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Vasorelaxant activity of phthalazinones and related compounds

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Abstract—Several series of dihydrostilbenamide, imidazo[2,1-*a*]isoindole, pyrimido[2,1-*a*]isoindole and phthalazinone derivatives were obtained and their vasorelaxant activity was measured on isolated rat aorta rings pre-contracted with phenylephrine (10^{-5} M). Some phthalazinones attained, practically, the total relaxation of the organ at micromolar concentrations. For the most potent compound **9h** (EC_{50} = 0.43 μM) the affinities for α_{1A}, α_{1B} and α_{1D} adrenergic sub-receptors were determined.

Hypertension is one of the most common cardiovascular diseases that can cause coronary disease, myocardial infarction, stroke and sudden death and is the major contributor to cardiac failure and renal insufficiency.[1] Because of that, great efforts are continuously being made searching for novel antihypertensive agents acting through different mechanisms.[2,3] Within the drugs in the market, hydralazine, one of the first antihypertensive agents developed in the 1950s, has attracted much attention in the last decade and it is newly considered as a lead for developing new drugs, due to its direct vasodilator action.[4] Structural modification of hydralazine led to the discovery of some pyridazinones and other phthalazine derivatives with broad spectra on the cardiovascular system,[5,6] including antihypertensive effects[7,8] and inhibition of platelet aggregation[9,10] and of phosphodiesterases.[11,12] Some of them also displayed antiasthmatic,[13,14] antipsychotic,[15] antidiabetic,[16] anticonvulsant,[17] antineoplastic,[18] antimicrobial,[19] antifungal[20] and antiparasitic[21] activities. Azelastine is a relevant member of this family of compounds that shows a fair bronchodilatory activity, useful for treating asthma,[22] and has also demonstrated to induce vasorelaxation in in vitro assays.[23]

Isonotholaenic acid (**1**) is a natural dihydrostilbenoid isolated, as the major component from CH_2Cl_2 extracts of the Andean fern *Notholaena nivea* var. *nivea*,[24] collected from the Peruvian Jurupe region. It was initially tested by us in vitro as a vasorelaxant, displaying moderate values of relaxation induction on pre-stimulated organs. Taking into account the above precedents, along with the experience of our research group on the chemistry of dihydrostilbenoids,[25,26] considering also the close structural relationship between stilbenoids and phthalazinones, we planned to obtain new antihypertensive drugs. With this aim in mind, we selected some types of stilbene-based heterocyclic compounds, including several 4-benzylphthalazinones, for testing their vasorelaxation during in vitro assays.

Isonotholaenic acid (**1**) was transformed into its amide **2** by treatment with piperidine and DCC.[25] The related heterocyclic derivatives containing two aromatic rings and an ethylene bridge were prepared (Scheme 1) through condensation of phthalic anhydride with several substituted phenylacetic acids to give the corresponding benzalphthalide[27] intermediates **3a**–**l**. These phthalides were further transformed into two series of ketostilbenamide (**4**) and diketostilbenamide (**5**) derivatives by treatment with piperidine in the presence of air and into imidazo[2,1-*a*]isoindoles (**6**) and pyrimido[2,1-*a*]isoindoles (**7**), by heating with ethylenediamine and propylenediamine, respectively. Phthalazinone derivatives of types **8**–**12** were obtained by direct condensation of the

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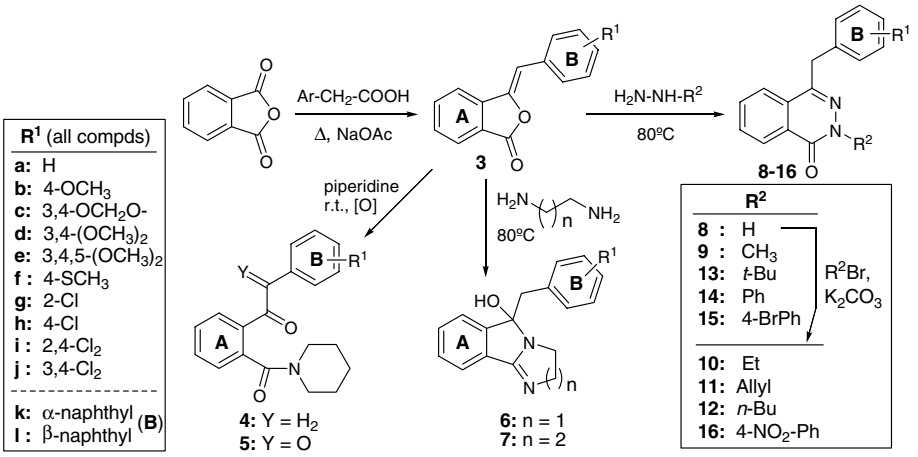
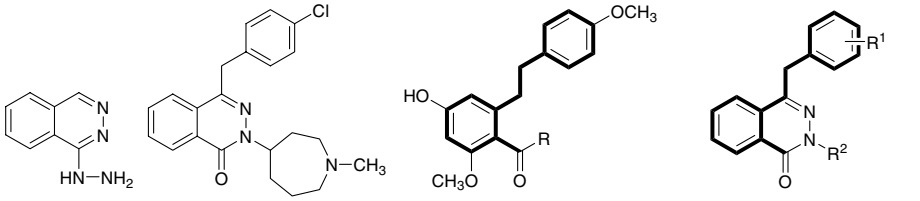


