SYNTHESIS OF 2-SUBSTITUED-5-NITRO-1[2-(1H-TETRAZOL-4-YLMETHYL]-1H-BENZOIMIDAZOLE WITH BIOLOGICAL EVALUATION OF BLOOD PRESSURE MEASURED BY INVASIVE METHOD AND TAIL-CUFF METHOD

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A series of substituted 5-nitro -benzimidazoles bearing a biphenyl tetrazole moiety at the 2-position was prepared via five synthetic routes and evaluated for angiotensin II (AII) receptor antagonistic antihypertensive activity by Invasive Method and Tail-Cuff Method . Structures of all the synthesized compounds have been corroborated on the basis of elemental IR, ^{1}H NMR, ^{13}C NMR and Mass spectra-analytical data.

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Keywords: 5-nitro-benzimidazole, biphenyl tetrazole, angiotensin II, Invasive**,** Tail cuff method

1. Introduction

The renin-angiotensin system (RAS) plays an important role in blood pressure regulation and electrolyte homeostasis. [1] Angiotensin II (AII) is the biologically active component of the RAS and is responsible for most of the peripheral effects of this system. There are two commonly described classes of effective inhibitors of the RAS renin inhibitors and angiotensin converting enzyme (ACE) inhibitors. In recent years, rennin inhibitors with high specificity and affinity for human renin have been reported,[2] but they have yet to be marketed. ACE inhibitors such as captopril, enalapril, and others are very effective for the treatment of most types of hypertension and congestive heart failure.[3]However, their lack of specificity provides a major reason for exploring alternative therapy. Some of the adverse effects of ACE inhibitors such **as** dry cough and angioedema have been attributed to the multisubstrate action of ACE[4] AII receptor antagonists would specifically affect the RAS independently of the source of AII[5] Saralasin was the first specific peptide antagonist of AIIadministered to humans, and it was found to reduce blood pressure in hypertensive patients with high renin levels. Unfortunately, long-term antihypertensive treatment was not possible because these peptide antagonists have low oral bioavailability and short duration of action.[6] 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 losratan, the first orally active AT1 selective nonpeptide AII antagonist that reached the market for the treatment of hypertension (1994, Cozaar). Whereas reports on effective replacements of the biphenyl tetrazole “tail” of losratan are scarce, the imidazolic “head” of the molecule, postulated to act mainly to link the required functionalities, has been successfully replaced by a wide variety of cyclic and acyclic structures, leading to a number of compounds currently in clinical trials.[10]AngII receptor antagonists are expected to have similar therapeutic

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effects and indications as the ACE inhibitors without unwanted side effects associated inhibition of other ACE mediated pathways, such as bradykinin metabolism.Intial research in this area led to the discovery of peptide analog such as saralasin ([sar1-Ala8]-AngII) which displayed potent and selective AngII receptor antagonist activity both *in vivo and in vitro*.However, these peptides had limited therapeutic utility due to partial agonist activity short duration of action and lack of appreciable oral bioavailability[11].Only in recent years a number of non peptides AngII antagonists that show promise as inhibitors of the RAS been reported[12].All these antagonists possess a central aromatic nucleus bearing the pharmacophores indispensable for activity and notably a polar function adjustant to biphenyl substituents while a polar function in this area of molecule seems to be necessary to maintain activity[13]. Sartans are appropriately substituted heterocyclic head coupled through a methylene linker to pendent biphenyl system bearing an acidic function; viz. candesartan is an effective competitive Ang II antagonist with benzimidazole nucleus as the heterocyclic head [14]. The substituent at 6-position on the nucleus increases the activity whereas small substituent at 5-position decreases the activity[15]. Compounds containing tetrazole nucleus are also reported as AT_1 receptor antagonists and their protypical derivative 3 exhibits non-competitive antagonism[16] and amino group attach with carboxylic group given good biological activity [17,18]

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.

Synthesis of 2-methylbenzimidazole

A mixture of 5.43g (0.03 mol) of o-phenylenediamine dihydrochloride, 20 ml of water and 5.4g (0.09 mol) of acetic acid was refluxed for 45 minutes. Then the reaction mixture was poured over crushed ice with stirring. The cooled mixture was made basic by the gradual addition of concentrated 90% ammonia solution. The precipitated product was then filtered and recrystallised from 10% aqueous ethanol, yield: 70%, m.p: 177-180^o C, Anal. Calcd for $C_8H_8N_2$ Found: C, 72.70; H, 6.10; N, 21.20%; IR (KBr) -1 cm: 3341(N-H), 1462(C=C), 1591(C=N), 789.75(CH), 1341(C-N), 1213(C-H). ^{1}H NMR (300 MHz, $CDCl_3$) δ:5.12, (s,1H,- NH);7.12-7.69 (m, 4H, ArH), 2.41(m, 3H, ArH). ^{13}C NMR ($CDCl_3$)δ:21.5, 112.4,113.1, 115.121.3, 125.1, 135.9, 137.0, FAB-MS, 132.69 (100%).

