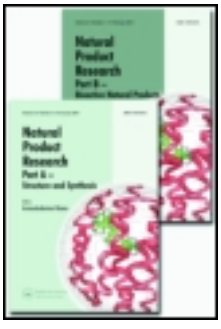

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Synthesis, phytotoxic, cytotoxic, acetylcholinesterase and butrylcholinesterase activities of N,N′-diaryl unsymmetrically substituted thioureas

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Synthesis, phytotoxic, cytotoxic, acetylcholinesterase and butrylcholinesterase activities of *N*,*N*′-diaryl unsymmetrically substituted thioureas

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Fourteen *N*,*N*′-diaryl unsymmetrically substituted thioureas were synthesised and their cytotoxic (*in vitro*), phytotoxic (*in vitro*), acetylcholinesterase and butrylcholinesterase activities were determined. Thiourea **16** exhibited high, and **1** and **3** showed significant phytotoxic activity. Thioureas **1**, **3**, **4**, **6** and **10** showed significant activity and **2**, **6** and **7** indicated moderate cytotoxic activities. Compound **12** exhibited butrylcholinesterase activity higher than a standard reference.

Keywords: *N*,*N*′-diaryl unsymmetrically substituted thiourea derivatives; phytotoxicity; cytotoxicity; acetylcholinesterase activity; butrylcholinesterase activity

1. Introduction

N,*N*′-Diarylsubstituted thioureas (symmetrical or unsymmetrical) have received a considerable amount of attention as a drug candidate against a variety of diseases, due to their broad spectrum of pharmacological activities. During the past few years, *N*-aryl-*N*′-phenyl thioureas, where aryls were *o*-anisyl, *m*-anisyl, *p*-tolyl, *m*-tolyl, 2-napthyl, *p*-chlorophenyl, 2-chlorophenyl, *p*-bromophenyl, etc. exhibited numerous biological activities, including fungicidal (Krause, Franke, & Vasilev, 1979; Ramadas, Suresh, Janarthanan, & Masilamani, 1998; Vasilev & Tomaleva, 1973), herbicidal (Vasilev & Davarski, 1986; Vasilev, Iliev, & Vasileva, 1969; Vasilev & Ionova, 1984), cytokinin (Bruce & Zwar, 1966; Bruce, Zwar, & Kefford, 1965; Izvorska, Vasilev, Lilov, & Belcheva, 1986; Mashev & Vasilev, 1974a, 1974b; Vasilev & Ionova, 1978; Vasilev & Mashev, 1974; G. Vassilev & N. Vassilev, 2002), antipolioviral (Galabov, Shindarov, Vasilev, & Vasileva, 1972), antiphytoviral (Vasilev & Schuster, 1986; Vasilev, Vasileva, Galabov, & Shindarov, 1972), and insecticidal (Kondo & Maekawa, 1976; Mathur, 1976) activities. They are also active against bacterial and microbial infections, in particular are potential anti-tubercular agents against mycobacterium tuberculosis (Sarkis & Faisal, 1985; Schroeder, 1951; Walpole et al., 1998). Some of them are found to be phenoloxidase enzyme inhibitors (Makhsumov, Safaev, & Abidova, 1968) and are also used as biomimic models (Smith, Liras, Schneider, & Anslyn, 1996; Tobe, Sasaki, Hirose, & Naemura, 1997). *N*,*N*′-diaryl

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thioureas are highly potent and selective human A_{2B} adenosine receptor antagonists (Baraldi et al., 2004) and cyclin-dependent kinase 2 (CDK2) inhibitors (Honma et al., 2001). They also find use as the building blocks for the synthesis of many valuable heterocyclic compounds (Griffin, Woods, & Klayman, 1975). Some thioureas are commercially used as accelerators in rubber vulcanisation (Alder, 1989; Debroy, Mazumdar, Barua, & Mahajan, 1984; Makhsumov et al., 1968).

These potential biological findings led us to synthesise 14 unsymmetrically substituted *N*-phenyl-*N*′-aryl thioureas to explore their cytotoxicity, phytotoxicity, acetylcholinesterase and butrylcholinesterase activities.

2. Results and discussion

Fourteen unsymmetrically substituted *N*,*N*′-diaryl thioureas, **1**–**14**, were prepared by thermal treatment of anilines in the solid phase with phenylisothiocynate at room temperature (Scheme 1). The reaction was completed within 1–2 min for all the thioureas, except **6**, **8** and **10**, where heating at 100°C was required for 10–15 min. The synthesised compounds were recrystallised from hot ethanol and were characterised by recording their spectral data.

