Protective Role of Piperazine Derivative in Ameliorating the Aluminium-induced Oxidative Stress and Neurobehavioral Impairments in Wistar Rats

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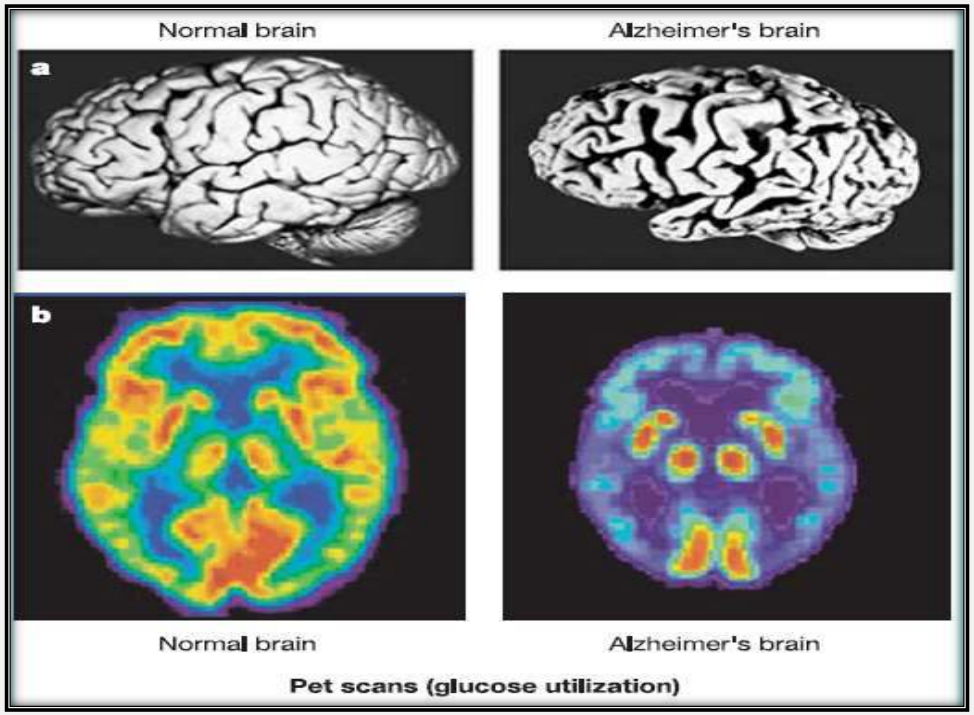
About Alzheimer's

Progressive and fatal neurodegenerative disorder

cognitive and memory deterioration

progressive impairment of activities of daily living

a variety of neuropsychiatric symptoms and behavioral disturbances

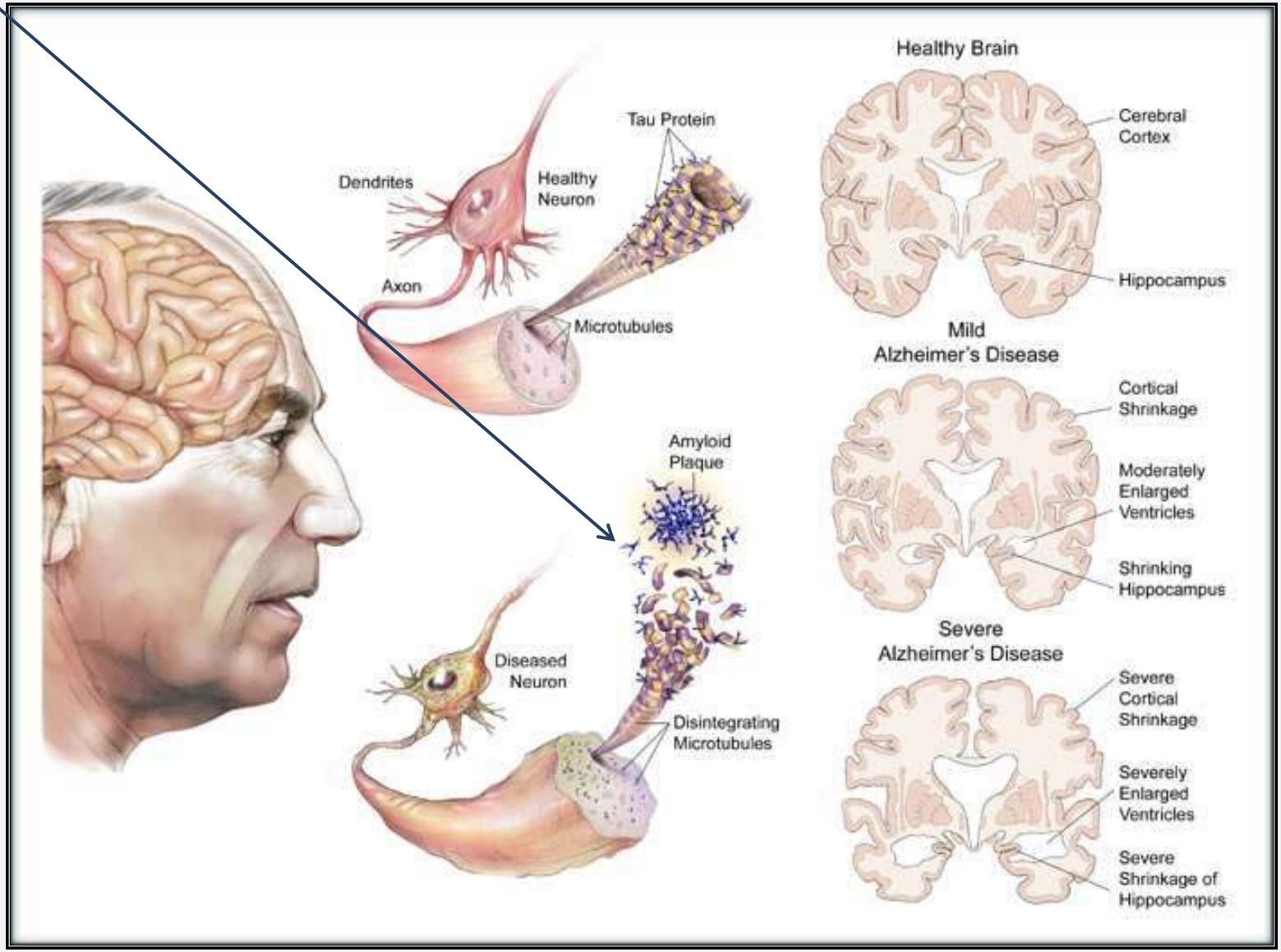


❖ Worldwide prevalence of the Alzheimer's disease (AD) is estimated to be more than 37 million cases (Mount, C. & Downton, C. 2006).

❖ In 2000, India had 3.5 million patients with AD as against US, which had 4.5 million patients with Alzheimer disease (Upadhyaya et al. 2010). But with an increase in the geriatric population in India, number of AD patients is growing at a phenomenal rate.

