



In Silico Screening, Synthesis and Pharmacological Screening of Quinazolinones Containing Oxazepinone Ring as NMDA Receptor Antagonists for Anticonvulsant Activity: Part –I

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ABSTRACT

Background: NMDA receptor specifically NR2B subunit plays a major role in eliptogenesis. Antagonists at NR2B receptor site have importance in design of anticonvulsant agents. Some quinazolinones and oxazepine have inherent drug likeliness for anticonvulsant activity. In this research work *in silico* biological activity spectrum (BAS), ADME prediction, Log P predictions and docking was carried out. A library of quinazolinones with oxazepinone ring was designed, from this library 3-(6-halo-2-methyl-oxoquinazolin-3-(4H-yl)-2-(substituted phenyl)-2, 3-dihydro-1,3-oxazepine-4,7-dione (AMQ₁₋₅) were prioritized for actual synthesis and pharmacological screening for NMDA receptor antagonistic activity. **Method:** The prioritized molecules were synthesized and characterized by melting point, IR, ¹H-NMR, TLC and elemental analysis. AOT was performed to determine LD₅₀ of prioritized molecules, further compounds were evaluated for their *in vivo* antagonistic activity on NMDA induced convulsions in mice. **Result:** Prioritized molecules AMQ₁₋₅ exhibited potent antagonistic activity on NMDA receptor. **Conclusion:** The compound of series AMQ₁ and AMQ₅ were showed significant activity compared to standard memantine used in the assay.

Key Words: *In silico*, NMDA receptors, Anticonvulsant activity, Quinazolinone, Oxazepinone, Docking.

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INTRODUCTION

A library of quinazolinone with oxazepinone ring was *in silico* screened for biological activity spectrum and partition coefficient predictions (Log P) using PASS server and mol inspiration software respectively. This gave biological

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Table 1: Prioritization of molecules (biological activity scores, pLog P) from the series AMQ₁₋₅

Code for comp.	Log P [†]	BAS [†] activity	MDCK ⁺⁺⁺ cell permeability	CaCO ₂ ⁺⁺ cell permeability	PPB [§]
Memantine	3.50	0.890	0.0076	25.89	98.89
AMQ ₁	3.40	0.554	0.054	24.83	93.87
AMQ ₂	3.41	0.444	0.069	18.63	96.61
AMQ ₃	2.99	0.331	0.064	18.75	97.85
AMQ ₄	3.87	0.336	0.141	23.88	92.10
AMQ ₅	3.11	0.235	0.075	23.52	95.42

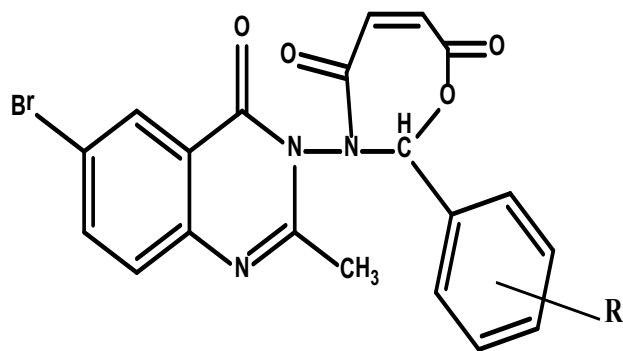
^{††}CaCO₂ cell permeability = human colon adenocarcinoma and possess multiple drug transport pathways through the intestinal epithelium. ⁺⁺⁺MDCK = Madin-Darby canine kidney cell. [§]PPB = Plasma Protein Binding, BAS[†] = Biological activity score.

Table 2: Ranges of BAS, pLogP, and ADME Prediction Values

Biological activity spectrum		Should Be greater than 0.55					
LogP Predictions		Should be greater than 2					
ADME Predictions		Have following ranges					
CaCo ₂ cells Permeability		MDCK ⁺⁺⁺ cells Permeability		BBB ^{sss} cells		Plasma protein binding ^{@@@} (%PPB)	
Low	less than 4	Low	less than 25	CNS active compounds (+)	More than 1	Chemicals strongly bound	More than 90%
Moderate	4 ~ 70	Moderate	25 ~ 500				
High	more than 70	High	more than 500	CNS inactive compounds (-)	Less than 1	Chemicals weakly bound	Less than 90%

