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Synthesis, vasorelaxant activity and antihypertensive effect of benzo[*d*]imidazole derivatives

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ABSTRACT

A series of 1*H*-benzo[*d*]imidazole analogues of Pimobendan, substituted at position 5 with either $-CF_3$ or $-NO_2$, were synthesized using a short synthetic route. All the nitro derivatives were potent, and exhibited a concentration- and partial endothelium-dependent vasorelaxant effects, with EC_{50}s <5 μM. 2-Methoxy-4-[5-nitro-1*H*-benzo[*d*]imidazol-2-yl]phenol (compound **13**) was the most potent derivative of the series, showing an EC_{50} value of 1.81 μM and E_{max} of 91.7% for ex vivo relaxant response in intact aortic rings, resulting in a 2.5-fold higher activity compared to Pimobendan. The closely related 5-CF_3 analogue (compound **8**), was 19 times less potent than **13**. The antihypertensive activity of compound **13** was evaluated at doses of 25, 50 and 100 mg kg^{-1}, using spontaneously hypertensive rats (SHR), showing a statistically significant dose-dependent effect.

1. Introduction

Hypertension is the most common cardiovascular disease, represents the major risk factor for endothelial dysfunction, metabolic syndrome, renal dysfunction, congestive heart failure, coronary artery disease, and stroke.[1] Therapeutic strategies to combat the consequent damage to the vascular endothelium are generally aimed at modulating the molecular and biochemical mechanisms underlying this dysfunction.[2] One approach to treat the affected endothelium involves improving endothelium-dependent vasodilatation, which is mediated by augmenting the influence of endothelial protective factors (prostacyclin, nitric oxide, cGMP). Alternatively, treatment of the damaged endothelium can be attempted by inhibiting the synthesis/release of pathogenic factors such as angiotensin II and endothelin, as well as prothrombotic factors, among others.[3] Great efforts have been made to obtain novel antihypertensive agents acting on different targets to control blood pressure although undesirable side-effects are still encountered. Therefore, new antihypertensive therapy will need to control hypertension more effectively, with fewer side-effects and neutral impact on known cardiovascular risk factors.[4]

In an effort to identify novel compounds with vasodilatory and antihypertensive activities, our group initially focused on the modestly selective structure of Pimobendan (Fig. 1), a dihydropyridazinone-benzo[*d*]imidazole derivative that acts as a calcium sensitizer, as well as a partial inhibitor of PDE-3. It is effective in

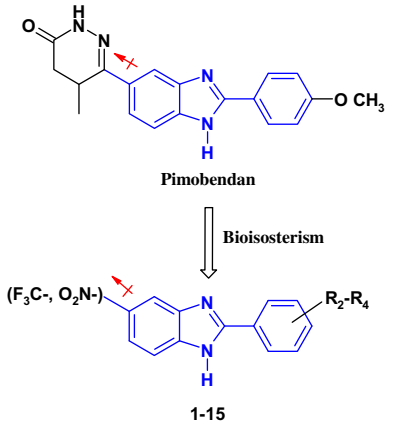

Figure 1. Drug design of compounds **1**–**15** through non-classical bioisosteric approach.

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both acute and chronic heart failure and it also causes peripheral vasodilation.[5]

We recently reported the vasorelaxant effect of six 5-substituted benzo[*d*]imidazole derivatives.[6] The present study extends the exploration of the vasorelaxant activity of 5-[nitro/(trifluoromethyl)]-2-(alkyloxyphenyl)-1*H*-benzo[*d*]imidazoles. As part of our search for basic information about the structural requirements for vasodilatory and antihypertensive action, we have designed and synthesized a series of benzo[*d*]imidazole derivatives reported in Table 1, based on the structure of Pimobendan, using non-classical bioisosteric electron-withdrawing substituents (pyridazinone /nitro/trifuoromethyl groups). The ex vivo vasorelaxant activity of these compounds in rat aorta rings pre-contracted with noradrenaline (NA) is also reported. A selected compound was investigated for its in vivo antihypertensive activity on spontaneously hypertensive rats (SHR) by tail cuff method.

2. Results and discussion

2.1. Chemistry

In this study, 15 benzo[*d*]imidazole derivatives have been designed, synthesized and tested as vasorelaxant agents in order to obtain potential antihypertensive compounds (Table 1). The reaction sequence shown in Scheme 1 was followed. Compounds **1**–**9** were prepared from 2-nitro-5-(trifluoromethyl) aniline (**16**), followed by catalytic nitroreduction to give **17,** and cyclo-condensation with the relevant aromatic aldehyde using conventional heating. Compounds **10**–**15** were prepared from 4-nitro-1,2-phenylenediamine (**18**). Reaction of **18** with the appropriately substituted benzaldehyde, sodium metabisulfite and dimethoxyethane (DME) was conducted at 70 °C, under microwave irradiation.

The reaction between 1,2-phenylenediamine **18** and the corresponding aldehyde was carried out in 35–90 s under microwave irradiation and afforded the corresponding products **10**–**15** in acceptable yields. When the microwave irradiation time was extended, it was possible to observe a decrease in the yield due to the formation of several byproducts. Solid compounds were purified by recrystallization or by column chromatography, and were all isolated in satisfactory yields. The chemical structures of the synthesized compounds were confirmed on the basis of their spectral data (NMR and mass spectra) and the purity was ascertained by microanalysis.

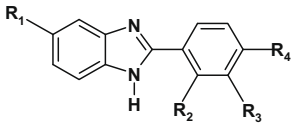
2.2. In silico PASS screening

We obtained predictive values of the biological activities of compounds (**1–15**) by comparing their structures with either the structures or substructures of more than 46,000 well-known biologically active drugs included in the database of the Prediction of Activity Spectra for Substances (PASS) software.[7] This software reports the predicted activity spectrum of a compound both as probable activity (*Pa*) and probable inactivity (*Pi*) with the accuracy of prediction reported to be as high as 85%.[8,9] The results presented in Table 2 describe two biological activities taken from PASS software: vasodilatory and phosphodiesterase inhibition effects. *Pa* values estimated for both effects ranged from 0.5 to 0.8. These results indicated that compounds exhibited chemical structures similar to known vasoactive drugs, and are likely to reveal this activity experimentally.

