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RESEARCH ARTICLE

IN SILICO PHARMACOPHORE VALIDATION OF ANTICONVULSANT ACTIVITY OF (*E*) (±)-3-MENTHONE DERIVATIVES

Jainendra Jain[1]*, Sandeep Kumar Bansal[2], Partha Chowdhury[1], Reema Sinha[1], Uttam Tripathi[3], Manav Malhotra[4]

[1]*Department of Pharmaceutical Chemistry, RamEesh Institute of Vocational and Technical Education, Greater Noida-201 310, Uttar Pradesh, India*
[2]*School of Pharmacy, Bharat Institute of Technology, Meerut-250 103, Uttar Pradesh, India*
[3]*D.A.V. Post Graduate College, Dehradun-248 121, Uttaranchal-248 121, India*
[4]*Department of Pharmaceutical Chemistry, ISF College of Pharmacy, Ferozpur Road, Moga-142 001, Punjab, India*

**E-mail*: jainendrem@gmail.com
Tel.: +91 9212449829.

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In an endeavor to pursuit new strategies beyond conventional ones, energetically optimized, structure based pharmacophore was used for *in silico* anticonvulsant screening in present investigation. The study combines pharmacophore perception with protein-ligand interactions (docking) computed by *BioPredicta* and *MolSign* of VLife Molecular Design Suite 4.3.0. We derived energy-optimized pharmacophoric features for six anticonvulsants relevant to (*E*)-(±) 3-menthone derivatives (J_6, J_{14}, J_{25}, J_{30}, J_{32}, J_{33}) to access novel and potent GABA-AT inhibitors. Docking produced the most cases (5/6) with score greater then Vigabatrin, a well known GABA-AT inhibitor with proven clinical anticonvulsant efficacy. The docking results suggest the probable mechanism of anticonvulsant action that leverages the strong H-bonding (LYS329A, ARG192A) and *π*-stacking interactions (PHE189A) with GABA-AT receptor.

Key words: Pharmacophore, Anticonvulsant, Menthone, GABA-AT, Docking.

INTRODUCTION

Target and lead discovery constitute the main components of today's early pharmaceutical research (Bansal *et al* 2011; Sharma *et al* 2011; Kumar, 2011). Today, there is a urgent need for new antiepileptic drugs, since long established antiepileptic drugs control seizures in 50% of patients developing partial seizures and in 60-70% of those developing generalized seizures (Duncan, 2002; Kulandasamy *et al* 2009a, b). During past decade numerous new drugs were approved but despite the advances, approved antiepileptic drugs have dose related toxicity and idiosyncratic side effects (Ghogare *et al* 2010). The field of antiepileptic drug development (AED) has become quite dynamic, affording many promising research opportunities. Mechanistic approaches are increasingly being facilitated by the wave of research in the epileptics (Eadie and Tyrer, 1989). The search continues for the ideal drug that should be potent, selective in raising seizure threshold and preventing seizure spread without causing serious side effects. Structural features of most of the currently used antiepileptic have indicated presence of hydrazones (=N-NH-), amides (-$CONH_2$), carbamides (-NHCONH-) and semicarbazido groups (=NNHCONH-). Majority of active compounds were found to contain large hydrophobic group in close proximity to two

electron donor atoms, thus fulfilling the MES structural requirements (Jones and Woodbury, 1982).

Several models have been proposed to investigate the probable mode of action of these compounds. One such pharmacophore model suggested for semicarbazones displaying anticonvulsant activity, has shown that four binding sites (an aryl hydrophobic binding site, hydrogen bonding domain, an electron donor group and hydrophobic-hydrophilic controlling domain; **Figure 1**) are essential to interact with a macromolecular complex, *in vivo* (Dimmock *et al* 1995; Pandeya *et al* 1998; 2002).

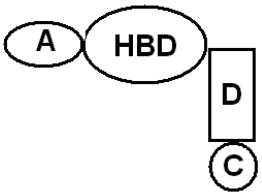
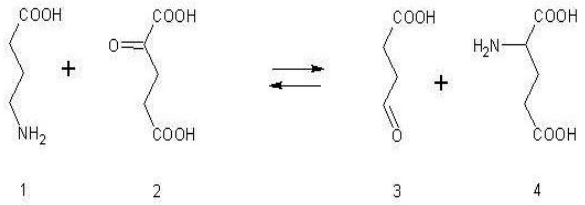


Fig. 1. Suggested pharmacophore model for semicarbazones displaying anticonvulsant activity

Another 2D model has suggested that at least one aryl unit, one or two electron donor atoms, and/or an NH group in a spatial arrangement are necessary for anticonvulsant activity (Yogeeswari *et al* 2005). A common pharmacophore model based on some well known voltage gated sodium channel blockers including phenytoin and lamotrigine has been identified (Unverferth *et al* 1998).

When the concentration of Gamma amino butyric acid (GABA) diminishes below a threshold level in the brain, convulsion results while, raising the brain GABA levels terminates the seizures. Gamma amino butyric acid amino transferase (GABA-AT) is also proposed as a validated target for antiepileptic drugs, because its selective inhibition raises GABA concentration in brain (Storici *et al* 1999). GABA-AT is a homodimer with each subunit containing an active site pyridoxal phosphate (PLP), covalently bound to Lys 329 of chain A *via* a schiff base (Storici *et al* 2004). It catalyzes reversible transfer of the amino group of GABA (**1**) to α-ketoglutarate (**2**) to yield succinic semialdehyde (**3**) and L-glutamate (**4**) (Toney *et al* 1995, **Scheme 1**).

