

DESIGN; SYNTHESIS AND PHARMACOLOGICAL INVESTIGATION OF SOME BENZIMIDAZOLE DERIVATIVES 4'-(5, 6-SUBSTITUED-2-TRIFLUOROMETHYL-BENZOIMIDAZOL-1-YLMETHYL)-BIPHENYL-2-CARBOXYLIC ACID AS POTENT ANTIHYPERTENSIVE AGENTS

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Synthesis of Some Benzimidazole derivatives 4'-(5, 6-substitued-2-trifluoromethyl-benzoimidazol-1-ylmethyl)-biphenyl-2-carboxylic acid were synthesized by condensation of various steps with different o-Phenylenediamine was condensed with in propylene glycol was added trifluoroacetic acid in the presence of $\text{BF}_3 \cdot \text{OEt}_2$ as catalysis. Elemental analysis, IR, ¹HNMR and mass spectral data confirmed the structure of the newly synthesized compounds. Synthesized and subjected to evaluate their antihypertensive activity. All the synthesized compounds of the series elicit remarkable activity in comparison to standard drug (Losartan).

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Keywords: Ang II, antihypertensive activity, $\text{BF}_3 \cdot \text{OEt}_2$, biphenyl-2-carboxylic acid

1. Introduction

The renin-angiotensin system (RAS) is known to play an important role in the regulation of blood pressure and electrolyte balance.¹ 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 reabsorption, and nor epinephrine release) and thus is an appropriate target for therapeutic intervention in hypertension². Inhibitors of the RAS would be effective for the treatment of hypertension and congestive heart failure. Although angiotensin-converting enzyme (ACE) inhibitors are highly effective and their use has become well-established for the treatment of hypertension and congestive heart failure, they suffer from some side effects such as dry cough and angioedema caused by the nonspecific action of ACE.³ On the other hand, angiotensin II (AII) (the primary effector component of the RAS) receptor antagonists block the RAS at the AII receptor level and are expected to be more specific and effective agents than ACE inhibitors. Angiotensin II (Ang II) α type 1 (AT1) receptor belongs to the G protein-coupled receptor super family and mediates virtually all the known physiological actions of Ang II through interaction with heterotrimeric G-protein and subsequent activation of several effector systems (phospholipases C, D, A2, adenylyl cyclase, etc.). AT1 receptor shows the seven hydrophobic Trans membrane domains forming R-helices in the lipid bilayer of the cell membrane and plays a key role in the renin angiotensin system involved in the regulation of cardiovascular functions and pathophysiology of hypertension.^{4,5} 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

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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 adjunct 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}

2. Experimental section

2.1 Materials and method

Synthesize benzimidazole derivatives. $\text{BF}_3 \cdot \text{OEt}_2$ is a Lewis acid catalyst used in a wide variety of applications, such as, in mild dehydration of tertiary alcohols to alkenes, in Diels-Alder reaction, in cleavage of ethers, in THP protection of alcohols, in rearrangement of epoxides to carbonyl compounds, in reaction of ally tin reagents with aldehyde and ketones *etc.* However, there are examples of the use of $\text{BF}_3 \cdot \text{OEt}_2$ as a catalyst for the preparation of benzimidazoles¹⁹. Herein, protocol for the rapid synthesis of a variety of biologically significant benzimidazoles using a catalytic amount of $\text{BF}_3 \cdot \text{OEt}_2$ under extremely mild solvent-free conditions. 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.

3. Preparation of various steps in synthesis compounds

Synthesis of 4-(5, 6-substitued-2-trifluoromethyl-benzoimidazol-1-ylmethyl)-biphenyl-2-carboxylic acid

Difference substituted [R₁ and R₂] o-Phenylenediamine was condensed with in propylene glycol (15 mL) was added trifluoroacetic acid (4.5 g,) in the presence of BF₃•OEt₂ (2.5 mmol) to this reaction mixture, CH₂Cl₂ (60 mL) was added and washed with water and the solution was heated at 110°C for 6 hrs. The organic phase was separated, dried (Na₂SO₄) and concentrated *in vacuo* to get the crude compound then cooled to room temperature. The crude compounds were purified by silica gel column chromatography using ethyl acetate: chloroform (95:5) as eluent. The reaction mixture was poured into crushed ice. Filtered, washed, dried and recrystallized [MCS-01]. To a solution of was dissolved in 50 ml of DMF (dimethyl formamide) and stirred vigorously with 28mg of potassium carbonate at 44 °C for 3hours. To the resulting mixture 1.2 gm of 4'-bromomethyl biphenyl-2-carboxylic acid dissolved in DMF and then was added drop wise with dropping funnel in three hour the reaction was allowed to proceed for further 11 hours at room temperature and solvent removed under vacuum. Residue was treated with 20ml of dilute HCl and extracted with ethyl acetate. After stirring for 12 h the mixture was poured into water (120 mL) and extracted with ethyl acetate (3 × 50 mL). The combined extracts were dried (MgSO₄) and evaporated. The organic layer was washed with brine solution, distilled water and dried over anhydrous sodium sulphate. (MCS-02) was obtained.

