Section I
SYNTHESIS OF 5-OXO-5H-INDENO[1,2-b]PYRIDINE-3 CARBONITRILE DERIVATIVES

6.1.1 Introduction

The highly conjugated, yellow alkaloid onychine was first isolated by De Almeida et al in 1976 from *Onychopetalum amazonicum* (Annonaceae) [1]. The indinopyridine moiety is like the 4-azafluorenone group of this alkaloid onychine. The naturally occurring onychine (Fig. 6.1) is having antimicrobial properties particularly against fungus *Candida albicans*.

![Onychine](image)

(Fig. 6.1) Onychine

The infections caused by this fungus are resistant to treatment and are opportunistic as they infect persons of weak immune system like AIDS patients and cancer patients undergoing chemotherapy [2]. The amphotericin-B which has used against *Candida albicans* has many side effects. Therefore the indinopyridines can be used as alternative to amphotericin-B.

Many indinopyridines are having the different biological activities like herbicidal, calcium antagonistic activators. They are also useful in the treatment of the neurological disorder as adenosine A2a receptor binding active and in the treatment of inflammation related disease as a phosphodiesterase inhibiting [3]. It has also anticandidal activity [4]. Along with these activities they are also show wide range of pharmaceutical activities such as antiepileptic, antimalarial, vasodilator, anesthetic, anticonvulsant and agricultural use such as fungicidal, pesticidal, herbicidal [5].
6. I.2 Literature Review

The 1-Methyl-4-azafluoren-9-one had been synthesized previously by Bowdcn et al [6] was prepared again by another route a year later by Prostakov et al [7]. There are several method are available for the synthesis of 5H-indeno[1,2-b]pyridin-5-ones. It can be synthesise by Knoevenengel condensation reaction of 1,3-indandione and aromatic aldehyde with phenyl acetonitrile and ammonium acetate [8]. The oxidative thermal rearrangement of 2-indanone oxime O-allyl ethers also give the 5H-indeno[1,2-b]pyridin-5-ones [9]. By the direct cyclization of 2-aryl-3-methylpyridines followed by oxidation gives the same product [10]. Most of these methods have serious drawbacks as more work-up and purification process, strong acid or base condition, multistep reactions, occurrence of side reactions and more expensive use of reagents. These reactions require harsh conditions.

The synthesis of substituted indeno[1,2-b]pyridine are made by multi-component reactions under microwave irradiation [11] (Scheme 6.1).

(Scheme 6.1)

The another methods are the cyclization of 2-aryl-3-nicotinic acids by the use of polyphosphoric acid [12], Pummerer reaction of imidosulfoxides [13] (Scheme 6.2), Pd(0)-catalyzed cross-coupling reaction between arylboronic acids and 2-halopyridines [14].
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Recently the synthesis of indeno[1,2-b]pyridine is made by use of catalyst L-proline [15] (Scheme 6.3).

The another way for preparation of indeno[1,2-b]pyridine is use of ceric ammonium nitrate (CAN) [16] (Scheme 6.4).
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The application of such MCRs toward the synthesis of 5H-indeno[1,2-b]pyridin-5-ones gives an important and subject in organic synthesis seeing as these products has wide applications in preparative organic chemistry. Due to elevated reactivity, simple procedure, exceptional efficiency, and atom-economy, multicomponent reactions (MCRs) have gained considerable interest [17-18] (Scheme 6.5).

(Scheme 6.5)

Other general method for the synthesis of indeno[1,2-b]pyridine is reaction of active methylene with amine and aldehyde. In next step oxidation is carried out by chromium (VI) oxide [19] (Scheme 6.6).

(Scheme 6.6)
The modification of Hantzsch reaction is done for three component reaction. Here oxidation is carried out with DDQ or MnO₂ instead of CrO₃ as shown below [20] (Scheme 6.7).

(Scheme 6.7)

The nanocrystalline metal oxides have great significance as catalyst in different organic reactions. Because of their coordination parts and high surface to volume ratio they provide a larger number of active sites for reactions [21-22]. Recent years CuO nanoparticles get interested because of their use as semiconductor [23] along with in preparation of organic and inorganic nanocomposites [24]. In addition, copper oxide nanoparticles cheaply available, requires only mild reaction conditions for producing high yields in short reaction time [25] (Scheme 6.8).

(Scheme 6.8)
The nanocrystalline copper(II) oxide-catalyzed one-pot four component synthesis of polyhydroquinoline derivatives are synthesized under solvent-free conditions as shown below [26] (Scheme 6.9).

![Scheme 6.9]

The Cu(II)O-NPs have been used as an efficient heterogeneous catalyst in different organic reactions as: cross-coupling reactions [27], C-acylation reaction [28] and CO and NO oxidation reactions [29]. The CuO-NPs are also used in the multi-component reactions [30] (Scheme 6.10).

![Scheme 6.10]

6. I.3 Present Work
6. I.3.1 Synthesis of CuO Nanoparticles

The CuO-nanoparticles (NPs) were prepared by the co-precipitation method [31]. A solution of copper acetate (1.0 gm) and acetic acid (1.0 mL) in 250 mL of distilled water was heated at 100°C. Then 0.8 gm of NaOH was added.
added quickly under vigorous starring. The reaction mixture being cooled to room temperature and the obtained black powder were separated by centrifugation. The collected precipitate then washed several times with distilled water, ethanol and dried at 100°C for 10 hrs.

