Journal of Computational Methods in Molecular Design, 2012, 2 (4):149-157

Scholars Research Library
(http://scholarsresearchlibrary.com/archive.html)

ISSN : 2231- 3176
CODEN (USA): JCMMDA

Design, Synthesis and Vasorelaxant activity of 5-Nitro Benzimidazole Derivatives

Radhika H. Datani, Suvarna G. Kini* , Muhammad Mubeen.

Department of Pharmaceutical Chemistry, Manipal College of Pharmaceutical Sciences, Manipal University, Manipal- 576104, Karnataka, India.

ABSTRACT

Docking studies of twenty novel 5-nitro benzimidazoles was done with the help of VLife MDS3.5software using GRIP batch docking method by taking AT-2 receptor model with bovine rhodopsin crystal structure as a target. Based on the docking scores of the designedmolecules which ranged from -14.39 to -36.16 , the 5-nitro benzimidazole derivatives were synthesized by refluxing 4-nitro-1,2-phenylenedimaine in dimethoxyethane with various substituted aromatic aldehydes in the presence of sodium metabisulphite as oxiding agent. The test compounds were characterized by TLC, melting point, UV, IR, ^{1}H NMR and mass spectroscopy.All the compounds were evaluated for ex-vivovasorelaxant activity in rat aorta rings pre-contracted with phenylepherine.Compounds BDZ3, BDZ6, BDZ12, BDZ18 and BDZ20 showed good vasorelaxant activity (EC_{50}<30 µM).

Keywords: Nitrobenzimidazoles, docking, angiotensin-II, Vasorelaxant.

INTRODUCTION

Hypertension is a major riskfactor for endothelial dysfunction, metabolic syndrome, diabetes,renal dysfunction, congestive heart failure, coronary artery disease and stroke [1]. These diseases are themost important causes of death in the world. Important classes of antihypertensive drugs are diuretics, sympatholytic drugs, vasodilators, calcium channel blockers,angiotensin converting enzyme inhibitors and angiotensin II receptor inhibitors[2]. Essential hypertension can no longer be considered simply as a state of elevated blood pressure. It is a syndrome ofmultiple abnormalities often characterized by cardiac and vascular remodelling [3], lipid abnormalities and defects in carbohydrate metabolism associated with insulin resistance/hyperinsulinemia. New antihypertensive therapy is needed to control hypertension more effectively, with fewer side effects and neutral impact on known cardiovascular risk factors [4]. In an attempt to identify novel antihypertensive compounds with vasodilator activity, we have tried to design and synthesize novel benzimidazoles derivatives as benzimidazoles are very useful intermediates or subunits in the development of pharmaceuticals and are compounds of biological interest. Fromliterature review benzimidazole derivatives have found application in diverse therapeutic areas including antihypertensive, antiviral, antiulcer, anticancer, anti-histaminic, antitubercular, antiallergic, antioxidant, antimicrobial and *in vitro* anti-HIV-1 activities [5-14]. 1-*H*-Benzimidazole rings, which exhibit remarkable basic characteristics due to their nitrogen content, comprise the active substances for several drugs. A number of biological activities have been attributed to these compounds [15]. There are many benzimidazole drugs that have clinical importance in market, like anthelmintic, antihypertensive, anticancer drugs etc [16]. The drugs which are in market as vasodilators having angiotensin-II receptor antagonist activity and having benzimidazole with vasodilatory and antihypertensive

activities are Candesartan, Telmisartan and Valsartan.Literature review has reported the vasorelaxant effect of six 5-substitutedbenzo[d]imidazolemoiety [5]. So in an effort to identify novel small moleculesderivatives, we also have tried to design and synthesizea series of benzo[d]imidazole derivatives based on the structure of Pimobendan [17], a dihydropyridazinone-benzo[d]imidazole derivative by using non-classical bioisosteric electron-withdrawing substituent (nitro groups). Pimobendan acts as a calcium sensitizer, as well as a partial inhibitor of PDE-3. It is effective in both acute and chronic heart failure and it also causes peripheral vasodilation. Pimobendan is a positive inotrope. It sensitizes and increases the binding efficiency of cardiac myofibril to the calcium ions that are already present without increasing the consumption of oxygen and energy. Pimobendan also causes peripheral vasodilation by inhibiting the function of phosphodiesterase III. This results in decreased pressure, translating into smaller cardiac preload and afterload (decreases the failing heart's workload). Twentytest compounds weredesigned based on molecular docking studies, synthesized and evaluated for the *ex-vivo*vasorelaxant activity in rat aorta rings pre-contracted with phenylephrine (PhE) [18].

