www.sphinxsai.com

PharmTech

International Journal of PharmTech Research
CODEN (USA): IJPRIF ISSN : 0974-4304
Vol.2, No.4, pp 2467-2481, Oct-Dec 2010

Synthesis and Pharmacological Evaluation of 4'-[2-(Phenyl-substituted amino-methyl)-benzoimidazol-1-ylmethyl] with Biphenyl Carboxylic acid Derivatives as Potent Antihypertensive Agents

Mukesh Chandra Sharma*[1], Dharm Veer Kohli[1], Smita Sharma[2]

***[1]Department of Pharmaceutical Sciences Dr.Hari Singh Gour Univ. Sagar (M.P) 470003 India**

[2]Department of Chemistry Yadhunath Mahavidyalya Bhind (M.P) 477001 India

Corres. author: mukeshcsharma@yahoo.com

Abstract : Some new 4'-[2-(Phenyl-substituted amino-methyl)-benzoimidazol-1-ylmethyl] biphenyl-2-carboxylic acid derivatives were synthesised by 2-(α -hydroxy benzyl) benzimidazole was converted to 2-(α-bromo benzyl) benzimidazole by reacting with HBr and Anhydrous $ZnCl_2$ schiff bases react with biphenyl carboxylic acid with different substituents amino group cyclocondensation with appropriate reagents. The compounds synthesised were identified by ^{1}H NMR, ^{13}C NMR, FAB Mass and FT-IR spectroscopic techniques. All compounds studied in this work were screened for their antihypertensive activity by tail cuff method and direct method measurement of Blood pressure.
Key word: Benzimidazole, Biphenyl Carboxylic acid, Angiotension-II, Blood pressure.

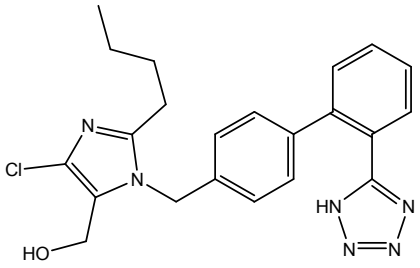
Introduction

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. The renin-angiotensin system (RAS) plays a key role in regulating cardiovascular homeostasis and electrolyte/ fluid balance in normotensive and hypertensive subjects.[1]Activation of the renin-angiotensin cascade begins with renin secretion from the juxtaglomerular apparatus of the kidney and culminates in the formation of the octapeptide angiotensin II (AII), which then interacts with specific receptors present in different tissues.[2] Two basic types of receptors, both having a broad distribution, have been characterized so far: the AT1 receptor, responsible for the majority of effects attributed to this peptide, and the AT2 receptor, with a functional role yet uncertain.[3] 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 antihypertensives.[4] Hypertension is one of the most important cardiovascular risk factor but its control is still Challenge for physicians all around the world. Antihypertensive are a class of drugs that are used in

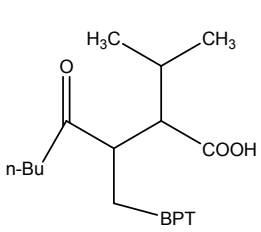
medicine and pharmacology to treat hypertension (high blood pressure). All Hypertensive drugs cause dizziness, ankle swelling, headache, fatigue, chest discomfort and cough. This review focus on the adverse effects of Antihypertensive drugs, severity of these adverse effects and attempts made to prevention and treatment of hypertension by non-pharmacological intervention.Substantial effort has been made to find renin inhibitors, although orally active agents have only recently been reported.[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 AT_1 receptor 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 adjustant 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] 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 in man[17-18.]

Experimental

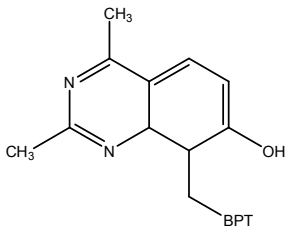
Melting points were determined in open capillary tubes and are uncorrected. The time required for completion of the reaction was monitored by TLC using Silica gel-G plates and spots were exposed in iodine chamber. IR spectra were recorded on a Perkin Elmer 1800 (FTIR) spectrometer 1H NMR spectra (DMSO) were taken on a DRX-300 spectrometer (300 MHz) using TMS as internal standard and chemical shifts are expressed in δ ppm.Prepared synthesis 2-(α-hydroxy benzyl) benzimidazole[19,20] and 2-(α-bromo benzyl) benzimidazole.[21]



