Chemical Methodologies 7(2023) 904-915

Chemical Methodologies

Journal homepage: http://chemmethod.com

Original Article

Investigation of Some Phenolic Compounds as iNOS Inhibitors: An *in silico* Approach

Emine Erdag*

Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Near East University, Nicosia 99138, Cyprus

ARTICLEINFO

Article history

Submitted: 2023-09-10
Revised: 2023-10-14
Accepted: 2023-11-18
Manuscript ID: CHEMM-2311-1738
Checked for Plagiarism: Yes
Language Editor:
Fatimah Ramezani
Editor who approved publication:
Dr. Sami Sajjadifar

DOI:10.48309/chemm.2023.424609.1738

KEYWORDS

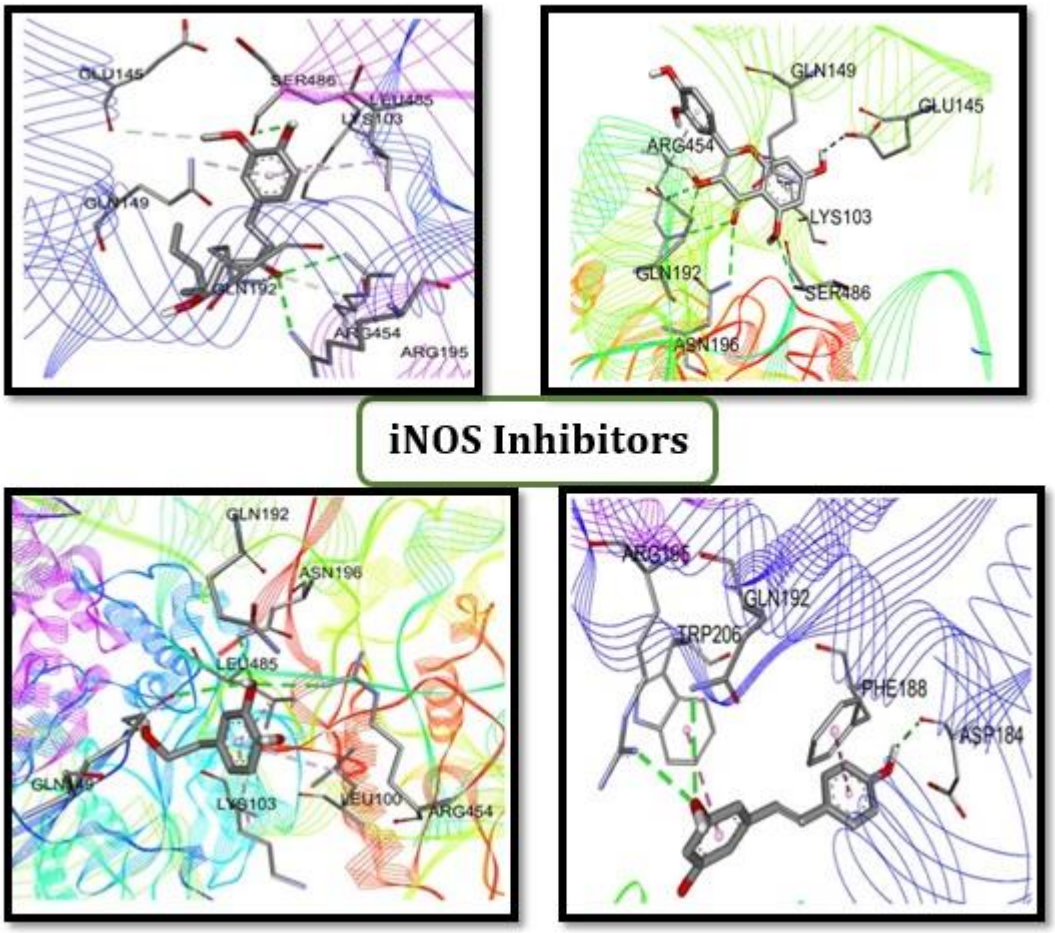
Phenolics
INOS inhibition
Molecular docking
Inflammation
Computational biology

ABSTRACT

Nitric oxide synthase (NOS) preferentially produces its inducible isoform (iNOS) in several cells, including macrophages, which are in charge of the immunological response. It is widely known that the expression of iNOS and the consequent production of nitric oxide (NO) have various biological benefits, such as anti-inflammatory and antioxidant properties. However, the overexpression or dysregulation of iNOS can result in the excessive release of NO, which may contribute to the pathogenesis of several disorders. Examining how bioactive plant components interact with iNOS may yield comprehensive and conclusive details on the processes of inhibition. The objective of this study was to employ molecular docking techniques for the investigation of molecular interactions between the iNOS enzyme and specific bioactive compounds, namely resveratrol, 6-gingerol, quercetin, and hydroxytyrosol. The Protein Data Bank (PDB) was the source of the crystal structure for the target protein, iNOS. In contrast, the three-dimensional (3D) structures of the bioactive compounds were acquired from the PubChem database. AutoDock Vina was employed for docking simulations of iNOS inhibitor compounds. The findings from the molecular docking analysis indicated that each of the chosen bioactive substances demonstrated inhibitory effects on iNOS. Furthermore, these compounds consistently formed interactions with the primary protease at the same binding site across all docking studies. The obtained results suggest that quercetin, among other phenolic compounds, could be a potential alternative for iNOS inhibition in a therapeutic context. Nevertheless, computer simulations represent the initial stage in the development of inhibitory compounds, and further research and clinical applications are essential.

* Corresponding author: Emine Erdag
E-mail: emine.erdag@neu.edu.tr

GRAPHICAL ABSTRACT



Introduction

Vascular endothelial cells are the source of nitric oxide (NO), a colorless gas that is soluble in water and has a brief half-life. Nitric oxide synthase (NOS) enzymes catalyse the formation of intracellular NO by converting the amino acid L-arginine to L-citrulline. There exist three types of NOS isoforms, namely Endothelial (eNOS), Neuronal (nNOS), and Inducible (iNOS). nNOS is involved in certain central, autonomic, and enteric nervous system cells, eNOS in vascular endothelial cells, and iNOS in immune defence alongside macrophages [1,2]. In their active forms, many cofactors, such as calmodulin, flavin adenine dinucleotide (FAD), flavin mononucleotide (FMN), and tetrahydrobiopterin (BH4), are present in all NOS enzymes. Both eNOS and nNOS require an increase in intracellular calcium (Ca^{2+}) levels to become active, whereas iNOS does not depend on changes in Ca^{2+} concentration for its activity [3].