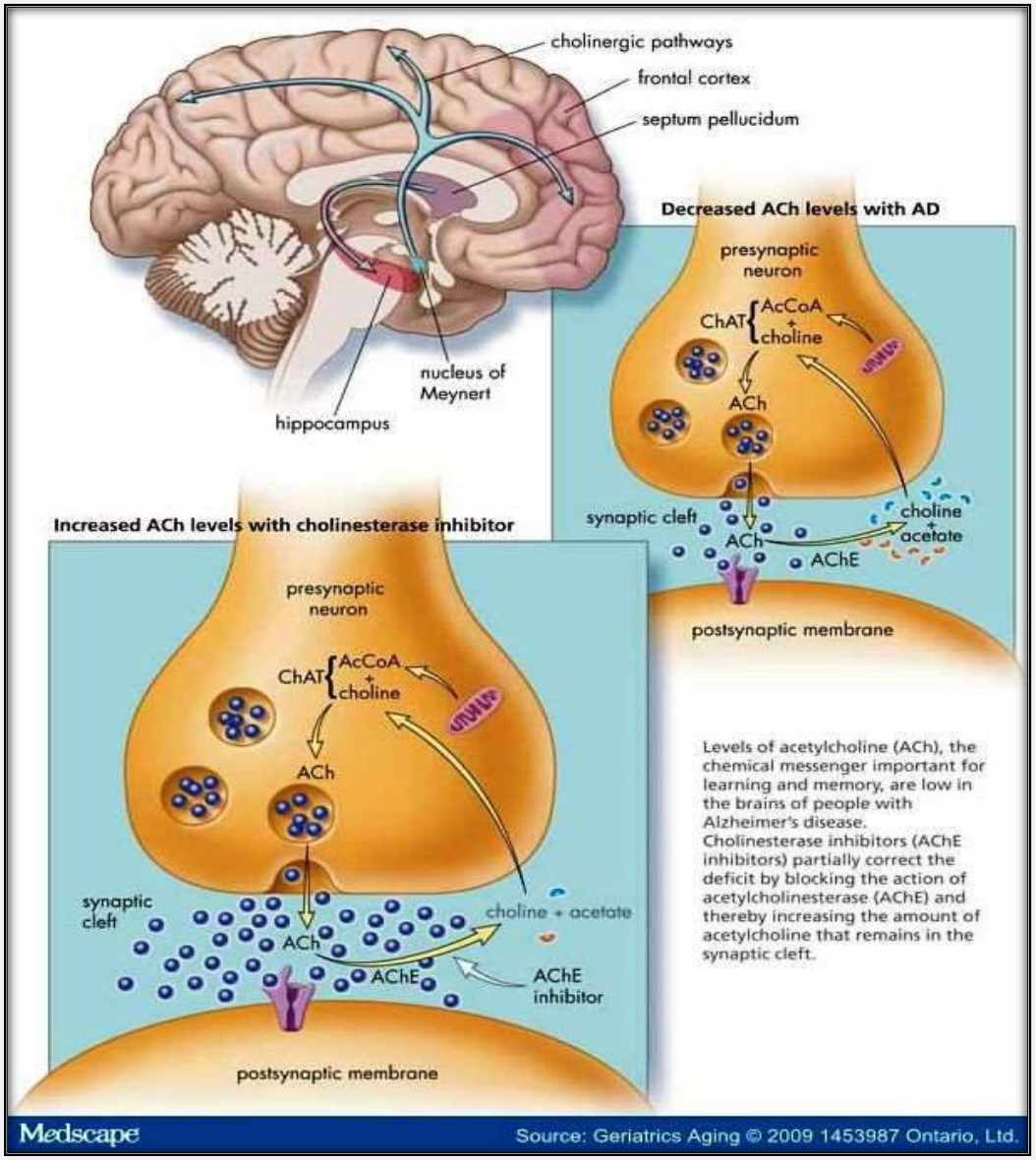
Brain Atrophy in AD

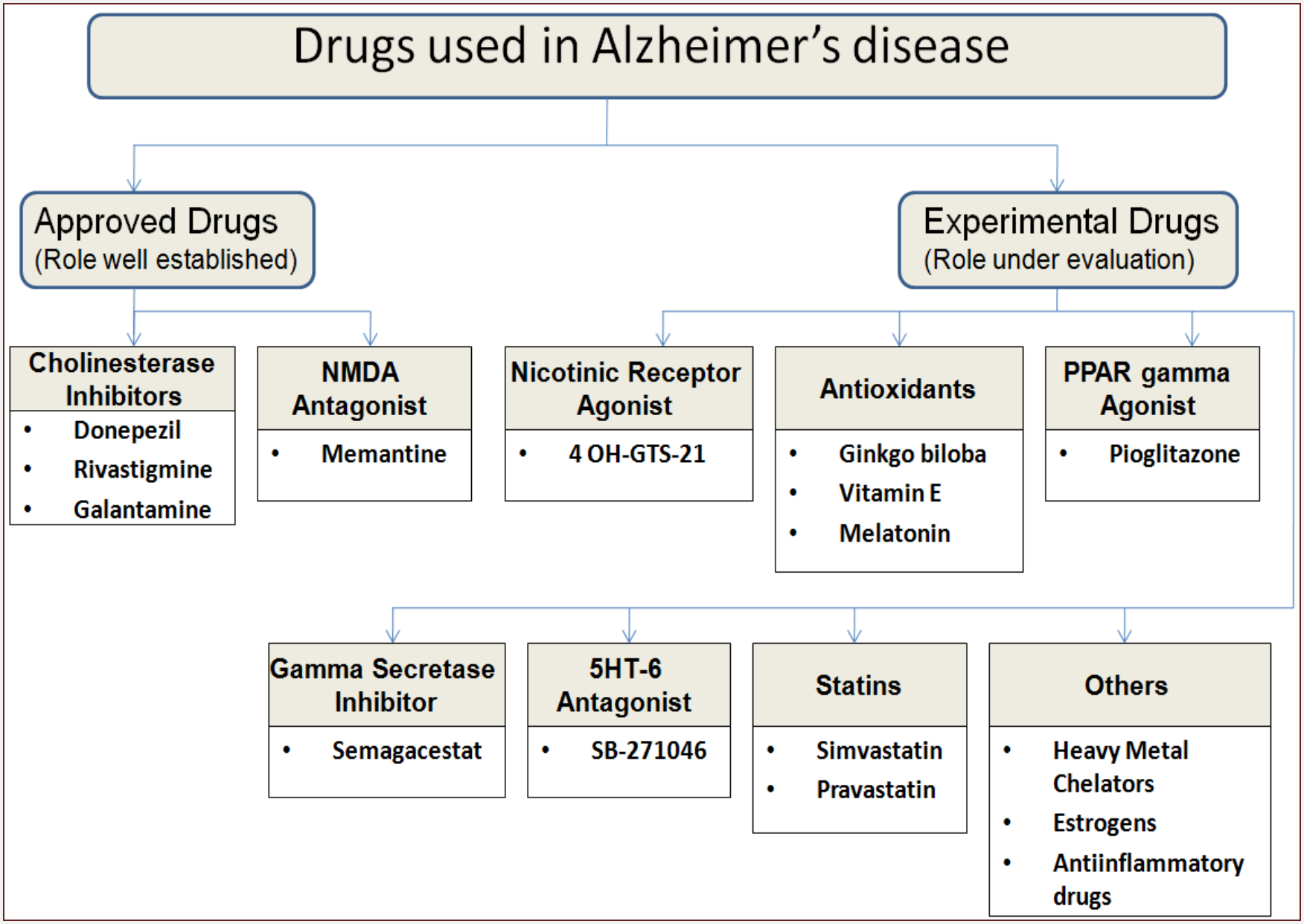
WRONG!



Cholinergic hypothesis

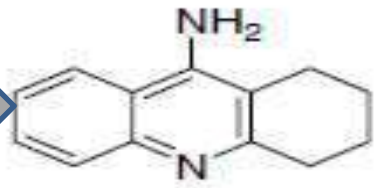
- The cholinergic hypothesis posits that a dysfunction in acetylcholine (ACh) - containing neurons substantially contributes to the cognitive decline observed in Alzheimer's disease (AD). This is based on the observation that cholinergic transmission has a fundamental role in cognition and is disrupted in patients with AD.



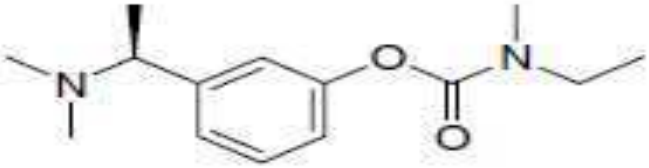

Drugs used in Alzheimer's disease
Approved Drugs
(Role well established)
Experimental Drugs
(Role under evaluation)
Cholinesterase Inhibitors
Donepezil
Rivastigmine
Galantamine
NMDA Antagonist
Memantine
Nicotinic Receptor Agonist
4 OH-GTS-21
Antioxidants
Ginkgo biloba
Vitamin E
Melatonin
PPAR gamma Agonist
Pioglitazone
Gamma Secretase Inhibitor
Semagacestat
5HT-6 Antagonist
SB-271046
Statins
Simvastatin
Pravastatin
Others
Heavy Metal Chelators
Estrogens
Antiinflammatory drugs