Yields range (%): Compounds Type **3**: 15-70; Types **4**, **5**: 10-60; Types **6, 7**: 10-90; Types **8**-**16**: 45-100.

Scheme 1. Phthalides, dihydrostilbenamides, imidazoisoindoles, pyrimidoisoindoles and phthalazinones tested as vasorelaxants.



benzalphthalides **3** either with hydrazine (type **8**) or with substituted hydrazines (types **9**, **13**–**15**), or through N-alkylation of compounds of type **8** with the corresponding alkyl bromide under basic conditions (types **10**–**12** and **16**). As it can be seen in Scheme 1, representative natural and nonnatural electron-donating and electron-withdrawing groups (**a**–**l**) were included as substituents, in order to determine their respective influences on the activity in every type of compound. All of the compounds were characterized by means of IR, MS, NMR and chemical analysis.[28]

The vasorelaxant activity of these compounds was evaluated in several series of assays in organ baths, on isolated rat aorta rings, previously stimulated with phenylephrine (PE).[29] PE was added to the bath up to a 10^{-5} M concentration to attain the maximal contraction of the organ. At the steady state, compounds to be tested, **1**, **2** and those of types **4**–**16**, were progressively added for a period of 30 min, to reach the 10^{-5} M or, in other cases, the 10^{-4} M concentration.

In the preliminary tests, **1** (10^{-4} M) induced a 34% relaxation[30] and its piperidide **2** increased the relaxation up to 51% under the same conditions.[30] Some 20 dihydrostilbenamides of types **4** and **5** were then tested[30,31] with similar (type **4**) or improved (type **5**) relaxation results, finding the maximum potency within both series, for the diketoderivative **5d**, displaying a 70% of induced relaxation at 10^{-5} M concentration (30% only for the monoketone analogue **4d**). In the case of imidazo- (type **6**) and pyrimidoisoindoles (type **7**), it was observed that imidazoline derivatives (**6**) were more potent than those containing the homologous tetrahydropyrimidine ring (data not shown), compound **6c** (47% of contraction inhibition at 10^{-5} M) being the best compound of both series.

Table 1. Vasorelaxant effects induced by phthalazinones of series **8** and **9** on PE pre-stimulated aorta rings

Ring B modification	Series **8** (R^2 = H)		Series **9** (R^2 = CH_3)	
	% relaxation[a] (10^{-5} M)	EC_{50} (μM)	% relaxation[a] (10^{-5} M)	EC_{50} (μM)
a	53.0 ± 7.9	⩽10	55.7 ± 7.4	⩽10
b	39.3 ± 3.1[b]	>10	61.6 ± 17.3[b]	<100
c	86.7 ± 3.6	2.9	**90.2 ± 4.1**	**0.93**
d	83.8 ± 9.4	3.7	67.7 ± 6.2	2.2
e	34.3 ± 4.9[b]	>100	16.7 ± 10.7	>10
f	66.0 ± 8.9	5.5	**87.2 ± 3.0**	**0.93**
g	76.3 ± 7.8	1.9	84.0 ± 5.7	1.4
h	**89.9 ± 4.0**	**0.79**	**99.3 ± 1.8**	**0.43**
i	61.3 ± 13.0	<10	80.7 ± 3.8	1.2
j	53.8 ± 5.7	⩽10	60.3 ± 6.4	4.9
k	79.2 ± 6.7	4.4	39.8 ± 5.1	14
l	43.9 ± 9.0	>10	44.6 ± 2.9	>10

Bold represents the most active compounds.
[a] % (± SEM) inhibition of the contraction induced by PE (10^{-5} M).
[b] Compound concentration 10^{-4} M.

