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Original article

Synthesis, biological evaluation and structure–activity relationships of new phthalazinedione derivatives with vasorelaxant activity

Javier Munín [a, b], Elías Quezada [b, *], Andrea Cuiñas [a], Manuel Campos-Toimil [a], Eugenio Uriarte [b], Lourdes Santana [b], Dolores Viña [a, *]

[a] Farmacología de las Enfermedades Crónicas, Centro de Investigación en Medicina Molecular y Enfermedades Crónicas, Universidad de Santiago de Compostela, 15782 Santiago de Compostela, Spain
[b] Departamento de Química Orgánica, Facultad de Farmacia, Universidad de Santiago de Compostela, 15782 Santiago de Compostela, Spain

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ABSTRACT

Five series of 1,4-phthalazinedione derivatives were synthesized in good yields. Vasorelaxant activity of these new derivatives was measured on either intact or endothelium-denuded isolated rat thoracic aortic rings pre-contracted with phenylephrine. Most of studied compounds, substituted in both nitrogen atoms, attained practically the total relaxation of the organ at low micromolar concentrations. The presence of functional endothelium significantly reduced the EC_{50} values for most of studied compounds. Some structure–activity relationships were established and compounds **2d** and **5d** can be considered as new leads for further modifications.

1. Introduction

Cardiovascular disorders are one of the main reasons for morbidity and death in recent years in developed countries. Among cardiovascular disorders, hypertension is the most common risk factor and it can cause coronary disease, myocardial infarction, stroke and sudden death and is the major contributor to cardiac failure and renal insufficiency [1].

Hypertension affects billions of people around the world. It is characterized by a persistently elevated blood pressure (BP) exceeding 140/90 mmHg or greater [2]. Hypertension induces an endothelial dysfunction via the release of superoxide anion by the vascular wall [3,4]. This free radical, together with its derivatives, counteracts the relaxing activity of endothelium-derived nitric oxide and prostacyclin (PGI_2) [5,6].

Due to the associated morbidity and mortality and cost to society, preventing and treating hypertension is an important public health challenge. Great efforts have been made on obtaining novel antihypertensive agents acting on different mechanisms to control blood pressure: diuretics [7], angiotensin-converting enzyme inhibitors [8,9], angiotensin II receptor blockers [9,10], calcium channel blockers [11], centrally sympathetic α_2-adrenoceptors [12] and drugs that prevent the action of peripheral sympathetic activity as β-adrenergic [13,14] and α-adrenergic [15] blocking agents.

Antihypertensive drugs such as prazosin (**I**), terazosin (**II**) and doxazosin (**III**), which have in their structure a quinazoline ring, are considered the most effective and clinically useful class of selective α_1-AR antagonists (Fig. 1) [16–18].

The phthalazine ring is the isostere and positional isomer of quinazoline ring and it is the nucleus of other well known vasodilator, hydralazine (**IV**) (Fig. 2). Hydralazine is an established antihypertensive drug that exerts its action via arterial dilation, although concentrations of hydralazine that present *in vitro* vasodilator effects in rat aorta (~1 mM) [19] are significantly higher than plasma concentrations which cause hypotension in awake rats (~100 nM) [20].

It is not used as a primary drug for treating hypertension because it also elicits sympathetic stimulation and salt retention, which may lead to the development of congestive heart failure and increases plasma rennin activity [21]. However, concomitant use of hydralazine together with a venodilatory nitrate has been shown to

* Corresponding authors.
E-mail addresses: elias.quezada@usc.es (E. Quezada), mdolores.vina@usc.es (D. Viña).

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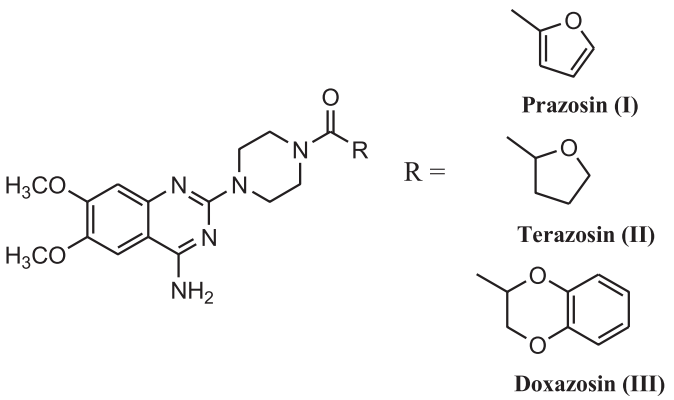


Fig. 1. Clinically used α_1-ARs antagonists **I**–**III**.

prevent the development of nitrate tolerance and to maintain the favorable hemodynamic effect of nitrates in patients with congestive heart failure [22]. Isosorbide dinitrate/hydralazine combination is advocated as a reasonable option in the treatment of patients with advanced, but stable heart failure and who remain symptomatic despite optimal standard therapy [23]. Furthermore, hydralazine (**IV**) is commonly used for severe pregnancy hypertension [24].

Structural modification of hydralazine led to the discovery of other phthalazine derivatives (**V**–**VII**, Fig. 2) [25–27] with vasorelaxant activity and some pyridazinone derivatives (**VIII**–**X**, Fig. 3) which are known drugs with interesting effects in cardiovascular system due to their platelet aggregation inhibition as well as their antihypertensive activity and cardiotonic properties [28–34].

Most of described pyridazinone derivatives, showing activity on cardiovascular system, include aryl residues at 6 position (Fig. 3). However, in the last few years new pyridazinone derivatives in which the phenyl group at C6 was removed or replaced (compound **X** in Fig. 3) were described such as α_1-ARs antagonists or platelet aggregation inhibitors [28,29,35,36].

Considering the pyridazinone residue as the pharmacophoric group for the activity, we focused on the synthesis of a series of new derivatives of its benzene condensed analogue, phthalazinone, with the aim of testing their vasorelaxant activity during *in vitro* assays and try to establish a preliminary SAR. Experiments were performed on both endothelium-intact and endothelium-denuded aortic rings in order to elucidate whether achieved relaxation is or not endothelium dependent.

Additionally, since overall these compounds showed interesting vasorelaxant activity, cytotoxicity test on cells of smooth muscle derived from rat aorta fibroblasts (line A7r5) were performed.

2. Results and discussion

2.1. Chemistry

A series of differently substituted 1,4-phthalazinedione derivatives were synthesized in good yield starting from phthalic anhydride (**A**) or 4-chlorophthalic anhydride (**B**) (Scheme 1) [37–39].

Firstly, 2,3-dihydro-2-methyl-1,4-phthalazinedione (**1a**) was synthesized by reaction of phthalic anhydride (**A**) and methylhydrazine under MW irradiation (320 W) and using Montmorillonite KSF as a catalyzer. The same procedure was used starting from **B** and yielded a mixture of 8-chloro-2,3-dihydro-2-methyl-1,4-phthalazinedione (**1b**) and 5-chloro-2,3-dihydro-2-methyl-1,4-phthalazinedione (**1c**) isomers in 1.8:1 proportion which were separated by HPLC using a Partisil Si 10 (Whatman) column.

Identification and characterization of both isomers was performed using 1D and 2D NMR experiments (HMQC and HMBC). For compound **1b** a long-distance correlation between C-1 (155.49 ppm) and protons of CH_3 (3.49 ppm) is observed by HMBC, however for compound **1c** this correlation is observed between C-1 (156.21 ppm) and protons of CH_3 (3.52 ppm) and H-8 (8.19 ppm) (Fig. 4).

tert-Butoxycarbonylaminealkyl-2,3-dihydro-1,4-phthalazinedione derivatives **2a**–**f** were obtained starting from 1,4-phthalanzinediones **1a**–**c** by reaction with *N*-Boc-n-bromoalkylamine and K_2CO_3/KI in DMF and heating at 125 °C [40]. Yields achieved for propyl derivatives **2b**, **2d** and **2f** (83%, 79% and 86% respectively) resulted slightly higher than those for the corresponding ethyl derivatives **2a**, **2c** and **2e** (67%, 78% and 78% respectively). Deprotection of the above mentioned derivatives was performed by treating with Et_2O/HCl, obtaining the corresponding aminoalkyl-2,3-dihydro-1,4-phthalazinedione derivatives **3a**–**f** in high yield [41].

Bromoalkyl-2,3-dihydro-1,4-phthalazinedione derivatives **4a**–**f** were obtained by reaction of 1,4-phthalazinediones **1a**–**c** and the corresponding alkyldibromide and K_2CO_3 in DMF and heating at 125 °C [42]. Afforded yields were, in all the cases, higher for bromopropyl-2,3-dihydro-1,4-phthalazinediones **4b**, **4d** and **4f** (46%, 86% and 80% respectively) than those afforded for the corresponding bromoethyl-2,3-dihydro-1,4-phthalazinediones **4a, 4c** and **4e** (32%, 60% and 17% respectively). Furthermore, reaction time had to be shorter from 24 h to obtain bromopropyl-2,3-dihydro-1,4-phthalazinediones **4b**, **4d** and **4f** to 2 h for the corresponding bromoethyl-2,3-dihydro 1,4-phthalazinediones **4a**, **4c** and **4e** because longer reaction time led to decomposition of these derivatives.

Reaction of bromoalkyl-2,3-dihydro-1,4-phthalazinedione derivatives **4a**–**f** with sodium azide and sodium iodide in DMSO at 100 °C afforded the azidoalkyl-2,3-dihydro-1,4-phthalazinedione derivatives **5a**–**f** in good yields [43]. Due to the instability showed for derivatives **4a**, **4c** and **4e**, the reaction with sodium azide was heating just for 3 h, while using derivatives **4b**, **4d** and **4f** time reaction was prolonged for 24 h.

