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Molecular Docking, ADMET, QSAR, Biological Activity and Toxicity Prediction of Selected Natural Products and Act as the Stimulators of Antioxidant Superoxide Dismutase Enzyme

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ABSTRACT

Objective: Antioxidants are substances that can prevent or delay cell damage caused by free radicals, which are unstable molecules produced by the body, in response to the environment and other pressures. Molecular docking is used to predict and analyze the interactions between protein receptors and ligands. In this study, efforts have been made to identify natural products with antioxidant activity using Autodock PyRx 0.8..

Methods: Autodock PyRx 0.8 was used to prepare for docking and was also used to calculate the binding energy. Virtual analysis of the docking site was analyzed by PyRx, PyMol & Discovery studio 2020 clients. The structure of the superoxide dismutase (1CB4) was obtained from the protein data bank and the structure of the ligand was collected from the PubChem database.

Results: All selected ligands (ligands 1, 3, 4, 5, 6) compared to the standard drug ascorbic acid satisfied Lipinski's rule of five and exhibited the highest binding affinity except ligand 2. The higher binding affinity and drug conductivity of all compounds suggest that it could be further explored as SOD promoters.

Conclusion: We found that selected natural products could be potential antioxidants.

Keywords: Natural products, antioxidants, superoxide dismutase, molecular docking, biological activity, ADMET study, QSAR, Toxicity

I. INTRODUCTION

Since very ancient times, herbal medicines had been used for alleviation of signs and symptoms of disease [1]. Despite the excellent advances found in current medicine in latest decades, plant life nevertheless make an essential contribution to health care. Herbal remedy has received reputation in health care; in step with the World Health Organization (WHO), approximately 65% to 80% of the world's populace which lives in growing countries relies upon basically on plant life for number one health care [2]. Since the center of the 19th century, unique lessons of bioactive compounds had been remoted and characterized. Many of those are used because the active ingredients of modern drugs, or because the lead compounds for brand new drugs discovery [3] .

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However, the great interest of people in medicinal plants is due to their long-term use in folk medicine and their prophylactic properties, especially in developing countries [4]. The antioxidant properties of a large number of medicinal plants have been studied. Natural antioxidants in the form of original extract or their chemical components are very effective in preventing the destructive process caused by oxidative stress. Although the toxicity characteristics of most medicinal plants have not been thoroughly evaluated, medicinal products derived from plant products are generally believed to be safer than their synthetic counterparts [5, 6].

Many natural products play an important role as antioxidants and represent useful scaffolds for the emergence of new drugs [7]. For a long time, polyphenols have been recognized especially for their strong chain-breaking effect and ability to scavenge free radicals, thereby protecting cells from reactive oxygen species [8]. These derivatives have been shown to be metal chelating agents and lipid peroxidation inhibitors. These properties have been related to its hepatoprotective, anti-inflammatory and cancer chemopreventive activities [9].

A phenolic compound derived from an aromatic ring containing at least one hydroxyl group. So far, 8000 natural phenolic compounds with the same general structure have been elucidated. Antioxidants can be divided into two groups: primary antioxidants and secondary antioxidants. Natural phenolic compounds isolated from plants are often considered major antioxidants. These phenolic compounds scavenge radicals by donating electrons to the medium. Plant phenol not only donates electrons to the medium to stop the radical reaction, but also provides the media with hydrogen radicals to stop the radical reaction [10]. Another role of plant phenol is to remove singlet oxygen, which accelerates radical reactions. Some phenolic compounds may be considered secondary antioxidants because they have the ability to chelate metals and form hydroxyl radicals through the Fenton reaction [11]. Flavonoids are one of the common bioactive groups of phenolic compounds [12]. So far, more than 4000 flavonoid compounds have been isolated from natural sources. Due to the location of other flavonoid substituents and their ability to bond electrons, these compounds can exhibit chelating and radical scavenging activity. Flavonoids and polyphenols are commonly found in the aerial part of plants [13]. The antioxidant capacity and mechanism of phenol and flavonoid compounds have been reported in several articles [14].

