Original Article

Molecular Docking, Pre-ADME, And Pre-Tox Analysis Of Swainsonine And Castanospermine As Potential Glucokinase Activators

Rita D. Chakole*, Manoj S. Charde

Government College of Pharmacy, Vidyanagar, Karad, Satara 415124, Maharashtra, India.
*Corresponding author email: kdcritu@gmail.com
DOI: 10.47750/pnr.2022.13.S10.805

Abstract

In this research, we investigated the efficacy of Swainsonine and Castanospermine as GK activators in the treatment of type 2 diabetes. It was determined to perform a comprehensive investigation on the absorption, distribution, metabolism, excretion, and toxicity (ADMET) characteristics of the substances. After that, molecular docking experiments were carried out in order to explore the possible binding affinity of these compounds with the GK enzyme. Based on this discovery, it can be deduced that both chemicals possess the capability to create conventional hydrogen bonds with the GK enzyme, which indicates that they have the potential to operate as activators of the enzyme. It is possible to improve the efficiency of GK activation by using the tactic of synthesising a variety of semisynthetic derivatives as part of the overall plan. It is acceptable to contemplate the possibility of either of these compounds being developed further into a GK activator given that both of them demonstrate a variety of drug-like features.

Keywords: Pre-ADMET; Swainsonine; Castanospermine; ADMET; Glucokinase; T2DM

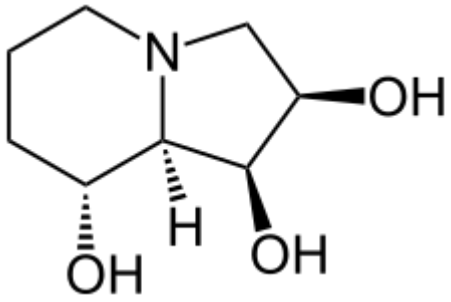
1. Introduction

Swainsonine, an indolizidine alkaloid, has been identified as the primary bioactive compound responsible for the manifestation of locoism. The compound in question was initially extracted and identified from Swainsona canescens, a plant species indigenous to Australia. Subsequently, it was also discovered in other plant species, namely Astragalus and Oxytropis spp, which are native to North America. The current findings suggest that the alkaloid is synthesized by an endophytic microorganism residing within the plant[1–3].

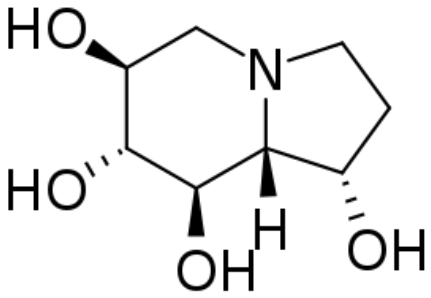
Castanospermine, an indolizidine alkaloid, was initially discovered and isolated from the seeds of Castanospermum australe, a plant species. This compound exhibits strong inhibitory effects on certain glucosidase enzymes and demonstrates antiviral activity both in vitro and in mouse models. The antiviral drug candidate celgosivir, which is a derivative of castanospermine, is currently undergoing development for its potential application in the treatment of hepatitis C virus (HCV) infection[3–5].

The primary liver dysregulation observed in individuals with Type 2 diabetes mellitus (T2DM) is an elevation in hepatic glucose output. Unfortunately, the currently available classes of glucose-lowering medications, with the exception of metformin, do not directly or adequately address this particular aspect of T2DM. The potential resolution of this unfulfilled requirement could potentially be achieved by means of the activation of a distinct enzyme belonging to the hexokinase family, specifically referred to as glucokinase (GK). GK functions as a glucose-sensor or glucose receptor within pancreatic cells, triggering insulin secretion in response to glucose stimulation[6–10]. Additionally, it acts as a glucose gate-keeper in hepatocytes, facilitating the uptake of glucose into the liver and promoting glycogen

synthesis and storage. Activation of glucokinase (GK) through the use of small molecules represents a potential alternative strategy for enhancing glycemic regulation in individuals diagnosed with type 2 diabetes mellitus (T2DM). GK activators (GKAs) have been shown to potentially enhance the secretion of insulin from the pancreas and facilitate the synthesis of glycogen in the liver. Consequently, these compounds may have the ability to mitigate hepatic glucose output. The GKA class has experienced various challenges during its development; however, recent advancements have sparked a renewed interest in this field[11]. In the current study, we have examined the potential of Swainsonine and Castanospermine as GK activators for the treatment of T2DM. An extensive analysis of the absorption, distribution, metabolism, excretion, and toxicity (ADMET) properties of the compounds was conducted. Subsequently, molecular docking studies were performed to investigate the binding affinity potential of these compounds with the GK enzyme. The structures of Swainsonine and Castanospermine are depicted in Figure 1.



