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Design, Synthesis, Molecular Docking, and Pharmacological Evaluation of 3H-Quinazolin-4-one Derivatives as Potential Antiepileptic Agents

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A B S T R A C T

Epilepsy is a chronic neurological condition characterized by abnormal electrical activity within clusters of neurons in the brain. These irregular signals may remain confined to one region (focal epilepsy) or spread across both hemispheres (generalized epilepsy). The term epilepsy originates from the Greek word epilepsia, meaning "falling sickness," and is often referred to as a seizure, ictus, or convulsion. Quinazoline derivatives have gained attention as promising scaffolds in the design of novel anticonvulsant and central nervous system (CNS) depressant agents. Their pharmacological potential is largely attributed to the presence of an aromatic or aliphatic group at the 2-position and a substituted aromatic ring at the 3-position of the quinazoline nucleus. Among the synthesized derivatives, compounds Zc, Zd, and Ze demonstrated notable antioxidant properties, with IC_{50} values of 7.48, 4.85, and 10.28 µg/mL, respectively. These compounds also displayed strong binding affinities toward carbonic anhydrase II, suggesting a possible structure–activity relationship (SAR). In vivo evaluation using a PTZ-induced seizure model in mice revealed that the same compounds exhibiting strong antioxidant potential also showed marked anticonvulsant activity, effectively mitigating tonic-clonic seizures. Furthermore, results from enzyme inhibition assays and computational docking studies support their potential as lead candidates for future drug development. Overall, the 3H-quinazoline-4-one core emerges as a valuable structural framework for designing new therapeutics with significant CNS and anticonvulsant activities.

Keywords: *Epilepsy, CNS depressant, Quinazoline, Tonic-clonic seizures, Computational studies*.

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INTRODUCTION

Epilepsy is a long-term neurological disorder marked by abnormal electrical signaling within clusters of neurons in the brain. These abnormal discharges may remain confined

to a specific area (focal epilepsy) or spread throughout the brain (generalized epilepsy). The term epilepsy originates from the Greek word epilepsia, meaning “falling sickness,” and is often used interchangeably with seizure, ictus, or convulsion. According to the World Health Organization (WHO), epilepsy affects over 60 million individuals globally. The electrical and behavioral manifestations of seizures vary greatly among patients, reflecting the disorder’s complex nature. The term active epilepsy refers to individuals who experience recurrent seizures, accounting for approximately 0.5–1% of the general population, while 3–5% are estimated to live with the condition at some point in their lives. Seizures may result from nearly any form of brain pathology, including structural, metabolic, or genetic abnormalities. To standardize diagnosis, the International Classification of Epileptic Seizures (ICES) was introduced in 1970 and later revised in 1981, dividing epilepsy into four principal categories based on clinical and electroencephalographic features. (Das et al., 2012). According to this classification epilepsy can be ordered such as idiopathic, cryptogenic, provocated and symptomatic (Erkec and Arihan, 2015).Approximately 60% of all epilepsies are idiopathic.Epilepsy causes neuronal damage preferentially in the hippocampal formation. Sprouting of mossy fibres also occur in epilepsy. These morphological changes may lead to various behavioural and cognitive impairments in epileptic patients (Pohleet al., 1997).

Another characteristic of epilepsy is recurrent seizures, which is caused due to disturbance of electrical discharge in cortical neurons.The ultimate goal is to cure seizures without adverse effects of medication and improved quality of life. The type of drug therapy prescribed depends on the type of seizure, the underlying cause of the epilepsy, age of the patient and possible side effects. Up to 80% of people with epilepsy are able to control their condition with anti-epileptic drugs (AEDs). Long-term AED therapy is required to treat epilepsy (Jayalekshmi et al., 2016). Treatment plan for epileptic patients involves general approaches including management, identification of goals and development of care plan. Empirical therapy is most common practice which involves usage of one drug at a low dose, than dose is increased slowly to achieve the devised goal. Over a period of years if patient is stabilized by dose of AEDs than dose is tampered off to avoid the symptoms of withdrawal. The cellular basis of the disease is still unexplained and still worse, around 30% of epileptic patients are pharmaco-resistant (Remy et al., 2005).

Patho-physiology of epilepsy also involves auto antibodies production via immune response, approximately 1% of patients etiology is of this origin. Already available antiepileptic drugs carry adverse effects which may lead to life threatening conditions and they offer only symptomatic treatment. Effective antiepileptic drugs available in the marketinclude phenobarbital, phenytoin, carbamazepine and valproic acid.These drugs provide improvement to about 70% of people with epilepsy, with satisfactory seizure control but on the other hand these drugs are reported to bear severe side effects including drowsiness, ataxia, gastrointestinal disturbance and megaloblastic anaemia (Zayed et al., 2013).A number of antiepileptic drugs (AEDs) have been licensed for epileptic patients which leads to life threatening conditions due to their dose related toxicities and serious side effects (Kwan et al., 2001).

The choice of AED treatment can be based on the chances of seizure recurrence, the chances of repeat seizures, and the beneficial and harmful effects of the pharmacologic agent chosen. Risk of recurrence is high (up to 90%) in patients with epileptic form discharges on an electroencephalogram or congenital neurologic defects and also in people having past symptomatic seizures. Some patients avoid taking AEDs because they don’t like to take medicines. Treatment may be difficult in drug abusers or who have less compliance with any therapy. These individuals should be counseled appropriately about consequences and expected sudden death due to epilepsy. AED mono therapy is preferred over combination due to benefits of more compliance, reduced side effects, decreased chances of drug related interactions, and less teratogenicity and cost effectiveness. Many antiepileptic drugs are available such as carbamazepine, phenytoin, vigabatrin, sodium valproate, gabapentin and oxcarbazepine which can worsen myoclonic jerks and absence seizures. Best anti epileptic drug must be well tolerated and efficient and having less un-wanted dose related effects like dizziness, ataxia and drowsiness (Stephen et al., 2009).

