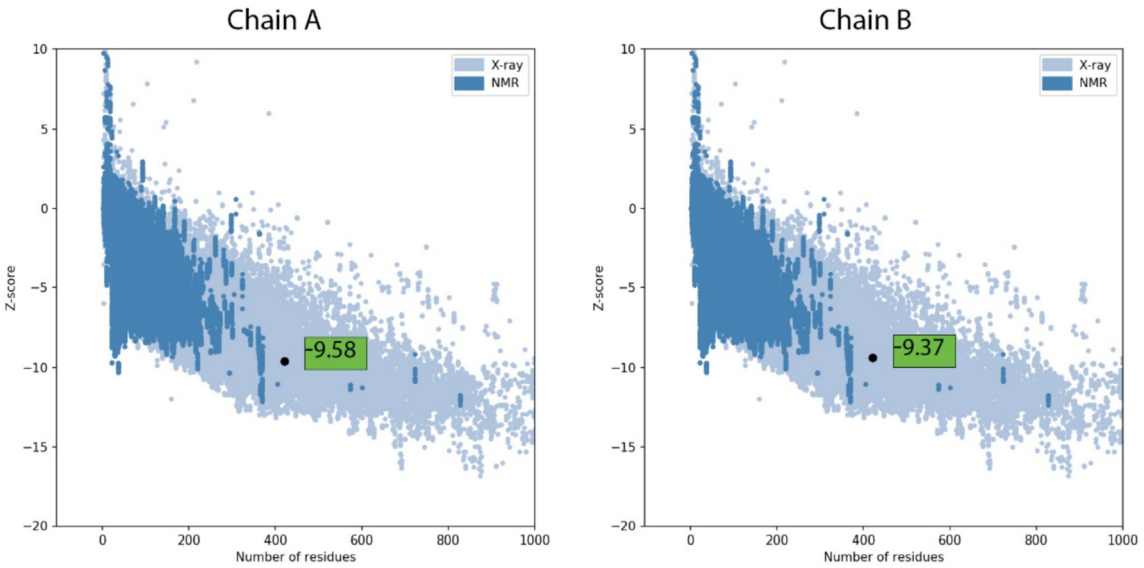

Fig. 3. Overall Z-score of the model's residues plotted with ProSA server.

Table 1
GlideSP docking data for all generated conformers of 10 selected ligands for docking against iNOS.

CID	Entry Name	rotatable bonds	docking score	ligand efficiency	ligand efficiency sa	gscore	hbond	energy	internal
5,281,672	myricetin	7	−6.676	−0.29	−0.825	−6.714	−0.16	−50.305	10.75
5,281,654	Isorhamnetin	6	−6.68	−0.29	−0.826	−6.712	−0.14	−43.917	7.4
5,280,445	luteolin	5	−6.34	−0.302	−0.833	−6.38	−0.138	−46.881	5.062
5,280,343	quercetin	6	−6.29	−0.286	−0.801	−6.322	−0.152	−48.048	6.854
5,281,614	fisetin	5	−6.259	−0.298	−0.822	−6.289	−0.152	−46.81	6.6
5,280,666	Chrysoeriol	5	−5.954	−0.271	−0.758	−5.994	−0.115	−42.239	1.313
5,281,605	Baicalein	4	−5.813	−0.291	−0.789	−5.865	0	−42.471	1.339
1880	7,8-Dihydroxyflavone	3	−5.766	−0.303	−0.81	−5.789	0	−40.255	1.994
5,281,691	Rhamnetin	5	−3.127	−0.136	−0.387	−5.711	−0.249	−37.961	8.071
5,281,612	Diosmetin	4	−3.884	−0.177	−0.495	−5.652	−0.307	−38.312	3.195
5,281,701	Tricetin	6	−5.502	−0.25	−0.701	−5.547	−0.302	−41.776	3.685
5,280,666	Chrysoeriol	4	−3.489	−0.159	−0.444	−5.201	−0.32	−42.199	5.364
5,281,612	Diosmetin	5	−5.164	−0.235	−0.658	−5.2	−0.16	−38.67	1.316
5,281,654	Isorhamnetin	5	−3.293	−0.143	−0.407	−5.159	−0.311	−45.276	3.976
5,281,605	Baicalein	3	−2.42	−0.121	−0.329	−5.028	0	−37.185	2.303
5,280,445	luteolin	4	−3.224	−0.154	−0.424	−4.936	−0.291	−33.667	0.694
1880	7,8-Dihydroxyflavone	2	−2.705	−0.142	−0.38	−4.878	−0.304	−32.203	1.553
5,281,614	fisetin	4	−2.879	−0.137	−0.378	−4.862	−0.136	−36.5	2.14
5,280,343	quercetin	5	−2.988	−0.136	−0.381	−4.854	−0.284	−38.169	6.124
5,281,701	Tricetin	5	−3.085	−0.14	−0.393	−4.803	−0.307	−38.083	3.184
5,281,691	Rhamnetin	6	−4.767	−0.207	−0.589	−4.775	−0.128	−42.578	5.416
5,281,672	myricetin	6	−2.835	−0.123	−0.351	−4.707	0	−43.888	6.431
5,281,614	fisetin	4	−2.024	−0.096	−0.266	−4.536	−0.263	−35.126	2.62
5,281,605	Baicalein	3	−2.831	−0.142	−0.384	−4.386	−0.089	−32.326	1.814
1880	7,8-Dihydroxyflavone	2	−1.225	−0.064	−0.172	−3.808	−0.097	−29.003	1.445

