IN SILICO INVESTIGATION OF THE POTENTIAL INHIBITORY EFFECTS OF ANTHRAQUINONE DERIVATIVES ON ALPHA SYNUCLEIN AGGREGATION

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Abstract. Parkinson's disease and Lewy body dementias are progressive neurodegenerative diseases accompanied by dementia and a wide array of symptoms such as bradykinesia, rigidity and postural instability. The hallmark of the disease is alpha synuclein aggregation, leading to the formation of Lewy. In this paper we investigate, using various *in silico* tools, the potential anti-aggregation effects of anthraquinone derivatives carminic, kermesic and laccaic acid and their potential future use as lead compounds for clinical applications. To this end, we employed a combination of predictors used for properties like absorption, distribution, metabolism and excretion (ADME), drug-likeness and lead-likeness. Additionally, we used a molecular docking to estimate the binding affinities of the three compounds to alpha synuclein. Our predictions indicate kermesic acid as the compound having the greatest therapeutical potential.

Key words: Parkinson's disease, alpha synuclein, carminic acid, kermesic acid.

INTRODUCTION

Parkinson's Disease and Lewy body dementias are progressive neurodegenerative diseases accompanied by dementia and a wide array of symptoms such as bradykinesia, rigidity and postural instability, among others. The main cause of the disease is considered to be the death of dopaminergic neurons in the *substantia nigra* with direct involvement of the alpha synuclein protein.

On a molecular level, neuronal death is driven by the accumulation and aggregation of the presynaptic protein alpha synuclein from the cytosol and possible low-affinity binding to neuronal membranes, leading to a loss of conformational entropy and leading to a more structured helical form that facilitates membrane interaction [5], pore formation [8] and subsequent genesis of Lewy bodies, abnormal intraneuronal accumulations of proteins, driven by its

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aggregation but also containing numerous other proteins responsible with ubiquitination and protein trafficking, oxidative stress, chaperones, kinases and many others. Structurally, alpha synuclein is a 140 aminoacid long intrinsically unstructured presynaptic protein and may suffer a series of post-traslational modifications as oxidation, nitrosylation, phosphorylation, glycation and glycosylation [16].

Structurally, alpha synuclein is divided into three main domains: the amphipathic N-terminus region, comprised of aminoacids 1–60 and containing numerous sites responsible for membrane binding, the hydrophobic NAC (non-amyloid-β component) region, comprised of aminoacids 61–95 and considered to be the main driver of protein aggregation [1] and an acidic intrinsically unstructured C-terminus tail involved in the inhibition of aggregation [4].

A number of highly conserved KTKEGV motif imperfect repeats are located in the N-terminus [13], with the KTKEGV motif itself [2] involved in the protein's tetramerization and the reduction of neurotoxicity.

Recently, a seven residue sequence (aminoacids 36–42) linker region was identified by Brockwell's group [3] that appears to control the aggregation behavior of alpha synuclein. A wide range of aggregation inhibitors have been identified by high throughput screening for the alpha synuclein monomer [7] mainly targeting the NAC region.

Carminic acid was recently shown to inhibit aggregation of human serum albumin [15]. In the present study, using various *in silico* methods, our aims are: (1) to evaluate the binding affinities of anthraquinone derivatives carminic, laccaic and kermesic acid on alpha synuclein using a blind molecular docking approach, (2) to evaluate the ADME (absorption, distribution, metabolism, excretion) profile of carminic, kermesic and laccaic acid, and (3) to evaluate the drug-likeness of the given molecules.

MATERIALS AND METHODS

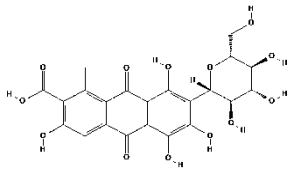
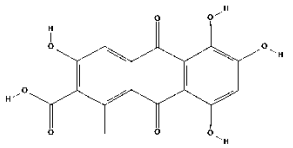
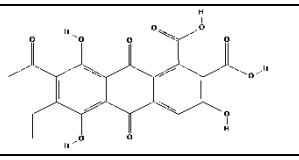
The structures of the anthraquinone derivatives carminic acid, kermesic acid and laccaic acid were downloaded from the PubChem database [11] in SDF (Spatial Data File) format. The files were then converted to the PDB (Protein Data Bank) and SMILES (Simplified Molecular Input Line Entry System) format using the online OpenBabel conversion tools [10].

