In Silico Analysis of Phytochemicals from Catharanthus roseus Targeting TGF-β1 Receptor for Anticancer Therapy

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Abstract

Objective

The research goal focuses on discovering potential blocking substances for the Transforming Growth Factor-beta 1 (TGF-β1) signalling pathway that functions as a critical regulator during cancer progression. A research analysis uses natural compounds from *Catharanthus roseus* (Madagascar Periwinkle) and Tranilast as reference compounds to determine their effects on TGF-β1 signalling pathways for developing new cancer treatments.

Methods

The research conducted a computational analysis using 59 active Catharanthus roseus compounds, including Tranilast. The ligand preparation sequence began with three-step processing that started with generating 3D conformers, followed by protonation state optimization, before finishing with energy minimization. Scientists minimized the energy of the TGF-β1 receptor (PDB ID: 1PY5) through heteroatom removal followed by residue addition. The docking method began with high-throughput virtual screening, followed by standard and extra precision docking procedures. The MM/GBSA protocol calculated binding free energy values during the analysis. The ADMET properties and drug-likeness evaluation occurred through SwissADME and ProTox-III platform analysis.

Results

The TGF-β1 receptor established strong chemical binding capabilities with multiple compounds. The five primary compounds evaluated for research included Petunidin 3-O-glucoside, Isorhamnetin, Quercetin, Kaempferol, and Hirsutidin 3-glucoside. These compounds exhibited favourable binding free energies and pharmacokinetic profiles. ADMET analysis confirmed their drug-like properties with minimal predicted toxicity. Tranilast served as a benchmark for comparison, validating the efficacy of the natural compounds.

Conclusion

This study highlights the therapeutic potential of Catharanthus roseus-derived compounds as inhibitors of the TGF-β1 signalling pathway. Their strong binding affinities and favourable pharmacokinetic profiles suggest their viability as candidates for further preclinical evaluation in cancer therapy. The findings underscore the importance of exploring natural products as sources for targeted cancer treatments.

Level of Evidence: Computational study; Level V.

1.0 INTRODUCTION

Cancer is a condition where cells can divide and grow uncontrolled, typically due to genetic changes in particular genes [1]. In a normal condition, cells in the human body are heavily regulated and controlled

by hormones and genes that code and synthesize proteins that are used for the stability and balance of the immune system [2]. These determine cell division, growth, and death of the cells. When an alteration to a gene or protein forms a codon for its pathway, an imbalance will lead to an excessive release or uncontrolled disasters [3].

Over the past ten years, advances in DNA sequencing have allowed us to rigorously analyse these genetic changes, leading to more excellent knowledge of the signalling pathways and processes that are frequently implicated [4][5]. DNA sequencing is a general laboratory procedure used to identify a DNA molecule's precise arrangement of bases or nucleotides [6]. The biological information that cells require to grow and function is encoded in the sequence of bases, which are frequently denoted by the initial letters of their chemical names: A, T, C, and G (*DNA Sequencing*, n.d.-b).

Advances in DNA sequencing technologies have, in large measure, increased the understanding of cancer genomics and molecular mechanisms of disease progression (Fig. 1).

DNA sequencing is becoming a crucial component of cancer treatment and a tool for studying the disease. Researchers are creating targeted therapies drugs that directly target cancer cells with specific mutations while sparing healthy cells as more genetic abnormalities are found. Precision medicine is the term for this method. For instance, physicians may recommend a medication that targets a particular genetic mutation in a patient's cancer, which may improve the patient's prognosis [7] [8] [9] [10].

However, cancer is a highly complex illness. Diverse cancer kinds and even individual tumours of the same type can have diverse changed genes and pathways [11]. This means scientists must completely grasp all the genetic alterations that can arise in cancer to create successful treatments. Finding new therapy targets and weaknesses in cancer cells is a complex but necessary undertaking.

The Transforming Growth Factor-beta (TGF-β) signalling pathway stands as one of the essential pathways that drive cancer formation and its subsequent spread, according to Boye (2021) [12]. TGF-β signalling is a tumour promoter or suppressor based on cancer development's disease stage and environmental factors. Successful cancer treatment requires a complete understanding of TGF-β pathway operations and metabolic relationships [13] [14].

Cells use their TGF-β1 pathway to regulate vital biological processes, including growth and differentiation, cell death, and immune responses. The process begins when TGF-β1 signalling molecules bind to particular receptors found on cell surfaces [15] [16] [17]. The inactive form of TGF-β1 requires activation before it can carry out its functions. The activated TGF-β1 molecule binds to TβRII receptors before they team up with TβRI receptors for activation. The receptors combine to create a complex that starts multiple cellular processes.

The pathway executes its functions by following multiple sequential steps. The first step involves TGF-β1 binding to TβRII followed by TβRII's recruitment and activation of TβRI according to Braczkowski et al. (2024) [18] and Yakymovych et al. (2022) [19] and Bertolio et al. (2021) [20]. TGF-β1 signals enter the cell

through this receptor complex that functions as a signal transmission messenger. The receptor complex activation leads to the activation of proteins named SMADs (Small Mothers Against Decapentaplegic). Activating SMAD2 and SMAD3 leads to their union with SMAD4, creating a functional team.

Within the cell nucleus, the SMAD proteins function as genetic switches that activate or deactivate genes that regulate cell growth, differentiation, and death. TGF-β1 controls cell growth in healthy tissues through its ability to activate cell-growth-inhibiting genes p21 and p15 and the death-promoting gene BIM. Tissues maintain their structure, and inflammation stays controlled through TGF-β1 activity, which enables proper system functionality [21] [22] [23].

