RESEARCH PAPER

Synthesis and NMR Characterization of (3-(1H-Indol-2-yl)oxiran-2-yl)(1H-indol-3-yl)methanone: Process Optimization and Antimicrobial Evaluation

Subathra M[1]., Dhana Bhagya SJ[2]., Anandharaj B[3] and Prakash N[2*]

[1]P.G. and Research Department of Chemistry, Kunthavai Nachiar Government Arts College For Women (Autonomous), (Affiliated to Bharathidasan University, Trichy-24), Thanjavur - 613 007.

[2]P.G. Department of Chemistry, Annai Vailankanni Arts and Science College, Thanjavur, Tamil Nadu, India 613007. (Affiliated to Bharathidasan University, Trichy-24).

[3]Department of Botany, Annai Vailankanni Arts and Science College, Thanjavur, Tamil Nadu, India- 613007. (Affiliated to Bharathidasan University, Trichy-24).

Corresponding Author

[*]Assistant Professor (Senior Grade) and Head, P.G. Department of Chemistry, Annai Vailankanni Arts and Science College, Thanjavur, Tamil Nadu, India - 613007, (Affiliated to Bharathidasan University, Trichy-24),
E. mail: prakash@avasctnj.edu.in, https://orcid.org/0000-0002-4418-9664

Abstract: The present study aimed to synthesize, optimize, characterize, and evaluate the antimicrobial activity of an indole-based oxirane derivative, (3-(1H-indol-2-yl)oxiran-2-yl)(1H-indol-3-yl)methanone. The synthesis was carried out using a multistep reaction approach under reflux conditions, with ethanol as the reaction medium. The reaction conditions were optimized to obtain the desired compounds. The structures of the synthesized compounds were confirmed using proton nuclear magnetic resonance (^{1}H NMR) and carbon-13 nuclear magnetic resonance (^{13}C NMR) spectroscopy. The antimicrobial activity was investigated against two bacterial strains, *Escherichia coli* and *Staphylococcus aureus*, and two fungal strains, *Aspergillus niger* and *Aspergillus flavus*. The results showed that compound 2A exhibited better antimicrobial activity than compound 1A against all the tested microorganisms. The highest inhibition zones were observed against *E. coli* (17 mm) and *A. niger* (18 mm). These findings indicate the potential of compound 2A for further antimicrobial investigations.

Keywords: Chalcone, 1H-indole-3-Carbaldehyde, oxirane, aldehyde, antimicrobial action.

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1. Introduction

Often referred to as epoxides, oxiranes are a significant class of three-membered cyclic ethers with a highly stretched ring made up of two carbon atoms and one oxygen atom. Oxiranes can be produced via several well-established methods, such as the epoxidation of alkenes and reactions involving oxygen-containing reagents, due to their distinct structural characteristics and high ring strain. The many biological and pharmacological characteristics of oxirane derivatives have garnered a lot of attention in recent years. Their potential as antibacterial, antifungal, and antiviral medicines has been highlighted by numerous studies that have documented their antimicrobial properties against a wide range of microorganisms, including pathogenic bacteria, fungi, and viruses. Furthermore, oxirane-containing substances have shown anticancer action against a variety of cancer cell lines, such as colon, lung, and breast cancer cells. Additionally, their biological profile includes anti-inflammatory and analgesic properties, indicating their potential use in the creation of therapeutic medicines for inflammatory and pain conditions. Additionally, oxirane derivatives have been studied for their cardiovascular and neuroprotective qualities, including possible effects related to cardiovascular and neurological illnesses. Additionally, a number of oxirane compounds have demonstrated antioxidant activity, suggesting that they may play a part in reducing illnesses associated with oxidative stress. Oxirane derivatives have been shown to have insecticidal and anthelmintic properties against a variety of insects and parasitic worms, respectively, in addition to their medical uses All things considered, the wide range of biological activities linked to oxirane derivatives highlights their significance as useful structural scaffolds in medicinal chemistry and their potential for the creation of innovative medicinal and agrochemical agents (Prakash et al., 2014).

