DESIGN, SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF SOME NOVEL 5, 6-SUBSTITUTE-1-[2'-(1H-TETRAZOL-5-YL)-BIPHENYL-4-YLMETHYL]-2-TRIFLUOROMETHYL-1H-BENZOIMIDAZOL FOR THEIR POTENTIAL ANTIHYPERTENSIVE AGENTS

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Series heterocyclic benzimidazole derivatives bearing of novel 5, 6-Substitute-1-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-2- trifluoromethyl-1H-benzoimidazol were designed and synthesized for their potential antihypertensive activity. All The structures of these compounds have been established by analytical data, IR, ^{1}H-NMR ^{13}C-NMR and Mass spectral data. The majority of the compounds were found active in the biological screening. The efforts were also made to establish structure activity relationships among synthesized compounds.

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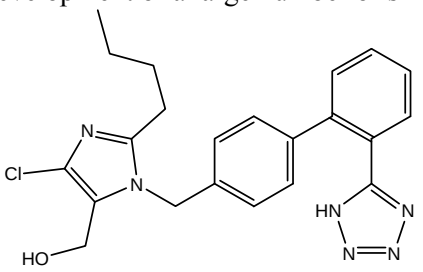
Keywords: Angiotensin II, Blood Pressure, antihypertensive activity, $BF_3 \cdot OEt_2$

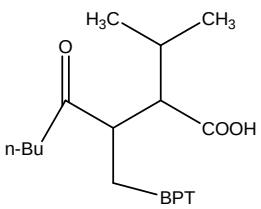
1. Introduction

The synthesis of novel benzimidazole derivatives remains a main focus of medicinal research. Recent observations suggest that substituted benzimidazoles and heterocyclic, 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 chemotherapeutic approach in man[1,2] . Moreover, these fused heterocylces were distinctively studied for their antitumor, antiviral and antimicrobial activities as the new nonnucleoside topoisomerase I poisons, human immunodeficiency virus-1 reverse transcriptase inhibitors and /or potent DNA gyrase inhibitors [3-5]. In addition, benzimidazole derivatives have played a crucial role in the theoretical development of heterocyclic chemistry and are also used extensively in organic synthesis. The rennin-angiotensin system (RAS) plays a major role in the regulation of blood pressure and electrolyte homeostasis [6]. RAS is a cascade of proteolytic enzymes (rennin and angiotensin converting enzyme (ACE)) that result in the production of the systemic hormone angiotensin II (AII).The blockade of RAS with inhibitors of ACE has demonstrated the effectiveness of the reduction of levels of AII on cardiovascular and kidney heamodynamics, aldosterone production and release, and the absorption of sodium. Antagonists of AII constitute an alternative method blocking the RAS. Several peptidic and nonpeptidic AII receptor antagonists are known. The therapeutic availability is less for the peptidic AII antagonist due to their poor bioavailability; short plasma half-life and partial agonist activity but the nonpeptidic AII receptors antagonist lack the defect of peptidic antagonist [7].The therapeutic profile of AII receptor antagonist is thought to be similar to that of angiotensin converting enzyme (ACE) inhibitors such as captopril, enalapril, and lisinopril. In addition, since AII receptor antagonist does not affect the metabolism of bradykinin so they may not have the side effect of ACE inhibitors, such as dry cough and angiodema. The main effects of AII are the regulation of blood pressure through vasoconstriction, thereby effecting an increase in vascular resistance, the regulation of volemia through the stimulated release of vasopressin and aldosterone, which induces saline retention, and the regulation of the adrenocorticotropic hormone (ACTH). Thus, reducing the levels of AII by

inhibition of one of the RAS enzymes or directly blocking the AII receptors is in theory a good approach for treating hypertension, confirmed by the success of angiotensin-converting enzyme (ACE) inhibitors as antihypertensive [8]. Substantial effort has been made to find renin inhibitors, although orally active agents have only recently been reported [9].The discovery of potent and orally active nonpeptide Angiotensin II antagonists such as losartan and eprosartan has encouraged the development of a large number of similar compounds. [10-12]



