

One-Pot Synthesis of Novel Imidazoline Amine Derivatives and Evaluation of their Anti Microbial Activity

Mudhurukolla Sudha¹, Payyaula Naresh², Kandhuri Srinidhi³

Assistant Professor¹ in Department of Pharmaceutical Chemistry, Avanthi Institute of Pharmaceutical Sciences, Gunthapally, Hyderabad, RR district

Email: [sudhamudhurukoll\[at\]gmail.com](mailto:sudhamudhurukoll[at]gmail.com)

Assistant Professor² in Department of Pharmaceutical Chemistry, Siddhartha Institute of Pharmacy, Narapally, Korremula, Hyderabad

Email: [nanipayyaula76\[at\]gmail.com](mailto:nanipayyaula76[at]gmail.com)

³Drug Safety Associate in Orcimed Life Sciences, Jubile Hills, Hyderabad

Email: [kandhuri.srinidhi\[at\]gmail.com](mailto:kandhuri.srinidhi[at]gmail.com)

Abstract: Infections are one of the major reasons for mortality. They cause one fourth of the deaths throughout the world and half of the deaths in developing countries. Since most of the current antibiotics got resistance towards the pathogens, the need for the development of novel antimicrobial agents became a vital task in the medicinal chemistry research. Current study focused on the synthesis and antimicrobial activity evaluation of the novel imidazole derivatives. The synthesis involves a simple one pot reaction between the aryl aldehyde, propane-1,2,3-triamine in the presence of NaI and aq 30% H₂O₂. The products were purified by column chromatography on silica gel using EtOAc–Hexane (10:1) as eluent. All the synthesized compounds were analysed by IR, NMR and Mass Spectral studies. The designed synthetic scheme delivered the targeted imidazoline amine hybrids in good yields. Analytical characterization with spectral techniques confirmed the structure of the synthesized compounds. Antimicrobial assay employed two gram-positive bacteria: *S. aureus* and *B. subtilis*; two gram-negative bacteria: *P. aeruginosa*, *E. coli* and two fungal strains - *Aspergillus niger* and *Candida albicans*. The agar plate disk diffusion method was used for the antimicrobial assay. The results reveal that all the compounds demonstrated potent antibacterial, antifungal properties especially the compounds 3d and 3e are more potent among the tested compounds.

Keywords: CDCl₃, DMSO, LC-MS, PPM, SEM, TMS

1. Introduction

Microbial infections and antimicrobial agents

Infections are one of the major reasons for mortality. They cause one fourth of the deaths throughout the world and half of the deaths in developing countries. Majority of the infants' deaths are because of infections only. Among the infectious diseases, majority of them are related to vital systems and organs of the body like respiratory tract. Because of the improper usage of antimicrobial agents, most of the developed countries have got resistance to the currently available antimicrobial agents. Majority of the infections caused by *Streptococcus pneumoniae*, *Haemophilus*

influenzae, and *Moraxella catarrhalis*, have got resistance to few antibiotic classes. *S. pneumoniae* which causes respiratory infections developed resistance most of the antibiotics. Because of these resistance, it is very difficult to estimate the exact treatment outcomes by using antimicrobial agents.

There are several categories of antibiotics currently in the market to kill bacteria which are depicted in the Figure 1.1. Antibiotics that act on the cell wall synthesis, nucleic acid synthesis and protein synthesis are the major class of antibiotics.

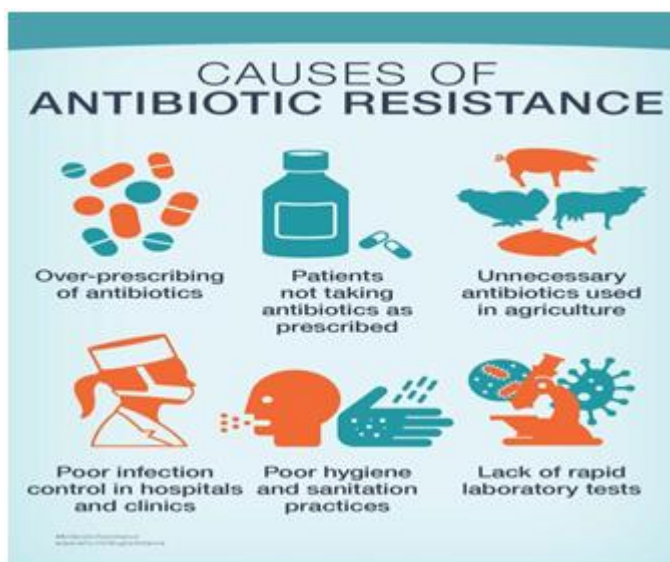
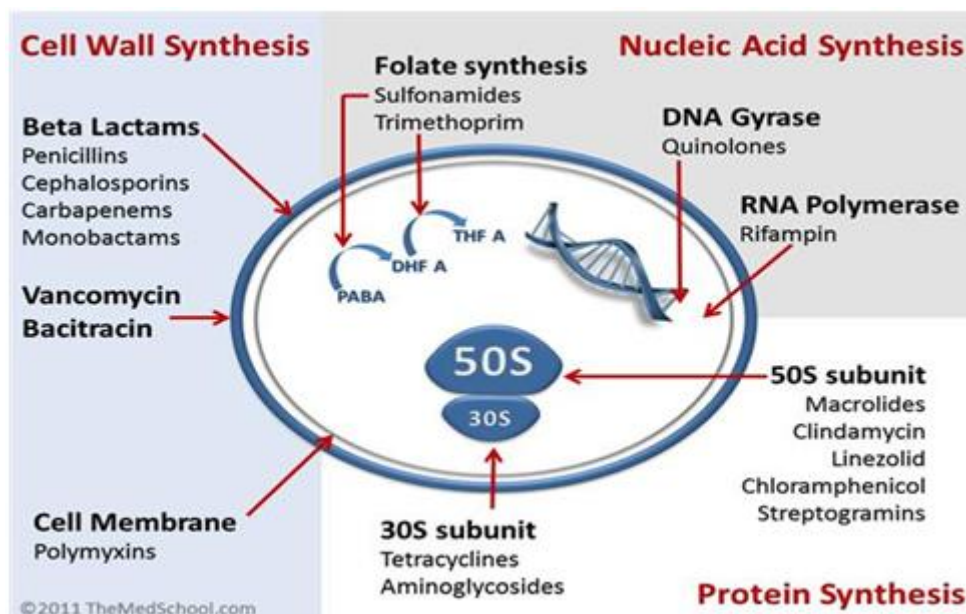


