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Research Article

# *IN SILICO DESIGN,* SYNTHESIS AND PHARMACOLOGICAL SCREENING OF NOVEL MONO AND DI- BROMO QUINAZOLINONE DERIVATIVES AS NMDA RECEPTOR ANTAGONISTS FOR ANTICONVULSANT ACTIVITY

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**ABSTRACT**

We report the *in silico* prioritization, synthesis and pharmacological evaluation of some prioritized mono and di –bromo quinazolines as NMDA receptor antagonists for anticonvulsant activity. AUTODOCK 1.5.4. software was used to dock mol files of the molecules from the mono bromo (**SKMBD$_{1-3}$**) and di-bromo series (**SKDBD$_{1-2}$**). Docking score for these molecules was analyzed. *In silico* prioritization was further performed with the help of Biological activity score (BAS), LogP prediction (pLogP) and ADME predictions obtained from pass server, mol inspiration and Pre-ADMET software respectively. Molecule having acceptable docking score, BAS as compared with Memantine and complying with ADME predictions were prioritized. LogP values for anticonvulsant activity being +2.00, molecules complying with this criteria were prioritized. Molecules were synthesized and characterized by TLC, FT-IR and $^1$H-NMR. Further these prioritized molecules were evaluated by antagonism of NMDA induced convulsions for anticonvulsant activity in mice. Pharmacological screening rendered $LD_{50}$ of all drugs same at a dose of 2000 mg/kg from AOT studies and it was observed that as dose increases time of prolongation of convulsion and percentage of inhibition also increases while percentage of death decreases. Prioritized molecules **(SKMBD$_{1-3}$ & SKDBD$_{1-2}$)** shown to have comparable results of BAS activity, Log p values and ADME properties with that of standard Memantine which is anticonvulsant agent.

**Keywords**: *In silico*, NMDA, Quinazolinones, Biological activity prediction, Anticonvulsant activity.

## INTRODUCTION

CADD is the use of computer technology for the process of drug designing used to calculate molecular properties and generate pharmacophore hypothesis. Computer aided drug designing now uses novel methods like Biological activity prediction or Biological activity score (BAS) [1], pLogP prediction[2] and ADME predictions.[3] Biological activity is the result of chemical compounds interaction with biological entity. The log P value for a compound is the logarithm (base 10) of the partition coefficient (P), which is defined as the ratio of the compound's organic (oil)-to-aqueous phase concentrations. ADME means absorption, distribution, metabolism and excretion being the major units of pharmacokinetics. Quinazolinone[4-13] is targeted in drug design due to its significant role in anticonvulsant activity.

**NMDA ( *N*-methyl-d- aspartate ) receptors:** NMDARs display a number of unique properties that distinguish them from other ligand-gated ion channels. Glutamate, the major excitatory neurotransmitter in the brain and spinal cord, exerts its postsynaptic effects via a diverse set of membrane receptors which are ionotropic and metabotropic in nature. Glutamate plays essential roles in the control of neuronal function which acts essentially in signal conduction for mediating excitatory synaptic transmission, by acting on *N*-methyl-d- aspartate (NMDA) receptors. It can induce synaptic plasticity and, if its levels go unchecked, it can stimulate excitotoxicity. NMDA receptor especially NR2B subunit plays an important role in eliptogenesis and Memantine the standard drug inhibiting convulsions have been introduced in the market as antiepileptic and antiprakinsonian drug. Hence study of anti-epileptic potential of this novel target is of prime importance.

Our main objective is to *In silico* prioritize molecules for actual synthesis and evaluation based upon BAS, pLogP and PreADMET prediction and further synthesize these prioritized molecules and pharmacologically screen them for NMDA receptor antagonistic activity as anticonvulsant agents.

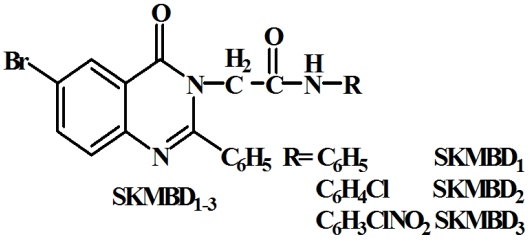
## MATERIALS AND METHODS

### *In silico screening*

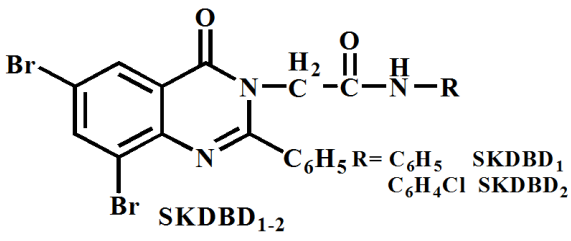
Chemdraw 8.0 was used to convert 2-D chemskech files into 3-D Mol Files. Further these were uploaded into the respective server to obtain BAS activity Predictions, pLogP values and ADME predictions respectively.

