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RESEARCH ARTICLE

In silico studies for the identification of potential SGLT2 inhibitors

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ABSTRACT

Sodium glucose cotransporter 2 (SGLT2) inhibitors work by controlling the blood glucose levels, through limiting reabsorption of glucose from the blood therefore, and promoting glucose excretion in the urine. As it is reason for the 90% of reabsorption of glucose through insulin-independent mechanism. The present study described the screening of potential SGLT2 inhibitors using docking studies. *In silico* studies were carried out with help of the Schrödinger software using PDB ID:3DH4. Inhibitors were docked which resulted that phlorizin is one of the most potent compound having highest docking score −12.118 kcal/mol showing binding interaction with the Asn64, Ser66, Ala63, Ser91, Tyr263, Glu88, and Gln 428 (PBD ID: 3DH4) amino acids. Various ADME properties were studied and numerous properties were also analyzed. The forecast model can also be used for the further development of the potential compounds against SGLT2.

KEY WORDS: Amino acids, Docking, *In silico*, PDB, Sodium glucose cotransporter 2

INTRODUCTION

Sodium glucose cotransporter 2 (SGLT2) inhibitors are a type of prescription drug that has been licensed by the FDA for the use in persons with type 2 diabetes in conjunction with diet and exercise.[1] Canagliflozin, dapagliflozin, and empagliflozin are examples of SGLT2 inhibitors.[2] In addition, to being combined with other diabetes drugs like metformin, they are also available as single-ingredient formulations.[3] Inhibitors of the SGLT2 enzyme cause the kidneys to eliminate sugar in the urine, lowering blood sugar levels. Because SGLT2 inhibitors' safety and effectiveness have not been shown, the FDA has not approved their use in people with type 1 diabetes.[4] SGLT2 inhibitors are drugs with a distinct mode of action that reduce blood glucose without the use of insulin.[5] Based on the current data on efficacy and benefits, these medications gradually solidifying their presence in the cure of the diabetes. People suffering from the type 2 diabetes additionally requires a glucose reduction,[6] therefore having the chances of the risk factor but cannot administer the insulin, so SGLT2 inhibitors are the substitutes.[7] Basic studies showed that the long uses of SGLT 2 inhibitors are favorable in various cardiovascular disorders.[8] Type 2 diabetes is a chronic illness that often necessitates the use of many drugs to maintain blood glucose control.[9] SGLT2 inhibitors work by inhibiting renal tubular glucose reabsorption, lowering blood glucose without triggering insulin release, as they have been approved through a FDA.[10] Other advantages could include lower blood pressure and weight loss. This review will concentrate on clinical studies published in the past year, particularly new safety issues that have resulted in repeated FDA cautions for SGLT2 inhibitors.[11] SGLT2 inhibitors, also known as gliflozins, affect the nephron's critical physiology, as opposed to SGLT1 inhibitors, which control sodium/glucose channels in the intestinal mucosa.[12] The delayed glucose reabsorption in the kidney and lowering blood sugar level as been recommended by this pharmacological class. They work by preventing sodium-glucose transport protein 2 from doing its job

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SGLT2 in T2DM.[13] Gliflozins have been demonstrated to help T2DM patients' cardiovascular health in addition to blood sugar control.[14] Several drugs in this category have been approved or are in the works. In research, it was discovered that canagliflozin is member of this family, which control the blood sugar level while simultaneously reduce the systolic and diastolic blood pressure as well as body weight.[15]

MATERIALS AND METHODS

Docking is a computational simulation method for predicting the preferred orientation of two molecules binding together to produce a stable complex.[16] Docking, thus, plays a significant part in drug rational design. Using scoring algorithms, docking is utilized to quantify the affinity and activity of small molecules binding the their protein targets.[17] The sensitivity of docking calculations to input ligand geometry demonstrates that even little changes in ligand conformation can result in significant alterations in the geometries and scores of the docked postures.[18] Here, we work with the module Glide v3.8 (Schrödinger, LLC, New York)[19] which provide us with detailed docking studies and essential docking parameters with satisfactory results which, further, can be used to determine the effectiveness of the testing ligand. The chemical structure of the SGLT2 inhibitor was opted for the antidiabetic activity for the type 2 diabetes. The online PubChem database was used to acquire the 2D structures of the SGLT2 inhibitors (Table 1). With use of the Ligprep wizard software from the Schrödinger, LLC in New York chemical structure was converted to 3D structure.

