Unlocking the Anticancer Efficacy of 4-Hydroxy-3-Methoxy Cinnamic acid: "A Predictive Computational Study for Drug Development."

ABSTRACT:

Background: Cancer remains a significant global health challenge, high failure rate in oncology therapeutic development necessitates novel approaches to model drug response and resistance. 4-Hydroxy-3-methoxy cinnamic acid (FA) is naturally occurring phenolic compounds found in various plants, have demonstrated significant in vitro and in vivo anticancer potential by targeting multiple molecular pathways, including apoptosis induction, tyrosine kinase inhibitor and cell cycle arrest.**Objective:** This study utilizes a sophisticated computational study approaches docking simulations were, to investigate the molecular interactions and mechanisms by which 4-Hydroxy-3-methoxy cinnamic acid derivative exert their anticancer activities, focusing on predicting drug responses as anticancer or identifying novel biomarkers. **Method:**A range of computational methods, including molecular docking, ROF, Pre-ADMET, Molinspiration analysis, were used to evaluate biological data. Molecular docking was performed to study the binding affinity and mechanism of 4-Hydroxy-3-methoxy cinnamic acid derivatives against key cancer-related targets Epidermal Growth Factor Receptor tyrosine kinase domain with 4-anilinoquinazoline inhibitor (PDB ID: 1M17) Additionally, 3D structures were optimized and in silico ADME and drug-likeness properties were assessed to determine their potential as drug candidates..**Result:**The molecular docking study revealed that the standard tyrosine kinase inhibitor (TKI) Erlotinib exhibited a docking score of −140.318, forming a hydrogen bond interaction with Ser630 and showing steric interactions with Trp629 and Lys554. In comparison, the compound MS8 demonstrated a docking score of −129.038 and formed multiple hydrogen bond interactions with Tyr752, Asn710, His740, Asp709, and Arg125. Additionally, MS8 showed steric interactions with Glu205 and Asp709. These interactions indicate that MS8 possess significant binding affinity toward the target protein, with Erlotinib showing comparatively stronger binding based on the docking score.4-Hydroxy-3-methoxy cinnamic acid derivative represents apromising candidate for translational oncology, particularly when integrated with nanotechnology-based targeted delivery and combination therapy designs.

Key words: In silico prediction, 4-hydroxy-3-methoxycinnamic acid, tyrosine kinase, lung carcinoma,

INTRODUCTION: Lung carcinoma: Globally, lung cancer is the most frequently diagnosed major cancer and the leading cause of cancer mortality worldwide, of which approximately 80% are non-small cell lung cancer (NSCLC). The dominant oncogenes that are frequently involved in lung cancer include c-MYC, KARS, EGFR, c-MET and c-KIT. There are several signal transduction molecules that are activated in lung cancer, such as receptor tyrosine kinase(RTKs), AKT etc.[(01)]. The expression of p53, a key tumor

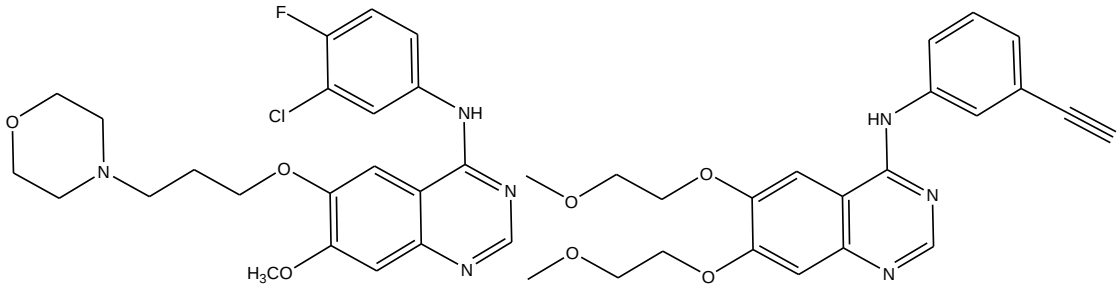
suppressor gene, is altered in most cancers. The loss of p53 function is often a prerequisite for the development of cancer. (02)

4-Hydroxy-3-methoxy cinnamic acid: Natural products have long been investigated and exploited for the development of new drugs(03). Recently, the antitumor effects of the active ingredients of natural plants have attracted extensive attention. 4-Hydroxy-3-methoxy cinnamic acid with the molecular formula is $C_{10}H_{10}O_4$, and molecular weight of 194.18 g. Its cis-form is a yellow liquid, and its trans-form is a solid (04) is a phenolic acid organic compound commonly found in medical plant (05).

4-Hydroxy-3-methoxy cinnamic acid role as anticancer: In the realm of oncology, 4-Hydroxy-3-methoxy cinnamic acid derivative has shown promising anti carcinogenic activities as it can inhibit the occurrence and development of various malignant tumor, such as liver cancer, lung cancer(05). 4-Hydroxy-3-methoxy cinnamic acid and its derivatives induces tumor cell apoptosis by upregulating the expression of P53, inhibits cell proliferation, and interferes with cancer cell signalling pathways. Moreover, 4-Hydroxy-3-methoxy cinnamic acid derivative has been reported to enhance the efficacy of specific chemotherapeutic agents, making it a valuable adjunct in cancer therapy .In silico approaches were used in the present study to investigate the probable activities of 4-Hydroxy-3-methoxy cinnamic acid in inhibiting the tyrosine kinase receptors(03).

Role of tyrosine kinase enzyme: Lung cancer is the leading type of cancer worldwide today in which Kinases play a crucial role in mediating the signalling pathways, and it directs to control several necessary cellular processes. Conversely, the deregulation of tyrosine kinases leads to oncogenic conversion, uncontrolled cell proliferation and tumorigenesis. Tyrosine kinases are largely deregulated in lung cancer and specifically in non-small cell lung cancer (NSCLC)(06).Therefore, the inhibition of pathogenic kinases is a breakthrough development in cancer research, treatment and care, which clinically improve the quality of life. (04)Tyrosine kinases (TKs) are a collective term for dozens of kinases encoded by multiple genes, which can phosphorylate tyrosine residues in cells. Based on varied cellular localizations, the TKs family is divided into receptor tyrosine kinases (RTKs)and non-RTKs(05).

Tyrosine kinase inhibitor: A key target in this context is the tyrosine kinase inhibitor (TKI) class, which currently includes several approved drugs for non-small cell lung cancer. Computational methods offer an efficient and cost-effective approach to identify 4-Hydroxy-3-methoxy cinnamic acid derivative is a potential drug candidate by predicting their effectiveness before extensive in vitro and preclinical testing.