2.3. Vasorelaxant activity

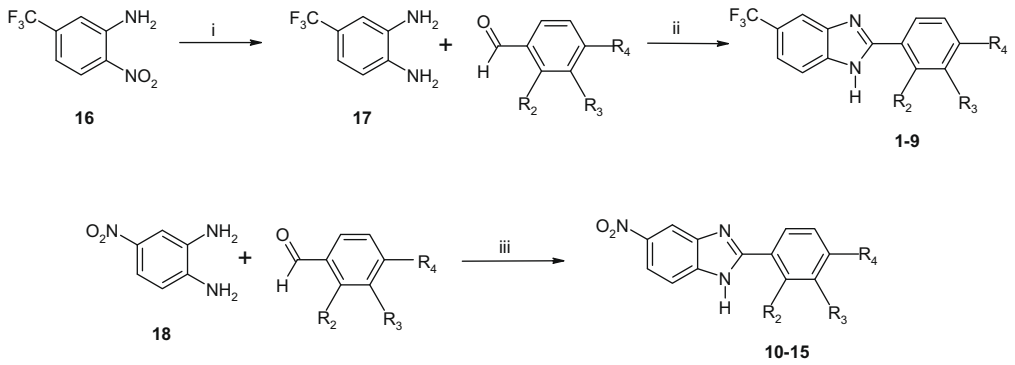
In order to study the vasorelaxant activity of compounds **1**–**15**, rat thoracic aorta rings with and without endothelium (+E and −E, respectively) pre-contracted with noradrenaline (NA, 0.1 μM) were employed. The effects of cumulative concentrations of the compounds were determined. Trifluoromethyl derivatives **1**–**3**, and **5**–**7,** did not show a significant vasorelaxant activity (EC_{50}s >200 μM). Compounds **8** and **9** showed low vasorelaxant potency (EC_{50}s <39 μM). However, compound **4** (with a nitro group attached at position 2 of the phenyl ring) showed the best activity

Table 1
EC_{50} and E_{max} values for the ex vivo vasorelaxant activity induced by derivatives **1**–**15**, in rat aorta rings pre-contracted with noradrenaline



Compound	R_1	R_2	R_3	R_4	Mp (°C)	Ex vivo vasorelaxant effect			
						With endothelium (+E)		Without endothelium (−E)	
						EC_{50} (μM)	E_{max} (%)	EC_{50} (μM)	E_{max} (%)
1	$-CF_3$	–H	–H	–H	56.0–58.0	369.37 ± 10.2	91.2 ± 1.18	467.75 ± 73.6	75.6 ± 6.31
2	$-CF_3$	–OMe	–H	–H	204.3–205.5	210.33 ± 11.3	75.14 ± 33.5	574.85 ± 30.3	45.7 ± 15.4
3	$-CF_3$	–OEt	–H	–H	121.5–122.9	548.5 ± 27.8	90.97 ± 2.30	548.51 ± 77.1	19.8 ± 8.13
4	$-CF_3$	$-NO_2$	–H	–H	155.8–157.1	3.18 ± 0.30	93.16 ± 3.52	15.03 ± 7.59	85.31 ± 2.63
5	$-CF_3$	–H	–H	–OH	263.6–265.4	219.20 ± 14.1	51.15 ± 20.6	219.20 ± 71.6	37.04 ± 10.6
6	$-CF_3$	–H	–H	–OPr	225.3–226.8	524.49 ± 25.4	51.0 ± 7.33	524.49 ± 19.3	19.0 ± 6.01
7	$-CF_3$	–H	–H	$-N(Me)_2$	222.1–224.4	550.27 ± 30.1	63.2 ± 4.81	550.27 ± 84.5	30.9 ± 7.53
8	$-CF_3$	–H	–OMe	–OH	214.4–216.6	34.84 ± 5.43	99.55 ± 1.23	140.14 ± 63.2	97.67 ± 3.26
9	$-CF_3$	–H	$-O-CH_2-O-$		99.5–102.2	38.53 ± 2.35	101.17 ± 5.83	77.42 ± 9.41	99.6 ± 13.5
10	$-NO_2$	–H	–H	–H	147.1–149.0	4.93 ± 0.30	73.82 ± 5.37	35.1 ± 5.21	60.53 ± 5.58
11	$-NO_2$	–OEt	–H	–H	128.3–131.2	3.71 ± 0.10	84.82 ± 3.73	15.0 ± 1.12	46.35 ± 7.85
12	$-NO_2$	–OiPr	–H	–H	158.9–163.2	4.89 ± 0.29	80.71 ± 9.41	14.12 ± 1.05	31.69 ± 1.32
13	$-NO_2$	–H	–OMe	–OH	303.4–306.3	1.81 ± 0.08	91.74 ± 2.35	19.49 ± 1.79	55.22 ± 8.85
14	$-NO_2$	–H	–OMe	–OMe	169.6–174.1	2.5 ± 0.10	75.0 ± 9.35	301.9 ± 10.2	36.33 ± 6.20
15	$-NO_2$	–OMe	–OMe	–OMe	153.2–155.3	3.23 ± 0.20	90.0 ± 4.56	43.65 ± 2.37	58.91 ± 7.81
Pimobendan						4.67 ± 0.83	93.22 ± 5.23	N.T.	N.T.
Carbachol						0.51 ± 1.9	106.3 ± 9.71	N.A.	N.A.
Nitrendipine						N.T.	N.T.	0.03 ± 0.003	98.90 ± 5.0

N.T.: Not tested; N.A.: Not active.



Scheme 1. Reagents and conditions: (i) H_2, Ni-Raney, EtOH; (ii) $Na_2S_2O_5$, DMF, reflux; (iii) $Na_2S_2O_5$, DME, μw, 70 °C.

of this series, with an EC_{50} = 3.18 μM. All the synthesized nitro-compounds (**10**–**15**) showed fully relaxant effects with EC_{50}s <5 μM. Compounds **10** and **12** were as potent as Pimobendan (EC_{50} = 4.67 ± 0.83 μM). However, compounds **13**–**15** exhibited an important vasodilatory effect in a concentration- and partially endothelium-dependent manner. These compounds were more potent than Pimobendan. 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 (Table 1). Compound **13** was the most potent in reducing the NA-induced contractile response, with EC_{50} of 1.81 μM and efficacy of 91.74%. This compound was 2.5 times more active than Pimobendan, but was not as active as carbachol. However, the E_{max} values of these compounds were slighter than the positive control. All the compounds were less potent than nitrendipine in aorta rings −E.

The relaxation effect of benzo[*d*]imidazole derivatives **10**–**15** in endothelium-intact aorta pre-contracted by NA was notably stronger than that in endothelium-denuded aorta (Table 1). In endothelium-denuded (−E) rings, the compounds produced a partial relaxation with a maximal effect of 60.53 ± 5.58% (vs endothelium-intact group with 91.74 ± 2.35%). 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,[10] such as Pimobendan. Further experiments are in progress in order to determine the possible mode of action of the most active compound. In order to establish a preliminary structure–activity relationship, we analyzed the contribution of substituents of the phenyl ring: bulky oxygenated radical at the C-2 position (compounds **11**: R = –OEt and **12:** R = –O*i*-Pr) 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. Interestingly, compound **8** substituted at position 5 with a CF_3, and with a pattern of substitution 3-MeO, 4-OH in the phenyl ring, showed less potent relaxant effect than compound **13,** which bears a nitro group in the same position of the benzo[*d*]imidazole core. This small change showed a 19-fold improvement in activity. The same response was observed with compounds **3** and **11** (–CF_3 and –NO_2 substituted, respectively), where **11** was 148 times more active than **3** (Fig. 2). From these results, we can observe that nitro group is essential for the potent vasodilatory action, suggesting a direct effect in the mechanism of vasorelaxation.