Essential oil containing menthone from flower heads of *Egletes viscosa,* has been reported to possess anticonvulsant properties in scPTZ test (Souza *et al* 1998). Simultaneously, a ten carbon amino acid derived from hydrolysis of (-)



Scheme 1. Reversible transfer of the amino group of GABA (**1**) to α-ketoglutarate (**2**) to yield succinic semialdehyde (**3**) and L-glutamate (**4**)

menthone oxime has emerged as potent inhibitor of GABA neuroreceptor (Lochyński *et al* 2000). Because (±) 3-menthone is a key component, to possess anticonvulsant properties, in addition to its pharmacokinetic profile according to MES structural requirement *i.e.* hydrophobicity, therefore (±) 3-menthone derivatives have been investigated (Jain *et al* 2007; 2010; 2011). We focused and reported the synthesis and antiepileptic properties of (±) 3-menthone Schiff bases (**J_1-J_8,** Figure 2), (±) 3-menthone aryl hydrazones (**J_9-J_{10}**), (±) 3-menthone semicarbazones (**J_{11}-J_{17}**) and thiosemicarbazones (**J_{18}-J_{27}**), (±) 3-menthone aryl acid hydrazones (**J_{28}-J_{37}**) and (±) 3-menthone oxime (**J_{38}**).

EXPERIMENTAL

Dataset

The data set comprises of previously reported (±) 3-menthone derivatives *viz.* (±) 3-menthone Schiff bases (**J_1-J_8**), (±) 3-menthone aryl hydrazones (**J_9-J_{10}**), (±) 3-menthone semi-carbazones (**J_{11}-J_{17}**) and thiosemicarbazones (**J_{18}-J_{27}**), (±) 3-menthone aryl acid hydrazones (**J_{28}-J_{37}**) and (±) 3-menthone oxime (**J_{38}**) and shown in **Table 1-5** respectively.

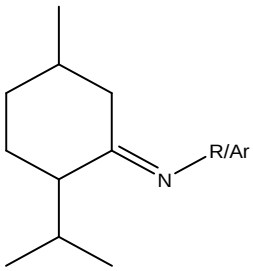
Ligand preparation

Structures of compounds **J_1-J_{38}** were built in 2D draw module of VLife MDS 4.3.0 Ligand geometry of all compounds was optimized by energy minimization using Merck Molecular Force Field (MMFF) and Gasteiger Marsili (GM) charges for the atoms till a gradient of 0.001 Kcal/mol/A° was reached. The conformation search was carried out to identify the lowest energy conformation, using the systematic search of MDS Engine. All these lowest energy conformations were saved as .mol2 file.

Docking studies

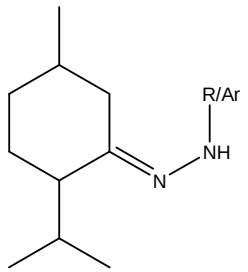
All synthesized compounds (**J_1-J_{38}**) were docked in crystal structure of GABA-AT (PDB ID: 1OHV)

Table 1. (±) 3-Menthone schiff bases (**J_1-J_8**)



Compound No.	R/Ar
J_1	*n*-butyl
J_2	cyclohexyl
J_3	phenylethyl
J_4	phenyl
J_5	4-chlorophenyl
J_6	4-bromophenyl
J_7	4-methoxyphenyl
J_8	2-methoxyphenyl

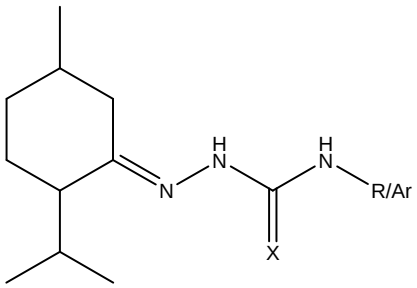
Table 2. (±) 3-Menthone aryl hydrazones (**J_9-J_{10}**)



Compound No.	R/Ar
J_9	phenyl
J_{10}	2,4-dinitrophenyl

complexed with co-crystal ligand pyridoxal-5-phosphate (PLP). It is a tetramer comprising of four chains A, B, C and D. The enzyme was purified by removing chain C, chain D and cofactors present in chain A and chain B.

