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Structure-based estimation of enzymatic hydrolysis rates and its application in computer-aided retrometabolic drug design

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After a brief review of the problems related to the description of enzymatic hydrolysis rates and the quantification of steric effects, a recently developed method that uses the inaccessible solid angle Ω_h calculated around different atoms as a novel steric parameter to estimate human blood *in vitro* enzymatic hydrolysis rates in noncongener ester series is summarized. Some illustrative results obtained by the integration of this method into the expert system developed for computer-aided soft drug design are also presented. Starting from a lead compound, the system can provide full libraries of possible new "soft" molecular structures, a ranking order of these candidates based on isosteric-isoelectronic analogy to the lead, and estimated hydrolytic half-lives for all structures of interest.

1. Introduction

As already summarized on a number of occasions by now, retrometabolic drug design approaches are systematic methodologies aimed to improve the therapeutic index of a drug molecule that thoroughly integrate structure-activity and structure-metabolism relationship in the design process [1, 2]. They include two distinct methods to design soft drugs [3, 4] and chemical delivery systems [5, 6], respectively. Because enzymatic hydrolysis plays an important role in the metabolism of therapeutic agents designed by these approaches, we were long interested in developing structure-based methods to describe and eventually predict the rate of these enzymatic reactions. Such methods are also of considerable general interest for anyone working with ester- or amide-containing compounds including prodrugs. Unfortunately, the complexity of the involved issues makes the development of generally applicable methods considerably difficult. In the present paper, after a review of the problems related to carboxylic ester hydrolases and the quantification of steric effects, we will briefly summarize a recently developed method [7] that uses the inaccessible solid angle as a novel steric parameter to estimate human blood *in vitro* enzymatic hydrolysis rates in non-congener ester series. We will also present some results obtained by the integration of this method into our expert system [4, 8, 9] for computer-aided soft drug design.

1.1. Carboxylic ester hydrolases

Carboxylic ester hydrolases (EC 3.1.1) efficiently catalyze the hydrolysis of a variety of ester-containing chemicals to the respective free acids. They exhibit broad and overlapping substrate specificity toward esters and amides, and the same substrate is often hydrolyzed by more than one enzyme. Consequently, their classification is difficult and still is in a somewhat confused state, despite the important roles that carboxylesterase (EC 3.1.1.1) and/or other carboxylic ester hydrolases, such as arylesterase (EC 3.1.1.2) and cholinesterase (EC 3.1.1.8), play in the metabolism of many xenobiotics [10–14]. Humans have been shown to express carboxylesterase in the liver, plasma, small intestine, brain, stomach, colon, macrophage, and monocytes [14].

Esterase activity varies quite strongly between species. The stability of acyloxyalkyl type esters, most frequently employed in prodrug and soft drug designs, usually increases in the rat < rabbit < dog < human order [15, 16], but there might be considerable variability. Rodents tend to metabolize ester-containing drugs much faster than humans. *In vitro* hydrolytic half-lives ($t_{1/2}$) measured in rat blood were often found orders of magnitude lower than those measured in human blood. The rank order of compounds tends to be similar in different biological systems, but even this cannot be considered a general rule [17, 18].

1.2. Quantitative structure-metabolism relationships

Unfortunately, it seems to be even more difficult to predict the rate of enzymatic hydrolysis ahead of synthesis, and only relatively limited useful data are available. As in quantitative structure-activity relationship (QSAR) studies [19], steric, lipophilicity, size/polarizability, and electronic parameters should be useful in establishing quantitative structure-metabolism relationships (QSMR). A number of QSMR studies limited to individual prodrug series have been made, but they did not establish any relationship of general validity [20–24]. To enhance computer-aided retrometabolic drug design, we needed a more general equation that can give structure-based estimates and that is not limited to congener series. Recently, we obtained one such equation that based on AM1-optimized structures was able to account for 80% of the variability in the log *in vitro* human blood hydrolysis half-lives of 67 compounds belonging to seven different classes [7]. Experimental data used for correlation were for two β-blocker series with ultrashort duration of action [15, 17, 25–27], short-acting antiarrhythmic agents [28, 29], ultra-short-acting angiotensin converting enzyme inhibitors [30], opioid analgetics [31], soft corticosteroids [32], and buprenorphine prodrugs [33]. Three, separate ester-containing drugs with available experimental data and completely unrelated structures were used to test the predictive power of the model: vinyl acetate [34], isocarbacyclin methyl ester (TEI-9090) [16], and glycovir [35]. The main novelty of this approach was the introduction of the inaccessible solid angle Ω_h as a novel, general steric parameter not yet explored in pharmaceutical studies.

1.2.1. Modeling steric effects

Steric effects obviously play an important role in chemical or enzymatic reactions, and numerous methods have been developed for their quantification; they have been reviewed on a number of occasions [19, 36–39]. However, steric effects are inherently difficult to characterize as they strongly depend on the three-dimensional structures involved, and these can vary considerably due to intramole-

cular motions and intermolecular interactions. In addition, 3D structures for drug receptors are rarely known with adequate accuracy.