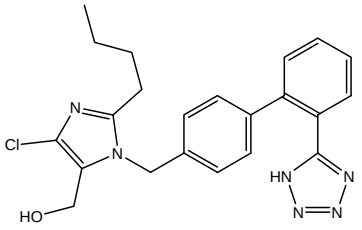
Synthesis of 2-benzylbenzimidazole

A mixture of 5.43g (0.03 mol) of o-phenylenediamine dihydrochloride, 20 ml of water and 12.3g (0.09 mol) of phenyl acetic acid was refluxed for 45 minutes. Then the reaction mixture was poured over crushed ice with stirring. The cooled mixture was made basic by the gradual addition of concentrated ammonia solution. The precipitated product was then filtered and recrystallised from 40% aqueous ethanol, yield: 74% m.p: 213-215 C, IR (KBr) cm: 3316 (N-H), 1543 (C=C), 1604.2 (C=N), 1138.1 (C-N), 2940.81 (C-H). Anal. Calcd for $C_{14}H_{12}N_2$ Found: C, 80.74; H, 5.81; N, 13.45%;^{1}H NMR (300 MHz, $CDCl_3$) δ:5.04, (s,1H,- NH);7.12-8.15 (m, 9H, ArH), 3.72(m, 2H, ArH). ^{13}C NMR ($CDCl_3$)δ:42.4, 112.1,115.1, 117.124.1, 127.1, 132.9, 135.0, FAB-MS, 208.1 (100%),$(M+H)^+$.

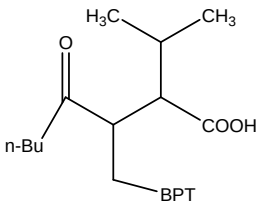
MCS- 2-phenyl Benzimidazole

o-Phenyl diamine (2.7 gm) was dissolved in a mixture of methanol/water (200 ml, v/v 1:1).To this, different aromatic aldehyde,alkyl and aryl compound (5.3gm) in absolute ethanol (50ml) and Cu $(OAc)_2$ H_2O (7gm in water (100ml) were added sequentially while stirring the solution. The reaction mixture was then heated to reflux under vigorous stirring for three hours after this a reddish pale precipitate was formed. The mixture was filtered hot and then washed with water to afford a yellow solid. The precipitate was dissolved in ethanol (150ml) and to this, HCl

(24 ml) and solution of Na_2SxH_2O was added in water (100 ml).At this stage, the reaction mixture was heated at reflux for 1 hour, resulting in the formation of black slurry. 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 *p*H 8-9 and then concentrated to yield a reddish pale precipitate. After filtration and vacuum evaporation, compound obtained as reddish solid.



Losartan



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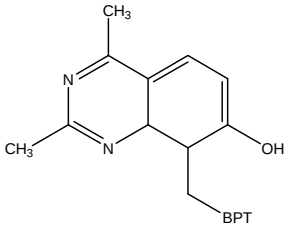
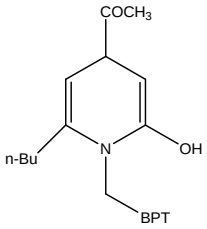
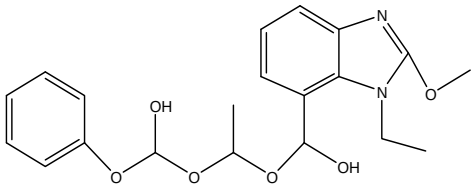


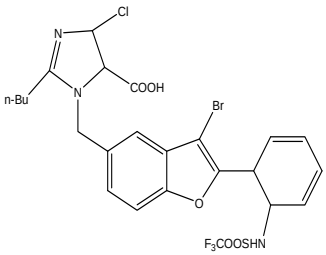
Tasosartan



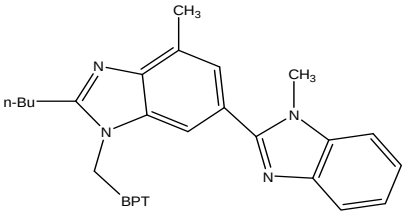
Candesartan



Milfasartan



Saprisartan



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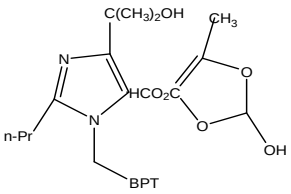
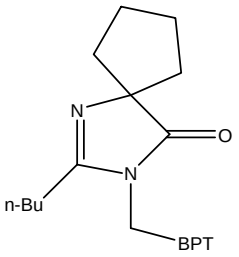
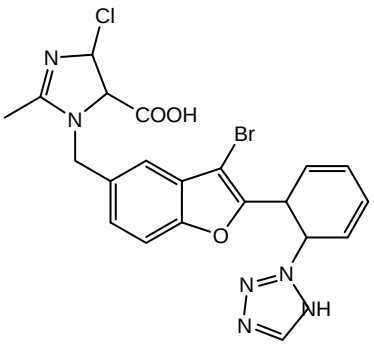