An accumulation of substantive evidence has marked the important role of reactive oxygen species (ROS) and other antioxidants in causing many disorders and diseases. Evidence has drawn scientist's attention to the evaluation of antioxidants for the prevention and treatment of disease, and for maintaining human health [15]. Many biological functions such as anti-mutagenic, anti-carcinogenic, anti-aging reactions, etc., which are intrinsic antioxidant mechanisms in the human body [16, 17]. Antioxidants stabilize or deactivate free radicals. Often they are done before they attack the target of living cells [18]. In recent years, interest in natural antioxidants has been very high, and is intended to be used in food, cosmetics, and pharmaceuticals. This is because they are multifaceted in their diversity and magnitude of activity and provide tremendous scope to address imbalances [19-21]. The role of radical reactions in the pathology of illness is well established and is involved in many acute and chronic diseases of individuals such as diabetes, atherosclerosis, aging, immunosuppression and neuroretraction is understood [22]. The imbalance between ROS and the body's specific antioxidant capacity has dictated the use of dietary and/or therapeutic adjuvants, especially during attacks of disease. Studies of medicinal herbs, vegetables, and fruits indicate the presence of antioxidants such as phenols, flavonoids, tannins, and proanthocyanidins. The antioxidant content of natural products can contribute to the protection they provide from disease.

Intake of natural antioxidants is inversely proportional to morbidity and mortality from degenerative diseases [16]. Free radicals are reported to damage tissues that result in cell damage through mechanisms of covalent bonding and lipid peroxidation [23]. Naturally-derived antioxidants have received special attention due to their ability to scavenge free radicals.

This study was designed to identify possible mechanisms underlying the antioxidant activity of natural products using molecular docking studies. Six natural ligands were randomly selected from the literature [24]. We used superoxide dismutase (SOD) as the target antioxidant enzyme for the purpose of docking, as superoxide dismutase is an enzyme that helps break down potentially harmful oxygen molecules within cells. This can prevent damage to tissues where free radical molecules are thought to be involved in disease. We also performed QSAR, ADMET bioactivity and toxicity risk predictions to confirm that the selected natural ligands of choice were safe and non-toxic.

II. MATERIALS AND METHOD

Docking study

Ligand preparation

In this study, we used ChemBio Office version 12.0 for PDB structure of ligand, which is a chemically intelligent drawing interface freeware developed by Advanced Chemistry Department. The Molecular Networks software package provides Chem3D, which was used to generate 3D coordinates in microscopic Again we used a converter on this same server to convert the 2D structure of the drug into PDB format. This is a format accepted by standard docking software. Finally, the ligand molecule was prepared by adding hydrogen atoms using the Discovery studio visualizer.

Preparation of Protein

Crystallographic structures of the target Superoxide dismutase (1CB4) [25] was obtained from PBD (Protein Data bank) and saved in standard 3D coordinate format (Table 2). Active site of the targets were possessing natural ligand and so active site residue identification was carried out. Preparation of active site explicit hydrogen atoms missing in the PDB structure were added using Discovery studio visualizer software [25].

Molecular Docking

Docking is a method of predicting the preferred orientation of one molecule to another as they bind to each other to form a stable complex. The docking protein was created by Autodock Pyrx 0.8, the proteins were refined by removing water molecules and polar hydrogen's and kollmann charges were added. A docking simulation grid box was created using the Autodock Pyrx program, AutoGrid utility with 40 points in the x, y, and z directions centered on the active site. Targeting ligand complexes received 2.5 million evaluations. The binding energy is compared to the docking score of the standard ligand, ascorbic acid.

Visual Inspection

The solution structures of each drug candidates against all the targets were visualized and inspected for their goodness of fit and orientation inside the active site. This was done with PYMOL (https://www.schrodinger.com/products/pymol) and Discovery studio visualizer. Also the conformation and contacts with all amino acids were checked manually.

Biological activity prediction of selective ligands

Computational screening of possible biological activities of the six identified major compounds were evaluated using Prediction of activity spectra for substances (PASS) (http://www.pharmaexpert.ru/PASSonline/predict.php) which provides quantitative structure-activity relationships of compounds and exhibits > 3750 kinds of biological activities based on decomposition of

chemical structures in 2D and/or 3D descriptors. This software estimates the predicted activity spectrum of a compound as probable activity (Pa) and probable inactivity (Pi). In the present study, a Pa value of more than 0.7 (pa > 0.7) is considered a probable activity of more than 70%. When Pa is more than Pi (Pa > Pi), and Pa value is more than 0.7, It indicates a higher possibility of finding a specific pharmacological activity in experimental methods.

