Novel 4-substituted-2(1H)-phthalazinone derivatives: synthesis, molecular modeling study and their effects on α-receptors

Nadia A. Khalil, Eman M. Ahmed, Hosam A. Elshihawy & Sawsan A. Zaitone

Archives of Pharmacal Research

ISSN 0253-6269
Volume 36
Number 6

Arch. Pharm. Res. (2013) 36:671-683
DOI 10.1007/s12272-013-0095-5

Springer

Arch. Pharm. Res. (2013) 36:671–683
DOI 10.1007/s12272-013-0095-5

Archives of Pharmacal Research
www.springer.com/12272

RESEARCH ARTICLE

Novel 4-substituted-2(1*H*)-phthalazinone derivatives: synthesis, molecular modeling study and their effects on α-receptors

Nadia A. Khalil · Eman M. Ahmed · Hosam A. Elshihawy · Sawsan A. Zaitone

Published online: 1 April 2013

Abstract Novel 4-(4-bromophenyl)phthalazine derivatives connected via an alkyl spacer to amine or *N*-substituted piperazine were designed and synthesized as promising α-adrenoceptor antagonists. The structures of the phthalazine derivatives were established using elemental and spectral analyses. Twelve of the tested compounds displayed significant α-blocking activity. Molecular modeling studies were performed to rationalize the biological results. Among the tested compounds, **7j** displayed the best-fitting score and the highest in vitro activity.

Keywords Phthalazinones · α-Adrenoceptor antagonists · Molecular modeling · Arylpiperazine

Introduction

The adrenergic system is an essential regulator of neuronal, endocrine, cardiovascular, and metabolic functions. Adrenergic receptors (ARs) are endogenously activated by adrenaline and noradrenaline and are members of the G-protein coupled receptors (GPCRs) super-family that transmit their signal across the plasma membrane (Calzada and de Artiñano 2001).

N. A. Khalil · E. M. Ahmed (✉)
Department of Pharmaceutical Organic Chemistry, Faculty of Pharmacy, Cairo University, Cairo, Egypt
e-mail: dr_eman2001@hotmail.com

H. A. Elshihawy
Organic Chemistry Division, Faculty of Pharmacy, Suez Canal University, Ismailia 41522, Egypt

S. A. Zaitone
Department of Pharmacology & Toxicology, Faculty of Pharmacy, Suez Canal University, Ismailia 41522, Egypt

α-Adrenergic receptors (α-ARs) play an important role in the regulation of a variety of physiological processes, particularly within the cardiovascular system and are divided into two main subtypes α_1- and α_2-ARs (Jain et al. 2008). The α_1-ARs mediate actions in the sympathetic nervous system through the binding of the catecholamines, epinephrine and norepinephrine (Romeo et al. 2011). Compounds acting as antagonists at various post-junctional α_1-ARs are frequently used in the therapy of hypertension, benign prostatic hyperplasia, lower urinary tract symptoms and cardiac arrhythmia (Frishman and Kotob 1999; Kojima et al. 2009). Prazosin **I**, a common antihypertensive drug, is considered the prototype of this class which selectively blocks postsynaptic α_1-ARs (Jain et al. 2008), (Fig. 1).

In this context, the search for selective α_1-adrenoceptor antagonists has been, and still is, an important goal in the medicinal chemistry. In recent decades, various new α_1-AR antagonists have been synthesized, many of them contain arylpiperazine moiety (Handzlik et al. 2008; Handzlik et al. 2010; Sharma et al. 2010). It has been reported that arylpiperazine moiety linked to different heterocyclic systems through polymethylene spacer is a key element for α_1-AR affinity (Barbaro et al. 2001; Kulig et al. 2009; Abou-Seri et al. 2011). Some known arylpiperazine derivatives such as SGB 1534 **II**, 5-methylurapidil **III**, RA36 **IV**, WAY-100635 **V** and the imidazopyridazinone derivative **VI** can be considered as classical examples of potent α_1-adrenoceptor antagonists which is useful in various pharmacological assays including binding studies, as well as in vivo tests (Betti et al. 2002) (Fig. 1). Besides, there are many reports in the literature regarding the effect of compounds containing a pyridazin-3(2*H*)-one fragment and their benzo analogue, phthalazinone, on the cardiovascular system (Demirayak et al. 2004a; Del Olmo et al. 2006; Strappaghetti et al. 2006). A phthalazine derivative, hydralazine, one of the first antihypertensive agents, is considered as a

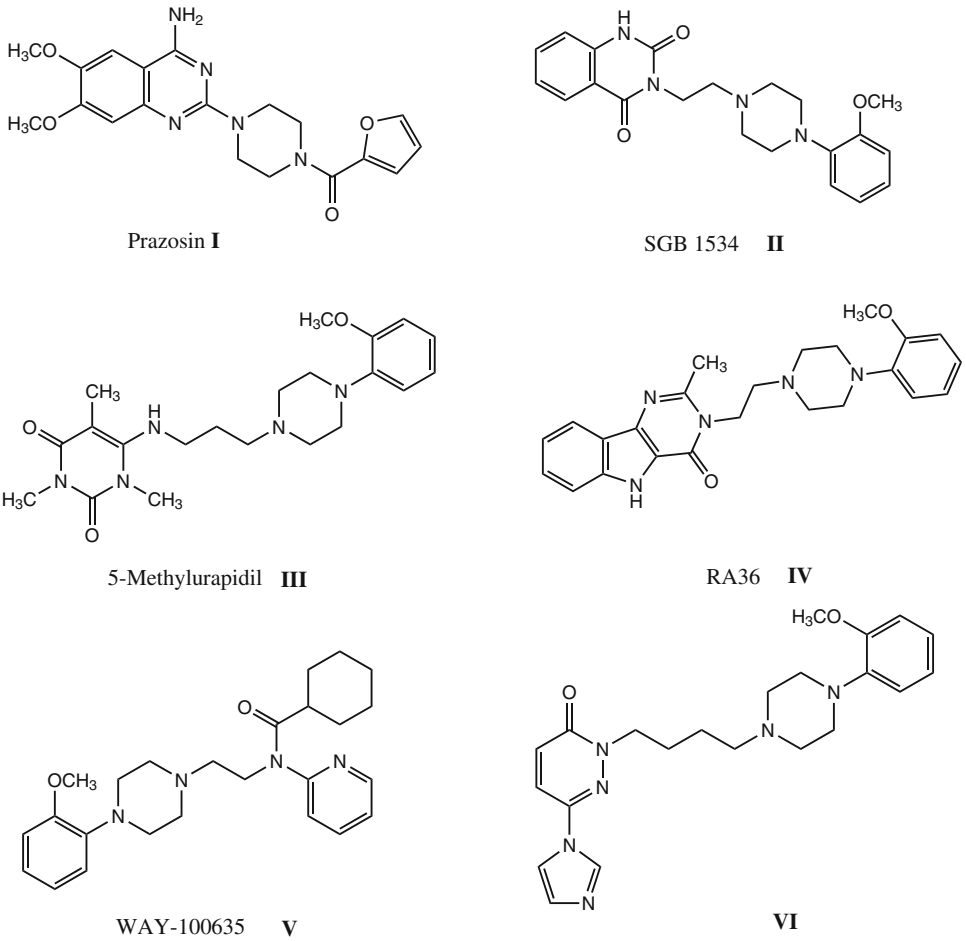


Fig. 1 Structures of potent selective α_1-adrenoceptor antagonists

lead for developing new drugs, due to its direct vasodilator action (Bang et al. 1998). Structural modification of hydralazine led to the discovery of phthalazine derivatives with broad spectra on the cardiovascular system including antihypertensive effects (Demirayak et al. 2004b), inhibition of platelet aggregation (Sotelo et al. 2002), and inhibition of phosphodiesterases (Kagayama et al. 2009).

A specially useful model considering the structural requirements for α_1-AR affinity concerning phenylpiperazine derivatives, has been postulated by Barbaro et al. (2001). Barbaro's model, has postulated five pharmacophore features: A positively ionizable group, corresponding to the more basic nitrogen atom of the arylpiperazine ring (A). An *ortho*- or *para* substituted phenyl ring, both of which constitute the arylpiperazine system (B) A polar group (corresponding to the phthalazinone ring) that provides a hydrogen bond acceptor (HBA) feature, filling one of the portions of the pharmacophore that is required at the edge of the molecule opposite the arylpiperazine moiety (C). Additionally, they possess an alkyl spacer between piperazine nitrogen and nitrogen placed close to carbonyl oxygen (HBA), which may consist of 2–7 carbons (D). Finally, a hydrophobic moiety, corresponding to the terminal molecular portions directly linked to the phthalazinone ring, was hypothesized to fit one of the five features of the model (E).

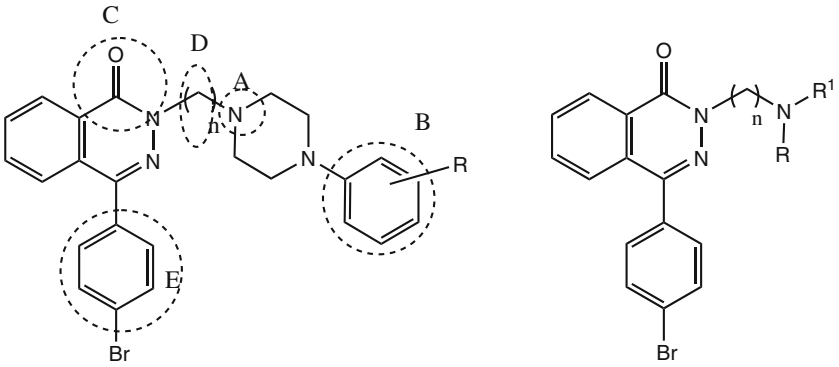
Taking in consideration the structural requirements for α_1-AR affinity, the final phthalazinone targets, **4a–f**, **5a–c**, **6** and **7a–j** were designed and synthesized. This study was directed at the modification of (i) a pharmacophoric portion constituted by the introduction of 2- or 4-methoxy; 2-ethoxy; 4-chloro into the phenyl ring or replacement the phenylpiperazine moiety by a tertiary amino function (ii) to probe the influence of the terminal cyclic substituent on α-AR blocking activity, a 4-bromophenyl moiety was placed at the 4-position of the phthalazin-1(*2H*)-one system (iii) an alkyl chain linker of one, two or three carbon atoms between these two substructures for investigation as α-AR antagonists, (Fig. 2).