Reduction of azidoalkyl-2,3-dihydro-1,4-phthalazinedione derivatives **5a**–**f** with Ph_3P in methanol at 80 °C for 1 h, let to obtain the aminoalkyl-2,3-dihydro-1,4-phthalazinedione derivatives **3a**–**f** with excellent yield (70–84%), following an alternative route to that previously described (Scheme 1) [44].

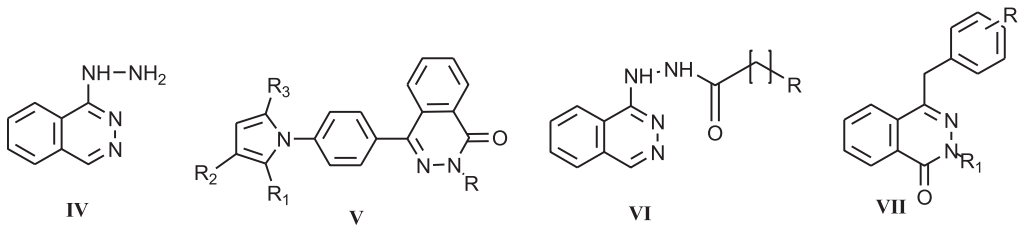


Fig. 2. Chemical structure of hydralazine (**IV**) and phthalazine derivatives (**V**–**VII**) with vasorelaxant activity.

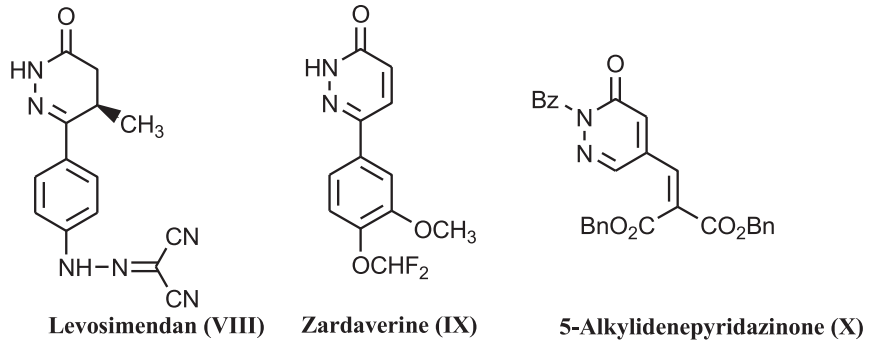
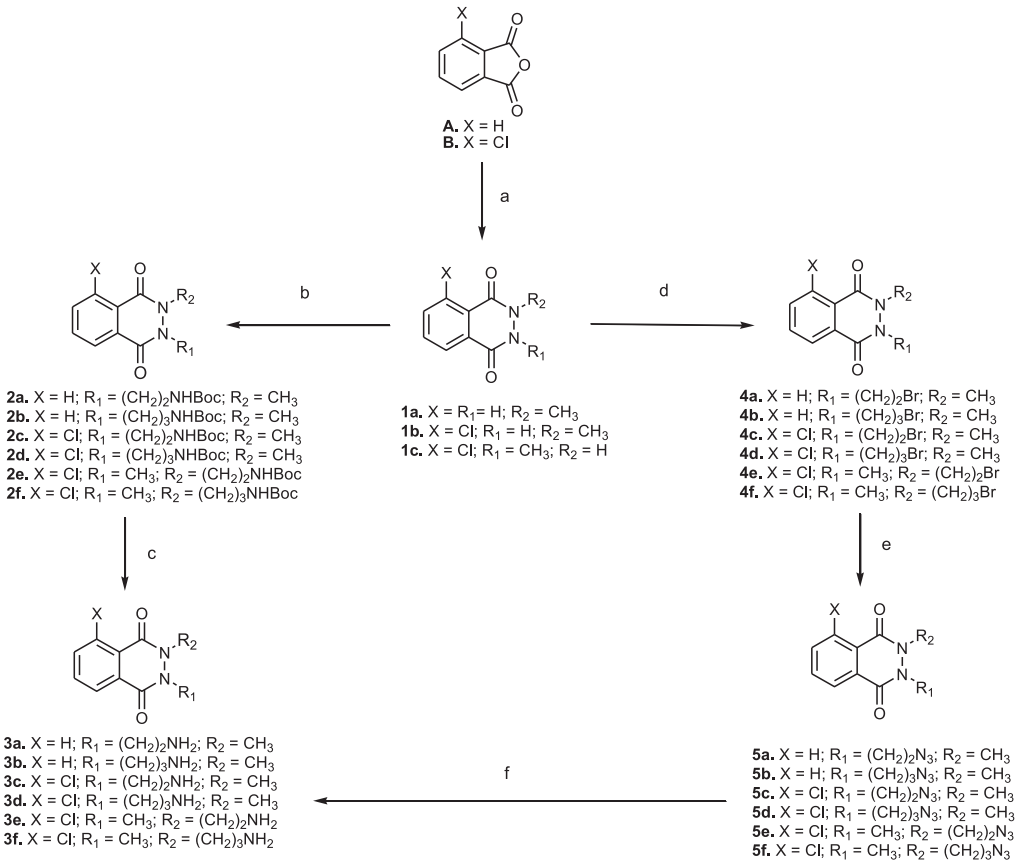


Fig. 3. Representative pyridazinone derivatives with activity on cardiovascular system.



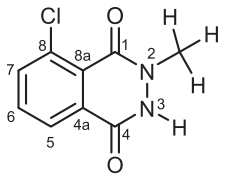
Scheme 1. Reagents and conditions: (a) CH_3NHNH_2, Montmorillonite KSF, MW, 15 min; (b) $Br(CH_2)_2NHBoc$ or $Br(CH_2)_3NHBoc$, K_2CO_3, KI, DMF, 125 °C, 24 h; (c) sat. HCl/Et_2O, CH_2Cl_2, 0 °C, 30 min, then rt, 1 h; (d) $Br(CH_2)_2Br$ or $Br(CH_2)_3Br$, K_2CO_3, DMF, 125 °C, 2 or 24 h; (e) NaN_3, NaI, DMSO, 100 °C, 3 or 24 h; (f) $(Ph)_3P$, CH_3OH, 80 °C, 1 h.

2.2. Pharmacology

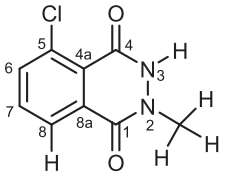
2.2.1. Vasorelaxant activity

Vasorelaxant properties of the synthesized 1,4-phthalazinedione derivatives (series **1**–**5**) were investigated using isolated rat thoracic aortic rings pre-contracted with phenylephrine (PE) following a standard procedure previously reported by us [45,46] and compared to the reference drugs hydralazine, amrinone [47] and diazepam [48]. Experiments were performed on both endothelium-intact and endothelium-denuded aortic rings. PE (1 μM) produced a sustained contraction in the rat isolated aortic rings with or without endothelium. The maximal tensions reached (mg) were 1491 ± 46 and 2048 ± 69, respectively ($p < 0.01$, $n = 50$).

Most of new 1,4-phthalazinedione derivatives bearing substituents in both nitrogen atoms significantly relaxed the aortic rings pre-contracted with PE, in a concentration-dependent manner, in the absence of endothelium but affording a major vasorelaxant effect in the presence of endothelium (Table 1). Our results suggest that the relaxation induced by the above mentioned compounds is caused by two mechanisms: a direct effect on vascular smooth muscle and a mechanism that is dependent on the presence of a functional endothelium. Most of the studied compounds resulted to be more potent than hydralazine and others vasorelaxant agents such as amrinone or diazepam. However, contrary to hydralazine or diazepam and as in the case of amrinone, the endothelium-dependent component resulted more significant.



