

**SYNTHESIS AND PHARMACOLOGICAL INVESTIGATION OF SOME  
BENZYLIDENE-(2-{5, 6-SUBSTITUTED-1-[2-(1H-TETRAZOL-5-yl)-BIPHENYL-4-  
YLMETHYL]-1H-BENZOIMIDAZOL-2-YL}-PHENYL-AMINE IN THE PRESENCES OF  
BF<sub>3</sub>·OET<sub>2</sub> CATALYSTS AS ANTIHYPERTENSIVE AGENTS**

M. C. SHARMA<sup>a\*</sup>, SMITA SHARMA<sup>b</sup>, D. V. KOHLI<sup>a</sup>, A. D. SHARMA<sup>c</sup>

<sup>a</sup>*Department of Pharmaceutical Sciences Dr.Hari Singh Gour University  
Sagar (M.P) 470003 India*

<sup>b</sup>*Department of Chemistry Yadhunath Mahavidyalya Bhind (M.P) 477001 India*

<sup>c</sup>*Oriental College of Pharmacy Ujjain-Saver Road Indore (M.P)-452001 India*

A series of Benzylidene-(2-{5, 6-substituted-1-[2-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-1H-benzoimidazol-2-yl}-phenyl)-amine have been synthesized by 4'-{2-[2-(Benzylidene-amino)-phenyl] 5, 6-substituted- benzimidazol-1-yl}-biphenyl-2-carbonitrile. Preliminary screening of the title compounds for antihypertensive activity was performed. Only one of the compound was found to possess antihypertensive activity.

(Received June 6, 2010; accepted June 30, 2010)

*Keywords:* angiotensin II, antihypertensive agents, biphenyl-2-tetrazol,

## 1. Introduction

The Renin Angiotensin Aldosterone System (RAAS) is a proteolytic cascade that plays an important role in electrolyte homeostasis and in the regulation of blood pressure [1], but it is also involved in the pathogenesis of hypertension and renal disease. The Angiotensin Converting Enzyme (ACE) to produce the octapeptide angiotensin II (All) which the main effectors hormone of the RAAS. The RAAS begins with the release of the aspartic protease renin from the juxtaglomerular cell of the kidney. This enzyme is responsible for the conversion of angiotensinogen to the inactive decapeptide angiotensin I. In turn, angiotensin I is cleaved by the angiotensin-converting enzyme to produce the octapeptide angiotensin II (All), which is the main effector hormone of the RAAS. All is the major regulator of the blood pressure, electrolyte balance and endocrine function related to cardiovascular disease such as hypertension. More ever, it has been shown that All plays a role in the various pathological situations involving tissue remodeling, such as cardiac hypertrophy. Recent finding indicate the involvement of this peptide also in cancer [2, 3]. All affect most of the biological function by activating selective membrane-bound receptor. Two distinct subtypes of All receptor [type 1(AT<sub>1</sub>) and type 2(AT<sub>2</sub>)] have been identified and both belongs to the G protein-coupled receptor super family. (GPCRs). AT<sub>1</sub> and AT<sub>2</sub> are seven-transmembrane-spanning receptors, comprising an extracellular glycosylated region connected to the seven transmembrane Alfa-helices, which are linked by three intracellular and three extracellular loops. The carboxy-terminal domain of the protein is cytoplasmic and is a regulatory site. AT<sub>1</sub> is a 359-amino acid protein, while AT<sub>2</sub> is made up of 363 aminoacids and is 30% homologous with ATI; both receptors are N-linked glycosylated post-translationally. AT<sub>1</sub> receptor are expressed in various parts of the body and mediate all of the known effects associated with All, such as vasoconstriction, aldosterone release and other function that tend to elevate blood pressure and cause hypertrophy and hyperplasia of target cells. The role AT<sub>2</sub> receptor is less folly understood. Recently studied [4] 3D-QSAR model was calculated based on the alignment obtained by docking several ligands into the AT<sub>1</sub> receptor. Given the important role played by the RAAS in hypertension, this system is the main target of any effective therapy, the first choice class of drugs to influence the RAAS targeting is that of ACE inhibitors. These drugs block the formation of All

