Original Article

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In silico Design, Synthesis and Pharmacological screening of Quinazolinones as NMDA receptor antagonists for Anticonvulsant activity: Part II

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ABSTRACT

Background: N-methyl-D-Aspartate (NMDA) receptor plays a main role in eliptogenisis and its inhibition has therapeutic significance in development of anticonvulsants. Prioritized quinazolinone molecules were synthesized, evaluated *in vivo* by AOT and then for anticonvulsant activity in NMDA induced convulsion model. **Method:** *In silico* Screening of prioritized molecule was done by biological activity predictions, partition coefficient predictions (Log P), molecular docking on NMDA receptors, *in silico* ADME predictions using PASS server and mol inspiration software, V Life MDS 4.3 software and Pre ADMET server respectively. This gave biological activity (BA) score for anticonvulsant activity and predicted Log P values (p Log P). The standard Log P required for anticonvulsant activities being more than 2.00, therefore molecules were also prioritized based on this p Log P criteria. Docking showed results of antagonism *in silico* as compared with Memnatine and molecules were prioritized for synthesis based on this criteria. **Result:** Quinazolinone molecules were prioritized based upon docking score, ADME and BA score, synthesized and pharmacologically screened for anticonvulsant activity. **Conclusion:** $SMMB_1$, $SMMB_2$, $SMMB_3$ showed the prominent anticonvulsant activity as compared with memantine used as standard for *in vivo* anticonvulsant activity. The compounds can serve as anticonvulsant Leads through NMDA antagonism.

Key words: Anticovulsant, Mol inspiration, NMDA, PASS, Pre ADMET, Quinazolinone.

INTRODUCTION

NMDA receptors in the mammalian central nervous system are only formed by combinations of NR1 and NR2 subunits, NR1 subunit express glycine and NR2 express glutamate. The NR2 subunit family is in turn divided into four individual subunit types: NR2A, NR2B, NR2C, and NR2D. In part-I of this series of research papers we published the data of some quinazolinones attached with oxazepinone rings as NMDA receptor antagonists for anti-convulsant activity which can serve as anti-convulsant "leads" with activity of 70% as compared with memantine, apotent anticonvulsant agent. In continuation of our work on discovery of potent NMDA receptor antagonists from quinazolinone series,[1-3] we here report the quinazolinones with thiobarbituric acid and barbituric acid moieties as potent NMDA receptor antagonists for anticonvulsant activity. This research work consists of *in silico* screening, synthesis, characterization and pharmacological screening of some Quinazolinones containing barbituric acid and

Access this article online	
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www.phcog.net	DOI: 10.5530/jyp.2015.4.4

