CHAPTER-3

Synthesis of Imidazole-fused nitrogen-bridgehead heterocycles catalysed by lipase and their antifungal and anti-microbial bioactivity

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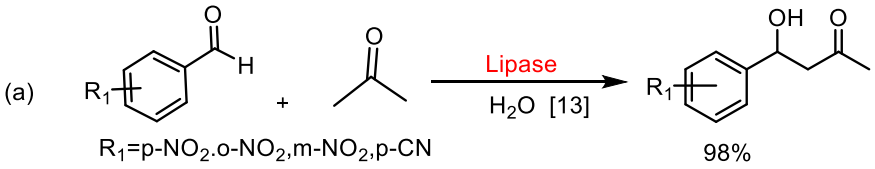
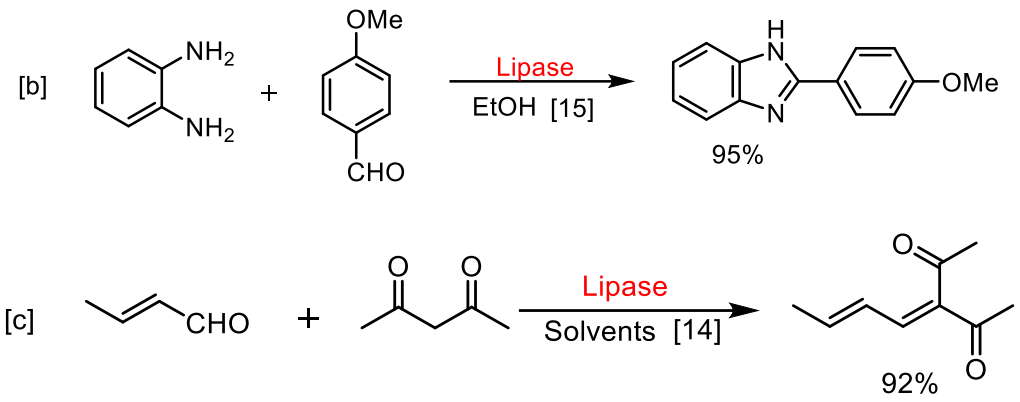
3.1 Introduction

In recent years, many efforts have been devoted to developing new synthetic strategies using enzymatic catalysts and have received much attention, especially in synthesizing heterocyclic compounds in synthetic organic chemistry [1-5]. Lipases are pervasive enzymes that are found in all living organisms. It is essential in food, flavour, beverages, biodiesel production, and the biopolymers industry. Lipase enzymes have many attractive applications, such as stability in organic solvents, catalytic activity without cofactors, broad substrate scope, and chemo, regio- and stereo-selectivity, making them the most versatile class of enzymes in organic synthesis [6-12]. Lipase as a catalyst has been successfully applied in nucleophilic substitution reactions like Aldol reaction [13], Knoevenagel condensation [14-15], Mannich reaction [16], Henry reaction [17], Morita– Baylis–Hillman reaction [18], Michael addition [19], Aza-Diels-Alder reaction [20] and few organic syntheses catalyzed by lipase reactions are shown in **Scheme 3.1(a-c)** which show catalytic activity.





Scheme 3.1 Few lipases catalysed reactions.

In this view, lipase is suitable for use in the nucleophilic substitution reaction described above and the bio-catalytic properties of lipase encouraged us to exploit the development of desired product. Various methods for synthesizing this targeted moiety have been published, owing to their diverse uses. Nitrogen-containing heterocyclic rings are found in many natural products and have many biological activities. Imidazo [1, 2-a] pyridines, imidazo [1, 2-a] pyrimidines, and imidazo [1, 2-a] pyrazines are a few essential core structures of this class that have many pharmacological activities [21-26]. For example, imidazo [1, 2-a] pyridine moiety which is found in some drugs like alpidem [27], necopidem, seripidem [28] (anxiolytics agent), zolimidine (gastroprotective agent) [29], zolpidem (hypnotic) [30], olprinone (cardiotonic agent) [31], GSK812397 (anti-HIV) [32], and rifaximinan [33-34] antibiotic used to prevent hepatic encephalopathy. Recently, imidazopyridine molecules have also been used as anti-inflammatory, anti-ulcerative, analgesic, anti-bronchospastic, and antiproliferative agents [35-38] (**Figure 3.1**). Moreover, these derivatives have remarkable applications in material science, polymer and dye synthesis [39], and also found pervasive

application in fluorescence [40], chemo sensing [41], crystal engineering [42], and corrosion science [43].

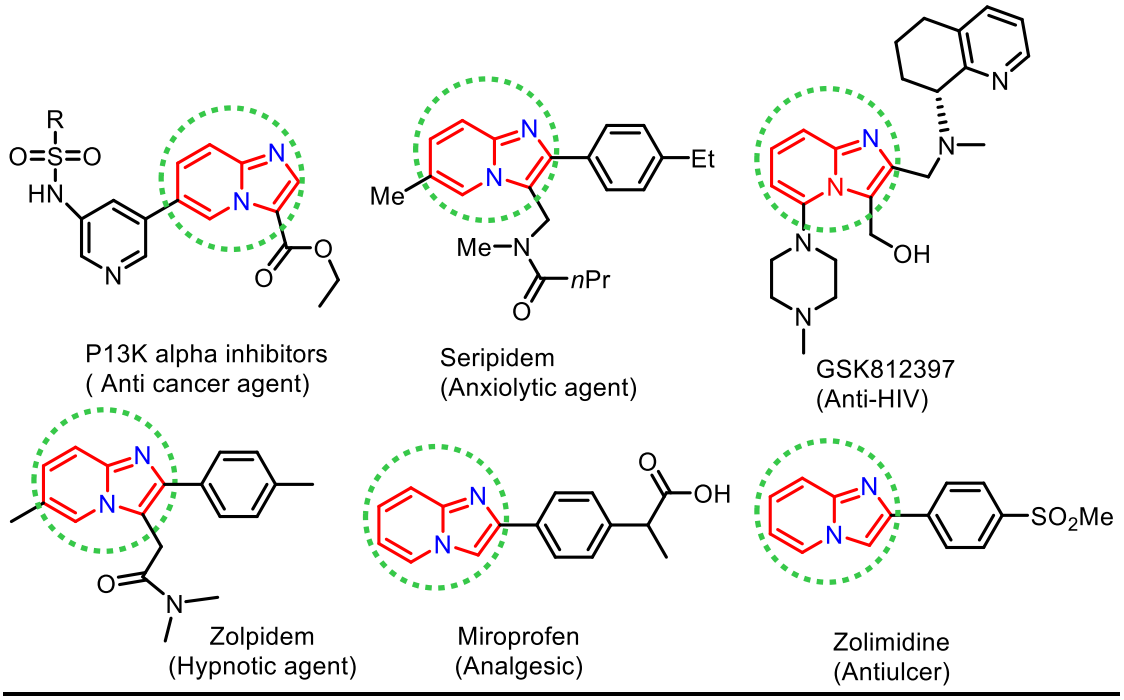


Figure 3.1 Biologically active imidazo [1, 2-a] pyridines

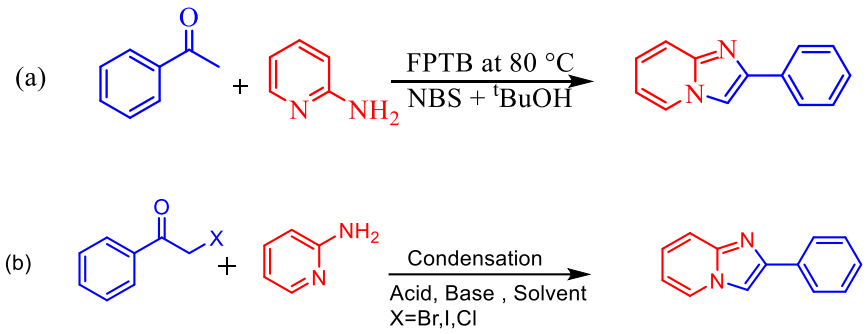
Therefore, numerous methods have been established for the synthesis of imidazo[1,2- a] pyridines scaffold by the reaction of 2-aminopyridine with numerous substrates such as methyl aryl ketone [44], 2-halo ketones [45]**,** alkynes derivatives [46]. These reactions generally perform in the presence of Lewis acid and base (**Scheme 3.2A**). Stasyuk *et al*; reported the iodine-mediated synthesis of imidazo[1,2-a] pyridine by the reaction of 2-aminopyridine and aryl methyl ketones in the presence of a base [47]. Synthesis of imidazo[1,2-a] pyridine has been reported by multicomponent reaction of 2-aminopyridines,

isonitrile and aldehydes, also known as Groebke-Blackburn-Bienayme reaction [48-52]. Vanya Kurteva et al. recently published a review article where they mentioned various methods that have already been reported for the synthesis of imidazole-fused nitrogen-bridgehead heterocycle scaffolds. These methods start from phenacyl bromide and 2-aminopyridine as substrates under different conditions such as DMF (Kwong et al.), ethanol (Liu et al.), t-butyl hydroperoxide (TBHP), $NaHCO_3$ (Rodriǵ uez et al.), and an ecologically favorable solventless grindstone procedure (GSP) performed at room temperature in a solvent-free condition (Godugu, K. et al.) (**Scheme3.2A)** [53 a-d]. Phenyl imidazole work found in the presence of earlier catalysts such as Ag_2CO_3, $FeCl_3$, Cu, and NBS to give which is a toxic metal-based catalyst given product using nucleophilic substitution reaction to perform this simple nucleophilic substitution reaction a metal-free and green biocatalyst is required without comprising the yield of the product. These approaches are appropriate for various substrates but have shortcomings, such as metal catalysts, acid/base, low yield, and tedious workup procedures. While most of these techniques have drawbacks, such as the employment of severe reaction conditions, high temperatures, expensive and toxic catalysts, long reaction times, and time-consuming work-ups, they all have certain disadvantages.

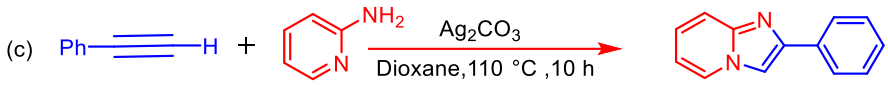
Based on the above conclusion, phenacyl bromide and 2-aminopyridine as substrates and ethanol as reaction medium are suitable for the synthesis of imidazo [1, 2-a], pyridines, which encouraged us to exploit the development of the desired product. Herein, we have synthesised imidazole-fused nitrogen-bridgehead heterocycles.

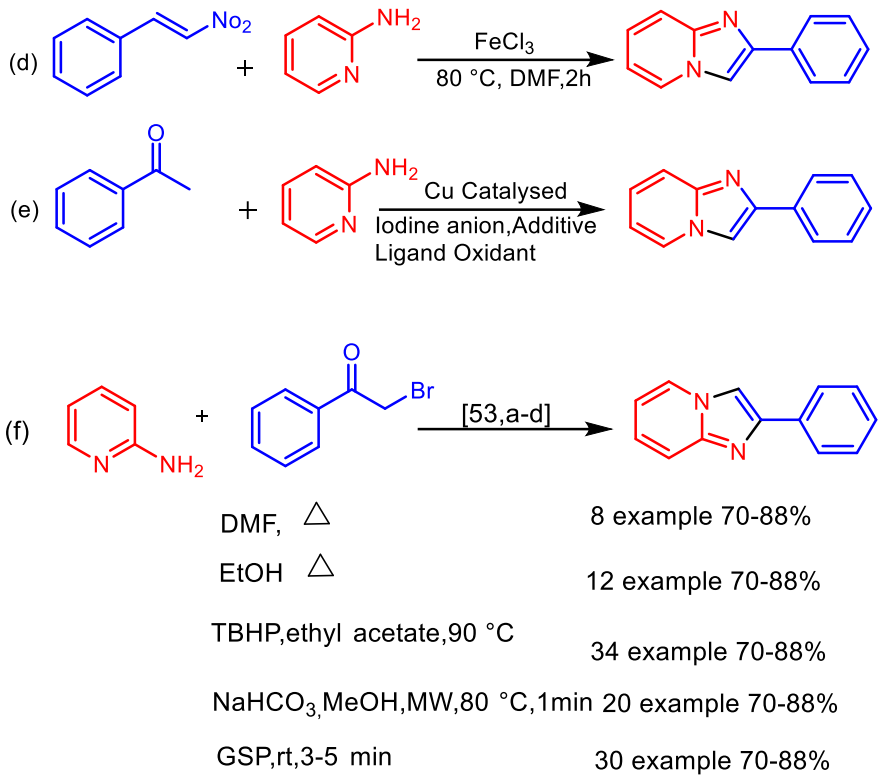
As a result, we devised a more practical and efficient synthetic procedure, a one-pot synthesis of imidazole-fused nitrogen-bridgehead heterocycles in ethanol as a reaction medium at 30 °C utilising a biocatalyst lipase enzyme (**Scheme 3.2B**). The present work describes the design, synthesis, physicochemical characterization and biological activity of 2-phenyl imidazo[1, 2-a] pyridine. The antimicrobial and antifungal activities of the **(3ha, 3ka, 3fa, 3hc, 3eb)** derivatives were observed and found to be biologically active for antimicrobial susceptibility test of gram-positive bacteria (Enterococcus faecalis ATCC 29212 and Staphylococcus auris ATCC 25923), gram-negative bacteria (Escherichia coli ATCC 25922 and Pseudomonas aeruginosa ATCC 27853), fungal strains (Candida albicans ATCC 90028 and Candida tropicalis ATCC 750), respectively, were also reported.

