Quantitative Structure−Metabolism Relationships: Steric and Nonsteric Effects in the Enzymatic Hydrolysis of Noncongener Carboxylic Esters

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An attempt to quantitatively describe human blood in vitro hydrolysis data for more than 80 compounds belonging to seven different noncongener series of ester-containing drugs is presented. A parameter not yet explored in pharmaceutical studies, the inaccessible solid angle Ω_h, calculated around different atoms was used as a measure of steric hindrance, and the steric hindrance around the carbonyl sp^2 oxygen ($\Omega_h^{O=}$) proved the most relevant parameter. The obtained final equation, $\log t_{1/2} = -3.805 + 0.172\Omega_h^{O=} - 10.146q_{C=} + 0.112\text{QLogP}$, also includes the AM1-calculated charge on the carbonyl carbon ($q_{C=}$) and a calculated log octanol−water partition coefficient (QLogP) as parameters and accounts for 80% of the variability in the log half-lives of 67 compounds. A number of structures are still mispredicted, but the equation agrees very well with a recently proposed mechanism for hydrolysis by carboxylesterases. The model, with a predictive power tested here on three unrelated structures, should be useful in estimating approximate rates of hydrolysis for prodrug or soft drug candidates ahead of their synthesis.

Introduction

Enzymatic hydrolysis plays an important role in the pharmacokinetic behavior of most therapeutic agents containing ester or amide bonds. Therefore, any relationship that can describe or eventually predict the rate of these enzymatic reactions would be of considerable interest for medicinal chemists and drug designers. This is especially true for prodrug and soft drug design, since these strategies mainly rely on enzymatic hydrolysis for drug activation and deactivation, respectively. Nevertheless, the search for such relationships proved unusually difficult and only a very limited number of related studies have been published. In the present paper, steric, electronic, and lipophilicity parameters based on three-dimensional molecular structures are investigated in an attempt to describe human blood in vitro stability data for a relatively large number of compounds belonging to seven different noncongener series of ester-containing drugs.

Background. 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.[1−7] Humans have been shown to express carboxylesterase in the liver, plasma, small intestine, brain, stomach, colon, macrophage, and monocytes.[7]

Esterase activity varies quite strongly between species.[1−7] The stability of acyloxyalkyl type esters, most frequently employed in prodrug and soft drug designs, usually increases in the rat < rabbit < dog < human order,[8,9] but there might be considerable variability. Rodents (rats, guinea pigs) tend to metabolize ester-containing drugs much faster than humans. Besides the usual problems related to the extrapolation of animal test results to man,[10,11] this is one additional aspect that can complicate early drug evaluations. 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, e.g., 2.3 min vs 26−27 min for esmolol (**2a**)[8,12] or 0.5 min vs 37 min for remifentanil (**4a**).[13] However, flestolol (**1q**), which contains an aromatic ester, showed an opposite trend ($t_{1/2}$ of 54 min in rat blood vs 1 min in human blood).[14] The rank order of compounds tends to be similar in different biological systems, but even this cannot be considered a general rule.[15,16] Furthermore, a recent investigation of the metabolism of flestolol and other esters found polymorphic rates of ester hydrolysis in New Zealand white rabbit blood and cornea.[14] Based on in vitro blood half-lives, about 30% of the animals studied ($n = 86$) were found as "slow" metabolizing ($t_{1/2} = 17$ min) and about 70% were found as "fast" metabolizing ($t_{1/2} < 1$ min). Interestingly, no such bimodal distribution of esterase activity was found in blood from rats, dogs, and humans or in the aqueous humor and iris−ciliary body complex of rabbits.[14]

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,[17−21] steric, lipophilicity, size/polarizability, and electronic parameters should be

