Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2017, 9(5):39-44

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Molecular Docking Studies of Quinones against Human Inducible Nitric Oxide Synthase (iNOS)

Radhakrishnan Narayanaswamy[1*], Lam Kok Wai[2] and Intan Safinar Ismail[3]

[1]Bio Waste Management Laboratory, Vel Tech Technology Incubator, Veltech Dr. RR & Dr. SR University, Chennai, Tamil Nadu, India
[2]Faculty of Pharmacy, Universiti Kebangsaan Malaysia (UKM), Jalan Raja Muda Abdul Aziz, Kuala Lumpur, Malaysia
[3]Laboratory of Natural Products, Institute of Bioscience (IBS), Universiti Putra Malaysia (UPM), 43400 Serdang, Selangor, Malaysia

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ABSTRACT

Quinone and its derivatives are well known to have various biological properties such as antineoplastic, antimalarial, antitumor, anticoagulant, herbicidal and antibiotic activities. They are also known to have inducible nitric oxide synthase (iNOS) inhibition activity. This prompted the present study to be carried out on 17 selected quinones which are menadione, thymoquinone, benzoquinone, 2,3-dichloro-5,6-dicyano-1,4-benzoquinone, 2,6-dimethyl-1,4-benzoquinone, 2,5-dimethyl-1,4-benzoquinone, duroquinone, hydroquinone, anthraquinone, 2-chloro-1,4-benzoquinone, 2-phenyl-1,4-benzoquinone, 2,3,5,6-tetrachloro-1,4-benzoquinone, 1,4-naphthoquinone, coenzyme Q_{10}, triptoquinone A, idebenone and dopaquinone. These quinones were evaluated on their docking behaviour on inducible nitric oxide synthase (iNOS) using Discovery Studio Version 3.1. In addition, molecular physicochemical, drug-likeness, ADMET (Absorption, Distribution, Metabolism, Excretion and Toxicity) and TOPKAT (Toxicity Prediction by Komputer Assisted Technology) analyses were done. The molecular physicochemical analysis revealed that all the tested ligands complied with Lipinski's rule of five. ADMET analysis showed that all the ligands except coenzyme Q_{10} exhibited good intestinal absorption property. Docking studies and binding free energy calculations exhibited that coenzyme Q_{10} gave the highest interaction energy (-71.05 kcal/mol) and benzoquinone in contrast showed the least interaction energy (-14.84 kcal/mol). Hence, the results of this present study exhibited the potential of these quinones as inducible nitric oxide synthase (iNOS) inhibitory agents.

Keywords: Inducible nitric oxide synthase; Thymoquinone; Coenzyme $Q_{10;}$ Triptoquinone A; Idebenone; Dopaquinone

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INTRODUCTION

Quinones represent an important class of naturally occurring compounds that are present in flowering plants, bacteria and fungi, primarily as essential components of the electron-transport chains involved in photosynthesis and cellular respiration. There are various kinds of biological activities such as antineoplastic [1], antimalarial [2], antitumor [3], anticoagulant [4], herbicidal [5] and antibiotic [6] attributable to this group of compounds including their derivatives. Synthetic or natural quinoid compounds such as 1, 4-benzoquinone and 1, 4-naphthoquinone have

been used as dyes [7]. Some quinones are widely applied in the fields of photochemistry [8], co-ordination chemistry [9] and synthetic chemistry [10].

Nitric oxide synthases (NOS) are family of enzymes catalyzing the production of nitric oxide (NO) from L-arginine (amino acid). It is an important cellular signalling molecule, playing vital role in various cellular processes [11]. Mammal has three isoforms of NOS, of which two are constitutive types of endothelium (eNOS) and neuron (nNOS) and another one is an inducible type (iNOS). Induction of iNOS by various stimuli contributes to the pathogenesis of septic shock [12], some inflammatory [13] and autoimmune diseases [14].

