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Original article

Synthesis, vasorelaxant activity, and molecular modeling study of some new phthalazine derivatives

Fadi M. Awadallah [a,*], Wafaa I. El-Eraky [b], Dalia O. Saleh [b]

[a] Pharmaceutical Chemistry department, Faculty of Pharmacy, Cairo University, 11562 Cairo, Egypt
[b] Pharmacology Department, National Research centre, 12622 Cairo, Egypt

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ABSTRACT

New phthalazine-based vasodilators were synthesized through the chloroacylation of the starting compound 1-hydrazinophthalazine **4** to give the two key intermediates **5** and **7**. These intermediates were used to alkylate various cyclic amines to furnish the final compounds **6a**–**h** and **8a**–**h**. Compounds were tested for their vasorelaxant activities against nor-adrenaline-induced spasm on thoracic rat aorta rings and compared to the reference drug, prazosin. Seven compounds showed higher activity than prazosin, especially compound **8d** having an IC_{50} = 0.10 mM. Molecular modeling studies, including fitting of the synthesized compounds to a 3D-pharmacophore and their docking into optimized homology model as α_1-AR antagonists showed high docking score and fit values. Most vasodilation activities of tested compounds are consistent with their molecular modeling results.

1. Introduction

Hypertension is the most common cardiovascular disease that represents the major risk factor for endothelial dysfunction, metabolic syndrome, renal dysfunction, congestive heart failure, coronary artery disease, and stroke [1]. Hypertension affects approximately billions of people all around the world. This is despite the great effort and progress in developing new drugs targeting the various mechanisms of hypertension [2].

Relaxation of vascular smooth muscles is one of the strategies for the treatment of hypertension [3]. Several agents have been developed; however they are all associated with side effects such as fatigue, mood change, sleep disturbances, etc [4]. Therefore, there is a continuous need to explore, search and develop new vasorelaxant agents with minimal side effects.

The α_1-adrenergic receptors (α_1-ARs), a family of G-protein coupled seven-transmembrane helix receptors, are mainly involved in the cardiovascular and central nervous system [5]. In the last two decades, the search for new selective α_1-AR antagonists has been intensified, mainly due to their importance in the treatment of hypertension, asthma, lower urinary tract symptoms (LUTS) and benign prostatic hypertrophy (BPH) [6–9]. Research efforts in the area of α_1-AR antagonists have led to the discovery of some clinically useful antihypertensive drugs such as prazosin **I**, terazosin **II** and doxazosin **III** that are considered the most effective and clinically useful class of selective α_1-AR antagonists (Fig. 1) [9–11].

In addition, 3-{2-[4-(2-methoxyphenyl)piperazin-1-yl]ethyl}-(1*H*,3*H*)-quinazoline-2,4-dione **V** (SGB 1534) proved effective as a potent α_1-antagonist [12]. Furthermore, certain anthranilides – the non-cyclic isosteres of quinazolines – linked to 4-arylpiperazines via propionyl spacer such as **VI** were reported to exhibit α_1-AR antagonist activity [13]. Recently, quinazolines linked to arylpiperazines through an acetamido or propionamido spacers **VII** have been designed and exhibited potent vasorelaxant activity (Fig. 2) [14].

Based on these findings and on previous reports that proclaimed that heterocyclic systems (A) linked to different arylpiperazine tails (C) through polymethylene spacer (B) is a key element for α_1-AR affinity [5], it was aimed to design and synthesize potential vasorelaxant agents having arylpiperazine moieties and other related non-classical bioisosteric groups linked to a phthalazine nucleus (Fig. 2). The phthalazine ring is the isostere and positional isomer of quinazoline ring and, it is also the nucleus of the well known vasodilator, hydralazine **IV** (Fig. 1) [15]. In other words, a hybrid pharmacophoric approach was adopted whereby the hydralazine structure was hybridized with different arylpiperazines and other related groups. Different N-(un)substituted phenyl–piperazines, N-

* Corresponding author. Tel.: +2 01223483941; fax: +20 2 2362 8426.
E-mail address: fadi_mae@hotmail.com (F.M. Awadallah).

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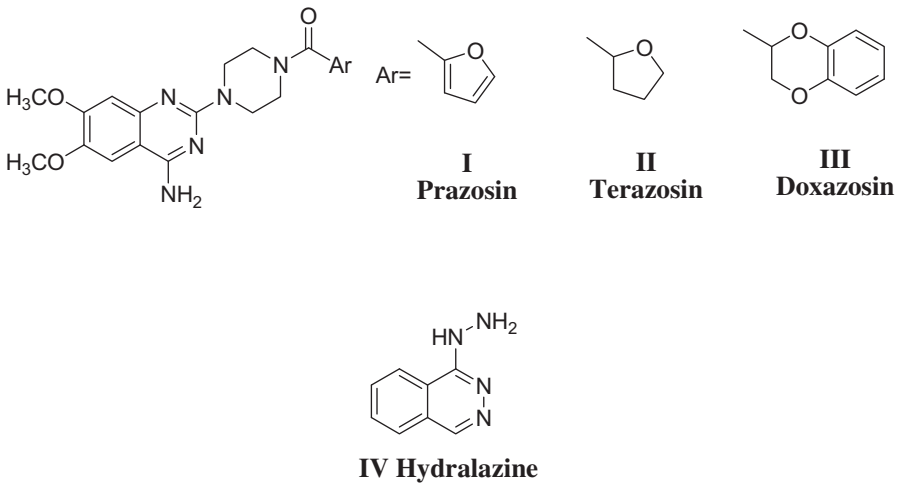


Fig. 1. Clinically used α_1-ARs antagonists **I**–**III** and hydralazine **IV**.

small groups substituted piperazines, the isosteric morpholine and the smaller aromatic imidazole ring were employed to study the effect of these moieties on the activity of the compounds. Moreover, Betti et al. reported the gradual increase in affinity to α_1-AR by increasing the length of the polymethene spacer between the arylpiperazine moiety and the pyridazinone nucleus from 2 up to 7 carbons [16]. Therefore, the second approach based on the previous experimental finding was to subject the ethylene spacer in the lead compound **V** to elongation using acetohydrazido and propionohydrazido linkers (Fig. 2.).

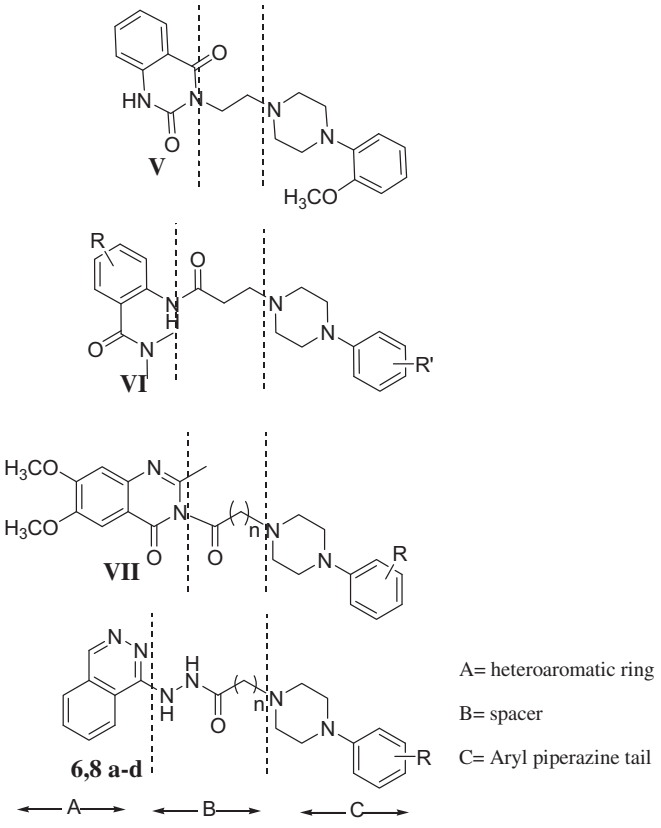


Fig. 2. Structure of lead compounds **V**–**VII** and strategy of the design of the target compounds **6**,**8a**–**d**.