Losartan



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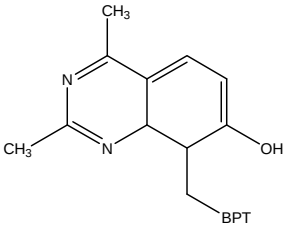

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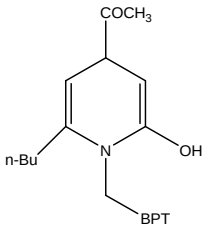

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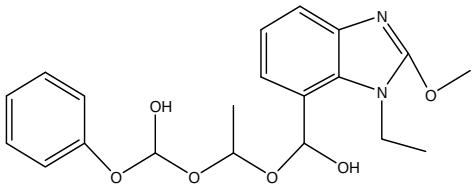

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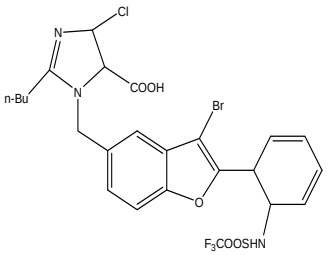
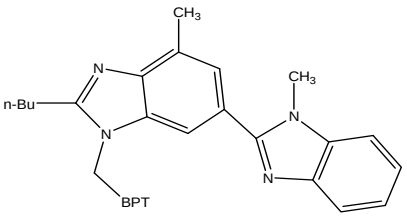
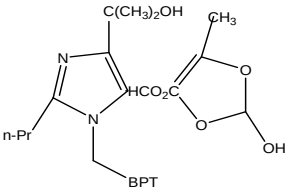


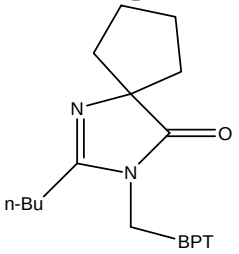
Saprisartan



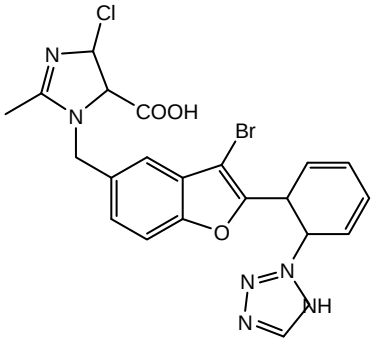
Telmisartan



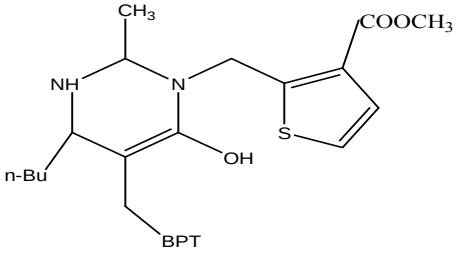
Olmesartan



Irbesartan



Zolzsartan



Eprosartan

Angiotensin II selective antagonists

2. Materials and method

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. 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 [13].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.

STEPS IN SYNTHESIS COMPOUNDS

5, 6-Substitute-1-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-2- trifluoromethyl-1H-benzoimidazol

Difference substituted [R_1 and R_2] o-Phenylenediamine was condensed with in propylene glycol (15 mL) was added trifluoroacetic acid [14] (2.5 gm) in the presence of $BF_3{\bullet}OEt_2$ (2.5 mmol) to this reaction mixture, CH_2Cl_2 (60 mL) was added and washed with water and the solution was heated at 110°C for 6 hrs. The organic phase was separated, dried (Na_2SO_4) and concentrated *in vacuo* to get the crude compound then cooled to room temperature. The crude compounds were purified by silica gel column chromatography using ethyl acetate: chloroform (95:5) as eluent.The reaction mixture was poured into crushed ice. Filtered, washed, dried and recrystalized [MCS-01]. To a solution of was dissolved in 50 ml of DMF (dimethyl formamide) and stirred vigorously with 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 ($MgSO_4$) and evaporated [MCS-02]. A mixture of different substituted [MCS-02] (80mg), sodium azide (35 mg), and Et3N·HCl (50 mg) in NH_4Cl (30 mL) is stirred at 40°C for 8 hours. After cooling, the mixture is diluted with distilled water (50 mL), acidified to pH 4.5with 4N HCl, and extracted with EtOAc (3 × 50 mL). The organic layer was washed with H_2O (3 × 50 mL), then the combined extracts were dried ($MgSO_4$) 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.

