SYNTHESIS, CHARACTERIZATION AND IN-VITRO ANTI-OXIDANT ACTIVITY OF SOME 1,4-DIHYDROPYRIDINES AND THEIR MANNICH BASES

A Dissertation submitted to

THE TAMIL NADU Dr.M.G.R.MEDICAL UNIVERSITY,
CHENNAI-600 032.

In partial fulfillment for the award of degree of

MASTER OF PHARMACY
IN
PHARMACEUTICAL CHEMISTRY

Submitted by
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Reg No.26081742

Under the supervision of
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VEL’S COLLEGE OF PHARMACY
OLD PALLAVARAM, CHENNAI-600 117.
Dedicated To
My Beloved Parents
and Teachers
First and foremost I express my deepest sense of gratitude and faithfulness to God’s grace, which has enabled me to finish this project work successfully.

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I bow to my affectionate my parents and brothers & sister for their blessings, support, love and prayers.

ARUMUGA NAVAMANI. K.
**LIST OF ABBREVIATIONS**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>mg</td>
<td>Milligram</td>
</tr>
<tr>
<td>%</td>
<td>Percentage</td>
</tr>
<tr>
<td>wt</td>
<td>Weight</td>
</tr>
<tr>
<td>hr</td>
<td>Hour</td>
</tr>
<tr>
<td>°C</td>
<td>Degree Centigrade</td>
</tr>
<tr>
<td>&gt;</td>
<td>Greater</td>
</tr>
<tr>
<td>&lt;</td>
<td>Lesser</td>
</tr>
<tr>
<td>std</td>
<td>Standard</td>
</tr>
<tr>
<td>DMSO</td>
<td>Dimethyl sulphoxide</td>
</tr>
<tr>
<td>TLC</td>
<td>Thin layer chromatography</td>
</tr>
<tr>
<td>KBr</td>
<td>Potassium bromide</td>
</tr>
<tr>
<td>m.p.</td>
<td>Melting Point</td>
</tr>
<tr>
<td>Rf</td>
<td>Retention Factor</td>
</tr>
<tr>
<td>µg</td>
<td>Microgram</td>
</tr>
<tr>
<td>mL</td>
<td>Millilitre</td>
</tr>
<tr>
<td>µL</td>
<td>Microlitre</td>
</tr>
<tr>
<td>HO</td>
<td>Hydroxylion</td>
</tr>
<tr>
<td>¹H NMR</td>
<td>Proton Nuclear Magnetic Resonance</td>
</tr>
<tr>
<td>IR</td>
<td>Infrared Spectroscopy</td>
</tr>
<tr>
<td>TMS</td>
<td>Tetra methyl silane</td>
</tr>
<tr>
<td>MS</td>
<td>Mass Spectroscopy</td>
</tr>
<tr>
<td>------</td>
<td>-------------------</td>
</tr>
<tr>
<td>TCA</td>
<td>Tricarboxylic acid</td>
</tr>
<tr>
<td>BHT</td>
<td>Butylated Hydroxy Toluene</td>
</tr>
<tr>
<td>EDTA</td>
<td>Ethylene diamine tetra acetic acid</td>
</tr>
<tr>
<td>q.s.</td>
<td>quantity sufficient</td>
</tr>
</tbody>
</table>
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Figure Name</th>
<th>Page No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>IR Spectra of synthesized Compounds 1a – 2e (fig. 1-10)</td>
<td>29</td>
</tr>
<tr>
<td>2.</td>
<td>Mass Spectra of synthesized Compounds 1a – 2e (fig. 11-20)</td>
<td>40</td>
</tr>
<tr>
<td>3.</td>
<td>$^1$H NMR Spectra of synthesized Compounds 1a – 2e (fig. 21-30)</td>
<td>52</td>
</tr>
<tr>
<td>4.</td>
<td>Antibacterial activity of synthesized Compounds (fig. 31)</td>
<td>72</td>
</tr>
<tr>
<td>5.</td>
<td>Antifungal activity of synthesized Compounds (fig. 32)</td>
<td>72</td>
</tr>
</tbody>
</table>
# LIST OF TABLES

<table>
<thead>
<tr>
<th>Table No.</th>
<th>Table Name</th>
<th>Page No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>List of Chemicals</td>
<td>17</td>
</tr>
<tr>
<td>2.</td>
<td>Various substitution and Physicochemical property of dihydropyridine derivatives</td>
<td>26</td>
</tr>
<tr>
<td>3.</td>
<td>Elemental analytical data of the synthesized compounds</td>
<td>27</td>
</tr>
<tr>
<td>4.</td>
<td>IR Spectral data of the synthesized compounds</td>
<td>28</td>
</tr>
<tr>
<td>5.</td>
<td>Mass Spectral data of the synthesized compounds</td>
<td>39</td>
</tr>
<tr>
<td>6.</td>
<td>$^1$H NMR Spectral data of the synthesized compounds</td>
<td>50</td>
</tr>
<tr>
<td>7.</td>
<td>Invitro Nitric oxide scavenging activity</td>
<td>65</td>
</tr>
<tr>
<td>8.</td>
<td>Invitro Hydroxyl scavenging activity</td>
<td>66</td>
</tr>
<tr>
<td>9.</td>
<td>Antimicrobial activity of synthesized compounds</td>
<td>71</td>
</tr>
</tbody>
</table>
## CONTENTS

<table>
<thead>
<tr>
<th>S.No.</th>
<th>TITLE</th>
<th>Page No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>2.</td>
<td>REVIEW OF LITERATURE</td>
<td>6</td>
</tr>
<tr>
<td>3.</td>
<td>OBJECTIVE AND PLAN OF WORK</td>
<td>13</td>
</tr>
<tr>
<td>4.</td>
<td>EXPERIMENTAL WORK</td>
<td>17</td>
</tr>
<tr>
<td>5.</td>
<td>CHARACTERIZATION</td>
<td>25</td>
</tr>
<tr>
<td>6.</td>
<td>PHARAMACOLOGICAL EVALUATION</td>
<td>62</td>
</tr>
<tr>
<td>7.</td>
<td>RESULTS AND DISCUSSION</td>
<td>75</td>
</tr>
<tr>
<td>8.</td>
<td>SUMMARY AND CONCULSION</td>
<td>77</td>
</tr>
<tr>
<td>9.</td>
<td>REFERENCE</td>
<td>i</td>
</tr>
</tbody>
</table>
INTRODUCTION
INTRODUCTION

For small organic molecules, simple nitrogen containing heterocyclic receive a large amount of attention in the literature, as a consequence of their exciting biological properties and they role as pharmacophores of considerable historical importance.

Heterocyclic compounds are stable cyclic compounds in which at least one atom other than carbon forms a part of the ring. The hetero atom is mostly nitrogen, oxygen or sulphur. The heterocyclic are usually five or six member cyclic compounds

Dihydropyridine\(^1\) is one of the heterocyclic compounds, which is simple derivative of pyridine. They are particularly well known in pharmacology as L-type calcium channel blockers. They are readily convertible to pyridines and are important role as intermediates in reactions of pyridine e.g in nucleophilic substitutions and reductions as well as acylations in the presence of pyridine. Dihydropyridines are of utmost importance in biological systems especially NADH\(^2\) which is involved in the biological redox reactions. It is water soluble B complex vitamin.

\[
\begin{align*}
\text{Pyridine} & \quad \text{Nicotinic acid} \\
\begin{tikzpicture}[scale=0.5]
\draw (0,0) -- (1,0) -- (1,1) -- (0,1) -- cycle;
\draw (0,1) -- (1,0);
\node at (0.5,0.5) {N};
\node at (0.5,0) {\text{Pyridine}};
\end{tikzpicture} & \quad \begin{tikzpicture}[scale=0.5]
\draw (0,0) -- (1,0) -- (1,1) -- (0,1) -- cycle;
\draw (0,0) -- (0,1);
\draw (1,0) -- (1,1);
\node at (0.5,0.5) {N};
\node at (0.5,0) {1,4-dihydropyridine};
\end{tikzpicture}
\end{align*}
\]

\(1, 4\text{-dihydropyridine}\)
Physical properties

Structure

![Structure diagram]

R-Hydroxy phenyl

R’-COOC₂H₅

R”-COOC₂H₅

Description

Dihydro pyridine is an amphoteric, colourless with a distinctive unpleasant fish-like odour. The pyridine ring occurs in many important compounds, including nicotinamides. Its PH in aqueous saturated solution is 8.5.