Materials and methods

Chemistry

Melting points were determined on Griffin apparatus and the values given are uncorrected. IR spectra were determined on Shimadzu IR 435 spectrophotometer (KBr, cm^{-1}). ^{1}H-NMR

Fig. 2 General structures of the newly designed compounds



4a-e, 5a-c, 7g-j **4f, 6, 7a-f**

spectra were carried out using Varian Mercury-300 (300 MHz) Spectrophotometer using TMS as internal standard. Chemical shift values are recorded in ppm on δ scale, Microanalytical Center, Cairo University, Egypt. Mass spectra were recorded on a GCMP-QP1000 EX Mass spectrometer, Microanalytical Center, Cairo University, Egypt. Elemental analyses were carried out at the Microanalytical Center, Cairo University, Egypt. Progress of the reactions was monitored using TLC sheets precoated with UV fluorescent silica gel Merck 60F 254 using acetone/benzene (1:9) and were visualized using UV lamp.

All chemicals were obtained from Aldrich, Fluka, or Merck chemicals.

4-(Bromophenyl)phthalazin-1(*2H*)-one was prepared as reported (Colotta et al. 1994).

4-(4-Bromophenyl)-2-(2-chloroethyl)phthalazin-1(2H)-one ***(2)***

A mixture of 4-(bromophenyl)phthalazin-1(*2H*)-one **(1)** (3.01 g, 0.01 mol), 1-bromo-2-chloroethane (7.17 g, 4.16 mL, 0.05 mol) and anhydrous K_2CO_3 (6.90 g, 0.05 mol) in anhydrous DMF (30 mL) was stirred at room temperature 2 h. The mixture was poured onto water, the resulting white solid was filtered, dried and crystallized from benzene-petroleum ether (60–80 °C).

Yield 62 %; mp 119–120 °C; IR (KBr) cm^{-1}: 3,097 (C–H aromatic), 2,950, 2,897 (C–H aliphatic), 1,658 (C=O), 1,589 (C=N); ^{1}HNMR (DMSO–d_6): 4.04 (t, 2H, $J = 6.3$ Hz, CH_2–N), 4.53 (t, 2H, $J = 6.3$ Hz, CH_2–Cl), 7.54–7.95 (m, 6H, ArH), 8.32–8.40 (m, 2H, ArH); MS (EI) *m/z* (% rel. Int.): 300 ($C_{14}H_9BrN_2O$, 69.82), 362 (M^+, 36.71), 364 (M + 2, 46.65), 366 (M + 4, 14.62). Anal. Calcd for $C_{16}H_{12}BrClN_2O$: C, 52.85; H, 3.33; N, 7.70. Found: C, 52.55; H, 3.15; N, 7.85.

4-(4-Bromophenyl)-2-(3-chloropropyl)phthalazin-1(2H)-one ***(3)***

The compound **3** was prepared following the procedure described for the compound **2** by using 4-(bromophenyl) phthalazin-1(*2H*)-one **(1)** (3.01 g, 0.01 mol) and 1-bromo-3-chloropropane (4.72 g, 2.96 mL, 0.03 mol). The reaction mixture was poured onto cold water, extracted with methylene chloride (3 × 10 mL) and dried (Na_2SO_4). The solvent was evaporated under reduced pressure, the residue was triturated with benzene, and the formed white solid product was filtered then crystallized from benzene-petroleum ether (60–80 °C).

Yield 47 %; mp 161–162 °C; IR (KBr) cm^{-1}: 3,070 (C–H aromatic), 2,978, 2,885 (C–H aliphatic), 1,654 (C=O), 1,604 (C=N); ^{1}HNMR (DMSO–d_6); 1.85–2.05 (m, 2H, –CH_2–), 2.68 (t, 2H, $J = 6.3$ Hz, CH_2–N), 3.98 (t, 2H, J=6.3 Hz, CH_2–Cl), 6.95–7.67 (m, 6H, ArH), 7.90–8.03 (m, 2H, ArH); MS (EI) *m/z* (% rel. Int.): 300 ($C_{14}H_9BrN_2O$, 100), 376 (M^+, 0.08), 378 (M + 2, 0.07), 380 (M + 4, 0.07). Anal. Calcd for $C_{17}H_{14}BrClN_2O$: C, 54.06; H, 3.74; N, 7.42. Found: C, 53.95; H, 3.88; N, 7.35.

General procedure for the synthesis of compounds ***4a–f*** *and* ***5a–c***

A mixture of an appropriate **2** or **3** (0.003 mol), anhydrous K_2CO_3 (2.07 g, 0.015 mol) and few specks of KI in dry acetonitrile (20 mL) was heated under reflux for 30 min. An appropriate secondary amine or *N*-arylpiperazine (0.009 mol) was added to the hot reaction mixture and the heating was continued for 10 h. After cooling, the reaction mixture was poured with continuous stirring onto ice-cold water (25 mL). The separated solid was filtered, and crystallized from isopropanol.

4-(4-Bromophenyl)-2-[2-(4-phenylpiperazin-1-yl)ethyl] phthalazin-1(2H)-one ***(4a)*** Yield: 58 %; mp 240–241 °C; IR (KBr) cm^{-1}: 3,074 (C–H aromatic), 2,947, 2,850 (C–H aliphatic), 1,651 (C=O), 1,600 (C=N); ^{1}HNMR (DMSO–d_6): 2.60–2.64 (m, 4H, piperazine), 2.80 (t, 2H, CH_2–N), 2.82–2.95 (m, 4H, piperazine), 4.37 (t, 2H, CH_2–N), 6.90–7.86 (m, 11H, ArH), 8.21–8.30 (m, 2H, ArH); EIMS (% rel. abundance): 175 ($C_{11}H_{15}N_2$, 78.67), 312 ($C_{15}H_9BrN_2O$, 27.06), 488 (M^+, 6.63), 490 (M + 2, 4.84).

Anal. Calcd for $C_{26}H_{25}BrN_4O$, C, 63.81; H, 5.15; N, 11.45; Found C, 63.55; H, 5.00; N, 11.54.

4-(4-Bromophenyl)-2-{2-[4-(4-chlorophenyl)piperazin-1-yl] ethyl}phthalazin-1(2H)-one ***(4b)*** Yield: 64 %; mp 141–142 °C; IR (KBr) cm^{-1}: 3,074 (C–H aromatic), 2,950, 2,850 (C–H aliphatic), 1,651 (C=O), 1,602 (C=N); ^{1}HNMR (DMSO–d_6): 2.58–2.62 (m, 4H, piperazine), 2.79 (t, 2H, CH_2–N), 2.98–3.15 (m, 4H, piperazine), 4.37 (t, 2H, CH_2–N), 6.91–6.95 (m, 2H, ArH), 7.19–7.22 (m, 2H, ArH), 7.56–7.92 (m, 6H, ArH), 8.31–8.39 (m, 2H, ArH); EIMS (% rel. abundance): 209 ($C_{11}H_{14}ClN_2$, 100), 522 (M^+, 3.97), 524 (M + 2, 3.77), 526 (M + 4, 1.51). Anal. Calcd for $C_{26}H_{24}BrClN_4O$, C, 59.61; H, 4.62; N, 10.70; Found C, 59.55; H, 4.82; N, 10.95.

4-(4-Bromophenyl)-2-{2-[4-(2-ethoxyphenyl)piperazin-1-yl] ethyl}phthalazin-1(2H)-one ***(4c)*** Yield: 65 %; mp 167–168 °C; IR (KBr) cm^{-1}: 3,043 (C–H aromatic), 2,935, 2,897 (C–H aliphatic), 1,654 (C=O), 1,604 (C=N); ^{1}HNMR (DMSO–d_6): 1.31 (t, 3H, J = 6.9 Hz, CH_3CH_2), 2.58–2.64 (m, 4H, piperazine), 2.79 (t, 2H, J = 6.9 Hz, CH_2–N), 2.85–3.01 (m, 4H, piperazine), 3.99 (q, 2H, J = 6.9 Hz, CH_3CH_2), 4.35 (t, 2H, J = 6.3 Hz, CH_2–N), 6.82–6.96 (m, 4H, ArH), 7.44–7.90 (m, 6H, ArH), 8.27–8.36 (m, 2H, ArH); EIMS (% rel. abundance): 219 ($C_{13}H_{19}N_2O$, 100), 532 (M^+, 5.39), 534 (M + 2, 5.44). Anal. Calcd for $C_{28}H_{29}BrN_4O_2$, C, 63.04; H, 5.48; N, 10.50; Found C, 62.90; H, 5.35; N, 10.68.

