Potential inhibitors of bromodomain-containing protein 4 (BRD4) against prostate cancer using molecular docking and network pharmacology

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ABSTRACT

Background: Bromodomain-containing protein 4 (BRD4) has emerged as a promising therapeutic target in prostate cancer (PCa). This chromatin reader protein, crucial for regulating gene expression through its bromodomains, plays a pivotal role in recognizing acetylated lysine residues on histones and recruiting RNA polymerase II. In the context of PCa, BRD4's overexpression and amplification are correlated with aggressive phenotypes, tumor progression, and the development of castration-resistant prostate cancer (CRPC), positioning BRD4 as a compelling target for therapeutic intervention.
Methods: The study conducted a thorough examination of protein and ligand interactions, focusing on 17 compounds. Ergosterol exhibited the most favorable binding affinity to the BRD4 receptor, and 2D visualizations provided detailed insights into dynamic interactions, revealing hydrogen bonds and key amino acid residues involved. Additionally, the research delved into network analyses, visualizing a network illustrating the interaction between bioactive components from marine sponges and their target receptors. Protein-protein interaction networks (PPINs) for prostate cancer disease and BRD4 were explored, highlighting the interconnectedness of proteins and shedding light on BRD4's potential roles in the broader context of PCa.
Results: The examination of 17 compounds revealed that ergosterol demonstrated the highest binding affinity to the BRD4 receptor. Detailed 2D visualizations unveiled the dynamic interactions, emphasizing specific hydrogen bonds and amino acid residues involved in the binding process. Network analyses depicted the intricate interactions between bioactive components and their target receptors, providing a comprehensive overview. PPINs for prostate cancer and BRD4 showcased the interconnectedness of proteins, elucidating potential roles of BRD4 beyond its primary function.
Conclusion: PCa BRD4 protein-ligand interactions were explored in this comprehensive study. Ergosterol is the most BRD4 receptor-affine of 17 ligands. Network analysis of marine sponge bioactive components and target receptors was also performed. Examining prostate cancer disease protein-protein interaction networks and BRD4 protein showed BRD4's complex role in PCa. Enrichment analysis revealed core target protein biological processes, molecular functions, cellular components, and signaling networks.

Keywords: Prostate cancer, molecular docking, network pharmacology, protein-protein interaction networks

Received: 13 October 2023 Revised: 24 November 2023 Accepted: 19 December 2023

INTRODUCTION

Prostate cancer (PCa) remains a leading cause of male mortality worldwide, posing a significant healthcare challenge [1]. PCa stands as a global health challenge, ranking as the second most common cancer and the fifth leading cause of cancer-related mortality in men worldwide [2]. Despite strides in early detection and treatment, a significant proportion of patients progress to castration-resistant prostate cancer (CRPC), a lethal stage with limited therapeutic options and a median survival of only 3-4 years [3]. This underscores the urgent need for innovative therapeutic strategies that address the underlying drivers of PCa progression [4]. Therefore, novel therapeutic strategies targeting key oncogenic drivers are urgently needed [5].

Bromodomain-containing protein 4 (BRD4) has emerged as a promising therapeutic target in various cancers, including PCa [6]. This chromatin reader protein regulates gene expression through its bromodomains, recognizing acetylated lysine residues on histones and recruiting RNA polymerase II [7]. In PCa, BRD4 overexpression and amplification are associated with aggressive phenotypes, tumor progression, and CRPC

development [8]. This oncogenic role makes BRD4 a compelling target for therapeutic intervention [9].

This study aimed to identify and validate potential BRD4 inhibitors for PCa therapy with a multi-pronged approach by combining molecular docking as computational tools to virtually screen and identify small-molecule ligands that bind to BRD4 with high affinity, potentially disrupting its oncogenic functions. Network pharmacology also used to analyze the gene networks regulated by BRD4 and its interaction with other signaling pathways in PCa, aiming to reveal novel therapeutic targets and predict potential synergisms with existing drugs. By integrating cutting-edge computational tools with rigorous experimental validation, this study holds significant promise for advancing the development of effective BRD4-targeted therapies and improving the clinical outlook for patients with PCa.

