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SYNTHESIS AND ANTICONVULSANT EVALUATION OF SOME NOVEL 2,5-DISUBSTITUTED 1,3,4-THIADIAZOLES: PHARMACOPHORE MODEL STUDIES

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Abstract: A novel series of N^1-{5-[(1*H*-indol-3-ylmethyl)-1,3,4-thiadiazol-2-yl}-N^4-(4-substituted benzaldehyde)-semicarbazones, N^1-{5-[(1*H*-indol-3-ylmethyl)-1,3,4-thiadiazol-2-yl}-N^4-[1-(4-substituted phenyl)ethanone]-semicarbazones and N^1-{5-[(1H-indol-3-ylmethyl)-1,3,4-thiadiazol-2-yl}-N^4-[1-(4-substituted phenyl)(phenyl) methanone]-semicarbazones were synthesized and evaluated for their anticonvulsant potential using maximal electroshock seizure (MES) and subcutaneous pentylenetrtrazole (*sc*PTZ) models. The minimal motor impairment (neurotoxicity) was determined by rotorod test. The results of the present study confirmed the requirements of various structural features of four binding site pharmacophore model for anticonvulsant activity.

Keywords: semicarbazones, 1,3,4-thiadiazoles, anticonvulsant activity

Epilepsy is a common neurological disorder characterized by recurrent unprovoked seizures. Approximately 60 million people worldwide suffer from epilepsy, making this condition the second leading neurological disorder (1). Epilepsy also affects about 4% of individuals over their lifetime. The most commonly used anticonvulsant drugs are associated with numerous side effects including ataxia, hepatotoxicity and megaloblastic anemia. Approximately 30% of the epileptic patients being treated with existing anticonvulsant drugs do not have complete control of their seizures and necessitate medication for the duration of their whole life (2). Thus there is a vast requirement for the development of more effective and safer antiepileptic drugs.

In the recent years, numerous reports have established the aryl semicarbazones as structurally novel class of compounds with anticonvulsant activity (3–5). On the other hand, several investigations revealed that 1,3,4-thiadiazole analogues possess considerable anticonvulsant activity (6–8). The search for better anticonvulsant drug and the importance of semicarbazones and 2,5-disubstituted 1,3,4-thiadiazoles as anticonvulsant pharmacophore encouraged us to carry out the synthesis of title compounds.

The conformational investigation of the existing anticonvulsant drugs such as phenytoin, carbamazepine, lamotrigine, rufinamide and phenobarbital has lead to the proposal of a general model for anticonvulsant activity (9, 10). This semicarbazones based pharmacophore model comprises of following four vital binding sites: (i) An aryl hydrophobic binding site (A) with halo substituent preferably at *para* position; (ii) A hydrogen bonding domain (HBD); (iii) An electron donor group (D) and (iv) Another hydrophobic-hydrophilic site controlling the pharmacokinetic properties of the anticonvulsant (C) (Fig. 1). In earlier studies on pharmacophoric model, our research group has reported a number of semicarbazones possessing considerable anticonvulsant activity (11).

EXPERIMENTAL

Chemistry

All the chemicals and solvents used in this study were purchased from E. Merck (Darmstadt,

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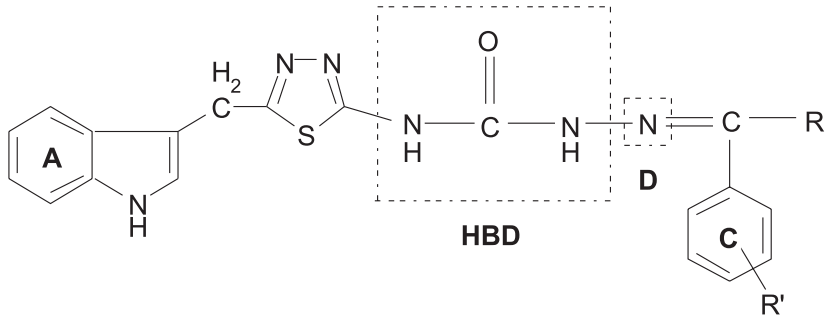


Figure 1. Pharmacophoric structural features in title compounds: (A) hydrophobic aryl ring system, (HBD) hydrogen binding domain, (C) distal aryl ring, (D) electron donor moiety

Germany), Aldrich (Steinheim, Germany) and Himedia (Mumbai, India). Melting points were determined by open capillary method and are uncorrected. Elemental analysis was done using an elemental analyzer Heraeus Carlo Erba-1108, the IR spectra were recorded on a Perkin Elmer IR spectrophotometer (KBr discs) (Perkin Elmer, Beaconsfield, UK); the ^{1}H-NMR at 300 MHz and ^{13}C-NMR at 75 MHz spectra on a Bruker DRX-300 NMR spectrometer (DMSO-d_6, TMS) (Bruker Bioscience, Billerica, MA, USA) and the electrospray mass spectra on a Micromass Quattro II triple-quadrupole mass spectrometer (methanol) (Micromass, Manchester, UK).

The title compounds were synthesized using the synthetic strategy described in Scheme 1. 1*H*-indol-3-ylacetic acid **I**, 3-[2-(hyrazinooxy)-2-oxoethyl]-1*H*-indole **II**, 2-[(1*H*-indol-3-ylacetyl) oxy]hyrazinecarbothioamide **III** and 5-(1*H*-indol-3-ylmethyl)-2-amino-1,3,4-thiadiazole **IV** were prepared according to the reported methods (12, 13). Compound **IV** was treated with sodium cyanate in the presence of glacial acetic acid, to yield 1-[5-(1*H*-indol-3-ylmethyl)-1,3,4-thiadiazol-2-yl]-urea **V**. N-[5-(1*H*-indol-3-ylmethyl)-1,3,4-thiadiazol-2-yl]-hydrazinecarboxamide **VI** was prepared by reaction of compound **V** with hydrazine hydrate in the presence of sodium hydroxide. Title compounds **1-14** were prepared by reaction of the appropriate aldehyde or ketone with compound **VI**.

