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IN SILICO NOVEL LUTEOLIN DERIVATIVES TARGETING EGFR IN NON-SMALL CELL LUNG CANCER COMPARED WITH ERLOTINIB

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ABSTRACT

Luteolin, a natural flavonoid abundantly present in *Lawsonia inermis* (Henna), exhibits anticancer activity by regulating key pathways including EGFR, PI3K/Akt, and MAPK. However, its clinical translation is limited by moderate EGFR affinity, low permeability, and rapid metabolism. This study applies rational Structure–Activity Relationship (SAR)-guided design to generate 12 novel luteolin derivatives and screen them for enhanced efficacy against Epidermal Growth Factor Receptor (EGFR), a major target in Non-Small Cell Lung Cancer (NSCLC). Ligands were designed in MolView, docked using SwissDock (Attracting Cavities 2.0), and evaluated for ADMET using SwissADME and toxicity using ProTox-3.0. Among all derivatives, 7-O-(4-pyridylmethyl)-3',4'-dichloro-5-O-phosphono-luteolin achieved the best binding score (−8.2684 kcal/mol), surpassing natural luteolin (−6.5928) and nearing Erlotinib (−7.6802). ADMET profiling confirmed high GI absorption, moderate lipophilicity, good drug-likeness, and low toxicity (Class 5). Mechanistic pathway mapping indicated that this compound potentially inhibits EGFR downstream signalling (RAS/RAF/MEK/ERK and PI3K/Akt), similar to clinically approved EGFR-TKIs. Thus, the designed derivative presents a promising scaffold for EGFR-targeted therapy in NSCLC.

KEYWORDS: Luteolin; EGFR; NSCLC; SwissDock; Erlotinib; SwissADME.

INTRODUCTION

Lung cancer remains the leading cause of cancer-related mortality worldwide, with approximately 2.2 million new cases and 1.8 million deaths reported annually according to WHO (2023). Among all lung cancer types, nearly 85% are classified as Non-Small Cell Lung Cancer (NSCLC).[1] The progression of NSCLC is strongly associated with dysregulation of Epidermal Growth Factor Receptor (EGFR) signalling, which plays a crucial role in uncontrolled cellular proliferation, inhibition of apoptosis, angiogenesis, and metastasis.[1,2] Due to its central role in tumour development, EGFR has emerged as a well-validated and clinically successful therapeutic target in NSCLC.[2] EGFR mutations, particularly L858R and exon 19 deletions, are frequently observed in 30–40% of NSCLC patients in Asian populations, highlighting the importance of targeted therapies.[1] Although EGFR inhibitors such as Erlotinib have demonstrated significant clinical efficacy, their use is often limited by the development of drug resistance, notably due to the T790M mutation, as well as adverse effects including rash, diarrhoea, and associated toxicity (ProTox class 3).[1,2] These limitations necessitate the exploration of novel therapeutic scaffolds with improved efficacy and reduced toxicity profiles. Luteolin, a naturally occurring flavonoid, has gained considerable attention for its broad-spectrum anticancer activity against various malignancies, including breast, lung, colon cancers, and leukaemia.[3] Its mechanisms of action include inhibition of EGFR signalling, blockade of the PI3K/Akt pathway, induction of apoptosis, and anti-inflammatory effects.[3] (Figure.01 as original luteolin compound structure). Despite its promising pharmacological properties, luteolin is not currently approved as an anticancer drug in modern allopathic medicine. However, luteolin-rich plants are widely utilized in traditional medicinal systems such as Ayurveda, Siddha, Traditional Chinese Medicine, and

folk medicine. For instance, *Lawsonia inermis* (henna), a known source of luteolin, has been traditionally used for anti-inflammatory purposes, wound healing, and immune modulation.[3] This combination of biological activity and traditional usage makes luteolin an attractive lead compound for novel drug development. However, luteolin exhibits several pharmacokinetic and physicochemical limitations, including a high number of hydrogen bond donors leading to poor membrane permeability, low hydrophobicity resulting in inadequate interaction with the EGFR binding pocket, rapid metabolism causing a short half-life, and moderate lipophilicity affecting solubility.[3] Structure–Activity Relationship (SAR) studies indicate that strategic modifications can enhance its therapeutic potential.[5,6,7] Specifically, substitution at the 5-OH position improves binding affinity and solubility, modification at the 7-OH position enhances lipophilicity, and the introduction of halogens at the 3′, 4′, and 5 positions increases binding stability within the receptor pocket.[4,5] Additionally, side-chain insertion can promote π–π stacking interactions, further strengthening ligand–receptor binding.[4,5] Based on these considerations, the present study aims to computationally design, screen, and evaluate twelve novel luteolin derivatives targeting EGFR using molecular docking, ADMET analysis, and toxicity prediction approaches, with a comparative assessment against the standard EGFR inhibitor Erlotinib.

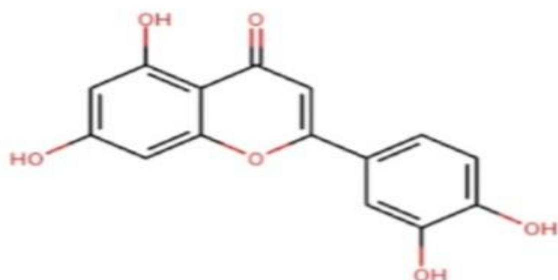

Figure 1: Chemical structure of original luteolin compound (3′,4′,5,7-tetrahydroxyflavone), the parent scaffold used for the design of novel derivatives targeting EGFR.

2. MATERIALS AND METHODS

2.1 Software used

Structure drawing -MolView
Protein retrieval - RCSB PDB (ID: 1M17)[8]
Docking -SwissDock (Attracting Cavities 2.0)[9]
ADME prediction – SwissADME[10]
Toxicity prediction - ProTox-3.0[11,12]

2.2 Ligand Preparation

Eleven modified structures were manually designed using SAR rationale.
Each structure was drawn in MolView and exported as SMILES.
SwissDock converted SMILES to 3D automatically.