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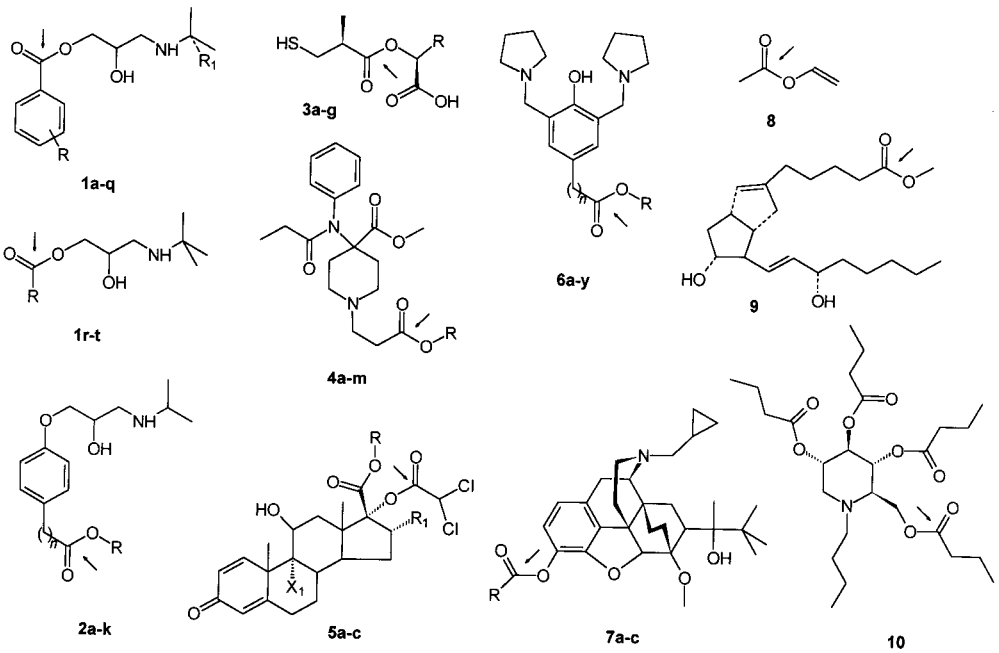

Figure 1. Structures included in the present study. Arrows indicate the sites of the enzymatic hydrolysis considered.

useful in establishing quantitative structure−metabolism relationships (QSMR).

The effect of structure on the enzymatic half-life was investigated in a number of prodrug and soft drug series.[8,12,13,15,16,22−43] An attempt by Charton[44] for quantitative lability prediction in a number of individual prodrug series,[22−25] using steric and polarizability parameters, resulted in some success, but failed to establish any relationship of general validity. Altomare and co-workers[36] and Testa and co-workers[30,39,40] also attempted to establish quantitative structure−metabolism relationships limited to the ester-containing prodrug series they examined. Separate studies on individual series may indicate the roles played by some of the involved effects, but they cannot be used to provide quantitative predictions in other, noncongener series. Additionally, the structural similarity present in such congener series usually makes it difficult to identify the exact source of variability, as many parameters may be strongly intercorrelated. In the present work, we attempted to obtain a more general equation based on human blood in vitro metabolism data of more than 80 compounds belonging to seven different classes: two β-blocker series with ultrashort duration of action **1a**−**t**[15] and **2a**−**k**,[8,12,35,41] ultra-short-acting angiotensin converting enzyme inhibitors **3a**−**g**,[42] opioid analgetics **4a**−**m**,[13] soft corticosteroids **5a**−**c**,[45] short-acting antiarrhythmic agents **6a**−**y**,[37,38] and buprenorphine prodrugs **7a**−**c**[43] (Table 1 in Supporting Information; Figure 1). Different hydrolytic half-life data were published; we selected in vitro human blood data because it was the data of interest for us available in the largest number over the widest range of structures under comparable experimental conditions. 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 (**8**),[46] isocarbacyclin methyl ester (TEI-9090, **9**),[9] and glycovir (**10**).[47] Following our general philosophy,[48−50] we avoided the use of individual substituent/fragment constants and attempted to identify useful parameters that are either derived from the three-dimensional structure of the molecules or can be obtained from quantum chemical calculations. While some of the existing steric parameters (E_s,[51,52] ν,[53,54] STERIMOL[55,56] L and B_1−B_5) may be useful in characterizing steric effects within congener series, we needed a more general concept for our attempt to establish a quantitative structure−metabolism relationship (QSMR) in noncongener series.

Steric Effects and the Inaccessible Solid Angle. 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.[19−21,57,58] 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 intramolecular motions and intermolecular interactions. In addition, three-dimensional structures for drug receptors are rarely known with adequate accuracy.