Table 3. (±) 3-Menthone (**J_{11}-J_{17}**) and thiosemicarbazones (**J_{18}-J_{27}**)

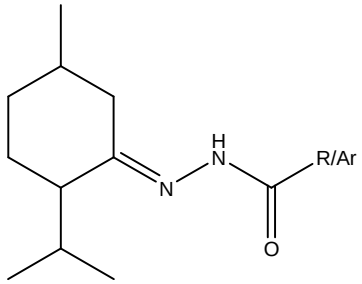


Compound No.	X	R/Ar	Compound No.	X	R/Ar
J_{11}	O	phenyl	**J_{18}**	S	H
J_{12}	O	4-chlorophenyl	**J_{19}**	S	Phenyl
J_{13}	O	4-bromophenyl	**J_{20}**	S	2-methylphenyl
J_{14}	O	4-fluorophenyl	**J_{21}**	S	4-methylphenyl
J_{15}	O	4-nitrophenyl	**J_{22}**	S	2-methoxyphenyl
J_{16}	O	4-methylhenyl	**J_{23}**	S	4-methoxyphenyl
J_{17}	O	2-methylphenyl	**J_{24}**	S	4-chlorophenyl
			J_{25}	S	4-bromophenyl
			J_{26}	S	4-fluorophenyl
			J_{27}	S	4-nitrophenyl

The incomplete residues were mutated by using *BioPredicta* module of VLife MDS. The co crystal ligand, PLP was extracted and both structures *i.e.* receptor and PLP were saved as .mol2 file separately.

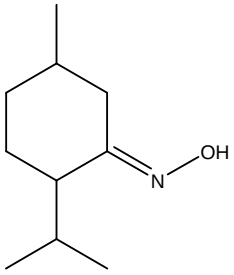
A systematic conformational search was performed to obtain the low energy conformations of the receptor and PLP. The low energy conformations, thus obtained were optimized till they reached r.m.s. gradient energy of 0.001 Kcal/mol/A°. In order to define ligand-receptor interactions, docking of all the low energy conformations within a range of 5 Kcal/mol/A° from the lowest energy conformation of each molecule, into the cavity of 1OHV was done by positioning the molecule appropriately in the active site of chain A and B by reference ligand, PLP, using GRIP docking. Docking score of **J_1-J_{38}** were compared with a well known GABA-AT inhibitor.

Table 4. (±) 3-Menthone aryl acid hydrazones (**J_{28}-J_{37}**)



Compound No.	R/Ar
J_{28}	phenyl
J_{29}	4-methylphenyl
J_{30}	4-aminophenyl
J_{31}	2-hydroxyphenyl
J_{32}	4-nitrophenyl
J_{33}	4-chlorohenyl
J_{34}	4-bromophenyl
J_{35}	4-fluorophenyl
J_{36}	3-pyridyl
J_{37}	4-pyridyl

Table 5. (±) 3-menthone oxime (**J_{38}**)

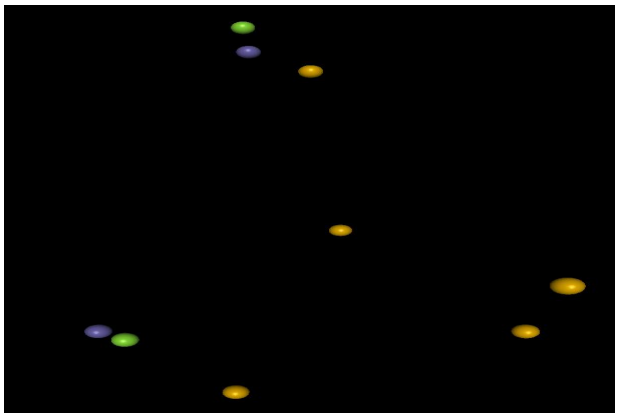


Pharmacophoric features analysis and interaction study

Complimentary "biophoric features" of 1OHV, "pharmacophoric features" and GABA-AT interactions of six most potent (±) 3-menthone derivatives including Vigabatrin were determined with *BioPredicta, MolSign* and *VLife Engine* modules of VLife MDS 4.3.0 and results are displayed in **Figure 2-4**. These interactions were analyzed in terms of charge transfer and hydrogen bond formation with active residues, Lysine 329 and Arginine 192 present in chain A of 1OHV.

RESULTS AND DISCUSSION

We developed "biophoric features" of GABA-AT enzyme (pdb code: 1OHV; *BioPredicta*, VLife Molecular Design Suite 4.3.0) and "pharmacophoric features" of six most potent anticonvulsant (±) 3-menthone derivatives (**J_6, J_{14}, J_{25}, J_{30}, J_{32}** and **J_{33}**; *MolSign*, VLife Molecular Design Suite 4.3.0) alongwith docking studies.

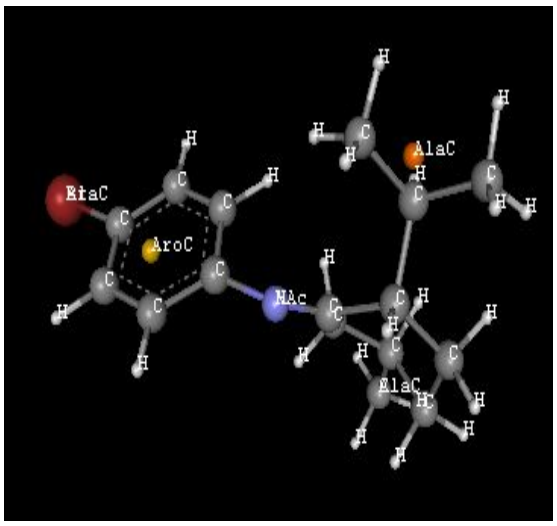


Hydrogen donor (HDr) in green, Hydrogen acceptor (HAc) in blue and Aromatic component (AroC) in yellow.

