DESIGN; SYNTHESIS AND PHARMACOLOGICAL INVESTIGATION OF SOME BENZIMIDAZOLE DERIVATIVES 4'-(5, 6-SUBSTITUED-2-TRIFLUOROMETHYL-BENZOIMIDAZOL-1-YLMETHYL)-BIPHENYL-2-CARBOXYLIC ACID AS POTENT ANTIHYPERTENSIVE AGENTS

SMITA SHARMA[a], M. C. SHARMA[*], D.V.KOHLI
Department of Pharmaceutical Sciences Dr.Hari Singh Gour University Sagar (M.P) 470003 India
[a]Department of Chemistry Chodhary Dilip Singh Kanya Mahavidyalya Bhind (M.P) 477001 India

Synthesis of Some Benzimidazole derivatives 4'-(5, 6-substitued-2-trifluoromethyl-benzoimidazol-1-ylmethyl)-biphenyl-2-carboxylic acid were synthesized by condensation of various steps with different o-Phenylenediamine was condensed with in propylene glycol was added trifluoroacetic acid in the presence of BF_3•OEt_2 as catalysis. Elemental analysis, IR, 1HNMR and mass spectral data confirmed the structure of the newly synthesized compounds. Synthesized and subjected to evaluate their antihypertensive activity. All the synthesized compounds of the series elicit remarkable activity in comparison to standard drug (Losartan).

(Received October 5, 2010; accepted October 26, 2010)

Keywords: Ang II, antihypertensive activity, BF_3•OEt_2, biphenyl-2-carboxylic acid

1. Introduction

The renin-angiotensin system (RAS) is known to play an important role in the regulation of blood pressure and electrolyte balance.[1]The renin-angiotensin system (RAS) plays an important role in the regulation of blood pressure through the actions of angiotensin II (AII) (vasoconstriction, aldosterone secretion, renal sodium reabsorption, and nor epinephrine release) and thus is an appropriate target for therapeutic intervention in hypertension[2]. Inhibitors of the RAS would be effective for the treatment of hypertension and congestive heart failure. Although angiotensin-converting enzyme (ACE) inhibitors are highly effective and their use has become well-established for the treatment of hypertension and congestive heart failure, they suffer from some side effects such as dry cough and angioedema caused by the nonspecific action of ACE.[3] On the other hand, angiotensin II (AII) (the primary effector component of the RAS) receptor antagonists block the RAS at the AII receptor level and are expected to be more specific and effective agents than ACE inhibitors.Angiotensin II (Ang II)*a* type 1 (AT1) receptor belongs to the G protein-coupled receptor super family and mediates virtually all the known physiological actions of Ang II through interaction with heterotrimeric G-protein and subsequent activation of several effector systems (phospholipases C, D, A2, adenyl cyclase, etc.). AT1 receptor shows the seven hydrophobic Tran membrane domains forming R-helices in the lipid bilayer of the cell membrane and plays a key role in the renin angiotensin system involved in the regulation of cardiovascular functions and pathophysiology of hypertension.[4,5] The discovery of potent and orally active nonpeptide Ang II antagonists such as losartan and eprosartan has encouraged the development of a large number of similar compounds.[6] Among them, irbesartan, candesartan, valsartan, telmisartan, tasosartan and olmesartan are on the market. Most of the developed AT1 receptor

