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SYNTHESIS AND SPECTROSCOPIC CHARACTERIZATION OF NEW QUINOXALINE DERIVATIVES

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ABSTRACT

Quinoxaline is a nitrogen-containing heterocyclic compound consisting ofa fused benzene and pyrazine ring, known for its wide range of pharmacological activities. Due to its stable aromatic structure and modifiable functional groups, quinoxaline has attracted significant attention in medical chemistry. Various quinoxaline derivatives have been reported to exhibit antimicrobial, anti-inflammatory, anticancer, and analgesic activities. The analgesic effect is mainly associated with inhibition of inflammatory mediators and modulation ofpain pathways. This present project involves the synthesis of novel quinoxaline derivatives with the aim of enhancing analgesic activity.

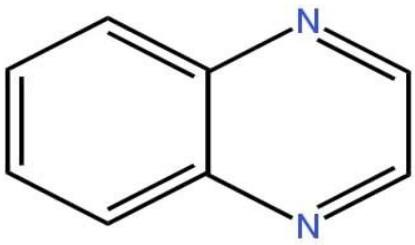
INTRODUCTION

QUINOXALINE

Quinoxaline is a well-known bicyclic nitrogenous heteroaromatic compound formed through the fusion of a benzene ring with a pyrazine ring. It contains a planar and rigid aromatic framework with nitrogen atoms positioned at the 1 and 4 locations, and its molecular formula is C_8 H_6 N_2. Because ofthis structural arrangement,

quinoxaline exhibits high chemical stability and serves as an important core structure in medicinal chemistry.

The quinoxaline nucleus was first described by Hinsberg in 1887. Since its discovery, it has received significant scientific attention due to its versatility in undergoing different structural modifications. The fused aromatic ring system provides excellent thermal and chemical stability, which helps quinoxaline derivatives maintain.



Molecular Formula: C8 H6 N2

Molecular Weight: 130.15g/mol

Melting Point: 27 – 29^{o}C

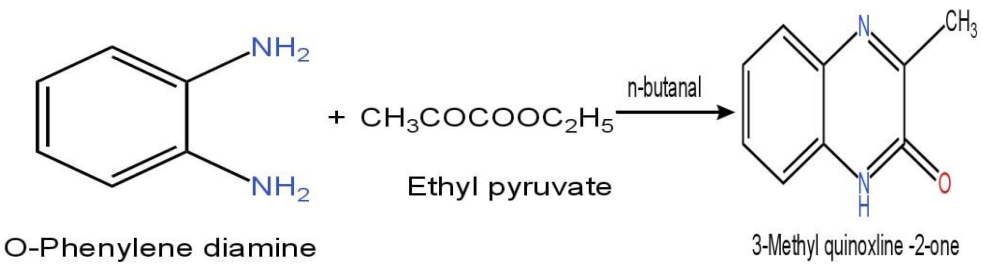
Boiling Point: Around 237-239^{o}C. The low melting point and moderate boiling point indicate that quinoxaline is a low-melting crystalline solid with good thermal stability Solubility: Soluble in organic solvents like ethanol, ether, benzene and chloroform. Slightly soluble in water.

Stability: Stable under normal conditions but sensitive to strong acids.

Appearance: Colorless to pale Yellow crystalline solid,

SCHEME AND MATERIALS METHOD

1. STEP 1: Synthesis of 3-methyl quinoxaline-2 (1H) – one

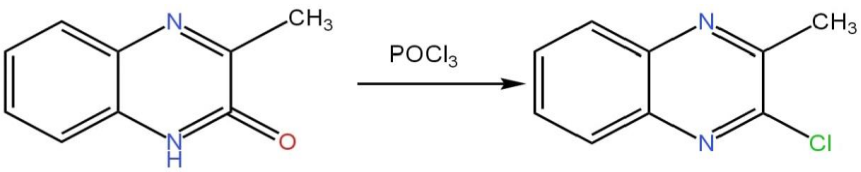


O-phenylenediamine (10.8 g, 0.10 mol) was dissolved in N-butanol (300 ml) with gentle warming.

Ethyl Pyruvate (11.6 g, 0.10 mol) was dissolved separately in N-butanol (100 ml) and added slowly to the above solution with constant stirring.

The reaction mixture Was kept aside for 30 minutes, then heated on a water bath for 1 hour. After cooling, the crystals formed were filtered, washed with n-hexane, and recrystallized from ethanol to obtain pure 3- methyl quinoxaline-2(1H)-one.

2. STEP-2: Synthesis of 2-chloro-3-methylquinoxaline



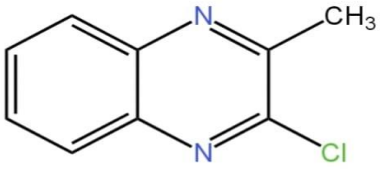
3-Methyl quinoxline -2-one 2-Chloro-3-Methyl quinoxline

3- methyl quinoxaline-2(1H)- one (11 g, 0.10mol) was treated with phosphoryl chloride (POCl3, 60 ml) and refluxed for 90 minutes.

Excess POCl3 was distilled off, and the residue was cooled to room temperature, then poured carefully onto crushed ice.

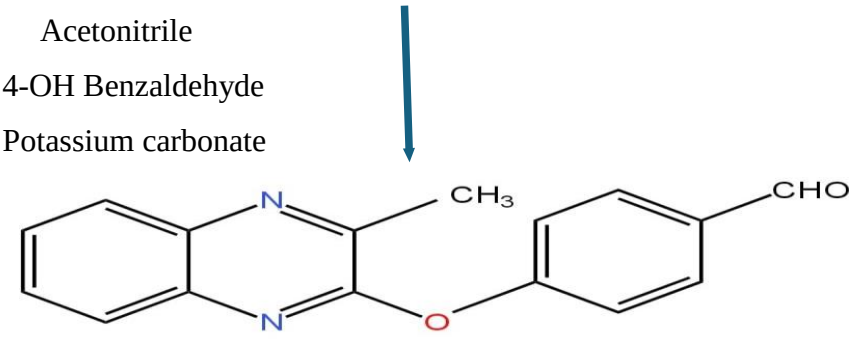
The solid obtained was filtered, washed with cold water, and dried to give 2-chloro-3-methyl quinoxaline.

