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REVIEW PAPER

Synthesis and evaluation of some new 1,2,4-triazolo(4,3-*a*) quinoxalin-4-5*H*-one derivatives as AMPA receptor antagonists

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Abstract This study involves the synthesis and anticonvulsant evaluation of 1-ethyl-3-hydrazinyl-quinoxalin-2-1*H*-one (**8**), and many other newly synthesized compounds (**9–14**). The structure of the synthesized compounds was confirmed by elemental analysis and spectral data (IR, ^{1}H NMR and Mass). Docking studies were performed to all the synthesized compounds in order to rationalize the anticonvulsant activity of the proposed compounds in a qualitative way. There is a strong correlation between the results of molecular modeling and the anticonvulsant activity of the synthesized compounds. The highest fitting value was noticed for compounds **9** and **10** "which showed the highest anticonvulsant activity".

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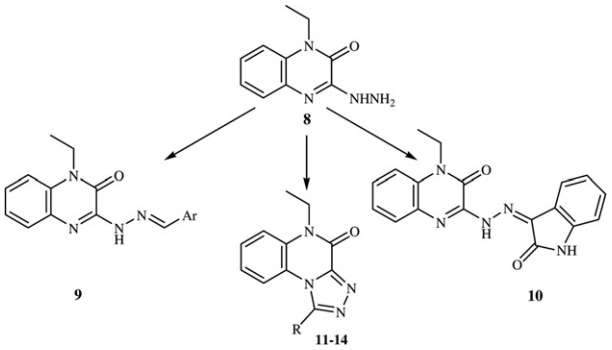


Contents

1. Introduction

Synthesis of quinoxaline derivatives has attracted a great deal of attention in view of their potent pharmacological activities.[1] Approximately 1% of the world's population is affected by epilepsy.[2] Therefore, investigation of new anticonvulsant is still a challenge.

Most of the currently used anticonvulsant drugs are associated with adverse effects, such as sedation, ataxia and weight loss or weight gain. Rare adverse effects can be life threatening such as aplastic anemia.[3] The development of safer and more effective new anticonvulsant drugs is necessary.

It was reported that, the majority of anticonvulsant agents mediates their effect through their action either by activation of the γ-aminobutyric acid (GABA) receptor or by inhibition of the glutamate receptor.[4] Glutamate receptors are classified into two main subtypes, *N*-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors.[5]

In fact, NMDA receptor antagonists may produce schizophrenia-like symptoms, perceptual alterations, and memory impairment; AMPA receptor antagonists have no such psychoactive properties.[6]

On the other hand, from the literature survey it was found that many quinoxaline derivatives showed anticonvulsant activity.[7,8]

Furthermore, Compounds **1** and $\mathbf{2_{a-d}}$ have shown high affinity toward AMPA receptor.[8] Similarly; compound **3** (Fig. 1) was reported as a potent AMPA receptor antagonist.[9] In the present work, it was decided to synthesize a series of 1,2,4-triazolo(4,3-*a*)quinoxalin-4-5*H*-one derivatives expected to have AMPA receptor antagonistic activity. Beside, new