NO is a signalling molecule with a wide range of functions in both health and disease. All biological membranes are easily penetrated by NO, a highly diffusible gas with a brief half-life of only a few seconds, which mediates both autocrine and paracrine activities [4]. Studies have revealed that NO plays vital roles in numerous systems, including the nervous, circulatory, respiratory, digestive, and immune systems. It is also a highly unstable free radical due to the unpaired electron it carries. While numerous free radicals pose harm at any level of concentration, nitric oxide (NO) plays a crucial role in vital physiological processes at low concentrations. However, unregulated and excessive synthesis of NO can be harmful to cells. These characteristics render NO an optimal physiological signalling molecule [5].

While initially identified in macrophages, iNOS is now acknowledged for its widespread expression across various human tissues, including insulin-sensitive organs like skeletal muscle, adipose tissue, and the liver [6, 2]. In a healthy state, iNOS

expression is absent. Unlike other isoforms, iNOS is produced anew in response to oxidative stress and inflammation. While iNOS is not naturally present in cells under normal physiological conditions, its activation occurs in response to stimuli such as bacterial lipopolysaccharides (LPS), interferon-gamma (IFN-γ), and cytokines (such as interleukin-1, -2, -6, and tumor necrosis factor). The iNOS enzyme gets activated and starts to be produced in macrophages and vascular endothelial cells [3]. The amount of NO produced by iNOS is secreted for an extended duration (hours to days) and at high concentrations (up to 40 times the normal level). Following its expression, iNOS has the capacity to generate substantial quantities of NO in nanomoles, sustaining this production over an extended duration, spanning hours to days [4]. The NO produced by this pathway acts as an effector of inflammation for clearing bacterial infections and also regulates adaptive immune responses [7]. The significance of NO generated by iNOS is heightened in inflammatory responses and diseases, including cancer [8]. In immune response pathways associated with inflammatory conditions, the high concentrations of NO produced by iNOS undergo rapid oxidation to form reactive nitrogen species (RNT), playing a pivotal role in mediating the immune system's response [9]. iNOS exhibits dual roles in cancer cells, acting both as a cytotoxic agent that destroys cancer cells and as a promoter of tumor development by inducing angiogenesis and metastasis in the tumor microenvironment [8]. Conversely, excessively elevated NO levels resulting from iNOS overexpression or dysregulation can lead to toxic effects. The NO overproduction is implicated in various diseases such as septic shock, cardiac dysfunction, pain, diabetes, and cancers [2]. In addition, iNOS induction may be linked to diabetes and insulin resistance associated with obesity. Notably, iNOS is expressed in insulin-sensitive tissues, and obesity contributes to elevated iNOS levels in muscle, adipose tissue, and vasculature [2]. Studies indicate that rat pancreatic cells are highly susceptible to NO toxicity, with increased iNOS expression observed in pancreatic macrophages of prediabetic rats [10]. Therefore, inhibiting iNOS expression has recently been proposed as a new mechanism of action for insulin sensitizers. In a previous study, it was reported that an iNOS inhibitor prevented fasting hyperglycemia and reduced insulin resistance in LPS-treated rats [11]. Inhibition of the expression or activity of iNOS has been considered as a strategy to mitigate the pathological roles of this enzyme in the onset and/or progression of iNOS-related diseases. To achieve this goal, researchers utilize iNOS inhibitors obtained from both natural and synthetic sources [8]. It has been reported that many plants traditionally used for their anti-inflammatory effects act by inhibiting NO production [12]. Information about the bioactive components derived from these plants can offer valuable insights to researchers in identifying potent and selective iNOS inhibitors. Computational studies on the binding of phenolic bioactive compounds obtained from plants to iNOS can provide detailed information about inhibitory mechanisms with a certain degree of accuracy. This study aimed to investigate the molecular interactions of resveratrol, quercetin, hydroxytyrosol, and 6-gingerol, which are widely recognized for their antioxidant and anti-inflammatory properties, with the iNOS enzyme through *in silico* methods, and to compare their effects at the molecular level.

Experimental

Preparation of dataset

The focal protein under investigation in this research was iNOS, a component of the human nitric oxide enzyme family with a readily available crystalline structure. The crystallographic configuration of the iNOS enzyme was sourced from the Protein Data Bank (PDB) (https://www.rcsb.org/). Ligands chosen for molecular docking were acquired from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). For the preparation of the human iNOS enzyme and the selected ligands, the Discovery Studio software was employed.

This involved eliminating all residues and water molecules from the environment, incorporating polar hydrogen bonds, and optimizing the enzyme for subsequent molecular docking analysis.

Molecular Docking

Docking simulations of iNOS inhibitor molecules were performed through AutoDock Vina software (version 1.1.2) using a three-way multiple reading approach and implementing the Lamarckian Genetic Algorithm [13] for the docking analysis. The initial inhibitor, extracted from the Protein Data Bank (PDB) database, was excluded from the docking site, and the molecular docking procedure was carried out in its stead. Visual representations were created using the Discovery Studio Visualizer software.

Drug similarity predictions

The realm of drug discovery benefits considerably from computer-based predictions of drug similarity [14]. Assessments of drug similarity were conducted utilizing the SwissAdme database, available at http://www.swissadme.ch/. Furthermore, various factors, including Lipinski, Ghose, and Veber rules, gastrointestinal system absorption, blood-brain barrier penetration, hepatotoxicity, skin permeability, and bioavailability scores were analysed in the drug similarity assessment process [15].

