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Synthesis, Characterization and Biological Evaluation of Benzimidazole And Benzindazole Derivatives as Anti-Hypertensive Agents

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Abstract

A substituted benzimidazole and benzindazole derivatives had been synthesized having antihypertensive activity through antagonizing the angiotensin II (Ang II) receptors. The in vivo antihypertensive activity of the compounds was done with acute renal hypertension model. Two compounds TG 1 and TG 3 were found to have antihypertensive activity comparable to Telmisartan which is a prototype for Angiotensin II receptor antagonists class of drugs.In an antihypertensive study the compounds TG 1, TG 2 and TG 3 had systolic blood pressures of 147.2 mm/Hg, 168.2 mm/Hg, and 126.3 mm/Hg, respectively. This systolic blood pressure was lower than the disease control vehicle-treated rodents, which had a systolic blood pressure of 167.2 mm/Hg. The diastolic blood pressure was 119.7 mm/Hg, 124.7 mm/Hg and 88.83 mm/Hg, respectively and that of the disease control vehicle-treated rodents was 122.3 mm/Hg. TG 3 had comparable decrease in the MABP to Telmisartan. These encouraging results make compound TG 3 effective anti-hypertensive drug candidate and worthy of further investigation.

Keywords: Hypertension;Synthesis;Chracterisation; Non-peptide AT1 Receptor Antagonist; Renin-Angiotensin System

1. Introduction

Hypertension is a common and serious illness that affects people all over the world. Hypertension affects about 30 percent of the world's population [1]. There are a wide variety of the synthetic and herbal medicines which are used for the treatment of hypertension [2,3]. Angiotensin (Ang) plays pivotal role in mediating hypertension where Angiotensin Converting Enzyme (ACE) inhibitors and Angiotensin Receptor Blockers (ARBs) are the drugs of choice to treat hypertension at present [4]. Though ACE inhibitors are used widely in the treatment of the hypertension but because of their side effects i.e. dry cough made ARBs as drug of choice. ARBs work by preventing Angiotensin-II (Ang-II) from binding to the Angiotensin type-1 (AT1) receptor. AT1 receptors are mainly found in the heart, kidneys, brain adrenal glands, brain, and liver. Angiotensin receptor Blockers (ARBs) are highly selective for the AT1 receptor. They show 10,000– 30,000 times greater affinity for the AT1 receptor than for the AT2 receptor. [6]. The rennin angiotensin system (RAS) present in the kidney plays an important role in controlling the blood pressure. Angiotensin II is an octapeptide produced in RAS from angiotensin I by angiotensin converting enzyme (ACE), and the component of the rennin-angiotensin system is responsible for one of the most powerful vasoconstrictors. Angiotensin II receptor blockers (ARBs) are the newest class of approved antihypertensive agents and have rapidly become established as one of the leading therapeutic drugs in

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the management of hypertension [6] .

Nowadays, many potent and safe ARBS are available in the market for the treatment of hypertension i.e., losartan, valsartan, irbesartan, candesartan, telmisartan etc [6]. Candesartan [7] and telmisartan are the two drugs have the benzimidazole ring and both are used in the treatment of the hypertension by blocking the AT1 receptors. Here a derivatives of benzimidazole and benzindazole are synthesized (compound TF1, TG2 and TG3) were synthesized taking telmisartan and candesartan as lead compounds. The anti-hypertension effects of these were evaluated and found that compound TG1 and TG3 were having the comparable antihypertensive activity as compared to telmisartan. *extent* from the presentation achieved in this Word® document.

