Research Article

Designing of wonder molecule for a polygenic disease: Diabetes Mellitus

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Abstract

Background: Diabetes mellitus is increasing globally affecting more than 180 million people worldwide. Thiazolidinediones (TZDs), Metformin, Sulfonylureas (SURs) are well-known drugs however, side-effects are associated with it which limits its use. Adiponectin Receptor 1 (AdipoR1) has been recently identified as an attractive anti-diabetic target as it plays a crucial role in regulating hyperlipidemic conditions which lead to obesity and increasing glucose transfer, gluconeogenesis and insulin sensitivity in pancreatic β-cells for the treatment of type 2 diabetes mellitus (T2DM). **Objective:** objective of present work was to design a drug molecule which can target adiponectin and Peroxisome proliferator-activated receptor as a target for treatment of diabetes mellitus. **Material and methods:** In this work, designing strategy relied on making a structural similarity having Peroxisome proliferator-activated receptor and Adiponectin Receptor 1 modulating activity. In this study to further investigate, the compounds were docked with individual receptor active sites, to study the stability of their complexes with both proteins and final binding orientations of these molecules. After the absorption, distribution, metabolism and excretion properties of the derivatives possessing a good binding affinity for all the receptors were evaluated. Later on, the in-silico toxicity studies were also performed. **Results and conclusion:** The positive results of these compounds showed that they can be further studied for the in-vitro and in-vivo activities after synthesis. Thus, these derivatives might be promising lead compounds for the treatment of diabetes mellitus without the allied risk of hypoglycemia and cardiovascular risks.

***Keywords*:** PPAR-G, AdipoR1, virtual screening, structural similarity, docking, Type 2 diabetes mellitus

Introduction

Diabetes mellitus is a chronic metabolic disorder characterized by hyperglycaemia, glycosuria, hyperlipidemia, negative nitrogen balance and sometimes ketonaemia. The World Health Organization has deemed the increased prevalence of obesity and diabetes as a "twenty-first century epidemic" (Golay and Ybarra, 2005). More than half of the world's population is considered overweight and being overweight is associated with several comorbidities such as T2DM, cardiovascular diseases, hypertension, dyslipidemia, respiratory diseases, osteoarthritis, and depression (Golay and Ybarra, 2005). According to Ford et al., for every kilogram of weight gain, the risk of diabetes increases between 4.5 and 9% (Ford, 1997). The relative risk for an obese individual to develop T2DM is 10-fold for women and 11.2-fold for men (Field, 2001). The most critical factor in the emergence of metabolic diseases is obesity. One of the causal links between obesity and T2DM is the development of insulin resistance and lipid metabolism on which the adiponectin works without alleviating the body weight or cardiovascular risks.

Material and Methods

Hardware and Software specifications

All computational studies were carried out using molecular modeling package from Schrödinger's Drug Discovery Suite 2019 (Schrödinger, Inc., LLC, New York, USA) installed on the platform of Lenovo workstation having 64-bit operating system, x-64 based Intel i3 core processor- 4005U CPU @ 1.70 GHz with 4Gb RAM.

Dataset and library preparation

In the virtual screening workflow, the database of 11,309 ligands was downloaded in SDF format from Asinex which is providing the library of datasets for structure-based drug designing and fragment-based drug designing.

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Ligand preparation

The ligands were prepared using the LigPrep module of Maestro in the Schrodinger suite. The possible ionization states were generated at cellular pH value 7+/-2 and the chirality was retained. Furthermore, the OPLS-2005 force field was used for optimization, which produces the lowest energy conformer of the ligand (Jorgensen et al., 1996). The resulting library of the prepared ligands was saved as the maestro file format.