Swainsonine



Castanospermine

Figure 1. The structures of Swainsonine and Castanospermine

2. Material and Methods

2.1 Pharmacokinetics predictions

The Lipinski rule of five and the pharmacokinetic (ADME) characteristics of molecules were investigated using PubChem[12], molinspiration[13], and SwissADME[14] servers. ADMETlab 2.0 is a totally revamped version of the AMDETlab web server, which is commonly used for predicting the pharmacokinetics and toxic characteristics of various compounds (https://admetmesh.scbdd.com/)[15].

2.2 Molecular docking studies

Molecular docking was performed on Lenovo ThinkPad with 64-bit operating system Processor: Intel(R) Core(TM) i5-4300M CPU @2.60 GHz, 2.59 GHz, RAM: 4GB by using Pyrx Virtual Screening Tool. The structures of all the designed novel derivatives and native ligand (mole. File format) were drawn in ChemDraw Ultra 8.0. The energy minimization (optimization) was performed by Universal Force Field (UFF)[16].

The elucidated crystal structure of human GK was obtained from the RCSB Protein Data Bank (PDB) as entry 1V4S (https://www.rcsb.org/structure/1V4S). The native ligand present in 1V4S was 5-(1-methyl-1H-imidazol-2-ylthio)-2-amino-4-fluoro-N-(thiazol-2-yl)benzamide. Autodock vina 1.1.2 in PyRx 0.8 was used to perform the docking studies of both compounds and native ligand against the crystal structure of GK[17]. The enzyme structure was optimized, purified, and prepared for docking with the help of Discovery Studio Visualizer 2019. The binding affinity studies were performed by using Vina Wizard Tool in PyRx 0.8. Molecules (PDBQT Files), both ligands as well as the target (human GK) were selected for the docking study. For molecular docking simulation, the three-dimensional grid box (size_x = 31.68A^o; size_y = 3.7901A^o; size_z = 64.27A^o) was designed using Autodock tool 1.5.6 with exhaustiveness value of 8[17]. The active amino acid residues in the protein were identified and noted using BIOVIA Discovery Studio Visualizer (version-19.1.0.18287)[18]. The identified cavity of the enzyme with co-crystallized ligand

molecule is represented in Figure 2. The complete molecular docking approach was carried out in accordance with the methods outlined by S. L. Khan et al.[19–24].

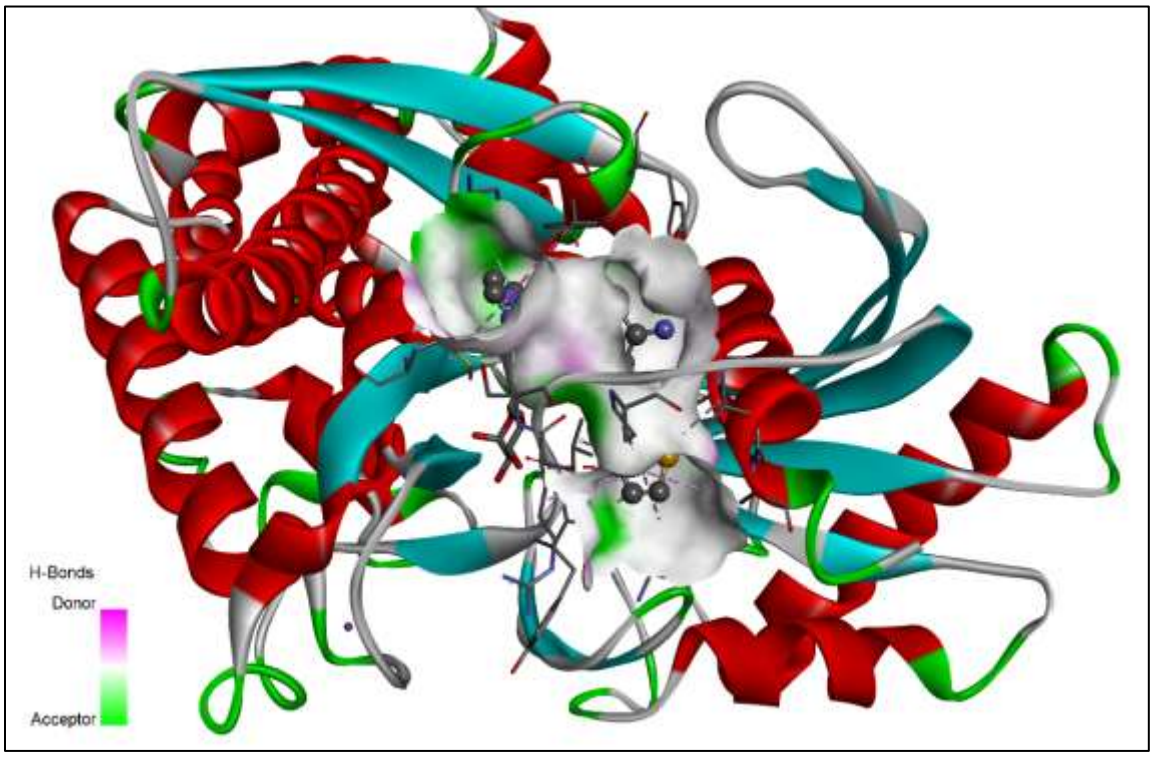


Figure 2. The identified active cavity with native ligand present in human GK (PDB ID: 1V4S)