Despite an interest in steric effects as early as the late 1800's [40–44], the first successful quantitative parameter, Taft's steric constant E_s, was introduced only in the 1950's [45, 46] following an earlier proposition of Ingold [47]. This steric constant was defined based on the change in the rate constant k of the acid catalyzed hydrolysis produced by a substituent X in X-CH_2COOR type esters:

$$E_s = \log \left(\frac{k_X}{k_H} \right)_A . \qquad (1)$$

Following a suggestion by Hansch et al., in order to reference the E_s scale to hydrogen, published values are usually re-scaled by subtraction of 1.24, a value obtained for the hydrolysis of HCOOR [36]. Despite a long-suspected contamination with electronic effects, this experimentally derived constant was the only available steric parameter that proved successful for a long time. A number of variations have been introduced; for example, Hancock introduced E_s^c values corrected for the number of α-hydrogen atoms, n_H, in an attempt to account for possible hyperconjugation effects [48]:

$$E_s^c = E_s + 0.306(n_H - 3) . \qquad (2)$$

To overcome problems with steric parameters of unsymmetrical substituents, Fujita et al. expressed the E_s^c values of $-CR^1R^2R^3$ type substituents as the weighted sum of the individual E_s^c values of R^1, R^2, and R^3 [49].

Charton was the first to introduce a general steric parameter based more closely on geometric consideration [50, 51]. For symmetrical substituents such as CX_3, he defined three different van der Waals radii: one along the group axis ($r_{ax} = d_{CX} \cos \varphi + r_X$) and two in perpendicular directions to the group axis ($r_{max} = d_{CX} \sin \varphi + r_X$, $r_{min} = d_{CX} \cos \varphi \cos 60° + r_X$). Noticing that they, and especially r_{min}, tend to correlate well with E_s, he defined a new steric parameter υ that was re-scaled using the radius of the hydrogen atom r_H:

$$\upsilon = r_{min} - r_H = r_{min} - 1.20 . \qquad (3)$$

However, generalization of this geometrical definition was not straightforward for unsymmetrical substituents. Consequently, he used correlations with experimental log $(k_X)_A$ values to calculate υ_{eff} values for such substituents. Taft's E_s and Charton's υ have indeed been shown to be strongly correlated [36]. Bowden and Young used a steric substituent constant R, calculated using molecular models as the distance from the atom to which the substituent is bonded to the periphery of the van der Waals radius of the substituent [52]. A set of more complex directional parameters, the STERIMOL parameters L, B_1–B_4, and B_5, were introduced based on quite similar geometric considerations [53, 54]. They were defined as the length of the substituent along the axis of substitution (L) and four width parameters perpendicular to this axis and forming 90° angles with each other ($B_1 < B_2 < B_3 < B_4$). Later, B_5, a width parameter having the largest value independent of the angle relative to B_1, was also introduced. The B_1 parameter showed high correlation with Charton's υ constant, which has a very similar definition, and also with Taft's E_s [38]. A substituent steric effect index (Ξ) based on the molecular graph and introduced by Kier [55] that also correlates well with E_s probably also should be mentioned here.

Such linear measures proved useful descriptors in a number of cases, but since steric effects result from three-dimensional structures, some measure of the spatial angle around the reaction center should give a less arbitrary and more accurate description of steric accessibility. Within this context, Tolman introduced cone angles obtained from CPK models to characterize steric effects of phosphorus ligands [56, 57]. The increase in computational power made it possible to calculate more rigorous measures of steric accessibility, and in 1984 Seeman et al. used the accessible solid angle Ω evaluated with a Monte Carlo sampling as a measure of the geometric accessibility factor for nitrogens in pyridines [58]. In an attempt to generalize this concept, Sakakibara, Hirota, et al. defined a steric substituent constant Ω_s, which basically represents the portion of the total solid angle that is hindered by the substituent considered [59–63]. They used molecular mechanics optimized structures and a population-weighted average value obtained from different possible conformers to account for conformational effects. A reasonable correlation was obtained between Ω_s and E_s ($r = 0.887$) that improved considerably when only alkyl substituents having no heteroatoms were considered ($r = 0.953$), suggesting that electronic contamination in E_s for heteroatom containing substituents may be a possible cause for deviation. White, Taverner et al. also attempted the quantification of steric effects by the use of solid angles while developing a different method of calculation [64–66].

1.2.2. The inaccessible solid angle

The solid angle Ω subtended at a point O by an arbitrary surface S is defined by the surface integral

$$\Omega = \int_S \frac{r \cdot dS}{r^3} , \qquad (4)$$

where r is the position vector of the element of surface dS with respect to O (Fig. 1). For a spherical surface centered at O, $\Omega = S/r^2$. As the angle α subtended by a circular arc of length l is $\alpha = l/r$, the solid angle can be considered a three-dimensional generalization of the two-dimensional (planar) angle concept. Ω is measured in steradians, and the steric angle subtended by a full sphere is 4π steradians (in contrast to the 2π radians angle subtended by a full circle). The area of the shadow of the surface S projected on a circumscribing sphere by a light placed in the center O is a good visual measure of the solid angle Ω, since, after all, Ω represents 4π times the ratio of this area to that of the whole sphere (Fig. 2).

