SYNTHESIS, DESIGN AND PHARMACOLOGICAL EVALUATION OF SOME DERIVATIVES 4'-{2-[2-BENZYLIDENE-AMINO)-PHENYL]-5, 6-SUBSTITUTED-BENZOIMIDAZOL-1-YLMETHYL} -BIPHENYL-2-CARBOXYLIC ACID AS POTENT ANTIHYPERTENSIVE AGENTS

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Series of Antihypertensive agents Some Derivatives 4'-{2-[2-Benzylidene-amino)-phenyl]-5, 6-substituted-benzoimidazol-1-ylmethyl} -biphenyl-2-carboxylic acid and Side chain in the using different aromatic aldehydes have been synthesized from substituted compounds [1-5] and tested for antihypertensive activity in induced hypertensive rats. Their structures were assigned with elemental analysis, melting point and spectral analysis like IR, ^{1}H NMR, ^{13}C NMR and FAB Mass. All the compounds have been found to be less active than Losartan.

(Received July 2, 2010; accepted July 20, 2010)

Keywords: Angiotensin II, Biphenyl Carboxylic acid, Blood Pressure, Antihypertensive activity

1. Introduction

The renin-angiotensin-aldosterone system (RAAS) is known to play an important role in electrolyte homeostasis and in the regulation of blood pressure and congestive heart failure[1]. The octapeptide angiotensin II (Ang II) is produced by the rennin angiotensin system (RAS) and is a potent vasoconstrictor and thus plays an important role in the pathophysiology of hypertension [2, 3] .For the last two decades, the focus of antihypertensive therapy has been on the rennin angiotensin system (RAS). RAS is a proteolytic cascade or one of the humoral mechanisms that plays an important role in electrolyte homeostasis and in the regulation of blood pressure, but it also is involved in the pathogenesis of hypertension and renal diseases [4] .Angiotensin II (AngII), an octapeptide produced from angiotensin I by the action of angiotensin converting enzyme (ACE) localized on the endothelium of blood vessels in the lungs, kidneys, and many other organs, is the primary effector component of the RAS [5].Two distinct subtypes of Ang II receptors [type 1(AT_1) and type 2 (AT_2)] have been identified, and belong to the G-protein-coupled receptor family (GPCRs). AT_1 and AT_2 are seven transmembrane spanning receptors, comprising an extra cellular 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. AT1 is a 359-amino acids protein, while AT2 is made up of 363 amino acids and is 30% homologous with AT_1. Both receptors are N-linked glycosylated post-translationally [6,7].The pioneering efforts of the Dupont Group have generated a promising first nonpeptide AT_1 antagonist, which represents losartan, the prototype of the sartans. In recent decades, several selective antagonists have been developed, which are used to treat both hypertension and damage associated with diseases such as arthrosclerosis and diabetes [8-12]. Losartan was the first agent of this category that served as lead for the development of newer AII

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AT1 receptor antagonist [13]. The therapeutic availability is less for the peptidic AII antagonist on account of their poor bioavailability, short plasma half-life, and partial agonist activity, but the nonpeptidic AII receptor antagonists lack the limitations of peptides antagonist. The substituent at 6-position on the nucleus increases the activity whereas small substituent at 5-position decreases the activity[14-16]. Compounds containing tetrazole nucleus are also reported as AT_1 receptor antagonists and their protypical derivative exhibits non-competitive antagonism and amino group attach with carboxylic group given good biological activity [14-16] .In recent years, attention has increasingly been given to the synthesis of benzimidazole derivatives as a source of new antimicrobial agents. The synthesis of novel benzimidazole derivatives remains a main focus of medicinal research. Recent observations suggest that substituted benzimidazoles and heterocycles, which are the structural isosters of nucleotides owing fused heterocyclic nuclei in their structures that allow them to interact easily with the biopolymers, possess potential activity with lower toxicities in the antihypertensive activity approach[14-15].

2. Materials and method

Herein, we would like to present unique approach to synthesize benzimidazole derivatives. $BF_3{\cdot}OEt_2$ 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 $BF_3{\cdot}OEt_2$ as a catalyst for the preparation of benzimidazoles[16]. Herein, protocol for the rapid synthesis of a variety of biologically significant benzimidazoles using a catalytic amount of $BF_3{\cdot}OEt_2$ under extremely mild solvent-free conditions. 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 δ ppm.