6.1.3.2 Optimization of Model Reaction

In earlier studies, to optimize the reaction conditions, reaction of 3-nitro benzaldehyde, malonitrile, 1,3-Indandione and ammonium acetate is chosen as model reaction for one-pot synthesis of corresponding 5-oxo-5H-indeno[1,2-b]pyridine-3-carbonitrile derivatives (Scheme 6.11).

![Scheme 6.11]

The reaction conditions were optimized on the basis of the solvent and concentration of catalyst (Table 6.1). The influence of solvent was studied when the model reaction was performing by using methanol, ethanol and water. Increasing the amount of catalyst does not show any significant changes in the yield and time of reaction. As the result of this experiment, the best results are obtained when the reactions were carried out in presence of CuO-NPs (0.1 mmol, 10 mol%, 0.0079 gm) under water as solvent at room temperature. The significant result of are related to the reactivity of catalytic nanoparticles which is largely determined by the energy of surface atoms that can be easily gauges with the number of neighboring atoms by the bonding modes and accompanying energies of small molecules to be transformed on the nanoparticles surface.
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(Table 6.1) The optimization of model reaction by using various solvents and concentrations of catalyst

<table>
<thead>
<tr>
<th>Entry</th>
<th>Catalyst (mol %)</th>
<th>Solvent</th>
<th>Time (min)</th>
<th>Yield (%)</th>
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<td>Methanol</td>
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<td>CuO-NPs (20)</td>
<td>Methanol</td>
<td>50</td>
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</tr>
<tr>
<td>6</td>
<td>CuO-NPs (20)</td>
<td>Water</td>
<td>30</td>
<td>84</td>
</tr>
</tbody>
</table>

a Reaction conditions: 3-nitro benzaldehyde (1mmol), malanonitrile (1mmol), 1,3-Indandione (1mmol) and ammonium acetate (1.5 mmol).

b Isolated yield.

In order to establish optimum ratio of reactants the model reaction is carried out several times in the presence of CuO nanoparticles. The best results were obtained when 3-nitro benzaldehyde, malanonitrile, 1,3-Indandione and ammonium acetate were employed as substrates in a 1:1:1:1.5 ratio. To study the scope of this process, we next utilize variety of aldehydes to investigate four-component reactions under optimal conditions (Scheme 6.11).

6. I.3.3 Reusability of CuO nanoparticles catalyst

Finely, we were interested in studying the reusability of the catalyst due to economic and environmental aspect. For this purpose, the reaction of 3-nitro benzaldehyde, malanonitrile, 1,3-Indandione and ammonium acetate was chosen as the model reaction in presence of CuO nanoparticles. At the end of each turn, catalyst was recovered from the reaction mixture by dilution of the mixture with chloroform, simple filtration and drying at 100°C. The catalyst was reused several times without significant loss of its activity (Table 6.2).
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(Table 6.2) Reusability of the CuO nanoparticles

<table>
<thead>
<tr>
<th>Run</th>
<th>Yield of 6A (%)</th>
<th>Catalyst recovery (%)</th>
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<tbody>
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<td>1</td>
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<td>97</td>
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<tr>
<td>2</td>
<td>80</td>
<td>94</td>
</tr>
<tr>
<td>3</td>
<td>78</td>
<td>91</td>
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<tr>
<td>4</td>
<td>75</td>
<td>90</td>
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6. 1.3.4 General Procedure for Synthesis of 5-Oxo-5H-Indeno[1,2-b]Pyridine-3-Carbonitrile Derivatives

The one-pot three-component condensation reactions is done in distilled water (5 ml) with 3-nitro benzaldehyde 1 (1 mmol, 0.151 gm) and malanoniitrile 2 (1.1 mmol, 0.072 gm) in presence of CuO-NPs (0.1 mmol, 10 mol%, 0.0079 gm) is stirred at room temperature for 15 minutes and addition of 1,3-Indandione (1 mmol, 0.146 gm) 3 was done in presence of ammonium acetate (1.5 mmol, 0.115 gm) 4, reaction is proceeded spontaneously at room temperature (Scheme 6.12).

The completion of the reaction is monitored on TLC. Then reaction mixture was dissolved in chloroform. The catalyst was insoluble in CHCl₃ and separated. The solvent was evaporated and desired solid material obtained was filtered and washed with distilled water and dried. The solid obtained was recrystallized with chloroform:ethanol mixture (1:2) to afford the pure 5-oxo-5H-indeno[1,2-b]pyridine-3-carbonitrile derivatives.

(Scheme 6.12)
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The plausible mechanism for this reaction is shown in below (Scheme 6.13). In the first step Knoevengel condensation reaction occurs between aldehyde 1 and malanoniitrile 2. In the second step 1,3-indandione 3 reacts with the Knoevengel product by Michael addition reaction to form intermediate 4. After addition of NH₄OAc oxygen is replaced by nitrogen, which on cyclization forms product 6. In the last step oxidation occurs followed by 1,4, elimination of water molecule giving final product 5-Oxo-5H-Indeno[1,2-b]Pyridine -3-Carbonitrile.

