

**GREEN SYNTHESIS AND ANTIHYPERTENSIVE ACTIVITY OF SOME MANNICH BASES OF 1, 4 DIHYDROPYRIDINE COMPOUNDS**

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**ABSTRACT**

Some new Mannich Bases of 1, 4 Dihydropyridine derivatives were synthesised by reaction of 1, 4 Dihydropyridine derivatives formaldehyde (HCHO) & secondary amines (e.g. morpholine, 1-methyl piperazine) in 1:1 ratio under acidic condition in presence of trace HCl. The compounds synthesised were identified by UV, <sup>1</sup>H NMR, and FT-IR spectroscopic techniques. All compounds studied in this work were screened for their antihypertensive activity by against DOCA salt induced hypertension.

**Keywords:** antihypertensive activity, 1, 4 Dihydropyridines, Mannich bases, green synthesis

**1. Introduction:**

Diseases of the arterial tree cause more premature deaths than all other diseases such as cancer and infections combined. Among the major risk factors for arterial diseases, high blood pressure has been identified as the most powerful one. Today hypertension represents a major public concern, affecting more than 20% of the adult population in Western countries and about 1 billion people worldwide<sup>1</sup>. Observational studies have shown a significant and continuous relation between high blood pressure levels and the burden of cardiovascular mortality and morbidity<sup>2</sup>. The presence of elevated blood pressure levels doubles the risk of ischemic heart disease, increases by fourfold the incidence of stroke, and accelerates the progression of renal disease<sup>3</sup>. In contrast, effective treatment of hypertension significantly reduces the incidence of coronary events and ischemic stroke and prevents the development or delays the progression of hypertension-related organ damage to congestive heart failure and end-stage renal disease<sup>4</sup>. In this view, even small reductions in blood pressure levels are associated with large reductions in the incidence of major cardiovascular events, especially in hypertensive patients at high risk, such as those with evidence of organ damage, metabolic syndrome, or diabetes<sup>5</sup>. Several classes of pharmacological agents have been used in the treatment of hypertension. One class of antihypertensive drugs known 1,4-dihydropyridines are associated with a low rate

of adverse side-effects and are the preferred class of anti-hypertensive agents for treating patients with concurrent secondary diseases<sup>6</sup>.

1,4-dihydropyridine is a six membered aromatic ring containing N at 1st position, which is saturated at 1 and 4<sup>th</sup> position are 1,4-DHP. The most feasible position for substitution is 4th which exhibit various activities i.e., as the calcium channel antagonists and the heterocyclic ring is the common feature for various pharmacological activities<sup>7</sup>. These chemical classes of compounds were shown to competitively block Ca<sup>2+</sup> movement through the slow channel and thus alter the cardiac action potential, hence called as slow channel blockers or calcium entry blockers or calcium antagonists. These agents work by blocking L-type voltage gated calcium channels (VGCC) in the heart and in the blood vessels. This prevents calcium levels from increasing as much in the cells when stimulated, leading to less contraction. This decreases total peripheral resistance by dilating the blood vessels, and decreases cardiac output by lowering the force of contraction. Because of resistance and output drop, so does blood pressure<sup>8</sup>.

The chemistry of dihydropyridines can be traced back to an 1882 paper in which Hantzsch described their utility as intermediates in the synthesis of substituted pyridines. Fifty years later, interest in this chemical class of compounds increased when it was discovered that 1, 4 DHP ring was responsible for the "hydrogen transfer" properties of the coenzyme

NADH. Numerous biochemical studies followed this discovery leading to identification of a variety of compounds that could block the inward movement of  $\text{Ca}^{2+}$  through slow cardiac channels occurred in the early 1960s. However, it was not until 1970s that the pharmacological properties of 1, 4-DHPs were fully investigated. The chemistry of dihydropyridine was reviewed in 1972 by Eisner and Kuthan & investigation of the activities of 1,4 DHPs known as "Hantzsch-type" compounds was systematically carried out by Love and coworkers at Smith, Klein and French laboratories. In analogy to the existing Dihydropyridine type of compounds e.g. - Nifedipine, Amlodipine, etc. used till today as effective calcium channel blockers; it was thought worthwhile to synthesize some new analogues of DHP. The success story of 1, 4 DHP as calcium antagonists has led to the development of novel synthetic strategies to improve their classical methods of preparation<sup>9,10</sup>.

Originally DHPs were synthesized by well established Hantzsch reaction; which entails condensation of an aldehyde with two equivalents of  $\beta$ -ketoester in presence of excess ammonia. It took long reaction time by refluxing in alcoholic solvent in presence of ammonia; which was not ecofriendly due to evolution of ammonia gas and energy consuming and yield was also less<sup>11</sup>. Hence, we thought it was worthwhile to attempt synthesis of DHP derivatives by Hantzsch reaction using Microwave irradiation technique under ecofriendly conditions called as Green synthesis. Furthermore, it appeared to offer better atom economy under solvent free condition than the conventional procedure to reduce reaction time from conventional for 12-16 hrs. to only a few minutes.

