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Synthesis and Antihypertensive Activity of Some N-{4-(6-Chloro-5-nitro-1-[2'--(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-1H-benzoimidazol-2yl-}-phenyl)-3-(substituted-phenyl)-acryl amides

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ABSTRACT

A new series of N-{4-(6-Chloro-5-nitro-1-[2'--(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-1H-benzoimidazol-2yl-}-phenyl)-3-(substituted-phenyl)-acryl amide derivatives [1-8] has been synthesized and subjected to evaluate their antihypertensive activity. All the synthesized compounds of the series displayed, remarkable activity in comparison to standard drug(losratan). Many Schiff bases were prepared by condensation reaction of nitro compound containing biphenyl tetrazole with aromatic aldehydes and ketone derivatives were prepared by Schiff base. The synthesized compounds were screened for AT1 Angiotension (A II) Receptor Antagonist activity.

Keywords: Angiotension (A II), antihypertensive, Schiff bases, biphenyl-tetrazole.

INTRODUCTION

Angiotensin 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 rennin 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 rennin 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 effecting an increase in vascular resistance, the regulation of volemia through the stimulated release of vasopressin and aldosterone, which induces saline retention, and the regulation of the adrenocorticotropic hormone (ACTH). Thus, reducing the levels of AII by inhibition of one of the RAS enzymes or directly blocking the AII receptors is in theory a good approach for treating hypertension, confirmed by the success of angiotensin-converting enzyme (ACE) inhibitors as 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 AT_1 receptor antagonists are characterized by the presence in their structure of the biphenyl fragment bearing an acidic moiety and differ in the nature of the pendent heterocyclic system (valsartan lacks the heterocyclic moiety) connected to the Para position of the proximal phenyl. Substantial effort has been made to find renin inhibitors, although orally active agents have only recently been reported [7-8].

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 substitute 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 AT_1 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 heterocyclic, which are the structural isosters of nucleotides owing fused heterocyclic nuclei in their structures that allow them to interact easily with the biopolymers, possess potential activity with lower toxicities in the 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.

MS-01-Synthesis of 4-(6-Chloro-1H-benzoimidazol-2-yl)-phenyl amine

A solution of 4-Chloro-1,2-phenylenediamine dihydrochloride (0.45 g, 2.5 mmol) in 5 ml of water was cooled to 0°C and treated with a solution of cyanogen bromide (0.60 ml, 5 M in acetonitrile, 3.0 mmol) and solid $NaHCO_3$ (0.41 mg, 4.9 mmol). The solution was stirred at ambient temperature for 40-45 h. The mixture was made basic with 1 M aqueous Na_2CO_3 and the solution was concentrated under reduced pressure. The residue was triturated with hot ethanol, and the ethanolic solution was filtered and concentrated under reduced pressure to obtain the compound **1** in appreciable yield.

Yield 80%; mp 142-145 ^{0}C; Anal Calcd for $C_{13}H_{10}ClN_3$ (R=H): C, 64.04; H, 4.14; N, 17.24%; IR (υ cm^{-1}): 3045 (C-H, sp^2), 3210 (NH, bonded), 3175 (NH, free), 1654 (C=N), 1626, 1586, 1444 (C⋯C, ring str) 958, 859, 742 (sub. phenyl) 647 (C-Cl); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 4.0 (s, 2H, NH_2), 5.0 (s,1H, NH), 7.3-7.8 (m, 7H, Ar-H); ^{13}C NMR ($CDCl_3$) δ: 111.6,115.1,119.7,126.1,141.6; FAB-MS: 243.692 $(M+H)^+$.

MS-02- *Synthesis of* 4-(6-Chloro -5-nitro-1H-benzoimidazol-2-yl)-phenyl amine

Twenty 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 (different R-aryl groups) (15.10 gm) was mixed in portions during 2 hour under room temperature. After stirred continuously for 8 hrs 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 recrystillzed from absolute ethanol.