4-(4-Bromophenyl)-2-{2-[4-(2-methoxyphenyl)piperazin-1-yl] ethyl}phthalazin-1(2H)-one ***(4d)*** Yield: 60 %; mp 234–235 °C; IR (KBr) cm^{-1}: 3,105 (C–H aromatic), 2,931, 2,893 (C–H aliphatic), 1,654 (C=O), 1,589 (C=N); ^{1}HNMR (DMSO–d_6): 2.56–2.59 (m, 4H, piperazine), 2.85 (t, 2H, CH_2–N), 3.00–3.09 (m, 4H, piperazine), 3.77 (s, 3H, OCH_3), 4.38 (t, 2H, CH_2–N), 6.85–6.94 (m, 2H, ArH), 7.54–7.91 (m, 8H, ArH), 8.32–8.35 (m, 2H, ArH); EIMS (% rel. Abundance): 205 ($C_{12}H_{17}N_2O$, 2.53), 313 ($C_{15}H_{10}BrN_2O$, 0.09), 518 (M^+, 0.13), 520 (M + 2, 0.12). Anal. Calcd for $C_{27}H_{27}BrN_4O_2$, C, 62.43; H, 5.24; N, 10.79; Found C, 62.11; H, 5.35; N, 10.66.

4-(4-Bromophenyl)-2-{2-[4-(4-methoxyphenyl)piperazin-1-yl] ethyl}phthalazin-1(2H)-one ***(4e)*** Yield: 70 %; mp 225–226 °C; IR (KBr) cm^{-1}: 3,090 (C–H aromatic), 2,943, 2,835 (C–H aliphatic), 1,654 (C=O), 1,596 (C=N); ^{1}HNMR (DMSO–d_6): 2.59–2.62 (m, 4H, piperazine), 2.80 (t, 2H, CH_2–N), 2.92–3.05 (m, 4H, piperazine), 3.66 (s, 3H, OCH_3), 4.35 (t, 2H, CH_2–N), 6.80–6.84 (m, 2H, ArH), 6.94 (d, 2H, ArH), 7.44 (d, 2H, ArH), 7.57–7.91 (m, 4H, ArH), 8.30–8.45 (m, 2H, ArH); EIMS (% rel. Abundance): 205 ($C_{12}H_{17}N_2O$, 100), 313 ($C_{15}H_{10}BrN_2O$, 4.10), 328 ($C_{16}H_{13}BrN_2O$, 27), (M^+, 10.94), 520 (M + 2, 10.76). Anal. Calcd for $C_{27}H_{27}BrN_4O_2$, C, 62.43; H, 5.24; N, 10.79; Found C, 62.15; H, 5.00; N, 10.98.

4-(4-Bromophenyl)-2-[2-(piperidin-1-yl)ethyl]phthalazin-1 (2H)-one ***(4f)*** Yield: 62 %; mp 99–100 °C; IR (KBr) cm^{-1}: 3,081 (C–H aromatic), 2,934, 2,863 (C–H aliphatic), 1,651 (C=O), 1,600 (C=N); ^{1}HNMR ($CDCl_3$): 1.21–1.26 (m, 2H, CH_2 piperidine), 1.50–1.75 (m, 4H, $2CH_2$ piperidine), 1.85–2.10 (m, 4H, $2CH_2$ piperidine), CH_2–N), 3.98 (t, 2H, J = 6.9 Hz, CH_2–N), 4.61 (t, 2H, J=6.3 Hz, CH_2–N), 7.27–8.52 (m, 6H, ArH), 8.49 (d, 2H, ArH); Anal. Calcd for $C_{21}H_{22}BrN_3O$, C, 61.17; H, 5.38; N, 10.19; Found C, 61.45; H, 5.15; N, 10.22.

4-(4-Bromophenyl)-2-{3-[4-(4-chlorophenyl)piperazin-1-yl] propyl}phthalazin-1(2H)-one ***(5a)*** Yield: 55 %; mp 143–144 °C; IR (KBr) cm^{-1}: 3,074 (C–H aromatic), 2,947, 2,816 (C–H aliphatic), 1,662 (C=O), 1,593 (C=N); ^{1}HNMR (DMSO–d_6) δ: 2.35–2.45 (m, 2H, CH_2), 2.60–2.70 (m, 4H, piperazine), 2.80 (t, 2H, J = 6.3 Hz, CH_2–N), 3.18–3.20 (m, 4H, piperazine), 4.20 (t, 2H, J = 6.3 Hz, CH_2–N), 6.89–7.97 (m, 10H, ArH), 8.32–8.35 (m, 2H, ArH); EIMS (% rel. abundance): 312 ($C_{15}H_9^{79}BrN_2O$, 16.97), 314 ($C_{15}H_9^{81}BrN_2O$,17.44), 536 (M^+, 0.06), 538 (M + 2, 0.02). Anal. Calcd for $C_{27}H_{26}BrClN_4O$, C, 60.29; H, 4.87; N, 10.42; Found C, 60.10; H, 4.55; N, 10.58.

4-(4-Bromophenyl)-2-{3-[4-(2-ethoxyphenyl)piperazin-1-yl] propyl}phthalazin-1(2H)-one ***(5b)*** Yield: 55 %; mp 198–199 °C; IR (KBr) cm^{-1}: 3,090 (C–H aromatic), 2,927, 2,855 (C–H aliphatic), 1,651 (C=O), 1,605 (C=N); ^{1}HNMR (DMSO–d_6) δ: 1.32 (t, 3H, CH_3CH_2), 2.40–2.45 (m, 2H, CH_2), 2.55–2.60 (m, 4H, piperazine), 2.85 (t, 2H, J = 6.3 Hz, CH_2–N), 3.22–3.28 (m, 4H, piperazine), 4.05 (q, 2H, CH_3CH_2), 4.32 (t, 2H, J = 6.3 Hz, CH_2–N), 7.48–7.83 (m, 10H, ArH), 8.24–8.26 (m, 2H, ArH); EIMS (% rel. abundance): 219 ($C_{13}H_{19}N_2O$, 8.17), 233 ($C_{14}H_{21}N_2O$, 36.74), 313 ($C_{15}H_{10}^{79}BrN_2O$, 3.19), 315($C_{15}H_{10}^{81}BrN_2O$, 3.55), 546 (M^+, 3.05), 548 (M + 2, 2.58). Anal. Calcd for $C_{29}H_{31}BrN_4O_2$, C, 63.62; H, 5.71; N, 10.23; Found C, 63.55; H, 5.82; N, 10.35.

4-(4-Bromophenyl)-2-{3-[4-(4-methoxyphenyl)piperazin-1-yl]propyl}phthalazin-1(2H)-one ***(5c)*** Yield: 67 %; mp 128–129 °C; IR (KBr) cm^{-1}: 3,100 (C–H aromatic), 2,927, 2,855 (C–H aliphatic), 1,657 (C=O), 1,620 (C=N); ^{1}HNMR (DMSO–d_6) δ: 2.32–2.40 (m, 2H, CH_2), 2.53–2.60 (m, 4H, piperazine), 2.80 (t, 2H, CH_2–N), 3.20–3.40 (m, 4H, piperazine), 3.66 (s, 3H, OCH_3), 4.40 (t, 2H, CH_2–N), 6.81–7.60 (m, 10H, ArH), 7.90–8.20 (m, 2H, ArH); EIMS

(% rel. abundance): 315 ($C_{15}H_{10}^{81}BrN_2O$, 32.15), 532 (M^+, 11.96), 534 (M + 2, 13.05). Anal. Calcd for $C_{28}H_{29}BrN_4O_2$, C, 63.04; H, 5.48; N, 10.50; Found C, 62.85; H, 5.25; N, 10.45.

2-{[4-(4-Bromophenyl)-1-oxophthalazin-2(1H)-yl]methyl} isoindoline-1,3-dione ***(6)*** A mixture of **1** (0.301 g, 0.001 mol), *N*-bromomethylphthalimide (0.24 g, 0.001 mol) and anhydrous K_2CO_3 (0.41 g, 0.003 mol) in dry DMF (10 mL) was stirred at room temperature for 24 h. The reaction mixture was poured onto ice-cold water, the resulting precipitate was filtered, washed with water and crystallized from ethanol.

Yield 75 %; mp 221–222 °C; IR (KBr) cm^{-1}: 3,097 (C–H aromatic), 2,927, 2,897 (C–H aliphatic), 1,662 (C=O), 1,589 (C=N); ^{1}HNMR (DMSO–d_6): 5.65 (s, 2H, N–CH_2), 7.54–7.91 (m, 10H, ArH), 8.32–8.35 (m, 2H, ArH); (EI) *m/z* (% rel. Int.): 459 (M^+, 0.35), 461 (M + 2, 0.32). Anal. Calcd for $C_{23}H_{14}BrN_3O_3$: C, 60.02; H, 3.07; N, 9.13. Found: C, 59.65; H, 3.16; N, 9.32.

General procedure for the compounds ***7a–j***

To a solution of **1** (0.301 g, 0.001 mol) in absolute ethanol (10 mL), a mixture of formalin (0.048 g, 0.001 mol) and the appropriate secondary amine or substituted piperazine (0.001 mol) in ethanol (5 mL) was added and the reaction mixture was heated under reflux for 4 h. The hot solution was filtered, the filtrate was concentrated to half its volume and cooled. The separated solid was filtered and crystallized from methanol.

4-(4-Bromophenyl)-2-(morpholinomethyl)phthalazin-1(2H) -one ***(7a)*** Yield 75 %; mp 184–185 °C; IR (KBr) cm^{-1}: 3,050 (C–H aromatic), 2,950, 2,855 (C–H aliphatic), 1,651 (C=O), 1,604 (C=N); ^{1}HNMR (DMSO–d_6): 2.67 (t, 4H, $J = 4.5$ Hz, $2CH_2$–N), 3.54 (t, 4H, $J = 4.5$ Hz, $2CH_2$–O), 5.04 (s, 2H, N–CH_2), 7.53–7.93 (m, 6H, ArH), 8.37–8.40 (m, 2H, ArH); Anal. Calcd for $C_{19}H_{18}BrN_3O_2$: C, 57.01; H, 4.53; N, 10.50. Found: C, 56.59; H, 4.64; N, 10.80.