The structure of human alpha synuclein, covering the full sequence of the protein (PDB ID: 1XQ8) was downloaded from the Protein Data Bank [14] and the first (lowest energy) NMR conformer was used in the subsequent docking runs.
Further, the generated files were visually inspected for potential structural errors and minimized using Swiss PDB Viewer version 4.1 [6] to remove any possible geometrical imperfections resulting from the conversion, and both protein and ligand files were submitted to MGL Tools version 1.5.7.rc1. Docking runs were

performed using Autodock Vina version 1.1.2 [12], using standard docking parameters (exhaustiveness = 8, number of generated hits = 9). The generated files were visualized with PyMOL Molecular Graphics System, Version 1.7.4, Schrödinger, LLC, New York, and interaction diagrams were obtained using LigPlot+ [9].

Table 1

Anthraquinone derivates, with corresponding chemical structures and SMILES codes

Name	Structure	SMILES code
Carminic acid		CC1=C2C(=CC(=C1C(=O)O)O)C(=O)C3=C(C2=O)C(=C(C(=C3O)O)C4C(C(C(C(O4)CO)O)O)O)O
Kermesic acid		CC1=C2C(=CC(=C1C(=O)O)O)C(=O)C3=C(C2=O)C(=CC(=C3O)O)O
Laccaic acid		CCC1=C(C(=C2C(=C1O)C(=O)C3=CC(=C(C(=C3C2=O)C(=O)O)C(=O)O)O)O)C(=O)C

Further, we used the SwissADME web server [17] to evaluate the absorption, distribution, metabolism and excretion profile along with drug and lead likeness, by providing the corresponding canonical SMILES codes for each of the three molecules of interest (Table 1).

RESULTS AND DISCUSSION

Our results show that carminic acid, along with kermesic and laccaic acid bind the linker region with high affinities (Table 2, 3 and 4 below), with carminic acid showing the highest affinity, followed by kermesic and laccaic acid, showing comparable binding affinities. Our results indicate that the interaction of carminic acid is stabilized by multiple hydrophobic contacts (for example with Gly41, Lys43, Glu35, Gly36, Tyr39) and two hydrogen bonds formed with aminoacids Lys32 and Val40 (as shown in Figure 1).

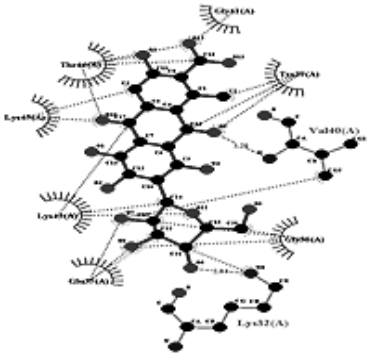


Fig. 1. Representation of carminic acid interaction with alpha synuclein.

Table 2

Carminic acid binding affinities

Mode	Affinity (kcal/mol)	Distance from best mode	
		RMSD l.b.	RMSD u.b.
1	–7.9	0	0
2	–6.8	2.689	4.659
3	–6.4	2.987	9.841
4	–6.4	4.21	7.194
5	–6.3	1.906	4.059
6	–5.8	24.292	27.946
7	–5.8	3.218	5.918
8	–5.7	45.26	50.307
9	–5.7	71.798	76.645

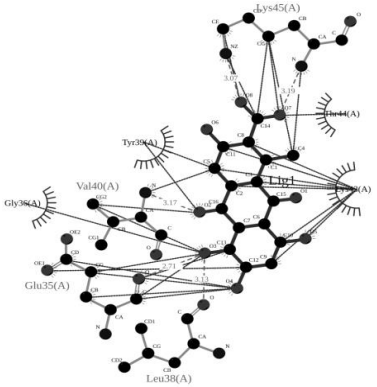


Fig. 2. Representation of kermesic acid interaction with alpha synuclein.

Table 3

Kermesic acid binding affinities

Mode	Affinity (kcal/mol)	Distance from best mode	
		RMSD l.b.	RMSD u.b.
1	–6.5	0	0
2	–6.2	2.015	2.712
3	–6.2	2.159	6.879
4	–6.1	1.416	6.324
5	–5.5	2.722	5.349
6	–5.3	3.307	7.378
7	–5.2	4.006	5.643
8	–5.2	4.778	8.978
9	–5.1	72.033	75.526

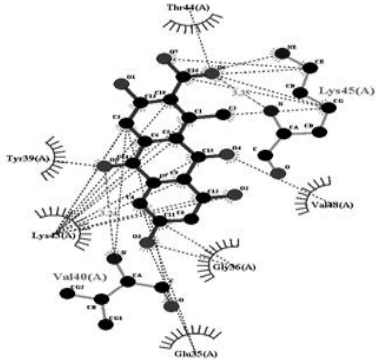


Fig. 3. Representation of laccaic acid interaction with alpha synuclein.