This solvent free green reaction technique of applying ultrasonic waves have been carried out according to the previous published data<sup>12</sup> but the green reaction technique by microwave irradiation using silica gel as catalyst for the synthesis of DHP derivative has not yet been reported as per our knowledge. Therefore this study was carried out to establish a solvent free Hantzsch reaction for preparing DHP derivatives by three component coupling reaction under microwave irradiation, and study the effect of using silica gel as novel catalyst on the product yield. This study also undertook a plan of synthesis of Mannich bases of some DHP derivatives under microwave irradiation and assessment of antihypertensive activity against

DOCA salt induced hypertension as literature survey indicated that mannich bases of DHP could enhance pharmacological activity of 1,4 DHP pharmacopore<sup>13</sup>.

## 2. Experimental

### 2.1 Synthesis:

**General procedure for synthesis of 4-substituted phenyl-1, 4-dihydropyridine:** Symmetrical dihydropyridines i.e. dihydropyridine 1-5 (DHP1-5) were synthesised by solvent free Hantzsch reaction by three component coupling reaction under microwave irradiation. A mixture of substituted benzaldehyde (20 mmol), ethyl acetoacetate (5.5ml) (42 mmol), approximately conc. ammonia solution (6ml) and silica gel (0.5 g) were taken in a 50 ml conical flask, mixed well by gentle shaking and then stoppered. The contents of the flask were irradiated in a microwave flask at power level 3, for 7-9 min. The progress of reaction was monitored by TLC (TLC- silica gel using chloroform: pet ether 4.5:0.5). The reaction mixture became a thick mass after completion of reaction. It was then cooled to room temperature; methanol (10-15 ml) was added into the reaction mixture and stirred well to break up the lumps. The contents were then filtered under suction to obtain the crude product. After washing several times with methanol the crude product was further purified by one or two recrystallisations (using methanol) to obtain the pure products DHP1-5 yielding about 75-80% whose m.p. and spectral (UV, IR, <sup>1</sup>H NMR) data has been reported below for the respective DHP derivatives. The melting point of the product in admixture with an authentic sample remained undepressed.

**General procedure for synthesis of Mannich bases of 1, 4 DHP:** A mixture of formaldehyde (8 mmol), Morpholine (6 mmol), were taken in a 50 ml conical flask, to this mixture HCl was added till solution becomes acidic (pH3). The solution was mixed well by stirring for 30 min. After stirring the solution was added to the 1, 4 DHP derivative (5 mmole) in 5 ml of methanol. Then the solution was taken in a two necked flask for microwave irradiation, the level of microwave instrument was kept at 3 (240 W) The progress of reaction was monitored by TLC after interval of 3 min. The reaction mixture showed colour change within 5 min & further reaction was allowed to continue for about 7-8 min (as specified below) till the completion of reaction (TLC). It was then cooled to room temperature and then extracted with chloroform

(10 ml), after addition of water (10mlx2). The organic layer was separated, washed with water (8 mlx2), dried over anhydrous sodium sulphate and concentrated under vacuum to obtain the crude product as semisolid mass, which was purified by two-three recrystallisations (methanol) to obtain the pure products i.e. Mannich bases 1-5 (MB1-5) yielding about 70-80 %. The m.p. and spectral (UV, IR, <sup>1</sup>H NMR) data of the product has been reported below for the respective mannich bases of 1, 4 DHP derivatives. The procedure of the reaction remains the same throughout with the change only in the type of 1, 4 DHP derivative, its quantity and the reaction time.

## 2.2 Physicochemical studies<sup>14-19</sup>:

**2.2.1 Melting points:** Melting points were determined on a Capillary Gallenkamp apparatus and are uncorrected.

**2.2.2 Thin layer chromatography:** In order to ascertain the purity and homogeneity of the synthesized compounds as reported in Tables (1, 2, 3); thin layer chromatography was carried out. The solvent system used for compounds were chloroform: pet ether (4.5:0.5) for the NIF 1-5 series; ethyl acetate: cyclohexane (1:1) for the hydrazine compounds and also for partial hydrolysis; Silica gel-G was used as an adsorbent. The spots were located using either iodine vapor or the UV lamp. Retention time ( $R_t$ ) were determined in minutes (min). Retention factor ( $R_f$ ) values were calculated for each derivative by the formula –

$$R_f = \frac{\text{Distance travelled by the compound}}{\text{Distance travelled by the solvent front}}$$

## 2.2.3 UV-visible spectrophotometric studies:

The synthesized compounds were checked for their characteristic absorption in UV-visible region (200-400nm) by using methanol as the solvent on the 1601 Shimadzu UV-visible spectrophotometer and the  $\epsilon_{\text{max}}$  was calculated by the standard procedure.

**2.2.4 Rotational and Vibrational absorption studies:** Infrared absorption spectra of each of the synthesized compounds were obtained by preparing KBr pellet, and then running it on Shimadzu 8400s FTIR spectrophotometer, to check the characteristic absorptions, as reported in the respective tables in the results section.

## 2.2.5 Nuclear Magnetic Resonance studies:

The synthesized compounds were subjected to <sup>1</sup>H NMR studies on the Shimadzu FT-NMR spectrophotometer (300 MHz) using CDCl<sub>3</sub>/ d<sub>6</sub>-

DMSO as the solvent and TMS as internal standard.

## 2.3 Pharmacology

**2.3.1 Acute Toxicity study:** Acute toxicities were carried out by using Wistar albino rats, following OECD text guideline 423(2001) usage in defined dosage and results allowed the substances to be ranked and classified according to globally harmonized system (GHS) for the classification of chemicals which can cause acute toxicities<sup>20</sup>.