Solubility

It is freely soluble in boiling water, alcohol, alkali hydroxide, carbonates and in propylene glycol. It is practically insoluble in diethylether.

Sources

It occurs in all living cells in small amounts.

Chemistry

The molecular formula is C₅H₇N.
HANTZSCH DIHYDRO PYRIDINE (PYRIDINE) SYNTHESIS

This reaction allows the preparation of dihydro pyridine derivatives by condensation of an aldehyde with two equivalents of a beta-ketoester in the presence of ammonia. Subsequent oxidation (or dehydrogenation) gives pyridine-3, 5-dicarboxylates, which may also be decarboxylated to yield the corresponding pyridines.

Mannich bases

A Mannich base is a β-amino ketone, which is formed by reacting of an amine, formaldehyde (or an aldehyde) and a carbon acid. The mannich base is an end product in the mannich reaction, which is a nucleophilic addition reaction of a non-enolizable aldehyde and primary or secondary amines to produce resonance stabilized imines (minimum ion or imines salt). The addition of a compound containing active hydrogen atom to the schiff’s base gives the Mannich base.

The essential feature of the Mannich reaction is the replacement of the active hydrogen atom by an amino methyl group.
Mannich bases are important compounds owing to their wide range of biological and industrial applications. They are also employed as intermediates in chemical synthesis and polymer chemistry. Several important therapeutic compounds have been synthesized via the Mannich reaction. They have also been found to possess pharmacological activities, such as anti-cancer, antiviral, anti-malarial, anti-tubercular, anti-bacterial, analgesic, anti-inflammatory activity etc.  

**Mechanism of the Hantzsch Dihydropyridine synthesis**

The reaction can be visualized as proceeding through a knoevenagel condensation product as a key intermediate.
Derivatives of 1, 4-dihydropyridine$^{5,35}$

$M_1$

Dimethyl 1,4-dihydro-4-(3,4-dichlorophenyl)-2,6-dimethyl-1-(3-phenylpropyl)-3,5-pyridine dicarboxylate

$M_2$

3,5-diethoxycarbonyl-1-[(4'-sulfamoyl-1'-aminomethyl)phenyl]-1,4-dihydro-2,6-dimethyl-4-(3'-methoxy-4'-hydroxy phenyl)-pyridine.

The dihydropyridine derivatives have variety of pharmacological activities like Analgesic, Anti-inflammatory, Anti-fungal, Anti-bacterial, Anti-convulsant, Anti-hypertensive.

Due to importance of 1, 4-dihydropyridine derivatives and its isomers, the aim of this dissertation is to evaluate the invitro antioxidant activity and antimicrobial activity against staphylococcus auereus of several compounds of this class.
REVIEW OF LITERATURE

Bu chanan et al\textsuperscript{6}, synthesised the structures of a number of manich bases which have been checked by NMR\textsuperscript{1} the deshielding effect of N-protonation being used to identify adjacent protons. whilst manich bases lacking a beta- proton can react Michael- wise via a rearrangement, but their quaternary methiodides do not. On this evidence, anomalous literature reports can be rationalized.

Jungjin suh et al\textsuperscript{7}, synthesised and carried out the anti hypertensive activity of 4-(2,4-Dioxo-5-pyrimidyl)-1,4-dihydro pyridine.

\[
\begin{array}{c}
\text{HN} \quad \text{NH} \\
\text{O} \\
\text{O} \\
\text{N} \\
\text{COOR}_2 \\
\text{R-C}_2\text{H}_5
\end{array}
\]

Denner et al\textsuperscript{8}, synthesised and carried out biological evaluation of new 1, 4-dihydro pyridines as anti-hypertensive agents in rats.

Kawashima.Y et al\textsuperscript{9}, synthesised and performed anti-hypertensive activities of 1, 4-dihydropyridine-5-phosphonate derivatives.

JAM Christians et al\textsuperscript{10}, synthesised and carried out in vitro pharmacology of new 1,4-dihydropyridines.2-(w-aminoalkylthiomethyl)-1,4-dihydropyridines as potent calcium channel blockers.


J M Vierfond et al\textsuperscript{11}, reported the synthesis, binding affinity and antioxidant activity of 1,4-dihydropyridine.

\[ \text{R-H} \]
\[ \text{Ar}_1 \text{-2-pyrazinyl} \]
\[ \text{Ar}_2 \text{-2-pyrazinyl} \]

M. Amir et al\textsuperscript{12}, reported 6-phenyl-1, 4-dihydro pyridine derivatives as potent and selective A3- adenosine receptor antagonist.

Kotecka et al\textsuperscript{13}, we had compared the ex-vivo anti-malarial activity of 12 new quinoline di-mannich base compounds containing the 7-dichloroquinoline (or) 7-trifluoro methylquinoline nucleus with amodiaquine, chloroquine and pyronaridine using the saimiri- bioassay model. In vitro activity against the multi drug-resistant K1 isolate of plasmodium falciparum was determined in serum samples by measuring the maximum inhibitory dilution at which the treated monkey serum inhibited schizont maturation in vitro of the 12 mannich bases tested, 8 were associated with levels of ex-vivo anti-malarial activity in serum greater than those of amodiaquine, chloroquine, or pyronaridine 1 to 7 days after drug administration. Further studies were carried out with four of these compounds, and results showed.

G. Diaz-Araya et al\textsuperscript{14}, synthesised and carried out the antioxidant effects of 1,4-dihydro pyridine and Nitroso aryl derivatives on the Fe\textsuperscript{3+}/ascorbate- stimulated lipid per oxidation in rat brain slice.
Tirzitis et al\textsuperscript{15}, synthesised some 2, 6-dimethyl-3, 5-dialkoxy carbonyl-1,4-dihydro pyridines in metal-ion catalyzed lipid per oxidation and screened for antioxidant activity.

\begin{center}
\includegraphics[width=0.4\textwidth]{structure1.png}
\end{center}

Kruk et al\textsuperscript{16}, performed the antioxidant activity of 4-flavonil-1, 4-dihydro pyridine derivatives.

\begin{center}
\includegraphics[width=0.4\textwidth]{structure2.png}
\end{center}

Pandeya et al\textsuperscript{17}, synthesised and done the spectral characterization, in vitro antibacterial and antifungal activities of N-mannich bases of 3(N-pyrimethaminylimino) isatin.

Khan et al\textsuperscript{18}, studied the mechanism- based inactivation of thioredoxin reductase from plasmodium falciparum by mannich bases implication for cytotoxicity.
Shafiee et al\textsuperscript{19}, reported the anticonvulsant activities of new 1, 4-dihydropyridine derivatives containing 4-nitroimidazolyl substituents.

\begin{center}
\includegraphics[width=0.5\textwidth]{structure.png}
\end{center}

R\textsubscript{1}-CH\textsubscript{2}C\textsubscript{6}H\textsubscript{5}

R\textsubscript{2}. CH\textsubscript{3}

Lopes et al\textsuperscript{20}, synthesised some novel N-mannich base-type derivatives of the antimalarial drug amodiaquine and their reaction with tertiary N-chloromethylamides with the exception of the derivative of ethyl hippurate, all the so-formed (1-amidomethyl-1H-quinolin-4-ylidine) arylamines displayed high chemical and enzymatic stability. These compounds displayed antimalarial activity against the multi-drug resistant plasmodium falciparum strain Dd2 (IC\textsubscript{50} values 15-31nm) and demonstrated no significant loss in activity compared to amodiaquine.