[*]Corresponding author: mukesh2206@rediffmail.com

antagonists are characterized by the presence in their structure of the biphenyl fragment bearing an acidic moiety and differ in the nature of the pendent heterocyclic system (valsartan lacks the heterocyclic moiety) connected to the para position of the proximal phenyl.Substantial effort has been made to find renin inhibitors, although orally active agents have only recently been reported.[7] No less effort has been devoted to finding AII antagonists, which besides being the most direct way of controlling the RAS could have the additional advantage of lacking the side effects, such as cough and angioedema, observed with ACE inhibitors, as these are probably caused by partial inhibition of the cleavage of bradykinin and substance P. Starting from the initial leads reported by Takeda,[9] researchers at DuPont discovered losartan, the first orally active AT1 selective nonpeptide AII antagonist that reached the market for the treatment of hypertension (1994, Cozaar). Whereas reports on effective replacements of the biphenyl tetrazole "tail" of losratan are scarce, the imidazolic "head" of the molecule, postulated to act mainly to link the required functionalities, has been successfully replaced by a wide variety of cyclic and acyclic structures, leading to a number of compounds currently in clinical trials.[10]AngII receptor antagonists are expected to have similar therapeutic effects and indications as the ACE inhibitors without unwanted side effects associated inhibition of other ACE mediated pathways, such as bradykinin metabolism.Intial research in this area led to the discovery of peptide analog such as saralasin ([sar1-Ala8]-AngII) which displayed potent and selective AngII receptor antagonist activity both *in vivo and in vitro*.However, these peptides had limited therapeutic utility due to partial agonist activity short duration of action and lack of appreciable oral bioavailability[11].Only in recent years a number of non peptides AngII antagonists that show promise as inhibitors of the RAS been reported[12].All these antagonists possess a central aromatic nucleus bearing the pharmacophores indispensable for activity and notably a polar function adjutant to biphenyl substituents while a polar function in this area of molecule seems to be necessary to maintain activity[13]. Sartans are appropriately substituted heterocyclic head coupled through a methylene linker to pendent biphenyl system bearing an acidic function; viz. candesartan is an effective competitive Ang II antagonist with benzimidazole nucleus as the heterocyclic head [14]. The substituent at 6-position on the nucleus increases the activity whereas small substituent at 5-position decreases the activity[15]. Compounds containing tetrazole nucleus are also reported as AT_1 receptor antagonists and their protypical derivative 3 exhibits non-competitive antagonism[16] and amino group attach with carboxylic group given good biological activity [17,18]

2. Experimental section

2.1 Materials and method

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[19].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.

3. Prepration of various steps in synthesis compounds

Synthesis of 4-(5, 6-substitued-2-trifluoromethyl-benzoimidazol-1-ylmethyl)-biphenyl-2-carboxylic acid

Difference substituted [R_1 and R_2] o-Phenylenediamine was condensed with in propylene glycol (15 mL) was added trifluoroacetic acid [20](4.5 g,) in the presence of BF_3•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 28mg of potassium carbonate at 44 ^{0}C for 3hours. To the resulting mixture 1.2 gm of 4'-bromomethyl biphenyl-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. After stirring for 12 h the mixture was poured into water (120 mL) and extracted with ethyl acetate (3 × 50 mL). The combined extracts were dried (MgSO4) and evaporated. The organic layer was washed with brine solution, distilled water and dried over anhydrous sodium sulphate. (MCS-02) was obtained.

Compounds spectral data analysis

[1] 3-(2'-Carboxy-biphenyl-4-ylmethyl)-6-chloro-2-trifluoromethyl-3H-benzoimidazole-5-carboxylic acid

Yield: 65%, m.p. = 166-169 C. $C_{23}H_{14}ClF_3N_2O_4$: Found: C,58.16;H, 2.99;N,5.94 %;IR (KBr): 3632,3583,3441,3311,3031 , 2937,2876 ,2316,1712, 1532 ,1147 ,1104,754.^{1}HNMR(300MHz,$CDCl_3$)13.11(1H,s,-NH Benzi),11.04(s,2H,COOH),7.3-8.5(m,9H,ArH),5.0(s,2H,CH_2).^{13}CNMR($CDCl_3$)δ:50.1,112.9,113.4,116.2,121.1,128.4,135.5,138.2, 142.6, FAB-MS, 476.054

[2] 4'-(5-Fluoro-2-trifluoromethyl-benzoimidazole-1-ylmethyl)-biphenyl-2-carboxylic acid

Yield: 73%, m.p. = 194-197 C. $C_{22}H_{14}F_4N_2O_2$: Found: C,63.74;H, 3.46;N,6.79 %;IR (KBr):3618,3597,3454,3031,2937,2311,1710,1542,1142,1097.^{1}HNMR(300MHz,$CDCl_3$)13.09(1H, s,NH,Benzim),11.21(s,1H,COOH),7.32-8.48(m,10H,ArH),5.05(s,2H,CH_2).^{13}CNMR($CDCl_3$)δ:52.6,112.9,113.4,116.2,121.1,128.4,135.5,138.2,142.6, FAB-MS, 414.11