TGF-β1 serves two functions during cancer development: it protects cells at early stages yet turns into a disruptive factor later. TGF-β1 is a protective agent for tumour growth during the initial cancer development. TGF-β1 controls cell overgrowth through its actions while helping damaged cells self-destruct [24]. The protective function of TGF-β1 signalling gets disrupted when genes containing mutations interfere with its mechanism [25]. The uncontrolled cell multiplication results in tumor formation because of this process. The biological behaviour of TGF-β1 transforms advanced cancers as it becomes a tumour-promoting factor.

TGF-β1 becomes a tool that cancer cells exploit after they learn to bypass its standard growth-limiting capabilities. Through TGF-β1 signalling, cancer cells undergo epithelial-to-mesenchymal transition (EMT), which results in their detachment while gaining mobility needed for tissue invasion. Through this process, cancer cells acquire the ability to migrate between different body regions, where they create new tumour growths. The immune system becomes vulnerable to cancer cell detection due to TGF-β1's ability to diminish its strength [26] [27] [28] [29] [30].

The TGF-β1 signalling pathway directly affects cellular energy consumption and nutrient utilization for cancer advancement [29]. The rapid growth of cancer cells requires metabolic reprogramming, leading them to modify their metabolic pathways. Multiple metabolic processes are affected by TGF-β1, according to Shi et al. (2022) [29]. Through this mechanism, TGF-β1 drives cells to perform glycolysis, breaking glucose for energy generation despite oxygen availability [31].

The Warburg effect results from this shift, allowing cancer cells to acquire fast growth through increased energy and materials. The cells that receive TGF-β1 signalling learn to process fats and lipids into essential membrane structures and molecular signals. TGF-β1 controls cellular utilization of amino acid glutamine as cancer cells require this substance for their growth. TGF-β1 modifies the mitochondria structure, so cells must use glycolysis as their primary energy source [32]. Through minor adjustments of cellular metabolic pathways, TGF-β1 enables cancer cells to survive and grow in challenging environments.

Scientific research indicates that the TGF-β1 pathway presents an attractive yet complex approach to cancer treatments. Scientists are currently investigating multiple methods to manage TGF-β1 signalling

[33]. The use of small molecules and antibodies functions as blockers for TGF-β1 receptors to prevent harmful signalling that promotes tumour development and metastasis.

Targeting SMAD proteins presents a potential method to help TGF-β1 suppress tumours during early cancer stages. When TGF-β1 inhibitors are combined with chemotherapy or immunotherapy treatments, they become stronger and more resistant to treatment failure [34]. Scientists explore natural compounds from the Madagascar Periwinkle plant to find potential blocking agents for the TGF-β1 receptor, which could provide an organic cancer-fighting method.

Scientists are searching for methods to control TGF-β1 since this protein plays a substantial role in cancer progression. The blocking of TGF-β1 receptors through drug administration stops the signalling pathway, which promotes cancer growth [35]. Scientists have explored SMAD proteins as targets because this approach might restore TGF-β1's tumour-suppressing properties in early-stage cancers.

Tranilast (PubChem ID: 5282230) (PubChem, n.d.) [36] was first developed as an allergy treatment medicine. Scientists found that Tranilast functions as a blocking agent of the TGF-β1 pathway, which could serve as a new therapy for cancer. Researchers utilize Tranilast as their benchmark compound to evaluate its results against other candidate TGF-β1 blocking agents.

The molecular docking results can be interpreted in relation to the TGF- β1 signalling pathway shown in Fig. 2. Activation of TGF-beta receptors activates SMAD-mediated signalling leading to regulation of gene expression involved in cell proliferation, apoptosis and tumour progression. The high binding affinities of some compounds such as Isorhamnetin, Kaempferol and Petunidin-3-O-glucoside imply that such phytochemicals could suppress TGF- β1 receptor activity which could interrupt downstream signalling events leading to cancer development.

Scientific research explores synthetic drugs such as Tranilast and natural compounds for cancer treatment. *Catharanthus roseus* from Madagascar is a well-known natural source of anticancer compounds [38]. The Madagascar Periwinkle plant synthesizes more than 100 compounds referred to as Monoterpenoid Indole Alkaloids (MIAs) that demonstrate strong biological properties [39].

Vinblastine and vincristine represent the most renowned MIAs that have served as cancer treatments for leukaemia and lymphoma patients since 1960. The therapeutic mechanism of these drugs consists of inhibiting cancer cell division until death occurs. Scientists investigate additional Monoterpenoid Indole Alkaloids from the Madagascar Periwinkle because of their initial success in cancer treatment [40] [41].

The Unique Chemistry of Catharanthus roseus

The Madagascar Periwinkle stands out as an exceptional plant because it generates an extensive range of MIAs, according to Sharma et al. (2022) [41], Nguyen & Dang (2021) [42], and Eng et al. (2022) [43]. The synthesized chemicals serve medical cancer treatment purposes and research on plant defence mechanisms. Advanced scientific methods such as genomics and metabolomics enabled researchers to discover the production mechanism of compounds by the Madagascar Periwinkle. The acquired

knowledge about drug production will enable more efficient manufacturing of valuable drugs in the upcoming years [44] [45].

The research investigates the inhibitory properties of 59 active compounds obtained from the Madagascar Periwinkle and Tranilast (PubChem ID: 5282230) against the TGF-β1 signalling pathway. The search focuses on discovering innovative methods that prevent cancer cell proliferation through direct TGF-β1 signalling pathway blockage. The research team will examine compound-TGF-β1 receptor binding through computer simulations of the TGF-β1 receptor (PDB ID: 1PY5) Crystal Structure of TGF-beta receptor I kinase with inhibitor (Fig. 3).

By comparing the effects of these natural compounds to Tranilast, we hope to discover new and effective treatments for cancer.