2.3 Protein Preparation

EGFR tyrosine kinase domain (PDB: 1M17) downloaded.
Water molecules and heteroatoms were removed.
Minimization automatically handled by SwissDock.

2.4 Molecular Docking (SwissDock)

Mode: Attracting Cavities 2.0
Box centre: (8, −7, 58)
Box size: 20 × 20 × 20 Å
Scoring: SwissParam energy
Interpretation: Lower score = stronger binding affinity.[9]

2.5 ADMET Prediction (SwissADME)

Evaluated: Physicochemical properties
Lipinski criteria
TPSA
Log P
Solubility
CYP profile
Bioavailability score
GI absorption
Log Kp
Synthetic accessibility[10]

2.6 Toxicity Prediction (ProTox-3.0)

Parameters assessed:
LD_{50} (mg/kg)
Toxicity class
Hepatotoxicity
Clinical toxicity[11,12]

2.7 Comparison

Novel luteolin derivative compared with standard drug Erlotinib.

3. SAR RATIONALE FOR DESIGNED LUTEOLIN DERIVATIVES

3.1. 7-(Benzyloxy)-Luteolin

The 7–OH is involved only in mild hydrogen bonding, but it can be replaced by a bulky aromatic substituent to increase π–π stacking and hydrophobic interactions with the EGFR binding pocket (which contains aromatic amino acids like Phe, Tyr, and Trp). So this change expands the interaction surface area and helps the molecule fit deeper into the pocket. Also, benzyl group increases lipophilicity (LogP) → better membrane permeability and cell entry — while other hydroxyl groups at 5, 3′, and 4′ are still available for hydrogen bonding. So it not losing binding capacity, only enhancing pocket affinity. (Figure.02 as modified compound chemical structure).

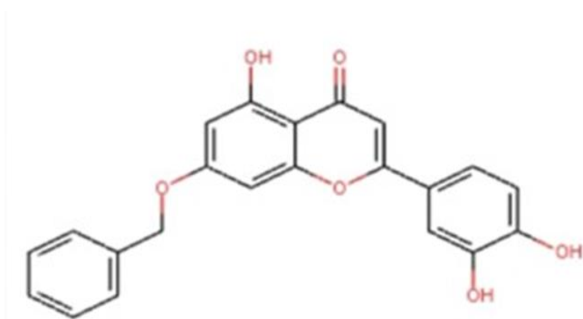

Figure 2: Chemical structure of modified luteolin derivative 7-(benzyloxy)-luteolin. The 7–OH is replaced by a bulky aromatic substituent to increase π–π stacking and hydrophobic interactions with the EGFR binding pocket.

3.2. 8-fluoro-3-(aminomethyl)-luteolin

The aminomethyl at C-3 introduces a new directional hydrogen bond donor/acceptor, which can reach the hinge region of EGFR and form an additional H-bond anchor — this is known to significantly improve binding affinity in kinase inhibitors. The 8-F substitution slightly increases lipophilicity and metabolic stability, helping the molecule survive longer in biological systems. (Figure.03 as modified compound chemical structure).

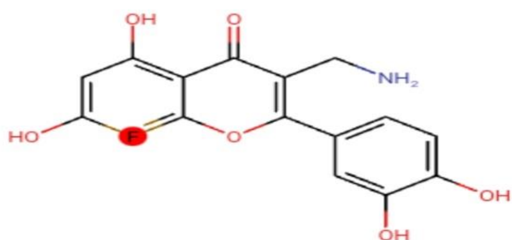

Figure 3: Chemical structure of modified luteolin derivative 8-fluoro-3-(aminomethyl)-luteolin. The aminomethyl at C-3 introduces a new directional hydrogen bond donor/acceptor, which can reach the hinge region of EGFR and form an additional H-bond anchor — this is known to significantly improve binding affinity in kinase inhibitors. The 8-F substitution slightly increases lipophilicity and metabolic stability.

3.3. 3-(carboxamide)-luteolin

C-3 is substituted to $–CONH_2$ (amide) instead of H. Carbonyl + NH can act as directional H-bond donor/acceptor and better mimic hinge binding patterns. Amide orientation often forms stable H-bonds. Predicted to be Increase in H-bond network improvement. (Figure.04 as modified compound chemical structure).

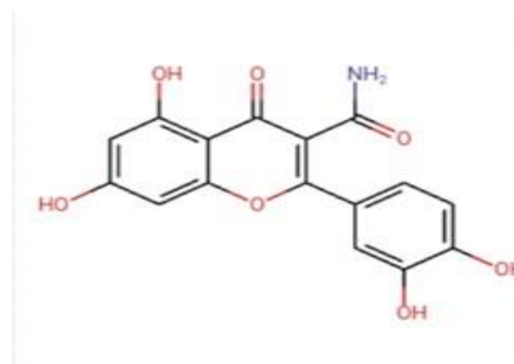

Figure 4: Chemical structure of modified luteolin derivative 3-(carboxamide)-luteolin. C-3 is substituted to $–CONH_2$ (amide) instead of H. Carbonyl + NH can act as directional H-bond donor/acceptor and better mimic hinge binding patterns.

3.4. 2-(pyridin-3-yl)-luteolin

Replace B-ring phenyl (at C-2) by pyridine. Pyridine N can form H-bond acceptor or coordinate with residues; changes electronics and can form new polar contacts. Predicted to be Increase or change binding pose — could improve affinity if N points to H-bond donor. (Figure.05 as modified compound chemical structure).

