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RESEARCH ARTICLE

Design and Synthesis of New Derivatives of (*E*)-3-(5-((phenylamino)methyl)-1,3,4-thiadiazol-2-yl)-2-styrylquinazolin-4(3H)-one for their Anticonvulsant Potential

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ABSTRACT

Objective: The objective of the paper was to design and synthesize new derivatives of (*(E)*-3-(5-((substitutedphenylamino)methyl)-1,3,4-thiadiazol-2-yl)-2-styrylquinazolin-4(3H)-one and evaluated for their anticonvulsant potential. **Materials and Methods:** Various synthesis of *(E)*-3-(5-(substitutedaminomethyl)-1,3,4-thiadiazol-2-yl)-2-styrylquinazolin-4(3H)-one derivatives has been synthesized by reacting 2-substituted benzoxazin-4-one with *(E)*-2-(4-Substituedstyryl)-4H-benzo[d] [1,3]oxazin-4-one. All synthesized compounds have been characterized by the infrared, ^{1}HNMR, and mass spectral analysis. Proposed compounds have been evaluated for anticonvulsant potential by subcutaneous pentylenetetrazole and maximal electroshock seizure model and compared with the reference drug phenytoin and carbamazepine. Neurotoxicity study of the synthesized compounds was also performed. **Results and Discussion:** The anticonvulsant evaluation of synthesized compound QNM-1, QNM-2, QNM-4, QNM-6, QNM-9, QNM-11, QNM-13, and QNM-15 has shown seizure protection at 100 mg/kg dose after 30 min and 4 h, so they have good onset of action as quickly reach brain and have prolonged action reveal that compound metabolized slowly. Whereas compound QNM-7, QNM-8, and QNM-12 were moderate active and reveal that their high concentration is required to cross blood brain barrier. Compounds QNM-3, QNM-5, QNM-10, and QNM-14 were less active. Compounds having chlorine, bromine, fluorine, and nitro in the phenyl moiety have shown good activity when attached to para group but the addition of meta and ortho group of the same may provide least active compounds and in last fluorine compounds have shown comparative less active compounds. **Conclusion:** The Pharmacological evaluation suggest that eight synthesized compounds have shown promising anticonvulsant potential and bulkier compounds can easily penetrate BBB to exert their effect.

Keywords: Anticonvulsant, carbamazepine, maximal electroshock seizure, neurotoxicity, phenytoin

INTRODUCTION

Medicinal chemistry has remains a unique position between chemistry and biology.[1] Medicinal chemistry plays a key role in the development of new molecules, their identification and interpretative correlate with their effective action at the molecular level as well as structure activity relationships, denoted the relationship between chemical structure[2] and pharmacological activity of the compounds.[3] A large number of heterocyclic compound are also used clinically, for example, penicillin, cephalosporin, morphine, nicotine, and 5-fluorouracil.[4] Heterocyclic compounds can be aliphatic or aromatic in character, depending on the electronic constitution.[5]

Epilepsy is a central nervous system (CNS) malfunction that leads either to generalized hyperactivity involving essentially all parts of the brain or hyperactivity of only a portion of the brain.[6] It has been estimated that adequate control of seizures could not be obtained in up to 20% of the patients with epilepsy using the first generation of antiepileptic drugs (phenobarbital, phenytoin, carbamazepine, sodium valproate, and diazepam).[7] The convulsions of approximately 25%

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of epileptics are adequately controlled by current clinically available drugs. The current drug therapy is accompanied by numerous side effects including drowsiness, ataxia, gastrointestinal disturbances, gingival hyperplasia, and megaloblastic anemia.

The activity of compounds depends on the substitution and small changes in position as well as atom may hinder or alter their pharmacological effect. This modification may be their position, replacement of electron withdrawing group as well as addition of new atom may alter their pharmacological action.[8] Slight change sometime completely reverse the action of the compound, as in the case, when the terminal methyl group of 5-(1-methyl amyl)-5-ethyl barbituric acid is moved one carbon atom nearer the nucleus to forms 5-(1,3-dimethyl butyl)-5-ethyl barbituric acid converted sedatives hypnotic activity toward anticonvulsant.[9]

2-methyl-3-o-tolyl; 4(3H)-quinazolinone is a potent hypnotic agent and other 4(3H)- quinazolinone and its derivatives have been reported to exhibits analgesic, anesthetic, antibacterial, anticancer, anticonvulsant, antihypertensive, anti-inflammatory, anti-tuberculosis,[10] anticonvulsant,[11] and antioxidant,[12] diuretic, muscle relaxant, sedative, anti-hepatitis-A virus,[13] and tranquilizer properties. Most of the drugs used in the primitive system of medicine were from the natural sources, for example, morphine, quinine, digitalis, ergot, and atropine were derived from plant sources and their therapeutic uses. The past decade has witnessed a continuous interest in the development of anticonvulsant drugs.

Literature survey revealed that the presence of substituted aromatic ring at 3rd position and methyl/ phenyl group at 2nd position of 4(3H)-quinazolinone is necessary requirement for the CNS depression and anticonvulsant activity. This hypothesis encourages us to build the modification of quinazolinone at 2nd and 3rd positions. The objective of the papers was to design, synthesize, and evaluation of synthesized compounds for anticonvulsant potential.

EXPERIMENTAL

Materials and methods

2-chloroacetyl chloride, thiosemicarbazide, and formaldehyde were purchased from Sigma-Aldrich, New Delhi. Substituted anilines (Aniline, p-fluoro aniline,o-fluoroaniline,p-chloroaniline,o-chloroaniline, m-chloro aniline, m-bromo aniline, p-bromo aniline, and p-nitro aniline) were purchased from HiMedia. Acetic anhydride, di-methyl formamide, glacial acetic acid substituted, and benzaldehyde (Benzaldehyde, p- fluorobenzaldehyde, p-Bromobenzaldehyde, and p-Tolualdehyde) were purchased from chemical drug house, New Delhi, India. The chemical used for experimental work was synthetic grade. The melting points of the synthesized compounds were determined in open glass capillaries. Infrared (IR) spectra were recorded on ALPHA (Bruker) Fourier-transform IR spectrometer. Elemental analysis was performed and found values were within 0.4% of theoretical values. 13C NMR spectra were recorded on Bruker Avance 400 spectrophotometer at 400 MHz, 5mm multi-nuclear inverse probe head, low- and high-temperature facility, and HRMAS accessory. Mass Spectra were recorded using Mass Spectrometers Jeol SX-102 (FAB) by ESI.

Chemistry

The synthesis of *(E)*-3-(5-(substitutedaminomethyl)-1,3,4-thiadiazol-2-yl)-2-styrylquinazolin-4(3H)-one is accompanied in Figure 1.

Present synthesis comprises

1. Synthesis of 1,3,4-thiadiazole
2. Synthesis of (*E*)-3-(5-(((4-Substitutedphenyl) amino)methyl)-1,3,4-thiadiazol-2-yl)-2-styrylquinazolin-4(3H)-one.

Synthesis-I

Synthesis of 1,3,4-thiadiazole

Step 1: Synthesis of 5-(chloromethyl)-1,3,4-thiadiazol-2-amine

In that reaction, substituted aminothiadiazole [3] was prepared by the conventional method by following procedure: In this reaction, 2-chloroacetyl chloride [2] (0.1M) and thiosemicarbazide [1] (0.1M) were mixed and refluxed with Conc. sulfuric acid for 2½ h. When the reaction is completed, reaction mixture was cooled in ice bath and neutralize with ammonia solution (2.5%).[14] The reaction was monitored by the thin-layer

