BIOMA
p-ISSN: 0126-3552
e-ISSN: 2580-9032

DOI:10.21009/Bioma20(1).1 Research article

Molecular docking of *Portulaca oleracea* L. alkaloid compounds for potential antidiabetic activity through Cdk5 protein expression

Alya Rahmaditya Arfan[1]* & Alif Rahman Habibi[2]

[1]*Blood Bank Technology Study Program, Kartini Batam Health Academy, Batam*
akkeskartini116@gmail.com, 0778 – 459037
[2]*Health Analyst Study Program, Putra Jaya Batam Health Analyst Academy, Batam*
*Corresponding author: alyarahmaditya@yahoo.com

ABSTRAK

Cyclin-dependent kinase 5 (Cdk5) adalah protein kinase serin/treonin, yang membentuk kompleks aktif p35 atau p39 yang diekspresikan secara dominan di neuron. Cdk5 memainkan peran penting dalam fungsi fisiologis dalam sel non-saraf seperti sekresi insulin yang distimulasi glukosa dalam sel pankreas. Penelitian ini bertujuan untuk mengeksplorasi dan mengetahui efektifitas senyawa kimia Oleracein E (OL-E) pada tumbuhan Portulaca oleracea *(PO) sebagai anti diabetes jika diekspresikan pada protein Cdk5. Pengujian dilakukan secara in silico dengan metode computer-aided drug design yang dimana proses molekuler docking menggunakan perangkat lunak berupa Pyrx 0.8. Hasil dari penelitian ini menunjukkan bahwa OL-E berpotensi sebagai antidiabetes dengan mengahambat Cdk5 dan mempunyai efektivitas 27,65% lebih baik dibandingkan metformin dalam menghambat Cdk5.*

Kata kunci: Portulaca oleracea, Oleracein E, Cyclin-dependent kinase 5

ABSTRACT

Cyclin-dependent kinase 5 (Cdk5) is a serine/threonine protein kinase, which forms the p35 or p39 active complex that is expressed predominantly in neurons. Cdk5 plays an important role in physiological functions in non-neural cells such as glucose-stimulated insulin secretion in pancreatic cells. This study aims to explore and determine the effectiveness of the chemical compound Oleracein E (OL-E) in the Portulaca oleracea *(PO) as an anti- diabetic when expressed on Cdk5 protein. The test was carried out in silico with a computer- aided drug design method in which the molecular docking process used software such as Pyrx 0.8. The results of this study indicate that OL-E has the potential as an antidiabetic by inhibiting Cdk5 and has 27.65% better effectiveness than metformin in inhibiting Cdk5.*

Keywords: Portulaca oleracea, Oleracein E, Cyclin-dependent kinase 5

INTRODUCTION

Portulaca oleracea L. (PO) or commonly known as purslane is a herbaceous plant that grows wild with many utilization including in traditional medicine. This PO is known

as diuretic, febrifuge, vermifuge, antiseptic, anti-spasmodic, and has pharmacological activities including analgesic, anti-bacterial, skeletal muscle relaxant, wound healing, anti-inflammatory, and anti-spasmodic (Xiang et al., 2005). This PO can also reduce various diseases such as diseases in the digestive tract, respiratory disorders, inflammation of the liver, kidneys, and urinary bladder. In addition, PO can also act as an antidiabetic by regulating lipids and blood sugar in the body (Rahimi et al., 2019).

FIGURE 1. Portulaca oleracea L.

Previous researchers showed that PO has many groups of bioactive compounds in the form of flavonoids, alkaloids, monoterpene glycosides, phenolic compounds, fatty acids, alpha-linoleic acid (Omega-3), vitamins, minerals and several other compounds (Rahimi et al., 2019). According to previous in vivo research PO seeds and metformin groups had the same results. PO seeds can be effective and safe as additional therapy for people with type-2 diabetes. These results indicate that PO seeds have hypoglycemic, hypolipidemic, and insulin resistance-reducing effects; perhaps because it contains polyunsaturated fatty acids, flavonoids and polysaccharides (El-Sayed, 2011).