(Scheme 6.13)

The physical and analytical data for synthesis of desired 5-oxo-5H-indeno[1,2-b]pyridine-3-carbonitrile derivatives are shown below (Table 6.3).

(Table 6.3) The physical and analytical data of 5-Oxo-5H-Indeno[1,2-b] Pyridine-3-Carbonitrile derivatives.
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<th>Entry</th>
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<th>Yield (%)</th>
<th>M. P. (°C)</th>
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<td>234</td>
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<td>6B</td>
<td>4-O₂NC₆H₄</td>
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<td>3</td>
<td>6C</td>
<td>4-FC₆H₄</td>
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<td>125</td>
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<td>4</td>
<td>6D</td>
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<tr>
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## Chapter 6: Synthesis of 5-oxo-5H-indeno[1,2-b]pyridine-3-carbonitrile derivatives and their antimicrobial activity

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<td>2,3,4-((H$_3$CO)$_3$C$_6$H$_2$</td>
<td><img src="image" alt="Structure 6R" /></td>
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<td>19</td>
<td>6S</td>
<td>C_{10}H_{7}</td>
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<td>79</td>
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<td>20</td>
<td>6T</td>
<td>C_{12}H_{9}</td>
<td>40</td>
<td>76</td>
<td>105</td>
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</table>

The compounds (6A to 6T) are confirmed by spectroscopic technique like IR, $^1$H NMR, $^{13}$C NMR. The data obtained is in good agreement with the proposed structure, which confirms formation of desired compounds.

6. I.4 Experimental

The melting points are uncorrected and were determined in an open capillary. Infrared spectra (in KBr pellets) were measured with on spectrophotometer of a Perkin Elmer Spectrum 100. The $^1$H and $^{13}$C NMR spectra were recorded on a Bruker Spectrospin Avance II-300 MHz spectrophotometer using DMSO-d$_6$ solvents and tetramethylsilane as an internal standard. Chemical shifts are given in the delta scale (ppm). Mass spectra were analyzed on a Shimadzu QP 2010 GCM. The 1,3-Indandione was purchased from Alfa Aesar chemicals. The purity of the compounds was checked by using TLC Silica gel 60-F254 plates. The XRD was done on X-ray Powder Diffractometer of Bruker AXS Analytical Instruments Pvt. Ltd. Germany Model: D2 PHASER. The SEM analysis was done using Scanning Electron Microscope of JEOL-JSM-6360, Germany.
6.1.5 Result and Discussion

The structures of 5-oxo-5H-indeno[1,2-b]pyridine-3-carbonitrile derivatives were confirmed from IR, $^{1}H$ NMR, $^{13}C$ NMR and Mass spectrometry data. The IR spectrum of compound (6I) (Fig. 6.7) was showed the broad peak at 3350 cm$^{-1}$ is for hydroxyl group of aldehyde, peaks of primary amine group at 3080 cm$^{-1}$, for nitrile group it shoes peak at 2226 cm$^{-1}$ for carbonyl ketone group it shows peak at 1722 cm$^{-1}$. It also shows peak for (C=N) group at 1666 cm$^{-1}$ and for (C-N) it shows peak at 1256 cm$^{-1}$ resembling the formation of desired derivative.

The $^{1}H$ NMR of same compound (6I) (Fig. 6.8) showed the phenolic broad proton of at 10.59 $\delta$ (ppm). It exhibited the signal of amine group (-NH$_2$) proton attached to aromatic ring at 8.43-8.41 $\delta$ (ppm), along with peak for aromatic protons at aromatic region of 7.86-7.68 $\delta$ (ppm) suggest the formation of desired multi-component product. Similarly the $^{1}H$ NMR of Also the compound (6Q) (Fig. 6.23) shows multiplet peaks for two methoxy group attached to aromatic (-OCH$_3$) of aldehyde at 3.91-3.88 $\delta$ (ppm). Similarly the $^{13}C$ NMR spectra are given all the corresponding carbo ns for carbonyl group and aromatic carbon presents in the structure.

Finally, the structure of formed compound (6I) (Fig. 6.10), (6L) (Fig. 6.13), (6N) (Fig. 6.19) and (6Q) (Fig. 6.25) was confirmed from the GCMS analysis. Compound (6L) (Fig. 6.13) shows exact mass peak at (m/z) 329M$^+$. For compound (6N) (Fig. 6.19) the peak at (m/z) 287 M$^+$ obtained due to loss of NH$_2$ group from the compound. For the compound (6Q) (Fig. 6.25) the peak at (m/z) 279 M$^+$ appear due to loss of one –OCH$_3$ and NH$_2$ loss. Due to bulky molecules exact mass peaks (M$^+$) was not obtained for some compounds. The data obtained is in good agreement with the proposed structure.

6.1.6 Conclusions

In the view of recent interest in the use of heterogeneous nanocatalysts we have developed CuO-NPs as recyclable, easy to handle, inexpensive, non-
volatile, non-explosive and eco-friendly catalyst which can be used in many organic transformations. The synthesis of desired 5-oxo-5H-indeno[1,2-b]pyridine-3-carbonitrile derivatives as shown in the (Scheme 6.2), were done by four component reaction of aldehyde with malononitrile, 1,3-indandione and ammonium acetate at room temperature in presence of CuO-NPs as catalyst and water as solvent. The reusability of catalyst is also an important aspect. The multi-component, single step, room temperature condition and water as eco-friendly solvent give the process valuable mark. The reaction gives good yield in short span of time.

