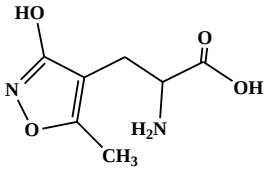
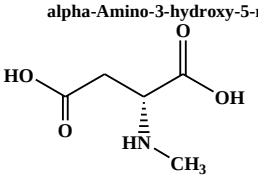
## 2. Literature Review

### 2.1. Glutamate Receptors

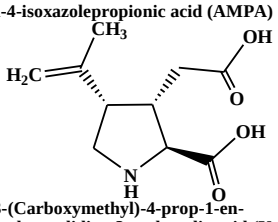
A number of neurotransmitter governs the neuronal processes in brain. The specific neurotransmitters that are associated with the event of epilepsy are GABA, norepinephrine, endogenous opioid peptides, and the excitatory amino acids, such as glutamate and aspartate. Among the various neurotransmitters, GABA and glutamate have been most widely studied with regard to epilepsy (De sarro *et al.*, 2005). The currently available anticonvulsant drugs predominantly target voltage gated cation channels (voltage–gated $Na^+$ and $K^+$ channels, T–type voltage gated $Ca^{2+}$ channels) or accentuate GABA–mediated inhibition. At present, the SV2A synaptic vesicle protein has been recognized as prospective targets (Meldrum and Rogawski, 2007). Drugs that specifically interact with this novel molecular target are levetiracetam and its analogs viz. seletracetam and brivaracetam (Luszczki, 2009). Further, the involvements of ionotropic glutamate receptors (iGluRs) in the pathophysiology of epilepsy have also gained considerable interest in recent years (Jensen *et al.*, 2007; Rogawski, 2011; Steinhoff, 2014). Taking into consideration the excitatory amino acids viz. aspartate and glutamate, glutamate is the primary neurotransmitter in the brain. It mediates excitatory action *via* ionotropic glutamate receptors (iGluRs) and metabotropic glutamate receptors (mGluRs) (Palmada and Centelles, 1998). The important physiological roles of these receptors are maintaining synaptic plasticity, neuronal development, learning and memory. There are three classes of iGluRs: i) *N*–methyl–D–aspartate (NMDA), ii) alpha–amino–3–hydroxy–5–methyl–4–isoxazolepropionate (AMPA) and iii) kainate cation channel receptors. All these receptors mimic the action of endogenous neurotransmitter glutamate (Traynelis *et al.*, 2010) whereas metabotropic receptors (mGluRs) are family G–protein coupled receptors (Niswender and Conn, 2010).




**alpha-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)**




***N*-Methyl-D-aspartic acid (NMDA)**



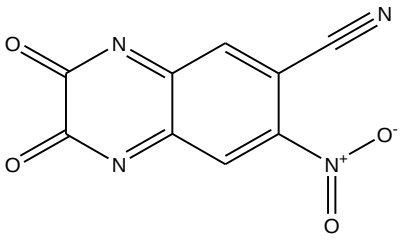

**3-(Carboxymethyl)-4-prop-1-en-2-ylpyrrolidine-2-carboxylic acid (Kainic Acid)**

The initial investigations to develop potential anticonvulsants based on interaction with ionotropic glutamate receptors such as NMDA and kainate receptor were unable to provide satisfactory therapeutic outcome. Later it was found that the ionotropic AMPA receptor is the predominant mediator of excitatory neurotransmission that lead to generation and spread of epileptic seizures (Lees, 2000). Moreover, overstimulation of AMPA receptors is one of the major causes of $Ca^{2+}$ overload in cells. It caused cell damage and death associated with neurodegenerative pathologies i.e. epilepsy, cerebral ischemia, stroke and Parkinson's disease (Russo *et al.*, 2012).

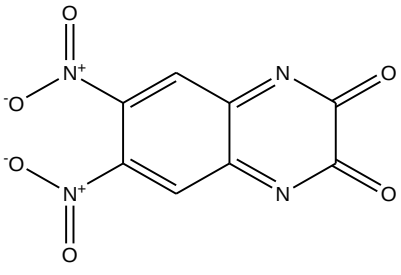
AMPA glutamate receptors are expressed into four subunits, GluR1 to GluR4 into ion channel tetramers. The *N*–terminal domain primarily facilitates the formation of dimer and subsequent tetramerisation involves the extracellular loop and the transmembrane segments (Greger *et al.*, 2007). The ligand binding core of AMPA receptors consists of two domains (S1 and S1) attached by linkers to the cation–channel domain consisting of three membrane–spanning segments (M1–3) and a pore loop (P). The C–terminal domain is located intracellularly. Based on the mechanism of action, drugs acting through AMPA receptors are broadly classified as competitive and non–competitive antagonist. The former class binds to the ligand binding core (glutamate recognition site) of the AMPA receptors and the latter act as negative allosteric modulators at a site that is different from the glutamate recognition site (Bialer *et al.*, 2007) (Figure 1.1.).

## 2.2. Competitive AMPA receptor antagonists

The first competitive AMPA receptor antagonist viz. 6–cyano–7–nitroquinoxaline–2,3–dione (CNQX) and 6,7–dinitro–quinoxaline–2,3–dione (DNQX) were reported in the year 1988.



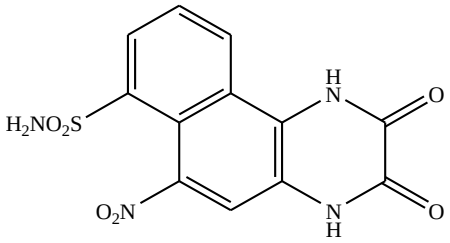
**6-cyano-7-nitroquinoxaline-2,3-dione (CNQX)**



**6,7-dinitro-quinoxaline-2,3-dione (DNQX)**

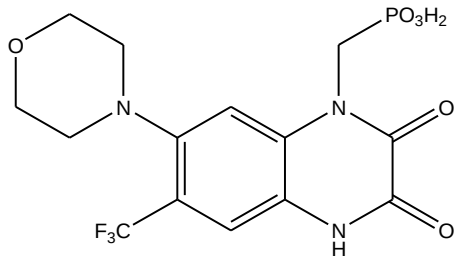
This was followed by the development of more selective antagonist 2,3–dihydroxy–6–nitro–7–sulfamoyl–benzo[*f*]quinoxaline–2,3–dione (NBQX) that demonstrated to have

useful therapeutic effects in animal models of neurological disease (Catarzi *et al.*, 2007).