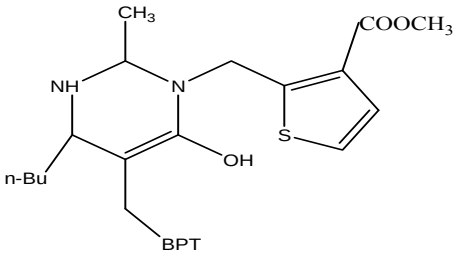
Olmesartan



Irbesartan



Zolzsartan



Eprosartan

Figure- Angiotensin II AT_1 selective antagonists

Yield: 65 %, Melting Point- 188-192°C, IR (KBr) (cm-1): 3315(N-H str.), 3231 (ArHstr.), 1621-1505(C=N and C=C str.), 811 (monosub.Benz.Ring). ^{1}H NMR (300 MHz, $CDCl_3$) δ:5.08, (s,1H,- NH);7.35-8.03 (m, 4H, ArH), 7.19-7.74 (m, 5H, ArH). ^{13}C NMR ($CDCl_3$)δ:115.24, 122.11, 123.05, 124.29, 128.18, 134.09, 137.56, 141.52, FAB-MS, 194.17(100%), 195.09(14.6) $(M+H)^+$.Anal. Calcd for $C_{13}H_{10}N_2$: C, 80.39; H, 5.19; N, 14.42%; Found: C, 80.29; H, 5.11; N, 14.25%;

MCS- (2-Substitued -5-Nitro Benzimidazole)

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(different R-methyl, ethyl, butyl) (15.10 gm) was mixed in portions during 2 hour under room temperature. After stirred continuously for 14 hours minutes 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 SR-2 was formed as yellowish pale.

MCS- 4' (2-Substitued -5-nitro-1-benzoimidazol-1-ylmethyl)-biphenyl-2-carbonitrile

To a solution of 2.0 g (10.12 mmol) of 2-Substitued -5-Nitro Benzimidazole in 65 mL of DMF was added potassium carbonate 0.5 g (5.52 mmol), the mixture was stirred for 30 min at ambient temperature, and 4-(bromomethyl) biphenyl-2'-nitrile 2.60 g (10.52 mmol) was added. After stirring for 24 h the mixture was poured into water (120 mL) and extracted with ethyl acetate (3 × 50 mL). The combined extracts were dried ($MgSO_4$) and evaporated.

MCS 2-Substitued -5-nitro-1[2'-(1H-tetrazol-4-ylmethyl]-1H-benzoimidazole

A mixture of (2-Substitued -5-nitro-1-benzoimidazol-1-ylmethyl)-biphenyl-2-carbonitrile (0.6 g, 1.59 mmol), sodium azide (0.47 g, 7.2 mmol), and Et3N·HCl (0.7 g, 5 mmol) in NH_4Cl (15 mL) is stirred at 160°C for 12 h. After cooling, the mixture is diluted with H2O (50 mL), acidified to pH 3 with 4N HCl, and extracted with EtOAc (3 × 50 mL). The organic layer was washed with H_2O (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 **5**(0.2 g, 30.3%) as a white solid. compounds were-

(a) **2-methyl -5-nitro-1[2'-(1*H*-tetrazol-5-yl)-biphenyl-4-methyl]-1H-benzoimidazole**

Yield: 61 %, m.p. = 243-246^{0}C. Anal.Calcd for $C_{22}H_{17}N_7O_2$:C,68.23;H, 4.16;N,23.83%; IR (KBr): KBr) 3341,2952, 1585, 1414, 1422, 1263, 753, cm.^{1}HNMR (300 Hz,$CDCl_3$) 2.38 (s,3H,CH_3),5.00(s,2H,CH_2),7.06-8.30(m,11H,ArH).
^{13}CNMR($CDCl_3$)δ:23.1,51.4,111.2,113.1,115.7,116.9,127.1,128.8,139.2,142.2,147.2,154,FAB-MS,411.14(100%)

(b) **2-ethyl-5-nitro-1[2'-(1*H*-tetrazol-5-yl)-biphenyl-4-methyl]-1H-benzoimidazole**

Yield: 55%, m.p. = 197^0-1200^{0}C. Anal.Calcd for $C_{23}H_{19}N_3O_4$:C,68.21;H, 4.42;N,10.52 %; Found: C,64.93;H, 4.50;N,23.05 %;IR (KBr): IR (KBr): KBr) 3403,2932, 1500, 1514, 1354, 1205, 792. ^{1}HNMR (300 Hz,$CDCl_3$) 2.28 (s,3H,CH_3),2.63 (s,2H,CH_2), 4.99(s,2H,CH_2),7.06-8.30(m,11H,ArH).
^{13}CNMR($CDCl_3$)δ:20.6,47.4,63.2,113.2,115.6,118.2,119.2,121.2,123.7,126.2,132.2,137.2, FAB-MS,425.1600(100%)

(c) 5-nitro -2- propyl-1[[2'-(1*H*-tetrazol-5-yl)-biphenyl-4-methyl]-1H-benzoimidazole

Yield:72%,m.p.=276-279^{0}C. Anal.Calcd for $C_{24}H_{21}N_7O_2$:C,65.59;H, 4.82;N,22.31 %; IR (KBr): 3429,2985, 1524, 1593, 1312, 1294, 788.2. ^{1}HNMR (300 Hz,$CDCl_3$) 1.08 (s,3H,CH_3),1.63 (s,2H,CH_2), 2.32 (s,2H,CH_2), 4.91(s,2H,CH_2),7.12-7.90(m,11H,ArH).
^{13}CNMR($CDCl_3$)δ:17.2,41.3,49.0,60.5,110.1,112.6,113.6,114.1,120.2,121.7,127.2,131.2,135.2,
FAB-MS, 439.17(100%)

