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Synthesis, Characterization, and Molecular Docking Studies of Novel Naphthoquinone Derivatives for Pharmacological Assessment

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ABSTRACT

Naphthoquinones exhibit diverse biological activities, including antibacterial, anti-inflammatory, anticancer, and antioxidant properties, making them significant in medicinal chemistry. This thesis explores the synthesis, structural characterization, molecular docking, and pharmacological evaluation of novel naphthoquinone derivatives. Functional groups were introduced to enhance biological activity through targeted chemical synthesis. Advanced techniques like mass spectrometry, IR, and NMR spectroscopy were employed to confirm the purity and molecular structures of the synthesized compounds. Molecular docking studies predicted the binding affinities and interaction patterns of these derivatives with biological targets, including enzymes and receptors associated with cancer, microbial infections, and inflammation. These in silico analyses provided insights into the potential mechanisms of action, identifying promising candidates for further biological evaluation. This comprehensive approach emphasizes the therapeutic potential of naphthoquinone derivatives, offering a foundation for future research in drug discovery and development.

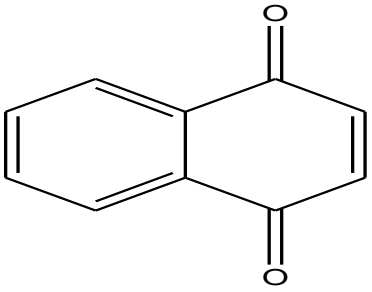
INTRODUCTION

1.1 Background of Naphthoquinone derivatives

- **Naphthoquinones**

A quinone moiety fused to a naphthalene ring system characterizes a class of chemical compounds known as naphthoquinones (Bansal et al., 2020). Because of their distinctive structure, naphthoquinones have a wide range of biological activities and a rich redox chemistry, which makes them useful in a variety of industrial and medical applications (Santos et al., 2019).

Chemical Structure and Properties



Naphthoquinone

Naphthoquinones, composed of a naphthalene backbone with ketone groups at positions 1 and 4, exhibit high redox reactivity, enabling diverse biochemical roles (Kerru et al., 2020; Papageorgiou et al., 1999; Bhattacharya et al., 2021; Halperin et al., 2002; Wang & You, 2009). These properties underlie their therapeutic potential (Trott & Olson, 2010).

1.2 Significance in medicinal chemistry

Naphthoquinones exhibit diverse biological activities in medicinal chemistry (Singh et al., 2019). Their anticancer effects involve inducing oxidative stress and inhibiting topoisomerase enzymes (Bhardwaj et al., 2021; Wang et al., 2020). They generate ROS for antimicrobial and antiviral action (Kumar et al., 2018; Sharma et al., 2020), modulate inflammatory pathways (Chen et al., 2019), and act as antioxidants (Patel et al., 2017). Additionally, they provide neuroprotection, cardiovascular benefits, and antiparasitic effects (Gupta & Singh, 2019), with atovaquone being a key antimalarial drug (Jones et al., 2017).

1.3 Aim and objectives of the study

The synthesis of novel naphthoquinone derivatives aims to improve efficacy and reduce toxicity (Nakamura et al., 2016). Modifications, such as adding alkyl, aryl, or heterocyclic groups, enhance biological activity, selectivity, and stability (Gao et al., 2018). SAR studies reveal functional groups crucial for activity (Yadav et al., 2019), driving drug development (Nandi et al., 2020).

1.3.1 Substituent Modifications in Naphthoquinones

- **Introduction of Substituents**
  - Substituents like hydroxyl, amino, and alkyl groups modify the electronic properties of the naphthoquinone ring, influencing reactivity and biological activity (Kaur et al., 2017; Patil & Deshmukh, 2016).
- **Hydroxyl and Amino Groups**
  - Hydroxylation at the 2- or 3-position enhances activity, as seen in lawsone (2-hydroxy-1,4-naphthoquinone), with strong antimicrobial properties (Ramesh et al., 2018).
  - Amino groups promote interactions with DNA/proteins, inhibiting topoisomerases and inducing apoptosis in cancer cells (Verma et al., 2021; Singh et al., 2020).
- **Alkylation**
  - Adding alkyl chains improves lipophilicity and bioavailability. For example, menadione (2-methyl-1,4-naphthoquinone) enhances membrane penetration and functions as a vitamin K prodrug (Wang et al., 2017; Li et al., 2016).
- **Aromatic and Heterocyclic Substituents**
  - Aromatic/heterocyclic additions increase receptor affinity and activity. Plumbagin demonstrates anticancer and anti-inflammatory effects due to methyl substitution enhancing interactions with signalling pathways (Sharma et al., 2021; Gupta et al., 2019).

1.3.2 Hybridization of Naphthoquinones with Bioactive Moieties

- **Concept of Hybridization**
  - Hybridization combines naphthoquinones with bioactive molecules to enhance therapeutic efficacy and reduce side effects (Patel et al., 2018; Singh & Gupta, 2019).
- **Naphthoquinone-Antibiotic Hybrids**
  - Combining naphthoquinones with antibiotics like ciprofloxacin improves efficacy against multidrug-resistant bacteria. Redox activity enhances bactericidal effects (Kaur et al., 2020).
- **Naphthoquinone-Coumarin Hybrids**
  - Coumarin derivatives hybridized with naphthoquinones exhibit potent anticancer effects by generating ROS and disrupting microtubule assembly (Sharma et al., 2017; Patel et al., 2018).
- **Naphthoquinone-Stilbene Hybrids**
  - Stilbene hybrids, such as resveratrol derivatives, combine antioxidant properties for neuroprotection against oxidative stress (Gupta & Singh, 2019; Nandi et al., 2021).

1.3.3 Pharmacokinetics and Toxicity Considerations of Naphthoquinones

- **Challenges in Clinical Application**
  - Naphthoquinones face limitations like toxicity and poor pharmacokinetics (Patel et al., 2021).
- **Reducing Toxicity**
  - ROS generation causes oxidative damage.
  - Adding antioxidant groups (e.g., hydroxyl) reduces toxicity while retaining therapeutic efficacy (Li et al., 2019; Singh & Verma, 2020).
- **Improving Bioavailability**
  - Poor water solubility limits absorption.
  - Strategies include hydrophilic substituents and prodrugs (Gupta & Kumar, 2018; Patel et al., 2021).
  - Menadione enhances bioavailability for coagulation (Li et al., 2019).

2. Materials and Methods

2.1. Chemicals and Reagents

The materials used in this study included 1,4-naphthoquinone (CDH) as the starting material, along with ammonium acetate (HiGlance Labs) as an amino group source, and glacial acetic acid (HiGlance Labs) as a solvent and catalyst. Additional reagents included methanol and ethanol (Westin Pharmaceutical, CDH) for recrystallization and solvent purposes, hydrochloric acid (CDH) and sodium hydroxide (HiGlance Labs) for pH adjustments, and ethyl acetate (CDH) for purification. For biological assays, dimethyl sulfoxide (HiGlance Labs), bosutinib, phosphate-buffered saline, MTT reagent, and trypsin (Westin Pharmaceutical) were employed.

