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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

4-Substitutedphthalazines and phthalazinones: synthesis, characterization and β-adrenergic blocking activity

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Abstract Novel 4-(4-bromophenyl)phthalazine and phthalazinone derivatives connected through 2-propanol spacer to *N*-substituted piperazine residue were synthesized. All the new compounds were screened for their effect on β-adrenergic blocking activity on the norepinephrine-induced precontracted aortic ring module. Most test compounds displayed appreciable β-adrenolytic activity compared to propranolol as a reference standard. The results have shown that compounds **3a**, **3d**, **3e** and **7c** displayed appreciable inhibition of norepinephrine-induced aortic ring contraction.

Keywords Phthalazines · Phthalazinones · Adrenergic β-blockers · Arylpiperazine

Introduction

Despite the significant progress made in prevention and treatment, cardiovascular diseases are still the main cause of death worldwide (Lopez *et al.*, 2006). The use of β-adrenoceptor antagonists is well-established in the treatment of various cardiovascular disorders. Since development of this class of drugs in the late 1950s of twentieth century, they are administered in the therapy of hypertension, coronary artery disease, arrhythmia, myocardial infarction and heart failure (Panjrath and Messerli, 2006). Also, much attention is being paid to β-blockers that possess vasodilator action produced through different mechanisms, such as release of nitric oxide (NO), antioxidant action, β_2-agonistic action, Ca entry blockade and α_1-blockade (Toda, 2003).

In the last decade, a new generation of β-blockers with additional α-adrenoceptor blocking activity was introduced to therapy. The α/β-blockers (bucindolol, carvedilol and labetalol) have vasodilating properties via relaxation of arterial smooth muscle, with no reflex tachycardia, as a result of β-adrenoceptor blockade (Matsuda *et al.*, 2000; Marona *et al.*, 2008). They have also beneficial effects on the regular circulation in contrast to classic β-blockers (Toda, 2003; Carella *et al.*, 2010).

Pyridazinone and phthalazinone derivatives have been reported to possess a variety of pharmacological effects on the cardiovascular system (Demirayak *et al.*, 2004a; Del Olmo *et al.*, 2006; Bansal *et al.*, 2009). Within the drugs in the market, hydralazine, one of the first antihypertensive agents, is considered as a lead for developing new drugs, due to its direct vasodilator effect (Del Olmo *et al.*, 2006). Structural modification of hydralazine led to the discovery of new phthalazine candidates possessing antihypertensive effect (Demirayak *et al.*, 2004b). However, in the long-term treatment of hypertension, the use of vasodilators alone does not suffice and it is the concomitant use of β-blockers which has proved to be useful for achieving adequate control of blood pressure (Bisi *et al.*, 2003).

β-Adrenergic blocking agents are very homogeneous in their chemical structures, which generally include the 2-aminoethanol basic skeleton to which the other groups of molecules are linked and which should be associated principally with the ability of these compounds to bind with the receptors (Saccomanni *et al.*, 2003).

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On the other hand, the nature of aromatic nucleus generally determines the blocking or stimulant properties of these compounds, (Macchia *et al.*, 1985).

β-Adrenergic antagonists containing phenylpiperazine moiety comprise a class of compounds with ancillary vasodilator properties and superior clinical efficacy compared to classical *β*-blockers (Toda, 2003; Gonec *et al.*, 2008; Racanska *et al.*, 2010).

In the past few decades, progress in understanding the biochemical pharmacology of *β*-blockers has lead to a more rational approach in designing new drug combinations involving this 2-hydroxylpropyl spacer. In this regard, keeping the basic 2-hydroxypropyl spacer for significant *β*-adrenoreceptor antagonistic activity in combination with the vasorelaxant-substituted phthalazine pharmacophore, two series of 4-(4-bromophenyl)phthalazine derivatives connected through 2-propanol spacer to N-substitutedpiperazine residue **3a–f** and **7a–f** were synthesized with the aim to elicit their *β*-adrenolytic activity.