3.1.A. Previous methods:

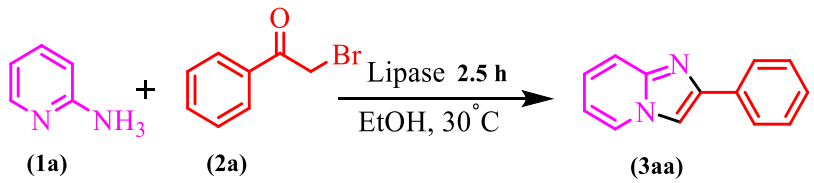


Metal catalyzed synthesis:





3. B. Present methods:

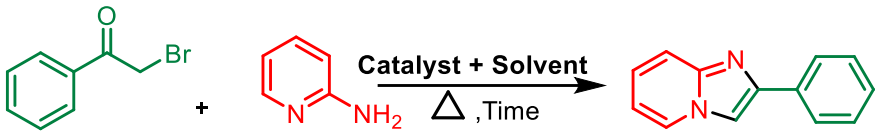


Scheme 3.2. Previous and Present methods synthesis of 2-phenyl imidazo[1, 2-a] pyridine

3.2. Result Discussion: We developed an improved, aided one-pot synthesis of 2-aryl imidazo fused heterocycles that are clean and efficient. To test the viability of our proposed approach, we used 2-aminopyridine (**1 a, 1 mmol**) and phenacyl bromide (**2a,1 mmol**) as substrates. Lipase is used as a catalyst, and ethanol is used as a reaction medium in this reaction.

The chemical reaction study was approved under numerous catalyst conditions (**Table 3.1**). For this synthesis, we first improved the catalyst system. In entries **1** and **2**, the reaction proceeds without a catalyst at room temperature and at **80 °C**, to give trace yields of the products. Further, in the presence of biocatalyst system α-amylase, trypsin, and amino lipase at **30 ˚C**, there were poor yields of the products **(Entry No. 3, 4 & 5)**; the next entry uses diastase enzyme to provide better yields of the product **(Entry No. 6)**. The yields of the product improved noticeably. When the reaction was carried out in the presence of lipase with ethanol as the reaction medium, much to our satisfaction, the yield reached 95%. Finally, we observed that lipase was the most influential promoter regarding reaction time and yield for forming the corresponding product.

Table 3.1. Optimization of catalyst system for the synthesis of compound (3aa)[a]



(1a) (2a) (3aa)

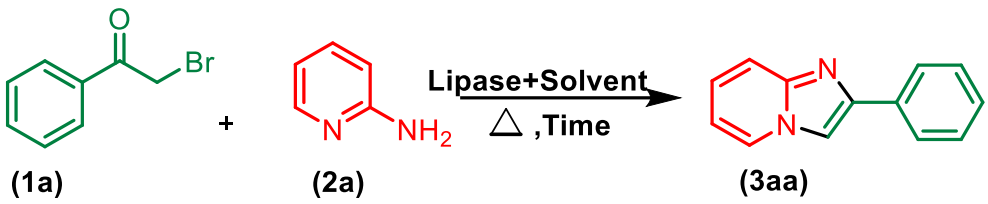
Entry No.	Catalyst	Temperature (°C)	Time (h)	Yields (%) [b]
1	No catalyst	RT	5	Trace
2	No Catalyst	80	5	Trace
3	α-Amylase	30	4	36
4	Trypsin	30	4	44

5	Amino Lipase	30	4	45
6	Diastase	30	4	58
7	**Lipase**	**30**	**2.5**	**95**

[a]**Reaction conditions:** All reactions were carried out with 2-amino pyridine (2a) (1 mmol) and phenacyl bromide (1a) (1 mmol) using the ethanol solvent system and the above several catalyst systems. [b] Isolated yields.

Additional experiments were carried out to identify the best reaction conditions, which included altering the solvent, and time. Various solvents were used, including toluene, xylene, benzene, 1,4-dioxane, $CHCl_3$, THF, DMF, DMSO, water, methanol, and ethanol (**Table 3.2**) contains a summary of the findings. Initially the reaction was done in non-polar, (toluene, xylene and benzene) and polar aprotic solvent (DMF and DMSO) which gave no yield/ trace amount of the product due to less solubility of lipase. When the experiment was done in water, a trace of the product was identified. This could be due to water's strong hydrogen bonding effects, which tend to pull constitutive polar molecules from the enzyme's inner structure, causing it to unfold and reduce its catalytic activity [54]. Other polar solvents have the same effect. Meanwhile, ethanol was shown to outperform the other solvents. As a result, ethanol was chosen as the best solvent for this reaction.

Table 3.2. Optimization of a solvent system for the synthesis of compound (3aa) [a]

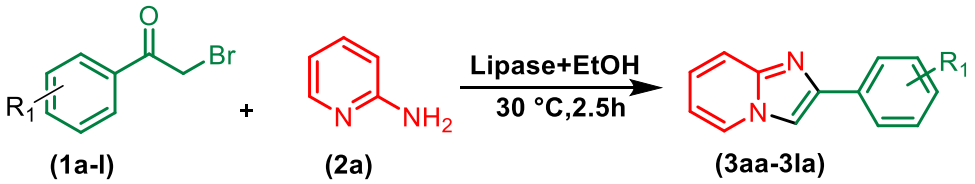


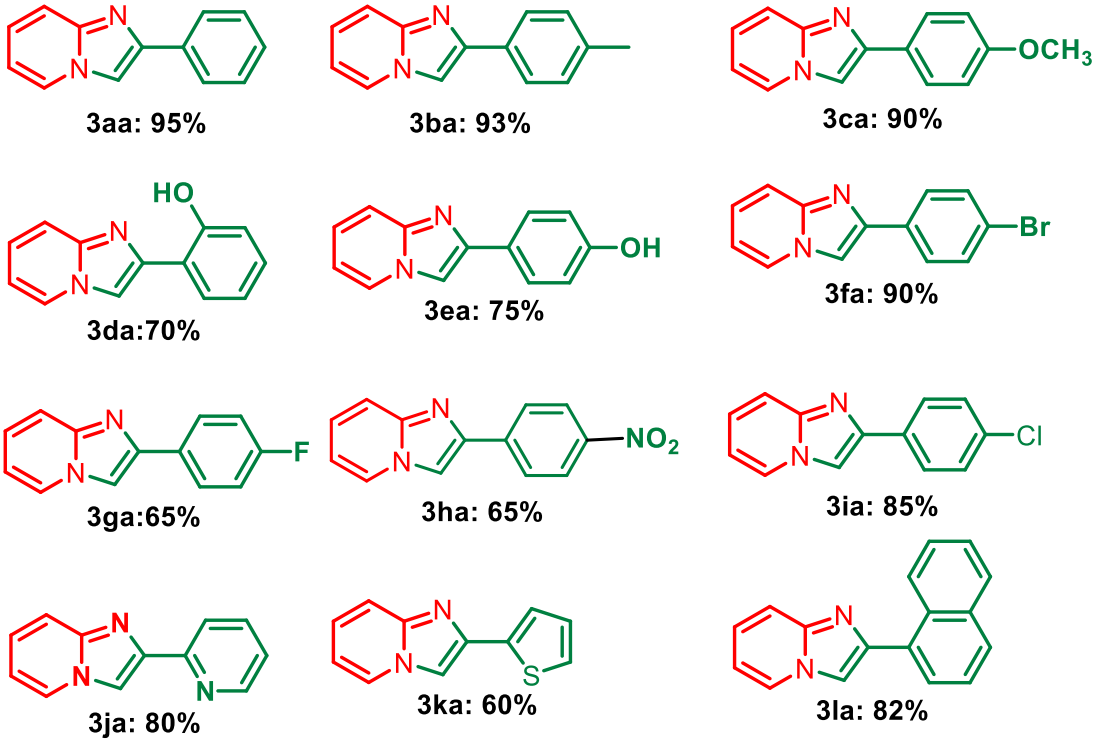
Entry No	Solvent	Time	Yield [b]
1	Toluene	5	Trace
2	Xylene	4	Trace
3	Benzene	4	Trace
4	1,4-Dioxane	4	32
5	Chloroform	3	40
6	THF	3	45
7	DMF	3	40
8	DMSO	3	50
9	Water	3	Trace
10	Methanol	3	68
11	**Ethanol**	**2.5**	**95**
12	Ethanol	3	95

[a]**Reaction conditions**: All reactions were carried out with 2-amino pyridine (2 a) (1 mmol) and phenacyl bromide (1 a) (1 mmol) using the solvent system, and lipase was used as a catalyst. [b] Isolated yields.

After optimizing the solvents' reaction condition, we have investigated substrate scopes for synthesizing a series of differently substituted imidazopyridines. All the target molecules were successfully synthesised with good to excellent yields and short reaction times (**Scheme 3.3**). In general, phenacyl bromides were observed bearing an electron-withdrawing group on the ortho or para position phenyl ring leading to lower yields (**3ca, 3fa, 3ga**). In contrast, the presence of an electron-donating group on the phenyl ring increased the yield of the desired product (**Scheme 3.3, 3aa-3la)**. Meanwhile, the electron donating group on the ortho or para position of 2-aminopyridine gave a higher yield (**Scheme 3.4, 3ab-ib**). At the same time, the electron-withdrawing group on ortho or para position of 2-aminopyridine gave a low yield (**Scheme 3.4, 3ac-3ad**). Synthesis of imidazopyridine using aliphatic ketone to give low yield (**Scheme 3.5,3ae-3de**). On the other hand, aliphatic ketone is less reactive in comparison to aryl ketone and gave only a **40-60%** yield of our desired products **(Scheme 3.5, 3ab-3ab)**.

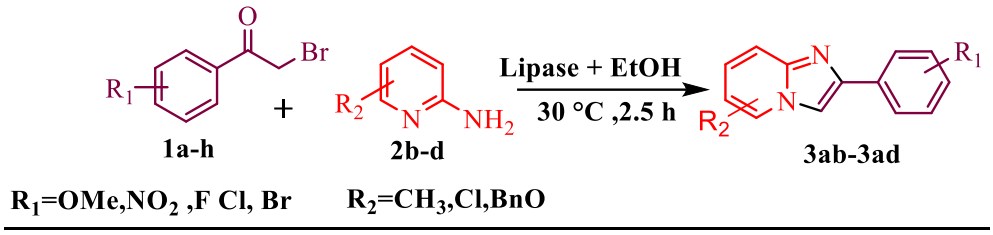
Scheme 3.3: Substrate scopes for synthesizing aryl series of imidazopyridines. [a,b]

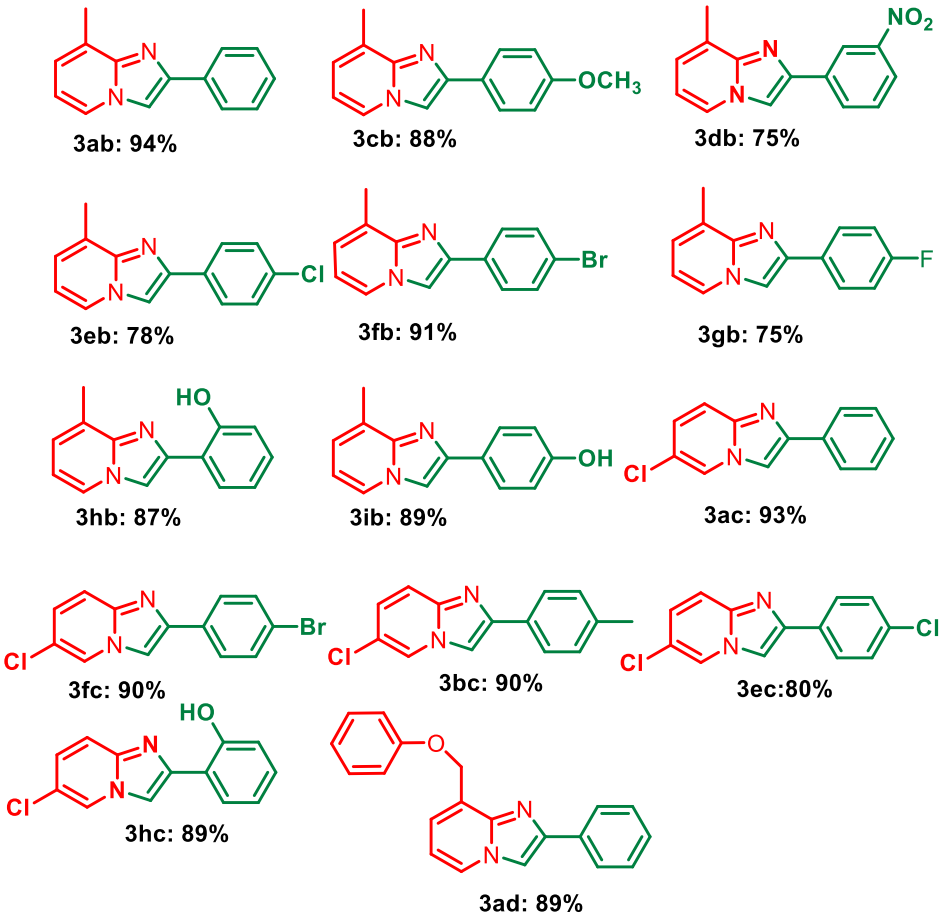




[a] **Reaction condition**: alkyl methyl ketone(1mmol) and 2-amino pyridine (2 a) (1 mmol) in the presence of PPL with EtOH (3 ml) at 30 °C. [b] Isolated yields