3. STEP-3: Synthesis of 4-(3-methylquin oxalin-2-yloxy) benzaldehyde



2-Chloro-3-Methyl quinoxline

Acetonitrile
4-OH Benzaldehyde
Potassium carbonate



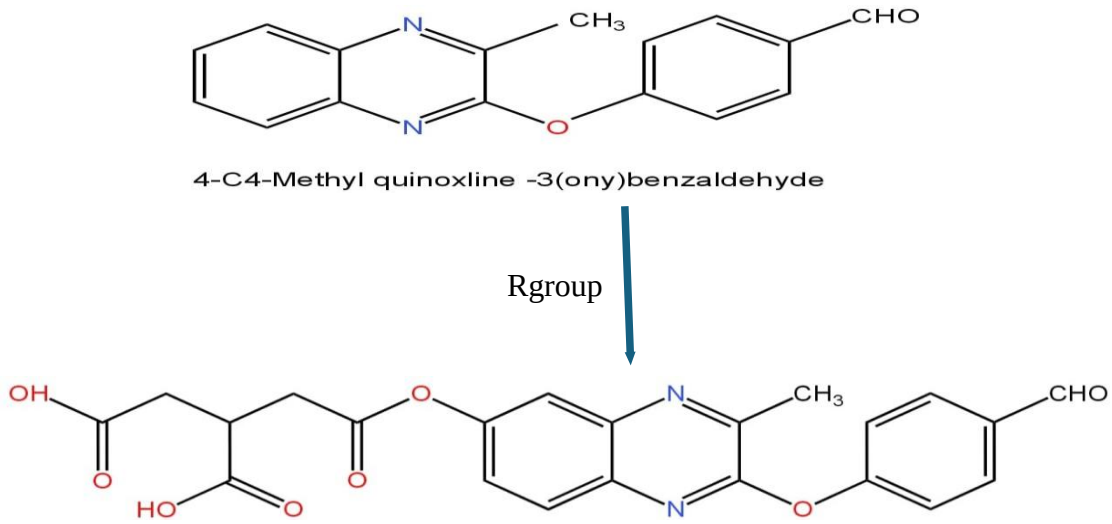
4-C4-Methyl quinoxline -3(ony)benzaldehyde

4-Hydroxybenzaldehyde (0.122 g, 0.01 mol) was dissolved in acetonitrile (50 ml) in a 250 ml round-bottom flask.

Anhydrous potassium carbonate (2.0 g) was added, and the mixture was refluxed for 1 hour. Then, 2-chloro-3-methyl quinoxaline (1.785 g, 0.01 mol) was added, and refluxing was continued for 30 minutes.

After completion of the reaction, the mixture was cooled, and the solid obtained was filtered, washed, dried, and recrystallized from ethanol to yield pure 4-(3-methyl quinoxaline-2-yloxy) benzaldehyde.

STEP 4: Preparation of Final Quinoxaline Derivatives.



3-{2-[(3-{4-formylphenoxy}-2-methylquinoxalin-6-yl)oxy]-2-oxoethyl}pentanedioic acid.

4-Hydroxy benzaldehyde (5 g) was dissolved in acetonitrile (50 mL) in a 250 mL round bottom flask. Anhydrous potassium carbonate (5 g) was added and the reaction mixture was refluxed at 80– 82° C for 1 hour with continuous stirring.

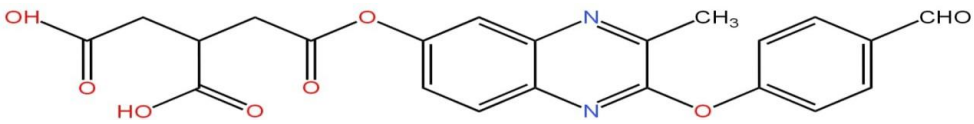
After 1 hour, 4-(2-methylquinoxaline-3-yloxy) benzaldehyde (5 g) and the derivative-forming chemical containing amide/acid functional groups (5 g) were added slowly to the reaction mixture. The mixture was further refluxed at 82– 85° C for 4– 5 hours for the attachment of the derivative at the 6th position of the quinoxaline ring, resulting in the formation of the final quinoxaline derivative.

The reaction mixture was cooled to room temperature and poured into ice-cold water (100

mL). The precipitated solid was filtered, washed with distilled water, dried, and purified by recrystallization using ethanol to obtain the final quinoxaline derivative.

DERIVATIVES

Q-01 (Quinoxaline citric acid derivative)



Molecular formula: C23H20 N2 O8

Molecular weight: 452.41 g/mol

IUPAC Name: 3-2-[(3-{4-formylphenoxy}-2-methylquinoxalin-6-yl) oxy]-2-oxoethyl} pentanedioic acid.

MATERIALS AND METHODS

1. Solubility

Solubility describes the ability of a solute to dissolve in a solvent until the solution reaches its maximum concentration under specific temperature and pressure conditions. At this point, the solution becomes saturated.

In certain cases, substances that form very fine, stable dispersions in a liquid may also be referred to as “soluble,” even though their exact solubility cannot be easily determined.

The degree of solubility is influenced by the chemical nature of both solute and solvent, the pH of the environment, temperature, pressure, and the presence of other dissolved materials.

