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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and vasorelaxant and antiplatelet activities of a new series of (4-Benzylphthalazin-1-ylamino)alcohol derivatives

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Abstract A new series of phthalazine derivatives was synthesized by reaction of phthalic anhydride and different substituted phenylacetic acids to yield the benzyliden-3H-isobenzofuran-1-one intermediates **2a–d**. Treatment of them with hydrazine afforded 4-benzyl-2H-phthalazin-1-one derivatives **3a–d**, which were substituted with the corresponding aminoalkylalcohol to obtain the (4-benzylphthalazin-1-ylamino)alcohol derivatives **4a–h**. In general, these phthalazine derivatives relaxed the contractions produced by phenylephrine both in intact or endothelium-denuded aortic rings. In addition, platelet aggregation induced by thrombin was also inhibited by compounds **4c** and **4g**.

Keywords Synthesis · Phthalazine · Vasorelaxant activity · Platelet aggregation · Hypertension · Thrombus

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Introduction

Relaxation of vascular smooth muscle is one of the useful strategies for the treatment of hypertension, which is the most common cardiovascular disease. Hypertension represents the major risk factor for endothelial dysfunction, congestive heart failure, coronary artery disease and stroke (Stokes 2004; Kümmerle et al. 2009). Other factor that contributes to the development of these diseases is the occlusion of a coronary artery by a thrombus affecting the normal blood flow (Mahmood et al. 2015). Platelets play a pivotal role in hemostasis forming a plug and limiting the blood loss at sites of vascular injury; nevertheless their involvement in pathological conditions such as thrombosis and atherosclerosis is also well established, making the platelets a key target in therapies to treat cardiovascular diseases (von Hundelshausen and Schmitt 2014). Therefore, there is a continuous need to explore, search and develop new agents with both vasorelaxant and antiplatelet activity in order to reduce the risk of cardiovascular complications associated to hypertension and thrombus formation.

The phthalazine ring is the isostere and positional isomer of quinazoline ring and it is also the nucleus of well known vasodilators such as hydralazine (**I**, Fig. 1) and budralazine (**II**, Fig. 1) (Zacest et al. 1972; Ellershaw and Gurney 2001; Yoshioka et al. 1987). Therefore, nowadays the phthalazine moiety continuous being used for the synthesis of new derivatives with vasodilator effect (Awadallah et al. 2012; del Olmo et al. 2006; Vila et al. 2015). Additionally, some phthalazine derivatives have shown antiplatelet activity. 4-Phenylphthalazines bearing an amine substituent at position 1 of phthalazine ring (**III**, Fig. 1) have been described by inhibiting platelet aggregation induced by both arachidonic acid and adenosine diphosphate (Eguchi et al. 1991). Recent modifications on phthalazine ring such as the fusion of a

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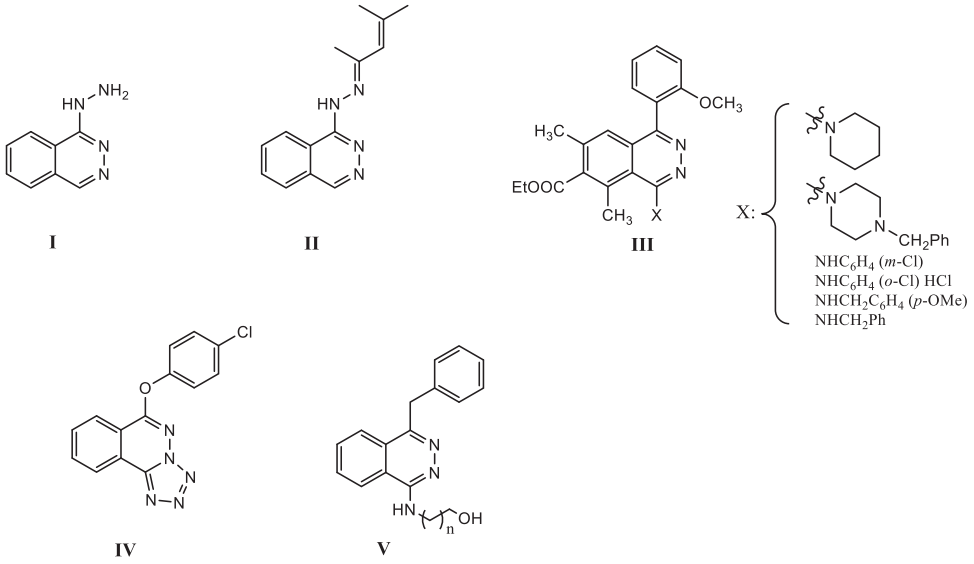