1-[5-(1H-indol-3-ylmethyl)-1,3,4-thiadiazol-2-yl]-urea V

Compound **IV** (0.01 mol, 2.30 g) was dissolved in 10–30 mL of glacial acetic acid and diluted to 50 mL with distilled water. The mixture was warmed for 3–5 min to dissolve **IV**. To this, sodium cyanate (0.01 mol, 0.65 g) in 20–30 mL of warm water was added with constant stirring. The reaction mixture was allowed to stand several hours followed by cooling on an ice bath. The precipitates obtained were collected by filtration, washed with cold water and recrystallized from 90% aqueous ethanol. **V**: yield 63%, m.p. 166–167^{o}C. IR (KBr, cm^{-1}): 3420 (sec. NH), 3319 (amide NH), 3087 (arom. C-H), 1686 (C=O str of amide), 1644 (C=N of thiadiazole), 738 (C-S of thiadiazole nucleus).

N-[5-(1H-indol-3-ylmethyl)-1,3,4-thiadiazol-2-yl]-hydrazinecarboxamide VI

1-[5-(1H-indol-3-ylmethyl)-1,3,4-thiadiazol-2-yl]-urea **V** (0.01 mol, 2.73 g) was dissolved in 30–40 mL of ethanol. To this was added solution of hydrazine hydrate (0.01 mol, 0.5 mL) in 5 mL of water. The reaction mixture was made alkaline by adding 4 g of NaOH pellets. The content was then heated to reflux for 2–8 h, followed by cooling on an ice bath. The product was filtered and recrystallized from 90% aqueous ethanol. **VI**: yield 60%, m.p. 188–189^{o}C. IR (KBr, cm^{-1}): 3419 (sec. NH), 3319 (amide NH), 3055 (arom. C-H), 1680 (C=O str. of amide), 1640 (C=N of thiadiazole), 740 (C-S of thiadiazole nucleus).

General procedure for synthesis of N^{1}-{5-[(1*H*-indol-3-ylmethyl)-1,3,4-thiadiazol-2-yl}-N^{4}-(4-substitutedbenzaldehyde)-semicarbazones 1-6, N^{1}-{5-[(1*H*-indol-3-ylmethyl)-1,3,4-thiadiazol-2-yl}-N^{4}-[1-(4-substitutedphenyl)ethanone]-semicarbazones 7-10 and N^{1}-{5-[(1*H*-indol-3-ylmethyl)-1,3,4-thiadiazol-2-yl}-N^{4}-[1-(4-substitutedphenyl) (phenyl) methanone]-semicarbazones 11-14

Compound **VI** (0.01 mol, 2.88 g) and appropriate carbonyl compound (0.01 mol) were dissolved in 20–30 mL of ethanol. To this, 5 mL of water was added. The turbidity, if appeared, was removed by adding ethanol with adequate stirring of the reaction

mixture. The pH of the reaction mixture was adjusted between 4 and 5, by adding glacial acetic acid. The reaction mixture was refluxed for 2–3 h with occasional stirring. Thereafter, the reaction mixture was cooled on an ice bath and the crystallized product was filtered under vacuum. The crude product was recrystallized from 90% aqueous ethanol.

N^1-{5-[(1H-indol-3-ylmethyl)-1,3,4-thiadiazol-2-yl}-N^4-(4-benzaldehyde)-semicarbazone **1**

Yield 56%, m.p. 154–156°C. IR (KBr, cm^{-1}): 3438, 3225, 3052, 1683, 1640, 1617, 1603, 1502, 741. ^{13}C-NMR (DMSO-d_6, δ, ppm): 29.4 (<u>C</u>H_2 connecting indole and thiadiazole rings), 111.3 (C-6'), 112.4 (C-3'), 119.7 (C-7'), 120.7 (C-9'), 121.5 (C-8'), 122.7 (C-2'), 128.6 (C-3'' and C-5''), 129.1 (C-2'' and C-6''), 130.8 (C-4''), 131.1 (C-1''), 131.2 (C-4'), 136.3 (C-5'), 154.7 (NHCONHN<u>C</u>H), 156.7 (C-5), 157.3 (NH<u>C</u>ONHNCH), 163.8 (C-2). ^{1}H-NMR (DMSO-d_6, δ, ppm): 3.7 (s, 2H, CH_2), 6.3 (s, 1H, N<u>H</u>CONH), 6.8 (H-2' of indole), 6.8 (s, 1H, imine H), 7.0–7.5 (m, 4H, indole protons), 7.2–7.6 (m, 5H, ArH), 9.4 (s, 1H, NHCON<u>H</u>), 10.1 (d, 1H, NH of indole). ESI-MS: *m*/*z* 377.8 ($[M+H]^+$).