MATERIALS AND METHODS

Materials

The BRD4 complex, which contains bromodomain, was obtained from the Protein Data Bank Repository (PDB) with the ID Code 5Z1R [10]. The files associated with it were downloaded in .pdb format. Furthermore, a 3D conformer file of the anticancer medication capecitabine was acquired from the original ligand 5-bromo-N-(2,2-dimethyl-3-oxo-3,4-dihydro-2H-1,4-benzoxazin-7-yl)-2-methoxybenzene-1-sulfonamide (EFL) in the 3D protein structure. Additionally, a total of 15 ligand files were obtained from PubChem [11]. These files include compounds such as 1,4-benzoquinone, 3,4-dihydroxybenzoic acid, antcin A, benzaldehyde hydrazone, cordycepin, ergosterol, erinacin A, eritadenine, homogentisic acid, mannitol, palmitic acid, phenylacetaldehyde, trehalose, triterpenoid, and vanillic acid. The ligand files were saved in the .sdf format.

Protein preparation

Following the elimination of initial ligands and water molecules using Discovery Studio Visualizer [12], the .pdb files of protein are obtained. Several critical stages are required to prepare a protein structure for molecular docking with PyRx [13] and guarantee that the protein is suitable for docking simulations. The process consists of several stages: acquiring the protein structure in a format that is compatible with PyRx, inputting the structure into the program, eliminating water molecules, if required, supplementing missing residues with hydrogen atoms, designating atom types to the residues, optimizing the structure, and ultimately saving the prepared protein structure. Researchers can ensure that their protein structures are sufficiently prepared for precise and dependable docking simulations by adhering to the subsequent procedures.

Molecular docking

The protein and ligand were prepared using the PyRx Tools software, which also converted them to the.pdbqt format. To conduct molecular docking simulations, adhere to the subsequent procedures: Import the protein structure into PyRx after obtaining it from a database such as the Protein Data Bank (PDB). In order to assemble the protein, it is necessary to remove water molecules, supplement any absent residues, and introduce hydrogen atoms. An alternative approach to improve precision is to allocate atom types and optimize the structure. Save the protein structure that has been prepared in PDB or PDBQT format. Subsequently, acquire the ligand structure through computational means or obtain it from a chemical database, guaranteeing its compatibility with PDB or SDF formats. Assign atom types, load the ligand into PyRx, supplement with hydrogen atoms as necessary, and convert to PDBQT format if necessary. Ensure that the ligand structure is saved in an appropriate format.

Protein and ligand interaction

Protein and ligand docking data were generated in accordance with.pdb files. The data integration process utilized the PyRx program in order to guarantee a consistent and standardized representation that could be built upon in subsequent analyses. Furthermore, a 3D structure visualization was performed using PyMOL [14], which allowed for a thorough examination of conformational changes, binding interfaces, and spatial arrangements. In addition, the binding energy (ΔG) value was employed to compute the magnitude of the ligand-target interaction during molecular docking. Using the formula $Ki = e^{-RT/\Delta G}$, the inhibition constants (Ki) were also calculated to determine the degree of binding affinity between a ligand and a target enzyme or receptor.

Network pharmacology

For ligand-protein interaction analysis, the proteins that were identified during the protein-protein interaction phase were utilized. Compounds obtained from marine sponges via ligands were obtained from the PubChem database and analyzed utilizing the STITCH webserver (https://stitch.embl.de/). The interaction networks resulting from the ligand-protein interactions were promptly generated. The interaction results were saved in.tsv format, whereas the visual outcomes were recorded in PNG format.

Gene enrichment analysis

In order to gain insight into the manner in which protein-protein interactions within the KEGG pathway and gene functional enrichment are organized into this gene ontology (GO), cellular components, molecular functions, and biological processes were incorporated. To forecast potential therapeutic pathways, the ShinyGO 0.77 webserver (http://bioinformatics.sdstate.edu/go/) was utilized. This analysis unveiled the precise proteins with which these compounds interact, thereby facilitating the investigation of novel therapeutic alternatives.

RESULTS

Protein and ligand interaction

The assessment employed a PyRx gridbox, which functioned as an intuitive interface to establish a personalized receptor docking gridbox for the purpose of molecular docking. The inhibition constant and binding energy of the interaction between the ligand inhibitors and bromodomain-containing protein 4 (BRD4) are depicted in Figures 1 and 2, respectively. Of the seventeen compounds examined, ergosterol exhibited the highest degree of affinity for binding to the BRD4 receptor. The original ligand 5-bromo-N-(2,2-dimethyl-3-oxo-3,4-dihydro-2H-1,4-benzoxazin-7-yl)-2-methoxybenzene-1-

sulfonamide (EFL), which was extracted from the three-dimensional structure of the protein-ligand complex, was utilized to validate the docking.

