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An *In Silico* Approach to Identify Solubilising Agents for Improving the Antidiabetic Activity of Verbenone

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ABSTRACT

Natural products are used globally to manage diseases, including diabetes mellitus. Verbenone, a component of *Daucus carota* seed, has been shown to exhibit antidiabetic activity; however, its low solubility due to its nonpolar nature limits its use. Enhancing verbenone's solubility will significantly improve its bioactivity as an antidiabetic agent. This study aims to employ an *in silico* approach to identify a soluble agent that enhances the antidiabetic activity of verbenone. The structures of verbenone, acarbose, and the identified chemical compounds (L-arginine, gelucire, lysine, eudragit, ethylene glycol, and poloxamer), all with the potential to increase solubility by more than 10-fold, were downloaded in SDF format from the PubChem database. The binding energies were computed using Autodock vina screening software with human salivary α-amylase, α-glucosidase, and pyruvate dehydrogenase kinase. At the same time, physicochemical properties and ADME parameters were predicted using the SwissADME server. The binding energies for L-arginine and gelucire ranged from -4.6 to -6.1 Kcal/mol compared with acarbose (-6.5 to -7.4 Kcal/mol) and verbenone (-5.7 to -6.1 Kcal/mol), respectively. Ethylene glycol and poloxamer have higher binding energies of -2.3 to -3.2 Kcal/mol, respectively. The ligands interacted with different amino acid residues. L-arginine, gelucire, and ethylene glycol are very soluble and do not violate Lipinski's rule of 5, with high gastrointestinal absorption except for L-arginine. Moreover, the compounds do not cross the blood-brain barrier. L-arginine, gelucire, lysine, and eudragit may be suitable polymers to enhance the antidiabetic activity of verbenone when used as solubilisers in diabetes treatments.

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Introduction

For many decades, natural products have played a critical role in the management and treatment of various diseases, including diabetes mellitus, one of the most prevalent and challenging metabolic disorders globally [1]. Diabetes mellitus is characterised by chronic hyperglycemia, resulting from defects in insulin secretion, insulin action, or both, leading to severe complications such as neuropathy, cardiovascular diseases, nephropathy, and retinopathy [2]. Effective management of diabetes typically involves lifestyle modifications and pharmacotherapy to maintain blood glucose levels within a normal range [3].

Verbenone, a compound known as 4,6,6-trimethylbicyclo[3.1.1]hept-3-en-2-one, a component found in the seeds of *Daucus carota*, has been extensively studied and has attracted significant attention for its potential as an antidiabetic and antioxidant [4,5]. Despite its promising therapeutic potential, the clinical application of verbenone is limited by its poor solubility, which affects bioavailability and therapeutic efficacy [6]. The nonpolar nature of verbenone contributes to its low aqueous solubility, posing a major challenge to its use as an effective antidiabetic agent. However, improving verbenone's solubility is crucial to enhancing its bioactivity. Various strategies,

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including the use of solubilising agents and formulation techniques, have been employed to address the solubility issues of poorly soluble drugs such as ibuprofen, cyclosporine, diazepam, itraconazole, griseofulvin, carbamazepine, and fenofibrate [7]. The use of solubilising agents, in particular, offers a promising approach to enhancing the solubility and bioavailability of verbenone without altering its chemical structure. Based on our previous research, we proposed in silico methods to identify novel soluble molecular agents for verbenone that could enhance its antidiabetic efficacy. Unlike prior studies, which focused solely on empirical testing, this computational approach has the potential to accelerate drug discovery, predict bioactivity with greater precision, and support the development of safer, more effective diabetes therapies. Thus, our use of molecular docking, a powerful computational technique that predicts the preferred orientation of a small molecule (ligand) when bound to a target protein (receptor), thereby forming a stable complex [8]. It is a widely used technique in drug discovery and development to elucidate the molecular interactions between potential drug candidates and their biological targets. This study employed molecular methods to evaluate interactions between verbenone and various solubilising agents, identifying soluble agents that enhance verbenone's biological activity.

Materials and methods

Ligand preparation

The structures of verbenone, acarbose, and selected chemical compounds (L-arginine, gelucire, lysine, eudragit, ethylene glycol, and poloxamer) with the potential to increase solubility by more than 10-fold [7] were retrieved in SDF format from www.pubchem.ncbi.nlm.nih.gov (PubChem database) [9]. These retrieved compounds were converted to the mol2 chemical format using Open Babel [10]. This was followed by setting the appropriate torsion centre, then saving the files in pdbqt format.

