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Original article

Pharmacological evaluation of some new 1-substituted-4-hydroxyphthalazines

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Abstract

In the present study, a series of 1-substituted-4-hydroxyphthalazines were synthesized and characterized by IR, ^{1}H-NMR and Elemental analysis. The compounds were assayed against seizures induced by maximal electroshock (MES) and pentylenetetrazole (scMet). Neurologic deficit was evaluated by the rotarod test. The decrease in the elevated motor activity by introceptive chemical stimuli (amphetamine antagonistic activity) was studied at the dose level of 25 and 50 mg kg^{-1} and cardiac activity was also studied. All the compounds exhibited significant anticonvulsant activity. Compounds **4**, **12**, **13** and **17** were most active of the seriesagainst MES-induced seizures. Compounds **2**, **4**, **13** and **17** exhibited significant decrease in the elevated motor activity at the dose of 50 mg kg^{-1}. Remarkable sympathetic blocking activity was observed with **3**, **5**, **6**, **7**, **9** and **15** only.

Keywords: Amphetamine antagonism; Anticonvulsant; Aryloxypropanolamines; Aryloxyaminopropanes; Phthalazine; Sympatholytic

1. Introduction

Hydralazine is reported to be associated with drug induced systemic lupus erythematous [1] because of the presence of hydrazine group on phthalazine nucleus. Phthalazine derivatives were reported to possess anticonvulsant [2–4], cardiotonic [5] and vasorelaxant [6,7] anti-inflammatory [8,9] activities. Aryloxypropanolamines were reported to be associated with β-adrenergic blocking [10,11], CNS depressant [12], hypotensive [13] activities. Aryloxyaminopropanes were reported to possess CNS depressant [14], neuroleptic [15], antiarrythmic [16], hypotensive [17] activities. In view of these potential nature of the compounds, it was thought worthwhile to study the effects of two pharmacophoric moieties like phthalazine and propanolamine/aminopropane in a single molecule, on the biological activity. We have reported the potential anticonvulsant activity of aminopropanes from our laboratory [18–20], to continue our work in the same direction it was envisaged that chemical entities with phthalazine, aryloxypropanolamine and aryloxyaminopropane moieties would result in compounds of interesting biological activities.

In this study, we report the synthesis, the pharmacological evaluation, and stucture–activity relationship of 1-(3′-substituted-2-hydroxy/unsubstituted-propyloxy)-4-hydroxyphthalazine. The compounds were characterized by IR, ^{1}H-NMR spectral and Elemental analysis. The compounds were investigated for anticonvulsant activity. The decrease in the elevated motor activity by introceptive chemical stimuli (amphetamine antagonistic activity) at the dose level of 25 and 50 mg kg^{-1} and cardiac activity on isolated frog heart.

2. Chemistry

In the present study, 1,4-dihydroxyphthalazine was reacted with epichlorohydrine and 1-bromo-3-chloropropane in presence of silver chloride and sodium iodide to yield 1-(2′,3′-epoxypropyloxy)-4-hydroxyphthalazine and 1-(3′-chloropropyloxy)-4-hydroxyphthalazine, respectively. These compounds were reacted with several primary and secondary amines in the presence of 10%

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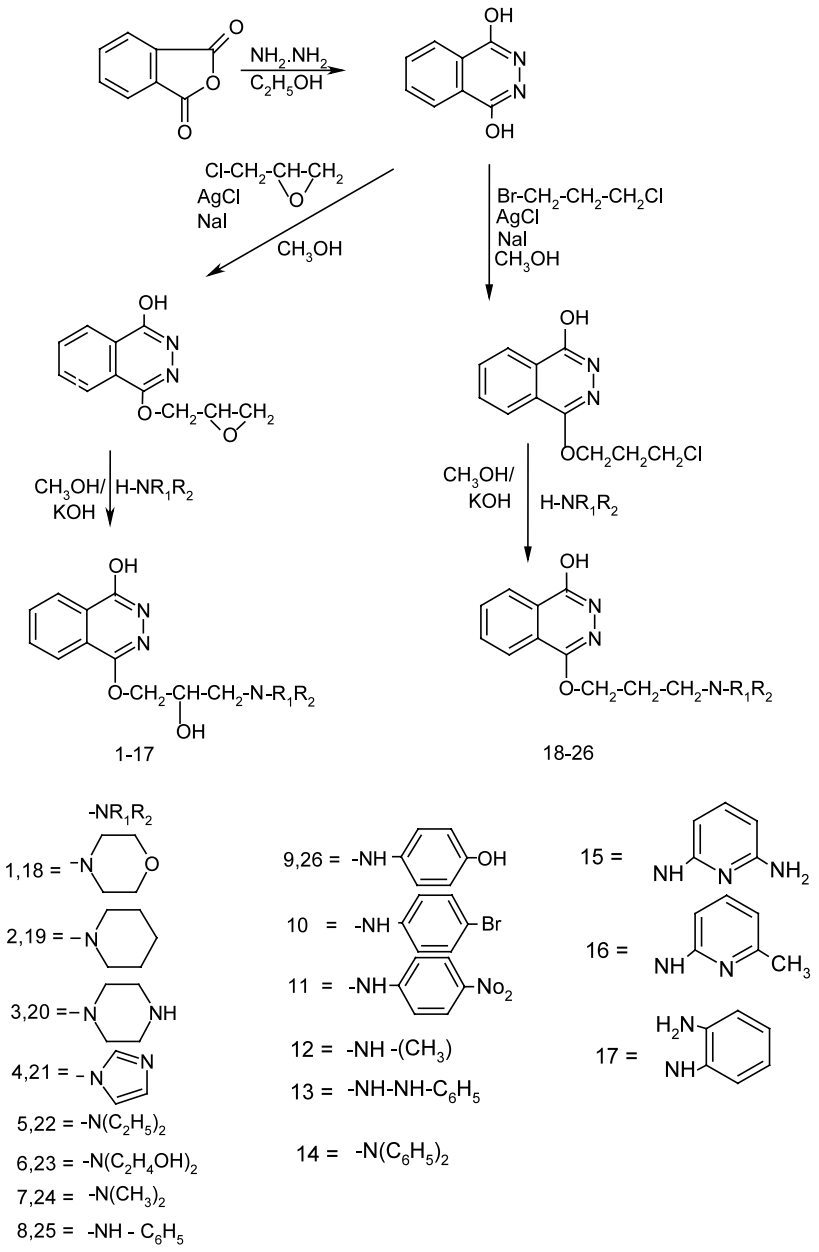

Fig. 1. Synthetic scheme.

methanolic potassium hydroxide to yield compounds **1**–**26** (Fig. 1).