Preparation of protein structure

For the determination of ligand-receptor interactions and the filtering of virtual screened molecules, the glide docking program of Schrödinger software was employed. The 3D-structure of the protein complex was retrieved from the protein data bank (Bernstein et al., 1977; Protein Data Bank, www.rcsb.org) and subjected to the protein preparation wizard available in Schrodinger suite 2019 (Sastry et al., 2013). At first, the protein was preprocessed by adding all hydrogen atoms to the structure, removal of crystallographic water molecules and assigning bond orders, creating disulfide bonds and filling missing side chains and loops. To optimize the hydrogen bond network, His tautomers and ionization states were predicted, and hydrogen atoms of hydroxyl and thiol groups were sampled. Finally, a restrained minimization was performed with the Impact refinement module, utilizing the OPLS-2005 force field to optimize the geometry and minimize the energy of the protein (Jorgensen et al., 1996). The minimization was terminated when the energy converged or the RMSD reached a maximum cut-off of 0.30 Å. In the case of adiponectin, PDB 3WXV is prepared using the same criteria for the selection of protein structure.

Receptor grid generation

Receptor grid file for prepared MDM2 protein was generated using Receptor Grid Generation option of Glide module (Grid-based Ligand Docking with Energetic) implemented in Maestro suite (Glide, 2019). In this way, a cubic grid box was defined at the centroid of the co-crystalized ligand so that roughly encompasses the active site. The grid box was adjusted based on a size capable of accommodating ligands with a length of 11*15*12 Å. In order to decrease the potential for nonpolar parts of receptor, a default value of 1.0 Å for the scaling factor was applied on the van der Waals radius of nonpolar atoms of protein having partial atomic charge cut-off of 0.25 Å.

Structure-based Virtual Screening

The VS workflow (VSW) module implemented in the Schrödinger suite was utilized to identify the potential inhibitors, amongst the 11,309 hits found through similarity search, against the active site of prepared proteins (Virtual Screening Workflow 2019-1, 2019; LigPrep, 2019; QikProp, 2019). As part of this multi-step workflow, two pre-filtering choices, Lipinski's Rule of 5 (RO5) and Reactive filters, were set to remove ligands with undesirable drug-likeness properties and reactive functional groups. The hits obtained from specified filtering criteria proceeded to the subsequent docking steps in the workflow.

The VSW exploits Glide docking protocol to rank the best compounds which can utilize three different levels of docking precision: high-throughput virtual screening (HTVS), standard precision (SP) and extra precision (XP) (Glide, 2015; Friesner et al., 2004, 2006). Docking calculations were first performed in HTVS mode and subsequently in XP mode using a pre-generated grid file for the receptor. The scaling factor for the van der Waals radii of the docked ligands was set to 0.80 Å, with a partial charge cut-off 0.15 Å. In every step of glide docking, 10% of the ligands were retrieved with a higher score of docking. Finally, ligands that are docked most favorably were sorted using the XP GlideScore scoring function. Glide Score values lower than −7.0 kcal mol^{-1} along with a visual inspection was considered in order to select top ranking compounds with the appropriate binding mode within the active site. This process resulted in the retrieval of 1,603 compounds employed in the next filtering stages.

ADMET properties and PAINS filter analysis

Drug development involves the assessment of efficacy and toxicity of the new drug candidates and includes generating a hypothesis of the target receptor for a particular disorder and screening the in vitro and/or in vivo biological activities of the new drug candidates. Therefore, with the aim of achieving drug-like molecules, the Qik-Prop module implemented in Maestro was applied as an extra filter for in silico assessment of ADMET properties of the potential hit molecules (QikProp, 2019). It provides a quick and detailed method for the calculation of a wide range of principal molecular descriptors that are useful in the prediction of physicochemical properties. Especially, membrane permeability, lipophilicity, human oral absorption, cardiotoxicity or potential interaction with hERG channels, were amongst important criteria investigated for filtering (Lipinski et al., 1997). Default settings were employed for these calculations. Subsequently, the selected compounds from Qikprop were further evaluated for “Pan Assay Interference Compounds” (PAINS) by means of the FAFDrugs 4.0 tool (FAFDrugs4.0, http://www.fafdrugs4.mti.univparis- diderot.fr; Lagorce et al., 2008, 2011).

