Identification and *in silico* analysis of phytochemicals from *Phyla nodiflora* (l.): Potential bioactive compounds for targeted therapy

Rajagopal Balasubramanian

rajagopal.b@tnau.ac.in

Tamil Nadu Agricultural University https://orcid.org/0000-0002-7828-9508

Prasanna Seenivasan

Tamil Nadu Agricultural University

Thangavelu AU

PSG College of Arts & Science

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Abstract

The increasing demand for safer, more sustainable alternatives to chemical-based medicines has prompted significant interest in plant-derived bioactive compounds, which are known to possess a wide range of therapeutic properties. Among these, Phyla nodiflora (L.), a medicinal plant used in traditional medicine, has garnered attention due to its potential for treating various ailments. However, a comprehensive understanding of the bioactive compounds in P. nodiflora and their mechanisms of action is lacking. In this backdrop, the present study aims to explore the therapeutic potential of phytochemicals derived from hexane and methanolic leaf extract of P. nodiflora. Through GC-MS analysis, we identified 50 distinct chemicals, among these the predominant 10 bioactive compounds were selected for further investigation based on their known pharmacological activities and subjected to molecular docking against protein targets: Protein Tyrosine Phosphatase 1B, Androgen Receptor, Cyclin A, and NKCC1. The results highlight n-Hexadecanoic acid, stigmasterol, and beta-sitosterol as potent candidates for drug development with promising therapeutic potential for diabetes, alopecia, cancer, and anti-diuresis. These findings provide valuable insights into the medicinal value of P. nodiflora and open avenues for further research, including in vitro and in vivo studies, to validate the efficacy of these compounds. The results also underscore the importance of plant-based drug discovery efforts in developing novel treatments for non-communicable diseases, thus contributing to the growing body of research in ethnopharmacology and natural product chemistry.

INTRODUCTION

The demand for safer, sustainable alternatives to synthetic medicines has driven increased interest in the therapeutic potential of plant-derived compounds. Phytochemicals—bioactive secondary metabolites in plants—offer promising prospects due to their diverse biological activities, including antioxidant, antimicrobial, and anti-inflammatory properties (Verpoorte et al., 2005). Numerous studies have demonstrated the efficacy of these compounds in treating chronic diseases such as diabetes, cancer, and cardiovascular conditions, with plant-based therapies often offering fewer side effects compared to conventional drugs (Sarker & Nahar, 2017).

Phyla nodiflora (L.), formerly known as Lippia nodiflora, is a perennial herb used in traditional medicine, has gained attention for its wide array of pharmacological activities, including anti-inflammatory, antimicrobial, and antipyretic properties (Arumanayagam & Arunmani, 2015; Khdera & Saad, 2024). Although several phytochemicals from P. nodiflora have been identified, their specific therapeutic potential in treating non-communicable diseases such as diabetes, alopecia, cancer, and anti-diuresis has yet to be thoroughly investigated, particularly at the molecular level (Alafnan et al., 2023; Sadgrove, 2018). Diabetes, characterized by chronic hyperglycemia, is often treated by targeting enzymes like Protein Tyrosine Phosphatase 1B (PTP1B), which negatively regulates insulin signaling (Teimouri et al., 2022). Similarly, alopecia can be managed by inhibiting the Androgen Receptor (AR), which influences hair follicle development (Zhuo et al., 2012). Cancer therapies often focus on regulating cell cycle proteins like Cyclin A, which plays a key role in tumor growth (Ding et al., 2020). Furthermore, the Na-K-Cl

Cotransporter 1 (NKCC1), crucial in fluid regulation, represents a target for addressing anti-diuretic conditions (Koumangoye et al., 2021).

Several studies have demonstrated the efficacy of molecular docking in identifying lead compounds from natural sources. Proven examples such as, phytochemicals isolated from Curcuma longa have been shown through docking studies to inhibit key proteins involved in cancer and inflammatory pathways, prompting further investigation into their therapeutic potential (Furlan et al., 2018). Similarly, molecular docking has been employed to identify natural inhibitors of enzymes such as acetylcholinesterase (involved in Alzheimer's disease) and angiotensin-converting enzyme (ACE), a target for antihypertensive therapies (Iman et al., 2018). These successes underscore the value of computational tools in accelerating the discovery of plant-based drugs.

