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New Drug Design to Suppress Nonalcoholic Steatohepatitis

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Abstract: A *de novo* designed biomolecule called INASHD was utilized through computer-aided drug design techniques to specifically target β2-spectrin, effectively suppressing and preventing NASH disease. Advanced computational software tools concerning the technologies of molecular docking and molecular dynamics (MD), were employed to showcase the drug's remarkable ability to efficiently suppress and control the α-helical topology of β2-spectrin. This protein is a vital component within the disease pathway. We successfully devised an effective design suppressing β2-spectrin, exhibiting an inhibition score surpassing any other molecule documented in scientific literature. With robust support from validated computational software, this bioorganic structure holds significant value and can be applied for a patent due to its innovative design. It shows promising potential for delivering positive outcomes in various stages, including *in vitro, in vivo, ex vivo*, and human phase studies.

Keywords: Nonalcoholic steatohepatitis, in silico drug design, molecular docking, molecular dynamics, β2-spectrin.

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1. INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is one of the most widespread disorders in the world. NAFLD is defined as a spectrum disorder affecting the liver that leads to hepatic steatosis. Nonalcoholic steatohepatitis (NASH) is described as an inflammatory subtype of NAFLD. In this condition, steatosis is accompanied by inflammation and hepatocyte damage, with or without fibrosis. It is also known that NASH can progress to cirrhosis in time, although it initially remains silent (1–5).

In a recent study by Rao *et al*., β2-spectrin (SPTBN1) stimulates sterol regulatory element-binding protein (SREBP)-related lipogenesis, and in this way, it promotes hepatic cancer development in an *in vivo* model. This study also reveals that SPTBN1 silencing in hepatocytes protects from lipid accumulation, fibrotic tissue formation, and hepatic damage in the same animal model. Furthermore, clinical samples from NASH patients indicate elevated SPTBN1 expression (6). Thus, SPTBN1 silencing and/or downregulation could provide a valuable route to develop novel therapeutic strategies for treating NASH. To completely eradicate NASH, it is essential to explore strategies such as gene silencing of SPTBN1, suppressing the expression of β2-spectrin, or inhibiting its overexpression. The initial approach involves regioselective drug design aimed at targeting particular repetitive nucleotide bases within the extensive 220,000-nucleotide sequence of SPTBN1 on chromosome 2 (7). Nevertheless, this proves to be a formidable challenge due to the demanding nature of modeling and simulation studies, requiring an immense amount of computational power, potentially involving thousands of GPUs in supercomputers, and spanning several months, if not years.

As an alternative approach to addressing the issue, a potential solution lies in the development of a siRNA-based drug that targets and suppresses the expression of β2-spectrin. However, caution must be exercised as this approach carries some risks, particularly concerning potential drug counter-indications. Spectrin proteins play a crucial role in maintaining the structural stability of cell membranes

and cell morphology. Excessive suppression of spectrin could lead to cell membrane instability and loss of cell structure (6,8,9).

A third strategy involves dealing with NASH, where there is an excessive accumulation of β2-spectrin due to inflammation. In this case, complete elimination of β2-spectrin, as proposed in the second approach, may not be desirable. Alternatively, *de novo* drug design can be employed to both inhibit and regulate the activity of β2-spectrin, enabling effective management of NASH symptoms and addressing the inflammatory issues within the affected tissue.

A new drug compound named INASHD has been computer-aided and designed with *in silico* techniques to specifically target β2-spectrin and effectively combat NASH disease. Computational software tools, including molecular docking and molecular dynamics (MD), were employed to demonstrate the drug's exceptional efficiency in suppressing and controlling the α-helical topology of β2-spectrin. This protein plays a crucial role in the progression of the disease.