Quantum-Mechanics polarized ligand docking

Polarization effect is a key term that is generally omitted from docking algorithms based on pure molecular mechanic calculations. It is now well recognized that the accuracy of electrostatic charges can contribute a critical role in determining the most energetically favorable ligand poses and thereof protein-ligand docking results (Cho et al., 2005). In the first step, compounds were initially docked into the

binding site of a protein. The initial docking calculations were carried out using Glide standard precision (SP) docking protocol, generating the top 5 poses per docked molecule. In the second step, the polarizable ligand charges induced by the protein field were calculated with QSite software which is coupled with the Jaguar quantum mechanics engine (Jaguar, 2015; QSite, 2015). In this regard, the QM charge calculations of the best scoring pose for each ligand were carried out using the density functional theory (DFT) method with the B3LYP/6-31G*/LACVP* basis set within the protein environment defined by the OPLS-2005 force field. The atomic charge assignment was made employing electrostatic potential (ESP) fitting. Finally, the ligands with modified partial charges were re-docked into the active site using Glide extra precision (XP) docking which reported 10 poses for each ligand as default. In this step of screening workflow, the potential inhibitors were selected based on lower values of XP GlideScore and the key interactions between the ligand and protein interacting region within the active site. Interaction of the best docked pose for each ligand was visually analyzed by ligand-interaction diagram implement in Maestro and PyMol visualizer (Maestro, 2019; PyMOL Molecular Graphics System, 2019).

Ligand-based affinity estimation based on MM-GBSA technique

Since the approximate scoring functions like Glide Score are not reliable predictive criteria to rank compounds in terms of their binding affinities, the best ligand-protein complexes obtained from QPLD studies were subjected to a subsequent analysis with MM-GBSA technique provided in Prime module of Schrödinger suite 2019 (Prime, 2019). This method offers a worthwhile post-scoring approach for prioritizing the screened hits by calculating the relative binding free energy (ΔGbind) between ligands and receptors with further accuracy.

The MM-GBSA approach combines the molecular mechanical (MM) energies with a continuum solvent generalized Born (GB) model for polar solvation as well as a solvent-accessible surface area (SASA) for non-polar solvation term (Huang et al., 2006; Kuhn and Kollman, 2000). For this purpose, the best-obtained ligand poses were first minimized using the local optimization feature in Prime and then energies of the ligand-protein complex were calculated using the OPLS-2005 force field and Generalized-Born/Surface Area continuum solvent model.

Results and discussion

The main purpose of the current study is to identify novel and promising scaffolds, interacting with adiponectin receptor AdipoR1, as potential anti-diabetic agents. To achieve this goal, an integrated and highly advantageous VS protocol combining various in silico methods (ligand and structure-based) was developed in a stepwise filtering approach (Figure 1).