Yield 82%; mp 135-138 ^{0}C; Anal Calcd for $C_{13}H_9N_4O_2$ (R=H): C, 54.09; H, 3.17; N, 19.41%; IR (υ cm^{-1}): 3041 (C-H, sp^2), 3216 (NH, bonded), 3171 (NH, free), 1653 (C=N), 1654 (NO_2),1629, 1589, 1449 (C⋯C, ring str) 952, 866, 773 (sub. phenyl), 649 (C-Cl); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 4.05 (s, 2H, NH_2), 5.01 (s, NH), 7.4-7.9 (m, 6H, Ar-H); ^{13}C NMR ($CDCl_3$) δ: 110.1,113.5,118.6,121.3, 131.2, 142.2; FAB-MS: 288.04 $(M+H)^+$.

MS-03- *Synthesis of* N-[4-(6-Chloro -5-nitro-1H-benzoimidazol-2-yl)-phenyl] acetamide

Dissolve 4-(6-Chloro -5-nitro-1H-benzoimidazol-2-yl)-phenyl amine (1.5 g, 0.01 mole) in absolute ethanol (100 mL) and acetyl chloride (1.5 g, 0.01 mole) was added drop wise with constant stirring at 0-5^{0}C. The reaction mixture was stirred for 6 hrs. The excess solvent was distilled off and the solid product was filtered, dried and recrystallised from ethanol to give compound yield

Yield 70 %; mp 156-158 ^{0}C; Anal Calcd for $C_{15}H_{11}N_4O_3$ (R=H): C, 54.49; H, 3.37; N, 16.94%; IR (υ cm^{-1}): 3045 (C-H, sp^2), 3210 (NH, bonded), 3179 (NH, free), 1652 (C=N), 1651(NO_2),1625, 1580, 1442 (C⋯C, ring str) 956, 861, 770 (sub. phenyl), 646 (C-Cl); ^{1}H NMR

(300 MHz, $CDCl_3$) δ: 4.01 (s, 2H, NH_2), 5.0 (s, NH), 7.2-7.7 (m, 6H, Ar-H),2.02(s3H,methyl; ^{13}C NMR ($CDCl_3$) δ: 111.2,112.1,115.8,127.5,139.8; FAB-MS: 330.05 $(M+H)^+$.

MS-04-N-[4-(6-Chloro -5-nitro-1H-benzoimidazol-2-yl)-phenyl]acetamide

Dissolve N-[4-(6-Chloro -5-nitro-1H-benzoimidazol-2-yl)-phenyl]acetamide (1.12g, 0.01 mole) in absolute ethanol (30 mL) and various aromatic aldehydes (1.06 g, 0.01 mole) were taken and then an aqueous solution of KOH (2%, 5 mL) added to it. The reaction mixture refluxed for 5 h and then the solvent was removed by vacuum distillation and then it was poured into crushed ice and acidified with HCl. The solid separated was filtered and recrystallised from ethanol (Scheme). Similarly remaining compounds [1-8] was prepared by above method.

MS-05-N-{4-(6-Chloro -1-(2-cyano-biphenyl-4-ylmethyl)-5-nitro-*1H*-benzoimidazol-2-yl)-phenyl]-3-substituted-phenyl-acrylamide

To a solution of 150 mg (2.5 mmol) compound aryl substitute 50 mL of DMF was added potassium carbonate 2.0 g (7.5 mmol), the mixture was stirred for 2.5 hours at room temperature, and 4-(bromomethyl) biphenyl-2'-nitrile 1.5 g (15.10 mmol) was added. After stirring for 14 hours the mixture was poured into distilled water (150 mL) and extracted with diethyl ether (3 × 50 mL). The combined extracts were dried ($MgSO_4$) and evaporated.

MS-06- N-{4-(6-Chloro -5-nitro-1-[2'--(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-1H-benzo imidazol-2yl-}-phenyl)-3-(substituted-phenyl)-acryl amide

A mixture of N-{4-(6-Chloro -1-(2-cyano-biphenyl-4-ylmethyl)-5-nitro-*1H* benzoimidazol-2-yl)-phenyl]-3-substituted-phenyl-acrylamide (0.70 g, 1.59 mmol), sodium azide (0.55 g, 7.2 mmol), and Et3N·HCl (0.89 g, 1 mmol) in NH_4Cl (50 mL) is stirred at 160^0C 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.

