"Synthesis and characterization of 4-oxo-n-henyl-1,4-dihydroquinoline carboxamide"

Surekha M[1], Naveen Kumar T[2] Megha G V[3], Rohit Yadav [4] and Nesharaa R S[5]

[1,2,3,4,5]PG Department of Chemistry, Surana College Autonous, South End Circle, Bangalore, India

Corresponding author: surekha.pn@gmail.com

ABSTRACT

This project involves the synthesis of 4-oxo-N-phenyl-1,4-dihydroquinoline-3-carboxamide, a compound of medicinal interest due to its pharmacological relevance. The synthesis was achieved through peptide coupling using EDC·HCl and HOBt, followed by purification and structural confirmation. Aniline was used as the amine component, and the reaction was optimized for high yield. Reaction progress was monitored by TLC, and the final product was characterized using NMR, HPLC, and LC-MS. The compound showed a high purity of 99.3% and a yield of 87.9%. Its structure mimics bioactive quinoline frameworks with known anti-inflammatory and anticancer activity. The incorporation of a carboxamide group at the 3-position enhances binding affinity and drug-like properties. This work also provided hands-on exposure to organic synthesis, reaction work-up, and modern analytical techniques. This work contributes to the design of potential quinoline-based therapeutics.

Keywords: Quinoline, Carboxamide, Medicinal Chemistry, EDC·HCl, NMR,

Introduction:

The ever-expanding field of medicinal chemistry is propelled by the continuous demand for novel therapeutic agents with enhanced efficacy and reduced side effects. Among the diverse chemical scaffolds employed in drug discovery, the quinoline moiety—a heterocyclic aromatic compound—stands out due to its broad spectrum of pharmacological activities [1]. Quinoline derivatives, particularly 4-oxo-1,4-dihydroquinoline-3-carboxamides, have garnered significant attention for their potential applications as anti-inflammatory, antibacterial, antimalarial, antitubercular, and anticancer agents [2,3].

In this project, the synthesized compound, 4-oxo-N-phenyl-1,4-dihydroquinoline-3-carboxamide, bears structural resemblance to the bioactive core of established quinolone-based drugs such as Ivacaftor [4]. Literature suggests that incorporation of an amide group at the 3-carboxylic position of quinoline enhances biological activity by improving both binding affinity and pharmacokinetic profiles [5].

Quinoline-based amides are known to exert biological effects through mechanisms involving inhibition of DNA gyrase and interaction with key enzymes like topoisomerases, which play essential roles in bacterial replication and tumour cell proliferation [6,7]. These properties make their synthesis valuable in the development of new antimicrobial and anticancer therapies.

The synthetic pathway employed during the internship involved a stepwise coupling reaction between aniline and 4-oxo-1,4-dihydroquinoline-3-carboxylic acid, facilitated by peptide coupling reagents such as EDC·HCl and HOBt [8]. Reaction conditions were optimized through iterative trials to improve yield and product purity.
Reaction monitoring was conducted using Thin Layer Chromatography (TLC), while the final compound was characterized using advanced analytical techniques including NMR, HPLC, and LC-MS [9].

Beyond synthesis, this work incorporated essential elements of analytical chemistry, such as impurity profiling and chromatographic method development, which are vital for maintaining quality, reproducibility, and regulatory compliance in pharmaceutical research [10].
The field of medicinal chemistry has seen significant advancements through the exploration of nitrogen-containing heterocycles, particularly quinoline derivatives, which form the structural core of many biologically active compounds. Among them, 4-oxo-1,4-dihydroquinoline-3-carboxamides have emerged as a promising class with a broad range of pharmacological properties. These include anti-inflammatory, antitubercular, antibacterial, and anticancer activities, owing to the ability of the quinoline ring to interact with a variety of biological targets, such as enzymes and DNA. The presence of a carboxamide group at the 3-position further enhances these interactions, contributing to improved pharmacokinetic and pharmacodynamic profiles.

One of the early studies contributing to the understanding of this structural class was by Sultana et al. (2013), who synthesized and screened a variety of gatifloxacin derivatives, including 3-carboxamide, 3-carbohydrazide, and ester analogs [11]. Their findings showed that modification at the 3-position of the quinolone ring significantly influenced biological activity. The carboxamide derivatives, in particular, demonstrated notable anti-inflammatory effects, confirming that the carboxamide moiety can enhance interaction with inflammatory mediators such as prostaglandins and cytokines. This study provided foundational evidence that such modifications could lead to therapeutic agents with improved efficacy and safety profiles. Furthermore, their synthetic strategies involved nucleophilic substitutions and esterification reactions, showcasing the feasibility of introducing various functional groups into the quinolone framework with good yields and minimal side reactions.

