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A pharmacophore screening approach of homeopathic phenols for a renovated design of fragment-optimized Bauhiniastatin-1 as a drug against acromegaly

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Abstract. – OBJECTIVE: Acromegaly is a fatal and chronic disease that is caused by the abnormal secretion of growth hormone (GH) by the pituitary adenoma or pituitary tumor, resulting in an increased circulated concentration of insulin-like growth factors 1 (IGF-1), where in most of the cases it is secreted by a pituitary tumor. Higher levels of GH cause an increase in IGF-1 in the liver leading to multiple conditions such as cardiovascular diseases, glucose imbalance, cancer, and sleep apnea. Medical treatments such as surgery and radiotherapy can be used as the first choice of patients; however, specified human growth hormone control should be an essential treatment strategy due to an incidence rate of 0.2-1.1 yearly. Therefore, the main focus of this study is to develop a novel drug for treating acromegaly by exploiting medicinal plants that have been screened using phenol as a pharmacophore model to identify target therapeutic medicinal plant phenols.

MATERIALS AND METHODS: The screening identified thirty-four pharmacophore matches of medicinal plant phenols. These were selected as suitable ligands and were docked against the growth hormone receptor to calculate their binding affinity. The candidate with the highest screened score was fragment-optimized and subjected to absorption, distribution, metabolism, and excretion (ADME) analysis, in-depth toxicity predictions, interpretation of Lipinski's rule, and molecular dynamic simulations to check the behavior of the growth hormone with the fragment-optimized candidate.

RESULTS: The highest docking energy was calculated as -6.5 K/mol for Bauhiniastatin-1. Enhancing the performance of Bauhiniastatin-1 against the growth hormone receptor with fragment optimization portrayed that human growth hormone inhibition can be executed in a more efficient and better way. Fragment-optimized Bauhiniastatin-1 (FOB) was predicted with high gastrointestinal absorption, a water solubility of -2.61 as soluble, and synthetic accessibility of 4.50, achieving Lipinski's rule of 5, with low organ toxicity prediction and interpreting a positive behavior against the targeted protein. The discovery of a *de novo* drug candidate was confirmed by the docking of fragment-optimized Bauhiniastatin-1 (FOB), which had an energy of -4,070 Kcal/mol.

CONCLUSIONS: Although successful and completely harmless, present healthcare treatment does not always eradicate the disease in some individuals. Therefore, novel formulas or combinations of currently marketed medications and emergent phytochemicals will provide new possibilities for these instances.

Key Words:
Growth hormone, Acromegaly, Fragment optimization, Bauhiniastatin-1, ADMET.

Introduction

Acromegaly is a rare chronic disease caused by the excessive secretion of the growth hormone (GH) mostly due to an adenoma of the anterior pituitary gland and resulting in an increased circulating concentration of insulin-like growth factor 1 (IGF-1), the main effector of GH activity. Acromegaly is caused by pituitary GH, which produces tumors in 95% of the cases, and it is part of a larger category of endocrine

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entities linked with hypophyseal tumors in the general population. It has an increasing ratio of incidence and prevalence of 3.9-7.4, respectively 76-116 cases per 100,000 annually, specifically for acromegaly with values of 0.2-1.1 and 2.8-1.37 respectively[1-7]. Characteristics of the disease include the enlargement of the acral, an increase in the sizes of visceral organs, and physical defacement. Its symptoms include pituitary tumor effects, like headache and bitemporal hemianopsia, or may cause the development of hypopituitarism as a result of the tumor itself. Acromegaly is also linked with other multiple health conditions, such as patients displaying the typical symptoms of arthritis, jaw overbite, pulmonary obstruction, hypertension, headache, vision abnormalities, and cranial nerve palsy as well[8]. The ratio of the spread of disease is between 3-10 cases per 100,000 individuals with an incidence rate of about 0.2-1.1 cases per year[9]. The imperative for screening for all individuals with elevated GH hormone levels is suggested due to an increase in morbidity and mortality rates.

The use of medicinal plants can be widely associated with the treatment of acromegaly, as medicinal plants contain several compounds and phytochemicals such as flavonoids, alkaloids, tannins, and other compounds, including phenols. The fact that herbal remedies are significantly healthier and safer to administer is merely one of their numerous virtues[10]. Medicinal plant phenols are rich in antioxidant activity as well as various health benefits. Plant phenolic compounds are viable components that work as antimicrobial, anti-cancer, anti-inflammatory, and anti-mutagenic agents[11]. These compounds essentially include simple phenols, flavonoids, phenolic acids, hydrolyzable, tannins, and lignans.

Phenols are characterized by organic compounds. The potential of traditional medicinal plants to produce aromatic compounds, the majority of which are phenols or their oxygen-substituted derivatives, is practically at its optimum[12]. A specific type of flowering plant called *Bauhinia purpurea* is used to treat a variety of diseases across several traditional medical practices. The herb is used to treat delirium febrile, convulsions, dropsy, ulcers, wounds, injuries, pain, rheumatism, thigh swelling, and blackness of the tongue or lips. It has anticancer qualities, and the decoction it produces stops tumor cells from growing[13]. Since phenols have anti-tumor and anti-inflammatory properties that can be used to combat various diseases, the potential of Bauhiniastatin-1 has been explored as an inhibitor in this study which is discovered in the leaf of *Bauhinia purpurea* with pharmacophore modeling technique. As phenols are found in nontoxic medicinal herbs, they can also be used as oral analgesics or in anesthetic products. They are preferable to use as they have relatively few side effects.