(d) 2-Butyl-5-nitro--1[[2'-(1*H*-tetrazol-5-yl)-biphenyl-4-methyl]-1H-benzoimidazole

Yield:54%,m.p.=269^0-273^{0}C. Anal.Calcd for $C_{25}H_{23}N_7O_2$:C,66.21;H, 5.11;N,21.62%; IR (KBr): 3287,2905, 1514, 1562, 1351, 1300, 780. ^{1}HNMR (300 Hz,$CDCl_3$) 1.34 (s,3H,CH_3),1.69 (s,2H,CH_2), 2.28 (s,2H,CH_2), 2.54 (s,2H,CH_2), 4.97(s,2H,CH_2),7.12-8.19

(m,11H,ArH).^{13}CNMR($CDCl_3$)δ:15.1,45.3,51.0,62.5,111.1,113.6,115.6,116.1,122.2,123.7,125.2,133.2,136.2,FAB-MS, 453.19(100%)

(e) 2-benzyl-5-nitro--1[[2'-(1*H*-tetrazol-5-yl)-biphenyl-4-methyl]-1H-benzoimidazole

Yield:49%,m.p.=293-296^{0}C. Anal.Calcd for $C_{28}H_{21}N_7O_2$:C,68.98;H, 4.34;N,20.11%; IR (KBr): 3331,2931, 1523, 1576, 1301, 1343, 786. ^{1}HNMR (300 Hz,$CDCl_3$) 3.59(s,2H,CH_2), 4.90(s,2H,CH_2),7.028.51(m,16H,ArH).^{13}CNMR($CDCl_3$)δ:15.1,45.3,51.0,62.5,111.1,113.6,115.6,116.1,122.2,123.7,125.2,133.2,136.2,FAB-MS, 487.17(100%)

(f) 5-nitro-2-phenyl--1[[2'-(1*H*-tetrazol-5-yl)-biphenyl-4-methyl]-1H-benzoimidazole

Yield:64%,m.p.=249^0-254^{0}C. Anal.Calcd for $C_{27}H_{19}N_7O_2$:C,68.49;H, 4.04;N,20.71%; IR (KBr): 3298,2900, 1508, 1521, 1342, 1311, 806. ^{1}HNMR (300 Hz,$CDCl_3$) 4.90(s,2H,CH_2),7.32-8.19(m,16H,ArH).^{13}CNMR($CDCl_3$)δ:55.1,
110.1,112.6,113.6,115.1,122.2,123.7,124.2,131.2,133.1, FAB-MS, 473.48(100%)

(g) 2-(2-Chloro-phenyl)-5-nitro-[[2'-(1*H*-tetrazol-5-yl)-biphenyl-4-methyl]-1H-benzoimidazole

Yield:61%,m.p.=231^0-235^{0}C. Anal.Calcd for $C_{27}H_{18}ClN_7O_2$:C,63.85;H, 3.57;N,19.30 %; IR (KBr): 3414, 2906 , 1663,1541, 1533-1315, 1108 , 822 ,633.6. ^{1}HNMR (300 Hz, CDCl3) 4.99(s,2H,CH_2),7.12-8.49 (m,15H,ArH). ^{13}CNMR($CDCl_3$)δ: 50.1, 112.1,113.6,115.6,116.1,123.2,126.7,129.2,135.2,137.8, FAB-MS, 507.12(100%)

(h) 2-(3-Chloro-phenyl)-5-nitro-[[2'-(1*H*-tetrazol-5-yl)-biphenyl-4-methyl]-1H-benzoimidazole

Yield:66%,m.p.=233^0-238^{0}C. Anal.Calcd for $C_{27}H_{18}ClN_7O_2$:C,63.85;H, 3.57;N,19.30 %; IR (KBr): 3417, 2913 , 1652,1541, 1522-1302, 1113 , 811 ,630. ^{1}HNMR (300 Hz,$CDCl_3$) 5.11(s,2H,CH_2),7.32-8.12 (m,15H,ArH). ^{13}CNMR($CDCl_3$)δ: 50.1, 112.1,113.6,115.6,116.1,123.2,126.7,129.2,135.2,137.8, FAB-MS, 506.12(100%) M^{+1}

(i) 2-(4-Chloro-phenyl)-5-nitro-[[2'-(1*H*-tetrazol-5-yl)-biphenyl-4-methyl]-1H-benzoimidazole

Yield:55%,m.p.=239^0-242^{0}C. Anal.Calcd for $C_{27}H_{18}ClN_7O_2$:C,63.85;H, 3.57;N,19.30 %; IR (KBr): 3398, 2965 , 1612,1561, 1543-1353, 1164 , 781 ,639. ^{1}HNMR (300 Hz,$CDCl_3$) 4.91(s,2H,CH_2),7.21-8.24 (m,15H,ArH). ^{13}CNMR($CDCl_3$)δ: 50.1, 112.1,113.6,115.6,116.1,123.2,126.7,129.2,135.2,137.8, FAB-MS, 508.12(100%)