The vasorelaxation results obtained for compounds of the phthalazinone series **8**–**16** were, in general, better than those observed for the above-mentioned series. They are shown in Tables 1 and 2 and expressed as percentages of the relaxation response with respect to the maximal contraction induced by 10^{-5} M PE, taken as the positive control, as well as their corresponding

Table 2. Effects of change in the N^2 substituent on the vasorelaxant activity of phthalazinones

Series: R^2	Substituent on ring B, EC_{50} (μM) values		
	3,4-$(OCH_3)_2$ (**d**)	4-SCH_3 (**f**)	4-Cl (**h**)
8: H	3.7	5.5	**0.79**
9: methyl	2.2	**0.93**	**0.43**
10: ethyl	5.3	>100	1.3
11: allyl	9.8	>100	2.6
12: *n*-butyl	5.4	>100	>100
13: *t*-butyl	10	>100	>100
14: phenyl	>100	>100	>100
15: *p*-bromophenyl	>100	>100	>30
16: *p*-nitrophenyl	>100	>100	29

Bold represents the most active compounds.

EC_{50} values. For the analysis of the results two main structural groups are being considered, namely, that of series **8**, without substitution at position N^2, and those alkylated (series **9**, **10**, **11**, **12** and **13**) or arylated (series **14**, **15** and **16**) at that position. The overall comparison of data in Table 1 for the series **8** and **9** clearly shows that N^2-methylated derivatives are some 1.1–3.2 (**9k** vs **8k**) times more potent as vasorelaxants than those unsubstituted at that position and also that the effects of B-ring substituents on the activity go in parallel for both series, with the exception of the α-naphthyl derivatives. Therefore, the SAR analysis will be mainly focused on the most potent methylated series **9**.

As it can be seen, the most potent vasorelaxants, with EC_{50} values under the μM level, are those 4-Cl (**9h**, **8h**) > 3,4-methylenedioxy (**9c**) ~ 4-SCH_3 (**9f**) derivatives, indicating a slight preference for electron-withdrawing rather than releasing groups. Change of the *p*-chloro (**h**) to the *o*-chloro (**g**) position or increasing the number of chlorine atoms (**i**, **j**) leads to a 3–10 times reduction of the relaxation. In contrast, it seems very interesting to compare the effect of the oxygenated substituents on ring B and to observe the alternated effect of this type of substituents. The presence of one 4-methoxyl group (**b**) on this ring decreases the vasorelaxant activity by almost one order of magnitude with respect to the unsubstituted derivative (**a**), while the 3,4-methylenedioxy (**c**) and the 3,4-dimethoxy (**d**) groups increase it by one order and the presence of an additional methoxy group (**e**) decreases the vasorelaxation potency of the phthalazinone. All these facts indicate that, apart from those mentioned electronic influences of the substituents, a certain spatial restriction around the 3–4–5 region of the phenyl ring should influence the activity. Compounds with naphthylmethyl groups attached to the phthalazinone nucleus instead of the benzylic groups became, in general, less potent.

In order to study the effect of the substituent at N^2 and taking into account the better activity found for the N-methylated derivatives, phthalazinones with larger 2-alkyl groups and several 2-aryl groups, in combination with three selected variations (**d**, **f** and **h**) of ring B, were synthesized (Scheme 1) and evaluated (Table 2). Data in Table 2 clearly demonstrate that none of the new modifications implemented on N^2 gave better vasorelaxation results than those found for the series **8** (hydrogen) and **9** (methyl). This will focus on the 2-methylphthalazinone family the future progression of our studies, addressed to establish the influence of ring A modifications on the vasorelaxant activity of these potential drugs.

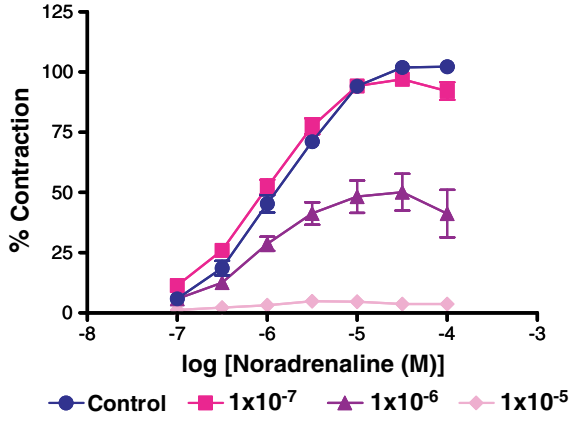


Figure 1. Contraction (%) of vas deferens induced by NA, in absence (control) and presence of **9h**.

