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Spectroscopic Characterization and Antimicrobial Studies of Synthesized *N*-Benzyl-1*h*-Indole-3-Carboxamide

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ABSTRACT

A four step synthetic approach afforded *N*-benzyl-1*H*-indole-3-carboxamide in a moderate yield. The process involved formylation with Vismeier-Haack reagents, followed by disproportionation through the Cannizzaro reaction, chlorination using thionyl chloride, and subsequent substitution with benzylamine, ultimately generating the target compound. The compound was analysed using, ^{1}H NMR (Proton nuclear magnetic resonance), ^{13}C NMR (carbon nuclear magnetic resonance) and FTIR (fourier transform infrared) spectrophotometry. The FT-IR spectrum of the compound show characteristics peaks at 1540 cm^{-1} for C=C of benzene ring, at 1660 cm^{-1} for C=O of amide, sp^{3} C-H stretching frequency, that is, the methylene bonded to the nitrogen of the amide at 2900 cm^{-1}, N-H. The proton and carbon data were obtained using the aforementioned nuclear magnetic resonance spectrometers, and the corresponding spectrum displaced chemical shifts that align with the proposed structure of the target molecule.

Keywords: Benzylamine, *N*-benzyl-1*H*-indole-3-carboxamide, synthesis, and spectroscopic studies.

INTRODUCTION

In 2007, the Global Risk Reports from the World Economic Forum highlighted that microbial resistance to commercial drugs is a significant challenge in the global health sector [1], [2]. The Food and Drugs administration (FDA) database has revealed that 59% of the approved prescribed drugs contain five membered nitrogen heterocycles, namely imidazole, benzimidazole, pyrimidines, quinoxaline and indoles [3]. Among these five membered nitrogen-heterocycles, indole and its derivatives were given special attention due to their prevalence as parent in natural products, especially alkaloids namely, serotonin, tryptophan, and indopan, as shown in Figure 1 [4].

Indole, a significant compound with versatile synthetic and therapeutic potential, represents one of the most prevalent nitrogen-based heterocyclic frameworks owing to its diverse biological and pharmaceutical activities [5]. Extensive research has been conducted on indole and its derivatives, focusing on their chemical properties, spectroscopic identification, and biological activity. For the latter properties, Owolabi and co-authors developed *N*-methylindole-3-acetic acid and *N*-methylindole-3-thiouronium iodide in a 10% NaOH solution under a hydrogen atmosphere. The synthesized compounds exhibited activity against tested pathogens but showed no herbicidal activity [6]. Synthetic pathways have been devised for the synthesis of indole derivatives with varying substituents [7].

Indole is an aromatic heterocyclic compound which could be regarded as benzopyr role due to the fusion of six-membered benzene ring and five membered pyrrole ring through 2 and 3 positions of the pyrrole structure (Figure 2). Molecularly, it has the molecular formula C_8H_7N [8].

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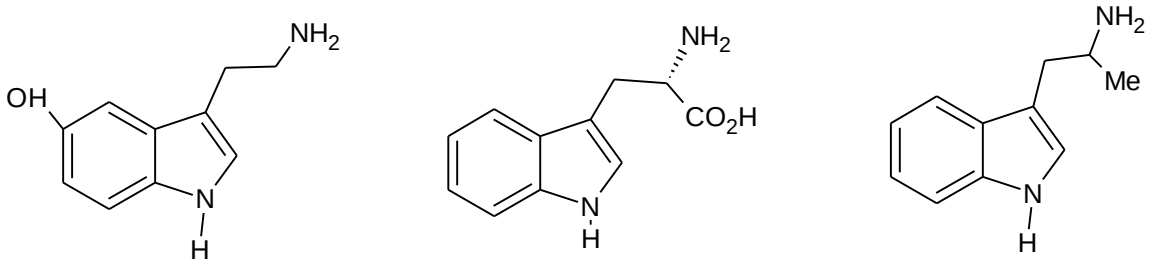
Indole appears as a colourless solid, characterized by a melting point of 52.5°C and emitting a pleasant fragrance in highly diluted solutions. It readily dissolves in methanol but is susceptible to oxidation, leading to the formation of isatin. Without proper precautions against atmospheric oxygen during the reaction process, it can further convert to oxindole. However, with adequate atmospheric guard in place, indole and its derivatives can be synthesized effectively [9].