3. Pharmacology

The protection offered by the synthesized compounds against clonic and tonic convulsions in wistar albino mice was screened at the dose levels of 30, 100 and 300 mg kg^{-1}. The decrease in the elevated motor activity induced by amphetamine (amphetamine antagonism) was also studied for the synthesized compounds at the dose levels of 25 and 50 mg kg^{-1}. The effects of the synthesized compounds on the rate and force of contraction was observed and the effects of these compounds with simultaneous administration of adrenaline were also studied on isolated frog heart.

4. Results and discussion

The compounds were tested for anticonvulsant activity by using the procedures described previously [21,22]. The initial evaluation (phase I) of anticonvulsant activity of synthesized compounds presented in Table 1. The compounds were administered intraperitoneally at three doses (30, 100 and 300 mg kg^{-1}). Three tests were performed for each compound; maximal electro-

Table 1
Anticonvulsant and toxicity screening data in mice (i.p.)

Compound	MES [a,b]		scMet [c]		Rotarod toxicity [d]	
	30 min	4 h	30 min	4 h	30 min	4 h
1	−	−	−	−	+	−
2	++	+	++	+	++	−
3	++	−	+	−	+	−
4	+++	+	++	+	++	−
5	++	+	+	−	++	−
6	+	−	+	−	+	++
7	++	+	++	−	+	−
8	+	−	+	−	+	+
9	+	−	−	−	++	+
10	++	+	+	−	++	−
11	+	−	+	−	++	−
12	+++	+	+	−	++	−
13	++	+	++	+	++	+
14	+	−	−	−	−	−
15	+	−	+	−	+	+
16	++	+	+	+	+	−
17	+++	+	++	+	++	−
18	+	−	+	−	+	−
19	++	+	++	+	++	−
20	++	+	++	−	+	−
21	+	−	+	−	−	−
22	+	−	−	−	+	−
23	+	−	−	−	−	−
24	+	−	−	−	+	−
25	+	−	−	−	−	−
26	+	−	−	−	++	+

[a] Key: +++, activity at 30 mg kg^{-1}; ++, activity at 100 mg kg^{-1}; +, activity at 300 mg kg^{-1}; −, no activity at 300 mg kg^{-1}.
[b] Maximal electroshock seizure test.
[c] Subcutaneous pentylenetetrazole seizure test.
[d] Neurologic toxicity (rotarod) test.

shock (MES)-induced convulsions, subcutaneous Metrozol (scMet)-induced convulsions and rotarod neurotoxicity test (Tox).

As result of preliminary screening, compounds **2**–**5**, **7**, **10**, **12**, **13**, **16**, **17**, **19** and **20** were considered for the phase II trails. This provides an evaluation of the median effective dose (ED_{50}) and median neurotoxic dose (TD_{50}). The slope of the regression line and the SE of the slope were then calculated. These data's are shown in Table 2. Some of these derivatives showed high degree of protection against MES-induced seizures. But they were found to be less effective aginast scMet-induced seizures. Compound **4** was the best in the MES test having ED_{50} of 23.8 mg kg^{-1}. In the MES test, the ED_{50} of compounds **12** (30.9 mg kg^{-1}), **13** (35 mg kg^{-1}) and **17** (37 mg kg^{-1}) were compared favourably with phenytoin.

The following stucture–activity relationships were observed. In the propanol series (**1**–**17**), only compounds **2**–**5**, **7**, **10**, **12**, **13**, **16** and **17** were found to have a high degree of protection against MES-induced convulsions. Imidazolo substituted compound at the 3′ position showed higher protection than the other heterocyclic substitution like morpholino, piperidino and piperazino compounds. Among the secondary amines methyl amine substituted at 3′ position offered more protection than substituted (*p*-hydroxy phenylamino, *p*-bromo phenylamino, *p*-nitro phenylamino, *o*-amino phenylamino) and unsubstituted (phenyl amino, phenyl hydrazino) aromatic compounds. In the propane series (**18**–**26**), compounds **19** and **20** were found to have high degree of protection against MES-induced seizures. Only heterocyclic (piperidino and piperazino) groups substituted at the 3′ position showed more protection than the other substitution. It may be suggested that the anticonvulsnat activity exerted by some of the compounds by blocking Ca^{2+} channels. Calcium influx via voltage-activated Ca^{2+} channels also plays a role in epileptogenesis and neurodegenerative events, raising the possibility that the blockade of Ca^{2+} channels may represent the mechanism of action of these compounds.

Compounds **2**, **3**, **4**, **9**, **10**, **13**, **16**, **17**, **19** and **20** exhibited significant decrease motor activity. The decrease in motor activity by the compounds with respect to propanol series (**1**–**17**) was found to be in the order of **17** > **4** > **2** > **13** > **3** > **10** > **16** > **9**. Among the heterocyclic groups substituted at the 3′ position imidazolo substituted compound is more active than piperidino and piperazino substituted compounds. Morpholino substituted compound was found to be very much less potent than all the heterocyclic substituted compounds. Among the secondary amines substituted at the 3′ position, substituted phenyl amino compounds showed better activity. Among these compounds 2-amino substituted is more active than 4-hydroxy, 4-bromo and the unsubstituted compounds. The decrease in motor activity by the compounds with respect to propane series (**18**–**26**) was found to be in the order of **20** > **19**. It was also observed that 2-OH substitution in the propane side chain plays a significant role in decrease of motor activity since hydroxyl group substituted compound **2** and **3** exhibited higher protection than the corresponding unsubstituted compounds **19** and **20**.

Compounds **3**, **5**, **7**, **8**, **9**, **13** and **15** exhibited potential cardiac activity. Piperazino substituted compound showed better blocking activity than the other heterocyclic compounds where as all other compounds are less active than piperazino compound. Among the tertiary amines substituted diethyl and dimethyl amino compounds are more active than the other compounds. *p*-Hydroxyphenylamino, phenylamino and phenylhydrazino compounds are more active among secondary amines. Electron pumping groups have a prominent role in this effect. Proton pumping groups significantly downplayed the effect of blocking adrenaline in the entire series. None of the propane series compounds were found to be active. The cardiac activity exhibited

Table 2
Quantitative anticonvulsant data in mice (test drug administered i.p.)

