One-pot Multicomponent Synthesis and Biological Activity of Oxindole- linked Isatin Schiff Bases Derivatives

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Abstract

The study focuses on the synthesis of isatin- oxindole compounds through a two-step process. In the first step, using mannich reaction, new derivatives of Isatin are synthesized by reacting Isatin with either oxindole, along with formaldehyde. This reaction results in the formation of Isatin- oxindole derivative. In the second step, these Isatin derivatives are further reacted with various aniline derivatives to generate new compounds containing Isatin. The synthesized compounds are characterized using spectroscopic techniques such as FTIR, ^{1}H NMR, and ^{13}C NMR. The study also explores the biological activity of some of the synthesized compounds against specific bacteria strains, such as Klebsiella pneumoniae and Staphylococcus aureus. These compounds are assessed for their potential antimicrobial properties.

Introduction

The Mannich reaction is a highly effective synthetic technique employed in the field of organic chemistry to generate β-amino carbonyl compounds. The process entails the amalgamation of a primary or secondary amine, an aldehyde or ketone, and a compatible enolizable compound through condensation. The aforementioned reaction exhibits extensive utility in various domains such as pharmaceuticals, agrochemicals, and materials chemistry. **(1)**

The first step involves the nucleophilic attack of the amine onto the carbonyl group present in the aldehyde or ketone, resulting in the formation of an intermediate iminium ion. Subsequently, the intermediate undergoes deprotonation by a base, leading to the generation of an enamine. The enamine has the ability to undergo a tautomeric conversion resulting in the formation of an imine. This imine can then react with another molecule of the amine. Ultimately, the Mannich reaction culminates in the protonation of the nitrogen, resulting in the formation of the intended β-amino carbonyl compound. **(2)**

The Schaffer base, also known as N,N-dimethylaminomethylene 2,2-dimethylpropanamide, is a frequently employed amine in the Mannich reaction. The compound in question is capable of promoting the formation of diverse β-amino carbonyl compounds owing to its notable stability and nucleophilic properties. The products that ensue from this process can manifest a wide range of biological activities and have been implemented in the field of medicinal chemistry. The potential of Schaffer base-derived Mannich bases as agents for anti-inflammatory, antiviral, and anticancer purposes has been investigated. **(3)**

The Mannich reaction is a commonly employed chemical process that utilises isatin and its derivatives, including oxindole, owing to their distinctive structural characteristics and reactivity. The heterocyclic compound Isatin, which contains a diketone moiety, is a highly suitable initial substrate for the production of β-amino carbonyl compounds that exhibit a wide range of substitution patterns. **(4)**

The aforementioned compounds have demonstrated favourable biological effects, such as antimicrobial, anti-inflammatory, and anticancer attributes. Oxindole, a chemical compound derived from isatin, is recognised as a significant precursor in the Mannich reaction. The Mannich products that ensue, which encompass an oxindole core, have been investigated as plausible therapeutic agents in the realm of drug discovery **(5)**

The utilization of Schiff base, isatin, and oxindole as starting materials in the Mannich reaction presents a diverse array of synthetic opportunities for the creation of innovative compounds possessing significant biological properties. The β-amino carbonyl compounds obtained can be subjected to additional modifications to optimize their characteristics and augment their effectiveness. Subsequent research endeavors could centre on investigating the utilization of these substances in drug development, materials engineering, and related domains. Furthermore, the enhancement of the Mannich reaction's synthetic potential and the production of intricate molecules with greater efficiency and atom economy could be facilitated by the creation of more effective and specific catalysts**. (6)**

The compound known as Isatin, which possesses a diketone structure and is classified as a heterocyclic compound, has garnered considerable interest in the field of medicinal chemistry owing to its multifaceted biological properties. The pharmacological properties of Isatin derivatives and their Mannich reaction products are diverse, rendering them desirable subjects for drug discovery **(7)**

The anticancer properties of Isatin derivatives have shown great promise, rendering them as viable contenders for cancer therapy. These compounds demonstrate cytotoxic properties against diverse cancer cell lines through the disruption of essential cellular mechanisms. Compounds based on Isatin have demonstrated the ability to impede the proliferation of cancer cells, trigger apoptosis or programmed cell death, and hinder tumour angiogenesis. Furthermore, it has been demonstrated that there are synergistic effects when these agents are utilised in conjunction with conventional chemotherapeutic treatments **(8)**

The anti-inflammatory and immunomodulatory properties exhibited by isatin compounds render them a promising therapeutic option for the management of inflammatory disorders. They have the ability to impede the generation of pro-inflammatory cytokines and enzymes, including tumour necrosis factor-alpha (TNF-α) and cyclooxygenase-2 (COX-2). **(9)**

The immunomodulatory effects of Isatin derivatives have been observed to involve the regulation of immune response and modulation of immune cell activity, including macrophages and lymphocytes. The aforementioned properties endow isatin compounds with the potential to serve as therapeutic agents for conditions that are typified by immune dysregulation and inflammation. Isatin derivatives have demonstrated noteworthy antimicrobial and antiviral properties against various pathogens **(10)**. The inhibitory effects against bacteria, fungi, and viruses have been demonstrated. Isatin compounds exert their antimicrobial effects through various mechanisms, including perturbation of microbial cell membranes, inhibition of crucial enzymes, and interference with critical viral proteins **(11)**

