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GRIND/ALMOND Based 3D Quantitative Structure-Activity Study of Dual Reversible Acetylcholinesterase Inhibitors. External Predictivity Assessed on PDB Ligands Conformation

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Introduction

Senile dementia of Alzheimer`s type (AD) is progressive neurodegenerative disorder that is followed by altered ability of memory and learning and behavioral disturbances. The main pathological hallmarks of the disease comprise: extracellular formation of senile Aβ-plaques (derived from APP protein), the loss of cholinergic neurons from basal forebrain and thereby decreased levels of neurotransmitter acetylcholine (ACh) and enzymes that are involved in its synthesis and hydrolysis, acetylcholinetransferase (ChAT) and acetylcholinesterase (AChE).

The current therapeutic strategy for AD is based on cholinergic hypothesis[1] according to which the increase in levels of available ACh in brain induces cognitive improvements in AD patients. One way to increase concentration of available ACh in synaptic cleft is inhibition of AChE. Until now FDA (Food and Drug Administration) has approved four AChE inhibitors for AD therapy: Tacrine (Cognex)[2], Donepezil (Aricept)[3], Rivastigmine (Exelon)[4] and Galanthamine (Reminyl)[5].

At the end of the past century a bivalent ligand strategy was applied to development of AChE reversible inhibitors. In this strategy, two identical (homodimers) or different (heterodimers) monomeric units, capable of binding to different sites on biological target (AChE) are bound to each other trough polymethylene tether. This resulted in marked enhancement in inhibitory activity. The primary sites of interaction of majority of synthesized dual binding site AChE inhibitors are two aromatic aminoacid residues, Trp 84 and Trp 279. The former residue is deeply buried inside active site gorge, found near catalytic triad (Ser 200, His 440 and Glu 327), this is the so-called "anionic site" since it interact with trimethylamino group of ACh. The later residue, Trp 279, which belongs to peripheral anionic site, is found at the entrance of the active site gorge. It was also shown that peripheral anionic site AChE inhibitors retard the process of Aβ aggregation. So, in addition to improvement of inhibitory activity, inhibition of Aβ-aggregation represents one more advantage of dual AChE inhibitors (which simultaneously bind to catalytic and peripheral anionic site) in respect to monomeric inhibitors that interacts only with enzyme's catalytic site. It was postulated that dual AChE inhibitors, in this way, could retard disease progression.

Within frame of one of our ongoing research aimed to design novel AChE inhibitors, nine sets of dual binding site AChE inhibitors, structurally related to tacrine, were extracted from literature for 3D-QSAR modeling. Inhibitor activity of all structures (**1-70**, Table 1) is determined under same conditions and on same enzyme sources (rat cortex homogenate). Reported IC_{50} values are molar concentrations of **1-70** that cause 50% inhibition of enzyme activity. Set of compounds comprise: homodimers of tacrine (I-IV),[#] 4-aminoquinoline (IX), 4-aminoquinaldine (VIII), 4-aminopyridine[6a)-d)] (X) and heterodimers of tacrine and hupridone[7] (V), phenyl-[9], amino-, dimethylamino-groups, 4-aminopyridine and 4-aminoquinoline[8]. All studied compounds are highly flexible, due to presence of polymethylene linker (as given in the Table 1). Therefore, three different conformations of each compound were used as inputs for modeling (See experimental). Finally, six dual AChE inhibitors were extracted from published inhibitor-AChE complexes (PDB* entries: 1ODC, 1UT6, 2CKM and 2CMF[9], 1ZGB and 1ZGC[10]) and used to examine external predictivity of models.