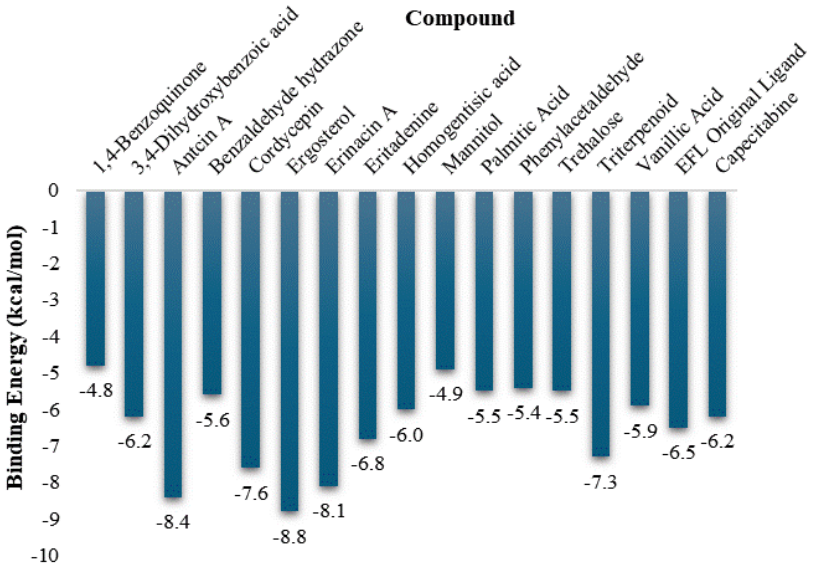

Figure 1. Inhibition constant value (Ki) of natural compounds derived from marine sponges towards pneumococcal surface protein A (PspA).

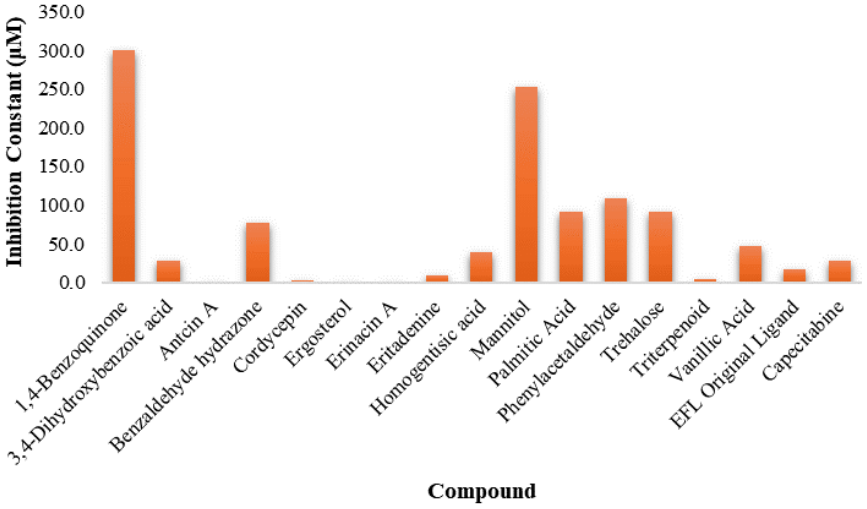

Figure 2. Binding energy between natural compounds from marine sponges and pneumococcal surface protein A (PspA).