Therefore, there is a great demand to develop potent and selective inhibitors of iNOS for various therapeutic applications. Niwa et al. [15] have reported the inhibition of iNOS expression by benzoquinones from which prompted the present study on a selected 17 quinones namely menadione, thymoquinone, benzoquinone, 2,3-dichloro-5,6-dicyano-1,4-benzoquinone, 2,6-dimethyl-1,4-benzoquinone, 2,5-dimethyl-1,4-benzoquinone, duroquinone, hydroquinone, anthraquinone, 2-chloro-1,4-benzoquinone, 2-phenyl-1,4-benzoquinone, 2,3,5,6-tetrachloro-1,4-benzoquinone, 1,4-naphthoquinone, coenzyme Q_{10}, triptoquinone A, idebenone and dopaquinone. These quinones were evaluated on the docking behaviour of human inducible nitric oxide synthase (iNOS) using Discovery Studio Version 3.1 whereby the results have given useful information for the future design of potent and selective iNOS inhibitors from quinoid compounds.

EXPERIMENTAL SECTION

Ligand preparation

Chemical structures of the ligands namely i) menadione [CID4055]; ii) thymoquinone [CID10281]; iii) benzoquinone [ID4489]; iv) 2,3-dichloro-5,6-dicyano-1,4-benzoquinone [ID6517]; v) 2,6-dimethyl-1,4-benzoquinone [ID61542]; vi) 2,5-dimethyl-1,4-benzoquinone [ID8394]; vii) duroquinone [CID68238]; viii) hydroquinone [CID785]; ix) anthraquinone [CID6780]; x) 2-chloro-1,4-benzoquinone [ID62872]; xi) 2-phenyl-1,4-benzoquinone [ID9307]; xii) 2,3,5,6-tetrachloro-1,4-benzoquinone [CID8371]; xiii) 1,4-naphthoquinone [ID8215]; xiv) coenzyme Q_{10} [CID5281915]; xv) triptoquinone A [CID132524]; xvi) idebenone [CID3686] and dopaquinone [CID439316] were retrieved from PubMed (www. pubmed.com) and Chemspider (www. chemspider.com).

Target protein identification and preparation

The three dimensional structure of the human inducible nitric oxide synthase (iNOS) (PDB ID: 4NOS with resolution of 2.3 Å) was obtained from the Research Collaborator for Structural Bioinformatics (RCSB) Protein data bank (Anonymous, www. rcsb.org). A chain of iNOS was pre-processed separately by deleting other chains, ligand, as well as the crystallographically observed water molecules (water without hydrogen bonds).

Molecular descriptors calculation

Molinspiration online database was used for all selected ligands to calculate thirteen descriptors (www.molinspiration.com) which are logP, polar surface area, molecular weight, number of atoms, number of O or N, number of OH or NH, number of rotatable bond, volume, drug likeness including G protein coupled receptors (GPCR) ligand, ion channel modulator, kinase inhibitor, nuclear receptor ligand, and number of violations to Lipinski's rule.

ADMET and TOPKAT test

ADMET (Absorption, Distribution, Metabolism, Excretion and Toxicity) and TOPKAT (Toxicity Prediction by Komputer Assisted Technology) tests were performed by using Discovery Studio® 3.1 (Accelrys, San Diego, USA). ADMET analysis was performed on human intestinal absorption (HIA), aqueous solubility (AS), blood brain barrier (BBB), cytochrome P450 2D6 (CYP2D6), plasma protein binding (PPB) and hepatotoxicity (HT) descriptors. As for the TOPKAT analysis, it was done on aerobic biodegradability (AB), Ames mutagenicity (AM), ocular irritancy (OI), skin irritancy (SI), skin sensitization(SS) and oral toxicity in rat (LD_{50} in g/Kg of body weight) descriptors.

Docking studies

Docking studies were carried out on the iNOS crystal structure retrieved from Protein Data Bank using the CDOCKER protocol under the protein-ligand interaction section in Discovery Studio® 3.1 (Accelrys, San Diego, USA). In general, CDOCKER is a grid-based molecular docking method that employs CHARMM force fields. This protein was firstly held rigid while the ligands were allowed to flex during the refinement. Two hundred random ligand conformations were then generated from the initial ligand structure through high temperature molecular

dynamics followed by random rotations, refinement by grid-based (GRID I) simulated annealing, and a final grid-based or full force field minimisation [16].

