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Dynamic 3D QSAR techniques: applications in toxicology

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Abstract

Two dynamic techniques recently developed to account for conformational flexibility of chemicals in three-dimensional (3D) quantitative structure–activity relationships (QSARs) are presented. A basic assumption underlying both methods is that chemical behavior in complex biological systems is context-dependent. A molecule can exist and interact in a variety of conformations. Selection of the appropriate 'active' conformer(s) in QSAR studies is a task as important as the selection of appropriate molecular parameters because multiple conformers of one chemical can differ significantly in the value of their calculated molecular descriptors. In the dynamic approaches for selection of active conformers in correlative QSAR studies, biological activity is modeled as a function of molecular descriptors derived from specifically selected active conformers, rather than as a property derived from the lowest-energy gas-phase conformer. In a recent pattern recognition approach all energetically reasonable conformers are taken into account to derive the common reactivity pattern (COREPA) of structurally diverse but biologically similar chemicals (and ultimately conformers). The COREPA method is based on the assumption that chemicals which elicit similar biological behavior through a common mechanism of interaction with the biological 'receptor' of interest, should possess a commonality in the values of their steric and/or electronic parameters, thus yielding a COREPA. Applicability of these techniques, based on the same underlying principles, is illustrated. In addition to the impact of conformational flexibility of chemicals in 3D QSAR models, the applicability of various molecular descriptors is discussed. The proposed classification could be useful as guidance for selection of appropriate molecular parameters for modeling a variety of toxicity endpoints according to the specificity of the underlying interactions.

Keywords: Three-dimensional quantitative structure–activity relationships; Conformational flexibility; Three-dimensional-screening; Molecular descriptors; Receptor binding

1. Introduction

Quantitative structure–activity relationships (QSARs or more general SARs) are based on a fundamental principle in chemistry, that chemical properties and the biological behavior elicited is a direct result of chemical structure. Once established, such SARs can be used to predict the activity of untested chemicals. Two conceptually different approaches have been implemented in SAR studies. In correlative approaches, the variation in molecular structure (assessed quantitatively by molecular descriptors) within a congeneric series of compounds is assumed correlated with the observed change in the endpoint under study (e.g. toxicity), thus providing

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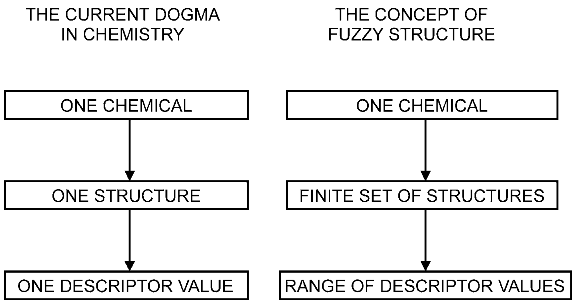
insight as to the molecular mechanisms controlling the modeled endpoint. On the other hand, pattern recognition approaches attempt to identify common stereoelectronic characteristics among structures that elicit similar biological activity. The recognized common structural characteristics can then be employed to screen large and structurally heterogeneous databases for identification of potentially active chemicals eliciting toxicity by the same mechanism of action. Both SAR approaches are successfully employed only if the modeled chemical or biological endpoint is a result of a common interaction mechanism.

Because chemical interactions are three-dimensional (3D) events, QSARs often depend on the 3D molecular models adopted for the chemicals under study. This certainly applies to receptor-site mapping models dealing directly with molecular shapes and fields. Correlative QSARs may also be influenced indirectly when employing electronic quantum-chemical descriptors that generally depend on 3D structure. The conventional 3D QSAR methods are based on the current 'one chemical–one structure–one parameter value' dogma (Scheme 1) where a single conformer characterized by point values of its parameters is used to represent a chemical under study, while all others are ignored. In the best case, the representative conformer is the one of lowest potential energy for the isolated molecule, or the one observed in crystal phase. Typically, one computationally derived low energy conformer is used, resulting in appreciable uncertainty due to the diversity of algorithms available for 2D–3D structure migration which provide different 3D structures from the same molecular connectivity.



Scheme 1.

To address the issue of selection of active conformers in QSAR studies with flexible molecules and complex biological interactions, Mekenyan et al. [1] introduced the 'dynamic' QSAR approach. The name dynamic was used to describe attempts at mimicing the infinite conformational space of a chemical by using a set of *static* (discrete) isomers. The approach assumes that in complex environments, such as biological tissues and fluids, chemicals can exist in conformations other than the lowest energy gas-phase state. The use of the lowest energy conformer in SAR studies is common, but inappropriate, because in complex systems such as biological tissues and fluids chemicals are likely to exist in a variety of conformational states. In fact the low-energy gas-phase conformation might be the least likely to interact with macromolecules [2], with solvation and binding interactions capable of compensating for energy differences among the conformers of a chemical [1,3–8]. Thus, at the macromolecular binding sites, conformational states can be populated which are substantially different than the isolated, lowest-energy or crystal-phase conformation. This holds true especially for enzyme-mediated reactions where enzyme-induced distortions in direction of the transition state can drive the molecules out of a local potential energy minima.

Employed in correlative SAR studies, the dynamic method was used for selection of active conformers. Using this approach biological activity is modeled as a function of molecular descriptors derived from specifically selected active conformers [1,3,4,6]. The inclusion of all conformers as structural representatives of the chemicals in the correlation samples was also found to be inappropriate because of potential statistical biases that could result due to differences in conformational flexibility of chemicals and thus number of conformers generated for each structure [8].

In pattern recognition studies the ad hoc selection of active conformers is hampered by the structural heterogeneity of the chemicals under study. For that reason, the distribution of all energetically reasonable conformations is analyzed when similarity between molecules is assessed. This approach was taken in developing a new pattern-recognition method, which is able to determine the common reactivity pattern (COREPA) for structurally diverse chemicals exerting high biological effect via the same mode of action [9,10]. It is assumed that chemicals which elicit similar biological behavior through a common mode of action should possess a commonality in their stereoelectronic (reactivity) pattern. Elucidation of this pattern requires examination of the conformational flexibility of the compounds in an attempt to reveal areas in the multidimensional descriptor space which are most populated by the conformers of the biologically active molecules and least populated by conformers of inactive chemicals, simultaneously (see also Ref. [6]). COREPA circumvents the problems of conformer alignment and selection, and initial assumptions concerning specific atoms/fragments in a pharmacophore are not obligatory. The method defines the COREPA by analyzing conformational distribution of chemicals across global and local reactivity parameter(s) potentially associated with the specific biological endpoint under study.

Examples of the QSAR techniques developed utilizing principles described above are presented in next sections.

2. Fuzzy logic and 3D structure modeling

Within the single (lowest energy) conformer representation of chemical structure, the corresponding geometric and electronic parameters have single, discrete point values. Such *truth-values* and the associated logic have been designated as *crisp* in the fuzzy logic theory [11,12]. These parameters are subject to classical prepositional logic, based on the *two-value principle*. According to this principle, every preposition is either true or false and can be treated as *Boolean truth-values* of classical logic. In other words, one chemical either can or cannot possess specific parameter values. This classical logic, however, does not match the real world due to conformational flexibility of chemicals and the fact that the lowest energy conformer is not necessarily the active one. Which conformer is active is context-dependent, influenced by the biological macromolecules and biochemical environment present, and therefore the specificity of the interaction partners and reaction media could significantly influence the choice of active conformer(s).

In a recent study, we have shown that even stereochemically rigid steroids are conformationally flexible within the retained stereochemistry of the natural enantiomer [9,13]. The range of 10.6 kcal/mol for formation enthalpies (within AM1 Hamiltonian) was found to be within the range of experimental receptor binding energies for these molecules (10–20 kcal/mol). Additionally, the conformer interconversion barrier was found to be no greater than 10.9 kcal/mol and thus comparable with the conformer formation enthalpies. Steroid conformer interconversions were therefore found to be feasible kinetically, as well as thermodynamically. Thus, conformational flexibility of these hormones should be taken into account when receptor or pharmacophore mapping approaches are employed [13].