### Generation of mol file

Mol files of series of mono bromo derivatives and dibromo derivatives were prepared. Mono bromo derivatives included 2-[6-bromo-2-phenyl-4-oxoquinazolin-3(4H)-yl]-N-substituted acetamides encoded as SKMBD$_1$, SKMBD$_2$, SKMBD$_3$ whereas dibromo derivatives included 2-[6, 8-dibromo-4-oxo-2-phenyl-4-oxoquinazolin-3(4H)-yl]-N-substituted acetamides encoded as SKDBD$_1$ and SKDBD$_2$. Structures used for *in silico* are shown in fig.1.




Monobromo derivatives




Dibromo derivatives

**Fig. 1: It shows General structures of compounds with substitutions used for *in silico*.**

**BAS:** The molecules were further subjected to predict BAS using PASS server. The structures of the compounds are shown in Fig 1. The values are shown in Table 1.

**Log P predictions (pLogP):** Mol files of all the series of molecules were subjected to predict Log P values (pLog P) using Molinspiration software available on line on www.molinspiration.com.

**ADME predictions:** After compilation of data from PASS and Molinspiration software the Mol files of the molecules were subjected to predict ADME properties mentioned earlier**.** All the above values are shown in values Table 2.

**Docking Study**

Computer-assisted simulated docking experiments were carried out in Auto Dock tools 1.5.4.for prioritized molecules on Windows OS. Validation of protein subunit NR2B of PDB( Protein data bank file ID: 3QEL) is 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. Docking score for prioritized molecules is shown in Table 2. Ramchandran plot of this PDB is shown in Fig 2.

**Table 1: It Shows the Upper and lower limits of BAS, LogP, and ADME Predictions**

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Biological activity spectrum** | | Should Be greater than 0.55 | | | | | | | |
| **LogP Predictions** | | Should be greater than 2 | | | | | | | |
| **ADME Predictions** | | Have following ranges | | | | | | | |
| **Caco2 cells Permeability** | | MDCK cells Permeability | | HIA Absorption | | BBB cells | | Plasma protein binding(%PPB) | |
| **Low** | less than 4 | Low | less than 25 | Poorly | 0 ~ 20 % | CNS active componds (+) | More than 1 | Chemicals strongly bound | More than 90% |
| **Moderate** | 4 ~ 70 | Moderate | 25 ~ 500 | Moderate | 20 ~ 70 % | | | | |
| **High** | more than 70 | High | more than 500 | Well | 70-100 % | CNS inactive compounds (-) | Less than 1 | Chemicals weakly bound | Less than 90% |

