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Synthesis of Thiadiazole Derivatives Bearing Hydrazone Moieties and Evaluation of Their Pharmacological Effects on Anxiety, Depression, and Nociception Parameters in Mice

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Novel thiadiazole derivatives bearing hydrazone moieties were synthesized through the reaction of 2-[(5-methyl-1,3,4-thiadiazol-2-yl)thio)]acetohydrazide with aldehydes/ketones. The chemical structures of the compounds were elucidated by ^{1}H-NMR, ^{13}C-NMR, MS-FAB spectral data, and elemental analyses. Behavioral effects of the test compounds in mice were examined by hole-board, activity cage, tail suspension and modified forced swimming tests (MFST). Antinociceptive activities were evaluated using the hot-plate and tail-clip methods. Results of the experiments indicated that the test compounds did not significantly change the exploratory behaviors or locomotor activities of animals in the hole-board and activity cage tests, respectively. Administration of the reference drug fluoxetine (10 mg/kg) and compounds **3a**, **3b**, **3c**, **3j**, **3k**, and **3l** significantly shortened the immobility times of animals in the tail suspension and MFST tests, indicating the antidepressant-like effects of these derivatives. Morphine (10 mg/kg) and compounds **3a**, **3b**, **3c**, **3d**, **3e**, **3j**, **3k**, and **3l** increased the reaction times of mice in both the hot-plate and tail-clip tests, indicating the antinociceptive effects of these compounds. To the best of our knowledge, this is the first study of central nervous system activities of chemical compounds carrying thiadiazole and hydrazone moieties together on their structures.

Key words: Thiadiazole, Hydrazone, Antidepressant, Antinociceptive, Tail suspension, Forced swimming test, Hot-plate, Tail-clip

INTRODUCTION

Thiadiazole, a 5-member diunsaturated ring structure containing 2 nitrogen atoms and 1 sulphur atom, occurs in 4 isomeric forms. These forms are 1,2,3-thiadiazole, 1,2,4-thiadiazole, 1,2,5-thiadiazole, and 1,3,4-thiadiazole.

A considerable amount of research has been carried out on the effects of thiadiazoles on the central nervous system (CNS). Among thiadiazole derivatives, 1,3,4-thiadiazoles have received a great deal of attention as important pharmacophores in drug design (Siddiqui et al., 2009). Numerous studies have confirmed that the 1,3,4-thiadiazole derivatives exhibit a broad CNS-related activity spectrum, including antidepressant (Brufani et al., 1994; Varvaresou et al., 1998; Clerici and Pocar, 2001; Yusuf et al., 2008; Pattanayak et al., 2009; Sharma et al., 2011), anxiolytic (Clerici and Pocar, 2001; Pattanayak et al., 2009; Sharma et al., 2011), and analgesic (Salgin-Gökşen et al., 2007; Pandey et al., 2011) effects. Anticonvulsant properties have also been reported for various thiadiazole derivatives (Chapleo et al., 1986; Stillings et al., 1986; Pattanayak et al., 2009; Rajak et al., 2009; Sharma et al., 2011). Moreover, acetazolamide, one of the currently used anticonvulsant drugs, has 1,3,4-thiadiazole nuclei in its structure (Chufán et al., 1999).

In addition to thiadiazoles, hydrazides and hydrazones show CNS-related effects, particularly antidepressant

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activity (Ergenç et al., 1998). Irreversible and non-selective monoamine oxidase (MAO) inhibitory drugs, iproniazid, isocarboxazid, and nialamide are examples of clinically used antidepressant agents with hydrazide groups (Rollas and Küçükgüzel, 2007). Moreover, anticonvulsant (Ragavendran et al., 2007) and analgesic (Silva et al., 2004; Salgin-Gökşen et al., 2007) activities have been reported for various hydrazide/hydrazone derivative compounds.

The CNS-related activities of both thiadiazole and hydrazone moieties prompted us to synthesize novel compounds bearing both of these biolabile components on their structures in our search for new pharmacologically active drug candidates. We combined thiadiazole and hydrazone moieties by a thioether group. This method increases lipophilicity of CNS-targeted drugs, thereby enhancing passage across the blood-brain barrier and improving drug delivery to sites of action (Waterbeemd and Mannhold, 1996; Silverman, 2004).

We used a bioisosteric replacement approach for designing the new compounds. Bioisosteric replacement can be defined as the rational modification of a part of a biologically active molecule to elicit similar biological activity. The bioisosteric replacement of benzene with a heteroaromatic ring resulted in analogues maintaining the same biological activity within a series of different pharmacological agents, lending great importance to ring-equivalent bioisosteres. Pyridine, furan, and thiophene, which are structurally related to benzene, are widely used as ring equivalents in drug development (Patani and LaVoie, 1996; Lima and Barreiro, 2005; Ciapetti and Giethlen, 2008). Therefore, we used these bioisosteres to derive our chemical compounds.

In summary, our research group aimed to synthesize novel thiadiazole derivatives bearing hydrazone moieties and examine their effects on pain perception and depression and anxiety parameters in mice. We also investigated structure-activity relationships in different bioisostere substitutions.

MATERIALS AND METHODS

Chemistry

Materials

All reagents were purchased from commercial suppliers and used without further purification. Melting points were determined with an Electrothermal 9100 melting point apparatus (Weiss-Gallenkamp) and were uncorrected. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Bruker 500 MHz and 125 MHz spectrometer (Bruker), respectively. Mass spectra were recorded on a VG Quattro mass spectrometer (Agilent). Elemental analyses were performed on a Perkin Elmer EAL 240 elemental analyser (Perkin-Elmer).

General procedure for synthesis of compounds

Ethyl 2-[(5-methyl-1,3,4-thiadiazol-2-yl)thio]acetate (1)

A mixture of 5-methyl-1,3,4-thiadiazole-2-thiol (0.05 mol) and ethyl chloroacetate (0.05 mol) in the presence of potassium carbonate (0.05 mol) in acetone was refluxed for 10 h. The reaction mixture was cooled and filtered, and the crude product was dissolved in water and then extracted with ether (Kidwai and Kumar, 1996).

2-[(5-Methyl-1,3,4-thiadiazol-2-yl)thio)]acetohydrazide (2)

A mixture of the ester (**1**) (0.05 mol) and hydrazine hydrate (0.1 mol) in ethanol was stirred at room temperature for 3 h and then filtered (Kidwai and Kumar, 1996).