Results and Discussion

In this *in silico* study, all the bioactive compounds demonstrated inhibitory activity against iNOS. In all the docking analyses, the molecules bound to the primary protease at the same binding site. The outcomes of the docking calculations performed by Autodock Vina, including the count of hydrogen bonds and the specific amino acid residues participating in these bonds are presented in Table 1. Quercetin, 6-gingerol, resveratrol, and hydroxytyrosol exhibited binding energies of -10.6, -8.6, -7.8, and -6.2 kcal/mol to iNOS, respectively. It is worth noting that lower negative binding energy values indicate stronger interactions between the iNOS enzyme and ligands (18). Among the examined bioactive compounds, quercetin, with a binding energy of -10.6 kcal/mol, was predicted to possess the most potent inhibitory effect due to its lowest binding energy.

In the results of molecular docking, quercetin formed six hydrogen bonds (Conventional and Pi-Donor) with the iNOS enzyme and the amino acids GLU-145, GLN-149, GLN-192, ASN196, ARG454, and SER486. Additionally, it established a Pi-Alkyl bond with the amino acid LYS-103

Table 1: Molecular docking results of the evaluated ligands

Ligands	PubChem ID	Binding Energy (kcal/mol)	Amino Acid Residues
Quercetin	5280343	-10.6	LYS-103, GLU-145, GLN-149, GLN-192, ASN196, ARG454, and SER486
6-Gingerol	442793	-8.6	GLU-145, GLN-149, GLN-192, ARG-195, ARG-454, and SER-486
Resveratrol	445154	-7.8	ASP-184, PHE-188, GLN-192, ARG195, and TRP206
Hydroxytyrosol	82755	-6.2	LEU-100, LYS-103, GLN-149, ASN-196, ARG-454, and LEU-485

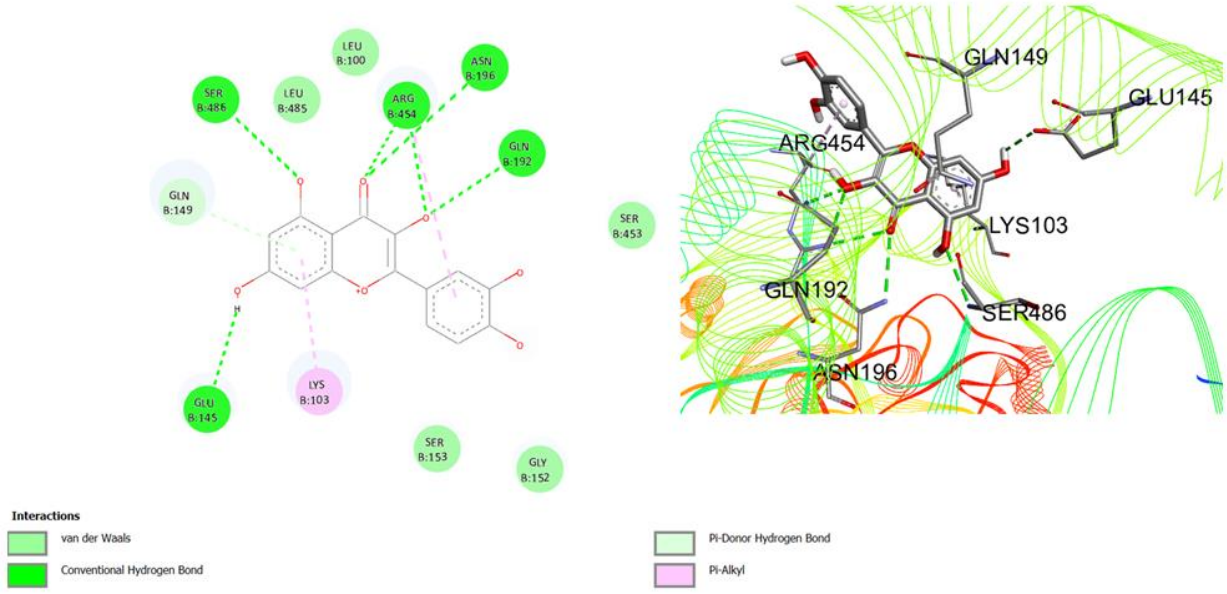


Figure 1: Binding interactions of Quercetin with amino acids in the binding region of iNOS

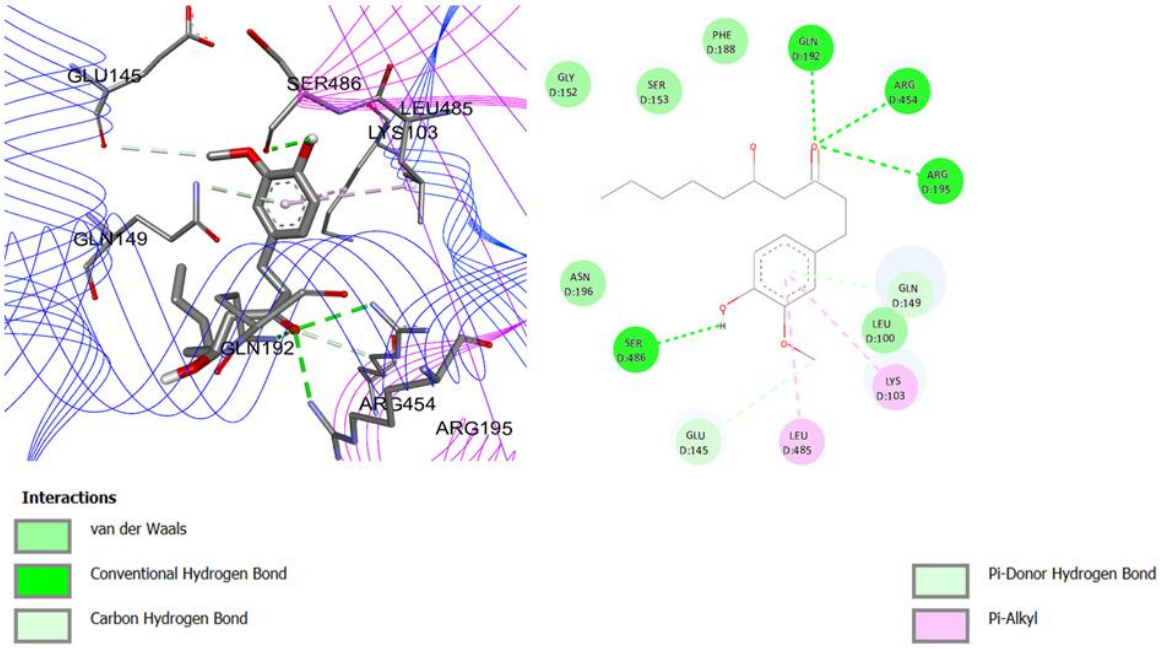


Figure 2: Binding interactions of 6-Gingerol with amino acids in the binding region of iNOS