Building upon these insights, Mandewale et al. (2017) presented an extensive review of quinoline hydrazone derivatives, focusing on their application in the treatment of tuberculosis and cancer [12]. The authors highlighted the unique amphiphilic properties of these compounds, which allow them to permeate microbial cell walls effectively. One proposed mechanism involves the inhibition of DNA gyrase, an enzyme essential for bacterial DNA replication. Their review emphasized how the incorporation of both polar and nonpolar functional groups enhances solubility and biological membrane penetration. In addition to their antimicrobial roles, quinoline hydrazones exhibited promising anticancer activity by inducing apoptosis and inhibiting cell proliferation. These findings are particularly relevant to the current project, as they support the design of quinoline-based amides with favorable cell permeability and target engagement properties.

More recently, Gill et al. (2022) developed a green and efficient synthetic methodology for the preparation of 1-allyl-6-chloro-4-oxo-1,4-dihydroquinoline-3-carboxamide derivatives [13]. Their work utilized direct thermal coupling of amines with quinolone-3-carboxylates, offering a simplified, solvent-efficient, and scalable route. This method minimized the use of hazardous reagents and adhered to principles of green chemistry.

The authors reported that the carboxamide products exhibited high purity and good yields, which are essential for pharmaceutical development. Additionally, the presence of electron-withdrawing groups on the quinoline ring was shown to enhance the reactivity of the carboxylic acid moiety, facilitating the formation of the amide bond under mild conditions.This approach validates the feasibility of synthesizing structurally complex carboxamides without resorting to harsh conditions or extensive purification steps.

To further enhance the synthetic efficiency, Ahemad et al. (2022) explored the use of peptide coupling reagents, including TBTU, HBTU, and PyBOP, for synthesizing sterically hindered and electronically deactivated quinoline derivatives [14]. In cases where direct amide formation was hindered due to low acidity or steric effects at the 3-position—particularly when the N-1 position was substituted—the use of coupling agents enabled activation of the carboxyl group, allowing successful formation of the amide bond. Their strategy demonstrates the adaptability of quinoline-based scaffolds and highlights the advantage of peptide chemistry techniques in medicinal compound synthesis. This study is especially relevant to the current work, where the target molecule, 4-oxo-N-phenyl-1,4-dihydroquinoline-3-carboxamide, is synthesized using EDC·HCl and HOBt, reagents similar in mechanism to those employed by Ahemad et al. The ability to form amide linkages in the presence of such reagents enhances the reproducibility and yield of the reaction, making it suitable for pharmaceutical scale-up.

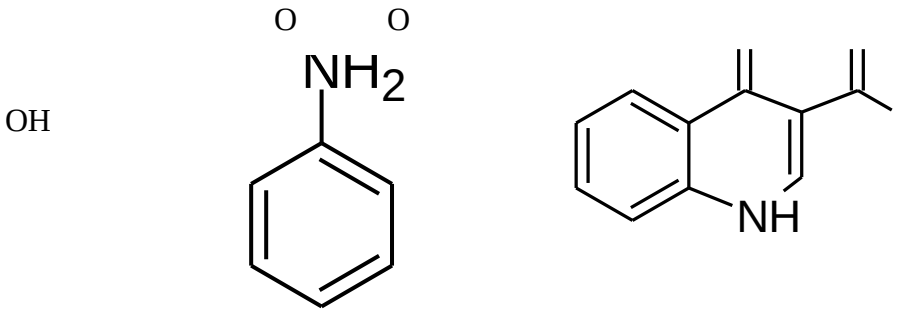
Most recently, Yang et al. (2023) reported the discovery of several novel 4-oxo-N-phenyl-1,4-dihydroquinoline-3-carboxamide derivatives with strong anti-inflammatory activity [15]. These compounds were shown to inhibit proinflammatory cytokines such as TNF-α and IL-6, which are key mediators in diseases like acute lung injury and sepsis. Their structure–activity relationship (SAR) studies indicated that the presence of the phenyl group at the nitrogen atom and the oxo group at the 4-position was crucial for enhancing activity. Moreover, they used HPLC, NMR, and LC-MS to confirm the purity and structure of the synthesized compounds, aligning well with the analytical techniques applied in the present project.