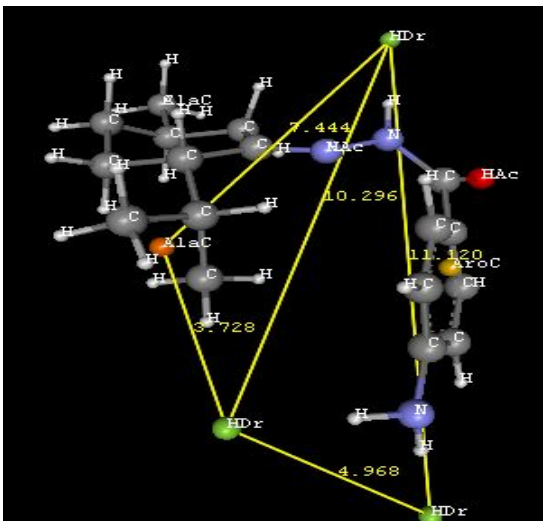
Fig. 2. Biophoric features of Gamma amino butyric acid amino transferase (GABA-AT; PDB ID: 1OHV)

BioPredicta identified three important biophoric features of GABA-AT enzyme, important for activity: one as hydrogen donor (HDr), the other as hydrogen acceptor (HAc) and third is represented by aromatic component (AroC). Docking produced the most cases (**5/6**) with score greater then Vigabatrin, a well known GABA-AT inhibitor with proven clinical anticonvulsant efficacy. Docking scoring function value and interactions observed, an indication of how well a ligand conformation is embedded in the binding site, was calculated for all (±) 3-menthone derivatives (**J_1-J_{38}**), Vigabatrin and PLP, are indicated in **Table 6**. Grip docking process, based on flexible ligand docking method was adopted for this purpose and hydrogen bond interactions with LYS329A, THR353B, SER137A, GLN301A and ARG192A; π-stacking interactions with PHE189A and charge interactions with LYS329 were observed. The bond lengths for hydrogen bond interactions with LYS329A and π-stacking interactions with PHE189A were identified in 1.666-2.542 A° and 4.891-5.377 A° respectively. Previously reported Phase-II quantitative anticonvulsant screening data (MES ED_{50}, scPTZ ED_{50} and 6Hz ED_{50}) of six most potent (±) 3-menthone derivatives (**J_6, J_{14}, J_{25}, J_{30}, J_{32}** and **J_{33}**) was used to correlate GABA-AT selectivity of these compounds and shown in **Table 7**. The docking results and binding interactions were in good agreement with

biological activities and suggest the probable mechanism of anticonvulsant action that leverages the strong H-bonding and π-stacking interactions with GABA-AT receptor.



a)



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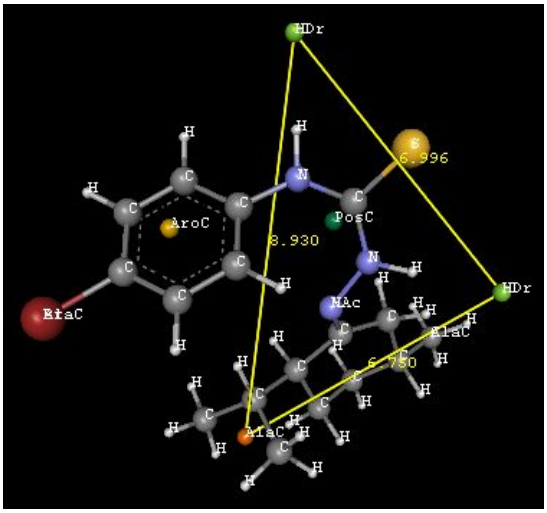

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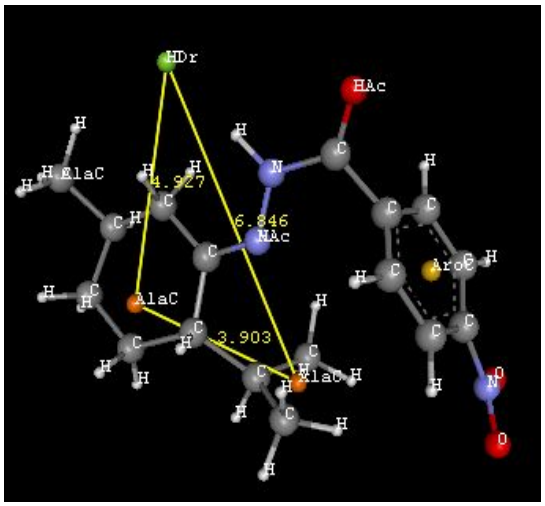

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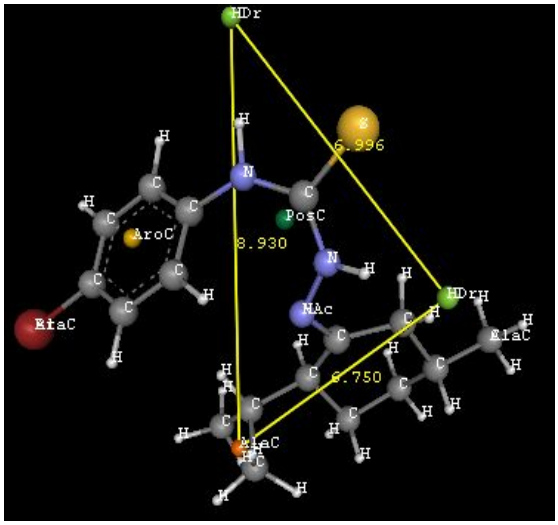