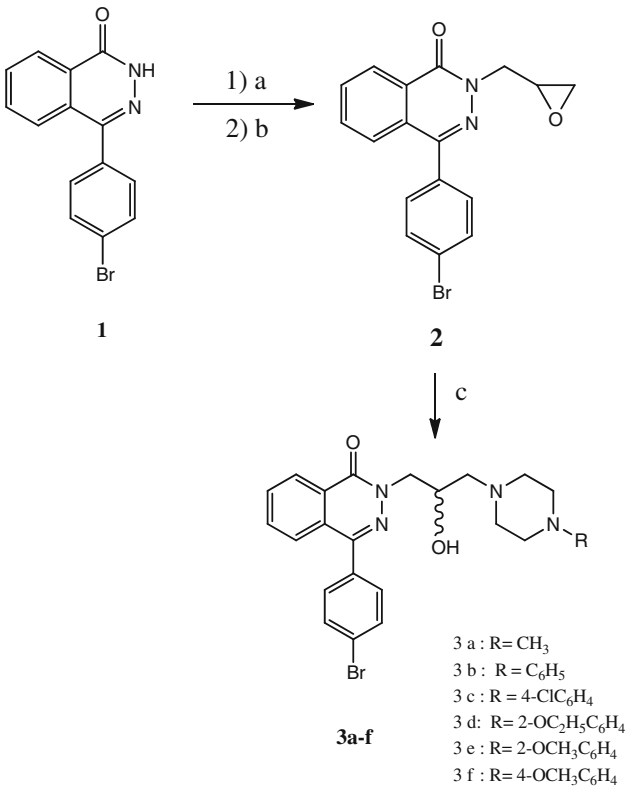
Results and discussion

Chemistry

The synthetic route used to synthesize the *N*-substituted derivatives **3a–f** is outlined in Scheme 1. 4-(4-Bromophenyl)-2(1*H*)-phthalazinone (**1**) was prepared by cyclization of 2-(4-bromobenzoyl)benzoic acid with hydrazine hydrate according to reported methods (Yamaguchi *et al.*, 1993; Colotta *et al.*, 1994). Reacting the sodium salt of **1** with epichlorohydrin in dimethylsulphoxide afforded the intermediate **2**. The product thus obtained was identified by its ^{1}HNMR spectrum that showed two doublet of doublets at *δ*2.76 and 2.83 ppm corresponding to two non-equivalent protons of N–CH_2. The oxirane ring was identified by the existence of the two doublet of doublets at *δ*4.43 and 4.46 ppm of the CH_2–O non-equivalent protons and a multiplet at 3.41–3.46 ppm due to CH–O proton. The mass spectrum revealed the $M^{\cdot}$ and M+2 fragments, in addition to the base beak at *m/z* 57 indicating the $C_3H_5O^{\cdot+}$ fragment.

The epoxy bridge of **2** was cleaved by reacting the crude product with an appropriate *N*-substituted piperazine in anhydrous acetonitrile and K_2CO_3 to give the target compounds **3a–f**. In this reaction, the epoxide opening takes place in a regioselective manner preferentially by terminal attack of the nucleophile to give the racemic compounds.

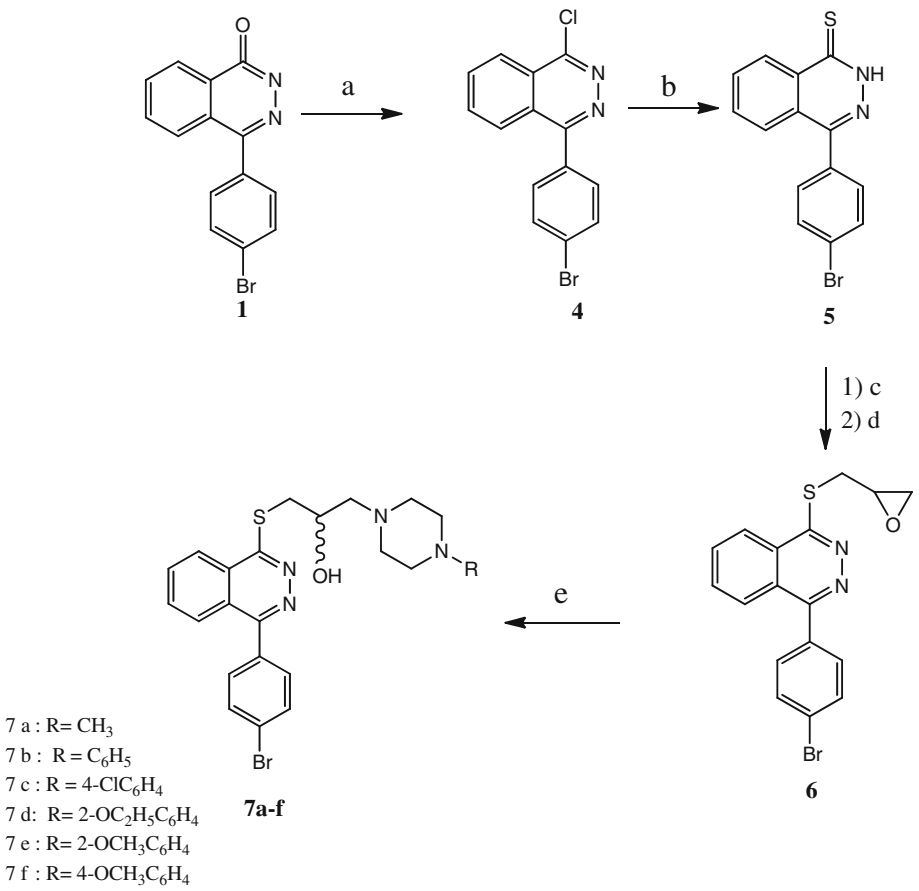
The structures of the new targets were established by IR, ^{1}HNMR and mass spectral assignments. The IR spectra displayed amide C=O stretching bands of phthalazinone ring system, which are characteristic for all compounds at 1,651–1,654 cm^{-1}. As expected, the stretching bands