These factors affect molecular interactions and can be explained through basic thermodynamic concepts.

Qualifiers used to describe extent of solubility

Solubility refers to the amount of a substance that can dissolve in a given solvent under specific conditions. The extent of solubility varies widely among different substances. Some materials dissolve in any proportion and are called miscible, such as ethanol mixing completely with water. In contrast, certain substances show extremely low solubility and are considered practically insoluble, like titanium dioxide in water.

Between these two limits, several descriptive terms such as freely soluble, very soluble, slightly soluble, sparingly soluble, and practically insoluble are used to indicate different levels of dissolving capacity depending on scientific or pharmaceutical needs.

S. No	Derivative	Hot water	Acetone	Glacial Acetic acid	Chloroform	Carbon tetra chloride	Ethyl acetate	Ethyl Aceto acetate	Nitric acid
1.	Step 1	+ + -	+ + -	+ + +	+ + -	+ + +	+ + -	+ + -	+ + +
2.	Step 2	- - -	- - -	++-	- - -	- - -	- - -	+ + -	+++
3.	Step 3	- - -	+ + -	+ + +	- - -	+ + +	- - -	+ + +	+ + -
4.	Q-1	+ + +	+ + -	+ + +	+ + +	- - -	- - -	+ + -	+ + +
5.	Q-2	+ + -	++ -	+ + +	+ + +	+ + -	- - -	- - -	+ + +
6.	Q-3	- - -	+ + +	---	+ + +	+ + -	+ + -	+ + -	- - -
7.	Q-4	+ + +	- - -	+++	+ + +	- - -	- - -	- - -	+ + +
8.	Q-5	+ + -	+ + +	+ + +	+ + +	+ + +	+ - -	+ + -	+ + +

(+ + +: Freely soluble; - -: Insoluble; + + -: Slightly soluble)

6.2 THIN LAYER CHROMATOGRAPHY

Thin Layer Chromatography (TLC) is an analytical separation technique widely used in chemistry to identify, isolate, and monitor compounds present in a mixture. In this method, a thin coating of adsorbent material, such as silica gel or alumina, is spread on a solid support like glass or aluminum to create the stationary phase. When a solution of the sample is applied and the plate is exposed to a suitable solvent, the components travel at different speeds over the surface due to variations in their interactions with the stationary and mobile phases, resulting in effective separation. TLC is valued for its simplicity, rapid results, minimal sample requirement, and ability to handle a range of organic substances. Owing to its flat surface operation, this technique is also referred to as planar chromatography, open chromatography, strip chromatography, surface chromatography, spread chromatography,

and drop chromatography. The concept of TLC was first introduced by Izmailov and Schreiber in 1938, marking the foundation of modern planar chromatography, and the method was later improved and systematically developed by Stahl in 1958, making it a standard tool in analytical laboratories.

Principle of thin layer chromatography

Thin Layer Chromatography (TLC) functions by separating substances in a mixture according to how they interact with two phases: a stationary adsorbent layer, typically silica gel, and a moving solvent. When the solvent travels upward through the plate by capillary action, each compound moves at its own pace based on its attraction to the stationary surface. Highly polar molecules attach more firmly to the adsorbent and therefore travel a shorter distance, while less polar molecules move further with the solvent, resulting in their separation on the plate.

METHODOLOGY

Preparation of TLC Plate

A pre-coated TLC plate containing a thin adsorbent layer—usually around 0.25 mm thick on a glass, plastic, or Aluminium sheet is used for the experiment. A light pencil mark is drawn about 1– 2 cm above the lower edge to serve as the baseline, since pencil does not interfere with the chromatographic result the way ink would.

Spotting the Sample

The sample is first dissolved in a volatile solvent to make a dilute solution. Using a capillary tube or micropipette, a very small amount of this solution is gently applied to the baseline. The spot should be compact and concentrated to achieve good separation during development.

Development of the Plate

The prepared plate is placed upright in a closed developing chamber containing a shallow layer of the mobile phase. It is important that the solvent does not touch the sample spots directly. As the solvent rises through the plate by capillary action, it carries the components at different rates depending on their interactions with the stationary layer, stopping when the solvent nears the upper region of the plate.

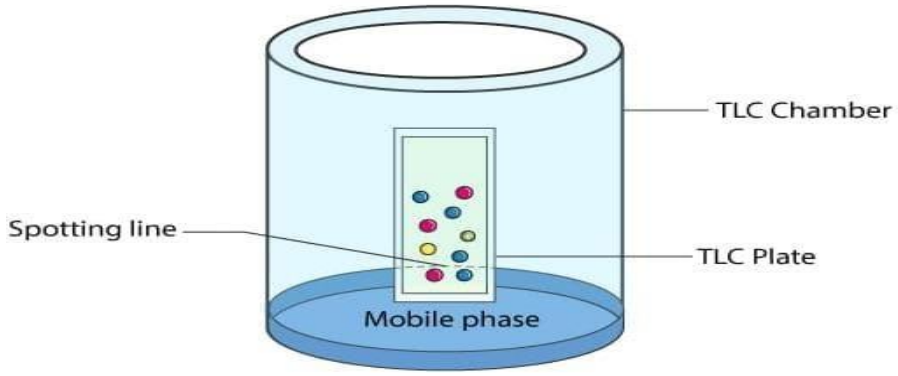
Visualization

If the separated substances are naturally coloured, they can be seen directly. For colourless compounds, detection methods such as ultraviolet light on fluorescent plates or chemical spraying agents like iodine, ninhydrin, or potassium permanganate are used to reveal the individual spots.

