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Design, synthesis, and pharmacological evaluation of 4'-{2-[4-[3-chloro-2-(substituted-phenyl)-4-oxo-azetidin-1-yl)-phenyl]-benzoimidazol-1-ylmethyl}-biphenyl-2-carboxylic acids

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ABSTRACT

Series of non peptide angiotensin (A-II) receptor antagonist has been prepared by 4'-{2-[4-[3-chloro-2-(substituted-phenyl)-4-oxo-azetidin-1-yl)-phenyl]-benzoimidazol-1-yl-methyl}-biphenyl-2-carboxylic acid were synthesised by 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 Chloroacetyl chloride (0.01mol) was added drop wise to a mixture of schiff base (0.01mol) and triethylamine (0.02 mol) in dioxane (25 ml) at room temperature Schiff bases react with biphenyl carboxylic acid with different substituents aryl group cyclocondensation with appropriate reagents. Different from the previously reported and related compounds in that they produce a potent hypertensive effect 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.

Keywords: Benzimidazole, Azetidinone, Biphenyl Carboxylic acid, Angiotensin II, Blood Pressure.

INTRODUCTION

Ang II receptor antagonists have proved to lower blood pressure effectively, and they are better tolerated than other classes of drugs. 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]. The octapeptide angiotensin II (Ang II) produced by the rennin angiotensin system (RAS) is a potent vasoconstrictor and thus plays an integral role in the pathophysiology of hypertension. This directed many researchers toward designing drugs to block the effects of Ang

II either by inhibiting the angiotensin converting enzyme (ACE) or renin or by blocking the Ang II receptors. 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 affecting an increase in vascular resistance, the regulation of volemia through the stimulated release of vasopressin and aldosterone, which induces saline retention, and the regulation of the adrenocorticotropic hormone (ACTH) Thus, reducing the levels of AII by inhibition of one of the RAS enzymes or directly blocking the AII receptors is in theory a good approach for treating hypertension, confirmed by the success of angiotensin-converting enzyme (ACE) inhibitors as antihypertensive[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 AT1 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). The substituent at 6-position on the nucleus increases the activity whereas small substituent at 5-position decreases the activity[10]. Compounds containing tetrazole nucleus are also reported as AT1 receptor antagonists and their protypical derivative exhibits non-competitive antagonism[11] and amino group attach with carboxylic group given good biological activity [11-12]. 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.

MATERIALS AND METHODS

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.

General Method for the Synthesis [13]
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.

MS-02 Synthesis of 2-[4-(azetidin-2-one) 3-chloro-4-phenyl]-1H-phenylbenzimidazole
Chloroacetyl chloride (0.01mol) was added drop wise to a mixture of schiff base (0.01mol) and triethylamine (0.02 mol) in dioxane (25 ml) at room temperature. The mixture was stirred for 8 h and allowed to stand at room temperature for 3 days. The contents were poured on crushed ice and the precipitate obtained was filtered, washed with 10 % w/v sodium bicarbonate solution, vacuum dried and recrystallised using absolute ethanol.

MS 03: (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-04 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%.

MS-04: (4'-Acetylamino methyl biphenyl-2-caboxylic acid)
5gm of MCS 03 was dissolved in 25 ml of concentrated H_2SO_4.After that acetamide (2.15 gm) and Paraformaldehyde (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), 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.

MS 05: (4'-Chloromethyl biphenyl-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): 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), 7.11-8.05(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 %.

MS-06-4'-{2-[4-(3-chloro-2-oxo-4-subsituted-azetidin-1-yl)-phenyl] benzoimidazol-1-yl-methyl}-biphenyl-2-carboxylic acid
115 mg of MS-02 was dissolved in 20ml of DMF (dimethyl formamide) and stirred vigorously with 5.78 gm of potassium carbonate at 35^{0}C for three half hours. To the resulting mixture 0.482gm of MCS-06 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 8 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 (MS-06) was obtained.