ligand efficiency: GlideScore/(number of heavy atoms); ligand efficiency sa: GlideScore / (number of heavy atoms)2/3. This efficiency metric approximates the effect of surface area; ligand efficiency ln: GlideScore / (1 + ln(number of heavy atoms).

4. Discussion

Quercetin is a secondary metabolite determined in various plants and is a polyphenoic compound (Wani et al., 2021). Quercetin is found as O-glycosides with hydroxyl group are generally substituted by various types of sugars. In the present study, we performed molecular docking studies on the inhibition of NOS by quercetin and its analogues. Diphenylpropane (C6–C3–C6) is a basic skeleton of flavonoids. Molecular docking was performed as described previously (Halgren et al., 2004). Quercetin and its analogues interacted with protein by hydrogen bonding, electrostatic interactions and Pi-Pi stacking interactions. Quercetin and its analogues with NOS have been reported for electrostatic, hydrogen bonding, Van der Waals and steric interactions (Thomsen and Christensen, 2006). MMGBSA data for quercetin revealed coulomb energy (-72.76), Covalent binding energy (-3.42), Van der Waals energy (-29.52), and ligand efficiency (1.772). MolDock scoring function has been derived from the PLP scoring systems which were proposed previously by Gehlhaar et al. (1995), Gehlhaar et al. (1998) and Yang and Chen (2004).

In the present study, natural ligand of NOS was screening using various previously published literature and ligands were identified. The best natural ligands of NOS were identified by screening ligands cout score, hydrogen bond acceptor, hydrogen bond donor, number of hydrophobic atoms interactions and polar atoms. In order to screen the natural compounds with lowest pose energy, docking was performed and the docking energy varied between – 50.305 and – 29.003 kcal/mol. Glide XP and Glid SP docking data

Table 2
GlideXP data for all generated conformers of 10 selected ligands for docking against iNOS.