Compound	ED_{50} [a]		TD_{50} [b]
	MES	ScMet	
2	85.2 (75–95)	186 (168–202)	224 (195–252)
3	98.1 (78–118)	> 250	256 (215–288)
4	23.8 (20–26)	78 (63–93)	132 (114–151)
5	51.6 (63–74)	> 100	154 (130–180)
7	42.2 (36–48)	92 (73–114)	129 (105–155)
10	59.7 (49–69)	> 150	184 (164–204)
12	30.9 (26–35)	> 150	96 (71–111)
13	35.8 (30–41)	62 (52–72)	122 (107–137)
16	112.2 (94–130)	144 (124–164)	318 (293–343)
17	37.5 (32–44)	78 (65–91)	152 (130–174)
19	63.4 (48–78)	67 (52–82)	144 (124–162)
20	71.8 (60–84)	96 (76–116)	170 (145–195)
Phenytoin	9.9 (6.3–13.1)	> 300	69.8 (57.2–80.7)
Carbamazepine	9.2 (6.9–11.7)	> 125	74.4 (59.1–87.5)
Valporate	264 (236–297)	157 (133–185)	408 (364–437)

[a] Doses measured in mg kg^{-1} at the peak effect.
[b] Doses (mg kg^{-1}) determined by rotarod test at the time of peak neuro toxic effect.

by these compounds may be correlated to the presence of the pharmacophore similarity to the chemical functionality present in β-adrenergic blocking agents. When administered concurrently with adrenaline, the compounds exhibited significant sympatholytic action. The compounds were able to block the effects of adrenaline (100, 200 and 400 μg).

5. Experimental

5.1. Chemistry

M.p. were determined in open capillary tubes and are uncorrected. IR spectra were recorded (in KBr) on Perkin–Elmer IR spectrophotometer 298. ^{1}H-NMR Spectra was recorded on 300 MHz Bruker DPX 200 using tetra methylsilane as internal standard. Elemental analysis was performed on Heraeus CHN rapid analyser. Analyses indicated by the symbols of the elements are within ±0.4% of the theoretical values.

5.1.1. Synthesis of 1,4-dihydroxyphthalazine

In the present study 1,4-dihydroxyphthalazine was prepared according to the procedure given by Fitton [23]. Phthalic anhydride (0.02 mol) was refluxed with hydrazine monohydrate (0.02 mol) in EtOH (25 mL) for half an hour. The reaction mixture was cooled and washed with petroleum ether 40–60 °C (3 × 40 mL) and recrystallised with MeOH–ether (1:1). Yield: 77%, m.p.: 334 °C. IR (KBr, cm^{-1}): 3240 (O–H), 1406 (C–H), 812, 790 (Ar–H). ^{1}H-NMR ($CDCl_3$, δ, ppm): 8.3–8.5 (m, 4H, Benzo protons of phthalazine), 7.41–7.29 (s, 2H, (phenolic $OH)_2$). Anal. Found (Calc.) for $C_8H_6N_2O_2$: C, 59.43 (59.25); H, 3.82 (3.70); N, 17.09% (17.28).

5.1.2. Synthesis of 1-(2′,3′-epoxy-propyloxy)-4-hydroxyphthalazine

1-(2′,3′-Epoxy-proploxy)-4-hydroxyphthalazine was prepared according to the procedure given by JCE Simpson [24]. A quantity of 1,4-dihydroxyphthalazine (0.1 mol) and epicholorohydrine (0.1 moles) were refluxed in the presence of AgCl (0.0007 mol) NaI (0.0006 mol) and MeOH (150 mL) for 20 h. The resultant product was purified by recrystallisation with $CHCl_3$–ether (1:1). Yield: 62%, m.p.: 206 °C. IR (KBr, cm^{-1}): 3240 (O–H), 1406 (C–H), 1240 (epoxide C–O), 1120 (ether C–O), 812, 790 (Ar–H). ^{1}H-NMR ($CDCl_3$, δ, ppm): 8.3–8.5 (m, 4H, Benzo protons of phthalazine), 7.38–7.16 (s, 1H, phenolic OH), 3.42–3.67 (d, $J = 5.2$ Hz, 2H, 3′-CH_2), 3.21–3.40 (d, $J = 6.3$ Hz, 2H; 1′-CH_2), 2.98–3.21 (s, 3H, 4-CH_3), 2.67–2.91 (m, 1H, 2′-CH). Anal. Found (Calc.) for $C_{11}H_{10}N_2O_3$: C, 60.21 (60.55); H, 4.34 (4.58); N, 12.62% (12.84).

5.1.3. Synthesis of 1-(3′-chloropropyloxy)-4-hydroxyphthalazine

1-(3′-Chloropropyloxy)-4-hydroxyphthalazine was prepared according to the procedure given by JCE Simpson [24]. A quantity of 1,4-dihydroxyphthalazine (0.1 mol) and 1-bromo-3-chloropropane (0.1 mol) were refluxed in the presence of AgCl (0.0007 mol), NaI (0.0006 mol) and MeOH (150 mL) for 20 h.The resultant product was purified by recrystallisation with $CHCl_3$–ether(1:1). Yield: 66%, m.p.: > 375 °C. IR

(KBr, cm^{-1}): 1409 (C–H), 1142 (C–O), 811, 792 (Ar–H), 742 (C–Cl). ^{1}H-NMR ($CDCl_3$, δ, ppm): 7.45–7.68 (m, 4H, benzo protons of phthalazine), 6.92–6.73 (s, 1H, phenolic OH), 3.42–3.90 (m, 4H, 1′,3′-CH_2), 1.15–1.23 (m, 2H, 2′-CH_2). Anal. Found (Calc.) for $C_{11}H_{11}ClN_2O_2$: C, 55.12 (55.46); H, 4.73 (4.62); N, 11.47% (11.76).

*5.1.4. General method synthesis of **1–26***

A mixture of 1-(3′-chloropropyloxy)-4-hydroxyphthalazine/1-(2′,3′-epoxypropyloxy)-4-hydroxyphthalazine (0.005 mol), amine (0.005 mol) were refluxed with 10% methanolic KOH (15 mL) for 5 h. The reaction mixture was filtered and the filtrate on concentration yielded the product. The product was dried under vacuum. The products were recrystallised using 1:1 MeOH–ether (**1**, **3**, **5**, **7**, **14**, **15**, **19**, **21**, **22**), 1:1 EtOH–ether (**2**, **9**, **10**, **11**, **13**, **16**, **20**, **23**, **24**, **25**), 1:1 C_3H_6O–ether (**4**, **6**, **8**, **12**, **18**, **26**) and 1:1 petroleum ether–ether (**17**).