Spectral data of synthesized compounds

MS-01- 4'-{2-[4-(3-Chloro-2-oxo-4- phenyl-azetidin-1-yl)-phenyl]-benzoimidazol-1-yl-methyl}-biphenyl-2-carboxylic acid
Yield: 65%, m.p.=123-126^{0}C. Molecular weight 584.06, Anal. Calcd for $C_{36}H_{26}ClN_3O_3$: C, 74.03; H, 4.49; N, 7.19%; IR (KBr): 3545, 3365, 3128, 1704, 1714, 1252, 894, 785, 705, 589. ^{1}HNMR (300 MHz, $CDCl_3$), 10.80(s, 1H, COOH), 6.77-8.46 (m, 21H, -ArH), 4.96 (s, 2H, CH_2), 5.34-5.41(s, 2H, CH-Cl). ^{13}CNMR ($CDCl_3$)δ: 53.5, 111.1, 112.5, 114.1, 121.2, 123.5, 128.2, 130.2, 133.1, 134.3, 135.2, 138.4, 140.5, FAB-MS, 583.166.

MS-02-4'-{2-[4-[3-Chloro-2-(2-chloro-phenyl)-4-oxo-azetidin-1-yl)-phenyl]-benzoimidazol-1-yl-methyl}-biphenyl-2-carboxylic acid
Yield:77%, m.p.=187-189^{0}C. Molecular weight 618.508, Anal. Calcd for $C_{36}H_{25}Cl_2N_3O_3$: C, 69.91; H, 4.07; N, 6.79%; IR (KBr): 3531, 3386, 3132, 1711, 1702, 1257, 890, 781, 652. ^{1}HNMR (300 MHz, $CDCl_3$), 10.85(s, 1H, COOH), 6.77-8.46 (m, 20H, -ArH), 4.98 (s, 2H, CH_2), 5.32-5.43(s, 2H, CH-Cl). ^{13}CNMR ($CDCl_3$)δ: 52.8, 111.1, 112.5, 114.1, 121.2, 123.5, 128.2, 130.2, 133.1, 134.3, 137.1, 139.4, 141, FAB-MS, 617.127.

MS-03- 4'-{2-[4-[3-Chloro-2-(3-chloro-phenyl)-4-oxo-azetidin-1-yl)-phenyl]-benzoimidazol-1-yl-methyl}-biphenyl-2-carboxylic acid
Yield: 73%, m.p.=193-195^{0}C. Molecular weight 618.508, Anal. Calcd for $C_{36}H_{25}Cl_2N_3O_3$: C, 69.91; H, 4.07; N, 6.79%; IR (KBr): 3531, 3386, 3132, 1711, 1702, 1257, 890, 781, 652. ^{1}HNMR (300 MHz, $CDCl_3$), 10.85(s, 1H, COOH), 6.77-8.46 (m, 20H, -ArH), 4.98 (s, 2H, CH_2), 5.32-5.43(s, 2H, CH-Cl). ^{13}CNMR ($CDCl_3$)δ: 52.8, 111.1, 112.5, 114.1, 121.2, 123.5, 128.2, 130.2, 133.1, 134.3, 137.1, 139.4, 141, FAB-MS, 617.127.

MS-04- 4'-{2-[4-[3-Chloro-2-(4-chloro-phenyl)-4-oxo-azetidin-1-yl)-phenyl]-benzoimidazol-1-yl-methyl}-biphenyl-2-carboxylic acid
Yield: 68%, m.p.=180-183^{0}C. Molecular weight 618.508, Anal. Calcd for $C_{36}H_{25}Cl_2N_3O_3$: C, 69.91; H, 4.07; N, 6.79%; IR (KBr): 3531, 3386, 3132, 1711, 1702, 1257, 890, 781, 652. ^{1}HNMR (300 MHz, $CDCl_3$), 10.85(s, 1H, COOH), 6.77-8.46 (m, 20H, -ArH), 4.98 (s, 2H, CH_2),

5.32-5.43(s, 2H, CH-Cl). ^{13}CNMR ($CDCl_3$)δ: 52.8, 111.1, 112.5, 114.1, 121.2, 123.5, 128.2, 130.2, 133.1, 134.3, 137.1, 139.4, 141, FAB-MS, 617.127.

