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Anti-Serine/threonine-protein kinase-4 (STK4) Potential of some *Cannabis* Extract compounds: *In Silico* Study

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Abstract

The increasing prevalence of diabetes is one of the most critical health challenges worldwide. Serine/threonine-protein kinase-4 (STK4) is vital not only in various cellular processes, but also in diabetes. *Cannabis* is a plant species that contains multiple medicinal compounds. This study examined whether the six compounds found in *Cannabis* extract can inhibit the STK4 protein present in diabetes. The crystallized structure (pdb format) of *Cannabis* extract compounds was obtained from the PubChem database and used as ligands. Using the mm^2 method, the ligand's structure was optimized. AutodockVina was employed to assess the ligand's effectiveness as an inhibitor against the active site of STK4 chains (A and B). The results generated were analyzed and evaluated using Discovery Studio v16.1.0 software. Toxicity prediction of the best inhibitor was done by ProTox-II. The best affinity was obtained against 6YAT -chain A by -9.1 kcal/mol. The highest diversity of links was also reported in Ligand C with 6YAT -chain A. Hydrogen bonds were established with 6YAT -chain A against Tyrosine: 104, Arginine: 245, and Phenylalanine: 244, indicating the effectiveness of delta (9)-Tetrahydrocannabinolic acid against chain A of 6YAT. Toxicity prediction showed that all pharmacokinetic parameters of the ligand C molecule are in the acceptable range. Our study provided valuable information about newly identified inhibitors for the treatment of diabetes. The findings of this study indicated that the delta (9)-Tetrahydrocannabinolic acid molecule could be used as a novel STK4 inhibitor in future studies.

1. Introduction

Diabetes mellitus, also known as sugar diabetes, is an endocrine disorder related to the insufficient production of insulin or the loss of sensitivity to secreted insulin (Type 2 diabetes), resulting in the disease of diabetes mellitus (Mukhtar et al., 2020). The most frequent kind of diabetes found in young individuals is Type 1 diabetes, and its occurrence is on the rise in both developed and developing nations (Magliano et al., 2020). However, 85 to 95 percent of diabetes in developing countries is Type 2 (Misra et al., 2019). About 285 million individuals aged 20 to 79 had diabetes in 2010, which is projected to increase to 438 million by 2030 (Altumairah et al., 2021). Immune response, genetic factors, and environmental factors are among the causes of Type 1 diabetes (Dedrick et

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al., 2020). Research has indicated that the depletion of beta cells in the pancreas is a crucial factor in the development of both Type 1 and Type 2 diabetes (Eizirik et al., 2020). Apoptosis induced in pancreatic beta cells leads to the loss of insulin-producing cells in Type 1 diabetes with a rapid progression and in Type 2 diabetes (with a slower progression) (Dettmer et al., 2022). Other mechanisms, such as loss of differentiation in pancreatic beta cells or disruption of replication, have also been suggested for the loss of these cells (Moin et al., 2019). One study has shown that complex mechanisms are involved in the disruption of beta cells (Roep et al., 2021). Several protein-inducing factors that activate apoptosis in beta cells through cascading signaling pathways have been identified. Therefore, there is an urgent need to discover and identify compounds that can inhibit apoptosis in pancreatic beta cells (Zhang et al., 2023).

Treatments currently prescribed for Type 1 and 2 diabetes only reduce the symptoms of the disease without preventing the loss of beta cells or curing the disease (Donath et al., 2019). For example, these drugs normalize blood sugar by increasing insulin secretion from the remaining beta cells or by improving cell sensitivity to insulin in Type 2 diabetes (Whitticar et al., 2020). STK4 is a vital enzyme involved in the signaling of the Hippo pathway, which has a significant function in cellular activities such as proliferation, stress response, and apoptosis (Bata et al., 2021). This enzyme, from the kinase family, leads to cell death by interacting with LATS1/2 proteins, histone H2B, FOXO family proteins, and Caspase 3 (Wei et al., 2023). Regarding tumor genesis, the STK4 enzyme plays a tumor suppressor role (Doghish et al., 2022). Another study has shown that high expression of the STK4 gene has a direct correlation with increased survival in breast cancer patients as a tumor suppressor, can form a complex with the MST2 enzyme and regulate oxidative stress and cellular regeneration. Therefore, STK4 can be a good target for drug design for the treatment of diabetes (Russell et al., 2022).

