Kragujevac J. Sci. 46 (2024) 59-71
doi: 10.5937/KgJSci2400000M

UDC 661.163.6:678.048:615.276:591.481
Original scientific paper

IN SILICO APPROACH IN THE DEVELOPMENT OF STRUCTURAL ANALOGUES OF RESVERATROL WITH IMPROVED DISTRIBUTION IN THE CENTRAL NERVOUS SYSTEM

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(Received Jul 19, 2023; Accepted November 03, 2023)

ABSTRACT. Resveratrol exerts neuroprotective effects that are not only due to anti-inflammatory and antioxidant activity but also by launched biogenesis via sirtuin-1 (SIRT1) protein that protects cells from oxidative stress. Resveratrol has low bioavailability due to low aqueous solubility and rapid metabolism. To improve the pharmacological profile, one of the strategies is the structural modification and selecting an analogue that would activate SIRT1 and improve pharmacokinetics. The aim was to examine the neuroprotective potential of resveratrol analogues through the analysis of the binding affinity to the sirtuin-1 receptor and with improved permeability through the blood-brain barrier. 15 of them have a higher affinity for the target. Four analogues are characterized by better properties including both higher permeability and higher binding affinity for SIRT1 compared to resveratrol. The selected compounds are assumed to have better bioavailability and CNS distribution, and further studies are proposed to confirm their effects and use as neuroprotective agents.

Keywords: molecular docking; resveratrol; sirtuin-1; neuroprotector.

INTRODUCTION

Resveratrol is a widely recognized biologically active compound that is naturally synthesized by plants that have been subjected to an infectious agent or ionizing radiation (Fig. 1). Renaud and de Lorgeril were the pioneers in linking wine polyphenols, including resveratrol, to potential health benefits associated with regular and moderate wine consumption, a phenomenon known as "the French paradox." Resveratrol has gained growing scientific attention, resulting in extensive research into its biological activity and numerous publications on the subject. Resveratrol possesses a diverse array of biological properties, encompassing antioxidant, cardioprotective, neuroprotective, anti-inflammatory, and anti-cancer effects (KALANTARI and DAS, 2010). Resveratrol plays multiple neuroprotective roles

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in various neurodegenerative disorders, including Alzheimer's, Huntington's, and Parkinson's disease. The protective effects of resveratrol extend beyond its anti-inflammatory and antioxidant properties; it has been demonstrated to enhance mitochondrial function and biogenesis through the Sirtuin-1 (SIRT1) protein. This mechanism helps counteract the detrimental effects induced by oxidative stress. Resveratrol diminishes cholinergic neurotransmission, lowers brain neurotrophic factor expression, and mitigates oxidative stress. Additionally, it enhances the clearance of β-amyloid peptides and reduces neuronal apoptosis (SALEHI *et al*., 2018).

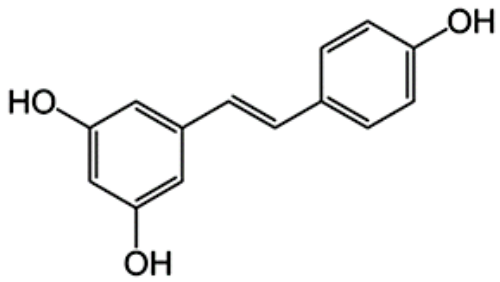

Figure 1. Chemical structure of trans-resveratrol

Sirtuins are evolutionarily preserved proteins that employ nicotinamide adenine dinucleotide (NAD+) as a co-substrate in their enzymatic reactions. The human sirtuin family comprises seven proteins (SIRT1-7), with SIRT1 being the most conserved and distinctive member. SIRT1, particularly in the brain, notably in the hypothalamus, plays a crucial role in regulating systemic energy balance and circadian rhythm. Furthermore, it has been discovered to facilitate advantageous outcomes in neurological disorders. SIRT1 is extensively expressed in the adult brain and predominantly localized within neuronal nuclei. Nevertheless, it is also detected in glial cells in postmortem human brain tissue, as well as in neural stem cells, microglia, and astrocytes in cultured environments (KORONOWSKI and PEREZ-PINZON 2015). Within the hypothalamus, the central hub for homeostasis, SIRT1 mRNA exhibits high expression in various nuclei, underscoring its vital role in regulating metabolic status within the brain. Apart from its physiological functions in the hypothalamus, SIRT1 is reported to provide neuroprotection in cases of neurological dysfunctions. In this paper, our focus will be on elucidating the roles of SIRT1 in the brain concerning the safeguarding against neurodegenerative disorders (XU *et al*., 2018).

The basic mechanisms of neurodegeneration include an increase in neuroinflammation, damage to mitochondria, and oxidative stress. It has been shown in the literature that sirtuin hyperactivity can reduce these negative outcomes both *in vivo* and *in vitro* due to its neuroprotective role. In the pathology of Alzheimer's disease, SIRT1 deacetylates substrates in favor of the non-amyloidogenic pathway or acts directly on Ab and Tau proteins. Molecular studies have shown that SIRT1 activation prevents the accumulation of Ab plaques and tau pathologies by regulating the ADAM10 gene (ELIBOL and KILIC, 2018).

