

SYNTHESIS AND BIOLOGICAL EVALUATION OF 1,4 DIHYDROPYRIDINES  
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## ABSTRACT

Heterocyclic chemistry is of great importance to the medicinal chemists because of their drug utility. Large number of heterocyclic compounds being used as therapeutic agents and these compounds are also essential for the human life. 1,4-Dihydropyridines are associated with broad spectrum of biological activities. A series of new Schiff bases containing the Hantzsch 1,4-dihydropyridine skeleton (1a-1g) has been synthesized by reaction of an appropriate aldehyde with 1, 4-dihydro-4-(substituted phenyl)-2,6-dimethylpyridine-3,5-dicarbohydrazide. The synthesized compounds were then characterized by TLC, melting point determination, IR, <sup>1</sup>H-NMR.

**KEYWORDS:** 3-Aryl-2-isobutanoyl-N-phenyl-acrylamide, Methyl-3-aminocrotonate, 1,4-Dihydropyridines, Antimicrobial activities.

## 1.1 INTRODUCTION

Dihydropyridine chemistry is of interest from the point of view of pure research on heterocyclic compounds and from a biological point of view (Sausins A 1998). Hantzsch 1,4-dihydropyridines (1,4-DHPs), a class of model compounds of the NADH coenzyme, have been extensively studied in view of the biological pertinence of these compounds to the NADH redox process (Swarnalatha G 2011). Since the early 1980's the presence of a DHP ring in the structure of 1,4-DHP derivatives has been regarded as a prerequisite for calcium (Ca<sup>2+</sup>) channel modulating properties (Stout DM 1982). So 1,4-DHPs classes of compounds are excellent starting synthons for the development of antitubercular agents (Boer R 1995). As a result, newly synthesized generations of 1,4-DHPs possess different pharmacological activities such as anticancer [Sadanandam YS 1994], antidiabetic [Cooper K 1992], antianginal (Briukhanov VM 1994), bronchodilating [Agudoawu SR 2000], neurotropic [Pattan SR 2007], antiallergic [Shafiee A 2004], anti-inflammatory [Wadher SJ, 2009], So the pharmacology of 1,4-DHPs derivatives is at the eve of a novel boom. From study of DHP compounds, it is found that both the ester groups present at 3 and 5 position play an important role in cardiovascular activity, removal of these groups may lead to reduction of the activity. This conclusion has opened up new scope for structural modifications at these positions.

Very wide range of literature regarding the structure, synthesis, stereochemistry and hydrogen transfer

mechanism of dihydropyridine is available. Some new Methyl-4-aryl-6-isopropyl-2-methyl-5-[n-phenyl-aminocarbonyl]-1,4-dihydropyridine-3-carboxylates. The Dihydropyridine derivatives of Type (1a-1) have been synthesized by the condensation of 3-Aryl-2-isobutanoyl-N-phenyl-acrylamide and Methyl-3-aminocrotonate. All the prepared compounds were characterized by their spectral (I.R., N.M.R., Mass) data and screened for their antimicrobial activities.

1,4-Dihydropyridines are now established as heterocycles having tremendous applications and still further scope for its pronounced drug activity like calcium channel antagonism and antihypertensive action. Since then tremendous study have been carried out on new synthesis & pharmacological activity of DHP. Some drugs currently used in market are nimodipine<sup>[1]</sup>, nicardipine<sup>[2]</sup>, isradipine<sup>[3]</sup>, nitrendipine<sup>[4]</sup>, flordipine<sup>[5]</sup>. 1,4-DHPs possess different pharmacological activities such as myocardial infarction<sup>[6]</sup>, stable<sup>[7]</sup> and unstable angina<sup>[8]</sup>, vasodilator<sup>[9]</sup>, coronary vasodilator and cardiopathic<sup>[10]</sup>, antiarrhythmic<sup>[11]</sup>, antiulcer<sup>[12]</sup>, anti-inflammatory<sup>[13]</sup>, subarachnoid haemorrhage<sup>[14]</sup>, ischemic brain damage<sup>[15]</sup>, atherosclerosis<sup>[16]</sup>, heart failure<sup>[17]</sup>, calcium channel antagonism<sup>[18]</sup>, antitumor<sup>[19]</sup>, antimayocardiac ischemic<sup>[20]</sup>, PAF antagonist<sup>[21]</sup>, adenosine A3 receptor antagonist<sup>[22]</sup>, antitubercular agents.<sup>[23]</sup>

