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Research Article

In silico analysis of bioactive compounds from ethanolic amber extract targeting BACE1: Potential implications for glucose metabolism

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Abstract

Ethanolic amber extract contains a mixture of terpenoids and other compounds with potential biological properties. This study aimed to explore *in silico* the interaction of selected constituents of amber extract on β-site amyloid precursor protein cleaving enzyme 1 (BACE1) since BACE1 inhibition, in addition to its influence on neurodegeneration, may affect insulin receptor levels in the liver, thereby contributing to the control of glucose homeostasis. By using the CB-Dock2 web-based software for protein-ligand interactions, auto blind docking on BACE1 was performed with compounds present in the amber extract, particularly terpenes (isopimaric acid methyl ester, borneol, camphor, 2-fenchanol, m-cymene and communic acid) and succinic acid derivatives. All six terpenes, as well as succinic acid and its ester, displayed an appreciable binding affinity, as documented by the respective Vina scores. In particular, isopimaric acid methyl ester and communic acid showed the strongest interactions. Computational ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) prediction allowed to evaluate the provisional properties of the compounds. The results obtained and presented here suggest that an interaction of terpenes and succinates, present in the ethanolic amber extract, with BACE1 protein is possible, potentially counteracting the loss of insulin receptor. Therefore, this finding may suggest a new approach to glycemic control.

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Amber, BACE1, diabetes, terpenes, *in silico* molecular docking, computational ADMET.

1. Introduction

In the process of identification of new pharmacological agents, an exciting method consists of exploring historical herbal and medicine texts which provide traditional uses of medicines [1, 2]. In this context, amber, a fossilized resin, besides its scientific relevance in palaeontology, and its widespread use as an ornament since antiquity, has been traditionally believed to be a remedy for many ailments, as documented by ancient pharmacopoeias that report a variety of formulations [3-5]. Most of the described remedies do not have pharmacological validation, however, in recent times, attempts have been made to undertake a scientific approach on amber-based products, and several activities of amber-based products have been reported, such as antioxidant, antimicrobial and anti-inflammatory

activities [6]. More recently, attention has been focused on the activity of amber extract against neurotoxicity [7]. Specifically, an ethanol-derived Baltic amber extract was found to inhibit the neurotoxic effects of β-amyloid *in vitro* by different mechanisms, such as a decrease in β-site amyloid precursor protein cleaving enzyme 1 (BACE1) [8], which is responsible for the first step in amyloid precursor protein proteolysis, leading to toxic β-amyloid peptide production, typical of neurodegeneration in Alzheimer's disease. This observed inhibitory effect of amber extract is relevant because the search for BACE1 inhibitors has raised wide interest as a therapeutic target in the pharmaceutical field [9-12], although with limited impact in the pharmacological clinical use. On another side, BACE1 has been found to be responsible for the regulation of the number of insulin receptors in the liver, playing a key role in the control of glucose homeostasis [13-16]. According to Meakin et al.'s results [13,14], in diabetes condition, where BACE1 determines an increased cleavage of the liver insulin receptor, the inhibition of the enzyme can partially counteract the loss of the insulin receptor. This finding therefore discloses a new target for the control of diabetes, although developed BACE1 inhibitors, such as LY2811376, besides an appreciable potency [17], have revealed potential toxicity in humans [18]. Most recently, new *in vitro* investigations in mouse C2C12 cells demonstrated the effects of ethanolic amber extract on glucose control [19], thereby opening new frontiers in the study of the antidiabetic effects of amber bioactive compounds.

Based on these observations, it is possible to hypothesize that the components of ethanolic amber extract, acting through BACE1 inhibition, may prove useful for the control of diabetes, in addition to potentially protecting against neurotoxic β-amyloid peptides. Amber is a complex mixture of secondary metabolites of fossilized plant origin with predominance of polymers, which has been extensively investigated [20-22]. In particular, Baltic amber (succinite) is considered a mixture of low molecular mass constituents with a prevailing polymeric structure made of terpenoids, and succinic acid as copolymerizing agent or succinate ester [6, 21, 23-25]. Table S1 (*supplementary Table S1)* illustrates the numerous chemical entities that have been described as constituents of Baltic amber, according to the current literature. The amber extract obtained with ethanol contains monomers or oligomers dissolved by depolymeric transesterification or hydrolytic reactions of the polymeric resin structure, or from clathrates included in the amber [6]. The most abundant phytoconstituents in the ethanolic amber extract are terpenes, particularly, borneol, isopimaric acid methyl ester, camphor, 2-fenchanol and m-cymene [26]. Baltic amber contains 3-8% succinic acid [27-29], but the amount present in an amber extract as a free dicarboxylic acid is very low, as it occurs combined as a succinate ester with terpenoids [26, 30, 31], or as mono- or di-methylester [21, 26]. Similarly, labdane-type (communic acid) polymers are among the constituents of Baltic amber, showing very low solubility [21].

Therefore, the aim of the present study was to evaluate the potential interaction between the main bioactive compounds of an ethanolic amber extract and the target protein BACE1, using an *in silico* molecular docking approach. A positive result would explain the reported activity in the control of diabetes [19]. Prediction of pharmacokinetic properties of the selected ligands was also performed.