Despite an interest in steric effects as early as the late 1800s, the first successful quantitative parameter, Taft's steric constant E_s, was introduced only in the 1950s[51,52] following an earlier proposition of Ingold.[59] This steric constant was originally 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. 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. Charton was the first to introduce a general steric parameter υ based more closely on geometric consideration.[53,54] 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.[19] Bowden and Young used a steric substituent constant R, calculated using molecular models as

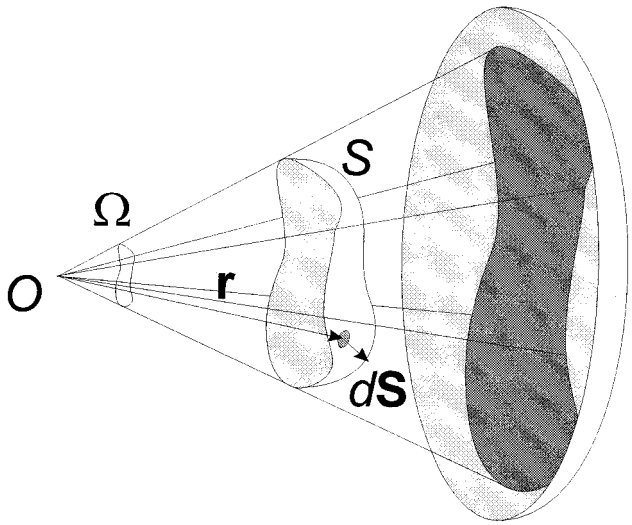

Figure 2. Solid angle Ω subtended at a center *O* by an arbitrary surface *S*.

the distance from the atom to which the substituent is bonded to the periphery of the van der Waals radius of the substituent.[60] 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.[55,56]

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.[61,62] The steady exponential development in computational power made it possible to calculate more rigorous measures of steric accessibility, and in 1984 Seeman and co-workers used the accessible solid angle Ω evaluated with a Monte Carlo sampling as a measure of the geometric accessibility factor for nitrogens in pyridines.[63] In an attempt to generalize this concept, Sakakibara, Hirota, and co-workers defined a steric substituent constant Ω_s, which basically represents the portion of the total solid angle that is hindered by the substituent considered.[64−68] 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, and co-workers also attempted the quantification of steric effects by the use of solid angles while developing a different method of calculation.[69−71]

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

$$\Omega = \int_S \frac{\mathbf{r}\,d\mathbf{S}}{r^3} \qquad (1)$$

where **r** is the position vector of the element of surface d**S** with respect to *O* (Figure 2). 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 (Figure 2).

On the basis of 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. Consequently, the inaccessible solid angle Ω_h, the solid angle at which access to a reaction center is hindered by substituents, 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. Such a calculated total value will, by definition, account for the effect of all the substituents avoiding, for example, problems related to the additivity of the different substituent contributions. Also, if reactivity at the center is not expected to be entirely isotropic, a directionally weighted form can be easily computed using, for example, a $\cos^2\theta$ type function that corresponds to the electron density of a p orbital. 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. Nevertheless, until now such measures of steric hindrance have been applied only in a few chemical studies[63−68,72] and, to our knowledge, not at all in pharmaceutical studies.

Using the van der Waals surface of the molecules, the total inaccessible solid angle around any atom can be relatively easily evaluated using numerical techniques. The van der Waals surface or volume concept already proved successful in many applications[50,73−76], and Bader and co-workers[77] have shown that contours of constant electronic density (0.001−0.002 electron/bohr^3) that contain over 96% of the total electronic charge gave good representations of the molecular van der Waals surfaces or of the smoother contact surfaces.