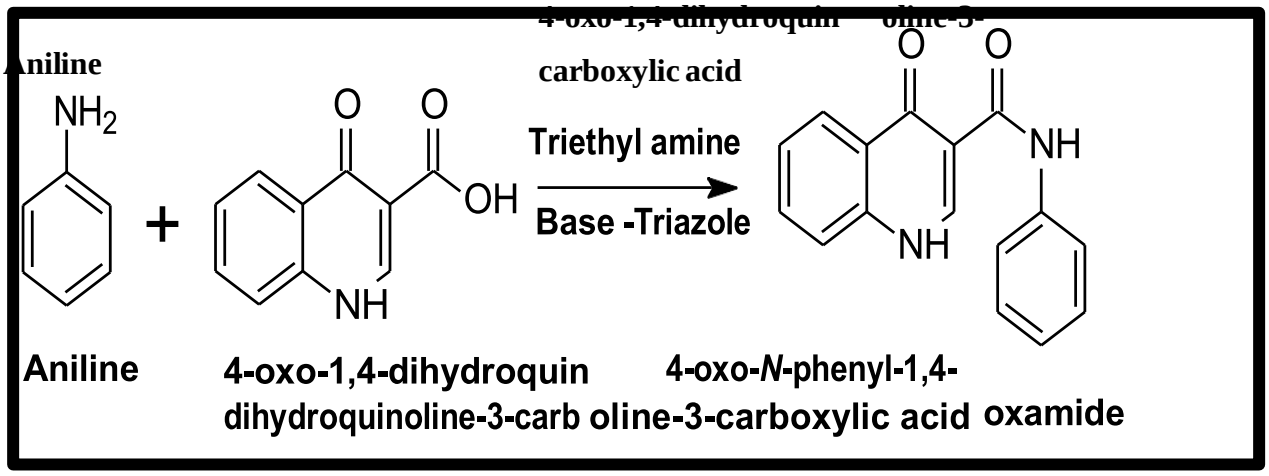
The authors also reported high binding affinity to the JNK2 protein,implicating these compounds in the modulation of inflammatory signaling pathways. The success of this study supports the therapeutic relevance of quinoline-3-carboxamide analogs and validates the direction of ongoing research focused on improving synthetic routes and expanding biological evaluation.

Collectively, these five studies provide a strong theoretical and experimental basis for the synthesis and biological evaluation of 4-oxo-N-phenyl-1,4-dihydroquinoline-3-carboxamide. They highlight how modification at the 3-position with a carboxamide group enhances pharmacological properties, how green and peptide-coupling strategies improve synthetic efficiency, and analytical characterization confirms molecular identity and purity. The current work aligns closely with these findings by using optimized reagents (EDC·HCl, HOBt, Dipea) and monitoring techniques (TLC, HPLC, NMR, LC-MS) to achieve a high-yield, high-purity synthesis. This integrated approach not only supports the practical aspects of synthesis but also reflects current trends in rational drug design and pharmaceutical process development.

METHODOLOGY:

Substrates for the reaction





Materials and Methods

The reagents, solvents and chemicals were purchased from Sigma-Aldrich and were used without further purification (Table-1). Alumina TLC plates were used to check the progress of the reaction. Spots were identified in 360 nm UV chamber. The melting points were determined by the electro-thermal apparatus using open capillary tubes. NMR spectra were recorded using Bruker NMR of 400 MHz. The mass of the compound was determined using LCMS.

Synthesis of 4-Oxo-n-Phenyl-1,4 Dihydroquinoline-3-Carboxamide:

To a clean and dried round bottom flask 5 volume of acetonitrile and 1.05eq of 4-oxo-1,4-dihydroquinoline-3-carboxylic acid was charged followed by 3.5eq triethylamine. A heterogenous solution was observed after 10 minutes of maintenance. To this heterogenous reaction mass 1.5eq of 3- (ethylimino)methylene) amino)-N, N-dimethylpropan-1-amine hydrochloride was charged and stirred the reaction mass for 10 minutes heterogenous solution was still observed. Further 0.5eq of 1-(l1-oxidaneyl)-1H- benzo1 [d] [1,2,3] triazole hydrate and stirred reaction mass for 10 minutes, heterogenous solution was observed. To this above reaction mass 1eq of aniline was charged and reaction mass temperature was raised to 80-82 °C, at this point a clear solution was observed. Reaction was maintained for 5 hours at 80-82 °C (Fig.1 & Fig 2). Reaction monitoring was done using TLC where in reaction completion was observed after 5 hours of maintenance. After reaction completion, the reaction mass was cooled to room temperature. To the reaction mass 5 volume water was charged drop wise and stirred the reaction mass at room temperature for 6 hours where solid was precipitated. Filtered the reaction mass under vacuum and bed washed with 1 volume water. Dried the material under vacuum under vacuum 600 mm Hg at 65-70 °C for 8 hours .