⁺⁺⁺MDCK = Madin-Darby canine kidney cell. ^{@@@}PPB = Plasma Protein Binding, ⁺⁺⁺HIA = Human Intestinal Absorption, BBB^{sss} = Blood brain barrier.

activity score¹ (BAS) for anticonvulsant activity for NMDA inhibition mechanism and predicted Log P value (p Log P). Quinazolinone nucleus with oxazepinone ring was selected in this research due to its significant anticonvulsant potential²⁻⁹ Docking was performed on AutoDock 1.5.3 software for screening of molecule. Synthesized molecules were evaluated by IR, ¹H-NMR, Mass spectroscopy and elemental analysis. Further synthesized prioritized molecules were evaluated for *in vivo* acute oral toxicity (AOT) and anticonvulsant activity. Anticonvulsant activity was established in antagonism of test compounds in NMDA induced convulsion methanistic model. Acute Oral Toxicity (AOT) was carried to determine LD₅₀ of the prioritized molecules according to OECD guideline 425.



R= Cl, Br, OCH₃, NO₂.

Figure 1: Structure of AMQ1-6 series

Further molecules were evaluated from 300-2000 mg/kg for AOT studies.

MATERIALS AND METHODS

In silico screening

Molecules were converted from 2-D chemsketch files into 3-D mol files using Marvin Sketch and then uploaded on PASS online server to get BAS prediction. Predicted Log P (pLogP) values were obtained from molinspiration online software¹⁰ and further ADME prediction were obtained PreADMET online server.¹¹

BAS Activity Prediction: The compounds from the quinazolinone with oxazepinone ring AMQ₁₋₅ series were subjected to predict BAS activity. These values are shown in Table 1, 2. Structures of these compounds are given in Figure 1.

Log P prediction: The ranges of log P values used for prioritization are shown in Table 1, 2.

ii) ADME predictions¹¹

a) MDCK cell permeability: The ranges of MDCK cell permeability used for *in silico* prioritization of molecule are shown in Table 2.

Table 3: Docking Score and Binding Energies of AMQ₁₋₅

Compound Code	Binding energy (k/cal mol)	Docking score (k/cal mol)	Electrostatic energy (k/cal mol)	Amino Acid	H bonding
Standard (Memantine)	-12.09	-13.63	-0.67	No	No
AMQ ₁	-11.04	-12.21	-0.03	ILE335:N	1
AMQ ₂	-10.65	-10.56	1.26	LEU135:N GLN110:NE2	2
AMQ ₃	-12.40	-12.55	-0.05	PHE113:N	No
AMQ ₄	-11.74	-10.56	-0.67	GIN110:NE2	1
AMQ ₅	-12.21	-13.15	0.0	No	No

Table 4: Anticonvulsant activity of AMQ₁ and AMQ₅ compound

Compound	Dose (mg/kg)	Death (minutes) mean \pm SEM	% potency
NMDA	125mg/kg	4.053 \pm 0.01	42.45
AMQ ₁	100mg/kg	5.776 \pm 0.60***	60.50
AMQ ₁	200mg/kg	6.790 \pm 0.52***	71.12
AMQ ₁	400mg/kg	6.907 \pm 0.64***	72.34
AMQ ₅	100mg/kg	7.512 \pm 0.27***	78.68
AMQ ₅	200mg/kg	8.173 \pm 0.39***	85.60
AMQ ₆	400mg/kg	7.870 \pm 0.33***	82.43

Each value represents the mean \pm SEM : Significance level $p^{***} < 0.001$

c) Blood Brain Barrier Penetration (BBB): The values of blood brain barrier predictions are shown in Table 1, 2.

d) Plasma Protein Binding (PPB): The values are shown in Table 1, 2.

Docking protocol

Computer-assisted simulated docking experiments were carried out in Auto Dock Tools 1.5.4 for prioritized molecules. Validation of protein subunit was done by the online server www.nihserver.mbi.ucla.edu/SAVES. The ChemOffice software was used to draw molecular structures and for the conversion of the 2D structure to

3D mol files.

Selection of the Protein File

For the docking purpose the PDB file 3QEL was selected after evaluating several files from the Protein Database bank www.rcsb.org. (Table 3)

Structure Validation of the Enzyme

The errata report, Ramachandran plot & mol probability ramachandran plot was obtained from the NIH MBI sever for evaluation of protein structures and are given in Figure 2, Figure 3 and Figure 4.