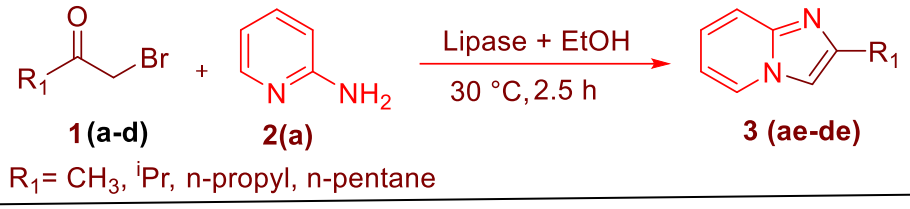
Scheme 3.4: Substrate scopes for one-pot conversion of 2-Bromoacetophenone and 2-aminopyrimidine into corresponding imidazopyridines [a, b]

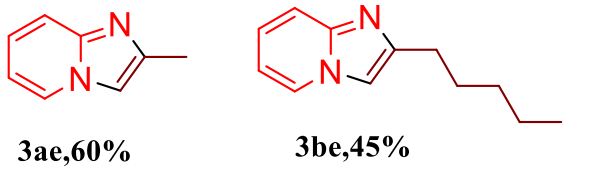


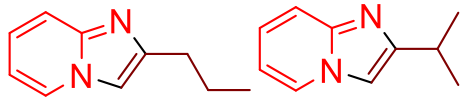


[a]**Reaction conditions:** All reactions were carried out with **2-amino pyridine (2a) (1 mmol)** and phenacyl bromide **(1a) (1 mmol)** using the ethanol solvent system and in the presence of **PPL** Lipase as catalyst systems. [b] Isolated yields.

Scheme 3.5: Substrate scopes for synthesizing alkyl series of imidazopyridines.

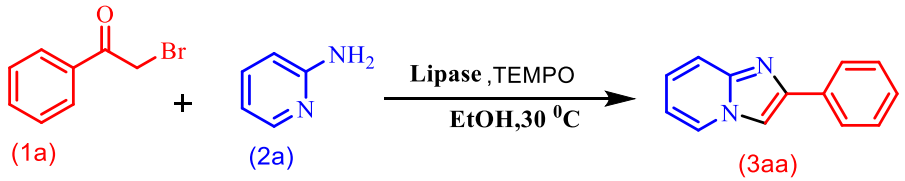






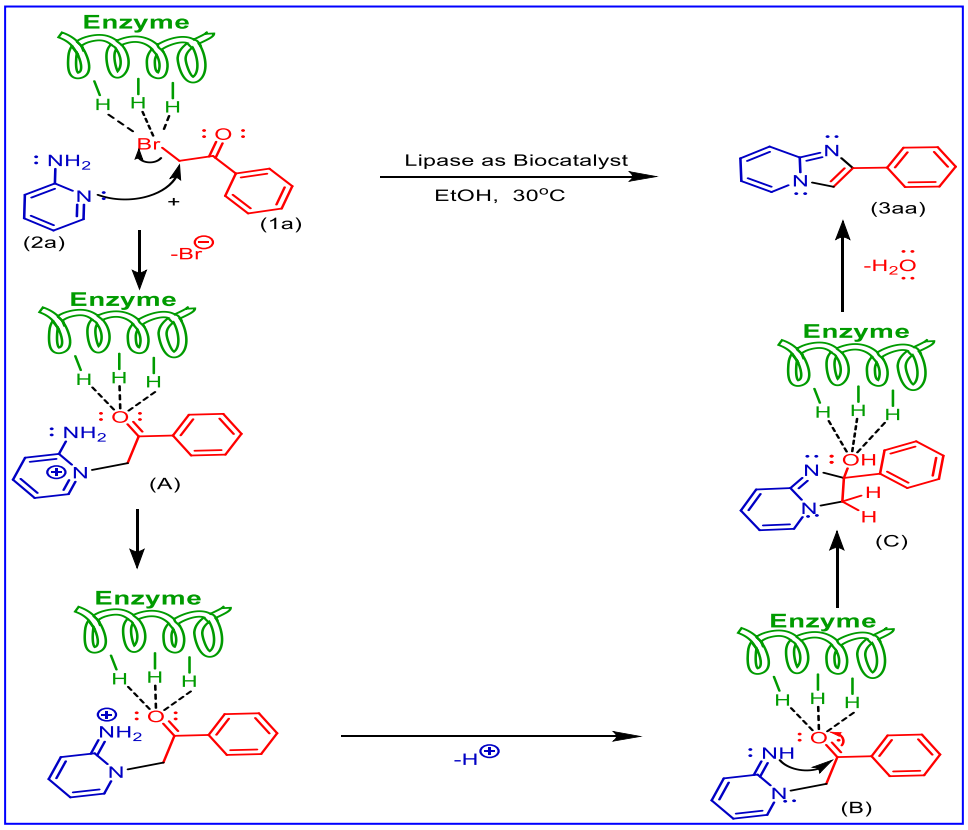
Reaction conditions: alkyl methyl ketone(1mmol) and 2-amino pyridine (2 a) (1 mmol) in the presence of PPL with EtOH(3ml) at 30°C.

3.2.1 Plausible Reaction Mechanism: A control experiment has been conducted to explore the reaction mechanism (**Scheme 3.6**). The reaction was performed with radical scavenger **TEMPO** (**2,2,6,6- tetramethylpiperidin-1-yloxyl**) under optimised conditions, but it did not quench the reaction. It rules out the possibility of a radical pathway of the reaction.



Scheme 3.6: Control experiment with TEMPO.

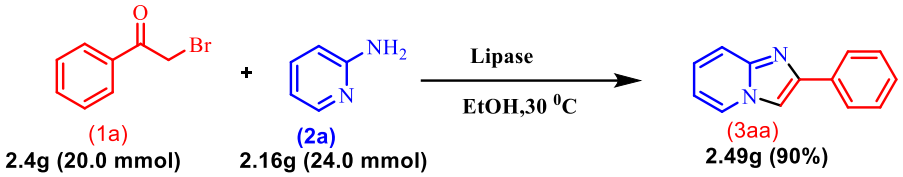
Based on literature reports [55] and our control experiment, a plausible mechanism has been shown in (**Scheme 3.7).** In the reaction, aminopyridine (**2a**) and phenacyl bromide (**1a**) were used as starting material. In this, the mechanism first step undergoes Br of phenacyl bromide activated by porcine pancreatic lipase (**PPL**), the lone pair of aminopyridine nitrogen (ring nitrogen) attacks nucleophilic substitution phenacyl bromide to form molecule (**A**), also activated by (**PPL**) to undergo cyclisation by the attack of nitrogen (**NH**) lone pair (**B**) as nucleophilic addition on carbonyl carbon to give (**C)** and followed by dehydration to the formation of imidazo [1, 2-a] pyridine derivatives (**3aa**).



Scheme 3.7: A plausible mechanism for the synthesis of 2-phenyl imidazo[1, 2-a] pyridine derivatives.

3.2.2 Scalability of the Protocol

After developing this methodology, gram scalability has been demonstrated for this method. The reaction was performed with acetophenone **(1a)** (2.4 g, 20.0 mmol), 2-amino pyridine **(2a)** (2.16 g, 24.0 mmol) under optimised reaction conditions, and it gave 90% (3.49 g) yield of the product **(3aa)** which is similar to the mmol scale synthesis. This indicates that our methodology is also effective for gram-scale synthesis **(Scheme 3.8.)**.



Scheme 3.8: Gram scale synthesis of imidazo[1,2-a] pyridine.

3.2.3 Reusability of the PPL Lipase

The reusability of PPL Lipase was also examined under the optimised reaction conditions up to 7 runs **(Figure. 3.2**). The catalyst was separated by a filter paper after completion of the reaction, first washed with water and then hexane (3 × 10 mL), dried and stored at 10 °C used for next reaction. The collected catalyst could be reused numerous times in the succeeding runs without a significant loss of catalytic activities. The conversion was decreased after the seven cycles, which may be due to the aggregation of particles of the used catalyst that may block the active sites present on the catalyst [56].

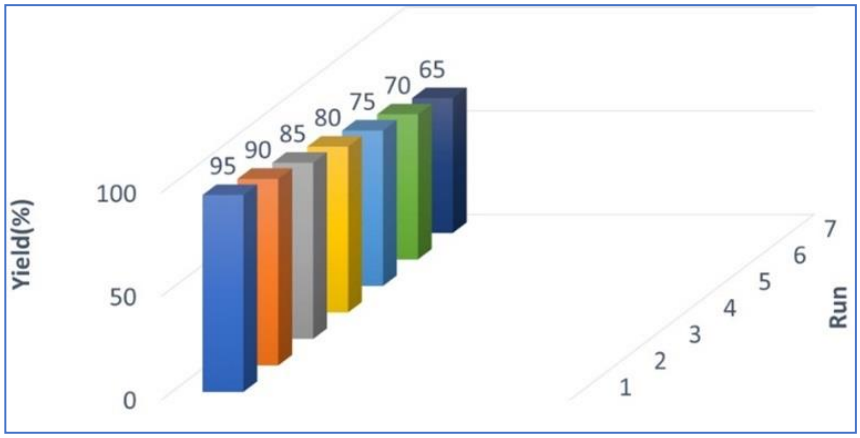


Figure 3.2: Reusability of catalyst

Significance of reusability of catalyst: Enzymes are very expensive, which will impact the overall synthesis process costs. Therefore, enzymes that can be recycled multiple times are more desirable for both industrial and laboratory purposes.

3.3. Antimicrobial activity of (3ha, 3ka, 3fa, 3hc, 3eb) compounds:

3.3.1 Materials and Methods

Microbial culture experiment required Potato Dextrose Agar (PDA), Nutrient Agar (NA), cation control Muller Hinton Broth and RPMI 1640 medium (without sodium bicarbonate supplemented with 0.165 moles per litter of morpholine propane sulfonic acid (MOPS) from Hi-Media Laboratories Limited, Mumbai, Maharashtra, India. The formulation of RPMI 1640 was developed at the Roswell Park Memorial Institute in Buffalo, NY, USA. Drugs (Amphotericin B, Mopenem, and Vancomycin) and solvents such as phosphate buffer saline (PBS) and sodium chloride (NaCl) were acquired from Sigma-Aldrich, St. Louis, MO, USA. Falcon, Eppendorf tubes, and other plastic ware were sourced from tarson Product Private

Limited, Kolkata, West Bengal, India. Moreover, 96-well flat-bottom and U-bottom microtiter plates were procured from Thermo Scientific, US. All reagents employed in this study were of analytical grades.

3.3.2 Microbial Strains and Growth conditions:

We have investigated the impact of synthesised compounds (**3ha, 3ka, 3fa, 3hc, 3eb**) by the antimicrobial susceptibility test of gram-positive bacteria (Enterococcus faecalis ATCC 29212 and Staphylococcus auris ATCC 25923), gram negative bacteria (Escherichia coli ATCC 25922 and Pseudomonas aeruginosa ATCC 27853) and against Yeast fungal cells (Candida albicans ATCC 90028 and Candida tropicalis ATCC 750). The lyophilized culture of all these bacteria and fungi was obtained from the american type culture collection (ATCC) and stored at -80℃. The bacterial strains were ssubculturesover nutrient agar (NA). The fungal strains were subcultured in subdural extrose agar (SDA) medium and incubated at 37 °C for Subsequently, the growth rate and microscopic characteristics were observed using Gram-stain for all strains. These strains were then preserved at -20℃ for further experiments.

3.3.3 Inoculum preparation:

Freeze-preserved culture strains of bacteria and fungi, namely (gram-positive bacteria: (Enterococcus faecalis ATCC 29212 and Staphylococcus auris ATCC 25923), gram-negative bacteria: (Escherichia coli ATCC 25922 and Pseudomonas aeruginosa ATCC 27853) and against Yeast fungal cells (Candida albicans ATCC 90028 and Candida tropicalis ATCC 750) were sub-culture over nutrient agar and sabouraud dextrose agar plates and incubated at 37℃

for 24 hours. Bacterial inoculum was prepared according to the CLSI (Clinical Laboratory Standard Institute) guidelines by adding 1-2 colonies in a normal saline tube, vortexed, and matched with MacFarland suspension of 0.5 OD at 565 nm wavelength. Subsequently, an inoculum suspension must have a concentration of 10^7 cells/ml for bacteria, 10^6 cells for yeast cells, and 10^5 cells for filamentous fungi.

