Journal of Integrated
SCIENCE & TECHNOLOGY

Computational identification of novel Leukotriene A4 Hydrolase (LTA4H) inhibitors as therapeutic candidates for colorectal cancer

Raje Siddiraju Upendra*, P. Hariprasad, Karun Chakki, Preetham R.S., Varun P. Kulkarni, Sanjay Shrinivas Nagar, R. Karthik

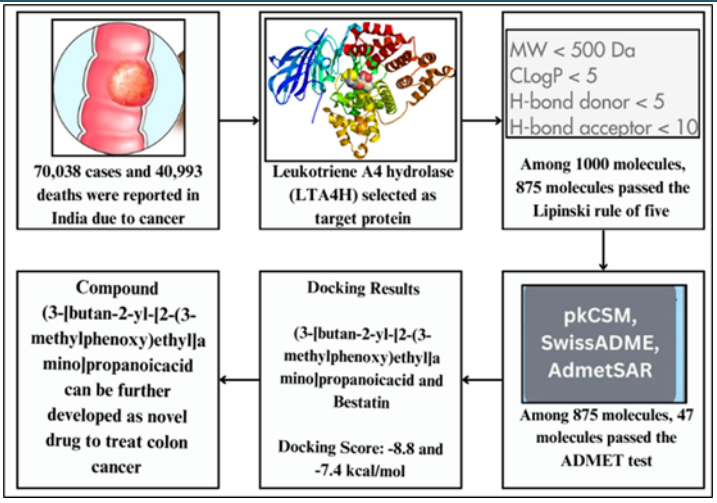
School of Electronics and Communication Engineering, REVA University, Bengaluru, Karnataka. India.

Submitted on: 12-Nov-2024, Accepted and Published on: 17-Mar-2025

Article

ABSTRACT

In recent years cancer cases have been increasing and according to the World Cancer Research Fund International, approximately 70,038 cancer cases were reported in India. There is a crucial need for the development of potential medications for the treatment of cancer patients. Among other target proteins, Leukotriene A4 hydrolase (LTA4H) is the target protein that plays a crucial role in the synthesis of leukotrienes. Leukotrienes belong to the inflammatory lipid mediators associated with colorectal cancer. The present study aims to explore potential inhibitors against the target protein LTA4H. Among 1000 molecules (PubChem), 875 molecules passed the Lipinski rule of five and were further evaluated for ADMET analysis. ADMET analysis indicated that 47 molecules out of 875 molecules were safe for human consumption and further docked with LTA4H through the PyRx software. Among the 47 ligand molecules assessed, the ligand molecule (3-[butan-2-yl-[2-(3-methyl)ethyl]propanoic acid showed a better docking score of -8.8 kcal/mol and formed 07 bonds with amino acids of target protein LTA4H. Meanwhile, the standard drug Bestatin displayed a docking score of -7.4 kcal/mol and formed 05 bonds with amino acids of target protein LTA4H. Hence the compound (3-[butan-2-yl-[2-(3-methylphenoxy)ethyl]amino]propanoicacid can be developed as the anticancer drug for colorectal cancer.



Keywords: Colorectal_cancer, Leukotriene_A4_hydrolase, ADMET, Molecular_docking, Bestatin.

INTRODUCTION

The human body is a complex system composed of trillions of cells that work together to maintain the health and well-being of the individual. These cells undergo a systematically regulated process of growth and division to ensure proper functioning and renewal of tissues. This intricate process is essential for maintaining homeostasis and supporting vital bodily functions. However, sometimes this carefully orchestrated process can go awry.[1] Uncontrolled cell proliferation, characterized by abnormal growth and division, can lead to cancer development. A significant feature of cancer is the uncontrolled growth and spread of abnormal cells that can invade and destroy healthy cells[2]. There are numerous types of cancer, each affecting different organs and tissues in the human body[3]. One of the prevalent and concerning types of cancer studied is colorectal cancer. Colorectal cancer originates in the colon or rectum, located in the large intestine and it is responsible for toxic and waste elimination from the human system. Colorectal cancer remains a significant public health concern globally. According to the World Health Organization (WHO), colon cancer is the second leading cause of cancer-related deaths worldwide. WCRF statistics indicated that worldwide 1,926,425 cases and 904,019 deaths were reported due to colorectal cancer in the year 2022.[4] In the present study, the target protein selected for finding novel anticancer drug molecules was LTA4H (3U9W)[5]. LTA4H plays a crucial role in the biosynthesis of leukotrienes, which are inflammatory lipid mediators associated with various diseases, including colorectal cancer. Inhibition of target protein LTA4H disrupts the production of leukotrienes and potentially hinders the

*Corresponding Author: Dr. Raje Siddiraju Upendra
Tel: +91-96111 39872; Email: upendra.rs@reva.edu.in

Cite as: *J. Integr. Sci. Technol., 2025, 13(5), 1115.*
URN:NBN:sciencein.jist.2025.v13.1115
DOI:10.62110/sciencein.jist.2025.v13.1115

cancer cell growth and progression. Current treatment options, including surgery, chemotherapy, vaccines,[6] and radiation therapy,[7] are accompanied by severe side effects and limited efficacy. Therefore, there is a pressing need for the development of novel effective therapeutic strategies. Figure 1 represents projected mortality rates (in thousands) for males and females from 2022 to 2050 for colorectal cancer.