3. Results and Discussion

3.1 Pre-ADMET Analysis

Table 1 summarizes the physical characteristics of common compounds. All of the compounds' physicochemical parameters were within the allowable range. Lipinski's rule of five includes logP and logS because of how important the drug's lipophilicity is. In the current study, all of these factors were found to be within the appropriate range, and they showed maximum oral bioavailability, suggesting they may be optimised for oral delivery[25,26].

The drug-likeness properties of molecules are exemplified in Table 2. The different parameters such as QED, NPscore, Lipinski rule, Pfizer rule, GSK rule, Golden Triangle, and Chelator rule were calculated. Most of the compounds showed attractive range of quantitative estimate of drug-likeness (QED) [27,28]. Typically, the natural product-likeness score, also known as the NPscore, falls somewhere in the range of -5 to 5[29,30]. All the molecules displayed NP-like properties. Both the compounds satisfy the GSK rule rule may have a more favorable ADMET profile. Both Swainsonine & Castanospermine rejected the Golden triangle rule. An absorption parameters of the molecules are illustrated in Table 3. As a model of how medications are absorbed by the human digestive tract, the human colon epithelial cancer cell line known as Caco-2 is employed. Fortunately both the compounds displayed optimum Caco-2 permeability[31]. Both the compounds displayed Pgp-substrate activity. None of the molecules displayed moderate inhibitory human intestinal absorption (HIA). F20% and F30% bioavailability of the molecules were within the range of acceptable values.

The distribution and metabolism profile of molecules are depicted in Table 4. Plasma protein binding (PPB, <90%), drugs with high protein-bound may have a low therapeutic index, both the compounds displayed PPB less

than 90%. Volume distribution (VD, optimal 0.04-20L/kg) of all the molecules were within the range of acceptable limit. Both of the molecules displayed strong BBB penetration potential. In present investigation, both the molecules showed CYP substrate potential[15].

Table 5 provides information on the molecular toxicity and excretion profile. Clearance rates were around average for both compounds. Each molecule only has a fleeting half-life. Many of the readings were within acceptable limits, and the compounds' toxicity profiles hinted to positive characteristics. Figure 3 displays the physicochemical radar of compounds, received from the ADMETlab 2.0 online server, which shows the favoured physicochemical characteristics of the molecules to be developed further [15]. An environmental toxicity profile (Bio concentration factors, IGC_{50}, $LC_{50}FM$, and $LC_{50}DM$) of molecules are demonstrated in Table 6. An environmental toxicity profile of the molecules was optimum and within the acceptable range.

Table 1. Physicochemical properties calculated for molecules

Code	Physicochemical Properties							
	Molecular Weight	Volume	nHA	nHD	nRot	TPSA	logS	logP
NL	349.50	310.799	6	3	4	89.060	-3.050	2.288
Swainsonine	173.110	167.179	4	3	0	63.93	0.038	-1.227
castanospermine	189.100	175.969	5	4	0	84.160	-0.069	-1.663

Table 2. Drug-likeness properties of molecules

Code	Medicinal Chemistry						
	QED	NPscore	Lipinski Rule	Pfizer Rule	GSK Rule	Golden Triangle	Chelator Rule
NL	0.711	-2.279	Accepted	Accepted	Accepted	Accepted	0 alert
Swainsonine	0.419	1.566	Accepted	Accepted	Accepted	Rejected	0
castanospermine	0.339	1.843	Accepted	Accepted	Accepted	Rejected	0

Table 3. An absorption parameters of molecules

Code	Absorption						
	Caco-2 Permeability	MDCK Permeability	Pgp-inhibitor	Pgp-substrate	HIA	F20%	F30%
NL	-4.358	2.8e-05	---	---	---	---	---
Swainsonine	-5.134	4.2e-05	---	+++	---	---	--
castanospermine	-5.255	7e-05	---	+++	--	---	+++