CID	Entry Name	XP GScore	XP HBond	XP LowMW	XPlophilic EvdW	XP Electro	Glide RMSD to input
5,281,691	Rhamnetin	−7.462	−3.305	−0.446	−2.635	−1.091	123.169
5,281,672	myricetin	−7.112	−2.708	−0.439	−2.145	−1.32	122.32
5,280,343	quercetin	−7.06	−2.235	−0.493	−2.406	−1.427	122.562
5,281,701	Tricetin	−6.524	−2.258	−0.493	−2.241	−1.032	122.144
5,281,672	myricetin	−6.462	−2.933	−0.443	−2.194	−0.392	122.663
5,281,654	Isorhamnetin	−6.423	−1.755	−0.446	−2.453	−1.269	122.506
5,281,614	fisetin	−6.399	−2.216	−0.5	−2.153	−1.53	122.216
5,280,343	quercetin	−6.258	−3.401	−0.496	−2.466	−0.896	123.18
5,281,701	Tricetin	−6.213	−2.759	−0.496	−2.169	−0.289	122.49
5,280,445	luteolin	−6.126	−1.769	−0.5	−2.309	−1.048	122.339
5,281,614	fisetin	−5.894	−2.531	−0.5	−2.228	−0.635	123.195
5,280,666	Chrysoeriol	−5.874	−1.292	−0.499	−2.7	−0.883	122.189
5,281,654	Isorhamnetin	−5.701	−2.568	−0.449	−2.141	−0.542	123.031
5,280,445	luteolin	−5.677	−2.876	−0.5	−2.561	−0.74	123.174
5,280,666	Chrysoeriol	−4.811	−1.66	−0.5	−2.229	0.079	119.366
5,281,612	Diosmetin	−4.638	−0.83	−0.499	−2.177	−0.632	122.379
5,281,614	fisetin	−4.225	−1.381	−0.5	−1.895	−0.449	122.515
5,281,691	Rhamnetin	−4.05	−2.133	−0.449	−2.454	−0.213	123.53
5,281,612	Diosmetin	−3.819	−1.66	−0.5	−2.295	−0.364	118.94
1880	7,8-Dihydroxyflavone	−3.267	−0.677	−0.5	−2.063	−0.145	121.058
1880	7,8-Dihydroxyflavone	−2.884	−1.18	−0.5	−2.041	−0.163	121.928
5,281,605	Baicalein	−2.431	−1.931	−0.5	−2.996	−0.504	120.106
5,281,605	Baicalein	−1.667	−1.959	−0.5	−2.96	−0.248	118.656
5,281,605	Baicalein	−0.964	−0.83	−0.5	−3.349	0.214	119.999
1880	7,8-Dihydroxyflavone	−0.439	−0.48	−0.5	−2.651	−0.807	121.296

Table 3
MMGBSA data for all generated conformers of 10 selected ligands for docking against iNOS.

