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Journal of Neonatal Surgery
ISSN(Online): 2226-0439
Vol. 14, Issue 19s (2025)
https://www.jneonatalsurg.com

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Design, Synthesis and Evaluation of Antimicrobial Potential of Dihydroimidazo Indole Complexes

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Cite this paper as: M.S. Vijayalakshmi, Patibandla Jahnavi, SG Raman, S. R. Sivakumar, Shraddha Patel, Deshraj Shyamkant Chumbhale, Manish R. Bhise, Praveen Sekar, (2025) Design, Synthesis and Evaluation of Antimicrobial Potential of Dihydroimidazo Indole Complexes. *Journal of Neonatal Surgery*, 14 (19s), 820-827.

ABSTRACT

This study aims to design and synthesize a series of novel dihydroimidazo indole complexes and evaluate their antimicrobial, antitubercular, and antifungal activities. The collection of a few novels 3, which is phenyl with a replacement of four By combining compounds with 2-substituted-indole analogues (1a–c) and 4-substituted anilines derivatives (2a–d), we were able to produce novel dihydroimidazo indole complexes (5a–l) derivatives. This allowed us to obtain 4-substituted- anilines analogues (3a–l). After that, these were combined with ammonium acetate and isatin (4) while glacial acetic acid was present in the mixture. To provide a description of each and every one of the molecules that were manufactured, we made use of spectrum data obtained from instruments such as IR, 1H NMR, FAB-Mass, and Elemental analysis. All of the chemicals that were manufactured were put through a series of tests to see whether or not they were capable of eliminating microorganisms. When tested against all of the diseases that were investigated, chemicals 5b, 5c, 5g, 5j, and 5k were found to be effective against them.

Keywords: *Dihydroimidazo indole, Indole, Isatin, Indolo-imidazole, Antibacterial, Antifungal, Anti-tubercular*

1. INTRODUCTION

When it comes to contemporary synthetic chemistry and medical science, heterocyclic compounds are of utmost significance [1]. Their importance is due to the fact that they are capable of performing a wide variety of biological acts and feature a tremendous amount of structural plasticity [2]. Among them, indole and imidazole scaffolds are of exceptional significance, displaying a wide variety of biological properties, including anti-inflammatory, antibacterial, antiviral, and anticancer activities [3]. The structural fusion of these heterocycles into novel frameworks, such as dihydroimidazo indole complexes, holds promising potential for the development of biologically active molecules with enhanced efficacy and selectivity [4].

Dihydroimidazo indole derivatives represent an emerging class of fused heterocyclic compounds characterized by the

integration of the indole nucleus with a partially saturated imidazole ring [5]. This fusion not only enhances the molecular rigidity and planarity but also introduces new sites for functionalization, which can be exploited in the design of metal complexes or pharmacophores with unique chemical and biological properties [6].

The synthesis of such compounds typically involves multistep reactions, including condensation, cyclization, and functional group modifications, often requiring precise control of reaction conditions. Furthermore, the design of these molecules can be tailored to explore structure-activity relationships (SAR), aiming to optimize their interaction with biological targets or catalytic systems [7, 8].

Microbial strains that are resistant to several drugs are becoming more prevalent, which poses a substantial risk to public health around the world. As a result, there is an ongoing need to find new therapeutic agents that are both effective and unique. Infectious diseases caused by bacteria, fungi, and mycobacteria remain a major concern, especially in regions with limited access to advanced healthcare. Among these, tuberculosis (TB), which is caused by Mycobacterium tuberculosis, continues to be one of the major causes of mortality around the world. An additional factor that makes matters more problematic is the proliferation of multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains of tuberculosis [9, 10].

Heterocyclic compounds, particularly those containing nitrogen, have been extensively studied due to their diverse pharmacological profiles. The imidazole ring system, known for its wide range of biological activities forms a crucial scaffold in medicinal chemistry. Similarly, indole derivatives have also demonstrated potent bioactivity, often serving as core structures in natural products and synthetic drugs [11, 12].

Recent studies have shown that hybrid molecules combining two or more pharmacophores can exhibit enhanced biological efficacy through synergistic effects. In this context, dihydroimidazo indole complexes emerge as promising candidates due to the potential dual-action mechanisms derived from both imidazole and indole moieties. Furthermore, the inclusion of metal complexes or appropriate substituents may enhance their stability, solubility, and bioavailability, further optimizing their therapeutic potential [13, 14].