Protein preparation

Carbohydrate-metabolising protein structures, including those of human salivary α-amylase (1SMD), α-glucosidase (3WY1), and pyruvate dehydrogenase kinase (4MP2), were obtained from www.rcsb.org (Protein Databank) [11] under the assigned PDB codes, respectively. Existing ligands and water molecules were removed, and polar hydrogen atoms were introduced using the AutoDock version 4.2 program, Scripps Research Institute. This was followed by merging nonpolar hydrogen atoms, and polar hydrogen atoms were added to each enzyme before saving in the dockable PDBQT format.

Molecular docking

The study used AutoDock Vina version 4.212 to evaluate molecular interactions between chemical compounds and protein targets. The position for the docking grid in the workflow was set at centre x, y, z 8.366, 58.672, 19.066, and size xyz at 40 for 1SMD, centre x, y, z -9.407, -12.188, 18.395 and size xyz at 40 for 3WY1, while centre x, y, z 4.476, 9.171, -39.372 and size xyz at 40 for 4MP2. The docking protocol was validated by checking the RMSD of redocked known ligands in protein binding pockets. The binding energy of the inhibitor (PA1) in pyruvate dehydrogenase kinase 4MP2 was -7.7 Kcal/mol, with a favourable docking pose. This was repeated for all proteins for validation. The superior binding compounds with high binding affinities for the selected diabetes proteins were recorded and visualised using Discovery Studio Visualizer, Version 21.1, BIOVIA, 2021.

Physicochemical and absorption distribution, metabolism, excretion, and toxicity (ADMET) properties

Compounds with the highest binding energies were screened for physicochemical and ADMET properties using SwissADME (http://www.swissadme.ch/index.php) [13] and the ADMETlab website (http://admet.scbdd.com/calcpre/index). Drug-likeness of each of the ligands was screened using “Lipinski’s rule of five” (Lipinski, 2000).

Results

The binding energies computed using human salivary α-amylase, α-glucosidase, and pyruvate dehydrogenase kinase for L-arginine and gelucire ranged from -4.6 to -6.1 Kcal/mol compared with acarbose (-6.5 to -7.4 Kcal/mol) and verbenone (-5.7 to -6.1 Kcal/mol), respectively (Table 1). Ethylene glycol and poloxamer have higher binding energies of -2.3 to -3.2 Kcal/mol, respectively. The ligands interacted with different amino acid residues (Table 2). Verbenone interacted with the dyad amino acid via non–hydrogen–bonding interactions (HIS299 and TYR^{62}), similar to acarbose. However, acarbose interacted with GLN^{8} and ARG^{398} for human salivary α-amylase. The binding pocket of verbenone and

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chemical compounds interacting with various amino acid residues is depicted in Figures 1-3. Physicochemical properties and ADME parameters were predicted using the SwissADME server, indicating that L-arginine, gelucire, and ethylene glycol are very soluble and do not violate the Lipinski rule of 5 (Table 3), with high gastrointestinal (GI) absorption, except for L-arginine (Table 4). Moreover, the compounds do not cross the blood-brain barrier.

Table 1. Binding energies of verbenone and chemical compounds against diabetic enzymes

S/N	Compounds	CIDs	Binding energy (Kcal/mol)		
			1SMD	3WY1	4MP2
Ref.	Acarbose	9811704	-7.4	-6.5	-6.8
1	Ethylene Glycol	174	-2.9	-3.2	-3.2
2	Eudragit	6658	-3.7	-4.2	-3.8
3	Gelucire	135169966	-4.6	-6.1	-5.0
4	L-arginine	6322	-4.9	-5.8	-5.1
5	Lysine	5962	-4.5	-4.9	-4.6
6	Poloxamer	24751	-2.3	-2.8	-3.1
7	Verbenone	29025	-5.7	-6.1	-5.7

Human salivary α-amylase (1SMD), α -glucosidase (3WY1), Pyruvate dehydrogenase kinase (4MP2)

Table 2. Verbenone and chemical compounds interacting with amino acid residues with diabetic enzymes (distance Å)