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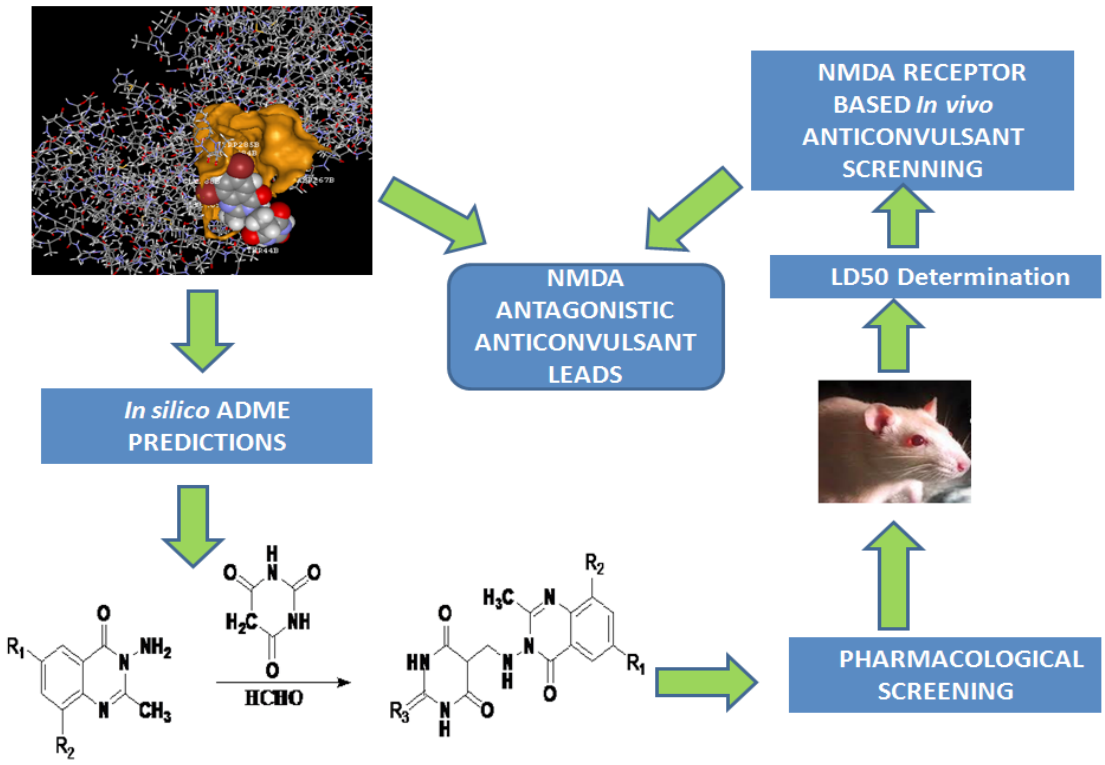

Graphical Abstract

thiobarbituric acid moieties as NMDA receptor antagonists for anticonvulsant activity. With a series of molecules as described (series $SMMB_{1-3}$) were *in silico* prioritized by molecular docking to obtain antagonism scores using Vlife sciences MDS 4.3 drug design software. Pre ADMET software was used for prioritization of molecules based upon ADME properties. Based on which Caco-2, MDCK, HIA (Human Intestinal Absorption), PBB (Plasma Protein Binding), BBB (penetration in Blood Brain Barrier) for the molecules *in silico*, these prioritized molecules were first synthesized and then characterized by TLC, IR, ^{1}HNMR and melting point. Caco-2 permeability is a determinant of apparent permeability, MDCK permeability value was considered for apparent permeability, HIA value is determinant of the apparent Human Intestinal absorption. PBB is determinant of binding of drug molecule to the plasma proteins. BBB values are determinant and essential for the molecules which are designed to be active on central nervous system. Prioritization was based on criteria of violation of the optimum upper limit of ranges in case of in silico ADME predictions and comparison with antagonism scores of Memantine used as standard for docking trials. Only these sufficient ADME predictions were considered during *in silico* predictions. Ranges of ADME predictions and obtained values are shown in Table 1. *In silico* p Log P values were also obtained to prioritize molecules based on Log P criteria. *In vivo* pharmacological evaluation was done by Acute Oral toxicity (AOT) followed by inhibition of NMDA induced convulsions mechanistic model for anticonvulsant activity. Acute Oral Toxicity (AOT) was carried to determine LD_{50} of the prioritized molecules according to OECD guideline 453. Further molecules were evaluated from 700 to 2000 mg/kg dose for AOT study. On the basis of AOT the dose was decided for anticonvulsant activity. Prioritized molecules were found to have potency between 40-70% as compared with Memantine used as standard in *in silico* and *in vivo* studies. Prioritized molecules are shown in Figure 1.

MATERIAL AND METHODS

In silico[4,5]

Biological Activity Scores

These were obtained from PASS server online for biological activity prediction.