Compounds Spectral Data Analysis

[1] 6-Chloro-3-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-2- trifluoromethyl-1H-benzoimidazol-5-carboxylic acid Yield: 77 %, m.p. = 248-251 C. $C_{23}H_{14}ClF_3N_6O_2$: Found: C,55.36;H, 2.87;N,16.82 %;IR(KBr):3618,3526,3411,3088,2943,2375,1527,896. cm^{-1}.^{1}HNMR(300MHz,$CDCl_3$) 12.86(1H,s,-NH-Benzimidazole),10.22(s,1H,tetrazole-NH), 11.06(s,1H,COOH),4.99(s,2H,CH_2),7.6-8.6(m,9H,ArH).^{13}CNMR($CDCl_3$)δ:51.6,112.9,113.4,116.2,121.1,128.4,135.5,138.2,140.4, FAB-MS, 499.41

[2] 5-Fluoro-1-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-2- trifluoromethyl-1H-benzoimidazol Yield: 72 %, m.p. = 225-227 C. $C_{22}H_{14}F_4N_6$: Found: C,60.28;H, 3.22;N,17.32 %;IR(KBr):3613,3521,3417,3249,3166,3088,2963,2371,1526,898 cm^{-1}.^{1}HNMR(300MHz,$CDCl_3$) 12.89(1H,s,-NH-Benzimidazole),10.21(s,1H,tetrazole-NH), 4.96(s,2H,CH_2),7.54-8.63(m,10H,ArH).^{13}CNMR($CDCl_3$)δ:50.2,111.5,113.2,115.4,116.2,121.1,128.4,135.5,138.2,142, FAB-MS, 439.05

[3] 5-Bromo-6-methyl-1-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-2-trifluoromethyl-1H-benzoimidazole Yield: 65 %, m.p. = 260-263 C. $C_{23}H_{16}BrF_3N_6$: Found: C,53.89;H, 3.12;N,11.16 %;IR(KBr):3609,3524,3411,3245,3139,3080,2967,1524,893 cm^{-1}.^{1}HNMR(300MHz,$CDCl_3$) 12.83(1H,s,-NH-Benzimidazole),10.22(s,1H,tetrazole-NH),

4.96(s,2H,CH_2),7.54-8.63(m,7H,ArH), 2.34(s,3H,CH_3).^{13}CNMR($CDCl_3$)δ: 20,11, 73.7,112.4,114.1,116.1,117.1,122.2,130.2,141.6, FAB-MS, 512.057

[4] 6-methyl-1-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-2-trifluoromethyl-1H-benzoimidazole-5-carboxylic acid

Yield: 69 %, m.p. = 211-213 C. $C_{24}H_{17}F_3N_6O_2$: Found: C,60.25;H, 3.54;N,17.56 %;IR(KBr):3617,3589,3417,3269,3152,3087,2961,1524,899 cm^{-1}.^{1}HNMR(300MHz,$CDCl_3$) 12.88(1H,s,-NH-Benzimidazole),10.26(s,1H,tetrazole-NH), 11.08(s,1H,COOH), 4.94(s,2H,CH_2),7.50-8.56(m,9H,ArH), 2.37(s,3H,CH_3).^{13}CNMR($CDCl_3$)δ: 20, ,65.1, 112.4,114.1,116.1,117.1,122.2,130.2,140.22, FAB-MS, 479.22

[5] 5-Fluoro-5-methyl-1-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-2-trifluoromethyl-1H-benzoimidazol