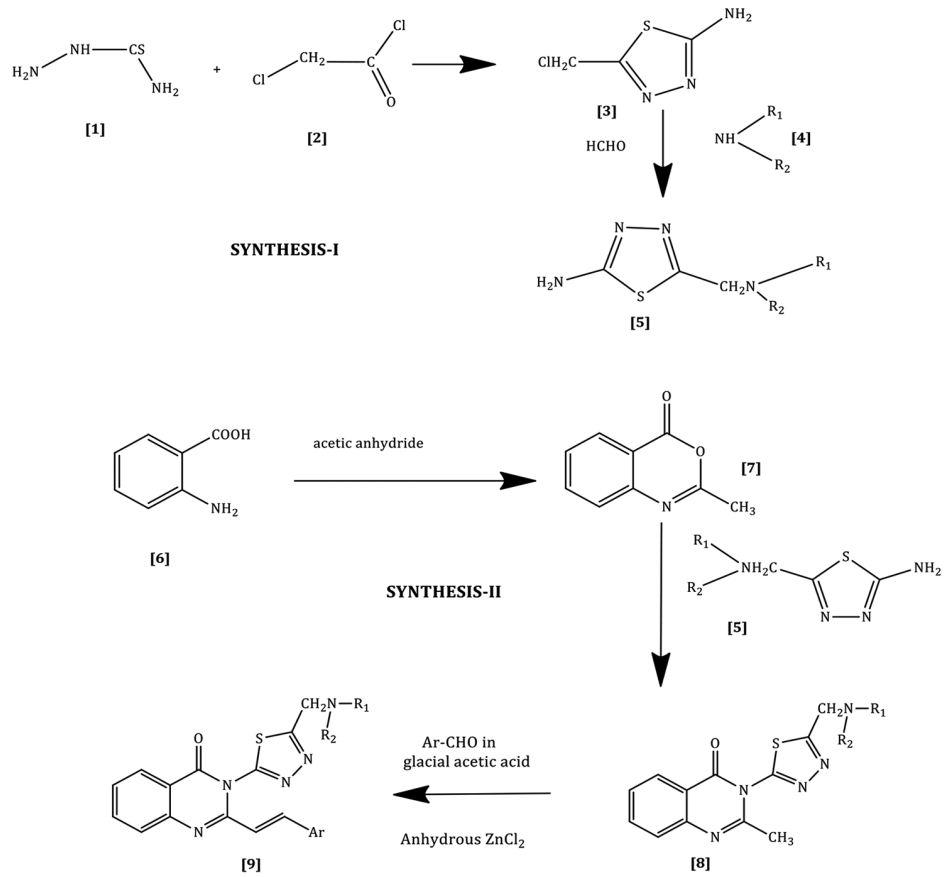


Figure 1: Schematic representation of Synthesis-I and Scheme-II

chromatography (TLC) method. The solid product thus obtained was filtration and re-crystallize by using 75% ethanol. The product is characterized by 1HNMR (6.99 ppm N-H; 4.62 ppm CH_2), and ultraviolet (UV)-spectral analysis. The compounds was shown peak at 280 nm by UV spectroscopic analysis.

Step 2: Synthesis of 5-(substituted-amino methyl)-1,3,4-thiadiazol-2-amine

In that reaction, 5-(chloromethyl)-1,3,4-thiadiazol-2-amine [3] (0.1 M) was taken in round bottom flask and formaldehyde was dissolved in methanol (3.0 ml) and then was added drop wise with continuous stirring. The resulting mixture was stirred during ½ h to complete the mixing. To this reaction mixture, methanol solution of aniline/p-fluoro, aniline/o-fluoro, aniline/p-chloro, aniline/o-chloro, aniline/m-chloro, aniline/m-bromo aniline/p-bromo, and aniline/p-nitro aniline (0.1M) [4] was mixed and reflux for 2 h at 65–70°C.[15] Then, after reaction mixture was cool at room temperature and solution poured in cold water. The solidification of compounds arises and obtained solid was filtered and washed with hot distilled water. The obtained solid product was air dried for further synthesis. Obtained compound N-((5-amino-1,3,4-thiadiazol-2-yl)methyl)nitramide [Figure 2] [5] was characterized by IR, 1HNMR and was found consistent with an expected structure. The IR data of 3270.5 (N-H str.); 3082.5 (Ar. C-H); 1515.3 (C=N str.); 642.5 (C-S str.) and 1466.9 (N=O asym. str.) confirm the compound N-((5-amino-1,3,4-thiadiazol-2-yl)methyl)nitramide. This compounds further confirmed by the 1HNMR (167 C_2-1,3,4-thiadiazole, 56 ppm CH_2-NH). TLC has been performed each and every steps to confirm the completion of the reaction.

Synthesis-II

***Synthesis of** (E)-3-(5-(((4-Substitutedphenyl)amino) methyl)-1,3,4-thiadiazol-2-yl)-2-styrylquinazolin-4(3H)-one*

Step 1: Synthesis of 2-methyl-4H-benzo[d][1,3]oxazin-4-one[16]

In this reaction, anthranilic acid [6] (0.01 M) was refluxed under anhydrous condition for

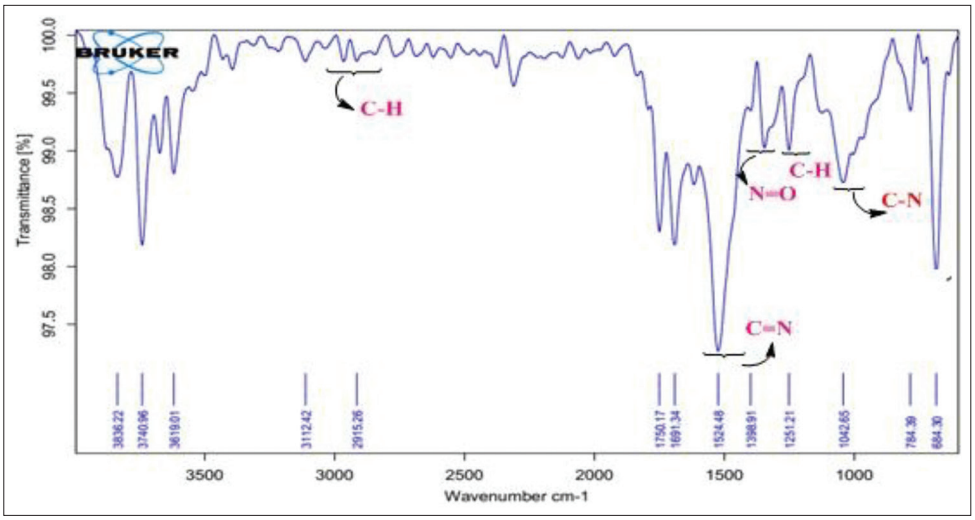


Figure 2: N-((5-amino-1,3,4-thiadiazol-2-yl)methyl)nitramide

4 h using acetic anhydride as a solvent. The remaining un-reacted acetic anhydride was distilled off to get product N-acetyl anthranilic acid. Then, N-acetyl anthranilic acid was further refluxed with acetic anhydride, under anhydrous condition for 4 h to obtained the solid mass of 2-methyl benzoxazin-4-one [7]. The products were dried and recrystallized from petroleum ether. The reaction was monitored by the TLC for the completion of the reaction. The compounds 7 (2-methyl benzoxazine-4-one) were characterized by 1H-NMR spectra (7.09–8.128 (δ ppm)=m, 4H (Ar); 2.511 (δ ppm)=s, 3H, CH_3). The 2-methyl benzoxazine-4-one was also confirmed by the IR analysis, IR peak shows at N-H str. (primary amine 3580 cm^{-1}), Ar-CH (3200 cm^{-1}) [Figure 3].

Step 2: Synthesis of 3-(5-((Substitutedamino)methyl)-1,3,4-thiadiazol-2-yl)-2 methylquina zolin-4(3H)-one

In that reaction, 2-methyl-4H-benzo[d][1,3]oxazin-4-one [7] (0.1 M) and obtained compounds [5] (0.1 M) was suspended in glacial acetic acid and refluxed for 4 h. After completion of reaction, the reaction mixture was cooled at room temperature and then it was poured into crushed ice and kept overnight in the refrigerator.[14] The obtained solid product [8] was filtered, washed with cold water and recrystallized from hot ethanol (75%). The synthesis was monitored by the TLC for the completion of the reaction.

Step 3: Synthesis of (E)-3-(5-(((4-Substitutedphenyl) amino)methyl)-1,3,4-thiadiazol-2-yl)-2-styrylquinazolin-4(3H)-one

In that reaction, equimolar quantity of compound [8] (0.2 M) was taken in round bottom flask, benzaldehyde and substituted benzaldehyde (p-fluorobenzaldehyde; p-Bromobenzaldehyde/p-Tolualdehyde) were dissolved in glacial acetic acid (0.2 M) and refluxed at 130–140°C for 2 h by the addition of anhydrous zinc chloride (0.1 g). After, reaction completion, mixture was washed with cold water to dissolve un-reacted zinc chloride. The obtained solid residue after filtration was washed with cold ethanol.[17] The purification of the synthesized compounds [9] was done by dissolving the compounds in minimum quantity of dimethylformamide (DMF) and then added this solution to distilled water. This synthesis was monitored by the TLC to confirm the completion of the reaction.

