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Research Letter

A Novel Series of 2,5-Disubstituted 1,3,4-oxadiazoles: Synthesis and SAR Study for their Anticonvulsant Activity

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In search for a better anticonvulsant drug and the importance of semicarbazones and 2,5-disubstituted 1,3,4-oxadiazoles as anticonvulsant pharmacophore, a series of novel substituted semicarbazones were designed, synthesized, and evaluated for their anticonvulsant activity. The chemical structures of the synthesized molecules were confirmed by elemental and spectral (IR, ^{1}H NMR, ^{13}C NMR and MS) analysis. The anticonvulsant activities of the compounds were investigated using maximal electroshock seizure and subcutaneous pentylenetetrazole (*sc*PTZ) models. Efforts were also made to establish structure–activity relationships among synthesized compounds. The results of the present study validated that the pharmacophore model with four binding sites is essential for anticonvulsant activity.

Key words: 1,3,4-oxadiazoles, anticonvulsant activity, semicarbazones

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Epilepsy is a chronic neurological disorder characterized by the periodic sudden loss or impairment of consciousness, often followed by convulsions. About 50 million people worldwide have epilepsy, with almost 90% of these people being in developing countries (1). Epilepsy also affects about 4% of individuals over their lifetime. Despite the development of several new anticonvulsants, over 30% of people with epilepsy do not have seizure control and others do so only at the expense of significant dose-related toxicity and peculiar adverse effects (2). Thus, there is an enormous need for the development of more effective and safer antiepileptic drugs.

In the recent years, aryl semicarbazones have emerged as structurally novel class of compounds with anticonvulsant activity (3–5). Also, several investigations have revealed that 1,3,4-oxadiazole analogs possess considerable anticonvulsant activity (6–8).

The conformational investigation into the clinically active anticonvulsant drugs such as phenytoin, carbamazepine, lamotrigine, rufinamide, and phenobarbitone has resulted in the proposal of a general model for anticonvulsant activity (9,10) (Figure 1). This semicarbazone-based pharmacophore model consists of the following four essential binding sites: (i) an aryl hydrophobic binding site (A) with halo substituent, preferably at para position; (ii) a hydrogen binding domain; (iii) an electron donor group (D) and (iv) another hydrophobic–hydrophilic site controlling the pharmacokinetic properties of the anticonvulsant (C) (Figure 2). In earlier pharmacophore model studies, our research group confirmed that the presence of aryl group (preferably halogen substituted) near the semicarbazone moiety is one of the indispensable parameters for anticonvulsant activity (11).

Materials and Methods

All the chemicals and solvents employed in this study were purchased from E-Merck (Darmstadt, Germany), Aldrich (Steinheim, Germany), and Himedia (Mumbai, India). Melting points were determined by open capillary method and are uncorrected. Elemental analysis was performed using an elemental analyzer Heraeus Carlo Erba-1108, IR spectra were recorded on a Perkin Elmer IR spectrophotometer (KBr disc) (Perkin Elmer, Beaconsfield, UK), NMR 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 prepared using the synthetic strategy described in Scheme 1. Methylsalicylate **I** on reaction with benzyl chloride in alkaline hydromethanolic solution resulted in corresponding 2-benzyloxy benzoic acid methyl ester **II** (12). 2-(Benzyloxy)benzohydrazide **III** was synthesized from compound **II** on treatment with hydrazine hydrate in methanol (13). Compound **III** was cyclized to 2-amino-5-(2-benzyloxyphenyl)-1,3,4-oxadiazoles **IV** using cyanogen bromide in methanol in the presence of sodium bicarbonate (14,15). Compound **IV** was treated with sodium cyanate in the presence of glacial acetic acid to yield 1-{5-[2-(benzyloxy)phenyl]-1,3,4-oxadiazol-2-yl}-urea **V**. *N*-{5-[2-(benzyloxy)phenyl]-1,3,4-oxadiazol-

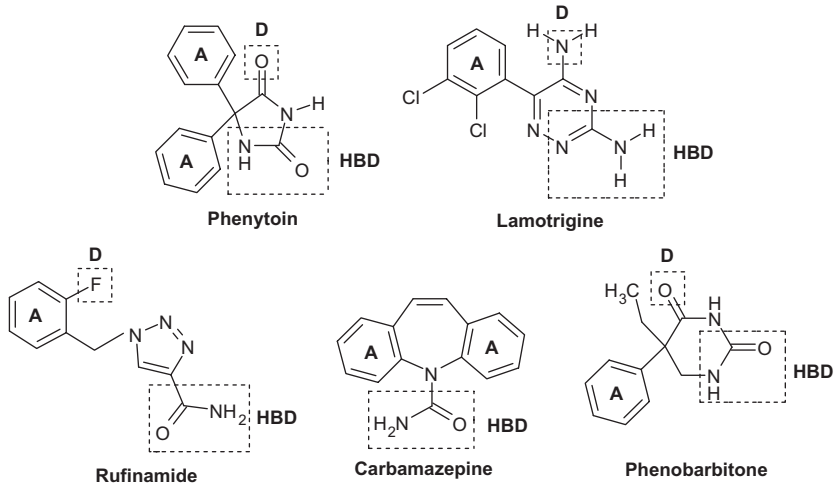


Figure 1: Commonly used anticonvulsant drugs with their vital structural features. (A) Hydrophobic aryl ring system, (HBD) hydrogen binding domain, (D) electron donor moiety.

2-yl}-hydrazine carboxamide **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**.

General procedure for synthesis of 1-{5-[2-(benzyloxy)phenyl)]-1,3,4-oxadiazol-2-yl}-urea V

The appropriate 2-amino-5-(benzyloxy)phenyl-1,3,4-oxadiazoles **IV** (0.01 mol) was dissolved in 10–30 mL of glacial acetic acid diluted to 50 mL with distilled water. To this, equimolar (0.01 mol) quantity of sodium cyanate in 20–30 mL of warm water was added with stirring. The reaction mixture was allowed to stand for 3 h followed by cooling on an ice bath. The precipitates obtained were collected by filtration, washed with cold water, and recrystallized from 90% aqueous ethanol.