FDA approved AChEIs

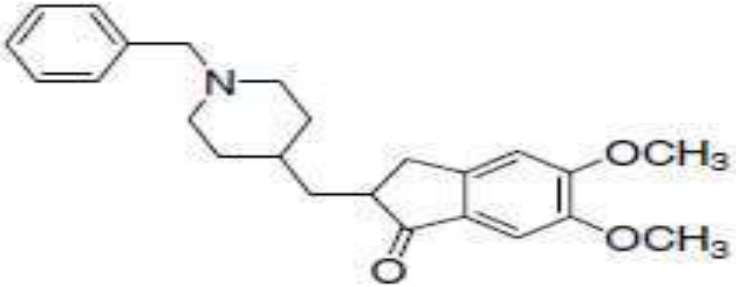
Withdrawal from the market



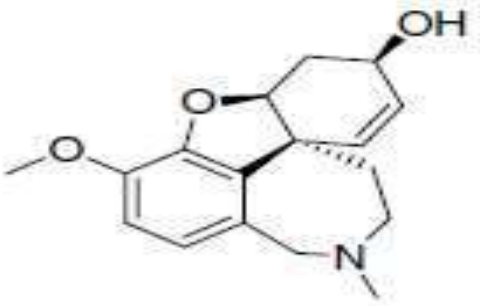
tacrine



rivastigmine



donepezil

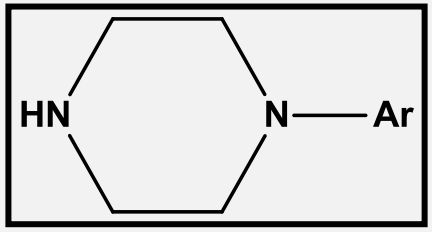


galantamine

Limited use in clinics : - Adverse peripheral effects
- Side effects such as confusion, hallucinations, extreme or sudden changes in behavior, nausea,or stomach pain
- Hepatotoxic effects

❖ Therefore, there is a continued interest in the development of novel AChEIs possessing less adverse effects and more beneficial effects in improving cognitive, functional and behavioural symptoms associated with AD.

Aryl piperazine derivatives for the study



Universal templates for designing CNS active agents

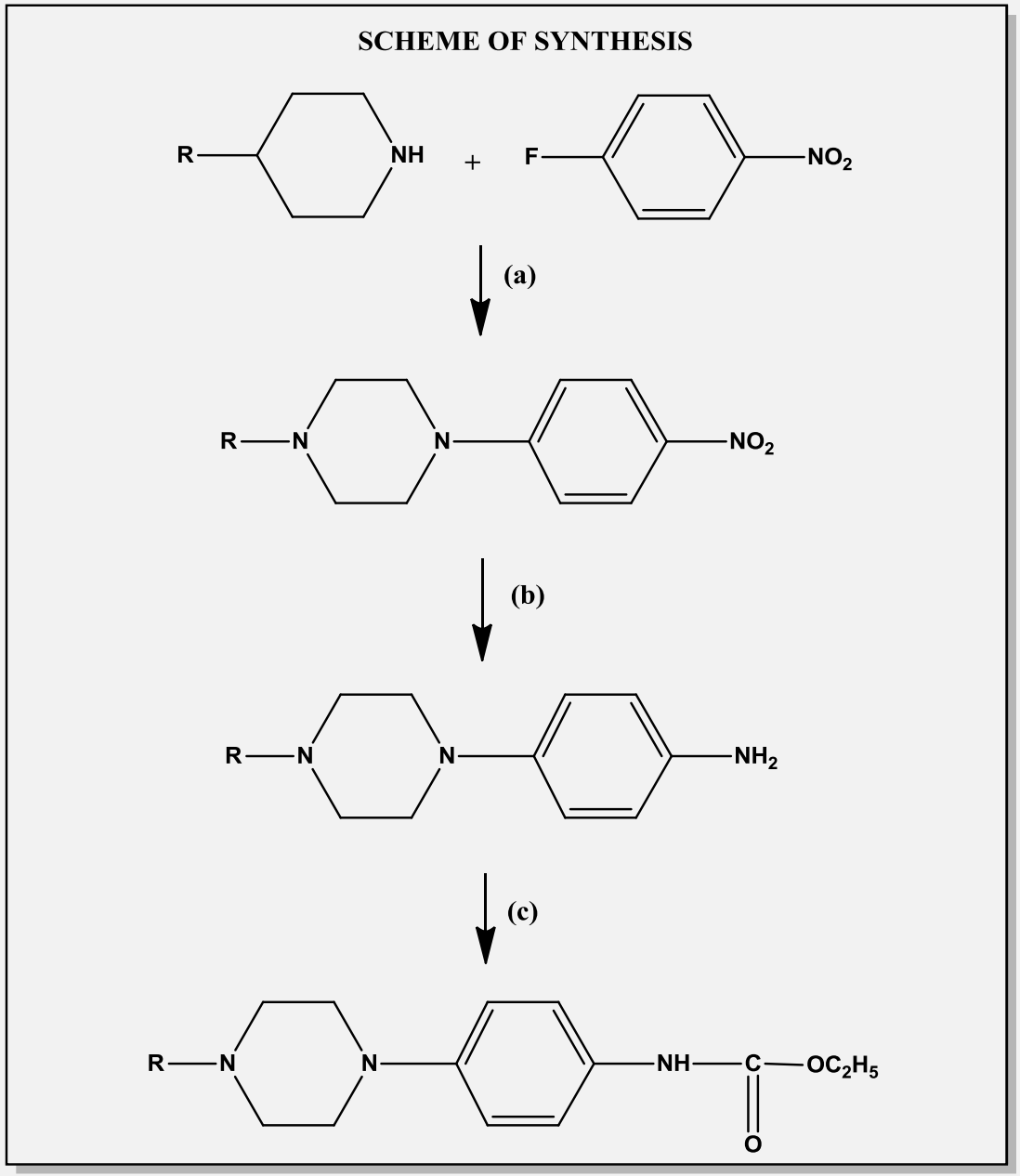
Known anticholinesterase activity in worms

Aryl piperazine

Treatment of depression, anxiety & related illness

FK-960, a novel putative anti-dementia drug candidate, and some other piperazine derivatives have been demonstrated to possess activities against AD

Synthesis of Aryl piperazine derivatives

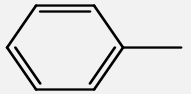
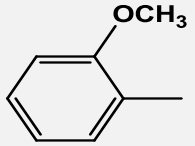
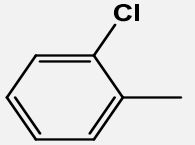
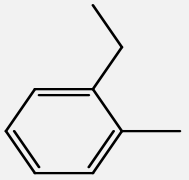


Reagents & conditions:

(a) DMSO,rt,1hr

(b) $CHCl_3$ Sn/HCl,60-70°, 3-4 hr

(c) Ethyl chloro formate, DCM, Et_3N, 2 hr

	R
D1	
D2	
D3	
D4	
D5	$-CH_3$
D6	$-CH_2CH_3$

OBJECTIVE 1

- ✓ Prediction of pharmacokinetic properties and drug-likeness of compounds D1-D6
- ✓ Selection of possible cognitive enhancers
- ✓ In vitro effects on AChE activity
- ✓ To explore a possible interacting mode of the piperazine derivatives with Torpedo californica (TcAChE) using molecular docking approach

Predicted ADMET properties of the target compounds (D1-D6)

Compound	Mol_MW (130–725)	HB donors (0–6)	HB acceptors (2–20)	QPlog Po/W (−2 to 6.5)	Rotable bonds (0–15)	PSA (7–200)	SASA (300–1,000)
D1	325.41	1	4.5	4.158	2	49.944	646.047
D2	355.436	1	5.25	4.273	3	53.821	679.826
D3	359.855	1	4.5	4.496	2	49.995	657.66
D4	353.463	1	4.5	4.728	3	49.496	687.477
D5	263.339	1	5.5	2.03	2	52.831	567.511
D6	277.366	1	5.5	2.371	3	52.44	597.505

Compound	CNS	QPPcaco (<25 poor, >500 great)	QPlogBB (−3 to 1.2)	QPPMDCK (<25 poor, >500 great)	QPlogKhSa (−1.5 to 1.5)	QPlogS (−6.5 to 0.5)	% Human oral absorption (>80 % high, <25 % poor)
D1	0	2024.458	−0.315	1060.312	0.652	−6.073	100
D2	0	2020.236	−0.387	1057.922	0.66	−6.233	100
D3	0	2020.26	−0.201	1915.403	0.732	−6.486	100
D4	0	2099.029	−0.368	1102.589	0.869	−6.653	100
D5	1	463.457	0.084	238.356	0.044	−3.076	86.55
D6	1	490.693	0.031	253.531	0.146	−3.471	88.99

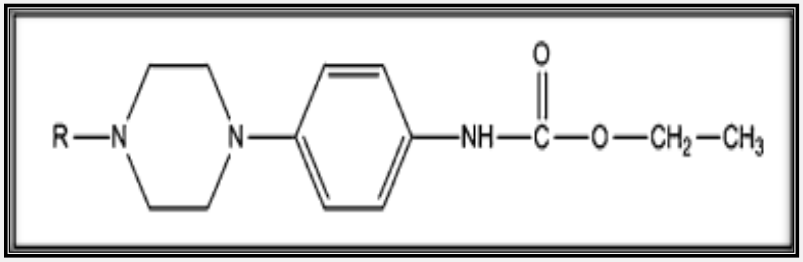
[a]MW: molecular weight, HBA: hydrogen-bond acceptor atoms, HBD: hydrogen-bond donor atoms, PSA: polar surface area, SASA: total solvent accessible surface area, QPlogPo/w: Predicted octanol/water partition coefficient, QPlogS: Predicted aqueous solubility, CNS: Predicted central nervous system activity on a –2 (inactive) to +2 (active) scale, QPPCaco: Caco-2 cell permeability in nm/sec, QPPMDCK: Predicted apparent MDCK cell permeability in nm/sec, QPlogBB: brain/blood partition coefficient, QPlogKhsa: binding to human serum albumin, Percent Human-Oral Absorption: human oral absorption on 0 to 100% scale.