Resveratrol can bind to SIRT1 at its N-terminal end and this interaction increases SIRT1 activity about twice, although this effect is achieved at high resveratrol concentrations. The main limitation of resveratrol is its low bioavailability due to its low water solubility and rapid metabolism to glucuronidated and sulfonated conjugated forms. As a consequence, a high oral dose of Resveratrol (3 or 5 g) in humans is required to achieve concentrations close to the active concentration described in animal models. The positive side of resveratrol as a neuroprotective biofactor is its ability to cross the blood-brain barrier in animal models. Resveratrol exhibits some interesting properties as a potential neuroprotective agent (primarily its action on SIRT1), but its mode of application and poor pharmacokinetics

require careful evaluation (ALBANI *et al*., 2010). After oral application to humans, 75% of resveratrol is absorbed, mainly by passive diffusion. However, oral bioavailability is low (<1%) due to rapid and extensive metabolism in the intestine and liver. These levels are maintained even after a repeated or increased dose (SERGIDES *et al*., 2016).

In recent times, various approaches have been employed to enhance the bioaccessibility of resveratrol. These approaches encompass Sericin nanoparticles, solid lipid nanoparticles, colloidal mesoporous silicon dioxide nanoparticles, and galactosylated PLGA nanoparticles. Nevertheless, the application of these formulations in clinical practice is constrained due to the high costs associated with the technology and the absence of pertinent pharmacokinetic data concerning their ability to penetrate the brain. Therefore, there is an evident requirement for an alternative formulation capable of enhancing the oral bioavailability of resveratrol and effectively permeating the blood-brain barrier (XIONG *et al*., 2020). Therefore, to improve the pharmacological profile of resveratrol, one of the strategies involves structural modification and selection of analogues that would activate the SIRT1 protein and have neuroprotective effects, which would have improved pharmacokinetic characteristics, which is the subject of research in this paper.

In recent times, *in silico* methods have become indispensable in the drug discovery process. This is primarily due to their ability to influence the entire drug development process, enabling the identification and discovery of new potential drugs while substantially cutting down costs and saving time. Furthermore, Computer-aided drug design (CADD) approaches play a crucial role in minimizing the need for experimental animal testing in vivo. They aid in designing safer drugs, repurposing known drugs, and assisting medical chemists at every stage, including design, discovery, development, and data optimization, throughout the drug discovery process. Conventional methods for drug discovery, on the one hand, entail costly and random testing of synthesized compounds or natural products. On the other hand, computational procedures can be highly diverse, necessitating interdisciplinary study and the application of computer science to strategically design effective drugs that have the potential for commercial availability (BROGI *et al*., 2020).

The working hypothesis is based on the assumption that *in silico* techniques, from the group of chemically related compounds, chemical entities with improved physicochemical and pharmacokinetic characteristics can be identified, which could be further investigated *in vitro* and *in vivo* studies as potential pharmacological agents. The objective of the study was to investigate the neuroprotective potential of structural analogues of the polyphenolic compound resveratrol by analyzing the binding affinity to the sirtuin-1 (SIRT1) receptor. Also, given the poor pharmacokinetic properties of resveratrol that limit its therapeutic use, the objective of the paper was to identify structural analogues of resveratrol with enhanced permeability across the blood-brain barrier. Based on the data on the binding affinity of resveratrol analogues to SIRT1 and their physicochemical and pharmacokinetic characteristics determined by modern computational techniques, a conclusion will be drawn on which compounds should be included in the further stages of the study, i.e., which have the greatest potential to achieve neuroprotective activity after application in biological systems.

MATERIALS AND METHODS

Protein and ligands preparation

The three-dimensional (3D) crystal structure of human sirtuin-1 (SIRT1) in a complex with resveratrol (PDB code: 5BTR) (CAO *et al*., 2015), which has been determined by X-ray diffraction at a resolution of 3.2 Å, was retrieved from the Protein Data Bank (BERMAN *et al*., 2000). ZINC database was used for screening of ligand structures (IRWIN *et al*., 2012). The

structure of resveratrol was imported in the standard SMILES format and the structural similarity search method was performed to retrieve the related compounds and analogues. The search parameters were set at 80% similarity, which resulted in the identification of 56 resveratrol analogues.

