Monoamino Oxidase A: An Interesting Pharmacological Target for the Development of Multi-Target QSAR

Enrique Molina[a,b,*], Eduardo Sobarzo-Sánchez[c], Alejandro Speck-Planche[b], Maria João Matos[b,*], Eugenio Uriarte[b], Lourdes Santana[b], Matilde Yáñez[d] and Francisco Orallo[d⊥]

[a]*Departamento de Química, Facultad de Química, Universidad de Camaguey, 74650 Camaguey, Cuba;* [b]*Departamento de Química Orgánica, Facultad de Farmacia, Universidad de Santiago de Compostela, 15782 Santiago de Compostela, Spain;* [c]*Departamento de Farmacia y Tecnología Farmacéutica, Facultad de Farmacia, Universidad de Santiago de Compostela, 15782 Santiago de Compostela, Spain;* [d]*Departamento de Farmacología, Facultad de Farmacia, Universidad de Santiago de Compostela, 15782 Santiago de Compostela, Spain*

Abstract: With the significant increase of life expectancy of populations in societies today, the importance of the discovery of drugs associated with neurodegenerative diseases has emerged. Therefore, neurodegenerative diseases are an important topic in Medicinal Chemistry. Although drug discovery is considered a complex and slow process, new approaches and methods have been developed with the intention of finding new chemical entities in more efficient ways. This work provides a review of virtual methodologies applied in drug discovery and especially a new model for the prediction of MAO-A inhibitors using a multi-target QSAR methodology. This model involves a mixed approach containing simple descriptors based on atom-centered fragments and functional groups (DRAGON) and topological substructural molecular design descriptors (MODESLAB). This unified multi-species QSAR model was validated through a virtual screening of a new series of oxoisoaporphine derivatives, taking into account the information in the calculated fragmental contributions. Therefore, this method represents a useful tool for the *in silico* screening of MAO-A inhibitors.

Keywords: Neurodegenerative diseases, Monoamine oxidase, MAO-A inhibitors, QSAR, Multi-target model, Drug design.

INTRODUCTION

Monoamine oxidase (MAO) is an enzyme bound to the outer mitochondrial membrane that contains flavin adenine dinucleotide (FAD) as a coenzyme. This enzyme has considerable physiological and pharmacological interest because it is implicated in the metabolism (oxidative deamination) of endogenous monoamine neurotransmitters [1] and several exogenous primary, secondary, and tertiary amines [2-5]. Two isoforms, namely MAO-A and MAO-B, have been identified based on their aminoacid sequences, three-dimensional structure, substrate preference, and inhibitor selectivity [6-11].

All of these findings determine the clinical importance of MAO inhibitors. Therefore, since major depression is basically related to the deficit of norepinephrine and 5-HT at critical synapses in the central nervous system (CNS), selective MAO-A inhibitors, e.g., clorgyline (irreversible) and moclobemide (reversible) are useful solutions in the treatment of the above-mentioned neurological disorder [12-17].

*Address correspondence to these authors at the Departamento de Química, Facultad de Química, Universidad de Camaguey, 74650 Camaguey, Cuba; E-mail: enrique.molina@reduc.edu.cu; and Departamento de Química Orgánica, Facultad de Farmacia, Universidad de Santiago de Compostela, 15782 Santiago de Compostela, Spain; E-mail: mariacmatos@gmail.com

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By contrast, selective and irreversible MAO-B inhibitors (e.g., *R*-(-)-deprenyl (selegiline) and rasagiline) are useful in the treatment of Parkinson's and Alzheimer's diseases either alone or in combination with L-DOPA, since the first neurodegenerative pathology is mainly due to a deficit of dopamine in the midbrain (corpus striatum) [14, 18-26].

In the 1950s, the discovery of the antidepressant properties of MAOIs was a major finding in the monoamine theory of depression. However, earlier MAOIs introduced into clinical practice were abandoned due to adverse effects such as hepatotoxicity, orthostatic hypotension, and the so-called "cheese effect", which was characterized by hypertensive crisis. These drawbacks were thought to be related to nonselective and irreversible enzyme inhibition [15, 17].

Several attempts to perform rational design of new inhibitors have been described by means of theoretical calculations, beginning with the crystalline structure of MAO-A [27,28]. Previous studies have explained some responsible factors for the selectivity of the above-mentioned compounds on the human monoamino oxidase A (hMAO-A) isoform. Some examples are the presence of electron-rich aromatic moieties [29] and the role that some amino acid residues (i.e., Ile335) play in the active sites [27].

Aporphinoid alkaloids are the active principles of a wide range of medicinal plants used for therapeutic purposes in oriental folk medicine. In previous studies, it has been described that some of these natural compounds with the

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aporphine skeleton display interesting pharmacological activity. The effectiveness and high selectivity as hMAO-A inhibitors of pyrrolyl-ethanolamine derivatives [30], pyrrolyl-oxazolidinones [31], α-alkyl-phenylethylamines [32], and quinoxaline derivatives [33] has been demonstrated. Nevertheless, a small group of little investigated isoquinoline alkaloids has been collected in Kyoto, Japan, and later in China. Their natural source resides in the creepers of the *Menispermum dauricum* DC (Menispermaceae) [34]. Several publications [35-41] have reported the isolation of seven new yellow-colored isoquinoline alkaloids.

The synthesis and chemical reactivity of these heterocycles have been studied using 2,3-dihydro- and oxoisoaporphine derivatives [42] by means of oxidizer agents and the use of metal and hydrogenation catalysis, unexpectedly affording some interesting analogues with the partial or complete reduction of the aromatic rings, lack of substituents, and a concomitant enolization of the carbonyl group [43].

