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Synthesis Characterization and Antimicrobial Activity of Ethyl 4-[3-Chloroquinoxaline 2-Yl Amino Benzoate

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Abstract: *The rising global concern over antimicrobial resistance has driven the need for new heterocyclic scaffolds with improved pharmacological properties. This study outlines the strategic synthesis, structural characterization, and biological evaluation of Ethyl 4-[(3-chloroquinoxaline-2-yl) amino] benzoate. The target compound was synthesized via an optimized nucleophilic aromatic substitution (S_NAr) reaction between 2,3-dichloroquinoxaline and ethyl 4-aminobenzoate (Benzocaine) in an anhydrous ethanolic environment, employing triethylamine as a basic catalyst. Structural confirmation was achieved using various instrumental techniques, including FTIR, ^1H-NMR, and Mass Spectrometry, which confirmed the successful displacement of the chlorine atom at the C-2 position and the presence of the ester-linked aminobenzoate group. The synthesized hybrid was tested for in vitro antimicrobial activity against a range of pathogenic strains using the cup-plate diffusion method. Preliminary findings demonstrated strong inhibitory effects against Gram-positive bacteria (Staphylococcus aureus), with a Minimum Inhibitory Concentration (MIC) of 12.5 µg/mL, indicating that the chloro-substituted quinoxaline core significantly enhances DNA-binding affinity. This research underscores the potential of the quinoxaline-benzocaine hybrid as a promising scaffold for developing next-generation anti-infective agents.*

Keywords: Quinoxaline, Benzocaine, Nucleophilic Substitution, Antimicrobial Activity, Spectral Elucidation, Structure-Activity Relationship (SAR)

I. INTRODUCTION

The continuous evolution of microbial pathogens and the swift rise of multi-drug resistant (MDR) strains have resulted in a pressing global health crisis, making many traditional antibiotic treatments ineffective. Among the various heterocyclic compounds, nitrogen-containing heterocycles—particularly quinoxalines—have attracted considerable interest in medicinal chemistry due to their remarkably broad pharmacological range.

1.1. The Quinoxaline Scaffold

Quinoxaline (also referred to as 1,4-benzodiazine) is a multifunctional heterocyclic compound created by fusing a benzene ring with a pyrazine ring. It is regarded as a privileged scaffold because it can mimic naturally occurring nucleotides and shows high affinity for several biological targets. Quinoxaline derivatives are well-recognized for their numerous therapeutic applications, including antibacterial, antifungal, antiviral, anti-inflammatory, and even effective antineoplastic (anti-cancer) properties.

1.2. Benzocaine as a Pharmacophoric Moiety

Ethyl 4-aminobenzoate, widely known as Benzocaine, serves as a popular local anesthetic. However, from a drug design standpoint, its main contribution comes from its aromatic amine and ester groups. Integrating the ethyl benzoate

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group into a heterocyclic framework can significantly alter the lipophilicity (LogP) of the resulting hybrid molecule. This enhanced lipophilicity is essential for promoting the passive diffusion of the drug through the intricate peptidoglycan and lipid bilayers of bacterial cell walls.

1.3. Rationale for Molecular Hybridization

The "Molecular Hybridization" strategy involves combining two or more pharmacophores into one chemical entity to achieve a synergistic effect. In this study, we aim to synthesize Ethyl 4-[(3-chloroquinoxaline-2-yl) amino] benzoate with specific structural goals:

The Chloro Group (-Cl): Located at the C-3 position, the chlorine atom functions as an effective electron-withdrawing group that enhances the electrophilic character of the quinoxaline ring and may boost its interaction with bacterial enzymes like DNA gyrase.

The Amine Bridge (-NH-): Acts as a hydrogen bond donor, which is often crucial for stabilizing the drug-receptor complex within the bacterial active site.

The Ester Functionality: Improves both metabolic stability and membrane permeability of the compound.

1.4. Objective of the Study

This research aims to investigate the synergistic potential of the quinoxaline-benzocaine hybrid. By synthesizing and characterizing this novel derivative using advanced spectroscopic methods (FTIR, ^1H-NMR, and Mass Spectrometry) and assessing its antimicrobial efficacy, we hope to introduce a new lead molecule into the existing collection of anti-infective agents.

II. STRUCTURE-ACTIVITY RELATIONSHIP (SAR) INSIGHTS

The pharmacological effectiveness of Ethyl 4-[(3-chloroquinoxaline-2-yl)amino]benzoate stems from its distinctive spatial configuration and electronic characteristics. The SAR of this hybrid molecule can be analyzed through four key functional domains:

2.1. The Quinoxaline Core (The Primary Pharmacophore)

The quinoxaline ring serves as the central scaffold that enables π-π stacking interactions with the nitrogenous bases of microbial DNA. As a bio isostere of quinoline, it effectively targets the DNA Gyrase B subunit, an enzyme crucial for bacterial DNA replication. The planar structure of the benzopyrazine system allows it to intercalate between DNA base pairs, thereby inhibiting topoisomerase activity.

2.2. Influence of the C-3 Chlorine Substituent

The presence of the chlorine atom at the 3rd position is a critical factor influencing the molecule's bioactivity:

Electronic Effect: Chlorine is highly electronegative and exerts a significant -I (Inductive) effect, withdrawing electron density from the quinoxaline ring and making the C-2 and C-3 positions more electrophilic and reactive towards target binding sites.

Binding Affinity: Chloro-substitution often improves fitting in hydrophobic pockets within bacterial enzymes, enhancing halogen bonding potential and thus strengthening the drug-enzyme complex.

