INTRODUCTION
Hypertension is considered to be one of the most common disease conditions. Even though it is common, it is a serious diagnosis. High blood pressure is the reason with regard to tens of thousands of deaths each year due to heart attack, cardiovascular disease, stroke, as well as kidney complications. The alpha-adrenergic receptors (α-ARs) play a pivotal role in the regulation of a variety of physiological processes, particularly within the cardiovascular system and are divided into two main subtypes: α1- and α2-ARs. The α1- and α2-ARs are located in the vascular smooth muscle cell membrane, and upon stimulation by an appropriate agonist mediate vasoconstriction. The simultaneous occurrence of both receptor subtypes on vascular smooth muscles makes it conceivable that α1- and α2-ARs can contribute to the maintenance of peripheral arterial tone and may play an important role in resistance seen in hypertension. The α1-ARs modulate intercellular biochemical processes in response to changes in extracellular concentrations of the neurotransmitter norepinephrine and the circulating hormone epinephrine.

Compounds acting as antagonists at various post-junctional α1-ARs are frequently used in the therapy of high blood pressure, prazosin being the most common drug. The α1-AR antagonists are also used in the treatment of benign prostatic hyperplasia, lower urinary tract symptoms and cardiac arrhythmia. Selective α1-adrenoceptor antagonist drugs which block α1-adrenergic receptors in arteries and smooth muscles though have many advantages and uses in the management of arterial hypertension. These receptors are responsible for the vasoconstrictive action of norepinephrine, which in turn raises blood pressure.

A literature survey reveals that a variety of antihypertensive drugs contain piperazine ring, for example, prazosin, doxazosin, naftopidil, urapidil, and Cdm-12 (Fig. 1) have been proven effective in the clinic, acting as α1-ARs. Moreover, many reports had shown that heterocyclic compounds containing an arylpiperazine moiety had high affinity for α1-ARs.

The medicinal value of benzofuran derivatives is significant among various heterocycles, as they are found to possess antibacterial, anti-arrhythmic, hypotensive and vasodilator effects. Visnagin (4-methoxy-7-methyl-5H-furo[3,2-g][1]benzopyran-5-one) is an active principle of the fruit of Ammi visnaga, a plant traditionally used in cardiovascular disorders and exhibited vasodilator effects. The benzofuran amiodarone and its analogue (KB130015) relax vascular smooth muscle. Dronedarone, SR33589, a benzofuran derivative, was approved by the FDA in 2009 for the treatment of atrial fibrillation and atrial flutter (Fig. 2).

In this context, in continued research efforts in the area of hypotensive agents, we have previously designed and synthesized benzofuran-piperazine hybridized compounds whose chemical structure incorporated both benzofuran nucleus and piperazine system bridged by methyl chain (Mannich bases) and substituted at 5-position with 4,5-dihydro-1H-pyrazoles, compounds 3a-c, 4a-c and 5a-c. Compounds 7a-c and 9a-c benzofuran and piperazine systems are linked through pyrazoline moiety. Pyrazolines are the important group of heterocyclic compounds, several derivatives of which have been marked as biologically and pharmaceutically active.
pharmacologically active products such as anti-inflammatory 17, analgesic, anti-tubercular 18 and antimicrobial 19 agents. Furthermore, pyrazolines were reported as they were efficient in patients with essential hypertension20-22.

In the present study we are just making the so called preliminary blind screening of the hypotensive activity for the new designed and synthesized compounds using normotensive cats’ method (in vivo) 23. In vitro, screening of the vasodilatation activity of the most potent compounds was investigated using isolated thoracic aortic rings of male Wistar rats pre-contracted with norepinephrine compounds was investigated using isolated thoracic aortic rings of male Wistar rats pre-contracted with norepinephrine. Furthermore, molecular modeling study using Accelrys Discovery Studio 2.1 software was performed by mapping the synthesized compounds to the α1-adrenoceptor antagonist hypothesis in order to further investigate their mechanism of action as α1-adrenoceptor antagonist 6-28.