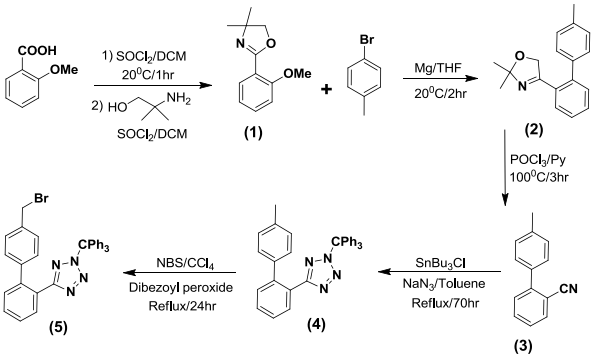
2. Experimental

2.1. Chemistry

The analytical grade chemicals, reagents, and solvents were procured from Merck Chemicals India Ltd., Germany; Sigma-Aldrich Ltd., Germany; and HiMedia Ltd., India.The melting point (mp) was measured on an melting point apparatus and were not accurated. Merck® silica gel coated aluminum sheets (silica gel 60 F254) thin layer chromatography was used to ensure the completion of the reaction. A JASCO FT/IR-4100 spectrophotometer was used to capture the Fourier-transformed Infrared (FT-IR) spectra (in KBr pellets). On a Bruker® 400 MHz setup, proton (1H)-Nuclear Magnetic Resonance (NMR) spectra were reported (through CDCl3) with trimethyl silane (TMS) as an internal standard. Mass spectra was recorded on JEOL JMS-D 300 spectrometer running at 70 eV.

2.1.1. Synthesis of C-1

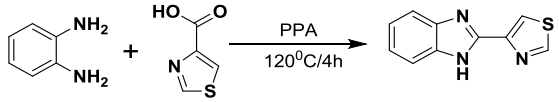
2-methoxybenzoic acid (17 g, 0.1mol) was treated with a solution of 2-amino-2-methyl-1-propanol (20g,0.224mol) in the 2-methoxybenzoic acid (17 g, 0.1mol) was treated with a solution of 2-amino-2-methyl-1-propanol (20g,0.224mol) in the presence of thionyl chloride(22ml) and dichloromethane to give compound (1). To this 1-bromo-4-methylbenzene (13 ml, 0.106 mol) was added to get compound (2) which was further treated with phosphorus oxy chloride to get compound (3) which was refluxed for 70 hours with tributyltinchloride, sodium azide, and toluene to get compound (4). This was refluxed for 3 hours with N-bromosuccinimide and dibenzoyl peroxide in carbon tetrachloride and cooled at 4°C to get C1 (**Scheme 1**)[8,9,10].



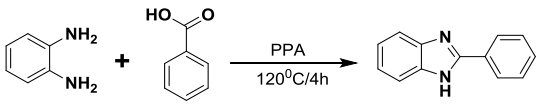
Scheme 1. Synthesis of Compound C-1.

2.1.2. General method for Synthesis of C-2a and C-2b

Benzene-1,2-diamine (3.34g,0.0310mol) was added to thiazole-4-carboxylic acid (4 g,0.0310 mmol) for the synthesis of C2a and benzoic acid for the synthesis of C2b in the presence of polyphosphoric acid [11]at 120°C for 4 hrs (**Scheme 2).**



C-2a

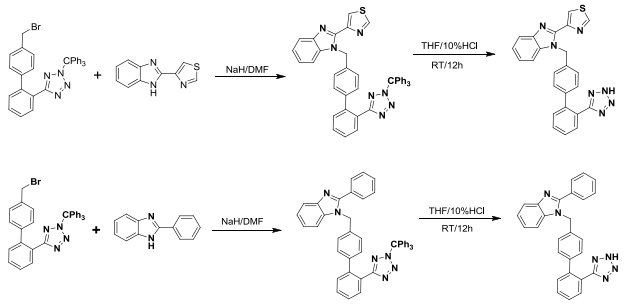


C-2b

Scheme2. Synthesis of C2a and 2b

2.1.3. General method for Synthesis of TG1 and TG2

C-1(17 g,0.1mol)was added to C-2a and C2b in the presence of sodium hydride and dimethylformamide to form an intermediate compound 5-{2-[1-(2-(thiazol-4-yl)-1*H*-benzo[d]imidazole)methyl) benzene]phenyl}-2-trityl-2*H*-tetrazole which was refluxed for 12 hrs with tetrahydrofuran in the presence of HCl to get TG 1and TG 2 respectively (Scheme 3)[12,13]



Scheme 3: Synthesis of TG1 and TG2

4-(1-((2'-(2H-tetrazol-5-yl)-[1,1'-biphenyl]-4-yl)methyl)-1H-benzo[d]imidazol-2-yl)thiazole: (TG1)

