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SYNTHESIS AND ANTIEPILEPTIC ACTIVITY OF SOME NOVEL SEMICARBAZONES CONTAINING 1,3,4-THIADIAZOLE AND QUINAZOLINE RING

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Abstract: The incomplete seizure control with frequent adverse effects of current anticonvulsant drugs and the importance of semicarbazones, quinazolines and 2,5-disubstituted 1,3,4-thiadiazoles as anticonvulsant pharmacophore prompted us to carry out synthesis of three novel series of semicarbazones containing 1,3,4-thiadiazole and quinazoline ring. The chemical structures of these compounds were elucidated 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 (MES) and subcutaneous pentylenetetrazole (*sc*PTZ) models. The rotorod test was conducted to evaluate neurotoxicity. The majority of the compounds were found active in the biological screening. The outcome of the present investigations proved that the four binding sites pharmacophore model is decisive for antiepileptic activity. An attempt has also been performed to establish structure-activity relationships among synthesized compounds.

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

Approximately 50 million people worldwide suffer from epilepsy, making this condition the second leading neurological disorder. Despite the optimal use of existing antiepileptic drugs, many patients fail to experience seizure control and others do so only at the expense of significant dose-related toxicity and peculiar adverse effects that range in harshness from minimal brain impairment to death from aplastic anemia or hepatic failure (1). These facts point towards urgent demand for the development of new antiepileptic drugs with better efficacy and minimal side effects.

In the last two decades, aryl semicarbazones have emerged as structurally novel class of compounds with anticonvulsant activity (2, 3). On the other hand, several research group have reported 1,3,4-thiadiazoles (4, 5) and quinazolines (6, 7) for their anticonvulsant activity. Thus, it was interesting to synthesize test compounds with semicarbazide moiety, 1,3,4-thiadiazole and quinazoline rings for their potential anticonvulsant activity.

A pharmacophore model has been put forward as a result of conformational investigations of the clinically established anticonvulsant drugs such as phenytoin, lamotrigine and phenobarbital (8) (Fig. 1). This semicarbazones based pharmacophore model is comprised of four vital binding sites namely: (i) an aromatic hydrophobic binding site (A); (ii) a hydrogen bonding domain (HBD); (iii) an electron donor group (D) and (iv) another hydrophobic-hydrophilic site regulating the pharmacokinetic properties of the anticonvulsant (C) (Fig. 2). In earlier studies on this pharmacophoric model, our research group has established that the presence of halogen substituted aryl group near the semicarbazone moiety is one of the crucial parameters for anticonvulsant activity (3–5).

EXPERIMENTAL

Chemistry

All the chemicals and solvents employed in this study were procured from E. Merck (Germany),

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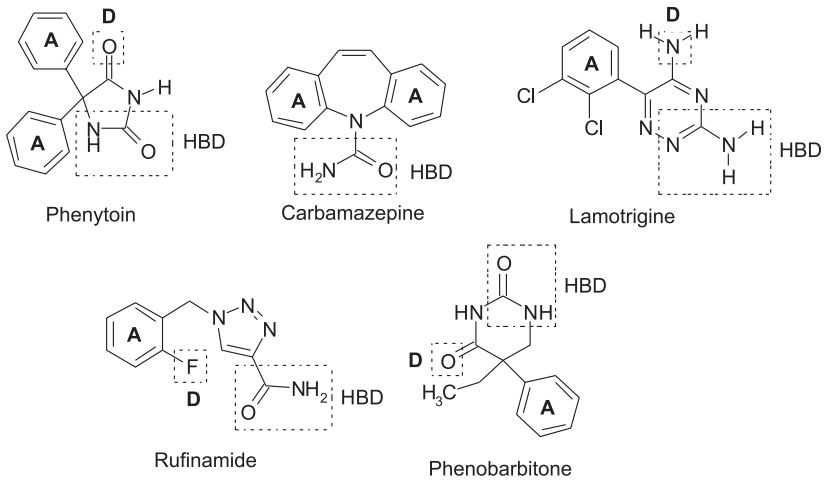


Figure 1. Commonly used anticonvulsant drugs with their vital structural features. **A** – hydrophobic aryl ring system, **HBD** – hydrogen binding domain, **D** – electron donor moiety

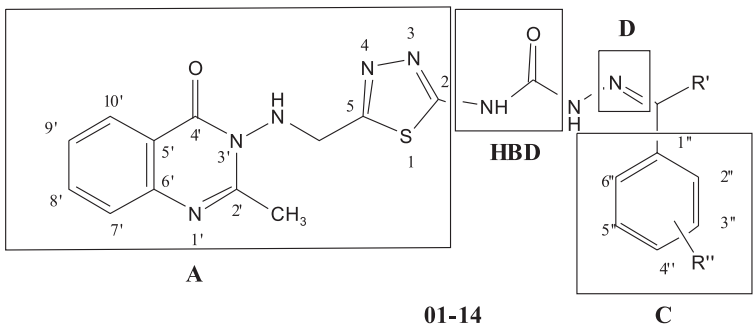


Figure 2. Pharmacophoric structural features in title compounds: **A** – hydrophobic aromatic system, **HBD** – hydrogen binding domain, **D** – electron donor moiety and **C** – hydrophobic-hydrophilic site