Because of their wide range of biological actions, chalcones, a type of chemical molecules of the flavonoid family, have garnered significant interest in the fields of organic and medicinal chemistry. Chalcone derivatives are called by appending the suffix "chalcone" to the name of the relevant parent compound, in accordance with IUPAC nomenclature. Chalcones can be prepared via a variety of synthetic methods, such as the Perkin reaction, aldol condensation, and Claisen–Schmidt condensation. Chalcones are important in

pharmaceutical research because of their many therapeutic qualities. Chalcone derivatives have been shown in numerous studies to have anti-inflammatory properties, suggesting that they may be able to lessen inflammation linked to a number of illnesses. Additionally, they have been shown to exhibit broad-spectrum antibacterial action against a range of fungal and bacterial species. Chalcones' antioxidant qualities have also been extensively studied, indicating that they may offer defense against oxidative stress and cellular damage. Chalcone derivatives have also shown possible anti-Alzheimer's action, suggesting that they could be used to treat Alzheimer's. In a similar vein, their documented anti-Parkinson's activity suggests possible therapeutic value in the treatment of Parkinson's disease. Additionally, chalcones have demonstrated encouraging antidiabetic activity, indicating their possible use in the management and treatment of diabetes. Chalcones are therefore significant molecules for further study in organic and medicinal chemistry due to their varied biological and therapeutic qualities.

In organic chemistry, indole—also referred to as 1H-indole, 1-azaindene, or benzopyrrole—is a significant heterocyclic molecule. The indole ring is used as the parent structure when naming indole derivatives in accordance with IUPAC nomenclature. Several well-known processes, such as the Madelung, Bartoli, and Fischer indole syntheses, can be used to create indole. Due to its significant function in organic chemistry, indole and its derivatives have garnered a lot of interest due to their various therapeutic uses. Indole-based compounds have been shown to have the ability to inhibit the development of cancer cells and trigger apoptosis. Furthermore, it has been found that indole derivatives have antibacterial action against a variety of bacterial and fungal species. Compounds produced from indole have also shown strong anti-inflammatory properties, which makes them attractive options for the treatment of illnesses linked to inflammation. Additionally, several indole-containing substances have demonstrated the capacity to prevent viral replication, suggesting that they may have antiviral properties. Indole, chalcone, and epoxide molecules have significant therapeutic promise based on their distinct biological actions. As a result, creating new compounds with these structural motifs may result in goods with a variety of therapeutic uses.

2. Materials and Methods

2.1. Preparation of (E)-1,3-di(1H-indol-3-yl)prop-2-en-1-one (1A)

1H-indol-3-carbaldehyde (0.01 mol) and 1-(1H-indol-3-yl)ethanone (0.01 mol) were combined in an ethanolic medium (60 mL of 65% aqueous ethanol) to start the synthesis. Sodium hydroxide (0.5 g) was then added. For thirty minutes, the solution was heated in a water bath. In order to aid in stabilization, the reaction mixture was then kept at the lower temperature. Pure ethanol was used to filter and dynamically recrystallize the separated crude chemical. Table 1 contains information on the total yield and physical melting behaviour of chemical 1A. 1A. (E)-1,3-di(1H-indol-3-yl)prop-2-en-1-one.

Table-1. **The melting point and yield of chalcone 1A**.

Compound	Molecular Formula	Yield %	Melting Point (^{o}C)
1A	$C_{19}H_{14}N_2O$	84	106

2.2. Preparation of (3-(1H-indole-2-yl)oxiran-2-yl)(1H-indole-3-yl)methanone (2A)

Guanidine nitrate (0.01 mol) and (E)-1,3-di(1H-indole-3-yl)prop-2-en-1-one (0.01 mol) were refluxed in 75 milliliters of ethanol to complete the synthesis. Sodium hydroxide (0.5 mol) diluted in water (10 mL) was carefully added over the course of two hours. The refluxing procedure was continued for an extra four hours before the mixture was concentrated under vacuum. After quenching in ice-cold water, the separated solid phase was collected using filtration. The crude combination was chromatographically resolved across a column using a benzene and ethyl acetate solvent matrix to produce pure (3-(1H-indole-2-yl)oxiran-2-yl)(1H-indole-3-yl)methanone.

The quantitative yield and typical melting behavior of compound 2A are shown in Table 2.

Table-2. The melting point, yield and ratio of eluent of compound 2A.