Dinh Thanh Hai et al\textsuperscript{21}, synthesised (5-nitrofurfural and m-nitro benzaldehydes) by the condensation of aromatic aldehydes with hydantoin. The derivatives I and VI were obtained. The compounds I VI underwent mannich reaction and gave 8 mannich base derivatives (II-V, VII-X). The structures of synthesised compounds have been characterized by IR, UV, and \textsuperscript{1}H-NMR, \textsuperscript{13}C-NMR and mass spectroscopy. The obtained compounds were tested for biological activities such as antibacterial, antifungal and anticancer. Compounds I and its mannich base derivatives showed high biological activities.
R.Sridhar, P.T.Perumal et al\textsuperscript{22}, reported a new protocol to synthesize 1,4-dihydropyridines by using 3, 4, 5-trifluorobenzenboronic acid as a catalyst in ionic liquid and synthesis of novel 4-(3-carboxyl-1H-pyrazol-4-yl)-1,4-dihydropyridines. 

R.Budriesi et al\textsuperscript{23}, synthesised the 1,4-dihydropyridine derivatives as calcium channel modulators: the role of 3-methoxy-flavones moiety was found to be angina and antihypertensive activity.

\begin{center}
\includegraphics[width=0.5\textwidth]{structure.png}
\end{center}

Negm Nobel's et al\textsuperscript{24}, prepared some novel series of cationic surfactants based on mannich base (produced from the condensation of piperidine and / or morpholine as secondary amine and para formaldehyde in the presence of 8-hydroxyl quinoline). The chemical structures of the synthesised cationic surfactants were confirmed using elemental analysis, FTIR spectroscopy and \textsuperscript{1}H NMR. Surface activities of the prepared surfactants were measured including surface tension (gamma), critical micelle concentration effectiveness (Pi\textsubscript{cmc}), efficiency(Pc 20), maximum surface excess (Gamma\textsubscript{max}), minimum surface area(A\textsubscript{min}), interfacial tension(gammaIT)), emulsification power and foaming power at 25\degree C. The structural influences on their surface activities and adsorption free energy were discussed.
A.K. Chhillar et al. reported microwave-assisted synthesis of antimicrobial dihydropyridines and tetrahydropyrimidin-2-ones and some novel compounds against *Aspergillus niger*.

![Chemical structure](image)

F. Mustata et al. synthesised and characterized p-amino benzoic acid/Cyclohexanone/formaldehyde resins as hardener for epoxy resins.

M.A. Zolfigol et al. synthesised iodine catalyzed synthesis of novel Hantzsch N-hydroxyethyl 1,4-dihydropyridines under mild conditions.

SR Pattan et al. synthesised some new substituted 1,4-dihydropyridine derivatives and their anti-inflammatory activity.

V. Sridharan et al. synthesised some new 3-component domino of 1,4-dihydropyridines.
R. Leon et al\textsuperscript{30}, synthesised 6-amino-1,4-dihydropyridines that prevent calcium overload and neuronal death to possess antihypertensive activity.

J. Carbajo et al\textsuperscript{31}, synthesised and carried antihypertensive activity of some C-substituted 2, 6-dimethyl-1,4-dihydropyridines.

M. Ashok et al\textsuperscript{32}, performed convenient one pot synthesis and antimicrobial evaluation of some new manich bases carrying 4-methylthio benzyl moiety.

Belle Ds and Singhvi et al\textsuperscript{33}, performed synthesis and antimicrobial activity of some manich bases of 6-substituted-2-amino benzothiazole.

Rakeshkumar et al\textsuperscript{34}, synthesised and performed antimicrobial activity of 4-(5-chloro -3-methyl-1-phenyl-1H-pyrazol-4-yl)–dihydropyridines and 4-(5-chloro -3-methyl-1-phenyl- 1H-pyrazol-4-yl) – 3,4-dihydropyrimidin-2-ones.
BBSubudhi et al., performed the synthesis and antiulcer activity study of 1,4-dihydropyridines and their mannich bases with sulphanilamide.
OBJECTIVE AND PLAN OF WORK

The Dihydropyridine have already proven to have variety of pharmacological activities like analgesic, anti-inflammatory, anti-fungal, anti-bacterial, anti-oxidant, anti-convulsant, anti-hypertensive, anti-ulcer, calcium channel antagonist, anti-tumor activity and many more activities.

Therefore, based on the previously reported information concerning 1,4.-dihydro pyridine derivatives, I have planned to synthesize some new 1,4dihydro pyridine derivatives with different aldehyde with more biological and chemotherapeutic efficacy as compared to some previously reported dihydro pyridine derivatives with good yield.

So an attempt was made to synthesize dihydro pyridine derivatives and prove their anti-oxidant and anti-microbial activities. The current study contains the following

- Synthesis of 1,4-dihydropyridine derivatives.
- Synthesis of mannich base of 1, 4- dihydropyridine derivatives.
- Characterization of synthesised compounds.
- Screening for Biological Activity
  a) Anti-oxidant activity
  b) Anti-microbial
PLAN OF WORK

Synthesis of the 1,4- dihydro pyridine was carried out in following steps

Step.1
- Synthesis of 1,4-dihydropyridine derivatives.

Step.2
- Synthesis of mannich base of 1,4- dihydropyridine derivatives.
SCHEME OF THE WORK

Step 1

\[
\begin{align*}
\text{C}_2\text{H}_5\text{OOC} & \quad \text{CH}_2 \\
\text{C} & \quad \text{C} \\
\text{O} & \quad \text{CH}_3 \\
\text{C}_2\text{H}_5\text{OOC} & \quad \text{CH}_2 \\
\text{C} & \quad \text{C} \\
\text{O} & \quad \text{CH}_3 \\
& + \\
\text{concnH}_4\text{OH} \\
& \text{RCHO} \quad \text{3hr} \\
\end{align*}
\]

Ethyl aceto acetate

\[
\begin{align*}
\text{R} & = \text{C}_8\text{H}_7, \ 3,4-\text{OCH}_3-\text{C}_6\text{H}_4, \ 2-4-\text{Cl-}\text{C}_6\text{H}_4, \\
& \ 4-\text{CH}_3-\text{C}_6\text{H}_4, \ 2-\text{OCH}_3-4-\text{OH-5-OCH}_3-\text{C}_6\text{H}_2
\end{align*}
\]
Step 2

Mannich base of 1,4-dihydropyridine derivatives

\[ R = C_8H_7, \text{ 3,4-OCH}_3\text{-C}_6\text{H}_4, \text{ 2-4-Cl-C}_6\text{H}_4, \]

\[ 4\text{-CH}_3\text{-C}_6\text{H}_4, \text{ 2-OCH}_3\text{-4-OH-5-OCH}_3\text{-C}_6\text{H}_2 \]
## EXPERIMENTAL WORK

### Table 1

#### LIST OF CHEMICALS

<table>
<thead>
<tr>
<th>S. No.</th>
<th>CHEMICALS USED</th>
<th>MANUFACTURES</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ethyl aceto acetate</td>
<td>Chemlabs</td>
</tr>
<tr>
<td>2</td>
<td>Ethanol</td>
<td>Chemlabs</td>
</tr>
<tr>
<td>3</td>
<td>Ammonia</td>
<td>Chemlabs</td>
</tr>
<tr>
<td>4</td>
<td>P-Tolualdehyde</td>
<td>Himedia</td>
</tr>
<tr>
<td>5</td>
<td>Cinnamaldehyde</td>
<td>Himedia</td>
</tr>
<tr>
<td>6</td>
<td>2,4-dichloro benzaldehyde</td>
<td>Himedia</td>
</tr>
<tr>
<td>7</td>
<td>Veratraldehyde</td>
<td>Himedia</td>
</tr>
<tr>
<td>8</td>
<td>Syringaldehyde</td>
<td>Himedia</td>
</tr>
</tbody>
</table>
Apparatus; Beaker, conical flask, round bottom flask, condenser made of Borosil glass, funnel, vacuum filter, magnetic stirrer.

PROCEDURE

Step I Preparation of the 1,4-dihydropyridine derivatives

General procedure

A mixture of aldehyde (0.2mole), ethyl acetoacetate (0.2mole) and concentrated ammonium hydroxide (8 ml) in ethanol (60ml) was heated under reflux for 3 hours. To the resulting mixture, warm water (40 ml) was added and then allowed to cool. The separated product was filtered off, washed with 60% aqueous ethanol and recrystallized from alcohol to give Product and it is used for the further reaction (compound 1a).