2.0 METHOD AND MATERIALS

2.1 Extraction of 2D Structures

The 2D structures of secondary metabolites from *Catharanthus roseus* were retrieved from PubChem, a publicly available chemical database, in Structure Data File (SDF) format. The SDF format provides detailed information about molecular geometries, including atom types, bond orders, and stereochemistry. A total of 59 compounds were downloaded for this study.

2.2.1 Preparation of Ligands

The SDF files of the 59 compounds were imported into Maestro (Schrödinger, Version 2021 – 4.8), a computational chemistry software. Ligand preparation was performed using the LigPrep module, which converts 2D structures into 3D conformers. The following steps were taken during ligand preparation:

Tautomer and Stereoisomer Generation

Multiple tautomeric and stereoisomeric forms were generated for each compound to account for all possible binding conformations.

Protonation States

Protonation states were assigned at a physiological pH of 7.0 ± 2.0 to simulate realistic biological conditions.

Energy Minimization

The OPLS4 force field was used to minimize the energy of each ligand, ensuring stable and clash-free conformations for docking simulations.

After preparation, 128 ligand conformers were generated and saved in Maestro format for further analysis.

Tranilast (PubChem ID: 5282230), a reference compound, was prepared separately using the same LigPrep protocol to ensure consistency in ligand preparation. This step was performed independently to allow for clear comparison and reproducibility.

2.2.2 Preparation of Protein Target

The crystal structure of the TGF-βRI receptor (PDB ID: 1PY5) was downloaded in PDB format from the Protein Data Bank **(**www.rcsb.org**).** The protein was prepared using the Protein Preparation Wizard in Maestro. The preparation process included the following steps:

1. Removal of Heteroatoms: Water molecules and other heteroatoms (e.g., sulfate ions) were removed to avoid artifacts during docking.
2. Addition of Missing Atoms: Missing atoms and residues in the protein structure were added using Maestro's default settings.
3. Protonation States: Protonation states were adjusted to a physiological pH of 7.0 using Epik.
4. Energy Minimization: The protein structure was energy-minimized using the OPLS4 force field to resolve unfavourable atomic interactions and improve geometry. Heavy atoms were restrained to a root-mean-square deviation (RMSD) of 0.3 Å.

A receptor grid was generated around the active site of TGF-βRI using the coordinates x = 4.21, y = 8.91, and z = 4.26. This grid box defined the docking search space for ligand binding.

2.3 Molecular Docking Simulations

Molecular docking simulations were performed using the Glide module in Maestro. The docking process involved the following steps:

1. High-Throughput Virtual Screening (HTVS): The 128 prepared ligands were initially docked using HTVS to identify compounds with favorable binding interactions. This step generated 52 potential ligands.
2. Standard Precision (SP) Docking: The top 52 ligands from HTVS were further docked using SP to refine the binding poses and improve accuracy. This step resulted in 51 ligands with effective binding interactions.
3. Extra Precision (XP) Docking: The final docking step used XP to evaluate the binding affinities and interactions of the 51 ligands in greater detail. This step provided highly accurate docking scores and binding poses.

Tranilast (PubChem ID: 5282230) was docked separately using the same protocol (HTVS → SP → XP) to ensure consistency and allow for independent evaluation. This approach ensures that the results for Tranilast can be easily replicated and compared with the *Catharanthus roseus* compounds.

2.4 Binding Free Energy Calculations

The binding free energies of the top-ranked ligands from the XP docking results were calculated using the Molecular Mechanics/Generalized Born Surface Area (MM/GBSA) method. This step provided an estimate of the binding affinity of each ligand for the TGF-βRI receptor.

2.5 Identification of Lead Compounds

Based on docking scores and binding free energies, the following compounds were identified as the most promising candidates for further analysis:

1. Petunidin 3-O-glucoside (PubChem ID: 443651)
2. Isorhamnetin (PubChem ID: 5281654)
3. Quercetin (PubChem ID: 5280343)
4. Hirsutidin 3-glucoside (PubChem ID: 44257023)
5. Kaempferol (PubChem ID: 5280863)
6. Vinpocetine (PubChem ID: 443955)
7. Vanillic acid (PubChem ID: 8468)
8. Tranilast (PubChem ID: 5282230)
9. Hydroxytyrosol(PubChem ID: 82755)

2.3 ADMET and Drug-Likeness Assessment

The pharmacokinetic and toxicity profiles of the lead compounds were evaluated using two computational tools: SwissADME (SwissADME, n.d.) [47] and ProTox-III (ProTox-3.0 - Prediction of Toxicity of Chemicals, n.d.) [48]. The critical parameters considered for the analysis include computational methods that assess the compounds' water solubility because solubility plays an essential role in bioavailability, illustrating the compound's ability to be absorbed and disseminated in biological systems. The drug-likeness of the compounds was then assessed based on established criteria, including Lipinski's Rule of Five, which evaluates key molecular properties such as molecular weight (≤ 500 Da), lipophilicity (LogP ≤ 5), hydrogen bond donors (≤ 5), and hydrogen bond acceptors (≤ 10). These criteria help predict the likelihood of a compound exhibiting favorable pharmacokinetic properties.

In addition, the primary pharmacokinetic parameters were assessed. The possibility of the compounds affecting the hERG potassium channel that is related to cardiac toxicity was determined, as this effect provokes severe side effects. The ability of compounds to permeate through the skin was evaluated, which is important for developing topical drug formulations. The capacity of compounds to cross the Blood-Brain Barrier (BBB) was analyzed, which is crucial for drugs targeting central nervous system disorders. The predicted absorption of compounds in the human intestine was evaluated, which is vital for oral drug formulations. Additionally, the potential inhibition of specific Cytochrome P450 (CYP) isoforms involved in drug metabolism was assessed to predict possible drug-drug interactions.