The potential of the tested substances as alternative or adjunctive therapeutic agents has been demonstrated by evaluating their antimicrobial activity against various drug-resistant strains. Furthermore, isatin derivatives have exhibited antiviral properties against various viruses, such as herpes simplex virus (HSV), human immunodeficiency virus (HIV), and influenza virus **(12)**.The observed neuroprotective activity of Isatin can be attributed to their demonstrated antioxidant and anti-inflammatory properties. Isatin derivatives have exhibited the capacity to mitigate oxidative stress, diminish neuro-inflammation, and regulate neurotransmitter systems **(13)**.The aforementioned characteristics render them promising contenders for the management of neurodegenerative ailments, including but not limited to Alzheimer's disease and Parkinson's disease **(14).** The modulation of serotonin and dopamine neurotransmission by Isatin derivatives has demonstrated potential as antidepressants and anxiolytics **(15)**

2. Experimental Part

2.1 General

Fourier transform infrared Alpha-Bruker (Germany) infrared spectrophotometer was used to record FTIR spectra in the Department of Chemistry, Faculty of Science, University of Kufa, Nuclear. Magnetic resonance spectra were recorded using a Bruker ARX400 (FT, 400 MHz for 1 H; 100 MHz for 13C), ^{1}H and ^{13}C NMR chemical shifts were referenced to solvent resonance of the solvent (DMSO-d_6). NMR spectroscopy were performed by The department of chemistry, Basra University.Melting point was recorded using Electrothermal ,made in the U.K.

2.2 One-pot three component reaction (Mannich reaction).(16)

2.2.1 Synthesis of 1-((2-oxoindolin-3-yl)methyl)indoline- 2,3-dione (compound A).

A mixture of isatin (0.735g ,0.005 mol), formaldehyde (37% , 5 ml, 0.06 mol) and oxindole (0.665g, 0.005 mol) in absolute ethanol (30 mL) was refluxed for 6 hours.

The mixture was allowed to cool somewhat and additional formaldehyde (37% ,5 ml, 0.06 mol) was added. The mixture was then refluxed for another 6 hours and allowed to stand overnight. During this time, the progress of the reaction was monitored by TLC. After the completion of the reaction, the resulting red solution was cooled in an ice bath, leading to the formation of brown precipitate. The product was collected and subjected to recrystallization using a minimum amount of absolute ethanol to obtain orange product (1.2g , 82.1% yield). M.P (196-198)℃. FT-IR cm^{-1}: 3326 (N-H), 3049(C-H aromatic), 2961 (C-H $_{aliph}$), 1690 (C=O), 1612(C=C).

2.2.2 Synthesis of compound C1

A solution of *p*-nitro aniline (0.069g ,0.0005 mol) in absolute ethanol (15 mL) was added to a solution of compound A (0.145g, 0.0005 mol) in absolute ethanol (15 mL). A few drops of glacial acetic acid were added to the mixture, which was then heated under reflux for 8 hours. After cooling, a precipitate formed, which was filtered and washed with ethanol. The resulting product was then recrystallized using ethanol to obtain a dark yellow powder (0.16g ,77.5% yield). M.P(223-225)℃ . FT-IR cm^{-1}: 3329 (N-H), 3187(C-H aromatic), 3049 (C-H $_{aliph}$), 1690 (C=O), 1615(C=N),1467(C=C).

2.2.3 Synthesis of compound C5

A solution of *p*-chloro aniline (0.063g ,0.0005 mol) in absolute ethanol (15 mL) was added to a solution of compound A(0.145g, 0.0005 mol) in absolute ethanol (15 mL). A few drops of glacial acetic acid were added to the mixture, which was then heated under reflux for 7 hours. After cooling, a precipitate formed, which was filtered and washed with ethanol. The resulting product was then recrystallized using ethanol to obtain a orange powder (0.15g ,74.6 % yield). M.P(194-196℃), FT-IR cm^{-1}: 3382 (N-H), 3055(C-H aromatic), 2967(C-H $_{aliph}$), 1699 (C=O), 1605(C=N),1475(C=C).

