Discovery of Potential Anti-Ischemic Stroke Agents Through Inhibiting Sulfonylurea Receptor 1 (SUR1): A Pharmacophore-based Screening, Docking, and Molecular Dynamic Simulation

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Abstract: Stroke is the leading cause of disability and death worldwide. Inhibition of sulfonylurea 1 receptor (SUR1) using glibenclamide previously has been studied in CNS ischemic tissues and faster recovery from stroke injury in different animal models of stroke. Unfortunately, glibenclamide cannot enter the brain through an intact brain membrane (BBB) due to its ionization at physiological pH. Therefore, it was hypothesized that compounds with structural properties similar to glibenclamide but with the ability to penetrate through BBB would be superior to glibenclamide in ischemic stroke. Docking energy and interactions of glibenclamide with SUR1 active site were assessed using AutoDock Vina. NCI databases search engines with limitations for penetration to CNS were used to find the best compounds with desired properties. Then two selected compounds were assessed with dynamic molecular studies. Two compounds called CID-415537 and CID-419074 with docking energies of -10.3 kcal/mol and -11 kcal/mol were identified. CID-415537 was selected as the best compound due to its proper interactions with SUR1 amino acids and stability in molecular dynamic simulation. Based on this study, compound CID-415537 would be a good candidate for a SUR1 inhibitor in ischemic stroke. However, further in vivo investigations are required to confirm these findings.

Keywords: Sulfonylurea receptor 1; stroke; glibenclamide; molecular dynamic simulation; docking; virtual screening.

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1. Introduction

Stroke is a complex neurological disease, which leads to sudden paralysis, speech impairments, or vision loss caused by thromboembolic ischemic events, which second leading cause of death worldwide [1-3]. Cerebral ischemia is divided into focal ischemia and global ischemia. Global ischemia occurs during cardiac failure and interruption of blood flow to the brain. A reduced amount of blood supply causes a lack of oxygen and glucose, resulting in mitochondrial dysfunction, and reduction of ATP synthesis results in cerebral edema and hemorrhage [3-5]. Briefly, after the cerebral ischemia, morphological change in injured neuronal, glial, and endothelial cells is occurred due to cell swelling. Increased Na+ and Ca2+...
influx into cells by membrane depolarization and the lack of ATP are responsible for several harmful factors such as glutamate accumulation and reactive oxygen species (ROS) production due to cytotoxic cell swelling and acute injury that affects cerebral functions [6-10].

In search of receptors or channels involved in the ischemic stroke process, were reported that sulfonylurea 1 receptors increase after ischemic stroke [11,12]. Transcriptional upregulation transient receptor potential melastatin 4 (Trpm4), the nonselective monovalent cation channel, occurred in neural and vascular cells due to brain infarction, which associates with sulfonylurea receptor 1 (Sur1) to produce Sur1-Trpm4 channels which in cytotoxic edema, cell death, blood-brain barrier (BBB) breakdown, and extravasation edema have life-threatening roles [10,13-17].

The SUR1-Trpm4 channel protects against the pathological increase in intracellular Ca\(^{2+}\) concentrations in normal conditions. However, extreme depletion in cellular ATP occurs during ischemic stroke deconditions due to reduced delivery of oxygen and glucose, two main sources for ATP production in the brain. Lower amounts of ATP constantly activate SUR1-Trpm4 channels and cause constant cell depolarization via Na\(^+\) influx, cytotoxic edema as a result of Cl\(^-\) and water influx through depolarization, and finally, cell destruction [18-21]. Therefore, it seems that inhibition of SUR1-Trpm4 assembly may be an approach for modulating the outcomes of ischemic stroke [22-26].

It has been previously demonstrated that the SUR1-Trpm4 channel is plugged by first and second-generation sulfonylureas, tolbutamide (EC\(_{50}\): 16.1 µM), and glibenclamide (EC\(_{50}\): 48 nM) [23,27-30]. In the early 2000s, in seminal work by Simard et al., identifying a channel SUR1-TRPM4 in injured CNS cells was described, inhibited by sulfonylurea. Therefore the critical role of GLY was established in CNS injury, and it has enhanced events of increased intracranial pressure (ICP), brain swelling, disruption of the BBB, hippocampal injury, and amnesia [23,31-37]. These drugs block the channel by prolonging a long-closed state without affecting channel opening or conductivity [14,29,38].