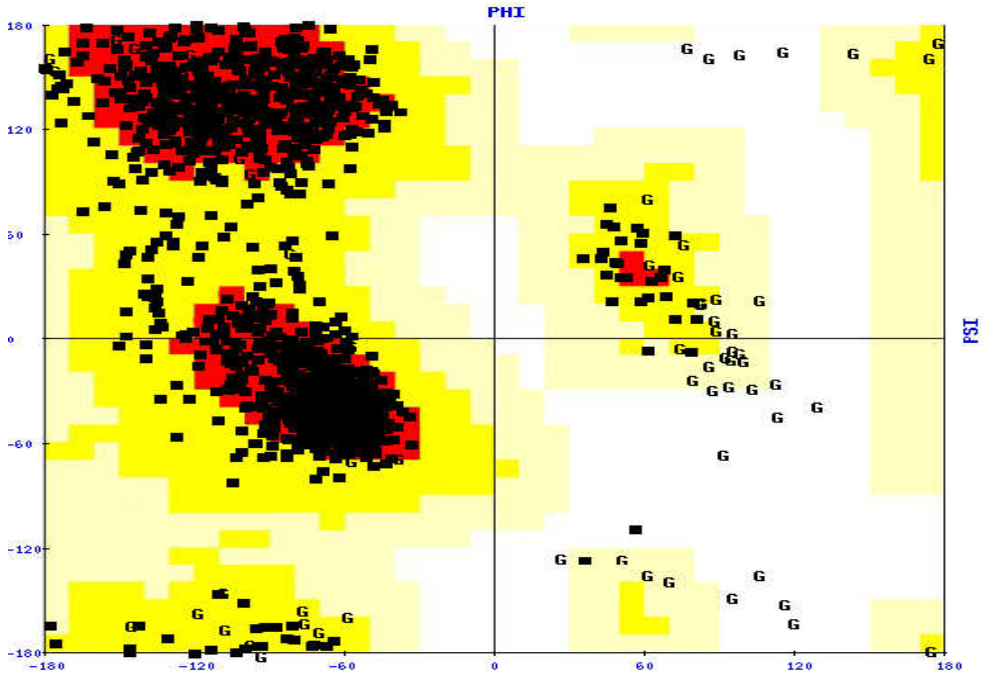



**Fig. 2: It shows Ramchandran plot of PDB Id: 3QEL**

**Table 2: It shows prioritization and docking score of library of molecules to prioritize the final molecules (biological activity scores i.e. BAS, Log P& ADME) from the series $SKMBD_{1-3}$, and $SKDBD_{1-2}$**

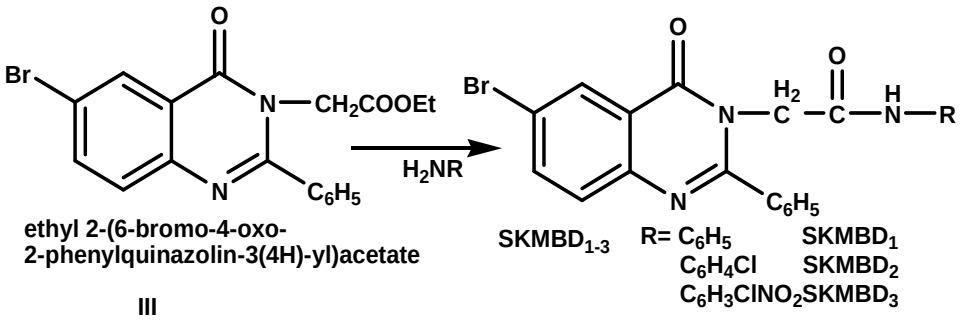
| Code | BAS@ | LP$ | B. E#. -12.09 | DS++ -13.63 | ADME Predictions CCP& | HIA† | PPB‡ | BBB!! |
|---|---|---|---|---|---|---|---|---|
| $SKMBD_1$ | 0.79 | 4.70 | -11.25 | -11.92 | 28.46 | 97.012 | 100.00 | 1.5 |
| $SKMBD_2$ | 0.82 | 4.54 | -12.07 | -13.46 | 30.78. | 97.30 | 97.69 | 1.84 |
| $SKMBD_3$ | 0.72 | 4.66 | -12.04 | -12.25 | 17.48 | 96.92 | 92.09 | 2.75 |
| $SKDBD_1$ | 0.73 | 4.12 | -10.46 | -11.33 | 33.02 | 97.44 | 95.14 | 2.80 |
| $SKDBD_2$ | 0.81 | 4.29 | -12.08 | -13.53 | 35.69 | 97.56 | 95.67 | 2.22 |

@: Biological Activity Score; $: Predicted Log P ; #: Predicted Binding Energy; ++: Docking score on AutoDock ; &: Predicted Caco-2 Cell permeability; †: Predicted Human Intestinal Absorption;‡: Predicted Plasma Protein Binding; !!: Predicted Blood Brain Barrier Prediction.