Protein	Compounds	Hydrogen bonding–related Interactions	Non-Hydrogen bonding–related Interactions
1SMD	Verbenone	---	HIS^{299} (4.73), TYR^{62} (3.52)
	L-arginine	ARG^{303} (2.92), ILE^{312} (1.97), ALA^{310} (2.73), ASN^{301} (2.43)	ARG^{346} (3.44), GLY^{304} (3.72)
	Gelucire	GLN^{63} (3.29)	ALA^{198} (4.54), TRP^{59} (4.80), TRP^{58} (5.36), TYR^{62} (4.09), LEU^{162} (4.42)
	Ethylene Glycol	ASN^{301} (2.55), ILE^{312} (1.81)	GLY^{309} (2.87)
	Acarbose	THR^{6} (2.10), SER^{4} (2.76), ASN^{5} (3.30), ARG^{10} (3.25), GLN^{7} (2.82), SER^{289} (2.89), ASP^{402} (2.24)	GLN^{8} (3.42), ARG^{398} (2.79)
3WY1	Verbenone	---	PHE^{166} (4.49)
	L-arginine	GLU^{271} (2.49), ASP^{202} (2.20), ASP^{333} (2.20), ARG^{400} (3.38), GLY^{228} (2.59)	---
	Gelucire	ASP^{202} (3.01), ASP^{333} (3.33), HIS^{332} (3.22), ARG^{400} (3.29)	PHE^{166} (4.61), ILE^{146} (4.77), PHE^{297} (5.17), TYR^{65} (3.67), TYR^{389} (4.98), VAL^{334} (4.11)
	Ethylene Glycol	ASP^{172} (2.74), PHE^{206} (2.30)	---
	Acarbose	GLU^{396} (2.07), GLU^{231} (2.00)	ASN^{301} (2.86), MET^{302} (3.29), PHE^{397} (3.26), PRO^{230} (2.19), PRO^{230} (2.99)
4MP2	Verbenone	---	LEU^{252} (4.90), ALA^{356} (4.47), LEU^{346} (4.48)
	L-arginine	ALA^{356} (3.23), ASP^{290} (2.35), THR^{354} (2.61)	---
	Gelucire	---	LYS^{287} (4.18), VAL^{357} (4.49), PHE^{347} (3.96), LEU^{297} (4.94)
	Ethylene Glycol	---	ASP^{290} (2.86), ASP^{355} (2.49)
	Acarbose	GLY^{294} (2.61), GLU^{262} (2.58), GLY^{292} (3.07)	ASN^{255} (2.70), GLY^{327} (3.54), LEU^{330} (2.00), LEU^{303} (3.77), SER^{263} (3.60)

Acarbose = Reference compound

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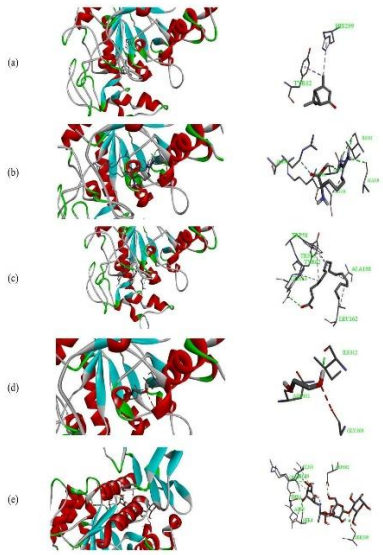

Figure 1: Binding pocket of verbenone and chemical compounds interacting with amino acid residues with human salivary α-amylase. (a) Verbenone (b) L-arginine (c) Gelucire (d) Ethylene Glycol (e) Acarbose

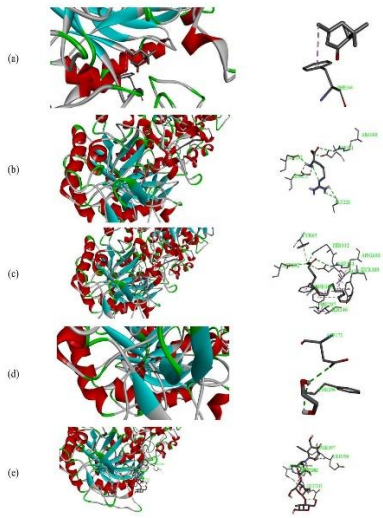

Figure 2: Binding pocket of verbenone and chemical compounds interacting with amino acid residues with α-glucosidase. (a) Verbenone (b) L-arginine (c) Gelucire (d) Ethylene Glycol (e) Acarbose