[1] N-{4-(6-Chloro -5-nitro-1-[2'--(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-1H-benzo imidazol-2yl-}-phenyl)-3-(2-chloro-phenyl)-acryl amide

Yield: 82 %, m.p. = 223-226^{0}C. Anal.Calcd for $C_{36}H_{24}Cl_2N_8O_3$: C,62.89; H, 3.52; N,16.31%; IR (KBr): 3391, 3219, 3167, 2810, 1576, 1611, 1687, 1554-1389, 1146.^{1}HNMR (300 MHz, $CDCl_3$) 12.16(1H, s, -NH-Benzimidazole), 10.14(s, 1H, tetrazole-NH), 4.99(s, 2H, CH_2), 6.9-8.6(m, 19H, Ar-H), 5.98(s, 1H-CH-Cl). ^{13}CNMR ($CDCl_3$)δ: 54.7, 58.2, 112.1, 113.3, 115.5, 122.2, 124.5, 127.3, 131.2, 133.1, 134.3, 135.2, 139.4, FAB-MS, 686.13

[2] N-{4-(6-Chloro -5-nitro-1-[2'--(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-1H-benzo imidazol-2yl-}-phenyl)-3-(2-bromo-phenyl)-acryl amide

Yield: 77 %, m.p. = 211-214^{0}C. Anal.Calcd for $C_{36}H_{24}ClBrN_8O_3$:C,59.07; H, 3.30; N,15.31%; IR (KBr): 3379, 3243, 3160, 2864, 1571, 1619, 1653, 1587-1319, 1155.^{1}HNMR (300 MHz, $CDCl_3$) 12.19(1H, s, -NH-Benzimidazole), 10.17(s, 1H, tetrazole-NH), 4.96(s, 2H, CH_2), 6.97-8.55(m, 19H, Ar-H), 5.94(s, 1H-CH-Cl). ^{13}CNMR($CDCl_3$)δ: 54.7, 58.2, 112.1, 113.3, 115.5, 122.2, 124.5, 127.3, 131.2, 133.1, 134.3, 135.2, 137.9, 142.6, FAB-MS, 730.08

[3] N-{4-(6-Chloro -5-nitro-1-[2'--(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-1H-benzo imidazol-2yl-}-phenyl)-3-(2-iodo-phenyl)-acryl amide

Yield: 66 %, m.p. = 254-257^{0}C. Anal.Calcd for $C_{36}H_{24}ClIN_8O_3$:C,55.51; H, 3.11; N,14.38%; IR (KBr): 3365, 3212, 3164, 2853, 1588, 1655, 1653, 1587-1319, 1155 .^{1}HNMR (300 MHz, $CDCl_3$) 12.06(1H, s, -NH-Benzimidazole), 10.33(s, 1H, tetrazole-NH), 4.92(s, 2H, CH_2), 6.87-8.43(m, 19H, Ar-H), 5.96(s, 1H-CH-Cl). ^{13}CNMR ($CDCl_3$)δ: 55.7, 110.1, 111.6, 113.1, 117.3, 123.1, 130.3, 131.3, 139.1, 139.5, 140.4, FAB-MS, 777.97

[4] N-{4-(6-Chloro -5-nitro-1-[2'--(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-1H-benzo imidazol-2yl-}-phenyl)-3-(2-fluoro-phenyl)-acryl amide

Yield: 60 %, m.p. = 265-267^{0}C. Anal.Calcd for $C_{36}H_{24}ClFN_8O_3$:C,64.43; H, 3.60; N,16.70%; IR (KBr): 3354, 3248, 3155, 2829, 1564, 1675, 1639, 1527-1377, 1159. ^{1}HNMR (300 MHz, $CDCl_3$) 12.06(1H, s, -NH-Benzimidazole), 10.33(s, 1H, tetrazole-NH), 4.92(s, 2H, CH_2), 6.87-8.43(m, 19H, Ar-H), 5.96(s, 1H-CH-Cl). ^{13}CNMR ($CDCl_3$)δ: 53.6, 110.1, 111.6, 113.1, 117.3, 123.1, 130.3, 131.3, 139.1, 139.5, 142.6, FAB-MS, 670.164.