Compound V

Yield 58%; MP (°C) 172–173; IR (KBr), 3071.3 (Aromatic C-H), 1091.8 (C-O of oxadiazole nucleus), 1667.2 (C = N of oxadiazole), 3429.5 (secondary NH), 3319.7 (amide NH), 1685.3 (C = O str of amide), 3432.8 (NH str of NH_2); ^{1}H NMR (300 MHz, DMSO-d_6, TMS, δ ppm): 5.3 (s, 2H, OCH_2), 6.8–7.4 (m, 9H, ArH), 6.3 (s, 2H, NH_2), 5.9 (s, 1H, NH); ESMS (Methanol) m/z 310 (M^+).

General procedure for synthesis of N-*{5-[2-(benzyloxy)phenyl)]-1,3,4-oxadiazol-2-yl}-hydrazinecarboxamide VI*

Required quantity of **V** (0.01 mol) was dissolved in 30–40 mL of ethanol. To this was added equimolar solution of hydrazine hydrate in 5 mL of water. The reaction mixture was made alkaline by adding 4 g of sodium hydroxide pellets. The contents were 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.

Compound VI

Yield 66%; MP (°C) 198–199; IR (KBr), 3086.2 (Aromatic C-H), 1094.8 (C-O of oxadiazole nucleus), 1661.6 (C = N of oxadiazole), 3423.1 (secondary NH), 3317.9 (amide NH) and 1682.4 (C = O str of amide), 3431.7 (NH str of NH_2); ^{1}H NMR (300 MHz, DMSO-d_6, TMS, δ ppm): 5.3 (s, 2H, OCH_2), 2.6 (d, 2H, NH_2), 6.2 (t, 1H, N<u>H</u>NH_2), 6.0 (s, 1H, N<u>H</u>CO), 6.8–7.4 (m, 9H, ArH); ESMS (Methanol) m/z 325 (M^+).

General procedure for synthesis of N^1*-{5-[(2-benzyloxy)phenyl]-1,3,4-oxadiazol-2-yl}-*N^4*-(4-substitutedbenzaldehyde)-semicarbazone 1-6,* N^1*-{5-[(2-benzyloxy)phenyl]-1,3,4-oxadiazol-2-yl}-*N^4*-[1-(4-substitutedphenyl)ethanone]-semicarbazone 7–10 and* N^1*-{5-[(2-benzyloxy)phenyl]-1,3,4-oxadiazol-2-yl}-*N^4*-[1-(4-substitutedphenyl) (phenyl) methanone]-semicarbazone 11–14*

Equimolar quantities of **VI** (0.01 mol) and carbonyl compound (0.01 mol) were dissolved in 20–30 mL of ethanol. To this, 5 mL of water was added. Whenever the solution became turbid, the turbidity was removed by adding ethanol. The pH of the reaction mixture was adjusted between 4 and 5, by adding glacial acetic acid. The reaction mixture was refluxed for a period of time ranging from 2 to 3 h. Thereafter, the reaction mixture was cooled on an ice bath, and the crystallized product so obtained was filtered under vacuum. The crude product was recrystallized from 90% aqueous ethanol.

Compound 1

Yield: 56%; MP: 173–175 °C; IR (per cm) (KBr) 3046.3 (Aromatic C-H str), 1602.7 & 1503.9 (Aromatic C-C str), 1090.2 (C-O of 1,3,4-oxadiazole nucleus), 1659.5 (C = N of 1,3,4-oxadiazole nucleus), 1685.2 (C = O str of amide), 3429.5 (N-H str of amide), 1615.8 (C = N group), 822.6 (C-H def disubstituted benzene ring); ^{1}H NMR (300 MHz, DMSO-d_6, TMS, δ ppm): 6.9–7.6 (m, 14H, ArH), 5.3 (OCH_2), 6.2 (s, 1H, N<u>H</u>CONH), 9.5 (s, 1H, NHCON<u>H</u>), 6.8 (s, 1H, imine H); ^{13}C-NMR (75 MHz, DMSO-d_6, TMS, δ ppm): 127.4 (C-2′ & C-6′), 128.8 (C-3′ & C-5′), 127.6 (C-4′), 140.8 (C-1′), 78.4 (OCH_2), 122.3 (C-1″), 160.5 (C-2″), 114.6 (C-3″) 129.5 (C-4″), 121.4 (C-5″), 128.2 (C-6″), 171.3 (C-2), 169.1 (C-5), 157.5 (NH<u>C</u>ONHNCH), 154.8 (NHCONHN<u>C</u>H), 129.2 (C-2‴ & C-6‴), 128.4 (C-3‴ & C-5‴), 130.9 (C-4‴), 131.3 (C-1‴); ESMS (Methanol) m/z 413 (M^+); Anal. Calcd for $C_{23}H_{19}N_5O_3$: C, 66.82; H, 4.63; N, 16.94. Found: C, 67.15; H, 4.73; N, 16.79.

