QSAR Applications During Last Decade on Inhibitors of Acetylcholinesterase in Alzheimer's Disease

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Abstract: This article reviews multi-criteria QSAR applications on Acetylcholinesterase inhibitors as palliative drugs for Alzheimer's Disease, published in the period 2001-2011. It includes QSAR models for different series of compounds, comparative studies, and advances in methodologies. This period is marked by a shift in focus from palliative treatment to pathogenesis. However, we believe that research into palliative treatment should continue. More comparative studies are desirable. In order to facilitate comparative and general studies on Acetylcholinesterase inhibitors, a standard experimental protocol for measuring an inhibitor's potency is needed. Finally, we recommend chemists to work closely with system and molecular biologists.

Keywords: Medicinal chemistry, QSAR theory, alzheimer's disease, acetylcholinesterase, molecular descriptors, CoMFA, CoMSIA.

1. BACKGROUND

Alzheimer's Disease (AD) is a neurodegenerative process characterized by a progressive memory loss, decline in language skills and other cognitive abilities [1]. It is common among the elderly, affecting around 7 % of the population above 65 years old [2]. Currently, this is an incurable disease without an effective therapeutic approach [3], and it is not surprising that research has been performed on both its palliative treatment and potential cure between 2001 and 2011. In this review, the focus is on the inhibition of the Acetylcholinesterase (AChE) enzyme as a palliative treatment for patients with Alzheimer's disease [4].

Patients with AD experience a selective loss of cholinergic neurons in the brain and decreasing levels of Acetylcholine [5]. The AChE enzyme is responsible for the termination of impulse signaling at cholinergic synapses by catalyzing the hydrolysis of Acetylcholine, a neurotransmitter [6]. As defined by the cholinergic hypothesis [7, 8], a palliative strategy works by enhancing cholinergic transmission. This should be achieved by inhibition of AChE.

Tacrine has been approved by the US Food and Drug Administration as the first drug for the treatment of AD in 1993. However, it is only one of many Acetylcholinesterase Inhibitors (AChEI), such as Donepezil, Rivastigmine, or Galantamine [9]. Fig. (**1**) includes the scaffold of these common AChEIs, which have different structural features. In order to facilitate the design of new and even more potent AChEIs, the use of mathematical frameworks linking specific molecular structures to potency is essential. The Quantitative Structure-Activity Relationships (QSAR) is a branch of Theoretical Chemistry which provides such frameworks [10].

The QSAR Theory depends on the main assumption that the biological activity of a chemical compound is solely determined by its molecular structure. This theory does not offer specific details on the usually complex mechanism/path of action involved. However, it is possible to get some insight on the underlying mechanism by means of the QSAR-based predicted activities [11, 12].

In the realms of QSAR, the molecular structure is quantified by using a set of suitable molecular descriptors, which are numbers carrying information on the constitutional, topological, geometrical, hydrophobic, and/or electronic aspects of the chemical structure [13-16]. A set of descriptors can then be statistically correlated to different experimental biological activities, resulting in a model which can be used to find out useful parallelisms.

Altogether, QSAR analyes are effected by various factors from which the most important are: (a) the selection of molecular descriptors that should include maximum information of structures and minimum colinearity between them; (b) the use of suitable multi-criteria modeling methods; (c) the number of descriptors to be included in the model; (d) the composition of the training and test sets; and (e) the employment of validation techniques to verify the predictive performance of the developed models [17-24]. The QSAR approach is an important tool in Medicinal Chemistry, which makes drug design more rational by minimizing the number of expensive, time consuming experiments.

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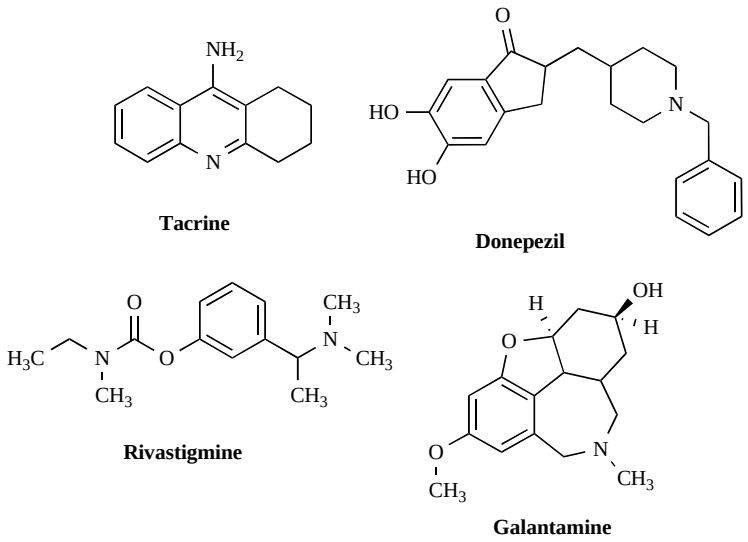


Fig. (1). Some common Acetylcholinesterase inhibitors.

2. MULTI-CRITERIA QSAR STUDIES ON ACHEI BY VARIOUS CHEMICAL STRUCTURES

In the last decade, many multi-criteria QSAR models have been built for various types of AChEIs using different modeling approaches. This section summarizes such studies and is categorized into subsections according to the involved chemical structures.

2.1. Tacrine Analogs

Although Tacrine is the oldest palliative drug designed based on the cholinergic hypothesis, new Tacrine derivatives are still being designed to treat AD.

In 2006, Akula *et al*. [25] have published 3D-QSAR studies on bis-tacrine compounds by using molecular docking scores calculated by FlexX [26], Flexidock [27] and Cscore [28], in addition to Comparative Molecular Field Analysis (CoMFA). The Sybyl [29] and Mopac [30] programs are both used in the optimization of structures and molecular alignment. The docking scores are used as molecular descriptors along with the steric and electrostatic field values obtained from CoMFA, and 16 molecules are set aside in the training set. The structure-activity model is validated on a test set having only 3 molecules, which we think it would not be able to describe the real predictive power of the QSAR model developed due to the limiting information used for validating it.

In another study of the same year, Fernández *et al*. [31] have applied Bayesian-Regularized Genetic Neural Networks (BRGNNs) to 136 Tacrine analogs. Their 3D structures are geometrically optimized using the semi-empirical quantum chemical method PM3 implemented in Mopac [30]. Here, the Bayesian-regularization avoids overtraining, while the Genetic Algorithm (GA) approach allows exploring an ample pool of 3D descriptors generated by the software Dragon [32]. The algorithms are implemented and the models are built in the Matlab environment [33]. The resulting model is evaluated by averaging multiple validation sets generated as members of diverse-training set Neural Network Ensembles (NNEs). When considering forty assembled members, the NNE provides reliable statistics. The employment of Artificial Neural Networks constitutes a common practice in QSAR studies for modeling non-linear relationships between the chemical structure and the considered biological property.

Jung *et al*. [34] have also worked in 2007 on the Tacrine scaffold: they build QSAR models using variable selections based on Multivariable Linear Regression (MLR): Genetic Algorithm (GA)-MLR and Simulated Annealing (SA)-MLR. For doing this, they place 68 molecules in the training set and leave 12 in the test set. The molecules are geometrically optimized using the Titan Pro software [35], and their molecular descriptors are calculated using PreADME on the web [36] and BioMedCAChe [37]. The stepwise multiple linear regression procedure is performed by the software package SPSS [38]. Simulated annealing and genetic algorithms are performed using the R statistical software package, Subselect [39]. The best model is obtained by SA-MLR with greater explanatory and prediction capability, and thus a smaller standard deviation (*S*). Based on their models, the authors suggest important roles for hydrophobic and electrostatic interactions in increasing the structure's AChE activity. They also suggest opposite effects for hydrophilic and topological features of molecules.

In 2008 Saracoglu *et al*. [40] have performed QSAR analyses of AChEIs related to Tacrine and 11 H-Indeno-[1,2-b]-quinolin-10-ylamine tetracyclic Tacrine analogs. The Electron-Topological Method (ETM) is applied with the ETM software [41] on a training set of 44 compounds, which we consider it as a valuable QSAR tool as this technique takes into account both geometrical and electronic characteristics of the molecules. Based on pharmacophores and anti-pharmacophores calculated as sub-matrices containing spatial and quantum chemistry characteristics, a system for the activity prognostication is developed. Some molecular fragments specific for active and inactive compounds are also revealed.

