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Novel Acetylcholinesterase Inhibitor as Potent and Selective anti-Alzheimer's Agents

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SUMMARY

The Acetylcholinesterase (AChE) inhibitor donepezil (E2020) was discovered by the molecular models studies and played significant roles in treatment of Alzheimer's disease (AD), which have become a novel target for the discovery of drugs against AD. QSAR analysis of benzylpipridines derivatives as AChE inhibitor has been performed using various physicochemical parameters on molecular operating environment (MOE) software. The docking score values used as molecular descriptors along with the steric and electrostatic field values of partially least square (PLS) analysis. Graphical illustrations and molecular interaction potentials were generated using MOE and Schrödinger softwares. Docking study, which provides a better understanding of the interaction between the inhibitors and AChE. Also, the term "ADMET" include any and all processes in the human system. absorption, distribution, metabolism, excretion and toxicity properties were calculated of the proposed new models as lipophilic blood brain barrier (BBB) using a toxicity estimation software tool (T.E.S.T). The high leave one out (LOO) cross-validated correlation coefficient (Q^2=0.85) reveals that the model is a useful tool for the prediction of test set as well as newly designed structures against AChE activity. The statistically significant and physically meaningful QSAR model provided better insight into understanding the inhibitory behaviors of those chemicals, which may provide useful information for the rational molecular design of N-benzylpiperidine derivatives anti-AChE and anti-AD agents. Consequently, based on these results, we got the best model **(29d)**, which has the glide score **(-15.3 Kcal/mol)** and exhibited relatively better affinity toward AChE compared to E2020 **(-14.3 Kcal/mol),** and we expected they active inhibitors for AChE.

Key words: Alzheimer disease, AChE inhibitors, MOE, molecular docking, ADMET, QSAR.

INTRODUCTION

Alzheimer's disease (AD), the most common form of neurodegenerative dementia, is associated with selective loss of cholinergic neurons and reduced levels of acetylcholine neurotransmitter[1,2]. The disease is characterized by progressive loss of memory and impairment in cognition, which is lead to a serious threat to life expectancy of elderly people[3,4]. The main pathological changes in the AD brain are the formation of extracellular senile plaques consisting of aggregated amyloid-β-peptide (Aβ) deposits and intracellular neurofibrillary tangles consisting of abnormally phosphorylated microtubule associated protein. In the past two decades, huge research effort has been done to understand the molecular pathogenesis of AD. Various medicinal chemistry approaches have been developed for the treatment and prevention of Alzheimer's disease[5]. Three main hypotheses (cholinergic, amyloid, and the tau hypothesis) have been proposed to explain the mechanism of the disease[6,7]. Deficit of cortical cholinergic neurotransmission and the reduction of cortical and hippocampal acetylcholine (ACh) levels showed a correlation with decline in cognitive and mental functions of Alzheimer's disease patients[8]. Hence, it has been hypothesized that elevation of ACh level, which could be achieved by inhibition of the ACh breakdown enzyme AChE, should reduce disease progression. Known AChEIs play an important role in alleviating mild to moderate progression of Therefore, Some drugs act as inhibitors of AChE, among them, tacrine first drug approved by FDA for the treatment of AD[9,10], that exhibits nonselective inhibition, has significant peripheral toxicity, which led to the development of second-generation drugs, such as donepezil[11], galanthamine[11], and rivastigmine[9,10], which act selectively on AChE over other cholinesterases or receptors. Other drugs have been clinically studied and tested for use in the treatment of AD, such as physostigmine[10-12]. Some others are being tested and are promising candidates for the approval, including, Huperzine A[13,14], metrifonate[11], and phenserine[10,11]. These drugs are indicated to treat mild to moderate stages of AD, when the patient still has independent cognitive activity. However, understanding of Alzheimer's disease is not clear, and effective drugs are still in need.

The goal of this study was designated new structures in order to develop 3D-QSAR models as AChE inhibitors[15,16], and study their physical parameters, and to identify potential inhibitors to AChE by using molecular modeling and docking method using automated docking and bioassay techniques.

Computational Methods and Software Packages[17,18]

All computational works were performed using the molecular operating environment (MOE 2009) software developed by Chemical computing group Inc., and Toxicity Estimation Software Tool (T.E.S.T, v.4.1), which was based on the Chemistry Development Kit, running on personal computer. A total of 30 Benzisoxazole analogs were reported recently were selected for the present study. all the compounds wear drown using builder module of MOE and energy minimized using PM3 semi empirical method[19]. steepest descent algorithm was used for minimization. this was subsequently followed by conjugate gradient method and finally truncated Newton, until it reached an RMS gradient of 0.0001 Kcal/mol/A°. the biological activity of the molecule depends on its conformation. thus, the lowest energy conformer of all the compounds was transferred to database viewer.

Descriptor refers to the 2-dimentional (2D) or 3-dimentional (3D) physiochemical property of a molecule. QuaSAR-descriptor module of MOE Was used to calculate a lot of molecular descriptors for each molecule. All the descriptors were calculated using MOE, and were classified in to three classes: 2D descriptors, based on the atoms and connection information of the molecule; i3D, internal 3D descriptors, based on the 3D coordinate information about each molecule and they are invariant to rotations and translation of the conformation; and x3D, external 3D descriptors, based on the 3D coordinate information, but also require an absolute frame of reference. All the 181 2D, 3D and inner 3D descriptors available in MOE were calculated for each molecule[20]. The Qua-cluster module of MOE was used to evaluate the diversity of the molecules based on the table of molecular descriptors. 3D-QSAR model was built using 22 training set compounds (Table1), which was based on the principles of structural diversity and activity range.

Molecular Docking [21-23]

Molecular docking(MD) is a computationally intensive structure- based virtual screening (SBVS) technique that generates and scores putative protein–ligand complexes according to their calculated binding affinities.The crystal structure of the target was given, molecular docking automatically samples ligand conformations and protein–ligand interactions with a specified region of the protein surface were measured. It has been successfully used for identifying active compounds by filtering out those that do not fit into the binding sites. In this study, molecular docking was performed at the gorge site and PAS of cholinesterase enzymes (AChE) by the program MOE. Donepezil, an FDA approved drug was used as a reference dual binding site inhibitor for docking analysis, using the human (HuAChE) crystal structure (PDB code: 1EVE), considering that the size and shape complementarily of donepezil is similar to the training data set.