Antiepileptic drugs (AEDs) primarily function to manage the symptoms of epilepsy rather than cure the underlying cause. Their mechanisms of action generally follow two major pathways: (1) modulation of ion channel activity and (2) indirect regulation of neurotransmitter synthesis, metabolism, or receptor function that influences channel gating. The principal classes of AEDs include sodium channel blockers, calcium channel inhibitors, GABAergic enhancers, and glutamate antagonists (Boshta et al., 2016). Antiepileptic drugs exert their therapeutic effects through multiple mechanisms, including: (a) enhancing γ-aminobutyric acid (GABA)-

mediated inhibitory signaling or acting on other components of the GABAergic system, (b) regulating voltage-gated sodium and calcium channels, (c) modulating the release of neurotransmitters at synapses, and (d) suppressing excitatory synaptic transmission mediated by ionotropic glutamate receptors (Dash et al., 2016).There is an evidence of excitotoxicity due to increase release of glutamate in the hippocampal region of the epileptic brain which contains a number of the kainite receptors (Vincent et al., 2009).

Pentylenetetrazole (PTZ) is utilized to study behavioral changes in brain excitability and different AEDs effect. PTZ induces seizure in animal model and to study drug resistant epilepsy, this model is preferred. Another model is of keen importance that is used for investigating neurochemical and long term structural changes in the brain and known as kindling model. Goddard in 1967, used kindling model as a chronic animal model for temporal lobe epilepsy (TLE). PTZ is claimed to exert its activity by inhibiting gammaaminobutyric acid (GABA) activated channels due to blockade of GABAA gated chloride receptors. It binds to the same site where picrotoxin (PTX) attaches to GABAA receptor and may perform its activity within (TM2) GABA-A receptor subunit second transmembrane domain. Seizure vulnerability and epileptogenesis can be found out by levels of GABA transporters. PTZ also causes an increase in density of glutamate neurotransmitter and formation of IP3 (Inositoltriphosphate) at the hippocampal region. It is reported that development of epileptogenesis result from changes in molecular expression in glutamate transporters. Kindling epileptogenesis also involve role of N-methyl-Daspartate (NMDA) (Figure 1). Activation of glutamate receptor and inhibition of inhibitor GABA neurotransmitters lead to transformation of induced convulsions into generalized form (Erkec and Arihan, 2015).

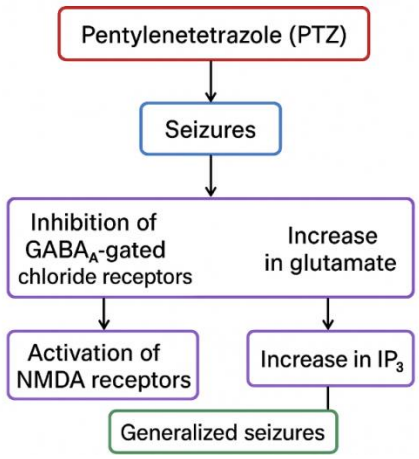


Figure 1: Mechanistic illustration of Pentylenetetrazole (PTZ)-induced seizure pathway showing inhibition of $GABA_a$-gated chloride receptors, increased glutamate release and IP_3 formation in the hippocampus, leading to NMDA receptor activation and progression from focal to generalized seizures.

Quinazoline and its derivatives have a number of medicinal activities like antihypertensive, anti- inflammatory, antitumor activities (Levin et al., 1994; Sharma et al., 2011), antihistaminic, antimicrobial, antifungal, antibacterial, antiallergic (El-Sawy et al., 2012), psychosedative, anticancer and antihypertensive activities (Zayed et al., 2013).

Quinazoline (1, 3-diazanaphthalene) was firstly prepared by Gabriel in 1903 but first derivative was formed by Griess et al. Above described properties show that the quinazoline derivatives may be a source of pharmacophore for new drug evaluation and improve inherent biological activity. It urges us towards synthesis of novel quinazoline derivatives (Chavan et al., 2014). Williamson reviewed the chemistry of quinazoline in 1957 and then Lindquist in 1959 and Armarego up to date the chemistry in 1963. It is stable in cold dilute acid and alkaline solutions (Asif et al., 2014).

Quinazoline derivatives have been involved in the design of new anti-convulsant and CNS depressant agents showed that the pharmacokinetics of the antiepileptic drug are regulated by the involvement of aryl hydrophobic binding site, hydrogen bonding domain and electron donor group. The anti-convulsant and CNS depressant activity is due to the presence of aromatic/aliphatic group at position 2 and

substituted aromatic ring at position 3 because lipophilicity is increased by the presence of aromatic ring. The CNS activity is increased by the methyl/methoxy group substitution in the aromatic ring (Dash et al., 2016). Aryl substitutions at the 3rd position of the quinozoline-Quinazolin-4(3H)-one derivatives have been shown to provide strong protection in various experimental seizure models (Amir et al., 2013). Historically, numerous compounds based on this scaffold have been synthesized and evaluated for anticonvulsant potential, with methaqualone being one of the most well-known examples (Vithal et al., 1964). Previous investigations have demonstrated that aryl substitution at the 3-position of the quinazolin-4(3H)-one nucleus significantly enhances anticonvulsant efficacy (Amir et al., 2013).

The present study aims to design and synthesize new quinazoline derivatives containing a hydrazone pharmacophore and to evaluate how different substituents at the 3-position of the quinazolin-4(3H)-one ring influence anticonvulsant activity.