and also prevent the conversion of bradykinin to inactive peptides. Although bradykinin may contribute to the beneficial effects of Ace inhibitor through its vesorelaxing effects, its accumulation determines some disadvantages such as the development of coughing and angioedema, which are side effect often associated with ACE-inhibitor therapy. Moreover, ACE inhibitor do not completely suppress All, because its formation ensured also by the ACE-independent pathways. For these reasons, it was particularly important when A II subtype 1 receptor antagonists were developed as a new class of antihypertensive agents clinically because of lesser side effects and better therapeutics profile than ACE inhibitors. A-II receptor antagonists have been investigated as an alternative approach in blocking the hypertensive response to endogenous A-II. The prototype of most advanced compound is Losartan (DuP 753), [5] a potent, specific, orally active non-peptide A-II receptor antagonists. In recent years, attention has increasingly been given to the synthesis of benzimidazole derivatives as a source of new antihypertensive agents.

## 2. Materials and method

The implementation in the laboratory of these synthetic technologies to increase the number of molecules generated by chemists is now a prerequisite to competitive advantage in the field. However, most of the existing methods to design benzimidazole skeleton requires the insertion of a carbon into a precursor with ortho heteroatom on a benzene ring. Moreover, most of the methods have not been found to be quite accessible from the viewpoints of both yield and economics of the reaction. Thus, in order to cater the needs associated with synthetic aspects, herein, we would like to present unique approach to synthesize benzimidazole derivatives. Benzimidazole structures are classified under several classes of drugs [6], based on the possible substitution at different positions of the benzimidazole nucleus. Methods of benzimidazole synthesis include the condensation of *o*-aryldiamines and aldehyde in refluxing nitrobenzene [7,8], the condensation of *o*-aryldiamines with carboxylic acids or their derivatives in the presence of strong acids such as polyphosphoric acid [9] or mineral acids [10]. Melting points were determined in open capillary tubes and are uncorrected. The time required for completion of the reaction was monitored by TLC using Silica gel-G plates and spots were exposed in iodine chamber. IR spectra were recorded on a Perkin Elmer 1800 (FTIR) spectrometer 1H NMR spectra (DMSO) were taken on a DRX-300 spectrometer (300 MHz) using TMS as internal standard and chemical shifts are expressed in  $\delta$  ppm. Moreover, most of the methods have not been found to be quite accessible from the viewpoints of both yield and economics of the reaction. Thus, in order to cater the needs associated with synthetic aspects, herein, we would like to present unique approach to synthesize benzimidazole derivatives.  $\text{BF}_3 \cdot \text{OEt}_2$  is a Lewis acid catalyst used in a wide variety of applications, such as, in mild dehydration of tertiary alcohols to alkenes, in Diels-Alder reaction, in cleavage of ethers, in THP protection of alcohols, in rearrangement of epoxides to carbonyl compounds, in reaction of ally tin reagents with aldehyde and ketones *etc.* However, there are examples of the use of  $\text{BF}_3 \cdot \text{OEt}_2$  as a catalyst for the preparation of benzimidazoles [10]. Herein, protocol for the rapid synthesis of a variety of biologically significant benzimidazoles using a catalytic amount of  $\text{BF}_3 \cdot \text{OEt}_2$  under extremely mild solvent-free conditions.