Data Interpretation (Rf Value)

To evaluate the separation, the Retention Factor (Rf) is measured, expressing how far each component moved compared to the distance travelled by the solvent front.

$$Rf = \frac{\text{Distance traveled by compound}}{\text{Distance traveled by solvent front}}$$



S.NO	COMPOUNDS	Rf VALUE
1	Step1	0.3
2	Step 2	0.5
3	Step 3	0.9
4	Q-01	0.7
5	Q-02	0.6

6.3 MELTING POINT

Melting point determination is an important method used to check the purity of a compound. It is measured using the open capillary tube method. The purity is confirmed by noting any change in the melting point, as recrystallized compounds may show a slight difference.

In this procedure, the sample was placed in a capillary tube, inserted into a Thiele's tube, and its melting point was recorded.

Application of melting point

1. Identifying Unknown Liquids: Since many liquids boil at similar temperatures,

converting them into solid derivatives makes identification easier through their melting points.

2. Telling Isomers Apart: Isomers produce derivatives with noticeably different melting points, helping distinguish them quickly.
3. Confirming New Syntheses: Creating standard derivatives of a newly made compound and checking their melting points helps verify that the correct structure was formed.
4. Checking Purity: A narrow melting point range signals a clean, pure derivative, while a wider range suggests impurities.
5. Fast Verification Method: When only a tiny amount of sample is available, forming a derivative and measuring its melting point gives a quick, low-cost confirmation.

Determination of Melting Point

1. Sample Preparation: Powder the dry substance thoroughly and place a small column (about 2– 3 mm) of it into a capillary tube that is sealed at one end by lightly tapping the tube.
2. Instrument Adjustment: Set the starting temperature a few degrees (5– 10° C) below the estimated melting point and choose a slow heating rate, usually between 0.5 and 1° C per minute.
3. Loading the Capillary: Insert the prepared tube into the heating chamber and begin the run once the instrument maintains the selected starting temperature.

4. Observing the Melting Process: Watch the sample using the built-in viewer or digital screen. The instrument will register the temperature at which melting begins and the temperature where the sample becomes fully liquid, using optical or video detection.

Molecular formula

The molecular formula is a concise chemical notation that specifies the exact count of each type of atom bonded together in a single molecule, reflecting its true composition rather than just ratios.

Example: Hydrogen peroxide = H_2 O_2

(shows 2 hydrogen atoms and 2 oxygen atoms in one molecule)

Principle of Molecular Formula

The molecular formula is based on the idea that elements combine in fixed whole-number ratios to form compounds. It is determined using the empirical formula and the molar mass, where the molecular formula is a multiple of the empirical formula. The multiplying factor is obtained by dividing the molecular weight by the empirical formula weight. For example, if the empirical formula is CH_2 and the molecular weight is 28, the molecular formula becomes C_2 H_4.

Molecular Weight

The molecular weight is the total mass of a molecule, obtained by adding the individual atomic masses of all constituent atoms, giving an idea of how heavy one molecule is relative to others.

Example: For carbon dioxide (CO_2):

$= (1 \times 12) + (2 \times 16)$

$= 12 + 32 = 44$ amu

Principle of Molecular Weight

The molecular weight is based on the principle that the total mass of a molecule is equal to the sum of the atomic masses of all its constituent atoms. These atomic masses are taken from standard values in the periodic table and can also be determined experimentally using techniques such as mass spectrometry or colligative property methods. For example, the molecular weight of H_2 O is 18 amu, calculated by adding the masses of hydrogen and oxygen atom.

S. No	DERIVATIVES	MELTING POINT (0C)	MOLECULAR FORMULA	MOLECULAE WEIGHT(g/mol)
1	Citric acid Derivatives	210-2150C	C23H20N2O8	452.4102
2	Benzamide Derivative	180-1850C	C23H17N3O3	383.403
3	Tartaric acid derivatives	220-2250C	C24H20N2O9	480.4204
4	Sulphanilamide Derivatives	165-1700C	C15H14N4O3S	330.3605
5	Benzoic acid Derivatives	190-1950C	C23H16N2O4	384.39

7. SPECTRAL ANALYSIS

The objective of spectral analysis is to identify and characterize chemical substances by studying their interaction with electromagnetic radiation. It aims to determine the structure, composition, and concentration of compounds through their unique spectral patterns. Spectral analysis is also used to detect impurities and ensure the purity and quality of pharmaceutical products. Additionally, it helps in understanding molecular structure, functional groups, and chemical bonding. Overall, its main objective is to provide accurate, rapid, and reliable analytical information for research, quality control, and drug development.

7.1 INFRARED SPECTROSCOPY

Introduction of IR

Infrared (IR) spectroscopy is a powerful analytical method that explores how molecules respond to infrared radiation by undergoing vibrational energy changes.

In modern analytical science, it is regarded as a “vibrational fingerprinting” technique because each compound exhibits a distinct absorption pattern corresponding to its molecular structure. When IR radiation interacts with a sample, specific frequencies are absorbed, leading to stretching and bending of chemical bonds, which are recorded as a spectrum.

This technique provides valuable insight into functional groups, bonding characteristics, and molecular interactions. Widely applied in pharmaceuticals, IR spectroscopy plays a crucial role in compound identification, quality control, and detection of impurities.

Its rapid, reliable, and non-destructive nature makes it indispensable in both research and industrial analysis.

Principle of IR Spectroscopy

Spectral analysis relies on the fact that every molecule absorbs, emits, or scatters light at

specific wavelengths. This "chemical fingerprint" is determined by the energy levels of the electrons and bonds within the molecule.

Range of IR Spectroscopy

1. Near IR: 0.78– 2.5 µm
2. Mid IR: 2.5– 25 µm
3. Far IR: 25– 1000 µm

Pressed Pellet Technique (IR Spectroscopy Method)

A small quantity of finely powdered sample is mixed with dry potassium bromide (KBr) and thoroughly ground to ensure uniform distribution.