Table 4

Laccaic acid binding affinities

Mode	Affinity (kcal/mol)	Distance from best mode	
		RMSD l.b.	RMSD u.b.
1	-5.8	0	0
2	-5.2	2.167	2.127
3	-5.2	2.133	6.879
4	-5.1	1.811	6.432
5	-4.5	2.587	5.349
6	-4.3	3.398	7.378
7	-4.2	5.033	5.436
8	-4.2	4.993	8.897
9	-4.1	73.056	76.294

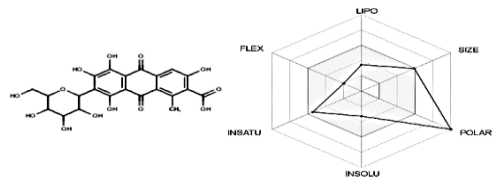
In the case of kermesic acid, the interaction is mediated by multiple hydrophobic contacts (for example with aminoacids Gly36, Gly41) and a hydrogen bond formed with Val40 (shown in Figure 2).

Laccaic acid forms hydrogen bonds with aminoacids Val40 and Lys32 and numerous hydrophobic interactions with adjacent residues (for example Gly41, Tyr39) as shown in Figure 3.

ADME EVALUATION OF CARMINIC ACID

In the case of carminic acid, the partition coefficient (logP) calculations consensus show a negative value, indicating the compound is not lipophilic. Our analysis shows that it has moderate water solubility with low gastro-intestinal absorption and does not permeate the blood-brain-barrier.

Carminic acid is not an inhibitor of the cytochrome P450 enzymes and is predicted as a possible P-glycoprotein 1 substrate. The molecule is not drug-like according to the full set of five predictors used by the SwissADME web-server. Carminic acid is also predicted to be a member of the pan-assay interference compounds class (PAINS), chemical compounds that have a high chance of giving a false positive result in high-throughput screening campaigns by reacting non-specifically with a wide range of biological targets. The compound shows an average synthetic accessibility of 5 (on a scale of 1 to 10, where 1 is easily accessible and 10 is difficult) and is not predicted to be lead-like (Fig. 4).



SMILES	OCC1OC(C(C(C1O)O)O)c1c(O)c2c(c(c1O)O)C(=O)c1c(C2=O)c(C)c(c(1)O)C(=O)O
Physicochemical Properties	
Formula	C22H20O13
Molecular weight	492.39 g/mol
Num. heavy atoms	35
Num. arom. heavy atoms	12
Fraction Csp3	0.32
Num. rotatable bonds	3
Num. H-bond acceptors	13
Num. H-bond donors	9
Molar Refractivity	112.39
TPSA	242.51 Å²
Lipophilicity	
Log $P_{o/w}$ (iLOGP)	-0.87
Log $P_{o/w}$ (XLOGP3)	0.53
Log $P_{o/w}$ (WLOGP)	-1.52
Log $P_{o/w}$ (MLOGP)	-2.95
Log $P_{o/w}$ (SILICOS-IT)	-0.55
Consensus Log $P_{o/w}$	-1.07

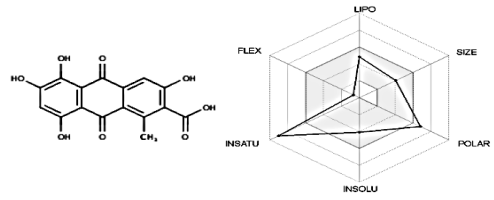
Water Solubility	
Log *S* (ESOL)	-3.28
Solubility	2.57e-01 mg/ml ; 5.22e-04 mol/l
Class	Soluble
Log *S* (Ali)	-5.19
Solubility	3.15e-03 mg/ml ; 6.40e-06 mol/l
Class	Moderately soluble
Log *S* (SILICOS-IT)	-0.62
Solubility	1.19e+02 mg/ml ; 2.42e-01 mol/l
Class	Soluble
Pharmacokinetics	
GI absorption	Low
BBB permeant	No
P-gp substrate	Yes
CYP1A2 inhibitor	No
CYP2C19 inhibitor	No
CYP2C9 inhibitor	No
CYP2D6 inhibitor	No
CYP3A4 inhibitor	No
Log K_p (skin permeation)	-8.93 cm/s
Druglikeness	
Lipinski	No; 2 violations: NorO>10, NHorOH>5
Ghose	No; 2 violations: MW>480, WLOGP<-0.4
Veber	No; 1 violation: TPSA>140
Egan	No; 1 violation: TPSA>131.6
Muegge	No; 3 violations: TPSA>150, H-acc>10, H-don>5
Bioavailability Score	0.11
Medicinal Chemistry	
PAINS	2 alerts: catechol_A, quinone_A
Brenk	2 alerts: catechol, hydroquinone
Leadlikeness	No; 1 violation: MW>350
Synthetic accessibility	5.04

Fig. 4. Various predicted properties for carminic acid (general physicochemical properties, lipophilicity, water solubility, pharmacokinetics, drug-likeness and medicinal chemistry characterization), provided by the SwissADME web-server.