Figure 1: Classification of antibiotics based on their mechanism of action and causes

1.2 Imidazoles

Imidazoles have occupied a unique position in heterocyclic chemistry, and its derivatives have attracted considerable

interests in recent years for their versatile properties in chemistry and pharmacology. Imidazole is nitrogen-containing heterocyclic ring which possesses biological and pharmaceutical importance. Thus, imidazole compounds have been an interesting source for researchers for more than a century¹.

These ring systems are key components of structural scaffolds occurring in modern medicinal chemistry, thus forming critical building blocks for new drug design. Compounds containing an imidazole ring display a wide range of pharmacological activities.

Imidazole is a 5-membered planar ring, which is soluble in water and other polar solvents. It exists in two equivalent tautomeric forms because the hydrogen atom can be located on either of the two nitrogen atoms. Imidazole is a highly polar compound, as evidenced by a calculated dipole of 3.61D, and is entirely soluble in water. Imidazole is amphoteric; that is, it can function as both an acid and a base. The compound is classified as aromatic due to the presence of a sextet of π -electrons, consisting of a pair of electrons from the protonated nitrogen atom and one from each of the remaining four atoms of the ring.

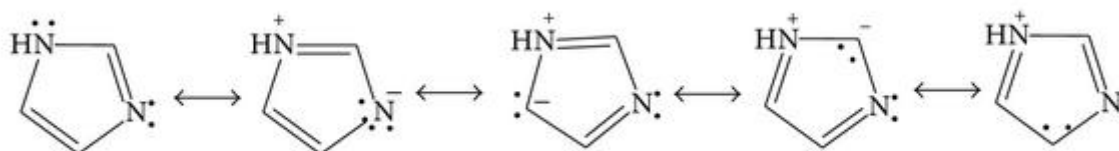


Figure 2: Resonance structures of imidazole

1.2.1 Imidazole Containing Antibacterial Agents

The 4, 5-diphenylimidazoles were identified as a class of potential candidates for optimisation as focused spectrum antibiotics during a high throughput screening campaign designed to detect compounds with activity at bacterial cell wall biosynthesis level [2]. Compound (1) (Fig. 1) is the most potent example of a recent series of

4,5-diphenylimidazoles bearing lipophilic electron withdrawing groups on the aryl rings and an electron withdrawing trifluoromethyl group in the 2-position. Its minimum inhibitory concentration (MIC) is 0.25 $\mu\text{g}/\text{mL}$ for Methicillin Resistant *Staphylococcus aureus*, 4 $\mu\text{g}/\text{mL}$ for MIC *Bacillus subtilis*, greater than 32 $\mu\text{g}/\text{mL}$ for MIC

Escherichia coli permeable mutant, and 160.25 µg/mL for MIC Escherichia coli permeable mutant + polymix.

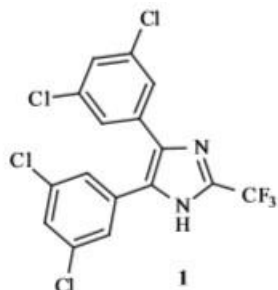


Figure 3.1: The structure of 4,5-bis(3,5-dichlorophenyl)-2(trifluoromethyl)-1H-imidazole

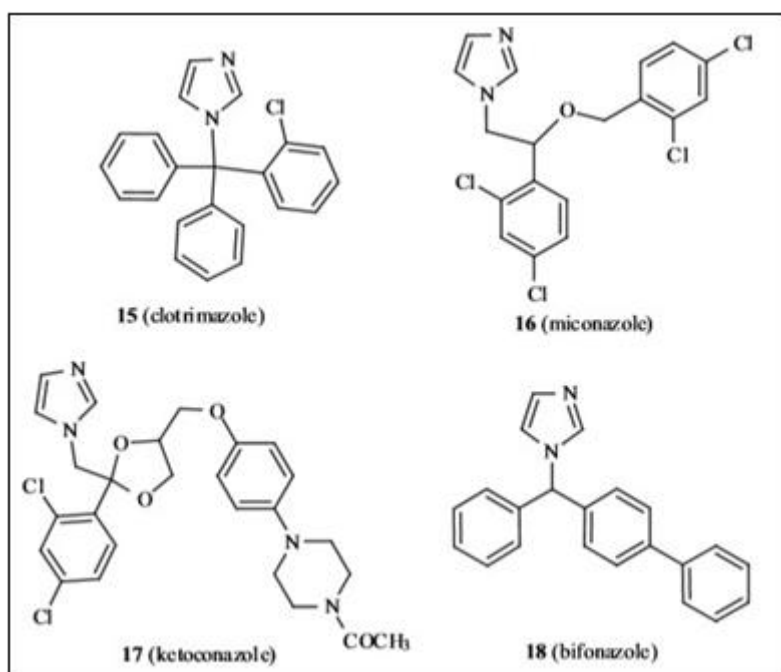


Figure 3.2: The structures of imidazole antifungal agents used in clinical practice

Imidazole nucleus forms the main structure of some well-known components of human organisms, that is, the amino acid histidine, Vit-B12, a component of DNA base structure and purines, histamine, and biotin. It is also present in the structure of many natural or synthetic drug molecules, that is, cimetidine, azomycin, and metronidazole. Imidazole-containing drugs have a broader scope in remedying various dispositions in clinical medicine³.

