Synthesis, characterization and antihypertensive activity of 2-phenyl substituted benzimidazoles

Muhammad Tariq Khan[1], Muhammad Tahir Razi[1], Syed Umer Jan[2*], Muhammad Mukhtiar[3], Rahman Gul[2], IzharUllah[3], Abid Hussain[3], Asif Mehmood Hashmi[3], Muhammad Taufiq Ahmad[4], Nisar Ahmed Shahwani[2] and Imran Rabbani[5]

[1]Faculty of Pharmacy, Bahuddin Zakariya University, Multan, Pakistan
[2]Faculty of Pharmacy and Heath Sciences, University of Balochistan, Quetta, Pakistan
[3]Department of Pharmacy, Faculty of Medical and Health Science, University of Poonch, Rawalakot, AJK
[4]Akson College of Pharmacy, University of Science and Technology, Mirpur, AJK
[5]Department of Pharmacy, Kohat University of Science and Technology, Kohat, Pakistan

Abstract: Hypertension is one of cardiovascular disease that is not sufficiently prevented and controlled at both hospital and community levels. Hypertension resulted in significant morbidity and mortality. The benz-imidazole ring is very important pharmacophore in modern drug discovery. The substituted benzimidazoles are the important for medicinal research. Researchers have reported that substituted Benzimidazoles are the structural isosteres of nucleotides, and easily allow them to interact with the different biopolymers, possess pharmacological activity especially antihypertensive activity. Angiotensin II Receptor Antagonists/Blockers (ARBs) compete with angiotensin II at the receptor site and block the contractile effect of angiotensin II in all vascular smooth muscles. Among all Angiotensin II Receptor Antagonists/Blockers (ARBs), Telmisartan, Milfasartan and many others have benzimidazole ring in their structure. In this study Angiotensin II Receptor Antagonists/Blockers (ARBs) have been prepared. Synthesized compounds were characterized by physical data and FTIR spectroscopic technique. Synthesized compounds studied were finally screened for their antihypertensive activity by tail cuff method of measurement of blood pressure by NIBP apparatus (None Invasive Blood Pressure) using Chart 5.0 software. The compounds synthesized were 2-(3-nitrophenyl)-1*H*-benzimidazole (1a), 3-(1*H* benzimidazol-2-yl)aniline (1b) and 5-(1H-benzimidazol-2-yl)-2-methoxyphenol (1c). The synthesized compounds have shown antihypertensive activity by taking Losartan as lead compound.

Keywords: Hypertension, benzimidazole, Telmisartan, Milfasartan, Losartan, 2-phenyl substituted benzimidazoles and Antihypertensive activity.

INTRODUCTION

Benzimidazole compounds are group of Heterocyclic compound with aromatic in nature: possess very prominent pharmacological activities and have drawn the attention of medicinal chemists and pharmacologists to explore it pharmacological and chemotherapeutic activities further. Benzimidazole and its derivatives are considered to be a very effective therapeutic agent due its extensive antimicrobial activity. Its nucleus is analogous to purine and hence has been used as biomimicry agent of guanine residue. N-ribosyl-dimethyl benzimidazole is considered to be the most prominent benzimidizole available in nature, having the activity of an axial ligand for cobalt in Vitamin B12 (Barker *et al*., 1960). It has been reported in literature that various benzimidazole compounds and its derivatives have shown a huge number of biological activities including antibacterial, antioxidants and anti-inflammatory (Ayhan-Kilcigil *et al*., 2014); (Yogita & Om, 2014); (Mentese *et al*., 2015). It is also found that other heterocyclic benzimidazole like pyrazole, quinoline and thiazolidine derivatives also possesses various biological activities (Desai *et al*., 2012). The literature review shows that the benzimidazole derivatives are proven to excellent effective compound and number of reviews available for biochemical and pharmacological studies conformed that their molecules are useful against a wide variety of pharmacological disorders like; a study was performed on the novel hybrid class of drug telmisartan-rosiglitazone molecules. These were prepared to discover the new dual PPARγ angiotensin antagonist. The prepared compounds showed the moderate PPARγ activity. (Chittiboyina *et al*., 2009). It was found that some new imidazole and imidazoline derivatives display the significant biological activities (Jignasa *et al*., 2011). Some new benzimidazole derivatives were prepared with heterocyclic ring having significant blocking activity for AT_1 receptor (Guo *et al*., 2008). Some highly potent AT_1 antagonist named as Fimasartan was discovered with an orally active AT_1 selective blocker and is more potent as compared to losartan and other analogues compounds. (Kim *et al*., 2012). Several new compounds were synthesized, were evaluated by for antihypertensive activity by the method of aortic ring. Some of the compounds showed significant antihypertensive activity (10YU 2006). Some Quinazoline