Scheme 1.Gefitinib Scheme 2.Erlotinib

TKs have the common activity to catalyse the transfer of γ-phosphate groups on adenosine triphosphate to the tyrosine residues of a variety of target proteins, and this process plays a key role in signal transduction within the cell. Abnormal activities of TKs are closely associated with proliferation, invasion, metastasis, apoptosis, and tumor angiogenesis in non-small–cell lung cancer (NSCLC), chronic myeloid leukaemia (CML), and many other tumors. Therefore, TKs have become excellent targets for tumor therapy. Tyrosine kinase inhibitors (TKIs) are a class of small-molecule compounds that can specifically inhibit TKs. They can penetrate through the cell membrane and block the signalling pathway of tumor proliferation, with some TKIs also capable of inhibiting angiogenesis. TKIs have revolutionized the treatment of a variety of tumors. For example, imatinib has been a typical pioneer in successfully translating oncogene research into molecular targeted therapy. TKIs are revolutionary targeted drugs that inhibit tumor proliferation by interfering with or inhibiting specific proteins within cancer cells, thus exerting prominent antitumor effect[(07).]

Rational for selecting EGFR tyrosine kinase domain: The selection of the Epidermal Growth Factor Receptor (EGFR) tyrosine kinase domain for lung cancer study is based on its critical role in regulating cell proliferation, survival, and differentiation. Mutations and overexpression of EGFR are frequently observed in non-small cell lung cancer (NSCLC), leading to uncontrolled tumor growth and progression. Targeting the EGFR tyrosine kinase domain has proven effective, as it directly inhibits aberrant signaling pathways involved in cancer development. Moreover, several clinically approved tyrosine kinase inhibitors, such as Erlotinib, validate EGFR as a well-established and therapeutically relevant molecular target for lung cancer treatment.

IN SILICO STUDY

The drug delivery and drug development is very tedious process. The pharmaceutical industry integrated with information technology for the development of new drug. The computer aided drug design is being utilized for the prediction of the ADME properties and also the toxicity of the new drug. The pre-development techniques for the new drug, improve the effectiveness and efficiency of the drug discovery. It decreases the animal, cost and time for the development of the new drug and also increasing the predictability. The drug discovery and the development of new drug are very costly up to multi-million dollars for the drug reach into the market. It required the huge investment and time for the development of new drug, but the success rates a very less i.e. only five out of 10,000 new compound make their way or reach at the human testing after preliminary evaluation on animals. The majority drug are failed at the later stages due to lack of the pharmacokinetic properties like, absorption, distribution, metabolism, excretion and toxicity. The drug designing and drug development process are speed up after the advanced computerized techniques. Due to this technique the pharmaceutical companies and research group done incredible work. The various methods are used for the development of the new drug are as follows: The computerized techniques are very useful for the development of the new drug. It is generally classified in to two parts one is the structure based drug design [SBDD] and another is the ligand based drug design [LBDD]. SBDD methods are used for the analysis of the macro-molecular target present in the 3-dimensional structural information, typically of proteins or RNA, for the biological function it is used to identify the key sites and interaction. Such

information can then be utilized to design new drugs that can compete with essential interactions involving the target and thus interrupt the biological pathways essential for survival of the microorganism(s). LBDD methods are used for the detection of the relationship between the physio-chemical properties and antibiotic activities for antibiotic ligands, referred to as a structure-activity relationship (SAR)[(08)].

Computational study

CADD: Computer-aided drug design is a computer technology that designs a product and documentsthe design's process. CADD may facilitate the manufacturing process by transferring detailed diagrams of a products materials, processes, tolerances and dimensions with specific conventions for the product in question [(09)]. It can be used to produce either two-dimensional or three-dimensional diagrams, which can then when rotated to be viewed from any angle, even from the inside looking out. The channel of drug discovery from idea to market consists of seven basic steps disease selection, target selection; lead compound identification, lead optimization, pre-clinical trial testing, and clinical trial testing and pharmacogenomics optimization. In practice, the last five steps required to pass repeatedly. The compounds for testing can be obtained from natural source (Plants, animals, microorganisms) and by chemical synthesis. These compounds can be rejected as perspectives owing to absence or low activity, existence of toxicity or carcinogenicity, complexity of synthesis, insufficient efficiency etc. As a result only one of 100000 investigation compounds may be introduced to the market and one average cost of development of new drug rose up to 800 million dollars. The reduction of time consuming and cost of the last stages of drug testing is unlikely due to strict state standard on their realization. Therefore main efforts to increasing efficiency of development of drugs are directed to stages of discovery and optimization of ligands[(10).]

Lipinski rule of five:The development of new drugs is a complex and challenging process that involves identifying molecules with desirable therapeutic properties while ensuring their safety and efficacy. In the late 1990s, Christopher Lipinski proposed a set of guidelines known as Lipinski's rule of five to assist in the identification of molecules with favourable pharmacokinetic and pharmaceutical properties. It provides a simple set of criteria to assess the potential for a molecule to become a drug candidate based on its physicochemical properties. Lipinski's Rule of Five is based on four key Parameters: molecular weight, hydrogen bond donors, hydrogen bond acceptors, and the octanol-water partition coefficient (log P). According to the rule, a compound is more likely to have desirable pharmacokinetic and pharmaceutical properties if it has a molecular weight below 500 Da, no more than five hydrogen bond donors, no more than 10 hydrogen bond acceptors, and a calculated octanol water partition coefficient (log P) less than 5[(11).]

ADMET:ADMET stands for absorption, distribution, metabolism, excretion and toxicity play key roles in the drug discovery and development. This covers the physicochemical properties of drugs, PH and solubility and approaches to improving aqueous solubility as well as drug metabolism and drug interactions. During drug discovery phase, chemical synthesis is guided toward potent compounds with physicochemical and absorption, distribution, metabolism and excretion properties that allow drug to reach effective concentration at the target. It also includes Swiss ADME which is a free web tool to

evaluate pharmacokinetics, drug likeness and medicinal chemistry friendliness of small molecules. ADMET covers pharmacokinetic issues determining whether a drug molecule will get to the target protein in body and how long it will stay in bloodstream. Parallel evaluation of efficiency and biopharmaceutical properties of drug candidates has been standardized and exhaustive studies of ADMET processes are nowadays routinely carried out at early stage of drug discovery to reduce attrition rate[12].

Molinspiration: Molinspiration is one such tool that provides researchers with the ability to perform rapid molecular property calculations and bioactivity predictions. Mol. inspiration is a widely utilized cheminformatics platform designed to assist in the prediction of molecular properties and biological activities of chemical compounds. Mol. inspiration offers broad range of cheminformatics software tools supporting molecule manipulation and processing, including SMILES and SDfile conversion, normalization of molecules, generation of tautomer , molecule fragmentation, calculation of various molecular properties needed in QSAR, molecular modelling and drug design, high quality molecule depiction, molecular database tools supporting substructure and similarity searches. The platform also predicts bioactivity scores for key pharmacological targets, such as G-protein coupled receptors (GPCRs), ion channel modulators, kinase inhibitors, nuclear receptor ligands, and general enzyme inhibitors. Additionally, the software evaluates drug-likeness based on Lipinski's Rule of Five, a widely accepted criterion for oral bioavailability. These predictions allow researchers to prioritize compounds for synthesis and in vitro testing[13].