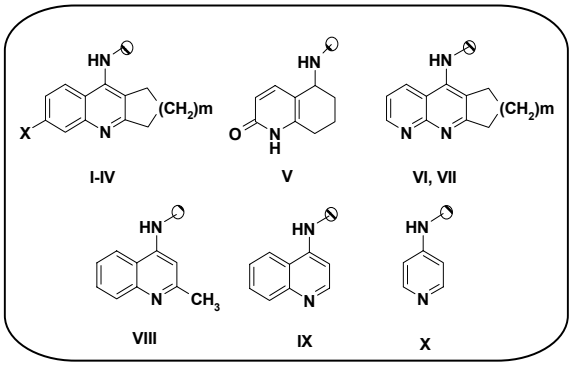
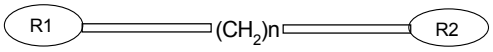


*Chart 1. Fragments of compounds **1-70** and L_1-L_5 R_1 and R_2: I: m=1, X=H; II: m=2, X=H; III: m=2, X=Cl; IV: m=2, X=F; VI: m=2; VII: m=3; XI= -Ph; XII= -NH_2; XIII= -$N(CH_3)_2$*

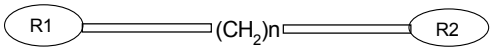
[#] As given on Chart 1.
* Protein Data Bank, *http://www.rcsb.org/pdb*

Table 1. Structures, literature activity data and calculated activities of compounds 1-70 and ligands L_1-L_5 R1 and R2 as given on Chart 1.



No.	R1	n	R2	-Log ($IC_{50\ exp}$)	-Log ($IC_{50\ calc}$) *	No.	R1	n	R2	-Log ($IC_{50\ exp}$)	-Log ($IC_{50\ calc}$) *
1	II	4	V	7.371	7.022	**36**	IX	7	IX	7.057	7.041
2	II	5	V	6.914	6.667	**37**	IX	8	IX	7.025	6.920
3	II	6	V	7.431	7.518	**38**	IX	9	IX	6.907	6.830
4	II	7	V	7.275	7.074	**39**	IX	10	IX	6.316	6.545
5	II	8	V	7.582	7.620	**40**	IX	11	IX	6.105	6.219
6	II	9	V	7.440	7.230	**41**	X	6	X	5.105	5.687
7	II	12	V	6.951	7.175	**42**	X	7	X	6.092	6.259
8	I	7	I	7.125	7.772	**43**	X	8	X	6.042	6.479
9	I	8	I	7.658	7.897	**44**	X	9	X	6.439	6.448
10	II	6	II	8.854	8.179	**45**	X	10	X	6.818	6.335
11	II	8	II	8.796	8.763	**46**	X	11	X	6.613	6.170
12	II	2	II	6.148	6.299	**47**	X	12	X	6.395	6.161
13	II	3	II	6.595	6.810	**48**	II	4	XI	5.854	5.717
14	II	4	II	6.804	6.498	**49**	II	5	XI	5.824	5.766
15	II	9	II	9.114	8.488	**50**	II	6	XI	6.215	6.076
16	II	10	II	8.509	8.442	**51**	II	7	XI	6.409	5.994
17	I	6	I	6.939	7.400	**52**	II	8	XI	6.678	5.982
18	IV	6	IV	9.046	8.872	**53**	II	9	XI	4.699	5.057
19	IV	7	IV	9.222	9.223	**54**	II	10	XI	4.569	4.827
20	IV	8	IV	9.155	9.135	**55**	II	7	XII	6.496	6.735
21	III	6	III	9.222	9.391	**56**	II	9	XII	6.883	7.275
22	III	7	III	10.155	9.801	**57**	II	10	XII	6.833	6.826
23	III	8	III	9.523	9.855	**58**	II	12	XII	6.745	6.977
24	VI	6	VI	8.319	8.356	**59**	II	7	XIII	7.284	7.198
25	VI	7	VI	8.886	8.955	**60**	II	8	XIII	7.328	7.516
26	VI	8	VI	8.721	8.834	**61**	II	9	XIII	7.149	7.418
27	VII	6	VII	8.602	8.456	**62**	II	10	XIII	7.347	7.468
28	VII	7	VII	8.569	8.916	**63**	II	12	XIII	6.578	6.995
29	VII	8	VII	8.796	8.771	**64**	II	7	X	7.893	7.422
30	VIII	7	VIII	6.830	7.180	**65**	II	8	X	7.866	7.702
31	VIII	8	VIII	6.793	7.124	**66**	II	9	X	7.078	6.894
32	VIII	9	VIII	7.267	7.133	**67**	II	10	X	6.754	6.496
33	VIII	10	VIII	6.845	6.729	**68**	II	12	X	6.583	7.017
34	VIII	11	VIII	6.506	6.315	**69**	II	7	IX	7.996	7.858
35	IX	6	IX	6.668	6.677	**70**	II	9	IX	7.412	7.124

