# Discovery of Anti-Alzheimer Agents: Current Ligand-Based Approaches toward the Design of Acetylcholinesterase Inhibitors

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**Abstract:** Alzheimer's disease (AD) is a neurodegenerative disorder characterized by progressive dementia and loss of cognitive abilities. Until now, AD remains incurable. The principal biological target for AD therapy is acetylcholinesterase (AChE). Thus, the search for new drug candidates like AChE inhibitors constitutes an essential part for the discovery of more potent anti-AD agents. In general terms, rational drug design methodologies have played a decisive role. The present work is focused on the current state of the Ligand-Based Drug Design (LBDD) methods which have been applied to the elucidation of new molecular entities with high anti-AChE activity. Also, as a contribution to this field, we suggest a promising fragment-based approach for the search and prediction of new AChE inhibitors and for the fast and efficient extraction of substructural alerts which are responsible for the anti-AChE activity.

**Keywords:** AChE inhibitors, QSAR, 3D-QSAR, linear discriminant analysis, fragments.

## 1. INTRODUCTION

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by progressive dementia, loss of cognitive abilities. Although AD is associated to the deposition of fibrillar amyloid proteins as intraneuronal neurofibrillary tangles, extracellular amyloid plaques and vascular amyloid deposits, the drugs commercially available are acetylcholinesterase (AChE) inhibitors (Fig. **1**). This fact is because reduction in the activity of the cholinergic neurons is a well-known feature of AD [1]. AChE inhibitors are employed to reduce the rate at which acetylcholine (ACh) is broken down, thereby increasing the concentration of ACh in the brain and combating the loss of ACh caused by the death of cholinergic neurons [2]. Thus, AChE continues being the principal target for the discovery of new compounds which could be employed against this primary degenerative dementia. Until now, AD remains incurable. Most often, it is diagnosed in people over 65 years of age [3], although the less-prevalent early-onset Alzheimer's can occur much earlier. An epidemiological study showed that an estimated between 10 to 15 new cases per thousand people and per year could suffer from any form of dementia and between 5 to 8 of these cases are due to the presence of AD [4]. For this reason, the search for new AChE inhibitors constitutes an essential part of all the efforts of the scientific community to discovery more efficient anti-AD agents.

All drug design methodologies which can be applied to the search AChE inhibitors can be divided in two great groups. The first group is constituted by methodologies which are based on the knowledge of the three-dimensional structure of the biological receptor. These methodologies are called Structure-Based Drug Design (SBDD). On the other hand, the second group of methodologies relies on the knowledge of molecules that bind to the biological target of interest, and these molecules can be used to generate a pharmacophore model, which defines the minimum necessary structural characteristics a molecule must possess in order to bind to the target. Alternatively, a Quantitative Structure-Activity Relationship (QSAR) in which a correlation between calculated properties of molecules and their experimentally determined biological activity can be derived. These QSA relationships in turn can be used to predict the activity of new analogs and can be used in an indirect way to have an idea of pharmacophore. This work is focused on the current state of all computational approaches related to ligand-based drug design (LBDD) methodologies toward the discovery of new AChE inhibitors. In this sense we will rely on those works which have made significant contributions to the search for new compounds and chemical families, and where the computational methods mentioned above have played a decisive role. Also, in order to make our contribution to this field, we suggest a promising fragment-based approach for the design of new AChE inhibitors and for the fast and efficient extraction of substructural alerts responsible for anti-AChE activity.