2. Materials and methods

2.1. Molecular docking

Molecular docking was performed using the CB-Dock2 web-based software (https://cadd.labshare.cn/cb-dock2/index.php) designed for cavity detection and protein-ligand blind docking in the field of computer-aided drug discovery (CADD). The details of the procedure have been published [32, 33]. Given the three-dimensional (3D) structure of a protein and ligand, the software predicts the binding sites and affinity. The software is an improved version of the AutoDock Vina-based procedure. The search for the interaction between a protein and ligand is performed on the entire protein surface in order to detect the most probable binding sites and poses (blind docking). The web server requires the input of a protein file in PDB format, and a ligand file in suitable format (SDF,

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MOL2 or MOL). The crystal structure of BACE1 (*Homo sapiens*) [34] at pH 7.0 was retrieved from the RCSB Protein Data Bank (PDB ID: 2ZHV; DOI: 10.2210/pdb2ZHV/pdb) (https://www.rcsb.org/structure/2ZHV).

Optimized structure of each ligand was obtained from PubChem [35] (https://pubchem.ncbi.nlm.nih.gov/) as SDF file. Chemical formulas were created using the web-based PubChem Sketcher V2.4 tool (https://pubchem.ncbi.nlm.nih.gov//edit3/index.html) from SMILES strings.

Using CB-Dock2, it was possible to obtain a rank for the potential binding sites, expressed as the Vina score (kcal/mol). The Vina score [36] represents a measure of binding energy [Gibbs free energy difference between the energy of the final state (protein-ligand complex) and the initial state (protein and ligand far away)], where a high negative score indicates a strong binding affinity.

2.2. Principal component analysis

Principal Component Analysis (PCA) was used to assess the relationship among Vina score, molecular weight and Log P (log of the partition coefficient) of the investigated compounds. A biplot was obtained, presenting the scores and loadings. The angle between the vectors in the biplot indicates correlation between the variables. A small angle suggests that the variables are positively correlated, an angle of 90 degrees indicates that the variables are not correlated, and an angle near 180 degrees suggests that the variables are negatively correlated.

2.3. Prediction of pharmacokinetic properties

Prediction of pharmacokinetic properties of the selected ligands was assessed by means of pkCSM web tool (https://biosig.lab.uq.edu.au/pkcsm/prediction) [37] and entering the structure as SMILES format. The software allows to estimate most relevant pharmacokinetic parameters for Absorption, Distribution, Metabolism, Excretion and Toxicity (ADMET). The theory underlying the prediction of parameters is available at https://biosig.lab.uq.edu.au/pkcsm/theory and described in Pires et al. [37]. The pkCSM workflow uses the general properties of molecule (among which: molecular characteristics, toxicophores and pharmacophore) as well as the graph-based structural signatures, to train and test machine learning-based predictors, to offer a range of estimated ADMET properties.

3. Results

In order to evaluate the possible interaction of amber extract phytoconstituents with the selected target, BACE1 protein, the most abundant constituents present in the ethanolic extract were selected from the literature. According to the current data, the following terpene compounds were considered: borneol, isopimaric acid methyl ester, camphor, 2-fenchanol and m-cymene [26] which have been reported in ethanolic amber extract in the range of 17-5%. Moreover, communic acid, dimethyl succinate and monomethyl succinate were also considered, as typical constituents of succinite [21, 26]. The chemical structures of the compounds of interest are presented in Fig. 1. The figure also shows the structure of known inhibitors of BACE1, such as hydroxyethylamine (HEA) compound 3 [38], LY2886721 and CNP-520 (Umibecestat) [39].

A preliminary exploration of possible BACE1 binding sites was performed with the structure-based search cavities procedure in CB-Dock2. As shown in Fig. 2, five binding pockets (C1–C5) were identified in the BACE1 structure, and their respective characteristics were summarized in terms of cavity volume, center coordinates, and dimensions. These pockets were ranked by volume, with pocket C1 being the largest, exhibiting a volume of 1528 Å^3 and approximate dimensions of 21 × 22 × 26 Å (x, y, z). The binding pockets were further analyzed for overlap with the catalytic residues Asp32 and Asp228, which define the active site of BACE1, as reported by Shimizu et al. [34]. Pocket C1 includes both of these residues, indicating that it corresponds to the catalytic active site. Additional key residues lining C1 include Thr72, Ser35, Ile110, and Tyr71, all of which have previously been associated with ligand binding, further supporting the relevance of this site. The remaining pockets (C2–C5) are spatially distinct and likely represent alternative allosteric binding regions, the functional roles of which remain to be evaluated. A detailed visualization and residue composition of all five pockets are shown in Fig. 2. The auto blind docking results of the selected ligands with the protein BACE1 structure (PDB code: 2ZHV) are reported