Lipophilicity and Octanol−Water log *P*. The usefulness of so-called lipophilicity parameters in describing solubility, permeability, receptor binding, and many other effects has been long recognized. They measure the affinity of a molecule or a moiety for a lipophilic environment, and the log octanol−water partition coefficient (log $P_{o/w}$), the parameter most commonly used to characterize such effects, has become one of the most successful physicochemical parameters used in medicinal or environmental chemistry.[20,78−81] It is, therefore, not surprising that a variety of predictive

methods were developed; they have been reviewed and compared in a number of papers.[80−82] In the present study, we investigated as predictive lipophilicity models both the AM1-based BLOGP model[48,83] and our molecular size-based (QLogP) model.[81,84,85] Calculated log $P_{o/w}$ values were also included from three other models: ACD/Log *P* (Advanced Chemistry Development Inc., Toronto, Ontario, Canada), AFC (KOWWIN),[86] and MLogP.[87] In addition, the AM1-based water solubility model BLOGW[88] was also included. Size-related parameters, such as molecular volume (*V*), molecular surface area (*S*), and ovality (*O*), that were computed for these models were also included in the study.

Electronic Effects and AM1-Calculated Properties. A wide variety of parameters can be used to characterize electronic properties. Those more frequently used include Hammett-type polar substituent constants σ,[89,90] Swain and Lupton-type field and resonance components F, R,[91] pK_a values, NMR chemical shifts δ, dipole moments D, charge distributions, or other quantum chemical descriptors.[92] In the present work, we investigated a number of AM1 (Austin Model 1)[93] derived parameters, such as ionization energies (I), dipole moments (D), average polarizabilities (α), and HOMO−LUMO energies (E_{HOMO}, E_{LUMO}). Absolute electronegativity (χ), calculated as the negative of the average HOMO and LUMO energies, and absolute hardness (η), calculated as half the HOMO−LUMO difference,[94,95] were also included as they proved successful in previous studies.[49,92] In addition, atomic charges ($q_{C=}$, $q_{O=}$, q_O) derived from Mulliken population analysis for the carbon and oxygen atoms of the ester bonds were also included. Advanced semiempirical molecular orbital calculations such as AM1 are sophisticated enough to yield structures that have molecular geometries and heats of formation that rival experimental accuracy. It is also true that semiempirical methods are mostly parametrized to reproduce heats of formation, ionization potentials, and geometric characteristics; therefore, calculated atomic charges, whose definitions are anyway arbitrary, might be less reliable. Nevertheless, they give a qualitative picture of the charge distribution in the molecule. AM1 semiempirical charges, dipoles, and bond lengths are usually considered more reliable than those obtained from low-quality ab initio methods.[92] Such AM1-based approaches are considerably easier to generalize than approaches based on substituent constants, and they also provide a more rigorous physicochemical basis. Nonetheless, the ability of the AM1 approach to reproduce substituent constants has been reported,[92,96,97] and AM1-based electronic parameters proved useful in a number of previous QSAR studies.[49,92,98,99]

Methods

Data Collection. Data for in vitro hydrolytic half-lives in human whole blood were collected from the original publications.[8,9,12−15,35,37,38,41−43,46,47] Understandably, different investigators used somewhat different experimental settings (e.g., different starting drug concentrations), but all studies were carried out in heparinized human whole blood at 37 °C.

Structure Optimization. Molecular structures used were optimized using the AM1 method of the MOPAC interface in Sybyl 6.3 (Tripos, Inc., St. Louis, MO) on a Silicon Graphics Origin2000 deskside server using the AM1 PRECISE POLAR keywords. Since for flexible molecular structures the computed inaccessible steric angles can be conformation sensitive, special care was given to start from structures with side chains folded away from the ester functions to ensure minimum steric hindrance around this function. If needed, several different starting conformations were investigated, and if different final conformations were found, the one with the lowest heat of formation was used in the correlation study. In all cases, conformations found to have the lowest AM1-calculated heats of formation and used in the present calculation were at least 5−10 kJ/mol more stable than the other possible conformations found for the same molecule that had significantly different Ω_h values. The only exception was the case of buprenorphine prodrugs, where two conformations having relatively close heats of formation but quite different Ω_h values were found depending on the position of the ester moiety above or below the plane of the aromatic ring in the **7a**−**c** structures. Data used here are for the conformer with the ester moiety below the plane of the aromatic ring, which is sterically less hindered but which is somewhat less stable (∼2 kJ/mol) according to the AM1-calculated heat of formation. For glycovir (**10**), where only the rate of disappearance was measured without determining or specifying which of the four ester groups is degraded, the Ω_h values determined at the less hindered ester of the primary alcohol were used, as it was assumed that this ester is metabolized with the fastest rate.