Moreover, validation of the observed pharmacological data was performed through studying the relationship between the chemical features of the synthesized compounds and their α_1-binding affinity data which were derived on the basis of a previously reported, five features pharmacophore model for α_1-AR antagonists which consisted of positive ionizable, three hydrophobic and a hydrogen bond acceptor pharmacophore features [17].

2. Results and discussion

2.1. Chemistry

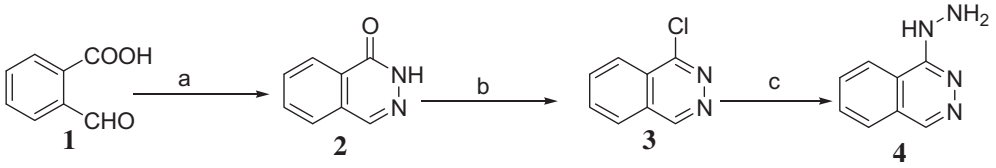
The target compounds **6a**–**h** and **8a**–**h** were prepared as depicted in Schemes 1 and 2. The key starting compound, 1-hydrazinophthalazine **4**, was prepared according to the published procedures starting from the commercially available 2-carboxybenzaldehyde **1** which upon reaction with hydrazine hydrate gave phthalazin-1(2*H*)-one **2**. Chlorination of **2** with phosphorous oxychloride resulted in 1-chlorophthalazine **3** which was converted to the corresponding 1-hydrazino derivative **4** upon treatment with hydrazine hydrate (Scheme 1) [18,19].

The intermediates 2-chloro-*N*′-(phthalazin-1-yl)acetohydrazide **5** and 3-chloro-*N*′-(phthalazin-1-yl)propanehydrazide **7** were prepared from 1-hydrazinophthalazine **4** through acylation reaction with chloroacetyl chloride and 3-chloropropionyl chloride, respectively, in dry methylene chloride (Scheme 2). Evidence for the reaction was drawn from the ^{1}H NMR spectrum of **5** that revealed the methylene protons at 5.20 ppm and that of **7** that showed the ethylene protons as two triplets at 2.62 and 3.78 ppm.

The final compounds **6a**–**h** and **8a**–**h** were synthesized through alkylation of the appropriate amine with the chloro-acetohydrazide **5** and the chloro-propanehydrazide **7**, respectively, in dry acetonitrile in the presence of catalytic amount of triethylamine (Scheme 2). The synthesized compounds were characterized by ^{1}H NMR analyses that showed signals corresponding to the introduced amine moieties. Other spectral and elemental analyses were also performed for additional characterization of the compounds.

2.2. Vasorelaxant activity

All final compounds **6a**–**h** and **8a**–**h** were tested for their vasorelaxant activities against nor-adrenaline-induced spasm on thoracic rat aorta rings [20–23] and compared to the reference drug, prazosin. The results were listed in Table 1 and illustrated in



Scheme 1. Reagents and reaction conditions. a: $NH_2NH_2 \cdot H_2O$, 1 h.; b: $POCl_3$, 2 min; c: $NH_2NH_2 \cdot H_2O$, ethanol, 1 h.

Fig. 3 as IC_{50} values in mM. Regarding the acetohydrazide derivatives, **6a**–**h**, the compounds with arylpiperazine substituents showed good activities comparable to prazosin, especially those which had an electron withdrawing groups on the phenyl ring such as compounds **6b** (IC_{50} 0.45 mM) and **6c** (IC_{50} 0.41 mM) which were more active than **6a**, **6d** and were only slightly more active than prazosin (IC_{50} 0.48 mM). Replacement of the aryl substitution on the piperazine ring with the small methyl group as in **6e** greatly enhanced the activity (IC_{50} 0.16 mM). This compound is the most active compound of this series and it is three times as potent as prazosin. On the other hand, introduction of an *N*-ethoxycarbonyl group on the piperazine ring negatively affected the activity as compound **6f** was the least active in this series ($IC_{50} > 1.00$ mM). Substitution of the bioisosteric morpholine group for the piperazine ring, in compound **6g**, retained good activity (IC_{50} 0.43 mM) being slightly better than prazosin. Finally, decline in activity was observed when the aromatic imidazole ring was introduced, compound **6h** (IC_{50} 0.61 mM).

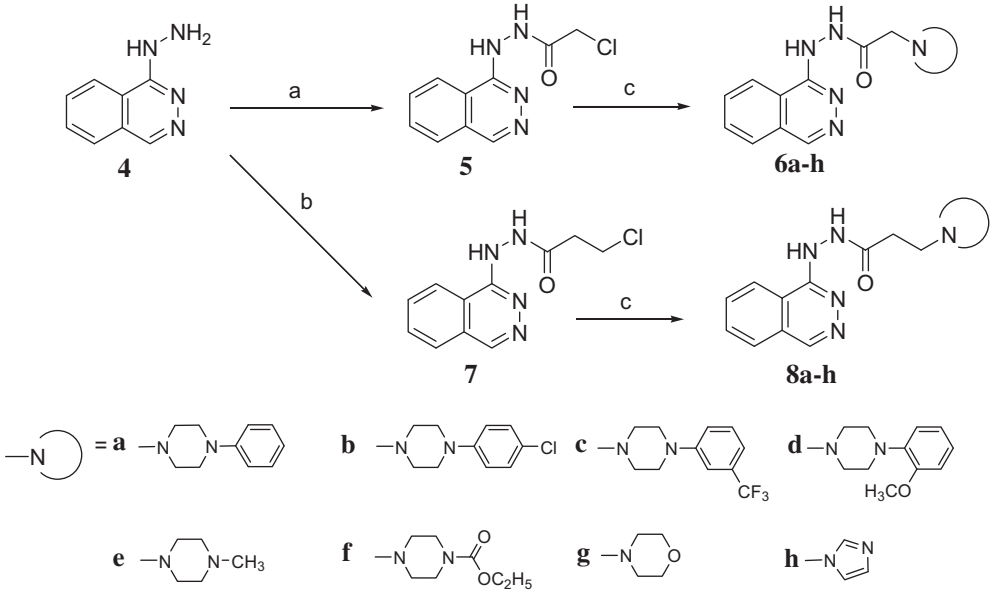
As for the propanehydrazide series **8a**–**h**, the activity of the arylpiperazinyl derivatives **8a**–**d**, unlike their respective analogs **6a**–**d**, showed inverse relationship with the electron withdrawing property of the substituent on the phenyl ring where compounds **8b** (IC_{50} 0.70 mM) and **8c** (IC_{50} 0.50 mM) were less active than both the unsubstituted derivative **8a** (IC_{50} 0.27 mM) and the 2-methoxyphenylpiperazinyl derivative **8d** (IC_{50} 0.10 mM) (c.f. **6a**–**d**). It is worth mentioned that compound **8d** is approximately 5 times more potent than prazosin and it is the most active of all tested compounds. The *N*-methylpiperazinyl derivative **8e** exhibited better activity (IC_{50} 0.36 mM) than prazosin. The morpholine derivative **8g** possessed moderate activity (IC_{50} 0.58 mM) being slightly lower than prazosin. Finally, compound **8f** with an *N*-ethoxycarbonyl substituent ($IC_{50} > 1.00$ mM) and compound **8h** with the imidazolyl substituent (IC_{50} 0.81 mM) were the least active of this series.