In another work published in 2010 [42], Chen *et al*. have studied multi-target-directed AChEIs of Tacrine-Nimodipine dihydropyridines. They establish 3D-QSAR models using CoMFA and Comparative Molecular Similarity Index Analysis (CoMSIA) methods. The compounds employed are very potent and selective AChEIs, and show an excellent neuroprotective profile and a moderate Ca^{2+} channel blockage effect. A training set of 60 compounds is used, and the resulting models are validated on a test set of 12 compounds. The structures of the investigated ligands are built and optimized using Sybyl [29], while the lowest energy structures are used during the alignment. The partial atomic charges required to estimate the electrostatic interaction are computed by semi-empirical molecular orbital methods using MOPAC [30] with an AM1 Hamiltonian. The AutoDock program [43] is used for docking. CoMFA and CoMSIA are performed on Sybyl. For the CoMSIA approach, descriptors of five physicochemical field properties are used to correlate with changes of ligands affinities, which explicitly define hydrophobic, hydrogen-bond donor and acceptor descriptors (in addition to the steric and electrostatic fields used in CoMFA).

2.2. Carbamates

Compounds related to carbamates (see Fig. (**2**)) have drawn a lot of attention from QSAR researchers in the last decade as well. G. Lin have published an article in 2004 [44] about substituted phenyl-N-butyl carbamates (1) and p-nitrophenyl-N-substituted carbamates (2). The author model virtual inhibition constants ($K_i^{'}$) of the protonated inhibitors from the equation, $-\log K_i^{'} = -\log K_i - pK_a + 14$ in pH 7.0 buffer solution, where K_i is the inhibition constant. The $-\log K_i^{'}$ and $\log k_c$ values (k_c is the carbamylation constant) for AChE inhibitions by carbamates (1) correlate with the Hammett equation ($\log k/k_0 = \rho.\sigma$); moreover, those by carbamates (2) correlate with the Taft equation ($\log k/k_0 = \rho^*.\sigma^*$). With modified Hammett-Taft cross-interaction variations, MLR models of the $-\log K_i^{'}$ and $\log k_c$ values of carbamates (1) and (2) give good correlations, and the cross-interaction constants ($\rho(\mathrm{XR})$) are 0.5 and 0.0, respectively. The $\rho(\mathrm{XR})$ value of 0.5 indicates that the carbamate moiety of the inhibitors stretches along the active site gorge of the enzyme but does not bind in the acyl binding site pocket of the enzyme. The $\rho(\mathrm{XR})$ value of 0.0 suggests that the transition states that lead to the carbamyl enzymes are breaking C-O bonds and are excluding the leaving groups, substituted phenols. In the same year, Lin *et al.* [45] have published their work on ortho effects in QSAR for acetylcholinesterase inhibition by a series of nine ortho-substituted phenyl-N-butyl carbamates, for which they model the virtual inhibition constants of the protonated inhibitors.

In a project published in 2008, Roy *et al.* [46] have studied structurally diverse carbamates for acetylcholinesterase inhibition. QSAR models are built and validated using a total of 78 molecules, using CoMFA, advanced CoMFA and CoMSIA on Sybyl [29]. The authors conclude that steric, electrostatic and hydrophobic interactions are important for describing the variation in binding affinity of the different structures. For a better inhibitory activity, the carbamoyl nitrogen should be more electropositive. Substitutions on it should have high steric bulk and hydrophobicity. The amino nitrogen, on the other hand, should be electronegative.

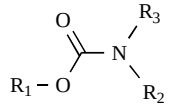


Fig. (2). Scaffold for AChEI of the carbamate type.

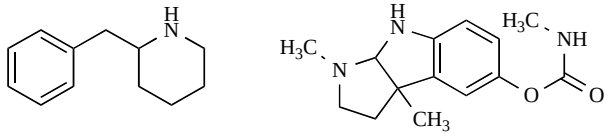
The work of Chadhaery *et al*. in 2009 [47] have carried out CoMFA and CoMSIA, using two different alignment methods, namely pharmacophore and maximum common substructure-based alignments. They place 52 structurally diverse carbamates covering a good range of AChE inhibitory activity in the training set, and resort to steric, electrostatic, hydrophobic, donor and acceptor field descriptors. All compounds are built using ISIS Draw [48], imported to Accelry's Discovery Studio window [49], and optimized using CHARMm force field. Pharmacophore modeling is performed using the Hip-Hop module in the software CATALYST [50], while the 3D-QSAR studies are done using Sybyl [29]. The resulting CoMFA and CoMSIA models with pharmacophore-based alignment are found to be in good agreement with each other. Additionally, the authors demonstrate that pharmacophore-based alignment has a significant superiority over maximum common substructure-based alignment in terms of leave-one-out statistical values. The best CoMFA and CoMSIA models based on pharmacophore-based alignment are then validated on a test set of 17 compounds. From the models, it is inferred that the hydrophobic factor has a major contribution to the AChE inhibitory activity modulation.

In 2010, another study of the same group [51] has developed a systematic virtual screening procedure, including development of 3D-pharmacophore, screening of virtual library, synthesis and pharmacology. Using a training set of 24 carbamates as AChEIs, these researchers develop a predictive pharmacophore model with one hydrogen-bond donor and three hydrophobic features. All molecular modeling analyzes are accomplished using the Window-based Accelerys Discovery Studio [49]. The Catalyst software [50] is used for pharmacophore modeling and virtual screening. Validation on a test set of 40 carbamates proves the model's significant predictive power, and therefore it provides a tool for searching new carbamates having specific properties.

2.3. Benzylpiperidine and Physostigmine Derivatives

In 2005, Kandermirli *et al.* [52] have applied electronic-topological and neural network methods for developing QSAR of AChE inhibition by a series of Physostigmine and N-benzylpiperidine derivatives (Fig. (**3**)). They calculate molecular fragments specific for active compounds and

breaks of activity for human AChE (HuAChE), formulating requirements for a compound to behave as active.



2-Benzylpiperidine **Physostigmine**

Fig. (3). Molecular structures of 2-Benzylpiperidine and Physostigmine.

In a publication of 2009, Haq *et al.* [53] have applied 3D-QSAR studies based on CoMFA and CoMSIA to a training set of 40 Physostigmine derivatives. All 3D structures are constructed on Gaussian software [54]. 3D-QSAR calculations are performed using the Sybyl package [29]. The study is conducted to obtain a highly reliable and extensive dynamic QSAR model based on the alignment procedure, for which the co-crystallized Ganstigmine is used as a template. The resulting QSAR is statistically significant and validated by an external test set composed of 8 compounds. Here again, we consider that the test set´s size employed is the limiting factor for the validation of the model.

In 2011 Araújo *et al.* [55] have built several receptor-dependent 3D-QSAR models using 60 Benzylpiperidine structures. A combined GA and Partial Least Squares (PLS) approach, available in the Wolf program [56], is used as computational methodology to develop and validate such models. The training set includes 47 molecules and the test set the remaining 13 ones. The 3D structures are built using the Spartan software [57]. In building the model of Human AChE, the program Modeller [58] is used for geometry optimization and Procheck [59] is used for validation. In complexes building and optimization, Sybyl is used. In molecular dynamics simulations of the complexes, each ligand/HuAChE complex is submitted to a preliminary optimization in the Gromacs program [60] then the molecular topologies of the ligands are generated by the Prodrg server [61]. The descriptors employed are steric (Lennard-Jones) and electrostatic (Coulomb) interaction energies, calculated between each ligand and the HuAChE residues within radii 10 Å around the ligand. According to the two best calculated models, the Lennard-Jones and the sum of Lennard-Jones and Coulomb contributions are more important than the Coulomb ones to the relationship between structure and activity. The authors also conclude that the hydrophobic residues of the active site of HuAChE are more important than the polar residues for this series of inhibitors.

2.4. Phosphate-Containing Compounds

In the last decade, enough research on Alzheimer's Disease has been carried out on compounds containing the phosphate group. For instance, Yazal *et al*. [62] have applied in 2001 a combination of conformational analysis and 3D-pharmacophore models on a collection of organophosphorous AChEIs (Fig. (**4**)), in order to rationalize their inhibitory potencies against the enzyme. The compounds are drawn in 2D and converted to 3D using the Sketch-and-Converter module in Insight 2000 [63]. The structures are optimized using Cerius2 [64]. Conformational analyses of the 6 inhibitors in the training set are carried out in the Catalyst package [50], generating pharmacophore models. The resulting 3D pharmacophore models are characterized by at least one hydrogen bond acceptor site and 2-3 hydrophobic sites. The models demonstrate a high degree of correlation between the calculated and experimentally measured inhibitory potencies. However, from our point of view, little information is used during the model's calibration.

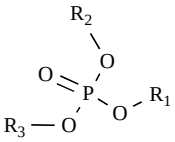


Fig. (4). Scaffold for AChEI compounds having the phosphate group.