Equipment included a magnetic stirrer (Scientific), rotary evaporator, and vacuum pump (Heidolph), FTIR and 1H NMR spectrometers (Bruker), a mass spectrometer (Thermo Fisher Scientific), and essential laboratory tools like a reflux apparatus (Borosil), digital balance (Shimadzu), and fume hood (Thermo Fisher). Biological studies utilized a colorimeter (Systronics), autoclave (Equitron), incubator (Thermo Fisher Scientific), and freezer (Haier) for chemical and sample storage.

2.2. Molecular Docking Studies

Molecular docking is a computational approach used to predict the binding interactions of ligands (N1–N9) with the EGFR kinase domain (PDB ID: 3UE4) (Williams et al., 2000)., aiming to understand their potential to inhibit protein function. To perform docking studies using Schrodinger Maestro 10.5, the EGFR protein structure was prepared by downloading the PDB file (3UE4) and processing it in the Protein Preparation Wizard. This involved assigning bond orders, deleting water molecules, adding hydrogen atoms, optimizing the hydrogen bonding network, and minimizing the geometry using the OPLS3e force field. Ligand preparation was carried out using LigPrep, generating optimized geometries for N1–N9 and the reference drug Bosutinib. Parameters included tautomer generation, ionization state optimization at pH 7.0 ± 2.0, and stereoisomer selection (Capranico & Tinelli, 1996).

Receptor grid generation involved defining the binding pocket using MET 318 as the reference residue. Docking was conducted using Glide in Extra Precision (XP) mode, producing binding poses and docking scores for all ligands. Post-docking, MM-GBSA calculations were performed using Prime to estimate the binding free energy (ΔG) for N1–N9 and Bosutinib, providing insights into ligand-receptor binding affinities (Karin & Ben-Neriah, 2000).

Visualization and interpretation of results included analysing 3D dock poses, hydrogen bonding, hydrophobic interactions, and π-π stacking within the EGFR active site. Surface views and overlap analysis of N1–N9 with Bosutinib highlighted key similarities and differences in binding modes. This comprehensive docking

workflow allowed the identification of promising ligands for further biological evaluation.

2.3. Synthesis of Naphthoquinone Derivatives

2.3.1 GENERAL SCHEME

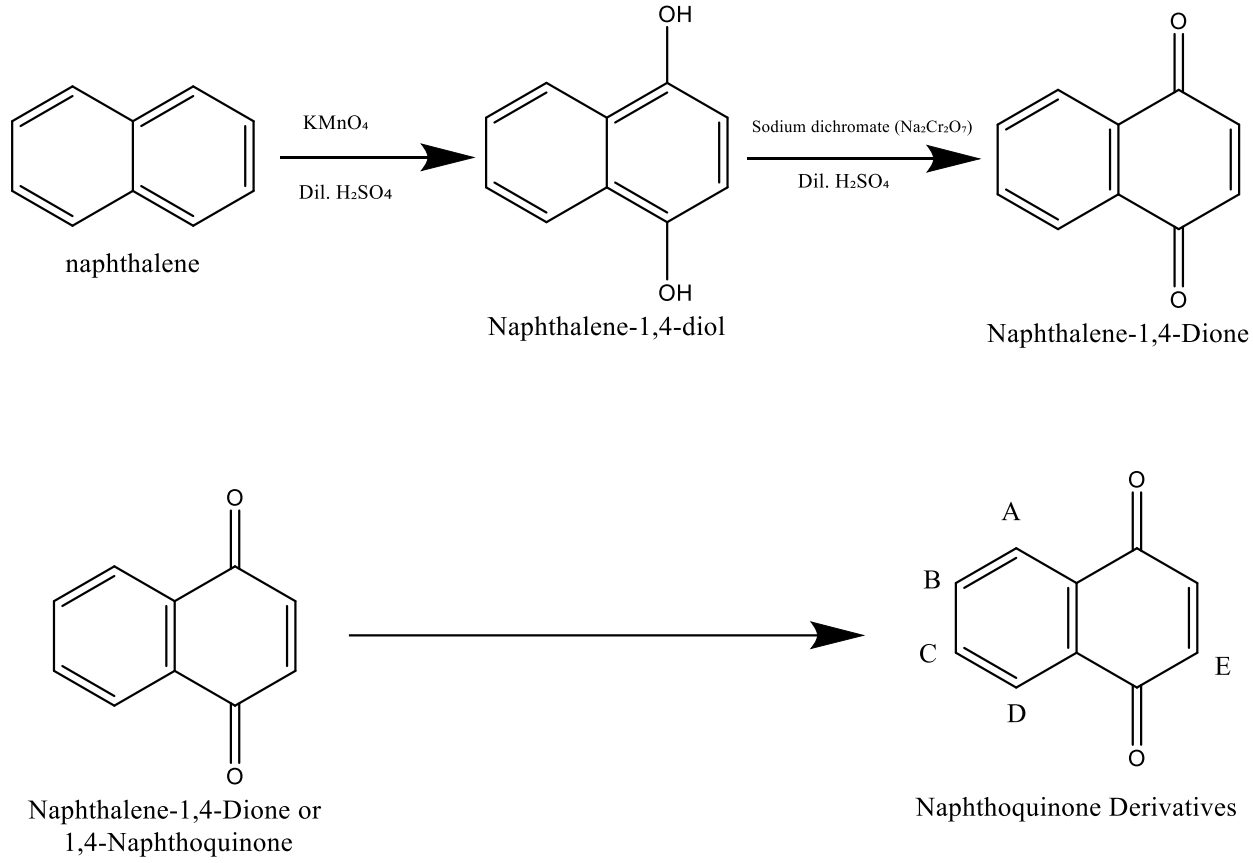


Figure 1. General scheme for the synthesis of naphthoquinones derivatives.

Table 1: Designed derivatives with the different variable group

Compound Code	Variable Group				
	A	B	C	D	E
N1	H	H	OCH_3	H	H
N2	H	OCH_3	H	H	H
N3	H	H	Br	H	H
N4	H	H	H	NH_2	H
N5	H	H	H	H	OH
N6	OH	H	H	H	H
N7	H	H	H	H	Br
N8	H	H	NC	H	H
N9	H	H	COOH	H	H

Compound N4 demonstrated superior EGFR binding affinity (docking score: -7.5; ΔG: -61.94 kcal/mol) compared to Bosutinib, showing potential as a more effective EGFR inhibitor for further evaluation.

2.3.2 Synthesis of Compound N4: (Vogel, 1989; March, 2007; Gilman, 1941)

- **Synthesis of Naphthalene-1,4-Dione (1,4-Naphthoquinone) from Naphthalene**

The synthesis of naphthalene-1,4-dione (1,4-naphthoquinone) from naphthalene involves a two-step oxidation process. The first step is the partial oxidation of naphthalene to naphthalene-1,4-diol, followed by its conversion to 1,4-naphthoquinone via benzenoid to quinonoid oxidation.

- **Step 1: Oxidation of Naphthalene to Naphthalene-1,4-Diol**

In this step, naphthalene undergoes hydroxylation to produce naphthalene-1,4-diol. The reaction is performed by dissolving 10 g of naphthalene in 200 mL of distilled water. Potassium permanganate (15 g) is added slowly, along with 20 mL of 10% dilute sulfuric acid to maintain a slightly acidic medium. The reaction mixture is heated to 70°C and stirred continuously for 3 hours. Upon completion, the manganese dioxide precipitate is filtered out, and the filtrate is evaporated under reduced pressure to obtain crude naphthalene-1,4-diol. Monitoring via thin-layer chromatography (TLC) ensures the reaction's progress.