[3] 4'-(5-Bromo-6-methyl-2-trifluoromethyl-benzoimidazole-1-ylmethyl)-biphenyl-2-carboxylic acid

Yield: 65%, m.p. = 216-219 C. $C_{23}H_{16}BrF_3N_2O_2$: Found: C,56.46;H, 3.27;N,5.77 %;IR(KBr):3605,3586,3451,3071,2987,2301,1706,1547,1140,1091.^{1}HNMR(300MHz,$CDCl_3$)13.07(1H,s,NH,Benzim),11.23(s,1H,COOH),7.24-8.51(m,9H,ArH), 2.33(s,3H,CH_3),5.03(s,2H,CH_2).^{13}CNMR($CDCl_3$)δ:52.6,112.9,113.4,116.2,121.1,128.4,135.5,138.2,142.6, FAB-MS, 488.034

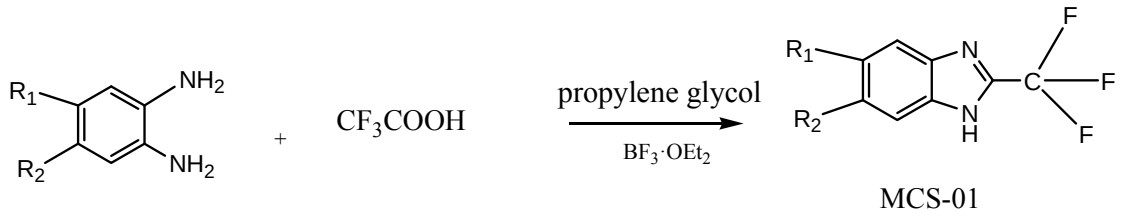
[4] 1-(2'-Carboxy-biphenyl-4-ylmethyl)-6-methyl-2-trifluoromethyl-3H-benzoimidazole-5-carboxylic acid

Yield: 73%, m.p. = 243-246 C. $C_{24}H_{17}F_3N_2O_4$: Found: C,63.43;H, 3.75;N,6.21 %;IR(KBr):3625,3581,3459,3063,2980,2336,1702,1540,1146,1088.^{1}HNMR(300MHz,$CDCl_3$)13.04(1H,s,NH,Benzim),11.20(s,2H,COOH),7.28-8.55(m,9H,ArH), 2.37(s,3H,CH_3),5.01(s,2H,CH_2).^{13}CNMR($CDCl_3$)δ:18.3,51.1,112.9,113.4,116.2,121.1,128.4,135.5, 138.2,142.6,148.76 FAB-MS, 455.31

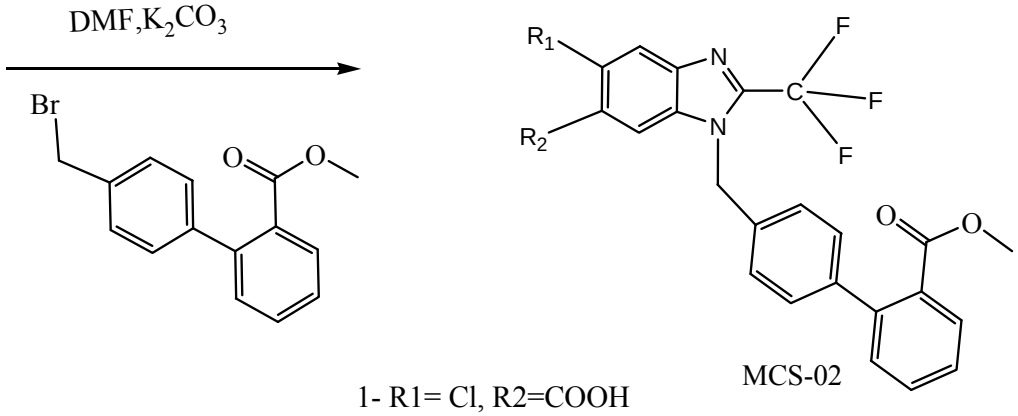
[5] 4'-(6-Fluoro-6-methyl-2-trifluoromethyl-benzoimidazole-1-ylmethyl)-biphenyl-2-carboxylic acid