*5.1.4.1. 1-(3′-Morpholino-2′-hydroxypropyloxy)-4-hydroxyphthalazine (**1**).* Yield: 72%, m.p. 351 °C. IR (KBr, cm^{-1}): 1455 (C–H), 1375 (C–N), 1071 (C–O), 834, 789 (Ar–H). ^{1}H-NMR ($CDCl_3$, δ, ppm): 8.3–8.54 (m, 4H, Benzo protons of phthalazine), 6.91–7.14 (s, 1H, phenolic OH), 3.68–3.8 (s, 1H, 2′-OH), 3.27–3.3 (m, 4H, 1′,3′-CH_2), 2.40–2.62 (m, 8H, 2″,3″,5″,6″-CH_2), 0.9–1.1 (m, 1H, 2′-CH). Anal. Found (Calc.) for $C_{15}H_{19}N_3O_4$: C, 58.92 (59.01); H, 6.31 (6.22); N, 13.83% (13.77).

*5.1.4.2. 1-(3′-Piperidino-2′-hydroxypropyloxy)-4-hydroxyphthalazine (**2**).* Yield: 79%, m.p. 316–317 °C. IR (KBr, cm^{-1}): 1454 (C–H), 1372 (C–N), 1156 (C–O), 879, 840 (Ar–H). ^{1}H-NMR ($CDCl_3$, δ, ppm): 7.94–8.15 (m, 4H, Benzo protons of phthalazine), 7.16–7.48 (s, 1H, phenolic OH), 3.76–3.92 (s, 1H, 2′-OH), 3.47–3.68 (m, 4H, 1′,3′-CH_2), 2.9–3.35 (m, 10H, 2″,3″,4″,5″,6″-CH_2), 1.22–1.42 (m, 1H, 2′-CH). Anal. Found (Calc.) for $C_{16}H_{21}N_3O_3$: C, 63.50 (63.36); H, 6.97 (6.93); N, 13.65% (13.86).

*5.1.4.3. 1-(3′-Piperazino-2′-hydroxypropyloxy)-4-hydroxyphthalazine (**3**).* Yield: 80%, m.p. 218–219 °C. IR (KBr, cm^{-1}): 1373 (C–N), 1305 (N–H), 1161 (C–O), 763, 689 (Ar–H). ^{1}H-NMR ($CDCl_3$, δ, ppm): 7.91–8.17 (m, 4H, Benzo protons of phthalazine), 7.24–7.41 (s, 1H, phenolic OH), 6.9–6.4 (m, 1H, NH), 3.78–3.92 (s, 1H, 2′-OH), 3.45–3.6 (m, 4H, 1′,3′-CH_2), 3.13–3.3 (m, 8H, 2″,3″,5″,6″-CH_2), 1.32–1.44 (m, 1H, 2′-CH). Anal. Found (Calc.) for $C_{15}H_{20}N_4O_3$: C, 59.35 (59.21); H, 6.69 (6.57); N, 18.6% (18.42).

*5.1.4.4. 1-(3′-Imidazolo-2′-hydroxypropyloxy)-4-hydroxyphthalazine (**4**).* Yield: 77%, m.p. 326 °C. IR (KBr, cm^{-1}): 3213 (O–H), 1473 (C–H), 1385 (C–N), 1142 (C–O), 877, 745 (Ar–H). ^{1}H-NMR ($CDCl_3$, δ, ppm): 7.79–7.92 (m, 4H, Benzo protons of phthalazine), 7.18–7.39 (s, 1H, phenolic OH), 6.7–6.9 (m, 1H, 2′-CH), 6.23–6.47 (m, 2H, 4″,5″(–CH)), 3.58–3.76 (s, 1H, 2′-OH), 3.35–3.42 (m, 4H, 1′,3′-CH_2), 1.26–1.38 (m, 1H, 2′-CH). Anal. Found (Calc.) for $C_{14}H_{14}N_5O_3$: C, 58.55 (58.74); H, 4.65 (4.89); N, 19.49% (19.58)

*5.1.4.5. 1-(3′-Diethylamino-2′-hydroxypropyloxy)-4-hydroxyphthalazine (**5**).* Yield: 82%, m.p. 264–265 °C. IR (KBr, cm^{-1}): 3010 (O–H), 1459 (C–H), 1372 (C–N), 1156 (C–O), 879, 840 (Ar–H). ^{1}H-NMR ($CDCl_3$, δ, ppm): 7.81–8.02 (m, 4H, Benzo protons of phthalazine), 7.09–6.84 (s, 1H, phenolic OH), 3.72–3.88 (s, 1H, 2′-OH), 3.43–3.59 (m, 4H, 1′,3′-CH_2), 1.64–1.83 (m, 1H, 2′-CH), 1.04–1.23 (s, 10H, $(C_2H_5)_2$). Anal. Found (Calc.) for $C_{15}H_{21}N_3O_3$: C, 61.65 (61.85); H, 7.35 (7.21); N, 14.55% (14.43).

*5.1.4.6. 1-(3′-Diethanolamino-2′-hydroxypropyloxy)-4-hydroxyphthalazine (**6**).* Yield: 87%, m.p. 294–295 °C. IR (KBr, cm^{-1}): 3118 (O–H), 1455 (C–H), 1368 (C–N), 1324 (N–H), 1161 (C–O), 834, 789 (Ar–H). ^{1}H-NMR ($CDCl_3$, δ, ppm): 7.89–8.11 (m, 4H, Benzo protons of phthalazine), 6.34–6.67 (s, 1H, phenolic OH), 3.84–3.97 (m, 4H, 1′,3′-CH_2), 3.44–3.58 (s, 2H, 2″-$(OH)_2$), 2.94–3.05 (m, 8H, $(C_2H_4)_2$), 2.23–2.54 (s, 1H, 2′-OH)), 1.48–1.62 (m, 1H, 2′-CH). Anal. Found (Calc.) for $C_{15}H_{21}N_3O_5$: C, 55.9 (55.72); H, 6.35 (6.5); N, 13.20% (13.00).