In a physiologically normal condition, the accumulation of glibenclamide and the other sulfonylurea drugs in the brain is not detectable [39,40]. The sulfonylurea drugs are passively transported into the brain and actively extruded out of the CNS cells. Extrusion of these compounds from BBB cells is active transport with an endothelial transporter similar to P-Glycoprotein (P-gp) and maybe the organic anion transporters. Although lipophilicity is a critical factor of passive transport across an intact BBB, however, glibenclamide and tolbutamide as weak acids have two forms: an ionized form that is hydrophilic and impermeable and a non-ionized form that is lipophilic and permeable [41,42]. For glibenclamide, the ratio of ionized to non-ionized state is 100:1 at pH 7.3. It means it cannot transport into the brain at neutral pH because of poor lipid solubility and strong, tight junctions of intact BBB. Nevertheless, there are different conditions in ischemic strokes and similar dysfunctions because lactic acidosis can drive extracellular pH to values near 6.5, which leads to concentrated weak acidic compounds in cells [43,44].

Therefore, the channels that contributed to the regulation of ionic homeostasis are considered promising as drug targets in ischemic stroke management. Considering the beneficial effect of glibenclamide on ischemic stroke and other types of neuroinflammation, it seems rational to reevaluate the interactions of glibenclamide with SUR1-Trpm4 to find other lead compounds through a pharmacophore-based virtual screening method without common side effects of sulfonylureas.
2. Materials and Methods

2.1. Methodology.

The overall procedures of this study are illustrated in Figure 1.

![Diagram showing the overall view of finding new anti-stroke agents.](image)

**Figure 1.** An overall view of finding new anti-stroke agents.

2.2. SUR1 homology modeling.

At the beginning of this study, the crystal structure of brain SUR1 was not available in Protein Data Bank (http://www.rcsb.org). The Swiss-model website (http://www.swissmodel.expasy.org) was used for homology modeling of SUR1 3D structure. Based on a survey showing structural similarities between the sulfonylurea drug-binding domain in P-glycoprotein (P-gp) and SUR1, the P-gp structure was used for the homology modeling procedure. For this purpose, the mouse P-gp crystal structure was obtained from Protein Data Bank with a PDB ID of 4KSB (www.rcsb.org).

Since it is demonstrated that comparative modeling is reliable when the sequence identity is above 30% [45], SUR1 amino acid sequence (FASTA, accession code: Q09428 (ABCC8_HUMAN)) was extracted from the Protein Database (http://www.ncbi.nlm.nih.gov/protein) and compared to P-gp sequence retrieved from Protein Data Bank (https://www.rcsb.org). The P-gp sequence was uploaded as a template, and the Swiss-model server then built a model of SUR1 based on the minimum energy level and the most stable structure. The comparison was made by pairwise sequence alignment using the local alignment tool and EBLOSUM62 algorithm.

Validation of the SUR1 model was done with the SAVES server, a meta server in which six different programs are available for validating a protein structure. PROCHECK is one of the SAVES server modules (http://www.ebi.ac.uk/thornton-srv/software/PROCHECK/) stereochemically evaluate a protein structure and generate several data such as the Ramachandran plot. Then, a Ramachandran plot was generated for the homology modeled structure of SUR1, and the resolution was estimated, and allowable regions were detected.
2.3. Molecular docking simulations.

AutoDockTools package (ADT, Ver. 1.5.6, The Scripps Research Institute, Florida, USA) was used to prepare all input files for docking simulations. To prepare protein files, polar hydrogens were added, non-polar hydrogens were merged, and the Kollman charges were assigned. All simulation parameters were set as default. Then, the protein input file was saved in PDBQT format. The chemical structure of glibenclamide and its derivatives were drawn using MarvinSketch (17.8.0) (ChemAxon) [46], explicit hydrogens were added and then saved in PDBQT format. Docking simulations were carried out by AutoDock Vina 1.1.2 program [47], with exhaustiveness set to 50. For both proteins, the search spaces were defined as 40×40×40 Å (x, y, and z coordinates: -40.081, 10.523, -18.087 for SUR1 while x, y, and z coordinates: 40.686, 5.067, and 2.75 for P-gp (4KSB)) with a spacing of 0.375 Å. Finally, 3D representations of the ligand-protein interactions have been provided by PyMol [48] and LigPlot+ [49].