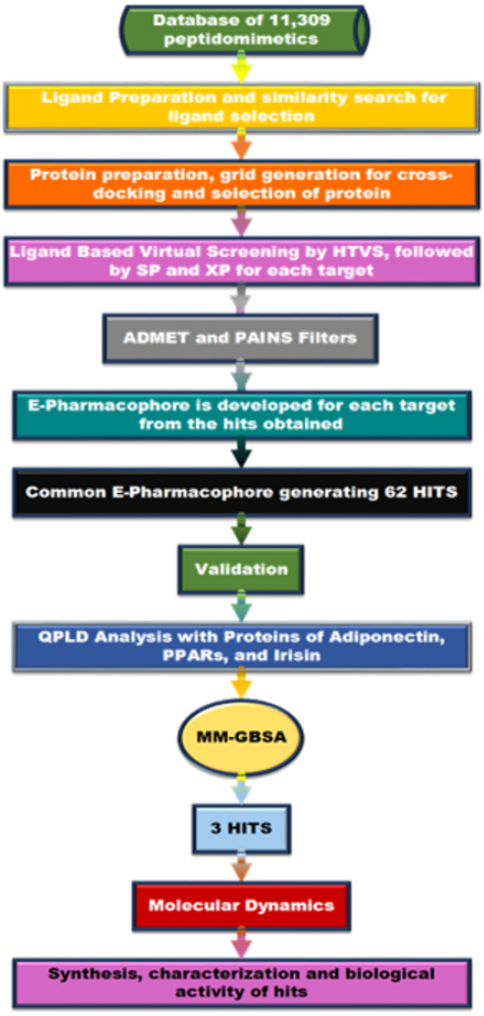

Figure 1. Schematic representation of the virtual screening process implemented in the identification of Adiponectin and PPAR-G inhibitors

Similarity search (ligand-based screening)

For this purpose, the online database of Asinex was screened for retrieving peptidomimetic compounds with respect to the AdipoR1 agonist BHD1028. This led to select a collection of 11,309 structurally similar compounds. These compounds were subjected to different hierarchical filtering steps.

Structure-based virtual screening

Structure-based VS is an extremely proficient method to recognize efficient lead compounds from chemical libraries. This method is quantitatively able to assess binding affinities of ligands towards the active site of a target protein through molecular docking technique and subsequent ranking them in decreasing order. It also predicts accurate binding modes and detailed molecular interactions between the ligand and receptor. Thus, the found 11,309 candidate compounds were screened against the putative active site of adiponectin protein which is involved in the PPARs activation using the VS