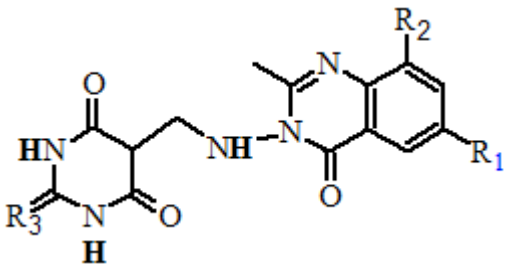

Figure 1: Structure of prioritized molecules

Table 1: ADME scores of prioritization molecules

Compound	ADME Predictions						
Standard ranges	BAS	pLog p	$Caco_2$ 4-70 nm/sec	MDCK 25-500 nm/sec	BBB ~ 1.00	HIA 20-70%	PPB 90%
$SMMB_1$	0.883	-1.74	19.79	0.15	0.05196	93.08	93.02
$SMMB_2$	0.965	-1.12	20.46	0.436	0.11710	93.96	67.79
$SMMB_3$	0.848	-1.04	19.70	0.48	0.059729	95.12	83.14
Memantine	0.890	3.500	25.89	0.078	1.5905	94.38	98.89

Table 2: Table shows the predicted scores and hydrogen bonding of ligands of series $SMMB_{1-3}$

Compound code	Autodock Docking Score	Amino Acid	H- bonding	Vlife MDS Docking Score
Standard (Memantine)	-13.63	HIS127B, GLY128B, ASP265B	No	-32.38
SMMB¬1	-8.38	HIS127B,SER131B, ILE133B	-	-54.34
SMMB2	-8.89	THR103B, GLY128B, MET132B	1	-61.23
SMMB3	-9.78	THR44B, ASP102B, HIS127B	2	-56.00
Barbituric acid	-	HIS127B, GLY128B, THR266B	3	-31.81
Thiobarbituric acid	-	HIS127B, ASP265B, GLU284B	2	-33.34

pLogP predictions[5]

Log P of a compound should be greater than 2.00 for compound to cross the blood brain barrier. pLog P values were also obtained to prioritize molecules based on Log P value. Scores were obtained by molinspiration software. Scores are given in Table 1.

ADME Predictions[6]

The *in silico* ADME parameters and their ranges used for prioritization are mentioned under each ADME property (Table 1). These were obtained *in silico* from Pre ADMET Server online.

Molecular Docking Studies

Docking study was performed on Vlife MDS 4.3 Drug design software and Auto dock 1.4.5. Marvin Bean and Chemdraw 12 software were used to draw molecular structures, for conversion of 2D structure to mole files. 2D structure of ligand were prepared in Marvin sketch and converted to 3D by Vlife sciences MDS 4.3 Drug Design Software. The 3D structure was stabilized by minimizing the energy using molecular mechanics followed by Merck Molecular Force Field (MMFF). Conformational changes in compound were obtained by Monte Carlo method. All the Conformers were then energetically minimized up to the rms gradient of 0.001. The PDB of NMDA receptor subunit NR2B was obtained from protein database with www.rscb.org. The PDB was subjected for docking study. All software scores are listed in Table 2.

Chemistry

General procedure for synthesis of the target compound ($SMMB_{1-3}$)

All chemicals were procured from, SD Fine, Spectrochem, Sigma Aldrich and Merck.

Apparatus: VEEGO - VMP I melting point apparatus was used to determine melting point. SHIMADZU Affinity-I spectrophotometer was for recording the IR spectrum. ^1H NMR were recorded at Diya labs Mumbai on 400 MHz Spectrophotometer facility, chemical shifts (δ) are reported in parts per million (ppm) with $CDCl_3$ and DMSO as solvent for NMR. TMS was used as internal standard for NMR. Splitting of signals is represented by s (singlet), d (doublet), t (triplet), q (quartet), m (multiplates). Thin layer chromatography (TLC) was performed on Merk GF_{254} precoated aluminium plate.