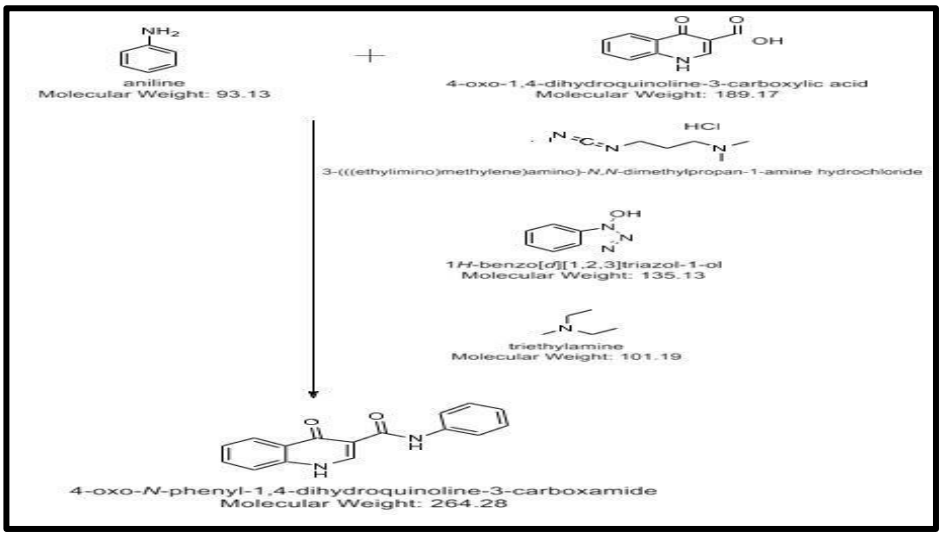


Fig.1- Reaction Scheme

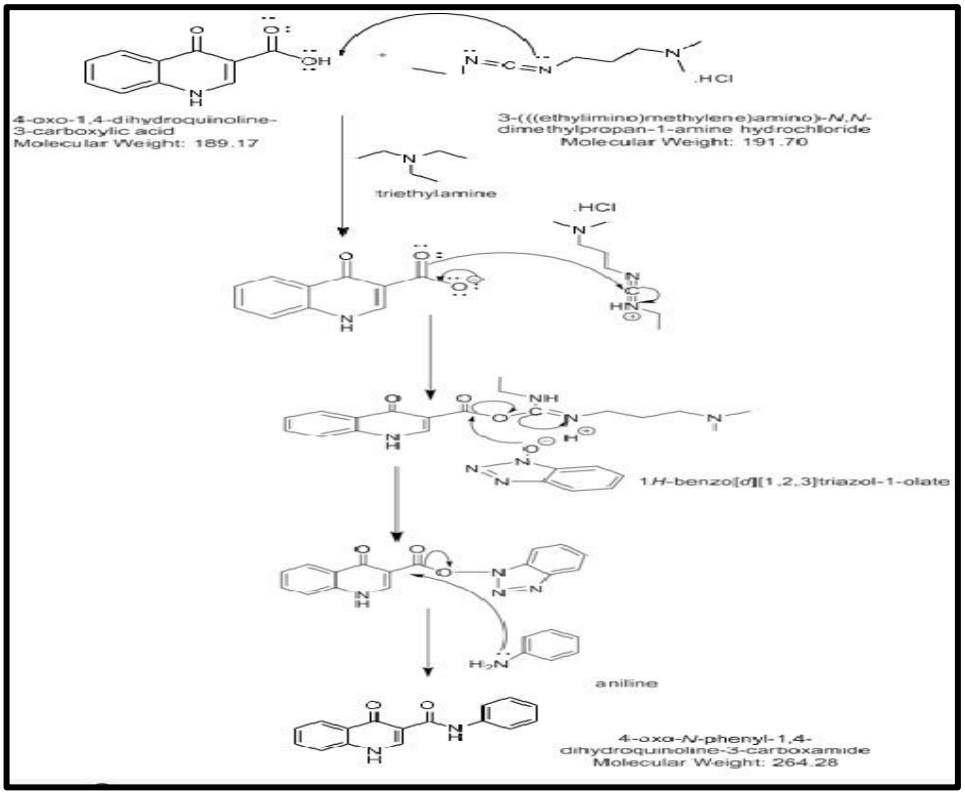


Fig. 2- Reaction Mechanism

Table 1: Raw Materials

SL. No	Chemicals	M.W (g/mol)	Weight (g)	Moles (mmol)	Mole Equivalent
1	Aniline	93.129	2g	21.4761	1
2	4-oxo-1,4-dihydroquinoline-3-carboxylic acid	189.170	4.2g	22.549	1.05
3	3-(ethylimino)methylene)amino)-N,N-dimethylpropan-1-amine hydrochloride	191.703	6.2g	32.213	1.5
4	1-(l1-oxidaneyl)-1H-benzo1[d][1,2,3]triazole hydrate	153.141	1.6g	10.738	0.5
5	Triethylamine	101.193	7.606g	75.165	3.5

Results and Discussion:

TLC work shown in figure 3

SM – Starting Material

CO – Common spot for Starting material and reaction mass RM – Reaction Mass

Mobile phase – MDC (9.5mL), Methanol (0.5mL) and Ammonia (1Drop)