Pharmacological evaluation of synthesized compounds

Anticonvulsant evaluation of *(E)*-3-(5-(substitutedaminomethyl)-1,3,4-thiadiazol-2-yl)-2-styryl quinazolin-4(3H)-one was done by the anticonvulsant drug development program protocol. The profile of anticonvulsant activity was established after injection by the i.p. maximal electroshock seizure (MES) pattern test.[18] Anticonvulsant potential of synthesized compounds have been evaluated by two methods i.e. maximal electroshock

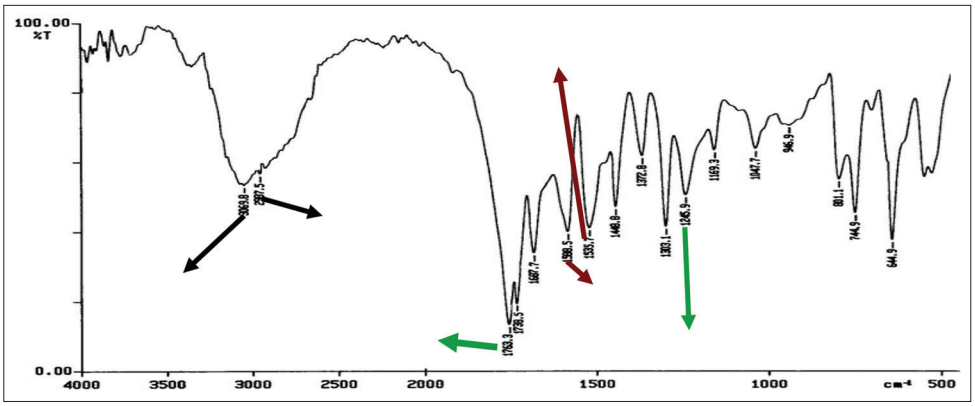


Figure 3: Infrared spectra of 2-methyl benzoxazinone

(MES) and subcutaneous pentylenetetrazole seizures (scPTZ) methods. Minimal motor impairment was measured by the rotorod (neurotoxicity, [NT]) test using doses of 30, 100, and 300 mg/kg at two different time intervals.[19]

Study protocol

Healthy young Swiss albino mice weighing between 20 and 30 g were used. Before the administration of the test samples, standard and control, the mice were first tested by giving current of 50 mA for 0.2 s using electro convulsometer. Those animals which showed characteristic course of convulsions were selected for experiment. The selected animals were divided into three groups of six animals each. After 1 h of the administration of the standard drug (phenytoin) and the test samples, the electric shock was induced. The different phases of convulsions, i.e., tonic flexion, tonic extensor, clonic convulsion, stupor, and recovery time or death were observed. The time (seconds) spent by the animals in each phase was recorded. The percentage protection provided by the standard and test samples were calculated. The scPTZ test was performed by administering PTZ dissolved in 0.9% NaCl solution in 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 least 5 s duration during this time period.

Experimental

Swiss albino mice of either sex (30–40 g) were used as experimental animals for anticonvulsant and neurotoxic activities. Animals were kept in wire-mesh cages in a restricted-access room for 1 week before the experiments. The animals were fed with standard lab pellets and purified water *ad libitum*. Before the experiments animals were fasted for 12 h. At 12 days, wash period was allowed before start of next study. All the test compounds were suspended in 30% aqueous polyethylene glycol 400. In each of the experiment, a control group was made which received the vehicle (30% PEG-400). All the experiments were carried out according to protocols approved by the Institutional Animal Ethical Committee., INSTITUT ..... NAME (Committee registration number CPCSEA/...../ YEAR/01 and Letter reference number is Animal ethical committee ...../IAEC/YEAR/01 dated...../...../.....2020).

Determination of NT

The test is used to evaluate whether any drug is interfering with established anticonvulsant activity. In 1957, Dunham and Miya suggested that the skeletal muscle relaxation induced by a test compound can be evaluated by testing the ability of Swiss albino mice to remain on a revolving rod. Many investigators have subsequently sued this forced motor activity. The dose that impairs, the ability of 50% of the Swiss albino mice to remain on the revolving rod is considered as the end point.

Method employed for NT evaluation

The method as adopted by Dunham and Miya was used. The Swiss albino mice were trained to stay on

an accelerating rotarod of diameter 3.2 cm rotating of a speed of 6 revolutions per minute. Only those animals showing ability to remain on the revolving rod for at least 1 min were selected for the test. These trained Swiss albino mice were divided into group of six animals each and were given test compounds by intra-peritoneal route in doses of 30, 100, and 300 mg/kg. Thirty minutes after intra-peritoneal administration, Swiss albino mice were placed on the rotating rod. The dose which indicated the inability of the animal to remain on the rod for at least 1 min in each of three trials was taken as the neurotoxic dose.

Methods employed for anticonvulsant evaluation

For the anticonvulsant evaluation of synthesized compounds, two methods were selected, i.e., MES and scPTZ seizure methods. Compounds affording protection in MES test usually prove to be useful in treating generalized tonic-clonic and complex partial seizures, i.e., generalized tonic-clonic seizure (grand mal epilepsy), while those showing activity in scPTZ test usually are of value in absence seizure (petit mal epilepsy).

Maximal electroshock method

Albino Swiss albino mice were used for this experiment. Food was withdrawn 12–15 h before the commencement of the experiments, while water was withdrawn immediately before the experiment. Maximum seizures were induced by applications of electrical current across the brain through corneal electrodes primed with normal saline (0.9 % NaCl). The stimulus parameters were 50 mA AC in a pulse of 60 Hz for 0.2 s. After applying shock Swiss albino mice were observed for the type of convulsion produced and the hind limb extensor response was taken as the end point.[20] Animals showing positive hind limb extensor response were used for testing drug substance. The animals were divided into groups of six animals each. The test compounds were suspended in 30% v/v aqueous polyethylene glycol 400 in concentrations so that the total volume injected to animals do not exceed 0.01ml/g. Animals were administered intraperitoneally the test compound in 30,100, and 300 mg/kg. After 30 min and 4 h of drug administration, electrical shock was given through corneal electrodes. Disappearance of the hind limb extensor component of convulsion if any was used as positive criteria.[21]

scPTZ method

Swiss albino mice of either sex were divided in groups of six animals each. The test compounds were administrated i.p. to all animals in a group in dose of 30,100, and 300 mg/kg. Pentylenetetrazole (85 mg/kg) was injected subcutaneously, 30 min and 4 h after the administration of the drugs. The absence or presence of an episode of clonic convulsion was taken as the end point. Standard drugs used for both the above studies were phenytoin and carbamazepine. The absence of tonic spasms in the observed time period indicates a compounds ability to abolish the effect of pentylenetetrazole on seizure threshold.[22]

RESULTS

Spectral analysis

Total 15 compounds were synthesized. The structures of the synthesized compounds (QNM-1 to QNM-15) were characterized by IR, 13C NMR spectra, and mass spectroscopy. The infrared spectra of the synthesized compounds showed characteristic absorption band between 1680 and 1700 cm^{-1} due to C=O str (quinazolinone ring); between 1600 and 1650 cm^{-1} due to C=C str. (vinyl group); between 1520 and 1560 cm^{-1} due to C=N str. (1,3,4-thiadiazole and quinazolinone ring), between 1210 and 1250 due to C-N str of quinazolinone ring; between 550 and 780 cm^{-1} due to C-S str. (1,3,4-thiadiazole ring); 1090 cm^{-1} due to Ar-Cl str.; and between 400 and 500 cm^{-1} due to aryl C-Cl in chloro containing compounds and 3163.3 C-H str. (Aromatic ring).

In 13C-NMR spectra of the synthesized compounds C-2 and C-4 of quinazolinone were observed between 160–165 and 167–168 (δ, ppm),

respectively, C-11 and C-5, C-6, C-7, C-8, C-9, C-10, C-12, C-13, C-14 and C18, C16, C15 and C17, C16 of quinazolinone were observed between 112–115 and 122.1–147.8 (δ, ppm), respectively. Methyl carbons were observed at 21.3 ppm. In addition peaks at δ 77.0 ppm for CDCl3 (solvent) and at δ 39.0 ppm for dimethyl sulfoxide-d6 (solvent) were also observed in respective cases. Elemental analysis of all synthesized compound was within the ±0.4% of the theoretical values. Generation of dense sooty flame and formation of oily layer after nitration of the compounds confirmed the presence of aromatic ring in all the synthesized compounds. In the FAB mass spectra, two prominent peaks were observed. TLC has been executed for the monitored of reaction and purity of the synthesized compounds using silica gel G in various solvent systems such as hexane/ethanol (95%)/chloroform/benzene, and iodine chamber has been used for the visualization and in some cases UV chamber used. All these characterization parameter showed that the structure of the synthesized compounds was near to expected.