## 2. LIGAND-BASED DRUG DESIGN METHODOLOGIES

Recently, several families of compounds have been synthesized and evaluated as possible AChE inhibitors [5-12]. In the last 15 years, some important works applying LBDD methodologies (Table **1**) have been reported for the design of new AChE inhibitors [13-21]. Some of them have been combined with structure based drug design methodologies which have played a determinant role for the study of the three-dimensional structure of AChE [13, 19, 22]. In essence, these methods have been based on classical

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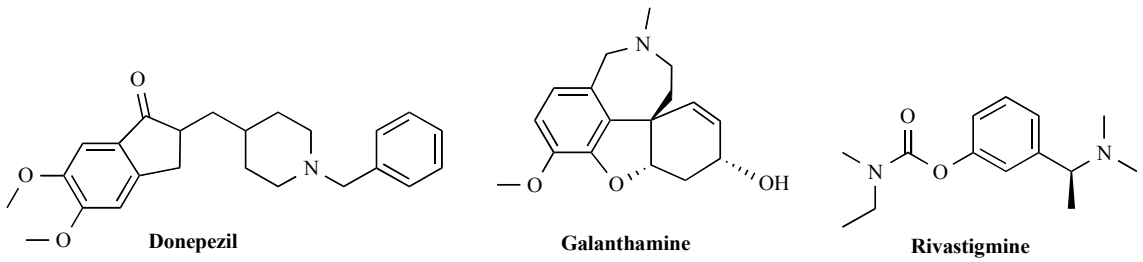


**Fig. (1).** Drugs like AChE inhibitors which have been currently approved for the treatment of AD.

QSAR or 3D-QSAR techniques such as Comparative Molecular Field Analysis (CoMFA), Comparative Molecular Similarity Indices Analysis (CoMSIA), GRID/GOLPE procedures and Ligand-Based Virtual Screening (LB-VS) [23-26], and generally they have been supported by methods such as Multiple Linear Regression (MLR) [24, 27], though it has been demonstrated that other innovative methodologies such as Complex Network theory [28-34], Artificial Neural Networks (ANN) analysis [35-38], Artificial Intelligence (AI) and Supporting Vector Machine (SVM) [38-43] have permitted along with MLR, to optimize the synthesis and evaluation of diverse families of compounds with a desired activity.

A good example of the application of LBDD methodologies for the design of AChE inhibitors is the work based on a receptor-dependent (RD) 3D-QSAR approach of a series of benzylpiperidine as AChE inhibitors [13]. Thus, RD-3D-QSAR models based on a series of 60 benzylpiperidine as AChE have been developed. The best two models (Table **1**), were validated by a combined Genetic Algorithm-Partial Least Squares (GA-PLS) approach. Residues of the aromatic gorge (Tyr341 and Trp439) and catalytic triad (His447) in AChE were related to both equations showing the consistency of these models with the SAR. Based on those models, four new benzylpiperidine derivatives were proposed and then, the inhibitory concentration at 50% predicted for each molecule (Fig. **2**). The good predicted potency of the benzylpiperidine derivative, **IIa** ($IC_{50}$ around sub-picomolar order), indicates that it could be a potential candidate as a new AChE inhibitor.

On the other hand, a virtual screening discovery of new AChE inhibitors has been issued from the CERMN Chemical Library [21]. The purpose of this research was to design new AChE inhibitors and, at the same time, to protect neurons from β-amyloid toxicity, *i.e.*, inhibitors interacting with the catalytic anionic subsite as well as with the peripherical anionic site of AChE. Thus, a virtual screening of the Centre d'Etudes et de Recherche sur le Médicament de Normandie (CERMN) chemical library has been carried out. [44]. Two complementary approaches were applied, *i.e.*, a LB-VS and a structure-based virtual screening (SB-VS). Each screening led to the selection of different compounds, but only two were present in both screening results. *In vitro* tests on AChE showed that one of those compounds presented a very good inhibition activity (Fig. **3**), of the same order as donepezil. This result showed the real complementary of both methods for the discovery of new ligands.