General synthesis scheme of the target compound[7-11] *($SMMB_{1-3}$)*

To a solution of barbituric acid (0.01 mol)/ thiobarbituric acid (0.01 mol) in methanol as solvent, formaldehyde (0.02 mol) and 3-amino-2-methylquinazolin-4(3H)-one (0.02 mol) were added drop wise. The reaction mixture was refluxed on a steam bath for 4 hr. The excess of solvent was distilled off. The solid thus obtained was washed with water and recrystallized from acetone. Impurities were removed by column chromatography.[6,7]

Synthetic scheme is given in (Figure 2).

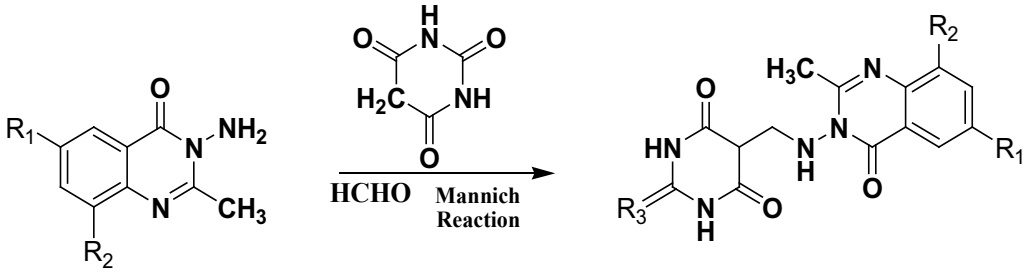

Figure 2: Synthetic scheme of prioritized molecules

Table 3: Synthesized Compounds

Compound Code	R1	R2	R3	Melting Point ^{0}C
SMMB$_1$	Br	H	O	105-110
SMMB$_2$	Br	Br	S	100-105
SMMB$_3$	Br	Br	O	105-115

R1=Bromine; R2=Bromine/Hydrogen; R3=Sulfer/Oxygen.

Synthesis of 2(methyl) 6-Bromo-3-amino(-5-methyl (Thiobarbituryl) quinazoline-4(3H) one (SMMB$_1$):[7,8] To a solution of thiobarbituric acid (0.01 mol) in methanol as solvent, formaldehyde (0.02 mol) and 6- bromo-3-amino-2-methylquinazolin-4(3H)-one (0.02 mol) was added drop wise. The reaction mixture was refluxed on a steam bath for 4 hr. The excess of solvent was distilled off. Obtained solid was washed with water and recrystallized from acetone. Impurities were removed by column chromatography.

2(methyl) 6-Bromo-3-amino(-5- methyl (Thiobarbituryl) quinazoline -4(3H) one (SMMB$_1$)

% Yield: 55%, Molecular Formula:$C_{14}H_{12}BrN_5O_3S$, Melting Point:100-105^{0}C, Molecular wt:408, R_f: 0.5 (Hexane:Ethyl acetate 70: 30). I.R. (KBr, cm^{-1}): 1679.55 (C=O, Str., M). 499.47(Ar., C=C, Str.), 2260 (S st.), 1374.03 (C-H, Str.), 2942.84(C-H str.), 3379 (2^{0}N-H str.) ^{1}H NMR (300 MHz, $CDCl_3$) δ [ppm]:7.71-7.79 (d, 2H, CH, J=0.52); 8.1(H,CH, J=0.029); 2.51(s, 1H,CH_3, J=0.805); 2.04 (NHs, H, CH_2, J=0.37);11.59-11.95 (d, 2H, NH, J=1.39).

Synthesis of 2(methyl), 6, 8-Bromo-3-amino(-5-methyl (Thioarbituryl) quinazoline-4(3H) one (SMMB$_2$)

To a solution of thiobarbituric acid (0.01 mol) in methanol as solvent, formaldehyde (0.02 mol) and 6, 8-dibromo-3-amino-2-methylquinazolin-4(3H)-one (0.02 mol) was added drop wise. The reaction mixture was refluxed on a steam bath for 4 hr. The excess of solvent was distilled off. Obtained solid was washed with water and recrystallized from acetone. Impurities were removed by column chromatography.