This study aims to design and synthesize a series of novel dihydroimidazo indole complexes and evaluate their antimicrobial, antitubercular, and antifungal activities. Through rational drug design, structure-activity relationship (SAR) analysis, and comprehensive biological screening, this research seeks to identify lead compounds with significant potential to combat resistant pathogens and contribute to the development of next-generation anti-infective agents [15, 16].

2. MATERIAL AND METHODS

Chemical, Reagents and Instruments:

The melting points were determined using open capillary tubes and are uncorrected. The acquisition of infrared spectra was accomplished by the utilization of KBr discs in conjunction with a Perkin-Elmer FT-IR spectrophotometer [17, 18]. In order to acquire proton nuclear magnetic resonance (NMR) spectra, a Bruker AMX spectrophotometer was utilized. The solvent utilized was DMSO-d6, and TMS was utilized as the reference. The utilization of a mass spectrometer allowed for the collection of mass spectra. Compounds were evaluated for purity via thin-layer chromatography on silica gel plates, utilizing iodine vapors as a visualization agent. Elemental analysis was performed using the FlashEA1112 series elemental analyzer [19, 20].

Procedure:

Physical and infrared spectral data verified that the starting materials, 4-substituted- anilines analogues (3a-l), were synthesized in accordance with the stated technique [21].

Step 1: General procedure for the synthesis of Dihydroimidazo indole (3a-l):

In a water bath, 20 ml of ethanol and a catalytic quantity of glacial acetic acid were refluxed with 0.001 mol of 2-substituted dihydroimidazo indole (1a-c) and 0.001 mol of 4-substituted anilines (2a-d) for 8 hours. Following filtration, washing with a minimal quantity of alcohol, drying, and crystallization from 1, 4-dioxane, the obtained solids were refined to produce 4-substituted aniline analogues (3a-l) [22, 23].

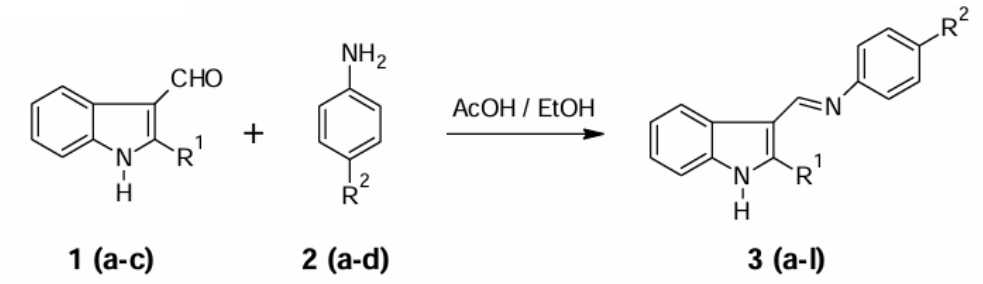

Figure 1: Steps 1 involved in the synthesis of dihydroimidazo indole complexes 5(a-l).

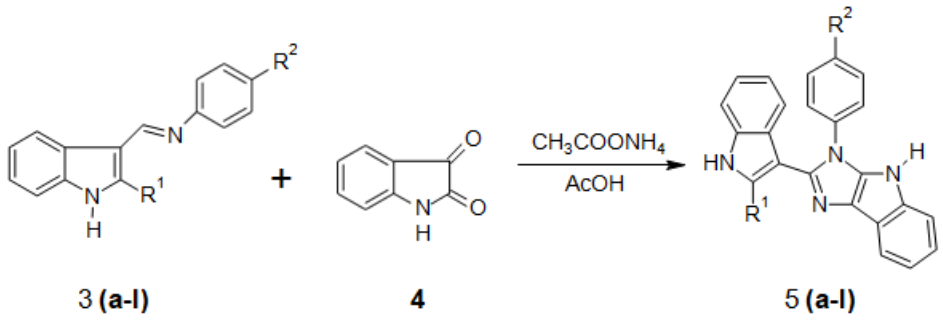

Figure 2: Steps 2 involved in the synthesis of dihydroimidazo indole complexes 5(a-l).