### [MCS 01] -2-(2- substituted amino phenyl) benzimidazole

Difference substituted [ $\text{R}_1$  and  $\text{R}_2$ ] *o*-Phenylenediamine was condensed with anthranilic acid in the presence of  $\text{BF}_3 \cdot \text{OEt}_2$  (0.5 mmol) to this reaction mixture,  $\text{CH}_2\text{Cl}_2$  (50 mL) was added and washed with water. The organic phase was separated, dried ( $\text{Na}_2\text{SO}_4$ ) and concentrated *in vacuo* to get the crude compound. The crude compounds were purified by silica gel column chromatography using ethyl acetate: ethanol (80:20) as eluent. The reaction mixture was poured into crushed ice. Filtered, washed, dried and recrystallized. The product 2-(2- substituted amino phenyl) benzimidazole was obtained.

### [MCS 02] – [2-(1H- substituted-Benzimidazol-2-yl)-phenyl]-benzylidene-amine

2-(2- substituted amino phenyl) benzimidazole treated with various aromatic aldehydes to obtain the Schiff bases compounds MCS-02.

### MCS-03- 4'-{2-[2-(Benzylidene-amino)-phenyl] 5, 6-substituted- benzimidazol-1-yl}-biphenyl-2-carbonitrile

To a solution of 65 mg (3.5 mmol) compound different substitute MCS-02 90 mL of DMF was added potassium carbonate 1.5 g (2.55 mmol), the mixture was stirred for 3 hours at room temperature, and 4-(bromomethyl) biphenyl-2'-nitrile 15 mg (3.6 mmol) was added. After stirring for 8 hours the mixture was poured into distilled water (50 mL) and extracted with diethyl ether (3 × 25 mL). The combined extracts were dried (MgSO<sub>4</sub>) and evaporated.

**MCS-04- Benzylidene-(2-{5, 6-substituted-1-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-1H-benzimidazol-2-yl}-phenyl)-amine**

A mixture of different substituted 4'-{2-[2-(Benzylidene-amino)-phenyl] 5, 6-substituted-benzimidazol-1-yl}-biphenyl-2-carbonitrile (115mg), sodium azide (450 mg.), and Et<sub>3</sub>N·HCl (50 mg.) in NH<sub>4</sub>Cl (30 mL) is stirred at 40°C for 8 hours. After cooling, the mixture is diluted with distilled water (50 mL), acidified to pH 4.5 with 4N HCl, and extracted with EtOAc (3 × 50 mL). The organic layer was washed with H<sub>2</sub>O (3 × 50 mL), then the combined extracts were dried (MgSO<sub>4</sub>) and evaporated and the solid residue was purified by silica gel column chromatography eluting with ethyl acetate/chloroform (80:20/v: v) to give solid Compounds.

**Spectral Data**

**[1] (2-Chloro-benzylidene)-(2-{5-chloro-1-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-1H-benzimidazol-2-yl}-phenyl)-amine**

Yield: 70 %, m.p. = 233-237c. C<sub>34</sub>H<sub>23</sub>Cl<sub>2</sub>N<sub>7</sub>:C,68.00;H, 3.86;N,16.33%; IR (KBr): 3598,3487,3311,3091,2951,1683,1537-1588,1204,898 cm<sup>-1</sup>.<sup>1</sup>HNMR(300MHz,CDCl<sub>3</sub>) 13.12(1H,s,-NH-Benzimidazole),10.25(s,1H,tetrazole-NH),4.93(s,2H,CH<sub>2</sub>),6.67-8.64(m,19H,Ar-H).<sup>13</sup>CNMR (CDCl<sub>3</sub>)δ: 51.1, 112.5, 124.32, 126.34, 129.59, 135.43, 142.11,FAB-MS.599.131

**[2] (2-Chloro-benzylidene)-(2-{5-Fluoro-1-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-1H-benzimidazol-2-yl}-phenyl)-amine**