MATERIALS AND METHODS
Chemistry

Remarks: All melting points are uncorrected and determined by the open capillary method using Electro thermal capillary melting point apparatus 9100. Microanalysis was carried out at the micro analytical unit, Faculty of Science, Cairo University. Infrared spectra were determined (KBr) using Shimadzu Infrared spectrometer (IR-435) and FT-IR 1650 (Perkin Elmer). 1H-NMR Spectra were carried out using Joel, FX90Q, NMR spectrometer at 200MHz and Fourier transform EM -390, 300 MHz NMR spectrometer. Mass spectra were carried out using Finnegan SQ 7000 Gas Chromatograph -Mass. TLC was carried out using Art. 5735, DC-Plastikfolien, kieselgel 60 F254 sheets (Merck), the developing solvent were carbon tetrachloride/methanol (9:1) and the spot were visualized by UV366, 254 nm. Log p for the synthesized compounds was calculated using Chemdraw ultra 8.0.

5-Acetyl-4-methoxy-7-[(4-methylpiperazin-1-yl)methyl] benzofuran-6-ol (1) Scheme 1.

Compound 1 was prepared according to the reported procedure 27.

General method for synthesis of 1-substituted-3-arylprop-2-en-1-one 2a-c (Scheme 1)

The warmed solution of 5-acetyl-4-methoxy-7-[(4-methylpiperazin-1-yl)methyl] benzofuran-6-ol 1 (3.18 g, 10 mmol) and the appropriate aromatic aldehyde (11 mmol) in EtOH (20 ml) was treated with NaOH (30%, 5 ml) and left for 48 hr at room temperature. The reaction mixture was diluted with ice water and acidified with AcOH. The precipitate was filtered off, washed with water and dried. The crude product was crystallized from MeOH.

Fig.2 Structures of some benzofurans have anti-arrhythmic, hypotensive and vasodilator effects.

Scheme 1: (a) Ar-CHO, NaOH, EtOH, 48 hr. (b) 98% NH4H2N2H, H2O, EtOH, reflux 3 hr. (c) 98% NH4H2N2H2, H2O, AcOH, reflux 6 hr. (d) C6H5NHCH2CH2N(CH3)2, EtOH, reflux 8 hr.

1-(6-Hydroxy-4-methoxy-7-[(4-methylpiperazin-1-yl)methyl]benzofuran-5-yl)-3-phenylprop-2-en-1-one (2a)

Yield 50%, mp 178-180°C. IR (KBr) cm⁻¹: 1630 (C=O), 3200 (OH). 1H-NMR (CDCl₃, D₂O) δ ppm: 5.03 (s, 3H, OCH3), 2.35-2.80 (broad, 8H, piperazine H), 4.13 (s, 3H, OCH3), 4.13 (s, 2H, CH), 6.69 (d, 1H, furan H; f2H), 6.90-7.00 (m, 8H, CH=CH, furan and ArH). 10.00 (s, 1H, OH, exch.). Anal. Calcd for C24H26N2O4: C, 70.56; H, 6.45; N, 6.99. Found: C, 70.86; H, 6.08; N, 7.05.

General method for synthesis of 5-(5-aryl-4,5-dihydro-1H-pyrizal-3-yl)benzofuran-6-ol 3a-c (Scheme 1)

Hydrazine hydrate 98% (0.5 ml, 10 mmol) was added to a solution of the appropriate propenoic derivative 2 (10 mmol) in absolute EtOH (20 ml) and the reaction mixture was refluxed for 3 h. The solvent was concentrated under reduced pressure and the residue was crystallized from EtOH.

5-(4,5-Dihydro-5-phenyl-1H-pyrizal-3-yl)-4-methoxy-7-[(4-methylpiperazin-1-yl)methyl] benzofuran-6-ol (3a)

The general method was adopted using 2a. Yield 60%, mp 150-152°C. IR (KBr) cm⁻¹: 2940 (C=H aliphatic), 3150 (OH), 3300 (NH). 1H-NMR (CDCl₃, D₂O) δ ppm: 6.00-6.60 (broad, 8H, piperazine H), 3.62 (s, 3H, CH₃), 3.70 (d, 2H, CH₂, pyrazole), 3.93 (s, 3H, OCH₃), 4.07 (s, 3H, OCH₂), 4.38 (s, 2H, CH₂), 4.84 (s, 1H, CH pyrazole), 5.95 (s, 1H, NH, exch.), 6.66-7.52 (m, 7H, furan and ArH). 12.09 (s, 1H, OH, exch.). Anal. Calcd for C31H₂₃N₅O₇: C, 68.55; H, 6.71; N, 13.32. Found: C, 68.82; H, N, 13.11.
5-[[4,5-Dihydro-5-(4-methoxyphenyl)-1H-pyrazol-3-yl]-4-methoxy-7-[[4-methylpiperazin-1-yl]methyl benzofuran-6-ol (3b)]

The general method was adopted using 2b. Yield 55%, mp 145-148°C. IR (KBr) cm⁻¹: 2900 (C-H aliphatic), 1530 (OH), 3300 (NH).