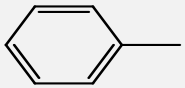
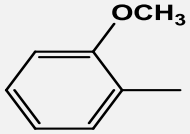
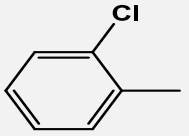
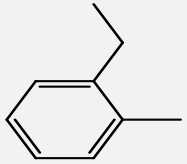
Selection of Possible Cognitive Enhancers

Compounds	Nootropic activity	
	Pa	Pi
D1	0.527	0.114
D2	0.569	0.091
D3	0.398	0.219
D4	0.565	0.093
D5	0.513	0.071
D6	0.537	0.108

Probable activities predicted for the targeted compounds D1–D6 using PASS server

Inhibition of AChE activity and their CDOCKER interaction energies by target compounds D1–D6



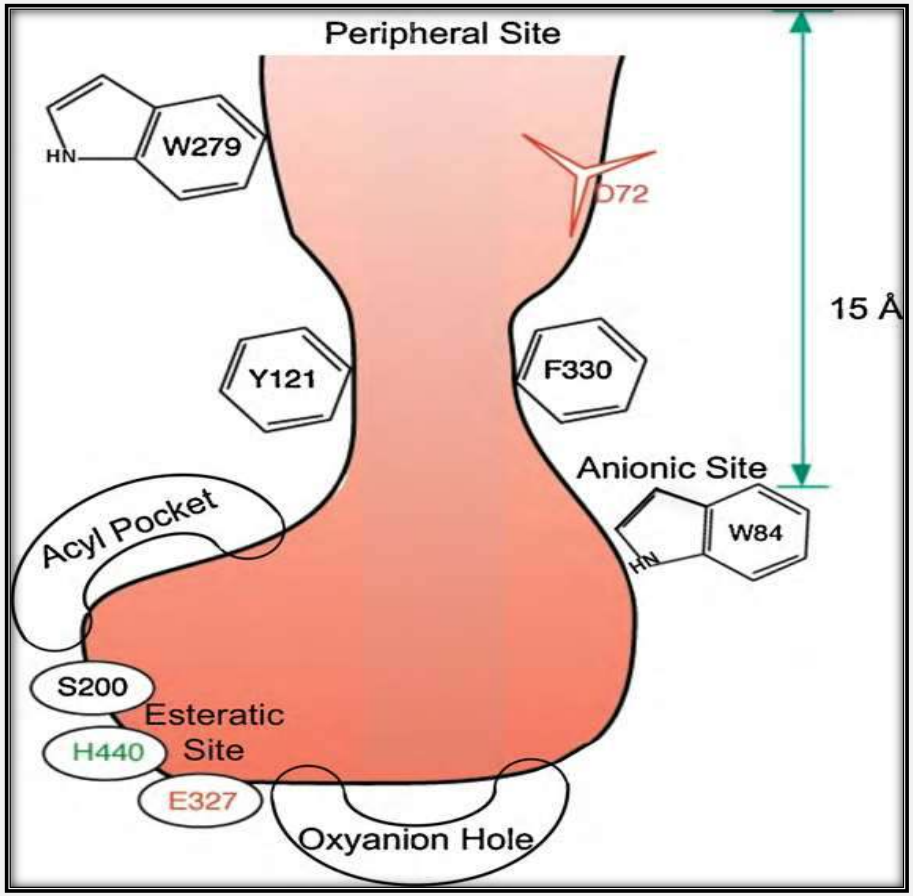
	R
D1	
D2	
D3	
D4	
D5	$-CH_3$
D6	$-CH_2CH_3$

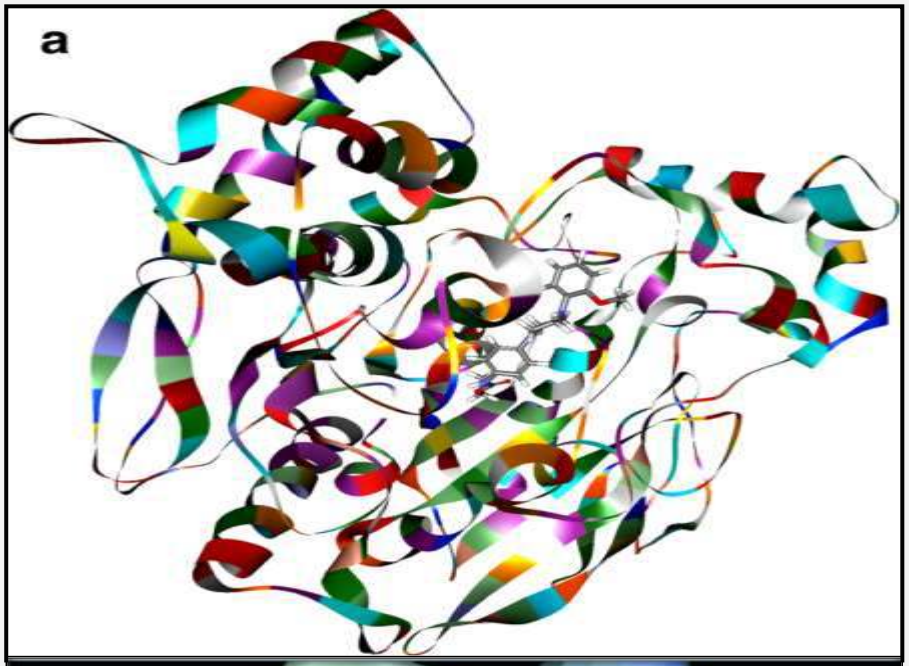
Compounds	AChE IC_{50} (nM)	CDOCKER interaction (CDI) energy (kcal/mol)
D1	168.28 ± 0.90	-43.99
D2	93.5 ± 0.55	-48.75
D3	139.52 ± 1.44	-46.97
D4	127.10 ± 3.22	-46.24
D5	284.67 ± 1.99	-39.30
D6	252.41 ± 0.71	-39.69
Donepezil	120.13 ± 0.23	-51.32
Rivastigmine	3360 ± 0.98	-

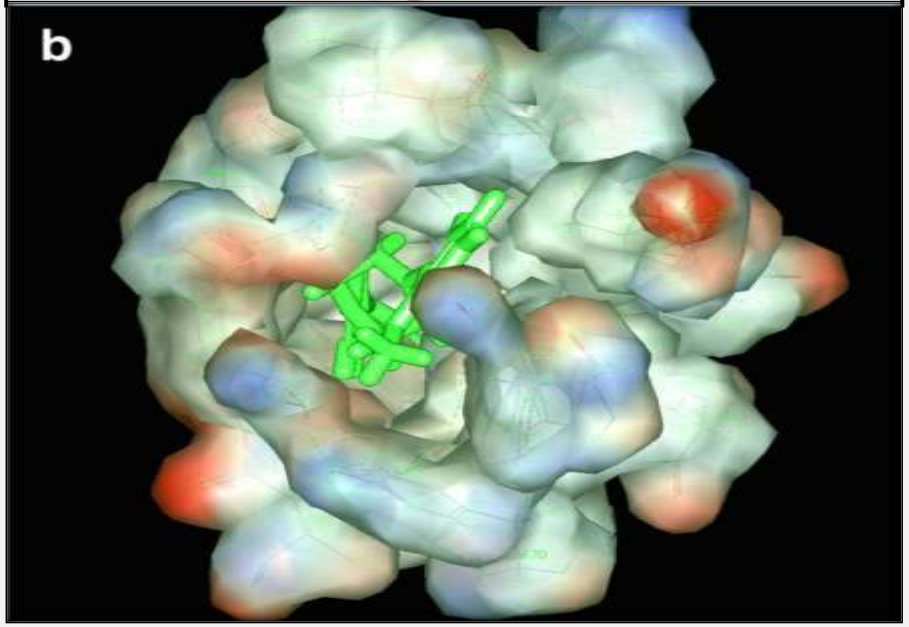
AChE from electric eel; IC50, inhibitor concentration (mean ± SEM of three independent experiments) resulting in 50% inhibition of AChE