The lead compound's toxicity profile was assessed regarding detrimental impacts on significant organs. The nephrotoxic effects were also determined to check the impact of the compounds on kidney function.

The impact on the neurological functions was evaluated as they cause severe side effects. The genotoxicity of the compounds was also tested due to the critical function of the liver in the metabolism of active pharmaceutical ingredients.

3.0 RESULT AND DISCUSSION

3.1.1 Molecular Docking Results

The docking scores and MMGBSA binding energies showed that several compounds showed to have strong binding affinities with the TGF- β receptor I. The detailed results of the docking score and the binding free energy of the evaluated compounds are provided in Table 1 and Fig. 4.

The bar chart depicts the XP-docking scores and MMGBSA binding free energies (ΔG bind) of selected phytochemicals from Catharanthus roseus compared with a reference compound Tranilast against the TGF-β receptor I (PDB ID: 1PY5). Lower docking scores and more negative MMGBSA binding energies show a stronger affinity between the ligand and the receptor. Among the studied compounds, Petunidin-3-O-glucoside, Isorhamnetin, Quercetin and Kaempferol were found to be more effective predicted binding compounds than the reference compound Tranilast. Isorhamnetin achieved the best binding free energy (−56.08 kcal/mol) which would indicate a very stable ligand-receptor complex.

2D and 3D Interaction Images

The detailed ligand-receptor interactions patterns and their binding conformations of the top ranked compounds are shown in Table 2 which displays their both 2D interaction maps and their 3D docking poses in binding pocket of TGF- β receptor I.

3.1.2 ADMET Profile Analysis

The predicted pharmacokinetic and drug-likeness properties of the selected compounds such as the molecular descriptors, absorption properties, and also the rule Lipinski are summarised in Table 3.

Protox 3. Result

Table 4 summarizes the toxicity endpoints obtained from Protox 3 for the selected compounds:

3.2 Discussion

3.2.1 Molecular Docking

The molecular docking analysis provides critical insights into the binding interactions between various bioactive compounds and TGF-β receptor I, offering a quantitative assessment of their potential as

therapeutic agents. The results reveal substantial differences in binding affinities as measured by XP-docking scores and MMGBSA binding free energies (ΔG bind), providing a comprehensive picture of molecular recognition and complex stability.

Petunidin 3-O-glucosides emerged as the strongest binder regarding initial docking affinity, achieving an exceptional XP-docking score of -9.553 kcal/mol. This remarkably high score indicates favourable geometric and chemical complementarity between the ligand and receptor binding site. The compound's glycoside moiety plays a crucial role in this interaction, forming an extensive network of hydrogen bonds with polar residues in the binding pocket, particularly with LYS 213, ASP 290, GLU 284, and HIE 283.

These findings align with previous studies demonstrating that glycosylated flavonoids often exhibit enhanced binding affinity due to additional hydrogen bonding capacity provided by their sugar units [49]. The MMGBSA ΔG bind value of -36.93 kcal/mol further confirms the thermodynamic stability of this complex, suggesting that the initial favorable docking orientation translates into a stable bound state.

The XP-docking score of Isorhamnetin (-8.915 kcal/mol) was slightly less favorable than Petunidin 3-O-glucosides yet the compound exhibited the most stable binding capacity with an MMGBSA ΔG bind of -56.08 kcal/mol. The difference between kinetic and thermodynamic measures provides valuable insights about the binding mechanism.

The presence of the methoxy group in Isorhamnetin likely stabilizes the compound through hydrophobic bond interactions with nonpolar residues within the pocket, according to recent molecular dynamics research on similar flavonoid-receptor systems [50] [51]. The substantial ΔG bind value demonstrates that although initial docking has some unfavourable aspects, the final bound position reaches remarkable stability, possibly because of site-binding conformational changes or water displacement.

Kaempferol demonstrates strong performance in the XP-docking score of -9.364 kcal/mol, along with a favourable ΔG bind of -43.61 kcal/mol. The balanced profile indicates that Kaempferol maintains superior initial binding and sand ex retention. The compound structure positions its hydroxyl groups effectively for hydrogen bonding while maintaining enough hydrophobic properties for stable van der Waals contacts. Removing the 3'-OH group from Quercetin does not significantly impact protein binding affinity. However, it may decrease unwanted interactions between the compound and other proteins, according to Veiko et al. (2021) [52], Carrillo-Martinez et al. (2024) [53], and Salehi et al. (2020) [54].

Quercetin and Hirsutidin 3-glucoside showed similar docking scores (-8.864 and -8.561 kcal/mol, respectively) but differed in their thermodynamic profiles (ΔG bind of -34.61 and -34.08 kcal/mol). While indicating favourable binding, these values suggest slightly fewer stable complexes than the top performers. For Quercetin, this may relate to suboptimal hydrophobic interactions despite its hydrogen bonding capacity, while for Hirsutidin 3-glucoside, the bulky glycoside group might limit optimal positioning in the binding pocket.

As the primary investigative compound in this study, "Tranilast" presents a unique binding profile that warrants particular attention. The docking results show a relatively modest XP-docking score of -4.153 kcal/mol and MMGBSA ΔG bind of -22.74 kcal/mol, significantly lower than the top-performing flavonoids. However, these values must be interpreted in the context of Tranilast's known clinical efficacy as an anti-fibrotic agent.