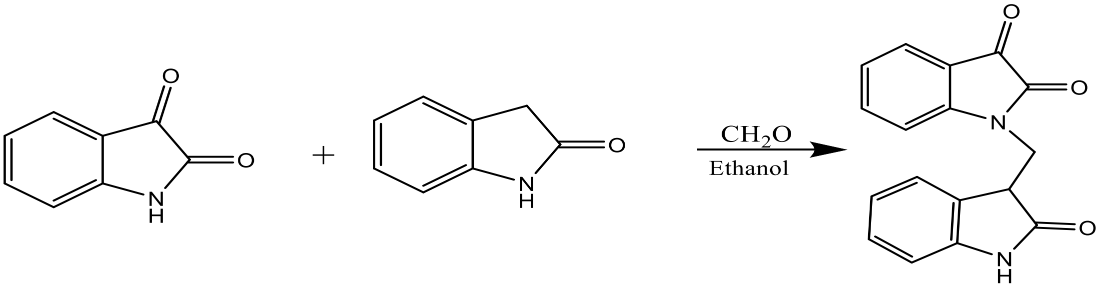
2.3 Study of biological activity

Antibacterial effects of all prepared compounds were examined using agar well diffusion methods against multidrug resistance. The Gram positive (*Staphylococcus aureus*) and Gram negative (*Klebsiela pneumonia*) were screened against two prepared compounds (C1, C5). Four isolated colony of fresh culture that suspended in five milliliters of Brain Heart Infusion Broth (BHIB) and incubate in 37°C for 3 hrs. The turbidity produced by growth culture was calibrated with sterilized broth to achieve optical density comparable to the 0.5 McFarland requirements (1.5 X 10^8 cells/ml the equivalent). Sterile cotton swab was dipped in the suspensions. The dipping cotton swabs was used to streak the whole surface of a Mueller Hinton Agar tray. Then using a sterile cork Pore, Pores (6 mm diameter) were created six holes with the corkscrew.(88). Dilute solutions of (100 and 200 µg mL^{-1}) were prepared for each compound at physiological pH(7). The prepared concentrated solutions from chemical compounds were put in holes to know their effectiveness for biological activity. And then, the paten dish was incubated at temperature 37 ^{o}C. for 24 hours. Finally, the diameter of inhibition zone was measured for each disc by the ruler to determine the effectiveness of each compound and compare with the standard limits of sensitivity of the same species of bacteria against azithromycin as a standard.

3. Results and discussion

3.1 Synthesis of Isatin linked-oxindole compound (Mannich reaction)

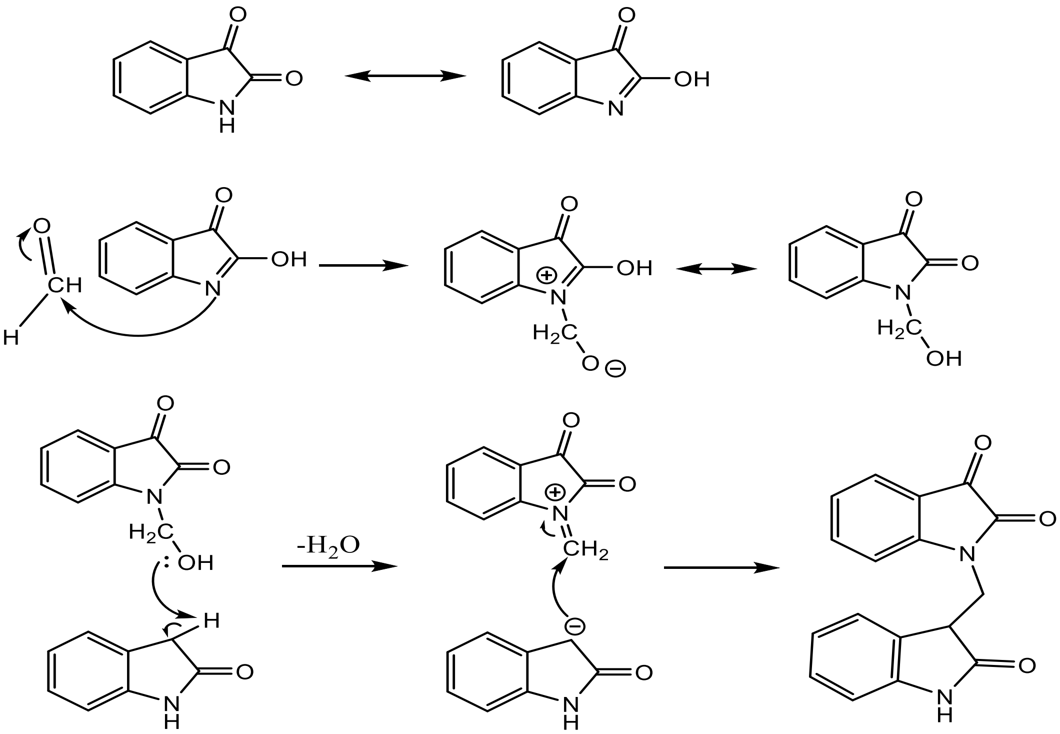
Isatin linked-oxindole compound (A) was synthesized by reacting of isatin, formaldehyde and oxindole in absolute ethanol (Scheme 1). The resulting product was recrystallized from a minimum amount of absolute ethanol to yield orange powder with a yield of 82.1% yield . The melting point of the compound was found to be within the range of (196-198)℃.



compound(A)

Scheme 1: Synthesis of Isatin linked-oxindole compound (A)

The mechanism of the reaction shows in Scheme 2 according to an Mannich reaction. In the first step, an intermediate iminium ion formed from substituted isatin and formaldehyde. Then, oxindole attaches to the intermediate obtain the main product.



Scheme 2: mechanism of synthesis of compound A

3.2 Identification of compound A

The FT-IR spectrum of the compound (A)showed characteristic peaks at various wavenumbers: 2961 cm^{-1} for C-H aliphatic stretching vibrations, 1690 cm^{-1} for C=O stretching vibrations, 3326 cm^{-1} for N-H stretching vibrations, 3049 cm^{-1} for C-H aromatic stretching vibrations, and 1612 cm-^{1} for C=C stretching vibrations (Figure 1).