Molecular docking analysis

Docking studies were performed using Molegro Virtual Docker (MVD) software, version 6.0 (THOMSEN and CHRISTENSEN, 2006). MVD is a widely applied docking program in the drug development process due to its high reliability, based on the application of differential evolution algorithms, which takes into account both the energy of the intermolecular interaction between the protein and ligand and the energy of the intramolecular interaction of the ligand itself. The scoring function, the most energetically favorable orientation of the studied ligand when interacting with the target macromolecule is based on a piecewise linear function involving both electrostatic interactions and hydrogen bond formation (SINGH and KONWAR, 2013). Analogues of resveratrol in mol2 format and SIRT1 protein in pdb format were imported into the MVD program. All solvent molecules were removed from the protein structure. The docking protocol was validated by "redocking" the co-crystalized ligand resveratrol at the active site of SIRT1. The Root Mean Square Distance (RMSD) of the docked ligand was within the reliable range of 2 Å, so it was verified after docking protocol that resveratrol could interact with the crystal structure of 5BTR similarly to the preexisting co-crystallized resveratrol.

Potential ligand binding sites for SIRT1 were predicted using MVD, and the cavity containing the co-crystallized resveratrol, was selected as the active site for further docking analyses. Different orientations of the ligands were searched and ranked based on their energy scores. All docking calculations were carried out using the grid based MolDock score (GRID) function with a grid resolution of 0.30 Å. The binding site on the receptor was defined as a spherical region that encompasses all protein atoms within 10 Å of the crystallographic ligand molecule. MolDock SE was used as a search algorithm and the number of runs was set to 10. A population size of 50 and a maximum iteration of 1500 was used for parameter settings. The number of generated poses was 5, and the best pose of each compound was selected for the subsequent ligand-protein interaction energy analysis.

Assessment of drug-like properties of resveratrol analogues

In order to examine the possibility of using the identified analogues of resveratrol as medicinal substances, as well as to compare their properties with resveratrol itself, the so-called resveratrol was performed. drug-like analyses that, based on set criteria, i.e., calculated molecular descriptors, determine whether a substance is a candidate for further testing as a potential drug. *In silico* preliminary screening of drug-likeness properties of resveratrol and its analogues was performed using DruLiTo (Drug-Likeness Tool) software. In addition to the Lipinski rule as the most commonly used filter for assessing drug-likeness properties of chemical entities, the Weber filter, Ghose filter, CMC-50 filter, and Quantitative Estimate of Drug-likeness (QED) were also applied within this software (KERNS and DI, 2008).

In silico prediction of CNS distribution

Molecular descriptors relevant to membrane permeability for resveratrol and its analogues were predicted using VolSurf+ software, version 1.0.4 (Molecular Discovery Ltd, UK) (CRUCIANI *et al.*, 2000). LogP octanol/water (LOGP n-Oct) and logP cyclohexane/water (LOGP c-Hex) as the logarithms of the partition coefficient between n-octanol or cyclohexane and water were computed via a linear equation derived by fitting GRID-derived atom type to

experimental data. Besides, the logarithm of the blood-brain barrier distribution (LgBB) was calculated and the values lower than -0.5 indicate poor brain permeation.

Permeation through BBB was also predicted using the SwissADME web tool, which includes the Brain or Intestinal EstimateD permeation method (BOILED-Egg evaluation) (DAINA *et al*., 2017). 'BOILED-Egg' is a graphical evaluation of human intestinal absorption as a function of the position of the small molecule in the WLOGP vs. TPSA plot. The white region of the 'BOILED-Egg' represents the high probability of passive absorption in the gastrointestinal tract, and the yellow region (yolk) represents the high probability of brain penetration (yolk and white areas are not mutually exclusive).

Eventually, interactions of resveratrol and its analogues with P-glycoprotein (P-gp) were predicted using PgpRules Server (WANG *et al*., 2019). PgpRules predict substrate and inhibitory properties of compounds towards P-gp. The prediction is based on the classification and regression tree (CART) algorithm. The rules are calculated based on PubChem 2D fingerprints and RDKit descriptors.

Principal Component Analysis (PCA)

The values of size/shape and physicochemical molecular descriptors for each compound in the data set were calculated using VolSurf+ software. Determined descriptors of the tested resveratrol and its analogues were: molecular volume (V), molecular globularity (G), amphiphilic moments (A), critical packing parameter (CP), polarizability (POL), molecular weight (MW), logP octanol/water (LOGP n-Oct), logP cyclohexane/water (LOGP c-Hex), polar surface area (PSA), hydrophobic surface area (HSA), and intrinsic solubility (SOLY).

Principal component analysis (PCA) was performed in order to substitute the representation of the objects, from the initial representation in the form of the n original intercorrelated variables, into the new principal component (PC) coordinate space. Each PC is characterized by loadings and scores, where scores are the new coordinates of the projected objects, and loadings reflect the direction concerning the original variables. Since the loadings plot represents relationships between variables, it was used to identify molecular descriptors that contribute to the positioning of the objects on the scores plot. In the scores plot, the grouping pattern of the tested compounds was analyzed and the potential outliers, i.e., compounds lying outside the Hotelling T2 ellipse, were identified.