**Obtained with 5 principal components (PC).*



Ligand	R1	n	R2	-Log (IC_{50}) Experimental	3PC#	4PC#	5PC#
L_1 (1ZGB)	II	10	V	8.056	7.830	7.690	7.761
$L_{1'}$ (1ZGC)	II	10	V	8.056	7.273	7.181	7.305
L_2 (2CKM)	II	7	II	9.699	6.902	6.939	7.216
L_3 (2CMF)	II	5	II	7.553	7.726	7.677	7.253
L_4 (1UT6)	II	8	XII	7.049	7.848	7.628	7.618
L_5 (1ODC)	II	7	IX	8.056	7.549	7.547	7.644

-Log (IC_{50}) calculated with 3, 4 and 5PC.

Methods

SMILES[11*] notation of (**1-70**) were converted to 3D by CORINA[12] and OMEGA[13] programs. Algorithms for conformer generation by both programs are described in literature. By our choose OMEGA generates 400 conformers of each studied compound. The most extended conformers of each compound generated by OMEGA and assessed by VegaZZ 2.2.0[14] analysis tool are collected in one set. Second set comprise of minimal energy conformers of each compound generated by OMEGA. Third set comprise of CORINA generated conformers. Three models were derived using ALMOND[15] suit of programs. ALMOND procedure for generation 3D QSAR models is based on GRID[16] molecular interaction fields (MIF), and alignment independent descriptors. Briefly, MIF's for four probes, namely O: (carbonyl oxygen) – hydrogen bond acceptor (HBA); N1 (flat amide NH) - hydrogen bond donor (HBD), DRY (hydrophobic probe) and TIP (shape probe) are generated and minima for each probe is extracted. The distances/energy products of every possible combination of each minima are temporary stored. Ten blocks of variables (descriptors) are generated in this way (DRY-DRY, N1-N1, O-O, TIP-TIP, DRY-N1, DRY-O, DRY-TIP, N1-O, N1-TIP, O-TIP). Alignment of every corresponding (same type) minima cluster of probes among all studied molecules (**1-70**) is performed. On the other words, program aligns pharmacophoric points of molecules and in this way shapes virtual receptor site. Consequently

* Simplified molecular input line entry specification.

there is no need for molecules alignment, that *per se* is one of main bottlenecks of 3D QSAR programs[17]. Descriptors for calculation obtained in this way were further processed by principal component analysis (PCA)[18]. Similarity and differences among compounds could be quantitatively observed on 2D and 3D PCA plots. Inclusion of compounds activity (−pk of activity = log ($1/IC_{50}$),where IC_{50} is in molar concentrations), allow partial least square analysis (PLS)[19] and generation of final models.

All three models were derived using same ALMOND settings (Probes: DRY, N1, O, TIP; GRID resolution 0.4 Å, number of nodes 120, % weight of field 60, smoothing window of maximum auto and cross covariance (MACC) 0.8 Å). The PCA and subsequent PLS models were derived by five components. All models were validated by maximal dimensionality of five and three random groups. Refining of models by using Fractional Factorial Design reduced number of x variables from initial 1200 to 783, but did not significantly improve predictivity. Model derived from CORINA input is best one among three generated. That model is described in present communication. Structures of ligands L_1-L_5 are extracted from corresponding PDB files (as given above), hydrogens were added in VegaZZ 2.2.0[19] and on all structures single point calculation by semiempirical MO PM6 method[20] were done using MOPAC2007[21]. All calculations were performed on AMD DualCore x86 4800+ (64 bit) processor in Linux or Windows environment.