Fisher Scientific (India), Himedia (India) and Spectrochem Pvt. Ltd. (India). Melting points were determined by open capillary method and are uncorrected. The IR spectra were recorded on a Perkin Elmer IR spectrophotometer (KBr discs) (Perkin Elmer, Beaconsfield, UK), the 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. The starting material, 3-amino-2-methylquinazolin-4(3*H*)-ones **I**, was synthesized according to the reported procedure (9). Other reaction intermediates i.e., ethyl [(2-methyl-4-oxoquinazolin-3(4*H*)-yl)amino] acetate **II**, 2-{[(2-methyl-4-oxoquinazolin-3(4*H*)-yl)amino]acetyl}hydrazinecarbothioamide **III** and 3-{[(5-amino-1,3,4-thiadiazol-2-yl)methyl]amino}-2-methylquinazolin-4(3*H*)-one **IV** were synthesized as per reported procedure (7). Compound **IV** reaction with phenyl formate in the presence of diethylamine resulted in the formation of N-(5-{[(2-methyl-4-oxoquinazolin-3(4*H*)-yl)amino] methyl}-1,3,4-thiadiazol-2-yl)-2-phenylacetamide **V**, which on 6 to 8 h refluxing with hydrazine hydrate in the presence of methylene dichloride yielded 2-amino-N-(5-{[(2-methyl-4-oxoquinazolin-3(4*H*)-yl)amino]methyl}-1,3,4-thiadiazol-2-yl)acetamide **VI**. The title compounds were prepared by 3 to 4 h refluxing of compound **VI** with appropriate aldehyde or ketone in the presence of ethanol.

General procedure for synthesis of *N*-(5-{[(2-methyl-4-oxoquinazolin-3(4*H*)-yl) amino]methyl}-1,3,4-thiadiazol-2-yl)-2-phenylacetamide V

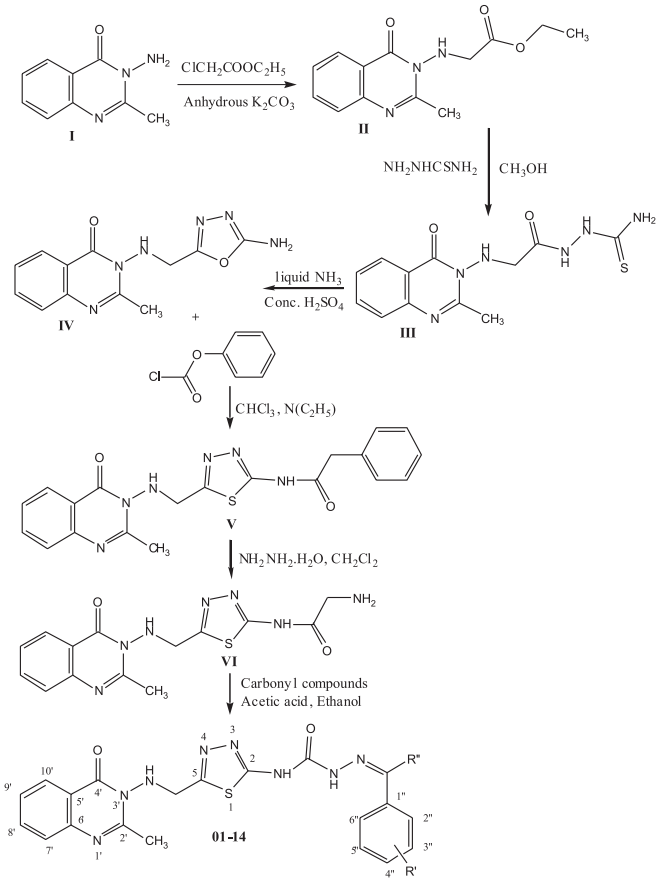
Compound **IV** (2.74 g; 0.01 mol) was dissolved in chloroform (40 mL) followed by addition of phenylchloroformate (1.7 mL; 0.01 mol) in a flat bottomed flask. Triethylamine (1.0 mL; 0.01 mol) was added dropwise to the reaction mixture which was then stirred using magnetic stirrer at the room temperature for 5 h. The solvent was evaporated under reduced pressure and the precipitate produced on addition of petroleum ether (30 mL) was washed with water, filtered and dried.

Yield 62%; m.p. 176–178°C; IR (KBr, cm^{-1}): 3430.7 (amide NH), 3066.2 (C-H arom.), 1688.1 (C=O of amide), 1670.5 (C=N of oxadiazole), 1094.4 (C-O of oxadiazole nucleus). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 1.3 (s, 3H, CH_3), 3.5 (s, 2H, $COCH_2$), 7.0–7.6 (m, 9H, ArH), 7.9 (s, 1H, NH); ESI-MS (methanol) m/z: 393.2 $[M+H]^+$.

General procedure for synthesis of 2-amino-*N*-(5-{[(2-methyl-4-oxoquinazolin-3(4*H*)-yl)amino] methyl}-1,3,4-thiadiazol-2-yl)acetamide VI

Compound **V** (3.92 g; 0.01 mol) was dissolved in dichloromethane (30–40 mL) followed by addition of hydrazine hydrate (0.5 mL; 0.01 mol). The reaction mixture was refluxed with occasional stirring for 16–20 h. The precipitate obtained was filtered and washed with dichloromethane and dried.