The imidazole ring is a constituent of several important natural products, including purine, histamine, histidine, and nucleic acid. Being a polar and ionisable aromatic compound, it improves pharmacokinetic characteristics of lead molecules and thus is used as a remedy to optimize solubility and bioavailability parameters of proposed poorly soluble lead molecules. There are several methods used for the synthesis of imidazole-containing compounds, and also their various structure reactions offer enormous scope in the field of medicinal chemistry. The imidazole derivatives possess extensive spectrum of biological activities such as

- Antibacterial,
- Anticancer,
- Anti-tubercular,

1.2.2. Imidazole Containing Antifungal Agents

The imidazoles are one class of antifungal azole derivatives, which have been shown to have a broad spectrum of antifungal activities both in vitro and in vivo. Since the identification of clotrimazole in 1972 a number of antifungal imidazole agents have been studied and are now used in clinical practice: miconazole and related derivatives, ketoconazole and bifonazole².

Figure 7: The structures of imidazole antifungal agents used in clinical practice.

- Antifungal,
- Analgesic, and
- Anti-hiv activities⁴.

2. Materials and Methodology

2.1 Chemicals

Synthetic grade (LR) chemicals used for the synthesis were purchased from sigma Aldrich, Bangalore, and SD fine chem ltd, Hyderabad. Silica gel 60 F254 grade coated aluminium TLC Plates were used for reaction monitoring. Spots visualized using Silica gel of mesh size 60/120 was used for the column chromatography to isolate the pure compounds.

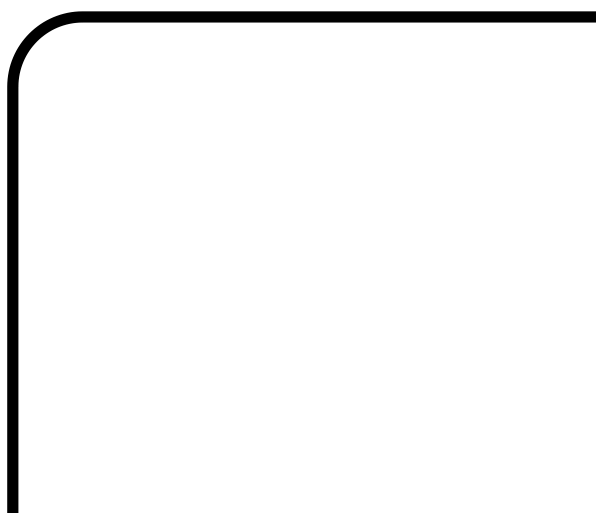
2.1.1 Instruments specifications

S.No	Instrument	Make
1	NMR – 400hz	Bruker AVANCE CORE
2	Mass – ESI -MS	Bruker MRMS
3	IR	Agilent Cary 630
4	Melting point apparatus	Remi-2000

2.2 Methodology

2.2.1 Synthesis

The scheme of synthesis for the designed imidazoline derivatives was depicted below in scheme.



Synthesis of Imidazole amine derivatives

In a 50ml round bottomed flask filled with 10ml of t-BuOH, 1 mmol of various substituted aryl aldehydes (1a-1j) were dissolved, then the solution is mixed with propane-1,2,3-triamine (1.1 mmol), and stirred for t-BuOH (10 mL) was stirred for 20 mins. To the above reaction mixture 0.4mmol of sodium iodide (NaI) and 0.5grams of anhydrous (MgSO₄) were added with continuous stirring and the temperature was maintained at 80°C. During the continuous heating and stirring 30% H₂O₂ solution was added dropwise from the addition funnel until the color of iodine not discharged. Reaction was monitored by TLC and once the reaction completed the reaction mass quenched with sodium thiosulfate solution (aq Na₂S₂O₄) work up procedure, then precipitate was collected by filtration. The crude precipitate was purified by column chromatography using EtOAc–Hexane (1:9) as eluent¹⁹.

3.2.2 Antimicrobial Activity

Microorganisms:

The department of Botany, Osmania University provided the four bacterial strains (Gram-positive bacteria: *B. subtilis* and *S. aureus*; Gram-negative bacteria: *E. coli* and *P. aeruginosa*) and two fungal (*Aspergillus niger* and *Candida albicans*) strains.

Preparation of stock solution:

A stock solution of (60µg/ml) for all the prepared compounds (3a-3j) is made by using DMSO.

Preparation of inocula:

Cultivation of gram-positive, gram-negative strains and fungi is maintained at 4°C on nutrient agar. Before starting the experiment, stock cultures were inoculated via a culture-filled ring into 8 ml of medium and incubated for 24 hours at 37°C for bacterial culture and 28°C for fungal culture. The cell density was adjusted to 0.5 Mc Farland standards. A final amount of 10⁵ CFU/ml was applied in the experiment.