Docking models of the compound-enzyme complex

Active-site gorge of TcAChE

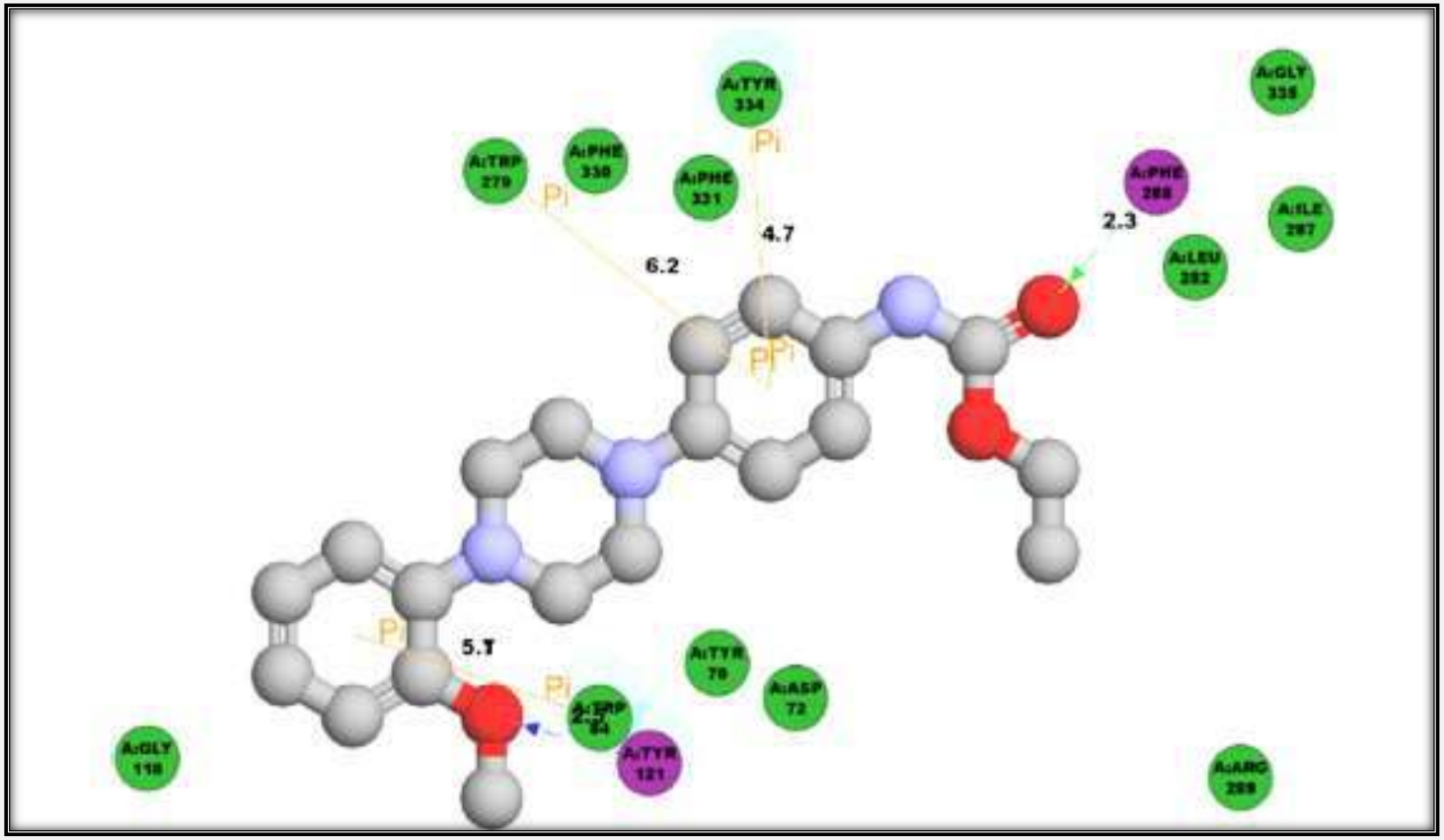






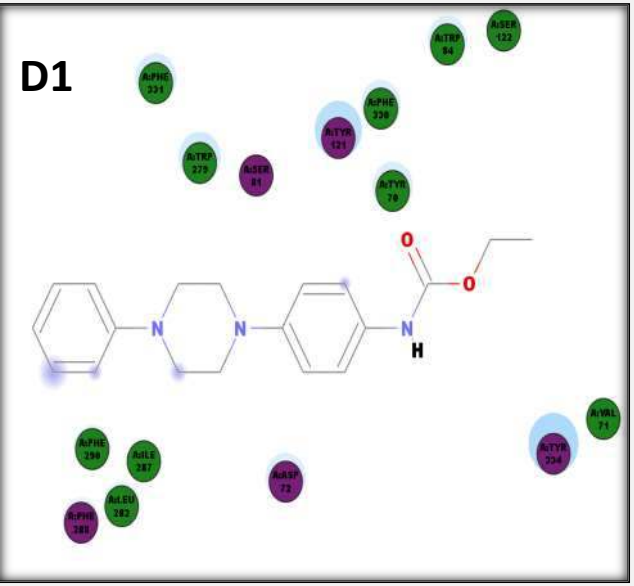
a) Compound D2 docked within the active site gorge of TcAChE (PDB: 1EVE)
b) Stereoviews looking down the gorge of TcAChE binding with D2

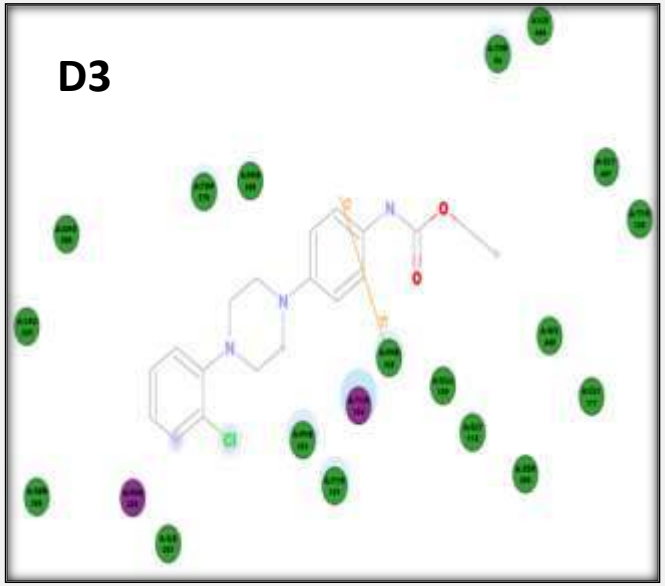
2D schematic diagram of docking model of compound D2 with TcAChE