1b



1c

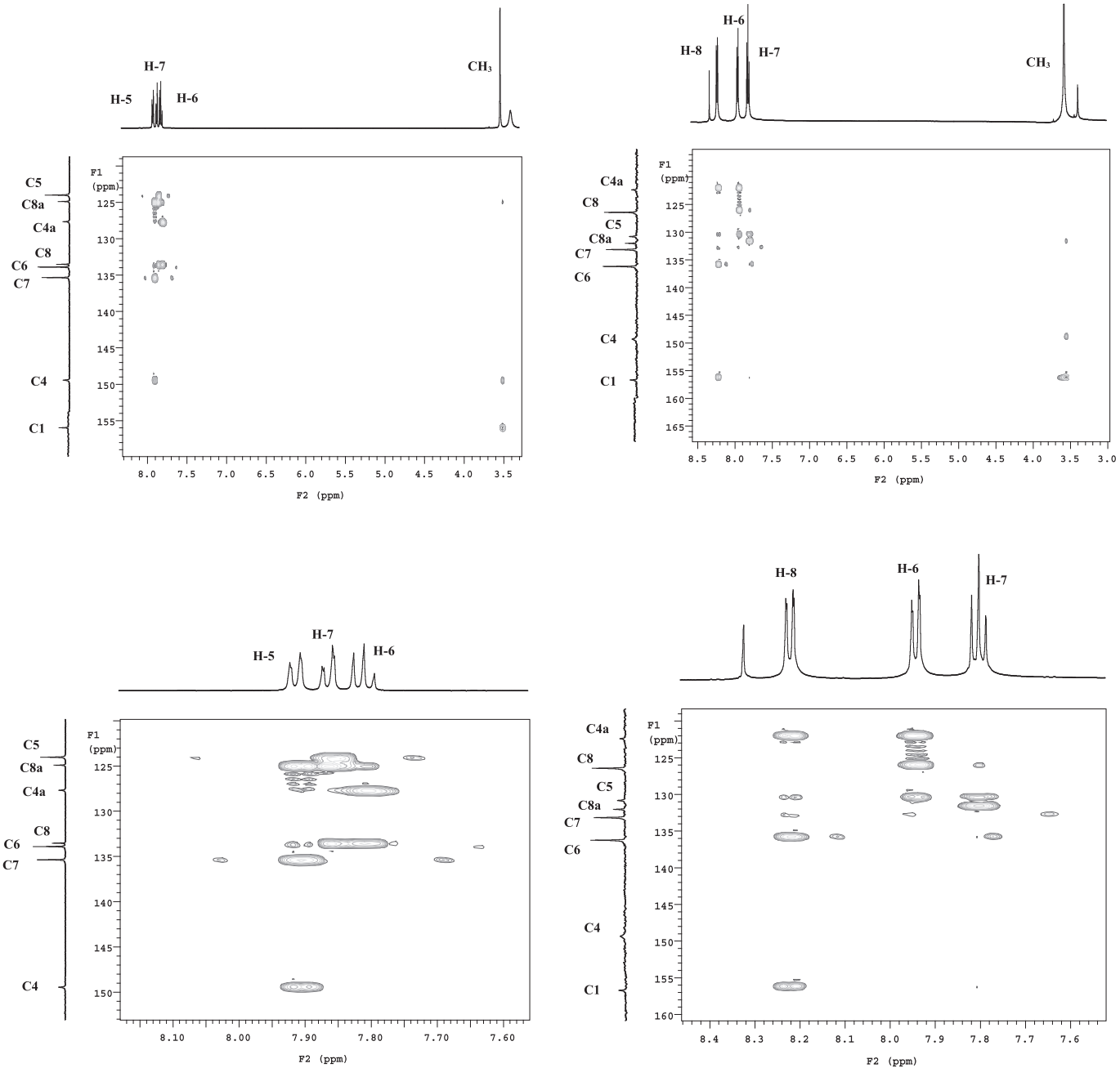


Fig. 4. HMBC spectra of compounds **1b** and **1c**.

The relaxation was expressed as a percentage of the reverse contraction produced by PE.

Analyzing structural features of the new molecules and their vasorelaxant activity on intact isolated rat aorta rings, some considerations of structure–activity relationship can be established. Compounds **1a**–**c** resulted inactive as vasorelaxant agents, demonstrating that position of the N-methyl substitution and the chloro atom on the phenyl ring of the 1,4-phthalazinedione moiety,

Table 1
Vasorelaxant activity of 1,4-phthalazinedione derivatives (series **1**–**5**) in rat aortic rings pre-contracted with PE (1 μM).

Compound	EC_{50} (μM) with endothelium	EC_{50} (μM) without endothelium
1a	*	*
1b	*	*
1c	*	*
2a	2.44 ± 0.57	14.56 ± 3.05[a]
2b	3.96 ± 1.02	23.61 ± 4.90[b]
2c	2.53 ± 0.57	5.57 ± 0.91[b]
2d	2.15 ± 0.30	4.85 ± 0.24[a]
2e	3.45 ± 0.78	13.30 ± 1.99[a]
2f	4.78 ± 1.13	18.23 ± 2.78[a]
3a	**	*
3b	**	*
3c	48.72 ± 10.64	*[a]
3d	8.51 ± 2.52	30.18 ± 2.81[a]
3e	**	*
3f	44.68 ± 4.13	*[a]
4a	1.70 ± 0.29	8.46 ± 1.59[a]
4b	3.10 ± 0.53	15.45 ± 2.55[a]
4c	7.35 ± 1.20	32.55 ± 5.90[a]
4d	6.61 ± 1.91	27.16 ± 4.96[a]
4e	7.95 ± 1.06	20.16 ± 2.51[a]
4f	3.36 ± 0.83	6.71 ± 1.15[a]
5a	10.00 ± 2.12	15.94 ± 2.52
5b	2.35 ± 0.70	3.71 ± 0.73
5c	4.12 ± 0.63	13.10 ± 1.40[a]
5d	2.33 ± 0.37	11.71 ± 0.97[a]
5e	6.35 ± 0.74	13.19 ± 2.39[b]
5f	4.31 ± 0.87	7.44 ± 1.00[b]
Hydralazine	(1.18 ± 0.09) × 10^3	(1.32 ± 0.12) × 10^3
Amrinone [47]	17 ± 6	28 ± 8[a]
Diazepam [48]	69.0 ± 6.5	77.2 ± 8.3

*Inactive at 200 μM (highest concentration tested). **200 μM produced relaxation about 50%. [a]$P < 0.01$ or [b]$P < 0.05$ *vs* rings with endothelium.

by themselves, are irrelevant to the activity. Potential interest of the presence of a chlorine atom on 1,4-phthalazinedione structure was studied because different compounds showing vasorelaxant activity as azelastine or diazepam show this substituent in their structure [26,48].

Introduction of an additional N-substitution on the second nitrogen atom of the phthalazinedione ring is necessary to afford active derivatives. With the incorporation of this second N-substitution, presence of chloro atom and position of the N-substituents allow to observe some differences on the activity of these derivatives.

In all of N,N-disubstituted studied series, activity of compounds is higher when alkyl linker is incorporated on N2. Only one exception is observed in bromo derivatives series in which compound **4f** resulted more potent than **4d** as vasorelaxant agent.

Length of the alkyl linker on N2 or N3 also modifies the activity of these derivatives. In general, when a chloro atom is present in the structure, a N-propyl linker resulted more effective for the substitution than an ethyl linker, with the only exception of compounds **2e** and **2f** (EC_{50} values resulted 3.45 and 4.78 μM for **2e** and **2f** respectively). When a chloro atom is not present in the structure, N-ethyl linker resulted more effective than the propyl one, with the exception of compounds **5a** and **5b** (EC_{50} values resulted 10.00 and 2.35 μM for **5a** and **5b** respectively).

Regarding to the nature of the alkyl linker, *tert*-butoxycarbonylamine, bromo and azide alkyl substituents on the one of the nitrogen atoms of the phthalazinedione ring, led to derivatives with interesting vasorelaxant activity on intact isolated rat aortic rings pre-contracted with PE.

However, aminoalkyl substitution in the same positions afforded new derivatives (compounds **3a**–**f**) which showed a

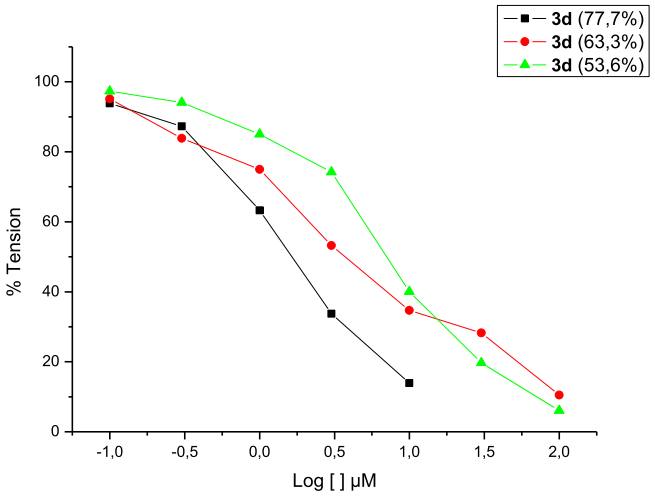


Fig. 5. Concentration–relaxation curves for compound **3d** in endothelium rat aortic rings precontracted with PE (1 μM). In brackets, % of the maximal contraction afforded after addition of ACh (1 μM) which is relative to the removed endothelium.

remarkable loss of activity and a high dependence on endothelium to produce vasorelaxation (Fig. 5). For these derivatives, the presence of a chloro atom on the phenyl ring resulted determinant for the activity, being compounds **3a** and **3b** practically inactive as vasorelaxant agents.

For all studied derivatives, the vasorelaxant activity was found lower on endothelium-denuded isolated rat aorta rings pre-contracted with PE, remaining the structural considerations above mentioned for all studied derivatives.

tert-Butoxycarbonylaminealkyl-2,3-dihydro-1,4-phthalazinedione derivatives **2a**–**f,** aminoalkyl-2,3-dihydro-1,4-phthalazinedione derivatives **3a**–**f,** bromoalkyl-2,3-dihydro-1,4-phthalazinedione derivatives **4a**–**f** and azidoalkyl-2,3-dihydro-1,4-phthalazinedione derivatives **5a**–**f** resulted more potent vasorelaxant agents than the reference compound hydralazine in both the presence or absence of endothelium (Fig. 6).

2.2.2. Cytotoxicity

The cytotoxic effects of two concentrations (25 and 50 μM) of compounds **1a**–**c**, **2a**–**f**, **3a**–**f**, **4a**–**f** and **5a**–**f** on A7r5, a well established vascular smooth muscle cell line obtained from embryonic rat aorta, were studied. As depicted in Fig. 7, compounds **2c**–**f** and **5f** (at 50 μM concentration) significantly decreased the viability of A7r5 cells. However for a lower concentration (25 μM), these compounds were well tolerated by the cells (Fig. 8). Considering the EC_{50} values obtained for vasorelaxant activity, this result justifies the interest of these derivatives.

3. Conclusion

Five series of 2 or 3-methyl-1,4-phthalazinedione derivatives N-monosubstituted (**1a**–**c**) or N,N-disubstituted with a variety of *tert*-butoxycarbonylaminealkyl (**2a**–**f**), aminoalkyl (**3a**–**f**), bromoalkyl (**4a**–**f**) or azidoalkyl (**5a**–**f**) groups linked to one of the N-atoms have been synthesized in good yields. Substituted N-monomethyl phthalazinediones lack of activity and additional substitution on the second nitrogen atom is necessary.