**Synthesis**

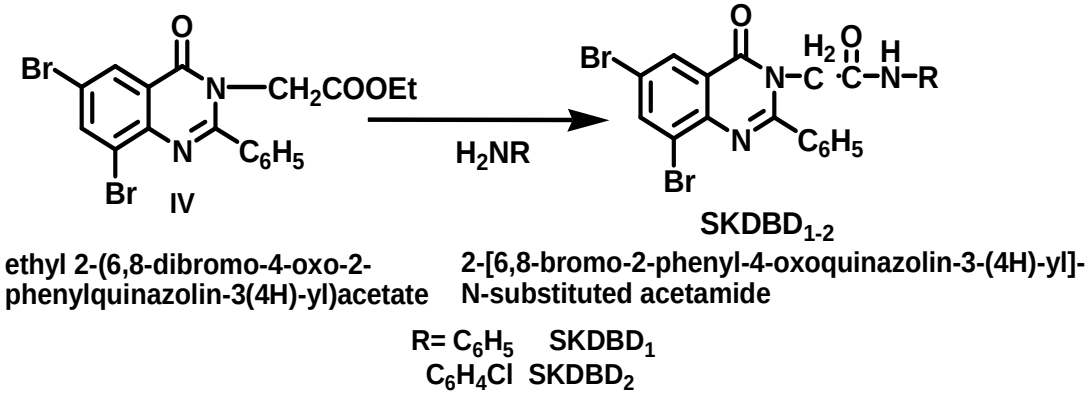
Melting points were determined on Veego VMP-1 melting point apparatus and are uncorrected. The $^1$H NMR spectra were recorded on a Varian XL 400 MHz FT spectrometer; chemical shifts are expressed in δ ppm with reference to TMS. The IR spectra of the synthesized compounds were recorded on Shimadzu IR- affinity-1 Spectrophotometer. Thin layer chromatography was performed on Merck 5-10 cm precoated (0.25 mm) silica gel GF254 plates (E. Merck, Germany).

$SKMBD_{1-3}$, $SKDBD_{1-2}$ were prepared as per reported literature[14].




**Synthesis of Ethyl (6-Bromo-3, 4 dihydro-2-phenyl-4-oxoquinazolin-3-yl) acetate [14] ( III ):** 6-Bromo 2-phenyl 1, 3, 4-benzoxazinone (0.01 mol) and glycine ethyl ester (0.01mol) were taken in a round bottom flask and then pyridine (freshly distilled and dried) was added slowly while shaking. The mixture was heated under reflux for 8 hrs. Excess of pyridine was distilled off under reduced pressure, then the solution was poured into a beaker contained crushed ice, to get the product. It was filtered under suction, washed with portions of ice cold water and dried at 100°C. The product was purified by recrystallization with ethanol to get a colorless crystalline solid.

**Synthesis of 2-[6-bromo-2-phenyl-4-oxoquinazolin-3(4H)-yl]-N-substituted acetamides ($SKMBD_{1-3}$)[14]:** Ethyl (6-Bromo-3, 4 dihydro-2-phenyl-4-oxoquinazolin-3-yl) acetate (0.01 mol) and corresponding primary amines (0.01 mol) were taken in round bottom flask and then glacial acetic acid was added slowly while shaking. The mixture was heated under reflux for 4-6 hrs. After cooling, the contents were poured into crushed ice. The resulting solid was washed with distill water, filtered, dried in vacuum and recrystallized from warm ethanol. The physiochemical characteristics for derivatives $SKMBD_{1-3}$ . $SKMBD_1$: M.pt: 126-130°C; TLC: Ethyl acetate: Pet ether (1:1) Yield: 55%, FT-IR ($cm^{-1}$): 3300 (-NH str.), 3050 (-CH-Ar str.), 1600 (C=C aromatic str.), 1700 (C=O of Characteristic quinazolinone peak,str. ), 1680-1630 (C=O of –CONH str), 3350 ( $2^0$ amine str.) and 510 ( C-Br str.). $^1$H-NMR (shift in δ ppm) : 7.5-8.7 [d, m Ar-H (4H) and Quinazolinone-H (3H) s, d], 8.906 (s, 1H, $CH_2$), 5.350 (s, 1H, NH). $SKMBD_2$: M.pt: 240-245°C; TLC: Ethyl acetate: Pet ether (1:1). Yield: 60%; FT-IR ($cm^{-1}$): 3350 ( $2^0$ amine str.), 3300 (-NH str.), 3000 (-CH-Ar str.), 1605 (C=C aromatic str.), 1700 (C=O of Characteristic quinazolinone peak,str. ), 1680-1630 (C=O of –CONH str), 510 (C-Cl str.) and 610 ( C-Br str.).$^1$H-NMR (shift in δ ppm): 7.390-8.343 [d, m Ar-H (4H) and Quinazolinone-H (3H) s, d], 8.926 (s, 1H, $CH_2$), 5.40 (s, 1H, NH). $SKMBD_3$: M.pt: 265-70°C; TLC : Ethyl acetate: Pet ether (1:1). Yield: 60%, FT-IR ($cm^{-1}$): 3300 (-NH str.), 3100 ( -CH-Ar str.), 1600 (C=C aromatic str.), 1700 (C=O of Characteristic quinazolinone peak), 1680 (C=O of -CONH). 530 (C-Br str.), 770 (C-Cl str). $^1$H-NMR (shift in δ ppm): H$^1$NMR (shift in δ ppm): 7.396-8.648 [d, m Ar-H (4H) and Quinazolinone-H (3H) s, d], 8.926 (s, 1H, $CH_2$), 5.09 (s,1H, NH).