RESULTS AND DISCUSSION

Results of drug-likeness analysis of resveratrol and its analogues

Based on the results obtained using DruLiTo software, the potential of a chemical compound to be used as a drug (drug-likeness) was analyzed. In 57 structural analogists of resveratrol, the following drug-likeness filters were applied: Lipinski rule, Ghose, Weber filters, CMC similarity rule, and mathematical models that quantitatively evaluate drug-likeness properties (QED filter). In Table 1. there is an overview of the applied filters, the requirements that must be met within these filters, as well as the number of analogues that violate these requirements. Drug-likeness analysis results showed that 1 compound did not meet the requirements of the Lipinski rule as the most used filter in preformulating studies. The compound ZINC38393465 violates the Lipinski rule due to the presence of 6 hydrogen donors, or 6 hydroxyl groups, while the requirements define a maximum of 5 hydrogen donors. Additionally, the 16 compounds failed to meet the criteria outlined in the CMC rule of similarity.

When compared to other exclusion-based filters, rules based on the number of certain structural parameters, QED uses complex mathematical models that calculate the contribution of a large number of structural parameters to the result of whether a given compound can potentially be used as a drug. Unlike the other filters listed above, QED can identify situations in which a generally unfavorable property can be accepted if all other parameters are nearly optimal (BICKERTON *et al.,* 2012). According to the QED filter, all the tested compounds exhibit the potential to be pharmacologically active substances suitable for oral administration, furthermore, all 57 analogues have been analyzed.

Table 1. Evaluation of drug-likeness properties of resveratrol and its analogues

Drug-likeness filter	Requirements that must be met within these filters*	The number of analogues that violate the requirements of the filter
Lipinski Rule	MW ≤ 500, logP ≤ 5, HBA ≤ 10, HBD ≤ 5	1
Ghose filter	MW: 160-480, MR: 40-130, logP: -0.4-5.6, NoA: 20-70	0
Webber filter	Number of rotating bonds ≤ 10, PSA ≤ 140	0
CMC-50-like rule	AlogP: 1.3-4.1, MR: 70-110, MW: 230-390, NoA: 30-55	16
QED filter	QED ≥ 0.5	0
All filters		17

*MW-molecular mass, HBA-hydrogen acceptors, HBD-hydrogen donors, MR-molar refractivity, NoA - number of atoms, PSA-polar surface, QED-mathematical models that quantitatively evaluate drug-likeness (includes MW, AlogP, HBA, HBD, rotating bonds, aromatic bonds, PSA, number of structural alerts)

Molecular docking results obtained by using Molegro virtual Docker software

When looking at the ligand binding energy for the active site of the SIRT1 protein, the potential energy of the resulting ligand-receptor complex, expressed in kcal/mol, falls within a binding energy range from -73.35 kcal/mol for 4-stirilphenol up to -103.14 kcal/mol for pterostilben. Resveratrol itself achieved a binding energy of -98.32 kcal/mol.

Of the observed 57 structural analogues, 15 of them have lower binding energy. This leads to a higher affinity for the target site and the formation of a more stable ligand-protein complex, while the remaining 41 analogues have a higher energy content of the resulting complex. Pterostilbene demonstrated the highest binding affinity for the active site of the SIRT1 protein. The method of binding pterostilbene to the active site SIRT1, in relation to the method of binding resveratrol, is shown in Figure 2.

Although resveratrol formed two hydrogen bonds with functional groups Pro 447 and Ser 229, the better binding energy of pterostilbene was due to better steric fit in the active site cavity and electrostatic interactions.

Molecular descriptors obtained using VolSurf+, PgpRules and SwissADME servers

Molecular descriptors calculated using the VolSurf + mathematical models are presented in the table in Annex 1. The calculated logP values in the octanol/water system range from 2,492 to 4,424, with the logP for resveratrol being 2,948 and 46 analogues being lipophilic at these values. Similar values are obtained for the LOGP cHex descriptor which describes the partition in the cyclohexane/water system, where only 6 compounds are more hydrophilic compared to resveratrol. The permeability through the blood-brain barrier is

shown by the LgBB descriptor, which goes from -1.402 for ZINC38393465 to 0.674 for ZINC01676024. Of the tested analogues, 19 showed very poor permeability, which is represented by a value less than -0.3. A value above 0.5 is considered high permeability, and it corresponds to only 6 analogues tested.