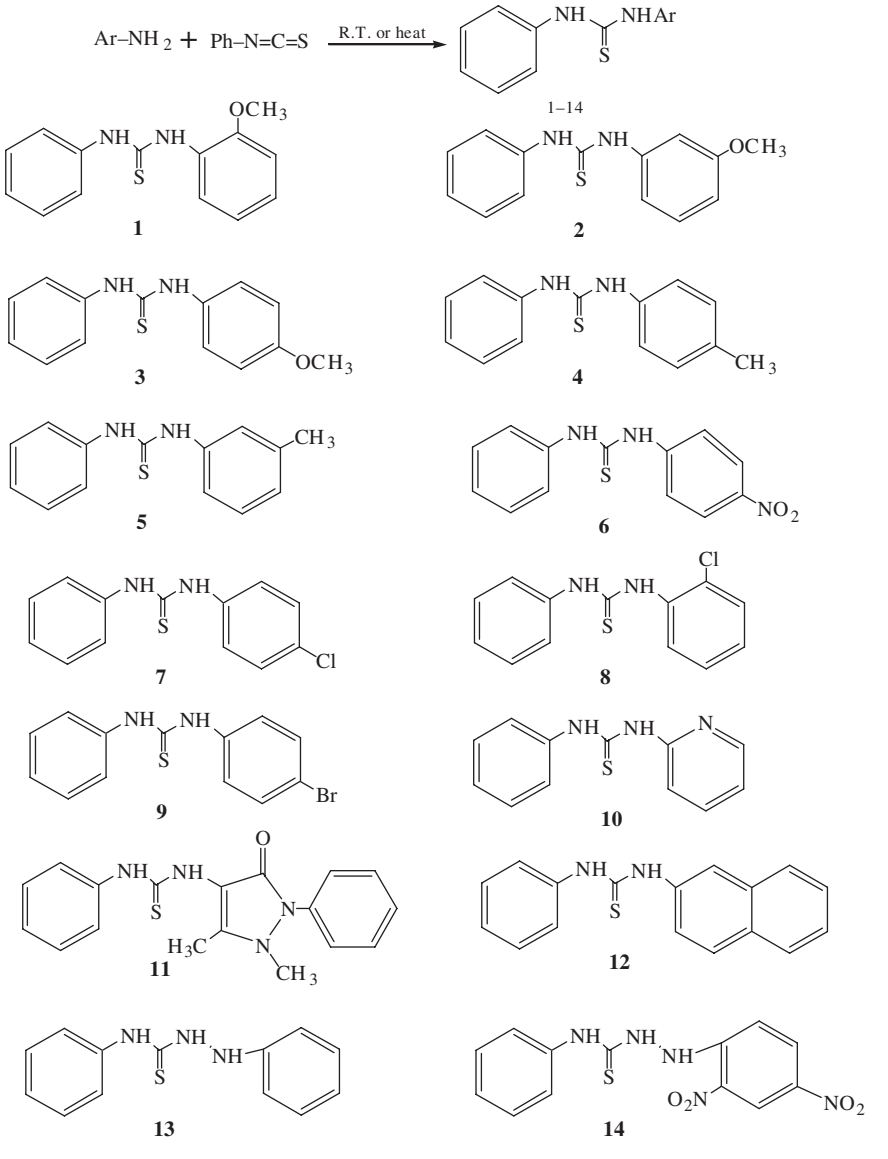
The IR spectra of all the synthesised thiourea derivatives exhibited N–H stretching vibrations in the region between 3470–3160 cm^{-1}. The vibrations in the region 1265–1225 and 1180–1120 cm^{-1} were assigned to C=S stretching. The N–H amide absorption bands were observed at 1380–1308 cm^{-1}. These IR bands were common to all the synthesised thiourea derivatives and are in agreement with the absorptions reported in the literature for thioureas (Alder, 1989; Sarkis & Faisal, 1985). Thiol-thione tautomerism was indicated by the signals between 1.5–2.4 ppm in the ^{1}H-NMR of all synthesised compounds, and were in accordance with the signals reported in the literature (Sarkis & Faisal, 1985). The molecular ion peak of all the synthesised compounds was observed in EIMS and HREIMS, except for compounds **6**, **11** and **14**. The calculated molecular masses were in good agreement with the values obtained. The fragments due to cleavage of ArNH (or Ar′NH), ArNHCS (or Ar′NHCS) and Ar (or Ar′) were prominent in the EIMS of all the synthesised thioureas.

All the synthesised thiourea derivatives were screened for their brine shrimp lethality (cytotoxicity), *Lemna minor* L. (phytotoxicity), acetylcholinesterase and butrylcholinesterase inhibition activities.

2.1. *Phytotoxic bioassays against* L. minor *L.*

Lemna minor L. (duckweed) is a miniature aquatic monocot. *L. minor* bioassays have been used to screen synthesised thioureas for their effect on plant growth (McLaughlin, Chang, & Smith, 1991). The results of the phytotoxicity of the synthesised thioureas are presented in Table 1.

The experiments were performed at 1000, 100 and 10 $\mu g\,mL^{-1}$ and all the compounds **1**–**14** were found to be active at higher concentration levels. *N*-(*p*-nitrophenyl)-*N*′-phenylthiourea (**6**) was found to exhibit significant activity, with 100% inhibition of plant growth at 1000 $\mu g\,mL^{-1}$. The high activity of **6** may be attributed to the presence of the *p*-nitro group at the aromatic ring. *N*-(*m*-methoxyphenyl)-*N*′-phenylthiourea (**1**) and



Scheme 1. Synthetic route for *N*,*N*′-diaryl unsymmetrically substituted thioureas **1**–**14**.