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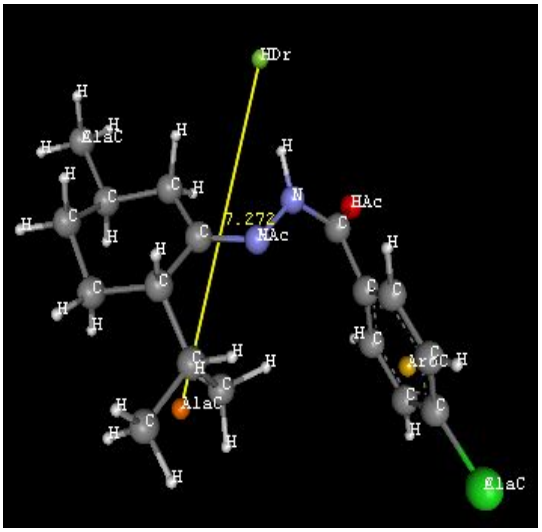

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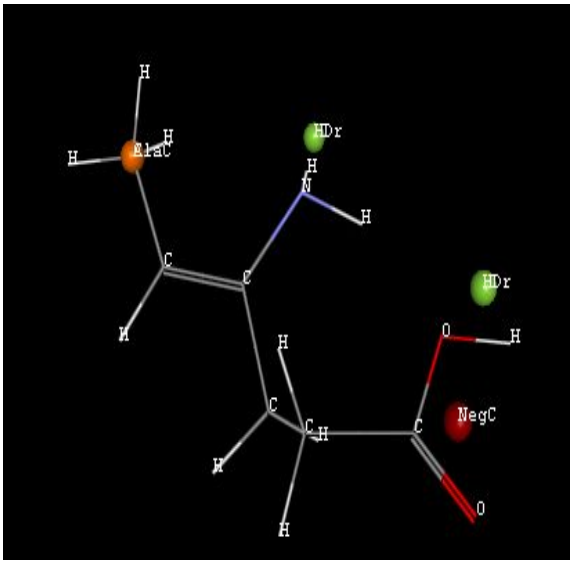
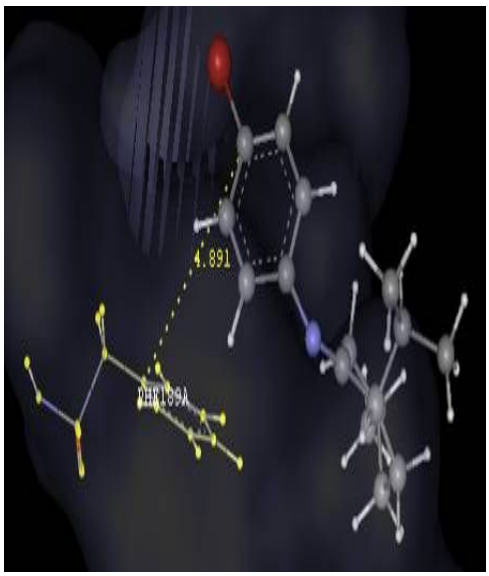
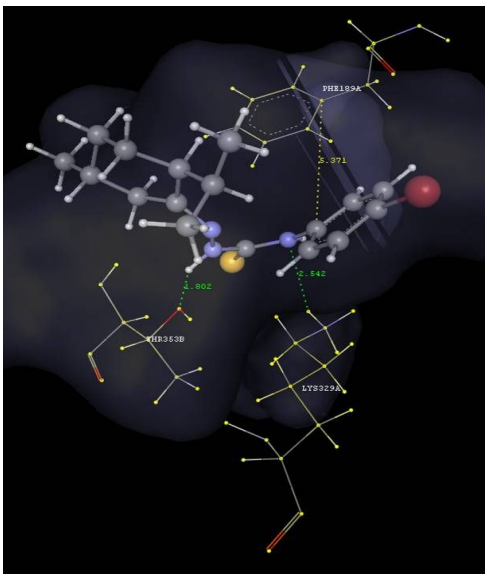


g)

Fig. 3. Pharmacophoric features of compounds a) J_6; b) J_{14}; c) J_{25}; d) J_{30}; e) J_{32}; f) J_{33}; g) Vigabatrin



a)



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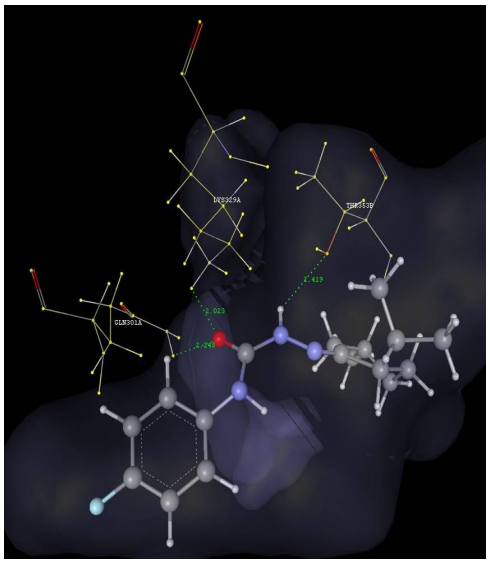