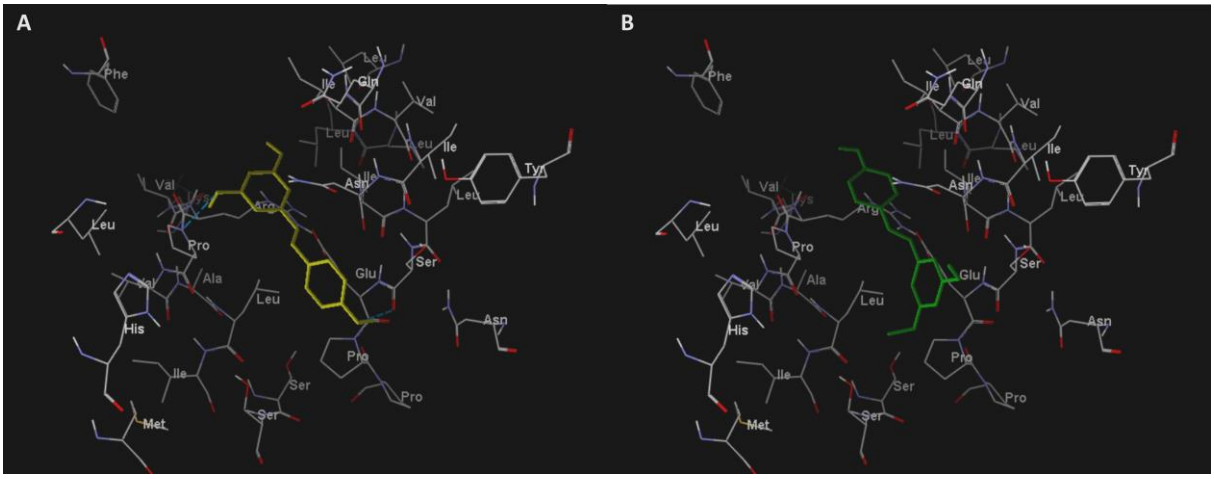

Figure 2. Location and the way to connection of (A) resveratrol [ZINC00006787] (yellow) and (B) pterostilbene [ZINC00899213] (green) in the active site of SIRT1

Annex 1 also presents the results of the PgpRules server for predicting two types of interactions of resveratrol analogues with P-glycoprotein (P-gp): substrate properties and inhibitor properties. The structural analogues used for the study did not show the properties of the P-gp substrate. The property of P-gp inhibitors has been shown by 6 analogues that show extremely high permeability through the blood-brain barrier where there is a possibility of interaction with other drugs and substances.

Permeation through the blood-brain barrier was calculated using the SwissADME web server “BOILED-Egg " (Brain or Intestinal EstimateD permeation) evaluation method (DAINA et al., 2017). 'BOILED-Egg chart' for gastrointestinal absorption and permeation through the blood-brain barrier of resveratrol and its analogues is presented in Figure 3. The white part represents a high degree of probability of passive absorption in the gastrointestinal tract, while the yellow area of the graph represents a high probability of penetration into the CNS (the White and yellow parts are not mutually exclusive). The graph shows that a pair of molecules are not located in the yellow part, which results in their less likely penetration into the CNS, while most of them are in the yellow part.

Comparative analysis of the results of molecular docking and LgBB descriptors

Certain resveratrol analogues were selected to obtain a compound with a potentially higher binding affinity but also with good permeability. Based on the obtained results of molecular docking, analogues with binding energies below -97 kcal/mol were selected, while analogues with a descriptor value above 0.2 were selected based on the LgBB descriptor.

Summing up the results, four analogues of resveratrol have been identified that are characterized by better properties both in terms of permeability and higher binding affinity for the SIRT1 protein compared to resveratrol itself and are presented in Table 2.

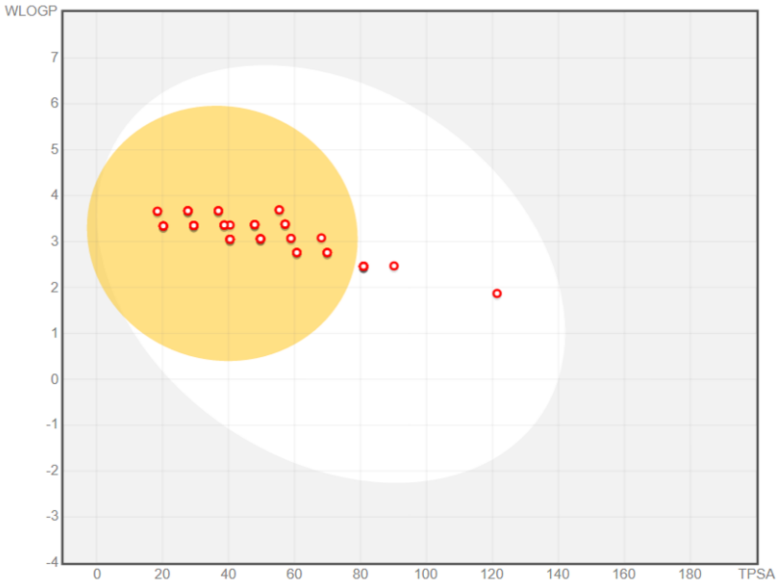

Figure 3. 'BOILED-Egg chart' gastrointestinal absorption and permeation through the blood-brain barrier of resveratrol and its analogues

Table 2. Resveratrol analogues with better permeability and higher binding affinity to the SIRT1 protein

Compound [ZINC ID]	MolDock score [kcal/mol]	LgBB
ZINC39110895	-99.2859	0.205
ZINC01650059	-97.7115	0.354
ZINC00899172	-99.8432	0.376
ZINC04847334	-99.678	0.478

The Principal Component Analysis Strategy

The Principal Component Analysis Strategy (PCA analysis) was used to obtain molecular descriptors that greatly affect the permeability of analogues through the blood-brain barrier. In the PCA score diagram (B), resveratrol (compound 1) is shown with a red dot, and compounds with LgBB descriptor values above 0.25 are shown with green dots (Fig. 4).