(Figure 1). Furthermore, 6-Gingerol established four conventional hydrogen bonds with amino acid residues GLN-192, ARG-195, ARG-454, and SER-486, as well as Pi-Donor and Carbon hydrogen bonds with amino acids GLU-145 and GLN-149 in the active binding pocket of iNOS enzyme (Figure 2). In addition, the Pi-Alkyl type of interactions was indicated with the amino acids LYS-103 and LEU-485. On the other hand, resveratrol formed three conventional hydrogen bonding interactions with amino acids ASP-184, GLN-192, and ARG195. However, it formed Pi-Pi T-shaped interactions with amino acids PHE-188 and TRP206 through aromatic rings (Figure 3). In addition, hydroxytyrosol formed three conventional hydrogen bonds with the amino acids GLN-149, ASN-196, and ARG-454. Furthermore, it established Pi-Alkyl bonds with amino acids LEU-100, LYS-103, and LEU-485 (Figure 4).

Several rules, including Lipinski, Veber, and Ghose rules, as well as parameters like

lipophilicity and water solubility, are valuable for evaluating the drug-likeness of ligands with biological activity. As per Lipinski's five rules, a potential drug molecule should have a molecular weight (MW) ≤500 g/mol, a lipophilicity coefficient LogP ≤5, ≤5 hydrogen bond donors, ≤ 10 hydrogen bond acceptors, and molar refractive values ranging between 4 and 130. Veber's rules specify that a drug candidate should have ≤12 hydrogen bonds, ≤10 rotatable bonds, a total polar surface area (TPSA) ≤140, and oral bioavailability ≥20% [16]. According to Ghose's rules, a drug candidate molecule should exhibit LogP (-0.45.6), a molar refractivity (MR) value of 40150, a molecular weight (MW) between 160480 g/mol, an atomic number between 2070, and a polar surface area (PSA) <140 [17].

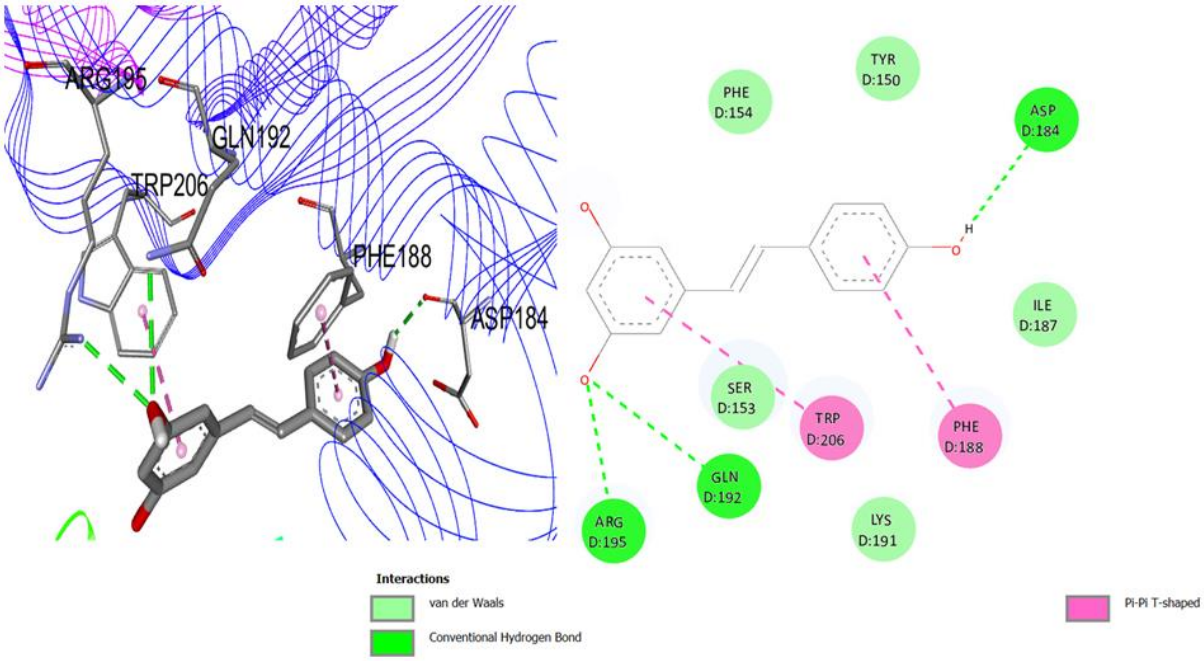


Figure 3: Binding interactions of Resveratrol with amino acids in the binding region of iNOS