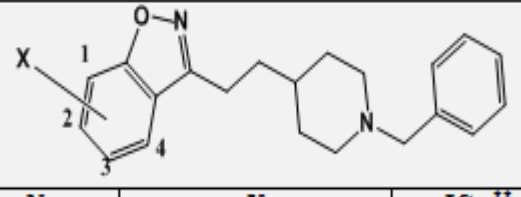
ADMET Properties[24-26]

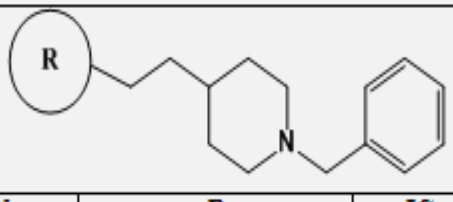
Absorption, distribution, metabolism, excretion and toxicity (ADMET) profile of FDA approved that the AD drugs, poor pharmacokinetic properties are one of the main reasons for terminating the development of drug candidates. Computed physicochemical properties associated with compounds that have good oral bioavailability, less or decrease toxicity and the capacity to be penetrate the BBB are key decision filter for CNS drug discovery. New compounds with good pharmacokinetic properties were needed. Also, these new models were analyzed in order to obtained new database search according to the rule-of five Lipinski hypothesis.

RESULT AND DISCUSSION

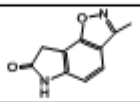
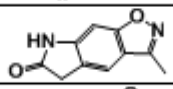
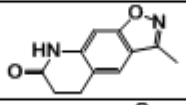
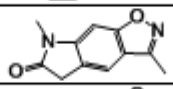
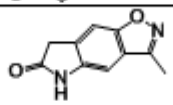
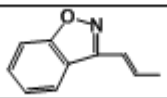
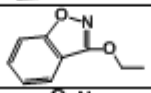
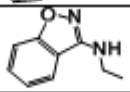
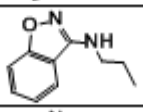
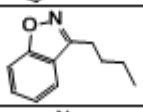
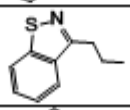
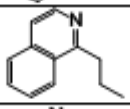
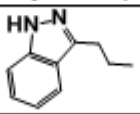
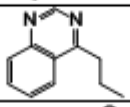
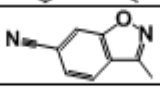
Our results were subjected to sequential multiple linear regression analysis, in order to develop QSAR between biological activity as dependent variables and substituent constants as independent variables. The series were run on auto mode for sequential multiple linear regression analysis it generate test set as 8 compounds (1b1, 1b9, 1b12, 1b19, 1b20, 1b23, 1b26 and 1b29) and remaining 22 compounds as training set. Several equations were obtained, the statistically significant equation was considered as best model as shown in (Table 1).

Table (1) Training and test groups of the benzisoxazole class of AChE inhibitors [1*,18]





No.	X	pIC_{50}**	No.	R	pIC_{50}**

E2020	Donepezil	**8.10**		1b13		8.44
1b1*	H	7.26		1b16		9.48
1b2	3-CH_3	8.11		1b17		9.24
1b3	2,3-diCH_3	8.24		1b18		9.32
1b4	3-OCH_3	8.14		1b19*		9.02
1b5	2-OCH_3	8.08		1b20*		6.68
1b6	1-OCH_3	8.15		1b21		5.59
1b7	2-$NHCOCH_3$	8.55		1b22		6.49
1b8	2-NHCOAr	8.03		1b23*		6.09
1b9*	2-$NHSO_2CH_3$	7.85		1b24		6.05
1b10	2-Cyclohexan	9.10		1b25		7.00
1b11	2-NH_2	7.70		1b26*		6.66
1b12*	2-Br	7.30		1b27		6.92
1b14	2-OH	7.59		1b28		6.47
1b15	2-$CONH_2$	8.06		1b29*		7.00

* The test group. ** IC50 values expressed in nM concentration, have been converted into M and processed in unit-Log IC50 (pIC50).

Bioisosteric replacement of the indanone ring in E2020 was attempted using a benzisoxazole moiety in the search of a AChE inhibitor with an improved in vivo profile. This was led to identified of CP118590 which is being evaluated in the clinic[28]. A number of analogues were synthesized in this series [17,18] and the QSAR model were developed for this series of 30 benzisoxazole analogues (Table1).

pIC50 = 4.1669 + 3.23044* LUMO + 1.10073* Std-dim1 +0.02423*HB1 +2.86469* PEOE_PC- **.....(1)**
Observations = 22, R= 0.924, R^2= 0.858, Descriptors = 4, **RMSE** = 0.393

The QSAR were suggested that the Electronic parameter for the benzisoxazole moiety as reflected by the LUMO Energy (lowest unoccupied molecular orbital) was shown a negative contribution towards the activity,Vsurf-HB1 is a hydrogen bond donor capacity, Std-dim1 is a standard dimension1 and PEOE_PC- is the total negative partial charge. The lack of a hydrophobic term in Equation (1) may result from the limited variation in these properties with this series. the predicted activity of the compounds were calculated using LOO method[29] and a comparison of these with the corresponding observed activities were calculated as shown in Figure1.

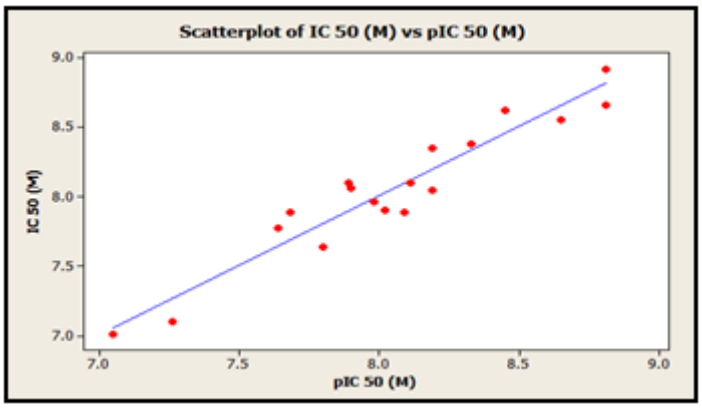


Figure (1): Correlation plot of actual (experimental) activities IC50 and predicted activities of the training set and test set compounds obtained using equation 1.