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

Difference substituted [R_1 and R_2] o-Phenylenediamine was condensed with anthranilic acid in the presence of $BF_3{\bullet}OEt_2$ (0.5 mmol) to this reaction mixture, CH_2Cl_2 (50 mL) was added and washed with water. The organic phase was separated, dried (Na_2SO_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 recrystalized. 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-benzoimidazol-1-ylmethyl} -biphenyl-2-carboxylic acid

72.5 mg of MCS-02 was dissolved in 50 ml of DMF (dimethyl formamide) and stirred vigorously with 28mg of potassium carbonate at 44 ^{0}C for 3hours. To the resulting mixture 113mg of 4'bromomethylbiphenyl-2-carboxylic acid dissolved in DMF and then was added drop wise with dropping funnel in three hour the reaction was allowed to proceed for further 11 hours at room temperature and solvent removed under vacuum. Residue was treated with 20ml of dilute HCl and extracted with ethyl acetate. The organic layer was washed with brine solution, distilled water and dried over anhydrous sodium sulphate. (MCS-03) was obtained.

Compounds Spectral Data Analysis

(a) 4'-(5-Chloro-2-{2-[(2-chloro-benzylidene-amino)-phenyl]- benzoimidazol-1-ylmethyl} -biphenyl-2-carboxylic acid

Yield: 75%, m.p. = 204-207 C. $C_{34}H_{23}Cl_2N_3O_2$: Found: C,70.84;H, 4.02;N,7.44 %;IR (KBr): 3376(Broad O-H str.), 3069 (C-H, sp^2), 3287 (NH, bonded), 3162(NH, free), 2817 (C-H

str., CH_2),1699 (carboxylic, C=O str.), 1648(CH=CH),1548(C=N and C=Cstr.), 1554-1355 ,1140 (C-N str.), 655.8(C-Cl str).^{1}H NMR (300 MHz, $CDCl_3$) 12.86(1H,s,-NH-Benzimidazole),10.24(s,1H,COOH), 6.95- 8.59 (m,19H,ArH), 5.08 (s,2H,CH_2).^{13}CNMR($CDCl_3$)δ:51.4,111.3,112.1,116.2,127.1,131.4,133.1,139.1,142.3, FAB-MS, 576.42

(b) 4'--2-{2-[(2-Chloro-benzylidene-amino)-phenyl}-5-fluoro-benzoimidazol-1-ylmethyl} -biphenyl-2-carboxylic acid

Yield: 68 %, m.p. = 233-237 C. $C_{34}H_{23}ClFN_3O_2$: Found: C,72.89;H, 4.12;N,7.53 %;IR (KBr): 3371(Broad O-H str.), 3075 (C-H, sp^2), 3283 (NH, bonded), 3168(NH, free), 2832 (C-H str., CH_2),1706 (carboxylic, C=O str.), 1644(CH=CH),1541(C=N and C=Cstr.), 1504-1328 ,1140 (C-N str.), 650 (C-Cl str).^{1}H NMR (300 MHz, $CDCl_3$) 12.84(1H,s,-NH-Benzimidazole),10.21(s,1H,COOH), 6.86- 8.71 (m,19H,ArH), 5.04 (s,2H,CH_2).^{13}CNMR($CDCl_3$)δ:46.4,53.2,55.3,67.1,71.3,112.2,113.5,117.1,118.1,124.1,132.2,134.1 ,140.8, FAB-MS, 559.74

(c) 4'-(5-Bromo-2-{2-[(2-nitro-benzylidene-amino)-phenyl] - benzoimidazol-1-ylmethyl} -biphenyl-2-carboxylic acid

Yield: 60%, m.p. = 212-214 C. $C_{34}H_{23}BrN_4O_4$: Found: C,64.67;H, 3.69;N,8.89 %;IR (KBr): 3386 (Broad O-H str.), 3075 (C-H, sp^2), 3283 (NH, bonded), 3168 (NH, free), 2812 (C-H str., CH_2),1711 (carboxylic, C=O str.), 1640(CH=CH),1541(C=N and C=Cstr.), 1551-1333 ,1148 (C-N str.), ^{1}H NMR (300 MHz, $CDCl_3$) 12.88(1H,s,-NH-Benzimidazole),10.20(s,1H,COOH), 6.88- 8.65 (m,19H,ArH), 5.08 (s,2H,CH_2).^{13}CNMR($CDCl_3$)δ:42.2,112.3,113.5,117.2,119.2,125.5,127.1,136.1,139.3,FAB-MS, 630.54