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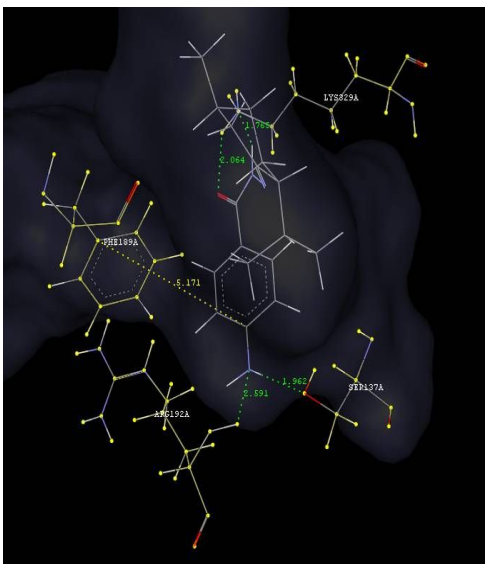

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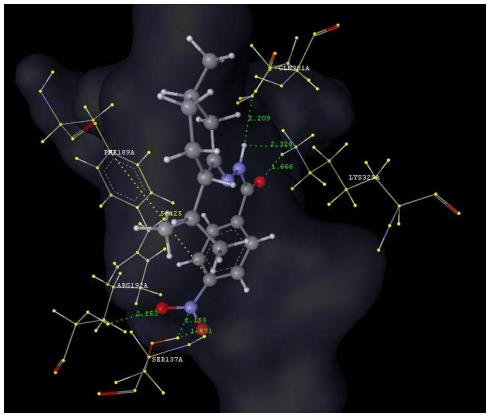

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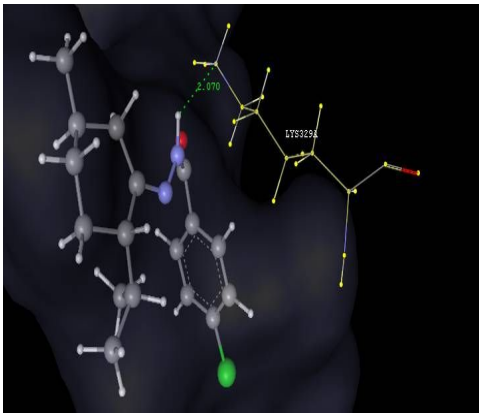

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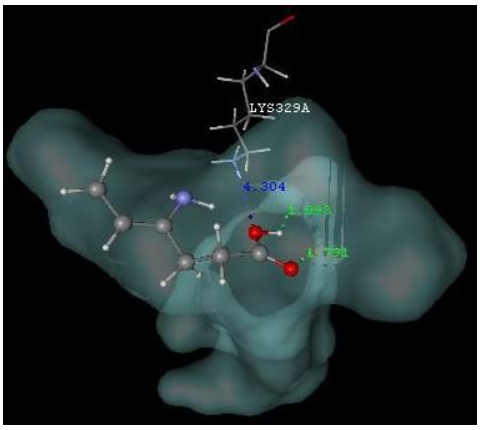


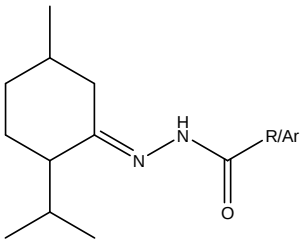
g)

(A dashed green line represents a hydrogen bond and a dashed yellow line represents a π-stacking interaction)

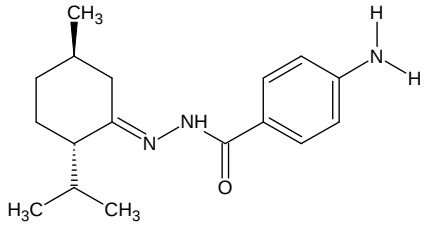
Fig. 4. GABA-AT interactions of compounds a) J_6; b) J_{14}; c) J_{25}; d) J_{30}; e) J_{32}; f) J_{33}; g) Vigabatrin

The results highlight (±) 3-menthone aryl acid hydrazones analogues (**Figure 5a**) as potential scaffold. The most promising molecule identified by these interaction studies was the 4-amino-N'-(2-isopropyl-5-methylcyclohexylidene) benzo hydrazide (**J_{30}**; **Figure 5b**) which has showed excellent binding affinity interaction with LYS329.

This compound was also potent *in vivo* anticonvulsant at 6Hz screen (onset of action in 0.25 h) with protective index greater than 10.96. The compound **J_{33}** exhibited the best therapeutic window, having protective index greater than 15.92. Relative to the native inhibitor vigabatrin, compound **J_{30}** yielded a higher docking score (-68.93 Kcal/mol). In **Figure 6d**, the binding conformation of **J_{30}** is compared with the binding conformation of vigabatrin. NH_2 group of **J_{30}** forms hydrogen bonding interaction with SER137A and ARG192A in contrast to vigabatrin, and aromatic box of the molecule, the aniline forms *π*-stacking interactions with PHE189A. Thus, compound **J_{30}** fulfills all the pharmacophore queries.