In this experiment, the ligand was heated to the temperature of 700 K in 2000 steps. The cooling steps were set to 5000 steps to 300 K cooling temperature. The grid extension was set to 10 Å. Hydrogen atoms were added to the structure and all ionisable residues were set at their default protonation state at a neutral pH. For each ligand, top ten ligand binding poses were ranked according to their CDOCKER energies, and the predicted binding interactions were analysed. The best among the ten ligand binding poses was chosen and carried out *in situ* ligand minimization using a standard protocol.

RESULTS AND DISCUSSION

Nowadays drug design and development strategies have progressively shifted from phenotypic screening to combinational chemistry and high throughput screening, whereby the physicochemical properties of exploratory drug have gained much importance. Lipinski rule of five is a rule applied to evaluate molecular physicochemical and drug-likeness properties of compounds, which when further determined of a lead compound having a certain pharmacological or biological activity could be made into an orally active drug for human [17].

Blake's [18] study has shown that for the five different clinical stages/phases from pre-clinical to approved, very few compounds violated the Lipinski's rule. Furthermore, the author indicated that compounds which violated this rule need to be modified completely and they have less probability to be drug candidates. Previously coenzyme $Q_{10,}$ dopaquinone, idebenone [19] and thymoquinone [20] have been reported for molecular physicochemical and drug-likeness properties. The main target compound will be that with violation of zero for all the tested ligands which complied very well with the Lipinski's rule of five as shown in Table 1.

Table 1: Molecular physicochemical descriptors analysis of thirteen ligands using Molinspiration online software tool

Ligand	Log A [a]	TPSA[b]	Natoms [c]	MW[d]	noN [e]	nOH NH [f]	Nviolations [g]	Nrotb [h]	Volume [i]
Menadione	4.62	74.6	21	294.4	4	2	0	10	295.2
Benzoquinone	0.56	34.1	8	108.1	2	0	0	0	94.6
2,3-dichloro-5,6-dicyano-1,4-benzoquinone	1.13	81.73	14	227	4	0	0	0	155.4
2,6-dimethyl-1,4-benzoquinone	1.31	34.14	10	136.2	2	0	0	0	127.7
2,5-dimethyl-1,4-benzoquinone	1.31	34.14	10	136.2	2	0	0	0	127.7
Duroquinone	2.06	34.14	12	164.2	2	0	0	0	160.8
Hydroquinone	0.98	40.46	8	110.1	2	2	0	0	100.1
Anthraquinone	3.67	34.14	16	208.2	2	0	0	0	182.6
2-chloro-1,4-benzoquinone	1.16	34.14	9	142.5	2	0	0	0	108.1
2-phenyl-1,4-benzoquinone	2.28	34.14	14	184.2	2	0	0	1	166
2,3,5,6-tetrachloro-1,4-benzoquinone	2.98	34.14	12	245.9	2	0	0	0	148.7
1,4-naphthoquinone	1.67	34.14	12	158.2	2	0	0	0	138.6
Triptoquinone A	3.56	71.4	24	328.4	4	1	0	2	310.9

[a]Octanol-water partition coefficient; [b]Polar surface area; [c]Number of non hydrogen atoms; [d]Molecular weight; [e]Number of hydrogen bond acceptors [O and N atoms]; [f]Number of hydrogen bond donors [OH and NH groups]; [g]Number of rule of 5 violations; [h]Number of rotatable bonds; [i]Molecular volume

Menadione, 2-phenyl-1, 4-benzoquinone and triptoquinone showed better drug-likeness score towards enzyme inhibitor descriptors. However, all the tested ligands exhibited active to moderate active score for other descriptors as shown in Table 2. Besides drug-likeness properties, in the view point of drug design and development strategy, ADMET drug properties are also essential for clinical success.

According to Li [21], the selection of drug candidates with the best ADMET properties drastically increase the clinical success rate. Table 3 shows the ADMET profile of the seventeen ligands in which all those except coenzyme Q_{10} showed a good intestinal absorption property. All the ligands were predicted to have cytochrome P_{450} 2D6 (CYP2D6) induction effect.