Fig. 1 Phthalazine derivatives with vasorelaxant or antiplatelet activities: hydralazine (**I**), budralazine (**II**), ethyl 1-(2-methoxyphenyl)-5,7-dimethylphthalazine-4-substituted-6-carboxylate (**III**), 6-(4-chlorophenoxy)tetrazolo [5,1-*a*]phthalazine (**IV**), (4-benzylphthalazin-1-ylamino) alcohols (**V**)

tetrazole ring at positions 1 and 2 as well as the introduction of a phenoxy group at position 4 (**IV**, Fig. 1) have lead to new derivatives showing anticoagulant and antithrombotic effects (Yu et al. 2014).

In a previous work, we have reported the vasorelaxant effects of a series of phthalazin-1,4-dione derivatives (Munín et al. 2014). Following our research and considering the background previously described, the aim of this work is the synthesis and study of the vasorelaxant and antiplatelet activities of new phthalazine derivatives bearing a benzyl ring at position 4 and a hydroxyalkylamino substituent at position 1 (**V**).

Experimental

Chemistry and pharmacology

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 dimethyl sulfoxide (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 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 followed by heating on a hot plate. Flash chromatography (FC) was performed with silica gel (60–200 mesh) under pressure. Nuclear magnetic resonance (NMR) spectra were recorded on Bruker-250 or AMX 500 spectrometers in $CDCl_3$ or DMSO-d_6 with Trimethyl silane 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.

High resolution mass spectrometry (HR-MS) and electrospray ionization mass spectrometry were carried out on a VG AutoSpec (Fision, Ipswich, United Kingdom) instrument.

*(Z)-3-(4-tert-Butylbenzyliden)-3*H*-isobenzofuran-1-one (**2d**)*

Phthalic anhydride (1.0 g, 6.75 mmol) and 2-(4-*tert*-butylphenyl)acetic acid (**1d**) (1.56 g, 8.10 mmol) were melted and subsequently KOAc (66 mg, 0.68 mmol) was added. The mixture was heated under molecular weight (MW) irradiation (350 W) for 15 min in 1 min periods, until change of color, keeping the temperature between 210 and 230 °C. Then it was extracted with ethyl acetate and washed with Na_2CO_3 10% and H_2O until pH 7. Residue was purified by FC with silica gel (35–70 mesh) using hexane/EtOAc (97:3) as eluent to give **2d** (79% yield), as a white solid, m.p. 109–110 °C. ^{1}H NMR (250 MHz, $CDCl_3$) $\delta =$ 1.34 (s, 9H, $3CH_3$), 6.41 (s, 1H, C=CH), 7.43 (d, $J = 8.6$, 2H, Ar–H), 7.53 (m, 1H, Ar–H), 7.75 (m, 4H, Ar–H), 7.92 (d, $J = 7.8$, 1H, Ar–H). ^{13}C NMR (62.9 MHz, $CDCl_3$) $\delta =$ 31.15, 34.72, 107.00, 119.69, 123.26, 125.47, 125.70, 129.50, 129.89, 130.24, 134.36, 140.63, 144.06, 151.72, 167.13. HR-MS: *m/z* 278.1304 $[M]^{+\bullet}$; calcd for $C_{19}H_{18}O_2$, 278.1307.