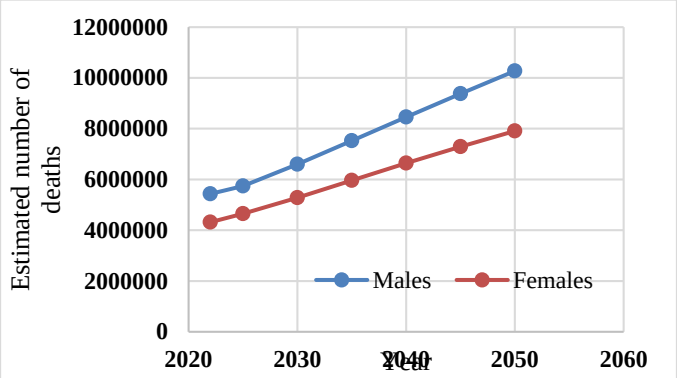


Figure 1. Cancer-related death rate predicted by WHO on a global scale[3].

The graph displays an upward trend in the mortality rates for both genders across the years, with consistently higher rates observed in males in comparison with females. These projections underscore the need to monitor gender-specific health trends and tailor public health strategies to address symptoms and improve the condition of the patients. The target protein LTA4H is selected due to its role in inflammation and immune response, which are key factors in the development and progression of cancer disease. LTA4H is involved in the leukotriene pathway, which can influence tumor growth, angiogenesis, and metastasis[8,9].

Table 1. Mechanism of target protein LTA4H in colorectal cancer[4]

Protein Name	Protein Function	Inhibition	Novelty
LTA4H (3U9W)	Bifunctional Zinc Enzyme: Epoxide Hydrolase and Aminopeptidase	Targeting LTA4H for prevention and control in colorectal cancer; Potential therapeutic target with unique dual enzymatic activities. Structural insights offer a foundation for drug design.	Discovery of LTA4H's role in LTB4 production in colon cancer cells; Structural insights into the binding site pave the way for drug development targeting LTA4H.

With the inhibition of the LTA4H, it is possible to reduce chronic inflammation, and potentially slow cancer progression. Targeting LTA4H offers a novel therapeutic approach against colorectal cancer and improves patient outcomes. Figure 2 Represents how LTA4H, a key enzyme in leukotriene metabolism, plays a vital role in colon cancer progression[10]. Initially, it catalyzes the conversion of LTA4 to LTB4, triggering inflammation within the colon cell. This inflammatory milieu, fueled by LTB4 and other mediators, fosters DNA damage, cell proliferation, and tumor initiation and progression. LTA4H also exerts profound effects on immune regulation by modulating the activity of various immune cells, leading to the suppression of anti-tumor immunity and the promotion of pro-tumor inflammation, further fueling tumor growth. Additionally, LTA4H-mediated production of LTB4 stimulates angiogenesis and facilitates the formation of new blood vessels that support tumor growth and metastasis (Table 1)[11].