Antibacterial assay:

The medium was prepared with the dissolution of meat extract, peptone, and sodium chloride in distilled water then the pH was adjusted to 7.2. The agar was dissolved and dispensed in 40 ml portions into 100 ml flasks and autoclaved at 121°C (15 lbs/sq.in) for 20 minutes. Eighteen-hour-old cultures of the above bacteria were inoculated into the medium at a 1% level and transferred to sterile Petri dishes 15 cm in diameter. The medium plate was placed at room temperature for 30 minutes. A 6 mm diameter hole was made using a sterile driller to prepare the cup agar plates. The solution of test compounds was placed in the cups through sterile pipettes. A cup was used for the control, with

2 drops (0.05 ml) of neomycin sulfate DMSO at a concentration of 10 µg/ml as a standard in each plate. The plate was incubated for 1 hour at room temperature for diffusion. The plates were then incubated for 24 hours at 37°C and the zone of inhibition was recorded. Experiments were performed in duplicate, and the mean diameter of inhibition zones was recorded and noted.

Antifungal assay:

The prepared compounds were also screened for their antifungal activity at 60µg/ml by agar plate method using the above protocols. Standard Nystatin and DMSO served as a positive and negative control, respectively. The zone of inhibition measured in mm was taken to evaluate antifungal activity.

Cut the peeled potatoes into small pieces and boil them in 200 ml of water for 30 minutes. During boiling, the chips are crushed and strained through cheesecloth to remove the pulp after cooling. Dextrose and agar were added while slowly stirring to 1000ml. Nystatin and DMSO at a concentration of 10 µg/ml were taken as sample and control respectively. After 48 hours of incubation at room temperature (30°C), plates were examined and zone diameters were recorded.

3. Results

Spectral Data

(2-phenyl-4,5-dihydro-1H-imidazol-4-yl) methanamine

3a:

¹H NMR: δ 2.91-3.02(2H, 2.96 (d, *J* = 3.3 Hz), 2.96(d, *J* = 3.3 Hz)), 3.38-3.61(2H, 3.46(dd, *J* = 13.5, 4.3 Hz), 3.53(dd, *J* = 13.5, 8.1 Hz)), 4.12(1H, ddt, *J* = 8.1, 4.3, 3.3 Hz), 7.37-7.53(3H, 7.44(dddd, *J* = 8.6, 7.7, 1.3, 0.4 Hz), 7.47(tt, *J* = 7.7, 1.4 Hz)), 8.29(2H, dtd, *J* = 8.6, 1.4, 0.4 Hz).

¹³C NMR: δ 40.6(1C, s), 52.9(1C, s), 56.6(1C, s), 127.1(2C, s), 127.8(1C, s), 128.4(2C, s), 131.8 (1C, s), 158.8 (1C, s).

ESI-MS: For C₁₀H₁₃N₃([M + H]⁺): 175.10, found m/z: 176.05.

IR (KBr):*v*_{max} in cm⁻¹: 1605.5(C=N), 1645.5(c=o), 3280.1(NH), 3060.3(=C-H), 1290.5(C-N),1540.3(C=C).

(2-(p-tolyl)-4,5-dihydro-1H-imidazol-4-yl) methanamine

¹H NMR: δ 2.27(3H, s), 2.91-3.02(2H, 2.96 (d, *J* = 3.2 Hz), 2.96(d, *J* = 3.2 Hz)), 3.42-3.57(2H, 3.49 (dd, *J* = 13.5, 8.1 Hz), 3.50(dd, *J* = 13.5, 4.3 Hz)), 4.19(1H, ddt, *J* = 8.1, 4.3, 3.2 Hz), 7.21(2H, ddd, *J* = 8.2, 1.3, 0.5 Hz), 7.65(2H, ddd, *J* = 8.2, 1.7, 0.5 Hz).

¹³C NMR: δ 21.3(1C, s), 40.6(1C, s), 52.9(1C, s), 56.6(1C, s), 127.3(2C, s), 129.1(2C, s), 131.8(1C, s), 141.5(1C, s), 158.8(1C, s).

ESI-MS: For C₁₁H₁₅N₃([M + H]⁺): 189.26, found m/z: 190.20.

IR (KBr): *v*_{max} in cm⁻¹: 1605.5(C=N), 1645.5(c=o), 3285.1(NH), 3040.5(=C-H), 1296.0(C-N),1525.3(C=C), 2895.4(C - H).

(2-(4-methoxyphenyl)-4,5-dihydro-1H-imidazol-4-yl) methanamine

3c:

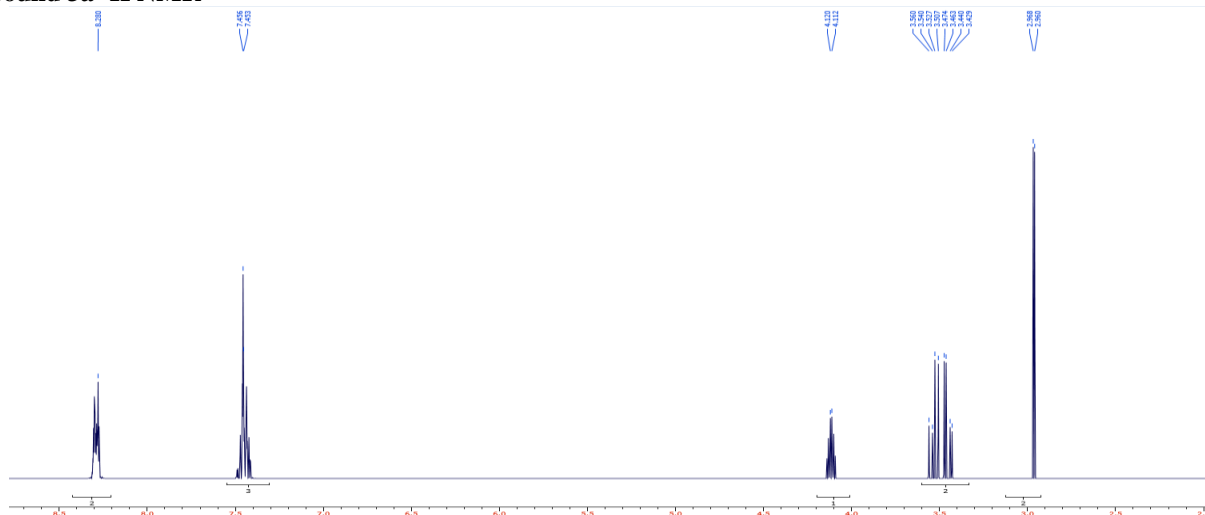
¹H NMR: δ 2.91-3.02(2H, 2.96(d, *J* = 3.3 Hz), 2.96(d, *J* = 3.3 Hz)), 3.35-3.57(2H, 3.42 (dd, *J* = 13.5, 4.3 Hz), 3.50(dd, *J* = 13.5, 8.1 Hz)), 3.86(3H, s), 4.23(1H, ddt, *J* = 8.1, 4.3, 3.3 Hz), 6.99(2H, ddd, *J* = 8.3, 1.1, 0.5 Hz), 7.55(2H, ddd, *J* = 8.3, 1.8, 0.5 Hz).

