

Structure-Guided Design and In Silico Evaluation of Proteolysis-Targeting Chimeras (PROTACs) for the Selective Ubiquitin-Mediated Degradation of Glycogen Synthase Kinase-3 Beta (GSK-3 β) as a Therapeutic Strategy in Alzheimer's Disease

MANYA KUMARI

John H Guyer High School
Denton, TX
Published Feb, 2026

Abstract: Alzheimer's disease (AD) is a neurodegenerative disorder that is caused by a buildup of amyloid-beta plaques and neurofibrillary tau tangles in the brain. Glycogen synthase kinase-3 beta (GSK-3 β) is an enzyme that contributes to tau phosphorylation and amyloid-beta production. Therefore, degrading this protein could be a potential therapeutic strategy targeting AD. Proteolysis-targeting chimeras (PROTACs) are bifunctional molecules designed to mediate the selective degradation of target proteins (such as GSK-3 β) by conjugating them to E3 ubiquitin ligases. This proximity-induced interaction facilitates the ubiquitination of the target protein, leading to its subsequent degradation by the ubiquitin-proteasome system. We hypothesize that PROTAC P1 interacts with both GSK-3 β and E3 ligases and facilitates the proteasomal degradation of GSK-3 β . The 3D models of GSK-3 β and E3 Ligase were predicted using AlphaFold, and the PROTAC structure was obtained from previous research and was designed using the YASARA software. Finally, the GSK-3 β protein and PROTAC interactions were computed using the HDock software. The interactions between the GSK-3 β -E3 ligase complex and the PROTAC molecule were identified using the Protein-Ligand Interaction Profiler (PLIP) analysis tool. The binding energy was calculated using the PRODIGY software. These results show that PROTAC P2 could be the most appropriate candidate for this research since it has the highest binding affinity of -9.2 kcal/mol. Finally, I have explored the basis of protein-protein interactions by considering the electrostatic surface potential and hydrophobicity as contributing factors. The current study will pave the way for PROTAC-based therapeutics targeting GSK-3 β as a treatment for AD.

1. INTRODUCTION:

Alzheimer's Disease (AD) is a chronic neurodegenerative condition that gradually destroys a person's memory, thinking abilities, and eventually the power to carry out simple everyday tasks. Some early symptoms include increased forgetfulness, confusion, language difficulties, and trouble with decision-making. [1] Current treatment can't cure the disease, but it tries to ease symptoms and slow the inevitable cognitive decline. [2] Some include medications like cholinesterase inhibitors, NMDA receptor antagonists, and non-drug strategies like cognitive therapy and lifestyle interventions. [2] They try to target the neurotransmitter symptoms that are disrupted to attempt to preserve neural function. Researchers are currently working on therapies that can target the direct causes of the disease by targeting

amyloid plaques, tau tangles, and brain inflammation. [3]

PROTACs ("Proteolysis Targeting Chimeras") are a special type of molecule that uses the body's own protein degradation mechanisms to eliminate specific harmful proteins. [4] They work by bringing a disease-related protein in close proximity with an E3 ubiquitin ligase, which then marks the protein for degradation. PROTACs are currently being explored for use in treating diseases like cancer, neurodegenerative conditions, and even viral infections. [5] They're used best when targeting proteins that traditional drugs are more ineffective against. [6]

Molecular docking is a computer-based method used to predict how two molecules, such as a drug and a protein, fit together. [7] It helps researchers understand the binding interactions and strength between the molecules. [8] This technique is