**Procedure:** Mannich bases of 1,4 dihydropyridine derivatives dissolved in DMSO was administered to group of animal (n=4) up to the dose of 200 mg/kg body weight orally, placed individually in plastic cages and observed at least one during first 30 minutes and periodically during 24 hours. Special attention given during first two hours treated animals observed by an observer blind to the treatment protocol. Since no mortality was observed in this dose range, hence this was considered as safer dose and no further toxicity study done at higher doses.

## 2.3.2 Antihypertensive activity<sup>21,22</sup>:

**Induction of hypertension:** Rats were housed temperature and humidity controlled, light cycled quarters. Animals were randomly divided into two groups including normotensive and hypertensive. Normotensive rats received saline injection 0.5ml/kg, twice weekly for 5 weeks, subcutaneously (s.c.) whereas hypertension was induced by DOCA-salt injection 20 mg/kg body weight ( b.w.) s.c., for 5 weeks and NaCl was added to the drinking water.

**Studies on anaesthetized rats:** Five weeks after saline or DOCA injection, animals were anaesthetized with sodium thiopental 30 mg/kg b.w. by intraperitoneal (i.p.) injection. The right common carotid artery was catheterized for the measurement of blood pressure, right and left jugular veins were cannulated for the administration of anesthetic (sodium thiopental, 10 mg/kg b.w.) throughout the experiment. The trachea was cannulated and the animals were allowed to breathe spontaneously. Body temperature was recorded using a rectal thermostat probe and was maintained at 37 + 0.5 °C using an incandescent lamp placed, over the abdomen. After 20 min. (for stabilization), arterial blood pressure (systolic, diastolic and mean) and heart rate were measured.

**Measurement of hypotensive effects:** All test compounds (Mannich Bases of 1, 4 DHP) were administered in a dose of 0.3, 3 and 30 mg/kg

b.w. to the normotensive rats through cannula in a volume of 0.3 ml/kg b.w. Equivolumetric injections of vehicle were administered to the control animals. Nifedipine was used as standard with the same doses.

**Measurement of antihypertensive effects:** All test compounds (Mannich Bases of 1, 4 DHP) were administered with the same doses as mentioned above to the hypertensive rats in a volume of 0.3 ml/kg b.w. Equivolumetric injections of vehicle were administered to the control animals. Nifedipine was used as standard with the same doses.

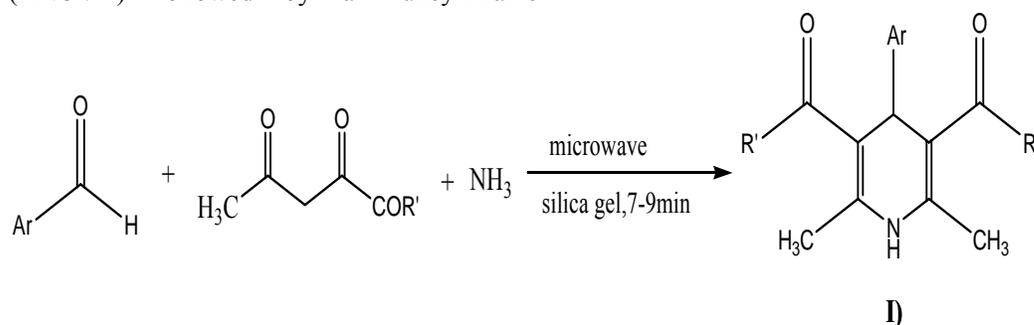
**2.4 Statistical analysis of data:** Results are expressed throughout as means + S.E.M. and were analyzed by one way analysis of variance (ANOVA) followed by a Tukey-Kramer

multiple comparison test (for comparison of responses to dihydropyridine in hypertensive rats). A 'P' value of less than 0.05 was considered to be significant.

### 3. Results and Discussion:

**3.1 Chemistry:** A solvent free Hantzsch reaction for synthesis of 4-substituted phenyl-1, 4- Dihydropyridine derivatives by three component coupling reaction under microwave irradiation using silica gel as novel catalyst was carried out to give the compounds DHP1-5 (Scheme 1).

The compounds were characterized by UV, IR and  $^1\text{H}$  NMR spectroscopy. The purity of all the compounds was determined by thin layer chromatography (Table 1 and 2).



Ar :- 2-(NO<sub>2</sub>)C<sub>6</sub>H<sub>4</sub>, 3-(NO<sub>2</sub>)C<sub>6</sub>H<sub>4</sub>, 3,4,5-(OCH<sub>3</sub>)C<sub>6</sub>H<sub>4</sub>, -C<sub>6</sub>H<sub>5</sub>,  
4-(OH)C<sub>6</sub>H<sub>4</sub>