The chemical compounds of *Cannabis* include Colin, Tergonelin, and various terpenoids such as Cannabinol and Cannabidiol (Radwan et al., 2021). Structures in *Cannabis* can induce relaxation, reduce pain, promote sleep, and improve appetite while alleviating symptoms of nausea, vomiting, inflammation, and other health issues. It has been used to treat various conditions such as multiple sclerosis, neurological disorders (such as Parkinson's disease, Huntington's disease, Tourette's syndrome, and Alzheimer's disease), epilepsy, glaucoma, osteoporosis, schizophrenia, cardiovascular disorders, cancer, and obesity (Brown et al., 2019), and metabolic syndrome-related disorders (Pérez-Acevedo et al., 2021). Due to the very high cost of drug production in the laboratory and the time-consuming nature of this process, computers and computational software are widely used for drug design and discovery.

Molecular docking is extensively used in the early stages of drug discovery to identify potential lead compounds. By simulating the binding of thousands of small molecules to a target protein, researchers can predict their binding affinity and select the most promising candidates for further experimental testing. This helps in reducing the time and cost involved in the drug development process. Also, Molecular docking can be used to predict the three-dimensional structure of proteins by simulating the binding of known ligands to the target protein. By analyzing the binding poses and interactions, researchers can obtain valuable insights into the protein's structure and function. This information is crucial for understanding disease mechanisms and designing targeted therapies. Since no research has been conducted on the important molecules of the *Cannabis* plant in inhibiting the Serine/threonine-protein kinase-4 enzyme in diabetic patients, the aim of this research was to use molecular simulation to examine the capacity of the six primary components of the *Cannabis* extract to inhibit the Serine/threonine-protein kinase-4 (STK4) enzyme present in diabetes.

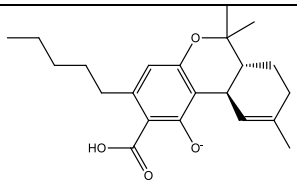
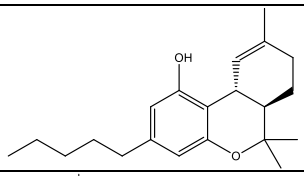
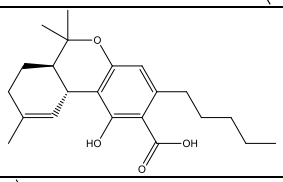
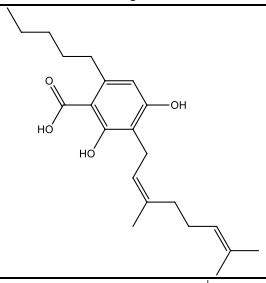
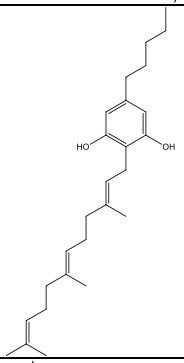
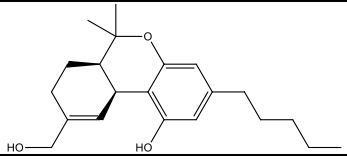
2. Materials and Methods

2.1. Ligands preparation

The PubChem database, an open chemistry database at the National Institutes of Health (NIH),

was utilized to obtain the SDF format of the crystalline structure of six significant molecules found in the *Cannabis* extract, which were then used as ligands in the study. Next, the optimized ligands and total energy were calculated using the MM^2 Job command in Chem3D v20.1.1.125 software (Table. 1) (SarveAhrabi, 2021).

Table 1: Name, Chemical Structure, Chemical Formula, Molecular Weight, PubChem CID, and Total Energy of ligands (A-F).

Name	Structure	Chemical Formula	Molecular Weight (g/mol)	PubChem CID	Total Energy (kcal/mol)
Delta(9)-Tetrahydrocannabinolate (ligand A)		$C_{22}H_{29}O_4^-$	357.5	70678828	24.2693
delta-9-cis-Tetrahydrocannabinol (ligand B)		$C_{21}H_{30}O_2$	314.5	12831993	18.3100
delta(9)-Tetrahydrocannabinolic acid (ligand C)		$C_{22}H_{30}O_4$	358.5	98523	34.7105
cannabinerolic acid (ligand D)		$C_{22}H_{32}O_4$	360.5	9998639	19.8859
Sesquicannabigerol (ligand E)		$C_{26}H_{40}O_2$	384.6	54669855	1.9274
11-Hydroxy-DELTA9-tetrahydrocannabinol (ligand F)		$C_{21}H_{30}O_3$	330.5	644022	15.8161

2.2. Protein preparation

The crystallized structure of STK-4 (PDB ID: 6YAT) was downloaded from the Protein Data Bank with an exactitude of 3 angstroms. The structure of the protein consists of a pair of chains, chain A and chain B, which were prepared for ligand binding with Discovery Studio 4.5. Gastiger charge and polar hydrogen were added with the AutoDockTools-1.5.6 software. All water molecules and ligands were deleted from the main chains of each structure. The grid box for 6YAT-chain A was 70×70×70 and 62×62×62 for 6YAT-chain B with spacing 1°A (Zarrabi Ahrabi et al., 2022).