**Synthesis of Ethyl 2-(6, 8-dibromo- 4-oxo-2-phenylquinazolin-3-(4H)-yl) acetate [14] (IV):** 6,8-Dibromo-2-phenyl-4H-benzo[d][1,3]oxazin-4-one (0.01 mol) and glycine ethyl ester (0.01mol) were taken in a round bottom flask and then pyridine (freshly distilled and dried) was added slowly while shaking. The mixture was heated under reflux for 8 hrs. Excess of pyridine was distilled off under reduced pressure, then the solution was poured into a beaker contained crushed ice, to get the product. It was filtered under suction, washed with portions of ice cold water and dried at 100°C. The product was purified by recrystallization with ethanol to get a colorless crystalline solid.

**Synthesis of 2-[6, 8-dibromo-4-oxo-2-phenyl-4-oxoquinazolin-3(4H)-yl]-N-substituted acetamides. [14] ($SKDBD_{1-2}$):** Ethyl 2-(6, 8-dibromo- 4-oxo-2-phenylquinazolin-3-(4H)-yl)acetate (0.01 mol) and corresponding primary amines (0.01 mol) were taken in round bottom flask and then glacial acetic acid was added slowly while shaking. The mixture was heated under reflux for 4-6 hrs. After cooling, the contents were poured into crushed ice. The resulting solid was washed with distill water, filtered, dried in vacuum and recrystallized from warm ethanol. **$SKDBD_1$ : M.pt: 185-190°C, TLC:** Pet Ether: Ethyl Acetate (1:1); **Yield:45%; H$^1$NMR (shift in δ ppm):** 7.5-8.720 [d, m Ar-H (4H) and Quinazolinone-H (2H), d], 8.906 (s,

1H, $CH_2$), 5.350 (s, 1H, NH). **SKDBD$_2$: M.pt: 260-65**℃C; **TLC:** Ethyl Acetate: Pet. Ether (1:1)**; H$^1$NMR (shift in δ ppm):** 7.513-8.343 [d, m Ar-H (4H) and Quinazolinone-H (2H) d], 8.926 (s, 1H, $CH_2$), 5.40 (s, 1H, NH).

**Pharmacological screening**[15,16]

Pharmacological screening is divided in two sections: 1) Acute oral toxicity Studies. 2) Anticonvulsant activity by antagonism of NMDA induced convulsions.

**Acute oral toxicity Studies:** Acute oral toxicity is done for determining the $LD_{50}$ of compounds. This experiment was performed following OECD guideline 425. According to OECD guidelines, all animals are given with dose of 2000 mg/Kg. And observed for 4 hrs and then after 24 hours for salivation, awareness, motor activity, muscle tone etc. After 24 hour not a single animal was dead. So 2000 mg/kg is safe dose for the test compounds.

**Anticonvulsant activity on NMDA receptor**

Mice of either sex were treated with test compound or the standard (e.g. Memantine) of dose 10 mg/Kg by oral or subcutaneous administration. Controls received NMDA of dose 200mg/Kg by intraperitoneal route .Test compound group received test compounds ($SKMBD_{1-3}$& $SKDBD_{1-2}$) orally with dose of 100 mg/Kg, 200 mg/Kg, 400 mg/Kg. After 30 min animals received NMDA 200 mg/Kg intraperitoneally. During the next 120 minutes the occurrence of clonic seizures, tonic seizures & death was recorded at three dose levels of the test 100mg/kg and 200mg/kg 400mg/Kg. The following observations are obtained at dose levels shown in Table 3. Time of tonic-clonic convulsions was noted.