*5.1.4.7. 1-(3′-Dimethylamino-2′-hydroxypropyloxy)-4-hydroxyphthalazine (**7**).* Yield: 83%, m.p. 287–288 °C. IR (KBr, cm^{-1}): 3321 (O–H), 1475 (C–H), 1373 (C–N), 1327 (N–H), 879, 840 (Ar–H). ^{1}H-NMR ($CDCl_3$, δ, ppm): 8.29–8.84 (m, 4H, Benzo protons of phthalazine), 7.29–7.56 (s, 1H, phenolic OH), 3.74–3.86 (s, 1H, 2′-OH), 3.34–3.47 (m, 4H, 1′,3′-CH_2), 2.26–2.42 (m, 1H, 2′-CH), 1.39–1.63 (s, 6H, $(CH_3)_2$). Anal. Found (Calc.) for $C_{13}H_{17}N_3O_3$: C, 59.50 (59.31); H, 6.56 (6.46); N, 15.79% (15.96).

*5.1.4.8. 1-(3′-Phenylamino-2′-hydroxypropyloxy)-4-hydroxyphthalazine (**8**).* Yield: 77%, m.p. 248–249 °C. IR (KBr, cm^{-1}): 3017 (O–H), 1445 (C–H), 1356 (C–N), 1149 (C–O), 854, 767 (Ar–H). ^{1}H-NMR ($CDCl_3$, δ, ppm): 7.75–8.39 (m, 4H, Benzo protons of phthalazine), 7.11–7.29 (s, 1H, phenolic OH), 6.64–6.75 (s, 1H, –NH), 5.4–5.9 (m, 5H, C_6H_5), 3.87–3.98 (s, 1H, 2′-OH), 3.35–3.49 (m, 4H, 1′,3′$(-CH_2)_2$), 1.32–1.52 (m, 1H; 2′-CH). Anal. Found (Calc.) for $C_{17}H_{17}N_3O_3$: C, 65.7 (65.59); H, 5.60 (5.46); N, 13.7% (13.5).

*5.1.4.9. 1-(3′-(4″-Hydroxyphenylamino)-2′-hydroxypropyloxy)-4-hydroxyphthalazine (**9**).* Yield: 80%, m.p. 344 °C. IR (KBr, cm^{-1}): 3231 (O–H),

1493 (C–H), 1375 (C–N), 1112 (C–O), 812, 736 (Ar–H). ^{1}H-NMR ($CDCl_3$, δ, ppm): 8.06–8.29 (m, 4H, Benzo protons of phthalazine), 7.32–7.58 (s, 2H, (phenolic $OH)_2$), 7.12–7.23 (m, 1H, NH), 6.4–6.7 (m, 4H, C_6H_4), 3.98–4.12 (s, 1H, 2′-OH), 3.73–3.88 (m, 4H, 1′,3′($-CH_2)_2$), 1.4–1.52 (m, 1H, 2′-CH). Anal. Found (Calc.) for $C_{17}H_{17}N_3O_4$: C, 62.50 (62.38); H, 5.25 (5.19); N, 12.79% (12.84).

*5.1.4.10. 1-(3′-(4″-Bromophenylamino)-2′-hydroxypropyloxy)-4-hydroxyphthalazine (**10**).* Yield: 68%, m.p. 301 °C. IR (KBr, cm^{-1}): 3035 (O–H), 1478 (C–H), 1394 (C–N), 1176 (C–O), 889, 778 (Ar–H). ^{1}H-NMR ($CDCl_3$, δ, ppm): 7.87–8.37 (m, 4H, Benzo protons of phthalazine), 7.26–7.48 (s, 1H, phenolic OH), 6.92–7.13 (s, 1H, –NH), 6.3–6.7 (m, 4H, C_6H_4), 3.78–3.92 (s, 1H, 2′-OH), 3.34–3.48 (m, 4H, 1′,3′($-CH_2)_2$), 1.43–1.62 (m, 1H, 2′-CH). Anal. Found (Calc.) for $C_{17}H_{16}N_3O_3Br$: C, 52.36 (52.3); H, 4.23 (4.1); N, 10.81% (10.76).

*5.1.4.11. 1-(3′-(4″-Nitrophenylamino)-2′-hydroxypropyloxy)-4-hydroxyphthalazine (**11**).* Yield: 67%, m.p. 198 °C. IR (KBr, cm^{-1}): 3201 (O–H), 1447 (C–H), 1395 (C–N), 1146 (C–O), 843, 756 (Ar–H). ^{1}H-NMR ($CDCl_3$, δ, ppm): 8.05–8.28 (m, 4H, Benzo protons of phthalazine), 7.61–7.88 (s, 1H, phenolic OH), 7.11–7.32 (m, 1H, NH), 6.32–6.45 (m, 4H, C_6H_4), 4.12–4.34 (s, 1H, 2′-OH), 3.65–3.82 (m, 4H, 1′,3′-CH_2), 1.32–1.48 (m, 1H, 2′-CH). Anal. Found (Calc.) for $C_{17}H_{16}N_4O_5$: C, 57.49 (57.30); H, 4.55 (4.49); N, 15.85% (15.73).

*5.1.4.12. 1-(3′-Methylamino-2′-hydroxypropyloxy)-4-hydroxyphthalazine (**12**).* Yield: 88%, m.p. 362 °C. IR (KBr, cm^{-1}): 3120 (O–H), 1474 (C–H), 1332 (C–N), 1134 (C–O), 812, 739 (Ar–H). ^{1}H-NMR ($CDCl_3$, δ, ppm): 8.43–8.62 (m, 4H, Benzo protons of phthalazine), 7.44–7.65 (s, 1H, phenolic OH), 6.37–6.45 (s, 1H, NH), 4.10–4.26 (s, 1H, 2′-OH), 3.23–3.41 (m, 4H, 1′,3′-CH_2), 2.12–2.34 (m, 1H, 2′-CH), 1.23–1.34 (s, 3H, CH_3). Anal. Found (Calc.) for $C_{12}H_{15}N_3O_3$: C, 57.95 (57.83); H, 6.23 (6.02); N, 16.95% (16.86).

*5.1.4.13. 1-(3′-Hydrazinophenyl-2′-hydroxypropyloxy)-4-hydroxyphthalazine (**13**).* Yield: 88%, m.p. 276–277 °C. IR (KBr, cm^{-1}): 3212 (O–H), 1487 (C–H), 1392 (C–N), 1124 (C–O), 878, 745 (Ar–H). ^{1}H-NMR ($CDCl_3$, δ, ppm): 8.13–8.36 (m, 4H, Benzo protons of phthalazine), 7.37–7.66 (s, 1H, phenolic OH), 6.4–6.9 (m, 5H, C_6H_5), 5.54–5.72 (m, 2H, NH–NH), 4.22–4.41 (s, 1H, 2′-OH), 3.78–3.92 (m, 4H, 1′,3′-CH_2), 1.06–1.24 (m, 1H, 2′-CH). Anal. Found (Calc.) for $C_{17}H_{18}N_4O_3$: C, 62.72 (62.57); H, 5.60 (5.52); N, 17.30% (17.17).