2-[(5-Methyl-1,3,4-thiadiazol-2-yl)thio]-N'-[(aryl) methylidene/ethylidene]acetohydrazide (3a-o)

A mixture of the hydrazide (**2**) (0.01 mol) and aldehydes/ketones (0.01 mol) was refluxed in ethanol for 5 h, filtered and crystallized from ethanol.

2-(5-Methyl-1,3,4-thiadiazol-2-ylthio)-N'-[(pyridin-2-yl)methylene]acetohydrazide (3a)

^{1}H-NMR (500 MHz, DMSO-d_6): 2.65-2.69 (3H, s, CH_3), 3.97 and 4.63 (2H, two s, S-CH_2), 7.42-8.81 (5H, m, -N =CH-, pyridine), 11.93 and 12.04 (1H, two s, N-H). ^{13}C-NMR (125 MHz, DMSO-d_6): 15.76 (CH_3), 36.44 (CH_2), 120.99 (CH), 126.11 (CH), 137.57 (CH), 139.91 (C), 145.67 (CH), 150.92 (CH), 154.16 (C), 165.93 (C), 170.55 (C). For $C_{11}H_{11}N_5OS_2$ calculated: C, 45.03; H, 3.78; N, 23.87; found: C, 45.00; H, 3.80; N, 23.85. MS (FAB) $[M+1]^+$: m/z 294.

2-(5-Methyl-1,3,4-thiadiazol-2-ylthio)-N'-[(pyridin-3-yl)methylene]acetohydrazide (3b)

^{1}H-NMR (500 MHz, DMSO-d_6): 2.67-2.69 (3H, s, CH_3), 4.20 and 4.61 (2H, two s, S-CH_2), 7.47-8.86 (5H, m, -N =CH-, pyridine), 11.90 (1H, s, N-H). ^{13}C-NMR (125 MHz, DMSO-d_6): 15.75 (CH_3), 36.54 (CH_2), 125.16 (CH), 131.13 (C), 134.75 (CH), 142.43 (C), 145.88 (CH), 149.93 (CH), 151.98 (CH), 164.76 (C), 169.98 (C). For $C_{11}H_{11}N_5OS_2$ calculated: C, 45.03; H, 3.78; N, 23.87; found: C, 45.04; H, 3.81; N, 23.88. MS (FAB) $[M+1]^+$: m/z 294.

2-(5-Methyl-1,3,4-thiadiazol-2-ylthio)-N'-[(pyridin-4-yl)methylene]acetohydrazide (3c)
^{1}H-NMR (500 MHz, DMSO-d_6): 2.67 (3H, s, CH_3), 4.21 and 4.63 (2H, two s, S-CH_2), 7.65-8.66 (5H, m, -N=CH-, pyridine), 12.03 (1H, s, N-H). ^{13}C-NMR (125 MHz, DMSO-d_6): 15.75 (CH_3), 36.43 (CH_2), 122.07 (2CH), 142.39 (C), 142.77 (C), 146.15 (CH), 151.64 (2CH), 165.06 (C), 170.23 (C). For $C_{11}H_{11}N_5OS_2$ calculated: C, 45.03; H, 3.78; N, 23.87; found: C, 45.05; H, 3.79; N, 23.90. MS (FAB) $[M+1]^+$: m/z 294.

2-(5-Methyl-1,3,4-thiadiazol-2-ylthio)-N'-[(furan-2-yl)methylene)]acetohydrazide (3d)
^{1}H-NMR (500 MHz, DMSO-d_6): 2.68 (3H, s, CH_3), 4.16 and 4.54 (2H, two s, S-CH_2), 6.63-8.09 (4H, m, -N=CH-, furan), 11.69 and 11.75 (1H, two s, N-H). ^{13}C-NMR (125 MHz, DMSO-d_6): 15.76 (CH_3), 36.61 (CH_2), 113.31 (CH), 115.06 (CH), 135.32 (CH), 138.31 (CH), 146.50 (C), 150.29 (C), 164.47 (C), 169.51 (C). For $C_{10}H_{10}N_4O_2S_2$ calculated: C, 42.54; H, 3.57; N, 19.84; found: C, 42.55; H, 3.60; N, 19.85. MS (FAB) $[M+1]^+$: m/z 283.

2-(5-Methyl-1,3,4-thiadiazol-2-ylthio)-N'-[(thiophen-2-yl)methylene)]acetohydrazide (3e)
^{1}H-NMR (500 MHz, DMSO-d_6): 2.68 (3H, s, CH_3), 4.15 and 4.50 (2H, two s, S-CH_2), 7.13-8.42 (4H, m, -N=CH-, thiophene), 11.72 and 11.76 (1H, two s, N-H). ^{13}C-NMR (125 MHz, DMSO-d_6): 15.76 (CH_3), 36.41 (CH_2), 129.12 (CH), 129.19 (CH), 129.97 (CH), 130.40 (CH), 140.36 (C), 143.69 (C), 164.37 (C), 169.35 (C). For $C_{10}H_{10}N_4OS_3$ calculated: C, 40.25; H, 3.38; N, 18.78; found: C, 40.27; H, 3.40; N, 18.75. MS (FAB) $[M+1]^+$: m/z 299.

2-(5-Methyl-1,3,4-thiadiazol-2-ylthio)-N'-[(5-methyl-furan-2-yl)methylene]acetohydrazide (3f)
^{1}H-NMR (500 MHz, DMSO-d_6): 2.34 (3H, s, CH_3), 2.68 (3H, s, CH_3), 4.17 and 4.53 (2H, two s, S-CH_2), 6.26-7.98 (3H, m, -N=CH-, furan), 11.60 and 11.66 (1H, two s, N-H). ^{13}C-NMR (125 MHz, DMSO-d_6): 14.11 (CH_3), 15.76 (CH_3), 36.66 (CH_2), 109.72 (CH), 116.86 (CH), 135.45 (CH), 138.17 (C), 148.78 (C), 156.06 (C), 164.29 (C), 169.30 (C). For $C_{11}H_{12}N_4O_2S_2$ calculated: C, 44.58; H, 4.08; N, 18.90; found: C, 44.60; H, 4.11; N, 18.93. MS (FAB) $[M+1]^+$: m/z 297.

