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Der Pharmacia Sinica, 2010, 1 (1): 58-73

Synthesis and antihypertensive activity of 4'-{2-[4-[2-(Substituted-phenyl)-4-oxo-thiazolidin-3-yl]-phenyl} benzoimidazol-1-yl-methyl}-biphenyl-2-carboxylic acids

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ABSTRACT

Structures of all the synthesized compounds have been corroborated on the basis of elemental IR, ^{1}H NMR, ^{13}C NMR and Mass spectro-analytical data. Many Schiff bases were prepared by condensation reaction of compounds containing biphenyl carboxylic acid with aromatic aryl aldehydes derivatives with Thiazoldine-4-one. The synthesized compounds 4'-{2-[4-[2-(Substituted-phenyl)-4-oxo-thiazolidin-3-yl]-phenyl} benzoimidazol-1-ylmethyl}-biphenyl-2-carboxylic acid react with mercaptoacetic acid and 4'-bromomethylbiphenyl-2-carboxylic acid and all synthesis compounds screened for Angiotension (A II) Receptor Antagonist antihypertensive activity with biphenyl carboxylic acid Schiff bases Thiazoldine-4-one shows good activity compared with losartan and Telmisartan.

Keywords: Biphenyl carboxylic acid, Thiazoldine-4-one, Angitotensin II, antihypertensive drug, Losartan, Telmisartan.

INTRODUCTION

The renin angiotensin system (RAS) is one of the most powerful regulators of blood pressure and volume homeostasis in mammals. Its effector peptide angiotensin II (ANG II) is cleaved from the decapeptide angiotensin I by the metalloprotease ACE.[1-2] ANG II mediates all the effects of RAS after binding to its G-protein-coupled angiotensin II type 1 (AT_1) receptor and thus plays a complex role in the regulation of blood pressure fluid and electrolyte homeostasis. More recently ANG II was shown to regulate vascular tone by delayed effects on vascular smooth muscle via growth stimulation aldosterone production and release leading to increased salt absorption in the kidney and gut and the induction of thirst and sodium appetite in the brain. It

also stimulates the release of vasopressin luteinizing hormone oxytocin and corticotropin. ANG II further induces vagus suppression and α-adrenergic potentiation and increases inotropy and chronotropy. Stimulation of the cardiac fibroblast matrix formation has also been described [3-5]. ANG II stimulates synthesis of prostaglandin [6] endothelin [7] and elicits procoagulatory effects by activating the plasminogen activator (PA) plasmin system [8-11]. The beneficial effect of a chronic RAS blockade was first shown for inhibitors of the angiotensin converting enzyme (ACE) such as captopril quinapril enalapril and ramipril in patients with ischemic heart disease congestive heart failure the development of potent drugs that interfered with the RAS: the angiotensin receptor type 1 (AT_1) antagonists[12]. To find a more specific blockade of ANG II at its AT_1 receptor highly selective non-peptidic AT_1-receptor antagonists were designed and developed as competitive antagonists with virtually no agonistic effect at the receptor level. Losartan was described as the first non-peptide AT_1 receptor antagonist and the coined group name was sartans. All major pharmaceutical companies embarked on a fast follower program immediately thereafter [13-16]. Today irbesartan candesartan and valsartan are all established in the market and others e.g. tasosartan and telmisartan are following closely. Some further 20 compounds are in development. Most of these compounds share the biphenyl tetrazole unit or replacements thereof with the original advanced lead Losartan [17]. Some 12 000 variations of the parent biphenyl tetrazole alone were reported in the meantime excluding the obvious variation of the biphenyl spacer. The carboxylic acid another common moiety of the sartans appears to establish another important interaction with the receptor but it often hampers oral absorption. Therefore several prodrug concepts had to be realized to mask the carboxylic acid as either a labile ester or an oxidatively labile precursor that delivers the acid after absorption. Recent findings [18-19] indicate the involvement of this peptide also in situations concerning tissue remodelling, such as cardiac hypertrophy and cancer. All these responses are mediated by two distinct subtypes of Ang II receptors [type 1 (AT_1) and type 2 (AT_2)]. In particular, AT_1 receptors mediate all of the known effects associated to Ang II that constitutes the principal target of an effectiveness therapy against the cardiovascular pathology. The Ang II effects may be reduced by inhibiting almost partially the enzyme responsible of biosynthesis of Ang II or through the interaction with AT_1 receptor. To date, many orally available sartans have been developed and are used in the treatment of both hypertension and damage associated with diseases like atherosclerosis and diabetes. In particular, the good properties of new non peptide Ang II antagonists, such as losartan, have stimulated the design of many different congeners. All these drugs contain some common structural features represented by a biphenyl fragment bearing an acidic moiety (i.e.: tetrazole, carboxylic- or sulphonamidocarboxyl- group), linked to a heteroaromatic or acyclic system by means of a methylene group. Almost all of the chemical manipulations within the fundamental skeleton of sartans concerned the substitution of the imidazole ring of losartan with several variously substituted heteroaromatic groups or acyclic structures[20].All these antagonists possess a central aromatic nucleus bearing the pharmacophores indispensable for activity and notably a polar function adjustant to biphenyl subsistent while a polar function in this area of molecule seems to be necessary to maintain activity[21]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 [22]. The subsistent at 6-position on the nucleus increases the activity whereas small substituent at 5-position decreases the activity [23] compounds containing tetrazole nucleus are also reported as AT_1 receptor antagonists and their protypical derivative exhibits non-competitive

antagonism[24] amino group attach with carboxylic group given good biological activity [25-27].In recent years, attention has increasingly been given to the synthesis of benzimidazole derivatives as a source of new antihypertensive agents. 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 [28-29]. Moreover, these fused heterocyclic were distinctively studied for their antihypertensive activity, 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 [30-31]. In addition, benzimidazole derivatives have played a crucial role in the theoretical development of heterocyclic chemistry and are also used extensively in organic synthesis.

MATERIALS AND METHODS

Moreover, most of the methods have not been found to be quite accessible from the viewpoints of both yield and economics of the reaction. Thus, in order to cater the needs associated with synthetic aspects, herein, we would like to present unique approach to synthesize benzimidazole derivatives. 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.