Table 4: Distribution and metabolism profile of molecules

Code	Distribution				Metabolism									
	PPB (%)	VD	BBB Penetration	Fu	CYP1A2		CYP2C19		CYP2C9		CYP2D6		CYP3A4	
					Inhibitor	Substrate	Inhibitor	Substrate	Inhibitor	Substrate	Inhibitor	Substrate	Inhibitor	Substrate
NL (1v4s)	69.060	1.156	+++	32.008	+++	+	+++	--	+	++	---	+	+	--
Swainsonine	12.137	1.003	--	84.070	---	---	---	++	----	--	---	+	---	--
Castanospermine	12.049	0.570	--	87.788	----	----	----	++	---	--	----	-	---	----

Table 5. Excretion and toxicity profile of molecules

Code	Excretion		Toxicity									
	CL	T1/2	H-HT	DILI	AMES Toxicity	Rat Oral Acute Toxicity	FDA MDD	Skin Sensitization	Carcinogencity	Eye Corrosion	Eye Irritation	Respiratory Toxicity
NL	6.091	0.260	+++	+++	+++	--	+++	--	++	---	---	+++
Swainsonine	2.588	0.829	--	---	---	---	--	--	---	---	-	++
Castanospermine	2.272	0.804	--	---	---	---	---	--	--	---	--	++

Table 6. Environmental toxicity profile of molecules

Code	Environmental toxicity			
	Bioconcentration Factors	IGC50	LC50FM	LC50DM
NL	0.635	3.825	3.952	4.896
Swainsonine	0.427	1.767	2.059	3.151
Castanospermine	0.279	1.5562	1.392	2.638

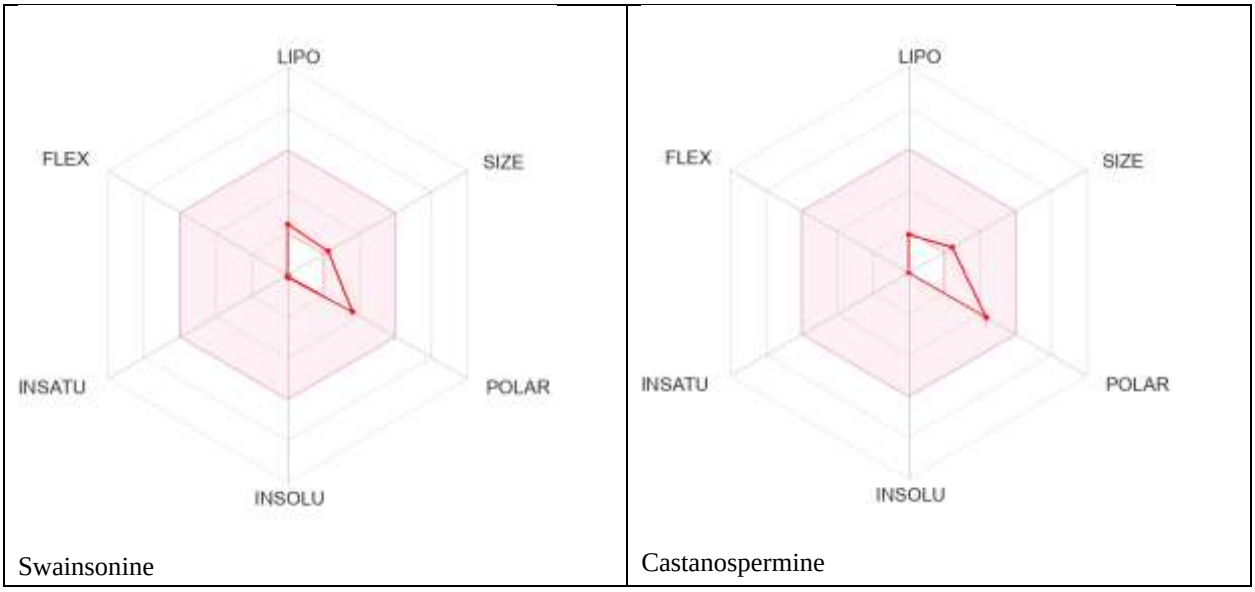


Figure 3. Physicochemical radar of the molecules obtained from ADMETlab 2.0 web server

3.2 Molecular Docking Studies

Molecular docking is a computer tool that enables us to digitally screen molecules to assess the ligand's preliminary activity potential against biological targets. This may be accomplished by determining the ligand's affinity for the target. The docking interactions of molecules are tabulated in Table 7 and the docking poses are exemplified in Table 8. The binding affinities of all the docked compounds have been compared with the binding mode of native ligand present in the crystal structure of GK enzyme (PDB ID: 1V4S).