2.4. Pharmacophore generation.

LigandScout v3.01 software was used to identify essential residues at the glibenclamide binding cassette for the pharmacophore model’s generation. This software allows to automatically check and set a map of interactions of macromolecules active site with ligands (glibenclamide) such as positive and negative ionizable area (PI and NI), H bond donor (HBD), H bond acceptor (HBA), aromatic ring (AR) and hydrophobic area (HA) [50].

2.5. Virtual screening.

After the pharmacophore generation, the PubChem search engine (http://pubchem.ncbi.nlm.nih.gov) was used for similarity search using glibenclamide as an input. For finding compounds with appropriate physicochemical properties that could cross through intact BBB, all filters were applied by default. All default filters were applied to finding the compounds with appropriate physicochemical properties that could cross through intact BBB [51]. The retrieved structures (481 compounds) were docked to the SUR1 binding cavity. Nine compounds with the lowest binding energy (-7.7 to -10.6 kcal/mol) and similar orientation to glibenclamide were considered for further investigations (Table 1).

Table 1. Nine compounds were chosen after pharmacophore-based NCI screening and docking simulations according to their docking binding free energy values and modes of interactions with SUR1.

| Compound | Structure | Docking energy (kcal/mol) |
|----------|-----------|---------------------------|
| Compound A (CID-319435) | ![Structure]( Compound A ) | -9.5 |
| Compound B (CID-215718) | ![Structure]( Compound B ) | -9.5 |
| Compound C (CID-205842) | ![Structure]( Compound C ) | -7.7 |
| Compound D (CID-216623) | ![Structure]( Compound D ) | -7.1 |
Of these compounds, compound H (CID-146771) and compound I (CID-110300) had the most similarities to glibenclamide in docking properties such as binding free energy, interactions with key residues, and RMSD value. These two compounds had the highest binding affinity and lowest free energy of binding alongside nearly similar docking sites to glibenclamide compared to other compounds.

To enlarge the number of potential hits for SUR1-Trpm4 assembly, compounds H and I were used as a new input for the PubChem search engine. All compounds obtained from PubChem’s second search were docked to the SUR1 binding cavity, and results were tabulated in Table 2.

Table 2. Fourteen compounds are chosen with BBB penetration and compounds H and I as inquiry in the PubChem database according to their docking binding free energy values and modes of interactions with SUR1.

| Compound        | Structure | Docking energy (kcal/mol) |
|-----------------|-----------|---------------------------|
| Compound E (CID-275971) | ![Structure](https://biointerfaceresearch.com/) | -8.4 |
| Compound F (CID-12488) | ![Structure](https://biointerfaceresearch.com/) | -8.3 |
| Compound G (CID-204262) | ![Structure](https://biointerfaceresearch.com/) | -8.3 |
| Compound H (CID-146771) | ![Structure](https://biointerfaceresearch.com/) | -10.6 |
| Compound I (CID-110300) | ![Structure](https://biointerfaceresearch.com/) | -9.7 |
| Glibenclamide | ![Structure](https://biointerfaceresearch.com/) | -10.7 |
| Compound 1 (CID-138944) | ![Structure](https://biointerfaceresearch.com/) | -9.2 |
| Compound 2 (CID-269825) | ![Structure](https://biointerfaceresearch.com/) | -10.4 |
| Compound 3 (CID-269834) | ![Structure](https://biointerfaceresearch.com/) | -10.5 |
| Compound 4 (CID-272220) | ![Structure](https://biointerfaceresearch.com/) | -9.2 |
| Compound 5 (CID-307288) | ![Structure](https://biointerfaceresearch.com/) | -8.9 |
| Compound 6 (CID-384802) | ![Structure](https://biointerfaceresearch.com/) | -9.0 |
2.6. Molecular dynamic simulation.