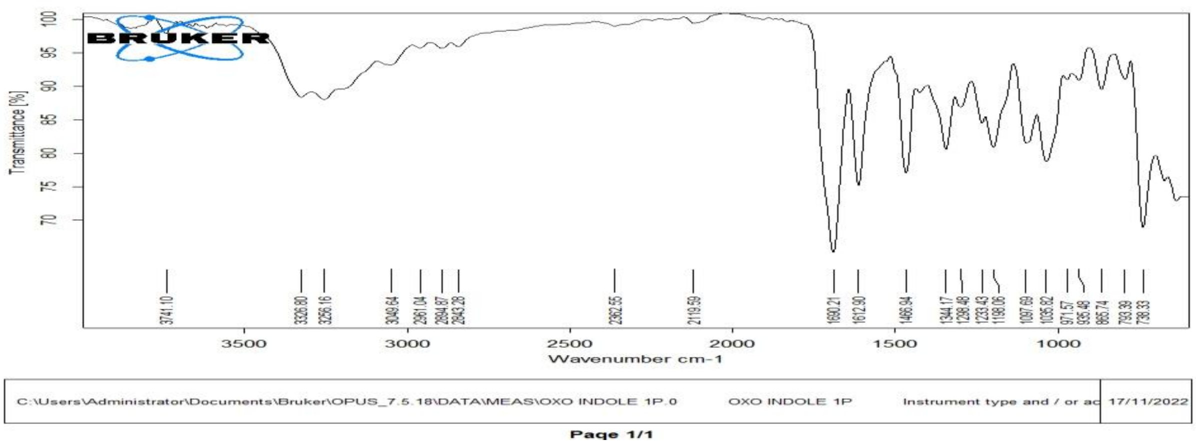


Figure 1: FTIR of compound A

The ^{1}H NMR spectrum of compound (A) in DMSO-d_6 is consistent with the identity of compound. The spectrum shows multiple signals at (7. 57- 6.17) ppm were assigned to the aromatic ring protons. Moreover, the signal at (10.36) ppm was assigned to (N-H) proton. Furthermore; the singlet at (5.21, 4.05)ppm non-equivalent CH_2 protons. Moreover, the signal at (4.90) ppm was assigned to (C-H) protons Figure (2).

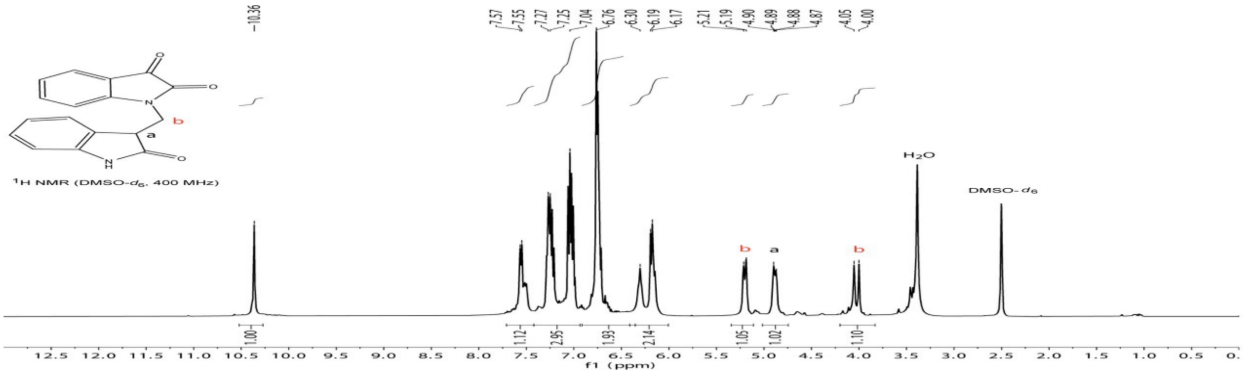


Figure 2: ^{1}H NMR of Compound A

In the ^{13}C-NMR spectrum of compound (A) obtained in DMSO-d_6, several signals were observed at characteristic chemical shifts. The signal at 174.36 ppm was assigned to the C=O$_{\text{amide}}$ oxindole group. Signals at δ 177.58 ppm and 175.81 ppm corresponded to the C=O$_{\text{ketone}}$ and C=O$_{\text{amide}}$ groups of the isatin moiety, respectively. The signals at 143.82-109.56 ppm were attributed to the aromatic ring carbons. The signal at 63.28 ppm was assigned to the carbon of the CH$_2$ group, while the signal at 52.21 ppm was attributed to the carbon of the CH group (Figure 3).

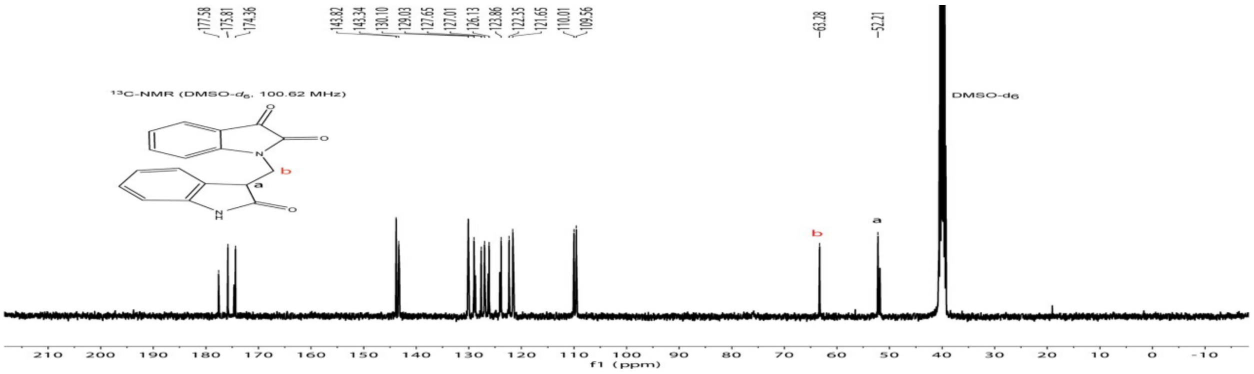
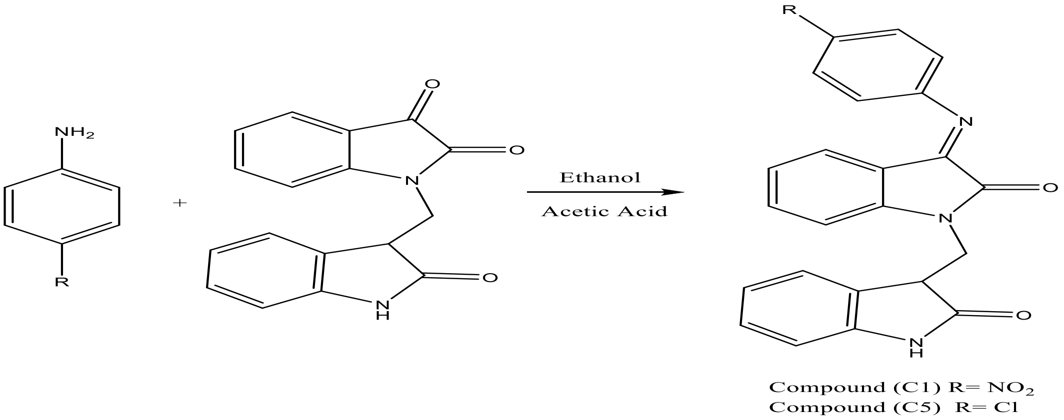