MATERIALS AND METHODS

Molecular Docking Simulations [19]

The 2Dstructure of the compounds were built and then converted in to the 3D with the help of VLifeMDS 3.5 software [18]. The 3D structures were then energetically minimized up to the rms gradient of 0.01using Merck Molecular Force Field (MMFF). Conformers of all the synthesized ligand selected and number of seeds used for searching the conformational space was set as 5. All conformers were then energetically minimized up to the rms gradient of 0.01 and then saved in separate folder. The bovine rhodopsin crystal structure (PDB Code: 1L9H) was obtained from the protein data bank [20].The crude PDB structure of receptor was then refined by completing the incomplete residues. The side chain hydrogen was added to the crystal structure and the ir positions were optimized up to the rms gradient 1 then saved as mol file. Docking was done by GRIP batch docking method. All the conformers were virtually docked at the define d cavity of the receptor. The parameter fixed for docking simulation was like, number of placements: 30,rotationangle: 30º, exhaustive method, scoring function: dockscore. In the result of docking, we have listed only best conformer and its dock score for each ligand in Table 1.The ligandformingmoststabledrug-receptorcomplexistheonewhichishavingminimum dock score. After docking simulation, the best docked conformer of each ligand and receptor were merged and their complexes were then energetically optimized by defining the radius of 10Å measured from the docked ligand. Stepwise energy optimization was done by first hydrogen, second side chains and finally the backbone of receptor [21]. The optimized complexes were then checked for various interaction of ligand with receptor like hydrogen bonding, hydrophobic bonding and van der Waal's interaction.

Table 1: Docking scores of the synthesized compounds

Compound code	Docking score
BDZ 1	-28.633060
BDZ 2	-19.357370
BDZ 3	-14.392149
BDZ 4	-29.399411
BDZ 5	-30.199752
BDZ 6	-36.162086
BDZ 7	-26.043721
BDZ 8	-23.119818
BDZ 9	-23.720553
BDZ 10	-28.779203
BDZ 11	-30.849361
BDZ 12	-30.148657
BDZ 13	-31.777840
BDZ 14	-31.837782
BDZ 15	-20.755062
BDZ 16	-28.225423
BDZ 17	-23.229933
BDZ 18	-27.181840
BDZ 19	-29.697274
BDZ 20	-30.514899

Compound BDZ6 was found highest negative dock score. It means that it forms most stable drug-receptor complex amongst all the compounds. It was found to form hydrophobic bonding, some of the residues involved in this type of

interaction are Lys67, Arg69, Thr70, Arg147, Ala246, Glu247, Val250, Ser334, Val345 and Pro347 (Fig.1). But it was not showing hydrogen bonding with the receptor. It was exhibited large number of van der Waal's bonding with wide range of residues. Some of the residues involved in this type of interaction are Lys66, Arg69, Glu247, Val345 and Pro347 etc. (Fig. 2).

Fig.1: Hydrophobic interaction (light blue coloured dotted line) of BDZ6 with receptor

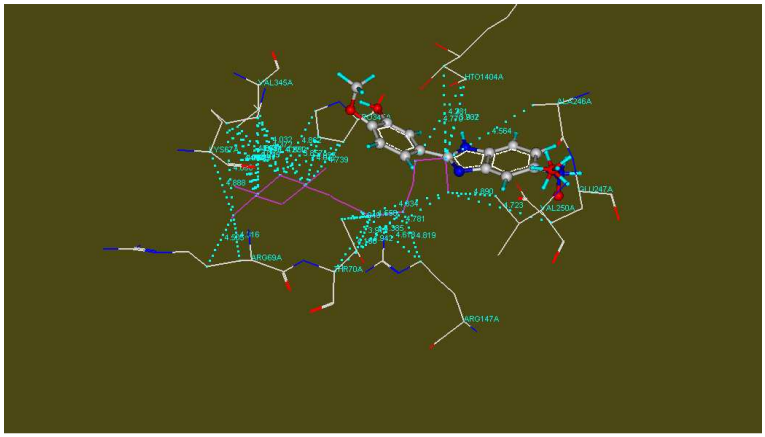
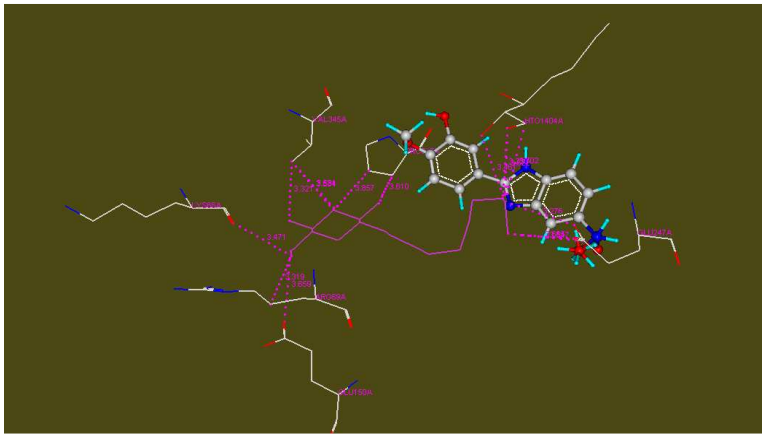


Fig.2: Vander Wall's interaction(pink colour dotted line) of BDZ6 with receptor



Synthesis

Reagents

All the chemicals and solvents used were of AR-grade and LR-grade and obtained from Sigma-Aldrich, Sisco Research Laboratories, Merck,Qualingens, Hi-media, Nice chemicals, Lobachemie, Spectrochem and were used without further purification.