### 6. I.7 Characterization of CuO Nanoparticles

The structural study of CuO nanoparticles (NPs) were done by using powder X-ray diffraction (PXRD) (Fig. 6.2) and morphological study using scanning electron microscope (SEM) (Fig. 6.3) respectively. The matching of powder XRD pattern with simulated XRD pattern of crystal structure data reported by Niggli et al [27] reveals the phase pure monoclinic structured CuO (II) nanoparticles. The nanocrystalline nature of CuO (II) powder also clarified by calculating the crystallite size for (111) reflection by using the Scherrer equation \(D = \frac{K\lambda}{\beta\cos\theta}\), and it is 8.04 nm. Where, \(K\) is the shape factor (0.9), \(\lambda\) is the wavelength of X-rays and \(\beta\) is the corrected full width at half-maximum. The SEM analysis of CuO nanoparticles carried out at different magnification shows the agglomeration nature.
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(Fig. 6.2) XRD Spectrum of CuO Nanoparticles,
(a) = simulated XRD pattern 
(b) = XRD pattern of the material
(Fig. 6.3) Scanning Electron Microscope (SEM) images of CuO(II)NPs at different magnification as A=500; B=2000; C=5000; D=10,000; E=15,000; F=20,000 for 20kV.
6. 1.8 Spectral Data of Synthesized Compounds

2-Amino-4-(3-nitrophenyl)-5-oxo-5H-indeno[1,2-b]pyridine-3-carbonitrile (Table 6.3, Entry 6A)

(Fig. 6.4)

(6A) Yellow solid; Yield=83%; M.P. = 234 °C; IR (KBr, cm⁻¹) 3094, 3069, 2945, 1730, 1686, 1530, 1350. ¹H NMR (300 MHz, DMSO d₆) δ (ppm): 9.57 (s, 1H, N-H), 8.69-8.68 (d, 1H, Ar-H), 8.44-8.42 (d, 1H, Ar-H), 8.05-8.01 (m, 5H, Ar-H), 7.87-7.83 (t, 1H, Ar-H), 3.32 (s, 2H, N-H). ¹³C NMR (300 MHz, DMSO d₆) δ (ppm): 189.25, 188.60, 158.00, 157.00, 148.42, 143.05, 138.45, 135.96, 135.83, 134.23, 131.60, 129.75, 127.98, 127.50, 126.90, 126.76, 123.81, 123.68, MS: (m/z) 249, 165, 104, 76.

2-amino-4-(2,4-dichlorophenyl)-5-oxo-5H-indeno[1,2-b]pyridine-3-carbonitrile (Table 6.3, Entry 6H)

(Fig. 6.5)

(6H) Brown solid; Yield=70%; M.P. = 130 °C; IR (KBr, cm⁻¹) 3101, 3049, 2921, 2229, 1693, 1579, 1218. ¹H NMR (300 MHz, DMSO d₆) δ (ppm): 8.71-
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8.69 (d, 1H, Ar-H), 8.26 (s, 1H, N-H), 8.19-8.14 (m, 1H, Ar-H), 8.05-8.00 (m, 2H, Ar-H), 7.90-7.85 (d, 2H, Ar-H), 7.52 (s, 1H, N-H), 7.45-7.38 (m, 1H, Ar-H), 13C NMR (300 MHz, DMSO d₆) δ (ppm): 197.38, 189.23, 154.52, 143.33, 142.40, 140.28, 138.95, 138.16, 135.63, 135.55, 134.47, 130.69, 130.08, 129.74, 128.32, 127.10, 123.54, 123.17, 86.01.

2-amino-4-(4-hydroxyphenyl)-5-oxo-5H-indeno[1,2-b]pyridine-3-carbonitrile (Table 6.3, Entry 6I)

(Fig. 6.6)

(6I) Yellow solid; Yield=74%; M.P. = 185 °C; IR (Fig. 6.7) (KBr, cm⁻¹) 3350, 3080, 2923, 2226, 1722, 1666, 1293. ¹H NMR (Fig. 6.8) (300 MHz, DMSO d₆) δ (ppm): 10.59 (s, 1H, Ar-OH), 8.43-8.41(d, 2H, N-H), 7.86-7.80 (m, 7H, Ar-H), 7.68 (d, 1H, Ar-H), 13C NMR (Fig. 6.9) (300 MHz, DMSO d₆) δ (ppm): 190.39, 189.28, 163.71, 146.95, 143.36, 142.13, 139.71, 137.80, 135.71, 135.37, 135.18, 134.10, 125.40, 125.00, 122.95, 122.86, 116.87, 116.33, 45.25, MS (Fig. 6.10):(m/z) 249, 165, 104, 76.

2-amino-4-(3,4-dihydroxyphenyl)-5-oxo-5H-indeno[1,2-b]pyridine-3-carbonitrile (Table 6.3, Entry 6L)
Chapter 6: Synthesis of 5-oxo-5H-indeno[1,2-b]pyridine-3-carbonitrile derivatives and their antimicrobial activity

(6L) Yellow solid; Yield=79%; M.P. = 255 °C; IR (Fig. 6.12) (KBr, cm⁻¹) 3459, 3247, 2923, 1670, 1554, 1294, MS (Fig. 6.13): (m/z) 329.30 (M⁺).