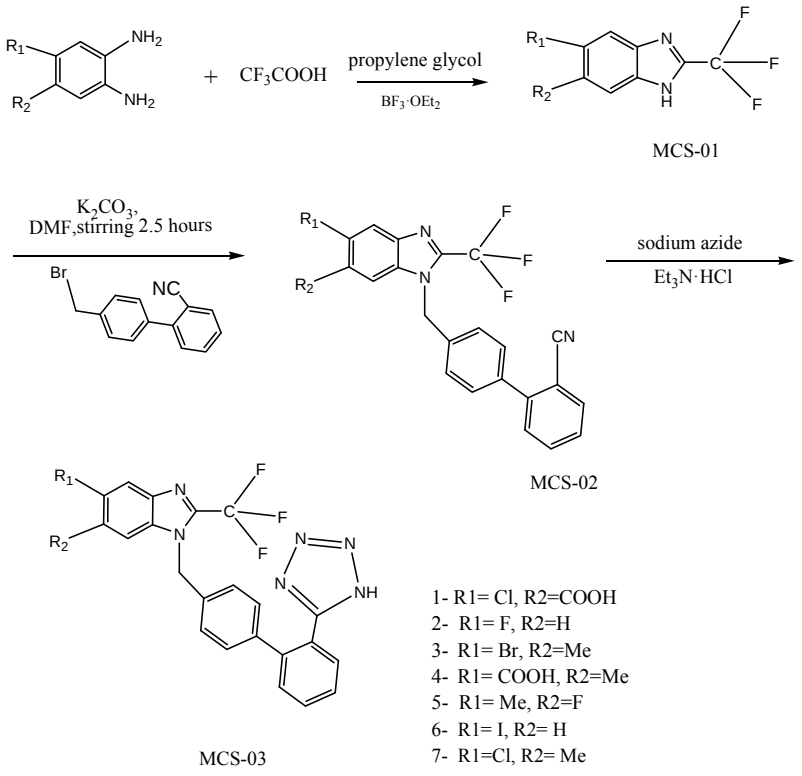
Yield: 55 %, m.p. = 235-237 C. $C_{23}H_{16}F_4N_6$: Found: C,61.11;H, 3.59;N,18.58 %;IR(KBr):3624,3582,3407,3276,3149,3081,2944,1574,895 cm^{-1}.^{1}HNMR(300MHz,$CDCl_3$) 12.87(1H,s,-NH-Benzimidazole),10.24(s,1H,tetrazole-NH), 4.98(s,2H,CH_2),7.45-8.59(m,9H,ArH),2.36(s,3H,CH_3).^{13}CNMR($CDCl_3$)δ:49.1,112,114.6,116.1,117.1,122.2,130.2,136, FAB-MS, 453.16

[6] 5-Iodo-1-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-2- trifluoromethyl-1H-benzoimidazole

Yield: 60 %, m.p. = 277-279 C. $C_{22}H_{14}F_3IN_6$: Found: C,48.37;H, 2.54;N,15.32 %;IR(KBr):3611,3564,3427,3286,3154,3087,2947,1543,889 cm^{-1}.^{1}HNMR(300MHz,$CDCl_3$) 12.84(1H,s,-NH-Benzimidazole),10.22(s,1H,tetrazole-NH), 4.95(s,2H,CH_2),7.6-8.5 (m,10H,ArH),^{13}CNMR($CDCl_3$)δ:21,6,53.1, 112.4,114.1,116.1,117.1,122.2,130.2,141.63, FAB-MS, 546.027

[7] 5-Chloro-6-methyl-1-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-2-trifluoromethyl-1H-benzoimidazol Yield: 67 %, m.p. = 289-292 C. $C_{22}H_{16}ClF_3IN_6$: Found: C,58.97;H, 3.48;N,17.87 %;IR(KBr):3654,3594,3429,3281,3168,3087,2947,1543,889 cm^{-1}.^{1}HNMR(300MHz,$CDCl_3$)12.88(1H,s,-NH-Benzimidazole),10.18(s,1H,tetrazoleNH),2.33(s,3H,CH_3),5.04(s,2H,CH_2),7.3-8.6(m,9H,ArH),^{13}CNMR($CDCl_3$)δ:18.76,52.1,112.4,114.1,116.1,117.1,122.2,130.2,143, FAB-MS, 469.06

SCHEME



Antihypertensive Activity: [15-21]

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 (50mg/kg i.v.)Table 3, 4.

Table 1. Hypertension induced in normotensive rat.