**Corresponding author:* e-mail: suj55@yahoo.com

derivatives developed, antihypertensive activity was investigated, screened for α1-adrenergic receptor antagonistic activity, have shown potential antihypertensive activity. (Patel *et al*., 2013). In the light of the above findings we extended our studies on Synthesis, Characterization and antihypertensive activity of 2-Phenyl Substituted Benzimidazoles

MATERIALS AND METHODS

Chemicals used in the synthesis of compounds

o-phenylenediamine (OPD) (MERCK), 3-nitrobenzldehyd (MERCK), Acetonitrile (MERCK)Vanillin (Gifted from Global Pharmaceuticals Ltd.), Losartan Potassium (Gifted from Pacific Pharmaceuticals Ltd**,** Normal saline (0.9% NaCl)(MERCK)**,** Distilled water, Sodium carboxy methyl cellulose (Gifted from Global pharmaceuticals Ltd.).

Equipments used in characterization of compounds

Open capillary Tubes (Kimble Chase), FTIR (Perkin Elmer 1800), Silica gel-G plates (Merck), Non-invasive / Direct blood pressure apparatus (NIBP). (Model No. ML 125AD instruments), Power lab Data Acquisition System and software. (Model No. ML 865 AD instruments), Chart 5.0 software (AD instruments), Restrainers, Weighing balance (Shimadzu Corporation, Japan) and Syringes (B.D).

Animals

Sprague dawley albino rats purchased from University of Health Sciences (UHS) Lahore. The animals were placed in the controlled room conditions of 25±1ºC and 12 hour light 12 hour dark cycles. The study was approved from the Animal Ethics committee, University of Sargodha (Approval diary No.25-A13/IEC/UOS).

Methods

Method of synthesis

Synthesis of 2-(3-nitrophenyl)-1H-benzimidazole (1a)

5gm (46 milimoles) of o-phenylenediamine (OPD) and 7gm (46 milimoles) 3-nitrobenzldehyde in 50ml of acetonitrile were refluxed for 14 hours. The mixture was cooled at room temperature. Then crystallized in ethanol and filtered off. The percentage yield obtained in this reaction was 40%.

Synthesis of 3-(1H-benzimidazol-2-yl) aniline (1b)

3.5gm (109 milimoles) of Hydrazine was added to the 0.5gm (2.3milimoles) 2(3-nitrophenyle)-1H-Benzimidazole and 0.05gm (0.47milimoles) Pd (Catalyst) in ethanol (25ml) and then refluxed it in inert atmosphere generated with argon gas. The reaction was completed in 10 hours. The reaction mixture was filtered from catalyst and solvent was evaporated on the rotary evaporator. Compound was purified with ethanol. The percentage yield obtained in this reaction was 58%.

Synthesis of 5-(1H-benzimidazol-2-yl)-2-methoxyphenol (1c)

7g (46 milimoles) Vanillin and 5gm (46 milimoles) OPD in 50ml of acetonitrile were refluxed for more than 18 hours. When the completion of reaction takes place, the final mixture was the cooled at room temperature and then filtered off. The crude product was crystallized in ethanol. The percentage yield obtained in this reaction was 30%.

Antihypertensive activity

Method for the development of hypertensions for normotensive rats

Sprague dawely albino rats weighing 200-300 gm were made hypertensive by cholinomimetic agent (physostigmine) for antihypertensive activity of synthesized substituted benzimidazoles (Badyal *et al*., 2003). Systolic blood pressure (SBP) and Mean blood pressure (MBP) was recorded from-invasive blood pressure apparatus (NIBP) by using the tail of animals (rats). For this, rats were placed in the restrainer. Then the cuff with sensor was finally fitted on the tails of the rats, tail was warmed to 35°C. Then the pressure in the cuff was inflated higher than the expected SBP and slowly decreased, by this the pulse rate was analyzed by the BP analyzer. Readings were taken after one hour and three hour time interval. The MBP and SBP were read from pulse tracings at Chart 5.0 software using NIBP. Heart rate was also recorded. The diastolic blood pressure (DBP) was calculated from Systolic blood pressure and Mean blood pressure by using the formula:

DBP = (3MBP-SBP)/2. (Ninahuaman *et al*., 2007) Observations are given in table 3 & 4.