Yield: 70%, m.p. = 226-229 C. $C_{23}H_{16}F_4N_2O_2$: Found: C,64.47;H, 3.78;N,6.60 %;IR(KBr):3621,3575,3453,3063,2989,2329,1707,1537,1146,1090.^{1}HNMR(300MHz,$CDCl_3$)13.01(1H,s,NH,Benzim),11.24(s,1H,COOH),7.28-8.55(m,9H,ArH), 2.38(s,3H,CH_3),5.07(s,2H,CH_2).^{13}CNMR($CDCl_3$)δ:20.04,51.1,112.9,113.4,116.2,121.1,128.4,135.5,138.2,142.12, FAB-MS, 429.43

SCHEME

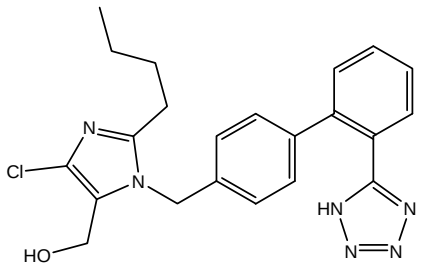


MCS-01

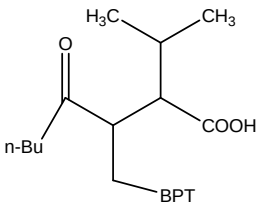


MCS-02

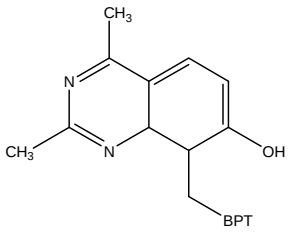
1- R1= Cl, R2=COOH
2- R1= F, R2=H
3- R1= Br, R2=Me
4- R1= COOH, R2=Me
5- R1= Me, R2=F



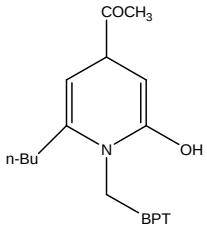
Losartan



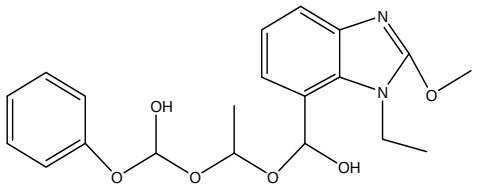
Valsartan



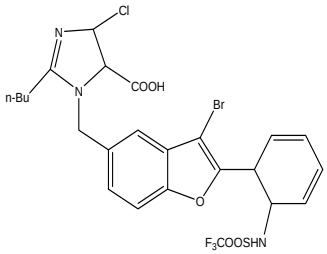
Tasosartan



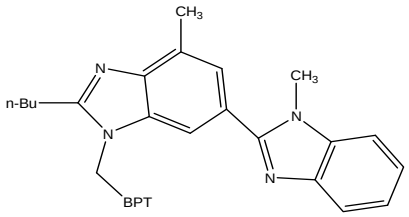
Candesartan



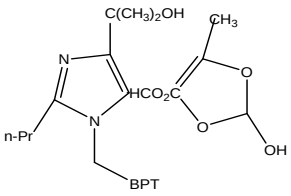
Milfasartan



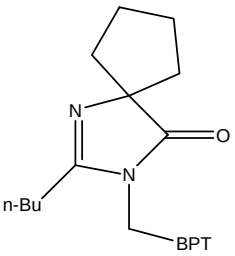
Saprisartan



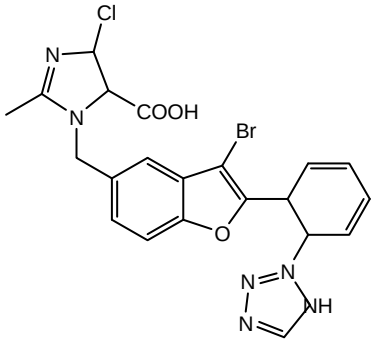
Telmisartan



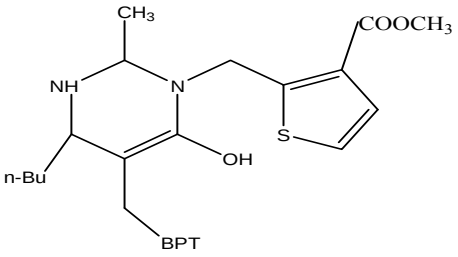
Olmesartan



Irbesartan



Zolzsartan



Eprosartan

Angiotensin II selective antagonists

Antihypertensive Activity: [18, 21-26]