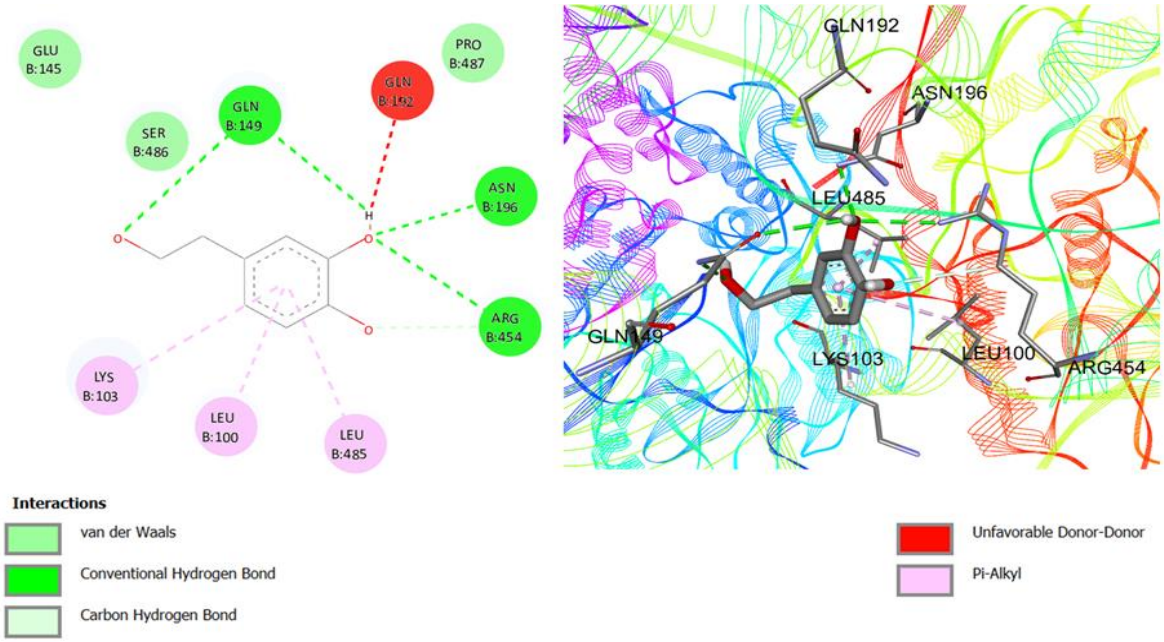


Figure 4: Binding interactions of Hydroxytyrosol with amino acids in the binding region of iNOS

The absorption percentages of the ligands from the intestinal tract were as follows: quercetin (99.9%), 6-gingerol (95.8%), resveratrol (90.9%), and hydroxytyrosol (90.3%) (Table 2). According to the drug-similarity results all tested ligands were found to comply with these rules (Table 3).

Molecular docking is a method employed to anticipate the three-dimensional configuration of the binding interaction between a protein (such as an enzyme or receptor) and a ligand (comprising bioactive compounds or drug molecules), facilitating the formation of a stable complex [18]. This approach enables the determination of molecular interactions before laboratory experiments, aiding in the discovery and development of new drugs for various diseases. Additionally, drug similarity prediction studies provide insights into the drug candidate properties of molecules analysed through molecular docking [19-23].

The scientific exploration of traditional medicinal plants holds significant importance for human health. In recent years, research on traditional medicinal plants has been on the rise globally, as natural resources and the diversity of such plants complement modern pharmacological approaches [24-32]. The ligands used in this study were selected from the active components of some of the most widely available and well-studied plants worldwide.

Stilbenes, a type of phenolic compound, serve as phytoalexins in plants [33]. Phytoalexins are low molecular weight secondary metabolites produced in response to stress factors. Resveratrol (trans-3,5,4'-trihydroxy-stilbene) was initially isolated from the roots of *Veratrum grandiflorum* by Takaoka in 1940, and later *Polygonum cuspidatum* roots were employed for this purpose [34]. Resveratrol has been suggested as an alternative to aspirin, which can lead to long-term gastric damage, in the current treatment of chronic inflammation [35]. An earlier investigation illustrated that resveratrol effectively suppressed inflammatory markers, including iNOS, cyclooxygenase-2 (COX-2), and tumor necrosis factor-alpha (TNFα), in a mouse colitis model induced by dextran sulfate sodium (DSS) [36]. Likewise, another study utilizing a DSS-induced colitis model observed that resveratrol supplementation mitigated chronic colon inflammation by modulating the down-regulation of interleukin-1 beta (IL-1b), IL-10, prostaglandin E synthase-1 (PGES-1), TNF-α, iNOS, COX-2, and the p38 MAPK (mitogen-activated protein kinases) signalling pathway [37]. Quercetin, on the other hand, is a compound with the potential to reduce the activity of iNOS [38]. Quercetin is a flavonoid naturally found in various plants, such as *Allium cepa*. It possesses anti-inflammatory and antioxidant properties and has been shown through research to have multiple biological effects, including the iNOS inhibition [39].

Moreover, curcumin is a yellow-coloured compound isolated from turmeric (*Curcuma longa*), a spice widely used in Asian countries [40]. Curcumin consists of two phenolic rings, each containing methoxy ether at the ortho position and joined at the para position by an aliphatic unsaturated heptene linker. Numerous scientific studies conducted over the last 30-40 years have emphasized its anti-inflammatory and anti-cancer effects [41]. Studies have indicated that curcumin promotes the breakdown of iNOS in murine macrophage-like RAW 264.7 cells when subjected to LPS stimulation. The mechanism behind this involves ubiquitination and dependence on the proteasome for degradation [42]. Furthermore, curcumin has been shown to decrease iNOS tyrosine phosphorylation by inhibiting the activation of ERK 1/2, subsequently leading to the suppression of iNOS enzyme activity [42]. Furthermore, hydroxytyrosol, an active ingredient found in olive (*Olea europaea L.*) leaves, has been observed to inhibit proinflammatory agents, such as iNOS, COX-2, and TNF-α, in human monocytic THP-1 cells under in vitro conditions [43]. In a separate investigation, hydroxytyrosol was identified as a regulator of iNOS and COX-2 gene expression, displaying anti-inflammatory effects by inhibiting NF-κB, STAT-1α, and IRF-1 activation, as well as reducing oxidative damage [44]. Another noteworthy compound exhibiting anti-

inflammatory properties is 6-gingerol, which was administered intraperitoneally to Wistar Albino rats at a dosage of 4 mg/kg. In this particular study, mRNA levels of COX-2, iNOS, and mPGES-1 enzymes were quantified using RT-PCR (real-time polymerase chain reaction). As a result, iNOS enzyme activity decreased in both liver and kidney tissues in the 6-gingerol-treated groups [45].