MS-05- 4'-{2-[4-[3-Chloro-2-(2-nitro-phenyl)-4-oxo-azetidin-1-yl)-phenyl]-benzoimidazol-1-yl-methyl}-biphenyl-2-carboxylic acid

Yield: 72%, m.p.=243-246^{0}C. Molecular weight 629.06, Anal. Calcd for $C_{36}H_{25}ClN_4O_5$: C, 68.74; H, 4.01; N, 8.91%; IR (KBr): 3575, 3380, 3072, 1689, 1716, 1263, 896, 788, 650. ^{1}HNMR (300 MHz, $CDCl_3$), 10.89(s, 1H, COOH), 6.96-8.40 (m, 20H, -ArH), 4.92 (s, 2H, CH_2), 5.28-5.41(s, 2H, CH-Cl). ^{13}CNMR ($CDCl_3$)δ: 53.1, 55.3, 61.1, 70.7, 111.4, 113.1, 113.5, 114.1, 121.2, 135.5, 139.5, FAB-MS, 628.151.

MS-06- 4'-{2-[4-[3-Chloro-2-(3-nitro-phenyl)-4-oxo-azetidin-1-yl)-phenyl]-benzoimidazol-1-yl-methyl}-biphenyl-2-carboxylic acid

Yield: 70%, m.p.=249-252^{0}C. Molecular weight 629.06, Anal. Calcd for $C_{36}H_{25}ClN_4O_5$: C, 68.74; H, 4.01; N, 8.91%; IR (KBr): 3575, 3380, 3072, 1689, 1716, 1263, 896, 788, 650. ^{1}HNMR (300 MHz, $CDCl_3$), 10.92(s, 1H, COOH), 6.96-8.40 (m, 20H, -ArH), 4.92 (s, 2H, CH_2), 5.28-5.41(s, 2H, CH-Cl). ^{13}CNMR ($CDCl_3$)δ: 53.1, 55.3, 61.1, 70.7, 111.4, 113.1, 113.5, 114.1, 121.2, 135.5, 139.5, FAB-MS, 630.21.

MS-07- 4'-{2-[4-[3-Chloro-2-(4-nitro-phenyl)-4-oxo-azetidin-1-yl)-phenyl]-benzoimidazol-1-yl-methyl}-biphenyl-2-carboxylic acid

Yield: 75%, m.p.=255-258^{0}C. Molecular weight 629.06, Anal. Calcd for $C_{36}H_{25}ClN_4O_5$: C, 68.74; H, 4.01; N, 8.91%; IR (KBr): 3575, 3380, 3072, 1689, 1716, 1263, 896, 788, 650. ^{1}HNMR (300 MHz, $CDCl_3$), 10.76 (s, 1H, COOH), 6.96-8.40 (m, 20H, -ArH), 4.92 (s, 2H, CH_2), 5.28-5.41(s, 2H, CH-Cl). ^{13}CNMR ($CDCl_3$)δ: 53.1, 55.3, 61.1, 70.7, 111.4, 113.1, 113.5, 114.1, 121.2, 135.5, 139.5, FAB-MS, 628.86.

MS-08- 4'-{2-[4-[3-Chloro-2-(2-hydroxy-phenyl)-4-oxo-azetidin-1-yl)-phenyl]-benzoimidazol-1-yl-methyl}-biphenyl-2-carboxylic acid

Yield: 58%, m.p.=276-278^{0}C. Molecular weight 600.062, Anal. Calcd for $C_{36}H_{26}ClN_3O_4$: C, 72.06; H, 4.01; N, 7.04%; IR (KBr): 3575, 3380, 3072, 1689, 1716, 1263, 896, 788, 650. ^{1}HNMR (300 MHz, $CDCl_3$), 10.55 (s, 1H, COOH), 6.67-8.64 (m, 20H, -ArH), 4.96 (s, 2H, CH_2), 5.07(s, 1H-arm, OH), 5.38(s, 2H, CH-Cl). ^{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, 138, FAB-MS, 599.162.

MS-09- 4'-{2-[4-[3-Chloro-2-(3-hydroxy-phenyl)-4-oxo-azetidin-1-yl)-phenyl]-benzoimidazol-1-yl-methyl}-biphenyl-2-carboxylic acid

Yield: 62%, m.p.=282-284^{0}C. Molecular weight 600.062, Anal. Calcd for $C_{36}H_{26}ClN_3O_4$: C, 72.06; H, 4.01; N, 7.04%; IR (KBr): 3575, 3380, 3072, 1689, 1716, 1263, 896, 788, 650. ^{1}HNMR (300 MHz, $CDCl_3$), 10.51 (s, 1H, COOH), 6.67-8.64 (m, 20H, -ArH), 4.96 (s, 2H, CH_2), 5.07(s, 1H-arm, OH), 5.38(s, 2H, CH-Cl). ^{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, 138, FAB-MS, 600.76.