ADME EVALUATION OF KERMESIC ACID

In the case of kermesic acid, logP calculations consensus show a positive value, indicating that the compound is lipophilic. Our analysis shows that it has moderate water solubility, with low gastro-intestinal absorption and does not permeate the blood-brain-barrier. Also, kermesic acid is not an inhibitor of the cytochrome P450 enzymes and is not a possible P-glycoprotein 1 substrate. The molecule is drug-like according to two out of the five predictors used by the SwissADME webserver.

Kermesic acid is also predicted to be a member of the pan-assay interference compounds class (PAINS), chemical compounds that have a high chance of giving a false positive result in high-throughput screening campaigns by reacting non-specifically with a wide range of biological targets. Importantly, the compound shows high synthetic accessibility of 2.79 (on a scale of 1 to 10, where 1 is easily accessible and 10 is difficult) and is predicted to be lead-like (Fig. 5).



SMILES OC(=O)c1c(O)cc2c(c1C)C(=O)c1c(C2=O)c(O)c(cc1O)O

Physicochemical Properties	
Formula	C16H10O8
Molecular weight	330.25 g/mol
Num. heavy atoms	24
Num. arom. heavy atoms	12
Fraction Csp3	0.06
Num. rotatable bonds	1
Num. H-bond acceptors	8
Num. H-bond donors	5
Molar Refractivity	79.77
TPSA	152.36 Å²

Lipophilicity	
Log $P_{o/w}$ (iLOGP)	0.27
Log $P_{o/w}$ (XLOGP3)	2.99
Log $P_{o/w}$ (WLOGP)	1.29
Log $P_{o/w}$ (MLOGP)	-0.52
Log $P_{o/w}$ (SILICOS-IT)	1.52
Consensus Log $P_{o/w}$	1.11

Water Solubility	
Log *S* (ESOL)	-4.08
Solubility	2.78e-02 mg/ml ; 8.41e-05 mol/l
Class	Moderately soluble
Log *S* (Ali)	-5.85
Solubility	4.63e-04 mg/ml ; 1.40e-06 mol/l
Class	Moderately soluble
Log *S* (SILICOS-IT)	-2.68
Solubility	6.93e-01 mg/ml ; 2.10e-03 mol/l
Class	Soluble

Pharmacokinetics	
GI absorption	Low
BBB permeant	No
P-gp substrate	No
CYP1A2 inhibitor	No
CYP2C19 inhibitor	No
CYP2C9 inhibitor	No
CYP2D6 inhibitor	No
CYP3A4 inhibitor	No
Log K_p (skin permeation)	-6.19 cm/s

Druglikeness	
Lipinski	Yes; 0 violation
Ghose	Yes
Veber	No; 1 violation: TPSA>140
Egan	No; 1 violation: TPSA>131.6
Muegge	No; 1 violation: TPSA>150
Bioavailability Score	0.11

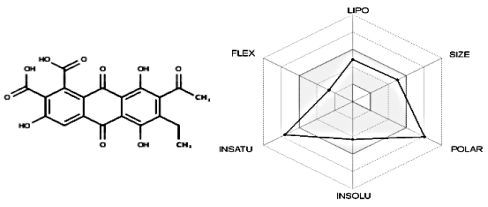
Medicinal Chemistry	
PAINS	2 alerts: catechol_A, quinone_A
Brenk	2 alerts: catechol, hydroquinone
Leadlikeness	Yes
Synthetic accessibility	2.79

Fig. 5. Various predicted properties for kermesic acid (general physicochemical properties, lipophilicity, water solubility, pharmacokinetics, drug-likeness and medicinal chemistry characterization), generated by the SwissADME web-server.

ADME EVALUATION OF LACCAIC ACID

In the case of laccaic acid, logP calculations consensus show a positive value of 1.33, indicating that the compound is lipophilic. It has poor gastro-intestinal absorption and low water solubility, with no permeation of the blood-brain-barrier

and is not an inhibitor of the cytochrome P450 enzymes and is not a possible P-glycoprotein 1 substrate. The molecule is drug-like according to according to two out of the five predictors used by the SwissADME webserver. Laccaic acid is also predicted to be a member of the pan-assay interference compounds class (PAINS), chemical compounds that have a high chance of giving a false positive result in high-throughput screening campaigns by reacting non-specifically with a wide range of biological targets. The compound shows high synthetic accessibility of 3.22 (on a scale of 1 to 10, where 1 is easily accessible and 10 is difficult) and is not predicted to be lead-like (Fig. 6).