QSAR properties

QSAR (quantitative structure activity relationship) descriptions of the six selected ligands have been studied using the molinspiration cheminformatics software tools (https://www.molinspiration.com). We used the molinspiration virtual screening engine v2018.10 for attribute investigation. We used the OSIRIS Property Explorer [25], a free tool for predicting molecular properties, to predict these properties. All QSAR explanations are joined to a single table and explained as part of the Lipinski parameters.

ADMET properties

Analysis of possible molecular absorption, distribution, metabolism, excretion, and toxicity (ADMET) properties using computerized screening techniques has significantly improved the success rate in drug discovery and development. These pharmacokinetic assessments provide descriptive ideas for target molecular activity in the human body and predict potential drug candidates. After molecular docking studies, preADMET, an online tool for predicting pharmacokinetic properties, was used to investigate the ADMET properties of six selected ligands [25]. Pharmacokinetic properties include Caco2 cell permeability, skin permeability, MadinDarby Canine Kidney (MDCK) Cell permeability, human absorption, blood brain barrier penetration, plasma protein binding (PPB) and toxic properties (eg, suddenly) Mutagenesis or stimulating effect is included. Penetration of the blood brain barrier (BBB) is an important part of drug distribution for drug candidate in the central nervous system (CNS). Drug candidates with optimal ability to penetrate the BBB are called CNS active. That BBB penetration should be at least 0.40 (>0.40). In contrast, CNS inactive candidate BBB penetration is less than 0.40 (<0>90%), weakly bound candidate BBB penetration is less than 90% (%PPB <90>70), and permeable high values are within 4-70. If the medium permeability value is specified, then the permeability of Caco2 cells is less than 4. Likewise, values of MDCK cell permeability above 500 are predicted to indicate high permeability of the compound, values within 25-500 are relatively permeable, and values below 25 are predicted to be low permeability. Through studies of skin permeability and human intestinal absorption (HIA), drug candidates suitable for oral administration can be identified. In *in-silico* studies, compounds exhibit properties such as skin permeability as negative values increase. Percent of Human Intestinal Absorption (% HIA) determines the bioavailability of a compound administered via the hepatic portal vein, and a %HIA value of 70-100 predicts that the compound has good absorbance. The risk of toxicity is a major issue, because although drug candidates have potential pharmacological properties, they can be excluded from the drug design process. Negative *in-silico* results from toxicity support the compound as a safe drug candidate.

Bioactivity and toxicity risks prediction

Six selected ligands were evaluated for multiple bioactivity and toxicity risk using molinspiration and Osiris property explorer. Biological activity assessment predicted the properties of kinase inhibitors (KI), protease inhibitors (PI), enzyme inhibitors (EI), ion channel modulators (ICM), G protein-coupled receptor ligands (GPCRL), and nuclear receptor ligand interactions (NRL). Toxicity risk was predicted for properties such as drug score and drag likeness. The analyzed predictions suggested that the selected ligands were non-toxic compound.

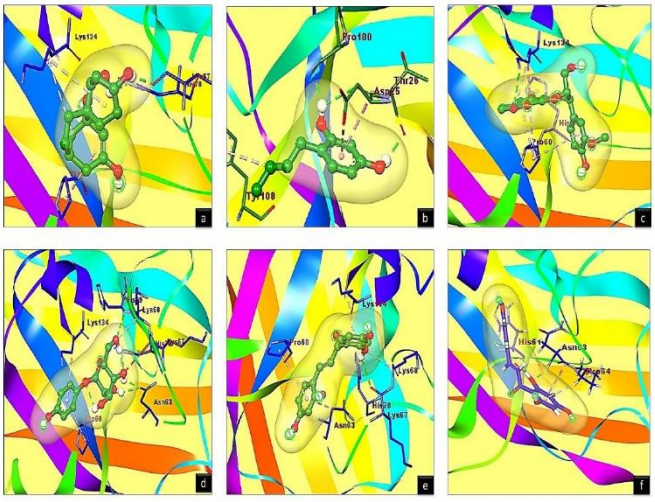
Results and discussion

Biological activity prediction

The PASS online prediction program was used to study the possible biological activities of confirmed phenolic compounds and their mechanism of action (MAO). All major biological activities of each compound were selected based on higher Pa values (Pa> 0.07). Table 1 shows the predicted biological properties and possible MAOs of the selective compounds.