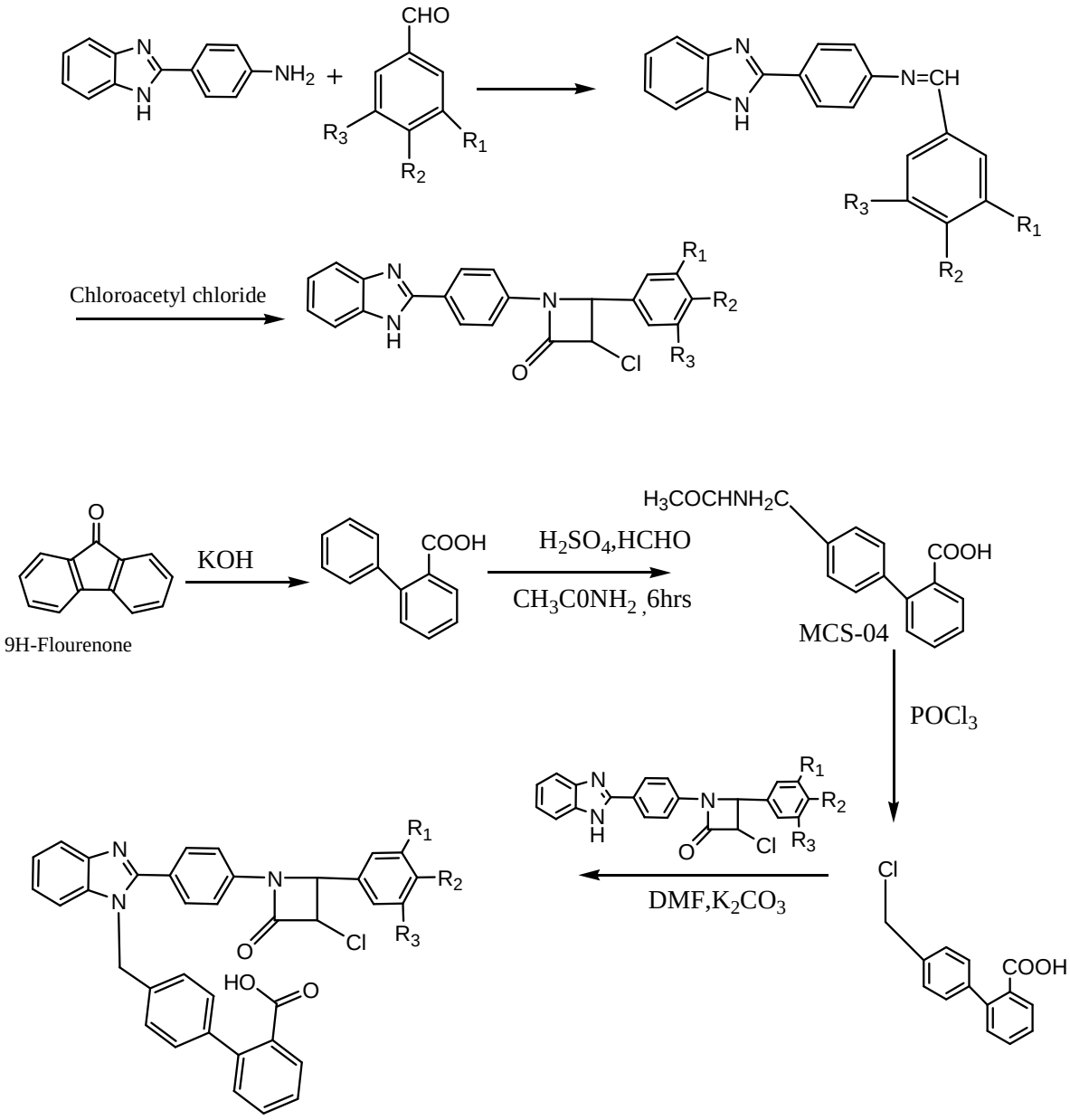
MS-10- 4'-{2-[4-[3-Chloro-2-(4-hydroxy-phenyl)-4-oxo-azetidin-1-yl)-phenyl]-benzoimidazol-1-yl-methyl}-biphenyl-2-carboxylic acid