Scheme 1 Reagents and conditions: *a* C_2H_5ONa/C_2H_5OH, *b* epichlorohydrin, DMSO, 25 °C, *c* 4-(substituted) piperazines, CH_3CN, K_2CO_3

corresponding to O–H were observed in the range of 3,450–3,300 cm^{-1}. The ^{1}HNMR spectra revealed signals arising due to typical secondary alcohol. The spectra displayed two doublet of doublets of the non-equivalent protons of N–CH_2 in the range of *δ* 2.59–2.64 ppm, and two multiplets at *δ*3.0–3.16 and *δ* 4.27–4.45 ppm corresponding to N–CH_2 and CH–O protons, respectively. The two multiplets of piperazine protons appeared at *δ*2.63–2.70 and *δ*2.74–2.86 ppm. The phthalazinone residue showed the characteristic phthalazinone C_8 protons resonated as doublet at about 8.5 ppm. The mass spectra showed $M^{\cdot}$, M+2 and in some cases M+4 fragments in addition to the characteristic fragmentation pattern. All compounds displayed notable peaks at *m/z* 345 and 343 due to $C_{16}H_{12}BrN_2O_2^+$ together with the corresponding 1-methylenepiperazin-1-ium fragment.

The synthesis of S-substituted derivatives **7a–f** is shown in Scheme 2. Chlorination of **1** by phosphorus oxychloride afforded the 1-chloro derivative **4** (Colotta *et al.*, 1994). The latter was reacted with thiourea to give the isothiouronium intermediate, which upon alkaline hydrolysis yielded the corresponding thione **5** following procedure reported for analogous compounds (Badran *et al.*, 2003). The sodium salt of **5** was stirred with epichlorohydrin in dimethylsulphoxide to give the intermediate methyloxirane



Scheme 2 Reagents and conditions: *a* $POCl_3$, *b* H_2NCSNH_2, *c* C_2H_5ONa/C_2H_5OH, *d* epichlorohydrin, DMSO, 25 °C, *e* 4-(substituted) piperazine, CH_3CN, K_2CO_3

derivative **6**. Subsequent ring opening with the relevant *N*-substituted piperazine afforded the target compounds **7a–f**.

The mass spectrum of **6** showed the characteristic $M^{\cdot+}$ and M+2 in 1:1 ratio. ^{1}HNMR of compounds **7a–f** displayed the characteristic aliphatic protons resonated in the expected regions.

Pharmacology

Assessment of potential β-blockade

The pharmacological evaluation of the possible *β*-blocking activity of the test compounds **3a–f** and **7a–f** has been carried out on the norepinephrine (NE)-induced precontracted aortic rings module. Blunting isoprenaline-induced relaxation was quantified as described in methodology section. Isoprenaline relaxed the NE-induced precontracted aortic rings by 19.75 % of the contracted tension. The reference drug, propranolol not only blunted the isoprenaline-induced relaxation, but also induced further 1.16 % contraction in the aortic ring preparation (negative sign indicates further contraction). Compounds **3a**, **3d**, **3e** and **7c** showed the most potent *β*-blocking activity by complete blunting and even further contracting the aortic ring preparation by 0.71 to 6.18 % of its pre-contracted tension. Compounds **3b** and **3c** displayed potentially strong *β*-blocking activity by decreasing the isoprenaline-induced relaxation to 2.2 and 3.2 %, respectively, of pre-contracted aortic tension compared to 19.75 % of control untreated aortic ring preparation. However, compounds **7b**, **7d** and **7f** exhibited mild to moderate *β*-blocking activity by decreasing the isoprenaline-induced relaxation to 12.05–15.43 % of pre-contracted aortic tension. On the other hand, compounds **7a**, **7e** and **7f** did not show tangible blocking of isoprenaline-induced relaxation. It is worth to mention that, the compound **3a** also strongly inhibited the NE-induced contraction of the aortic ring preparation to 9.52 % compared to 51.18 % of control untreated preparation. However, the test compounds **3c**, **7d**, **7e** and **7f** showed similar but weaker inhibition effects to NE-induced aortic ring contraction (Table 1).

The pharmacological screening revealed that the *N*-substituted derivatives **3a–f** displayed more potent *β*-adrenergic blocking activity than the *S*-substituted analogues **7a–f**. In the first series of compounds, it is obvious that the compounds with methyl or *o*-substituted phenyl groups on the piperazine nitrogen **3a**, **3d**, **3e** showed the highest *β*-blocking activity. Moreover, the unsubstituted and *p*-chloro substituted analogues **3b**, **3c** were found to

Table 1 Change in aortic muscle tension after NE and isoprenaline exposure

	NE-induced change in aortic muscle tension (%)	Isoprenaline-induced change in precontracted aortic muscle (%)
Control	51.18	19.75
Propranolol	63.91	−1.16
3a	9.52	−6.18
3b	42.03	2.2
3c	31.05	3.2
3d	55.2	−3.87
3e	54.57	−0.71
3f	47.36	18.01
7a	46.6	18.51
7b	45.32	12.79
7c	42.35	−1.83
7d	31.55	12.05
7e	19.37	17.4
7f	18.9	15.43

Change in tension is expressed as average muscle tone over duration of 1–2 min of recording

possess appreciable β-blocking activity. In contrast, the *p*-methoxyphenyl derivative **3f** did not show promising β-blocking effect.