Yield 55%, m.p. 159–161°C. IR (KBr, cm^{-1}): 3446.2 (NH of NH_2), 3380.6 (amide NH), 3055.6 (C-H arom.), 1672.9 (C=O of amide), 1669.4 (C=N of oxadiazole), 1093.7 (C-O of oxadiazole nucleus). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 1.3 (s, 3H, CH_3), 2.5 (t, 2H, CH_2NH_2), 3.6 (t, 2H, CH_2NH_2),



1: R' = H; R'' = H **2**: R' = H; R'' = 4-NO_2 **3**: R' = H; R'' = 4-OH **4**: R' = H; R'' = 4-CH_3 **5**: R' = H; R'' = 4-OCH_3
6: R' = H; R'' = 4-Cl **7**: R' = CH_3; R'' = 4-OH **8**: R' = CH_3; R" = 4-OCH_3 **9**: R' = CH_3; R" = 4-NO_2 **10**: R' = CH_3; R" = 4-Cl
11: R' = C_6H_5; R" = H **12**: R' = C_6H_5; R" = 4-OH **13**: R' = C_6H_5; R'' = 4-NO_2 **14**: R' = C_6H_5; R" = 4-OCH_3

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

7.2–7.8 (m, 4H, ArH), 8.1 (s, 1H, NHCO). ESI-MS (methanol) m/z: 346.2 $[M+H]^+$.

General procedure for synthesis of N^1-{5-(2-methyl-4-oxoquinazolin-3(4*H*)-yl)amino] methyl}-1,3,4-thiadiazol-2-yl)}-N^4-(4-substituted benzaldehyde)-semicarbazones 1–6, N^1-{5-(2-methyl-4-oxoquinazolin-3(4*H*)-yl)amino] methyl}-1,3,4-thiadiazol-2-yl)}-N^4-[1-(4-substituted phenyl)ethanone]-semicarbazones 7–10 and N^1-{5-(2-methyl-4-oxoquinazolin-3(4*H*)-yl) amino]methyl}-1,3,4-thiadiazol-2-yl)}-N^4-[1-(4-substituted phenyl) (phenyl) methanone]-semicarbazones 11–14

Compound **VI** (0.01 mol) and appropriate quantity of required carbonyl compound (0.01 mol) were dissolved in 25–30 mL of ethanol and 1–2 drops of glacial acetic acid was added. The reaction mixture was refluxed for 3–4 h with occasional stirring. The resulting precipitate was filtered under vacuum, dried and recrystallized from 90% aqueous ethanol.

Compound **1**: m.p 194–196°C, yield 55%. IR (KBr, cm^{-1}): 3438.3 (N-H amide), 3044.7 (C-H arom.), 2928.2 (C-H aliph.), 1721.6 (C=O attached to quinazoline ring), 1669.2 (C=O amide), 1626.0 (C=N group), 1601.6 and 1504.5 (C-C arom.), 1641.4 (C=N of 1,3,4-thiadiazole nucleus), 1431.8 (C-H aliph.), 742.2 (C-S of 1,3,4-thiadiazole nucleus). ^{13}C NMR (75 MHz, DMSO-d_6, δ, ppm): 15.6 (CH_3 attached to quinazoline ring); 46.5 ($NHCH_3$), 122.8 (C-7'), 127.2 (C-5'), 127.5 (C-9'), 128.4 (C-3'' & C-5''), 128.8 (C-10'), 129.3 (C-2'' & C-5''), 131.3 (C-4''), 132.3 (C-1''), 133.7 (C-8'), 147.6 (C-6'), 154.7 (NHCONHNCCH), 157.5 (NHCONHNCH), 164.7 (C-2'), 167.7 (C-4'), 169.8 (C-5), 171.4 (C-2). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 2.3 (s, 3H, CH_3 attached to quinazoline ring), 4.3 (d, 2H, $NHCH_2$), 6.6 (s, 1H, imine H), 6.9 (s, 1H, NHCONH), 7.3–7.9 (m, 9H, ArH), 8.7 (t, 1H, $NHCH_2$), 9.5 (s, 1H, NHCONH). ESI-MS (methanol) m/z: 435.2 $[M+H]^+$.

Compound **2**: m.p. 178–179°C, yield 65%. IR (KBr, cm^{-1}): 3445.2 (N-H amide), 3048.3 (C-H arom.), 2933.8 (C-H aliph.), 1725.2 (C=O attached to quinazoline ring), 1673.0 (C=O of amide), 1647.1 (C=N of 1,3,4-thiadiazole nucleus), 1629.3 (C=N group), 1602.8 & 1504.3 (C-C arom.), 1521.4 & 1355.7 (N=O of Ar-NO_2 group), 1437.0 (C-H aliph.),; 738.5 (C-S of 1,3,4-thiadiazole nucleus). ^{13}C-NMR (75 MHz, DMSO-d_6, δ, ppm): 15.8 (CH_3 attached to quinazoline ring), 46.8 ($NHCH_3$), 122.5 (C-7'), 123.9 (C-3'' & C-5''), 127.3 (C-9'), 127.5 (C-5'), 128.9 (C-10'), 130.2 (C-2'' & C-5''), 133.4 (C-8'), 137.5 (C-1''), 147.8 (C-6'), 150.9 (C-4''), 154.6 (NHCONHNCCH), 157.8 (NHCONHNCH), 164.9 (C-2'), 167.4 (C-4'), 169.6 (C-5), 171.3 (C-2), ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 2.3 (s, 3H, CH_3 attached to quinazoline ring), 4.2 (d, 2H, $NHCH_2$), 6.7 (s, 1H, imine H), 7.1 (s, 1H, NHCONH), 7.3–8.3 (m, 8H, ArH), 8.6 (t, 1H, $NHCH_2$), 9.4 (s, 1H, NHCONH). ESI-MS (methanol) m/z: 489.4 $[M+H]^+$.