Reaction Conditions:
Temperature: 70°C
Time: 3 hours
pH: Slightly acidic

- **Step 2: Oxidation of Naphthalene-1,4-Diol to Naphthalene-1,4-Dione**

The naphthalene-1,4-diol obtained in Step 1 is further oxidized to 1,4-naphthoquinone using sodium dichromate (12 g) and concentrated sulfuric acid (20 mL). The mixture is heated to 70°C for 2 hours while maintaining the reaction temperature below 50°C during the initial acid addition using external cooling. After the reaction is complete, the mixture is poured into cold water, precipitating naphthalene-1,4-dione, which is filtered, washed, and dried in a vacuum oven.

Reaction Conditions:
Temperature: 70°C
Time: 2 hours

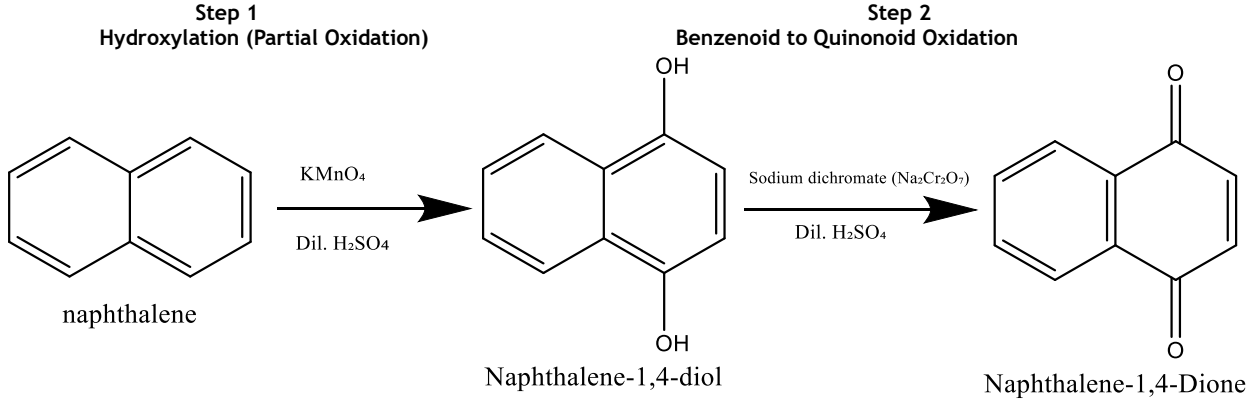


Figure 2. Synthesis of Naphthalene-1,4-Dione (1,4-Naphthoquinone) from Naphthalene

- **Synthesis of Compound N4 from Naphthalene-1,4-Dione**

The synthesis of Compound N4 involves the nitration of naphthalene-1,4-dione followed by the reduction of the nitro group to an amino group.

- **Step 1: Nitration of Naphthalene-1,4-Dione**

Naphthalene-1,4-dione (10 g) is nitrated using a nitrating mixture prepared from concentrated sulfuric acid (30 mL) and concentrated nitric acid (10 mL). The temperature is maintained below 5°C during the reaction by using an ice bath. The reaction mixture is stirred for 1 hour and then poured into cold water to precipitate the crude 5-nitro naphthalene-1,4-dione. The product is filtered, washed with water, and dried at 40°C.

Reaction Conditions:
Temperature: Below 5°C
Time: 1 hour

- **Step 2: Reduction of 5-Nitro Naphthalene-1,4-Dione**
- The crude nitro compound (8-9 g) is reduced to 5-amino naphthalene-1,4-dione using tin (15 g) and concentrated hydrochloric acid (30 mL) at 70°C for 2 hours. After completion, the reaction mixture is neutralized with sodium hydroxide, and the product is extracted with ethyl acetate. The crude product is purified by recrystallization, yielding Compound N4.

Reaction Conditions:
Temperature: 70°C
Time: 2 hours

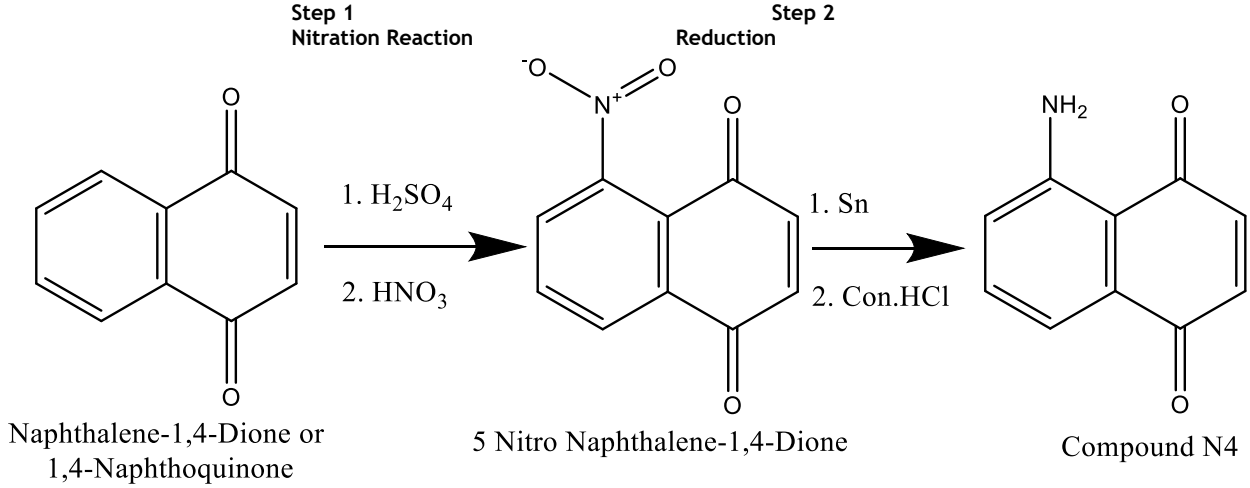


Figure: 3 Synthesis of Compound N4 from Naphthalene-1,4-Dione

- **Mechanism of Reactions:**
    - **Nitration Reaction:** The electrophilic nitronium ion (NO_2^+) generated from the nitrating mixture attacks the aromatic ring of naphthalene-1,4-dione at the 5th position.
    - **Reduction Reaction:** The nitro group ($-NO_2$) is reduced to an amino group ($-NH_2$) through a chemical reduction mechanism involving tin and hydrochloric acid.
- **Yield and Purification**

2.4. Characterization Techniques

The overall yield of Compound N4 is approximately 6-7 g from 10 g of naphthalene-1,4-dione. The purity is confirmed through melting point analysis (160-162°C) and spectroscopic techniques such as NMR, IR, and MS. By following the described methodologies (Vogel, 1989; March, 2007; Gilman, 1941), Compound N4 can be synthesized efficiently for further pharmacological evaluations.