Equipment

Melting points were measured on anelectro thermal melting point apparatus (Shital scientific industries, Mangalore, India). Infrared (IR) spectra were recorded as KBr pellets with an FTIR-8310 spectrophotometer (Shimadzu, Japan). Mass spectra were recorded on a GCMS (QP 5050A, Shimadzu Corporation, Japan). Absorption maxima were taken on a UV-Visible spectrophotometer-1650 (Shimadzu, Japan).

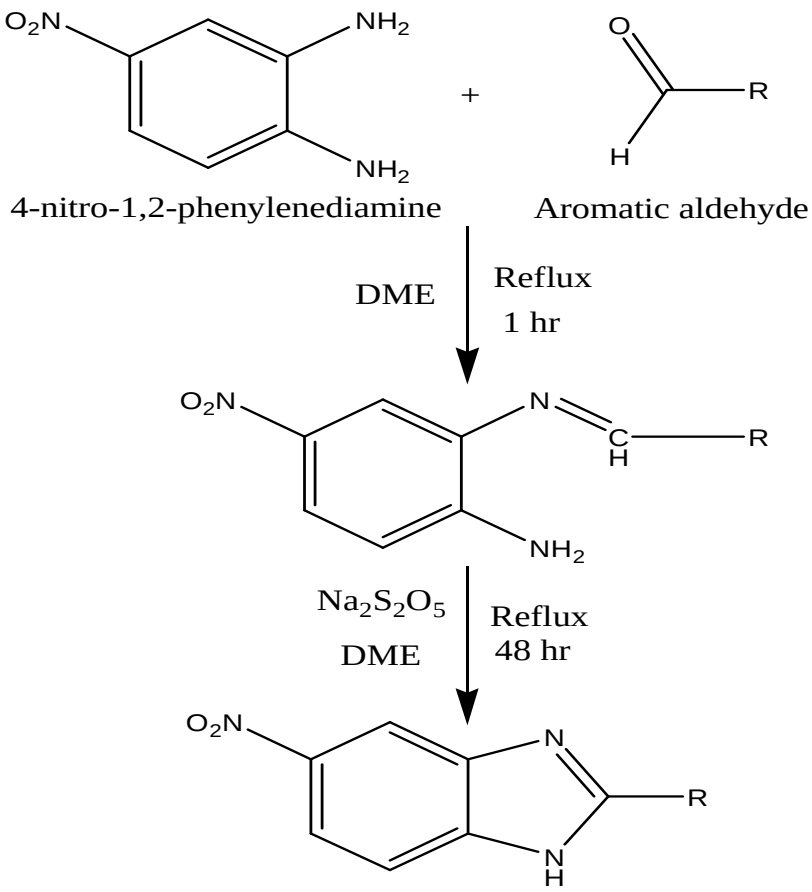
TLC analysis

Thin-layer chromatography (TLC) was performed on pre-coated aluminium plates (silica gel 60 F254, Merck). Plates were visualized by UV light and iodine vapor.

Synthesis of 5-Nitro-Benzoimidazole derivatives [17]

0.004 moles of 4-nitro-1, 2-phenylenediamine in appropriate amount of solvent dimethoxyethanewas mixed with 1.01 equivalent of aromatic aldehyde. It was kept for stirring at 0°C in ice bath for 2 hours. Then it was refluxed for 1 hour to get Schiff base as an intermediate.Then this Schiff base was cyclized by the addition of further dimethoxyethane and 1.01 equivalent of sodium metabisulphite which is an oxidant and stirred under reflux for 48 hours. Completion of the reaction was monitored by TLC in chloroform: methanol 9:1 solvent system.After completion of reaction, the reaction mixture was poured on to ice cold water. The precipitate was collected by filtration, washed with water, dried and recrystallized with methanol.In cases where compounds did not precipitate, the mixture was extracted with ethyl acetate. The organic layer was removed under vacuum using rotary evaporator. Purification was done by column chromatography using chloroform and methanol or petroleum ether and ethyl acetate.Fig.1 Yields and physical characteristics are listed in Table 1.