**Table 1. Some of the Most Promising Works for the Discovery of AChE Inhibitors**

| Methodology | Family | Technique [a] | *N*(T/P) | Statistical indices | Authors | Ref. |
|---|---|---|---|---|---|---|
| RD-3D-QSAR [b] | Benzylpiperidine | *GA-PLS* | 47/13 | $r^2$=0.900, $q^2$=0.753, *SEE*=1.301, *s*=0.431 [d] | *Araujo et al.* | [13] |
| 3D-QSAR (CoMFA) | Physostigmine analogues | *PLS* | 32/8 | $r^2$=0.989, $q^2$=0.762, *SEE* =0.089, *F*=388.64, *SEP*=0.399, $r^2$=0.730 [d] | *Ul-Haq et al.* | [14] |
| 3D-QSAR (CoMSIA) | Physostigmine analogues | *PLS* | 32/8 | $r^2$=0.988, $q^2$=0.754, *SEE* =0.095 *F*=360.00, *SEP*=0.429, $r^2$=0.720 [d] | *Ul-Haq et al.* | [14] |
| Classical QSAR | *N*-aryl derivatives | *GA-MLR* | 53/26 | $r^2$=0.862, $q^2$=0.803, *LOF*=0.021, $r^2$=0.857 [d] | *Solomon et al.* | [15] |
| 3D-QSAR (GRID/GOLPE) [b] | Aminopyridazine compounds | *PLS* | 24/24 | $r^2$=0.990, $q^2$=0.910, *SDEP*=0.409, *SDEP*=0.0.440 [d] | *Sippl et al.* | [19] |
| LB-VS | Multiple families of compounds | - | 6626/29/10 [c] | 34% [e] | *Sopkova et al.* | [21] |

[a]Statistical techniques used to generate the models. [b]Only the best models are presented. [c]Referring to the total number of compounds in the chemical library, number of compounds biologically tested and predicted by LB-VS, and number of compounds highly active. [d]Statistical indices used for the test set. [e]Percentage for the success rate in LB-VS. ***N*(T/P)**: number of compounds in training and prediction series, respectively; **RD**: Receptor Dependent; **LB-VS**: Ligand-Based Virtual Screening; **GA**: Genetic Algorithm; **PLS**: Partial Least Squares; **MLR**: Multiple Linear Regression; $r^2$: coefficient of determination; $q^2$: cross-validation coefficient; ***SEP***: Standard Error of Prediction; ***F***: *F*-test ratio; ***SEE***: Standard Error of Estimate; ***s***: standard deviation; ***SDEP***: Standard Deviation of Error of Prediction.

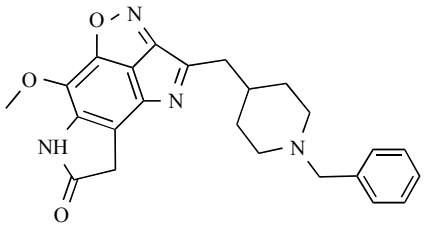

**Fig. (2).** Structure of **IIa** as possible highly active AChE inhibitor.

## 3. DESIGNING NEW INHIBITORS USING SUBSTRUCTURAL APPROACHES

Even though the current LBDD methodologies have had a vital importance for the design of new molecular pattern which will be able to inhibit AChE, they suffer from two disadvantages. Firstly, these methodologies employ only homogenous series of compounds. This drawback makes almost impossible to explore in a deeper way, the molecular patterns related to AChE inhibitors to a higher level of chemical diversity and complexity. This problem may be resolved with techniques such as LB-VS. However, this last technique generates a second inconvenient: rigorous computational requirements in terms of time of calculation and condition of the processors. In an effort to overcome this problem, we present a fragment-based approach using a heterogeneous database of compounds for the efficient and fast design of AChE inhibitors. Herein, we developed a QSAR discriminant model in order to extract the principal fragments responsible for the activity, and in so doing then suggest new molecular entities as possible potent AChE inhibitors.

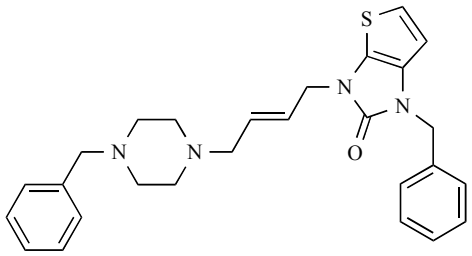

**Fig. (3).** Structure of compound **3** as the most potent AChE inhibitor.

### 3.1. Methods

#### *3.1.1. Functional Group Counts*

These are types of descriptors which express certain fragmental features. Functional group counts (FGC) constitute simple molecular descriptors defined as the number of specific functional groups in a molecule and they are also calculated from the molecular composition and atom connectivity. The functional groups defined here, are those which are traditionally used in Organic Chemistry. Functional group counts are descriptors that keep relation with the indicator variables in a Free-Wilson analysis [23].