Figure 3 illustrated a two-dimensional representation of receptor-ligand interactions, specifically the formation of complexes among various entities. The dynamic interaction between ergosterol and the receptor of bromodomain-containing protein 4 (BRD4) was depicted in Figure 3A. Five hydrogen bonds are formed during this interaction with Tyr-97, Phe-136, Pro-82, Val-87, Ile-146, and Pro-82. A comprehensive illustration of the interaction between the BRD4 receptor and the anticancer agent capecitabine was presented in Figure 3B. The illustration depicted seven hydrogen bonds connecting the two components: Tyr-98, Pro-45, Pro-104, Lys-102, Arg-113, Glu-49, and Thr-103. Finally, the intricate interaction between the ligand EFL and the BRD4 receptor was depicted in Figure 3C. This analysis offers a holistic viewpoint on the molecular connections present in the system, encompassing four hydrogen bonds with Asp-128, Pro-48, Arg-113, Glu-49, and Pro-48. In addition, the 3D visualization of complex interactions between the following substances was presented in Figure 4: A. BRD4 receptor and ergosterol; B. BRD4 receptor and capecitabine drug; and C. BRD4 receptor and the initial ligand. The network illustrating the correspondence between target receptors and bioactive components obtained from marine sponges is represented graphically represented in Figure 5. Rectangular nodes

symbolize the bioactive compounds, while circle nodes symbolize the receptors.

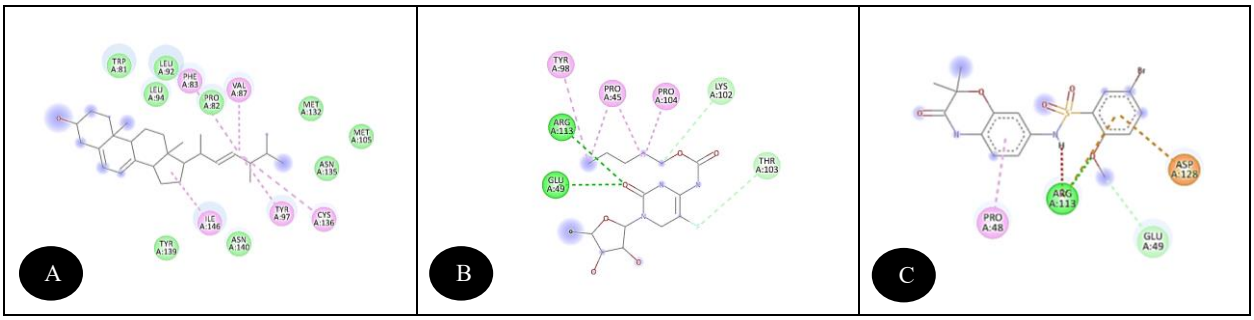

Figure 3. The 2D visualization of interactions complex between: A. BRD4 receptor and the ergosterol, B. BRD4 receptor and capecitabine drug, BRD4 receptor and the original ligand.

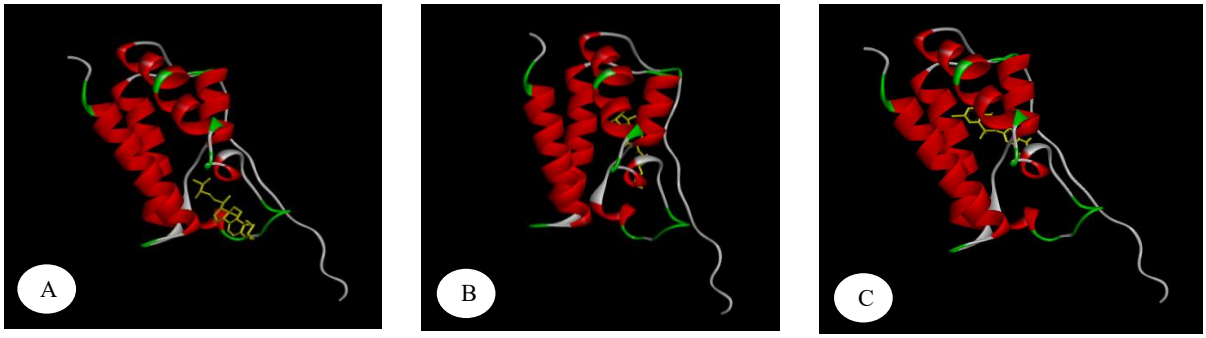

Figure 4. The 3D visualization of interactions complex between: A. BRD4 receptor and the ergosterol, B. BRD4 receptor and capecitabine drug, BRD4 receptor and the original ligand.

Network pharmacology

The network showed the relationship between bioactive components from marine sponges and their target receptors. Receptors are represented by circle nodes, while bioactive compounds are represented by rectangle nodes. There are a total of 11 nodes and 12 edges in the network. On average, each node is connected to 2.18 other nodes. The clustering coefficient, which measures the degree of interconnectedness, is 0.909. The expected number of edges in the network is 12. Additionally, the PPI enrichment p-value, which indicates the significance of protein-protein interactions, was 0.536 (Figure 5).