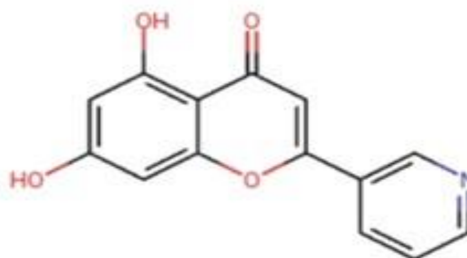

Figure 5: Chemical structure of modified luteolin derivative 2-(pyridin-3-yl)-luteolin. Replace B-ring phenyl (at C-2) by pyridine. Pyridine N can form H-bond acceptor or coordinate with residues; changes electronics and can form new polar contacts. Predicted to be Increase or change binding pose — could improve affinity if N points to H-bond donor.

3.5. 7-O-(2-pyridylmethyl)-luteolin

The introduction of a 2-pyridylmethyl substituent at the 7-O position incorporates a heteroaromatic pyridine ring, where the nitrogen atom functions as a hydrogen-bond acceptor. This modification enhances π–π stacking interactions within the EGFR binding pocket and improves polarity and pharmacokinetic properties compared to unsubstituted luteolin. (Figure.06 as modified compound chemical structure).

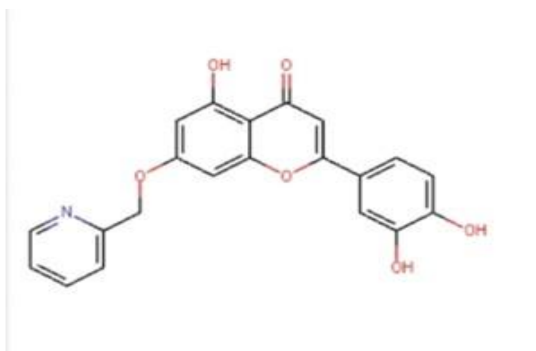

Figure 6: Chemical structure of modified luteolin derivative 7-O-(2-pyridylmethyl)-luteolin. The introduction of a 2-pyridylmethyl substituent at the 7-O position incorporates a heteroaromatic pyridine ring, where the nitrogen atom functions as a hydrogen-bond acceptor. This modification enhances π–π stacking interactions within the EGFR binding pocket.

3.6. 7-(benzyloxy)-3',4'—dichloroluteolin

Replace the 3′-OH and 4′-OH on the B-ring with Cl atoms. Convert the 7-OH (A-ring) into a benzyloxy group (7–O–CH_2–Ph). Keep the 5-OH on the A-ring and the flavone core (C2=C3 double bond and C4=O) intact. Overall these changes were expected to improve binding affinity and docking score while maintaining the structural integrity of luteolin. (Figure.07 as modified compound chemical structure).

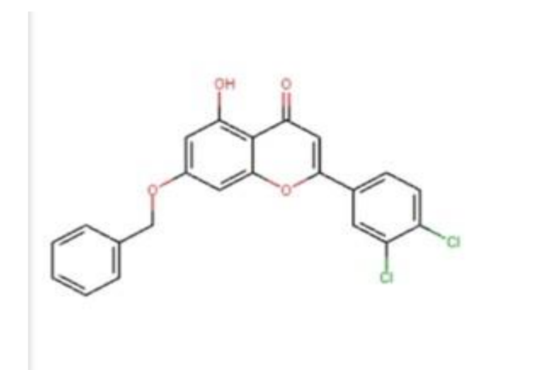

Figure 7: Chemical structure of modified luteolin derivative 7-(benzyloxy)-3',4'—dichloroluteolin. Replace the 3′-OH and 4′-OH on the B-ring with Cl atoms. Convert the 7-OH (A-ring) into a benzyloxy group (7–O–CH_2–Ph). Keep the 5-OH on the A-ring and the flavone core (C2=C3 double bond and C4=O) intact. Overall these changes were expected to improve binding affinity and docking score while maintaining the structural integrity of luteolin.

3.7. 3-(aminomethyl)-flavone luteolin

C2=C3 double bond reduced → flavone converted to flavanone, increasing molecular flexibility. Amino group introduced at C-3 improves polarity and potential hydrogen-bonding ability. Net effect to be better solubility and additional H-bond interactions, but slightly reduced planarity may lower π–π stacking. (Figure.08 as modified compound chemical structure).

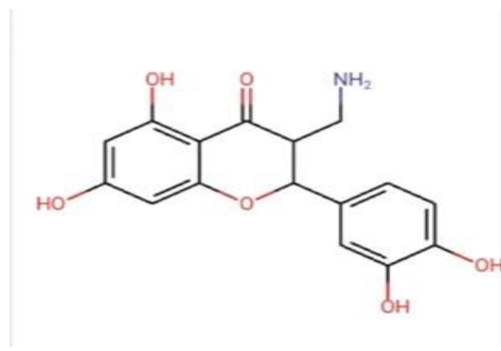

Figure 8: Chemical structure of modified luteolin derivative 3-(aminomethyl)-flavone luteolin. C2=C3 double bond reduced → flavone converted to flavanone, increasing molecular flexibility. Amino group introduced at C-3 improves polarity and potential hydrogen-bonding ability.

3.8. 7-O-(4-pyridylmethyl)-3′,4′-dichloro-5-O-phosphono-luteolin

7-OH → 7-O-(4-pyridylmethyl) and then 3′-OH and 4′-OH → 3′,4′-dichloro substitution and also 5-OH → 5-O-phosphate ester are changed. The pyridine nitrogen functions as a polar hydrogen-bond acceptor, subtly reducing overall lipophilicity compared to a benzyl group. This modification enhances aqueous solubility, lowers plasma protein binding, and reduces metabolic liability by decreasing the likelihood of CYP enzyme inhibition associated with electron-rich aromatic systems. Importantly, the aromatic ring system is retained, preserving π–π stacking interactions within the hydrophobic region of the EGFR ATP-binding pocket. The methylene spacer maintains spatial geometry, allowing optimal occupation of the binding cavity, while the pyridine nitrogen can establish additional polar or hydrogen-bond interactions with nearby amino acid residues, further stabilizing the ligand–protein complex.