*4-(4-Methylbenzyl)-2*H*-phthalazin-1-one (**3b**)*

3-(4-Methylbenzyliden)-3*H*-isobenzofuran-1-one (**2b**) (1.0 g, 4.2 mmol) was dissolved in hydrazine 1M in THF (8.5 mL, 8.50 mmol). The mixture was stirred for 2 h at room

temperature following for 4 h at 60 °C, in a sealed tube. The mixture was cooled to room temperature and evaporated to dryness. The resulting solid was purified by crystallization in EtOAc, to give **3b** (98% yield), as a white solid, m.p. 203–204 °C. ^{1}H NMR (250 MHz, $CDCl_3$) $\delta = 2.29$ (s, 3H, CH_3), 4.28 (s, 2H, CH_2–Ar), 7.10 (d, $J = 8.0$, 2H, Ar–H), 7.18 (d, $J = 8.0$, 2H, Ar–H), 7.74 (m, 3H, Ar–H), 8.48 (d, $J = 7.6$, 1H, Ar–H), 11.59 (bs, 1H, NH). ^{13}C NMR (62.9 MHz, $CDCl_3$) $\delta = 20.97$, 38.45, 125.38, 126.91, 128.22, 128.31 (2C), 129.36 (2C), 129.79, 131.19, 133.36, 134.52, 136.28, 146.58, 161.00. HR-MS: *m/z* 250.1114 $[M]^{+\bullet}$; calcd for $C_{16}H_{14}N_2O$: 250.1106.

*4-(4-Isopropylbenzyl)-2H-phthalazin-1-one (**3c**)*

The compound **3c** was obtained from **2c** and hydrazine 1M in THF, following the previously described procedure to obtain **3b** (0.52 g, 98%) as a white solid; m.p. 197–198 °C. ^{1}H NMR (250 MHz, $CDCl_3$) $\delta = 1.21$ (d, $J = 7.5$, 6H, $2CH_3$), 2.86 (m, 1H, $(CH_3)_2CH$), 4.28 (s, 2H, CH_2–Ar), 7.18 (d, $J = 8.2$, 2H, Ar–H), 7.22 (d, $J = 8.2$, 2H, Ar–H), 7.76 (m, 3H, Ar–H), 8.47 (d, $J = 7.2$, 1H, Ar–H), 10.93 (bs, 1H, NH). ^{13}C NMR (62.9 MHz, $CDCl_3$) $\delta = 23.93$ (2C), 33.64, 38.43, 125.51, 126.77 (2C), 126.95, 128.30, 128.38 (2C), 129.88, 131.28, 133.44, 134.82, 146.51, 147.35, 160.65. HR-MS: *m/z* 278.1417 $[M]^{+\bullet}$; calcd for $C_{18}H_{18}N_2O$: 278.1419.

*4-(4-tert-Butylbenzyl)-2H-phthalazin-1-one (**3d**)*

The compound **3d** was obtained from **2d** and hydrazine 1M in THF following the previously described procedure to obtain **3b** (99%), as a white solid, m.p. 218–219 °C. ^{1}H NMR (250 MHz, $CDCl_3$) $\delta = 1.28$ (s, 9H, $3CH_3$), 4.29 (s, 2H, CH_2–Ar), 7.23 (d, $J = 8.5$, 2H, Ar–H), 7.31 (d, $J = 8.5$, 2H, Ar–H), 7.76 (m, 3H, Ar–H), 8.50 (d, $J = 7.5$, 1H, Ar–H), 11.50 (bs, 1H, NH). ^{13}C NMR (62.9 MHz, $CDCl_3$) $\delta = 31.28$ (3C), 34.36, 38.30, 125.49, 125.59 (2C), 126.92, 128.09 (2C), 128.24, 129.88, 131.21, 133.39, 134.53, 146.59, 149.55, 160.96. HR-MS: *m/z* 292.1572 $[M]^{+\bullet}$; calcd for $C_{19}H_{20}N_2O$: 292.1576.