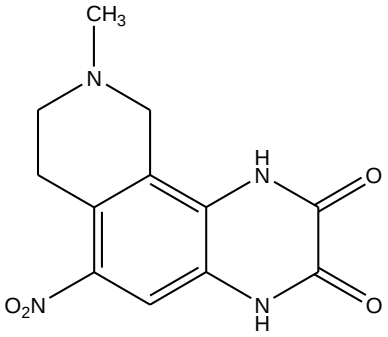
**2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo[f]quinoxaline-2,3-dione (NBQX)**

NBQX showed anticonvulsant activity in the MES and PTZ induced seizure test, but the compound was found ineffective against seizures and lethality induced by 4–aminopyridine, kainate and AMPA (Yamaguchi *et al.*, 1993). NBQX failed in subsequent clinical trials because of nephrotoxicity on account of limited water solubility. Thereafter, the template 2,3–quinoxalinedione became the framework for the design of more potential competitive glutamate antagonists such as ZK200775 with favourable pharmacokinetic profile. It is a water soluble phosphonate quinoxalinedione AMPA antagonist and displayed neuroprotection in stroke and trauma as it alleviated both cortical and hippocampal damage induced by head trauma in the rat (Turski *et al.*, 1998).

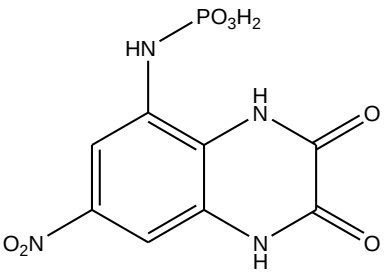


**ZK200775**

Another selective AMPA receptor antagonist PNQX was tested in a model of temporary focal ischemia and was found to demonstrate neuroprotection in rats (Schielke *et al.*, 1999).

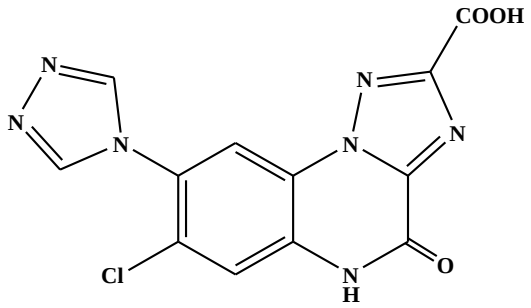


**PNQX**



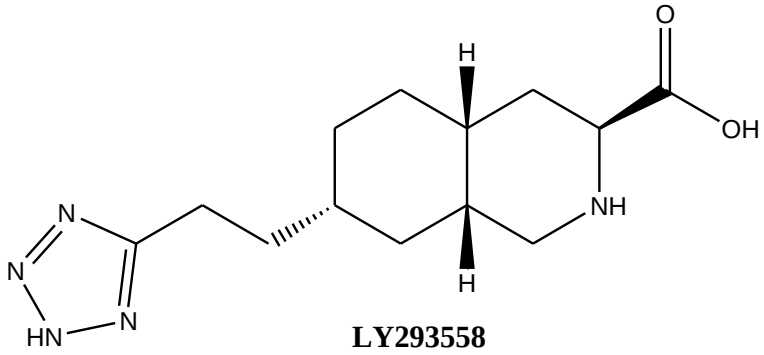
**AMP397A**

With a goal to increase the water solubility of 2,3–quinoxalinedione derivatives, another orally active compound AMP397A having phosphonate functionality was developed with high affinity and selectivity for AMPA receptor. It showed good *in vivo* potency in different animal models of epilepsy (Suter *et al.*, 2002). Besides 2,3–quinoxalinedione, a 1,2,4–triazolo[1,5–*a*]quinoxaline–2–carboxylates derivative (TQX–173) moiety also exhibited potent and selective AMPA antagonistic activity (Catarzi *et al.*, 2000).




**TQX-173**

Further, a decahydroisoquinoline derivative (LY293558) with carboxylic acidic function in the 3–position have also been reported as a potential compound in the treatment of seizures and brain damage (Figueiredo *et al.*, 2011).

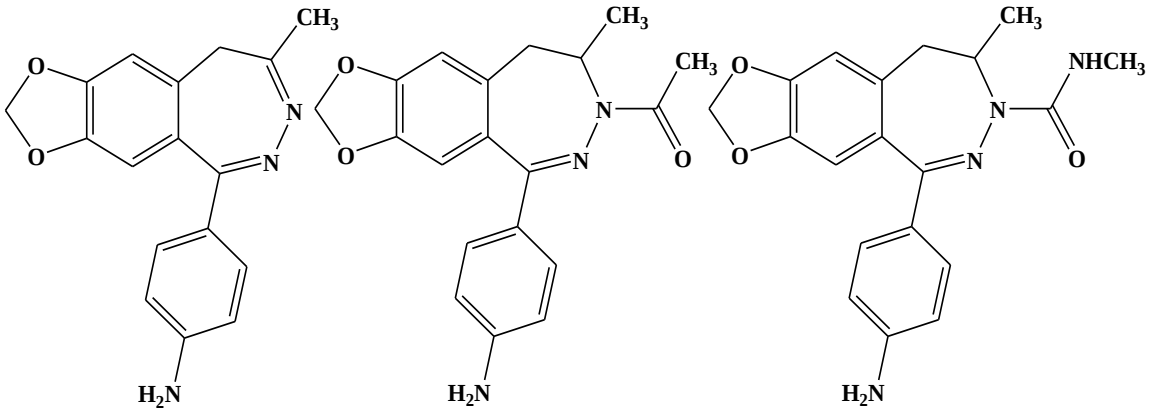



**LY293558**

The molecules that have been developed as selective AMPA receptor antagonist showed their efficacy as neuroprotectant and anticonvulsants. However, majority of the potential AMPA antagonist anticonvulsant developed till date are non–competitive antagonists. The rationality behind this is that in the presence of high glutamate concentration, the competitive antagonist gets displaced from the agonist binding site. In the contrary, non–competitive antagonists can remain effective independently of the level of glutamate. Further, they do not influence the normal glutamatergic activity even after prolonged use (Chimirri *et al.*, 2004).