Calculation of Ω_h. 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.[50,81,84] 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 are needed for sufficient accuracy. We used about 25 000 during the evaluation of the model, and about 60 000 for the final results published here. 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 (H 1.08, C 1.53, N 1.40, O 1.36, F 1.29, Cl 1.60, Br 1.83, I 2.05, S 1.70, P 1.75, Si 2.10; all values in Å),[50,84] 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. In addition, the inaccessible solid angle can also be computed using a test atom of arbitrary size. As a further, computationally more demanding development, we also explored a "second generation" type accessibility descriptor intended to give a better overall characterization for the accessibility of the surface and not for the center of the selected atom. In this case, for each sampling point on the surface, instead of simply determining whether the corresponding direction is hindered or not (a value of 0 or 1), the actual inaccessible solid angle of the surface point was computed and the inaccessible to total solid angle ratio (value between 0 and 1) was used in the summation. Size and lipophilicity-related parameters were computed with our previously published method.[50,81,84] Multiple linear regression analyses were performed with a standard spreadsheet program (Microsoft Excel 97).

Results and Discussion

The present quantitative study 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 $t_{1/2}$ ($r^2 = 0.58$, with all data included $n = 79$), followed by Ω_h around the carbonyl sp^2 carbon ($\Omega_h^{C=}$, $r^2 = 0.29$), ACD/Log P ($r^2 = 0.27$), MLogP ($r^2 = 0.21$), and absolute hardness (η, $r^2 = 0.17$). Because these Ω_h values are computed based on the van der Waals surface of the molecules, $\Omega_h^{O=}$ may be more sensitive to changes in steric hindrance than $\Omega_h^{C=}$, which is measured around the carbon atom considerably more buried inside other atoms. With the radii set used here, on average about 75% of the surface of this carbon atom is already buried inside its neighbors, whereas only about 45% of the oxygen surface is buried inside its immediate neighbors (Table 1 in Supporting Information). However, since the correlation is much better and is also considerably better than that for the overall ester group ($r^2 = 0.42$ for $\Omega_h^{COO} = \Omega_h^{O=} + \Omega_h^{C=} + \Omega_h^{O}$), we believe that it 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. These differences are also present even if we drop the three removed steroid data, which can considerably alter the involved statistics, as the corresponding r^2 become 0.38, 0.12, and 0.14 for $\Omega_h^{O=}$, $\Omega_h^{C=}$, and Ω_h^{COO}, respectively.

Ω_h varies over a relatively small range, and a 10% variation in $\Omega_h^{O=}$ seems to cause a change of almost 2 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. On the basis of theoretical considerations, log Ω_h should have been used in these correlations, but because the range of variability was relatively small, Ω_h and log Ω_h gave similar correlations. Since Ω_h gave a somewhat better correlation and it varied over a relatively larger range, we used simply Ω_h in these equations.

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 $\Omega_h^{O=}$ ($r^2 = 0.73$ vs 0.59). 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, and co-workers and taken from their publication ($r^2 = 0.78$).[67]

Since the algorithm allows the evaluation of Ω_h values using an arbitrary radii scaling factor to uniformly increase or decrease all atomic radii employed in the calculation, we investigated how this changes the correlation between Ω_h and log $t_{1/2}$. Considering the quality of the experimental data, small changes (<20%) in the van der Waals radii do not cause significant changes in the correlation as shown by the changes in r^2 for 65

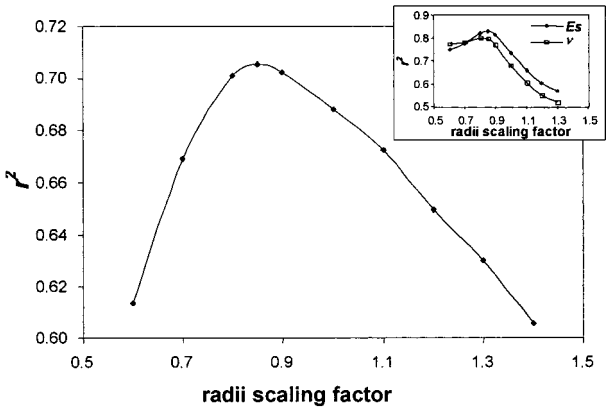

Figure 3. Change in correlation between enzymatic hydrolysis log $t_{1/2}$ and $\Omega_h^{O=}$ as the radii scaling factor used in the computation is varied. The insert shows the same change in correlation between E_s or ν derived from chemical hydrolysis rates and $\Omega_h^{C=}$ for 40 methyl esters.