Comp.	Exp. Animal Albino (Wistar) Rat	After 1hour			After 3 hour		
		SBP	DBP	MABP	SBP	DBP	MABP
[1]	1	142	112	127	140	103	121
	2	140	110	125	139	107	123
	3	137	110	123	138	105	121
	4	140	105	122	137	105	122
	5	140	110	125	134	105	119
[2]	1	142	105	122	142	101	126
	2	139	111	126	141	102	119
	3	144	104	124	144	101	120
	4	142	101	123	142	100	121
	5	144	114	129	138	103	121
[3]	1	148	104	126	142	100	121
	2	144	112	127	141	102	121
	3	142	114	128	144	101	122

Comp.	Exp. Animal Albino (Wistar) Rat	After 1hour			After 3 hour		
		SBP	DBP	MABP	SBP	DBP	MABP
	4	148	108	126	144	102	123
	5	151	112	133	146	101	124
[4]	1	146	106	126	142	104	123
	2	142	110	126	140	116	128
	3	148	102	125	144	106	125
	4	140	106	123	142	106	124
	5	141	114	128	142	104	123
[5]	1	142	106	124	140	102	123
	2	140	105	128	138	104	121
	3	139	108	124	141	103	122
	4	142	113	128	142	104	123
	5	141	109	125	144	103	124
[6]	1	142	104	123	141	104	122
	2	138	104	121	140	106	123
	3	141	109	125	143	106	124
	4	136	112	124	141	103	122
	5	144	114	128	141	102	121
[7]	1	140	105	123	141	105	124
	2	143	101	122	140	110	125
	3	142	103	122	139	104	121
	4	137	109	123	140	110	125
	5	139	111	125	138	108	123
Control	**Losartan**	118	-	-	-	-	-

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

Comp.	AnimalAlbino (Wistar) Rat	After 1hour			After 3 hour		
		SBP	DBP	MABP	SBP	DBP	MABP
[1]	1	126	103	114	126	96	111
	2	129	101	115	119	104	111
	3	124	106	115	127	102	114
	4	126	104	115	125	105	115
	5	124	104	114	121	100	110
[2]	1	134	112	123	135	107	121
	2	140	105	122	137	103	120
	3	141	106	124	141	101	121
	4	141	110	121	143	115	124
	5	140	105	123	138	104	121
[3]	1	126	105	116	127	101	114
	2	126	109	117	122	106	114
	3	124	103	115	125	101	113
	4	128	105	114	127	102	114
	5	132	104	118	127	107	117

Comp.	AnimalAlbino (Wistar) Rat	After 1hour			After 3 hour		
		SBP	DBP	MABP	SBP	DBP	MABP
[4]	1	123	103	116	124	110	117
	2	125	104	115	125	106	116
	3	129	108	119	124	104	114
	4	122	112	117	122	103	112
	5	126	114	120	128	107	117
[5]	1	123	103	113	124	103	114
	2	122	106	114	123	107	115
	3	127	101	114	126	106	116
	4	136	101	118	122	104	113
	5	135	105	120	129	102	116
[6]	1	133	103	117	127	105	116
	2	129	108	118	124	104	114
	3	122	112	121	122	103	112
	4	126	114	120	128	107	117
	5	124	111	118	123	104	113
[7]	1	129	102	116	124	101	113
	2	134	100	117	126	104	115
	3	123	102	113	128	103	112
	4	121	101	113	123	102	111
	5	125	101	113	128	102	115
Control	**Losartan**	101	-	-	-	-	-

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	**170**	**166**	**161**	**155**	**149**	**142**	**134**	**128**	**122**	**110**
1	175	171	168	162	157	151	148	142	138	132
2	173	169	163	159	151	147	140	138	134	130
3	178	174	170	165	158	151	146	141	133	128
4	181	177	172	169	163	158	152	147	144	137
5	183	178	174	168	164	159	151	145	139	132
6	180	174	168	162	157	152	148	140	134	127
7	177	172	166	159	153	148	143	138	130	125

Table: 4 Antihypertensive Activity of synthesized compounds

Compound. No	Minimum Blood pressure value(mm Hg)	Duration of hypertension effect(min.)
Losratan	**110**	**90**
1	122	115
2	120	110
3	119	100
4	123	113
5	123	115
6	121	100
7	122	95