*2-(4-(4-Methylbenzyl)phthalazin-1-ylamino)ethanol (**4b**)*

4-(4-Methylbenzyl)-2H-phthalazin-1-one (**3b**) (0.30 g, 1.20 mmol) was dissolved in 2-aminoethanol (0.70 mL, 12.00 mmol) and refluxed for 48 h. The mixture was cooled to room temperature, CH_2Cl_2 (100 mL) was added and washed with H_2O and brine. Organic phase was dried over Na_2SO_4, filtered and evaporated to dryness. The resulting solid was purified by crystallization using CH_3CN, to give **4b** (0.25 g, 72%), as a white solid, m.p. 201–202 °C. ^{1}H NMR (250 MHz, DMSO-d_6) $\delta = 2.18$ (s, 3H, CH_3), 3.66 (m, 4H, CH_2–CH_2), 4.37 (s, 2H, CH_2–Ar), 4.95 (bs, 1H, OH), 7.02 (d, $J = 8.0$, 2H, Ar–H), 7.14 (d, $J = 8.0$, 2H, Ar–H), 7.37 (bs, 1H, NH), 7.76 (m, 2H, Ar–H), 7.92 (d, $J = 7.0$, 1H, Ar–H), 8.28 (d, $J = 7.0$, 1H, Ar–H). ^{13}C NMR (62.9 MHz, DMSO-d_6) $\delta = 20.56$, 38.11, 43.98, 59.76, 118.34, 122.45, 124.78, 125.68, 128.23 (2C), 128.97 (2C), 130.77, 131.38, 135.05, 136.61, 150.04, 153.46. HR-MS (ESI): *m/z* 294.1590 (M^+H^+); calcd for $C_{18}H_{20}N_3O$: 294.1601.

*2-(4-(4-Isopropylbenzyl)phthalazin-1-ylamino)ethanol (**4c**)*

The compound **4c** was obtained from **3c** and 2-aminoethanol following the previously described procedure to obtain **4b** (98%), as a white solid, m.p. 137–138 °C. ^{1}H NMR (250 MHz, DMSO-d_6) $\delta = 1.11$ (d, $J = 7.0$, 6H, $2CH_3$, 2.76 (m, 1H, $(CH_3)_2CH$), 3.67 (m, 4H, CH_2–CH_2), 4.38 (s, 2H, CH_2–Ar), 5.0 (bs, 1H, OH), 7.08 (d, $J = 8.1$, 2H, Ar–H), 7.19 (d, $J = 8.1$, 2H, Ar–H), 7.38 (bs, 1H, NH), 7.79 (m, 2H, Ar–H), 7.99 (d, $J = 7.6$, 1H, Ar–H), 8.26 (d, $J = 7.9$, 1H, Ar–H). ^{13}C NMR (62.9 MHz, DMSO-d_6) $\delta =$ 23.90 (2C), 33.01, 38.02, 44.00, 59.77, 118.36, 122.50, 124.81, 125.74, 126.31 (2C), 128.33 (2C), 130.83, 131.49, 136.99, 146.11, 150.11, 153.47. HR-MS (ESI): *m/z* 322.1904 (M^+H^+); calcd for $C_{20}H_{24}N_3O$: 322.1913

