RESEARCH PAPER

Phenanthrene-based Pyrazoline Derivatives as Emerging Anticancer Agents: Synthetic Strategy, Molecular Targets, Structure Activity relationships and *in-silico* Approaches

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ABSTRACT

Cancer remains one of the leading causes of mortality worldwide and continues to pose a major challenge to healthcare systems despite significant advances in diagnosis and treatment. Conventional chemotherapy is frequently associated with poor selectivity, systemic toxicity, multidrug resistance and disease recurrence, highlighting the need for safer and more effective targeted anticancer agents. Hybrid molecules containing multiple pharmacologically active scaffolds have emerged as a promising strategy for overcoming these limitations. Among these, phenanthrene, pyrazoline, carboxamide and phenolic pharmacophores have attracted considerable attention because of their diverse biological activities and their ability to interact with multiple molecular targets involved in tumor initiation and progression. Phenanthrene derivatives exhibit strong DNA-binding properties and interfere with cell proliferation, whereas pyrazoline derivatives possess remarkable antiproliferative, antiangiogenic and pro-apoptotic activities. Carboxamide moieties enhance receptor binding through hydrogen-bonding interactions and contribute to improved pharmacokinetic properties, while phenolic substituents provide antioxidant activity and regulate intracellular signaling pathways associated with oxidative stress and apoptosis.

This review summarizes recent developments in phenanthrene-based pyrazoline carboxamide derivatives as potential anticancer agents. Particular emphasis is placed on synthetic strategies, structure–activity relationships(SAR), molecular mechanisms and important therapeutic targets including Epidermal Growth Factor Receptor (EGFR), Vascular Endothelial Growth Factor Receptor-2 (VEGFR-2), Carbonic Anhydrase IX/XII (CA IX/XII), Topoisomerase II, BRAF kinase, Aurora kinases, Cyclin-Dependent Kinase-2 (CDK2) and tubulin. The role of molecular docking, virtual screening, drug-likeness evaluation and ADME/ADMET prediction in lead optimization is also discussed. Finally, current challenges and future perspectives for developing multifunctional phenanthrene–pyrazoline hybrids are highlighted. The integration of experimental medicinal chemistry with computational drug discovery is expected to accelerate the development of novel targeted anticancer therapeutics.

Keywords: Phenanthrene, pyrazoline, carboxamide, phenolic group, Anticancer agent, structure-activity relationships, Molecular Docking, ADME, drug discovery

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1. INTRODUCTION

1.1. Global burden of cancer

Cancer remains one of the leading causes of morbidity and mortality worldwide, posing a significant public health challenge. Cancer is a condition characterised by the abnormal behaviour of certain cells within the body.(1) In a healthy person, cells follow a specific pattern of growth, division and eventually, natural death, which is essential to maintain proper body function. However, in the case of cancer, these cells deviate from this normal behaviour, undergoing. Millions of new cancer cases and cancer-related deaths are recorded worldwide each year, according to recent estimates and the burden is predicted to rise as a result of population expansion, ageing, urbanisation and risk factors connected to lifestyle. Conventional therapeutic techniques like chemotherapy and radiotherapy are frequently linked to serious side effects, poor

selectivity, multidrug resistance and limited efficacy against advanced-stage cancers, despite significant advancements in diagnostic and treatment.(2)

1.2. Importance of heterocyclic compounds in medicinal chemistry

In medical chemistry and pharmaceutical research, heterocyclic compounds are among the most significant types of molecules. These substances have one or more heteroatoms, like sulphur, nitrogen or oxygen, integrated into a cyclic structure that gives them special biological and physicochemical characteristics.(3) Heterocyclic compounds can interact with a variety of biological targets due to their structural diversity and synthetic accessibility. Heterocyclic moieties are found in a sizable percentage of clinically approved medications, underscoring their significance in the management of several illnesses, such as cancer, infectious diseases, cardiovascular diseases and neurological disorders. Heterocyclic systems are especially useful in the search for anticancer drugs because they can improve receptor binding affinity, reduce enzyme activity and modify important signalling pathways. Because of their wide range of biological activities, including anticancer, anti-inflammatory, antibacterial, antioxidant and enzyme-inhibitory properties, pyrazolines have garnered significant attention among the many heterocyclic scaffolds studied.(4)

1.3 Role of Phenanthrene in drug development

Three fused benzene rings placed in an angular configuration make up phenanthrene, significant polycyclic aromatic hydrocarbon (PAH). It is a colourless crystalline compound naturally present in coal tar, crude oil, fossil fuels and combustion products. Due to its rigid aromatic framework and unique physicochemical properties, phenanthrene has gained considerable importance in organic chemistry, medicinal chemistry, pharmaceutical research, material science and environmental studies.(5) Phenanthrene serves as a valuable structural scaffold for the synthesis of biologically active compounds and pharmaceutical intermediates. Many natural products, drugs, steroids, alkaloids and heterocyclic compounds contain phenanthrene or phenanthrene-like ring systems. Because of its planar aromatic structure, phenanthrene derivatives exhibit strong interactions with biological targets such as DNA, enzymes and receptors.(6)

1.4 Role of in Pyrazoline in drug development-

Pyrazoline is an important five-membered nitrogen-containing heterocyclic scaffold widely used in drug discovery due to its diverse pharmacological activities and favourable biological properties. Pyrazoline derivatives have demonstrated significant anticancer, antimicrobial, anti-inflammatory, antioxidant, antitubercular, anticonvulsant and antiviral activities. The presence of two nitrogen atoms and the ease of structural modification allow pyrazoline molecules to interact effectively with various biological targets, enhancing their potency and selectivity.(7) In anticancer research, pyrazoline derivatives have been reported to inhibit key targets involved in cell proliferation, apoptosis, angiogenesis and metastasis, making them promising candidates for the development of novel targeted therapeutics. Consequently, the pyrazoline scaffold continues to attract considerable attention in medicinal chemistry as a versatile pharmacophore for designing new drug candidates.(8)

1.5 Role of Phenolic Group in Drug Development

One of the most significant functional groups in medicinal chemistry, pharmaceutical chemistry and drug development is the phenolic group. A hydroxyl group (-OH) is directly bonded to an aromatic benzene ring in a phenolic group. Compounds containing phenolic groups are called phenolic compounds or phenols.(9) These compounds are naturally present in plants, natural products, secondary metabolites, vitamins, hormones and many pharmaceutical drugs. Phenolic compounds possess remarkable biological and pharmacological activities because of their ability to participate in hydrogen bonding, electron transfer, free radical scavenging and molecular interactions with biological targets. Due to these properties, phenolic groups are widely incorporated into drug molecules to improve potency, selectivity, pharmacokinetics, and therapeutic activity**.**(10)

1.6 Chemistry of Carboxamide

Carboxamides are an important class of organic compounds containing the amide functional group. They are widely used in medicinal chemistry, pharmaceutical chemistry, organic synthesis, polymers, dyes, agrochemicals and biological systems. The carboxamide group is present in proteins, peptides, vitamins, antibiotics and many drug molecules. A carboxamide contains a carbonyl group (**C=O**) attached to a nitrogen atom (**–NH_2**, –NHR or –NR_2). Due to resonance stabilization and hydrogen bonding ability, carboxamides possess unique chemical and biological properties.(11)