Its native ligand displayed -7.2 kcal/mol of binding affinity and generated three conventional hydrogen bonds with Asp78, Lys169, Thr228, in addition to one carbon-hydrogen bond with Thr228. It established many electrostatic

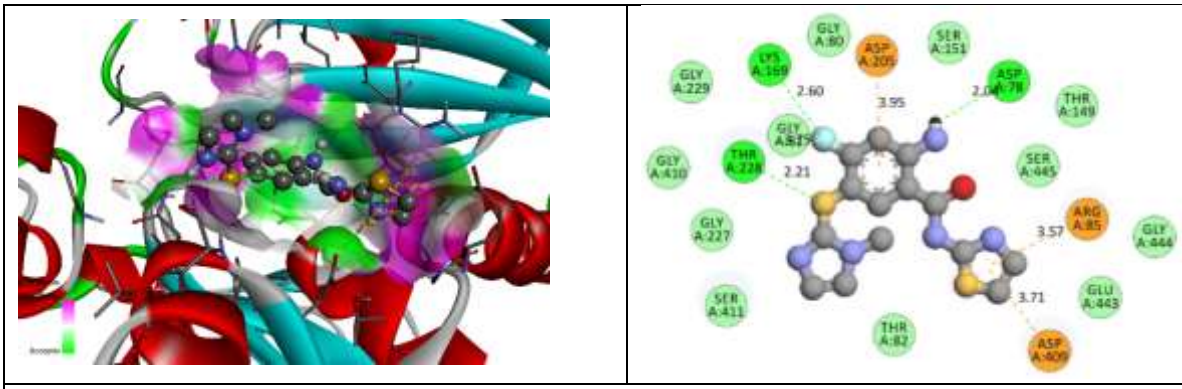
interactions, such as Pi-cation, Pi-anion bonds with Arg85, Asp205 and Asp409. Swainsonine exhibited -6 kcal/mol of binding affinity and formed hydrogen bonds three conventional and three carbon hydrogen bonds with Ser151, Lys169 and Asp205. It also showed hydrophobic interactions (Alkyl) with Ile225. Castanospermine displayed -5.7 kcal/mol of binding affinity and formed two conventional hydrogen bonds with Asp205 and Asp78. The observation revealed that both compounds exhibited the ability to form conventional hydrogen bonds with the GK enzyme, suggesting their potential as activators of the enzyme. By employing the strategy of synthesizing diverse semisynthetic derivatives, it is plausible to enhance the efficacy of GK activation. Given that both of these molecules exhibit a range of drug-like properties, it is reasonable to consider their potential for further development as GK activator.

Table 7. The binding interactions of molecules with GK enzyme

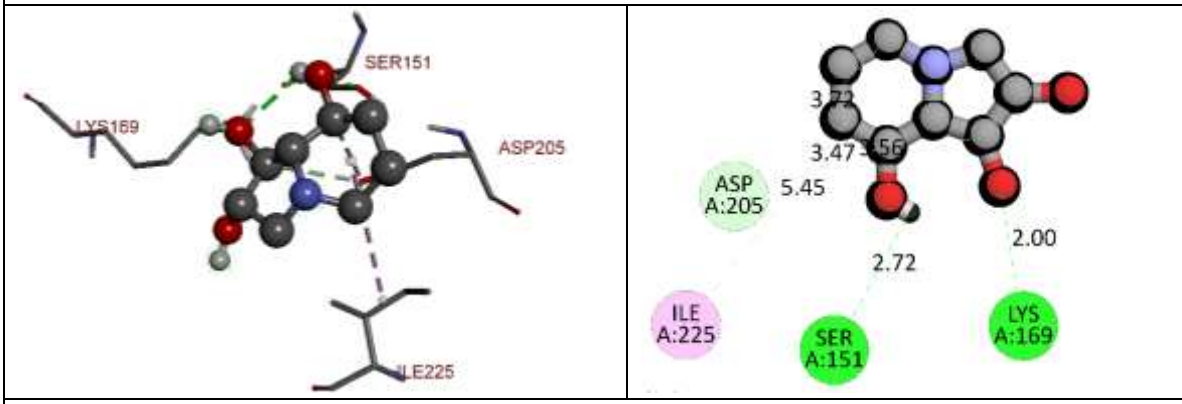
Active amino acid residues	**Bond length (A0)**	**Bond category**	**Bond types**	**Ligand energy (kcal/mol)**	**Binding affinity (kcal/mol)**
NL					
ASP78	2.04258	Hydrogen Bond	Conventional Hydrogen Bond	689.51	-7.2
LYS169	2.60065	Hydrogen Bond; Halogen	Conventional Hydrogen Bond; Halogen (Fluorine)		
THR228	2.20855	Hydrogen Bond	Conventional Hydrogen Bond		
THR228	3.75081		Carbon Hydrogen Bond		
ARG85	3.56863	Electrostatic	Pi-Cation		
ASP205	3.9455		Pi-Anion		
ASP409	3.70544				
Swainsonine					
SER151	2.56518	Hydrogen Bond	Conventional Hydrogen Bond	193.54	-6
	2.72383				
LYS169	2.0038				
ASP205	3.55985		Carbon Hydrogen Bond		
ASP205	3.71608				
ASP205	3.4733				
ILE225	5.4484	Hydrophobic	Alkyl		
Castanospermine					
ASP205	2.87033	Hydrogen Bond	Conventional Hydrogen Bond	166.4	-5.7
ASP78	2.34048				