2.3. Molecular docking

AutoDockVina software and Discovery Studio 4.5 were utilized to conduct the ligand docking process to the binding sites of chain A and chain B of 6YAT. Discovery Studio 4.5 Client software was employed to investigate and analyze the interactions between the ligand and the junction. The docking calculations were performed using a genetic optimization algorithm and Lamarck traits, with specific configurations such as a maximum of 25,000,000 energy assessments, an initial population of 150 randomly assigned a maximum of 27,000 generations, a 0.02 mutation rate, a 0.8 crossover rate, and an elitism value. The Solis algorithm was used for local search, with a maximum of 1000 repetitions per search. The protein was considered inflexible, while the ligand was considered flexible during the process (Morris and Lim-Wilby, 2008).

2.4. Prediction of toxicity

The toxicity of the compounds was calculated using the ProTox-II web server. This involved predicting and determining the probability of various forms of toxicity, including hepatotoxicity, carcinogenicity, immunotoxicity, mutagenicity, cytotoxicity, and the activation of various receptors such as the Aryl hydrocarbon Receptor (AhR), Androgen Receptor (AR), Androgen Receptor Ligand Binding Domain (AR-LBD), Aromatase, Estrogen Receptor Alpha (ER), Estrogen Receptor Ligand Binding Domain (ER-LBD), Peroxisome Proliferator Activated Receptor Gamma (PPAR-Gamma), Nuclear factor (erythroid-derived 2)-like 2/antioxidant responsive element (nrf2/ARE), Heat shock factor response element (HSE), Mitochondrial Membrane Potential (MMP), Phosphoprotein (Tumor Suppressor) p53, and ATPase family AAA domain-containing protein 5 (ATAD5). These calculations were carried out to determine the best ligand (Banerjee et al. 2018).

3. Results and Discussion

3.1. Molecular docking

Computational and molecular modeling methods have achieved significant success in solving biological problems and challenges. Identifying and optimizing lead compounds are essential steps in discovering and developing drugs (Alber et al., 2019). This investigation utilized computer simulations to explore the potential of the six primary compounds present in Cannabis extract to hinder the STK4 enzyme associated with diabetes. STK4, also referred to as MST1 (mammalian sterile 20-like kinase 1), is a serine/threonine-protein kinase encoded by the STK4 gene in humans. It belongs to the serine/threonine kinase family and is involved in regulating cellular functions like cell cycle regulation, apoptosis, and cellular stress response. The disruption of STK4 has been linked to the emergence and advancement of different illnesses, including neurodegenerative disorders and cancer. A study revealed that STK4 contributes to the control of insulin sensitivity and glucose metabolism in muscle cells. The study demonstrated that mice lacking STK4 exhibited better glucose tolerance, insulin sensitivity, and less adiposity when consuming a high-fat diet in comparison to wild-type mice.

Another study suggested that STK4 might be associated with the regulation of pancreatic beta-cell activity, which is essential in maintaining glucose homeostasis (Cagdas et al., 2021). According to the research, mice without STK4

exhibited enhanced glucose-stimulated insulin secretion and glucose tolerance in contrast to wild-type mice. Overall, these studies suggest that STK4 may indirectly contribute to the development of diabetes through its effects on insulin sensitivity, glucose metabolism, and pancreatic beta-cell function. Further investigation is required to gain a comprehensive understanding of the function of STK4 in the development of diabetes.

Cannabis is a plant species that contains various psychoactive compounds, most notably delta-9-tetrahydrocannabinol (THC) and cannabidiol (CBD) (Pombo et al., 2019). Cannabis has been used for medicinal, spiritual, and recreational purposes for thousands of years. THC, the main psychoactive constituent found in cannabis, is accountable for the euphoric sensation linked with the consumption of marijuana. THC binds to cannabinoid receptors in the brain and nervous system, leading to a range of effects, including altered perception, mood, and cognition. CBD, on the other hand, does not produce a high and has been studied for its potential therapeutic benefits. CBD interacts with the endocannabinoid system, which plays a role in regulating a range of physiological functions such as pain, inflammation, and anxiety. Cannabis is utilized for various medical purposes, such as alleviating pain, reducing nausea and vomiting caused by chemotherapy, and easing muscle spasms in individuals with multiple sclerosis (Fragoso et al., 2020).