A conclusion of the above analysis is that structural information derived from a single conformer may be insufficient for a complete structural description of a chemical and could cause a subsequent failure in SAR analysis. It appears that the classical prepositional logic of two options, namely true and false, when associated with a single geometrical representation of a chemical needs to be replaced by the fuzzy logic of an infinite set of geometries describing conformational space of molecules. In order to overcome the infinity problem, however, one could make conformational space discrete using a finite number of conformers to represent the whole space. Thus, by analogy with the notion of *fuzzy molecular graphs* [11, 12], we propose the use of *fuzzy chemical structures* where the atoms, bonds and valence angles are assumed to carry crisp information, whereas the torsion angles carry fuzzy information. In other words, when describing conformational space, the atoms, bonds and valence angles are fixed, thus forming the static elements of the molecule, whereas torsion angles are varied, thus forming its flexible components.

It has been found that conformational flexibility has significant impact on molecular electronic structure and associated properties, as has been illustrated, for example, with several series of androgen and estrogen receptor ligands [9,10,13–15]. For a given compound, conformers within the formation enthalpy range of 20 kcal/mol exhibited significant variation in potentially relevant electronic descriptors (the 20 kcal/mol threshold for selection of potentially active conformers falling within experimental binding energies). To illustrate the point, conformers of hydroxyflutamide (again, those conformers within the energetically reasonable formation enthalpy range of 20 kcal/mol) had calculated parameter values spanning over 0.63 eV for E_{LUMO}, a range of 1.09 eV for E_{HOMO}, 0.61 eV for $E_{\mathrm{HOMO-LUMO}}$ gap, and 6.51 D for dipole moment (μ). The conformers of steroid structures included in the same analysis spanned somewhat smaller parameter ranges (likely due to their stereochemical rigidity which was maintained during conformer generation), but were also noteworthy. Conformers of methyltriendone had a range of 0.30 eV for E_{LUMO}, 0.18 eV for E_{HOMO}, 0.46 eV for $E_{\mathrm{HOMO-LUMO}}$, and 1.60 D for μ. The observation that relatively small energy differences between conformers of a chemical can yield significant variation in electronic structure highlights the necessity to represent the molecular parameter values as finite ranges for each chemical, instead of single point values. Hence, the application of fuzzy logic in the handling of chemical structures requires the one chemical–one structure–one parameter value principle to be modified to a ‘one chemical–finite set of structures–range of parameter values’ principle (Scheme 1). Currently in textbooks, catalogues, and databases, the property values listed are for one conformer of a chemical, usually in a specific solution or immobilized in a solid phase. Significant amounts of structural and property (activity) information are therefore missing, due to the lack of experimental and/ or theoretical studies on the relationship of conformational change and associated variability in parameter values.

It should also be noted that true values in fuzzy logic, i.e. the parameter values corresponding to conformationally multiplied chemicals, should be considered as *possibility* values rather than as *stochastic*. The possibility character is derived from the assumption that active conformers are reaction and environment specific, and that the selection of conformers in structure–activity studies should be considered in the context of the specificity of the interaction rather than as a result of random choice.

There are many attempts to solve the issue of conformational freedom of chemicals in QSAR studies for drug design and for screening of 3D structure databases. In the former case, the traditional QSAR packages provide conformational analysis in an attempt to solve the problem of generating the lowest energy conformer. However, as mentioned previously, the selection of active conformers in QSAR analysis is as important as the selection of significant molecular descriptors. Similarly, the use of a *rigid search*, based on single conformer representation of chemicals is likely to fail to identify structures that, due to their flexibility, could adopt the conformation of a query pharmacophore. The alternative *flexible search* using the query to direct the conformational changes needed to find a match are based on *distance geometry* [16,17], *systematic search* [18–20], *random-search* [21], *genetic* [22, 23], and *directed tweak* [24] algorithms. These algorithms for searching conformational space are discussed and compared by Clark et al. [25]. For 3D flexible searching, Murrall and Davies [26] have suggested the direct application of distance range screens with user defined tolerances.

Newly developed modeling approaches and associated techniques allowing interpretation of fuzzy molecular structure are presented in Section 3, with examples of QSAR applications discussed in Section 4.

3. Dynamic techniques for 3D-structure manipulation in QSAR analysis

The main feature of the 3D QSAR approaches presented in this work is the consideration of conformational flexibility of chemicals. Techniques for exploring conformational space of chemicals are included as an initial step in the development of 3D QSARs. Two algorithms have been developed within the OASIS software system to handle conformer generation and evaluation for QSAR application. Following is a short description of these algorithms, however, readers are encouraged to consult the respective references for a detailed presentation of the approaches.

3.1. Algorithms for conformer generation

3.1.1. Systematic search by the 3DGEN algorithm

The 3DGEN technique [27] is a combinatorial procedure for systematic search of conformational space. It initiates from molecular topology and generates all conformers in the context of steric constraints (e.g. distances between non-bonded atoms, ring-closure limits, torsional resolution) and expert rules (e.g. likelihood of intramolecular hydrogen bonds, *cis/trans* or L/D isomers). A unique aspect of the approach involves the initial propagation of an acyclic 3D model of the molecular skeleton. The construction of this skeleton initiates from a specified atom of the molecule based on its topochemical ranking (a ranking based on connectivity and atom type). A bond 'under construction' in this acyclic model is positioned in space by using a recursive procedure based on the 3D information of previously established bonds. This includes the atom type and hybridization of the atoms incident to the bond being constructed as well as the two atoms associated with the previously completed bond. Cyclic fragments incident to the bond being constructed are also addressed. Bond lengths and valence angles are determined through a molecular mechanics parameterization. During the propagation of the acyclic model, cyclic character is gained through defined ring-closure constraints. Rotamers associated with all torsional angles that meet hybridization and specified geometric constraints are retained. In summary, the approach incorporates the conformational flexibility of saturated cyclic molecular fragments, as opposed to other techniques, that explore conformational space formed by rotations around acyclic single bonds only.

With strained molecules the possible violation of imposed geometric constraints are corrected with a strain-relief procedure (pseudo molecular mechanics, PMM) based on a truncated force field energy-like function, where the electrostatic terms are omitted [27]. In fact, the PMM force field involves additive interatomic interactions for bond lengths, valence angles, dihedral angles, out-of-plane bends of sp^2 conjugated sites and Lennard-Jones repulsions of non-bonded sites. The basic form and parameterization of the interatomic interactions mentioned above was taken from the Chem-X force field [28,29]. Geometry optimization is further completed by quantum-chemical methods. Usually, MOPAC 93 [30,31] is employed by making use of the AM1 Hamiltonian. Next, the conformers are screened to eliminate those, whose heat of formation, ΔH_f^0, is greater from the ΔH_f^0 associated with the conformer with absolute energy minimum by user defined threshold. Usually, 20 kcal/mol (or 15 kcal/mol) threshold is employed based on experimental evidence that the free energy of binding to some steroid hormones is in the range of -10 to -20 kcal/mol [7,32,33], which would provide the necessary energy to elevate conformers from the low(est) energy state during binding. Subsequently, conformational degeneracy, due to molecular symmetry and geometry convergence is detected within a user defined torsion angle resolution.

3.1.2. Genetic algorithm for conformational coverage

A new approach for coverage of chemical conformational space by a limited number of conformers [34] was developed to evaluate conformational flexibility of molecules. Instead of using the systematic search whose time-complexity increases exponentially with degrees of freedom, a genetic algorithm (GA) is employed to minimize 3D similarity among the generated conformers. This makes the problem computationally feasible even for large, flexible molecules. The 3D similarity of a pair of conformers is assumed reciprocal to the root-mean-square (RMS) distance between identical atomic sites, using an alignment which minimizes this distance. Thus, in contrast to traditional GA, the fitness of a conformer is not quantified individually, but only in conjunction with the population it belongs to. The approach handles the following stereochemical and conformational degrees of freedom: rotation around acyclic single and double bonds, inversion of stereocenters, flip of free corners in saturated rings, reflection of pyramids on the junction of two or three saturated rings. The latter two were particularly introduced to encompass structural diversity of polycyclic structures. However, they generally affect valence angles and can be restricted up to a certain level of severity of such changes. Stereochemical modifications are totally/selectively disabled when the stereochemistry is exactly/partially specified on input. For the chemicals under study, the stereochemistry of the active enantiomer is maintained during conformer generation. The reproducibility and robustness of GA runs, and subsequent density of coverage of conformational space can be controlled by the ratio between parents and children.