The 3-(2-(1-benzylpiperidin-4-yl) ethyl) benzo[d]isoxazole (benzisoxazole) derivatives were tested for their ability to inhibit acetylcholinesterase (AChE). A human brain homogenate was used as the AChE source and the activities were measured according to the method of Ellman et al.[30]. The benzisoxazole derivative was divided into four parts as shown in Fig.(2): Part 1 (benzisoxazole moiety), part 2 as spacer (linkage moiety), part 3 (piperidine moiety), and part 4 (benzyl moiety). All data were obtained as racemic isomers.

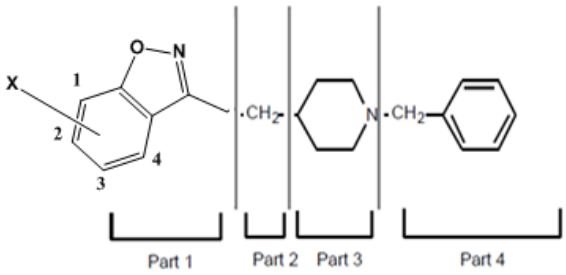
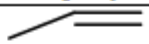
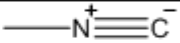
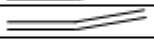
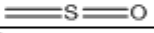
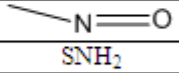
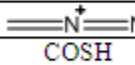
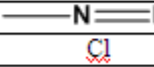
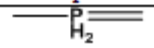
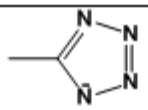
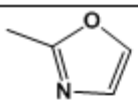
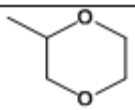
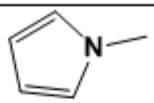
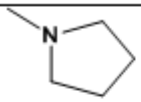
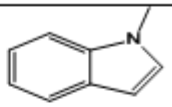
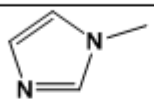
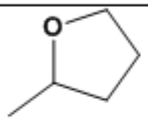
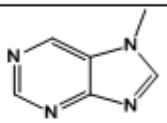
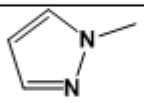
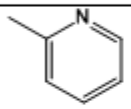
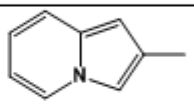
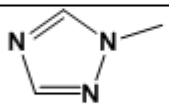
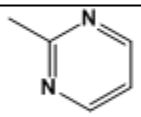
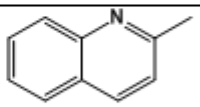
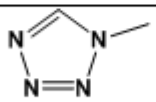
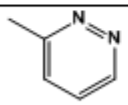
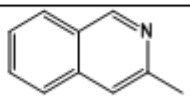
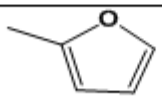
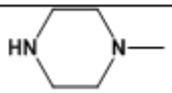
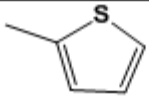
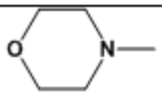


Figure (2): Structure of benzisoxazole derivatives

Firstly, different substitutions in the benzisoxazole moiety in part1 were gave different anti-AChE active as listed in Table2.

Table (2) part-1 substitutions of benzisoxazole moiety					
1	H	21	ethylamine	41	CF_2CH_3
2	CH_3	22	OCH_3	42	
3	CH_2CH_3	23	COOH	43	
4	Propyl	24	NHCOR	44	CHNH
5	Butyl	25	OCOR	45	CHS
6	isopropyl	26	$CONH_2$	46	Cyano
7	isobutyl	27	$OCOO^-$	47	
8	phenyl	28	COH	48	
9	neopentyl	29	$COCH_3$	49	
10	OH	30	$COOCH_3$	50	
11	dimethylether	31	SO_2H	51	
12	methylethylether	32	NO_2	52	SNH_2
13	diethylether	33		53	$N^+H_2CNH_2$
14	ethylether	34	COSH	54	$N^+HC(NH_2)_2$
15	NH_2	35		55	Sulfone
16	NHR	36	Cl	56	Sulfonamide
17	trimethylamine	37	F	57	Sulfiximide
18	dimethylamine	38	Br	58	Phosphate
19	methylethylamine	39	CF_3	59	
20	diethylamine	40	CCl_3	60	Nitrate

61		69		77	
62		70		78	
63		71		79	
64		72		80	
65		73		81	
66		74		82	
67		75			
68		76			

The physical parameters as steric, hydrophobic and electronic properties were related with substitutions on benzisoxazole moiety, a qualitative analysis of this series were suggested that electron donating groups(EDG) (10,15-20,26,etc.) and moderate in electron withdrawing groups (EWG) (27-30). Also, their effects were appeared to be a preference for hydrophobic substituents, but that steric effect of the substituents have no effect for their activities (2-9). An electron-donating X substituent for the benzisoxazoles has been suggested to be favoured for AChE activity by increasing the electron density on the oxygen atom, which was formed a hydrogen bond with the enzyme (receptor).