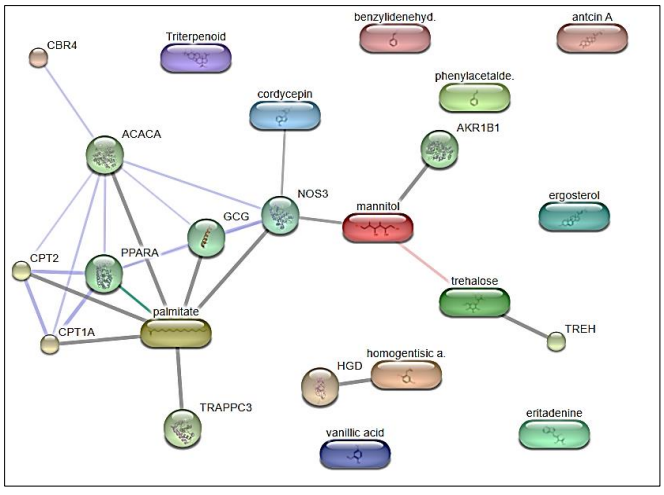

Figure 5. The bioactive compounds-target network, and their intersection are represented as circle nodes for receptors and rectangle nodes for bioactive compounds, respectively.

The analysis of protein-protein interaction networks (PPINs) of prostate cancer disease and BRD4 protein were depicted in Figure 6. Figure 6A showed the PPINs of prostate cancer disease with the backbone network consisted of 97 nodes with a high betweenness centrality (BC) value, 1849 number of edges, average node degree was 38.1, avg. local clustering coefficient was 0.725 and PPI enrichment p-value was < 1.0 x 10^{-16}. Figure 6B. depicted the associated interaction with BRD4 gene. This sub-network is formed by PPIs between connected proteins. Color refers to different functional groups. Size of nodes indicate the number of interactions for BRD4 protein derived from the global PPINs. The connected proteins were sorted and ordered based on degrees with number of nodes were 11, number of edges were 48, average node degree: was 8.73, avg. local clustering coefficient was 0.894, expected number of edges were 21, and PPI enrichment p-value was 2.94 x 10^{-7}.