Within the series of *S*-substituted compounds **7a–f**, the *p*-chloro-substituted derivative **7c** was the only compound that displayed potent β-adrenergic blockade. Other derivatives showed from weak to moderate activities.

Conclusion

In summary, certain 4-(4-bromophenyl)phthalazine and phthalazinone derivatives connected through 2-propanol spacer to *N*-substituted piperazine residue were synthesized and screened for their β-adrenergic blocking activity on the norepinephrine-induced precontracted aortic ring module. All compounds were obtained and tested as racemates. The results revealed that *N*-substituted derivatives **3a–f** generally displayed more potent β-adrenergic blocking activity than the *S*-substituted analogues **7a–f**. The test compounds **3a**, **3d**, **3e** and **7c** showed the most potent β-blocking activity by complete blunting and even further contracting the aortic ring preparation by 0.71 to 6.18 % of its precontracted tension.

Experimental

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}). ^1H NMR spectra were carried out using a Varian Gemini 200 MHz Spectrophotometer and 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.

2-(4-Bromodbenzoyl)benzoic acids (Yamaguchi *et al.*, 1993), 4-(4-bromophenyl) phthalazin-1(2*H*)-ones **(1)** and 1-chloro-4-(4-bromophenyl)phthalazines **(4)** (Colotta *et al.*, 1994) were prepared as reported.

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

4-(4-Bromophenyl)-2-(oxiran-2-ylmethyl)phthalazin-1(2*H*)-one (**2**)

A solution of **1** (1 g, 0.0033 mol) in absolute ethanol (10 ml)-containing metallic sodium (0.1 g, 0.043 mol) was heated under reflux for 30 min, then the solvent was distilled off under reduced pressure. The resulting sodium salt was dissolved in dimethylsulphoxide (10 ml), epichlorohydrin (1.0 g, 0.84 ml, 0.0117 mol) was added and the mixture was stirred overnight at room temperature. The precipitated solid was filtered, washed with water, dried and crystallized from benzene–petrolium ether mixture (60–80 °C).

Yield 85 %; mp 112–113 °C; IR (KBr) cm^{-1}: 3059 (C–H aromatic), 2927, 2850 (C–H aliphatic), 1658 (C=O); ^{1}HNMR ($CDCl_3$) δ ppm: 2.76 (dd, 1H, N–CH_2), 2.83 (dd, 1H, N–CH_2), 3.41–3.46 (m, 1H, CH-oxiranic), 4.43 (dd, 1H, CH_2-oxiranic), 4.46 (dd, 1H, CH_2-oxiranic), 7.45 (dd, 2H, Ar–H, J = 8.4 Hz), 7.64 (dd, 2H, Ar–H, J = 8.4 Hz), 7.67–7.80 (m, 3H, Ar–H), 8.50 (d, 1H, Ar–H_5); MS (EI) *m/z* (% rel. Int.): 358 (M+2, 33.73), 356 (M$^+$, 34.81), 315 (14.62), 313 (15.33), 301 (25.36), 299 (24.23), 57 (100). Anal. Calcd for $C_{17}H_{13}BrN_2O_2$, Mwt. (357.20): C, 57.16; H, 3.67; N, 7.84; Found: C, 57.00; H, 3.35; N, 8.05.

(R/S)4-(4-Bromophenyl)-2-[2-hydroxy-3-(4-substitutedpiperazin-1-yl)propyl]- phthalazin-1(2*H*)-ones (**3a–f**)

A mixture of **2** (0.0016 mol), anhydrous K_2CO_3 (0.276 g, 0.002 mol) and few specs of KI in anhydrous acetonitrile (20 ml) was heated under reflux for 30 min. An appropriate substituted piperazine, (0.002 mol), commercially

available, was added to the hot reaction mixture was heated under reflux for 10 h. After dilution with ice-cold water (15 ml), the mixture was extracted with dichloromethane (3 × 10 ml), then the solvent was removed in vacuo to give the final targets **3a–f**. The compounds were purified by trituration with petroleum ether (60–80 °C), then crystallized from benzene–petroleum ether mixture (60–80 °C).

(R/S)4-(4-Bromophenyl)-2-[2-hydroxy-3-(4-methylpiperazin-1-yl)propyl]-phthalazin-1(2*H*)-one (**3a**)

Yield 60 %; mp 101–102 °C; IR (KBr) cm^{-1}: 3421 (O–H), 3082 (C–H aromatic), 2951, 2823 (C–H aliphatic), 1651 (C=O); ^{1}HNMR (DMSO-d_6) δ ppm: 1.90–2.05 (br, 1H, OH, D_2O exchangeable), 2.44 (s, 3H, CH_3), 2.60 (dd, 1H, N–CH_2), 2.64 (dd, 1H, N–CH_2), 2.66–2.70 (br, 4H, piperazine), 2.74–2.80 (br, 4H, piperazine), 3.13–3.16 (m, 2H, CH_2N), 4.27–4.34 (m, 1H, CH–O), 7.50–7.90 (m, 7H, Ar–H), 8.36 (d, 1H, Ar–H); MS (EI) *m/z* (% rel. Int.): 458 (M+2, 0.23), 456 (M⁺, 0.31), 440 (3.83), 438 (3.09), 345 (2.18), 343 (1.86), 113 (100). Anal. Calcd for $C_{22}H_{25}BrN_4O_2$, Mwt. (457.36): C, 57.77; H, 5.51; N, 12.25; Found: C, 57.56; H, 5.62; N, 12.32.