[5] N-{4-(6-Chloro -5-nitro-1-[2'--(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-1H-benzo imidazol-2yl-}-phenyl)-3-(2-hydroxy-phenyl)-acryl amide

Yield: 65 %, m.p. = 234-237^{0}C. Anal.Calcd for $C_{36}H_{25}ClN_8O_4$:C,64.62; H, 3.77; N, 16.75%; IR (KBr): 3398, 3241, 3150, 2854, 1547, 1670, 1643, 1527-1343, 1151. ^{1}HNMR (300 MHz, $CDCl_3$) 12.02(1H, s, -NH-Benzimidazole), 10.27(s, 1H, tetrazole-NH), 4.98(s, 2H, CH_2), 6.9-8.41(m, 19H, Ar-H), 5.01(s, 1H, armO-H) 5.89(s, 1H-CH-Cl). ^{13}CNMR ($CDCl_3$)δ: 55.8, 110.1, 111.6, 113.1, 117.3, 123.1, 130.3, 131.3, 137.1, 140.3, FAB-MS.668.16

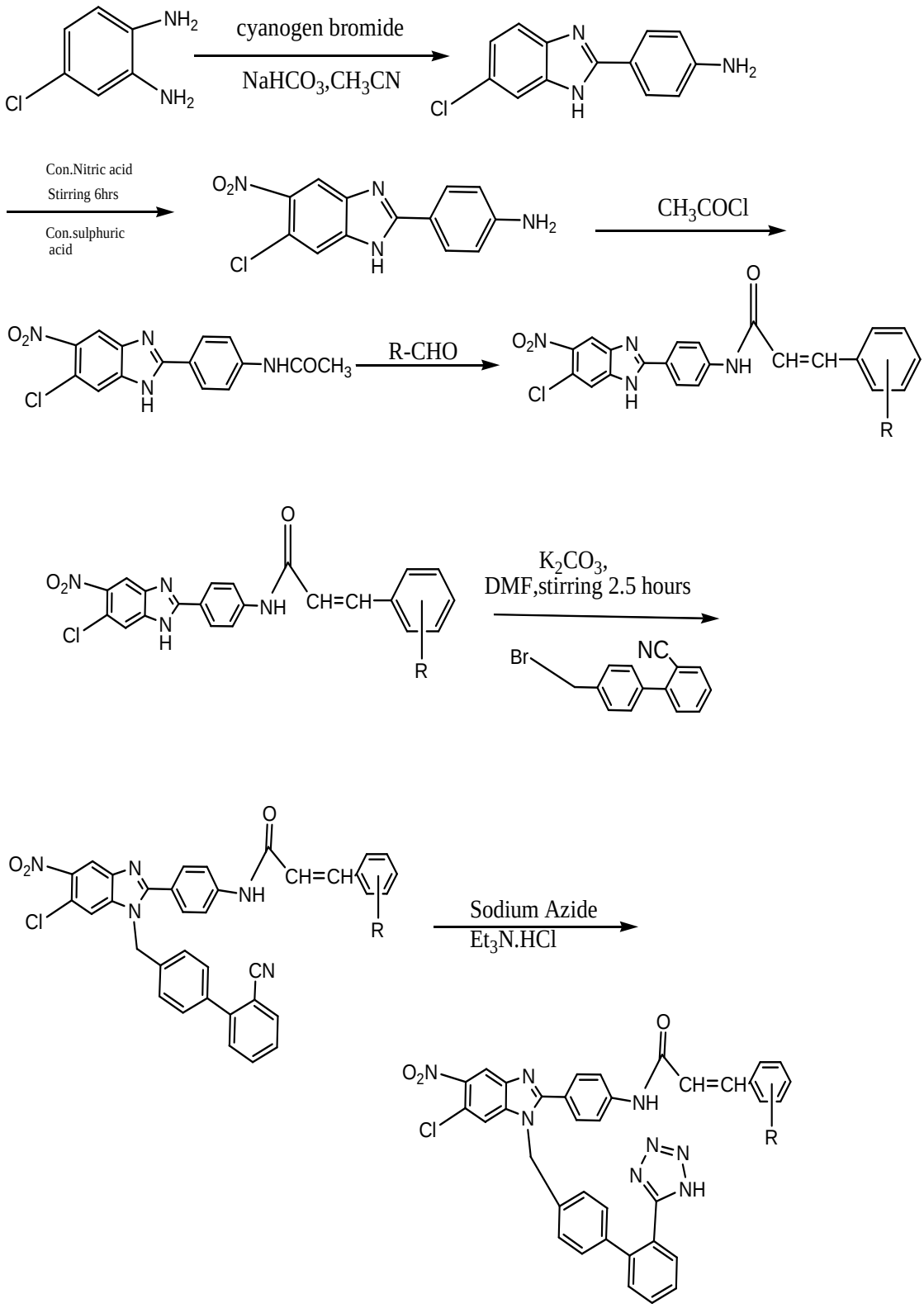
[6] N-{4-(6-Chloro -5-nitro-1-[2'--(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-1H-benzo imidazol-2yl-}-phenyl)-3-(2-methoxy-phenyl)-acryl amide