An efficient method for creating strong and specific anticancer drugs is the integration of heterocyclic pharmacophores into physiologically active molecular frameworks. As a result, research into new heterocyclic compounds is still essential to the development of tailored cancer treatments.(12)

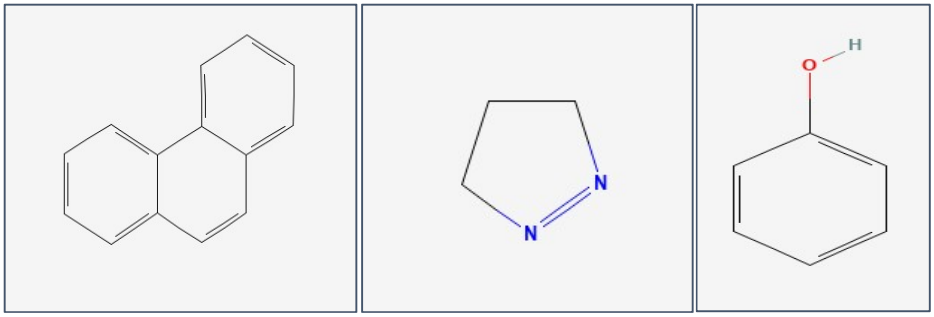


Fig. 1. Structure of Phenanthrene, Pyrazoline and Phenol compound

2. PHENANTHRENE SCAFFOLD IN ANTICANCER DRUG DISCOVERY:

2.1. Introduction

Phenanthrene ($C_{14}H_{10}$) features a planar, angularly fused, tricyclic aromatic hydrocarbon skeleton. Its planar geometry enables it to intercalate between DNA base pairs, while structural derivatization with specific functional groups creates potent anticancer agents that halt cell division, trigger apoptosis, and inhibit tumour kinases. (13)

To maximise cytotoxicity, the base phenanthrene core by itself is frequently insufficient and necessitates particular chemical modifications.

2.2. Structure-activity relationship of phenanthrene

1. Planar Core: The molecule can glide between DNA base pairs, interfering with DNA synthesis, thanks to the conjugated 14π-electron system of the three fused benzene rings.
2. Substituent Groups: Target selectivity and potency are determined by the addition of electron-donating (such as methoxy or hydroxyl groups) and electron-withdrawing (such as carboxylates) groups to particular rings.
3. Quinone Modifications: Cytotoxicity against epithelial and colon carcinomas is greatly increased by oxidizing the core into phenanthrenequinone, especially at the 9,10-positions.
4. Nitrogen-Containing Side Chains: Strong inhibitors that prevent DNA/RNA synthesis and causes apoptosis are produced by fusing the skeleton with pyrrolidine, piperidine, or other alkaloid-like nitrogen chains.(14)

Structural Classes of phenanthrene derivatives with their Potential Mechanisms are mentioned in the table. 1.

Table 1. Representative Phenanthrene derivatives with anticancer activity.(15)

Sr. no.	Author name	Phenanthrene compound	Target	Cancer Cell line	Mechanism	IC_{50}
1.	Wang et.al.	Phenanthrene based Tylophorine (PBT's)	Human lung cancer	H460	Induce cell cycle arrest and apoptosis by suppressing At and NF-kB signalling pathway	6.1 uM
2.	H. Guedour et. al.	Phenanthrenequinone	Colon cancer and epithelial carcinoma	Caco-2, Hep-2	Functions as NA intercalator and generates reactive oxygen species, induce cell death	0.97 ug/ml against Caco-2, 2.81 ug/ml against Hep-2
3.	Chia-Ching Liaw et. al.	Naturally occurring plant based Phenanthrene	Breast cancer, cervical cancer, non-small cell lung cancer	MCF-7, HeLa, A549	Destabilize microtubules, triggering mitotic arrest during M-phase	0.02ug/ml against MCF-7, 0.5 ug/ml against all
4.	Y, Y, Wang et. al.	Phenanthrene-9-benzimidazole	Prostate cancer	PC-3	Directly bind to DNA by intercalation	Nanomolar to micromolar

3. PYRAZOLINE SCAFFOLD IN ANTICANCER DRUG DISCOVERY

Pyrazolines are five-membered heterocyclic rings that include nitrogen and are very useful scaffolds in the search for anticancer drugs. To target cancer cells through a variety of biological pathways, their

fundamental structure, more especially, the stable 2-pyrazoline isomer, can be significantly altered by electron-donating or withdrawing groups.(16)

3.1. Pharmacological Action and Core Structure

A pyrazoline's fundamental structure is a five-membered ring with one endocyclic double bond, three carbon atoms and two nearby nitrogen atoms.(17)

3.2. Mechanism of Action

Pyrazolines exhibit strong antiproliferative activity. They cause apoptosis (programmed cell death), function as tyrosine kinase inhibitors (targeting VEGFR and EGFR) and block enzymes to stop the growth of cancer cells.(7)

3.3. Structure-activity relationship of Pyrazoline

Pyrazoline's antitumor effectiveness is strongly influenced by its substituents:

1. Ring A (3-position): This is where aryl groups, such as substituted phenyl rings, are typically found. Methoxy (-OCH_3) and halogen (Cl, F) modifications frequently show the greatest inhibition against breast and cervical cancer cell types.
2. Ring B (5-position): This location is often where furan, thiophene, or indole moieties are integrated, improving the molecule's capacity to connect with target receptors such as cyclooxygenase-2 (COX-2).
3. N-1 position: Adding a phenyl or acetyl group to the nitrogen usually increases its lipophilicity, making it easier to pass through the membranes of cancer cells.(18)

Structural Classes of Pyrazoline derivatives with their Potential Mechanisms are mentioned in the table. 1.