## 1. 2 METHODS AND MATERIALS

### MATERIALS AND INSTRUMENTS

The chemicals used for the experiments were Benzaldehyde, Ethyl acetate, Ammonium acetate, Pyridine, 4-nitro aniline, Para di amino diphenyl, Diphenyl amine, Acetanilide, 4-Bromo acetanilide, Ethylene diamine tetra acetic acid di sodium salt (EDTA) and 1-Amino -2-naphthal-4-sulfonic acid. The chemicals used for the experiments are Sodium nitroprusside, Phosphate buffer, Griess Reagent (mixing the equal volume of 1% sulphalinamide in 2% phosphoric acid & 0.1% naphthyl ethylene diamine dihydrochloric acid in water) Hydrogen peroxide. The Reagents used were Alsiever's solution-(dextrose+sodium citrate+citric acid+sodium chloride), HRBC solution (blood + Alsiever's solution and centrifuge at 3000rpm for 20 minutes then packed cells were washed with isotonic saline & later 10%v/v suspension of the packed cells was made with isotonic saline) and Hypotonic solution. Solvents used are Ethanol, Chloroform, DMF, DMSO, Ether, methanol and diethyl ether.

**Equipments:** purity of compounds was checked by thin layer chromatography. Melting points of synthesized compounds was determined by VEEGO Digital melting point apparatus. The IR Spectra of synthesized compounds were recorded on **Fourier-Transform** spectrophotometer (Perkin Elmer Spectrum Version), in the range of 400-4000 $\text{cm}^{-1}$  using KBr pellets. The  $^1\text{H-NMR}$  spectra of synthesized compounds were recorded on **BRUKER Advanced II 400 MHz** NMR Spectrometer.

### 1.3 PROCEDURE

#### STEP-1: GENERAL PROCEDURE FOR 1,4-DIHYDROPYRIDINE SYNTHESIS

- A mixture of aldehydes(0.1M), Ethyl acetoacetate(0.1M), Ammonium acetate (0.2M) and acetonitrile(5ml) as catalyst is stirred at 25°C for 4hrs.
- After stirring for a specified time the reaction mixture is diluted with distilled water and extracted with ethyl acetate.
- The solution is moistened with sodium bicarbonate [ $\text{NaHCO}_3$ ] solution.
- The crude product obtained above is recrystallized from methanol with dimethyl formamide solution to obtain 1,4-dihydropyridine.

#### STEP-2: SYNTHESIS OF VARIOUS AMINES

- **1,4-DHP** (0.1M), Various amines(0.1M), Formaldehyde(3.0031ml) were refluxed in methanol(25ml) for 6hrs.
- On cooling solid appeared which is recrystallized from mixture of chloroform with petroleum ether.

### VARIOUS AMINES USED:-

- A<sub>1</sub>** 4-Nitro aniline
- A<sub>2</sub>** P-Diamino diphenyl
- A<sub>3</sub>** Diphenyl amine
- A<sub>4</sub>** Acetanilide
- A<sub>5</sub>** Ethylene diamine tetra acetic acid disodium salt
- A<sub>6</sub>** 4-Bromo acetanilide
- A<sub>7</sub>** 1-Amino-2-naphthol-4-sulfonic acid

### 1.4 PROCEDURE

#### STEP-1: SYNTHESIS OF 2-[1-(2-PHENYL HYDRAZONO)ETHYL]1H BENZIMIDAZOLE

- A mixture of benzoimidazol ethanone 3.2gms and phenyl hydrazine (0.1mole), 50 ml of acetic acid and methanol (25ml) was refluxed for 3 hours.
- At the end of this period the mixture was cooled and poured into ice cold water.
- The separated solid was filtered, washed with water and dried to get crude product which on recrystallized from hot methanol gives pure 2-[1-(2-phenyl hydrazono)ethyl]1H benzoimidazole (compound-1).