SMILES CCc1c(O)c2C(=O)c3cc(O)c(c(c3C(=O)c2c(c1C(=O)C)O)C(=O)O)C(=O)O

Physicochemical Properties	
Formula	C20H14O10
Molecular weight	414.32 g/mol
Num. heavy atoms	30
Num. arom. heavy atoms	12
Fraction Csp3	0.15
Num. rotatable bonds	4
Num. H-bond acceptors	10
Num. H-bond donors	5
Molar Refractivity	99.70
TPSA	186.50 Å²

Lipophilicity	
Log $P_{o/w}$ (iLOGP)	0.28
Log $P_{o/w}$ (XLOGP3)	2.99
Log $P_{o/w}$ (WLOGP)	1.74
Log $P_{o/w}$ (MLOGP)	-0.73
Log $P_{o/w}$ (SILICOS-IT)	2.35
Consensus Log $P_{o/w}$	1.33

Water Solubility	
Log *S* (ESOL)	-4.32
Solubility	1.96e-02 mg/ml ; 4.74e-05 mol/l
Class	Moderately soluble
Log *S* (Ali)	-6.57
Solubility	1.11e-04 mg/ml ; 2.69e-07 mol/l
Class	Poorly soluble
Log *S* (SILICOS-IT)	-3.30
Solubility	2.06e-01 mg/ml ; 4.97e-04 mol/l
Class	Soluble

Pharmacokinetics	
GI absorption	Low
BBB permeant	No
P-gp substrate	No
CYP1A2 inhibitor	No
CYP2C19 inhibitor	No
CYP2C9 inhibitor	No
CYP2D6 inhibitor	No
CYP3A4 inhibitor	No
Log K_p (skin permeation)	-6.70 cm/s

Druglikeness	
Lipinski	Yes; 0 violation
Ghose	Yes
Veber	No; 1 violation: TPSA>140
Egan	No; 1 violation: TPSA>131.6
Muegge	No; 1 violation: TPSA>150
Bioavailability Score	0.11

Medicinal Chemistry	
PAINS	1 alert: quinone_A
Brenk	1 alert: hydroquinone
Leadlikeness	No; 1 violation: MW>350
Synthetic accessibility	3.22

Fig. 6. Various predicted properties for laccaic acid (general physicochemical properties, lipophilicity, water solubility, pharmacokinetics, drug-likeness and medicinal chemistry characterization), generated by the SwissADME web-server.

Our predictions cover a number of important molecular characteristics, spanning lipophilicity and water solubility, which influence both gastro-intestinal (G.I.) absorption and cell membrane penetration of the evaluated compounds, various pharmacokinetic parameters such as the affinity for the cytochrome P complex, essential for drug metabolism and possible drug-drug interaction and blood-brain-barrier penetration (B.B.B.), which is especially relevant in the case of pharmacological intervention in neuronal pathologies. We also evaluate drug-likeness – the similarity between our compounds and other compounds already classified as drugs. Finally, we evaluate the possibility of our compounds could interfere with various assays (PAINS-pan-assay interference compounds), their lead- likeness – the probability of the compounds to have therapeutic value, but

with suboptimal structural characteristics, that may require further optimization and their synthetic accessibility, an indication of how facile the compounds can be obtained by synthetic chemistry routes.

Considering our results, we can conclude that kermesic acid is the most promising molecule that can be used for the inhibition of alpha synuclein aggregation with potential clinical benefit – it shows the most promising ADME profile out of the three investigated candidates, with the highest logP, no affinity to cytochrome P450 and is not a P-glycoprotein 1 substrate. It also has excellent synthetic accessibility and is shown to be both drug and lead-like. Also, considering that all three molecules investigated in the present study show no blood-brain-barrier penetration, which would be paramount for their potential clinical applications, the fact that kermesic acid shows lead-like properties is very important, as it would most likely require additional structural optimization.

CONCLUSIONS

Our *in silico* investigation shows that kermesic acid, an anthraquinone derivative, has good potential for the inhibition of alpha synuclein aggregation, a hallmark of Parkinson's disease. Further, we show that kermesic acid has an advantageous absorption, distribution and excretion profile and drug-like and lead-like properties. We surmise that further experimental investigation is necessary, to confirm and possibly extend our predictions.

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