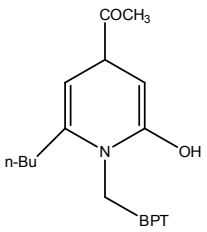
Losartan



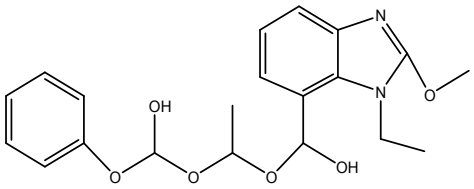
Valsartan



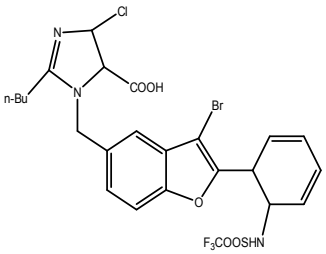
Tasosartan



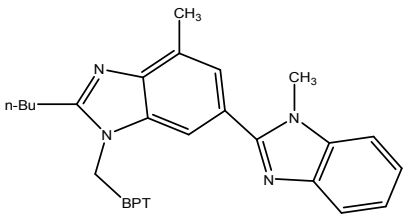
Candesartan



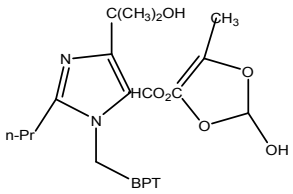
Milfasartan



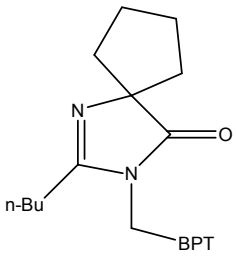
Saprisartan



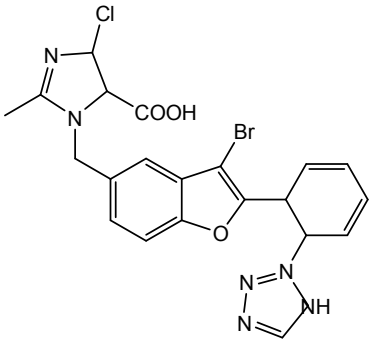
Telmisartan



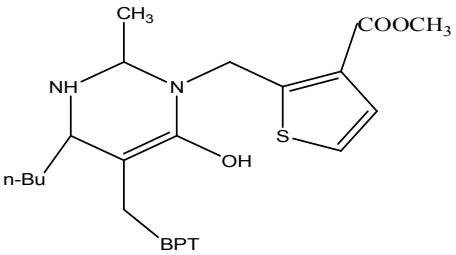
Olmesartan



Irbesartan



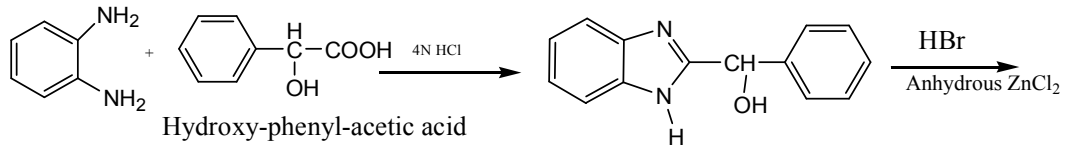
Zolzsartan



Eprosartan

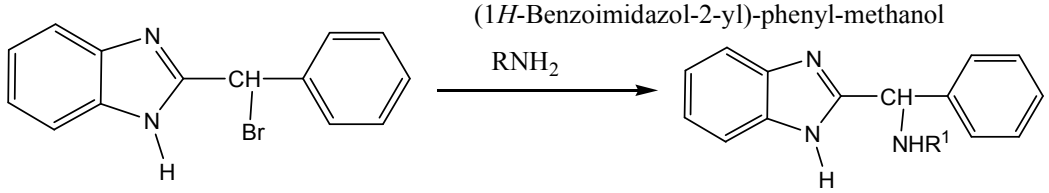
Figure- Angiotensin II selective antagonists

SCHEME

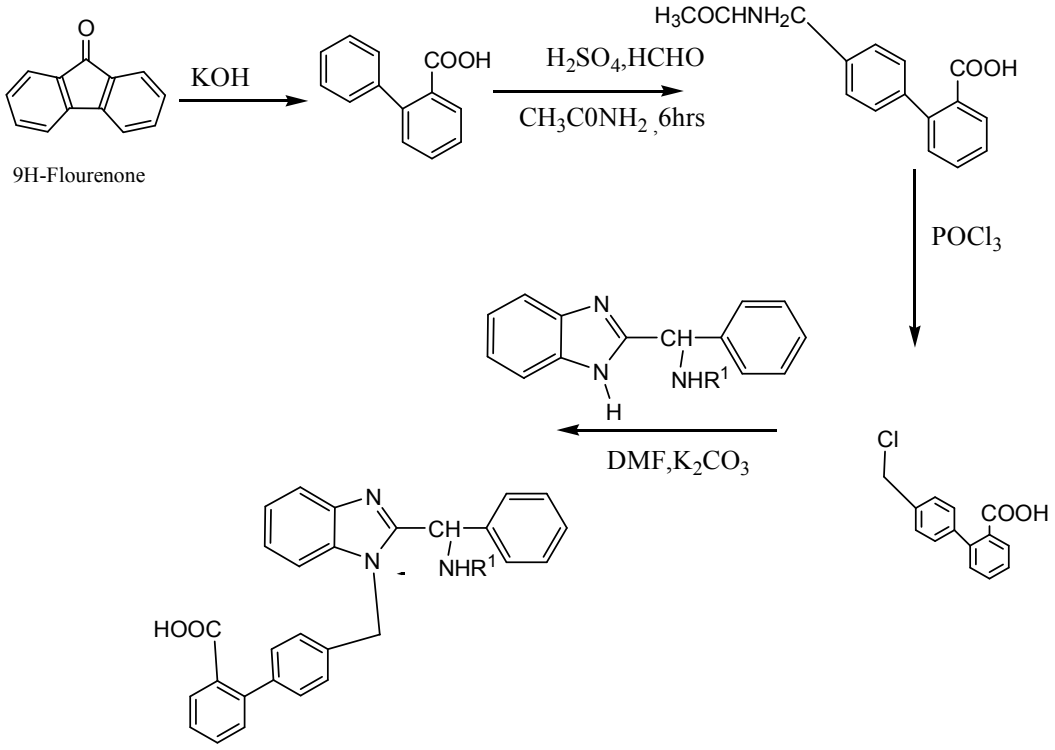


Hydroxy-phenyl-acetic acid

(1*H*-Benzoimidazol-2-yl)-phenyl-methanol



2-(Bromo-phenyl-methyl)-1*H*-benzoimidazole



9H-Flourenone

4`-[2-(Phenyl-substituted amino-methyl)-benzoimidazol-1-ylmethyl] biphenyl-2-carboxylic acid

Step- 2-(α-hydroxy benzyl) benzimidazole

In a 500ml of round bottom flask, equimolar amount of o-phenylene diamine (0.01 mol) and Mandelic acid (0.01 mol) were placed. 18 ml of 4n HCL was than added followed by a few porcelain chips and the mixture was refluxed gently on an oil-bath at 135-140^{o}C for 2 hours. The reaction mixture was then allowed to cool to room temperature, which was further neutralized with 20% Na2CO3 solution and was kept overnight. The crude product filtered at the vacuum pump, was recrystallized from ice-cold water or acetone. The formation of 2-(**α** -hydroxy benzyl) benzimidazole was confirmed by comparing its melting point with that reported in the literature, by thin layer chromatography and spectral analysis.

Yield: 85%, m.p. =122^{0}-125^{0}C. Anal.Calcd for $C_{14}H_{12}N_2O$:C,74.98;H, 5.39 ;N,12.49 %;IR(KBr):1954,1297.6,1581,1375,1965.^{1}HNMR(300 Hz,CDCl3):2.54(s,1H,OH),4.98(s,1H,NH)5.26(m,1H, C-H),7.10-8.23(m,9H,ArH), FAB-MS, 224.09

Step- Synthesis of 2-(α-bromo benzyl) benzimidazole

In a 250 ml round bottom flask fitted with a reflux condenser, (the top of which is connected to a device for absorbing hydrogen chloride gas) Zinc chloride (o.5 mole) and HBr (3ml) were placed and 2-(**α** – hydroxy benzyl) benzimidazole (0.25 mol) was added. The mixture was refluxed gently at 125-130^{o}C for 3 hours on an oil bath. The reaction mixture was then allowed to cool and the resulting bromide compound was separated. Refrigerated overnight and recrystallized successively from ice-cold water or acetone.