### 2.3. Non–competitive AMPA receptor antagonists

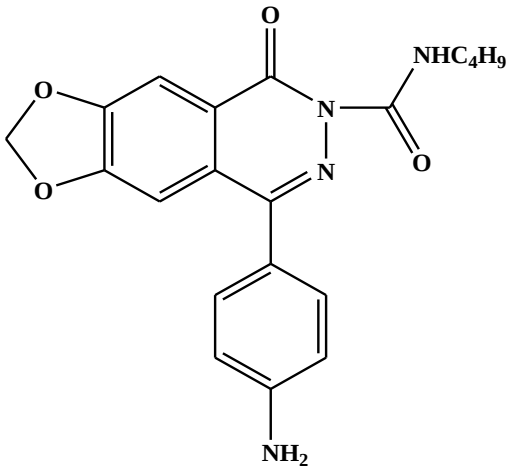
The 2,3–benzodiazepine derivative (GYKI 52466), was the prototype non–competitive AMPA receptor antagonist (Donevan and Rogawski, 1993). This was followed by the discovery of two other *N*–substituted analogs of 2,3–benzodiazepine viz. GYKI 53405 and GYKI 53655. The $ED_{50}$ values of GYKI 52466, GYKI 53405 and GYKI 53655 against MES induced seizure were found to be 6.9, 2.6, and 2.2 mg/kg, respectively.




**GYKI 52466** **GYKI 53405** **GYKI 53655**

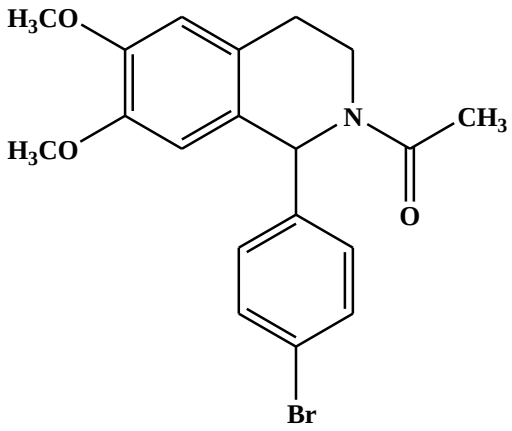
The differences in activity of the analogs indicate that a minor changes in the chemical structure of the compounds result in significant improvement in their pharmacological action (Szabados *et al.*, 2001).

Additionally, based on the framework of 2,3–benzodiazepine derivatives, a series of novel substituted 4–aryl–6,7–methylene–dioxyphthalazin–1(2*H*)–ones have been synthesized and developed as negative AMPAR modulators and observed that most of the reported molecules were active than GYKI 52466. Among the phthalazin–1(2*H*)–ones, the 4–(4–aminophenyl)–2–butylcarbamoyl–6,7–methylene–dioxyphthalazin–1(2*H*)–one showed a longer lasting anticonvulsant activity. This particular compound was reported effective to suppress seizures induced by MES and PTZ and the anticonvulsant activity was 11 fold higher than that of GYKI 52466 (Grasso *et al.*, 2000).



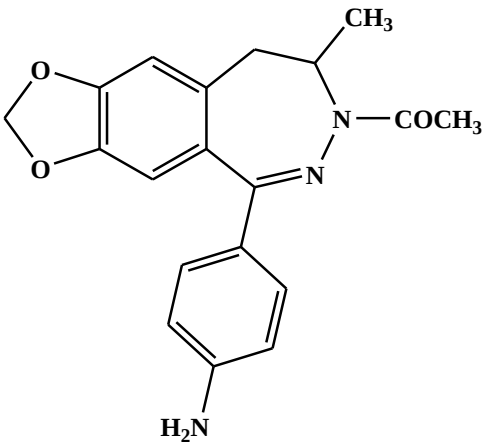

**4-(4-aminophenyl)-2-butylcarbamoyl-6,7-methylene-dioxyphthalazin-1(2*H*)-one**

In another instance, a comparative studies of four *N*–acetyl–1–aryl–6,7–dimethoxy–1,2,3,4–tetrahydroisoquinoline derivatives with established AMPA receptor antagonist for anticonvulsant activities were carried out by Ferreri *et al.* In particular, THIQ–10c was found effective than both competitive (NBQX) and non–competitive antagonist (GYKI 52466) in preventing seizures induced by MES and AMPA with an $ED_{50}$ of 5.17 μmol/kg and 7.9 μmol/kg respectively (Ferreri *et al.*, 2004).

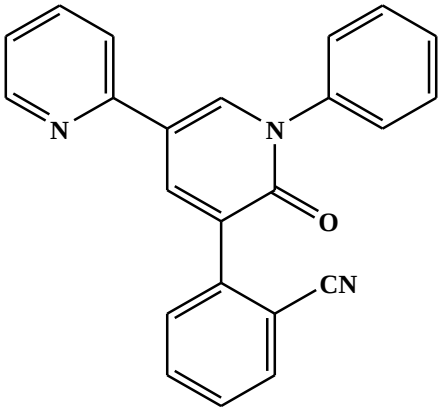



**THIQ-10c**

Thereafter, a major breakthrough was obtained with the development of talampanel and subsequent discovery of perampanel having a 2,3'–bipyridin–6'–one nucleus (Gitto *et al.*, 2003; Hibi *et al.*, 2012).