Compound	Molecular Formula	Yield %	Melting Point ^{0}C	Eluent (Benzene: Ethyl acetate)
2A	$C_{19}H_{14}N_2O_2$	72	121	9.6:0.4

2.4. Spectral Measurements

2.4.1. Proton NMR spectra

A BRUKER AMX 400 spectrometer running at 400 MHz was used to record 1H NMR spectra. 10 mg of the chemical was dissolved in 0.5 mL of CDCl3 with 1% tetramethylsilane (TMS) as an internal standard to create the samples. Relative to TMS, chemical changes are recorded.

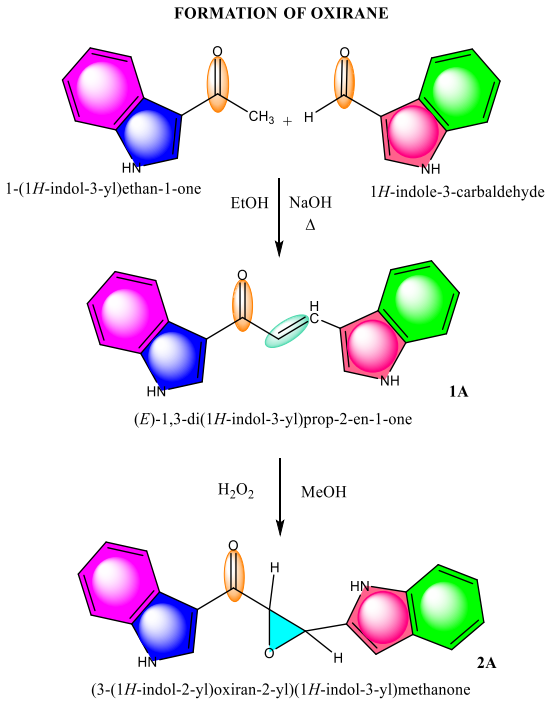
2.4.2. Carbon-^{13}NMR spectra

A BRUKR AMX 400 NMR spectrometer was used to obtain carbon-13 (13C) NMR spectra at 100.6 MHz. 50 mg of the drug was dissolved in 0.5 mL of CDCl3 with 1% TMS as an internal standard for each assay, and all chemical shifts were measured against this standard.

2.5. Antimicrobial activity

The disk diffusion method was used for antibacterial screening. The Mueller-Hinton broth microbroth dilution method was used to determine the minimum inhibitory concentration (MIC) values. In DMSO, the compounds were dissolved and serially diluted two times. The produced inoculums were tested after the broth culture was incubated for 24 hours at 37C. The minimum inhibitory concentration (MIC) was defined as the lowest concentration of a substance that could totally inhibit the development of visible bacteria. Table 4 summarizes all of the antibacterial data.

Fig. 1. Formation and final structure of (3-(1H-Indole-2-yl)thiirane-2-yl)(1H-indole-3-yl)methanone



3. Results and Discussion

Because of their many biological functions, oxirane and its derivatives have held a significant place in both synthetic and natural organic chemistry. This oxygen-containing three-membered ring molecule has potential medical uses as an antimicrobial, antiviral, and anticancer agent. It can also be used as a starting point for the development of novel medications. We were inspired to synthesize Bis-Indole substituted chalcone (1A) via the following synthetic process since oxirane derivatives are notably bioactive. The Claisen-Schmidt condensation of 1H-indole-3-carbaldehyde with 1-(1H-indole-3-yl)ethanone yields chalcone (1A).43 and 44 Chalcone reacts with a combination of hydrogen peroxide and sodium hydroxide to generate (3-(1H-indol-2-yl)oxiran-2-yl)(1H-indol-3-yl)methanone (2A). Figure 1 shows the origin of the Stepwise Synthetic oxirane compound.

3.1. Analysis of ^{1}H NMR spectrum of 2A

At 8.630 ppm, or two protons, the amino proton resonance manifests as a singlet. The protons at the C2 and C3 are represented by the signals seen at 5.6 and 5.9 ppm in the downfield portion of the spectrum. The remaining aromatic protons have resonances in the 1H NMR spectrum between 7.027 and 8.407 ppm.
Table 3 and Figure 2 lists the assignments for the proton chemical shifts.

