

SYNTHESIS AND BIOLOGICAL EVALUATION 6-CHLORO-2-SUBSTITUED-1-[2-(1H-TETRAZOL-5-YL)-BIPHENYL-4-YLMETHYL]-1H-BENZOIMIDAZOL-5-YLAMINE ANTIHYPERTENSIVE AGENTS

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A series of methyl 6-Chloro-2-Substitued-1-[2-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-1H-benzoimidazol-5-ylamine were successfully synthesized. Benzimidazoles were prepared by condensation of 4-chloro-o-phenylenediamine with substituted aryl, aldehyde condensation with biphenyl tetrazole group and reduction stannous chloride dihydrate. An insertion-cyclization strategy was involved during synthetic path sequences. Structures of all the synthesized compounds have been corroborated on the basis of elemental IR, ¹H NMR, ¹³C NMR and Mss spectra-analytical data.

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Keywords: 5-amino-benzimidazole; AT₁ receptor antagonists; Antihypertensive activity, Blood pressure

1. Introduction

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. 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 re-absorption, 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 adrenocorticotrophic 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 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 Amongthem, 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). Whereas reports on effective replacements of the biphenyl tetrazole "tail" of losartan 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. Initial 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 bioavailability¹¹. Only in recent years a number of non peptides AngII antagonists that show promise as inhibitors of the RAS been reported¹². All these antagonists possess a central aromatic nucleus bearing the pharmacophores indispensable for activity and notably a polar function adjacent to biphenyl substituents while a polar function in this area of molecule seems to be necessary to maintain activity¹³. 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 head¹⁴. The substituent at 6-position on the nucleus increases the activity whereas small substituent at 5-position decreases the activity¹⁵. Compounds containing tetrazole nucleus are also reported as AT₁ receptor antagonists and their prototypical derivative 3 exhibits non-competitive antagonism¹⁶ 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 isomers 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 man¹⁷⁻¹⁸.

2. 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.

MCS-01- 6-chloro-2-substitued -1H-benzoimidazole²⁰

4-chloro-benzene-1, 2-diamine (5.0 gm) was dissolved in a mixture of substituted benzaldehydes, aryl groups using sodium hydrogen sulfite in dimethylacetamide. The reaction mixture was then heated to reflux under vigorous stirring for three hours after this a white precipitate was formed. The mixture was filtered hot and then washed with water to afford a yellow solid. The precipitate was again dissolved in absolute ethanol (50ml) and to this; HCl (25 ml) was added in water (100 ml). At this stage, the reaction mixture was heated at reflux for 4.0 hours. Reaction mixture was allowed to cool to room temperature and filtered through a pad of celite to remove the precipitated

CuS. The filtrate was treated with ammonia solution to pH 7.5-8.2 and then concentrated to yield precipitate. After filtration and vacuum evaporation, compound obtained as solid product.

MCS-02: 6-chloro-2-substitued -5-nitro-1H-benzoimidazole

65.0 ml of concentrated nitric acid was placed in three necked flask and equal quantity of concentrated sulphuric acid (1:1) was added slowly. The mixture was kept in the ice cold water then compound MCS-01 (1.5 gm) was mixed in portions during 3.5 hours under room temperature. After stirred continuously for 10 hours and then the reaction mixture was poured slowly over crushed ice with stirring. The precipitated product was filtered out and washes with cold water. The final product recrystallized from absolute ethanol.

MCS-03-(6-chloro-2-Substitued-5-nitro-1-benzoimidazol-1-ylmethyl)-biphenyl-2-carbonitrile

To a solution of 2.0 g (10.12 mmol) of 6-chloro-2-substitued -5-nitro-1H-benzoimidazole in 65 mL of DMF was added potassium carbonate 0.5 g (5.52 mmol), the mixture was stirred for 1.3 hours at room temperature, and 4-(bromomethyl) biphenyl-2'-nitrile 6.50 g (20.12 mmol) was added. After stirring for 18 hours the mixture was poured into distilled water (120 mL) and extracted with diethyl ether (6 × 20 mL). The combined extracts were dried (MgSO₄) and evaporated.

MCS-04-6-Chloro-2-Substitued-5-nitro-1-[2-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-1H-benzoimidazol-

A mixture of (6-chloro-2-Substitued-5-nitro-1-benzoimidazol-1-ylmethyl)-biphenyl-2-carbonitrile (2.5 g, 3.08 mmol), sodium azide (1.21 g, 13.43 mmol), and Et₃N·HCl (2.1 g, 10.05 mmol) in NH₄Cl (15 mL) is stirred at 160°C for 15 hours. After cooling, the mixture is diluted with distilled water (50 mL), acidified to pH 4.5 with 4N HCl, and extracted with EtOAc (3 × 50 mL). The organic layer was washed with H₂O (3 × 50 mL), then the combined extracts were dried (MgSO₄) and evaporated and the solid residue was purified by silica gel column chromatography eluting with ethyl acetate/ethanol (80:20/v: v) to give solid. Compounds.