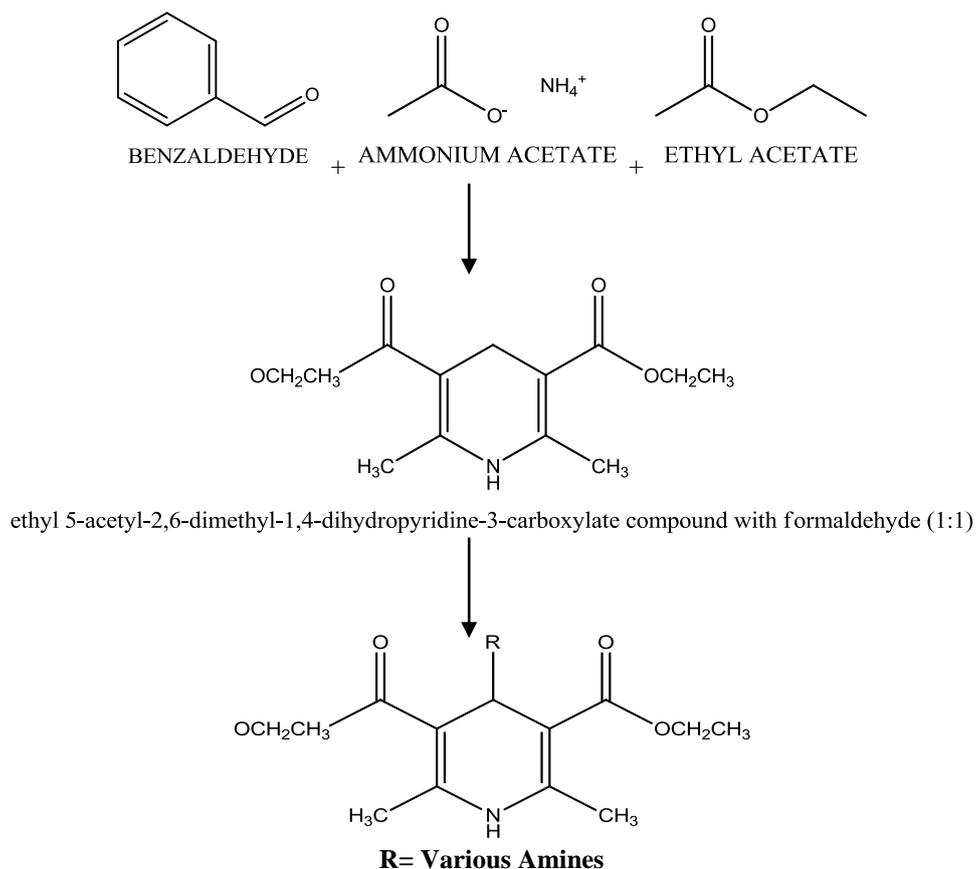
#### STEP-2: SYNTHESIS OF 2-(1H INDOLE-2YL)1-H BENZIMIDAZOLE

- A mixture of polyphosphoric acid (PPA) 25 ml and 2-[1-(2-phenyl hydrazono)ethyl]1H benzoimidazole (compound-1) 0.1 mole in a 100ml round bottomed flask was heated with occasional stirring at 80°C for 4 hours.
- At the end of this period, the mixture was cooled and poured into ice cold water.
- The separated solid was filtered.
- The filtered solid was treated with a few drops of ammonia solution.
- The resulting solid was filtered and dried to obtain 2-(1H indol-2yl)1-H benzoimidazole (compound-2).
- The crude product obtained above was recrystallized from methanol-DMF solution to obtain pure compound-2.