Scheme for the synthesis of 5-Nitro-Benzoimidazole derivatives



2-(3-Chloro-phenyl)-5-nitro-1*H*-benzoimidazole (BDZ1)IR: (KBr) 3304 cm^{-1} (NH), 2922 cm^{-1} (CH), 1626 cm^{-1} (C=N), 1446 cm^{-1} (C-N), 1573 cm^{-1} (N-O). GC-MS: 273$[M]^+$

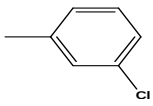
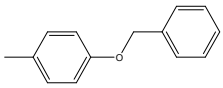
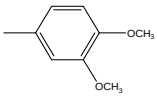
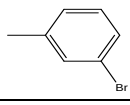
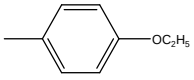
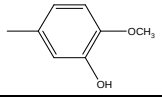
2-(4-Benzyloxy-phenyl)-5-nitro-1*H*-benzoimidazole (BDZ2) IR: (KBr) 3321 cm^{-1} (NH), 2922 cm^{-1} (CH), 1614 cm^{-1} (C=N), 1446 cm^{-1} (C-N), 1491 cm^{-1} (N-O).^{1}H NMR (DMSO): δ 5.2 (s, 2H, -CH_2), δ 7.22 (d, *J* = 8 Hz, 2H, Ar-H), δ 7.48-7.32 (m, 5H, Ar-H), δ 7.71 (d, *J* = 8 Hz, 1H, Ar-H), δ 8.15-8.08 (m, 3H, Ar-H), δ 8.41 (s, 1H, N-H) ppm.

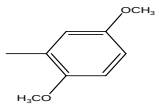
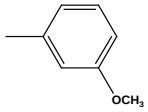
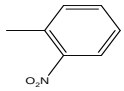
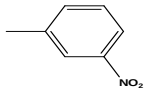
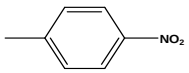
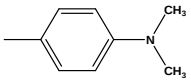
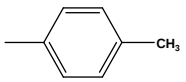
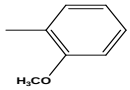
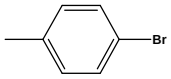
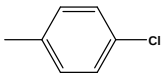
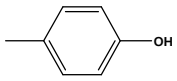
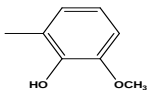
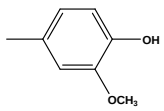
2-(3, 4-Dimet- hoxy-phenyl)-5-nitro-1*H*-benzoimidazole (BDZ3) IR: (KBr) 3319 cm^{-1} (NH), 3105 cm^{-1} (CH), 1599 cm^{-1} (C=N), 1438 cm^{-1} (C-N), 1500 cm^{-1} (N-O). ^{1}H NMR: (DMSO) δ 3.86 (d, *J* = 16 Hz, 6H, 3'& 4' OCH_3), δ 7.16 (d, J = 8 Hz, 1H, Ar-H), δ 7.79-7.71 (m, 3H, Ar-H), δ 8.12-8.09 (dd, J = 8 Hz, 4 Hz, 1H), δ 8.42 (s, 1H, N-H) ppm. GC-MS: 299$[M]^+$

2-(3-Bromo-phenyl)-5-nitro-1*H*-benzoimidazole (BDZ4) IR: (KBr) 3300 cm^{-1} (NH), 2920 cm^{-1} (CH), 1627 cm^{-1} (C=N), 1442 cm^{-1} (C-N), 1566 cm^{-1} (N-O). GC-MS: 317$[M]^+$