Oleracein is the main bioactive component in PO. Oleracein in PO is mostly found in plant stems and consists of A-L oleracein. Oleracein E (OL-E) and Oleracein L (OL-L) are known to evaluate the antidiabetic effect, and when compared OL-E is more potent than OL. The findings of the present study indicate that as primary isoquinoline alkaloids in PO, both OL-E and OL-L, at low to moderate concentrations, significantly stimulate insulin secretion and enhance glucose uptake. These results are consistent with those of other related studies. It is crucial to note that high doses of Oleracein derivatives are linked to increased ceramide production, cellular toxicity, and reduced insulin secretion. Therefore, OL-E and OL-L show promise as natural antidiabetic agents for preventing or treating diabetes mellitus (DM) and its various complications (Roozi et al., 2021).

Diabetes mellitus (DM) is a chronic metabolic disease caused by abnormalities in insulin secretion or insulin action (ADA, 2015). Cyclin-dependent kinase 5 (Cdk5) is a serine/threonine protein kinase, which forms the p35 or p39 active complex that is expressed predominantly in neurons. Cdk5 plays an important role in physiological functions in non-neural cells such as glucose-stimulated insulin secretion in pancreatic

cells. Current evidence suggests that Cdk5 may be a potential drug target for the treatment of neurodegenerative diseases, drug abuse and diabetes mellitus. Cdk5 is known to regulate insulin secretion in cells. Under normal conditions, Cdk5 negatively regulates insulin secretion through voltage-dependent phosphorylation of calcium channels. Inhibition of Cdk5 can increase glucose-stimulated insulin secretion. When cells are exposed to high glucose for a long time, Cdk5 can inhibit insulin gene transcription through the regulation of the PDX-1 protein (Wei & Tomizawa, 2007).

According to Kitani et al., (2007) and Wei & Tomizawa (2007) The mechanism of insulin secretion induction by Cdk5 inhibitors has been shown to be as follows: insulin secretion is induced by calcium via L-type voltage-dependent calcium channels (L-VDCCs) in response to elevated extracellular glucose levels. Cdk5 phosphorylates loops II-III of the 1c subunit of L-VDCC and inhibits channel activity, resulting in inhibition of glucose-stimulated insulin secretion. A transient increase in extracellular glucose improves pancreatic cell function and survival, whereas a chronic increase in glucose has the opposite effect, impairing cell function and survival. The deleterious effect of chronic glucose elevation is referred to as glucotoxicity. Glucotoxicity is an important component of the pathophysiology of type 2 diabetes because it interferes with the effects of insulin on peripheral tissues and insulin secretion from cells. An increase in p35 expression was observed in cells undergoing glucotoxicity. It is known that Cdk5 plays a role in the loss of pancreatic cell function in type 2 diabetes. Chronic exposure of cells to high glucose reduces insulin mRNA levels and insulin promoter reporter gene activity. Inhibition of Cdk5 prevents decreased insulin gene expression through inhibition of nuclear translocation of PDX-1, which is a transcription factor for the insulin gene.

PO has shown that the compound myricetin from this plant exhibits in vitro effects on high glucose-induced β-cell apoptosis, likely by inhibiting cyclin-dependent kinase 5 (CDK5). The data demonstrated that myricetin at a concentration of 20 μM significantly protected β cells by reducing apoptosis in INS-1 cells and mouse islets exposed to 30 mM glucose for 24 and 48 hours, respectively. Docking studies further suggested that myricetin inhibits the activation of CDK5 (Kang et al., 2015).

Metformin is the most widely used drug for type 2 diabetes mellitus, by suppressing Cdk5-dependent hyper-activation and Cdk5-dependent hyper-phosphorylation. Metformin prevents Cdk5 hyper-activation by inhibiting calpain-dependent cleavage of p35 to p25 (Wang et al., 2020). Based on this background, researchers are interested in knowing the effectiveness of the OL-E alkaloids in PO plants when compared to metformin in inhibiting Cdk5 by using the molecular docking method. Molecular docking is a technique that predicts the preferred orientation, affinity, and interactions of ligands at protein binding sites. Information from the preferred orientation in turn can be used to predict the strength of the binding affinity between the drug target and the ligand molecule using a scoring function (Saileela et al., 2017).