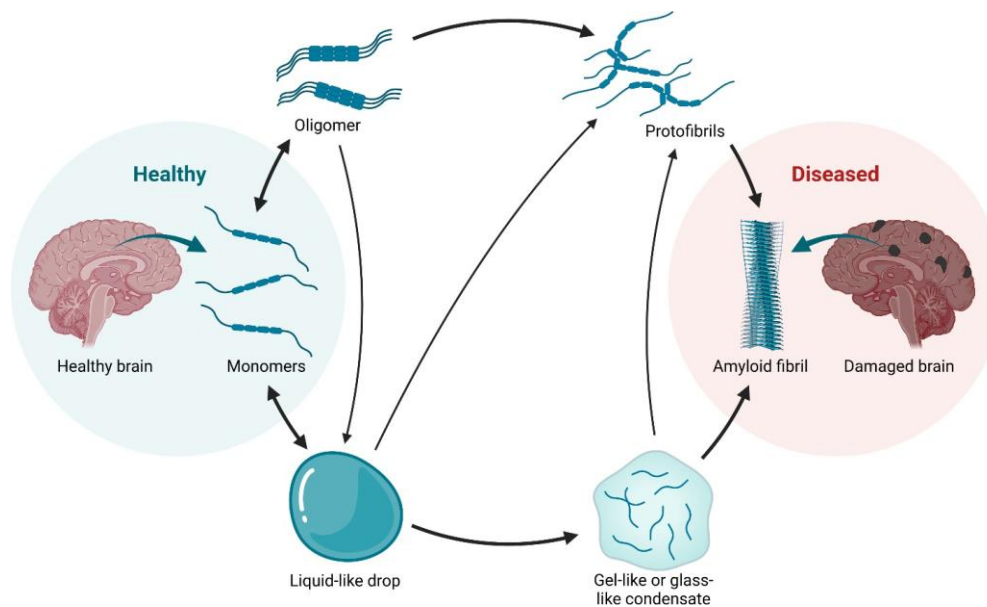


Fig. 1. Illustrates the transition of a protein monomer into a pathological amyloid fibril, a hallmark of neurodegenerative diseases. On the left side, the diagram shows a healthy brain monomer that first aggregates into an oligomer and then forms liquid-like droplets. These droplets can further transition into protofibrils or gel-like condensates, ultimately leading to the formation of amyloid fibrils. The accumulation of these fibrils is associated with neural degradation, as seen in conditions such as AD. The Figure was generated using the BioRender.com

widely used in drug discovery, especially for identifying potential drug candidates. [9] It can also be applied to study protein-protein interactions and enzyme inhibition. Molecular docking helps scientists design more effective and targeted therapies. Overall, it plays a crucial role in modern medicine and biological research. [9]

2. METHOD:

Uniprot is a comprehensive protein database that provides detailed information on protein sequences, structures, and functions. [10, 11] We used it to retrieve accurate sequences and functional details for E3 ligase and GSK-3 β , P49841. PROTTER is an interactive visualization tool that displays protein sequence features, such as transmembrane regions, signal peptides, and post-translational modifications, using data from UniProt and experimental proteomics studies. [12] It helped us visualize the locations of the proteins within a sample cell. AlphaFold 3 is an AI tool that predicts 3D protein structures with high accuracy. [10] We used it to visualize the structures of both proteins, helping us assess whether a PROTAC could bind to and bring them into close proximity to trigger the degradation of the target protein. P2Rank is a machine learning-based tool used to predict ligand-binding sites on protein structures. [13] ScanNet is a similar web-based tool that utilizes geometric deep learning to predict protein binding sites from 3D structures. [14] Both analyzed the surface of the proteins to identify likely “pockets” where the PROTAC may bind. HDock is a web-based

molecular docking tool used to predict how proteins and small molecules interact. [15] We used HDock to simulate interactions between various PROTACs, GSK-3 β , and the E3 ligase. This helped identify the best potential binding sites and interaction strengths of the different PROTACs with the proteins.

3. RESULTS

In this research work, we utilized computational simulations to identify a PROTAC that can bind to the protein and facilitate the proteosomal degradation of the GSK-3 protein. To identify the most effective PROTAC, we utilized computational software, such as P2Rank, to predict the binding sites of the proteins (GSK-3 and E3 ligase). Understanding the binding site of the proteins is essential for understanding the protein-PROTAC interactions. Figure 3 depicts the GSK-3 β and E3 Ligase proteins used in this research. The red regions (in the box) show where the PROTAC is predicted to bind to each protein (shown in Figure 3a and b). Figure 3c is shown for reference. These results will help select the PROTAC in the molecular docking simulations.