*5.1.4.14. 1-(3′-Diphenylamino-2′-hydroxypropyloxy)-4-hydroxyphthalazine (**14**).* Yield: 62%, m.p. 213 °C. IR (KBr, cm^{-1}): 3211 (O–H), 1459 (C–H), 1345 (C–N), 1134 (C–O), 858, 792 (Ar–H). ^{1}H-NMR ($CDCl_3$, δ, ppm): 7.86–8.06 (m, 4H, Benzo protons of phthalazine), 6.76–6.92 (s, 1H, phenolic OH), 6.3–6.54 (m, 10H, $(C_6H_5)_2$), 4.40–4.64 (s, 1H, 2′-OH), 3.4–3.58 (m, 4H, 1′,3′-CH_2), 1.42–1.52 (m, 1H, 2′-CH). Anal. Found (Calc.) for $C_{23}H_{21}N_3O_3$: C, 71.42 (71.31); H, 5.60 (5.42); N, 10.69% (10.85).

*5.1.4.15. 1-(3′-(6″-Aminopyridyl-2″-amino)-2′-hydroxypropyloxy)-4-hydroxyphthalazine (**15**).* Yield: 67%, m.p. 336 °C. IR (KBr, cm^{-1}): 3160 (O–H), 1454 (C–H), 1385 (C–N), 1174 (C–O), 814, 756 (Ar–H). ^{1}H-NMR ($CDCl_3$, δ, ppm): 7.2–7.7 (m, 4H, Benzo protons of phthalazine), 6.72–6.87 (s, 1H, phenolic OH), 5.34–6.13 (m, 2H, NH_2), 4.9–5.1 (m, 1H, NH), 3.78–3.89 (s, 1H, 2′-OH), 3.28–3.42 (m, 4H, 1′,3′-CH_2), 1.34–1.56 (m, 1H, 2′-CH). Anal. Found (Calc.) for $C_{16}H_{17}N_5O_3$: C, 58.55 (58.71); H, 5.3 (5.19); N, 21.55% (21.4).

*5.1.4.16. 1-(3′-(6″-Methylpyridyl-2″-amino)-2′-hydroxypropyloxy)-4-hydroxyphthalazine (**16**).* Yield: 73%, m.p. 308–309 °C. IR (KBr, cm^{-1}): 3270 (O–H), 1494 (C–H), 1334 (C–N), 1176 (C–O), 837, 783 (Ar–H). ^{1}H-NMR ($CDCl_3$, δ, ppm): 8.65–8.41 (m, 4H, Benzo protons of phthalazine), 7.22–7.56 (s, 1H, phenolic OH), 6.5–6.9 (m, 3H, 3″,4″,5″(–CH)), 4.09–3.98 (s, 1H, 2′-OH), 3.72–3.83 (m, 4H, 1′,3′-CH_2), 2.48–2.65 (s, 3H, CH_3), 1.45–1.66 (m, 1H, 2′-CH). Anal. Found (Calc.) for $C_{17}H_{18}N_4O_3$: C, 62.7 (62.51); H, 5.65 (5.52); N, 17.35% (17.17).

*5.1.4.17. 1-(3′-(2″-Aminophenylamino)-2′-hydroxypropyloxy)-4-hydroxyphthalazine (**17**).* Yield: 80%, m.p. 109–110 °C. IR (KBr, cm^{-1}): 3102 (O–H), 1478 (C–H), 1351 (C–N), 1193 (C–O), 873, 729 (Ar–H). ^{1}H-NMR ($CDCl_3$, δ, ppm): 7.42–7.61 (m, 4H, Benzo protons of phthalazine), 7.09–7.23 (s, 1H, phenolic OH), 6.7–6.9 (m, 5H, C_6H_5), 5.63–5.76 (s, 2H, NH_2), 4.31–4.45 (m, 1H, NH), 3.80–3.92 (s, 1H, 2′-OH), 3.5–3.6 (m, 4H, 1′,3′-CH_2), 0.92–1.12 (m, 1H, 2′-CH). Anal. Found (Calc.) for $C_{17}H_{18}N_4O_3$: C, 62.70 (62.51); H, 5.75 (5.52); N, 17.05% (17.17).

*5.1.4.18. 1-(3′-Morpholinopropyloxy)-4-hydroxyphthalazine (**18**).* Yield: 62%, m.p. 234 °C. IR (KBr, cm^{-1}): 3207 (O–H), 1456 (C–H), 1349 (C–N), 1185 (C–O), 851, 730 (Ar–H). ^{1}H-NMR ($CDCl_3$, δ, ppm): 7.49–7.78 (m, 4H, Benzo protons of phthalazine), 6.59–6.87 (s, 1H, phenolic OH), 5.9–6.16 (m, 1H, NH), 3.23–3.42 (m, 4H, 1′,3′-CH_2), 2.40–2.62 (m, 8H, 2″,3″,5″,6″-CH_2), 1.36–1.47 (m, 2H, 2′-CH_2). Anal.

Found (Calc.) for $C_{15}H_{19}N_3O_3$: C, 62.3 (62.28); H, 6.65 (6.57); N, 14.4% (14.53).

*5.1.4.19. 1-(3′-Piperidinopropyloxy)-4-hydroxyphthalazine (**19**).* Yield: 80%, m.p. 282 °C. IR (KBr, cm^{-1}): 3107 (O–H), 1423 (C–H), 1343 (C–N), 1175 (C–O), 845, 775 (Ar–H). ^{1}H-NMR ($CDCl_3$, δ, ppm): 8.13–8.36 (m, 4H, Benzo protons of phthalazine), 7.16–7.38 (s, 1H, phenolic OH), 3.80–3.92 (s, 10H, 2′,3′,4′,5′,6′-CH_2), 3.73–3.88 (m, 6H, 1′,3′-CH_2), 1.56–1.72 (m, 2H, 2′-CH_2). Anal. Found (Calc.) for $C_{16}H_{21}N_3O_2$: C, 66.6 (66.89); H, 7.37 (7.31); N, 14.5 (14.63).