Results and discussion

Analysis of numerical and visual output (representation of spatial arrangement of MIF minima and distances between them) for the discussed model, revealed facts that are in accordance with those already known about structure of R1, R2 and linker length. The nodes having higher weights (see Figure 1) describe distances/energies interactions between R1 and R2 that are connected through polymethyene chain of optimal length (7, 8 or 9 methylene units depending on structure of R1 and R2). The highest impacts on model (the weight that are most positive correlated with activity) are noted for DRY-DRY 51 node (as exemplified on Figure 2 a) and N1-N1 73 node. Graphycal presentation of 5PC weights is given on Figure 1. This is also in accordance with known interactions within central and peripheral anionic site of AChE dual biding site inhibitors. More precisely, stacking of R1 positioned between Trp 84 and Phe 330 in active, as well as of R2 positioned between Trp 279 and Tyr 70 in peripheral anionic site.

Partial least square analysis explains ~ 40% of variance within studied set, having r^2 0.82 and q^2 ~ 0.60 with 3PC. Three PC were enough to exactly predict activity of L3 (ligand/conformation taken from 2CMF PDB entry), while ligands/conformations L_4 and L_5 taken from 1UT6 and 1ODC respectively, have predicted values in fair agreement with experimentally ones. L_2 ligand/conformation are wrongly predicted, while of rest two entries (L_1 and L_1'), which in fact are same ligand cocrystallized with the AChE in different conformation, only for L_1' predicted activity is not to far for experimentally obtained one. The same could be stated to predicted activity values obtained with 4PC (that explain ~ 49% of variance within studied set). Significant improvement could be observed for L_1 ligand/conformation in model with 5PC (that explains ~ 51% of variance within studied set).

Table 2. PCA model for 1-70, 1200 x variables.

Comp.	X variance explanation	X accumulation
1	24.625	24.625
2	13.262	37.888
3	9.982	47.870
4	4.553	52.423
5	3.738	56.153

Table 3. PLS model for 1-70, 783 x variables, one y variable.

Comp.	X variance explanation	X accumulation	SDEC	r^2
1	20.983	20.983	0.796	0.504
2	13.956	34.938	0.652	0.667
3	5.488	40.426	0.484	0.816
4	9.085	49.511	0.431	0.854
5	1.495	51.005	0.293	0.933

Table 4. PLS validation, 3 random groups, 20 SDEP calculations

Components	SDEP	SDEV (sdep)	q^2
1	0.868	0.0255	0.411
2	0.776	0.0393	0.528
3	0.719	0.0598	0.596
4	0.699	0.0631	0.617
5	0.678	0.0604	0.640

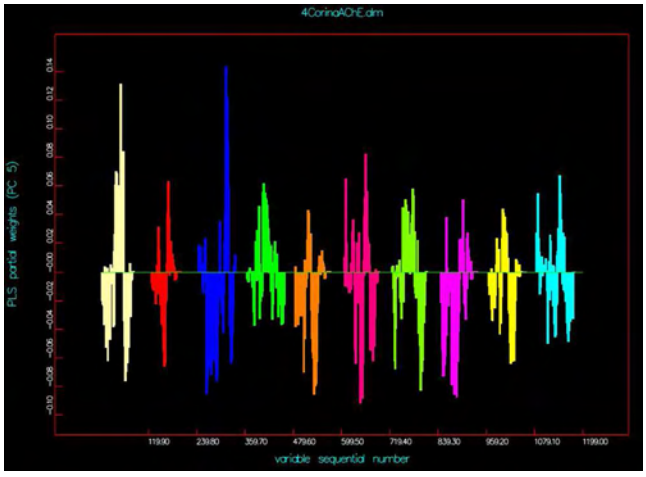


Figure 1. PLS weights plot obtained with 5PC. Ivory (DRY-DRY), Red (O-O), Blue (N1-N1), Left Green (TIP-TIP), Orange (DRY-O), Purple (DRY-N1), Right Green (DRY-TIP), Violet (O-N1), Yellow (O-TIP) and Turquoise (N1-TIP)