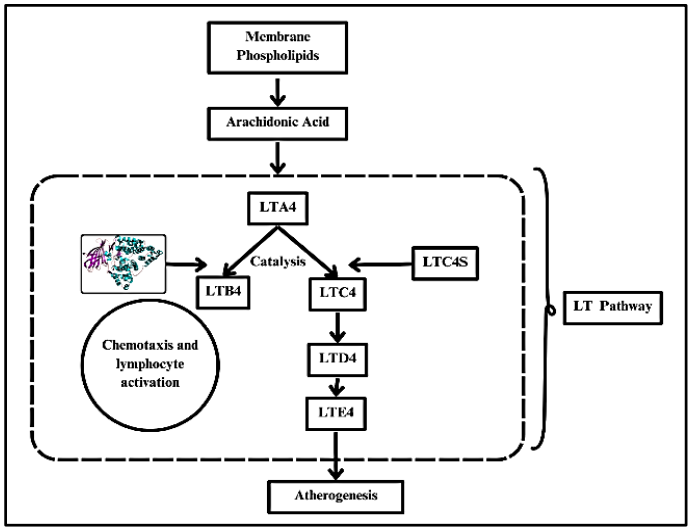


Figure 2. Function of LTA4H enzyme in colon cancer development

Due to the complex structure of the target protein, it is important to analyze the structure properly to discover the drugs from natural sources such as *Epiphyllum oxypetalum*[12], *fungal species*[13], *Toddalia asiatica*[14], *Monascus ruber*[15] could be explored as anticancer drugs for colorectal cancer. The study conducted by Saputra et al. 2021, employed *insilco* molecular docking to investigate the potential of quercetin as the potential drug against colorectal cancer with LTA4H as the target protein. Their findings suggested that quercetin exhibited promise in inhibiting LTA4H, indicating its potential as a therapeutic agent against colorectal cancer[5]. In 2021, Gupta and Jain focused on bio-isosteric modification of the compound 4-cyano-3-(trifluoromethyl)phenyl in enzalutamide using MolOpt. They conducted screening for pharmacokinetic and toxicological properties with ADMET Lab 2.0 and performed molecular docking with Auto Dock Vina 1.5.6. Authors developed novel analogs of enzalutamide with potential improvements in pharmacokinetics and reduced toxicity. Molecular docking further revealed promising binding affinities of the analogs with the androgen receptor, providing insights into potential advancements in prostate cancer therapy[16]. Hence there is a need to discover potential novel compounds to fight against colorectal cancer.

MATERIALS AND METHODOLOGY

Optimization of Query Molecule Structures

The optimization of query molecule structures commenced with the selection of Bestatin as the query molecule from a published

research paper[17]. The publicly available PubChem database was used for the similarity search to find compounds similar to Bestatin. All the molecules obtained from search results were screened according to Lipinski's rule of five (i.e.: Molecular weight > 500, Hydrogen bond donors > 5, Hydrogen bond acceptor > 10, Log p value > 5). All the optimized query molecules were finalized and saved as the CSV file for drug-likeness and ADMET test[18].

Drug likeness and ADMET analysis of chemical compounds

SwissADME and ADMET LAB 2.0 web servers were used for performing drug likeliness and ADMET analysis.[19,20] The isosmiles of the molecules clearing Lipinski's rule of five were provided as the input to both SwissADME and ADMET LAB 2.0[21] for drug-likeness and ADMET tests. At the end of the analysis, the results provided by SwissADME[22] and ADMET LAB 2.0 were saved as a CSV file for further analysis. Compounds were screened for properties such as Hydrogen bond acceptor (less than 10), Hydrogen bond donor (less than 5), RO5 violation (0 violation), Human intestinal absorption (HIA) (High), Caco-2 permeability (Permeable), Blood-Brain Barrier (BBB) (Non-permeable), permeability (0 to 0.3) and Protein Plasma Binding (PPB), CYP inhibition (Non-inhibitor), CYP-substrate (Non-substrate), clearance (CL), half-life (T12), P-glycoprotein inhibition (Pgp-inh) and substrate status (Pgp-sub), helps in evaluating the potential side effects and safety of the drugs inside the human body. Compounds displaying both drug likeliness and ADMET within the accepted range are further selected for molecular docking against the target protein LTA4H[23].

Preparation of target protein LTA4H structure and ligand structures for molecular docking

LTA4H protein structure (3U9W)[24] is prepared using Chimera X software[25]. The 3D structure of LTA4H is imported and all the nonstandard residues are selected using the “select” option from the dropdown menu. Further nonstandard residues such as water molecules and negative ligands were removed from the protein structure using the “Atoms” option of the action’s menu. After the removal of unwanted residues “Dock Prep” option was availed to add both hydrogens and charges to the protein structure and the final file was saved in PDBQT format for the molecular docking process. The SDF structures of ligand molecules were retrieved from PubChem and converted into PDBQT format through the OpenBabel package. The final protein and ligand structure were processed as input for the molecular docking process[26].

Molecular Docking between selected Ligand Compounds and Protein LTA4H

The open-source AutoDock Vina-based PyRx software[27] was used for the molecular docking process. The prepared protein molecule was loaded into the PyRx software and the "AutoDock" method was chosen from the menu of PyRx software. After loading the protein structure, the protein was prepared using the "Make Macromolecule" option. After choosing "Insert New Item" all the selected ligands were loaded into PyRx software to initiate the docking process. After uploading, all the selected ligand molecules were converted from SDF to PDBQT format. After loading both ligand and protein molecules, search space was set to define the grid box dimensions (x = 55.35 Å, y = 92.42 Å, z = 71.99 Å) with a centered grid (x = 35.08 Å, y = 4.94 Å, z = 2.97 Å) in PyRx software. Finally, docking was performed for all the ligand molecules against protein LT4AH using the “Forward button” present in the docking menu[28].

Analysis of protein-ligand interaction of docked complex structures

At the end of the docking process, the results were analyzed and the molecule that showed the best docking score in comparison with the standard drug Bestatin was provided as input to Discovery Studio Visualizer[28] and PyMOL software[29] for the protein-ligand interaction analysis. The protein-ligand complex files are generated through Discovery Studio Visualizer and PyMOL to analyze the amino acids interacting with the ligand molecule in both 2D and 3D formats.