Where,

\[ R = C_8H_7, \ 3,4-OCH_3-C_6H_4, \ 2-4-Cl-C_6H_4, \]
\[ 4-CH_3-C_6H_4, \ 2-OCH_3-4-OH-5-OCH_3-C_6H_2 \]
Similarly, compounds (1a-e) were prepared by condensation of ethylacetoacetate and ammonium hydroxide with other aromatic aldehyde.

**Step II Preparation of 1,4-dihydropyridine derivatives**

A mixture of compound 1a, p-aminobenzoic acid (0.01 mole) and p-formaldehyde (0.02 mole) was taken in 15 ml of rectified spirit and heated under reflux for 4 hours. The reaction mixture was poured on to crushed ice. The product was filtered and recrystallized from aqueous ethanol to give product (**compound 2a**).

Where,

\[
R = C_8H_7, \ 3,4-OCH_3-C_6H_4, \ 2-4-Cl-C_6H_4, \\
4-CH_3-C_6H_4, \ 2-OCH_3-4-OH-5-OCH_3-C_6H_2
\]
Similarly, compounds (2a-e) were prepared by condensation of p-aminobenzoic acid and p-formaldehyde with product (1a-e).

**SYNTHESISED Derivatives**

1a

\[
\text{Diethyl2,6-dimethyl-4-styryl-1,4-dihydro pyridine-3,5-dicarboxylate}
\]

1b
Diethyl4-(3,4-dimethoxyphenyl)2, 6-dimethyl- 1,4-dihydropyridine3,5- dicarboxylate.
**1c**

Diethyl 4-(2,4-dichloro phenyl)- 2,6-dimethyl- 1, 4 –dihydropyridine-3,5-dicarboxylate.

**1d**

Diethyl 2, 6-dimethyl-4-p-tolyl-1,4-dihydropyridine-3,5-dicarboxylate
Diethyl 4-(4-hydroxy-3, 5-dimethoxy-2-phenyl)-2, 6-dimethyl-1,4-dihydro pyridine-3, 5-dicarboxylate

4-((3,5-bis(ethoxycarbonyl)-2,6-dimethyl-4-styrylpyridine-1(4H)-yl)methylamino)benzoic acid.
4-((3,5-bis(ethoxycarbonyl)-2,6-dimethyl-4-(3,4-dimethoxy phenyl)pyridine-1(4H)-yl) methylamino) benzoic acid.

4-((3,5-bis(ethoxycarbonyl)-2,6-dimethyl-4-(3,4-dichlorophenyl)pyridine-1-(4H)-yl)methylamino) benzoic acid.
2d

\[
4-((3,5\text{-bis(ethoxycarbonyl)}-2,6\text{-dimethyl-4-tolylpyridine-1-(4H)-yl})\text{ methylamine})\text{benzoic acid.}
\]

2e

\[
4-(3,5\text{-bis(ethoxycarbonyl)2,6-dimethyl-4-(4hydroxy3,5dimethoxypheny)pyridine-1-(4H)-yl})\text{ methylamino)benzoic acid.}
\]
CHARACTERIZATION

The synthesized compounds were purified by recrystallization and thin layer chromatography. The compounds were then subjected to spectral characterization and elemental analysis.

The melting point was determined by open capillary tube method and it was uncorrected. Analysis was performed in Heraeus CHN Rapid analyzer (division of catalysis and kinetics, Department of Chemistry, Indian Institute of Technology, Chennai,). The data is presented in Table 2.

IR Spectra was recorded (KBr) on ABB BOMEM FTIR spectrophotometer MB serial II–Canada (Mical Lab, Chennai-32). The data is presented in Table 4.

1H NMR Spectrum (DMSO) was recorded on 400 MHZ-Joel DPX (Indian Institute of Technology, Chennai, Tamil Nadu, India) using tetra methyl Silane as internal standard. The data is presented in a Table 6.

Mass spectra were recorded on Joel GC mate-II, GCMS system (Sophisticated Analytical Instrument facility, Indian Institute of Technology, Chennai, Tamil Nadu). The data is presented in a Table 5.
Table 2

Various substitution and physicochemical property of dihydropyridine derivatives

<table>
<thead>
<tr>
<th>Compound</th>
<th>R</th>
<th>R&lt;sub&gt;F&lt;/sub&gt; value</th>
<th>M..P (°C)</th>
<th>% Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>C&lt;sub&gt;8&lt;/sub&gt;H&lt;sub&gt;7&lt;/sub&gt;</td>
<td>0.69</td>
<td>131-132</td>
<td>65</td>
</tr>
<tr>
<td>1b</td>
<td>3,4-OCH&lt;sub&gt;3&lt;/sub&gt;-C&lt;sub&gt;6&lt;/sub&gt;H&lt;sub&gt;4&lt;/sub&gt;</td>
<td>0.62</td>
<td>126-128</td>
<td>68</td>
</tr>
<tr>
<td>1c</td>
<td>2,4-Cl – C&lt;sub&gt;6&lt;/sub&gt;H&lt;sub&gt;4&lt;/sub&gt;</td>
<td>0.71</td>
<td>123-125</td>
<td>67</td>
</tr>
<tr>
<td>1d</td>
<td>4-CH&lt;sub&gt;3&lt;/sub&gt;-C&lt;sub&gt;6&lt;/sub&gt;H&lt;sub&gt;4&lt;/sub&gt;</td>
<td>0.23</td>
<td>146-149</td>
<td>70</td>
</tr>
<tr>
<td>1e</td>
<td>2-OCH&lt;sub&gt;3&lt;/sub&gt;-4-OH-5-OCH&lt;sub&gt;3&lt;/sub&gt;-C&lt;sub&gt;6&lt;/sub&gt;H&lt;sub&gt;2&lt;/sub&gt;</td>
<td>0.7</td>
<td>120-125</td>
<td>63</td>
</tr>
<tr>
<td>2a</td>
<td>C&lt;sub&gt;8&lt;/sub&gt;H&lt;sub&gt;7&lt;/sub&gt;</td>
<td>0.31</td>
<td>123-124</td>
<td>62</td>
</tr>
<tr>
<td>2b</td>
<td>3,4-OCH&lt;sub&gt;3&lt;/sub&gt;-C&lt;sub&gt;6&lt;/sub&gt;H&lt;sub&gt;4&lt;/sub&gt;</td>
<td>0.39</td>
<td>131-135</td>
<td>63</td>
</tr>
<tr>
<td>2c</td>
<td>2,4-Cl-C&lt;sub&gt;6&lt;/sub&gt;H&lt;sub&gt;4&lt;/sub&gt;</td>
<td>0.67</td>
<td>145</td>
<td>62</td>
</tr>
<tr>
<td>2d</td>
<td>4-CH&lt;sub&gt;3&lt;/sub&gt;-C&lt;sub&gt;6&lt;/sub&gt;H&lt;sub&gt;4&lt;/sub&gt;</td>
<td>0.71</td>
<td>148-155</td>
<td>65</td>
</tr>
<tr>
<td>2e</td>
<td>2-OCH&lt;sub&gt;3&lt;/sub&gt;-4-OH-5-OCH&lt;sub&gt;3&lt;/sub&gt;-C&lt;sub&gt;6&lt;/sub&gt;H&lt;sub&gt;2&lt;/sub&gt;</td>
<td>0.20</td>
<td>125-134</td>
<td>60</td>
</tr>
</tbody>
</table>