R' :- OEt

#### SCHEME 1

**Table-1: The physicochemical data of 1, 4-dihydropyridines (DHP- 1 to 5)**

Compound	Ar	Molecular Formula	Yield (%)	m.p. (°C)	$\lambda_{\text{max}}$ ( $\epsilon_{\text{max}}$ )	Rf	Rt (min)
DHP -1	2-(NO <sub>2</sub> ) C <sub>6</sub> H <sub>4</sub>	C <sub>19</sub> H <sub>21</sub> O <sub>6</sub> N <sub>2</sub>	78	160-162	272(9119)	0.63	13.78
DHP -2	3-(NO <sub>2</sub> ) C <sub>6</sub> H <sub>4</sub>	C <sub>19</sub> H <sub>21</sub> O <sub>6</sub> N <sub>2</sub>	75	155-158	276(3671)	0.56	10.94
DHP -3	3,4,5 (-OMe) C <sub>6</sub> H <sub>4</sub>	C <sub>22</sub> H <sub>19</sub> O <sub>7</sub> N	76	160-162	274(7142)	0.58	20.33
DHP-4	-C <sub>6</sub> H <sub>5</sub>	C <sub>19</sub> H <sub>23</sub> NO <sub>4</sub>	86	151-152	275(2631)	0.60	15.00
DHP -5	4-(OH) C <sub>6</sub> H <sub>4</sub>	C <sub>19</sub> H <sub>22</sub> O <sub>5</sub> N	74	220-222	272(6482)	0.57	12.70

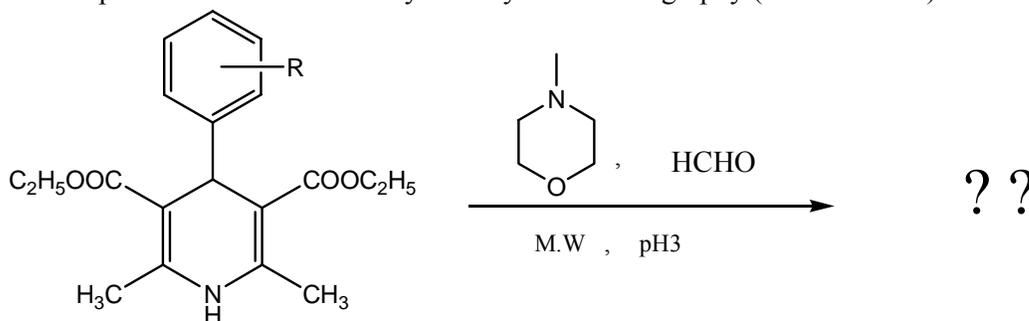
**Table-2: The Spectral data of 1, 4-dihydropyridines (DHP- 1 to 5)**

Compound	IR Data: Absorption in cm <sup>-1</sup> (Signal characteristics)	$^1\text{H}$ NMR Data (CDCl <sub>3</sub> ): Absorption in cm <sup>-1</sup> (Peak Characteristics)
DHP -1	3323 (NH stretching), 2989 (CH aliphatic), 1701 (C=O ester), 1535 (NO <sub>2</sub> ), 3105 (CH aromatic), 1130 (-C-O- ester), 1363 (-C-CH <sub>3</sub> )	0.9 (t, 6H, -OCH <sub>2</sub> CH <sub>3</sub> ), 2.45 (s, 6H, methyl grs), 3.9 (q, 3H, -OCH <sub>2</sub> CH <sub>3</sub> ), 5.7 (s, H, at C <sub>4</sub> ), 5.9 (s, NH), 7.3 (d, 1H, Ar), 7.4 (m, 1H, Ar), 7.6 (d, 1H, Ar) 7.8 (m, 1H, Ar)
DHP -2	3344 (NH stretching), 2989 (CH aliphatic), 1701 (C=O ester), 1523 (NO <sub>2</sub> ), 3191 (CH aromatic), 1118 (-C-O- ester), 1369 (C-CH <sub>3</sub> )	1.3 (t, 6H, -OCH <sub>2</sub> CH <sub>3</sub> ), 2.37 (s, 6H, methyl grs), 4.1 (q, 3H, -OCH <sub>2</sub> CH <sub>3</sub> ), 5.1 (s, 1H, at C <sub>4</sub> ), 5.82 (s, NH), 7.3 (t, 2H, Ar), 7.6 (d, 1H, Ar)

DHP -3	3355 (NH stretching), 2977 (CH aliphatic), 1693 (C=O ester), 3101 (CH aromatic), 1128 (-C-O- ester), 1379 (-C-CH <sub>3</sub> ).	1.3 (t, 6H, -OCH <sub>2</sub> CH <sub>3</sub> ), 2.38 (s, 6H, methyl grs), 3.8 (s, 9H, methoxy grs), 4.1 (q, 3H, -OCH <sub>2</sub> CH <sub>3</sub> ), 4.99 (s, 1H, at C <sub>4</sub> ), 5.8 (s, NH), 6.58 (s, 2H, Ar)
DHP-4	3340 (NH stretching), 2981 (CH aliphatic), 1685 (C=O ester), 3068 (CH aromatic), 1130 (-C-O- ester), 1367 (-C-CH <sub>3</sub> )	1.2 (t,6H,-OCH <sub>2</sub> CH <sub>3</sub> ), 2.4 (6H,2CH <sub>3</sub> ), 4.2 (3H,OCH <sub>2</sub> CH <sub>3</sub> ), 5.6 (1H at C <sub>4</sub> ), 6.6 (H,NH), 7.2 (2H,Ar)
DHP -5	3344 (NH stretching), 2985 (CH aliphatic), 1670 (C=O ester), 3028 (CH aromatic), 1130 (-C-O- ester), 1367 (-C-CH <sub>3</sub> )	1.21 (t, 6H, -OCH <sub>2</sub> CH <sub>3</sub> ), 2.34 (s, 6H, methyl grs), 4.1 (q, 3H, -OCH <sub>2</sub> CH <sub>3</sub> ), 4.9 (s, -OH gr), 5.45 (s, 1H, at C <sub>4</sub> ), 6.81 (s, NH), 7.1 (d, 2H, Ar), 7.21 (s, 2H, Ar)

DHP derivatives (DHP 1- 5) were allowed to react with formaldehyde (HCHO) & secondary amines (e.g. morpholine ,1-methyl piperazine) in 1:1 ratio under acidic condition in presence of trace HCl to obtain Mannich bases (Scheme 2).