Administration of test compounds to hypertensive rats

For this the suspension of the test compound was prepared in the sodium carboxy methyl cellulose (1% w/v) and administered orally to the animals at dose of 50 µgm/kg animal body weight to five rats of the each group. Same quantity of the drug was administered to the control group (Siddiqui and Wani 2004). After administration of oral dose to the rats, the BP was analyzed by tail of the animals by the use of NIBP apparatus. The readings were taken after one hour and three hour. Observations are shown in table 5, 6 & 7.

RESULTS

Physical data

The Melting points of all synthesized compounds were determined by open capillary tubes. R_f values were calculated from TLC (Slica gel-G) plates. The Spots were exposed in the Iodine Chamber. Observations were shown in table (1). The percentage yield obtained 40%, 58%, 30% for compound 1a, 1b and 1c respectively.

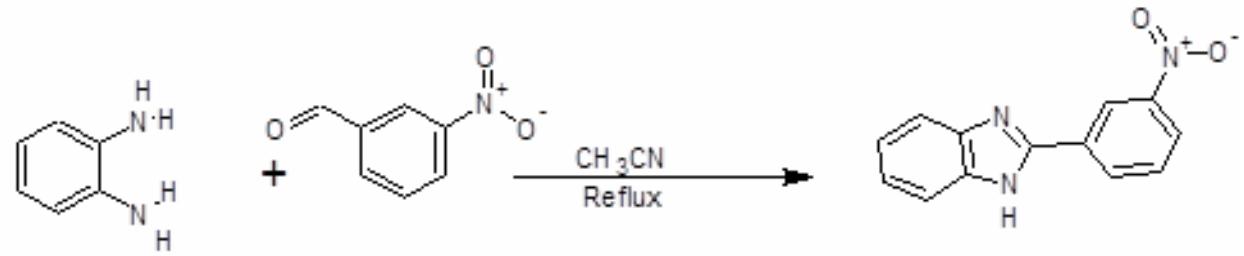

Fig. 1: Synthesis of compound 1a (Scheme 1a).

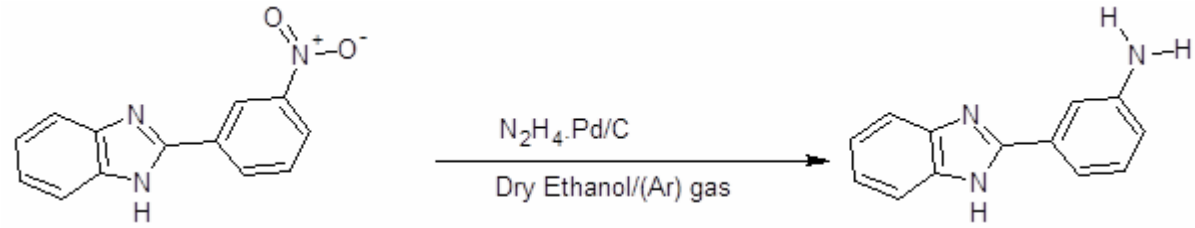

Fig. 2: Synthesis of compound 1b (Scheme 1b)

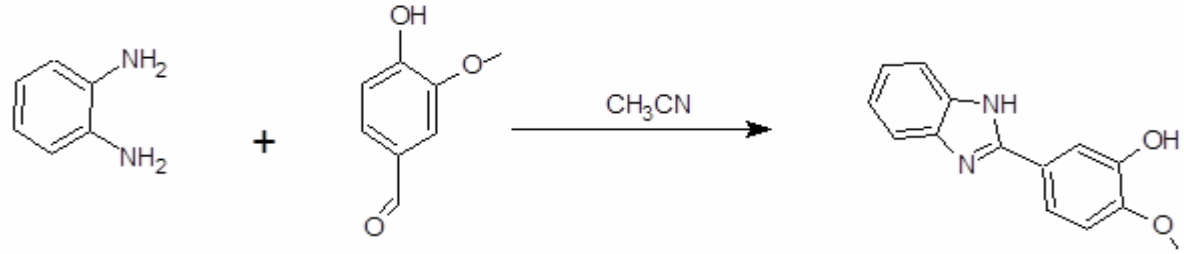

Fig. 3: Synthesis of compound 1c (Scheme 1c)