(j) 2-(2-fluoro-phenyl)-5-nitro-[[2'-(1*H*-tetrazol-5-yl)-biphenyl-4-methyl]-1H-benzoimidazole

Yield:51%,m.p.=263^0-267^{0}C. Anal.Calcd for $C_{27}H_{18}FN_7O_2$:C,63.98;H, 3.87;N,19.95 %; IR (KBr): 3254.6, 2894.2 , 1632,1511, 1517-1303, 1265 , 754 ,697.1. ^{1}HNMR (300 Hz,$CDCl_3$) 5.09(s,2H,CH_2),7.38-8.63 (m,15H,ArH). ^{13}CNMR($CDCl_3$)δ: 48.1, 111.5,112.6,114.6,117.1,122.2,124.7,127.2,132.2,133.1, FAB-MS, 491.15(100%)

(k)2-(3-fluoro-phenyl)-5-nitro-[[2'-(1*H*-tetrazol-5-yl)-biphenyl-4-methyl]-1H-benzoimidazole

Yield:58%,m.p.=269^0-274^{0}C. Anal.Calcd for $C_{27}H_{18}FN_7O_2$:C,63.98;H, 3.87;N,19.95 %; IR (KBr): 3274.6, 2909 , 1639,1516, 1511-1323, 1260 , 751 ,689.1. ^{1}HNMR (300 Hz,$CDCl_3$) 5.09(s,2H,CH_2),7.38-8.63 (m,15H,ArH). ^{13}CNMR($CDCl_3$)δ: 48.1, 111.5,112.6,114.6,117.1,122.2,124.7,127.2,132.2,133.1, FAB-MS, 491.15(100%)

(l)2-(4-fluoro-phenyl)-5-nitro-[[2'-(1*H*-tetrazol-5-yl)-biphenyl-4-methyl]-1H-benzoimidazole

Yield:45%,m.p.=286^0-287^{0}C. Anal.Calcd for $C_{27}H_{18}FN_7O_2$:C,63.98;H, 3.87;N,19.95 %; IR (KBr): 3254.6, 2894.2 , 1632,1511, 1517-1303, 1265 , 754 ,697.1. ^{1}HNMR (300 Hz,$CDCl_3$) 5.09(s,2H,CH_2),7.38-8.63 (m,15H,ArH). ^{13}CNMR($CDCl_3$)δ: 48.1, 111.5,112.6,114.6,117.1,122.2,124.7,127.2,132.2,133.1, FAB-MS, 491.15(100%)

(m)2-(2-methoxy-phenyl)-5-nitro-[[2'-(1*H*-tetrazol-5-yl)-biphenyl-4-methyl]-1H-benzoimidazole

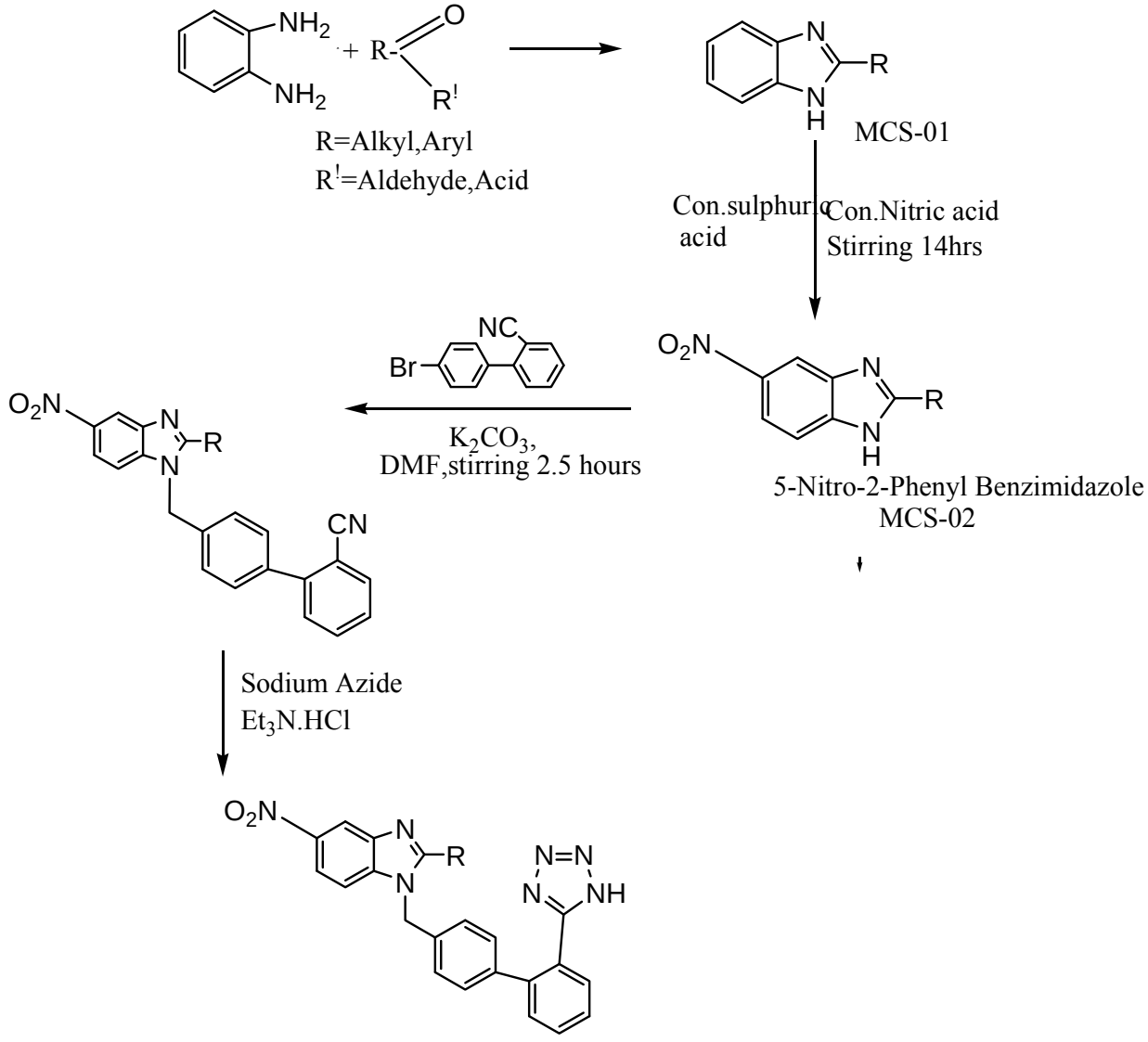
Yield: 50 %, m.p. =205^0-208^{0}C. Anal.Calcd for $C_{27}H_{21}N_7O_3$:C,63.98;H, 3.87;N,19.95 %; IR (KBr): 3497.9,2991.3 2875 , 1600,1554, 1507-1323, 1132,1432. ^{1}HNMR (300 Hz, CDCl3) 2.99(s,3H, CH_3), 4.87(s,2H,CH_2),7.28-8.52 (m,15H,ArH). ^{13}CNMR($CDCl_3$)δ: 21.3,46.4, 112.1,113.6,115.6,116.1,125.2,127.2,127.6,135.2,139.1, FAB-MS, 503.53(100%)