Yield: 73 %, m.p. = 269-272 c. C<sub>34</sub>H<sub>23</sub>ClFN<sub>7</sub>:C,69.93;H, 3.98;N,16.82%; IR (KBr): 3591,3484,3355,3064,2976,1689,1532-1577,1221,892 cm<sup>-1</sup>.<sup>1</sup>HNMR(300MHz,CDCl<sub>3</sub>) 13.17(1H,s,-NH-Benzimidazole),10.11(s,1H,tetrazole-NH),4.97(s,2H,CH<sub>2</sub>),6.84-8.69(m,19H,Ar-H).<sup>13</sup>CNMR (CDCl<sub>3</sub>)δ: 52.4, 115.5, 121.32, 124.34, 127.59, 135.43, 142.11,FAB-MS.583.187

**[3] (2-{5-Bromo-1-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-1H-benzimidazol-2-yl}-phenyl)-(2-nitro-benzylidene)-amine**

Yield: 66 %, m.p. = 286-289 c. C<sub>34</sub>H<sub>23</sub>BrN<sub>8</sub>O<sub>2</sub>:C,62.28;H, 3.58;N,17.14%; IR (KBr): 3574,3461,3315,3077,2947,1634,1521-1587,1215,898 cm<sup>-1</sup>.<sup>1</sup>HNMR(300MHz,CDCl<sub>3</sub>) 13.18(1H,s,-NH-Benzimidazole),10.09(s,1H,tetrazole-NH),4.91(s,2H,CH<sub>2</sub>),6.82-8.57(m,19H,Ar-H).<sup>13</sup>CNMR (CDCl<sub>3</sub>)δ: 50.1, 113.5, 120.32, 121.34, 125, 136.6, 140,FAB-MS.655.22

**[4] (2-{5-Chloro-6-iodo-1-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-1H-benzimidazol-2-yl}-phenyl)-(2-nitro-benzylidene)-amine**

Yield: 63 %, m.p. = 211-214 c. C<sub>34</sub>H<sub>22</sub>ClIN<sub>8</sub>O<sub>2</sub>:C,55.42;H, 3.07;N,15.24%; IR (KBr): 3611,3524,3419,3387,3017,2996,1522-1565,1245,889 cm<sup>-1</sup>.<sup>1</sup>HNMR(300MHz,CDCl<sub>3</sub>) 13.12(1H,s,-NH-Benzimidazole),10.04(s,1H,tetrazole-NH),4.99(s,2H,CH<sub>2</sub>),6.85-8.65(m,18H,Ar-H).<sup>13</sup>CNMR (CDCl<sub>3</sub>)δ: 54.7, 113.0, 120.32, 122.34, 122.36, 124.59, 137.43, 148.48, 149.28, FAB-MS.737.064

**[5] (2-Chloro-benzylidene)-(2-{5-iodo-6-methyl-1-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-1H-benzimidazol-2-yl}-phenyl)-amine**

Yield: 77 %, m.p. = 253-256 c. C<sub>35</sub>H<sub>25</sub>Cl<sub>2</sub>IN<sub>7</sub>:C,59.51;H, 3.56;N,13.91%; IR (KBr): 3617,3586,3449,3291,3054,2991, 1532-1565,1261,895 cm<sup>-1</sup>.<sup>1</sup>HNMR(300MHz,CDCl<sub>3</sub>) 13.08(1H,s,-NH-Benzimidazole),10.21(s,1H,tetrazole-NH),4.96(s,2H,CH<sub>2</sub>),6.88-8.73(m,18H,Ar-H), 2.36(t,3H,CH<sub>3</sub>).<sup>13</sup>CNMR (CDCl<sub>3</sub>)δ: 21.1, 55.2,111.5, 113.2,115.4,116.2,121.1,128.4,135.5,138.2,148.0,FAB-MS.706.094