Overall, we have successfully designed the molecule with a pharmaceutical organic formulation, and its inhibitory capabilities surpass those of any molecule documented in scientific literature. By substantiating the chemistry of this molecule with extensively validated computational software, it becomes a valuable bioorganic structure that was sent for patenting. Its innovative design holds the potential to yield promising results in various stages of testing, including *in vitro*, *in vivo*, *ex vivo, etc*.

2. MATERIALS AND METHODS

2.1. Theoretical Geometric Optimization

To accurately determine the active sites of a molecule and investigate its interactions with receptors, it is essential to establish its optimal geometric structure. In our present study, we utilized INASHD as the ligand's organic chemical structure and analyzed its most stable molecular geometry using the Gaussian 09 program (10) with density functional theory (DFT)/B3LYP functional (11) and the 6-31G(d,p) basis set. This process resulted in the formation of the most stable molecular structures of INASHD with β2-spectrin (PDB ID: isolated from 6M3P, AnkG was removed), intended for further computational and simulation-based research, as depicted in Figures 1 and 2. To prepare input files for molecular docking, molecular dynamics computations, and post-processing of output files, we employed GaussView 6.0 and Avogadro 1.95 softwares (12,13).

2.2. Molecular Docking Procedure

Molecular docking simulations were conducted using AutoDock Vina 1.1.2 software (14), renowned for its exceptional precision and accuracy in biochemical docking simulations. A total of 800 poses were generated, comprising 100 poses for each simulation. Blind docking was employed, and the grid box dimensions were set to 80 × 80 × 100 $Å^3$ (as the x, y, and z grid parameters, respectively). The ligand INASHD, designed *de novo*, was studied for its interactions with the receptor structure of β2-spectrin, which was downloaded with the PDB id of 6M3P and then isolated from the protein complex for analysis. Gauss View 6.0 and Avogadro 1.95 software were utilized for the optimization of the ligands and 1BNA structures. The simulations effectively illustrated the interactions and binding of the drug to the receptor, with the docking scores represented in kcal/mol signifying the Gibbs free binding energy. From all the simulations, the initial structure and input file for the subsequent molecular dynamics (MD) simulations were selected as the docking pose with the most accurate and favorable binding energy, identified within the best-clustered data.

2.3. Molecular Dynamics (MD) Simulations

The initial structures for the MD simulations were chosen based on the docking poses with the most favorable binding energies (15–18), as previously reported in scientific literature. The molecular dynamics (MD) simulations were conducted using Schrödinger's Maestro Desmond software (19), each spanning 50 ns with 5000 poses at 10 ps intervals. To ensure accuracy, each MD simulation was repeated five times with different seed numbers, confirming the correctness of the simulation parameters and the structures of the INASHD-β2 spectrin complexes formed.

During the MD simulations, the dynamic properties of the ligand-receptor complexes were continuously evaluated over time. The simulation area was defined by a grid box measuring 130 × 130 × 130 $Å^3$ with a spacing of 0.5 Å, providing an extensive grid area for the simulations. TIP3P-type water molecules were included within the box, and 0.15 M NaCl ions were added to neutralize the system. The temperature and pressure conditions were set as follows: NPT at 310 K with Nose-Hoover temperature coupling (20) and a constant pressure of 1.01 bar using Martyna Tobias−Klein pressure coupling (21). The system was unconstrained, and the default fitting for OPLS 3.0 standards provided the initial velocity values for the forcefield calculations.

Furthermore, the formation of hydrogen bonds during the interaction of ligands with β2 spectrin was investigated during the MD simulations.

3. RESULTS AND DISCUSSION

This *in silico* study offers a further comprehension of the non-covalent interactions between the drug and protein, aiming to unveil the inhibition efficiency and the role of hydrogen bonding in the drug binding mechanism.

The objective is to initiate the research using computational and simulation tools to effectively illustrate how the drug aligns with the domains of β2-spectrin (Figure 1) and subsequently assess the protein binding affinity, inhibition, binding mechanism, and overall efficacy of the drug.