Molinspiration Parameter

MILOGP (Mi Log P) is used for its robustness and reliable lipophilicity prediction, widely applied in ZINC database screening and validated against experimental values. TPSA represents the surface area of polar atoms (mainly oxygen and nitrogen with attached hydrogens), indicating molecular polarity and transport properties. NATOMS refers to the total number of atoms, while molecular weight (in Daltons) defines the mass of a molecule. The parameters nON and nOHNH denote hydrogen bond acceptors and donors, respectively. The number of rotatable bonds (nrotb) measures molecular flexibility and is an important predictor of oral bioavailability, excluding rigid amide bonds.[14]

Molecular docking:A type of computational modelling known as "molecular docking" is used to represent the complexes produced when two or more molecules interact.The words "ligand" and "protein" are mostly linked to the concept of molecular docking[15]. It is a computational technique for determining the architecture of compounds made up of two or more different molecules. Predicting the desirable 3D structures is the objective of molecular docking studies. In computational drug design and molecular structural biology, it is helpful[16]. The goal of molecular docking is to achieve an optimal conformation for both the protein and the ligand as well as the fundamental direction between the protein and the ligand in order to reduce the overall method's free energy. Molecular docking is one of the most often utilized techniques in structure-based drug design. Because of its capacity to predict he binding conformation of small molecule ligands to the appropriate target binding site [17]. It is possible to anticipate the preferred binding orientation of a molecule (such as a ligand) to a different one (such as a receptor) when they interact to produce a stable complex through a type of computational modelling known as molecular docking[18].

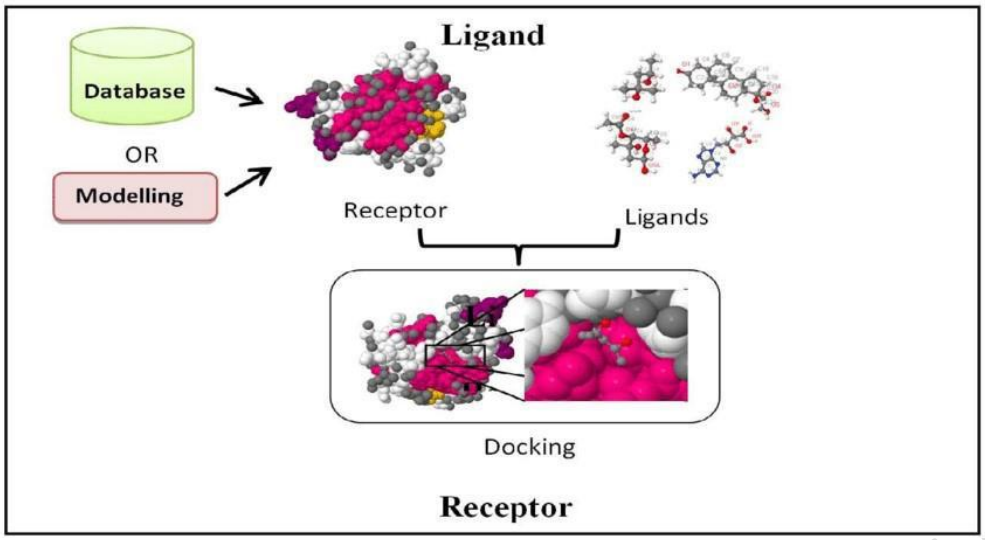


Fig -1 Molecular Docking Flow Chart

The receptor targets for the anti-lung cancer study used are Epidermal Growth Factor Receptor tyrosine kinase domain with 4-anilinoquinazoline inhibitor(PDBID: 1M17)

- **Classification: Transferase**
- **Organism(s):** Homo sapiens
- **Expression System:** Spodopterafrugiperda
- **Mutation(s):** No
- **Resolution:** 2.60 Å

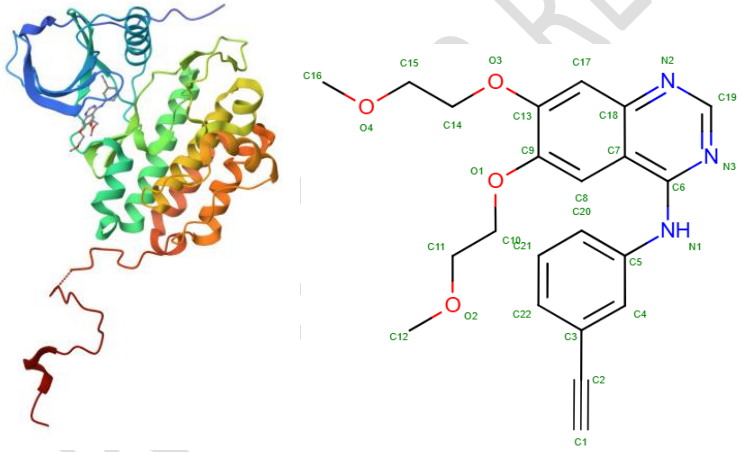
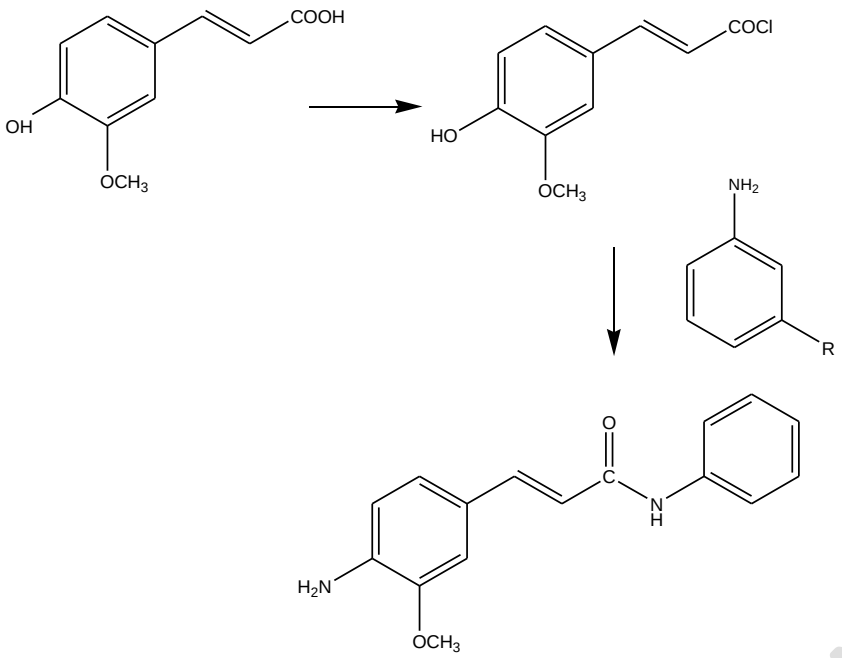