N^1-{5-[(1H-indol-3-ylmethyl)-1,3,4-thiadiazol-2-yl}-N^4-(4-nitrobenzaldehyde)-semicarbazone **2**

Yield 66%, m.p. 189–191°C. IR (KBr, cm^{-1}): 3429, 3222, 3038, 1682, 1640, 1617, 1602, 1521, 1502, 1353, 741. ^{13}C-NMR (DMSO-d_6, δ, ppm): 29.3 (<u>C</u>H_2 connecting indole and thiadiazole rings), 111.2 (C-6'), 112.3 (C-3'), 119.8 (C-7'), 120.6 (C-9'), 121.7 (C-8'), 122.8 (C-2'), 123.6 (C-3'' & C-5''), 129.9 (C-2'' & C-6''), 131.5 (C-4'), 136.4 (C-5') 137.2 (C-1''), 150.9 (C-4''), 154.6 (NHCONHN<u>C</u>H), 156.5 (C-5), 157.4 (NH<u>C</u>ONHNCH), 163.9 (C-2). ^{1}H-NMR (DMSO-d_6, δ ppm): 3.7 (s, 2H, CH_2); 6.2 (s, 1H, N<u>H</u>CONH), 6.7 (s, 1H, imine H), 6.8 (H-2' of indole), 7.0–7.5 (m, 4H, indole protons), 7.8–8.3 (m, 4H, ArH), 9.3 (s, 1H, NHCON<u>H</u>), 10.1 (d, 1H, NH of indole). ESI-MS: *m*/*z* 422.5 ($[M+H]^+$).

N^1-{5-[(1H-indol-3-ylmethyl)-1,3,4-thiadiazol-2-yl}-N^4-(4-hydroxybenzaldehyde)-semicarbazone **3**

Yield 59%, m.p. 181–182°C. IR (KBr, cm^{-1}): 3462, 3426, 3223; 3038, 1676, 1643, 1617, 1602, 1504, 1165, 824, 741. ^{13}C-NMR (DMSO-d_6, δ, ppm): 29.2 (<u>C</u>H_2 connecting indole and thiadiazole rings), 111.2 (C-6'), 112.1 (C-3'), 115.6 (C-3'' and C-5''), 119.6 (C-7'), 120.7 (C-9'), 121.6 (C-8'), 122.9 (C-2'), 123.7 (C-1''), 130.3 (C-2'' and C-6''), 131.5 (C-4'), 136.4 (C-5'), 154.9 (NHCONHN<u>C</u>H), 156.8 (C-5), 157.2 (NH<u>C</u>ONHNCH), 159.8 (C-4''), 163.7 (C-2). ^{1}H-NMR (DMSO-d_6, δ, ppm): 3.7 (s, 2H, CH_2), 5.2 (ArOH); 6.3 (s, 1H, N<u>H</u>CONH), 6.7 (s, 1H, imine H), 6.8 (H-2' of indole), 6.8–7.4 (m, 4H, ArH), 7.0–7.5 (m, 4H, indole protons), 9.5 (s, 1H, NHCON<u>H</u>), 10.1 (d, 1H, NH of indole). ESI-MS: *m*/*z* 393.6 ($[M+H]^+$).

N^1-{5-[(1H-indol-3-ylmethyl)-1,3,4-thiadiazol-2-yl}-N^4-(4-methylbenzaldehyde)-semicarbazone **4**

Yield 61%, m.p. 160–162°C. IR (KBr, cm^{-1}): 3430, 3220; 3041, 2910, 1671, 1647, 1617, 1604, 1501, 1442, 828, 742. ^{13}C-NMR (DMSO-d_6, δ, ppm): 21.1 (<u>C</u>$H_3C_6H_5$), 29.3 (<u>C</u>H_2 connecting indole and thiadiazole rings); 111.2 (C-6'), 112.2 (C-3'), 119.7 (C-7'), 120.6 (C-9'), 121.6 (C-8'), 122.8 (C-2'), 128.3 (C-1''), 128.9 (C-2'' and C-6''), 129.4 (C-3'' and C-5''), 131.5 (C-4'), 136.4 (C-5'), 140.2 (C-4''), 154.7 (NHCONHN<u>C</u>H) 156.8 (C-5), 157.3 (NH<u>C</u>ONHNCH), 163.7 (C-2). ^{1}H-NMR (DMSO-d_6, δ, ppm): 2.3 ($ArCH_3$); 3.7 (s, 2H, CH_2), 6.4 (s, 1H, N<u>H</u>CONH), 6.8 (H-2' of indole), 6.9 (s, 1H, imine H), 7.0–7.5 (m, 4H, indole protons), 7.1–7.5 (m, 4H, ArH), 9.4 (s, 1H, NHCON<u>H</u>), 10.1 (d, 1H, NH of indole), ESI-MS: *m*/*z* 390.4 ($[M+H]^+$).

N^1-{5-[(1H-indol-3-ylmethyl)-1,3,4-thiadiazol-2-yl}-N^4-(4-methoxybenzaldehyde)-semicarbazone **5**

Yield 63%, m.p. 202–204°C. IR (KBr, cm^{-1}): 3426, 3219, 3043, 1685, 1648, 1619, 1609.9, 1501, 1268, 826, 743. ^{13}C-NMR (DMSO-d_6, δ, ppm): 29.3 (<u>C</u>H_2 connecting indole and thiadiazole rings), 56.1 (O<u>C</u>$H_3C_6H_5$), 111.2 (C-6'), 112.2 (C-3'), 114.3 (C-3'' and C-5''), 119.8 (C-7'), 120.6 (C-9'), 121.6 (C-8'), 122.9 (C-2'), 123.5 (C-1''), 131.5 (C-4'), 136.4 (C-5'), 154.8 (NHCONHN<u>C</u>H), 156.8 (C-5), 157.4 (NH<u>C</u>ONHNCH), 163.6 (C-2), 164.5 (C-4''). ^{1}H-NMR (DMSO-d_6, δ, ppm): 3.7 (s, 2H, CH_2), 3.8 ($ArOCH_3$), 6.4 (s, 1H, N<u>H</u>CONH), 6.8 (H-2' of indole), 6.8–7.5 (m, 4H, ArH), 6.9 (s, 1H, imine H), 7.0–7.5 (m, 4H, indole protons), 9.4 (s, 1H, NHCON<u>H</u>), 10.1 (d, 1H, NH of indole). ESI-MS: *m*/*z* 419.5 ($[M+H]^+$).