To reach this objective, it is essential to understand

that the efficacy of interactions seems to rely on several factors, such as the drug's external groups' affinity towards the protein and the binding topology.

The primary concept involves the simulation and calculation of the theoretical stability of β2-spectrin under inflammatory conditions within a liver cell, specifically at a pH of 7.3. This stability will then be compared to the Δ(ΔG) (Gibbs free energy change) when β2-spectrin forms a complex with INASHD, leading to INASHD-β2-spectrin.

Figure 2 illustrates the main pose of the INASHD-β2-spectrin, taken under 3D video within Schrödinger's Maestro Desmond MD software, where each MD run had 5000 frames. INASHD is strongly attached to the groove active site of β2-spectrin. Along with the MD results in Schrödinger's Maestro Desmond and according to the cluster analyses of 100 posed trials from the molecular docking studies in Autodock Vina, it forms strong H-bonds β2-spectrin, and the obtained docking energies are around -14 kcal/mol. The drug bends the morphological alpha-helical structures of β2-spectrin. INASHD inhibits and bends the alpha-helical structure and morphology of β2-spectrin via very strong H-bonds.

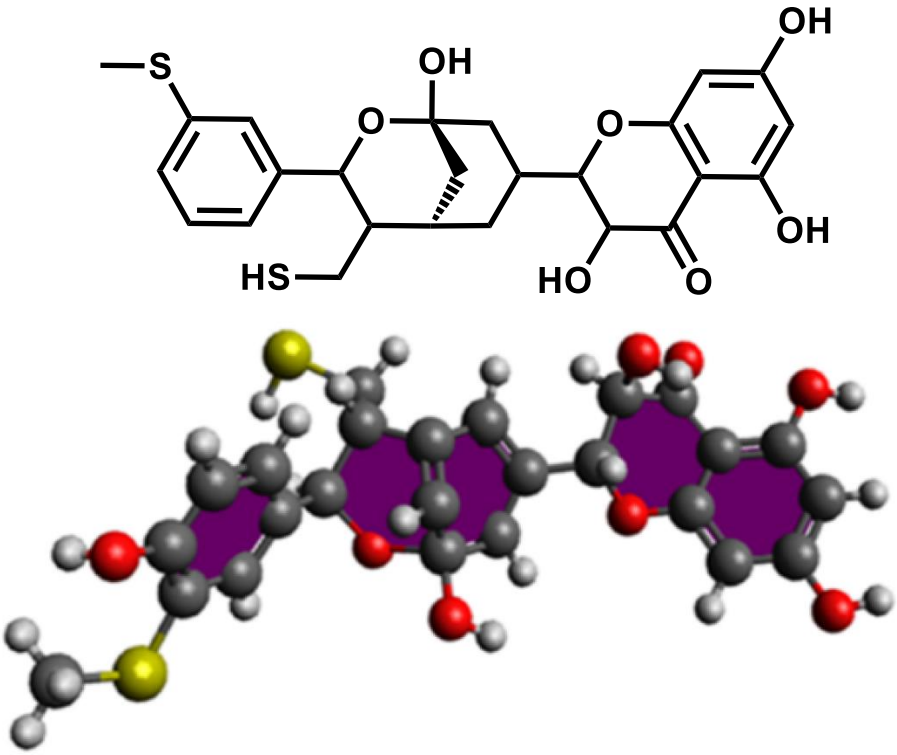

Figure 1: *De novo* designed INASHD (patent pending) with 2D and 3D illustrations, geometrically optimized under OPLS 3.0 Force field and a pH of 7.3 to suppress NASH disease.