Preparation of ligand for docking

The ligand was prepared using Schrodinger software.[20] The ligand processed through various ways such as addition of hydrogen, removal of water molecules, and energy minimization.

Protein preparation for docking

The preparation wizard was used for the preparation of protein (Schrodinger, LLC, New York). The downloaded crystal structure (PBD ID: 3DH4) was processed[21] and converted into single entity by removal of water molecule. Furthermore, optimization of hydrogen bonds, removal of water molecule, and minimization were performed using OPLS_2005 force field.

Docking method

The docking study was performed for the determining the binding interaction between ligand and receptor molecule.[22] Docking is the main tool for the virtual screening methods, where a library of different compounds is docked to the single drug target and displays the best match.[23-25] The docking scores predicted in the form of extra precision (XP) and standard precision (SP) has been demonstrated to enhance the actual binding positions.[26] The docked complexes were rescored using MM/GBSA-based method in the form of their relative binding free energies.[27-30]

MM/GBSA based redocking

MM/GBSA was used to determination of binding free energy of docked ligand complexes.[31]

Pharmacokinetics properties (ADME)

Schrodinger ADME QikProp tool has been employed to determine the ADME properties of drugs QikProp tool. Properties such as QPP Caco-2, QP logBB, QPPMDCK, percentage human oral absorption, QP log Po/w, and QP logKhsa have been determined to assist the ligands profile. Various properties such as hydrogen bond donor, rotatable bonds, hydrogen bond acceptor, molecular weight, and polar surface area are incluled under Lipinski Rule of Five.[32-34]

RESULTS AND DISCUSSION

Computer-assisted drug design (Molecular docking)

The molecular docking has been performed to determine the interactions of SGLT2 inhibitors with PDB ID: 3DH4.[35] To produce the antidiabetic activity through interaction of compounds, all the inhibitors were docked to their active sites.

The docking scores in the form of SP and XP are shown in Table 2. The compounds docking investigations revealed a complementary match in the protein's allosteric location. The docking interactions of the ligand with amino acids were determined in allosteric region.[36]

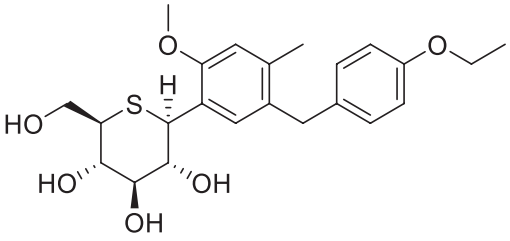
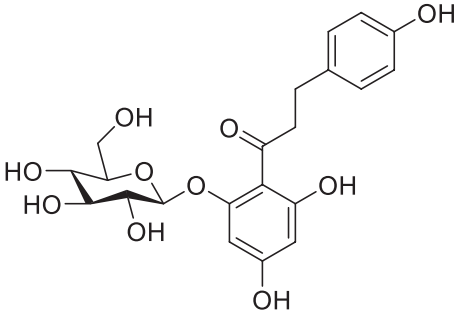
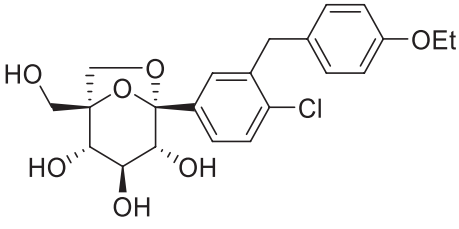
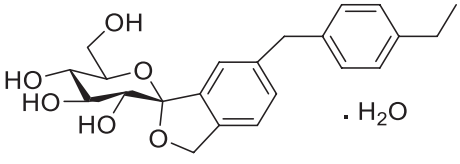
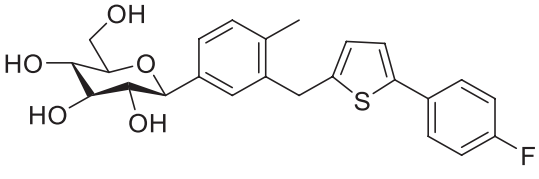
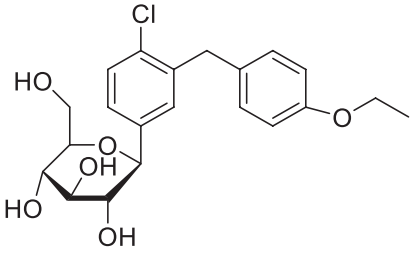
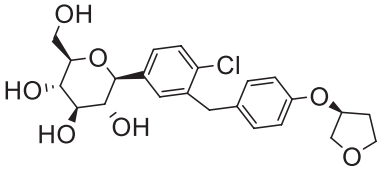
Phlorizin exhibited the best glucose lowering effect with the docking score (−12.118). Compound interaction with active site residue Asn64, Ser66, Ala63, Ser91, Tyr263, Glu88, Gln 428 are described in Figure 1. The compound empagliflozin showed the interaction with the active site Ala63, Tyr263, and Ser91 (Figure 2). The compound canagliflozin showed the interaction with active site Asn267 (Figure 3).