*5.1.4.20. 1-(3′-Piperazinopropyloxy)-4-hydroxyphthalazine (**20**).* Yield: 76%, m.p. 210 °C. IR (KBr, cm^{-1}): 3087 (O–H), 1424 (C–H), 1332 (C–N), 1142 (C–O), 835, 724 (Ar–H). ^{1}H-NMR ($CDCl_3$, δ, ppm): 7.93–8.21 (m, 4H, Benzo protons of phthalazine), 6.89–7.12 (s, 1H, phenolic OH), 6.13–6.47 (m, 1H, NH), 4.24–4.08 (m, 8H, 2″,3″,5″,6′-CH_2), 3.78–3.91 (m, 6H, 1′,3′-CH_2), 1.56–1.83 (m, 2H, 2′-CH_2). Anal. Found (Calc.) for $C_{15}H_{20}N_4O_2$: C, 62.4 (62.5); H, 6.89 (6.94); N, 19.3% (19.44).

*5.1.4.21. 1-(3′-Imidazolopropyloxy)-4-hydroxyphthalazine (**21**).* Yield: 66%, m.p. 313 °C. IR (KBr, cm^{-1}): 3176 (O–H), 1458 (C–H), 1386 (C–N), 1169 (C–O), 845, 787 (Ar–H). ^{1}H-NMR ($CDCl_3$, δ, ppm): 8.19–8.34 (m, 4H, Benzo protons of phthalazine), 7.65–7.84 (s, 1H, phenolic OH), 4.5–4.9 (m, 1H, CH), 4.1–4.3 (m, 2H, $(-CH)_2$), 3.32–3.48 (m, 4H, 1′,3′-CH_2), 1.12–1.36 (m,1H, 2′-CH_2). Anal. Found (Calc.) for $C_{14}H_{14}N_4O_2$: C, 62.1 (62.22); H, 5.16 (5.18); N, 20.5% (20.74).

*5.1.4.22. 1-(3′-Diethylaminopropyloxy)-4-hydroxyphthalazine (**22**).* Yield: 72%, m.p. 147 °C. IR (KBr, cm^{-1}): 3198 (O–H), 1429 (C–H), 1373 (C–N), 1191 (C–O), 829, 782 (Ar–H). ^{1}H-NMR ($CDCl_3$, δ, ppm): 8.21–8.37 (m, 4H, Benzo protons of phthalazine), 7.65–7.91 (s, 1H, phenolic OH), 4.23–4.46 (m, 4H, 1′,3′-CH_2), 2.32–2.43 (m, 2H, 2′-CH_2), 1.72–1.92 (m, 10H, $(C_2H_5)_2$). Anal. Found (Calc.) for $C_{15}H_{22}N_3O_2$: C, 65.19 (65.21); H, 7.91 (7.97); N, 15.1% (15.21).

*5.1.4.23. 1-(3′-Diethanolaminopropyloxy)-4-hydroxyphthalazine (**23**).* Yield: 58%, m.p. 187 °C. IR (KBr, cm^{-1}): 3061 (O–H), 1484 (C–H), 1335 (C–N), 1134 (C–O), 859, 746 (Ar–H). ^{1}H-NMR ($CDCl_3$, δ, ppm): 8.08–8.21 (m, 4H, Benzo protons of phthalazine), 7.48–7.69 (s, 1H, phenolic OH), 4.23–4.45 (m, 4H, 1′,3′-CH_2), 3.15–3.36 (s, 2H, 2″-$(OH)_2$), 2.94–3.05 (m, 8H, $(C_2H_4)_2$), 1.32–1.53 (m, 2H, 2′-CH_2). Anal. Found (Calc.) for $C_{15}H_{22}N_3O_4$: C, 57.4 (57.32); H, 6.91 (7.00); N, 15.3% (15.28).

Table 3
Amphetamine antagonism of the compounds

Compound	Dose (mg kg^{-1})	% Reduction of motor activity
1	25	12.46*
	50	23.78*
2	25	44.48**
	50	89.54*
3	25	43.26*
	50	86.52*
4	25	45.76*
	50	91.6*
5	25	39.62**
	50	77.26*
6	25	39.38*
	50	80.28*
7	25	39.62*
	50	78.26*
8	25	24.62**
	50	49.58***
9	25	39.62*
	50	80.28**
10	25	43.72**
	50	85.74**
11	25	21.44**
	50	43.48**
12	25	33.28**
	50	66.62*
13	25	44.52*
	50	87.48**
14	25	8.14*
	50	17.88*
15	25	38.82*
	50	96.76**
16	25	41.84**
	50	82.72*
17	25	46.64*
	50	93.88***
18	25	35.24**
	50	69.44*
19	25	41.24*
	50	83.46*
20	25	42.66*
	50	84.52*
21	25	23.82**
	50	49.34*
22	25	38.68*
	50	75.52**
23	25	13.12*
	50	25.94**
24	25	22.32**
	50	43.76*
25	25	23.82*
	50	46.34**
26	25	32.34**
	50	63.76*

Table 3 (*Continued*)

Compound	Dose (mg kg^{-1})	% Reduction of motor activity
Phenobarbitone	10	98.32*

* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$ compared to control.

*5.1.4.24. 1-(3′-Dimethylaminopropyloxy)-4-hydroxyphthalazine (**24**).* Yield: 85%, m.p. 159 °C. IR (KBr, cm^{-1}): 3108 (O–H), 1454 (C–H), 1396 (C–N), 1164 (C–O), 867, 739 (Ar–H). ^{1}H-NMR ($CDCl_3$, δ, ppm): 7.79–7.93 (m, 4H, Benzo protons of phthalazine), 6.56–6.82 (s, 1H, phenolic OH), 4.35–4.49 (s, 1H, 2′-OH), 3.67–3.88 (m, 4H, 1′,3′-CH_2), 2.92–3.12 (m, 2H, 2′-CH_2), 2.31–2.45 (m, 6H, $(CH_3)_2$). Anal. Found (Calc.) for $C_{13}H_{18}N_3O_2$: C, 63.1 (62.9); H, 7.3 (7.25); N, 16.8% (16.93).