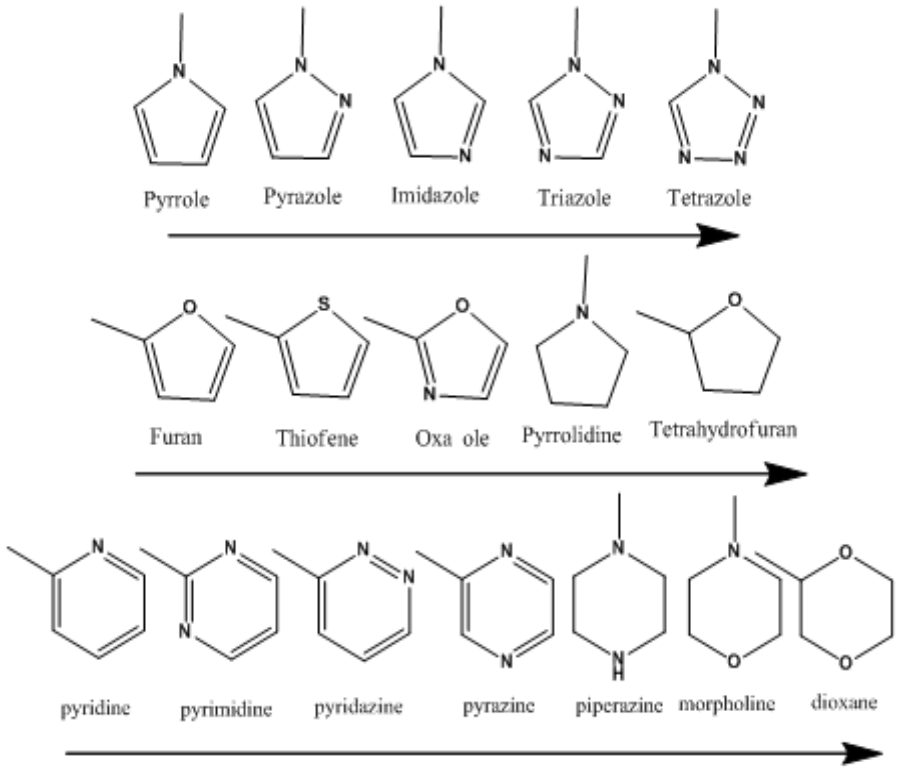
It was found that 2,3- and 4- compensation sites of X substituents are favoured for activity, whereas 1 and 1,4- (ortho) substituented are detrimental. In addition, The qualitative SAR available for these AChE inhibitor classes also suggest that they all share functional groups (carbonyl groups) which have been shown to be important for activity.

In the same series, the compounds (31-60), only disodium and carbonyl group substituents at the 2,3- sites have been showed high activity compare with other withdrawing substituents. The favoured disodium and carbonyl groups for X within this series was attributed to its ability to form a hydrogen bond to bind with the active site residue of the enzyme. The effect of replacement of H of benzisoxazole moiety at 2,3-position with series of methyl group, leads to a slight increased in the rank order of activity. H < Me < ethyl < propyl < isopropyl< butyl < isobutyl < neopentyl..

While, the effect of different types of amine substitutions on potency varied in the following order NH_2 <$NHCH_3$ <$N(CH_3)_2$ <dimethyl amine < methyl ethyl amine < diethyl amine <ethyl amine of ortho, meta, para ,1,4- and 2,3- sites of the benzisoxazole moiety. The aromatic amines were given significantly higher potency than aliphatic amines. But, the introduction of the oxygen groups to the site of the benzisoxazole moiety lead to increase of the biological activity more than nitrogen groups, OH <dimethyl ether < methyl ethyl ether < diethyl ether <ethyl ether. Introduction of NO_2 group as new substitution leads to a significantly loss in AChE activity, thereby highlighting the importance of the increasing electron density on the oxygen and nitrogen atoms of benzisoxazole moiety toward the interaction with AChE enzyme.

The effect of saturated(aliphatic) and unsaturated cyclic(aromatic) substitutions on the benzisoxazole moiety are favored for activity such as in the different series , which it was appearing that saturated ring increase biological

activity more than unsaturated ring and six heterocyclic the best compare with five heterocyclic because it is stable the chair form configuration.



Finally, the aromatic hetero bicyclic substitutions , Indole, , indolizine, quinoline, isoquinoline were tested (78-82), and the purine is the best potency, but increasing the number of N more than 10 and the molecular weight becames more than 500, This leads to deviated from Lipinski role[26]. We performed sequential virtual screening of part 1 by means of 3D pharmacophore search and virtual screening hit prioritization using molecular docking, and QSAR. The most active compounds being X= (OCOR, $OCOO^-$, $CONH_2$, $COCH_3$, NHCOR, Ethylamine, CHN^+N^-, tetrazole, pyrimidine, piperazine, morpholine,) as shown in Table3 below.

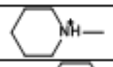
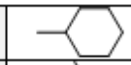
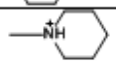
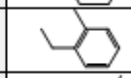
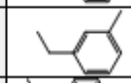
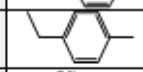
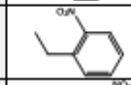
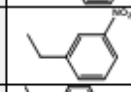
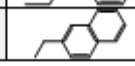
Table (3) the best models of part 1 substitutions

		pIC_{50} *	E-Doc **
E2020	Donepezel	8.6646	-11.3653
a	2,3- OCOR	9.5002	-15.0259
b	2,3- CHN^+N	9.1697	-15.1906
c	2,3- pyrimidine	8.6821	-16.4072
d	2,3- piperazine	8.7156	-14.0347

* IC50 values expressed in nM concentration, have been converted into M and processed in unit-Log IC50 (pIC50) . ** Interaction energy (kcal / mol).

Table (4) The 3-(2-(1-benzylpiperidin-4-yl)ethyl)benzo[d]isoxazole-5,6-diyl diacetate

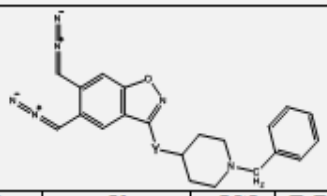
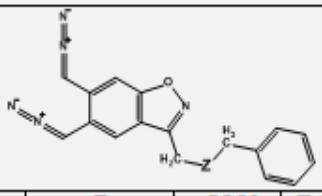
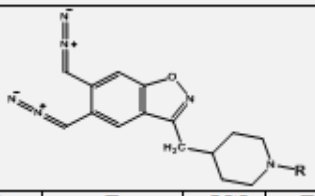
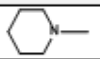
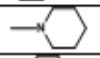
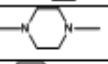
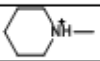
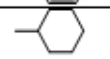
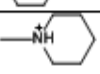
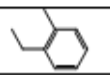
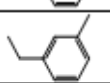
Part 2				Part 3				Part 4			
No.	Y	pIC50	E-Doc	No.	Z	pIC50	E-Doc	No.	R	pIC50	E-Doc
a	CH_2CH_2	9.50	-15.7	A		9.50	-15.7	A		9.50	-15.7
1a	CH_2	8.57	-13.9	13a		9.64	-14.2	25a	H	9.07	-12.8
2a	-	8.05	-13.2	14a		9.43	-14.4	26a		9.08	-13.2