2-(4-Ethoxy- phenyl)-5-nitro-1*H*-benzoimidazole (BDZ5) IR: (KBr) 3410 cm^{-1} (NH), 2974 cm^{-1}(CH), 1606 cm^{-1} (C=N), 1438 cm^{-1} (C-N), 1519 cm^{-1} (N-O). GC-MS: 283[M] $^{+}$
2-Methoxy5-(5-nitro-1*H*-benzoimidazole-2-yl)-phenol (BDZ6) IR: (KBr) 3327 cm^{-1} (NH), 2912 cm^{-1} (CH), 1620 cm^{-1} (C=N), 1444 cm^{-1} (C-N), 1502 cm^{-1} (N-O).
2-(2, 5-Dimeth- oxy-phenyl)-5-nitro-1*H*-benzoimidazole (BDZ7) IR: (KBr) 3390 cm^{-1} (NH), 2947 cm^{-1} (CH), 1591 cm^{-1} (C=N), 1487 cm^{-1} (C-N), 1531 cm^{-1} (N-O). GC-MS: 299[M] $^{+}$
2-(3-Methoxy- phenyl)-5-nitro-1*H*-benzoimidazole (BDZ8) IR: (KBr) 3429 cm^{-1} (NH), 2939 cm^{-1} (CH), 1585 cm^{-1} (C=N), 1483 cm^{-1} (C-N), 1517 cm^{-1} (N-O).
5-Nitro-2-(2-nitro-phenyl)-1*H*-benzoimidazole (BDZ9) IR: (KBr) 3365 cm^{-1} (NH), 2913 cm^{-1} (CH), 1620 cm^{-1} (C=N), 1481 cm^{-1} (C-N), 1510 cm^{-1} (N-O).
5-Nitro-2-(3-nitro-phenyl)-1*H*-benzoimidazole (BDZ10) IR: (KBr) 3360 cm^{-1} (NH), 3099 cm^{-1} (CH), 1624 cm^{-1} (C=N), 1473 cm^{-1} (C-N), 1521 cm^{-1} (N-O). GC-MS: 284[M] $^{+}$
5-Nitro-2-(4-nitro-phenyl)-1*H*-benzoimidazole (BDZ11) IR: (KBr) 3381 cm^{-1} (NH), 3110 cm^{-1} (CH), 1614 cm^{-1} (C=N), 1483 cm^{-1} (C-N), 1517 cm^{-1} (N-O).
Dimethyl-[4-(5-nitro-1*H*-benzoimidazole-2-yl)-phenyl]-amine (BDZ12) IR: (KBr) 3423 cm^{-1} (NH), 3095 cm^{-1} (CH), 1608 cm^{-1} (C=N), 1438 cm^{-1} (C-N), 1504 cm^{-1} (N-O).
5-Nitro-2-p-tolyl-1*H*-benzoimidazole (BDZ13) IR: (KBr) 3323 cm^{-1} (NH), 3061 cm^{-1} (CH), 1627 cm^{-1} (C=N), 1456 cm^{-1} (C-N), 1506 cm^{-1} (N-O).
2-(2-Methoxy-phenyl)-5-nitro-1*H*-benzoimidazole (BDZ14) IR: (KBr) 3377 cm^{-1} (NH), 3099 cm^{-1} (CH), 1595 cm^{-1} (C=N), 1471 cm^{-1} (C-N), 1517 cm^{-1} (N-O).
2-(4-Bromo-phenyl)-5-nitro-1*H*-benzoimidazole (BDZ15) IR: (KBr) 3290 cm^{-1} (NH), 3101 cm^{-1} (CH), 1631 cm^{-1} (C=N), 1473 cm^{-1} (C-N), 1506 cm^{-1} (N-O).
2-(4-Fluoro-phenyl)-5-nitro-1*H*-benzoimidazole (BDZ16) IR: (KBr) 3371 cm^{-1} (NH), 3116 cm^{-1} (CH), 1639 cm^{-1} (C=N), 1446 cm^{-1} (C-N), 1500 cm^{-1} (N-O).
2-(4-Chloro-phenyl)-5-nitro-1*H*-benzoimidazole (BDZ17) IR: (KBr) 3435 cm^{-1} (NH), 3101 cm^{-1} (CH), 1635 cm^{-1} (C=N), 1483 cm^{-1} (C-N), 1557 cm^{-1} (N-O).
4-(5-Nitro-1*H*-benzoimidazole-2-yl)-phenol (BDZ18) IR: (KBr) 3288 cm^{-1} (NH), 3111 cm^{-1} (CH), 1604 cm^{-1} (C=N), 1446 cm^{-1} (C-N), 1502 cm^{-1} (N-O).
2-Methoxy-6-(5-nitro-1*H*-benzoimidazole-2-yl)-phenol (BDZ19) IR: (KBr) 3361 cm^{-1} (NH), 3082 cm^{-1} (CH), 1622 cm^{-1} (C=N), 1477 cm^{-1} (C-N), 1521 cm^{-1} (N-O).
2-Methoxy-4-(5-nitro-1*H*-benzoimidazole-2-yl)-phenol (BDZ20) IR: (KBr) 3342 cm^{-1} (NH), 3105 cm^{-1} (CH), 1597 cm^{-1} (C=N), 1444 cm^{-1} (C-N), 1504 cm^{-1} (N-O).

Table 2:Physical data of the synthesized 5-Nitro-Benzoimidazole derivatives

Compound	R	M.P °C	%Yield	Rf*
BDZ1		110-115	66	0.51
BDZ2		185-190	86	0.48
BDZ3		168-171	75	0.40
BDZ4		180-184	86	0.52
BDZ5		260-265	79	0.57
BDZ6		235-238	53	0.41

BDZ7		205-208	80	0.72
BDZ8		105-110	76	0.54
BDZ9		190-193	90	0.73
BDZ10		265-271	81	0.46
BDZ11		225-230	91	0.77
BDZ12		195-199	79	0.48
BDZ13		198-202	87	0.51
BDZ14		170-173	82	0.65
BDZ15		250-254	76	0.54
BDZ16		220-225	77	0.45
BDZ17		270-276	54	0.57
BDZ18		316-320	75	0.43
BDZ19		302-305	70	0.55
BDZ20		295-298	87	0.46