**Mobile phase** Hexane: Ethyl acetate (2:1)
## Table 3

### Elemental analytical data of synthesized compounds

<table>
<thead>
<tr>
<th>Compound</th>
<th>Mol. Formula</th>
<th>Molecular Weight</th>
<th>Yield in percentage</th>
<th>Elemental analysis Calculated (Experimental)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>C</td>
</tr>
<tr>
<td>1a</td>
<td>C_{29}H_{32}N_{2}O_{6}</td>
<td>355.43</td>
<td>65</td>
<td>70.96</td>
</tr>
<tr>
<td>1b</td>
<td>C_{28}H_{32}N_{2}O_{7}</td>
<td>389.44</td>
<td>68</td>
<td>64.16</td>
</tr>
<tr>
<td>1c</td>
<td>C_{27}H_{28}N_{2}O_{6}Cl_{2}</td>
<td>361.64</td>
<td>67</td>
<td>62.90</td>
</tr>
<tr>
<td>1d</td>
<td>C_{28}H_{32}N_{2}O_{6}</td>
<td>343.42</td>
<td>70</td>
<td>69.95</td>
</tr>
<tr>
<td>1e</td>
<td>C_{29}H_{34}N_{2}O_{9}</td>
<td>450.16</td>
<td>63</td>
<td>62.21</td>
</tr>
<tr>
<td>2a</td>
<td>C_{29}H_{32}N_{2}O_{6}</td>
<td>504.57</td>
<td>62</td>
<td>69.03</td>
</tr>
<tr>
<td>2b</td>
<td>C_{28}H_{32}N_{2}O_{7}</td>
<td>508</td>
<td>63</td>
<td>64.67</td>
</tr>
<tr>
<td>2c</td>
<td>C_{27}H_{28}Cl_{2}N_{2}O_{6}</td>
<td>510.70</td>
<td>62</td>
<td>59.24</td>
</tr>
<tr>
<td>2d</td>
<td>C_{28}H_{32}N_{2}O_{6}</td>
<td>492</td>
<td>65</td>
<td>68.28</td>
</tr>
<tr>
<td>2e</td>
<td>C_{29}H_{34}N_{2}O_{9}</td>
<td>554</td>
<td>60</td>
<td>62.81</td>
</tr>
</tbody>
</table>
Table 4

IR spectra of the compounds

<table>
<thead>
<tr>
<th>S.No.</th>
<th>COMPOUNDS</th>
<th>WAVE NUMBER (cm(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1a</td>
<td>3369,3173,1648,1587,1465,1443,1396,1315,1300, 1283,1147,1069,724,694.</td>
</tr>
<tr>
<td>2</td>
<td>1b</td>
<td>3348,2982,2715,1776,1746,1669,1589,1388,1234,1198,712,518,478.</td>
</tr>
<tr>
<td>3</td>
<td>1c</td>
<td>3380,2696,2062,1621,1466,1400,1269,693,505.</td>
</tr>
<tr>
<td>4</td>
<td>1d</td>
<td>3409,3199,1649,1581,1444,1393,1315,1298,1282,1067,842,724,694.</td>
</tr>
<tr>
<td>5</td>
<td>1e</td>
<td>3667,3643,3624,3606,3582,3541,3497,3457,3440,3026,1744,1728,1712,1694,1681536,1445,1393,1283.</td>
</tr>
<tr>
<td>6</td>
<td>2a</td>
<td>3177,2053,1776,1665,1590,1509,1399,1371,1263,1231,1200,713,673,476.</td>
</tr>
<tr>
<td>7</td>
<td>2b</td>
<td>3333,1651,1605,1580,1511,1475,1443,1360,1280,1217,1166,567.</td>
</tr>
<tr>
<td>8</td>
<td>2c</td>
<td>3464,3172,3062,2976,1721,1662,1579,1530,1447,1346,1234,1150,928,830,735,567.</td>
</tr>
<tr>
<td>9</td>
<td>2d</td>
<td>3365,3267,3166,2061,1647,1587,1466,1444,1393,1299,1283,1068,724,693.</td>
</tr>
<tr>
<td>10</td>
<td>2e</td>
<td>3369,3173,1648,1582,146,1443,1396,1315,1300,1283,1147,694.</td>
</tr>
</tbody>
</table>
Fig. 1 IR Spectra of compound-1a
Fig. 2 IR Spectra of compound-1b
Fig. 3 IR Spectra of compound-1c

Fig. 4 IR Spectra of compound-1d
Fig. 5 IR Spectra of compound-1e
Fig. 6 IR Spectra of compound-2a
Fig. 7 IR Spectra of compound-2b
Fig. 8 IR Spectra of compound-2c
Fig. 9 IR Spectra of compound-2d
Fig. 10 IR Spectra of compound-2e
Table 5

MASS spectral data of the synthesized compounds

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Compounds</th>
<th>Molecular Weight</th>
<th>M/Z (%Relative Abundance)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1a</td>
<td>355.43</td>
<td>355.58 M⁺(5%), 353.55(10%), 340.51(4%), 326.52(6%), 308.58(5%), 292.50(25%), 286.49(8%), 215.44(6%), 206.45(7%), 196.43(8%), 185.40(5%).</td>
</tr>
<tr>
<td>2</td>
<td>1b</td>
<td>389.44</td>
<td>387.66 M⁺(57%), 378.52(5%), 363.50(6%), 252.51(5%), 236.49(8%), 215.44(6%), 206.45(7%), 196.43(8%), 185.40(5%).</td>
</tr>
<tr>
<td>3</td>
<td>1c</td>
<td>361.64</td>
<td>362.50 M⁺(5%), 316.60(20%), 270.63(5%), 251.46(100%), 205.40(40%), 175.29(40%), 58.29(10%).</td>
</tr>
<tr>
<td>4</td>
<td>1d</td>
<td>343.42</td>
<td>343.65 M⁺(5%), 312.62(6%), 270.60(18%), 252.57(99%), 236.51(15%), 215.44(6%), 206.45(7%), 196.43(8%), 185.40(5%).</td>
</tr>
<tr>
<td>5</td>
<td>1e</td>
<td>450.16</td>
<td>450.65 M⁺(3%), 316.60(20%), 270.63(5%), 251.46(100%), 205.40(40%), 175.29(40%), 58.29(10%).</td>
</tr>
<tr>
<td>6</td>
<td>2a</td>
<td>504.57</td>
<td>504.92 M⁺(3%), 467.70(3%), 252.51(58%), 236.51(18%), 196.43(20%), 110.42(5%).</td>
</tr>
<tr>
<td>7</td>
<td>2b</td>
<td>508</td>
<td>508.37 M⁺(18%), 475.30(20%), 455.53(18%), 398.58(16%), 316.60(18%), 264.46(12%), 252.57(55%), 149.39(25%), 137.37(55%).</td>
</tr>
<tr>
<td>8</td>
<td>2c</td>
<td>510.70</td>
<td>511.37 M⁺(7%), 483.07(7%), 358.13(7%), 299.56(5%), 251.46(32%), 205.40(15%), 75.36(15%), 120.43(8%), 83.36(9%).</td>
</tr>
<tr>
<td>9</td>
<td>2d</td>
<td>492</td>
<td>492.81 M⁺(3%), 341.60(8%), 252.54(98%), 224.49(19%), 120.39(70%), 65.32(35%).</td>
</tr>
<tr>
<td>10</td>
<td>2e</td>
<td>554</td>
<td>555.87 M⁺(3%), 527.67(9%), 403.59(18%), 372.22(18%), 232.57(98%), 224.54(22%), 154.41(22%), 137.42(52%), 120.40(90%).</td>
</tr>
</tbody>
</table>
Fig-11 MASS Spectra of Compound- 1a