¹³C NMR: δ 40.6(1C, s), 52.9(1C, s), 56.0(1C, s), 56.6(1C, s), 114.3(2C, s), 128.9(2C, s), 131.8(1C, s), 158.8(1C, s), 159.8(1C, s).

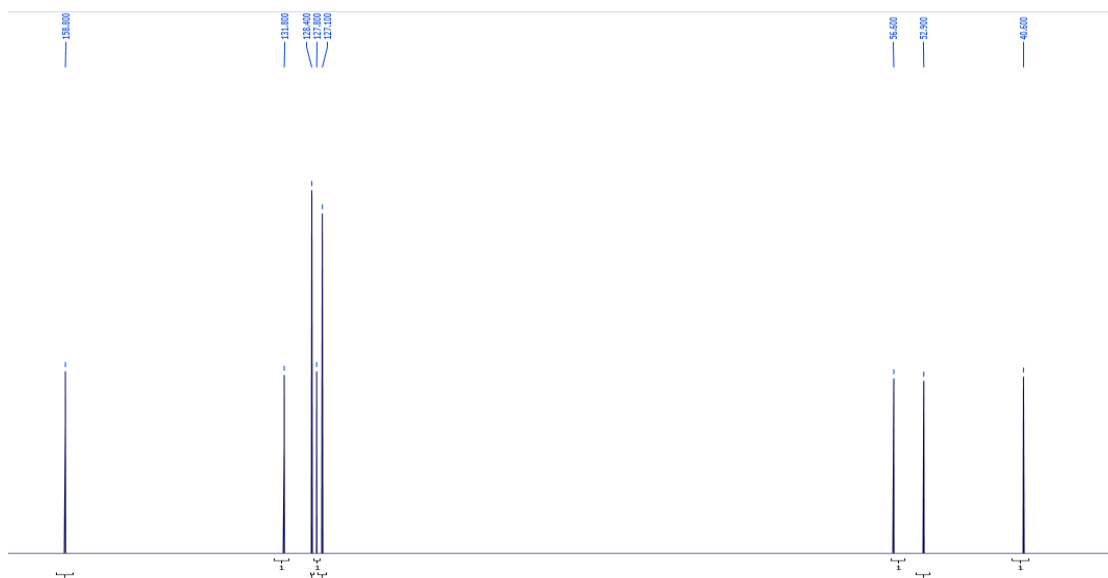
ESI-MS: For C₁₁H₁₅N₃O([M + H]⁺): 205.26, found m/z: 206.15.

IR (KBr): *v*_{max} in cm⁻¹: 1603.5(C=N), 1645.5(c=o), 3282.1(NH), 3050.5(=C-H), 1292.0(C-N),1530.3(C=C), 1090.5(C - O).