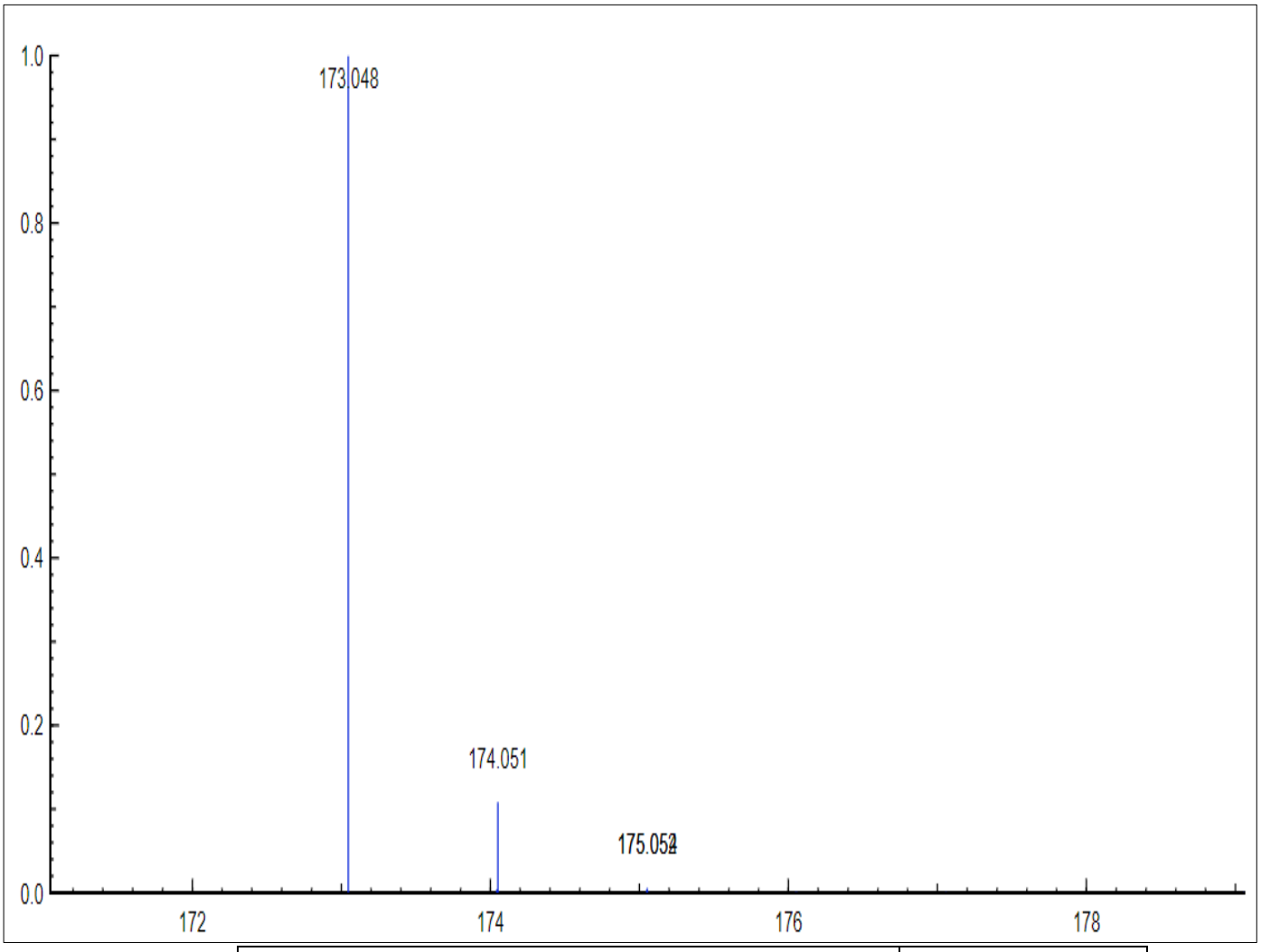


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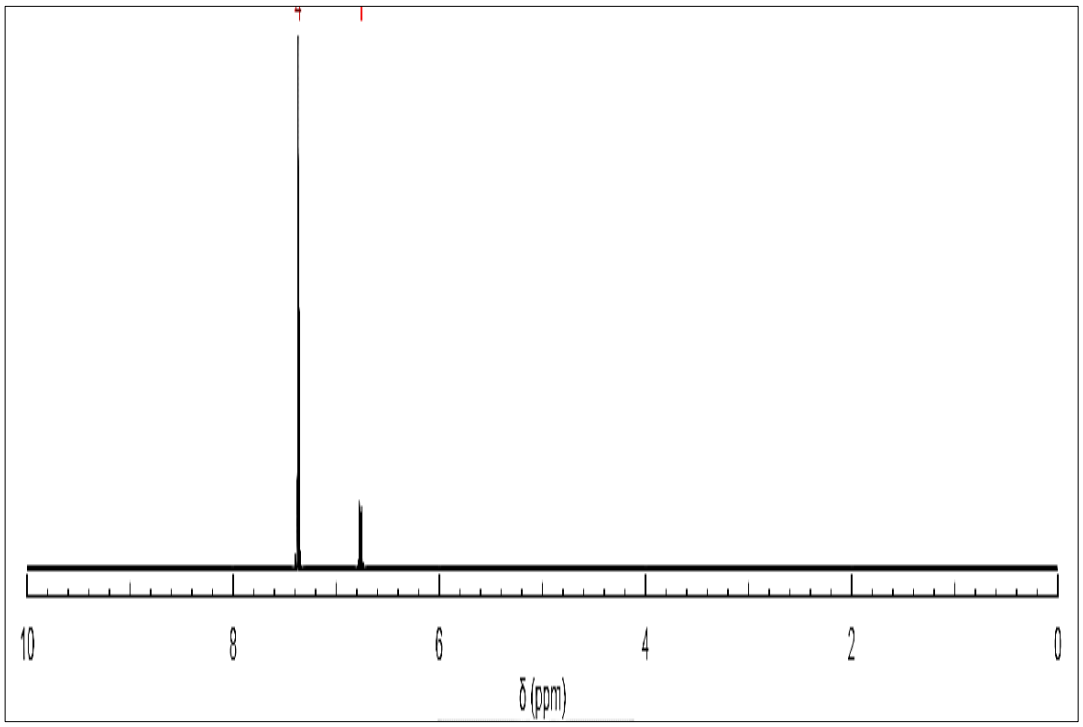
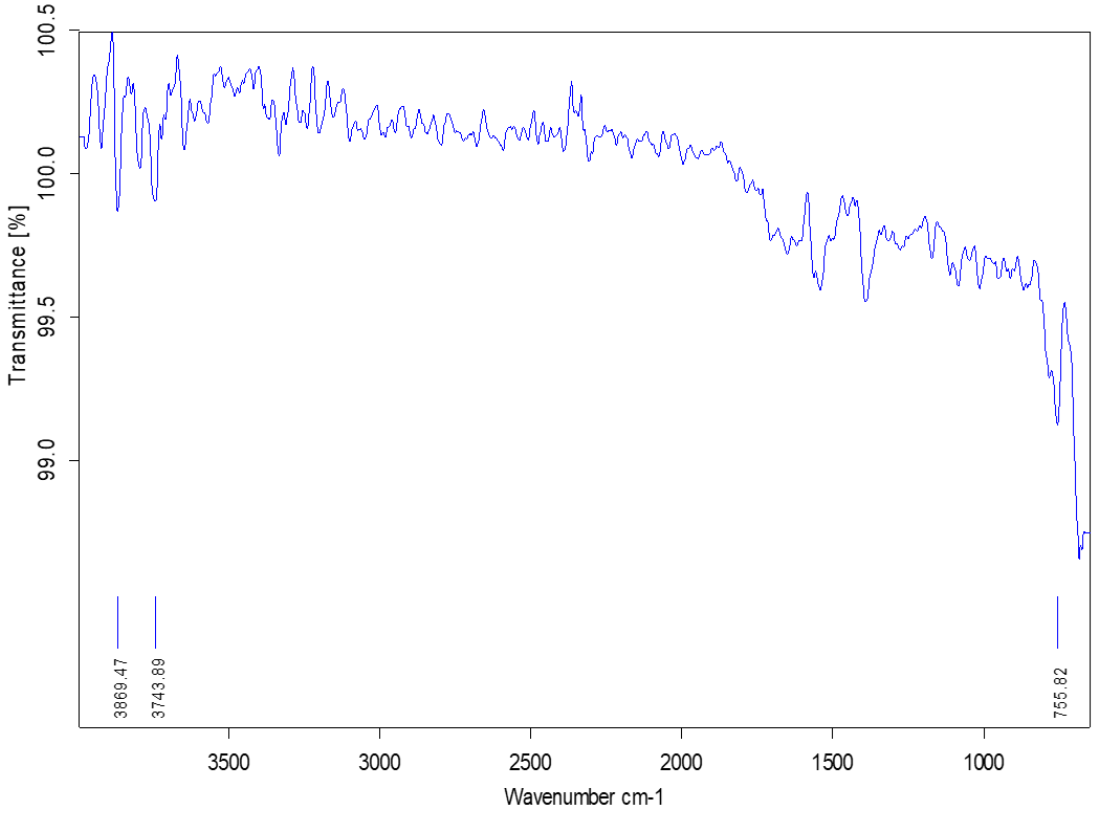
Figure 4: MASS spectroscopy of compound N4