In silico prediction of ADME properties SGLT2 inhibitors

Table 3 displayed ADME attributes. The surface and rotatable bond of all SGLT2 inhibitors were within the permitted range of medication resemblance attributes.

Here, in this present study, we have studied various physicochemical properties because one of the key factors

Table 1: The compounds used as SGLT2 inhibitors

S. No.	Compounds	Chemical structure	Reference code
1	Luseogliflozin		11988953
2	Phlorizin		6072
3	Steglatro		44814423
4	Tofogliflozin		46908929
5	Canagliflozin		24812758
6	Dapagliflozin		9887712
7	Empagliflozin		11949646

SGLT2: Sodium glucose cotransporter 2

Table 2: The description of docking scores with binding energy of potent compounds

SGLT2 Inhibitors	PDB ID: 3DH4		
	Standard precision (SP) kcal/mol	Extra precision (XP) kcal/mol	MM/GBSA dG bind (xp complex) kcal/mol
Phlorizin	−7.761	−12.118	−42.3791
Empagliflozin	−7.664	-	-
Canaglifozin	−5.901	−10.289	−57.1803

SGLT2: Sodium glucose cotransporter 2

Table 3: Predicted *in silico* LogP and ADME properties of SGLT2 inhibitors

Compounds	Mol.Wt.	PSA	QPlogPo/w[a]	QPP Caco[b]	QPlog BB[c]	QPP MDCK[d]	SASA[e]	Percent Human Oral Absorption[f]
Luseogliflozin	448.57	94.87	3.051	321.22	−1.66	172.63	757.904	89.67
Phlorizin	436.41	184.75	−0.545	8.69	−3.27	2.931	653.4	27.60
Steglatro	436.88	107.90	2.442	268.35	−1.58	224.14	712.43	84.71
Tofogliflozin	384.47	91.89	2.55	270.29	−1.56	120.29	681.70	85.4
Canagliflozin	444.51	90.98	3.15	256.92	−1.49	272.64	747.41	88.55
Dapagliflozin	408.878	100.63	2.13	223.40	−1.66	183.47	700.32	81.46
Empagliflozin	450.915	109.82	1.95	223.38	−1.66	183.51	729.07	80.40

[a]Predicted octanol/water partition coefficient (Range= −2.0−6.5); [b]Predicted apparent Caco-2 cell permeability in nm/sec. Caco-2 cells (<25% is poor, >500 great). [c]predicted brain/blood partition coefficient. [d]Predicting passive permeability of Caco-2 and MDCK cell. [e]Total solvent accessible surface area (SASA) in 1.4 Å radius (range=300−1000); [f]Predicted human oral absorption on 0 to 100% scale. >80% is high<25% is poor

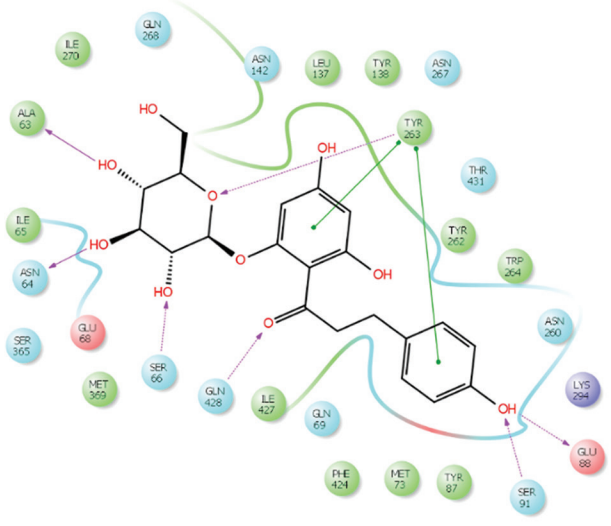


Figure 1: 2D interaction of Phlorizin using PDB ID: 3DH4.