The incorporation of a phosphate ester group introduces strong polar functionality that significantly enhances ligand–receptor interactions. The negatively charged phosphate moiety enables favourable electrostatic interactions with positively charged residues (such as lysine and arginine) present in the EGFR kinase domain. In addition, the multiple oxygen atoms of the phosphate group act as efficient hydrogen-bond acceptors, increasing the number and strength of non-covalent interactions within the active site. This group also contributes to improved ligand orientation by restricting excessive rotational freedom, effectively anchoring the flavone scaffold in a bioactive conformation that aligns optimally with key binding residues in the ATP-binding pocket. Collectively, these structural features result in a ligand that achieves stronger and more stable binding through a combination of hydrophobic interactions, π–π stacking, hydrogen bonding, and electrostatic forces, while simultaneously improving solubility, reducing protein binding, and maintaining an acceptable toxicity profile. This rational design explains the observed enhancement in docking score and overall in-silico

pharmacokinetic performance against EGFR. (Figure.09 as modified compound chemical structure).

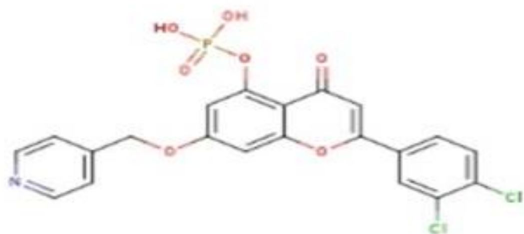

Figure 9: Chemical structure of modified luteolin derivative 7-O-(4-pyridylmethyl)-3′,4′-dichloro-5-O-phosphono-luteolin. 7-OH → 7-O-(4-pyridylmethyl) and then 3′-OH and 4′-OH → 3′,4′-dichloro substitution and also 5-OH → 5-O-phosphate ester are changed. Collectively, these structural features result in a ligand that achieves stronger and more stable binding through a combination of hydrophobic interactions, π–π stacking, hydrogen bonding, and electrostatic forces, while simultaneously improving solubility, reducing protein binding, and maintaining an acceptable toxicity profile.

3.9. 7-O-(propargyl)- luteolin

Structure of a luteolin derivative modified at the 7-O position with a propargyl group (7-O-CH_2–C≡CH). This electrophilic substitution is designed to explore potential covalent interactions with nucleophilic residues (e.g., cysteine) in the EGFR active site, which may enhance binding affinity but carries a risk of off-target reactivity. (Figure.10 as modified compound chemical structure).

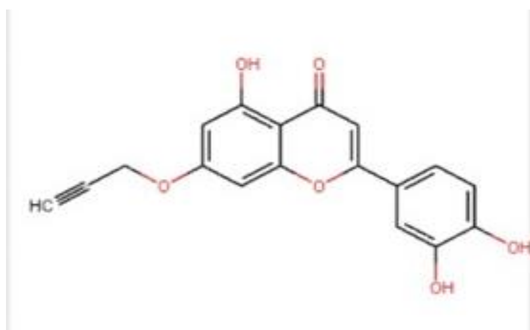

Figure 10: Chemical structure of modified luteolin derivative 7-O-(propargyl)- luteolin. Modified at the 7-O position with a propargyl group (7-O-CH_2–C≡CH). This electrophilic substitution is designed to explore potential covalent interactions with nucleophilic residues (e.g., cysteine) in the EGFR active site, which may enhance binding affinity but carries a risk of off-target reactivity.

3.10. 8-carboxy luteolin

Introducing a carboxylate group at position 8 of luteolin increases the polarity and creates new hydrogen-bonding or electrostatic anchoring possibilities. The 8-position faces the solvent-accessible region in many kinase pockets; hence, polar groups here can enhance binding specificity without severely disturbing the flavone's π-system. (Figure.11 as modified compound chemical structure).

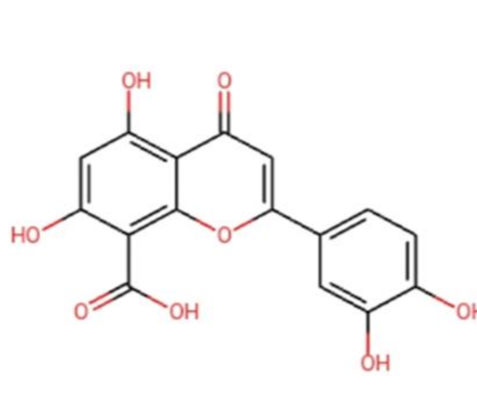

Figure 11: Chemical structure of modified luteolin derivative 8-carboxy luteolin. Introducing a carboxylate group at position 8 of luteolin increases the polarity and creates new hydrogen-bonding or electrostatic anchoring possibilities.

3.11. 7-O-(4-cyanobenzyl)-luteolin

In this derivative, the 7-OH group is replaced with a 4-cyanobenzyl moiety to form 7-O-(4-cyanobenzyl)-luteolin. This modification introduces a polar aprotic cyano (–CN) functional group, which acts as a hydrogen bond acceptor and enhances electron-withdrawing character without significantly increasing steric bulk. The substitution is expected to improve molecular orientation within the binding site and promote additional dipole interactions, while preserving the aromatic π–π stacking interactions of the flavone core. (Figure.12 as modified compound chemical structure).

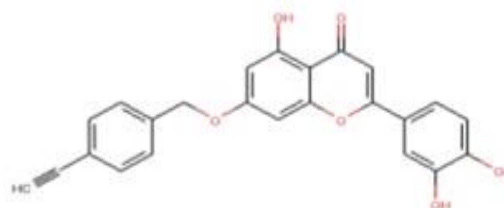

Figure 12: Chemical structure of modified luteolin derivative 7-O-(4-cyanobenzyl)-luteolin. In this derivative, the 7-OH group is replaced with a 4-cyanobenzyl moiety to form 7-O-(4-cyanobenzyl)-luteolin. This modification introduces a polar aprotic cyano (–CN) functional group, which acts as a hydrogen bond acceptor and enhances electron-withdrawing character without significantly increasing steric bulk. The substitution is expected to improve molecular orientation within the binding site and promote additional dipole interactions, while preserving the aromatic π–π stacking interactions of the flavone core.