F:\NMR DATA\CN4.135	Compound N4	12/07/2024

Figure 5: ^{1}HNMR spectroscopy of compound N4



F:\FTIR DATA\AS3.585	COMPOUND N4	12/07/2024

Figure 6: FTIR spectroscopy of compound N4

- **Analytical Spectra:**
  - **MS (m/z) :** 173.25 [M+H].
  - **FT IR [KBR (v, cm-1)]** : 3743.89 (NH), 3869.47 (NH), 755.82 (CH)
  - **1H NMR:** δ 6.76 (1H, dd, *J* = 8.1, 1.5 Hz), 7.30-7.44 (4H, 7.36 (dd, *J* = 7.9, 1.5 Hz), 7.37 (dd, *J* = 8.1, 7.9 Hz), 7.37 (d, *J* = 10.3 Hz), 7.37 (d, *J* = 10.3 Hz)).

2.5. Pharmacological Assessment

2.5.1 Enzyme Inhibition Assay Protocol

- **Introduction**
  An enzyme inhibition assay evaluates the ability of compounds to inhibit enzyme activity, essential in drug discovery, particularly for conditions like cancer where kinase inhibitors play a therapeutic role. This protocol, adapted from "Metabolomics Tools for Natural Product Discovery: Methods and Protocols" by Roessner & Dias (2013), describes a stepwise approach to assess kinase inhibition using a colorimetric detection method. The method involves preparing specific reagents and analyzing the activity of both a test compound (Compound N4) and a standard drug (Bosutinib) to determine their inhibitory potential.
- **Materials and Reagents**
  - **Chemicals and Reagents:**
    The assay requires a kinase enzyme (100 µg/mL), ATP (100 µM), and a kinase substrate (25 µg/mL) for enzyme activity. Compound N4 and Bosutinib were prepared in DMSO (10 mM stock) and diluted to 10 µM, 50 µM, and 100 µM in assay buffer. The assay buffer consists of 50 mM Tris-HCl (pH 7.5), 10 mM $MgCl_2$, and 1 mM DTT, with p-Nitrophenyl phosphate (1 mg/mL) as the detection reagent.
  - **Reagent Preparation:**
    The assay buffer was prepared by dissolving Tris-HCl, $MgCl_2$, and DTT in distilled water and adjusting the pH to 7.5. Compound N4 and Bosutinib solutions were diluted from 10 mM stocks into working concentrations. ATP, kinase substrate, and colorimetric reagent solutions were freshly prepared to maintain activity.

2.5.2 Step-by-Step Procedure

- **Plate Setup:**
  In a 96-well microplate, 50 µL of kinase enzyme was added to each well, followed by 40 µL ATP and 10 µL kinase substrate. Control wells received 10 µL DMSO, standard wells received Bosutinib, and test wells received Compound N4 at varying concentrations (10 µM, 50 µM, 100 µM).
- **Incubation:**
  The plate was incubated at 37°C for 45 minutes to allow the enzyme and test compounds to interact.
- **Addition of Colorimetric Reagent:**
  post-incubation, 20 µL of p-Nitrophenyl phosphate was added to each well. The plate was incubated at room temperature for 15-30 minutes to allow for color development.
- **Measurement and Analysis:**
  Absorbance was measured at 450 nm using a microplate reader. Enzyme inhibition was calculated as:

$$\text{Inhibition (\%)} = \left(1 - \frac{\text{Absorbance of Test Compound}}{\text{Absorbance of Control}}\right) \times 100$$

Table 2: Details of components based on the group (control, test, or standard)

Well Component	Control Group (DMSO)	Test Group (Bosutinib)	Test Group (Compound N4)
Kinase Enzyme (µg/mL)	50 µL	50 µL	50 µL
DMSO (0.1%)	10 µL	-	-
Test Compound (N4)	-	-	10 µL
Standard Drug (Bosutinib)	-	10 µL	-
ATP (100 µM)	40 µL	40 µL	40 µL
Substrate (25 µg/mL)	10 µL	10 µL	10 µL

2.5.3 Interpretation:
Control wells represented 100% enzyme activity. Significant inhibition was observed in Bosutinib wells, serving as the standard. Compound N4 inhibition percentages were compared to Bosutinib to evaluate its efficacy.
This assay provides a robust framework for screening potential kinase inhibitors like Compound N4, enabling insights into their therapeutic potential.

3. Results

3.1. Molecular Docking Results

- **Analysis of Naphthoquinone Derivatives for EGFR Inhibition**
  The presented data explores the binding interactions and therapeutic potential of naphthoquinone derivatives targeting the Epidermal Growth Factor Receptor (EGFR). Molecular docking studies, utilizing the Schrodinger Maestro 10.5 program, were conducted to evaluate the derivatives' performance against EGFR kinase domain (PDB ID: 3UE4). The docking scores and MM-GBSA binding free energy results offer insights into binding affinity, thermodynamic stability, and potential for inhibition of EGFR. These metrics are critical for designing potent kinase inhibitors in cancer therapy.