Each of the generated conformations is submitted to geometry optimization procedure as described in Section 3.1.1.

3.2. Classification of molecular descriptors in 3D QSARs

Besides selection of active conformers to represent chemicals in 3D QSAR studies, one needs to assess the molecular geometric and electronic structure as well as the physicochemical parameters of the chemicals under investigation. The selection of molecular descriptors is not a trivial task. Usually, this problem is addressed by applying robust statistical tools in an attempt to avoid the frequently faced conflict between a small number of observations and a large number of molecular parameters in the descriptor pool. The latter could result in chance correlations according to the criteria of Topliss and Edwards [35]. Conventionally 3:1 or 5:1 ratio between observations and molecular parameters is assumed as optimal. However, it should be emphasized that often the number of modeling parameters evaluated in this ratio is erroneously counted from the ultimate model, and not reflective of the initial data matrix.

Traditionally only statistical tools are relied upon to reduce the cardinality of descriptors in the initial data matrix. Our belief is, however, that informal assessments should be used first to reduce this cardinality, with statistical tools applied afterwards. These informal assessments should be based on known, or hypothesized, mechanisms of chemical–biological interaction, which would allow elimination of molecular descriptors unlikely to be associated with the endpoint under study, i.e. would eliminate the 'noise' in the descriptor pool. To accomplish this we propose a classification of toxic endpoints and molecular descriptors according to the specificity of the associated interaction mechanism (Fig. 1). The core of this classification scheme is the segregation of chemical–biological interactions prerequisite to a biological effect into three types: non-specific, specific and receptor-mediated. The interactions loosely termed 'non-specific' include chemical processes such as perfusion or diffusion-controlled chemical partitioning across biological membranes leading to predictable endpoints such as chemical bioconcentration (of non-metabolizable compounds) [36] aquatic toxicity mechanisms such as non-polar narcosis [37], etc. Somewhat more 'specific' interactions might include chemical–biological interactions leading to aquatic acute toxicity from exposure to reactive (electrophile/proelectrophile) chemicals [37], many of the reactions leading to mutations, chemical alterations accomplished during microbial biodegradation, etc. Finally, we have

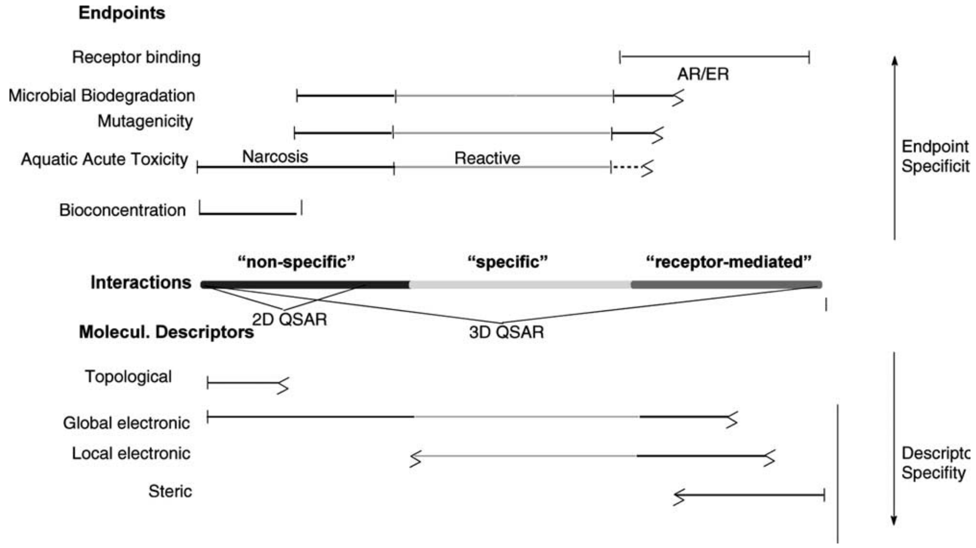


Fig. 1. Classification of toxic endpoints according to specificity of underlying interaction mechanisms and associated molecular descriptors.

considered 'receptor-mediated' interactions that induce subsequent conformational change in receptor proteins and are associated with endpoints such as gene activation, cell proliferation, etc.

The chemical processes associated with non-specific interactions include reversible processes which may be associated with underlying weak electrostatic (Van der Waals) forces, and include interactions during which chemical bonding in molecules is not irreversibly altered. As a result, these non-specific interactions yield reversible toxic effects. Note that these are not strict categorizations, but represent a continuum of chemical–biochemical interactions. For instance, weak interactions (Van der Waals, H-bonding) also underlie reversible receptor-mediated events. Moreover, if we take into account the interacting targets (e.g. nucleophilic sites targeted by electrophilic chemicals producing acute aquatic reactive toxicity), these effects can have pronounced specificity. A specific interaction, for example, includes interactions involving formation of covalent bonds. In contrast to electrostatic effects, these interactions have specific character due to directionality (i.e. well-defined orientation) of covalent bonds. The bond rearrangements in these interactions produce irreversible toxic effects. The receptor-mediated interactions have been elegantly described as reversible ligand–receptor–effector interactions with conformationally flexible ligands binding with the receptor and inducing conformational change such that the new complex allows interaction with effector sites [38]. The site–site binding interactions between reaction centers are weak, i.e. with non-specific character in accordance with underlying chemical (electrostatic) forces.

The molecular descriptors that are calculable from chemical structure assess different aspects of molecular structure and hence ability of chemicals to take part in different interactions. For example, topological indices describe non-metric geometry and therefore could describe non-specific interactions and related endpoints, such as chemical penetration (diffusion), acute aquatic non-polar narcosis, etc. The same holds to some extent for global electronic indices, which in addition to the non-specific interactions, encompass the area of specific interactions as well, strongly dependent upon molecular geometry. Hence, these molecular descriptors should be considered in developing QSARs for mutagenicity, aquatic reactive toxicity, etc. The global electronic descriptors also extend to the realm of receptor-mediated interactions, and have been found useful in quantifying the global reactivity of receptor ligands. Local electronic reactivity parameters describe the ability of atomic sites and fragments to take part mostly in specific interactions dependent upon the reactive groups present, and subsequently should be used for modeling toxic endpoints such as reactive acute toxicity, biodegradation, receptor mediated effects, etc. These parameters, however, could also be used for assessing propensity of chemicals to take part in weak interactions; hence, they are also included in the list of parameters available to describe non-specific interactions. Finally, specific steric parameters, such as distances between fragments and/or atomic sites have also shown to be critically important for deriving mechanistically reasonable models for receptor-mediated effects.

The list of parameters used routinely in OASIS system [39] includes:

(i) Non-specific molecular descriptors, such as topological indices;

(ii) Specific electronic descriptors: Global electronic parameters such as heat of formation (ΔH_f), LUMO energy (E_{LUMO}), HOMO energy (E_{HOMO}), HOMO–LUMO energy gap (E_{gap}), electronegativity (EN); dipole moment (μ), volume polarizability (VolP), degree of stretching or compactness (quantified as sum of interatomic steric distances, GW), greatest interatomic distance ($L_{\max}$), planarity (normalized sum of torsion angles in a molecule) are included, here. VolP is defined as a sum of atomic self-polarizabilities, and describe the averaged ability of a compound to change electron density as its atoms during chemical interactions (lower VolP values reflect higher charge localizations and more polarizable, i.e. less lipophilic are molecules). Van der Waals surface, solvent accessible surface (SAS1.5; assuming water as a solvent) calculated by making use of Connoly algorithm [40] and charged partial surface areas (CPSAs) as introduced in Ref. [41] by Stanton and Jurs could be included in the group of specific physicochemical parameters.

Local electronic descriptors include atomic charges (q_i), frontier atomic charges (f_i^{HOMO} and f_i^{LUMO}), donor and acceptor superdelocalizabilities (S_i^{E} and S_i^{N}), and self-atomic polarizability (π_i), where i denotes a given atomic site of the molecule.

(iii) Specific steric descriptors, such as the steric distance between atoms i and j (d_{ij}).

All molecular descriptors described above except topological indices, could be used for deriving 3D QSAR.