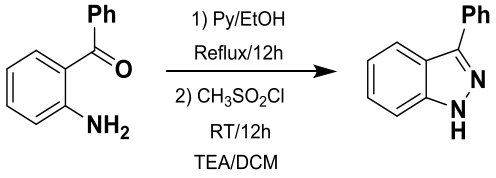
IR (cm^{-1}): 3501.21, 3059.93, 1569.92, 1260.65, 901.78, 869.64. ^{1}H NMR (400 MHz, $CDCl_3$, ppm) 9.27 (s,1H),) 8.03 (s,1H), 7.57-7.84 (m, 9H), 7.03-7.25 (m, 4H), 6.21 (s,2H).13C NMR (400 MHz, CDCl3, ppm) 156.52, 144.33, 140.84, 140.47,138.78,135.03,131.0(2), 129.85,,127.81,127.09(2),126.85,125.15(2),123.34, 116.33, 112.56,75.20,48.12,. MS(ESI): calcd 436.01; found 436.06 Anal.Calcd for C24H17N7S: C, 66.19; H, 3.93; N, 22.51; S, 7.36

1-((2'-(2H-tetrazol-5-yl)-[1,1'-biphenyl]-4-yl)methyl)-2-phenyl-1H-benzo[d]imidazole (TG 2)

IR (cm^{-1}): 3432.21, 3060.66, 1559.32, 1240, 869.64. ^{1}H NMR (400 MHz, $CDCl_3$, ppm) 8.23 (s,1H), 7.9 (d, 1H),7.64-7.83 (m, 8H), 7.5-7.58 (m, 4H), 7.02-7.12 (m,4H), 5.75 (s,2H).^{13}C NMR (400 MHz, $CDCl_3$, ppm) 151.52, 140.97, 138.86, 134.68,133.80,131.86,131.05,129.63,129.63,129.16,128.06,127.90,127.74,127.66,127.48, 126.60,126.43,125.25,124.88,123.34,116.51,114.31, 112.59, 47.81 MS(ESI): calcd 429.50; found 429.25 .C27H20N6: C, 75.68; H, 4.70; N, 19.61

2.1.4. Synthesis of Compound 3a

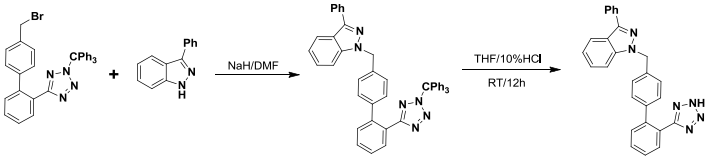
2-aminophenyl)(phenyl)methanone is treated with methane sulfonyl chloride and ethanol for 12 hrs for the formation of 3-phenyl-1H-indazole (Scheme 4).



Scheme 4: Synthesis of Compound 3a

2.1.5. Synthesis of TG 3

5-{2-[1-(bromomethyl)benzene]phenyl}-2*H*-tetrazole is treated with 3-phenyl-1*H*-imidazole with sodium hydride and dimethylformamide to form an intermediate which on treating with tetrahydrofuran in the presence of HCl for 12 hrs gives an compound .(scheme 5)



Scheme 5. Synthesis of TG 3

1-((2'-(2H-tetrazol-5-yl)-[1,1'-biphenyl]-4-yl)methyl)-3-phenyl-1H-indazole (TG 3)

IR (cm^{-1}): 3415.85, 3056.01, 1604.04, 1259.46, 821.53. ^{1}H NMR (400 MHz, $CDCl_3$, ppm) 8.03 (s,1H), 7.9 (d, 3H),7.34-7.58 (m, 10H), 7.20-7.33 (m, 4H), 5.55 (s,2H).13C NMR (400 MHz, CDCl3, ppm) 154.52, 144.97, 141.262, 140.47,138.80,136.86,132.88,131.27,130.68,130.68,129.85,128.87,128.29(2),128.03,127.57,127.08,123.34, 121.73(3),109.28,75.20,76.90,77.01, 77.23, 51.97 MS(ESI): calcd 428.50; found 428.26 .C27H20N6: C, 75.68; H, 4.70; N, 19.61

2.2. Biological Evaluation

Male Wistar albino rats of either sex weighing 100-150 g were procured from MM University's central animal house in Ambala, Haryana. The animals were stabilized for one week and held in regular conditions at room temperature with a 12-hour light-dark period. Throughout the study, they were fed on a normal pellet diet and had access to unlimited water. The animals were gently treated to prevent any discomfort, which could lead to rise in adrenal activity.