N^1-{5-[(1H-indol-3-ylmethyl)-1,3,4-thiadiazol-2-yl}-N^4-(4-chlorobenzaldehyde)-semicarbazone **6**

Yield 60%, m.p. (207–209°C. IR (KBr, cm^{-1}): 3422, 3216, 3033, 2912, 1692, 1637, 1606.4, 1605, 1507, 1441, 825, 749, 717. ^{13}C-NMR (DMSO-d_6, TMS, δ, ppm): 3.7 (s, 2H, CH_2), 29.4 (<u>C</u>H_2 connecting indole and thiadiazole rings); 111.2 (C-6'), 112.3 (C-3'), 119.8 (C-7'), 120.8 (C-9'), 121.6 (C-8'), 122.9 (C-2'), 129.1 (C-3'' & C-5''), 129.2 (C-1''), 131.4 (C-4'), 130.5 (C-2'' and C-6''), 136.2 (C-4''), 136.4 (C-5') 156.6 (C-5), 154.7 (NHCONHN<u>C</u>H), 157.2 (NH<u>C</u>ONHNCH), 163.7

(C-2). ^{1}H-NMR (DMSO-d_6, δ, ppm): 6.3 (s, 1H, NHCONH), 6.8 (s, 1H, imine H), 6.8 (H-2' of indole), 7.0–7.5 (m, 4H, indole protons), 7.3–7.6 (m, 4H, ArH), 9.4 (s, 1H, NHCONH), 10.1 (d, 1H, NH of indole). ESI-MS: *m/z* 411.9 ([M+H]$^+$).

N^1-{5-[(1H-indol-3-ylmethyl)-1,3,4-thiadiazol-2-yl}-N^4-[1-(4-hydroxyphenyl)ethanone]-semicarbazone **7**

Yield 64%, m.p. 215–216^{o}C. IR (KBr, cm^{-1}): 3447, 3423, 3219, 3042, 1681, 1640, 1605.2, 1604, 1504, 1144, 827, 744. ^{13}C-NMR (DMSO-d_6, δ, ppm): 17.4 (NHCONHNC$\underline{C}H_3$), 29.3 ($\underline{C}H_2$ connecting indole and thiadiazole rings); 111.1 (C-6'), 112.2 (C-3'), 115.7 (C-3'' and C-5''), 119.6 (C-7'), 120.6 (C-9'), 121.6 (C-8'), 122.7 (C-2'), 123.8 (C-1''), 130.4 (C-2'' and C-6''), 131.5 (C-4'), 136.5 (C-5'), 155.8 (NHCONHN$\underline{C}CH_3$), 156.7 (C-5), 157.2 (NH$\underline{C}$ONHNCCH$_3$), 159.8 (C-4''), 163.8 (C-2). ^{1}H-NMR (DMSO-d_6, δ, ppm): 1.1 (s, 3H, carbimino CH_3), 3.7 (s, 2H, CH_2), 6.3 (s, 1H, N$\underline{H}$CONH), 6.8 (H-2' of indole), 6.8–7.4 (m, 4H, ArH), 7.0–7.5 (m, 4H, indole protons), 9.4 (s, 1H, NHCON$\underline{H}$), 10.1 (d, 1H, NH of indole). ESI-MS: *m/z* 407.5 ([M+H]$^+$).

N^1-{5-[(1H-indol-3-ylmethyl)-1,3,4-thiadiazol-2-yl}-N^4-[1-(4-methoxyphenyl)ethanone]-semicarbazone **8**

Yield 65%, m.p. 167–169ş C. IR (KBr, cm^{-1}): 3433, 3218; 3042, 2915, 1681, 1643, 1620, 1603.7, 1502, 1442, 1273, 820, 740. ^{13}C-NMR (DMSO-d_6, δ, ppm): 17.3 (NHCONHNC$\underline{C}H_3$), 29.4 ($\underline{C}H_2$ connecting indole and thiadiazole rings), 56.2 (O$\underline{C}H_3C_6H_5$), 111.3 (C-6'), 112.4 (C-3'), 114.3 (C-3'' and C-5''), 119.7 (C-7'), 120.7 (C-9'), 121.6 (C-8'), 122.8 (C-2'), 123.6 (C-1''); 130.1 (C-2'' and C-6''), 131.6 (C-4'), 136.7 (C-5'), 155.9 (NHCONHN$\underline{C}CH_3$), 156.7 (C-5), 157.3 (NH$\underline{C}$ONHNCCH$_3$), 163.6 (C-2), 164.4 (C-4''), ^{1}H-NMR (DMSO-d_6, δ, ppm): 1.1 (s, 3H, carbimino CH_3), 3.7 (s, 2H, CH_2), 3.8 (ArOCH_3), 6.4 (s, 1H, N$\underline{H}$CONH), 6.8 (H-2' of indole), 6.8–7.5 (m, 4H, ArH), 7.0–7.5 (m, 4H, indole protons), 9.3 (s, 1H, NHCON$\underline{H}$), 10.1 (d, 1H, NH of indole). ESI-MS: *m/z* 433.5 ([M+H]$^+$).