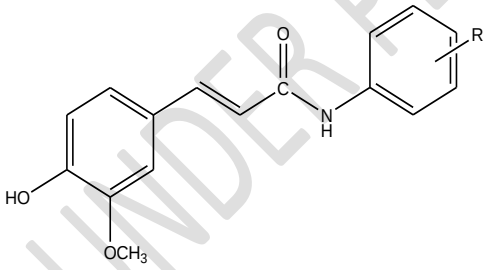
Figure 02: Crystal structure of Epidermal Growth Factor Receptor tyrosine kinase domain with 4-anilinoquinazoline inhibitor and Chemical structure of Co-crystallized ligand

Material and method:



Scheme: steps for the the synthesis of 4-Hydroxy-3-methoxy cinnamic acid derivatives

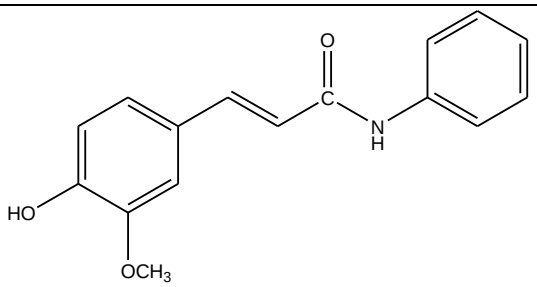
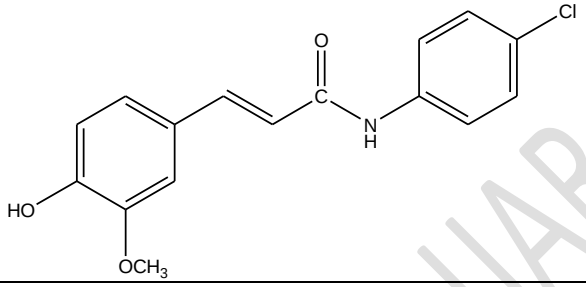
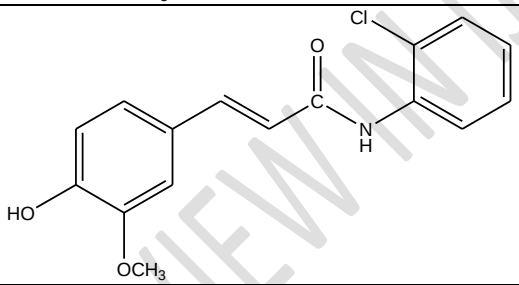
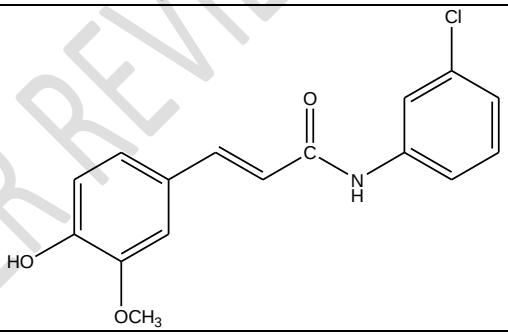
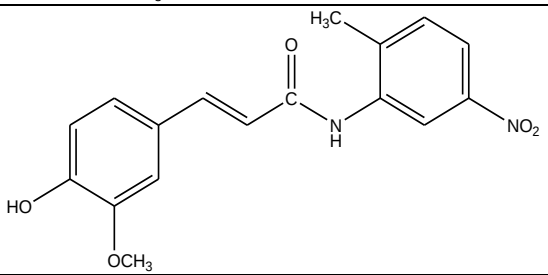
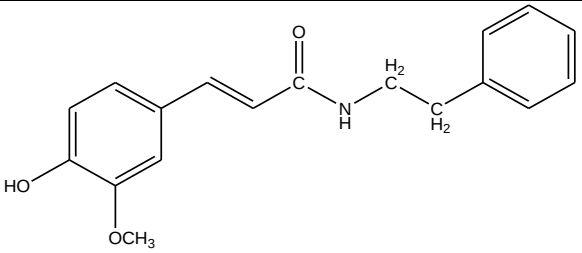
Dataset of compounds

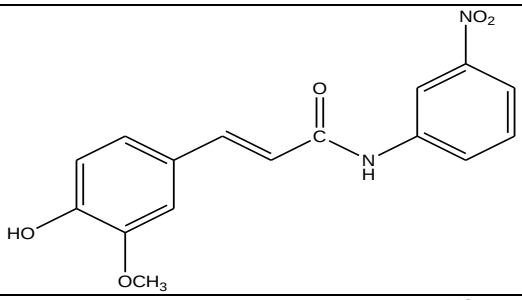
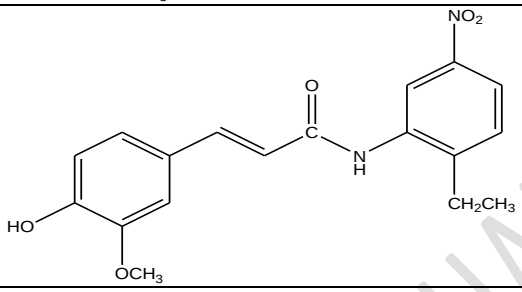
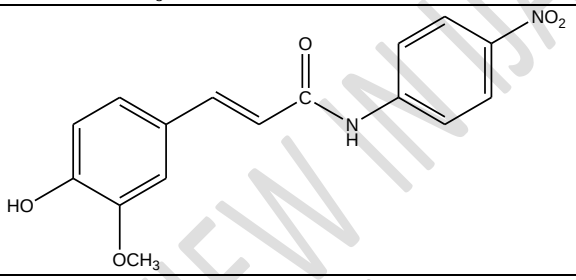
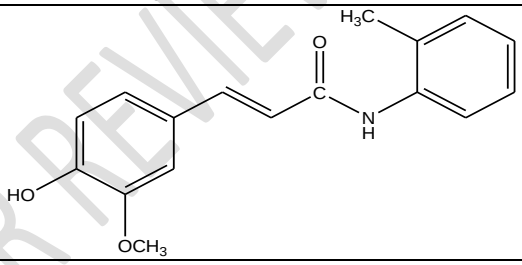


Where R: Cl, NH_2, CH_3, C_2H_5,

Table 01: 10 derivatives of 4-Hydroxy-3-methoxycinnamic acid with aromatic amines

CODE	AROMATIC AMINE	STRUCTURE

MS1	Aniline	
MS2	4-chloro Aniline	
MS3	2-chloro aniline	
MS4	3-chloro Aniline	
MS5	2 methyl 5nitro Aniline	
MS6	2-Phenylethyl Amine	

MS7	m- nitro aniline	
MS8	2-ethyl 5-nitro Aniline	
MS9	4-nitro Aniline	
MS 10	o-toluidine	

PROTEIN DATA BANK

The RCSB PDB provides a variety of tools and resources for studying the structures of biological macro molecules and their relationships to sequence, function, and disease. The RCSB is a member of the .Whose mission is to ensure that the PDB archive remains an international resource with uniform data. This site offers tools for browsing, searching, and

reporting that utilize the data resulting from ongoing efforts to create a more consistent and comprehensive [19].