A broad consensus exists concerning the necessity of searching for novel MAOIs and the study of MAOs mechanism of action [9, 44]. In any case, despite considerable progress in understanding the interactions of both enzyme isoforms with their preferred substrates and inhibitors, few general parameters are available for the rational design of potent and selective inhibitors [45, 46].

Bearing in mind all of these considerations and, as the aporphine derivatives exhibit a number of interesting biological effects, we consider that oxoisoaporphines may be promising lead compounds for developing new and effective MAOIs. Therefore, these compounds are an interesting family to study in a multi-target QSAR model.

Consequently, computational approaches are important tools in an efficient search for new inhibitors. Although a number of reports have been published on the quantitative structure-activity relationships (QSAR) for MAOIs, in general, these are restricted to the study of congeneric families of compounds [47-53].

In the past 15 years, new approaches considered to be a part of ligand-based drug design have emerged as powerful tools for the design of new pharmacological agents. Therefore, massive screenings of databases of heterogeneous series of compounds have been rapidly developed with the aim of extracting maximum structural information at different levels of chemical complexity and diversity, supported by computer-aided drug design methods [54]. In this sense, some promising methodologies based on graph-theoretical descriptors have been developed and applied in the design of compounds with different biological activities [55, 56].

Many studies that employ graph-theoretical approaches have been reported regarding the design of new drugs, the modeling of toxicities, and in general, the search for common structural or substructural patterns of known drugs [57-64]. Other descriptors such as the indicator variables in the Free-Wilson analysis have also been used for modeling some pharmacological activities [65]. These variables are based on fragment contribution approaches and they have proved to be very useful in combination with other descriptors [66, 67].

Almost all QSAR techniques are based on the use of molecular descriptors which are numerical series that codify useful chemical information and enable correlations between statistical and biological properties [68-71]. The principal deficiency in the use of some molecular indices concerns their lack of physical meaning. Regarding this limitation, the introduction of novel molecular indices must obey physicochemical laws in order to ensure a theoretically rigorous interpretation of the results [69].

The development of rational approaches for the discovery of MAO-A selective inhibitory drugs is of great interest. The combination of methodologies based on virtual screening (applying graph-theoretical descriptors) with the structure-based drug design methods would be ideal for the development of new MAO-A inhibitor agents. This combined study would be perfect taking into consideration the different mechanisms of action, i.e., to find a relationship between the ability of a given compound to inhibit more than one biological receptor.

More than 3000 compounds have been reported as potential MAOIs in the past years [72]. However, the massive screening of compounds as possible MAO inhibitory agents is not yet sufficiently explored. As the ideal drug candidate has not been attained, an intensive search for new and innovative MAOIs is still needed. Only two studies using graph-theoretical approaches have been conducted in this area [51, 52], one of them published by some of the co-authors of the present paper.

MOLECULAR DESCRIPTORS

Fragment Descriptors

In a previous publication [73], we pointed out that the molecular descriptors based on fragments are focused on obtaining information concerning which fragments have positive (favourable) contributions and which have negative (undesirable) contributions on the activity. This allows redirecting the design of bioactive compounds in order to minimize the number of fragments with negative contributions and maximize the number of fragments with positive contributions.

Atom-Centered Fragments

Atom-centered fragments have been demonstrated to be useful molecular descriptors and have been employed in some QSAR studies [74, 75], providing important information about the hydrophobic and dispersive interactions that are involved in processes such as transport and distribution of the drug through the membrane as well as drug–receptor interactions. For these reasons, these descriptors could give an idea of the energetic outgo of this process in terms of free energy [74]. This is an essential thermodynamic property that provides a clear understanding about the nature of the entire process of drug (ligand)–receptor interaction [76, 77]. Atom-centered fragments are simple molecular descriptors defined as the number of specific atom types in a molecule. They are calculated from molecular composition and atom bonds. Each type of atom in the molecule is

described taking into account its neighbouring atoms. Hydrogen and halogen atoms are classified by the hybridization and oxidation state of the carbon atom to which they are bound; heteroatoms attached to a carbon atom in α-position are further considered. Carbon atoms are classified by their hybridization state and whether their neighbours are carbon or heteroatoms [74, 75]. Considering that these descriptors depend on their atom bonds for their definition, we can say that the presence of an atom defines the presence of a fragment.

Functional Groups Count

Another type of descriptor expresses certain fragmental features. These are simple molecular descriptors defined as the number of specific functional groups in a molecule and are also calculated from the molecular composition and atom bonds. The functional groups defined by these descriptors are those traditionally used in Organic Chemistry. Both atom-centered fragment and functional group count are descriptors that are related with indicator variables in a Free-Wilson analysis.

Spectral Moments of the Bond Adjacency Matrix

The approach that encompasses the calculation of the spectral moments of the bond adjacency matrix is known as the TOPS-MODE (TOPological Substructural MOlecular DEsign) approach. It has been applied for the description of some physicochemical properties of organic compounds [78-80] in quantitative structure-toxicity relationship (QSTR) [81, 82]. It has also been utilized for the modeling of pharmacological activities [83-86]. Some ideas in spectral moments have been generalized and extended to biomolecules by Estrada [87-90]. The theoretical background of the spectral moments of bond adjacency matrix has been described in many articles [78-84]. In order to codify information concerning heteroatoms, the TOPS-MODE approach uses $\boldsymbol{B}(W_{ii})$ weighted matrices instead of $\boldsymbol{B}$. The weights (W_{ii}) are chemically meaningful numbers such as bond distances, bond dipoles, bond polarizabilities, or mathematical expressions involving atomic weights [91, 92]. Weights are introduced in the main diagonal of matrix $\boldsymbol{B}(W_{ii})$. 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_{ii}) in the natural powers of $\boldsymbol{B}(W_{ii})$. As such, the spectral moment of order k (μ_k) is the sum of the main diagonal elements (e_{ii}) of matrix $\boldsymbol{B}(W_{ii})$. The total spectral moments of the bond matrix [89-91] are defined as:

$$\mu_k = \mathrm{Tr}\left(\mathrm{B}^k\right) = \sum_{i=1}^{s}\left(\mathrm{e}_{ii}\right)_k \quad (1)$$

where *Tr* represents 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 kth power of the bond matrix. *Local spectral moments* [87] are defined as the sum of the diagonal entries of different powers of the bond matrix corresponding to a given molecular fragment, using a similar expression:

$$\mu_k(\mathrm{f}) = \sum_{i=1}^{f}\left(\mathrm{e}_{ii}\right)_k \quad (2)$$

where f is the corresponding fragment for which the spectral is defined and the sum is carried out over all bonds that form the fragment, f.

The simplest case is when f corresponds to a single bond in which case the kth local moment is defined as the diagonal entry that belongs to this bond in the matrix, raised to the kth power. The spectral moments of the bond matrix have a topological nature (they are 2D descriptors). However, their principal advantage is that they make it possible to calculate the relative contribution of the fragments to a desired activity [71-84] because they can be expressed as linear combinations of the number of times that a fragment appears in the molecules. Another advantage of spectral moments of the bond matrix is that they can reasonably explain a considerable part of spatial phenomena [93, 94] which is a particular characteristic of 3D descriptors.

MULTI-TARGET QSAR METHODOLOGY IN DRUG DESIGN

New graph-theoretical 2D and 3D descriptors have been developed and and they are proving to be promising tools for the modeling of biological activities. Thus, stochastic and nonstochastic descriptors have been employed for the modeling of antitrypanosomal and antimalarial activities [95]. Markov chain invariants for simulation and design (MARCH-INSIDE) can be considered another promising approach in drug design and it has been extended to bioinformatics [96-98]. At the same time, another powerful tool has emerged: multi-target QSAR methodology.

This methodology was first applied by González-Díaz and co-workers in order to obtain unified models for the design and prediction of compounds with antimicrobial activity against several pathogen agents [99-102]. Using this methodology, atomic average properties were introduced as characteristics of a given group of compounds tested (being active) against a specific pathogen agent, as weighted in the topological (MARCH-INSIDE) descriptors. For this reason, the newly constructed descriptors were tested for differentiating the species of pathogen agents against a given compound. Those are called *multi-target descriptors.*

Later, the same author improved and extended the same methodology to the study of proteins in microbial agents [103], calculating the original descriptor of each protein (that only depends on the molecular structure of each protein) and then calculating the average of each original descriptor of the proteins with the same enzymatic activity, constituting in that way, the first set of multi-target descriptors. Finally, the second set of multi-target descriptors was created and the difference between the original descriptors corresponding to each protein and the average descriptor corresponding to each group of protein with the same activity was determined. The second set of multi-target descriptors represents the deviation from the average multi-target descriptors.

Another advantage of multi-target QSAR methodologies is that they have proved to be very useful in combination with complex networks [102, 103], being high power tools

for the analysis of different phenomena [104]. In this study, we used the multi-target QSAR methodology reported in reference [103] to calculate multi-target descriptors from spectral moments of the bond adjacency matrix, taking into account that this methodology can be applied to almost all classes of descriptors. In this specific case, we gave the name, *multi-species descriptors*, to those descriptors used for the prediction of MAO-A inhibitory activity through the inhibition of the enzyme in different species.

IN SILICO EVALUATION OF NOVEL MAO-A INHIBITORS

As previously described, a specific QSAR methodology expected to lead to new discoveries involves several general common steps [51]: (a) construction of a suitable molecular database of compounds (with or without MAO-A inhibitory activity), (b) calculation of the molecular descriptors, (c) construction of the model, (d) estimation of the biological activity using QSAR, (e) synthesis and characterization of selected compounds, and (f) testing of the candidate compounds in order to corroborate the predicted biological activity. As mentioned above, the aporphine analogues are a family of natural and/or synthetic compounds with different pharmacological activities, including MAO inhibitory activity. They have shown their effectiveness and high selectivity as hMAO-A inhibitors [30-37, 39-43]. In many cases, it is known that activity and selectivity are determined and modulated by the nature of the substituents in the scaffold.

Based on the above information, and considering our experience in this family of compounds, we designed and calculated the molecular descriptors for new aporphine analogues. The model developed in this study was created for the identification of novel MAO-A inhibitors.

UNIFIED MULTI-TARGET QSAR MODEL FOR MAO-A INHIBITORS

In the last years, Linear Discriminant Analysis (LDA) [105, 106] has been one of the most common statistical techniques in many QSAR studies: [83-85, 99, 101-105]

$$A\ MT_{mao\text{-}a} = a_0 + a_1D_1 + ... + a_kD_k \quad (3)$$

In the above equation, $A\ MT_{mao\text{-}a}$ (multi-target MAO-A inhibitor activity due to the inhibition of enzymatic activity) is a dummy indicator variable, having values of 1 (active compounds) or -1 (inactive ones). The a_k terms are the coefficients in the discriminant function and the D_k terms are the descriptors. The discriminant function was obtained by employing the LDA modules of STATISTICA 6.0 (http://www.statsoft.com). The default parameters of this program were used in the development of the model. The variables included in the equations were selected using a forward stepwise procedure as the variable selection strategy. The selection was subjected to the principle of parsimony. Then, the function with high statistical significance, but as few parameters as possible, was chosen.