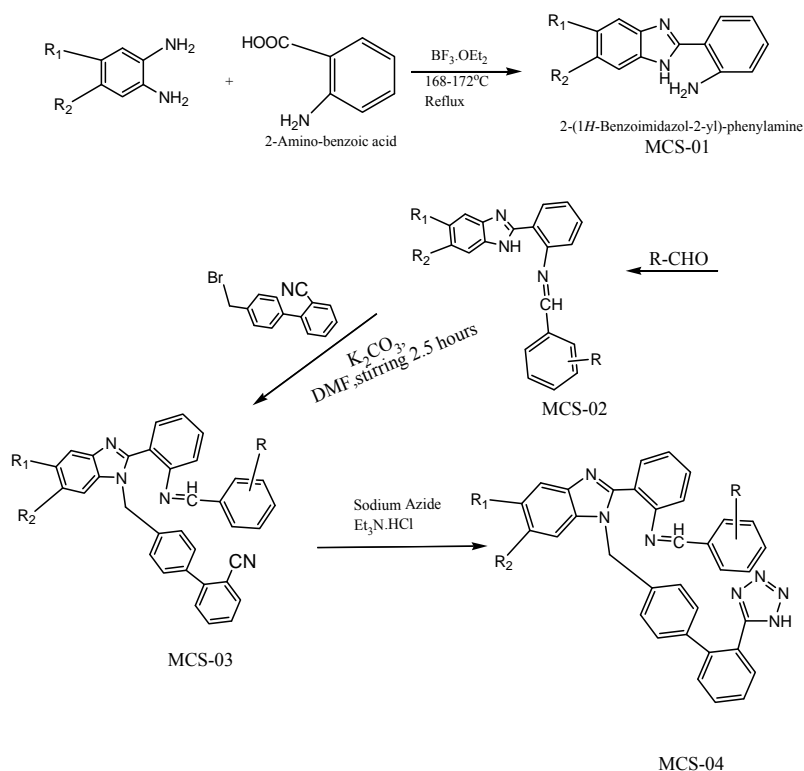
**Biological Activity:**<sup>11-17</sup>

**Non-invasive Method (Indirect Method)** Albino rats weighing 150-250 gm were used to screening for all the synthesizes benzimidazoles derivatives for antihypertensive activity. Suspension of test compound was prepared in 1% w/v sodium carboxy methyl cellulose and administered at dose level of 50 mg/kg animal body weight to different of five rats each group. Contorl group received an equal quantity of 1% w/v sodium carboxy methyl cellulose suspension. After administration of dose to animal, blood pressure was measured by Non-invasive