Studies have shown that indole and its derivatives are active against fungal, bacterial, cancer, diabetic, and tubercular, depressant, and inframmatory conditions [10], [11].

On account of spectral analysis, Yuichi and co-authors, listed the principal infrared bands of indole and generalized that N-H absorption of indole compounds are in the range of 3472-3378 cm^1 and the methyl derivatives they show bands within the range of 3450-3375 cm^1. Aromatic absorption is in the range of 1650-1000cm^{-1}, while region of 2000-1650 cm^{-1} are very weak combination or overtone vibration. Notably, the strong bands in the region of 900-700cm^{-1} are characteristics of indole compounds with no substituents attached to the benzene ring [12], [13].

N-benzyl-1*H*-indole-3-carboxamide is a derivative of indole. Although, it is commercially available, there is no report of its antimicrobial properties to the best of our knowledge, despite having indole and amide in its structure. We aim to fill this gap and also show its spectroscopic characteristics. Therefore, this article presents the spectroscopic characterization and antimicrobial studies on the synthesized indole-3-carboxamide representing the primary, secondary, and application stages of the compound.



Serotonin Tryptophan Indopan

Figure 1: Indoles in alkaloids

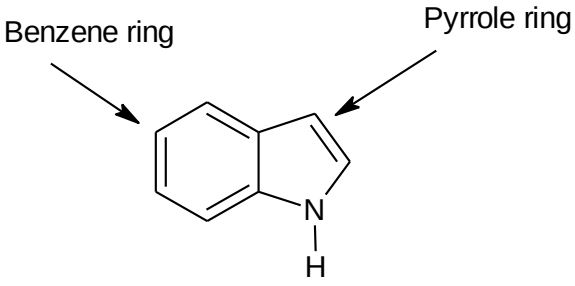


Figure 2: The structure of an indole

MATERIALS AND METHODS

Reagents

All reagents were analytical grade and used with further purification. The solvents were properly dried before usage. Reactions were monitored by thin layer chromatography (TLC). TLC spots were visualized

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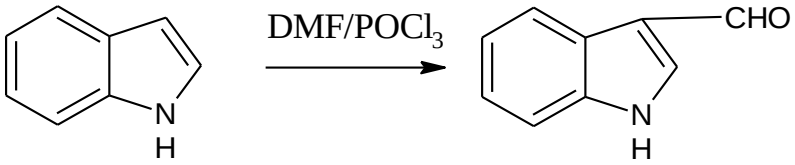
using iodine chamber. Melting point was determined using the Kofler Electro thermal melting point apparatus. ^{1}H NMR and ^{13}C NMR spectra were recorded at 600 MHz, calibrated using residual undeuterated solvent as an internal reference ($CHCl_3$, δ 7.27 ppm and 77.2 ppm), relative to trimethylsilane (TMS, δ 0.00 ppm) and presented as follows; Chemical shift (δ, ppm) multiplicity (s= singlet, d=doublet, dd= double of doublets, ddd= doublet of doublet of doublets, dt= doublets of triplets, t= triplet, m=multiplet, p=pentent, coupling constant (J, Hz). An IR spectrum was measured on FT-IR (Cary 630 Agilent Technologies) spectrophoto meter.

Synthesis of indole-3-carbaldehyde

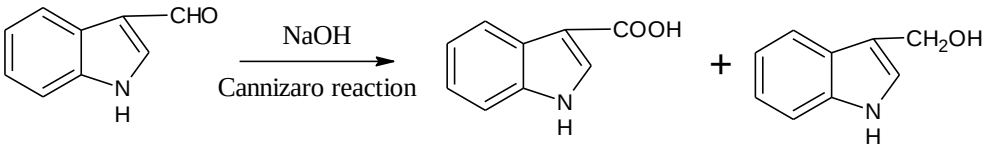
The synthesis of indole aldehyde was carried out using a modified procedure [ ] by (Masomeh, 2009).