Based on spatial considerations, the accessible solid angle probably represents the most rigorous structure-related measure of the probability that a reagent molecule can access the reaction center in a given configuration. Conse-

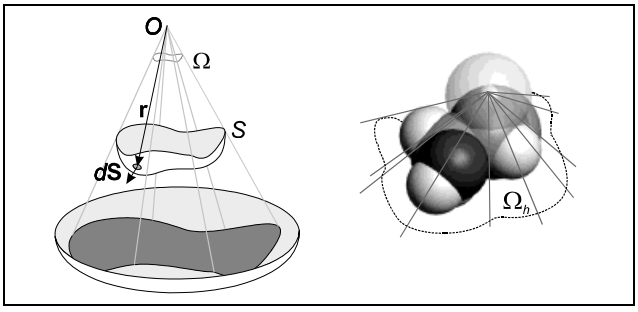


Fig. 1: Definition of the solid angle Ω subtended at a center O by an arbitrary surface S. The figure on the right side illustrates the inaccessible solid angle Ω_h hindered around a selected atom (shown at top, as a transparent surface with a white center) by a 1-chloro-2-hydroxyethyl substituent (chlorine in the back). See also Fig. 2

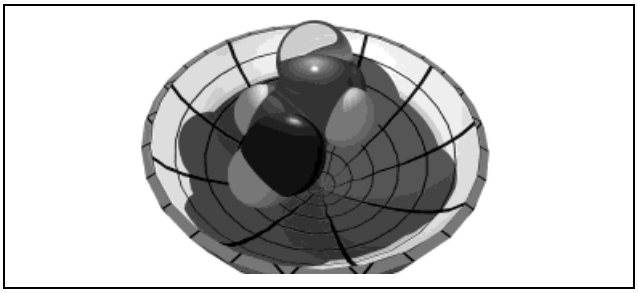


Fig. 2: A three-dimensional illustration (made using Ray Dream Designer 3.0) of the inaccessible solid angle Ω_h hindered around a selected atom (shown at top as a transparent "glass" sphere) by a 1-chloro-2-hydroxyethyl substituent (see also Fig. 1). The area of the shadow projected by the light placed in the center of the selected atom on a circumscribing sphere is proportional with Ω_h. For transparency, this atom had to be made of "glass", which also caused a distortion of the light-rays passing through it (top of the figure)

quently, the inaccessible solid angle Ω_h, the solid angle at which access to a reaction center is hindered by substituents (Figs. 1, 2), should be a good measure of steric hindrance. A main advantage over other previously used steric parameters is that the solid angle is calculable for any molecular structure and is, by definition, independent of electrical or transport-related effects. Sufficiently accurate evaluations of the total accessible or inaccessible solid angle around a center of interest can be done reasonably fast using computer models. The only major problem that has to be adequately treated is the conformation dependency of Ω_h. For sufficiently flexible molecules, the value of Ω_h as defined here may vary considerably; therefore, some energetic considerations have to be included in any reasonable treatment. Molecular structures used for the present calculations were optimized using the AM1 method [67] of the MOPAC interface in Sybyl 6.3 (Tripos, Inc., St. Louis, MO) on a Silicon Graphics Origin 2000 deskside server.

The total inaccessible steric angle Ω_h used here was computed with a numerical algorithm implemented by us and integrated within our previous computer software package [7]. It is evaluated using a numerical procedure where the directional sampling points are obtained with a regular sampling grid using spherical coordinates. We found that at least 20,000 sampling directions were needed for sufficient accuracy. We used about 25,000 during the evaluation of the model, and about 60,000 for the final results. A 2D projection of a sampling grid on the spherical surface obtained at a much lower sampling density is presented in Fig. 3. For every considered atom, any direction that somewhere crossed the van der Waals surface of another atom in the molecule was considered as hindered, and the Ω_h values used here simply represent the percentage of hindered direction points: $\Omega_h = 100 \times N_{hindered}/N_{total}$. Calculations were performed with a van der Waals radii set identical to that employed in our previous studies [68, 69], but the algorithm also allows the evaluation of Ω_h values with an arbitrary radii scaling factor that uniformly increases or decreases all atomic radii employed in the calculation.

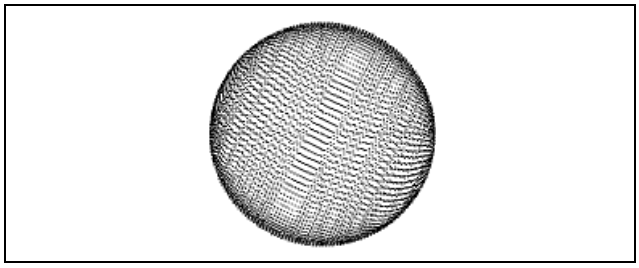


Fig. 3: A 2D projection of a sampling grid used on the spherical surface by the present algorithm for the numerical evaluation of the total inaccessible solid angle Ω_h. The actual grid used in the computation was considerably denser

2. Investigations, results, and discussion

2.1. Modeling log $t_{1/2}$ for enzymatic hydrolysis

Using the mentioned data [7], we found steric effects having the most generally valid influence on the rate of enzymatic hydrolysis as measured by the *in vitro* half-life in human blood. Lipophilicity and some of the electronic parameters, such as the charge on the carbonyl C (q_C), also proved informative, but to a much lesser degree. As expected, the half-life was found to increase with increasing steric hindrance around the ester moiety. Interestingly, the steric hindrance as measured by Ω_h around the carbonyl sp^2 oxygen ($\Omega_h^{O=}$) had by far the most significant correlation with the rate of metabolism as measured by log $t_{1/2}$ [7]. We believe that this provides evidence for the important role played by hydrogen bonding at this oxygen atom in the mechanism of this reaction, as will be discussed later.