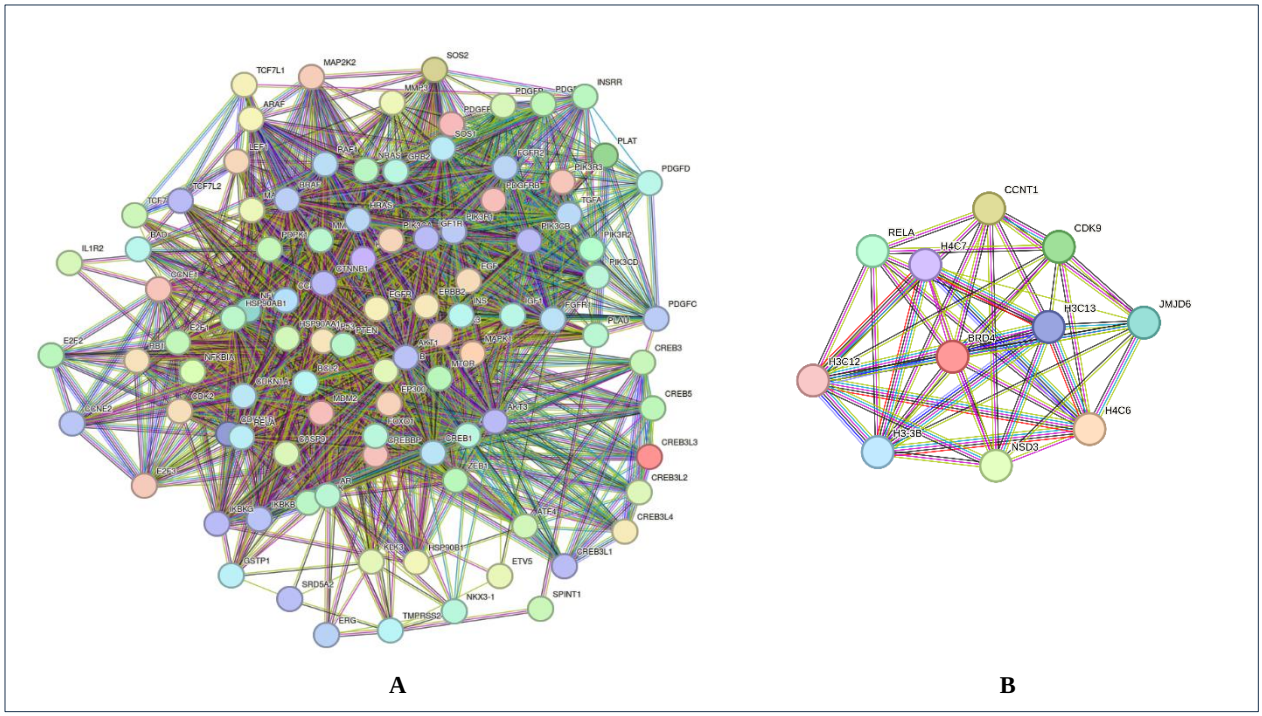

Figure 6. STRING analysis which indicates protein-protein interaction network (PPI) of A). prostate cancer disease, and B). associated interaction with BRD4 gene.

Gene enrichment analysis

Following the identification of core target proteins, our study proceeded to conduct comprehensive enrichment analysis utilizing ShinyGO 0.77 charts. This rigorous analysis was particularly centered on elucidating the enriched biological processes (BPs), molecular functions (MFs), cellular components (CCs), and signaling pathways associated with the identified core proteins. Notably, our focus in this analysis was exclusively on entities exhibiting a fold enrichment of less than 8, as depicted in Figure 7. The enrichment analysis yielded significant insights across various domains. In terms of KEGG pathways, our investigation delved into six distinct pathways. The gene ontology (GO) profiles related to biological processes encompassed a rich spectrum of 20 pathways, reflecting the diverse functional aspects influenced by the core target proteins. Similarly, the analysis of cellular components revealed involvement in 14 pathways, shedding light on the specific cellular locales influenced by the identified core proteins. Molecular functions, as elucidated by the enrichment analysis, implicated 20 pathways, further enhancing our understanding of the functional roles these proteins play at the molecular level. This meticulous exploration of diverse pathways and functions not only broadens our comprehension of the regulatory networks orchestrated by the core target proteins but also provides a nuanced perspective on their potential roles in biological processes, cellular organization, and molecular functionalities. The emphasis on entities with a fold enrichment less than 8 ensures a focused investigation into enriched pathways without overwhelming the analysis with less significant associations.