(a)



(b)

Figure 5. a) (±) 3-menthone aryl acid hydrazones analogues a potential scaffold; **b)** 4-amino-N'-(2-isopropyl-5-methylcyclohexylidene)benzohydrazide (**J_{30}**) showed excellent binding affinity and interaction with LYS329

Table 6. Docking score interactions observed of (±) 3-menthone derivatives (J_1-J_{38})

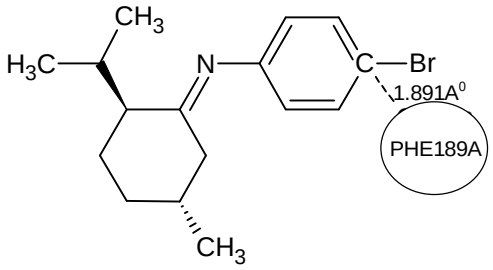
Compound No.	Docking score (PLP Score)	H-bonding interactions (Bond length; A°)	π-stacking interactions (Bond length; A°)	Charge interactions with Lys 329A
J_1	-68.35	-	-	-
J_2	-75.75	-	-	-
J_3	-66.17	-	-	-
J_4	-69.72	-	-	-
J_5	-89.87	-	-	-
J_6	-75.49	-	PHE189A(4.891)	-
J_7	-93.69	-	-	-
J_8	-66.29	-	-	-
J_9	-73.94	-	-	-
J_{10}	-78.60	-	-	-
J_{11}	-70.00	-	-	-
J_{12}	-56.82	-	-	-
J_{13}	-52.03	-	-	-
J_{14}	-67.87	LYS329A(2.023) THR353B(2.419) GLN301A(2.245)	-	-
J_{15}	-55.93	-	-	-
J_{16}	-72.40	-	-	-
J_{17}	-74.58	-	-	-
J_{18}	-65.43	-	-	-
J_{19}	-55.99	-	-	-
J_{20}	-62.02	-	-	-
J_{21}	-65.77	-	-	-
J_{22}	-55.43	-	-	-
J_{23}	-60.00	-	-	-
J_{24}	-62.49	-	-	-
J_{25}	-60.17	LYS329A(2.542) THR353B(1.802)	PHE189A (5.371)	-
J_{26}	-54.47	-	-	-
J_{27}	-74.16	-	-	
J_{28}	-74.72	-	-	-
J_{29}	-81.49	-	-	-
J_{30}	-68.93	LYS329A(1.765, 2.064) ARG192A(2.591) SER137A(1.962)	PHE189A (5.171)	-
J_{31}	-68.13	-	-	-
J_{32}	-61.81	LYS329A(2.328, 1.666) ARG192A(2.263) SER137A(2.155, 1.471) GLN301A(2.209)	PHE189A (5.425)	-
J_{33}	-61.94	LYS329A(2.070)	-	-
J_{34}	-71.67	-	-	-
J_{35}	-70.08	-	-	-
J_{36}	-69.45	-	-	-
J_{37}	-60.03	-	-	-
J_{38}	-50.01	-	-	-
Vigabatrin	-47.36	LYS329A(4.304)	-	LYS329A (1.993, 1.791)
PLP	-76.28	-	-	-

*** '-'** indicates absence of interactions

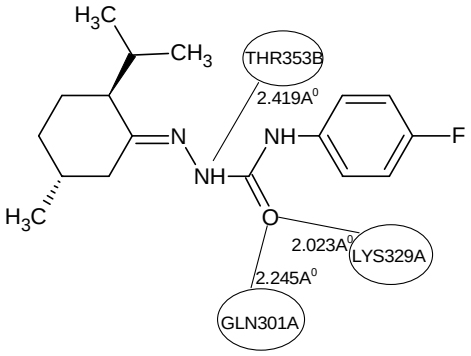
Table 7. Quantitative anticonvulsant screening data of six most potent (±) 3-menthone derivatives

Compound No.	Test	Time (h)	ED_{50} (mg/kg)	TD_{50} (mg/kg)	Protective index (pI)
J_6	scPTZ	0.5	67.83	125.25	1.84
J_{14}	MES	0.25	44.15	73.5	1.66
	scPTZ	0.25	38.68	145.39	3.75
J_{25}	scPTZ	0.25	58.62	-	-
J_{30}	6Hz	0.25	45.6	>500	>10.96
	MES	0.5	80.0	>500	>6.25
	scPTZ	0.5	>250	-	-
J_{32}	6Hz	0.5	88.12	-	-
J_{33}	6Hz	0.25	16.1	-	-
	MES	0.5	31.4	>500	>15.92
	scPTZ	0.5	>250	-	-

* '-' No activity



a)