Blood pressure was measured non-invasively using the tail cuff procedure with an LE 5002 storage pressure meter (PAN LAB, Harvard Apparatus). The NIBP tail-cuff procedure was used to measure systolic blood pressure in each rodent . Before each systolic blood pressure measurement, the conscious rats were warmed with the cuff and the pulse wave transducer placed across the tail for 10 minutes.The mean arterial pressure (MABP) was calculated by the formula: MABP = (SBP − DBP)/3 + DBP.[6]

48 male Wistar rats were divided into eights groups, each comprising of 6 animals.

Group-1 Normal Control (NC): Rats treated with vehicle as distilled water by intravenous route at a dose of 1 ml/kg without any drug.

Group-2 Disease Control (DC): Rats treated with Angiotensin-II (120 ng/kg) in distilled water by intravenous route.

Group-3 Positive Control (PC): Rats treated with simultaneous *Telmisartan (2 mg/kg)* and Ang-II in distilled water by intravenous route.

Group-4 Test Group-1 (TG1): Rats treated with simultaneous TG 1 *(2 mg/kg) a*nd Ang-II in distilled water by intravenous route.

Group-5 Test Group-2 (TG2): Rats treated with simultaneous TG 2 *(2 mg/kg)* and Ang-II in distilled water by intravenous route.

Group-6 Test Group-3 (TG3): Rats treated with simultaneous TG 3 *(2 mg/kg)* and Ang-II in distilled water by intravenous route.

3. Results And Discussions

3.1. Chemistry

The spectroscopic study highlighted some key features which supported the formation of the proposed compound. The amine and amide groups

were ascertained from the FT-IR spectra at 3391 cm^{-1} and 3282 cm^{-1}. Furthermore, the N-H bending and C-N stretching at 1594 cm^{-1} and 1271 cm^{-1}, respectively, corroborate the amide constituent in the molecule. The linkage was predominantly observed from the vibrational peaks at 1686 cm^{-1}. The methylene was verified from the $–CH_2$ part which appeared in both vibrational and rotational spectroscopic spectra. Additionally, the stretching noticed in the proton-NMR spectra revealed the peak at 9.27 or at 8.03 ppm was due to the presence of the NH of the tetrazole, whereas all the aromatic proton peaks were in between the 7.1 to 7.9. . The two methylene protons were appeared at 5.2 ppm. Moreover, the peaks at 3155 (C-H, aromatic) and 1633 (C=C, aromatic) reflected the presence of the aromatic components. The mass spectra substantiated the formation of the compound as noticed from the base peak which corresponded exactly with the molecular mass.

3.2. Anti-hypertensive potentials

The antihypertensive activity was measured as a decrease in MABP (mm of Hg) (**Table 1**). The data were statistically analyzed by performing one way ANOVA followed by Dunnets multiple comparison Test. In Dunnets multiple comparison test, group DC was compared with NC and other groups i.e. the test group were compared with DC.

Table 1.*In-vivo* effects of test drugs on angiotensin-II induced spontaneous hypertension in rats.

Groups	Systolic Blood Pressure	Diastolic Blood Pressure	Mean Blood Pressure	Heart Rate
Normal Control (NC)	116.3±1.1160	87.33±0.7149	109.3±1.783	85.50±0.8466
Disease Control (DC)	167.7±0.7601	122.3±1.874	172.8±1.276	93.50±2.487
Positive Control (PC)	114.2±1.0782	82.28±1.167	105.2±1.327	75.17±1.662
Test Group-1 (TG1)	147.2±1.7011	119.7±1.430	170.5±1.232	88.17±2.358
Test Group-2 (TG2)	168.2±1.0464	124.7±1.626s	162.2±1.537	93.67±1.563
Test Group-3 (TG3)	126.3±0.7608	88.83±2.750	123.7±0.6667	77.67±1.085

Data are expressed as Mean ± SEM and analyzed statistically by One way ANOVA followed by Dunnett's Multiple Comparison Test, using Graph Pad Prism Software trial version. IN Dunnett's Multiple Comparison Test, Group DC was compared with NC and other treated groups were compared with DC. P value considered as P<0.05 Significant (*), P<0.01 Very Significant (**), P<0.001 Highly Significant (***).