The absence of a reliable treatment for neurodegenerative diseases presents a formidable challenge for researchers, prompting extensive efforts to discover and create improved therapeutic remedies. Compounds that target multiple pathways in the neurodegeneration cascade, ultimately contributing to disease development, are especially intriguing. Resveratrol and its synthetic and natural derivatives serve as noteworthy examples of such substances. These compounds have gained popularity in recent years and offer a novel potential pharmacological approach to the treatment of these diseases. However, the extensive metabolism of resveratrol in the body and its low bioavailability substantially restricts its clinical application (WICIŃSKI *et al*., 2020).

By analyzing the results based on which we evaluate the drug-similarity properties of the given analogues, it was observed that 1 compound does not meet the Lipinski rule, and 16 of them do not meet the requirements of the CMC rule of similarity. However, all compounds meet the requirements of the QED filter. Lipinski's rule is quite significant in the early stages of drug discovery, due to its simplicity and predictability. Conversely, when contrasted with other exclusion filters relying on specific structural parameter counts, QED uses complex mathematical models that calculate the contribution of a large number of structural parameters to the outcome of whether a given compound can potentially be used as a drug. Unlike the

other filters listed above, QED can identify scenarios in which a generally unfavorable property can be accepted if all other parameters are close to ideal values (BICKERTON *et al*., 2012). As all compounds have met the QED filter, all of them are included in further analysis, but high caution is required for those compounds that do not meet the Lipinski and CMC rule of similarity rules.