Evaluation of Antimicrobial activity

Antibacterial and antifungal activity

For the purpose of conducting in vitro biological screening of compounds against the bacteria S. aureus and E. coli, as well as the fungus A. niger and A. flavus, the cup-plate method was utilized. Nutritional agar was used as the medium. A sterile cork borer was used to carefully perforate holes with a diameter of 6 millimeters. After that, test solutions containing 5 and 10 milligrams per milliliter of DMF were added, with DMF serving as the control for the experiment. A total of 24 hours were spent incubating the plates at 37 degrees Celsius to test the antibacterial activity, and 72 hours were spent doing the same for the antifungal activity. The diameter of the inhibitory zone for each test compound was measured in order to make a comparison with the results obtained from conventional pharmaceuticals like Gentamycin, which has antibacterial action, and Nystatin, which has antifungal activity, both of which were administered at the same concentrations [24, 25]. A presentation of the findings may be found in Table 2.

Anti-tuberculosis activity:

With the intention of carrying out antituberculosis tests in vitro, a human virulent strain of Mycobacterium tuberculosis known as H37Rv was utilized. This testing was carried out by utilizing the dispersion culture technique in conjunction with Kirchner's media approach, which was treated with Tween-80. After heating the sterile normal bovine serum at a temperature of 56 degrees Celsius for a period of thirty minutes, 0.5 milliliters of the serum was added to 4.5 milliliters of the sterile Kichner dispersion medium that was contained within a borosilicate test tube that measured 150 millimeters by 20 millimeters. This was done in order to conduct the experiment. Following the dissolution of the compounds that were being examined in DMF, the final concentrations of 100, 50, 25, 12.5, 6.25, 3.12, and 1.56 µg/ml were eventually attained. Subsequently, the solution was added. A standard culture of M. tuberculosis (H37Rv) with 10^6 bacilli per milliliter was used to create the inoculum, which was 0.1 milliliters in volume. After an incubation period of eight days at 37 degrees Celsius, the tubes were analyzed to establish whether or not the organism under test had successfully multiplied. The endpoint was determined to be the lowest concentration at which there was no discernible increment in the growth of the organism. In order to conduct a comparison analysis, the data were compiled and presented in Table 1. Additionally, a conventional drug control tube containing streptomycin and a tube containing just DMF were also included in consideration. Each chemical that was put through its paces was evaluated, and the lowest inhibitory concentration was found [26, 27-30].

3. RESULTS AND DISCUSSION:

Using the approach that our group had previously presented to the general public, we were able to successfully synthesize the various Schiff bases, which are indicated by the letters 3a–l. The compounds (3a-l) created a greater number of

dihydroimidazo indole complexes than they had previously produced as a result of their interaction with isatin (4) and sodium acetate in acetic acid. The results of the synthesis of dihydroimidazoindole complexes (5a-l) were of an exceptionally high quality. There were two indole-NH functionalities and an imidazole-C=N- functionality that were responsible for the generation of absorption bands in the infrared spectra of compound 5a. At the frequencies of 1622, 3295, and 3302 cm-1, these bands were observed. A multiplet at 6.69-8.15 parts per million was observed in the 1H nuclear magnetic resonance (NMR) spectrum of the chemical 5a, along with two singlets at 10.31 and 10.51 parts per million. At the time, it was thought that the thirteen aromatic protons and the two indole NH protons were responsible for these, respectively. In the course of the analysis of the mass spectrum of chemical 5a, the molecular ion peak M+1, which is representative of the molecular weight of the chemical, was found to be located at 349, which is 72% of the total. The synthesis of compound 5a from compound 4a is evidenced by all of these elements of the link between the two compounds.

Table 1: Various design derivatives synthesis of dihydroimidazo indole complexes 5(a-l).

	a	**b**	**c**	**d**	**e**	**f**	**g**	**h**	**i**	**j**	**k**	**l**
R^1	-H	-H	-H	-H	$-CH_3$	$-CH_3$	$-CH_3$	$-CH_3$	$-C_6H_5$	$-C_6H_5$	$-C_6H_5$	$-C_6H_5$
R^2	-H	-Br	-Cl	$-OCH_3$	-H	-Br	-Cl	$-OCH_3$	-H	-Br	-Cl	$-OCH_3$