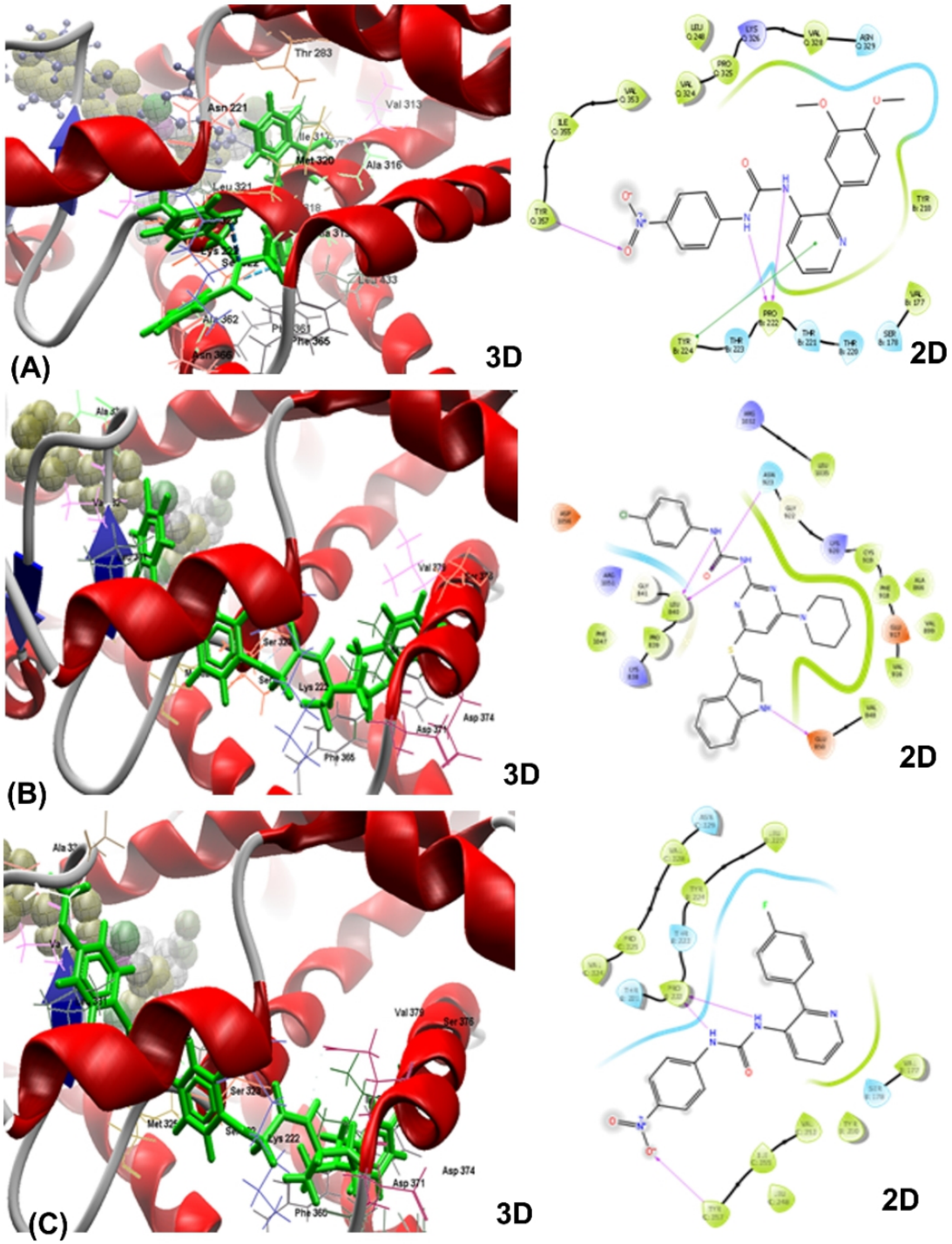

Figure 2. 2D and 3D docking images of (A) compound 19 in complex with Adiponectin Adipor1 (PDB:3WXV); (B) compound 7 in complex with Adiponectin Adipor1 (PDB:3WXV); (C) compound 8 in complex with Adiponectin Adipor1 (PDB:3WXV)

workflow (VSW) procedure implemented in Maestro molecular modelling environment. This workflow allows performing a fast preliminary screening (HTVS), followed by more accurate docking protocols (SP or XP). Molecular docking protocol requires carefully optimized 3D-structure of protein and ligands for the accurate binding affinity prediction. Therefore, the X-ray structure of adiponectin protein and PPARs were optimized in protein preparation wizard applying OPLS-2005 force field to remove bad atomic contacts in the 3D-structure and to obtain a structure with lower energy state. Furthermore, ligand preparation at the cellular pH value (7.4 ± 0.5) using LigPrep generated 12,530 derivatives from 11,309 compounds taking into account possible ionization states and guaranteed that all ligands are in the lowest energy conformation. Since, there were no other filters implemented in the library generation process, the absolute similarity, Lipinski's RO5 and Reactive pre-filters, at

Table 1. Top 28 hits obtained after structure based virtual screening based on docking score, glide energy and glide Emodel