3.12. 7-O-(4-pyridylmethyl)-3’,4’-dichloro luteolin

Modified at the 7-O position to a 7-O-(4-pyridylmethyl) derivative (–CH_2–C_5H_4N). The pyridine nitrogen acts as a hydrogen-bond acceptor, enabling additional polar interactions with the EGFR binding site, while the aromatic ring preserves π–π stacking. This modification reduces lipophilicity, lowering plasma protein binding, and enhancing metabolic stability.(Figure.13 as modified compound chemical structure).

Figure 13: Chemical structure of modified luteolin derivative 7-O-(4-pyridylmethyl)-3’,4’-dichloro luteolin Modified at the 7-O position to a 7-O-(4-pyridylmethyl) derivative (–CH_2–C_5H_4N). The pyridine nitrogen acts as a hydrogen-bond acceptor, enabling additional polar interactions with the EGFR binding site, while the aromatic ring preserves π–π stacking. This modification reduces lipophilicity, lowering plasma protein binding, and enhancing metabolic stability.

4. RESULTS

Table 1: Docking Results of All 12 Modified Compounds, Original compound and the standard drug compound.

S.NO:	COMPOUND NAME	DOCKING SCORE
1.	Original Luteolin	-6.5928
2.	Standard drug – Erlotinib	-7.6802
3.	7-(benzyloxy)-luteolin	-7.2342
4.	8-fluoro-3-(aminomethyl)-luteolin	-6.6821
5.	3-(carboxamide)-luteolin	-7.0793
6.	2-(pyridin-3-yl)-luteolin	-6.6034
7.	7-O-(2-pyridylmethyl)-luteolin	-7.3710
8.	7-(benzyloxy)-3’,4’—dichloro luteolin	-7.3291
9.	3-(aminomethyl)-flavone luteolin	-6.7660
10.	7-O-(4-pyridylmethyl)-3’,4’-dichloro-5-O-phosphono-luteolin	-8.2684
	7-O-(propargyl)- luteolin	-7.1681
11.	8-carboxy luteolin	-6.5542
12.	7-O-(4-cyanobenzyl)-luteolin	-7.1528
13.	7-O-(4-pyridylmethyl)-3’,4’-dichloro luteolin	-7.5406

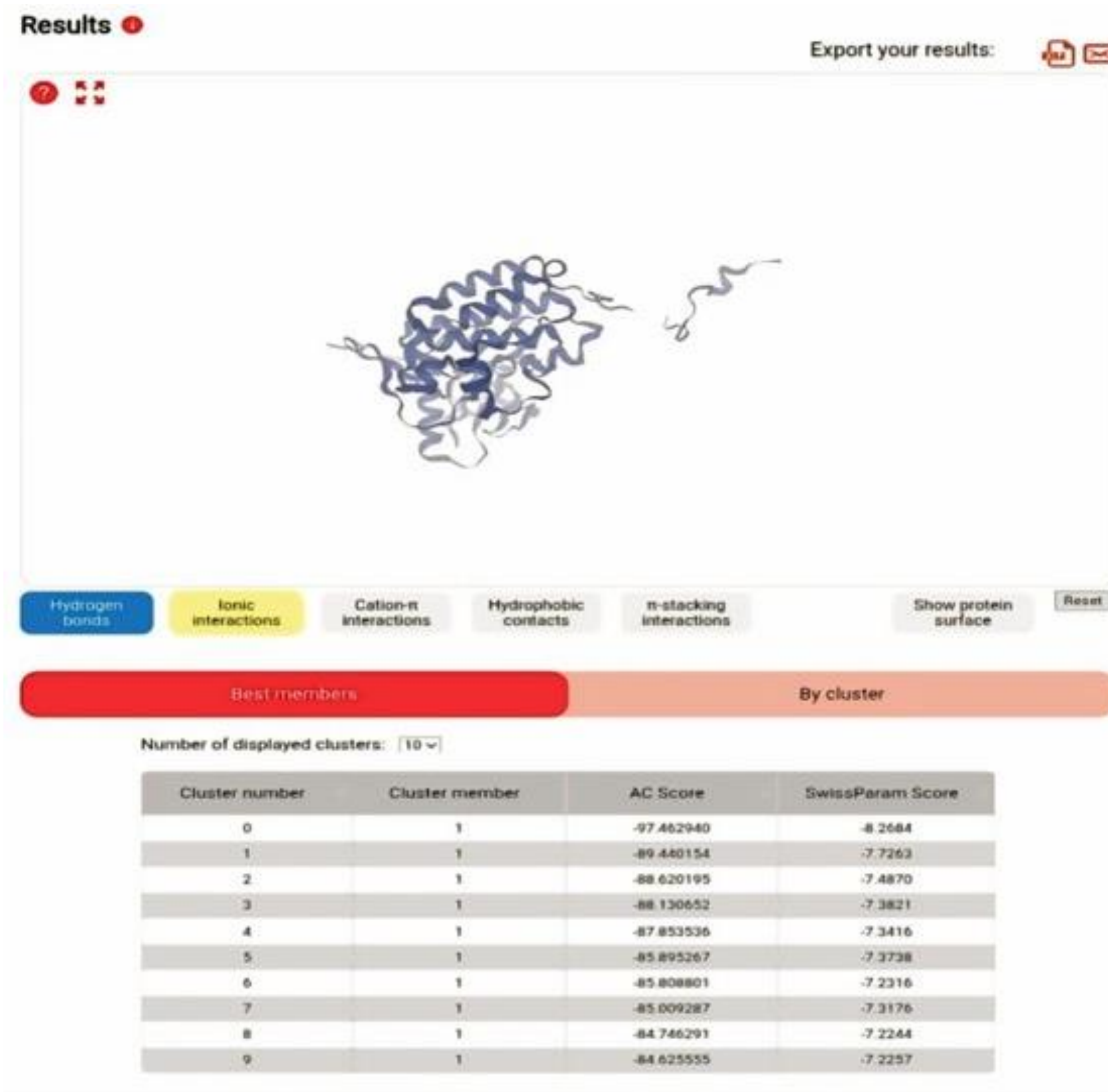

Figure 14: As Docking Result of best modified compound 7-O-(4-pyridylmethyl)-3’,4’-dichloro-5-O-phosphono-luteolin.