Five ligands which are menadione, 1, 4-naphthoquinone, coenzyme Q_{10}, idebenone and dopaquinone) were predicted to have no hepatotoxicity effect compared to the other ligands. The toxicity profiles of seventeen ligands as depicted in Table 4 wherein six ligands (2,3-dichloro-5,6-dicyano-1,4-benzoquinone, hydroquinone, anthraquinone, 2-chloro-1,4-benzoquinone, 2,3,5,6-tetrachloro-1,4-benzoquinone and 1,4-naphthoquinone) exhibited as non-degradable towards aerobic biodegradability nature compared to the other ligands. All of the ligands were predicted to have ocular irritancy and skin sensitization effect in human.

Nitric oxide (NO) is synthesised from L-arginine and can be produced by three distinct enzyme systems. Out of the previously mentioned three, inducible nitric oxide synthase (iNOS) is harmful and a specific inhibition of this enzyme is beneficial in clinical point of view [22]. Benzoquinones were reported as potent drug candidates against inducible nitric oxide synthase (iNOS) over-expressed diseases [15].

Table 2: Drug-likeness property analysis of thirteen ligands using Molinspiration online software tool

Ligand	GPCR ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor
Menadione	-0.31	-0.22	0.03	0.01	-0.16	0.33
Benzoquinone	-3.59	-2.94	-3.17	-3.48	-3.52	-2.87
2,3-dichloro-5,6-dicyano-1,4-benzoquinone	-0.62	-0.24	-0.44	-0.58	-0.68	-0.13
2,6-dimethyl-1,4-benzoquinone	-1.65	-0.64	-1.58	-1.75	-1.75	-0.68
2,5-dimethyl-1,4-benzoquinone	-1.76	-0.71	-1.53	-1.97	-1.78	-0.69
Duroquinone	-0.87	-0.5	-1.11	-1.06	-1.07	-0.32
Hydroquinone	-3.02	-2.48	-3.07	-2.84	-3.2	-2.66
Anthraquinone	-0.4	-0.16	-0.3	-0.44	-0.51	-0.03
2-chloro-1,4-benzoquinone	-2.78	-1.94	-2.45	-2.79	-2.88	-1.76
2-phenyl-1,4-benzoquinone	-0.7	-0.31	-0.32	-0.59	-0.67	0.17
2,3,5,6-tetrachloro-1,4-benzoquinone	-0.94	-0.43	-0.91	-1.08	-1.03	-0.29
1,4-naphthoquinone	-0.94	-0.46	-0.77	-1	-1.1	-0.34
Triptoquinone A	0.04	0.31	-0.4	0.5	-0.06	0.43

Table 3: ADMET analysis of seventeen ligands

Ligand	HIA			AS		BBB		PPB	CYP2D6	HT
	PSA	ALogP98	L*	Log (SW)	L**	Log BB	L***	Prediction		
Menadione	34.6	2.2	0	-3.25	3	-0.02	2	T	F	F
Thymoquinone	34.6	2.29	0	-3.02	3	0.01	1	F	F	T
Benzoquinone	34.6	0.69	0	-1.29	4	-0.49	2	F	F	T
2,3-dichloro-5,6-dicyano-1,4-benzoquinone	80.5	1.1	0	-2.08	3	-1.09	3	F	F	T
2,6-dimethyl-1,4-benzoquinone	34.6	1.58	0	-2.31	3	-0.21	2	F	F	T
2,5-dimethyl-1,4-benzoquinone	34.6	1.58	0	-2.31	3	-0.21	2	F	F	T
Duroquinone	34.6	2.47	0	-3.33	3	0.06	1	T	F	T
Hydroquinone	41.6	1.35	0	-0.78	4	-0.4	2	F	F	T
Anthraquinone	34.6	2.81	0	-4.15	2	0.17	1	T	F	T
2-chloro-1,4-benzoquinone	34.6	1.06	0	-1.73	4	-0.38	2	F	F	T
2-phenyl-1,4-benzoquinone	34.6	2.17	0	-3.13	3	-0.03	2	F	F	T
2,3,5,6-tetrachloro-1,4-benzoquinone	34.6	2.16	0	-3.06	3	-0.03	2	T	F	T
1,4-naphthoquinone	34.6	1.76	0	-2.75	3	-0.16	2	T	F	F
Coenzyme Q10	52.5	18.77	3	-3.77	3	0	4	T	F	F
Triptoquinone A	72.7	4.11	0	-5.33	2	-0.03	2	T	F	T
Idebenone	73.3	3.84	0	-3.44	3	-0.13	2	T	F	F
Dopaquinone	99.3	-0.31	0	-0.8	4	-1.82	3	F	F	F