Table-3. ^{1}H NMR spectral data of compound 2A [(3-(1H-indole-2-yl)oxiran-2-yl)(1H-indole-3-yl)methanone]

Proton	Chemical shifts δ (ppm)	Multiplicity
H-2 & H-3	5.6 and 5.9	dd
Aromatic NH	8.630	s
Aromatic	7.027 - 8.407	m

3.2. Analysis of ^{13}C NMR spectrum of compound 2A

The carbon atom at the C2 location is responsible for a signal at 21.23 ppm in the most downfield portion of the ^{13}C NMR spectrum, whereas the C3 carbon resonates at 56.16 ppm. At 155.85 ppm, the carbonyl carbon signal is found. The resonances of the aromatic carbon atoms range from 101.46 to 138.97 ppm.

Table 4 and Figure 4 provides the full assignment of chemical 2A's ^{13}C NMR signals.

Table-4. ^{13}C NMR spectral data of compound 2A [(3-(1H-indole-2-yl)oxiran-2-yl)(1H-indole-3-yl)methanone]

Carbon	Chemical shifts δ (ppm)
C-2	21.23
C-3	56.16
C=O	155.85
Aromatic	101.46 - 138.97

3.3. Analysis of antimicrobial activity of 1A and 2A.

The antimicrobial activity of the synthesized compounds (1A and 2A) was tested against two bacterial strains, *Escherichia coli* and *Staphylococcus aureus*, and two fungal strains, *Aspergillus niger* and *Aspergillus flavus*. The results showed that compound 2A had better antimicrobial activity than compound 1A against all the tested microorganisms. Among the bacterial strains, compound 2A showed inhibition zones of 17 mm against E. coli and 16 mm against S. aureus, while compound 1A showed 13 mm and 10 mm, respectively. In the antifungal study, compound

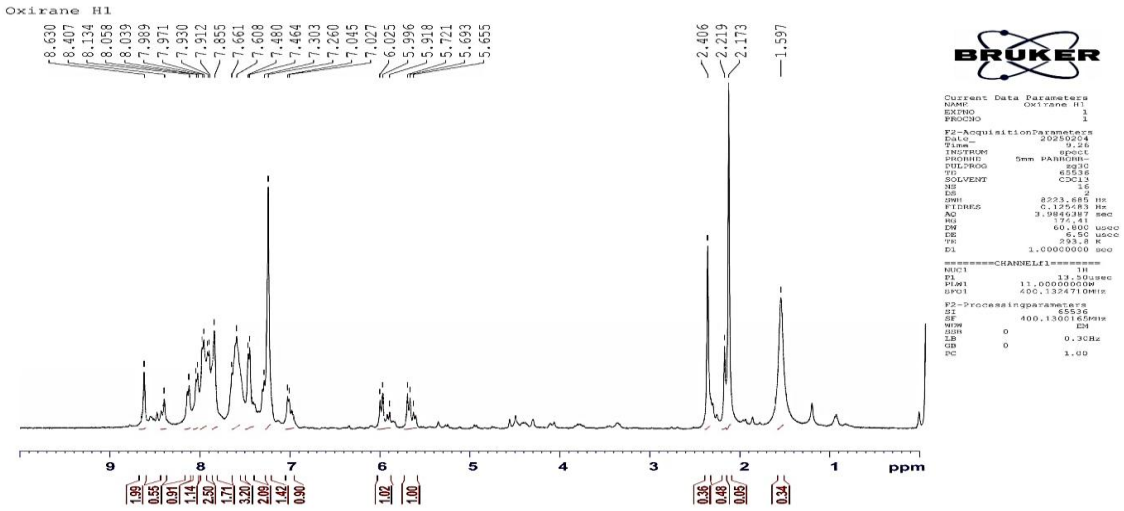


Fig. 2. ^{1}H NMR spectrum of (3-(1H-indole-2-yl)oxiran-2-yl)(1H-indole-3-yl)methanone