N^1-{5-[(1H-indol-3-ylmethyl)-1,3,4-thiadiazol-2-yl}-N^4-[1-(4-nitrophenyl)ethanone]-semicarbazone **9**

Yield 53%, m.p. 228–230^{o}C. IR (KBr, cm^{-1}): 3431, 3220; 3043, 2913, 1684, 1641, 1618, 1603.2, 1533.2, 1503, 1432, 1357, 829, 741. ^{13}C-NMR (DMSO-d_6, δ, ppm): 17.4 (NHCONHNC$\underline{C}H_3$), 29.2 ($\underline{C}H_2$ connecting indole and thiadiazole rings), 111.3 (C-6'), 112.3 (C-3'), 119.8 (C-7'), 120.7 (C-9'), 121.7 (C-8'), 122.8 (C-2'), 123.8 (C-3'' and C-5''), 129.8 (C-2'' and C-6''), 131.6 (C-4'), 136.7 (C-5') 137.5 (C-1''), 150.9 (C-4''), 155.7 (NHCONHN$\underline{C}CH_3$), 156.8 (C-5), 157.3 (NH$\underline{C}$ONHNCCH$_3$), 163.7 (C-2). ^{1}H-NMR (DMSO-d_6, δ, ppm): 1.1 (s, 3H, carbimino CH_3); 3.7 (s, 2H, CH_2), 6.3 (s, 1H, N$\underline{H}$CONH), 6.8 (H-2' of indole), 7.0–7.5 (m, 4H, indolic protons), 7.9–8.2 (m, 4H, ArH), 9.4 (s, 1H, NHCON$\underline{H}$), 10.1 (d, 1H, NH of indole). ESI-MS: *m/z* 436.5 ([M+H]$^+$).

N^1-{5-[(1H-indol-3-ylmethyl)-1,3,4-thiadiazol-2-yl}-N^4-[1-(4-chlorophenyl)ethanone]-semicarbazone **10**

Yield 60%, m.p. 176–178^{o}C. IR (KBr, cm^{-1}): 3422, 3218; 3041, 2910, 1682, 1648, 1620, 1605, 1503, 1441, 822, 745, 720. ^{13}C-NMR (DMSO-d_6, δ, ppm): 17.3 (NHCONHNC$\underline{C}H_3$), 29.4 ($\underline{C}H_2$ connecting indole and thiadiazole rings), 111.3 (C-6'), 112.2 (C-3'), 119.7 (C-7'), 120.8 (C-9'), 121.8 (C-8'), 122.9 (C-2'), 129.2 (C-3'' and C-5''), 129.4 (C-1''), 130.5 (C-2'' and C-6''), 131.6 (C-4'), 136.2 (C-4''), 136.7 (C-5'), 155.8 (NHCONHN$\underline{C}CH_3$), 156.8 (C-5), 157.3 (NH$\underline{C}$ONHNCCH$_3$), 163.7 (C-2). ^{1}H-NMR (DMSO-d_6, δ, ppm): 1.1 (s, 3H, carbimino CH_3), 3.7 (s, 2H, CH_2), 6.3 (s, 1H, N$\underline{H}$CONH), 6.8 (H-2' of indole), 7.0–7.5 (m, 4H, indole protons), 7.3–7.6 (m, 4H, ArH), 9.4 (s, 1H, NHCON$\underline{H}$), 10.1 (d, 1H, NH of indole). ESI-MS: *m/z* 425.8 ([M+H]$^+$).

N^1-{5-[(1H-indol-3-ylmethyl)-1,3,4-thiadiazol-2-yl}-N^4-[1-(diphenyl) methanone]-semicarbazone **11**

Yield 59%, m.p. 256–257^{o}C. IR (KBr, cm^{-1}): 3428, 3218; 3036, 1671, 1644, 1626, 1605, 1503, 766, 742, 708.4. ^{13}C-NMR (DMSO-d_6, δ, ppm): 29.3 ($\underline{C}H_2$ connecting indole and thiadiazole rings), 111.3 (C-6'), 112.3 (C-3'), 119.6 (C-7'), 120.5 (C-9'), 121.9 (C-8'), 122.7 (C-2'), 128.6 (C-3''' and C-5'''), 128.7 (C-3'' and C-5''), 129.1 (C-2''' and C-6'''), 129.1 (C-2'' and C-6''), 130.8 (C-4'''), 130.9 (C-4''), 131.3 (C-1''), 131.4 (C-1'''), 131.7 (C-4'), 136.7 (C-5'), 155.7 (NHCONHN$\underline{C}C_6H_5$), 156.8 (C-5), 157.4 (NH$\underline{C}$ONHNCC$_6$H$_5$), 163.7 (C-2). ^{1}H-NMR (DMSO-d_6, δ, ppm): 3.7 (s, 2H, CH_2); 6.3 (s, 1H, N$\underline{H}$CONH), 6.8 (H-2' of indole), 7.0–7.5 (m, 4H, indole protons), 7.3–7.8 (m, 10H, ArH), 9.3 (s, 1H, NHCON$\underline{H}$), 10.1 (d, 1H, NH of indole). ESI-MS: *m/z* 453.6 ([M+H]$^+$).

N^1-{5-[(1H-indol-3-ylmethyl)-1,3,4-thiadiazol-2-yl}-N^4-[1-(4-hydroxyphenyl) (phenyl) methanone]-semicarbazone **12**

Yield 54%, m.p. 237–239^{o}C. IR (KBr, cm^{-1}): 3473, 3440, 3217; 3044, 1681, 1640, 1617, 1602,