(n)2-(3-methoxy-phenyl)-5-nitro-[[2'-(1*H*-tetrazol-5-yl)-biphenyl-4-methyl]-1H-benzoimidazole

Yield: 53 %, m.p. =215^0-218^{0}C. Anal.Calcd for $C_{27}H_{21}N_7O_3$:C,63.98;H, 3.87;N,19.95 %; IR (KBr): 3491.4,2981.0 2889 , 1612,1559, 1512-1325, 1131,1421. ^{1}HNMR (300 Hz, CDCl3) 3.12(s,3H, CH_3), 4.98(s,2H,CH_2),7.13-8.14 (m,15H,ArH). ^{13}CNMR($CDCl_3$)δ: 21.3,46.4, 112.1,113.6,115.6,116.1,125.2,127.2,127.6,135.2,139.1, FAB-MS, 503.53(100%)

(o)2-(4-methoxy-phenyl)-5-nitro-[[2'-(1*H*-tetrazol-5-yl)-biphenyl-4-methyl]-1H-benzoimidazole

Yield: 45 %, m.p. =200^0-204^{0}C. Anal.Calcd for $C_{27}H_{21}N_7O_3$:C,63.98;H, 3.87;N,19.95 %; IR (KBr): 3490,2976, 2898 , 1625.6,1524, 1500-1303, 1168,1398. ^{1}HNMR (300 Hz, CDCl3) 3.29(s,3H, CH_3), 5.17(s,2H,CH_2),7.28-8.52 (m,15H,ArH). ^{13}CNMR($CDCl_3$)δ: 21.3,46.4, 112.1,113.6,115.6,116.1,125.2,127.2,127.6,135.2,139.1, FAB-MS, 503.53(100%)



2-Substitued -5-nitro-1-[2'-(1*H*-tetrazol-5-yl)-biphenyl-4-ylmethyl]-1*H*-benzoimidazole

SCHEM -2-Substitued-5-nitro-1[2-(1H-tetrazol-4-ylmethyl]-1H-benzoimidazole

Biological Evaluation

Procedure for development of hypertention for normotensive rats [22]

Albino normotensive rats (Wistar Strain) were taken and they were hypertensized by cholinomimetic agents for screening of all the synthesized benzimidazole derivatives for there anti-hypertensive activity. Suspension of test compounds was prepared in 1% w/v sodium carboxy methyl cellulose (sodium CMC) and was administered at dose level of 50 and 100 microgram/kg animal body weight to different groups of six 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:

Angiotensin II induced Hypertension: [23] (i) Invasive method (Direct method). (ii) Non-invasive Tail cuff method (Indirect method).