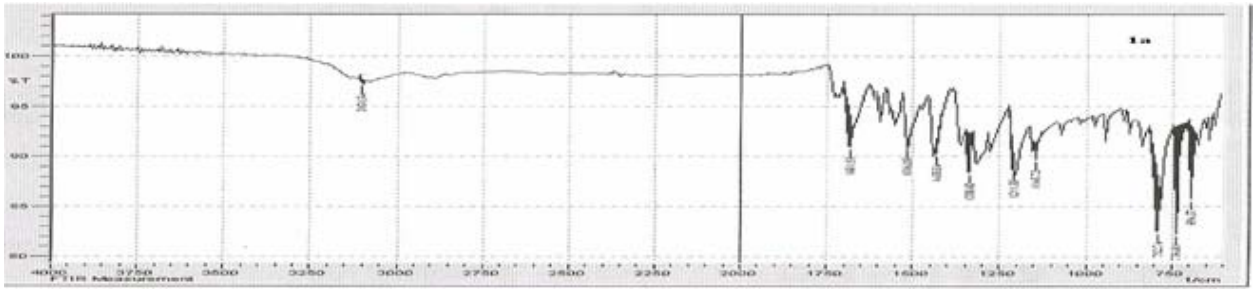

Fig. 4: FTIR Spectrum of Compound 1a

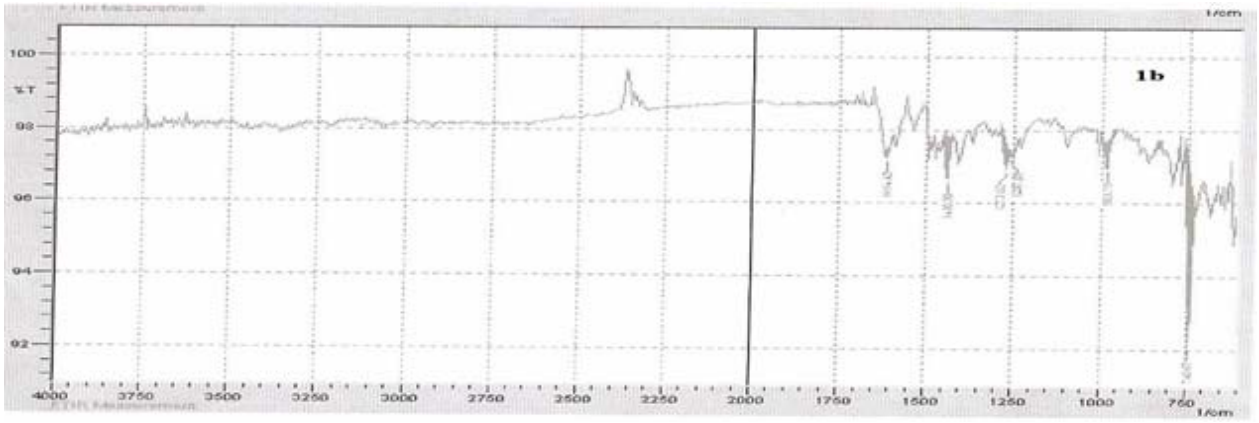

Fig. 5: FTIR Spectrum of Compound 1b

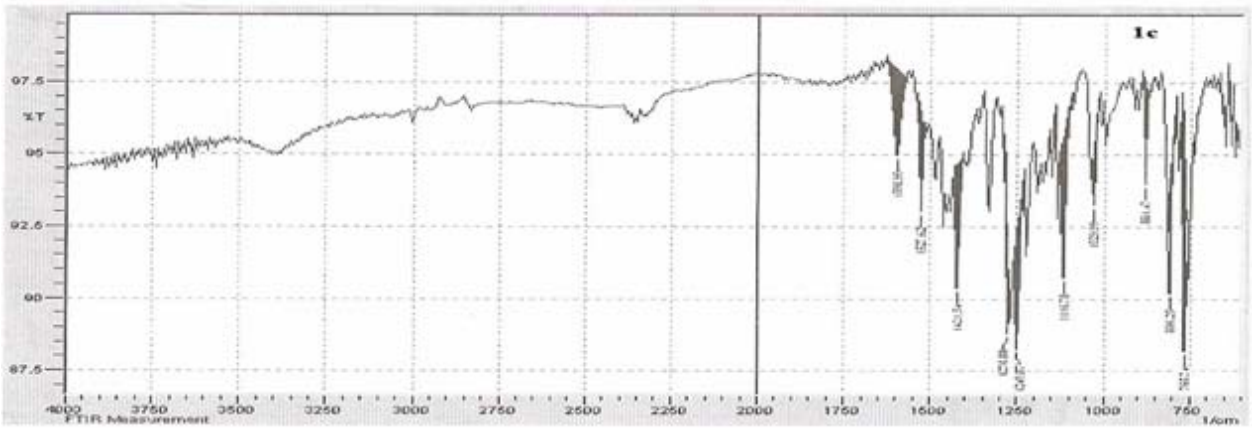

Fig. 6: FTIR Spectrum of Compound 1c

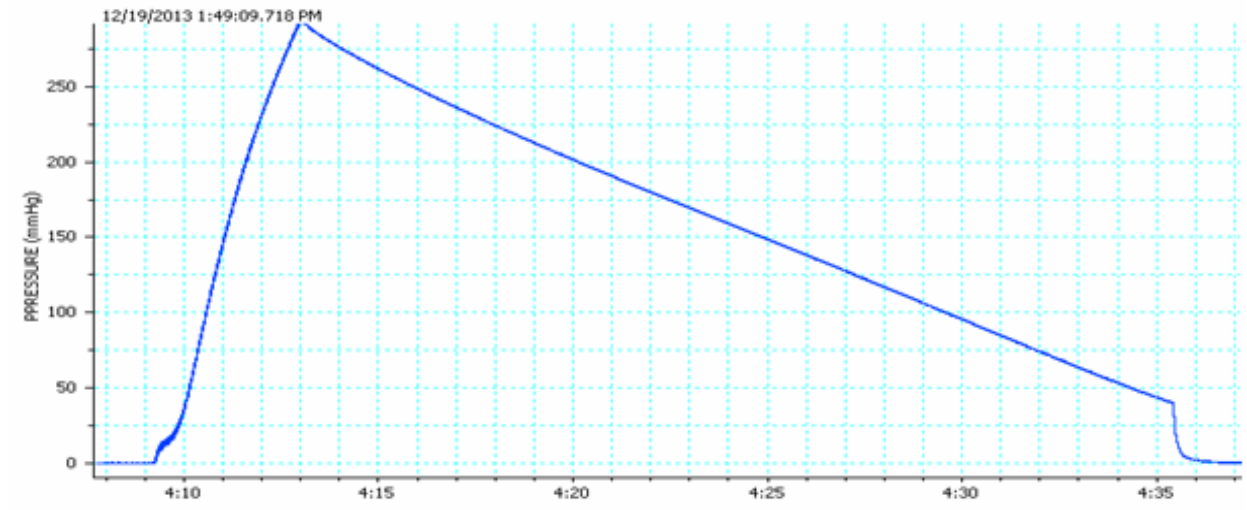

Fig. 7: Cuff inflation and deflation in NIBP chart 5.0 Software

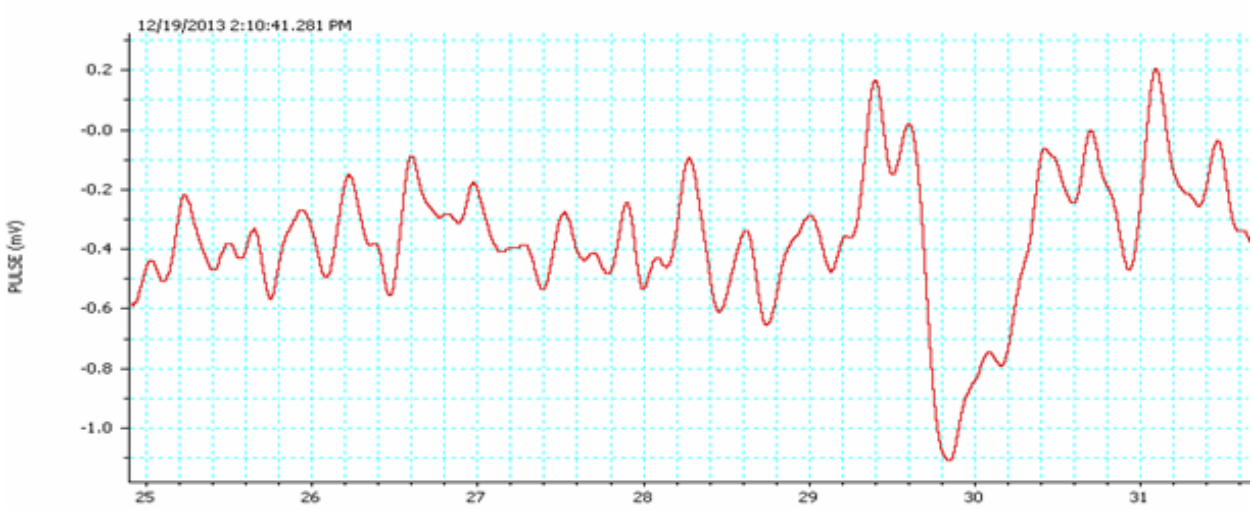

Fig. 8: Recording of NIBP showing pulse and pressure signals

Fig. 9: A Recording showing Heart rate (BPM) in Chart 5.0

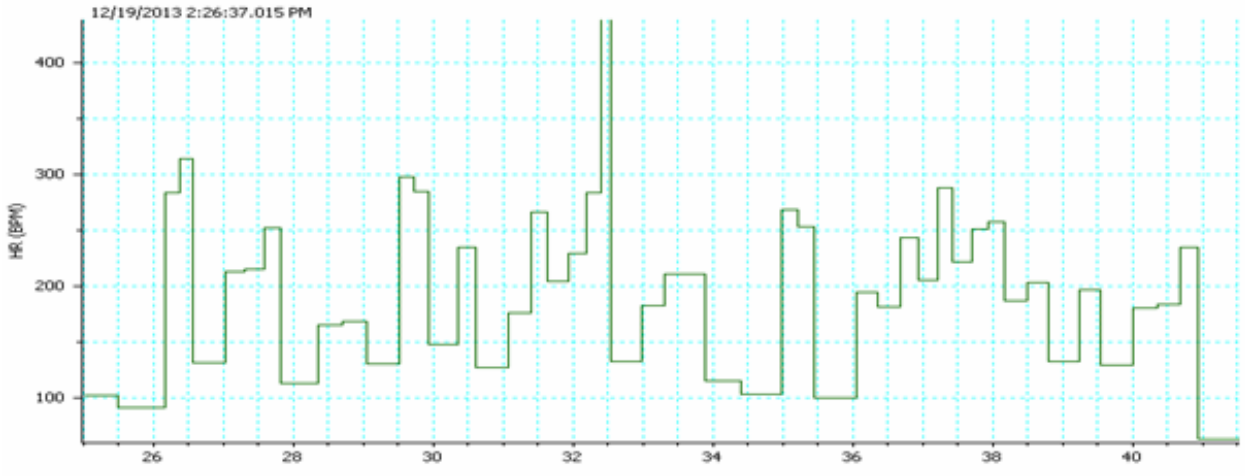

Fig. 10: A recording of Chart 5.0 showing Pulse, Pressure, and Heart rate

Table 1: Physical data

Compound	Colour	State	Melting Point ºC	% yield	Rf
1a	Light Brown	Solid	190	40%	0.51
1b	Dark Yellow	Solid	246	58%	0.63