Compound **3**: m.p. 208–210°C; yield 58%. IR (KBr, cm^{-1}): 3478.8 (O-H of alcoholic group), 3426.4 (N-H amide), 3037.9 (C-H arom.), 2938.0 (C-H aliph), 1723.5 (C=O attached to quinazoline ring, 1671.3 (C=O amide), 1642.9 (C=N of 1,3,4-thiadazole nucleus), 1601.5 & 1505.2 (C-C arom.), 1442.2 (C-H aliph.), 1634.8 (C=N group), 1163.7 (C-O of alcoholic group) 734.7 (C-S of 1,3,4-thiadazole nucleus). ^{13}C-NMR (75 MHz, DMSO-d_6, δ, ppm): 15.4 (CH_3 attached to quinazoline ring), 46.8 ($NHCH_3$), 116.4 (C-3'' & C-5''), 122.5 (C-7'),), 123.9 (C-1''), 127.2 (C-5'), 127.9 (C-9'), 129.1 (C-10'), 130.6 (C-2'' & C-5''), 133.6 (C-8'), 147.8 (C-6'), 154.8 (NHCONHNCCH), 157.7 (NHCONHNCH), 159.8 (C-4''), 164.3 (C-2'), 167.6 (C-4'), 169.9 (C-5), 171.2 (C-2). ^{1}H NMR (300 MHz, DMSO-d_6, TMS, δ ppm): 2.4 (s, 3H, CH_3 attached to quinazoline ring), 4.2 (d, 2H, $NHCH_2$), 5.2 (s, 1H, ArOH), 6.5 (s, 1H, imine H), 6.9 (s, 1H, NHCONH), 7.1–7.9 (m, 8H, ArH), 8.8 (t, 1H, $NHCH_2$), 9.5 (s, 1H, NHCONH). ESI-MS (methanol) m/z: 473.2 $[M+Na]^+$.

Compound **4**: m.p. 211–213°C, yield 63%. IR (KBr, cm^{-1}): 3448.4 (N-H amide), 3041.6 (C-H arom.), 2926.9 (C-H aliph.), 1725.9 (C=O attached to quinazoline ring), 1667.8 (C=O amide), 1639.7 (C=N group), 1636.3 (C=N of 1,3,4-thiadiazole nucleus), 1602.8 & 1504.5 (C-C arom.), 1437.4 (C-H aliph.), 737.9 (C-S of 1,3,4-thiadiazole nucleus). ^{13}C-NMR (75 MHz, DMSO-d_6, δ, ppm): 15.4 (CH_3 attached to quinazoline ring), 47.2 ($NHCH_3$), 122.5 (C-7'), 127.1 (C-5'), 127.8 (C-9'), 128.4 (C-10'), 128.4 (C-1''), 128.9 (C-2'' & C-5''), 129.8 (C-3'' & C-5''), 133.8 (C-8'), 141.1 (C-4''), 147.7 (C-6'), 154.6 (NHCONHNCCH), 157.7 (NHCONHNCH), 164.5 (C-2'), 167.7 (C-4'), 169.6 (C-5), 171.3 (C-2). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 2.1 (s, 3H, CH_3 attached to quinazoline ring), 2.6 (s, 3H, $ArCH_3$), 4.3 (d, 2H, $NHCH_2$), 6.6 (s, 1H, imine H), 6.9 (s, 1H, NHCONH), 7.2–7.9 (m, 8H, ArH), 8.7 (t, 1H, $NHCH_2$), 9.5 (s, 1H, NHCONH),). ESI-MS (methanol) m/z: 447.3 $[M-H]^?$.

Compound **5**: m.p. 222–224°C, yield 62%. IR (KBr, cm^{-1}): 3432.7 (N-H amide), 3039.2 (C-H arom.), 2927.7 (C-H aliph.), 1729.6 (C=O attached

to quinazoline ring), 1677.8 (C=O amide), 1644.6 (C=N of 1,3,4-thiadiazole nucleus), 1639.2 (C=N group), 1602.7 & 1505.7 (C-C arom.), 1435.0 (C-H aliph.), 1262.5 (C-O of OCH_3 group) 730.4 (C-S of 1,3,4-thiadiazole nucleus). ^{13}C-NMR (75 MHz, DMSO-d_6, δ ppm): 15.2 (CH_3 attached to quinazoline ring), 48.3 ($NHCH_3$), 56.3 ($OCH_3C_6H_5$), 114.8 (C-3'' & C-5''), 122.6 (C-7'), 124.2 (C-1''), 127.1 (C-5'), 127.1 (C-9'), 128.6 (C-10'), 130.5 (C-2'' & C-5''), 133.6 (C-8'), 147.6 (C-6'), 154.5 (NHCONHNCCH), 157.8 (NHCONHNCH), 164.2 (C-2'), 164.2 (C-4''), 167.8 (C-4'), 169.8 (C-5), 171.5 (C-2). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 2.2 (s, 3H, CH_3 attached to quinazoline ring), 3.8 (s, 3H, $ArOCH_3$), 4.3 (d, 2H, $NHCH_2$), 6.6 (s, 1H, imine H), 6.8 (s, 1H, NHCONH), 7.0–8.1 (m, 8H, ArH), 8.6 (t, 1H, $NHCH_2$), 9.5 (s, 1H, NHCONH). ESI-MS (methanol) m/z: 465.1 $[M+H]^+$.