Ω_h varies over a relatively small range, and a 10% variation in $\Omega_h^{O=}$ seems to cause a change of almost two log units in the rate of hydrolysis. Therefore, for each compound, considerable care had to be given to find the AM1-optimized conformation that has the sp^2 oxygen in its most accessible position.

Meanwhile, for a set of 40 structurally diverse simple methyl esters used to develop and test our program, $\Omega_h^{C=}$ actually gave a better correlation with Taft's E_s steric constant than $\Omega_h^{O=}$ [7]. This suggests that the ability of this new approach to compute steric hindrance at different atoms might be useful in distinguishing between different mechanisms. It also should be mentioned that the inaccessible solid angles computed here for this set of 40 structures did not give very good correlations with Taft's steric constant. Multiple halosubstituted compounds gave the largest deviations, suggesting again that electronic effect may indeed be still contaminating E_s. The correlation obtained with our calculated values on these 40 data were about the same quality as those obtained with the Ω_s steric substituent as defined by Sakakibara, Hirota et al. ($r^2 = 0.78$) and taken from their publication [62].

The obtained final equation (eq. 5) that will be used to estimate log $t_{1/2}$ values also includes the AM1-calculated charge on the carbonyl carbon ($q_{C=}$) and a calculated log octanol-water partition coefficient (QLogP) [68, 70, 71] as parameters and accounts for 80% of the variability in the log half-lives of 67 compounds (Fig. 4). All its parameters are statistically relevant ($p < 0.01$), but the present form was obtained after omission of twelve outliers. However, eight out of the twelve compounds omitted from the final correlation have very short half-lives that are difficult to determine and the corresponding experimental error might be considerable especially on a log scale.

$$\log t_{1/2} = -3.805(\pm 1.412) + 0.172(\pm 0.012)\ \Omega_h^{O=} - 10.146(\pm 3.439)\ q_{C=} + 0.112(\pm 0.044)\ \mathrm{QLogP}\,,$$
$$n = 67\,, \quad r = 0.899\,, \quad \sigma = 0.356\,, \quad F = 88.1 \qquad (5)$$

All of the parameters in this equation are calculable from the molecular structure making possible estimates even for compounds that have not been synthesized yet. This equa-

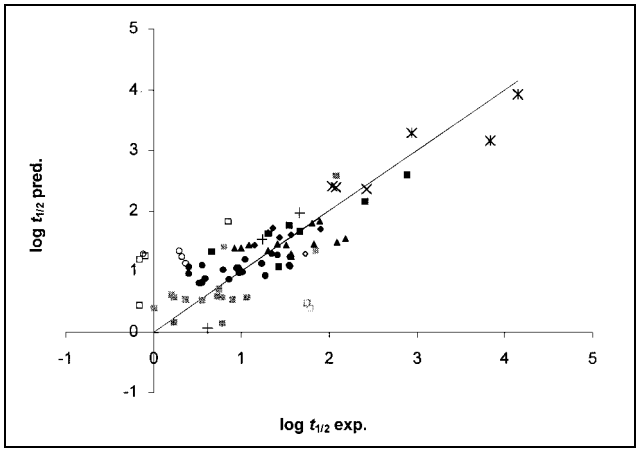


Fig. 4: Predicted vs. experimental log half-lives for data included in the present study. Compounds not included in the final correlation were denoted with an open symbol. ■ β-blockers, ■ β-blockers, ◆ ACE inhibitors, ▲ opioid analgetics, ✳ soft corticosteroids, ● antiarrhythmics, × pro-buprenorphines, + others, predicted

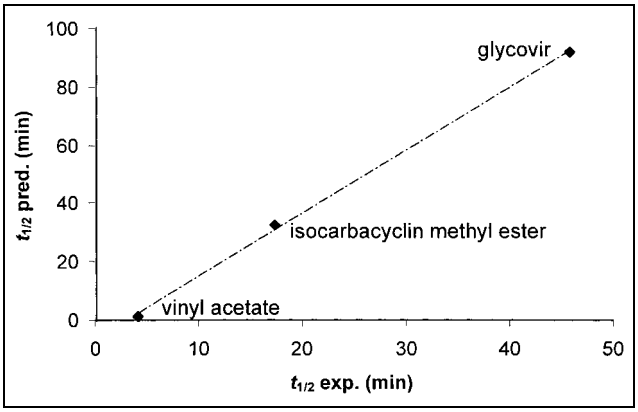


Fig. 5: Predicted (eq. 5) vs. observed in vitro human blood hydrolysis half-lives for three ester-containing compounds with unrelated structures used to test the predictive power of the QSMR model