Table 2. Representative Pyrazoline derivatives with anticancer activity.(19)

Sr. no.	Author name	Pyrazoline compound	Target	Cancer Cell line	Mechanism	IC_{50}
1.	Basma S Gabr et.al.	3-naphthyl-5-phenylpyrazoline-1-phenylthioamide (influenced by methoxy groups at C-5)	Colorectal	HCT-116	AurA and AurB kinases	210.55 uM
2.	Rasha Hassan et. al.	Pyrazoline hybrid	Ovarian & Breast	OVCAR-4, MDA-MB-468	VEGFR-2 pathway	0.29 uM
3.	Wang et. al.	Substituted pyrazoline	Hepatoma & Cervical	HepG-2, HeLa	Destabilize microtubules, triggering mitotic arrest during M-phase	6.78 uM
4.	Menier Al-Anazi et. al.	Benzo[b]thiophen-2-yl-[5-(4-hydroxy-3,5-dimethoxy-phenyl)-3-(2-hydroxy-phenyl)	Hepatocellular Carcinoma	Hep-G2	Apoptosis/G2/M Arrest	3.57 uM

4. CARBOXAMIDE SCAFFOLD IN ANTICANCER DRUG DISCOVERY:

Carboxamide derivatives are privileged scaffolds in modern drug discovery. Their structure features a carbonyl group linked to an amine and can be broadly categorised into (N)-substituted and (N)-unsubstituted variants. They show potent anticancer activity through a modular structure that can be optimised for various targets essential structural elements. (11)

4.1. Mechanism of action

Depending on their specific chemical architecture, carboxamide derivatives exhibit strong activity against several key pathways:

1. Enzyme Inhibition**:** They interact with cancer-related targets, including DNA topoisomerase, EGFR, and \(PI3K\alpha\), often triggering apoptosis in tumor cells.
2. Microtubule Disruption: By binding to the tubulin-colchicine pocket, they force cell cycle arrest and inhibit cancer progression.(20)

4.2. Structure-activity relationship of Carboxamide

Certain structural substitutions play a major role in carboxamides' anticancer efficacy-

1. **The Linkage:** Important hydrogen bonding and hydrophilic interactions with target enzymes are provided by the α-CONH-) (carboxamide) bond.
2. **Heterocyclic Cores:** Quinoline/quinolone, indole, thiazole or thiophene are examples of favoured aromatic or heterocyclic scaffolds to which the carboxamide group is usually linked. The molecule is propelled into particular receptor binding sites by these cores.
3. **N-Terminus Substitutions:** Modifying the

nitrogen on the carboxamide with aryl (e.g., fluorophenyl) or bulky aliphatic groups drastically alters binding affinity.

4. **Bioisosteric Mimicry:** Thiophene carboxamide structures have been designed to mimic naturally occurring tubulin inhibitors (like Combretastatin A-4), disrupting cancer cell spheroid formation.(21)

Structural classes of carboxamide derivatives with their potential mechanisms are mentioned in the table. 1.

Table 3. Representative carboxamide derivatives with anticancer activity.(22)

Sr. no.	Author name	Carboxamide compound	Target	Cancer Cell line	Mechanism	IC_{50}
1.	Shaoyong Ke et al. (2022)	Diarylamine-based carboxamides	Gastric, liver cancer	SGC-7901 (gastric), A875 (melanoma), HepG2 (liver)	Induction of apoptosis and inhibition of cancer cell proliferation	0.29 uM
2.	Tao Qian Zhao et al. (2018)	Purine-carboxamide derivatives	lung cancer	A549 (lung), MGC803 (gastric), PC-3 (prostate)	Selective anti-lung cancer activity; inhibition of tumor cell growth	6.78 uM
3.	Xiaopei Yang et al. (2025)	Various carboxamide scaffolds	cell-cycle arrest	Multiple cancer cell lines	Kinase inhibition, enzyme inhibition, apoptosis induction, cell-cycle arrest	3.57 uM

5. PHENOLIC SCAFFOLD IN ANTICANCER DRUG DISCOVERY

Phenolic compounds, which contain one or more aromatic benzene rings with hydroxyl (-OH) substitutions, exhibit powerful anticancer properties. They exert cytotoxic, apoptotic and anti-angiogenic effects on cancer cells. Their potency strongly correlates with the number and position of hydroxyl groups on the ring.(15)

5.1. Mechanism of action

1. Autophagy and apoptosis: By boosting tumour suppressor proteins (like p53) and controlling proteins like Bcl-2, they encourage programmed cell death in tumour cells.
2. Cell Cycle Arrest: They stop cancer cells at different phases of the cell cycle by blocking oncogenic signalling pathways that regulate cell proliferation.
3. Anti-Angiogenesis & Metastasis: They limit the growth of tumours by stopping the migration of cancer cells and the production of new blood vessels, or angiogenesis.(20)

5.2. Structure-activity relationship of the phenolic group

1. Hydroxyl Group Count: Higher antiproliferative and cytotoxic actions are usually associated with more hydroxyl substitutions on the aromatic ring.
2. Electron/Hydrogen Donation: The ring's hydroxyl groups offer superior electron or hydrogen donation capabilities. This enables the molecule to neutralize reactive oxygen species, scavenge free radicals and lessen oxidative stress, which is a key factor in the development of cancer.(23)
3. Ortho-Dihydroxy Groups: Compounds with two contiguous hydroxyl groups on the ring or an ortho-dihydroxy arrangement are very effective at scavenging free radicals and reducing the survival of cancer cells.
4. Chain Length and Substituents: Adding ester or methoxy groups to the phenolic ring or changing the side chains might change the cytotoxicity and bio-selectivity of some synthetic or modified derivatives, making them more selective to particular cancer receptors. (24)

6. STRUCTURE–ACTIVITY RELATIONSHIP (SAR) OF PHENANTHRENE–PYRAZOLINE CARBOXAMIDE DERIVATIVES

Structure–Activity Relationship (SAR) analysis is an indispensable component of medicinal chemistry because it establishes the relationship between the chemical structure of a molecule and its biological activity. Through systematic structural modification, medicinal chemists identify functional groups that enhance potency, selectivity, pharmacokinetic behaviour and safety while minimising toxicity.(25) In recent years, hybrid molecules containing phenanthrene, pyrazoline, carboxamide, and phenolic pharmacophores have demonstrated remarkable anticancer activity owing to their ability to interact with multiple molecular targets simultaneously.(26) The biological activity of these hybrid molecules depends not only on the presence of individual pharmacophores but also on the nature, number and position of substituents present on each scaffold.

The phenanthrene ring contributes a rigid, planar aromatic system that facilitates DNA intercalation and hydrophobic interactions within enzyme active sites. The pyrazoline ring provides structural flexibility and multiple hydrogen-bonding sites through its adjacent nitrogen atoms.(27) Incorporation of a carboxamide moiety significantly improves receptor binding by

forming hydrogen bonds with catalytic amino acid residues, whereas hydroxyl or methoxy substituents further modulate lipophilicity, antioxidant activity, and receptor selectivity. Consequently, optimisation of these structural features has become the principal strategy for designing multifunctional anticancer agents.(28)

6.1. Effect of Halogen Substitution

Halogen atoms, particularly fluorine and chlorine, are widely employed in medicinal chemistry to optimise pharmacological activity. Fluorine substitution increases metabolic stability by reducing oxidative metabolism while simultaneously improving receptor affinity through enhanced hydrophobic interactions. Chlorine substitution frequently increases cytotoxic potency by improving lipophilicity; however, excessive halogenation may decrease aqueous solubility. Among halogens, fluorinated pyrazoline derivatives consistently exhibit superior docking scores against EGFR, BRAF, VEGFR-2 and Carbonic Anhydrase IX compared with unsubstituted analogues.(29)