The test drugs group 1-3 showed systolic blood pressure 147.2 mm/hg, 168.2 mm/hg, 126.3 mm/hg respectively whereas systolic blood pressure was 167.2 mm/Hg of disease control vehicle treated rats. TG-3 and TG-1 showed lower systolic blood pressure level 126.3 mm/hg and 147.2 mm/hg as compared to 114.2 mm/hg of positive control telmisartan treated rats (**Fig 1**).

The test drugs group 1-3 showed diastolic blood pressure 119.7 mm/hg, 124.7 mm/hg, 88.83 mm/hg respectively. whereas the diastolic blood pressure was 122.3 mm/hg for the disease control vehicle treated rats. TG-3 and TG-1 showed diastolic blood pressure level 88.83 mm/hg and 119.7±1.430 mm/hg as compared to 82.28 mm/hg of positive control telmisartan treated rats (**Fig 2**).

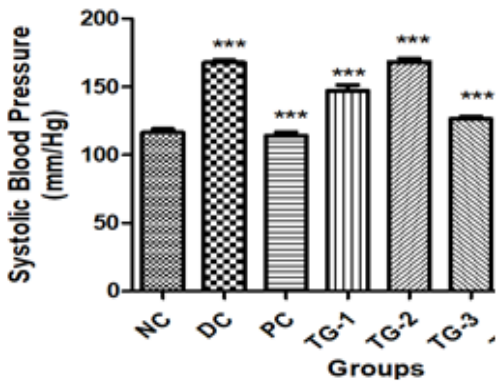


Fig 1. Systolic Blood Pressure of various test drugs vs. control groups.

Data are expressed as Mean ± SEM and analyzed statistically by One way ANOVA followed by Dunnett's Multiple Comparison Test, using Graph Pad Prism Software trial version. IN Dunnett's Multiple Comparison Test, Group DC was compared with NC and other treated groups were compared with DC. P-value considered as P<0.05 Significant (*), P<0.01 Very Significant (**), P<0.001 Highly Significant (***).

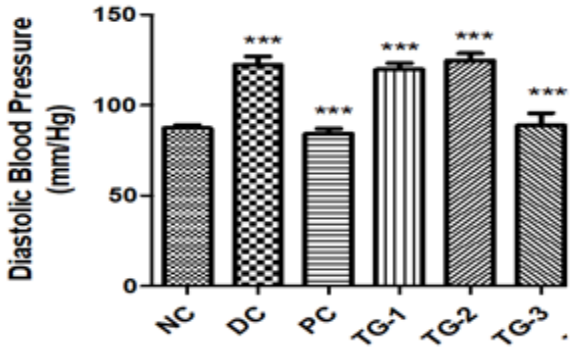


Figure 2. Diastolic Blood Pressure of various test drugs vs. control groups

Data are expressed as Mean ± SEM and analyzed statistically by

One way ANOVA followed by Dunnett's Multiple Comparison Test, using Graph Pad Prism Software trial version. IN Dunnett's Multiple Comparison Test, Group DC was compared with NC and other treated groups were compared with DC. P-value considered as P<0.05 Significant (*), P<0.01 Very Significant (**), P<0.001 Highly Significant (***).

The antihypertensive effect can be evaluated on the maximam fall in the MABP as compared to the DC. Telmisartan i.,e. the positive control showed significant fall in the MABP i.e. if we compare the positive control with the DC there was maximum fall in BP to 114mm/Hg i.e change in 53mm/Hg . In TG2 and TG 3 the maximum fall in BP was 162.2. and 123.7 mm/Hg i.e. there is a change of 10 and 49mm/Hg. This showed that the TG3 had comparable decrease in the MABP to Telmisartan.The test drug 3 (TG-3) and test drug 5 (TG-5) showed lower systolic blood pressure level 123.7 mm/hg and 101.8 mm/hg as compared to 105.2 mm/Hg of positive control telmisartan treated rats (**Fig 3**).