Yield: 72 %, m.p. =133^0-135^{0}C. Anal.Calcd for $C_{14}H_{13}N_3$:C,75.68;H, 5.46 ;N,18.91 %;IR(KBr):1923,1562,1437,1353,1870,1933.^{1}HNMR(300Hz,CDCl3):2.89(s,2H,NH)5.22(m,1H,C-H),7.10-8.23(m,10H,ArH),FAB-MS, 222.11

Step-3 -2-(α-bromo benzyl) benzimidazole (0.01mole) was dissolved in anhydrous dioxane (45 ml) and excess of various substituted aromatic amines were added. The reaction mixtures were allowed to stand overnight at room temperature, the solutions were evaporated to dryness and gummy solid obtained was washed with cold water. The solids were then extracted with 2M HCl. The acidic extracts were made alkaline with NH4OH and the resultant products were recrystallized from ice-cold water or acetone. Some of these final derivatives were characterized as picrates. The melting points of all the title compounds were recrystallized by open capillary method and were found to be uncorrected. The compounds were obtained in comparatively good yield of 50-70%.

Step- (Biphenyl Carboxylic acid)

35 gm of potassium hydroxide was heated at 170°-192 °C in a three necked flask until fusion. 12.5gm of finely powdered of 9H-Fluorenone was added in five portions over one and half hour with vigorous stirring and the temperature was maintained at 170°-192°C for further one half hour. The fusion mixture was then poured in ice cold water with stirring. The obtained suspension was filtered at vacuum pump and then filtrate was acidify with HCl to pH-4.5 resulting in precipitation of by product which was filtered under suction wash with distilled water and the filtrate was again acidify with Con.HCl. The precipitated product was filtered under suction and dried in air. The product was recrystallized from Absolute ethanol. Product MCS-03 was formed.

Yield:81%.m.p.=145-148°C.IR(KBr):3598-3069(O-Hstr),1675.4(C=O Carboxylic, str), 1393, 1364.3(C-O-H in-plane bend); 1H NMR(CDCl3): d 10.03(1H,s,COOH),7.41-8.21(1H,m,9H),^{13}CNMR($CDCl_3$)δ:112.4,116.8,126.8,133.5,162.8,FABMS,198.08(100%),199.06(14.5%),200.12(1.%).Anal. Calcd for $C_{13}H_7N_{10}O_2$: C, 78.71; H, 5.05%,N,16.14; Found: C, 78.54; H, 4.97%,N,16.03

Step- (4' Acetylamino methyl biphenyl-2-caboxylic acid)

5 gm of MCS 03 was dissolved in 25 ml of concentrated H_2SO_4.After that acetamide (2.15 gm) and Paraform aldehyde (0.560) gm were added subsequently. The solution was heated at 70°C along with stirring for 4.5 hours. The hot mixture was poured over ice and cold water. The precipitated product was filtered under suction and dried in air. The product was recrystallized from Absolute ethanol. The resulting solid was filtered out.

Yield: 58% m.p.-165^o-169^{o}C IR (KBr) (cm^{-1}): 3397.4 (N-H str.), 3262.7(O-H, str), 2986 (C-H str), 2945 (aliphatic C-H str), 1675.2 (C=O str of), 1587.5 (N-H bend of amide), 1495.9(C-N str) 784.6(Benz. Ring); ^{1}H NMR (300 MHz, $CDCl_3$) δ:2.03(s,3H,CH_3), 9.76(1H,s,COOH),4.32(2H,s,CH_2),7.98(s,1H,-NH);7.09 8.24(m,8H,ArH).^{13}CNMR($CDCl_3$)δ:19.5(CH_3),53.7(CH_2)112.4,116.1,122.1,125.7,133.5,139.2,144.1,155.7,170.2,FAB-MS, 269.12(100%), 270.03(18.6),271.07 (2.2%).Anal. Calcd for $C_{16}H_{15}NO_3$: C, 71.36; H, 5.61; N, 5.20%; Found: C, 71.27; H, 5.54; N, 5.12

Step- (4'chloromethylbiphenyl-2-carboxylic acid)

1.4gm of MCS-04 was taken in a RBF. 1.598 gm of phosphorus oxy chloride was added to 4ml of DMF and further addition of xylene (4ml). The reaction mixture was refluxed for 7 ½ hours. The cold solution was washed with water and evaporated to give a light yellow crystalline product.

Yield: 52 % m.p.-133^o-136^{o}C IR (KBr) (cm-1): IR (KBr): 3354 (O-H str.), 2902(C-H str., CH_2), 1679.4 (Carboxylic, C=O str.), 1676-1413 (C=N, C=C str.), 1189 (C-O str), 854.2 (benz. ring), 598.7(C-Cl str.) ^{1}H NMR (300 MHz, $CDCl_3$) 10.07(s, 1H, OH), 7.118.05(m,8H,ArH),4.64(s,2H,CH_2)..^{13}CNMR($CDCl_3$)δ:33.8(CH_2)115.9,117.2,123.4,128.2,136.1,139.2,142.4,151.2,,FABMS,289.12(100%)291.14(97.11%),270.03(18.6),271.07 (2.2%).Anal.Calcd for $C_{14}H_{11}ClO_2$: C, 57.76; H, 3.81; %; Found: C, 57.71; H, 3.80; %.

Step- 4'-[2-(Phenyl-substituted amino-methyl)-benzoimidazol-1-ylmethyl] biphenyl-2-carboxylic acid

150mg of MCS-03 was dissolved in 20ml of DMF (dimethyl formamide) and stirred vigorously with 2.0gm of potassium carbonate at 32^{0}C for one half hour. To the resulting mixture 0.482gm of MCS-05 first dissolved in 20 ml of DMF and then was added drop wise with dropping funnel in 1 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 water, distilled water and dried over anhydrous sodium sulphate. The solvent was evaporated and solid (MCS-06) was obtained.