In this study, six important compounds of Cannabis,including Delta(9)-Tetrahydrocannabinolate,delta-9-cis-Tetrahydrocannabinol,delta(9)-tetrahydrocannabinolic acid, cannabinerolic acid, Sesquicannabigerol, and 11-Hydroxy-DELTA9-tetrahydrocannabinol, were used to predict the STK4 enzyme. After calculating all affinities, the best affinity for each receptor with a low ΔG (-ΔG bind) was chosen from Table 1 to proceed with AutoDock interactions. Ligand (A to F) interactions within the active sites of 6YAT -chain A and 6YAT -chain B are shown in (Table 1 and Fig 2). Among all the ligands, Ligand C showed the best performance in inhibiting 6YAT-chain A. This ligand also had the highest binding affinity, with an affinity of -9.1, compared to other ligands. Ligand C links in order with three strong hydrogen bonds with the amino acids Tyrosine: 104, Arginine: 245, and Phenylalanine: 244, forming the 6YAT-chain A receptor. Docking results showed that Ligand A with 6YAT-chain B, Ligand D with 6YAT-chain A, and Ligand E with 6YAT-chain B formed acceptable hydrogen bonds. Ligand A inhibited the active site of 6YAT-chain B with amino acids Leucine: 36 and Aspartic acid: 112. Ligand D inhibited the active site of 6YAT-chain A with amino acids Cysteine: 105 and Aspartic acid: 112, and Ligand E inhibited the active site of 6YAT-chain B with amino acids Aspartic acid: 112 and Cysteine: 105. According to the results of the predicted conformational conformations, the Ligand C combination shows valuable inhibitory potential and can be used as an agent to develop alternative drug structures for treating different forms of diabetes in future research.

The best ligand to inhibit the STK4 was delta (9)-Tetrahydrocannabinolic acid, which inhibited chain A of the whole STK4 structure with an affinity of -9.1. In the molecular docking part, ligand C inhibited chain A of the whole STK4 by interacting with Tyrosine 104, Arginine 245, and Phenylalanine 244.

3.2. Prediction of toxicity

Ligand C was selected for this section. As shown in (Table 3), Prediction of Carcinogenicity, Mutagenicity and Cytotoxicity were inactive with a probability of 0.62, 0.97, and 0.93, respectively. Prediction of Hepatotoxicity and Immunotoxicity were active with a probability of 0.69.

Toxicity studies showed that the prediction of Carcinogenicity, Mutagenicity, and Cytotoxicity were inactive, while Hepatotoxicity and Immunotoxicity were active.

Table 2: AutoDockVina results of ligands (A-F) as an inhibitor of chain A and chain B of 6YAT

-	Receptor	Affinity (kCal/mol)	Hydrogen Bond	Pi-Alkyl Bond	Pi-Sigma Bond
Ligand A	***6YAT -chain A***	-7.6	Glutamine: 38	Leucine: 36 Leucine: 156 Alanine: 57 Alanine: 166 Valine: 44 Methionine: 102 Lysine: 59	-
	6YAT -chain B	-7.7	Leucine: 36 Aspartic acid: 112	Leucine: 156 Cysteine: 105 Tyrosine: 104 Alanine: 57 Valine:44	-
Ligand B	***6YAT -chain A***	-6.4	-	Leucine: 36 Alanine: 166 Alanine: 57 Valine: 44 Tyrosine: 104	Leucine: 156
	6YAT -chain B	-6.7	-	Leucine: 36 Leucine: 156 Alanine: 57 Alanine: 166 Tyrosine: 104 Methionine: 102	Valine: 44
Ligand C	***6YAT -chain A***	-9.1	Tyrosine: 104 Arginine: 245 Phenylalanine:244	-	-
	6YAT -chain B	-7.1	Cysteine: 105	Lysine: 301 Lysine: 46 Leucine: 116 Leucine: 156 Alanine: 166 Alanine: 57 Methionine: 102 Valine: 44	Leucine: 36
Ligand D	***6YAT -chain A***	-7.6	Cysteine: 105 Aspartic acid: 112	Leucine: 116 Arginine: 304 Lysine: 301 Valine: 44	-
	6YAT -chain B	-7.1	Cysteine: 105	Leucine: 156 Leucine: 116 Leucine: 36 Lysine: 301 Valine: 44	-
Ligand E	***6YAT -chain A***	-6.8	Aspartic acid: 112	Lysine: 301 Leucine: 116 Valine: 44 Methionine: 102	Leucine: 36
	6YAT -chain B	-7.4	Aspartic acid: 112 Cysteine: 105	Lysine: 301 Methionine: 102 Methionine: 298 Alanine: 57 Tyrosine: 104 Leucine: 156 Valine: 44	Leucine: 36
Ligand F	***6YAT -chain A***	-6.0	-	Cysteine: 203 Alanine: 272	-
	6YAT -chain B	-6.8	-	Valine: 29 Valine: 56 Tyrosine: 89	Valine: 101