With respect to the spacer between the phthalazine ring and the amine moiety, results indicated that increasing the length of the spacer from acetohydrazido in compounds **6b**, **6c**, **6e**, **6g** and **6h** to propanehydrazido in **8b**, **8c**, **8e**, **8g** and **8h** was associated with decrease in activity. The opposite was true for compounds **8a** and **8d** which were more active than their respective analogs.

In summary, the results of the in-vitro vasorelaxant activity testing indicated that the presence of a piperazine group bearing an aryl or a small alkyl group was befitting to good activity. Also, the bioisosteric morpholine group is a good substitute for the piperazine ring. In case of the arylpiperazine derivatives, the vasorelaxant activity was not in a consistent relation with the nature of the substituent on the phenyl ring. On the other hand, incorporation of a piperazine ring substituted with an ethoxycarbonyl group markedly decreased the activity and the same held true when the piperazine ring was replaced by the smaller imidazole group. However, no precise rule could be attained through the screening results concerning the role of the length of the linker group (either acetohydrazido or propanehydrazido) in affecting the observed pharmacological properties.

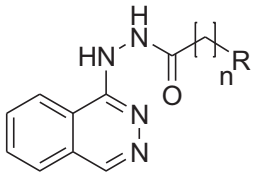
2.3. Molecular modeling

Pharmacophore generation as well as docking of antagonists into minimized homology model of receptor were performed using

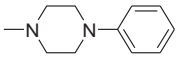
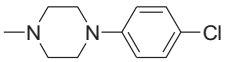
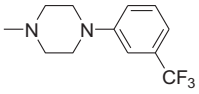
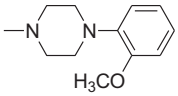
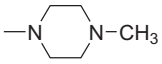
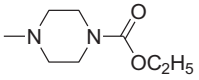
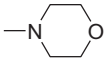
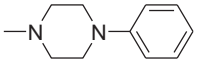
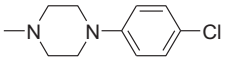
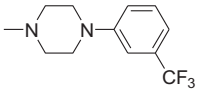
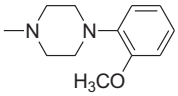
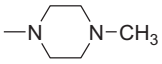
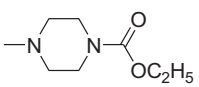
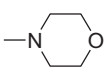


Scheme 2. Reagents and reaction conditions. a: chloroacetyl chloride, CH_2Cl_2, 1 h.; b: 3-chloropropionyl chloride, CH_2Cl_2, 1 h.; c: appropriate amine, acetonitrile, TEA, 3 h.

Table 1
Vasorelaxant potency (IC_{50}), and best fit and docking conformer for each compound in the test set (**6a**–**h** and **8a**–**h**) mapped with the generated α_1-AR antagonist pharmacophore and active binding pocket of homology model.



6a-h, 8a-h

Compound	R	n	IC_{50} (mM)	Fit value	E (Kcal mol^{-1})
6a		1	0.56	3.35	−67.35
6b		1	0.45	3.90	−83.34
6c		1	0.41	4.35	−92.87
6d		1	0.55	3.65	−72.91
6e		1	0.16	3.95	−105.11
6f		1	>1.00	2.97	−51.12
6g		1	0.43	3.99	−50.24
6h		1	0.61	2.85	−59.45
8a		2	0.27	3.99	−102.10
8b		2	0.70	2.99	−68.50
8c		2	0.50	3.76	−79.43
8d		2	0.10	4.87	−103.24
8e		2	0.36	3.00	−95.32
8f		2	>1.00	2.96	−72.57
8g		2	0.58	2.79	−52.01
8h		2	0.81	2.75	−52.21

Prazosin: IC_{50} = 0.48 mM, docking E = −98.2 kcal mol^{-1}, and fit value = 4.55.

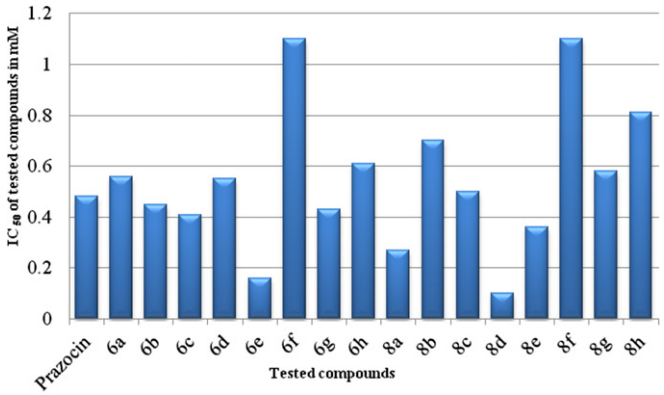


Fig. 3. IC_{50} values of the tested compounds compared to prazosin on contracture induced by norepinephrine hydrochloride on thoracic rat aortic rings compared to prazosin HCl.

the Discovery Studio 2.5 software (Accelrys Inc., San Diego, CA, USA).

2.3.1. Studies of the pharmacophore/receptor mode of α_1-AR antagonist

The simulation compare/fit study of compounds **6a**–**h**, **8a**–**h** was carried out using reported common feature pharmacophore model that encompassed five features namely; positive ionizable (red), hydrogen bond acceptor (green) and three hydrophobic features (blue) (Fig. 4a) [20,21]. The fitting of the tested compound was performed using best fit during the compare/fit process. Different mappings for all the conformers of each compound of the test set to the pharmacophore hypothesis were visualized and the fit values of the best fitting conformers were determined (Table 1, see Figure 1 of supplementary meterials). Most of the fit value derived from fitting of our compounds into the pharmacophore hypothesis were in good agreement with the vasodilation experimental data. Alignment study of compound **8d** (the most promising vasodilation active agent) and prazosin (highly selective α_1-AR antagonist) in the pharmacophore model of α_1-AR antagonist revealed that: (i) The *o*-methoxyphenyl residue attached to the N^4-piperazinyl function of **8d** was aligned perfectly with the dimethoxyquinazoline moiety of prazosin exhibiting hydrophobic interaction through both the phenyl ring and the CH_3 of methoxy group. (ii) The N^1 of piperazinyl moiety of **8d** is aligned with the N^1 of piperazinyl residue of prazosin, mapping the same feature (positive ionizable) on pharmacophore model. (iii) Both carbonyl functions in **8d** and prazosin have the same orientation and mapped into hydrogen bond acceptor feature of pharmacophore. (iv) The phthalazine ring system was aligned with the furanyl moiety of prazosin exhibiting hydrophobic interaction feature (Fig. 4a).