Most of the compounds (**2a**–**f**, **3c**–**d** and **3f**, **4a**–**f**, **5a**–**f**) have been found to cause relaxation of the aortic rings pre-contracted with PE in a concentration-dependent manner, both in the presence or in the absence of endothelium. The relaxation caused by our compounds was reduced after removal of the endothelium

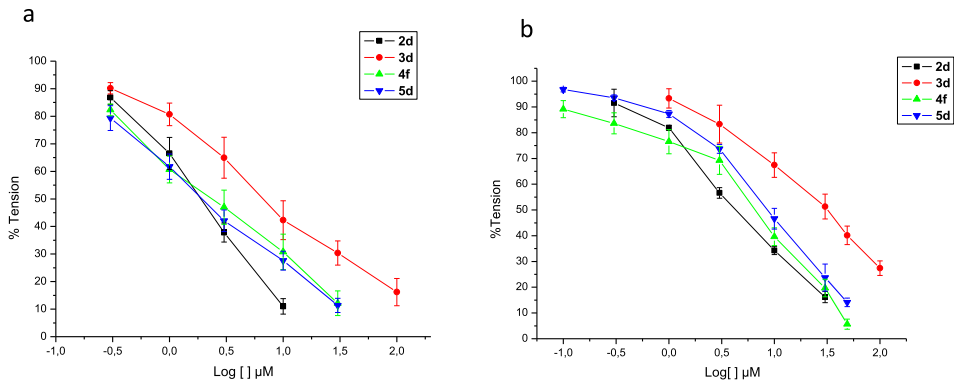


Fig. 6. Concentration–relaxation curves for some of most active compounds (**2d**, **3d**, **4f** and **5d**) in the described series on endothelium-denuded (a) and intact (b) rat thoracic aortic rings precontracted with PE (1 μM). Each point represents the mean value ± SEM (indicated by vertical bars) from, at least, five experiments.

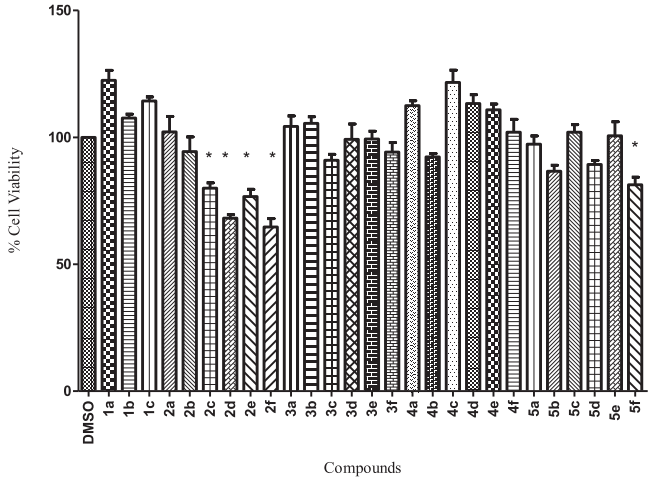


Fig. 7. Cytotoxic effects of a 24 h incubation with the studied compounds (50 μM) on A7r5 vascular myocytes. Results are expressed as mean ± S.E.M from at least 5 experiments. *Compounds decreased the viability versus the corresponding control group treated with DMSO ($P < 0.05$).

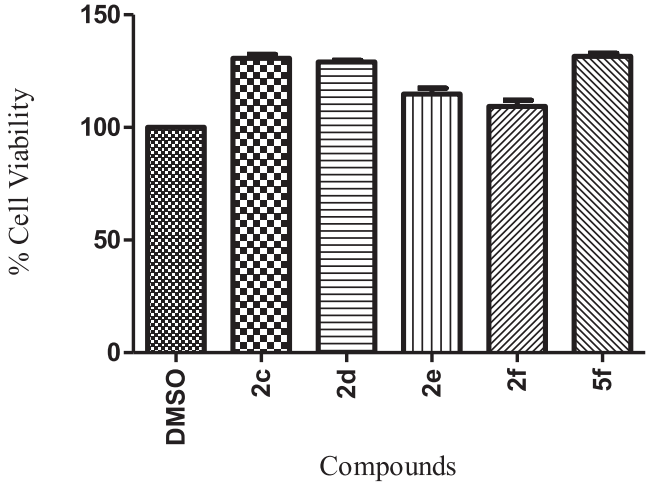


Fig. 8. Cytotoxic effects of a 24 h incubation with compounds **2c**–**f** and **5f** (25 μM) on A7r5 vascular myocytes. Results are expressed as mean ± S.E.M from at least 5 experiments.

layer. Further experimentation is needed in order to understand the precise mechanism of action involved in the vascular smooth muscle effects of these derivatives.

All compounds are non-toxic on A7r5 cells in concentration showing vasorelaxant effects.

Compounds **2d** and **5d** can be considered as new leads for further modifications. Both derivatives have a chloro atom at 5 position, methyl on the N3 and NHBoc group or azide group on N2 separated by a propyl linker. Replacement by different functional groups of the chlorine atom as well as NHBoc and azide in the propyl linker, will be performed in the future in order to obtain new and more potent vasorelaxant derivatives.

4. Experimental section

4.1. Chemistry

All reactions utilizing air- or moisture-sensitive reagents were carried out in flame dried glassware under an argon atmosphere, unless otherwise stated. CH_2Cl_2, $(CH_3)_2CO$, MeOH and DMSO were distilled prior to use according to the standard protocols. Other reagents were purchased and used as received without further purification unless otherwise stated. Reactions were magnetically stirred and monitored by thin layer chromatography (TLC) with 0.15–0.2 mm pre-coated silica gel (10–40 μm) plates. Compounds were visualized with UV light and/or by staining with ethanolic phosphomolybdic acid (PMA) followed by heating on a hot plate whilst ninhydrin was used as developer for amines. Flash chromatography (FC) was performed with silica gel (60–200 mesh) under pressure. NMR spectra were recorded on Bruker-250 or AMX 500 spectrometers in $CDCl_3$ or DMSO with TMS as the internal standard. Chemical shifts (δ) are given in ppm and coupling constants (J) in Hz. Multiplicity is indicated as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad.

EIMS and HR-EIMS were carried out on a VG AutoSpec (Fison, Ipswich, United Kingdom) instrument; the data are reported as *m/z* (percentage of relative intensity of the most important fragments).

4.1.1. 2,3-Dihydro-2-methyl-1,4-phthalazinedione (*1a*)

Phthalic anhydride (**A**, 1.50 g, 10.13 mmol), methylhydrazine (0.53 ml, 0.46 g, 10.13 mmol) and montmorillonite KSF (10.0 g, previously activated at 120 °C for 8 h), were mixed and ground in a mortar and placed in a clean and dry beaker. The reaction mixture was irradiated in a microwave oven for 15 min in 1-min period (320 W), the reaction was monitored by TLC. After completion of the reaction, it was cooled to room temperature and the resulting product was extracted into CH_2Cl_2 (4 × 50 ml). The KSF

Montmorillonite was filtered out and the solvent was evaporated under vacuum. The residue was purified by FC (CH_2Cl_2/CH_3OH 95:05, 90:10) affording **1a** [39] (1.46 g, 82% yield) as a white solid, m.p. 231–232 °C. ^{1}H NMR (250 MHz, DMSO-d_6) δ = 11.75 (br, 1H, NH), 8.17–8.21 (m, 1H, Ar–H), 7.84–7.96 (m, 3H, Ar–H), 3.38 (s, 3H, CH_3). ^{13}C NMR (62.9 MHz, DMSO-d_6) δ = 157.41, 150.28, 132.98, 132.31, 128.83, 126.36, 124.83, 124.25, 37.73 (CH_3). EIMS: *m/z* 176 $[M]^{+\bullet}$.

4.1.2. 8-Chloro-2,3-dihydro-2-methyl-1,4-phthalazinedione (*1b*) and 5-chloro-2,3-dihydro-2-methyl-1,4-phthalazinedione (*1c*)

Following the previously described procedure to obtain **1a**, reaction of 3-chlorophthalic anhydride (**B**, 1.50 g, 8.22 mmol), methylhydrazine (0.43 ml, 0.38 g, 8.22 mmol) and montmorillonite KSF (8.0 g), afforded a mixture of **1b** and **1c** (0.56 g, 48% yield). Mixture was purified by HPLC using a Partisil Si 10 column (Whatman) and $CHCl_3$/*i*PrOH (99:1), isolating **1b** (0.36 g) and **1c** (0.20 g) as white solids, ratio1.8:1. (**1b**): m.p. 223–224 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ = 11.72 (br, 1H, NH), 7.89 (d, J = 7.8, 1H, H-5), 7.84 (d, J = 7.8, 1H, H-7), 7.78 (t, J = 7.8, 1H, H-6), 3.49 (s, 3H, CH_3). ^{13}C NMR (125.7 MHz, DMSO-d_6) δ = 155.49, 148.98, 134.92, 133.46, 133.08, 127.25, 124.49, 123.60, 38.24 (CH_3). HR-EIMS: *m/z* 210.0192 $[M]^{+\bullet}$; calcd for $C_9H_7ClN_2O_2$, 210.0196. (**1c**): m.p. 222–223 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ = 11.72 (br, 1H, NH), 8.19 (d, J = 7.8, 1H, H-8), 7.92 (d, J = 7.8, 1H, H-6), 7.78 (t, J = 7.8, 1H, H-7), 3.52 (s, 3H, CH_3). ^{13}C NMR (125.7 MHz, DMSO-d_6) δ = 156.21, 148.84, 135.78, 132.72, 131.59, 130.38, 126.00, 121.97, 37.47 (CH_3). HR-EIMS: *m/z* 210.0196 $[M]^{+\bullet}$; calcd for $C_9H_7ClN_2O_2$, 210.0196.