*2-(4-(4-tert-Butylbenzyl)phthalazin-1-ylamino)ethanol (**4d**)*

The compound **4d** was obtained from **3d** and 2-aminoethanol following the previously described procedure to obtain **4b** (83%), as a white solid, m.p. 202–203 °C. ^{1}H NMR (250 MHz, DMSO-d_6) $\delta = 1.19$ (s, 9H, $3CH_3$), 3.64 (m, 4H, CH_2–CH_2), 4.38 (s, 2H, CH_2–Ar), 4.96 (bs, 1H, OH), 7.19 (d, $J = 8.4$, 2H, Ar–H), 7.24 (d, $J = 8.4$, 2H, Ar–H), 7.38 (bs, 1H, NH), 7.79 (m, 2H, Ar–H), 7.99 (d, $J = 9.3$, 1H, Ar–H), 8.26 (d, $J = 8.9$, 1H, Ar–H). ^{13}C NMR (62.9 MHz, DMSO-d_6) $\delta = 31.14$ (3C), 34.06, 37.88, 43.99, 59.76, 118.34, 122.49, 124.80, 125.15 (2C), 125.74, 128.07 (2C), 130.83, 131.49, 136.59, 148.35, 150.09, 153.45. HR-MS (ESI): *m/z* 336.2000 (M^+H^+); calcd for $C_{21}H_{26}N_3O$: 336.2070.

*3-(4-(4-Methylbenzyl)phthalazin-1-ylamino)propan-1-ol (**4f**)*

4-(4-Methylbenzyl)-2H-phthalazin-1-one (**3b**) (0.50 g, 2.00 mmol) was dissolved in 3-aminopropan-1-ol (1.50 mL, 20.00 mmol) and refluxed for 48 h. The mixture was cooled to room temperature, CH_2Cl_2 (100 mL) was added and washed with H_2O and brine. Organic phase was dried over Na_2SO_4, filtered and evaporated to dryness. The resulting solid was purified by crystallization using CH_3CN, to give **4f** (0.45 g, 74%), as a white solid, m.p. 167–168 °C. ^{1}H

NMR (250 MHz, DMSO-d_6) $\delta = 1.85$ (m, 2H, CH_2–CH_2–CH_2), 2.18 (s, 3H, CH_3), 3.52 (t, $J = 6.3$, 2H, CH_2–N), 3.61 (t, $J = 6.4$, 2H, CH_2–O), 4.37 (s, 2H, CH_2–Ar), 4.72 (bs, 1H, OH), 7.02 (d, $J = 8.0$, 2H, Ar–H), 7.14 (d, $J = 8.0$, 2H, Ar–H), 7.34 (bs, 1H, NH), 7.76 (m, 2H, Ar–H), 7.91 (d, $J = 7.2$, 1H, Ar–H), 8.24 (d, $J = 7.1$, 1H, Ar–H). ^{13}C NMR (62.9 MHz, DMSO-d_6) $\delta = 20.57$, 32.06, 38.12, 38.27, 59.78, 118.33, 122.37, 124.79, 125.66, 128.24 (2C), 128.98 (2C), 130.76, 131.34, 135.06, 136.63, 149.89, 153.43. HR-MS (ESI): *m/z* 308.1749 (M^+H^+); calcd for $C_{19}H_{22}N_3O$: 308.1757.

*3-(4-(4-Isopropylbenzyl)phthalazin-1-ylamino)propan-1-ol (**4g**)*

The compound **4g** was obtained from **3c** and 3-aminopropan-1-ol following the previously described procedure to obtain **4f** (95%), as a white solid, m.p. 109–110 °C. ^{1}H NMR (250 MHz, DMSO-d_6) $\delta = 1.10$ (d, $J = 6.9$, 6H, $2CH_3$), 1.83 (m, 2H, CH_2–CH_2–CH_2), 2.76 (m, 1H, $(CH_3)_2CH$), 3.52 (t, $J = 6.2$, 2H, CH_2–N), 3.60 (t, $J = 6.1$, 2H, CH_2–O), 4.38 (s, 2H, CH_2–Ar), 4.8 (bs, 1H, OH), 7.08 (d, $J = 8.2$, 2H, Ar–H), 7.19 (d, $J = 8.2$, 2H, Ar–H), 7.36 (bs, 1H, NH), 7.77 (m, 2H, Ar–H), 7.98 (d, $J = 9.4$, 1H, Ar–H), 8.23 (d, $J = 9.4$, 1H, Ar–H). ^{13}C NMR (62.9 MHz, DMSO-d_6) $\delta = 23.90$ (2C), 32.07, 33.00, 38.02, 38.28, 58.79, 118.34, 122.42, 124.80, 125.71, 126.31 (2C), 128.33 (2C), 130.82, 131.44, 137.00, 146.11, 149.93, 153.42. HR-MS (ESI): *m/z* 336.2000 (M^+H^+); calcd for $C_{21}H_{26}N_3O$: 336.2070.