Synthesized compounds

QNM-1: (E)-3-(5-((phenylamino)methyl)-1,3,4-thiadiazol-2-yl)-2-styrylquinazolin-4(3H)-one

Molecular formula: $C_{25}H_{19}N_5OS$; molecular weight: 437.52; TLC (Rf value): 0.45; element analysis found (Calculated): Nitrogen (%) 16.01 (15.98); sulfur (%) 7.33 (7.31); oxygen (%) 3.66 (3.64). IR (cm-1): 3020 (C-H str.); 760 (C-H def.); 1700 (C=O str.); 1174 (-C_6H_5); 1516 (C=C str.) 2856 (C-H str.) 3120 (C-H str.); 1461 (C-H str.); 1580 (C-C str.); 1614 (C=C str.); 1326 (C-N str.) 1555 (C=N str.) 760 (C-S str.) 13C NMR (ppm): 113.3 (C_{11}due to styryl group attached to 4-quinazolinone ring); 126.7 (C8 due to 4-quinazolinone ring); 128.5 (C14 and C18 due to phenyl substituted styryl group attached to 4-quinazolinone ring); 145.5 (C9, due to 4-quinazolinone ring and phenyl ring attached to 1,3,4 thiadiazole ring); 127.9 (C_{16} due to phenyl substituted styryl group attached to 4-quinazolinone ring); 127.3 (C6 due to 4-quinazolinone ring; 128.6 (C15 and C17, due to phenyl substituted styryl group attached to 4-quinazolinone ring and phenyl ring attached to 1,3,4-thiadiazole ring); 129.6 (due to phenyl ring attached to 1,3,4 thiadiazole ring); 126.6 (C5 due to 4-quinazolinone ring); 133.4 (C7 due to 4-quinazolinone ring); 135.2 (C13 due to phenyl substituted styryl group attached to 4-quinazolinone ring); 138.1 (C12 due to styryl group attached to 4-quinazolinone ring); 147.4 (C14 due to phenyl ring attached to 1,3,4 thiadiazole ring); 120.8 (C10 due to 4-quinazolinone ring); 158.9 (C2 due to 4-quinazolinone ring); 160.6 (C4 due to 4-quinazolinone ring); 51.3 a, (due to CH2–NH attached to 1,3,4 thiadiazole ring); and FAB Mass (m/z): 437.11.

QNM-2:*(E)*-3-(5-(((4-chlorophenyl)amino) methyl)-1,3,4-thiadiazol-2-yl)-2-styrylquinazol in-4(3H)-one

Molecular formula: $C_{25}H_{18}ClN_5OS$; molecular weight: 471.96; TLC (Rf value): 0.65; elemental analysis: Found (calculated): Nitrogen (%) 14.82 (14.84); sulfur (%) 6.72 (6.79); oxygen (%) 3.37 (3.39); IR (cm^{-1}): 3020 (C-H str.); 1700 (C=O str.) 1174 (-C_6H_5); 1516 (C=C str.); 2856 (C-H str.); 3120 (C-H str.); 1461 (C-H str.); 1580 (C-C str.); 1614 (C=C str.); 1326 (C-N str.); 1555 C=N str.; 760 C-S str.; 1542 C-Cl str.; 13C NMR (ppm): 113.3 C11 due to styryl group attached to 4 quinazolinone ring; 126.7 C8 due to 4-quinazolinone ring; 128.5; C14 and C18 due to phenyl substituted styryl group attached to 4-quinazolinone ring; 145.5 (C9, due to 4-quinazolinone ring and phenyl ring attached to 1,3,4 thiadiazole ring); 127.9 (C16 due to phenyl substituted styryl group attached to 4-quinazolinone ring); 127.3 (C6 due to 4-quinazolinone ring); 128.6 (C15 and C17 due to phenyl substituted styryl group attached to 4-quinazolinone ring and phenyl ring attached to 1,3,4-thiadiazole ring); 129.6 (due to phenyl ring attached to 1,3,4 thiadiazole ring); 126.6 (C5 due to 4-quinazolinone ring); 133.4 (C7 due to 4-quinazolinone ring); 135.2 (C13 due to phenyl substituted styryl group attached to 4-quinazolinone ring); 138.1 (C12 due to styryl group attached to 4-quinazolinone ring); 147.4 (due to phenyl ring attached to 1,3,4 thiadiazole ring); 120.8 (C10 due to 4-quinazolinone ring); 158.9 (C2 due to 4-quinazolinone ring); 160.6 (C4 due

to 4-quinazolinone ring); 51.3 a, (due to CH_2–NH attached to 1,3,4 thiadiazole ring); and FAB Mass (m/z): 472.

QNM-3: *(E)*-3-(5-(((4-chlorophenyl) amino)methyl)-1,3,4-thiadiazol-2-yl)-2-styrylquinazolin-4(3H)-one

Molecular formula: $C_{25}H_{18}ClN_5OS$; molecular weight: 471.96; TLC (Rf value): 0.65; elemental analysis found (Calculated): Nitrogen (%) 14.82 (14.84); sulfur (%) 6.72 (6.79); oxygen (%) 3.37 (3.39); IR (cm^{-1}): 3020 C-H str.; 760 C-H def.; 1700 C=O str.; 1174 -C6H5; 1516 C=C str.; 2856 C-H str.; 3120 C-H str.; 1461 C-H str.; 1580 C-C str.; 1614 C=C str.; 1326 C-N str.; 1555 C=N str.; 760 C-S str.; 1538 C-Cl str.; 13C NMR (ppm):113.3 (C11 due to styryl group attached to 4-quinazolinone ring); 126.7 (C8 due to 4-quinazolinone ring); 128.5 (C14 and C18 due to phenyl substituted styryl group attached to 4-quinazolinone ring); 145.5 (C9, due to 4-quinazolinone ring and phenyl ring attached to 1,3,4 thiadiazole ring); 127.9 (C16 due to phenyl substituted styryl group attached to 4-quinazolinone ring); 127.3 (C6 due to 4-quinazolinone ring); 128.6 (C15 and C17, due to phenyl substituted styryl group attached to 4-quinazolinone ring and phenyl ring attached to 1,3,4-thiadiazole ring); 129.6 (due to phenyl ring attached to 1,3,4 thiadiazole ring); 126.6 (C5 due to 4-quinazolinone ring); 133.4 (C7 due to 4-quinazolinone ring); 135.2 (C13 due to phenyl substituted styryl group attached to 4-quinazolinone ring); 138.1 (C12 due to styryl group attached to 4-quinazolinone ring); 147.4, due to phenyl ring attached to 1,3,4 thiadiazole ring; 120.8, C10 due to 4-quinazolinone ring; 158.9, C2 due to 4-quinazolinone ring; 160.6 C4 due to 4-quinazolinone ring; 51.3 a, due to CH2–NH attached to 1,3,4 thiadiazole ring; and FAB Mass (m/z): 472.

QNM-4:*(E)*-3-(5-(((4-chlorophenyl) amino)methyl)-1,3,4-thiadiazol-2-yl)-2-styrylquinazolin-4(3H) -one

Molecular formula: $C_{25}H_{18}ClN_5OS$; molecular weight: 471.96; TLC (Rf value): 0.65; elemental analysis: Found (calculated): Nitrogen (%) 14.82 (14.84); sulfur (%) 6.72 (6.79); oxygen (%) 3.37 (3.39); IR (cm^{-1}): 3020, C-H str.; 760 C-H def; 1700 C=O str.; 1174 -C6H5; 1516 C=C str.; 2856 C-H str.; 3120 C-H str.; 1461 C-H str.; 1580 C-C str.; 1614 C=C str.; 1326 C-N str.; 1555 C=N str.; 760 C-S str.; 1542 C-Cl str.; 13C NMR (ppm): 113.3, C11 due to styryl group attached to 4-quinazolinone ring; 126.7, C8 due to 4-quinazolinone ring; 128.5, C14 and C18 due to phenyl substituted styryl group attached to 4-quinazolinone ring; 145.5, C9 due to 4-quinazolinone ring and phenyl ring attached to 1,3,4 thiadiazole ring; 127.9, C16 due to phenyl substituted styryl group attached to 4-quinazolinone ring; 127.3, C6 due to 4-quinazolinone ring; 128.6, C15, and C17 due to phenyl substituted styryl group attached to 4-quinazolinone ring and phenyl ring attached to 1,3,4-thiadiazole ring; 129.6, due to phenyl ring attached to 1,3,4 thiadiazole ring; 126.6 C5 due to 4-quinazolinone ring; 133.4 C7 due to 4-quinazolinone ring; 135.2 C13 due to phenyl substituted styryl group attached to 4-quinazolinone ring; 138.1, C12 due to styryl group attached to 4-quinazolinone ring; 147.4, due to phenyl ring attached to 1,3,4 thiadiazole ring; 120.8, C10 due to 4-quinazolinone ring; 158.9, C2 due to 4-quinazolinone ring; 160.6, C4 due to 4-quinazolinone ring; 51.3 a, due to CH2–NH attached to 1,3,4 thiadiazole ring; and FAB Mass (m/z): 472.