Figure 3: ^{13}C NMR spectrum of Compound A

3.3 Synthesis of Isatin linked-Oxindole derivatives (Schiff bases)

Compounds (C1 and C5) were synthesized by reaction of substituted aniline and compound (A) in absolute ethanol and a few drop from acetic acid (Scheme 3). The resulting products were recrystallized from a minimum amount of absolute ethanol to yield dark yellow powder with a yield of 77.5% for compound (C1) and orange powder with a yield of 74.6% for compound (C5). The melting point of the compounds were found to be within the range of (225-223 °C) for compound (C1) and (196-194) ℃ for compound (C5).



Scheme 3: Synthesis of Compound C1 and C5

3.4 Identification of Compounds C1 and C5

The FT-IR spectrum of the compound (C1) showed characteristic peaks at various wave numbers: 3049 cm^{-1} for C-H aliphatic stretching vibrations, 1690 cm^{-1} for C=O stretching vibrations, 3329 cm^{-1} for N-H stretching vibrations, 3187 cm^{-1} for C-H aromatic stretching vibrations, and 1467cm^{-1} for C=C stretching vibrations, and 1615cm^{-1} for C=N stretching vibrations (Figure 4). For compound C5, FTIR spectrum showed peak at 2967cm^{-1} for C-H aliphatic stretching vibrations, 1699cm^{-1} for C=O stretching vibrations, 3382 cm^{-1} for N-H stretching vibrations, 3055cm^{-1} for C-H aromatic stretching vibrations, and 1475cm^{-1} for C=C stretching vibrations, , and 1605cm^{-1} for C=N stretching vibrations (Figure 5).