Table 2: Pharmacokinetic predictions of tested ligands

Ligands	Gastrointestinal absorption (%)	Acute Oral Toxicity	Maximum Tolerable Dose (log mg/kg/day)
Quercetin	99.945	2.480	0.435
6-Gingerol	95.830	2.524	0.332
Resveratrol	90.930	2.020	1.120
Hydroxytyrosol	90.385	1.730	0.85

Table 3: Drug likeness and compatibility of tested ligands

Ligands	Lipinski's Rule of Five	Ghose Rule	Veber Rule
Quercetin	Yes	Yes	Yes
6-Gingerol	Yes	Yes	Yes
Resveratrol	Yes	Yes	Yes
Hydroxytyrosol	Yes	Yes	Yes

Conclusion

To sum up, this *in silico* study delving into the inhibitory potential of selected phenolic compounds on iNOS presents promising insights into the realm of drug discovery for inflammatory conditions. The molecular docking simulations revealed that quercetin, 6-gingerol, resveratrol, and hydroxytyrosol all exhibited inhibitory effects on iNOS, with quercetin demonstrating the most potent inhibitory activity, as evidenced by its lower binding energy. The detailed analysis of molecular interactions provided by the docking studies, including hydrogen bonding and specific amino acid residues involved, adds granularity to our understanding of how these bioactive compounds interact with the iNOS enzyme. Quercetin, for instance, not only displayed a strong binding affinity, but also formed six hydrogen bonds with crucial amino acids, suggesting a robust inhibitory potential. Moreover, the drug similarity predictions and adherence to established drug-likeness rules further support the potential utility of these compounds in therapeutic applications. The favorable pharmacokinetic profiles, including high gastrointestinal absorption percentages and compliance with Lipinski, Ghose, and Veber rules, contribute to the overall drug-like characteristics of the studied ligands. While the results from this computational exploration are promising, it is essential to acknowledge the preliminary nature of *in silico* studies. Further experimental validations and clinical investigations are warranted to corroborate the inhibitory effects observed *in silico*. In addition, exploring the potential synergistic effects of these phenolic compounds and their broader impact on cellular pathways associated with inflammation could be avenues for future research. In summary, the findings suggest that quercetin, 6-gingerol, resveratrol, and hydroxytyrosol hold promise as iNOS inhibitors and warrant further exploration in experimental and clinical settings. This study contributes valuable information to the ongoing efforts in identifying novel therapeutic agents for conditions associated with iNOS dysregulation, opening avenues for future research in the dynamic field of molecular pharmacology.

Acknowledgments

None.

Disclosure Statement

No potential conflict of interest was reported by the authors.

Funding

This study did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Authors' contributions

All authors contributed toward data analysis, drafting, and revising the paper and agreed to responsible for all the aspects of this work.

Conflict of interest

The authors declare that they have no conflicts of interest in this article.

ORCID

Emine Erdag
https://orcid.org/0000-0002-1431-935X

References

[1]. Liu V.W., Huang P.L., Cardiovascular roles of nitric oxide: a review of insights from nitric oxide synthase gene disrupted mice, *Cardiovascular Research*, 2008, **77**:19 [Crossref], [Google Scholar], [Publisher]
[2]. Paakkari I., Lindsberg P., Nitric oxide in the central nervous system, *Annals of Medicine*, 1995, **27**:369 [Crossref], [Google Scholar], [Publisher]
[3]. Förstermann U., Sessa W.C., Nitric oxide synthases: regulation and function, *European Heart Journal*, 2012, **33**:829 [Crossref], [Google Scholar], [Publisher]
[4]. a) Peter M., Gayathry R., Peter V.S., Inducible Nitric Oxide Synthase/Nitric Oxide System as a Biomarker for Stress and Ease Response in Fish: Implication on Na^+ Homeostasis During Hypoxia, *Frontiers in Physiology*, 2022, **13**:795 [Crossref], [Google Scholar], [Publisher] b) Swami M.B., Nagargoje G.R., Mathapati S.R., Bondge A.S., Jadhav A.H., Panchgalle S.P., More V., A Magnetically Recoverable and Highly Effectual Fe_3O_4 Encapsulated MWCNTs Nano-Composite for Synthesis of 1,8-Dioxo-octahydroxanthene Derivatives, *Journal of Applied Organometallic Chemistry*, 2023, **3**:184 [Crossref], Publisher] c) Olaleye O., Oladipupo A., Oyawaluja B., Coker H., Chemical composition, antioxidative and antimicrobial activities of different extracts of the leaves of parquetina nigrescens (asclepiadaceae), *Progress in Chemical and Biochemical Research*, 2021, **4**:359 [Crossref], [Google Scholar], [Publisher] d) Kasim S., Abdulaziz N., Jasim M., Mustafa Y., Resveratrol in cancer chemotherapy: Is it a preventer, protector, or fighter?, *Journal of Medicinal and Pharmaceutical Chemistry Research*, 2023, **5**:576 [Google Scholar], [Publisher]
[5]. a) Phaniendra A., Jestadi D.B., Periyasamy L., Free radicals: properties, sources, targets, and their implication in various diseases, *Indian Journal of Clinical Biochemistry*, 2015, **30**:11 [Crossref], [Google Scholar], [Publisher] b) Vaeli N., Laboratory study of effective factors on how to extract carvacrol from oliveria decumbens plant with the help of supercritical fluid CO_2 and using ultrasound waves, *Eurasian Journal of Science and Technology*, 2022, **2**:32 [Crossref], [Google Scholar], [Publisher]
[6]. Soskić S.S., Dobutović B.D., Sudar E.M., Obradović M.M., Nikolić D.M., Djordjevic J.D., Radak D.J., Mikhailidis D.P., Isenović E.R., Regulation of inducible nitric oxide synthase (iNOS) and its potential role in insulin resistance, diabetes and heart failure, *The open Cardiovascular Medicine Journal*, 2011, **5**:153 [Crossref], [Google Scholar], [Publisher]
[7]. Cronkite D.A., Strutt T.M., The regulation of inflammation by innate and adaptive lymphocytes, *Journal of Immunology Research*, 2018, **2018** [Crossref], [Google Scholar], [Publisher]
[8]. Vannini F., Kashfi K., Nath N., The dual role of iNOS in cancer, *Redox Biology*, 2015, **6**:334 [Crossref], [Google Scholar], [Publisher]
[9]. a) Coleman J.W., Nitric oxide in immunity and inflammation, *International Immunopharmacology*, 2001, **1**:1397 [Crossref], [Google Scholar], [Publisher] b) Mostafa H., Risan, M., Al-Faham M., A study of Mycobacterium