4.1.3. 2-[2-(tert-Butoxycarbonyl)amine]ethyl-2,3-dihydro-3-methyl-1,4-phthalazinedione (*2a*)

A mixture of **1a** (0.10 g, 0.57 mmol) and K_2CO_3 (0.08 g, 0.57 mmol) in anhydrous DMF (6 ml) was stirred at room temperature for 2 h and kept under argon. Likewise, a solution of N-Boc-2-bromoethylamine (0.13 g, 0.57 mmol) and KI (0.09 g, 0.57 mmol) in DMF (8 ml) was stirred for 2 h and kept under argon. Then, this solution was added on the first one and the reaction mixture was stirred at 125 °C for 24 h and kept under argon. The mixture was cooled to room temperature, filtered and the precipitate was washed several times with anhydrous acetone. The solvent was removed under vacuum and the residue was purified by FC (CH_2Cl_2/MeOH 99:1, 98:2) to provide **2a** (0.12 g, 67% yield) as a white solid, m.p. 170–171 °C. ^{1}H NMR (250 MHz, $CDCl_3$) δ = 8.30–8.34 (m, 1H, Ar–H), 7.90–7.96 (m, 1H, Ar–H), 7.70–7.75 (m, 2H, Ar–H), 5.1 (br, 1H, NH), 4.33 (t, J = 4.9, 2H, N–CH_2), 3.68 (s, 3H, N–CH_3), 3.59 (m, 2H, NH–CH_2), 1.43 (s, 9H, $3CH_3$). ^{13}C NMR (62.9 MHz, $CDCl_3$) δ = 158.58, 155.77, 149.27, 132.39, 131.74, 128.80, 126.81, 124.37, 123.21, 79.42 ((CH_3)$_3C$), 66.12 (N–CH_2), 39.65 (NH–CH_2), 38.63 (N–CH_3), 28.25 ((CH_3)$_3$C). HR-EIMS: *m/z* 319.1523 $[M]^{+\bullet}$; calcd for $C_{16}H_{21}N_3O_4$, 319.1532.

4.1.4. 2-[3-(tert-Butoxycarbonyl)amine]propyl-2,3-dihydro-3-methyl-1,4-phthalazinedione (*2b*)

The compound **2b** was obtained from **1a** and N-Boc-3-bromopropylamine in a similar manner as for the preparation of **2a** (83%), as a white solid, m.p. 153–154 °C. ^{1}H NMR (250 MHz, $CDCl_3$) δ = 8.35–8.41 (m, 1H, Ar–H), 7.91–8.00 (m, 1H, Ar–H), 7.74–7.78 (m, 2H, Ar–H), 4.80 (br, 1H, NH), 4.36 (t, J = 6.0, 2H, N–CH_2), 3.72 (s, 3H, CH_3), 3.34 (m, 2H, NH–CH_2), 2.04 (m, 2H, (CH_2–CH_2–CH_2)), 1.43 (s, 9H, $3CH_3$). ^{13}C NMR (62.9 MHz, $CDCl_3$) δ = 158.55, 155.86, 149.47, 132.39, 131.67, 128.76, 126.76, 124.49, 123.17, 79.49 ((CH_3)$_3C$), 64.46 (N–CH_2), 38.60 (N–CH_3), 37.74 (NH–CH_2), 29.03 (CH_2–CH_2–CH_2), 28.26 ($3CH_3$). HR-EIMS: *m/z* 333.1685 $[M]^{+\bullet}$; calcd for $C_{17}H_{23}N_3O_4$, 333.1689.

4.1.5. 2-[2-(tert-Butoxycarbonyl)amine]ethyl-5-chloro-2,3-dihydro-3-methyl-1,4-phthalazinedione (*2c*)

The compound **2c** was obtained from **1b** and N-Boc-2-bromoethylamine in a similar manner as for the preparation of **2a** (78%) as a white solid, m.p. 114–115 °C. ^{1}H NMR (250 MHz, $CDCl_3$) δ = 7.91 (d, J = 7.8, 1H, Ar–H), 7.74 (d, J = 7.8, 1H, Ar–H), 7.62 (t, J = 7.8, 1H, Ar–H), 5.11 (br, 1H, NH), 4.34 (t, J = 5.0, 2H, N–CH_2), 3.68 (s, 3H, N–CH_3), 3.62 (m, 2H, NH–CH_2), 1.45 (s, 9H, $3CH_3$). ^{13}C NMR (62.9 MHz, $CDCl_3$) δ = 156.82, 155.85, 148.33, 134.88, 134.81, 132.60, 126.91, 124.79, 122.49, 79.53 ((CH_3)$_3C$), 66.38 (N–CH_2), 39.59 (NH–CH_2), 39.14 (N–CH_3), 28.29 ((CH_3)$_3$C). HR-EIMS: *m/z* 353.1130 $[M]^{+\bullet}$; calcd for $C_{16}H_{20}ClN_3O_4$, 353.1142.

4.1.6. 2-[3-(tert-Butoxycarbonyl)amine]propyl-5-chloro-2,3-dihydro-3-methyl-1,4-phthalazinedione (*2d*)

The compound **2d** was obtained from **1b** and N-Boc-3-bromopropylamine (0.11 g, 0.46 mmol) in a similar manner as for the preparation of **2a** (79%) as a white solid, m.p. 161–162 °C. ^{1}H NMR (250 MHz, $CDCl_3$) δ = 7.83 (d, J = 7.8, 1H, Ar–H), 7.67 (d, J = 7.8, 1H, Ar–H), 7.56 (t, J = 7.8, 1H, Ar–H), 5.00 (br, 1H, NH), 4.29 (t, J = 6.1, 2H, N–CH_2), 3.61 (s, 3H, N–CH_3), 3.33 (m, 2H, NH–CH_2), 2.00 (m, 2H, CH_2–CH_2–CH_2), 1.40 (s, 9H, $3CH_3$). ^{13}C NMR (62.9 MHz, $CDCl_3$) δ = 156.71, 155.90, 148.46, 134.77, 134.75, 132.53, 127.05, 124.80, 122.41, 79.05 ((CH_3)$_3C$), 64.65 (N–CH_2), 39.02 (N–CH_3), 37.70 (NH–CH_2), 29.11 (CH_2–CH_2–CH_2), 28.31 ((CH_3)$_3$C). HR-EIMS: *m/z* 367.1299 $[M]^{+\bullet}$; calcd for $C_{17}H_{22}ClN_3O_4$, 367.1299.

4.1.7. 3-[2-(tert-Butoxycarbonyl)amine]ethyl-5-chloro-2,3-dihydro-2-methyl-1,4-phthalazinedione (*2e*)

The compound **2e** was obtained from **1c** and N-Boc-3-bromoethylamine in a similar manner as for the preparation of **2a** (78%) as a white solid, m.p. 107–108 °C. ^{1}H NMR (250 MHz, $CDCl_3$) δ = 8.37 (d, J = 7.9, 1H, Ar–H), 7.78 (d, J = 7.9, 1H, Ar–H), 7.64 (t, J = 7.9, 1H, Ar–H), 5.16 (br, 1H, NH), 4.32 (t, J = 5.0, 2H, N–CH_2), 3.70 (s, 3H, N–CH_3), 3.63 (m, 2H, NH–CH_2), 1.46 (s, 9H, $3CH_3$). ^{13}C NMR (62.9 MHz, $CDCl_3$) δ = 157.52, 155.77, 147.95, 135.74, 131.82, 131.50, 130.45, 126.36, 121.90, 79.42 ((CH_3)$_3C$), 66.59 (N–CH_2), 39.48 (NH–CH_2), 38.67 (N–CH_3), 28.31 ((CH_3)$_3$C). HR-EIMS: *m/z* 353.1134 $[M]^{+\bullet}$; calcd for $C_{16}H_{20}ClN_3O_4$, 353.1142.

4.1.8. 3-[3-(tert-Butoxycarbonyl)amine]propyl-5-chloro-2,3-dihydro-2-methyl-1,4-phthalazinedione (*2f*)

The compound **2f** was obtained from **1c** and N-Boc-3-bromopropylamine in a similar manner as for the preparation of **2a** (86%) as a white solid, m.p. 142–143 °C. ^{1}H NMR (250 MHz, $CDCl_3$) δ = 8.39 (d, 1H, J = 7.9, Ar–H), 7.80 (d, 1H, J = 7.9, Ar–H), 7.65 (t, J = 7.9, 1H, Ar–H), 4.80 (br, 1H, NH), 4.35 (t, J = 6.0, 2H, N–CH_2), 3.71 (s, 3H, N–CH_3), 3.39 (m, 2H, NH–CH_2), 2.05 (m, 2H, CH_2–CH_2–CH_2), 1.43 (s, 9H, $3CH_3$). ^{13}C NMR (62.9 MHz, $CDCl_3$) δ = 157.48, 155.94, 148.25, 135.71, 131.70, 131.43, 130.52, 126.25, 122.00, 78.99 ((CH_3)$_3C$), 65.14 (N–CH_2), 38.62 (N–CH_3), 37.99 (NH–CH_2), 28.83 (CH_2–CH_2–CH_2), 28.31 ((CH_3)$_3$C). HR-EIMS: *m/z* 367.1312 $[M]^{+\bullet}$; calcd for $C_{17}H_{22}ClN_3O_4$, 367.1299.