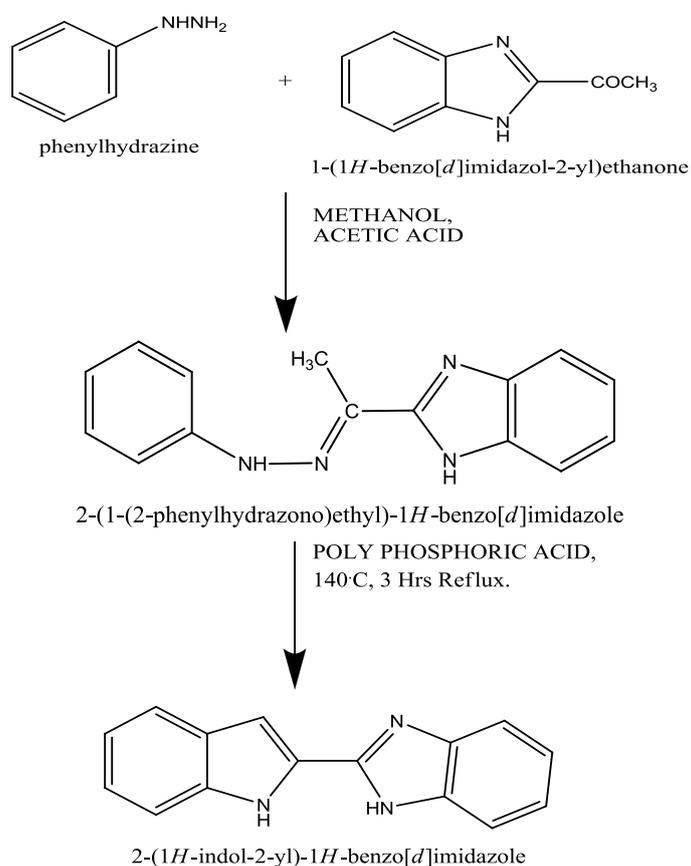
#### STEP-3: SYNTHESIS OF 2-(1H INDOL-2YL)1-ARYL-H BENZIMIDAZOLE

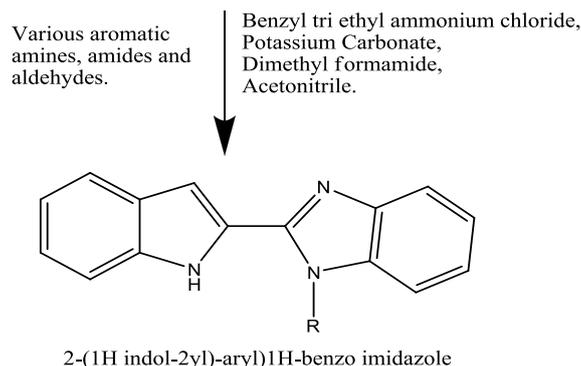
- A mixture of compound-2 (0.1 mole),  $\text{K}_2\text{CO}_3$  (Potassium permanganate) 0.1 mole, benzyl triethyl ammonium chloride (TEBAC) 10mg, acetonitrile ( $\text{CH}_3\text{CN}$ ) 20ml, and alkylating agent (0.1 mole) in a round bottomed flask was heated with occasional stirring for 5 hours.
- At the end of this period the mixture was poured into ice cold water. The separated solid was filtered and dried to obtain 2-(1H-indol-2yl)-1-aryl-1H-benzoimidazole (compound-3), which are recrystallized from hot methanol to obtain compound-3.

## 1.5: SCHEME OF WORK



## 1.6: SCHEME OF WORK 2





## RESULTS

**1.7: Melting Points and Appearance:** Melting points were determined using Veego Digital melting point

apparatus and are uncorrected. The melting point and physical characterization of all synthesized compounds is given in the Table No: 3.

Table No: 7.

S. No	Compound code	Melting Point(°C)	Appearance	% of yield
1.	C	103.37°C	White color	88%
2.	A <sub>1</sub>	105°C	Pale yellow	86%
3.	A <sub>2</sub>	109°C	Dark green	81%
4.	A <sub>3</sub>	102.12°C	White color	85%
5.	A <sub>4</sub>	100°C	Yellow	89%
6.	A <sub>5</sub>	101.23°C	Pure white	83%
7.	A <sub>6</sub>	106°C	Cream color	84%
8.	A <sub>7</sub>	109.83°C	Brown	87%

## 1.8: Thinlayer Chromatography

The purity of all synthesized compounds was monitored on TLC.