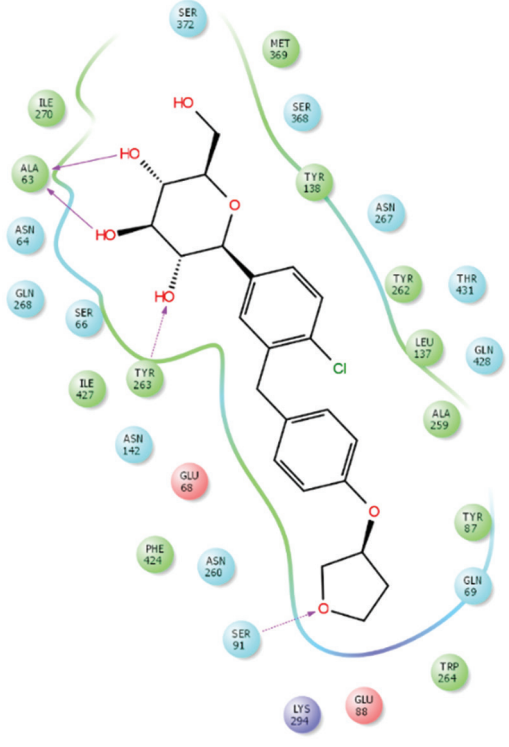


Figure 2: 2D interaction of empagliflozin using PDB ID: 3DH4.

in maximizing a drug's capacity to enter cells is its polar surface area. Some compounds having value >140 Å^2 which is the reason for the poor cell membrane permeability.

QPPCaco signifies the apparent permeability across the Caco-2 cell, whereas, in this study, all the compounds were in a range 25–500 showing moderate to good Caco-2 values.

SASA stands for square angstroms of solvent accessible surface area with a radius of 1.4 Å radius. The compound canagliflozin showed the highest value, whereas the phlorizin showed the lowest value. As per Qikprop projections, inhibitors have optimal properties for antidiabetic action and can be used as a lead molecule for further development.

The effectiveness of binding of ligands with receptors in terms of energy was assessed by MM/GBSA-based

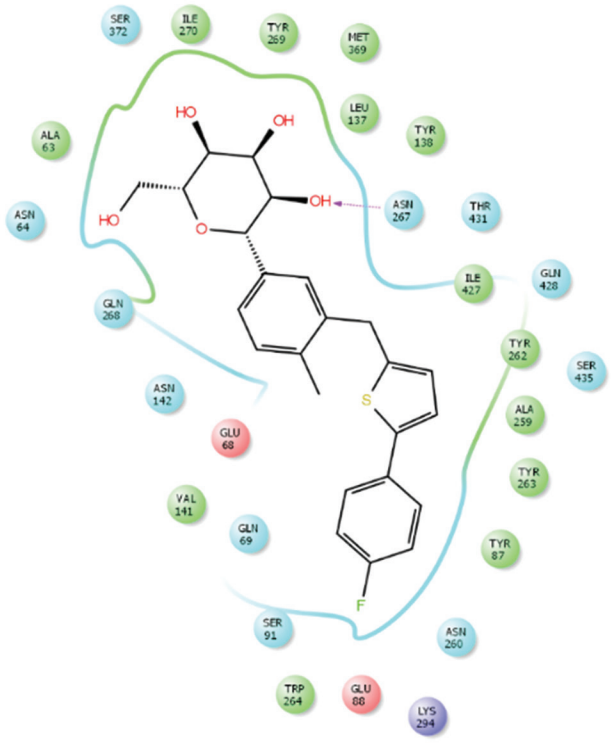


Figure 3: 2D interaction of canagliflozin using PDB ID: 3DH4.

redocking. In the present study, the compound phlorizin and canagliflozin (PDB ID: 3DH4) showed interaction with SGLT2 with the highest binding free energy as the dG bind = −42.3791 and −57.1803 kcal/mol, respectively [Table 2]. The compound canagliflozin showed the highest dG binding (dGbind = −57.1803 kcal/mol) in MM/GBSA data redocking.