Compounds spectral data analysis

[1] 3-(2'-Carboxy-biphenyl-4-ylmethyl)-6-chloro-2-trifluoromethyl-3H-benzoimidazole-5-carboxylic acid

Yield: 65%, m.p. = 166-169 C. C₂₃H₁₄ClF₃N₂O₄: Found: C,58.16;H, 2.99;N,5.94 %;IR (KBr): 3632,3583,3441,3311,3031, 2937,2876, 2316,1712, 1532, 1147, 1104,754. ¹HNMR(300MHz,CDCl₃)13.11(1H,s,-NH Benzi),11.04(s,2H,COOH),7.3-8.5(m,9H,ArH),5.0(s,2H,CH₂). ¹³CNMR(CDCl₃)δ:50.1,112.9,113.4,116.2,121.1,128.4,135.5,138.2, 142.6, FAB-MS, 476.054

[2] 4'-(5-Fluoro-2-trifluoromethyl-benzoimidazole-1-ylmethyl)-biphenyl-2-carboxylic acid

Yield: 73%, m.p. = 194-197 C. C₂₂H₁₄F₄N₂O₂: Found: C,63.74;H, 3.46;N,6.79 %;IR (KBr):3618,3597,3454,3031,2937,2311,1710,1542,1142,1097. ¹HNMR(300MHz,CDCl₃)13.09(1H, s,NH,Benzim),11.21(s,1H,COOH),7.32-8.48(m,10H,ArH),5.05(s,2H,CH₂). ¹³CNMR(CDCl₃)δ:52.6,112.9,113.4,116.2,121.1,128.4,135.5,138.2,142.6, FAB-MS, 414.11

[3] 4'-(5-Bromo-6-methyl-2-trifluoromethyl-benzoimidazole-1-ylmethyl)-biphenyl-2-carboxylic acid

Yield: 65%, m.p. = 216-219 C. C₂₃H₁₆BrF₃N₂O₂: Found: C,56.46;H, 3.27;N,5.77 %;IR(KBr):3605,3586,3451,3071,2987,2301,1706,1547,1140,1091. ¹HNMR(300MHz,CDCl₃)13.07(1H,s,NH,Benzim),11.23(s,1H,COOH),7.24-8.51(m,9H,ArH), 2.33(s,3H,CH₃),5.03(s,2H,CH₂). ¹³CNMR(CDCl₃)δ:52.6,112.9,113.4,116.2,121.1,128.4,135.5,138.2,142.6, FAB-MS, 488.034