CID	Name	dG Bind (NS)	dG Bind (NS) Coulomb	dG Bind (NS) Hbond	dG Bind (NS) Solv GB	dG Bind (NS) vdW	ligand efficiency	ligand efficiency sa	Prime MMGBSA ligand efficiency ln
5,281,691	Rhamnetin	−11.6	−31	−3.39	73.52	−29.79	−0.119	−0.179	−0.662
5,281,672	myricetin	2.82	−52.63	−2.17	117.13	−35.02	0.886	1.329	4.927
5,280,343	quercetin	−1.25	−50.4	−1.9	108.46	−34.4	0.31	0.466	1.669
5,281,701	Tricetin	2.35	−47.8	−1.18	107.84	−34.76	0.438	0.657	2.357
5,281,654	Isorhamnetin	1.76	−40.69	−1.71	105.74	−35.79	0.692	1.038	3.847
5,281,614	fisetin	−8.88	−52.91	−1.64	101.92	−33	0.277	0.416	1.44
5,280,445	luteolin	6.36	−41.06	−1.15	103.91	−33.64	0.609	0.914	3.164
5,280,666	Chrysoeriol	−0.41	−38.97	−1.09	97.71	−34.59	0.49	0.735	2.635
5,281,612	Diosmetin	5.68	−30.34	−0.9	92.15	−34.28	0.543	0.815	2.923
5,281,672	myricetin	51.78	−79.6	−1.75	190.19	−35.58	3.1	4.65	17.241
5,281,701	Tricetin	53.29	−75.46	−1.29	187.17	−35.76	2.726	4.089	14.661
5,280,343	quercetin	29.59	−72.76	−3.42	155.11	−29.52	1.772	2.658	9.529
5,280,445	luteolin	26.07	−80.2	−1.6	157.99	−30.95	1.389	2.084	7.214
5,281,654	Isorhamnetin	32.65	−88.65	−2.05	178.92	−33.49	1.734	2.601	9.643
5,281,614	fisetin	39.62	−73.63	−0.78	164	−31.04	1.53	2.296	7.946
5,280,666	Chrysoeriol	46.64	−78.57	−1.68	187.23	−38.09	2.41	3.615	12.958
5,281,605	Baicalein	5.01	−27.86	−2.86	97.9	−37.36	0.437	0.655	2.187
5,281,614	fisetin	50.77	−1.42	−1.18	110.91	−33.67	2.7	4.049	14.017
5,281,612	Diosmetin	53.18	−72.27	−0.87	181.85	−34.84	2.655	3.982	14.277
5,281,691	Rhamnetin	43.69	−11.17	−2.1	118.64	−39.01	2.142	3.213	11.914
1880	7,8-Dihydroxyflavone	43.37	−60.54	−1.13	154.75	−29.67	2.367	3.551	11.402
1880	7,8-Dihydroxyflavone	17.41	−15	−0.87	79.63	−28.59	1.014	1.52	4.883
1880	7,8-Dihydroxyflavone	22.11	−19.31	−1.54	104.62	−36.12	1.316	1.974	6.339
5,281,605	Baicalein	59.51	−43.22	−1.21	170.77	−40.41	3.104	4.656	15.536
5,281,605	Baicalein	48.24	−62.85	−3.16	176.81	−36.2	2.62	3.93	13.113

dGBind(NS) = Complex – Receptor(from optimized complex) – Ligand(from optimized complex) = MMGBSA dG Bind – Rec Strain – Lig Strain; "NS" means "no strain"; Coulomb—Coulomb energy; Covalent—Covalent binding energy; vdW—Van der Waals energy; Lipo—Lipophilic energy; Solv GB—Generalized Born electrostatic solvation energy; Hbond—Hydrogen-bonding correction.

were generated for all conformers of the selected natural ligands against NOS. Glid program has been frequently used to optimize the ligand performance and analyzing the scoring function (Repasky et al., 2012; Friesner et al., 2004). Among the best 10 natural ligands, myricetin showed very least energy in Glide SP (-50.305 kcal/mol). Various selected natural compounds showed affinity values revealed that these molecules may be efficient ligands for NOS. The interactions revealed in the NOS / quercetin complexes, as predicted by molecular docking. The most suitable interacting residues with quercetin (CID: 5280343) on Glu 296 and HEM 5 (hydrogen bond donor) and luteolin (CID: 5280445) on Glu 296 and HEM 5 (hydrogen bond donor)

The complexes formed between quercetin and structural analogues and NOS structures involved various interactions. This