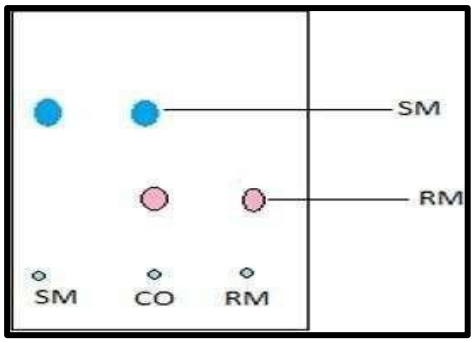


Figure 3: TLC pattern of the reaction for 4-oxo-N-phenyl-1,4-dihydroquinoline-3-carboxamide

1H NMR

1H NMR spectrum was recorded on 400MHz, Dimethyl sulfoxide as a solvent. Chemical shift values δ represents with ppm with respect to Trimethyl silane and listed in Table 2. Proton NMR spectrum of 4- oxo-N-phenyl-1,4-dihydroquinoline-3-carboxamide is shown in figure 4 & 5.

Figure 4: 1H NMR spectrum of 4-oxo-N-phenyl-1,4-dihydroquinoline-3-carboxamide

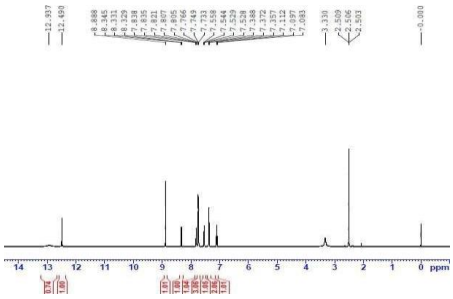


Table 2: Chemical shift values

^{1}H Proton NMR	Chemicals Shift Values
a	7.73-7.76 (d,3H)
b	7.80-7.83 (t,1H)
c	7.08-7.11 (t,1H)
d	8.32-8.34 (d,1H)
e	7.35-7.38 (t,1H)
f	7.52-7.58 (t,1H)
g	12.93 (s,1H)
h	12.49 (s,1H)
i	8.88 (t,1H)