HIA-Human intestinal absorption; AS- Aqueous solubility, BBB-Blood brain barrier; PPB-Plasma protein binding; CYP2D6- cytochrome P450 2D6; HT-hepatotoxicity; L-Level, F-False & T-True *- (0-good; 1-moderate; 2-poor & 3-very poor); **- (0-extremely low; 1-very low; 2-low; 3-good; 4-optimal; 5-too soluble & 6-warning); ***-(0-very high penetrate; 1-high; 2-medium; 3-low & 4-undefined)

The docking studies and binding free energy calculations as in Table 5 shows coenzyme Q_{10} with the highest interaction energy (-71.05 kcal/mol) while benzoquinone gave the least interaction energy (-14.84 kcal/mol). Interaction with Trp194 amino acid residue was shown by nine ligands namely menadione, 2,3-dichloro-5,6-dicyano-1,4-benzoquinone, 2,6-dimethyl-1,4-benzoquinone, duroquinone, anthraquinone, 2-phenyl-1,4-benzoquinone, 2,3,5,6-tetrachloro-1,4-benzoquinone, triptoquinone A and idebenone (Table 5). Similarly, Wang et al. [23] reported that 6-methyl-1, 3, 8-trihydroxyanthraquinone (emodin) inhibited the inducible nitric oxide synthase (iNOS) activity in lipopolysaccharide (LPS) activated RAW 264.7 cells. The iNOS protein expression was reported to be suppressed by a few quinone types which are shikonin/alkannin (natural naphthoquinone derivatives), 2-(1-methoxy carbonyl-4, 6-dihydroxyphenoxy)-3-methoxy-5, 6-di-(3-methoxyl-2-butenyl)-1, 4-benzoquinone

(atrovirinone), and 1, 2, 4-trihydroxyanthraquinone (purpurin) [24-26]. While, Hirakawa et al. [27] reported on that pyrroloquinoline quinone (PQQ) attenuated the iNOS gene expression in the injured spinal cord. The coenzyme Q_{10} was observed to reduce both inducible and endothelial nitric oxide synthase in rat ischemia/reperfusion injury model [28].

Table 4: Toxicity prediction analysis of seventeen ligands

Ligand	AB*	AM**	OI#	SI##	SS♦	Oral toxicity▲
Menadione	Degradable	Mutagen	Irritant	Irritant	Sensitizer	1.72
Thymoquinone	Degradable	Non-mutagen	Irritant	Irritant	Sensitizer	0.88
Benzoquinone	Degradable	Non-mutagen	Irritant	Irritant	Sensitizer	0.73
2,3-dichloro-5,6-dicyano-1,4-benzoquinone	Non-degradable	Non-mutagen	Irritant	Irritant	Sensitizer	0.13
2,6-dimethyl-1,4-benzoquinone	Degradable	Non-mutagen	Irritant	Irritant	Sensitizer	0.82
2,5-dimethyl-1,4-benzoquinone	Degradable	Non-mutagen	Irritant	Irritant	Sensitizer	0.82
Duroquinone	Degradable	Non-mutagen	Irritant	Irritant	Sensitizer	1.05
Hydroquinone	Non-degradable	Non-mutagen	Irritant	Non-irritant	Sensitizer	0.96
Anthraquinone	Non-degradable	Mutagen	Irritant	Non-irritant	Sensitizer	2.48
2-chloro-1,4-benzoquinone	Non-degradable	Non-mutagen	Irritant	Irritant	Sensitizer	0.3
2-phenyl-1,4-benzoquinone	Degradable	Non-mutagen	Irritant	Irritant	Sensitizer	0.94
2,3,5,6-tetrachloro-1,4-benzoquinone	Non-degradable	Mutagen	Irritant	Irritant	Sensitizer	0.46
1,4-naphthoquinone	Non-degradable	Mutagen	Irritant	Irritant	Sensitizer	2.54
Coenzyme Q10	Degradable	Non-mutagen	Non-irritant	Irritant	Sensitizer	9.68
Triptoquinone A	Degradable	Non-mutagen	Irritant	Irritant	Sensitizer	1.4
Idebenone	Degradable	Non-mutagen	Irritant	Irritant	Sensitizer	1.76
Dopaquinone	Degradable	Non-mutagen	Irritant	Irritant	Sensitizer	0.44