N-(*o*-methoxyphenyl)-*N*′-phenylthiourea (**3**), both carrying methoxy groups, showed significant activities of 75.0 and 85.7, respectively, at 1000 μg mL^{-1}. *N*-(*p*-methylphenyl)-*N*′-phenylthiourea (**4**) exhibited a good activity of 64.28 μg mL^{-1} at 1000 μg mL^{-1} and moderate activity of 57.14 μg mL^{-1} at 100 μg mL^{-1} concentrations. *N*-(*p*-methoxyphenyl)-*N*′-phenylthiourea (**2**), *N*-(*o*-chlorophenyl)-*N*′-phenylthiourea (**8**), *N*-(*p*-bromophenyl)-*N*′-phenylthiourea (**9**), *N*-phenyl-*N*′-(2-pyridinyl)thiourea (**10**) and *N*-(1-naphthyl)-*N*′-phenylthiourea (**12**) exhibited moderate *L. minor* growth inhibition at higher concentrations. Other thiourea derivatives showed low or insignificant activities at all (1000, 100, and 10 μg mL^{-1}) concentration levels.

2.2. *Cytotoxic brine shrimp lethality bioassays*

Brine shrimp bioassays are rapid and inexpensive for screening the physiologically active chemical substances for their cytotoxic effects (Finney, 1971; Meyer et al., 1982). Tiny crustacean, brine shrimp, *Artemia salina* Leach eggs are utilised in bioassays and pharmacological activity is manifested as the toxicity towards newly hatched nauplii.

All the synthesised compounds were subjected to brine shrimp bioassays against *A. salina* Leach, the results are collected in Table 2.

Thioureas *N*-(*m*-methoxyphenyl)-*N*′-phenylthiourea (**1**), *N*-(*p*-methoxyphenyl)-*N*′-phenylthiourea (**2**), *N*-(*o*-methoxyphenyl)-*N*′-phenylthiourea (**3**), *N*-(*p*-methylphenyl)-*N*′-phenylthiourea (**4**), *N*-(*m*-methylphenyl)-*N*′-phenylthiourea (**5**), *N*-(*p*-nitrophenyl)-*N*′-phenylthiourea (**6**), *N*-(*p*-chlorophenyl)-*N*′-phenylthiourea (**7**), *N*-phenyl-*N*′-(2-pyridinyl)thiourea (**10**) and 2-phenyl-*N*-phenyl-1-hydrazinecarbothioamide (**13**) were found

Table 1. Results of *L. minor* phytotoxic bioassay (growth inhibition).

Compound no.	1000 μg mL^{-1}	100 μg mL^{-1}	10 μg mL^{-1}
1	75.00	53.57	39.28
2	53.57	50.00	42.42
3	85.71	46.42	42.85
4	64.28	57.14	25.00
5	28.20	19.0	23.25
6	100	6.70	39.28
7	42.85	35.71	28.57
8	50.00	32.14	28.57
9	53.57	46.57	39.28
10	53.57	10.71	7.14
11	32.14	28.57	21.42
12	57.14	35.71	28.57
13	26.00	10.00	Inactive
14	30.00	16.00	6.00

Table 2. Results of brine shrimp (*A. salina*) bioassay.

Compound no.	LD_{50} μg mL^{-1}
1	48.029
2	86.10
3	34.70
4	22.96
5	2.54
6	122.00
7	120.53
8	Inactive
9	Inactive
10	35.61
11	Inactive
12	Inactive
13	51.69
14	Inactive

to be active. The LD_{50} values were found to be highest for compound **5**, with an LD_{50} value = 2.54 μg mL^{-1}. Compound **4** was also found to be significantly active, with an LD_{50} = 22.96 μg mL^{-1}. Compounds **1**, **3** and **10** exhibited significant activities, with LD_{50} values of 48.02, 34.69 and 35.61 μg mL^{-1}, respectively. Thioureas **2**, **6**, **7** and **13** were found to be less active. Compounds **8**, **9**, **11**, **12** and **14** were inactive to brine shrimp bioassay. The structure activity relationships of the aforesaid compounds revealed that the activity of these compounds may be attributed to the presence of methoxy, methyl, chlorine and pyridyl groups, respectively, at the phenyl ring, especially **4** and **5** (with methyl groups), exhibited excellent activities against *A. salina*.

2.3. *Acetylcholinesterase and butrylcholinesterase bioassays*

Acetylcholinesterase is the key component of cholinergic brain synapses and neuromuscular junctions (Tougu, 2001). According to the cholinergic hypothesis, memory impairments in patients with senile dementia are due to a selective and irreversible deficiency in the cholinergic functions in the brain. This serves as a rationale for the use of acetylcholinesterase inhibitors for the symptomatic treatment of Alzheimer's disease in its early stages. The role of butrylcholinesterase in normal ageing and brain diseases is still elusive. It has been found that butrylcholinesterase is found in significantly higher quantities in Alzheimer's plaques than in plaques of normal age related non-demented brains (Yu, Holloway, Utsuki, Brossi, & Grieg, 1999).

All the synthesised compounds were also screened against acetylcholinesterase and butrylcholinesterase activities at 0.2 mM, and results are depicted in Table 3.

All thiourea derivatives **1**–**14** were found to be inactive against acetylcholinesterase, whereas 2-(2,4-dinitrophenyl)-*N*-phenyl-1-hydrazinecarbothioamide (**14**) exhibited butrychlolinesrease activity (IC_{50} = 17.1 μM), which is slightly lower than the standard

Table 3. Results of acetylcholinesterase and butrylcholinesterase bioassays of compounds **1**–**14**.