Zhao *et al.* have published an article in 2004 [65] about their 3D-QSAR studies on the acute toxicity of 35 dialkyl phenyl phosphate compounds to houseflies. Aided by the software Sybyl [29], they use CoMFA and CoMSIA methods, which provide more comprehensive and accurate perspectives on the reaction mechanism between organophosphate compounds and AChE than classical QSAR methods. They find that steric and electronic properties of the substituent on the phenyl of an organophosphate compound, like the length of alkyl and the electronegativity, respectively, have a dominant influence on its potency against AChE. Hydrophobicity has little influence.

A 2009 study of Kuzmin *et al*. [66] has performed consensus QSAR modeling of AChE inhibition by various organophosphate compounds. Simplex representation of molecular structure (SiRMS) and Lattice Model (LM) techniques are used to generate the molecular descriptors. In order to avoid chance correlations, 1000 rounds of Y-scrambling are performed. Leverage and Ellipsoid Applicability Domain (DA) approaches estimate the quality of prognosis. A successful consensus model is obtained which is applied to predict AChE inhibition of new compounds. It is revealed that both the atom's individuality and stereochemistry of chiral surroundings of the asymmetric atom of phosphorus are vital for AChE inhibition. Thus, the (*R*)-isomers are always less active than the (*S*) isomers and the racemate. The quantum chemical calculations are carried out using the Gaussian-03 package [54]. Dragon software is also used in this work [32].

2.5. Ketones

Our bibliographic search identifies four QSAR studies about the inhibitory potency of ketones during the last decade (Fig. (**5**)). A study published by Liu *et al.* in 2007 [67] has analyzed 26 compounds with the phenyl pentenone scaffold to construct a 3D-QSAR model using CoMFA, performed with the QSAR module of Sybyl for each combination of steric and electrostatic molecular fields. The molecular docking program Dock [68] is used to determine the conformation of the active compound **5** in the active

pocket of AChE. This model possesses an ability to predict the activities of new inhibitors, and would be useful for the future design of new AChE inhibitors.

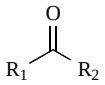


Fig. (5). Scaffold for AChEI containing the ketone group.

In 2007, Sheng *et al.* [69] have conducted 3D-QSAR studies using CoMFA and CoMSIA [29], on 2-phenoxy-indan-1-one derivatives bearing AChE inhibitory activities. The results show that the contributions to the activity of steric fields are greater than that of electrostatic fields. Addition of CoMSIA has elucidated the role of hydrophobic and hydrogen bonding along with the effect of steric and electrostatic properties revealed by CoMFA. In conclusion, the two models demonstrate a good fit, the analysis of CoMFA and CoMSIA contour maps provide insight into the possible modification of the molecules with better activity.

Another study of the same year has seen Shen *et al*. [70] to explore the binding mode of 2-substituted 1-indanone derivatives with AChE. The 3D structures of these compounds are sketched and optimized with the molecular modeling software package Sybyl [29]. Aided by Gold [71], they employ the Gold-docking conformations of the compounds in the active site of AChE. Highly reliable and predictive 3D-QSAR models are established by CoMFA and CoMSIA methods, which are afterwards successfully validated with an external test set. As a result of such mathematical models, a better understanding of the interaction between the inhibitors and AChE is provided.

In their work published in 2010, Sharma *et al*. [72] have applied QSAR on indanone and aurone derivatives by using various physicochemical parameters calculated with the software ChemOffice [73]. The structure-activity relationship is established by means of sequential multiple linear regression analysis, resorting to the Valstat program [74]. A set of 23 compounds is used in the training set, and the validation is performed with 9 compounds. The best model found includes the Lowest Unoccupied Molecular Orbital (LUMO) energy, diameter and the Gibbs Free Energy as molecular descriptors, and it is found to be statistically significant.

2.6. Other Chemical Structures

In 2001, Sippl *et al*. [75] have modeled aminopyridazine derivatives (Fig. (**6**)) by threedimensional analyses using Sybyl [29], Grid [76] and Golpe [77] programs. They initially use four X-ray structures of AChE complexed with small, non-specific inhibitors to create a model of the binding of some recently developed aminopyridazine derivatives. Combined automated and manual docking methods are applied to dock the co-crystalized inhibitors into the binding pocket, using AutoDock [43]. The ionization states of the protein residues are determined from pK_a calculations using the UHBD program [78]. The program Grid [76] is used to study the interaction potentials of the protein and inhibitor structures. A training set of 42 aminopyridazine compounds derived by the docking procedure is used to build a 3D-QSAR model, using the Grid/Golpe method. A test set of 7 designed molecules is used to validate the model. The modeling process is validated by comparing the predicted enzyme-bound conformation with the known conformation in the X-ray structure. The successfully validated model is then used to evaluate the binding conformation of the aminopyridazine compounds under consideration.

In another study of the same year, Spassova and Singh [79] have analyzed Methamidophos (Met), which is a weak inhibitor of housefly head AChE. Acephate (Ace), like Met, is a poor inhibitor of AChE *in vitro* and has a comparable to Met insect toxicity *in vivo*. Contrary to Met, though, Ace has much lower mammalian toxicity. Understanding the structural properties which make insecticides toxic to insects but not to mammals is of great importance, since mammals (including humans) are inadvertently exposed to these compounds. The QSAR results found are consistent with the model in which both the *in vitro* and *in vivo* toxicity of Met depends on the inhibition of the active center of AChE by the unchanged Met. An optimal susceptibility to hydrolysis is needed for Met and its analogs to have high insecticidal activity since in order to phosphorylate AChE they need to be hydrolyzed and at the same time their stability is of great importance *in vivo* for accumulating at the site of action. The insecticidal activity of Ace analogs may be due to direct interaction with the active center of the AChE. The mammalian toxicity of Ace analogs may be due to interaction with an 'allosteric' reaction center in the AChE. In terms of computational tools, the 2D structures of the molecules are transformed into 3D structures in a conformational analysis by the 3DGEN sub-program of Oasis [80]. After the conformational analysis, the geometry of each conformer is optimized using Mopac [30]. The hydrophobic parameters are calculated using Molecular Modeling Pro [81].

In 2003, Haq *et al*. [82] have derived a comprehensive structure-activity relationship model for a series of natural AChEIs isolated from *Sarcococca saligna*. All structures are initially generated by Gaussian View [83] and minimization is performed with the Austin Model 1. The statistically significant CoMFA models are established by atom-based alignment, using Sybyl [29]. The training set consists of 28 previously isolated and tested pregnane-type steroidal alkaloids inhibitors, while 4 molecules form the test set. In our opinion, the accuracy of this model should be further verified using more experimental data.

In 2005, Chiou *et al*. [84] have carried out a QSAR analysis for Acetylcholinesterase and Butyrylcholinesterase inhibition by cardiovascular drugs and benzodiazepines, including Lovastatin, Simvastatin, Amlodipine Besylate, Nifedipine, Hydralazine hydrochloride, Diazepam and Chlordiazepoxide hydrochloride. The pK_i values for Acetylcholinesterase and Butyrylcholinesterase inhibitions by these drugs are linearly correlated with the molecular weights, with slopes of 0.005 and 0.0021, respectively. Therefore, van der Waals' interactions between Acetylcholinesterase and these drugs are stronger than those between Butyrylcholinesterase probably due to a small