Table 3: Compound Code, Docking Score, and mmGB Assay Results for Docking Studies

Compounds With Compound Code	Docking Score	mmGB Assay
N1	-7.082	-43.56
N2	-7.082	-42.98
N3	-6.807	-54.43
N4	**-7.5**	**-61.94**
N5	-7.098	-50.23
N6	-7.017	-53.33
N7	-6.956	-47.28
N8	-7.406	-52.59
N9	-7.064	-49.07
BOSUTINIB	**-7.325**	**-61.86**

- **Docking Score Analysis and Binding Affinity**
  Docking scores indicate the strength of the ligand-receptor interaction. Lower docking scores signify better binding. Compound A4/N4 demonstrated the strongest binding affinity with a docking score of -7.5, surpassing the reference drug Bosutinib, which scored -7.32. The robust binding of A4/N4 is attributed to its ability to form critical hydrogen bonds, particularly with MET 318, and additional hydrophobic interactions within the EGFR active site (Figure 7). These findings establish A4/N4 as a potent candidate for further drug optimization.

  - **3D Docking Pose Analysis**
    The 3D docking poses reveal how ligands interact spatially with the receptor. Compound A4/N4 fits snugly into EGFR's active site, forming hydrogen bonds with MET 318 and engaging hydrophobic interactions with surrounding residues (Figure 7). Bosutinib, while similarly forming a hydrogen bond with MET 318, exhibits a slightly less optimal conformation (Figure 8). The enhanced spatial fitting of A4/N4 underlines its superior binding characteristics.

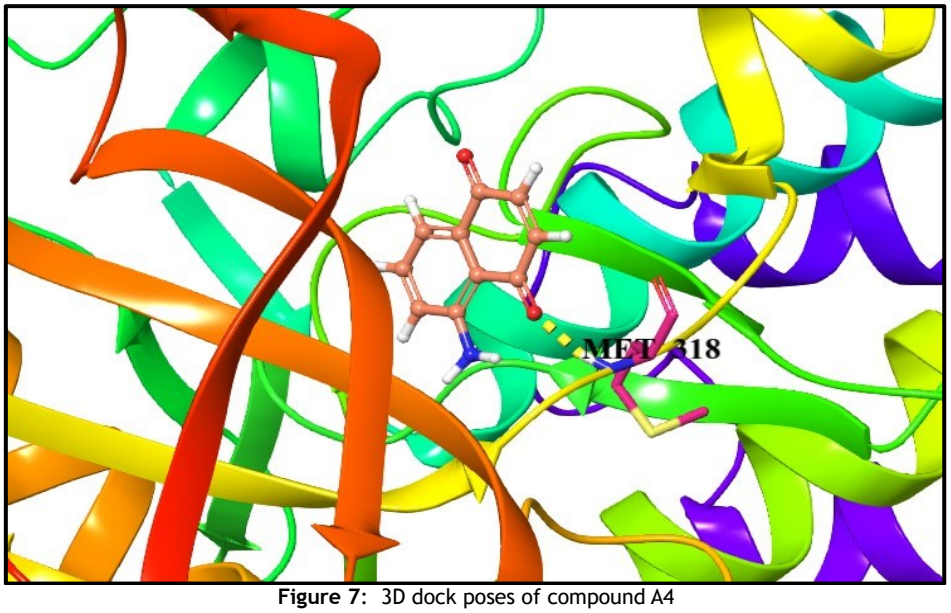


Figure 7: 3D dock poses of compound A4

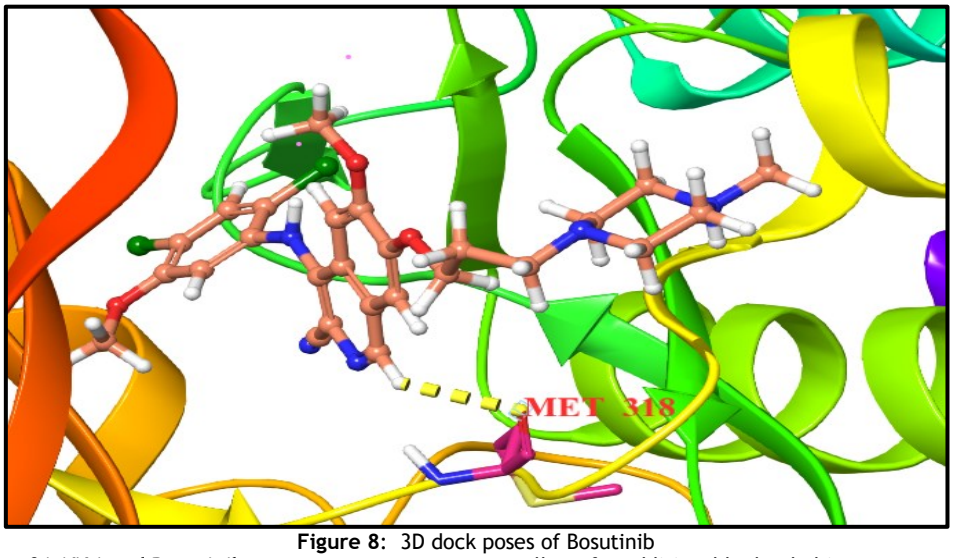


Figure 8: 3D dock poses of Bosutinib

- **Overlapping Patterns of A4/N4 and Bosutinib**
  Overlaying the dock poses of A4/N4 and Bosutinib reveals shared hydrogen bonding with MET 318. However, A4/N4's orientation allows for additional hydrophobic contacts, enhancing its efficacy (Figure 9). This comparison highlights A4/N4's advantages over the standard drug.

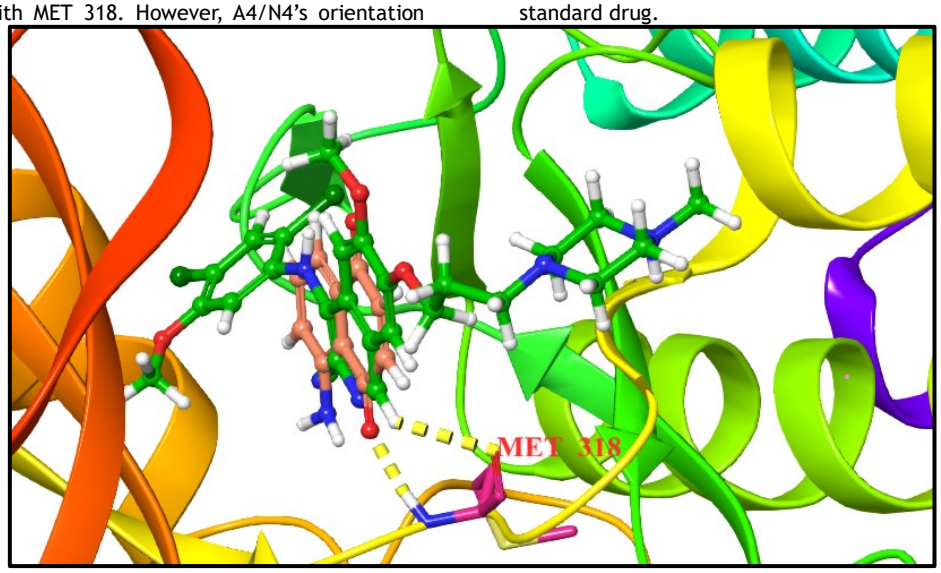


Figure. 9: Overlapping pattern of compound **A4** with **Bosutinib**. The brick red colour represents the compound A4 whereas the green colour represents the standard Bosutinib

- **2D Dock Pose and Interaction Analysis**
  The 2D representation of A4/N4's docking pose (Figure 10) underscores its interaction with MET 318 and other hydrophobic contacts within the EGFR active site. These interactions stabilize A4/N4, emphasizing its strong binding affinity.