2-amino-4-(4-methylphenyl)-5-oxo-5H-indeno[1,2-b]pyridine-3-carbonitrile (Table 6.3, Entry 6M)

(6M) Yellowish brown solid; Yield=94%; M.P. = 117 °C; IR (KBr, cm⁻¹) 3036, 2922, 2223, 1686, 1557, 1189. ¹H NMR (300 MHz, DMSO d₆) δ (ppm): 8.41-8.39 (d, 1H, Ar-H), 8.00-7.99 (d, 1H, Ar-H), 7.89-7.80 (d, 4H, Ar-H), 7.72 (s, 1H, N-H), 7.34-7.33 (d, 2H, Ar-H), 3.25 (s, 1H, N-H), 2.46 (s, 3H, Ar-CH₃), ¹³C NMR (300 MHz, DMSO d₆) δ (ppm): 197.41, 159.74, 147.09, 146.33, 143.33, 135.57, 135.24, 135.03, 134.45, 130.88, 130.59, 130.34, 129.62, 128.44, 123.21, 123.17, 113.97, 112.82, 81.21, 21.98.

2-amino-5-oxo-4-(thiophen-2-yl)-5H-indeno[1,2-b]pyridine-3-carbonitrile (Table 6.3, Entry 6N)
Chapter 6: Synthesis of 5-oxo-5H-indeno[1,2-b]pyridine-3-carbonitrile derivatives and their antimicrobial activity

(6N) Green solid; Yield=86%; M.P. = 150 °C; IR (Fig. 6.16) (KBr, cm\(^{-1}\)) 3065, 2921, 2224, 1688, 1585, 1206. \(^1\)H NMR (Fig. 6.17) (300 MHz, DMSO \(d_6\)) \(\delta\) (ppm): 8.27 (m, 3H, N-H & Ar-H), 8.06 (s, 1H, N-H), 7.91-7.90 (m, 4H, Ar-H), 7.38-7.31 (m, 1H, Ar-H). \(^1^3\)C NMR (Fig. 6.18) (300 MHz, DMSO \(d_6\)) \(\delta\) (ppm): 189.49, 188.93, 153.50, 143.58, 141.41, 139.92, 138.69, 136.75, 135.90, 135.72, 135.54, 129.23, 128.93, 124.11, 122.86, 122.76, 75.96, MS (Fig. 6.19): (m/z) 242, 212, 165, 158.

2-amino-4-(3-methoxyphenyl)-5-oxo-5H-indeno[1,2-b]pyridine-3-carbonitrile (Table 6.3, Entry 6P)

(6P) Brown solid; Yield=86%; M.P. = 140 °C; IR (KBr, cm\(^{-1}\)) 3078, 2930, 2229, 1721, 1681, 1229. \(^1\)H NMR (300 MHz, DMSO \(d_6\)) \(\delta\) (ppm): 8.46 (s, 1H, N-H), 8.036-8.032 (d, 1H, Ar-H), 7.85-7.82 (m, 3H, Ar-H), 7.46-7.40 (m, 3H, Ar-H), 7.16-7.13 (m, 2H, Ar-H), 3.80 (s, 3H, O-CH\(_3\)). \(^1^3\)C NMR (300 MHz, DMSO \(d_6\)) \(\delta\) (ppm): 190.25, 189.14, 159.72, 147.15, 142.58, 140.01, 135.41,
Chapter 6: Synthesis of 5-oxo-5H-indeno[1,2-b]pyridine-3-carbonitrile derivatives and their antimicrobial activity

135.23, 132.04, 130.57, 129.62, 127.71, 123.91, 123.35, 121.35, 120.77, 116.91, 114.07, 82.97, 55.52.

2-amino-4-(3,4-dimethoxyphenyl)-5-oxo-5H-indeno[1,2-b]pyridine-3-carbonitrile (Table 6.3, Entry 6Q)

(Fig. 6.21)

(6Q) Green solid; Yield=73%; M.P. = 190 °C; IR (Fig. 6.22) (KBr, cm⁻¹) 3088, 2922, 2222, 1713, 1676, 1275. ¹H NMR (Fig. 6.23) (300 MHz, DMSO d₆) δ (ppm): 8.69 (s, 1H, N-H), 8.35 (s, 1H, Ar-H), 8.05-8.02 (d, 1H, Ar-H), 7.97-7.94 (m, 2H, Ar-H), 7.63-7.59 (d, 1H, Ar-H), 7.23-7.15 (m, 2H, Ar-H), 3.79 (s, 1H, N-H), 3.91-3.88 (m, 6H,CH₃ ), ¹³C NMR (Fig. 6.24) (300 MHz, DMSO d₆) δ (ppm): 189.82, 189.20, 160.66, 154.44, 153.92, 148.74, 148.32, 146.41, 135.68, 135.54, 131.10, 127.27, 126.10, 124.13, 122.78, 115.78, 114.04, 112.01, 76.70, 56.06, 55.49, MS (Fig. 6.25):(m/z) 293, 279, 263, 251.