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	102	121
	2	144	116	130	141	101	122
	3	150	123	136	103	119	137
	4	147	125	138	105	122	140
	5	146	114	12	143	101	122
[2]	1	146	104	132	142	100	121
	2	148	104	125	145	102	123
	3	140	106	123	142	101	121
	4	142	108	125	141	102	120
	5	139	110	125	143	101	120
[3]	1	146	114	12	143	101	122
	2	142	112	124	141	103	122
	3	142	112	127	140	103	121
	4	148	107	123	140	101	120
	5	132	104	128	142	102	122
[4]	1	143	105	124	143	105	124
	2	142	112	127	140	102	121
	3	144	116	130	141	101	122
	4	142	102	124	143	101	122
	5	140	105	122	137	103	120
[5]	1	144	114	129	146	106	126
	2	142	111	125	146	104	125
	3	142	112	127	140	102	121
	4	144	116	130	141	101	122
	5	141	106	124	141	101	121
Control	**Losartan**	125	-	-	-	-	-

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
[a]	1	122	104	118	124	98	113
	2	127	108	119	126	103	109
	3	128	106	117	123	100	112
	4	127	101	116	125	105	110
	5	124	102	119	128	102	111
[b]	1	126	103	114	126	96	111
	2	129	101	115	119	104	111
	3	123	107	115	121	99	110
	4	126	103	114	125	104	113
	5	132	105	119	121	102	110
[c]	1	126	104	115	125	105	115
	2	124	104	114	121	100	110
	3	129	102	116	124	101	113
	4	133	103	117	127	105	116
	5	130	108	118	124	100	112
[d]	1	126	103	114	126	96	111
	2	129	101	115	119	104	111
	3	123	107	115	121	99	110
	4	131	106	118	119	103	107
	5	122	104	112	125	101	113
[e]	1	123	102	113	128	103	112
	2	126	104	110	123	106	116
	3	122	106	114	129	101	113
	4	124	106	115	127	102	114
	5	133	103	118	126	100	110
Control	**Losartan**	104		-	-	-	-

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	**163**	**158**	**152**	**147**	**142**	**137**	**132**	**126**	**123**	**113**
[1]	169	164	158	151	152	148	144	138	133	129
[2]	175	170	166	159	153	149	142	139	135	132
[3]	167	164	159	153	148	143	139	136	133	128
[4]	169	164	159	154	149	144	140	135	131	130
[5]	173	167	163	160	152	148	142	139	136	134

Table: 4 Antihypertensive Activity of synthesized compounds

Compound. No	Minimum Blood pressure value(mm Hg)	Duration of hypertension effect(min.)
Losratan	**113**	**90**
[1]	118	110
[2]	121	115
[3]	122	100
[4]	120	112
[5]	118	120