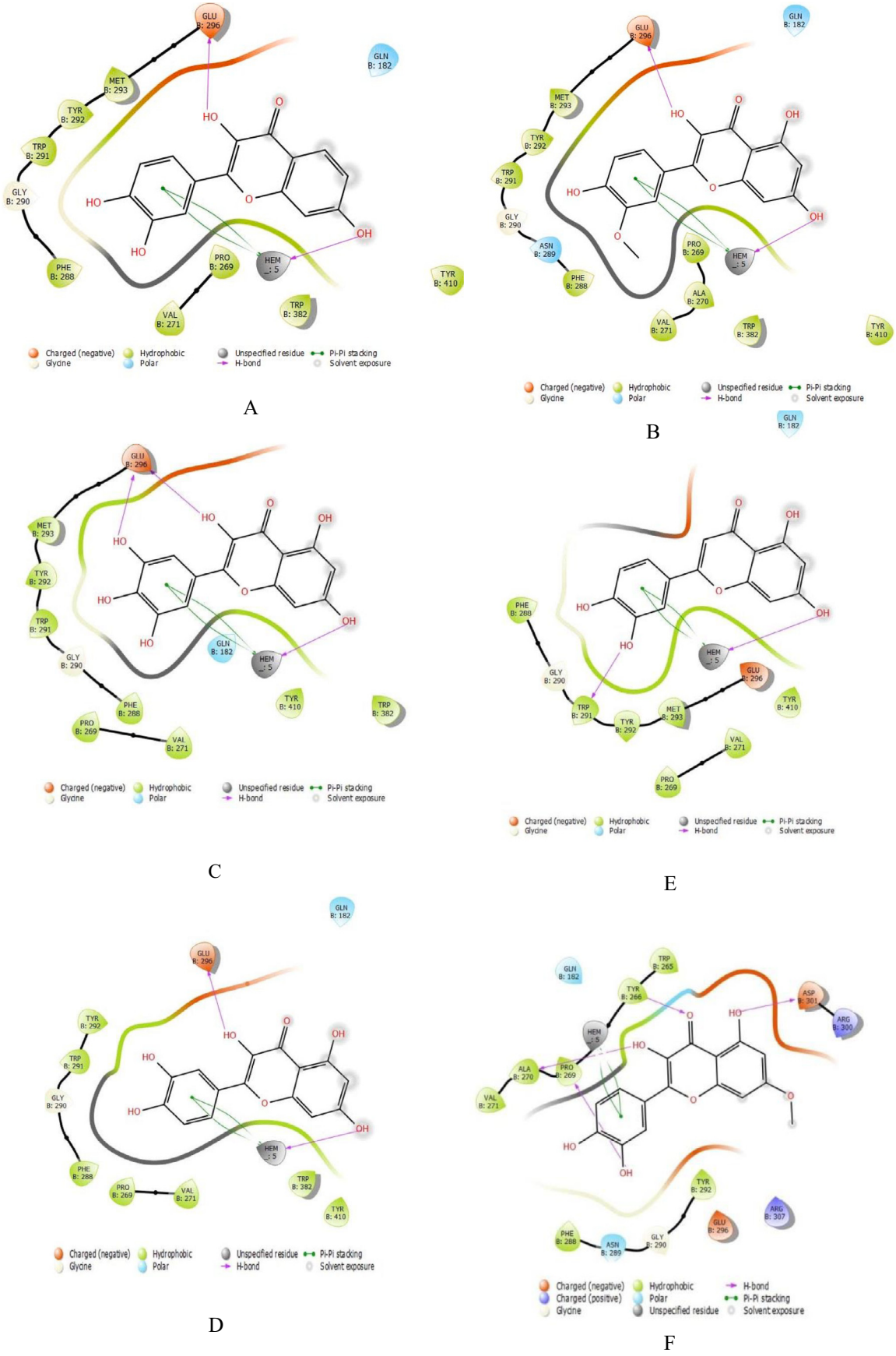

Fig. 4. Atom level interactions of selected analogs of (a) fisetin, (b) isorhamnetin, (c) myricetin (d) Quercetin with hydrogen bon on Glu 296 and HEM 5 receptor, (e) luteolin interacted with Trp 291 and HEM 5 by hydrogen bond, and (f) Rhamnetin interacted with Ala 270, Pro 269, Tyr 266 and Asp 301 residues of the protein in the active site by hydrogen bond.

Table 4
ADMET data for all generated conformers of 10 selected ligands for docking against iNOS.