**Table 3: It Shows evaluation of AOT and antagonism of NMDA induced convulsions at dose 100, 200 and 400mg/Kg**

| Code | AOT at 2000 mg / Kg dose | % Inhibition 100 mg/Kg | % Inhibition 200 mg/Kg | % Inhibition 400 mg/Kg |
|---|---|---|---|---|
| $SKMBD_1$ | Safe | 50 | 50 | 33.33 |
| $SKMBD_2$ | Safe | 16.66 | 16.66 | 16.66 |
| $SKMBD_3$ | Safe | 16.66 | 16.66 | ----- |
| $SKDBD_1$ | Safe | 16.66 | 33.33 | 16.66 |
| $SKDBD_2$ | Safe | 33.33 | 50 | 50 |

**RESULTS AND DISCUSSION**

***In silico* Screening**

From the library of designed molecules, five molecules ($SKMBD_{1-3}$ & $SKDBD_{1-2}$) were prioritized by using BAS prediction from PASS online server, Log p prediction from mol inspiration online software, ADME prediction from PreADMET online server. In that compound $SKMBD_1$ and $SKDBD_2$ had good results out of five compounds.

***In silico* Docking**

The library of designed molecule were subjected to *in silico* docking by Auto Dock Tools 1.5.4 on NR2B subunit of target receptor i.e. NMDA receptor. PDB 3QEL of NR2B subunit of NMDA receptors were taken from pdbsum online server. That pdb were subjected to validation by two parameters ramachandran plot, errata report (not included in this communication). Enzyme structure was checked for missing atoms, bonds and contacts. Ramchandran plot was plotted to check the health of protein. Hydrogen atoms were added to enzyme structure. Bound ligands were manually deleted from the enzyme. The ligands were constructed using marvin view and Chem Draw softwares and optimized structure was used for docking. The active site was generated and ligands were docked within the 3QEL active site using AutoDock tools. Before molecular docking, the 3D structure of ligands was optimized. The conformation of ligands were selected and subjected to energy minimization using MMFF94 force field. The PDB 3QEL was also subjected to energy and residue optimization. After docking, results of docking score, binding energy, hydrogen bonding were compared with standard Memantine. The five molecules ($SKMBD_{1-3}$ & $SKDBD_{1-2}$) which were prioritized from *in silico* method shown comparable results with that of standard Memantine. Visualizations of Docked poses were done.

**Synthesis & Characterization**

Five prioritised molecules ($SKMBD_{1-3}$ & $SKDBD_{1-2}$) were synthesized by the proposed scheme. Step 1 was bromo-anthranilic acid was cyclised by addition of benzoyl chloride in equimolar quantity. Cyclised molecule i.e. benzoxazine were reacted with glycine ethyl ester in equimolar quantity to form oxoquinazolynyl ring which further reacted with corresponding aromatic amines in equimolar quantities to give final derivatives. ($SKMBD_{1-3}$ and $SKDBD_{1-2}$). All the reactions were monitored by TLC with suitable solvent system. Final test compounds were characterized with FT-IR, $^1$H-NMR for characteristic peaks. Uncorrected melting points were reported which were sharp.

**Pharmacological screening**

**Acute Oral Toxicity (AOT):** Studies were carried out on 6 groups of animals i.e. Swiss albino mice according to OECD guideline 425. Test compounds ($SKMBD_{1-3}$ & $SKDBD_{1-2}$) were subjected orally to albino mice and observed for 24 hrs. All five test compounds ($SKMBD_{1-3}$ & $SKDBD_{1-2}$) were observed to be safe at dose of 2000 mg/Kg.

**Antagonism of NMDA induced convulsion:** As 2000 mg/Kg dose of test compounds were observed to be safe so according to OECD guideline 425, dose of 100 mg/Kg, 200 mg/Kg, 400 mg/Kg were selected and injected /orally administered to test group of animals of weight around 25-30 gms. After half an hour NMDA 200 mg/Kg injected to test group of animals intraperitoneally. Time of tonic-clonic convulsions was noted. From that it was observed that as dose increases time of prolongation of convulsion and percentage of inhibition also increases while percentage of death decreases.

**CONCLUSION**

From the present we came to the conclusion that the prioritized molecules ($SKMBD_{1-3}$ & $SKDBD_{1-2}$) shown to have comparable results of BAS activity, Log p Values and ADME properties with that of standard Memantine which is anticonvulsant agent specifically acting on NR2B subunit of NMDA receptor. Also the docking score of these molecules were almost similar to that of Memantine. From the characterization of synthesized molecules ($SKMBD_{1-3}$ & $SKDBD_{1-2}$) , it found was that FT-IR and $^1$H-NMR of synthesized molecules contain characteristic peak which concludes that derivative is synthesized. Pharmacological activity also shows that there is prolongation of convulsion period. Out of five $SKMBD_1$, $SKDBD_2$ have given good and comparable results for BAS activity, ADME properties, Log P values, docking score, % inhibition, % death.

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