MCS-05- 6-Chloro-2-Substitued-1-[2-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-1H-benzoimidazol-5-ylamine

1.5 gm of compounds (MCS-04) was placed in three necked RBF and dissolved in absolute ethanol and heated to 60°C under reflux. To this, 0.5 gm stannous chloride dihydrate was added with slow stirring during 3.0 hours and reaction conditions were maintained for further 10 hours. The mixture was cooled to room temperature and pH adjusted to 8.5 with 5% sodium hydroxide solution. The organic layer was washed with brine, distilled water then dried over anhydrous sodium sulphate. Solvent removed under vacuum and product was obtained.

[1]6-Chloro-2-phenyl-1-[2-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-1H benzoimidazol-5-ylamine

Yield: 73 %, m.p. =143⁰-145⁰C. Anal. Calcd for C₂₇H₂₀ClN₇: C, 67.85; H, 4.22; N, 20.51 %; IR (KBr): 3611, 2899, 2806, 1633, 1675, 1529, 1522-1341, 1104, 1249. 796.3, 674. ¹HNMR (300Hz, CDCl₃) 9.62(s, 1H, tetrazole-NH), 4.87(s, 2H, CH₂), 7.11-8.74(m, 15H, ArH), 4.55(s, 2H, aromatic C-NH). ¹³CNMR(CDCl₃) δ: 55.3, 112.2, 113, 114.1, 115.6, 122, 127, 128, 129, 133, 134.3, 135.8, 138.4 FAB-MS, 477.16

[2]6-Chloro-2-propyl-1-[2-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-1H-benzoimidazol-5-ylamine

Yield: 66%, m.p. =129.4-131⁰C. Mol. weight 444.14, Anal. Calcd for C₂₄H₂₂ClN₇: C, 64.93; H, 5.01; N, 22.09%. IR (KBr): 3564, 29983, 2873.2, 2384.1, 1678-1632, 1511.1321, 1187, 799.6, 652. ¹HNMR (300Hz, CDCl₃) 9.72(s, 1H, tetrazole-NH), 0.87(s, 3H, CH₃), 4.94(s, 2H, CH₂), 1.88(s, 2H, CH₂), 2.65(s, 2H, CH₂), 7.19-8.24(m, 10H, ArH), 4.55(s, 2H, aromatic C-NH). ¹³CNMR(CDCl₃) δ: 17, 53.1, 55.3, 61.1, 70.7, 111.4, 113.1, 113.5, 114.1, 121.2, 132.2, 145.1, 148.1, FAB-MS, 443.21

[3]2-Butyl-6-Chloro-1-[2-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-1H-benzoimidazol-5-ylamine

Yield: 61%, m.p. =129.4-131⁰C. Mol. weight 457.17, Anal. Calcd for C₂₅H₂₄ClN₇: C, 65.57; H, 5.28; N, 21.41%. IR (KBr): 3498, 3104, 2983.4, 28878.4, 2305, 1673.8, 1563-

1321.8, 1187.0, 799.4, 650.6¹HNMR(300Hz, CDCl₃). 9.89(s, 1H, tetrazole-NH), 0.99(s, 3H, CH₃), 5.04(s, 2H, CH₂), 1.65(s, 2H, CH₂), 2.34s, 2H, CH₂, 7.10-8.14(m, 10H, ArH), 4.22(s, 2H, aromatic C-NH) 1.85(s, 2H, CH₂).
¹³CNMR(CDCl₃)δ: 20, 42.4, 50.2, 58.3, 60.1, 75.3, 112.7, 114.5, 116.1, 119.1, 123.1, 136.2, 139.1, 145.0, FAB-MS, 456.05

[4] 6-Chloro-2-(2-chloro-phenyl)-1-[2-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-1H-benzoimidazol-5-ylamine

Yield: 69%, m.p. = 164-169^oC. Mol. weight 512.392, Anal. Calcd for C₂₅H₁₉Cl₂N₇. C, 63.29; H, 3.78; N, 19.14%. IR(KBr): 3485.5, 3284, 2984, 2847, 2305, 1677, 1532.6, 1549, 1322, 1184, 799.9, 643.2.¹H NMR(300Hz, CDCl₃). 9.65(s, 1H, tetrazole-NH), 5.34(s, 2H, CH₂), 7.15-8.48(m, 14H, ArH), 4.22(s, 2H, aromatic C-NH)
¹³CNMR(CDCl₃)δ: 47, 111.1, 115.3, 119.5, 121.2, 127.5, 129.3, 133.2, 142.0, FAB-MS, 511.079