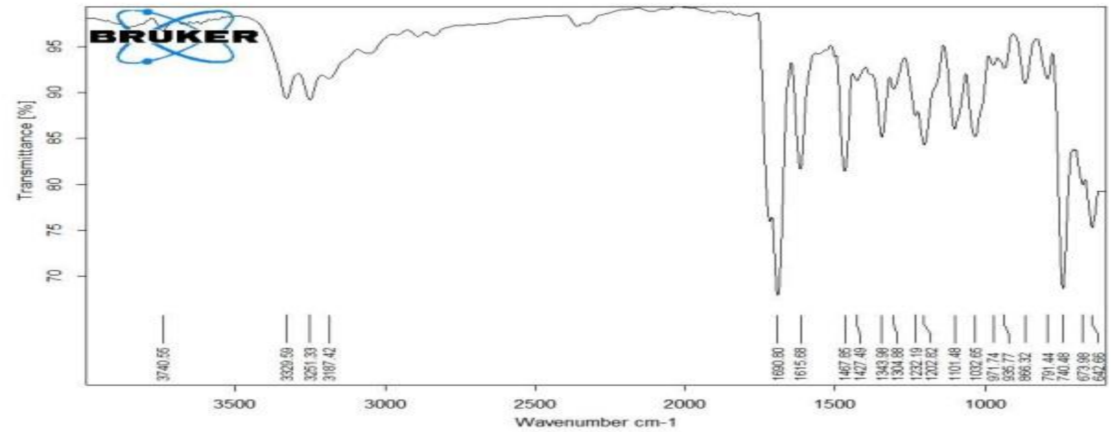


Figure 4: FT-IR spectrum of Compound C1

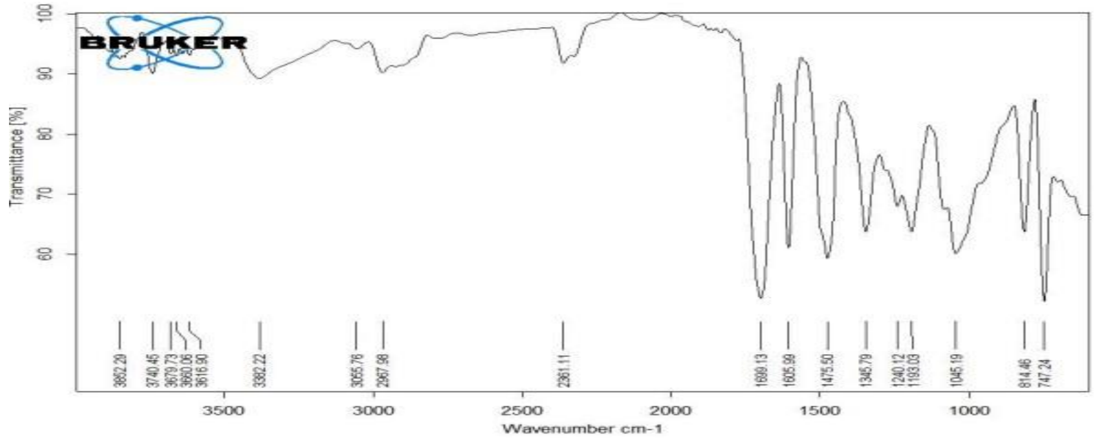


Figure 5: FT-IR spectrum of Compound C5

The ^{1}H NMR spectrum of compound (C1) in DMSO-d_6 showed singlet signal at (10.44) ppm assigned to (N-H) and at (7.45-6.19) ppm assigned to (C-H aromatic, 12 H). The spectrum showed a triplet signal at (5.19) ppm assigned to aliphatic (C-Ha, 2H) , and a doublet signals obtained at (4.05 and 5.3) ppm assigned for unequivalent CH_2b (Figure 6). For the compound (C5), The ^{1}H NMR spectrum showed a single signal at (10.11) ppm assigned for (N-H), a triplet and quadruplet signals at (7.53-6.13)ppm assigned for the (C-H) of the aromatic ring. A triplet signal assigned for the (C-Ha) at (4.85)ppm, and a doublet signal assigned for unequivalent CH_2b at (4.13 and 5.17) ppm (Figure 7).