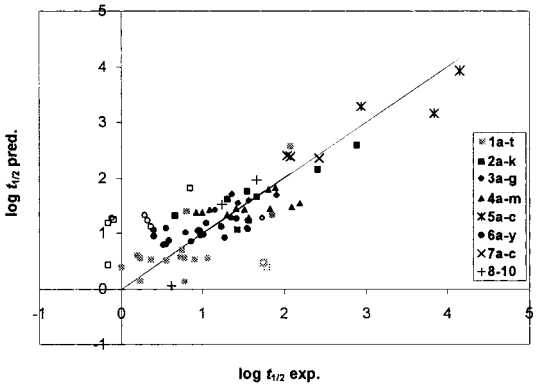

Figure 4. Predicted vs experimental log half-lives for data included in the present study. Compounds not included in the final correlation are denoted with an open symbol.

structures in Figure 3. This can be considered an additional proof that the van der Waals surface as computed here is a realistic and adequate measure of molecular size. Maximum correlation was obtained with a slight reduction in the radii set (scaling factor of 0.85). Interestingly, the correlation for the mentioned 40 methyl ester structures between Ω_h and E_s or ν, parameters derived from chemical hydrolysis rates, also had a maximum at about the same value as shown on the insert in Figure 3. This probably happens because this small reduction in radii allows the Ω_h computed here to better incorporate the hindering effect of atoms that are farther away while the corresponding size is still more or less realistic.

Searching for other informative parameters to be used in our QSMR model, we looked at the correlation with the residuals after taking away the dependence on $\Omega_h^{O=}$ (log $t_{1/2} - 0.185\Omega_h^{O=}$). At first sight, no parameter gave significant correlation, but after analyzing the data and dropping eight outliers (**1h**−**i**, **1o**, **2b**−**d**, **2i**, **6i**), the charge on the carbonyl C, $q_{C=}$, seemed promising ($r^2 = 0.26$). In addition, lipophilicity (QLogP, $r^2 = 0.17$) or size (e.g., V, $r^2 = 0.16$) could also be considered. Among calculated log $P_{o/w}$ values, QLogP gave the best correlation here, followed very closely by AFC (KOWWIN) ($r^2 = 0.16$), these two calculated series (QLogP and KOWWIN) being very closely intercorrelated ($r^2 = 0.96$). For the structures included, the AM1-calculated charge