METHODOLOGY

Tools and materials

The test was carried out in silico using a computer-aided drug design method. The hardware used is the HP Pavilion 13 Aero Laptop with OS specifications: Windows 11, AMD Ryzen 5 5600U Processor with 16 GB RAM. The software used are PyMOL and PyRX applications. Toxicity testing was predicted through the link site (http://lmmd.ecust.edu.cn) , and to predict the physicochemistry, pharmacokinetics, similarity and safety of the drug, SwissAdme (http://www.swissadme.ch) and Lipinski Rule of Five (http://www.scfbio-iitd.res.in/software/drugdesign/lipinski.jsp). The material used is the 3D structure of the ligand obtained from the Pubchem link site to obtain the chemical structure and SMILE (PubChem (nih.gov), and PCSB PDB (RCSB PDB: Search) protein data bank website. Structure and regulation of the CDK5-p25(nck5a), PDB code selected as a receptor in the RCSB database is 1H4L, Classification: Kinase/Kinase Activator, Organism(s): *Homo sapiens* and Released on 2002-08-14.

Research procedure

Compounds OL-E and metformin (control) acted as ligands where the structure of both was obtained through the Pubchem website which was downloaded in 3D SDF format. The target protein was matched through two data banks on the Super-PRED and Swiss Target Prediction sites by looking at the probability of the compound against the target protein. The structure of the target protein is then downloaded in PDB format via the RCSB site, before the docking process is carried out, the water molecules present in the target protein are removed first so as not to affect the docking process.

Molecular docking procedure

The molecular docking process is carried out with the Pyrx application with the Autodock Control Wizard and Autodock Navigator. The structure of the target protein (Cdk5) which has been removed the water molecule and the structure of the ligands (herbs and control compounds) are optimized to be stored in the autodock navigator. The protein and ligand structures are then docked by clicking forward after a while the vina search space will display the center and dimensions of the two structures, click maxime after that click forward again to display the binding affinity number of the docking results. The docking results are then saved in pdb format, to view visually through the Pymol application.

RESULTS AND DISCUSSION

Physicochemical, pharmacokinetic, drug similarity and safety tests

Before doing molecular docking, first we check the nature of the ligand used, whether it meets the rules. The criteria for a good drug must follow the Lipinski Five Rules developed by Christopher A. Lipinski (Tallei et al., 2020). Lipinski et al., (2001) stated that a molecule can proceed with a docking simulation if (1) The molecular mass is less than 5000 daltons; (2) No more than 10 hydrogen bond acceptors; (3) No more

than 5 hydrogen bond donors (total number of N-H and O-H bonds); and (4) The octanol-water partition coefficient (log-P) does not exceed 5.

TABLE 1. Bioaviability score of OL-E compounds

Physicochemical Properties	
Formula	C12H13NO3
Molecular weight	219,24 g/mol
Num. heavy atoms	16
Num. arom. heavy atoms	6
Fraction Csp3	0.42
Num. rotatable bonds	0
Num. H-bond acceptors	3
Num. H-bond donors	2
Molar refractivity	62.43
TPSA	60.77 A^2
Druglikeness	
Lipinski	Yes; 0 violation
Ghose	Yes
Veber	Yes
Egan	Yes
Muegge	Yes
Biovailabillty score	0.55

Based on the results using the Swissadme website and the Lipinski Rule of Five, it can be seen in the table and figure that the compound OL-E with SMILE C1CC(=O)N2C1C3=CC(=C(C=C3CC2)O)O meets these criteria and has pharmacokinetic properties of drugs in the human body, including absorption, distribution, metabolism, and excretion (ADME).

Toxicity Test

TABLE 2. Toxicity test of OL-E compounds based on AdmetSAR data

Model	**Result**	**Probability**
Human Ether-a-go-go-related gene	Weak inhibitor	0,9580
AMES toxicity	Non-inhibitor	0,5465
Carcinogens	Non-carcinogens	0,9539
Fish toxicity	High FHMT	0,6856
Tetrahymena pyriformis toxicity	High TPT	0,8265
Toxicity		
Rat acute toxicity	2,5947 mol/kg LD50	
Fish toxicity	1,2247 mg/L PLC50	
Tetrahymena pyriformis toxicity	0,2011 ug/L pIGC50	

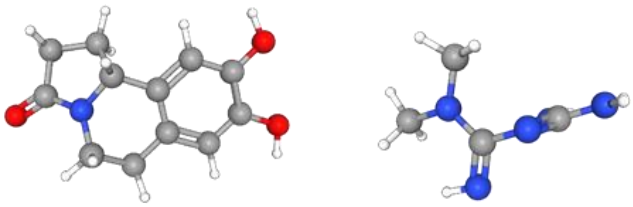
Source : AdmetSAR

Based on the **Table 2** it can be seen that the Human Ether-a-go-go-related gene (hERG) value indicates this compound is likely to inhibit the hERG channel with a probability of 95.8%, but the inhibition is relatively weak. The AMES test, used to evaluate the mutagenic potential of a compound, shows that this compound is not a

mutagen with a probability of 54.65%. From the data obtained, OL-E compounds are not carcinogenic, the results show that this compound is most likely not a carcinogen with a probability of 95.39%. The value of the level of toxicity in rats is LD50 2.5947 mol/kg the substance falls into the "Practically Non-Toxic" category, indicating that it has relatively low toxicity (Ahammad et al., 2020), but LC50 value of 1.2247 mg/L indicates that the substance has a toxic level of toxicity to fish (El-Harbawi, 2014).