Synthesis of 1H-benzimidazol-2-amine

A solution of 1,2-phenylenediamine dihydrochloride (0.45 g, 2.5 mmol) in 5 ml of water was cooled to 0°C and treated with a solution of cyanogen bromide (0.60 ml, 5 M in acetonitrile, 3.0 mmol) and solid $NaHCO_3$ (0.41 mg, 4.9 mmol). The solution was stirred at ambient temperature for 40-45 h. The mixture was made basic with 1 M aqueous Na_2CO_3 and the solution was concentrated under reduced pressure. The residue was triturated with hot ethanol, and the ethanolic solution was filtered and concentrated under reduced pressure to obtain the compound **1** in appreciable yield.

Yield 85%; mp 135-136 ^{0}C; Anal Calcd for $C_7H_7N_3$ (R=H): C, 63.14; H, 5.30; N, 31.56%; Found: C, 63.10; H, 5.28; N, 31.53%; IR (υ cm^{-1}): 3045 (C-H, sp^2), 3210 (NH, bonded), 3175 (NH, free), 1654 (C=N), 1626, 1586, 1444 (C⋯C, ring str) 958, 859, 742 (sub. phenyl); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 4.0 (s, 2H, NH_2), 5.0 (s, NH), 7.6-7.9 (m, 4H, Ar-H); ^{13}C NMR ($CDCl_3$) δ: 117.41, 124.34, 136.66, 158.62; FAB-MS: 134 $(M+H)^+$.

Synthesis of 4-(1H-Benzimidazole-2-yl)-phenyl]-benzylidene-amine)

A mixture of 2-(4-aminophenyl) Benzimidazole (0.01 mol), substituted Benzaldehyde (0.01 mol) and a drop of acetic acid was dissolved in ethanol (25 ml) and heated on a steam bath for 45-60 min. The reaction mixture was allowed to stand at room temperature for 24 h; the product separated out was filtered, dried under vacuum and recrystallized by using warm Absolute ethanol.

Synthesis of 3-[4-(1H-Benzimidazole-2-yl)-phenyl]-substituted -phenyl-thiazolidin-4-one
To a mixture of Schiff base (0.01 mol) and mercaptoacetic acid (0.05 mol) dissolved in dioxane (50 ml), anhydrous zinc chloride (0.008 mol) was added and refluxed for 12 hrs. The reaction mixture was cooled, filtered, washed with 10 % w/v sodium bicarbonate solution, vacuum dried and recrystallised using absolute ethanol, Compounds [1-18].

Synthesis of (Biphenyl Carboxylic acid) [37-38]
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 Conc.HCl. The precipitated product was filtered under suction and dried in air. The product was recrystallized from Absolute ethanol. Product 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): 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.

Synthesis of (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); 7.0-8.24(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.

Synthesis of 4'-chloromethylbiphenyl-2-carboxylic acid [37-38]
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 $C_{14}H_{11}BrO_2$: C, 57.76; H, 3.81; Found: C, 57.71; H, 3.80.

Synthesis of 4'-{2-[4-[2-(Substituted-phenyl)-4-oxo-thiazolidin-3-yl]-phenyl} benzoimidazol-1-ylmethyl}-biphenyl-2-carboxylic acid

100 mg of Schiff base (different substituted aryl aldehydes MCS-02) was dissolved in 50 ml of DMF (dimethyl formamide) and stirred vigorously with 12.5 gm of potassium carbonate at 39^{0}C for 4 hours. To the resulting mixture 0.482gm of MCS-03 first dissolved in 15 ml of DMF and then was added drop wise with dropping funnel in 4 hours the reaction was allowed to proceed for further 12 hours at room temperature and solvent removed under vacuum. Residue was treated with 20ml of Conc.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-04) was obtained.

[1] **4'-{2-[4-[2-(2-Methoxy-phenyl)-4-oxo-thiazolidin-3-yl]-phenyl} benzoimidazol-1-yl-methyl}-biphenyl-2-carboxylic acid**

Yield: 55%, m.p.=246-248^{0}C, Mol. wt 611.71, Analysis. Calculated: $C_{37}H_{29}N_3O_4S$: C, 72.65; H, 4.79; N, 6.87, S; 5.24 %; IR (KBr): 3611, 3482, 3035, 2949, 2806, 1665, 1704, 1236, 1176, 894, 659. ^{1}HNMR (300MHz, $CDCl_3$), 10.43(s, 1H, COOH), 4.96 (s, 2H, CH_2), 3.85 (d, 2H, methylene-CH_2), 3.76(s, 3H- CH_3), 6.84-8.56 (m, 21H, -ArH). ^{13}CNMR($CDCl_3$)δ: 17.4, 51.1, 110.1, 112, 114.2, 115.1, 116.6, 120.1, 122.5, 122.9, 133, 135.1, 140.2, 142.6, FAB-MS, 611.54.

[2] **4'-{2-[4-[2-(3-Methoxy-phenyl)-4-oxo-thiazolidin-3-yl]-phenyl} benzoimidazol-1-yl-methyl}-biphenyl-2-carboxylic acid**

Yield: 50%, m.p.=243-245^{0}C, Mol.wt 611.71, Analysis. Calculated:$C_{37}H_{29}N_3O_4S$: C,72.65; H,4.79; N,6.87,S; 5.24 %; IR (KBr): 3611, 3482, 3035, 2949, 2806, 1665, 1704, 1236, 1176, 894, 659. ^{1}HNMR (300 MHz, $CDCl_3$), 10.40(s, 1H, COOH), 4.96 (s, 2H, CH_2), 3.85 (d, 2H, methylene-CH_2), 3.76(s, 3H- CH_3), 6.84-8.56 (m, 21H, -ArH). ^{13}CNMR($CDCl_3$)δ: 17.4, 51.1, 110.1, 112, 114.2, 115.1, 116.6, 120.1, 122.5, 122.9, 133.1, 135.1, 140.2, 141.3, FAB-MS, 610.37