Table 4
Cardiac activity of the compounds

Compound	Concentration	Effect on systole
1	1–50 μg of **1**	Normal
	100 μg of **1**	Negative ionotropic
	100 μg of **1** + 100 μg of adrenaline	Positive ionotropic
	400 μg of **1** + 100 μg of adrenaline	Normal
3	1–4 μg of **3**	Negative ionotropic
	1 μg of **3** + 100 μg of adrenaline	Positive ionotropic
	2 μg of **3** + 100 μg of adrenaline	Normal
4	1–2 μg of **4**	Normal
	4–100 μg of **4**	Negative ionotropic
	100 μg of 4 + 100 μg of adrenaline	Positive ionotropic
	400 μg of **4** + 100 μg of adrenaline	Normal
5	1–2 μg of **5**	Negative ionotropic
	40 μg of **5** + 100 μg of adrenaline	Positive ionotropic
	50 μg of **5** + 100 μg of adrenaline	Normal
7	1–8 μg of 7	Normal
	10–20 μg of **7**	Negative ionotropic
	50 μg of **7** + 100 μg of adrenaline	Positive ionotropic
	100 μg of **7** + 100 μg of adrenaline	Normal
8	1–40 μg of **8**	Normal
	100 μg of **8**	Negative ionotropic
	100 μg of **8** + 100 μg of adrenaline	Positive ionotropic
	200 μg of **8** + 100 μg of adrenaline	Normal
9	1–8 μg of **9**	Normal
	10–40 μg of **9**	Negative ionotropic
	20 μg of **9** + 100 μg of adrenaline	Positive ionotropic
	100 μg of **9** + 100 μg of adrenaline	Normal
10	1–100 μg of **10**	Normal
	200–400 μg of **10**	Negative ionotropic
	200 μg of **10** + 100 μg of adrenaline	Positive ionotropic
	400 μg of **10** + 100 μg of adrenaline	Normal
13	1–40 μg of **13**	Normal
	50–200 μg of **13**	Negative ionotropic
	200 μg of **13** + 100 μg of adrenaline	Normal
15	2–10 μg of **15**	Normal
	20 μg of **15**	Negative ionotropic
	100 μg of **15** + 100 μg of adrenaline	Positive ionotropic
	200 μg of **15** + 100 μg of adrenaline	Normal

*5.1.4.25. 1-(3′-Aminophenylpropyloxy)-4-hydroxyphthalazine (**25**).* Yield: 90%, m.p. 203 °C. IR (KBr, cm^{-1}): 3190 (O–H), 1438 (C–H), 1332 (C–N), 1187 (C–O), 836, 739 (Ar–H). ^{1}H-NMR ($CDCl_3$, δ, ppm): 7.67–7.84 (m, 4H, Benzo protons of phthalazine), 7.43–7.57 (s, 1H, phenolic OH), 7.13–7.27 (m, 1H, NH), 6.18–6.54 (m, 5H, C_6H_5), 4.45–4.69 (m, 4H, 1′,3′-CH_2), 1.54–1.74 (m, 2H, 2′-CH_2). Anal. Found (Calc.) for $C_{17}H_{17}N_3O_2$: C, 69.24 (69.15); H, 5.65 (5.76); N, 14.31% (14.23).

*5.1.4.26. 1-(3′-(4″-Hydroxyphenylamino)propyloxy)-4-hydroxyphthalazine (**26**).* Yield: 82%, m.p. 255 °C. IR (KBr, cm^{-1}): 3047 (O–H), 1457 (C–H), 1378 (C–N), 1138 (C–O), 829, 735 (Ar–H). ^{1}H-NMR ($CDCl_3$, δ, ppm): 7.69–7.88 (m, 4H, Benzo protons of phthalazine), 7.14–7.37 (s, 2H, $(\text{phenolic OH})_2$), 6.2–6.67 (m, 4H, C_6H_4), 4.23–4.4 (m, 1H, NH), 3.78–3.92 (m, 4H, 1′,3′-CH_2), 1.92–2.12 (m, 2H, 2′-CH_2). Anal. Found (Calc.) for $C_{17}H_{17}N_3O_3$: C, 65.2 (65.59); H, 5.37 (5.46); N, 13.61% (13.50).

5.2. Pharmacology

All the synthesized compounds were screened for anticonvulsant activity. The amphetamine antagonism was done at the dose of 25 mg and 50 mg kg^{-1}. The experimental dose for the amphetamine antagonism was selected between the minimal effective and maximal non-lethal dose. The compounds were also screened for the cardiac activity on isolated frog heart. All the compounds were soluble in water and administered to the animals as a solution in water for injection and triple glass distilled water. Wistar albino mice (20–30 g) of either sex were procured from King Institute, Guindy, Chennai. They were kept in colony cages at ±2 °C, relative humidity 45–55% under 12 h light and dark cycle. All the animals were acclimatized for a week before use. Small frogs (Rana trigana, 80–120 g) were procured locally and used on the same day. Unpaired student's *t*-test [25] was performed to ascertain the significance of the exhibited activity.

5.2.1. Anticonvulsant activity

All compounds were tested for anticonvulsant activity with Wistar albino mice. Each compound was administered intraperitoneally at three dose levels (30, 100 and 300 mg kg^{-1}). The compounds were made solution with water for injection.

Maximal electroshock seizures (MES) were induced 30 min after drug treatment by application of 50 mA current for 0.2 s via corneal electrodes into the eyes. The

protection was defined as the abolition of hind-leg tonic maximal extension component of the seizure. The subcutaneous pentylenetetrazole (Metrozol) seizure threshold test (scMet) was carried out by an intraperitoneal administration of pentylenetetrazole (85 mg kg^{-1}). Animals were observed for over 30 min. Failure to observe the generalised clonic seizure is defined as protection.

Minimal neurotoxicity (TD_{50}) was measured by the rotarod test (Tox). Mice were placed in 1-in diameter knurled plastic rod rotating 6 rpm after administration of the drug, and their ability to maintain their balance was tested. Neurological deficit was indicated by the inability of the animal to maintain the equilibrium for 1 min on the rotating rod in each of three trials. The results are tabulated in Tables 1 and 2.

5.2.2. Amphetamine antagonistic activity

The decrease in the motor activity [26] induced by introceptine stimuli of the compounds was studied by amphetamine antagonism. Wistar albino mice ($n = 6$) of either sex were selected by random sampling technique. The compounds were administered at a dose level 25 mg and 50 mg kg^{-1} i.p 30 min prior to the administration of amphetamine (5 mg kg^{-1}, i.p.). Phenobarbitone (10 mg kg^{-1}, i.p) was used as the standard drug. The motor activity of the compounds was observed for 15 min in an octophotometer after 10 min of amphetamine administration. The percentage reduction of motor activity by the compounds is presented in Table 3.

5.2.3. Cardiac activity

Isolated frog heart [27] was mounted using frog ringer locke solution. The effect of the compounds on the rate and force of the contraction was observed from 1 to 100 μg. The effect of the compounds at different concentration (1–800 μg) with simultaneous administration with adrenaline (100, 200 and 400 μg) was also studied (Table 4).

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