2-(5-Methyl-1,3,4-thiadiazol-2-ylthio)-N'-[(5-methyl-thiophen-2-yl)methylene]acetohydrazide (3g)
^{1}H-NMR (500 MHz, DMSO-d_6): 2.47 (3H, s, CH_3), 2.68 (3H, s, CH_3), 4.14 and 4.47 (2H, two s, S-CH_2), 6.82-8.32 (3H, m, -N=CH-, thiophene), 11.64 and 11.67 (1H, two s, N-H). ^{13}C-NMR (125 MHz, DMSO-d_6): 15.76 (CH_3), 19.15 (CH_3), 36.46 (CH_2), 127.58 (CH), 132.33 (CH), 137.65 (CH), 140.55 (C), 143.92 (C), 144.46 (C), 164.22 (C), 169.22 (C). For $C_{11}H_{12}N_4OS_3$ calculated: C, 42.29; H, 3.87; N, 17.93; found: C, 42.31; H, 3.89; N, 17.91. MS (FAB) $[M+1]^+$: m/z 313.

2-(5-Methyl-1,3,4-thiadiazol-2-ylthio)-N'-[(1H-pyrrol-2-yl)methylene]acetohydrazide (3h)
^{1}H-NMR (500 MHz, DMSO-d_6): 2.68 (3H, s, CH_3), 4.20 and 4.52 (2H, two s, S-CH_2), 6.10-8.11 (4H, m, -N=CH-, pyrrole), 11.20 (1H, br, pyrrole N-H), 11.42 and 11.68 (1H, two s, N-H). ^{13}C-NMR (125 MHz, DMSO-d_6): 17.98 (CH_3), 38.74 (CH_2), 112.56 (CH), 116.11 (CH), 125.49 (CH), 130.44 (C), 140.04 (C), 143.87 (CH), 165.94 (C), 171.34 (C). For $C_{10}H_{11}N_5OS_2$ calculated: C, 42.69; H, 3.94; N, 24.89; found: C, 42.72; H, 3.94; N, 24.84. MS (FAB) $[M+1]^+$: m/z 282.

2-(5-Methyl-1,3,4-thiadiazol-2-ylthio)-N'-[(1-methyl-1H-pyrrol-2-yl)methylene]acetohydrazide (3i)
^{1}H-NMR (500 MHz, DMSO-d_6): 2.68 (3H, s, CH_3), 3.85 (3H, s, CH_3), 4.13 and 4.52 (2H, two s, S-CH_2), 6.10-8.11 (4H, m, -N=CH-, pyrrole), 11.42 (1H, s, N-H). ^{13}C-NMR (125 MHz, DMSO-d_6): 20.52 (CH_3), 41.67 (CH_3), 41.97 (CH_2), 114.01 (CH), 121.24 (CH), 132.65 (CH), 134.34 (C), 143.40 (CH), 146.53 (C), 168.53 (C), 173.79 (C). For $C_{11}H_{13}N_5OS_2$ calculated: C, 44.73; H, 4.44; N, 23.71; found: C, 44.75; H, 4.42; N, 23.72. MS (FAB) $[M+1]^+$: m/z 296.

2-(5-Methyl-1,3,4-thiadiazol-2-ylthio)-N'-[(1H-indol-3-yl)methylene]acetohydrazide (3j)
^{1}H-NMR (500 MHz, DMSO-d_6): 2.69 (3H, s, CH_3), 4.28 and 4.74 (2H, two s, S-CH_2), 7.08-8.50 (6H, m, -N=CH-, indole), 10.72 (1H, s, indole N-H), 11.51 (1H, s, N-H). ^{13}C-NMR (125 MHz, DMSO-d_6): 15.76 (CH_3), 37.10 (CH_2), 110.40 (C), 111.10 (CH), 119.80 (CH), 121.70 (CH), 121.80 (CH), 126.30 (C), 130.70 (CH), 137.10 (C), 142.70 (C), 143.30 (CH), 164.20 (C), 170.20 (C). For $C_{14}H_{13}N_5OS_2$ calculated: C, 50.74; H, 3.95; N, 21.13; found: C, 50.72; H, 3.97; N, 21.11. MS (FAB) $[M+1]^+$: m/z 332.

2-(5-Methyl-1,3,4-thiadiazol-2-ylthio)-N'-[(naphthalen-1-yl)methylene]acetohydrazide (3k)
^{1}H-NMR (500 MHz, DMSO-d_6): 2.68 (3H, s, CH_3), 4.22 and 4.67 (2H, two s, S-CH_2), 7.58-8.85 (8H, m, -N=CH-, naphthalene), 11.78 (1H, br, N-H). ^{13}C-NMR (125 MHz, DMSO-d_6): 20.55 (CH_3), 41.64 (CH_2), 129.69 (CH), 130.39 (CH), 131.54 (CH), 131.59 (CH), 132.34 (CH), 133.31 (CH), 133.44 (C), 134.48 (C), 134.84 (CH), 134.90 (C), 139.60 (C), 149.76 (CH), 169.37 (C), 174.46 (C). For $C_{16}H_{14}N_4OS_2$ calculated: C, 56.12; H, 4.12; N, 16.36; found: C, 56.13; H, 4.10; N, 16.39. MS (FAB) $[M+1]^+$: m/z 343.

2-(5-Methyl-1,3,4-thiadiazol-2-ylthio)-N'-[(biphenyl-4-yl)methylene]acetohydrazide (3l)
^{1}H-NMR (500 MHz, DMSO-d_6): 2.67 (3H, s, CH_3), 4.19 and 4.62 (2H, two s, S-CH_2), 7.39-8.25 (10H, -N=CH-, biphenyl), 11.79 (1H, s, N-H). ^{13}C-NMR (125 MHz, DMSO-d_6): 15.76 (CH_3), 36.63 (CH_2), 127.93 (2CH), 128.30 (CH), 128.78 (2CH), 129.01 (2CH), 129.16 (2CH), 134.31 (C), 140.63 (C), 142.84 (C), 144.85 (C), 148.12 (CH), 167.07 (C), 169.77 (C). For $C_{18}H_{16}N_4OS_2$ calculated: C, 58.67; H, 4.38; N, 15.21; found: C, 58.70; H, 4.41; N, 15.19. MS (FAB) $[M+1]^+$: m/z 369.

2-(5-Methyl-1,3,4-thiadiazol-2-ylthio)-N'-[1-(furan-2-yl)ethylidene]acetohydrazide (3m)
^{1}H-NMR (500 MHz, DMSO-d_6): 2.22 (3H, s, CH_3), 2.67 (3H, s, CH_3), 4.27 and 4.56 (2H, two s, S-CH_2), 6.60-7.81 (3H, m, furan), 10.71 and 10.94 (1H, two s, N-H). ^{13}C-NMR (125 MHz, DMSO-d_6): 13.88 (CH_3), 15.75 (CH_3), 37.17 (CH_2), 112.26 (CH), 113.07 (CH), 142.12 (CH), 145.84 (2C), 152.96 (C), 164.91 (C), 170.38 (C). For $C_{11}H_{12}N_4O_2S_2$ calculated: C, 44.58; H, 4.08; N, 18.90; found: C, 44.60; H, 4.11; N, 18.88. MS (FAB) $[M+1]^+$: m/z 297.