(R/S)4-(4-Bromophenyl)-2-[2-hydroxy-3-(4-phenylpiperazin-1-yl)propyl]-phthalazin-1(2*H*)-one (**3b**)

Yield 52 %; mp 95–96 °C; IR (KBr) cm^{-1}: 3421 (O–H), 3066 (C–H aromatic), 2927, 2850 (C–H aliphatic), 1654 (C=O); ^{1}HNMR (DMSO-d_6) δ ppm: 1.62–1.80 (br, 1H, OH, D_2O exchangeable), 2.61 (dd, 1H, N–CH_2), 2.63 (dd, 1H, N–CH_2), 2.66–2.70 (br, 4H, piperazine), 2.75–2.79 (br, 4H, piperazine), 3.08–3.11 (m, 2H, CH_2N), 4.28–4.35 (m, 1H, CH–O), 7.23–7.80 (m, 12H, Ar–H), 8.51 (d, 1H, Ar–H); MS (EI) *m/z* (% rel. Int.): 520 (M+2, 1.72), 518 (M⁺, 1.62), 345 (8.47), 343 (8.29), 315 (1.89), 313 (1.77), 175 (100). Anal. Calcd for $C_{27}H_{27}BrN_4O_2$, Mwt. (519.43): C, 62.43; H, 5.24; N, 10.79; Found: C, 62.66; H, 5.05; N, 10.45.

(R/S)4-(4-Bromophenyl)-2-{2-hydroxy-3-[4-(4-chlorophenyl)piperazin-1-yl]propyl}- phthalazin-1(2*H*)-one (**3c**)

Yield 55 %; mp 175–176 °C; IR (KBr) cm^{-1}: 3429 (O–H), 3082 (C–H aromatic), 2951, 2823 (C–H aliphatic), 1651 (C=O); ^{1}HNMR ($CDCl_3$) δ ppm: 1.62–1.80 (br, 1H, OH, D_2O exchangeable), 2.59 (dd, 1H, N–CH_2), 2.61 (dd, 1H, N–CH_2), 2.63–2.66 (br, 4H, piperazine), 2.76–2.80 (br, 4H, piperazine), 3.13–3.16 (m, 2H, CH_2–N), 4.32–4.42 (m, 1H, CH–O), 6.80–7.82 (m, 11H, Ar–H), 8.53 (d, 1H, Ar–H); MS (EI) *m/z* (% rel. Int.): 556 (M+4, 1.93), 554 (M+2, 7.03), 552 (M⁺, 5.57), 345 (10.18), 343 (11.08), 209 (100), 166 (16.15). Anal. Calcd for $C_{27}H_{26}BrClN_4O_2$, Mwt. (553.88): C, 58.55; H, 4.73; N, 10.12; Found: C, 58.26; H, 4.65; N, 10.44.

(R/S)4-(4-Bromophenyl)-2-{2-hydroxy-3-[4-(2-ethoxyphenyl)piperazin-1-yl]propyl}- phthalazin-1(2*H*)-one (**3d**)

Yield 50 %; mp 135–136 °C; IR (KBr) cm^{-1}: 3422 (O–H), 3051 (C–H aromatic), 2950, 2816 (C–H aliphatic), 1651 (C=O); ^{1}HNMR ($CDCl_3$) δ ppm: 1.44 (t, 3H, CH_2CH_3), 1.60–1.70 (br, 1H, OH, D_2O exchangeable), 2.60 (dd, 1H, N–CH_2), 2.64 (dd, 1H, N–CH_2), 2.66–2.68 (br, 4H, piperazine), 2.82–2.86 (br, 4H, piperazine), 3.06–3.11 (m, 2H, CH_2N), 4.05 (q, 2H, CH_2CH_3), 4.32–4.41 (m, 1H, CH–O), 6.83–7.82 (m, 11H, Ar–H), 8.55 (d, 1H, Ar–H); MS (EI) *m/z* (% rel. Int.): 564 (M+2, 12.95), 562 (M⁺, 13.97), 345 (18.63), 343 (18.89). Anal. Calcd for $C_{29}H_{31}BrN_4O_3$, Mwt. (563.49): C, 61.81; H, 5.55; N, 9.94; Found: C, 61.58; H, 5.78; N, 10.24.