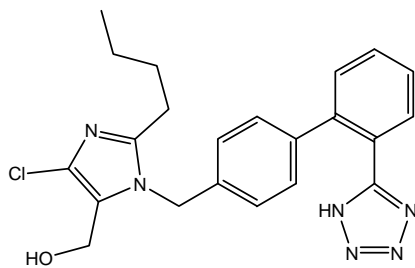
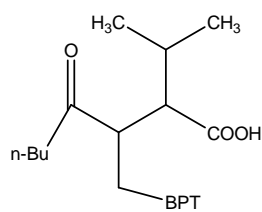
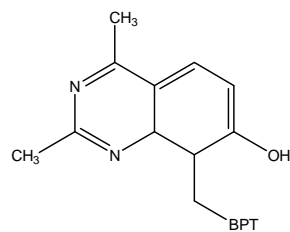
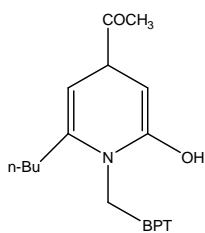
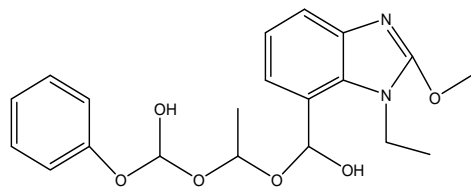
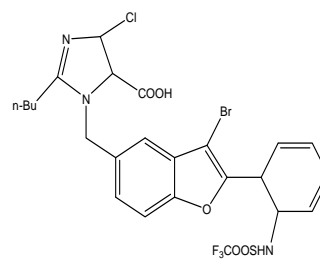
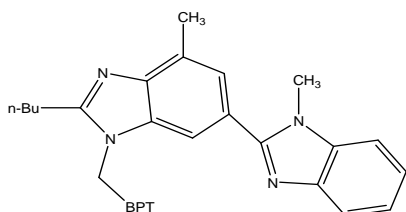
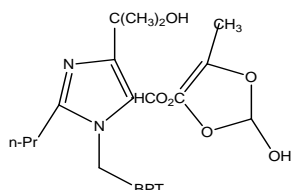
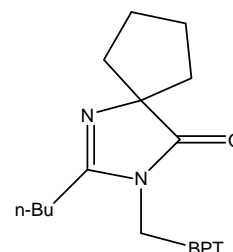
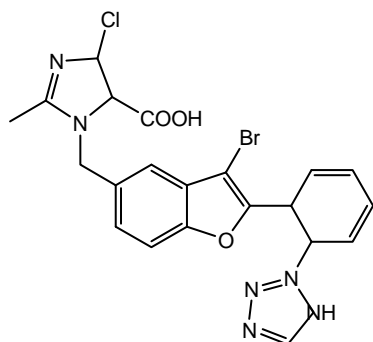
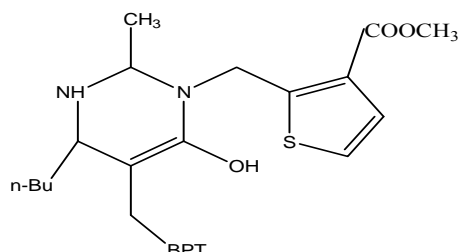
Tail cuff Method using pressure meter. Measurements were done after 1 hour and 3 hour time interval intensive stepwise. One hour after administration of drug sample, animal was shifted to the restrainers, which restricts the movement of animal. The tail was cleaned with moist cotton to remove the dirty matter and talcum powder was sprayed on tail to make its surface smooth. A tail cuff and pulse transducer was fixed around the tail. Initially animal shows particular pulse level, when the pulse rate is within the normal range. 'STRAT' switch is put on and the recorder records the blood pressure as SBP (systolic blood pressure), DBP (Diastolic blood pressure) and MABP (mean arterial blood pressure), which are displayed on monitor. The pressure can be easily read from the pre-calibrated monitor. Once all the values are displayed the recorder is switched off and for next measurement. Some procedures are allowed once when sufficient pulse level is attained. [Table1, 2]

**Invasive Method (Direct Method):** Male albino wistar (150-250 gm) rats were used and housed at  $24 \pm 1^{\circ}\text{C}$  room temperature. The rats were anaesthetized with sodium chloride 0.9% solution, Drug solution  $10\text{-}\mu\text{g}/100\text{ml}$ , and Heparin 500 I.U. solution urethane hydrochloride 50% w/v solution  $80\text{ mg}/\text{kg}$  i.p. To set up the instrument firstly the level of mercury in the left arm of manometer was adjusted to 90-100 mm of Hg (normal blood pressure of rat). this was done in steps of 10mm at a time and the physiogram so obtained was used as calibration graph for calculations. The Jugular vein and carotid artery were surgically cannulated for drug administration for recording the blood pressure respectively. The trachea was cannulated in order to provide artificial respiration to rat during the experiment. By means of three way stop cock and a stainless steel needle at the end of P.E. tubing was attached to arterial cannula for B.P., Transducers and the Venus cannula to a syringe. Then both the cannula were filled by heparinized saline before the administration. Arterial cannula was connected via transducer to physiograph recorder. Several baseline readings of systolic and diastolic pressures were recorded. The physiograph shows the reduction of the blood pressure with compare to losartan. Synthesized compounds were screened in presence of Angiotensin II induced hypertension ( $0.5\text{ }\mu\text{g}/\text{kg}$  i.v.) Table 3, 4.