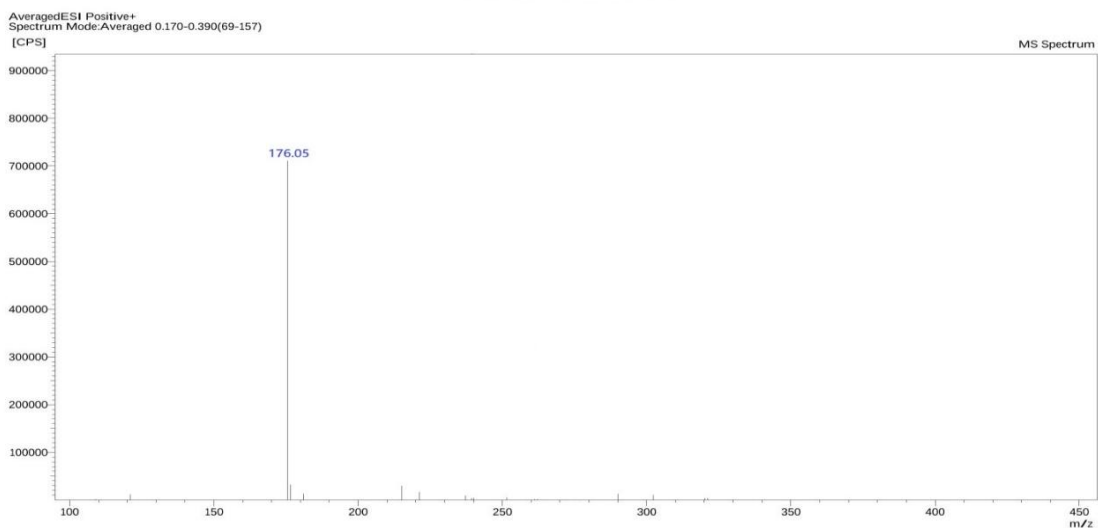
Compound 3a ¹H NMR



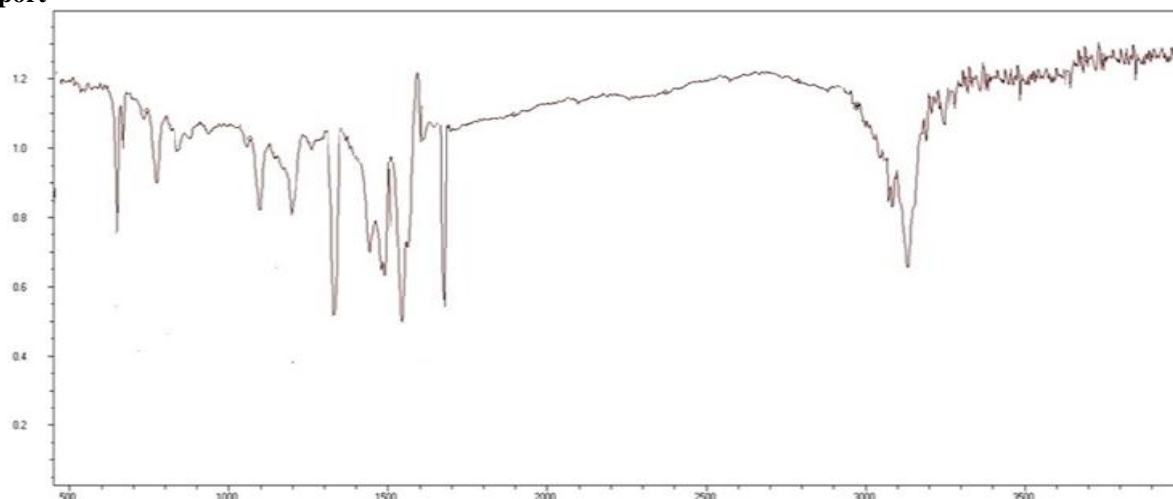
C¹³ NMR



MASS REPORT



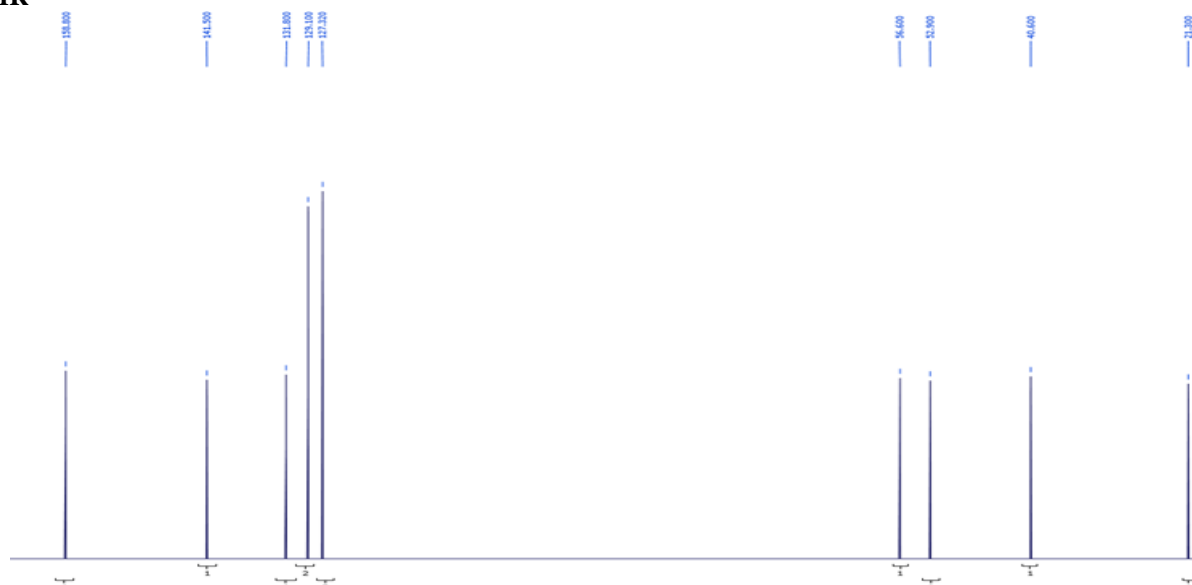
IR Report