Molecular docking analysis

The docking scores were obtained from selective ligands against superoxide dismutase (1CB4). The output of all ligands was provided as an energy value in kcal / mol units as shown in Table 3. All selected ligands except ligand 2 showed excellent docking scores compared to standard drugs. Ascorbic acid showed free binding affinity – 5.2 Kcal/mol with SOD. Ligands 1,3,4,5 and 6 showed higher binding affinity compared to standard drugs. The interactions between the amino acid residues of the target protein and the atoms of the selected ligand are shown in Table 3, and the interaction poses of the protein ligands generated in the docking study are shown in Figures 1 and 2.



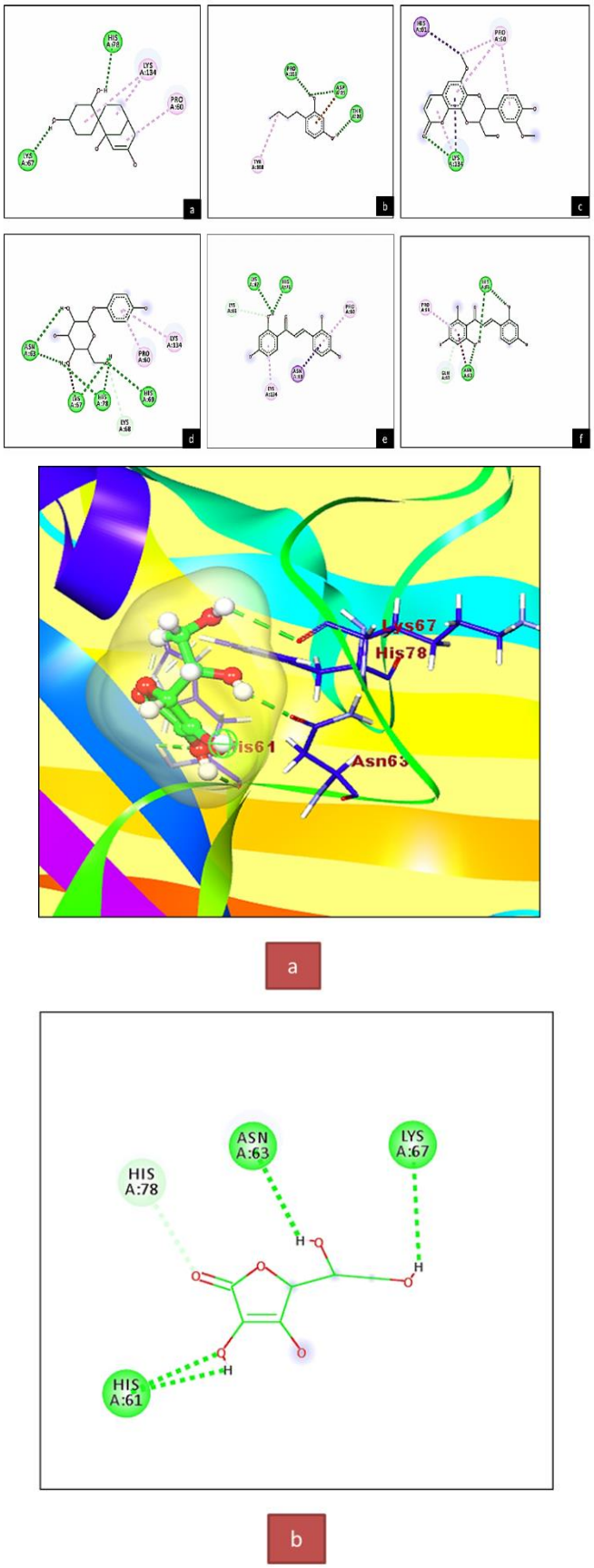


Figure 1: Possible 3D and 2D interactions of Ascorbic acid and SOD

QSAR Properties

A critical step in determining the drag-like properties of a compound is to analyze the physicochemical interactions of the target domains. QSAR studies predict possible biochemical interaction properties of selected compounds. This study evaluated six ligands selected for some QSAR descriptors from the five-parameter Lipinski's law (Table 4). The molecular weight of the ligand has been reported in the range of 166.1 to 386.1 Da. This value is less than 500 Da, thus following Lipinski's first rule for effective and safe drug delivery. Another rule is that no more than 5 hydrogen bond donation criteria must exist. Hydrogen bond acceptor groups were less than or equal to 10 in the range of 2 to 8, and logP measurements were less than or equal to 5 from -0.238 to 1.338. In addition, the molecular refractive index was calculated in the range of 49.88 to 98.82 cm3/mol. This suggested high potential as a drug candidate. Zero Lipinski violation was observed for six ligands.