4.1.9. 2-(2-Aminoethyl)-2,3-dihydro-3-methyl-1,4-phthalazinedione (*3a*)

To a solution of **2a** (0.10 g, 0.31 mmol) in CH_2Cl_2 (25 ml) stirring at 0 °C, sat. HCl/Et_2O (2 ml) was added. Resulted solution was kept to 0 °C for 30 min. Then, it was carried out to room temperature and stirred for 1 h. After the reaction, ion exchange Ambersep 900 OH resin (3 g) was added and kept under stirring for another 30 min. Finally, it was filtered and solvent was evaporated under vacuum. The residue was purified by FC (CH_2Cl_2/MeOH 90:10, 85:15) affording **3a** (0.06 g, 88% yield) as a white solid, m.p. 100–101 °C. ^{1}H NMR (250 MHz, $CDCl_3$) δ = 8.40 (d, J = 9.3, 1H, Ar–H), 7.98 (d,

J = 9.3, 1H, Ar–H), 7.77 (m, 2H, Ar–H), 4.34 (t, J = 5.3, 2H, N–CH_2), 3.72 (s, 3H, N–CH_3), 3.30 (t, J = 5.3, 2H, NH_2–CH_2), 1.25 (br, 2H, NH_2). ^{13}C NMR (62.9 MHz, $CDCl_3$) δ = 158.71, 149.60, 132.48, 131.80, 128.93, 126.93, 124.63, 123.21, 69.18 (N–CH_2), 41.09 (NH_2–CH_2), 38.74 (N–CH_3). HR-EIMS: m/z 219.1003 $[M]^{+\bullet}$; calcd for $C_{11}H_{13}N_3O_2$, 219.1008.

4.1.10. 2-(3-Aminopropyl)-2,3-dihydro-3-methyl-1,4-phthalazinedione (**3b**)

The compound **3b** was obtained from **2b**, following the previously described procedure to obtain **3a**, (87%) as a white solid, m.p. 97–98 °C. ^{1}H NMR (250 MHz, $CDCl_3$) δ = 8.35 (d, J = 9.0, 1H, Ar–H), 7.91 (d, J = 9.0, 1H, Ar–H), 7.72 (m, 2H, Ar–H), 4.35 (t, J = 6.8, 2H, N–CH_2), 3.69 (s, 3H, N–CH_3), 2.91 (t, J = 6.8, 2H, NH_2–CH_2), 1.97 (m, 2H, CH_2–CH_2–CH_2), 1.72 (br, 2H, NH_2). ^{13}C NMR (62.9 MHz, $CDCl_3$) δ = 158.60, 149.62, 132.40, 131.66, 128.76, 126.75, 124.49, 123.16, 64.55 (N–CH_2), 39.01 (NH_2–CH_2), 38.66 (N–CH_3), 32.43 (CH_2–CH_2–CH_2). HR-EIMS: m/z 233.1167 $[M]^{+\bullet}$; calcd for $C_{12}H_{15}N_3O_2$ 233.1164.

4.1.11. 2-(2-Aminoethyl)-5-chloro-2,3-dihydro-3-methyl-1,4-phtalazinedione (**3c**)

The compound **3c** was obtained from **2c** following the previously described procedure to obtain **3a**, (85%) as a white solid, m.p. 127–128 °C. ^{1}H NMR (250 MHz, $CDCl_3$) δ = 7.99 (d, J = 7.9, 1H, Ar–H), 7.80 (d, J = 7.9, 1H, Ar–H), 7.70 (t, J = 7.9, 1H, Ar–H), 4.37 (t, J = 5.2, 2H, N–CH_2), 3.70 (s, 3H, N–CH_3), 3.16 (t, J = 5.2, 2H, NH_2–CH_2), 1.26 (br, 2H, NH_2). ^{13}C NMR (62.9 MHz, $CDCl_3$) δ = 156.93, 148.62, 134.87, 134.51, 132.77, 126.76, 124.42, 122.30, 68.25 (N–CH_2), 40.05 (NH_2–CH_2), 38.97 (N–CH_3). HR-EIMS: m/z 253.0617 $[M]^{+\bullet}$; calcd for $C_{11}H_{12}ClN_3O_2$, 253.0618.

4.1.12. 2-(3-Aminopropyl)-5-chloro-2,3-dihydro-3-methyl-1,4-phthalazinedione (**3d**)

The compound **3d** was obtained from **2d** following the previously described procedure to obtain **3a**, (82%) as a white solid, m.p. 111–112 °C. ^{1}H NMR (250 MHz, $CDCl_3$) δ = 7.90 (d, J = 7.8, 1H, Ar–H), 7.74 (d, J = 7.8, 1H, Ar–H), 7.63 (t, J = 7.8, 1H, Ar–H), 4.37 (t, J = 6.2, 2H, N–CH_2), 3.69 (s, 3H, N–CH_3), 2.94 (t, J = 6.2, 2H, NH_2–CH_2), 2.00 (m, 2H, CH_2–CH_2–CH_2), 1.33 (br, 2H, –NH_2). ^{13}C NMR (62.9 MHz, $CDCl_3$) δ = 156.80, 148.59, 134.78, 134.74, 132.53, 127.18, 124.85, 122.40, 64.81 (N–CH_2), 39.09 (N–CH_3), 39.06 (NH_2–CH_2), 32.50 (CH_2–CH_2–CH_2). HR-EIMS: m/z 267.0767 $[M]^{+\bullet}$; calcd for $C_{12}H_{14}ClN_3O_2$, 267.0775.

4.1.13. 3-(2-Aminoethyl)-5-chloro-2,3-dihydro-2-methyl-1,4-phthalazinedione (**3e**)

The compound **3e** was obtained from **2e** following the previously described procedure to obtain **3a**, (70%) as a white solid, m.p. 54–55 °C. ^{1}H NMR (250 MHz, $CDCl_3$) δ = 8.35 (d, J = 7.9, 1H, Ar–H), 7.82 (d, J = 7.9, 1H, Ar–H), 7.67 (t, J = 7.9, 1H, Ar–H), 4.33 (t, J = 5.1, 2H, N–CH_2), 3.71 (s, 3H, N–CH_3), 3.16 (t, J = 5.1, 2H, NH_2–CH_2), 1.25 (br, 2H, NH_2). ^{13}C NMR (62.9 MHz, $CDCl_3$) δ = 157.64, 148.31, 135.85, 131.92, 131.24, 130.36, 126.17, 121.82, 68.69 (N–CH_2), 40.25 (NH_2–CH_2), 38.64 (N–CH_3). HR-EIMS: m/z 253.0617 $[M]^{+\bullet}$; calcd for $C_{11}H_{12}ClN_3O_2$, 253.0618.

4.1.14. 3-(3-Aminopropyl)-5-chloro-2,3-dihydro-2-methyl-1,4-phthalazinedione (**3f**)

The compound **3f** was obtained from **2f** following the previously described procedure to obtain **3a**, (68%) as a white solid, m.p. 79–80 °C. ^{1}H NMR (250 MHz, $CDCl_3$) δ = 8.32 (d, J = 7.8, 1H, Ar–H), 7.79 (d, J = 7.8, 1H, Ar–H), 7.51 (t, J = 7.8, 1H, Ar–H), 4.36 (t, J = 5.9, 2H, N–CH_2), 3.70 (s, 3H, N–CH_3), 3.03 (t, J = 5.9, 2H, NH_2–CH_2), 2.05 (m, 2H, CH_2–CH_2–CH_2), 1.25 (br, 2H, NH_2). ^{13}C NMR (62.9 MHz, $CDCl_3$) δ = 157.59, 148.43, 135.80, 131.79, 131.10, 130.47, 126.00, 121.83, 64.79 (N–CH_2), 38.61 (N–CH_3), 38.50 (NH_2–CH_2), 30.92 (CH_2–CH_2–CH_2). HR-EIMS: m/z 267.0777 $[M]^{+\bullet}$; calcd for $C_{12}H_{14}ClN_3O_2$, 267.0775.

4.1.15. 2-(2-Bromoethyl)-2,3-dihydro-3-methyl-1,4-phthalazinedione (**4a**)

A mixture of **1a** (0.20 g, 1.14 mmol) and K_2CO_3 (0.24 g, 1.73 mmol) in anhydrous DMF (10 ml) was kept under argon and stirring for 2 h at room temperature. Then, 1,2-dibromoethane (0.15 ml, 0.32 g, 1.70 mmol) was added and resulted solution was stirred for 2 h at 125 °C. The reaction mixture was then cooled to room temperature, filtered and washed with anhydrous acetone several times. Solvent was removed under vacuum and residue was purified by FC (hexane/AcOEt 85:15), affording **4a** (0.10 g, 32% yield) as a white solid, m.p. 105–106 °C. ^{1}H NMR (250 MHz, $CDCl_3$) δ = 8.40 (d, J = 9.7, 1H, Ar–H), 8.00 (d, J = 9.7, 1H, Ar–H), 7.79 (m, 2H, Ar–H), 4.63 (t, J = 6.0, 2H, N–CH_2), 3.75 (t, J = 6.0, 2H, Br–CH_2), 3.70 (s, 3H, N–CH_3). ^{13}C NMR (62.9 MHz, $CDCl_3$) δ = 158.66, 148.82, 132.57, 131.92, 128.88, 126.84, 124.27, 123.30, 66.09 (N–CH_2), 38.71 (N–CH_3), 28.90 (Br–CH_2). HR-EIMS: m/z 281.9993 $[M]^{+\bullet}$; calcd for $C_{11}H_{11}BrN_2O_2$, 282.0004.