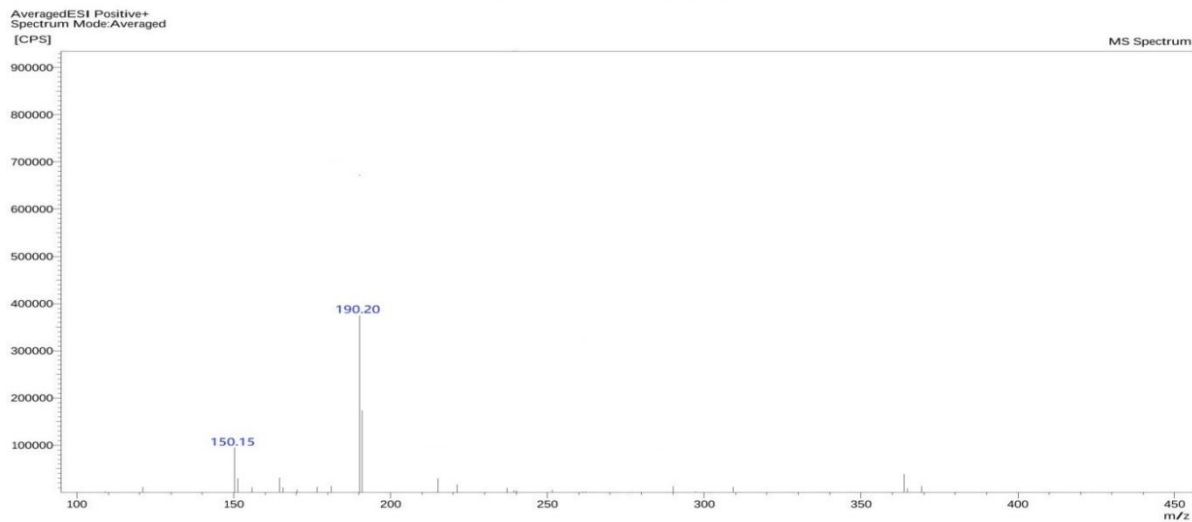
Compound 3b ¹H NMR



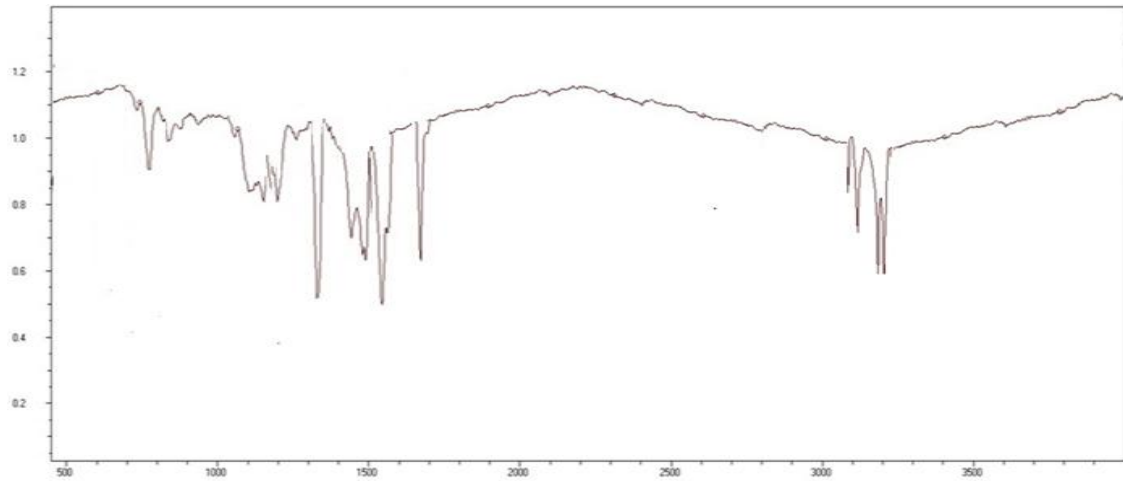
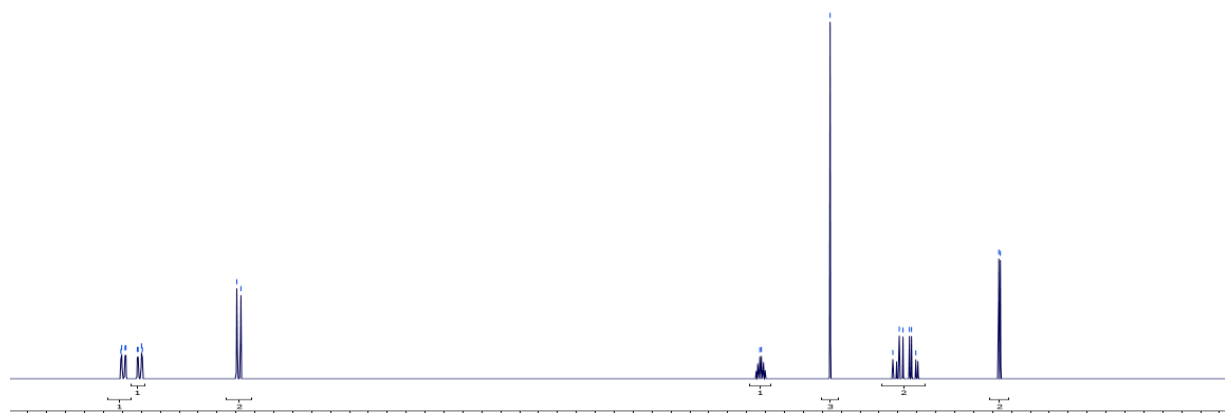
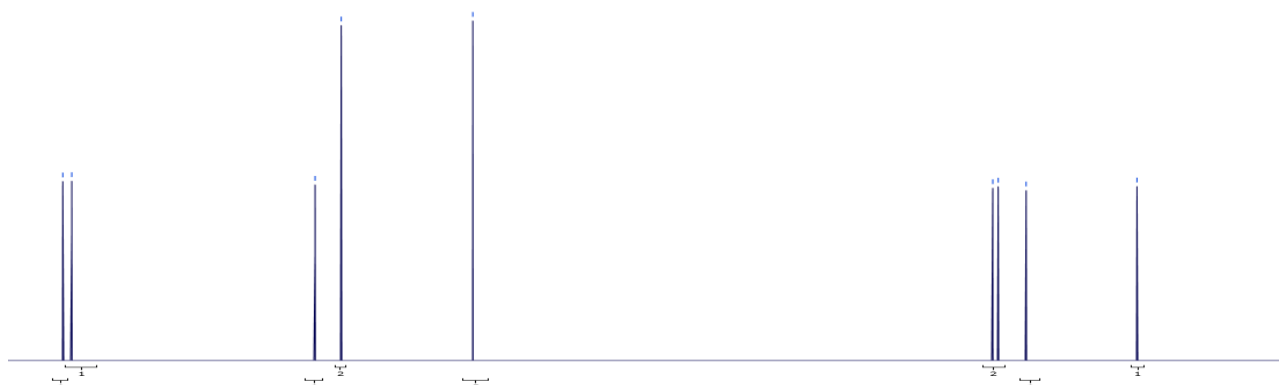
¹³C NMR