**Talampanel**

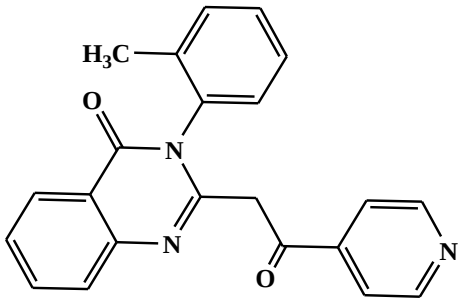


**Perampanel**

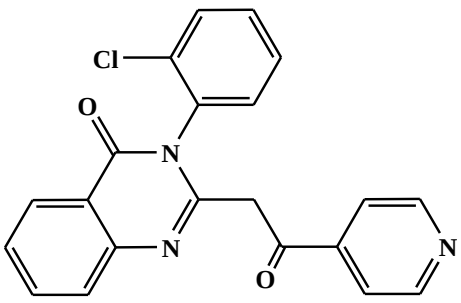
Talampanel has been reported to be well tolerated in adults with refractory complex partial seizures. The activity of talampanel was comparable with the newer anticonvulsant drugs (Howes and Bell, 2007). In the case of perampanel, the drug has been found to be safe as an anticonvulsant after 3 years exposure in 39 countries. Further, seizure responses remained stable with reductions of secondarily generalized seizures (Krauss *et al.*, 2014).

### 2.4. Quinazolinones as anticonvulsants

Quinazolinone derivative such as methaqualone was initially explored for its sedative and hypnotic properties. Methaqualone was found to have sedative and muscle relaxant properties at high dose and at low dose it exhibits an anticonvulsant activity (Boggan *et al.*, 1977). Later, two compounds possessing *N*3 3–*ortho*–tolyl (**I**) and *N*3 3–*ortho*–chlorophenyl (**II**) groups were evaluated for anticonvulsant activity. It showed good protection against MES and scPTZ induced seizures with relatively low neurotoxicity after *i.p.* administration in mice (Wolfe *et al.*, 1990).

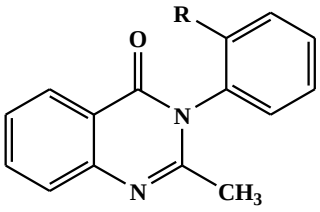


**(I)**

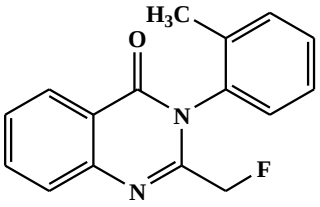


**(II)**

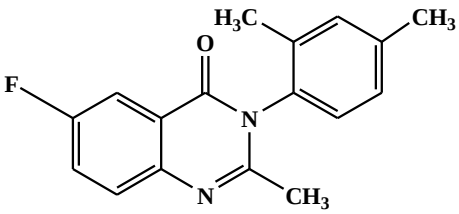
Several other methaqualone analogs have been reported so far such as ethaloqualone, mecloqualone, mebroqualone, afloqualone, methylmethaqualone and piriqualone (Ugale and Bari, 2014). Most of them were utilized for their sedative, hypnotic and muscle relaxant properties, and few were found to causes drug induced photosensitivity related dermatitis (Tokura *et al.*, 1994). The anticonvulsant properties of methaqualone derivatives however were not much exploited because of their associated neurotoxicity.




**Methaqualone, R=$CH_3$**
**Etaqualone, R=$C_2H_5$**
**Mecloqualone, R=Cl**
**Mebroqualone, R=Br**

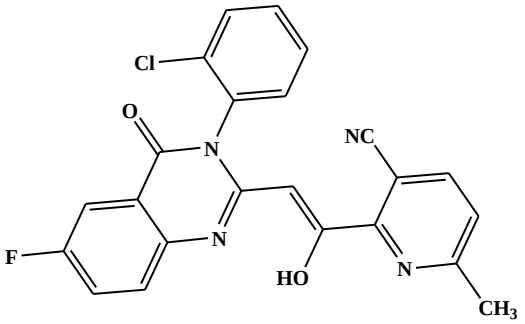



**Afloqualone**




**Methylmethaqualone**

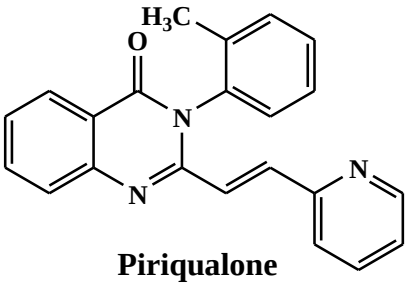
Nevertheless, the activity of the molecules substantiated the CNS active nature of quinazolinone scaffold but the exact mechanism *via* which the methaqualone and its derivatives elicit anticonvulsant activity was unknown. The researchers of Pfizer Inc. finally established that quinazolin–4–one derivative of methaqualone substituted at C2 position showed potential non–competitive antagonistic activity at AMPA receptors. Among the various compounds synthesized and evaluated, the combination of the cyano and methyl substituents was reported as the most potent AMPA receptor antagonist (Chenard *et al.*, 2000).



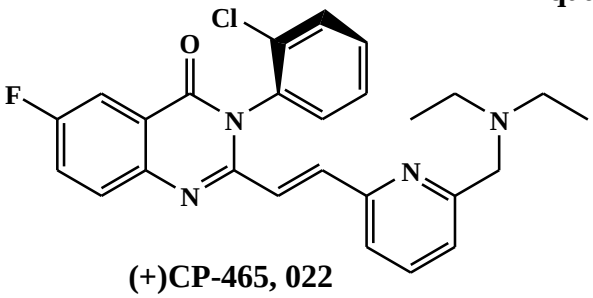

**Quinazolin-4-one derivatives of methaqualone substituted at C2 position**

In an effort to increase the potency and specificity of another methaqualone derivative piraquilone for AMPA receptor inhibition resulted in the identification of a series of novel quinazolinone AMPA receptor antagonists by Groton Laboratories of Pfizer Inc. Among the compounds including CP–465, 022 and CP–526,427, the latter was reported

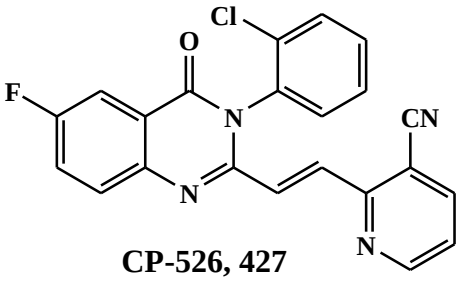
as one of the most potent analog that interact with the allosteric site on the AMPA receptor established through receptor binding assay (Menniti *et al.*, 2000).