(R/S)4-(4-Bromophenyl)-2-{2-hydroxy-3-[4-(2-methoxyphenyl)piperazin-1-yl]propyl}- phthalazin-1(2*H*)-one (**3e**)

Yield 80 %; mp 160–161 °C; IR (KBr) cm^{-1}: 3433 (O–H), 3059 (C–H aromatic), 2935, 2819 (C–H aliphatic), 1654 (C=O); ^{1}HNMR ($CDCl_3$) δ ppm: 1.73–1.82 (br, 1H, OH, D_2O exchangeable), 2.60 (dd, 1H, N–CH_2), 2.64 (dd, 1H, N–CH_2), 2.66–2.69 (br, 4H, piperazine), 2.82–2.86 (br, 4H, piperazine), 3.00–3.08 (m, 2H, CH_2N), 3.85 (s, 3H, OCH_3), 4.32–4.45 (m, 1H, CH–O), 6.84–7.83 (m, 11H, Ar–H), 8.53 (d, 1H, Ar–H); MS (EI) *m/z* (% rel. Int.): 550 (M+2, 1.58), 548 (M⁺, 1.75), 345 (1.58), 343 (1.73), 315 (1.14), 313 (1.08), 205 (100). Anal. Calcd for $C_{28}H_{29}BrN_4O_3$, Mwt. (549.46): C, 61.21; H, 5.32; N, 10.20; Found: C, 61.46; H, 5.25; N, 10.34.

(R/S)4-(4-Bromophenyl)-2-{2-hydroxy-3-[4-(4-methoxyphenyl)piperazin-1-yl]propyl}- phthalazin-1(2*H*)-one (**3f**)

Yield 65 %; mp 122–123 °C; IR (KBr) cm^{-1}: 3426 (O–H), 3100 (C–H aromatic), 2929, 2823 (C–H aliphatic), 1651 (C=O); ^{1}HNMR ($CDCl_3$) δ ppm: 1.70–1.85 (br, 1H, OH, D_2O exchangeable), 2.59 (dd, 1H, N–CH_2), 2.64 (dd, 1H, N–CH_2), 2.66–2.70 (br, 4H, piperazine), 2.77–2.83 (br, 4H, piperazine), 3.07–3.09 (m, 2H, CH_2N), 3.77 (s, 3H, OCH_3), 4.34–4.42 (m, 1H, CH–O), 6.82–7.82 (m, 11H, Ar–H), 8.56 (d, 1H, Ar–H); MS (EI) *m/z* (% rel. Int.): 550 (M+2, 7.29), 548 (M⁺, 6.95), 345 (2.25), 343 (2.49), 205 (100). Anal. Calcd for $C_{28}H_{29}BrN_4O_3$, Mwt. (549.46): C, 61.21; H, 5.32; N, 10.20; Found: C, 61.55; H, 5.65; N, 10.48.

4-(4-Bromophenyl)phthalazine-1(2*H*)-thione (**5**)

A mixture of **4** (0.63 g, 0.002 mol) and thiourea (0.23 g, 0.003 mol) was heated under reflux in absolute ethanol (20 ml) for 3 h. The reaction mixture was allowed to cool to room temperature and the excess solvent was removed under diminished pressure. The crude isothiouronium salt was combined with sodium hydroxide solution (10 %, 20 ml) and the mixture was heated under reflux for 2 h, cooled to room temperature then acidified with glacial acetic acid. The resulting orange precipitate was filtered, washed with water and crystallized from ethanol.

Yield 97 %, mp 229–230 °C; IR (KBr) cm^{-1}: 3100 (C–H aromatic); ^{1}HNMR (DMSO-d_6) δ ppm: 7.12–7.88 (m, 8H, Ar–H); MS (EI) *m/z* (% rel. Int.): 318 (M+2, 100), 316 ($M^{+\cdot}$, 98.78). Anal. Calcd for $C_{14}H_9BrN_2S$, Mwt. (317.20): C, 53.01; H, 2.86; N, 8.83; Found: C, 53 05; H, 3.00; N, 8.75.

1-(4-Bromophenyl)-4-(oxiran-2-ylmethylthio)phthalazine (**6**)

The title compound was prepared from **5** and epichlorohydrin following the procedure described for the compound **2**.

Yield 88 %; mp 101–102 °C; IR (KBr) cm^{-1}: 3062 (C–H aromatic), 2920, 2850 (C–H aliphatic); ^{1}HNMR ($CDCl_3$) δ ppm: 2.47 (dd, 1H, N–CH_2), 2.72 (dd, 1H, N–CH_2), 3.26-3.29 (m, 1H, CH-oxiranic), 3.62 (dd, 1H, CH_2-oxiranic), 3.72 (dd, 1H, CH_2-oxiranic), 7.19-7.73 (m, 7H, Ar–H); 8.45 (d, 1H, Ar–H); MS (EI) *m/z* (% rel. Int.): 374 (M+2, 7.19), 372 ($M^{+\cdot}$, 7.42), 313 (6.92). Anal. Calcd for $C_{17}H_{13}BrN_2OS$, Mwt. (373.27): C, 54.70; H, 3.51; N, 7.50; Found: C, 54.55; H, 3.63; N, 7.88.

(R/S)1-[4-(4-Bromophenyl)phthalazin-1-ylthio]-3-(4-substitutedpiperazin-1-yl) propan-2-ols (**7a**–**f**)

The title compounds were prepared from **6** and an appropriate 4-substituted piperazines following the procedure described for the compounds **3a**–**f**.