2-(5-Methyl-1,3,4-thiadiazol-2-ylthio)-N'-[1-(thiophen-2-yl)ethylidene]acetohydrazide (3n)
^{1}H-NMR (500 MHz, DMSO-d_6): 2.29 (3H, s, CH_3), 2.68 (3H, s, CH_3), 4.26 and 4.52 (2H, two s, S-CH_2), 7.09-7.61 (3H, m, thiophene), 10.75 and 11.00 (1H, two s, N-H). ^{13}C-NMR (125 MHz, DMSO-d_6): 14.68 (CH_3), 15.76 (CH_3), 37.05 (CH_2), 128.79 (CH), 129.48 (CH), 129.77 (CH), 144.52 (C), 146.49 (C), 150.64 (C), 164.71 (C), 170.19 (C). For $C_{11}H_{12}N_4OS_3$ calculated: C, 42.29; H, 3.87; N, 17.93; found: C, 42.31; H, 3.85; N, 17.91. MS (FAB) $[M+1]^+$: m/z 313.

2-(5-methyl-1,3,4-thiadiazol-2-ylthio)-N'-[1-(1H-indol-3-yl)ethylidene]acetohydrazide (3o)
^{1}H-NMR (500 MHz, DMSO-d_6): 2.31 (3H, s, CH_3), 2.69 (3H, s, CH_3), 4.28 and 4.74 (2H, two s, S-CH_2), 7.08-8.50 (5H, m, indole), 10.72 (1H, s, indole N-H), 11.47 and 11.51 (1H, two s, N-H). ^{13}C-NMR (125 MHz, DMSO-d_6): 14.75 (CH_3), 15.76 (CH_3), 37.10 (CH_2), 111.10 (CH), 112.50 (C), 119.80 (CH), 121.70 (CH), 121.80 (CH), 126.30 (C), 127.10 (CH), 137.10 (C), 142.70 (C), 164.20 (C), 165.80 (C), 170.20 (C). For $C_{15}H_{15}N_5OS_2$ calculated: C, 52.15; H, 4.38; N, 20.27; found: C, 52.13; H, 4.40; N, 20.25. MS (FAB) $[M+1]^+$: m/z 346.

Pharmacology

Animals

Adult BALB/c mice weighing 30-35 g were used for all tests. Animals were housed in a room with controlled temperature (25 ± 1°C) and a 12-h light/dark cycle. Temperature, sound, and light conditions were not altered during the course of the experiments.

All animals were acclimatised to the laboratory environment at least 48 h before the experiments. Food was withdrawn 12 h before experiments in order to avoid food interference with substance absorption, although water was allowed *ad libitum*. The experimental protocols were approved by the Local Ethical Committee on Animal Experimentation of Eskişehir Anadolu University, Turkey.

Administration of compounds

Animals were randomly divided into the following groups: control group, reference drug-treated group and test compound-treated groups (n = 6 animals/group).

Behavioral tests and nociceptive measurements were performed in different groups of animals. In behavioral tests, control solution, reference drug fluoxetine (Flx, 10 mg/kg), and the test compounds (100 mg/kg) were injected intraperitoneally (*i.p*) for 3 times; 24, 5 and 0.5 h before testing (Can et al., 2009).

For nociceptive measurements, control solution, the reference drug morphine sulphate (10 mg/kg), and the test compounds (100 mg/kg) were injected (*i.p*) once, 30 min before testing (Kaplancikli et al., 2009).

Behavioral tests

Assessment of exploratory behavior

Exploratory behavior of mice was screened using hole-board tests. The hole-board apparatus (Ugo Basile, No. 6650) consisted of gray Perspex panels (40 cm × 40 cm, 2.2 cm thick) with 16 equidistant holes 3 cm in diameter in the floor. Head-dipping was measured by infrared cells placed under the holes. In hole-board testing, each mouse was individually placed in the centre of the apparatus facing away from the observer and allowed to explore freely for 5 min. Total number of head-dips, latencies prior to the first head-dips, and total number of explored holes were recorded during the test session (Takeda et al., 1998; Can et al., 2010).

Assessment of spontaneous locomotor activity

Spontaneous locomotor activities of mice were monitored in activity cage apparatus (Ugo Basile, No. 7420) containing 2 pairs of 16 photocells placed 3 cm and 12 cm above the floor under a transparent cover. Interruptions of light beams during vertical and horizontal movements of the animals were automatically recorded for 5 min (Can et al., 2009).

Assessment of antidepressant activity

Antidepressant-like activity of the test compounds

was screened using tail suspension and modified forced swimming tests (MFST). Tail suspension tests were performed similarly to tests described by Steru et al. (Steru et al., 1985). Mice were dangled from their tails using adhesive tape placed approximately 1 cm from the tip of the tail and attached to an applicator stick. Then, the mice were hung approximately 30 cm above a table and were considered immobile only when they did not make struggling movements and hung passively. Immobility time for each animal was recorded by a stopwatch during the last 4 min of a 6-min test (Can et al., 2011).

In MFST tests, mice were forced to swim individually in a glass cylinder (diameter, 12 cm; height, 30 cm) containing 20 cm of water at 25 ± 1°C. Twenty-four hours prior to the MFST, animals were exposed to a pre-test for 15 min. In test session, swimming (horizontal movement on the surface of the water and crossing into another quadrant), climbing (upward-directed movements of the forepaws along the side of the swim chamber), and immobility (the minimum movement required to keep the head above water) times over 5-sec intervals were recorded for 5 min by a stopwatch (Cryan et al., 2002; Tanaka and Telegdy, 2008; Kaplancikli et al., 2010).

The water in the cylinder was changed after testing each animal to minimise the influence of secreted alarm substances. Following the training and the test sessions, the animals were dried in a heated enclosure.

Assessment of antinociceptive activity

The antinociceptive action of the tested compounds in mice was evaluated using hot-plate and tail-clip tests. The response to thermal stimuli was tested using a hot-plate analgesia meter (Ugo Basile, 7280) maintained at 55 ± 1°C. Delay of nociceptive responses, such as licking or shaking the paws was recorded as the reaction time. A cut-off latency time of 30 sec was imposed for each measurement to avoid lesions to the skin and unnecessary suffering (Woolfre and MacDonald, 1944; Pavin et al., 2011).