**Piriqualone**



**(+)CP-465, 022**



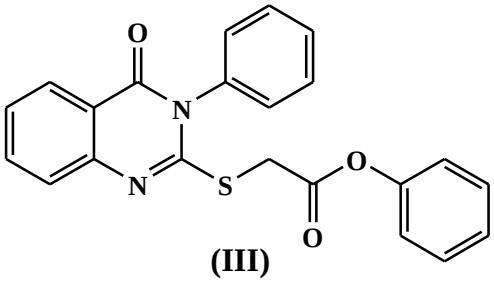
**CP-526, 427**

Subsequently, in a separate study by Groton Laboratories of Pfizer Inc., the atropisomer of CP–465,022, $[\alpha]_D$ = +43.5° was very effective at blocking seizures in mice induced by *sc*PTZ or AMPA and also displayed potent anticonvulsant activity (Welch *et al.*, 2001).

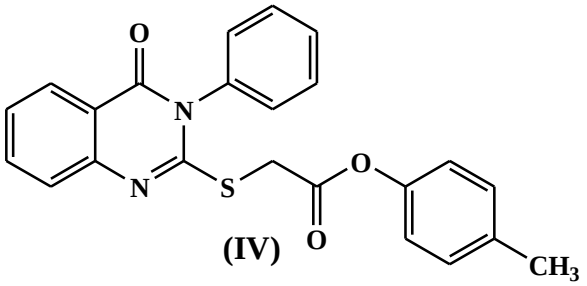
Based on the activity of a series of 6–fluoro–3–(2–chlorophenyl)quinazolin–4–ones, a pharmacophoric model (Chenard *et al.*, 2001) for the quinazolin–4–one class of noncompetitive AMPA receptor antagonists has been proposed which may be summarized as

a) The quinazolin–4–one ring with its small C6 substituent,
b) The orthogonal *N*3 phenyl ring, which must contain a single *ortho* substituent, and
c) The aryl ring attached to C2 through a two–atom spacer.

However, it was found that the presence of small C6 substituent is not that much important for showing anticonvulsant activity as far as substitution at C2 position of quinazolin–4–one is concerned. Some 2–sulfanyl–3*H*–quinazolin–4–one compounds with *N*3 phenyl ring were tested for their anticonvulsant activity against PTZ induced convulsions and compounds **III** and **IV** exhibited the highest anticonvulsant with a relative potency to phenobarbitone sodium of 0.30 and 0.31 respectively (El–Helby *et al.*, 2003).

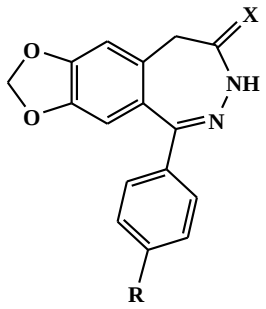


(III)

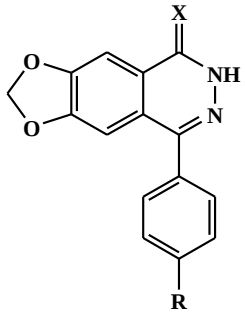


(IV)

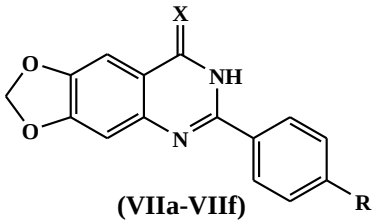
Six 2–aryl–6,7–methylenedioxy–3*H*–quinazolin–4–ones (**VIIa–VIIf**) were designed as structural hybrids considering the scaffold of reported derivatives (**Va–Vf)**, (**VIa–VIf)** and **CP–526,427**.



(Va-Vf)



(VIa-VIf)



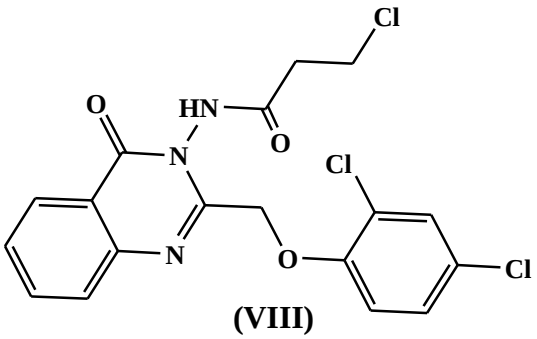
(VIIa-VIIf)

**Va, VIa, VIIa: R = H; X= O**
**Vb, VIb, VIIb: R = F; X = O**
**Vc, VIc, VIIc: R = Cl; X = O**
**Vd, VIe, VIIf: R = Br; X = O**
**Ve, VIe, VIIe: R = $NH_2$; X = O**
**Vf, VIf, VIIf: R = $NH_2$; X = S**

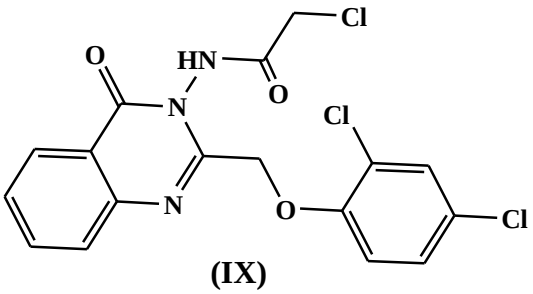
The anticonvulsant activity of **VIIa**–**VIIf** against audiogenic seizures was evaluated 30 min after *i.p.* administration at DBA/2 mice. It was found that the insertion of halogen (fluoro and chloro) in the 4 position of the phenyl ring (VIIb–VIId) increased the potency due to electronic effects. The derivative having more lipophilic thiocarbonyl moiety (**VIIf**) exhibited a decrease in potency. Most of the derivatives reported elicit anticonvulsant activity comparable to that of GYKI 52466. Nevertheless, receptor displacement binding assay with AMPA receptor complexed with radioligand [$^3$H]CP–526,427 revealed that the derivatives were totally inactive (Zappala *et al*., 2003).