Step 2: General procedure synthesis of dihydroimidazo indole complexes (5a-l):

In a round-bottom flask that already contained glacial acetic acid and four substituted aniline analogues (3a-l), isatin and ammonium acetate were added to the mixture The charged compounds were then added to the flask, which was subsequently charged. The reaction mixture was refluxed and stirred continuously on a heating plate that was controlled by a thermostat for around ten to twelve hours. This process might take anywhere from ten to twelve hours. The utilization of thin-layer chromatography was done in order to guarantee that the reaction was carried out all the way through to its conclusion. The reaction mixture was moved into a beaker that contained 250 milliliters of water in order to make the process of creating dihydroimidazo indole complexes easier. This was done in order to remove acetic acid and ammonium acetate from the combination that was being used. Following this, the substance was filtered, dried in a furnace that utilized hot air, and then recrystallized with the assistance of ethyl acetate. All of these stages were performed in the subsequent phases. A compound that has a two-unit substituent and has a dihydroimidazo indole as its basic component.

Characterization of synthesized molecules:

Infrared (IR) Spectroscopy:

The IR spectra of the synthesized dihydroimidazo-indole complexes exhibit characteristic vibrational bands confirming the presence of key functional groups and their involvement in coordination. A strong absorption band around **3400–3200 cm^{-1}** corresponds to the **N–H stretching vibration**, which shows a slight shift compared to the free ligand, indicating possible involvement of the nitrogen atom in coordination with the metal center. The **C=N stretching** band, typically observed near **1600 cm^{-1}**, also shifts upon complexation, supporting metal coordination through the imine nitrogen. In the complexes, the appearance of new bands in the **500–600 cm^{-1}** region suggests **M–N** and **M–O** bond formation, further confirming successful metal–ligand complexation.

Nuclear Magnetic Resonance (NMR) Spectroscopy:

^{1}H NMR spectra of the ligands and their metal complexes reveal notable changes upon coordination. In the free ligand, signals corresponding to the aromatic protons of the indole ring typically appear in the **δ 6.5–8.0 ppm** range. Upon complexation, these signals exhibit downfield shifts due to the electron-withdrawing effect of the metal center. The singlet corresponding to the **NH proton** of the indole ring (around **δ 10.5–11.5 ppm**) either disappears or broadens significantly, implying its involvement in hydrogen bonding or metal coordination. Additionally, signals corresponding to aliphatic methylene protons in the dihydroimidazo moiety (around **δ 2.5–4.5 ppm**) also show slight shifts upon complexation.

^{13}C NMR spectra further support these findings, where the carbon atoms adjacent to the donor nitrogen show noticeable chemical shift variations, typically shifting downfield upon complexation, indicating deshielding due to coordination.

Mass Spectrometry (ESI-MS):

The ESI-MS spectra of the dihydroimidazo-indole complexes show molecular ion peaks consistent with their proposed molecular formulas. The appearance of **$[M+H]^+$** or **$[M+Na]^+$** peaks confirms the molecular weights of the complexes. Additionally, fragmentation patterns provide insight into the structural stability and possible decomposition pathways. The observed fragmentation peaks correlate with the loss of neutral ligand molecules or metal-associated fragments, confirming the ligand-to-metal stoichiometry.

Evaluation of Antimicrobial activity:

Evaluation of Antibacterial and antifungal activity:

Compounds 5b, 5c, 5g, 5j, and 5k were discovered to have extraordinarily high levels of activity against S. aureus, whereas compounds 5d, 5f, 5h, and 5l were found to have moderate levels of activity. These findings were made public in the scientific literature. When compared to the standard antibiotic Gentamycin, which was proven to be ineffective against S. aureus, this was a huge accomplishment. Contrary to compounds 5b, 5c, 5g, 5j, and 5k, which displayed outstanding effectiveness against E. coli, compounds 5f, 5h, and 5l demonstrated only moderate activity. These compounds were found to be effective against E. coli. By comparing the effectiveness of these compounds to that of the pharmaceutical that is generally regarded as the gold standard, which is gentamycin, we were able to arrive at this particular figure. In the fight against A. niger, compounds 5b, 5c, 5g, 5j, and 5k have demonstrated remarkable effectiveness. When compared to the conventional medication, Nystatin, which has shown just a moderate amount of action against the same pathogen, this is a significant difference. Compounds 5d, 5f, 5h, and 5l are shown to have a moderate level of action, according to scientific research. Compounds 5d, 5f, and 5h were found to have a moderate effect against A. flavus when they were tested against the conventional medication Nystatin. This was determined through the process of testing. After conducting the evaluation, this was the conclusion that was obtained. However, compounds 5b, 5c, 5g, 5j, and 5k have demonstrated remarkable antibacterial activity against A. flaus. This is due to the fact that these compounds possess antibacterial characteristics. In comparison to the bacteria that were the subject of the experiment, the antimicrobial activity of the remaining compounds was significantly lower. The data shown in Table 1 make it abundantly evident that the chemical that served as the control, N, N-dimethylformamide, did not exhibit any antibacterial activity when subjected to these conditions.