Figure.14 Molecular docking interaction of best modified luteolin derivative 7-O-(4-pyridylmethyl)-3′,4′-dichloro-5-O-phosphono-luteolin with EGFR protein using SwissDock. The compound demonstrated a strong binding affinity with a docking score of −8.2684 kcal/mol, indicating a stable ligand–protein interaction. The ligand was observed to interact with key amino acid residues within the active binding site through hydrogen bonding and hydrophobic interactions, contributing to the stabilization of the complex. The binding conformation suggests effective accommodation of the ligand within the EGFR active pocket, supporting its potential as an anticancer agent targeting EGFR.

Table 2: ADMET evaluation results of Original compound, Best modified compound and the standard drug.

CATEGORIES	ORIGINAL LUTEOLIN	BEST MODIFIED LUTEOLIN	STANDARD DRUG ERLOTINIB
Molecular weight (< 500)	286.24g/mol	494.22g/mol	393.44g/mol
Log P (consensus) [2-5]	1.73	3.32	3.23
TPSA(Å²) (<140Å²)	111.13Å²	128.90Å²	74.73Å²
H bond donors (<5)	4	2	1
H bond receptors	6	8	6

(<10)			
GI absorption	High	Low	High
Solubility	Moderately soluble	Moderately soluble	Moderately soluble
CYP2C19 & CYP2C9 inhibition	No	Yes	Yes
Log Kp (skin permeation) (-1 to -6)	-6.25cm/s	-6.91cm/s	-6.35cm/s
Drug likeness Rules	Yes	Yes	Yes
Bioavailability score	0.55	0.56	0.55
Synthetic accessibility	3.02	3.72	3.19
Predicted LD50 (2000-5000)	3919mg/kg	4000mg/kg	1250mg/kg
Predicted Toxicity Class	5	5	3
Hepatotoxicity	No	No	Yes
Clinical Toxicity	No	No	No

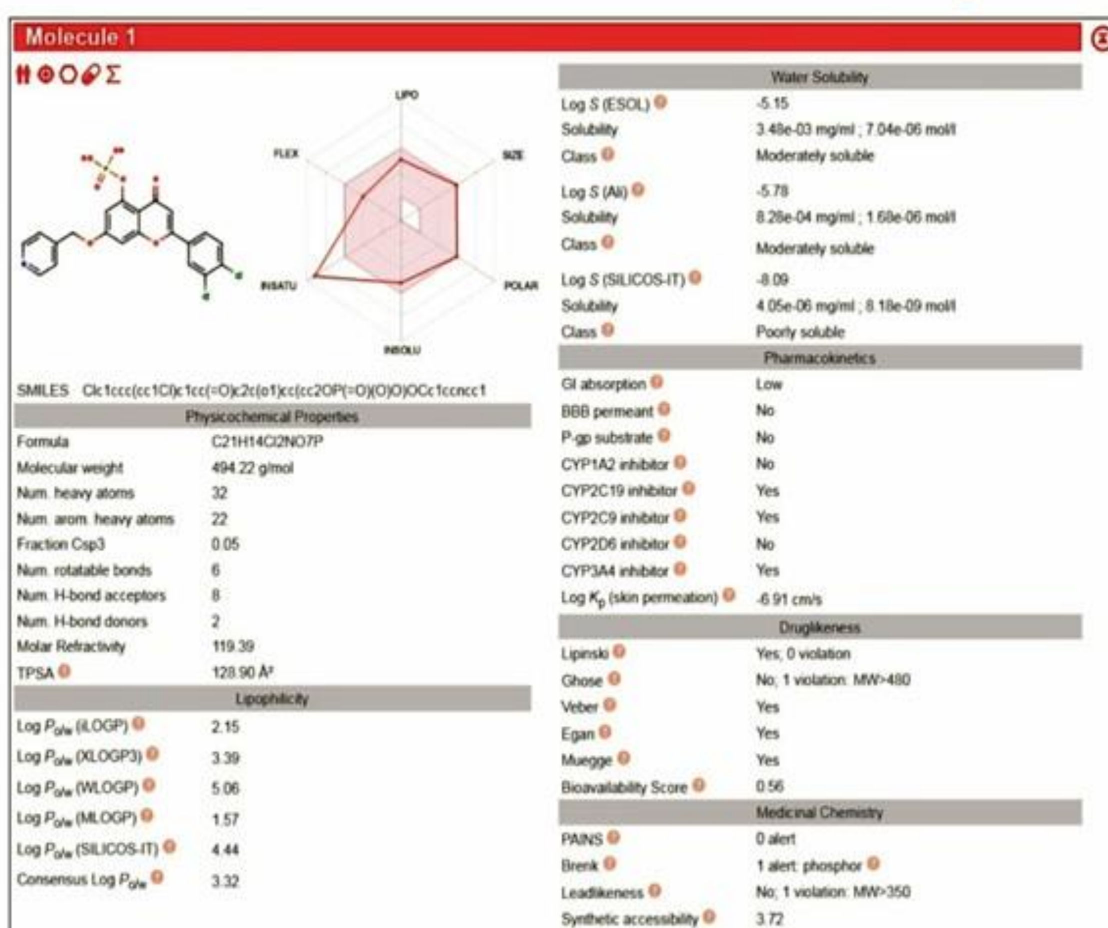

Figure 15: as SwissADME results for best modified compound 7-O-(4-pyridylmethyl)-3',4'-dichloro-5-O-phosphono-luteolin.