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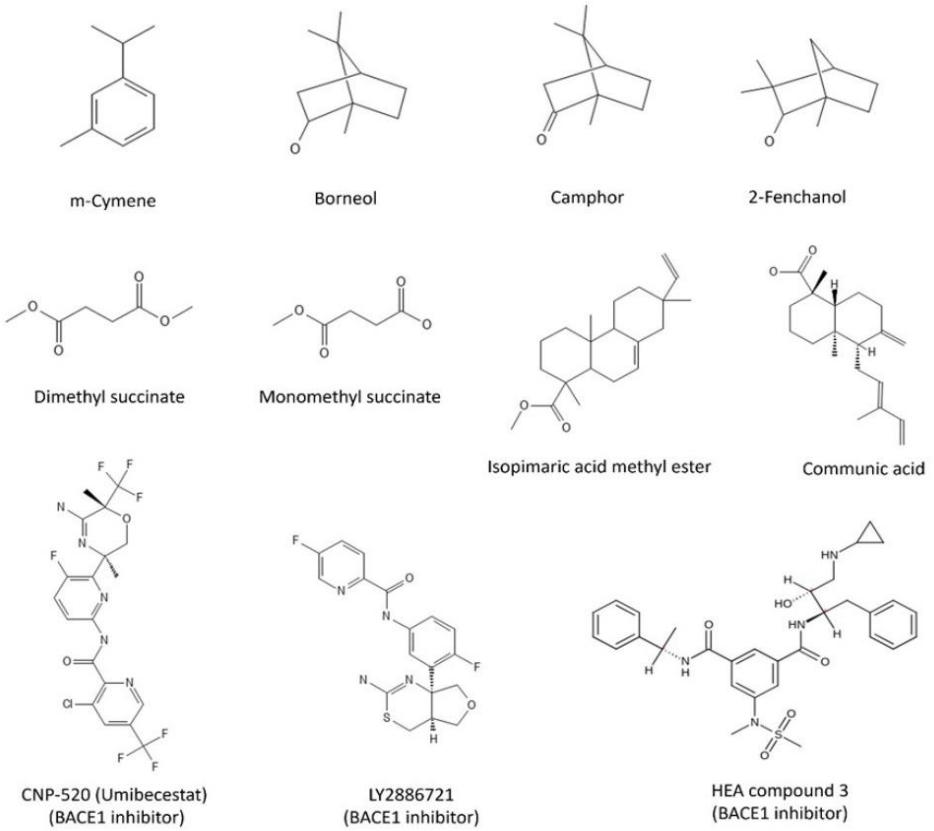

Figure 1. Chemical structure of the considered constituents of amber extract, and of three BACE1 inhibitors.

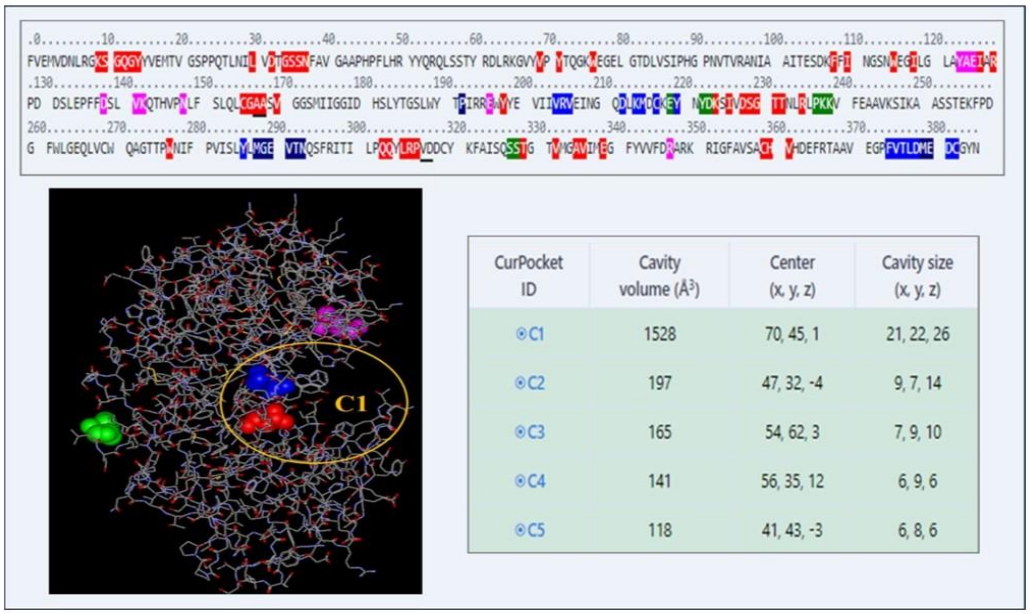

Figure 2. Pockets determined in the BACE1 protein structure, using the search cavity procedure of CB-Dock2. The upper part of the figure presents the residues of the BACE1 protein sequence detected in each cavity (indicated with C and consecutive number), color coded (red: C1; blue: C2; pink: C3; green: C4; dark blue: C5). Pocket C1 includes the catalytic residues Asp32 and Asp228 of the BACE1 aspartic protease, as reported by Shimizu et al. [34], and is the site where both known inhibitors and components of the ethanolic amber extract bind. Left: 3D representation of the BACE1 protein structure with the C1 cavity highlighted. The catalytic aspartic acids are colored red (Asp32) and blue (Asp228) within the C1 catalytic cavity. The C-terminal region of the protein is shown in magenta and the N-terminal in green. Right: table showing the characteristics of the pockets, sorted by volume.

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Table 1. Docking results (Auto BlindDock) for the selected compounds typical of amber extract, on BACE1 (PDB code: 2ZHV). Vina score represents the rank for the potential binding sites, indicated for the identified C1-C5 pockets. In parentheses is the determined pocket size.