The statistical quality of a model can be determined by examining some statistical indices such as the Wilks' lambda (λ), the square of the Mahalanobis distance (D^2), the F ratio, the corresponding p-level, the percentage of classification inside each group (for each case), and the proportion between cases and variables. The Wilks λ is a multivariate measure of group differences over several variables, having values in the range from 0 (perfect discrimination) to 1 (no discrimination). The D^2 statistic indicates the separation between two groups, demonstrating if the model has an appropriate discriminatory power for the differentiation of those groups. In the case of probabilities for the classification of each compound/species, they were considered as unclassified by the model when the difference between probabilities to be active and to be inactive was less than 5%. Another important aspect is that the compounds used in the external prediction set were never used to develop the discrimination function.

On the other hand, to confirm the quality of a model and to validate it, some statistical indices such as sensitivity (sens), the ability to classify active cases, specificity (spec) or the ability to classify inactive cases, accuracy (acc), and overall predictability were calculated. These parameters were determined according to the following equations:

$$Sens = (TP/C^+) \cdot 100\ \% \quad (4)$$

$$Spec = (TN/C^-) \cdot 100\ \% \quad (5)$$

$$Acc = (TP + TN)/[(C^+) + (C^-)] \cdot 100\ \% \quad (6)$$

where TP represents the cases (compounds) correctly classified by the model as active, C+ signifies the total of correctly classified active compounds, TN indicates the cases correctly classified by the model as inactive, C- represents the total inactive compounds, FP are the false positives, and FN are the false negatives.

In the present approach, the development of a discriminant function [107] that classifies organic compounds as active or inactive is the key step for the discovery of MAO-A inhibitors. Therefore, it was necessary to select a training data set of MAO-A inhibitors containing wide structural variability.

PRELIMINARY RESULTS IN THE DEVELOPMENT OF A MAO-A MODEL SUITABLE FOR VIRTUAL SCREENING

The data set [51, 52, 72] was formed by 2,246 different compounds. Not all the compounds found in the literature were tested against the MAO-A enzymes of the four studied species (Bos taurus, Mus musculus, Rattus norvegicus or Homo sapiens). We were able to collect 2,246 cases between active and inactive (2,246 combinations of compound/species) instead of 4 x 1,000 cases. According to the previous criteria, 590 of 2,246 cases were considered to be active. In order to perform a rigorous, rapid, and rational design, the compounds were chosen according to the following biochemical criteria: the compounds chosen as active had $IC_{50} \leq 25\ \mu M$, taking into consideration that some MAO-A inhibitor drugs such as *clorgyline* and *rasagiline* had IC_{50} smaller than this value for more than one species. IC_{50} is the half maximal inhibitory concentration and it is a measure of the effectiveness of a compound in inhibiting the MAO-A inhibitor activity. This quantitative measure indicates how much of a particular drug is needed to inhibit a given biological process by half. It is important to point out

that the compounds chosen as active had to comply with this condition. Then, if the criteria failed, the compound was considered to be inactive.

The atom-centered fragment and functional group count descriptors were calculated using DRAGON 5.3 (www.talete.mi.it/dragon.htm). The spectral moments of the weighted bond adjacency matrix were calculated using MODESLAB 1.5 software (www.modeslab.com). In this case, the spectral moments were weighted by bond dipole moments, hydrophobicity, polar surface, and Abraham dipolarity/polarizability terms. For these weighted spectral moments, we calculated the average spectral moments that depend on the group of compounds that were tested, which turned out to be active according to our criteria against given species and in MAO-A inhibitory activity. This provides specificity and differentiation between the distinct species and their inhibitors. Also, we calculated the so-called *difference spectral moment*. It is equal to the difference between the original and the average spectral moment of each molecule. It indicates the deviation of a molecule (in structural terms) from the average value to be active, depending on both the structure of each molecule and the species against each the molecule was tested.

QSAR models can be used for various purposes. The most important and most used is the prediction of the activity of new molecules. However, if the model is used in order to design new molecular entities, the descriptors employed to construct it should have a meaningful physicochemical and/or structural interpretation. Also, each descriptor should provide an idea of the physicochemical processes in terms of structural features of the molecules.

Once the training series had been designed (with a total of 443 compounds considered to be active), forward stepwise linear discriminant analysis (LDA) was carried out in order to derive the multi-species QSAR model for the MAO-A inhibitory activity score (inhibitor-MAO-$A_{i\text{-}a\text{-}sc}$):

$$\begin{aligned} MAO-A_{i-a-sc} &= -1.437(nArCO) - 2.046(nS(=O)2) - 1.753(C-022) \\ &-3.457\cdot 10^{-15} AVG\mu_8(Ato) + 0.279(nArOR) + 0.125(C-006) \\ &+0.178(nCt) + 0.210(nROH) - 0.531(N-073) + 0.220(O-057) \\ &-1.529\cdot 10^{-29}\mu_{15}(Ato) - 2.909(nR=CHX) + 0.536(nCONN) \\ &-0.789(nPyrroles) - 0.207(N-066) + 0.587 \end{aligned} \quad (7)$$

$$N = 2246 \quad \lambda = 0.573 \quad D^2 = 3.902 \quad F(15, 2230) = 84.951 \quad p<0.0000001$$