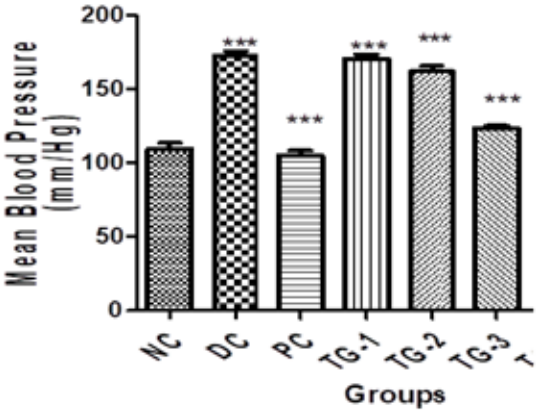


Fig 3. Mean Blood Pressure of various test drugs vs. control groups

Data are expressed as Mean ± SEM and analyzed statistically by One way ANOVA followed by Dunnett's Multiple Comparison Test, using Graph Pad Prism Software trial version. IN Dunnett's Multiple Comparison Test, Group DC was compared with NC and other treated groups were compared with DC. P value considered as P<0.05 Significant (*), P<0.01 Very Significant (**), P<0.001 Highly Significant (***).

In anti-hypertensive study the test drugs group 1-3 showed heart rate 88.17, 93.67, 77.67, 96.83, and 71.50, respectively. This heart rate was significantly lower than 93.50 of disease control vehicle treated rats. TG-3 and TG-1 showed lower heart rate level 77.67 and 88.17 as compared to 75.17 of positive control telmisartan treated rats (**Fig 4**)

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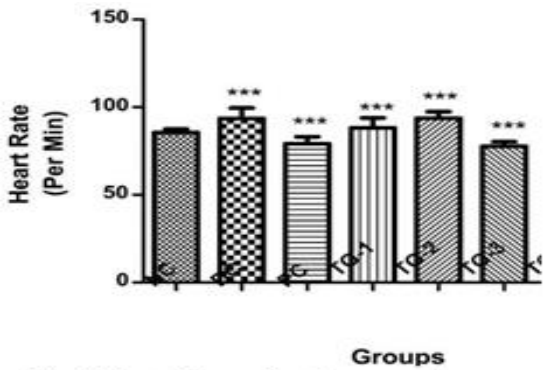

Fig 4. Heart Rate of various test drugs vs. control groups

Data are expressed as Mean ± SEM and analyzed statistically by One way ANOVA followed by Dunnett's Multiple Comparison Test, using Graph Pad Prism Software trial version. IN Dunnett's Multiple Comparison Test, Group DC was compared with NC and other treated groups were compared with DC. P value considered as P<0.05 Significant (*), P<0.01 Very Significant (**), P<0.001 Highly Significant (***).

In the reported anti-hypertensive study amongst all newly synthesized TG 3 and TG 1 showed more beneficial anti-hypertensive effect as compared to all treated and control rats. During comparison of anti-hypertensive effect of test drug TG-3 and TG-1, the TG-3 treated rats showed most comparable lower blood pressure and heart rate with standard treated and vehicle treated rats.

4. Conclusion

A number of novel non-peptide ARBs have been engineered, synthesized, and tested. Three compounds TG1, TG2 and TG 3 were synthesized and a dosage of 2 mg/kg, all three compounds were shown to induce a significant reduction in blood pressure. However, TG-1 and TG-3, had a greater anti-hypertensive activity than the other compounds. .These encouraging results make **TG 3** a potent anti-hypertensive drug candidate deserving for further investigation.

5. Abbreviation section

Ang	Angiotensin
ACE	Angiotensin Converting Enzyme
Ang-II	Angiotensin-II
AT1	Angiotensin type-1
ARBs	Angiotensin receptor Blockers
NIBP	Non-invasive blood pressure
MABP	The mean arterial pressure
SBP	Systolic blood prsessure
DBP	Diastolic blood pressure

6. Conflicts of interest

"There are no conflicts to declare".

7. Acknowledgments

We acknowledge Amity University, Noida and K.R. Mangalam University, Gurugram India.