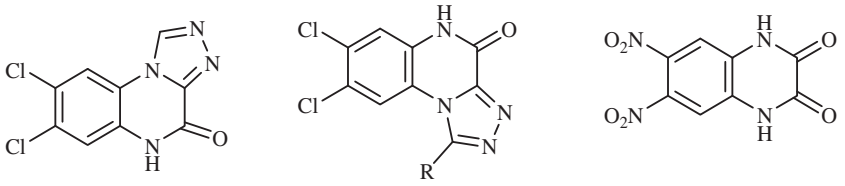
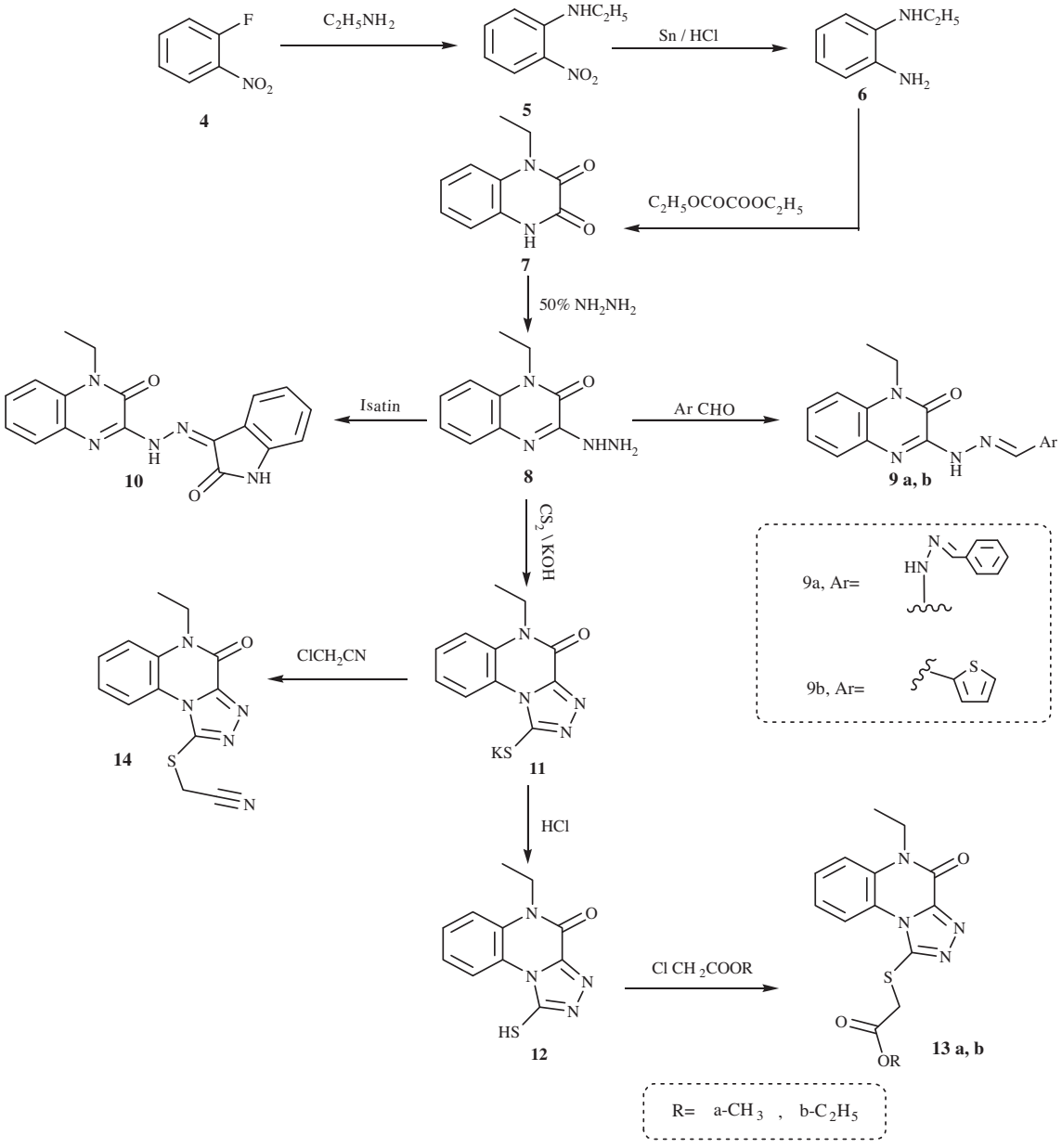


Fig. 1 Compounds have shown high affinity toward AMPA Receptor.

Schiff's bases $\mathbf{9_{a,b}}$ and the indolone derivative **10** were prepared to confirm the chemical structure of the hydrazino derivative **8** in addition to its spectral data. For preparation of the new derivatives Scheme 1 was adopted.