3.3.4 Antifungal susceptibility test by well diffusion:

The agar well diffusion method assessed synthesised compounds' in vitro antibacterial and antifungal activity (**3ha, 3ka, 3fa, 3hc, 3eb**). All ATCC strains of bacteria Gram-positive bacteria (Enterococcus faecalis ATCC 29212 and Staphylococcus auris ATCC 25923), gram-negative bacteria (Escherichia coli ATCC 25922 and Pseudomonas aeruginosa ATCC 27853) were sub-cultured on nutrient agar. Fungal strains (Candida albicans ATCC 90028 and Candida tropicalis ATCC 750) were subculture on sabouraud dextrose agar and incubated for 24 hours at 37°C temperatures. The inoculum was prepared according to the previously described method. Subsequently, the fungal suspension was evenly spread over MHA plates, and the bacterial suspension was evenly spread over plain MHA plates. Wells were created in each inoculated culture plate using a sterile cork-borer, and then 50µl of various substances were added into each well. Specifically, 10 µg/ml of Meropenem served as a positive control for gram-negative bacteria, 2twoµg/ml of aa vancomycin was taken as a positive control for gram-positive bacteria and 32 µg/ml of Amphotericin B as a positive control for fungal Strains. Distilled water was taken as a negative control. Additionally, 50µl of 32 µg/ml

solution of each produced compound (**3ha, 3ka, 3fa, 3hc, 3eb**) were transferred into separate wells. The culture plates were subsequently incubated for 24 hours at a temperature of 37℃.The above-described method was again applied for fungal strain. Following incubation, the zones were measured and recorded. The entire experiment was conducted in triplicate to ensure the reliability of the results.

3.3.5 Minimal Inhibitory concentration determination of synthesized compounds:

Minimal Inhibitory concentration of synthesised compounds against bacterial strains gram-Positive (Enterococcus faecalis ATCC 29212 and Staphylococcus auris ATCC 25923), gram-negative bacteria (Escherichia coli ATCC 25922 and Pseudomonas aeruginosa ATCC 27853) and fungal strains (Candida albicans ATCC 90028 and Candida tropicalis ATCC 750) utilizing the broth micro-dilution method in 96 well U-shaped bottom microtiter plates and 96 well-flat bottom microtiter plates as per CLSI guidelines. A 1mg/ml stock solution of each synthesised compound was prepared. Inoculum was prepared using a previously described method, and the resulting suspension was diluted to achieve a concentration of 10^7 cells/ml for bacterial cells and 10^6 for fungal cells. We use a 96-well microtiter plate for MIC determination. Initially, we conducted a two-fold serial dilution of the synthesized compounds in the RPMI 1640 with MOPS buffer. Subsequently, we added 100µl of the inoculum suspension to each well. As a control, we take 100 µl of meropenem for (gram-negative bacteria) as a positive control, 100 µl of vancomycin as a positive control for (gram-positive bacteria), and 100 µl of Amphotericin B for fungal strain as a positive control. The

microtiter plates were incubated for 24 hours at a temperature of, 37℃ and MIC values were determined the following day by visually assessing turbidity.

3.3.6 Results:

3.3.7 Antimicrobial activity by well diffusion method:

In our investigation, we assessed the antimicrobial efficacy of synthesized compounds using the well diffusion method against bacterial gram-positive (*Enterococcus faecalis ATCC 29212* and *Staphylococcus auris ATCC 25923*), gram-negative bacteria (*Escherichia coli ATCC 25922* and *Pseudomonas aeruginosa ATCC 27853*) and fungal strains (*Candida albicans ATCC 90028* and *Candida tropicalis ATCC 750*). The outcomes of this suitable diffusion assay are illustrated in **(Table 3).** Notably, (**3ha, 3ka, 3fa, 3hc, 3eb**) did not manifest any significant antimicrobial activity against the tested. In contrast, amphotericin employed as a positive control shows a 15 mm zone of inhibition against fungal strain of *Candida albicans* and 18 mm zone of inhibition against *C. tropicalis*, Meropenem shows 30 mm zone of inhibition in *E. coli* and a 40 mm zone of inhibition in *Pseudomonas*, and Vancomycin shows 25 mm zone of inhibition in *Staphylococcus auris*, 24 mm of a zone of inhibition in *Enterococcus faecalis* and shown in ***Table*.3.3 A and 3.3B.**

Table 3.3 A: Well diffusion against gram-positive and gram-negative bacteria

Bacteria (+, -)	L1	L2	L3	L4	L5	Positive Control
S. auris	**0**	**0**	**0**	**0**	**0**	**25mm**
E. faecalis	**0**	**0**	**0**	**0**	**0**	**24mm**
P. aeruginosa	**0**	**0**	**0**	**0**	**0**	**40mm**
E. coli	**0**	**0**	**0**	**0**	**0**	**30mm**

Antimicrobial efficacy of synthesised compounds using the well diffusion method against bacterial gram-positive (Enterococcus faecalis ATCC 29212 and Staphylococcus auris ATCC 25923), gram-negative bacteria (Escherichia coli ATCC 25922 and Pseudomonas aeruginosa ATCC 27853)

Table 3.3.B: Well diffusion against *Candida sp*. (fungal strain)

Bacteria (+, -)	3h	3ka	3fa	3hc	3eb	Positive Control
C. albicans	**0**	**0**	**0**	**0**	**0**	**15mm**
C. tropicalis	**0**	**0**	**0**	**0**	**0**	**18mm**

Antimicrobial efficacy of synthesised compounds using the well diffusion method fungal strains (Candida albicans ATCC 90028, Candida tropicalis ATCC 750).

3.3.8 Determination of MIC of synthesized compounds (3ha, 3ka, 3fa, 3hc, 3eb):

MIC of synthesised compound was determined against bacterial gram-positive (*Enterococcus faecalis ATCC 29212* and *Staphylococcus auris ATCC 25923*), gram-negative bacteria (*Escherichia coli ATCC 25922* and *Pseudomonas aeruginosa ATCC 27853*) and

fungal strains (*Candida albicans ATCC 90028* and *Candida tropicalis ATCC 750*) utilizing the broth micro-dilution method in 96 well microtiter plates. The MIC results are presented in (**Table 3.4**). Specifically, in bacterial strains, we observed MIC values of 2 µg/ml of **(3ha)** and **(3ka)**, **(3fa)** shows ≥16 µg/ml, **(3hc)** shows resistance in *E. faecalis,* and **(3eb)** shows 2 µg/ml while in fungal strains **(3ha)** and **(3ka)** shows MIC value of 1 & 2 µg/ml, **(3fa)** shows 16 µg/ml MIC value, **(3hc)** was resistance in ***C****. albicans* species and 2 µg/ml MIC value in C. tropicalis and last L5 shows 2 µg/ml MIC value in C. albicans and 1 µg/ml MIC value in C. tropicalis, respectively, as shown in **(Table 3.4A and 3.4B.)**

3.3.9 MIC against gram-positive and gram-negative bacteria:

Table 3.4.A MIC value against gram positive.

Bacteria (+, -)	3ha	3ka	3fa	3hc	3eb
S. auris	**2 µg/ml**	**2 µg/ml**	**16 µg/ml**	**16 µg/ml**	**2 µg/ml**
E. faecalis	**2 µg/ml**	**2 µg/ml**	**>16 µg/ml**	**R**	**4 µg/ml**
P. aeruginosa	**2 µg/ml**	**2 µg/ml**	**16 µg/ml**	**16 µg/ml**	**2 µg/ml**
E. coli	**1 µg/ml**	**2 µg/ml**	**16 µg/ml**	**2 µg/ml**	**2 µg/ml**
MIC (mg/mL) of imidazopyridine compounds (3ha, 3ka, 3fa, 3hc, 3eb)					

Table 3.4.B MIC against *Candida* sp.

Fungi	3ha	3ka	3fa	3hc	3eb	Positive Control
C. albicans	**1 µg/ml**	**2 µg/ml**	**16 µg/ml**	**R**	**2 µg/ml**	**0.25 µg/ml**
C. tropicalis	**2 µg/ml**	**2 µg/ml**	**16 µg/ml**	**2 µg/ml**	**1 µg/ml**	**<0.015µg/ml**
Minimum inhibition concentration (mg/mL) of imidazopyridine compounds (3ha, 3ka, 3fa, 3hc, 3eb)						

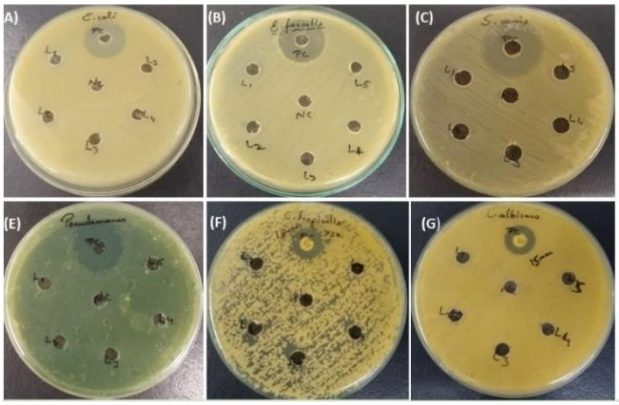


Figure 3.3: Shows the results of the antimicrobial susceptibility test by agar well diffusion method against gram-positive, gram-negative and fungal strains **(A)** *Enterococcus faecalis* ATCC 29212:- positive control, (**3ha**),**(3ka)**,**(3fa),(3hc**)and**(3eb) (B)** *Staphylococcus auris* ATCC 25923:- positive control**(3ha),(3ka),(3fa),(3hc)and(3eb) (C)** *Escherichia coli ATCC* 25922:- positive control, (**3ha**),**(3ka)**,**(3fa),(3hc**)and**(3eb) (D)** *Pseudomonas aeruginosa* ATCC 27853:- positive control, (**3ha**),**(3ka)**,**(3fa),(3hc**)and**(3eb) (E)** Candida albicans ATCC 90028:- positive control, (**3ha**),**(3ka)**,**(3fa),(3hc**)and**(3eb) (F)** *Candida tropicalis* ATCC 750:- positive control, L1, L2, L3, L4, L5 (**3ha**),**(3ka)**,**(3fa),(3hc**)and**(3eb).**

3.3.10 Discussion: -

Antimicrobial susceptibility testing (AST) is a crucial laboratory technique conducted by clinical laboratory scientists to determine the most effective antimicrobial treatment for individual patients. Additionally, it plays a vital role in assessing the quality of treatment offered by healthcare facilities and national programs to manage and prevent infectious diseases [57]. In our study, the absence of any inhibition zones in antimicrobial susceptibility testing indicates that the synthesised compound cannot likely hinder microorganism growth under controlled laboratory conditions. Various factors, including the compound's properties like (polarity and its volatile nature [58-59], its interactions with microorganisms, its resistance mechanism and the experimental procedure can contribute to this outcome. The MIC is another quantitative antimicrobial susceptibility method [60]. We observed that MIC for bacterial strains was 2µg/ml for compounds **(3ha)** and **(3ka)**, while **(3fa)** exhibited a MIC value of ≥16 µg/ml, and **(3hc)** demonstrated resistance in *E. faecalis*. **(3eb)** showed a MIC value of 2 µg/ml. For fungal strain, **(3ha)** had a MIC value of 1µg/ml, **(3ka)** had a MIC value of 2 µg/ml, and **(3fa)** had a MIC value of 16µg/ml, **(3hc)** exhibited resistance in *C. albicans*, while it showed a MIC value of 2µg/ml in *C. tropicalis*. Last, **(3eb)** displayed a MIC value of 2µg/ml in *C. albicans* and 1µg/ml in *C. tropicalis*. It provides a better understanding of the compound's antimicrobial activity, even if it doesn't produce a clear or visible zone in the well diffusion test. It still possesses some level of antimicrobial effectiveness, as indicated by the MIC value. It is essential to systematically investigate and discuss these factors to

comprehend the compound's limitation in antimicrobial treatment—this information is valuable for guiding future research efforts.

3.4.0 Experimental Section:

3.4.1 General procedure for the synthesis of products:

The mixture of 1.0 mmol 2-bromoacetophenone, 1.0 mmol 2-aminopyridine, 3 ml C_2H_5OH and 30 mg PPL was introduced to an RB (50 ml), then the mixture was subjected to shaking at 160 rpm with end-over-end rotation at 30 °C for a specific time. The reaction was monitored by TLC (petroleum ether–ethyl acetate ¼ 5: 1, v/v), and 5 ml ethyl acetate was added into the reaction mixture to dissolve any solids if necessary. Then, the mixture was filtered through a paper filter to remove the enzymes, and the solvent was evaporated. The solid residue was recrystallized with ethanol, yielding the target compounds. ^{1}H NMR spectra were recorded on a Bruker Avance 500 spectrometer at 500 MHz in DMSO, using TMS as an internal standard.