3.3. Selection of active conformers in correlative QSAR studies

According to basic assumptions, in complex reaction environments or with solvents of different polarity one should expect that a molecule can populate different conformational states depending on the particular interaction step, such as tissue partitioning, substrate–receptor complex formation, etc. [1]. Moreover, the specificity of the processes allows for particular conformers to be active for the different endpoints under investigation. The identification of those conformers, however, is a difficult problem, especially when the equilibrium among conformational states is easily attained. At the present time there is no general approach identifying the 'real' conformers among computed ones.

The method combines a conformation generation routine with a module for conformer screening. It begins with the generation of an exhaustive set of conformers and allows one to select conformers based on the problem being addressed, by using a hierarchical set of screening rules. Thus, one selects a specific conformer among the finite set of isomers describing the *fuzzy molecular structure*. The screening stage is based on the stereoelectronic molecular structure of selected conformers assessed by quantum chemical methods. Then, in an interactive mode, the user has the opportunity to introduce working hypotheses in the selection of conformers. As was mentioned, great variation in stereoelectronic indices among different conformers of a given molecule often results, which again points to the conclusion that the selection of active conformers appears to be as crucial for QSAR analysis as the selection of suitable molecular descriptors.

Conformers can be selected based on their distribution across specific physico-chemical and/or stereoelectronic parameters. The conformer distribution (number of conformers belonging to a certain range of parameter values) is displayed as a selected number of parameter windows, evenly dividing the whole parameter range. There are a variety of selection schemes, which can be applied after examining the conformer distributions, providing:

(i) Prevailing values of the parameter of interest (select conformers belonging to the most populated window of the relevant electronic or geometric property).

(ii) Extreme values of the parameters of interest (select conformers providing the values of maximum or minimum relevant molecular property). Thus for example, one can select lowest energy conformers having minimum values of heats of formation. One can also obtain the conformers with the highest electron acceptor (donor) properties, taking those having minimum (maximum) values of E_{LUMO} (E_{HOMO}) or maximum (minimum) values of acceptor (donor) superdelocalizabilities or the respective frontier charges at specific atomic sites.

(iii) Certain Boltzmann-weighted populations according to

$$P^i[\%] = \exp[-(\Delta H_{\mathrm{f}}^i - \Delta H_{\mathrm{f}}^1)/RT]/ \Sigma^n \exp[-(\Delta H_{\mathrm{f}}^i - \Delta H_{\mathrm{f}}^1)/RT]100 \quad (1)$$

with $P^i[\%]$ denoting the percent level of population of the conformation i with heat of formation ΔH_{f}^i at 298 K (as quantified by AM1).

(iv) Descriptor values weighted according to conformer-specific values for each compound. Different averaging methods including arithmetic, geometric, quadratic, and harmonic methods are provided.

3.4. COREPA method

COREPA is a pattern recognition type of QSAR approach providing visualization and interpretation of reactivity pattern of biologically similar chemicals [9,10]. The approach is based on the premise that the similar biological behavior of topologically dissimilar chemicals is due to a commonality in their stereoelectronic (3D) structures, as described by common ranges of selected stereoelectronic descriptors. The core of the approach is the procedure for evaluation of 3D similarity between chemicals accounting for their conformational flexibility (referred to as the "dynamic" 3D similarity method in Mekenyan et al. [42]). The commonly used 3D similarity methods compare 3D structural patterns of fixed molecular geometries and do not account for structural differences due to conformational flexibility of the molecules. Thus, two conformers each representing compared molecules could deviate significantly in their stereoelectronic structure in terms of reactivity parameter values whereas other conformers of both molecules could populate a common range of variation for those parameters.

To employ the COREPA method, conformer generation routines (Sections 3.1 and 3.2) are used to establish conformers of each chemical within a certain energy range of the lowest energy structure. The set of conformers of each chemical could be considered as statistical ensembles that, in turn, could impose weighing of the associated distributions (e.g. according to the Boltzman's statistics). Presently, however, we are reluctant to overestimate the gas-phase energetic assessment of conformers accounting for the complexity of biological interactions.

All conformers of a given chemical are plotted across a molecular descriptor axis, thus forming a discrete distribution for the chemical relative to the selected descriptor. For the global molecular descriptors, each conformer is represented by single point value of the parameter. For atomic parameters, several descriptor values associated with various local sites (atoms) of the conformer are allocated across the parameter axis. Each parameter point value is considered to be a midpoint of continuous probability distribution. Lorenz distribution has been used in the original COREPA implementation [10]

$$\phi(\chi) = \frac{\left(\frac{\mathit{\Gamma}}{2}\right)^2}{\left(\frac{\mathit{\Gamma}}{2}\right)^2 + (\chi - \chi_?)^2}\mathit{\Gamma} \tag{2}$$

where $\mathit{\Gamma}$ is the half-width of the distribution function around the probability maxima. The higher the values of $\mathit{\Gamma}$ the flatter are the density function $\varphi(x)$. Various probabilistic functions are in use in the present version of COREPA, including but not limited to:

Gaussian distribution:

$$\phi(x) = \frac{1}{h\sqrt{2\pi}}\mathrm{e}^{-(x-x_0)^2/2h^2} \tag{3}$$

Laplace distribution:

$$\phi(x) = \tfrac{1}{2}\mathrm{e}^{-x}. \tag{4}$$

The conformer distribution of a chemical is obtained as a normalized sum of these probability distributions. The normalization is performed by dividing the sum of individual distributions by the number of conformers. This normalization ensures the area of the obtained distribution to be unified, and hence the resulting distribution function to be considered as a probability density. Thus, for a sample of descriptor values $x_1, x_2, \ldots, x_n$ associated with a chemical a probability density function is constructed as

$$p(x) = \frac{1}{nh}\sum_{i=1}^{n}\phi\left(\frac{x - x_i}{h}\right) \tag{5}$$

known as the Kernel density estimation, where $\varphi(x)$ is any probability density function (called 'kernel') for which

$$\int \phi(x)\mathrm{d}x = 1, \qquad \int \phi(x)\mathrm{d}x = 0 \tag{6}$$

and h is a smoothing parameter defining the smoothness (wideness) of individual probability functions ($\mathit{\Gamma} \cong h$, for Lorenz density function). Apparently, the probability density estimation $\varphi(x)$ and resulting $p(x)$ depend on the value of the smoothing parameter h. The higher the h values the flatter are $\varphi(x)$ and $p(x)$. The relation between kernels $\varphi(x)$ and resulting probability density function $p(x)$ constructed as a sum of the Kernel density estimation is shown in Fig. 2.

Different methods could be used to define the optimal h values. Traditionally, parametric density estimations are employed when the type of distribution is predetermined and the fitting attempts to determine the parameters of an already specified distribution. For example, if a Gaussian probability

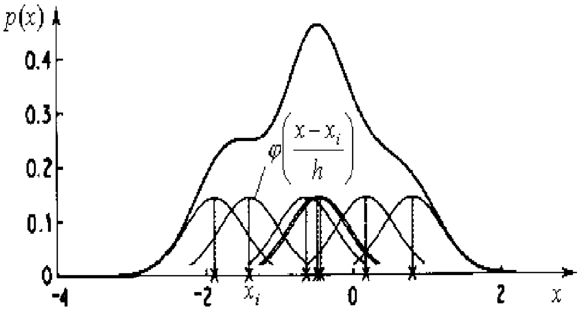


Fig. 2. Kernel density estimation.

density function is specified the fitting is aimed at determining the mean and variance of the data set. The obvious shortcoming of this approach is the need for proper specification of the density function. Thus, the smoothing parameter Γ associated with the Lorenz density function was defined empirically in the original COREPA implementation as 0.1–0.125 of the variation range for global molecular parameters, and 0.01–0.05 of the variation for local descriptors [10,14,15,43]. The non-parametric density estimation used in the present version of COREPA is the Kernel density estimation [44–46]. Here, the smoothing parameter can be determined using cross-validation or other available algorithms [47]. In COREPA, the h value is optimized by empirical approximation of data points, as specified in Ref. [47], according to equation

$$h = 3.5\sigma n^{-1/3} \tag{7}$$

where σ is the deviation over the data set points and n is the cardinality of the data set.

Fast calculation of kernel density is available through the use of fast Fourier transform [47].