Fig-12 MASS Spectra of Compound- 1b
Fig-13 MASS Spectra of Compound- 1c
Fig-14  MASS Spectra of Compound- 1d
Fig-15  MASS Spectra of Compound- 1e
Fig-16 MASS Spectra of Compound- 2a
Fig-18  MASS Spectra of Compound- 2c
Fig-19  MASS Spectra of Compound- 2d
Fig-20 MASS Spectra of Compound- 2e
<table>
<thead>
<tr>
<th>Compounds</th>
<th>Nature of proton</th>
<th>Aromatic proton (Ar –H) (m)</th>
<th>N=CH-Ar(s)</th>
<th>-CH$_3$ (s)</th>
<th>C-O-CH$_3$(s)</th>
<th>Ar-OH</th>
<th>N(CH$_3$)$_2$ (s)</th>
<th>-OCH$_3$ (s)</th>
<th>-NH$_2$ (s)</th>
<th>Total No. of proton</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>No. of proton $\delta$ value (ppm)</td>
<td>5H 7.14-7.30</td>
<td>-</td>
<td>2H 1.71</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>7</td>
</tr>
<tr>
<td>1b</td>
<td>No. of proton $\delta$ value (ppm)</td>
<td>5H 114.1-149.7</td>
<td>-</td>
<td>2H 16.3</td>
<td>-</td>
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<td>2H 56.2</td>
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<tr>
<td>1c</td>
<td>No. of proton $\delta$ value (ppm)</td>
<td>4H 4.43-7.16</td>
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<td>2H 1.71</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>6</td>
</tr>
<tr>
<td>1d</td>
<td>No. of proton $\delta$ value (ppm)</td>
<td>2H 4.43-6.94</td>
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<td>3H 1.71-2.35</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>5</td>
</tr>
<tr>
<td>1e</td>
<td>No. of proton $\delta$ value (ppm)</td>
<td>2H 5.96</td>
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<td>2H 1.71</td>
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<td>-</td>
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<td>-</td>
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<tr>
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<td>No. of proton $\delta$ value (ppm)</td>
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<td>2H 1.71</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<td>-</td>
<td>10</td>
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<tr>
<td>2b</td>
<td>No. of proton $\delta$ value (ppm)</td>
<td>7H 6.46-8.05</td>
<td>-</td>
<td>2H 1.71</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2H 3.73</td>
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<td>9</td>
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<tr>
<td>2c</td>
<td>No. of proton $\delta$ value (ppm)</td>
<td>7H 6.94-8.05</td>
<td>-</td>
<td>2H 1.71</td>
<td>-</td>
<td>-</td>
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<td>2d</td>
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<td>3H 1.7-2.78</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>10</td>
</tr>
<tr>
<td>2e</td>
<td>No. of proton $\delta$ value (ppm)</td>
<td>6H 5.96-8.05</td>
<td>-</td>
<td>2H 1.71</td>
<td>-</td>
<td>1H 5.0</td>
<td>-</td>
<td>2H 3.73</td>
<td>-</td>
<td>10</td>
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</table>
Fig.-21 $^1$H NMR Spectra of compound 1a

Fig.-22 $^1$H NMR Spectra of compound 1b
Fig.-23 $^1$H NMR Spectra of compound 1c
Fig.-24 $^1$H NMR Spectra of compound 1d
Fig.-25 $^1$H NMR Spectra of compound 1e
Fig. - 26 $^1$H NMR Spectra of compound 2a
Fig.-27 $^1$H NMR Spectra of compound 2b
Fig.-28 $^1$H NMR Spectra of compound 2c
Fig.-29 $^1$H NMR Spectra of compound 2d
Fig.-30 $^1$H NMR Spectra of compound 2e
PHARMACOLOGICAL EVALUATION

INVITRO ANTI-OXIDANT ACTIVITY

Antioxidants\textsuperscript{36} are substances whose presence in relatively low concentration significantly inhibits the rate of oxidation of the major targets of oxidative activity viz., cell membranes and components, proteins and other cellular constituents.

Potential role of anti-oxidant in preventing the two important causes of premature death, cardiovascular diseases and cancer is partly attractive synthesis of some novel 1, 4-dihydro pyridine by using Biginelli condensation one pot-multi component reaction which are emerged as a novel anticancer agents.

ANTI-OXIDANT STUDIES

FREE RADICAL SCAVENGING ACTIVITY BY NITRIC OXIDE SCAVENGING METHOD\textsuperscript{37}

Chemicals used

- Sodium nitro prusside
- Phosphate buffer
- Methanol
- Griess reagent

Procedure

Nitric oxide scavenging activity was measured by spectrophotometry method. Sodium nitroprussicle (5mmol) in phosphate-buffered saline was mixed with a control test compound, but with an equivalent amount of methanol. Test solution at different concentrations (5- 100 mg/ml) were dissolved in methanol and incubated at 25\textdegree c for 30mts. After 30min, 1.5ml of the incubated solution was removed and diluted with 1.5ml of griess reagent (1% sulphanalimide, 2% phosphoric acid, 0.1% naphthyl
ethylenediamine hydrochloride). The absorbance of the chromophore formed during the diazotization of the nitrite with sulphanilamide and the subsequent coupling value is the concentration of sample required to inhibit 50% of nitric oxide radical all determinations were performed in triplicates % inhibition of nitric oxide radical was calculated by following formula.

$$\text{Nitric oxide scavenged (\%) } = \frac{A_{\text{cont}} - A_{\text{test}}}{A_{\text{cont}}} \times 100$$

Where,

$A_{\text{cont}}$ is the absorbance of the control reaction mixture.

$A_{\text{test}}$ is the absorbance of the control sample of the synthesized compounds at different concentrations.

The antioxidant activity of the synthesized compounds was expressed as IC$_{50}$Values.

**Hydroxyl radical scavenging activity**

**Chemicals used**

- EDTA
- FeCl$_3$
- H$_2$O$_2$
- Thio barbituric acid
- Tri chloro acetic acid
- Distilled water
- Ascorbic acid
- Deoxy ribose
- Phosphate buffer
Procedure

The scavenging capacity for hydroxyl radical was measured according to the modified method. The assay was performed by adding 0.1 ml EDTA, 0.01 ml Fecl₃, 0.1ml H₂O₂, 0.36ml of deoxy ribose. 1.0ml of test solutions (5 – 100 kg/ml) dissolved in distilled water, 0.33ml of phosphate buffer (50mm, pH 7.4), and 0.1ml of ascorbic acid in sequence. The mixture was then incubated at 37⁰ for 1 hour. A 1 ml portion of the incubated mixture was mixed with 1 ml of 10% TCA and 1 ml of 0.5% TBA to develop the chromogen which was measured at 532nm. BHT was used as a positive control.

% inhibition of hydroxyl radical was calculated by following formula.

\[
\text{Hydroxyl radical scavenged (\%) } = \frac{A_{\text{cont}} - A_{\text{test}}}{A_{\text{cont}}} \times 100
\]

Where,

\( A_{\text{cont}} \) is the absorbance of the control reaction mixture.

\( A_{\text{test}} \) is the absorbance of sample of the synthesized compounds at different concentrations.
Table 7

*In Vitro* Nitric oxide scavenging activity of compounds

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Compounds</th>
<th>%RSC 25μg/ml</th>
<th>%RSC 50μg/ml</th>
<th>%RSC 75μg/ml</th>
<th>%RSC 100μg/ml</th>
<th>IC$_{50}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1a</td>
<td>7.15±0.07</td>
<td>13.59±0.09</td>
<td>22.61±0.13</td>
<td>39.02±0.01</td>
<td>&gt;100</td>
</tr>
<tr>
<td>2</td>
<td>1b</td>
<td>18.71±0.25</td>
<td>29.00±0.32</td>
<td>40.19±0.08</td>
<td>51.00±0.001</td>
<td>98.03</td>
</tr>
<tr>
<td>3</td>
<td>1c</td>
<td>14.86±0.23</td>
<td>28.57±0.09</td>
<td>39.48±0.12</td>
<td>50.30±0.19</td>
<td>99.22</td>
</tr>
<tr>
<td>4</td>
<td>1d</td>
<td>19.43±0.14</td>
<td>30.22±0.1</td>
<td>43.00±0.37</td>
<td>54.22±0.23</td>
<td>92.21</td>
</tr>
<tr>
<td>5</td>
<td>1e</td>
<td>26.39±0.12</td>
<td>52.79±0.2</td>
<td>72.28±0.19</td>
<td>82.00±0.09</td>
<td>47.35</td>
</tr>
<tr>
<td>6</td>
<td>2a</td>
<td>13.28±0.12</td>
<td>27.86±0.09</td>
<td>38.27±0.2</td>
<td>51.82±0.3</td>
<td>96.48</td>
</tr>
<tr>
<td>7</td>
<td>2b</td>
<td>16.90±0.08</td>
<td>31.00±0.019</td>
<td>44.08±0.13</td>
<td>66.12±0.09</td>
<td>75.62</td>
</tr>
<tr>
<td>8</td>
<td>2c</td>
<td>31.71±0.07</td>
<td>50.64±0.12</td>
<td>67.95±0.19</td>
<td>81.00±0.24</td>
<td>49.36</td>
</tr>
<tr>
<td>9</td>
<td>2d</td>
<td>34.00±0.09</td>
<td>59.81±0.21</td>
<td>73.39±0.25</td>
<td>84.56±0.16</td>
<td>41.79</td>
</tr>
<tr>
<td>10</td>
<td>2e</td>
<td>40.43±0.23</td>
<td>65.28±0.13</td>
<td>78.80±0.09</td>
<td>90.73±0.24</td>
<td>38.29</td>
</tr>
</tbody>
</table>
### Table 8