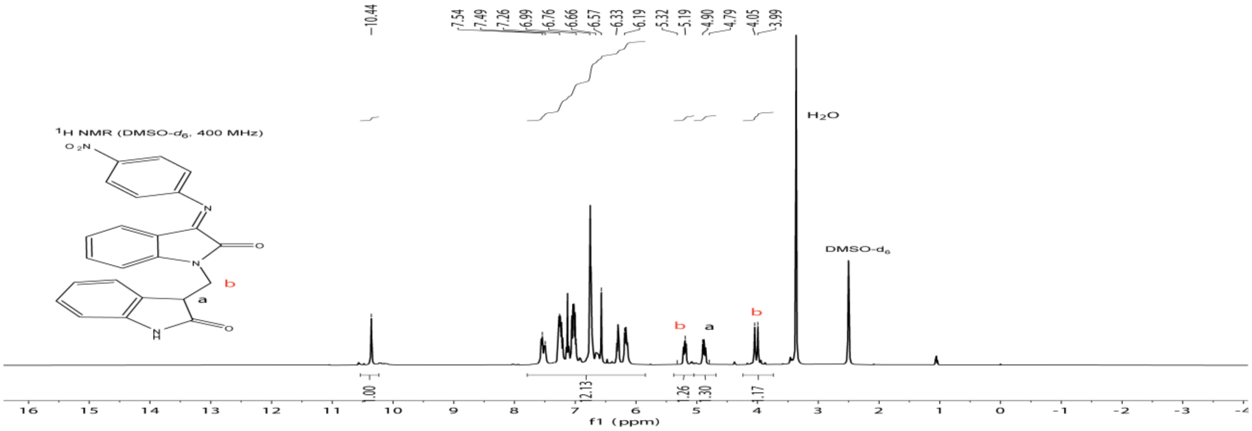


Figure 6: ^{1}HNMR spectrum of Compound C1

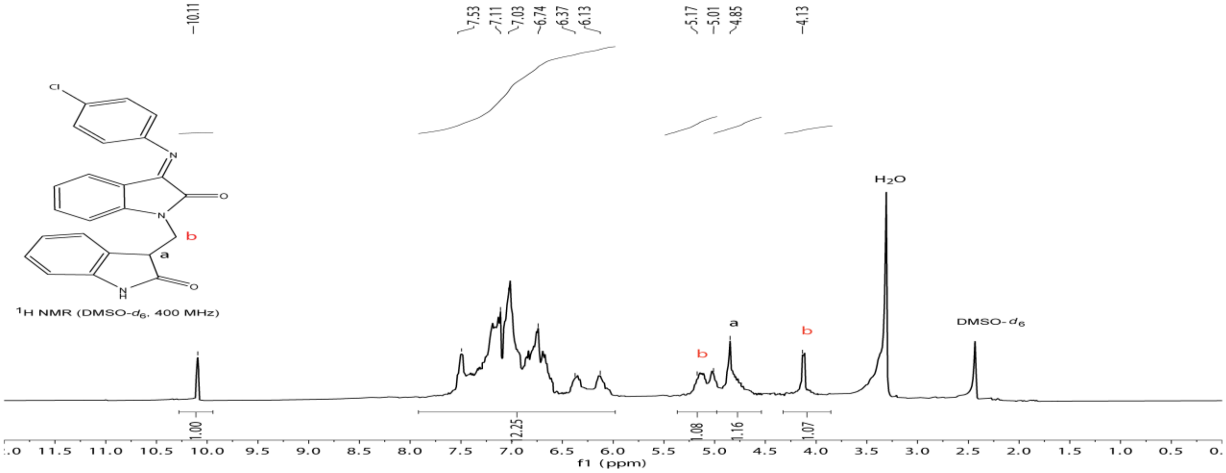


Figure 7: ^{1}HNMR spectrum of Compound C5

In the ^{13}C-NMR spectrum of compound (C1) obtained in DMSO-d_6, several signals were observed at characteristic chemical shifts. The spectrum showed singlet signal assigned to (C=O) of oxindol at (174.82)ppm. At (175.92) ppm refer to singlet signal for the (C=O) of Isatin. Another signal obtained at (162.79) ppm assigned to (C=N). A signals noticed at (158.52-109.49)ppm assigned for carbons of the aromatic ring. The two signals were noticed at (51.83 and 63.58)ppm assigned for (CH) and (CH_2) respectively (Figure 8). For the compound (C5), the spectrum showed a signal at (175.39) ppm assigned for (C=O) of Isatin, another signal at (174.35) ppm assigned for (C=O) of oxindole. At (161.32) ppm a signal was found and assigned for (N=C). The signals at (158.73-109.63) ppm assigned for (C-H) of the aromatic ring. The two signals were noticed at (51.97 and 62.63) ppm assigned for (CH) and (CH_2) respectively (Figure 9).

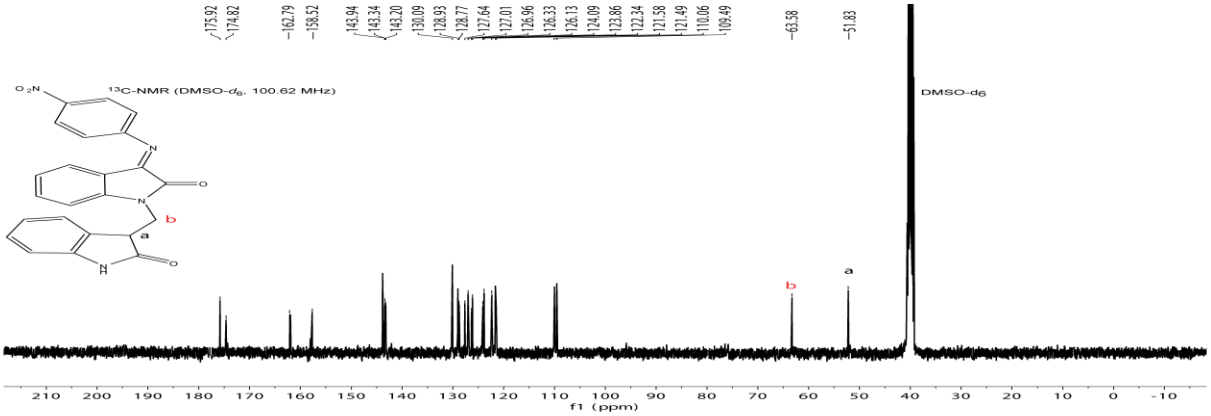


Figure 8: ^{13}C NMR spectrum of Compound C1

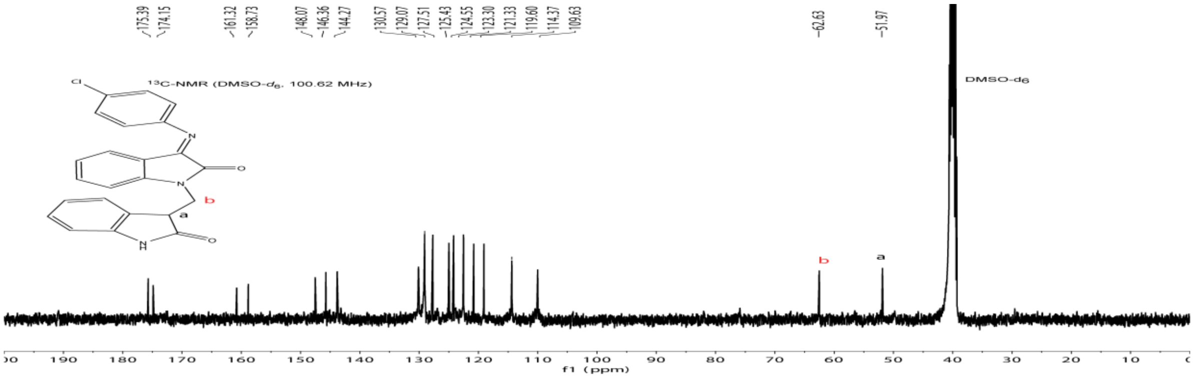


Figure 9: ^{13}C NMR spectrum of Compound C5

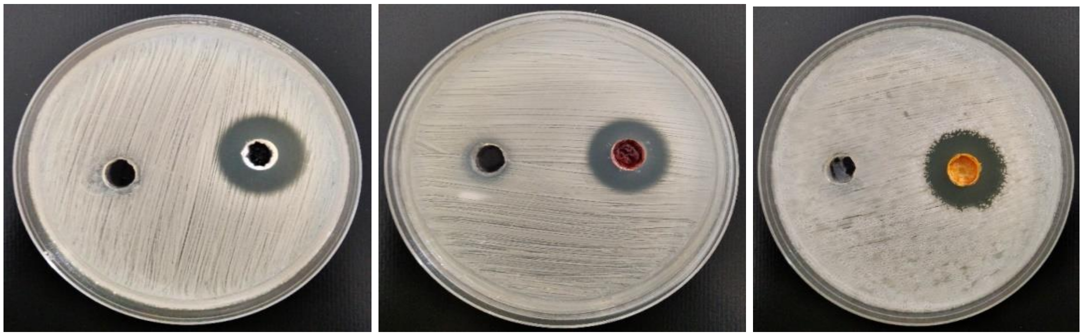
3.5 Biological Activity of prepared compounds

The susceptibility of pathogenic bacteria to antimicrobial agents was performed on Muller Hinton agar by the modified Kirby Bauer disc diffusion method by the measuring the diameter of inhibition zones around antibiotic discs according to CLSI (2018).(17) The Gram positive (*Staphylococcus aureus*) and Gram negative (*Klebsiela pneumonia*) were screened against eight prepared compounds (A , C1,C5) (Figure 10).