From the aforementioned studies, it may be inferred that the *N*3 phenyl ring is an important moiety for eliciting anticonvulsant activity with AMPA antagonistic activity. Certain other quinazolin–4(3*H*)–one derivatives devoid of *N*3 phenyl ring were also reported to sustain the activity. The anticonvulsant activity of the compounds

substituted with phenoxy methyl ring (**VIII** and **IX**) at C2 position without a small C6 substitution retain the activity but the mechanism was not elucidated (Georgey *et al.*, 2008).

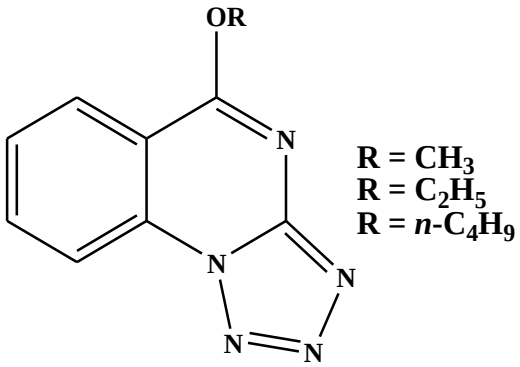



**(VIII)**



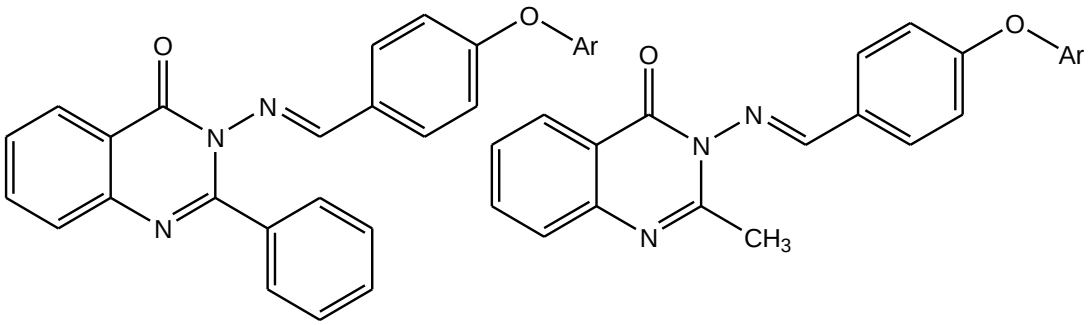

**(IX)**

Although, the compounds (**VIII** and **IX**) does not possess a small C6 substituent, but as a matter of fact the compounds retains the C2 substituent with two atoms spacer. However, in general, any rigidness with the structure of the quinazoline nucleus at this position and without the *N*3 substitution failed to retain the anticonvulsant activity. The fact has been demonstrated by the weak anticonvulsant activity profile of 5–alkoxy–tetrazolo[1,5–*a*]quinazoline derivatives even at a dose of 300mg/kg (Wang *et al.*, 2009).



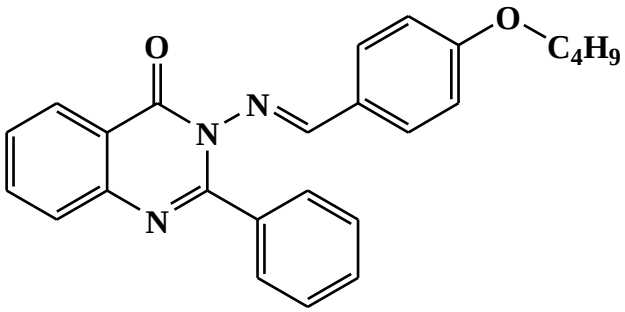

**5-Alkoxy-tetrazolo[1,5-*a*]quinazolines**

On the other hand, some exception to the trend has also been reported as compound having alternative C2 phenyl (**PhQZ 7**) and methyl (**MtQZ 3**) substituent with aryloxyphenyl ring attached with two atom spacer at the *N*3 position displayed potent activity. The compound PhQZ showed 100% protection (4/4, 0.5 h) and 75% protection (3/4, 0.25 h) at a dose of 100 mg/kg in mice against 6 Hz psychomotor seizure test. Computational studies revealed that **MtQZ 3** has exhibited good binding properties at glutamate receptor (-7.8 kcal/mol) while the affinity of **PhQZ 7** was found as -7.1 kcal/mol for $GABA_A$ delta receptor (Kumar *et al.*, 2011).




**PhQZ 7; Ar = 3-$CH_3$, 4-Cl phenyl** **MtQZ 3; Ar = 4-$CH_3$ phenyl**

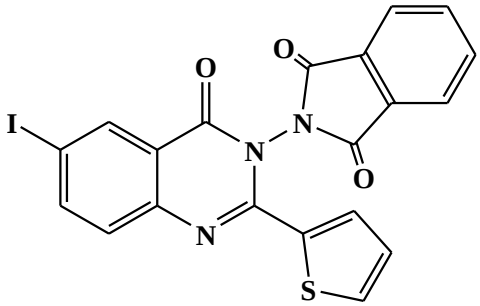
Similar results were obtained with compounds having C2 phenyl substitution and incorporating Schiff base pharmacophore at the *N*3 position of quinazolin–4–(3*H*)–ones. Herein instead of aryloxy phenyl, a series of compounds bearing variable alkyloxy phenyl substituents were attached at the *N*3 position and the anticonvulsant activities were evaluated using the MES and scPTZ tests. The compound 3–[(4–butoxy–benzylidene)–amino]–2–phenyl–3*H*–quinazolin–4–one showed good protection at 30 mg/kg (0.5h) against MES and scPTZ induced seizures. Further, mechanism based studies on the whole brain GABA estimation suggests that the anticonvulsant activity of quinazolinone derivatives having Schiff base pharmacophore might be due to an increased GABA concentration and GABA mediated inhibitory action (Amir *et al*., 2013).