4. Results and discussion

The compounds are recommended for the screening of antihypertensive activity. Synthesis compounds were screened for their antihypertensive activity by methods using 200-250 gm male either sex.the rats having hypertension more than 160 mm of Hg were taken for the experiment. All the twelve compounds synthesized [1-5] showed antihypertensive activity and with compared the standard drug. The maximum activity has been observed with carboxylic, chlorine and fluorine group. 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 antagonists. 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] Ferrario, C. M. J. Cardiovasc. Pharmacol., **15b**(Suppl. 3), S1-S5 (1990).
[2] Naka, T.; Inada, Y. Eur. Pat. 520423, 1993; Chem Abstr., **119**, 49388x (1993).
[3] Coulter, M. D.; Edwards, I. R. Br. Med. J., **294**, 1521 (1987)
[4] De Gasparo, M.; Catt, K. J.; Inagami, T.; Wright, J. W.; Unger, T. Pharmacol. ReV. **52**, 415 (2000).
[5] Inoue, Y.; Nakamura, N.; Inagami, T. J. Hypertens., **15**, 703 (1997).
[6] (a) Wexler, R. R.; Greenlee, W. J.; Irvin, J. D.; Goldberg, M. R.; Prendergast, K.; Smith, R. D.; Timmermans, P. B. M. W. M. J. Med. Chem., **39**, 625–656 (1996). (b) Schmidt, B.; Schieffer, B. J. Med. Chem. **46**, 2261 (2003), and references cited therein.
[7] (a) J.R.McEwan,R.W. Fuller. J. Cardiovasc. Pharmacol. **13** (Suppl. 3), S67 (1989). (b) R.A.Skidgel, S.Engelbrecht, A.R. Johnson, E.G. Erdos. Peptides. **5**, 769 (1984).
[8] Y.Furukawa, S.Kishimoto, S.Nishikawa. U.S. Patent 4340598, 1982.
[9] D. J.Carini, J.V.Duncia, P. E. Aldrich,A.T. Chiu,A.L. Johnson, M.E.Pierce, W.A. Price, J.B. Santella, G.J. Wells, R.R. Wexler, P.C. Wong, S. Yoo, P.B.M.W. Timmermans. J. Med. Chem. **34**, 2525 (1991).
[10] Wexler, R. R.; Greenlee, W. J.; Irvin, J. D.; Goldberg, M. R.; Prendergast, K.; Smith, R. D.; Timmermans, P. B. M. W. M. J. Med. Chem. **39**, 625 (1996)
[11] J.A.Keiser, F.A. Bjork , Pharmaco. Exp Ther. **262**, 1154 (1992).
[12] P.Juniak, A. Pillon. Hypertension. **20**(6):737(1992).
[13] D.A.Scheur, M.H. Perrone, Am J Physiol Regul Integr Comp Physiol. 264: 917-923(1993)

[14] K. Kubo, Y. Kohara, E. Imamiya, Y. Sugiura, Y. Inada, Y. Furukawa, K. Nishikawa, T. Naka, J. Med. Chem. **36**(1993) 2182e2195(1993).
[15] U.J. Ries, G. Mihm, B. Narr, K.M. Hasselbach, H. Wittneben, M. Entzreoth, J.C.A. Van Meel, M. Wienen, N.H. Hauel, J. Med. Chem. **36**, 4040 (1993).
[16] W.T. Ashton, C.L. Cantone, L.L. Chang, S.M. Hutchins, R.A. Strelitz, M. Maccoss, R.S.L. Chang, V.J. Lotti, K.A. Faust, T. Chen, P. Bunting, T.W. Schorn, S.D. Kivlighn, P.K.S. Siegel, J. Med. Chem. **36,** 591 (1993).
[17] A. Bali, Y. Bansal, M. Sugumaran, J.S. Saggu, P. Balakumar, G. Kaur, G. Bansal, A. Sharma, M. Singh, Bioorg. Med. Chem. Lett. **15**, 3962 (2005).
[18] R.K.Jat, J.L.Jat, D.P.Pathak., Synthesis of Benzimidazole Derivatives: As Anti-hypertensive Agents.E Journal of Chemistry **3(**13), 278 (2006).
[19] M. R. Grimmett, In Comprehensive Heterocyclic Chemistry, Eds,Pergamon: Oxford, **5**, 457 (1984).
[20] Weifa Yu, Zhiming Zhou, Xinqi Zhao, Congxuan Yu. Journal of Chemical Crystallography, **34**, No. 9, (2004).
[21] Gupta S.K.Drug Screening methods, Jaypee Brothers Medical Publisher, New Delhi, 236-246, 2004.
[22] Shreenivas M.T, Chetan B.P, Bhat A.R, J. of Pharma.Sci. and Technology.,; **1**(2), 88 (2009).
[23] Vogel G.H.Drug Discovery and Evaluation, Pharmacological Assay, 2002 ; (Springer. Berlin), 122.
[24] A.A.Siddiqui, M.S.Wani.Indian.J.Chemistry.**43B**, 1574 (2004).
[25] Badyal D.K, Lata H, Dadhich A.P, Indian J of Pharmacology; **35**(66), 349 (2003).
[26].Bunag R.D, McCubbin,J.W, Page I.H, Cardiovasc. Res. **5**(1): 24 (1971).