Figure.15 ADME and drug-likeness properties of the modified luteolin derivative predicted using SwissADME. The compound demonstrated moderate water solubility with Log S (ESOL) of −5.15 and consensus Log P of 3.32, indicating balanced lipophilicity. Pharmacokinetic analysis revealed absence of blood–brain barrier permeability. The compound was predicted as a non-substrate for P-glycoprotein and showed selective inhibition of CYP2C19, CYP2C9, and CYP3A4 enzymes. Drug-likeness evaluation indicated compliance with Lipinski, Veber, Egan, and Muegge rules, with one violation in Ghose criteria due to molecular weight exceeding 480 g/mol. The bioavailability score was found to be 0.56.

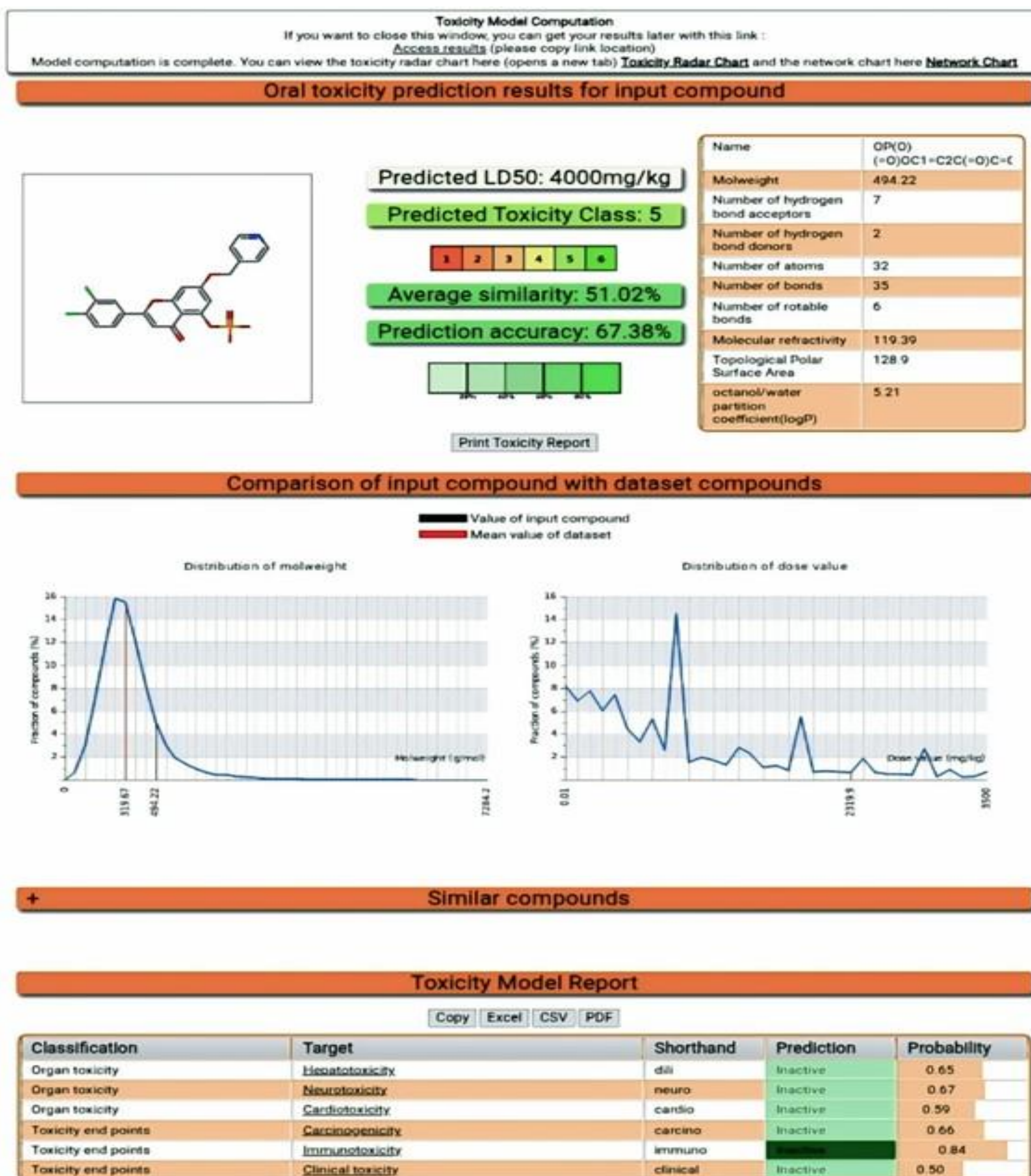

Figure 16: as ProTox3.0 Toxicity prediction results for modified best compound 7-O-(4-pyridylmethyl)-3',4'-dichloro-5-O-phosphono-luteolin.

Figure 16: In silico toxicity prediction profile of the modified luteolin derivative using pkCSM tool. The compound exhibited a predicted oral LD_{50} value of 4000 mg/kg, indicating low acute toxicity and was classified under toxicity class 5. The model showed an average similarity of 51.02% and prediction accuracy of 67.38%. Comparative analysis with dataset compounds demonstrated acceptable distribution patterns for molecular weight and dose values. Toxicity endpoint evaluation revealed inactive predictions for hepatotoxicity, neurotoxicity, cardiotoxicity, carcinogenicity, immunotoxicity, and clinical toxicity with moderate probability scores, suggesting a favourable safety profile.