[3] **4'-{2-[4-[2-(4-Methoxy-phenyl)-4-oxo-thiazolidin-3-yl]-phenyl} benzoimidazol-1-yl-methyl}-biphenyl-2-carboxylic acid**

Yield: 57%, m.p.=243-245^{0}C, Mol.wt 611.71, Analysis. Calculated: $C_{37}H_{29}N_3O_4S$: C,72.65; H,4.79; N,6.87, S; 5.24 %; IR (KBr): 3611, 3482, 3035, 2949, 2806, 1665, 1704, 1236, 1176, 894, 659. ^{1}HNMR (300 MHz, $CDCl_3$), 10.46(s, 1H, COOH), 4.96 (s, 2H, CH_2), 3.85 (d, 2H, methylene-CH_2), 3.76(s, 3H- CH_3), 6.84-8.56 (m, 21H, -ArH). ^{13}CNMR($CDCl_3$)δ: 17.8, 53.1, 110.1, 112, 114.2, 115.1, 116.6, 120.1, 122.5, 122.9, 133, 135.1, 140.2, 141.2, FAB-MS, 612.16.

[4] **4'-{2-[4-[2-(2-Chloro-phenyl)-4-oxo-thiazolidin-3-yl]-phenyl} benzoimidazol-1-yl-methyl}-biphenyl-2-carboxylic acid**

Yield: 69%, m.p.=202-204^{0}C. Mol.wt 616.13, Analysis. Calculated: $C_{36}H_{26}ClN_3O_3S$: C,74.03; H,4.49; N,7.19 %; IR (KBr): 3611, 3422, 3386, 3038, 1705, 1687, 1258, 1164, 897, 788, 651. ^{1}HNMR (300 MHz, $CDCl_3$), 10.67(s, 1H, COOH), 6.97-8.73 (m, 20H, -ArH), 3.85 (d, 2H,

methylene-CH_2), 4.99 (s, 2H, CH_2), 5.96(s, 1H, CH-Cl). ^{13}CNMR($CDCl_3$)δ: 49.9, 52.5, 111.1, 112, 114.2, 115.1, 116.6, 120.1, 122.5, 122.9, 133.135.1, 141.5, FAB-MS, 615.74.

[5] **4'-{2-[4-[2-(3-Chloro-phenyl)-4-oxo-thiazolidin-3-yl]-phenyl} benzoimidazol-1-yl-methyl}-biphenyl-2-carboxylic acid**
Yield: 72%, m.p.=200-201^{0}C. Mol.wt 616.13, Analysis. Calculated: $C_{36}H_{26}ClN_3O_3S$: C,74.03; H,4.49; N,7.19%; IR (KBr): 3611, 3422, 3386, 3038, 1705, 1687, 1258, 1164, 897, 788, 651. ^{1}HNMR (300 MHz, $CDCl_3$), 10.61(s, 1H, COOH), 6.97-8.73 (m, 20H, -ArH), 3.85 (d, 2H, methylene-CH_2), 4.99 (s, 2H, CH_2), 5.96(s, 1H, CH-Cl). ^{13}CNMR ($CDCl_3$)δ: 49.9, 52.5, 111.1, 112, 114.2, 115.1, 116.6, 120.1, 122.5, 122.9, 133.135.1, 141.5, FAB-MS, 616.58.

[6] **4'-{2-[4-[2-(4-Chloro-phenyl)-4-oxo-thiazolidin-3-yl]-phenyl} benzoimidazol-1-yl-methyl}-biphenyl-2-carboxylic acid**
Yield: 77%, m.p.=208-211^{0}C. Mol.wt 616.13, Analysis. Calculated: $C_{36}H_{26}ClN_3O_3S$: C,74.03; H,4.49; N,7.19%; IR (KBr): 3611, 3422, 3386, 3038, 1705, 1687, 1258, 1164, 897, 788, 651. ^{1}HNMR (300 MHz, $CDCl_3$), 10.70(s, 1H, COOH), 6.97-8.73 (m, 20H, -ArH), 3.85 (d, 2H, methylene-CH_2), 4.99 (s, 2H, CH_2), 5.96(s, 1H, CH-Cl). ^{13}CNMR($CDCl_3$)δ: 49.9, 52.5, 111.1, 112, 114.2, 115.1, 116.6, 120.1, 122.5, 122.9, 133.135.1, 141.5, FAB-MS, 615.19.

[7] **4'-{2-[4-[2-(2-nitro-phenyl)-4-oxo-thiazolidin-3-yl]-phenyl} benzoimidazol-1-yl-methyl}-biphenyl-2-carboxylic acid**
Yield: 80%, m.p.=238-241^{0}C. Mol.wt 626.68, Analysis. Calculated:$C_{36}H_{26}N_4O_5S$: C,69.04; H,4.18; N,8.94; S 4.12%; IR (KBr): 3643, 3454, 3316, 3078, 1707, 1681, 1265, 1198, 891, 780, 647. ^{1}HNMR (300 MHz, $CDCl_3$), 10.54(s, 1H, COOH), 7.11-8.54 (m, 21H, -ArH), 3.88 (d, 2H, methylene-CH_2), 4.94 (s, 2H, CH_2). ^{13}CNMR($CDCl_3$)δ: 47, 48.8, 110.5, 111.1, 115.3, 119.5, 121.2, 127.5, 129.3, 133.2, 140.3, FAB-MS, 626.15.

[8] **4'-{2-[4-[2-(3-nitro-phenyl)-4-oxo-thiazolidin-3-yl]-phenyl} benzoimidazol-1-yl-methyl}-biphenyl-2-carboxylic acid**
Yield: 84%, m.p.=243-246^{0}C, Mol.wt 626.68, Analysis.Calculated: $C_{36}H_{26}N_4O_5S$: C,69.04; H,4.18; N,8.94; S 4.12%; IR (KBr): 3643, 3454, 3316, 3078, 1707, 1681, 1265, 1198, 891, 780, 647. ^{1}HNMR (300 MHz, $CDCl_3$), 10.57(s, 1H, COOH), 7.11-8.54 (m, 21H, -ArH), 3.88 (d, 2H, methylene-CH_2), 4.94 (s, 2H, CH_2). ^{13}CNMR($CDCl_3$)δ: 46.4, 47, 111.1, 115.3, 119.5, 121.2, 127.5, 129.3, 133.2, 141.5, FAB-MS, 625.87.