4-(4-Bromophenyl)-2-(piperidin-1-ylmethyl)phthalazin-1 (2H)-one ***(7b)*** Yield 60 %; mp 219–220 °C; IR (KBr) cm^{-1}: 3,050 (C–H aromatic), 2,935, 2,893 (C–H aliphatic), 1,651 (C=O), 1,604 (C=N); ^{1}HNMR (DMSO–d_6): 1.31–1.47 (m, 2H, CH_2 piperidine), 2.48–2.50 (m, 4H, $2CH_2$ piperidine), 2.63–2.67 (m, 4H, $2CH_2$ piperidine), 5.03 (s, 2H, N–CH_2), 7.53–7.92 (m, 6H, ArH), 8.36–8.39 (m, 2H, ArH); MS (EI) *m/z* (% rel. Int.): 301 ($C_{14}H_{10}BrN_2O$, 16.59), 314 ($C_{15}H_{11}BrN_2O$, 1.77), 397 (M^+, 0.77), 399 (M + 2, 0.72). Anal. Calcd for $C_{20}H_{20}BrN_3O$: C, 60.31; H, 5.06; N, 10.55. Found: C, 60.59; H, 4.80; N, 10.85.

4-(4-Bromophenyl)-2-(pyrrolidin-1-ylmethyl)phthalazin-1 (2H)-one ***(7c)*** Yield 65 %; mp 164–165 °C; IR (KBr) cm^{-1}: 3,078 (C–H aromatic), 2,947, 2,873 (C–H aliphatic), 1,651 (C=O), 1,604 (C=N); ^{1}HNMR (DMSO–d_6): 1.62 (t, 4H, $2CH_2$ pyrrolidine), 2.75 (t, 4H, $2CH_2$–N pyrrolidine), 5.14 (s, 2H, N–CH_2), 7.54–7.93 (m, 6H, ArH), 8.36–8.39 (m, 2H, ArH); Anal. Calcd for $C_{19}H_{18}BrN_3O$: C, 59.39; H, 4.72; N, 10.94. Found: C, 59.50; H, 4.78; N, 11.22.

4-(4-Bromophenyl)-2-[(diethylamino)methyl]phthalazin-1 (2H)-one ***(7d)*** Yield 75 %; mp 139–140 °C; IR (KBr) cm^{-1}: 3,030 (C–H aromatic), 2,966, 2,855 (C–H aliphatic), 1,651 (C=O), 1,605 (C=N); ^{1}HNMR (DMSO–d_6): 1.06 (t, 6H, $2CH_3$), 2.70 (q, 4H, $2CH_2$), 5.13 (s, 2H, N–CH_2), 7.76–7.89 (m, 6H, ArH), 8.30–8.37 (m, 2H, ArH); Anal. Calcd for $C_{19}H_{20}BrN_3O$: C, 59.08; H, 5.22, N, 10.88. Found: C, 59.25; H, 4.93; N, 10.94.

4-(4-Bromophenyl)-2-[(dibenzylamino)methyl]phthalazin-1 (2H)-one ***(7e)*** Yield 60 %; mp 261–262 °C; IR (KBr) cm^{-1}: 3,060 (C–H aromatic), 2,950, 2,847 (C–H aliphatic), 1,647 (C=O), 1,600 (C=N); ^{1}HNMR (DMSO–d_6): 5.46 (s, 4H, 2N–CH_2), 5.49 (s, 2H, N–CH_2), 6.67–7.95 (m, 16H, ArH), 8.36–8.42 (m, 2H, ArH); MS (EI) *m/z* (% rel. Int.): 510 (M^+, 6.40), 512 (M + 2, 7.26). Anal. Calcd for $C_{29}H_{24}BrN_3O$: C, 68.24; H, 4.74; N, 8.23. Found: C, 68.43; H, 4.92; N, 8.00.

4-(4-Bromophenyl)-2-[(diphenylamino)methyl]phthalazin-1(2H)-one ***(7f)*** Yield 65 %; mp 244–245 °C; IR (KBr) cm^{-1}: 3,050 (C–H aromatic), 2,968, 2,846 (C–H aliphatic), 1,643 (C=O), 1,605 (C=N); ^{1}HNMR (DMSO–d_6): 5.46 (s, 2H, N–CH_2), 6.67–7.94 (m, 16H, ArH), 8.32–8.41 (m, 2H, ArH); MS (EI) *m/z* (% rel. Int.): 301 ($C_{14}H_{10}BrN_2O$, 75.53), 314 ($C_{15}H_{11}BrN_2O$, 0.13), 481(M^+, 0.06), 483 (M + 2, 0.05). Anal. Calcd for $C_{27}H_{20}BrN_3O$: C, 67.23; H, 4.18; N, 8.71. Found: C, 67.30; H, 4.33; N, 8.50.

4-(4-Bromophenyl)-2-[(4-methylpiperazin-1-yl)methyl] phthalazin-1(2H)-one ***(7g)*** Yield 80 %; mp 252–253 °C; IR (KBr) cm^{-1}: 3,074 (C–H aromatic), 2,955, 2,865 (C–H aliphatic), 1,647 (C=O), 1,605 (C=N); ^{1}HNMR (DMSO–d_6): 2.09 (s, 3H, N–CH_3), 2.27–2.32 (m, 4H, piperazine), 2.68–2.72 (m, 4H, piperazine), 5.48 (s, 2H, N–CH_2), 7.54–7.93 (m, 6H, ArH), 8.32–8.41 (m, 2H, ArH).

Anal. Calcd for $C_{20}H_{21}BrN_4O$: C, 58.12; H, 5.12; N, 13.56. Found: C, 58.30; H, 5.20; N, 13.75

4-(4-Bromophenyl)-2-[(4-phenylpiperazin-1-yl)methyl] phthalazin-1(2H)-one ***(7h)*** Yield 60 %; mp 187–188 °C; IR (KBr) cm^{-1}: 3,051 (C–H aromatic), 2,944, 2,850 (C–H aliphatic), 1,647 (C=O), 1,597 (C=N); ^{1}HNMR (DMSO–d_6): 2.80–2.83 (m, 4H, piperazine), 3.10–3.13 (m, 4H, piperazine), 5.13 (s, 2H, N–CH_2), 6.85–7.92 (m, 11H, ArH), 8.30–8.40 (m, 2H, ArH); MS (EI) *m/z* (% rel. Int.): 301 ($C_{14}H_{10}BrN_2O$, 13.14), 314 ($C_{15}H_{11}BrN_2O$, 3.71), 474 (M^+, 24.84), 476 (M + 2, 25.23). Anal. Calcd for $C_{25}H_{23}BrN_4O$: C, 63.16; H, 4.88; N, 11.79. Found: C, 63.50; H, 4.80; N, 12.05.

4-(4-Bromophenyl)-2-{[4-(2-methoxyphenyl)piperazin-1-yl]methyl}phthalazin-1(2H)-one ***(7i)*** Yield 65 %; mp 199–200 °C; IR (KBr) cm^{-1}: 3,051 (C–H aromatic), 2,950, 2,855 (C–H aliphatic), 1,654 (C=O), 1,597 (C=N); ^{1}HNMR (DMSO–d_6): 2.83–2.84 (m, 4H, piperazine), 2.92–2.94 (m, 4H, piperazine), 3.76 (s, 3H, OCH_3), 5.12 (s, 2H, N–CH_2), 6.85–7.94 (m, 10H, ArH), 8.31–8.40 (m, 2H, ArH); MS (EI) *m/z* (% rel. Int.): 301 ($C_{14}H_{10}BrN_2O$, 30.35), 314 ($C_{15}H_{11}BrN_2O$, 0.84), 504 (M^+, 6.05), 506 (M + 2, 6.13). Anal. Calcd for $C_{26}H_{25}BrN_4O_2$: C, 61.79; H, 4.99; N, 11.09. Found: C, 61.50; H, 4.75; N, 11.00.

4-(4-Bromophenyl)-2-{[4-(4-methoxyphenyl)piperazin-1-yl] methyl}phthalazin-1(2H)-one ***(7j)*** Yield 72 %; mp 219–220 °C; IR (KBr) cm^{-1}: 3,051 (C–H aromatic), 2,943, 2,831 (C–H aliphatic), 1,651 (C=O), 1,604 (C=N); ^{1}HNMR (DMSO–d_6): 2.65–2.70 (m, 4H, piperazine), 2.90–2.95 (m, 4H, piperazine), 3.91 (s, 3H, OCH_3), 5.07 (s, 2H, N–CH_2), 6.75–7.89 (m, 10H, ArH), 8.33–8.41 (m, 2H, Ar–H); Anal. Calcd for $C_{26}H_{25}BrN_4O_2$: C, 61.79; H, 4.99; N, 11.09. Found: C, 61.66; H, 4.85; N, 11.23.