Compound	PubChem CID	Molecular weight	Log P	Vina score, kcal/mol				
				C1 (1528 Å^3)	C2 (197 Å^3)	C3 (165 Å^3)	C4 (141 Å^3)	C5 (118 Å^3)
Isopimaric acid methyl ester	519327	316.48	5.2946	-6.8	-6.1	-5.4	-5.2	-5.1
Communic acid	637125	302.46	5.3723	-6.3	-5.9	-5.5	-5.4	-5.6
m-Cymene	10812	134.22	3.1184	-5.2	-4.2	-4.1	-5.0	-3.8
Borneol	64685	154.25	2.1935	-4.7	-4.4	-4.0	-3.1	-3.5
Camphor	2537	152.24	2.4017	-4.7	-4.5	-4.0	-3.1	-3.5
2-Fenchanol	15406	154.25	2.1935	-4.7	-4.9	-4.3	-3.4	-3.6
Dimethyl succinate	160419	146.14	0.1126	-4.6	-3.9	-3.4	-3.2	-3.1
Monomethyl succinate	77487	132.12	0.0242	-4.5	-4.4	-3.6	-3.6	-3.4
HEA compound 3 (BACE1 inhibitor)	---	578.74	3.0274	-8.0	-7.3	-6.5	-6.0	-7.3
LY2886721 (BACE1 inhibitor)	49837968	390.42	2.5153	-7.7	-8.2	-6.8	-6.5	-6.4
CNP-520 (Umibecestat) (BACE1 inhibitor)	88602735	513.80	4.4638	-8.2	-7.8	-6.9	-6.7	-7.1

in Table 1. All six terpenes displayed an appreciable binding affinity, as well as succinic acid and its ester, as documented by the respective Vina scores particularly for isopimaric acid methyl ester, which had the highest score among the terpenes. Also communic acid yielded a Vina score similar to that of isopimaric acid. Notably, affinity was observed for all five pockets identified. To contextualize the docking results, three known BACE1 inhibitors were included as reference compounds, with Vina scores resulted as –8.2, –8.0, and –7.7 kcal/mol (Table 1). These values indicate stronger predicted binding affinities compared to the amber extract constituents, whose scores ranged from –6.8 to –4.5 kcal/mol. Therefore, the amber compounds showed weaker binding *in silico* relative to the positive controls.

A specific close-up evaluation of the BACE1 interaction with isopimaric acid methyl ester is presented in Fig. 3, which depicts the main pocket C1 with the binding of the ligand and the contact residues. Isopimaric acid methyl ester was predicted to bind within pocket C1 of BACE1, forming interactions with multiple residues in and around the catalytic site (Fig. 3). Notably, it engaged both catalytic residues, Asp32 and Asp228, suggesting direct involvement in active site binding. The ligand also contacted several residues lining the binding cleft, including Gly11, Gln12, Gly13, Leu30, Gly34, Ser35, and Pro70. Additional interactions were observed with residues involved in substrate recognition and ligand stabilization, such as Tyr71, Thr72, Gln73, and Ile110. Hydrophobic contacts were formed with nonpolar residues including Leu30, Ile110, Trp115, and Ile118, while polar and charged residues such as Lys75, Lys107, Arg128, and Arg235 may contribute to electrostatic or hydrogen bonding interactions. These findings suggest a stable binding conformation involving both the catalytic core and peripheral residues of the active site. Similarly, communic acid was predicted to bind within pocket C1, establishing interactions with the catalytic residues Asp32 and Asp228 (Fig. 4). The ligand engaged multiple residues in the active site region, including Gly11, Gln12, Gly13, Leu30, Gly34, and Ser35. Notable contacts were also observed with Tyr71, Thr72, and Gln73, which are involved in the ligand recognition. Hydrophobic interactions were likely contributed by Ile110, Trp115, Ile118, and Tyr198, while polar and charged residues such as Lys75, Arg128, Arg235, and Glu339 may form hydrogen bonds or electrostatic contacts. These interactions suggest that communic acid adopts a binding orientation that stabilizes its position in close proximity to the catalytic dyad, supporting its potential as an active site-directed ligand.

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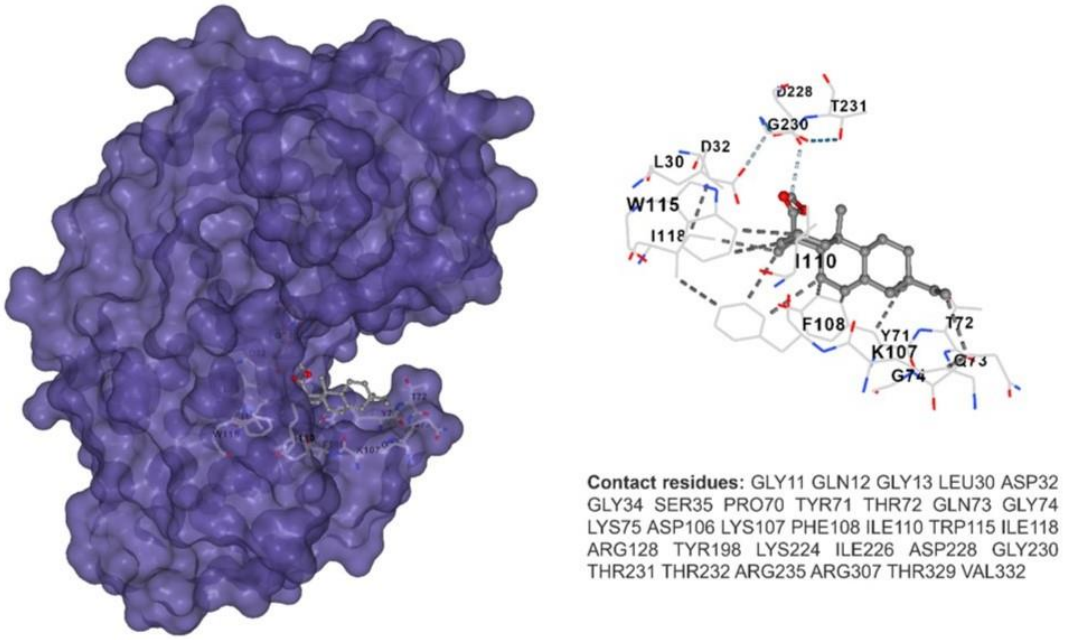