tion is especially promising since it agrees very well [7] with a mechanism for hydrolysis by carboxylesterases recently proposed based on conserved motifs in various carboxylesterases and following other, similar mechanisms [14]. This mechanism involves Ser^{203}, Glu^{335}, and His^{448} as a catalytic triad, where low-barrier hydrogen bonds facilitate a general base mechanism for the acylation of Ser^{203}, and Gly^{123}-Gly^{124} as part of an oxyanion hole, where weak hydrogen bonds stabilize the tetrahedral adduct. The sequences required for the hydrolytic capability at the catalytic triad of carboxylesterase, acetylcholinesterase, butyrylcholinesterase, and cholesterol esterase are highly conserved [14]. A more positive carbon ($q_{C=}$) is more prone to the nucleophilic attack by the serine oxygen decreasing log $t_{1/2}$, and a less accessible carbonyl oxygen ($\Omega_h^{O=}$) is more difficult to stabilize by hydrogen bonds in the oxyanion hole increasing log $t_{1/2}$. The strong influence on the rate of reaction of the steric hindrance around the carbonyl sp^2 oxygen atom may suggest that the hydrogen bonds in the oxyanion hole play an important, possibly even rate-determining, role during the reaction [7].

The correlations obtained are not very good, but considering that we have biological data on seven different drug series from a number of different investigators, they can be considered quite informative. In addition, since most likely a number of different enzymes are involved in the hydrolysis of these compounds, one can hardly expect any general description at this level to give a significantly better overall fit. It has to be mentioned, however, that within some of the series a number of compounds were found not to be metabolized in any significant amount and the corresponding (large) $t_{1/2}$ were not reported at all. For most of them, our model failed to predict a half-life significantly larger than those of their structurally similar analogues. It is possible that some of their structural features hinder the fit into the active site of the metabolizing enzyme(s), but no such features were obvious.

The predictive power of the model was tested on three separate drugs with completely unrelated structures and available experimental data. For vinyl acetate [34], isocarbacyclin methyl ester [16], and glycovir [35] measured vs. predicted *in vitro* human blood half-lives (min) are 4.1, 17.3, and 45.9 vs. 1.2, 32.6, and 92.0 min, respectively, in good linear agreement with the experimental data (Fig. 5). These prove that even if at present one cannot predict accurate hydrolytic half lives for arbitrary structures, an unrealistic goal that we never expected to achieve, the present method is useful in distinguishing among compounds whose hydrolysis is fast, medium, or slow based on chemical structure alone.

2.2. Applications in computer-aided retrometabolic drug design

Soft drug design aims to design safer drugs with an increased therapeutic index by integrating metabolism considerations into the drug design process [3, 4]. Soft drugs are active isosteric-isoelectronic analogues of a lead compound that are deactivated in a predictable and controllable way after achieving their therapeutic role. Hence, they are obtained by building into the molecule, in addition to the activity, the most desired way in which the molecule is to be deactivated and detoxified. Owing to the considerable flexibility of retrometabolic drug design, for certain lead compounds a large number of possible soft structural analogues can be designed, and finding the best drug candidate among them may prove tedious and difficult.

Fortunately, computer methods developed to calculate various molecular properties, such as molecular volume, surface area, charge distribution, polarizability, aqueous solubility, or partition coefficient make possible more quantitative design [68−75]. The capabilities of quantitative design have been further advanced by developing expert systems that combine the various structure-generating rules of soft drug design with the developed predictive softwares to provide an ranking order based on isosteric/isoelectronic analogy [4, 8, 9]. This is taken one step further now by integrating within this system the above QSMR equation to predict hydrolytic lability. The interface of a recent version of the corresponding computer program is shown in Fig. 6. The overall approach is general in nature and can be used starting with essentially any lead. The system can provide full libraries of possible new "soft" molecular structures and an analogy-based ranking of these candidates. With such software, more thorough and more quantitative design is possible. Candidates that are unlikely to have reasonable activity can be eliminated ahead of synthesis and experimental testing, saving considerable amount of laboratory time and expense.

2.2.1. Structure generation and candidate ranking

The expert system designed to aid the generation and ranking of novel soft drug candidates has been described in the literature [4, 8, 9]. As an important part, the system

contains the rules to transform certain substructures of the lead compound according to the principles of retrometabolic design. For the soft drug approach, the two most successful strategies, the inactive metabolite-based and the soft analogue strategies, were implemented. The program can generate common oxidative metabolites of the lead compound; for example, it can find $-CH_3$, $-CH_2OH$, or other alkyl groups in the lead and replace them by $-COOH$ or $-(CH_2)_n-COOH$ groups, or it can find phenyl ($-C_6H_5$) groups and replace them by phenol ($-C_6H_4OH$) groups, etc. Accordingly, the computer can then generate new soft drugs by derivatization of the "oxidized" metabolite to regenerate active soft drugs. The program can also generate soft analogues by looking for the presence of neighboring methylene/hydroxymethylene groups and by replacing them in an isosteric/isoelectronic fashion with corresponding esters $-O-C-$, reversed esters $-CO-O-$, or other functions.