8. References

[1] Han X.F., He X., Wang M., Xu D., Hao L.P., Liang A.H., Zhang J.and Zhou Z.M. Discovery of novel, potent and low-toxicity angiotensin II receptor type 1 (AT1) blockers: Design, synthesis and biological evaluation of 6-substituted aminocarbonyl benzimidazoles with a chiral center. *European Journal of Medicinal Chemistry*, 103:473-487(2015).

[2] Banerjee S., Bose S., Mandal S.C., Dawn S., Sahoo U., Ramadan M.A.M. and Mandal S.K. Pharmacological Property of Pentacyclic Triterpenoids. *Egyptian Journal of Chemistry*, 62, 13-35(2019).

[3] Mandal S.K., Das A., Dey S., Sahoo U., Bose S., Bose A., Dhiman N., Madan S. and Ramadan M.A.M. Bioactivities of Allicin and Related Organosulfur Compounds from Garlic: Overview of the Literature Since 2010. *Egyptian Journal of Chemistry*, *62*, 1-11(2019).

[4] Siragy H. Angiotensin II receptor blockers: review of the binding characteristics. *The American Journal of Cardiology*,84,3-8(1999).

[5] Wan Y., Wallinder C., Plouffe B., Beaudry H., Mahalingam A.K., Wu X., Johansson B., Holm M., Botoros M., Karlén A. and Pettersson A. Design, synthesis, and biological evaluation of the first selective nonpeptide AT2 receptor agonist. *Journal of medicinal chemistry*,47(24),5995-6008(2004).

[6] Bao X.L., Zhu W.B., Shan T.L., Wu Z., Zhang R.J., Liao P.Y., Zheng M.Z., Tang H.S., Yan Y.J. and Chen Z.L. Design, synthesis and evaluation of novel angiotensin II receptor 1 antagonists with antihypertensive activities. *RSC Advances*,7(42),26401-26410(2017).

[7] Asma S. Al-Wasidi , Moamen S.R., Ahmed M.N.and Ahmed A. E. Different Potential Biological Activities of Benzimidazole. Derivatives. Egyptian Journal of Chemistry, 64(5),2631 - 2646 (2021).

[8] Bali A., Bansal Y., Sugumaran M., Saggu J.S., Balakumar P., Kaur G., Bansal G., Sharma A. and Singh M. Design, synthesis, and evaluation of novelly substituted benzimidazole compounds as angiotensin II receptor antagonists. *Bioorganic & Medicinal Chemistry Letters*, 15(17),3962-3965(2005).

[9] Shah D.I., Sharma M., Bansal Y., Bansal G. and Singh M. Angiotensin II–AT1 receptor antagonists: Design, synthesis and evaluation of substituted carboxamido benzimidazole derivatives. *European Journal of Medicinal Chemistry*, 43(9),1808-1812(2008).

[10] Kaur N., Kaur A., Bansal Y., Shah D.I., Bansal G. and Singh M. Design, synthesis, and evaluation of 5-sulfamoyl benzimidazole derivatives as novel angiotensin II receptor antagonists. *Bioorganic & medicinal chemistry*,6(24),10210-10215(2008).

[11] Mandal S.K.and Ray S.M. Synthesis and Biological Evaluation of (5,6-Dialkoxy-3-Oxo-2,3 Dihydro-1*H*-Inden-1-yl)Acetic Acid Esters as Anti- Inflammatory Agents with Much Reduced Gastrointestinal Ulcerogenic Potential. *Indo American Journal of Pharmaceutical Research*, 4, 3796-3807(2014).

[12] Wang J.L., Zhang J., Zhou Z.M., Li Z.H., Xue W.Z., Xu D., Hao L.P., Han X.F., Fei F., Liu T.and Liang A.H. Design, synthesis and biological evaluation of 6-substituted aminocarbonyl benzimidazole derivatives as nonpeptidic angiotensin II AT1 receptor antagonists. *European Journal of Medicinal Chemistry*, 49,183-190(2012).

[13] Zhang J., Wang J.L., Zhou Z.M., Li Z.H., Xue W.Z., Xu D., Hao L.P., Han X.F., Fei F., Liu T. and Liang A.H. Design, synthesis and biological activity of 6-substituted carbamoyl benzimidazoles as new nonpeptidic angiotensin II AT1 receptor antagonists. *Bioorganic & Medicinal Chemistry*, 20(14),4208-4216(2012).