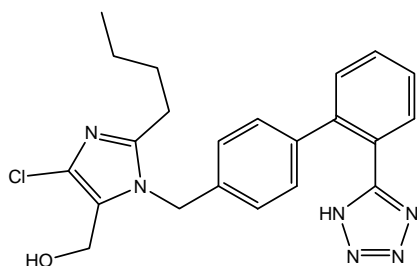
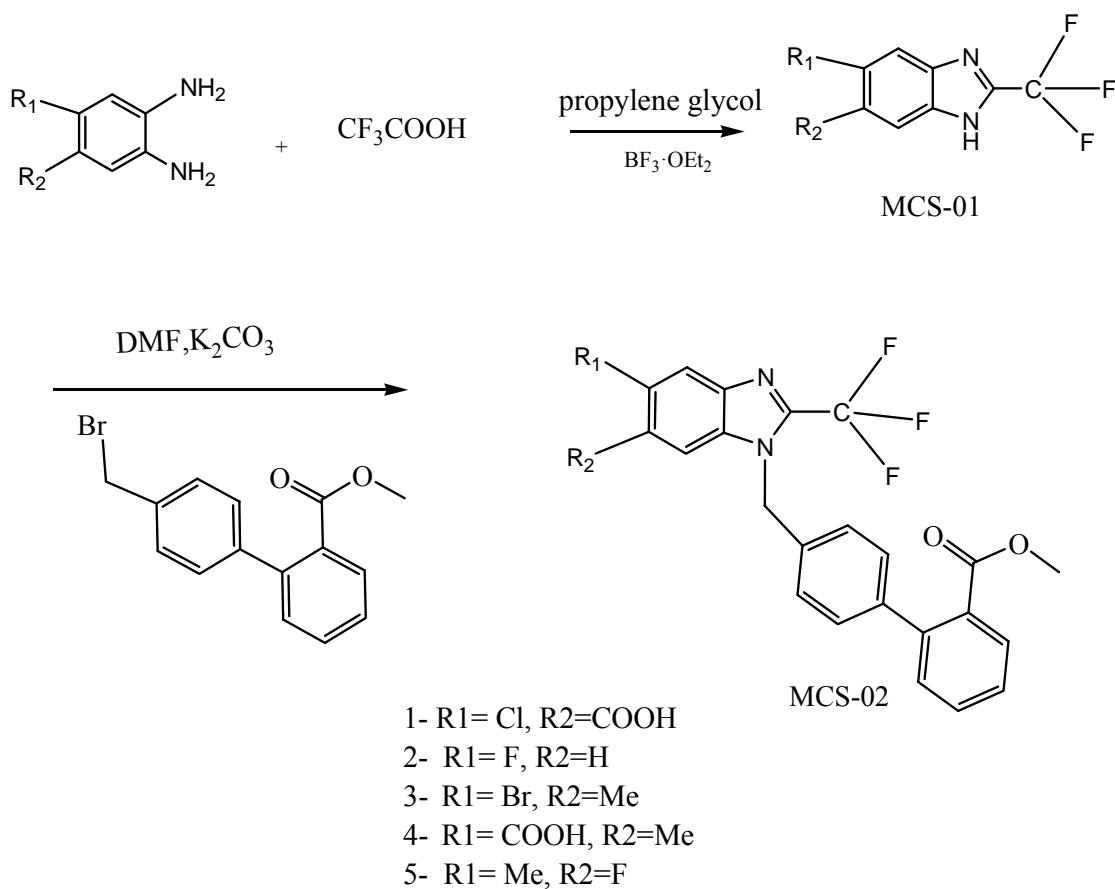
[4] 1-(2'-Carboxy-biphenyl-4-ylmethyl)-6-methyl-2-trifluoromethyl-3H-benzoimidazole-5-carboxylic acid

Yield: 73%, m.p. = 243-246 C. C₂₄H₁₇F₃N₂O₄: Found: C,63.43;H, 3.75;N,6.21 %;IR(KBr):3625,3581,3459,3063,2980,2336,1702,1540,1146,1088. ¹HNMR(300MHz,CDCl₃)13.04(1H,s,NH,Benzim),11.20(s,2H,COOH),7.28-8.55(m,9H,ArH), 2.37(s,3H,CH₃),5.01(s,2H,CH₂). ¹³CNMR(CDCl₃)δ:18.3,51.1,112.9,113.4,116.2,121.1,128.4,135.5, 138.2,142.6,148.76 FAB-MS, 455.31

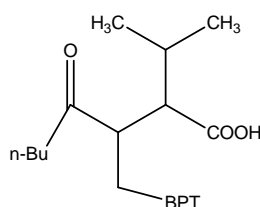
[5] 4'-(6-Fluoro-6-methyl-2-trifluoromethyl-benzoimidazole-1-ylmethyl)-biphenyl-2-carboxylic acid

Yield: 70%, m.p. = 226-229 C. $C_{23}H_{16}F_4N_2O_2$: Found: C, 64.47; H, 3.78; N, 6.60 %; IR(KBr): 3621, 3575, 3453, 3063, 2989, 2329, 1707, 1537, 1146, 1090. 1H NMR(300MHz, $CDCl_3$) 13.01 (1H, s, NH, Benzim), 11.24 (s, 1H, COOH), 7.28-8.55 (m, 9H, ArH), 2.38 (s, 3H, CH_3), 5.07 (s, 2H, CH_2). ^{13}C NMR($CDCl_3$) δ : 20.04, 51.1, 112.9, 113.4, 116.2, 121.1, 128.4, 135.5, 138.2, 142.12, FAB-MS, 429.43