Molecular dynamic simulations were used to monitor probable interaction behavior of selected ligands (Compound 9, CID-415537 and Compound 12, CID-419074) and glibenclamide with SUR1 binding pocket. GROMACS package 5.0.1 was used, and the best docking posing of the selected compounds (9 and 12) and glibenclamide with SUR1 was chosen and used as the inputs of MD simulations [52]. The accurate protonation state of the residues was provided by the PROPKA server in pH 7.4 (http://propka.org/) [53]. The topology files of SUR1 and the ligands were generated using the AMBER force field and the PRODRG server (http://prodrg1.dyndns.org), respectively [54]. Afterward, ligand-protein complexes were individually placed in a dodecahedron-shaped box, and the minimum distances between the protein surface and box walls were set to 1 nm. This box was filled with water model molecules through GROMACS’ solvation method. In the next step, complexes were energy minimized by the steepest descent algorithm with 1000 kJ/mol/nm tolerance. The system went through an NVT condition for 20 ps and NPT until a constant pressure of 1 bar was obtained. Finally, the MD simulations were carried out for a period of 60 ns. Other MD parameters were assigned based on our previous study [55].

2.7. ADMET analysis.

The ADME properties and potential toxicity of the final hits were predicted using SwissADME (http://www.swissadme.ch/) and ProTox-II (ProTox-II - Prediction of TOXicity of chemicals (charite.de)) web server, respectively [56,57].
3. Results and Discussion

3.1. SUR1 homology modeling.

To generate the SUR1 3D structure, homology modeling was carried out using the P-gp structure as a template and SUR1 sequence (Figure 2). Comparative modeling can be reliable since pairwise alignment showed a 39.1% similarity between P-gp and SUR1 sequences. After modeling the Swiss-model webserver, the modeled structure was stereochemically checked and validated by the PROCHECK server and the Ramachandran plot.

Figure 2. 3D representations of crystalized P-gp (PDB ID: 4KSB) (right) and SUR1 generated by homology modeling (left).

Figure 3. Ramachandran plot of SUR1 protein. Disallowed residues are specified in the plot.
Besides, the estimated resolution was 1.5 Å, only about 0.9% of SUR1 residues were recognized as disallowing, including Gly 1406, Gly 1383, Gly 672, Gly 692, Gly 410, Gly 823, Gly 735, Gly 1478, and Gly 911. None of them were in the glibenclamide binding region (Figure 3).

3.2. Docking of glibenclamide on SUR1 and P-gp.

Glibenclamide was docked to SUR1 and P-gp to validate the probable SUR1 binding region and the interactions involved. Glibenclamide is bound to both proteins in a similar binding region (Figure 4) with binding energy values of -9.3 kcal/mol and -10.7 kcal/mol, suggesting that glibenclamide better affinity for SUR1 than P-gp. It was consistent with this theory that SUR1 ligands have more affinity for SUR1 than P-gp. Comparison of docking poses showed that glibenclamide was docked similarly in two receptors. As a confirmation for this method, the docking site was close to the amino acids mentioned above.

![Figure 4](https://biointerfaceresearch.com/)  
**Figure 4.** Overall representations of glibenclamide binding regions to P-gp (Left) and SUR1 (right). The glibenclamide molecule is presented as a blue stick.

![Figure 5](https://biointerfaceresearch.com/)  
**Figure 5.** 2D and 3D illustrations of glibenclamide interactions with P-gp. Dotted arrows represent hydrogen bonding.
The residues involved in binding glibenclamide to P-gp are as follows: Asp 184, Lys 883, and Lys 185 make hydrogen bonds while Phe 938, Val 129, Trp 132, Thr 937, Lys 930, Phe 934, Asn 179, Asp 882, Ser 176, Ala 876, Leu 875, and Glu 871 contribute to hydrophobic interactions (Figure 5). However, the SUR1-glibenclamide complex is formed only by hydrophobic interactions made by Tyr 377, Phe 433, Val 1183, Ile 381, Ile 385, Met 429, Leu 1240, Trp 430, Leu 434, Tyr 1180, Arg 388, Ser 1237, Asn 1244, Phe 1181, Met 1251, and Glu 1248 residues (Figure 6).

Figure 6. 2D and 3D illustrations of glibenclamide interactions with SUR1 active site.

Figure 7. 3D illustration of the best-docked conformations of glibenclamide and its methylated derivatives in complex with SUR1.
3.3. Docking of methylated derivatives of glibenclamide on SUR1.