MATERIALS AND METHODS

Chemicals and solvents

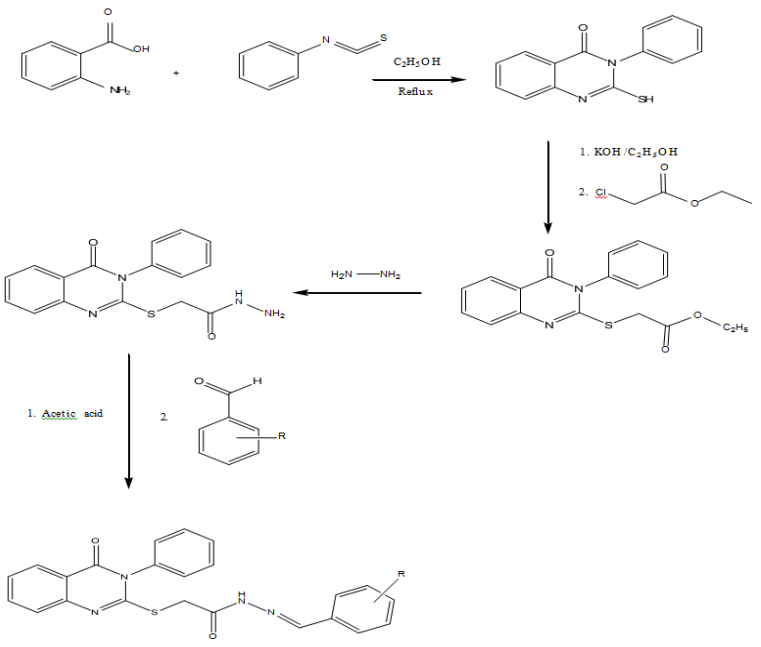
Diazepam (Valium, 10 mg/2 mL injection) was obtained from Roche Pharmaceuticals (Karachi), Anthranilic acid, phenyl isothiocyanite, ethanol, ethyl chloroacetate, potassium hydroxide, pentylenetetrazole, dimethylsulphoxide (DMSO), ascorbic acid, hydrazine, acetic acid and salicylaldehyde were purchased from SigmaAldrich Co. LLC U.S.A. All chemicals used were of analytical grade.

Characterization & Purification

Purity of individual compounds was verified by carrying out TLC technique. Spectrophotometric analysis was used to characterize all the synthesized compounds by including FTIR done on Burker ALPHA FTIR spectrometer with Eco ATR module and ^{1}HNMR on Burker AM300 spectrometer. The NMR spectra will be recorded using deuterated dimethyl sulphoxide (DMSO-d6) or deuterated chloroform ($CDCl_3$) as solvents. Gallenkamp melting point apparatus was used to record melting points.

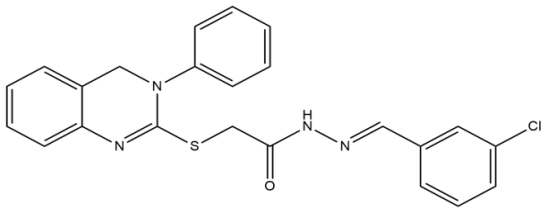
General procedure for preparation of 3H-quinazoline-4-one derivatives

3H-quinazoline-4-one derivativewill be synthesized by following reported method (Amir et al., 2013).The intermediate product 2-mercapto-3-propyl-3*H*- quinazolin-4-ones will be prepared by treating anthranilic acid with phenylisothiocyanate in the presence of ethanol. Treatment of resultant compound with ethylchloroacetate in the presence ofpotassium hydroxide and ethanol and further with hydrazine will yield the hydrazide. Finally hydrazide will be condensed with different aldehydes to furnish the target hydrazone derivatives in the presence of glacial acetic acid (Figure 2).



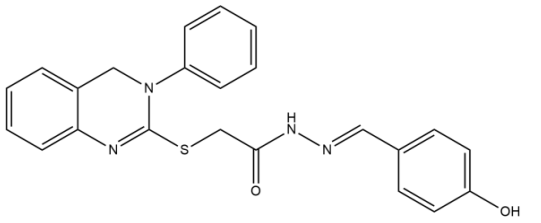
Compound	R
Za	3-Cl
Zb	4-NO_2
Zc	4-Cl
Zd	3-OCH3
Ze	4-OH

Figure 2: Synthesis of 3H-quinazoline-4-onederivatives.



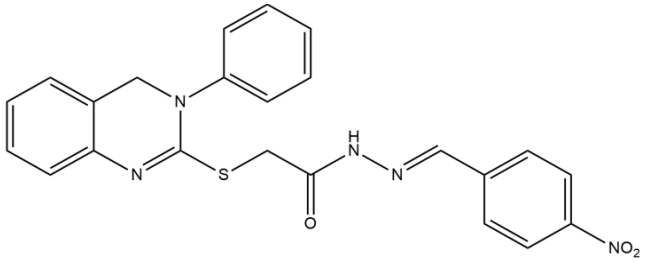
3-chlorobenzylidene-2-(3-phenyl-3,4-dihydroquinazolin-2-yl) thioacetohydrazide

Za



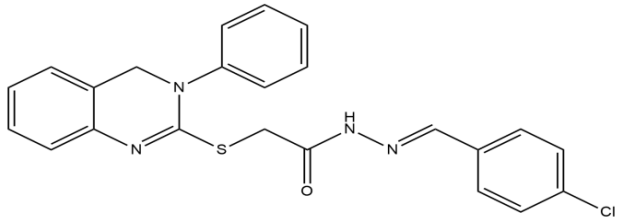
4-hydroxybenzylidene-2-(3-phenyl-3,4-dihydroquinazolin-2-yl) thioacetohydrazide

Ze



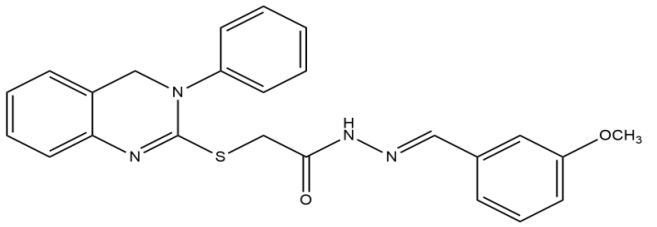
4-nitrobenzylidene-2-(3-phenyl-3,4-dihydroquinazolin-2-yl) thioacetohydrazide

Zb



4-chlorobenzylidene-2-(3-phenyl-3,4-dihydroquinazolin-2-yl) thio acetohydrazide

Zc



3-methoxybenzylidene-2-(3-phenyl-3,4-dihydroquinazolin-2-yl) thioacetohydrazide

Zd

Figure 2: Structures of synthesized compounds.