[1]4'-[2-(Phenyl-phenyl amino-methyl)-benzoimidazol-1-ylmethyl] biphenyl-2 carboxylic acid

Yield:64%,m.p.=202^0-205^{0}C.Molecular weight 509.21, Anal.Calcd for $C_{34}H_{27}N_3O_2$:C,80.82;H, 5.34;N,8.25 %; Found: C,68.75;H, 4.70;N,10.43 %; IR (KBr): 3487,

3404,3056, 1510,1654, ^{1}HNMR (300 MHz, $CDCl_3$) 9.98(s, 1H, COOH), 4.0 (s, 2H,NH_2),7.05-8.75(m,22H,-ArH),4.98(s,2H,methylene). ^{13}CNMR($CDCl_3$)δ:56.8,111.3,113,114,115.2,116.3,129.5,175.4,FAB-MS, 508.98

[2]4'-{2-[2-chloro-phenylamino)-phenyl-methyl]-benzoimidazol-1-ylmethyl} biphenyl-2-carboxylic acid

Yield:71%,m.p.=197^0-200^{0}C.Molecular weight 543.17, Anal.Calcd for $C_{34}H_{26}ClN_3O_2$:C,75.06;H, 6.54;N,7.72 %; IR (KBr): 3517, 3465,3021, 1518,1634,677.4 ^{1}HNMR (300 MHz, $CDCl_3$) 10.18(s, 1H, COOH), 4.03 (s, 1H,NH),6.55-8.42(m,22H,-ArH),4.99(s,2H,methylene). ^{13}CNMR ($CDCl_3$)δ:54.1,110.1,111,112,114.2, 118.3,123.5,167.5,FAB-MS, 543.12

[3]4'-{2-[3-chloro-phenylamino)-phenyl-methyl]-benzoimidazol-1-ylmethyl} biphenyl-2-carboxylic acid

Yield:65%,m.p.=197^0-200^{0}C.Molecular weight 543.17, Anal.Calcd for $C_{34}H_{26}ClN_3O_2$:C,75.06;H, 6.54;N,7.72 %; IR (KBr): 3519, 3461,3065, 1511,1643,678.1 ^{1}HNMR (300 MHz, $CDCl_3$) 10.11(s, 1H, COOH), 4.06 (s, 1H,NH),6.55-8.42(m,22H,-ArH),4.96(s,2H,methylene). ^{13}CNMR ($CDCl_3$)δ:54.1,110.1,111,112,114.2, 118.3,123.5,167.5,FAB-MS, 542.02

[4]4'-{2-[4-chloro-phenylamino)-phenyl-methyl]-benzoimidazol-1-ylmethyl} biphenyl-2-carboxylic acid

Yield:57%,m.p.=205^0-208^{0}C.Molecular weight 543.17, Anal.Calcd for $C_{34}H_{26}ClN_3O_2$:C,75.06;H, 6.54;N,7.72 %; IR (KBr): 3526, 3460,3088, 1532,1621,677.4 ^{1}HNMR (300 MHz, $CDCl_3$) 10.14(s, 1H, COOH), 4.00 (s, 1H,NH),6.59-8.41(m,22H,-ArH),4.89(s,2H,methylene). ^{13}CNMR ($CDCl_3$)δ:54.1,110.1,111,112,114.2, 118.3,123.5,167.5,FAB-MS, 544.1

[5]4'-{2-[2,4-Dichloro-phenylamino)-phenyl-methyl]-benzoimidazol-1-ylmethyl} biphenyl-2-carboxylic acid

Yield:52%,m.p.=215^0-219^{0}C.Molecular weight 577.13, Anal.Calcd for $C_{34}H_{25}Cl_2N_3O_2$:C,70.59;H, 4.36;N,7.26 %; IR (KBr): 3603, 3432,2993, 1527,1654,647. ^{1}HNMR (300 MHz, $CDCl_3$) 10.88(s, 1H, COOH), 4.14 (s, 1H,NH),6.28-8.15(m,21H,-ArH),4.97(s,2H,methylene).^{13}CNMR($CDCl_3$)δ:52.5,111.1,112,114,115.2,119.3,124.5,126.0,127.2,127.5,127.9,133.2,134.2,136,167.5,FAB-MS, 576.89

[6]4'-{2-[3,4-Dichloro-phenylamino)-phenyl-methyl]-benzoimidazol-1-ylmethyl} biphenyl-2-carboxylic acid

Yield:59%,m.p.=223^0-226^{0}C.Molecular weight 577.13, Anal.Calcd for $C_{34}H_{25}Cl_2N_3O_2$:C,70.59;H, 4.36;N,7.26 %; IR (KBr): 3611, 3439,2965, 1515,1634,652. ^{1}HNMR (300 MHz, $CDCl_3$) 10.81(s, 1H, COOH), 4.11 (s, 1H,NH),6.38-8.35(m,21H,-ArH),4.87(s,2H,methylene).^{13}CNMR($CDCl_3$)δ:52.5,111.1,112,114,115.2,119.3,124.5,126.0,127.2,127.5,127.9,133.2,134.2,136,167.5,FAB-MS, 577.32

[7]4'-{2-[2,5-Dichloro-phenylamino)-phenyl-methyl]-benzoimidazol-1-ylmethyl} biphenyl-2-carboxylic acid

Yield:62%,m.p.=221^0-224^{0}C.Molecular weight 577.13, Anal.Calcd for $C_{34}H_{25}Cl_2N_3O_2$:C,70.59;H, 4.36;N,7.26 %; IR (KBr): 3578, 3466,2937, 1586,1645,639. ^{1}HNMR (300 MHz, $CDCl_3$) 10.80(s, 1H, COOH), 4.06 (s, 1H,NH),6.21-8.10(m,21H,-ArH),4.97(s,2H,methylene).^{13}CNMR($CDCl_3$)δ:52.5,111.1,112,114,115.2,119.3,124.5,126.0,127.2,127.5,127.9,133.2,134.2,136,167.5,FAB-MS, 578.03

[8]4'-{2-[2,6-Dichloro-phenylamino)-phenyl-methyl]-benzoimidazol-1-ylmethyl} biphenyl-2-carboxylic acid

Yield:52%,m.p.=223^0-226^{0}C.Molecularweight577.13,Anal.Calcd-$C_{34}H_{25}Cl_2N_3O_2$:C,70.59;H, 4.36;N,7.26 %; IR (KBr): 3578, 3466,2937, 1586,1645,639. ^{1}HNMR (300 MHz, $CDCl_3$) 10.84(s, 1H, COOH), 4.00 (s, 1H,NH),6.25-8.18(m,21H,-ArH),4.99(s,2H,methylene).^{13}CNMR($CDCl_3$)δ:52.5,111.1,112,114,115.2,119.3,124.5,126.0,127.2,127.5,127.9,133.2,134.2,136,167.5,FAB-MS, 577.32

[9]4'-{2-[2-nitro-phenylamino)-phenyl-methyl]-benzoimidazol-1-ylmethyl} biphenyl-2-carboxylic acid

Yield:76%,m.p.=278^0-282^{0}C.Mol.wt-554.59, Anal.Calcd for $C_{34}H_{26}N_4O_4$:C,70.59;H, 4.36;N,7.26 %; IR (KBr): 3544, 3475,2912, 1543,1624.4.^{1}HNMR (300 MHz, $CDCl_3$) 10.73(s,1H,COOH),4.01(s,1H,NH),6.28-8.18(m,22H,-ArH),5.0 (s,2H,methylene).^{13}CNMR($CDCl_3$)δ:52.5,111.1,112,114.2,115.1,116.6,120.1,122.5,122.9,133.2,134.2,136,167.5,FAB-MS, 553.94.