4.1.16. 2-(3-Bromopropyl)-2,3-dihydro-3-methyl-1,4-phthalazinedione (**4b**)

The compound **4b** was obtained from **1a** and 1,3-dibromopropane in a similar manner as for the preparation of **4a** but it was purified by FC using hexane/AcOEt 8:2, (46%) as a white solid, m.p. 86–87 °C. ^{1}H NMR (250 MHz, $CDCl_3$) δ = 8.39 (d, J = 9.3, 1H, Ar–H), 7.93 (d, J = 9.3, 1H, Ar–H), 7.76 (m, 2H, Ar–H), 4.45 (t, J = 5.9, 2H, N–CH_2), 3.73 (s, 3H, N–CH_3), 3.62 (t, J = 6.5, 2H, Br–CH_2), 2.42 (m, 2H, CH_2–CH_2–CH_2). ^{13}C NMR (62.9 MHz, $CDCl_3$) δ = 158.64, 149.28, 132.45, 131.77, 128.94, 126.92, 124.54, 123.12, 64.39 (N–CH_2), 38.70 (N–CH_3), 31.84 (CH_2–CH_2–CH_2), 29.63 (Br–CH_2). HR-EIMS m/z 296.0167 $[M]^{+\bullet}$; calcd for $C_{12}H_{13}BrN_2O_2$, 296.0160.

4.1.17. 2-(2-Bromoethyl)-5-chloro-2,3-dihydro-3-methyl-1,4-phthalazinedione (**4c**)

The compound **4c** was obtained from **1b** and 1,2-dibromoetane in a similar manner as for the preparation of **4a** but it was purified by FC using hexane/AcOEt 9:1, (60%) as a white solid, m.p. 110–111 °C. ^{1}H NMR (250 MHz, $CDCl_3$) δ = 7.95 (d, J = 7.9, 1H, Ar–H), 7.77 (d, J = 7.9, 1H, Ar–H), 7.66 (t, J = 7.9, 1H, Ar–H), 4.62 (t, J = 5.9, 2H, N–CH_2), 3.74 (t, J = 5.9, 2H, Br–CH_2), 3.69 (s, 3H, N–CH_3). ^{13}C NMR (62.9 MHz, $CDCl_3$) δ = 156.87, 147.84, 135.03, 134.93, 132.75, 126.85, 124.95, 122.52, 66.30 (N–CH_2), 39.13 (N–CH_3), 28.83 (Br–CH_2). HR-EIMS: m/z 315.9619 $[M]^{+\bullet}$; calcd for $C_{11}H_{10}BrClN_2O_2$, 315.9614.

4.1.18. 2-(3-Bromopropyl)-5-chloro-2,3-dihydro-3-methyl-1,4-phthalazinedione (**4d**)

The compound **4d** was obtained from **1b** and 1,3-dibromopropane in a similar manner as for the preparation of **4c** (86%) as a white solid, m.p. 115–116 °C. ^{1}H NMR (250 MHz, $CDCl_3$) δ = 7.89 (d, J = 7.9, 1H, Ar–H), 7.76 (d, J = 7.9, 1H, Ar–H), 7.64 (t, J = 7.9, 1H, Ar–H), 4.44 (t, J = 5.9, 2H, N–CH_2), 3.70 (s, 3H, N–CH_3), 3.61 (t, J = 6.5, 2H, Br–CH_2), 2.40 (m, 2H, CH_2–CH_2–CH_2). ^{13}C NMR (62.9 MHz, $CDCl_3$) δ = 156.88, 148.29, 134.97, 134.91, 132.63, 127.06, 124.96, 122.33, 64.62 (N–CH_2), 39.17 (N–CH_3), 31.73 (CH_2–CH_2–CH_2), 29.60 (Br–CH_2). HR-EIMS: m/z 329.9770 $[M]^{+\bullet}$; calcd for $C_{12}H_{12}BrClN_2O_2$, 329.9771.

4.1.19. 3-(2-Bromoethyl)-5-chloro-2,3-dihydro-2-methyl-1,4-phthalazinedione (**4e**)

The compound **4e** was obtained from **1c** and 1,2-dibromoethane in a similar manner as for the preparation of **4c** (17%) as a white solid, m.p. 89–90 °C. ^{1}H NMR (250 MHz, $CDCl_3$) δ = 8.37 (d, J = 7.8, 1H, Ar–H), 7.80 (d, J = 7.8, 1H, Ar–H), 7.65 (t, J = 7.8, 1H, Ar–H), 4.62 (t, J = 6.0, 2H, N–CH_2), 3.75 (t, J = 6.0, 2H, Br–CH_2), 3.71 (s, 3H, N–CH_3). ^{13}C NMR (62.9 MHz, $CDCl_3$) δ = 157.65, 147.69, 135.90, 131.96, 131.57, 130.91, 126.26, 121.91, 66.73 (N–CH_2), 38.70 (N–CH_3), 28.55 (Br–CH_2). HR-EIMS: m/z 315.9609 $[M]^{+\bullet}$; calcd for $C_{11}H_{10}BrClN_2O_2$, 315.9614.

4.1.20. 3-(3-Bromopropyl)-5-chloro-2,3-dihydro-2-methyl-1,4-phthalazinedione (**4f**)

The compound **4f** was obtained from **1c** and 1,3-dibromopropane in a similar manner as for the preparation of **4c** (80%) as a white solid, m.p. 113–114 °C. ^{1}H NMR (250 MHz, $CDCl_3$) δ = 8.38 (d, J = 7.9, 1H, Ar–H), 7.79 (d, J = 7.9, 1H, Ar–H), 7.64 (t, J = 7.9, 1H, Ar–H), 4.42 (t, J = 5.7, 2H, N–CH_2), 3.72 (s, 3H, N–CH_3), 3.70 (t, J = 6.4, 2H, Br–CH_2), 2.40 (m, 2H, CH_2–CH_2–CH_2). ^{13}C NMR (62.9 MHz, $CDCl_3$) δ = 157.61, 148.16, 135.80, 131.82, 131.59, 130.56, 126.37, 122.08, 64.75 (N–CH_2), 38.73 (N–CH_3), 31.72 (CH_2–CH_2–CH_2), 30.24 (Br–CH_2). HR-EIMS: m/z 329.9755 $[M]^{+\bullet}$; calcd for $C_{12}H_{12}BrClN_2O_2$, 329.9771.

4.1.21. 2-(2-Azidoethyl)-2,3-dihydro-3-methyl-1,4-phthalazinedione (**5a**)

To a stirred solution of **4a** (0.10 g, 0.35 mmol) in DMSO (2 ml) were added NaN_3 (0.03 g, 0.46 mmol) and NaI (5 mg, 0.03 mmol). Then, the mixture was kept under stirring for 3 h at 100 °C. After cooling, water was added (20 ml) and the mixture was extracted with ether (4 × 20 ml). The ether extracts were washed with brine (3 × 10 ml) and dried over Na_2SO_4. The solvent was removed under vacuum and the crude oil obtained was purified by FC (hexane/AcOEt 90:10–85:15), affording **5a** (0.08 g, 93% yield) as a white solid, m.p. 115–116 °C. ^{1}H NMR (250 MHz, $CDCl_3$) δ = 8.39 (d, J = 9.2, 1H, Ar–H), 7.95 (d, J = 9.2, 1H, Ar–H), 7.78 (m, 2H, Ar–H), 4.50 (t, J = 5.0, 2H, N–CH_2), 3.73 (s, 3H, N–CH_3), 3.67 (t, J = 5.0, 2H, N_3–CH_2). ^{13}C NMR (62.9 MHz, $CDCl_3$) δ = 158.61, 148.93, 132.60, 131.87, 128.82, 126.79, 124.17, 123.30, 65.64 (N–CH_2), 49.82 (N_3–CH_2), 38.62 (N–CH_3). HR-EIMS: m/z 245.0910 $[M]^{+\bullet}$; calcd for $C_{11}H_{11}N_5O_2$, 245.0913.

4.1.22. 2-(3-Azidopropyl)-2,3-dihydro-3-methyl-1,4-phthalazinedione (**5b**)

Following the previously described procedure to obtain **5a**, but keeping the stirring for 24 h, reaction of **4b** afforded **5b** (100%) as a white solid, m.p. 62–63 °C. ^{1}H NMR (250 MHz, $CDCl_3$) δ = 8.38 (d, J = 9.2, 1H, Ar–H), 7.92 (d, J = 9.2, 1H, Ar–H), 7.76 (m, 2H, Ar–H), 4.39 (t, J = 6.1, 2H, N–CH_2), 3.72 (s, 3H, N–CH_3), 3.54 (t, J = 6.7, 2H, N_3–CH_2), 2.13 (m, 2H, CH_2–CH_2–CH_2). ^{13}C NMR (62.9 MHz, $CDCl_3$) δ = 158.49, 149.17, 132.35, 131.64, 128.78, 126.75, 124.39, 123.01, 63.48 (N–CH_2), 48.21 (N_3–CH_2), 38.56 (N–CH_3), 28.07 (CH_2–CH_2–CH_2). HR-EIMS: m/z 259.1060 $[M]^{+\bullet}$; calcd for $C_{12}H_{13}N_5O_2$, 259.1069.