Figure 3. Left: Binding of isopimaric acid methyl ester to the C1 pocket of the BACE1 protein. Right: 3D schematic representation of the BACE1 protein highlighting the C1 pocket and its interaction with the ligand. Inset: contact residues involved in ligand binding within the C1 pocket. Compared to the inhibitors shown in Figure 6 of Shimizu et al. [34], isopimaric acid methyl ester occupies a smaller region of the catalytic pocket, resulting in fewer molecular interactions and consequently a less negative binding energy.

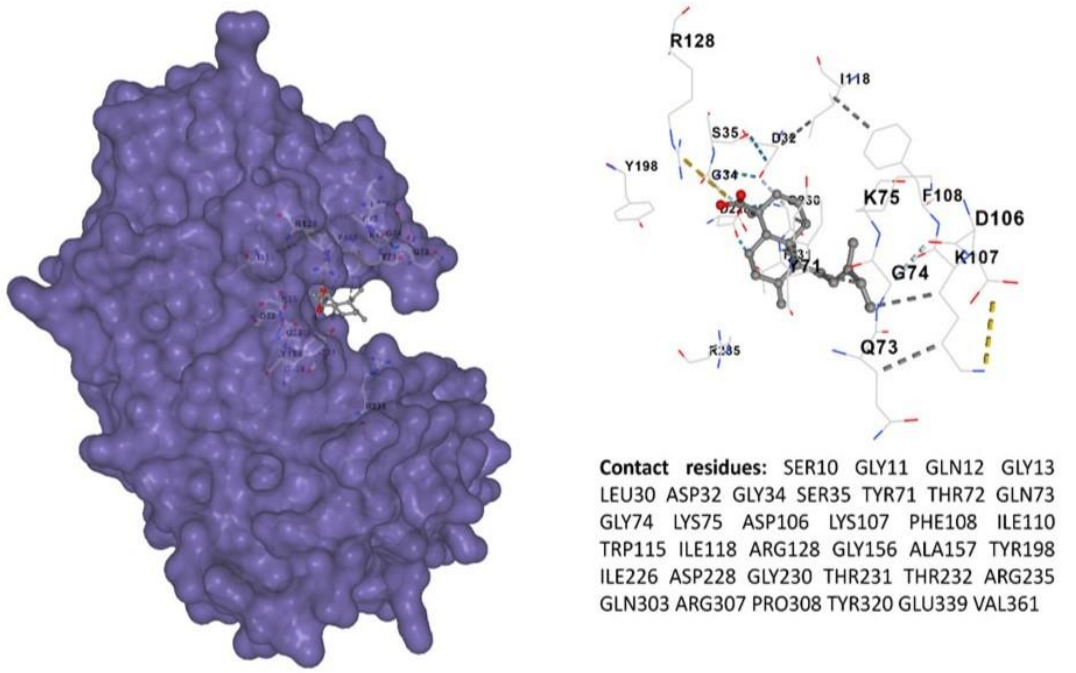

Figure 4. Left: Binding of communic acid to the C1 pocket of the BACE1 protein. Right: 3D schematic representation of the BACE1 protein highlighting the C1 pocket and its interaction with the ligand. Inset: contact residues involved in ligand binding within the C1 pocket. Compared to the inhibitors illustrated in Figure 6 of Shimizu et al. [34], communic acid occupies a more limited region of the catalytic pocket, leading to fewer molecular interactions and a correspondingly less negative binding energy.