ADMET properties

Studies of absorption, distribution, metabolism, excretion, and toxicity (ADMET) of various compounds are important for determining physicochemical interactions with specific targets. This helps assess potential drug candidates in the process of discovering and developing new drugs, and ultimately finding new lead compounds in specific target domains [26]. In the current study, we seek to determine these pharmacokinetic properties of seven selected ligands in order to understand their interactions within the body. Human absorption (HIA) is a major rate limiting step in which a drug is transported to a target site to exhibit a pharmacological response. Predicting HIA is difficult because several factors are involved. After oral administration, the drug is absorbed into the bloodstream and distributed to other organs by systemic circulation at the site of absorption. When the absorbed drug is distributed to the target site, the blood concentration decreases. Some drugs are metabolized by enzymatic reactions, and the activated metabolites exert pharmacological responses. Conversely, inactivated metabolites minimize the drug's response and the kidneys excrete inactive metabolites.

ADMET characterization (Table 5) predicted that all selected ligands had blood brain barrier (BBB) penetration in the range of 0.020539 to 5.40383 in vivo. This shows a higher effect, distributing to the central nervous system. All selected ligands except ligands 3 and 4 exhibited higher BBB penetration than standard ascorbic acid (0.11727). The *in-vitro* Caco2 cell permeability values of the six selected ligands were found within the range of 0.2834232 to 0.0068 nm/s, and the standard ascorbic acid found only 2.48366. Ligands 2 and 4 are less permeable to Caco2 cells, while other ligands are highly permeable. This Caco2 cell permeability indicates the continuous permeability of PPB that allows it to invade the blood brain barrier system. This assessment was further enhanced by the percentage of *in-vitro* plasma protein binding of the selected ligands observed in the range of 36.049960 to 100.000000%. Ligand 1 (100%), Ligand 2 (100%), Ligand 3 (93.23%), Ligand 4 (36.049960), Ligand 5 (100.000000) and Ligand 6 (100.000000) are very high compared to 2.79009 ascorbic acid Showed plasma protein binding affinity. The *in-vitro* MadinDarby Canine Kidney (MDCK) cell permeability values of these ligands were recorded within the range of 2.39284 to 44.3542 nm/sec, while ascorbic acid was recorded as 5.303528, and as a result, it became clear that they were relatively permeable. Rates of human intestinal absorption (HIA) were identified within the range of 42.139925 to 93.808460. This was significantly higher than standard ascorbic acid (33.157259). The toxic outcome was also recognized as negative and recommended that seven ligands are safe and non-toxic. Finally, this ADMET characterization of six ligands predicted its micro physicochemical interactions and drug like activity.

Bioactivity and toxicity risk studies

The analyzed bioactivity and toxicity risk profile predictions of the six ligands are summarized in Table 6. GPCRL properties were detected within the range of -0.08 to 0.05, ICM properties were reported as -0.01 to 0.12, KI properties were evaluated as -0.13 to -0.87, and NRL interaction properties were varied. In the range of -0.07 to 0.11, PI properties were recorded within the limits of -0.09 to 0.26 and EI properties were recognized within the range of -0.20 to 0.46. All bioactive properties of the selected ligands were higher than standard ascorbic acid. Likewise, all selected ligands were within the range of -1.67 to 0.51 drag likeness, which was higher than the standard ascorbic acid of 0.02. In addition, drug scores for these ligands were estimated within the range of 0.29 to 0.77 compared to the standard (0.75) drug score for ascorbic acid. Finally, this assessment of bioactivity and toxicity concludes that all selected ligands are safe as drug candidates and potentially bioactive in reactive species.

III. Conclusion

Adaptive binding of ligands to receptor particles is a promising and widely used method to reduce the cost and time of drug discovery. Based on the results, it can be concluded that the selected natural ligands 1, 3, 4, 5 and 6, with the exception of ligand 2, exhibit excellent binding affinity to target receptors which contribute to the removal of free radicals and exhibit antioxidant properties. Further studies are underway to reveal other active biomolecules and their biological properties.