2.4. Antihypertensive assay

In order to evaluate the oral antihypertensive activity, we selected compound **13**, which showed the highest potency, as well as good E_{max} in the rat aortic contraction assay with +E (EC_{50} = 1.81 μM) and −E (EC_{50} = 19.49 μM). The in vivo antihypertensive activity of compound **13** was evaluated in spontaneously hypertensive rats (SHR), by the tail cuff method. Oral dosing (25, 50, and 100 mg kg^{-1}) of **13,** led to a robust, sustained and dose-dependent decrease in systolic blood pressure as shown in Figure 3B. Thus, 50 mg kg^{-1} of compound **13** caused a moderate descent in systolic blood pressure, while 100 mg kg^{-1} provoked a marked and sustained decrease in systolic blood pressure, which lasted for more than 6 h (Fig. 3). Interestingly, the antihypertensive effect of compound **13** (50 mg kg^{-1}) was similar to captopril (30 mg kg^{-1}), which is widely used in clinical settings for the treatment of hypertension and some types of congestive heart failure.

The results of the antihypertensive activity study indicate that compound **13** reduces blood pressure at 50 and 100 mg kg^{-1}; however, there was no significant effect on heart rate (Fig. 3A). The lack of activity for a low dose of compound **13** (25 mg kg^{-1}) may be due to low oral bioavailability, which could be the consequence of either extensive liver metabolism or limited gastrointestinal absorption. Furthermore, many other reasons could affect the in vivo activity of this compound.

The benzo[*d*]imidazoles prepared in this work are fully compatible with Lipinski's rule of five (Table 2),[11] which should allow for the development of additional vasorelaxant analogues. Their advantages include: (i) physical properties known to be compatible with desirable pharmacokinetic (low molecular weight, favorable Clog *P*, favorable hydrogen bond donating and accepting capabilities), (ii) potency and efficacy, with EC_{50} values at the low micromolar level, and (iii) simple synthetic access and thus low production costs.

Interestingly, a good correlation was found between the calculated log *P* and the topological polar surface area (TPSA) of the title compounds. Both parameters were compared in order to analyze the biological results obtained. The relatively higher hydrophobicity of the compounds (*mi* log *P* >4) and lower TPSA values might explain the low potency of the $-CF_3$ compounds **1**–**9** comparing with those shown by nitroderivatives **10**–**15** (Table 2). TPSA is a descriptor that shows good correlation with passive molecular transport through membranes, and so allows estimation of transport properties of drugs. These results are consistent with the activities predicted by PASS: the trifluoromethyl derivatives showed lower probabilities of biological effects than the nitro-analogues.

Table 2
Rule of five properties and predictive values of biological activities calculated with PASS for compounds **1–15**

Rule	MW	*mi* log *P*	No. of H bond acceptors	No. of H bond donors	TPSA (Å^2)	Volume (Å^3)	Violations	PASS probability			
								Vasodilatory effect		Phosphodiesterase inhibition	
Compd	<500	<5	<10	<5	<140		<2	*Pa*	*Pi*	*Pa*	*Pi*
1	262	4.41	2	1	28.68	211.57	0	0.648	0.005	0.623	0.007
2	292	4.42	3	1	37.91	237.11	0	0.570	0.005	0.725	0.036
3	306	4.80	3	1	37.91	253.91	0	0.793	0.003	0.793	0.003
4	307	4.32	5	1	74.50	234.90	0	0.634	0.004	0.582	0.009
5	278	3.93	3	2	48.91	219.58	0	0.663	0.010	0.568	0.010
6	270	5.35	3	1	37.91	270.72	1	0.758	0.004	0.758	0.004
7	305	4.51	3	1	31.92	257.47	0	0.659	0.019	0.514	0.015
8	308	3.75	4	2	58.14	245.13	0	0.698	0.007	0.521	0.014
9	306	4.30	4	1	47.15	235.50	0	0.651	0.005	0.536	0.013
10	239	3.48	5	1	74.50	203.60	0	0.763	0.003	0.763	0.004
11	283	3.86	6	1	83.74	245.95	0	0.846	0.003	0.846	0.003
12	297	4.28	6	1	83.74	264.54	0	0.781	0.004	0.759	0.004
13	285	2.81	7	2	103.96	237.17	0	0.788	0.059	0.631	0.006
14	299	3.12	7	1	92.97	254.69	0	0.792	0.003	0.683	0.005
15	329	3.30	8	1	102.20	280.24	0	0.837	0.003	0.742	0.004

Rule of five parameters were calculated on line at: http://www.molinspiration.com/cgi-bin/properties; PASS probability values were calculated on line at: http://195.178.207.233/PASS/.

3. Conclusion

Compounds **1–15** have been characterized as agents with clear relaxant effects on isolated rat aorta, although further studies are needed to determine the mechanisms underlying such activity. The potent vasorelaxant action of nitro-compounds did not persist on denuded aortic rings.

The potency of these compounds was dramatically improved by the addition of a bioisosteric nitro group instead of the trifluoromethyl cluster in the benzo[*d*]imidazole core, and several oxygenated substituents at the phenyl ring. In addition, compound **13** was orally active, showed an important decrease in blood pressure in a rat model of hypertension at doses of 50 and 100 mg kg^{-1}. In summary, we have demonstrated that the 5-nitrobenzo[*d*]imidazole structure is a bioisostere of Pimobendan, and it can be a useful template for the development of vasorelaxant compounds with better and higher potency. This core could be a promising lead for future optimizations as an antihypertensive agent. Further studies on the mode of action and pharmacology of these compounds and SAR around the core template will be reported in due course.