[10] 4'-{2-[3-nitro-phenylamino)-phenyl-methyl]-benzoimidazol-1-ylmethyl} biphenyl-2-carboxylic acid

Yield:66%,m.p.=274^0-276^{0}C.Mol.wt-554.59, Anal.Calcd for $C_{34}H_{26}N_4O_4$:C,70.59;H, 4.36;N,7.26 %; IR (KBr): 3547, 3471,2944, 1522,1643.^{1}HNMR (300

MHz,$CDCl_3$) 10.64(s,1H,COOH),4.09(s,1H,NH),6.34-8.29(m,22H,-ArH),5.0 (s,2H,methylene).^{13}CNMR($CDCl_3$)δ:52.5,111.1,112,114.2,115.1,116.6,120.1,122.5,122.9,133.2,134.2,136,167.5,FAB-MS, 553.94.

[11]4'-{2-[4-nitro-phenylamino)-phenyl-methyl]-benzoimidazol-1-ylmethyl} biphenyl-2-carboxylic acid

Yield:62%,m.p.=278^0-282^{0}C.Mol.wt-554.59, Anal.Calcd for $C_{34}H_{26}N_4O_4$:C,70.59;H, 4.36;N,7.26 %; IR (KBr): 3544, 3475,2912, 1543,1624.4.^{1}HNMR (300 MHz, $CDCl_3$) 10.73(s,1H,COOH),4.01(s,1H,NH),6.19-8.37(m,22H,-ArH),5.0 (s,2H,methylene).^{13}CNMR($CDCl_3$)δ:52.5,111.1,112,114.2,115.1,116.6,120.1,122.5,122.9,133.2,134.2,136,167.5,FAB-MS, 553.94.

[12] 4'-{2-[2,5-nitro-phenylamino)-phenyl-methyl]-benzoimidazol-1-ylmethyl} biphenyl-2-carboxylic acid

Yield:71%,m.p.=254^0-257^{0}C.Mol.wt-599.59,Anal.Calcd for $C_{34}H_{25}N_5O_6$:C:68.11;H, 4.20;N,11.68 %; IR (KBr): 3573-3111, 3448,2976, 1576,1643.0.^{1}HNMR (300 MHz, $CDCl_3$)10.58(s,1H,COOH),4.16(s,1H,NH),6.43-8.73(m,21H,-ArH),5.06 (s,2H,methylene).^{13}CNMR($CDCl_3$)δ:55.4,112.1,113.2, 114.1,115,120.5,121.1,127.4,133.2,144.5,FAB-MS, 599.11.

[13] 4'-{2-[2,4-nitro-phenylamino)-phenyl-methyl]-benzoimidazol-1-ylmethyl} biphenyl-2-carboxylic acid

Yield:81%,m.p.=259^0-263^{0}C.Mol.wt-599.59 Anal.Calcd for $C_{34}H_{25}N_5O_6$:C:68.11;H, 4.20;N,11.68 %; IR (KBr): 3597-3121, 3444,2912, 1532,1630.1.^{1}HNMR (300 MHz, $CDCl_3$)10.64(s,1H,COOH),4.11(s,1H,NH),6.35-8.25(m,21H,-ArH),5.09 (s,2H,methylene).^{13}CNMR($CDCl_3$)δ:55.4,112.1,113.2, 114.1,115,120.5,121.1,127.4,133.2,144.5,FAB-MS, 599.11.

[14]4'-{2-[2,Hydroxy-5-nitro-phenylamino)-phenyl-methyl]-benzoimidazol-1-ylmethyl} biphenyl-2-carboxylic acid

Yield:78%,m.p.=294^0-296^{0}C.Mol.wt-570.594 Anal.Calcd for $C_{34}H_{26}N_4O_5$:C:71.57;H, 4.59;N,9.82 %; IR (KBr): 3613-3164, 3476,2954, 1574,1621.5.^{1}HNMR (300 MHz, $CDCl_3$)11.05(s,1H,COOH),4.04(s,1H,NH),6.35-8.25(m,21H,-ArH),5.20(s,1H,armC-OH),4.93 (s,2H,methylene).^{13}CNMR($CDCl_3$)δ:55.4,112.1,113.4, 114.1,116.3,119.2,128.2,134.2,139.7,142.0,FAB-MS, 570.19

[15]4'-{2-[2-Hydroxy-4-nitro-phenylamino)-phenyl-methyl]-benzoimidazol-1-ylmethyl} biphenyl-2-carboxylic acid

Yield:70%,m.p.=287^0-289^{0}C.Mol.wt-570.594 Anal.Calcd for $C_{34}H_{26}N_4O_5$:C:71.57;H, 4.59;N,9.82 %; IR (KBr): 3613-3150, 3443,2975, 1523,1645.5.^{1}HNMR (300 MHz, $CDCl_3$)11.01(s,1H,COOH),4.08(s,1H,NH),6.32-8.29(m,21H,-ArH),5.24(s,1H,armC-OH),4.97 (s,2H,methylene).^{13}CNMR($CDCl_3$)δ:55.4,112.1,113.4, 114.1,116.3,119.2,128.2,134.2,139.7,142.0,FAB-MS, 569.12

[16]4'-{2-[2-Hydroxy-3-nitro-phenylamino)-phenyl-methyl]-benzoimidazol-1-ylmethyl} biphenyl-2-carboxylic acid

Yield:78%,m.p.=292^0-293^{0}C.Mol.wt-570.594 Anal.Calcd for $C_{34}H_{26}N_4O_5$:C:71.57;H, 4.59;N,9.82 %; IR (KBr): 3613-3164, 3476,2954, 1574,1621.5.^{1}HNMR (300 MHz, $CDCl_3$)10.95(s,1H,COOH),4.0(s,1H,NH),6.30-8.33(m,21H,-ArH),5.12(s,1H,armC-OH),4.99 (s,2H,methylene).^{13}CNMR($CDCl_3$)δ:55.4,112.1,113.4, 114.1,116.3,119.2,128.2,134.2,139.7,142.0,FAB-MS, 571.05

[17] 4'-{2-[2-Chloro-5-nitro-phenylamino)-phenyl-methyl]-benzoimidazol-1-ylmethyl} biphenyl-2-carboxylic acid