In the original COREPA implementation, the probability density function could also be constructed as a product of Kernel probability distributions:

$$\rho(x) = \frac{1}{nh}\prod_{i-1}^{n}\phi\left(\frac{x-x_i}{h}\right). \tag{8}$$

A variation of the Kernel density formula is used to account for conformer multiplicity of chemicals, in COREPA:

$$\alpha_{ij} = \frac{p(C_j^{s_i})}{N_{ij}} = \frac{\mathrm{e}^{-\Delta E_j/RT}}{k\sum_{j=1}^{N}\mathrm{e}^{-\Delta E_k/RT}} \tag{9}$$

$$p(x\backslash \mathrm{class}_m) = \frac{1}{M_m}\sum_{i=1}^{M_m}\sum_{j=1}^{R_i}\sum_{k=1}^{N_{ij}}\frac{\alpha_{ij}}{h}\varphi\left(\frac{x-x_{ijk}}{h}\right) \tag{10}$$

where M_m is the number of chemicals in class m (chemicals with similar biological effect), R_i is the number of conformers of the ith chemical, N_{ij} is the number of parameter point values for the jth conformer of ith compound. $C_j^{S_i}$ denotes the jth conformer of ith chemical, and α_{ij} is the weighting coefficient of a conformer (with energy ΔE_j), based on Boltzman statistics. For the probability density estimation of a chemical M_m is set to 1.

The probabilistic distribution obtained by Eq. (4) is referred to as a COREPA of the group of chemicals across a selected molecular descriptor. Well-defined or distinct reactivity patterns are observed when the conformer distributions for the individual chemicals from the group are harmonized (in phase). In the present version of COREPA the probabilistic distributions are estimated across single stereoelectronic parameter. Currently, work on a multidimensional reactivity patterns is in progress. To date, the effect of various probabilistic functions on resultant reactivity patterns has not been studied, but will be included in further formulations of the approach.

The COREPA algorithm, according to its original version, consists of three steps. First, two subsets of chemicals are selected as training sets (step 1). The first subset consisted of chemicals having activity above a user-defined high activity threshold. The second subset includes chemicals having activity below a predetermined non-active threshold. (The generalization of the algorithm according to Eq. 9, allows more than one class of biologically similar chemicals to be selected and analyzed.) Next, a set of parameters, associated with biological activity is established (step 2) by evaluating the degree of overlap (in %) between the distributions associated with those thresholds. The parameters are evaluated based on the normalized sum of similarity indices between each pair of molecules in the training set [42]. The cutoffs, i.e. the part of the non-active area in common with the active pattern maximum can also be used as a measure of similarity [10]. The stereoelectronic parameters that provide the maximal measure of similarity among chemicals in the training subsets of active and inactive chemicals, and least overlap between overall patterns associated with those subsets (i.e. most distinct patterns) are assumed to be related to biological activity and used in the subsequent step of the algorithm. The COREPAs are described in terms of molecular descriptor ranges around the probability maxima of the probability distributions across relevant molecular descriptors (step 3). The width of these ranges depends on values of above discussed smoothing parameter. Ultimately, the general reactivity pattern for the biological activity of concern (SAR model) is obtained as a hierarchically ordered collection of the parameter ranges determined from reactivity patterns associated with each relevant molecular descriptor (see Section 3.5).

The dissimilarity between overall patterns of active and non-active chemicals, as well as between overall patterns and chemical-specific distributions, can be evaluated by an Euclidean distance metric (D) based on the squared differences between distribution densities over the entire range of the parameter variation. The Euclidean distance metric will also be used to compare distributions derived from different chemical training sets or for training sets derived from different weighting schemes (e.g. different energy thresholds for conformer selection; see Section 3.1.). The Euclidean distance metric can also be used to ascertain the extent to which an overall probability distribution of active or non-active chemicals is influenced by a specific chemical(s). In this respect, the 'stability' of a pattern is assessed statistically by a 'leave-one-out' procedure. The Euclidean distance metric is used to iteratively assess differences between patterns derived for n vs. $n-1$ chemicals in the training subsets. Variation of similarity indices, cut-offs between overall patterns of active and inactive chemicals, and associated parameter ranges can also be quantified. More stable patterns are associated with smaller Euclidean distances, variations in similarity indices, and corresponding parameter ranges.

The COREPA algorithm circumvents the problems in existing 3D pharmacophore mapping methods such as the:

- selection of active conformers (all energetically reasonable conformers are taken into account);
- predetermination of toxicophores (atomic sites and fragments with differing levels of generality can be used in establishing reactivity patterns);
- alignment of conformers to a lead compound (conformer distributions of chemicals are naturally ordered across descriptor axis with no necessity to overlap templates).

3.5. COREPA model decision tree and rule interpreter

The ultimate SAR model was described as a logistical decision tree. The latter consisted of multiple hierarchically ordered rules based on parameter ranges that comprise COREPAs. Boolean logic operators were used to establish 'rules' in the decision tree. If the value of a parameter calculated for a conformer is found in a range of the molecular descriptor defined by a confidence limit with a probability of $P\%$, around the pattern maximum, then it is assumed that the conformer meets the specific requirement with a probability $P\%$. If a chemical had to meet two successive stereoelectronic requirements to equal or exceed an activity threshold (with probabilities P_A and P_B, respectively), the total probability of meeting both requirements ($P_{A \text{ and } B}$) is obtained as a product of the probabilities of meeting the two requirements separately, i.e. $P_{A \text{ and } B} = P_A \cdot P_B$. If the value of the parameter calculated for a conformer falls outside of at least one of the parameter ranges, then the overall probability of having an activity above that specified threshold is 0. As seen, the approach offered flexibility in establishing hazard ranking protocols for unknown compounds based on choices of activity thresholds and confidence limits around pattern maxima. It must be stressed that the probability outcomes from the decision tree should not be viewed in absolute terms. Rather, the output from the algorithm permits a relative ranking of unknown chemicals in terms of their likelihood to have an activity above the user-defined threshold.

To simplify presentation of the rule interpreter, a binary version of the decision tree could be used. In the binary version, a value of 100% was assigned to chemical if at least one of its conformers fall within the range of the molecular descriptor defined by a confidence limit with a probability of $P\%$ around the pattern maximum. This simplified version provided a discrimination of chemicals as being active or non-active. Thus, the chemicals with similar binding affinity have at least one conformer that meets all the specified parameter ranges, whereas those from the other activity ranges should have no conformers that meet all the multi-parameter requirements simultaneously. The binary version of the rule interpreter is usually employed in the COREPA screening applications.

4. Dynamic techniques applied to toxicity studies

4.1. Selection of active conformers in 3D QSAR studies

Dynamic QSAR techniques for selection of active conformers were successfully employed for modeling the toxicity of unsaturated alcohols [1], semicarbazides [4], and α-terthienyls [48]. More recently these techniques have been applied to development of QSARs for the aryl hydrocarbon receptor (AhR) [3], and estrogen receptor (ER) [5] binding of congeneric chemicals. The results obtained are illustrated below by presenting the different schemes of deriving QSAR for AhR binding (log(1/EC50) of 14 polychlorinated biphenyls (PCBs) [3]. Multiple conformers (103), optimized by the PM3 Hamiltonian, were generated for the 14 compounds analyzed. A stacking type of interaction with AhR was hypothesized and the receptor affinity of PCBs was hypothesized to be correlated with the energies of frontier orbitals, particularly the energy of the lowest unoccupied molecular orbital (E_{LUMO}). This was chosen as a relevant molecular descriptor due to the experimental findings that charge-transfer complexes are obtained with a charge delocalization toward PCBs. A poor correlation between AhR binding and E_{LUMO} was found $r^2 = 0.38$ when all conformers were included in the correlation sample. It was not significantly improved ($r^2 = 0.43$; see Fig. 3a) when the most stable conformers were selected from the conformation sample (occupying 10% of E-level). If the most planar conformers were selected, however, the variance r^2 increased significantly, $r^2 = 0.72$ (Fig. 3b). The planarity of conformers was assessed quantitatively using the planarity index [3] obtained by a simple (normalized) summation of the dihedral angles. The conformers with minimum values of planarity index were specified as most planar.

Similar results were obtained for non-optimized conformers whose electronic structure was assessed by single point (1SCF) calculations. The selection of the most planar conformers yielded again a significant QSAR model, $r^2 = 0.78$.