4. Experimental

4.1. Chemistry

Melting points were determined on an EZ-Melt MPA120 automated melting point apparatus from Stanford Research Systems and are uncorrected. Reactions were monitored by TLC on 0.2 mm precoated Silica Gel 60 F254 plates (E. Merck). Catalytic hydrogenation was carried out in a Parr shaker hydrogenation apparatus.^{1}H NMR spectra were measured with a Varian EM-390 (300 MHz) spectrometer. Chemical shifts are given in ppm relative to tetramethylsilane (TMS, $\delta = 0$) in DMSO-d_6; *J* values are given in Hz. The following abbreviations are used: s, singlet; d, doublet; q, quartet; dd, doublet of doublet; t, triplet; m, multiplet; br s, broad signal. Electron impact (EIMS) and FAB$^+$ mass spectra (*m*NBA matrix), were recorded on a JEOL JMS-SX102A spectrum. Starting material was commercially available (Aldrich). Carbamoylcholine HCl (carbachol) and noradrenaline HCl (NA) were purchased from Sigma–Aldrich Co. All other reagents were analytical grade from local sources. Reactions under microwave irradiation were performed in a CEM Discovery Microwave System apparatus (2450 MHz, 300 W). Predictive values of biological activities were also investigated using the chemistry software server PASS (http://195.178.207.233/PASS/).

4.1.1. General method of synthesis of 5-(trifluoromethyl)-1*H*-benzo[*d*]imidazoles 1–9

A mixture of 2-nitro-5-(trifluoromethyl)aniline (5 g, 0.0242 mol), EtOH (100 mL) and 10% Ni-Raney (500 mg) was hydrogenated at 25 °C until cessation of H_2 uptake. The catalyst was filtered off on a Whatman paper number 2, washed with EtOH, and the filtrate concentrated to provide a dark purple-colored liquid, which was used immediately in a subsequent step without purification. A mixture of the last reduction product [4-(trifluoromethyl)-1,2-phenylenediamine (0.0313 mol)], 1.01 equiv of appropriate aldehyde, and 1.01 equiv of sodium metabisulfite in 10 mL of DMF was heated under reflux for 3–4.5 h. After cooling, water (20 mL) was added and the mixture was extracted with EtOAc (3 × 15 mL). The organic layer was dried over magnesium sulfate and removed under vacuum. Purification was done by chromatography on silica gel eluting with chloroform and/or recrystallization from adequate solvent.

4.1.1.1. 2-Phenyl-5-(trifluoromethyl)-1*H*-benzo[*d*] imidazole (1). Recrystallized from methanol. Yield 0.62 g (67%) of an white solid. Mp 56.0–58.0 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ6.89–7.54 (m, 5H, H-2′, H-3′, H-4′, H-5′, H-6′), 7.70 (d, 1H, H-4, *J* = 2.0 Hz), 7.94 (dd, 1H, H-6, *J* = 8.4, *J* = 2.0 Hz), 8.41 (d, 1H, H-7, *J* = 8.4 Hz), ppm. ^{13}C NMR (50.28 MHz, DMSO-d_6): δ 113.9 (C-7), 114.1 (q, C-4, *J* = 6.8 Hz), 119.7 (q, C-6, *J* = 6.8 Hz), 123.8 (q, CF_3, *J* = 271.8 Hz), 126.3 (q, C-5, *J* = 32.2 Hz), 127.7 (C-3′, C-5′), 130.0 (C-2′, C-6′), 131.1 (C-4′), 132.6 (C-1′), 137.8 (C-3a), 143.0 (C-7a), 152.1 (C-2) ppm; EIMS: *m/z* 262 (M$^+$, 100), 243 (25); Anal. Calcd for $C_{14}H_9N_2F_3$: C, 64.12; H, 3.46; N, 10.68. Found: C, 64.55; H, 3.77; N, 10.01.

4.1.1.2. 2-(2-Methoxyphenyl)-5-(trifluoromethyl) -1*H*-benzo[*d*] imidazole (2). Recrystallized from ethanol. Yield 0.85 g (65.3%) of brown solid. Mp 204.3–205.5 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.1 (s, 1H, H–CH_3), 7.21–7.26 (m, 1H, H-6′), 7.73 (dd, 1H, H-3′, *J* = 8.2, *J* = 2.2 Hz), 7.54–7.56 (m, 2H, H-4′, H-5′), 7.97 (d, 1H, H-4, *J* = 2.2 Hz), 8.30 (d, 1H, H-7, *J* = 8.2), 8.62 (dd, 1H, H-6, *J* = 7.7, *J* = 1.9 Hz) ppm. ^{13}C NMR (50.28 MHz, DMSO-d_6): δ 55.6 (CH_3O), 113.7 (C-7), 114.2 (q, C-4, *J* = 6.7 Hz), 116.4 (C-3′), 119.5 (q, C-6, *J* = 6.8 Hz), 121.5 (C-1′), 122.7 (C-5′), 123.8 (q, CF_3, *J* = 271.6 Hz),

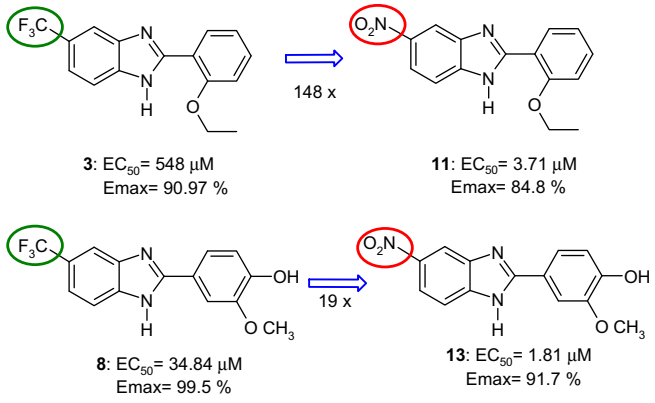


Figure 2. Comparison between potencies and chemical structures of selected analogues. The improvement of activity is represented under the arrow.

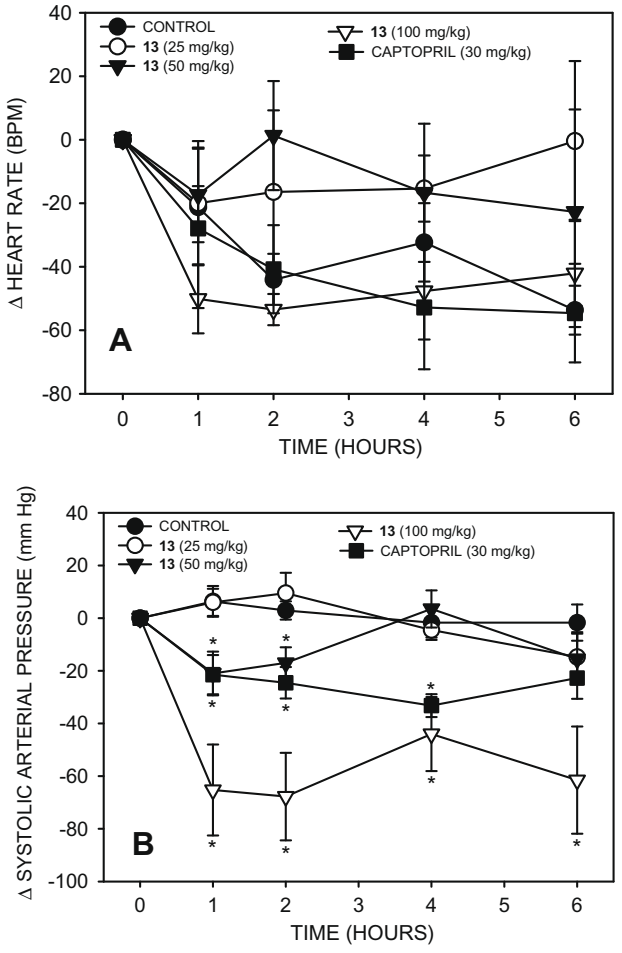


Figure 3. Effect of compound **13** on heart rate (A) and systolic arterial pressure (B) in conscious SHR at 25, 50, and 100 mg kg^{-1} po ($n = 5$). Compound was orally administrated at time zero. Each point represents mean ± SEM from five experiments. * p <0.05, as compared with the control.