2.3.2. Molecular docking studies and binding conformation

All dock runs were conducted to investigate the detailed intermolecular interactions between the ligand and the target protein according to the previously reported homology modeling technique [20,24,25]. The observed binding energies and the corresponding binding mode of interaction taking place between the ligand (test compound) and the receptor (active site of the protein homology model) are listed in Table 1 (see Figure 2 of supplementary materials). As indicated in previous reports, Asp106 is the key amino acid involved in the interaction [20,24,25]. From the mode of interactions of the tested compounds with the assigned active site it was noticed that the distance between the positive ionizable center of compound **8d** which is the piperazinyl nitrogen (N^1) and negative edge of Asp106, which is the carboxylate

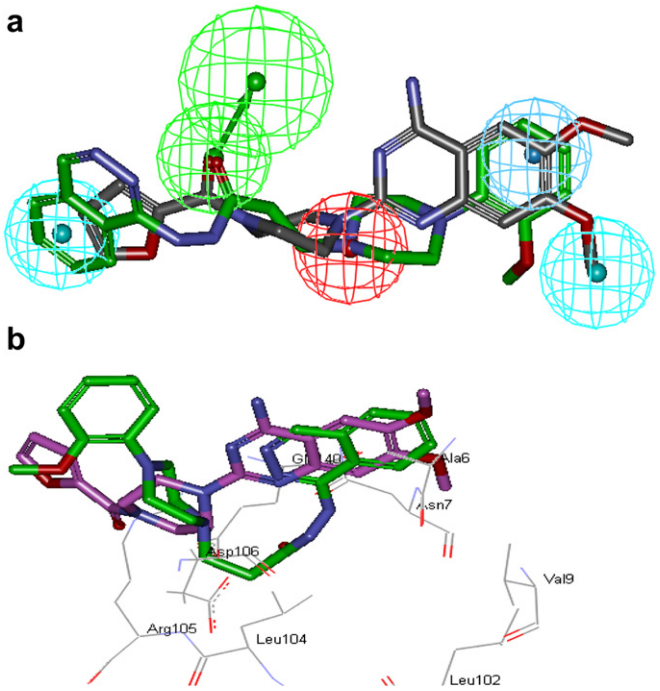


Fig. 4. (a) Alignment of compound **8d** (green) and prazosin (gray) on the α_1-AR antagonist pharmacophore hypothesis. (b) Alignment of compound **8d** (green) and prazosin (violet) in the binding site of α_1-AR homology model. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

oxygen was more or less similar to that between the nitrogen atom of the N^1-piperazinyl moiety of prazosin and the same Asp106 carboxylate oxygen (5.72 and 4.64 A, respectively) (Fig. 4b).

The involvement of N^1 rather than N^4 of piperazine ring in the binding interaction with Asp106 can be explained based on the fact that the negative charge accumulated on the N^1-piperazinyl nitrogen is greater than that on the N^4-piperazinyl nitrogen probably due to the +I effect of the 3 methylene groups surrounding the N^1 compared to the -M effect of the phenyl ring on N^4 which reduces the negative charge on it. This makes N^1 of piperazine ring the most protonatable center of the molecule (positive ionizable feature of the generated pharmacophore) and thus, can strongly interact with the negative carboxylate of Asp106.

Also, by observing the binding mode of the rest of the compounds, it can be assumed that the N^1 of piperazine or its equivalent nitrogen atom in the morpholine and imidazole derivatives is crucial for interacting with Asp106.

3. Conclusion

Two series of phthalazine derivatives hybridized to a variety of N-substituted piperazines, morpholine and imidazole moieties through either an acetohydrazido linker, compounds **6a**–**h**, or a propanehydrazido spacer, compounds **8a**–**h**, were synthesized. The synthesized compounds were tested in vitro for their vaso-relaxant activities against nor-adrenaline-induced spasm on thoracic rat aorta rings and compared to the reference drug, prazosin. Seven out of the sixteen tested compounds showed vaso-relaxant activity higher than that of prazosin viz. **6b**, **6c**, **6e**, **6g**, **8a**, **8d** and **8e**. Compound **8d** is the most active among all derivatives being 5 times more potent than prazosin. Molecular modeling studies, including fitting of the synthesized compounds to a 3D-pharmacophore and their docking into optimized homology model as α_1-AR antagonists show good docking score and fit values. The experimental vasodilation activities of tested compounds are consistent with their molecular modeling results. Docking studies explains the importance of the N^1 of piperazine or its equivalent nitrogen atom in the morpholine and imidazole derivatives in interacting with Asp106.

4. Experimental

4.1. Chemistry

Melting points were determined with Stuart SMP3 version 5 apparatus and were uncorrected. FT-IR spectra were recorded on Bruker FT-IR spectrophotometer using KBr cell. Unless otherwise noted, ^{1}H NMR spectra were recorded in DMSO-d_6 on Varian mercury 300BB at 300 MHz. NMR of some compounds were recorded in DMSO-d_6 on JOEL (Eclipse) 400 at 400 MHz for ^{1}H NMR and at 100 MHz for ^{13}C NMR; using tetramethylsilane (TMS) as internal reference. The electron impact (EI) mass spectra were recorded on Finnigan Mat SSQ 7000 (70 ev) mass spectrometer. Elemental microanalysis was performed at Microanalytical Center, Cairo University and Al-Azhar University. TLC was monitored on FLUKA silica gel TLC aluminum cards (0.2 mm thickness) with fluorescent indicator 254 nm using chloroform/methanol (9:1) as eluent to follow the course of reactions and to check the purity of products. All reagents and solvents were purified and dried by standard techniques.

4.1.1. General procedure for the synthesis of compounds (**5**) and (**7**)

To a solution of 1-hydrazinophthalazine **4** (1 g; 6.2 mmol) in dry methylene chloride (25 ml), the appropriate chloroacyl chloride (6.8 mmol) was added and the mixture was refluxed for 1 h. After cooling, the solid formed was filtered, washed thoroughly with methylene chloride and air dried. The crude product was crystallized from ethanol.

*4.1.1.1. 2-Chloro-N′-(phthalazin-1-yl)acetohydrazide (**5**).* Mp 187–189 °C; yield 97%. IR ν_{max}/cm^{-1}: 3425, 3217 (NHs), 3074 (aromatic CH), 2947 (aliphatic CH), 1674 (C=O), 1635 (NHs). ^{1}H NMR: δ 5.20 (s, 2H, CH_2), 7.88 (t, 1H, J = 8 Hz, CH-6), 7.93 (t, 1H, J = 8 Hz, CH-7), 8.20 (d, 1H, J = 8 Hz, CH-5), 8.45 (d, 1H, J = 8 Hz, CH-8), 9.07 (s, 1H, CH-4), 10.20 (br s, 2H, NHs, exchanged with D_2O). Anal. Calcd. for $C_{10}H_9ClN_4O$ (236.66): C, 50.75; H, 3.83; N, 23.67. Found: C, 50.74; H, 379; N, 24.05.