Methodology: An in-silico investigation of a 4-Hydroxy-3-methoxy cinnamic acid derivative as a possible lung cancer treatment was part of the technique. The molecule was initially assessed fordesigning of compound-2D structures and 3D structures, drug-likeness using the Rule of Five (ROF), and then its bioactivity was predicted using molinspiration. Pharmacokinetic and toxicity profiles were estimated using pre-ADMET analysis. In order to ensure therapeutic relevance, molecular docking studies were carried out to examine binding affinity and interactions with target proteins associated with lung cancer[20].

Designing of compound:

2D Structure -

The chemical structures were drawn using ChemDraw Ultra 8.0 software, which was preinstalled on the system. The software provides a main toolbar containing essential tools for structure drawing, including selection and bond tools, which were utilized to construct the molecules. [21].

3D Structure -

The 2D structures were imported into ChemDraw Ultra 8.0 software, preinstalled on the system, and refined using the "Clean Up Structure" option available in the main toolbar. The optimized structures were then subjected to energy minimization using MM2 and MOPAC tools provided within the software. Following optimization, the finalized structures were saved in both PDB and MOL file formats for subsequent computational studies.[22].

Methodology of ROF:

The drug-likeness of the designed compounds was evaluated using Lipinski's Rule of Five. 4-Hydroxy-3-methoxy cinnamic acid derivatives in pdb format were subsequently uploaded to the ROF web tool, where the pH was set to 7 prior to analysis. The tool generated detailed physicochemical parameters, enabling assessment of drug-likeness and providing insights into the behavior and suitability of the compounds as potential drug candidates.[24].

Methodology of Molinspiration:

Molinspiration offers a user-friendly web interface that allows for the calculation of various molecular descriptors. These include LogP (octanol-water partition coefficient), topological polar surface area (TPSA), molecular weight, number of hydrogen bond donors and acceptors, and the number of rotatable bonds. Additionally, the software evaluates drug-likeness based on Lipinski's Rule of Five, a widely accepted criterion for oral bioavailability[25].

The Molinspiration cheminformatics platform, which forecasts drug-likeness and receptor binding potential, was used to evaluate the activity of 4-Hydroxy-3-methoxy cinnamic acid derivatives. The coding in SMILES notation was transferred into the platform's input box after structures were saved in the mol. format and opened in Notepad. The Submit button

started computational analysis to produce activity predictions once the tool rebuilt the compound for visual confirmation.

Methodology of ADME

The methodology of ADME prediction through the PreADMET website is carried out in a structured and professional manner. Initially, the compound's mol. doc file is opened in Notepad to extract its structural data. This data is then carefully copied and pasted into the designated input section of the PreADMET platform. Once the information is verified, the submission process begins, allowing the tool to analyse the compound thoroughly[26]. The PreADMET system evaluates key pharmacokinetic parameters, including absorption, distribution, metabolism, and excretion, thereby generating comprehensive ADME results. This systematic workflow ensures accuracy and reliability, making it a valuable approach for assessing the pharmacokinetic behaviour of newly designed molecules. By following these precise steps, researchers can obtain meaningful insights into drug-likeness and optimize compounds for further development in pharmaceutical research[27].

Methodology of Toxicity

The methodology of toxicity prediction through the PreADMET website is executed in a structured and professional sequence. To begin, the compound's mol.doc file is opened in Notepad to extract its structural data. This information is then carefully copied and pasted into the toxicity section of the PreADMET platform. Once the data is verified, it is submitted for computational analysis. The PreADMET tool processes the compound and generates toxicity readings across multiple parameters, including mutagenicity, carcinogenicity, and other toxicological endpoints relevant to drug safety evaluation. This systematic workflow ensures precision and reliability, providing comprehensive toxicity profiles that support the assessment of compound safety during drug discovery and development. By following these steps, researchers can obtain meaningful insights into potential risks, thereby guiding the optimization of molecular structures and enhancing the overall safety profile of candidate drugs [28].

Methodology of molecular docking

The molecular docking procedure was performed using Mole gro. Virtual Docker (MVD) software using a structured workflow to ensure accuracy and reproducibility. Initially, the receptor protein structures using "Epidermal Growth Factor Receptor tyrosine kinase domain with 4-anilinoquinazoline inhibitor"(PDB ID:1M17) were retrieved from the Protein Data Bank and imported into the docking software interface. A surface was generated by right-clicking on the protein and confirming the operation. The protein was then processed by detecting cavities, with five cavities identified for analysis[29]. The surface option was unticked, and two cavities were removed by right-clicking, leaving the relevant binding sites. Subsequently, the protein option was unticked, and ligand molecules were imported through the "File → Import Molecule" function. Ligand preparation was performed to optimize the structures for docking. Docking was initiated using the docking wizard (RezardF1), where the cavity was arranged and highlighted with a green ball. The ball size was minimized to refine the binding site, and the docking process was repeated three times to ensure consistency and reliability of the results. This systematic approach

provided accurate binding affinity scores and interaction profiles, supporting the evaluation of ligand–protein interactions for drug discovery [30].

Result and discussion

Result of ROF: Result of ROF of derivatives of 4-Hydroxy-3-methoxy cinnamic acid with aromatic aminesis detailed in table no.01

Table 01: Result of ROF of derivatives of 4-Hydroxy-3-methoxy cinnamic acid with aromatic amines

CODE	MW	HBA	HBD	MolLogP	MolPSA	MolVol	Drug likeness model score
MS1	269.11	3	2	2.90	47.29	277.68	-0.27
MS2	303.07	3	2	3.69	47.29	294.87	0.17
MS3	303.07	3	2	3.46	46.60	294.07	-0.05
MS4	303.07	3	2	3.85	47.29	294.95	-0.16
MS5	328.11	5	2	3.23	79.98	323.49	-0.53
MS6	297.14	3	2	3.02	48.46	313.39	0.16
MS7	314.09	5	2	3.20	80.68	302.74	-0.67
MS8	342.12	5	2	3.99	79.98	342.39	-0.36
MS9	314.09	5	2	3.03	80.68	302.67	-0.61
MS10	283.12	3	2	3.23	46.60	298.43	-0.15

Discussion: The Rule of Five (ROF) analysis was performed to evaluate the drug-likeness of 4-Hydroxy-3-methoxy cinnamic acid derivatives conjugated with aromatic amines. All compounds demonstrated molecular weights below the threshold of 500 Da, indicating favourable compliance with Lipinski's criteria. The number of hydrogen bond donors (HBD) ranged consistently at 2, while hydrogen bond acceptors (HBA) varied between 3 and 5, remaining within acceptable limits. LogP values were between 2.90 and 3.99, suggesting moderate lipophilicity conducive to oral bioavailability. Topological polar surface area (TPSA) values ranged from 46.60 to 80.68 $Å^2$, supporting adequate permeability. Molecular volumes were within 277–342 $Å^3$, reflecting compact structures suitable for drug design. Drug-likeness model scores varied across the series, with MS2 and MS6 showing positive values (0.17 and 0.16, respectively), indicating higher potential as drug candidates. In contrast, derivatives such as MS5, MS7, and MS9 exhibited negative scores, suggesting comparatively lower drug-likeness. Overall, the dataset highlights MS2 and MS6 as promising candidates for further pharmacological evaluation, while other derivatives may require structural optimization to enhance drug-like properties.