Since in certain cases a large number of analogue structures may be designed, it is desirable to have some prediction regarding their activities and/or toxicities to avoid the time consuming synthesis and testing of all these structures. The analogue structures that are better isosteric/isoelectronic analogues are also more promising drug candidates. The present expert system provides a ranking-order based on the closeness of calculated properties to those of the lead compound using fully optimized geometries obtained from advanced semi-empirical AM1 calculations [67] for all the compounds that are of interest. These semi-empirical molecular orbital calculations are sophisticated enough to yield structures that have molecular geometries and heats of formation that rival experimental accuracy. As the number of transistors that can be integrated into a microchip, and as a result of this processor speed, seems to double about every 18 months (Moore's law, which held true for more than three decades by now [76]), the steady exponential increase of computational power put the molecules that are of interest for medicinal chemists well within the reach of such quantum chemical calculations.

Because most new structures are close structural analogues (often exact isomers), it is important to include as many and as relevant properties as possible. In the present version, four parameter categories are used with equal weights to describe isosteric-isoelectronic relations: molecular size/shape descriptors (V, volume; S, surface area; O, ovality); electronic properties (D, dipole moment; α, average polarizability; I, ionization potential); predicted solubility/partition properties (log W, log $P_{o/w}$); and atomic charge distribution on the unchanged portions. Each of these parameters can be obtained from the optimization output, and they all play important roles in determining binding and transport properties; they should give a relatively good description of the isosteric/isoelectronic analogy.

Since all these properties are measured in different units and vary over different ranges, a ranking factor (RF) is computed using the following procedure: All differences compared with the lead are computed. For each property P, a RF of one ($RF_P^j = 1$) is assigned to the analogue i that has the smallest absolute difference compared with the lead, $\Delta_i = Abs(P_i - P_0)$. For all other analogues j, the corresponding relative differences are used as RFs ($RF_P^j = \Delta_j/\Delta_i$). Within each of the four categories (S, size/shape; E, electric/electronic; P, solubility/partition; Q, atomic charge distribution) the average of the pertinent RFs is computed, e.g., $RF_E = (RF_D + RF_\alpha + RF_I)/3$. The final value used is the average of these four, $RF = (RF_S + RF_E + RF_P + RF_Q)/4$. Smaller values indicate better analogy. This method might favor a bit too strongly compounds where some parameter has a value very close to that of the lead compound, but by using many predictors, this possibility is at least partly compensated.

2.2.2. *Esmolol*

A formal computer-aided soft drug design that uses homometoprolol (**1**) as lead compound has already been used in a number of occasions to illustrate the process of computerized structure-generation and candidate ranking [8, 9]. Starting with structure **1**, four different soft drug analogues were generated by oxidizing the methoxymethyl function to its corresponding carboxylic acid type metabolites and converting them into various esters (R methyl or ethyl) (**2a**, **2b**), respectively by replacing neighboring methylene groups with –O–CO– or –CO–O– functions (**2c**, **2d**), as shown in Scheme 1. Mentioned properties

Scheme 1

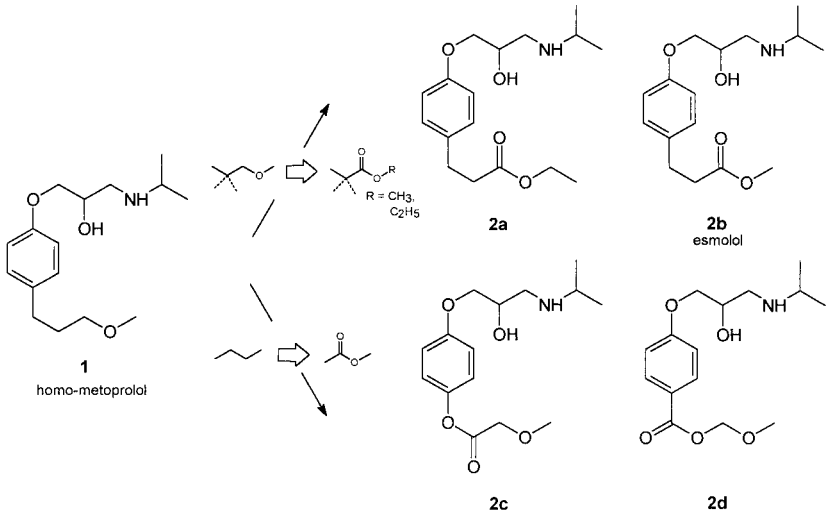


Illustration of a formal soft drug design process that uses homo-metoprotol (**1**) as lead to generate four soft drug analogues including esmolol (**2b**)