Evaluation of Anti-tuberculosis activity:

According to the findings of the study, chemicals 5b, 5c, 5g, 5j, and 5k have the ability to inhibit the growth of mycobacterium at a concentration of 6.5 µg/ml. During the course of the research, chemicals 5d, 5f, 5h, and 5l were investigated and compared to conventional medical practices. Streptomycin exposed the fact that the former had a level of effectiveness against M. tuberculosis that was somewhere in the middle. In terms of M. tuberculosis, the remaining compounds exhibited a lower level of efficiency than the compounds that were tested. Regular streptomycin demonstrated efficacy under these conditions when administered at a concentration of 6.25 mg/ml; however, the control drug N, N-dimethylformamide did not demonstrate any capacity to combat tuberculosis (Table 2).

Table 2: Zone of inhibition of synthesized compounds

Mol. No.	**Zone of inhibition in mm**								**Anti-TB activity in MIC (mg / ml)**
	Antibacterial activity				**Antifungal activity**				
	S. aureus		***E. coli***		***A. niger***		***A. flaus***		
	5 mg/ml	10 mg/ml	5 mg/ml	10 mg/ml	5 mg/ml	10 mg/ml	5 mg/ml	10 mg/ml	
5a	13	15	11	13	10	11	11	14	55
5b	16	19	18	19	19	20	18	19	7.25
5c	17	20	19	19	19	19	18	18	7.25
5d	13	16	14	15	15	14	16	17	15
5e	13	15	12	14	13	15	10	13	55
5f	16	17	14	16	14	16	15	17	15
5g	19	19	18	18	15	18	18	19	7.25
5h	15	17	16	17	14	16	17	17	15
5i	14	13	12	14	11	12	12	14	55
5j	18	19	19	18	16	18	18	19	7.25
5k	17	20	18	19	17	18	17	18	7.25

5l	16	17	15	16	14	17	13	15	15
GTN	17	19	17	18	-	-	-	-	-
NST	-	-	-	-	18	19	17	19	-
STM	-	-	-	-	-	-	-	-	7.25
DMF	-	-	-	-	-	-	-	-	-

GTN: Gentamycin,

NST: Nystatin,

STM: Streptomycin,

DMF: Dimethyl formamide (Control)

4. CONCLUSIONS

The objective of synthesizing novel chemicals 3-Phenyl (4-substituted) A 2-substituted variant of indole analogues was synthesized following the procedures outlined in Scheme I to produce dihydroimidazoindole molecules (5a-l). Their systems are novel and unprecedented, and their reactions are direct and uncomplicated to execute under standard reaction conditions. The antibacterial and antifungal efficacy of all freshly synthesized compounds (5a–l) was assessed against S. aureus, E. coli, A. niger, and A. flavus. In comparison to the standards Gentamycin and Nystatin, administered at the same quantities (5 and 10 mg/ml in DMF) as the test compounds, compounds 5b, 5c, 5g, 5j, and 5k showed significant efficacy against the microorganisms evaluated. The antituberculosis efficacy of the recently synthesized compounds (5a–l) against M. tuberculosis was assessed. In comparison to the conventional medicine streptomycin, compounds 5b, 5c, 5g, 5j, and 5k demonstrate significant efficacy.

DECLARATIONS:

Ethics approval and consent to participate:

Not applicable.

Consent for publication:

All the authors approved the manuscript for publication.

Availability of data and material:

All required data is available.

Competing interests:

All authors declare no competing interests.

Funding:

Not applicable.

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