**3-[(4-butoxy-benzylidene)-amino]-2-phenyl-3*H*-quinazolin-4-one**

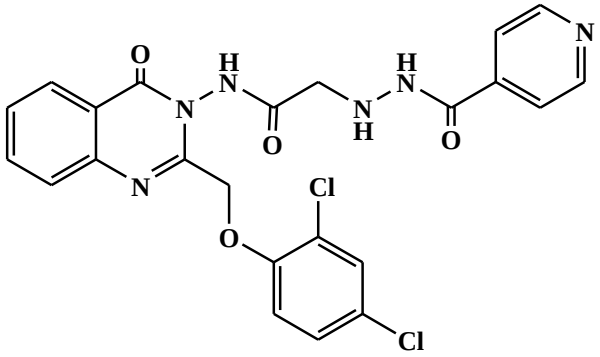
Therefore, an interesting feature of compounds substituted with Schiff base at *N*3 position of quinazolin–4(3*H*)–ones enables PhQZ 7 and 3–[(4–butoxy–benzylidene)–amino]–2–phenyl–3*H*–quinazolin–4–one) to elicit their anticonvulsant activity *via* GABA mediated inhibition instead of conventional suppression of glutamate excitation. Henceforth, it may be suggested that appropriate substitution at C2 and *N*3 position of quinazolin–4(3*H*)–ones is necessary for AMPA receptor inhibition.

Recently a new series of quinazolinone analogs was screened for their anticonvulsant activity and it was reported that the compound with *N*3 isoindoline–1,3–dione displayed a two fold increase in activity than the standard drug sodium valproate with an $ED_{50}$ value of 251 mg/kg and a high therapeutic index of 5.50 (El–Azab *et al*., 2013).



**2-(6-iodo-4-oxo-2-(thiophen-2-yl)quinazolin-3(4*H*)-yl)isoindoline-1,3-dione**

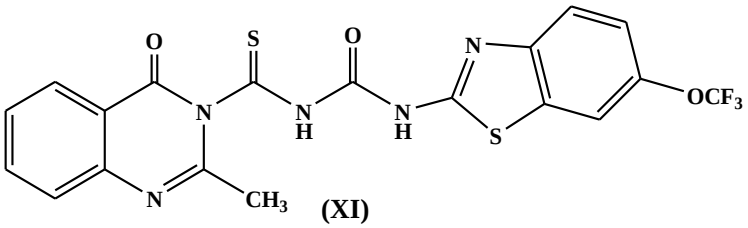
In another studies, among various 2,3–disubstituted quinazolinone derivatives evaluated for anticonvulsant activity, the most active compound was the *N*3 hydrazino acetamide derivative (**X**) having a protective dose 50 ($PD_{50}$) of 200.53 µmol/kg and possess a much higher safety profile ($LD_{50}$ >3000 mg/kg) when tested against *sc*PTZ induced seizures (Abbas *et al*., 2013).



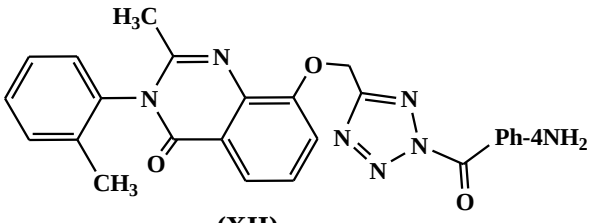
**(X)**

Malik *et al.* designed and synthesized several 4–oxoquinazoline–3(4*H*)–carbothioamide derivatives on the basis of hybrid pharmacophore approach by connecting benzothiazole nuclei and quinazolin–4(3*H*)–one within a single molecular framework by urea and carbothio linkage. Among the various derivatives assessed after oral administration in rats, 2–methyl–4–oxo–*N*–(6–(trifluoromethoxy)benzo[*d*]thiazol–2–ylcarbamoyl)quinazoline–3(4*H*)–carbothioamide (**XI**) emerged as the most active compound with an $ED_{50}$ values of 82.5 mmol/kg (MES) and 510.5 mmol/kg (scPTZ). Further, the compound was found effective in showing protection against AMPA induced seizures with an $ED_{50}$ of 28.5 µmol/kg and also increase the GABA

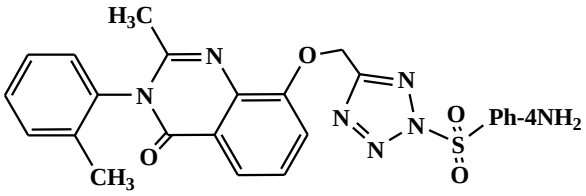
concentration as determined by GABA assay. This suggests that the derivatives under consideration interact with multiple receptors. Additionally, two more potent molecules (**XII** and **XIII**) having quinazolino–tetrazole as a hybrid structure with potent anticonvulsant activity was also reported (Malik *et al*., 2013; Malik and Khan, 2014).




**(XI)**




**(XII)**




**(XIII)**

As indicated above, over a period several quinazolin–4(3*H*)–ones were reported to have anticonvulsant activity ranging from moderate to high potency. The available literatures intriguingly link the epileptic seizure with glutamate. Establishing a potent drug to specifically and effectively antagonize AMPA receptor and devoid of potential adverse effect would emerge as successful therapies for epilepsy as exemplified by the drug perampanel. However, substantiation on the mechanism reinforcing the relationship between structure of quinazolinones and AMPA receptor antagonism is sparse. To date, majority of the approaches in developing quinazolinones as anticonvulsant is through modification at C2 and *N*3 position. Moreover, it has been observed that compounds lacking the *N*3 phenyl ring were not as much effective from those having an aryl ring at that particular position of quinazolin–4(3*H*)–ones. With a plethora of molecules available, prospects of structural developments are still amenable to design and fructify quinazolinone based anticonvulsants with high therapeutic index over the coming years.