tuberculosis Zopf, 1883 (Mycobacteriaceae) in Iraq, *International Journal of Advanced Biological and Biomedical Research*, 2022, **10**:1 [Crossref], [Publisher] c) Milani Fard A. M., Milani Fard M., Evaluation of Office Stones in Kidney Patients and How to form and Treat Them, *Eurasian Journal of Science and Technology*, 2022, **2**:111 [Crossref], [Publisher]

[10]. Tyka K., Jörns A., Turatsinze J.V., Eizirik D.L., Lenzen S., Gurgul-Convey, E., MCPIP1 regulates the sensitivity of pancreatic beta-cells to cytokine toxicity, *Cell Death & Disease*, 2019, **10**:29 [Crossref], [Google Scholar], [Publisher]

[11]. Fujimoto M., Shimizu N., Kunii K., Martyn J.J., Ueki K., Kaneki M., A role for iNOS in fasting hyperglycemia and impaired insulin signaling in the liver of obese diabetic mice, *Diabetes*, 2005, **54**:1340 [Crossref], [Google Scholar], [Publisher]

[12]. Nunes C.d.R., Barreto Arantes M., Menezes de Faria Pereira S., Leandro da Cruz L., de Souza Passos M., Pereira de Moraes L., Vieira I.J.C., Barros de Oliveira D., Plants as sources of anti-inflammatory agents, *Molecules*, 2020, **25**:3726 [Crossref], [Google Scholar], [Publisher]

[13]. Fuhrmann J., Rurainski A., Lenhof H.P., Neumann D., A new Lamarckian genetic algorithm for flexible ligand-receptor docking, *Journal of Computational Chemistry*, 2010, **31**:1911 [Crossref], [Google Scholar], [Publisher]

[14]. Sliwoski G., Kothiwale S., Meiler J., Lowe E.W., Computational methods in drug discovery, *Pharmacological Reviews*, 2014, **66**:334 [Crossref], [Google Scholar], [Publisher]

[15]. Ononamadu C., Ibrahim A., Molecular docking and prediction of ADME/drug-likeness properties of potentially active antidiabetic compounds isolated from aqueous-methanol extracts of Gymnema sylvestre and Combretum micranthum, *BioTechnologia*, 2021, **102**:85 [Crossref], [Google Scholar], [Publisher]

[16]. Veber D.F., Johnson S.R., Cheng H.Y., Smith B.R., Ward K.W., Kopple K.D., Molecular properties that influence the oral bioavailability of drug candidates, *Journal of Medicinal Chemistry*, 2002, **45**:2615 [Crossref], [Google Scholar], [Publisher]

[17]. Ghose A.K., Viswanadhan V.N., Wendoloski J.J., A knowledge-based approach in designing combinatorial or medicinal chemistry libraries for drug discovery. 1. A qualitative and quantitative characterization of known drug databases, *Journal of Combinatorial Chemistry*, 1999, **1**:55 [Crossref], [Google Scholar], [Publisher]

[18]. Agu P., Afiukwa C., Orji O., Ezeh E., Ofoke I., Ogbu C., Ugwuja E., Aja P., Molecular docking as a tool for the discovery of molecular targets of nutraceuticals in diseases management, *Scientific Reports*, 2023, **13**:13398 [Crossref], [Google Scholar], [Publisher]

[19]. Meng X.Y., Zhang H.X., Mezei M., Cui M., Molecular docking: a powerful approach for structure-based drug discovery, *Current Computer-aided Drug Design*, 2011, **7**:146 [Crossref], [Google Scholar], [Publisher]

[20]. Geromichalos G.D., Importance of molecular computer modeling in anticancer drug de velopment, *Journal of BUON*, 2007, **12**:S101 [Google Scholar], [Publisher]

[21]. Kroemer R.T., Structure-based drug design: docking and scoring, *Current Protein and Peptide Science*, 2007, **8**:312 [Crossref], [Google Scholar], [Publisher]

[22]. Naqvi A.A., Mohammad T., Hasan G.M., Hassan M.I., Advancements in docking and molecular dynamics simulations towards ligand-receptor interactions and structure-function relationships, *Current Topics in Medicinal Chemistry*, 2018, **18**:1755 [Crossref], [Google Scholar], [Publisher]

[23]. Zivkovic M., Zlatanovic M., Zlatanovic N., Golubović M., Veselinović A.M., The application of the combination of Monte Carlo optimization method based QSAR modeling and molecular docking in drug design and development, *Mini Reviews in Medicinal Chemistry*, 2020, **20**:1389 [Crossref], [Google Scholar], [Publisher]

[24]. Salmerón-Manzano E., Garrido-Cardenas J.A., Manzano-Agugliaro F., Worldwide research trends on medicinal plants, *International Journal of Environmental Research and Public Health*, 2020, **17**:3376 [Crossref], [Google Scholar], [Publisher]