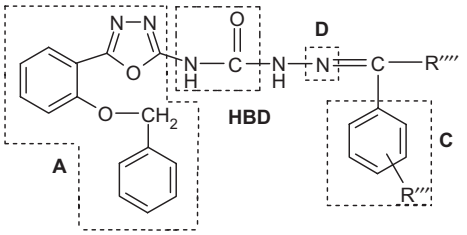


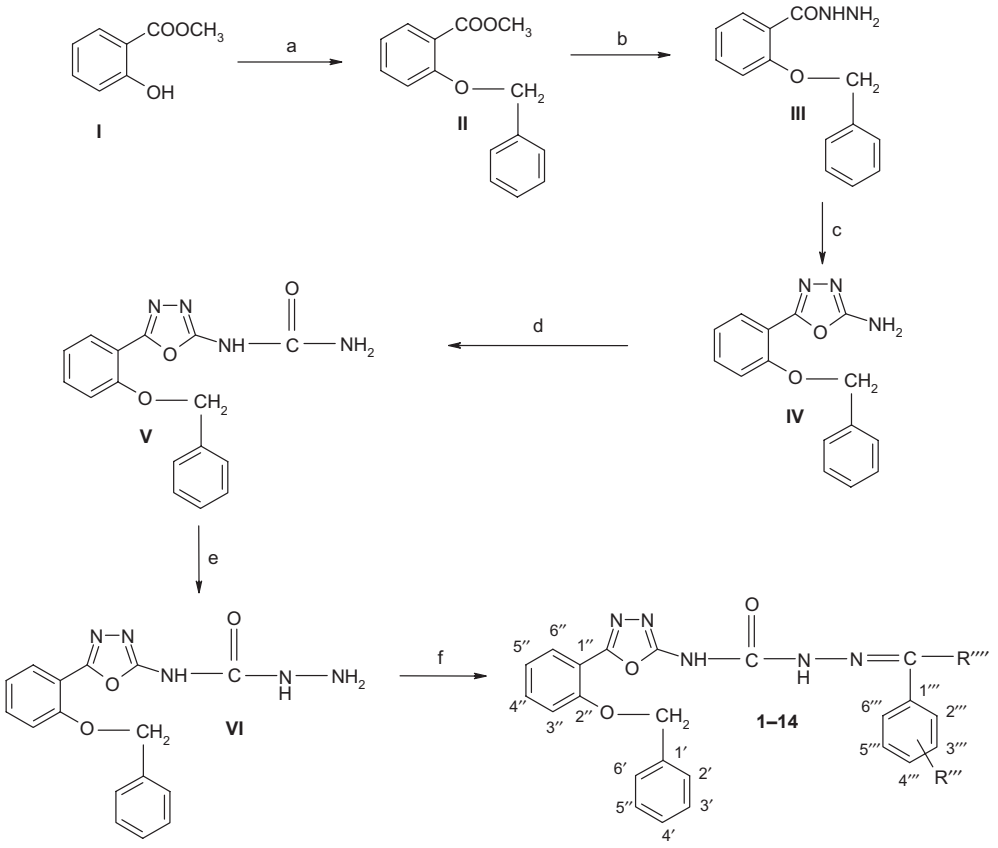
Figure 2: Pharmacophoric structural features in title compounds: (A) hydrophobic aryl ring system, (HBD) hydrogen binding domain, (D) electron donor moiety, (C) distal aryl ring.

Compound 2

Yield: 67%; MP: 180–182 °C; IR (per cm) (KBr) 3043.7 (Aromatic C-H str), 1602.9 & 1505.4 (Aromatic C-C str), 1090.6 (C-O of 1,3,4-oxadiazole nucleus), 1656.3 (C = N of 1,3,4-oxadiazole nucleus), 1682.9 (C = O str of amide), 3428.8 (N-H str of amide), 1607.2 (C = N group), 821.6 (C-H def disubstituted benzene ring), 1521.8 & 1354.5 (N = O str of Ar-NO_2 group); ^{1}H NMR (300 MHz, DMSO-d_6, TMS, δ ppm): 7.0–8.2 (m, 13H, ArH), 5.2 (OCH_2), 6.3 (s, 1H, NHCONH), 9.6 (s, 1H, NHCONH), 6.8 (s, 1H, imine H); ^{13}C-NMR (75 MHz, DMSO-d_6, TMS, δ ppm): 127.3 (C-2′ & C-6′), 128.6 (C-3′ & C-5′), 127.5 (C-4′), 140.7 (C-1′), 78.3 (OCH_2), 122.4 (C-1″), 160.7 (C-2″), 114.5 (C-3″) 129.6 (C-4″), 121.5 (C-5″), 128.2 (C-6″), 171.2 (C-2), 169.0 (C-5), 157.4 (NHCONHNCH), 154.7 (NHCONHNCH), 129.8 (C-2‴ & C-6‴), 123.6 (C-3‴ & C-5‴), 150.9 (C-4‴), 137.3 (C-1‴); ESMS (Methanol) *m/z* 458 (M^+); Anal. Calcd for $C_{23}H_{18}N_6O_5$: C, 60.26; H, 3.96; N, 18.33. Found: C, 60.19; H, 4.02; N, 18.45.

Compound 3

Yield: 56%; MP: 165–166 °C; IR (per cm) (KBr) 3041.4 (Aromatic C-H str), 1601.7 & 1504.9 (Aromatic C-C str), 1091.6 (C-O of



Compound Code	R‴	R⁗	Compound Code	R‴	R⁗	Compound Code	R‴	R⁗
1	H	H	6	4-Cl	H	11	H	C_6H_5
2	4-NO_2	H	7	4-OH	CH_3	12	4-OH	C_6H_5
3	4-OH	H	8	4-OCH_3	CH_3	13	4-NO_2	C_6H_5
4	4-CH_3	H	9	4-NO_2	CH_3	14	4-OCH_3	C_6H_5
5	4-OCH_3	H	10	4-Cl	CH_3			

Reaction conditions: (a) BzCl, KOH 10%; MeOH, rt, 6 h; (b) $NH_2NH_2.H_2O$; MeOH, rt, 5 h; (c) BrCN, $NaHCO_3$; MeOH, rt, 3 h; (d) NaOCN, CH_3COOH, rt, 3 h; (e) $NH_2NH_2.H_2O$, NaOH, reflux 2-8 h; (f) Aldehydes or ketone, CH_3COONa, reflux, 2-3 h.