[9] **4'-{2-[4-[2-(4-nitro-phenyl)-4-oxo-thiazolidin-3-yl]-phenyl} benzoimidazol-1-ylmethyl}-biphenyl-2-carboxylic acid**
Yield: 82%, m.p.=242-244^{0}C, Mol.wt 626.68, Analysis. Calculated:$C_{36}H_{26}N_4O_5S$: C,69.04; H,4.18; N,8.94; S4.12; IR (KBr): 3643, 3454, 3316, 3078, 2843, 2831, 1707, 1681, 1265, 1198, 891, 780, 647. ^{1}HNMR (300 MHz, $CDCl_3$), 10.50(s, 1H, COOH), 7.11-8.54 (m, 21H, -ArH), 3.88 (d, 2H, methylene-CH_2), 4.94 (s, 2H, CH_2). ^{13}CNMR($CDCl_3$)δ: 47, 111.1, 115.3, 119.5, 121.2, 127.5, 129.3, 133.2, 142.6, FAB-MS, 627.36.

[10] **4'-{2-[4-[2-(2-fluoro-phenyl)-4-oxo-thiazolidin-3-yl]-phenyl} benzoimidazol-1-ylmethyl}-biphenyl-2-carboxylic acid**
Yield: 60%, m.p.=273-276^{0}C. Mol.wt 599.67, Analysis. Calculated: $C_{36}H_{26}FN_3O_3S$: C,72.10; H,4.37; N,7.04; S5.35; IR (KBr): 3620, 3484, 3353, 3070, 2871, 2836, 1713, 1658, 1236, 1167, 886, 660. ^{1}HNMR (300 MHz, $CDCl_3$), 10.89(s, 1H, COOH), 6.87-8.65(m, 21H, -ArH), 3.84 (d,

2H, methylene-CH_2), 4.91 (s, 2H, CH_2). ^{13}CNMR($CDCl_3$)δ: 55.4, 111.3, 111.8, 114.3, 117.5, 120.2, 128.5, 129.3, 133.2, 144.1, FAB-MS, 599.36.

[11] **4'-{2-[4-[2-(3-fluoro-phenyl)-4-oxo-thiazolidin-3-yl]-phenyl} benzoimidazol-1-ylmethyl}-biphenyl-2-carboxylic acid**
Yield: 65%, m.p.=279-282^{0}C. Mol.wt 599.67, Analysis. Calculated: $C_{36}H_{26}FN_3O_3S$: C,72.10; H,4.37; N,7.04; S5.35; IR (KBr): 3620, 3484, 3353, 3070, 2871, 2836, 1713, 1658, 1236, 1167, 886, 660. ^{1}HNMR (300 MHz, $CDCl_3$), 10.84(s, 1H, COOH), 6.87-8.65 (m, 21H, -ArH), 3.84 (d, 2H, methylene-CH_2), 4.91 (s, 2H, CH_2). ^{13}CNMR($CDCl_3$)δ: 49.0, 111.3, 111.8, 114.3, 117.5, 120.2, 128.5, 129.3, 133.2, 142.2, FAB-MS, 600.49.

[12] **4'-{2-[4-[2-(4-fluoro-phenyl)-4-oxo-thiazolidin-3-yl]-phenyl} benzoimidazol-1-ylmethyl}-biphenyl-2-carboxylic acid**
Yield: 58%, m.p.=266-269^{0}C. Mol.wt 599.67, Analysis. Calculated: $C_{36}H_{26}FN_3O_3S$: C,72.10; H,4.37; N,7.04; S5.35; IR (KBr): 3620, 3484, 3353, 3070, 2871, 2836, 1713, 1658, 1236, 1167, 886, 660. ^{1}HNMR (300 MHz, $CDCl_3$), 10.93(s, 1H, COOH), 6.87-8.65 (m, 21H, -ArH), 3.84 (d, 2H, methylene-CH_2), 4.91 (s, 2H, CH_2). ^{13}CNMR($CDCl_3$)δ: 48.7, 111.3, 111.8, 114.3, 117.5, 120.2, 128.5, 129.3, 133.2, 139.3, FAB-MS, 598.70.

[13] **4'-{2-[4-[2-(2-iodo-phenyl)-4-oxo-thiazolidin-3-yl]-phenyl} benzoimidazol-1-ylmethyl}-biphenyl-2-carboxylic acid**
Yield: 57%, m.p.=298-301^{0}C. Mol.wt 707.58, Analysis. Calculated: $C_{36}H_{26}IN_3O_3S$: C,61.14; H,3.70; N,5.94; S,4.54; IR (KBr): 3646, 3472, 3356, 3061, 2871, 2884, 1714, 1658, 1265, 1161, 889, 654. ^{1}HNMR (300 MHz, $CDCl_3$), 11.00(s, 1H, COOH), 6.96-8.70 (m, 21H, -ArH), 3.84 (d, 2H, methylene-CH_2), 4.98(s, 2H, CH_2). ^{13}CNMR($CDCl_3$)δ: 55.1, 112.1, 113.4, 114.1, 116.3, 119.2, 128.2, 134.2, 139.7, 140.7, FAB-MS, 708.53.

[14] **4'-{2-[4-[2-(3-iodo-phenyl)-4-oxo-thiazolidin-3-yl]-phenyl} benzoimidazol-1-ylmethyl}-biphenyl-2-carboxylic acid**
Yield: 57%, m.p.=295-298^{0}C. Mol.wt 707.58, Analysis. Calculated: $C_{36}H_{26}IN_3O_3S$: C,61.14; H,3.70; N,5.94; S,4.54; IR (KBr): 3646, 3478, 3351, 3064, 2871, 2884, 1710, 1658, 1265, 1161, 889, 657. ^{1}HNMR (300 MHz, $CDCl_3$), 10.97(s, 1H, COOH), 6.97-8.76 (m, 21H, -ArH), 3.89 (d, 2H, methylene-CH_2), 4.98(s, 2H, CH_2). ^{13}CNMR($CDCl_3$)δ: 55.1, 112.1, 113.4, 114.1, 116.3, 119.2, 128.2, 134.2, 139.7, 140.7, FAB-MS, 706.12.