Yield:62%,m.p.=297^0-300^{0}C.Mol.wt-589.03 Anal.Calcd for $C_{34}H_{25}ClN_4O_4$:C:69.33;H, 4.28;N,9.51 %; IR (KBr): 3561-3280, 3448,2966.5,1565.3,1623.2.^{1}HNMR (300 MHz, $CDCl_3$)10.76(s,1H,COOH),4.10(s,1H,NH),6.32-8.45(m,21H,-ArH),5.00 (s,2H,methylene).^{13}CNMR($CDCl_3$)δ:54.4,112.1,113.5, 115.1,119.1,126,133.1,139.3,144.3,FAB-MS, 589.03

[18]4'-{2-[(3-Carboxy-phenylamino)-phenyl-methyl]-benzoimidazol-1-ylmethyl} biphenyl-2-carboxylic acid

Yield:54%,m.p.=322^0-325^{0}C.Mol.wt-553.606 Anal.Calcd for $C_{35}H_{27}N_3O_4$:C:75.93;H, 4.92;N,7.59 %; IR (KBr): 3543-3112, 3474,2948,1577.8,1654.1.^{1}HNMR (300 MHz, $CDCl_3$)11.06(s,1H,COOH), 10.99(s,1H,COOH),4.10(s,1H,NH),7.09-8.79(m,22H,-ArH),5.10 (s,2H,methylene).^{13}CNMR($CDCl_3$)δ:54.4,110.8,112.3, 114.2,123.2,124.2,129.2,139.3,148.1,FAB-MS, 553.022

[19]4'-{2-[(4-Carboxy-phenylamino)-phenyl-methyl]-benzoimidazol-1-ylmethyl} biphenyl-2-carboxylic acid

Yield:54%,m.p.=322^0-325^{0}C.Mol.wt-553.606 Anal.Calcd for $C_{35}H_{27}N_3O_4$:C:75.93;H, 4.92;N,7.59 %; IR (KBr): 3540-3118, 3443,2966,1548.8,1634.5.^{1}HNMR (300 MHz, $CDCl_3$)11.00(s,1H,COOH), 11.13(s,1H,COOH),4.14(s,1H,NH),7.14-8.70(m,22H,-ArH),5.00 (s,2H,methylene).^{13}CNMR($CDCl_3$)δ:54.4,110.8,112.3, 114.2,123.2,124.2,129.2,139.3,148.1,FAB-MS, 554.15

Pharmacological Activity

Procedure for development of hypertension for normotensive rats.[27]

Albino normotensive rats (Wistar Strain) were taken and they were hypertensized by cholinomimetic agents for screening of all the synthesized benzimidazole derivatives for there anti-hypertensive activity. Suspension of test compounds was prepared in 1% w/v sodium carboxy methyl cellulose (sodium CMC) and was administered at dose level of 50 and 100 microgram/kg animal body weight to different groups of five rats each. After administration of dose to animal blood pressure was measured by normotensive tail and cuff method using pressure meter. Measurements were done after one hour and three hours interval in step-wise manner as follows:

Screening Methods for Anti-hypertensive Activity:

(a) Angiotensin II induced Hypertension: (i) Invasive method (Direct method). (ii) Non-invasive Tail cuff method (Indirect method).

(b) In-vitro determination of vasodilator activity by aortic rings.

Experimental Techniques:

(i) Invasive Method (Direct Method):[28-32]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 cannulas 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.). Observations are given in the table 1, 2.

Table: 1 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	**160**	**152**	**148**	**140**	**132**	**127**	**122**	**116**	**113**	**104**
1	175	170	166	159	150	144	137	131	127	122
2	180	176	170	166	161	154	148	140	135	131
3	177	171	167	159	151	145	138	131	128	124
4	170	166	160	154	148	145	141	137	135	133
5	168	164	160	157	154	150	146	139	134	130
6	165	162	156	150	145	139	135	131	129	126
7	178	173	166	161	155	148	140	136	133	130
8	165	160	156	151	147	143	138	134	130	125
9	168	164	159	152	147	141	137	132	129	123
10	172	167	163	160	152	148	142	138	132	127
11	181	177	170	162	153	147	138	133	129	125
12	175	170	164	156	150	143	138	130	127	125
13	163	158	154	149	145	140	136	131	129	126
14	165	158	151	145	140	136	132	129	126	122
15	170	164	158	152	147	140	133	127	124	121
16	167	163	158	151	146	141	137	132	129	125
17	165	160	156	151	146	140	134	130	126	123
18	176	170	164	158	151	144	138	133	129	127
19	179	176	172	167	163	159	153	148	142	138

Table: 2 Antihypertensive Activity of synthesized compounds

Compound. No	Minimum Blood pressure value(mm Hg)	Duration of hypertension effect(min.)
Losratan	**104**	**90**
1	118	105
2	121	110
3	118	100
4	122	102
5	120	103
6	117	100
7	122	101
8	116	100
9	114	105
10	116	112
11	114	99
12	113	105
13	117	102
14	115	95
15	110	95
16	121	102
17	117	100
18	120	100
19	123	120

(ii) Non-invasive Tail cuff Method (Indirect Method):[30-32] Albino rats weighing 200-250 gm were used to screening for all the synthesizes benzimidazole 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.Measurment 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. Observations are given in the table3, 4.

Table 3. Hypertension induced in normotensive rat

Comp.	Exp. Animal Albino (Wistar) Rat	After 1hour			After 3 hour		
		SBP	DBP	MABP	SBP	DBP	MABP
[1]	1	139	112	122	142	108	125
	2	135	109	124	138	102	120
	3	140	106	121	137	102	120
	4	144	106	125	142	104	123
	5	146	108	124	140	103	120
[2]	1	139	102	122	143	100	121