Given the therapeutic promise of Phyla nodiflora and the demonstrated utility of computational methods in drug discovery, this study aims to investigate the phytochemicals present in the hexane and methanolic extract of P. nodiflora and assess their potential for treating diabetes, alopecia, cancer, and anti-diuresis. Using GC-MS analysis, we identified the chemical constituents of the plant extract, followed by in silico molecular docking studies to evaluate their interactions with key protein targets: Protein Tyrosine Phosphatase 1B (PTP1B), Androgen Receptor (AR), Cyclin A, and NKCC1. These proteins are implicated in the pathogenesis of the diseases under investigation, making them ideal targets for therapeutic intervention. By identifying compounds with strong binding affinities to these proteins, we aim to provide new insights into the medicinal potential of P. nodiflora and its role in plant-based drug discovery.

MATERIALS AND METHODS

2.1 Plant Material Collection and Preparation

Leaves of Phyla nodiflora were collected from the Herbal Garden, Department of Spices and Medicinal Crops, Tamil Nadu Agricultural University, Coimbatore, India, and authenticated by the horticulturist. Ten grams of leaves were shade dried for 48 h. Then the leaf samples were macerated with 50 ml Hexane, until no visible large particles remained. The macerated plant samples were kept soaked in hexane for 24 h at room temperature. The extracts were filtered using Whatmann filter paper labelled as hexane extract. The sediment was then steeped in methanol and extracted at room temperature for 24h, which was the methanol extract. Filtrates were then condensed separately using a rotary evaporator at 40°C to yield the respective crude extracts. One microlitre of each sample were injected in GC-MS for fractionation.

2.2 GC-MS Analysis

The crude methanolic extract was analyzed using gas chromatography-mass spectrometry (GC-MS) to identify the chemical constituents. Using a Perkin-Elmer GC Clarus 500 system equipped with an Elite-

5MS fused silica column (5% biphenyl 95% dimethylpolysiloxane, 30 m 0.25 mm ID 250m df). The components were separated at a steady flow rate of 1 ml/min using Helium as a carrier gas. Then, 1µL of sample was injected into the system at 260°C injection temperature. The oven temperature was initially set at 60°C for 2 minutes before gradually increasing to 300°C at a rate of 10°C/min. The temperature was then increased to 300°C for 6 minutes. The mass detector's transfer line and ion source temperatures were both 240°C. The National Institute of Standards and Technology (NIST) database was used to identify compounds based on mass spectral data.

2.3 In Silico Molecular Docking

Docking studies were conducted using “AutoDock Vina” software to evaluate the interaction between selected bioactive compounds and the target proteins. The 3D structures of the target proteins: Tyrosine phosphatase 1B (PDB ID: 4Y14) for diabetes, Androgen receptor (PDB ID: 4K7A) for dandruff, Cyclin A (PDB ID: 6GUE) for cancer, and Human NKCC1 (PDB ID: 6PZT) for anti-diuresis treatments, were retrieved from the Protein Data Bank (PDB). Ligands were prepared using the PubChem database and optimized using MM2 force fields. ADMETLAB 2.0 and Lipinski's rule of five criteria, which include molecular weight, log P, the number of hydrogen bond donors, and the number of hydrogen bond acceptors, were used to select these compounds.

2.4 Target Proteins and Their Role in Disease

• Tyrosine Phosphatase 1B (PTP1B): A critical enzyme implicated in the regulation of insulin signaling and is a therapeutic target for type 2 diabetes (Teimouri et al., 2022).

• Androgen Receptor (AR): Involved in hair follicle development and its dysregulation is linked to alopecia (Zhuo et al., 2012).

• Cyclin A: A key regulator of the cell cycle, with overexpression often observed in cancerous tissues (Ding et al., 2020).

• NKCC1 (Na-K-Cl Cotransporter 1): Plays a role in fluid balance and is a therapeutic target for diuretics (Koumangoye et al., 2021).