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

1,3,4-oxadiazole nucleus), 1664.0 (C = N of 1,3,4-oxadiazole nucleus), 1681.7 (C = O str of amide), 3426.9 (N-H str of amide), 1614.5 (C = N group), 821.8 (C-H def disubstituted benzene ring), 3459.1 (O-H str of alcoholic group), 1155.4 (C-O str of alcoholic group); ^{1}H NMR (300 MHz, DMSO-d_6, TMS, δ ppm): 6.9–7.5 (m, 13H, ArH), 5.2 (OCH_2), 6.3 (s, 1H, NHCONH), 9.7 (s, 1H, NHCONH), 6.7 (s, 1H, imine H), 5.2 (ArOH); ^{13}C-NMR (75 MHz, DMSO-d_6, TMS, δ ppm): 127.4 (C-2′ & C-6′), 128.6 (C-3′ & C-5′), 127.5 (C-4′), 140.8 (C-1′), 78.3 (OCH_2), 122.3 (C-1″), 160.8 (C-2″), 114.7 (C-3″) 129.6 (C-4″), 121.6 (C-5″), 128.2 (C-6″), 171.1 (C-2), 169.2 (C-5), 157.2 (NHCONHNCH), 154.6 (NHCONHNCH), 130.6 (C-2‴ & C-6‴), 115.8 (C-3‴ & C-5‴), 150.8 (C-4‴), 123.5 (C-1‴); ESMS (Methanol) *m/z* 429 (M^+); Anal. Calcd for $C_{23}H_{19}N_5O_4$: C, 64.33; H, 4.46; N, 16.31. Found: C, 64.47; H, 4.44; N, 16.48.

Compound 4

Yield: 68%; MP: 171–173 °C; IR (per cm) (KBr) 3042.7 (Aromatic C-H str), 1601.7 & 1508.5 (Aromatic C-C str), 1089.3 (C-O of 1,3,4-oxadiazole nucleus), 1664.7 (C = N of 1,3,4-oxadiazole nucleus), 1675.2 (C = O str of amide), 2908.0 (aliphatic C-H str), 1443.3 (aliphatic C-H def), 3422.7 (N-H str of amide), 1612.8 (C = N group), 819.6 (C-H def disubstituted benzene ring); ^{1}H NMR (300 MHz, DMSO-d_6, TMS, δ ppm): 7.0–7.5 (m, 13H, ArH), 5.2 (OCH_2), 6.3 (s, 1H, NHCONH), 9.8 (s, 1H, NHCONH), 6.9 (s, 1H, imine H), 2.4 ($ArOCH_3$); ^{13}C-NMR (75 MHz, DMSO-d_6, TMS, δ ppm): 127.4 (C-2′ & C-6′), 128.7 (C-3′ & C-5′), 127.6 (C-4′), 140.8 (C-1′), 78.4 (OCH_2), 122.2 (C-1″), 160.6 (C-2″), 114.6 (C-3″) 129.7 (C-4″), 121.5 (C-5″), 128.3 (C-6″), 171.3 (C-2), 169.2 (C-5), 157.3 (NHCONHNCH), 154.9 (NHCONHNCH), 129.9 (C-2‴ & C-6‴), 129.5 (C-3‴ & C-5‴), 140.2 (C-4‴), 128.3 (C-1‴), 21.2 ($CH_3C_6H_5$); ESMS (Methanol) *m/z* 427 (M^+); Anal. Calcd for $C_{24}H_{21}N_5O_3$: C, 67.44; H, 4.95; N, 16.38. Found: C, 67.28; H, 4.85; N, 16.31.

Compound 5

Yield: 58%; MP: 202–204 °C; IR (per cm) (KBr) 3040.5 (Aromatic C-H str), 1602.6 & 1507.4 (Aromatic C-C str), 1094.2 (C-O of 1,3,4-oxadiazole nucleus), 1665.7 (C = N of 1,3,4-oxadiazole nucleus), 1681.2 (C = O str of amide), 3421.5 (N-H str of amide), 1613.7 (C = N group), 827.5 (C-H def disubstituted benzene ring), 1256.3 (C-O of OCH_3 group); ^{1}H NMR (300 MHz, DMSO-d_6, TMS, δ ppm): 6.9–7.5 (m, 13H, ArH), 5.3 (OCH_2), 6.4 (s, 1H, NHCONH), 9.7 (s, 1H, NHCONH), 6.7 (s, 1H, imine H), 3.8 ($ArOCH_3$); ^{13}C-NMR (75 MHz, DMSO-d_6, TMS, δ ppm): 127.4 (C-2′ & C-6′), 128.8 (C-3′ & C-5′), 127.5 (C-4′), 140.7 (C-1′), 78.3 (OCH_2), 122.3 (C-1″), 160.5 (C-2″), 114.7 (C-3″) 129.6 (C-4″), 121.4 (C-5″), 128.1 (C-6″), 171.4 (C-2), 169.2 (C-5), 157.3 (NHCONHNCH), 154.8 (NHCONHNCH), 130.0 (C-2‴ & C-6‴), 114.4 (C-3‴ & C-5‴), 164.5 (C-4‴), 123.5 (C-1‴), 56.2 ($OCH_3C_6H_5$); ESMS (Methanol) *m/z* 443 (M^+); Anal. Calcd for $C_{24}H_{21}N_5O_4$: C, 65.00; H, 4.77; N, 15.79. Found: C, 65.16; H, 4.72; N, 15.88.