Molecular modeling procedure

Molecular modeling was carried out on Schrodinger computational software workstation using Maestro 7.5 graphic user interface (GUI) and Red Hat Linux nash Enterprise version V 4.1.18 on Batchmin V 9.1 modeling engine. The atomic coordinates for the polypeptide segments of MTHF and Hcy binding domains of MetS enzyme extracted from human liver were obtained from the protein bank database [PDB (Brookhaven protein database)]. Explicit calcium ion counter ions were included instead of cadmium and positioned at a 4 Å distance from the homocysteine binding site. The basic and acidic amino acids were neutralized at pH 7 ± 2 by protonation of the terminal amino groups such as histidine, lysine and arginine. Moreover, the terminal carboxylic acid groups of amino acids and acidic amino acids such as glutamic acid, aspartic acid, asparagine and glutamine were deprotonated. Prime 1.5 was used to check the energy content of the protein segments and loops. The docking process involved the standard precision docking (SP) in which ligand poses that were expected to have unfavourable energies would be rejected. The presumption is that only active compounds will have available poses that avoid these penalties. SP docking is appropriate for screening ligands of unknown quality in large numbers. SP is a soft docking programme that was adept at identifying ligands that have a reasonable propensity to bind and 20 % of the final poses produced from the SP docking were subjected to the Extra Precision mode of Glide docking (XP) to perform the more expensive docking simulation on worthwhile poses. XP docking mode is harder than SP docking mode in that it penalizes the poses that violate established charges. Flexible docking was selected to generate conformations of all possible ligand poses, which is more realistic as this occurs in reality because the protein undergoes side chain and back bone movement or both, upon ligand binding. Five and six-membered rings were allowed to flip and amide bonds which were not *cis* or *trans* configuration were penalised. 5,000 Poses per ligand for the initial phase of docking with a scoring window for keeping poses of 100 kJ/mol were set up. The best poses which fulfil those conditions were subjected to energy minimization on the OPLS-AA non-bonded interaction grid with a distance dielectric constant of 2 and maximum number of conjugate gradient steps of 500 iterations. The ligands of the poses selected by the initial screening were subsequently minimized in the field of the receptor using a standard molecular mechanics energy function (OPLS-AA force field) in conjunction with a distance-dependant dielectric model. Finally, the lowest energy poses obtained in this fashion were subjected to a Monte Carlo procedure that examines nearby torsion minima. The complex was minimized using the conjugate gradients algorithm until an energy convergence criterion of 0.1 kJ/mol was reached with iteration cycle of 10,000. Molecular dynamics (MD) at 300 K were then performed on the solvated system for a 10 ps equilibration and 100 ps of production employing a 1 fs time step using OPLS-2005 force field, from which 100 structures were sampled at 1 ps intervals and averaged. The final averaged structure was then finally minimized (Cairns et al. 2001). The free energy of binding was calculated from the following equation.

$$\Delta E_{Bind} = \Delta E_{Complex} - (\Delta E_{Receptor} + \Delta E_{Ligand})$$

Pharmacological assessment of α-adrenergic blocking activity

Drugs

Acetylcholine hydrochloride, noradrenaline and prazosin hydrochloride were purchased from Sigma-Aldrich (MO, USA). All other chemicals were of analytical grades and obtained from ADWIC Company (Cairo, Egypt).

Isolated rabbit intestine preparation

It has been reported that α-receptors mediate the inhibitory effects in the isolated small intestine of the rabbit (Cutler et al. 1985). The novel final targets **4a–f**, **5a–c**, **6** and **7a–j** were tested for their blocking effect on the α-receptors. The α-blocking activity was determined by measuring the degree of blocking of the inhibitory action of norepinephrine (NE), which is mediated via α-ARs by a method described by Cutler et al. (1980). A segment of small intestine (2–3 cm) was suspended in oxygenated Ringer–Locke solution of the following composition (g/L): 9.0 g NaCl, 0.42 g KC1, 0.17 g $CaCl_2$, 0.5 g $NaHCO_3$ and 1.0 g glucose at 37 °C.

Muscle contractions were recorded by a transducer and displayed on a calibrated oscillograph. The preparation was allowed to equilibrate for 30–40 min, then 0.4 mL of 0.01 % acetylcholine (Ach) was added and the contraction amplitude was measured over a 1 min period. The bath was then washed out three times with Ringer–Locke solution. The response of the same dose of Ach was re-evaluated after 3 min of pre-treatment with 0.8 mL of 0.01 % norepinephrine solution. The bath was then washed out three times with Ringer–Locke solution and the effect of test compounds (antagonists) to be assayed was measured by their ability to attenuate the NE-induced inhibition on the amplitude of contraction induced by Ach.

The test compounds as well as prazosin hydrochloride (a reference standard) were dissolved in dimethylsulfoxide (DMSO) as stock solution (20 mL, 1 %). DMSO was used in control experiments. The % decrease in the amplitude of contraction induced by Ach was recorded in case of pre-treatment with NE alone as well as in case of pre-treatment with NE and the test compounds (or prazosin) (0.01 % solution, 1 mL) (Table 2).

Compared to Ach + NE treatment, compounds that exhibited significant change in % decrease in the amplitude of contraction relative to Ach treatment were considered to have promising α-blocking activity. Different concentrations of prazosin (standard compound) or the test compounds (antagonists) to be assayed (0.01 % solution) were added to the bathing solution 3 min before addition of norepinephrine. Ach was added to the bathing solution and the response was recorded.

For all of the suggested α-adrenergic blocking agents, the percentage decrease in the amplitude of contraction was calculated as follows:

% decrease in amplitude of contraction = (amplitude of contraction due to Ach-amplitude of contraction due to Ach after treatment with the test compound and NE)/ amplitude of contraction due to Ach. The mean of six measures was taken and expressed as mean ± SEM. The results were statistically analyzed using one-way ANOVA followed by Bonferroni's multiple comparisons test. $P \leq 0.05$ was considered as a significant difference. All statistical tests were done employing SPSS program version 17 (SPSS Software, SPSS Inc., Chicago, USA).

The IC_{50} values (a concentration necessary for 50 % inhibition of maximal inhibitory effect of norepinephrine hydrochloride upon Ach contraction) was determined by plotting the % inhibition for NE effect versus the concentration of the test compounds or prazosin. Dose–response curve was plotted and IC_{50} values were calculated using Microsoft EXCEL, version 2007.

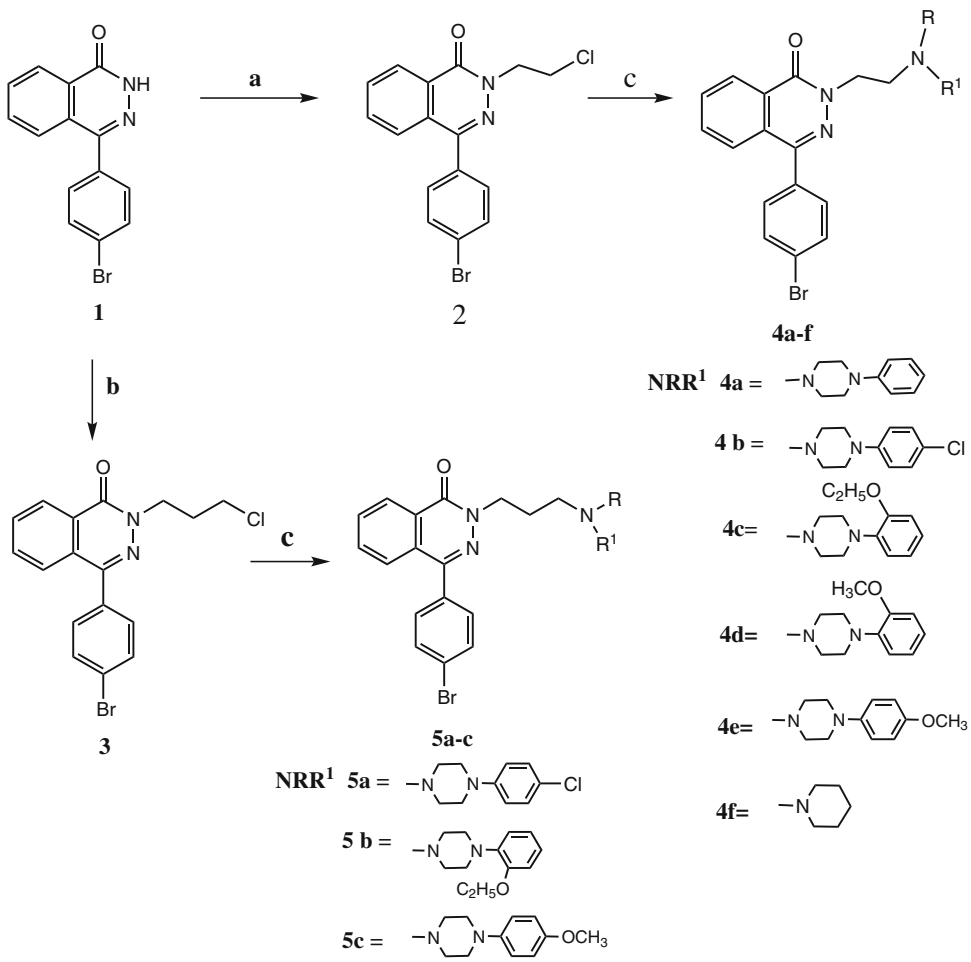
Results and discussion

Chemistry

The synthetic pathways to compounds **4a–f** and **5a–c** are shown in Scheme 1. The new intermediates 4-(4-bromophenyl)-2-(2-chloroethyl)phthalazin-1(2*H*)-one **(2)** and 4-(4-bromophenyl)-2-(3-chloropropyl)phthalazin-1(2*H*)-one **(3)** were obtained by alkylation of 4-(bromophenyl)phthalazin-1(*2H*)-one **(1)** (Colotta et al. 1994) with the respective bromochloroalkane in dry DMF in presence of anhydrous potassium carbonate. Nucleophilic displacement of chlorine in **2** or **3** with various *N*-(substituted)arylpiperazines or piperidine gave the relevant **4a–f** or **5a–c** respectively in 55–70 % yields. The structures of the new compounds were confirmed by elemental analyses and spectral data. ^{1}HNMR spectra of compounds **4a–e** and **5a–c** displayed two broad signals of piperazine ring in the range of δ 2.55–2.70 and 2.82–3.40 ppm. All the other signals in ^{1}HNMR were observed in the expected regions.