Result of ADME profiling: Result of ADME Profiling of derivatives of 4-Hydroxy-3-methoxy cinnamic acid with aromatic amines is represented in table 02

Table 02:Result of ADME Profiling of derivatives of 4-Hydroxy-3-methoxy cinnamic acid with aromatic amines

Comp.	BBB	CaCO2	CY2D6	HIA	MDCK	Pgp	PPB	Skin permeability
MS1	1.020725	25.35	NON	92.98	28.82	NON	79.73	-2.8402

MS2	2.134	24.57	NON	93.99	1.84	NON	86.23	-2.88
MS3	2.081	23.54	NON	93.99	0.162	NON	86.39	-2.85
MS4	2.027	24.57	NON	93.99	1.81	NON	87.59	-2.88
MS5	0.042	19.62	NON	91.59	0.078	INHIBITOR	84.99	-2.80
MS6	1.61	27.83	NON	93.42	183.46	NON	84.56	-2.58
MS7	0.028	18.97	NON	90.63	0.843	INHIBITOR	84.32	-2.95
MS8	0.071	19.72	NON	92.40	1.061	INHIBITOR	87.46	-2.61
MS9	0.023	20.77	NON	90.63	0.317	INHIBITOR	85.86	-2.95
MS10	1.682	26.13	NON	93.21	0.170	NON	82.83	-2.70

Discussion: The ADME profiling of 4-Hydroxy-3-methoxy cinnamic acid derivatives was conducted to evaluate their pharmacokinetic behaviour and drug-likeness. Blood–brain barrier (BBB) penetration values varied across the series, with MS2 (2.134), MS3 (2.081), and MS4 (2.027) showing relatively higher permeability, suggesting potential central nervous system activity, while MS5, MS7, MS8, and MS9 exhibited minimal BBB penetration. $CaCO_2$ permeability values ranged between 18.97 and 27.83, indicating moderate intestinal absorption potential. All compounds were classified as non-inhibitors of CYP2D6, reducing the likelihood of metabolic drug–drug interactions. Human intestinal absorption (HIA) was consistently high (>90%) across all derivatives, confirming favourable oral bioavailability. MDCK cell permeability showed significant variation, with MS6 displaying exceptionally high permeability (183.46), while others remained low to moderate. P-glycoprotein (Pgp) inhibition was observed in MS5, MS7, MS8, and MS9, which may affect efflux transport and bioavailability. Plasma protein binding (PPB) values were generally high (79–87%), indicating strong binding affinity and potential influence on free drug concentration. Skin permeability values ranged from –2.58 to –2.95, reflecting limited transdermal absorption. Overall, MS2, MS3, MS4, and MS6 demonstrated favourable ADME characteristics, particularly in terms of BBB penetration, HIA, and permeability, making them promising candidates for further pharmacological evaluation. In contrast, derivatives such as MS5, MS7, MS8, and MS9 showed limitations due to low BBB penetration and suggesting the need for structural optimization.

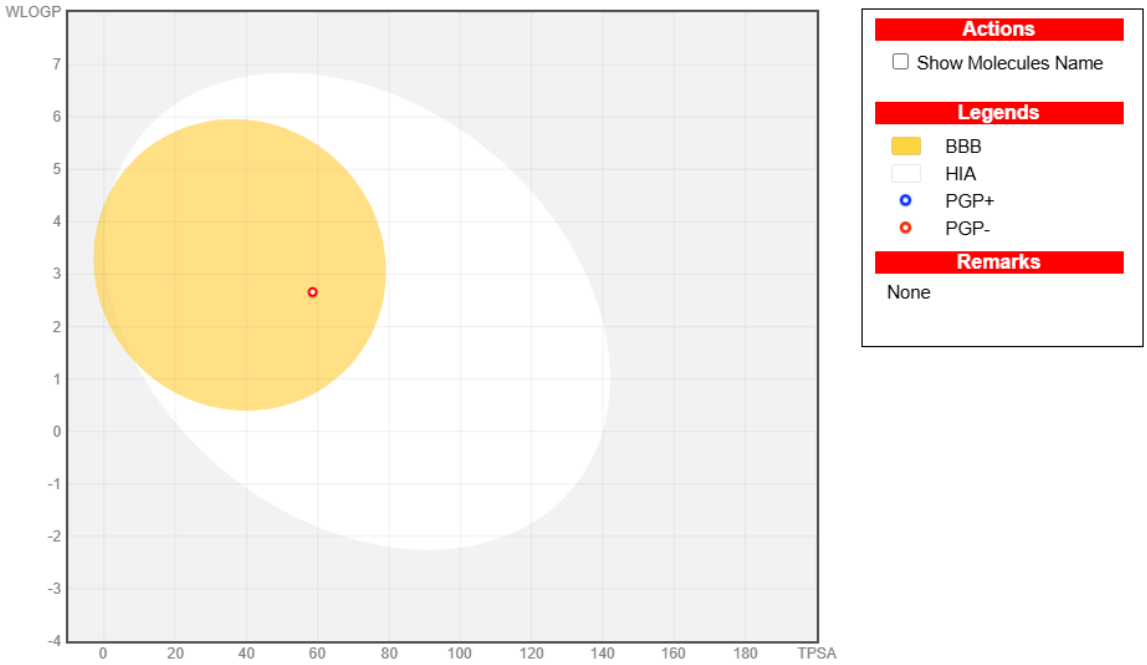


Fig 03: boiled egg model representing compound MS7

Result of toxicity: Result of toxicity of derivative of 4-Hydroxy-3-methoxy cinnamic acid with aromatic amine as shown in table 03

Table 03: Result of toxicity of derivative of 4-Hydroxy-3-methoxy cinnamic acid with aromatic amine

COMP	AMES TEST	CARCINO MOUSE	CARCINO RAT	HERG
MS1	mutagen	-ve	+ve	Medium risk
MS2	mutagen	+ve	-ve	Medium risk
MS3	mutagen	+ve	-ve	Medium risk
MS4	mutagen	+ve	-ve	Medium risk
MS5	mutagen	-ve	+ve	Medium risk
MS6	mutagen	+ve	-ve	Medium risk
MS7	mutagen	-ve	+ve	Medium risk
MS8	mutagen	-ve	+ve	Medium risk
MS9	mutagen	+ve	+ve	Medium risk
MS10	mutagen	-ve	+ve	Medium risk

Discussion: Toxicity profiling of the 4-Hydroxy-3-methoxy cinnamic acid derivatives revealed consistent mutagenic potential across all compounds, as indicated by positive Ames test outcomes. Carcinogenicity assessments demonstrated variability between mouse and rat models. Several derivatives (MS1, MS5, MS7, MS8, MS10 were negative in mice but positive in rats, whereas MS2, MS3, MS4, and MS6 showed the opposite trend, being positive in mice but negative in rats. Notably, MS9 exhibited carcinogenicity in both species, suggesting a higher toxicological concern. HERG inhibition analysis classified all derivatives as medium risk, indicating a potential liability for cardiotoxicity through QT interval prolongation. Overall, while the compounds demonstrate mutagenicity and moderate HERG-associated risk, differences in species-specific carcinogenicity highlight the need for careful evaluation. Among the series, MS9 appears the least favourable due to

dual carcinogenicity, whereas compounds with single-species carcinogenicity may warrant further optimization to mitigate toxicity concerns.