*4.1.1.2. 3-Chloro-N′-(phthalazin-1-yl)propanehydrazide (**7**).* Mp 141–143 °C; yield 90%. IR ν_{max}/cm^{-1}: 3425, 3194 (NHs), 3074 (CH aromatic), 2966 (CH aliphatic), 1682 (C=O), 1635 (NHs). ^{1}H NMR: δ 2.62 (t, 2H, J = 6.3 Hz, CH_2Cl), 3.78 (t, 2H, J = 6.3 Hz, $COCH_2$), 7.93 (t, 1H, J = 8 Hz, CH-6), 8.08 (t, 1H, J = 8 Hz, CH-7), 8.26 (d, 1H, J = 8 Hz, CH-5), 8.52 (d, 1H, J = 8 Hz, CH-8), 9.11 (s, 1H, CH-4), 10.23 (br s, 2H, NHs, exchanged with D_2O). Anal. Calcd. for $C_{11}H_{11}ClN_4O.H_2O$ (268.70): C, 49.17; H, 4.88; N, 20.85. Found: C, 48.88; H, 4.85; N, 21.31.

4.1.2. General procedure for the synthesis of compounds (**6a**–**h**) and (**8a**–**h**)

A mixture of the corresponding chloro derivative **5** or **7** (4.2 mmol), the appropriate amine (4.2 mmol) and triethylamine (1.16 ml, 8.4 mmol) in dry acetonitrile was heated under reflux for 3 h. The mixture was cooled, poured on ice–water and filtered. The residue was washed with cold water and dried. The crude product was crystallized from the appropriate solvent.

*4.1.2.1. 2-(4-Phenylpiperazin-1-yl)-N′-(phthalzin-1-yl)acetohydrazide (**6a**).* Mp 222–224 °C (ethanol); yield 90%. IR ν_{max}/cm^{-1}: 3350

(NHs), 3059 (CH aromatic), 2954, 2843 (CH aliphatic), 1680 (C=O), 1597 (NHs). ^{1}H NMR: δ 2.69 (br s, 4H, CH_2-2 and CH_2-6 piperazine), 3.11 (br s, 4H, CH_2-3 and CH_2-5 piperazine), 4.14 (s, 2H, CH_2), 6.75–7.15 (m, 5H, phenyl H), 7.94 (t, 1H, J = 8 Hz, CH-6), 8.04 (t, 1H, J = 8 Hz, CH-7), 8.21 (d, 1H, J = 8 Hz, CH-5), 8.50 (d, 1H, J = 8 Hz, CH-8), 9.11 (s, 1H, CH-4), 11.60 (br s, 2H, NHs, exchanged with D_2O). Anal. Calcd. for $C_{20}H_{22}N_6O$ (362.43): C, 66.28; H, 6.12; N, 23.19. Found: C, 66.50; H, 5.89; N, 23.42.

*4.1.2.2. 2-(4-(4-Chlorophenyl)piperazin-1-yl)-N′-(phthalzin-1-yl)acetohydrazide (**6b**).* Mp 234-235 °C (isopropanol); yield 86%. IR ν_{max}/cm^{-1}: 3441 (NHs), 3050 (CH aromatic), 2958, 2920 (CH aliphatic), 1690 (C=O), 1630 (NHs). ^{1}H NMR: δ 2.71 (br s, 4H, CH_2-2 and CH_2-6 piperazine), 3.12 (br s, 4H, CH_2-3 and CH_2-5 piperazine), 4.15 (s, 2H, CH_2), 6.88 (d, 2H, J = 9 Hz, CH-2′ and CH-6′ phenyl), 7.17 (d, 2H, J = 9 Hz, CH-3′ and CH-5′ phenyl), 7.90 (t, 1H, J = 8 Hz, CH-6), 8.07 (t, 1H, J = 8 Hz, CH-7), 8.21 (d, 1H, J = 8 Hz, CH-5), 8.50 (d, 1H, J = 8 Hz, CH-8), 9.10 (s, 1H, CH-4), 10.35 (br s, 2H, NHs, exchanged with D_2O). ^{13}C NMR (100 MHz): δ 47.8 (C3 + C5 piperazine), 49.6 (C2 + C6 piperazine), 52.1 (CO*CH*$_2$), 116.8–134.3 (C aromatic), 142.1 (C4-phthalazine), 146.9 (C1-phenyl), 149.6 (C1-phthalazine), 165.4 (CO). MS, m/z: 396.10 [M$^+$], 398.40 [M$^+$+2]. Anal. Calcd. for $C_{20}H_{21}ClN_6O$ (396.87): C, 60.53; H, 5.33; N,21.18. Found: C, 60.29; H, 5.47; N, 21.56.

*4.1.2.3. 2-(4-(3-Trifluoromethylphenyl)piperazin-1-yl)-N′-(phthalzin-1-yl)acetohydrazide (**6c**).* Mp 164–166 °C (ethanol); yield 85%. IR ν_{max}/cm^{-1}: 3425 (NHs), 3062 (CH aromatic), 2958, 2927 (CH aliphatic), 1670 (C=O), 1608 (NHs). ^{1}H NMR: δ 2.71 (br s, 4H, CH_2-2 and CH_2-6 piperazine), 3.22 (br s, 4H, CH_2-3 and CH_2-5 piperazine), 4.15 (s, 2H, CH_2), 7.03–7.41 (m, 4H, phenyl), 7.94 (t, 1H, J = 8 Hz, CH-6), 8.04 (t, 1H, J = 8 Hz, CH-7), 8.21 (d, 1H, J = 8 Hz, CH-5), 8.50 (d, 1H, J = 8 Hz, CH-8), 9.11 (s, 1H, CH-4), 10.24 (br s, 2H, NHs, exchanged with D_2O). ^{13}C NMR (100 MHz): δ 47.4 (C3 + C5 piperazine), 49.6 (C2 + C6 piperazine), 52.1 (CO*CH*$_2$), 118.7–134.3 (C aromatic), 142.1 (C4–phthalazine), 146.9 (C1–phenyl), 148.3 (C1–phthalazine), 167.0 (CO).Anal. Calcd. for $C_{21}H_{21}F_3N_6O$ (430.43): C, 58.60; H, 4.92; N, 19.52. Found: C, 59.02; H, 5.11; N, 19.80.