2.5 Molecular Docking and Visualization

Molecular docking between the receptor and ligands was performed using the "Vina Wizard" software in PyRx-Python Prescription 0.8. The ligands and receptor were imported into the program, categorized appropriately as either ligands or macromolecules. After completing the docking procedure, the data and docked structures were acquired. The analysis included examining bond energies, van der Waals interactions, hydrogen bonds (H-bonds), and electrostatic energy. The results were visualized using Discovery Studio to identify hydrogen bonds and the nearest amino acid residues.

2.5.1 Validation of molecular docking method

Six out of the ten docked ligands showed the best performance with four proteins. Table 4.1 displays the analysis results of the generated bonds.

Results

3.1 GC-MS Analysis of Extracts

GC–MS chromatogram of hexane and methanol extract of Phyla nodiflora is shown in Fig. 1. GC-MS analysis of hexane leaf extract and methanol leaf extract indicated the presence of approximately 50 compounds (Tables 1 and 2). Ten bioactive compounds (Table 3), each with an area percentage greater than 1%, were selected and subjected to molecular docking with selected four protein targets. Ligand structures of the ten bioactive compounds selected for molecular docking are shown in Table 3. Figure 2 shows the 3D crystal structure of four target proteins taken from the PDB for dicking studies: Tyrosine phosphatase 1B complexed with inhibitor (PDB ID: 4Y14), androgen receptor (PDB ID: 4K7A), Cyclin A (PDB ID: 6GUE), and human NKCC1 (PDB ID: 6PZT).

3.2 In silico docking analysis

Figure 3 depicts the docking results of bioactive chemicals. Protein-ligand binding studies indicated that the binding patterns varied based on the nature of the ligands. The docking results, in terms of binding energy values, number of H-bonds, interacting residues of selected compounds are shown in Table 4.

3.2.1 Docking with Tyrosine Phosphatase 1B

The docking of n-Hexadecanoic acid with tyrosine phosphatase 1B (PDB ID: 4Y14), which implicated in pathogenesis of diabetes, showed promising results, with a binding energy of -4.5 kcal/mol and the formation of two hydrogen bonds with key residues LYS A 197 and ASN A 193. This indicates that n-Hexadecanoic acid effectively interacts with the active site, suggesting its potential as an anti-diabetic agent by modulating tyrosine phosphatase 1B activity.

3.2.2 Docking with Androgen Receptor

The androgen receptor (PDB ID: 4K7A), which implicated in pathogenesis of dandruff, demonstrated significant binding interactions both with 1'-Hydroxy-4,3'-dimethyl-bicyclohexyl-3,3'-dien-2-one and n-Hexadecanoic acid. 1'-Hydroxy-4,3'-dimethyl-bicyclohexyl-3,3'-dien-2-one achieved a binding energy of -8 kcal/mol with one hydrogen bond, while n-Hexadecanoic acid had a binding energy of -5.2 kcal/mol with one hydrogen bond. These results underscore the potential of these compounds in influencing androgen receptor activity, which could be leveraged for anti-dandruff treatments.

3.2.3 Docking with Cyclin A

Cyclin A (PDB ID: 6GUE), which implicated in pathogenesis of cancer, showed the strongest docking interaction with stigmasterol and phytol. Stigmasterol exhibited a superior binding energy of -8.4 kcal/mol with one hydrogen bond at HIS A 84, compared to phytol with – 4.9 kcal/mol and multiple hydrogen bonds. This suggests that stigmasterol may be more effective in targeting Cyclin A, a key player in cancer progression, thereby supporting its potential use in cancer therapy.

3.2.4 Docking with Human NKCC1

Beta-Sitosterol and 1'-Hydroxy-4,3'-dimethyl-bicyclohexyl-3,3'-dien-2-one displayed strong interactions with human NKCC1 (PDB ID: 6PZT), which is implicated in pathogenesis of anti-diuresis. Beta-Sitosterol had a binding energy of -9.3 kcal/mol and formed a hydrogen bond with GLU B 363, while 1'-Hydroxy-4,3'-dimethyl-bicyclohexyl-3,3'-dien-2-one had a binding energy of -7.8 kcal/mol with two hydrogen bonds. These findings suggest that beta-Sitosterol might be a more effective diuretic agent due to its stronger binding affinity and interaction with the active site.