**Chloroform: Methanol (9:1)*

Biological Activity

Vasorelaxant activity [18]

Male Wistar weighing 250–350g each were anesthetized with Ketamine rats hydrochloride (100 mg/kg, ip). The descending thoracic region aorta was dissected free, and the surrounding connective tissue and fat were removed under a microscope while the blood vessel (aorta) was bathed in Kreb's solution. The aorta was then cut into 2.5-mm rings, and these rings were mounted in a 10ml organ bath containing Kreb's solution maintained at 37°C that was continuously gassed with 95% O_2 and 5% CO_2 with a resting tension of 2g. Isometric contractions and relaxations were recorded using force displacement transducer (model MLT050/A, AD Instruments). The transducer

signals were displayed and stored on a computer for further analysis using AD Instruments Power Lab software. The rings were equilibrated at 2g resting tension for 120 min, during which time the bathing solution was changed every 15 min. Only one concentration–response curve was elicited by the contractile agonist (phenylephrine, PhE: 300 nM) for each ring in all the experiments.

In denuded aortic rings, the endothelium was intentionally removed by inserting a 25-gauge needle tip into the lumen of ring and gently rolling the ring for a few seconds. The absence of the endothelium was confirmed by a less than 10% relaxation upon acetylcholine challenge. After pre-contraction with PhE, the test samples (compounds 1–20) were added to the bath in a volume of 0.1 ml; then cumulative concentration-response curves were obtained for each ring (0.3–100 µM). In order to avoid fatigue of the arterial preparation, a 60 min recovery period was allowed between the curves. The relaxant effect was determined by comparing contraction before and after application of the test compounds. All the concentration of samples was prepared in DMSO. This solvent was determined to have no effect on PhE-induced contractions at 1% of concentration.

The concentration-response curves (CRC) were plotted and data adjusted by the nonlinear curve fit method using commercially available software (Prism version 4.0; Graph Pad Software, San Diego, CA, USA). EC_{50} was calculated from each concentration-contraction (or relaxation) curve by a curve-fitting equation: E=MAp/ (Ap+Kp) where E is response, M is maximal contraction (or relaxation), A is concentration, K is EC_{50} concentration, and p is the slope. From this equation, the mean EC_{50} value ± SEM were calculated in each group (n=3). Maximal relaxations are expressed as E_{max}. Animal ethical clearance was taken from Institutional Animal ethics Committee (IAEC) KMC Manipal, Manipal Universty, Manipal for this *ex-vivo* study.

The contraction of the aorta was induced by submaximal concentration of PhE (300nM) in the aortic rings with or without endothelium. Cumulative doses of BDZ1 to BDZ20 (0.3–100 µM) were added directly to the organ bath and the effect of BDZ1 to BDZ20 were recorded on precontracted aorta in Table 3.

Table 3: *Ex-vivo* relaxant effect of synthesized 5-Nitro-Benzoimidazole derivatives

Compound	Ex-vivo vasorelaxant effect			
	With endothelium (+E)		Without endothelium(-E)	
	EC50 (µM)	Emax (%)	EC50 (µM)	Emax (%)
BDZ 1	225.67 ± 10.25	80.17 ± 9.53	456.57 ± 34.63	65.03 ± 9.75
BDZ 2	217.59 ± 14.96	84.28 ± 8.34	478.58 ± 41.71	69.56 ± 12.55
BDZ 3	22.48 ± 5.80	97.24 ± 1.58	53.10 ± 6.14	83.51 ± 3.13
BDZ 4	298.41 ± 11.03	79.41 ± 6.35	544.85 ± 49.59	60.54 ± 11.28
BDZ 5	310.28 ± 9.22	75.28 ± 7.53	512.52 ± 67.32	52.52 ± 9.85
BDZ 6	25.99 ± 4.37	98.77 ± 4.39	57.69 ± 8.48	80.76 ± 5.23
BDZ 7	257.60 ± 13.31	82.05 ± 9.17	396.19 ± 71.65	67.93 ± 10.19
BDZ 8	277.74 ± 10.29	83.22 ± 7.32	408.63 ± 44.02	61.08 ± 12.50
BDZ 9	305.48 ± 14.57	76.1 ± 8.65	470.46 ± 51.08	55.32 ± 10.97
BDZ 10	281.09 ± 12.66	80.44 ± 6.33	429.50 ± 36.35	59.44 ± 7.03
BDZ 11	297.25 ± 13.90	78.56 ± 7.18	451.36 ± 37.23	61.16 ± 8.62
BDZ 12	23.92 ± 3.73	96.58 ± 2.27	51.99 ± 7.37	87.17 ± 11.81
BDZ 13	354.88 ± 15.21	68.23 ± 5.91	543.60 ± 66.56	50.46 ± 15.28
BDZ 14	301.23 ± 14.94	71.08 ± 8.93	501.07 ± 61.79	55.91 ± 12.20
BDZ 15	276.67 ± 11.80	76.60 ± 9.28	416.19 ± 40.10	68.22 ± 10.71
BDZ 16	299.91 ± 16.59	78.42 ± 8.26	471.39 ± 46.37	61.19 ± 11.66
BDZ 17	264.36 ± 12.46	84.37 ± 7.07	455.53 ± 38.48	65.58 ± 8.58
BDZ 18	29.48 ± 6.31	100.41 ± 6.55	61.40 ± 11.32	82.32 ± 4.04
BDZ 19	220.33 ± 10.13	90.15 ± 9.83	394.96 ± 31.05	73.71 ± 9.13
BDZ 20	21.08 ± 5.22	93.94 ± 1.97	49.92 ± 12.65	85.49 ± 7.12
Acetylcholine	0.69 ± 0.97	104.51 ± 8.10	Not Active	Not Active
Sodiumnitroprusside	0.47 ± 1.24	101.47 ± 7.64	1.27 ± 0.84	99.83 ± 5.22