## SCHEME



- 1-  $\text{R} = \text{Cl}$   $\text{R}_1 = \text{Cl}$ ,  $\text{R}_2 = \text{H}$
- 2-  $\text{R} = \text{Cl}$   $\text{R}_1 = \text{F}$ ,  $\text{R}_2 = \text{H}$
- 3-  $\text{R} = \text{NO}_2$   $\text{R}_1 = \text{Br}$ ,  $\text{R}_2 = \text{H}$
- 4-  $\text{R} = \text{NO}_2$   $\text{R}_1 = \text{Cl}$ ,  $\text{R}_2 = \text{I}$
- 5-  $\text{R} = \text{Cl}$   $\text{R}_1 = \text{I}$ ,  $\text{R}_2 = \text{CH}_3$

**Losartan****Valsartan****Tasosartan****Candesartan****Milfasartan****Sapisartan****Telmisartan****Olmesartan****Irbesartan****Zolzasartan****Eprosartan**

Angiotensin II selective antagonists

Table 1. Hypertension induced in normotensive rat.

Comp.	Exp. Animal Albino (Wistar) Rat	After 1hour			After 3 hour		
		SBP	DBP	MAB P	SBP	DBP	MABP
[1]	1	149	101	125	143	101	121
	2	144	109	131	140	100	120
	3	142	115	127	135	98	118
	4	140	106	123	142	101	121
	5	142	108	125	141	102	120
[2]	1	143	110	127	134	102	118
	2	138	107	128	143	101	121
	3	140	108	125	141	104	120
	4	144	111	126	143	112	116
	5	144	106	125	144	109	128
[3]	1	142	109	126	143	111	126
	2	140	102	123	140	100	120
	3	144	111	126	143	112	116
	4	144	106	125	144	109	128
	5	145	112	126	139	100	124
[4]	1	133	114	124	139	101	120
	2	142	105	124	135	107	121
	3	141	102	121	139	103	121
	4	136	105	123	142	104	119
	5	135	102	122	140	97	119
[5]	1	140	108	125	141	104	120
	2	144	111	126	143	112	116
	3	144	106	125	144	109	128
	4	145	112	126	139	100	124
	5	142	109	126	143	111	126
Control	<b>Losartan</b>	123	-	-	-	-	-

Table 2. Reduction in blood pressure (mm Hg) at a dose of 50 mg/kg animal body weight.

Comp.	Exp. Animal Albino (Wistar) Rat	After 1hour			After 3 hour		
		SBP	DBP	MABP	SBP	DBP	MABP
[1]	1	126	102	111	124	101	112
	2	121	100	110	125	102	111
	3	126	103	115	122	103	112
	4	123	102	113	128	103	112
	5	126	102	111	124	101	112
[2]	1	122	100	111	126	104	115
	2	125	100	112	121	107	114
	3	128	102	115	130	103	116
	4	129	101	115	119	104	111
	5	123	107	115	121	99	110
[3]	1	122	112	117	122	103	112

Comp.	Exp. Animal Albino (Wistar) Rat	After 1 hour			After 3 hour		
		SBP	DBP	MABP	SBP	DBP	MABP
	2	125	105	115	122	100	112
	3	124	100	112	128	101	113
	4	130	104	117	128	102	115
	5	125	105	115	124	101	112
[4]	1	128	102	115	130	103	116
	2	123	102	113	128	103	112
	3	121	101	113	123	102	111
	4	126	102	111	124	101	112
	5	121	100	110	125	102	111
[5]	1	122	102	119	124	102	113
	2	126	104	118	125	102	114
	3	125	101	113	128	102	115
	4	123	103	116	126	100	113
	5	126	102	113	123	103	113
Control	<b>Losartan</b>	103	-	-	-	-	-

Table: 3 Blood pressure values for synthesized compounds over duration of 90 minutes