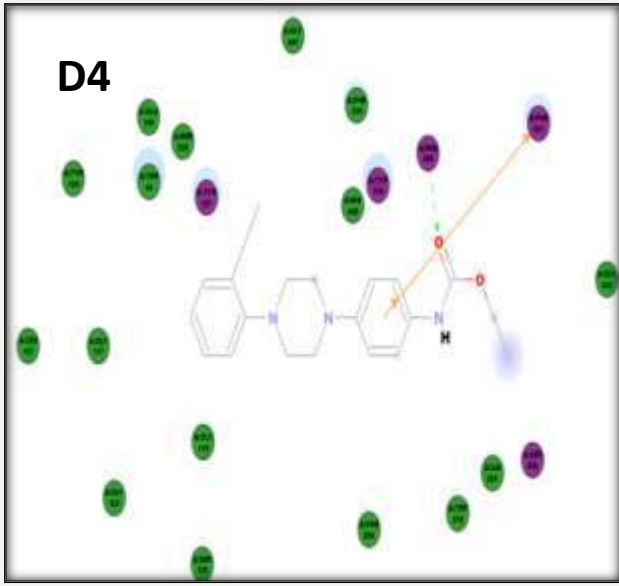


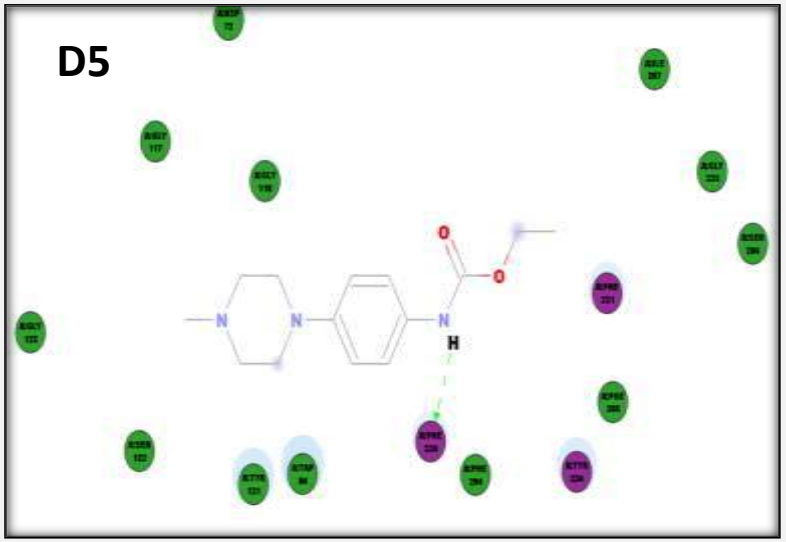
Residues involved in hydrogen bonding, charge or polar interactions are represented by magenta-coloured circles. Residues involved in van der Waals interactions are represented by green circles. The solvent accessible surface of an atom is represented by a blue halo around the atom. Hydrogen-bond interactions with amino acid side chains are represented by a blue dashed line with an arrow head directed towards the electron donor. p–p interactions are represented by an orange line with symbols indicating the interaction.

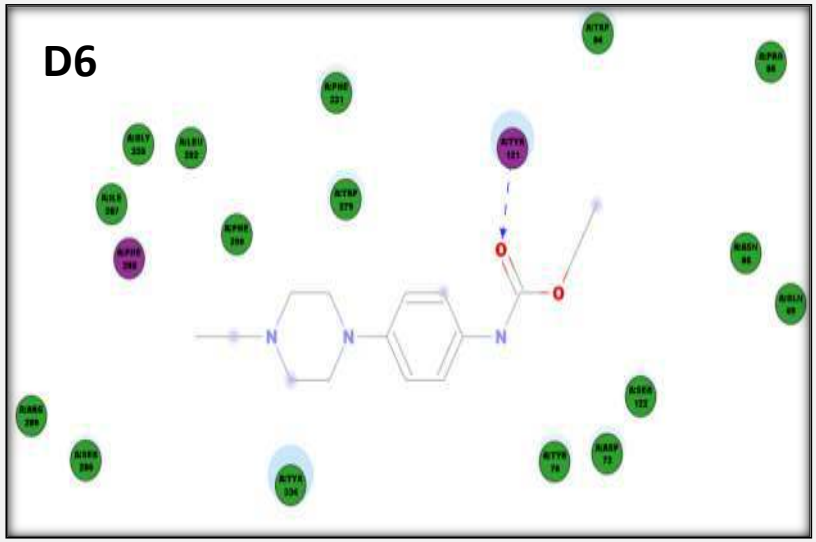
Continued.....











OBJECTIVE 2

- To investigate the neuroprotective effect of Piperazine Derivative [N-{4-[4-(2-methoxyphenyl)-piperazin-1-yl]-phenyl} Carbamic Acid Ethyl Ester] against aluminium-induced

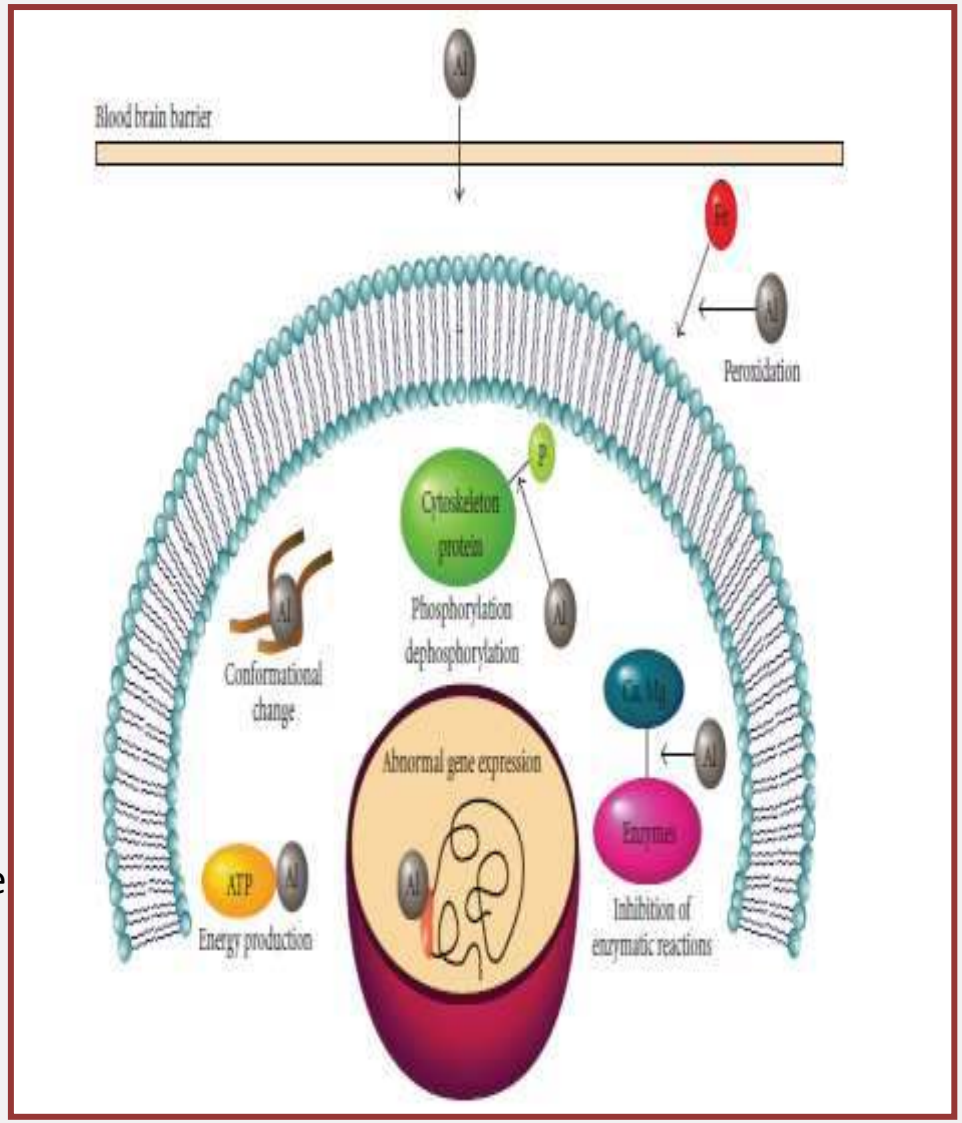
  - Cholinergic and Neurobehavioral impairment
  - associated oxidative damage in Wistar rats

Neurotoxicity of Aluminium (Al)

- Aluminum (Al) is a well established neurotoxin and is suspected to be linked with various neurodegenerative diseases. Al exposure in rodents indicated impaired cholinergic system in different brain regions (Yellamma et al. 2010), which mainly leads to neurobehavioural alterations (Kumar and Gill 2009).

- Al is considered one of the contributing factors to oxidative stress, as it generates ROS. Al has been shown to cause oxidative damage to neurons through Iron (Fe^{2+}) (Zatta et al. 2002, Gutteridge et al. 1985).

- Different salts of Al have been reported to accelerate oxidative damage to biomolecules like lipids, proteins and endogenous antioxidant enzyme activity & it also affects the cholinergic neurotransmission in terms of AChE activity.