QNM-5: *(E)*-3-(5-(((2-fluorophenyl)amino) methyl)-1,3,4-thiadiazol-2-yl)-2-styrylquinazolin-4(3H)-one

Molecular formula: $C_{25}H_{18}FN_5OS$; molecular weight: 455.51; TLC (Rf value): 0.68; elemental analysis: Found (Calculated): Nitrogen (%) 15.35 (15.37); sulfur (%) 7.02 (7.04); oxygen (%) 3.45 (3.51); IR (cm^{-1}): 3120 (C-H str.); 775 (C-H def.); 1737 (C=O str.); 1598 (C=C str.); 2975 (C-H str.); 3020 (C-H str.); 1378 (C-H def.); 1458 (C-C str.); 1610 (C=C str.); 1269 (C-N str.); 733 (C-S str.); 650 (C-F str.); 13C NMR (ppm): 113.3, C11 due to styryl group attached to 4-quinazolinone ring; 126.7, C8 due to 4-quinazolinone ring; 128.5, C14 and C18 due to phenyl substituted styryl group

attached to 4-quinazolinone ring; 114.9, C9 due to 4-quinazolinone ring and phenyl ring attached to 1,3,4 thiadiazole ring; 127.9, C16 due to phenyl substituted styryl group attached to 4-quinazolinone ring; 127.3, C6 due to 4-quinazolinone ring; 115.1, C15, and C17 due to phenyl substituted styryl group attached to 4-quinazolinone ring and phenyl ring attached to 1,3,4-thiadiazole ring; 132.4, phenyl ring attached to 1,3,4 thiadiazole ring; 126.6, C5 due to 4-quinazolinone ring; 133.4, C7 due to 4-quinazolinone ring; 135.2, C13 due to phenyl substituted styryl group attached to 4-quinazolinone ring; 138.1, C12 due to styryl group attached to 4-quinazolinone ring; 148.3, due to phenyl ring attached to 1,3,4 thiadiazole ring; 120.8, C10 due to 4-quinazolinone ring; 158.9, C2 due to 4-quinazolinone ring; 160.6, C4 due to 4-quinazolinone ring; 51.3a, due to CH2–NH attached to 1,3,4 thiadiazole ring; and FAB Mass (m/z): 455.31.

QNM-06: *(E)*-3-(5-(((4-nitrophenyl) amino)methyl)-1,3,4-thiadiazol-2-yl)-2-styrylquinazolin-4(3H)-one

Molecular formula: $C_{25}H_{18}N_6O_3S$; molecular weight: 482.51; TLC (Rf value): 0.67; elemental analysis: Found (Calculated): Nitrogen (%) 17.38 (17.42); sulfur (%) 6.60 (6.65); oxygen (%) 9.90 (9.95); IR (cm^{-1}): 3261 (C-H str.); 812 (C-H def.); 1700 (C=O str.); 1174 (-C6H5); 1540 (N = 0 str.) 1320 (N-O str.); 1593 (C=C str.); 1076-C6H5; 2856 (C-H str.); 3057 (C-H str.); 1382 (C-H def.); 1442 (C-C str.); 1620 (C=C str.); 1274 (C-N str.); 740 (C-S str.); 13C NMR (ppm): 113.5 (C11 due to styryl group attached to 4-quinazolinone ring); 126.9 (C8 due to 4-quinazolinone ring); 128.1, C14, and C18 due to phenyl substituted styryl group attached to 4-quinazolinone ring; 114.4, C9, due to 4-quinazolinone ring and phenyl ring attached to 1,3,4 thiadiazole ring; 127.9, C16 due to phenyl substituted styryl group attached to 4-quinazolinone ring; 127.3, C6 due to 4-quinazolinone ring; 136.3, C15 and C17 due to phenyl substituted styryl group attached to 4-quinazolinone ring and phenyl ring attached to 1,3,4 thiadiazole ring; 127.5 due to phenyl ring attached to 1,3,4 thiadiazole ring; 126.3, C5 due to 4-quinazolinone ring; 133.6, C7 due to 4-quinazolinone ring; 135.1, C13 due to phenyl substituted styryl group attached to 4-quinazolinone ring; 138.6, C12 due to styryl group attached to 4-quinazolinone ring; 155.4, C due to phenyl ring attached to 1,3,4 thiadiazole ring; 120.3, C10 due to 4-quinazolinone ring; 158.7, C2 due to 4-quinazolinone ring. 160.9, C4 due to 4-quinazolinone ring; 55.36a, due to CH_2–NH attached to 1,3,4 thiadiazole ring; and FAB Mass (m/z): 482.32.

QNM-7: *(E)*-3-(5-(((4-nitrophenyl) amino)methyl)-1,3,4-thiadiazol-2-yl)-2-styrylquinazolin-4(3H)-one

Molecular formula: $C_{25}H_{18}N_6O_3S$; molecular weight: 482.51; TLC (Rf value): 0.67; elemental analysis: Found (Calculated): Nitrogen (%) 17.38 (17.42); sulfur (%) 6.60 (6.65); oxygen (%): 9.90 (9.95); IR (cm^{-1}): 3125 (C-H str.); 808 (C-H def (oop); 1700 (C=O str.; 1590 C=C str.; 2945 C-H str.; 3050 C-H str.; 1450 C-H def.; 1570 C-C str.; 1630 C=C str.; 1348 C-N str.; 1560 C=N str.; 575 C-S str.; 520 C-Br str.; 13C NMR (ppm): 113.3, C11 due to styryl group attached to 4-quinazolinone ring; 126.2, C8 due to 4-quinazolinone ring: 128.5, C14 and C18 due to phenyl substituted styryl group attached to 4-quinazolinone ring; 118.9, C9 due to 4-quinazolinone ring and phenyl ring attached to 1,3,4 thiadiazole ring; 127.8, C16 due to phenyl substituted styryl group attached to 4-quinazolinone ring; 127.9, C6 due to 4-quinazolinone ring; 155.2, C15 and C17 due to phenyl substituted styryl group attached to 4-quinazolinone ring and phenyl ring attached to 1,3,4-thiadiazole ring; 116.3, phenyl ring attached to 1,3,4 thiadiazole ring; 126.3, C5 due to 4-quinazolinonering; 133.7, C7 due to 4-quinazolinone ring; 135.2, C13 due to phenyl substituted styryl group attached to 4-quinazolinone ring; 138.5, C12 due to styryl group attached to 4-quinazolinone ring; 144.9, phenyl ring attached to 1,3,4 thiadiazole ring; 120.5, C10 due to 4-quinazolinone ring 158.2, C2 due to 4-quinazolinone ring; 160.1, C4 due to 4-quinazolinone ring; 51.5a, due to CH_2–NH attached to 1,3,4 thiadiazole ring; and FAB Mass (m/z): 482.51.