2. Chemistry

1-Ethyl-2-oxoquinoxalin-3-yl hydrazine (**8**) is the key intermediate for the synthesis of the new compounds.[10] Reaction of



Scheme 1

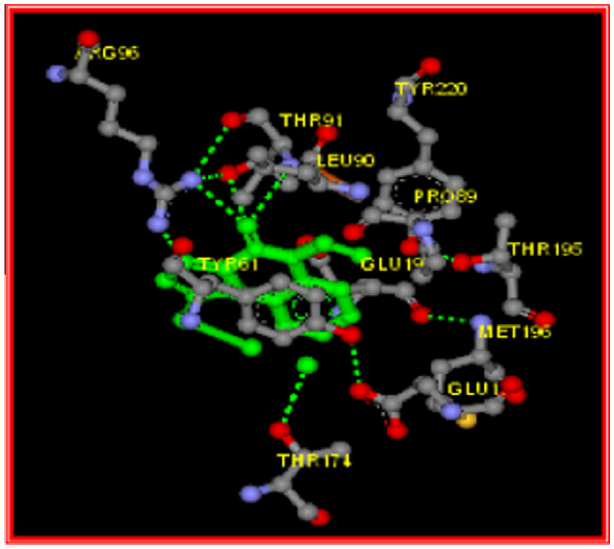


Fig. 2 Compound **12** drawn in green color and docked inside active site. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

intermediate (**8**) with a solution of the appropriate aldehydes in ethanol afforded the crystalline products of (**9**$_{a,b}$), while reaction with isatin in glacial acetic afforded (**10**). 5-Ethyl-1-mercapto-1,2,4-triazolo[4,3-*a*]quinoxalin-4-5*H*-one (**12**), was prepared by reaction of 1-ethyl-2-oxoquinoxalin-3-yl hydrazine (**8**) with carbon disulphide and potassium hydroxide in ethanol and then treated with HCl. The reaction mixture was treated with appropriate alkyl chloroacetate and anhydrous potassium carbonate in DMF to afford the corresponding alkyl (5-ethyl-4,5-dihydro-4-oxo-1,2,4-triazolo [4,3-*a*]quinoxalin-1-ylthio) acetate derivatives (**13**$_{a,b}$). Potassium salt of 5-ethyl-1-mercapto-1,2,4-triazolo[4,3-*a*]quinoxalin-4-5*H*-one (**11**) when treated with chloroacetonitrile in DMF afforded 5-ethyl-4,5-dihydro-4-oxo-1,2,4-triazolo[4,3-*a*]-quinoxalin-1-ylthioacetonitrile (**14**).

2.1. Docking study

All the target compounds were subjected to docking study to explore their binding mode to AMPA receptor, since AMPA is a target for a remarkable variety of anticonvulsant agents.[8,11] All modeling experiments were performed using Discovery Studio program version 2.5. Each experiment used the biological target coordinates downloaded from the Brookhaven website (PDB: 1FTL).[10] In order to qualify the docking results in terms of accuracy of the predicted binding conformations in comparison with the experimental procedure, the internal ligand (7, 8-dichloro-1-propyl-1,2,4-triazolo[4,3-*a*]quinoxalin-4-5*H*-one) **2c** was used as a reference molecule. The docking study has been conducted to predict the binding mode and to rationalize the observed biological activity.

From the synthesized compounds, those having good binding opportunities according to docking experiments were subjected to *in vivo* anticonvulsant evaluation on different groups of mice. Compound **12** found to have the highest potency in protection of animals against induced seizures. In order to rationalize the high potency of this compound, docking into the active site is illustrated in Fig. 2. The carbonyl group at position 4 established critical H-bond to THR-90 and ARG-96.