126.0 (C-6′), 126.3 (q, C-5, J = 32.2 Hz), 132.5 (C-4′), 139.8 (C-3a), 145.1 (C-7a), 154.1 (C-2′), 157.2 (C-2) ppm; EIMS: *m/z* 292 (M^+, 100), 273 (33); Anal. Calcd for $C_{15}H_{11}F_3N_2O$: C, 61.64; H, 3.79; N, 9.59. Found: C, 60.93; H, 3.75; N, 9.45.

4.1.1.3. 2-(2-Ethoxyphenyl)-5-(trifluoromethyl)-1*H*-benzo[*d*] imidazole (3). Recrystallized from ethanol–water. Yield 0.73 g (61.4%) of yellow solid. Mp 121.5–122.9 °C. ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.56 (t, 3H, *CH*$_3$), 4.41 (q, 2H, *CH*$_2$), 7.15 (t, 1H, H-5′, J = 8.2, J = 1.1 Hz), 7.26 (d, 1H, H-3′, J = 8.2 Hz), 7.51–7.54 (m, 2H, H-6′, H-4′), 7.80 (d, 1H, H-7, J = 8.2 Hz), 7.98 (d, 1H, H-4, J = 1.1 Hz), 8.53 (dd, 1H, H-6, J = 1.6, J = 7.7 Hz) ppm. ^{13}C NMR (50.28 MHz, DMSO-d_6): δ 14.7 (CH_3), 62.6 (CH_2–O), 113.7 (q, C-4, J = 6.7 Hz),113.9 (C-7), 115.5 (C-3′), 119.7 (q, C-6, J = 6.8 Hz), 121.5 (C-1′), 122.7 (C-5′), 124.3 (q, CF_3, J = 271.8 Hz),125.7 (C-6′), 125.9 (q, C-5, J = 32.2 Hz), 132.1 (C-4′), 139.9 (C-3a), 145.0 (C-7a), 153.9 (C-2), 154.6 (C-2′) ppm; EIMS: *m/z* 306 (M^+, 50), 291 (100); Anal. Calcd for $C_{16}H_{13}F_3N_2O$: C, 62.74; H, 4.28; N, 9.15. Found: C, 62.93; H, 4.55; N, 9.48.

4.1.1.4. 2-(2-Nitrophenyl)-5-(trifluoromethyl)-1*H*-benzo[*d*]imidazole (4). Recrystallized from acetonitrile. Yield 0.59 g (61.1%) of yellow solid. Mp 155.8–157.1 °C. ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.15 (t, 1H, H-4′, J = 8.2, J = 7.7, J = 1.1 Hz), 7.26 (d, 1H, H-7, J = 8.2 Hz), 7.5 (d, 1H, H-5′, J = 8.2, J = 7.7,), 7.67 (d, 2H, H-6′, J = 8.2), 7.78 (d, 1H, H-4, J = 2.7 Hz), 7.79 (d, 2H, H-6, J = 2.7, J = 8.2 Hz), 8.0 (d, 1H, H-3′, J = 7.7 Hz), ppm. ^{13}C NMR (50.28 MHz, DMSO-d_6): δ 111.2 (C-7), 111.4 (q, C-4, J = 6.7 Hz), 119.5 (C-6), 119.7 (q, C-6, J = 6.7 Hz), 123.5 (C-1′), 123.2 (q, CF_3, J = 271.8 Hz), 125.3 (C-6′), 125.6 (q, C-5, J = 32.2 Hz), 126.6 (C-3′), 131.9 (C-4′), 132.4 (C-5′), 140.2 (C-3a), 145.3 (C-7a), 146.4 (C-2′), 151.2 (C-2) ppm; EIMS: *m/z* 307 (M^+, 90), 290 (100); Anal. Calcd for $C_{14}H_8F_3N_3O_2$: C, 54.73; H, 2.62; N, 13.68. Found: C, 55.03; H, 2.65; N, 13.95.

4.1.1.5. 4-[5-(Trifluoromethyl)-1*H*-benzo[*d*] imidazol-2-yl]phenol (5). Recrystallized from methanol–water. Yield 0.90 g (71%) of beige solid. Mp 300.2–303.4 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.66 (d, 1H, H-7, J = 8.2 Hz), 7.84 (dd, 1H, H-6, J = 8.2, J = 1.6 Hz), 7.85 (s, 1H, H-4, J = 1.6 Hz), 8.01 (dd, 2H, H-2′, H-6′, J = 8.7, J = 1.6 Hz), 8.31 (dd, 2H, H-3′, H-5′, J = 8.7, J = 1.6 Hz) ppm. ^{13}C NMR (50.28 MHz, DMSO-d_6): δ 114.1 (C-7), 113.6 (q, C-4, J = 6.1 Hz), 117.8 (C-3′,C-5′), 119.1 (q, C-6, J = 6.2 Hz), 123.5 (q, CF_3, J = 271.8 Hz), 126.3 (q, C-5, J = 32.0 Hz), 126.5 (C-1′), 127.9 (C-2′, C-6′), 136.9 (C-3a), 143.3 (C-7a), 152.2 (C-2), 161.8 (C-4′) ppm; EIMS: *m/z* 278 (M^+, 100), 249 (33); Anal. Calcd for $C_{14}H_9F_3N_2O$: C, 60.44; H, 3.26; N, 10.07. Found: C, 60.15; H, 3.57; N, 10.25.