Using molecular docking simulation software (HDock), we generated various structures of docked protein-PROTAC models with E3 Ligase (left, purple) and GSK-3 β (right, green), and five different PROTAC molecules binding at the proteins' predicted binding sites (Figures 3a, 3b). The docking gives us the GSK3 β -PROTAC-E3 ligase complex as shown in Figure 4. The box depicts an example chemical structure of the tertiary complex with PROTAC P2.

Pharmaceutical properties (I-V) of the five designed PRO-

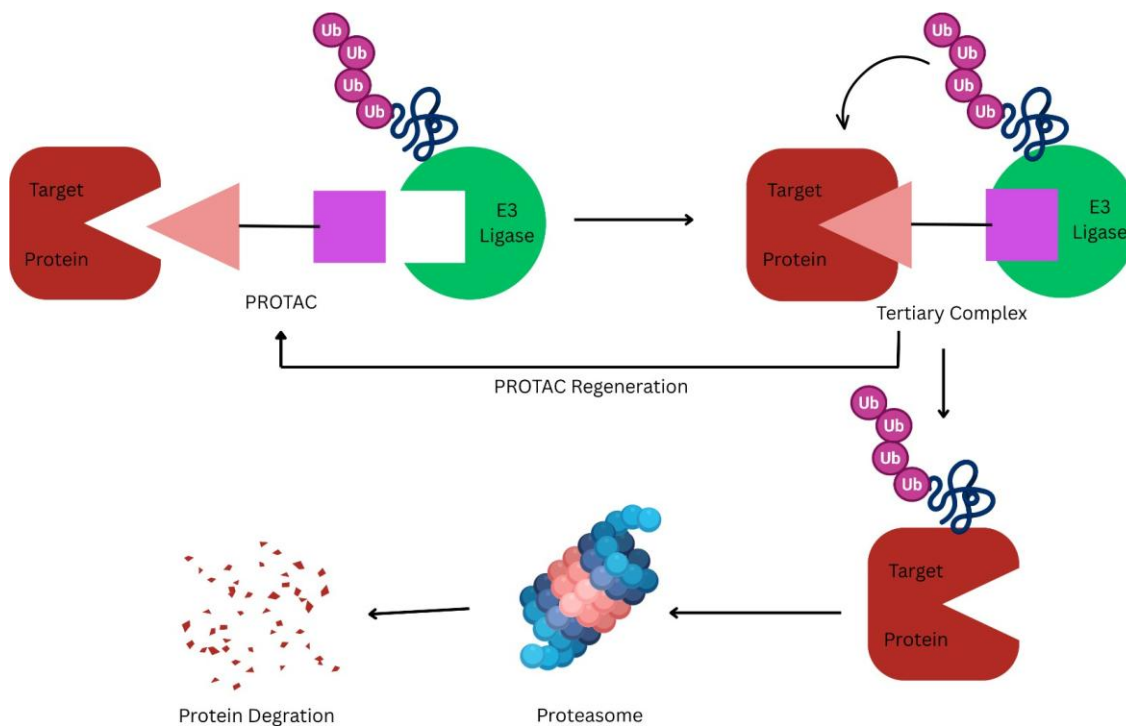


Fig. 2. The PROTAC binds to the GSK-3 β (Target Protein) and the E3 Ligase. This facilitates the formation of a tertiary complex, which enables the E3 Ligase to tag the GSK-3 β with ubiquitin molecules. This marks the GSK-3 β for degradation and recognition by the proteasome, and breaks the GSK-3 β into small peptides. The PROTAC can be reused to repeat this process with other GSK-3 β proteins.