[15] **4'-{2-[4-[2-(4-iodo-phenyl)-4-oxo-thiazolidin-3-yl]-phenyl} benzoimidazol-1-ylmethyl}-biphenyl-2-carboxylic acid**
Yield: 57%, m.p.=293-296^{0}C. Mol.wt 707.58, Analysis. Calculated: $C_{36}H_{26}IN_3O_3S$: C,61.14; H,3.70; N,5.94; S,4.54; IR (KBr): 3646, 3478, 3351, 3064, 2871, 2884, 1710, 1658, 1265, 1161, 889, 657. ^{1}HNMR (300 MHz, $CDCl_3$), 11.05(s, 1H, COOH), 6.97-8.76 (m, 21H, -ArH), 3.89 (d, 2H, methylene-CH_2), 4.98(s, 2H, CH_2). ^{13}CNMR($CDCl_3$)δ: 55.1, 112.1, 113.4, 114.1, 116.3, 119.2, 128.2, 134.2, 139.7, 140.7, FAB-MS, 707.82.

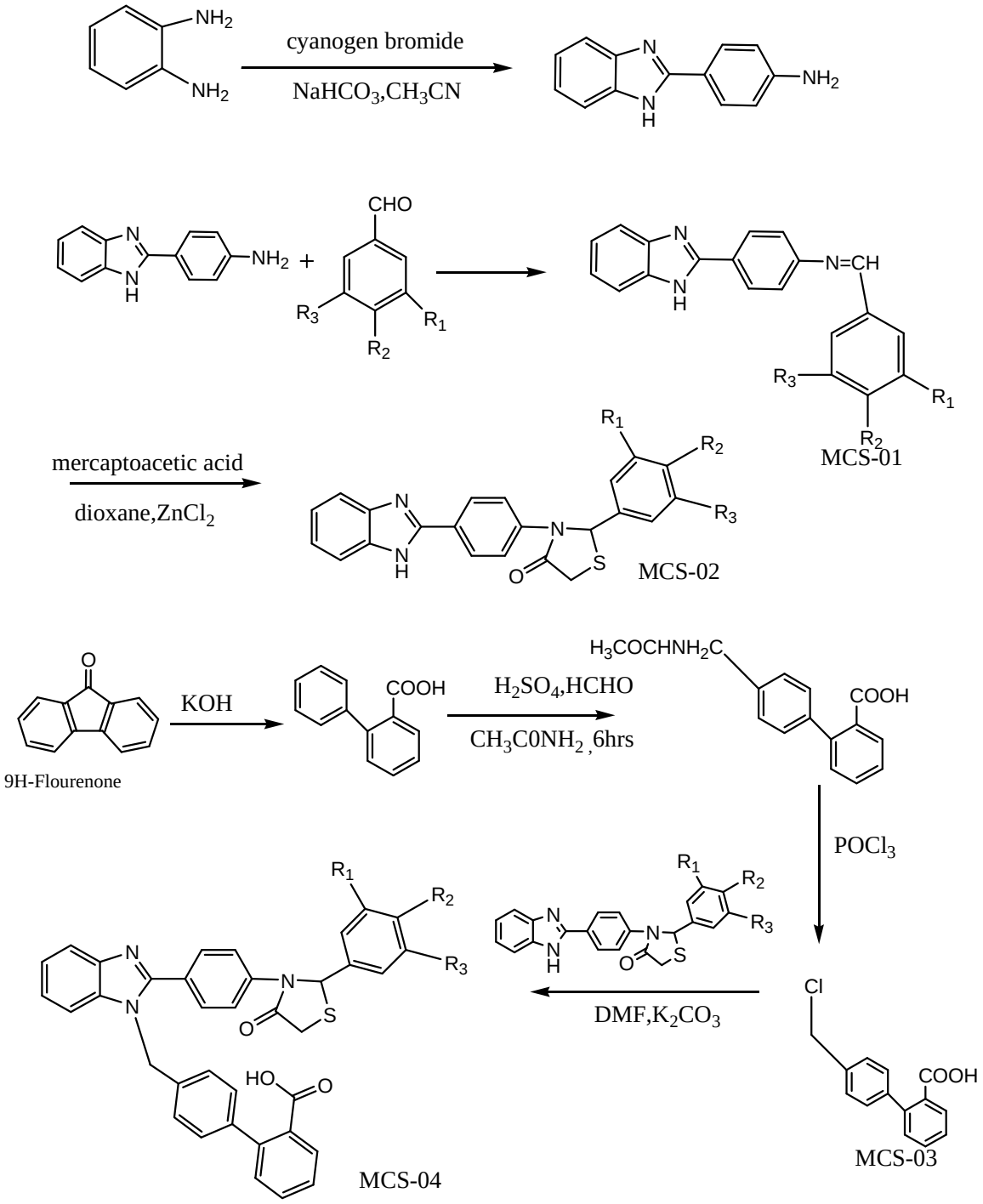
[16] **4'-{2-[4-[2-(2-hydroxy-phenyl)-4-oxo-thiazolidin-3-yl]-phenyl} benzoimidazol-1-ylmethyl}-biphenyl-2-carboxylic acid**
Yield: 61%, m.p.=265-267^{0}C. Molecular weight 597.68, Anal. Calcd for $C_{36}H_{27}N_3O_4S$: C,72.32; H,4.55; N,7.04; S,5.36; IR (KBr): 3597, 3490, 3343, 3074, 2858, 2861, 1708, 1674, 1287, 1116,

882, 654.6. ^{1}HNMR (300 MHz, $CDCl_3$), 10.21(s, 1H, COOH), 6.94-8.86 (m, 21H, -ArH), 5.08(s, 1H-arm, OH), 3.80 (d, 2H, methylene-CH_2), 4.93(s, 2H, CH_2). ^{13}CNMR ($CDCl_3$)δ: 49.1, 55.4, 112. 110.8, 112.3, 114.2, 123.2, 124.2, 129.2, 139.3, 145.4, FAB-MS, 596.62