4.1.1.6. 2-(4-Propoxyphenyl)-5-(trifluoromethyl)-1*H*-benzo[*d*] imidazole (6). Recrystallized from ethanol. Yield 0.60 g (37.1%) of yellow solid. Mp 225.3–226.8 °C. ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.04 (t, 3H, *C–CH*$_3$), 1.82 (sext, 2H, *C–CH*$_2$), 4.05 (t, 2H, *O–CH*$_2$), 7.11 (d, 2H, H-3′, H-5′, J = 8.7, J = 1.6 Hz), 7.48 (dd, 1H, H-6, J = 8.2, J = 1.6 Hz), 7.73 (s, 1H, H-4), 7.89 (s, 1H, H-7), 8.31 (d, 2H, H-2′, H-6′, J = 8.7, J = 1.6 Hz) ppm. ^{13}C NMR (50.28 MHz, DMSO-d_6): δ 10.4 (CH_3), 22.2 (CH_2), 69.1 (CH_2–O), 114.1 (C-7), 113.9 (q, C-4, J = 6.7 Hz), 116.8 (C-3′,C-5′), 119.5 (q, C-6, J = 6.7 Hz), 123.4 (q, CF_3, J = 271.8 Hz), 124.7 (C-2′, C-6′), 126.3 (q, C-5, J = 32.2 Hz), 126.4 (C-1′), 137.8 (C-3a), 143.1 (C-7a), 152.1 (C-2′), 162.1 (C-4′) ppm; EIMS: *m/z* 320 (M^+, 45), 278 (100); Anal. Calcd for $C_{17}H_{15}F_3N_2O$: C, 63.75; H, 4.72; N, 8.75. Found: C, 63.93; H, 4.75; N, 8.46.

4.1.1.7. *N,N*-Dimethyl-4-[5-(trifluoromethyl)-1*H*-benzo[*d*]imidazol-2-yl]aniline (7). Recrystallized from methanol. Yield 0.51 g (45.3%) of yellow solid. Mp 222.1–224.4 °C. ^{1}H NMR (200 MHz, $CDCl_3$): δ 3.04 (s, 6H, *N-(CH*$_3$*)*$_2$), 6.86 (d, 2H, H-3′, H-5′, J = 8.7 Hz), 7.45 (d, 1H, H-7, J = 8.2 Hz), 7.69 (dd, 1H, H-6, J = 2.2, J = 8.2 Hz), 7.85 (d, 1H, H-4, J = 2.2), 8.12 (d, 2H, H-2′, H-6′, J = 8.7) ppm. ^{13}C NMR (50.28 MHz, DMSO-d_6): δ 39.9 (N(CH_3)$_2$), 113.3 (C-3′,C-5′), 113.1 (C-7), 114.1 (q, C-4, J = 6.7 Hz), 119.9 (q, C-6, J = 6.7 Hz), 127.1 (q, C-5, J = 32.2 Hz), 121.5 (q, CF_3,

J = 271.8 Hz), 127.3 (C-2′, C-6′), 128.6 (C-1′), 137.8 (C-3a), 143.1 (C-7a), 151.5 (C-4′), 152.1 (C-2) ppm; EIMS: *m/z* 307 (M^+, 90), 290 (100); Anal. Calcd for $C_{16}H_{14}F_3N_3$: C, 62.95; H, 4.62; N, 13.76. Found: C, 62.93; H, 4.65; N, 13.85.

4.1.1.8. 2-Methoxy-4-[5-(trifluoromethyl)-1*H*-benzo[*d*]imidazol-2-yl]phenol (8). Recrystallized from ethanol. Yield 1.02 g (56.1%) of white solid. Mp 214.4–216.6 °C. ^{1}H NMR (200 MHz, $CDCl_3$): δ 3.91 (s, 3H, CH_3O), 6.99 (d, 1H, H-7, *J* = 8.2 Hz), 7.5 (dd, 1H, H-6, *J* = 8.2, *J* = 1.6 Hz), 7.73 (dd, 1H, H-6′, *J* = 8.3, *J* = 2.2 Hz), 7.81 (d, 1H, H-5′, *J* = 2.2 Hz), 7.87 (d, 1H, H-2′, *J* = 2.2 Hz), 7.9 (d, 1H, H-4, *J* = 1.6 Hz) ppm. ^{13}C NMR (50.28 MHz, DMSO-d_6): δ 55.67 (CH_3O), 110.24 (C-2′), 113.9 (C-7), 114.2 (q, C-4, *J* = 6.7 Hz), 117.3 (C-5′), 119.6 (q, C-6, *J* = 6.7 Hz), 122.3 (q, CF_3, *J* = 271.8 Hz), 124.7 (C-6′), 125.1 (C-1′), 125.6 (q, C-5, *J* = 32.2 Hz), 137.8 (C-3a), 142.9 (C-7a), 146.8 (C-4′), 150.2 (C-3′), 151.6 (C-2) ppm; EIMS: *m/z* 308 (M^+, 100), 279 (20); Anal. Calcd for $C_{15}H_{11}F_3N_2O_2$: C, 58.45; H, 3.60; N, 9.09. Found: C, 58.40; H, 3.75; N, 9.28.

4.1.1.9. 2-(Benzo[*d*][1,3]dioxol-5-yl)-5-(trifluoro methyl)-1*H*-benzo[*d*]imidazole (9). Recrystallized from methanol. Yield 0.47 g (55.8%) of white solid. Mp 99.5–102.2 °C. ^{1}H NMR (200 MHz, $CDCl_3$): δ 6.11 (s, 2H, *O–CH*$_2$*–O*), 7.0 (d, 1H, H-7, *J* = 8.2 Hz), 7.49 (dd, 1H, H-6, *J* = 8.2, *J* = 2.2 Hz), 7.74 (d, 1H, H-2′, *J* = 1.6 Hz), 7.79 (dd, 1H, H-5′, *J* = 8.8, *J* = 1.6 Hz), 7.80 (d, 1H, H-6′, *J* = 8.8 Hz), 7.89 (d, 1H, H-4, *J* = 2.2 Hz) ppm. ^{13}C NMR (50.28 MHz, DMSO-d_6): δ 101.6 (OCH_2O), 106.8 (C-2′), 107.4 (C-5′), 113.8 (C-7), 113.9 (q, C-4, *J* = 6.7 Hz), 119.6 (q, C-6, *J* = 6.7 Hz), 120.2 (C-6′), 123.4 (q, CF_3, *J* = 271.8 Hz), 125.2 (C-1′), 125.6 (q, C-5, *J* = 32.2 Hz), 137.9 (C-3a), 143.8 (C-7a), 148.8 (C-4′), 151.3 (C-3′), 151.3 (C-3′), 151.7 (C-2) ppm; EIMS: *m/z* 306 (M^+, 100), 287 (10); Anal. Calcd for $C_{15}H_9F_3N_2O_2$: C, 58.83; H, 2.96; N, 9.15. Found: C, 58.53; H, 3.05; N, 9.45.