The compounds were characterized by UV, IR and H<sup>1</sup> NMR spectroscopy. The purity of all the compounds was determined by thin layer chromatography (Table 3 and 4).

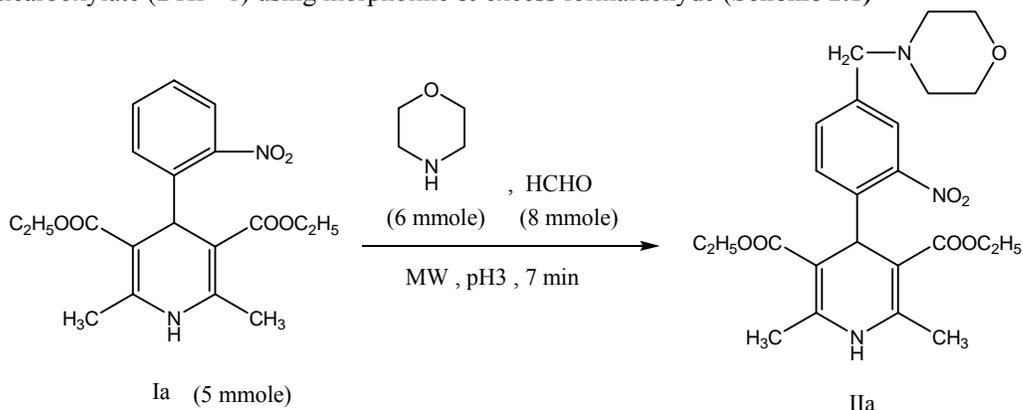


R-2NO<sub>2</sub>,3NO<sub>2</sub>,3-4-5 OCH<sub>3</sub>,OH

#### SCHEME 2

**Synthesis of Mannich bases of some 4-substituted phenyl-1, 4- Dihydropyridines under the microwave Irradiation: (Scheme 2.1- 2.6)**

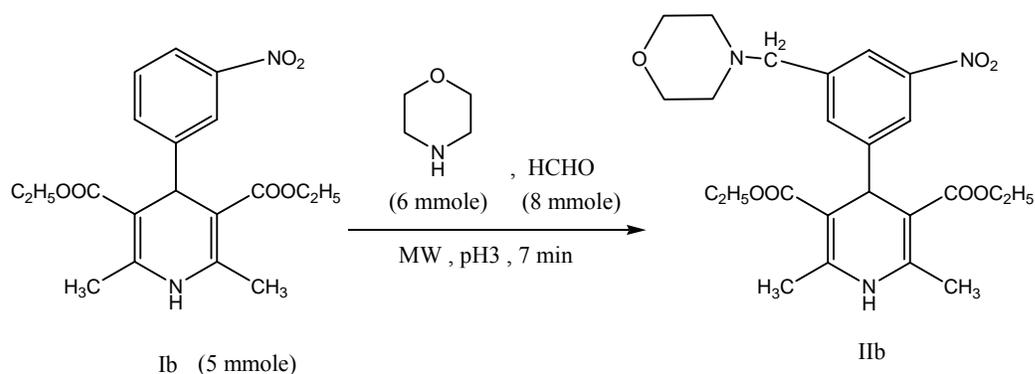
**MB 1:** Mannich reaction of 4-(2-Nitrophenyl)-diethyl, 2, 6-dimethyl-1, 4- dihydropyridine-3, 5-dicarboxylate (DHP -1) using morpholine & excess formaldehyde (**Scheme 2.1**)



diethyl 1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)pyridine-3,5-dicarboxylate

diethyl 1,4-dihydro-2,6-dimethyl-4-[4-(morpholinomethyl)-2-nitrophenyl]pyridine-3,5-dicarboxylate

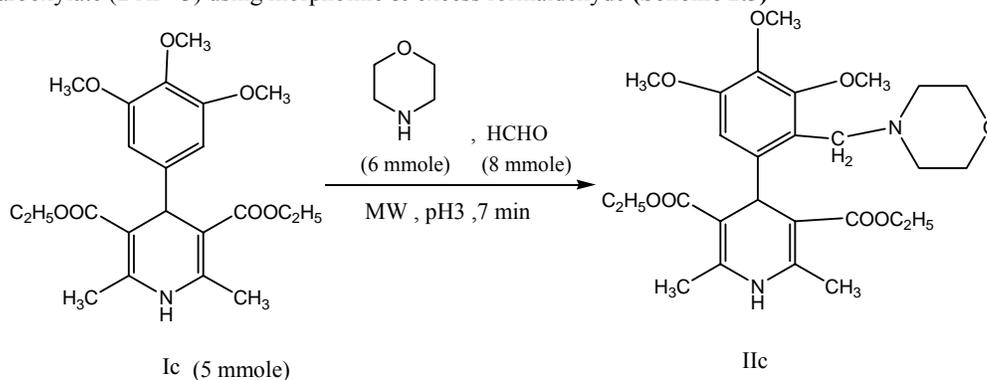
**MB 2:** Mannich reaction of diethyl 1, 4 dihydro-2, 6-dimethyl-4(3-nitrophenyl) pyridine- 3, 5-dicarboxylate (DHP -2) using morpholine & excess formaldehyde (**Scheme 2.2**)



diethyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)pyridine-3,5-dicarboxylate

diethyl 1,4-dihydro-2,6-dimethyl-4-[3-(morpholinomethyl)-5-nitrophenyl]pyridine-3,5-dicarboxylate

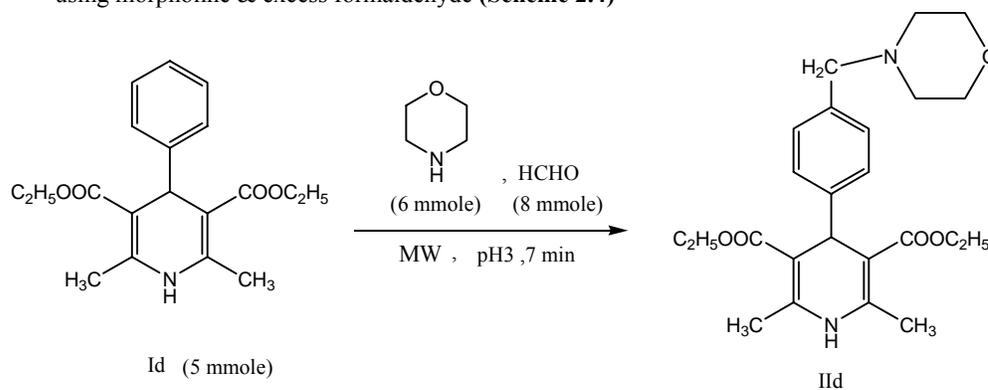
**MB 3:** Mannich reaction of 4-(3, 4, 5- Trimethoxyphenyl) - diethyl, 2, 6-dimethyl-1, 4- dihydropyridine-3, 5-dicarboxylate (DHP -3) using morpholine & excess formaldehyde (**Scheme 2.3**)



diethyl 1,4-dihydro-4-(3,4,5-trimethoxyphenyl)-2,6-dimethylpyridine-3,5-dicarboxylate

diethyl 1,4-dihydro-4-[3,4,5-trimethoxy-2-(morpholinomethyl)phenyl]-2,6-dimethylpyridine-3,5-dicarboxylate

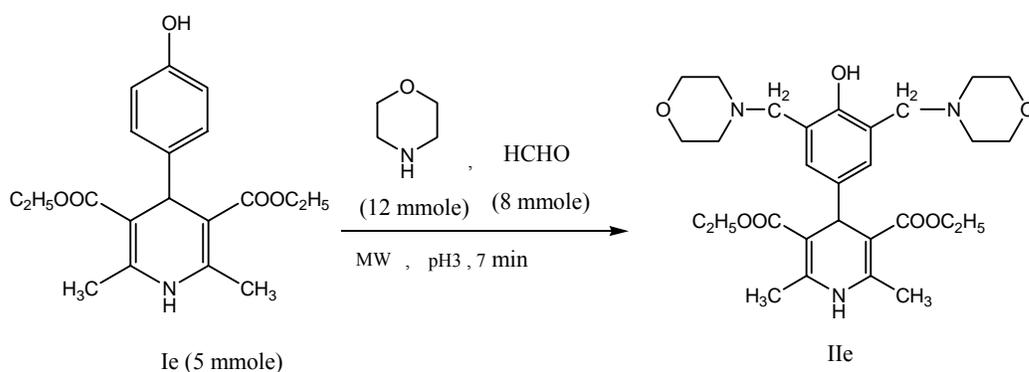
**MB 4:** Mannich reaction of 4-phenyl- diethyl- 2, 6-dimethyl-1, 4-dihydropyridine-3, 5- Dicarboxylate (DHP -4) using morpholine & excess formaldehyde (**Scheme 2.4**)



4-phenyl-diethyl-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate

diethyl 1,4-dihydro-2,6-dimethyl-4-[4-(morpholinomethyl)phenyl]pyridine-3,5-dicarboxylate