*4.1.2.4. 2-(4-(2-Methoxyphenyl)piperazin-1-yl)-N′-(phthalzin-1-yl) acetohydrazide (**6d**).* Mp 178–180 °C (ethanol); yield 87%. IR ν_{max}/cm^{-1}: 3444 (NHs), 3059 (CH aromatic), 2939, 2938, 2831 (CH aliphatic), 1680 (C=O), 1589 (NHs). ^{1}H NMR (400 MHz): δ 2.68 (br s, 4H, CH_2-2 and CH_2-6 piperazine), 2.92 (br s, 4H, CH_2-3 and CH_2-5 piperazine), 3.72 (s, 3H, OCH_3), 4.14 (s, 2H, CH_2), 6.83–6.89 (m, 4H, phenyl), 7.93 (t, 1H, J = 8 Hz, CH-6), 8.05 (t, 1H, J = 8 Hz, CH-7), 8.20 (d, 1H, J = 8 Hz, CH-5), 8.51 (d, 1H, J = 8 Hz, CH-8), 9.10 (s, 1H, CH-4), 10.25 (br s, 2H, NHs, exchanged with D_2O). ^{13}C NMR (100 MHz): δ 49.7 (C3 + C5 piperazine), 50.9 (C2 + C6 piperazine), 52.5 (OCH_3), 55.3 (CO*CH*$_2$), 117.8–134.3 (C aromatic), 141.1 (C4–phthalazine), 146.9 (C1–phenyl), 148.5 (C2–phenyl), 152.0 (C1–phthalazine), 165.2 (CO).Anal. Calcd. for $C_{21}H_{24}N_6O_2$ (392.45): C, 64.27; H, 6.16; N, 21.41. Found: C, 64.54; H, 6.03; N, 21.67.

*4.1.2.5. 2-(4-Methylpiperazin-1-yl)-N′-(phthalzin-1-yl)acetohydrazide (**6e**).* Mp 235–237 °C (isopropanol); yield 65%. IR ν_{max}/cm^{-1}: 3441, 3329 (NHs), 3059 (CH aromatic), 2966, 2920, 2850 (CH aliphatic), 1608 (C=O), 1585 (NHs). ^{1}H NMR: δ 2.48 (s, 3H, CH_3), 3.29 (br s, 4H, CH_2-3 and CH_2-5 piperazine), 3.40 (br s, 4H, CH_2-2 and CH_2-6 piperazine), 4.35 (s, 2H, CH_2), 7.56 (t, 1H, J = 8 Hz, CH-6), 8.06 (t, 1H, J = 8 Hz, CH-7), 8.26 (d, 1H, J = 8 Hz, CH-5), 8.64 (d, 1H, J = 8 Hz, CH-8), 9.22 (s, 1H, CH-4), 11.58 (br s, 2H, NHs, exchanged with D_2O). MS, m/z: 300.30 [M$^+$]. Anal. Calcd. for $C_{15}H_{20}N_6O$ (300.36): C, 59.98; H, 6.71; N, 27.98. Found: C, 59.42; H, 6.08; N, 28.37.

*4.1.2.6. 2-(4-Ethoxycarbonylpiperazin-1-yl)-N′-(phthalzin-1-yl)acetohydrazide (**6f**).* Mp 142–143 °C (methanol); yield 58%. IR ν_{max}/cm^{-1}: 3421 (NHs), 3020 (CH aromatic), 2978, 2900, 2866 (CH aliphatic), 1712 (C=O), 1685 (C=O), 1627 (NHs).

^{1}H NMR: δ 1.15 (t, 3H, J = 7.5 Hz, CH_2CH_3), 2.50 (br s, 4H, CH_2-2 and CH_2-6 piperazine), 3.37 (br s, 4H, CH_2-3 and CH_2-5 piperazine), 3.96 (q, 2H, J = 7.5 Hz, CH_2CH_3), 4.11 (s, 2H, CH_2), 7.91 (t, 1H, J = 8 Hz, CH-6), 8.03 (t, 1H, J = 8 Hz, CH-7), 8.12 (d, 1H, J = 8 Hz, CH-5), 8.48 (d, 1H, J = 8 Hz, CH-8), 9.09 (s, 1H, CH-4), 9.89 (br s, 2H, NHs, exchanged with D_2O). Anal. Calcd. for $C_{17}H_{22}N_6O_3$ (358.39): C, 56.97; H, 6.19; N, 23.45. Found: C, 57.26; H, 6.29; N, 23.79.

*4.1.2.7. 2-(1-Morpholinyl)-N′-(phthalzin-1-yl)acetohydrazide (**6g**).* Mp 192–193 °C (isopropanol); yield 60%. IR ν_{max}/cm^{-1}: 3387, 3332 (NHs), 3059 (CH aromatic), 2954, 2924 (CH aliphatic), 1690 (C=O), 1608 (NHs). ^{1}H NMR: δ 2.44 (br s, 4H, CH_2-2 and CH_2-6 morpholine), 3.54 (br s, 4H, CH_2-3 and CH_2-5 morpholine), 4.15 (s, 2H, CH_2), 7.85 (t, 1H, J = 8 Hz, CH-6), 7.97 (t, 1H, J = 8 Hz, CH-7), 8.13 (d, 1H, J = 8 Hz, CH-5), 8.47 (d, 1H, J = 8 Hz, CH-8), 9.09 (s, 1H, CH-4), 11.57 (br s, 2H, NHs, exchanged with D_2O). Anal. Calcd. for $C_{14}H_{17}N_5O_2$ (287.32): C, 58.52; H, 5.96; N, 24.37. Found: C, 58.80; H, 5.72; N, 24.73.

*4.1.2.8. 2-(1-Imidazolyl)-N′-(phthalzin-1-yl)acetohydrazide (**6h**).* Mp 251–254 °C (methanol); yield 52%. IR ν_{max}/cm^{-1}: 3394, 3332 (NHs), 3059 (CH aromatic), 2974, 2924 (CH aliphatic), 1612 (C=O), 1570 (NHs). ^{1}H NMR (400 MHz): δ 3.48 (s, 2H, CH_2-N), 7.57 (m, 2H, CH-4′ and CH-5′ imidazole), 7.98 (s, 1H, CH-2′ imidazole), 8.10 (t, 1H, J = 7.6 Hz, CH-6), 8.26 (t, 1H, J = 7.8 Hz, CH-7), 8.28 (d, 1H, J = 8 Hz, CH-5), 8.42 (d, 1H, J = 8 Hz, CH-8), 8.83 (s, 1H, CH-4), 9.22 (br s, 2H, NHs, exchanged with D_2O). ^{13}C NMR (100 MHz): δ 35.3 (CO*CH*$_2$), 119.1–131.2 (C aromatic), 138.5 (C2′–imidazole), 143.2 (C4–phthalazine), 151.9 (C4–phthalazine), 162.2 (CO). Anal. Calcd. for $C_{13}H_{12}N_6O$ (268.27): C, 58.20; H, 4.51; N,31.33. Found: C, 57.64; H, 4.43; N, 31.65.