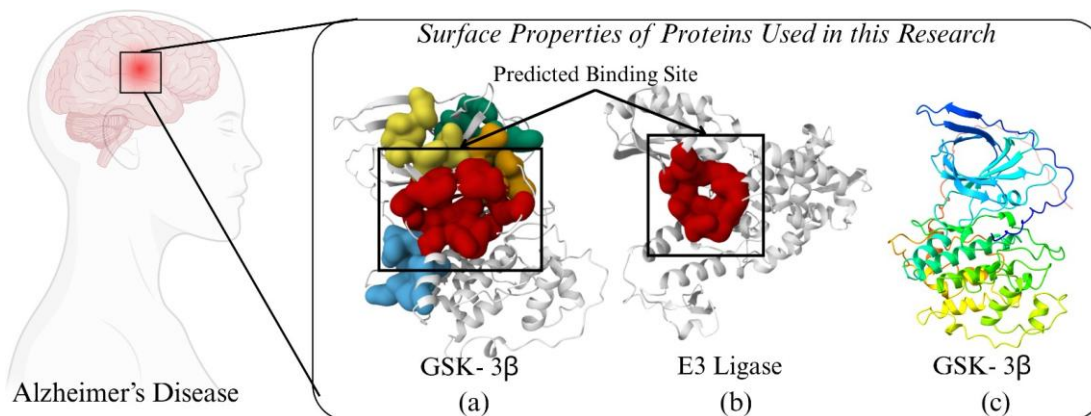


Fig. 3. (a) Red region is the binding site for the GSK-3 β (P2Rank); (b) Red region is the binding site for E3 Ligase; and (c) The ribbon structure to visualize GSK-3 β