#### *3.1.2. Atom-Centered Fragments*

Atom-centered fragments (ACF) have been demonstrated to be very useful descriptors, and have been employed in some QSAR studies [45-48]. They provide important information about hydrophobic and dispersive interactions which are involved in biological processes such as transport and distribution of drugs through the membrane. Also, they give information about hydrophobic and dispersive interactions [47]. ACF are simple molecular descriptors which are defined as the number of specific atom types in a molecule. They are calculated from the molecular composition and atom connectivities. Each type of atom in the molecule is described in terms of its neighboring atoms. Hydrogen and halogen atoms are classified by the hybridization and oxidation states of the carbon atom to which they are attached. For hydrogen atoms, heteroatoms which are attached to a carbon in $\alpha$-position are further considered. Carbon atoms are classified by their hybridization state and depending on whether their neighbors are carbon or heteroatoms.

#### *3.1.3. Spectral Moments of the Bond Adjacency Matrix*

The approach that encloses the calculation of the spectral moments of the bond adjacency matrix, is known as the TOPS-MODE (**TOP**ological **S**ubstructural **MO**lecular **DE**sign) approach and it has been applied for the description of some physicochemical properties of organic compounds [49-51], in Quantitative Structure-Toxicity Relationships (QSTR) [52-57], and also have been reported for the modeling of pharmacological activities [58-60]. The theoretical background about the spectral moments of bond adjacency matrix has been described in many papers; however, we will focus our explanation on the most important aspects. In this approach the molecular structure is encoded by mean of the edge adjacency matrix ***E*** (commonly called the bond adjacency matrix ***B***). The ***E*** or ***B*** matrix is a square table of order $m$ (the number of chemical bonds in the molecule). The elements of this matrix ($e_{ij}$) are equal to 1 if bonds $i$ and $j$ are adjacent (which means that $i$ and $j$ are incident in the same vertex or atom) and 0 otherwise. In order to encode information of heteroatoms, the TOPS-MODE approach uses $\boldsymbol{E}(w_{ij})$ weighted matrices instead of ***E***. The weights ($w_{ij}$) are chemically meaningful numbers such as bond distances, bond dipoles, or mathematical expressions involving atomic weights. The weights are introduced in the main diagonal of matrix $\boldsymbol{E}(w_{ij})$. Then, the spectral moments of this matrix can be used as molecular fingerprints in QSAR studies for the codification of molecular structures. By mathematical definition, the term spectral moments must be understood as the sum of the elements ($e_{ij}$) in the natural powers of $\boldsymbol{E}(w_{ij})$ [61-63]. Then the spectral moment of order $k$ ($\mu_k$) is the sum of the main diagonal elements ($e_{ii}$) of matrix $\boldsymbol{E}(w_{ij})^k$. The spectral moments of the bond matrix are defined as:

$$\mu_k = \mathrm{Tr}(E^k) = \sum_{i=1}^{s} (e_{ii})^k \qquad (1)$$

where **Tr** means the trace of the matrix, that is the sum of the diagonal entries of the matrix and the elements $(e_{ii})^k$ are the diagonal entries of the $k$th power of the bond matrix. The spectral moments of the bond adjacency matrix have topological nature. The principal advantage of these descriptors is the possibility to calculate the relative contribution of any fragment to the desired activity [64]. That is possible because they can be expressed as linear

combinations of the number of times in which a fragment appears in the molecule. Another advantage of the spectral moments of the bond adjacency matrix is the ability to explain in a reasonable way, a considerable part of spatial phenomena [65]. That is a particular characteristic of topographical descriptors.