RESULTS AND DISCUSSION

Optimization of Query Molecule Structures

At the end of PubChem screening, 1000 molecules similar to query molecule Bestatin were obtained, and these molecules were further filtered through Lipinski's rule of five. Among 1000 molecules evaluated, 875 molecules cleared Lipinski's rule of five, and data related to these molecules was stored in both CSV and SDF format. The SMILE format of these molecules was further provided as input for drug likeliness and ADMET analysis.

Drug likeness and ADMET analysis of chemical compounds

Table 2 obtained as output from SwissADME provides key parameters to assess the potential of a compound as a drug. Molecular weight (MW) influences the compound's solubility and absorption in the body. Hydrogen bond acceptors (HBA) and hydrogen bond donors (HBD) measure the ability of the compound to form hydrogen bonds, affecting its interactions with biological targets. Total Polar Surface Area (TPSA) is an indicator of a molecule's polarity and its ability to permeate cell membranes. The number of rotatable bonds shows the molecule's flexibility, which can influence its ability to bind effectively to targets. Rule of Five (RO5) violations assess how well a compound adheres to established drug-likeness criteria. Lead likeness violations evaluate how closely the compound aligns with characteristics common in lead-like molecules, useful as starting points in drug development. Table 3 shows the ADMET analysis which evaluates a compound's absorption, distribution, and metabolism, indicating how well a drug is absorbed. The distribution excretion, and toxicity. Absorption involves Human Intestinal Absorption (HIA) and Caco-2 permeability, including blood-brain barrier (BBB) permeability and protein plasma binding (PPB), affecting drug delivery to tissues. Metabolism assesses enzyme interactions such as CYP inhibition and substrate status, impacting how the body processes the drug. Excretion measures clearance (CL) and half-life (T12), determining how quickly a drug is eliminated. Toxicity predictions, such as P-glycoprotein inhibition and substrate help to evaluate the potential side effects and safety of drugs on the human body. Among 875 molecules, 47 ligand molecules cleared both drug-likeness and ADMET tests and these molecules were selected as the ligand molecules for the molecular docking process.

Table 2. Drug likeness prediction results for selected ligands

Sl No	Compound Name	MW	HBA	HBD	TPSA	Rotatable bonds	RO5 violation	Lead likeness Violations
1	3-[3-[3-(Hydroxymethyl)phenoxy]propyl-methylamino]-4,4-dimethylpentanoic acid	323.43	5	2	70	10	0	1
2	3-[3-(3-Methylphenoxy)propylamino]propanoic acid	237.29	4	2	58.56	8	0	2
3	2-methyl-3-[2-(3-methylphenoxy)ethylamino]propanoic acid	237.29	4	2	58.56	7	0	1
4	3-[3-(3,5-dimethylphenoxy)propanoyl-ethylamino]propanoic acid	293.36	4	1	66.84	9	0	1
5	3-[ethyl-[4-(3-methylphenoxy)butanoyl]amino]propanoic acid	293.36	4	1	66.84	10	0	1
6	3-[ethyl-[3-(3-methylphenoxy)propyl]amino]butanoic acid	279.37	4	1	49.77	9	0	1
7	3-[2-(3,5-dimethylphenoxy)ethyl-ethylamino]butanoic acid	279.37	4	1	49.77	8	0	1
8	3-[ethyl-[2-(3-methylphenoxy)ethyl]amino]butanoic acid	265.35	4	1	49.77	8	0	1
9	3-[(3-ethoxyphenyl)methyl-propan-2-ylamino]propanoic acid	265.35	4	1	49.77	8	0	1
10	3-[propan-2-yl-[(3-propan-2-yloxyphenyl)methyl]amino]propanoic acid	279.37	4	1	49.77	8	0	1
11	3-[propan-2-yl-[(3-propoxyphenyl)methyl]amino]propanoic acid	279.37	4	1	49.77	9	0	1
12	3-[(3-ethoxyphenyl)methyl-ethylamino]butanoic acid	265.35	4	1	49.77	8	0	1
13	3-[ethyl-[(3-propoxyphenyl)methyl]amino]butanoic acid	279.37	4	1	49.77	9	0	1
14	3-[ethyl-[(3-propan-2-yloxyphenyl)methyl]amino]butanoic acid	279.37	4	1	49.77	8	0	1
15	3-[ethyl-[(3-propoxyphenyl)methyl]amino]propanoic acid	265.35	4	1	49.77	9	0	1
16	3-[(3-ethoxyphenyl)methyl-ethylamino]propanoic acid	251.32	4	1	49.77	8	0	1
17	3-[ethyl-[(3-propan-2-yloxyphenyl)methyl]amino]propanoic acid	265.35	4	1	49.77	8	0	1
18	3-[methyl-[(3-phenoxyphenyl)methyl]amino]propanoic acid	285.34	4	1	49.77	7	0	0
19	2-[3-(3,5-dimethylphenoxy)propyl-prop-2-enylamino]acetic acid	277.36	4	1	49.77	9	0	1
20	2-[2-(3-methylphenoxy)ethyl-prop-2-enylamino]acetic acid	249.31	4	1	49.77	8	0	2
21	2-[3-(3-methylphenoxy)propyl-prop-2-enylamino]acetic acid	263.33	4	1	49.77	9	0	1
22	2,2-dimethyl-3-[2-(3-methylphenoxy)ethylamino]propanoic acid	251.32	4	2	58.56	7	0	0
23	3-[4-(3-methylphenoxy)butanoyl-propan-2-ylamino]propanoic acid	307.38	4	1	66.84	10	0	1
24	3-[3-[3-(2-methylpropoxy)phenyl]propylamino]propanoic acid	279.37	4	2	58.56	10	0	1
25	3-[[(E)-3-[4-(4-methylpentoxy)phenyl]prop-2-enyl]amino]propanoic acid	305.41	4	2	58.56	11	0	1
26	3-[3-(3,5-dimethylphenoxy)propylamino]propanoic acid	251.32	4	2	58.56	8	0	1
27	3-[2-(3-methylphenoxy)ethylamino]propanoic acid	223.27	4	2	58.56	7	0	1
28	3-[2-(4-methylphenoxy)ethyl-propan-2-ylamino]propanoic acid	265.35	4	1	49.77	8	0	1
29	3-[2-(3-methylphenoxy)ethyl-propan-2-ylamino]propanoic acid	265.35	4	1	49.77	8	0	1
30	4-[2-(3-methylphenoxy)ethyl-propan-2-ylamino]butanoic acid	279.37	4	1	49.77	9	0	1