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Declaration

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Conflict of interest statement

The authors declare no conflict of interest.

Availability of data and material

We have used online tools for our data sheet which are very much authentic and transparent.

Code availability

Not applicable.

Authors' contributions

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome. We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us. We understand that the Corresponding Author is the sole contact for the Editorial process (including Editorial Manager and direct communications with the office). He is responsible for communicating with the other authors about progress, submissions of revisions and final approval of proofs. We confirm that we have provided a current, correct email address which is accessible by the Corresponding Author. The contribution of all authors is equally evaluated.

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Table 1. Predicted biological activities of selective compounds

SI No. (Ligand)	IUPAC	Predicted Biological Activity/MOA
1.	4-(3,5-dihydroxystyryl)benzene-1,3-diol	• Anti-inflammatory • TNF expression inhibitor • Oxygen scavenger • Antioxidant
2.	4-butylbenzene-1,3-diol	• Anti-inflammatory, intestinal • Phosphatase inhibitor • Free radical scavenger • Antiulcerative
3.	(2R,3R)-3-(4-hydroxy-3-methoxyphenyl)-2-(hydroxymethyl)-5-methoxy-2H-[1,4]dioxino[2,3-h]chromen-9(3H)-one	• Antioxidant • Free radical scavenger • TP53 expression enhancer • Hepatoprotectant • Anticarcinogenic

4.	(3R,4R,5S,6R)-2-(hydroxymethyl)-6-(4-hydroxyphenoxy)tetrahydro-2H-pyran-3,4,5-triol	• Anti-inflammatory • Vasoprotector • Anticarcinogenic • TP53 expression enhancer • Antioxidant
5.	(E)-1,3-bis(2,4-dihydroxyphenyl)prop-2-en-1-one	• Antineoplastic • Antiulcerative • Antioxidant • Anthelmintic (Nematodes) • Anti-inflammatory, ophthalmic
6.	(E)-3-(2,4-dihydroxyphenyl)-1-(2,4,6-trihydroxyphenyl)prop-2-en-1-one	• Antileukemic • Anti-inflammatory • Chemopreventive • Lipid peroxidase inhibitor • Antioxidant

Table 2. Protein with active site and 3D structure

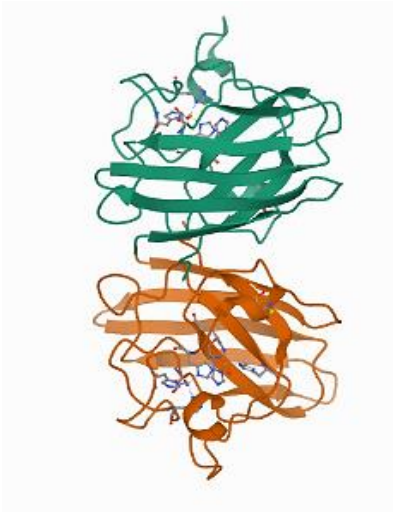
Name	Active site	3D structure
Suoeroxide dimutase (1CB4)	Chain A	

Table 3

Docking scores and H-bonding interactions involved in selective ligands and target protein SOD (1CB4)

Compounds	MW	HBA	HBD	logP(O/W)	MR	Lip Vio
Ligand-1	244.07	4	4	0.641	69.90	0
Ligand-2	166.1	2	2	2.689	49.88	0
Ligand-3	386.1	8	2	1.183	98.82	0
Ligand-4	272.09	7	5	-0.238	62.61	0
Ligand-5	272.07	5	4	1.338	74.34	0
Ligand-6	288.06	6	5	0.804	76.36	0
Ascorbic acid	176.12	6	4	-1.40	35.12	0

Table 4. QSAR properties of selective ligands

Name	Docking Score	H-Bonding Amino acid
Ligand-1	-5.9	HIS78, LYS67
Ligand-2	-4.5	ASP25, PRO100,THR26
Ligand-3	-6.6	LYS134
Ligand-4	-5.7	HIS69, LYS67, ASN63, HIS78
Ligand-5	-6.0	LYS67, HIS78
Ligand-6	-6.0	HIS61, ASN63
Ascorbic acid	-5.2	HIS61, ASN63, LYS67