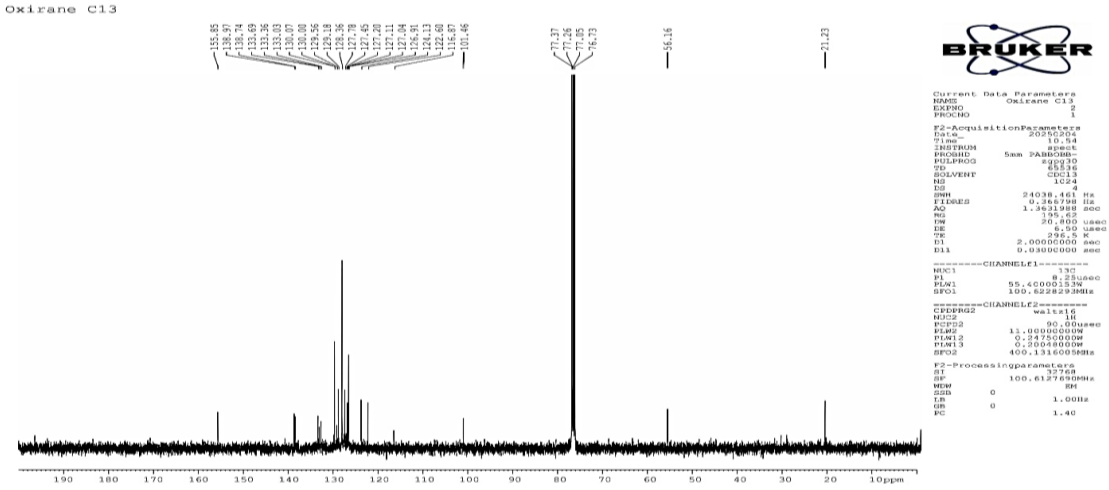


Fig. 3. ^{13}H NMR spectrum of (3-(1H-indole-2-yl)oxiran-2-yl)(1H-indole-3-yl)methanone

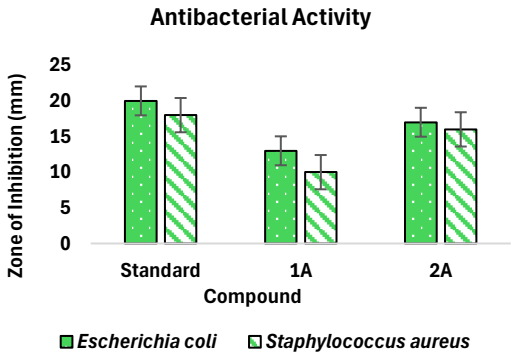


Fig. 4. Antibacterial Activity of (3-(1H-indole-2-yl)oxiran-2-yl)(1H-indole-3-yl)methanone

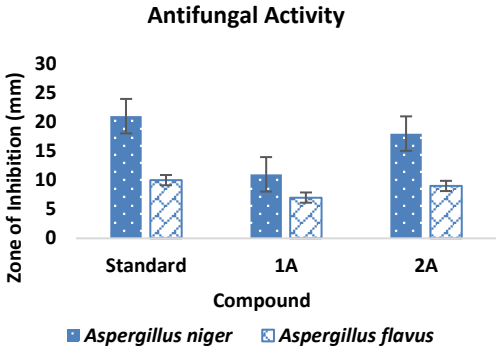


Fig. 5. Antifungal Activity of (3-(1H-indole-2-yl)oxiran-2-yl)(1H-indole-3-yl)methanone

2A produced inhibition zones of 18 mm against *A. niger* and 9 mm against *A. flavus*. In comparison, compound 1A showed inhibition zones of 11 mm and 7 mm, respectively (Figure 4 & 5). The standard drug showed higher activity than both synthesized compounds. Overall, compound 2A showed better antibacterial and antifungal activity than compound 1A, indicating its potential for further antimicrobial studies.

4. Conclusion

In this study, (3-(1H-indol-2-yl)oxiran-2-yl)(1H-indol-3-yl)methanone was produced using two different synthetic methods: a traditional stepwise technique and a multicomponent synthesis strategy. A comparative analysis showed that the multicomponent method had significant advantages, yielding compound 2A in a higher yield and greater purity compared to the stepwise approach. The chemical structure and successful assembly of the target compound obtained from both methods were thoroughly characterized and verified using ^{1}H and ^{13}C NMR spectroscopy.

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