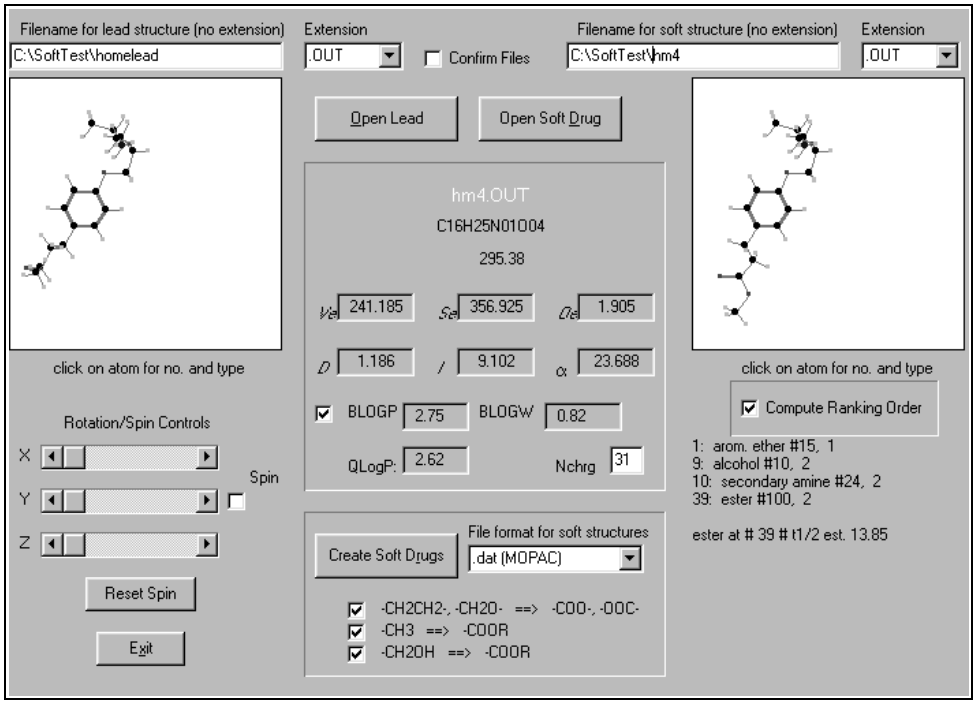


Fig. 6:
Interface of the current version of the program for computer-aided soft drug design

were then calculated based on AM1 optimized structures. Values obtained for the lead compound are: V: 290.71 Å^3, S: 371.35 Å^2, O: 1.75, D: 1.50 D, α: 23.15 Å^3, I: 9.01 eV, BLogP: 3.09, BLogW: 0.77. Table 1 summarizes the analogy-ranking in order of decreasing overall analogy together with the half-lives estimated using the present program (Fig. 6). Charge distributions were compared for atoms in the aromatic ring and the β-amino alcohol side chain (including hydrogens). The obtained results clearly show **2b** as the outstanding best analogue in practically all categories.

The nice aspect of this hindsight design is that structure **2b** corresponds to esmolol (Brevibloc®), an ultra-short-acting (USA) β-blocker designed by researchers at American Critical Care (McGaw Park, IL) [77, 78] that elegantly demonstrates the concept of controlling plasma drug levels by rapid metabolism via serum esterases. In the late 1970s, it was already noticed that for the usual β-blocker structure, insertion of an ester moiety between the aromatic ring and the β-amino alcohol side chain [79] or at the more remote *para* position [80] might not affect significantly β-adrenergic blocking activity. It was also shown that the acid metabolites formed by hydrolysis are devoid of β-adrenoceptor activity [80, 81]. Somewhat later, esmolol was selected as the best candidate for development from different series of β-blocker analogues that contained ester moieties systematically inserted at different positions of the molecular structure [17, 77, 82, 83]. Esmolol (**2b**) was approved by the FDA in 1986 for the acute, temporary control of ventricular rate in certain supraventricular arrhythmias such as sinus tachycardia and atrial flutter and/or fibrilation in the perioperative, postoperative, or emergency setting. It is the fourth β-blocker to be approved for intravenous clinical use, but it differs from the other three (propranolol, metoprolol, labetalol) because its pharmacological effects dissipate within 15–20 min after stopping the drug [84].

Table 1: Ranking order and predicted hydrolytic half-lives for homo-metoprolol (1) analogues

Compound	RF_S*	RF_E	RF_P	RF_Q	RF	Pred. $t_{1/2}$ (min)
2b	1.00	3.30	2.12	1.00	1.85	13.9
2c	13.12	2.59	9.94	18.99	11.16	21.3
2a	36.94	5.37	4.56	1.47	12.09	14.7
2d	17.11	16.18	13.88	15.89	15.76	1.8

* Ranking factors (RF) were computed as described in the text. Smaller values indicate better isosteric/isoelectronic analogy