2.2. Experimental

All melting points were taken on electrothermal (IA 9000 SERIS) digital melting point apparatus and are uncorrected. IR spectra were recorded on Pye Unicam SP 1000 IR spectro-photometer at Microanalytical Center, Cairo University. The ^{1}H NMR spectra were recorded in DMSO-d_6 at 300 MHz, and ^{13}C NMR spectra were recorded in DMSO-d_6 at 75 MHz on a Varian Mercury VXR-300 NMR spectrometer at Research Services Unit, Faculty of Science, Cairo University. Chemical shifts were related to those of the solvent. Tetramethylsilane (TMS) was used as a standard. Mass spectra were recorded on Hewlett Packard 5988 spectrometer at Regional Center for Mycology and Biotechnology, Al-Azhar University. Microanalyses were carried out at Microanalytical Center, Cairo University. Progresses of the reaction were monitored by tlc using tlc sheets precoated with UV fluorescent silica gel Merck 60 F254 plates and were visualized using UV lamp and n-hexane: ethyl acetate 9:1 as mobile phase.

N-Ethyl-2-nitroaniline (**5**), *N*-ethyl-*O*-phenylendiamine (**6**) and 1-ethylquinoxaline-2,3(1*H*,4*H*)-dione (**7**) were prepared according to the directions of Lin.[12]

*2.2.1. 1-Ethyl-2-oxoquinoxalin-3-yl hydrazine (**8**)*

A mixture of 1-ethylquinoxaline-2,3(1*H*,4*H*)-dione (**7**) (1.90 g, 0.01 mol) and hydrazine hydrate (10 mL) (50%), was refluxed for 2 h. After cooling, a crystalline product was obtained which collected by filtration, washed with water several times and dried. Recrystallization of the resulting product from ethanol afforded faint yellow crystalline needles, 1.93 g, (95%); mp: 165–167 °C. IR: NH_2 3339; CO 1645 cm^{-1}; ^{1}H NMR: δ 1.17 (t, 3H, C**H$_3$**—CH_2, J = 7.20 Hz), 4.15 (q, 2H, C**H$_2$**—CH_3, J = 7.20 Hz), 4.52 (s, 2H, N**H$_2$**) (D_2O exchangeable), 7.17–7.44(m, 4H, aromatic), and 8.68 (s, 1H, N**H**), (D_2O exchangeable); ^{13}C NMR: δ 12.30, (CH_3, aliphatic), 36.44, (CH_2, aliphatic), 113.93, 123.34, 123.43, 125.13, 127.80, 133.87, (benzene ring), 148.84 (amidic C=O), 149.96 (C3 in quinoxaline ring); Ms: m/z 204 (molecular ion) (100%), 189 (3.90%), 175 (13.62%), 145 (5.90%), 147 (28.47%). *Anal.* Calcd. for $C_{10}H_{12}N_4O$: C, 58.81; H, 5.92; N, 27.43. Found: C, 58.63; H, 5.80; N, 27.65.

*2.2.2. General procedure for the reaction of 1-ethyl-2-oxoquinoxalin-3-yl hydrazine (**8**) with different aromatic aldehydes*

All these reactions were carried out in ethanol. To a solution of 1-ethyl-2-oxoquinoxalin-3-yl hydrazine (**8**) (2.04 g, 0.01 mol) in ethanol (10 mL), a solution of the appropriate aldehyde (0.01 mol) in ethanol (5 mL), was added. The reaction mixture was refluxed for 5 h. The reaction mixture was filtered while hot and the filtrate was allowed to stand overnight to give the crystalline products of (**9**)$_{a\&b}$.

*2.2.3. 3-(-2-Benzylidenehydrazinyl)-1-ethylquinoxalin-2-1H-one (**9$_a$**)*

This compound was obtained as dark yellow needles, 2.90 g, (90%); mp: 234–236 °C; IR: NH 3240, CO 1642 cm^{-1}; ^{1}H NMR: δ 1.24 (t, 3H, C**H$_3$**—CH_2, J = 7.20 Hz), 4.31 (q, 2H, C**H$_2$**—CH_3, J = 6.90 Hz), 7.28–7.73(m, 9H, aromatic), 8.60 (s, 1H, NCH) and11.99 (s, 1H, NH); Ms: m/z 292 (molecular

ion) (9.62%), 263 (M + —CH_2CH_3) (11.83%), 215 (M + —C_6H_5) (11.98%). *Anal*. Calcd. for $C_{17}H_{16}N_4O$: C, 58.81; H, 5.92; N, 27.43. Found: C, 58.63; H, 5.80; N, 27.65.