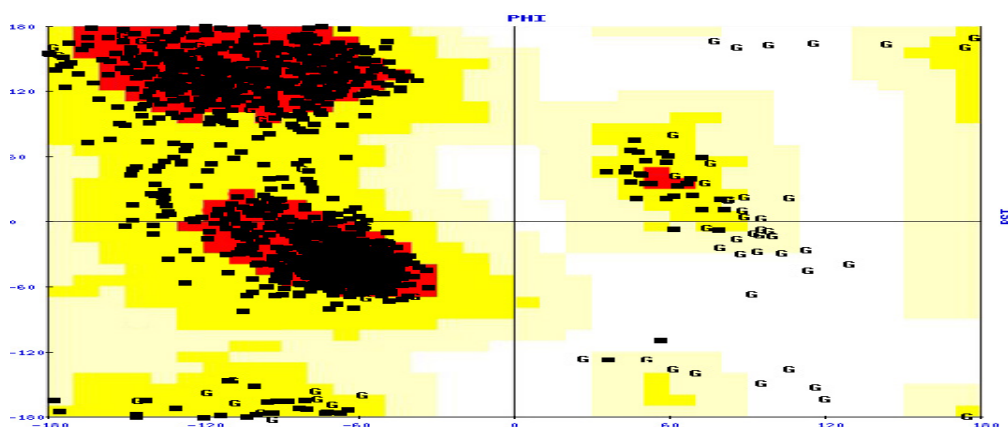
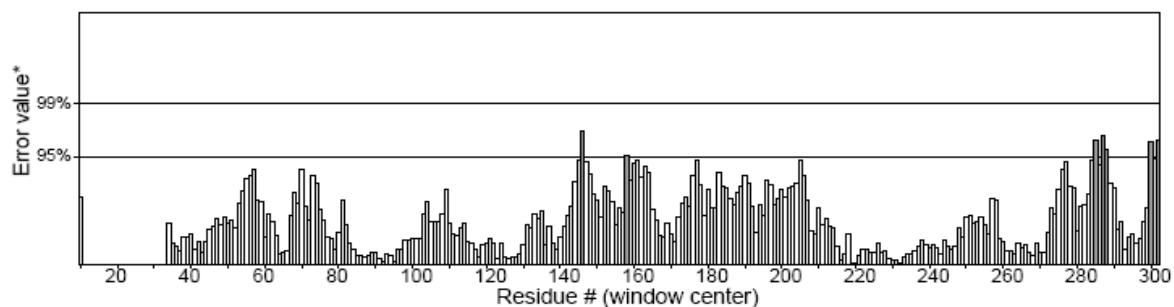


Figure 2: Ramachandran plot

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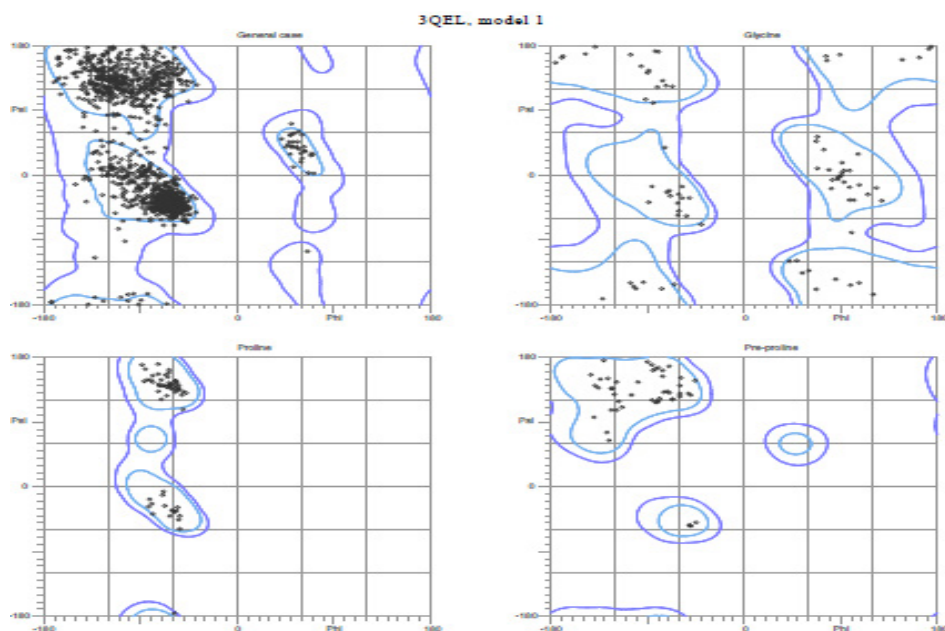
Overall quality factor**: 93.866



*On the error axis, two lines are drawn to indicate the confidence with which it is possible to reject regions that exceed that error value.