[5] 6-Chloro-2-(3-chloro-phenyl)-1-[2-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-1H-benzoimidazol-5-ylamine

Yield: 92%, m.p. = 160-162^oC. Mol. weight 512.392, Anal. Calcd for C₂₅H₁₉Cl₂N₇. C, 63.29; H, 3.78; N, 19.14%. IR(KBr): 3497.2, 3280, 2963, 2840, 2312, 1645, 1524.2, 1503, 1320, 1194, 797, 643.0.¹H NMR(300Hz, CDCl₃). 9.69(s, 1H, tetrazole-NH), 5.30(s, 2H, CH₂), 7.05-8.08(m, 14H, ArH), 4.29(s, 2H, aromatic C-NH)
¹³CNMR(CDCl₃)δ: 47, 111.1, 115.3, 119.5, 121.2, 127.5, 129.3, 133.2, 142.0, FAB-MS, 513.21

[6] 6-Chloro-2-(4-chloro-phenyl)-1-[2-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-1H-benzoimidazol-5-ylamine

Yield: 88%, m.p. = 165-167^oC. Mol. weight 512.392, Anal. Calcd for C₂₅H₁₉Cl₂N₇. C, 63.29; H, 3.78; N, 19.14%. IR(KBr): 3481.5, 3277, 2995, 2888, 2333, 1632, 1527.2, 1532, 1321, 1190, 800.3, 645.1.¹H NMR(300Hz, CDCl₃). 9.59(s, 1H, tetrazole-NH), 5.21(s, 2H, CH₂), 7.22-8.17(m, 14H, ArH), 4.27(s, 2H, aromatic C-NH).¹³CNMR(CDCl₃)δ: 47, 111.1, 115.3, 119.5, 121.2, 127.5, 129.3, 133.2, 142.0, FAB-MS, 510.54

[7] 6-Chloro-2-(2-methoxy-phenyl)-1-[2-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-1H-benzoimidazol-5-ylamine

Yield: 73 %, m.p. = 189-194^oC. Mol. weight 507.15, Anal. Calcd for C₂₈H₂₂Cl₂N₇O. C, 66.20; H, 4.37; N, 19.30%. IR(KBr): 3532.2, 2911, 2890, 2325, 1644, 1512.2, 1505, 1355, 1185, 804.5, 646.¹H NMR(300Hz, CDCl₃). 9.52(s, 1H, tetrazole-NH), 5.11(s, 2H, CH₂), 6.94-8.22(m, 14H, ArH), 4.43(s, 2H, aromatic C-NH) 3.98 (s, 3H, OCH₃),¹³CNMR(CDCl₃)δ: 18.6, 47, 111.1, 115.3, 119.5, 121.2, 127.5, 129.3, 133.2, 141.1, 144.6. FAB-MS, 507.11

[8] 6-Chloro-2-(3-methoxy-phenyl)-1-[2-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-1H-benzoimidazol-5-ylamine

Yield: 76%, m.p. = 181-184^oC. Mol. weight 507.15, Anal. Calcd for C₂₈H₂₂Cl₂N₇O. C, 66.20; H, 4.37; N, 19.30%. IR(KBr): 3532.2, 2911, 2890, 2325, 1644, 1512.2, 1505, 1355, 1185, 804.5, 646.¹H NMR(300Hz, CDCl₃). 9.56(s, 1H, tetrazole-NH), 5.16(s, 2H, CH₂), 6.94-8.22(m, 14H, ArH), 4.43(s, 2H, aromatic C-NH) 3.98 (s, 3H, OCH₃),¹³CNMR(CDCl₃)δ: 18.6, 47, 111.1, 115.3, 119.5, 121.2, 127.5, 129.3, 133.2, 141.1, 144.6. FAB-MS, 507.854

[9] 6-Chloro-2-(4-methoxy-phenyl)-1-[2-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-1H-benzoimidazol-5-ylamine