Experimental Techniques

(i) Invasive Method (Direct Method):[23-25] Male albino wistar (150-250 gm) rats were used and housed at 22 ± 1^0C room temperature. The rats were anaesthetized with sodium chloride 0.9% solution, Drug solution 10-µg/100ml, and Heparin 500 I.U.solution urethane hydrochloride 50% w/v solution 80 mg/kg i.p. To set up the instrument firstly the level of mercury in the left arm of manometer was adjusted to 90-100 mm of Hg (normal blood pressure of rat).this was done in steps of 10mm at a time and the physiogram so obtained was used as calibration graph for calculations. The Jugular vein and carotid artery were surgically cannulated for drug administration for recording the blood pressure respectively. The trachea was cannulated in order to provide artificial respiration to rat during the experiment. By means of three way stop cock and a stainless steel needle at the end of P.E. tubing was attached to arterial cannula for B.P., Transducers and the Venus cannula to a syringe. Then both the cannulas were filled by heparinized saline before the administration. Arterial cannula was connected via transducer to physiograph recorder. Several baseline readings of systolic and diastolic pressures were recorded. The physiograph shows the reduction of the blood pressure with compare to losratan. Synthesized compounds were screened in presence of Angiotensin II induced hypertension (0.5 µg/kg i.v.). Observations are given in the table 1, 2.

(ii) Non-invasive Tail cuff Method (Indirect Method):[24-25] 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 six 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 table1, 2, 3.

Table 1. Hypertension induced in normotensive rat.

Comp.	Exp. Animal Albino (Wistar) Rat	After 1hour			After 3 hour		
		SBP	DBP	MABP	SBP	DBP	MABP
[a]	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
	6	141	116	127	139	104	122
[b]	1	139	112	122	142	108	125
	2	135	109	124	138	102	120
	3	140	106	121	137	102	120
	4	144	106	125	142	104	123
	5	146	108	124	140	103	120
	6	138	112	125	138	100	119
[c]	1	139	102	122	143	100	121
	2	148	104	124	143	102	122
	3	146	112	128	137	101	118
	4	143	108	126	140	103	121
	5	147	104	124	141	104	120
	6	145	106	123	136	97	116
[d]	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	149	101	125	143	101	121
	6	144	109	131	140	100	120
[e]	1	142	102	124	143	101	122
	2	145	105	125	145	100	121
	3	136	113	124	142	101	121
	4	139	113	122	140	100	120
	5	146	116	127	143	101	122
	6	144	113	128	142	101	123
[f]	1	139	105	123	138	198	118
	2	138	112	125	141	102	121
	3	143	114	125	142	102	122
	4	146	102	124	143	101	120
	5	144	114	124	141	100	119
	6	143	105	123	140	101	120
[g]	1	145	107	126	146	96	119
	2	142	115	127	135	98	118
	3	140	106	123	142	101	121
	4	142	108	125	141	102	120
	5	139	110	125	143	101	120
	6	146	105	126	142	101	118
[h]	1	140	113	124	143	100	121

	2	142	105	122	142	101	120
	3	139	111	126	141	102	119
	4	144	104	124	144	101	120
	5	142	101	123	142	100	121
	6	143	100	125	139	100	117
[i]	1	143	105	122	142	100	121
	2	141	106	125	144	99	119
	3	140	111	124	139	97	120
	4	144	114	126	141	100	120
	5	141	112	123	139	96	117
	6	140	103	124	145	98	119
[j]	1	141	108	124	140	103	121
	2	145	113	128	144	102	123
	3	143	111	125	143	100	121
	4	141	114	126	139	102	120
	5	140	112	126	143	100	122
	6	144	116	130	145	98	119
[k]	1	144	106	125	144	100	122
	2	145	112	126	139	100	120
	3	142	109	126	143	97	120
	4	140	102	123	140	100	120
	5	137	101	124	146	100	123
	6	142	108	125	142	102	120
[l]	1	143	105	124	139	104	121
	2	141	101	126	143	104	120
	3	141	110	126	143	104	119
	4	142	102	125	141	102	121
	5	139	111	124	138	102	120
	6	142	104	126	139	105	122
[m]	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	142	114	123	142	103	122
	6	139	105	126	146	106	120
[n]	1	139	109	123	142	102	123
	2	140	101	125	140	101	124
	3	138	107	128	143	101	121
	4	140	108	125	141	104	120
	5	144	111	126	143	100	119
	6	147	114	127	140	100	120
[o]	1	142	116	125	139	101	121
	2	141	116	126	140	101	120
	3	140	113	124	143	102	121
	4	146	108	126	141	101	120
	5	142	111	124	143	100	121
	6	143	105	128	141	101	121
Control	**Losartan**	113	-	-	-	-	-
	Telmisartan	115	-	-	-	-	-

Table 2. Reduction in blood pressure (mm Hg) at a dose of 50 µgm/kg animal body weight.

Comp.	**AnimalAlbino (Wistar) Rat**	**After 1hour**			**After 3 hour**		
		SBP	**DBP**	**MABP**	**SBP**	**DBP**	**MABP**
[a]	1	123	104	112	124	101	112
	2	124	102	113	126	102	113
	3	122	103	112	122	101	111
	4	121	106	113	124	101	112
	5	124	103	111	125	102	113
	6	122	102	114	123	100	111
[b]	1	120	103	112	119	101	110
	2	122	106	114	127	102	111
	3	124	103	114	126	105	113
	4	125	102	113	124	101	112
	5	127	103	115	125	102	114
	6	124	104	114	128	101	113
[c]	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
	6	126	101	113	128	102	115
[d]	1	123	101	112	125	100	112
	2	122	100	111	126	102	115
	3	124	102	112	126	102	111
	4	126	101	113	124	104	114
	5	128	102	115	126	104	115
	6	125	105	115	122	100	112
[e]	1	124	101	112	124	100	112
	2	122	100	111	121	103	112
	3	124	102	113	124	106	115
	4	122	103	112	122	105	114
	5	124	102	111	125	102	114
	6	126	100	113	121	101	111
[f]	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	122	101	111	126	102	114
	6	124	102	113	125	102	112
[g]	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	121	100	110	125	102	111