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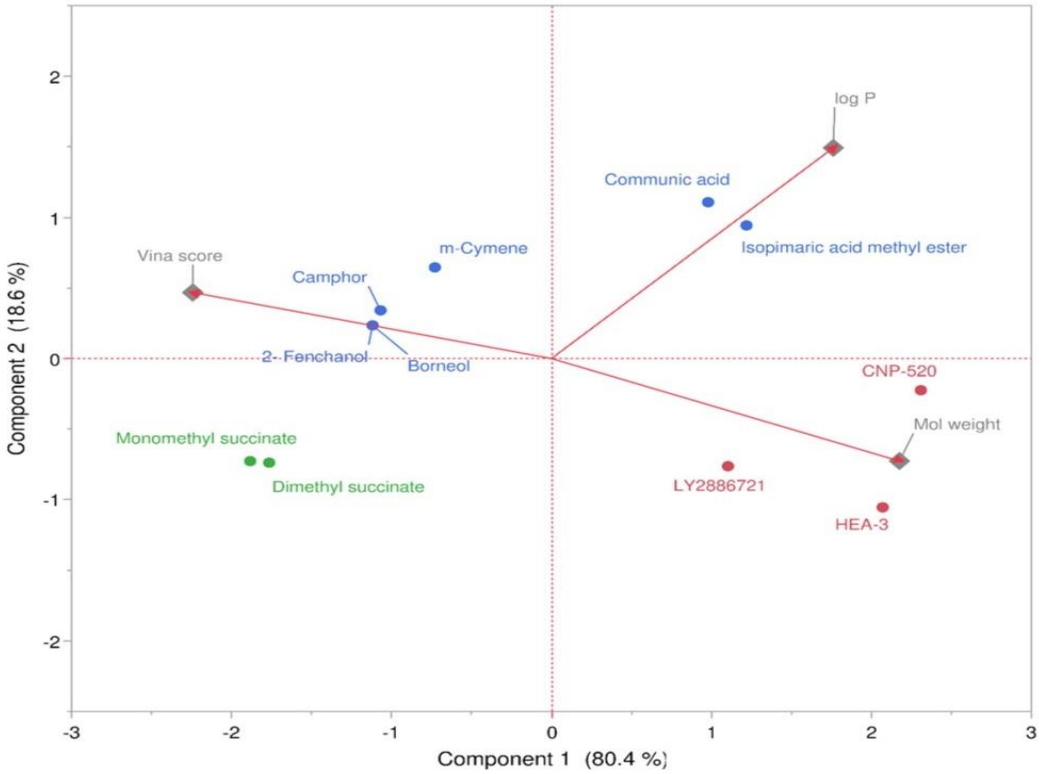

Figure 5. Biplot of PCA analysis, presenting the score for the considered ligands (points with label), and the loadings components (red vectors). Vina score refers to the value obtained at pocket C1.

Together, these results indicate that the high-affinity ligand binding site corresponds to the catalytic active site of BACE1, reinforcing the hypothesis that the selected compounds may exert inhibitory effects by directly interfering with enzymatic activity.

Succinic acid esters, namely monomethyl and dimethyl esters, showed a binding affinity that is lower than that of isopimaric acid methyl ester and of communic acid, but were still evaluable in the five cavities of the protein (Table 1). The inclusion of known BACE1 inhibitors as reference compounds provided a benchmark for interpreting the docking results. As expected, these inhibitors showed stronger predicted binding affinities (Vina scores below –7.5 kcal/mol) than the amber extract constituents. While the amber compounds did not outperform the reference inhibitors in terms of binding strength, some displayed moderate affinities, suggesting potential as BACE1-interacting molecules.

An attempt to correlate the docking characteristics with Lipinski's parameters, such as molecular weight and Log P, was performed by means of principal component analysis (PCA) multivariate technique. As shown in the biplot of Fig. 5 isopimaric acid methyl ester and communic acid are located in a unique quadrant, distinct from the other ligands, suggesting a peculiar property in comparison to the other terpenes and succinates. Overall, the amber extract bioactive components appear to have a peculiar behaviour, with respect to reference BACE1 inhibitors. The relative direction of the three loading vectors suggests that the Vina score is inversely correlated with the molecular weight of the compounds, while Log P seems less related to the other parameters, since the angle between the vectors is closer to 90 degrees.

As a complement of the *in silico* ligand binding evaluation, a computational analysis of the pharmacokinetic properties of the compounds was conducted based on their ADMET profiles. Table 2 presents the panel of ADMET properties. In general, some properties of the compounds are favourable, although the water solubility of terpenes, in particular those of isopimaric acid methyl ester and communic acid, is low, which conditions the absorption-distribution process.

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Table 2. Prediction of pharmacokinetic (PK) properties (ADMET) of the selected ligands in amber extract, and of three reference BACE1 inhibitors.