#### *3.1.4. Selection of the Data Set: Calculation of the Descriptors and Development of the Model*

The data set was formed by 1326 compounds (see Suppl. Inf. 1 for codes and/or names), 820 AChE inhibitors with $IC_{50} \leq 50\mu M$ [66], being $IC_{50}$ the concentration of the compound which decreases the enzymatic activity by 50%. We had also 506 drugs which have been reported in the Merck Index. These drugs present other activities that do not include anti-AChE activity and have been used as inactive [67]. The FGC and ACF were calculated using DRAGON version 5.3 [68]. The $\mu_k$ descriptors (from order 1 to 15), were calculated using MODESLAB version 1.5 [69]. In this case, the spectral moments were weighted by physicochemical properties such as van der Waals atomic radii, Gasteiger-Marsili charges, atomic weights and by the Abraham molar refractivity. Linear Discriminant Analysis (LDA) has been a technique applied in many QSAR studies [70-75], and it was used to develop the QSAR classifier model. In order to perform a rigorous design, the dataset was randomly split into two series: training and prediction series. The training set was formed by 996 compounds, 616 AChE inhibitors and 380 inactive compounds, and the external prediction set was composed by 330 compounds (~24.9% of the total data), 204 AChE inhibitors and 126 inactive compounds. The general expression for the developed LDA model is as follows:

$$A_{AChE} = a_0 + \sum b_k \cdot \mathrm{FGC} + \sum c_k \cdot \mathrm{ACF} + \sum d_k \cdot \mu_k \quad (2)$$

where $A_{AChE}$ is not the probability, but a real value score that predicts the propensity of a compound to have anti-AChE activity. The term denoted as $a_0$ is the constant, while $b_k$, $c_k$, and $d_k$ are the corresponding coefficients of the variables in the model. The discriminant function was obtained by employing the LDA modules of STATISTICA [76]. The variables which were included in the discriminant model were selected using a forward stepwise as the variable selection strategy. The selection of the best model was subjected also, to the principle of parsimony. Thus, the model with high statistical significance, but having as few parameters as possible, was chosen.

The statistical quality of the model was determined, examining some statistical indices such as the Wilks' lambda ($\lambda$), the square of Mahalanobis distance ($D^2$), the chi-square ($\chi^2$), and the $p$-level. Another important aspect is that, the compounds used in the prediction set were never used to develop the discrimination function. On the other hand, to confirm the quality of the model, and to validate it, we employed other statistics such as: the sensitivity (*sens*) – *i.e.* the ability for the classification of active cases; the specificity (*spec*)– *i.e.* the ability for the classification of inactive cases; and the accuracy (*acc*)– *i.e.* the overall predictivity. These statistical indices were calculated for both, training and prediction series according to the following equations:

$$sens = \frac{\mathrm{TP}}{\mathrm{TA}} \cdot 100\% \quad (3)$$

$$spec = \frac{\mathrm{TN}}{\mathrm{TI}} \cdot 100\% \quad (4)$$

$$acc = \frac{\mathrm{TP} + \mathrm{TN}}{\mathrm{TA} + \mathrm{TI}} \cdot 100\% \quad (5)$$

where **TP** means the cases (compounds) classified correctly by the model as active, **TA** the total active compounds, **TN** means the cases classified correctly by the model as inactive and **TI** represents the total inactive compounds.

The sensitivity and the specificity can describe adequately the quality of a model. However, these two statistical indices have disadvantages. The most important one is that they cannot provide information about how many times the probabilities indicate that a compound, observation or case will be predicted more as positive (active) than negative (inactive), and this is very important since it confirms together with the positive predictive value if a given case is active. However, that information can be provided by a Receiver–Operating Characteristic (ROC) analysis. ROC is a classic methodology from signal detection theory [77]. The ROC curve is created by plotting the true-positive rate against false-positive rate, or sensitivity against (1 − specificity). The ROC curve going along the diagonal from bottom left to upper right represents pure-chance performance. In the following, we will see that the present QSAR derived model has an appropriate statistical quality taking into account all these statistical indices, alike other QSAR reports in the literature [70, 72, 78-84].