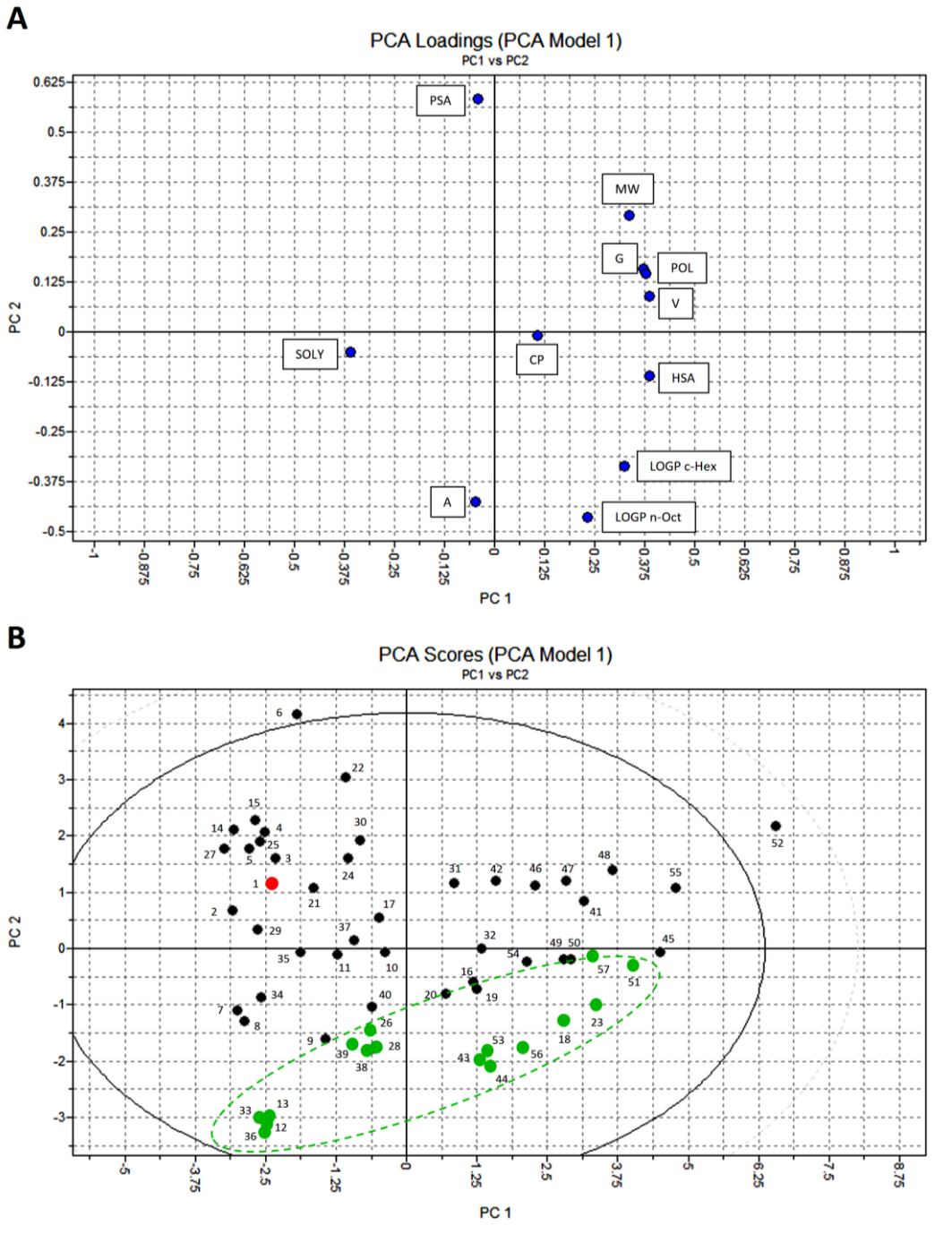

Figure 4. PCA load diagram (A) and score diagram (B) of molecular descriptors for resveratrol and its analogues

Molecular docking results obtained using Molegro Virtual Docker software showed that from the analyzed analogues of resveratrol, 15 of them have a lower binding energy for the active site of the SIRT1 protein compared to resveratrol, which means that the complexes formed between these compounds and the active site have a lower energy content. These

complexes are thermodynamically more stable, which improves the realization of their effects at the target binding site. However, the deviations from the binding energy of resveratrol are not big, so we can say that the binding energies of analogues are preserved relative to resveratrol.

With VolSurf+, more conclusions were drawn based on the results. Numerical values that describe a particular property of a molecule are molecular descriptors and are obtained as a result of a logical-mathematical procedure starting from the chemical information encoded in the symbolic representation of the molecule. Molecular descriptors are used to interpret and better understand some properties of molecules or to form a mathematical model by which a property can be predicted for other molecules.

Based on the calculated logP values in the octanol/water system, 45 analogues are more lipophilic according to these values, and similar values are obtained for LOGP cHex descriptor describing the partition in the cyclohexane/water system, where only 6 compounds are more hydrophilic compared to resveratrol, where their structures contain a higher number of hydroxyl groups compared to other analogues.

Permeability through the blood-brain barrier is shown by an LgBB descriptor. Values of this descriptor below -0.5 indicate weak penetration of the compound into the CNS, while higher values indicate moderate and good permeation through the blood-brain barrier. From the tested analogues, 19 showed very poor permeability, which is represented by a value less than -0.3. A value above 0.5 is considered high permeability, and it corresponds to only 6 analogues tested.

The property to act as an inhibitor of P-gp transport protein has been shown by 6 analogues that show extremely high permeability through the blood-brain barrier where there is the possibility of interaction with other drugs and substances. Their use is limited in neurodegenerative diseases, as they can lead to the accumulation of these drugs and side effects in the central nervous system. The structural analogues used for the study did not show the properties of P-gp substrates, so their efflux extrusion from the CNS will not occur.

The permeation through the blood-brain barrier was calculated via the SwissADME web server. The white part represents a high degree of probability of passive absorption in the gastrointestinal tract, while the yellow area of the graph represents a high probability of penetration into the CNS. Through the analysis of the data from the chart, a pair of molecules is obtained that show a lower probability of penetration into the CNS. The isolated analogues are also repeated in the previously analyzed data where they show poor permeability and more pronounced hydrophilic properties.

Out of the 57 analogs analyzed, 4 can be distinguished which we believe will have better pharmacokinetic profiles *in vivo* experimental conditions, namely: 1,3-benzenediol,5-[(1E)-2-(4-methylphenyl)ethenyl] (1); 4-[(E)-2-(3-methoxyphenyl)ethenyl]phenol (2); 5-methoxy-3-stilbenol (3); 3,4',5-trimethoxy-3'-hydroxystylbene (4) (Fig. 5).

When we look at the selected analogues, we notice that the binding energies of the analogues given to sirtuin-1 are approximately similar to resveratrol, which means that they form a stable complex with the receptor. All 4 selected analogues show higher permeability values in both the cyclohexane/water system and the octanol/water system, which is important for the permeability of molecules. Like resveratrol, the selected analogues have not shown binding affinity as P-gp substrates and P-gp inhibitors, thus indicating that their efflux ejection from the CNS will not occur, and also interaction and accumulation of other drugs/substances in the CNS which may exhibit adverse effects. In terms of permeability through the blood-brain barrier, all show better affinity transitions, which is complemented by the fact that some of these 4 molecules have the greatest potential to achieve neuroprotective activity after application in biological systems.