6.2. Influence of Hybrid Pharmacophore Design

Hybridisation of phenanthrene, pyrazoline, carboxamide and phenolic pharmacophores represents an effective strategy for developing multitarget anticancer agents. The phenanthrene nucleus provides DNA-binding capability, pyrazoline contributes enzyme inhibition, carboxamide enhances receptor recognition, and phenolic substituents regulate oxidative stress and apoptosis. Simultaneous incorporation of these pharmacophores frequently produces synergistic biological effects, including increased apoptosis, inhibition of angiogenesis, suppression of metastasis and improved selectivity toward malignant cells.(30)

Table 4. Summary of Structure–Activity Relationship

Structural modification	Observed biological effect
Phenanthrene nucleus	DNA intercalation and hydrophobic binding
Hydroxyl substitution	Increased hydrogen bonding and apoptosis
Methoxy substitution	Improved membrane permeability
Fluoro substitution	Enhanced metabolic stability and kinase inhibition
Carboxamide group	Strong hydrogen bonding and receptor affinity
N-Methyl carboxamide	Improved lipophilicity
Hydroxyphenyl group	Increased antioxidant and antiproliferative activity
Bulky aromatic substituents	Enhanced tubulin inhibition

7. DESIGNING A NEW DRUG

Drug design is a rational approach used to discover biologically active molecules with desired therapeutic effects. The process involves designing compounds that can bind selectively with biological targets and modulate their activity. Drug design mainly focuses on improving efficacy, minimising toxicity and enhancing pharmacokinetic properties such as absorption, distribution, metabolism and excretion (ADME). Drug design has been transformed by the advent of sophisticated computational tools that allow for molecular modelling, virtual screening and ligand-target interaction prediction. These methods shorten the time and expense needed to find new therapeutic candidates.

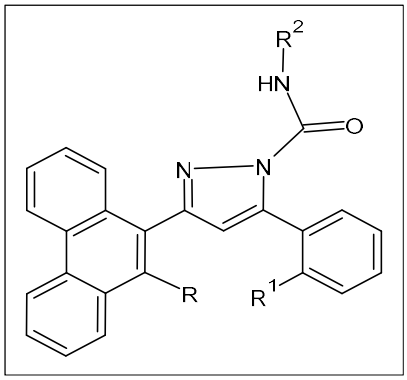


Fig. 2. Pyrazoline phenanthrene carboxamide phenol- main compound

The present hybrid design integrates four pharmacologically important structural motifs within a single molecular framework: a phenanthrene nucleus, a pyrazoline ring, a carboxamide linker and phenolic substituents. Each pharmacophore contributes a distinct biological function that collectively enhances anticancer activity.

The phenanthrene scaffold provides a rigid planar aromatic system that promotes π–π stacking interactions with nucleic acids and hydrophobic amino

acid residues. This characteristic facilitates DNA intercalation and enhances affinity toward enzymes such as Topoisomerase II and tubulin. The pyrazoline ring functions as the central heterocyclic pharmacophore responsible for broad-spectrum biological activity. The presence of adjacent nitrogen atoms enables strong hydrogen-bond formation and electrostatic interactions with catalytic residues present in kinase active sites. Consequently, pyrazoline derivatives frequently inhibit EGFR, VEGFR-2, Aurora kinase, CDK2, and Carbonic Anhydrase IX/XII. Incorporation of a carboxamide group further strengthens receptor binding by acting as both a hydrogen-bond donor and acceptor. Numerous kinase inhibitors currently approved for clinical use contain carboxamide functionality because of its ability to improve target selectivity and pharmacokinetic behaviour.

Finally, hydroxyl and methoxy substituents provide antioxidant properties, regulate intracellular reactive oxygen species, and enhance receptor recognition through hydrogen-bond interactions. The integration of these pharmacophores is therefore expected to generate multifunctional molecules capable of inhibiting proliferation, angiogenesis, metastasis, and tumour progression simultaneously.

8. SYNTHESIS

The synthesis involves three key reactions: first, **Claisen–Schmidt condensation** between substituted phenanthrene and aromatic benzaldehyde forms the chalcone via enolate formation and dehydration. Next, the chalcone undergoes **condensation with semicarbazide** to produce the corresponding semicarbazone through nucleophilic attack on the carbonyl group, followed by water elimination. Finally, the semicarbazone reacts with hydrazine hydrate via **Michael addition and intramolecular cyclisation** to afford the desired pyrazoline derivative. Thus, the overall process comprises condensation, dehydration, cyclisation and ring-closure reactions.

Step 1: Synthesis of Chalcone

Take equimolar quantities of 9-acetylanthracene and benzaldehyde in a round-bottom flask.

Add ethanol to it and mix well. Add 10% alcoholic sodium hydroxide solution slowly with continuous stirring. Stir the reaction mixture at room temperature for about 4–6 hours. Keep the reaction mixture overnight for completion of the reaction. Pour the mixture into crushed ice and acidify with dilute HCl. The yellow colored chalcone precipitate, obtained was filtered, washed with distilled water and dried. Recrystallise the product using ethanol.

Step 2: Synthesis of Semicarbazone Intermediate

Dissolve the synthesised chalcone derivative in ethanol. Add semicarbazide hydrochloride and a few drops of glacial acetic acid, and reflux the reaction mixture for about 5–6 hours. Monitor the progress of the reaction by TLC. After completion, cool the reaction mixture at room temperature and pour it into ice-cold water to obtain the precipitate. Filter the solid product, wash with water and dry. Purify the compound by recrystallisation using ethanol.

Step 3: Synthesis of Pyrazoline Derivative from Semicarbazone Intermediate

The synthesised semicarbazone intermediate was dissolved in ethanol in a round-bottom flask. A few drops of glacial acetic acid were added to the reaction mixture and refluxed for about 6–8 hours with continuous stirring. After completion of the reaction, the mixture was allowed to cool to room temperature. The reaction mixture was then poured into crushed ice-cold water to obtain the precipitated product. The solid pyrazoline derivative formed was filtered, washed with distilled water, and dried. The crude product was purified by recrystallization using ethanol to obtain pure pyrazoline derivative.