1H-NMR (CDCl₃, D₂O) δ ppm: 1.25 (3H, CH₂CH₃), 1.94-2.40 (m, 8H, pyrazole), 2.67 (3H, CH₃), 3.07 (3H, OCH₃), 3.27 (3H, CH₃), 3.57 (3H, OCH₃), 3.75 (3H, OCH₃), 3.80 (2H, CH₂), 5.50 (1H, CH pyrazole), 6.60-7.10 (m, 6H, furan and ArH), 8.57 (1H, OH, exch.). Anal. Calc'd for C₂₂H₂₁N₂O₄: (462.54) C, 73.28; H, 6.32; N, 12.22.

5-[[Acetly-1, 5-dihydro-1H-pyrazol-3-yl]-4-methoxy-7-[[4-methylpiperazin-1-yl]methyl benzofuran-6-ol (4a)]

The general method was adopted using 2a. Yield 65%, mp 260-262°C. IR (KBr) cm⁻¹: 1650 (C=O), 2900 (C-H aliphatic), 1300 (OH).

1H-NMR (CDCl₃, D₂O) δ ppm: 1.25 (3H, CH₂CH₃), 1.94-2.40 (m, 8H, pyrazole), 2.67 (3H, CH₃), 3.07 (3H, OCH₃), 3.27 (3H, CH₃), 3.57 (3H, OCH₃), 3.75 (3H, OCH₃), 3.80 (2H, CH₂), 5.50 (1H, CH pyrazole), 6.60-7.10 (m, 6H, furan and ArH), 8.57 (1H, OH, exch.). Anal. Calc'd for C₂₂H₂₁N₂O₄: (462.54) C, 73.28; H, 6.32; N, 12.22.

5-[[Acetly-1, 5-dihydro-5-(4-methoxyphenyl)-1H-pyrazol-3-yl]-4-methoxy-7-[[4-methylpiperazin-1-yl]methyl benzofuran-6-ol (4b)]

The general method was adopted using 2b. Yield 60%, mp 242-244°C. IR (KBr) cm⁻¹: 1650 (C=O), 2900 (C-H aliphatic), 1300 (OH).

1H-NMR (CDCl₃, D₂O) δ ppm: 1.24 (3H, CH₂CH₃), 1.74-2.40 (m, 8H, pyrazole), 2.67 (3H, CH₃), 3.07 (3H, OCH₃), 3.27 (3H, CH₃), 3.57 (3H, OCH₃), 3.75 (3H, OCH₃), 3.80 (2H, CH₂), 5.50 (1H, CH pyrazole), 6.60-7.70 (m, 6H, furan and ArH), 9.57 (1H, OH, exch.). Anal. Calc'd for C₂₂H₂₁N₂O₄: (492.57) C, 75.84; H, 6.55; N, 11.37. Found: N, 11.38.

5-[[Acetly-5-[[4-chlorophenyl-4,5-dihydro-1H-pyrazol-3-yl]-4-methoxy-7-[[4-methylpiperazin-1-yl]methyl benzofuran-6-ol (4c)]

The general method was adopted using 2c. Yield 70%, mp 320-322°C. IR (KBr) cm⁻¹: 730 (C=O), 1660 (C=O), 2950 (C-H aliphatic), 1300 (OH). 1H-NMR (CDCl₃, D₂O) δ ppm: 2.01-2.68 (m, 8H, pyrazole), 3.31 (s, 3H, NCH₃), 3.45 (2H, CH₂ pyrazole), 3.99 (3H, OCH₃), 4.30 (2H, CH₂), 5.06 (1H, CH pyrazole), 6.60-7.75 (m, 6H, furan and ArH), 12.00 (1H, OH, exch.). Anal. Calc'd for C₂₂H₁₉ClN₂O₄: (531.05) C, 67.85; H, 5.88; N, 10.55. Found: C, 67.80; H, 5.85; N, 10.89.