Compound **6**: m.p. 238–240°C, yield 56%. IR (KBr, cm^{-1}): 3449.2 (N-H amide), 3027.6 (C-H arom.), 2937.4 (C-H aliph.), 1727.3 (C=O attached to quinazoline ring), 1676.2 (C=O amide), 1640.3 (C=N of 1,3,4-thiadiazole nucleus), 1638.3 (C=N group), 1602.6 & 1504.8 (C-C arom.), 1439.0 (C-H aliph.), 734.7 (C-Cl), 732.6 (C-S of 1,3,4-thiadiazole nucleus). ^{13}C-NMR (75 MHz, DMSO-d_6, δ, ppm): 15.6 (CH_3 attached to quinazoline ring), 47.7 ($NHCH_3$), 122.7 (C-7'), 127.2 (C-5'), 127.9 (C-9'), 128.8 (C-10'), 129.1 (C-3'' & C-5''), 129.6 (C-1''), 130.6 (C-2'' & C-5''), 133.9 (C-8'), 136.3 (C-4''), 147.5 (C-6'), 154.7 (NHCONHNCCH), 157.8 (NHCONHNCH), 164.5 (C-2'), 167.7 (C-4'), 169.4 (C-5), 171.5 (C-2). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 2.5 (s, 3H, CH_3 attached to quinazoline ring), 4.2 (d, 2H, $NHCH_2$), 6.8 (s, 1H, imine H), 7.1 (s, 1H, NHCONH), 7.3–7.9 (m, 8H, ArH), 8.7 (t, 1H, $NHCH_2$), 9.3 (s, 1H, NHCONH). ESI-MS (methanol) m/z: 469.0 $[M+H]^+$.

Compound **7**: m.p. 198–199°C, yield 60%. IR (KBr, cm^{-1}): 3471.6 (O-H), 3441.8 (N-H amide), 3038.3 (C-H arom.), 2933.5 (C-H aliph.), 1719.7 (C=O attached to quinazoline ring), 1673.6 (C=O amide), 1640.2 (C=N of 1,3,4-thiadiazole nucleus), 1633.9 (C=N group), 1602.2 & 1504.8 (C-C arom.), 1444.8 (C-H aliph.), 1155.2 (C-O); 736.4 (C-S of 1,3,4-thiadiazole nucleus). ^{13}C-NMR (75 MHz, DMSO-d_6, δ, ppm): 15.3 (CH_3 attached to quinazoline ring), 19.2 ($NHCONHNCCH_3$), 47.6 ($NHCH_3$), 115.6 (C-3'' & C-5''), 122.4 (C-7'), 123.6 (C-1''), 127.0 (C-5'), 127.8 (C-9'), 128.9 (C-10'), 130.5 (C-2'' & C-5''), 133.6 (C-8'), 147.9 (C-6'), 154.9 ($NHCONHNCCH_3$), 157.8 ($NHCONHNCCH_3$), 159.8 (C-4''), 164.6 (C-2'), 167.8 (C-4'), 169.7 (C-5), 171.4 (C-2). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 1.2 (s, 3H, carbimino CH_3), 2.4 (s, 3H, CH_3 attached to quinazoline ring), 4.3 (d, 2H, $NHCH_2$), 5.2 (s, 1H, ArOH), 6.6 (s, 1H, imine H), 7.0 (s, 1H, NHCONH), 7.2–7.9 (m, 8H, ArH), 8.8 (t, 1H, $NHCH_2$), 9.5 (s, 1H, NHCONH). ESI-MS (methanol) m/z: 465.5 $[M+H]^+$.

Compound **8**: m.p. 279–280°C, yield 63%. IR (KBr, cm^{-1}): 3429.1 (N-H amide), 3046.1 (C-H arom.), 2941.8 (C-H aliph.), 1726.9 (C=O attached to quinazoline ring), 1676.6 (C=O amide), 1640.5 (C=N of 1,3,4-thiadiazole nucleus), 1631.4 (C=N group), 1601.4 & 1504.9 (C-C arom.), 1438.0 (aliphatic C-H def), 1262.5 (C-O of OCH_3 group), 732.8 (C-S of 1,3,4-thiadiazole nucleus). ^{13}C-NMR (75 MHz, DMSO-d_6, δ, ppm): 14.9 (CH_3 attached to quinazoline ring), 18.8 ($NHCONHNCCH_3$), 47.8 ($NHCH_3$), 56.4 ($OCH_3C_6H_5$), 115.0 (C-3'' & C-5''), 122.6 (C-7'), 123.8 (C-1''), 127.2 (C-5'), 127.8 (C-9'), 128.7 (C-10'), 130.6 (C-2'' & C-5''), 133.4 (C-8'), 147.8 (C-6'), 154.8 ($NHCONHNCCH_3$), 157.6 ($NHCONHNCCH_3$), 164.4 (C-2'), 165.2 (C-4''), 167.7 (C-4'), 169.7 (C-5), 171.3 (C-2). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 1.1 (s, 3H, carbimino CH_3), 2.5 (s, 3H, CH_3 attached to quinazoline ring), 3.9 (s, 3H, $ArOCH_3$), 4.3 (d, 2H, $NHCH_2$), 6.6 (s, 1H, imine H), 6.9 (s, 1H, NHCONH), 7.1–7.9 (m, 8H, ArH), 8.9 (t, 1H, $NHCH_2$), 9.6 (s, 1H, NHCONH). ESI-MS (methanol) m/z: 501.6 $[M+Na]^+$.