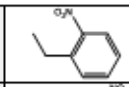
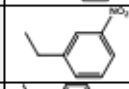
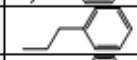
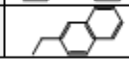
3a	CH=CH	9.42	-12.1	15a		0.04	-14.3	27a		10.0	-13.2
4a	$CH_2HC=$	9.42	-13.8	16a		-0.83	-14.6	28a		9.51	-14.7
5a	$-(CH_2)_3-$	8.14	-13.5	17a		10.1	-12.3	29a		9.84	-13.4
6a	$-(CH_2)_4-$	8.83	-15.5	18a	_	9.09	-12.0	30a		9.73	-13.8
7a	$-(CH_2)_5-$	8.31	-14.7	19a	CH_2	9.51	-11.9	31a		9.90	-12.1
8a	O- CH_2	8.81	-13.6	20a	CH_2CH_2	9.11	-11.7	32a		9.67	-14.8
9a	CH_2-O	9.23	-12.5	21a	CH=CH	8.94	-12.7	33a		9.21	-16.7
10a	O	8.47	-11.6	22a	O	8.98	-13.6	34a		9.91	-13.2
11a	N	8.16	-12.9	23a	N	9.06	-13.0	35a		9.55	-14.4
12a	S	8.69	-13.1	24a	S	9.63	-12.2	36a		9.95	-13.3

E-Doc= Kcal/mole

Secondly. many various bridging groups between the benzisoxazole moiety and the piperidine moiety were tested of in part 2 for the compounds a,b,c,d. The results were listed in Tables (4,5,6,7). In Table (4) shows direct connection of the benzisoxazole moiety and the piperadine rings were dramatically decreased in potency (**2a**). The effect of the length of the bridging moiety (spacer) on potency varied in the following order: ethylene(**a**) > butylene (**6a**) > methylene(**1a**) > pentylene(**7a**) > propylene(**5a**). The replacement of an ethylene by cabon-carbon double bond on both the indanone and piperidine moiety slightly decreased the activity (**3a**, **4a**). The free energy of docking (Kcal/mole) was given of **(a)** compound. In Tables (5,6,7) were showed the similar behavior of variety Y of table(4), The effect of the chain length of the Y moiety has also been investigated. The optimal spacer length for AChE activity when Y=$\mathbf{CH_2CH_2}$. Increasing the chain length Y to 2,3 or 4 leads to a drop in AChE affinity. An unusual observation is that in the analogue for which Y= $\mathbf{CH_2CH_2CH_2}$ of **(b)** model, the free energy of docking an affinity (-16.2) is observed that is comparable to the analogue in which Y=$\mathbf{CH_2CH_2}$ (-15.9). One explanation for this observation is that the propyl chain may adopt a conformation that allows good interaction with the enzyme. In all analogues studied within this benzisoxazole series, the benzisoxazole moiety was always linked to the benzylpiperdine ring via an ethyl linker.

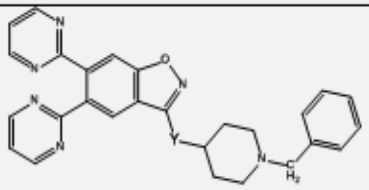
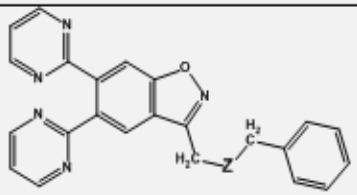
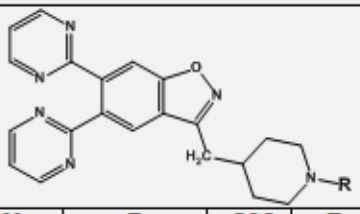
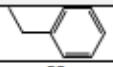
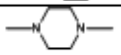
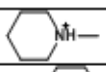
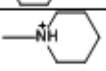
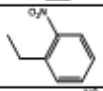
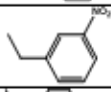
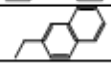
Table (5) The 3-(2-(1-benzylpiperidin-4-yl)ethyl)-5,6-bis(diazomethyl)benzo [d]isoxazole

Part 2				Part 3				Part 4			
											
No.	**Y**	**pIC50**	**E-Doc**	**No.**	**Z**	**pIC50**	**E-Doc**	**No.**	**R**	**pIC50**	**E-Doc**
b	$\mathbf{CH_2CH_2}$	**9.16**	**-15.9**	**B**		**9.16**	**-15.9**	**b**		**9.16**	**-15.9**
1b	CH_2	8.19	-12.5	13b		9.23	-14.0	25b	H	8.87	-12.9
2b	-	7.88	-13.0	14b		8.94	-13.5	26b		8.55	-12.4
3b	CH=CH	9.00	-11.4	15b		-2.00	-13.2	27b		9.76	-12.9
4b	$CH_2HC=$	8.80	-13.5	16b		-2.37	-13.5	28b		8.92	-15.1
5b	$-(CH_2)_3-$	8.00	-16.2	17b		9.21	-13.2	29b		9.20	-14.3
6b	$-(CH_2)_4-$	8.75	-13.0	18b	_	8.08	-11.9	30b		9.26	-13.4

7b	$(CH_2)_5$	8.25	-15.2	19b	CH_2	7.88	-12.4	31b		9.41	-17.5
8b	O- CH_2	8.46	-12.7	20b	CH_2CH_2	8.18	-10.9	32b		9.38	-15.8
9b	CH_2-O	8.75	-14.5	21b	CH=CH	8.48	-12.6	33b		8.08	-15.3
10b	O	7.99	-14.2	22b	O	7.43	-13.1	34b		9.50	-16.3
11b	N	7.13	-12.9	23b	N	7.38	-13.5	35b		10.2	-12.7
12b	S	7.58	-13.9	24b	S	7.95	-12.1	36b		9.50	-14.9

Thirdly, the Tables 4,5,6,7 were showed the relationships between the location and the number of nitrogen atom and activity in part 3 moiety. The nitrogen atom at 4-position of the benzylpiperidine moiety was very important since the activity of 1-benzylpiperidine derivative (**13a,13b,13d**) slightly increased activity. While, the piperidine group with a piperazine group (**14a,14b,14c,14d**) were decreased in potency. Replacement of the piperidine moiety with a phenyl ring lead to an increase in the AChE inhibitory potency (**17a,17b,17d**). This result suggests that a hydrophobic group at this position in the molecule is favourable, but the free energy value of interaction of these models with receptor more decreased than piperidine moiety. the protonation of nitrogen piperidine moiety result in a huge decrease in the potency (**15a,15b,15c,15d**), The basicity of the nitrogen atom in the piperidine ring appear to have increase activity. The lower affinity may be attributed to protonation of nitrogen may, and decrease in the binding between the molecule and the receptor.