Comp. No.	Mean Arterial Pressure After									
	0 min.	10 min.	20 min.	30 min.	40 min.	50 min.	60 min.	70 min.	80 min.	90 min.
Losartan	166	164	158	151	147	140	136	131	126	122
1	174	168	160	155	149	145	141	137	131	128
2	184	175	167	161	156	152	147	142	137	133
3	173	169	164	159	153	148	142	138	134	129
4	174	168	163	157	152	148	144	139	135	131
5	178	171	167	160	155	149	145	141	138	135

Table: 4 Antihypertensive activity of synthesized compounds

Compound. No	Minimum Blood pressure value(mm Hg)	Duration of hypertension effect(min.)
Losratan	122	90
1	123	110
2	121	115
3	123	105
4	120	120
5	122	120

### 3. Result and discussion

The compounds [1-5] were obtained in fair to good yield, the structure assigned was confirmed by spectral data (IR, NMR and Mass) and the purity was ascertained by microanalysis. Among the compounds tested for antihypertensive activity, the compounds 3 were found to reduce blood pressure significantly that is compared with standard.

### Acknowledgement

The authors are thankful to Prof. Pratibha Sharma School of Chemical Sciences DAVV Indore, to given valuable suggestion to experimental work, authors also thankful to CDRI Lucknow providing NMR, Mass spectral analysis and Head of Department School of Pharmacy D.A.V.V Indore to providing the facilities for IR spectra.

### Reference

- [1] G. Goodman, *The Pharmacological Basis of Therapeutics*, 7<sup>th</sup> Ed., McGraw-Hill, New York ed., pp.639–659, 1985.
- [2] J. L Juillerat, J. Celerier, B. C. Chapuis, G. Nguyen, W. Wostle, H.P. Maerki, R.C Janzer, *Br .J. Cancer*; **90**, 1059 (2004).
- [3] D. J. Fogarty, M. V. Sanchez-Gomez, C. Matute, *Glia*; **39**, 304 (2002)
- [4] T. Tiziano, C Vincenzo, R Simona, M Adriano, *J Med Chem* **49**, 4305 (2006).
- [5] Wong PC, Barnes TB, Chiu AT, *Cardiovasc Drug Rev.* **9**, 317 (1991).
- [6] J. Velik, V. Baliharova, J. Fink-Gremmels, *Res. Vet. Sci.* **76**, 95 (2004).
- [7] B. Yadagiri, J. W. Lown, *Synth. Commun.* **20**, 955 (1990).
- [8] Q. Sun, B. Yan, *Bioorg. Med. Chem. Lett.* **8**, 361 (1998).
- [9] P. N. Preston, In *The Chemistry of Heterocyclic Compounds*, Part 1, Vol. 40, Eds.: Weissberger, A. Taylor, E. C., Wiley: New York, pp. 6-60. 1981
- [10] Rahul R. Nagawade, Devanand B. Shinde. *Chinese Chemical Letters*, **17**(4), 453 2006
- [11] Gupta S.K. *Drug Screening methods*, Jaypee Brothers Medical Publisher, New Delhi, 236-246, 2004.
- [12] Shreenivas M.T, Chetan B.P, Bhat A.R, *J. of Pharma.Sci. and Technology.* **1**(2), 88 (2009).
- [13] Vogel G.H. *Drug Discovery and Evaluation, Pharmacological Assay*, 2002; (Springer. Berlin), 122.
- [14] A. A. Siddiqui, M. S. Wani. *Indian. J. Chemistry.* **43B**, 1574 (2004).
- [15] Badyal D.K, Lata H, Dadhich A.P, *Indian J of Pharmacology*; **35**(66), 349 (2003).
- [16] Bunag R.D, McCubbin J.W, Page I.H, *Cardiovasc. Res.* **5**(1), 24 (1971).
- [17] Jat R.K, Jat J.L, Pathak D.P, *E. Journal. of Chemistry.* **3**(13), 278 (2006).

---

\*Corresponding author: mukesh2206@rediffmail.com