Discussion

The results of this study provide significant insights into the therapeutic potential of phytochemicals from P. nodiflora. The in silico docking analysis identified n-Hexadecanoic acid as a potent inhibitor of PTP1B, which plays a crucial role in insulin regulation and has been considered a promising target for type 2 diabetes management (Teimouri et al., 2022). Previous studies have demonstrated the antidiabetic properties of plant-derived fatty acids, and our findings support the continued investigation of n-Hexadecanoic acid as a potential therapeutic agent (Purushothaman et al., 2024).

Additionally, androgen receptor inhibition by 1'-Hydroxy-4,3'-dimethyl-bicyclohexyl-3,3'-dien-2-one points to its potential role in treating androgen-related alopecia (Balakrishna et al., 1996; Sadgrove, 2018). Stigmasterol, a well-known phytosterol, exhibited strong binding to Cyclin A, a key player in cancer cell proliferation. This finding aligns with earlier studies suggesting that phytosterols can inhibit cancer cell growth by modulating cell cycle pathways (Ding et al., 2020; Teoh et al., 2019).

Beta-sitosterol, a widely studied phytosterol, showed significant binding affinity for NKCC1, suggesting its potential role in anti-diuresis. This is consistent with studies highlighting the role of plant sterols in fluid balance and kidney function (Koumangoye et al., 2021).

Conclusion

Our in silico docking study demonstrates the therapeutic potential of phytochemicals derived from Phyla nodiflora for treating diabetes, alopecia, cancer, and anti-diuresis. The findings from this research provide a strong foundation for further experimental validation and drug development efforts. Further in vitro and in vivo studies would validate the therapeutic potential of these compounds and explain their mechanisms of action in greater detail.

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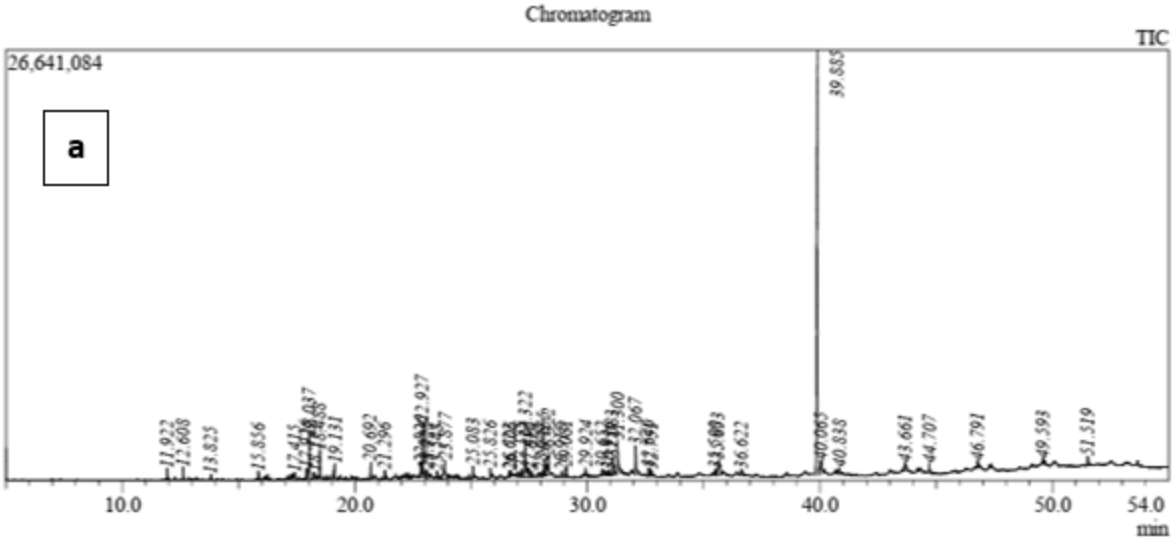
Tables