The primary objective of this study is to treat acromegaly by inhibiting the elevated growth hormone by utilizing a *de novo* drug candidate developed from Bauhiniastatin-1.

This phenol is found in homeopathic *Bauhinia purpurea*, a medicinal plant that can potentially treat various diseases. To enhance the inhibitory effect for the human growth hormone receptor, the target phenol Bauhisnistatin-1 was fragment-optimized. The successful development of natural medicinal phenol-based therapies is projected to increase the demand for *Bauhisnistatin-1* production on a large scale at the *in-vitro* level.

Materials and Methods

Protein Retrieval

The UniProt id P10912 of the target protein for the human growth hormone receptor that this study addressed was obtained from UniProt (Universal Protein Source, available at: https://www.uniprot.org). An AI algorithm called Alphafold (available at: https://alphafold.ebi.ac.uk/) was used to obtain the 3D structure, and Discovery Studio (available at: https://discover.3ds.com/discovery-studio-visualizer-download) created a 3D representation of the chosen protein's structure.

Binding Pocket Identification

The online tool ConSurf (available at: http://consurf.tau.ac.il),examines the evolutionary dynamics of amino- and nucleic acid substitutions among homologous sequences, and functional regions in the macromolecules. ConSurf calculates the amino/nucleic acid evolutionary rates and maps them to the sequence and/or structure of the query macromolecule[14]. ConSurf analysis can identify extremely crucial sites within the query macromolecule because gradually evolving sites on the query surface are typically important for function. In this study, this tool was used for the analysis of binding pockets in the human growth hormone receptor protein.

Binding Site Prediction

Locating, defining, and measuring concave surface regions on three-dimensional protein structures was made possible online by the Computed Atlas of Surface Topography of Proteins (CASTp) (available at: http://cast.engr.uic.edu). This online tool, CASTp, finds and measures pockets and voids on 3D protein structures. It was utilized for identifying and measuring the accessible surface pockets and inaccessible inner cavities of human growth hormone receptor protein. Both in solvent-accessible surface and molecular surface, it quantified the area and volume of each pocket and cavity.

Selection of Pharmacophore Model

The pharmacophore selection aimed to look for a similar match in the lead library, considering the 3D complementarity. Due to its therapeutic properties, Phenol is the main pharmacophore model chosen in this study. In an attempt to acquire data about medicinal chemistry to use in the development of pharmaceutical products, chEMBL (available at: https://www.ebi.ac.uk/chembl/), a sizable, free-access database for drug discovery was accessed[15]. The structure of phenol was retrieved from chEMBL with id 14060.

Pharmacophore Screening

Pharmacophore modeling offers some distinctive possibilities for finding new applications for known chemicals and discovering new molecules. Pharmacophore-based search retrieves molecules with the specified 3D arrangement of pharmacophore properties[16]. It is based on the combination of chemical functionalities and the subsequent alignment of the training compound set's shared characteristics. In order to quickly elucidate the therapeutic mechanism of natural products, MedPServer (available at: http://bif.uohyd.ac.in/medserver/) offers an open-access platform for computational analyses. Natural product-based leads were found using a technique based on structure-based pharmacophore screening employing phenol (chEMBL id: 14060) as the pharmacophore model. The libraries of small molecules obtained were then optimized and refined using the pharmacophore model's sensitivity and specificity check to find only active hits and discard the inactive ones from the initial screening.

Medicinal Phenol Selection

The thirty-four plant phenols determined by MedPServer through pharmacophore modeling were evaluated using Pyrx's single ligand docking. To select the most suitable interacting molecules for experimental analysis, it was employed to investigate tiny molecules' behavior in the targets' binding pocket. The molecular docking software with the highest preference is Autodock Vina in PyRx. The molecular docking method can be used to interpret how small molecules behave at the binding sites of target proteins, model the interaction between small molecules and proteins at the atomic level, and shed light on basic biochemical processes[17-19]. The molecule with the lowest energy was chosen for subsequent toxicity-removal optimizations.

Fragment Optimization

DeepFrag (available at: http://durrantlab.com/deepfragmodel) is a deep convolutional neural network that directs the optimization of ligands by expanding them with chemical fragments that are highly complementary to receptors[20]. This tool was utilized to predict how the ligand would be chemically modified to increase its binding affinity by fragment optimization with the addition of different chemical chains at a specific site of the ligand.

Pre-Clinical Testing

To estimate the individual absorption, distribution, metabolism, and excretion (ADME) behaviors of the compounds, the Swiss ADME tool (available at: www.swissadme.ch) of the Swiss Institute of Bioinformatics (available at: http://www.sib.swiss) was accessed on a web server. The results are presented for each input molecule in tables, graphs, and an Excel spreadsheet. The list is designed to contain one input molecule per line with multiple inputs, defined by the simplified molecular input line entry system (SMILES). Pharmacophore properties, along with drug likeliness and medicinal chemistry of the drug candidate, were attained and studied.