Table 8. The docking poses of molecules

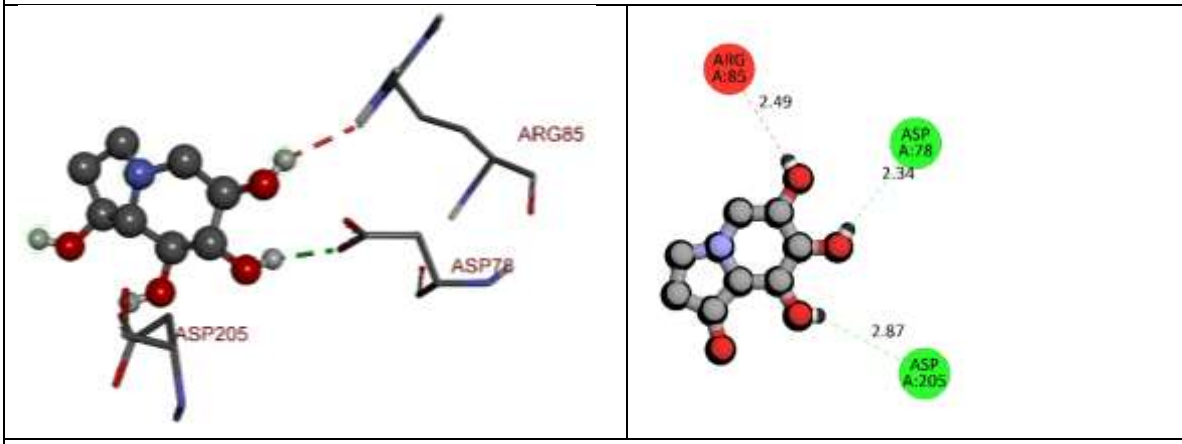
3D-docking poses	2D-docking poses



Native ligand



Swainsonine



Castanospermine

Conclusion

Glucokinase, also known as GK, is an enzyme that plays an important role in the process of glucose sensing and is responsible for the synthesis of glucose into glucose-6 phosphate. As a result, substances that activate GK might be used as a potential treatment for type 2 diabetes. In this research, we investigated the efficacy of Swainsonine and Castanospermine as GK activators in the treatment of type 2 diabetes. It was determined to perform a comprehensive investigation on the absorption, distribution, metabolism, excretion, and toxicity (ADMET) characteristics of the substances. After that, molecular docking experiments were carried out in order to explore the possible binding affinity of these compounds with the GK enzyme. Based on this discovery, it can be deduced that both chemicals possess the capability to create conventional hydrogen bonds with the GK enzyme, which indicates that they have the potential to

operate as activators of the enzyme. It is possible to improve the efficiency of GK activation by using the tactic of synthesising a variety of semisynthetic derivatives as part of the overall plan. It is acceptable to contemplate the possibility of either of these compounds being developed further into a GK activator given that both of them demonstrate a variety of drug-like features.

References

(1) Mendonça, F. S.; Filho, G. B. S.; Chaves, H. A. S.; Aires, L. D. A.; Braga, T. C.; Gardner, D. R.; Cook, D.; Buril, M. T. Detection of Swainsonine and Calystegines in Convolvulaceae Species from the Semiarid Region of Pernambuco. Pesqui. Vet. Bras. **2018**, 38 (11), 2044–2051. https://doi.org/10.1590/1678-5150-PVB-5945.

(2) Ren, Z.; Song, R.; Wang, S.; Quan, H.; Yang, L.; Sun, L.; Zhao, B.; Lu, H. The Biosynthesis Pathway of Swainsonine, a New Anticancer Drug from Three Endophytic Fungi. J. Microbiol. Biotechnol. **2017**, 27 (11), 1897–1906. https://doi.org/10.4014/jmb.1709.09003.

(3) Neyaz, M.; Das, S.; Cook, D.; Creamer, R. Phylogenetic Comparison of Swainsonine Biosynthetic Gene Clusters among Fungi. J. Fungi **2022**, 8 (4). https://doi.org/10.3390/jof8040359.