AB*- Aerobic biodegradability; AM**- Ames mutagenicity; OI#- Ocular irritancy; SI##- Skin irritancy; SS♦- Skin sensitization and Oral toxicity▲- Oral toxicity in rat (LD_{50} in g/Kg of body weight-rat).

Table 5: The interaction energy analysis of seventeen ligands with that of iNOS using Discovery Studio® 3.1

Ligand name	-cDocker interaction energy (kcal/mol)	Interaction amino acid residue	Bond distance (Å)
Menadione	20.91	Trp194▪	6.3 & 6.6
		Phe369▪	4.2 & 5.9
Thymoquinone	22.64	Nil	Nil
Benzoquinone	14.84	Nil	Nil
2,3-dichloro-5,6-dicyano-1,4-benzoquinone	25.11	Trp194▪	5.9
2,6-dimethyl-1,4-benzoquinone	17.99	Trp194▪	6.1
		Phe369▪	4.9
2,5-dimethyl-1,4-benzoquinone	17.5	Nil	Nil
Duroquinone	21.6	Trp194▪	6.5
		Phe369▪	5.2
Hydroquinone	20.06	Cys200	1.4
		Ile201	3
		Gly202	3.1
		Trp372	1.6 & 3.1
Anthraquinone	25.3	Trp194▪	4.0 & 4.5
		Phe369▪	4.5, 6.0 & 6.7
2-chloro-1,4-benzoquinone	17.34	Nil	Nil
2-phenyl-1,4-benzoquinone	24.47	Trp194▪	4.2
2,3,5,6-tetrachloro-1,4-benzoquinone	26.46	Trp194▪	6.1
1,4-naphthoquinone	17.47	Nil	Nil
Coenzyme Q_{10}	71.05	Asn354	3.2
		Lys497▪	6.3
Triptoquinone A	40.56	Trp194▪	5.7
		Phe369▪	5.6
		Trp372	3.2
Idebenone	49.26	Trp194▪	6.8
		Ser242	2.7
Dopaquinone	33.02	Trp372	3.2
		Glu377	1.7 & 1.8

▪-π-π interaction

Recently, Wang et al. [29] reported that 5-hydroxy-2-methyl-1, 4-napthoquinone (plumbagin) also suppressed the iNOS activity in lipopolysaccharide (LPS) induced RAW 264.7 cells.

CONCLUSION

In the present study, all of the tested ligands have shown to dock with iNOS. However, five ligands which are thymoquinone, benzoquinone, 2, 5-dimethyl-1, 4-benzoquinone, 2-chloro-1, 4-benzoquinone and 1, 4-naphthoquinone did not interact with any amino acid residues of iNOS. Coenzyme Q_{10} exhibited the highest interaction energy (-71.05 kcal/mol) and benzoquinone in contrast showed the least interaction energy (-14.84 kcal/mol). The results from the present study provide new insight in understanding these quinones as potential iNOS inhibitors in which the molecular docking studies could contribute for further development and understanding of the iNOS inhibitors for the prevention of inducible nitric oxide synthase associate disorders.

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