Effects of the test compounds on nociceptive perception were calculated by converting hot-plate reaction times to percentage antinociceptive activity according to the following previously described equation (Can et al., 2010):

$$\text{Analgesia \%} = [(\text{post drug latency} - \text{pre drug latency}) / \text{pre-drug latency}] \times 100$$

Tail-clip tests were applied for measuring mechanical antinociceptive activities of the compounds (D'Amour and Smith, 1941). A metal artery clamp was applied to the tail of mouse, and the time that passed before the mouse began biting the clamp was recorded by a stopwatch. A sensitivity test was carried out before the experimental session, and animals that did not respond to the clamp within 10 sec were excluded from the experiments. Maximum latency time (cut-off time) for the tail-clip tests was chosen as 10 sec to avoid possible tissue damage. Analgesia was expressed as a percentage of the maximum possible effect (MPE %), according the following equation (Can et al., 2010):

$$\text{MPE \%} = [(\text{post drug latency} - \text{pre drug latency}) / (\text{cut-off time} - \text{pre drug latency})] \times 100.$$

Statistical analyses

Statistical analyses of the experimental data were performed using GraphPad Prism 3.0 software (GraphPad Software). Experimental data were evaluated by one-way analysis of variance (ANOVA) followed post hoc by Tukey's honestly significantly different (HSD) test. The results are presented as mean ± S.E.M. Differences between data sets were considered significant when the p value was less than 0.05.

RESULTS

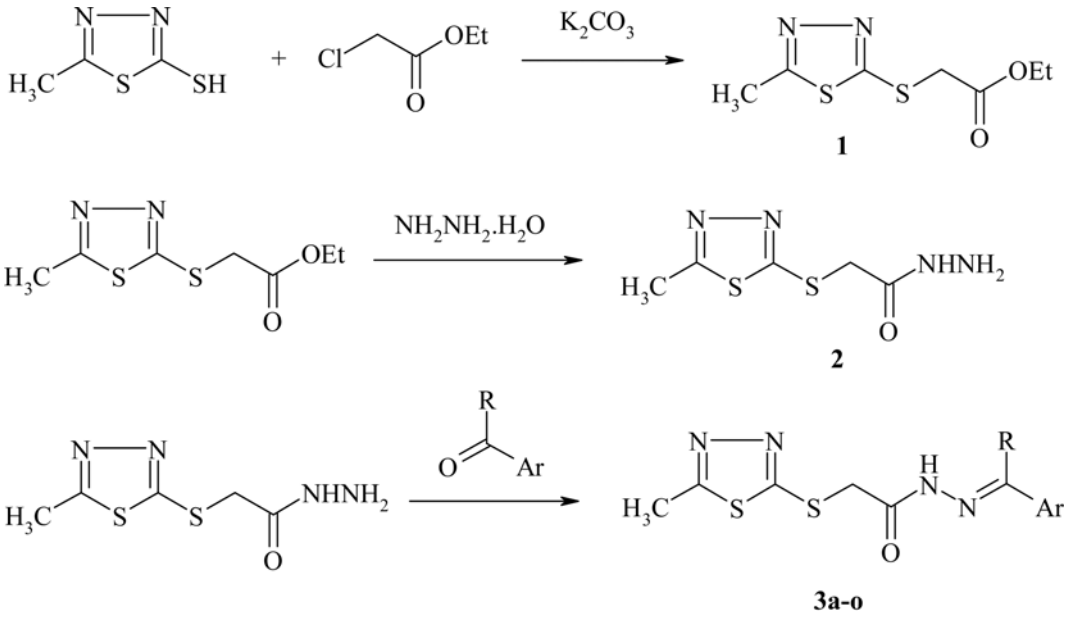
Chemistry

Initially, ethyl 2-[(5-methyl-1,3,4-thiadiazol-2-yl)thio)] acetate (**1**) was synthesized by the reaction of 5-methyl-1,3,4-thiadiazole-2-thiol with ethyl chloroacetate in the presence of potassium carbonate. Then, this ester (**1**) was converted to the corresponding hydrazide derivative (**2**). The treatment of the hydrazide derivative (**2**) with various aldehydes/ketones produced the target compounds (**3a-o**). These reactions are summarized in Scheme 1, and some properties of the compounds (**3a-o**) are given in Table I.

The structures of the compounds (**3a-o**) were confirmed by ^{1}H-NMR, ^{13}C-NMR, mass spectral data, and elemental analyses.

In the ^{1}H-NMR spectra of the compounds (**3a-o**), the signal due to the hydrazone proton appeared in the region of 11.0-13.8 ppm. The signal due to the -S-CH_2- protons was observed in the region of 4.0-5.0 ppm. In the ^{1}H-NMR spectra of some compounds, N-H and -S-CH_2- protons gave rise to 2 singlet peaks in accordance with the presence of the *E* and *Z* isomers (Özdemir et al., 2009; Despaigne et al., 2010). The signal due to the methyl protons attached to the thiadiazole ring was observed in the region of 2.6-2.7 ppm. The other aromatic and aliphatic protons were observed at the expected regions.

In ^{13}C-NMR spectra, the signal due to the -S-CH_2-



Scheme 1. Synthesis of thiadiazole derivatives (**3a-o**).

Table I. Some properties of the synthesized compounds (**3a-o**)

Compound	R	Ar	Yield (%)	M.p. (°C)	Molecular formula	Molecular weight
3a	H	2-pyridyl	70	137-138	$C_{11}H_{11}N_5OS_2$	293
3b	H	3-pyridyl	80	170-171	$C_{11}H_{11}N_5OS_2$	293
3c	H	4-pyridyl	90	183-185	$C_{11}H_{11}N_5OS_2$	293
3d	H	2-furyl	85	164-165	$C_{10}H_{10}N_4O_2S_2$	282
3e	H	thiophen-2-yl	95	190-191	$C_{10}H_{10}N_4OS_3$	298
3f	H	5-methyl-2-furyl	85	138-139	$C_{11}H_{12}N_4O_2S_2$	296
3g	H	5-methylthiophen-2-yl	70	154-156	$C_{11}H_{12}N_4OS_3$	312
3h	H	1H-pyrrol-2-yl	72	207-208	$C_{10}H_{11}N_5OS_2$	281
3i	H	1-methyl-1H-pyrrol-2-yl	80	151-152	$C_{11}H_{13}N_5OS_2$	295
3j	H	1H-indol-3-yl	90	249-250	$C_{14}H_{13}N_5OS_2$	331
3k	H	1-naphthyl	95	170-172	$C_{16}H_{14}N_4OS_2$	342
3l	H	biphenyl-4-yl	90	161-163	$C_{18}H_{16}N_4OS_2$	368
3m	CH_3	2-furyl	70	140-141	$C_{11}H_{12}N_4O_2S_2$	296
3n	CH_3	thiophen-2-yl	78	139-140	$C_{11}H_{12}N_4OS_3$	312
3o	CH_3	1H-indol-3-yl	75	242-246	$C_{15}H_{15}N_5OS_2$	345