5-[[4-Aryl-4,5-dihydro-1H-pyrazol-3-yl]-4-methoxybenzofuran-6-ol (6a-c)]

Compounds 6a-c were prepared according to the reported procedure 28.
General method for synthesis of 5-[5-aryl-4,5-dihydro-1-((4-
methylpiperazin-1-yl)methyl)-1H-pyrazol-3-yl]4-
 methoxybenzofuran-6-ol 7a-c (Scheme 2)

To a solution of 6a-c (10 mmol) in absolute ethanol (20 mL), N-
methylpiperazin hydrochloride (1.5 g, 11 mmol-) and paraformaldehyde (0.6 g, 20 mmol) were added. The mixture was
refluxed for 24 h. Excess solvent was evaporated under vacuum,
cooled and water was added. The mixture was neutralized with
dil. NH4OH and extracted with CHCl3. The chloroformed extract was
filtered through anhydrous Na2SO4 and the filtrate was concentrated
under vacuum. The residue was purified using preparative TLC.

Preparative TLC

TLC plates are prepared by mixing silica gel, with a small amount of
calcium sulfate (gypsum) and water. This mixture is spread as thick
slurry on glass plates (20 cm x 20 cm). The resultant plate is dried
and activated by heating in an oven for thirty minutes at 110 °C.

The mixture (about 0.5 g was dissolved in chloroform) is applied
to the plate as a thin even layer horizontally to and just above the
solvent level. When developed with solvent (chloroform/methanol
9:1), the compounds separate in horizontal bands with yellow color.
Lower band (Rf = 0.18-0.20) and upper band (Rf = 0.65-0.70) were
scraped off the backing material. The backing material is then
extracted with dichloromethane and filtered to give the isolated
material upon removal of the solvent. Lower bands yielded
compounds 3a-c (yield 60-70%) while, upper bands yielded
compounds 7a-c (yield 40-30%)

Fig. 3 Mapping of α1-AR antagonist hypothesis and Prazosin. The two α-
methoxy groups of quinazoline moiety occupied both Hy1 and
Hy2 (blue sphere) and the quinazoline N-1 atom is located
inside the positive ionizable feature IB (red sphere). The oxygen of
the carbonyl group attached to piperazine overlapped HBA (green
sphere), while the furan ring matched Hy3 (blue sphere). (For
interpretation of the references to colour in this figure legend,
the reader is referred to the web version of this article).

5-[4,5-Dihydro-1-((4-methylpiperazin-1-yl)methyl)-5-phenyl-
1H-pyrazol-3-yl]4-methoxybenzofuran-6-ol (7a)
The general method was adopted using 6a, mp 215-217°C. IR (KBr)
cm−1: 2937, 2940 (C-H aliphatic), 3150 (OH). ‘H-NMR
(DMSO-d6, D2O) δ ppm: 1.92-2.60 (broad, 8H, piperazine H),
3.10 (s, 3H, NCH3), 3.75 (d, 2H, CH2, pyrazole), 4.00 (s, 3H, OCH3),
4.30 (s, 2H, CH2), 4.80 (t, 1H, CH pyrazole), 6.90-7.98 (m, 8H, furan and ArH),
12.10 (s, 1H, OH, exch.). Anal. Calcd for C24H28N4O3
424 (M+). 426 (M+2) C, 68.55; H, 6.71; N, 13.32. Found: C, 68.77; H, 6.70; N, 13.11.

5-[4,5-Dihydro-5-(4-methoxyphenyl)-1-((4-methylpiperazin-1-
yl)methyl)-1H-pyrazol-3-yl]4-methoxybenzofuran-6-ol (7b)
The general method was adopted using 6b, mp 270-272°C. IR (KBr)
cm−1: 2950 (C-C aliphatic), 3200 (OH). ‘H-NMR
(DMSO-d6, D2O) δ ppm: 1.92-2.60 (m, 8H, piperazine H),
2.80 (s, 3H, NCH3), 3.24 (d, 2H, CH2, pyrazole), 3.81 (s, 3H, OCH3),
3.95 (s, 3H, OCH3), 4.35 (s, 2H, CH2), 5.06 (t, 1H, CH pyrazole),
6.60-7.95 (m, 7H, furan and ArH), 11.99 (s, 1H, OH, exch.). Anal. Calcd for C25H27NO3
444 (M+). 456 (M+2) C, 66.65; H, 6.71; N, 12.44. Found: C, 66.84; H, 6.30; N, 12.70.

Fig. 4 Mapping of α1-AR antagonist hypothesis and the most potent
compound 7b.