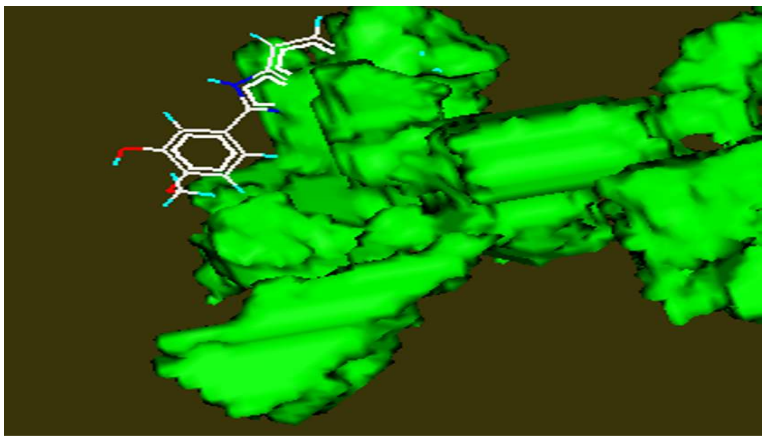
RESULTS AND DISCUSSION

Docking studies was carried out by taking bovine rhodopsin crystal structure (PDB Code: 1L9H)as a target for vasorelaxant activity. The compound BDZ6 was found to have highest negative dock score (-36.16) Fig.3. It means that it can fit well in the receptor cavity forming energetically most stable drug receptor complex.In order to study the vasorelaxant activity of compounds, rat thoracic aorta rings with and without endothelium (+E and -E,

respectively) pre-contracted with phenylephrine (PhE, 300 nM) were employed. The effects of cumulative concentrations of the compounds were determined. Most of the synthesized benzimidazolederivatives did not show a significant vasorelaxant activity (EC_{50}>200 µM). Compounds BDZ3, BDZ6, BDZ12, BDZ18 and BDZ20 showed good vasorelaxant potency (EC_{50}<30 µM). However, these compounds exhibited vasodilatory effect in a concentration and partially endothelium-dependent manner. The EC_{50} values for the relaxant response of the active compounds were different in rings with a functional endothelium, and in those without endothelium. Compound BDZ20 was the most potent in reducing the PhE-induced contractile response, with EC_{50} of 21.08µM and efficacy of 93.94%.This compound was active, but was not as active as sodium nitroprusside.

The relaxation effect of benzo[d]imidazole derivatives in endothelium-intact aorta pre-contracted by PhE was notably stronger than that in endothelium-denuded aorta. The partial endothelium dependent relaxation showed by these compounds indicates that their vasorelaxant effect is through endothelium derived factors such as cyclooxygenase, endothelium-dependent hyperpolarization factor (EDHF) or nitric oxide synthase pathways, including PDE-3 inhibition. In order to establish a preliminary structure activity relationship, we analyzed the contribution of substituents on the phenyl ring.Halogen group at the C-2 and C-4 position (Cl / Br) were non-selective and exhibited less potent vasorelaxation. On the other hand, the presence of two or even three oxygenated radicals in the rest of compounds increased the relaxant effect. This suggests that the bioactivity of these compounds depends on the presence of nitro groups and several small oxygenated radicals attached at different positions of the phenyl ring. When the docking score and vasorelaxant activity results were compared, derivatives BDZ6, BDZ12, BDZ18 and BDZ 20 were found to be ideal compounds with good dock score as well as good vasorelaxant activity .