	Acetylcholinesterase		Butrylcholinesterase	
Compound no.	Percentage inhibition	$IC_{50} \pm SEM$ (μM)	Percentage inhibition	$IC_{50} \pm SEM$ (μM)
1	−128.5	–	5.5	–
2	−28.1	–	34.29	–
3	−56.3	–	28.79	–
4	−37.8	–	18.20	–
5	−73.9	–	25.69	–
6	0.88	–	0.74	–
7	4.70	–	9.58	–
8	−11.27	–	33.67	–
9	−5.4	–	31.45	–
10	−10.8	–	12.30	–
11	−6.74	–	31.42	–
12	1.72	–	27.28	–
13	−14.65	–	18.12	–
14	34.58	–	94.05	17.1 ± 1.32
Standard (galanthamine)	–	0.5 ± 0.01	–	8.5 ± 0.5

galathamine, having an IC_{50} value of 8.5 μM. Compound **13**, which contains an unsbstituted aromatic ring, was found to be completely inactive. This comparison clearly indicates that nitro groups present on an aromatic moiety dramatically enhanced the butrychlolinesrease inhibitory activity. This interesting finding invites us to synthesise compounds that are structurally closer to compound **14**, which may contain one more nitro group or some other electron withdrawing groups. Conclusively, hydrazinecarbothioamide compound **14** may serve as a lead molecule for further research on this class of compounds as butrychlolinesrease inhibitors.

3. Experimental

Melting points were recorded using Gallenkamp melting point apparatus and are uncorrected. IR spectra were measured as KBr discs on a JASCO spectrophotometer. Electron impact mass spectra (EIMS) were performed on MAT-312 spectrophotometer. ^{1}H-NMR were recorded in $CDCl_3$ or DMSO-d_6 with a Bruker AM 400 spectrometer operating at 400 MHz. The purity of the compounds were checked on TLC plates coated with silica gel GF_{254} (Merck, Darmstadt, Germany) and the spots were visualised under ultraviolet light at 254 nm.

3.1. *General procedure for the preparation*

Different *N*-phenyl-*N*′-aryl thioureas were prepared by mixing the corresponding anilines in solid phase with phenyl isothiocynate at room temperature, whereupon anilines were first dissolved and then solidified within 1–2 min on swirling the reaction flask. The reaction was exothermic in all cases except VI, VIII and X, where heating to 100°C for 10–15 min was required. The cold mass was disintegrated and washed thrice with hexane and then thrice with 1 : 1 aqueous ethanol to wash out unreacted anilines and phenylisothiocyante. The crude thioureas were purified by recrystallisation from hot ethanol. All the compounds were characterised by recording their spectral data.

***N*-(*m*-Methoxyphenyl)-*N*′-phenylthiourea (1)**. $C_{14}H_{14}N_2OS$ (Calcd: 258.0828; found: 258.0810), yield 88.7%; white crystals; m.p. 94–96°C (lit. not available) (El-Din, 1986); IR λ_{max} (cm^{-1}): 3450 and 3211.3 (N–H stretching), 3011.6, 2960.5, 2806.2, 1602.7, 1546.8, 1492.8, 1450.4, 1340 (N–H amide stretching), 1234.0 and 1166.9 (C=S disubstituted stretching), 1068.5, 1024 and 929.6; EIMS *m*/*z* (rel. int.%): 257.8 (1.83) [M^+], 227.8 (25.6) [$M^+ - OCH_2$], 135.9 (10.64), 92.9 (100), 76.9(43.26); ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.0 (2H, broad s, NH), 7.35 (1H, d, $J = 7.85$ Hz, H-6), 7.312 (1H, d, $J = 5.5$ Hz, H-4), 7.30 (1H, s, $J = 5.5$ Hz, H-2), 7.27 (1H, dd, $J = 10.30$, 6.54 Hz, H-5), 7.20 (5H, m, C_6H_5), 7.24 (1H, s, H-2), 3.7 (3H, s, OCH_3), 1.7, (1H, broad s, SH).

***N*-(*p*-Methoxyphenyl)-*N*′-phenylthiourea (2)**. $C_{14}H_{14}N_2OS$ (Calcd: 258.0828; found: 258.0812), yield 75.5%; white crystalline solid; m.p. 140–143°C (lit. 144°C) (Furniss, Hannaford, Rogers, Smith, & Tatchel, 1986); IR, λ_{max} (cm^{-1}): 3450 and 3211.3 (N–H stretching), 3012.6, 2960.5, 2806.2, 1602.7, 1546.8, 1456.2, 1336.0 (N–H amide stretching), 1244.0 and 1176.5 (C=S disubstituted), 1101, 1031.8 and 927.7; EIMS *m*/*z* (rel. int.%): 257.9 (22.66) [M^+], 164.8 (16.9), 122.9 (63.72), 108 (100), 93 (40.33), 76.9 (53.9); ^{1}H-NMR (400 MHz, $CDCl_3$); δ 8.0 (2H, broad s, NH), 7.36 (5H, m, C_6H_5), 7.26 (2H, d, $J = 8.94$ Hz, H-2,6), 6.915 (2H, d, $J = 6.18$ Hz, H-3,5), 3.7 (3H, s, OCH_3), 2.14 (1H, broad s, SH).