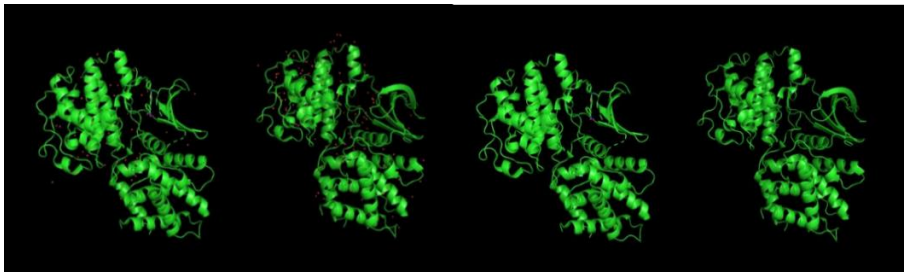
Molecular Docking

Molecular docking is used to predict bond strength, complex energy, type of signal generated and calculate binding affinity between two molecules using a scoring function. Binding Affinity is an indicator of measuring the ability of a drug to bind to a receptor (Saputri., et al., 2016). In the fields of computational chemistry and molecular modeling, grading function is a fast approximation mathematical method used to predict the strength of the non-covalent interaction (also referred to as binding affinity) between two molecules after they are bonded. Affinity is estimated by adding up the intermolecular van der Waals forces and the electrostatic interactions between all atoms of the two molecules in the complex using a force field. Intramolecular energies (also referred to as strain energies) of the two binding pairs are also frequently included (Saileela et al., 2017). The principle used in the assessment of binding affinity is a physics-based molecule with a mechanical force field that predicts energy properties, low (negative) energy indicates the stability of the system and thus the better the possibility of affinity interactions between receptors that bind the two molecules (Monika et al., 2010).



(a) (b)

FIGURE 2. 3D visualization of (a) Oleracein-E and (b) control ligand (metformin)



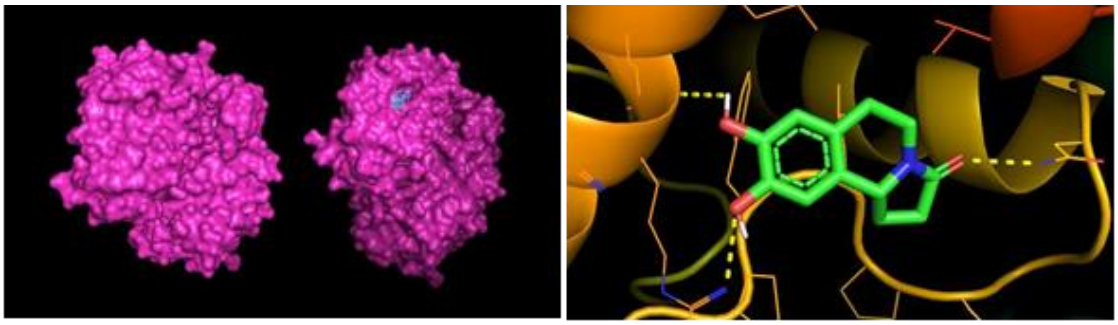
(a) (b)

FIGURE 3. Visualization of CDK5 target protein (a) with water molecules and (b) without water molecules

(a) (b)

FIGURE 4. Visualization of 3D Cdk5 with test compound (a) OL-E and (b) Metformin

Based on the results obtained, the compound OL-E showed antidiabetic activity with the mechanism of inhibiting Cdk5 through the formation of hydrogen bonds with negative affinity can be seen in **Figure 8**.