Analysis of the docking pose reveals that Tranilast binds in a distinct region of the TGF-β receptor I kinase domain compared to the flavonoids, occupying a more peripheral site near the N-lobe. This binding location suggests potential allosteric modulation rather than competitive inhibition of the ATP-binding site. The interaction appears to be stabilized primarily through hydrophobic contacts with GLU 245, GLU 218, and HIE 283, along with a key hydrogen bond to ASP351. This limited interaction profile explains its lower binding metrics but may confer advantages regarding receptor selectivity, as demonstrated in crystallographic studies of similar kinase inhibitors [55].

The moderate binding affinity of Tranilast aligns with its established polypharmacology. While showing only modest direct binding to TGF-β receptor I, Tranilast is known to exert additional effects, including Inhibition of TGF-β secretion from mast cells [56]; Modulation of PPARγ activity (Makoto Kurano et al., 2018) [57]; Suppression of inflammatory mediator release

This multi-target activity likely explains its clinical efficacy despite our study's relatively weak receptor binding. The ΔG bind value of -22.74 kcal/mol suggests that Tranilast's receptor interaction may be transient or context-dependent, potentially requiring specific conformational states of the receptor for optimal binding [58].

Comparative analysis with the flavonoid compounds reveals important structure-activity insights. Tranilast's more straightforward structure lacks the extended aromatic systems and multiple hydroxyl groups that enable the flavonoids to achieve such strong binding (ΔG bind < -30 kcal/mol). However, this structural simplicity contributes to its favourable pharmacokinetic properties, including high oral bioavailability (85%) and minimal CYP450 interactions - advantages not shared by all the high-affinity flavonoids in our study [59].

Vinpocetine and Vanillic acid showed intermediate binding characteristics, with XP-docking scores of -7.096 and -6.551 kcal/mol, respectively. Their ΔG bind values (-13.02 and -23.62 kcal/mol) indicate relatively unstable complexes, likely due to suboptimal complementarity with the binding site. Hydroxytyrosol presented an interesting profile with a moderate docking score (-7.116 kcal/mol) but relatively favorable ΔG bind (-36.03 kcal/mol), suggesting that while initial binding may be less intense, the final complex achieves good stability, possibly through unique interaction patterns worth further investigation.

3.2.2 ADMET And Protox 3.0

The molecular characteristics and pharmacokinetics of Petunidin 3-o-glucosides ($C_{22}H_{23}O_{12}^+$, MW: 479.41) highlight its high hydrogen-bonding capacity with 12 acceptors and eight donors. Its large topological polar surface area (TPSA) of 202.67 suggests poor cell permeability, correlating with low gastrointestinal (GI) absorption and non-permeability to the blood-brain barrier (BBB). However, it does not block CYP3A4, and it is not a P-glycoprotein (Pgp) substrate; however, this is a violation of the Lipinski rule of 2 times so it may pose a problem with bioavailability, and indeed it holds a very low bioavailability score of 0. 17.

In contrast, Isorhamnetin ($C_{16}H_{12}O_7$, MW: 316. 26) which shows less ADMET properties that is high GI absorption and no BBB permeability. It only affects the CYP3A4 enzyme. However, it does not violate Lipinski's or Ghose's rules, which gives the Bioavailability Score of 0. 55. In contrast, Isorhamnetin ($C_{16}H_{12}O_7$, MW: 316.26) exhibits a more favourable absorption, distribution, metabolism, excretion, and toxicity (ADMET) profile, with high GI absorption and no BBB permeability. It inhibits CYP3A4 but does not violate Lipinski's or Ghose's rules, resulting in a higher bioavailability score of 0.55. Its intrinsic log partition coefficient (iLOGP) of 2.35 and TPSA of 120.36 further support its potential for good bioavailability.

Similarly, Quercetin ($C_{15}H_{10}O_7$, MW: 302.24) exhibits high GI absorption and CYP3A4 inhibition without violations of Lipinski's or Ghose's rules, indicating good drug-likeness and bioavailability, though it shows active nephrotoxicity and mutagenicity. Quercetin's bioavailability score is 0.55. Hirsutidin 3-glucoside ($C_{24}H_{27}O_{12}^+$, MW: 507.46), with a high hydrogen-bonding capacity and TPSA of 180.67, shows low GI absorption and non-BBB permeability. With three Lipinski violations and one Ghose violation, its bioavailability score is significantly reduced to 0.17.

Kaempferol ($C_{15}H_{10}O_6$, MW: 286.24) stands out with high GI absorption, no BBB permeability, and CYP3A4 inhibition. It does not violate Lipinski's or Ghose's rules and has a favourable bioavailability score of 0.55, supported by its iLOGP of 1.7 and TPSA of 111.13. Tranilast ($C_{18}H_{17}NO_5$, MW: 327.33) exhibits high GI absorption and a relatively high bioavailability score of 0.56, with no BBB permeability and no inhibition of CYP3A4, aligning with its absence of violations in Lipinski's and Ghose's rules.

Vinpocetine ($C_{22}H_{26}N_2O_2$, MW: 350.45) demonstrates high GI absorption and BBB permeability, distinguishing it from other studied molecules. It inhibits CYP3A4 and does not violate any drug-likeness rules, resulting in a bioavailability score 0.55. Vanillic acid ($C_8H_8O_4$, MW: 168.15) exhibits high GI absorption, no BBB permeability, and no inhibition of CYP3A4. It has a high bioavailability score of 0.85, with no violations of drug-likeness rules.

In terms of toxicity profiles, predicted using Protox 3, Petunidin 3-o-glucosides and Hirsutidin 3-glucoside are classified as Class 5, with inactive hepatotoxicity and neurotoxicity but active nephrotoxicity and cytotoxicity. They both have high predicted LD50 values of 5000 mg/kg. Isorhamnetin, also a Class 5, shows inactive hepatotoxicity and neurotoxicity but active nephrotoxicity and carcinogenicity. With an LD50 of 5000 mg/kg. Quercetin, categorized as Class 3, indicates higher toxicity, with active nephrotoxicity, carcinogenicity, and mutagenicity, and a significantly lower LD50 of 159 mg/kg.