It was summarized that the description of the most planar conformers of PCBs provides a correspondence to the binding data. One could suppose that the active conformers of PCBs taking part in the AhR receptor binding are the most planar rather than the most stable (ground state) ones, which also supports the experimental fact of charge-transfer complex formation as well as the hypothesis for a stacking type of interactions.

4.2. COREPA method for predicting estrogen receptor binding affinity

Recently, the COREPA approach has been evaluated for prediction of ER binding affinity in two companion papers [14,15].

The COREPA method has been used to develop a model to predict hERα binding affinity ranges using a training set of 45 chemicals (26 steroids and 19 non-steroidal ligands) [14]. Reactivity patterns were established for identifying and ranking compounds within a series of RBA ranges, of >150, 100–10, 10–1, and 1–0.1%. Local, global, and steric descriptors used in the COREPA model were restricted to those hypothesized to be associated with ER binding, based on previous studies with a variety of model receptors [7,41,42,49–55]. Through the COREPA analysis, the authors determined that RBA could be predicted based on three parameters, global nucleophilicity (represented by energy of the highest occupied molecular orbital, E_{HOMO}), interatomic distances between nucleophilic atomic sites, and electron donor capability of these sites. The reactivity profiles for one of the three descriptors associated with ER binding affinity, E_{HOMO}, is illustrated in Fig. 4, where the pattern of non-active ligands ($0.01 > \mathrm{RBA} > 0.00\%$) (white distribution in Fig. 4a–d) is compared with the pattern of active ligands of decreasing binding affinity, $\mathrm{RBA} > 150\%$ (Fig. 4a), $150 > \mathrm{RBA} > 10\%$ (Fig. 4b), $10 > \mathrm{RBA} > 1\%$ (Fig. 4c), and $1 > \mathrm{RBA} > 0.1\%$ (Fig. 4d). The E_{HOMO} ranges (in eV), obtained as a function of the confidence limit [%] around the E_{HOMO} probability maximum, clearly show a shift in the E_{HOMO} pattern toward higher global nucleophilicity with increasing

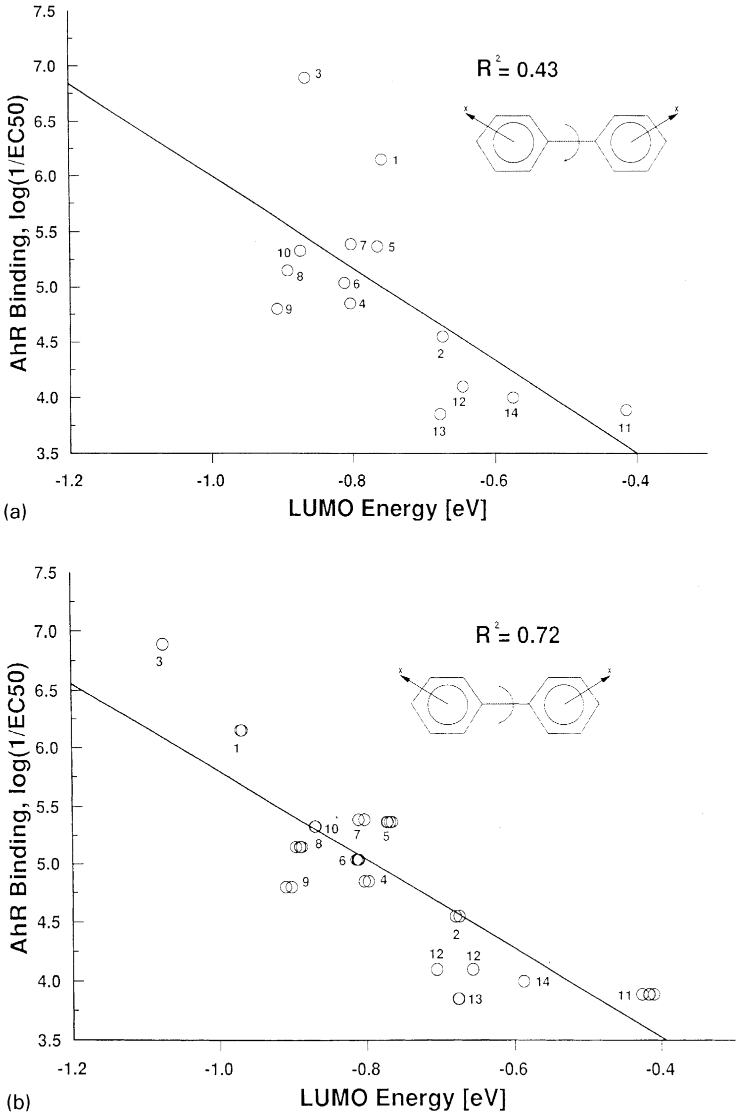


Fig. 3. Variation of observed AhR binding affinity versus energy of lowest unoccupied molecular orbitals (E_{LUMO}) for PCBs: (a) lowest energy conformers; (b) most planar conformers.

RBA values. Thus, the lower boundaries of these ranges −8.82, −9.6, −9.7, and −9.8 eV (one-sided E_{HOMO} ranges are used in the decision tree) correspond to the above four activity ranges. This result is consistent with the hypothesis that more active ER ligands have higher nucleophilicity [5,53].

The steric and electronic requirements associated with each RBA range were organized in a hierarchical decision tree, whose output is an estimated probability that a conformer would bind to the hER within a given RBA range (Fig. 5). Prescreen criteria, based on the necessary structural requirements for eliciting minimal ER binding affinity, i.e. RBA ≥ 0.1% are used to accelerate the screening. Conformers which have E_{HOMO} values of less than −10.0 eV, nucleophilic sites R: C, O, N, Cl, F, and S not meeting the least conservative charge screen of −0.33 to −0.22 a.u., at least one cyclic fragment or steroids not conforming stereochemical requirements of