In Eq. 7, *nARCO* represents the number of ketones (aromatic); *nS(=O)2* corresponds to the number of sulfones; *C-022* takes into consideration a triple bond or cumulative double bond; *AVG*μ_8*(Ato)* symbolizes the average descriptor of the spectral moments of order 8, weighted by the Atomic weight term; *nROH* characterizes the number of aliphatic hydroxyl groups; *C-006* symbolizes the CH_2RX where X represents any electronegative atom (O, N, S, P, Se, halogens); *nCt* represents the number of terminal primary carbon, C(sp3); *nArOR* signifies the number of ethers (aromatic); *N-073* represents the secondary and tertiary amine (aromatic) and aromatic single bonds such as the C-N bond in pyrrole; *O-057* corresponds to phenol, enol, and carboxyl OH; μ_{15}*(Ato)* symbolizes the spectral moments of order 15, weighted by the atomic weight term; *nR=CHX* represents the number of R=CHX where X is halogen; *nCONN* corresponds to the number of urea (-thio) derivatives; *nPyrroles* represents the number of pyrroles, and *N-066* signifies the primary aliphatic amine.

In the model represented by Eq. 7, the atom-centered fragments and the functional group count descriptors are easily interpreted because they indicate certain types of group of atoms that form fragments and/or functional groups. In this sense, the information provided by these descriptors depends clearly on the structure of the fragments or functional groups. For this reason, a strong relationship will exist with reactivity, i.e., according to its structure, each fragment will undergo some reactions such as nucleophilic and/or electrophilic addition (described by *nArCO*, *C-022* or *nR=CHX*) or basicity and acidity (described by *N-066* and *O-057*), that has only been cited in some cases. However, atom-centered fragment and functional group count descriptors can provide information about physicochemical properties of each specific fragment in particular, such as, polarizability, hydrophobicity, atomic weight, and many others. The most important element is the contribution to the activity associated to these descriptors because the contribution is the result of all possible factors which have an influence on the structure of the fragment or the functional group under study.

In the case of the spectral moments of the bond adjacency matrix, they provided very important information about some structural features related to the drug-biological receptor interaction. The descriptors represented by *AVG*μ_8*(Ato)* and μ_{15}*(Ato)* gave information about the atomic weight, related with the molecular size of the molecules. These descriptors have a negative magnitude in Eq. 7. This indicates that a decrease of the atomic weight, in terms of molecular size, will cause an increase of activity. Moreover, the descriptor *AVG*μ_8*(Ato)* has a greater meaning than the descriptor μ_{15}*(Ato)*. This implies that the influence of the molecular size is greater among the different species than within one species, where *AVG*μ_8*(Ato)* constitutes a multi-species descriptor.

With the descriptors employed, the principal advantage of the model obtained by us is that we can calculate the relative contribution (for the development of the MAO-A activity) of any fragment to the enzymatic inhibition in any species. In the first step, all of the substructures (fragments) whose contribution we wanted to calculate, were selected. The relative contribution of any fragment to the MAO-A inhibitory activity was determined by substitution of the values of all the descriptors for each substructure in Eq. 7. In order to give an idea of the calculation of fragment contributions, we represented the fragments that were found in compounds that are MAO-A inhibitors. Thus, taking into account the model represented by Eq. 7 and the information provided by the different descriptors, we obtained fragments with favourable (positive) influence to develop MAO-A activity as well as fragments with undesirable (negative) contribution to this activity. These are sensitive to small structural variations in the molecules and, for this reason, they can be very useful in the identification process (or

design) of MAO-A compounds by the inhibition of one or several biological receptors.

Calculation of the fragment contributions provides useful information about the molecular patterns that can be determinant for the development of MAO-A activity as a consequence of the binding of the compound with the biological receptor of each species. The most important aspect is that, with the knowledge of the desirable fragments for MAO-A activity, the molecule can be designed according to the interest of the analyst. Thus, when the fragment contributions are calculated, some fragments will have positive contributions to the MAO-A activity against several receptors. Then, new molecules designed from those fragments will theoretically cause the inhibition of several receptors. In this case, those new molecules would be interesting multi-target inhibitors. On the other hand, other fragments will have positive contributions to only one receptor. Thus, new molecules will be designed as MAO-A inhibitory agents but, taking into consideration one biological receptor, and for this reason, one mechanism of action. Another important element is that some fragments with positive contributions can be present in inactive molecules (e.g., the OH aliphatic groups, represented by the *nROH* descriptor). At the same time, some fragments with negative contributions can be present in active molecules (like the aromatic carbonyl groups, represented by the *nArCO* descriptor). Only the combination of the different fragments will determine if the molecule will effectively be active or inactive.

The contributions of the different fragments depend on the targets for which they were calculated. In this sense, a fragment will have different contributions depending on the species against which the contribution to the activity was calculated. This fact confirms our suggestion stated above concerning the sensitivity of the multi-target descriptors which were used to generate the model.

The sensibility of the multi-target descriptors can not only be found in the fragments but also at the molecular level. In this case, only the drugs included in this study for each one of the species are shown. The probability of good classification generated by Eq. 7 was considered.

The aim of our work was to develop a unified multi-target (multi-species) QSAR model based on substructural-topological and fragment descriptors. This investigation not only focused on the search and prediction of the MAO-A inhibitor compounds but also on demonstrating that is possible, at the same time, and with the use of one model, to predict possible mechanisms of action. Therefore, in this study we have synthesized some nitro and bromo oxoisoaporphine derivatives and investigated the inhibitory activity against hMAO.

The designed compounds were evaluated by the QSAR model. We used the multi-target QSAR methodology reported in reference 99 to calculate multi-target descriptors from spectral moments of the bond adjacency matrix, taking into account that this methodology can be applied to almost all classes of descriptors. In this specific case, we gave the name, *multi-species descriptors*, to those descriptors used for the prediction of MAO-A inhibitory activity through the inhibition of the enzyme in different species.