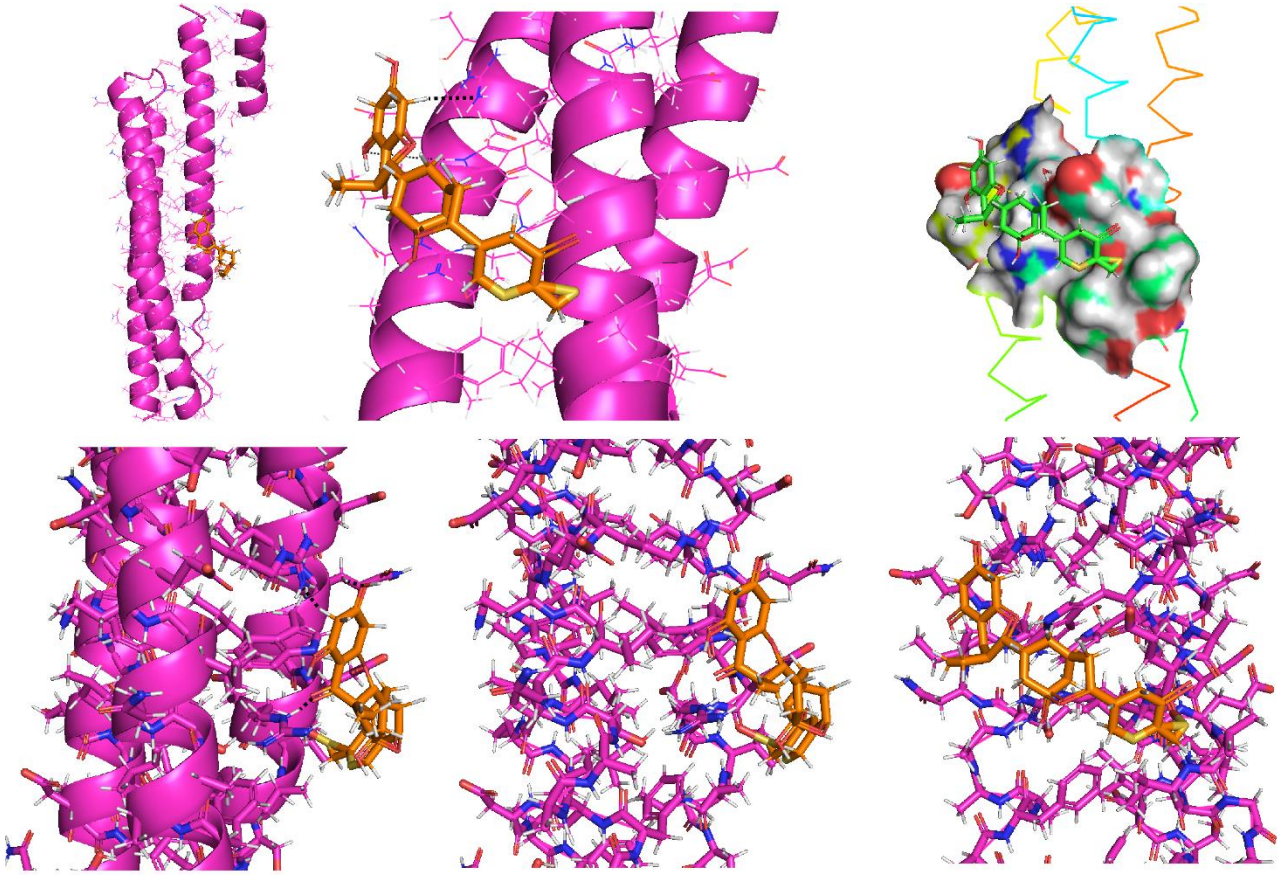

Figure 2: INASHD-β2-spectrin complex with the inhibition Δ(ΔG) binding energy of -13.9 kcal/mol.

Depending on the cluster analyses in Figure 3, there were two main clusters, which are good result indicators and expected to happen for strong binding, inhibiting drugs as in the scientific literature. The two main clusters have similar binding energies that are around -14 kcal/mol.

With such a high docking score, α-helical modulation, and a bioactive molecule, along with great cluster results that can pass *in vitro* and clinical tests depending on the data at hand and our research paper publishing past in this area, the molecular formula should be patented before going into further pre-clinical and clinical studies.