Compound **9**: m.p. 244–245°C, yield 55%. IR (KBr, cm^{-1}): 3452.7 (N-H amide), 3032.5 (C-H arom.), 2943.7 (C-H aliph.), 1727.1 (C=O attached to quinazoline ring), 1674.3 (C=O amide), 1640.5 (C=N of 1,3,4-thiadiazole nucleus), 1638.2 (C=N group), 1602.9 & 1505.1 (C-C arom.), 1530.6 & 1354.3 (N=O of Ar-NO_2 group); 1439.0 (C-H aliph.), 736.2 (C-S of 1,3,4-thiadiazole nucleus). ^{13}C-NMR (75 MHz, DMSO-d_6, δ, ppm): 17.5 (CH_3 attached to quinazoline ring), 19.0 ($NHCONHNCCH_3$), 47.2 ($NHCH_3$), 122.6 (C-7'), 123.8 (C-3'' & C-5''), 127.2 (C-5'), 127.8 (C-9'), 128.5 (C-10'), 130.4 (C-2'' & C-5''), 133.4 (C-8'), 137.7 (C-1''), 147.8 (C-6'), 151.9 (C-4''), 154.7 ($NHCONHNCCH_3$), 157.3 ($NHCONHNCCH_3$), 164.5 (C-2'), 167.8 (C-4'), 169.8 (C-5), 171.4 (C-2). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 1.3 (s, 3H, carbimino CH_3), 2.3 (s, 3H, CH_3 attached to quinazoline ring), 4.3 (d, 2H, $NHCH_2$), 6.6 (s, 1H, imine H), 7.1 (s, 1H, NHCONH), 7.3–8.2 (m, 8H, ArH), 8.7 (t, 1H, $NHCH_2$), 9.5 (s, 1H, NHCONH). ESI-MS (methanol) m/z: 494.2 $[M+H]^+$.

Compound **10**: m.p. 217–219°C; yield 55%. IR (KBr, cm^{-1}): 3442.7 (N-H amide), 3033.4 (C-H

arom.), 2926.3 (C-H aliph.), 1724.9 (C=O attached to quinazoline ring), 1673.1 (C=O amide), 1646.3 (C=N of 1,3,4-thiadiazole nucleus), 1627.2 (C=N group), 1602.7 & 1503.8 (C-C arom.), 1440.5 (C-H aliph.), 741.6 (C-Cl) 740.8 (C-S of 1,3,4-thiadiazole nucleus). ^{13}C-NMR (75 MHz, DMSO-d_6, δ, ppm): 15.4 (CH_3 attached to quinazoline ring); 17.2 ($NHCONHNCCH_3$), 46.9 ($NHCH_3$), 122.6 (C-7'), 126.7 (C-9'), 127.4 (C-5'), 128.9 (C-10'), 129.2 (C-3'' & C-5''), 129.5 (C-1''), 130.6 (C-2'' & C-5''), 133.9 (C-8'), 136.8 (C-4''), 147.2 (C-6'), 154.5 ($NHCONHNCCH_3$), 157.6 ($NHCONHNCCH_3$), 163.6 (C-2'), 167.5 (C-4'), 169.5 (C-5), 171.3 (C-2). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 1.7 (s, 3H, carbimino CH_3); 2.2 (s, 3H, CH_3 attached to quinazoline ring), 4.2 (d, 2H, $NHCH_2$), 7.1 (s, 1H, NHCONH), 7.3–7.9 (m, 8H, ArH), 8.9 (t, 1H, $NHCH_2$), 9.5 (s, 1H, NHCONH). ESI-MS (methanol) m/z: 481.2 $[M-H]^-$.

Compound **11**: m.p. 253–255°C, yield 61%. IR (KBr, cm^{-1}): 3453.8 (N-H amide), 3034.2 (C-H arom.), 2943.1 (C-H aliph.), 1727.1 (C=O attached to quinazoline ring), 1674.9 (C=O amide), 1640.3 (C=N of 1,3,4-thiadiazole nucleus), 1639.2 (C=N group), 1602.7 & 1504.9 (C-C arom.), 1438.6 (C-H aliph.), 733.8 (C-S of 1,3,4-thiadiazole nucleus). ^{13}C-NMR (75 MHz, DMSO-d_6, δ, ppm): 15.5 (CH_3 attached to quinazoline ring), 47.8 ($NHCH_3$), 122.4 (C-7'), 127.2 (C-5'), 127.8 (C-9'), 128.7 (C-3'' & C-5''), 128.8 (C-3''' & C-5'''), 128.9 (C-10'), 129.3 (C-2''' & C-6'''), 129.4 (C-2'' & C-6''), 130.8 (C-4''), 130.9 (C-4'''), 131.0 (C-1'''), 131.5 (C-1''), 133.7 (C-8'), 147.8 (C-6'), 154.7 ($NHCONHNCC_6H_5$), 157.3 ($NHCONHNCC_6H_5$), 164.5 (C-2'), 167.9 (C-4'), 169.6 (C-5), 171.3 (C-2). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 2.2 (s, 3H, CH_3 attached to quinazoline ring), 4.2 (d, 2H, $NHCH_2$), 6.9 (s, 1H, NHCONH), 7.3–7.9 (m, 14H, ArH), 8.6 (t, 1H, $NHCH_2$), 9.5 (s, 1H, NHCONH). ESI-MS (methanol) m/z: 511.4 $[M+H]^+$.