4.1.23. 2-(2-Azidoethyl)-5-chloro-2,3-dihydro-3-methyl-1,4-phthalazinedione (**5c**)

The compound **5c** was obtained from **4c**, following the previously described procedure to obtain **5a** and after purification by FC using hexane/AcOEt 9:1, (75%) as a white solid, m.p. 112–113 °C. ^{1}H NMR (250 MHz, $CDCl_3$) δ = 7.93 (d, J = 7.8, 1H, Ar–H), 7.78 (d, J = 7.8, 1H, Ar–H), 7.67 (t, J = 7.8, 1H, Ar–H), 4.49 (t, J = 5.0, 2H, N–CH_2), 3.70 (s, 3H, N–CH_3), 3.67 (t, J = 5.0, 2H, N_3–CH_2). ^{13}C NMR (62.9 MHz, $CDCl_3$) δ = 156.99, 148.08, 135.11, 135.04, 132.86, 126.85, 122.59, 65.86 (N–CH_2), 49.88 (N_3–CH_2), 39.17 (N–CH_3). HR-EIMS: m/z 279.0519 $[M]^{+\bullet}$; calcd for $C_{11}H_{10}ClN_5O_2$, 279.0523.

4.1.24. 2-(3-Azidopropyl)-5-chloro-2,3-dihydro-3-methyl-1,4-phthalazinedione (**5d**)

The compound **5d** was obtained from **4d** following the previously described procedure to obtain **5b** and after purification by FC using hexane/AcOEt 9:1 (67%) as a white solid, m.p. 115–116 °C. ^{1}H NMR (250 MHz, $CDCl_3$) δ = 7.89 (d, J = 7.9, 1H, Ar–H), 7.76 (d, J = 7.9, 1H, Ar–H), 7.65 (t, J = 7.9, 1H, Ar–H), 4.39 (t, J = 6.1, 2H, N–CH_2), 3.70 (s, 3H, N–CH_3), 3.54 (t, J = 6.6, 2H, N_3–CH_2), 2.14 (m, 2H, CH_2–CH_2–CH_2). ^{13}C NMR (62.9 MHz, $CDCl_3$) δ = 156.86, 148.29, 134.95, 134.90, 132.63, 127.05, 124.94, 122.31, 63.83 (N–CH_2), 48.29 (N_3–CH_2), 39.14 (N–CH_3), 28.13 (CH_2–CH_2–CH_2). HR-EIMS: m/z 293.0679 $[M]^{+\bullet}$; calcd for $C_{12}H_{12}ClN_5O_2$, 293.068.

4.1.25. 3-(2-Azidoethyl)-5-chloro-2,3-dihydro-2-methyl-1,4-phthalazinedione (**5e**)

The compound **5e** was obtained from **4e** following the previously described procedure to obtain **5c** (56%) as a white solid, m.p. 106–107 °C. ^{1}H NMR (250 MHz, $CDCl_3$) δ = 8.37 (d, J = 7.9, 1H, Ar–H), 7.80 (d, J = 7.9, 1H, Ar–H), 7.65 (t, J = 7.9, 1H, Ar–H), 4.44 (t, J = 5.0, 2H, N–CH_2), 3.72 (m, 5H, N–CH_3 + N_3–CH_2). ^{13}C NMR (62.9 MHz, $CDCl_3$) δ = 157.59, 147.80, 135.86, 131.94, 131.51, 130.71, 126.26, 121.87, 65.33 (N–CH_2), 49.84 (N_3–CH_2), 38.65 (N–CH_3). HR-EIMS: m/z 279.0517 $[M]^{+\bullet}$; calcd for $C_{11}H_{10}ClN_5O_2$, 279.0523.

4.1.26. 3-(3-Azidopropyl)-5-chloro-2,3-dihydro-2-methyl-1,4-phthalazinedione (**5f**)

The compound **5f** was obtained from **4f** following the previously described procedure to obtain **5c** (67%) as a white solid, m.p. 61–62 °C. ^{1}H NMR (250 MHz, $CDCl_3$) δ = 8.38 (d, J = 7.9, 1H, Ar–H), 7.79 (d, J = 7.9, 1H, Ar–H), 7.64 (t, J = 7.9, 1H, Ar–H), 4.37 (t, J = 5.7, 2H, N–CH_2), 3.72 (s, 3H, N–CH_3), 3.61 (t, J = 6.7, 2H, N_3–CH_2), 2.13 (m, 2H, CH_2–CH_2–CH_2). ^{13}C NMR (62.9 MHz, $CDCl_3$) δ = 157.55, 148.10, 135.77, 131.79, 131.55, 130.50, 126.33, 122.03, 63.89 (N–CH_2), 48.37 (N_3–CH_2), 38.67 (N–CH_3), 28.11 (CH_2–CH_2–CH_2). HR-EIMS: m/z 293.0690 $[M]^{+\bullet}$; calcd for $C_{12}H_{12}ClN_5O_2$, 293.068.

4.1.27. General procedure for reduction from azides (**5a**–**f**) to amines (**3a**–**f**)

Triphenylphosphine (0.75 mmol, 74 mg) was added to a solution of the corresponding azide derivatives **5a**–**f** (0.5 mmol) in dry methanol (7 ml). Reaction mixture was heated under refluxing at 80 °C for 1 h. After completion of the reaction, it was cooled to room temperature and the solvent was removed under vacuum. The residue was purified by FC (CH_2Cl_2/MeOH 90:10; 85:15), to obtain **3a** (82%), **3b** (84%), **3c** (73%), **3d** (70%), **3e** (76%), **3f** (71%). Chemical structures of these compounds were confirmed by ^{1}H and ^{13}C RMN experiments, obtaining similar spectra for the compounds **3a**–**f** to those obtained by deprotecting of compounds **2a**–**f**.

4.2. Animals

The animals used throughout this study (Male Wistar–Kyoto (WKY) rats (Iffa-Credo)), purchased from Criffa (Barcelona, Spain) were housed, cared for and acclimatized (before the experiments) as indicated previously [49].

4.2.1. Ethical approval

All experiments were carried out in accordance with European regulations on the protection of animals (Directive 2010/63/UE), the Spanish Real Decreto 53/2013 (1 February) and/or the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the US.

4.2.2. Vasorelaxant activity

Vasorelaxant activity of newly synthesized compounds was studied using thoracic aortic rings of Wistar rats precontracted with PE (1 μM). Male Wistar rats weighing 300–400 g were killed by a blow to the head and exsanguinated. The thoracic aorta was rapidly dissected and transferred to a Petri dish with Krebs bicarbonate solution (KBS composition: 119 mM NaCl, 4.7 mM KCl, 1.5 mM $CaCl_2 \cdot 2H_2O$, 1.2 mM $MgSO_4 \cdot 7H_2O$, 25 mM $NaHCO_3$, 0.03 mM EDTA-Na_2, 11 mM glucose; pH = 7.4). Excess of fat and connective tissue was removed and for the endothelium free experiments was made a scraping of the arterial lumen with thick cotton thread. The aorta was cut into rings (4–5 mm in length, 0.9–1.0 mm in thickness) and each ring was placed in a 10 ml organ-bath in KBS, maintained at 37 °C and bubbled with carbogen. Each aortic ring was mounted between two stainless steel hooks passed through its lumen. The lower hook was fixed between two plates and the upper one was attached to a force displacement transducer (Pioden Controls Ltd., Canterbury, UK) to record the isometric tension with a MacLab/16s and Chart v.3.6.3 software or a PowerLab/8sp and Chart v.4.1.2 software Data Acquisition system (AD Instruments Castle Hill, Australia).

Preparations were equilibrated at a resting tension of 2 g for at least 1 h. Thereafter, isometric contractions were induced by the addition of PE (1 μM). Once the contraction stabilized, a single concentration of ACh (1 μM) was added to the bath in order to assess the endothelial integrity of the preparations. Endothelium was considered to be intact when this drug elicited a vasorelaxation >50% of the maximal contraction obtained in vascular rings precontracted with PE. The absence of ACh relaxant action in the vessels indicated the total removal of endothelial cells. After assessing the integrity of the endothelium, vascular tissues were allowed to recuperate for 1 h, during which the physiological solution was replaced every 15 min, before any experiment protocol was started.

After equilibration aortic rings were contracted by single concentration of PE (1 μM). Once the contractions stabilized, compounds of series **1**–**5** were added in progressively increasing cumulative concentrations (10 nM-200 μM) at 10 min intervals. Only one compound was tested in each ring. All compounds were initially dissolved in DMSO to prepare a 100 mM stock solution. Further solutions were made in KBS. Control experiments were performed in presence of DMSO alone, at the same concentration as those used with the derivatives tested, which demonstrated that the solvent did not affect the contractile response of isolated aorta.

All the results are expressed as means ± the standard error of the mean (S.E.M). The response of the aortic rings to the all compounds was expressed as a percentage of the initial contraction to 1 μM PE. Dose-response curves were analyzed by a sigmoidal curve fitting analysis to give EC_{50} values (50% of the Emax) obtained in the presence of blockers and the Emax value (the maximal effect).

4.2.3. Cell proliferation assay

A7r5, a vascular smooth muscle cell line obtained from embryonic rat aorta (American Type Culture Collection, CRL-1444, Rockville, MD, USA) were grown and kept in culture as described elsewhere [50].

For experiments A7r5 cells ($20 \times 10^3/cm^2$) were seeded in each well of 96-well culture plate. After overnight incubation, DMSO (1%) was added to the cells in the presence or absence of various concentrations of compounds **1a**–**c**, **2a**–**f**, **3a**–**f**, **4a**–**f** and **5a**–**f**. Cells without the addition of compounds were used as negative control. After further incubation for 24 h, 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyltetrazolium (MTT) assay was performed to measure cell viability [51]. Briefly, 10 μl of MTT solution (5 mg/ml in PBS) was added to each well. After incubation for 2 h at 37 °C, MTT solution was removed and 100 μl of dimethyl sulfoxide was added to dissolve the crystals formed. Then, absorbance at 540 nm was read using a microplate reader. The percentage cell viability was calculated as $[Absorbance_{(treatment)}/Absorbance_{(negative\ control)}] \times 100\%$.

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