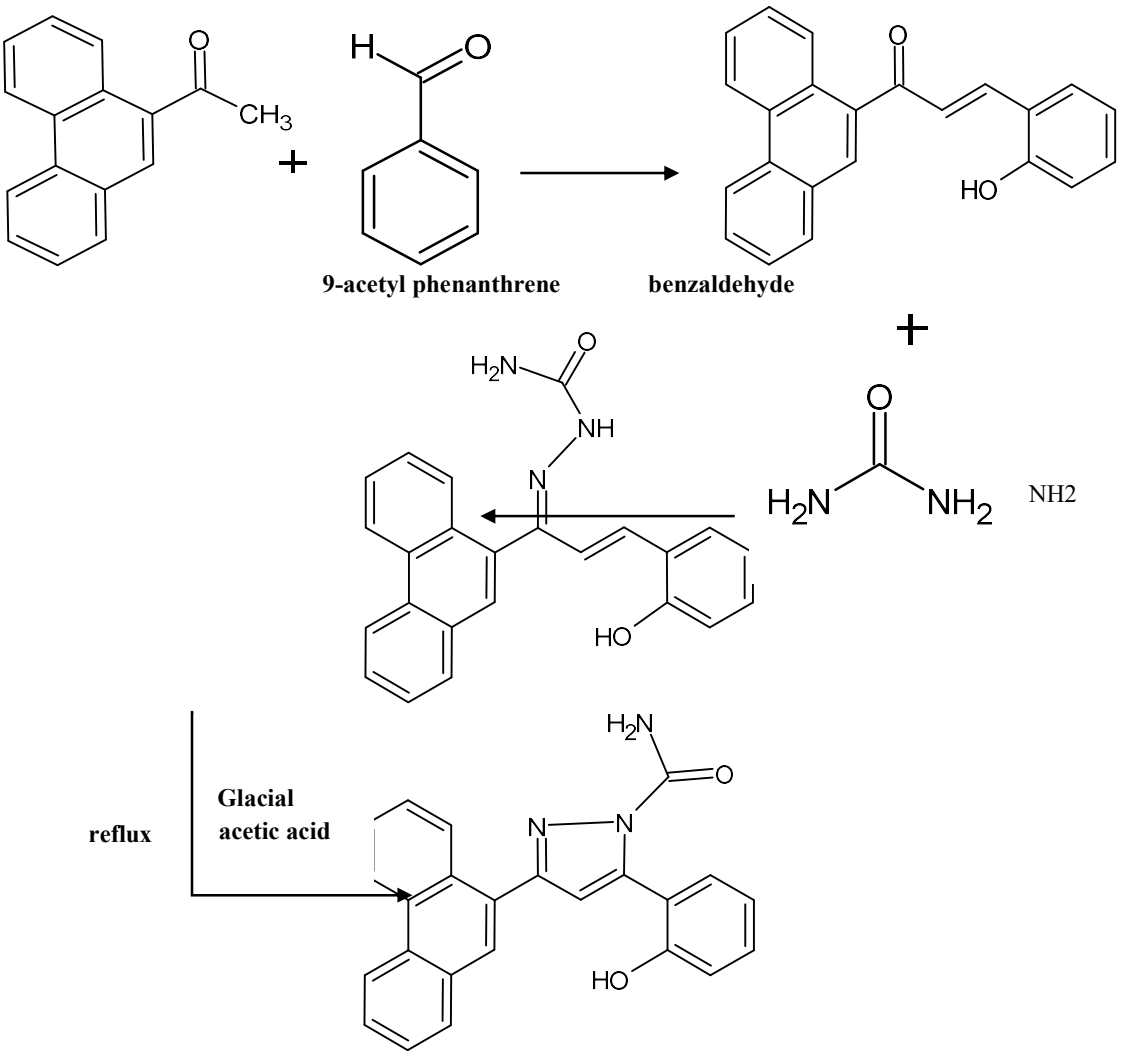
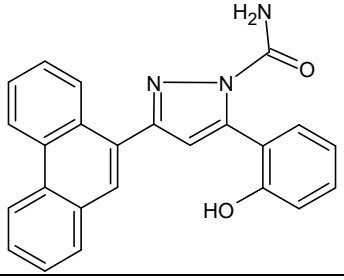
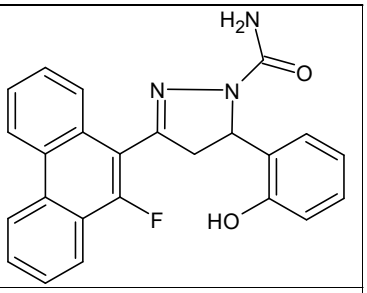
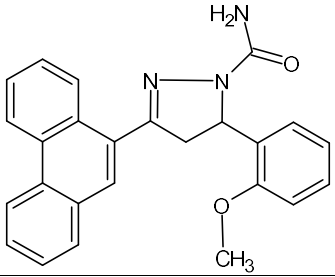
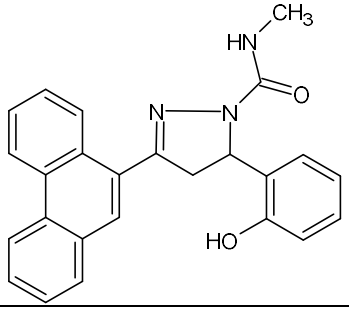
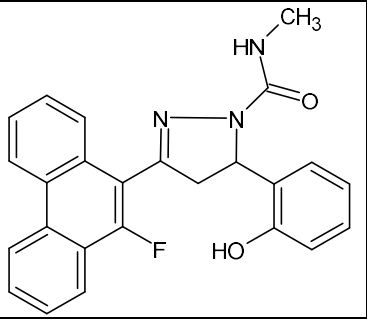


Fig. 3. Schematic flow of Phenanthrene pyrazoline derivative synthesis

Table 5. List of chemical compounds synthesized

Compound Code	Chemical Name	Structure
S1	**5-(2-hydroxyphenyl)-3-(phenanthren-9-yl)-1*H*-pyrazole-1-carboxamide**	

S2	**3-(10-fluorophenanthren 9-yl)-5- (2-hydroxyphenyl)-4,5-dihydro-1*H*-pyrazole-1-carboxamide**	
S3	**5-(2-methoxyphenyl)-3-(phenanthren-9-yl)-4,5-dihydro-1*H*-pyrazole-1-carboxamide**	
S4	**5-(2-hydroxyphenyl)-*N*-methyl-3-(phenanthren-9-yl-4,5-dihydro-1*H*-pyrazole-1-carboxamide**	
S5	**3-(10-fluorophenanthren-9-yl)-5-(2 hydroxyphenyl)-*N*-methyl-4,5-dihydro-1*H*-pyrazole-carboxamide**	

9. MOLECULAR TARGETS FOR ANTICANCER ACTIVITY

1. **Carbonic Anhydrase (CA) IX and XII:** Pyrazoline-1-carboxamide derivatives are identified as potent and selective inhibitors of tumour-associated carbonic anhydrases, specifically isoforms CA IX and XII. These enzymes are overexpressed in many cancer types and are critical for tumor survival in acidic microenvironments. Pyrazoline-1-carboxamide scaffolds are recognized as potent and selective inhibitors of these enzymes.(23)

2. **EGFR (Epidermal Growth Factor Receptor):** Some derivatives target EGFR tyrosine kinase to inhibit the signalling pathways that promote rapid cell proliferation. Pyrazole-based hybrids frequently exhibit inhibitory activity against receptor tyrosine kinases like EGFR. The 5-substituted 2-pyrazoline core is a common scaffold for targeting EGFR-mediated proliferation.(31)

3. **Tubulin Polymerization:** Certain pyrazole derivatives act as tubulin inhibitors, binding to the colchicine site to disrupt microtubule dynamics, thereby preventing cancer cells from dividing. Many 3,5-diaryl-4,5-dihydro-1H-pyrazoles with bulky substituents at C-3 (like phenanthrene) are known to bind to tubulin, disrupting microtubule formation, which leads to cell cycle arrest in the G2/M phase and induces apoptosis.(32)