Scheme 1. Vilsmeier-Haack Formylation of indole

A freshly distilled phosphorus oxychloride (8.60 mL) was reacted with 28.80 mL of dimethyl formamide (DMF) in a conical flask immersed in an ice-bath for 30 minutes until a pinkish colouration of the formylation complex was observed. Afterward, indole (0.085 mol, 9.99g) was dissolved in 10 mL of DMF and was added slowly with stirring to the flask containing DMF and $POCl_3$ at a temperature below 10^0C. After the solution was completely added and thoroughly mixed over a period of an hour, the temperature of the viscous solution was increased to 35^0C. The syrup was stirred efficiently for an hour at this temperature to form a canary-yellow paste. 30 g of crushed ice was added to the paste with careful stirring producing a clear, cherry-red aqueous solution. Furthermore, to the above mixture, 10 mL of water and 20 g of crushed ice were added. The solution was hydrolyzed by adding 2M sodium carbonate solution drop wise with continuous stirring. The resulting product was filtered using a vacuum pump, then washed with 30 mL of water, and finally recrystallized from methanol.

Preparation of indole-3-carboxylic acid



Scheme 2: Disproportionation reaction of indole-3-carbaldehyde

The solution of indole-3-carbaldehyde (0.075 mol, 0.300 g) in 20% NaOH in a round bottom flask at room temperature was stirred vigorously for 24 hours using magnetic stirrer. The progress of reaction monitored on TLC plate. Afterward, the solution was neutralized with concentrated HCl to afford the indole-3-carboxylic acid as precipitate. The precipitate was filtered through vaccuo and washed with dry chloroform to afford the acid, indole-3-carboxylic as a white crystal.

Synthesis of indole-3-carbonylchloride

Indole-3-carboxylic acid (0.1g, 0.001 mol) was heated to reflux in freshly distilled $SOCl_2$ (10 mL) for 2 hours. The progress of the reaction was monitored on TLC plate. The unreacted $SOCl_2$ was distilled-off with dry $CHCl_3$ (10 mL x 3). The reaction mixture was allowed to cool at room temperature and protected with anhydrous calcium chloride guard tube.

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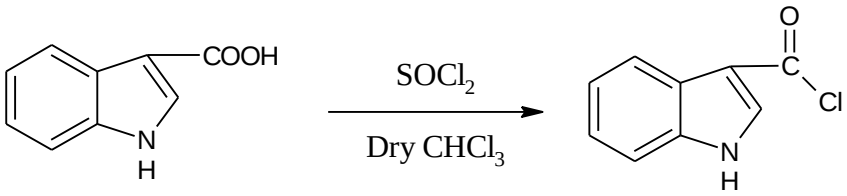
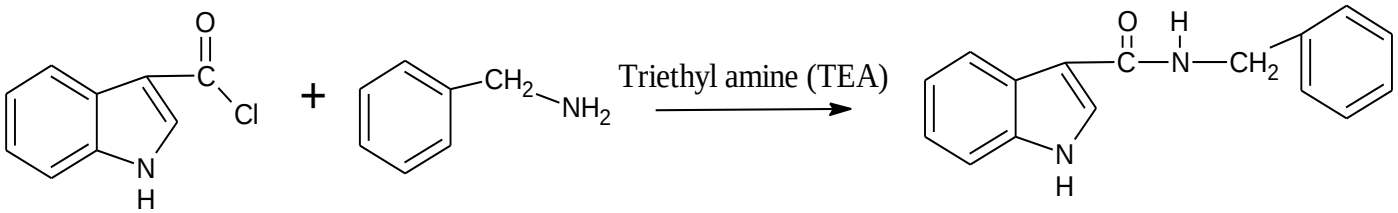


Scheme 3: Chlorination reaction of indole-3-carboxylic acid

Synthesis of *N*-benzyl-1*H*-indole-3-carboxamide

Indole-3-carbonylchloride (0.003 mol, 0.500 g) was dissolved in freshly distilled triethylamine (10 mL). After stirring for 20 minutes at room temperature, freshly distilled benzylamine (0.5 mL) was added, and stirred for an additional 4 hours. The mixture was left to stand for 24 hours, diluted with distilled water to dissolve the by-product, triethylamine hydrochloride and extracted with $CHCl_3$ (20 mL x 2). The organic layer was distilled under reduced pressure, and dried in a desiccator over calcium chloride anhydrous .