2-amino-4-(naphthalen-1-yl)-5-oxo-5H-indeno[1,2-b]pyridine-3-carbonitrile (Table 6.3, Entry 6S)

(Fig. 6.21)
Chapter 6: Synthesis of 5-oxo-5H-indeno[1,2-b]pyridine-3-carbonitrile derivatives and their antimicrobial activity

(Fig. 6.26)

(6S) Yellow solid; Yield=79%; M.P. = 155°C; IR (KBr, cm\(^{-1}\)) 3051, 2923, 1680, 1591, 1229. \(^1\)H NMR (300 MHz, DMSO d\(_6\)) \(\delta\) (ppm): 8.59 (s, 1H, N-H), 8.54-8.52 (d, 1H, Ar-H), 8.20-8.18 (d, 2H, Ar-H), 8.08-8.04 (m,2H, Ar-H), 7.99-7.97 (m, 3H, Ar-H), 7.74-7.61 (m, 4H, Ar-H), \(^13\)C NMR (300 MHz, DMSO d\(_6\)) \(\delta\) (ppm): 189.49, 188.93, 153.50, 143.58, 141.41, 140.49, 139.92, 139.81, 138.69, 136.75, 135.90, 135.72, 135.54, 135.34, 129.23, 128.93, 124.11, 122.86, 122.76, 114.38, 113.00, 75.96.

2-amino-4-(anthracen-9-yl)-5-oxo-5H-indeno[1,2-b]pyridine-3-carbonitrile (Table 6.3, Entry 6T)

(Fig. 6.27)

(6T) Red solid; Yield=76%; M.P. = 105°C; IR (Fig. 6.28) (KBr, cm\(^{-1}\)) 3050, 2923, 1687, 1552, 1249. \(^1\)H NMR (Fig. 6.29) (300 MHz, DMSO d\(_6\)) \(\delta\) (ppm): 11.44 (s, 1H, N-H), 9.01-8.95 (m, 4H, Ar-H), 8.72 (s, 1H, N-H), 8.20-8.14 (m, m, 3H, Ar-H), 7.63-7.49 (m, 6H, Ar-H), \(^13\)C NMR (Fig. 6.30) (300 MHz, DMSO d\(_6\)) \(\delta\) (ppm): 194.27, 188.39, 187.41, 142.00, 140.58, 140.01, 136.06, 136.00, 135.29, 134.75, 131.38, 130.68, 130.52, 129.37, 129.31, 129.12, 128.80, 127.34, 126.75, 125.87, 125.74, 125.65, 124.42, 123.50, 123.42, 123.01.
Section II
ANTIMICROBIAL ACTIVITY

6. II.1 Introduction

The consumption of pesticides for pest control in agriculture picked after the introduction of high yielding varieties in 1966-67. The earliest fungicides were inorganic materials like sulphur, lime-sulphur & mercury compounds. Elemental sulphur has been recognized as fungicide for at least 170 years. The latter is the most important of the copper fungicide; Bordeaux mixture was discovered by Millarded (1882). The majority of protestants fungicides are directly toxic to fungi & so will show up as active against spore germination in-vitro tests. Development of systemic fungicides largely arisen from antibacterial action of Penicillium mould by Fleming (1929) & of Prontosil by Domagt (1935) & number of bactericides & antibiotics were examined as potential systemic fungicides eg. Sulpholamides.

This discovery of organic pesticides provides man with new & powerful weapon against insect pests diseases & weeds. So, there is need of work on new synthetic derivatives. The specific aim of the study is, due to toxic pesticides & their hazards in ecosystem some less toxic derivatives to the non target organisms to be synthesized.

They cause damage of millions of dollars to crop by causing plat diseases. It is necessary to apply certain chemical which is toxic to certain target fungi but harmless to human beings.

6. II.2 Materials and Method

6. II.2.1 Strains used for Antifungal Activity

The evaluation of antifungal activity we have used Aspergillus niger and Candida albicans. The details of the strains are given in the (chapter 5).
6. II.2.2 Strains used for Antibacterial Activity

The evaluation of antibacterial activity we have used *Staphylococcus aureus* (ATCC 6538) from gram-positive group of bacteria and *Escherichia coli* (ATCC 8739) selecting from gram-negative bacteria. The details of the strains are given in the (Chapter 4).

6. II.2.3 Compounds Selected for Activity

The compounds with structural variability which showing good result in preliminary study are selected for antifungal and antibacterial activity. The compounds 6A, 6I, 6N, 6Q and 6T (Table 6.4) are used for further detail study.

(Table 6.4) The compounds selected for antifungal and antibacterial activity.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Entry</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6A</td>
<td><img src="image" alt="6AStructure" /></td>
</tr>
<tr>
<td>2</td>
<td>6I</td>
<td><img src="image" alt="6IStructure" /></td>
</tr>
<tr>
<td>3</td>
<td>6N</td>
<td><img src="image" alt="6NStructure" /></td>
</tr>
</tbody>
</table>
6. II.3 Experimental Procedure for Antifungal Screening

**Agar Well Diffusion Method:**

Antifungal activity was tested by agar well diffusion method. The procedure is discussed in the (Chapter 5).

6. II.4 Experimental Procedure for Antibacterial Screening

**Agar Well Diffusion Method:**

Antibacterial activity was tested by agar well diffusion method on nutrient agar plates. The organisms used in the present study were obtained from the laboratory stock. The procedure is discussed in the (Chapter 4).