4. **DNA and Topoisomerase:** The bulky phenanthren-9-yl group in your molecule is

often associated with DNA cleavage and the inhibition of Topoisomerase I and II, which are essential for DNA replication. Pyrazoline analogs are frequently docked against the Topoisomerase II receptor to explore their potential as cell cycle inhibitors. This interaction is particularly relevant for activity against A549 (Lung Adenocarcinoma) and HeLa (Cervical Cancer) cell lines.(33)

5. **Kinase Inhibitors:** Substituted pyrazoles are also explored as FGFR (Fibroblast Growth Factor Receptor) inhibitors. For instance, some 1H-pyrazole-4-carboxamide derivatives have shown nanomolar activity against FGFR1 (modeled with PDB structures like 5EVV) to suppress growth in lung and gastric cancer cells.(34)

6. **Heat Shock Protein 90 (HSP90)-** Description: Certain pyrazoline derivatives have been identified as novel inhibitors of HSP90, causing degradation of client proteins responsible for tumor survival.(35)

7. **BRAF(V600E):** BRAF is a serine/threonine kinase that participates in the MAPK signaling pathway. Mutation of BRAF, particularly the V600E variant, results in constitutive activation of cell proliferation and is strongly associated with melanoma, colorectal cancer, and thyroid carcinoma. Specifically, 4,5-dihydropyrazoles containing a hydroxyphenyl moiety (like your product) have been designed as potential inhibitors of this mutant kinase, which is a major driver in melanoma and breast cancer.

8. **Aurora Kinases A and B:** Aurora kinase A and Aurora kinase B regulate centrosome maturation, chromosome alignment, spindle assembly and cytokinesis during mitosis. Overexpression of Aurora kinases results in chromosomal instability and uncontrolled proliferation. Pyrazoline-carboxamide derivatives inhibit ATP binding within Aurora kinases, producing mitotic arrest and apoptotic cell death.(36)

Table 6. Molecular targets and signaling pathways involved in cancer progression (EGFR, VEGFR-2, PI3K/Akt, MAPK, Topoisomerase II, Carbonic Anhydrase IX/XII, Aurora kinase, CDK2, and tubulin).

Sr. no.	Protein target	PDB ID	Significance
1.	Topo isomerase II	1ZXM	Inhibition prevents DNA replication and repair, used to study drugs against Cancer (A549) and HeLa cells.
2.	Carbonic Anhydrase IX (hCA IX)	6G9U	A critical anticancer target; inhibitors disrupt the pH regulation of tumor cells, especially in hypoxic environments.
3.	Carbonic Anhydrase XII (hCA XII)	1JD0	Similar to CA IX, this is a susceptibility target for aryl-pyrazoline-1-carboxamides, with some derivatives reaching sub nanomolar inhibitory constants.
4.	EGFR (Epidermal Growth Factor Receptor)	1M17	Targeted by pyrazoline hybrids to inhibit cell proliferation signaling in breast and lung cancers.
5.	VEGFR-2	4ASD	Targeted to inhibit angiogenesis (blood vessel formation) and breast cell lines.
6.	BRAF	3OG7	Specifically, 4,5-dihydropyrazoles containing a hydroxyphenyl moiety (like your product) have been designed as potential inhibitors of this mutant kinase, which is a major driver in melanoma and breast cancer.
7.	Aurora Kinases	4AF3	These are critical regulators of cell division; pyrazole-carboxamides can selectively inhibit them, leading to abnormal mitosis and cell death.
8.	Tubulin alpha-1A chain	6J8F	Certain pyrazole derivatives act as tubulin inhibitors, binding to the colchicine site to disrupt microtubule dynamics, thereby preventing cancer cells from dividing.
9.	CDK-2	1HCL	CDK2 is an enzyme that drives cancer cell division and growth. Hyperactive CDK2 (often linked to Cyclin E) causes tumor growth and resistance to CDK4/6 inhibitors.Target: Inhibiting CDK2 blocks the cell cycle and triggers cancer cell death.

10. MOLECULAR DOCKING STUDIES

10.1. Protein selection

A comprehensive literature survey was conducted to identify the molecular mechanisms and therapeutic targets associated with skin cancer. Published research articles and review papers were systematically screened to understand the disease pathogenesis and to explore potential drug candidates for anticancer activity. (37)

Research on 4,5-dihydro-1H-pyrazole-1-carboxamides and related pyrazoline derivatives has identified several key protein targets through molecular docking and *in-vitro* assays. Analysis of published studies revealed that several signalling pathways, including the topo isomerase II, carbonic anhydrase (IX, XII), EGFR, VEGFR-2, BRAF, Aurora kinase, Tubulin α 1A chain, CDK-2 this are all protein targets are primarily involved in DNA regulation, cell cycle management and tumor metabolism.(38) The protein target, PDB ID and their significance are mentioned in table 1

10.2. Detection of the active site of the protein target

The active site of a protein is where the ligands interact with the protein. So, to perform *an in-silico* study, it is important to know the cavity where the ligand fits and interacts with the amino acids of the active sites.(39) The active site of the protein target was determined by using CavityPlus 2022: Protein cavity detection and beyond. This site analysed the protein 3D structure to identify potential binding sites, ranking them by ligand-ability and druggability. CavPharmer detected pharmacophore features of fluconazole and its chloro-analogue within cavities to understand ligand interactions. (40)

10.3. Molecular docking

The identified hub targets from literature were selected for molecular docking studies.

10.3.1. Protein Preparation

The targets for Pyrazoline and their derivatives were found by a literature survey. The relevant protein was obtained from the RCSB Protein Data Bank (PDB). The PDB IDs of the selected genes were obtained by applying specific filters, including *Homo sapiens* as the organism, X-ray crystallography as the experimental method and a resolution range of 2.0–2.5 Å.(41) After applying these criteria, the corresponding protein structures were selected from the filtered results. The protein was prepared using BIOVIA Discovery Studio Visualizer, in which water, co-crystallised ligands and chains (except interacting chains) were removed. PyRx software is used to convert the PDB format to a PDBQT file.(42)

10.3.2. Ligand Preparation

The compound of interest, Pyrazoline and their derivatives are to be docked with selected protein targets. The 3-dimensional model of 2 ligands was downloaded from PubChem as SDF file. The PyRx software is used to convert the SDF format to a PDBQT file.(43)

10.3.3. Molecular Docking

Molecular docking analysis is used to predict how two molecules, usually a drug and a target protein, interact with each other. Molecular docking was carried out using PyRx to predict binding orientations and estimate binding affinities within the active sites of the target proteins.(44) The best-ranked docking poses were selected based on binding energy and interaction profiles. Protein–ligand interactions were further analysed and visualised using BIOVIA (Discovery Studio Visualizer) to identify key hydrogen bonds, hydrophobic contacts, and other non-covalent interactions.(45) Pyrazoline and their derivatives docked against all skin cancer targets to determine the anticancer potential by the binding score of ligand and target interactions, expressed in kcal mol^{-1}.(46)

Table 7. Binding scores of all derivatives with cancer targets as compared with standard drug(Dabrafenib)