The mixture is then compressed under high pressure using a die to form a thin, transparent pellet of about 1– 2 mm thickness.

This pellet is placed in the beam path of an IR spectrophotometer for analysis, while a blank KBr pellet of equal thickness is used as the reference. Finally, the IR spectrum is recorded and interpreted for the identification of functional groups.

Instrumentation of IR Spectroscopy

The main components of an IR spectrometer are:

Radiation Source

Provides IR radiation with sufficient intensity and stability.

Examples: Incandescent lamp, Nernst glower, Globar source, Mercury arc.

Sample Cells

Hold solid, liquid, or gas samples. Materials used must be transparent to IR radiation (e.g., NaCl, KBr).

Monochromator

Separates polychromatic light into a single wavelength.

Functions include dispersion of radiation and maintaining constant energy.

Types: Prism, grating, filters.

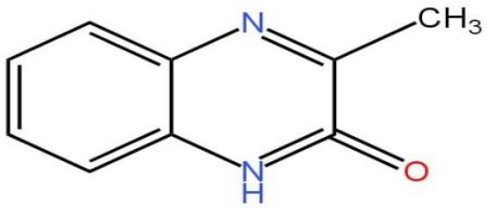
Detector

Detects transmitted IR radiation and converts it into an electrical signal.

Recorder

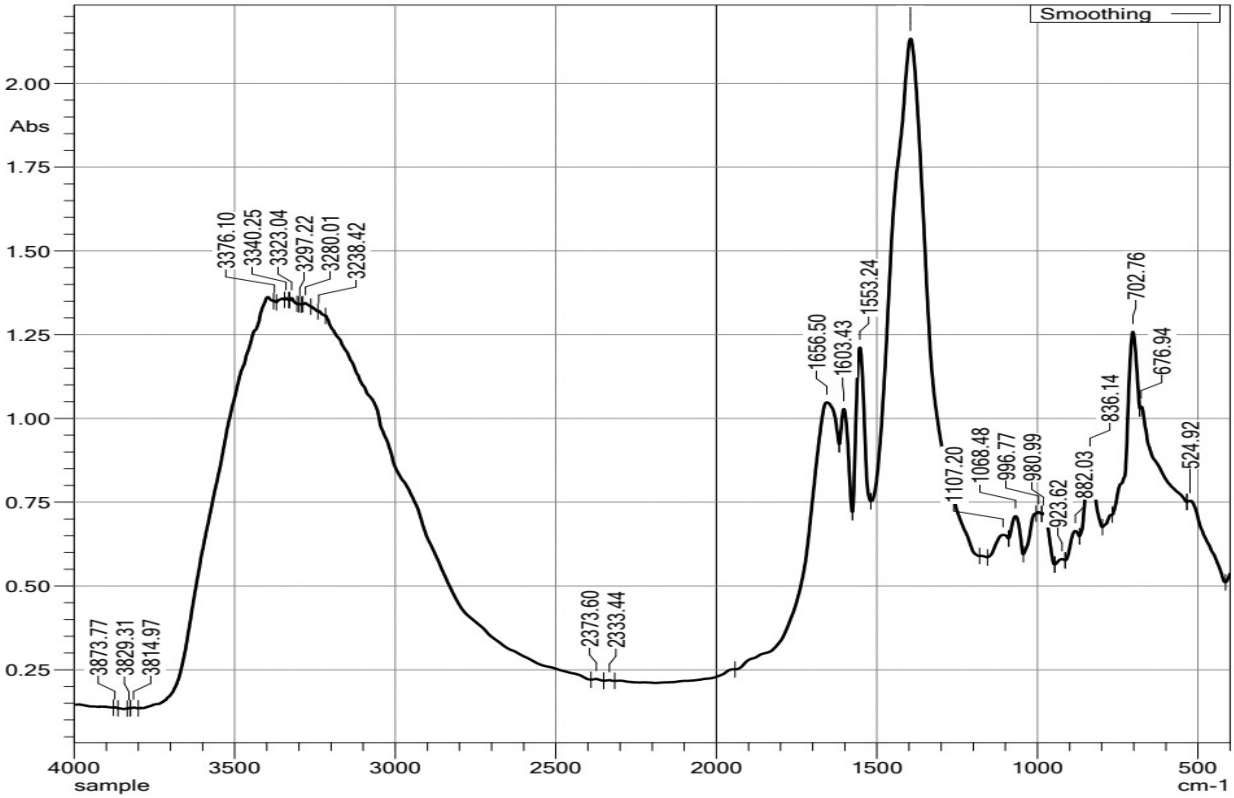
Displays the IR spectrum for analysis.

STEP 1: Product



3-Methyl Quinoaliine -2-One

IR Spectral data (KBr Disc) (in cm-1)	
C-NH	3323.04 cm-1
C=O	1656.50
C-H	882.03



S. No	Items	Value
1	Sample Name	Quinoxaline
2	Sample ID	Sample
3	Option	
4	Intensity mode	%Transmittance
5	Apodization	Happ-Genzel
6	No. of scans	45
7	Resolution	4 cm-1

HIGH PRESSURE LIQUID CHROMATOGRAPHY

1. Definition

High-Performance Liquid Chromatography (HPLC) is an advanced analytical technique used to separate, identify, and quantify components in a mixture. It is a modernized form of column liquid chromatography that uses high pressure (400– 600 bar) to push a liquid mobile phase through a column packed with a stationary phase, achieving faster and higher-resolution separations than classical chromatography.

HPLC is widely used in pharmaceutical, food science, biochemical, environmental, and clinical laboratories. It can analyze thermally labile, non-volatile, and high-molecular-weight compounds that cannot be analyzed by Gas Chromatography (GC), making it one of the most powerful and versatile analytical tools in modern science.