(4) Whitby, K.; Pierson, T. C.; Geiss, B.; Lane, K.; Engle, M.; Zhou, Y.; Doms, R. W.; Diamond, M. S. Castanospermine, a Potent Inhibitor of Dengue Virus Infection In Vitro and In Vivo. J. Virol. **2005**, 79 (14), 8698–8706. https://doi.org/10.1128/jvi.79.14.8698-8706.2005.

(5) Clarke, E. C.; Nofchissey, R. A.; Ye, C.; Bradfute, S. B. The Iminosugars Celgosivir, Castanospermine and UV-4 Inhibit SARS-CoV-2 Replication. Glycobiology **2021**, 31 (4), 378–384. https://doi.org/10.1093/glycob/cwaa091.

(6) Gao, Q.; Zhang, W.; Li, T.; Yang, G.; Zhu, W.; Chen, N.; Jin, H. The Efficacy and Safety of Glucokinase Activators for the Treatment of Type-2 Diabetes Mellitus: A Protocol for Systematic Review and Meta-Analysis. Medicine (Baltimore). **2021**, 100 (7), e24873. https://doi.org/10.1097/MD.0000000000024873.

(7) Patel, H.; Pawara, R.; Surana, S. In-Silico Evidences for Binding of Glucokinase Activators to EGFR C797S to Overcome EGFR Resistance Obstacle with Mutant-Selective Allosteric Inhibition. Comput. Biol. Chem. **2018**, 74, 167–189. https://doi.org/10.1016/j.compbiolchem.2018.03.026.

(8) Filipski, K. J.; Futatsugi, K.; Pfefferkorn, J. A.; Stevens, B. D. Glucokinase Activators. Pharm. Pat. Anal. **2012**, 1 (3), 301–311. https://doi.org/10.4155/ppa.12.26.

(9) Coghlan, M.; Leighton, B. Glucokinase Activators in Diabetes Management. Expert Opin. Investig. Drugs **2008**, 17 (2), 145–167. https://doi.org/10.1517/13543784.17.2.145.

(10) Pal, M. Recent Advances in Glucokinase Activators for the Treatment of Type 2 Diabetes. Drug Discov. Today **2009**, 14 (15–16), 784–792. https://doi.org/10.1016/j.drudis.2009.05.013.

(11) Khan, A.; Unnisa, A.; Sohel, M.; Date, M.; Panpaliya, N.; Saboo, S. G.; Siddiqui, F.; Khan, S. Investigation of Phytoconstituents of Enicostemma Littorale as Potential Glucokinase Activators through Molecular Docking for the Treatment of Type 2 Diabetes Mellitus. Silico Pharmacol. **2021**, 10 (1). https://doi.org/10.1007/s40203-021-00116-8.

(12) Kim, S.; Chen, J.; Cheng, T.; Gindulyte, A.; He, J.; He, S.; Li, Q.; Shoemaker, B. A.; Thiessen, P. A.; Yu, B.; Zaslavsky, L.; Zhang, J.; Bolton, E. E. PubChem in 2021: New Data Content and Improved Web Interfaces. Nucleic Acids Res. **2021**, 49 (D1), D1388–D1395. https://doi.org/10.1093/nar/gkaa971.

(13) Molinspiration Cheminformatics. Choice Rev. Online **2006**, 43 (11), 43-6538-43–6538. https://doi.org/10.5860/choice.43-6538.

(14) Daina, A.; Michielin, O.; Zoete, V. SwissADME: A Free Web Tool to Evaluate Pharmacokinetics, Drug-Likeness and Medicinal Chemistry Friendliness of Small Molecules. Sci. Rep. **2017**, 7. https://doi.org/10.1038/srep42717.

(15) Xiong, G.; Wu, Z.; Yi, J.; Fu, L.; Yang, Z.; Hsieh, C.; Yin, M.; Zeng, X.; Wu, C.; Lu, A.; Chen, X.; Hou, T.; Cao, D. ADMETlab 2.0: An Integrated Online Platform for Accurate and Comprehensive Predictions of ADMET Properties. Nucleic Acids Res. **2021**, 49 (W1), W5–W14. https://doi.org/10.1093/nar/gkab255.

(16) Rappé, A. K.; Casewit, C. J.; Colwell, K. S.; Goddard, W. A.; Skiff, W. M. UFF, a Full Periodic Table Force Field for Molecular Mechanics and Molecular Dynamics Simulations. J. Am. Chem. Soc. **1992**, 114 (25), 10024–10035. https://doi.org/10.1021/ja00051a040.

(17) Dallakyan, S.; Olson, A. J. Small-Molecule Library Screening by Docking with PyRx. Methods Mol. Biol. **2015**, 1263 (1263), 243–250. https://doi.org/10.1007/978-1-4939-2269-7_19.

(18) San Diego: Accelrys Software Inc. Discovery Studio Modeling Environment, Release 3.5. Accelrys Softw. Inc. **2012**.