3.4.2 Procedure for gram-scale synthesis: A mixture of acetophenone (2.4 g, 20.0 mmol), 2-amino pyridine (2.16 g, 24.0 mmol), PPL (30 mg.) and C_2H_5OH (30, ml.) were added in 50 ml RB and steerer continuously, and the progress of the reaction was monitored by TLC. The reaction was monitored by TLC (petroleum ether–ethyl acetate ¼ 5: 1, v/v), and 5 ml ethyl acetate was added into the reaction mixture to dissolve any solids if necessary. Then, the mixture was filtered through a paper filter to remove the enzymes, and the solvent was evaporated. The solid residue was recrystallized with ethanol, yielding the target compounds.

^{1}H NMR spectra were recorded on a Bruker Avance 500 spectrometer at 500 MHz in DMSO, using TMS as an internal standard.

3.5 Conclusions: In this work, a completely regioselective, environment-friendly technique for the straightforward synthesis of physiologically active imidazole-fused nitrogen-bridgehead heterocycles have been developed by the use of 2-halocarbonyl compounds with 2-aminopyridines as starting materials. This strategy involves an efficient biocatalytic route, using lipase as a biocatalyst and ethanol as an eco-friendly reaction medium. This reaction avoids toxic catalysts and volatile organic solvents. The merits of this protocol are high atom economy, operational simplicity, mild reaction conditions, easy workup and purification process, and good yields of desired products in short reaction times. After studying the antimicrobial and anti-fungal activity of desired products to understand the compound's limitations in antimicrobial treatment completely, it is important to conduct a thorough investigation and discourse on these issues. This data is important for directing future investigations.

3.6 Analytical data

3.6.1 Analytical data of ^{1}H and ^{13}CNMR of the 2-phenylimidazo[1,2-a] pyridine derivative

^{1}H and ^{13}CNMR of the 2-phenylimidazo[1,2-a] pyridine (3aa) Purified by recrystallisation using aqueous ethanol; white solid; yield: 960 mg (95%); mp 130- 132 °C. **^{1}H NMR** (500 MHz, DMSO): δ 8.05 (d, J =7.0 Hz, 1H, arom H), 7.95 (dd, J =8.5 Hz, J =1.5 Hz, 2H, arom H), 7.71 (s, 1H, arom H), 7.59 (d, J =9.0 Hz, 1H, arom H), 7.42 (t, J =7.4 Hz, 2H, arom H),

7.28 (t, J =7.5 Hz, 1H, arom H), 7.14-7.10 (m, 1H, arom H), 6.57 (td, J =7.0 Hz, J =1.0 Hz, 1H, arom H). **^{13}CNMR** (126 MHz, $CDCl_3$): 144.91, 143.83, 134.89, 127.90, 129.15, 125.21, 126.77, 125.85, 116.68, 111.60, 107.30.IR (KBr, v, cm^{-1}): 3433, 3140, 3042, 1945, 1658, 1636, 1223, 1067, 847, 765 **HRMS** (ESI): Anal. Calc. For $C_{13}H_{11}N_2$ $[M+H]^+$ 195.0922; Obser.: 195.0882

^{1}H and ^{13}CNMR of the 2-(p-Tolyl) imidazo [1,2-a] pyridine (3ba) White solid; yield 193 mg (93%); m.p. 143-144 °C; **^{1}H NMR** (500 MHz, DMSO) δ (ppm): 8.93 (d, 1H), 7.84 (d, 2H), 7.77 (s, 1H),7.43 (d, 1H), 7.37 (dd, 1H), 7.26 (d, 2H), 2.44 (s, 3H); **^{13}C NMR** (126 MHz, $CDCl_3$) δ (ppm): 167.38, 143.2, 139.3, 133.5, 131.5, 129.4, 128.6, 126.1, 119.5, 106.5, 14.33; **HRMS** (ESI):Anal. Calc. for $C_{14}H_{12}N_2[M+H]^+$: 209.1073, Obser.: 209.1077.

^{1}H and ^{13}CNMR of the 2-(4-methoxyphenol) imidazo[1,2-a]pyridine (3ca) White solid; yield 210 mg (90%); m.p. 138-139 °C; **^{1}H NMR** (500 MHz, DMSO) δ (ppm): 8.63 (dd, 1H), 8.05 (d, 2H), 7.98 (s, 1H), 7.62 (d, 1H), 7.17 – 7.14 (m, 1H), 6.96 (d, 2H), 6.74 (t, 1H), 3.84 (s, 3H); **^{13}CNMR** (126 MHz, $CDCl_3$) δ (ppm):161.32,144.84, 128.74, 126.13, 125.5, 124.5, 123.6, 117.4, 114.2, 110.4, 107.3, 56.4.**HRMS** (ESI): Anal. Calc. for $C_{14}H_{12}N_2O[M+H]^+$: 225.1022, Obser.: 225.1025.

^{1}H and ^{13}C NMR of the 2-(4-nitrophenyl) imidazo[1,2-a]pyridine (3da) Yellow solid; yield 227 mg (70%); m.p. 202-203 °C; **^{1}H NMR** (500 MHz, DMSO) δ (ppm): 8.54 – 8.44 (m, 1H), 7.93 (d, 1H), 7.92 (dd, 2H), 7.64 (s, 1H), 7.63 (d, 1H), 7.25 (t, 1H), 7.21 (d, 1H), 6.91 (t, 1H**); ^{13}C NMR** (126 MHz, $CDCl_3$) δ (ppm): 145.37, 143.67, 133.70, 132.12, 128.03,

127.44, 125.68, 121.15, 117.16, 112.91,109.98. **HRMS** m/z (ESI): calc. for [$C_{13}H_9N_3O_2^+$] [M+H] $^+$:240.0768 Obser.: 240.0769

^{1}H and ^{13}CNMR of the 4-(Imidazo[1,2-a]pyridin-2-yl)phenol (3ea) Brown solid; yield 185 mg (75%); m.p. 230-231 °C; **^{1}H NMR** (500 MHz, DMSO) δ (ppm): 8.80 (s, 1H), 8.45 (d, 1H), 8.02 (s, 1H), 7.98 (d, 2H), 7.96 (d, 1H), 7.84 – 7.79 (m, 1H), 7.26 (d, 2H), 6.06 (s, 1H); **^{13}C NMR** (126 MHz, $CDCl_3$) δ (ppm): 156.18, 143.95, 128.01, 127.37, 126.6, 125.49, 117.09, 116.13, 115.96, 112.78, 109.41, 107.46 **HRMS (ESI):** Calc. for $C_{13}H_{10}N_2O$[M+H] $^+$: 211.0866, Obser.: 211.0870.

^{1}H and ^{13}C NMR of the 2-(4-Bromophenyl) imidazo[1,2-a] pyridine (3fa) Yellow solid; yield 211 mg (90%); m.p. 206-207 °C; **^{1}H NMR** (500 MHz, DMSO) δ (ppm): 8.53 (d, 1H), 8.44 (d, 2H), 8.00 (s, 1H), 7.98 (d, 1H), 7.41 (d, 2H), 7.59-726 (dd, 1H), 6.91 (t, 1H); **^{13}C NMR** (126 MHz, $CDCl_3$) δ (ppm): 145.78, 143.8, 133.24, 132.18, 129.05, 126.24, 125.59, 117.24, 116.7, 113.7, 109.96; **HRMS (ESI):** Calc. for $C_{13}H_9BrN_2$[M+H] $^+$: 273.0027,Obser.: 275.0009.

^{1}H and ^{13}CNMR of the 2-(4-Fluorophenyl) imidazo[1,2-a] pyridine (3ga) Pale yellow solid; yield 199 mg (65%); m.p. 164-165 °C; **^{1}H NMR** (500 MHz, DMSO) δ (ppm): 8.52 (d, 1H), 7.99 – 7.57 (m, 2H), 7.28 (s, 1H), 7.26 (d, 1H), 7.24 (t, 1H), 7.13 – 7.08 (m, 2H), 6.93 (t,1H),**^{13}CNMR**(126MHz,$CDCl_3$)δ(ppm):163.06,147.18,145.25(J=97Hz.),129.84(J=3.2Hz) ,128.96,127.09(J=8.06Hz),125.68,125.47,116.74116.132,(J=21.5Hz),111.78,107.81.**^{19}F NMR** (471 MHz, DMSO)δ-108.75.**HRMS** (ESI): Calc. for $C_{13}H_9FN_2$[M+H]$^+$: 213.0823, Obser.: 213.0815.

^{1}H and ^{13}CNMR of the 2-(4-nitrophenyl) imidazo[1,2-a]pyridine (3ha) Colorless solid; yield 187 mg (65%); m.p. 200-201 °C; **^{1}H NMR** (500 MHz, DMSO) δ (ppm): 12.73 (s, 1H), 8.52 (d, 1H), 8.44 (s, 1H), 7.94 (d, 2H), 7.92-791 (t, 2H), 7.64-7.63 (dd, 1H), 7.26 – 6.911(m, 2H); **^{13}C NMR** (126 MHz, $CDCl_3$) δ (ppm):145.37, 143.67, 133.70, 132.02, 128.03, 127.53, 127.44, 125.68, 121.15, 117.16, 112.91, 109.98. **HRMS (ESI):** Calc. for $C_{14}H_9N_3[M+H]^+$: 220.0869, Obser.: 220.0869.

^{1}H and ^{13}CNMR of the 2-(4-chlorophenyl) imidazo[1,2-a] pyridine (3ia) Yellow solid; yield 227 mg (85%); m.p. 202-203 °C; **^{1}H NMR** (500 MHz, DMSO) δ (ppm): 8.53 – 8.44 (m, 1H), 8.00 (d, 1H), 7.98 (dd, 2H), 7.53 (s, 1H), 7.49 (d, 1H), 7.27 (t, 1H), 7.22 (d, 1H), 6.91 (t, 1H); **^{13}C NMR** (126 MHz, $CDCl_3$) δ (ppm): 145.37, 145.0, 143.64, 133.55, 129.21, 127.71, 127.43, 125.5, 123.66, 117.16, 112.96, 114.1, 109.96 **HRMS (ESI)**: Calc. for $C_{13}H_9ClN_2$ $[M+H]^+$: 229.0527,Obser.: 229.0531.

^{1}H and ^{13}C NMR of the 2-(Pyridin-2-yl)imidazo[1,2-a]pyridine (3ja) Brown solid; yield 166 mg (80%); m.p. 240-241 °C; **^{1}H NMR** (500 MHz, DMSO) δ (ppm): 8.64 – 8.63 (m, 2H), 8.24 (s, 1H), 8.23 (dd, 1H), 7.86 (d, 1H), 7.84 (d, 1H), 7.71 – 7.57 (m, 2H), 6.93 (d, 1H); **^{13}C NMR** (126MHz, DMSO) δ (ppm): 153.38, 151.02, 147.86, 141.19, 139.60, 127.09, 125.68, 123.22, 116.74, 116.71, 111.00, 100.86. **HRMS** (ESI): Calc. For $C_{12}H_9N_3$ $[M+H]^+$195.08; Obser.: 196.08

^{1}H and ^{13}CNMR of the 2-(Thiophen-2-yl)imidazo[1,2-a]pyridine (3ka) Yellowish white solid; yield 184 mg (60%); m.p. 135-136 °C; **^{1}H NMR** (500 MHz, DMSO) δ (ppm): 8.51 (d, 1H), 8.76 (s, 1H), 8.28 (d, 1H), 7.25 – 7.14 (m, 1H), 7.61 – 7.56 (m, 1H), 7.54 – 7.51 (m,

1H), 7.25 – 7.14 (m, 1H), 6.90 (t, 1H); **^{13}C NMR** (126 MHz, $CDCl_3$) δ (ppm): 145.04, 140.23, 138.26, 128.45, 127.26, 125.92, 125.59, 124.06, 116.79, 112.88, 108.67. **HRMS (ESI)**: Calc. for $C_{11}H_8N_2O$ $[M+H]^+$: 185.0709, Obser.: 185.0695.

^{1}H and ^{13}CNMR of the2-(Naphthalen-1-yl) imidazo[1,2-a] pyridine (3la) Yellowish liquid; yield 227 mg (82%); **^{1}H NMR** (500 MHz,) δ (ppm): 8.62 – 8.55 (m, 1H), 8.16 (d, 1H), 7.91 – 7.86 (m, 2H), 7.82 (d, 2H), 7.70 (d, 1H), 7.57 – 7.48 (m, 3H), 7.22 – 7.17 (m, 1H), 6.80 (s, 1H); **^{13}C NMR** (126 MHz, DMSO) δ (ppm): 146.48, 144.34, 138.01, 130.87, 132.63, 127.59, 129.47, 126.83, 125.55, 127.06, 124.89, 126.66, 124.52, 123.71, 116.85, 116.74, 111.00. **HRMS (ESI):** calculated for $C_{17}H_{12}N_2[M+H]^+$: 245.1073, Obser.: 245.1076.