QNM-8: *(E)*-2-(4-bromostyryl)-3-(5-(((4-fluorophenyl)amino)methyl)-1,3,4-thiadiazol-2-yl)quinazolin-4(3H)-one

Molecular formula: $C_{25}H_{17}BrFN_5OS$; molecular weight: 534.40; TLC (Rf value): 0.68; elemental analysis: Found (calculated): Nitrogen (%) 13.10 (13.11); sulfur (%) 5.97 (6.00); oxygen (%) 2.96 (2.99); IR (cm^{-1}): 3092 C-H str.; 758 C-H def (oop); 1708 C=O str.; 1578 C=C str.; 2945 C-H str.; 3008 C-H str.; 1442 C-H def.; 1466 C-C str.; 1608 C=C str.; 1278 C-N str.; 1505 C=N str.; 620 C-S str.; 520 C-Br str.; 650 C-F str.; 13C NMR (ppm): 113.3, C11 due to styryl group attached to 4-quinazolinone ring; 126.2, C8 due to 4-quinazolinone ring; 128.4, C14 and C18 due to phenyl substituted styryl group attached to 4-quinazolinone ring; 113.7, C9 due to 4-quinazolinone ring and phenyl ring attached to 1,3,4 thiadiazole ring; 127.5, C16 due to phenyl substituted styryl group attached to 4-quinazolinone ring; 127.7, C6 due to 4-quinazolinone ring; 129.6, C15 and C17 due to phenyl substituted styryl group attached to 4-quinazolinone ring and phenyl ring attached to 1,3,4-thiadiazole ring; 129.8, due to phenyl ring attached to 1,3,4 thiadiazole ring; 126.2, C5 due to 4-quinazolinone ring; 133.7, C7 due to 4-quinazolinone ring; 135.2, C13 due to phenyl substituted styryl group attached to 4-quinazolinone ring; 138.5, C12 due to styryl group attached to 4-quinazolinone ring; 146.4, due to phenyl ring attached to 1,3,4 thiadiazole ring; 120.1, C10 due to 4-quinazolinone ring; 158.3, C2 due to 4-quinazolinone ring; 160.2, C4 due to 4-quinazolinone ring; 48.3a, due to CH2–NH attached to 1,3,4 thiadiazole ring; and FAB Mass (m/z): 534.40.

QNM-9: *(E)*-2-(4-bromostyryl)-3-(5-(((4-nitrophenyl)amino)methyl)-1,3,4-thiadiazol-2-yl)quinazolin-4(3H)-one

Molecular formula: $C_{25}H_{17}BrN_6O_3S$; molecular weight: 561.41; TLC (Rf value): 0.65; elemental analysis found (calculated): Nitrogen (%) 14.92 (14.97); sulfur (%) 5.68 (5.71); oxygen (%) 8.45 (8.55); IR (KBr, cm^{-1}): 3159 C-H str.; 761 C-H def (oop); 1701 C=O str.; 1562 C=C str.; 2907 C-H str.; 3010 C-H str.; 1375 C-H def; 1439 C-C str.; 1693 C=C str.; 1274 C-N str.; 754 C-S str.; 520 C-Br str.; 1540 N = 0 str.; 1320 N-O str.; 13C NMR (ppm): 113.3, C11 due to styryl group attached to 4-quinazolinone ring; 126.2, C8 due to 4-quinazolinone ring; 128.4, C14 and C18 due to phenyl substituted styryl group attached to 4 quinazolinone ring; 113.4, C9due to 4-quinazolinone ring and phenyl ring attached to 1,3,4 thiadiazole ring; 127.1, C16 due to phenyl substituted styryl group attached to 4-quinazolinone ring; 127.4, C6 due to 4-quinazolinone ring; 136.7, C15 and C17 due to phenyl substituted styryl group attached to 4-quinazolinone ring and phenyl ring attached to 1,3,4-thiadiazole ring; 128.5, due to phenyl ring attached to 1,3,4 thiadiazole ring; 126.2 C5 due to 4-quinazolinone ring; 133.5, C7 due to 4-quinazolinone ring; 135.5, C13 due to phenyl substituted styryl group attached to 4-quinazolinone ring; 138.7, C12 due to styryl group attached to 4-quinazolinone ring; 146.5, due to phenyl ring attached to 1,3,4 thiadiazole ring; 120.2, C10 due to 4-quinazolinone ring; 158.3, C2 due to 4-quinazolinone ring; 160.6, C4 due to 4-quinazolinone ring; 51.5a, due to CH2–NH attached to 1,3,4 thiadiazole ring; and FAB Mass (m/z): 561.41.

QNM-10: *(E)*-3-(5-(((2-fluorophenyl)amino) methyl)-1,3,4-thiadiazol-2-yl)-2-(4-fluorostyryl) quinazolin-4(3H)-one

Molecular formula: $C_{25}H_{17}F_2N_5OS$; molecular weight: 473.50; TLC (Rf value): 0.62; elemental analysis found (calculated): Nitrogen (%) 14.75 (14.79); sulfur (%) 6.74 (6.77); oxygen (%) 3.32 (3.38); IR (KBr, cm^{-1}): 3157 C-H str.; 819 C-H def (oop); 1703 C=O str.; 1080 C-O-C str.; 1559 C=C str.; 2909 C-H str.; 3050 C-H str.; 1417 C-H def.; 1450 C-C str.; 1609 C=C str.; 1252 C-N str.; 1519 C=N str.; 615 C-S str.; 650 C F str.; 13C NMR (ppm): 113.1, C11 due to styryl group attached to 4-quinazolinone ring; 126.2, C8 due to 4-quinazolinone ring; 128.3, C14 and C18 due to phenyl substituted styryl group attached to 4-quinazolinone ring; 113.3, C9 due to 4-quinazolinone ring and phenyl ring attached to 1,3,4 thiadiazole ring; 127.6, C16 due to phenyl substituted styryl group attached to 4-quinazolinone ring; 127.3, C6 due to 4-quinazolinone ring;

151.7, C15 and C17 due to phenyl substituted styryl group attached to 4-quinazolinone ring and phenyl ring attached to 1,3,4-thiadiazole ring; 126.5, C5 due to 4-quinazolinone ring; 133.4, C7 due to 4-quinazolinone ring; 135.7, C13 due to phenyl substituted styryl group attached to 4-quinazolinone ring; 138.8, C12 due to styryl group attached to 4-quinazolinone ring; 120.3, C10 due to 4-quinazolinone ring; 158.6, C2 due to 4-quinazolinone ring; 160.8, C4 due to 4-quinazolinone ring; 51.5a, due to CH_2–NH attached to 1,3,4 thiadiazole ring; and FAB Mass (m/z): 473.16.

QNM-11: *(E)*-3-(5-(((4-fluorophenyl)amino) methyl)-1,3,4-thiadiazol-2-yl)-2-(4-fluorostyryl) quinazolin-4(3H)-one

Molecular formula: $C_{25}H_{17}F_2N_5OS$; molecular weight: 473.50; TLC (Rf value): 0.68; elemental analysis: Found (calculated): Nitrogen (%) 14.76 (14.79); sulfur (%) 6.75 (6.77); oxygen (%) 3.37 (3.38); IR (KBr, cm^{-1}): 3159 C-H str.; 822 C-H def (oop); 1692 C=O str.; 1210 C-O-C str.; 1562 C=C str.; 2907 C-H str.; 3031 C-H str.; 1438 C-H def; 1450 C-C str.; 1650 C=C str.; 1313 C-N str.; 1520 C=N str.; 670 C-S str.; 650 C-F str.; 13C NMR (ppm): 113.1, C11 due to styryl group attached to 4-quinazolinone ring; 126.3, C8 due to 4-quinazolinone ring; 128.4, C14 and C18 due to phenyl substituted styryl group attached to 4-quinazolinone ring; 112.9, C9 due to 4 quinazolinone ring and phenyl ring attached to 1,3,4 thiadiazole ring; 127.3, C16 due to phenyl substituted styryl group attached to 4-quinazolinone ring; 127.5, C6 due to 4-quinazolinone ring; 150.4, C15 and C17 due to phenyl substituted styryl group attached to 4-quinazolinone ring and phenyl ring attached to 1,3,4-thiadiazole ring; 115.2, due to phenyl ring attached to 1,3,4 thiadiazole ring; 126.1, C5 due to 4-quinazolinone ring; 133.3, C7 due to 4-quinazolinone ring 135.3, C13 due to phenyl substituted styryl group attached to 4-quinazolinone ring; 138.6, C12 due to styryl group attached to 4-quinazolinone ring; 140.9, due to phenyl ring attached to 1,3,4 thiadiazole ring; 120.1, C10 due to 4-quinazolinone ring; 158.2, C2 due to 4-quinazolinone ring; 160.5, C4 due to 4-quinazolinone ring; 51.3a, due to CH2–NH attached to 1,3,4 thiadiazole ring; and FAB Mass (m/z): 473.16

QNM-12: *(E)*-2-(4-fluorostyryl)-3-(5-(((4-nitrophenyl)amino)methyl)-1,3,4-thiadiazol-2-yl) quinazolin-4(3H)-one