Pharmacological Evaluation

Animals

Adult balb-C mice of either gender were housed at controlled temperature (22–25℃). Animals having mass in the range of 25-40g were used; these were kept in neat and clean plastic cages and maintained on a standard diet and water. Activities were carried out by dividing animals into different groups.

Antioxidant Assay

FRSA (Free radical scavenging assay)

A DPPH stock solution was prepared by dissolving 9.2 mg of DPPH in 100 mL of methanol. Similarly, an ascorbic acid

stock solution was made by dissolving 1 mg/mL of ascorbic acid in DMSO. For the test compounds, 4 mg of each was dissolved in 1 mL of DMSO to obtain the respective stock solutions.

The assay was conducted following the procedure reported by Bibi et al., to evaluate the free radical scavenging activity of the synthesized compounds, as indicated by the decolorization of the purple DPPH solution. In a 96-well microplate, 10 µL of each test sample was added to the designated wells, followed by 190 µL of DPPH reagent, resulting in a final sample concentration of 40 µg/mL. The plate was then incubated in the dark at 37°C for 60 minutes. Ascorbic acid served as the positive control, while DMSO was used as the negative control. After incubation, the absorbance was recorded at 517 nm using a microplate reader. A decrease in absorbance signified the antioxidant (radical scavenging) potential of the tested compounds. All test samples and controls were further diluted threefold to ensure accurate measurement during the assay (Wazir et al., 2025).

To calculate % radical scavenging activity following formula was used:

$$\%\ \text{scavenging activity} = \frac{1 - \text{Abs}}{Abc} \times 100$$

Where; Abs (Absorbance of the sample), Abc (Absorbance of negative control)

Three-fold serial dilution having concentrations of 20, 10 and 5µg/ml were further tested for samples having %DPPH scavenging activity greater than 50%.

Anticonvulsant activity

All experimental mice were divided into five groups, each comprising five animals (n = 5). Group I received an intraperitoneal injection of normal saline (10 mL/kg) and served as the control. Groups II, III, and IV were administered different synthesized compounds at a dose of 100 mg/kg, while Group V was treated with diazepam (1 mg/kg, i.p.) as a reference standard. After 30 minutes of saline, test compound, or diazepam administration, pentylenetetrazole (PTZ) was injected intraperitoneally at a dose of 50 mg/kg to induce seizures. Each mouse was observed for 30 minutes to record the latency to onset and duration of tonic-clonic seizures, as well as the onset of myoclonic jerks. Compounds exhibiting anticonvulsant potential were identified based on their ability to prolong seizure onset and shorten the duration of tonic-clonic convulsions compared to the control group (Bum et al., 2008). Finally, the percentage (% age) mortality of mice was observed.

$$\%\text{Mortality} = \frac{\text{Number of mice dead after seizures}}{\text{Total number of mice used}} \times 100$$

Molecular docking

All the proposed synthesized ligands were docked with the protein using Auto Dock Vina. Preparation steps are as follow

Preparation of target protein

Docking analysis of synthesized compounds was performed against Carbonic Anhydrase II (PDB ID 1a42), protein structure was downloaded from RCSB Protein Data Bank site, and further structure was cleaned with the help of DSV (Discovery Studio Visualizer). This clean protein molecule was saved in pdb format.

Ligand preparation

Individual ligands were drawn in the Chem Draw software, 3-D optimization of the ligand structures were done and saved in pdbqt format by using Auto Dock Tools.

Docking analysis and visualization of binding affinity

Molecular docking studies were performed using AutoDock Vina to predict the interaction of ligands with the target protein. The software generated the binding energies (kcal/mol) and identified the most favorable binding conformations (Di Muzio, Toti, & Polticelli, 2017). The resulting docked complexes were further examined using Discovery Studio Visualizer, which facilitated visualization of the ligand–protein interactions at the lowest energy poses. The amino acid residues participating in these interactions were identified and the complexes were saved in both 2D and 3D formats to enhance interpretability (Ali Kharl et al., 2025).

RESULTS

The synthesis of 3H-quinazoline-4-ones derivatives was accomplished by scheme 1. Purity of the compounds was confirmed with the help of TLC and structures of the synthesized compounds were elucidated through FTIR and ^{1}H NMR spectroscopy. Synthesized compounds were evaluated for their antioxidant, anticonvulsant and docking studies.

Physical data and Chemo-informatics of synthesized compounds

All the synthesized compounds were solid and obtained in pure crystal form after recrystallization. Melting point of all the compounds, percentage yield and Lipinski's rule was determined. Comprehensive physical chemo-informatic properties are given in Table 1 and 2 respectively. All of the synthesized compounds were analyzed for Lipinski's rule of five, by using SWISS ADME an online tool, none of these compounds deviate from this rule hence these compounds are suitable candidate for oral route.

Table 2 shows different parameters including % absorption,

TPSA, molecular weight, Log P value, number of hydrogen bond donors and acceptors.

FTIR (cm^{-1}) Spectral data of synthesized compounds (Za-Ze)

Functional groups of synthesized compounds were verified using FTIR. Stretching frequencies for were observed at the expected wave number. Oxadiazole C=N peak was present in the range of 1622-1636 cm^{-1}. Table 3 shows the individual IR frequencies of synthesized compounds.