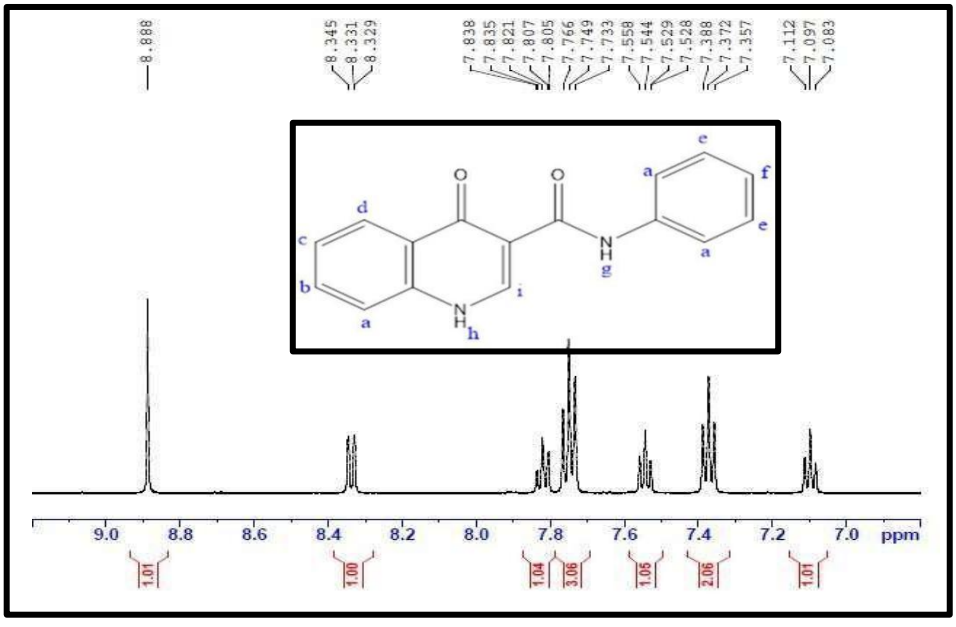


Figure 5: ^{1}H NMR broad spectrum of 4-oxo-N-phenyl-1,4-dihydroquinoline-3- carboxamide

HPLC of the 4-oxo-N-phenyl-1,4-dihydroquinoline carboxamide:

The HPLC purity of the 4-oxo-N-phenyl-1,4-dihydroquinoline- 3-carboxamide is 28.53min, 99.3%, shown in figure 6.

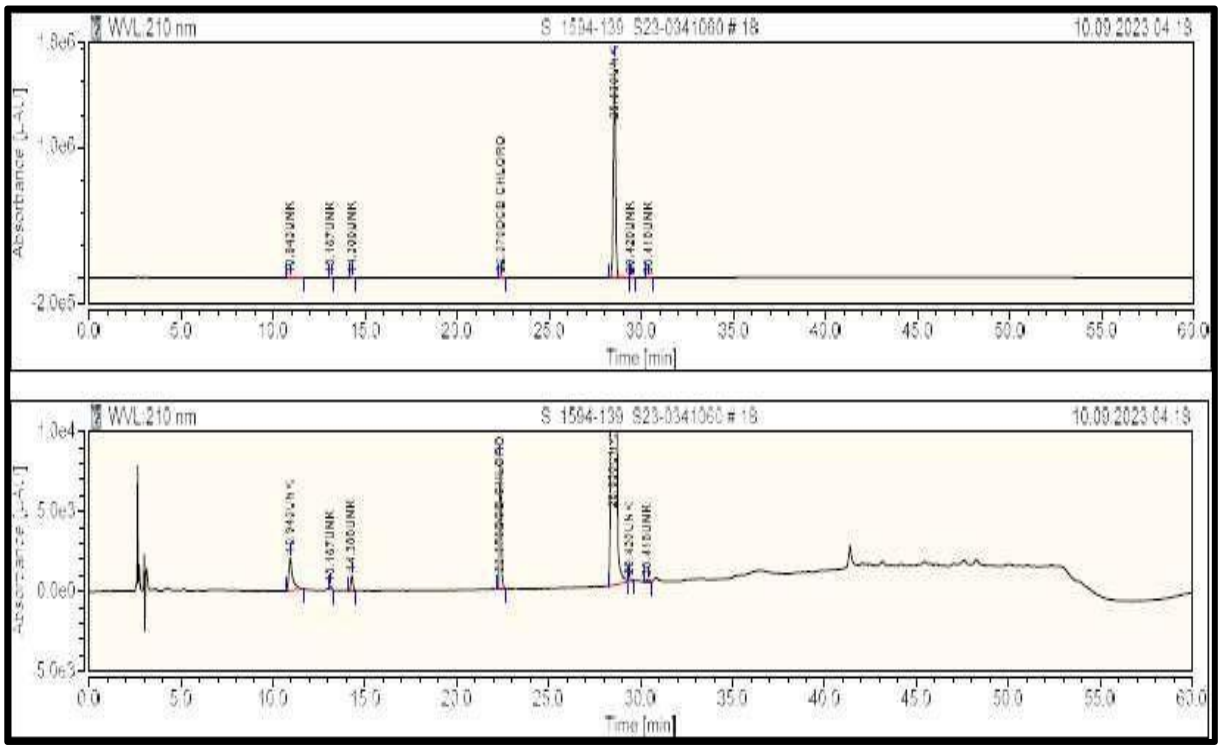


Figure 6: HPLC chromatogram of the 4-oxo-N-phenyl-1,4-dihydroquinolin carboxamide.