4.1 Interpretation

The best derivative: 7-O-(4-pyridylmethyl)-3',4'-dichloro-5-O-phosphono-luteolin
Outperformed natural luteolin by ~1.68 kcal/mol
Approaches Erlotinib (difference only 0.59 kcal/mol)
Indicates enhanced EGFR binding affinity (better than earlier derivatives)[9]
Improved aqueous solubility[10]
Acceptable lipophilicity balance
Favourable toxicity profile (ProTox Class 5)[11,12]

Thus, the final compound achieved an optimal balance between binding strength, physicochemical properties, and predicted safety, making it superior to both the parent luteolin and earlier modified analogues.

5. DISCUSSION

This study successfully demonstrates how rational structural modification of luteolin improves its anticancer potential against EGFR, a key driver of Non-Small Cell Lung Cancer, the most prevalent lung cancer type.[1]

5.1 Luteolin as a Promising Lead Scaffold

Although luteolin is not a conventional allopathic anticancer drug, it exhibits intrinsic anticancer properties through inhibition of multiple oncogenic pathways, including EGFR phosphorylation, PI3K/Akt/mTOR signalling, MAPK/ERK signalling, and NF-κB-mediated inflammatory responses.[3]

These multi-target effects make luteolin a valuable starting scaffold for drug design. However, its clinical applicability is limited by suboptimal pharmacokinetic properties, necessitating structural modifications to enhance its bioavailability and efficacy.[4,5,6]

5.2 Structural Basis for Enhanced Binding and ADMET Profile of the Optimized Compound

The final optimized compound, 7-O-(4-pyridylmethyl)-3′,4′-dichloro-5-O-phosphono-luteolin, demonstrated the highest EGFR binding affinity and an improved pharmacokinetic profile due to the synergistic contribution of heteroaromatic substitution, halogenation, and phosphate ester incorporation. Key structural contributions are described below.

5.2.1 4-Pyridylmethyl substitution at the 7-O position

The introduction of a 4-pyridylmethyl group preserved the aromatic π-system necessary for strong π–π stacking interactions within the EGFR ATP-binding pocket. The pyridine nitrogen acts as a polar hydrogen-bond acceptor, enabling additional stabilizing interactions with hinge-region residues. Compared to a benzyl group, this heteroaromatic substitution moderately reduces excessive lipophilicity, lowers plasma protein binding, and improves solubility while maintaining optimal binding geometry through the flexible $–O–CH_2–$ linker.

5.2.2 3′,4′-Dichloro substitution on the B-ring

Halogenation at the 3′ and 4′ positions significantly enhanced hydrophobic and van der Waals interactions within the lipophilic regions of the EGFR active site. Chlorine atoms improve cavity filling, increase binding stability, and reduce metabolic vulnerability of the aromatic ring, contributing to both higher docking scores and improved metabolic robustness.

5.2.3 5-O-Phosphono substitution

Incorporation of a phosphate ester group at the 5-OH position markedly improved the interaction profile of the ligand. The phosphate moiety introduces strong electrostatic interactions with positively charged residues (such as Lys and Arg) in the kinase domain. Additionally, multiple phosphate oxygen atoms function as powerful hydrogen-bond acceptors, anchoring the ligand more firmly within the ATP-binding site. This group also restricts excessive conformational flexibility, stabilizing the flavone core in an orientation favourable for high-affinity binding.[4,5,6]

5.3 Mechanistic Pathway of EGFR Inhibition by the Final Best Compound

The optimized compound 7-O-(4-pyridylmethyl)-3′,4′-dichloro-5-O-phosphono-luteolin is predicted to exert its anticancer effect through potent inhibition of EGFR-mediated signalling pathways, which are critically dysregulated in Non-Small Cell Lung Cancer (NSCLC).[1,2]

5.3.1 Proposed Mechanism of Action

Step 1: High-affinity binding to EGFR ATP-binding site
The compound occupies the ATP-binding pocket of EGFR with strong electrostatic, hydrogen-bonding, and hydrophobic interactions, effectively competing with ATP.
Step 2: Inhibition of EGFR autophosphorylation
By blocking ATP access, EGFR tyrosine kinase activity is suppressed, preventing receptor autophosphorylation.
Step 3: Downregulation of downstream oncogenic pathways
↓ PI3K/Akt pathway → suppression of cell survival and anti-apoptotic signalling
↓ RAS/RAF/MEK/ERK pathway → inhibition of uncontrolled cell proliferation
↓ VEGF signalling → reduction in angiogenesis
↑ Mitochondrial apoptotic signalling → cytochrome-c release and programmed cell death.[5,6]

This mechanism is highly relevant to NSCLC, The strategic incorporation of heteroaromatic substitution, halogenation, and phosphate ester functionality significantly enhanced both pharmacodynamic and pharmacokinetic properties while maintaining structural integrity of the luteolin scaffold.

6. CONCLUSION

This in silico investigation successfully designed and evaluated twelve novel luteolin derivatives targeting EGFR in Non-Small Cell Lung Cancer using molecular docking, ADMET profiling, and toxicity prediction approaches.[1] Among them, 7-O-(4-pyridylmethyl)-3′,4′-dichloro-5-O-phosphono-luteolin emerged as the most promising candidate.

The compound demonstrated

Superior EGFR binding affinity
Improved solubility compared to earlier derivatives
Balanced lipophilicity
Low predicted toxicity (ProTox Class 5)
Strong mechanistic relevance to NSCLC via EGFR pathway inhibition

The strategic incorporation of heteroaromatic substitution, halogenation, and phosphate ester functionality significantly enhanced both

pharmacodynamic and pharmacokinetic properties while maintaining structural integrity of the luteolin scaffold. Overall, this study highlights the successful transformation of a traditional flavonoid lead into a potent, drug-like EGFR inhibitor with strong potential for further preclinical development in NSCLC therapy.[4,5,6]

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