Yield: 82%, m.p. = 183-187^oC. Mol. weight 507.15, Anal. Calcd for C₂₈H₂₂Cl₂N₇O. C, 66.20; H, 4.37; N, 19.30%. IR(KBr): 3532.2, 2911, 2890, 2325, 1644, 1512.2, 1505, 1355, 1185, 804.5, 646.¹H NMR(300Hz, CDCl₃). 9.52(s, 1H, tetrazole-NH), 5.11(s, 2H, CH₂), 6.89-8.39(m, 14H, ArH), 4.48(s, 2H, aromatic C-NH) 3.85 (s, 3H, OCH₃),¹³CNMR(CDCl₃)δ: 18.6, 47, 111.1, 115.3, 119.5, 121.2, 127.5, 129.3, 133.2, 141.1, 144.6. FAB-MS, 508.14

[10] 6-Chloro-2-(2-nitro-phenyl)-1-[2-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-1H-benzoimidazol-5-ylamine

Yield: 79%, m.p. = 148-152^oC. Mol. weight 522.94, Anal. Calcd for C₂₇H₁₉ClN₈O₂. C, 62.01 %; H, 3.66; N, 21.43%. IR(KBr): 3615.5, 2988.3, 2875, 2354, 1625, 1504.1, 1526, 1325, 1154, 811.4, 656.¹H NMR(300Hz, CDCl₃). 9.68(s, 1H, tetrazole-NH), 5.00(s, 2H, CH₂), 6.99-8.25(m, 14H, ArH), 4.48(s, 2H, aromatic C-NH),¹³CNMR(CDCl₃)δ: 50.2, 111., 113.1, 115.2, 123.1, 130.4, 135.1, 138.1, 141.2, 143.2, 146.3 FAB-MS, 521.03

[11]6-Chloro-2-(3-nitro-phenyl)-1-[2-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-1H-benzoimidazol-5-ylamine

Yield:81%,m.p.=144-146^oC.Mol.weight522.94,Anal.Calcd for C₂₇H₁₉ClN₈O₂:C,62.01 0;H,3.66;N,21.43%.IR(KBr):3615.5,2988.3,2875,2354,1625,1504.1,1526,1325,1154,811.4,656.¹HNM R(300Hz,CDCl₃).9.75(s,1H,tetrazole-NH),4.96(s,2H,CH₂),7.07-8.14(m,14H,ArH),4.48(s,2H,aromaticCNH),¹³CNMR(CDCl₃)δ:50.2,111.,113.1,115.2,123.1,130.4,135.1,138.1,141.2,143.2,146.3FAB-MS, 521.65

[12]6-Chloro-2-(4-nitro-phenyl)-1-[2-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-1H-benzoimidazol-5-ylamine

Yield:65%,m.p.=146-149^oC.Mol.weight522.94,Anal.Calcd for C₂₇H₁₉ClN₈O₂:C,62.01 0;H,3.66;N,21.43%.IR(KBr):3615.5,2988.3,2875,2354,1625,1504.1,1526,1325,1154,811.4,656.¹HNM R(300Hz,CDCl₃).9.63(s,1H,tetrazole-NH),5.09(s,2H,CH₂),7.03-8.21(m,14H,ArH),4.48(s,2H,aromaticCNH),¹³CNMR(CDCl₃)δ:50.2,111.,113.1,115.2,123.1,130.4,135.1,138.1,141.2,143.2,146.3FAB-MS, 522.14

[13]6-Chloro-2-(2-fluoro-phenyl)-1-[2-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-1H-benzoimidazol-5-ylamine

Yield:59%,m.p.=207-210^oC.Mol.weight495.32,Anal.Calcd for C₂₇H₁₉ClFN₇:C,65.39;H,3.86;N,19.77%.IR(KBr):3503.6,2954,2824,1648,1515,1552,1304,1143,801,652.6.¹HNM R(300Hz,CDCl₃).9.95(s,1H,tetrazole-NH),5.13(s,2H,CH₂),7.43-8.75(m,14H,ArH),4.48(s,2H,ar-CNH),¹³CNMR(CDCl₃)δ: 58.0,112.9,113.4,116.2,121.1,128.4,135.5,137.2,140.3FAB-MS, 495.13

[14]6-Chloro-2-(3-fluoro-phenyl)-1-[2-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-1H-benzoimidazol-5-ylamine

Yield:66%,m.p.=204-206^oC.Mol.weight495.32,Anal.Calcd for C₂₇H₁₉ClFN₇:C,65.39;H,3.86;N,19.77%.IR(KBr):3503.6,2954,2824,1648,1515,1552,1304,1143,801,652.6.¹HNM R(300Hz,CDCl₃).9.95(s,1H,tetrazole-NH),5.13(s,2H,CH₂),7.43-8.75(m,14H,ArH),4.48(s,2H,ar-CNH),¹³CNMR(CDCl₃)δ: 58.0,112.9,113.4,116.2,121.1,128.4,135.5,137.2,140.3FAB-MS, 494.04