As a weak acid, the major amount of glibenclamide (pK\textsubscript{a} 6.3) ionizes in pH 7.4 while it exerts its inhibitory effect in its unionized form. In this study, we added one, two, and three methyl substituents to the nitrogen atoms of glibenclamide to improve its permeability. These three designed ligand molecules were docked on SUR1 to assess their possible docking energy and interactions with SUR1. Docking energies of methylated glibenclamide derivatives to SUR1 were -9.1 kcal/mol for methyl glibenclamide, -8.2 kcal/mol for dimethyl glibenclamide, and -8 kcal/mol for trimethyl glibenclamide. It suggests that adding methyl substituents on nitrogen atoms decreases the potential inhibitory effect of glibenclamide against SUR1. Moreover, these derivatives showed to be bound in different regions than the glibenclamide binding region (Figure 7).

3.4. Pharmacophore generation.

A pharmacophore map based on glibenclamide-SUR1 interactions was generated using LigandScout [50]. Then, pharmacophore-based screening was done on the National Cancer Institution’s Natural Product database by PubChem search engine. Nine compounds were obtained, and all inhibitors were docked over the SUR1 binding cavity (Table 1). Compounds H and I were chosen due to their structural similarity to glibenclamide, similar binding pocket, and lowest binding energies. Figure 8 shows the interactions made by compounds H and I against SUR1 and the residues involved.

![Figure 8](https://biointerfaceresearch.com/)

**Figure 8.** 2D and 3D views of the interactions made by compound H (A) and compound I (B).
Compound H shows hydrogen bonding with residues Thr 548, Ser 1137, Arg 1299, Asn 1300, and Thr 1138, while hydrophobic is made by Phe 1067, Ile 1029, Ile 544, Arg 1144, Cys 1078, Cys 1141, Thr 540 (Figure 8 A). However, there are only two H-bonds in compound I binding formed by Thr 540 and Gln 485. Hydrophobic interactions in Cys 1078, Ile 542, Ser 1082, Ser 1137, Glu 1086, Phe 536, Asp 1132, Leu 1090, and Pro 1136 are involved in compound I and SUR1 (Figure 8 B).

To find more potential inhibitors, similarity screening was carried out in the PubChem database based on the 90% similarity to compounds H and I. Fourteen compounds (1-14) were obtained and evaluated for their binding mode and interactions by docking to the SUR1 cavity (Table 2). We observed that all of the ligands exhibited a similar binding region to glibenclamide. However, except for compound 12 (CID-149074), all ligands have lower binding energy than glibenclamide. Compound 9 (CID-415537) and compound 12 (CID-419074) were chosen for MD simulations due to their structural similarity to glibenclamide and desirable (lowest) docking energy. Figure 9 represents 2D and 3D conformations of compound 9 and 12-SUR1 complexes.

**Figure 9.** 3D and 2D representations of compound 9 (A) and compound 12 (B) generated by PyMol and LigPlot* software, respectively. The ligands are shown in the sticks in 3D pictures, while surrounded residues are shown in green stick forms.
3.5. Molecular dynamic studies.

Molecular dynamic simulations were performed on the lowest docked conformations of compounds 9, 12, and glibenclamide over SUR1 to evaluate their structural stability over 60 ns of simulation. The root means square deviation (RMSD) plots of compounds 9, 12, and glibenclamide indicate that these compounds tightly bind to the SUR1 binding pocket (Figure 10 A).

It was expected that the protein bound to the ligand is more stable than the protein without the ligand. However, the RMSD plots of the protein backbone show that SUR1 is stable both without and in complex with the ligand. SUR1 is bound to glibenclamide, compounds 9 and 12, and their complex reaches the steady-state after 15 ns (Figure 10 B). The free binding energy of ligands is much less than the overall energy of the whole protein (because of the large size of the protein). Therefore, ligand-protein interaction has no impact on the faster stability of the protein. To have a deep insight into the movements of active site residues in the presence of ligands, root means square fluctuation (RMSF) plots were calculated (Figure 11).

![Figure 10](image_url)

**Figure 10.** Molecular dynamic simulation graphs; (A) RMSD plot of protein in complex with glibenclamide (magenta), compound 9 (green), compound 12 (red), and protein without ligand (blue). (B) RMSD graph of glibenclamide, compound 9 and 12 in magenta, green and red colors, respectively. (C) several hydrogen bonds between ligands and protein active site residues. The color of ligands is the same as explained above.