S. N.	Title	Docking score	Glide ligand efficiency	XPG Score	Glide G score	Glide energy	Glide E model	XPH Bond
1.	19	-7.241	-0.25	-7.242	-7.242	-40.796	-60.178	-1.069
2.	7	-7.069	-0.272	-7.07	-7.07	-42.303	-55.809	-1.271
3.	8	-6.998	-0.28	-6.999	-6.999	-39.881	-56.058	-1.19
4.	4	-6.897	-0.287	-6.899	-6.899	-40.423	-53.622	-1.14
5.	16	-6.874	-0.264	-6.876	-6.876	-42.007	-56.129	-1.145
6.	15	-6.83	-0.253	-6.831	-6.831	-41.819	-57.885	-0.482
7.	17	-6.737	-0.25	-6.739	-6.739	-41.712	-54.159	-1.177
8.	5	-6.346	-0.264	-6.347	-6.347	-38.701	-50.196	-0.7
9.	1	-6.169	-0.268	-6.171	-6.171	-37.943	-53.939	-1.219
10.	2	-6.141	-0.267	-6.143	-6.143	-40.392	-52.77	-1.292
11.	20	-5.924	-0.212	-5.926	-5.926	-42.709	-61.766	-1.042
12.	14	-5.881	-0.235	-5.883	-5.883	-40.744	-60.104	-1.042
13.	6	-5.844	-0.243	-5.845	-5.845	-38.955	-55.489	0
14.	23	-5.551	-0.179	-5.552	-5.552	-51.413	-65.447	-0.479
15.	3	-5.408	-0.216	-5.41	-5.41	-44.271	-65.306	-1.442
16.	24	-5.295	-0.177	-5.296	-5.296	-51.465	-66.705	-1.176
17.	9	-5.185	-0.192	-5.195	-5.195	-39.267	-60.483	-0.711
18.	13	-5.045	-0.202	-5.046	-5.046	-40.341	-58.974	-0.629
19.	12	-4.928	-0.176	-4.939	-4.939	-44.949	-62.191	-0.7
20.	21	-4.883	-0.168	-4.885	-4.885	-48.876	-62.686	-0.7
21.	10	-4.849	-0.18	-4.859	-4.859	-45.387	-62.098	-0.998
22.	22	-4.641	-0.16	-4.642	-4.642	-49.924	-63.517	-0.7
23.	18	-4.554	-0.169	-4.555	-4.555	-46.947	-61.923	-0.7
24.	11	-4.506	-0.155	-4.515	-4.515	-44.48	-59.57	-0.7
25.	9	-4.137	-0.153	-6.552	-6.552	-40.51	-59.555	-0.7
26.	11	-3.846	-0.133	-6.319	-6.319	-44.414	-63.342	-0.858
27.	12	-3.707	-0.132	-6.095	-6.095	-45.111	-61.951	-1.33
28.	10	-3.658	-0.135	-6.081	-6.081	-41.94	-61.175	-1.031

the dawn of the screening workflow, were applied to remove less relevant ligands in the dataset. In fact, the RO5 filter evaluates the potential drug-like profile of compounds belonging to a given dataset, helping to make an easier selection of molecules with better physicochemical properties (Lipinski et al., 1997). This rule states that a molecule to be considered as druglike should have:

1) A partition coefficient values (logP) ≤5;

2) A molecular weight (MW) ≤500 g mol−1;

3) A number of hydrogen bond acceptors ≤10;

4) A number of hydrogen bond donors ≤5.

Any value differing from these values is considered a violation. The acceptable value of violations for a drug-like molecule is 1. Results displayed that 10,625 out of 12,530 compounds fitted with the Lipinski's properties and thus proved to possess satisfactory drug-like profiles. Moreover, after Reactive filtering, a total of 8,230 hits in the dataset were identified to have no reactive functional groups which were kept to proceed to the next steps. Subsequently, the pre-filtering process was followed by structure-based searching in order to dock the retrieved 8,230 hits into the binding site of protein and to score their binding affinities. In the first step, 6,723 compounds as top 10% of the ligands with the lowest docking score were obtained by application of the Glide HTVS method. Further screening of these compounds in highly accurate Glide XP mode of docking resulted in 270 compounds which were top-ranked based on XP GlideScore values. Lower XP GlideScore represents the higher binding affinity of the ligand towards protein. Therefore, 270 top-ranked compounds with XP GlideScore values lower or equal to -7.0 kcal mol^{-1} were finally selected as potential hits with a satisfactory drug-like profile to continue to the next filters.