N-Methyl group attached to piperazine moiety occupied Hy1 and
OCH3, attached to phenyl occupied Hy2 (blue sphere). The third
hydrophobic Hy3 occupied by furan of benzofuran. The pyrazoline
N-1 atom is located inside the positive ionizable feature IB (red
sphere). The hydroxyl oxygen of benzofuran system overlapped HBA
(green sphere). (For interpretation of the references to colour in
this figure legend, the reader is referred to the web version of this
article).
5-[4-(Chlorophenyl)-4,5-dihydro-1-(4-methylpiperezin-1-yl)methyl]-1H-pyrazol-3-y1]-4,7-dimethoxybenzofuran-6-ol (9c)

The general method was adopted using 8c. Yield 60%, mp 196-198°C. IR (KBr) cm⁻¹: 739 (C–Cl), 2838, 2936 (C–H aliphatic), 3124 (C–H aromatic), 3055 (C–H aliphatic), 1654 (C=O), 1589 (N–H). 1H-NMR (CDCl₃, D₂O) δ ppm: 2.95 (6H, CH₃), 3.30 (s, 3H, NCH₃), 3.71 (d, 2H, CH₂pyrazole), 3.88 (s, 3H, OCH₃), 4.04 (s, 3H OCH₂), 4.31 (s, 2H, CH₂), 4.55 (t, 1H, CH pyrazole), 4.68 (s, 2H, CH₂), 7.60 (m, 7H, furan and ArH). Anal Calcd for C₃₂H₂₉ClN₄O₄: C, 61.91; H, 5.03; N, 11.55. Found: C, 62.25; H, 5.08; N, 11.51.

Computer modeling

In this work, we utilized phamacophore model for a set of potent α1-AR antagonists developed in a previous publication to prioritize the designed compounds by mapping them to the generated pharmacophore model²⁹.

The pharmacophore hypothesis was produced using Accelrys Discovery Studio 2.1, (Accelrys Inc., San Diego, CA, USA). Hip‐Hop algorithm, which identifies common chemical features from a set of ligands without the use of affinity data, was used to develop the pharmacophore model. The set of conformational models of each structure of the lead compounds was performed and was used to generate the common feature hypotheses. The ideal hypothesis encompassed five features; positive ionizable nitrogen, three hydrophobic pockets and a hydrogen bond acceptor group.

The calculation of fitting and relative energy Values of the best fitted compounds of the target compounds preceded as follows:

1) The structures of the test set of the target compounds were built using the Discovery studio software.
2) Their conformational models were generated in the energy range of 20 kcal/mol above the estimated global energy minimum to ensure conformational diversity.
3) The fitting of the tested compounds was performed using ligand pharmacophore mapping protocol. The best fit option has been selected which manipulate conformers of each compound to find, when possible, different mapping modes of the ligand within the model. Different mappings for all the conformers of each compound of the test set to the hypothesis were visualized and the fit values of the best-fitting conformers were found and listed in Table 1.

Pharmacology

In vivo biological evaluation

In vivo biological evaluation of the hypertensive effect of the tested compounds on the arterial blood pressure of normotensive adult cats was conducted adopting the reported method. Male cats weighing 2–3 kg were housed in the animal facility for 7 days prior to the experiment. Animals were kept at 22 ± 2 °C and 12 h light / 12 h dark cycle. Stressful condition or manipulation was avoided. Experiments were performed between 8-10 a.m. Cats were divided into groups, each of six animals. Cats were anesthetized with phenoxybarbitone sodium. The right femoral artery of the leg was exposed, cleared from connective tissue for a distance of about 2 cm and was used for blood pressure determination. The left femoral vein was exposed and then connected to saline infusion through a cannula. Tested drugs were all dissolved in 1 ml DMSO and then diluted with water to the final volume. Tested compounds were injected in 2 mmol / Kgdose through the femoral vein and washed with 1 ml saline. Blood pressure was recorded using Washington 400 MD2C mercury manometers on a kymograph. The effects of prazosin (reference drug) and saline/DMSO (control) were compared those of the tested compounds. The results are given in Table 2.

In vitro vasodilatation activity

The vasodilatation activity screening procedures were carried out according to the standard reported techniques by testing the effects of the most potent hypotensive derivatives 3c and 7b on isolated thoracic aortic rings of male Wistar rats (250–350 g). After light ether anesthesia, the rats were sacrificed by cervical dislocation and bleeding. The aorta was immediately excised, freed of extraneous tissues and prepared for isometric tension recording.