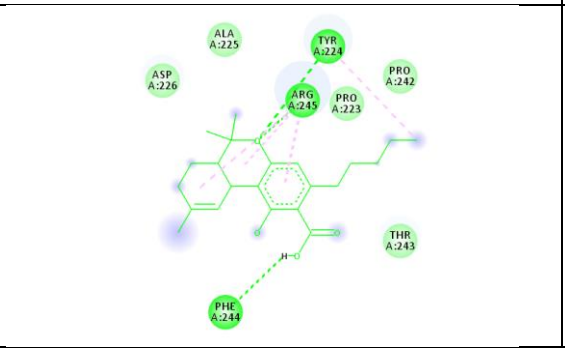
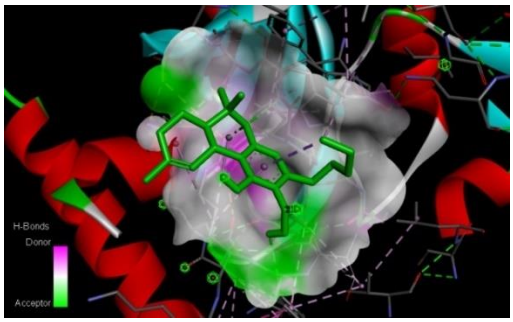
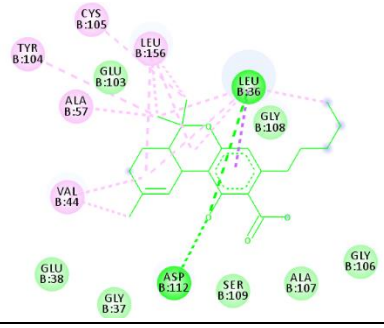
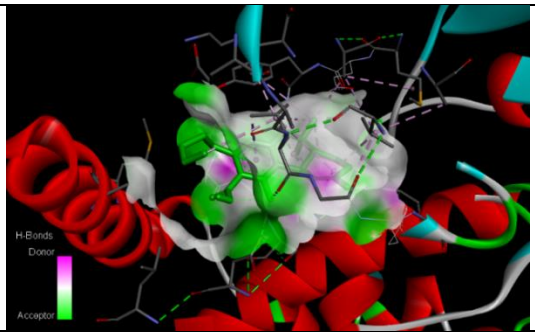
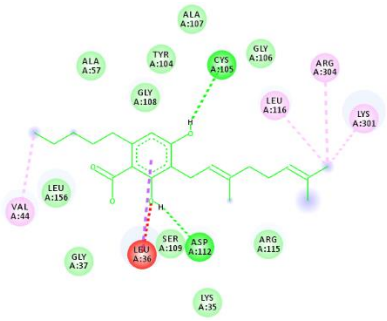
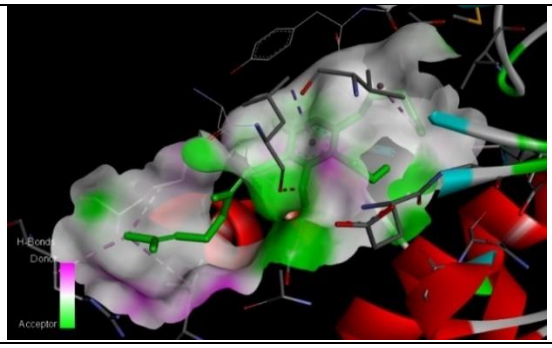
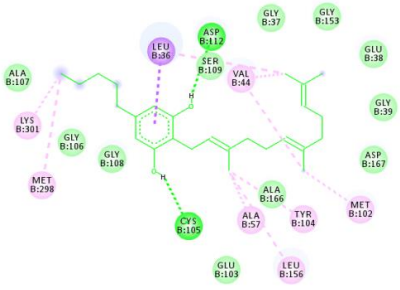
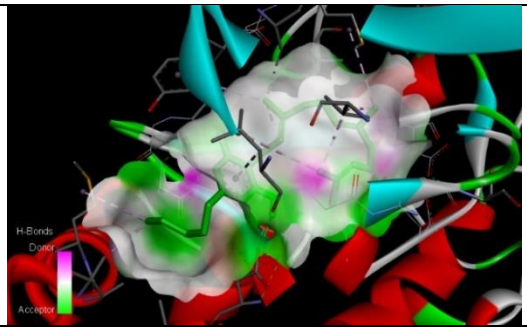
6YAT-Chain A with Ligand C	
2D	3D
	