Table 1: Physical properties of synthesized compounds.

Compound	Molecular formula	Color	Physical State	Melting Point	Molecular Weight	% Yield
Za	$C_{23}H_{19}ClN_4OS$	Off-White	Solid	218 °C	434.94g/mol	71%
Zb	$C_{23}H_{19}N_5O_3S$	Green	Solid	176 °C	445.12 g/mol	62%
Zc	$C_{23}H_{19}ClN_4OS$	Off-White	Solid	203 °C	434.94 g/mol	67%
Zd	$C_{24}H_{22}N_4O_2S$	Brown	Solid	187 °C	430.52 g/mol	74%
Ze	$C_{23}H_{20}N_4O_2S$	White	Solid	192°C	416.49 g/mol	58%

Table 2: Chemo-informatics analysis of synthesized compounds.

Compound	% Abs	TPSA(Aº)	Molecular weight	Log p(o/w)	# of H bond donors	# of H bond acceptors
Rule		<140	<500	<5	<5	<10
Za	82.28	82.36	434.94	2.99	1	3
Zb	62.28	128.18	445.12	2.50	1	5
Zc	82.28	82.36	434.94	2.99	1	3
Zd	77.32	91.59	430.52	3.27	1	4
Ze	75.23	102.59	416.49	2.42	2	4

Table 3: FTIR spectral data of synthesized compounds.

Compound	(N-H)	(O-H)	(C=C)	(C=N)	(C-S)
Za	3140	-	1515	1624	661
Zb	3156	-	1567	1616	657
Zc	3178	-	1582	1618	665
Zd	3275	-	1534	1626	667
Ze	3367	3342	1547	1610	658

^{1}H NMR (σ ppm) spectral data of synthesized compounds

3-chlorobenzylidene-2-(3-phenyl-3,4-dihydroquinazolin-2-yl)thioacetohydrazide (Za)

^{1}H-NMR (DMSO, 300MHz, σ ppm): 7.19-8.03(m, 13H, Ar-H), 3.99 (s, 2H, S-CH_2), 8.00 (s, 1H, N- H)

4-nitrobenzylidene-2-(3-phenyl-3,4-dihydroquinazolin-2-yl)thioacetohydrazide (Zb)

^{1}H-NMR (DMSO, 300MHz, σ ppm): 7.19-8.44(m, 13H, Ar-H), 3.99 (s, 2H, S-CH_2), 8.00 (s, 1H, N- H)

4-chlorobenzylidene-2-(3-phenyl-3,4-dihydroquinazolin-2-yl)thioacetohydrazide (Zc)

^{1}H-NMR (DMSO, 300MHz, σ ppm): 6.90-8.03(m, 13H, Ar-H), 3.99 (s, 2H, S-CH_2), 8.00 (s, 1H, N- H)

3-methoxybenzylidene-2-(3-phenyl-3,4-dihydroquinazolin-2-yl)thioacetohydrazide (Zd)

^{1}H-NMR (DMSO, 300MHz, σ ppm): 6.90-8.03(m, 13H, Ar-H), 3.99 (s, 2H, S-CH_2), 8.00 (s, 1H, N- H)

4-hydroxybenzylidene-2-(3-phenyl-3,4-dihydroquinazolin-2-yl)thioacetohydrazide (Ze)

^{1}H-NMR (DMSO, 300MHz, σ ppm): 3.83 (s, 3H, OCH_3), 6.90-8.03(m, 13H, Ar-H), 3.99 (s, 2H, S- CH_2), 8.00 (s, 1H, N-H)

Antioxidant activity

Purple color of 2,2-diphenyl-1-picrylhydrazyl was changed to yellow color due to antioxidant activity of synthesized compounds, those compounds which showed more than

50% scavenging activity were screened out for DPPH IC_{50} and showed significant results. Antioxidant activity shown by synthesized compounds was tabulated in table 4 and figure 3 shows scavenging activity and IC_{50}.

Table 4: Antioxidant activity (% Inhibition) of synthesized compounds.

Compounds	(Concentration µg/ml) (% Inhibition)				IC50 µg/ml
	40 µg/ml)	20 µg/ml)	10 µg/ml)	5 µg/ml)	
Za	43.4	0	0	0	0
Zb	28.7	0	0	0	0
Zc	83.8	71.2	63.2	0	7.48
Zd	67.2	61.3	57.4	52.3	4.85
Ze	93.1	64.3	0	0	10.28

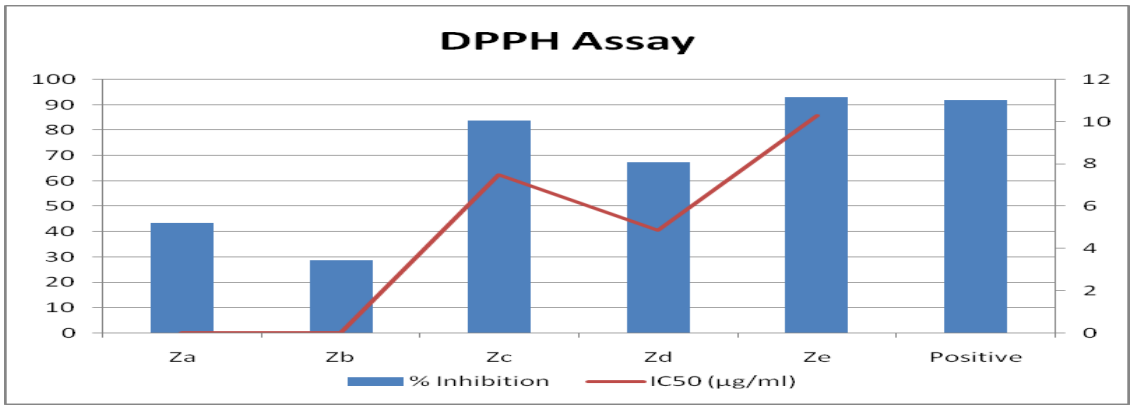


Figure 3: % Inhibition of synthesized compounds and their IC_{50} $^{+}$values.