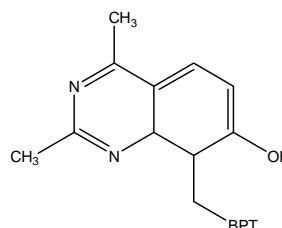
SCHEME



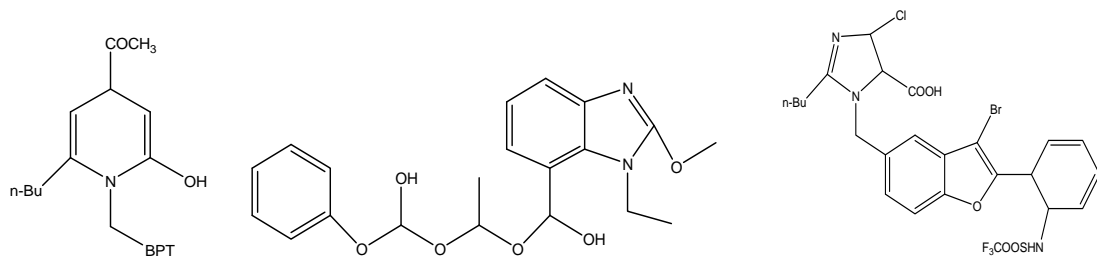
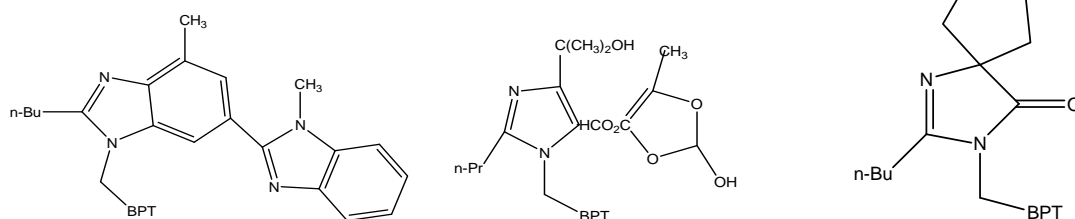
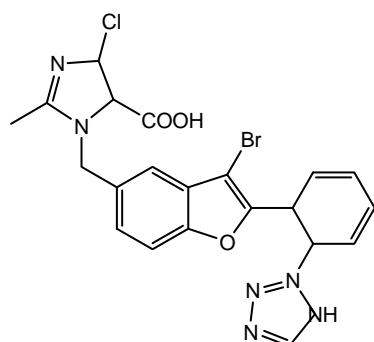
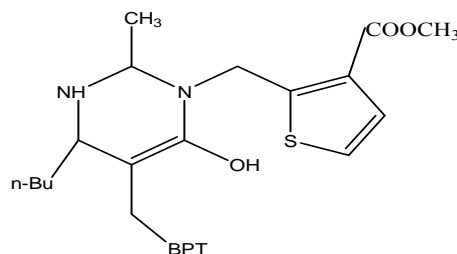
Losartan



Valsartan



Tasosartan

**Candesartan****Milfasartan****Sapisartan****Telmisartan****Olmesartan****Irbesartan****Zolzsartan****Eprosartan**

Angiotensin II selective antagonists

Antihypertensive Activity:^{18, 21-26}

Non-invasive Method (Indirect Method) Albino rats weighing 150-250 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. Control 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. Measurements 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]

Invasive Method (Direct Method): 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- $\mu\text{g}/100\text{ml}$, 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 cannula 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 (50mg/kg i.v.)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 | 142 | 112 | 127 | 140 | 102 | 121 |
| | 2 | 144 | 116 | 130 | 141 | 101 | 122 |
| | 3 | 150 | 123 | 136 | 103 | 119 | 137 |
| | 4 | 147 | 125 | 138 | 105 | 122 | 140 |
| | 5 | 146 | 114 | 12 | 143 | 101 | 122 |
| [2] | 1 | 146 | 104 | 132 | 142 | 100 | 121 |
| | 2 | 148 | 104 | 125 | 145 | 102 | 123 |
| | 3 | 140 | 106 | 123 | 142 | 101 | 121 |
| | 4 | 142 | 108 | 125 | 141 | 102 | 120 |
| | 5 | 139 | 110 | 125 | 143 | 101 | 120 |
| [3] | 1 | 146 | 114 | 12 | 143 | 101 | 122 |
| | 2 | 142 | 112 | 124 | 141 | 103 | 122 |
| | 3 | 142 | 112 | 127 | 140 | 103 | 121 |
| | 4 | 148 | 107 | 123 | 140 | 101 | 120 |
| | 5 | 132 | 104 | 128 | 142 | 102 | 122 |
| [4] | 1 | 143 | 105 | 124 | 143 | 105 | 124 |
| | 2 | 142 | 112 | 127 | 140 | 102 | 121 |
| | 3 | 144 | 116 | 130 | 141 | 101 | 122 |
| | 4 | 142 | 102 | 124 | 143 | 101 | 122 |
| | 5 | 140 | 105 | 122 | 137 | 103 | 120 |
| [5] | 1 | 144 | 114 | 129 | 146 | 106 | 126 |
| | 2 | 142 | 111 | 125 | 146 | 104 | 125 |
| | 3 | 142 | 112 | 127 | 140 | 102 | 121 |
| | 4 | 144 | 116 | 130 | 141 | 101 | 122 |
| | 5 | 141 | 106 | 124 | 141 | 101 | 121 |
| Control | Losartan | 125 | - | - | - | - | - |