***N*-(*o*-Methoxyphenyl)-*N′*-phenylthiourea (3)**. $C_{14}H_{14}N_2OS$ (Calcd: 258.0828; found: 258.0810), yield 87.1%, white crystalline solid, m.p. 125°C (lit. 136°C) (Furniss et al., 1986); IR (KBr), IR, λ_{max} (cm^{-1}): 3334 and 3128 (N–H stretching), 2931.6, 289, 1593, 1517, 1456.2, 1350 (N–H amide stretching), 1261 and 1163 (C=S disubstituted); EIMS *m*/*z* (rel. int.%): 258 (5.6) [M^+], 226.8 (42.0), 107.8 (100), 92.9 (46.2); ^{1}H-NMR (400 MHz, $CDCl_3$); δ 8.12 (2H, s, 2 NH), 7.91 (2H, d, $J = 7.47$ Hz, H-2′,H-6′), 7.41 (1H, m, H-4′), 7.37 (2H, m, H-3′,5′), 7.24 (1H, m, H-4), 7.166 (1H, d, $J = 7.62$ Hz, H-3), 6.98 (1H, m, H-5), 6.915 (1H, d, $J = 8.304$ Hz, H-6), 3.7 (3H, s, OCH_3), 1.8 (1H, broad s, SH).

***N*-(*p*-Methylphenyl)-*N′*-phenylthiourea (4)**. $C_{14}H_{14}N_2S$ (Calcd: 242.0897; found: 242.0575), yield 74.28%, m.p, 141°C (sharp), (lit. 141°C) (Furniss et al., 1986) white crystalline solid; IR (KBr), λ_{max} (cm^{-1}): 3500 and 3155.3 (N–H stretching), 2950.9, 1591, 1552, 1442.7, 1311.5 (N–H amide), 1245.9 and 1139.9 (C=S), 1074.3; EIMS *m*/*z* (rel. int.%): 241.9 (19.04) [M^+], 208 (4.95), 149.9 (9.37), 107.0 (100), 93.0 (47.7) 76.8 (40.78). ^{1}H-NMR (400 MHz, DMSO-d_6); δ 9.66 (2H, s, 2 NH), 7.45 (2H, d, $J = 7.74$ Hz, H-2,6), 7.316 (5H, m, C_6H_5), 7.176 (2H, d, $J = 8.26$ Hz, H-3, 5), 2.26 (3H, s, CH_3), 2.49 (1H, broad s, SH).

***N*-(*m*-Methylphenyl)-*N′*-phenylthiourea (5)**. $C_{14}H_{14}N_2S$ (Calcd: 242.0897; found: 242. 0889), yield 54.42%, m.p. 104°C (sharp) (lit. 104°C) (Furniss et al., 1986) white crystalline solid; IR (KBr), λ_{max} (cm^{-1}): 3454.3 and 3203.5 (N–H stretching), 3010.7, 2925.8, 2856.4, 1595.0, 1546.8, 1494.7, 1448.4, 1448.4 and 1342.4, (N–H amide), 1236.3 and 1139.9 (C=S), 1074.3, 1024.1; EIMS *m*/*z* (rel. int.%): 241.8 [M^+] (2.1), 227.8 (19.7), 92.9 (100), 76.9 (44.1) ^{1}H-NMR (400 MHz, $CDCl_3$); δ 7.99 (2H, s broad, N–H), 7.39 (5H, m, C_6H_5), 7.37 (1H, d, $J = 7.28$ Hz, H-6), 7.27 (1H, m, H-5), 7.15 (1H, s, H-2), 7.09 (1H, m, H-4), 2.34 (3H, s, CH_3), 1.83 (1H, broad s, SH).

***N*-(*p*-Nitrophenyl)-*N′*-phenylthiourea (6)**. $C_{13}H_{11}N_3O_2S$ (Calcd: 273.0573), yield 1.3%, m.p. 188–189°C, (lit. 191°C) (Otterbacher & Whitemore, 1929) yellow crystalline solid; IR (KBr), λ_{max} (cm^{-1}): 3457.3 and 3206.6 (N–H stretching), 3031.7, 2925.8, 2856.4, 1594.0, 1527.4, 1447.9 and 1340.3, (N–H amide), 1237.1 and 1139.9 (C=S), 1069, 931; EIMS *m*/*z* (rel. int.%): 228.1 [$M^+ + 1 - NO_2$] (16.87) [$M^+ - NO_2$], 195.1 (11.09) [$M^+ - NO_2 - SH$], 136.0 (20.87) [$NH - C_6H_4 - NO_2$], 93.1(100) [$NH - C_6H_5$], 77.0 (49.84) [C_6H_5]; negative ion FAB-MS *m*/*z*: [$M - H]^+$; positive ion FAB-MS *m*/*z*: [$M + H]^+$, 274; ^{1}H-NMR (400 MHz, $CDCl_3$); δ 7.98 (2H, s, NH), 7.39 (2H, d, $J = 8.24$ Hz, H-3,5), 7.36 (5H, m, C_6H_5), 7.24 (2H, d, $J = 6.4$ Hz, H-2,6), 1.66 (1H, broad s, SH).