[17] **4'-{2-[4-[2-(3-hydroxy-phenyl)-4-oxo-thiazolidin-3-yl]-phenyl} benzoimidazol-1-ylmethyl}-biphenyl-2-carboxylic acid**
Yield: 53%, m.p.=269-272^{0}C. Molecular weight 597.68, Anal. Calcd for $C_{36}H_{27}N_3O_4S$: C, 72.32; H, 4.55; N, 7.04; S, 5.36; IR (KBr): 3597, 3490, 3343, 3074, 2858, 2861, 1708, 1674, 1287, 1116, 882, 654.6. ^{1}HNMR (300 MHz, $CDCl_3$), 10.26(s, 1H, COOH), 6.94-8.86 (m, 21H, -ArH), 5.08(s, 1H-arm, OH), 3.80 (d, 2H, methylene-CH_2), 4.93(s, 2H, CH_2). ^{13}CNMR ($CDCl_3$)δ: 49.6, 55.4, 110.8, 112.3, 114.2, 123.2, 124.2, 129.2, 139.3, 146.1, FAB-MS, 598.09.

[18] **4'-{2-[4-[2-(4-hydroxy-phenyl)-4-oxo-thiazolidin-3-yl]-phenyl} benzoimidazol-1-ylmethyl}-biphenyl-2-carboxylic acid**
Yield: 68%, m.p.=260-262^{0}C. Molecular weight 597.68, Anal. Calcd for $C_{36}H_{27}N_3O_4S$: C, 72.32; H, 4.55; N, 7.04; S, 5.36; IR (KBr): 3597, 3490, 3343, 3074, 2858, 2861, 1708, 1674, 1287, 1116, 882, 654.6. ^{1}HNMR (300 MHz, $CDCl_3$), 10.24(s, 1H, COOH), 6.94-8.86 (m, 21H, -ArH), 5.08(s, 1H-arm,OH), 3.80 (d, 2H, methylene-CH_2), 4.93(s, 2H, CH_2). ^{13}CNMR ($CDCl_3$)δ: 46.8, 55.4, 112, 110.8, 112.3, 114.2, 123.2, 124.2, 129.2, 139.3, 147.1, FAB-MS, 597.26.

SCHEME



COMPOUNDS-[1-18]

Biological Activity

Non-invasive Tail cuff Method (Indirect Method) [25, 33-37]-Albino rats weighing 150-200 gm were used to screening for all the synthesized 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.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. [Table1, 2]

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	140	106	123	138	101	119
	2	138	104	121	140	106	123
	3	151	109	130	146	104	125
	4	146	104	125	142	104	123
	5	144	106	125	140	102	121
[2]	1	137	102	124	135	102	118
	2	139	107	123	140	101	120
	3	142	108	125	146	104	125
	4	146	106	126	142	104	123
	5	142	110	126	140	116	128
[3]	1	135	102	122	140	97	119
	2	146	103	125	139	105	120
	3	144	109	131	140	100	120
	4	141	104	123	138	104	121
	5	139	111	125	135	103	119
[4]	1	148	104	125	145	102	123
	2	144	116	130	141	101	122
	3	142	110	126	139	104	123
	4	146	106	126	144	104	124
	5	142	114	127	140	101	122
[5]	1	141	106	124	141	101	121
	2	143	105	124	143	105	124
	3	139	105	126	146	106	120

	4	148	106	127	142	106	124
	5	151	109	130	146	104	125
[6]	1	138	105	122	139	109	124
	2	132	104	118	142	106	124
	3	142	103	123	140	106	123
	4	142	112	127	140	102	121
	5	144	116	130	141	101	122
[7]	1	145	105	125	145	105	124
	2	136	113	124	142	101	121
	3	139	113	122	140	106	126
	4	143	105	124	139	104	121
	5	141	101	126	143	104	120
[8]	1	139	107	123	140	108	124
	2	143	109	126	137	114	128
	3	141	109	125	145	106	117
	4	141	103	122	135	109	122
	5	133	113	123	141	109	125
[9]	1	144	103	124	141	107	125
	2	141	111	126	140	108	124
	3	135	101	118	136	107	121
	4	140	110	125	138	112	125
	5	141	103	122	135	109	122
[10]	1	144	112	127	141	107	127
	2	142	105	124	135	107	121
	3	141	102	121	139	103	126
	4	140	105	123	141	105	123
	5	143	101	122	140	117	129
[11]	1	139	107	123	140	106	124
	2	143	109	126	137	108	127
	3	141	109	125	139	103	122
	4	149	105	125	143	107	127
	5	144	103	131	140	109	126
[12]	1	144	112	127	141	102	121
	2	142	114	128	144	101	122
	3	146	110	126	142	104	122
	4	140	108	124	138	101	125
	5	146	105	126	142	114	127
[13]	1	142	102	124	143	108	125
	2	145	112	126	139	103	122
	3	142	109	126	143	111	126
	4	140	102	123	140	106	123
	5	137	101	124	146	108	125
[14]	1	143	106	125	139	104	121
	2	146	110	128	140	104	122
	3	144	114	129	146	106	126

	4	142	108	125	146	104	125
	5	146	106	126	142	104	123
[15]	1	152	112	133	145	103	124
	2	150	111	131	146	104	125
	3	142	110	126	140	116	128
	4	148	102	125	144	106	125
	5	144	109	129	146	109	127
[16]	1	148	104	124	143	102	122
	2	146	112	128	137	101	118
	3	143	108	126	140	103	121
	4	145	106	123	136	97	116
	5	138	106	122	141	103	122
[17]	1	142	112	127	139	102	121
	2	140	108	124	143	101	122
	3	137	104	121	140	103	121
	4	140	101	125	140	101	124
	5	138	107	128	143	101	121
[18]	1	140	106	121	137	102	126
	2	144	106	125	142	104	129
	3	146	108	124	140	103	124
	4	139	102	122	143	100	122
	5	148	104	124	143	102	128
Control	**Losartan**	120	-	-	-	-	-
	Telmisartan	116	-	-	-	-	-