Compound 6

Yield: 63%; MP: 219–220 °C; IR (per cm) (KBr) 3043.5 (Aromatic C-H str), 1604.2 & 1506.2 (Aromatic C-C str), 1092.3 (C-O of 1,3,4-oxadiazole nucleus), 1653.6 (C = N of 1,3,4-oxadiazole nucleus), 1691.5 (C = O str of amide), 3429.3 (N-H str of amide), 1618.3 (C = N group), 817.8 (C-H def disubstituted benzene ring), 719.8 (C-Cl str); ^{1}H NMR (300 MHz, DMSO-d_6, TMS, δ ppm): 7.0–7.6 (m, 13H, ArH), 5.2 (OCH_2), 6.3 (s, 1H, NHCONH), 9.6 (s, 1H, NHCONH), 6.8 (s, 1H, imine H); ^{13}C-NMR (75 MHz, DMSO-d_6, TMS, δ ppm): 127.3 (C-2′ & C-6′), 128.8 (C-3′ & C-5′), 127.4 (C-4′), 140.8 (C-1′), 78.3 (OCH_2), 122.2 (C-1″), 160.6 (C-2″), 114.5 (C-3″) 129.6 (C-4″), 121.4 (C-5″), 128.2 (C-6″), 171.4 (C-2), 169.1 (C-5), 157.4 (NHCONHNCH), 154.7 (NHCONHNCH), 130.4 (C-2‴ & C-6‴), 129.2 (C-3‴ & C-5‴), 136.2 (C-4‴), 129.4 (C-1‴); ESMS (Methanol) *m/z* 448 (M^+); Anal. Calcd for $C_{23}H_{18}N_5O_3Cl$: C, 61.68; H, 4.05; N, 15.64. Found: C, 61.60; H, 4.12; N, 15.58.

Compound 7

Yield: 62%; MP: 188–190 °C; IR (per cm) (KBr) 3037.7 (Aromatic C-H str), 1603.2 & 1506.8 (Aromatic C-C str), 1092.8 (C-O of 1,3,4-oxadiazole nucleus), 1659.4 (C = N of 1,3,4-oxadiazole nucleus), 1681.6 (C = O str of amide), 3421.6 (N-H str of amide), 1617.3 (C = N group), 822.5 (C-H def disubstituted benzene ring), 3451.4 (O-H str of alcoholic group), 1151.0 (C-O str of alcoholic group); ^{1}H NMR (300 MHz, DMSO-d_6, TMS, δ ppm): 6.8–7.5 (m, 13H, ArH), 5.2 (OCH_2), 6.3 (s, 1H, NHCONH), 9.7 (s, 1H, NHCONH), 5.4 (ArOH), 1.1 (s, 3H, Carbimino CH_3); ^{13}C-NMR (75 MHz, DMSO-d_6, TMS, δ ppm): 127.3 (C-2′ & C-6′), 128.7 (C-3′ & C-5′), 127.5 (C-4′), 140.8 (C-1′), 78.4 (OCH_2), 122.3 (C-1″), 160.7 (C-2″), 114.6 (C-3″) 129.5 (C-4″), 121.4 (C-5″), 128.1 (C-6″), 171.4 (C-2), 169.2 (C-5), 157.3 (NHCONHNCH), 154.7 (NHCONHNCH), 130.6 (C-2‴ & C-6‴), 115.6 (C-3‴ & C-5‴), 159.7 (C-4‴), 123.6 (C-1‴), 12.3 ($NHCONHNCCH_3$); ESMS (Methanol) *m/z* 443 (M^+); Anal. Calcd for $C_{24}H_{21}N_5O_4$: C, 65.00; H, 4.77; N, 15.79. Found: C, 65.15; H, 4.71; N, 15.84.

Compound 8

Yield: 64%; MP: 176–177 °C; IR (per cm) (KBr) 3037.5 (Aromatic C-H str), 1603.6 & 1509.3 (Aromatic C-C str), 1091.8 (C-O of 1,3,4-oxadiazole nucleus), 1656.4 (C = N of 1,3,4-oxadiazole nucleus), 1679.3 (C = O str of amide), 3422.8 (N-H str of amide), 2906.9 (aliphatic C-H str), 1442.4 (aliphatic C-H def), 1614.1 (C = N group), 817.7 (C-H def disubstituted benzene ring), 3460.8 (O-H str of alcoholic group), 1166.2 (C-O str of alcoholic group), 1262.9 (C-O of OCH_3 group); ^{1}H NMR (300 MHz, DMSO-d_6, TMS, δ ppm): 6.8–7.5 (m, 13H, ArH), 5.3 (OCH_2), 6.3 (s, 1H, NHCONH), 9.8 (s, 1H, NHCONH), 1.1 (s, 3H, Carbimino CH_3), 3.8 ($ArOCH_3$); ^{13}C-NMR (75 MHz, DMSO-d_6, TMS, δ ppm): 127.2 (C-2′ & C-6′), 128.5 (C-3′ & C-5′), 127.6 (C-4′), 140.8 (C-1′), 78.2 (OCH_2), 122.2 (C-1″), 160.7 (C-2″), 114.7 (C-3″) 129.6 (C-4″), 121.4 (C-5″), 128.3 (C-6″), 171.3 (C-2), 169.1 (C-5), 157.3 (NHCONHNCH), 154.7 ($NHCONHNCCH_3$), 12.3 ($NHCONHNCCH_3$), 129.9 (C-2‴ & C-6‴), 114.4 (C-3‴ & C-5‴), 164.6 (C-4‴), 123.3 (C-1‴), 56.1 ($OCH_3C_6H_5$); ESMS (Methanol) *m/z* 457 (M^+); Anal. Calcd for $C_{25}H_{23}N_5O_4$: C, 65.63; H, 5.07; N, 15.31. Found: C, 65.54; H, 5.14; N, 15.37.