**Expressed as the percentage of the protein for which the calculated error value falls below the 95% rejection limit. Good high resolution structures generally produce values around 95% or higher. For lower resolutions (2.5 to 3Å) the average overall quality factor is around 91%.

Figure 3: Errata Report of PDB 3QEL



95.5 % (1312/1374) of all residues were in favoured (98%) regions. 100.0 % (1374/1374) of all residues were in allowed (>99.8%)

Figure 4: Favoured region for binding of molecule with receptor

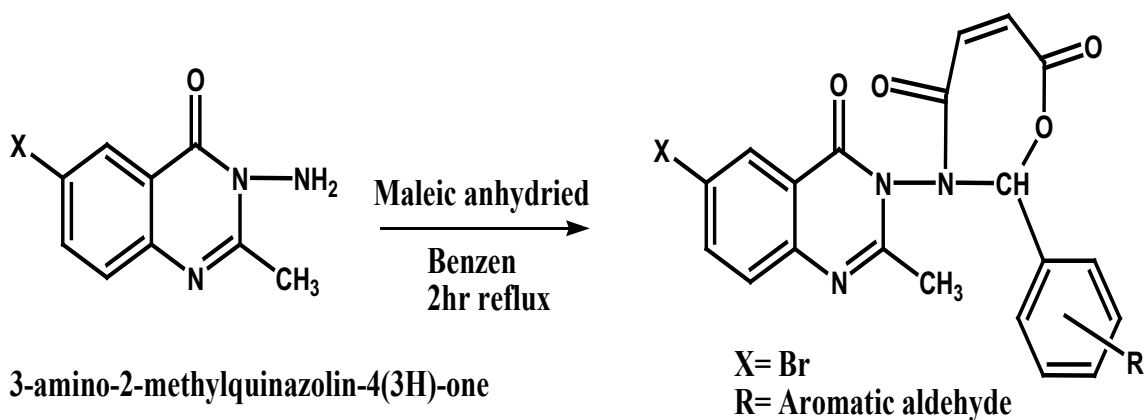
Chemistry

General procedure for synthesis of the target compound (AMQ₁₋₅ series)

All chemicals were purchased from Sigma Aldrich, SD Fine, Spectrochem and Merck. Yields refer to purified products and are not optimized

Synthesis of Schiff bases¹²⁻²⁰

A mixture of 0.01 mole of 3-amino-2-methylquinazolin-4(3H)-one and 0.01 mole of aldehyde in (10 ml) absolute ethanol was refluxed in water bath for (30 min) then left to cool in ice-water. The solid was filtered, washed with 2% HCl and water. Recrystallized the precipitate twice from ethanol. Figure 1

Figure 5: Synthetic scheme for AMQ_{1,5}

Cyclization of Schiff base (Synthesis of (Z)-2-(3-hydroxyphenyl)-3-(2-methyl-4-oxoquinazolin-3(4H)-yl)-2,3-dihydro-1,3-oxazepine-4,7-dione)

Mixture of 0.01 mole of Schiff base ((E)-3-(3-hydroxybenzylideneamino)-2-methylquinazolin-4(3H)-one) with 0.01 mole of maleic anhydride in 10mL of dry benzene was refluxed on water bath for 2 hours. Solvent was evaporated in reduced pressure on rotatory evaporator. Remained separated solid was recrystallized using THF. Figure 5

Yield: 52.17%, m.p. 206-208°C. IR (KBr, cm⁻¹): 1690 (C=O str. Of quinazolinone), 1598 (N-C=O str.), 1342, 1476, 2900, (-CH str. of hetero aromatic ring), 1600 (C=C str. Aromatic), 604-700 (C-Cl, C-Br str. Of chlorophenyl, quinazolinone respectively). ¹H-NMR (300MHz, CDCl₃ shift in ppm): 9.6-9.65 (s, 1H, N-CH of oxazepine), 8.4-8.45 (s, 1H, CH of quinazolinone), 7.45-7.6 (d, J=8.7Hz, 2H, CH of quinazolinone), 7.8 (d, J=2.4, 2H), 7.4 (t, J=9, 1H), 6.4-6.8 (d, J=7.2, 2H, vinylic proton of oxazepine), 1.32 (s, 3H, methyl of quinazoline). Anal. Calcd for C₂₀H₁₂O₄N₃BrCl₂: C, 47.18%; H, 2.38%; N, 8.25%; O, 12.57%. Found: C, 47.20%; H, 2.36%; N, 8.23%; O, 12.6%.