***N*-(*p*-Chlorophenyl)-*N′*-phenylthiourea (7)**. $C_{13}H_{11}N_2ClS$ (Calcd: 262.0333; found: 262.0300), yield 83.1%, m.p. 150–152°C (lit. 152°C) (Furniss et al., 1986; Otterbacher & Whitemore, 1929), white crystalline solid; IR (KBr), λ_{max} (cm^{-1}): 3470.7 and 3206.0 (N–H stretching), 3026.8, 1591, 1527.6, 1488.1 and 1337.8 (N–H amide), 1225.6 (C=S), 1089.8, 1015.2, 970.2, 811.1, 770.7; EIMS *m*/*z* (rel. int.%): 262 (83.0) [M^+], 227.2 (6.5), 126.9 (98.1), 93.0 (100); ^{1}H-NMR (400 MHz, $CDCl_3$); δ 8.1 (2H, s, N–H), 7.40 (2H, d, $J = 6.76$ Hz, H-3,5), 7.328 (5H, m, C_6H_5), 7.2 (2H, d, $J = 6.69$ Hz, H-2,6), 1.59 (1H, broad s, SH).

***N*-(*o*-Chlorophenyl)-*N′*-phenylthiourea (8)**. $C_{13}H_{11}N_2ClS$ (Calcd: 262.0333; found: 261.9978), yield 42.5%, m.p. 154–155°C (lit. 156°C) (Furniss et al., 1986; Otterbacher & Whitemore, 1929) white crystalline solid; IR (KBr) λ_{max} (cm^{-1}): 3450.9 and 3170.4 (N–H stretching), 3000.3, 1591.3, 1543.7, 1363.7, 1315 (N–H amide), 1291.0, 1249.2 (C=S), 1124; EIMS *m*/*z* (rel. int.%): 228 [$M^+ - Cl + 1H$] (3.7), 169 (85.5), 135 (100),

93 (70), 77 (49.9) ^{1}H-NMR (400 MHz, $CDCl_3$); δ 8.1 (2H, s, 2 N–H), 7.46 (1H, d, $J = 7.8$ Hz, H-6), 7.44 (2H, d, $J = 7.54$ Hz, H-2′,6′), 7.39 (1H, m, H-4′), 7.36 (1H, d, $J = 6.6$ Hz, H-3), 7.32 (1H, dd, $J = 8.4$, 8.9 Hz, H-4), 7.28 (1H, dd, $J = 7.7$, 7.6 Hz, H-5), 7.15 (2H, m, H-3′,5′),1.55 (1H, broad s, SH).

***N*-(*p*-Bromophenyl)-*N*′-phenylthiourea (9)**. $C_{13}H_{11}N_2BrS$ (Calcd: 307.9900; found: 307.9805), yield 77.8%, m.p. 148°C (sharp) (lit. 148°C) (Furniss et al., 1986; Otterbacher & Whitemore, 1929), pale yellow crystalline solid; IR (KBr), λ_{max} (cm^{-1}): 3433.1 and 3209.3 (N–H stretching), 2927.7, 2806.2, 1591.2, 1542.9, 1332.7 (N–H amide), 1292.2, 1238.2 (C=S), 1064.6, 1010.6, 925.8; EIMS *m*/*z* (rel. int.%): 307.8 [M^+] (26.8), 271.9 (9.97), 170.8 (100), 93.0 (90.58), 76.9 (53.0) ^{1}H-NMR (400 MHz, $CDCl_3$); δ 8.2 (2H, s, NH), 7.46 (5H, m, C_6H_5), 7.32 (2H, d, $J = 7.2$ Hz, H-3,5), 7.27 (2H, d, $J = 7.32$ Hz, H-2,6), 1.72 (1H, s, SH).

***N*-Phenyl-*N*′-(2-pyridinyl)thiourea (10)**. $C_{12}H_{11}N_3S$ (Calcd: 229.0675; found: 229.0684), yield 75.9%, m.p. 171–172°C (Lit. 172°C) (Sarkis & Faisal, 1985), white shiny crystalline solid; IR (KBr), λ_{max} (cm^{-1}): 3462.3 and 3220.2 (N–H stretching), 1601.1, 1598.1, 1537.9, 1473.6, 1431.5, 1353.9, 1342.2 (N–H amide), 1265.1, 1184.8 and 1142.4 (C=S),; EIMS *m*/*z* (rel. int.%): 229.0 (71.43) [M^+], 196.1 (27.13), 137.0 (36.29), 94.0 (100), 78.0 (50.47); ^{1}H-NMR (400 MHz, $CDCl_3$); δ 13.68 (1H, s, N–H), 9.39 (1H, s, N′–H), 8.19 (1H, d, $J = 4.25$ Hz, H-6), 7.65 (2H, d, $J = 7.86$ Hz, H-2′,6′), 7.62 (1H, m, H-4), 7.39 (2H, dd, $J = 7.6$, 7.8 Hz, H-3′,5′), 7.24 (1H, dd, $J = 7.4$, 7.3 Hz, H-4′), 6.97 (2H, d, $J = 6.92$ Hz, H-3), 6.94 (1H, m, H-5), 1.67 (1H, s, SH).