Aorta was cut in 3-5 mm long rings and placed in a vertical chamber "10 ml jacketed automatic multi-chamber organ bath system (model no. MB87086/C, Panlab, Spain)" filled with modified Krebs Henseleit solution composed of (in mM): NaCl, 118.0; KCl, 4.7; NaHCO₃, 25.0; CaCl₂, 1.8; NaH₂PO₄, 1.2; MgSO₄, 1.2; glucose, 11.0 and oxygenated with carbogen gas (95% O₂/5% CO₂) at 37±0.5 °C. Each aorta 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.

Fig. 5 Mapping of α1-AR antagonist hypothesis and the second potent compound 3c. The first hydrophobic H1 did not occupy. Halogen atom attached to phenyl moiety occupied H2, while methyl of OCH₃ attached to benzofuran occupied H3 (blue sphere). The oxygen atom of OH group attached to benzofuran is located inside the positive ionizable feature IB (red sphere). The oxygen of benzofuran system overlapped HBA (green sphere). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

Preparations were stabilized fewer than 2 g resting tension during 2 h. The lack of endothelium was confirmed by the absence of acetylcholine (1μM) vasorelaxation effect in aortic rings precontracted by noradrenaline (0.1μM). The contractile response to norepinephrine hydrochloride (10⁻⁵ M) was measured before and after exposure to increasing concentrations of the tested compounds. The compounds 3c and 7b as well as prazosin hydrochloride (as reference standard) were dissolved in dimethyl sulfoxide (DMSO) as stock solution (10 ml of 0.01M). 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 data are reported (Table 2, Fig. 6) and the potency (IC₅₀, concentration necessary for 50 % reduction of maximal norepinephrine hydrochloride induced contracture) was determined by the best fit line technique.

RESULTS AND DISCUSSION

Chemistry

α,β-Unsaturated carbonyl compounds 2 were synthesized by condensation of 1, R = H, OCH₃, 30 or CH₂N(CH₃)₂NCH₃, 27 and the appropriate aromatic aldehydes in ethanolic sodium hydroxide. In the present investigation, 2 were reacted with different nuclophiles as hydrazine hydrate and phenyl hydrazine. When, 2 were reacted with hydrazine hydrate in ethanol the corresponding pyrazolines 3 were obtained through 1,4-addition to the carbonyl group, followed by dehydration and rearrangement. On the other hand, when 2 were reacted with hydrazine hydrate in glacial acetic acid, N-acetylpyrazolines 4 were obtained. N-phenyl-2-pyrazolines 5 were obtained through the reaction of 2 with phenyl hydrazine (Scheme 1).

Reactions of 5-[5-(4-aryl-4,5-dihydro-1H-pyrazol-3-yl)-4-methoxybenzofuran-6-ol 6a-c with N-methylpiperezine hydrochloride and paraformaldehyde, Mannich reactin, two positional isomers were obtained 3a-c and 7a-c. The later compounds were separated by
application of preparative TLC technique (Scheme 2). On the other hand, hydrochloride and paraformaldehyde, yielded one product 9a-c (Scheme 3).

5-(5-aryl-4,5-dihydro-1H-pyrazol-3-yl)-4,7-dimethoxybenzofuran-6-ol 8a-c reacted with N-methylpiperazine and paraformaldehyde, yielded one product 9a-c (Scheme 3).

Table 1: Number and types of contacts, fitting and relative energy values of the best-fitted conformers of prazosin and compounds 3-5, 7 and 9 by mapping onto the pharmacophore model of the α1-AR antagonist hypothesis. Also, effect of test compounds on blood pressure (% inhibition) of anaesthetized normotensive cats compared to prazosin at dose 2 mmol./kg

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<th>Compound</th>
<th>Number of contacts</th>
<th>Types of contacts</th>
<th>Fitting values</th>
<th>Relative energy (kcal. mol⁻¹)</th>
<th>% Inhibition ± SE</th>
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<td>Prazosin</td>
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<td>7c</td>
<td>3</td>
<td>Hy₂ = 1</td>
<td>2.99</td>
<td>44.1588</td>
<td>14.7 ± 0.58</td>
</tr>
<tr>
<td>9a</td>
<td>3</td>
<td>HBA₅ = 1, Hy₃ = 1</td>
<td>2.99</td>
<td>23.563</td>
<td>10.5 ± 0.53</td>
</tr>
<tr>
<td>9b</td>
<td>4</td>
<td>Hy₂ = 1</td>
<td>3.73</td>
<td>39.6844</td>
<td>14.2 ± 0.43</td>
</tr>
<tr>
<td>9c</td>
<td>4</td>
<td>Hy₂ = 1</td>
<td>3.21</td>
<td>18.045</td>
<td>13.1 ± 0.57</td>
</tr>
</tbody>
</table>

HBA (green) = Hydrogen bond, acceptor feature.
Hy (blue) = Hydrophobic bond.
IB (red) = Ionizable bond = Ionizable Positive.