Title	Entry Name	mol MW	HOA	Percent Human Oral Absorption	PSA	Rule Of Five	Rule Of Three
5,281,691	Rhamnetin.1	316.267	3	66.899	123.298	0	0
5,281,672	myricetin.1	318.239	2	28.177	158.86	1	1
5,280,343	quercetin.1	302.24	2	53.024	137.306	0	1
5,281,701	Tricetin.1	302.24	2	49.777	141.343	0	1
5,281,654	Isorhamnetin.1	316.267	3	67.898	122.452	0	0
5,281,614	fisetin.1	286.24	3	60.191	118.762	0	0
5,280,445	luteolin.1	286.24	3	61.713	120.03	0	0
5,280,666	Chrysoeriol.1	300.267	3	77.601	105.256	0	0
5,281,612	Diosmetin.1	300.267	3	74.217	106.184	0	0
5,281,672	myricetin.1	318.239	2	28.942	157.808	1	1
5,281,701	Tricetin.1	302.24	2	50.106	140.698	0	1
5,280,343	quercetin.1	302.24	2	53.019	136.906	0	1
5,280,445	luteolin.1	286.24	3	61.907	119.192	0	0
5,281,654	Isorhamnetin.1	316.267	3	67.601	123.274	0	0
5,281,614	fisetin.1	286.24	3	60.19	117.901	0	0
5,280,666	Chrysoeriol.1	300.267	3	75.43	105.558	0	0
5,281,605	Baicalein.1	270.241	3	76.696	96.64	0	0
5,281,614	fisetin.1	286.24	3	60.409	118.916	0	0
5,281,612	Diosmetin.1	300.267	3	77.319	104.878	0	0
5,281,691	Rhamnetin.1	316.267	3	67.076	123.53	0	0
1880	7,8-Dihydroxyflavone.1	254.242	3	84.647	77.488	0	0
1880	7,8-Dihydroxyflavone.1	254.242	3	84.687	77.387	0	0
1880	7,8-Dihydroxyflavone.1	254.242	3	84.554	78.182	0	0
5,281,605	Baicalein.1	270.241	3	77.023	95.543	0	0
5,281,605	Baicalein.1	270.241	3	76.695	96.45	0	0

Human oral absorption (HOA).

analysis reveals these naturally available compounds respond as efficient and versatile ligands for NOS. It could be noted that the NOS interacted with quercetin and analogues and this interaction on this enzyme may be highly related to competitive inhibition with the suitable substrate in the binding sites. The results described here revealed that some flavonoids with suitable quantity from natural sources regulating NOS expression, are the effective ligands for this protein. Resveratrol and quercetin have been showed inhibitory effect on inducible nitric oxide synthase pathway (Chan et al., 2000). NOS inhibitors considerably reduced total infarct volume, and reduced brain lesion size in animal models (Willmot et al., 2005). NOS inhibitors decreased brain injury and the reactive oxygen species-sensitive nitric oxide synthase inhibitor has been used for the treatment of ischemic stroke (Nash et al., 2018). Gahm et al. (2005) reported that co-administration of nitrones with NOS inhibitors used to treat neuronal pathology. Traynham et al. (2012) revealed that administration of nitrone to NOS knockout cardio mycocytes in experimental animals. The affinity score of natural quercetin and its derivatives showed better than inhibitors such as, etiron, targinine, nintroarginine, L-NIL, AMT and pimagedine. Theoretically, flavonoids and anthocyanins showed good ability to bind and inhibited NOS and docking simulations shows pharmacological benefits of natural flavonoids on stroke prevention. NOS is also a heme containing enzyme and hemedependent reactions in proteins has attracted much more attention in recent years and various NOS inhibitors have been reported. iNOS caused ischemic brain damage in mouse models, however deficient in iNOS has been less susceptible to cerebral ischemia (Iadecola et al., 1997).

5. Conclusions

The molecular docking analysis with quercetin and its derivatives showed highly favourable interactions on nitric oxide synthase involving ligand–protein interaction and favourable docking scores. It is therefore concluded that quercetine and its derivatives could be suitable molecule for testing as antistroke agents. Glide SP docking revealed that the compound myricetin (CID 5281672) showed least gscore (-6.714), and very low energy (-50.305) than selected ligands. ADME toxicity analysis revealed high percent human oral absorption of isorhamnetin (67.8%), rhamnetin (66.89%) and luteolin (61.7%) was determined. From the results we conclude that quercetin and its derivatives could be a novel lead molecules and assists for *in vivo* testing against NOS as antistroke agent.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

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