3-(6-bromo-methyl-oxoquinazolin-3(4H)-yl)-2-(4-nitrophenyl)-2,3-dihydro-1,3-oxazepine-4,7-dione. (AMQ₃)

Yield: 46.83%, m.p. 200-202°C. IR (KBr, cm⁻¹): 1677 (C=O Str. of quinazolinone), 1640 (C=N str.), 1342, 1476, 1349, 1400, 2985, 3057, 3012.4 (-CH str. of hetero aromatic ring), 1600 (C=C str. Aromatic); ¹H-NMR (shift in ppm) 9.45 (s, 1H, N-CH of oxazepine), 8.2-8.4 (d, J=8.7Hz, 2H, CH of quinazolinone), 7.6-7.8 (m, 4H, CH of aromatic), 6.4-6.6 (d, J=12.3, 2H, vinylic proton of oxazepine), 1.25 (s, 3H, methyl of quinazoline). Anal. Calcd for C₂₀H₁₃O₆N₄Br: C, 49.50%; H, 2.70%; N, 11.55%; O, 19.78. Found: C, 49.53%; H, 2.68%; N, 11.50%; O, 19.75%.

3-(6-bromo-methyl-oxoquinazolin-3(4H)-yl)-2-(4-bromophenyl)-2,3-dihydro-1,3-oxazepine-4,7-dione. (AMQ₃)

Yield: 52.84%, m.p. 196-198°C. IR (KBr, cm⁻¹): 1677 (C=O str. of quinazolinone), 1597 (C=N str.), 1327, 1470, 1349, 2960, 2985, 3012.4 (-CH str. of hetero aromatic ring), 1640 (C=N str.). ¹H-NMR (shift in ppm): 9.6 (s, 1H, N-CH of oxazepine), 8.8 (s, 1H, CH of quinazolinone), 7.8 (d, J=9.3Hz, 2H), 7.4 (d, J=8.7Hz, 2H, CH of quinazolinone), 7.45-7.6 (d, J=9, 2H, quinazolinone), 6.4-6.6 (d, J=12, 2H, vinylic proton of oxazepine), 1.32 (s, 3H, methyl of quinazoline). Anal. Calcd for C₂₀H₁₃O₄N₃Br₂: C, 46.27%; H, 2.52%; N, 8.09%; O, 12.33%. Found: C, 46.25%; H, 2.50%; N, 8.12%; O, 12.36%.

3-(6-bromo-methyl-oxoquinazolin-3(4H)-yl)-2-(2-nitrophenyl)-2,3-dihydro-1,3-oxazepine-4,7-dione. (AMQ₄)

Yield: 43.23%, m.p. 186-188°C. IR (KBr, cm⁻¹): 1677 (C=O str. Of quinazolinone), 1597 (C=N str.), 1327, 1470, 1349, 2960, 2985, 3012.4 (-CH str. of hetero aromatic ring), 1640 (C=N str.). ¹H-NMR (shift in ppm): 9.2 (s, 1H, N-CH of oxazepine), 8.0 (s, 1H, CH of quinazolinone), 7.8 (d, J=1.8Hz, 2H), 7.4 (t, J=15Hz, 14.7Hz, 1H, CH of quinazolinone), 7.45-7.6 (d, J=7.8, 2H, quinazolinone), 6.4-6.8 (d, J=12.3, 2H, vinylic proton of oxazepine), 1.56 (s, 3H, methyl of quinazoline). Anal. Calcd for C₂₀H₁₂O₆N₄Br: C, 49.50%; H, 2.70%; N, 11.55%; O, 19.78%. Found: C, 49.52%; H, 2.72%; N, 11.53%; O, 19.76%.