Yield: 55 %, m.p. = 221-224^{0}C. Anal.Calcd for $C_{36}H_{27}ClN_8O_4$:C,65.05; H, 3.98; N,16.75%; IR (KBr): 3391, 3254, 3143, 2822, 1547, 1670, 1643, 1527-1343, 1151. ^{1}HNMR (300 MHz, $CDCl_3$), 12.37(1H, s, -NH-Benzimidazole), 10.05(s, 1H, tetrazole-NH), 5.03(s, 2H, CH_2),7.11-8.45(m, 18H, Ar-H), 5.01(s, 1H, armO-H), 3.75(t, 3H-OCH_3), 5.87(s, 1H-CH-Cl). ^{13}CNMR ($CDCl_3$)δ: 58.0, 112.9, 113.4, 116.2, 121.1, 128.4, 135.5, 138.2, 142.6, FAB-MS.682.18

[7] N-{4-(6-Chloro -5-nitro-1-[2'--(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-1H-benzo imidazol-2yl-}-phenyl)-3-(3-methoxy-phenyl)-acryl amide

Yield: 60 %, m.p. = 226-228^{0}C. Anal.Calcd for $C_{36}H_{27}ClN_8O_4$: C,65.05; H, 3.98; N,16.75%; IR (KBr): 3391, 3254, 3143, 2822, 1547, 1670, 1643, 1527-1343, 1151.^{1}HNMR (300 MHz, $CDCl_3$), 12.37(1H, s, NH-Benzimidazole), 10.01(s, 1H, tetrazole-NH), 5.03(s, 2H, CH_2), 7.14-8.40(m, 18H, Ar-H), 5.04(s, 1H, armO-H), 3.77(t, 3H-OCH_3), 5.87(s, 1H-CH-Cl). ^{13}CNMR ($CDCl_3$)δ: 58.0, 112.9, 113.4, 116.2, 121.1, 128.4, 135.5, 138.2, 140.2, FAB-MS.683.16

SCHEME



Compound [1-8]

[8] N-{4-(6-Chloro -5-nitro-1-[2'--(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-1H-benzo imidazol-2yl-}-phenyl)-3-(2-ethoxy-phenyl)-acryl amide