Yield: 60%, m.p.=284-287^{0}C. Molecular weight 600.062, Anal. Calcd for $C_{36}H_{26}ClN_3O_4$: C, 72.06; H, 4.01; N, 7.04%; IR (KBr): 3575, 3380, 3072, 1689, 1716, 1263, 896, 788, 650. ^{1}HNMR (300 MHz, $CDCl_3$), 10.59 (s, 1H, COOH), 6.67-8.64 (m, 20H, -ArH), 4.96 (s, 2H, CH_2), 5.07(s, 1H, arm-OH), 5.38(s, 2H, CH-Cl). ^{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, 138, FAB-MS, 601.32.

MS-10- 4'-{2-[4-[3-Chloro-2-oxo-4-*p*-tolyl-azetidin-1-yl)-phenyl]-benzoimidazol-1-yl-methyl}-biphenyl-2-carboxylic acid

Yield: 55%, m.p.=243-245^{0}C. Molecular weight 598.32, Anal. Calcd for $C_{36}H_{28}ClN_3O_3$: C, 74.30; H, 4.72; N, 7.03%; IR (KBr): 3541, 3366, 3043, 1695, 1712, 1272, 843, 781, 646. ^{1}HNMR (300 MHz, $CDCl_3$), 10.57 (s, 1H, COOH), 6.78-8.55 (m, 20H, -ArH), 4.97 (s, 2H, CH_2), 2.37(s, 3H, -CH_3), 5.38(s, 2H, CH-Cl). ^{13}CNMR ($CDCl_3$)δ: 22.3, 58.0, 112.9, 113.4, 116.2, 121.1, 128.4, 135.5, 137.2, 138.8, FAB-MS, 599.37.

SCHEME



MS-11-4'-{2-[4-[3-Chloro-2-(4-dimethylamino-phenyl)-4-oxo-azetidin-1-yl)-phenyl]-benzo imidazol-1-yl-methyl}-biphenyl-2-carboxylic acid

Yield: 57%, m.p.=266-268^{0}C. Molecular weight 627.15, Anal. Calcd for $C_{38}H_{31}ClN_4O_3$: C, 72.78; H, 4.98; N, 8.93%; IR (KBr): 3569, 3363, 3076, 1698, 1718, 1270, 846, 786, 648.

^{1}HNMR (300 MHz, $CDCl_3$), 10.34 (s, 1H, COOH), 6.70-8.57 (m, 20H, -ArH), 4.99 (s, 2H, CH_2), 2.37-2.41 (s, 6H, -CH_3), 5.34(s, 2H, CH-Cl). ^{13}CNMR ($CDCl_3$)δ: 17.5, 22.3, 55.4, 112.1, 113.4, 114.1, 116.3, 119.2, 128.2, 134.2, 139, FAB-MS, 626.65.

MS-12- 4'-{2-[4-[3-Chloro-2-(2-fluoro-phenyl)-4-oxo-azetidin-1-yl)-phenyl]-benzoimidazol-1-yl-methyl}-biphenyl-2-carboxylic acid

Yield: 62%, m.p.=295-296^{0}C. Molecular weight 602.43, Anal. Calcd for $C_{36}H_{25}ClFN_3O_3$: C, 71.82; H, 4.19; N, 6.98%; IR (KBr): 3556, 3368, 3087, 1690, 1719, 1267, 899, 782, 654. ^{1}HNMR (300 MHz, $CDCl_3$), 10.99(s, 1H, COOH), 6.79-8.54 (m, 20H, -ArH), 4.96 (s, 2H, CH_2), 5.23-5.33(s, 2H, CH-Cl). ^{13}CNMR ($CDCl_3$)δ: 54.1, 55.3, 61.1, 70.7, 111.4, 113.1, 113.5, 114.1, 121.2, 135.5, 144.2, FAB-MS, 601.22.