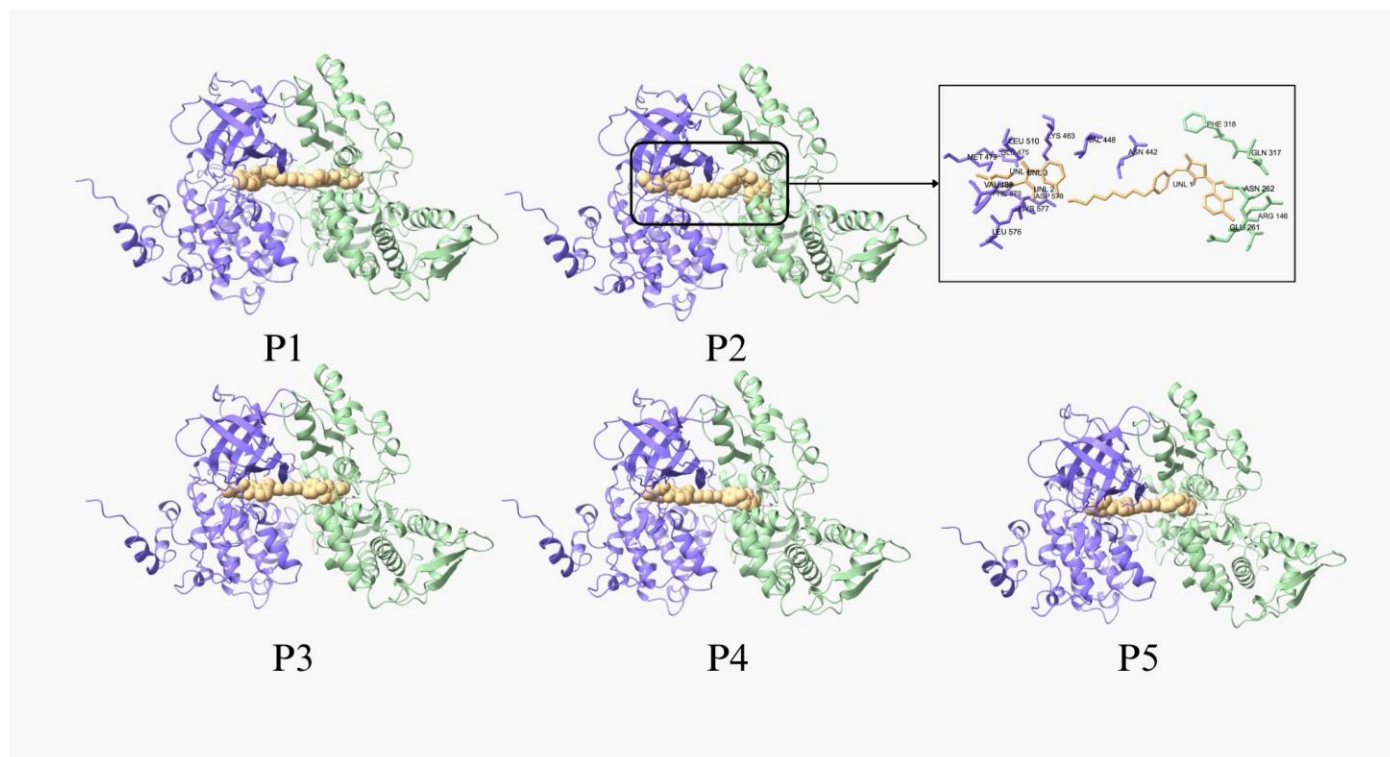


Fig. 4. The figure shows the interaction of various PROTACs (P1-P5) bound to the protein complex. The box shows active residues binding to the PROTAC molecules.

Table 1. Pharmaceutical properties (I-V) of the five designed PROTACs. The binding energy shows that PROTAC P2 has the strongest binding (binding energy = -9.22 kcal/mol). Since the PROTAC has a higher molecular weight compare to most of the small molecule drugs

	I	II	III	IV	V
Formula, molecular weight	C ₃₈ H ₃₆ ClN ₆ O ₈ S, 772.25 g/mol	C ₃₈ H ₃₇ Cl ₂ N ₆ O ₇ S, 792.71 g/mol	C ₃₄ H ₃₆ ClN ₆ O ₇ S, 708.20 g/mol	C ₂₉ H ₃₄ ClN ₆ O ₇ S, 646.13 g/mol	C ₃₀ H ₃₇ N ₆ O ₇ S, 625.72 g/mol
GI absorption	Low	Low	Low	Low	Low
BBB permeation	No	No	No	No	No
Drug likeliness (Lipinski)	No; 2 violations: MW>500, NorO>10	No; 2 violations: MW>500, NorO>10	No; 2 violations: MW>500, NorO>10	No; 2 violations: MW>500, NorO>10	No; 2 violations: MW>500, NorO>10
Binding Energy (kcal/mol)	-8.70	-9.22	-7.58	-7.34	-7.55

TACs. The binding energy shows that PROTAC P2 has the strongest binding (binding energy = -9.22 kcal/mol). Since, the PROTAC has a higher molecular weight compare to most of the small molecule drugs

In the next step, we have computed the ADMET properties of the selected ligands as displayed in Figure 5. ADMET properties describe how a drug behaves in the body and are critical in drug development. Absorption refers to how well and how quickly a drug enters the bloodstream from its site of administration. Once absorbed, distribution talks about how the drug spreads throughout the body's tissues and organs. Metabolism involves how the body chemically alters the drug, typically in the liver, to make it easier to excrete. Excretion is the process by which the drug and its metabolites are eliminated from the body, usually via urine or feces. Lastly, Toxicity refers to the degree to which a drug can cause harmful effects in the body at therapeutic or higher doses.

4. DISCUSSION:

The current research will help in developing a novel therapeutic strategy against AD, unlike current drugs, which mainly manage symptoms, PROTACs target and remove the disease-causing protein (GSK-3 β), potentially producing longer-lasting effects. [4] Existing treatments include cholinesterase inhibitors, NMDA receptor antagonists, monoclonal antibodies against amyloid-beta, and lifestyle interventions. These often slow progression but do not eliminate pathogenic proteins. PROTACs offer high specificity and targeted degradation but face challenges such as poor GI absorption, limited BBB penetration, and potential off-target effects. [16]