2(methyl), 6, 8-Bromo-3-amino(-5-methyl (Thioarbituryl) quinazoline-4(3H) one (SMMB$_2$)

% Yield: 60%, Molecular Formula:$C_{14}H_{11}Br_2N_5O_3S$, Melting Point:100-105^{0}C, Molecular wt:489, Rf: 0.5 (Hexane:Ethyl acetate 70: 30). I.R. (KBr, cm^{-1}): 1679.55 (C=O, Str.). 660 (Br st.), 499.47(Ar., C=C, Str.),2260 (S st.), 1374.03 (C-H, Str..), 2942.84(C-H str.), 3379 (2^{0}N-H str.) ^{1}H NMR (300 MHz, $CDCl_3$) δ [ppm]:8.17-8.3 (d, 2H,CH, J=0.16); 2.62 (H,CH_3, J=0.16); 10.96 (s, 1H,NH, J=1.13); 11.92-12.69(d, H,NH, J=2.24).

Synthesis of 2(methyl), 6, 8-Bromo-3- amino (-5-methyl (barbituryl) quinazoline-4(3H) one (SMMB$_3$)

To a solution of barbituric acid (0.01 mol) in methanol as solvent, formaldehyde (0.02 mol) and 6, 8-dibromo-3-amino -2-methylquinazolin-4(3H)-one (0.02 mol) was added drop wise. The reaction mixture was refluxed on a steam bath for 4 hr. The excess of solvent was distilled off. Obtained solid was washed with water and recrystallized from acetone. Impurities were removed by column chromatography.

2(methyl), 6, 8-Bromo -3-amino(-5-methyl (barbituryl) quinazoline -4(3H) one (SMMB$_3$)

% Yield: 60%, Molecular Formula:$C_{14}H_{11}Br_2N_5O_4$, Melting Point:105-115^{0}C, Molecular wt:473, Rf: 0.5 (Hexane:Ethyl acetate 70: 30). I.R. (KBr, cm^{-1}): 1710 (C=O, Str.). 680 (Br st.), 499.47(Ar., C=C, Str.),2260 (S st.),1374.03 (C-H, Str..), 2942.84(C-H str.), 3600 (2^{0}N-H str.) ^{1}H NMR (300 MHz, $CDCl_3$) δ [ppm]:8.17-8.34 (d, 2H,CH, J=0.009); 2.61 (S, H,CH_3, J=0.037); 9.65 (s, 1H, NH J=1.36); 4.35 (S, CH_2, J=0.34), 10.11-10.24(d, H,NH, J=0.17).

Pharmacology Screening[11-16]

Albino mice of either sex weighing between 20-25 gm, obtained from National Institute of Biomedical Sciences, Pune, India, were used in the present study. Animals were kept in wire-mesh cages under the laboratory conditions

Table 4: Data of acute oral toxicity (AOT)

Code	300 mg/kg	700 mg/kg	1000 mg/kg	2000 mg/kg
$SMMB_1$	Safe	Safe	Safe	Safe
$SMMB_2$	Safe	Safe	-----	-----
$SMMB_3$	Safe	Safe	Safe	-----

Table 5: It shows series $SMMB_{1-3}$ evaluated at dose 2000 mg/Kg

Compound Code	No. of Animals	No. of dead Animals	% Death	% Inhibition
$SMMB_1$	6	5	90	16.66
$SMMB_2$	6	4	85	33.33
$SMMB_3$	6	5	90	16.66
Memantine	6	0	0	100

Table 6: It shows % Death and % Inhibition of standard (Memantine) and control (NMDA)

Compound Code	No. of Animals	No. of dead Animals	% Death	% Inhibition
Standard (Memantine)	6	0	0	100
Control (NMDA)	6	6	100	0

(23 ± 2°C), 12 h light. Animals were provided with food (Hindustan Lever Ltd. Mumbai) and water for 24 hrs period before testing in a constant light-dark cycle. During the period of the experiment, the general behaviour of the animal was normal. The homogenous suspension of the test compounds and the standard drugs (Memantine) were prepared in tween 20 or tween 80 and distilled water (1:9 ML). Appropriate dose of NMDA was prepared in water.