31	2-[butan-2-yl-[3-(3-methylphenoxy)propyl]amino]acetic acid	279.37	4	1	49.77	9	0	1
32	2-[3-(3-methylphenoxy)propyl-propan-2-ylamino]acetic acid	265.35	4	1	49.77	8	0	1
33	3-[3-(3-methylphenoxy)propyl-propan-2-ylamino]propanoic acid	279.37	4	1	49.77	9	0	1
34	3-[cyclopropyl-[3-(3-methylphenoxy)propanoyl]amino]propanoic acid	291.34	4	1	66.84	9	0	1
35	3-[butan-2-yl-[2-(3-methylphenoxy)ethyl]amino]propanoic acid	279.37	4	1	49.77	9	0	1
36	3-[carboxymethyl-[2-(3-methylphenoxy)ethyl]amino]propanoic acid	281.3	6	2	87.07	9	0	1
37	3-[2-(3-methylphenoxy)ethyl-prop-2-enylamino]propanoic acid	263.33	4	1	49.77	9	0	1
38	3-[butan-2-yl-[2-(4-methylphenoxy)ethyl]amino]propanoic acid	279.37	4	1	49.77	9	0	1
39	3-[2-(4-methylphenoxy)ethyl-prop-2-enylamino]propanoic acid	263.33	4	1	49.77	9	0	1
40	3-[3-(4-methylphenoxy)propyl-propan-2-ylamino]propanoic acid	279.37	4	1	49.77	9	0	1
41	3-[3-(4-methylphenoxy)propyl-prop-2-enylamino]propanoic acid	277.36	4	1	49.77	10	0	1
42	3-[3-(3-methylphenoxy)propyl-prop-2-enylamino]propanoic acid	277.36	4	1	49.77	10	0	1
43	3-[methyl-[1-(3-propoxyphenyl)butan-2-yl]amino]propanoic acid	293.4	4	1	49.77	10	0	1
44	3-[1-(3-ethoxyphenyl)propan-2-yl-methylamino]propanoic acid	265.35	4	1	49.77	8	0	1
45	3-[methyl-[1-(3-propoxyphenyl)propan-2-yl]amino]propanoic acid	279.37	4	1	49.77	9	0	1
46	3-[methyl-[1-(3-propan-2-yloxyphenyl)propan-2-yl]amino]propanoic acid	279.37	4	1	49.77	8	0	1
47	(E)-3-[3-[2-[ethyl(3-hydroxypropyl)amino]ethoxy]phenyl]prop-2-enoic acid	293.36	5	2	70	10	0	1