(a) (b)

FIGURE 5. (a) 3D visualization of OL-E compound docking results with Cdk5 and (b) Visualization of hydrogen bonds shaped

Based on the results obtained in silico, it can be seen in **Table 3**, that the OL-E compound has the potential as an antidiabetic by inhibiting Cdk5 and has a better effectiveness than metformin in inhibiting Cdk5 by looking at the binding affinity of the docking results. The binding affinity of the OL-E ligand has a lower value than the metformin ligand, which is -6.0 which indicates that the bond and the reaction that occurs is stronger and more efficient. The results of this study indicate that OL-E has the potential as an antidiabetic by inhibiting Cdk5 and has 27.65% better effectiveness than metformin in inhibiting Cdk5.

This is also supported by research by Roozi et al., (2021) in which the OL-E alkaloid compound from the PO plant is known to increase the stimulation of insulin secretion and glucose uptake which can help the recovery of diabetic patients. The mechanism of action of this plant according to research (Lei et al., 2010) is associated with increased insulin secretion through the closure of K+-ATP, which causes membrane depolarization and stimulation of calcium penetration into the membrane which is the first step of insulin secretion.

TABLE 3. Affinity Binding Results

Ligand	Binding Affinity
Oleracein-E	-6.0
Metformin	-4.7

Consistent with previous research PO has revealed that the compound myricetin from this plant has in vitro effects on high glucose-induced β-cell apoptosis, potentially through the inhibition of cyclin-dependent kinase 5 (CDK5). The findings showed that myricetin at a concentration of 20 μM significantly protected β cells by reducing apoptosis in INS-1 cells and mouse islets exposed to 30 mM glucose for 24 and 48 hours, respectively. Additionally, docking studies suggested that myricetin inhibits CDK5 activation (Kang et al., 2015).

Not only that according to (Lee et al., 2012) treatment with PO plants carried out using experimental rats can reduce blood glucose, levels of plasma triglycerides, cholesterol, and LDL, and systolic blood pressure in rats with type 2 diabetes. it also enhances the function of acetylcholine. Therefore, it is suggested that consumption of this plant can inhibit hyperglycemia, inflammation of blood vessels, and endothelial dysfunction in diabetics (Roozi et al., 2021).

CONCLUSIONS

The binding affinity of the OL-E ligand has a lower value than the metformin ligand, which is -6.0 which indicates that the bond and the reaction that occurs is stronger and more efficient. The results of this study indicate that OL-E has the potential as an antidiabetic by inhibiting Cdk5 and has 27.65% better effectiveness than metformin in inhibiting Cdk5.

AUTHOR CONTRIBUTIONS

A.R.A. conceived the presented idea, developed the theory, verified the methods, to investigate [a specific aspect], and supervised the findings of this work. A.R.A contributed to the research design and implementation and the manuscript's writing. A.R.A and A.R.H. discussed the results and contributed to the final manuscript.

ACKNOWLEDGMENTS

Thank you to the Kartini Batam Health Academy and the Putra Jaya Batam Health Analyst Academy for the convenience and support provided to carry out this research.

CONFLICTS OF INTEREST STATEMENT

There are no conflicts to declare.

REFERENCES

AdmetSAR. http://lmmd.ecust.edu.cn/admetsar2/. accessed on April 25, 2024.

Ahammad, I., Sarker, M. R. I., Khan, A. M., Islam, S., & Hossain, M. (2020). Virtual Screening to Identify Novel Inhibitors of Pan ERBB Family of Proteins from Natural Products with Known Anti-tumorigenic Properties. *International Journal of Peptide Research and Therapeutics*, *26*(4), 1923–1938. https://doi.org/10.1007/s10989-019-09992-3

American Diabetes Association. (2015). Introduction. *Diabetes Care*, *38*(1). https://doi.org/10.2337/dc15-S001

El-Harbawi, M. (2014). Toxicity Measurement of Imidazolium Ionic Liquids Using Acute Toxicity Test. *Procedia Chemistry*, *9*, 40–52. https://doi.org/10.1016/j.proche.2014.05.006

El-Sayed, M. I. K. (2011). Effects of Portulaca oleracea L. seeds in treatment of type-2 diabetes mellitus patients as adjunctive and alternative therapy. *Journal of Ethnopharmacology*, *137*(1), 643–651. https://doi.org/10.1016/j.jep.2011.06.020

Kang, S. J., Park, J. H. Y., Choi, H. N., & Kim, J. I. (2015). A-Glucosidase Inhibitory Activities of Myricetin in Animal Models of Diabetes Mellitus. *Food Science and Biotechnology*, *24*(5), 1897–1900. https://doi.org/10.1007/s10068-015-0249-y