Tables 1 to 4 are available in the Supplementary Files section

Figures



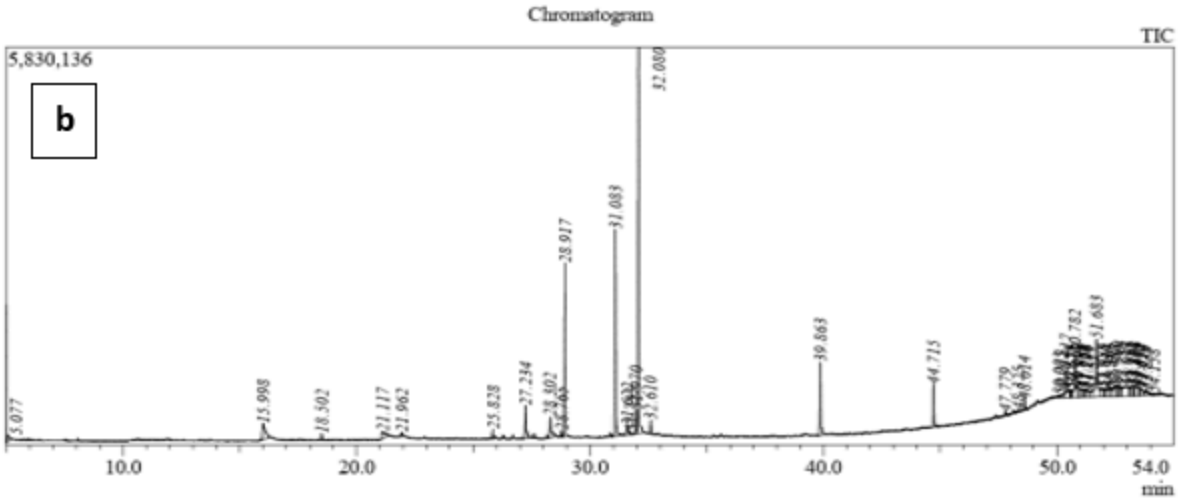


Figure 1

GC–MS chromatogram of (a) hexane and (b) methanol extract of Phyla nodiflora

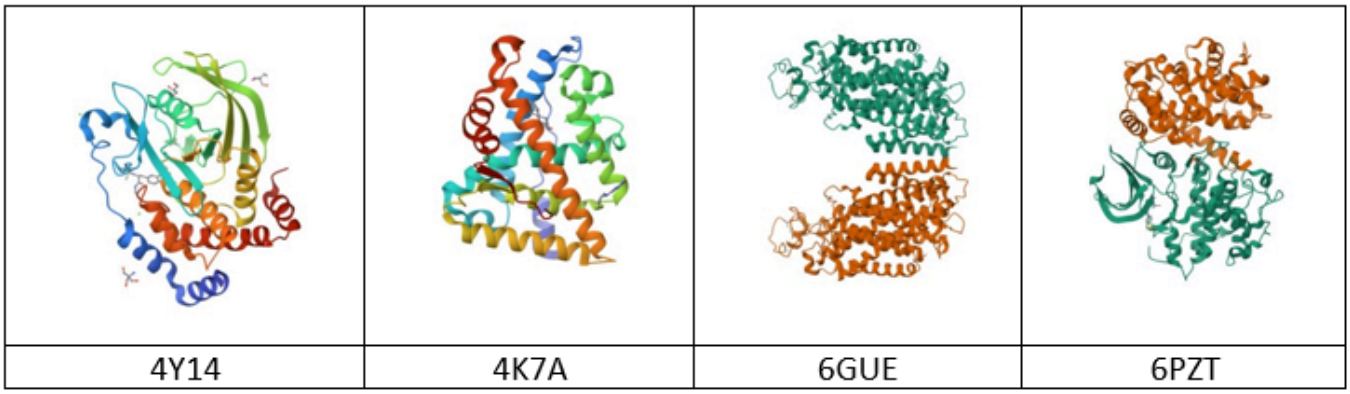


Figure 2

3D crystal structure of four proteins taken from the PDB: Tyrosine phosphatase 1B complexed with inhibitor (PDB ID: 4Y14), androgen receptor (PDB ID: 4K7A), Cyclin A (PDB ID: 6GUE), and human NKCC1 (PDB ID: 6PZT).