Table 3. ADMET Screening for selected Ligands

Compound CID	**Absorption**			**Distribution**				**Metabolism**		**Elimination**	
	Pgp-inh	**Pgp-sub**	**HIA**	**Caco-2**	**BBB**	**PPB**	**VDss**	**CYP-inh**	**CYP-sub**	**CL**	**T12**
10860416	0	0.002	0.016	-5.46	0.244	86.08	1.301	0.301	0.19	9.916	0.797
10425734	0	0.002	0.022	-5.482	0.209	81.81	1.365	0.275	0.149	10.641	0.807
54303570	0.017	0.005	0.921	-5.34	0.241	44.47	3.103	0.222	0.677	7.504	0.943
23121697	0.001	0.003	0.093	-5.345	0.167	88.1	0.873	0.488	0.156	12.056	0.84
60833297	0.001	0.01	0.005	-4.924	0.289	62.53	1.107	0.252	0.312	11.048	0.788
65062362	0.001	0.028	0.008	-4.868	0.275	62.32	0.578	0.063	0.846	10.877	0.77
82329428	0.001	0.005	0.004	-4.765	0.259	82.99	1.298	0.12	0.615	10.975	0.731
82329437	0	0.001	0.017	-5.32	0.278	81.77	1.269	0.255	0.761	12.158	0.759
82329486	0.001	0.003	0.004	-5.012	0.243	56.25	1.337	0.28	0.511	11.546	0.766
82329488	0	0.001	0.016	-5.365	0.279	78.83	1.474	0.28	0.847	12.708	0.707
82329501	0	0.001	0.018	-5.328	0.273	79.08	1.502	0.297	0.697	12.387	0.74
117042268	0	0.002	0.004	-5.086	0.275	48.22	0.92	0.132	0.846	10.146	0.793
131886055	0	0.003	0.005	-5.133	0.19	24.82	0.781	0.096	0.814	16.492	0.783

Protein preparation of target protein LTA4H and ligand structures for molecular docking

The target protein LTA4H (3U9W) was retrieved from the Protein Data Bank (PDB) database. The miscellaneous molecules (water and natural co-crystallized ligand molecules) were removed from the protein structure and the dehydrated protein structure was prepared for the molecular docking process through PyMOL software. The initial structure contains water molecules and small molecules and the final protein structure which is prepared for the docking study displays the removal of miscellaneous molecules as seen in Figure 3.

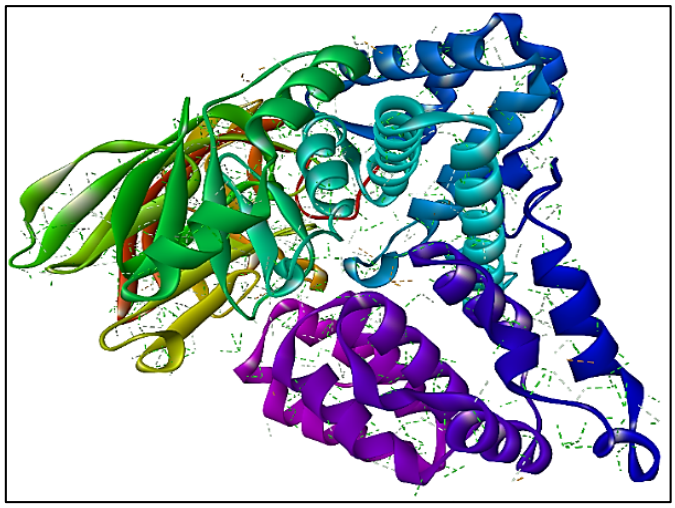


Figure 3. Visualization of LTA4H protein indicating hydrogen bond addition (Green line)

Molecular docking between selected ligand compounds and protein LTA4H

Among 47 compounds, 7 compounds displayed the best docking score, and 04 out of 7 compounds displayed a docking score more than the standard drug Bestatin (i.e. 7.4 kcal/mol). Among these 05 compounds, compound 3-[butan-2-yl-[2-(3-ethylphenoxy)ethyl]amino]propanoic acid displayed the highest docking score of -8.8 kcal/mol, and the lowest RMSD value of 0.042 in comparison with the standard drug Bestatin which displayed the docking score of -7.4 kcal/mol. Hence both compounds 3-[butan-2-yl-[2-(3-ethylphenoxy)ethyl]amino]propanoicacid and Bestatin were selected as input for 2D protein-ligand interaction analysis (Table 4).

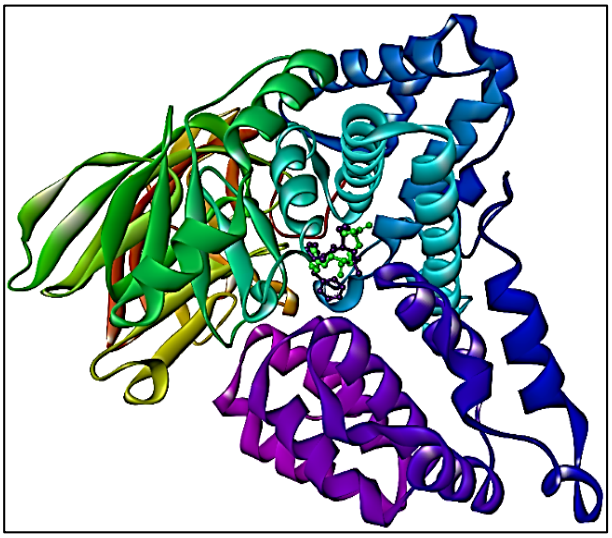


Figure 4. 3D interaction of ligand molecules (3-[butan-2-yl-[2-(3-methylphenoxy)ethyl]amino]propanoic acid (Green) and Bestatin (Dark blue) with target protein LTA4H