Kaempferol is Class 5, with inactive hepatotoxicity, neurotoxicity, and cytotoxicity and a high LD50 of 3919 mg/kg.

Tranilast and Vinpocetine are Class 4, with Tranilast showing active hepatotoxicity and neurotoxicity and Vinpocetine showing neurotoxicity, with LD50 values of 1190 mg/kg and 503 mg/kg, respectively. Vanillic acid, classified as Class 4, exhibits inactive hepatotoxicity, neurotoxicity, and carcinogenicity but active nephrotoxicity, with an LD50 of 2000 mg/kg.

4.0 CONCLUSION

This study demonstrates that *Catharanthus roseus* compounds, particularly petunidin 3-O-glucosides, isorhamnetin, and kaempferol, exhibit strong binding to TGF-β receptor I, with kaempferol showing optimal balance between affinity (-9.364 kcal/mol docking score) and drug-like properties. While glycosylated compounds displayed superior binding, their poor bioavailability limits therapeutic potential. Isorhamnetin's exceptional thermodynamic stability (-56.08 kcal/mol) and kaempferol's favourable ADMET profile (0.55 bioavailability score, Class 5 toxicity) highlight their promise as lead compounds. Comparative analysis with Tranilast revealed that moderate binders can achieve clinical efficacy through polypharmacology, emphasizing the need to consider multiple pharmacological parameters. However, universal nephrotoxicity signals and CYP3A4 inhibition by top candidates necessitate structural optimization. These findings position kaempferol as the most promising candidate for further development while underscoring the value of natural products in anticancer drug discovery.

Declarations

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Author Contributions: Eze, Charles, Precious, and Sakariyau: Conceptualization, Methodology, Writing - Original Draft

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Tables

Tables 1 to 4 are available in the supplementary files section

Figures

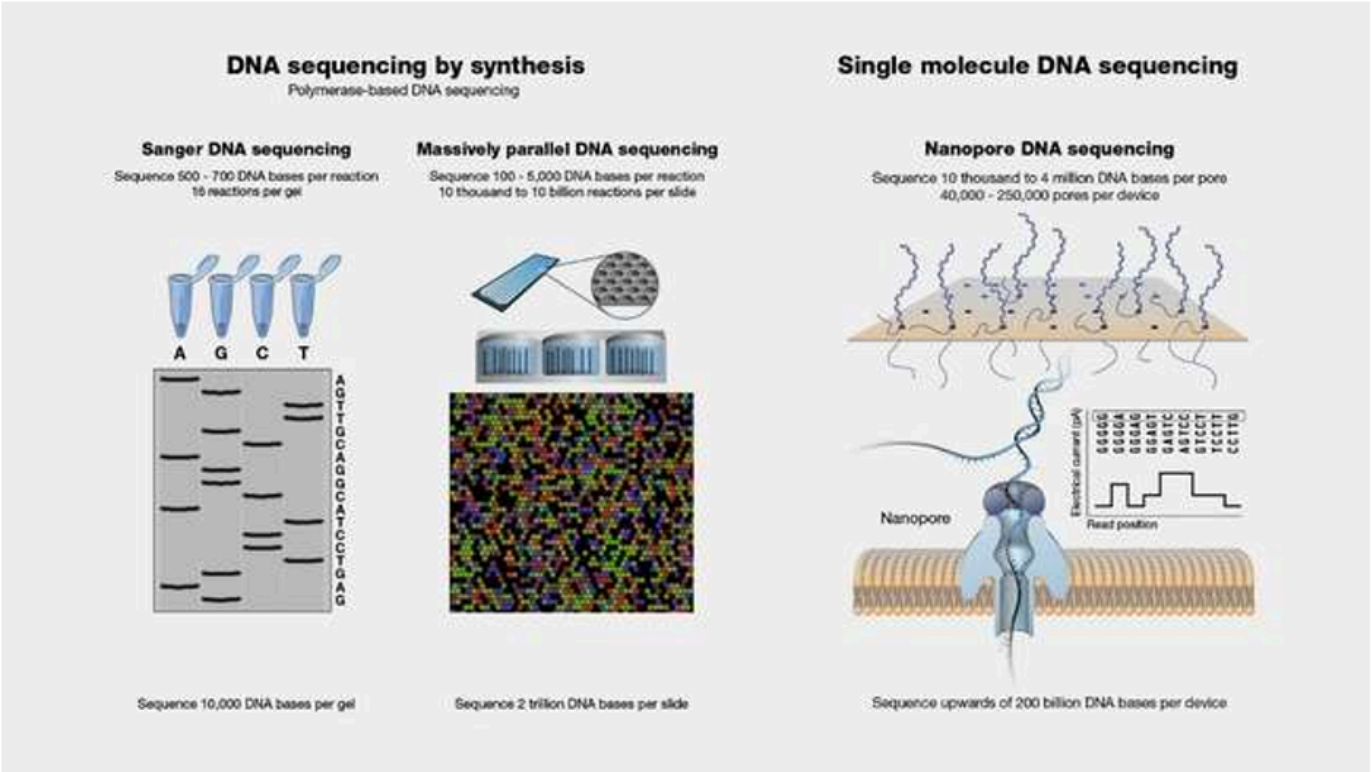

Figure 1

Overview of major DNA sequencing technologies used in genomic research. Source: Adapted from Malone et al., Genome Medicine (2020) and related literature on DNA sequencing technologies.