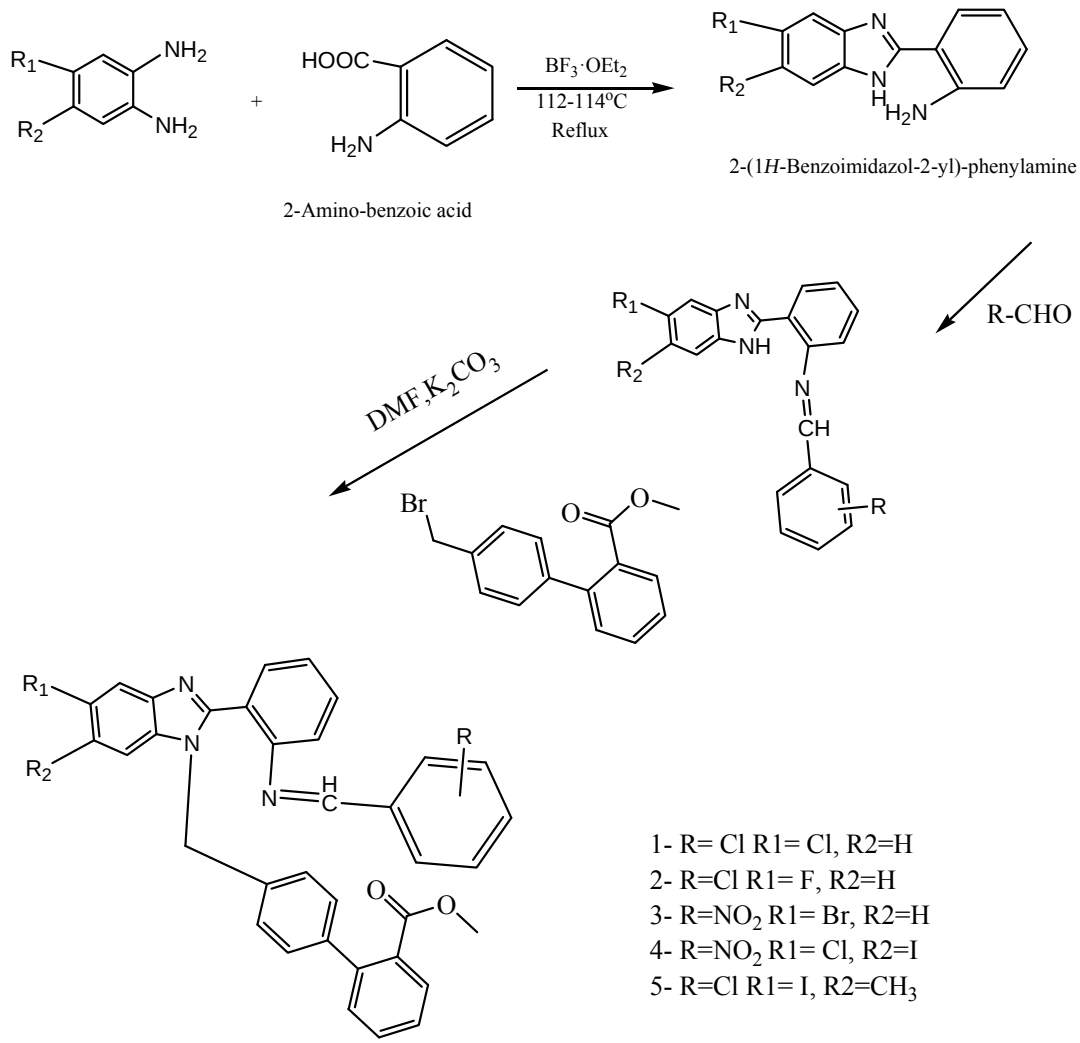
(d) 4'-(5-Chloro-6-iodo-2-{2-[(2-nitro-benzylidene-amino)-phenyl] - benzoimidazol-1-ylmethyl} -biphenyl-2-carboxylic acid

Yield: 64 %, m.p. = 264-267 C. $C_{34}H_{22}ClIN_4O_4$: Found: C,57.24;H, 3.14;N,7.88 %;IR (KBr): 3386(Broad O-H str.), 3043 (C-H, sp^2), 3244 (NH, bonded), 3193(NH, free), 2865 (C-H str., CH_2),1703 (carboxylic, C=O str.), 1632(CH=CH),1548(C=N and C=Cstr.), 1511-1326 ,1144 (C-N str.), 651.3 (C-Cl str).^{1}H NMR (300 MHz, $CDCl_3$) 12.85(1H,s,-NH-Benzimidazole),10.20(s,1H,COOH), 6.84- 8.66 (m,18H,ArH), 5.05 (s,2H,CH_2).^{13}CNMR($CDCl_3$)δ:46.4,53.2,55.3,67.1,71.3,112.2,113.5,117.1,118.1,124.1,132.2,134.1 ,140.8, FAB-MS, 711.63