1c	Yellow	Solid	176	30%	0.67

Table 2: FTIR Data

COMPOUND	C=C(ARO)	C-H(ARO)	N-H	C-N	C=N
1a	1435, 1516	3100	3350	1338	1681
1b	1438, 1500	3101	3448	1273	1614
1c	1421, 1527	3000	3421	1276	1598

FTIR

The Infra-Red spectra were recorded on Perkin Elmer (FTIR). FTIR spectra of all final synthesized compounds (1a, 1b, 1c) were recorded and interpreted. Observations of IR spectra were recorded and given in fig. 4, 5, & 6 for 1a, 1b, 1c compounds respectively. Furthermore the Interpretation of IR spectra for identification of compounds have been given in table 2. Purity of all compounds has been established through appropriate spectral and chromatographic techniques. The synthesized compounds were characterized on the basis of chemical and spectral data (IR).

Table 3: Hypertension induced in normotensive rat (Sprague dawley) (mmHg).

Compound	Experimental animal (Sprague dawley)rat	After 1 hour			After 3 hour		
		SBP	DBP	MBP	SBP	DBP	MBP
1a	1	149	102	118	142	102	115
	2	145	105	118	139	103	115
	3	135	109	118	137	109	118
	4	144	112	123	142	105	117
	5	142	116	125	145	113	124
1b	1	148	112	124	149	113	125
	2	144	109	121	143	105	118
	3	149	112	124	145	110	122
	4	139	104	116	143	106	118
	5	150	101	117	148	104	119
1c	1	152	109	123	150	109	123
	2	147	106	120	144	100	115
	3	146	103	117	140	101	114
	4	133	103	113	140	102	115
	5	142	113	123	140	110	120
(Control) Losartan	1	141	110	120	144	112	123
	2	143	105	118	140	103	115
	3	134	109	117	138	109	119
	4	145	107	120	146	103	117
	5	149	110	123	145	105	118