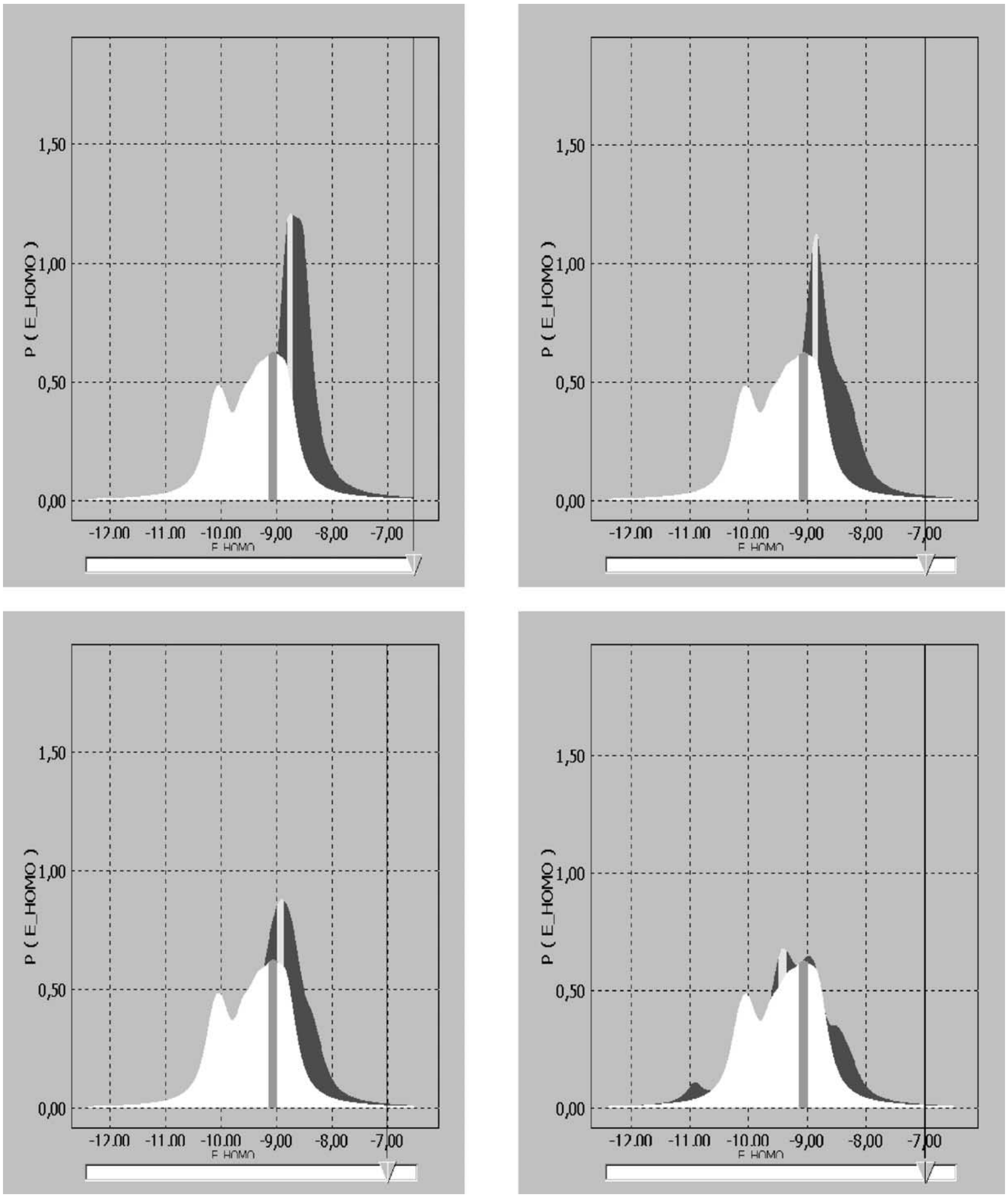


Fig. 4. The reactivity patterns based on E_{HOMO} for active ER ligands: RBA $>$ 150% (a), 10 $<$ RBA $<$ 100% (b), 1 $<$ RBA $<$ 10% (c), and 0.1 $<$ RBA $<$ 1% (d), compared with the pattern for inactive ligands with 0 $<$ RBA $<$ 0.01%; the integral reactivity pattern of active ligands is in red, whereas the pattern of non-active ligands—in white.

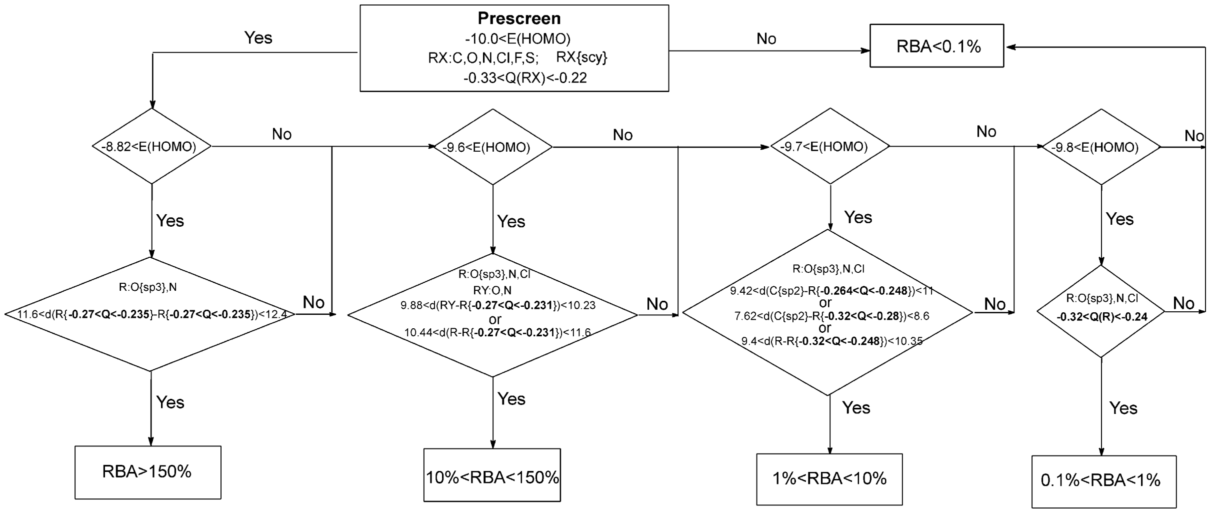


Fig. 5. A decision tree for identification of estrogen ligands with RBA values $> 0.1\%$. The decision tree is based on a prescreen followed by a sequence of rules based on reactivity patterns derived from ligands with RBA values $>150\%$, between 10 and 100%, between 1 and 10% and between 0.1 and 1%.

the natural enantiomer were assigned a 0% probability to bind to ER with a RBA $> 0.01\%$. Conformers that passed these absolute requirements are then compared consecutively to the combined E_{HOMO}/distance/charge screens associated with RBA ranges, in decreasing order of activity. If a compound is identified as belonging to an RBA range it is not further screened for lower activity ranges. Thus, the decision reflects a sequential ordering of the reactivity patterns derived from different RBA ranges.

Conformers that passed these absolute requirements were then compared to the E_{HOMO} (-8.99 eV $< E_{\mathrm{HOMO}}$), interatomic distance ($11.77 < d(\mathrm{R{-}R}) < 12.22$), and charge ($-0.272 < Q(\mathrm{R}) < -0.233$) screens associated with the activity pattern of chemicals having RBA $> 150\%$. The identification of a ligand with a binding affinity within a RBA range requires that at least one conformer meets all three specified parameter ranges. If a compound was not identified as having an RBA $> 150\%$, it was then screened to determine if it had an RBA between 10 and 100% (E_{HOMO} (-9.44 eV $< E_{\mathrm{HOMO}}$), interatomic distance ($10.62 < d(\mathrm{R{-}R}) < 10.95$), and charge ($-0.273 < Q(\mathrm{R}) < -0.236$)), and so on until all activity range screens are applied.

Recently, much larger and structurally more diverse training sets were used to extend the COREPA model for ER binding affinity. For that purpose, data for 242 chemicals evaluated for rER affinity, and another dataset of about 318 chemicals with ERs from several mammalian species (human, rat, and mouse) were collected. As an extension of previous hER models, the rat and mammalian models have also been expended to allow predictions to the lower ranges of RBA $= 0.0001$ (log RBA $= -4$) by using two sided distance rules and widening the range of acceptable global nucleophilicity, allowing untested chemicals to be sorted by predicted ER binding affinity into ranges, or 'bins', from $>100\%$ RBA to <0.0001. The same molecular descriptors were found to be used in the models, regardless of the size of training set and bioassays used. The modeling approach has also been applied to a more homologous series of chemicals (alkylphenols) with relatively weak binding affinity, to determine more precise predictors of activity within a confined range, and, in this instance, for the more biologically complex endpoint of gene activation [43].

4.3. COREPA method for discrimination of estrogen receptor antagonists from agonists

The models developed to predict the relative binding affinity of chemicals to ER can be used for prioritization for further tiered biological testing to assess their potential for endocrine disruption. These

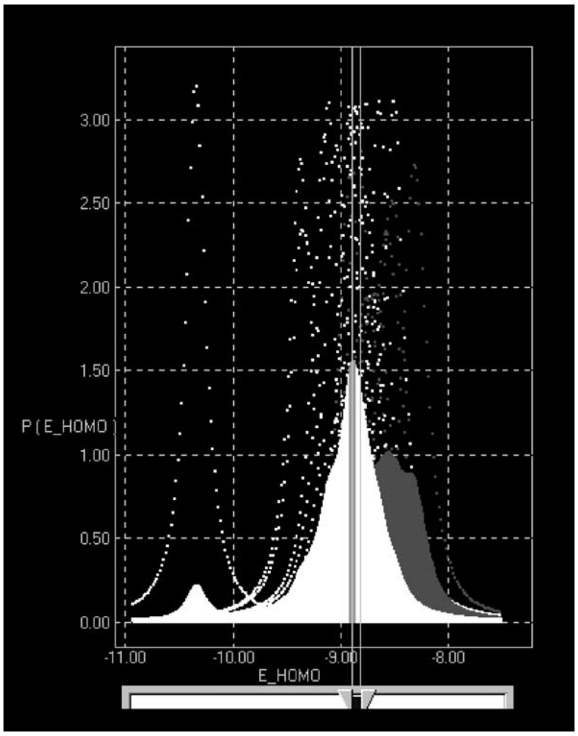


Fig. 6. Reactivity pattern for ER agonists (white) and ER antagonists (red) based on E_{HOMO}.

models, however, are not able to distinguish potential receptor antagonism from agonism, and hence the type of in vivo response. Recently, the COREPA approach has also been employed as a discrimination tool for ER antagonists from agonists [56]. It has been suggested that steroid receptor antagonists are less compact than agonists, which eventually prohibits proper alignment of the former by the receptor protein helices. As a result of the alignment prohibition the transactivation process is prevented. The COREPA model developed a reactivity pattern for ER antagonists and agonists by employing chemical bulk as a defining parameter of antagonism. A dataset of 23 potent ER ligands (16 agonists, seven antagonists) has been used. The molecular parameters previously found to be associated with ER binding affinity, namely global (E_{HOMO}) and local (donor delocalizabilities and charges) nucleophilicity and steric distances between nucleophilic sites, were found insufficient to discriminate ER antagonists from agonists (Fig. 6). However, parameters related to molecular bulk, including solvent accessible surface and negatively charged Van der Waal's surface, provided reactivity patterns that were 100% successful in discriminating antagonists from agonists in the limited data set tested (Fig. 7). The model also shows potential to discriminate pure antagonists from partial agonist/antagonist structures. The derived model could be useful for predicting additional chemicals for their ability to bind but inactivate the ER, providing a further tool for hypothesis testing to elucidate chemical structural characteristics associated with estrogenicity and anti-estrogenicity.