	6	126	103	115	122	103	112
[h]	1	124	102	113	126	100	113
	2	122	101	112	126	103	112
	3	126	104	115	124	102	113
	4	128	102	115	126	104	115
	5	131	103	117	124	102	113
	6	128	104	118	122	106	114
[i]	1	129	103	119	129	104	111
	2	126	101	117	123	102	112
	3	131	100	123	121	106	110
	4	129	103	124	122	100	111
	5	133	105	118	127	104	114
	6	130	108	113	123	102	113
[j]	1	127	105	118	126	102	114
	2	124	106	122	122	101	111
	3	123	102	119	127	101	110
	4	122	104	118	124	98	113
	5	127	108	119	126	103	109
	6	128	102	116	125	101	106
[k]	1	127	101	114	122	103	112
	2	125	106	117	127	101	112
	3	123	104	114	125	104	111
	4	129	102	119	121	102	110
	5	130	104	118	119	103	104
	6	132	102	121	129	101	111
[l]	1	123	101	119	122	101	113
	2	127	103	117	127	102	112
	3	122	102	119	124	102	113
	4	126	104	118	125	102	114
	5	125	101	113	128	102	115
	6	123	103	116	126	100	113
[m]	1	126	102	113	123	103	113
	2	123	101	112	122	106	116
	3	124	102	113	124	102	113
	4	122	102	112	126	100	111
	5	124	102	113	128	100	114
	6	128	102	115	129	101	115
[n]	1	128	106	117	123	100	112
	2	127	101	116	125	105	110
	3	124	102	119	128	102	111
	4	129	104	117	124	101	112
	5	133	103	118	126	100	110
	6	131	102	114	124	102	111
[o]	1	130	102	116	126	103	112
	2	128	101	114	124	104	114
	3	126	102	114	122	102	111
	4	125	104	118	123	101	112
	5	128	105	116	128	102	115

	6	129	101	117	126	104	115
Control	**Losartan**	118	-	-	-	-	-
	Telmisartan	113	-	-	-	-	-

Table 3. Reduction in blood pressure (mean ± SEM) at a dose of 50 µgm/kg animal body weight

Comp	**After 1hour**			**After 3 hour**		
	SBP	**DBP**	**MABP**	**SBP**	**DBP**	**MABP**
[a]	129.2	103.8	119.3	122.5	101.8	111.2
[b]	126.4	104.3	117.2	121.5	101.6	111.6
[c]	128.5	100.6	116.6	125.6	100.3	112.1
[d]	124.5	101.2	114.3	123.5	99.12	109.4
[e]	126.4	104.6	115.6	122.4	104.2	113.0
[f]	123.2	102.1	117.4	120.1	101.5	111.9
[g]	132.1	102.5	1151	122.3	102.5	112.4
[h]	129.3	102.2	118.2	124.2	96.43	110.2
[i]	126.4	103.6	116.2	124.6	98.94	109.3
[j]	122.3	109.4	119.1	122.8	102.5	112.3
[k]	125.7	107.0	115.3	123.5	101.3	113.4
[l]	129.5	105.1	119.2	126.3	100.4	112.1
[m]	123.7	101.5	121.5	121.4	103.2	114.3
[n]	124.2	102.3	117.1	122.6	101.4	111.6
[o]	125.4	100.3	116.72	121.2	101.3	110.2

3. Results and discussion

All the synthesized benzimidazole incorporated antihypertensive activity with standard drug compared all synthesized compounds (a-o). Almost all the newly synthesized substituted 5-nitro-benzimidazole showed good antihypertensive activity. With the goal of investigating the structure–activity relationships of benzimidazole, based molecules, fifteen analogs compounds were synthesized (Scheme). Our initial efforts of optimizing the benzimidazole structures were focused on either replacing the 2-substituted 5-nitro groups on different substituents at different positions of the benzimidazole derivatives. We recently determined the significance of the 5 position of the benzimidazole ring for inhibitory activity. We also investigated the possible effect of any change in the linker between both aromatic rings upon the bioactivity. The structures of the synthesized compounds were confirmed using IR, NMR and elemental analysis methods. The comparative data of the synthesized compounds are provided in experimental section. Overall approximately a 95 to 98 % decrease in reaction times and a 3% to 113% increase in the yields were obtained. 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 substituent at 5-position. Presence of nitro group has increased the activity substantially over the substituted one (A and O). The maximum activity has been observed with nitro group (Compound b,c e,g,I,j,l,m,n,o). The higher activity of Compound b,c e,g,I,j,l,m,n,o suggests that this group at 5-position should be H-bond acceptor. The higher activity of 5-nitro derivatives may be ascribed to the ability of nitro group to act as H-bond acceptor with respect to the receptor site.

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