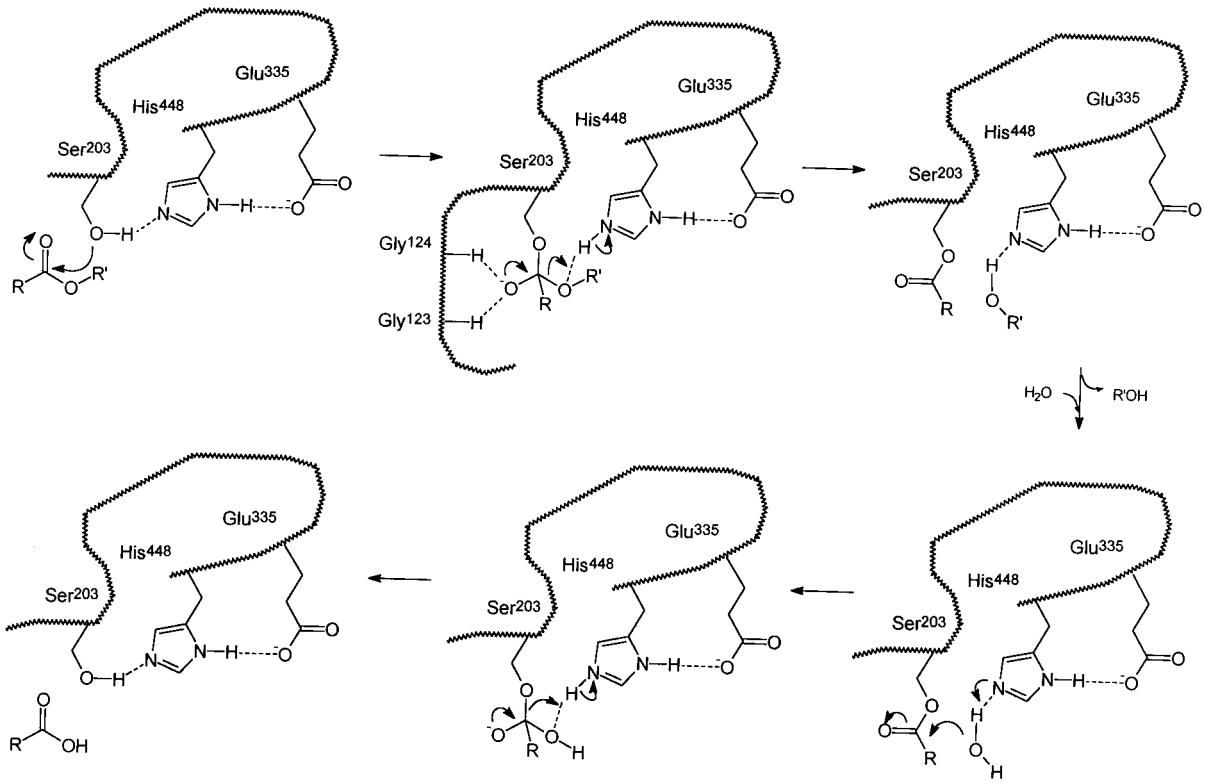

Figure 5. Illustration of the mechanism proposed for hydrolysis by carboxylesterases based on analogy with other, similar mechanisms and a study of highly conserved motifs.[7] It involves Ser[203], Glu[335], and His[448] as a catalytic triad and Gly[123]-Gly[124] as part of an oxyanion hole. Equations 2 and 3 obtained here also agree well with such a mechanism.

turned out not to be very sensitive to substitutions; it mainly distinguishes the aromatic substituted carbonyls of structure **1** and those of the other structures (Table 1); therefore, some precaution with its use is suggested. Nevertheless, even if structures **1a**−**t** are dropped from the correlation, $q_{C=}$ still gives the best correlation with the residuals. The value of $q_{C=}$ may also not be entirely adequate when multivalent sulfur is present or when N- or O-substituted aromatic rings are attached, and this may account, at least partially, for the larger deviations seen in some of the structures omitted (**1h**−**i**, **1o**, **2c**−**d**).

For these data, we obtained the following correlation:

$$\log t_{1/2} = -2.272(\pm 1.162) + 0.176(\pm 0.013)\Omega_h^{O=} - 14.374(\pm 2.876)q_{C=}$$

$$n = 71, \quad r = 0.876, \quad \sigma = 0.390, \quad F = 112.5 \quad (2)$$

This equation is especially promising since it agrees very well with a recently proposed mechanism for hydrolysis by carboxylesterases (Figure 5). As will be discussed later, 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 increasing log $t_{1/2}$.

Finally, the dependence of log $t_{1/2}$ on lipophilicity is less clear, but for most structures, except some of the antiarrhythmic series **6**, there is a tendency for more lipophilic compounds to be more slowly hydrolyzed. Protein binding might represent part of the reason for this dependence as extensive protein binding might considerably alter the rate of hydrolysis by reducing the available substrate concentration. We present, therefore, a final equation in which all parameters are statistically relevant ($p < 0.01$), but that was obtained after omission of four more structures (**3a**, **6h**, **6j**, **6w**) (Figure 4):

$$\log t_{1/2} = -3.805(\pm 1.412) + 0.172(\pm 0.012)\Omega_h^{O=} - 10.146(\pm 3.439)q_{C=} + 0.112(\pm 0.044)\mathrm{QLogP}$$

$$n = 67, \quad r = 0.899, \quad \sigma = 0.356, \quad F = 88.1 \quad (3)$$

There are no strong intercorrelations among the parameters included (Table 2 in Supporting Information) making the model sufficiently stable. We found no need to include second or higher order terms. 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 equation will be integrated into the expert system developed in our center for computerized generation and ranking of soft drug candidates[100−103] as the metabolic degradation rate is an important parameter to be considered in the design of soft drugs.