1506, 1157, 822, 765, 745, 709. ^{13}C-NMR (DMSO-d_6, δ, ppm): 29.3 ($\underline{C}H_2$ connecting indole and thiadiazole rings); 111.2 (C-6'), 112.2 (C-3'), 115.8 (C-3'' & C-5''), 119.5 (C-7'), 120.6 (C-9'), 121.7 (C-8'), 122.8 (C-2'), 123.7 (C-1''), 128.6 (C-3''' and C-5'''), 129.1 (C-2''' and C-6'''), 130.4 (C-2'' and C-6''), 130.9 (C-4'''), 131.4 (C-1'''), 131.6 (C-4'), 136.8 (C-5'), 155.8 (NHCONHN$\underline{C}C_6H_5$), 156.9 (C-5), 157.3 (NH$\underline{C}$ONHNCC_6H_5), 159.8 (C-4''), 163.9 (C-2). ^{1}H-NMR (DMSO-d_6, δ, ppm): 3.7 (s, 2H, CH_2), 5.3 (ArOH), 6.3 (s, 1H, N$\underline{H}$CONH), 6.8 (H-2' of indole), 6.8–7.9 (m, 9H, ArH), 7.0–7.5 (m, 4H, indole protons), 9.4 (s, 1H, NHCON$\underline{H}$), 10.1 (d, 1H, NH of indole). ESI-MS: *m*/*z* 469.6 ($[M+H]^+$).

N^1-{5-[(1H-indol-3-ylmethyl)-1,3,4-thiadiazol-2-yl}-N^4-[1-(4-nitrophenyl) (phenyl) methanone]-semicarbazone **13**

Yield 64%, m.p. 172–173°C. IR (KBr, cm^{-1}): 3426, 3220, 3043, 1682, 1646, 1620, 1603, 1521, 1511, 1354, 828, 767, 761, 708. ^{13}C-NMR (DMSO-d_6, δ, ppm): 29.3 ($\underline{C}H_2$ connecting indole and thiadiazole rings); 111.4 (C-6'), 112.3 (C-3'), 119.6 (C-7'), 120.3 (C-9'), 121.9 (C-8'), 122.7 (C-2'), 123.8 (C-3'' and C-5''), 128.7 (C-3''' and C-5'''), 129.1 (C-2''' & C-6'''), 130.1 (C-2'' and C-6''), 130.9 (C-4'''), 131.3 (C-1'''), 131.5 (C-4'), 136.9 (C-5'), 137.4 (C-1''), 150.8 (C-4''), 155.7 (NHCONHN$\underline{C}C_6H_5$), 156.8 (C-5), 157.3 (NH$\underline{C}$ONHNCC_6H_5), 163.7 (C-2). ^{1}H-NMR (DMSO-d_6, δ, ppm): 3.7 (s, 2H, CH_2), 6.3 (s, 1H, N$\underline{H}$CONH), 6.8 (H-2' of indole), 7.0–7.5 (m, 4H, indole protons), 7.5–8.2 (m, 9H, ArH), 9.3 (s, 1H, NHCON$\underline{H}$), 10.1 (d, 1H, NH of indole). ESI-MS: *m*/*z* 498.5 ($[M+H]^+$).

N^1-{5-[(1H-indol-3-ylmethyl)-1,3,4-thiadiazol-2-yl}-N^4-[1-(4-methoxyphenyl) (phenyl) methanone]-semicarbazone **14**

Yield 57%, m.p. 243–245°C. IR (KBr, cm^{-1}): 3429, 3221, 3044, 1689, 1649, 1618, 1609, 1501, 1256, 822, 768, 744, 708. ^{13}C-NMR (DMSO-d_6, δ, ppm): 29.2 ($\underline{C}H_2$ connecting indole and thiadiazole rings); 56.2 (O$\underline{C}H_3C_6H_5$), 111.3 (C-6'), 112.2 (C-3'), 114.3 (C-3'' & C-5''), 119.6 (C-7'), 120.8 (C-9'), 121.9 (C-8'), 122.8 (C-2'), 123.6 (C-1''), 128.6 (C-3''' and C-5'''), 129.1 (C-2''' & C-6'''), 130.2 (C-2'' and C-6''), 130.8 (C-4'''), 131.4 (C-1''') 131.7 (C-4'), 136.5 (C-5') 155.6 (NHCONHN$\underline{C}C_6H_5$), 156.8 (C-5), 157.3 (NH$\underline{C}$ONHNCC_6H_5), 163.7 (C-2), 164.5 (C-4''), ^{1}H-NMR (DMSO-d_6, δ, ppm): 3.7 (s, 2H, CH_2), 3.8 (ArOCH_3), 6.2 (s, 1H, N$\underline{H}$CONH), 6.8 (H-2' of indole), 6.8–7.9 (m, 9H, ArH), 7.0–7.5 (m, 4H, indole protons), 9.4 (s, 1H, NHCON$\underline{H}$), 10.1 (d, 1H, NH of indole). ESI-MS: *m*/*z* 495.6 ($[M+H]^+$).

Pharmacology

The anticonvulsant screening (14, 15) was performed using male albino mice (Swiss, 18–25 g) and rats (Wistar 100–150 g). The anticonvulsant activity of the test compounds were evaluated by two models namely, maximal electroshock seizure (MES) and subcutaneous pentylenetetrazole (*sc*PTZ) models. Phenytoin, carbamazepine and sodium valproate were used as the standard drugs for comparison. The acute neurological toxicity was determined in the rotorod test (16). Procedures employed for evaluation of anticonvulsant activity and neurotoxicity were reviewed and approved by the University Animal Ethical Committee.

The maximal electroshock seizure were elicited with a 60 cycle alternating current of 50 mA intensity delivered for 0.25 s *via* ear clip electrodes. The maximal seizure usually consists of a short period of tonic extension of the hind limbs and a final clonic episode. After 30 min and 4 h of drug administration electroshock was applied *via* corneal electrodes. Disappearance of the hind limb tonic extensor component of convulsion was considered as positive criterion.