We selected 13 oxoisoaporphine derivatives with different levels of structural complexity. Nine of them were determined to be active and one, inactive. The 13 selected compounds were prepared and evaluated *in vitro* as potential hMAO-A inhibitors. The theoretical prediction was compared with the experimental results and the model correctly predicted eight compounds with only two mistakes on compounds with activities above and below the cutoff point established for the model (IC_{50} = 25 μM). Most of the tested compounds inhibited hMAO-A isoform activity in the micro and nanomolar range. The theoretical model is able to predict the MAO-A activity of the oxoisoaporphine derivatives with 84.6% certainty. This information matches the predictive capacity of the model which was 83.4 %.

We studied our MAO-A inhibitors against four species (or organisms: Bos taurus, Mus musculus, Rattus norvegicus, or Homo sapiens) of MAO. Only a few compounds were reported against that species. This fact is applicable to any enzyme in any species. For this reason, the database would be limited, essentially considering analogue compounds. This fact is almost the same for all of the organisms reported and characterized as MAO-A inhibitors. As a consequence, the specialist in QSAR/drug design methodologies will need to develop as many QSAR models as combinations of families of compounds versus species necessary to be predicted. In this sense, the development of one single unified mathematical model explaining the MAO-A inhibitory activity of structurally heterogeneous series of compounds through the inhibition of those compounds against several species in MAO-A inhibitory activity is a topic of major interest.

It was, therefore, necessary to select a training data set of MAO-A inhibitors extracted from international databases (www.ebi.ac.uk/chembl/) [72]; these include benzamide (moclobemide analogues), coumarin, diazoheterocyclic derivatives, indole, phenylethylamine, thioxanthene, oxadiazolidone, propargyl (clorgyline analogues), and other families of compounds (Fig. (**1**)).

The training set was formed by 1,725 cases (76.8% of the total data), with 443 compounds considered as active compounds. The external prediction set was composed by 521 compounds (23.2% of the total data), with 147 compounds being active. This series was composed at random of the most representative families of MAO-A inhibitors. Compounds with $IC_{50} \leq 25$ μM were considered active and those with $IC_{50} > 25$ μM were considered inactive. It is common for compounds with even higher IC_{50} values to be considered as active or moderately active but we considered 25 μM to be a reasonable limit. The selection of higher break point values to cluster chemicals by their MAO-A IC_{50} may generate a series with a clearly disproportionate size and, therefore, a vastly reduced number of active compounds. As reported in different sources, numerous IC_{50} values lie within a range rather than a single value.