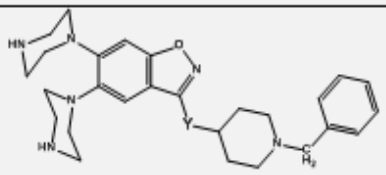
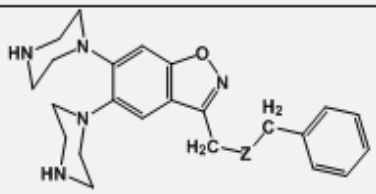
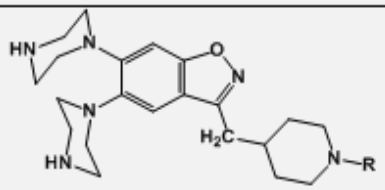
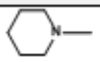
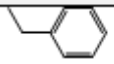
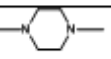
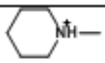
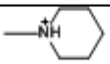
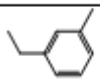
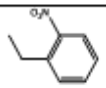
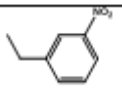
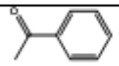
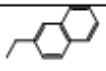
Table (6) The 3-(2-(1-benzylpiperidin-4-yl)ethyl)-5,6-di(pyrimidin-2-yl)benzo[d]isoxazole

Part 2				Part 3				Part 4			
											
No.	**Y**	**pIC50**	**E-Doc**	**No.**	**Z**	**pIC50**	**E-Doc**	**No.**	**R**	**pIC50**	**E-Doc**
c	**CH_2CH_2**	**8.68**	**-16.8**	**C**		**8.68**	**-16.8**	**c**		**8.68**	**-16.8**
1c	CH_2	6.28	-14.0	13c		7.91	-13.3	25c	H	7.60	-10.9
2c	-	6.44	-14.7	14c		7.43	-13.1	26c		7.17	-13.5
3c	CH=CH	6.86	-13.4	15c		-3.17	-14.3	27c		7.74	-14.7
4c	$CH_2HC=$	7.72	-13.8	16c		-3.41	-14.6	28c		7.93	-13.4
5c	$(CH_2)_3$	7.44	-14.6	17c		7.90	-11.4	29c		8.19	-13.5
6c	$(CH_2)_4$	7.12	-12.8	18c	_	6.83	-13.6	30c		8.00	-13.9
7c	$(CH_2)_5$	7.17	-16.0	19c	CH_2	6.13	-11.9	31c		7.94	-16.5
8c	O- CH_2	7.14	-15.3	20c	CH_2CH_2	5.62	-13.6	32c		7.49	-14.7
9c	CH_2-O	7.79	-14.3	21c	CH=CH	5.74	-13.4	33c		6.94	-13.6
10c	O	6.42	-14.2	22c	O	5.82	-14.3	34c		8.17	-13.5
11c	N	5.66	-15.1	23c	N	5.60	-12.4	35c		8.24	-14.8
12c	S	6.24	-14.4	24c	S	6.18	-12.8	36c		8.56	-12.5

Fourthly, the compounds in Tables 4,5,6,7, were showed the relationships between the benzyl moieties. Replacement of the benzyl group (**25a,25b,25c,25d**) was caused a great reduction in the potency of the compound, and the free energy value of interaction of these models with receptor was decreased too. This results were suggested that a hydrophobic group at this position in the molecule is favorable. The activity was slightly decreased after replacement with phenyl group in the compounds **(26a,26b,26c,26d)**.

Another replacement of the benzyl moieties with a cyclohexyl ring may leads to an increase in the AChE inhibitory potency. The energies of the docking values were decreased after replacement with cyclohexyl ring, due to the lack of □-□ interaction with the enzyme. The effect of substituted at position 3 of benzyl derivatives were showed the highest potency among the 2-, 3-, and 4-substituted regioisomers. There is no any significant differences between the donating and withdrawing substitutions. The replacement of benzyl moiety with phenethyl in the compounds **(35a,35b)** and 2-naphthyl group in **(36a,36b,36d)** were increased in potency. Among the benzisoxazole derivatives, four models **(a,34b,c,29d)** are the most potent compounds in terms of anti-AChE activity.

Table (7) The 3-(2-(1-benzylpiperidin-4-yl)ethyl)-5,6-di(piperazin-1-yl)benzo[d]isoxazole

Part 2				Part 3				Part 4			
											
No.	**Y**	**pIC50**	**E-Doc**	**No.**	**Z**	**pIC50**	**E-Doc**	**No.**	**R**	**pIC50**	**E-Doc**
d	**CH_2CH_2**	**8.71**	**-14.3**	**D**		**8.71**	**-14.3**	**d**		**8.71**	**-14.3**
1d	CH_2	6.99	-13.3	13d		8.73	-13.0	25d	H	8.05	-12.5
2d	-	7.52	-14.6	14d		8.40	-14.2	26d		8.04	-14.4
3d	CH=CH	7.90	-13.0	15d		-2.82	-17.0	27d		9.29	-13.9
4d	CH_2HC=	8.00	-12.9	16d		-2.89	-14.5	28d		8.59	-14.7
5d	$(CH_2)_3$	7.64	-13.0	17d		8.80	-14.1	29d		8.78	-15.3
6d	$(CH_2)_4$	6.54	-16.3	18d	–	7.54	-14.0	30d		8.88	-13.6
7d	$(CH_2)_5$	7.07	-14.4	19d	CH_2	8.11	-13.3	31d		8.74	-14.9
8d	O- CH_2	7.89	-14.0	20d	CH_2CH_2	8.10	-14.1	32d		7.91	-14.0
9d	CH_2-O	7.93	-15.6	21d	CH=CH	8.12	-13.7	33d		7.34	-14.2
10d	O	7.17	-14.7	22d	O	7.75	-14.3	34d		8.85	-14.4
11d	N	7.53	-15.0	23d	N	7.74	-15.4	35d		8.43	-15.4
12d	S	7.17	-14.9	24d	S	8.06	-13.9	36d		8.81	-14.6