Implementation of Lipinski's Rule

"Lipinski Drug Filters" were used to predict ligand properties. The Lipinski's rule of 5 aids in differentiating between drug-like and non-drug-like features and forecasts a high likelihood of success or failure due to a molecule's drug-likeliness. By assisting in the early preclinical assessment, the Lipinski filter helps to avoid expen-

sive late-stage preclinical and clinical failures[21]. SCFBio (available at: http://www.scfbioiitd.res.in/utility/LipinskiFilters.jsp) was accessed to estimate hydrogen bond donors, molar refractivity, acceptors, and logP values of the drug candidate.

Toxicity Examination

Toxicologists, regulatory bodies, computational chemists, and medicinal chemists can use the free web server provided by ProTox-II (available at: http://tox.charite.de/protox_II) to estimate the toxicity *in silico*[22]. The web server ProTox-II received a two-dimensional chemical structure of the drug candidate as input and the results included 33 models of potential toxicity profiles together with their confidence scores, an overall toxicity radar chart, and the top three compounds that are most comparable and have known acute toxicity.

Docking Assessment

A molecular docking algorithm called PatchDock (available at: http://bioinfo3d.cs.tau.ac.il/PatchDock/) predicts the docked transformation, which results in a good complementarity of molecular shape[23]. It is a very effective approach for protein-small ligands and protein-protein docking. The receptor and ligand molecules on PatchDock were provided by uploading a file in PDB format. The 20 top-scoring solutions' geometric scores and interface area dimensions were displayed on the solutions page.

Bonding Interaction Analysis

The interaction of amino acids of human growth hormone receptors with the inhibitors at active sites was visualized with the help of BIOVIA Discovery Studio Visualizer (DSV) version 4.0. BIOVIA Discovery Studio can visualize, profile, and analyze various chemical library sources in order to design and optimize compound selection[24]. It also offers additional comprehensive and scalable tools for lead optimization, virtual screening, and hit and lead identification.

MD Simulation

IMODs (available at: http://imods.chaconlab.org/) is a platform for understanding and viewing three-dimensional biological image data. This server simulates the representation of macromolecules' intricate domain dynamics and identifies feasible conformational changes, elastic network possibilities, resolution with numerous coarse-grained atomic interpretations, and accuracy modelling[25]. This software enables various methods for visualizing image data. Models of the image data can produce quantitative data and can be rendered as a volume or contour surface.

Results

Identification of the Human Growth Hormone Receptor

The human growth hormone receptor protein with UniProt id P10912, was obtained from UniProt and further the 3D structure was retrieved from Alphafold. In all, the protein contains 371 amino acids. The 3D structure visualized by Discovery Studio is shown in Figure 1.

Binding Pocket Identification

The identification of the cavities and pockets present in the human growth hormone receptor protein structure is calculated by ConSurf. The colored scale shown in Figure 2A depicts the range of variability of protein conservancy accordingly. The variable regions are shown in blue colored whereas the phylogenetically conserved regions are shown in dark pink shade. Figure 2B below shows that the protein has more conserved regions colored in dark pink shade; hence it maintains its evolutionary conservancy by sustaining structural integrity. Thus, it is a suitable candidate for binding matching receptor molecules as a drug candidate to inhibit the human growth hormone.

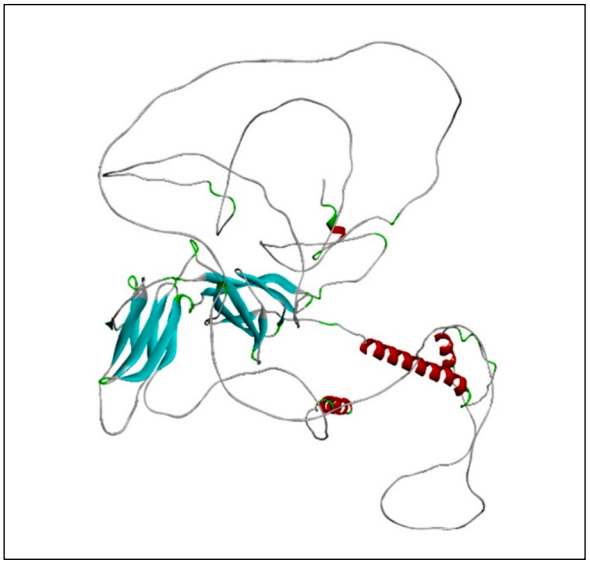

Figure 1. Human growth hormone receptor protein visualized by Discovery Studio.