Absorbent used : Precoated Silica gel-G Plate

Mobile Phase : Chloroform ,n-butan0l ,water (7:3:4)

Detecting Technique : Iodine chamber

$$R_f = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent}}$$

Table No. 8.

S. No.	Compound Code	R <sub>f</sub> Value
1.	A <sub>1</sub>	0.08
2.	A <sub>2</sub>	0.41
3.	A <sub>3</sub>	0.78
4.	A <sub>4</sub>	0.35
5.	A <sub>5</sub>	0.10
6.	A <sub>6</sub>	0.76
7.	A <sub>7</sub>	0.5

## 1.9: Molecular Formula and Molecular Weight

The molecular formula and molecular weight of all synthesized compounds are given in Table No: 6.

Table No: 9.

S. No	Compound code	Molecular Formula	Molecular weight (gm)
1.	C	C <sub>5</sub> H <sub>7</sub> N <sub>1</sub>	81.118
2.	A <sub>1</sub>	C <sub>19</sub> H <sub>27</sub> N <sub>3</sub> O <sub>6</sub>	393.43
3.	A <sub>2</sub>	C <sub>25</sub> H <sub>33</sub> N <sub>3</sub> O <sub>4</sub>	439.55
4.	A <sub>3</sub>	C <sub>25</sub> H <sub>32</sub> N <sub>2</sub> O <sub>4</sub>	424.53
5.	A <sub>4</sub>	C <sub>21</sub> H <sub>30</sub> N <sub>2</sub> O <sub>5</sub>	390.47
6.	A <sub>5</sub>	C <sub>23</sub> H <sub>37</sub> N <sub>3</sub> O <sub>12</sub>	547.55
7.	A <sub>6</sub>	C <sub>21</sub> H <sub>29</sub> BrN <sub>2</sub> O <sub>5</sub>	469.37
8.	A <sub>7</sub>	C <sub>23</sub> H <sub>30</sub> N <sub>2</sub> O <sub>8</sub> S	494.56

## INVITRO ANTIOXIDANT ACTIVITY

An antioxidant is a molecule capable of slowing or preventing the oxidation or preventing the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing agent. Oxidation reactions can produce free radicals, which start chain reactions that damage cells. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reaction by being oxidized themselves. Hydrogen peroxide scavenging activity is one of the methods for determining antioxidant *in-vitro* activity.

**Nitric oxide radical-Scavenging Activity****Nitric oxide Scavenging Effect (% inhibition) of Compounds(A<sub>1</sub>-A<sub>7</sub>)**

Table No. 7.

Compound code	50µg/ml	100µg/ml	150µg/ml	200µg/ml	250µg/ml
A <sub>1</sub>	9.34	7.46	18.17	28.47	21.72
A <sub>2</sub>	38.83	58.13	43.86	45.50	47.10
A <sub>3</sub>	44.10	58.72	48.25	48.58	43.25
A <sub>4</sub>	39.18	32.09	44.08	62.23	40.87
A <sub>5</sub>	54.83	41.26	57.13	62.71	71.41
A <sub>6</sub>	63.20	51.60	49.08	53.29	67.95
A <sub>7</sub>	42.76	60.94	55.18	42.95	51.57
STD	53.96	43.17	47.11	48.58	44.69

**Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) Scavenging Activity****Reagents**

- Hydrogen peroxide
- Methanol
- Phosphate buffer saline (PH-7.4)