MS-13- 4'-{2-[4-[3-Chloro-2-(3-fluoro-phenyl)-4-oxo-azetidin-1-yl)-phenyl]-benzoimidazol-1-ylmethyl}-biphenyl-2-carboxylic acid

Yield: 66%, m.p.=292-295^{0}C. Molecular weight 602.43, Anal. Calcd for $C_{36}H_{25}ClFN_3O_3$: C, 71.82; H, 4.19; N, 6.98%; IR (KBr): 3556, 3368, 3087, 1690, 1719, 1267, 899, 782, 654. ^{1}HNMR (300 MHz, $CDCl_3$), 10.99(s, 1H, COOH), 6.79-8.54 (m, 20H, -ArH), 4.96 (s, 2H, CH_2), 5.23-5.33(s, 2H, CH-Cl). ^{13}CNMR ($CDCl_3$)δ: 54.1, 55.3, 61.1, 70.7, 111.4, 113.1, 113.5, 114.1, 121.2, 135.5, 144.2, FAB-MS, 603.54.

MS-14- 4'-{2-[4-[3-Chloro-2-(4-fluoro-phenyl)-4-oxo-azetidin-1-yl)-phenyl]-benzoimidazol-1-ylmethyl}-biphenyl-2-carboxylic acid

Yield:56% ,m.p.=289-292^{0}C. Molecular weight 602.43, Anal. Calcd for $C_{36}H_{25}ClFN_3O_3$: C, 71.82; H, 4.19; N, 6.98%; IR (KBr): 3556, 3368, 3087, 1690, 1719, 1267, 899, 782, 654. ^{1}HNMR (300 MHz, $CDCl_3$), 10.99(s, 1H, COOH), 6.79-8.54 (m, 20H, -ArH), 4.96 (s, 2H, CH_2), 5.23-5.33(s, 2H, CH-Cl). ^{13}CNMR ($CDCl_3$)δ: 54.1, 55.3, 61.1, 70.7, 111.4, 113.1, 113.5, 114.1, 121.2, 135.5, 144.2, FAB-MS, 603.41.

Biological Activity: [12-19]

Method [A]

Albino rats weighing 200-250 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.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 table1, 2.

Method [B]

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 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	143	106	125	139	104	121
	2	146	110	128	140	104	122
	3	149	111	130	143	106	124
	4	152	112	133	145	103	124
	5	150	111	131	146	104	125
[2]	1	144	114	129	146	106	126
	2	142	108	125	146	104	125
	3	146	106	126	142	104	123
	4	142	110	126	140	116	128
	5	148	102	125	144	106	125
[3]	1	149	101	125	143	101	121
	2	144	109	131	140	100	120
	3	142	102	124	143	101	122
	4	145	105	125	145	100	121
	5	136	113	124	142	101	121
[4]	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]	1	141	112	123	139	96	117
	2	140	103	124	145	98	119
	3	141	108	124	140	103	121
	4	145	113	128	144	102	123
	5	143	111	125	143	100	121
[6]	1	141	114	126	139	102	120
	2	140	112	126	143	100	122
	3	144	116	130	145	98	119

	4	144	106	125	144	100	122
	5	145	112	126	139	100	120
[7]	1	140	102	123	140	100	120
	2	141	108	124	140	103	121
	3	145	113	128	144	102	123
	4	143	111	125	143	100	121
	5	141	114	126	139	102	120
[8]	1	140	112	126	143	100	122
	2	139	109	123	142	102	123
	3	140	101	125	140	101	124
	4	138	107	128	143	101	121
	5	140	108	125	141	104	120
[9]	1	144	111	126	143	100	119
	2	140	111	124	139	97	120
	3	144	114	126	141	100	120
	4	141	112	123	139	96	117
	5	140	103	124	145	98	119
[10]	1	143	102	121	142	103	122
	2	133	117	124	143	102	121
	3	137	105	123	140	104	122
	4	140	105	124	139	104	120
	5	143	108	123	138	103	121
[11]	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	142	108	125	138	100	119
[12]	1	136	105	123	142	104	119
	2	135	102	122	140	97	119
	3	146	103	125	139	105	120
	4	149	101	125	143	101	121
	5	144	109	131	140	100	120
[13]	1	142	115	127	135	98	118
	2	140	106	123	142	101	121
	3	142	108	125	141	102	120
	4	139	110	125	143	101	120
	5	146	105	126	142	101	118
[14]	1	149	111	130	143	106	124
	2	152	112	133	145	103	124
	3	150	111	131	146	104	125
	4	142	103	122	134	102	118
	5	140	106	123	138	101	119
Control	**Losartan**	118	-	-	-	-	-