### 3.2. Results and Discussion

#### *3.2.1. Discriminant Model*

Following the strategy outlined before, the resulting best-fit model derived for predicting the AChE inhibitory activity contains 13 descriptors. This model has the three types of descriptors referred to above, *i.e.* functional group counts, topological distances and spectral moments of the bond adjacency matrix, and it is given below together with the statistical parameters of the LDA analysis.

$$\begin{aligned} A_{AChE} = {} & +0.367\,\mathbf{Crt} - 1.550\,(\mathbf{R\#C\text{-}}) + 2.324\,(\mathbf{ArOCON}) + 0.332\,(\mathbf{ARCO}) + 0.833\,(\mathbf{Pyrid}) \\ & - 0.280\,(\mathbf{C\text{-}001}) + 0.475\,(\mathbf{C\text{-}028}) - 1.453\,(\mathbf{C\text{-}030}) + 0.666\,(\mathbf{N\text{-}068}) + 1.219\,\mu_1^{(\mathrm{Gas})} \\ & - 1.489 \cdot 10^{-4}\,\mu_2^{(\mathrm{Ato})} + 1.288 \cdot 10^{-3}\,\mu_4^{(\mathrm{Van})} - 6.106 \cdot 10^{-6}\,\mu_9^{(\mathrm{Ab\text{-}R2})} - 1.018 \\ & N = 996 \quad \lambda = 0.567 \quad D^2 = 3.227 \quad \chi^2 = 559.97 \quad p < 0.001 \end{aligned} \quad (6)$$

In this equation, the descriptor **Crt** is the number of tertiary $Csp^3$ atoms which are present in rings, while descriptor **R#C-** means the number of non-terminal C(sp) atoms, **ArOCON** indicates the presence or absence of aromatic carbamates (or thiocarbamates), **ArCO** is the number of aromatic carbonyls, **Pyrid** is related to the number of pyridine rings, **C-001** represents the number of primary $Csp^3$ atoms attached to an aliphatic group, **C-028** indicates the number of fragments in which an aromatic carbon atom is bound to another aromatic carbon, to a $Csp^3$

atom and to an electronegative atom (O, N, S, Se, or halogens) through an "aromatic bond", **C-030** means the presence or absence of fragments in which an aromatic carbon is bound to two electronegative atoms through "aromatic bonds", and **N-068** indicates the number of tertiary aliphatic amine fragments; whereas descriptor $\mu_1^{(Gas)}$ is the spectral moment of order 1 weighted by Gasteiger-Marsili charges, $\mu_2^{(Ato)}$ is the spectral moment of order 2 weighted by the atomic masses, $\mu_4^{(Van)}$ represents the spectral moment of order 4 weighted by the sum of the van der Waals atomic radii, and $\mu_9^{(Ab\text{-}R2)}$ is the spectral moment of order 9 weighted by the Abraham molar refractivity.

It is necessary to point out that with 13 descriptors we raised a good performance of the model. The increment in the number of variables did not improve the quality of the model in a significant way, while a diminution in the number of variables, led to an appreciable lack of quality and predictive ability. Judging also from the statistical indices in Eq. **6**, the model has a good quality. The sensitivity of the model is 82.79% and the specificity 87.37% in the training series, for an accuracy of 84.54%. We examined all the compounds, searching for misclassified cases because they can be outliers and this fact can have influence in the quality of a model. We checked the Mahalanobis distance of each molecule with respect to the two centroids of both groups (active and inactive compounds). Generally, in the case of abnormal values, the case should be excluded from the model. In our case, no outliers were detected and the deletion of the misclassified compounds did not improve the quality of the model. In order to validate our model, we took into consideration the sensitivity, the specificity and the accuracy in the prediction series. The sensitivity of the model in the prediction series is 82.35% and the specificity is 86.51%, for an accuracy of 83.94%. The names or codes, and probabilities as AChE inhibitors of each compound (expressed as percentages) are recorded in the supplementary material file (Suppl. Inf. **2**). The ROC curve had a vital role to demonstrate the quality and the predictivity of the model. The areas under the ROC curves are 0.91 and 0.89 for the training and prediction series, respectively (Fig. **4**). These values of area can be interpreted as follows: such value of area (=0.91) means that a randomly selected compound or case from the active group (AChE inhibitor) will have a larger value of probability than a randomly selected compound or case from the inactive group, 91% of the times. A similar deduction can be made from the area under the ROC curve for prediction series. Altogether, this proves that our model is not a random classifier because the areas under the ROC curves are different and statistically significant from those obtained by random classifiers (area = 0.5).