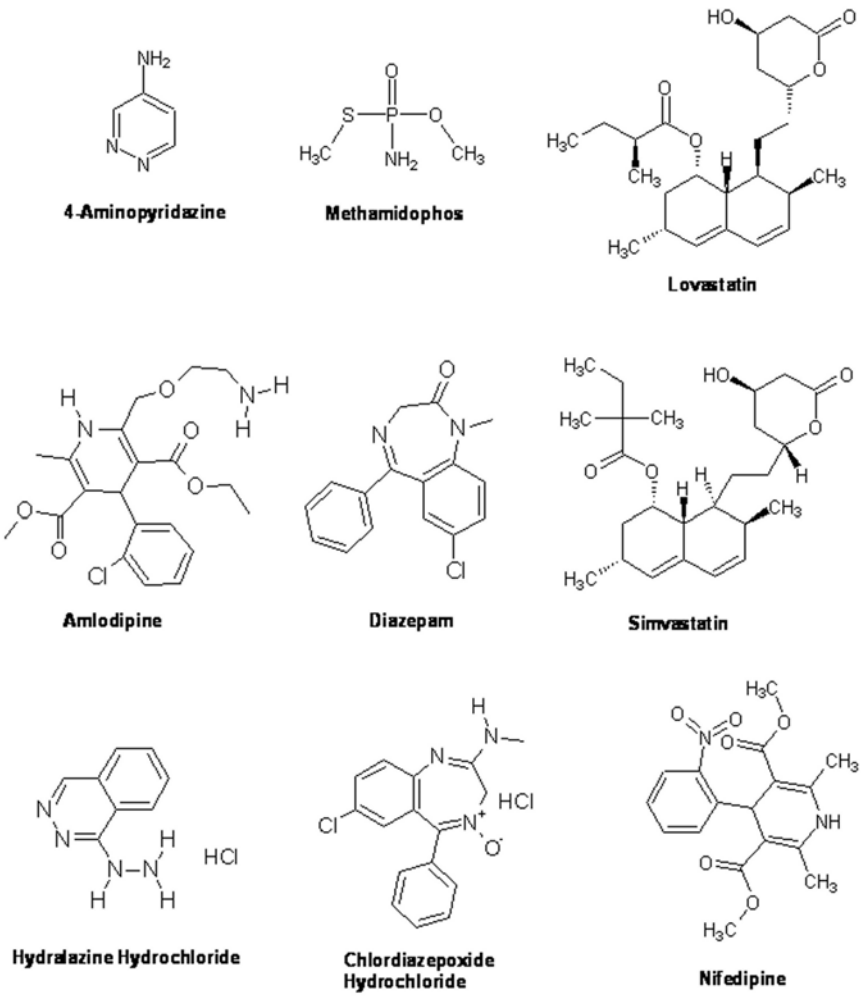


Fig. (6). Other chemical structures involved during the Acetylcholinesterase inhibition.

active site gorge and a significant peripheral anionic site for Acetylcholinesterase. The fact that the pK_i values for both cholinesterase inhibitions are linearly correlated with each other suggests that both enzyme inhibitions proceed via a common mechanism. Since Amlodipine Besylate is a very potent inhibitor of both cholinesterases, Amlodipine Besylate may, like Donepezil, be useful in AD treatment.

In 2009, Solomon *et al.* [85] have studied the QSAR of a series of 88 N-aryl derivatives which display varied inhibitory activity towards both Acetylcholinesterase and Butyrylcholinesterase. All the N-aryl derivatives are built using Insight-II software [86]. The QSAR model for AChE inhibition is derived for a training set of 53 compounds, with the aid of the GA technique using topological, molecular shape, electronic and structural descriptors. Here, a test set of 26 compounds is used to successfully validate the resulting model.

3. INTEGRATIVE STUDIES ON DIFFERENT CLASSES OF MOLECULES

As summarized in Section 2, there are numerous, structurally diverse AChEIs. Specialized QSAR models for individual classes of inhibitors are quite common, although an integration of these models is the logical next step. Instead of focusing on specific structures like Tacrine derivatives, Carbamates or Benzylpiperidines, such an integrative study should identify common and different physicochemical properties leading to the inhibitory activity. This would help researchers to design novel drugs. A couple of studies in this direction have been published recently in 2011.

Gupta *et al*. [87] have developed comparative QSAR models for 42 AChE inhibitors binding at the catalytic and peripheral anionic site, identified on the basis of the molecular docking approach. The compounds under study are built using Sybyl [29]. Molecular docking at the dual site of AChE is performed using the Gold software [71], while Cerius2 [64] is used to compute the molecular descriptors. They select the dataset from diverse chemical classes such as piperidines, tetra hydro acridines, tetrahydroazepines and carbamates instead of focusing on any particular series. QSAR models are developed using GA, Genetic PLS, Support Vector Machine (SVM) and Artificial Neural Network (ANN) techniques, using a training set that includes 31 molecules. The robustness and significance of each model

is critically assessed on an external test data set of 11 molecules. In conclusion, the generated models using thermodynamic, electrotopological and electronic descriptors show that nonlinear methods are more robust than linear ones.

In their work, Lu *et al*. [88] have developed both qualitative and quantitative 3D-pharmacophore models based on AChEIs, collected from nine publications reported by the same laboratory. This covers a range of molecules including polyamines, ketones, Donezil-based inhibitors, Tacrine and benzofuran-based hybrid compounds. Pharmacophore modeling correlates activities with the spatial arrangement of various chemical features in a set of active analogs. From the diverse compounds, 62 are selected as part of the training set, and the test set contains 26 compounds. The 2D chemical structures of these AChE inhibitors are sketched using CS ChemDraw Ultra [73]. The resulting files are imported into Discovery Studio [49] and converted into the corresponding standard 3D structures. Aided by the same software, two different methods, HipHop and HypoGen, are used to generate ligand based pharmacophore models. The best five-features pharmacophore model includes one hydrogen bond donor and four hydrophobic features, and is applied to identify nine novel inhibitors.

4. ADVANCES IN RELEVANT METHODOLOGIES BETWEEN 2001 AND 2011

Research into methodologies used to establish QSAR models for AChE inhibition potency is reviewed in this section. Interestingly, these studies have been performed recently in the last three years.

A study performed by Asadabadi *et al*. [89] in 2009 extracts the most effective structural features of AChEIs from a large number of molecular descriptors. An efficient feature selection method is emphasized in such approach, which uses the confirmative results of different routines and novel feature selection methods. The proposed methods generate quite consistent results ensuring the effectiveness of the selected structural features. In this study, all structures are drawn and optimized in HyperChem [90]. Statistical analyses are performed using the statistical software SPSS [38]. The programming and implementation of the algorithms is performed in Matlab environment [33].

In 2010, Tsai *et al*. [91] have performed a comparative study on different methods employed for assigning electrostatic potentials to atoms in a molecule. This choice of methodology is critical for QSAR studies although, however, no systematic comparison of the effects of electrostatic potentials on the model's quality has previously been done. Twelve semiempirical and empirical charge-assigning methods, AM1, AM1-BCC, CFF, Del-Re, Formal, Gasteiger, Hückel, Gasteiger-Hückel, MMFF, PRODRG, Pullman, and VC2003 charges are compared for their performances in CoMFA and CoMSIA modeling, using several standard datasets. Del-Re, PRODRG and Pullman are excluded from the study because they are specific to certain atom and bond types. The commonly used Gasteiger-Hückel charge performs poorly in prediction accuracy. The AM1-BCC method is better than most charge-assigning methods based on prediction accuracy but unsuccessful in yielding overall higher cross-validation correlation coefficient values than others. The CFF charge model is found to work best in prediction accuracy when the cross-validation correlation coefficient is used as the evaluation criterion. The programs used for assigning charges and modeling include QuACPAC [92], Sybyl [29], Discovery Studio [49] and Dundee PRODRG2 online server [93].

5. BEYOND INHIBITING ACHE: OTHER FUNCTIONS OF THE SAME MOLECULES

In the course of reviewing the current topic, we come across with many theoretical and experimental studies on molecules other than AChE, but which are still related to Alzheimer's Disease. As far as the authors are aware, specific QSAR studies on the inhibition of Acetylcholinesterase by AChEIs form a relatively small part of AD research. This reflects a shift in focus in the field of neurodegenerative disease. This shift can even be seen in some AChEI studies which focus on the inhibitors' effect on the pathogenetic cascade leading to AD instead of their palliative functions.

One such function is the modulation of Amyloid Precursor Protein (APP) processing. The brain tissue of AD patients shows the presence of neuropathologic markers such as neurofibrillary tangles and neurotic or senile plaques. The latter are characterized by the accumulation of proteins in the form of β-pleated sheet fibrils, which consist mainly of a 39-43 amino acids peptides called β-Amyloid (Aβ) [9]. The Amyloid hypothesis of AD is focused on the toxic effect of excessive Aβ on neurons and suggests the aberrant mechanism of the APP processing is a central pathogenetic mechanism for AD [94, 95].

In a 2004 review, Racchi *et al*. [9] have explored the experimental evidence which suggests a role for AChEIs in APP processing. They conclude that evidence pointed to a mechanism of interaction of AChEIs and the mechanism of APP. Multiple complex mechanisms are suggested to modulate APP processing, involving cholinergic agonist effect, coupled to multiple signal transduction pathways, or post-transcriptional effects modulating the expression of cellular APP.

Fu *et al*. [96] have carried out one such study on AChEIs in 2008. α-, β- and γ-Secretase are involved in the processing of APP to form Aβ. This group experimentally shows that Bis(7)-Tacrine could substantially reduce the generation of Aβ by inhibiting β-Secretase (BACE-1) and activating α-Secretase activity. Citing the group's previous study showing bis(7)-Tacrine's role in attenuating Aβ -induced neuronal apoptosis [97], the authors argue that this inhibitor has multiple targets in the Amyloid pathological cascade of AD.