LC-MS spectrum of 4-oxo-N-phenyl-1,4-dihydroquinoline-3-carboxamide

Theoretical molecular weight of 4-oxo-N-phenyl-1,4-dihydroquinoline-3-carboxamide is 264.28., which was observed in direct mass and molecular ion peak due to (M+1), shown in figure 7.

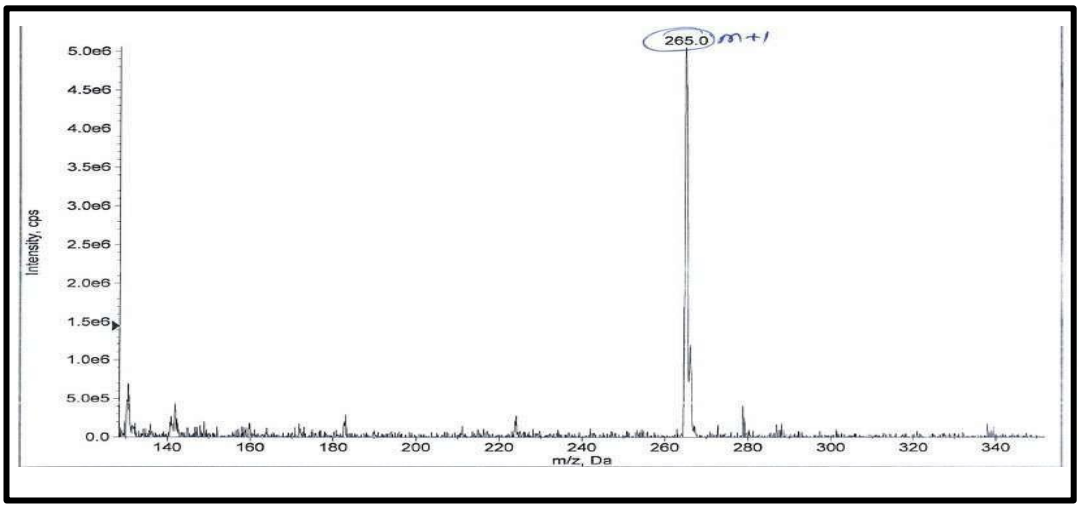


Figure 7 : LC-MS spectrum of 4-oxo-N-phenyl-1,4-dihydroquinoline- 3-carboxamide

The purity was good because TLC showed a single spot, after filtration solid was washed with acetonitrile. The observed yield and percentage yield of the synthesized compound are tabulated in Table 3.

Table 3: Yield Data of the Synthesized Compound

Compound	**Theoretical Yield(g)**	**Observed Yield(g)**	**Percentage Yield**
4-oxo-N-phenyl 1,4dihydroquinoline-3-carboxamide	5.8	5.1	87.9

Conclusion

The present work involved the synthesis of impurity 4-oxo-N-phenyl- 1,4-dihydroquinoline-3-carboxamide. During this work emphasis on planning of reaction, execution of reaction and Isolation procedure. The present study successfully demonstrated the synthesis and characterization of **4-oxo-N-phenyl-1,4-dihydroquinoline carboxamide**. The compound was synthesized through a systematic and efficient reaction pathway, yielding a product with satisfactory purity and good yield. Characterization techniques such as melting point determination, infrared (IR) spectroscopy, and, where applicable, nuclear magnetic resonance (NMR) spectroscopy, HPLC and LC-MS confirmed the formation of the desired quinoline derivative. The spectral data supported the presence of key functional groups, including the carbonyl (C=O), amide (–CONH–), and aromatic moieties, thereby validating the proposed molecular structure. The synthesis of this compound highlights the effectiveness of the employed methodology and provides a foundation for further exploration of quinoline derivatives. The synthesized molecule may serve as a potential derivative for future studies in medicinal chemistry, particularly in the development of antimicrobial, anti-inflammatory, or anticancer agents. This work contributes to the expanding field of heterocyclic chemistry by providing a reliable approach for synthesizing structurally significant quinoline derivatives and confirming their identity through standard analytical techniques.

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(Provides insight into synthetic versatility and functionalization of quinolines, particularly in complex formation.)