Compound **12**: m.p. 228–230°C, yield 59%. IR (KBr, cm^{-1}): 3468.4 (O-H), 3439.7 (N-H amide), 3044.6 (C-H arom.), 2939.3 (C-H aliph.), 1721.5 (C=O attached to quinazoline ring), 1676.8 (C=O str of amide), 1644.2 (C=N of 1,3,4-thiadiazole nucleus), 1636.1 (C=N group), 1602 & 1505.7 (C-C arom.), 1437.4 (C-H aliph.), 1177.2 (C-O) 733.4 (C-S of 1,3,4-thiadiazole nucleus). ^{13}C-NMR (75 MHz, DMSO-d_6, δ, ppm): 15.3 (CH_3 attached to quinazoline ring), 47.5 ($NHCH_3$), 115.9 (C-3'' & C-5''), 122.6 (C-7'), 127.1 (C-5'), 127.7 (C-9'), 128.6 (C-10'), 128.9 (C-3''' & C-5'''), 129.4 (C-2''' & C-6'''), 130.7 (C-2'' & C-6''), 130.8 (C-4'''), 131.3 (C-1'''), 131.5 (C-1''), 133.8 (C-8'), 147.7 (C-6'), 154.8 ($NHCONHNCC_6H_5$), 157.4 ($NHCONHNCC_6H_5$), 159.8 (C-4''), 164.7 (C-2'), 167.5 (C-4'), 169.8 (C-5), 171.5 (C-2). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 2.2 (s, 3H, CH_3 attached to quinazoline ring), 4.3 (d, 2H, $NHCH_2$), 5.3 (s, 1H, ArOH), 7.1 (s, 1H, NHCONH), 7.4–7.9 (m, 13H, ArH), 8.7 (t, 1H, $NHCH_2$), 9.5 (s, 1H, NHCONH). ESI-MS (methanol) m/z: 549.2 $[M+Na]^+$.

Compound **13**: m.p. 237–238°C, yield 67%. IR (KBr, cm^{-1}): 3427.6 (N-H amide), 3044.7 (C-H arom.), 1724.9 (C=O attached to quinazoline ring), 1684.1 (C=O amide), 1638.2 (C=N of 1,3,4-thiadiazole nucleus), 1622.8 (C=N group), 1602.5 & 1504.0 (C-C arom.), 1524.7 & 1360.2 (N=O Ar-NO_2 group) 746.3 (C-S of 1,3,4-thiadiazole nucleus). ^{13}C-NMR (75 MHz, DMSO-d_6, δ, ppm): 15.2 (CH_3 attached to quinazoline ring), 47.3 ($NHCH_3$), 122.4 (C-7'), 123.9 (C-3'' & C-5''), 126.8 (C-9'), 127.3 (C-5'), 128.7 (C-10'), 129.1 (C-3''' & C-5'''), 130.2 (C-2'' & C-6''), 130.9 (C-4'''), 131.0 (C-1'''), 131.5 (C-2''' & C-6'''), 133.6 (C-8'), 137.0 (C-1''), 147.5 (C-6'), 152.7 (C-4''), 154.5 ($NHCONHNCC_6H_5$), 157.3 ($NHCONHNCC_6H_5$), 163.9 (C-2'), 167.3 (C-4'), 169.4 (C-5), 171.1 (C-2). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 2.1 (s, 3H, CH_3 attached to quinazoline ring), 4.4 (d, 2H, $NHCH_2$), 7.0 (s, 1H, NHCONH), 7.3–8.4 (m, 13H, ArH), 8.8 (t, 1H, $NHCH_2$), 9.4 (s, 1H, NHCONH). ESI-MS (methanol) m/z: 556.2 $[M+H]^+$.