An extension of this shift in focus from Acetylcholinesterase inhibition to other aspects of AD is the emergence of multi-target drugs. The use of multi-potent drugs to treat AD has been reviewed by Zhang in 2005 [98]. The idea behind this strategy is that most human diseases involve multiple pathogenetic factors and therefore, a one-drug-one-target paradigm in drug discovery is flawed [99,

100]. For the case of AD, important factors include aggregation of amyloid-β and tau proteins, excessive metal ions, oxidative stress and reduced level of acetylcholine [101-104]. As reviewed by Zhang, there are various natural and synthetic structures which influence different combinations of these factors. Details about such individual hybrid molecules or natural structures can be found in Zhang's review and will not be iterated here. However, some examples include dual AChE and monoamine oxidase (MAO) inhibition, simultaneous AChE inhibition and AChE-induced Aβ aggregation, or metal chelators with radical scavenging potential. When multiple structure dependent factors are under consideration, experiments become more costly and time consuming, if they are feasible at all. Therefore, techniques used in computer-aided drug design such as QSAR are essential in this paradigm shift.

6. FUTURE RESEARCH DIRECTIONS

It is understandable that the focus on AD research has shifted away from the inhibition of Acetylcholinesterase. It is just a palliative, symptomatic treatment which does not stop the progression of neurodegeneration. However, there are a few reasons why research into this branch of research should continue.

First, the pathogenesis and progression of Alzheimer's disease remain partially understood at best. Therefore, it is unwise and premature to abandon effort to develop more effective symptomatic drugs before substantial results are obtained in the discovery of a cure which attacks the root of AD. Second, any emerging cure does not necessarily supersede palliative drugs. They are still needed while neurodegeneration is being stopped and perhaps reversed in a patient. The third case is the complexity of the nervous system. It is regulated by multiple, interwoven pathways which consist of many and sometimes common biomolecules and receptors. The AChEIs may play a role in other pathways involved in AD too, some of which may be behind the onset of the disease. There is also evidence which suggests that multi-functional compounds may provide greater therapeutic benefits by concurrently targeting different sites in the brain [98, 105].

The two comparative studies reviewed in Section 3 are both published in 2011. We encourage this new trend in AD research. Considering there are so many specialized multi-criteria QSAR studies in the literature, each modeling a specific class of inhibitors, there is a need to integrate these separate studies. It helps designing the most potent AChEI. QSAR studies covering diverse structures allow researchers to pick out molecular features which play important roles in Acetylcholinesterase inhibition, but which are found in separate classes of inhibitors. Then, researchers can design and synthesize new compounds with different combinations of these separate features.

In order to facilitate QSAR studies into AChE inhibition in general, and comparative studies of different types of inhibitors in particular, we call for the establishment of a standard experimental protocol to measure the inhibitory activity of AChEIs. Like all multi-criteria QSAR models, the predictive ability of a QSAR model of AChEIs strongly depends on the size and quality of datasets of inhibitors' biological activity. In most experimental studies measuring the inhibitory activity, the number of structures measured does not exceed twenty or so. Furthermore, although the general experimental method is largely consistent in different studies, parameters like concentration and amount of chemicals used, or temperature vary slightly. This lack of consistency makes combination of datasets problematic. This problem is particularly relevant to comparative studies because datasets are often obtained by combining data collected by different researchers.

Finally, we call for multi-criteria QSAR chemists to work more closely with system biologists and molecular biologists. As reviewed by Katayam *et al.* [106], several authors have reported that neuronal death has its origin in the Endoplasmic Reticulum (ER). Accumulation of unfolded proteins in the ER due to genetic mutations or exogenous factors leads to ER stress. Normal cells respond to ER stress in a mechanism called the Unfolded Protein Response (UPR). Dysfunction of ER down regulates UPR. This leads to vulnerability to ER stress and hence neuronal apoptosis and neurodegeneration. This development is a step forward in the understanding of AD and the search for a cure. Nevertheless, like many biological system, UPR and its associated bioCical and physiological responses are highly complex. They involve many interdependent components, forming a dynamic network. They span across many spatial and temporal levels. A holistic approach is more appropriate than scientific reductionism in its study.

Through multi-scale modeling of interactions between the components of AD, system biologists can identify emergent properties. This leads to hypotheses about AD. Just as molecular biologists are needed to prove these hypotheses experimentally, chemists' expertise in QSAR is needed to design drugs once their targets are identified by the other two groups. Therefore, better communication between all three parties is called for. It allows theoretical chemists to be more selective and efficient in the use of multi-criteria QSAR, facilitating the discovery of a cure for Alzheimer's Disease.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflicts of interest.

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PATIENT CONSENT

Declared none.

ABBREVIATIONS

Aβ	=	β-Amyloid
AChE	=	Acetylcholinesterase
AChEI	=	Inhibitors of AChE

AD	=	Alzheimer's Disease
ANN	=	Artificial Neural Network
APP	=	Amyloid Precursor Protein
BACE-1	=	β-Secretase
BRGNNs	=	Bayesian-Regularized Genetic Neural Networks
CoMFA	=	Comparative Molecular Field Analysis
CoMSIA	=	Comparative Molecular Similarity Index Analysis
DA	=	Application Domain
ER	=	Endoplasmic Reticulum
ETM	=	Electron-Topological Method
FSI	=	Forward Stepwise Inclusion
G	=	Gibbs Free Energy
GA	=	Genetic Algorithm
GFA	=	Genetic Function Approximation
HuAChE	=	Human Acetylcholinesterase
LM	=	Lattice Model
LUMO	=	Lowest Unoccupied Molecular Orbital
MLR	=	Multivariable Linear Regression
NNE	=	Neural Network Ensemble
PLS	=	Partial Least Squares
QSAR	=	Quantitative Structure-Activity Relationships
S	=	Standard Deviation
SA	=	Simulated Annealing
SiRMS	=	Simplex Representation of Molecular Structure
SVM	=	Support Vector Machine
UPR	=	Unfolded Protein Response.

REFERENCES

[1] Goedert, M., Spillantini, M. G. A Century of Alzheimer's Disease. *Science*, **2006**, *314*, 777-781.

[2] McDowell, I. Alzheimer's Disease: Insights from Epidemiology. *Aging: Milano*, **2001**, *13*, 143-162.

[3] Schmidt, R., Neff, F., Lampl, C., Benke, T., Anditsch, M., Bancher, C., Dal-Bianco, P., Reiseker, F., Marksteiner, J., Rainer, M., Kapeller, P., Dodel, R. Therapy of Alzheimer's disease: current status and future development. *Neuropsychiatrie*, **2008**, *22*, 153-171.

[4] Haq, Z.u., Uddin, R. In: *Alzheimer's Disease Pathogenesis-Core Concepts, Shifting Paradigms and Therapeutic Targets*. De La Monte, S., Ed.; InTech, http://www.intechweb.org/, **2011**.

[5] Alcala, M.D.M., Vivas, N. M., Hospital, S., Camps, P., Munoz-Torrero, D., Badia, A. Characterisation of the anticholinesterase activity of two new tacrine-huperzine A hybrids. *Neuropharmacology*, **2003**, *44*, 749-755.

[6] Quinn, D.M., Feaster, S. R., Nair, H. K., Baker, N. A., Radic, Z., Taylor, P. Delineation and decomposition of energies involved in quaternary ammonium binding in the active site of acetylcholinesterase. *J. Am. Chem. Soc.*, **2000**, *122*, 2975-2980.

[7] Bartus, R.T., Dean III, L. D., Beer, B., Lippa, A. S. The cholinergic hypothesis of geriatric memory dysfunction. *Science*, **1982**, *217*, 408-417.

[8] Gualtieri, F., Dei, S., Manetti, D., Romanelli, M. N., Scapecchi, S., Teodori, E. The medicinal chemistry of Alzheimer's and Alzheimer-like diseases with emphasis on the cholinergic hypothesis. *Farmaco*, **1995**, *50*, 489-503.

[9] Racchi, M., Mazzucchelli, M., Porrello, E., Lanni, C., Govoni, S. Acetylcholinesterase inhibitors: novel activities of old molecules. *Pharmacol. Res.*, **2004**, *50*, 441-451.

[10] Hansch, C., Leo, A. *Exploring QSAR. Fundamentals and Applications in Chemistry and Biology*. American Chemical Society: Washington, D. C., **1995**.

[11] Kubinyi, H. *QSAR: Hansch Analysis and Related Approaches*. Wiley-Interscience: New York, **2008**.

[12] Puzyn, T., Leszczynski, J., Cronin. M. T. *Recent Advances in QSAR Studies: Methods and Applications*. First Edition ed. Springer: New York, **2009**.

[13] Trinajstic, N. *Chemical Graph Theory*. CRC Press: Boca Raton (FL), **1992**.