CONCLUSION

In the current *in silico* study, potential SGLT2 inhibitors have been identified. SGLT2 inhibitors are the blood glucose level lowering inhibitors through a insulin-independent mechanism for type 2 diabetes. Selected SGLT2 inhibitors were docked and resulted in the good potency of the compound and desired ADME properties with help of XP, SP, and MM/GBSA redocking, using Glide v5.8 (Schrodinger, LLC, New York, NY). From the above study, it has been concluded that phlorizin having highest docking score −12.118 kcal/mol showing binding interaction with the Asn64, Ser66, Ala63, Ser91, Tyr263, Glu88, Gln 428, and other compounds empagliflozin showed that the interaction with Ala63, Tyr263, Ser91, and canagliflozin showed the interaction with Asn267 (PBD ID:3DH4) amino acid. The result of ADME studies were also good making the compound well-suited for the further studies. The results of the current investigation might assist in the continued development of advanced SGLT2 inhibitors.

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REFERENCES

1. Ndefo UA, Anidiobi NO, Basheer E, Eaton AT. Empagliflozin (Jardiance): A novel SGLT2 inhibitor for the treatment of Type-2 diabetes. P T 2015;40:364-8.
2. Singh M, Kumar A. Risks associated with SGLT2 inhibitors: An overview. Curr Drug Saf 2018;13:84-91.
3. Vallon V, Thomson SC. Targeting renal glucose reabsorption to treat hyperglycaemia: The pleiotropic effects of SGLT2 inhibition. Diabetologia 2017;60:215-25.
4. El Masri D, Ghosh S, Jaber LA. Safety and efficacy of sodium-glucose cotransporter 2 (SGLT2) inhibitors in Type 1 diabetes: A systematic review and meta-analysis. Diabetes Res Clin Pract 2018;137:83-92.
5. Hsia DS, Grove O, Cefalu WT. An update on SGLT2 inhibitors for the treatment of diabetes mellitus. Curr Opin Endocrinol Diabetes Obes 2017;24:73-9.
6. Inzucchi SE, Bergenstal RM, Buse JB, Diamant M, Ferrannini E, Nauck M, *et al*. Management of hyperglycemia in Type 2 diabetes, 2015: A patient-centered approach: Update to a position statement of the American diabetes association and the European association for the study of diabetes. Diabetes Care 2015;38:140-9.
7. Afrin N. Risk Factors of Diabetes Mellitus; A Review. East West University; 2016. Available from: https://www.muysalud.com/en/diseases/causes -and-risk-factors-of-type-2-diabetes [Last accessed on 2022 Feb 12].
8. Taylor SI, Blau JE, Rother KI, Beitelshees AL. SGLT2 inhibitors as adjunctive therapy for Type 1 diabetes: Balancing benefits and risks. Lancet Diabetes Endocrinol 2019;7:949-58.
9. Opie LH. Sodium glucose co-transporter 2 (SGLT2) inhibitors: New among antidiabetic drugs. Cradiovasc Drugs Ther 2014;28:331-4.
10. DeFronzo RA, Norton L, Abdul-Ghani M. Renal, metabolic and cardiovascular considerations of SGLT2 inhibition. Nat Rev Nephrol 2017;13:11-26.
11. Blau JE, Tella SH, Taylor SI, Rother KI. Ketoacidosis associated with SGLT2 inhibitor treatment: Analysis of FAERS data. Diabetes Metab Res Rev 2017;33:e2924.
12. Faillie JL. Pharmacological aspects of the safety of gliflozins. Pharmacol Res 2017;118:71-81.
13. Peene B, Benhalima K. Sodium glucose transporter protein 2 inhibitors: Focusing on the kidney to treat Type 2 diabetes. Ther Adv Endocrinol Metab 2014;5:124-36.
14. Salvatore T, Caturano A, Galiero R, Di Martino A, Albanese G, Vetrano E, *et al*. Cardiovascular benefits from gliflozins: Effects on endothelial function. Biomedicines 2021;9:1356.
15. Neal B, Perkovic V, Matthews DR, Mahaffey KW, Fulcher G, Meininger G, *et al*. Rationale, design and baseline characteristics of the CANagliflozin