Kitani, K., Oguma, S., Nishiki, T. I., Ohmori, I., Galons, H., Matsui, H., Meijer, L., & Tomizawa, K. (2007). A Cdk5 inhibitor enhances the induction of insulin secretion by exendin-4 both in vitro and in vivo. *Journal of Physiological Sciences*, *57*(4), 235–239. https://doi.org/10.2170/physiolsci.RP006607

Lee, A. S., Lee, Y. J., Lee, S. M., Yoon, J. J., Kim, J. S., Kang, D. G., & Lee, H. S. (2012). Portulaca oleracea ameliorates diabetic vascular inflammation and endothelial dysfunction in db/db mice. *Evidence-Based Complementary and Alternative Medicine*, *2012*, 1–10. https://doi.org/10.1155/2012/741824

Lei, X., Barbour, S. E., & Ramanadham, S. (2010). Group VIA Ca2+-independent phospholipase A2 (iPLA2β) and its role in β-cell programmed cell death. *Biochimie*, *92*(6), 627–637. https://doi.org/10.1016/j.biochi.2010.01.005

Lipinski, C. A., Lombardo, F., Dominy, B. W., & Feeney, P. J. (2001). Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Advanced Drug Delivery Reviews*, *23*, 2–26. https://doi.org/10.1016/j.addr.2012.09.019

Lipinski Rule of Five. http://www.scfbio-iitd.res.in/software/drugdesign/lipinski.jsp. accessed on April 25, 2024.

Monika, G., Punam, G., Sarbjot, S., & D., G. G. (2010). An overview on Molecular Docking International Journal of Drug Development & Research. *International Journal of Drug Development & Research*, *2*(2), 219–231. https://www.ijddr.in/drug-development/an-overview-on-molecular-docking.pdf

Rahimi, V. B., Ajam, F., Rakhshandeh, H., & Askari, V. R. (2019). A pharmacological review on Portulaca oleracea L.: Focusing on anti-inflammatory, anti- oxidant, immuno-modulatory and antitumor activities. *Journal of Pharmacopuncture*, *22*(1), 7–15. https://doi.org/10.3831/KPI.2019.22.001

Roozi, H., Boojar, M. M. A., Eidi, A., & Khavari-Nejad, R. (2021). The effect of portulaca oleracea alkaloids on antidiabetic properties through changes in ceramide metabolism. *Egyptian Journal of Basic and Applied Sciences*, *8*(1), 156–166. https://doi.org/10.1080/2314808X.2021.1877889

Saileela, M. V., Rao, M. V., & Vutla, V. R. (2017). A Review on Applications of Molecular Docking in Drug Designing. *Indo American Journal of Pharmaceutical Research*, *7*(04), 8391–8402. https://doi.org/10.5281/zenodo.2526083

Tallei, T. E., Tumilaar, S. G., Niode, N. J., Fatimawali, Kepel, B. J., Idroes, R., Effendi, Y., Sakib, S. A., & Emran, T. Bin. (2020). Potential of Plant Bioactive Compounds as SARS-CoV-2 Main Protease (Mpro) and Spike (S) Glycoprotein Inhibitors: A

Molecular Docking Study. *Scientifica*, *2020*(6307457), 18 pages. https://doi.org/10.1155/2020/6307457

Wang, Y. L., Zhao, J. H., Guo, F. L., Gao, X. H., Xie, X., Liu, S. Q., Yang, X., Yang, X. F., Zhang, L. Y., Ye, Y. X., Fan, L. B., & Wang, J. G. (2020). Metformin Ameliorates Synaptic Defects in a Mouse Model of AD by Inhibiting Cdk5 Activity. *Frontiers in Cellular Neuroscience*, *14*(June), 1–10. https://doi.org/10.3389/fncel.2020.00170

Wei, F.-Y., & Tomizawa, K. (2007). Cyclin-Dependent Kinase 5 (Cdk5): A Potential Therapeutic Target for the Treatment of Neurodegenerative Diseases and Diabetes Mellitus. *Mini-Reviews in Medicinal Chemistry*, *7*(10), 1070–1074. https://doi.org/10.2174/138955707782110114

Xiang, L., Xing, D., Wang, W., Wang, R., Ding, Y., & Du, L. (2005). Alkaloids from Portulaca oleracea L. *Phytochemistry*, *66*(21), 2595–2601. https://doi.org/10.1016/j.phytochem.2005.08.011