### *3.2.2. Extracting Substructural Alerts Responsible for the Anti-AChE Activity*

Here, it should be emphasized that the model obtained by the present approach has two principal advantages. First, the model can determine quickly and efficiently the probability of a compound to be AChE inhibitor. The second advantage of the model is related to the nature and interpretation of the descriptors. Some of them express specific contributions of characteristic fragments which are present in AChE inhibitors. This is the case of the FGC and ACF. These descriptors contain structural information that may show the ability of a specific fragment to interact with the biological receptor. The signs of the coefficients in the equation will express the influence (favorable or unfavorable) of corresponding fragments to the inhibitory activity against AChE. On the other hand, descriptors like the spectral moments take into account some physicochemical properties. For example, the descriptor $\mu_1^{(Gas)}$ encodes information related to the increment in the number of electronegative atoms. This will increase the number of polar regions and for this reason, the ability of the molecule to form important interactions like hydrogen bonds will be increased. On the other hand, descriptors such as $\mu_2^{(Ato)}$ and $\mu_4^{(Van)}$ encode aspects related to steric factors in regions of different sizes in the molecule. The first of these descriptors is related to the diminution of the molecular size while the other embodies the increment in accessibility. Finally, $\mu_9^{(Ab\text{-}R2)}$ takes into consideration the diminution in molecular polarizability and also the diminution in the interaction of the molecules with the receptor using regions of electrons **n** and $\pi$. As all the descriptors employed in the model comply with the rule of linear additively, one can then extract structural alerts responsible for the inhibition against AChE by calculating the contribution of any fragment to the anti-AChE activity. Thus, some fragments were identified (Fig. **5**) and their contributions to the inhibition against AChE calculated (Table **2**). Some fragments such as F1, F3 and F11 that have high positive contributions appear in the three drugs which are currently used as anti-AD agents because of their AChE inhibitory activity.

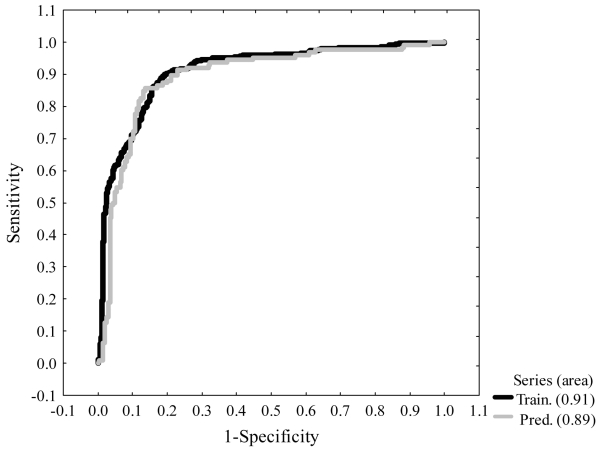


**Fig. (4).** ROC curve.

Additionally, it should be noticed that from the fragments with positive contributions, new molecular entities can be designed, synthesized and evaluated as possible potent AChE inhibitors. For this reason the present fragment-based approach comprises several advantages, that is to say:

1. Bears the possibility for well-predicting the anti-AChE activity;
2. Constitutes a tool for the fast and efficient extraction of structural alerts responsible for the anti-AChE