*In Vitro* Hydroxyl scavenging activity of compounds

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Compounds</th>
<th>%RSC</th>
<th></th>
<th></th>
<th>IC$_{50}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>25µg/ml</td>
<td>50µg/ml</td>
<td>75µg/ml</td>
<td>100µg/ml</td>
</tr>
<tr>
<td>1</td>
<td>1a</td>
<td>10.23±0.12</td>
<td>20.32±0.32</td>
<td>34.19±0.10</td>
<td>49.12±0.01</td>
</tr>
<tr>
<td>2</td>
<td>1b</td>
<td>17.71±0.21</td>
<td>28.59±0.32</td>
<td>39.19±0.83</td>
<td>50.00±0.83</td>
</tr>
<tr>
<td>3</td>
<td>1c</td>
<td>10.49±0.02</td>
<td>19.96±0.4</td>
<td>33.83±0.09</td>
<td>51.96±0.1</td>
</tr>
<tr>
<td>4</td>
<td>1d</td>
<td>16.90±0.28</td>
<td>30.69±0.42</td>
<td>43.18±068</td>
<td>58.12±0.1</td>
</tr>
<tr>
<td>5</td>
<td>1e</td>
<td>23.28±0.56</td>
<td>67.86±0.35</td>
<td>79.27±0.81</td>
<td>92.82±0.3</td>
</tr>
<tr>
<td>6</td>
<td>2a</td>
<td>18.63±0.25</td>
<td>29.60±0.32</td>
<td>42.00±0.08</td>
<td>51.00±0.001</td>
</tr>
<tr>
<td>7</td>
<td>2b</td>
<td>19.43±0.14</td>
<td>31.22±0.1</td>
<td>48.36±0.34</td>
<td>62.22±0.28</td>
</tr>
<tr>
<td>8</td>
<td>2c</td>
<td>32.92±0.05</td>
<td>62.83±0.12</td>
<td>73.19±0.10</td>
<td>81.31±0.20</td>
</tr>
<tr>
<td>9</td>
<td>2d</td>
<td>38.23±0.03</td>
<td>65.19±0.17</td>
<td>75.28±0.3</td>
<td>84.31±0.9</td>
</tr>
<tr>
<td>10</td>
<td>2e</td>
<td>53.77±0.24</td>
<td>66.41±0.37</td>
<td>79.07±0.19</td>
<td>89.92±0.2</td>
</tr>
</tbody>
</table>
ANTIMICROBIAL ACTIVITY

Antibacterial Evaluation

The antibacterial activity of different sample is done in disc diffusion method against the following organisms as directed by Ellen Jo Boron.

- **Escherichia coli** - Gram negative
- **Pseudomonas aeruginosa** - Gram negative
- **Bacillus cereus** - Gram positive
- **Staphylococcus aureus** - Gram positive

**Media employed** - M.H.AGAR

**Solvent control Used** - Dimethyl Sulfoxide

**Test Samples**

1a Diethyl2,6-dimethyl-4-styryl-1,4-dihydro pyridine-3,5-dicarboxylate.

1b Diethyl 4-(3, 4-dimethoxy phenyl) 2, 6-dimethyl-1, 4-dihydropyridine3,5-dicarboxylate.

1c Diethyl4-(2,4-dichloro phenyl)- 2,6-dimethyl- 1, 4 –dihydropyridine-3,5-dicarboxylate.

1d Diethyl 2, 6-dimethyl-4-p-tolyl-1,4-dihydropyridine-3,5-dicarboxylate

1e Diethyl4-(4-hydroxy -3, 5-dimethoxy-2-phenyl)-2, 6-dimethyl-1,4dihydropyridine-3, 5-dicarboxylate

2a 4-((3,5-bis(ethoxy carbonyl)-2,6-dimethyl-4-styryl pyridine-1(4h)-yl)methylamino)benzoic acid
2b 4-((3,5-bis(ethoxy carbonyl)-2,6-dimethyl-4- (3,4-dimethoxy phenyl)pyridine-1(4H)-yl) methylamino) benzoic acid.

2c 4-((3,5-bis(ethoxy carbonyl)-2,6-dimethyl-4-(3,4-dichlorophenyl)pyridine-1(4H)-yl) methylamino) benzoic acid.

2d 4-((3,5-bis(ethoxy carbonyl)-2,6-dimethyl-4-p-tolyl pyridine-1(4H)-yl)methylamino) benzoic acid.

2e 4-(3,5-bis(ethoxy carbonyl)2, 6-dimethyl(4hydroxy3,5dimethoxypheny)pyridine1(4H)-yl)methylamino)benzoic acid.

**Standard Used** - Ciprofloxacin

The test samples used in concentration 1 mg / µl using dimethyl sulfoxide as solvent and 1mg/µl using their respective solvent Gentamicin, Ciprofloxacin and Tetracycline were used as standards for *Staphylococcus auereus* and *Escherichia coli*.

**Preparation of Media**

**Preparation of Nutrient agar**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptone</td>
<td>0.5%</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>0.5%</td>
</tr>
<tr>
<td>Meat of beef extract</td>
<td>0.5%</td>
</tr>
<tr>
<td>Agar</td>
<td>3.0%</td>
</tr>
<tr>
<td>Distilled water</td>
<td>q.s</td>
</tr>
<tr>
<td>pH adjusted to</td>
<td>7.2-7.4</td>
</tr>
</tbody>
</table>

Then the media is distributed in 5ml quantity into culture tubes and sterilized by autoclaving.

**DISC DIFFUSION METHOD**
To the sterile nutrient agar suspension of E.coli was added to 45°C and transferred to sterile petri dishes and allowed to solidify. Sterile discs 5 mm in diameter (made from filter paper sterilized in isopropyl alcohol) were dipped in solution containing compound samples and standard and blank placed on surface of agar plates.

Leave the plates standing for one hour at room temperature as a period of preincubation diffusion to minimize the effect of variation in time between the application of different solutions. Then the plates were incubated at 37°C for 18 hrs and observed for antibacterial activity. The diameters of zones of inhibition were measured for plates in which zone of inhibition was observed and presented in Table 9.

The average area of zone of inhibition was calculated and compared with that of standard.

A similar procedure was carried out for studies of anti-bacterial activity of other sample against staphylococcus aureus, the results were tabulated in Table 9.

**ANTIFUNGAL EVALUATION**

The antifungal activities of different samples were done in disc diffusion method against the following organism as described by Ellen Jo Boron.

1) *Aspergillus niger*

2) *Candida albicans*

Media employed - savouraud dextrose agar media

Solvent control used - dimethyl sulfoxide

The sample used in 1mg/µl concentration, using dimethyl sulfoxide as solvent and 1mg/µl concentration using their respective solvent Ketoconozole were used as standard against *Aspergillus niger.*
**Test Samples**

1a  Diethyl 2,6-dimethyl-4-styryl-1,4-dihydro pyridine-3,5-dicarboxylate.

1b  Diethyl 4-(3, 4-dimethoxy phenyl) 2, 6-dimethyl-1, 4-dihydropyridine3,5-dicarboxylate.

1c  Diethyl 4-(2,4-dichloro phenyl)- 2,6-dimethyl-1, 4-dihydropyridine-3,5-dicarboxylate.