carbon appeared at 35-40 ppm. The signal due to the hydrazone carbon was observed at 168-172 ppm. The signal due to the methyl carbon attached to the thiadiazole ring was observed in the region of 15.0-20.0 ppm. The other aromatic and aliphatic carbons were observed in the expected regions.

In the mass spectra of all compounds (**3a-o**), M+1 peaks were observed. All compounds provided satisfactory elemental analysis results.

Pharmacology

Results obtained from the hole-board tests are given in Table II. In this test, none of the test compounds significantly changed the total numbers of head-dipping behaviors, latency to first head-dippings and total number of explored holes. Similarly, activity cage tests showed no specific changes between the spontaneous locomotor activities of animals injected with control solution and those injected with test compounds (Table III).

Fig. 1 illustrates the effects of the test compounds on immobility times of mice in the tail-suspension test. Administration of the reference drug (Flx) and compounds **3a**, **3b**, **3c**, **3j**, **3k**, and **3l** significantly shortened the immobility times of animals as compared to controls. Fig. 2 shows that the same test compounds significantly decreased immobility and increased the swimming times in the MFST. Climbing times in

Table II. Effects of the test compounds on exploratory behavior of mice in hole-board tests

Groups	Dose	Total number of head-dips	Latency to first head-dip (s)	Total numbers of holes explored
Control	-	22.3 ± 1.9	5.9 ± 1.2	8.3 ± 0.9
3a	100 mg/kg	21.3 ± 1.8	5.3 ± 0.8	8.8 ± 1.0
3b	100 mg/kg	19.0 ± 1.6	7.4 ± 1.1	9.7 ± 1.2
3c	100 mg/kg	19.7 ± 2.1	8.4 ± 0.9	8.5 ± 0.9
3d	100 mg/kg	22.0 ± 2.1	7.3 ± 1.4	7.5 ± 1.2
3e	100 mg/kg	20.5 ± 2.6	7.8 ± 0.9	8.0 ± 1.6
3f	100 mg/kg	19.0 ± 2.7	6.7 ± 1.0	8.2 ± 1.5
3g	100 mg/kg	20.3 ± 2.4	7.5 ± 1.1	9.2 ± 1.4
3h	100 mg/kg	20.0 ± 2.5	6.7 ± 1.2	7.8 ± 1.2
3i	100 mg/kg	20.5 ± 1.7	8.3 ± 1.3	8.5 ± 1.1
3j	100 mg/kg	19.8 ± 3.4	7.3 ± 1.6	7.2 ± 1.0
3k	100 mg/kg	22.5 ± 1.2	6.3 ± 1.1	7.5 ± 0.6
3l	100 mg/kg	20.8 ± 1.9	8.2 ± 1.3	8.5 ± 0.8
3m	100 mg/kg	19.2 ± 2.9	7.2 ± 0.9	6.8 ± 1.6
3n	100 mg/kg	18.7 ± 2.0	7.4 ± 1.2	7.5 ± 0.7
3o	100 mg/kg	20.5 ± 2.4	7.9 ± 1.1	8.0 ± 0.9

Values are given as mean ± S.E.M. One-way ANOVA, post-hoc Tukey's test, n = 6.

Table III. Effects of the test compounds on spontaneous locomotor activity parameters of mice in activity cage tests

Groups	Dose	Number of horizontal movements	Number of vertical movements
Control	-	464.3 ± 63.5	94.7 ± 11.6
3a	100 mg/kg	457.7 ± 57.7	83.2 ± 14.4
3b	100 mg/kg	544.5 ± 62.9	102.7 ± 16.2
3c	100 mg/kg	361.5 ± 52.3	71.2 ± 12.3
3d	100 mg/kg	468.7 ± 65.5	65.8 ± 16.3
3e	100 mg/kg	563.7 ± 45.1	94.8 ± 13.5
3f	100 mg/kg	415.2 ± 77.7	78.2 ± 11.6
3g	100 mg/kg	407.0 ± 52.1	73.7 ± 13.1
3h	100 mg/kg	447.5 ± 63.1	84.7 ± 11.0
3i	100 mg/kg	524.8 ± 43.1	89.7 ± 14.0
3j	100 mg/kg	378.0 ± 49.9	74.8 ± 16.0
3k	100 mg/kg	551.5 ± 54.1	87.5 ± 21.2
3l	100 mg/kg	366.7 ± 70.1	70.8 ± 12.1
3m	100 mg/kg	392.8 ± 54.3	67.0 ± 14.3
3n	100 mg/kg	414.2 ± 44.0	94.5 ± 11.8
3o	100 mg/kg	494.7 ± 47.9	68.8 ± 10.6

Values are given as mean ± S.E.M. One-way ANOVA, post-hoc Tukey test, n = 6.

the MFST were not changed. Flx exhibited an antidepressant effect, evidenced by decreased immobility and increased swimming times, as expected (Cryan et al., 2002).

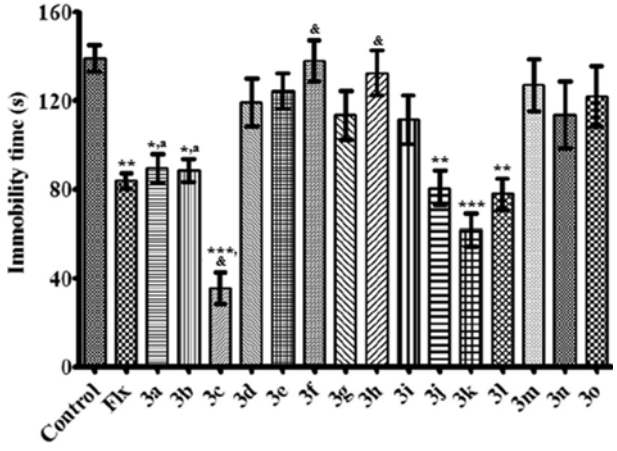


Fig. 1. Effects of the test compounds on immobility time of mice in tail suspension tests. Values are given as mean ± S.E.M. Significance against control values, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$; significance against Flx group, $^{\&}p < 0.05$; significance against 3c group, $^{a}p < 0.05$. One-way ANOVA, post-hoc Tukey test, n = 6.