^{1}H and ^{13}CNMR of the_8-Methyl-2-phenylimidazo[1,2-a]pyridine (3ab)[1] Pale yellow solid; yield 189 mg (94%); m.p. 119-120 °C; **^{1}H NMR** (500 MHz, $CDCl_3$) δ (ppm): 8.69 (t, 1H), 7.98 (d, 2H), 7.62 (s, 1H), 7.82 (dd, 2H), 7.33 – 7.14 (m, 1H), 6.84 (d, 1H), 3.85 (t, 1H), 2.65 (s, 3H); **^{13}CNMR** (126 MHz, DMSO) δ (ppm): 161.06, 143.38, 142.69, 131.69, 128.67, 126.66, 123.47, 122.89, 117.4, 55.93, 19.2.**HRMS (**ESI): Calc. for $C_{14}H_{12}N_2$ $[M+H]^+$: 209.1073, Obser.: 209.1074.

^{1}H and ^{13}CNMR of the 2-(4-Methoxyphenyl)-8-methylimidazo[1,2-a] pyridine (3cb) Pale yellow solid; yield 209 mg (88%); m.p. 132-133 °C; **^{1}H NMR** (500 MHz, DMSO) δ (ppm): 7.94 (d, 1H), 7.87 (d, 2H), 7.72 (s, 1H), 6.96 (d, 2H), 6.91 (d, 1H), 6.65 (t, 1H), 3.83 (s, 3H), 2.64 (s, 3H); **^{13}C NMR** (126 MHz, $CDCl_3$) δ (ppm): 160.5, 147.2, 144.2, 126.5, 126.4, 125.9, 122.4, 124.2, 115.1, 113.2, 106.8. 54.4, 16.2.

HRMS (ESI**):** Calc. for $C_{15}H_{14}N_2O$ $[M+H]^+$: 239.1179, Obser.: 239.1173.

^{1}H and ^{13}CNMR of 8-Methyl-2-(3-nitrophenyl)imidazo[1,2-a]pyridine (3db) Yellow solid; yield 233 mg (75%); m.p. 168-169 °C**; ^{1}H NMR** (500 MHz, DMSO) δ (ppm):8.63 (m, 1H), 8.25 (dd, 1H), 8.14 – 8.10 (m, 1H), 7.97 (d, 1H), 7.84 (s, 1H), 7.68 (t, 1H), 7.05 (dd, 1H), 7.84 (t, 1H), 2.64 (s, 3H); **^{13}C NMR** (126 MHz, $CDCl_3$) δ (ppm): 148.44, 141.24, 140.73, 140.1, 134.76, 132.79, 129.69, 128.10, 125.92, 124.49, 123.23, 123.18, 108.92, 108.46,19.11 **HRMS** (ESI):Calc. For $C_{14}H_{11}N_3O_3$ $[M+H]^+$ 253.0922; Obser: 254.0882

^{1}H and ^{13}C NMR of the2-(4-Chlorophenyl)-8-methylimidazo[1,2-a]pyridine (3eb) White solid; yield 226 mg (78%); m.p. 119-120 °C; **^{1}H NMR** (500 MHz, DMSO) δ (ppm): 8.44 (d, 1H), 8.37 (d, 2H), 8.01 (s, 1H), 7.99 (d, 2H), 7.06 (d, 1H), 6.83 (t, 1H), 2.53 (s, 3H); **^{13}C NMR** (126 MHz, $CDCl_3$) δ (ppm): 145.86, 143.01, 133.47, 132.43, 129.35, 127.70, 126.5, 125.6, 124.5, 123.6, 110.7, 17.09. **HRMS** (ESI): Calc. For $C_{14}H_{11}ClN_2[M+H]^+$ 242.0922; Obser.: 242.0621

^{1}H and ^{13}CNMR of the**2-(4-Bromophenyl)-8-methylimidazo[1,2-a]pyridine (3fb)** Yellow solid; yield 261 mg (91%); m.p. 131-132°C; **^{1}H NMR** (500 MHz, DMSO) δ (ppm): 8.43 (d, 1H), 7.86 – 7.81 (m, 2H), 7.78 (s, 1H), 7.54 (d, 2H), 6.95 (d, 1H), 6.72 – 6.63 (m, 1H), 2.64 (s, 3H); **^{13}CNMR** (126 MHz, $CDCl_3$) δ (ppm): 155.06,146.25,142.61, 137.13, 133.51, 132.40, 128.01, 121.00, 112. 64, 110.40, 17.04 **HRMS (ESI):** Calc. for $C_{14}H_{11}BrN_2[M+H]^+$ 287.0178 Obser.: 287.0166.

^{1}H & ^{13}CNMR of the2-(4-Fluorophenyl)-8-methylimidazo[1,2-a] pyridine (3gb) White solid; yield 199 mg (75%); m.p. 128-129 °C**; ^{1}H NMR** (500 MHz, DMSO) δ (ppm): 8.14 (s, 1H), 7.70 (dd, 2H), 7.58 (s, 1H), 7.16 – 7.04 (m, 2H), 6.97 – 6.93 (m, 1H), 6.84 (t, 1H), 2.59

(s, 3H); **^{13}CNMR** (126 MHz, CDC_{l3}) δ (ppm): 163.06, 141.37, 141.24(d, J=16.3Hz.),129.84,128.96,(d, J=15.1Hz). 128.10, 125.92, 124.49, 116.32, 108.46, 108.45, (sJ=1.26Hz.) 19.11. **^{19}F NMR** (471 MHz, DMSO) δ -121.66. **HRMS (ESI):** Calc. for $C_{14}H_{11F}N_2$ $[M+H]^+$: 226.0178 Obser.: 227.0166.

^{1}H and ^{13}CNMR of the 2-(8-Methylimidazo[1,2-a]pyridin-2-yl) phenol (3hb) White solid; yield 195 mg (87%); **^{1}H NMR** (500 MHz, DMSO) δ (ppm): 12.94 (s, 1H), 8.47 (s, 1H), 8.46 (dd, 1H), 7.87 (d, 1H), 7.25 – 7.18 (m, 1H), 6.94 – 6.92 (m, 2H), 6.90 (t, 1H), 6.89 (d, 1H), 2.50 (s, 3H); **^{13}C NMR** (126 MHz, $CDCl_3$) δ (ppm): 156.67, 143.80, 143.08, 129.55, 126.88, 125.71, 125.09, 124.86, 118.52, 117.49, 117.24, 113.49, 110.01, 16.81.**HRMS** (ESI): Anal. Calc. For $C_{14}H_{12}N_2O$ $[M+H]^+$ 224.0917; Obser.: 225.0100

^{1}H and ^{13}C NMR of the 4-(8-Methylimidazo[1,2-a]pyridin-2-yl)phenol (3ib) Brown solid; yield 199 mg (89%); **^{1}H NMR** (500 MHz, DMSO) δ (ppm): 10.51 (s, 1H), 8.39 (s, 1H), 8.08(dd, 2H), 7.96 (s, 1H), 7.41 (s, 1H), 7.81 – 7.02 (m, 3H), 6.91 – 6.91 (m, 1H), 6.83 (d, 1H), 6.76 – 6.71 (m, 1H), 2.60 (s, 3H); **^{13}C NMR** (126 MHz, DMSO) δ (ppm): 157.99, 141.77, 141.24, 128.43, 128.10, 125.92, 125.07, 124.49, 116.47, 108.46, 19.11. **HRMS** (ESI): Calc. For $C_8H_8N_2O$ $[M+H]^+$ 148.0617; Obser.: 148.0701.

^{1}H and ^{13}CNMR of the 6-Chloro-2-phenylimidazo[1,2-a]pyridine (3ac) White solid; yield 213 mg (93%); m.p. 207-208 °C; **^{1}H NMR** (500 MHz, DMSO) δ (ppm): 8.28 (d, 1H), 7.83 (d, 2H), 7.66 (s, 1H), 7.62 (d, 1H), 7.45 (t, 2H), 7.39 (d, 1H), 7.32 (dd, 1H); **^{13}C NMR** (126 MHz, $CDCl_3$) δ (ppm): 147.17, 145.57, 132.27, 132.26, 129.78, 128.03, 127.06, 126.65,

115.91,109.0,107.53.**HRMS(**ESI**):** Calc. for $C_{13}H_9ClN_2[M+H]^+$: 229.0527, Obser.:229.0515.

^{1}H and ^{13}CNMR of the 2-(4-Bromophenyl)-6-chloroimidazo[1,2-a]pyridine (3fc), Yellow solid; yield 277 mg (90%); m.p. 199-200 °C; **^{1}H NMR** (500 MHz, DMSO) δ (ppm): 8.27 (dd, 1H), 7.82 – 7.65 (m, 3H), 7.60 (d, 3H), 7.51-739 (dd, 1H); **^{13}C NMR** (126 MHz, $CDCl_3$) δ (ppm): 147.17, 145.57, 132.44, 131.25, 129.78, 128.22, 129.03, 123.88, 115.91, 109.47, 107.53 **HRMS (ESI**): Calc. for $C_{13}H_8BrClN_2$ $[M+H]^+$: 306.9632, Obser.: 306.9624

^{1}H &^{13}C NMR of the 6-Chloro-2-(p-tolyl) imidazo[1,2-a] pyridine (3bc) Pale yellow solid; yield 228 mg (90%); m.p. 125-126 °C; **^{1}H NMR** (500 MHz, DMSO) δ (ppm): 8.27 (d, 1H), 7.82 (d, 2H), 7.66 (s, 1H), 7.57 (d, 1H), 7.51 (d, 2H), 7.39 (d, 1H), 2.33 (s, 3H); **^{13}C NMR** (126 MHz, $CDCl_3$) δ (ppm): 147.17, 145.57, 137.20, 132.42, 129.78, 129.49, 128.08, 115.91,109.47, 107.53, 107.12, 21.13.**HRMS (**ESI**):**Calc. for $C_{13}H_9ClN_2$ $[M+H]^+$: 229.0527, Obser.: 229.0515.

^{1}H and ^{13}C NMR of the 6-Chloro-2-(4-chlorophenyl) imidazo[1,2-a] pyridine (3ec) Pale yellow solid; yield 242 mg (80%); m.p. 206-207 °C; **^{1}H NMR** (500 MHz, DMSO) δ (ppm): 8.27 (d, 1H), 7.81 (d, 2H), 7.66 (s, 1H), 7.56 (d, 1H), 7.44 (d, 2H), 7.39 (dd, 1H); **^{13}C NMR** (126 MHz, $CDCl_3$) δ (ppm): 147.17, 145.57, 132.24, 131.39, 129.78, 129.36, 128.72, 128.03, 115.91, 109.47, 107.5396 **HRMS (**ESI**)**: Calc. for $C_{13}H_8Cl_2N_2$ $[M+H]^+$: 262.0101,Obser.: 262.0531

^{1}H and ^{13}C NMR of the 2-(6-Chloroimidazo[1,2-a]pyridin-2-yl)phenol (3hc) White solid; yield 218 mg (89%); m.p. 197-199 °C; **^{1}H NMR** (500 MHz, DMSO) δ (ppm): 8.26 (d, 1H),

7.77 (s, 1H), 7.64 – 7.56 (m, 2H), 7.42 (dd, 2H), 7.38 (d, 1H), 6.98 (t, 1H), 6.91 (t, 1H), 1.27 (s, 1H); **^{13}C NMR** (126 MHz, $CDCl_3$) δ (ppm): 160.10, 145.32, 140.95, 130.76, 129.78, 128.03, 127.15, 121.02, 120.31, 119.66, 115.91, 109.47, 105.41. **HRMS** (ESI): Calcd. For $C_{13}H_9ClN_2O$ $[M+H]^+$ 244.0241; Obser.: 243.0321

^{1}H and ^{13}C NMR of the 8-(Phenoxymethyl)-2-phenylimidazo[1,2-a] pyridine (3ad) Pale yellow solid; yield 337 mg (89%); m.p. 130-131 °C**; ^{1}H NMR** (500 MHz, DMSO) δ (ppm): 8.16 – 7.84 (m, 2H), 7.63 (s, 1H), 7.42 (dd, 1H), 7.33 – 7.23 (m, 4H), 6.99 (dd, 2H), 6.91 (d, 1H), 6.89 (dd, 1H), 6.23 (d, 1H), 5.23 (s, 2H); **^{13}C NMR** (126 MHz, $CDCl_3$) δ (ppm): 159.22, 141.77, 140.85, 129.54, 129.09, 127.06, 126.52, 121.46, 120.45, 115.72, 108.45, 108.10, 112.5, 109.3, 103.4, 69.44 **HRMS** (ESI): Calc. For $C_{13}H_9ClN_2O$ $[M+H]^+$ 244.0241; Obser.: 243.0321.