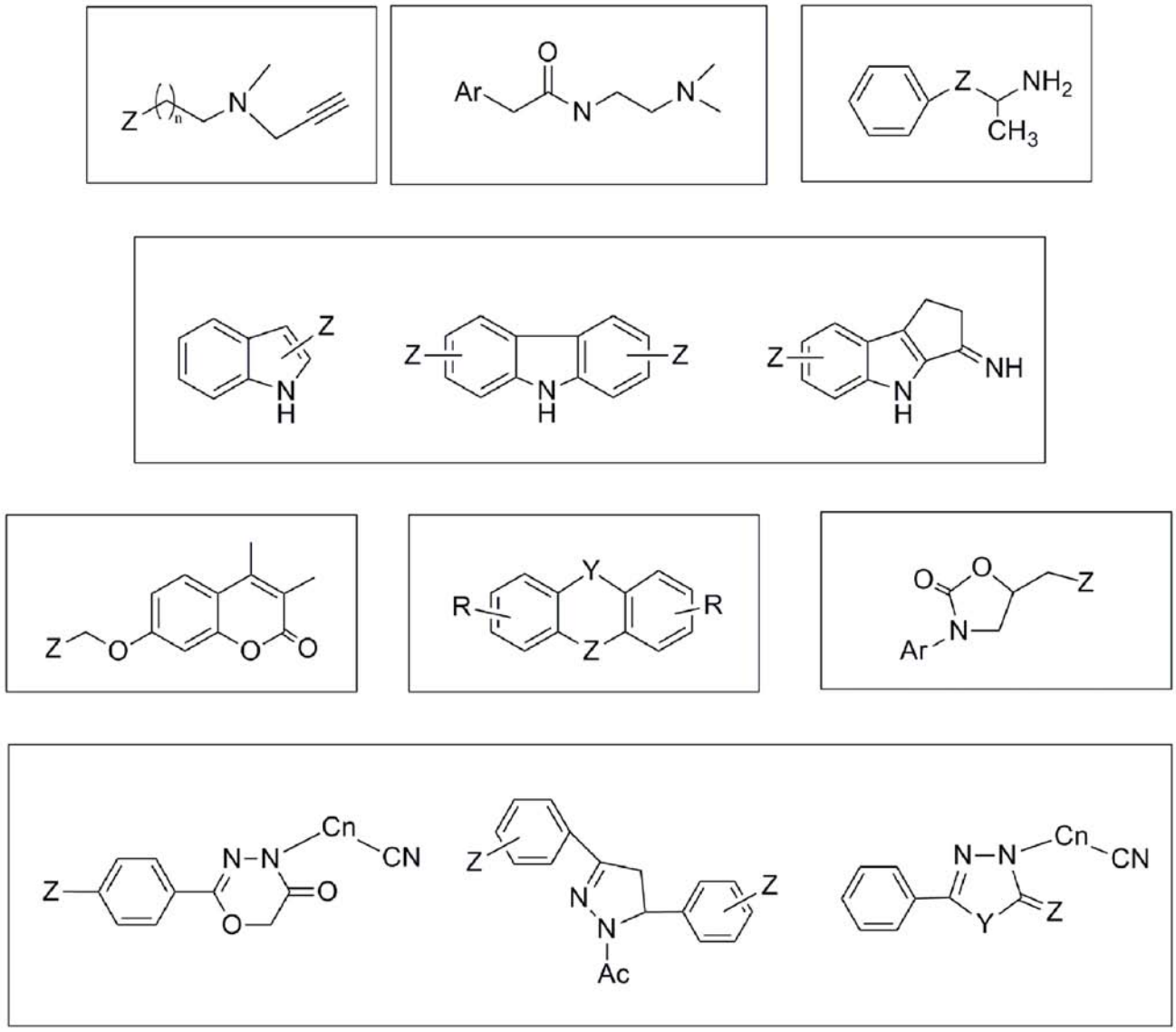

Fig. (1). General structures of the data base MAO-A inhibitory compounds.

According to the statistical indices, the model has an appropriate quality. The sensitivity of the model was 83.5% and the specificity was 84.9 % in the training series, with an accuracy of 84.5 %. We examined all of the compounds, searching misclassified cases because they can be outliers and they may influence the quality of a model. We checked the Mahalanobis distance of each molecule respecting the two centroids of both groups (active and inactive compounds). Generally, in the case of abnormal values, the compound should be excluded of the model. In this sense, no outliers were detected.

In order to validate our model, we considered the sensitivity, the specificity, and the accuracy (all statistical indices in the prediction series). The sensitivity of the model in the prediction series was 84.4% and the specificity was 83.2%, with an accuracy of 83.5% which means that, if the QSAR model predicts that a compound is active against a species, the probability of this compound to be really active is 84.4%. Similarly, if the model predicts that the compound is inactive against a species, the probability of the compound to be really inactive is 83.2% (Table **1**).

In this study we have synthesized some nitro and bromo oxoisoaporphine derivatives and investigated the inhibitory activity against hMAO of 2,3-dihydro-7*H*-dibenzo[*de,h*]quinolin-7-one (dihydrooxoisoaporphine, **C**), 7*H*-dibenzo[*de,h*]quinolin-7-one (oxoisoaporphine, **D**), 2,3,8,9,10,11-hexahydrooxoisoaporphine (hexahydrooxoisoaporphine, **E**), and 7-hydroxy-1,2,3,11b-tetrahydro-7*H*-dibenzo[*de,h*]quinoline (isoaporphine, **F**) derivatives (Fig. **2**).

From a total of 13 compounds, nine were predicted to be active in the *in silico* evaluation carried out using the QSAR model developed here. As previously mentioned, compounds were considered active when they had an IC_{50} value below

Table 1. Training and Validation Results

	Percent	MAO-A Inhibitors	Inactive	Total
Training results				
MAO-A inhibitors	83.5	370	73	443
Inactive	84.9	194	1088	1282
Total	84.5	564	1161	1725
Validation results				
MAO-A inhibitors	84.4	124	23	147
Inactive	83.2	63	311	374
Total	83.5	187	334	521