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	126	103	114	126	96	111
	2	129	101	115	119	104	111
	3	123	107	115	121	99	110
	4	127	105	119	123	103	113
	5	123	101	113	124	103	112
[2]	1	131	105	118	124	101	115
	2	126	103	114	128	106	117
	3	124	106	115	127	104	116
	4	127	105	116	125	105	115
	5	132	96	114	130	101	116
[3]	1	129	108	119	124	104	114
	2	122	112	117	122	103	112
	3	126	114	124	128	107	117
	4	124	111	121	123	104	113
	5	126	104	115	127	107	117
[4]	1	127	105	122	126	105	115
	2	129	108	121	124	104	114
	3	122	112	117	122	103	112
	4	126	114	120	128	107	117
	5	124	111	118	123	104	113
[5]	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	122	100	112
[6]	1	124	100	112	128	101	113
	2	130	104	117	128	102	115
	3	125	105	115	124	101	112
	4	122	100	111	126	104	115
	5	128	102	115	130	103	116
[7]	1	128	105	114	121	103	112
	2	126	100	113	124	101	112
	3	123	102	112	123	102	111
	4	122	101	111	126	102	114
	5	124	102	113	125	102	112
[8]	1	126	103	114	126	96	111
	2	129	101	115	119	104	111
	3	123	107	115	121	99	110
	4	127	105	119	123	103	113
	5	123	101	113	124	103	112
[9]	1	131	105	118	124	101	115
	2	126	103	114	128	106	117
	3	124	106	115	127	104	116
	4	127	105	116	125	105	115

	5	132	96	114	130	101	116
[10]	1	122	102	111	123	102	112
	2	128	103	115	125	101	113
	3	126	104	115	122	100	111
	4	123	103	113	123	102	112
	5	124	104	114	124	104	114
[11]	1	126	101	113	128	102	115
	2	123	101	112	125	100	112
	3	122	100	111	126	102	115
	4	124	102	112	126	102	111
	5	126	101	113	124	104	114
[12]	1	128	102	115	126	104	115
	2	125	105	115	122	100	112
	3	124	101	112	124	100	112
	4	122	100	111	121	103	112
	5	124	102	113	124	106	115
[13]	1	122	103	112	122	105	114
	2	124	102	111	125	102	114
	3	126	100	113	121	101	111
	4	124	101	112	122	102	114
	5	128	105	114	121	103	112
[14]	1	126	100	113	124	101	112
	2	123	102	112	123	102	111
	3	122	101	111	126	102	114
	4	124	102	113	125	102	112
	5	122	104	112	125	101	113
Control	**Losartan**	102	-	-	-	-	-

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	**165**	**161**	**152**	**146**	**141**	**137**	**132**	**122**	**115**	**109**
1	172	163	156	149	141	133	129	127	125	123
2	177	170	167	161	156	148	142	137	133	130
3	174	168	160	155	149	141	134	129	126	123
4	177	169	160	152	145	140	132	129	126	123
5	175	168	159	150	146	141	136	132	129	126
6	176	170	162	159	150	143	137	134	131	128
7	170	164	159	152	145	139	135	132	129	125
8	173	166	160	153	146	139	132	129	126	124
9	169	153	149	144	141	137	132	128	125	119
10	179	172	168	163	159	153	148	145	139	133
11	177	169	161	156	150	144	138	130	128	125
12	181	176	170	165	159	151	143	137	130	122
13	170	164	158	152	149	143	139	136	131	128
14	169	160	154	146	142	139	135	131	129	126

Table: 4 Antihypertensive Activity of synthesized compounds

Compound. No	Minimum Blood pressure value(mm Hg)	Duration of hypertension effect(min.)
Losratan	109	90
1	117	105
2	116	110
3	118	96
4	115	111
5	121	100
6	119	105
7	118	110
8	121	100
9	118	110
10	117	115
11	120	107
12	118	105
13	118	100
14	114	100