*4.1.2.9. 3-(4-Phenylpiperazin-1-yl)-N′-(phthalazin-1-yl)propanehydrazide (**8a**).* Mp 211–212 °C (isopropanol); yield 84%. IR ν_{max}/cm^{-1}: 3217 (NHs), 3035 (CH aromatic), 2947, 2823 (CH aliphatic), 1610 (C=O), 1579 (NHs). ^{1}H NMR: δ 2.34 (t, 2H, J = 6 Hz, $COCH_2$), 2.62 (br s, 4H, CH_2-2 and CH_2-6 piperazine), 3.10 (br s, 4H, CH_2-3 and CH_2-5 piperazine), 3.34 (t, 2H, J = 6 Hz, CH_2-N), 6.74–7.17 (m, 5H, phenyl), 7.61 (t, 1H, J = 7.6 Hz, CH-6), 7.98 (t, 1H, J = 7.8 Hz, CH-7), 8.02 (d, 1H, J = 8 Hz, CH-5), 8.50 (d, 1H, J = 8 Hz, CH-8), 9.00 (s, 1H, CH-4), 11.60 (br s, 2H, NHs, exchanged with D_2O). ^{13}C NMR (100 MHz): δ 37.4 (CO*CH*$_2$CH$_2$–N), 48.0 (C3 + C5 piperazine), 52.3 (–$COCH_2$*CH*$_2$–N), 54.5 (C2 + C6 piperazine), 118.7–128.8 (C aromatic), 143.6 (C4–phthalazine), 147.8 (C1–phenyl), 150.1 (C1–phthalazine), 168.3 (CO). Anal. Calcd. for $C_{21}H_{24}N_6O$ (376.45): C, 67.00; H, 6.43; N, 22.32. Found: C, 66.82; H, 6.70; N, 22.78.

*4.1.2.10. 3-(4-(4-Chlorophenyl)piperazin-1-yl)-N′-(phthalazin-1-yl) propanehydrazide (**8b**).* Mp 132–134 °C (isopropanol); yield 78%. IR ν_{max}/cm^{-1}: 3394, 3329 (NHs), 3035 (CH aromatic), 2924, 2823 (CH aliphatic), 1610 (C=O), 1589 (NHs). ^{1}H NMR (400 MHz): δ 2.35 (t, 2H, J = 6 Hz, $COCH_2$), 2.70 (br s, 4H, CH_2-2 and CH_2-6 piperazine), 3.07 (br s, 4H, CH_2-3 and CH_2-5 piperazine), 3.33 (t, 2H, J = 6 Hz, CH_2–N), 6.92 (d, 2H, J = 9 Hz, CH-2′ and CH-6′ phenyl), 7.18 (, 2H, J = 9 Hz, CH-3′ and CH-5′ phenyl), 7.61 (t, 1H, J = 8 Hz, CH-6), 7.93 (t, 1H, J = 8 Hz, CH-7), 8.20 (d, 1H, J = 8 Hz, CH-5), 8.47 (d, 1H, J = 8 Hz, CH-8), 9.05 (s, 1H, CH-4), 11.50 (br s, 2H, NHs, exchanged with D_2O). ^{13}C NMR (100 MHz): δ 37.5 (CO*CH*$_2$CH$_2$–N), 48.0 (C3 + C5 piperazine), 52.3 (–$COCH_2$*CH*$_2$–N), 54.7 (C2 + C6 piperazine), 116.7–131.1 (C aromatic), 143.9 (C4–phthalazine), 148.1 (C1–phenyl), 149.8 (C1–phthalazine), 169.9 (CO). Anal. Calcd. for $C_{21}H_{23}ClN_6O$

(410.90): C, 61.38; H, 5.64; N, 20.45. Found: C, 61.47; H, 5.58; N, 20.59.

*4.1.2.11. 3-(4-(3-Trifluoromethylphenyl)piperazin-1-yl)-N′-(phthalzin-1-yl)propanehydrazide (**8c**).* Mp 267–269 °C (isopropanol); yield 68%. IR ν_{max}/cm^{-1}: 3390, 3329 (NHs), 3035 (CH aromatic), 2920, 2850 (CH aliphatic), 1608 (C=O), 1581 (NHs). ^{1}H NMR (400 MHz): δ 2.51 (t, 2H, J = 6 Hz, $COCH_2$), 2.75 (br s, 4H, CH_2-2 and CH_2-6 piperazine), 3.27 (br s, 4H, CH_2-3 and CH_2-5 piperazine), 3.43 (t, 2H, J = 6 Hz, CH_2–N), 7.10–7.45 (m, 4H, phenyl), 7.57 (t, 1H, J = 8 Hz, CH-6), 7.77 (t, 1H, J = 8 Hz, CH-7), 8.20 (d, 1H, J = 8 Hz, CH-5), 8.64 (d, 1H, J = 8 Hz, CH-8), 9.15 (s, 1H, CH-4), 11.57 (br s, 2H, NHs, exchanged with D_2O). ^{13}C NMR (100 MHz): δ 37.9 ($COCH_2CH_2$–N), 46.5 (C3 + C5 piperazine), 52.5 (–$COCH_2CH_2$–N), 53.2 (C2 + C6 piperazine), 124.4–136.4 (C aromatic + CF_3), 143.8 (C4–phthalazine), 148.2 (C1–phenyl), 153.4 (C1–phthalazine), 168.1 (CO). Anal. Calcd. for $C_{22}H_{23}F_3N_6O$ (444.45): C, 59.45; H, 5.22; N, 18.91. Found: C, 59.63; H, 5.29; N, 19.20.

*4.1.2.12. 3-(4-(2-Methoxyphenyl)piperazin-1-yl)-N′-(phthalzin-1-yl) propanehydrazide (**8d**).* Mp 150–153 °C (ethanol); yield 62%. IR ν_{max}/cm^{-1}: 3387, 3332 (NHs), 3059 (CH aromatic), 2920, 2831 (CH aliphatic), 1612 (C=O), 1589 (NHs). ^{1}H NMR: δ 2.64 (t, 2H, J = 6 Hz, $COCH_2$), 2.80 (br s, 4H, CH_2-2 and CH_2-6 piperazine), 3.57 (br s, 4H, CH_2-3 and CH_2-5 piperazine), 3.76 (t, 2H, J = 6 Hz, CH_2–N), 3.81 (s, 3H, OCH_3),6.78–6.97 (m, 4H, phenyl), 7.58 (t, 1H, J = 7.6 Hz, CH-6), 8.04 (t, 1H, J = 7.8 Hz, CH-7), 8.22 (d, 1H, J = 8 Hz, CH-5), 8.64 (d, 1H, J = 8 Hz, CH-8), 9.31 (s, 1H, CH-4), 11.60 (br s, 2H, NHs, exchanged with D_2O). MS, m/z: 406.55 [M^+]. Anal. Calcd. for $C_{22}H_{26}N_6O_2$ (406.48): C, 65.01; H, 6.45; N, 20.68. Found: C, 65.14; H, 6.52; N, 21.07.

*4.1.2.13. 3-(4-Methylpiperazin-1-yl)-N′-(phthalzin-1-yl)propanehydrazide (**8e**).* Mp 252–254 °C (ethanol); yield 55%. IR ν_{max}/cm^{-1}: 3398, 3329 (NHs), 3055 (CH aromatic), 2924, 2850 (CH aliphatic), 1620 (C=O), 1589 (NHs). ^{1}H NMR: δ 2.49 (s, 3H, CH_3), 2.50 (t, 2H, J = 6 Hz, $COCH_2$), 3.35 (br s, 4H, CH_2-2 and CH_2-6 piperazine), 3.41 (br s, 4H, CH_2-3 and CH_2-5 piperazine), 3.56 (t, 2H, J = 6 Hz, CH_2–N), 7.56 (t, 1H, J = 7.6 Hz, CH-6), 7.82 (t, 1H, J = 7.8 Hz, CH-7), 8.23 (d, 1H, J = 8 Hz, CH-5), 8.67 (d, 1H, J = 8 Hz, CH-8), 9.09 (s, 1H, CH-4), 11.60 (br s, 2H, NHs, exchanged with D_2O). Anal. Calcd. for $C_{16}H_{22}N_6O$ (314.39): C, 61.13; H, 7.05; N, 26.73. Found: C, 61.43; H, 6.77; N, 27.15.