It is also important that omission of the three steroid data, which are about 2 orders of magnitude removed from most other data points and can, therefore, considerably effect the final regression equation, worsens the statistics but does not have a significant effect on the regression coefficients of eq 2 or 3. Equation 3 accounts for about 80% of the variance in log $t_{1/2}$ (58% with all data included). The correlation between predicted (eq 3) and experimental log half-lives is presented in Figure 4, and it is interesting to note that most of the unexplained variance remained within the different

series and not between the different series. As can be seen in Figure 4, 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.

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 (e.g., **1d**, **1m**, **6a**). For most of them, our model fails to predict a half-life significantly larger than those of their structurally similar analogues (Table 1). 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.

Based on conserved motifs in various carboxylesterases and following other, similar mechanisms,[104,105] a mechanism for hydrolysis by carboxylesterase was recently proposed.[7] It 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 (Figure 5). The sequences required for the hydrolytic capability at the catalytic triad of carboxylesterase, acetylcholinesterase, butyrylcholinesterase, and cholesterol esterase are highly conserved.[7] As mentioned, eq 2 or 3 agrees very well with such a mechanism. A more positive carbon ($q_{C=}$) is more prone to the nucleophilic attack by the serine oxygen, and a less accessible carbonyl oxygen ($\Omega_h^{O=}$) is more difficult to stabilize by hydrogen bonds in the oxyanion hole. 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. We would like to suggest, therefore, that they not only help stabilize the tetrahedral adduct in the second step, but they already play an important role in the first step of the mechanism presented in Figure 5 by a partial proton transfer that makes the sp^2 carbon more susceptible to the nucleophilic attack.

Finally, to test the predictive power of the present model, we calculated the half-lives of three separate drugs with completely unrelated structures and experimental data available (Table 1). For vinyl acetate (**8**), isocarbacyclin methyl ester (**9**), and glycovir (**10**) measured vs predicted (eq 2) in vitro human blood half-lives (min) are 4.1, 17.3, and 45.9 vs 2.3, 21.4, and 57.4, respectively. For these compounds, eq 3 predicts values that are somewhat larger, 1.2, 32.6, and 92.0 min, respectively, but still in good linear agreement with the experimental data (Figure 6). 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.

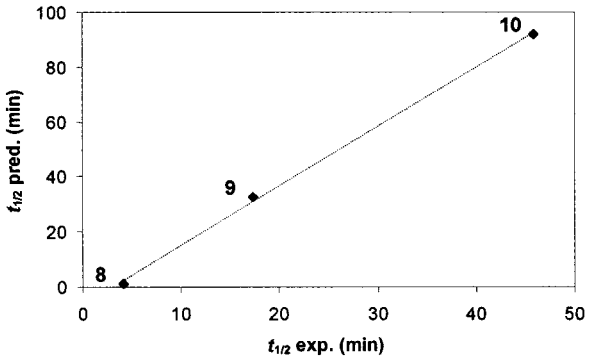

Figure 6. Predicted (eq 3) vs observed in vitro human blood hydrolysis half-lives for three compounds (vinyl acetate **8**, isocarbacyclin methyl ester **9**, and glycovir **10**) used to test the predictive power of the present model.

Conclusions

Using the inaccessible solid angle Ω_h calculated around different atoms as a novel measure of steric hindrance, a quantitative structure−metabolism relationship was developed that can account for a large part of the variance in the log half-lives of a variety of noncongener carboxylic ester-containing drugs. In agreement with a recently proposed mechanism for hydrolysis by carboxylesterases, steric hindrance around the sp^2 oxygen and charge on the sp^2 carbon of the ester moiety were found having the most important influence on the rate of in vitro human blood enzymatic hydrolysis. While a number of structures are still mispredicted, most predictions are reasonable, and the present method may become useful in distinguishing among compounds whose hydrolysis is fast, medium, or slow based on chemical structure alone.

Supporting Information Available: Tables 1 and 2 listing data included in this study and correlation matrix for data. This material is available free of charge via the Internet at http://pubs.acs.org.

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