Table 4. Molecular Docking Results

S. No	Name of Compound	PubChem	RMSD (A^0)	Score (Kcal/mol)	Protein-Ligand Interaction
1	3-[butan-2-yl-[2-(3-methyl phenoxy)ethyl]amino]propanoic acid	82329405	0.042	-8.8	**Hydrogen** ((GLU (1325), GLU (1384), GLY (1268))), **Alkyl** (ARG (1326), TYR (1267), LYS (1565), ARG (1563)).
2	3-[butan-2-yl-[2-(4-methylph enoxy)ethyl]amino]propan oic acid	82329428	0.045	-8.1	**Hydrogen** (ASN (1291)) & **Alkyl** (HIS (1295), VAL (1292), TYR (1383, GLU (1296))
3	3-[methyl-[(3-phenoxyphenyl)methyl]amino]propanoicacid	65063210	0.042	-7.6	**Hydrogen** (GLY (1269), ARG (1568))
4	3-[2-(3-methyl phenoxy)ethyl-propan-2-ylam ino]propanoic acid	60833298	0.059	-7.5	**Alkyl** (TYR (1378))
5	3-[cyclopropyl-[3-(3-methyl phenoxy)propanoyl]amino]propanoic acid	60844005	0.045	-7.3	**Alkyl** (MET (1564))
6	3-[4-(3-methyl phenoxy)butanoyl-propan-2-ylamino]propanoic acid	119207335	0.063	-7.2	**Hydrogen** (GLY (1268), GLY (1269)) **Alkyl** (GLU (1296), MET (1564, TYR (1378), LYS (1565), HIS (1295))
7	3-[ethyl-[(3-propan-2-ylo xyphenyl)methyl]amino]butanoic acid	65061150	0.064	-7.2	**Hydrogen** ((TYR (1383), GLY (1268), GLY (1269), MET (1564), TYR (1378)), **Alkyl** (TYR (1267))
8	Bestatin (Standard)	72172	0.000	-7.4	**Hydrogen** (ARG (1563), GLY (1269), GLU (1325)), **Alkyl** (TYR (1378) and TYR (1383))

Analysis of protein-ligand interaction of docked complex structures

The compound 3-[butan-2- yl-[2-(3-ethyl phenoxy)ethyl]amino]pr
opanoicacid formed 07 bonds (04 hydrogens and 03 alkyls) with the 07 amino acids (GLU (1325), GLU (1384), GLY (1268), ARG (1326), TYR (1267), LYS (1565), ARG (1563)) present in the active site of the target protein LTA4H. On the other hand, the standard ligand Bestatin formed 05 bonds (03 hydrogens and 02 alkyl) with 05 amino acids (ARG (1563), GLY (1269), GLU (1325), TYR (1378) and TYR (1383)) present in the active site of the target protein LTA4H (Figure 4) (Figure 5).