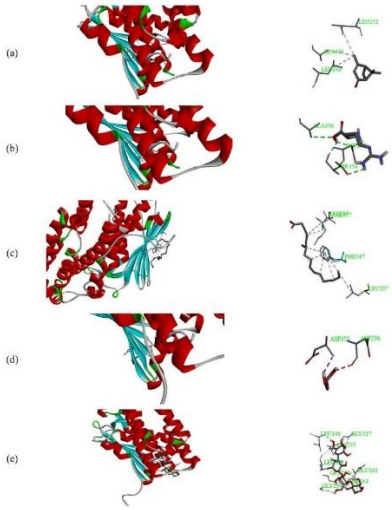

Figure 3: Binding pocket of verbenone and chemical compounds interacting with amino acid residues with pyruvate dehydrogenase kinase. (a) Verbenone (b) L-arginine (c) Gelucire (d) Ethylene Glycol (e) Acarbose

Discussion

Verbenone has demonstrated promising antidiabetic and antioxidant activities *in vitro*, *in vivo,* and *in silico*[4,5]. However, the solubility of verbenone has hindered its therapeutic usage as an antidiabetic compound. Thus, the search for a soluble agent to improve its solubility continues. Gelucire, Molecular interactions suggest that L-arginine, lysine, and Eudragit possess features that enable favourable molecular interactions with human salivary α-amylase, α-glucosidase, and pyruvate dehydrogenase kinase via hydrogen bonding, electrostatic attraction, or hydrophobic interactions. These interactions may enhance the compounds' ability to modulate enzymatic activity by stabilising enzyme–ligand complexes or influencing substrate accessibility. The agent interacted with various amino acids in protein-binding pockets, such as glycine and tyrosine, similar to those reported for type 2 diabetes mellitus inhibitors [15]. However, ethylene glycol and poloxamer showed lower binding affinities than the reference compound, acarbose. α-amylase, an enzyme located

Table 3. Physicochemical properties and Lipinski filter analysis of verbenone and chemical compounds

Properties	Verbenone	L-arginine	Gelucire	Ethylene Glycol
Formula	$C_{10}H_{14}O$	$C_6H_{14}N_4O_2$	$C_{11}H_{12}N_6O_3$	$C_2H_6O_2$
Molecular weight (g/mol)	150.22	174.20	276.25	62.07
No. heavy atoms	11	12	20	4
No. aromatic heavy atoms	0	0	6	0
No. rotatable bonds	0	5	5	1
No. H-bond acceptors	1	4	7	2
No. H-bond donors	0	4	2	2
Molar Refractivity	45.42	44.54	79.00	14.05
Lipinski violation	No	No	No	No

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Table 4. ADME and SAR of verbenone and chemical compounds

Sub-properties		Verbenone	L-arginine	Gelucire	Ethylene Glycol
Water solubility	Log *S* (ESOL)	-2.18	2.05	-1.77	0.70
	Solubility (mg/mL)	1.00×10^{0}	1.95×10^{4}	4.67×10^{0}	3.10×10^{2}
	Class	Soluble	Highly soluble	Very soluble	Highly soluble
Pharmacokinetics	Skin permeation (Log K_p, cm/s)	-5.63	-10.34	-7.62	-7.64
	P-gp substrate	No	No	No	No
	GI absorption	High	Low	High	High
	CYP3A4 inhibitor	No	No	No	No
	CYP2D6 inhibitor	No	No	No	No
	CYP2C9 inhibitor	No	No	No	No
	CYP2C19 inhibitor	No	No	No	No
	CYP1A2 inhibitor	No	No	No	No
	BBB permeant	Yes	No	No	No
Lipophilicity	Log $P_{o/w}$ (iLOGP)	2.19	0.27	1.68	0.70
	Log $P_{o/w}$ (XLOGP3)	2.23	-4.19	0.52	-1.36
	Consensus Log $P_{o/w}$	2.25	-2.04	-0.08	-0.70