Effects of Al on the central nervous system

Experimental design

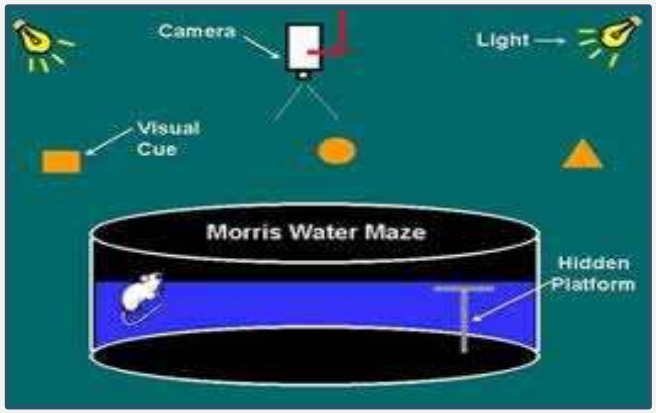
Wistar rats

(n = 8)

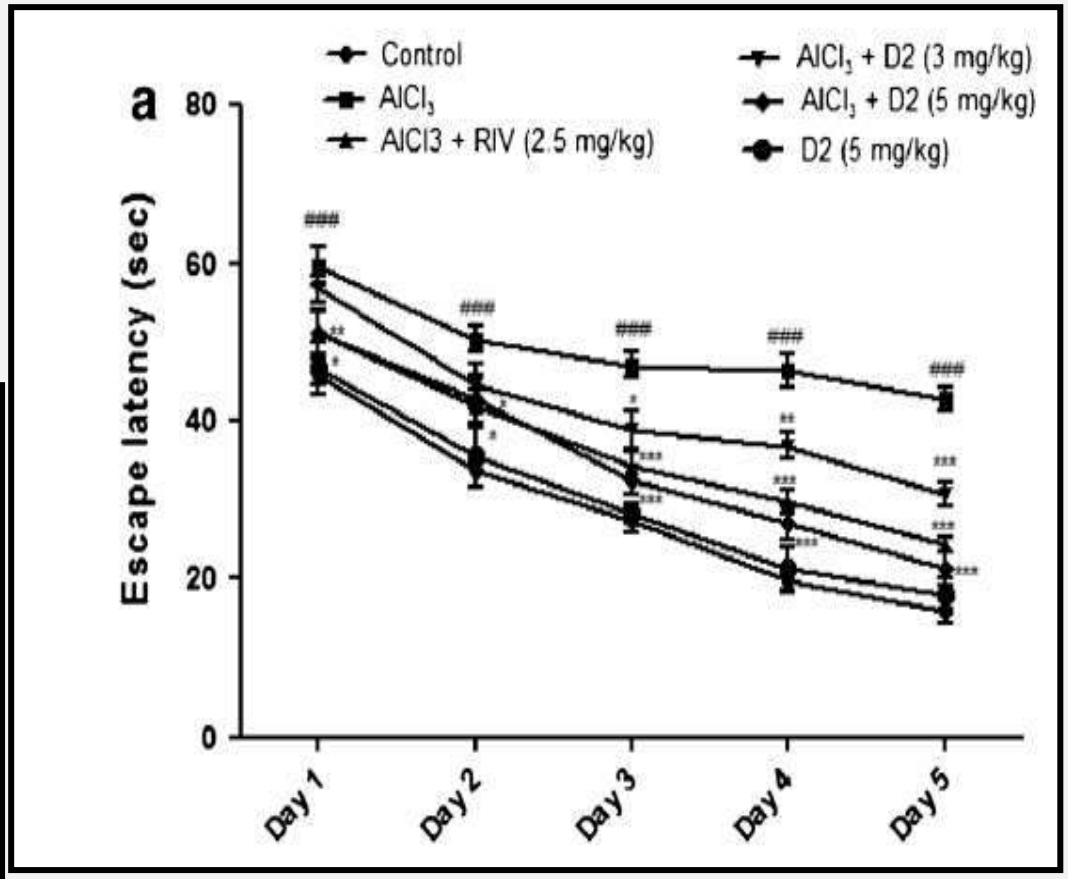
Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Control	$AlCl_3$ treated (100 mg/kg b.wt./day, p.o.)	$AlCl_3$ treated + Rivastigmine tartrate (2.5 mg/kg b.wt./day, p.o.)	$AlCl_3$ treated + D2 (3 mg/kg b.wt./day, i.p.)	$AlCl_3$ treated + D2 (5 mg/kg b.wt./day, i.p.)	D2 (5 mg/kg b.wt./day, i.p.)

- ✓ The study was carried out for a period of 42 days (6 weeks).
- ✓ At the end of treatment schedules, behavioural studies were carried out. The dose of piperazine derivative was selected based on the previous studies in the laboratory (Khatri et al. 2009).

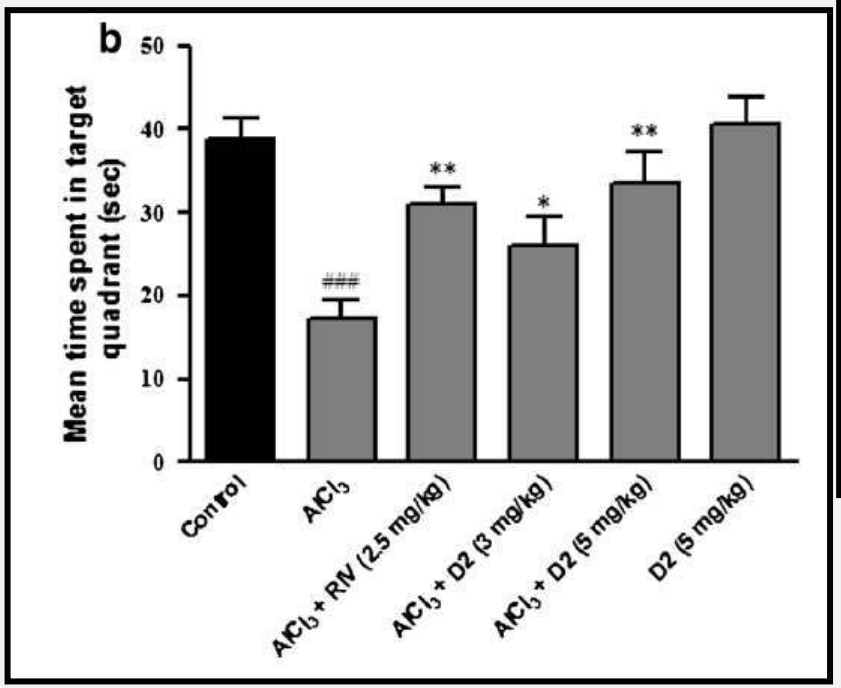
Effects of D2 and rivastigmine tartrate on the spatial learning and memory of rats in Morris water maze test



(A) Effect on Spatial Memory (Acquisition Test)

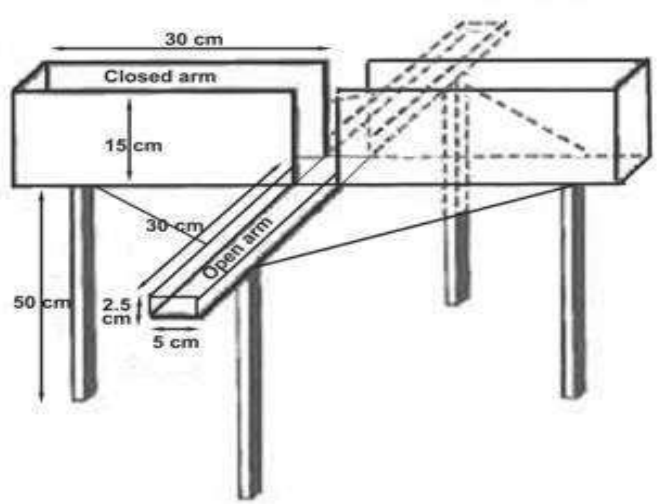


(B) Effect on Spatial Memory (Retrieval Test)



All values are expressed as mean ± SEM (n = 8). #p<0.05, ##p<0.01 and ###p<0.001 as compared with control group. *p<0.05, **p<0.01 and ***p<0.001 as compared with $AlCl_3$-treated group