Compound 9

Yield: 56%; MP: 214–215 °C; IR (per cm) (KBr) 3041.7 (Aromatic C-H str), 1601.5 & 1503.3 (Aromatic C-C str), 1091.0 (C-O of 1,3,4-oxadiazole nucleus), 1653.8 (C = N of 1,3,4-oxadiazole nucleus), 1685.3 (C = O str of amide), 3429.3 (N-H str of amide), 2909.5 (aliphatic C-H str), 1445.8 (aliphatic C-H def), 1611.6 (C = N group),

821.1 (C-H def disubstituted benzene ring), 3462.5 (O-H str of alcoholic group), 1162.6 (C-O str of alcoholic group), 1526.9 & 1358.7 (N = O str of Ar-NO_2 group); ^{1}H NMR (300 MHz, DMSO-d_6, TMS, δ ppm): 6.8–8.2 (m, 13H, ArH), 5.3 (OCH_2), 6.3 (s, 1H, NHCONH), 9.8 (s, 1H, NHCONH), 1.1 (s, 3H, Carbimino CH_3); ^{13}C-NMR (75 MHz, DMSO-d_6, TMS, δ ppm): 127.4 (C-2′ & C-6′), 128.4 (C-3′ & C-5′), 127.5 (C-4′), 140.9 (C-1′), 78.2 (OCH_2), 122.3 (C-1″), 160.6 (C-2″), 114.8 (C-3″) 129.6 (C-4″), 121.5 (C-5″), 128.2 (C-6″), 171.2 (C-2), 169.3 (C-5), 157.3 (NHCONHNCH), 154.8 (NHCONHNCCH$_3$), 12.3 (NHCONHNCCH$_3$), 129.8 (C-2‴ & C-6‴), 123.6 (C-3‴ & C-5‴), 150.8 (C-4‴), 137.5 (C-1‴); ESMS (Methanol) *m/z* 472 (M^+); Anal. Calcd for $C_{24}H_{20}N_6O_5$: C, 61.01; H, 4.27; N, 17.79. Found: C, 61.21; H, 4.33; N, 17.68.

Compound 10

Yield: 58%; MP: 182–184 °C; IR (per cm) (KBr) 3040.8 (Aromatic C-H str), 1602.9 & 1503.6 (Aromatic C-C str), 1682.3 (C = O str of amide), 3429.7 (N-H str of amide), 2911.8 (aliphatic C-H str), 1444.6 (aliphatic C-H def), 1621.5 (C = N group), 826.1 (C-H def disubstituted benzene ring) 719.8 (C-Cl str); ^{1}H NMR (300 MHz, DMSO-d_6, TMS, δ ppm): 6.8–7.6 (m, 13H, ArH), 5.3 (OCH_2), 6.4 (s, 1H, NHCONH), 9.7 (s, 1H, NHCONH); ^{13}C-NMR (75 MHz, DMSO-d_6, TMS, δ ppm): 127.3 (C-2′ & C-6′), 128.7 (C-3′ & C-5′), 127.5 (C-4′), 140.9 (C-1′), 78.3 (OCH_2), 122.3 (C-1″), 160.6 (C-2″), 114.7 (C-3″) 129.5 (C-4″), 121.4 (C-5″), 128.1 (C-6″), 171.2 (C-2), 169.3 (C-5), 157.4 (NHCONHNCH), 154.6 (NHCONHNCCH$_3$), 12.2 (NHCONHNCCH$_3$), 130.6 (C-2‴ & C-6‴), 129.2 (C-3‴ & C-5‴), 136.3 (C-4‴), 129.5 (C-1‴); ESMS (Methanol) *m/z* 462 (M^+); Anal. Calcd for $C_{24}H_{20}N_5O_3Cl$: C, 62.41; H, 4.36; N, 15.16. Found: C, 62.31; H, 4.43; N, 15.32.

Compound 11

Yield: 59%; MP: 220–221 °C; IR (per cm) (KBr) 3041.6 (Aromatic C-H str), 1601.3 & 1502.9 (Aromatic C-C str), 1672.7 (C = O str of amide), 3431.9 (N-H str of amide), 1629.4 (C = N group), 709.5 & 766.7 (C-H def monosubstituted benzene ring); ^{1}H NMR (300 MHz, DMSO-d_6, TMS, δ ppm): 6.8–7.8 (m, 19H, ArH), 5.2 (OCH_2), 6.3 (s, 1H, NHCONH), 9.7 (s, 1H, NHCONH); ^{13}C-NMR (75 MHz, DMSO-d_6, TMS, δ ppm): 127.3 (C-2′ & C-6′), 128.4 (C-3′ & C-5′), 127.5 (C-4′), 140.8 (C-1′), 78.3 (OCH_2), 122.3 (C-1″), 160.8 (C-2″), 114.8 (C-3″) 129.6 (C-4″), 121.5 (C-5″), 128.2 (C-6″), 171.2 (C-2), 169.4 (C-5), 157.3 (NHCONHNCC$_6$H$_5$), 154.8 (NHCONHNCC$_6$H$_5$), 129.2 (C-2‴ & C-6‴), 128.5 (C-3‴ & C-5‴), 130.7 (C-4‴), 131.3 (C-1‴), 129.2 (C-2⁗ & C-6⁗), 128.5 (C-3⁗ & C-5⁗), 130.7 (C-4⁗), 131.3 (C-1⁗); ESMS (Methanol) *m/z* 489 (M^+); Anal. Calcd for $C_{29}H_{23}N_5O_3$: C, 71.15; H, 4.74; N, 14.31. Found: C, 71.15; H, 4.79; N, 14.25.