Sr. no.	Target	PDB ID	S1	S2	S3	S4	S5	Std drug (Dabrafenib)
1.	**CDK2**	1HCL	-9.8	**-9.9**	-9.6	-9.3	-9.7	-7.7
2.	**Carbonic 11**	1JDO	-9.6	-9.9	**-10**	**-10**	-8.8	-7.7
3.	**EGFR**	1M17	-10.2	-10.3	-10.5	**-10.6**	-10.1	-7.7
4	**Topo 2**	1ZXM	-11.1	**-11.4**	-10.7	-10.5	-8.8	-7.7
5	**BRAF**	3OG7	-9.2	**-10.9**	-10.2	-10.1	-10.6	-7.7
6	**Aurora kinase**	4AF3	-10.6	-10.5	-8.4	-11.3	**-11.6**	-7.7
7	**VEGFR**	4ASD	-10.1	-11.1	**-11.4**	-10.9	-10.4	-7.7
8	**Carbonic 9**	6G9U	-8	**-10.5**	**-10.5**	-10.1	-9.1	-7.7
9	**Tubulin alpha-1A chain**	6J8F	**-10.9**	-8.2	-8.2	-8.1	-7.9	-7.7

Among these targets, S2 demonstrated the highest binding affinity with CDK2, Topo2 and BRAF targets, each displaying a higher binding score than that of the standard drug dabrafenib. The docking analysis showed that S2 had a binding affinity of -9.9, -11.4 and -10.9 kcal/mol for CDK2, Topo2 and BRAF targets, which

was stronger than that of the reference drug dabrafenib (-7.7 kcal/ mol), suggesting greater potential for interaction.

Compound S2, S3 showed the same binding scores with carbonic 9 and carbonic 11 targets (-10 kcal/mol and -10.5 kcal/mol). Compound S1, S3, S4 and S5 show scores of -10.9, -11.4, -10.6 and -11.6 kcal/mol with targets tubulin α 1A chain, VEGFR, EGFR and Aurora kinase, respectively. All derivatives showed higher binding score than the standard drug dabrafenib (-7.7 kcal/mol) with all respective targets.

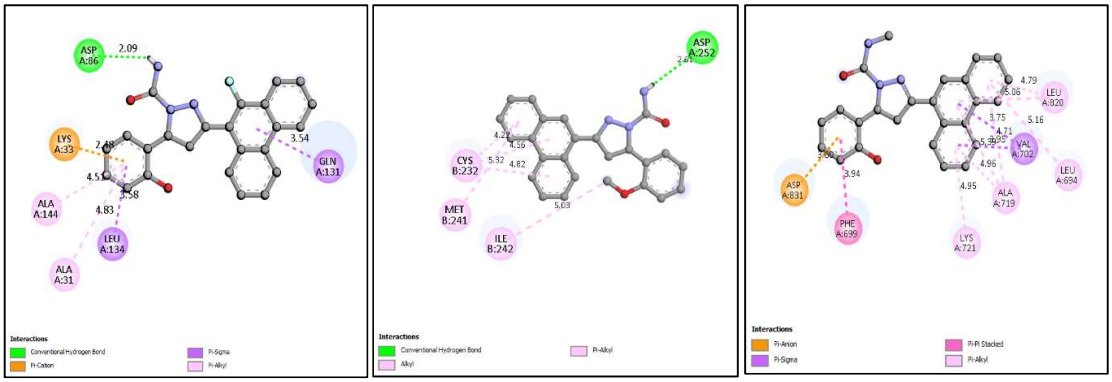


Fig. 4. 2D images of compound S2, S3 and S4 with CDK2, Carbonic11 and EGFR.

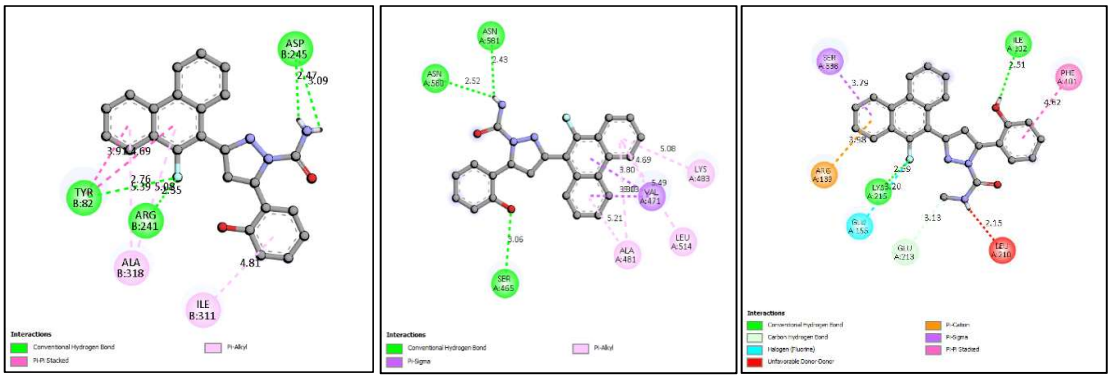


Fig. 5. 2D images of compound S2, S2 and S5 with Topoisomerase 2, BRAF and Aurora kinase.

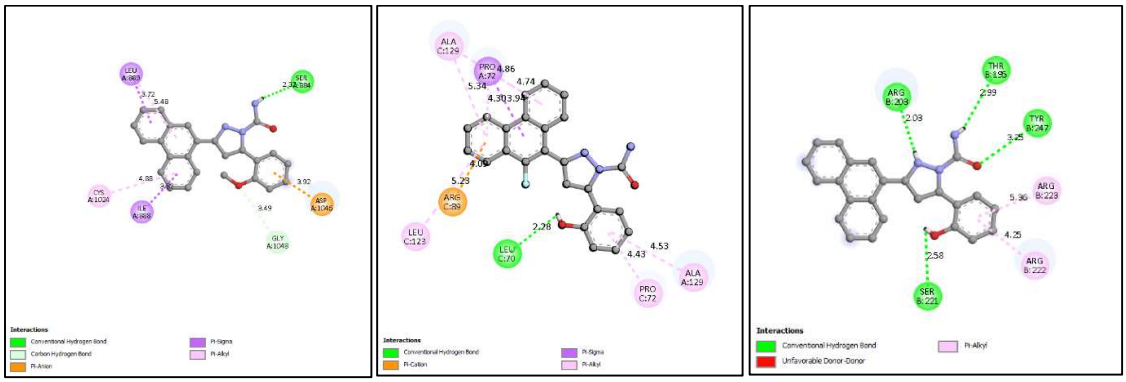


Fig. 6. 2D images of compound S3, S2 and S1 with VEGFR, carbonic 9 and Tubulin α-1 A chain

Compound S2 and S3 showed the highest binding affinities with different targets, including CDK2, Carbonic11, Topo2, BRAF, VEGFR and tubulin alpha-1A chain, than the other derivatives.