Molecular formula: $C_{25}H_{17}FN_6O_3S$; molecular weight: 500.50; TLC (Rf value): 0.75; elemental analysis: Found (calculated): Nitrogen (%) 16.78 (16.79); sulfur (%) 6.39 (6.41); oxygen (%) 9.57 (9.59); IR (KBr, cm^{-1}): 3160 C-H str; 760 C-H def (oop); 1693 C=O str.; 1590 C=C str.; 2902 C-H str.; 3020 C-H str.; 1373 C-H def.; 1437 C-C str.; 1580 N = 0 str.; 1370 N-O str.; 1610 C=C str.; 1316 C-N str.; 1568 C=N str.; 667 C-S str.; 650 C-F str.; 13C NMR (ppm): 113.1, C11 due to styryl group attached to 4-quinazolinone ring; 126.3, C8 due to 4-quinazolinone ring; 129.0, C14 and C18 due to phenyl substituted styryl group attached to 4-quinazolinone ring; 114.9, C9due to 4-quinazolinone ring and phenyl ring attached to 1,3,4 thiadiazole ring; 147.1, C16 due to phenyl substituted styryl group attached to 4-quinazolinone ring; 127.3, C6 due to 4-quinazolinone ring; 123.8, C15 and C17 due to phenyl substituted styryl group attached to 4-quinazolinone ring and phenyl ring attached to 1,3,4-thiadiazole ring; 129.6, due to phenyl ring attached to 1,3,4 thiadiazole ring; 126.1, C5 due to 4-quinazolinone ring; 133.7, C7 due to 4-quinazolinone ring; 141.3, C13 due to phenyl substituted styryl group attached to 4-quinazolinone ring; 138.2, C12 due to styryl group attached to 4-quinazolinone ring; 147.4, due to phenyl ring attached to 1,3,4 thiadiazole ring; 120.8, C10 due to 4-quinazolinone ring; 158.2, C2 due to 4-quinazolinone ring; 160.3, C4 due to 4-quinazolinone ring; 51.3 a, due to CH_2–NH attached to 1,3,4 thiadiazole ring; and FAB Mass (m/z): 500.50.

QNM-13: *(E)*-3-(5-(((4-chlorophenyl)amino) methyl)-1,3,4-thiadiazol-2-yl)-2-(4-methyl styryl) quinazolin-4(3H)-one

Molecular formula: $C_{26}H_{20}ClN_5OS$; molecular weight: 485.99; TLC (Rf value): 0.62; elemental

analysis found (calculated): Nitrogen (%) 14.35 (14.41); sulfur (%) 6.56 (6.60); oxygen (%) 3.25 (3.29); IR (KBr, cm^{-1}): 3117.3 C-H str.; 752.8 C-H def (oop); 1689.5 C=O str.; 1594 C=C str.; 2917 C-H str.; 3020 C-H str.; 1448.3 C-H def.; 1240 C-C str.; 1610 C=C str.; 1346 C-N str.; 1519 C=N str.; 608 C-S str.; 464.4 C-Cl str.; 13C NMR (ppm): 113.4, C11 due to styryl group attached to 4-quinazolinone ring; 127.3, C8 due to 4-quinazolinone ring; 128.5, C14 and C18 due to phenyl substituted styryl group attached to 4-quinazolinone ring; 114.4, C9, due to4-quinazolinone ring and phenyl ring attached to 1,3,4 thiadiazole ring; 137.6, C16 due to phenyl substituted styryl group attached to 4-quinazolinone ring; 127.1, C6 due to 4-quinazolinone ring; 128.9, C15 and C17 due to phenyl substituted styryl group attached to 4-quinazolinone ring and phenyl ring attached to 1,3,4-thiadiazole ring; 129.4, due to phenyl ring attached to 1,3,4 thiadiazole ring; 126.5, C5 due to 4-quinazolinone ring; 133.4, C7 due to 4-quinazolinone ring; 132.2, C13 due to phenyl substituted styryl group attached to 4-quinazolinone ring; 138.4, C12 due to styryl group attached to 4-quinazolinone ring; 147.4, due to phenyl ring attached to 1,3,4 thiadiazole ring; 120.3, C10 due to 4-quinazolinone ring; 158.1, C2 due to 4-quinazolinone ring; 160.7, C4 due to 4-quinazolinone ring; 51.1a, due to CH2–NH attached to 1,3,4 thiadiazole ring; 15.4, CH3; phenyl substituted styryl group attached to 4-quinazolinone ring; and FAB Mass (m/z): 486.13.

QNM-14: *(E)*-3-(5-(((4-fluorophenyl)amino) methyl)-1,3,4-thiadiazol-2-yl)-2-(4-methyl styryl) quinazolin-4(3H)-one

Molecular formula: $C_{26}H_{20}FN_5OS$; molecular weight: 469.53; TLC (Rf value): 0.80; elemental analysis: Found (calculated): Nitrogen (%) 14.95 (14.92); sulfur (%) 6.82 (6.83); oxygen (%) 3.38 (3.41); IR (KBr, cm^{-1}): 3163.3 C-H str.; 822 C-H def (oop); 1691.4 C=O str.; 1568.1 C=C str.; 2911 C-H str.; 3032.5 C-H str.; 1374.6 C-H def. 1438.2 C-C str.; 1600 C=C str.; 1313.7 C-N str.; 1520 C=N str.; 614 C-S str.; 650 C-F str.; 13C NMR (ppm): 113.3, C11 due to styryl group attached to 4-quinazolinone ring; 127.5, C8 due to 4-quinazolinone ring; 128.3, C14 and C18 due to phenyl substituted styryl group attached to 4-quinazolinone ring; 114.5, C9 due to 4-quinazolinone ring and phenyl ring attached to 1,3,4 thiadiazole ring; 137.5, C16 due to phenyl substituted styryl group attached to 4-quinazolinone ring; 127.6, C6 due to 4-quinazolinone ring; 118.7, C15 and C17 due to phenyl substituted styryl group attached to 4-quinazolinone ring and phenyl ring attached to 1,3,4-thiadiazole ring; 132.4, due to phenyl ring attached to 1,3,4 thiadiazole ring; 126.2, C5 due to 4-quinazolinone ring; 133.1, C7 due to 4-quinazolinone ring; 132.1, C13 due to phenyl substituted styryl group attached to 4-quinazolinone ring; 138.2, C12 due to styryl group attached to 4-quinazolinone ring; 148.5, due to phenyl ring attached to 1,3,4 thiadiazole ring; 120.1, C10 due to 4-quinazolinone ring; 158.7, C2 due to 4-quinazolinone ring; 160.2, C4 due to 4-quinazolinone ring; 51.3a, due to CH2–NH attached to 1,3,4 thiadiazole ring; 15.4 CH3 phenyl substituted styryl group attached to 4-quinazolinone ring; and FAB Mass (m/z): 469.23.

QNM-15: *(E)*-3-(5-(((4-bromophenyl) amino)methyl)-1,3,4-thiadiazol-2-yl)-2-(4-methylstyryl)quinazolin-4(3H)-one

Molecular formula: $C_{26}H_{20}BrN_5OS$ molecular weight: 530.44; TLC (Rf value): 0.63; elemental analysis: Found (calculated): Nitrogen (%) 13.19 (13.20); sulfur (%) 6.02 (6.04); oxygen (%): 2.97 (3.02); IR (KBr, cm-1): 3163 C-H str.; 824 C-H def (oop); 1695 C=O str.; 1565 C=C str.; 2909 C-H str.; 3050 C-H str.; 1368 C-H def; 1440 C-C str.; 1600 C=C str.; 1322 C-N str.; 1520 C=N str.; 619 C-S str.; 720 C-Br str.; 13C NMR (ppm): 113.3, C11 due to styryl group attached to 4-quinazolinone ring; 127.5, C8 due to 4-quinazolinone ring; 130.3, C14 and C18 due to phenyl substituted styryl group attached to 4-quinazolinone ring; 114.5, C9 due to 4-quinazolinone ring and phenyl ring attached to 1,3,4 thiadiazole ring; 159.6, C16 due to phenyl substituted styryl group attached to 4-quinazolinone ring; 127.3, C6 due to 4-quinazolinone ring; 115.1, C15 and C17 due to phenyl substituted styryl

group attached to 4-quinazolinone ring and phenyl ring attached to 1,3,4-thiadiazole ring; 132.4, due to phenyl ring attached to 1,3,4 thiadiazole ring; 126.6, C5 due to 4-quinazolinone ring; 133.1, C7 due to 4-quinazolinone ring; 127.6, C13 due to phenyl substituted styryl group attached to 4-quinazolinone ring; 138.7, C12 due to styryl group attached to 4-quinazolinone ring; 148.3, due to phenyl ring attached to 1,3,4 thiadiazole ring; 120.3, C10 due to 4-quinazolinone ring; 158.1, C2 due to 4-quinazolinone ring; 160.7, C4 due to 4-quinazolinone ring; 51.3a, due to CH_2–NH attached to 1,3,4 thiadiazole ring; 15.4, CH3; phenyl substituted styryl group attached to 4-quinazolinone ring; and FAB Mass (m/z): 530.42.