Limitations: Common limitations related to PROTACs are the solubility in blood. [16] Since the molecular weight of PROTACs is higher than that of other small-molecule drugs, the solubility and BBB crossing capability of PROTACs increases substantially. This limitation can be overcome by altering the chemical structure of the PROTAC without changing its therapeutic capability. Increasing the bioavailability of a PROTAC involves optimizing its absorption, distribution, metabolism, and excretion (ADME) properties. PROTACs are typically large and polar, which can lead to poor bioavailability, especially for oral delivery. [6] The bioavailability can be increased by reducing the molecular weight and increasing the lipophilicity. In addition, linker optimization can also play a crucial role in PROTAC solubility. Another limitation of this study is the use of computational tools and the lack of experimental validation. Therefore, in future studies, we will be performing molecular dynamics simulations to understand the effect of water on the protein-PROTAC interactions. [17] Finally, experimental studies like surface plasmon resonance (SPR) and isothermal calorimetry (ITC) need to be performed to validate the docking interactions further. [18] Finally, the in vitro and in vivo studies will help in determining their therapeutic potential. In vitro studies involve cell-based or biochemical assays that assess the degradation efficiency, mechanism of action, and selectivity of the PROTAC molecule.

5. CONCLUSION:

The study demonstrates the potential of structure-guided, in silico-designed PROTACs as a therapeutic strategy for AD by selectively targeting and degrading GSK-3 β , a key contributor to tau hyperphosphorylation and amyloid-beta production. Using

advanced computational tools, we have identified and evaluated five candidate PROTACs; among them, PROTAC P2 exhibited the highest binding affinity and favorable protein-protein interaction profiles. While all candidate showed limited GI absorption and blood-brain barrier permeability, further structural optimization may enhance their drug-like properties. The results support the desirability; further structure optimization may improve their drug-like properties. This research highlights a promising direction for the development of next-generation targeted therapies against neurodegenerative disorders.

REFERENCES

1. P. Scheltens, B. D. Strooper, M. Kivipelto, H. Holstege, G. Ch  telat, C. E. Teunissen, . . V. D. Flier, and W. M (2021).
2. J. M. Long and D. M. Holtzman, "Alzheimer Disease: An Update on Pathobiology and Treatment Strategies," *Cell* **179**, 312–339 (2019).
3. Y. R. Alugubelli, J. Xiao, K. Khatua, S. Kumar, L. Sun, Y. Ma, . . Xu, and S, "Discovery of First-in-Class PROTAC Degraders of SARS-CoV-2 Main Protease," *Journal of Medicinal Chemistry* **67**, 6495–6507 (2024).
4. R. Pujari, S. Bhatt, U. Soni, S. Sharma, and S. Patil, "Advantages and Disadvantages of PROTACs," in "PROTAC-Mediated Protein Degradation: A Paradigm Shift in Cancer Therapeutics," M. N. P. Jain, ed. (Springer Nature, 2024), pp. 67–88.
5. Y. Xu, Y. Yuan, D. Q. Fu, Y. Fu, S. Zhou, W. T. Yang, . . Tang, and Z, "The aptamer-based RNA-PROTAC," *Bioorganic & Medicinal Chemistry* **86**, 117299–117299 (2023).
6. M. S. Gadd, A. Testa, X. Lucas, K. H. Chan, W. Chen, D. J. Lamont, . . Ciulli, and A, "Structural basis of PROTAC cooperative recognition for selective protein degradation," *Nat Chem Biol* **13**, 514–521 (2017).
7. L. Ferreira, D. R. Santos, G. Oliva, and A. Andricopulo, "Molecular Docking and Structure-Based Drug Design Strategies," *Molecules* **20**, 13384–13421 (2015).
8. J. Aghajani, P. Farnia, P. Farnia, J. Ghanavi, and A. A. Velayati, "Molecular Dynamic Simulations and Molecular Docking as a Potential Way for Designed New Inhibitor Drug without Resistance," *Tanaffos* **21**, 1–14 (2022).
9. J. Fan, A. Fu, and L. Zhang, "Progress in molecular docking," *Quantitative Biology* **7**, 83–89 (2019).
10. J. Abramson, J. Adler, J. Dunger, R. Evans, T. Green, A. Pritzel, . . Jumper, and J. M, "Accurate structure prediction of biomolecular interactions with AlphaFold 3," *Nature* **630**, 493–500 (2024).
11. T. U. Consortium, "UniProt: a hub for protein information," *Nucleic acids research* **43** (2014).
12. U. Omasits, C. H. Ahrens, S. M  ller, and B. Wollscheid, "Protter: interactive protein feature visualization and integration with experimental proteomic data," *Bioinformatics* **30**, 884–886 (2013).
13. R. Kriv  k and D. Hoksza, "P2Rank: machine learning based tool for rapid and accurate prediction of ligand binding sites from protein structure," *Journal of Cheminformatics* **10**, 39–39 (2018).
14. J. Tubiana, D. Schneidman-Duhovny, and H. J. Wolfson, "ScanNet: an interpretable geometric deep learning model for structure-based protein binding site prediction," *Nature Methods* **19**, 730–739 (2022).
15. Y. Yan, D. Zhang, P. Zhou, B. Li, and S. Y. Huang, "HDock: a web server for protein-protein and protein-DNA/RNA docking based on a hybrid strategy," *Nucleic Acids Res* **45**(2017).
16. H. Gao, X. Sun, and Y. Rao, "PROTAC Technology: Opportunities and Challenges," *ACS Medicinal Chemistry Letters* **11**, 237–240 (2020).
17. S. A. Hollingsworth and R. O. Dror, "Molecular Dynamics Simulation for All," *Neuron* pp. 1129–1143 (2018).
18. W. Hou and S. B. Cronin, "A Review of Surface Plasmon Resonance-Enhanced Photocatalysis," *Advanced Functional Materials* **23**, 1612–1619 (2013).