6. II.5 Results and Discussion

The zone of inhibition at two different concentrations was recorded. These are reported in the (Table 6.5) and (Table 6.6) as shown in the below.
(Table 6.5) The Antifungal Screening: zone of inhibition for different compounds.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Entry</th>
<th>Aspergillus niger</th>
<th>Candida albicans</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>50 (µg/ml)</td>
<td>100 (µg/ml)</td>
</tr>
<tr>
<td>1</td>
<td>6A</td>
<td>6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>14</td>
</tr>
<tr>
<td>2</td>
<td>6I</td>
<td>--</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>6N</td>
<td>--</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>6Q</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>5</td>
<td>6T</td>
<td>--</td>
<td>4</td>
</tr>
<tr>
<td>8</td>
<td>Standard (Bavistin) (10µg/ml)</td>
<td>30</td>
<td>34</td>
</tr>
<tr>
<td>9</td>
<td>Solvent (DMSO)</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>10</td>
<td>Control</td>
<td>--</td>
<td>--</td>
</tr>
</tbody>
</table>

<sup>a</sup>Zone inhibition values are given in millimeters, ‘--’ no inhibition, <sup>b</sup>NT= Not taken
(Table 6.6) The Antibacterial screening: zone of inhibition for different compounds.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Entry</th>
<th>Zone of Inhibition (mm)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><strong>Staphylococcus aureus</strong></td>
<td><strong>Escherichia coli</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td>50 (µg/ml)</td>
<td>100 (µg/ml)</td>
</tr>
<tr>
<td>1</td>
<td>6A</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>2</td>
<td>6I</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>3</td>
<td>6N</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>4</td>
<td>6Q</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>5</td>
<td>6T</td>
<td>8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12</td>
</tr>
<tr>
<td>8</td>
<td>Standard Ampicillin (10µg/ml)</td>
<td>23</td>
<td>20</td>
</tr>
<tr>
<td>9</td>
<td>Solvent (DMSO)</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>10</td>
<td>Control</td>
<td>--</td>
<td>--</td>
</tr>
</tbody>
</table>

<sup>a</sup> Zone inhibition values are given in millimeters, ‘--’ no inhibition.

The photograph showing zone of inhibition of compound (6A) (Fig. 6.31) against fungus *Aspergillus niger*, compounds (6I and 6T) (Fig. 6.32) against fungus *Candida albicans* and compound (6I) (Fig. 6.33) against fungus *Escherichia coli* for 50 (µg/ml) and 100 (µg/ml) concentrations.
(Fig. 6.31) The Zone of Inhibition for Compound 6A (Table 6.3, Entry 6A)

(Fig. 6.32) The Zone of Inhibition for Compound 6I and 6T (Table 6.3, Entry 6I and 6T)
(Fig. 6.33) The Zone of Inhibition for Compound 6I
(Table 6.3, Entry 6I)

The structure of most active compounds (6A) against *Aspergillus niger*, compound (6I) (Fig. 6.34) active against *Candida albicans* and *Escherichia coli*, and (6T) against *Staphylococcus aureus* are shown.

(Fig. 6.34) The Compound 6I active against
*Candida albicans* and *Escherichia coli*. (Table 6.3, Entry 6I)
6. II.6 Conclusions

First the synthesis of desired 5-oxo-5H-indeno[1,2-b]pyridine-3-carbonitrile were done by four component reaction of aromatic aldehydes, malanoniitrile, 1,3-indandione and ammonium acetate at room temperature using CuO-NPs as catalyst in water as eco-friendly solvent.

In the section second the antifungal activity and antibacterial activities were carried out. For antifungal activity was screened against the two fungal species *Aspergillus niger* and *Candida albicans*. The agar well diffusion method is used for the antifungal activity. The zone of inhibition at two different concentrations was recorded after the 48 hr. The compound 2-amino-4-(3-nitrophenyl)-5-oxo-5H-indeno[1,2-b]pyridine-3-carbonitrile (6A) is good against *A. niger* with zone of inhibition 14 mm and compound 2-amino-4-(4-hydroxyphenyl)-5-oxo-5H-indeno[1,2-b]pyridine-3-carbonitrile (6I) against *C. albicans* with 18 mm zone of inhibition at 100 µg/ml concentration.

The antibacterial activity was carried out against two bacterial species *S. aureus* and *E. coli*. The disc diffusion method is used for the antibacterial activity. The zone of inhibition at two different concentrations was recorded after the 24 hr. The compounds 2-amino-4-(4-hydroxyphenyl)-5-oxo-5H-indeno[1,2-b]pyridine-3-carbonitrile (6I) is active against *E. coli* with zone of inhibition 17 mm and compound 2-amino-4-(anthracen-9-yl)-5-oxo-5H-indeno[1,2-b]pyridine-3-carbonitrile (6T) is most active against *S. aureus* with zone of inhibition 12 mm at 100µg/ml concentration.
Chapter 6: Synthesis of 5-oxo-5H-indeno[1,2-b]pyridine-3-carbonitrile derivatives and their antimicrobial activity

(Fig. 6.3) IR Spectrum of 2-Amino-4-(3-nitrophenyl)-5-oxo-5H-indeno[1,2-b]pyridine-3-carbonitrile (Table 6.3, Entry 6A)