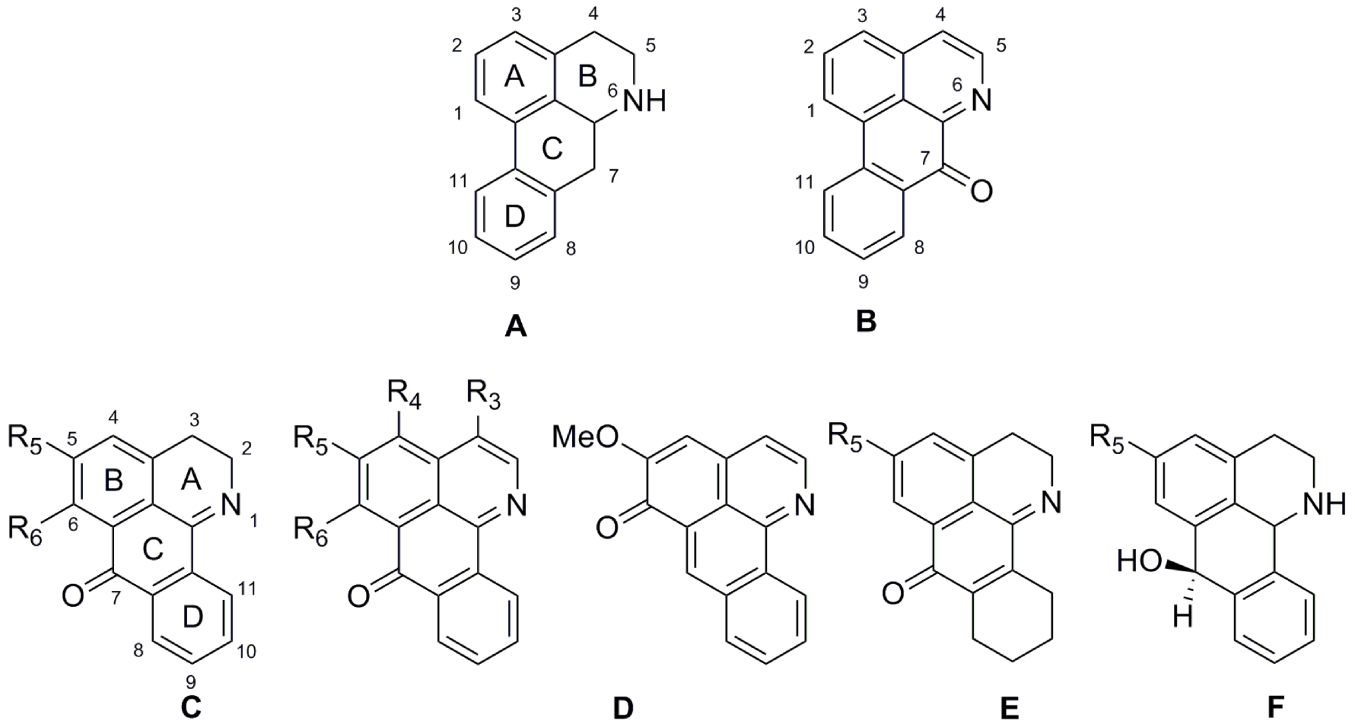

Fig. (2). General chemical structure of aporphine (**A**), oxoaporphine (**B**), 2,3-dihydro-oxoisoaporphine (**C**), oxoisoaporphine (**D**), 2,3,8,9,10,11-hexahydrooxoisoaporphine (**E**) and isoaporphine (**F**).

25 μM. Ten of the studied compounds were efficient and highly selective inhibitors of the hMAO-A isoform activity in the micromolar and nanomolar range. From these series, an oxoisoaporphine with a **D** structure was the most potent inhibitor experimentally identified (IC_{50} = 0.83 nM). This data was corroborated by the model (Eq. 7). The inhibition of the MAO-A by this compound, due to the existence of the reversible inhibition, consequently produces an inhibition by a non-competitive mechanism.

Two compounds with the only differences being a methoxy group in R_5 and the aromatization of the A had IC_{50} values of 0.72 μM and 0.83 nM, respectively. This corresponds with the positive contribution of this group represented by the descriptor *nArOR* in Eq. 7. Moreover, additional substituents attached to the quinoline framework in the oxoisoaporphine derivatives clearly affected the inhibitory activity of the compounds studied on hMAO-A. Thus, the demethoxylation of the most active compound considerably diminished MAO-A inhibition in both the C and D structure patterns. Also, the presence of electron-withdrawing groups such as bromine or nitro decreased the MAO-A inhibitory activity. This fact proves the importance of the molecular size of those groups (negative influence of

the descriptors, $AVG\mu_8(Ato)$ and $\mu_{15}(Ato)$). Other electron-withdrawing groups had an inverse contribution, that is, a positive contribution. Therefore, small chemical modifications on the oxoisoaporphine skeleton could give important information concerning the key points for the design of selective and highly effective inhibitors of the hMAO-A isoform.

The results obtained in this study indicate that these derivatives may have interesting therapeutic potential as original chemical models for the design and subsequent development of new drugs (selective and efficient MAO-A inhibitors) useful for improving the pharmacological treatment of depression (major depressive disorders). In this sense, these results were recently presented as a Spanish Patent [107] due to the novel and surprising pharmacological data for this type of alkaloid.

ROC Curve

Although the sensitivity and the specificity can better describe the quality of a model, these two statistical indices have disadvantages. The most important is that they cannot provide information about how many times the probabilities indicate that a compound, observation, or case will be predicted more to be positive (active) rather than negative (inactive). This element is very important because it confirms, together with the positive predictive value, if a given case is active. This information is provided through the receiver–operating characteristic (ROC) analysis. ROC is a classic methodology from signal detection theory [108, 109].

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. When the variable (or variables) under study cannot distinguish between the two groups, i.e., when there is no difference between the two distributions, the area will be equal to 0.5 (the ROC curve will coincide with the diagonal) and the classifier is considered to be random. When there is a perfect separation of the values of the two groups, i.e., there is no overlapping of the distributions, the area under the ROC curve equals 1. The areas under the ROC curves were 0.92 and 0.91 for the training and prediction series, respectively. Thus, these areas can be interpreted in the following manner. In the case of the training series, the value of area 0.92 means that a randomly selected compound or case from the active group will have a larger value of probability than a randomly selected compound or case from the inactive group (92% of the time). A similar conclusion can be deduced from the value of the area under the ROC curve in the prediction series. This fact proves that our model is not a random classifier because the areas under the ROC curves are different and statistically significant from those obtained in random classifiers (area = 0.5).

Correlation Among Independent Variables

When a model is developed, sometimes the independent variables appear to be highly correlated. This fact can be a consequence of the inherent properties of the used descriptors. That can be a disturbing factor which is frequently overlooked and can lead to instability of the model. The correlation coefficient between independent variables should not exceed the value of r = 0.7, with r being the Pearson correlation coefficient.

CONCLUDING REMARKS

As we described in this review, in the last years many advances have been reported in the development of new MAO-A inhibitors. As the ideal drug candidate has not been attained, an intensive search for innovative and selective MAO inhibitors is still needed. This effort has considerably increased in recent years. Different research groups are working on the search for novel inhibitors of this enzyme. The results achieved in the design of new MAO-A inhibitors shows that the rational design approaches significant advances in this area.

In this work, a unified multi-target QSAR model for classification and prediction of MAO-A compounds through the inhibition against four species enzymes was obtained. Taking into account the inhibition of different enzymes by several compounds, our model has an appropriate statistical quality and provides a guide for the development of molecular patterns to be used in the design of MAO-A compounds. Our model has proved to be very useful for the design, search, and prediction of novel MAO-A drugs in a quantitative, rapid, and easy way, considering the inhibition of several enzymes and, for this reason, several mechanisms of action. In addition, the significance of these approaches is that a unified multi-target QSAR model for MAO inhibitors was able, for the first time, to correctly classify (84.6%) series of compounds with different structural patterns. This ability demonstrates that this is a general model. The methodology can be extended to other species and it can also be extended to the study of specificities of many phenomena.

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