It was observed that movements of active site residues in contact with ligands are lower than their movement without ligands. Also, the number of hydrogen bond between ligands and protein residues were studied (Figure 10 C). It is seen that compound 9 makes more hydrogen bonds than the other two compounds. In conclusion, compound 9 makes efficient interactions with the SUR1 binding region compared to glibenclamide and compound 12. Regarding both graphs, compound 9 is as stable as glibenclamide. So, compound 9 can be a potential candidate for mimicking glibenclamide interaction to inhibit the SUR1 receptor without hypoglycemic side effects.

3.6. ADMET analysis.

The prediction of drug-likeliness and toxicity of the candidates not only does ensures their safety but also affords the opportunity for further improvements in proposed compounds.
The prediction analysis showed that both compounds had favorable properties according to various filters and no toxicity, as shown in Table 3.

| Compound       | Drug-likeness criteria | Bioavailability Score | Predicted Toxicity Class |
|----------------|------------------------|-----------------------|--------------------------|
|                | Lipinski\textsuperscript{a} | Ghose\textsuperscript{b} | Veber\textsuperscript{c} | Egan\textsuperscript{d} | Muegge\textsuperscript{e} |                     |
| Compound 9    | Yes*                   | No                    | Yes                     | Yes                     | Yes                     | 0.55                  | 4                      |
| Compound 12   | Yes                    | No                    | Yes                     | Yes                     | Yes                     | 0.55                  | 4                      |

\textsuperscript{a} Lipinski’s rule of 5: MW < 500 Dalton, Log P < 5, hydrogen-bond donors < 5, hydrogen-bond acceptors < 10, rotatable bonds < 10.
\textsuperscript{b} 160 ≤ MW ≤ 480, -0.4 ≤ WLOGP ≤ 5.6, 40 ≤ MR ≤ 130, 20 ≤ atoms ≤ 70.
\textsuperscript{c} Rotatable bonds < 10, TPSA ≤ 140.
\textsuperscript{d} WLOGP ≤ 5.88, TPSA ≤ 131.6.
\textsuperscript{e} 200 ≤ MW ≤ 600, -2 ≤ XLOGP ≤ 5, TPSA ≤ 150, Num. rings ≤ 7, Num. carbon > 4, Num. heteroatoms > 1, Num. rotatable bonds ≤ 15, H-bond acc ≤ 10, H-bond donor ≤ 5.
\textsuperscript{f} Probability of F>10% in the rat.
\textsuperscript{g} Carried out by ProTox-II.
\* 1 Violation.

Glibenclamide is a weak acid; a significant amount of glibenclamide (pK\textsubscript{a} 6.3) ionizes at pH 7.4. At the same time, it exerts its inhibitory effect in its unionized form [58,59]. On the other hand, the ischemic condition in which the cerebral pH is decreased [41] increases the lipid solubility of glibenclamide and facilitates the penetration through the blood-brain barrier (BBB) [60]. Simard \textit{et al}., in their studies on non-lethal and lethal rat models of ischemic stroke, estimated a time for delivery of glibenclamide. They observed that glibenclamide must be delivered up to 4-6 hours after non-lethal ischemic stroke and 6-10 hours after a lethal ischemic stroke to be beneficial [61-63]. Also, Weih and Favila, in independent studies, observed that the use of glibenclamide before ischemic stroke did not affect mortality, morbidity, and severity of stroke [64-65]. All these observations confirmed sulfonfonyurea drugs application in the CIA, especially glibenclamide, in a few hours reduces CNS pH, BBB disintegration, and up-regulation of SUR1.

The SUR1 protein is a calcium and ATP-sensitive nonselective cation channel that is upregulated in ischemic conditions and is responsible for brain edema and cell death [11]. SUR1-Trpm4 channels are inhibited by glibenclamide and tolbutamide, two sulfonfonyurea drugs [27]. These drugs can reduce mortality and edema, and decrease infarct volume and increase cerebral blood flow in stroke conditions through binding to the SUR1 subunit [58], but hypoglycemic side effects of sulfonfonyurea prevent their general use in daily clinic safely.

SUR1 ligands (SUR1 openers such as SR47063, P1075, diazoxide, and SUR1 blockers such as glibenclamide, tolbutamide, meglitinide) have been reported to interact with P-glycoproteins (P-gp) but with less affinity [66].