[15]6-Chloro-2-(4-fluoro-phenyl)-1-[2-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-1H-benzoimidazol-5-ylamine

Yield:70%,m.p.=211-213^oC.Mol.weight495.32,Anal.Calcd for C₂₇H₁₉ClFN₇:C,65.39;H,3.86;N,19.77%.IR(KBr):3503.6,2954,2824,1648,1515,1552,1304,1143,801,652.6.¹HNM R(300Hz,CDCl₃).9.95(s,1H,tetrazole-NH),5.13(s,2H,CH₂),7.43-8.75(m,14H,ArH),4.48(s,2H,ar-CNH),¹³CNMR(CDCl₃)δ: 58.0,112.9,113.4,116.2,121.1,128.4,135.5,137.2,140.3FAB-MS, 496.17

[16]6-Chloro-2-(2-Iodo-phenyl)-1-[2-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-1H-benzoimidazol-5-ylamine

Yield:53%,m.p.=279-283^oC.Mol.weight603.04,Anal.Calcd for C₂₇H₁₉ClIN₇:C,53.70;H,3.17;N,16.24%.IR(KBr):3521.4,2912,2885,1636,1529,1500,1317,1136,791,646.5.¹HNM R(300Hz,CDCl₃).9.84(s,1H,tetrazole-NH),5.05(s,2H,CH₂),7.34-8.63(m,14H,ArH),4.43(s,2H,ar-CNH),¹³CNMR(CDCl₃)δ: 53.1,55.3,61.1,70.7,111.4,113.1,113.5,114.1,121.2,142.3,FAB-MS, 604.046

[17]6-Chloro-2-(3-Iodo-phenyl)-1-[2-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-1H-benzoimidazol-5-ylamine

Yield:53%,m.p.=276-278^oC.Mol.weight603.04,Anal.Calcd for C₂₇H₁₉ClIN₇:C,53.70;H,3.17;N,16.24%.IR(KBr):3521.4,2912,2885,1636,1529,1500,1317,1136,791,646.5.¹HNM R(300Hz,CDCl₃).9.80(s,1H,tetrazole-NH),5.05(s,2H,CH₂),7.30-8.61(m,14H,ArH),4.41(s,2H,ar-CNH),¹³CNMR(CDCl₃)δ: 53.1,55.3,61.1,70.7,111.4,113.1,113.5,114.1,121.2,142.3,FAB-MS, 603.17

[18]6-Chloro-2-(4-Iodo-phenyl)-1-[2-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-1H-benzoimidazol-5-ylamine

Yield:53%,m.p.=278-282^oC.Mol.weight603.04,Anal.Calcd for C₂₇H₁₉ClIN₇:C,53.70;H,3.17;N,16.24%.IR(KBr):3521.4,2912,2885,1636,1529,1500,1317,1136,791,646.5.¹HNM R(300Hz,CDCl₃).9.89(s,1H,tetrazole-NH),5.01(s,2H,CH₂),7.24-8.53(m,14H,ArH),4.48(s,2H,ar-CNH),¹³CNMR(CDCl₃)δ: 53.1,55.3,61.1,70.7,111.4,113.1,113.5,114.1,121.2,142.3,FAB-MS, 603.68

Pharmacological Activity

Procedure for development of hypertension for normotensive rats ²¹

Albino normotensive rats (Wistar Strain) were taken and they were hypertensitized by cholinomimetic agents for screening of all the synthesized benzimidazole derivatives for their 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: ²²(i) Invasive method (Direct method).
 (ii) Non-invasive Tail cuff method (Indirect method).
 (b) In-vitro determination of vasodilator activity by aortic rings.