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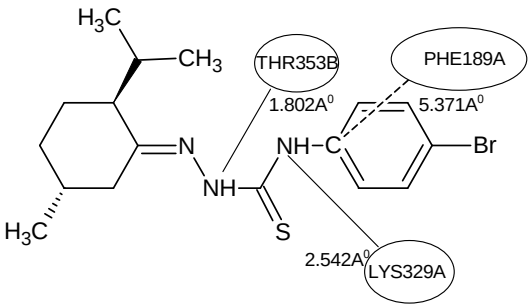
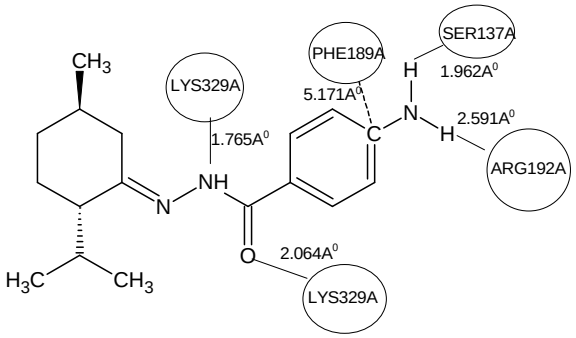


c)



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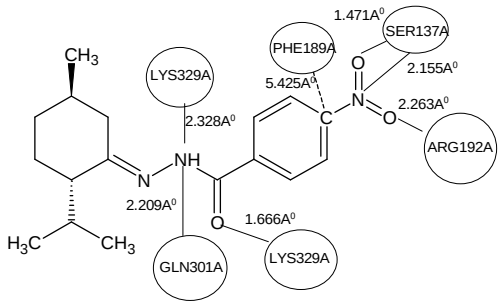

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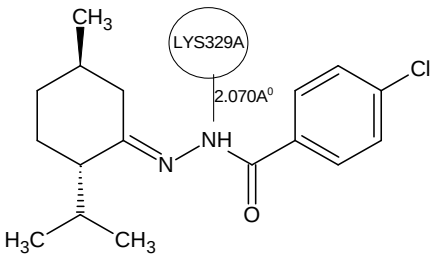

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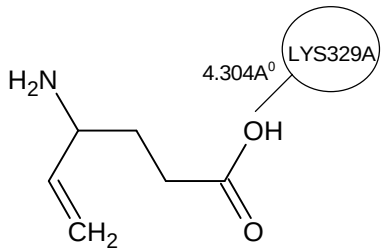

g)

(A dashed line represents π-stacking interactions, and a solid line represents H-bonding interaction)

Fig. 6. GABA-AT interactions of compounds a) J_6; b) J_{14}; c) J_{25}; d) J_{30}; e) J_{32}; f) J_{33}; g) Vigabatrin

The compound **J32**, (±) 3-menthone aryl acid hydrazones analogue was received a docking score of -68.93 Kcal/mole. In **J32**, pharmacophore query are fulfilled by nitro group in place of NH_2, exhibited same interaction as observed in **J30**, in addition two more H-bonding interactions with GLN301A and SER137A were observed. With docking score of **J14** stood rank third and lacks π-stacking interactions. On the contrary **J6** and **J33** void H-bonding interactions and π-stacking interactions respectively.

Compound **J6**; (*N*-(4-bromophenyl)-5-methyl-2-(propan-2-yl) cyclohexanimine) was found the most potent (scPTZ, ED_{50} = 67.83 mg/kg at 0.5 h; 38.68 mg/kg at 0.5 h interval) among the series of (±) 3-menthone Schiff bases. Simultaneously, the series of (±) 3-menthone semicarbazones and thiosemicarbazones have produced the compounds, **J14**; N^1-(4-fluorophenyl)-N^4-(menth-3-one) semicarbazide and **J25**; N^1-(4-bromophenyl)-N^4-(menth-3-one) thiosemicarbazide. **J14** has been evaluated for anti-MES and anti-scPTZ screening at corresponding ED_{50} values of 44.15 mg/kg and 38.68 mg/kg respectively; while, **J25** was found active in scPTZ screen only at ED_{50} value of 58.62 mg/kg. Three compounds (**J30, J32** and **J33**) from the series of (±) 3-menthone aryl acid hydrazones have shown potent activity in MES, scPTZ and minimal clonic seizure test. Compounds **J30**; 4-amino-N'-(menth-3-one) benzohydrazide and **J32**; 4-nitro-N'-(menth-3-one) benzohydrazide showed 6Hz ED_{50} values of 45.6 mg/kg at 0.25hr and 88.12 mg/kg at 0.5hr respectively. While the compound **J30** was also active in MES test at 0.5hr with ED_{50} value of 80.0 mg/kg. Compound **J33**; 4-chloro-N'-(menth-3-one) benzohydrazide, the most active analog among the series, exhibited MES ED_{50} value of 31.4 mg/kg at 0.5 h and 6Hz ED_{50} value of 16.1 mg/kg at 0.25 h.

CONCLUSION

In summary, inspired by the anticonvulsant potential of (±) 3-menthone derivatives, inhibitory behavior towards GABA-AT receptor, we envisioned (±) 3-menthone aryl acid hydrazones analogues as a promising scaffold. Molecular docking simulations predicted six compounds to bind with better affinity than the native inhibitor, vigabatrin. GABA-AT binding interactions of these six compounds revealed three compounds that satisfied pharmacophore queries.

It is interesting to note that 4-amino-N'-(2-isopropyl-5-methylcyclohexylidene) benzo hydrazide **J30** showed excellent binding affinity and interaction with LYS329 (H-bond = 1.765 A°). Thus the identified (±) 3-menthone aryl acid hydrazones can be developed in to lead structure with therapeutic anticonvulsant because of its simple structure.

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