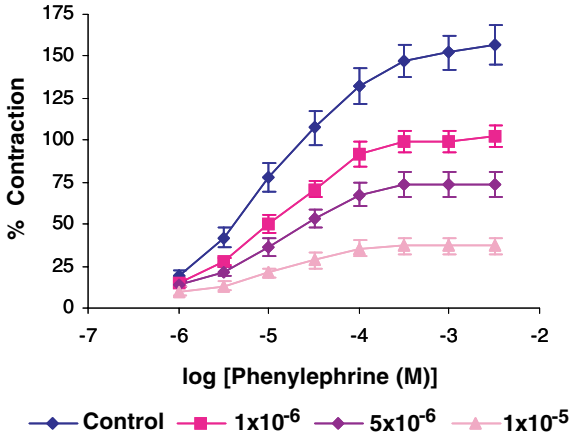


Figure 2. Contraction (%) of rat spleen tissue induced by PE, in absence (control) and presence of **9h**.

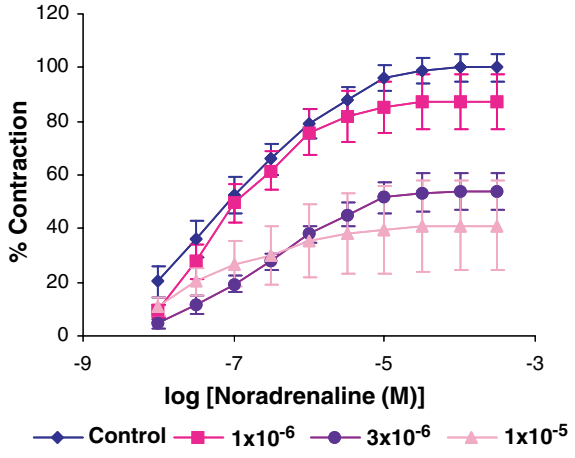


Figure 3. Contraction (%) of aorta rings induced by NA, in absence (control) and presence of **9h**.

Table 3. Contraction inhibition by several phthalazinones on rat vas deferens

Phthalazinone	R^1	R^2	Inhibitory effect[a]		EC_{50} (μM)	pD'_2
			1 μM	10 μM		
8c	3,4-O-CH_2-O-	H	24.3 ± 5.8	78.8 ± 5.3	3.0	5.5 ± 0.1
8h	4-Cl	H	16.3 ± 6.9	76.3 ± 3.6	3.9	5.4 ± 0.1
9a	H	CH_3	36.6 ± 2.9	98.8 ± 1.2	1.5	5.8 ± 0.1
9h	4-Cl	CH_3	**61.5 ± 3.2**	**95.4 ± 0.5**	**0.81**	**6.1 ± 0.1**
11h	4-Cl	Allyl	16.9 ± 3.7	70.4 ± 13.5	4.4	5.4 ± 0.1

Bold represents the most active compounds.
[a] % (± SEM) inhibition of the contraction induced by NA (10^{-5} M).

Aiming to establish the mechanism of the vasorelaxant action of phthalazinones, compound **9h** was subjected to several experiments to define its selectivity to α_1-adrenergic sub-receptors α_{1A}, α_{1B} and α_{1D} on tissues enriched in them (rat vas deferens, spleen and aorta, respectively).

Affinity to α_{1A} sub-receptor was studied on vas deferens of Wistar–Harlan rats[30] stimulated with progressive amounts of noradrenaline (NA) in absence (control) and in presence of the phthalazinone. The graphical shapes of Figure 1 suggest that compound **9h** displays an allosteric behaviour, since it binds strongly to the receptor, impeding NA to reach its maximal contraction effect. The affinity of **9h**, to the α_{1A} receptor, in this type of tissue, was calculated as $pD'_2 = 6.1 \pm 0.1$.

Affinity to α_{1B} sub-receptor was studied, similarly, on portions of rat spleen tissue,[31] stimulated with progressive amounts of PE in absence (control) and in presence of **9h** (Fig. 2). As in the first experiment, PE cannot reach the maximal contraction effect, and **9h** displayed an allosteric behaviour, with a value of $pD'_2 = 5.55$, which is too low to be considered representative.

Affinity to α_{1D} sub-receptor was studied on rat aorta rings stimulated with progressive amounts of noradrenaline (NA),[31] in absence (control) and in presence of the phthalazinone **9h**. The affinity of **9h** to the α_{1D} receptor was calculated as $pD'_2 = 5.4$, also a low value to be considered representative (Fig. 3).