Anticonvulsant activity

It is important to highlight that this experimental model effectively mimics human tonic-clonic and partial seizures, with or without secondary generalization, and is suitable for identifying compounds capable of preventing seizure propagation. In the current study, test compounds Za–Ze were administered intraperitoneally at a dose of 100 mg/kg, followed 30 minutes later by pentylenetetrazole (PTZ, 50 mg/kg, subcutaneous) injection. This PTZ dose was previously established as the minimum concentration required to induce 100% clonic convulsions in mice.

Compounds that either inhibited seizure onset or prolonged latency to convulsions were considered to exhibit anticonvulsant potential. The phase-I screening results, presented as percentage protection, demonstrated that compound Zd produced the highest protection (82%), followed by Zc (78%) and Ze (73%), as illustrated in Figure 4.

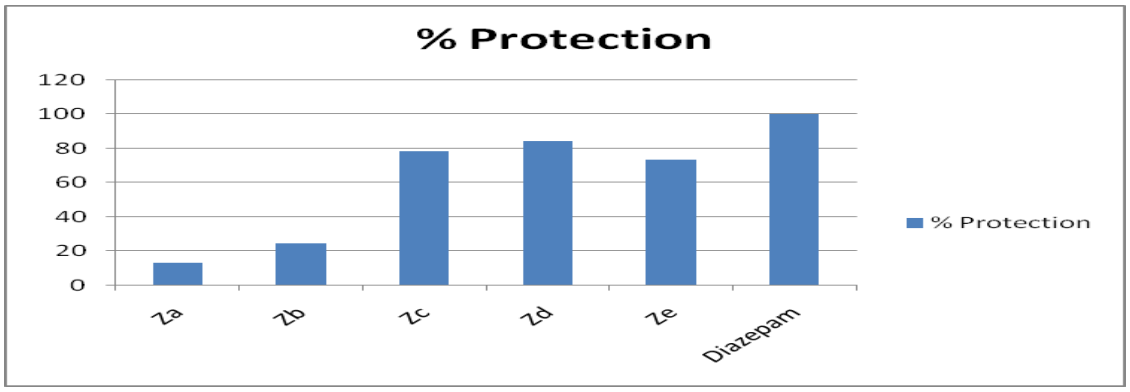


Figure 4: % Protection of synthesized compounds.

Docking of compounds with Carbonic Anhydrase II (PDB ID: 1a42)

Enzyme Carbonic Anhydrase (PDB ID: 1a42) was used for docking the synthesized compounds as discussed in methodology. Binding energies and their interaction with different amino acids were calculated. Binding energies and interaction is shown in table 5.

DISCUSSION

The present study aimed to synthesize and evaluate a new series of 3H-quinazoline-4-one derivatives for their antioxidant, anticonvulsant, and in-silico binding potential. The design strategy was based on incorporating hydrazone pharmacophores and substituting various electron-donating and electron-withdrawing groups at the 3rd position of the quinazoline nucleus, which is known to influence CNS activity through modulation of lipophilicity and receptor affinity. All synthesized compounds (Za–Ze) were successfully characterized by FTIR and ^{1}H-NMR spectroscopy, confirming the formation of the expected hydrazone linkages. The analytical data aligned well with reported values, ensuring structural reliability. The compounds also fulfilled Lipinski's Rule of Five, suggesting their suitability for oral bioavailability and drug-likeness.

Table 5: Binding energy and ligand interaction with amino acids.

Compounds	Binding Energy	Amino acids involved
Za	-7.0	ALA 166, ASP 70, LEU 194, VAL 118
Zb	-7.2	LEU 194, VAL 118, PRO 197, THR 196, HIS 61
Zc	-8.5	ALA 212, VAL 118, 139, PRO197, THR 196
Zd	-7.6	LEU 194, VAL 118, PRO 197, THR 196, HIS 61, 91
Ze	-8.4	LEU 194, VAL 118, THR 196, HIS 61, 91, ALA 62