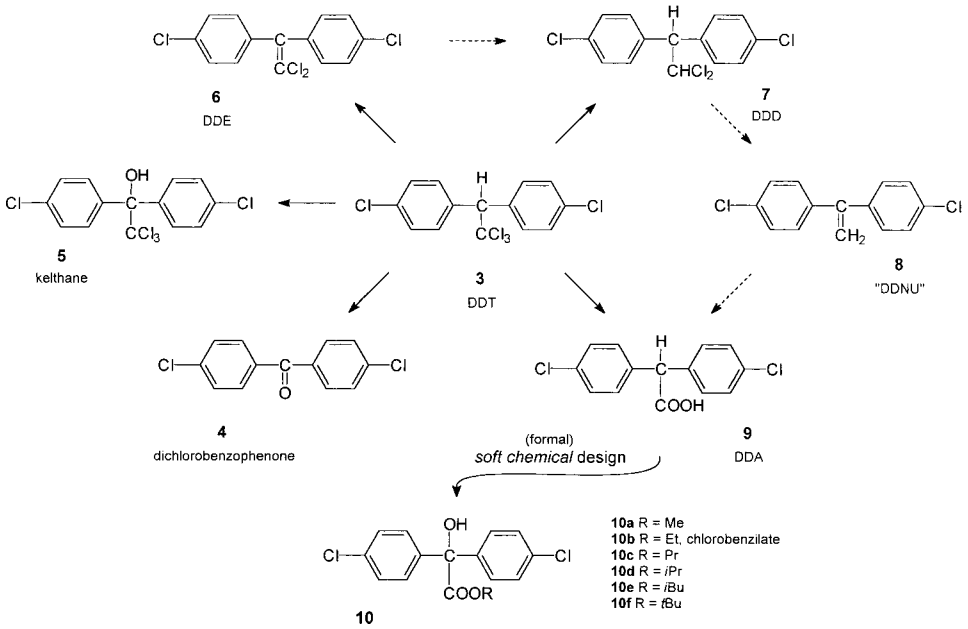
Using eq. 5, the estimated *in vitro* human blood hydrolytic half-life of esmolol is 13.9 min (Table 1) in reasonable agreement with the experimentally determined 26–27 min [15, 25]. Even if in hindsight only, the above example for computer-aided retrometabolic design illustrates that the method might be useful in designing and selecting drugs with an improved therapeutic index based on the calculated isosteric-isoelectronic ranking order and the predicted hydrolytic labilities.

2.2.3. *Chlorobenzilate*

One of the few instances in which the general principles of soft drug design have been accidentally used for the design of nonpharmaceutical products is the well-known pesticide dichlorodiphenyltrichloroethane (DDT, **3**, Scheme 2) with its analogues and metabolites. DDT was the first chemical that revolutionalized pest control. Although already synthesized in 1874, its insecticidal properties were discovered by Paul Müller only in 1939 in the Basel laboratories of the Swiss company J. R. Geigy S. A. [85]. During World War II, it was demonstrated that DDT also could be used to control typhus and malaria. It was widely used as a pesticide in the United States, but it was banned in 1972 for all but essential public health use and for a few minor uses to protect crops for which no effective alternatives were available. The decision was prompted by the prospect of ecological imbalance from continued use of DDT, by the development of resistant strains of insects, and by suspicions that it causes a variety of health problems, including cancer.

DDT undergoes *in vivo* oxidative dehaloformation (**4**), oxidation (**5**), iterative dehydrohalogenation/reduction cycles

Scheme 2



Metabolism of DDT (**3**) and chlorobenzilate (**10b**) as a soft chemical obtained by using a formal inactive metabolite-based approach

Table 2: Ranking order and predicted hydrolytic half-lives for kelthane (5) analogues

Compound	RF_S*	RF_E	RF_P	RF_Q	RF	Pred. $t_{1/2}$ (min)
10a, R = Me	1.87	1.15	1.26	1.00	1.32	172.4
10b, R = Et	3.72	1.86	1.19	1.23	2.00	247.3
10c, R = Pr	8.24	2.63	1.20	1.04	3.28	230.8
10d, R = iPr	8.07	2.82	1.20	1.13	3.30	690.7
10f, R = tBu	11.87	3.91	1.25	1.20	4.56	4164.5
10e, R = iBu	12.49	3.50	1.28	1.02	4.57	258.4

* Ranking factors (RF) were computed as described in the text. Smaller values indicate better isosteric/isoelectronic analogy

(**6**–**8**), and hydrolysis (**9**) (Scheme 2) [86]. The inactive acid metabolite **9** is of low toxicity, can be excreted as a water-soluble species, and is, indeed, a major metabolite detected in feces and urine. Therefore, it is an ideal lead compound for a formal inactive metabolite approach (Scheme 2). It was found that ethyl-4,4'-dichlorobenzilate (chlorobenzilate, **10b**, R = Et) is also active as a pesticide, but has much lower carcinogenicity than DDT (**3**) or kelthane (dicofol, **5**). For example, carcinogen concentrations determined for mice are 10, 264, and 6,000 mg/kg for DDT, kelthane, and chlorobenzilate, respectively [87, 88]. The ethyl ester moiety apparently functions similarly to that of the trichloromethyl group of DDT in restoring pesticidal activity. However, chlorobenzilate is considerably less toxic than DDT because its labile ethyl ester group enables rapid metabolism in exposed subjects to the free, nontoxic carboxylic acid.

Using the metabolite **9** as starting point, we generated six possible esters (**10a**–**10f**) and compared them with the analogous structure represented by kelthane (**5**), which had V: 264.79 Å^3, S: 304.60 Å^2, O: 1.53, D: 1.85 D, α: 23.29 Å^3, I: 9.74 eV, BLogP: 4.90, BLogW: −5.55. Among these structures, the present method clearly ranks the methyl and ethyl (**10a**, **10b** chlorobenzilate) esters as best analogues, as indicated in Table 2. They also are predicted to have the shortest half-lives. All these represent a nice illustration for the possibilities inherent to a soft chemical approach, a generalization of the soft drug approach, and also for the advantages introduced by a method that allows quantified comparison by using corresponding computer software.

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