NT screening

Minimal motor impairment was measured in mice by the rotorod test. The mice were trained to stay on an accelerating rotorod that rotates at six revolutions per minute. The rod diameter was 3.2 cm. NT was indicated by the inability of the animal to maintain equilibrium on the rod for at least 1 min in each of the three trials. The data are presented in Table 1.

Anticonvulsant activity of synthesized compounds

Maximal electroshock method

Only 11 compounds were shown protection against MES convulsion at dose level used in the study. Compounds QNM-1, QNM-2, QNM-4, QNM-6, QNM-9, QNM-11, QNM-13, and QNM-15 have shown the effect 30 min after administration of 30 mg/kg of the drug [Table 1]. From the point of view of potency, these drugs can be claimed to be better as comparable to phenytoin and carbamazepine, but failed to show the effect after 4 h of administration. These 11 compounds showed quick onset of action. It is highlighted that the presence of electron rich atom/group attached at the para position of the aryl ring showed increased potency in the MES screen. All the synthesized compounds were active in MES screen for a long duration of time (after 4 h).

scPTZ method

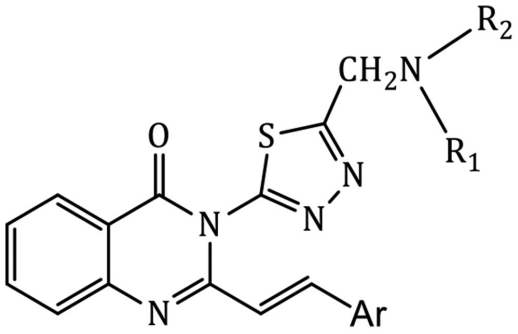
In the ScPTZ model, only six compounds, i.e., QNM-3, QNM-4, QNM-7, QNM-10, QNM-13, and QNM-15 has showed protection of the convulsions but that too at the dose level of 300 mg/kg. Out of these compounds, all other compounds, i.e., QNM-1, QNM-2, QNM-5, QNM-6, QNM-8, QNM-9, QNM-12, and QNM-14 have failed to protect the convulsion after 4 h of administration of the drugs [Table 1].

DISCUSSION

MES test screening involves in various phase of convulsion, compounds QNM-1, QNM-2, QNM-4, QNM-6, QNM-9, QNM-11, QNM-13, and QNM-15 were found most active at anticonvulsant screening when compared with phenytoin (standard drug). Out of all the compounds QNM-7, QNM-8, and QNM-12 have exhibited medium activity whereas rest of the compounds QNM-3, QNM-5, QNM-10, and QNM-14 were found inactive or least active. Compounds QNM-6, QNM-9, and QNM-11 have shown broad level of activity, which is advantageous as compared to phenytoin. QNM-6

Table 1: Anticonvulsant effect of the synthesized compounds (QNM-1 to QNM-15)

QNM-1	30	100
QNM-2	30	-
QNM-4	100	100
QNM-6	30	100
QNM-9	30	100
QNM-11	100	-
QNM-13	30	100
QNM-15	100	300



S. No.	Code No.	Ar	R_1	R_2	Minimum active dose (mg/kg)*				*Neurotoxicity Dose (mg/kg)	
					Maximal electroshock seizure test		scPTZ test			
					0.5 h	4 h	0.5 h	4h	0.5 h	4 h
	QNM-1	$-C_6H_5$	$-C_6H_5$	H	30	100	-	-	-	-
	QNM-2	$-C_6H_5$	$-C_6H_5Cl$ (p)	H	30	-	-	-	-	-
	QNM-3	$-C_6H_5$	$-C_6H_5Cl$ (o)	H	-	300	300	-	-	100
	QNM-4	$-C_6H_5$	$-C_6H_5Cl$ (m)	H	100	100	-	300	-	-
	QNM-5	$-C_6H_5$	$-C_6H_5F$ (o)	H	-	-	-	-	300	-
	QNM-6	$-C_6H_5$	$-C_6H_5Br$ (p)	H	30	100	-	-	-	-
	QNM-7	$-C_6H_5Br$	$-C_6H_5Br$ (p)	H	-	300	300	-	-	-
	QNM-8	$-C_6H_5Br$	$-C_6H_5F$ (p)	H	-	-	-	-	100	-
	QNM-9	$-C_6H_5Br$	$-C_6H_5NO_2$ (p)	H	30	100	-	-	-	-
	QNM-10	$-C_6H_5F$	$-C_6H_5F$ (o)	H	-	300	300	-	100	300
	QNM-11	$-C_6H_5F$	$-C_6H_5F$ (p)	H	100	-	-	-	300	-
	QNM-12	$-C_6H_5F$	$-C_6H_5NO_2$ (p)	H	-	300	-	-	-	-
	QNM-13	$-C_6H_5CH_3$	$-C_6H_5Cl$ (p)	H	30	100	-	300	-	-
	QNM-14	$-C_6H_5CH_3$	$-C_6H_5F$	H	-	-	-	-	100	-
	QNM-15	$-C_6H_5CH_3$	$-C_6H_5Br$	H	100	300	300	-	300	-
Phenytoin					30	30	-	-	100	100
Carbamazepine					30	100	100	300	100	300

*Dose in mg/kg at which bioactivity was observe in majority of the animals. The (−) sign indicates absence of protection of convulsion at the maximum dose administered, i.e., 300 mg/kg.

and QNM-11 have shown the almost similar activity as compare to phenytoin. Thus, we can say that these could block MES thereby suggesting that these could be of use in clonic-tonic (grand mal) seizures.

The anticonvulsant evaluation of synthesized compound QNM-1, QNM-2, QNM-4, QNM-6, QNM-9, QNM-11, QNM-13, and QNM-15 has shown seizure protection at 100 mg/kg dose after 30 min and 4 h, so they have good onset of action as quickly reach brain and have prolonged action reveal that compound metabolized slowly, whereas compound QNM-5, QNM-8, and QNM-14 were somewhat less active and reveals that their high concentration is required to cross blood–brain barrier. Compounds QNM-5, QNM-8, and QNM-14 were less active; this may be due to the presence of bromine group at ortho position and fluorine groups at para and meta position at phenyl ring. In other cases, the substitution of $C_6H_5CH_3$ and $-C_6H_5F$ group attached to 2-position in 4(3H)-benzoxazin-4-one may diminish the activity. After evaluation of minimum active dose of the synthesized compounds in anticonvulsant activity, we further selected 100 mg/kg body weight dose to observe the effect of compounds on different phases of convulsion.

In that pharmacological testing result concluded that phenyl ring at Ar position may provide the actives compound with R_1 position $-C_6H_5$, $-C_6H_5Cl$ (p), $-C_6H_5Cl$ (m), $-C_6H_5NO_2$ (p), $-C_6H_5Br$ (p), in spite that with phenyl ring at Ar position and at R_1 position $-C_6H_5F$ (m), $-C_6H_5Br$ (m) may provide medium activity and but at R_1 position $-C_6H_5Cl$ (o), $-C_6H_5F$ (o), $-C_6H_5Br$(o) may provide the least activity. Compound in which R is electron releasing group such as p-Cl, p-Br, and p-NO_2 groups has shown better activity when compared to o-Cl, o-Br and o-NO_2 substitution at R_1 position that promote the compounds least active. Compounds having chlorine, bromine, fluorine, and nitro in the phenyl moiety have shown good activity when attached to para group but the addition of meta and ortho group of the same may provide least active compounds and in last fluorine compounds have shown comparative less active compounds.

CONCLUSION

This study concluded that these synthesized compounds have potential anticonvulsant activity and other pharmacological activity also prompted. Bulkier compounds are more lipophilic and can

cross blood–brain barrier to exert their effect on CNS. The present study explored that substitution of 4(3H)-quinazolinone at the second and third H position of 4(3H)-quinazolinone leads to the development of new chemical entities with potent sedative-hypnotic as compared to anticonvulsant activity.

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CONFLICTS OF INTEREST

The author declares that they have no conflicts of interest.

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