***N*-(1,5-Dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)-*N*′-phenylthiourea (11)**. $C_{18}H_{18}N_4OS$ (Calcd: 338.1203; HRFAB-MS 339.1290 (339.1281 for $C_{18}H_{19}N_4OS$), yield 98.9%, m.p. 198°C (sharp) (lit. 198°C) (Cuhna et al., 2005) crystalline solid; IR (KBr), λ_{max} (cm^{-1}): 3441.4 and 3272.9 (N–H stretching), 3034.3 (Ar–H stretching), 1632.8, 1600.3, 1585.9, 1532.6, 1494.8, 1450.7, 1416.3, 1313.5 (N–H amide), 1293.1S (C=S); EIMS *m*/*z* (rel. int.%): 304 (15.1) [$M^+ - H_2S$], 244.96 (100) [$M^+ - NH - C_6H_5$], 203 (46.8) [$M^+ - CSNHC_6H_5$], 134.97 (64.2) [$CSNHC_6H_5$], 93.1 (43.1) [NHC_6H_5], 77.0 (38.5) [C_6H_5]; positive ion FAB-MS *m*/*z*: [$M + H$]$^+$, 339.1, HRFAB-MS *m*/*z*: 339.1290 (Calcd: 339.1281 for $C_{18}H_{19}N_4OS$); ^{1}H-NMR (400 MHz, DMSO-d_6); δ 9.7 (2H, broad s, NH), 7.51 (4H, d, $J = 7.6$ Hz, H-2′,2″, 6′,6″), 7.45 (1H, m, H-4′), 7.33 (4H, dd, $J = 7.73$, 8.85 Hz, H-3′,3″,5′,5″), 7.13 (1H, m, H-4″), 3.1 (3H, s, N–CH_3), 2.4 (3H, s, C–CH_3), 2.18 (1H, broad s, SH); ^{13}C-NMR ($CDCl_3$): 181.45 (C-8), 161.72 (C-3), 153.05 (C-1′), 139.7 (C-1″), 134.94 (C-5), 129.18 (C-3′,C-5′), 128.32 (C-3″,C-5″), 126.6 (C-2′,C-6′), 124.818 (C-4′,4″), 124.04 (C-2″,C-6″), 113.4 (C-4), 35.59 (C-6), 10.90 (C-7).

***N*-(1-Naphthyl)-*N*′-phenylthiourea (12)**. $C_{17}H_{14}N_2S$ (Calcd: 278.0879; found: 277.9988), yield 88.6%, m.p. 196°C (sharp) (Lit. 165°C) (Furniss et al., 1986), white crystalline solid; IR (KBr), λ_{max} (cm^{-1}): 3450.76 (N–H stretching), 1627.91, 1594.28 1526.50, 1493.43, 1435.97, 1393.9, 1329.17 (N–H amide), 1274.1, 1218.7 (C=S); EIMS *m*/*z* (rel. int.%): 278 (2.9) [M^+], 244 (39.7) [$M^+ - H_2S$], 185 (82.6)[$M^+ - C_6H_5NH$], 143 (68.6) [$M^+ - C_6H_5NHCS$], 127 (33.4) Naphthyl group; ^{1}H-NMR (400 MHz, DMSO-d_6); δ 9.79 (2H, broad s, NH), 8.00 (2H, d, $J = 7.2$ Hz, H-4, 5), 7.96 (2H, d, $J = 7.5$ Hz, H-2′, 6′), 7.84 (2H, d, $J = 7.2$ Hz, H-3′, 5′), 7.54 (6H, m, H-3, 4, 4′ 6, 7, 8), 2.4 (1H, broad s, SH).

2-Phenyl-*N*-phenyl-1-hydrazinecarbothioamide (13). $C_{13}H_{13}N_3S$ (Calcd: 243.0832; found: 243.0802), yield 55.5%, m.p. 171–172°C (lit. 172°C) (Furniss et al., 1986), crystalline solid;

IR (KBr), λ_{max} (cm^{-1}): 3452.3 and 3280.7 (N–H stretching), 3168.8, 1596.0, 1542.0, 1492.8, 1440.7, 1300.0 (N–H amide), 1271.0 1234.4, 1203.5, 1101.3 (C=S); EIMS *m/z* (rel. int.%): 242.9 (37.9) [M^+], 149.9 (2.1), 135.9 (14.3), 124.8 (100), 107.9 (47.0), 91.9 (13.9), 76.9 (18.9); ^{1}H-NMR (400 MHz, $CDCl_3$); δ 8.9 (3H, broad s, H–N), 7.58 (2H, d, $J = 7.74$ Hz, H-2″, 6″), 7.35 (2H, m, H-3″,5″), 7.31 (2H, m, H-3′,5′), 7.21 (1H, dd, 7.4, 7.2 Hz, H-4′), 7.02 (1H, m, H-4″), 6.92 (2H, d, $J = 7.612$ Hz, H-2′,6′), 1.65 (1H, broad s, SH).