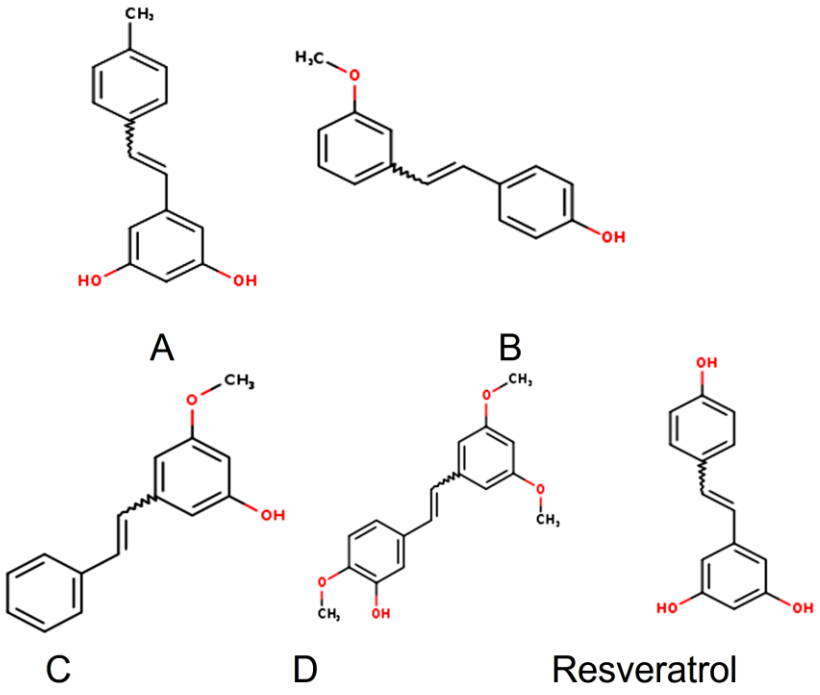

Figure 5. Resveratrol structures and resveratrol analogues have a high affinity for the SIRT1 protein and improved ADME characteristics.

Some results have shown that trans- and cis-resveratrol and hydroxylated analogues exhibit similar antioxidant activity and inhibit eicosanoid synthesis and Caco-2 cell growth. These effects may be associated with an increase in the percentage of cells in the S phase as a result of cell damage in the G0/G1 phase. Moreover, these molecules have been observed to induce apoptosis at concentrations below 100 mM. These effects were associated with changes in the potential of the mitochondrial membrane involved in the internal apoptosis pathway. However, 3,4',5-trimethoxy-3'-hydroxystylbene (4) inhibits Caco-2 cell growth more intensively than resveratrol, but without effects on oxidative stress and the arachidonic acid cascade [21].

Except for 3,4',5-trimethoxy-3'-hydroxystylbene (4) which has been investigated for the Prevention of oxidative stress in intestinal epithelial cancer cells (STORNIOLO and MORENO, 2019), no significant studies in the field of antioxidant and neuroprotective effects have been observed for other tested analogues of resveratrol.

By selecting resveratrol analogues with these physico-chemical characteristics, we have significantly overcome the factors that limit the bioavailability of resveratrol after oral administration, which makes these compounds important candidates for further *in vitro* and *in vivo* studies.

By analyzing the data obtained from the PCA chart, we get factor loads that reflect the direction regarding the original variables. It has been observed that compounds that exhibit better pharmacokinetic properties, primarily in terms of penetration through the blood-brain barrier, are directed in the direction of descriptor A (amphiphilic moment), as well as towards SOLY (intrinsic solubility) and LogP cHex and LogP nOct descriptors. SOLY and LogP descriptors must be partially in balance in order for the molecules to have adequate solubility in water and lipophilicity, i.e., the possibility of better bioavailability in the body. The PCA chart completes the fact that the selected 4 analogues of resveratrol, in addition to having a high affinity for binding to the SIRT1 protein, have better pharmacokinetics and better passing through the blood-brain barrier, and therefore a greater possibility for the manifestation of neuroprotective effect.

CONCLUSION

The improvement of therapy and the reduction of the frequency of adverse reactions can be achieved today by using *in silico* techniques. The goal is to provide much more information about the drug and its impact on biological systems during the process of discovery and development of drugs. The principle of these techniques is to identify from a large group of compounds those that have the greatest chance of success in later stages of research. These techniques shorten the time and reduce the number of experiments required when choosing potential drugs, which is a great advantage over the classic way of developing potential drugs. Based on the analysis of the results of our pharmacoinformatic study, it can be concluded that there exist structural analogues of resveratrol that are likely to exhibit a high affinity for binding to the sirtuin-1 protein and good permeability through the blood-brain barrier on the one hand, and on the other hand have improved pharmacokinetic properties and therefore a greater likelihood of success in further research in biological systems. In particular, they stand out: 1,3-benzenediol, 5-[(1E)-2-(4-methylphenyl)ethenyl]; 4-[(E)-2-(3-methoxyphenyl)ethenyl] phenol;5-methoxy-3-stilbenol; 3,4',5-trimethoxy-3'-hydroxystylbene due to their favorable binding energy but also better distribution in the CNS. Based on the analysis of all the results, it is hypothesized that these compounds may offer superior oral bioavailability compared to resveratrol. Further in vitro and in vivo studies are suggested to confirm this hypothesis, paving the way for their potential clinical application as agents that bind and activate the SIRT1 protein to lead to neuroprotective effects after administration in biological systems.

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