	2	148	104	124	143	102	122
	3	146	112	128	137	101	118
	4	143	108	126	140	103	121
	5	147	104	124	141	104	120
[3]	1	142	102	124	143	101	122
	2	145	105	125	145	100	121
	3	136	113	124	142	101	121
	4	139	113	122	140	100	120
	5	146	116	127	143	101	122
[4]	1	143	105	124	139	104	121
	2	141	101	126	143	104	120
	3	141	110	126	143	104	119
	4	142	102	125	141	102	121
	5	139	111	124	138	102	120
[5]	1	139	109	123	142	102	123
	2	140	101	125	140	101	124
	3	138	107	128	143	101	121
	4	140	108	125	141	104	120
	5	144	111	126	143	100	119
[6]	1	144	106	125	144	100	122
	2	145	112	126	139	100	120
	3	142	109	126	143	97	120
	4	140	102	123	140	100	120
	5	137	101	124	146	100	123
[7]	1	103	123	140	103	122	142
	2	110	122	140	102	121	134
	3	108	123	136	103	119	137
	4	110	125	138	105	122	140
	5	146	114	12	143	101	122
[8]	1	144	108	126	142	104	123
	2	148	106	127	142	106	124
	3	151	109	130	146	104	125
	4	146	104	125	142	104	123
	5	144	106	125	140	102	121
[9]	1	140	106	123	138	102	120
	2	144	112	127	142	104	123
	3	142	114	127	140	101	122
	4	148	104	126	144	104	124
	5	148	104	126	142	100	121
[10]	1	146	108	127	142	106	124
	2	143	106	125	139	104	121
	3	146	110	128	140	104	122
	4	149	111	130	143	106	124
	5	152	112	133	145	103	124
[11]	1	139	102	122	143	100	121
	2	148	104	124	143	102	122
	3	146	112	128	137	101	118
	4	143	108	126	140	103	121
	5	145	106	123	136	97	116
[12]	1	142	113	125	143	100	121

	2	136	105	123	142	104	119
	3	135	102	122	140	97	119
	4	146	103	125	139	105	120
	5	144	109	131	140	100	120
[13]	1	140	106	123	138	102	120
	2	144	112	127	142	104	123
	3	142	114	127	140	101	122
	4	148	104	126	144	104	124
	5	154	108	132	144	102	123
[14]	1	144	112	127	141	102	121
	2	142	114	128	144	101	122
	3	146	110	126	142	100	120
	4	140	108	124	138	102	120
	5	144	106	125	142	101	123
[15]	1	143	110	127	134	102	118
	2	137	102	124	135	102	118
	3	139	107	123	140	101	120
	4	143	109	126	137	104	120
	5	141	109	125	139	102	120
[16]	1	138	106	122	141	103	122
	2	132	110	121	143	105	124
	3	141	111	126	139	104	121
	4	144	105	124	139	103	122
	5	140	113	127	142	107	124
[17]	1	141	104	123	137	106	121
	2	135	101	118	136	107	121
	3	140	110	125	138	112	125
	4	141	103	122	135	109	122
	5	134	106	120	143	114	129
[18]	1	140	102	121	140	103	121
	2	138	104	122	137	104	120
	3	142	112	127	139	102	121
	4	140	108	124	143	101	122
	5	137	104	121	140	103	121
[19]	1	135	102	122	140	97	122
	2	146	103	125	139	105	126
	3	144	109	131	140	100	124
	4	146	104	125	142	104	125
	5	144	106	125	140	102	127
Contro	**Losartan**	119	-	-	-	-	-
	Telmisartan	117	-	-	-	-	-

Table 4. Reduction in blood pressure (mm Hg) at a dose of 50 µgm/kg animal body weight.

Comp.	Exp. Animal Albino (Wistar) Rat	After 1hour			After 3 hour		
		SBP	DBP	MABP	SBP	DBP	MABP
[1]	1	118	104	111	115	103	114
	2	125	105	115	124	102	113
	3	128	102	115	127	104	115

	4	126	101	112	124	100	111
	5	125	103	114	122	103	116
[2]	1	125	105	115	127	101	114
	2	120	102	111	123	101	112
	3	125	103	114	126	100	113
	4	122	100	111	128	103	114
	5	125	101	113	121	101	110
[3]	1	124	106	115	127	102	114
	2	126	104	115	125	105	115
	3	124	104	114	121	100	110
	4	125	102	112	128	100	114
	5	120	100	120	130	95	112
[4]	1	122	106	114	122	103	112
	2	128	107	117	127	101	114
	3	126	103	114	125	104	113
	4	132	105	119	121	102	110
	5	131	106	118	119	103	107
[5]	1	122	104	112	125	101	113
	2	123	102	113	128	103	112
	3	121	101	113	123	102	111
	4	126	102	111	124	101	112
	5	126	103	115	122	103	112
[6]	1	144	114	129	142	102	121
	2	139	114	127	135	103	119
	3	142	106	124	140	102	123
	4	144	108	126	142	100	121
	5	148	104	126	145	104	124
[7]	1	144	106	125	144	100	122
	2	145	112	126	139	100	120
	3	142	109	126	143	97	120
	4	140	102	123	140	100	120
	5	137	101	124	146	100	123
[8]	1	123	101	112	125	100	112
	2	122	100	111	126	102	115
	3	124	102	112	126	102	111
	4	126	101	113	124	104	114
	5	128	102	115	126	104	115
[9]	1	124	101	112	124	100	112
	2	122	100	111	121	103	112
	3	124	102	113	124	106	115
	4	122	103	112	122	105	114
	5	124	102	111	125	102	114
[10]	1	124	102	113	126	100	113
	2	122	101	112	126	103	112
	3	126	104	115	124	102	113
	4	128	102	115	126	104	115
	5	131	103	117	124	102	113
[11]	1	126	103	114	122	109	115
	2	124	107	115	127	106	117
	3	127	104	116	124	95	109

	4	129	108	118	130	102	116
	5	126	101	117	123	97	110
[12]	1	128	103	115	120	103	112
	2	124	96	110	124	106	115
	3	127	101	114	123	102	112
	4	121	103	112	121	97	109
	5	120	100	115	128	100	114
[13]	1	127	105	118	126	102	114
	2	124	106	122	122	101	111
	3	123	102	119	127	101	110
	4	122	104	118	124	98	113
	5	127	108	119	126	103	109
[14]	1	128	106	117	123	100	112
	2	127	101	116	125	105	110
	3	124	102	119	128	102	111
	4	129	104	117	124	101	112
	5	133	103	118	126	100	110
[15]	1	124	102	113	126	104	115
	2	121	101	111	129	103	116
	3	124	105	115	123	106	115
	4	135	102	119	124	101	112
	5	136	101	118	122	104	113
[16]	1	127	104	116	124	105	114
	2	125	108	117	122	108	115
	3	124	105	115	125	106	116
	4	122	109	116	126	106	116
	5	125	104	114	122	106	114
[17]	1	120	102	111	125	102	113
	2	122	106	114	122	100	111
	3	123	101	113	126	102	113
	4	121	107	114	127	103	115
	5	122	102	112	123	107	115
[18]	1	124	111	115	122	102	112
	2	127	109	123	124	102	113
	3	125	110	122	127	101	114
	4	124	112	118	121	102	112
	5	126	105	116	127	101	114
[19]	1	124	108	119	129	111	126
	2	128	105	117	125	109	122
	3	126	108	119	121	106	121
	4	129	111	119	126	108	125
	5	128	106	117	125	108	119
Control	**Losartan**	102		-	-	-	-
	Telmisartan	107	-	-	-	-	-