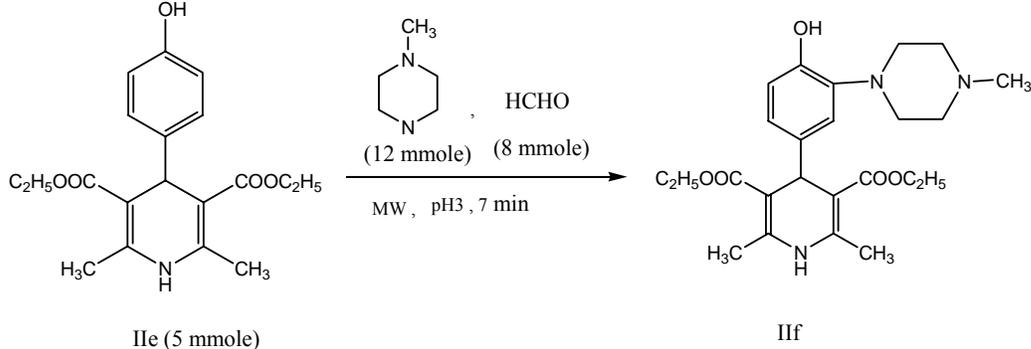
**MB 5** Mannich reaction of 4-(4- Hydroxyphenyl)-diethyl, 2, 6-dimethyl-1, 4- dihydropyridine-3, 5-dicarboxylate (DHP -5) using excess morpholine & excess formaldehyde (**Scheme 2.5**)



diethyl 1,4-dihydro-4-(4-hydroxyphenyl)-2,6-dimethylpyridine-3,5-dicarboxylate

diethyl 1,4-dihydro-4-[4-hydroxy-3,5-bis(morpholinomethyl)phenyl]-2,6-dimethylpyridine-3,5-dicarboxylate

**MB 6** Mannich reaction of 4-(4-Hydroxyphenyl)-diethyl, 2, 6-dimethyl-1, 4- dihydropyridine-3, 5-dicarboxylate (DHP -5) using 1- methyl piperazine & excess formaldehyde (**Scheme 2.6**)



diethyl 1,4-dihydro-4-(4-hydroxyphenyl)-2,6-dimethylpyridine-3,5-dicarboxylate

diethyl 1,4-dihydro-4-[4-hydroxy-3-(4-methylpiperazin-1-yl)phenyl]-2,6-dimethylpyridine-3,5-dicarboxylate

**Table 3: The physicochemical data of Mannich bases of 1, 4 DHP derivatives**

Compound	Molecular Formula	Yield (%)	M.p. (°C)	$\lambda_{\max}$ ( $\epsilon_{\max}$ )	Rf	Rt (min)
(MB 1)	C <sub>24</sub> H <sub>31</sub> N <sub>3</sub> O <sub>7</sub>	75	130-132	275 (4544)	0.63	13.78
(MB 2)	C <sub>23</sub> H <sub>29</sub> N <sub>3</sub> O <sub>7</sub>	78	125-127	278 (4024)	0.56	10.94
(MB 3)	C <sub>27</sub> H <sub>38</sub> N <sub>2</sub> O <sub>8</sub>	68	140-142	274 (4736)	0.58	20.33
(MB 4)	C <sub>24</sub> H <sub>32</sub> N <sub>2</sub> O <sub>5</sub>	80	160-162	280 (3084)	0.60	15.00
(MB 5)	C <sub>29</sub> H <sub>41</sub> N <sub>3</sub> O <sub>7</sub>	78	170-172	275 (4444)	0.57	12.70
(MB 6)	C <sub>24</sub> H <sub>33</sub> N <sub>3</sub> O <sub>5</sub>	80	166-168	272 (3272)	0.62	10.00

**Table 4: Spectral data Mannich bases of 1, 4 DHP derivatives**

Compound	IR Data: Absorption in cm <sup>-1</sup> (Signal characteristics)	<sup>1</sup> H NMR Data (CDCl <sub>3</sub> ): Absorption in cm <sup>-1</sup> (Peak Characteristics)
(MB 1)	3323 (NH stretching), 2989 (CH aliphatic), 1635 (C=O ester), 1531 (NO <sub>2</sub> ), 3105 (CH aromatic), 1363 (-C-CH <sub>3</sub> )	1.1 (d, 6H, -2CH <sub>3</sub> ), 1.2 (t, 6H, -OCH <sub>2</sub> CH <sub>3</sub> ), 2.3 (m, 8H, 2CH <sub>2</sub> N), 3.8 (m, 8H, 2CH <sub>2</sub> O), 4.0 (m, 8H, -O(CH <sub>2</sub> ) <sub>2</sub> ), 5.4 (s, 1H, NH), 7.4 (m, 1H, Ar)
(MB 2)	3340 (NH stretching), 2989 (CH aliphatic), 2360 (CH aromatic), 1635 (C=O ester), 1531 (NO <sub>2</sub> ), 1363 (-C-CH <sub>3</sub> )	1.03 (d, 6H, -2CH <sub>3</sub> ), 1.2 (t, 6H, -OCH <sub>2</sub> CH <sub>3</sub> ), 2.4 (m, 8H, 2CH <sub>2</sub> N), 3.8 (m, 8H, 2CH <sub>2</sub> O), 5.4 (s, 1H, NH), 7.5-7.6 (m, 1H, Ar)