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

| Comp. | Exp. Animal Albino (Wistar) Rat | After 1hour | | | After 3 hour | | |
|---------|---------------------------------------|-------------|-----|------|--------------|-----|------|
| | | SBP | DBP | MABP | SBP | DBP | MABP |
| [a] | 1 | 122 | 104 | 118 | 124 | 98 | 113 |
| | 2 | 127 | 108 | 119 | 126 | 103 | 109 |
| | 3 | 128 | 106 | 117 | 123 | 100 | 112 |
| | 4 | 127 | 101 | 116 | 125 | 105 | 110 |
| | 5 | 124 | 102 | 119 | 128 | 102 | 111 |
| [b] | 1 | 126 | 103 | 114 | 126 | 96 | 111 |
| | 2 | 129 | 101 | 115 | 119 | 104 | 111 |
| | 3 | 123 | 107 | 115 | 121 | 99 | 110 |
| | 4 | 126 | 103 | 114 | 125 | 104 | 113 |
| | 5 | 132 | 105 | 119 | 121 | 102 | 110 |
| [c] | 1 | 126 | 104 | 115 | 125 | 105 | 115 |
| | 2 | 124 | 104 | 114 | 121 | 100 | 110 |
| | 3 | 129 | 102 | 116 | 124 | 101 | 113 |
| | 4 | 133 | 103 | 117 | 127 | 105 | 116 |
| | 5 | 130 | 108 | 118 | 124 | 100 | 112 |
| [d] | 1 | 126 | 103 | 114 | 126 | 96 | 111 |
| | 2 | 129 | 101 | 115 | 119 | 104 | 111 |
| | 3 | 123 | 107 | 115 | 121 | 99 | 110 |
| | 4 | 131 | 106 | 118 | 119 | 103 | 107 |
| | 5 | 122 | 104 | 112 | 125 | 101 | 113 |
| [e] | 1 | 123 | 102 | 113 | 128 | 103 | 112 |
| | 2 | 126 | 104 | 110 | 123 | 106 | 116 |
| | 3 | 122 | 106 | 114 | 129 | 101 | 113 |
| | 4 | 124 | 106 | 115 | 127 | 102 | 114 |
| | 5 | 133 | 103 | 118 | 126 | 100 | 110 |
| Control | Losartan | 104 | | - | - | - | - |

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 | 163 | 158 | 152 | 147 | 142 | 137 | 132 | 126 | 123 | 113 |
| [1] | 169 | 164 | 158 | 151 | 152 | 148 | 144 | 138 | 133 | 129 |
| [2] | 175 | 170 | 166 | 159 | 153 | 149 | 142 | 139 | 135 | 132 |
| [3] | 167 | 164 | 159 | 153 | 148 | 143 | 139 | 136 | 133 | 128 |
| [4] | 169 | 164 | 159 | 154 | 149 | 144 | 140 | 135 | 131 | 130 |
| [5] | 173 | 167 | 163 | 160 | 152 | 148 | 142 | 139 | 136 | 134 |

Table: 4 Antihypertensive Activity of synthesized compounds

| Compound. No | Minimum Blood pressure value(mm Hg) | Duration of hypertension effect(min.) |
|-----------------|-------------------------------------|---------------------------------------|
| Losratan | 113 | 90 |
| [1] | 118 | 110 |
| [2] | 121 | 115 |
| [3] | 122 | 100 |
| [4] | 120 | 112 |
| [5] | 118 | 120 |

4. Results and discussion

The compounds are recommended for the screening of antihypertensive activity. Synthesis compounds were screened for their antihypertensive activity by methods using 200-250 gm male either sex. The rats having hypertension more than 160 mm of Hg were taken for the experiment. All the twelve compounds synthesized [1-5] showed antihypertensive activity and with compared the standard drug. The maximum activity has been observed with carboxylic, chlorine and fluorine group. There are some sites in the receptor pocket, which can interact with the functional groups at position 5 and 6. Substituted benzimidazole nucleus coupled to carboxylbiphenyl methyl group has been designed, synthesized and evaluated for angiotensin II antagonists. Among the compounds tested for antihypertensive activity, the compounds 3 were found to reduce blood pressure significantly that is compared with standard.

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