Since there is no crystallized 3D structure for brain SUR1, we generated its 3D structure by homology modeling. The structural superposition revealed that there are close structural similarities between the binding site of SUR1 and P-gp. This comparison showed that Ser 1237 in SUR1 is equivalent to Ser 931 in P-gp, Thr 1285, and Met 1289 in SUR1 equivalent to Ser 979 and Phe 983 were associated with binding of different ligands. Evaluations of the SUR1 proposed model showed that the glibenclamide binding site based on the articles mentioned above is associated with Ala 927 in P-gp equivalent to Ser 1237 in SUR1. This implies a structural similarity between SUR1 and P-gp binding pockets. Ser 1237 in the SUR1 sequence was identified as an equivalent residue to Ser 931 in P-gp which both are associated with
glibenclamide binding [67]. Therefore, we used the 3D structure of P-gp for homology modeling, and Ser 1237 was considered for determining the approximate SUR1 binding pocket. Moreover, their findings are consistent with our results. Glibenclamide shows a better affinity to SUR1 than P-gp due to its lower binding energy (-10.7 and -9.3 kcal/mol in complex with SUR1 and P-gp, respectively (Figures 5 and 6).

Figure 11. Root mean square fluctuation (RMSF) graphs; the above picture shows amino acids (range of 350-1585) movements in contact with glibenclamide (magenta), compound 9 (green), compound 12 (red), and protein without ligand (blue). The other two graphs indicate the fluctuation of amino acids in ranges of 375-500 and 1150-1250 to focus on essential amino acids inside the active site.

4. Conclusions

In this study, we used the structural features of glibenclamide to find new potential inhibitors of SUR1 without potential hypoglycemic side effects of sulfonylurea drugs through docking study. Therefore, we added one, two, and three methyl substituents to the nitrogen atoms of glibenclamide to increase its lipophilicity and improve its permeability and possible inhibitory potential. These three designed molecules were docked on SUR1 to assess their possible docking energy and interactions with SUR1. However, results show that lipophilicity is not essential for binding ligands to the SUR1 active site. We observed that increasing the lipophilic properties by adding methyl groups decreases the inhibitor affinity and different binding regions than glibenclamide (Figure 7). It can be realized that there is not enough space in the SUR1 binding cavity for enlarged nitrogen groups to accommodate.

In the next step, several filters that are believed to be necessary for BBB penetration were applied for screening the NCI database. We choose compounds H and I due to structural similarity to glibenclamide and appropriate binding energy after virtual screening and docking simulations. Subsequent similarity screening was carried out based on a 90% similarity with these two compounds using the PubChem structure search engine. In other words, this
screening was done to enlarge the potential inhibitors based on the maximum similarity to glibenclamide and the ability for BBB penetration. Among fourteen compounds retrieved from the NCI database, compounds 9 and 12 were chosen for the next study step due to their higher binding energy and appropriate binding pattern against SUR1 (Table 2).

Molecular dynamic simulation results confirmed that these compounds tightly bind to the SUR1 binding pocket (Figure 10 A). SUR1 is bound to glibenclamide, compounds 9 and 12, and their complex reaches the steady-state after 15 ns of simulation (Figure 10 B). Moreover, SUR1 is stable both without and in complex with the ligand, and binding to ligands has no impact on the faster stability of the protein. It was observed that movements of active site residues in contact with ligands are lower than their movement without ligands (Figure 11).

All these reports and observations suggest that the most similar compound in structure to glibenclamide also improved the ability of BBB penetration in neutral pH and relatively normal conditions that will be superior to glibenclamide in ischemic strokes and may prevent the expansion of ischemic lesions, morbidity, and mortality, consistent with “time in brain” principle. In conclusion, it seems that compound 9 has the most similar structure to glibenclamide and can penetrate through the BBB and enter the brain at neutral pH and a relatively normal situation. Therefore, glibenclamide may excel in ischemic strokes and prevent the expansion of ischemic lesions, morbidity, and mortality. It might be expected that compound 9 decreases the ischemic consequences through disrupting SUR1 contacts forming SUR1-Trpm4. Failure in developing ion channels results in a decrease in sodium ions input and prevents brain swelling. Although further experiments are needed to approve these data however we believe that our computer-aided simulations would help lead to compound consideration.

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Conflicts of Interest

The authors declare no conflict of interest. The funders had no role in the study's design, in the collection, analyses, or interpretation of data, in the writing of the manuscript, or in the decision to publish the results.

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