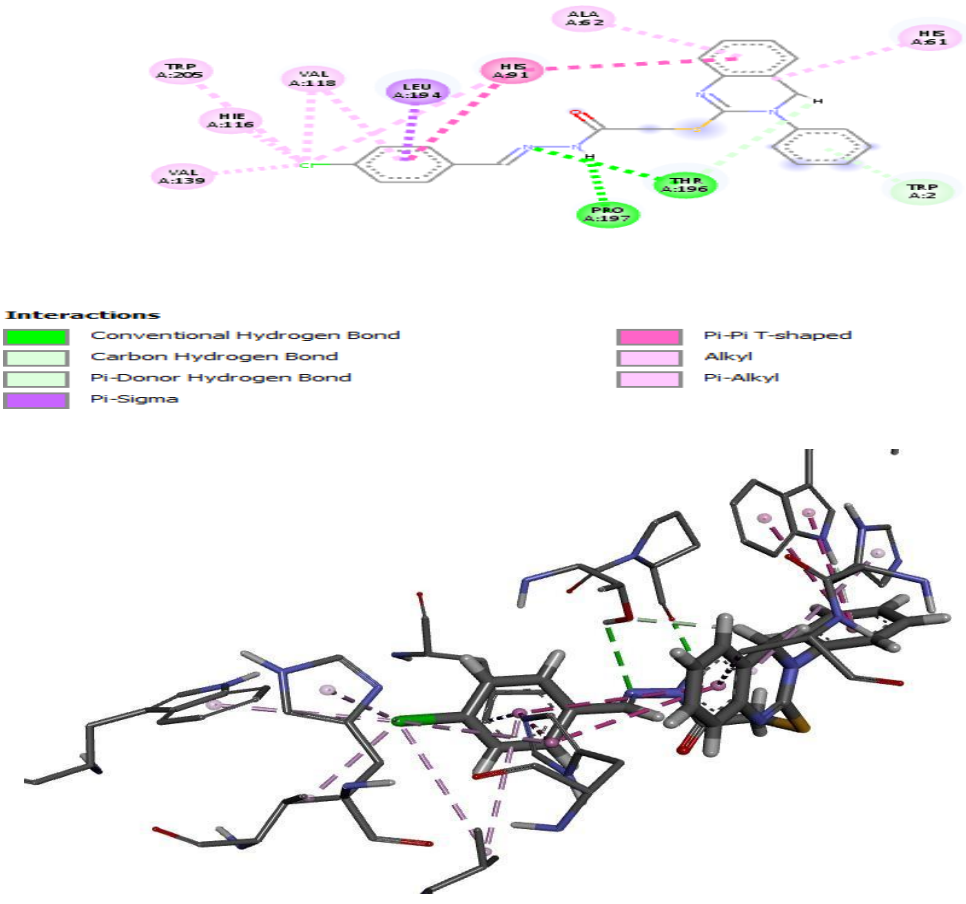


Figure 5: Compound Zc interaction with the active site of carbonic anhydrase II (1a42).

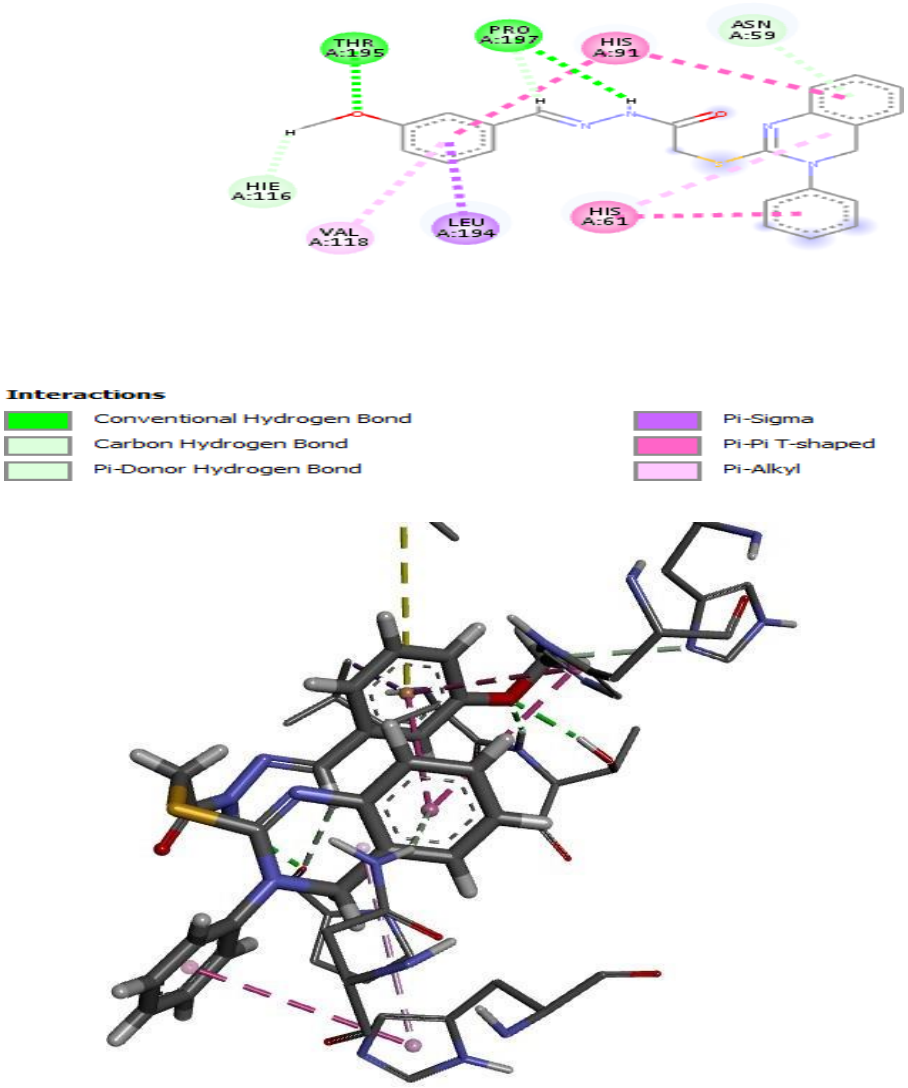
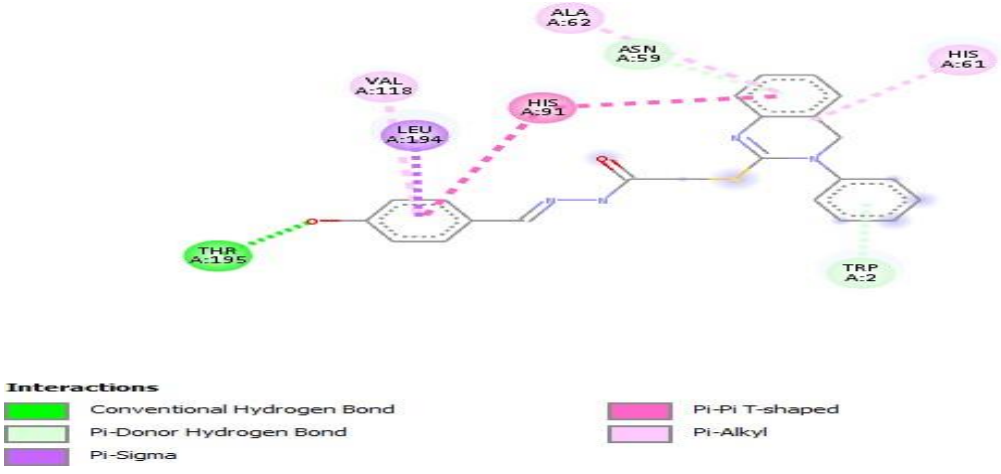


Figure 6: Compound Zd interaction with the active site of carbonic anhydrase II (1a42).