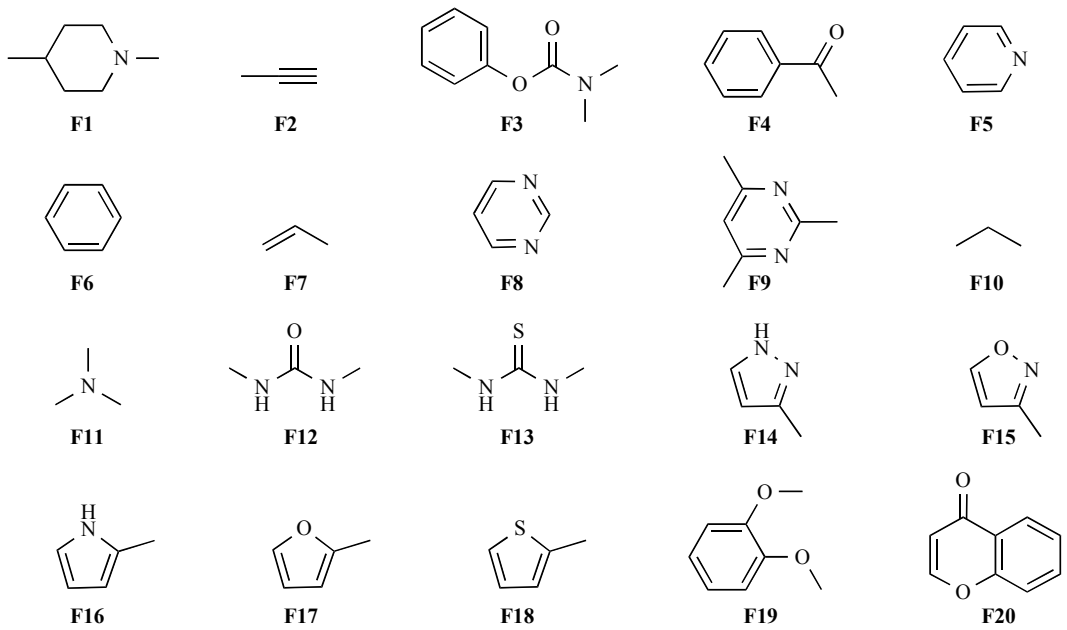


**Fig. (5).** Different fragments which were found in the molecules.

**Table 2. Quantitative Contributions to the Anti-AChE Activity**

| Fragment | Contribution | Fragment | Contribution |
|---|---|---|---|
| F1 | +1.115 | F11 | +0.668 |
| F2 | –1.651 | F12 | –0.271 |
| F3 | +2.464 | F13 | –0.371 |
| F4 | +0.422 | F14 | +0.380 |
| F5 | +0.669 | F15 | +0.537 |
| F6 | –0.156 | F16 | –0.112 |
| F7 | –0.391 | F17 | +0.016 |
| F8 | –1.622 | F18 | –0.011 |
| F9 | +0.757 | F19 | –0.141 |
| F10 | +0.004 | F20 | +0.408 |

activity and of those with negative contributions for that activity;

3. Requires less time and computational requirements.

## 4. CONCLUSION

LBDD methodologies have contributed in a determinant way toward the discovery and development of AChE inhibitors to be used as AD therapy. However, it is necessary to extend the existing techniques in order to explore a major chemical diversity and complexity in order to design more potent AChE inhibitors. Our model that resorts to a substructural approach, and is based on a large heterogeneous database of compounds, is an attempt to overcome this problem. Regarding future perspectives toward the design of new and highly active AChE inhibitors, those should take more into consideration the following aspects:

- Application of new approaches based in QSAR models to combine strategies that resort to graph-theoretical descriptors for an easily, rapidly and rationally prediction of the anti-AChE activity of several compounds.
- Extend and increase the application of methodologies for drug design which take into account the three-dimensional structure of the biological receptor, combining more those methodologies with the existing LBDD approaches. This will permit not only the design of new AChE inhibitors, but also will provide extremely important information about the binding mode of the

ligands and consequently, better understanding about the affinity of the molecules to AChE.

## CONFLICT OF INTEREST

None declared.

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## SUPPLEMENTARY MATERIALS

Supplementary material is available on the publishers web site along with the published article.

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