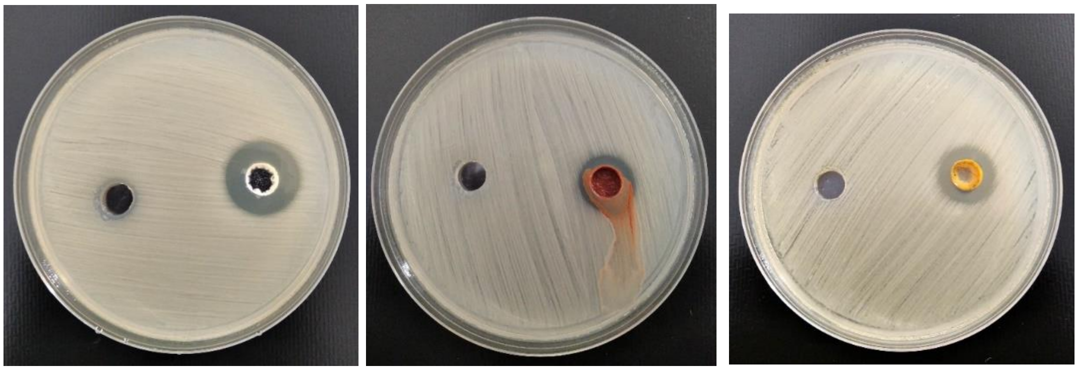
The results showed that the gram positive bacteria which were highly sensitive to chemical compounds that include (A, D5) with the diameter of inhibition zone (23, 21,) mm respectively. Notably, gram negative bacteria showed sensitive to all chemical compounds with the diameter of inhibition zone as shown in (Table 1).

Table(1)Antibiotic susceptibility of the Gram positive and negative isolates 0.12g/mol

Tested bacteria		**Inhibitions zone (mm)**	
	A	**C1**	**C5**
S. aureus	23	21	21
Klebsiela pneumonia	20	18	15



S.auruse (A) S.auruse (C5) s.auruse (C1)



Klebsiella paneumoniae (A) Klebsiella paneumoniae (C5) klebsiella paneumoniae(C1)

Figure 10: Antibacterial activity of prepared compounds against(*Staph aureus and Klebsiella pneumoniae)*

Bacterial degradation of several compounds has been studied in a variety of bacteria, which utilizes these materials as their sole source of carbon and energy. Several degradation pathways have been proposed and the related enzymes and genes have also been characterized (**18**). Various isatin derivatives have been screened for their anti-bacterial activities, and some of them demonstrated promising in vitro and in vivo potency. Treatment of bacterial infections is challenging, mainly attributed to the rapidly evolving resistance mechanisms, creating an urgent demand to develop novel antibacterial agents (**19**). The prepared compounds contain isatin moiety (A and C5) distributed widely in nature, may exert antibacterial properties by acting on diverse enzymes, proteins, and receptors .The morphology and particle size depend on several chemical and physical parameters, e.g., incubation time, pH, composition of the culture medium, and growth in light or darkness (**20**). *Klebsiela pneumonia* less sensitive than s. aureus because the cell walls of gram-negative bacteria are more complex than those of gram positive bacteria, both structurally and chemically. The structure of gram-negative microorganisms cell contains two layers outside the cytoplasmic membrane , which would represent a greater physical barrier to overcome(**21**). Gram-negative bacteria have an outer membrane that contains lipopolysaccharides that provide a hydrophilic surface to the bacterium. The lipid components present inside the lipopolysaccharide molecules contain anionic groups (phosphate, carboxyl), which contribute to the stability of the lipopolysaccharide layer through electrostatic interactions with divalent cations (**22**).

Conclusion

To summarize, the study successfully achieved the synthesis of Isatin-oxindole compounds using a two-step process. In the initial step, Isatin was reacted with oxindole and formaldehyde via the Mannich reaction, resulting in the formation of Isatin-oxindole derivatives. In the subsequent step, these derivatives were further reacted with different aniline derivatives to generate novel compounds containing Isatin. The synthesized compounds underwent characterization using spectroscopic techniques such as FTIR, ^{1}H NMR, and ^{13}C NMR. These methods provided valuable data on the chemical properties and structural features of the compounds, enabling their identification and analysis. Furthermore, the study investigated the biological activity of selected synthesized compounds against specific bacterial strains, including Klebsiella pneumoniae and Staphylococcus aureus. The compounds were assessed for their potential antimicrobial properties, aiming to identify promising candidates for further development as antimicrobial agents. The results obtained from the biological activity evaluation revealed that certain synthesized compounds exhibited notable antimicrobial activity against the tested bacteria strains. This finding suggests that the Isatin-oxindole compounds synthesized in this study hold promise for future development as effective antibacterial agents.

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