Computer-based exploration of potential inhibitors of FTO protein by structure-based virtual screening and molecular docking studies.

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Abstract: *In this study, our goal was to screen novel potential FTO1inhibitors by structure-based virtual screening, ADME prediction, molecular dynamics simulation, and binding free energy calculations. We loaded the FTO target protein (PDB ID: 3LFM) at 2.57Å from the RSBC Protein Data Bank. We performed a virtual screening of a large compound library using it as a receptor. We selected seven top ranked compounds and further evaluated their binding modes and energies to FTO by molecular docking, molecular dynamics simulations, and binding free energy calculation methods. In addition, we predicted their ADME properties and stability using online computational tools. Among them, we identified a selection of potential compounds that showed high binding affinity, favorable ADME characteristics, and good stability with FTO. These candidates have the potential to be future herald compounds in the development of novel FTO drug targets. Our study provides new insights into FTO inhibitor discovery and demonstrates the feasibility and efficiency of using computational approaches for FTO inhibitor discovery*.

***Key Words*: Docking, Fat mass and obesity associated (FTO) protein, Ant obesity, ADME prediction, Computational studies.**

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1. INTRODUCTION

Studies have shown fat mass and obesity-associated (FTO) protein contributes to non-syndromic human obesity which refers to excessive fat accumulation in human body and results in health risk. FTO protein has become a promising target for anti-obesity medicines as there is an immense need for the rational design of potent inhibitors to treat obesity..Dysfunction of FTO1 leads to imbalances in multiple signaling pathways, resulting in imbalanced immune responses and severe inflammation. FTO1 has an essential part in the regulation of cell death and inflammation mediated by the tumor necrosis factor (TNF), including apoptosis, proliferation-induced activation of NF-κBs and mitogen activated protein kinases (MAPKs), and caspase-8-mediated apoptosis. FTO1 is also involved in various physiological and pathophysiological functions including embryonic development, immune homeostasis, and neurodegeneration.

1.1. Obesity

Obesity and weight gain are the main risk factors associated with diseases like diabetes mellitus, cardiovascular disease, and non-alcoholic fatty liver disease, with an increased risk of disability . In 2007, several studies described that a cluster of single nucleotide polymorphism (SNPs) in the first intron of fat mass and obesity-associated (FTO) protein was highly correlated with obesity-related trait. FTO protein has been shown to influence obesity and energy utilization in human up to half the world's population. Additionally, FTO protein has been identified to be involved in various disease processes which include cardiovascular diseases , Alzheimer disease , type II diabetes , and breast cancer . This makes FTO protein an interesting target to study with respect to its involvement in human diseases.

1.2. Structure of FTO Protein

FTO protein contains a double-stranded β-helix fold which is typical for the member in the Fe(II) and 2-oxoglutarate (2OG)-dependent AlkB dioxygenase family which also includes human homologues ALKBH1-8 . FTO protein can oxidatively demethylate single-stranded nucleic acids in vitro , but it has relatively lower repair activities compared to other member proteins of AlkB family . However, the physiological function and in vivo substrates of FTO protein have remained largely unclear. The function of obesity-risk factor FTO protein is to demethylate N6-methyladenosine (m6A) in mRNA. And this apparently indicates a novel and reversible regulatory mechanism in mammalian cells. Additionally, FTO protein has been defined to demethylate diverse mRNAs which shows that the regulation of N6-methyladenosine (m6A) by FTO protein likely influences various biological pathways related to diseases and cellular signaling. This knowledge suggests that FTO protein plays an important role in controlling other gene expression and protein translation processes involved in the regulation of diseases like obesity and cancer. These studies have advanced the possibilities of

novel therapeutics which involves, targeting FTO protein with small molecules.

Recently revealed crystal structure of FTO protein provides basis for its substrate specificity and binding sites (Han et al., 2010). Moreover, these studies enable the rational design of new inhibitors targeting FTO protein. Inhibiting activity of FTO protein by small molecules has been proposed as a potential treatment for extreme obesity. However, validation of this approach requires the development of small molecule inhibitors for this protein. The natural product rhein has been identified as the first inhibitor of FTO protein through structure-based virtual screening. Rhein competitively disrupts binding of FTO protein to the m6A substrate, and enhances thermal stability of FTO protein by direct binding. Rhein actively increases cellular levels of m6A in mRNA and the structural complex illustrate that rhein does indeed bind to the nucleic acid binding site . However, rhein characterized little selectivity for the AlkB subfamily which eclipses its applicability as a specific functional probe of FTO protein inside the cell. The 2OG-tethering approach was also applied to develop the cell active FTO protein inhibitors and these inhibitors proved highly selective in vitro for the AlkB subfamily. However, selectivity of these inhibitors remains unclear in vivo . Moreover, the molecular modeling of FTO/rhein complex suggests that rhein has a smaller and more compact chemical structure to achieve potency and selectivity through favorable interactions at the active site