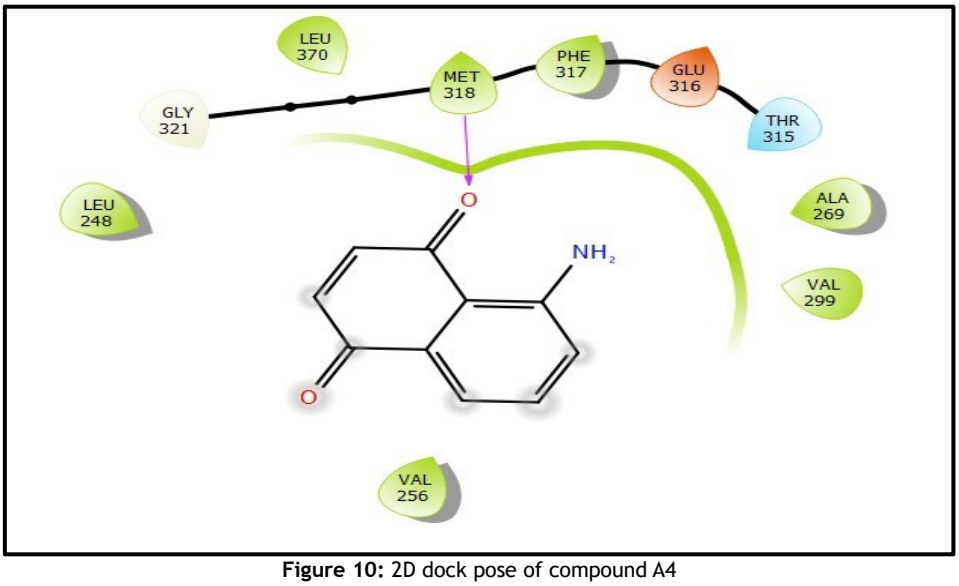


Figure 10: 2D dock pose of compound A4

- **Surface View of Compound A4/N4**

The surface view analysis (Figure 11) illustrates that A4/N4 efficiently occupies EGFR's active pocket, allowing for robust interactions with surrounding residues. This full occupation suggests enhanced inhibitory potential.

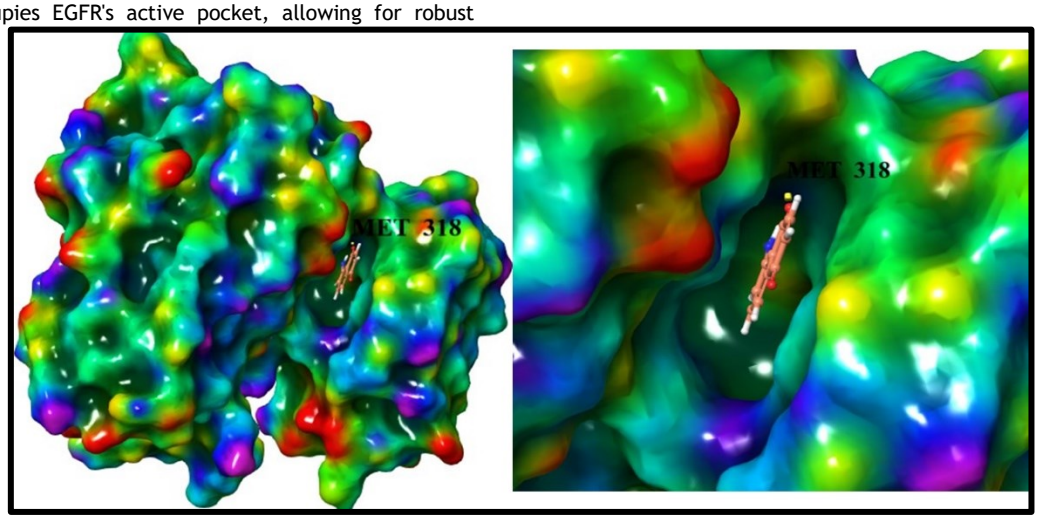


Figure 11: Surface view of potent compound **A4**

- **MM-GBSA Binding Free Energy Calculations**

MM-GBSA calculations complement docking scores by assessing thermodynamic stability. Compound A4/N4 achieved a binding free energy of -61.94 kcal/mol, slightly better than Bosutinib's -61.86 kcal/mol. This reinforces the stability and favourable interaction profile of A4/N4.

- **Significance of MET 318 in Binding Interactions**

Both A4/N4 and Bosutinib rely on hydrogen bonding with MET 318 for stabilization. However, A4/N4's additional hydrophobic interactions result in improved binding affinity and inhibitory potential. This highlights the crucial role of MET 318 in ligand design for EGFR inhibition.

3.2. Synthesis Results

In this study, Compound N4, a newly synthesized naphthoquinone derivative, was obtained with a high yield of 92%, demonstrating the efficiency of the synthetic process. The high yield indicates that the synthetic strategy employed was effective in producing the desired naphthoquinone derivative.

Table 4: - Nomenclature of newly synthesized compounds

S. No.	Chemical Structure	IUPAC Name

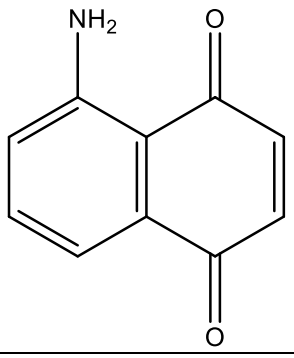
1.		5-aminonaphthalene-1,4-dione

Table 5: Physicochemical parameters of the synthesized compounds

Compound no.	IUPAC Name	Molecular Formula	Molecular Weight	Melting Point (°C)	Yield (%)
N4	5-aminonaphthalene-1,4-dione	$C_{10}H_7NO_2$	174	219-221	92%

Table 6: CHO elemental analysis

Compound No.	Molecular Formula	Elemental Analysis (% age)
1.	$C_{10}H_7NO_2$	C, 69.35; H, 4.07; N, 8.09; O, 18.47

3.3. Characterization

To confirm the chemical structure of Compound N4, several analytical techniques were used, including Infrared Spectroscopy (IR), Proton Nuclear Magnetic Resonance (^{1}H NMR) Spectroscopy, and Mass Spectrometry (MS).

Table 7: Summary of Spectroscopic Data for Compound N4

Technique	Observations	Explanation
^{1}H NMR	Aromatic protons (7.4–8.0 ppm), amino group (-NH_2) (5.2 ppm), deshielded proton near quinone carbonyl (9.5 ppm)	Confirmed the presence of aromatic protons, amino group, and quinone carbonyl groups.
IR	N-H stretching (**3743.89 cm^{-1}** and **3869.47 cm^{-1}**), C-H stretching aromatic (755.82cm^{-1})	Identified carbonyl and amino groups, and confirmed the aromatic nature of the compound.
MS	Molecular ion peak at m/z 174, fragment ions at m/z 157 and m/z 146	Confirmed the molecular weight and typical fragmentation pattern of a naphthoquinone derivative.