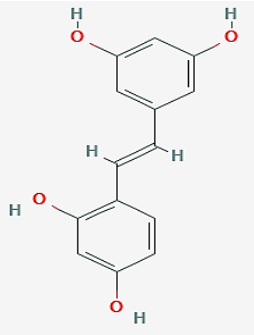
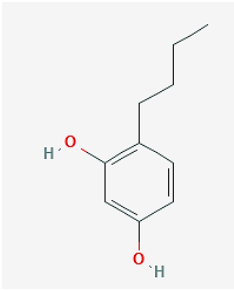
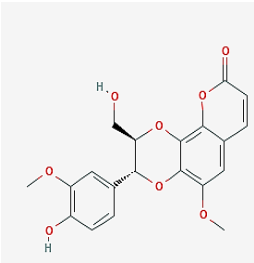
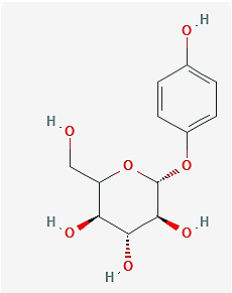
MW: Molecular weight; HBD: Hydrogen bond donors; HBA: Hydrogen bond acceptors; logP: octanol to water partition coefficient; MR: Molecular refractivity; (cm3/mol); Lip Vio: Lipinski Violations.

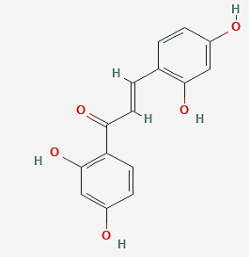
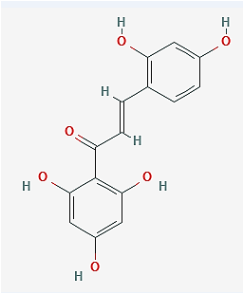
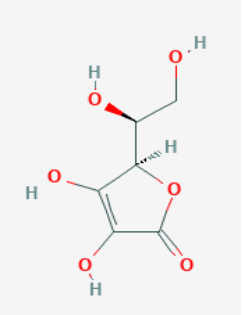
Table 5. ADMET properties of selective ligands

Compounds	BBB[a]	Caco2[b]	HIA[c]	MDCK[d]	PPB[e]	Toxicity[f]
Ligand-1	0.925582	19.8473	81.965955	44.3542	100.000000	Negative
Ligand-2	5.40383	0.283423	89.199801	31.082	100.000000	Negative
Ligand-3	0.020539	17.7686	93.808460	6.49589	83.395347	Negative
Ligand-4	0.0768102	0.436035	42.139925	2.39284	36.049960	Negative
Ligand-5	0.625451	20.0068	80.612013	30.4337	100.000000	Negative
Ligand-6	0.348009	19.3972	66.615460	40.2555	100.000000	Negative
Ascorbic acid	0.11727	2.48366	33.157259	0.881902	5.303528	Negative

a Blood-Brain Barrier (BBB) penetration = [Brain]/[Blood].
b Caco-2 cells are derived from human colon adenocarcinoma, possess multiple drug transport pathways through intestinal epithelium.
c Human intestinal absorption is the sum of bioavailability and absorption evaluated from ratio of excretion or cumulative excretion in urine, bile and feces.
d MDCK cell system used as tool for rapid permeability screening.
e % of drug binds to plasma protein.
f In vitro Ames test by Metabolic & Non-metabolic activated TA100 & TA1535 strains collected from rat liver homogenate.

Table 6. Bioactivity and toxicity risks of compounds ligand 1-7 & Ascorbic acid

Compounds	Structure	Bioactivity						Toxicity risk	
		GPCRL	ICM	KI	NRL	PI	EI	Drug-likeness	Drug score
Ligand-1		-0.16	-0.02	-0.14	0.08	-0.36	0.04	-3.59	0.29
Ligand-2		-0.54	-0.18	-0.87	-0.36	-0.83	-0.20	-7.33	0.47
Ligand-3		-0.11	-0.18	-0.15	-0.07	-0.19	0.23	-1.67	0.31
Ligand-4		0.05	0.12	-0.13	0.04	-0.09	0.46	-6.97	0.29

Ligand-5		-0.09	-0.13	-0.27	0.03	0.26	0.08	0.34	0.36
Ligand-6		-0.08	-0.01	-0.17	0.11	-0.17	0.11	0.51	0.77
Ascorbic acid		-0.53	-0.24	-1.09	-1.01	-0.81	0.20	0.02	0.75