Acute oral toxicity (AOT) Studies[11]

Acute oral toxicity was performed for determining the LD_{50} of compounds. This experiment was performed under the OECD guideline 453 adopted: 7th September 2009.[10] 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 mortality was observed in animals. The test procedure was used to minimize the number of animals required for estimation of acute oral toxicity of compound as per OECD guidelines. Signs of toxicity were assured by LD_{50} and confidence interval. AOT study data shown in Table 4.

Acute Oral Toxicity (AOT) studies were carried out on 6 groups of animal i.e. Swiss albino mice according to OECD guideline 453. Test compounds ($SMMB_{1-3}$) were administered orally to albino mice and mice were observed for 24 hrs. All three test compounds ($SMMB_{1-3}$) were observed to be safe at dose of 2000 mg/Kg.

Anticonvulsant activity (Inhibition of NMDA induced convulsion)[13-16]

NMDA can precipitate convulsions in patients with seizure disorders. The compound is regarded as a NMDA synthesis inhibitor. Tonic Clonic seizures induced in mice are antagonized by anticonvulsant agents.[11,12] The animals were divided into three groups. Each group contained six mice of either sex having 20-28 gm weight. The test groups received a series of synthesized compound ($SMMB_{1-3}$) and at appropriate doses based upon its individual LD_{50} values. The standard group received (Memantine 10 mg/kg) by oral or subcutaneous administration. Controls received the vehicle only. After 60 minutes subcutaneously (s.c) or animals were injected with dose of 125 mg/kg NMDA (N-methyl-D-aspartate) intraperitonial. The occurrence of clonic seizure,[15] tonic seizures & death was recorded at three dose levels of the test compound. Activity results are given in Table 5, 6, 7.

Albino mice of either sex (weighing about 25-30 gm) were used in study in which memantine was used as standard at a dose of 10 mg/Kg by oral or subcutaneous administration. Control group received NMDA of dose 125 mg/Kg by intraperitoneal route (I.P.) and all test groups received test compounds ($SMMB_{1-3}$) orally. Based upon their AOT studies $SMMB_3$ was administered orally at a dose of 50-150 mg/Kg body weight 30 min before to start the experiment. After 30 minute animals received NMDA at a dose of 125 mg/Kg intraperitoneal.[16]

During the next 120 minutes the occurrence of clonic seizures, tonic seizures & death was recorded at three dose high medium and low doses respectively. Time for inhibition of convulsions was observed and recovered (Table 4).

RESULT AND DISCUSSION

In silico Studies

Compounds with the code $SMMB_{1-3}$ shown to have BAS score is 0.883, 0.965 and 0.848 respectively which

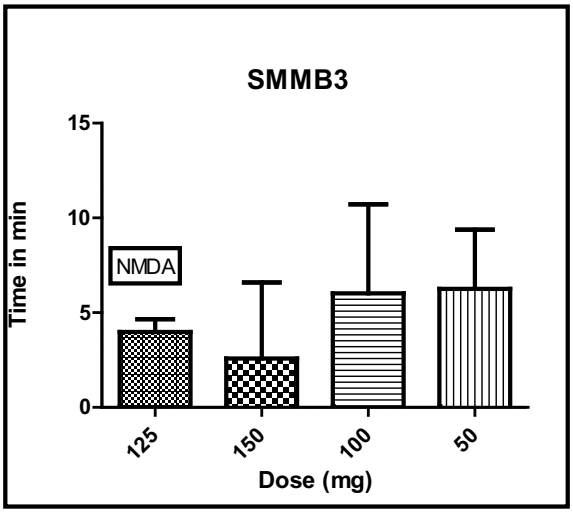

Figure 3: It shows one way ANOVA for inhibition of clonic convulsion of $SMMB_3$ at different dose level