*2.2.4. 1-Ethyl-3-[2-(thiophen-2-ylmethylidene)hydrazinyl]quinoxalin-2-1H-one (**9b**)*

This compound was obtained as yellow crystals, 1.86 g, (60%); mp: 218–220 °C; IR: NH 3263, CO 1647 cm^{-1}; ^1H NMR: δ 1.23 (t, 3H, C**H**$_3$—CH_2, J = 7.20 Hz), 4.28 (q, 2H, C**H**$_2$—CH_3, J = 6.90 Hz), 7.11–7.65(m, 7H, aromatic), 8.79 (s, 1H, NC**H**) and 11.21 (s, 1H, N**H**); Ms: m/z 298 (molecular ion) (14.95%), 269 (M + —CH_2CH_3) (12.96%), 189 (M + —C_4H_{3SCN}) (100%). *Anal*. Calcd. for $C_{15}H_{14}N_4OS$: C, 60.38; H, 4.73; N, 18.78. Found: C, 60.08; H, 4.56; N, 18.50.

*2.2.5. (3Z)-1H-Indole-2,3-dione3-[(4-ethyl-3-oxo-3,4-dihydroquinoxalin-2-yl)hydrazone] (**10**)*

A mixture of 1-ethyl-2-oxoquinoxalin-3-yl hydrazine (**8**) (2.04 g, 0.01 mol) and isatin, (1.47 g, 0.01 mol) was refluxed in glacial acetic acid (15 mL) for 6 h. The reaction mixture was cooled to the room temperature. The orange precipitated product was filtered, washed with distilled water, dried and crystallized from ethanol 80% to afford a faint yellow crystalline product, 3.09 g, (93%); mp: 275–277 °C; IR: NH 3332, CO 1653, 1708 cm^{-1} (quinoxalinone and indolone, respectively); ^1H NMR: δ 1.27 (t, 3H, C**H**$_3$-CH_2, J = 6.60 Hz), 4.25 (q, 2H, C**H**$_2$—CH_3, J = 7.20 Hz), 11.20 (s, 1H, N**H**), 6.94–7.73 (m, 8H, aromatic) and 13.67 (s, 1H, N**H** exchangeable); Ms: m/z 333 (molecular ion) (18.97%), 304 (M + —C_2H_5) (100%), 276 (M + —COC_2H_5) (8.83%). *Anal*. Calcd. for $C_{18}H_{15}N_5O_2$: C, 64.86; H, 4.54; N, 21.01. Found: C, 64.92; H, 4.54; N, 20.90.

*2.2.6. Potassium salt of 5-ethyl-1-mercapto-1,2,4-triazolo[4,3-a]quinoxalin-4-5H-one (**11**)*

A mixture of 1-ethyl-2-oxoquinoxalin-3-yl hydrazine (**8**) (2.04 g, 0.01 mol), carbon disulphide (0.76 g, 0.71 mL, 0.01 mol) and potassium hydroxide (0.56 g, 0.01 mol) was refluxed in ethanol (20 mL) for 2 h. The mixture was then allowed to reach the room temperature to afford a faint yellow precipitated product, 2.80 g, (98%); mp: > 300 °C; IR: CO 1656 cm^{-1}; *Anal*. Calcd. for $C_{11}H_9KN_4OS$: C, 46.46; H, 3.19; N, 19.70. Found: C, 46.58; H, 3.25; N, 19.84.