[14] Katritzky, A.R., Lobanov, V. S., Karelson, M. QSPR: the correlation and quantitative prediction of chemical and physical properties from structure. *Chem. Soc. Rev.*, **1995**, *24*, 279-287.

[15] Diudea, M.V. *QSPR/QSAR Studies by Molecular Descriptors*. Nova Science Publishers: New York, **2001**.

[16] Todeschini, R., Consonni, V. *Molecular Descriptors for Chemoinformatics*. Wiley-VCH: Weinheim, **2009**.

[17] Golbraikh, A., Tropsha, A. Beware of q2! *J. Mol. Graphics Modell.*, **2002**, *20*, 269-276.

[18] Hawkins, D.M., Basak, S. C., Mills, D. Assessing Model Fit by Cross Validation. *J. Chem. Inf. Model.*, **2003**, *43*, 579-586.

[19] Goodarzi, M., Duchowicz, P. R., Wu, C. H., Fernández, F. M., Castro, E. A. New Hybrid Genetic Based Support Vector Regression as QSAR Approach for Analyzing Flavonoids-GABA(A) Complexes. *J. Chem. Inf. Model.*, **2009**, *49*, 1475-1485.

[20] Chicu, S.A., Putz, M. V. Köln-Timişoara Molecular Activity Combined Models toward Interspecies Toxicity Assessment *Int. J. Mol. Sci.*, **2009**, *10*, 4474-4497.

[21] Putz, M.V., Putz, A. M., Lazea, M., Lenciu, L., Chiriac, A. Quantum-SAR Extension of the Spectral-SAR Algorithm. Application to Polyphenolic Anticancer Bioactivity *Int. J. Mol. Sci.*, **2009**, *10*, 1193-1214.

[22] Lacrămă, A.M., Putz, M. V., Ostafe, V. A Spectral-SAR Model for the Anionic-Cationic Interaction in Ionic Liquids: Application to Vibrio fischeri Ecotoxicity. *Int. J. Mol. Sci.*, **2007**, *8*, 842-863.

[23] Putz, M.V., Ionaşcu, C., Putz, A. M., Ostafe, V. Alert-QSAR. Implications for Electrophilic Theory of Chemical Carcinogenesis. *Int. J. Mol. Sci*., **2011**, *12*, 5098-5134.

[24] Putz, M.V. Residual-QSAR. Implications for Genotoxic Carcinogenesis. *Chem. Cent. J.*, **2011**, *5*, doi: 10.1186/1752-1153X-1185-1129.

[25] Akula, N., Lecanu, L., Greeson, J., Papadopoulos. V. 3D QSAR studies of AChE inhibitors based on molecular docking scores and CoMFA. *Bioorg. Med. Chem. Lett.*, **2006**, *16*, 6277-6280.

[26] Rawal, R.K.; Prabhakar, Y.S.; Katti, S.B.; De Clercq, E. 2-(Aryl)-3-furan-2-ylmethyl-thiazolidin-4-ones as selective HIV-RT Inhibitors. *Bioorg Med Chem*, **2005**, *13*, (24), 6771-6776.

[27] Yao, X.; Liu, H.; Zhang, R.; Liu, M.; Hu, Z.; Panaye, A.; Doucet, J.P.; Fan, B. QSAR and classification study of 1,4-dihydropyridine calcium channel antagonists based on least squares support vector machines. *Mol Pharm*, **2005**, *2*, (5), 348-356.

[28] Dapson, R.W. Dye-tissue interactions: mechanisms, quantification and bonding parameters for dyes used in biological staining. *Biotech Histochem*, **2005**, *80*, (2), 49-72.

[29] Rodriguez-Morales, S.; Compadre, R.L.; Castillo, R.; Breen, P.J.; Compadre, C.M. 3D-QSAR, synthesis, and antimicrobial activity of 1-alkylpyridinium compounds as potential agents to improve food safety. *Eur J Med Chem*, **2005**, *40*, (9), 840-849.

[30] Maurici, D.; Aardema, M.; Corvi, R.; Kleber, M.; Krul, C.; Laurent, C.; Loprieno, N.; Pasanen, M.; Pfuhler, S.; Phillips, B.; Prentice, D.; Sabbioni, E.; Sanner, T.; Vanparys, P. Carcinogenicity. *Altern Lab Anim*, **2005**, *33 Suppl 1*, 177-182.

[31] Fernández, M., Carreiras, M. C., Marco, J. L., Caballero, J. Modeling of acetylcholinesterase inhibition by tacrine analogs

using Bayesian-regularized Genetic Neral Networks and ensemble averaging. *J. Enzym. Inhib. Med. Ch.,* **2006**, *21*, 647-661.

[32] Dragon *Milano Chemometrics and QSAR Research Group*, http://michem.disat.unimib.it/chm.

[33] Zuang, V.; Alonso, M.A.; Botham, P.A.; Eskes, C.; Fentem, J.; Liebsch, M.; van de Sandt, J.J. Skin irritation and corrosion. *Altern Lab Anim,* **2005**, *33 Suppl 1*, 35-46.

[34] Jung, M., Tak, J., Lee, Y. N., Jung, Y. G. Quantitative structure-activity relationship (QSAR) of tacrine derivatives against acetylcholinesterase (AChE) activity using variable selections. *Bioorg. Med. Chem. Lett.,* **2007**, *17*, 1082-1090.

[35] Vedani, A.; Dobler, M.; Lill, M.A. Virtual test kits for predicting harmful effects triggered by drugs and chemicals mediated by specific proteins. *Altex,* **2005**, *22*, (3), 123-134.

[36] Figueiredo, L.J.; Garrido, F.M.; Kunisawa, V.Y.; Aboim, C.E.; Licks, O.B. A chemometric study of phosphodiesterase 5 inhibitors. *J Mol Graph Model,* **2005**.

[37] Bak, A.; Polanski, J. A 4D-QSAR study on anti-HIV HEPT analogs. *Bioorg Med Chem,* **2006**, *14*, (1), 273-279.

[38] Jojart, B.; Martinek, T.A.; Marki, A. The 3D structure of the binding pocket of the human oxytocin receptor for benzoxazine antagonists, determined by molecular docking, scoring functions and 3D-QSAR methods. *J Comput Aided Mol Des,* **2005**, *19*, (5), 341-356.

[39] Riviere, J.E.; Brooks, J.D. Predicting skin permeability from complex chemical mixtures. *Toxicol Appl Pharmacol,* **2005**, *208*, (2), 99-110.

[40] Saracoglu, M., Kandemirli, F. The Investigation of Structure-Activity Relationships of Tacrine Analogs: Electronic-Topological Method. *Open Med. Chem. J.,* **2008**, *2*, 75-80.

[41] Dimoglo, A.S., Shvets, N. M., Tetko, I. V., Livingstone, D. J. Electronic-topological investigation of the structure-acetylcholinesterase inhibitor activity relationship in the series of n-benzylpiperidine derivatives. *Quant. Struct.-Act. Rel.,* **2001**, *20*, 31-45.

[42] Chen, N., Liu, C. K., Zhao, L. Z., Zhang, H. B. 3D-QSAR study of multi-target-directed AchE inhibitors based on autodocking. *Med. Chem. Res.,* **2010**, 1-12 doi:10.1007/s00044-00010-09516-x Key: citeulike:8597115.

[43] Raska, I., Jr.; Toropov, A. QSAR of the testosterone binding globulin affinity by means of correlation weighting of local invariants of the graph of atomic orbitals. *Bioorg Med Chem,* **2005**, *13*, (24), 6830-6835.

[44] Lin, G. Structure-reactivity Relationships as Probes to Acetylcholinesterase Inhibition Mechanisms by Aryl Carbamates. II. Hammett-Taft Cross-interaction Correlations. *J. Chin. Chem. Soc.-Taip,* **2004**, *51*, 423-429.

[45] Lin, G., Liu, Y. C., Lin, Y. F., Wu, Y. G. Ortho Effects in Quantitative Structure-activity Relationships for Acetylcholinesterase Inhibition by Aryl Carbamates. *J. Enzym. Inhib. Med. Ch.,* **2004**, *19*, 395-401.

[46] Roy, K.K., Dixit, A., Saxena, A. K. An investigation of structurally diverse carbamates for acetylcholinesterase (AChE) inhibition using 3D-QSAR analysis. *J. Mol. Graphics Modell.,* **2008**, *27*, 197-208.

[47] Chadhaery, S.S., Roy, K. K., Saxena, A. K. Consensus Superiority of the Pharmacophore-Based Alignment, Over Maximum Common Substructure (MCS): 3D-QSAR Studies on Carbamates as Acetylcholinesterase Inhibitors. *J. Chem. Inf. Model.,* **2009**, *49*, 1590-1601.