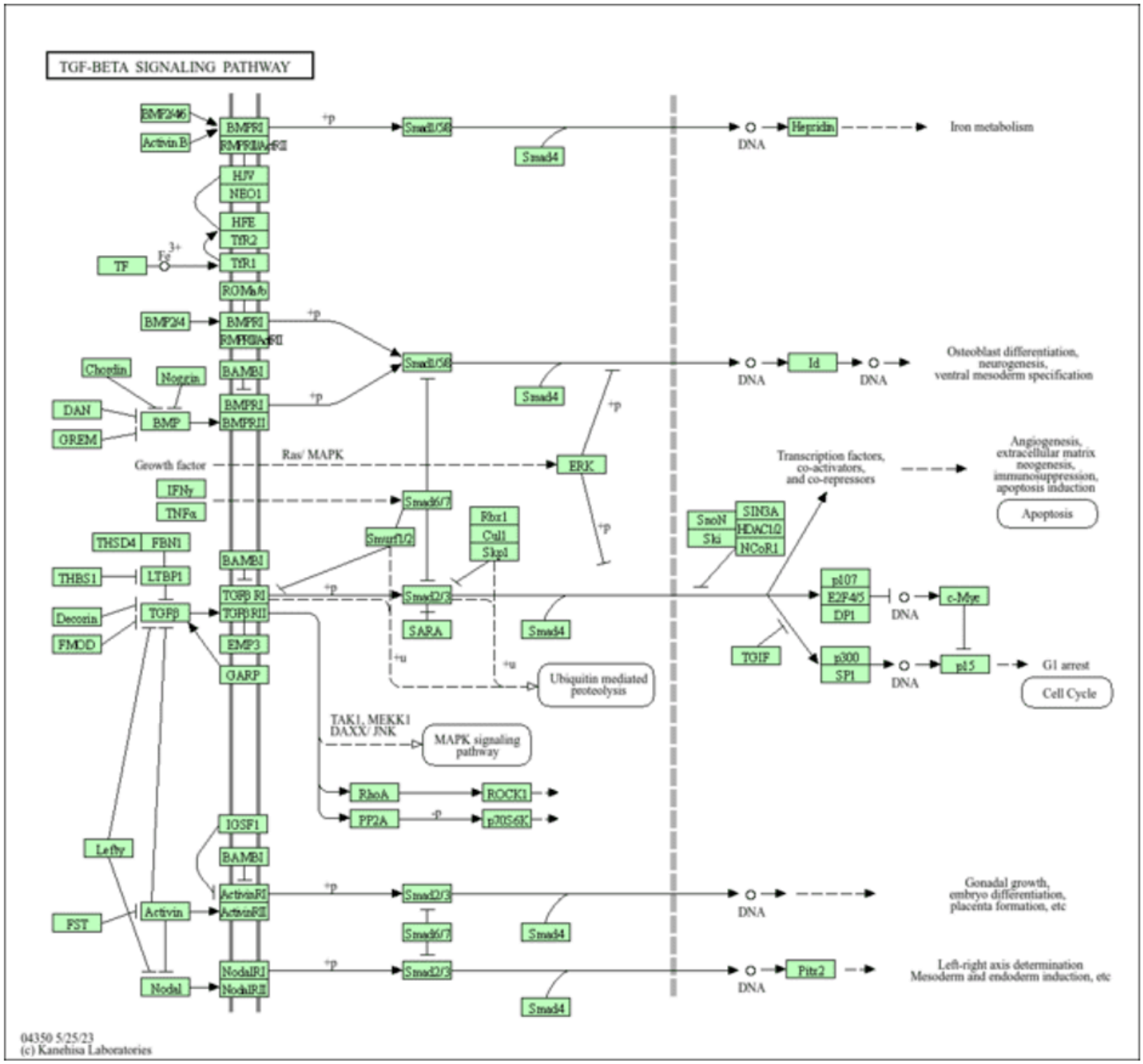

Figure 2

KEGG PATHWAY. Source TGF-beta Signaling Pathway - *Homo sapiens*(Human), n.d.-c) [37]