(19) Khan, Sharuk L; Siddiui, F. A. Beta-Sitosterol: As Immunostimulant, Antioxidant and Inhibitor of SARS-CoV-2 Spike Glycoprotein. Arch. Pharmacol. Ther. **2020**, 2 (1). https://doi.org/10.33696/pharmacol.2.014.

(20) Siddiqui, F. A.; Khan, S. L.; Marathe, R. P.; Nema, N. V. Design, Synthesis, and In Silico Studies of Novel N-(2-Aminophenyl)-2,3-Diphenylquinoxaline-6-Sulfonamide Derivatives Targeting Receptor- Binding Domain (RBD) of SARS-CoV-2 Spike Glycoprotein and Their Evaluation as Antimicrobial and Antimalarial Agents. Lett. Drug Des. Discov. **2021**, 18 (9), 915–931. https://doi.org/10.2174/1570180818666210427095203.

(21) Chaudhari, R. N.; Khan, S. L.; Chaudhary, R. S.; Jain, S. P.; Siddiqui, F. A. B-Sitosterol: Isolation from Muntingia Calabura Linn Bark Extract, Structural Elucidation And Molecular Docking Studies As Potential Inhibitor of SARS-CoV-2 Mpro (COVID-19). Asian J. Pharm. Clin. Res. **2020**, 13 (5), 204–209. https://doi.org/10.22159/ajpcr.2020.v13i5.37909.

(22) Khan, S. L.; Siddiqui, F. A.; Shaikh, M. S.; Nema, N. V.; Shaikh, A. A. Discovery of Potential Inhibitors of the Receptor-Binding Domain (RBD) of Pandemic Disease-Causing SARS-CoV-2 Spike Glycoprotein from Triphala through Molecular Docking. Curr. Chinese Chem. **2021**, 01. https://doi.org/10.2174/2666001601666210322121802.

(23) Khan, S. L.; Sonwane, G. M.; Siddiqui, F. A.; Jain, S. P.; Kale, M. A.; Borkar, V. S. Discovery of Naturally Occurring Flavonoids as Human Cytochrome P450 (CYP3A4) Inhibitors with the Aid of Computational Chemistry. Indo Glob. J. Pharm. Sci. **2020**, 10 (04), 58–69. https://doi.org/10.35652/igjps.2020.10409.

(24) Khan, S.; Kale, M.; Siddiqui, F.; Nema, N. Novel Pyrimidine-Benzimidazole Hybrids with Antibacterial and Antifungal Properties and Potential Inhibition of SARS-CoV-2 Main Protease and Spike Glycoprotein. Digit. Chinese Med. **2021**, 4 (2), 102–119. https://doi.org/10.1016/j.dcmed.2021.06.004.

(25) Waring, M. J. Lipophilicity in Drug Discovery. Expert Opin. Drug Discov. **2010**, 5 (3), 235–248. https://doi.org/10.1517/17460441003605098.

(26) Lobo, S. Is There Enough Focus on Lipophilicity in Drug Discovery? Expert Opin. Drug Discov. **2020**, 15 (3), 261–263. https://doi.org/10.1080/17460441.2020.1691995.

(27) Bickerton, G. R.; Paolini, G. V.; Besnard, J.; Muresan, S.; Hopkins, A. L. Quantifying the Chemical Beauty of Drugs. Nat. Chem. **2012**, 4 (2), 90–98. https://doi.org/10.1038/nchem.1243.

(28) Kosugi, T.; Ohue, M. Quantitative Estimate Index for Early-Stage Screening of Compounds Targeting Protein-Protein Interactions. Int. J. Mol. Sci. **2021**, 22 (20). https://doi.org/10.3390/ijms222010925.

(29) Ertl, P.; Roggo, S.; Schuffenhauer, A. Natural Product-Likeness Score and Its Applications in the Drug Discovery Process. Chem. Cent. J. **2008**, 2 (S1). https://doi.org/10.1186/1752-153x-2-s1-s2.

(30) Menke, J.; Massa, J.; Koch, O. Natural Product Scores and Fingerprints Extracted from Artificial Neural Networks. Comput. Struct. Biotechnol. J. **2021**, 19, 4593–4602. https://doi.org/10.1016/j.csbj.2021.07.032.

(31) Lee, J. B.; Zgair, A.; Taha, D. A.; Zang, X.; Kagan, L.; Kim, T. H.; Kim, M. G.; Yun, H. yeol; Fischer, P. M.; Gershkovich, P. Quantitative Analysis of Lab-to-Lab Variability in Caco-2 Permeability Assays. Eur. J. Pharm. Biopharm. **2017**, 114, 38–42. https://doi.org/10.1016/j.ejpb.2016.12.027.