Fig.3: Docked position of BDZ6 with receptor in cavity form (green colour)



CONCLUSION

Docking studies of the synthesized compounds was done for all twenty derivatives.In docking study thirteencompounds BDZ1, BDZ4, BDZ5, BDZ6, BDZ10, BDZ11, BDZ12,BDZ13, BDZ14, BDZ16, BDZ18, BDZ19 and BDZ20 were found to have highest negative dock score ranging from -36.16 to -27.18. All the designed twentysubstituted 5-nitro benzimidazoles derivatives were synthesized and characterized by IR, 1H NMR and mass spectroscopy. All the compounds were screened for their *ex-vivo*vasorelaxant activity in rat aorta rings pre-contracted with phenylephrine. In *ex-vivo*vasorelaxant activity five compounds BDZ3, BDZ6, BDZ12, BDZ18 and BDZ20 showed good vasorelaxantpotency (EC_{50}<30 µM).The compounds possessing good dock score as well as good vasorelaxant activity can be explored further.

Acknowledgements
We are thankful to Head, Dept. of Pharmacology, MCOPS, Manipal for helping and providing materials to perform biological activity.We are thankful to NIPER, Chandigarh, for providing the NMR spectra for our compounds in time and DST and FIST facility in MCOPS for spectral details.

REFERENCES

[1] T.Ogihara,M. Matsuzaki, H.Matsuoka, K.Shimamoto, K.Shimada *et al*, *Hypertension Research*. **2005**, 28, 331–338.
[2] B.A.Staffileno, *Journal of Cardiovascular Nursing*.**2005**, 20, 354–364.
[3] K.T Weber,P. Anversa, , P.W Armstrong, C.G Brilla *et al*, *Journal ofthe AmericanCollege of Cardiology*. **1992**, 20, 3–16.
[4] A.Cogolludo, F.Perez-Vizcaino, J. Tamargo, C*urrent Opinion in Nephrology andHypertension*.**2005**, 14, 423–427.
[5] S.Estrada-Soto, R. Villalobos-Molina,F. Aguirre-Crespo, J. Vergara-Galicia, H.Moreno-Diaz, M. Torres-Piedra, G. Navarrete-Vázquez, *Life Sci*.**2006,** 79, 430.
[6] Y.F Li; G.F Wang; Y. Luo; W.G Huang; W. Tang; C.L Feng; L.P Shi; Y.D Ren; J.P Zuo; W. Lu.,*Eur. J. Med. Chem*.**2007**, 42, 1358-1364.
[7] E. Carlsson, P. Lindberg, S. Unge. *Chem. Br*. **2002**, 5, 42.
[8] M.M Ramla, M.A Omar, H. Tokuda, HI.El.Diwani. *Bioorg. Med. Chem.* **2007**, 15, 6489- 6496.
[9] H. Goker, G. Ayhan-Kilcigil, M Tuncbilek, C Kus, R Ertan, E Kendi, S Ozbey, M Fort, C Garcia, A.J Farre.,*Heterocycles*, **1999**, 51, 2561-2573.
[10] I .Kuchkguzel; G. Kuchkguzel; S. Rollas; M. Kiraz. *Bioorg. & Med. Chem. Lett*. **2001**, 11, 1703.
[11] H. Nakano; T. Inoue; N. Kawasaki; H. Miyataka; H. Matsumoto; T. Taguchi; N. Inagaki; H .Nagai; T. Satoh. *Chem. Pharm. Bull*. **1999**, 47, 1573-1578.
[12] G.Ayhan-Kilcigil.,C.Kus, T.Coban, B.Can-Eke, M. Iscan, *Journal of Enzyme Inhibition and Medicinal Chemistry*. **2004**, 19, 129-135.
[13] K Sztanke; K Pasternak; A Sidor-Wojtowicz; J Truchlinska; K Jozwiak. *Bioorg. Med*. **Chem**. **2006**, 14, 3635-3642.
[14] R Samia; A Soda; M El-Hesham; TY Fahmy. *Arch. Pharm. Res*. 2006, 29, 826-833.
[15] Jafar A.A et al. *Orbital***2009**; 1(4): 306-309.
[16] Ramanpreet Walia, Md. Hedaitullah, Syeda Farha Naaz, Khalid Iqbal and HS. Lamba ,*IJRPC***2011**, 1(3), 256-274.
[17] G.NVázquez, S.H Figueroa, M .T Piedra, J.V Galicia, *Bioorg. Med. Chem*.**2010**; 18: 3985–3991.
[18] A.OHernandez , P.Castillo Espana, I.León Rivera , M Ibarra Barajas . *Biochem. Pharmacol*.**2009**; 78: 54-61.
[19] VLife MDS 3.5 documentation tutorial: Molecular docking using MDS; 1-26.
[20] M.C.Sharma,D.V. Kohli, S.C.Chaturvedi, Smita Sharma. *Digest Journal of Nanomaterialsand Biostructures***2009**; 4: 843 – 856.
[21] VLife MDS 3.5 documentation tutorial: Protein Complex Optimization using MDS; 1-12.