Table 2. 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	124	101	112	122	102	114
	2	124	102	113	125	102	112
	3	126	101	113	125	100	113
	4	126	106	116	122	100	111
	5	126	103	114	126	96	111
[2]	1	129	101	115	119	104	111
	2	123	107	115	121	99	110
	3	127	105	119	123	103	113
	4	123	101	113	124	103	112
	5	131	105	118	124	101	115
[3]	1	129	108	119	124	104	114
	2	122	112	117	122	103	112
	3	125	105	115	122	100	112
	4	124	100	112	128	101	113
	5	130	104	117	128	102	115
[4]	1	125	105	115	124	101	112

	2	122	100	111	126	104	115
	3	128	102	115	130	103	116
	4	123	102	113	128	103	112
	5	121	101	113	123	102	111
[5]	1	126	102	111	124	101	112
	2	121	100	110	125	102	111
	3	126	103	115	122	103	112
	4	123	102	113	128	103	112
	5	125	100	112	121	107	114
[6]	1	128	102	115	130	103	116
	2	129	101	115	119	104	111
	3	123	107	115	121	99	110
	4	127	105	119	123	103	113
	5	129	100	111	126	104	115
[7]	1	124	111	118	123	104	113
	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
[8]	1	127	101	114	123	102	112
	2	121	103	112	121	97	109
	3	120	100	115	128	100	114
	4	127	105	118	126	102	114
	5	124	106	122	122	101	111
[9]	1	123	102	119	127	101	110
	2	122	104	118	124	98	113
	3	127	108	119	126	103	109
	4	128	106	117	123	100	112
	5	127	101	116	125	105	110
[10]	1	124	102	119	128	102	111
	2	129	104	117	124	101	112
	3	133	103	118	126	100	110
	4	137	101	124	146	100	123
	5	123	101	112	125	100	112
[11]	1	122	100	111	126	102	115
	2	124	102	112	126	102	111
	3	126	101	113	124	104	114
	4	128	102	115	126	104	115
	5	125	105	115	124	101	112
[12]	1	122	100	111	126	104	115
	2	125	100	112	121	107	114
	3	128	102	115	130	103	116
	4	125	105	115	127	101	114
	5	120	102	111	123	101	112
[13]	1	125	103	114	126	100	113

	2	122	100	111	128	103	114
	3	125	101	113	121	101	110
	4	123	107	115	125	100	112
	5	126	103	114	126	96	111
[14]	1	129	101	115	119	104	111
	2	123	107	115	121	99	110
	3	127	105	119	123	103	113
	4	129	100	111	126	104	115
	5	123	101	113	124	103	112
[15]	1	126	102	114	128	104	116
	2	132	104	118	122	101	111
	3	128	102	115	127	104	114
	4	125	105	110	126	103	115
	5	126	104	110	123	106	116
[16]	1	122	106	114	129	101	113
	2	124	106	115	127	102	114
	3	126	104	115	125	105	115
	4	124	104	114	121	100	110
	5	125	102	112	128	100	114
[17]	1	120	100	120	130	95	112
	2	125	105	115	124	101	112
	3	126	103	114	125	104	113
	4	132	105	119	121	102	110
	5	131	106	118	119	103	107
[18]	1	136	107	121	129	101	115
	2	126	103	114	122	109	115
	3	124	107	115	127	106	117
	4	127	104	116	124	95	109
	5	129	108	118	130	102	116
Control	**Losartan**	107	-	-	-	-	-
	Telmisartan	1122	-	-	-	-	-

RESULTS AND DISCUSSION

Tail-cuff non-invasive blood pressure measurements can be consistent, accurate and reproducible when studying awake and anesthetized mice and rats. Care must be exercised to properly handle the animals. Training the animals and monitoring the animal's temperature may also be beneficial. The volumetric pressure recording method provides the highest degree of correlation with telemetry and direct blood pressure and is clearly the preferred tail-cuff sensor technology. Non-invasive blood pressure devices that utilize Volume Pressure Recording are a valuable tool in research and will continue to be beneficial in many study protocols. The main advantages are: (1) they require no surgery; (2) they are significantly less expensive than other blood pressure equipment, such as telemetry; (3) they can screen for systolic and diastolic BP changes over time in large numbers of animals; and (4) they provide the researcher with the ability to obtain accurate and consistent blood pressure measurements over time in long-term studies.1,2-

phenylenediamine dihydrochloride (0.45 g, 2.5 mmol) in 5 ml of water was cooled to 0°C and treated with a solution of cyanogen bromide (0.60 ml, 5 M in acetonitrile, 3.0 mmol) and solid $NaHCO_3$ (0.41 mg, 4.9 mmol). The solution was stirred at ambient temperature for 40-45 h. The mixture was made basic with 1 M aqueous Na_2CO_3 and the solution was concentrated under reduced pressure. 2-(4-aminophenyl) Benzimidazole (0.01 mol), substituted Benzaldehyde (0.01 mol) and a drop of acetic acid was dissolved in ethanol (25 ml) and heated on a steam bath for 45-60 min, To a mixture of Schiff base (0.01 mol) and mercaptoacetic acid (0.05 mol) dissolved in dioxane (50 ml), anhydrous zinc chloride (0.008 mol) was added and refluxed for 12 hrs. The reaction mixture was cooled, filtered, washed with 10 % w/v sodium bicarbonate solution. 100 mg of Schiff base (different substituted aryl aldehydes) was dissolved in 50 ml of DMF (dimethyl formamide) and stirred vigorously with 12.5 gm of potassium carbonate at 39^0C for 4 hours. To the resulting mixture 0.482gm of MCS-03 first dissolved in 15 ml of DMF and then was added drop wise with dropping funnel in 4 hours the reaction was allowed to proceed for further 12 hours at room temperature and solvent removed under vacuum. Residue was treated with 20ml of Conc.HCl and extracted with ethyl acetate. The benzimidazole derivatives were able to increases Angiotensin-II induced hypertension and also vasoconstriction, compared with Losartan and Telmisartan. The similar results were noticed with synthesized Schiff bases and Thiazoldine -4-one. From the preliminary screening the synthesized molecules shows good Angiotensin II induced anti hypertension activity and above molecules can be taken as a lead molecule for Angiotensin II induced hypertension. Therefore we conclude in our preliminary screening that it may not to be necessary to go for a synthesis of big molecule [compounds numer-2, 6, 8, 9, 11, 13, 15, 17]. Further study of these molecules and its analogues under progress in our laboratory.