[25]. Huang L., Shi X., Zhang N., Gao Y., Bai Q., Liu L., Zuo L., Hong B., Bibliometric analysis of trends and issues in traditional medicine for stroke research: 2004–2018, *BMC Complementary Medicine and Therapies*, 2020, **20**:1 [Crossref], [Google Scholar], [Publisher]
[26]. Tandon N., Yadav S.S., Contributions of Indian Council of Medical Research (ICMR) in the area of Medicinal plants/Traditional medicine, *Journal of Ethnopharmacology*, 2017, **197**:39 [Crossref], [Google Scholar], [Publisher]
[27]. Du J., Tang X.L., Natural products against cancer: A comprehensive bibliometric study of the research projects, publications, patents and drugs, *Journal of Cancer Research and Therapeutics*, 2014, **10**:C27 [Google Scholar], [Publisher]
[28]. Yuan H., Ma Q., Ye L., Piao G., The traditional medicine and modern medicine from natural products, *Molecules*, 2016, **21**:559 [Crossref], [Google Scholar], [Publisher]
[29]. Ngo L.T., Okogun J.I., Folk W.R., 21st century natural product research and drug development and traditional medicines, *Natural Product Reports*, 2013, **30**:584 [Crossref], [Google Scholar], [Publisher]
[30]. Dai W., Feng K., Sun X., Xu L., Wu S., Rahmand K., Jia D., Han T., Natural products for the treatment of stress-induced depression: Pharmacology, mechanism and traditional use, *Journal of Ethnopharmacology*, 2022, **285**:114692 [Crossref], [Google Scholar], [Publisher]
[31]. Wang M.W., Hao X., Chen K., Biological screening of natural products and drug innovation in China, *Philosophical Transactions of the Royal Society B: Biological Sciences*, 2007, **362**:1093 [Crossref], [Google Scholar], [Publisher]
[32]. Mukherjee P.K., Harwansh R.K., Bahadur S., Banerjee S., Kar A., Chanda J., Biswas S., Ahmmed, S.M., Katiyar C., Development of Ayurveda–tradition to trend, *Journal of Ethnopharmacology*, 2017, **197**:10 [Crossref], [Google Scholar], [Publisher]
[33]. Valletta A., Iozia L.M., Leonelli F., Impact of environmental factors on stilbene biosynthesis, *Plants*, 2021, **10**:90 [Crossref], [Google Scholar], [Publisher]
[34]. Kaur A., Tiwari R., Tiwari G., Ramachandran V., Resveratrol: A vital therapeutic agent with multiple health benefits, *Drug Research*, 2021, 5 [Google Scholar], [Publisher]
[35]. Salla M., Pandya V., Bhullar K.S., Kerek E., Wong Y.F., Losch R., Ou J., Aldawsari F.S., Velazquez-Martinez C., Thiesen A., Resveratrol and resveratrol-aspirin hybrid compounds as potent intestinal anti-inflammatory and anti-tumor drugs, *Molecules*, 2020, **25**:3849 [Crossref], [Google Scholar], [Publisher]
[36]. Tsai H.Y., Ho C.T., Chen Y.K., Biological actions and molecular effects of resveratrol, pterostilbene, and 3′-hydroxypterostilbene, *Journal of Food and Drug Analysis*, 2017, **25**:134 [Crossref], [Google Scholar], [Publisher]
[37]. Cui X., Jin Y., Hofseth A.B., Pena E., Habiger J., Chumanevich A., Poudyal D., Nagarkatti M., Nagarkatti P.S., Singh U.P., Resveratrol suppresses colitis and colon cancer associated with colitis, *Cancer Prevention Research*, 2010, **3**:549 [Crossref], [Google Scholar], [Publisher]
[38]. Xu D., Hu M.J., Wang Y.Q., Cui Y.L., Antioxidant activities of quercetin and its complexes for medicinal application, *Molecules*, 2019, **24**:1123 [Crossref], [Google Scholar], [Publisher]
[39]. Aghababaei F., Hadidi M., Recent advances in potential health benefits of quercetin, *Pharmaceuticals*, 2023, **16**:1020 [Crossref], [Google Scholar], [Publisher]
[40]. Sharifi-Rad J., Rayess Y.E., Rizk A.A., Sadaka C., Zgheib R., Zam W., Sestito S., Rapposelli S., Neffe-Skocińska K., Zielińska D., Turmeric and its major compound curcumin on health: bioactive effects and safety profiles for food, pharmaceutical, biotechnological and medicinal applications, *Frontiers in Pharmacology*, 2020, **11**:1021 [Crossref], [Google Scholar], [Publisher]
[41]. Wilken R., Veena M.S., Wang M.B., Srivatsan E.S., Curcumin: A review of anti-cancer properties and therapeutic activity in head and neck squamous cell carcinoma, *Molecular Cancer*, 2011, **10**:1 [Crossref], [Google Scholar], [Publisher]

[42]. Kolodziejski P.J., Musial A., Koo J.S., Eissa N.T., Ubiquitination of inducible nitric oxide synthase is required for its degradation, *Proceedings of the National Academy of Sciences*, 2002, **99**:12315 [Crossref], [Google Scholar], [Publisher]
[43]. Vilaplana-Pérez C., Auñón D., García-Flores L.A., Gil-Izquierdo A., Hydroxytyrosol and potential uses in cardiovascular diseases, cancer, and AIDS, *Frontiers in Nutrition*, 2014, **1**:18 [Crossref], [Google Scholar], [Publisher]
[44]. Pérez de la Lastra J.M., Curieses Andrés C.M., Andrés Juan C., Plou F.J., Pérez-Lebeña E., Hydroxytyrosol and Arginine as Antioxidant, Anti-Inflammatory and Immunostimulant Dietary Supplements for COVID-19 and Long COVID, *Foods*, 2023, **12**:1937 [Crossref], [Google Scholar], [Publisher]
[45]. Li X.H., McGrath K.C., Tran V.H., Li Y.M., Duke C.C., Roufogalis B.D., Heather A.K., Attenuation of proinflammatory responses by S-[6]-gingerol via inhibition of ROS/NF-kappa B/COX2 activation in HuH7 cells, *Evidence-Based Complementary and Alternative Medicine*, 2013, **2013** [Crossref], [Google Scholar], [Publisher]

HOW TO CITE THIS ARTICLE
Emine Erdag*. Investigation of Some Phenolic Compounds as iNOS Inhibitors: An *in silico* Approach. *Chem. Methodol.*, 2023, 7(12) 904-915
DOI: https://doi.org/10.48309/chemm.2023.424609.1738
URL: https://www.chemmethod.com/article_183357.html