Yield: 65 %, m.p. = 246-248^{0}C. Anal.Calcd for $C_{38}H_{29}ClN_8O_4$: C,65.47; H, 4.19; N, 16.07%; IR (KBr): 3384, 3257, 3179, 2838, 1566, 1649, 1637, 1526-1396, 1144. ^{1}HNMR (300 MHz, $CDCl_3$), 12.30(1H, s, NH-Benzimidazole), 10.09(s, 1H, tetrazole-NH), 5.08(s, 2H, CH_2), 7.22-8.49(m, 18H, Ar-H), 5.06(s, 1H, armO-H), 1.36(t, 3H-CH_3), 3.96(s, 2H, CH_2), 5.85(s, 1H-CH-Cl). ^{13}CNMR ($CDCl_3$)δ: 17.9, 53.1, 55.3, 61.1, 70.7, 111.4, 113.1, 113.5, 114.1, 121.2, 135.5, 137.2, 142.5, FAB-MS.696.21

Pharmacological Activity: [14, 15, 17, 19-20]

Non-invasive Method (Indirect Method)

Albino rats weighing 150-200 gm were used to screening for all the synthesized 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. Measurement were done after 1 hour and 3 hour time interval intensive stepwise.

One hour after administration of drug sample, animal was shifted to the restrainers, which restricts the movement of animal. The tail was cleaned with moist cotton to remove the dirty matter and talcum powder was sprayed on tail to make its surface smooth. A tail cuff and pulse transducer was fixed around the tail.

Initially animal shows particular pulse level, when the pulse rate is within the normal range. 'STRAT' switch is put on and the recorder records the blood pressure as SBP (systolic blood pressure). DBP (Diastolic blood pressure) and MABP (mean arterial blood pressure), which is displayed on monitor. The pressure can be easily read from the pre-calibrated monitor. Once all the values are displayed the recorder is switched off and for next measurement. Some procedures are allowed once when sufficient pulse level is attained. [Table1, 2]

Table 1. Hypertension induced in normotensive rat

Comp.	Exp. Animal Albino (Wistar) Rat	After 1hour			After 3 hour		
		SBP	DBP	MABP	SBP	DBP	MABP
[1]	1	140	102	121	140	103	121
	2	138	104	122	137	104	120
	3	142	112	127	139	102	121
	4	140	108	124	143	101	122
	5	137	104	121	140	103	121
[2]	1	138	106	122	137	101	119
	2	143	110	127	134	102	118
	3	137	102	124	135	102	118
	4	139	107	123	140	101	120
	5	143	109	126	137	104	120

[3]	1	141	109	125	139	102	120
	2	143	103	123	141	103	122
	3	140	106	123	138	101	119
	4	138	104	121	140	106	123
	5	141	109	125	143	106	124
[4]	1	136	112	124	141	103	122
	2	142	112	127	140	103	121
	3	140	110	125	139	107	123
	4	138	106	122	141	103	122
	5	132	110	121	143	105	124
[5]	1	141	111	126	139	104	121
	2	144	105	124	139	103	122
	3	140	113	127	142	107	124
	4	138	104	121	143	103	123
	5	138	105	122	143	107	123
[6]	1	136	113	124	142	101	121
	2	142	112	127	140	103	121
	3	140	110	125	139	107	123
	4	138	106	122	141	103	122
	5	132	110	121	143	105	124
[7]	1	140	108	124	138	102	120
	2	144	106	125	142	101	123
	3	143	110	127	134	102	118
	4	138	107	128	143	101	121
	5	140	108	125	141	104	120
[8]	1	144	111	126	143	112	116
	2	144	106	125	144	109	128
	3	145	112	126	139	100	124
	4	142	109	126	143	111	126
	5	140	102	123	140	100	120
Control	**Losartan**	125	-	-	-	-	-

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**	**MAB P**	**SBP**	**DBP**	**MABP**
[1]	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	125	100	112	121	107	114
[2]	1	128	102	115	130	103	116
	2	125	105	115	127	101	114
	3	120	102	111	123	101	112
	4	125	103	114	126	100	113