All the results are significant different from control value at p < 0.05 using one-way ANOVA test.
Table 2: Concentration of prazosin, 3c and 7b necessary to reduce the maximal norepinephrine hydrochloride induced contracture by 50% (IC50) in thoracic rat aortic rings

<table>
<thead>
<tr>
<th>Compound</th>
<th>Prazosin</th>
<th>3c</th>
<th>7b</th>
</tr>
</thead>
<tbody>
<tr>
<td>IC50 (mM)</td>
<td>0.48</td>
<td>0.49</td>
<td>0.21</td>
</tr>
</tbody>
</table>

Computer modeling

Pharmacophore modeling method has been widely used as a key tool of computer aided drug design in the lead discovery and optimization. Pharmacophore can also be used to rationalize the relationship between the structural features and pharmacological activity. In this study, the generated α1-AR antagonist hypothesis was carried out adopting reported method by using Accelrys Discovery Studio 2.1 software and Hip-Hop modules.

The ideal hypothesis encompassed five features namely; positive ionizable (IB, red sphere), hydrogen bonding acceptor (HBA, green sphere) and three hydrophobic features (Hy_1, Hy_2 and Hy_3, blue sphere). Molecular modeling simulation studies were then conducted by measuring the fit values, separately, between the conformational models of prazosin (reference), 3a-c, 4a-c, 5a-c, 7a-c and 9a-c, and the α1-AR antagonist hypothesis. The results of number and types of contacts, fitting and relative energy values of the best-fitted conformers with this hypothesis are given in Table 1.

The fitting values may be a guide for estimating relative affinities of these compounds to their receptor while the relative energy is a guide of stability of the compounds. Also, the mapping of α1-AR antagonist hypothesis with prazosin and the most active compound 7b and 3c illustrated that all chemical functionalities of the model are all matched by the chemical groups of them (Figs. 3-5).

Evaluation of how well the prepared compounds were able to fit the α1-AR pharmacophore hypothesis and the correlation of the fitting values with the hypotensive activity highlighted that:

1- Prazosin has the five features; that means number of its contacts is five. Fig.3 shows mapping of α1-AR antagonist hypothesis and prazosin. The two α-methoxy groups of quinazoline moiety occupied both Hy_1 and Hy_2 (blue sphere) and the quinazoline N-1 atom is located inside the positive ionizable feature IB (red sphere). The oxygen of the carbonyl group attached to piperazine overlapped HBA (green sphere), while the furan ring matched Hy_3 (blue sphere).

2- Based on the hypotensive activity, the most potent compound 7b occupied the five features. Fig.4 shows its mapping with α1-AR antagonist hypothesis. N-Methyl group attached to piperazine moiety occupied Hy_1 and OCH3 attached to phenyl occupied Hy_3 (blue sphere). The third hydrophobic Hy_3 occupied by furan of benzofuran. The pyrazoline N-1 atom is located inside the positive ionizable feature IB (red sphere). The hydroxyl oxygen of benzofuran system overlapped HBA (green sphere).

3- Compound 7b which showed high fit value (3.48) closely related to that of Prazosin (3.53). Although, compounds 4b, 4c, and 9b have higher fit values (3.79, 3.64 and 3.73, respectively), exhibited less hypotensive activity. The later compounds have higher relative energy (91.0642, 51.0548.0, 39.6844 kcal. mol⁻¹, respectively).
The first hydrophobic $\text{Hy}_1$ did not occupy. Halogen atom attached to phenyl moiety occupied $\text{Hy}_2$, while methyl of OH attached to benzo-furan occupied $\text{Hy}_3$ (blue sphere). The oxygen atom of OH group attached to benzo-furan is located inside the positive ionizable feature IB (red sphere). The oxygen of benzo-furan system overlapped HBA (green sphere).

5. The presence of pyrazoline moiety spacer between benzo-furan and piperazine systems is a key parameter affecting the fitting efficiency of each compound to the pharmacophore (cf. 3c and 7b).