*2.2.7. 5-Ethyl-1-mercapto-1,2,4-triazolo[4,3-a]quinoxalin-4-5H-one (**12**)*

A mixture of 1-ethyl-2-oxoquinoxalin-3-yl hydrazine (**8**) (2.04 g, 0.01 mol), carbon disulphide (0.76 g, 0.71 mL, 0.01 mol) and potassium hydroxide (0.56 g, 0.01 mol) was refluxed in ethanol (20 mL) for 2 h. The mixture was then allowed to reach the room temperature and poured onto 1N HCl (20 mL). The yellow precipitated product was filtered, washed with distilled water and crystallized from ethanol 80% to afford 2.23 g (90%) of compound **12** as faint yellow crystalline needles; mp: 285–287 °C; IR: SH 2630, CO 1683 cm^{-1}; ^1H NMR: δ 1.25 (t, 3H, C**H**$_3$—CH_2, J = 7.20 Hz), 4.24 (q, 2H, C**H**$_2$—CH_3, J = 7.20 Hz), 7.32–7.63 (m, 4H, aromatic) and 14.77 (s, 1H, S**H** exchangeable); Ms: m/z 246 (molecular ion) (100%), 218 (M + —CO) (74.81%), 204 (M + —C_2H_5) (5.75%). *Anal*. Calcd. for $C_{11}H_{10}N_4OS$: C, 53.64; H, 4.09; N, 22.75. Found: C, 53.29; H, 3.96; N, 22.52.

*2.2.8. General procedure for preparation of alkyl 2-(5-ethyl-4,5-dihydro-4-oxo-1,2,4-triazolo[4,3-a]quinoxalin-1-ylthio)acetates (**13**)*

A mixture of 5-ethyl-1-mercapto-[1,2,4]triazolo[4,3-a]quinoxalin-4(5H)-one (**12**) (2.46 g, 0.01 mol), appropriate alkylchloroacetate (0.012 mol) and anhydrous potassium carbonate 1 g was stirred in DMF (20 mL) for 1 h on a water bath. The mixture was then poured onto ice cold water (100 mL) with continuous stirring. The white crystalline product was filtered and washed with distilled water (100 mL). Recrystallization from ethanol 70% afforded the corresponding Alkyl 2-(5-ethyl-4,5-dihydro-4-oxo-[1,2,4]triazolo[4,3-a]quinoxalin-1-ylthio)acetate derivatives (**13$_{a\&b}$**).

*2.2.9. Methyl-5-ethyl-4,5-dihydro-4-oxo-1,2,4-triazolo[4,3-a]quinoxalin-1-ylthioacetate (**13$_a$**)*

2.38 g, (75%); mp: 157–159 °C; IR: CO 1679, 1743 cm^{-1} (amide and ester, respectively); ^1H NMR: δ 1.29 [t, 3H, NCH_2—C**H**$_3$, J = 1.50 Hz], 3.70 [s, 3H, OC**H**$_3$], 4.32 [q, 2H, NC**H**$_2$—CH_3, J = 1.50 Hz], 4.46 [s, 2H, SC**H**$_2$] and 7.50–7.75 [m, 4H, aromatic]; Ms: m/z 318 (molecular ion) (32.10%), 259 (M + —$COOCH_3$) (100%), 231 (M + —$COOCH_3CO$) (60.06%); *Anal*. Calcd. for $C_{14}H_{14}N_4O_3S$: C, 52.82; H, 4.43; N, 17.60. Found: C, 52.53; H, 4.77; N, 17.44.

*2.2.10. Ethyl-5-ethyl-4,5-dihydro-4-oxo-1,2,4-triazolo[4,3-a]quinoxalin-1-ylthioacetate (**13$_b$**)*

3.51 g, (95%); mp: 165–167 °C; IR: CO 1677, 1737 cm^{-1} (amide and ester, respectively); ^1H NMR: δ 1.14 [t, 3H, NCH_2—C**H**$_3$, J = 1.50 Hz], 1.20 [t, 3H, OCH_2CH_3, J = 1.20 Hz], 4.08 [q, 2H, NC**H**$_2$—CH_3, J = 1.50 Hz], 4.26 [q, 2H, OC**H**$_2$$CH_3$, J = 1.20 Hz], 4.40 [s, 2H, SC**H**$_2$] and 7.43–7.73 [m, 4H, aromatic]; Ms: m/z 332 (molecular ion) (27.83%), 259 (M + —$COOC_2H_5$) (100%), 231 (M + —$COOC_2H_5CO$) (22.40%); *Anal*. Calcd. for $C_{15}H_{16}N_4O_3S$: C, 54.20; H, 4.85; N, 16.86. Found: C, 52.53; H, 4.60; N, 16.94.