ADMET prediction and PAINS filter analysis

Consequently, as a step of the developed screening workflow, 270 compounds selected from the last step were evaluated for ADMET properties using QikProp software. This step allowed to select only ligands possessing key pharmacokinetic properties within permissible ranges, as defined by QikProp, for 95% approved drugs. Caco-2 and MDCK cell permeability in nm/sec (QPPCaco-2 and QPPMDCK > 500), blood-brain barrier permeability (QPlogBB: -3 to 1.2), percentage of human oral absorption (HOA > 80%), lipophilicity (QPlogP: -2 to 6.5) and capability of blocking hERG K+ channels and so cardiotoxicity (QPlogHERG<-5) were special parameters considered in this step of filtering. 86 out of 270 compounds with predicted appropriate pharmacokinetic properties passed the screening of ADMET prediction. Moreover, the resulting compounds were fruitfully screened for their potential capability to behave as PAINS by FAFDrugs 4.0 webserver (FAFDrugs4.0, http://www.fafdrugs4.mti.univ-paris-diderot.fr). Based on these criteria, 24 molecules among 86 compounds contain substructural features that marked them as "frequent hitters" in high throughput screens. Finally, this step greatly reduced the number of screened hits, thereby highly enriching the library with 62 more promising virtual hits.

Quantum-mechanics polarized ligand docking (QPLD)

The resulting 62 compounds were subjected to QPLD calculations for a better prediction of their binding mode inside the binding site of the AdipoR1 protein. It was presumed that this docking protocol provides a more accurate prediction of electronic interactions, improving the accuracy of docking results (Cho et al., 2005; Illingworth et al., 2008). The obtained QPLD results for all compounds were sorted based on XP GlideScore values. Ultimately, a total number of 28 candidates (Table 1) were identified with docking score within the range from -7.24 to -3.65 kcal mol^{-1}, glide energy from -73.71 to -37.06 kcal mol^{-1} and glide Emodel energy from -65.47 to -50.19 kcal mol^{-1}

Prioritization of hit compounds based on MM-GBSA ΔG binding energy

Although docking calculations are highly successful in offering the best ligand pose within the protein binding site, they often don't render reliable measuring criteria to rank compounds with respect to their binding affinities (Taylor et al., 2002). It appeared that the incorporation of more physically relevant energy terms such as solvation energy and surface accessibility area with an appropriate force field provides ligand binding energy calculations with more acceptable accuracy to prioritize the relative potencies of screened hit compounds (Huang et al., 2006; Kuhn and Kollman, 2000). Thus for each ligand selected from the QPLD studies, the pose with the lowest GlideScore was rescored using a subsequent MM-GBSA post docking protocol (Lyne et al., 2006). Rescoring using MM-GBSA leads to minor changes of the ligand conformations within the receptor site (Figure 2).

These changes result from minimization of the ligand in the receptor's environment and consequent stabilization of receptor-ligand complex.

The final ranking of the ligands was carried out based on obtained free binding energy values (ΔGbind). The ΔGbind values lower than -64 kcal mol−1 were considered to retrieve the final set of compounds, leading to the recognition of 10 top-ranked hits with different scaffolds. This suggests that these compounds were the most stable ligands within the protein active site, thereby possessing the highest in silico binding affinity for adiponectin binding site of AdipoR1.

Conclusion

In the present study, an integrated VS protocol with the combination of different in silico methods for the efficient identification of novel small-molecule inhibitors of adiponectin AdipoR1 interaction was developed. In this respect, computational evaluation of a library of 11,309 compounds, was conducted in several hierarchical steps. The exploited filtering criteria for the selection of potential adiponectin disruptors include: 1) the estimated GlideScore values lower than −7.0 kcal mol−1 obtained by the VS workflow; 2) the prediction of pharmacokinetic and ADMET properties using QikProp software and FAF-Drugs webserver; 3) the lowest GlideScore values and the best interaction pattern with key hydrophobic pocket of the adiponectin binding site obtained from QPLD calculations; 5) the best estimated binding free energy values calculated using Prime/MM-GBSA simulation. The discussed filtering process resulted in elicitation of 10 top ranked hits. These compounds displayed satisfactory ADMET properties and passed the false-positive evaluation during PAINS analysis. Additionally, the results of QPLD protocol coupled with MM-GBSA rescoring method were indicative of higher binding affinities of selected hits towards three critical hydrophobic pockets in the adiponectin binding site. As a final point, three compounds 9a, 13e and 19b amongst selected final hits exhibited the best stability profiles and binding pattern in the adiponectin active site. In view of all these observations, the presented potential hits can be considered as promising scaffolds for the development of novel anti-diabetic agents targeting adiponectin AdipoR1interaction. To further substantiate it, the experimental explorations of these compounds will allow to provide explicit indications for the design of analogues with improved pharmacological profile.