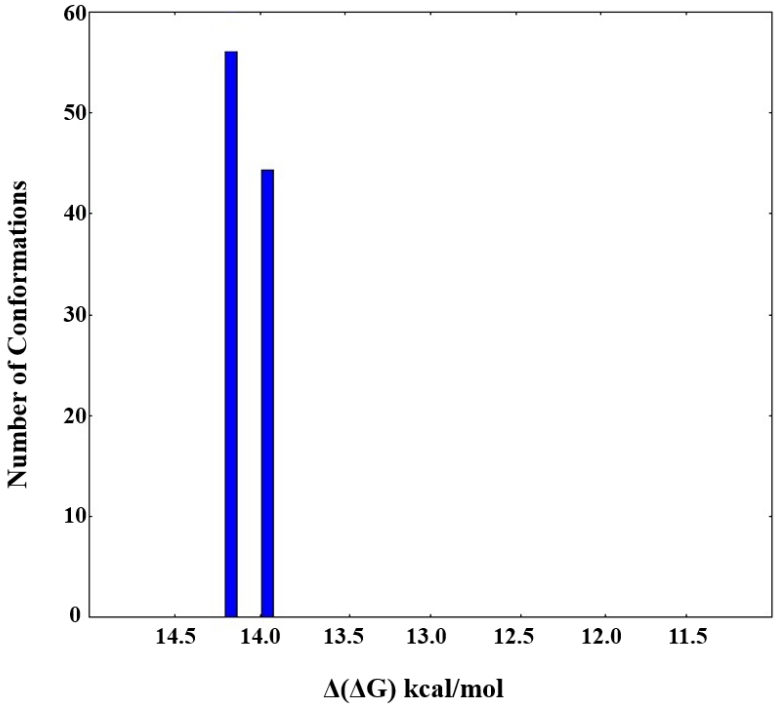

Figure 3: The cluster analysis of all the docked poses.

4. CONCLUSION

A new drug compound called INASHD was created using computer-based techniques to specifically target β2-spectrin and effectively combat NASH disease. Computational tools, including molecular docking and molecular dynamics (MD), were employed to demonstrate the drug's remarkable efficiency in inhibiting and regulating the α-helical topology of β2-spectrin, a protein critical in the disease pathway.

In conclusion, we successfully designed this molecule with a pharmaceutical organic formulation, and its inhibitory potential surpasses that of any molecule documented in scientific literature. Given the strong support from validated computational software, this bioorganic structure holds significant value and has already been sent for patenting. Its innovative design shows promising potential for success in various stages, including *in vitro*, *in vivo*, *ex vivo*, and human phase studies.

5. ACKNOWLEDGMENTS

i. Funding statement
This study was funded by Molecular Cancer Research Association at ISTU under the oncology & medical biochemistry team of Prof. Dr. EU (M.D. Ph.D.).

ii. Patent application
The patent application was done for the drug molecule and its mechanism within the research article via Technology Transfer Office of IU with the application number of P23-0899, and it was accepted.

iii. Any conflict of interest
The authors, Assistant Professor SA (Ph.D.), Teaching & Research Fellow BA (Ph.D.), and Prof. Dr. EU (M.D. Ph.D.), declare that there is no conflict of interest for this research paper and its datasets.

iv. How the ethical issue was handled (name the ethical committee that approved the research)
All *in silico* data was studied and represented with honest work, and since it was an *in silico* study and not an experimental study, there was no need for ethical committee approval for this research paper, which is compatible with the laws of the national ethical committee.

v. Authors contribution
SA: Writing the draft, methodology and *in silico* simulations; BA: Writing the draft, proofreading; EU: Conceptualization, checking the draft.

vi. Availability of data (if apply to your research)
It can be shared with open access in case of journal asks of us.

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