(MB 3)	3365- NH stretching, 2837- CH aliphatic- 3040, CH aromatic, 1693- C=O ester, 1000- C-O stretching, 1363 - C-CH <sub>3</sub>	1.1 (d, 6H, -2CH <sub>3</sub> ), 1.28 (t, 6H, -OCH <sub>2</sub> CH <sub>3</sub> ), 1.6, (s, 2H, -OCH <sub>2</sub> ), 2.6, (t, 8H, CH <sub>2</sub> -N), 3.8 (m, 6H, 2OCH <sub>3</sub> ), 4.1 (m, 8H, -O <sub>2</sub> CH <sub>2</sub> ), 5.6 (s, H, NH)
(MB 4)	3323 (NH stretching), 2837 (CH aliphatic), 3040 (CH aromatic), 1635 (C=O ester), 1211 (C-N amine), 1690 (-C=C-)	1.2 (t, 6H, -OCH <sub>2</sub> CH <sub>3</sub> ), 2.2 (s, 6H, 2CH <sub>3</sub> ), 4.05 (br, m, 5H, 2CH <sub>2</sub> N), 4.95 (s, 1H, -CH), 5.4 (s, 1H, NH), 7.2 (d, 2H, Ar)
(MB 5)	3291 (NH stretching), 2837 (CH aliphatic), 3040 (CH aromatic), 1635 C=O ester, 1207 C-N amine, 1691 (-C=C-)	1.22 (t, 6H, OCH <sub>2</sub> CH <sub>3</sub> ), 2.2 (s, 6H, -2CH <sub>3</sub> ), 2.4 (m, 8H, -CH <sub>2</sub> N), 3.5 (s, 4H, -NCH <sub>2</sub> ), 3.7 (m, 8H, -O <sub>2</sub> CH <sub>2</sub> ), 4.10-4.16 (q, 4H, OCH <sub>2</sub> CH <sub>3</sub> ), 4.8 (s, 1H, -CH), 5.67 (s, 1H, NH), 6.9 (d, 2H, Ar)
(MB 6)	3340 (NH stretching), 2981 (CH aliphatic), 3040 (CH aromatic), 1687 (C=O ester), 1220 (C-N amine), 1690 (-C=C-)	1.2 (t, 6H, -OCH <sub>2</sub> CH <sub>3</sub> ), 1.6 (s, 1H, -CH <sub>3</sub> ), 2.32 (m, 8H, -2CH <sub>2</sub> N), 4.2 (m, 5H, -OCH <sub>2</sub> CH <sub>3</sub> ), 4.9 (s, 1H, -CH), 5.56, (s, 1H, NH D <sub>2</sub> O exchange), 7.1-7.2 (br.m, 4H, Ar)

### 3.2 Biological studies:

#### Effects of test agents on normotensive rats:

Intravenous administration of test compounds (at a doses 0.3, 3, and 30mg/kg b.w.) produced blood pressure lowering effects in thiopental-anesthetized normotensive rats. After 20 min (for stabilization), mean arterial blood pressure fall was measured.

#### Effects of test agents on hypertensive rats:

Intravenous administration of test compounds (at a doses 0.3, 3, and 30mg/kg b.w.) produced blood pressure lowering effects in thiopental-anesthetized hypertensive rats. After 20 min (for stabilization), mean arterial blood pressure fall was measured.

**Table 5: Fall in blood pressure after administration of Mannich bases in Normotensive and Hypertensive rats**

Compound	Mean Arterial Blood Pressure fall (SEM) <sup>a</sup>					
	Normotensive			Hypertensive		
	Dose in mg/kg b.w., i.v.					
	0.3	3	30	0.3	3	30
MB1	19.45(3.2)	45.23(2.7)	41.2(2.3)	28.4(5.3)	29.5(2.1)	30.2(5.6)
MB2	25.24(2.9)	42.34(2.9)	42.8(3.2)	28.65(4.5)	30.2(5.2)	34.62(5.9)
MB3	22.23(3.5)	44.24(2.8)	49.48(2.1)	22.5(3.5)	32(4.6)	32.32(5.3)
MB4	23.52(2.3)	49.15(4.1)	49.63(1.2)	28.20(5.6)	32.00(4.5)	34.56(4.3)
MB5	23.26(3.4)	50.26(3.6)	52.26(2.1)	31.32(3.3)	33.23(5.6)	35.23(6.5)
MB6	20.40(2.3)	51.6(4.2)	55.22(2.3)	30(4.7)	34.40(5.6)	38.92(5.6)
Nifedipine	22.2 (3.1)	52.53(5.2)	nd	31.4(5.3)	32.46(6.8)	nd
DMSO	12.30(2.3)	12.30(2.3)	12.30(2.3)	15.20(5.3)	15.20(5.3)	15.20(5.3)

<sup>a</sup> Mean arterial blood pressure fall: standard errors of mean (SEM) are indicated in the parenthesis. All the results were analysed for statistically significant differences from control DMSO (0.3 ml/kg b. w., i.v.) by analysis of variance and all showed significant difference (p< 0.05), nd: not determined

Where, \* p< 0.05, \*\* p< 0.01, \*\*\* p< 0.001.

**0.3 Normotensive-** 0.3 mg/kg b.w in Normotensive rats.

**3.0 Normotensive-** 3.0 mg/kg b.w in Normotensive rats.

**30.0 Normotensive-** 30.0 mg/kg b.w in Normotensive rats.

**0.3 Hypertensive-** 0.3 mg/kg b.w in Hypertensive rats.

**3.0 Hypertensive-** 3.0 mg/kg b.w in Hypertensive rats.

**30.0 Hypertensive-** 30.0 mg/kg b.w in Hypertensive rats.

The results of this study exhibited that all the Mannich bases significantly lowered the arterial blood pressure in normotensive and hypertensive rats in comparison with the solvent DMSO.

They were found to be more potent and effective as compared to Nifedipine. However, further experiments are needed to investigate the effects

of these test compounds on vascular tonicity in different vasculatures.

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