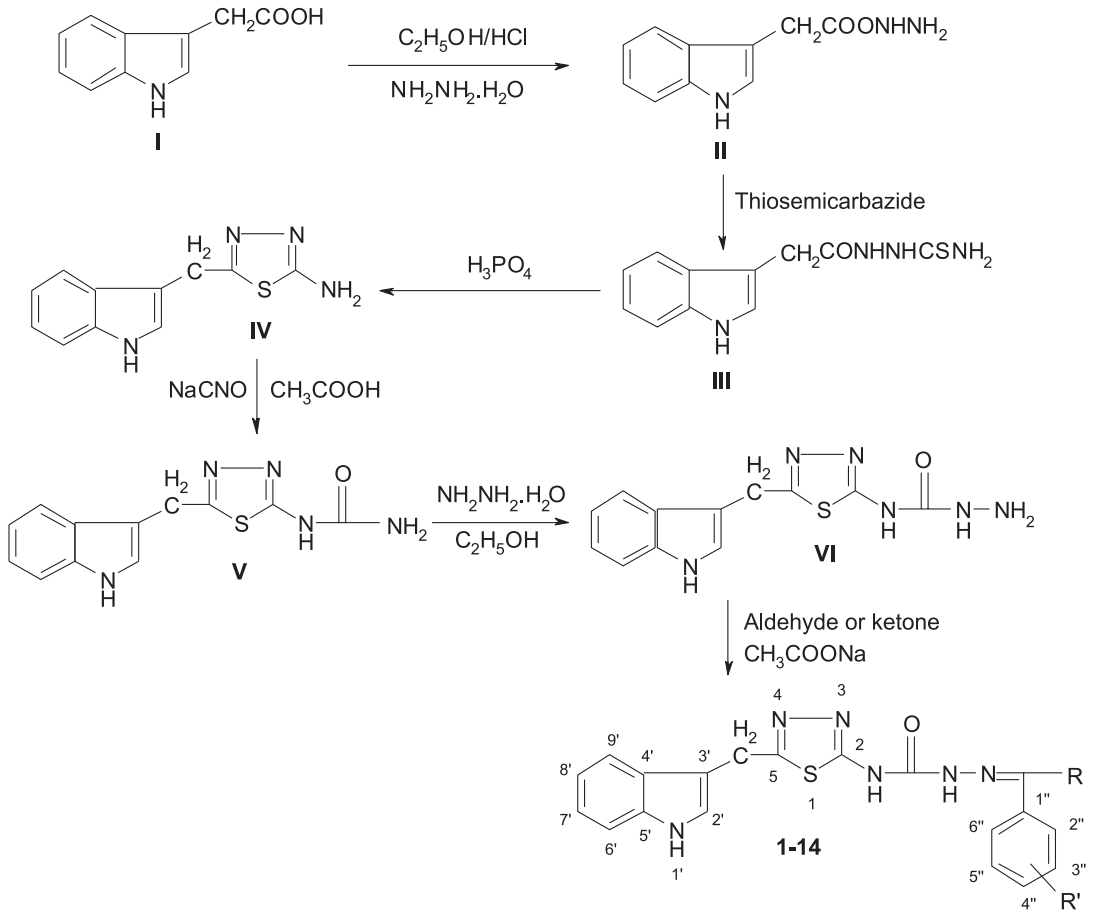
The *sc*PTZ test was performed by administering PTZ dissolved in 0.9% NaCl solution in the posterior midline of the animals. A minimal time of 30 min consequent to administration of PTZ was used for seizure detection. Protection was referred to as the failure to observe an episode of clonic convulsions of at least 5 s duration during this time period.

The rotorod test was conducted to evaluate neurotoxicity. Minimal motor impairment was measured in mice by the rotorod test. The mice were trained to stay on an rotating rotorod of diameter 3.2 cm that rotates at 6 revolutions per min. Only those animals which have demonstrated their capability to remain on the revolving rod for at least 1 min were considered for the test. Previously trained mice were given test compounds *i.p.* in doses of 30, 100 and 300 mg/kg. Thirty minutes after *i.p.* administration the mice were placed on the rotating rod. Neurotoxicity was indicated by the failure of the animal to sustain equilibrium on the rod for at least 1 min in each of three trials.

RESULT AND DISCUSSION

The structures of the compounds were elucidated on the basis of elemental analysis, IR, ^{1}H NMR, ^{13}C NMR and mass spectroscopy. C, H and N determinations were found to be within 0.4% of the calculated values.

All the compounds were screened for their anticonvulsant potential through MES and *sc*PTZ



Compound no.	R	R'	Compound no.	R	R'	Compound no.	R	R'
1	H	H	**6**	H	4-Cl	**11**	C_6H_5	H
2	H	4-NO_2	**7**	CH_3	4-OH	**12**	C_6H_5	4-OH
3	H	4-OH	**8**	CH_3	4-OCH_3	**13**	C_6H_5	4-NO_2
4	H	4-CH_3	**9**	CH_3	4-NO_2	**14**	C_6H_5	4-OCH_3
5	H	4-OCH_3	**10**	CH_3	4-Cl			

Scheme 1. Synthesis of 2,5-disubstituted-1,3,4-thiadiazole derivatives **1–14**

models in doses of 30, 100, 300 mg/kg by intraperitoneal (*i.p.)* injection. The data indicate that 50% of the compounds i.e., **2, 4, 7, 9, 10, 12** and **13** were active in the MES screening as compared to 35% of the compounds i.e., **4, 5, 7, 12** and **14** in the *sc*PTZ test. Thus the compounds exhibited some MES selectivity. In the present studies, N^1-{5-[(1H-indol-3-ylmethyl)-1,3,4-thiadiazol-2-yl}-N^4-[1-(4-hydroxyphenyl) (phenyl) methanone]-semicarbazone **12** come out as the most active compound, showing considerable activity in maximal electroshock seizure (at 100 mg/kg after 0.5 h and at 300 mg/kg after 4.0 h) and subcutaneous pentylenetetrazole model (at 300 mg/kg after 4.0 h) without any neurotoxicity (up to 300 mg/kg after 4.0 h). The majority of the compounds showed activity after 4 h, indicating that the synthesized compounds are slow acting anticonvulsants (Table 1 and 2).