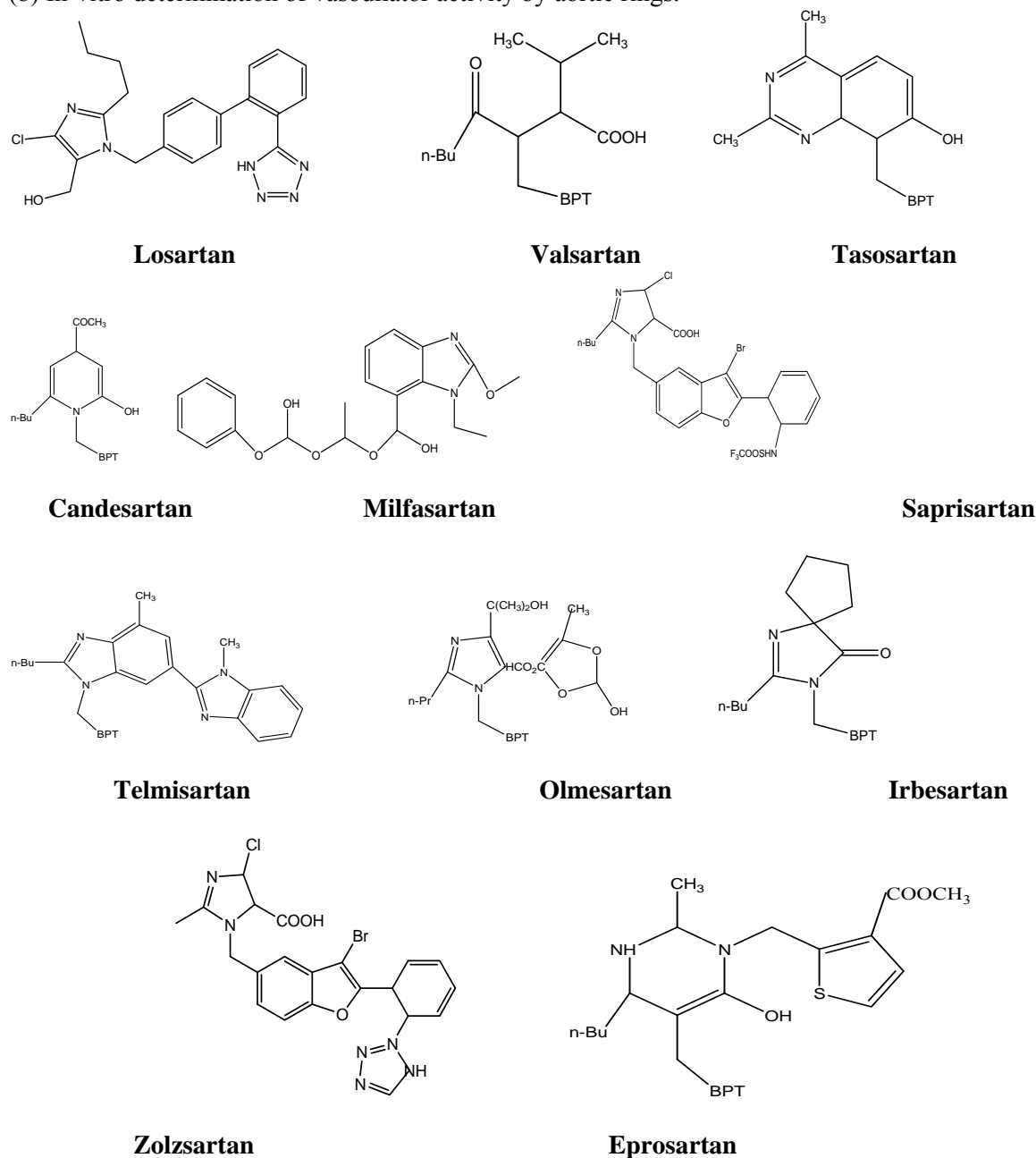
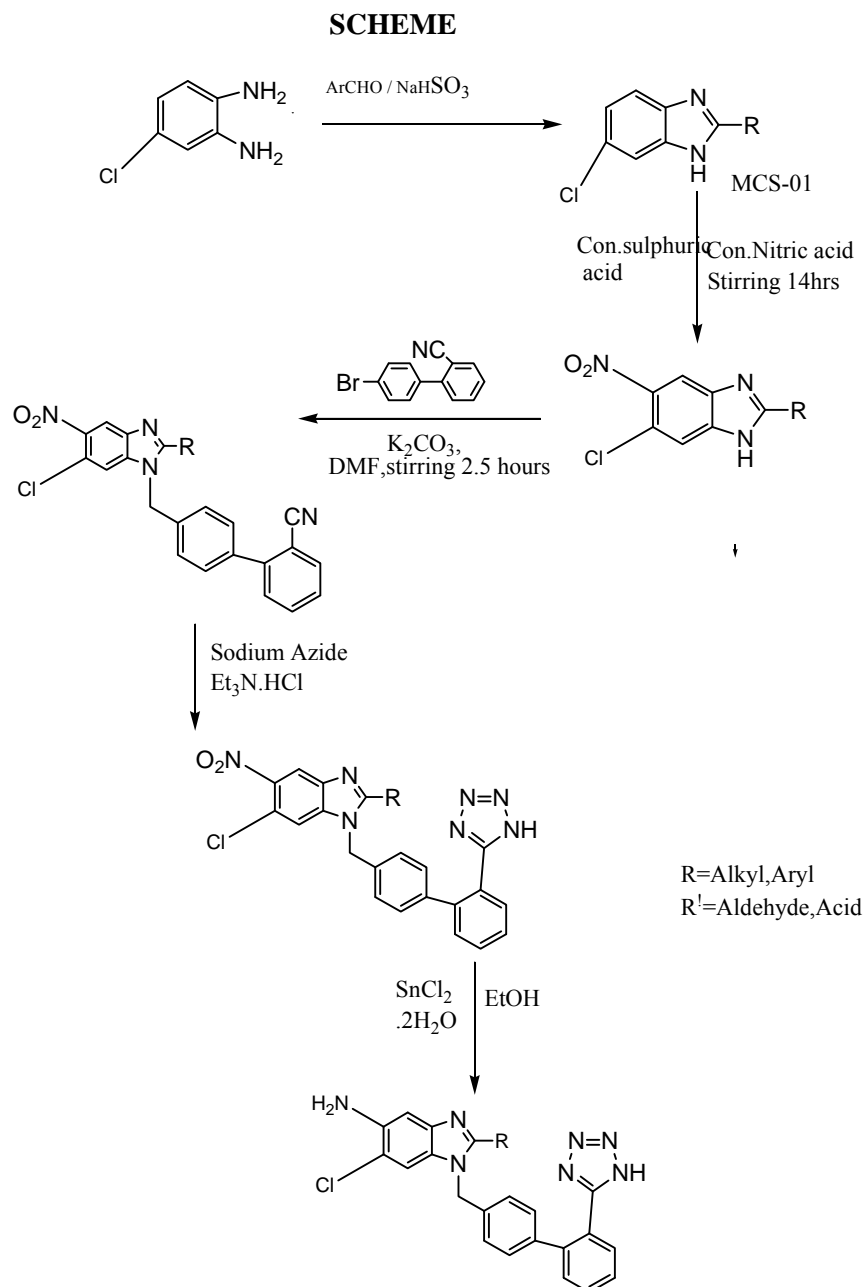