2-(2′,4′-Dinitrophenyl)-*N*-phenyl-1-hydrazinecarbothioamide (14). $C_{13}H_{11}N_5O_4S$ (Calcd: 333.18444); (Calcd%: C, 46.820; H, 3.327; N, 21.01, S, 9.6) (found%: C, 46.423; H, 4.09, N, 19.83; S, 12.09), yield 100%, m.p. 177.6°C (sharp) (lit. not available) (Truong & Ngo, 1999), pale yellow crystalline solid; IR (KBr) λ_{max} (cm^{-1}): 3460.1, 3319.3, 3244.0 and), 3087.8 (N–H stretching), 1598.9, 1533.3, 1500, 1438.8, 1352.0, 1309.6 (N–H amide sretching), 1247.9, 1197.7 and 1143.7 (C=S), 1097.4; EIMS *m/z* (rel. int.%): 182.9 (3) [$M^+ - C_6H_5NHCS$], 152.9 (4.2) [$M^+ - NHC_6H_3(NO_2)_2$], 134.9 (48.1), 121.9 (2.9), 108.0 (6.7), 93.0 (81.9), 77.0 (100); ^{1}H-NMR (400 MHz, DMSO-d_6); δ 10.279 (2H, d, $J = 52.3$ Hz, NH-1, NH-2), 9.9 (1H, broad s, NH), 8.86 (1H, d, $J = 2.55$ Hz, H-3′), 8.45 (1H, d, $J = 8.86$ Hz, H-5′), 7.36 (2H, d, $J = 9.7$ Hz, H-2″,6″), 7.32 (2H, m, H-3″,5″), 7.23 (1H, d, $J = 9.46$ Hz, H-6), 7.16 (1H, m, H-4″), 2.4 (1H, broad s, SH); ^{13}C-NMR (100 MHz, DMSO-d_6): 180.80 (CS), 147.99 (C′-1), 138.93 (C-4′), 137.15 (C-1″), 131.0 (C-2′), 130.29 (C-5′), 128.53 (C-3″,C-5″), 125.55 (C-4″), 123.81 (C-2″, C-6″), 122.86 (C-3′), 115.17 (C-6′).

4. Determination of biological activities

4.1. *Phytotoxic bioassay* (Lemna *Welv.*)

Phytotoxic bioassays were performed at 5, 50 and 500 $\mu g\,mL^{-1}$ concentration in methanol. These tests were conducted using a modified protocol by McLaughlin et al. (1991). The compounds of desired concentrations from stock solution were inoculated in sterilised conical flasks, and solvent was evaporated overnight. Each flask was inoculated with sterilised E-medium (20 mL) and 10 *Lemna aequinocitalis* Welv., each containing a roselle of three fronds. Negative controls were taken with flasks containing methanol and a reference inhibitor, i.e. parquet, serving as the positive control. The flasks were incubated at 30°C in a fisons Fi-Tortran 600 H growth cabinet for 7 days, 9000 lux light intensity, 56 ± 10 rh (relative humidity), and 12 h day length. The growth of *L. aequinocitalis* in the flasks containing compounds was determined by counting the number of fronds per dose and growth inhibition was calculated with reference to the negative control.

4.2. *Cytotoxic brine shrimp bioassay*

Brine shrimp (*A. salina* Leach) eggs were hatched in a shallow rectangular plastic dish (22×32 cm) filled with artificial seawater, which was prepared with commercial salt mixture (Instant Ocean Aquarium System, Inc., Mentor, Ohio, USA) and doubly distilled water. A plastic divider with several 2 mm holes was clamped in the dish to make two unequal compartments. Approximately 50 mg of eggs were sprinkled into the large compartment, which was darkened, while the smaller compartment was opened to ordinary light. After 2 days, nauplii was collected by a pipette from the side in light. A sample of the test compound was prepared by dissolving 20 mg of each compound in

2 mL of methanol. The solvent was allowed to evaporate overnight. After 2 days, when shrimp larvae were ready, 1 mL of seawater and 10 shrimps were added to each vial (30 shrimps dilution^{-1}) and the volume was adjusted with seawater to 5 mL per vial. After 24 h, the number of the survivors was counted. Data were analysed with the Finney program to determine the LD_{50} (Finney, 1971; Meyer et al., 1982).

4.3. *Acetylcholinesterase and butrylcholinesterase bioassays*

Acetylcholinesterase and butrylcholinesterase inhibition activities were measured by a spectrophotometric method (Ellman, Courtney, Andres, & Featherstone, 1961). The reaction mixture, containing 150 μL of (100 mM) sodium phosphate buffer (pH 8.0), 10 μL of 5,5-dithiobis-2-nitrobenzoic acid, 10 μL of the test compound solution and 20 μL of the acetylcholinesterase or butrylcholinesterase solution, was mixed and incubated for 15 min at 25°C. The reaction was then initiated by the addition of 20 μL acetylthiocholine iodide or butyrylthiocholine chloride, in that order. The hydrolysis of these substrates was monitored by the formation of yellow 5-thio-2-nitrobenzoate anion as the result of the reaction of DTNB with thiocholine, released by the enzymatic hydrolysis of acetylthiocholine iodide or butyrylthiocholine chloride, respectively, at a wavelength of 412 nm for 15 min. Test compounds and the positive control (galanthamine and eserine) were dissolved in EtOH. All the reactions were performed in triplicate in 96-well microplates in Spectrmax 340 (Molecular Devices, USA).

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