Effect on EPM Test

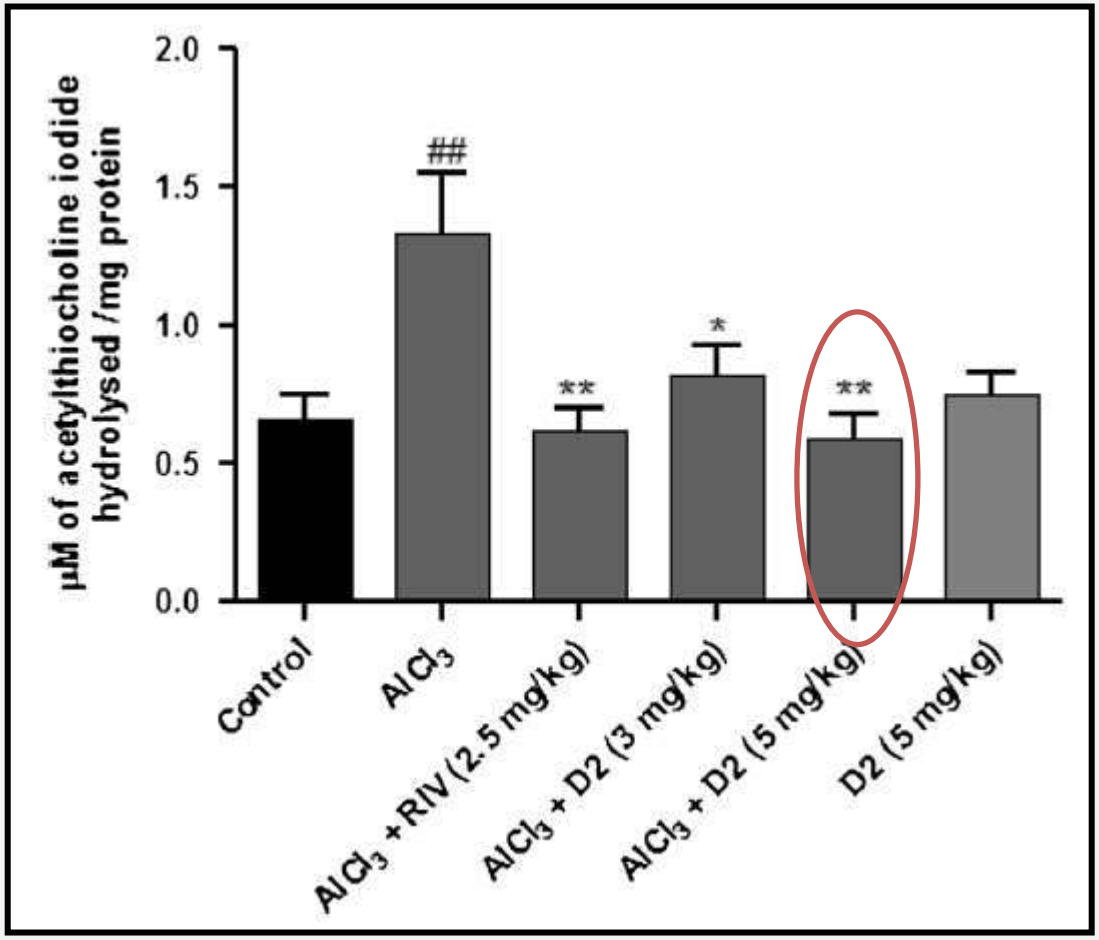


Effect of D2 and rivastigmine tartrate treatment on average time spent and number of entries in open and closed arm by rats (anxiety test) subjected to $AlCl_3$ treatment

Groups	Time spent in open arm (sec)	Time spent in closed arm (sec)	No. of entries in open arm	No. of entries in closed arm
Control	62.61 ± 2.05	38.05 ± 4.41	17.83 ± 2.18	7.50 ± 0.76
$AlCl_3$	25.39 ± 2.80$^{###}$	65.94 ± 4.19$^{###}$	6.67 ± 1.08$^{###}$	14.50 ± 1.26$^{##}$
$AlCl_3$ + RIV (2.5 mg/kg)	44.97 ± 6.01**	44.80 ± 4.38**	16.50 ± 1.65**	7.83 ± 0.87**
$AlCl_3$ + D2 (3 mg/kg)	39.73 ± 4.61*	49.73 ± 3.65*	11.83 ± 1.62*	10 ± 1.42*
$AlCl_3$ + D2 (5 mg/kg)	48.66 ± 2.36**	41.00 ± 4.50**	14.83 ± 1.30**	8.66 ± 1.28**
D2 (5 mg/kg)	58.90 ± 3.25	42.90 ± 4.02	14.30 ± 3.40	8.09 ± 2.09

All values are expressed as mean ± SEM (n = 8). ## $p<0.01$ and ### $p<0.001$ as compared with control group. *$p<0.05$ and ** $p<0.01$ as compared with $AlCl_3$-treated group

Effect of D2 and rivastigmine tartrate administration on AChE activity in $AlCl_3$-treated rats



All values are expressed as mean ± SEM (n = 8). ##p<0.01 as compared to control group; *p<0.05 and **p<0.01 as compared to the $AlCl_3$-treated group

BIOCHEMICAL MARKERS OF OXIDATIVE STRESS

Effect of D2 and rivastigmine tartrate treatment on $AlCl_3$-induced lipid peroxidation and protein oxidation in cerebral cortex region of rats

Treatment (mg/kg)	TBARS levels (µmol /mg protein)	Protein oxidation (nmol carbonyl / mg protein)
Control	0.36 ± 0.03	2.08 ± 0.23
$AlCl_3$	0.54 ± 0.03$^{\#\#}$	5.02 ± 0.42$^{\#\#\#}$
$AlCl_3$ + RIV (2.5 mg/kg)	0.38 ± 0.02**	3.41 ± 0.16**
$AlCl_3$ + D2 (3 mg/kg)	0.46 ± 0.03	4.46 ± 0.24
$AlCl_3$ + D2 (5 mg/kg)	0.36 ± 0.02**	3.65 ± 0.29**
D2 (5 mg/kg)	0.42 ± 0.05	2.25 ± 0.35

Values are represented as mean ± S.E.M. ## $p<0.01$, ### $p<0.001$ in comparison to untreated control rats. ** $p<0.01$ in comparison to $AlCl_3$-treated rats

Effect of D2 and rivastigmine tartrate supplementation on antioxidant parameters in $AlCl_3$-induced neurotoxicity in cerebral cortex region of rats

Treatment (mg/kg)	GPx (nmol NADPH oxidized/ min/mg protein)	GST (nmol CDNB conjugate formed/min/mg protein)	GR (nmol NADPH oxidized/min/ mg protein	GSH (µmol/mg of protein)
Control	22.59 ± 1.61	92.59 ± 3.88	16.15 ± 1.57	0.46 ± 0.002
$AlCl_3$	15.54 ± 1.31$^{\#\#}$	37.20 ± 3.15$^{\#\#\#}$	8.47 ± 1.05$^{\#\#}$	0.27 ± 0.004$^{\#\#}$
$AlCl_3$ + RIV (2.5 mg/kg)	20.77 ± 1.40*	56.60 ± 3.41**	14.0 ± 1.24*	0.42 ± 0.003**
$AlCl_3$ + D2 (3 mg/kg)	17.04 ± 0.86	44.04 ± 3.65	11.02 ± 1.28	0.38 ± 0.002*
$AlCl_3$ + D2 (5 mg/kg)	20.21 ± 1.10*	58.37 ± 4.191**	13.47 ± 1.62*	0.44 ± 0.003**
D2 (5 mg/kg)	25.67 ± 1.89	86.32 ± 3.29	13.90 ± 1.70	0.40 ± 0.007

All the values are expressed as mean ± SEM. ## p<0.01, ### p<0.001 in comparison to untreated control rats.
* p<0.05, ** p<0.01 in comparison to $AlC1_3$-treated rats

CONCLUSION

$AlCl_3$ induced cognitive impairments

MWM test

Spatial learning and memory

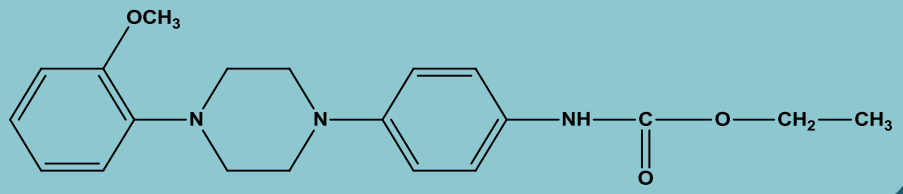
- Significant decrease in escape latency
- Increase in time spent in the target quadrant

EPM test

Anti-anxiety effects

- Increase in time spent in open arm
- Increase in no. of entries in open arm

D2



- Decrease in AChE activity

Cholinergic dysfunction

- Decrease in TBARS & protein carbonyl levels
- Restoration of altered levels of viz. GPx, GST, GR & GSH enzymes

Oxidative damage

$AlCl_3$ induced biochemical changes

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Publications

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