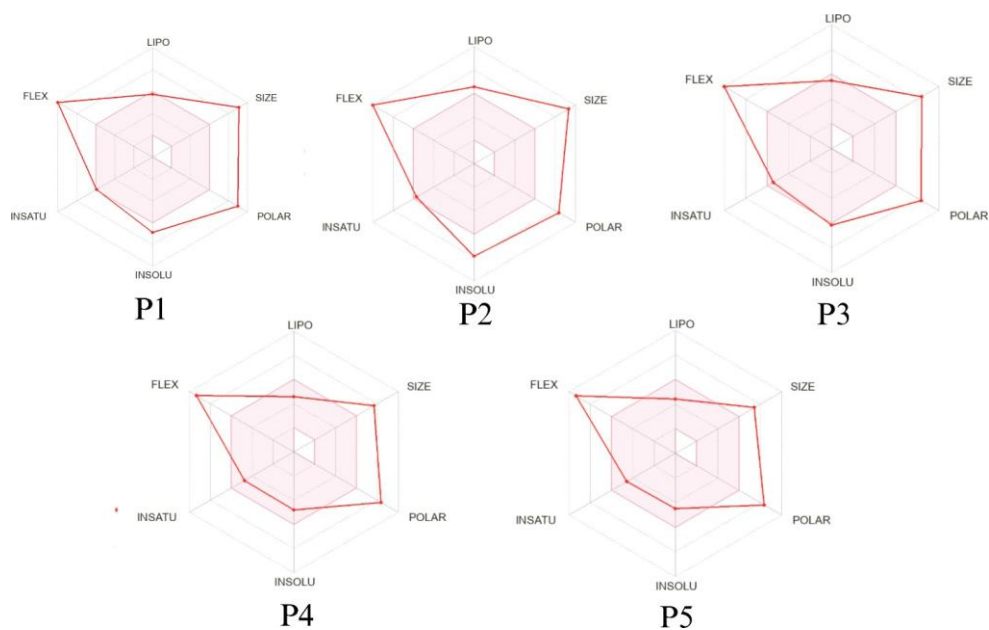


Fig. 5. The image shows the radar spider web graph of the different PROTACs used in this research. It depicts ADMET properties of the PROTACs, including unsaturation, insolubility, polarity, size (molecular weight), lipophilicity, and flexibility.