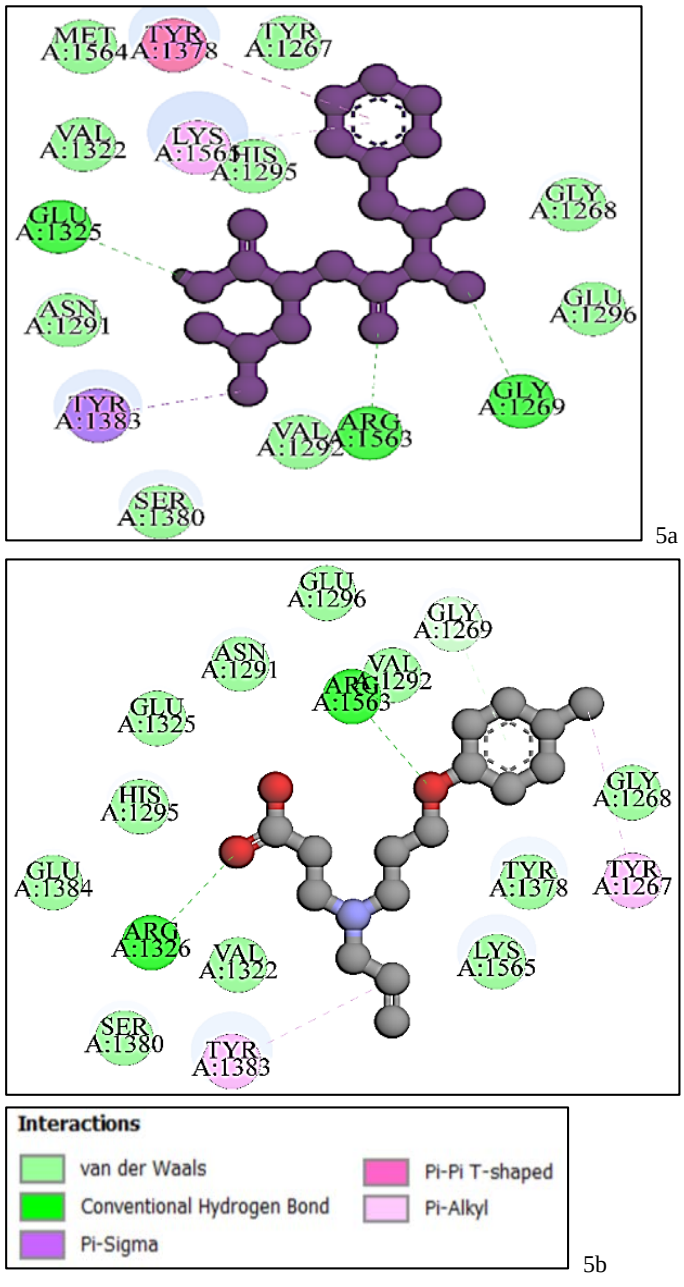


Figure 5. Protein-ligand interaction between protein LTA4H and ligand compounds. (**5a**) 2D interaction between (3-[butan-2-yl-[2-(3-methylphenoxy)ethyl]amino]propanoicacid and LTA4H. (**5b**) 2D interaction analysis between Bestatin and LTA4H

DISCUSSION

The human body is a complex system consisting of trillions of cells. Uncontrolled cell growth with damaged DNA is the main indication for identifying cancer cells. Damaged DNA makes the individual weak and prone to diseases like pandemics[30]. One of the major types of cancer cases identified in the year 2022 is colorectal cancer. According to WCRF, more than 1,926,425 cases and 904,019 deaths were reported due to colorectal cancer in the year 2022. Researchers have studied many potential targets for colorectal cancer and among them, LTA4H (3U9W) plays a significant role in the growth and proliferation of the cancer cells. LTA4H plays a crucial role in the biosynthesis of leukotrienes, which are important for the growth of cancer cells, hence it was selected as the target protein for finding novel drugs against colorectal cancer. In the present study, Bestatin was explored as the query molecule through PubChem screening, and 1000 query-like molecules as the result of PubChem screening. These 1000 molecules were further filtered through Lipinski's rule of five to filter out the molecules with more probability of becoming potential lead molecules. Among 1000 molecules, 875 molecules were obtained after applying Lipinski's rule of five and these 875 molecules were further processed for both druglikeness and ADMET tests through SwissADME and ADMETlab software. Among 875 molecules, 47 molecules displaying both druglikeness and ADMET properties within the accepted range will be selected as the ligand molecules for molecular docking against the target protein LTA4H. At the end of the druglikeness and ADMET, tests target protein LTA4H, 47 ligand molecules, and standard ligands were prepared for the molecular docking process with the help of AutoDock vina package of UCSF Chimera and OpenBabel. The prepared ligand structures were docked against the protein LTA4H through batch docking in PyRx software. Docking results depicted that among 47 ligand molecules, 07 out of 47 ligand molecules displayed the best docking results in comparison with the standard drug Bestatin. Among 07 ligand molecules, compound 3-[butan-2-yl-[2-(3-methylphenoxy)ethyl]amino]propanoicacid displayed the highest docking score of -8.8 kcal/mol, lowest RMSD value of 0.042 A°, and formed 07 bonds (03 hydrogens and 04 alkyl) with the active site residue amino acids ((GLU (1325), GLU (1384), GLY (1268), (ARG (1326), TYR (1267), LYS (1565), ARG (1563)) with the target protein LTA4H. Meanwhile, the standard drug Bestatin displayed a docking score of -7.4 kcal/mol and formed 05 bonds (03 hydrogens and 02 alkyls) with the active site residue amino acids (ARG (1563), GLY (1269), GLU (1325), (TYR (1378) and TYR (1383)) with LTA4H protein. Hence compound 3-[butan-2-yl-[2-(3-methylphenoxy)ethyl]amino]propa
noicacid can be further developed as the anticancer drug to fight colorectal cancer with LTA4H as the target protein.

CONCLUSION

From the present study, it was evident that the compound (3-[butan-2-yl-[2-(3-methylphenoxy)ethyl]amino]propanoicacid displayed the highest docking score of -8.8 kcal/mol, lowest RMSD of 0.042 A^0 and formed 07 bonds with amino acids (GLU (1325), GLU (1384), GLY (1268), ARG (1326), TYR (1267), LYS (1565),

ARG (1563)) present in the active site residue of the target protein LTA4H. Hence the compound (3-[butan-2-yl-[2-(3-methylphenoxy)ethyl]amino]propanoicacid can be further studied as an effective and safe medication to treat colorectal cancer through in-vitro and in-vivo studies.

ACKNOWLEDGMENTS

The authors acknowledge support from REVA University for the facilities provided to conduct the present research work.

CONFLICT OF INTEREST STATEMENT

The authors declare that there is conflict of interest for this work.

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