6. Hydrogen bond pattern (HBA) is essential features for the relationship between structure properties and $\alpha_1$-binding affinity. The least potent compounds 3a, 5a, 5b, and 5c do not occupy this feature (fit value 2.99).

**Pharmacology**

The experimental tests on animals have been performed in accordance with the Institutional Ethical Committee approval, Faculty of Pharmacy, Cairo University.

**In vivo hypotensive activity**

All the newly synthesized final compounds 3a-c, 4a-c, 5a-c, 7a-c, 9a-c and prazosin were evaluated for their in vivo hypotensive activity on blood pressure of normotensive cats at equimol doses (2 mmol/kg)\textsuperscript{23}. Furthermore, the molecular modeling simulation study predicted that, most of these compounds would have probable affinity for the $\alpha_1$-AR antagonist hypothesis. The results of hypotensive evaluation indicated that most of the tested compounds have the ability to reduce the blood pressure with different degrees (Table 1).

Compounds 7b and 3c showed the most potent hypotensive activity, but less than prazosin. Moreover, compounds 3b, 4b, 7a, 7a, 7c, 9a, and 9c elicited moderate activity. Compounds 3a, 4a, 5a, 5b and 5c had weak hypotensive effect.

**Structure activity relationship (SAR)**

- The data in table 1 indicates that compounds 7b and 3c produced low hypotensive effect compared to prazosin. The presence of pyrazoline moiety spacer between benzo-furan and piperazine systems is a crucial element that accounts for the relationship between structure properties and pharmacologically potency (3c and 7b).
- Replacement of the pyrazoline spacer by methylene linker (only one carbon) led to several compounds characterized by lower conformational flexibility, which might be responsible for low fit to the $\alpha_1$-AR pharmacophore model and consequently less potent hypotensive activity. Moreover, Betti et al.\textsuperscript{25} reported the gradual increase in affinity to $\alpha_1$-ARs by increasing the length of the polymethylene spacer between the arylpiperazine moiety and the pyridazineone from 2 up to 7 carbons.
- Blocking of NH of pyrazoline with phenyl abolishes the hypotensive activity (5a, 5b and 5c). Free NH (3a, 3b, 3c, 9a, 9b, and 9c) or substitution with acetyl group (4b, and 4c) was favorable for hypotensive activity. The higher hypotensive potency was obtained through N-substituted with piperazine moiety (7b).

3.3.3. In vitro hypotensive activity

Based on the hypotensive activity, the most potent compounds 7b and 3c were subjected to functional bioassay to evaluate their $\alpha_1$-adrenoceptors antagonist activity, according to the standard procedure.\textsuperscript{24,25} The antagonistic activity was assessed by inhibition of (±)norepinephrine-induced contraction on isolated rat aorta tissues which predominantly express the $\alpha_1$-AR subtype.\textsuperscript{26,27} Stimulation of $\alpha_1$-AR subtype is known to cause blood vessels contraction and control blood pressure. From the observed data (Table 2, Fig. 6), it can be noticed that the two tested compound 7b and 3c had lower or equal IC50 relative to prazosin. Furthermore, the results of the antagonistic potency were in agreement with the in vivo hypotensive activity, where the most potent hypotensive agents 7b and 3c revealed the lowest IC50 (0.21 and 0.49 mM, respectively).

In summary, the consistency between the in vivo hypotensive results and the functional bioassay data proved that the obtained hypotensive effect is mediated through the blockage of $\alpha_1$-adrenoceptors.

**CONCLUSION**

Some compounds belonging to a class of piperazinomethyl benzo-furan linked to pyrazoline moiety have been synthesized. Fitting of the proposed compounds to previously built pharmacophore model\textsuperscript{20} was done to prioritize the synthesized compounds and to correlate the chemical structure of the studied compounds to their pharmacological data.

All the newly synthesized final compounds 3a-c, 4a-c, 5a-c, 7a-c, 9a-c and prazosin were evaluated for their in vivo hypotensive activity on blood pressure of normotensive cats. Moreover, the most potent hypotensive compounds 7b and 3c were tested for their $\alpha_1$-adrenoceptors antagonist activity on isolated thoracic rat aorta. The two tested compounds revealed activity, but less than prazosin, reference drug. Some structural features have been demonstrated to markedly affect the affinity of $\alpha_1$-AR antagonists and hypotensive activity. The presence of pyrazoline moiety as a spacer between benzo-furan and piperazine systems is essential for the relationship between structure properties and pharmacologically potency.

**ACKNOWLEDGEMENT**

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**REFERENCES**

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