(Fig. 6.7) IR Spectrum of 2-Amino-4-(4-hydroxyphenyl)-5-oxo-5H-indeno[1,2-b]pyridine-3-carbonitrile (Table 6.3, Entry 6I)
(Fig. 6.8) $^1$H NMR Spectrum of 2-Amino-4-(4-hydroxyphenyl)-5-oxo-5H-indeno[1,2-b]pyridine-3-carbonitrile (Table 6.3, Entry 6I)
Chapter 6: Synthesis of 5-oxo-5H-indeno[1,2-b]pyridine-3-carbonitrile derivatives and their antimicrobial activity

(Fig. 6.9) $^{13}$C NMR Spectrum of 2-Amino-4-(4-hydroxyphenyl)-5-oxo-5H-indeno[1,2-b]pyridine-3-carbonitrile (Table 6.3, Entry 6I)
Chapter 6: Synthesis of 5-oxo-5H-indeno[1,2-b]pyridine-3-carbonitrile derivatives and their antimicrobial activity

(Fig. 6.10) Mass Spectrum of 2-Amino-4-(4-hydroxyphenyl)-5-oxo-5H-indeno[1,2-b]pyridine-3-carbonitrile (Table 6.3, Entry 6I)
(Fig. 6.12) IR Spectrum of 2-Amino-4-(3,4-dihydroxyphenyl)-5-oxo-5H-indeno[1,2-b]pyridine-3-carbonitrile (Table 6.3, Entry 6L)
(Fig. 6.13) Mass Spectrum of 2-Amino-4-(3,4-dihydroxyphenyl)-5-oxo-5H-indeno[1,2-b]pyridine-3-carbonitrile
(Table 6.3, Entry 6L)
Chapter 6: Synthesis of 5-oxo-5H-indeno[1,2-b]pyridine-3-carbonitrile derivatives and their antimicrobial activity

(Fig. 6.17) IR Spectrum of 2-Amino-5-oxo-4-(thiophen-2-yl)-5H-indeno[1,2-b]pyridine-3-carbonitrile (Table 6.3, Entry 6N)
Chapter 6: Synthesis of 5-oxo-5H-indeno[1,2-b]pyridine-3-carbonitrile derivatives and their antimicrobial activity

(Fig. 6.17) $^1$H NMR Spectrum of 2-Amino-5-oxo-4-(thiophen-2-yl)-5H-indeno[1,2-b]pyridine-3-carbonitrile (Table 6.3, Entry 6N)
(Fig. 6.18) $^{13}$C NMR Spectrum of 2-Amino-5-oxo-4-(thiophen-2-yl)-5H-indeno[1,2-b]pyridine-3-carbonitrile
(Table 6.3, Entry 6N)
(Fig. 6.19) Mass Spectrum of 2-Amino-5-oxo-4-(thiophen-2-yl)-5H-indeno[1,2-b]pyridine-3-carbonitrile
(Table 6.3, Entry 6N)
(Fig. 6.22) IR Spectrum of 2-Amino-4-(3,4-dimethoxyphenyl)-5-oxo-5H-indeno[1,2-b]pyridine-3-carbonitrile (Table 6.3, Entry 6Q)
Chapter 6: Synthesis of 5-oxo-5H-indeno[1,2-b]pyridine-3-carbonitrile derivatives and their antimicrobial activity

(Fig. 6.23) 1H NMR Spectrum of 2-Amino-4-(3,4-dimethoxyphenyl)-5-oxo-5H-indeno[1,2-b]pyridine-3-carbonitrile (Table 6.3, Entry 6Q)

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Chapter 6: Synthesis of 5-oxo-5H-indeno[1,2-b]pyridine-3-carbonitrile derivatives and their antimicrobial activity

(Fig. 6.24) $^{13}$C NMR Spectrum of 2-Amino-4-(3,4-dimethoxyphenyl)-5-oxo-5H-indeno[1,2-b]pyridine-3-carbonitrile (Table 6.3, Entry 6Q)
(Fig. 6.25) Mass Spectrum of 2-Amino-4-(3,4-dimethoxyphenyl)-5-oxo-5H-indeno[1,2-b]pyridine-3-carbonitrile
(Table 6.3, Entry 6Q)
(Fig. 6.28) IR Spectrum of 2-Amino-4-(anthracen-9-yl)-5-oxo-5H-indeno[1,2-b]pyridine-3-carbonitrile
(Table 6.3, Entry 6T)
Chapter 6: Synthesis of 5-oxo-5H-indeno[1,2-b]pyridine-3-carbonitrile derivatives and their antimicrobial activity

(Fig. 6.29) 1H NMR Spectrum of 2-Amino-4-(anthracen-9-yl)-5-oxo-5H-indeno[1,2-b]pyridine-3-carbonitrile (Table 6.3, Entry 61)
Chapter 6: Synthesis of 5-oxo-5H-indeno[1,2-b]pyridine-3-carbonitrile derivatives and their antimicrobial activity

(Fig. 6.30) $^{13}$C NMR Spectrum of 2-Amino-4-(anthracen-9-yl)-5-oxo-5H-indeno[1,2-b]pyridine-3-carbonitrile (Table 6.3, Entry 6T)
Chapter 6: Synthesis of 5-oxo-5H-indeno[1,2-b]pyridine-3-carbonitrile derivatives and their antimicrobial activity

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