In order to synthesize a diverse range of title compounds, nitro, hydroxy, methyl, methoxy and chloro substitutions around the benzene ring on the right hand side of the molecules were employed. In

Table 1. Anticonvulsant activity and minimal motor impairment of 2,5-disubstituted 1,3,4-thiadiazoles

Compound	Intraperitoneal injection in mice[a]					
	MES screenin		**scPTZ screening**		**NT screening**	
	0.5 h	4.0 h	0.5 h	4.0 h	0.5 h	4.0 h
1	–	–	–	–	100	–
2	–	300	–	–	–	300
3	–	–	–	–	–	300
4	–	300	–	300	–	300
5	–	–	300	–	300	–
6	–	–	–	–	–	–
7	–	300	–	300	–	–
8	–	–	–	–	–	–
9	100	300	–	–	–	300
10	–	300	–	–	–	–
11	–	–	–	–	300	–
12	100	300	–	300	–	–
13	100	300	–	–	–	300
14	–	–	–	300	300	–
Phenytoin	30	30	–	–	100	100
Carbamazepine	30	100	100	300	100	300
Na valproate	300	–	300	–	–	–

[a]30, 100 and 300 mg/kg of doses were administered *i.p.* in mice. The data of the table indicate the minimal dose whereby biological activity was demonstrated in half or more of the mice. The activity was measured after 0.5 and 4.0 h of dose administration of test compounds. The sign – (dash) represents an absence of activity at maximum dose administered (300 mg/kg).

Table 2. Anticonvulsant evaluation of compounds after oral administration in rats

Compound	Oral administration in rats[a]									
	MES screening					NT screening				
	0.25 h	0.5 h	1 h	2 h	4 h	0.25 h	0.5 h	1 h	2 h	4 h
7	0	0	1	0	1	0	0	0	0	1
9	0	0	1	0	1	0	0	0	1	0
12	0	0	0	1	1	0	0	0	0	0
13	0	0	1	0	1	0	0	0	0	1

[a]The compounds were administered in a dose of 30 mg/kg. The data in the Table indicate the number of rats out of four, which were protected.

general, compounds bearing hydroxy and nitro group on distant phenyl ring showed high potency in MES and *sc*PTZ tests, whereas replacement of these groups with methoxy and chloro groups on the distant phenyl ring has resulted in compounds with a decrease in anticonvulsant activity with the exception of compound **8** containing methoxy group and compound **6** containing chloro group as they were devoid of biological activity. It is pertinent to mention here that compounds other than **6** and **8** containing methoxy or chloro group were biologically active. On comparison of results, it has been found that anticonvulsant activity of test compounds changes on varying *p*-substituted group on aryl moiety as follows: hydroxy > nitro > methoxy > chloro > methyl group. It is well known that the hydroxy group is electron withdrawing and methyl group is electron donating group. Thus observed changes in biological activity with change in substitutions in the title compounds might be attributed to the electronegativity effect. Replacement of the proton on the carbimino carbon atom by methyl group i.e., **7** to

10 or phenyl ring i.e., **11** to **14** has demonstrated variation in activity due to an increase in the dimension of the group at this position of the molecule. Compounds with phenyl ring were found to possess considerable activity in comparison to methyl group. The increase in the anticonvulsant activity with phenyl substitution might be due to additional van der Waals bonding to the binding site.

In the present studies, we have designed and synthesized the title compounds with keeping two major facts in mind. Firstly, a number of clinically active anticonvulsants possess a nitrogen hetero atomic system with one or two phenyl rings and at least one carbonyl group in their structure. Secondly, several investigations concluded that at least one aryl group, one or two-electron donor atom and/or an NH group in a special spatial arrangement appears to be vital for anticonvulsant activity (17). Our earlier work confirmed the requirements of these structural features i.e., substituted semicarbazones of lipophilic carbonyl molecules yielded compounds with excellent anticonvulsant activity (11).

The structure of the title compounds fulfilled all the pharmacophoric structural requirements i.e., the presence of [5-(1*H*-indol-3-ylmethyl)-1,3,4-thiadiazol-2-yl] moiety as hydrophobic portion, nitrogen atom as electron donor system and another hydrophobic distal aryl ring responsible for metabolism. The results show that a majority of the compounds exhibited anticonvulsant activity. Thus these results confirmed the four binding site hypothesis for semicarbazones.

CONCLUSION

A few novel series of N^1-{5-[(1*H*-indol-3-ylmethyl)-1,3,4-thiadiazol-2-yl}-N^4-(4-substitutedbenzaldehyde)-semicarbazones, N^1-{5-[(1*H*-indol-3-ylmethyl)-1,3,4-thiadiazol-2-yl}-N^4-[1-(4-substitutedphenyl)ethanone]-semicarbazones and N^1-{5-[(1H-indol-3-ylmethyl)-1,3,4-thiadiazol-2-yl}-N^4-[1-(4-substitutedphenyl) (phenyl) methanone]-semicarbazones were synthesized to meet structural requirement essential for anticonvulsant activity. The results of the present studies confirmed that the pharmacophore model with four binding sites is crucial for anticonvulsant activity. These new details might be valuable in the future development of semicarbazones as novel anticonvulsants.

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