11. CHALLENGES IN DEVELOPMENT OF PHENANTHRENE-PYRAZOLINE CARBOXAMIDE ANTICANCER AGENT

Despite significant progress in the design and synthesis of phenanthrene-based pyrazoline carboxamide derivatives, several scientific and translational challenges remain before these compounds can progress toward clinical application. Addressing these

limitations through rational medicinal chemistry and multidisciplinary research will be essential for developing safer and more effective anticancer therapeutics.

11.1 Limited Selectivity toward cancer cells

Many heterocyclic anticancer agents exhibit potent cytotoxicity against cancer cells but also affect rapidly dividing normal tissues such as bone marrow, gastrointestinal epithelium, and hair follicles. This lack of selectivity often results in severe adverse effects including myelosuppression, gastrointestinal toxicity, hepatotoxicity, nephrotoxicity, and cardiotoxicity. Future research should therefore focus on designing derivatives with enhanced selectivity for tumor-specific molecular targets, thereby minimizing toxicity to healthy tissues.

11.2 Drug Resistance

Acquired multidrug resistance (MDR) remains one of the greatest obstacles in chemotherapy. Cancer cells develop resistance through multiple mechanisms, including overexpression of ATP-binding cassette (ABC) transporters such as P-glycoprotein, increased DNA repair capacity, mutation of therapeutic targets, activation of compensatory signaling pathways, inhibition of apoptosis, and epithelial–mesenchymal transition. Since phenanthrene–pyrazoline hybrids possess multitarget inhibitory activity, they may reduce resistance; however, extensive biological validation is still required.

11.3 Poor Water Solubility

The phenanthrene scaffold is highly hydrophobic because of its extended aromatic system. Consequently, many derivatives exhibit limited aqueous solubility, leading to poor oral absorption and reduced bioavailability. Incorporation of hydrophilic substituents, prodrug strategies, salt formation, and nanoformulation approaches may improve physicochemical properties without compromising biological activity.

11.4 Pharmacokinetic Limitations

Although computational ADME prediction provides valuable preliminary information, experimental pharmacokinetic studies remain necessary. Factors such as plasma protein binding, first-pass metabolism, cytochrome P450 interactions, tissue distribution, and elimination rates can substantially influence therapeutic efficacy. Comprehensive in vivo pharmacokinetic investigations should therefore accompany future lead optimization.

11.5 Synthetic Complexity

Hybrid pharmacophore design often requires multistep synthesis involving chalcone formation, semicarbazone preparation, cyclization, purification and structural characterization. Increasing molecular complexity may reduce synthetic yield and increase production costs. Development of efficient one-pot syntheses, microwave-assisted synthesis, green chemistry approaches, and continuous-flow techniques may improve scalability.

11.6 Limited *in-vivo* and Clinical Evidence

Most published investigations of phenanthrene-based pyrazoline derivatives remain limited to in vitro cytotoxicity assays and computational studies. Only a small number of compounds have progressed to animal studies, and clinical investigations are currently very limited. Future research should emphasize pharmacokinetics, efficacy in xenograft and orthotopic tumor models, chronic toxicity, and eventual clinical evaluation.

12. FUTURE PROSPECTS

The future of anticancer drug discovery lies in integrating medicinal chemistry, computational biology, molecular pharmacology, and artificial intelligence to accelerate the identification of highly selective therapeutics. Phenanthrene-based pyrazoline carboxamide derivatives represent an attractive platform for developing multifunctional anticancer agents because they combine several pharmacologically active scaffolds within a single molecular framework.

Future research should focus on:

- Rational design of multitarget-directed ligands capable of simultaneously inhibiting EGFR, VEGFR-2, Carbonic Anhydrase IX/XII, Topoisomerase II, Aurora kinase, CDK2, and BRAF.
- Application of artificial intelligence and machine learning algorithms for virtual screening, quantitative structure–activity relationship (QSAR) modeling, de novo molecular design and prediction of biological activity.
- Integration of molecular dynamics simulations and free-energy calculations with molecular docking to obtain more reliable predictions of ligand–protein interactions.
- Development of targeted drug delivery systems such as liposomes, polymeric nanoparticles, dendrimers and antibody-drug conjugates to improve tumor selectivity and reduce systemic toxicity.
- Exploration of combination therapy using hybrid molecules together with immunotherapy, radiotherapy or conventional chemotherapy to overcome multidrug resistance.
- Investigation of structure optimization using bioisosteric replacement, scaffold hopping, fragment-based drug design and lead optimization strategies.
- Comprehensive evaluation of pharmacokinetics, metabolism, chronic toxicity and efficacy in advanced preclinical models before clinical translation.

Continued interdisciplinary collaboration among medicinal chemists, computational scientists, pharmacologists and oncologists will facilitate the development of safer and more effective targeted anticancer therapeutics.

13. CONCLUSION

Cancer remains one of the most devastating diseases worldwide despite remarkable advances in diagnosis and treatment. Conventional chemotherapy continues to suffer from limitations including poor selectivity, severe systemic toxicity, and multidrug resistance, emphasizing the need for innovative therapeutic strategies. Hybrid pharmacophore design has emerged as an effective approach for developing multifunctional molecules capable of simultaneously modulating multiple signaling pathways involved in tumor progression.

Phenanthrene-based pyrazoline carboxamide derivatives integrate several biologically active pharmacophores into a single molecular framework. The phenanthrene scaffold contributes DNA-binding capability and hydrophobic interactions, while the pyrazoline ring provides broad-spectrum biological activity through interactions with multiple molecular targets. The carboxamide moiety enhances receptor binding and pharmacokinetic properties by facilitating hydrogen-bond formation, whereas phenolic substituents improve antioxidant activity and molecular recognition. Structure–activity relationship studies clearly demonstrate that appropriate substitution patterns substantially influence biological activity. Hydroxyl and methoxy groups generally improve receptor binding and cytotoxicity, whereas fluorine substitution enhances metabolic stability and kinase inhibition. Carboxamide functionality plays a central role in increasing affinity toward protein active sites through hydrogen-bond interactions.

These hybrid molecules have demonstrated promising inhibitory activity against several clinically important molecular targets, including Topoisomerase II, EGFR, VEGFR-2, Carbonic Anhydrase IX/XII, Aurora kinase, BRAF, CDK2, and tubulin. Their multitarget mode of action offers the potential to overcome drug resistance while improving therapeutic efficacy. Computational approaches such as molecular docking, virtual screening, drug-likeness evaluation, and ADMET prediction have become indispensable tools for accelerating lead optimization and reducing the cost of drug discovery. Integration of computational methods with synthetic medicinal chemistry provides a rational pathway for developing novel targeted anticancer agents.

Overall, phenanthrene-based pyrazoline carboxamide derivatives represent a highly promising class of hybrid molecules for future anticancer drug development. Continued optimization of molecular structure, comprehensive biological evaluation, and clinical translation will be essential for transforming these compounds into effective therapeutic agents.

14. DISCLOSURE STATEMENT

- The authors declare no conflicts of interest with respect to the authorship, research work and/or publication of this work.

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