Docking results

The enzyme-based models have also been useful in rationalizing the QSAR models generated in order to understand further the AChE-inhibitor interactions. Such information available from QSAR and enzyme-docking models has been used successfully to predict more potent analogues within a particular series.

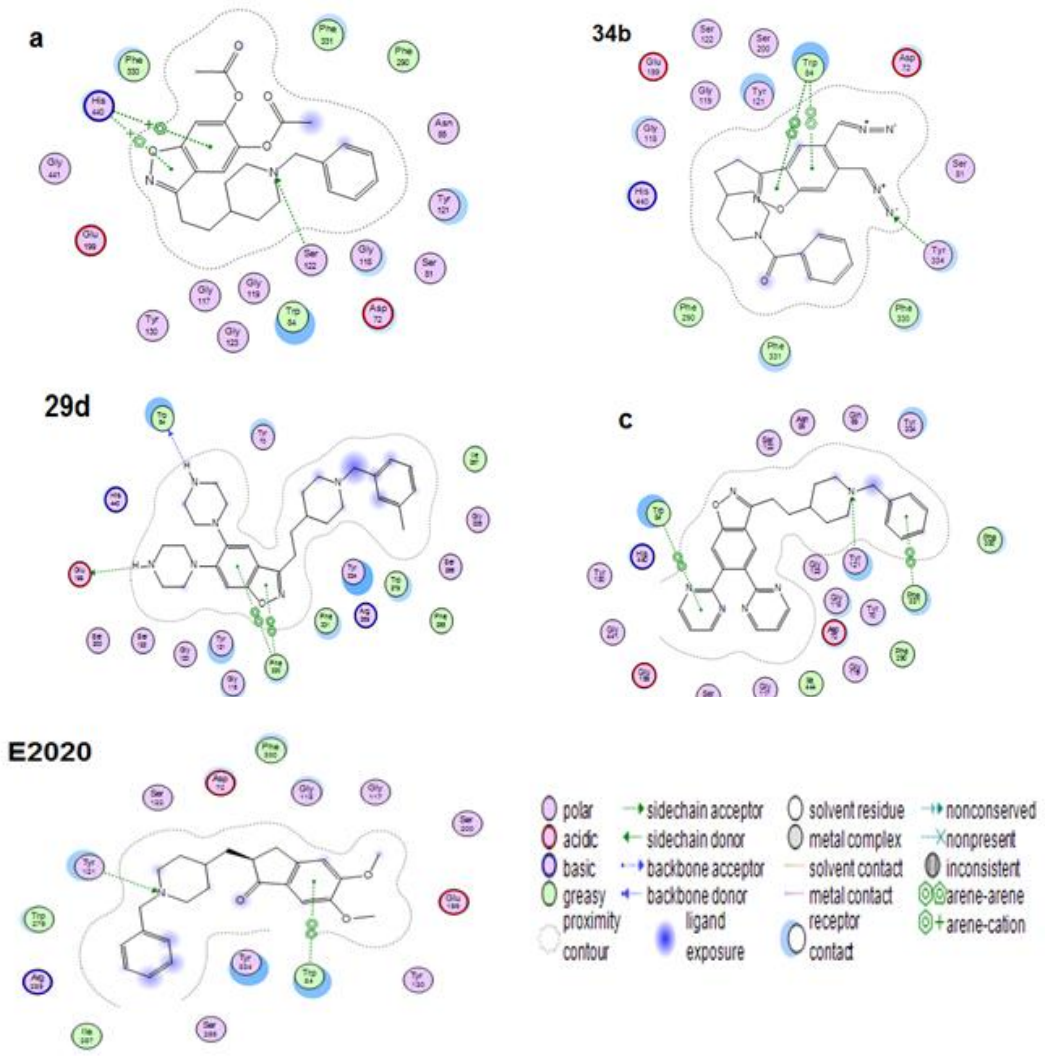


Figure 3. Binding mode of top ranked most active (a,34b,c,29d) models in the binding site of AChE enzyme.

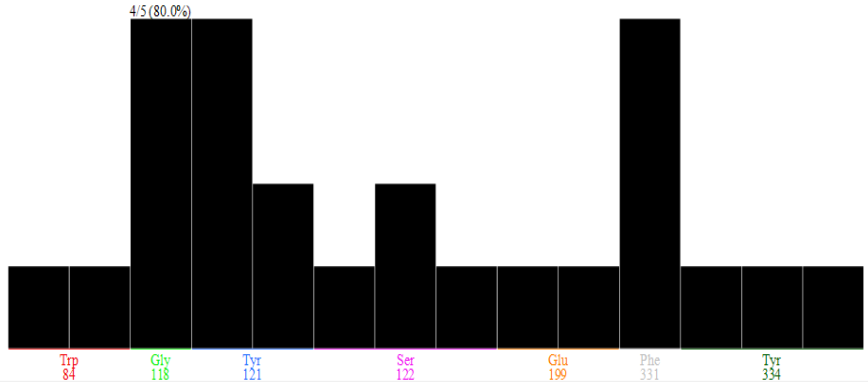


Figure 4. The coefficients of the 7 most important residues for electrostatic and VDW interactions.