*3-(4-(4-tert-Butylbenzyl)phthalazin-1-ylamino)propan-1-ol (**4h**)*

The compound **4h** was obtained from **3d** and 3-aminopropan-1-ol following the previously described procedure to obtain **4f** (82%), as a white solid, m.p. 130–131 °C. ^{1}H NMR (250 MHz, DMSO-d_6) $\delta = 1.18$ (s, 9H, $3CH_3$), 1.84 (m, 2H, CH_2–CH_2–CH_2), 3.52 (t, $J = 6.2$, 2H, CH_2–N), 3.60 (t, $J = 6.2$, 2H, CH_2–O), 4.38 (s, 2H, CH_2–Ar), 4.78 (bs, 1H, OH), 7.19 (d, $J = 8.5$, 2H, Ar–H), 7.24 (d, $J = 8.5$, 2H, Ar–H), 7.36 (sa, 1H, NH), 7.78 (m, 2H, Ar–H), 7.99 (d, $J = 9.3$, 1H, Ar–H), 8.25 (d, $J = 9.3$, 1H, Ar–H). ^{13}C NMR (62.9 MHz, DMSO-d_6) $\delta = 31.15$ (3C), 32.07, 34.07, 37.90, 38.29, 58.79, 118.35, 122.43, 124.82, 125.16 (2C), 125.72, 128.09 (2C), 130.84, 131.48, 136.60, 148.36, 149.94, 153.41. HR-MS (ESI): *m/z* 350.2215 (M^+H^+); calcd for $C_{22}H_{28}N_3O$: 350.2226.

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 (Orallo et al. 2002).

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). The experimental protocols were approved by the Bioethics Committee of the University of Santiago de Compostela (Spain) and the Bioethics Committee for Research (CEIC) of the Xunta de Galicia (Spain).

Vasorelaxant activity

Vasorelaxant activity of newly synthesized compounds **4a–h** was studied using thoracic aortic rings of WKY rats pre-contracted with phenylephrine (PE) (1 μM). Male WKY 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 $CaCl_2$ • $2H_2O$, 1.2 mM $MgSO_4$ • $7H_2O$, 25 mM $NaHCO_3$, 0.03 mM EDTA-Na_2, 11 mM glucose; pH 7.4). Excess of fat and connective tissue was removed. For the endothelium free experiments a scraping of the arterial lumen with a thick cotton thread was made. 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 acethylcholine (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 pre-contracted 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 **4a–h** were added in progressively increasing cumulative concentrations (1–200 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 solvent did not affect the contractile response of isolated aorta.

All the results are expressed as means ± the standard error of the mean. 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 EC50 values (50% of the Emax) obtained in the presence of the tested compounds and the Emax value (the maximal effect).