Table 4: Blood pressure (mm Hg) (Mean ± SEM) in hypertensive rats (Sprague dawley)

Group	After 1 hour			After 3 hour		
	SBP	DBP	MABP	SBP	DBP	MABP
1a	143+2.32	108.8±2.47	120.4±1.50	141.4±1.12	106.4±2.03	117.8±1.65
1b	146±2.02	107.6±2.20	120.4±1.69	145.6±1.24	107.6±1.69	120.4±1.36
1c	144±3.17	106.8±1.90	119.2±1.90	142.8±1.95	104.4±2.11	117.4±1.74
Control	142.4±2.48	108.2±0.97	119.6±1.02	142.6±1.53	106.4±1.78	118.4±1.32

Table 5: Reduction in blood pressure (mm Hg) at a dose of 50 µgm/kg animal body weight

Compound	Experimental animal (Sprague dawley)rat	After 1 hour			After 3 hour		
		SBP	DBP	MBP	SBP	DBP	MBP
1a	1	124	100	108	122	100	107
	2	122	102	109	121	101	108
	3	127	103	111	125	103	110
	4	125	104	111	123	102	109
	5	123	105	111	121	101	108
1b	1	127	101	110	122	100	107
	2	124	103	110	123	101	108
	3	123	100	108	120	96	104
	4	121	102	108	119	100	106
	5	126	101	109	122	99	107
1c	1	129	106	114	125	104	111
	2	126	103	111	123	102	109
	3	125	102	110	123	100	108
	4	124	102	109	121	101	108
	5	126	104	111	125	103	110
(Control) Losartan	1	125	104	111	123	102	109
	2	125	103	110	121	100	107
	3	121	102	108	119	101	107
	4	124	104	111	122	101	108
	5	122	102	109	120	100	107

Table 6: Reduction in blood pressure (Mean ± SEM) at a dose of 50 µgm/kg animal body weight

Compound	After 1 hour			After 3 hour		
	SBP	DBP	MBP	SBP	DBP	MBP
1a	124.2±0.86	102.8±0.86	110±0.63	122.4±0.75	101.8±0.51	108.4±0.51
1b	124.2±1.06	101.4±0.51	109±0.45	121.2±0.73	99.2±0.86	106.4±0.68
1c	126±0.83	103.4±0.74	111±0.84	123.4±0.75	102±0.71	109.2±0.58
Control (Losartan)	123.4±0.81	103±0.45	110±0.58	121±0.71	100.8±0.37	108±0.40

Table 7: Percentage reduction in blood pressure

Compound	After 1 hour			After 3hour		
	SBP	DBP	MABP	SBP	DBP	MABP
1a	13%	6%	8%	13%	4%	8%
1b	15%	5%	9%	17%	8%	12%
1c	13%	3%	7%	14%	2%	7%
Control	13%	5%	8%	15%	5%	9%

Antihypertensive activity (NIBP)

In this study all the synthesized 2-phenyl substituted Benzimidazoles showed good antihypertensive activity by using None Invasive blood Pressure apparatus (NIBP). The present work was mainly intended to establish the moieties which are responsible for Angiotensin-II inhibition. Biological activity of synthesized compounds was carried out using spontaneous hypertensive rats (SHR) using None Invasive blood Pressure apparatus (NIBP). Cuff inflation and deflation has shown in fig. 7. Pulse and pressure signals have shown fig. 8. Heart rate signals have been shown in shown in fig. 9.

Oral administration of suspension of the test compounds (1a,1b,1c) in 1% sodium carboxy methyl cellulose caused an immediate and time dependant fall in systolic blood pressure (SBP) and diastolic Blood pressure (DBP), and mean blood Pressure (MBP) values after one hour and three hours of the drug administration. An oral dose of 50 µg/kg of compound 1a reduced the systolic blood pressure significantly from 143+2.32mmHg to 124.2±0.86mmHg and the diastolic pressure from 108.8±2.47mmHg to 102.8±0.86 mmHg after 1hour. An oral dose of 50µg/kg of compound 1b reduced the systolic blood pressure significantly from 146±2.02mmHg to 124.2±1.06mmHg and the diastolic pressure from 107.6±2.20mmHg to101.4±0.51 mmHg after 1hour. An oral dose of 50µg/kg of compound 1c reduced the systolic blood pressure significantly from 144±3.17mmHg to 126±0.83mmHg and the diastolic pressure from 106.8±1.90mmHg to 103.4±0.74mmHg after 1hour.