BBB = Blood-Brain Barrier

Discussion

Verbenone has demonstrated promising antidiabetic and antioxidant activities *in vitro*, *in vivo,* and *in silico* [4,5]. However, the solubility of verbenone has hindered its therapeutic usage as an antidiabetic compound. Thus, the search for a soluble agent to improve its solubility continues. Gelucire, Molecular interactions suggest that L-arginine, lysine, and Eudragit possess features that enable favourable molecular interactions with human salivary α-amylase, α-glucosidase, and pyruvate dehydrogenase kinase via hydrogen bonding, electrostatic attraction, or hydrophobic interactions. These interactions may enhance the compounds' ability to modulate enzymatic activity by stabilising enzyme–ligand complexes or influencing substrate accessibility. The agent interacted with various amino acids in protein-binding pockets, such as glycine and tyrosine, similarly to reported type 2 diabetes mellitus inhibitors [15]. However, ethylene glycol and poloxamer showed lower binding affinities than the reference compound, acarbose. α-amylase, an enzyme located in the oral cavity and intestine, is the first major enzyme that initiates the hydrolysis or breakdown of carbohydrates, more specifically starch, into disaccharides, while α-glucosidase acts on the glycosidic bonds in disaccharides and hydrolyses them to monosaccharides [16]. Both α-amylase and α-glucosidase contribute to postprandial hyperglycaemia; therefore, inhibiting these enzymes can slow glucose digestion, reduce glucose absorption, and assist in the management of diabetes [16]. Verbenone interacted with the evaluated enzymes. Aside from that, the observed interaction could be improved by gelucire, L-arginine, lysine, and Eudragit. Improving the solubility of the test compound by several folds [7].

The physicochemical properties are important parameters for screening potential drug candidates. Verbenone presented favourable pharmacokinetic properties. Some of which had molecular weights less than 500 g/mol and a few heavy atoms with one hydrogen-bond acceptor. Similarly, L-arginine, gelucire, and ethylene glycol exhibited comparable physicochemical properties and no Lipinski violations, indicating strong drug potential. Lipinski's rule indicates drug-likeness for oral bioavailability for small molecules [14,17]. Compounds with two or more violations of the rule are said to be poorly soluble/permeable. Verbenone, L-arginine, gelucire, and ethylene glycol violated none of the rules, thus indicating their potential for drug formulation.

Verbenone is known for its poor solubility, as indicated by its solubility in the ADME test. Verbenone has a relatively high skin permeation rate, indicating its permeability through the skin. It is not a P-gp substrate, implying it may not be actively pumped out of cells, facilitating its intracellular activity [18]. Verbenone has high GI absorption, making it suitable for oral administration. Moreover, verbenone does not

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inhibit major CYP enzymes, minimising the risk of drug-drug interactions; it is versatile, with good pharmacokinetic properties across various routes of administration, and can cross the blood-brain barrier. Similar properties were observed with arginine, gelucire, and ethylene glycol, except that arginine and ethylene glycol showed higher solubility than verbenone and gelucire. Moreover, these compounds are highly soluble, have favourable ADME properties, and may not outcompete verbenone but rather be complementary.

Gelucire has good potential for absorption and bioavailability, moderate skin permeation, and high GI absorption, making it suitable for oral delivery. It does not inhibit CYP enzymes, reducing the potential for metabolic drug interactions, and does not cross the BBB, limiting its use for CNS-related treatments [19]. Meaning gelucire is ideal for oral administration and has a low risk of drug interactions. L-arginine has a high Log S value, promising solubility, and excellent absorption and bioavailability. L-arginine has poor skin permeation. It is not a P-gp substrate. It has low GI absorption, which might limit its effectiveness in oral formulations. Furthermore, ethylene glycol has good absorption characteristics and low lipophilicity, reducing the risk of metabolic interactions and making it suitable for various formulations.

The limitations of the current report include the use of purely theoretical methods (MM-PBSA/MM-GBSA) without molecular dynamics simulations, as well as reliance on *in vitro* or *in vivo* experimental studies. Based on these findings, we are currently using wet-laboratory procedures to validate the claims reported in this study.

Conclusion

Verbenone demonstrates significant potential for *in silico* antidiabetic activity through inhibition of human salivary α-amylase, α-glucosidase, and pyruvate dehydrogenase kinase, exhibiting strong binding interactions with key amino acid residues in these enzymes. Although its binding energies and profiles are not as extensive as acarbose, verbenone's favourable pharmacokinetic profile, including high gastrointestinal absorption, blood-brain barrier permeation, and moderate lipophilicity, absence of CYP inhibition, and P-gp substrate properties, could be improved by its co-formulations with promising candidates for experimental solubility (gelucire, L-arginine, lysine, and eudragit). Thus, these compounds may be suitable as polymers to enhance the solubility of verbenone and its biological activities when used as solubilisers in diabetes treatments. The results require further validation, as the current study is purely computational and requires experimental confirmation.

Competing interest

The authors declare that they have no competing interests.

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