6YAT-Chain B with Ligand A	
2D	3D
	
6YAT-Chain A with Ligand D	
2D	3D
	
6YAT-Chain B with Ligand E	
2D	3D
	

Figure 2. 2D and 3D results of AutoDockVina

Table 3: Results related to predicting the toxicity of the ligand C.

Classification	Target	Shorthand	Prediction	Probability
Organ toxicity	Hepatotoxicity	dili	Active	0.69
Toxicity end points	Carcinogenicity	carcino	Inactive	0.62
Toxicity end points	Immunotoxicity	immuno	Active	0.69
Toxicity end points	Mutagenicity	mutagen	Inactive	0.97
Toxicity end points	Cytotoxicity	cyto	Inactive	0.93
Tox21-Nuclear receptor signalling pathways	Aryl hydrocarbon Receptor (AhR)	nr_ahr	Inactive	0.97
Tox21-Nuclear receptor signalling pathways	Androgen Receptor (AR)	nr_ar	Inactive	0.99
Tox21-Nuclear receptor signalling pathways	Androgen Receptor Ligand Binding Domain (AR-LBD)	nr_ar_lbd	Inactive	0.99
Tox21-Nuclear receptor signalling pathways	Aromatase	nr_aromatase	Active	1.0
Tox21-Nuclear receptor signalling pathways	Estrogen Receptor Alpha (ER)	nr_er	Active	0.99
Tox21-Nuclear receptor signalling pathways	Estrogen Receptor Ligand Binding Domain (ER-LBD)	nr_er_lbd	Active	1.0
Tox21-Nuclear receptor signalling pathways	Peroxisome Proliferator Activated Receptor Gamma (PPAR-Gamma)	nr_ppar_gamma	Inactive	0.99
Tox21-Stress response pathways	Nuclear factor (erythroid-derived 2)-like 2/antioxidant responsive element (nrf2/ARE)	sr_are	Inactive	0.88
Tox21-Stress response pathways	Heat shock factor response element (HSE)	sr_hse	Inactive	0.88
Tox21-Stress response pathways	Mitochondrial Membrane Potential (MMP)	sr_mmp	Inactive	0.70
Tox21-Stress response pathways	Phosphoprotein (Tumor Supressor) p53	sr_p53	Inactive	0.96
Tox21-Stress response pathways	ATPase family AAA domain-containing protein 5 (ATAD5)	sr_atad5	Inactive	0.99

4. Conclusion

Diabetes remains a major health problem in this century and no drug treatment has yet been developed for this disease. This study used biocomputational methods such as molecular docking and toxicity prediction to target the STK4 enzyme, a protein target whose role in the occurrence of diabetes has recently been discovered, in order to identify an inhibitor. This enzyme plays an essential role in both apoptosis and Type 1 and 2 diabetes. With the use of computer hardware and software, the costs and time needed for the complex process of drug discovery are significantly reduced. At the end of the calculation phase, one molecule was proposed as an inhibitor, based on the binding free energy of delta(9)-Tetrahydrocannabinolic acid as the strongest inhibitor studied by molecular docking against the A chain. The results of molecular

docking showed that this ligand inhibits the A chain of this enzyme by forming a hydrogen bond with the amino acids Tyrosine: 104, Arginine: 245, and Phenylalanine: 244. Binding of this inhibitor to the enzyme did not cause a significant change in the secondary structure of STK4, which suggests that the enzyme-inhibitor complex is stable. Molecular simulation data indicate that delta (9)-Tetrahydrocannabinolic acid inhibitor can be tested experimentally and developed as a drug against Type 1 and 2 diabetes.

Conflict of interest

There is no conflict of interests.

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Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

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