(R/S)1-[4-(4-Bromophenyl)phthalazin-1-ylthio]-3-(4-methylpiperazin-1-yl) propan-2-ol (**7a**)

Yield 70 %; mp 125–126 °C; IR (KBr) cm^{-1}: 3414 (O–H), 3100 (C–H aromatic), 2924, 2850 (C–H aliphatic); ^{1}HNMR ($CDCl_3$) δ ppm: 1.67–1.85 (br, 1H, OH, D_2O exchangeable), 2.41 (s, 3H, CH_3), 2.55 (dd, 1H, CH_2), 2.58 (dd, 1H, CH_2), 2.68–2.73 (br, 4H, piperazine), 2.80–2.84 (br, 4H, piperazine), 3.34–3.42 (m, 2H, CH_2), 4.18–4.35 (m, 1H, CH–O), 7.19–7.67 (m, 7H, Ar–H), 8.23 (d, 1H, Ar–H); MS (EI) *m/z* (% rel. Int.): 474 (M+2, 14.48), 456 (M-18, 16.01), 342 (22.08), 316 (74.22). Anal. Calcd for $C_{22}H_{25}BrN_4OS$, Mwt. (473.43): C, 55.81; H, 5.32; N, 11.83; Found: C, 55.65; H, 5.18; N, 11.48.

(R/S)1-[4-(4-Bromophenyl)phthalazin-1-ylthio]-3-(4-phenylpiperazin-1-yl) propan-2-ol (**7b**)

Yield 75 %; mp 140–141 °C; IR (KBr) cm^{-1}: 3421 (O–H), 3080 (C–H aromatic), 2924, 2845 (C–H aliphatic); ^{1}HNMR (DMSO-d_6) δ ppm: 1.55–1.60 (br, 1H, OH, D_2O exchangeable), 2.62–2.67 (br, 4H, piperazine), 2.78 (dd, 1H, CH_2), 2.82 (dd, 1H, CH_2), 3.20–3.25 (br, 4H, piperazine), 3.58 (dd, 1H, CH_2), 3.77 (dd, 1H, CH_2), 3.85–4.00 (m, 1H, CH–O), 7.59–7.99 (m, 12H, Ar–H), 8.26 (d, 1H, Ar–H); MS (EI) *m/z* (% rel. Int.): 534 ($M^{+\cdot}$, 7.37), 342 (72.13), 174 (13.64). Anal. Calcd for $C_{27}H_{27}BrN_4OS$, Mwt. (535.50): C, 60.56; H, 5.08; N, 10.46; Found: C, 60.25; H, 5.35; N, 10.78.

(R/S)1-[4-(4-Bromophenyl)phthalazin-1-ylthio]-3-[4-(4-chlorophenyl)piperazin-1-yl]propan-2-ol (**7c**)

Yield 73 %; mp 158–159 °C; IR (KBr) cm^{-1}: 3425 (O–H), 3062 (C–H aromatic), 2939, 2823 (C–H aliphatic); ^{1}HNMR ($CDCl_3$) δ ppm: 1.60–1.70 (br, 1H, OH, D_2O exchangeable), 2.53–2.60 (m, 2H, CH_2), 2.65–2.68 (br, 4H, piperazine), 3.05–3.08 (br, 4H, piperazine), 3.24–3.29 (m, 2H, CH_2), 3.98–4.06 (m, 1H, CH–O), 7.10–7.71 (m, 11H, Ar–H), 8.45 (d, 1H, Ar–H); MS (EI) *m/z* (% rel. Int.): 566 (M−2, 0.17), 342 (9.51), 340 (11.17), 209 (12.56). Anal. Calcd for $C_{27}H_{26}BrClN_4OS$, Mwt. (569.94): C, 56.90; H, 4.60; N, 9.83; Found: C, 56.75; H, 4.67; N, 10.12.

(R/S)1-[4-(4-Bromophenyl)phthalazin-1-ylthio]-3-[4-(2-ethoxyphenyl)piperazin-1-yl]propan-2-ol (**7d**)

Yield 68 %; mp 130–131 °C; IR (KBr) cm^{-1}: 3444 (O–H), 3062 (C–H aromatic), 2931, 2812 (C–H aliphatic); ^{1}HNMR ($CDCl_3$) δ ppm: 1.17 (t, 3H, CH_2CH_3), 1.50–1.57 (br, 1H, OH D_2O exchangeable), 2.64 (dd, 1H, CH_2), 2.67 (dd, 1H, N–CH_2), 2.69–2.72 (br, 4H, piperazine), 2.84–2.88 (br, 4H, piperazine), 3.10–3.17 (m, 2H, CH_2N), 4.15 (q, 2H, CH_2CH_3), 4.23–4.28 (m, 1H, CH–O), 6.98–7.89 (m, 11H, Ar–H), 8.35 (d, 1H, Ar–H); MS (EI) *m/z* (% rel. Int.): 578 ($M^{+\cdot}$, 11.53), 342 (8.78), 219 (7.40). Anal. Calcd for $C_{29}H_{31}BrN_4O_2S$, Mwt. (579.55): C, 60.10; H, 5.39; N, 9.67; Found: C, 60.34; H, 5.58; N, 9.48.