Acknowledgement
The authors are thankful to Head of Department School of Pharmacy D.A.V.V Indore to providing the facilities for IR spectra.

REFERENCES

[1] Puri PL,Avantaggiati L,BurgioVL,Chirillot P, Collepardo D. *J. Biol. Chem,* **1995,** *270,* 22129-22134.
[2] Benigni A, Perico N,Remuzzi G.*J. Cardiovasc. Pharmacol,* **2000,** *35* ,S75-S78.
[3] Wolny A ,Clozel JP, Rein J ,Mory P ,*Vogt P. Circ. Res*, **1997,** *80,* 219-227.
[4] Levine B, Kalman J ,MayerL, Fillit H M, Packer M. N. *Engl. J. Med*, **1990,** *323* ,236-241.
[5] Griendling KK, Minieri C A, Ollerenshaw JD, Alexander RW.*Circ. Res*, **1994,** *74,* 1141-1148.
[6] Mayer A MS, Brenic S, Glaser K. B. *J. Pharmacol. Exp. Ther*, **1996,** *279,* 633-644.
[7] Beckman JS, Beckman TW, Chen J, Marshall P A, Freeman BA, *Proc. Natl. Acad. Sci*. U.S.A, **1990,** 87 1620- 1624.
[8] Wong PC, Price W A, Chiu A T, Duncia J V, Carini D J, *J. Pharmacol. Exp. Ther*, **1990,** 252, 719.
[9] Raij L, Baylis C, *Kidney Int*., **1995,** 48, 20-32.
[10] Pollman M J, Yamada T, Horiuchi M, Gibbons G. H, *Circ. Res*, **1996,** 79, 748-756.
[11] Kagami S, Border W, Miller DE, Noble N A, *J. Clin. Invest*, **1994,** 93. 2431-2437.
[12] Wilkes B C, Masaro L, Schiller PW, Carpenter KA. *J. Med. Chem*, **2002,** *45* ,4410-4418.

[13] Schmidt B, Lindman S, Tong W, Lindeberg G, Gogoll A.*J. Med. Chem*, **1997** *40* 903-919.
[14] Spear KL, Brown MS, Reinhard EJ, McMahon EG, Olins GM. *J.Med.Chem*, **1990** ,33 , 1935-1940.
[15] Duncia J V, Carini D J, Chiu A T, Johnson A L, Price WA, *Med. Res. Rev*, **1992,** 12, 149-191.
[16] Duncia J V, Chiu A T, Carini D J, Gregory G B, *Med. Chem*, **1990,** 33, 1312-1329.
[17] Israili Z H, J. Hum. *Hypertension*, **2000,** 14 (Suppl. 1) S73-S86.
[18] JuilleratJ L, Celerier J, Chapuis Bernasconi C, Nguyen G, Wostl W, Maerki HP, Janzer RC, Corvol P, Gasc JM. *Br. J. Cancer*, **2004,** *90*, 1059.
[19] Fogarty DJ ,Sanchez-Gomez MV, Matute C. *Glia*, **2002,** *39*, 304.
[20] Ashton W T, Hutchins SM, Greenlee WJ, Doss GA, Chang RS, Lotti V J,Faust K A, Chen T B, Zingaro G J, Kivlighn SD.*J . Med. Chem*, **1993,** *36* ,3595.
[21] Scheur DA, Perrone MH. *Am. J. Physio.l Regu.l Integ.r Comp. Physiol*,**1993**, 264: 917-923.
[22] Kubo K, Kohara Y, Imamiya E, Sugiura Y, Inada Y, Furukaw Y, Nishikawa K,Naka T, *J. Med. Chem*, **1993**,36, 2182-2195.
[23] Ries UJ, Mihm G, Narr B, Hasselbach K.M, Wittneben H, Entzreoth M, Van Meel JCA, Wienen M, Hauel NH. *J. Med. Chem*, **1993**, 4040-4051.
[24] Bali A, Bansal Y, Sugumaran M, Saggu J.S, Balakumar P, Kaur G, Bansal G, Sharma A, Singh M, *Bioorg. Med. Chem. Lett*, **2005,** 15, 3962-3965.
[25] Jat RK, Jat JL, Pathak DP, *Euro. Journal. of Chemistry*., **2006**,3:(13), 278-285
[26] Dhvanit I S, Sharma M, Bansal Y,Bansal G, M. Singh, *European Journal of Medicinal Chemistry*, **2008**,43, 1808-1812.
[27] Saggu JS, Sharma R,Dureja H,Kumar V, J. *Indian. Inst. Sci*, **2002**,82, 177–182.
[28] Haugwitz RD. *J. Med. Chem*, **1982**, 25, 969-974.
[29] Hisano T. *Chem. Pharm.Bull*, **1982**, 30, 2996-3004.
[30] Hubschwerlen C. *J .Med.Chem*, **1992**, 35, 1385.
[31] Kim JS. *Bio. Med. Chem*, **1996**,4, 621-630.
[32] Badyal DK, Lata H, Dadhich AP,. *Indian J of Pharmacology*, **2003**, 35(66), 349-362.
[33] Bunag RD, McCubbin JW, Page IH, *Cardiovasc. Res*, **1971**,5(1): 24-31.
[34] Gupta SK, Drug Screening methods, Jaypee Brothers Medical Publisher, New Delhi, **2004**, pp 236-246.
[35] Shreenivas MT, Chetan BP, Bhat AR, *J. of Pharma.Sci. And Technology*, **2009**, 1 (2), 88-94.
[36] Siddiqui AA, Wani M.S, *Indian.J.Chemistry*, **2004**, 43B,pp. 1574-1579.
[37] Vogel G.H.Drug Discovery and Evaluation, Pharmacological Assay, **2002** ;(Springer. Berlin), 122.