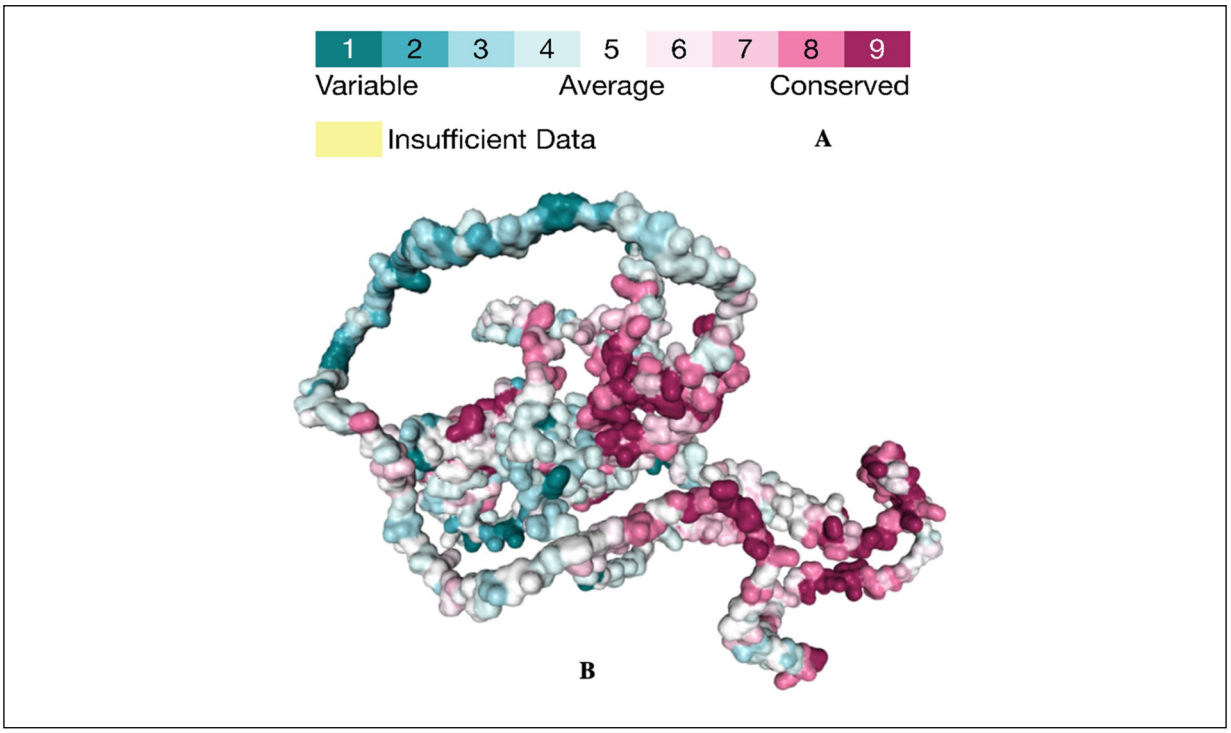

Figure 2. **A**, The color scale of protein variability and conservancy obtained from ConSurf. **B**, The surface view of human growth hormone receptor depicting variable regions in blue and conserved regions in pink and dark pink, respectively.

Binding Site Prediction

CASTp calculated the binding sites of the human growth hormone receptor by measuring concave surface regions on 3D protein structure, showing that an area of 23,332.322 Å^2 and volume of 244,206.320 (SA) Å^3 are potential binding sites of the protein as shown in Figure 3A, below in purple color. The figures show front and sideways views of the probable binding sites of the protein.

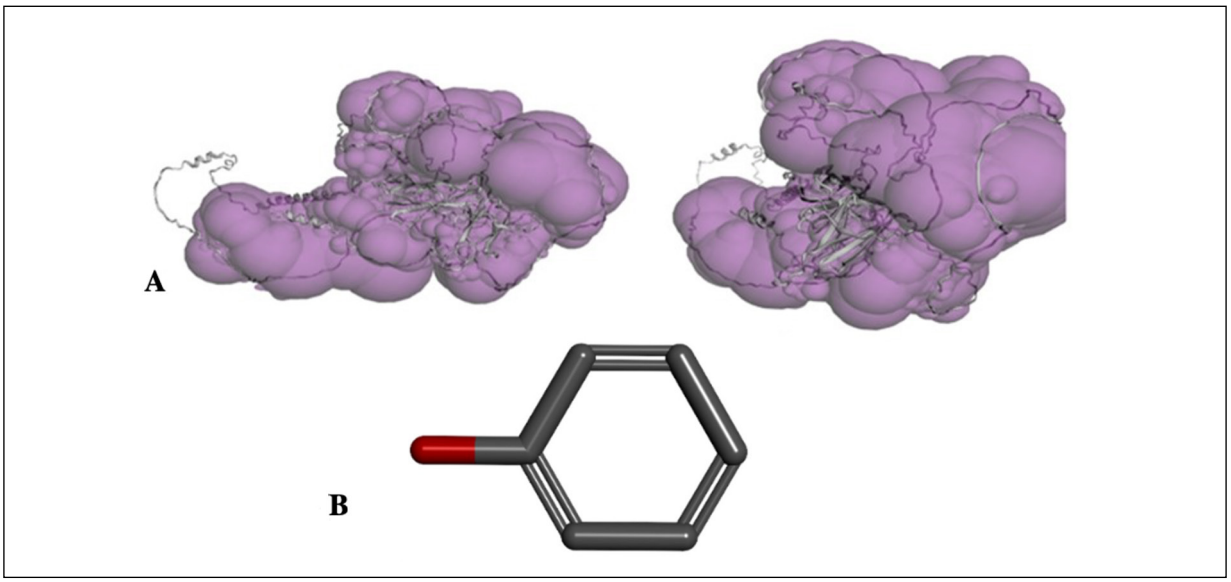

Figure 3. **A**, The front and side-ways view of human growth hormone receptor protein binding pockets shown in purple predicted by CASTp. **B**, The 3D structure of phenol chEMBL id: 14060 visualized by Discovery Studio.