Compound **14**: m.p. 257–259°C, yield 57%. IR (KBr, cm^{-1}): 3441.7 (N-H amide), 3042.5 (C-H arom.), 2941.1 (C-H aliph.), 1726.2 (C=O attached to quinazoline ring), 1675.1 (C=O amide), 1647.2 (C=N of 1,3,4-thiadiazole nucleus), 1639.4 (C=N group), 1601.6 & 1505.8 (C-C arom.), 1435.1 (C-H aliph.), 1267.9 (C-O of OCH_3 group); 739.3 (C-S of 1,3,4-thiadiazole nucleus). ^{13}C-NMR (75 MHz, DMSO-d_6, δ, ppm): 15.3 (CH_3 attached to quinazoline ring), 47.6 ($NHCH_3$), 56.5 ($OCH_3C_6H_5$); 114.6 (C-3'' & C-5''), 122.2 (C-7'), 123.7 (C-1''), 127.2 (C-5'), 127.8 (C-9'), 128.6 (C-10'), 128.9 (C-3''' & C-5'''), 129.4 (C-2''' & C-6'''), 130.3 (C-2'' & C-6''), 130.7 (C-4'''), 131.4 (C-1'''), 133.9 (C-8'), 147.5 (C-6'), 154.6 ($NHCONHNCC_6H_5$), 157.5 ($NHCONHNCC_6H_5$), 164.6 (C-2'), 164.8 (C-4''), 167.8 (C-4'), 169.6 (C-5), 171.4 (C-2). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 2.2 (s, 3H, CH_3 attached to quinazoline ring), 3.8 (s, 3H, $ArOCH_3$), 4.5 (d, 2H, $NHCH_2$), 6.9 (s, 1H, NHCONH), 7.1–7.9 (m, 13H, ArH), 8.7 (t, 1H, $NHCH_2$), 9.6 (s, 1H, NHCONH). ESI-MS (methanol) m/z: 541.2 $[M+H]^+$.

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

Compound	Intraperitoneal injection in mice[a]					
	MES screening		scPTZ screening		NT screening	
	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	–	–	–	–	–	100
5	–	–	300	–	100	–
6	–	300	–	300	300	–
7	–	300	–	300	–	–
8	–	300	–	–	100	–
9	100	300	–	300	–	300
10	–	300	–	300	–	300
11	100	300	–	–	–	–
12	–	300	–	300	–	300
13	100	300	–	300	–	–
14	100	300	–	–	300	–
Phenytoin	30	30	–	–	100	100
Carbamazepine	30	100	100	300	100	300
Na valproate	300	–	300	–	–	–

[a] Doses of 30, 100 and 300 mg/kg were administered *i.p.* to 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 maximal 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
9	0	0	0	1	1	0	0	0	0	1
10	0	0	1	1	0	0	0	0	1	0
13	0	0	0	1	1	0	0	0	1	1

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

Pharmacology

The anticonvulsant screening (10, 11) of the test compounds was performed using maximal electroshock seizure (MES) and subcutaneous pentylenetetrazole (*sc*PTZ) models. Phenytoin, carbamazepine and sodium valproate were used as the standard drugs for comparison. The rotorod test was conducted to evaluate neurotoxicity (12). Procedures employed for evaluation of anticonvulsant activity and neurotoxicity were reviewed and approved by the University Animal Ethical Committee.

MES test

The maximal electroshock seizures were elicited with a 60 cycle altering current of 50 mA intensity delivered for 0.25 s *via* ear clip electrodes. The maximal seizures usually consist 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.

scPTZ seizure threshold test

The scPTZ test 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 after 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.

Neurotoxicity screening

Minimal motor impairment was measured in mice by the rotorod test. The mice were trained to stay on an accelerating 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 min 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.

RESULTS AND DISCUSSION

The results indicate that 72% of the compounds i.e., **2** and **6**–**14** were active in the MES screening as compared to 57% of the compounds i.e., **3**, **5**–**7**, **9**, **10**, **12** and **13** in the *sc*PTZ test. Thus, the compounds exhibited some MES selectivity indicating their effectiveness in generalized seizures of the tonic-clonic (grandmal) type. The majority of the compounds showed activity after 4 h, indicating that the synthesized compounds are slow acting anticonvulsants. In the present studies, N^1-{5-{[(2-methyl-4-oxoquinazolin-3(4*H*)-yl) amino]methyl}-1,3,4-thiadiazol-2-yl}-N^4-[1-(4-nitrophenyl) (phenyl)methanone]-semicarbazone **13** emerged out as the most active compound, demonstrating 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) (Tables 1 and 2).

On correlating the structure of the test compounds with their biological activity, it has been found that compounds bearing the groups like nitro or chloro on distant phenyl ring possess high potency in MES and *sc*PTZ tests. However, replacement of these substituents with methyl or methoxy groups on the distant phenyl ring has resulted in compounds with a 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: nitro > chloro > hydroxy > methoxy > methyl group. Replacement of the proton on the carbimino carbon atom by methyl group *i.e.*, in **7**–**10** or phenyl ring *i.e.*, in **11**–**14** has shown variation in activity due to an increase in the dimension of the group at this position of the molecule. The amplified anticonvulsant activity of compounds **11**–**14** may be due to the presence of phenyl substitution, which might be attributed to additional dispersive forces or van der Waals-London forces bonding to the binding site. Compounds with phenyl ring exhibited considerable anticonvulsant activity in comparison with those with methyl group. The structure of the title compounds fulfilled all the structural requirements i.e., the presence of hydrophobic aromatic ring system, N atom as electron donor system and [(2-methyl-4-oxoquinazolin-3(4*H*)-yl)amino-methyl]-1,3,4-thiadiazol-2-yl moiety as hydrophobic-hydrophilic portion responsible for metabolism.

CONCLUSION

Three novel series of semicarbazones containing 1,3,4-thiadiazole and quinazoline rings were synthesized and their anticonvulsant activity was evaluated using MES and *sc*PTZ models. The present study confirmed the requirements of various structural features of four binding sites of pharmacophore model for anticonvulsant activity. These new findings might be beneficial in the future research and development of semicarbazones containing 1,3,4-thiadiazole and/or quinazoline nucleus as novel anticonvulsants.

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