Figs. 3 and 4 illustrate the effects of the test compounds on the reaction times of mice to noxious stimuli in hot-plate and tail-clip tests, respectively. Significant increases in reaction times were observed following administration of compounds **3a**, **3b**, **3c**, **3d**, **3e**, **3j**, **3k**, and **3l** at 100 mg/kg doses. Morphine sulphate (10 mg/kg) also increased reaction times in both tests, as expected.

Tested compounds exhibited negligible toxicity; they incurred neither deaths nor undesirable side effects such as ataxia, paralysis, convulsions, and diarrhea when administered at dose of 100 mg/kg.

DISCUSSION

We synthesized thiadiazole derivatives bearing hydrazone moieties and investigated their effects on behavioral and nociceptive parameters in mice. Putative effects of the test compounds on exploratory behavior and spontaneous locomotor activity were examined by hole-board and activity cage tests, respectively. MFST and tail suspension tests were used for assessments of depression parameters. Potential antinociceptive effects of the compounds were evaluated using the hot-plate and tail-clip methods.

As illustrated in Table II, tested compounds did not cause any significant changes in the exploratory parameters of mice in hole-board tests. These results indicated that neither anxiolytic nor sedative effects were induced by these derivatives when administered at a dose of 100 mg/kg. The results obtained from the hole-board tests were recapitulated by the findings of the activity cage tests. In activity cage tests, administra-

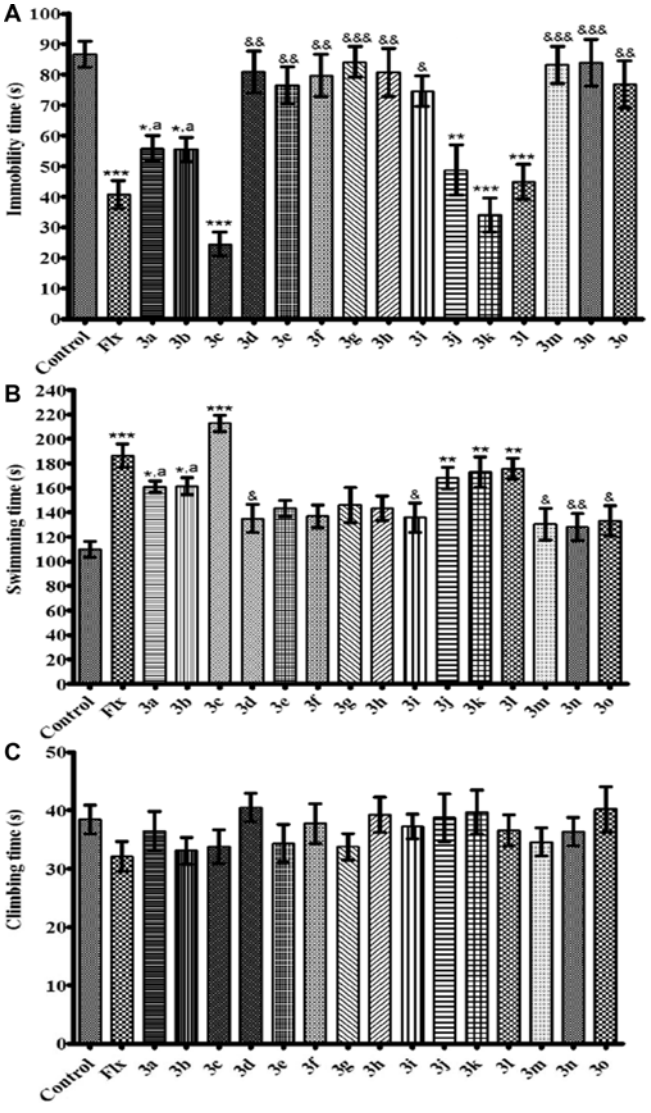


Fig. 2. Effects of the test compounds on (**A**) immobility, (**B**) swimming, and (**C**) climbing time of mice in MFST. Values are given as mean ± S.E.M. Significance against control values, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$; significance against Flx group, $^{\&}p < 0.05$, $^{\&\&}p < 0.01$, $^{\&\&\&}p < 0.001$, significance against 3c group, $^{a}p < 0.05$. One-way ANOVA, post-hoc Tukey test, n = 6.

tion of the test compounds did not alter the total numbers of the horizontal or vertical locomotor activities (Table III). Collectively, these data indicate that the behavioral experimental results in this study were not attributable to motor alterations caused by the test compounds.

Tail suspension tests showed that compounds **3a**, **3b**, **3c**, **3j**, **3k**, and **3l** significantly shortened immobility times of animals and showed antidepressant-like activities (Fig. 1). MFST results confirmed these findings and provided additional information related to a possible mechanism of activity. Decreased immobility

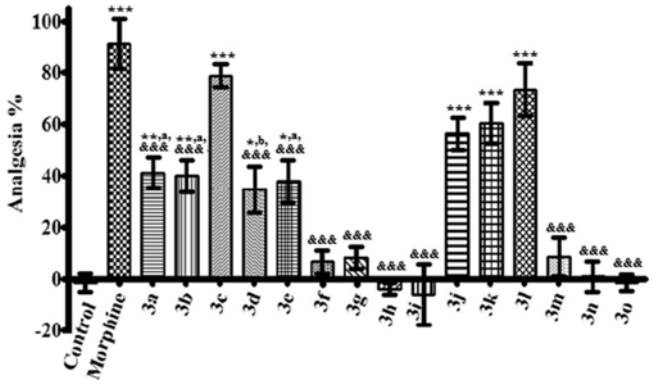


Fig. 3. Effects of the test compounds on response latency of mice in hot-plate test. Values are given as mean ± S.E.M. Significance against control values, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$; significance against morphine group $^{\&\&\&}p < 0.001$; significance against 3c group, $^{a}p < 0.05$, $^{b}p < 0.01$. One-way ANOVA, post-hoc Tukey test, n = 6.