Figure 1 Angiotensin II AT₁ selective antagonists



6-Chloro-2-Substitued -1-[2'-(1H-tetrazol-5-yl)]-biphenyl-4-ylmethyl]-1H-benzoimidazol-5-ylamine

3. Experimental techniques

(i) Invasive Method (Direct Method).²³⁻²⁴ Male albino wistar (150-250 gm) rats were used and housed at $24 \pm 1^\circ\text{C}$ room temperature. The rats were anaesthetized with sodium chloride 0.9% solution, Drug solution $10\text{-}\mu\text{g}/100\text{ml}$, and Heparin 500 I.U. solution urethane hydrochloride 50% w/v solution $80\text{ mg}/\text{kg}$ 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 losartan. 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	172	169	158	145	133	129	121	116	111	108
1	183	172	169	157	143	142	134	128	126	119
2	178	173	165	155	150	142	138	130	125	122
3	174	168	157	152	147	138	130	126	122	117
4	169	162	155	149	145	134	130	124	116	109
5	174	169	164	157	150	144	138	130	123	107
6	177	171	165	159	150	146	143	139	135	131
7	169	165	160	155	149	142	138	134	130	128
8	151	147	143	136	131	128	123	118	113	108
9	146	142	138	133	129	124	122	119	115	110
10	158	153	149	144	141	137	132	128	125	121
11	171	167	164	158	151	148	142	137	134	127
12	163	157	151	146	140	136	128	122	117	114
13	152	150	146	142	138	132	129	125	118	113
14	143	141	138	135	130	126	123	118	115	111
15	166	163	160	155	149	142	138	134	127	120
16	175	169	165	159	154	148	142	136	130	127
17	173	166	162	156	148	141	138	131	126	116
18	168	162	155	147	140	133	128	121	117	110

Table: 2 Antihypertensive Activity of synthesized compounds

Compound. No	Minimum blood pressure value(mm Hg)	Duration of hypertension effect(min.)
Losartan	108	90
1	115	100
2	112	98
3	107	110
4	105	100
5	113	90
6	124	120
7	122	105
8	108	90
9	110	90
10	116	120
11	122	110
12	112	120
13	111	100
14	107	96
15	116	110
16	121	120
17	114	115
18	106	100