Scheme 4: Reaction of indole-3-carbonylchloride and benzylamine

Spectral data for the compound

Brownish solid; **mp**: 118-120^{0}C, **Yield**: 43.8%; **R_f**: 0.82 (DMF and $CHCl_3$, 1:3); **^{1}H NMR:** (600 MHz, $CDCl_3$, ppm): δ 1.25 (s, 1H, HC=C in the pyrrole ring), δ 4.7 (s, 2H, NH (CH_2)) δ 7.3-7.8 (m, 4H, Ar-H, benzopyrrole), δ 7.8 (d, 5H, Ar-H phenyl), δ 8.3 (s, 1H, NH(C=C)). **^{13}C NMR** (600 MHz. $CDCl_3$, ppm): 162 (carbonyl-carbon), 133-128 (Ar-carbon), 77.2 ($CDCl_3$), 65 (C=C), 30 (CH_2). **FT-IR (KBr, cm^{-1}):** 875-745 (str, phenyl monosubstituted), 1729.48 (str, C=O), 1796.57 (str, C=C), 2959.51 (str, CH_2), 3369.51 (str, NH).

Microbial Analysis

The synthesized compound was screened for antibacterial and antifungi activities against *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis, Klebsiella pneumonia, Candida albicans, and Aspergillus niger.* Agar well diffusion method as described by Murray et al., (1995) was used [14]. Ciprofloxacin and ketoconazole was used as standards. A sensitivity test was carried out to evaluate the zones of inhibition, MIC and MBC of the compound using the agar-well dilution method.

RESULT AND DISCUSSION

The synthesized compound was purified through column chromatography. We have confirmed the structure of the compound by spectroscopic techniques such as FT-IR, ^{1}H NMR, and ^{13}C NMR. The infrared spectrum displayed characteristic absorption bands at 1540 cm-1 for the C=C of the benzene ring, 1660 cm^{-1} for the C=O of the amide, 2900 cm^{-1} for the sp^3 C-H stretching frequency of the saturated compound (the methylene bonded to the amide nitrogen), 3150 cm^{-1} for the N-H (the secondary amide in the pyrrole ring), and 3080 cm^{-1} for the aromatic C-H stretch in the benzene ring. The infrared spectrum of the compound show characteristics peaks at 1540 cm^{-1} for C=C of benzene ring, at 1660 cm^{-1} for C=O of amide, sp3 C-H stretching frequency of saturated compound that is the methylene bonded to the nitrogen of the amide at 2900 cm^{-1}, N-H, that is the secondary amide in the pyrrole ring at 3150 cm^{-1} and the aromatic C-H stretch in the benzene ring at3080 cm^{-1}. In ^{1}H NMR spectra of the compound showed characteristic signals of the

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entire aromatic proton between 7.25-7.75 δppm in the spectrum. The characteristic peak at 7.8 δppm is for NH proton in the pyrrole ring while the amide NH proton bonded to the methylene has peak near about 8.45 δppm.

In the ^{13}C NMR spectrum, the compound displayed a chemical shift value of 162 δppm for the carbonyl carbon, while the aromatic carbons were observed in the range of 120-140 δppm. Additionally, the solvent peak appeared at 77.2 δppm, with the alkene and methylene signals observed at 65 and 30 δppm, respectively.

Antimicrobial Evaluation

The in vitro antimicrobial activities of the compound showed that at 3 mg/mL concentration that the compound has no zone of inhibition against the tested bacterial and fungi isolates.

CONCLUSION

This research work presents the synthesis of *N*-benzyl-1H-indole-3-carboxamide. The synthetic pathway was structured to proceed by three sequential steps, with the reaction progress being monitored by TLC. The obtained range of yield is moderately good.

Analysis of the compound via spectroscopic techniques revealed the presence of distinct peaks and functional groups in the compound. This study holds promising potential for further bio-evaluations and applications.

Antimicrobial screening reveals that the compound was inactive against the bacterial strains; *E. coil, B. subtilis, K. pneumonia, S. aureus,* and *P. aeruginosa*, and fungi strains are *C. albicans and A. niger*. However, further antimicrobial screening against more bacteria and fungi is needed to establish that *N*-benzyl-1*H*-indole-3-carboxamide has no antimicrobial properties.

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