ADMET predicted characteristics			Predicted value											
PK property	Model name	Unit of measure	Isopimaric acid methyl ester	Communic acid	m-Cymene	Borneol	Camphor	2- Fenchanol	Dimethyl succinate	Monomethyl succinate	HEA 3 (BACE1 inhibitor)	LY2886721 (BACE1 inhibitor)	CNP-520 (BACE1 inhibitor)
Absorption	Water solubility	log mol/L	-6.147	-3.854	-4.098	-2.462	-2.895	-2.648	0.446	0.113	-4.232	-3.311	-5.054
Absorption	Caco2 permeability	log Papp in 10^{-6} cm/s	1.764	1.746	1.526	1.484	1.499	1.486	1.16	0.496	0.701	1.442	0.74
Absorption	Intestinal absorption (human)	% Absorbed	96.935	98.49	93.643	93.439	95.965	93.288	100	84.385	78.697	93.823	87.579
Absorption	Skin Permeability	log Kp	-2.735	-2.73	-1.206	-2.174	-2.002	-2.034	-3.075	-2.735	-2.735	-2.876	-2.883
Absorption	P-glycoprotein substrate	Yes/No	No	No	No	No	No	No	No	No	Yes	Yes	No
Absorption	P-glycoprotein I inhibitor	Yes/No	Yes	No	No	No	No	No	No	No	Yes	No	No
Absorption	P-glycoprotein II inhibitor	Yes/No	No	No	No	No	No	No	No	No	Yes	No	No
Distribution	VDss (human)	log L/kg	0.335	-0.827	0.724	0.337	0.331	0.346	-0.45	-0.9	0.629	0.447	-0.728
Distribution	Fraction unbound (human)	Fu	0	0.047	0.156	0.486	0.459	0.45	0.721	0.731	0.029	0.173	0.202
Distribution	BBB permeability	log BB	0.638	0.065	0.475	0.646	0.612	0.658	-0.27	-0.364	-0.988	-0.897	-1.014
Distribution	CNS permeability	log PS	-1.803	-1.746	-1.397	-2.331	-2.158	-2.16	-2.947	-2.993	-3.236	-3.073	-3.136
Metabolism	CYP2D6 substrate	Yes/No	No	No	No	No	No	No	No	No	No	No	No
Metabolism	CYP3A4 substrate	Yes/No	Yes	Yes	No	No	No	No	No	No	Yes	Yes	Yes
Metabolism	CYP1A2 inhibition	Yes/No	No	No	Yes	No	No	No	No	No	No	No	No
Metabolism	CYP2C19 inhibition	Yes/No	No	No	No	No	No	No	No	No	Yes	No	No
Metabolism	CYP2C9 inhibition	Yes/No	No	Yes	No	No	No	No	No	No	No	No	No
Metabolism	CYP2D6 inhibition	Yes/No	No	No	No	No	No	No	No	No	No	No	No
Metabolism	CYP3A4 inhibition	Yes/No	No	No	No	No	No	No	No	No	Yes	No	No
Excretion	Total Clearance	log ml/min/kg	0.78	1.23	0.249	1.035	0.109	0.946	0.824	0.78	1.014	0.068	0.058
Excretion	Renal OCT2 substrate	Yes/No	No	No	No	No	No	No	No	No	No	No	No
Toxicity	AMES toxicity	Yes/No	No	No	No	No	No	No	No	No	No	No	No
Toxicity	Max. tolerated dose (human)	log mg/kg/day	-0.539	-0.282	0.896	0.577	0.473	0.5	0.975	1.27	-0.614	-0.632	0.116
Toxicity	Max. tolerated dose (human)	mg/kg/day	0.29	0.52	7.87	3.78	2.97	3.16	9.44	18.62	0.24	0.23	1.31
Toxicity	hERG I inhibitor	Yes/No	No	No	No	No	No	No	No	No	No	No	No
Toxicity	hERG II inhibitor	Yes/No	No	No	No	No	No	No	No	No	Yes	No	No
Toxicity	Oral Rat Acute Toxicity (LD50)	mol/kg	1.915	1.789	1.83	1.707	1.653	1.662	1.978	1.785	2.545	2.576	2.394
Toxicity	Oral Rat Chronic Toxicity (LOAEL)	log mg/kg bw/day	2.082	2.472	2.339	1.877	1.981	1.89	2.458	2.677	3.187	2.018	0.971
Toxicity	Oral Rat Chronic Toxicity (LOAEL)	mg/kg bw/day	120.78	296.48	218.27	75.34	95.72	77.62	287.08	475.34	1538.15	104.23	9.35
Toxicity	Hepatotoxicity	Yes/No	Yes	Yes	No	No	No	No	No	No	Yes	Yes	Yes
Toxicity	Skin Sensitisation	Yes/No	Yes	No	Yes	Yes	Yes	Yes	No	No	No	No	No
Toxicity	*Tetrahymena pyriformis* toxicity	log µg/L	1.377	0.311	0.446	0.175	0.233	0.303	-0.698	0.019	0.285	0.346	0.30
Toxicity	Minnow toxicity	log mM	-0.633	-0.692	0.794	1.727	1.458	1.634	2.36	2.744	2.479	2.46	2.916

This finding is confirmed by the Log P value (Table 1), which is the log of the n-octanol-water partition coefficient, indicating lipophilicity; indeed, the value is quite elevated for the considered terpenes. In the case of isopimaric acid methyl ester and communic acid, Log P slightly exceeds the value of 5, that is

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considered a limit for the druglikeness of a chemical compound [40].

4. Discussion

The present study attempted to evaluate the potential of some selected constituents of a Baltic amber alcoholic extract as possible pharmacological modulators of BACE1 activity, a protein target that has been considered relevant not only in the management of Alzheimer's disease, but also in glucose metabolism. Considering that BACE1 increases the cleavage of the liver insulin receptor, it is possible that inhibition of the enzyme activity may be beneficial for glucose control [13-15], also on the basis of *in vitro* and *in vivo* data by Hettich et al. [41], who demonstrated that the antidiabetic drug metformin can decrease BACE1 protein expression by interfering with mRNA.

Several effective BACE1 inhibitors have already been identified [39], and entered even in clinical studies, however, almost all molecules with BACE1 inhibitor activity have failed clinical expectations, because of the development of significant toxicity. The search for new chemical entities in this field is still a challenging issue. The results obtained here suggest that an interaction of terpenes and succinates, present in ethanolic amber extract, with BACE1 protein is possible, thereby counteracting the loss of insulin receptors.

Although the reference BACE1 inhibitors exhibited stronger binding affinities for the active site (AutoDock Vina scores between −8.2 and −7.7 kcal/mol), compounds from amber extract showed moderate predicted affinities, with isopimaric acid methyl ester scoring −6.8 kcal/mol and communic acid −6.3 kcal/mol. Despite their lower scores, both natural compounds bound at the catalytic site and interacted with key residues, including Asp32, Asp228, Thr72, Ile110, and Tyr71, suggesting a binding orientation similar to that of known inhibitors. The reduced affinity may indicate weaker interactions or a suboptimal fit, however, these compounds of natural origin may still serve as scaffolds for structural optimization or derivative development to improve potency.