Result of molinspiration: Result of derivative of 4-Hydroxy-3-methoxy cinnamic acid with aromatic amine is detailed in table 04

Table 04: Result of derivative of 4-Hydroxy-3-methoxy cinnamic acid with aromatic amine

code	miLogP	TPSA	natoms	MW	nON	nOHNH	nviolations	nrotb	volume
MS1	2.81	58.56	20	269.30	4	2	0	4	247.82
MS2	3.48	58.56	21	303.75	4	2	0	4	261.35
MS3	3.44	58.56	21	303.75	4	2	0	4	261.35
MS4	3.46	58.56	21	303.75	4	2	0	4	261.35
MS5	3.14	104.38	24	328.32	7	2	0	5	187.71
MS6	2.91	58.56	22	297.35	4	2	0	6	281.42
MS7	2.74	104.38	23	314.30	7	2	0	5	271.15
MS8	3.61	134.38	25	342.35	7	2	0	6	304.51
MS9	2.77	104.38	23	314.30	7	2	0	5	271.15
MS10	3.21	58.56	21	283.33	4	2	0	4	264.38

Discussion: Mol. inspiration analysis was performed to evaluate the physicochemical properties and drug-likeness of 4-Hydroxy-3-methoxy cinnamic acid derivatives conjugated with aromatic amines. All compounds demonstrated compliance with Lipinski's Rule of Five, showing no violations. The molecular weights (MW) ranged between 269.30 and 342.35 Da, well below the 500 Da threshold, supporting favourable oral bioavailability. LogP values varied from 2.74 to 3.61, indicating moderate lipophilicity suitable for membrane permeability. Topological polar surface area (TPSA) values were distributed between 58.56 and 134.38 $Å^2$, with derivatives MS5, MS7, MS8, and MS9 exhibiting higher TPSA (>100 $Å^2$), suggesting reduced permeability compared to other compounds. The number of hydrogen bond donors (nOHNH) remained constant at 2 across all derivatives, while hydrogen bond acceptors (nON) ranged from 4 to 7. Rotatable bonds (nrotb) varied between 4 and 6, reflecting moderate molecular flexibility. Molecular

volumes ranged from 187.71 to 304.51 Å^3, with MS5 showing the lowest volume and MS8 the highest, consistent with their atom counts. Overall, derivatives MS2, MS3, MS4, MS6, and MS10 demonstrated balanced physicochemical profiles with moderate lipophilicity, optimal TPSA, and favourable molecular weights, making them promising candidates for further drug development. In contrast, MS5, MS7, MS8, and MS9, with elevated TPSA values, may require structural optimization to enhance permeability and bioavailability.

Result of molecular docking with 1M17 (Epidermal Growth Factor Receptor tyrosine kinase domain with 4-anilinoquinazoline inhibitor) **:**Result of molecular docking of derivatives of 4-Hydroxy-3-methoxy cinnamic acidis shown in table 5

Table 05: **Result of molecular docking of derivatives of 4-Hydroxy-3-methoxy cinnamic acid**

S.NO.	COMPOUND	Docking score	Hydrogen bond	Steric hindrance
01.	Co-crystallized ligand	-136.53	Tyr662, Tyr547, Tyr 631, Ser 630, Glu 205, Glu 206	Tyr 547,Tyr 631
02.	MS1	-105.367	Gly 141	Asn710, Asp709, His740
03	MS2	-107.81	Tyr 752, Arg 125	Arg125,His140, Asp709
04	MS3	-105.626	Tyr 752, Arg 125	Asp739, His740
05	MS4	-111.978	Arg125	Asn710,Asp739, His 740
06	MS5	-131.724	Ser 360, Ser 209, Arg 382	Arg358,Ile374,Arg356,Glu26,Glu374
07	MS6	-121.133	Asp 739, Lys 122, Trp 629, Arg 125, Trp 124	His740, Asp709
08	MS7	-121.747	Tyr 631, Asp 709, Arg 125, Ser 630	Ser630, His740, Arg125,Glu205, Asp739,Asp709
09	MS8	-129.038	Tyr 752, Asn 710,His 740, Asp 709, Arg 125	Glu 205, Asp709
10	MS9	-111.827	Lys 122, Tyr 752, Arg125	Trp124,His740, Arg 125
11	MS10	-104.952	Tyr 662	Tyr631,Val 546, Tyr 547, Trp 629

Discussion: Overall, **MS8** emerge as the most promising 4-Hydroxy-3-methoxy cinnamic acid derivatives EGFR inhibition, given their strong docking scores, favourable hydrogen bonding interactions, and relatively low steric hindrance. These compounds warrant further **in vitro and in vivo validation** to confirm their potential as lead candidates in the development of novel anticancer drugs.