^{1}H and ^{13}CNMR of the 2-methylimidazo[1,2-a] pyridine of (3ae) cherry-colored liquid; yield 237 mg (60%); m.p. 39-42 °C**; ^{1}H NMR** (500 MHz, $CDCl_3$) δ 8.20 (d, J = 6.7 Hz, 1H), 7.56 (d, J = 9.9 Hz, 1H), 7.49 (s, 1H), 7.17-7.06 (m, 1H), 6.90 (t, J = 6.7 Hz, 1H), 2.45 (s, 3H**) ^{13}C NMR** (126 MHz, $CDCl_3$) δ 143.46, 125.25, 124.04, 116.90, 111.85, 109.54, 14.51 **HRMS m/z** (ESI): calc. for $[C_8H_8N_2]$ $[M+H]^+$ 133.0767 Obser.:133.0759

^{1}H and ^{13}C NMR of the 2-pentylimidazo[1,2-a] pyridine of (3be) Pale yellow liquid; yield 137 mg (45%); m.p. 40-45 °C; **^{1}H NMR** (500 MHz, $CDCl_3$) δ (ppm): δ 8.20 (m, 1H), 7.57 (d, J = 9.0 Hz, 1H), 7.49 (s, 1H), 7.17 (m,1H), 6.90 (t, J = 6.8 Hz, 1H), 2.71 (t, J = 7.6 Hz, 2H), 1.79 (m, 2H), 1.32 (t, J = 7.4 Hz, 3H) **^{13}C NMR** (126 MHz, $CDCl_3$) δ 148.08, 144.91,

125.24, 123.91, 116.81, 111.71, 108.88, 29.70, 28.92, 22. **HRMS** m/z (ESI)**:**calc. for $[C_{10}H_{12}N_2]$ $[M+H]^+$: 161.1072,Obser.: 161.1067

^{1}H and ^{13}CNMR of the 2-propyllimidazo[1,2-a]pyridine of (3ce) Pale yellow liquid; yield 127 mg (48%); m.p. 45-50 °C**; ^{1}H NMR** (500 MHz, $CDCl_3$) δ (ppm): 8.20 (m, 1H), 7.57 (d, J = 9.0 Hz, 1H), 7.49 (s, 1H), 7.17 (m,1H), 6.90 (t, J = 6.8 Hz, 1H), 2.70 (t, J = 7.6 Hz, 2H), 1.78 (m, 2H), 1.04 (t, J = 7.4 Hz, 3H) **^{13}C NMR** (126 MHz, $CDCl_3$) δ (ppm): δ 147.88,144.96, 125.27, 123.96, 116.86, 111.76, 109.02, 30.99, 22.59, 14.05 **HRMS** (ESI): calc. for $[C_{10}H_{12}N_2$ $[M+H]^+$: 161.1073 Obser.: 161.1068

^{1}H and ^{13}CNMR of the 2-isopropylimidazo[1,2-a] pyridine (3de) White solid; yield 157 mg (40%); m.p. 80-100 °C**; ^{1}H NMR** (500 MHz, $CDCl_3$) δ (ppm): δ 8.20 (dt, J = 6.8, 1.2 Hz, 1H), 7.57 (dd, J = 9.1, 0.7 Hz, 1H), 7.49 (s, 1H),7.17 (m, 1H), 6.90 (td, J = 6.8, 1.1 Hz, 1H), 2.97 (m, 1H), 1.35 (d, J = 4 Hz, 6H) **^{13}C NMR** (126 MHz, $CDCl_3$) δ (ppm): 154.13, 144.96, 125.43, 123.97, 117.03, 111.72, 107.43, 28.44, 22.51 **HRMS** (ESI): Anal. Calcd. For HRMS m/z (ESI): calc. for $[C_{10}H_{12}N_2]$ $[M+H]^+$: 161.1073 Obser.: 161.1076

3.7Some spectrum of 2-phenyl-1H-benzo[d]imidazole derivatives of ^{1}HNMR,^{13}C NMR and HRMS.

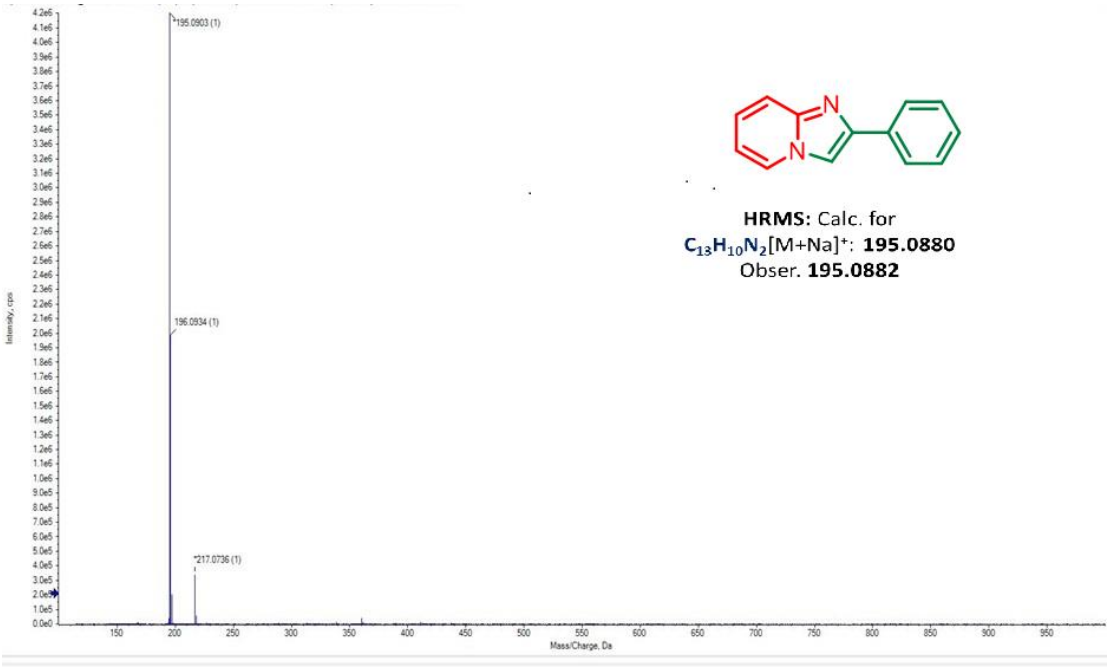


Figure 3.4 Mass spectra of the 2-phenylimidazo[1,2-a] pyridine (3a)

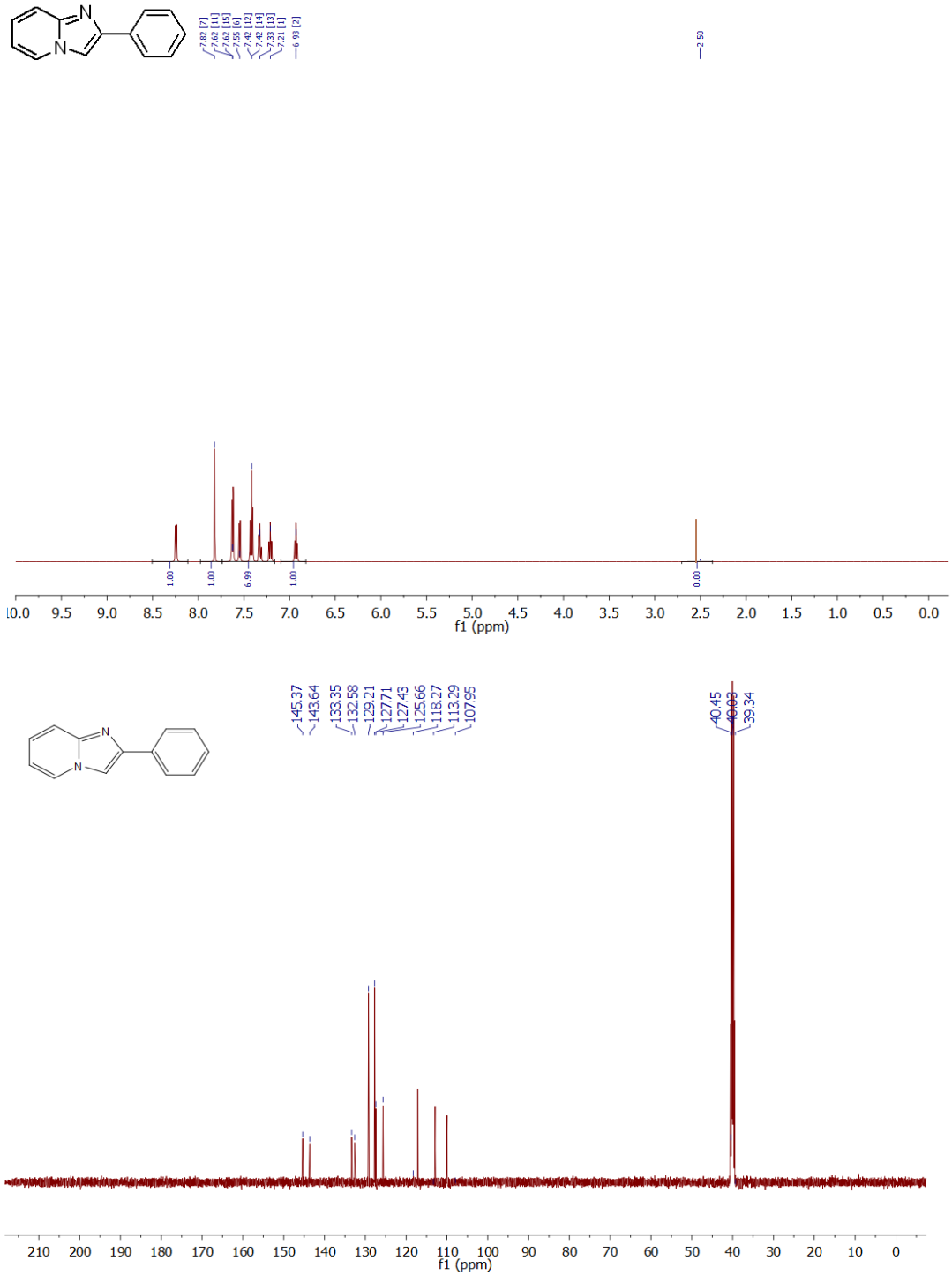


Figure 3.5 ^{1}H NMR and ^{13}C NMR of the 2-phenylimidazo[1,2-a] pyridine (3a)

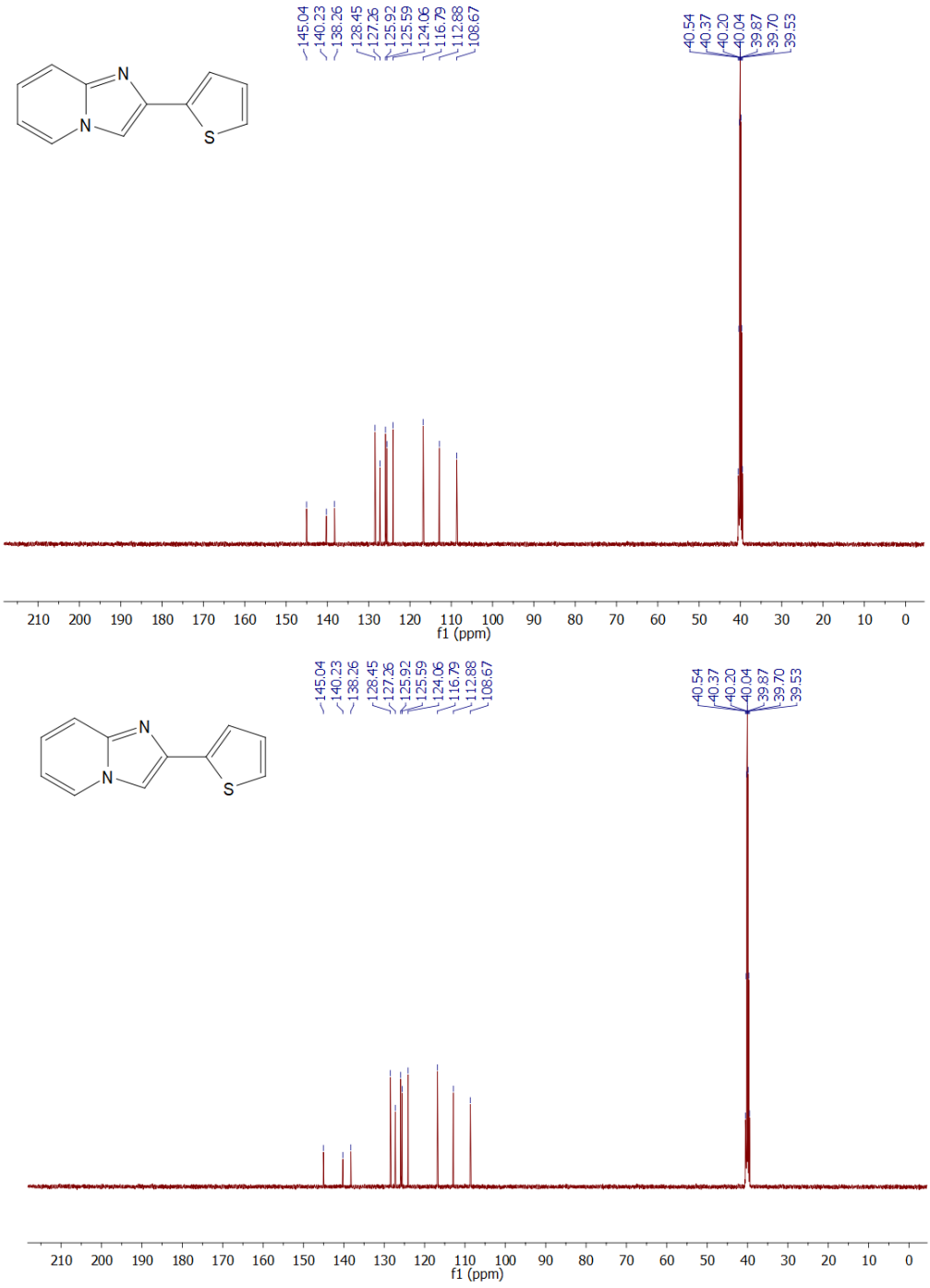


Figure 3.6 ^{1}H NMR and ^{13}C NMR 2-(Thiophen-2-yl)imidazo[1,2-a]pyridine (3ka)