1d  Diethyl 2, 6-dimethyl-4-p-toly1-1,4-dihydropyridine-3,5-dicarboxylate

1e  Diethyl 4-(4-hydroxy -3, 5-dimethoxy-2-phenyl)-2, 6-dimethyl-1,4dihydropyridine-3,5-dicarboxylate

2a  4-((3,5-bis(ethoxy carbonyl)-2,6-dimethyl-4-styryl pyridine-1(4h)-yl)methylamino)benzoic acid

2b  4-((3,5-bis(ethoxy carbonyl)-2,6-dimethyl-4- (3,4-dimethoxy phenyl)pyridine-1(4H)-yl) methylamino) benzoic acid.

2c  4-((3,5-bis(ethoxy carbonyl)-2,6-dimethyl-4- (3,4dichlorophenyl)pyridine-1(4H)-yl) methyamino) benzoic acid.

2d  4-((3,5-bis(ethoxy carbonyl)-2,6-dimethyl-4-p-tolyl pyridine-1(4 H)-yl)methylamino) benzoic acid.

2e  4-(3,5-bis(ethoxy carbonyl)2, 6-dimethyl (4hydroxy3,5dimethoxypheny)pyridine1(4H)-yl)methylamino)benzoic acid.

**Standard used**  Ketoconozole

**Preparation of Media used**

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Savouraud Dextrose Agar medium</td>
<td>gm/l</td>
</tr>
<tr>
<td>Mycological procedure</td>
<td>10</td>
</tr>
<tr>
<td>Dextrose</td>
<td>40.0</td>
</tr>
<tr>
<td>Agar</td>
<td>15.0</td>
</tr>
</tbody>
</table>
Final pH at 25°C: 5.6 ± 0.2

**DISC DIFFUSION METHOD**

Suspensions of *Aspergillus niger* were added to sterile nutrient agar at 45°C. The mixture was transferred to sterile petri dishes and allowed to solidify. Sterile discs 5 mm in diameter (made from whatman filter paper sterilized in U.V lamp) were dipped in solution containing compound samples and standards and blank samples were placed on the surface of agar plates.

Leave the plates standing for one hour at room temperature as a period of preincubation diffusion to minimize the effect of variation in time between the application of different solutions.

The average area of zone of inhibition was calculated and compared with that of the standards and the results were tabulated.

**Table 9**

Antimicrobial activity of synthesized 1,4-dihydropyridine derivatives

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Compound</th>
<th>Antibacterial activity zone of inhibition (MM)</th>
<th>Antifungal activity zone of inhibition (MM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>B.cereus</td>
<td>S.aureus</td>
</tr>
<tr>
<td>1</td>
<td>1a</td>
<td>14</td>
<td>12</td>
</tr>
<tr>
<td>2</td>
<td>1b</td>
<td>17</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>1c</td>
<td>13</td>
<td>15</td>
</tr>
<tr>
<td>4</td>
<td>1d</td>
<td>20</td>
<td>16</td>
</tr>
<tr>
<td>5</td>
<td>1e</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>2a</td>
<td>13</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>2b</td>
<td>24</td>
<td>19</td>
</tr>
<tr>
<td>8</td>
<td>2c</td>
<td>25</td>
<td>12</td>
</tr>
<tr>
<td>9</td>
<td>2d</td>
<td>18</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>2e</td>
<td>14</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>Ciprofloxacin</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>-----</td>
<td>---------------</td>
<td>----</td>
<td>----</td>
</tr>
<tr>
<td>12</td>
<td>Ketikonazole</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Fig. 31  Antibacterial activity of synthesised compounds 1a – 2e

![Antibacterial Activity Chart]

\[BC = \text{Bacillus Cereus}\]
\[SA = \text{Staphylococcus auereus}\]
\[EC = \text{Escherichia Coli}\]
\[PA = \text{Pseudomonas Aeruginosa}\]

Fig. 32  Antifungal activity of synthesised compounds 1a – 2e

![Antifungal Activity Chart]
CA = Candida Albicans

AN = Aspergillus Niger

Photograph showing the effect of Cpd 1d against S.aureus and A.niger
Photograph showing the effect of Cpd 2d against *S.aureus* and *A.niger*
Photograph showing the effect of Cpd 2c against *S.aureus* and *A.niger*
RESULT S AND DISCUSSION

I have synthesized the 1, 4 dihydro pyridine derivatives with 65-70 % yield. It was further converted as substituted mannich base of 1, 4- dihydro pyridine derivative with an of 70-80% yield.

The melting point of all synthesized compounds was found in open capillary tubes and readings were uncorrected .the elemental analysis was determined and the results were tabulated in the Table 3. The found value of the element by elemental analysis closer to calculated values

The IR spectra of the compounds were done in a schimadzu FT 8300 infrared spectrophotometer (Vmaxcm-1) by using KBr discs. The results of IR spectra given in the Table 4 shows that the functional groups such as phenolic hydroxyl, chlorine, amine, phenyl and methoxy groups may be present in the synthesized compound.

The $^1$HNMR spectra of the synthesized 1, 4-dihydropyridine derivative were recorded on JEOL GSX 400 spectrometer using TMS as internal standard (chemical shifts in $\delta$, ppm) and DMSO as the solvent The results of the $^1$HNMR spectra given in Table 6 shows that the numbers of hydrogen atoms present in all the synthesized compounds were exact when compared to the number of hydrogen atoms in the expected compounds.

The Mass spectra of the all synthesized compounds were done on a JEOL MSMATE spectrometer. The results were presented in Table 5 show that molecular mass of the synthesized compounds were nearer to the molecular mass of expected compounds.

The synthesized compounds were screened for there in vitro antioxidant activity by Nitrous oxide, Hydroxyl radical scavenging activity. The results obtained were tabulated in Table 7, 8 were given as mean IC$_{50}$. All the synthesized compounds showed good anti-oxidant activity, out of all the synthesized compounds 1e, 2c, 2d, 2e showed
significant anti-oxidant activity, in all the method except the compound 1a which showed less when compared to that of the standard butylated hydroxyl toluene (BHT).

The bleaching of Nitric oxide, hydroxyl ion absorption is representative of the capacity of the test compounds to scavenging free radical independently. The result of my investigation revealed that the test compound is electron donor and could react with free radicals to convert them to more stable product and terminate radical chain reaction.

The compound (1e, 2c, 2d, 2e) substituted with electron donating groups like methoxy and hydroxyl showed higher anti-oxidant activity compared to others.

The synthesized compounds were screened for their anti-microbial activity by Disc diffusion method. The results were tabulated in Table 9. The results showed that the compounds 1d, 2b 2c having very good activity when compare that of standard drug (ciprofloxacin). Because of the presence of methoxy group in 3&4\textsuperscript{th} position (2b), similarly (2c) presence of chlorine in 2&4\textsuperscript{th} position and presence of methyl group in p-position (1d).
SUMMARY AND CONCLUSION

Molecular modification of simple and complex chemical entities may lead to biological active compounds. Different types of approaches are made to derive such compounds which exhibit selective pharmacological activity. Pyridine and their related compounds exhibited potent antioxidant and antimicrobial activity.

This research work was oriented towards the finding of newly 1, 4-dihydro pyridine derivatives with antioxidant and anti microbial activities. The different substitution of some 1, 4- dihydro pyridine was synthesized by aromatic aldehydes and ethyl acetoacetate followed by condensation reactions. Ten compounds have been synthesized and all the compounds were tabulated.

Using different analytical techniques, elemental analysis, IR 1HNMR and mass spectrosopes. The results of this analysis showed that the expected different substituted some dihydro pyridine derivative were prepared.

The newly synthesized some dihydro pyridine derivatives were evaluated for their antioxidant and antimicrobial activity. The synthesized compounds 1e,1c,2d,2e showed effective antioxidant activity, also all the compound expect compound 1d,2b,2c was found to exhibit good antimicrobial activity . This clearly indicates that new dihydro pyridine derivatives can be effectively synthesized by the method mentioned in this study and these compounds exhibited significant antimicrobial and anti oxidant activities.

FUTURE PLAN

In conclusion, the present study reveals that some 1,4-dihydropyridine derivatives could be used as template for the future development through modification or derivatization to design more potent therapeutic agents.
REFERENCES