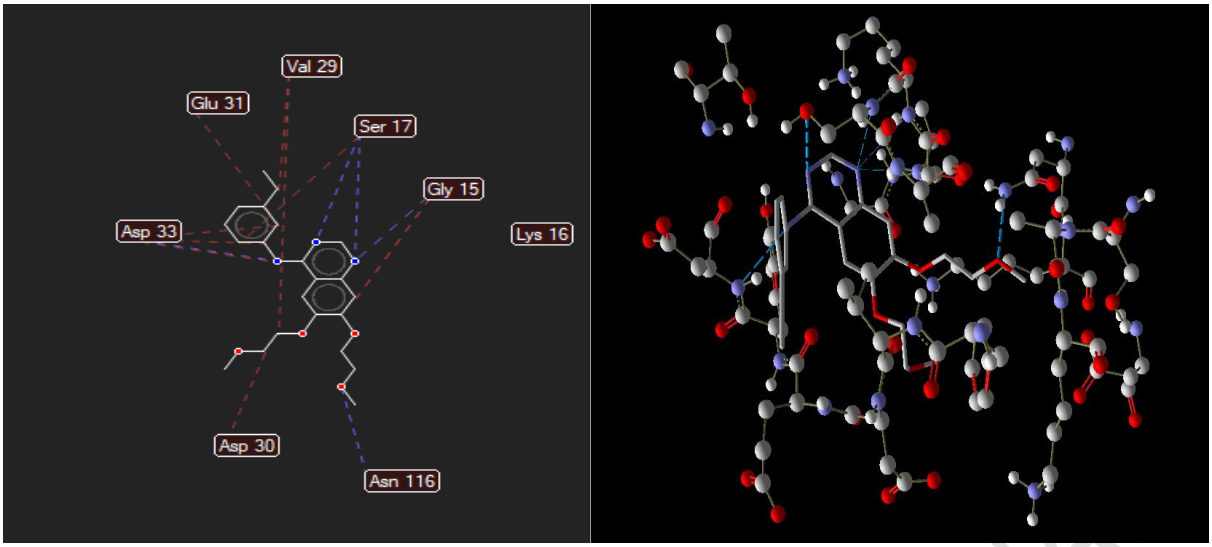


Fig 04: H-bong interaction and docking pose of MS7

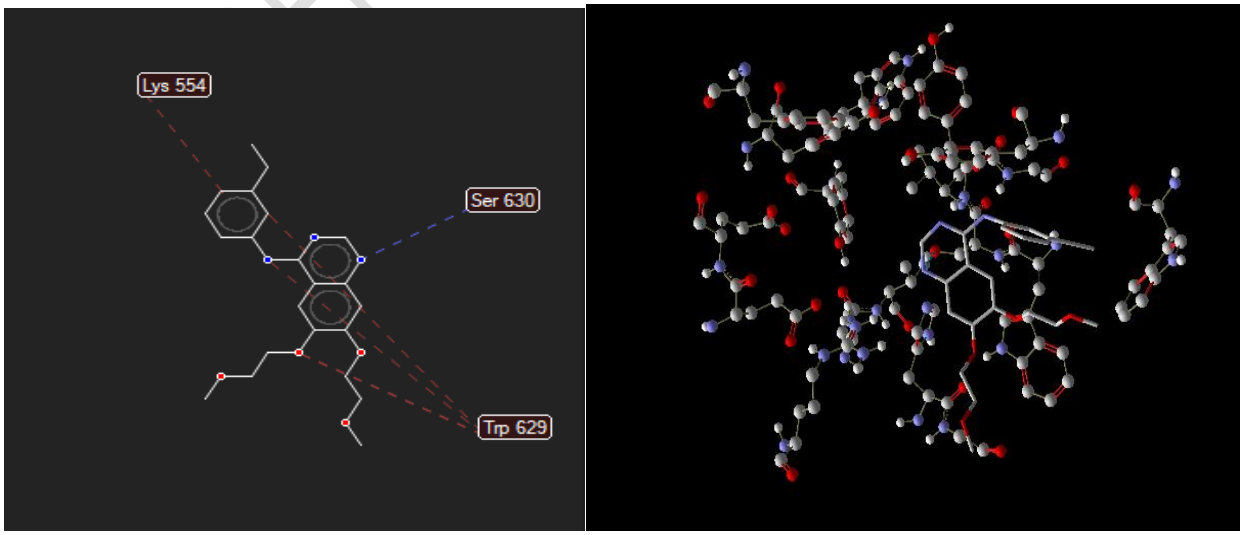


Fig 05: H-bond interaction and docking pose of erlotinib(standard tyrosine kinase inhibitor)

Result of molecular docking of standard TKI (erlotinib) with (Epidermal Growth Factor Receptor tyrosine kinase domain with 4-anilinoquinazoline inhibitor **pdb1M17)**: Result of molecular docking of standard TKI (erlotinib) is shown in table 6.

Table 06: result of molecular docking of standard TKIs

Sr. no.	Standard TKI	Mol. dock score	H bond	Steric interaction
01.	Erlotinib	-140.318	Ser 630	Trp 629, lys 554

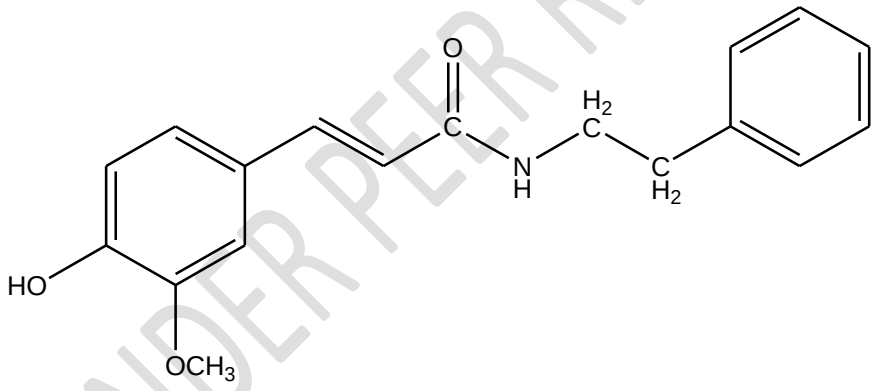


Figure 4.4-Hydroxy-3-methoxy cinnamic acid with 2-Phenylethyl amine

Among all the 10 derivatives compound MS7 which is derivative of 4-hydroxy-3-methoxycinnamic acid with 2- phenylethyl amine shows the relatable results with standard tyrosine kinase inhibitor in terms of binding affinity and interaction.

Conclusion: This study demonstrates the significant potential of 4-hydroxy-3-methoxycinnamic acid derivatives as promising anticancer agents through a comprehensive in silico approach. Molecular docking analysis against the EGFR tyrosine kinase domain (PDB ID: 1M17) revealed strong binding affinities for several derivatives, particularly MS8, which exhibited notable hydrogen bonding interactions and stable ligand–protein conformations. Although the standard drug erlotinib showed superior binding energy, selected derivatives displayed comparable interaction profiles, indicating their potential as alternative or complementary therapeutic candidates.

Drug-likeness evaluation based on Lipinski's Rule of Five confirmed that most compounds possess favourable physicochemical properties, including optimal molecular weight, hydrogen bonding capacity, and lipophilicity. ADME profiling further supported their suitability, demonstrating high human intestinal absorption, acceptable permeability, and minimal risk of CYP2D6-mediated drug interactions. Compounds such as MS2, MS3, MS4, and MS6 exhibited particularly balanced pharmacokinetic characteristics, suggesting strong potential for oral bioavailability. However, toxicity assessment indicated mutagenic tendencies and moderate HERG inhibition risk across the series, highlighting the need for structural optimization to improve safety profiles. Additionally, variations in carcinogenicity between species emphasize the importance of further biological validation.

Overall, this study establishes a strong computational foundation for the development of 4-hydroxy-3-methoxycinnamic acid derivatives as EGFR-targeted anticancer agents, particularly for lung cancer. Future work should focus on in vitro and in vivo validation, structural modification to reduce toxicity, and formulation strategies such as nanocarrier-based delivery systems to enhance stability and bioavailability. These findings contribute to the growing evidence supporting natural product-derived compounds in modern anticancer drug discovery and provide a strategic direction for the development of safer and more effective targeted therapies.

Authors' Contributions

All authors collaboratively contributed to this project. The design of the study, literature review, data compilation, and manuscript preparation were undertaken jointly. Each author participated in drafting, revising, and approving the final version of the manuscript. All authors have reviewed and approved the submitted version.

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this manuscript.

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