[48] Rackova, L.; Firakova, S.; Kostalova, D.; Stefek, M.; Sturdik, E.; Majekova, M. Oxidation of liposomal membrane suppressed by flavonoids: Quantitative structure-activity relationship. *Bioorg Med Chem,* **2005**, *13*, (23), 6477-6484.

[49] Khlebnikov, A.I.; Schepetkin, I.A.; Quinn, M.T. Quantitative structure-activity relationships for small non-peptide antagonists of CXCR2: Indirect 3D approach using the frontal polygon method. *Bioorg Med Chem,* **2005**.

[50] Liu, J.; Li, Y.; Pan, D.; Hopfinger, A.J. Predicting permeability coefficient in ADMET evaluation by using different membranes-interaction QSAR. *Int J Pharm,* **2005**, *304*, (1-2), 115-123.

[51] Chadhaery, S.S., Roy, K. K., Shakya, N., Saxena, G., Sammi, S. R., Nazir, A., Nath, C., Saxena, A. K. Novel Carbamates as Orally Active Acetylcholinesterase Inhibitors Found to Improve Scopolamine-Induced Cognition Impairment: Pharmacophore-Based Virtual Screening, Synthesis and Pharmaycology. *J. Med. Chem.,* **2010**, *53*, 6490-6505.

[52] Kandemirli, F., Saraçoglu, M., Kovalishyn, V. Human Acetylcholinesterase Inhibitors: Electronic-Topological and Neural Network Approaches to the Structure-Activity Relationships study. *Mini Rev. Med. Chem.,* **2005**, *5*, 479-487.

[53] Haq, Z.u., Mahmood, U., Jehangir, B. Ligand-based 3D-QSAR Studies of Physostigmine Analogs as Acetylcholinesterase Inhibitors. *Chem. Biol. Drug. Des.,* **2009**, *74*, 571-581.

[54] Swaan, P.W.; Ekins, S. Reengineering the pharmaceutical industry by crash-testing molecules. *Drug Discov Today,* **2005**, *10*, (17), 1191-1200.

[55] Araújo, J.Q., de Brito, M. A., Hoelz, L. V., de Alencastro, R. B., Castro, H. C., Rodrigues, C. R., Albuquerque. M. G. Receptor-dependent (RD) 3D-QSAR approach of a series of benzylpiperidine inhibitors of human acetylcholinesterase (HuAChE). *Eur. J. Med. Chem.,* **2011**, *46*, 39-51.

[56] Rogers, D., Hopfinger, A. J. Application of genetic function approximation to quantitative structure-activity-relationships and quantitative structure-property relationships. *J. Chem. Inf. Comput. Sci.,* **1994**, *34*, 854-866.

[57] Snyder, R.D.; Smith, M.D. Computational prediction of genotoxicity: room for improvement. *Drug Discov Today,* **2005**, *10*, (16), 1119-1124.

[58] Pratuangdejkul, J.; Schneider, B.; Jaudon, P.; Rosilio, V.; Baudoin, E.; Loric, S.; Conti, M.; Launay, J.M.; Manivet, P. Definition of an uptake pharmacophore of the serotonin transporter through 3D-QSAR analysis. *Curr Med Chem,* **2005**, *12*, (20), 2389-2406.

[59] Netzeva, T.I.; Worth, A.; Aldenberg, T.; Benigni, R.; Cronin, M.T.; Gramatica, P.; Jaworska, J.S.; Kahn, S.; Klopman, G.; Marchant, C.A.; Myatt, G.; Nikolova-Jeliazkova, N.; Patlewicz, G.Y.; Perkins, R.; Roberts, D.; Schultz, T.; Stanton, D.W.; van de Sandt, J.J.; Tong, W.; Veith, G.; Yang, C. Current status of methods for defining the applicability domain of (quantitative) structure-activity relationships. The report and recommendations of ECVAM Workshop 52. *Altern Lab Anim,* **2005**, *33*, (2), 155-173.

[60] Gerner, I.; Liebsch, M.; Spielmann, H. Assessment of the eye irritating properties of chemicals by applying alternatives to the Draize rabbit eye test: the use of QSARs and *in vitro* tests for the classification of eye irritation. *Altern Lab Anim,* **2005**, *33*, (3), 215-237.

[61] Gieleciak, R.; Magdziarz, T.; Bak, A.; Polanski, J. Modeling robust QSAR. 1. Coding molecules in 3D-QSAR--from a point to surface sectors and molecular volumes. *J Chem Inf Model,* **2005**, *45*, (5), 1447-1455.

[62] Yazal, J.E., Rao, S. N., Mehl, A., Slikker Jr., W. Prediction of Organophosphorus Acetylcholinesterase Inhibition Using Three-Dimensional Quantitative Structure-Activity Relationship (3D-QSAR) Methods. *Toxicol. Sci.,* **2001**, *63*, 223-232.

[63] Clare, B.W.; Supuran, C.T. Predictive flip regression: a technique for QSAR of derivatives of symmetric molecules. *J Chem Inf Model,* **2005**, *45*, (5), 1385-1391.

[64] Roy, K.; Leonard, J.T. QSAR analyses of 3-(4-benzylpiperidin-1-yl)-N-phenylpropylamine derivatives as potent CCR5 antagonists. *J Chem Inf Model,* **2005**, *45*, (5), 1352-1368.

[65] Zhao, J.S., Wang, B., Dai, Z. X., Wang, X. D., Kong, L. R., Wang, L. S. 3D-quantitative structure-activity relationship study of organophosphate compounds. *Chinese Sci. Bull.,* **2004**, *49*, 240-245.

[66] Kuzmin, V.E., Muratov, E. N., Artemenko, A. G., Varlamova, E. V., Gorb, L., Wang, J., Leszczynski, J. Consensus QSAR Modeling of Phosphor-containing Chiral AChE Inhibitors. *QSAR Comb. Sci.,* **2009**, *28*, 664-677.

[67] Liu, A., Guang, H. M. , Zhu, L. Y. , Du, G. H. , Lee, S. M. Y. , Wang, Y. T. 3D-QSAR analysis of a new type of acetylcholinesterase inhibitors. *Sci. China Ser. C* **2007**, *50*, 726-730.

[68] Wei, H.Y.; Tsai, K.C.; Lin, T.H. Modeling ligand-receptor interaction for some MHC class II HLA-DR4 peptide mimetic inhibitors using several molecular docking and 3D QSAR techniques. *J Chem Inf Model,* **2005**, *45*, (5), 1343-1351.

[69] Sheng, R., Shen, Y. H., Lin, X., Luo, Y., Fan, Y. J., Li, J. Y., Xia, H. R., Hu, Y. Z. 3D-QSAR studies on AChE inhibitory activities of 2-phenoxy-indan-1-one derivatives. *Chin. J. Med. Chem.,* **2007**, *17*, 348-353.

[70] Shen, L.L., Liu, G. X., Tang, Y. Molecular docking and 3D-QSAR studies of 2-substituted 1-indanone derivatives as acetylcholinesterase inhibitors. *Acta Pharm. Sinic.*, **2007**, *28*, 2053-2063.

[71] Papa, E.; Villa, F.; Gramatica, P. Statistically validated QSARs, based on theoretical descriptors, for modeling aquatic toxicity of organic chemicals in Pimephales promelas (fathead minnow). *J Chem Inf Model*, **2005**, *45*, (5), 1256-1266.

[72] Sharma, A., Mishra, A., Prajapat, R. P., Jain, S., Bhandari, A. QSAR analysis of indanone and aurone derivatives as acetylcholinesterase inhibitors. *J. Chem. Pharm. Res.*, **2010**, *2*, 682-689.

[73] Debnath, A.K. Application of 3D-QSAR techniques in anti-HIV-1 drug design--an overview. *Curr Pharm Des*, **2005**, *11*, (24), 3091-3110.

[74] Burlet, S.; Pietrancosta, N.; Laras, Y.; Garino, C.; Quelever, G.; Kraus, J.L. Prospects for the resistance to HIV protease inhibitors: current drug design approaches and perspectives. *Curr Pharm Des*, **2005**, *11*, (24), 3077-3090.

[75] Sippl, W., Contreras, J. M., Parrot, I., Rival, Y. M., Wermuth, C. G. Structure-based 3D QSAR and design of novel acetylcholinesterase inhibitors. *J. Comput. Aided Mol. Des.*, **2001**, *15*, 395-410.

[76] Lin, G.; Chen, G.H.; Yeh, S.C.; Lu, C.P. Probing the peripheral anionic site of acetylcholinesterase with quantitative structure activity relationships for inhibition by biphenyl-4-acyoxylate-4'-N-Butylcarbamates. *J Biochem Mol Toxicol*, **2005**, *19*, (4), 234-243.