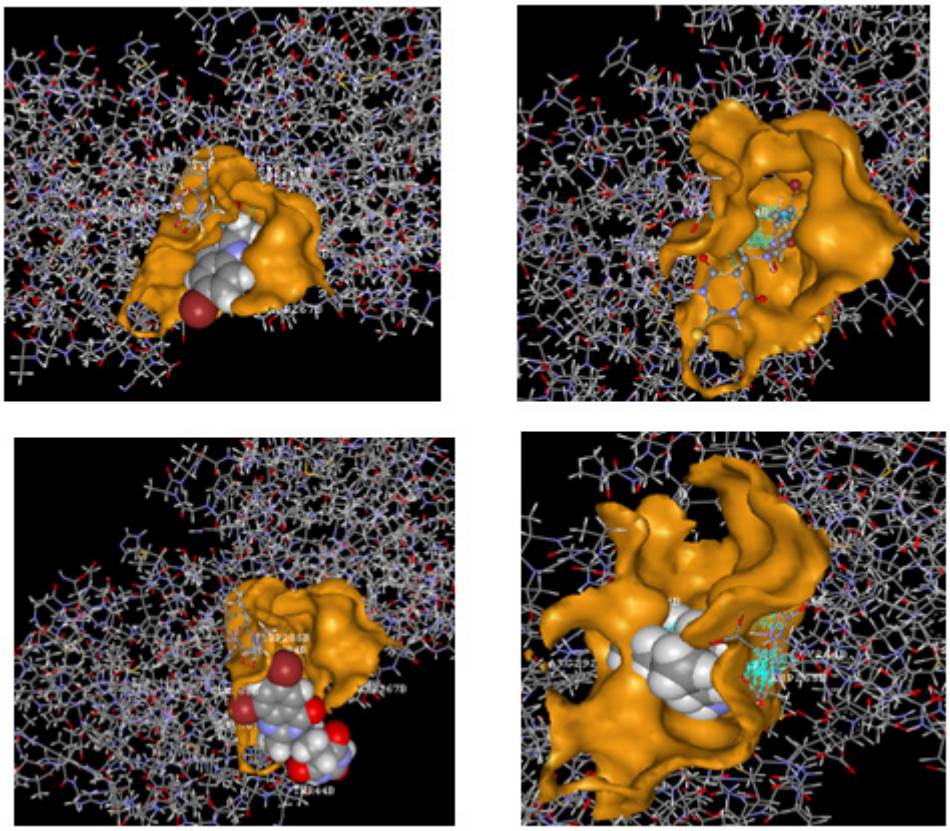

Figure 3a: CPK space fill model of SMMB1-3 and Std. memantine (posses of molecules with receptor and amino acid interaction)

is indicative of 88%, 96% and 84% chances of being anticonvulsant leads. ADME properties of these compounds were also found to be satisfactory. Limit for MDCK cell permeability is in the limit of less than 500 indicates medium permeability; all the compounds have Caco2 cell permeability in the range of middle permeability. Compounds shown to have plasma protein binding in the range of more than 90% shown strongly bound. Thus the molecules $SMMB_{1-3}$ were prioritized based on the basis of BAS & ADME before actual synthesis. The results in this paper indicate that compounds $SMMB_2$ and $SMMB_3$ shown the existence of a ligand with receptor hydrogen bond

Table 7: It shows one way ANOVA result for inhibitoin of clonic convulsions of SMMB3 at different doses (mg/kg)