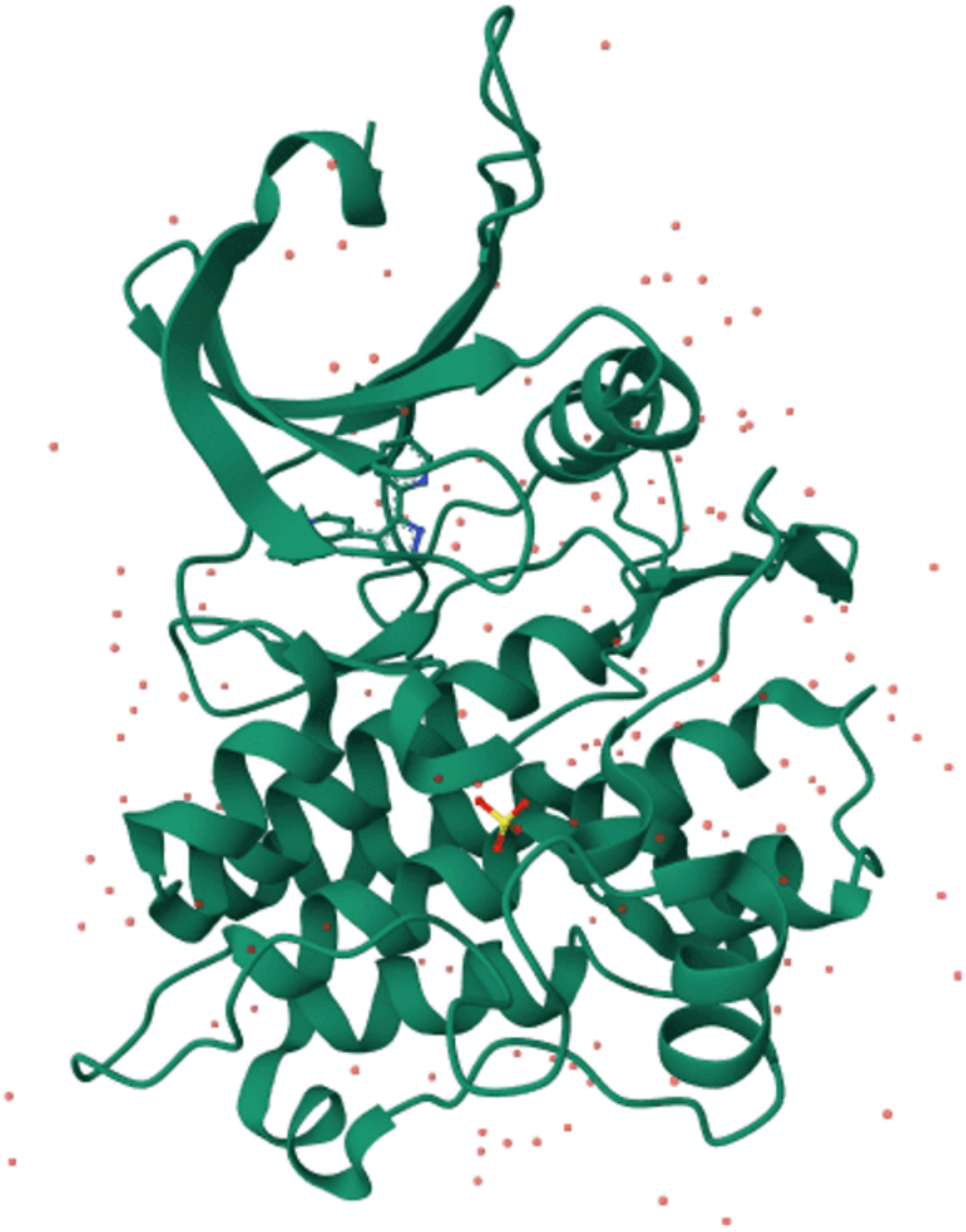

Figure 3

TGF-beta receptor I (Bank, n.d.-a) [46]

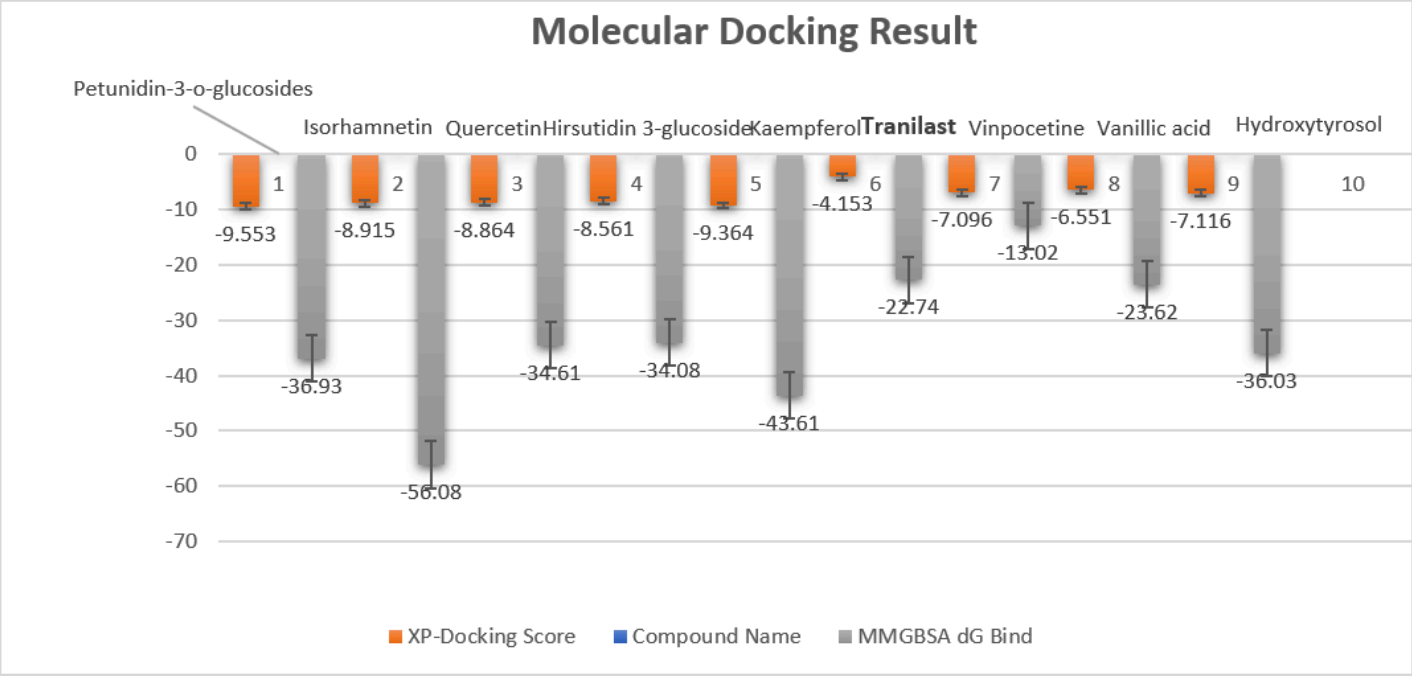

Figure 4

Molecular docking binding affinity comparison of selected phytochemicals with TGF-β receptor I. Source: Generated from the molecular docking analysis conducted in this study using Schrödinger Maestro (Glide docking and MMGBSA calculations)

Supplementary Files

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