(e) 4'--2-{2-[(2-Chloro-benzylidene-amino)-phenyl}-5-iodo-6-methyl-benzoimidazol-1-ylmethyl} -biphenyl-2-carboxylic acid

Yield: 60 %, m.p. = 242-244 C. $C_{35}H_{25}ClIN_3O_2$: Found: C,61.66;H, 3.74;N,6.18 %;IR (KBr): 3383(Broad O-H str.), 3046 (C-H, sp^2), 3243 (NH, bonded), 3196(NH, free), 2869 (C-H str., CH_2),1700 (carboxylic, C=O str.), 1637(CH=CH),1543(C=N and C=Cstr.), 1511-1321 ,1145 (C-N str.), 653(C-Cl str).^{1}H NMR (300 MHz, $CDCl_3$) 12.87(1H,s,-NH-Benzimidazole),10.26(s,1H,COOH), 6.43- 8.67 (m,18H,ArH), 2.33 (s,3H,CH_3),5.01(s,2H,CH_2).^{13}CNMR($CDCl_3$)δ:22.1,50.1,53.3,65.1,73.7,112.4,114.1,116.1,117.1,1 22.2,130.2,141, FAB-MS, 682.25

SCHEME



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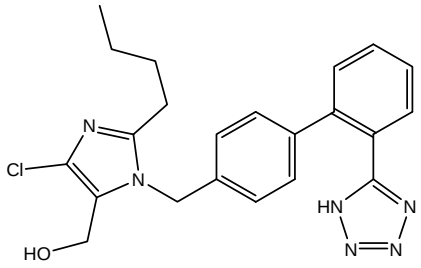

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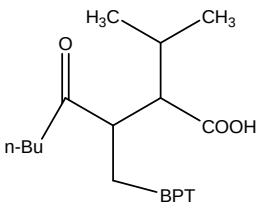

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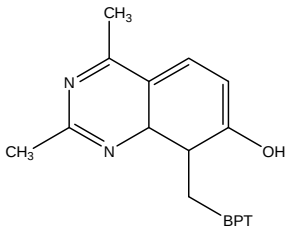

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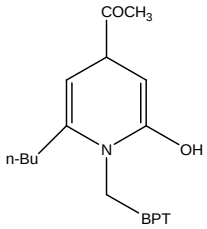

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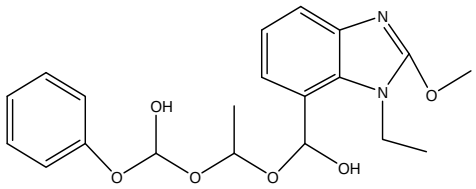

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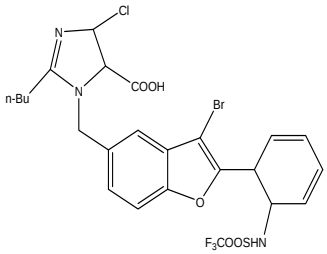

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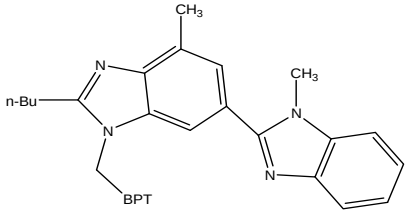

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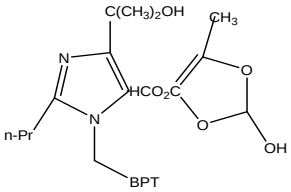

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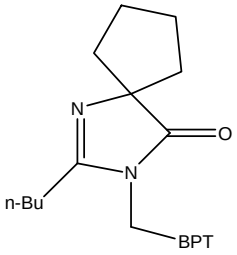

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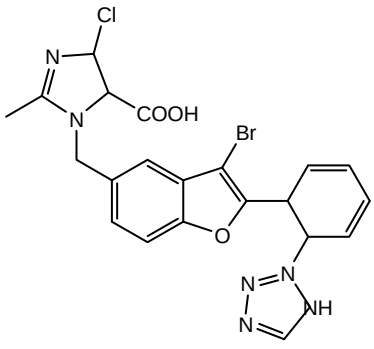