3-(6-bromo-methyl-oxoquinazolin-3(4H)-yl)-2-(4-methoxyphenyl)-2,3-dihydro-1,3-oxazepine-4,7-dione. (AMQ₅)

Yield: 48.42%, m.p. 192-194°C. IR (KBr, cm⁻¹): 1600 (C=C str. aromatic), 1677 (C=O str. Of quinazolinone), 1597 (C=N str.), 1347, 1467, 2985, 3020 (-CH str. of hetero aromatic ring) ¹H-NMR (shift in ppm): 8.9 (s, 1H, N-CH

of oxazepine), 8.4 (s, 1H, CH of quinazolinone), 7.6-7.89 (d, J=7.5, 2H, quinazolinone), 7.2 (d, J=9Hz, 2H), 7.0(d, J=9Hz, 1H, CH of quinazolinone), 6.4-6.8 (d, J=12.3, 2H, vinylic proton of oxazepine), 3.8 (s, 3H, OCH₃ of phenyl ring), 1.25 (s, 3H, methyl of quinazolinone). Anal. Calcd for C₂₀H₁₆O₅N₃Br: C, 49.50%; H, 2.70%; N, 11.55%; O, 19.78%. Found: C, 49.52%; H, 2.72%; N, 11.53%; O, 19.76%.

Pharmacological screening

Swiss albino mice of either sex weighing between 20-25 gm, obtained from National Toxicological Centre, Pune, India. During the course of the experiment, the general behaviour of the animal was normal.²¹⁻²⁴ To ensure proper testing, prior to the testing for 24hr period mice had free access to food and water. All the experimental protocols were approved by the institutional animal ethical committee according to OECD guidelines 425.²¹

Acute Oral Toxicity (AOT) Studies

AOT studies were performed to determine the LD₅₀ of the compounds following the OECD 425 guidelines. According to OECD guidelines the dose should be 300-2000 mg/kg for AOT study.²² Animal were observed for 4 hr and also observed for salivation, lacrimation and motor activity of animals. Further animals were observed for 24 hr and note the death rate of animals.

Antagonism of NMDA induced convulsions (Anticonvulsant Activity)

Six mice of either sex with a weight of 20 to 25 g were treated with test compound or the standard (Memantine) by oral or subcutaneous administration. Controls received the vehicle only 30 minutes after subcutaneous (s. c.) or 60 minutes after p.o treatment the animals were injected with subcutaneous dose of 125 mg/kg^{23,24} NMDA (N-methyl-D-aspartate). During the next 120 minutes the occurrence of clonic seizures, tonic seizures & death was recorded at dose levels of the test 100 mg/kg to 4000 mg/kg. Test group received synthesized (test) compounds (AMQ₁₋₅). Standard group received Memantine 10 mg/kg. (Table 4)

RESULT AND DISCUSSION

In silico study was performed by Biological activity prediction Spectrum (BAS), Log P, Auto dock tool 1.5.4 and Pre ADMET software. Novel series of 4-Oxo-Quinazolines with oxazepinone (AMQ₁₋₅) ring showed the interaction with ILE335:N, GLN110:NE2, PHE113:N and GIN110:NE2. AMQ₁, AMQ₂ and AMQ₃ molecules showed good hydrogen bonding with receptor. In general

the obtained scores are in between -10.5 and -15.56 and compared with standard Memantine with the score of -13.63. All the ligands docked deeply within the binding pocket region suggested their shape complementarily with 3QEL. LogP values are between 2.99 to 3.87 suggested that prioritized molecules can cross Blood brain barrier (BBB). Further synthesized compounds of series AMQ₁₋₅ were evaluated for pharmacological screening by acute oral toxicity (AOT) and anticonvulsant activity on NMDA receptor by mechanistic model. AOT was carried at a dose of 300-2000 mg/kg on mice for determining the LD₅₀ of compounds based on OECD 425 guidelines. On the basis of AOT (shown in table) the dose for anticonvulsant activity was chosen between 50-400 mg/kg. Compound AMQ₁ and AMQ₅ showed 60.5% and 85% of percentage potency respectively as compared with Memantine taken as standard in the anticonvulsant pharmacological screening.

CONCLUSION

Compounds AMQ₁ and AMQ₅ can serve as lead for as NMDA receptor antagonists for anticonvulsant activity.

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CONFLICTS OF INTEREST

Authors declare none conflicts of intrests

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