4.1.2. General method of synthesis of 1*H*-benzo[*d*]imidazoles 10–15

A mixture of 4-nitro-1,2-phenylenediamine (0.0065 mol), 1.01 equiv of appropriate aldehyde, 1.01 equiv of sodium metabisulfite, and DME as solvent, was taken in a 25 mL round bottom flask. The flask was shaken well and heated under microwave irradiation system (CEM) fitted with reflux condenser for 35–90 s at 70 °C. After irradiation, the mixture was poured onto cold water. The precipitate was collected by filtration, washed with water, dried and recrystallized. In cases where compounds did not precipitate, the mixture was extracted with EtOAc (3 × 15 mL). The organic layer was dried over magnesium sulfate and removed under vacuum. Purification was done by chromatography on silica gel eluting with chloroform and recrystallization from adequate solvent.

4.1.2.1. 5-Nitro-2-phenyl-1*H*-benzo[*d*]imidazole (10). Recrystallized from ethanol. Yield 0.97 g (63%) of brown solid. Mp 147.1–149.0 °C. ^{1}H NMR (200 MHz, DMSO-d_6): δ 7.46–7.57 (m, 5H, H-2′, H-3′, H-4′, H-5′, H-6′) 7.67 (d, 1H, *J* = 8.5 Hz), 8.12 (dd, 1H, H-6, *J* = 2.0, *J* = 8.5 Hz), 8.21 (d, 1H, H-4, *J* = 2.0 Hz). ^{13}C NMR (50 MHz, DMSO-d_6): δ 114.1 (C-4), 115.6 (C-7), 119.4 (C-6), 127.7 (C-3′, C-5′), 128.1 (C-1′), 130.1 (C-2′, C-6′), 131.2 (C-4′), 141.7 (C-7a), 141.9 (C-3a), 144.8 (C-5), 152.3 (C-2) ppm; EIMS: *m/z* (% rel. int.) 239 (M+, 100), 223 (2), 193 (30), 166 (20). Anal. Calcd for $C_{13}H_9N_3O_2$: C, 65.27; H, 3.79; N, 17.56. Found: C, 65.53; H, 3.85; N, 17.46.

4.1.2.2. 2-(2-Ethoxyphenyl)-5-nitro-1*H*-benzo[*d*] imidazole (11). Recrystallized from methanol. Yield 1.17 g (62%) of beige solid. Mp 128.3–131.2 °C. ^{1}H NMR indicates a mixture of tautomers (200 MHz, DMSO-d_6): δ 1.39 (t, 3H, *CH*$_3$), 4.21 (q, 2H, *CH*$_2$), 6.8 (d, 1H, H-2′, *J* = 6.9 Hz), 7.26 (dd, 1H, H-6′, *J* = 7.6, *J* = 1.6 Hz), 7.54 (dd, 2H, H-5′, *J* = 8.2, *J* = 1.6 Hz), 7.7 (d, 1H, H-4, *J* = 2.1 Hz), 7.94 (dd, 1H, H-6, *J* = 10.2, *J* = 2.4 Hz), 8.4 (d, 1H, H-7, *J* = 8.4 Hz), 7.5 (t, 1H, H-3′, *J* = 8.24, *J* = 6.8, *J* = 1.6 Hz) ppm; ^{13}C NMR (50 MHz, DMSO-d_6): δ 14.76 (CH_3), 65.6 (CH_2), 114.1 (C-4), 115.4 (C-7), 115.5 (C-3′), 116.9 (C-1′), 119.4 (C-6), 122.7 (C-5′), 125.6 (C-6′), 132.1 (C-4′), 143.7 (C-7a), 143.8 (C-3a), 144.9 (C-5), 153.9 (C-2), 154.6 (C-2′) ppm; MS (FAB^+): *m/z* 283 $(M+H)^+$; Anal. Calcd for $C_{15}H_{13}N_3O_3$: C, 63.60; H, 4.63; N, 14.83. Found: C, 63.81; H, 4.58; N, 14.95.

4.1.2.3. 2-(2-Isopropoxyphenyl)-5-nitro-1*H*-benzo[*d*]imidazole (12). Recrystallized from methanol. Yield 6.31 g (90%) of white solid. Mp 158.9–163.2 °C. ^{1}H NMR (200 MHz, DMSO-d_6): δ 1.36 (d, 6H, $(CH_3)_2$), 6.76 (dd, 1H, H-3′, *J* = 7.8, *J* = 1.2 Hz), 7.13–7.22 (m, 2H, H-4, H-5), 7.63 (d, 1H, H-7, *J* = 7.7 Hz), 7.72 (dd, 1H, H-6′, *J* = 7.8, *J* = 1.8 Hz), 8.02 (dd, 1H, H-6, *J* = 8.6, *J* = 1.8 Hz), 8.39 (d, 1H, H-4, *J* = 1.8 Hz), 10.88 (br s, 1H, N–H) ppm; ^{13}C NMR (50 MHz, DMSO-d_6): δ 21.8 $(CH_3)_2$, 72.1 (CH), 114.1 (C-4), 114.9 (C-3′), 115.5 (C-7), 116.7 (C-1′), 118.2 (C-6), 122.7 (C-5′), 125.4 (C-6′), 131.6 (C-4′), 143.7 (C-7a), 143.8 (C-3a), 144.9 (C-5), 154.6 (C-2′), 156.0 (C-2) ppm; EIMS: *m/z* (% rel. int.) 297 (M^+, 100); Anal. Calcd for $C_{16}H_{15}N_3O_3$: C, 64.64; H, 5.09; N, 14.13. Found: C, 65.10; H, 5.12; N, 14.38.

4.1.2.4. 2-Methoxy-4-[5-nitro-1*H*-benzo[*d*] imidazol-2-yl]phenol (13). Purified by column chromatography on silica gel, eluted with CH_2Cl_2/acetone (85:15). Yield 1.5 g (81%) of yellow solid. Mp 303.4–306.3 °C. ^{1}H NMR (200 MHz, $CDCl_3$): δ 3.97 (s, 3H, *–OCH*$_3$), 6.96 (d, 1H, H-5′, *J* = 8.2 Hz), 7.54–7.67 (m, 2H, H-7, H-6′), 7.76 (d, 1H, H-2′, *J* = 1.65 Hz), 8.07 (dd, 1H, H-6, *J* = 8.8, *J* = 2.2 Hz), 8.41 (s, 1H, H-4), 9.14 (br s, 2H, N–H, O-H) ppm; ^{13}C NMR (50 MHz, DMSO-d_6): δ 55.7 (CH_3O), 110.2 (C-2′), 114.3 (C-4), 114.9 (C-7), 117.3 (C-5), 119.4 (C-6), 120.5 (C-1′), 124.7 (C-6′), 141.7 (C-7a), 141.8 (C-3a), 144.9 (C-5), 146.8 (C-4′), 150.2 (C-3′), 151.6 (C-2) ppm; EIMS: *m/z* (% rel. int.) 285 (M^+, 100); Anal. Calcd for $C_{14}H_{11}N_3O_4$: C, 58.95; H, 3.89; N, 14.73. Found: C, 58.07; H, 3.73; N, 14.55.