4.4. Screening of EU chemical inventories for ER ligands

Models derived to predict ER binding affinity were preliminarily validated by screening of the EU chemical inventories for potentially active ER ligands and subsequent experimental testing of a limited number of selected chemicals using a series in vitro and in vivo assays [57,58], including an hERα-binding assay and a reporter-gene assay (ERE-CALUX). The EU inventories included 908 chemicals in a high production volume chemicals (HPVCs)

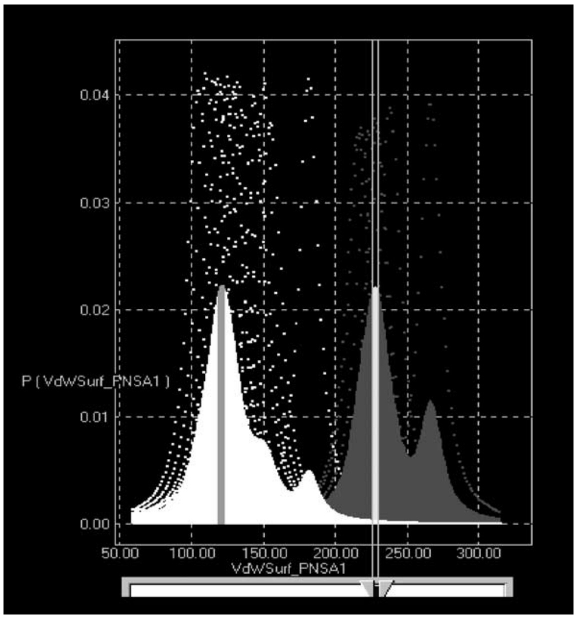


Fig. 7. Reactivity pattern for ER agonists (white) and antagonists (red) based on the calculated global 3D parameter of Van der Waal's partial negative surface area.

database and 65,232 individual records in a low production volume chemicals (LPVCs) database. The numerous chemicals were organized in 3D databases with quantum-chemically (AM1) optimized geometries and with assessed electronic structure. While the model was developed using all energetically-reasonable conformers for each chemical in the training set, the rapid screening of $>65{,}000$ chemicals for ER binding potential was based on a single optimized conformer representing each chemical. The 'directed tweak' [24] flexible conformer search algorithm (see Section 2), was used for conformer selection, to avoid a possible computational explosion when screening tens of thousands of chemicals where 10–100 conformers might be generated per chemical. (New approaches, using a combination of the GA method for computational coverage (Section 3.1) with the tweak approach, are currently under investigation for application to extremely large datasets.) Each chemical from the databases was processed through the decision tree by making use of the interpreter that permits the use of stereoelectronic structure-based rules based on an extended SMILES notation. Chemicals predicted to be within a RBA range had at least one conformer that met parameter ranges associated with that range, whereas those from the other RBA ranges had no conformers within parameter requirements.

The screening of the HPVC database predicted seven chemicals to be active binders, i.e. having ER binding affinity greater that 1% relative to 17β-estradiol, with one of these chemicals to have affinity in the range of $10\% < \text{RBA} < 100\%$ (bisphenol A diglicidyl ether, CAS 1675543). In vitro tests were then done based on these predictions. Eighteen (18) chemicals were tested in an hERα-binding assay (four of the seven predicted as active ER binders were chosen), and 14 chemicals predicted to be inactive (in this case inactives were those predicted to have $<1\%$ binding affinity). An ERE-CALUX reporter gene assay was also used to test 16 of the chemicals (including the four chemicals predicted active). Out of the four chemicals predicted to be active ($\text{RBA} > 1\%$), two were found to be weakly estrogenic in the hERα-binding assays [bisphenol A (CAS 80057); and bisphenol A diglycidyl (CAS 1675543)] and two were found active, i.e. activated the reporter gene, in the ERE-CALUX assay [bisphenol A (CAS 80057); and 2,2′-dithiodibenzoic acid (CAS 119802)]. Fourteen chemicals predicted to be inactive by the QSAR model (i.e. have $\text{RBA} < 1\%$) were also found to be inactive in the hERα-binding assay; and among these, 13 chemicals were also identified as non-active in the ERE-CALUX assay.

The above screening predictions did not indicate any highly potent ER ligands (with $\text{RBA} > 150\%$ of 17β-estradiol) among HPV chemicals. In an attempt to test the predictions of higher potency, the LPVC inventory was screened for predicted higher ER binding potency ($\text{RBA} > 10\%$). An additional experimental validation was undertaken in which 10 chemicals predicted by the QSAR model to be have $\text{RBA} > 10\%$ were selected from a list of 200. The 10 chemicals were testing in the ERE-CALUX and hERα-binding assays. The results showed that ERE-CALUX and hERα-binding assays validated QSAR predictions with no outliers (false negatives or false positives), i.e. all 10 chemicals were found to bind to the ER and turn on gene expression. Moreover, these tests confirmed identification of some extremely active ER ligands, such as meso-3,4-bis(4-hydroxyphenyl)hexane (CAS 84162) 3,4-bis-(4-acetoxyphenyl)-2,4-hexadiene (CAS 84195), and fenoterol hydrobromide (CAS 1944123), which appear to be more potent than estradiol, according to theoretical (QSAR) and experimental data. The results from ERE-CALUX and hERα-binding assays were confirmed by additional in vitro assays. For more details on this QSAR screening and validation exercise refer to Ref. [58].

5. Conclusions

The principle difficulties in developing mechanistically sound SARs for receptor-mediated events in toxicology, with application to additional mechanisms, are discussed. Development of such models requires one to account for the 3D character of the interaction causing the toxic event as well as the selection of active conformations to represent the chemical in SAR models. Techniques we have recently developed to address these issues were reviewed in the present work. The approaches taken follow the basic assumption that chemical behavior in complex biological systems is context-dependent.

A molecule can exist and interact in a variety of conformations, with solvation and binding interactions capable of compensating for energy differences among the conformers. It was shown that selection of active conformers in QSAR studies is as important as the selection of appropriate molecular descriptors because the conformers of same chemicals differ significantly in their descriptor values.

In the approach for selection of active conformers in QSAR studies, biological activity is modeled as a function of molecular descriptors derived from specifically selected active conformers, rather than as a property derived from the lowest-energy gas-phase conformer. In a recent pattern-recognition approach the COREPA of structurally dissimilar chemicals acting via the same mechanism of action is determined. This is based on the assumption that chemicals, which elicit similar biological behavior through a common mechanism should possess a commonality in their stereoelectronic (reactivity) pattern. Elucidation of this pattern requires examination of the conformational flexibility of the compounds to allow evaluation of molecular similarity in the context of specific interaction.

Applicability of both techniques were illustrated by presenting QSAR models derived for Ah binding affinity of PCBs and ER binding affinity of structurally diverse chemicals. COREPA models were found to be promising in 3D screening of large chemical inventories, as demonstrated by experimental validation within the EDAEP project.

Besides the impact of conformational flexibility of chemicals in QSAR the role of different molecular descriptors is discussed. In this respect, a classification of descriptors according to their ability to describe molecular interaction is suggested. This classification could be useful as guidance for selection of appropriate molecular parameters for modeling different toxic endpoints according to specificity of the underlying interactions.

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