Results and discussion

The synthesized compounds were characterized on the basis of chemical and spectral data. Synthetic scheme for target compounds was divided into two steps. Step I involved synthesis of aniline substituents benzimidazoles by condensation reaction of o-phenylenediamine with the respective hydroxy phenyl acetic acid then corresponding react with hydrogen

bromide in presence of anhydrous zinc chloride with phenyl amino derivatives were prepared by under controlled temperature conditions.[19-21] Step II include the novel sequential combination of three routine reactions to synthesize 2'- carboxybiphenyl methylene chloride. Biphenyl –2-carboxylic acid was prepared by potash fusion of 9H flourenone[22] which was then subjected to aromatic substitution reaction using paraformaldehyde and acetamide in conc. sulphuric acid[20] to affect intermediate,4-acetamidomethyl biphenyl-2'-carboxylic acid. The required component was identified as third fraction which was subjected to substitution reaction with phosphorus oxychloride in xylene and dimethyl formamide[23-26] to produce the pendant moiety 4-(chloromethyl) biphenyl-2'-carboxylic acid. 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 position has been found to be a function of substitute at 5-position. Presence of amino group has increased the activity substantially over the substituted one ([1] to [19]). The maximum activity has been observed with amino group, this suggests that there are some sites in the receptor pocket, which can interact with the functional groups at position 2-Substituted benzimidazole nucleus coupled to carboxylic 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 160mm Hg to 104 mm Hg over a period 90 minutes .

Acknowledgement

The authors are thankful to Prof.Pratibha Sharma School of chemical sciences DAVV Indore to given valuable suggestion and Central Drugs Research Institute (CDRI), Lucknow (India) for providing spectro-analytical facilities, respectively.

References:

[1] Ferrario, C. M. The Renin-Angiotensin System: Importance in Physiology and Pathology. *J. Cardiovasc. Pharmacol.* 1990, *15* (Suppl. 3), 51-55.

[2] Vallotton, M. B. The Renin-Angiotensin System. *Trends Pharmacol. Sci.* 1987, *8*, 69.

[3] Nahmias, C.; Strosberg, A. D. The angiotensin AT2 Receptor: Searching for Signal-Transduction Pathways and Physiological Function. *Trends Pharmacol. Sci.* 1995, *16*, 223-225.

[4] Berecek, K. H.; King, S. J.; Wu, J. N. *Angiotensin-Converting Enzyme and Converting Enzyme Inhibitors. Cellular and Molecular Biology of the Renin-Angiotensin* System; CRC Press: Boca Raton, FL, 1993; pp 183-220.

[5] Kleinert, H. D. Recent Developments in Renin Inhibitors. *Exp. Opin. Invest. Drugs* 1994, *3*, 1087-1104.

[6] A.S.Dutta, Design and Therapeutic Potential of Peptides. In Advances in Drug Research; Testa, B., Ed.Academic Press: London, 21, 147-286(1991).

[7] (a) J.R.McEwan, R.W. Fuller, Angiotensin Converting Enzyme Inhibitors and Cough. *J. Cardiovasc. Pharmacol. 13* (Suppl. 3), S67-S69 (1989).

(b) R.A.Skidgel, S.Engelbrecht, A.R. Johnson, E.G. Erdos. Hydrolysis of Substance P and Neurotensin by Converting Enzyme and Neutral Endopeptidase. *Peptides*. *5*: 769-776(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,* 2525-2547(1991).

[10] R.R.Wexler,W.J. Greenlee,J.D. Irvin,M.R. Goldberg,K. Prendergast,R.D.Smith, P. B. M. W. M. Timmermans. *J. Med. Chem. 39*, 625-656(1996).

[11] J.A.Keiser, F.A. Bjork . *Pharmaco. Exp Ther.* 262, 1154-1160 (1992).

[12] P.Juniak, A. Pillon. *Hypertension*. 20(6):737-745(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, 2182-2195(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 , 4040-4051(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 ,591-609(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. 15,3962-3965(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, 278-285, (2006).

[19] Preston, Heterocyclic Compounds: Benzimidazoles and Congeneric Tricyclic compounds, Part-I, Vol-40, p-7.
[20] Solomon Marmor, Laboratory methods in Organic Chemistry, 1986, Surjeet publications, p-284-285.
[21] F.Gualtieri, G.Brody, A.H.Filedsteel and W.A.Skinar, *J.Med.Chem*., 1971, 14, 546.
[22] Sarhan , Hozien, Mahmoud, *J. Chinese chem. soc.*, Vol. 47, PP 1279 – 1286(2000).
[23] M.R.Grimmett. In Comprehensive Heterocyclic Chemistry. The Structure, Reactions, Synthesis and Uses of Heterocyclic Compounds. Potts, K.T., Ed.; Pergamon: Oxford, Vol. 5; 429.
[24]B.S.Furniss,A.J.Hannaford,W.G.Peter,T.Smith, A.R.Tetchell, Vogel's Text book of Practical Organic Chemistry, 5 ed.,Longman Scientific and Technical, England,1989; 1163.
[25] G.W.Kenner, M.J.T.Robinson, C.M.B.Tylor, B. R. Webster. Reactions between strongly basic nucleophiles and fluorenones J. Chem. Soc. 1756(1962).
[26] D.R.Manlding, K.D.Lotts, S.A. Robinson. J. Org. Chem. 48, 2938(1983).
[27]D.K. Badyal, H. Lata, A.P.Dadhich.Indian. J. of pharmacology 35,66, 349-362, (2003).
[28] R.D.Bunag.J.W.McCubbin, I.H.Page. Lack of correlation between direct and indirect measurements of arterial pressure in unanaesthetized rats. Cardiovascular Res. 5(1): 24-31 (1971).
[29] A.A.Siddiqui, M.S.Wani.Indian.J.Chemistry.43B, 1574, 1579(2004).
[30] S.K.Gupta.Drug Screening methods, Jaypee Brothers Medical Publisher, New Delhi, pp 236-246, (2004).
[31] Vogel G.H.Drug Discovery and Evaluation, Pharmacological Assay, 2002(Springer. Berlin), 122.
[32] Shanmugapandiyan P, Denshing KS, Ilavarasan N R, Anbalagan RN. International Journal of Pharma Science and Drug Research, 2(2) 115-119, (2010)

*****