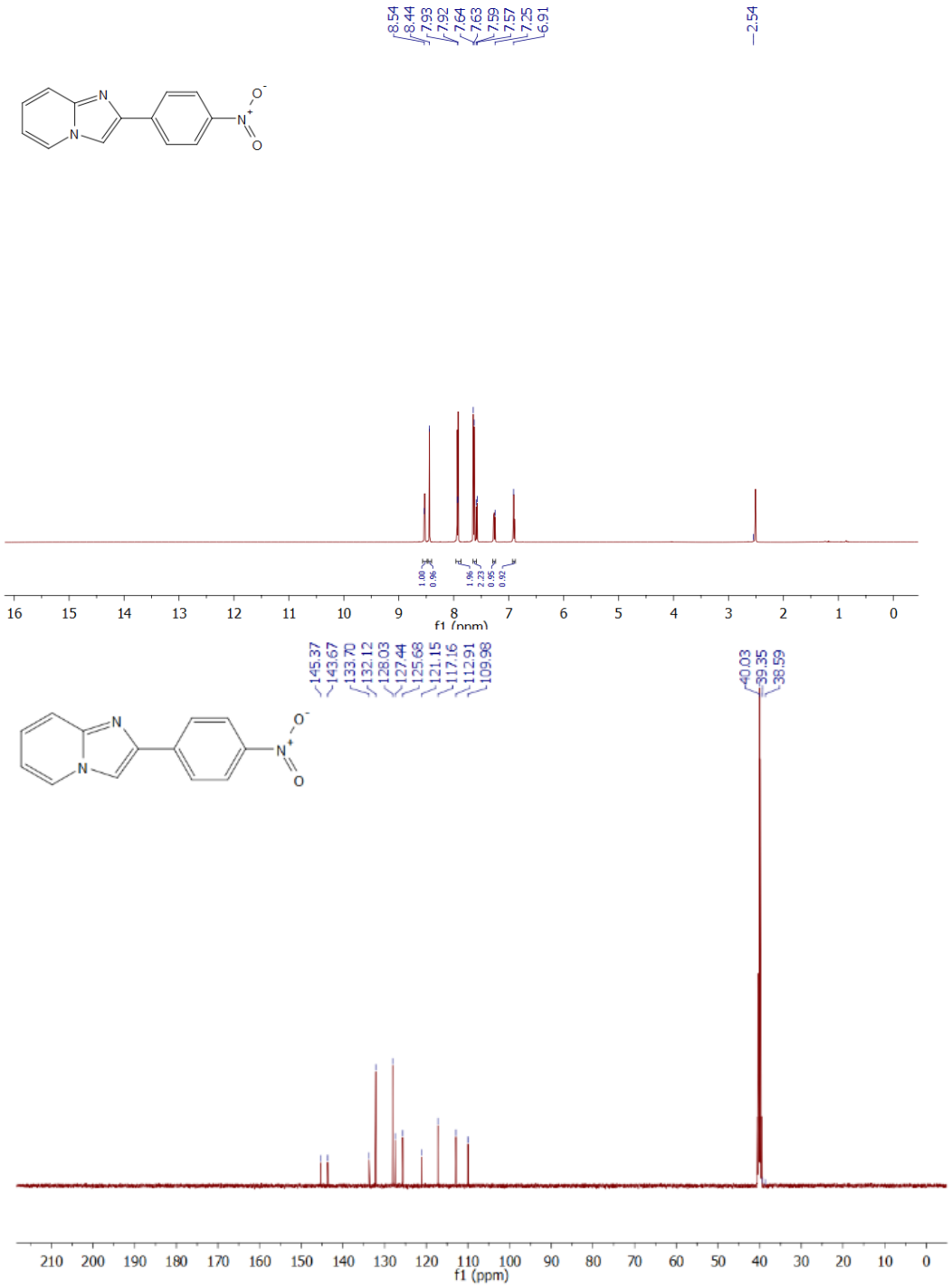


Figure 3.7 ^{1}H NMR and ^{13}CNMR spectra of 2-(4-nitrophenyl) imidazo[1,2-a]pyridine (3ha)

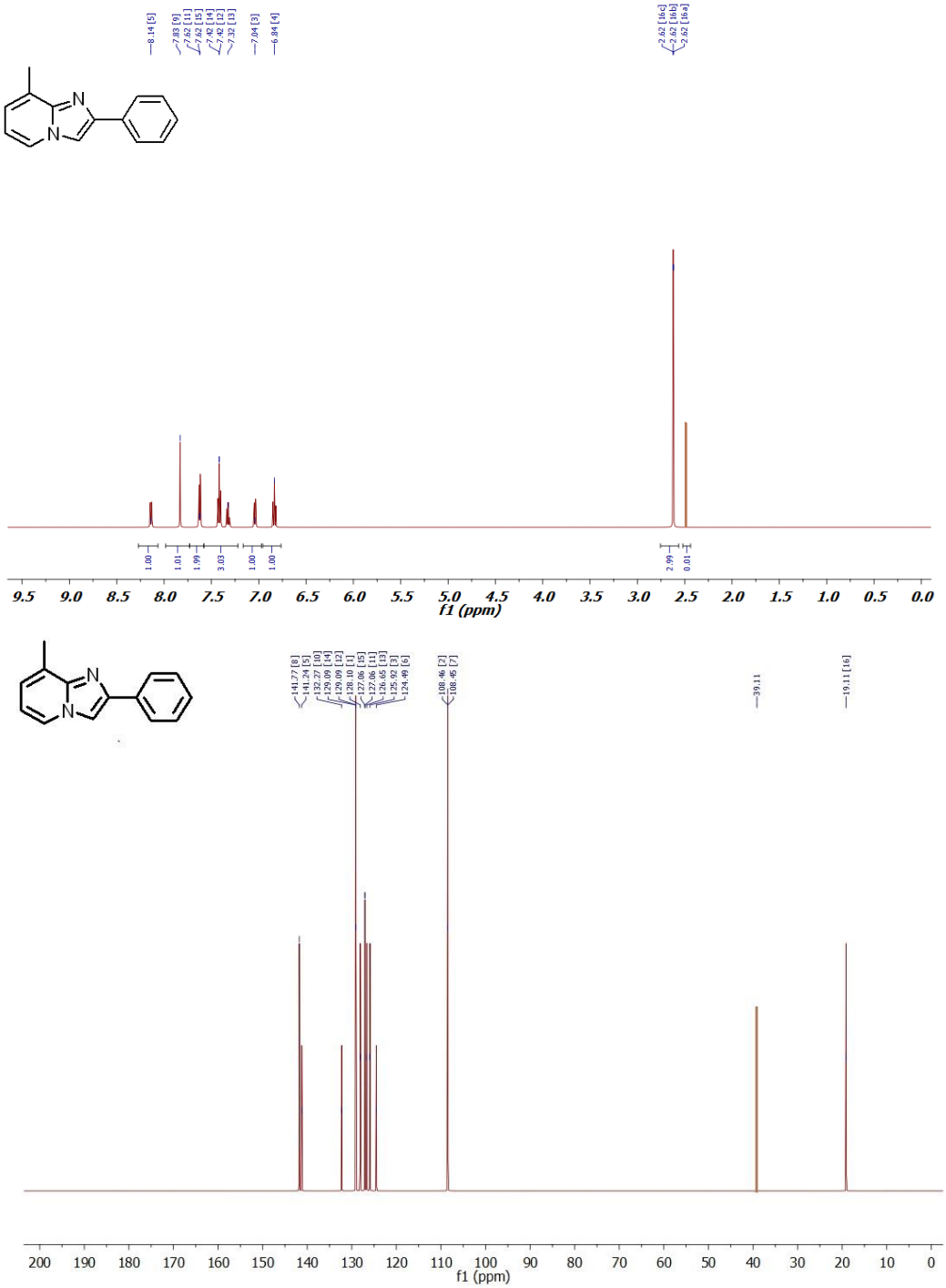


Figure 3.8 ^{1}H NMR and ^{13}CNMR spectra of 8-Methyl-2-phenylimidazo[1,2-a]pyridine (3ab)

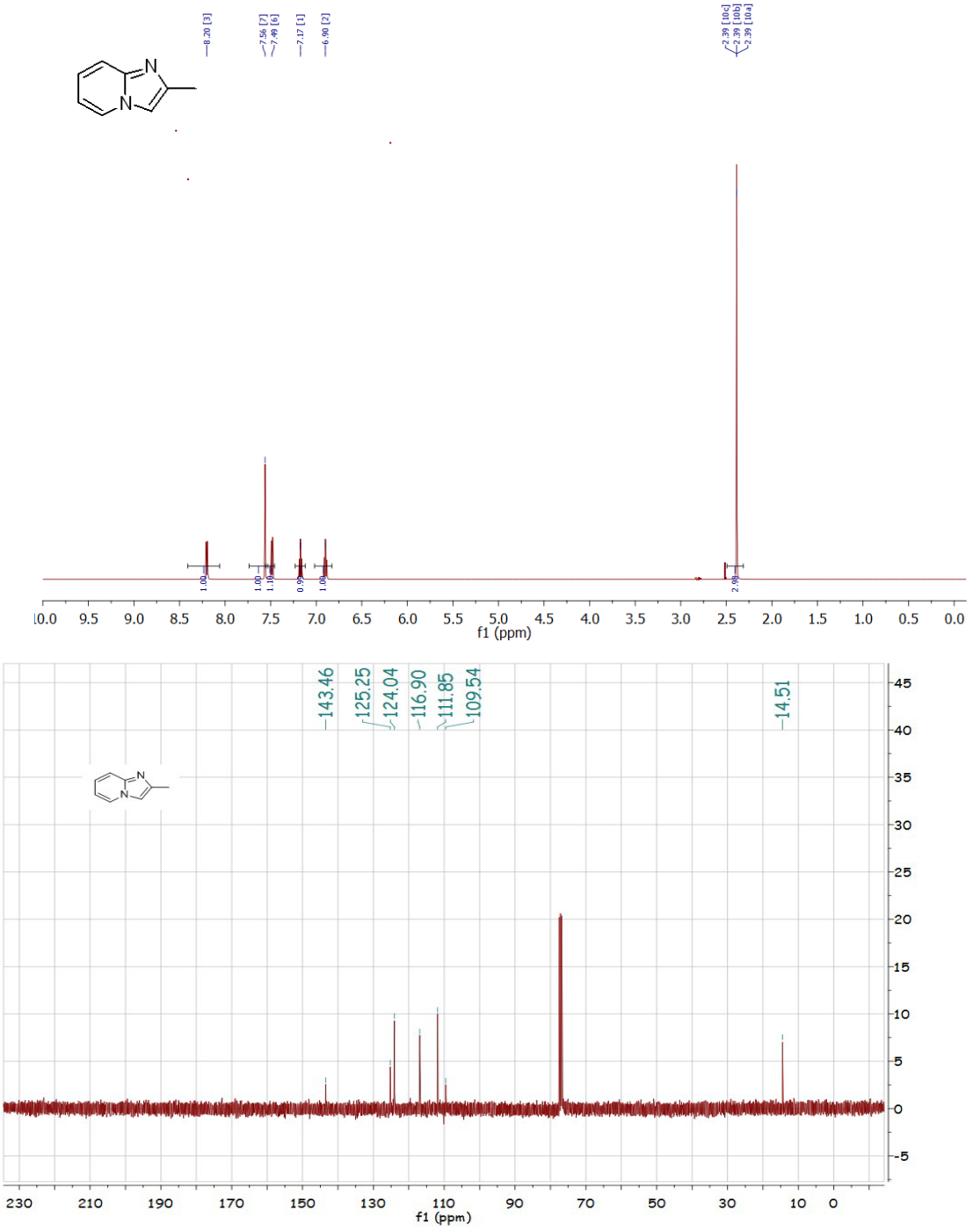


Figure 3.9 ^{1}H NMR and ^{13}CNMR spectra of 2-methylimidazo[1,2-a] pyridine of (3ae)

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