2. Principle

HPLC is based on the principle of differential migration. A sample dissolved in a mobile phase is injected into the system and pushed through a column packed with stationary phase particles (1.7– 5 µm in diameters). Different components in the sample interact with the stationary phase to varying degrees:

Components with strong interaction with the stationary phase travel slowly and elute later.

Components with weak interaction travel faster and elute earlier.

This difference in migration rates causes the components to separate into distinct peaks recorded at different retention times, producing a chromatogram. The area or height of each peak is used for quantification.

3. Types of HPLC

Normal Phase HPLC (NP-HPLC)

Polar stationary phase (silica) with a non-polar mobile phase (hexane). Polar compounds are retained longer. Used for isomers and polar organic molecules.

Reverse Phase HPLC (RP-HPLC)

Non-polar stationary phase (C18-bonded silica) with a polar mobile phase (water/methanol/acetonitrile). The most commonly used HPLC mode (over 70% of analyses). Non-polar compounds are retained longer.

Ion Exchange HPLC

Separates ionic species based on charge interactions. Used for amino acids, proteins, and inorganic ions.

Size Exclusion Chromatography (SEC)

Separates molecules by size. Larger molecules elute first. Used for polymers and protein molecular weight estimation.

4. Step-by-Step HPLC Procedure

Step 1 — Mobile Phase Preparation

Prepare the mobile phase using HPLC-grade solvents. Filter through a 0.45 µm membrane filter to remove particulates. Degas thoroughly using vacuum or sonication. Common mobile phases include water, methanol, and acetonitrile, often with buffers to control pH.

Step 2 — Column Selection and Equilibration

Select the appropriate column based on the analyte's polarity and nature. Install the column in the correct flow direction (marked on the column). Equilibrate the column by pumping 10–20 column volumes of mobile phase at the working flow rate until a stable baseline is obtained.

Step 3 — Sample Preparation

Dissolve the sample in the mobile phase or a weaker solvent at an appropriate concentration (typically 0.01– 1 mg/mL). Filter the sample solution through a 0.22 µm or 0.45 µm syringe filter to remove particulates that could block the column or injector.

Step 4 — Instrument Setup and Parameter Setting

Set the following parameters in the HPLC software: Flow rate (e.g., 1.0 mL/min)

Detection wavelength (e.g., 254 nm or 280 nm for UV)

Column temperature (commonly 25° C– 40° C)

Injection volume (e.g., 10– 20 µL)

Run time (based on expected retention times)

Gradient or isocratic mode

Step 5 — System Suitability Testing

Before injecting actual samples, inject a standard solution and verify:

Theoretical plate count (N) — measures column efficiency (should be > 2000) Resolution (Rs) between peaks — should be > 1.5

Tailing factor — should be between 0.8 and 1.5

Retention time reproducibility — %RSD should be <1%.

Step 6 — Calibration

Prepare at least 3– 5 standard solutions of known concentrations. Inject each standard and record peak areas or heights. Plot a calibration curve (peak area vs. concentration) to establish the linear range and calculate the response factor.

Step 7 — Sample Injection and Analysis

Inject the prepared sample using the autosampler or manual injector. The system records the chromatogram as components elute and are detected. Each peak represents a different compound identified by its retention time.

Step 8 — Data Analysis and Quantification

Compare sample retention times with reference standards for identification. Use the calibration curve or external/internal standard method to calculate concentration. Review peak shape, resolution, and baseline for analytical validity.

Step 9 — System Shutdown

After all analyses, flush the column with an appropriate solvent (e.g., 100% methanol or acetonitrile) for 20– 30 minutes to remove any residual sample or buffer. Store the column in recommended storage solvent (typically 100% organic). Rinse all tubing and clean the system.

5. Applications of HPLC

Pharmaceuticals — Purity testing, potency determination, stability studies, impurity profiling of drugs

Food & Beverage — Detection of additives, preservatives, vitamins, pesticide residues, and contaminants

Clinical & Biomedical — Analysis of hormones, amino acids, nucleotides, and metabolites in biological fluids

Environmental — Detection of pesticides, heavy metal chelates, and pollutants in water and soil

Forensics — Identification of drugs of abuse, toxins, and explosive residues.