[77] Pasha, F.A.; Srivastava, H.K.; Singh, P.P. Comparative QSAR study of phenol derivatives with the help of density functional theory. *Bioorg Med Chem*, **2005**, *13*, (24), 6823-6829.

[78] Gonzalez-Diaz, H.; Uriarte, E. Proteins QSAR with Markov average electrostatic potentials. *Bioorg Med Chem Lett*, **2005**, *15*, (22), 5088-5094.

[79] Spassova, D.P., Singh, A. K. QSAR for acetylcholinesterase inhibition and toxicity of two classes of phosphoramidothioates. *SAR QSAR Environ. Res.*, **2001**, *11*, 453-471.

[80] Aptula, A.O.; Patlewicz, G.; Roberts, D.W. Skin sensitization: reaction mechanistic applicability domains for structure-activity relationships. *Chem Res Toxicol*, **2005**, *18*, (9), 1420-1426.

[81] Medina-Franco, J.L.; Golbraikh, A.; Oloff, S.; Castillo, R.; Tropsha, A. Quantitative structure-activity relationship analysis of pyridinone HIV-1 reverse transcriptase inhibitors using the k nearest neighbor method and QSAR-based database mining. *J Comput Aided Mol Des*, **2005**, *19*, (4), 229-242.

[82] Haq, Z.u., Wellenzohn, B., Tonmunphean, S., Khalid, A., Choudhary, M. I., Rode, B. M. 3D-QSAR Studies on Natural Acetylcholinesterase inhibitors of Sarcococca saligna by comparative molecular field analysis (CoMFA). *Bioorg. Med. Chem. Lett.*, **2003**, *13*, 4375-4380.

[83] Li, Y.; Liu, J.; Pan, D.; Hopfinger, A.J. A Study of the Relationship between Cornea Permeability and Eye Irritation Using Membrane-Interaction QSAR Analysis. *Toxicol Sci*, **2005**, *88*, (2), 434-446.

[84] Chiou, S.Y., Lai, G. W., Tsai, Y. H., Lin, L. Y., Lin, G. Qsar for Acetylcholinesterase and Butyrylcholinesterase Inhibition by Cardiovascular Drugs and Benzodiazepines. *Med. Chem. Res.*, **2005**, *14*, (5), 297-308.

[85] Solomon, K.A., Sundararajan, S., Abirami, V. QSAR Studies on N-aryl Derivative Activity Towards Alzheimer's Disease. *Molecules*, **2009**, *14*, 1448-1455.

[86] Kotsikorou, E.; Song, Y.; Chan, J.M.; Faelens, S.; Tovian, Z.; Broderick, E.; Bakalara, N.; Docampo, R.; Oldfield, E. Bisphosphonate inhibition of the exopolyphosphatase activity of the Trypanosoma brucei soluble vacuolar pyrophosphatase. *J Med Chem*, **2005**, *48*, (19), 6128-6139.

[87] Gupta, S., Fallarero, A., Vainio, M. J., Saravanan, P., Puranen, J. S., Järvinen, P., Johnson, M. S., Vuorela, P. M., Mohan, C. G. Molecular Docking Guided Comparative GFA, G/PLS, SVM and ANN Models of Structurally Diverse Dual Binding Site Acetylcholinesterase Inhibitors. *Mol. Inf.*, **2011**, *30*, 689-706.

[88] Lu, S.H., Wu, W. J., Liu, H. L., Zhao, J. H., Liu, K. T., Chuang, C. K., Lin, H. Y., Tsai, W. B., Ho, Y. The discovery of potential acetylcholinesterase inhibitors: A combination of pharmacophore modeling, virtual screening, and molecular docking studies. *J. Biomed. Sci.*, **2011**, *18*, doi:10.1186/1423-0127-1118-1188.

[89] Asadabadi, E.B., Abdolmaleki, P., Barkooie, S. M. H., Jahandideh, S., Rezaei, M. A. A combinatorial feature selection approach to describe the QSAR of dual site inhibitors of acetylcholinesterase. *Comput. Biol. Med.*, **2009**, *39*, 1089-1095.

[90] Keizers, P.H.; de Graaf, C.; de Kanter, F.J.; Oostenbrink, C.; Feenstra, K.A.; Commandeur, J.N.; Vermeulen, N.P. Metabolic regio- and stereoselectivity of cytochrome P450 2D6 towards 3,4-methylenedioxy-N-alkylamphetamines: in silico predictions and experimental validation. *J Med Chem*, **2005**, *48*, (19), 6117-6127.

[91] Tsai, K.C., Chen, Y. C., Hsiao, N. W., Wang, C. K., Lin, C. L., Lee, Y. C., Li, M. Y., Wang, B. H. A comparison of different electrostatic potentials on prediction accuracy in CoMFA and CoMSIA studies. *Eur. J. Med. Chem.*, **2010**, *45*, 1544-1551.

[92] Ersmark, K.; Nervall, M.; Hamelink, E.; Janka, L.K.; Clemente, J.C.; Dunn, B.M.; Blackman, M.J.; Samuelsson, B.; Aqvist, J.; Hallberg, A. Synthesis of malarial plasmepsin inhibitors and prediction of binding modes by molecular dynamics simulations. *J Med Chem*, **2005**, *48*, (19), 6090-6106.

[93] Procopio, A.; Alcaro, S.; Cundari, S.; De Nino, A.; Ortuso, F.; Sacchetta, P.; Pennelli, A.; Sindona, G. Molecular modeling, synthesis, and preliminary biological evaluation of glutathione-S-transferase inhibitors as potential therapeutic agents. *J Med Chem*, **2005**, *48*, (19), 6084-6089.

[94] Gasparini, L., Racchi, M., Binetti, G., Trabucchi, M., Solerte, S. B., Alkon, D. Peripheral markers in testing pathophysiological hypotheses and diagnosing Alzheimer's disease. *Faseb J.*, **1998**, *12*, 17-34.

[95] Racchi, M., Govoni, S. Rationalizing a pharmacological intervention on the amyloid precursor protein metabolism. *Trends. Pharmacol. Sci.*, **1999**, *20*, 418-423.

[96] Fu, H.J., Li, W. M., Luo, J. L., Lee, N. T. K., Li, M. T., Tsim, K. W. K., Pang, T. P., Youdim, M. B. H., Han, Y. F. Promising anti-Alzheimer's dimer bis(7)-tacrine reduces β-amyloid generation by directly inhibiting BACE-1 activity. *Biochem. Biophys. Res. Commun.*, **2008**, *366*, 631-636.

[97] Fu, H., Li, W., Lao, Y., Luo, J., Lee, N. T., Kan, K. K., Tsang, H. W., Tsim, K. W., Pang, Y., Li, Z., Chang, D. C., Li, M., Han, Y. Bis(7)-tacrine attenudates beta amyloid-induced neuronal apoptosis by regulating L-type calcium channels. *J. Neurochem.*, **2006**, *98*, 1400-1410.

[98] Zhang, H.Y. One-compound-multiple-targets strategy to combat Alzheimer's disease. *FEBS Lett.*, **2005**, *79*, 5260-5264.

[99] Buehler, L.K. Advancing drug discovery-beyond design. *PharmaGenomics*, **2004**, *4*, 24-26.

[100] Keith, C.T., Borisy, A. A., Stockwell, B. R. Multicomponent therapeutics for networked systems. *Nat. Rev. Drug Discov.*, **2005**, *4*, 71-78.

[101] Barnham, K.J., Masters, C. L., Bush, A. I. Neurodegenerative diseases and oxidative stress. *Nat. Rev. Drug Discov.*, **2004**, *3*, 205-214.

[102] Cummings, J.L. Alzheimer's Disease. *N. Engl. J. Med.*, **2004**, *351*, 56-67.

[103] Mattson, M.P. Pathways towards and away from Alzheimer's disease. *Nature*, **2004**, *430*, 631-639.

[104] Brown, D.R., Kozlowski, H. Biological inorganic and bioinorganic chemistry of neurodegeneration based on prion and Alzheimer diseases. *Dalton Trans.*, **2004**, *13*, 1907-1917.

[105] Youdim, M.B., Buccafusco, J. J. Multi-functional drugs for various CNS targets in the treatment of neurodegenerative disorders. *Trends Pharmacol. Sci.*, **2005**, *26*, 27-35.

[106] Katayama, T., Imaizumi, K., Manabe, T., Hitomi, J., Kudo, T., Tohyama, M. Induction of neuronal death by ER stress in Alzheimer's disease. *J. Chem. Neuroanat.*, **2004**, *28*, 67-78.

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