*2.2.11. 5-Ethyl-4,5-dihydro-4-oxo-1,2,4-triazolo[4,3-a]quinoxalin-1-ylthioacetonitrile (**14**)*

A mixture of the potassium salt (**11**) (2.84 g, 0.01 mol) and chloroacetonitrile (0.68 mL, 0.01 mol) in DMF (20 mL) was heated on a water bath for 2 h. After cooling to the room temperature, the reaction mixture was added to cold water (200 mL) with continuous stirring. The white precipitated product was filtered, washed with water and crystallized from methyl alcohol to produce 1.85 g (85%); mp: 231–233 °C; IR: CO 1666 (amide), C≡N 2243 cm^{-1}; ^1H NMR: δ 1.27 (t, 3H, C**H**$_3$—CH_2, J = 6.90 Hz), 4.33 (q, 2H, C**H**$_2$—CH_3, J = 6.90 Hz), 4.62 (s, 2H, S**CH**$_2$) and 7.43–8.26 (m, 4H, aromatic); Ms: m/z 285 (molecular ion) (100%), 257 (M + —CO) (61.66%), 242 (M + —C_2H_5N) (46.06%); *Anal*. Calcd. for $C_{13}H_{11}N_5OS$: C, 55.61; H, 4.67; N, 18.53. Found: C, 55.84; H, 4.74; N, 18.87.

Table 1 Results of anticonvulsant evaluation for compounds **8** and **11–13**.

Cpd. ID	Dose (mg/kg)	Protection (%)	ED_{50} (mg/kg)	ED_{50} (mmol/kg)	R. P.*
Standard	12.5	100.00	6.25	0.02	1.00
	6.25	50.00			
	3.12	16.67			
8	200	83.33	129	0.63	0.04
	100	33.33			
	50	16.67			
11	25	66.67	12.5	0.05	0.48
	12.5	50.00			
	6.25	16.67			
12	25	66.67	12.5	0.04	0.60
	12.5	33.33			
	6.25	16.67			
13_a	200	66.67	150	0.47	0.05
	100	33.33			
	50	16.67			
13_b	200	100.00	95	0.29	0.09
	100	66.67			
	50	16.67			

* R. P. = Relative potency

3. Pharmacology

3.1. Anticonvulsant evaluation

Five compounds of the newly synthesized derivatives were selected to be screened for their anticonvulsant activity on different groups of mice. Phenobarbitone sodium was used as a reference standard. The compounds to be tested or the standard phenobarbitone sodium were given by intraperitoneal injection to a group of adult mice each group containing 6 animals. After 45 min pentylenetetrazole (as convulsion inducing drug) was given intramuscularly in a dose of (60 mg/kg) body weight. Convulsions began with jerks of the head and body of the mouse consisting chiefly of clonic contractions. The seizures ended either by depression or by complete recovery.[13,14]

The criterion of anticonvulsant activity is complete protection against convulsions of any kind. Observations were made at least 60 min after the administration of pentylenetetrazole. Doses that gave full protection against the induced convulsions and that which exhibited 50% protection in addition to the relative potencies of the test compounds to phenobarbitone sodium were recorded in Table 1.

4. Conclusion

From the results of docking study and biological evaluation, it was noticed that, the presence of another aromatic system attached to quinoxaline nucleus like 1,2,4-triazolo nucleus increases the binding affinity with AMPA receptor due to the formation of favorable kind of interaction with the active site. This effect was clearly seen in the higher relative potencies of compounds **11** and **12**. Compound **8** showed the lowest active compound during biological evaluation. The higher reactivity of the potassium salt **11** over compound **12** may be attributed to the higher solubility of compound **11**. Elongation of the aliphatic side chain in the ester derivatives **13** has a moderate effect on the activity.

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