Nominated Pharmacophore Model Retrieval

The phenol with chEMBL id 14060, was retrieved from chEMBL and further the 3D structure was downloaded. The 3D structure visualized by Discovery Studio is shown in Figure 3B.

Pharmacophore Screening of Drug Candidates

Medicinal plants were screened using phenol as the pharmacophore model at MedPServer to identify target medicinal plant phenols from a total of one thousand, one hundred and eighteen compounds. The screening identified thirty-four pharmacophore matches of medicinal plant phenols. The screened compounds' lead library is shown in **Supplementary Table I**.

These were selected as suitable ligands and were docked with Pyrx against human growth hormone receptor to calculate their binding affinity with the purpose of being employed as a competitive inhibitor. The lowest docking energy was calculated as -6.5 K/mol for Bauhiniastatin-1 and the highest docking energy was -4.3 K/mol for 5-Hydroxymethyl-2 furancarboxaldehyde respectively, as displayed in **Supplementary Table II**.

Chemical Characterization of Bauhiniastatin-1

The following Table I retrieved from PubChem shows the chemical properties of Bauhiniastatin-1 for the assessment of its potential to be a drug candidate.

Fragment Optimization of Bauhiniastatin-1

The 0:C8 fragment, which is the cysteine residue of Bauhiniastatin-1, was chosen as the optimization point in DeepFrag online server. The results showed that the addition of hydroxide ion at the selected position scored 1 proving the position to be the right fit for optimization, as displayed in **Supplementary Figure 1**. **Supplementary Figure 2** shows the optimization results and the Bauhinisatin-1 structures before and after optimization.

Table I. The chemical properties of Bauhisnistatin-1 accessed from PubChem.

Property name	Property value
Molecular Weight	284.26
XLogP3-AA	2.5
Hydrogen Bond Donor Count	1
Hydrogen Bond Acceptor Count	5
Rotatable Bond Count	1
Exact Mass	284.06847348
Monoisotopic Mass	284.06847348
Topological Polar Surface Area	72.8 Å²
Heavy Atom Count	21
Formal Charge	0
Complexity	596

Table II. ADMET profiling, along with concerning physiochemical properties of fragment-optimized Bauhinistatin-1 (FOB) is shown in the table below.

ADMET parameters	Parametric values
Formula	C16H14O6
Molecular weight	302.28 g/mol
Num. heavy atoms	22
Num. arom. heavy atoms	0
Fraction Csp3	0.25
Num. rotatable bonds	1
Num. H-bond acceptors	6
Num. H-bond donors	2
Molar Refractivity	76.68
TPSA (Topological Polar Surface Area)	93.06 Å²
Water Solubility Log S (ESOL)	-2.61
GI absorption	High
BBB permeant	No
Skin Permeation (Log Kp)	-7.29 cm/s
Bioavailability Score	0.56
Synthetic Accessibility	4.50
Drug likeness (Ghose)	Yes i.e., 0 violations

Pre-Clinal Testing

Drug development includes the calculation of absorption, distribution, metabolism, and excretion (ADME), which is executed gradually in the early discovery phase. The fragment-optimized Bauhiniastatin-1 (FOB) pharmacokinetics, drug-ability, and curative approachability were assessed using Swiss ADMET. The ligand (FOB) was studied and selected as the utmost suitable drug candidate based on its fulfillment of the drug-like properties (Table II). The International Standard drug likeness rules were observed since no violations can be found in this drug candidate.

Evaluation of Lipinski's Rule

Lipinski's rule's characteristics, such as logP, mass, hydrogen bond donors, hydrogen bond acceptors, and molar refractivity, were predicted using SCFBio. The results demonstrated that the ligand (FOB) justifies the Lipinski's rule of 5, as shown in Table III.

Table III. Lipinski's rule of 5 for selected fragment-optimized ligand, FOB.

Lipinski rule of 5	logP	Molecular weight	Hydrogen bond donor	Hydrogen bond acceptor	Molar refractivity
Ligand FOB	1.572	302.28 g/mol	1	6	76.698

In-Depth Toxicity Analysis

The calculation of toxic properties of the ligand FOB is examined and results depict that no toxicity is found. A high probability of nontoxicity of the ligand as an antagonist of all organs and toxic signaling pathways in humans is witnessed. **Supplementary Table III** below shows that the prediction of hepatotoxicity, mutagenicity, and cytotoxicity are inactive with a high probability for FOB. Figure 4 shows the radar chart of diverse toxicity models of the already available active compounds. The regular probability of their toxicity in comparison to that of FOB is shown in the chart, where FOB bears no toxicity. Figure 5 demonstrates the graph of the daily dose intake recommendation 2,319.9 mg/Kg of FOB as a drug

Docking Evaluation

The docking was performed with a patch dock for fragment-optimized ligand, FOB, and human growth hormone receptor protein scored 4,070 K/mol with an area of 480.50 and ACE (Angiotensin-I-converting enzyme) value of -87.31,