Our results were explained a four structures that can inhibited the AChE and demonstrate the most characteristic ligand-protein interactions will be discussed. In general, it may be evidenced the process of inhibitors takes upon an unfolded shape and form a bridge between the 'anionic' (Tyr121) and 'peripheral' (Trp84) sites of AChE. While the *N*-benzylpiperidine moiety neighbors the 'anionic site', the benzisoxazole group and its derivatives are localized close to the 'peripheral site'. A thorough probing inside the protein by means of our automated docking routine shows that these inhibitors would in no way be able to adopt a different orientation. These results are in agreement with the concept of a 'deep and narrow gorge', issued from the crystallographic study [27-32]. The most potent inhibitors, e.g compounds **(a,32b,c,29d)** are longer than E2020. This suggests that these inhibitors have favorable electrostatic and hydrophobic interactions throughout the gorge.

The coefficients of the most important residues in terms of electrostatic and VDW contributions are shown in Figure 4. In those residues, Gly118, Tyr121, Glu199 and Ser122 are the most important residues in the active site for VDW interactions. The amino acid at Trp84 functions can form a □-□ interaction with ligand aryl groups (when available), while the other residues define the shape of the gorge base, serving to discriminate according to ligand shape, and the other residues of Tyr334, Trp84 and Phe331 are responsible for providing hydrophobic contacts.

Many of the models studied, which are not able to create a H-bonds, are not active. The binding of the substitutions of benzisoxazole group to residues of the enzyme may also explain why the absence of such a group decreases the activity. Moreover, the substituent attached to the benzisoxazole moiety in the compounds **(a,32b,c,29d)** seem to form one more hydrogen bond with different residual owing to their H-bond acceptor groups, while the benzisoxazole nucleus has a □-□ interaction with residual aryl groups. The 'peripheral' and 'catalytic' sites of AChE complexed with compound **(a,32b,c,29d)** are presented in Figure 3. The indanone ring of E2020 forms a □-□ interaction with the indole ring of Trp84 in AChE. The nitrogen of the piperidine ring of the E2020 interacts with the hydroxyl groups of the Tyr121 within distances of 3.16Å relative to the oxygen atom of the respective hydroxyl groups. The rings of benzisoxazole of **(a)** model is almost perpendicular to the His440 ring and forms a blocking wall to prevent the ligand ring from moving away from the position where it forms a □-□ interaction with the His440 ring. The nitrogen of the piperidine ring of the **(a)** model interacts with the hydroxyl groups of the Ser122 within distances of 3.49Å.

Based on docking simulations, we can explain that strong binding affinity of **(c)** model with AChE may be because of the hydrogen bonding interaction of the nitrogen of the piperidine ring with O-H of Tyr121 residue within distances of 2.26 Å. and ring of pyrimidine with side chain of Trp84 residue, also the benzyl ring of benzylpiperidine moiety forms a □-□ interaction with the benzyl ring of Phe331 residue at distances of 3.98 Å. All these interactions in this model has the largest value of docking with AChE. shown in Figure 3.

Finally, the most active compound **(29d)** for AChE interacts with enzyme through hydrogen bonding interaction, acidic hydrogen of piperazine substitution of benzisoxazole moeity makes an hydrogen binds with COO- of Glu199 residue within distances of 2.84 Å, and another hydrogen of piperazine makes hydrogen binds of Trp84 residue at distances of 1.98 Å. Also, the rings of benzisoxazole moiety interaction with the ring of Phe330 residue where it forms a □-□ interaction at distances of 5.03Å. as shown in Figure 3. It has been observed that **(C)** model have strong binding affinities over **(E2020,a,32b,29d)** models with AChE enzymes, which is in contrast to observed activities. This might be due to inabilities or poor performance of the docking scoring functions[33].

ADMET Analysis

For further evaluation by Lipinski's rule and some other physico-chemical filtering relevant for CNS activity. The more than 1000 compounds were further submitted to an in silico evaluation for bioavailability and BBB penetration filter. Specifically, some key computed parameters that we examined as part of multiple property filter of lead compounds are toxicity, octanol/water partition coefficient (LogP), distribution coefficient (LogD), computed aqueous solubility (LogS), polar surface area (PSA), percent human oral absorption, BBB penetration and CNS activity, to prioritize compounds with higher probabilities of possessing favorable tissue absorption distribution profiles, bioavailability and BBB penetration. On applying all the above mentioned properties, we filtered the new model design from 1000 compounds, which have good pharmacokinetic property and CNS activity. After carefully analyzing the toxicity of those compounds, we got four models, which were not showing any toxicity predicted by the T.E.S.T.

Table 8: ADMET prediction on FDA approved drugs, The most potent compounds from the data set.

Molecular Property	M.W	R.B	H.A	H.D	CLogp	Log D	Log S	PSA	PPb	ACyp450	Oral rat LD_{50} mg/kg
Acceptable Range	**<500**	**<10**	**<5**	**<5**	**<5**	**<5**	**>-5**	**>30**	**1**	**1**	**>32.6***
E2020	**379.5**	**6**	**4**	**0**	**3.6**	**3.2**	**-4.2**	**38.77**	**1**	**1**	**496.27**
A	436.5	9	7	0	3.8	3.3	-4.3	77.43	1	1	1440.79
32b	400.5	7	7	0	4.1	3.9	-3.2	97.63	1	1	1686.04
C	490.5	7	8	0	2.0	3.6	-4.9	91.34	1	1	571.19
29d	502.7	7	5	2	4.9	4.2	-3.8	55.37	1	1	1805.46

M.W: molecular weight, **R.B:** Rotatable Bonds, **H.A:** H-B Accepters, **H.D:** H-B Donors, **logP**: between 0.8 and 4.9, **logS** between -5.0 and -1.5, **PPb:** A docking with plasma protein binding, **ACyp450:** A docking with Cy p 450, **PSA:** polar surface area between, toxicity of E2020, Oral rat LD_{50} **32.6**mg/kg. * the experimental value of toxicity.

CONCLUSIONS

Several models were designated to study the quantitive structure activity relationship of benzoxazole- N-benzylpipridines derivatives as inhibitory of acetylcholinesterase (AChE) as well as evaluated their inhibitory concentrations (IC_{50}). The good match of predicted and experimental structures gave confidence that such a strategy provides important information for the drug design process. Using a combination of receptor-based alignment and 3D QSAR yielded a significant and predictive model, indicated by the high square correlation coefficients and the low RMSE values.

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