Based on the docking results, the amber extract constituents exhibited a range of predicted affinities for the BACE1 catalytic pocket, with Vina scores ranging from –6.8 to –4.5 kcal/mol. To better interpret these differences, the compounds can be grouped as strong binders (scores ≤ –6.0: isopimaric acid methyl ester and communic acid), moderate binders (–6.0 to –5.0: m-cymene), and weak binders (> –5.0: borneol, camphor, 2-fenchol, dimethyl succinate, and monomethyl succinate). While this classification offers a preliminary indication of relative inhibitory potential, it is important to note that docking scores are predictive and do not directly measure enzymatic inhibition. Therefore, further *in vitro* validation is essential to confirm these findings and determine the actual inhibitory strength.

The present observations may suggest a new additional approach in the scenario of glycemic control and are in agreement with a study conducted in a rat model of Type-2 Diabetes (T2D), reporting that succinic acid and its esters may be effective in the control of T2D as insulinotropic agents [42, 43], and also confirm the findings that succinic acid, together with oleic acid, synergistically mitigates symptoms of T2D in streptozotocin-induced diabetic rats [44]. Moreover, succinate has previously been proven as a metabolic stimulus-coupling signal in the mechanism of proinsulin biosynthesis promoted by glucose [45]. Also, a recent *in vitro* study on adipocytes [46] indicated that an amber extract can modulate lipid and glucose metabolism. In this context, the inclusion of a terpene moiety as "tails" in the structure of glitazars has recently been suggested as an approach in medicinal chemistry for the development of new analogs for the treatment of metabolic syndrome [47].

The present study is only an exploratory attempt to identify the possible role of some selected amber extract constituents on a promising new target, BACE-1, involved in glucose metabolism regulation. The PCA correlations revealed that the Vina score was inversely correlated with the molecular weight of the compounds, suggesting that larger molecules have a higher binding affinity (larger negative docking score) to the identified C1 pocket of the BACE-1 molecule. Conversely, for the whole set of compounds considered, Log P appears to be less related to docking affinity, although for the amber extract

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components, higher affinities are found for the more lipophilic compounds, i.e. isopimaric acid methyl ester and communic acid. However, these two molecules appear to have a better affinity for the pocked C1 of the target enzyme. It is of note that both compounds clustered together in the PCA space, indicating that they have similar physicochemical and docking properties. These observations may become of interest in future research pointing to the optimizing of the binding efficacy of molecules, as in the case of natural origin, on the selected target BACE-1.

On the pharmacokinetic point of view, a limitation is that isopimaric acid methyl ester and communic acid have a high Log P value (~5.3), at the limit of drug-likeness, which may argue against a direct role of the compounds in drug development. Additionally, the predicted water solubility values across the tested amber extract compounds ranged from −6.147 to +0.113 log mol/L, indicating a broad spectrum of solubility, from very poorly soluble to moderately soluble. Isopimaric acid methyl ester and communic acid, in particular, fall within the poorly soluble range (−6.147 and −3.854, respectively), which may restrict oral bioavailability despite their high predicted intestinal absorption (~97%). Although such physicochemical properties do not fully align with classical oral drug-likeness criteria (e.g., Lipinski's Rule of Five), many natural products with similar profiles have been successfully developed into therapeutic agents. These compounds may still represent promising bioactive scaffolds, with their pharmacokinetic limitations potentially addressable through medicinal chemistry optimization or formulation approaches aimed at enhancing their solubility and absorption. Moreover, hepatotoxicity predicted for isopimaric acid methyl ester and communic acid, together with their provisional values of maximal tolerated dose, limit the possible direct application in therapy.

The potential inhibitory activity of amber extract constituents on BACE1 remains theoretical and reflects a preliminary effort to explore the effects of the extract on lipid and glucose metabolism [41]. Specific *in vitro* assays are needed to confirm BACE1 inhibition. Although this study focused on compound–BACE1 interactions, BACE1's role in insulin receptor processing and glucose homeostasis—particularly in the liver—offers a promising direction for future research on metabolic disorders linked to neurodegeneration.

5. Conclusions

The present findings indicate that some terpenoid constituents of an ethanolic amber extract may interact with a biological target, i.e. BACE1, which is involved in glucose metabolism control and may open new future perspectives for pharmaceutical and pharmacological exploration.

It is important to note that the *in silico* prediction of the binding of amber extract constituents to the protein BACE1 does not indicate an absolute inhibitory effect on enzyme activity, but it may suggest a possible interaction that deserves further investigation. The degree of ligand binding to BACE1 and its patho-physiological relevance must be verified by *in vitro* and *in vivo* evaluations. Computational ADMET prediction suggests that the provisional properties found for the compounds, although promising, must be carefully evaluated to determine appropriate dosing, limit toxicity and identify possible practical applications.

Supplementary material

Table S1.

Supplementary material related to this article can be found online at https://leafletpub.com/images/articles File/ supplementary.1749722077.pdf

Authors' contributions

Conceptualization, data curation, formal analysis, methodology, software, writing–original draft, writing – review and editing, E.R.; Conceptualization, data curation, methodology, writing – review and editing, G.Z.; Conceptualization, data curation, writing – review and editing, G.S.

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Availability of data and materials

All data will be made available on request according to the journal policy.

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Conflicts of interest

The authors have no competing interests to declare that are relevant to the content of this article.

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