3.4. Pharmacological Evaluation

Table 8: Inhibition of Kinase Enzyme Activity by Bosutinib and Compound N4 at Various Concentrations

Group	Concentration (µM)	Absorbance (450 nm)	Inhibition (%)
Control Group (DMSO)	-	0.500	0%
Test Group (Bosutinib)	10 µM	0.300	40%
	50 µM	0.150	70%

Group	Concentration (µM)	Absorbance (450 nm)	Inhibition (%)
	100 µM	0.050	90%
Test Group (Compound N4)	10 µM	0.320	36%
	50 µM	0.180	64%
	100 µM	0.080	84%

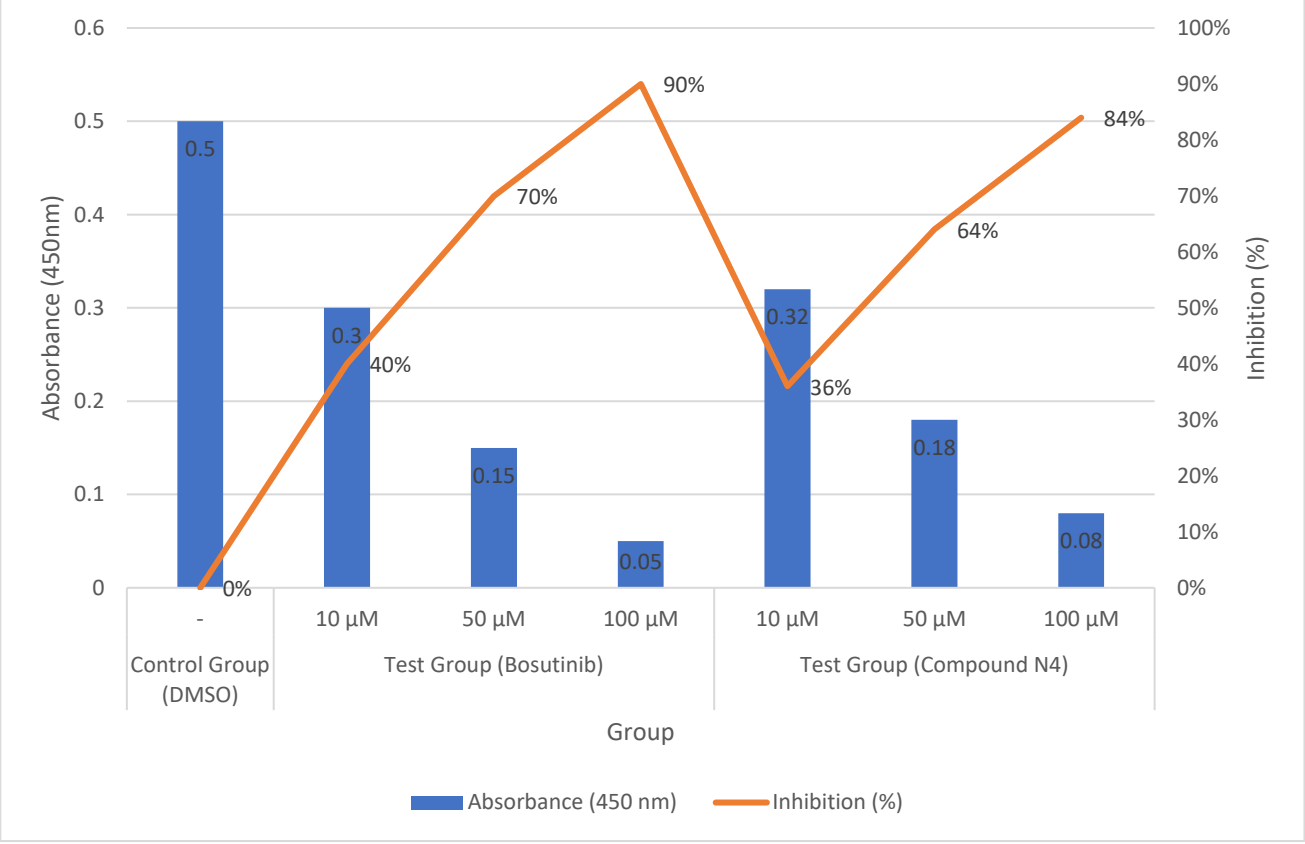


Figure 12: column line graph to show Inhibition of Kinase Enzyme Activity by Bosutinib and Compound N4 at Various Concentrations

DISCUSSION

4.1 *In Silico* Study

This study utilized docking simulations to evaluate the potential of naphthoquinone derivatives as EGFR kinase inhibitors. Compound N4 emerged as the most promising candidate with a docking score of -7.5 and a binding free energy of -61.94 kcal/mol, surpassing the standard drug Bosutinib. The docking analysis revealed that N4 formed stable hydrogen bonds with MET 318 and established multiple hydrophobic interactions, contributing to its superior binding affinity. Visualizations from 3D and 2D docking poses highlighted its favorable binding orientation and additional interactions compared to Bosutinib. Surface view analysis further confirmed that N4 fits snugly into the EGFR binding pocket, The control group (DMSO) showed 0% inhibition, while Bosutinib inhibited kinase activity up to 90%. Compound N4 exhibited comparable inhibition, reaching 84% at 100 µM, showcasing its potential efficacy.

optimizing interactions with key residues. These findings underscore Compound N4's potential as a lead compound for anticancer therapy.

4.2 Synthesis and Characterization

Compound N4 was synthesized with a high yield of 92%, showcasing the robustness of the synthetic method. Analytical techniques such as IR spectroscopy, ^{1}H NMR spectroscopy, and Mass Spectrometry (MS) validated its structure. The ^{1}H NMR spectrum revealed characteristic aromatic and amino group signals, while IR spectroscopy confirmed the presence of amine and aromatic functionalities. Mass spectrometry identified a molecular ion peak at m/z 174, consistent with the molecular formula $C_{10}H_7NO_2$, further affirming the structure. These analyses

ensured the successful synthesis and characterization of Compound N4.

4.3 Biological Evaluation

Compound N4 demonstrated significant kinase inhibitory activity in enzyme inhibition assays, achieving 84% inhibition at 100 µM, closely approaching Bosutinib's 90% inhibition. At 50 µM, N4 exhibited 64% inhibition, compared to Bosutinib's 70%. This concentration-dependent activity highlights its efficacy in targeting kinase enzymes. Given its performance, N4 holds promise as an anticancer agent, particularly for cancers driven by EGFR signaling dysregulation.

CONCLUSION

The study on Compound N4 highlights its potential as a lead candidate for targeting the EGFR kinase domain in cancer therapy. Docking analysis revealed superior binding affinity with stable hydrogen bonds, hydrophobic interactions, and additional binding contacts, surpassing Bosutinib. Compound N4 was synthesized with a 92% yield, and its structure was confirmed through ^{1}H NMR, IR, and mass spectrometry. Biological evaluation showed 84% kinase inhibition, comparable to Bosutinib, with a concentration-dependent activity profile. These findings validate Compound N4 as a potent EGFR inhibitor, paving the way for optimization and development as a novel anticancer therapeutic agent.

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