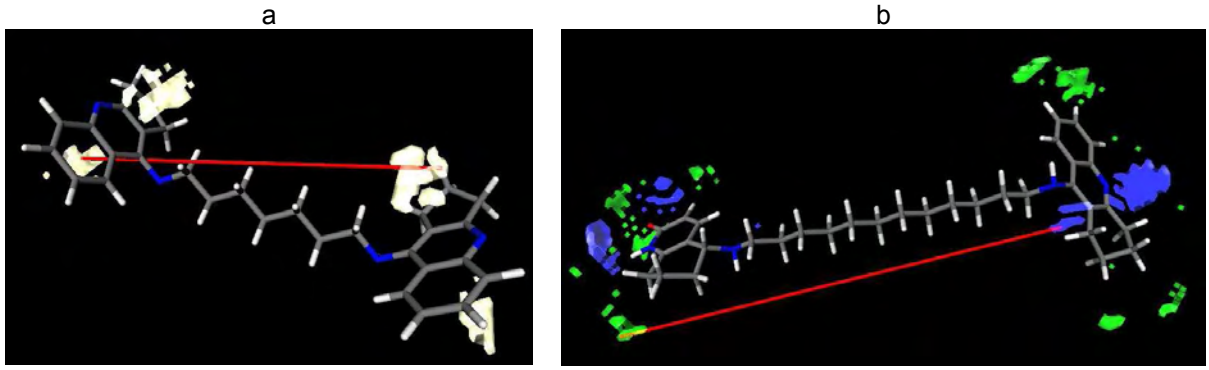


Figure 2. a) DRY-DRY node 51 for 1, b) N1-TIP node 62 for 8

Concluding remarks

Obtained model is not the best possible one, and is derived from training set of 70 compounds having determined activity values that are not uniformly distributed throughout the set. No one model could be better than the set from which is derived. On the other hand, ligand conformation observed within enzyme active site by x-ray crystallography is the best one, and widespread approximation of ligand conformation within enzyme. The conditions of crystallization, crystal packing, engineering and resolution of solved structures significantly influence final results that could be taken from PDB, or found in literature. Therefore from results reported in previous rows, as well as from data that can be found in literature[22], should be concluded that applied method (having advantages and disadvantages, as every method) could offer the good predictivity. Further extension of model by inclusion of novel literature data is in progress.

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ГРИНД/АЛМОНД Тродимензонална анализа квантитативног односа структуре и активности дуалних реверзибилних инхибитора ацетилхолинестеразе. Процена екстерне предиктивности на конформацијама лиганада из ПДБ кристалних структура

У оквиру дизајнирања нових инхибитора ензима ацетилхолинестеразе, девет сетова дуалних реверзибилних инхибитора предходно поменутог ензима, структурно сродних такрину, је преузето из литературе. Тако добијени сет (70 једињења) садржи довољно различитих структурних детаља и обухвата широк опсег активности да је погодан за квантитативну 3Д анализу односа структуре и активности. Анализа је урађена применом релативно новог приступа заснованог на преклапању отиска фармакофорних тачака молекула, применом програма АЛМОНД. Добијени модел је показао да се може користити за предвиђање активности сличних молекула који нису укључени у сет из којих је модел изведен. Конформације лиганада које су коришћене за процену спољње предиктивности модела (6 једињења) су преузете из кристалних структура протеина кокристалисаних са инхибиторима.

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