**Standard**

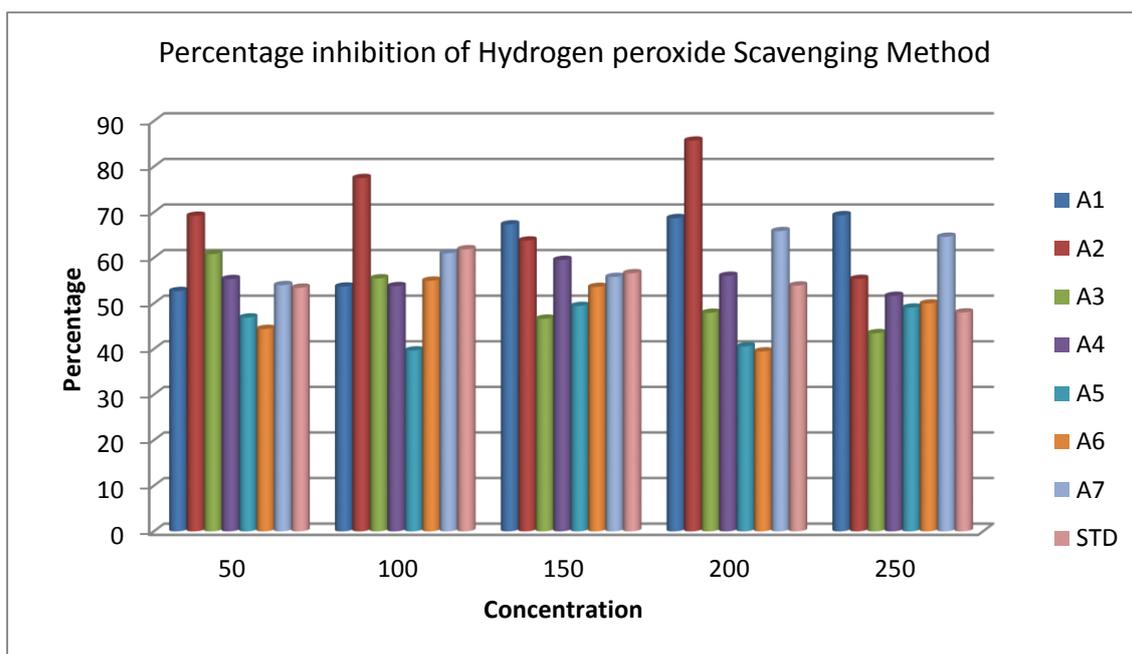
All the compound and the standard are dissolved in methanol and the various concentration of sample ranging from (50-250µg/ml) was prepared using methanol in different 10ml volumetric flasks. To each solution 2ml hydrogen peroxide (2ml) was be added and

the volume made 10ml with phosphate buffer saline (PH-7.4). A control solution was prepared with methanolic solution in phosphate buffer saline without hydrogen peroxide solution. The absorbance at 230nm was recorded using a UV-visible spectrophotometer against blank samples. The percentage inhibition of Hydrogen peroxide scavenging activity will be calculated using the following formula

$$\% \text{ inhibition} = \frac{\text{Abs.control} - \text{Abs.of test}}{\text{Abs.control}} \times 100$$