The synthesis of compounds **6** and **7a–j** is illustrated in Scheme 2. The synthesis of compound **6** was achieved by alkylation of **1** with *N*-bromomethylphthalimide in dry DMF at room temperature. On the other hand, the phthalazinone Mannich bases **7a–j** were obtained through Mannich reaction of **1** with formaline and a various secondary amines or arylpiperazines following standard reaction conditions. IR spectral data showed the disappearance of the *NH* absorption band at 3,250 cm^{-1} of the starting phthalazinone **1**. Moreover, the formation of **6** and the Mannich bases **7a–j** was confirmed through appearance of a singlet peak corresponding to methylene group flanked by two nitrogen atoms in the range of δ 5.03–5.65 ppm.

In addition, ^{1}HNMR spectra of compounds **7g–j** showed the appearance of two characteristic broad multiplets of piperazine ring in the range δ 2.27–2.84 and 2.68–3.13 ppm. All the other aromatic and aliphatic protons were observed in the expected regions.



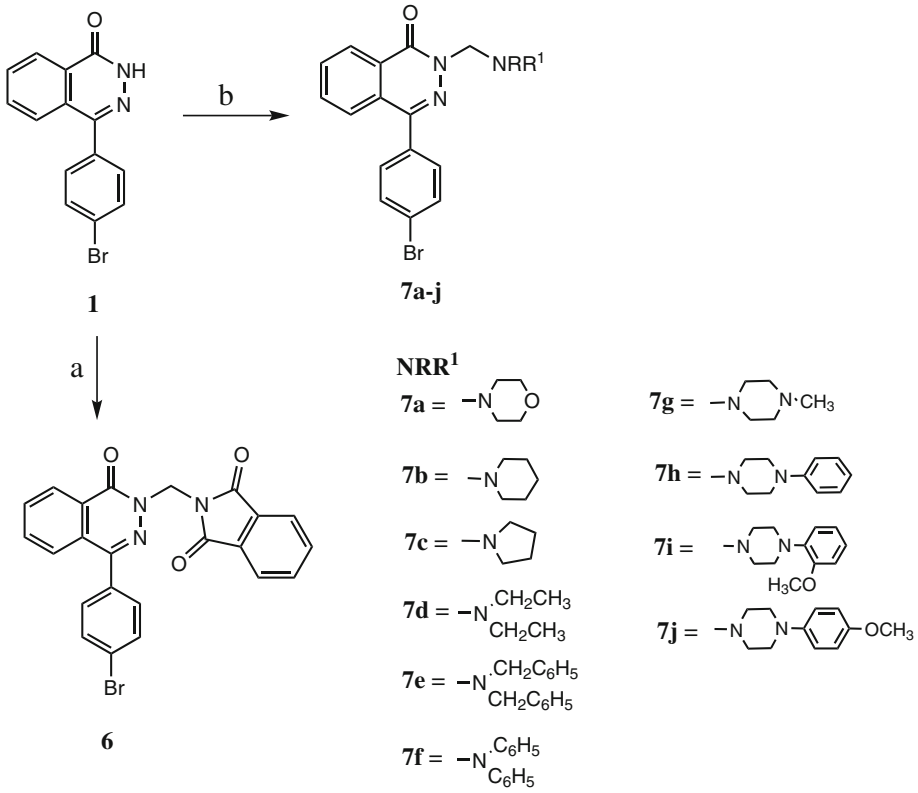
Scheme 1 Reagents and conditions: **a** 1-bromo-2-chloroethane, anhydrous K_2CO_3, DMF, rt; **b** 1-bromo-3-chloropropane, anhydrous K_2CO_3, DMF, rt; **c** HNRR[1], K_2CO_3, CH_3CN, reflux

Molecular modeling studies

Molecular modeling investigations were done on α_1-adrenergic receptor and the designed inhibitors including the known drug prazosin were docked into the receptor. There is a direct proportionality between the free energy of binding of the inhibitors (vasodilators) and the percentage decrease in the degree of contraction induced by them. The smaller the free energy of binding, the more plausible and tight is the binding of the inhibitors to their binding domain with the suppression of the vasoconstriction action (Cairns et al. 2001). The active site map analysis clearly showed the presence of a large hydrophobic region deep inside the binding domain. Prazosin was docked inside the α_1-adrenergic receptor as illustrated in Figs. 3 and 4. Prazosin docking poses showed a maximum fitting to the receptor cavity where the hydrophobic side chain at N-2 docked deeply inside the binding domain and the quinazoline moiety at the surface of the receptor as shown in Fig. 4. The free energy of binding and the percentage decrease in the degree of contraction collected were quiet small (−257.81 kJ/mol, 5.2 ± 0.6). The thermodynamic enthalpy parameterization set up with reference drug prazosin was used with subsequent synthesized compounds in this article. Compound **7j** showed low free energy of binding −223.79 kJ/mol and a high reduction percentage in the contraction of the intestine (3.3 ± 0.3 %). The root mean square deviation (RMSD) for the compound was 1.96 Å. The docking poses of the compound **7j** showed a similar docking poses of prazosin, where the phthalazine moiety docked at outside part of the receptor and the hydrophobic side chain at N-2 of the phthalazine nucleus at the hydrophobic pocket at deep part of the receptor (Figs. 5, 6). Methoxy group at the side chain of the ligand made a hydrogen bond with lysine 267. Therefore, the ligand showed a higher docking score and a better electrostatic and van der Waals energy interactions with the receptor.

Ligand **4a** showed a moderate free energy of binding −125.51 kJ/mol and a moderate reduction percentage in the contraction of the intestine (37.5 ± 7.0). The RMSD for the ligand was 2.03 Å (Table 1). The ligand formed a hydrogen bond with threonine 1050 and the amino group of the side chain at N-2 of the phthalazine nucleus (Figs. 7, 8). The ligand produced a moderate type of electrostatic interactions between lysine 1050 and threonine 1050 from one side and

Scheme 2 Reagents and conditions: **a** *N*-bromomethylphthalimide, K_2CO_3, DMF, rt; **b** HCHO, $HNRR^1$, ethanol, reflux



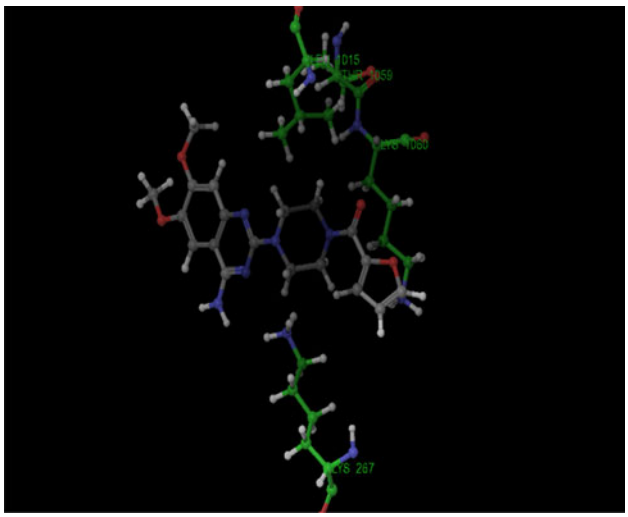


Fig. 3 The docked prazosin in the α_1-adrenergic receptor

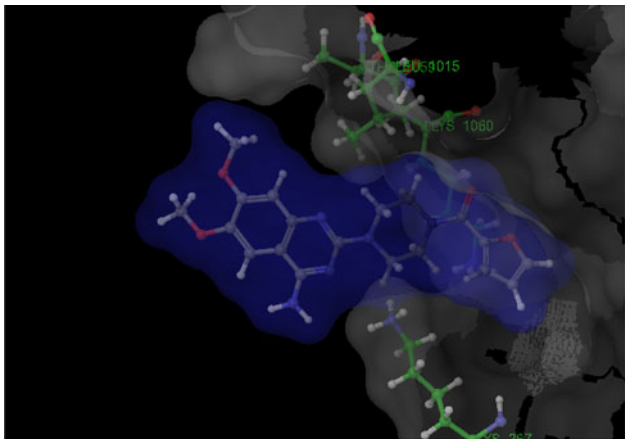


Fig. 4 The docked prazosin in the α_1-adrenergic receptor (receptor-prazosin binding site surfaces

the side chain at N-2 of the phthalazine nucleus. The phthalazine moiety tended to point away from the receptor due to high energy barrier present between the nucleus threonine 1050 and this contributes unfavourably to the free energy of binding of the ligand into its binding domain.

The ligand **5a** showed the highest free energy of binding 6.54 kJ/mol and poor reduction percentage in the contraction of the intestine (62.5 ± 7.0 %). The RMSD for the ligand was 4.42 Å therefore the docking poses of ligand 5a and prazosin are different (Figs. 9, 10). The side chain of the ligand started to fold up on itself to avoid the high energy barrier between the ligand and receptor. This led to the ligand docked at the surface of the domain. The hydrophobic chain of the ligand resulted in the expulsion of the ligand from the receptor to avoid the high steric clashes with the hydrophobic residues inside the receptor pocket

Fig. 5 The docked pose of compound **7j** in the α_1-adrenergic receptor

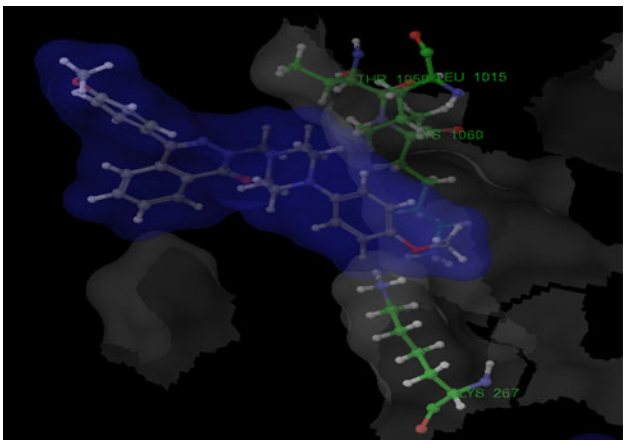


Fig. 6 The docked compound **7j** in the α_1-adrenergic receptor (receptor-ligand binding site surfaces)

and stacked to the surface of the binding domain. Therefore the free binding energy is enormous due to unfavourable ligand–receptor interactions.