Control NMDA	Standard MEMANTINE	Doses (mg/kg)		
		150 mg/kg	100 mg/kg	50 mg/kg
3.2*	10.5	8.0	10	6.5
3.5	12.5	7.5	8.5	7.2
4.0	15.5	0.0	9.3	7.8
5.0	9.8	0.0	8.3	8.5
4.5	10.7	0.0	0.0	7.5
3.7	9.8	0.0	0.0	0.0
3.98±0.66	11.43±2.1	2.58±4.00	6.017±4.7	6.25±3.1
33.85%	100%	48.63%	79.20%	69.10%

Each value represent the mean ± SEM. Significance level p***<0.05 *Time in minutes for inhibition of convulsions. +SD = Standard deviation, n = 6.

interaction between an acceptor attached to the ligand and a hydrogen bond donor attached to the receptor shown good result compared with standard (Figure 3). $SMMB_1$ showed the interaction with His127b, Ser131b, Ile133b, $SMMB_2$ showed the interaction with Thr103b, Gly128b, Met132b and $SMMB_3$ showed the interaction with Thr44b, Asp102b, His127b amino acids.

Synthesis

In synthetic scheme barbituric acid and thiobarbituric acid condensed with quinazolinone scaffold in presence of methanol and formaldehyde called as mannich base or mannich reaction.

The compounds were characterized on the basis of, TLC, Melting point, ^{1}H-NMR and IR. The ^{1}H-NMR showed characteristic peak at 4.2-4.4 ppm which is conclusive of the linkage between the thiobarbituric acid, barbituric acid and Quinazonlinone scaffolds.

Pharmacological Screenings

Synthesized compounds of series $SMMB_{1-3}$ were evaluated for pharmacological screening by acute oral toxicity (AOT) and anticonvulsant activity on NMDA receptor.

AOT: According to OECD 425 guidelines based upon their AOT studies $SMMB_{1-3}$ was administered orally at a dose of 700-2000 mg/Kg body weight. At AOT compounds were found to safe at 2000 mg/kg dose and can be considered as maximum safe dose of the compounds. For anti-convulsant activity proper dose was selected based upon the LD50 values obtained from AOT.

Anticonvulsant Screenings: NMDA induced convulsion model in which the LD_{50} of molecules were divided into three dose level and memantine was given orally as the standard or as antagonist. It was observed that as dose increases time of prolongation of convulsion and percentage of inhibition also increases while percentage of death decreases. $SMMB_3$ showed the % potency of about 79.2%, as compared with memnatine at a dose of 100 mg/kg (Table 7).

CONCLUSION

This research work concludes that, compounds were prioritized on the basis of the docking study by Vlife MDS 4.3 software. AOT was carried at a dose of 2000 mg/kg on mice for determining the LD_{50} of compounds. Compounds $SMMB_3$ showed potent anticonvulsant activity as NMDA receptor antagonist compared with standard as memnatine and can be considered as "Lead" for NMDA antagonism.

At conclusion, Mannich bases of barbituric acid and thiobarbituric acid were *in silico* designed, synthesized and pharmacologically evaluated. Compound $SMMB_3$ showed potent anti-convulsant activity of 79.2% through NMDA receptor antagonism, as compared with memnatine. The compound can serve as future lead for anticonvulsant activity.

ACKNOWLEDGEMENT

We acknowledge the Board of College and University Development (BCUD), Savitribai Phule Pune University for funding the project vide sanction grant number 13PH000882.

CONFLICT OF INTERESTS

There are no conflicts of interests.

Highlights of Paper

- NMDA Receptor is an important target for development and discovery of Anticonvulsants.
- A series of quinazolinones condensed with Barbiturates and thiobarbiturates/barbiturates was *in silico* prioritized, synthesized and pharmacologically screened for NMDA antagonism.
- Acute Oral Toxicity studies revealed LD50 dose of compounds and Compound SMMB3 was found safe at 2000Mg/Kg dose and has 79.2 %potentcy as compared with Memantine.

Author Profile

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