4.1.2.5. 2-(3,4-Dimethoxyphenyl)-5-nitro-1*H*-benzo[*d*]imidazole (14). Purified by column chromatography on silica gel, eluted with CH_2Cl_2/acetone (85:15). Yield 1.6 g (85%) of yellow solid. Mp 169.6–174.1 °C. ^{1}H NMR (200 MHz, $CDCl_3$): δ 3.82 (s, 3H, $C_{4'}$–*OCH*$_3$), 3.88 (s, 3H, $C_{3'}$–*OCH*$_3$), 6.74 (d, H-7, *J* = 8.7 Hz), 7.14 (d, 1H, H-5′, *J* = 8.2 Hz), 7.51 (dd, 1H, H-6′, *J* = 8.2, *J* = 1.6 Hz), 7.71 (d, 1H, H-2′, *J* = 1.6 Hz), 7.88 (dd, 1H, H-6, *J* = 8.7, *J* = 2.7 Hz), 7.92 (d, 1H, H-4, *J* = 2.7 Hz), 8.66 (s, 1H, N–H) ppm; ^{13}C NMR (50 MHz, DMSO-d_6): δ 55.7 (CH_3O), 56.0 (CH_3O) 108.2 (C-2′), 111.4 (C-5′), 114.7 (C-4), 115.6 (C-7), 119.4 (C-6), 120.7 (C-6′), 121.4 (C-1′), 141.5 (C-7a), 142.1 (C-3a), 144.9 (C-5), 151.6 (C-2), 152.1 (C-4′), 153.6 (C-3′) ppm; MS (FAB^+): *m/z* 300 $(M+H)^+$; Anal. Calcd for $C_{15}H_{13}N_3O_4$: C, 60.20; H, 4.38; N, 14.04. Found: C, 59.97; H, 4.43; N, 14.25.

4.1.2.6. 5-Nitro-2-(3,4,5-trimethoxyphenyl)-1*H*-benzo[*d*]imidazole (15). Recrystallized from methanol. Yield 1.62 g (76%) of an orange solid. Mp 153.2–155.3 °C. ^{1}H NMR (200 MHz, $CDCl_3$): δ 3.73 (s, 3H, $C_{4'}$–OCH_3), 3.86 (s, 6H, $C_{3'}$–OCH_3, $C_{5'}$–OCH_3), 6.72–6.75 (m, 1H, H-7, *J* = 8.7 Hz), 7.38 (s, 2H, H-2′, H-6′), 7.87 (dd, 1H, H-6, *J* = 2.7, *J* = 8.8 Hz), 7.94 (s, 1H, H-4, *J* = 2.7), 8.68 (s, 1H, N–H) ppm; ^{13}C NMR (50 MHz, DMSO-d_6): δ 56.3 $(CH_3O)_2$, 60.6 (CH_3O) 102.4 (C-2′, C-6′), 114.2 (C-4), 115.5 (C-7), 119.2 (C-6), 127.4 (C-1′), 141.4 (C-7a), 142.1 (C-3a), 143.0 (C-4′), 144. 9 (C-5), 151.6 (C-2), 152.6 (C-3′, C-5′) ppm; MS (FAB^+): *m/z* 330 $(M+H)^+$; Anal. Calcd for $C_{16}H_{15}N_3O_5$: C, 58.36; H, 4.59; N, 12.76. Found: C, 58.85; H, 4.77; N, 13.01.

4.2. Pharmacological assays

4.2.1. Animals and preparation of aortic rings

Thoracic aorta was removed from healthy male Wistar rats (250–350 g), maintained under standard laboratory conditions with free access to food and water. Animals were killed by exposure to ether. All animal procedures were conducted in accordance with our Federal Regulations for Animal Experimentation and Care (NOM-062-ZOO-1999, Ministry of Agriculture, México), and were approved by the Institutional Animal Care and Use Committee. The aorta was cleaned of adhering connective tissue and cut into 4–5 mm length rings. Endothelium was removed in some rings by gently rubbing intraluminally with a metallic forceps; in these preparations, absence of the endothelium was confirmed by a less than 10% relaxation upon carbachol challenge (1 μM). Then, tissue segments were mounted by stainless steel hooks, under an optimal tension of 3 g, in 10 mL organ baths containing warmed (37 °C) and oxygenated (O_2/CO_2, 19:1) Krebs solution (composition, mM: NaCl, 118; KCl, 4.7; $CaCl_2$, 2.5; $MgSO_4$, 1.2; KH_2PO_4, 1.2; $NaHCO_3$, 25.0; EDTA, 0.026 and glucose, 11.1, pH 7.4). Changes in tension were recorded by Grass-FT03 force transducers connected to a MP100 analyzer, as previously described.[12,13]

4.2.2. Experimental protocol

After equilibration, rings were contracted by NA (0.1 μM) and washed every 30 min for 2 h. The absence of endothelium was confirmed by the lack of a relaxing response to carbachol (1 μM). After pre-contraction with NA, the test samples (compounds **1–15**, vehicle and positive control) were added to the bath in a volume of 100 μL; then cumulative concentration–response curves were obtained for each ring (0.31–100 μM). In order to avoid fatigue of the arterial preparation, a 60 min recovery period was allowed between curves. The relaxant effect of compounds and positive controls [carbachol (+E) and nitrendipine (−E)] were determined by comparing contraction before and after application of the test materials. NA, carbachol and nitrendipine were dissolved in distilled water, whereas compounds **1–15** and Pimobendan in DMSO. This solvent was determined to have no effect on NA-induced contractions at 1% of concentration. Muscular tone was calculated from the tracings, using BIOPAC Aqcknowledge software.

4.2.3. Antihypertensive activity of compound 13

In vivo antihypertensive activity study of compound **13** was performed on spontaneous hypertensive rats (SHR) by tail cuff method, using LE 5007 automatic blood pressure computer (Letica). In each group five rats were taken. Test compound **13** at doses of 25, 50, and 100 mg kg^{-1} was administered orally as suspension in 1% of Tween 20. Measurements (blood pressure and heart rate) were recorded before and after the treatment of test compound at the interval of 1 h for 6 h. The decrease in blood pressure (BP) was calculated and is plotted in Figure 3.

4.3. Data analysis

All the results are expressed as the mean of five or six experiments ± SEM. Concentration- responses curves (CRC) were plotted, and the experimental data from the CRC were adjusted by the nonlinear curve fitting program (ORIGIN 6.0). EC_{50} values were calculated separately for each concentration–response curve by probit analyses. Maximal relaxations are expressed as E_{max}. Student's *t*-test was performed to ascertain the significance of the antihypertensive exhibited activity.

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