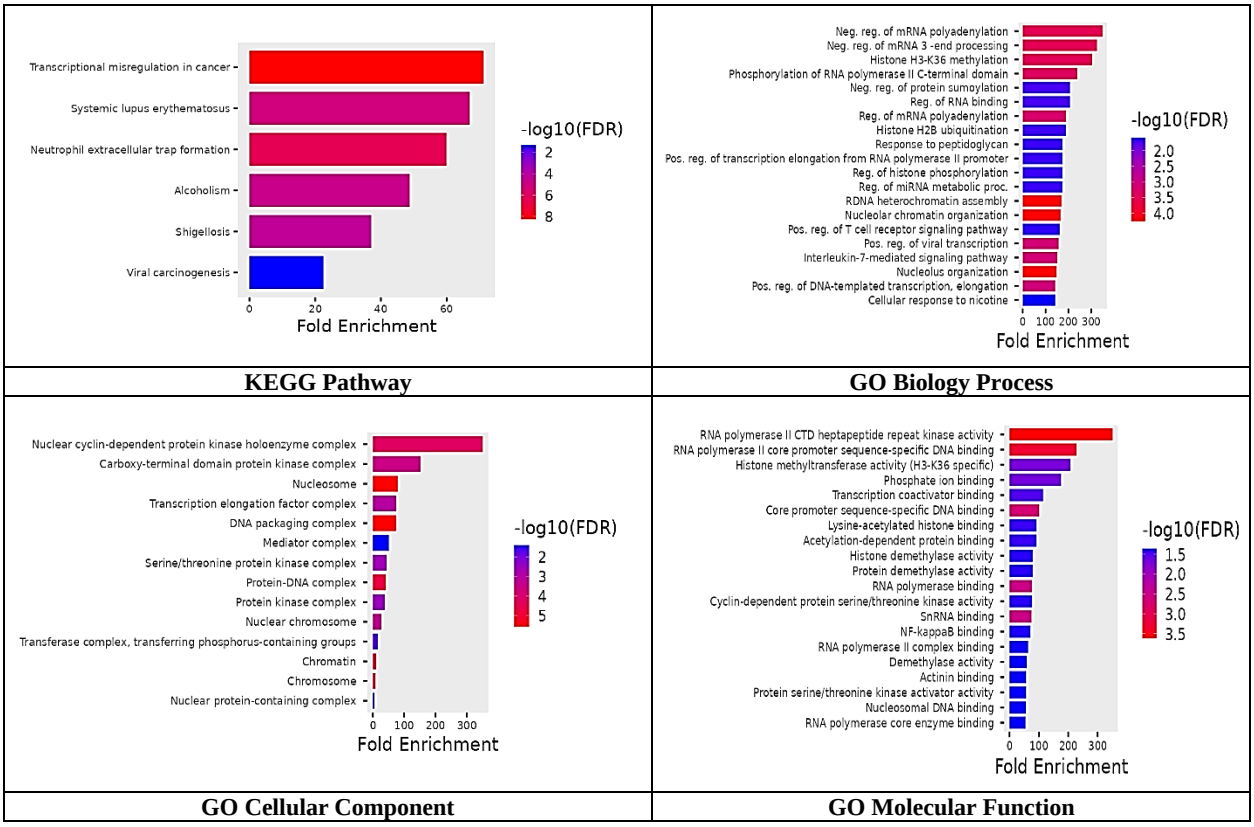

Figure 7. A bar graph representing the gene ontology (GO) of Biological Processes (BPs), Molecular Functions (MFs), Cellular Component, and KEGG pathways.

DISCUSSION

Bromodomain-containing protein 4 (BRD4) emerges as a promising therapeutic target, particularly in the context of PCa [15]. This chromatin reader protein plays a crucial role in regulating gene expression through its bromodomains, recognizing acetylated lysine residues on histones and recruiting RNA polymerase II [7]. In PCa, BRD4 overexpression and amplification are associated with aggressive phenotypes, tumor progression, and the development of castration-resistant prostate cancer (CRPC) [16]. The oncogenic role of BRD4 positions it as a compelling target for therapeutic intervention [17].

The examination of protein and ligand interactions analysis focused on 17 compounds, among which ergosterol exhibited the most favorable binding affinity to the BRD4 receptor. The 2D visualizations provided a detailed insight into the dynamic interactions, showcasing hydrogen bonds and key amino acid residues involved.

Beyond molecular interactions, the study delved into network analyses. A network depicting the interaction between bioactive components derived from marine sponges and their target receptors was visualized, offering a comprehensive overview. Additionally, protein-protein interaction networks (PPINs) for prostate cancer disease and BRD4 protein were explored. These networks revealed the interconnectedness of proteins and shed light on the potential roles of BRD4 in the broader context of PCa.

Enrichment analysis provided significant insights into the biological processes, molecular functions, cellular components, and signaling pathways associated with the identified core target proteins. The emphasis on entities with a fold enrichment of less than 8 ensured a focused investigation into enriched pathways, avoiding overwhelming the analysis with less significant associations. The exploration of diverse pathways and functions enhances our understanding of the regulatory networks orchestrated by core target proteins, offering potential therapeutic avenues and novel targets for intervention in PCa.

CONCLUSION

BRD4 protein-ligand interactions in prostate cancer (PCa) were examined in this comprehensive investigation. Ergosterol has the highest affinity for the BRD4 receptor among 17 ligands. The study also examined network analysis of marine sponge bioactive components and their target receptors. Prostate cancer disease protein-protein interaction networks (PPINs) and BRD4 protein were also examined, demonstrating BRD4's complex role in PCa. Enrichment analysis exposed

core target protein biological processes, molecular functions, cellular components, and signaling networks. Entities with a fold enrichment of fewer than 8 were focused on studying enriched pathways and gain a deeper understanding of the main target proteins' regulatory networks.

ACKNOWLEDGEMENT

We express our gratitude for the ongoing support provided by the Directorate of Research and Development UI, and the Institute for Research and Community Service UNAS.

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