*4.1.2.14. 3-(4-Ethoxycarbonylpiperazin-1-yl)-N′-(phthalzin-1-yl)propanehydrazide (**8f**).* Mp 177–179 °C (methanol); yield 50%. IR ν_{max}/cm^{-1}: 3441, 3329 (NHs), 3051 (CH aromatic), 2920, 2850 (CH aliphatic), 1630 (C=O), 1612 (C=O), 1589 (NHs). ^{1}H NMR: δ 1.21 (t, 3H, J = 7.8 Hz, CH_2CH_3), 2.46 (t, 2H, J = 6 Hz, $COCH_2$), 2.62 (br s, 4H, CH_2-2 and CH_2-6 piperazine), 3.07 (br s, 4H, CH_2-3 and CH_2-5 piperazine), 3.36 (t, 2H, J = 6 Hz, CH_2–N), 4.22 (q, 2H, J = 7.8, CH_2CH_3), 7.61 (t, 1H, J = 7.6 Hz, CH-6), 7.95 (t, 1H, J = 7.8 Hz, CH-7), 8.02 (d, 1H, J = 8 Hz, CH-5), 8.68 (d, 1H, J = 8 Hz, CH-8), 9.21 (s, 1H, CH-4), 11.82 (br s, 2H, NHs, exchanged with D_2O). Anal. Calcd. for $C_{18}H_{24}N_6O_3$ (372.42): C, 58.05; H, 6.50; N, 22.57. Found: C, 58.30; H, 6.38; N, 22.93.

*4.1.2.15. 3-(1-Morpholinyl)-N′-(phthalzin-1-yl)acetohydrazide (**8g**).* Mp 265–266 °C (isopropanol); yield 62%. IR ν_{max}/cm^{-1}: 3398, 3332 (NHs), 3059 (CH aromatic), 2920, 2840 (CH aliphatic), 1608 (C=O), 1589 (NHs). ^{1}H NMR: δ 2.50 (t, 2H, J = 6 Hz, $COCH_2$), 2.85 (br s, 4H, CH_2-2 and CH_2-6 morpholine), 3.31 (br s, 4H, CH_2-3 and CH_2-5 morpholine), 3.42 (t, 2H, J = 6 Hz, CH_2–N), 7.55 (t, 1H, J = 7.6 Hz, CH-6), 7.85 (t, 1H, J = 7.8 Hz, CH-7), 8.25 (d, 1H, J = 8 Hz, CH-5), 8.67 (d, 1H, J = 8 Hz, CH-8), 9.12 (s, 1H, CH-4), 11.60 (br s, 2H, NHs, exchanged with D_2O). Anal. Calcd. for $C_{15}H_{19}N_5O_2$ (301.34): C, 59.79; H, 6.36; N, 23.24. Found: C, 59.54; H, 6.09; N, 23.61.

*4.1.2.16. 3-(1-Imidazolyl)-N′-(phthalzin-1-yl)acetohydrazide (**8h**).* Mp 225–227 °C (ethanol); yield 62%. IR ν_{max}/cm^{-1}: 3402, 3332 (NHs), 3062 (CH aromatic), 2924, 2850 (CH aliphatic), 1615 (C=O), 1589 (NHs). ^{1}H NMR: δ 2.64 (t, 2H, J = 6 Hz, $COCH_2$), 3.82 (t, 2H, J = 6 Hz, CH_2–N), 7.59 (m, 2H, CH-4′ and CH-5′ imidazole), 7.87 (s, 1H, CH-2′ imidazole), 7.98 (t, 1H, J = 7.6 Hz, CH-6), 8.15 (t, 1H, J = 7.8 Hz, CH-7), 8.28 (d, 1H, J = 8 Hz, CH-5), 8.64 (d, 1H, J = 8 Hz, CH-8), 9.15 (s, 1H, CH-4), 10.18 (br s, 2H, NHs, exchanged with D_2O). ^{13}C NMR (100 MHz): δ 38.1 ($COCH_2CH_2$–N), 48.1 (C3 + C5 piperazine), 51.8 (–$COCH_2CH_2$–N), 55.0 (C2 + C6 piperazine), 119.8–130.8 (C aromatic), 135.6 (C2′′–imidazole), 143.3 (C4–phthalazine), 146.9 (C1–phenyl), 149.2 (C1–phthalazine), 167.0 (CO). MS, m/z: 282.00 [M^+]. Anal. Calcd. for $C_{14}H_{14}N_6O$ (282.30): C, 59.56; H, 5.00; N, 29.77. Found: C, 59.68; H, 5.16; N, 30.12.

4.2. Vasorelaxant activity

The vasodilation activity screening procedures were carried out according to the standard reported techniques [20–23] by testing the effects of the synthesized compounds **6a**–**h**, **8a**–**h** on isolated thoracic aortic rings of male Wister rats (250–350 g). After light ether anesthesia, the rats were sacrificed by cervical dislocation. The aorta were immediately excised, freed of extraneous tissues and prepared for isometric tension recording. Aorta was cut into (3–5 mm width) rings and each ring was placed in a vertical chamber "10 ml jacketed automatic multi-chamber organ bath system (Model no. ML870B6/C, Panlab, Spain)" filled with Krebs solution composed of (in mM): NaCl, 118.0; KCl, 4.7; $NaHCO_3$, 25.0; $CaCl_2$, 1.8; NaH_2PO_4, 1.2; $MgSO_4$, 1.2; glucose, 11.0 and oxygenated with carbogen gas (95% O_2/5% CO_2) at 37 ± 0.5 °C. Each aortic ring was mounted between two stainless steel hooks passed through its lumen. The lower hook was fixed between two plates, while the upper one was attached to a force displacement transducer (Model no. MLT0201, Panlab, Spain) connected to an amplifier (PowerLab, AD Instruments Pty. Ltd.) which is connected to a computer. The Chart for windows (v 3.4) software was used to record and elaborate data.

Preparations were stabilized under 2 g resting tension during 2 h and then the contracture response to norepinephrine hydrochloride (10^{-6} M) was measured before and after exposure to increasing concentrations of the tested synthesized compounds. The tested compounds **6a**–**h**, **8a**–**h** were dissolved in dimethylsulfoxide (DMSO) as stock solution (10 ml of 0.01 M). Control experiments were performed in the presence of DMSO alone, at the same concentrations as those used with the derivatives tested, which demonstrated that the solvent did not affect the contractile response of isolated aorta. The observed vasodilatation activity screening data are reported (Table 1, Fig. 3) and the potency (IC_{50}, concentration necessary for 50% reduction of maximal norepinephrine hydrochloride induced contracture) was determined by the best-fit line technique.

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Appendix. Supplementary material

Supplementary data related to this article can be found online at doi:10.1016/j.ejmech.2012.02.051.

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