Antiplatelet activity

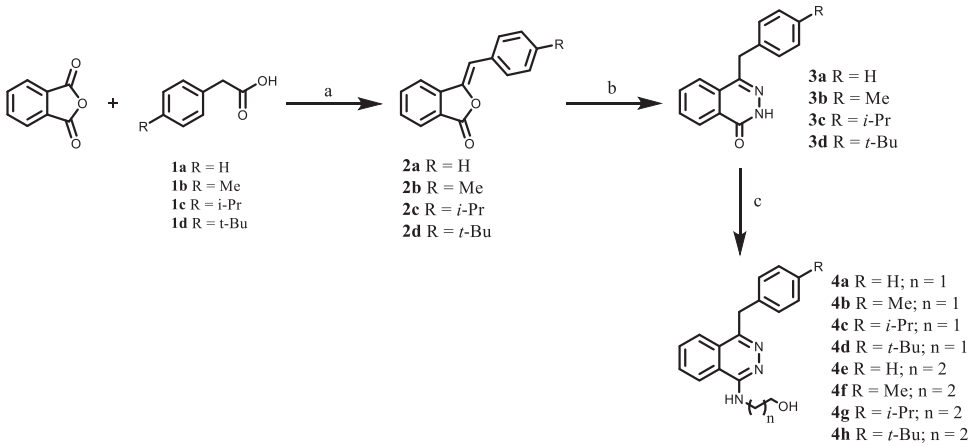
Preparation of washed platelets Washed human platelets were prepared from blood anticoagulated with citrate-phosphate-dextrose, which was obtained from Centro de Transfusion de Galicia (Santiago de Compostela, Spain). Bags containing buffy coat from individuals donors were diluted with the same volume of washing buffer (NaCl, 120 mM; KCl, 5 Mm; trisodium citrate, 12 mM; glucose, 10 mM; sucrose, 12.5 mM; pH = 6) and centrifuged at 400×*g* for 8 min. The upper layer containing platelet (platelet-rich plasma) was removed and centrifuged at 1100×*g* for 18 min. The resulting platelet pellet was recovered, resuspended with washing buffer, and centrifuged again at 1100×*g* for 15 min. Finally, the platelet pellet from this step was resuspended in a modified Tyrode-HEPES buffer (HEPES, 10 mM; NaCl, 140 mM; KCl, 3 mM; $MgCl_2$, 0.5 mM; $NaHCO_3$, 5 mM; glucose, 10 mM; pH 7.4) to afford a cell density of $2.5–3.5 \times 10^8$ platelet/ml. The calcium concentration in the extracellular medium was adjusted to 2 mM by the addition of the appropriate amount of $CaCl_2$.

Platelet aggregation studies Platelet aggregation was measured using a dual channel aggregometer (Chrono-log, Havertown, PA, USA). Each tested compound, dissolved in DMSO, was incubated with washed platelets at 37 °C for 5 min. A platelet activator (thrombin) was then added to induce platelet aggregation and the light transmission was monitored over 5 min period. Platelet aggregation is measured as the maximum change in light transmission during this period. The 100% aggregation value was obtained when vehicle (DMSO) was added instead of the compounds. The final DMSO concentration was below 1% (v/v) in all cases.

Results and discussion

Chemistry

A series of differently substituted (4-benzylphthalazin-1-ylamino)alcohols were synthesized in good yields. Reaction under microwave irradiation of phthalic anhydride and different substituted phenylacetic acids **1a–d** yielded the corresponding benzyliden-3*H*-isobenzofuran-1-ones **2a–d** (Viña et al. 2009; Munín et al. 2015; Kumar et al. 2013; Hjelmencrant et al. 2000). These benzyliden-3*H*-isobenzofuran-1-one derivatives were further transformed into 4-benzyl-2*H*-phthalazin-1-one derivatives **3a–d** (Munín et al. 2015) by treatment with hydrazine in THF (del Olmo et al. 2006; Viña et al. 2009). Finally, reaction under reflux of **3a–d** derivatives with an excess of either 2-aminoethan-1-ol or 3-aminopropan-1-ol lead to the desired (4-benzylphthalazin-1-ylamino)alcohol derivatives **4a–h** (Scheme 1) (Munín et al. 2015).



Scheme 1 General synthetic route to obtain compounds **4a–h**. Reagents and conditions: **a** AcOK, MW (350W), 210–230 °C; **b** H_2NNH_2 1M THF, rt to 60 °C; **c** $H_2NCH_2CH_2OH$ or $H_2NCH_2CH_2CH_2OH$, reflux

Table 1 Vasorelaxant activity of (4-benzylphthalazin-1-ylamino) alcohol derivatives in rat aortic rings pre-contracted with PE (1 μM)