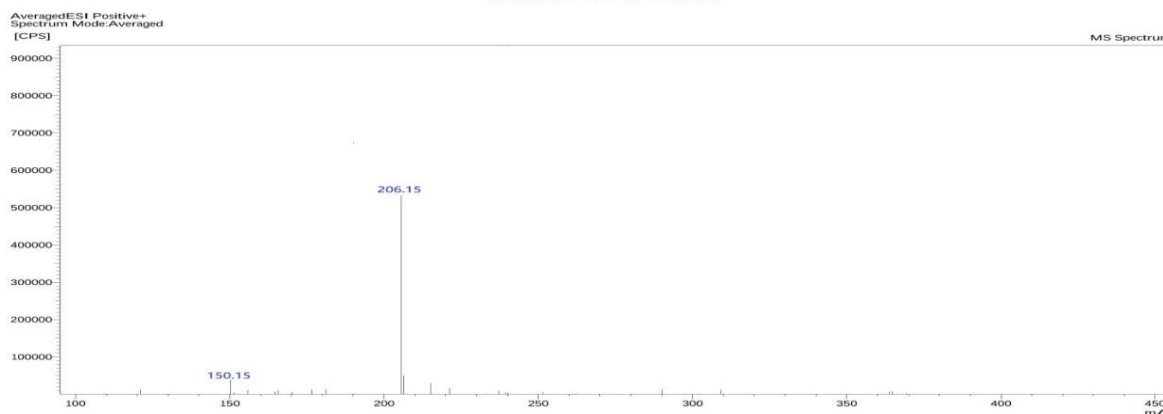
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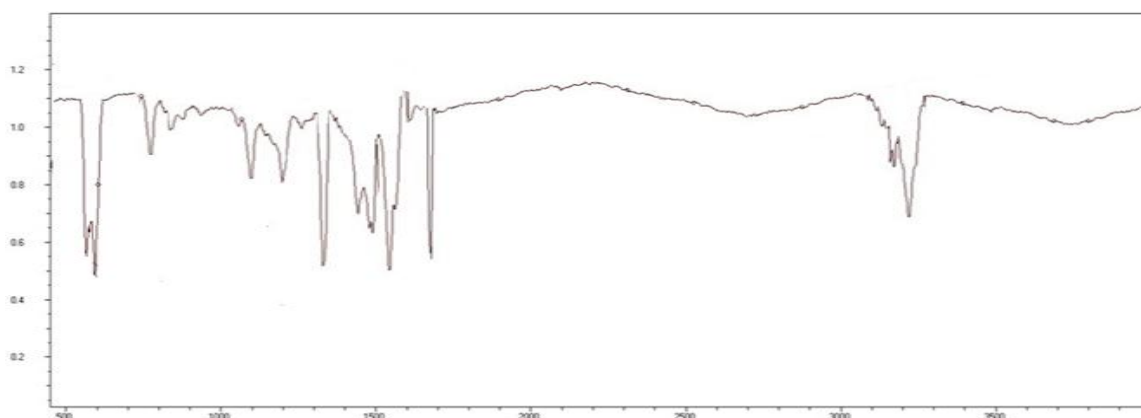
IR Report

Compound 3c ^1H NMR C^{13} NMR

MASS REPORT



IR Report



Antimicrobial activity

All the synthesized five novel series of title compounds (**3a-3j**) were evaluated for their antibacterial activity in vitro against Gram-positive bacteria: *B. subtilis*, *S. aureus*; Gram-negative bacteria: *E. coli*, *P. aeruginosa*, and antifungal activity against *Aspergillus niger* and *C. albicans*. The results of the antimicrobial assay of the tested compounds revealed the antimicrobial potential of the all compounds. (Table 4.2). Among the tested compounds, the compounds possessing electron withdrawing groups -F, -Cl such as **3d** and **3e** are showing comparatively higher inhibition to both gram-positive and gram-negative bacteria relative to remaining compounds.

Table 4.4.1: Antibacterial activity of the imidazoline amines (3a-3j)

Test Compound	Zone of inhibition of imidazoline amines (60µg/ml) in mm			
	<i>B. subtilis</i>	<i>S. aureus</i>	<i>E. coli</i>	<i>P. aeruginosa</i>
<i>3a</i>	14	16	11	14
<i>3b</i>	13	14	14	14
<i>3c</i>	15	15	13	12
<i>3d</i>	18	19	17	18
<i>3e</i>	20	21	19	18
<i>3f</i>	13	14	12	13
<i>3g</i>	13	12	12	11
<i>3h</i>	12	13	13	10
<i>3i</i>	10	12	09	08
<i>3j</i>	12	12	10	10
Solvent Control (DMSO)	4	3	3	3
Neomycin sulphate (10µg/ml)	24	23	22	20

4. Conclusion

The designed synthetic scheme delivered the targeted imidazoline hybrids in good yields. Synthesis of targeted imidazoline derivatives accomplished through a single step condensation reaction between aryl aldehyde and propane-1,2,3-triamine. The reactions were carefully monitored by TLC. All the synthesized derivatives have been purified by column chromatography and re-crystallized with ethanol. The thorough spectral analysis revealed the structures of the synthesized compounds. These synthesized substances were examined for their potential antibacterial and antifungal effects. The findings show that every chemical examined exhibited strong antibacterial and antifungal characteristics, but the compounds **3d** and **3e** were particularly effective. Further studies are need to establish the mechanism of these developed imidazoline derivatives.

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