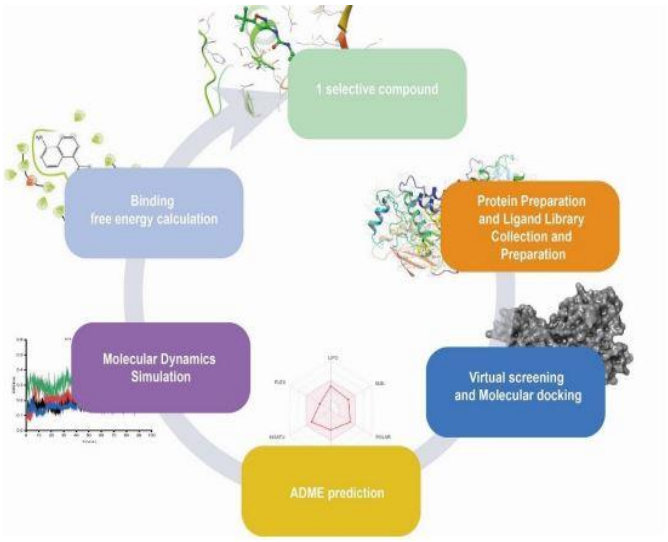

FIGURE 1 The workflow for computer-assisted discovery of potential inhibitors of RIPK1 kinase was carried out by virtual screening, molecular docking of the seven scored compounds obtained and MD simulations with the homologous ligand compound27 to study the stability of their binding and mode of interaction.

2. MATERIALS AND METHODS

We have obtained the 3D structure of the protein kinase RIPK1 target protein. (PDB ID: 3LFM) using the RSBC Protein Data Bank (https://www.rcsb.org), and other strands, heteroatoms, as well as water molecules in the PDB file were deleted using the PyMOL visualization software, preserving the A chain of 3LFM. Compound libraries for screening and docking studies were obtained from the ZINC-FDA database, a publicly available and accessible database, and a total of 71,069 ligands were downloaded for subsequent virtual screening using Lipinski's "rule of five".

Virtual screening and Molecular docking

Computer-aided drug discovery can significantly reduce the time to drug discovery. To identify potential FTO1 inhibitors, we used structure-based virtual screening. TPO1 kinase was screened using Openbabel to convert ligand SDF files downloaded from the ZINC-FDA database into PDBQT format. The TPO1 kinase structure was first hydrogenated, calculated, and charged, and energy was minimized using UCSF Chimera 1.17.1. The input files required for protein molecule docking were then generated using the LePro module of LeDock win32.

Molecular docking was then performed using LeDock, leading directly to the identified potential TPO1 inhibitors, and the LeDock docking results were split into multiple PDB files, which were imported into Maestro 13.5 along with the protein structures for visualization, and analyzed using the The top compounds were selected by sequencing the compounds according to their docking scores, and the ligand-receptor interactions were plotted using the ligand interaction visualization tool Ligplot+ to correctly analyze the interacting residues .

ADME prediction

To understand their 'drug-like' properties, the seedling compounds derived by molecular docking were submitted to ADME analysis. Seven small molecules were selected as suitable for molecular dynamics simulations by comparing predicted properties within an acceptable range using predicted parameters such as the number of molecular weight, number of heavy atoms, number of aromatic heavy atoms, number of rotational bonds, number of hydrogen bond acceptors, cost of hydrogen bond donors, topological polarity surface area, solubility class, gastrointestinal absorption, blood-brain barrier penetration and Lipinski's five laws from Swiss ADME. cost number of hydrogen bond donors, topological polarity surface area, solvent classification, absorption in the stomach, penetration through the human blood-brain barrier and the Lipinski five laws from Swiss ADME (http://www.swissadme.ch/index.php)

3. RESULTS AND DISCUSSION

Many commercial and non-commercial software programs are now available for molecular docking studies, and to screen the database for potentially biologically active molecules, we used the molecular docking technique; the Ledock molecular docking software was used to virtually

screen the ZINC database and sort the scores. The final screen yielded seven suitably scored compounds, their structure schematic as well as parameters are shown. On the basis of the final scores, we selected seven top-ranked compounds for further evaluation and predicted their ADME properties with the aid of various computational tools, we also performed docking analyses of the compounds with FTO1, calculated their binding modes and energies, and performed binding free energy.

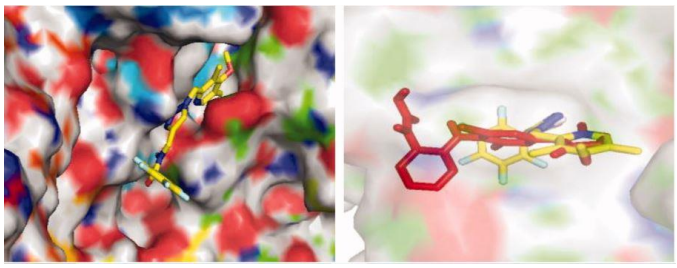

Figure 2 Binding interactions with the receptor with the protein.