3. Results and discussion

Synthesized compounds were characterized on the basis of chemical and spectral data. The present work was mainly intended to establish the moieties which are responsible for Angiotension-II inhibition. Ang II antagonism by compounds with same functional group at 2, 5, 6 position has been found to be a function of substitute at 5-position. Presence of COOH, Cl and CH3 group has increased the activity substantially over the substituted one ([1] to [7]). The maximum antihypertensive activity has been observed with COOH, CH_3 group Compound. This suggests that there are some sites in the receptor pocket, which can interact with the functional groups at position 6-Substituted benzimidazole nucleus coupled to tetrazole biphenyl group has been designed, synthesized and evaluated for angiotensin II antagonism. Biological activity of synthesized compounds was carried out using rat blood pressure measurement experiment; the maximum fall blood pressure produced by Losartan is from value 170 mm Hg to 110 mm Hg over a period 90 minutes.

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Reference

[1] Haugwitz RD.J Med Chem; **25**, pp. 969-974. (1982)
[2] Hisano T. Chem PharmBull; **30**, pp.2996-3004 (1982)
[3] Hubschwerlen C. J MedChem; 35, pp. 1385. (1992)
[4] Kim J.S. Borg Med Chem.; **4**, pp. 621-630. (1996).
[5] Shi D.F. J .Med. Chem,; **39**, 3375-3384. (1996).
[6] Gilman G, Goodman SL, Rall TW, Murad F.In: The Pharmacological Basis of Therapeutics.7th ed.New York: Macmillan Publishing Co, 639–659 (1985).
[7] Smith RD, Chiu AT, Wong PC, Herblin WF.Ann RevPharmacol Toxicol.; **32**, 135–165. (1992).
[8] Berecek K. H, King, S. J, WuJ. N, Angiotensin-Converting Enzyme and Converting Enzyme Inhibitors. Cellular and Molecular Biology of the Renin-Angiotensin System; CRC Press: Boca Raton, FL,; 183-220 (1994).
[9] KleinertH. D. Drugs,; **3**, 1087-1104 (1994).
[10] Dutta A.S, Design and Therapeutic Potential of Peptides. In Advances in Drug Research; Testa, B., Ed.Academic Press: London,; **21**, 147-286 (1991).
[11] Bali A, Bansal Y, Sugumaran M, Saggu J.S, Balakumar P, Kaur G, Bansal G, Sharma A, Singh M. Bio Med Chem Lett;**15**,3962-3965. (2005).
[12] Dhvanit I. S, Sharma M, Bansal Y,Bansal G, M. Singh. European Journal of Medicinal Chemistry; **43**, 1808-1812. (2008).
[13] Rahul R. Nagawade, Devanand B. Shinde. Chinese Chemical Letters, **17**(4), 453-456, 2006
[14]Weifa Yu, Zhiming Zhou, Xinqi Zhao, Congxuan Yu. Journal of Chemical Crystallography, Vol. 34, No. 9, 2004
[15] R.K.Jat, J.L.Jat, D.P.Pathak., Synthesis of Benzimidazole Derivatives: As Anti-hypertensive Agents.E Journal of Chemistry .**3**(13), 278-285, (2006)
[16] Gupta S.K.Drug Screening methods, Jaypee Brothers Medical Publisher, New Delhi, 236-246,2004.
[17] Shreenivas M.T, Chetan B.P, Bhat A.R, J. of Pharma.Sci. and Technology. **1**(2), 88-94 (2009).
[18] Vogel G.H.Drug Discovery and Evaluation, Pharmacological Assay, 2002 ;(Springer. Berlin), 122.

[19] A.A.Siddiqui, M.S.Wani.Indian.J.Chemistry.,**43B**, 1574, 1579 (2004).
[20] Badyal D.K, Lata H, Dadhich A.P, Indian J of Pharmacology; **35**(66), 349-362. (2003).
[21] Bunag R.D, McCubbin J.W, Page I.H, Cardiovasc. Res., **5**(1): 24-31 (1971).