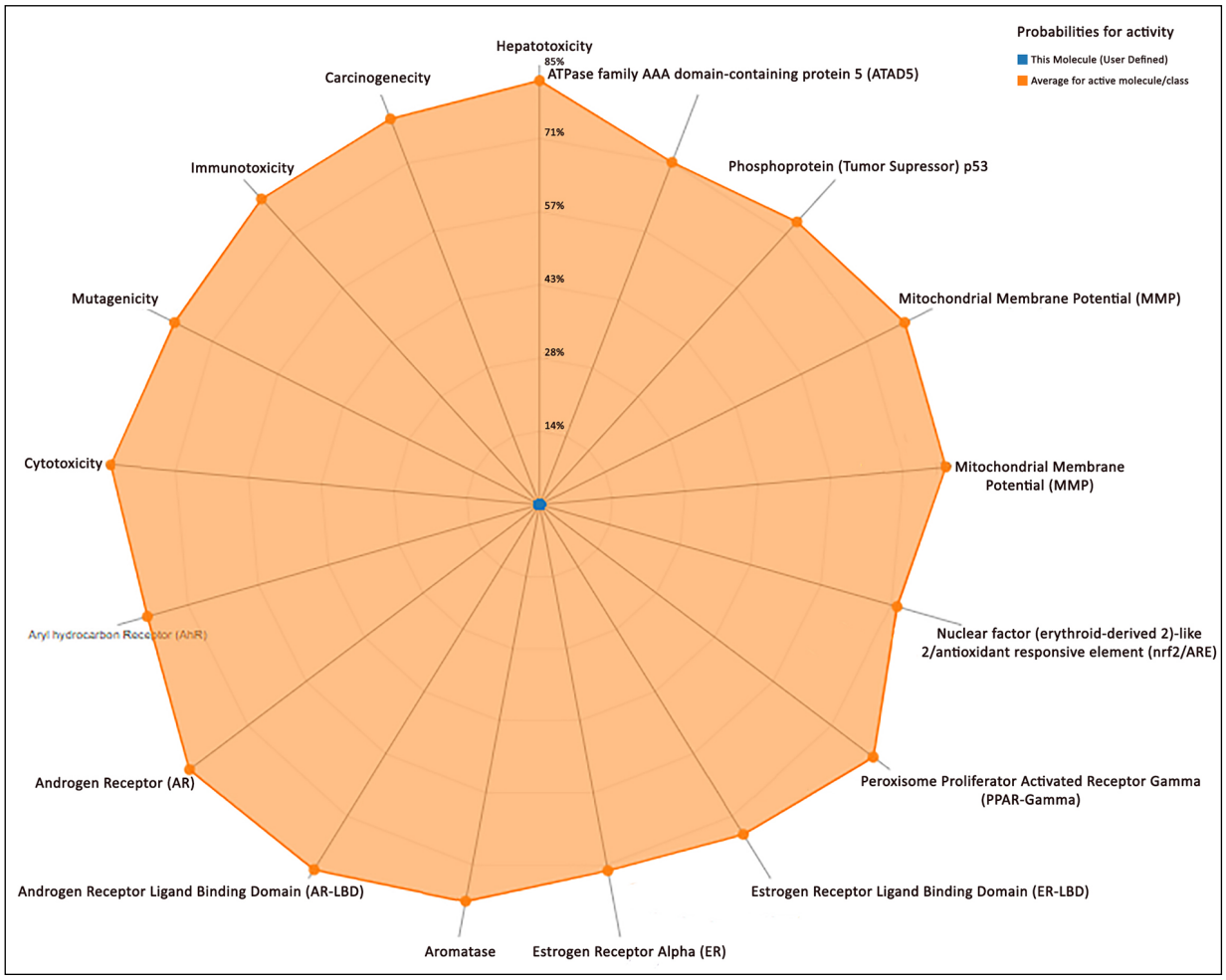

Figure 4. The radar chart of fragment-optimized Bauhiniastatin-1 (FOB) displays no toxicity against any pathway when compared to other bioactive molecules in Pro Tox II.