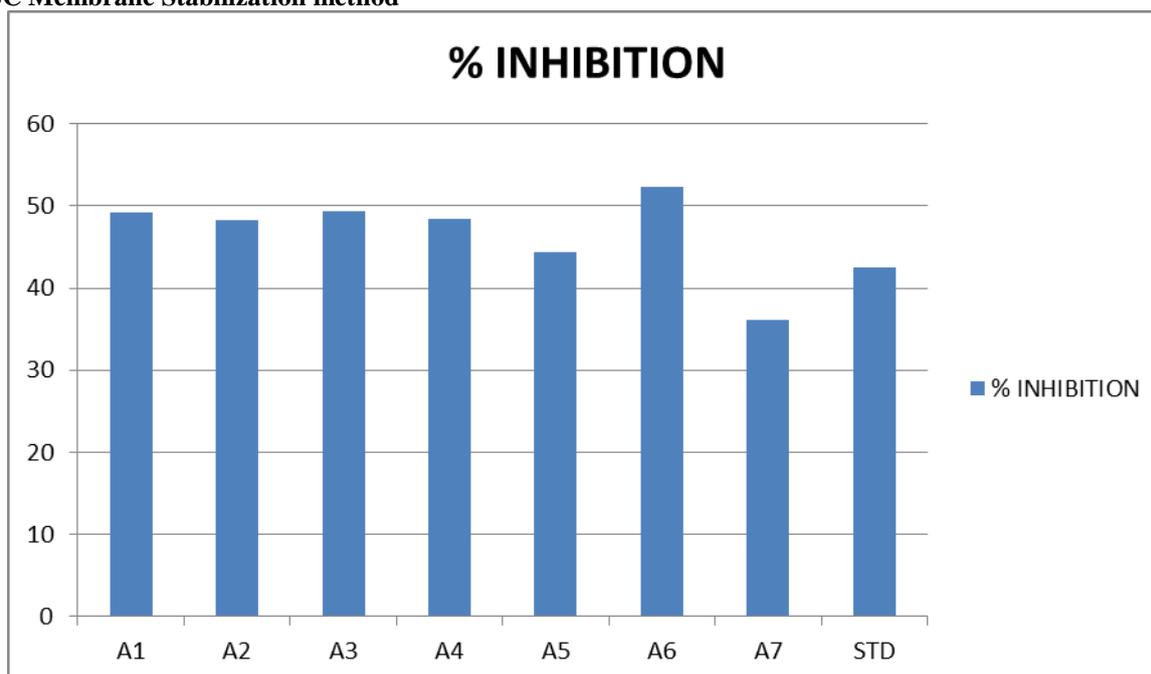
**Hydrogen peroxide Scavenging Effect (% inhibition) of Compounds**(A<sub>1</sub>-A<sub>7</sub>)

Compound code	50µg/ml	100µg/ml	150µg/ml	200µg/ml	250µg/ml
A <sub>1</sub>	52.71	53.68	67.32	68.71	69.34
A <sub>2</sub>	69.22	77.49	63.78	85.70	55.36
A <sub>3</sub>	60.83	55.48	46.64	47.93	43.46
A <sub>4</sub>	55.33	53.77	59.53	56.04	51.65
A <sub>5</sub>	46.90	39.68	49.43	40.58	49.07
A <sub>6</sub>	44.39	54.98	53.60	39.46	49.97
A <sub>7</sub>	54.00	61.00	55.81	65.85	64.61
STD	53.43	61.87	56.61	53.90	48.00



**Anti-Inflammatory Activity of compounds A<sub>1</sub>-A<sub>7</sub> in µg/ml****HRBC Membrane Stabilization Method****Table No. 9.**

S. No	Compound code	Percentage Stabilization
1	A <sub>1</sub>	49.19
2	A <sub>2</sub>	48.26
3	A <sub>3</sub>	49.38
4	A <sub>4</sub>	48.36
5	A <sub>5</sub>	44.30
6	A <sub>6</sub>	52.34
7	A <sub>7</sub>	36.09
8	STD	42.49

**HRBC Membrane Stabilization method****CONCLUSION****Structural confirmation**

The Infra red spectroscopy was performed with KBr on Perkin Elmer Spectrum Version 10.03.07 instrument. Presence of stretching in the range  $1500\text{ cm}^{-1}$  to  $1800\text{ cm}^{-1}$  indicating the presence of C=N functional group. C=O stretching between at  $1300\text{ cm}^{-1}$  to  $1700\text{ cm}^{-1}$ .

**In-vitro Anti-oxidant activity**

All the compounds were subjected to *in-vitro* anti-oxidant activity using ascorbic acid as a standard by two methods i.e. by Hydrogen peroxide scavenging method and nitric oxide radical scavenging method. Antioxidant activity revealed that all the synthesized compounds have shown significant anti-oxidant activity when compared with that of standard drug. The compound A4 showed more activity as compared to the other derivatives.

**In-vitro Anti-inflammatory activity**

The synthesized compounds were subjected to *in-vitro* anti-inflammatory activity using HRBC membrane stabilizing method. The method involves the stabilization

of human red blood cell membrane by hypotonicity induced membrane lysis. The prevention of hypotonicity induced HRBC membrane lysis is taken as a measure of anti-inflammatory activity of the drug. The compound A3 showed better activity as compared to the standard diclofenac. Rest of the compounds showed moderate activity.

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