In summary, the thermodynamic algorithms were operated on the different synthesized ligands revealed that the hydrophobic side chain at position 2 increased the van der Waals repulsive interactions. In addition, the presence of polar groups attached to the hydrophobic side chain at position 2 improved the free energy of docking.

Pharmacological evaluation

Norepinephrine caused inhibition of the spontaneous contraction as well as contractions induced by Ach in the isolated preparation of the small rabbit intestine, suspended in Ringer–Locke solution. The amplitude of spontaneous contraction was inhibited by norepinephrine by 65 ± 7 % in comparison to the amplitude of contraction due to Ach. Pre-treatment with prazosin significantly reduced the percentage inhibition of contraction after norepinephrine by

Table 1 The calculated energy terms for the interaction of the synthesized compounds and % decrease in amplitude of contraction relative to acetylcholine their RMSD values

Ligand	Energy term (KJ/mol)				RMSD (Å)
	ΔE_{Bind}	$\Delta E_{Complex}$	$\Delta E_{Receptor}$	ΔE_{Ligand}	
Prazosin	−257.81	−360.51	−222.90	120.20	1.56
4a	−125.51	222.72	−186.09	88.880	2.03
4b	−162.93	−267.08	−190.24	86.090	1.90
4c	4.07	−301.15	−385.62	80.400	4.42
4d	−100.00	−321.50	−209.90	114.20	5.09
4e	−105.89	−340.55	−289.94	109.20	5.13
4f	−84.80	−321.50	−259.91	23.210	4.11
5a	6.54	−301.22	−388.22	80.460	4.42
5b	−140.81	−345.55	−299.94	114.48	3.12
5c	−131.73	−341.55	−298.90	89.080	6.11
6	−125.71	−341.55	−303.02	87.180	6.11
7a	−111.81	−321.50	−279.90	70.210	5.09
7b	−84.80	−321.50	−259.91	23.210	4.11
7c	−150.19	−331.05	−280.94	100.08	1.9
7d	−131.73	−341.55	−298.90	89.080	6.11
7e	−152.54	−340.50	−269.04	81.080	6.01
7f	−75.52	−321.52	−269.01	23.010	4.11
7g	−90.62	−390.03	−326.11	26.700	1.6
7h	−29.81	−210.55	−289.94	109.200	5.13
7i	−195.93	−301.15	−185.62	80.400	2.42
7j	−223.79	−240.51	−120.92	104.20	1.96

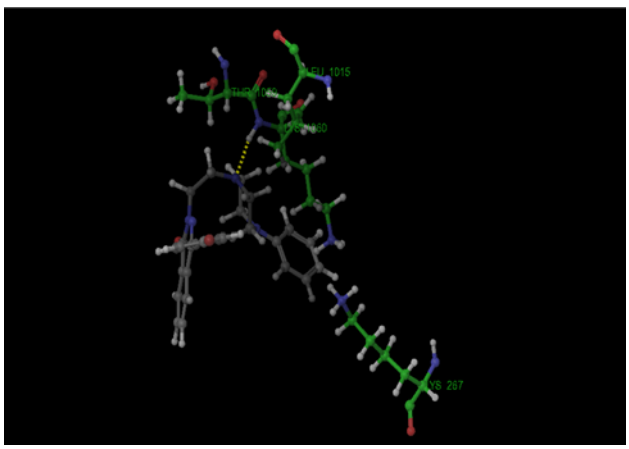


Fig. 7 The docked pose of compound **4a** in the α_1-adrenergic receptor

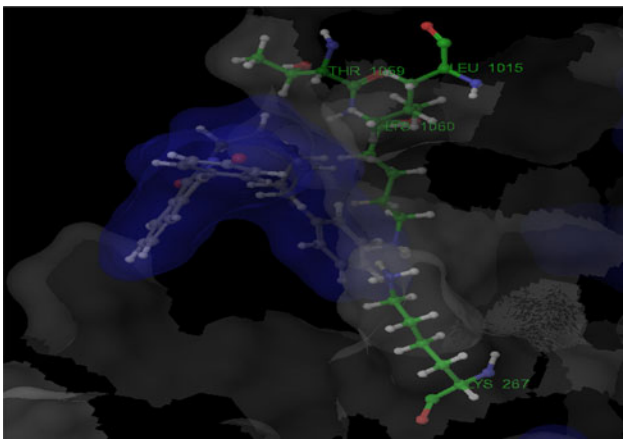


Fig. 8 The docked compound **4a** in the α_1-adrenergic receptor (receptor-ligand binding site surfaces)

Fig. 9 The docked pose of compound **5a** in the α_1-adrenergic receptor

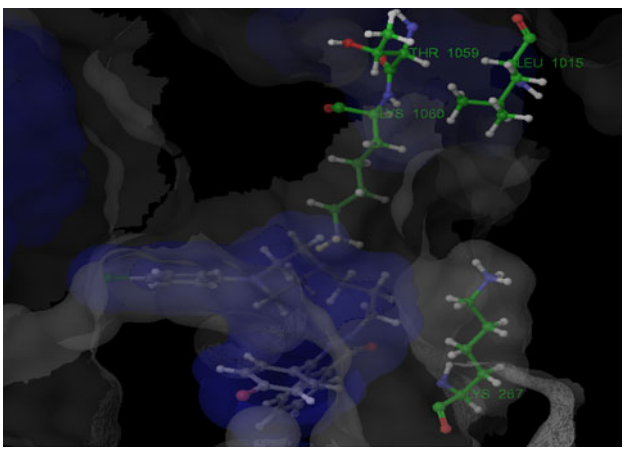


Fig. 10 The docked compound **5a** in the α_1-adrenergic receptor (receptor-ligand binding site surfaces)

Table 2 IC_{50} values and % decrease in amplitude of contraction relative to acetylcholine

Compounds	% Decrease in amplitude of contraction relative to Ach	IC_{50} (μg/mL)
Ach	0	–
Ach + NE	65 ± 7.0#	–
Ach + NE + Prazosin	5.2 ± 0.6*	0.498
Ach + NE + **4a**	37.5 ± 7.0*	1.101
Ach + NE + **4b**	50 ± 6.0	–
Ach + NE + **4c**	62.5 ± 8.0	–
Ach + NE + **4d**	20 ± 3.0*	1.42
Ach + NE + **4e**	21.9 ± 2.5*	0.696
Ach + NE + **4f**	16 ± 2.0*	0.915
Ach + NE + **5a**	62.5 ± 7.0	–
Ach + NE + **5b**	13.3 ± 2.0*	0.679
Ach + NE + **5c**	26.6 ± 3.0*	1.614
Ach + NE + **6**	26.6 ± 3.0*	1.605
Ach + NE + **7a**	39 ± 7.0	–
Ach + NE + **7b**	40 ± 3.6	–
Ach + NE + **7c**	16.6 ± 2*	0.692
Ach + NE + **7d**	23.3 ± 3*	0.70
Ach + NE + **7e**	12 ± 1.7*	0.81
Ach + NE + **7f**	59.4 ± 6.0	–
Ach + NE + **7g**	53.2 ± 4.0	–
Ach + NE + **7h**	80 ± 6.0	–
Ach + NE + **7i**	0.093 ± 0.01*	0.621
Ach + NE + **7j**	3.3 ± 0.3*	0.51

Data are expressed as mean ± SEM and analyzed using one-way ANOVA followed by Bonferroni's multiple comparison's test. Each individual compound was compared with the first three groups separately. IC_{50} (concentration necessary for 50 % inhibition of maximal inhibitory effect of norepinephrine hydrochloride upon Ach contraction)

Ach acetylcholine, *NE* norepinephrine

Significantly different from Ach at $P < 0.05$

* Significantly different from Ach + NE at $P < 0.05$, $n = 6$

5.2 ± 0.6 %. Therefore, prazosin blocked the action of norepinephrine on the isolated rabbit intestine.

Contraction due to Ach was returned near the baseline value in case of pre-treatment with various tested compounds before NE addition. Those compounds that resulted in significant changes in the amplitude of contraction relative to that recorded after treatment with Ach + NE were considered to possess α-antagonistic activity (Table 2). Compound **7j** produced α-antagonistic activity that is fairly similar to that produced by prazosin. However, compounds **4b**, **4c**, **5a**, **7a**, **7b**, **7f**, **7g**, **7h** did not show any significant activity.