Compound	EC_{50} (μM) with endothelium	EC_{50} (μM) without endothelium
4a	26.41 ± 4.29	59.54 ± 4.82**
4b	31.35 ± 2.65	48.79 ± 5.42**
4c	16.41 ± 3.35	21.89 ± 4.75
4d	43.34 ± 4.62	a
4e	15.02 ± 3.05	63.23 ± 1.10*
4f	23.09 ± 4.34	44.34 ± 6.59**
4g	21.47 ± 2.71	23.92 ± 2.27
4h	b	a
Hydralazine	$(1.18 \pm 0.09) \times 10^3$	$(1.32 \pm 0.12) \times 10^3$
Amrinone[c]	17 ± 6	28 ± 8*

*$P < 0.01$

**$P < 0.05$ vs. ring with endothelium as determined by ANOVA/Dunnet's test. All reported *EC*50 values are the mean of at least five experiments

[a] Inactive at 200 μM (highest concentration tested)

[b] 100 μM produced relaxation about 50%

[c] Mori et al. (1996)

Pharmacology

The vasorelaxant effects of compounds **4a–h** were studied using isolated rat thoracic aortic rings pre-contracted with PE following a standard procedure previously reported by us (Cuíñas et al. 2013; Quezada et al. 2010) and compared to the reference drugs hydralazine and amrinone (Mori et al. 1996). The cumulative addition of the new compounds (1–200 μM) caused a sustained concentration-dependent relaxation of the contractions induced by PE (1 μM) in intact rat aortic rings. In the absence of endothelium, this vasorelaxant effect decreases significantly, except for the compounds **4c** and **4g** which showed nearly the same relaxing activity in both cases. Both derivatives contain in their structure an isopropyl group at the *para* position of 4-benzyl ring, which seems determinant to show independent endothelium activity. However, when this isopropyl group was replaced by a *tert*-butyl group in the same position, these derivatives **4d** and **4h**, lack vasorelaxant activity in the absence of endothelium at the highest concentration tested (200 μM). Most of these derivatives present a vasorelaxant effect in intact aortic rings similar to the amrinone and they are much more potent vasorelaxant agents than hydralazine in the in vitro studies. The described effects seem to be reversible because a 60 min washout period allows a progressive recovery of the functionality of muscle and endothelial cells of the rings. The corresponding EC_{50} values are shown in Table 1.

Platelet aggregation studies were also performed about (4-benzylphthalazin-1-ylamino)alcohol derivatives. Only the compounds **4c** and **4g** inhibited platelet aggregation induced by thrombin (0.25 U/ml) (Table 2). This suggests that both the substitution at *para* position of the 4-benzyl ring as its nature is responsible for the antiplatelet activity. Also the alkyl chain length at 1 position is important to inhibitory activity, because the compound **4g** bearing a propyl chain is more than three times more potent than the compound **4c** which links an ethyl chain.

Table 2 Antiplatelet activity of (4-benzylphthalazin-1-ylamino) alcohol derivatives using thrombin (0.25 U/ml) as stimulating agent for aggregation

Compound	IC_{50} (μM)
4a	*
4b	*
4c	110.6 ± 5.4
4d	**
4e	*
4f	*
4g	31.59 ± 1.88
4h	**
Milrinone	4.70 ± 0.50

*Inactive at 200 μM (highest concentration tested). All reported IC_{50} values are the mean of at least five experiments employing human blood from different individuals

**At 200 μM, platelet aggregation was produced by the compounds

Conclusion

A new series of (4-benzylphthalazin-1-ylamino)alcohol derivatives has been synthesized in good yields. All the compounds have been found to cause a concentration-dependent relaxation of aortic rings pre-contracted with PE in the μM range in the presence of an intact endothelium. However, this relaxant effect was significantly reduced after removal of the endothelium layer, except for compounds **4c** and **4g**. Both compounds bearing an isopropyl group at the *para* position of benzyl ring. Moreover, only these two compounds also showed antiplatelet activity against thrombin, being compound **4g** the most potent derivative in this series.

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Conflict of interest The authors declare that they have no competing interests.

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