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Conflict of interest

There is no conflict of interest in the work so conducted.

References

Bernstein FC, Koetzle TF, Williams GJB, Meyer EF, Brice MD, Rodgers JR. 1977. The protein data bank: a computer- based archival file for macromolecular structures. European Journal of Biochemistry 80: 319–324.

Cho AE, Guallar V, Berne BJ, Friesner R. 2005. Importance of accurate charges in molecular docking: quantum mechanical/molecular mechanical (QM/MM) approach. Journal of Computational Chemistry 26: 915–931.

Field AE, Coakley EH, Must A, Spadano JL, Laird N, Dietz WH. 2001. Impact of overweight on the risk of developing common chronic diseases during a 10-year period. Archives of Internal Medicine: 161(13): 1581–1586.

Ford ES, Williamson DF, Liu S. 1997, Weight change and diabetes incidence: findings from a national cohort of US adults. American Journal of Epidemiology, 146(3): 214–222.

Friesner RA, Banks JL, Murphy RB, Halgren TA, Klicic JJ, Mainz DT. 2004. Glide: a new approach for rapid, accurate docking and scoring. 1. Method and assessment of docking accuracy. Journal of Medicinal Chemistry, 47:1739–1749.

Friesner RA, Murphy RB, Repasky MP, Frye LL, Greenwood JR, Halgren TA, Sanschagrin PC, Mainz DT. 2006. Extra precision glide: Docking and scoring incorporating a model of hydrophobic enclosure for protein-ligand complexes. Journal of Medicinal Chemistry, 49: 6177–6196.

Golay A, Ybarra J. 2005. Link between obesity and type 2 diabetes. Best Practice & Research: Clinical Endocrinology & Metabolism, 19(4): 649–663.

Huang N, Kalyanaraman C, Irwin JJ, Jacobson MP. 2006. Physics-based scoring of protein-ligand complexes: enrichment of known inhibitors in large-scale virtual screening. Journal of Chemical Information and Modeling, 46: 243–253.

Jorgensen WL, Maxwell DS, Tirado-Rives J. 1996. Development and testing of the OPLS all-atom force field on conformational energetics and properties of organic liquids. Journal of the American Chemical Society, 118: 11225–11236.

Kuhn B, Kollman PA. 2000. Binding of a diverse set of ligands to avidin and streptavidin: an accurate quantitative prediction of their relative affinities by a combination of molecular mechanics and continuum solvent models. Journal of Medicinal Chemistry, 43:3786–3791.

Lagorce D, Sperandio O, Galons H, Miteva MA, Villoutreix BO. 2008. FAF-Drugs2: free ADME/tox filtering tool to assist drug discovery and chemical biology projects. BMC Bioinformatics, 9: 396.

Lagorce D, Maupetit J, Baell J, Sperandio O, Tuffery P, Miteva MA, Galons H, Villoutreix BO. 2011. The FAF-Drugs2 server: a multistep engine to prepare electronic chemical compound collections. Bioinformatics, 27: 2018–2020.

Sastry GM, Adzhigirey M, Day T, Annabhimoju R, Sherman W. 2013. Protein and ligand preparation: parameters, protocols, and influence on virtual screening enrichments. Journal of Computer-Aided Molecular Design, 27: 221–234.