7. ADVANTAGES AND LIMITATIONS

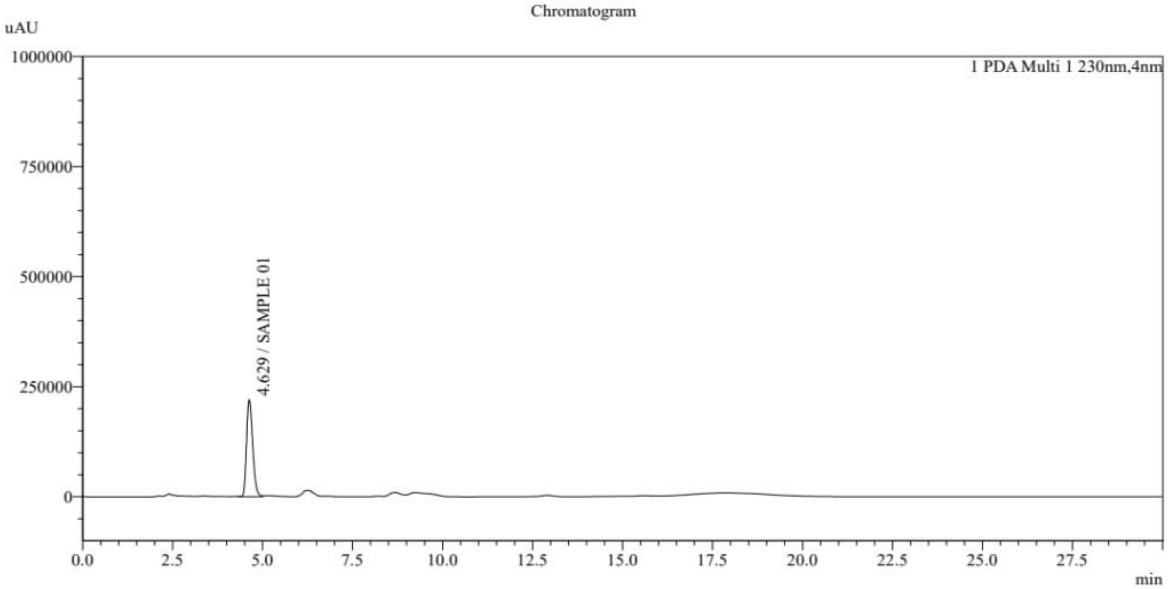
ARUNAI COLLEGE OF PHARMACY

Picture

Sample Information

Acquired by	: System Administrator
Sample Name	: SAMPLE 01
Sample ID	: QUINO
Tray#	: 1
Vial#	: 5
Injection Volume	: 20
Data File	: SAMPLE 01.lcd
Method File	: AS PER DEVELOPMENT 01.lcm
Batch File	: AS PER DEVELOPMENT 01.lcb
Report Format File	: Summary Data Report.lsr
Date Acquired	: 30-01-2026 13:07:29
Date Processed	: 30-01-2026 14:39:29

Chromatogram



Peak Table

PDA Ch1 230nm

Peak#	Ret. Time	Name	Area	Theoretical Plate	Tailing Factor	Capacity Factor(k')
1	4.629	SAMPLE 01	2627769	3488	1.270	--
Total			2627769			

Peak Purity Index	S/N	Resolution(USP2)
Not calculated	285.84	--

Analysed By:

NUCLEAR MAGNETIC RESONANCE

1. Definition

Nuclear Magnetic Resonance (NMR) Spectroscopy is a powerful analytical technique used to determine the structure, composition, dynamics, and purity of chemical compounds. It is based on the principle that certain atomic nuclei, when placed in a strong external magnetic field and exposed to radiofrequency (RF) radiation, absorb and re-emit electromagnetic energy at characteristic frequencies. NMR provides detailed information about the number, type, and connectivity of atoms within a molecule — particularly hydrogen (^{1}H) and carbon (^{13}C) atoms — making it the most definitive technique for elucidating the complete structure of organic and inorganic compounds without destroying the sample.

It is extensively used in organic chemistry, pharmaceuticals, biochemistry, food science, polymer science, and medical imaging (as MRI — Magnetic Resonance Imaging, the clinical application of NMR principles).

2. Principle of NMR

Principle of NMR (Nuclear Magnetic Resonance) Spectroscopy

Nuclear Magnetic Resonance (NMR) spectroscopy is based on the absorption of radiofrequency radiation by certain atomic nuclei when placed in a strong external magnetic field. Nuclei such as hydrogen (^1H) and carbon (^13C), which possess spin, behave like tiny magnets. In the presence of a magnetic field, these nuclei align either with or against the field, creating different energy levels.

When radiofrequency energy corresponding to the energy difference between these levels is applied, the nuclei absorb the energy and undergo resonance. The absorbed energy is measured and recorded as an NMR spectrum. The position of signals in the spectrum depends on the chemical environment surrounding the nuclei, providing detailed information about the molecular structure, functional groups, and arrangement of atoms within the compound.

3. Procedure

Step 1: Sample Preparation

About 5– 20 mg of the sample is taken in a clean vial and dissolved in 0.5– 0.7 mL of a suitable deuterated solvent such as $CDCl_3$, DMSO-d_6, or CD_3 OD. The solution is transferred into a clean 5 mm NMR tube. Tetramethylsilane (TMS) may be added as an internal standard.

Step 2: Placement of Sample

The NMR tube is placed inside the NMR spectrometer. The instrument subjects the sample to a strong external magnetic field.

Step 3: Alignment of the Nuclei

In the magnetic field, nuclei such as ^1H or ^13C align in two spin states: parallel (low energy) and antiparallel (high energy). This creates an energy difference between the two states.

Step 4: Application of Radiofrequency Pulse

A radiofrequency (RF) pulse is applied to the sample. The nuclei absorb energy and move from the lower energy state to the higher energy state when resonance occurs.

Step 5: Relaxation of Nuclei

After the RF pulse is stopped, the excited nuclei return to their original state. During relaxation, energy is released in the form of signals.

Step 6: Detection of Signal

The released energy is detected by the receiver coil as a signal called Free Induction Decay (FID).

Step 7: Fourier Transformation

The FID signal is converted into an NMR spectrum using Fourier transformation.

Step 8: Interpretation of Spectrum

The obtained spectrum is analyzed based on chemical shift, integration, multiplicity, and coupling constants to determine the molecular structure and purity of the compound.

4. Types of NMR Experiments

1. Proton NMR (^1H NMR)

Proton NMR studies the hydrogen atoms present in a molecule. It provides information about the number of hydrogen atoms, their chemical environment, and neighboring proton interactions.

2. Carbon-13 NMR (^13C NMR)

Carbon-13 NMR analyzes carbon atoms in a compound. It helps identify the carbon skeleton

and different types of carbon environments present in the molecule.

3. DEPT NMR

Distortionless Enhancement by Polarization Transfer (DEPT) NMR is used to distinguish between CH, CH_2, and CH_3carbon atoms in organic compounds.

4. Two-Dimensional NMR (2D NMR)

Two-dimensional NMR techniques such as COSY, HSQC, and HMBC provide detailed structural information by showing interactions between nuclei in a molecule.