RESULTS AND DISCUSSION

StepI, 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 Chloroacetyl chloride (0.01mol) was added drop wise to a mixture of schiff base (0.01mol) and triethylamine (0.02 mol) in dioxane (25 ml) at room temperature. The mixture was stirred for 8 h and allowed to stand at room temperature for 3 days. 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 which was then subjected to aromatic substitution reaction using paraformaldehyde and acetamide in conc. sulphuric acid 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 to produce the pendant moiety 4-(bromomethyl) biphenyl-2'-carboxylic acid and synthesis biphenyl with carboxylic compound [12, 18,]. Almost all the newly synthesized substituted aryl group showed good antihypertensive activity with the goal of investigating the structure–activity relationships of benzimidazole and purity have been established through appropriate spectral and chromatographic techniques. 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 positions has been found to be a function of substitute aryl groups 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. Taking Losartan as lead compound we had fused the benzene ring with imidazole and coupling reaction with 4-chloromethyl biphenyl 2'- carboxylic acid to get the resulting compounds which shows hypertensive standard compared with our synthesis molecules activity. In the biphenyl ring carboxylic group at ortho position is necessary for pharmacological activity.

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REFERENCES

[1] Ferrario CM, *J. Cardiovasc. Pharmacol*, **1990**, 15 (Suppl. 3), 51-55.
[2] Vallotton M B, *Trends Pharmacol. Sci*, **1987**, 8, 69.
[3] Nahmias C, Strosberg A. D, *Trends Pharmacol. Sci*, **1995**, 16, 223-225.
[4] Berecek K H, King S J, Wu JN, Angiotensin-Converting Enzyme and Converting Enzyme Inhibitors. Cellular and Molecular Biology of the Renin-Angiotensin System; CRC Press: Boca Raton, FL, **1993**, 183-220.
[5] Griendling KK, Minieri CA, Ollerenshaw JD, Alexander RW, Circ. Res., **1994,** 74, 1141-1148.
[6] Dutta AS, Testa B, Ed.Academic Press: London, 21, 147-286, **1991**.
[7] McEwan JR, FullerRW. *J. Cardiovasc. Pharmacol*, **1989**,*13* (Suppl. 3), S67-S69.
[8] Furukawa Y, Kishimoto S, Nishikawa S. *U.S. Patent* 4340598,(**1982**).
[9] Carini DJ, Duncia JV, Aldrich PE, Chiu AT, Johnson AL, Pierce ME, Price WA, Santella JB, Wells GJ, Wexler RR, Wong PC, Yoo SE, Timmermans PBMWM, *J. Med. Chem*, **1991**,34, 2525-2547.
[10] 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.
[11] Dhvanit I S, Sharma M, Bansal Y,Bansal G, M. Singh, European Journal of Medicinal Chemistry, **2008**,43, 1808-1812.
[12] Jat RK, Jat JL, Pathak DP, *Euro. Journal. of Chemistry*., **2006**,3:(13), 278-285.
[13] Badyal DK, Lata H, Dadhich AP, Animal models of hypertension and effect of drugs. *Indian J of Pharmacology*, **2003**, 35(66), 349-362.
[14] Bunag RD, McCubbin JW, Page IH, Lack of correlation between direct and indirect measurements of arterial pressure in unanaesthetized rats, *Cardiovasc. Res*, **1971**, 5(1): 24-31.
[15] Gupta SK, Drug Screening methods, Jaypee Brothers Medical Publisher, New Delhi, **2004**, 236-246.
[16] Shreenivas MT, Chetan BP, Bhat AR, *J. of Pharma.Sci. And Technology*, **2009**, 1 (2), 88-94.
[17] Siddiqui AA, Wani M.S, *Indian.J.Chemistry*, **2004**, 43B, 1574-1579.
[18] Vogel G.H.Drug Discovery and Evaluation, Pharmacological Assay, **2002**;(Springer. Berlin), 122.
[19] Shanmugapandiyan P, Denshing KS, Ilavarasan N R, Anbalagan RN. *Int. J. of Pharma. Scien., and Drug Research* **2010**, 2(2): 115-119.