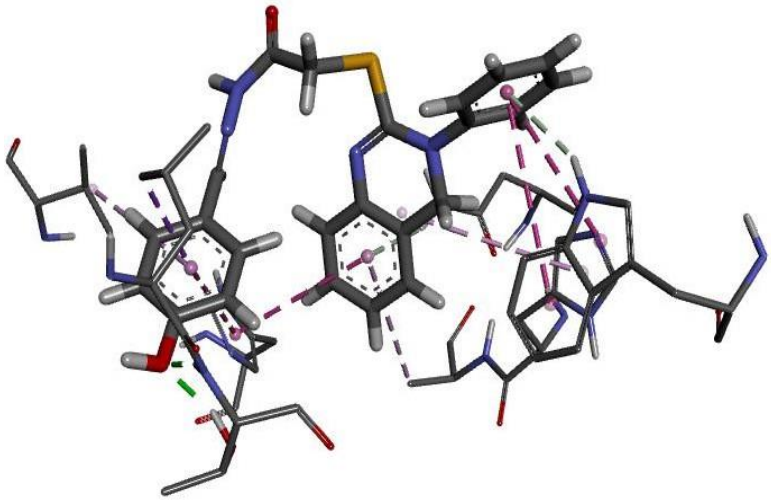


Figure 7: Compound Ze interaction with the active site of carbonic anhydrase II (1a42).

Among the tested molecules, Zc (4-Cl), Zd (3-OCH_3), and Ze (4-OH) exhibited significant free-radical scavenging activity with IC_{50} values of 7.48, 4.85, and 10.28 µg/mL**,** respectively, compared with negligible activity from Za and Zb. This pattern indicates a positive correlation between antioxidant potential and the presence of electron-donating groups (–OCH_3, –OH) or moderately electron-withdrawing substituents (–Cl). These groups may stabilize phenoxy radicals and enhance hydrogen-donating ability, explaining their superior performance. Similar findings have been reported in other heterocyclic antioxidant studies, where methoxy and hydroxyl substitutions enhanced radical-scavenging efficiency via resonance stabilization of reactive intermediates.

The pentylenetetrazole (PTZ)-induced seizure model was employed to evaluate anticonvulsant potential. Compounds Zd, Zc, and Ze exhibited the highest protection against tonic–clonic seizures (82%, 78%, and 73%, respectively). Their superior anticonvulsant activity aligns with their strong antioxidant capacity, supporting the hypothesis that attenuation of oxidative stress contributes to seizure suppression. Moreover, the presence of hydrazone and quinazoline moieties may facilitate GABAergic modulation or carbonic anhydrase inhibition, both of which are established mechanisms in antiepileptic drug design.

Docking simulations against human Carbonic Anhydrase II (PDB ID: 1a42) revealed favorable binding energies for the same compounds—Zc (−8.5 kcal/mol), Ze (−8.4 kcal/mol), and Zd (−7.6 kcal/mol). The key amino acid residues involved in hydrogen bonding and hydrophobic interactions included LEU-194, VAL-118, THR-196, HIS-61**,** and ALA-212. These interactions suggest potential enzyme inhibition that could modulate neuronal excitability via pH and ion-transport regulation, providing a mechanistic link between in-silico and in-vivo results.

The SAR analysis thus implies that moderate lipophilicity and the presence of polar functional groups improve both target binding and pharmacological activity. The combined experimental and computational findings indicate that the 3H-quinazoline-4-one framework provides a promising scaffold for developing multifunctional antiepileptic agents with antioxidant and enzyme-inhibitory profiles.

Although the study provides strong preliminary evidence, further in-vitro enzyme assays and in-vivo pharmacokinetic/toxicity studies are needed to substantiate the proposed mechanism. Expanding docking analysis to additional epilepsy-related targets (e.g., GABA-A receptor subunits, voltage-gated sodium channels) would clarify the mode of action. Future work should also include QSAR modeling to refine substituent effects and optimize potency toxicity balance.

CONCLUSION

In summary, a novel series of 3H-quinazoline-4-one derivatives were synthesized, characterized, and evaluated for pharmacological potential. Compounds Zc, Zd, and Ze demonstrated notable antioxidant and anticonvulsant activities, consistent with their strong binding affinities to carbonic anhydrase II in molecular docking studies. The introduction of methoxy and hydroxyl substituents significantly enhanced biological activity, supporting their role in stabilizing free radicals and improving enzyme

binding. These findings confirm that the quinazoline-hydrazone hybrid nucleus serves as a viable structural template for future antiepileptic drug development. Further optimization through biological screening, toxicity profiling, and multi-target docking could lead to the identification of lead candidates with superior efficacy and safety profiles. The present study therefore provides both a synthetic and mechanistic foundation for the rational design of next-generation anticonvulsant agents.

DECLARATIONS

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Conflict of Interest/Competing Interests

There are no conflicts of interest, whether of a financial or non-financial nature, that could influence the impartiality of the research.

Ethics Approval

It is noted that this investigation did not entail the involvement of either animal subjects or human participants, thereby rendering ethics approval unnecessary.

Consent to Participate

The concept of obtaining consent for participation does not apply to the scope of this study.

Consent for Publication

All the authors have diligently examined and provided their approval for the final version of the manuscript, endorsing its readiness for publication.

AUTHORS' CONTRIBUTIONS

The research was conceptualized and designed through the collective efforts of all authors.

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