### 2.5. Quinazoline derivatives in other diseases

Quinazolin–4–one derivatives have also been reported as possessing a wide range of biological activities e.g. inflammation, anti–Alzheimer, anticancer, antihypertensive,

etc. Some of the recently reported activities showed by quinazolin–4–one derivatives are listed in Table 2.1.

**Table 2.1.** Quinazoline derivatives and their biological activities

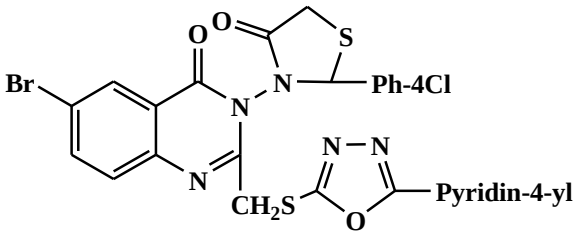
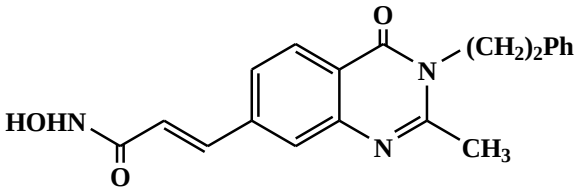
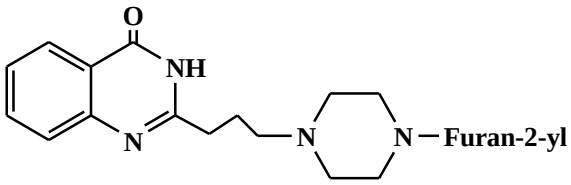
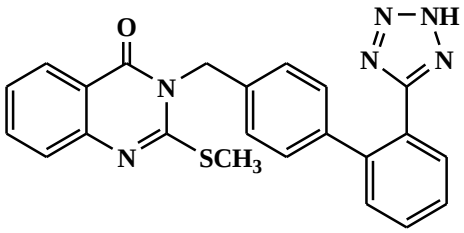
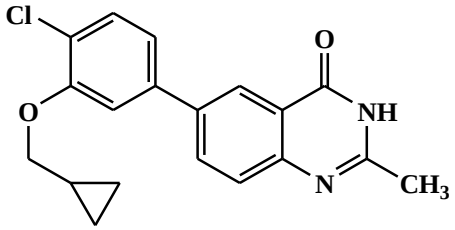
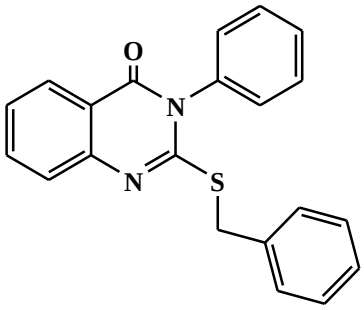
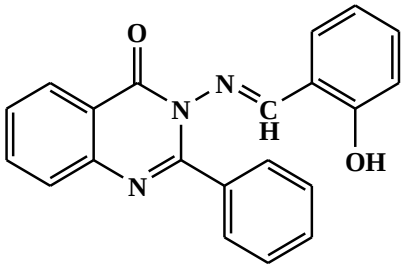
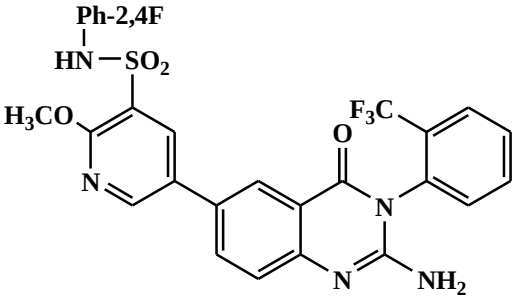
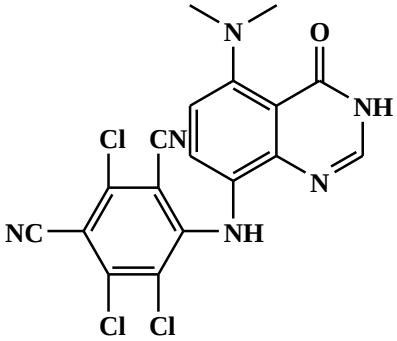
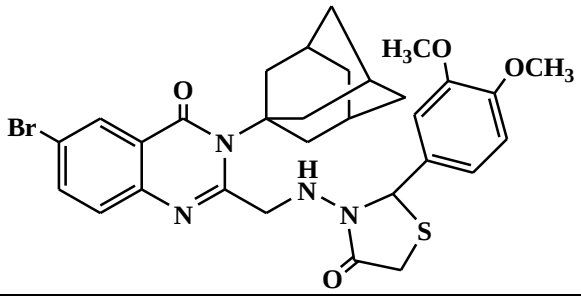
| Sl. No. | Compound | Biological Activity | Reported By |
|---|---|---|---|
| 1. |  | Anti–inflammatory activity. | Kumar and Rajput, 2009 |
| 2. |  | Selective histone deacetylase–6 inhibitor for the treatment of Alzheimer's disease. | Yu *et al.*, 2013 |
| 3. |  | Selective poly(ADP–ribose)polymerase–I inhibitor for the treatment of cancer. | Giannini *et al.*, 2014 |
| 4. |  | Angiotensin II $AT_1$ receptor antagonists for the treatment of hypertension. | Ismail *et al.*, 2006 |
| 5. |  | Vanilloid receptor–1 blocker for the treament of chronic nociceptive and neuropathic pain. | Culshaw *et al.*, 2006 |
| 6. |  | Antiplatelet aggregation activity for the treatment of thromboembolic disorders. | Eskandariyan *et al.*, 2014 |

**Table 2.1.** (Contd.)

| | | | |
|---|---|---|---|
| 7. |  | Antiviral activity. | Kumar *et al.*, 2010 |
| 8. |  | Type III phosphatidylinositol 4–kinase alpha inhibitors as anti hepatitis C (HCV) agents. | Leivers *et al.*, 2014 |
| 9. |  | Antimicrobial activities | Shi *et al.*, 2013 |
| 10 |  | Antiparkinsonian activity | Kumar *et al.*, 2012 |