5. Solid State NMR

Solid state NMR is used for studying solid materials, polymers, catalysts, and crystalline substances.

6. Fluorine NMR (^19F NMR)

Fluorine NMR is used to analyze fluorine-containing compounds and is widely applied in pharmaceutical and organic chemistry research.

5. Applications of NMR Spectroscopy

Structural Elucidation

NMR is widely used to determine the molecular structure and arrangement of atoms in organic and inorganic compounds.

Identification of Functional Groups

It helps identify functional groups present in a molecule based on characteristic chemical shifts.

Purity Analysis

NMR is used to determine the purity of compounds and detect impurities in pharmaceutical and chemical samples.

Pharmaceutical Research

It is extensively used in drug discovery, medicinal chemistry, and characterization of pharmaceutical compounds such as quinoxaline derivatives.

Study of Stereochemistry

NMR helps determine stereochemical configuration and spatial arrangement of atoms in

molecules.

Biomolecular Analysis

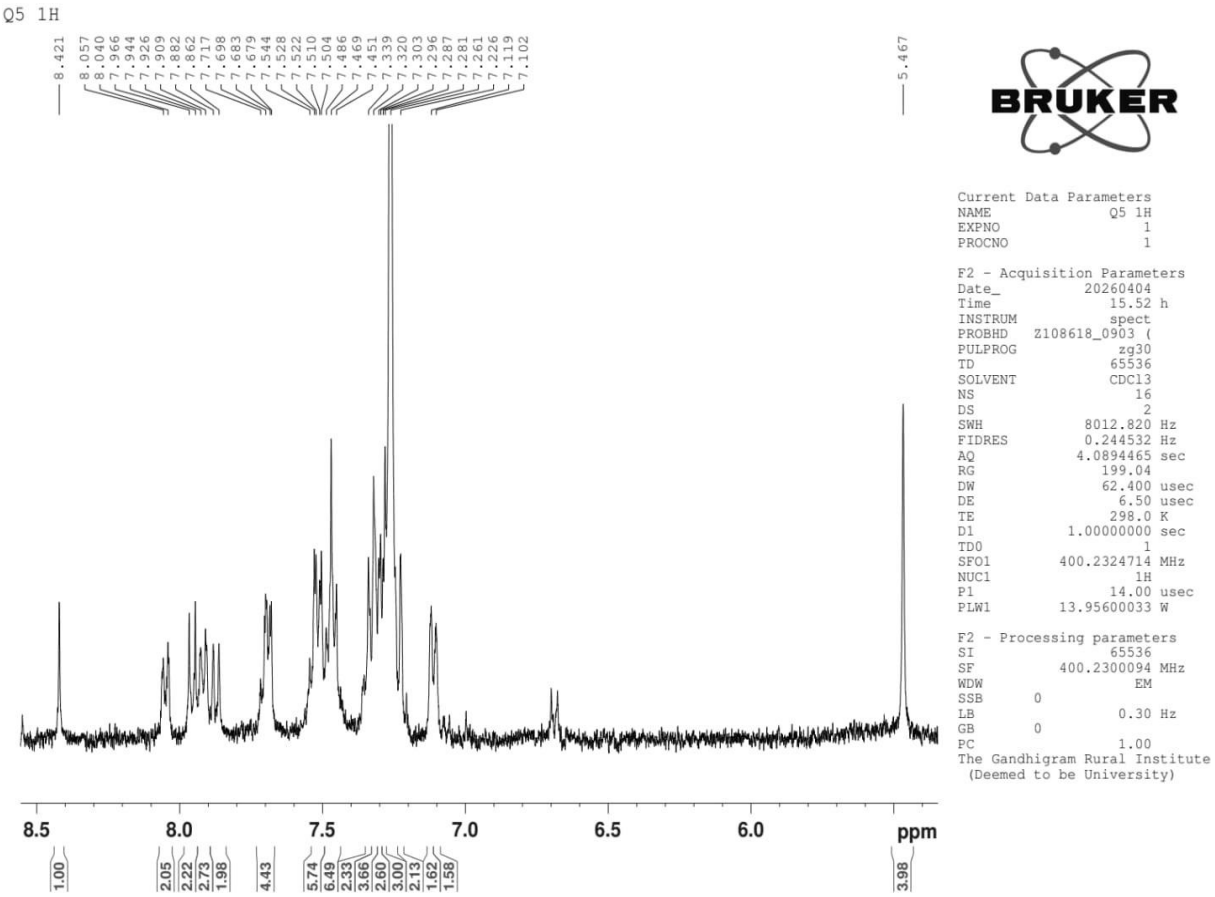
It is used in the study of proteins, nucleic acids, enzymes, and other biological molecules.

Quantitative Analysis

NMR can measure the concentration of compounds in a mixture without separating the components.

INDUSTRIAL APPLICATIONS

NMR is applied in polymer chemistry, petrochemical analysis, food analysis, and quality control studies.



CONCLUSION

In the present study, a series of novel quinoxaline derivatives were successfully designed, synthesized, and characterized with the aim of identifying new compounds with promising pharmacological potential. The rational design strategy, based on structural modification of the quinoxaline scaffold, enabled the incorporation of various substituents that significantly

influenced the spectral and biological properties of the synthesized compounds.

The synthesized compounds were characterized using standard analytical and spectroscopic techniques, confirming the proposed chemical structures and purity. Notably, derivatives containing electron-withdrawing and heteroaryl substituents demonstrated enhanced biological activity, suggesting a strong structure– activity relationship (SAR) within this series. The results of the study indicate that quinoxaline derivatives may serve as promising lead molecules for further development in medicinal chemistry and drug discovery research.

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