	5	122	100	111	128	103	114
[3]	1	125	101	113	121	101	110
	2	123	107	115	125	100	112
	3	126	103	114	126	96	111
	4	129	101	115	119	104	111
	5	123	107	115	121	99	110
[4]	1	127	105	119	123	103	113
	2	129	100	111	126	104	115
	3	123	101	113	124	103	112
	4	126	102	114	128	104	116
	5	132	104	118	122	101	111
[5]	1	128	102	115	127	104	114
	2	125	105	110	126	103	115
	3	126	104	110	123	106	116
	4	124	100	112	128	101	113
	5	130	104	117	128	102	115
[6]	1	140	108	124	138	102	120
	2	144	106	125	142	101	123
	3	143	110	127	134	102	118
	4	137	102	124	135	102	118
	5	139	107	123	140	101	120
[7]	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
[8]	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
Control	**Losartan**	116	-	-	-	-	-

Invasive Method (Direct Method)

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.

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	**167**	**164**	**158**	**151**	**145**	**139**	**131**	**125**	**120**	**115**
1	169	153	149	144	141	137	132	128	125	122
2	179	172	166	160	155	149	142	138	133	127
3	177	169	164	159	153	149	142	137	131	125
4	181	176	170	165	159	154	147	141	135	129
5	177	170	167	161	156	148	142	137	130	123
6	178	171	163	157	151	145	138	131	125	121
7	176	170	165	157	151	142	137	130	122	122
8	179	173	168	164	159	152	148	143	139	136

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.)

Table: 4 Antihypertensive Activity of synthesized compounds

Compound. No	Minimum Blood pressure value(mm Hg)	Duration of hypertension effect(min.)
Losratan	115	90
1	117	100
2	124	100
3	121	105
4	116	115
5	117	100
6	119	105
7	120	100
8	113	120

RESULTS AND DISCUSSION

Training the animals and monitoring the animal's temperature may also be beneficial. The volumetric pressure recording method provides the highest degree of correlation with telemetry and direct blood pressure and is clearly the preferred tail-cuff sensor technology. Non-invasive blood pressure devices that utilize Volume Pressure Recording are a valuable tool in research and will continue to be beneficial in many study protocols.

The main advantages are: (1) they require no surgery; (2) they are significantly less expensive than other blood pressure equipment, such as telemetry; (3) they can screen for systolic and diastolic BP changes over time in large numbers of animals; and (4) they provide the researcher with the ability to obtain accurate and consistent blood pressure measurements over time in long-term studies.4-chloro-1,2-phenylenediamine dihydrochloride (0.45 g, 2.5 mmol) in 5 ml of water was cooled to 0°C and treated with a solution of cyanogen bromide (0.60 ml, 5 M in acetonitrile, 3.0 mmol) and solid $NaHCO_3$ (0.41 mg, 4.9 mmol).

The solution was stirred at ambient temperature for 40-45 h. N-[4-(6-Chloro -5-nitro-1H-benzoimidazol-2-yl)-phenyl]acetamide (1.12g, 0.01 mole) in absolute ethanol (30 mL) and various aromatic aldehydes (1.06 g, 0.01 mole) were taken and then an aqueous solution of KOH (2%, 5 mL) added to. the mixture was stirred for 2.5 hours at room temperature, and 4-(bromomethyl) biphenyl-2'-nitrile 1.5 g (15.10 mmol) was added. N-{4-(6-Chloro -1-(2-cyano-biphenyl-4-ylmethyl)-5-nitro-*1H* benzoimidazol-2-yl)-phenyl]-3-substituted-phenyl-acrylamide (0.70 g, 1.59 mmol), sodium azide (0.55 g, 7.2 mmol), and Et3N·HCl (0.89 g, 1 mmol) in NH_4Cl (50 mL) is stirred at 160^0C for 12 h.

The maximum activity has been observed with nitro group (Compound 4,7,8 and 9). there are some sites in the receptor pocket, which can interact with the functional groups at position 5. Substituted benzimidazole nitro group nucleus coupled to biphenyl tetrazole group has been designed, synthesized and evaluated for angiotensin II antagonism. Compound with amino group at 5-position and aromatic, aryl, alkyl compounds at 2- position have been found to be more potent than losratan.

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