Compound 12

Yield: 60%; MP: 212–214 °C; IR (per cm) (KBr) 3047.5 (Aromatic C-H str), 1601.9 & 1504.4 (Aromatic C-C str), 1088.1 (C-O of 1,3,4-oxadiazole nucleus), 1659.0 (C = N of 1,3,4-oxadiazole nucleus), 1686.2 (C = O str of amide), 3426.5 (N-H str of amide), 1613.8 (C = N group), 821.8 (C-H def disubstituted benzene ring), 3461.8 (O-H str of alcoholic group), 1165.2 (C-O str of alcoholic group), 1526.3 & 1357.9 (N = O str of Ar-NO_2 group); ^{1}H NMR (300 MHz, DMSO-d_6, TMS, δ ppm): 6.8–7.6 (m, 18H, ArH), 5.2 (OCH_2), 6.3 (s, 1H, NHCONH), 9.7 (s, 1H, NHCONH), 5.4 (ArOH); ^{13}C-NMR (75 MHz, DMSO-d_6, TMS, δ ppm): 127.3 (C-2′ & C-6′), 128.6 (C-3′ & C-5′), 127.4 (C-4′), 140.9 (C-1′), 78.2 (OCH_2), 122.2 (C-1″), 160.7 (C-2″), 114.7 (C-3″) 129.7 (C-4″), 121.5 (C-5″), 128.3 (C-6″), 171.3 (C-2), 169.4 (C-5), 157.4 (NHCONHNCC$_6$H$_5$), 154.9 (NHCONHNCC$_6$H$_5$), 130.3 (C-2‴ & C-6‴), 115.7 (C-3‴ & C-5‴), 159.8 (C-4‴), 123.9 (C-1‴), 129.1 (C-2⁗ & C-6⁗), 128.7 (C-3⁗ & C-5⁗), 130.8 (C-4⁗), 131.3 (C-1⁗); ESMS (Methanol) *m/z* 506 (M^+); Anal. Calcd for $C_{29}H_{23}N_5O_4$: C, 68.90; H, 4.59; N, 13.85. Found: C, 68.76; H, 4.51; N, 13.80.

Compound 13

Yield: 66%; MP: 226–227 °C; IR (per cm) (KBr) 3042.6 (Aromatic C-H str), 1603.7 & 1507.9 (Aromatic C-C str), 1091.4 (C-O of 1,3,4-oxadiazole nucleus), 1657.3 (C = N of 1,3,4-oxadiazole nucleus), 1684.2 (C = O str of amide), 3425.7 (N-H str of amide), 1612.7 (C = N group), 826.2 (C-H def disubstituted benzene ring), 3460.6 (O-H str of alcoholic group), 1162.8 (C-O str of alcoholic group), 1259.3 (C-O of OCH_3 group); ^{1}H NMR (300 MHz, DMSO-d_6, TMS, δ ppm): 6.8–8.4 (m, 18H, ArH), 5.2 (OCH_2), 6.3 (s, 1H, NHCONH), 9.7 (s, 1H, NHCONH); ^{13}C-NMR (75 MHz, DMSO-d_6, TMS, δ ppm): 127.4 (C-2′ & C-6′), 128.6 (C-3′ & C-5′), 127.5 (C-4′), 140.8 (C-1′), 78.3 (OCH_2), 122.3 (C-1″), 160.7 (C-2″), 114.8 (C-3″) 129.7 (C-4″), 121.4 (C-5″), 128.2 (C-6″), 171.2 (C-2), 169.5 (C-5), 157.3 (NHCONHNCC$_6$H$_5$), 154.7 (NHCONHNCC$_6$H$_5$), 130.0 (C-2‴ & C-6‴), 123.8 (C-3‴ & C-5‴), 150.8 (C-4‴), 137.5 (C-1‴), 129.2 (C-2⁗ & C-6⁗), 128.7 (C-3⁗ & C-5⁗), 130.9 (C-4⁗), 131.3 (C-1⁗); ESMS (Methanol) *m/z* 534 (M^+); Anal. Calcd for $C_{29}H_{22}N_6O_5$: C, 65.16; H, 4.15; N, 15.72. Found: C, 65.34; H, 4.22; N, 15.63.

Compound 14

Yield: 57%; MP: 240–242 °C; IR (per cm) (KBr) 3041.8 (Aromatic C-H str), 1601.7 & 1504.8 (Aromatic C-C str), 1091.5 (C-O of 1,3,4-oxadiazole nucleus), 1660.2 (C = N of 1,3,4-oxadiazole nucleus), 1681.5 (C = O str of amide), 3421.4 (N-H str of amide), 1613.0 (C = N group), 821.5 (C-H def disubstituted benzene ring), 3457.7 (O-H str of alcoholic group), 1161.0 (C-O str of alcoholic group), 1259.6 (C-O of OCH_3 group); ^{1}H NMR (300 MHz, DMSO-d_6, TMS, δ ppm): 6.9–7.8 (m, 18H, ArH), 5.2 (OCH_2), 6.3 (s, 1H, NHCONH), 9.7 (s, 1H, NHCONH), 3.8 (ArOCH_3); ^{13}C-NMR (75 MHz, DMSO-d_6, TMS, δ ppm): 127.4 (C-2′ & C-6′), 128.5 (C-3′ & C-5′), 127.5 (C-4′), 140.9 (C-1′), 78.3 (OCH_2), 122.3 (C-1″), 160.8 (C-2″), 114.8 (C-3″) 129.6 (C-4″), 121.4 (C-5″), 128.1 (C-6″), 171.3 (C-2), 169.3 (C-5), 157.4 (NHCONHNCC$_6$H$_5$), 154.7 (NHCONHNCC$_6$H$_5$), 130.2 (C-2‴ & C-6‴), 114.4 (C-3‴ & C-5‴), 164.5 (C-4‴), 123.7 (C-1‴), 129.2 (C-2⁗ & C-6⁗), 128.8 (C-3⁗ & C-5⁗), 130.9 (C-4⁗), 131.2 (C-1⁗), 56.2 ($OCH_3C_6H_5$); ESMS (Methanol) *m/z* 520 (M^+); Anal. Calcd for $C_{30}H_{25}N_5O_4$: C, 69.35; H, 4.85; N, 13.48. Found: C, 69.45; H, 4.77; N, 13.42.

The structures of the compounds were confirmed on the basis of elemental analysis, IR, ^{1}H-NMR, ^{13}C-NMR, and mass spectroscopy.