(ii) Non-invasive Tail cuff Method (Indirect Method).²⁵⁻²⁶ 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 restrainer, 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	140	102	124	140	103	117
	2	138	104	122	137	104	120
	3	142	112	127	139	102	122
	4	140	108	124	143	101	121
	5	137	104	121	140	103	121
[2]	1	143	110	127	134	102	113
	2	137	102	124	135	102	111
	3	139	107	123	140	101	120
	4	143	109	126	137	104	120
	5	141	109	125	139	102	110
[3]	1	140	106	123	138	101	119
	2	138	104	121	140	106	123
	3	141	109	125	143	106	124
	4	136	112	124	141	103	122
	5	142	112	127	140	103	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
	6	133	113	123	141	109	125
[5]	1	141	110	126	140	108	124
	2	138	105	122	139	109	124
	3	132	104	118	142	106	124
	4	142	103	123	140	106	123
	5	140	105	123	138	104	121
[6]	1	139	108	124	141	103	122

	2	140	113	127	136	104	120
	3	144	103	124	141	103	122
	4	141	111	126	140	104	122
	5	141	104	123	137	106	121
[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	142	112	127	140	102	121
	2	144	116	130	141	101	122
	3	142	110	126	139	104	123
	4	146	106	126	144	104	124
	5	144	104	124	140	100	120
[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	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
[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	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	139	105	123	138	198	118
[14]	1	138	112	125	141	102	121
	2	143	114	125	142	102	122
	3	146	102	124	143	101	120
	4	144	114	124	141	100	119
	5	139	102	122	143	100	121
[15]	1	142	113	125	142	102	122
	2	141	109	123	144	101	121
	3	144	114	129	141	104	120
	4	146	104	132	142	100	121

	5	148	104	125	145	102	123
[16]	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	142	104	126	139	105	122
[17]	1	140	118	128	143	110	122
	2	135	116	125	142	104	120
	3	139	112	124	146	102	121
	4	144	116	126	144	101	121
	5	139	105	126	146	106	120
[18]	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	147	114	127	140	100	120
Control	Losartan	112	-	-	-	-	-
	Telmisartan	113	-	-	-	-	-

Table 4. Reduction in blood pressure (mm Hg) at a dose of 50 $\mu\text{g}/\text{kg}$ animal body weight.

Comp.	Exp. Animal Albino (Wistar) Rat	After 1hour			After 3 hour		
		SBP	DBP	MABP	SBP	DBP	MABP
[1]	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	126	101	113	128	102	115
[2]	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	125	105	115	122	100	112
[3]	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	126	100	113	121	101	111
[4]	1	124	101	112	122	102	114
	2	128	105	114	121	103	112
	3	126	100	113	124	101	112
	4	123	102	112	123	102	111
	5	124	102	113	125	102	112
[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	126	101	113	125	100	113
	2	131	105	118	125	100	115
	3	128	104	116	123	101	112
	4	125	105	115	126	104	115
	5	126	106	116	122	100	111
[7]	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	127	103	115	130	102	115
[8]	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
[9]	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	123	107	115	125	100	112
[10]	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
[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	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
[14]	1	127	105	118	126	105	115
	2	129	108	124	124	104	114
	3	122	112	121	122	103	112
	4	126	114	120	128	107	117
	5	124	111	118	123	104	113
[15]	1	127	105	114	126	105	115
	2	129	108	119	124	104	114
	3	122	112	118	122	103	112

	4	126	114	123	128	107	117
	5	124	111	118	123	104	113
	6	126	104	117	127	107	117
[16]	1	127	105	119	126	105	115
	2	129	108	119	124	104	114
	3	122	112	117	122	103	112
	4	126	114	124	128	107	117
	5	124	111	121	123	104	113
	6	126	104	115	127	107	117
[17]	1	127	105	114	126	105	115
	2	129	108	118	124	104	114
	3	122	112	117	122	103	112
	4	126	114	123	128	107	117
	5	126	104	115	127	107	117
[18]	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
Control	Losartan	112	-	-	-	-	-
	Telmisartan	113	-	-	-	-	-

4. Results and discussion

The synthesized compounds were characterized on the basis of chemical and spectral data. The present work was mainly intended to establish the moieties which are responsible for Angiotension-II inhibition. Ang II antagonism by compounds with same functional group at 2 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 [18]). The maximum antihypertensive activity has been observed with amino group Compound number 2, 4, 6, 7, 9, 10, 12,13,14,18. This suggests that there are some sites in the receptor pocket, which can interact with the functional groups at position 2-Substitued benzimidazole nucleus coupled to tetrazole biphenyl group has been designed, synthesized and evaluated for angiotensin II antagonism. Biological activity of synthesized compounds was carried out using rat blood pressure measurement experiment; the maximum fall blood pressure produced by Losartan is from value 172mm Hg to 108 mm Hg over a period 90 minutes .compounds number, 5, 8, 9 also duration 90 minutes

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