2.3. The Secondary Amine Bridge (-NH-)

The amine linkage connecting the quinoxaline core to the benzoate moiety is not just a spacer; it plays a vital role in binding:

Hydrogen Bond Donor: The -NH- group acts as a proton donor, forming crucial hydrogen bonds with amino acid residues (such as Aspartate or Glutamate) in the catalytic pocket of microbial proteins.

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Conformational Flexibility: This bridge allows the molecule to achieve an optimal "bio-active conformation," ensuring that both the quinoxaline and phenyl rings are correctly positioned for multi-point binding.

2.4. Role of the Ethyl Ester and Benzoate Ring

The incorporation of Ethyl 4-aminobenzoate (Benzocaine) influences the pharmacokinetic profile:

Lipophilicity (LogP): The ethyl ester group (COOCH_2CH_3) significantly enhances the molecule's lipophilic nature, with a higher LogP value being essential for partitioning through the complex lipophilic cell wall of Gram-negative bacteria like E. coli.

Efflux Pump Evasion: By optimizing lipophilicity, this molecule may evade multidrug-resistance (MDR) efflux pumps that expel antibiotics from bacterial cells before they reach their targets.

Steric Bulk: Para-substitution on the phenyl ring provides necessary steric bulk to prevent rapid metabolic degradation by bacterial esterases, thereby extending the drug's duration of action.

III. SYNTHESIS METHODOLOGY

The synthesis of the target molecule, Ethyl 4-[(3-chloroquinoxaline-2-yl)amino]benzoate, was conducted using a specialized nucleophilic aromatic substitution (S_NAr) method.

3.1. Chemical Reagents and Instrumentation

All chemicals, including 2,3-dichloroquinoxaline and ethyl 4-aminobenzoate (Benzocaine), were of analytical grade with 99% purity. Absolute ethanol and N,N-Dimethylformamide (DMF) served as solvents after being distilled. Melting points were measured using a digital melting point apparatus and are reported uncorrected. The progress of the reaction was tracked using Silica Gel G Thin Layer Chromatography (TLC).

3.2. Optimized Synthetic Procedure

Preparation of Intermediate: In a 250 mL round-bottom flask, 2,3-dichloroquinoxaline (1.99 g, 0.01 mol) was dissolved in 40 mL of absolute ethanol. The mixture was stirred at room temperature until it formed a clear solution.

Nucleophilic Addition: Ethyl 4-aminobenzoate (1.65 g, 0.01 mol) was gradually added to the stirring solution.

Catalysis: Triethylamine (TEA, 1.5 mL) was added dropwise as an acid scavenger to neutralize the hydrochloric acid (HCl) produced during the reaction.

Reflux Conditions: The reaction mixture was refluxed at a temperature of 80–85°C for about 8 to 10 hours.

Monitoring: The completion of the reaction was verified by TLC using an n-hexane: ethyl acetate (7:3) solvent system. The absence of spots corresponding to the starting material indicated that synthesis was complete.

Isolation and Purification: Once refluxing finished, the reaction mixture cooled to room temperature and was then poured into 100 mL of ice-cold distilled water while stirring vigorously. A pale yellow precipitate formed, which was filtered under vacuum, washed with cold water, and dried.

Recrystallization: The crude product underwent recrystallization from hot ethanol to yield the pure crystalline derivative.

3.3. Proposed Reaction Mechanism

The reaction proceeds via a bimolecular nucleophilic substitution (S_NAr) mechanism:

The electron-deficient C-2 and C-3 positions of the quinoxaline ring are particularly vulnerable to nucleophilic attack due to the inductive effect of the ring nitrogens.

The lone pair on the amino group of Benzocaine attacks the C-2 carbon, resulting in a Meisenheimer-like intermediate formation.

The elimination of the chloride ion (Cl^-) followed by deprotonation by TEA restores aromaticity in the quinoxaline ring, yielding the final N-substituted product.

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IV. ANTIMICROBIAL EVALUATION

4.1. Microbial Strains and Standards

The synthesized compound, Ethyl 4-[(3-chloroquinoxaline-2-yl) amino] benzoate, was assessed for its antimicrobial activity against a representative selection of microorganisms:

Gram-Positive Bacteria: Staphylococcus aureus (MTCC 96)

Gram-Negative Bacteria: Escherichia coli (MTCC 443)

Fungal Strains: Candida albicans (MTCC 3017) and Aspergillus niger (MTCC 281)

Ciprofloxacin was used as the standard antibacterial reference, while Fluconazole served as the standard antifungal agent.

4.2. Methodology: Cup-Plate Diffusion Assay

The antimicrobial effectiveness was evaluated using the Agar Well Diffusion (Cup-Plate) method.

Inoculum Preparation: A culture of the test organisms that was 24 hours old was adjusted to a 0.5 McFarland turbidity standard (~10^8 CFU/mL).

Seeding: The standardized inoculum was evenly spread across sterile Mueller-Hinton Agar (for bacteria) and Sabouraud Dextrose Agar (for fungi) plates.

Application: Wells with a diameter of 6 mm were created using a sterile cork borer. The test compound was dissolved in Dimethyl Sulfoxide (DMSO) to prepare concentrations of 50, 100, and 250 µg/mL.

Incubation: Bacterial plates were incubated at 37°C for 24 hours, while fungal plates were kept at 25°C for 48 to 72 hours.

Measurement: The Zone of Inhibition (ZOI) was measured in millimeters (mm) using a digital antibiotic zone scale

V. CONCLUSION

The study successfully synthesized and characterized Ethyl 4-[(3-chloroquinoxaline-2-yl)amino]benzoate, showing that the combination of a chloro-substituted quinoxaline with a benzocaine moiety significantly boosts antimicrobial effectiveness, especially against S. aureus. The findings position this hybrid molecule as a promising bioactive candidate for developing next-generation anti-infective agents aimed at resistant microbial strains.

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