The α_{1A} affinity values for other potent vasorelaxant phthalazinones, **8c**, **8h**, **9a** and **11h**, were also determined with similar results (Table 3). Compound **9h** still remaining the best compound in these assays.

From all these data it can be concluded that the most potent phthalazinone, **9h**, shows an allosteric behaviour on the three α_1-adrenergic receptors, with a greater affinity towards the α_{1A} sub-receptor. On the basis of these pharmacological results, further chemical and pharmacological research is being carried out, in order to synthesize new compounds and to analyze the influence of substituents and structural changes of ring A on the vasodilating activity. Also, further pharmacological and biochemical assays will be applied with the aim of establishing more precisely the mechanism of action of phthalazinones.

Acknowledgments

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28. Analytical data for compound **9h**. IR ν_{max}: 3068, 2920, 1650, 1587, 815, 797, 749 and 700 cm^{-1}. ^{1}H RMN (200 MHz, $CDCl_3$): δ (ppm) 8.42–7.70 (4H, *m*, H-5 to H-8); 7.26 (2H, d, $J = 8.7$ Hz, H-11 +H-15); 7.20 (2H, d, $J = 8.7$ Hz, H-12 +H-14); 4.25 (2H, s, H-9); 3.87 (3H, s, NCH_3). ^{13}C RMN (50.3 MHz, $CDCl_3$): δ 160.0, 145.1, 137.9, 132.7, 131.2, 129.4, 128.7, 128.3, 127.0, 126.7, 125.3, 39.4, 38.9 ppm. MS (CI) $m/z = 286$. Anal. Calcd for $C_{16}H_{13}N_2OCl$: C, 67.13; H, 4.50; N, 9.79. Found: C, 67.05; H, 4.52; N, 9.71.
29. Wistar rats (300–350 g) were lightly anaesthetised with ether and killed. Thoracic aortas were removed and placed in Krebs–Henseleit solution (mmol/L: NaCl, 118; KCl, 4.7; $CaCl_2$, 2.5; KH_2PO_4, 1.2; $MgSO_4$, 1.2; $NaHCO_3$, 25; glucose, 11). The solution, after saturation with carbogen (95% O_2, 5% CO_2), was adjusted to pH 7.4. After the excess fat and connective tissue were dissected out, the aorta was cut into rings (4 mm length). Rings were placed between stainless-steel hooks and set up in organ baths filled with 5 mL Krebs, gassed with carbogen and kept at 37 °C. One of the hooks was fixed to the bath and the other connected to an isometric force transducer (UF1; Harvard Apparatus Inc., South Natick, MA, USA). Force was recorded on a PC computer using Chart version 3.4 software and a PowerLab/800 data acquisition system (AD Instruments, Cibertec, Madrid, Spain). All rings were allowed to equilibrate for 1 h at a resting tension of 2*g*. The Krebs solution was periodically changed and tension was reset during this period. Then, the vessels were exposed to 10^{-5} M phenylephrine (PE) and, at the steady maximal contraction, compounds to be tested were added. Responses were measured after 30 min and given as relaxation percentages of PE contraction. EC_{50} was calculated when relaxant response was greater than 60%.
30. *Specificity to α_{1A} receptor*. Vas deferens from Wistar–Harlan–Nossan rats were quickly removed, cleaned from the connective tissue and placed in 10 mL organ bath filled with Krebs–Henseleit physiological solution, oxygenated (95% O_2–5% CO_2), heated at 37 °C ±0.5 and under a resting tension of 1*g*. After control concentration–response curve to NA was obtained, each tissue was equilibrated with a fixed concentration of test compound for at least 30 min, before determining a new concentration–response curve to the agonist. Preliminary experiments pointed out that the reproducibility of the response to NA was good. All the compounds or solvent (DMSO) tested was used in concentrations that did not alter the normal quiescent tone of the preparation (data not shown). Each experimental set has its own time-matched experimental set (control). Responses are expressed as % of the maximum effect obtained in the control curve. All the data are expressed as means ± SE.
31. *Specificity to α_{1B}- and α_{1D}-receptors.* Longitudinal strips of spleen were placed in organ baths under 1*g* tension with Krebs at 37 °C. After stabilisation, a single dose of PE (10^{-4} M) was added, then washed and the dose–response curves were constructed with two separate strips, one for control experiments and the other for compound **9h**, after 30 min incubation with the phthalazinone. Values are expressed as % with respect to the first response to PE. Aorta rings were prepared as described above.[29] After stabilisation, increasing amounts of the vasoconstrictor were added until a plateau was reached. The contractile response was expressed as percentages of the control response. The same protocol was repeated after 30 min incubation with compound **9h**.