For compounds that produced significant α-blocking activity, IC_{50} values were determined, and the results showed that compound **7j** exhibited the lowest IC_{50} value

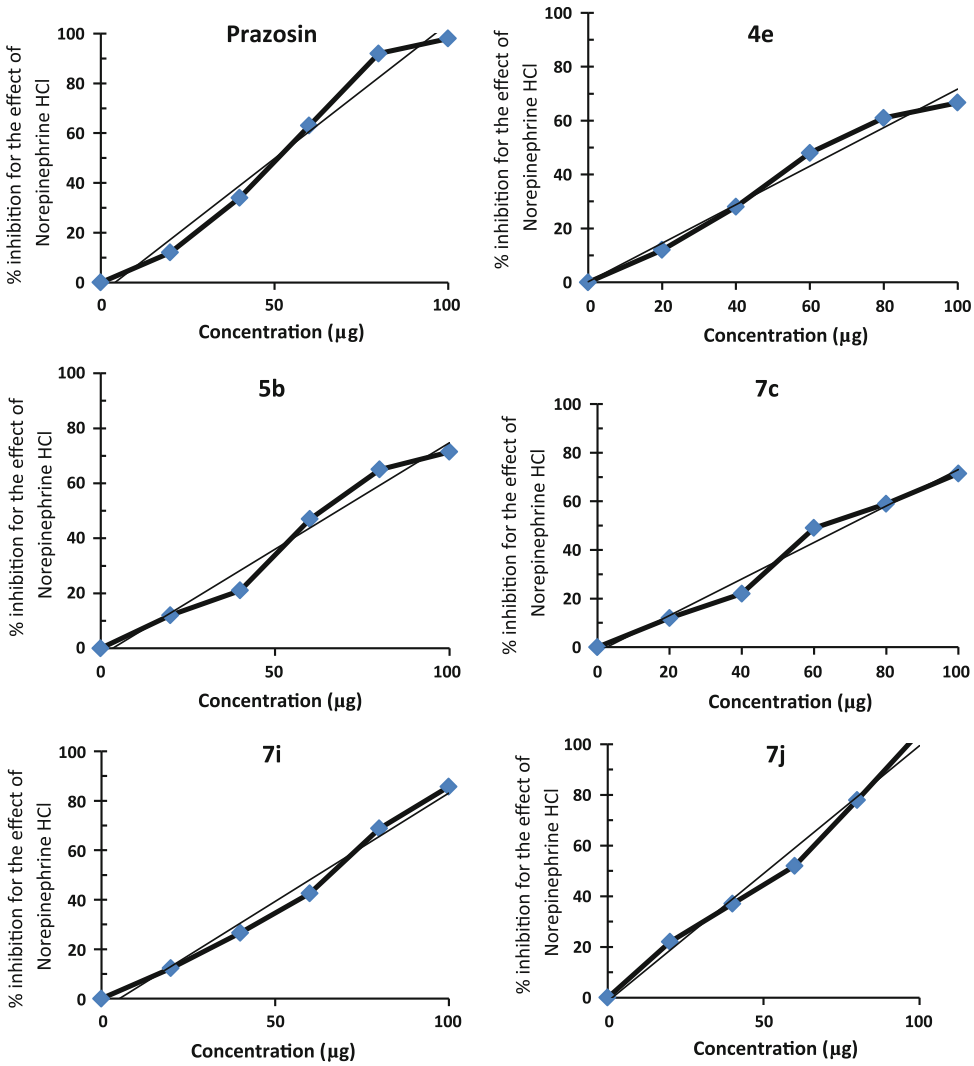


Fig. 11 Dose-response curve for the most active compounds in comparison to prazosin

(0.51 µg), which was comparable to that showed by prazosin (0.498 µg) (Figs. 6, 11; Table 2).

References

Abou-Seri, S.M., K. Abouzid, and D.A. Abou El Ella. 2011. Molecular modeling study and synthesis of quinazolinone-arylpiperazine derivatives as α_1-adrenoreceptor antagonists. *European Journal of Medicinal Chemistry* 46: 647–658.

Bang, L., J.E. Nielsen-Kudsk, N. Gruhn, S. Trautner, S.A. Theilgaard, S.P. Olesen, S. Boesgaard, and J. Aldershvile. 1998. Hydralazine-induced vasodilation involves opening of high conductance Ca^{2+}-activated K^+ channels. *European Journal of Pharmacology* 361: 43–49.

Barbaro, R., L. Betti, M. Botta, F. Corelli, G. Giannaccini, L. Maccari, F. Manetti, G. Strappaghetti, and F. Corsano. 2001. Synthesis, biological evaluation and pharmacophore generation of new pyridazinone derivatives with affinity toward α_1- and α_2-adrenoceptors. *Journal of Medicinal Chemistry* 44: 2118–2132.

Betti, L., M. Botta, F. Corelli, M. Floridi, G. Giannaccini, L. Maccari, F. Manetti, G. Strappaghetti, A. Tafi, and S. Corsano. 2002. Alpha(1)-adrenoceptor antagonists. Pharmacophore-based design, synthesis, and biological evaluation of new imidazo-, benzimidazo-, and indoloarylpiperazine derivatives. *Journal of Medicinal Chemistry* 45: 3603–3611.

Cairns, D., E. Michalitsi, and C. Terence. 2001. Molecular modelling and cytotoxicity of substituted anthraquinones as inhibitors of human telomerase. *Bioorganic & Medicinal Chemistry* 10: 803–807.

Calzada, B.C., and A.A. de Artiñano. 2001. Alpha-adrenoceptor subtypes. *Pharmaceutical Research* 44: 195–208.

Colotta, V., L. Cecchi, D. Catarzi, G. Filacchioni, A. Galli, and F. Mori. 1994. Synthesis and structure-activity relationships of 1-aminophthalazinium salts as GABA 318 A receptor antagonists. *European Journal of Medicinal Chemistry* 29: 95–105.

Cutler, M.G., M.R. Moore, and J.M. Dick. 1980. Effects of delta-aminolaevulinic acid on contractile activity in rabbit duodenum. *European Journal of Pharmacology* 64: 221–230.

Cutler, M.G., M. Mclaughlin, E. Mcneil, and M.R. Moorer. 1985. Effects of delta-amrnolaevulinic acid on contractile activity in the isolated small intestine of the rabbit. *Neuropharmacology* 24(10): 1005–1009.

Del Olmo, E., B. Barboza, M. Ybarra, J.L. Lopez-Perez, R. Carron, A. Sevilla, C. Boselli, and A. San Feliciano. 2006. Vasorelaxant activity of phthalazinones and related compounds. *Bioorganic & Medicinal Chemistry Letters* 16: 2786–2790.

Demirayak, S., A.C. Karaburun, I. Kayagil, K. Erol, and B. Sirmagul. 2004a. Some pyridazinone and phthalazinone derivatives and their vasodilator activities. *Archives of Pharmacal Research* 27: 13–18.

Demirayak, S., A.C. Karaburun, and R. Beis. 2004b. Some pyrrole substituted aryl pyridazinone and phthalazinone derivatives and their antihypertensive activities. *European Journal of Medicinal Chemistry* 39: 1089–1095.

Frishman, W.H., and F. Kotob. 1999. Alpha-adrenergic blocking drugs in clinical medicine. *Journal of Clinical Pharmacology* 39: 7–16.

Handzlik, J., D. Maciag, M. Kubacka, S. Mogliski, B. Filipek, K. Stadnicka, and K. Kiec-Kononowicz. 2008. Synthesis, alpha 1-adrenoceptor antagonist activity, and SAR study of novel arylpiperazine derivatives of phenytoin. *Bioorganic & Medicinal Chemistry* 16: 5982–5998.

Handzlik, J., H.H. Pertz, T. Görnemann, S. Jähnichen, and K. Kieć-Kononowicz. 2010. Search for influence of spatial properties on affinity at α_1-adrenoceptor subtypes for phenylpiperazine derivatives of phenytoin. *Bioorganic & Medicinal Chemistry* 20: 6152–6156.

Jain, K.S., J.B. Bariwal, M.K. Kathiravan, M.S. Phoujdar, R.S. Sahne, B.S. Chauhan, A.K. Shah, and M.R. Yadav. 2008. Recent advances in selective α_1-adrenoreceptor antagonists as antihypertensive agents. *Bioorganic & Medicinal Chemistry* 16: 4759–4800.

Kagayama, K., T. Morimoto, S. Nagata, F. Katoh, X. Zhang, N. Inoue, A. Hashino, K. Kageyama, J. Shikaura, and T. Niwa. 2009. Synthesis and biological evaluation of novel phthalazinone derivatives as topically active phosphodiesterase 4 inhibitors. *Bioorganic & Medicinal Chemistry* 17: 6959–6970.

Kojima, Y., S. Sasaki, N. Oda, T. Koshimizu, Y. Hayashi, M. Kiniwa, G. Tsujimoto, and K. Kohri. 2009. Prostate growth inhibition by subtype-selective alpha(1)-adrenoceptor antagonist naftopidil in benign prostatic hyperplasia. *Prostate* 69: 1521–1528.

Kulig, K., J. Sapa, A. Nowaczyk, B. Filipek, and B. Malawska. 2009. Design, synthesis and pharmacological evaluation of new 1 -[3-(4-arylpiperazin-1-yl)-2-hydroxy-propyl]-3,3-diphenylpyrrolidin-2-one derivatives with antiarrhythmic, antihypertensive, and α-adrenolytic activity. *European Journal of Medicinal Chemistry* 44: 3994–4003.

Romeo, G., L. Materia, M.N. Modica, V. Pittala, L. Salerno, M.A. Siracusa, F. Manetti, M. Botta, and K.P. Minneman. 2011. Novel 4-phenylpiperidine-2,6-dione derivatives. Ligands for α_1-adrenoceptor subtypes. *European Journal of Medicinal Chemistry* 46: 2676–2690.

Sharma, B.K., K. Sarbhai, and P. Singh. 2010. A rationale for the activity profile of arylpiperazinylthioalkyls as 5-HT1A-serotonin and α_1-adrenergic receptor ligands. *European Journal of Medicinal Chemistry* 45: 1927–1934.

Sotelo, E., N. Fraiz, M. Yáñez, V. Terades, R. Laguna, E. Cano, and E. Raviña. 2002. Pyridazines. Part XXIX: synthesis and platelet aggregation inhibition activity of 5-substituted-6-phenyl-3(2*H*)-pyridazinones. Novel aspects of their biological actions. *Bioorganic & Medicinal Chemistry* 10: 2873–2882.

Strappaghetti, G., C. Brodi, G. Giannaccini, and L. Betti. 2006. New 4-(4-methyl-phenyl)phthalazin-1(2*H*)-one derivatives and their effects on α_1-receptors. *Bioorganic & Medicinal Chemistry Letters* 16: 2575–2579.