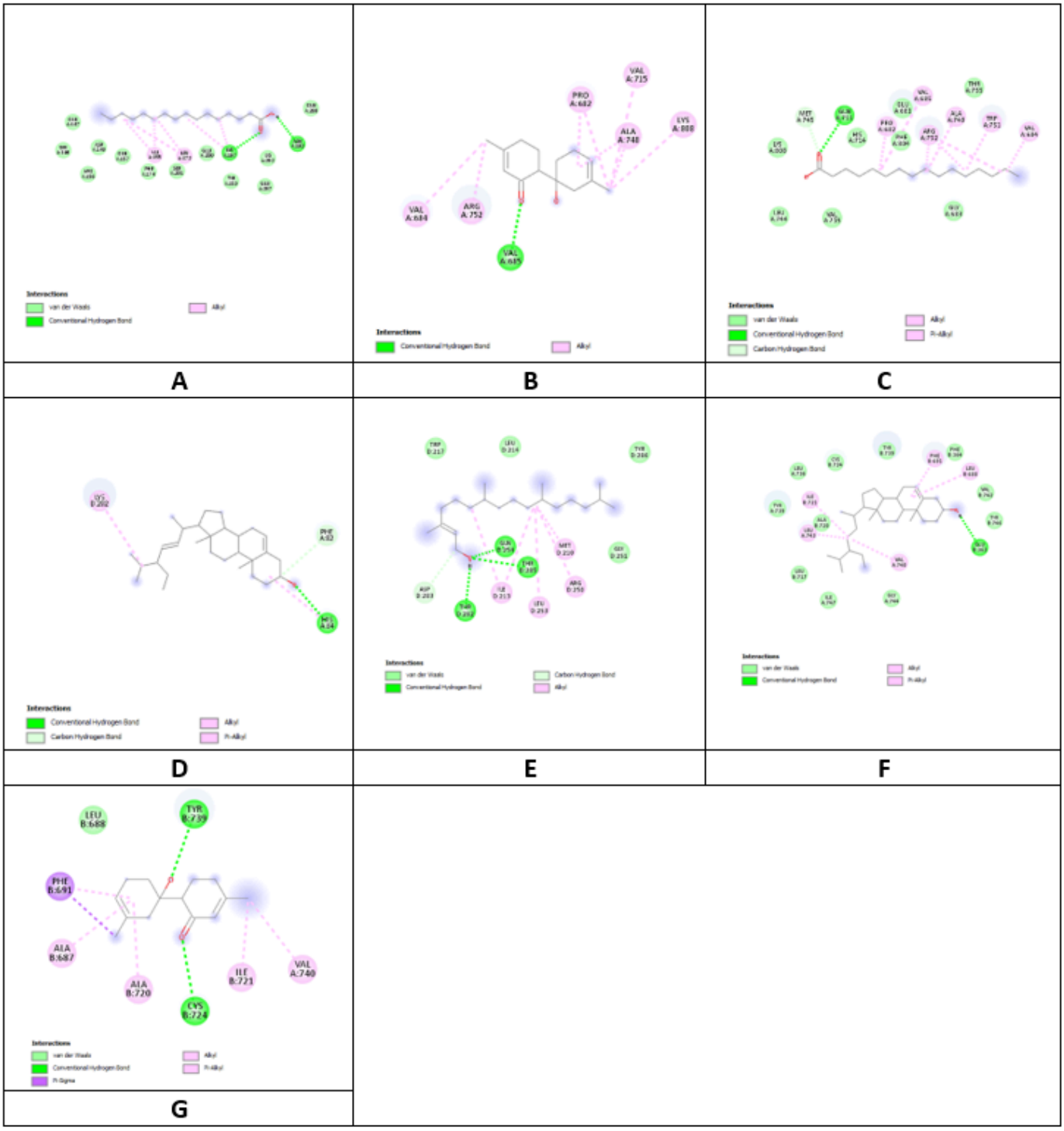


Figure 3

Visualisation of molecular docking between tyrosine phosphatase 1B with (A) - n-Hexadecanoic acid; androgen receptor with (B) - 1'-Hydroxy-4,3'-dimethyl-bicyclohexyl-3,3'-dien-2-one and (C) - n-Hexadecanoic acid; cyclin A with (D) - Stigmasterol, (E) – Phytol; cryo-EM structure of human NKCC1 with (F) - beta-Sitosterol, (G) - 1'-Hydroxy-4,3'-dimethyl-bicyclohexyl-3,3'-dien-2-one.

Supplementary Files

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