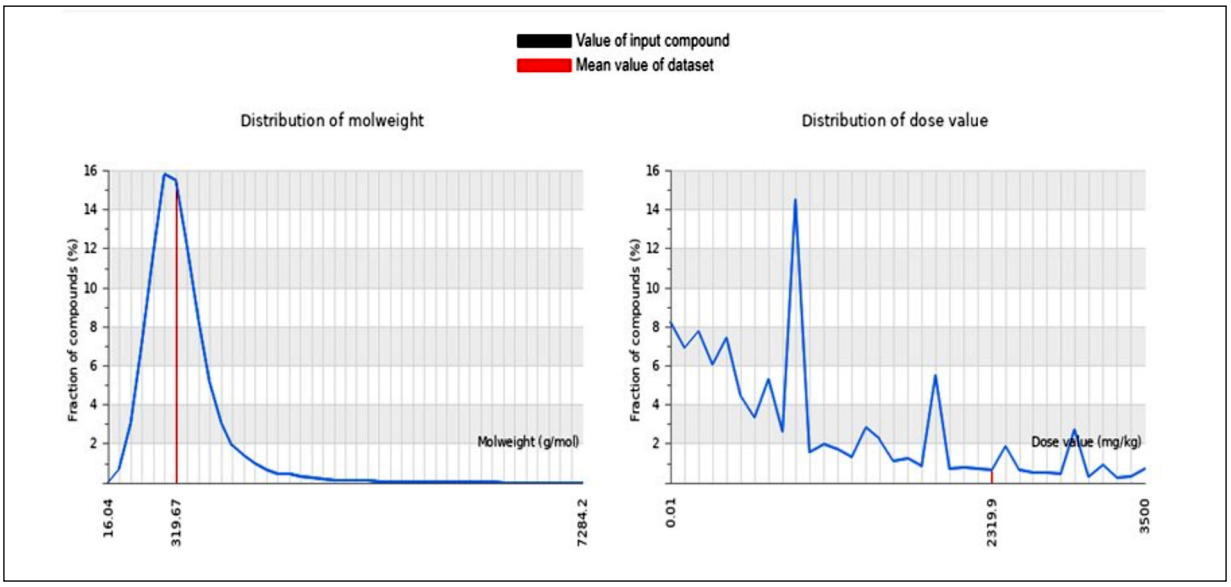

Figure 5. The daily recommended intake dose of FOB is shown as 2,319.9 mg/Kg in the graph analysis from Pro Tox II.

respectively. The docked complex bonding, hydrogen bonding, and hydrophobic interactions are visualized with Discovery Studio, as shown in Figure 6A-C.

MD Simulations

Multiple parameters were taken into account by iMODS to calculate and characterize the molecular simulation of the docked complex of human

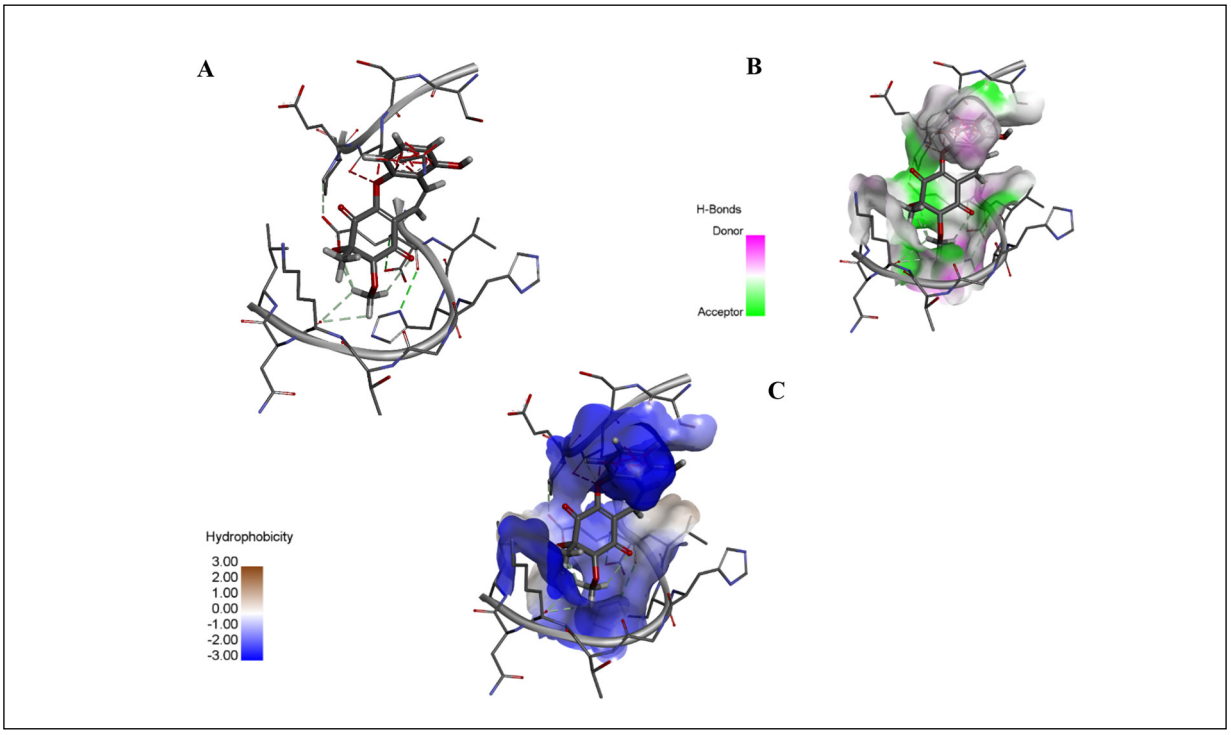

Figure 6. **A**, The docking complex of human growth hormone receptor and FOB retrieved from PatchDock. The interactions are visualized with Discovery Studio. **B**, The hydrogen bond donors and acceptors of the docked complex of human growth hormone receptor protein and FOB visualized with Discovery Studio. **C**, The hydrophobicity of the docked complex of human growth hormone receptor protein and FOB visualized with Discovery Studio.

growth hormone receptor and FOB. The results were retrieved. The heat map depicts that there are high co-related areas, as observed. A low RMSD (Root Mean Square Deviation) value indicates improved interactions among the different residues of the structure. The resultant Eigon value for the docked complex was 1.045431e-08. **Supplementary Figure 3** depicts the results calculated below.