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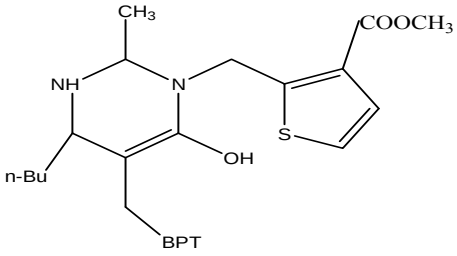

Eprosartan

Angiotensin II selective antagonists

Antihypertensive Activity: [17-23]

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 is 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±1^{0}C room temperature. The rats were anaesthetized with sodium chloride 0.9% solution, Drug solution 10-µg/100ml, and Heparin 500 I.U.solution urethane hydrochloride 50% w/v solution 80 mg/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 losratan. Synthesized compounds were screened in presence of Angiotensin II induced hypertension (0.5 µg/kg i.v.)Table 3, 4.

Table 1. Hypertension induced in normotensive rat.

Comp.	Exp. Animal Albino (Wistar) Rat	After 1hour			After 3 hours		
		SBP	DBP	MABP	SBP	DBP	MABP
[a]	1	142	106	124	140	102	123
	2	140	105	128	138	104	121
	3	143	105	124	139	107	121
	4	141	101	126	143	102	120
	5	141	110	126	143	108	119
[b]	1	142	113	128	142	104	123
	2	142	102	125	141	105	121
	3	139	111	124	138	106	120
	4	144	114	129	142	102	121
	5	139	114	127	135	103	119
[c]	1	144	114	128	141	102	121
	2	146	104	125	142	102	122
	3	140	106	123	142	106	124
	4	141	114	128	142	104	123
	5	146	108	127	144	104	124

Comp.	Exp. Animal Albino (Wistar) Rat	After 1hour			After 3 hours		
		SBP	DBP	MABP	SBP	DBP	MABP
[d]	1	141	102	121	139	103	121
	2	140	105	123	141	105	124
	3	141	110	129	142	108	125
	4	138	105	125	139	107	123
	5	132	104	128	142	102	122
[e]	1	144	116	130	141	101	122
	2	142	110	126	139	104	123
	3	146	106	126	144	104	124
	4	148	106	127	146	102	124
	5	151	112	133	146	101	124
Control	**Losartan**	124	-	-	-	-	-

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 hours		
		SBP	DBP	MABP	SBP	DBP	MABP
[1]	1	129	108	119	124	104	114
	2	122	112	117	122	103	112
	3	126	114	120	128	107	117
	4	125	103	114	126	102	114
	5	127	104	116	124	105	114
[2]	1	124	105	115	125	106	116
	2	122	109	116	126	106	116
	3	136	101	118	122	104	113
	4	134	100	117	126	104	115
	5	122	102	112	122	100	111
[3]	1	124	103	111	125	102	113
	2	122	102	114	123	100	111
	3	123	111	118	128	104	116
	4	127	105	116	126	105	115
	5	128	105	116	128	102	115
[4]	1	129	101	117	126	104	115
	2	128	102	115	126	104	115
	3	131	103	117	124	102	113
	4	123	103	116	124	110	117
	5	125	104	115	125	106	116
[5]	1	131	100	123	121	106	110
	2	129	103	124	122	100	111
	3	127	103	115	125	102	114
	4	124	104	114	128	101	113
	5	122	102	111	123	102	112
Control	**Losartan**	105	-	-	-	-	-

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	**169**	**163**	**158**	**153**	**148**	**141**	**136**	**131**	**127**	**119**
1	181	175	168	162	158	152	147	142	138	133
2	171	167	163	158	153	149	144	139	135	131
3	174	168	163	157	152	148	142	138	133	129
4	177	173	167	161	157	152	146	141	138	134
5	174	168	164	161	156	151	147	141	137	133

Table: 4 Antihypertensive Activity of synthesized compounds

Compound. No	**Minimum Blood pressure value(mm Hg)**	**Duration of hypertension effect(min.)**
Losratan	119	90
1	124	115
2	121	110
3	121	100
4	123	98
5	122	100

3. Result and discussion

The maximum activity has been observed with Chlorine, nitro group (Compound 4). There are some sites in the receptor pocket, which can interact with the functional groups at position 5 and 6. Substituted benzimidazole nucleus coupled to carboxylbipheny methyl group has been designed, synthesized and evaluated for angiotensin II antagonism. Among the compounds tested for antihypertensive activity, the compounds 4 were found to reduce blood pressure significantly that is compared with standard. Compound with Chlorine, nitro group at 5, 6 and 2 position and aromatic, aryl, alkyl compounds at 2- position have been found to be more potent than losratan.

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.

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