DISCUSSION

The results in the current research indicated that the all the blood pressure reductions by the newly synthesized compounds are comparable to Losartan which was used as controlled drug. Compound 1b had shown more significant antihypertensive activity in the spontaneous hypertensive rats (SHR). However all the newly synthesized compounds have no effect on the heart rate in the hypertensive rats. Losartan has also shown no effect on the Heart rate (HR). The percentage reduction in blood has been shown in table 7. The compound 1b had shown maximum reduction in systolic and diastolic blood pressure.

CONCLUSION

It is concluded from our finding that substituted Benzimidazoles and their derivatives possess potential antihypertensive activity; our study is also supported by literatures from modern research that Benzimidazole possesses marked antihypertensive activity (Kedar *et al* 2011). It is found that all the synthesized compounds (1a, 1b, 1c) have antihypertensive effects in spontaneous hypertensive rats (SHR).

REFERENCES

Ayhan KG, Kus C, Coban T, Can EB and Iscan M (2014). Synthesis and antioxidant properties of novel benzimidazole derivatives. *J. Enzyme Inhib. Med. Chem*., **19**(2): 129-135.

Badyal D, Badyal K, Lata H and Dadhich AP (2003). Animal models of hypertension and effect of drugs. *Ind. J. Pharmacol.,* **35**(6): 349-362.

Barker HA, Smyth RD, Weissbach H, Toohey JI, Ladd JN and Volcani BE (1960). Isolation and properties of crystalline cobamide coenzymes containing benzimidazole or 5,6-dimethyl benzimidazole. *J. Biol. Chem.,* **235**(2): 480-488.

Chittiboyina AG, Mizunot SA and Falgun H (2009). Design, synthesis, and docking studies of novel telmisartan–glitazone hybrid analogs for the treatment of metabolic syndrome. *Med. Chem. Res*., **18**(7): 589-610.

Desai NC, Pandya DD, Joshi VV, Rajpara KM, Vaghani HV, Satodiya HM (2012). Synthesis, characterization and antimicrobial screening of hybrid molecules

containing benzimidazole pyrazole and pyridine nucleus. *Med. Chem. Res*., **21**: 4463-4472.

Guo XZ, Shi L and Wang R (2008). Synthesis and biological activities of novel nonpeptide angiotensin II receptor antagonists based on benzimidazole derivatives bearing a heterocyclic ring. *Bioorg. Med. Chem*., **16**(24): 10301-10310.

Jignasa K, Savjani and Anuradha KG (2011). Pharmaceutical importance and sythetic strategies for Imidazole-2-thione and Imidazole 2-thione Derivatives. *Pak. J. Biol. Sci*., **14**(24): 1076-1089.

Kedar MS (2011). Benzimidazole in medicinal chemistry: An overview. *Chem. Inform*. **42**(10): 249-256.

Kim TW, Yoo BW and Lee JK (2012). Synthesis and antihypertensive activity of pyrimidin-4 (3^{H})-one derivatives as losartan analogue for new angiotensin II receptor type 1 (AT_1) antagonists. *Bioorg. Med. Chem. Lett.,* **22**(4): 1649-1654.

Mentese E, Ulker S and Kahveci B (2015). Synthesis and study of a a-glucosidase inhibitory, antimicrobial and antioxidant activities of some benzimidazole derivatives containing triazole, thiadiazole, oxadiazole and morpholine rings. *Chem. Heterocycl. Compd*. **50**(12): 1671-1682.

Patel HU, Patel RS and Patel CN (2013). Synthesis and Antihypertensive Activity of Some Quinazoline Derivatives. *J. Appl. Pharm. Sci*., **3**(03): 171-174.

Siddiqui AA and Wani SM (2004). Synthesis and hypotensive activity of some 6-(substituted aryl)-4-methyl-2, 3-dihydropyridazin-3-ones. *Ind. J. Chem. Sect*., **43**(7): 1574-1579.

Yogita B and Om S (2014). Synthesis and pharmacological evaluation of polyfunctional benzimidazole-NSAID chimeric molecules combining anti-inflammatory, immunomodulatory and antioxidant activities. *Arch. Pharm. Res*., **37**:1426-1436.

Yu WF, Zhou Zhi-Ming, Zhu, Hai-Boc HE and Ling-Shuaic (2006). Synthesis of Telmisartan and its derivatives and evaluation of their biological activities. *Chin. J. Org. Chem.,* **3**: 125-130.