Discussion

Acromegaly is a chronic condition linked to higher mortality rates and morbidity. With appropriate therapeutic interventions, the majority of these ailments can be prevented or minimized. There are certain individuals who continue to have disease activity, even though there are three treatment modalities (surgery, medicinal treatment, and radiotherapy) that are available[26]. As a primary therapy or adjuvant therapy for acromegaly, medical care is crucial since few individuals have clinical contraindications to surgery or refuse to undergo it. Novel treatments for acromegaly targeting growth hormone inhibition are a dire need to overcome its perilous effects.

Three drugs are currently available as somatostatin receptor ligands which are the furthermost persistently pre-scribed therapy for acromegaly. In a study conducted by Gadelha et al[27] these are divided into two classes which include first-generation SRLs (fg-SRLs), which are regarded as first-line therapy, represented by octreotide long-acting re-lease (OCT-LAR) and lanreotide auto gel; and second-generation SRL, represented by pasireotide (PAS). Cabergoline, a type of dopamine agonist, is used as a supplementary therapy in situations with moderately high IGF-1 levels [2.5 times the upper limit of normal (ULN)] in acromegaly[28]. In clinical trials[29], there is currently only one selective growth hormone receptor antagonist, pegvisomant (PEGV), which is typically used as second-line therapy, which attained biochemical control rates of 90% or higher and a complete control rate of nearly 70%. Hence, this research aimed to develop a novel growth hormone receptor inhibitor drug as a potential long-term treatment for the targeted suppression of human growth hormone utilizing optimized phytochemicals. Therefore, to overcome the gap of specified growth receptor, this study was undertaken.

The use of natural chemicals obtained from medicinal plants substantially aids in the development of novel, successful curative treatments. Therapeutically defined plant-derived substances like phenolic compounds can increase pharmacological efficacy while also eradicating harmful side effects. In an attempt to discover relevant compounds in this study, the homeopathic plant phenols were subjected to pharmacophore screening with MedPServer employing phenol as a pharmacophore model. With the help of Pyrx, the resultant thirty-four medicinal plant phenols were docked with the human growth hormone receptor, which determined Bauhiniastatin-1 with the lowest energy score of -6.4 K/mol as the optimal lead compound with the requisite biocompatibility.

The fragment-optimization strategy, according to Wasko et al[32] and some other studies[30-33], aims to consider weakly binding molecules that should be altered to become high-affinity lead compounds. Therefore, it was implemented in this study. This approach helps boost the most viable compounds' potency, minimize their toxic effects, or maximize their absorption[21]. In an effort to elevate the innovative drug candidate, Bauhiniastatin-1 efficiency, the DeepFrag tool was selected as the optimization method. The chosen fragment of 0:C8 was optimized with the addition of a hydroxide chain resulting in the highest score of 1 post-optimization. The fragment-optimized Bauhiniastatin-1 (FOB) was carefully examined for its ADMET characteristics and for compliance with Lipinski's rule of 5. The outcomes demonstrated that FOB achieved all criteria demonstrating that it is the most favorable candidate for human growth hormone receptor inhibitor. Furthermore, the compound's value of 4,070 K/mol energy anticipated by Patchdock supports the findings[34-39].

A more potent and nontoxic inhibitory *de novo* homeopathic drug has been developed and is thus indicated in this research as a mean of combating the excess expression of human growth hormone in acromegaly. The research findings discussed in this article provide adequate computational pharmacological evidence to allow the creation of a novel formulation of FOB medication since emerging natural phenol molecules will open up new possibilities for the cure of acromegaly. The gap in past investigations for direct human growth hormone inhibition can be filled by this proposed drug with the collaboration of supporting data provided in this study. It is advised that this medication should be tested in *in-vitro* experiments to demonstrate the viability of the proposed design.

Conclusions

Acromegaly is a chronic, systemic condition with numerous consequences that, if not properly managed, is linked to a higher mortality rate.. Excessive human growth hormone expression is suspected to promote the growth of tissues and cells in the body. This has been circumvented by the discovery of the homeopathic phenol known as "Bauhiniastatin-1", which is also fragment-optimized to a significant therapeutic candidate, FOB. In this study, a *de novo* drug has been developed that has the potential to be an effective inhibitor alongside its pharmacophore properties. Clinicians need to be careful when designing treatment plans to account for the complete clinical disease spectrum, taking into account tumor characteristics, glucose metabolism, and biochemical control rates. In order to overcome the excess production of human growth hormone, and thus to ensure better outcomes by its inhibition, it is imperative to do an *in vitro* testing of the proposed drug for acromegaly treatment.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Authors' Contribution

Conceptualization, M.N, N.A, K.J, H.I, S.K and T.A.; methodology, T.A., M.G, and A.S; software, M.A.; validation, M.G and A.S.; formal analysis, M.N, N.A, K.J, H.I, S.K and T.A; investigation, M.N, N.A, K.J, H.I and S.K. resources, M.A, and T.L.N.; data curation, A.H.; writing and original draft preparation, T.A and M.A; writing, review and editing, A.H and M.W.A; visualization, M.W.A and M.N.; supervision, M.N and T.A.; project administration, A.S.A.; funding acquisition, T.A.

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Ethics Approval

Not applicable.

Informed Consent

Not applicable.

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