(R/S)1-[4-(4-Bromophenyl)phthalazin-1-ylthio]-3-[4-(2-methoxyphenyl) piperazin-1-yl]propan-2-ol (**7e**)

Yield 72 %; mp 125–126 °C; IR (KBr) cm^{-1}: 3440 (O–H), 3062 (C–H aromatic), 2931, 2816 (C–H aliphatic);

^{1}HNMR ($CDCl_3$) δ ppm: 1.50–1.61 (br, 1H, OH, D_2O exchangeable), 2.62–2.66 (m, 2H, CH_2), 2.70–2.74 (br, 4H, piperazine), 2.81–2.85 (br, 4H, piperazine), 3.10–3.16 (m, 2H, CH_2N), 3.77 (s, 3H, OCH_3), 4.33–4.42 (m, 1H, CH–O), 7.16–7.80 (m, 11H, Ar–H), 8.55 (d, 1H, Ar–H); MS (EI) *m/z* (% rel. Int.): 566 (M+2, 19.75), 564 ($M^{\pm}$, 16.02). Anal. Calcd for $C_{28}H_{29}BrN_4O_2S$, Mwt. (564.12): C, 59.47; H, 5.17; N, 9.91; Found: C, 59.35; H, 5.05; N, 10.26.

(R/S)1-[4-(4-Bromophenyl)phthalazin-1-ylthio]-3-[4-(4-methoxyphenyl) piperazin-1-yl]propan-2-ol (**7f**)

Yield 80 %; mp 150–151 °C; IR (KBr) cm^{-1}: 3421 (O–H), 3062 (C–H aromatic), 2985, 2866 (C–H aliphatic); ^{1}HNMR ($CDCl_3$) δ ppm: 1.58–1.65 (br, 1H, OH, D_2O exchangeable), 2.35–2.40 (m, 2H, CH_2), 2.55–2.62 (br, 4H, piperazine), 2.85–2.95 (br, 4H, piperazine), 3.35–3.42 (m, 2H, CH_2), 3.64 (s, 3H, OCH_3), 4.12–4.30 (m, 1H, CH–O), 6.85–7.80 (m, 11H, Ar–H), 8.25 (d, 1H, Ar–H); MS (EI) *m/z* (% rel. Int.): 566 (M+2, 10.36), 360 (6.72), 343 (55.39), 205 (11.48), 193 (22.04). Anal. Calcd for $C_{28}H_{29}BrN_4O_2S$, Mwt. (564.12): C, 59.47; H, 5.17; N, 9.91; Found: C, 59.58; H, 5.26; N, 9.65.

Assessment of potential β-blocking activity

Drugs and chemicals Norepinephrine hydrochloride (NE) and isoprenaline were purchased from Sigma-Aldrich (St Louis, MO, USA). All other chemicals were of the highest commercially available grade.

Laboratory animals Adult male albino rats, weighing 180–250 g, were used in all the experiments of this study. They were obtained from the Animal House Colony of the National Research Center (Dokki, Giza, Egypt), and were housed under conventional laboratory conditions throughout the period of experimentation. The animals were fed a standard rat pellet diet and allowed free access to water. All the experimental techniques were approved by the ethical committee of the National Research Center.

Isolation and suspension of rat aortic rings Rats were euthanized by cervical dislocation and the thoracic aorta was carefully exposed and isolated. The isolated aorta was cut into rings (3–5 mm width) and each ring was then vertically 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 (Power Lab, AdInstruments Pty. Ltd.) which is connected to a computer. The chart for windows (v 3.4) software was used to record and elaborate data.

The isolated aortic ring was mounted in 10 ml water jacketed automatic multi-chamber organ bath system (Model no. ML870B6/C, Panlab, Spain). The organ bath contained Krebs' solution of the following composition (g/l): NaCl 6.9, KCl 0.35, KH_2PO_4 0.16, $MgSO_4{\cdot}7H_2O$ 0.3, $CaCl_2{\cdot}2H_2O$ 0.37, $NaHCO_3$ 2.1 and glucose 1.05. The organ bath solution was continuously aerated with carbogen (a mixture of 95 % O_2 and 5 % CO_2) and its temperature was kept at 37 °C. The preparation was allowed to equilibrate for about 120 min under a resting tension of 2 g. During that time any change in the resting tension was readjusted. The aortic ring was contracted by norepinephrine (10 M^{-6}) then relaxed by exposure to β-agonist, isoprenaline (10 M^{-6}). Pre-exposure of the aortic ring to β-blocker is supposed to suppress isoprenaline- induced aortic relaxation. Percent change in aortic tension after isoprenaline treatment was calculated for compounds under investigation (10 M^{-6}) and compared to standard β-blocker, propranolol (10 M^{-6}). Change in aortic tension was recorded and calculated over a period of one minute and average tension was used for calculation (Girgis *et al.*, 2010).

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