cardioVascular Assessment Study-Renal (CANVAS-R): A randomized, placebo-controlled trial. Diabetes Obes Metab 2017;19:387-93.
16. Agarwal S, Mehrotra R. An overview of molecular docking. JSM Chem 2016;4:1024-8.
17. Khamis MA, Gomaa W, Ahmed WF. Machine learning in computational docking. Artif Intell Med 2015;63:135-52.
18. Feher M, Williams CI. Effect of input differences on the results of docking calculations. J Chem Inf Model 2009;49:1704-14.
19. Asati V, Bharti SK, Budhwani AK. 3D-QSAR and virtual screening studies of thiazolidine-2, 4-dione analogs: Validation of experimental inhibitory potencies towards PIM-1 kinase. J Mol Struct 2017;1133:278-93.
20. Das SK, Mahanta S, Tanti B, Tag H, Hui PK. Identification of phytocompounds from *Houttuynia cordata* Thunb. As potential inhibitors for SARS-CoV-2 replication proteins through GC–MS/LC–MS characterization, molecular docking and molecular dynamics simulation. Mol Divers 2022;26:365-88.
21. Krishnamurthy H, Piscitelli CL, Gouaux E. Unlocking the molecular secrets of sodium-coupled transporters. Nature 2009;459:347-55.
22. Guedes IA, de Magalhães CS, Dardenne LE. Receptor-ligand molecular docking. Biophys Rev 2014;6:75-87.
23. Yuriev E, Agostino M, Ramsland PA. Challenges and advances in computational docking: 2009 in review. J Mol Recognit 2011;24:149-64.
24. Cramer RD, Patterson DE, Bunce JD. Comparative molecular field analysis (CoMFA). 1. Effect of shape on binding of steroids to carrier proteins. J Am Chem Soc 1988;110:5959-67.
25. Garden DP, Zhorov BS. Docking flexible ligands in proteins with a solvent exposure-and distance-dependent dielectric function. J Comput Aided Mol Des 2010;24:91-105.
26. Friesner RA, Murphy RB, Repasky MP, Frye LL, Greenwood JR, Halgren TA, *et al*. Extra precision glide: Docking and scoring incorporating a model of hydrophobic enclosure for protein-ligand complexes. J Med Chem 2006;49:6177-96.
27. Sun H, Li Y, Shen M, Tian S, Xu L, Pan P, *et al*. Assessing the performance of MM/PBSA and MM/GBSA methods. 5. Improved docking performance using high solute dielectric constant MM/GBSA and MM/PBSA rescoring. Phys Chem Chem Phys 2014;16:22035-45.
28. Thomsen R, Christensen MH. MolDock: A new technique for high-accuracy molecular docking. J Med Chem 2006;49:3315-21.
29. Meng EC, Shoichet BK, Kuntz ID. Automated docking with grid-based energy evaluation. J Comput Chem 1992;13:505-24.
30. Graves AP, Shivakumar DM, Boyce SE, Jacobson MP, Case DA, Shoichet BK. Rescoring docking hit lists for model cavity sites: Predictions and experimental testing. J Mol Biol 2008;377:914-34.
31. Hou T, Wang J, Li Y, Wang W. Assessing the performance of the molecular mechanics/Poisson Boltzmann surface area and molecular mechanics/generalized Born surface area methods. II. The accuracy of ranking poses generated from docking. J Comput Chem 2011;32:866-77.
32. Pajouhesh H, Lenz GR. Medicinal chemical properties of successful central nervous system drugs. NeuroRx 2005;2:541-53.
33. Leung SS, Mijalkovic J, Borrelli K, Jacobson MP. Testing physical models of passive membrane permeation. J Chem Inf Model 2012;52:1621-36.
34. Shelke SM, Bhosale SH. Synthesis, antidepressant evaluation and QSAR studies of novel 2-(5H-[1, 2, 4] triazino [5, 6-b] indol-3-ylthio)-N-(substituted phenyl) acetamides. Bioorg Med Chem Lett 2010;20:4661-4.
35. Bisignano P, Ghezzi C, Jo H, Polizzi NF, Althoff T, Kalyanaraman C, *et al*. Inhibitor binding mode and allosteric regulation of Na+-glucose symporters. Nat Commun 2018;9:5245.
36. Abolhasani H, Zarghi A, Hamzeh-Mivehroud M, Alizadeh AA, Mojarrad JS, Dastmalchi S. *In-silico* investigation of tubulin binding modes of a series of novel antiproliferative spiroisoxazoline compounds using docking studies. Iran J Pharm Res 2015;14:141-7.