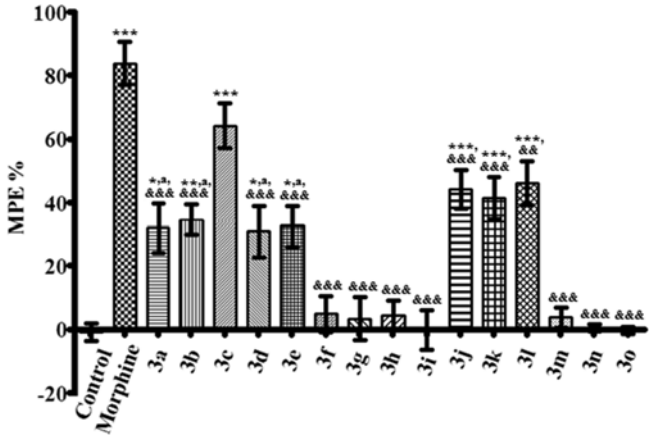


Fig. 4. Effects of the test compounds on response latency of mice in tail-clip test. Values are given as mean ± S.E.M. Significance against control values, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$; significance against morphine group $^{\&\&}p < 0.01$, $^{\&\&\&}p < 0.001$; significance against 3c group, $^{a}p < 0.05$. One-way ANOVA, post-hoc Tukey test, n = 6.

and increased swimming times without changes in the climbing durations indicated that the antidepressant-like effects exhibited by **3a**, **3b**, **3c**, **3j**, **3k**, and **3l** in this study (Fig. 2) may be related to serotonergic, rather than noradrenergic mechanisms in the CNS (Cryan et al., 2002). Involvement of the serotonergic system in the antidepressant activity should be confirmed with further studies, such as depleting neuronal serotonin by p-chlorophenylalanine pretreatment or measuring serotonin levels in limbic areas of the brain.

Significant increases in reaction times of animals against noxious stimuli after administration of compounds **3a**, **3b**, **3c**, **3d**, **3e**, **3j**, **3k**, and **3l** indicated the presence of antinociceptive activities in hot-plate (Fig. 3) and tail-clip tests (Fig. 4). Hot-plate and tail-clip

tests have been reported as a measure centrally mediated, transient pain. As reported previously, the hot-plate test predominantly measures responses organized supraspinally, whereas the tail-clip test mainly measures spinal reflexes (Wong et al., 1994; Gabra and Sirois, 2003). The tested compounds prolonged the response times of animals in both hot-plate and tail-clip tests. We therefore suggest that their antinociceptive activities are related to both supraspinal and spinal mechanisms.

In the present study, 100 mg/kg doses of compounds **3a**, **3b**, **3c**, **3j**, **3k**, and **3l** showed both antidepressant and centrally mediated antinociceptive activities. Antidepressants that increase monoamine levels in CNS synaptic clefts activate descending inhibitory nociceptive pathways and thus show analgesic activity (Korzeniewska-Rybicka and Plaznik, 2000). We may speculate that compounds **3a**, **3b**, **3c**, **3j**, **3k**, and **3l** exert both antidepressant and antinociceptive effects by activating the monoaminergic system in the CNS. This hypothesis is supported by the results of the MFST, which suggested that test compounds activate central serotonergic mechanisms. However, the exact mecha- nism underlying the test compound activity must be clarified with further detailed studies.

In contrast to compounds **3a**, **3b**, **3c**, **3j**, **3k**, and **3l,** compounds **3d** and **3e** did not exhibit significant effects in the depression tests. On the other hand, these two compounds increased the reaction times in hot-plate and tail-clip tests and exhibited significant antinociceptive activities, which were related to both supraspinal and spinal mechanisms. Their mechanisms of action in the CNS seems to be different from those of compounds **3a**, **3b**, **3c**, **3j**, **3k**, and **3l**, which showed both antidepressant and antinociceptive activities in the present study. The exact mode of action for test compounds **3d** and **3e** should be clarified with further detailed investigations.

Among these pharmacologically active compounds, **3j** is substituted by 1H-indol-3-yl, **3k** by 1-naphthyl, and **3l** by biphenyl-4-yl. Additionally, compounds **3a**, **3b**, and **3c** are 2-, 3-, and 4-pyridyl group substituted derivatives, respectively. The results of the depression tests showed that substitution by 1H-indol-3-yl, 1-naphthyl, biphenyl-4-yl, or pyridyl groups adds an antidepressant activity potential to the tested compounds. Among pyridyl substituted derivatives, the 4-pyridyl group substituted derivative **3c** was found to be statistically more active than **3a** and **3b**. These results suggest that substitution with a 4-pyridyl group, but not 2 or 3-pyridyl, increased the antidepressant activity of the compounds.

Another interesting structure-antidepressant activity relationship was observed in compounds **3j** and **3o**. Compound **3j**, having a 1H-indol-3-yl group substitution only, exhibited significant antidepressant-like effects, whereas compound **3o**, with a methyl substitution in addition to a 1H-indol-3-yl group, was ineffective. We concluded that substitution of the hydrazone carbon with an additional methyl group caused a loss of antidepressant activity.

Structure-antinociceptive activity relationships were similar to the structure-antidepressant activity relationships observed for compounds **3a**, **3b**, **3c**, **3j**, **3k**, and **3l**. In terms of the antinociceptive effect, compound **3c** was statistically more active than **3a** and **3b**. Compounds **3d** and **3e**, which were substituted by 2-furyl and thiophen-2-yl groups, respectively, also showed antinociception effects. This finding indicated that substitution by furyl and thiophen-2-yl groups did not add to the strength of antidepressant activity, but did add an antinociceptive potential. For compounds **3m** and **3n**, which are further methyl substituted derivatives of compounds **3d** and **3e** respectively, lack of an antinociceptive effect indicated that methyl substitution abolished antinociceptive activity. These results illustrated once more the importance of substitutions on chemical structure for pharmacological activity.

In summary, the results of the present study provide scientific evidence of the antidepressant and antinociceptive activity potential of thiadiazole derivatives with hydrazone moieties, supporting our initial hypothesis. Furthermore, our results suggest that the active test compounds show serotonergic system-related antidepressant activities and centrally mediated antinociceptive activities. To the best of our knowledge, this is the first study of the CNS activities of chemical compounds carrying both thiadiazole and hydrazone moieties on their structures.

Antidepressants with antinociceptive effects are currently used to treat various neuropathic pain syndromes (Watson et al., 2011; Dharmshaktu et al., 2012). Therefore, our results may have implications for the synthesis and development of new drugs targeted to treat chronic neuropathic pain.

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