The anticonvulsant screening (16,17) was performed using male albino mice (swiss, 18–25 g) and rats (wistar 100–150 g). The anticonvulsant activity of the test compounds was evaluated by two models, namely maximal electroshock seizure (MES) and subcutaneous pentylenetetrazole (*sc*PTZ) models. The MES test identifies those compounds that stop seizure spread, and it is used to

evaluate drug effectiveness in tonic-clonic (grand mal) type of epilepsy. The *sc*PTZ seizure test primarily detects compounds that elevate seizure threshold. The *sc*PTZ-induced convulsion is not typical of absence epilepsy but clonic in nature. Phenytoin, carbamazepine and sodium valproate were used as the standard drugs for the comparison. Acute neurological toxicity was determined in the rotorod test (18). Procedures employed for the evaluation of anticonvulsant activity and neurotoxicity were reviewed and approved by the University Animal Ethical Committee.

Maximal electroshock seizure (MES test)

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

*Subcutaneous pentylenetetrazole (sc*PTZ) seizure threshold test

It was performed by administering PTZ dissolved in 0.9% sodium chloride solution in the posterior midline of the animals. A minimal time of 30 min subsequent to administration of PTZ was employed for seizure detection. Protection was indicated as there was a failure to notice any episode of clonic convulsions of at least 5 seconds duration during this time period.

Neurotoxicity screening

Minimal motor impairment (neurotoxicity) was evaluated in mice by the rotorod test. The mice were trained to stopover an accelerating rotorod of diameter 3.2 cm that rotates at six revolutions per min. Only those animals that had proven their competence 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. The mice were placed on the rotating rod 30 min after i.p. administration. Neurotoxicity was indicated by the failure of the animals to maintain equilibrium on the rod for at least 1 min in each of the three trials.

Result and Discussion

All the compounds were screened for their anticonvulsant potential through MES and *sc*PTZ models in doses of 30, 100, and 300 mg/kg by intraperitoneal (i.p.) injection. The data indicate that 64% of the compounds were active in the MES screening when compared to 28% in the *sc*PTZ test. Thus, the compounds exhibited some MES selectivity. The majority of the compounds showed activity after 4 h, indicating that the synthesized compounds were slow-acting anticonvulsants (Tables 1 and 2).

A variety of title compounds were synthesized using various substitutions i.e., hydroxy, nitro, methoxy, and chloro groups around the phenyl ring on the right-hand side of the molecules. On critical overview of synthesized compounds, it has been found that compounds bearing the groups like hydroxy or nitro on distant phenyl ring present on right-hand side of molecules possess high potency in MES and *sc*PTZ tests. Conversely, replacement of these groups with methoxy and chloro groups on the distant phenyl ring has resulted in compounds with decrease in anticonvulsant activity. On comparison of results, it has been found that antiepileptic activity of test compounds changes on varying *p*-substituted group on aryl moiety as follows: hydroxy > nitro > chloro > methoxy group. Replacement of the proton on the carbimino carbon atom by methyl group i.e., **7–10** or phenyl ring i.e., **11**–**14** has demonstrated variation in activity because of increase in the dimension of the group

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

	Intraperitoneal injection in mice[a]					
	MES screening		*sc*PTZ screening		NT screening	
Compound	0.5 h	4.0 h	0.5 h	4.0 h	0.5 h	4.0 h
1	–	–	–	–	300	–
2	–	300	–	–	–	300
3	–	300	–	–	–	300
4	–	–	–	–	–	–
5	–	–	–	–	–	–
6	–	–	300	–	300	–
7	100	300	–	–	–	300
8	–	300	–	–	100	100
9	–	300	–	300	–	–
10	–	–	–	300	–	–
11	–	300	–	–	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).
MES, maximal electroshock seizure; NT, Neurotoxicity.

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

	Oral administration in rats[a]									
	MES screening					NT screening				
Compound	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	1	0	0	1	0	0	0	1	0
9	0	0	0	1	1	0	0	0	0	1
12	0	0	0	1	1	0	0	0	0	0
13	0	0	1	0	1	0	0	0	1	0

[a]The compounds were administered in a dose of 30 mg/kg. The data in table indicate the number of rats out of four, which were protected.
MES, maximal electroshock seizure; NT, Neurotoxicity.

at this position of the molecule. The increase in the anticonvulsant activity with phenyl substitution i.e., compounds **11–14** might be because of additional van der Waals-London forces bonding to the binding site. Compounds with phenyl ring were found to possess considerably more activity in comparison with methyl group.

In the present study, we have designed and synthesized the title compounds keeping in mind that 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. The structure of the title compounds fulfilled all the pharmacophore structural requirements i.e., presence of 5-[2-(benzyloxy)phenyl]-1,3,4-oxadiazol-2-yl moiety as hydrophobic portion, N as electron donor system, and another hydrophobic distal aryl ring responsible for metabolism. In the present study, N^1-{5-[(2-benzyloxy)phenyl]-1,3,4-oxadiazol-2-yl}-N^4-[1-(4-hydroxyphenyl) (phenyl) methanone]-semicarbazone **12** came out as the most active compound, showing considerable activity in MES (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 results obtained showed that the majority of the compounds exhibited anticonvulsant activity. Thus, the results confirmed that four pharmacophore elements in semicarbazones are vital for their anticonvulsant activity.

Conclusion

A series of novel substituted semicarbazones containing 1,3,4-oxadiazole nucleus were synthesized to meet structural requirements essential for anticonvulsant activity. The structure–activity relationship studies indicate that anticonvulsant activity changes on varying *p*-substituted group on aryl moiety in the following manner: hydroxy > nitro > chloro > methoxy group. While anticonvulsant activity changes on varying the substitution attached to carbimino carbon atom as follows: $C_6H_5 > CH_3 > H$. Our results supported that the pharmacophore model with four structural features is vital for anticonvulsant activity. These new data might be expedient in the future development of semicarbazones as novel anticonvulsants.

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