ADME prediction

Since the ADME quality of compounds is essential for discovering and developing medicines, we used an internet service, Swiss ADME (http://www.swissadme.ch/index.php), to analyze the characteristics associated with selected seedling compounds, and the seedling compounds obtained after molecular docking were evaluated for their drug physicochemical properties. The prediction of the ADME metrics of the screened ligands indicated that they could be used in the future as parameters for the use of drug candidates, and only compounds fulfilling all the criteria were selected for molecular modeling studies. The prepared libraries underwent structure based virtual screening with the use of Ledock molecular docking software, and the best-scoring ligands were further investigated. A total of seven compounds were identified through screening and docking, of which ZINC000055224617 (-8.52 kcal/mol), ZINC000048990080 (-8.50 kcal/mol), ZINC000065373080 (-8.60 kcal/mol) and ZINC000222934382 (-8.46 kcal/mol) 4 compounds with good scores and essential features were identified as the best drug candidates.

4. CONCLUSIONS

Molecule docking and computational simulation allow faster and more cost effective testing of potent drug candidates without using live animals. In short, there were 71,069 compounds downloaded from the ZINC-FDA database with Lipinski's "Rule of Five ."

In summary, we provide sufficient evidence that the above drugs can effectively exert inhibitory effects on RIPK1 kinase. These candidates represent potential reasonable candidates for the generation of RIPK1 kinase inhibitors. However, we acknowledge the limitations of our study and the lack of basic experimental data, which will require additional in vitro and in vivo experiments, as well as clinical trials, to confirm these compounds' inhibitory effects on RIPK1 kinase, so that they can be applied in the clinic as possible future therapeutic agents

REFERENCES

1] Damayanti CA1 , Harahap MAY , Wibowo S , Sjofjan , Djunaidi IH Computational Insight into Anti-Obesity Effects of Combined Some Phytobiotics to GLP1R (Receptor) Protein in Anas javanica on April 29, 2023(https://doi.org/10.1101/2023.04.26.538390)

2] Mengrong Cheng, Bing Mei, Qian Zhou, Manling Zhang, Han Huang, Lanchun Han, Qingyang Huang Computational analyses of obesity associated loci generated by genome-wide association studies Published: July 2, 2018 (https://doi.org/10.1371/journal.pone.0199987)

3] Lavanya P, Dicky John, Davis G.on Computational study of potential inhibitors for fat mass and obesity-associated protein from seaweed and plant compounds Department of Bioinformatics, Faculty of Engineering and Technology, Sri Ramachandra Institute of Higher Education and Research, Chennai, Tamil Nadu Published 21 October 2022. (http://dx.doi.org/10.7717/peerj.14256)

4] Ji-Hyuk Park, Wona Jee, So-Mi Park, Ye-Rin Park, Seok Woo Kim, Hanbit Bae, Won-Suk Chung, Jae-Heung Cho, Hyungsuk Kim, Mi-Yeon Song,and Hyeung-Jin Jang Timosaponin A3 Induces Anti-Obesity and Anti-Diabetic Effects In Vitro and In Vivo Published: 2 March 2024 (https://doi.org/10.3390/ijms25052914)

5] Manobharathi, Surya, Shobanadevi. Umadevi, Rabiya, Vedhapriya, Sangavai .Pshytochemical Screening and Antiobesity Activity of morinda citrifolia leaves extract VOLUME 18, ISSUE 2, 2024 (http://xadzkjdx.cn)

6] Hyuck Kim, Jihwan Lee, Seyeon Chang , Dongcheol Song , Jaewoo , Sehyun Park ,Kyeongho Jeon , Jungseok Choi, Jinho Cho on Anti-obesity effects of two herbal extracts in C57BL/6N mice fed high-fat diet Department of Animal Science, Chungbuk National University, Cheongju 28644, Korea and Accepted: February 21, 2024 (https://doi.org/10.7744/kjoas.510108)

7] Doaa Salah Eldin Abdelfattah, Mervat Fouad, Aliaa Elmeshad, Mohamed A. El-Nabarawi and Sammar Fathy Elhabal National Nutrition Institute, Cairo 11435, Egypt; Published: 20 January 2024 (https://doi.org/10.3390/nu16020310)

8] Kumaraswamy Athesh , Nayagam Agnel Arul John, Gurunagarajan Sridharan, Pemiah Brindha, Amer M. Alanazi, Kannan R.R. Rengasamy, Balasubramanian Balamuralikrishnan,Wen-Chao Liu and Arumugam Vijaya Anand on Protective Effect of Dolichos biflorus Seed Extract on 3T3-L1 Preadipocyte Differentiation andHigh-Fat Diet-Induced Obesity in Rats At Department of Biochemistry, Srimad Andavan Arts and Science to Bharathidasan University, Tiruchirappalli 620005, Tamil Nadu, India ; Published 10 February 2023. (https://doi.org/10.1155/2023/6251200)