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Physicochemical aspects of the enzymatic hydrolysis of carboxylic esters

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Considering the important role played by enzymatic hydrolysis in the metabolism of therapeutic agents designed by retrometabolic approaches (soft drugs and chemical delivery systems), the present article offers a review of a number of issues related to the enzymatic hydrolysis of carboxylic esters. Current knowledge regarding interorgan- and interspecies variability, stereospecificity, activation energy, proposed mechanism, and quantitative structure-metabolism relationship is summarized. The effects of chain-length and branching in the alcohol or acyl substituent on the rate of hydrolysis in congener series are also summarized. Available *in vitro* human blood data suggest that shortest half-lives are achieved with sterically non-hindered chains that are neither too short nor too long and are of around four carbon-atom long.

1. Introduction

Many carboxylic ester-containing chemicals are very efficiently hydrolyzed into the respective free acids by a class of enzymes designated as carboxylic ester hydrolases (EC 3.1.1). These ubiquitous enzymes exhibit broad and overlapping substrate specificity toward esters and amides. Meanwhile, the same substrate is often hydrolyzed by more than one enzyme. Hence, despite a long-standing interest in the characterization of these enzymes and despite the important roles they play in the metabolism of many xenobiotics, their classification is difficult and still is in a confused state [1–7]. More important subclasses include carboxylesterase, EC 3.1.1.1 (carboxylic-ester hydrolase, ali-esterase, B-esterase, monobutyrase, cocaine esterase); arylesterase, EC 3.1.1.2 (A-esterase, paraoxonase); acetylcholinesterase, EC 3.1.1.7 (choline esterase I); and cholinesterase, EC 3.1.1.8 (choline esterase II, pseudocholinesterase, butyryl-choline esterase, benzoylcholinesterase...) [8]. A possible solution to the existing confusion might come from accumulation of adequate sequence information and emergence of a novel classification system based on this information [7]. It is also intriguing that, in many cases, the physiological role of these enzymes still remains unclear [6]. From the perspective of retrometabolic drug design [9], a characterization of the related physicochemical aspects and a review of structure-metabolism relationships is of special interest since these strategies, as well as those related to prodrug design, mainly rely on enzymatic hydrolysis for drug activation (prodrugs [10–12], chemical delivery systems [13]) or deactivation (soft drugs) [14].

2. Interspecies variability

Esterase activity varies considerably between species [1–7, 15], and, as extreme examples, even compounds that are metabolized in one species but not in others are known. For example, atropine is not hydrolyzed in human serum, but is hydrolyzed in the serum of some rabbits [16]. Aliphatic esters tend to be metabolized much faster by rodents (rats, guinea pigs) than by humans (Fig. 1) [17–22]. Aromatic esters, however, might show an opposite trend, as exemplified by flestolol (Fig. 1) [23] and by nicotinate esters [24]. Aliphatic esterases, which are presumably absent from dog and human plasma, but present in rat and guinea pig plasma, were suspected as causes of the observed large differences [17]. For ester-containing compounds, the strong interspecies variability of hydrolytic rates might complicate early drug evaluations.

On the other hand, the differential distribution of these enzymes can be exploited to provide metabolism-based selectivity. Malathion provides an elegant example for the selectivity that can be achieved for pesticides by exploit-

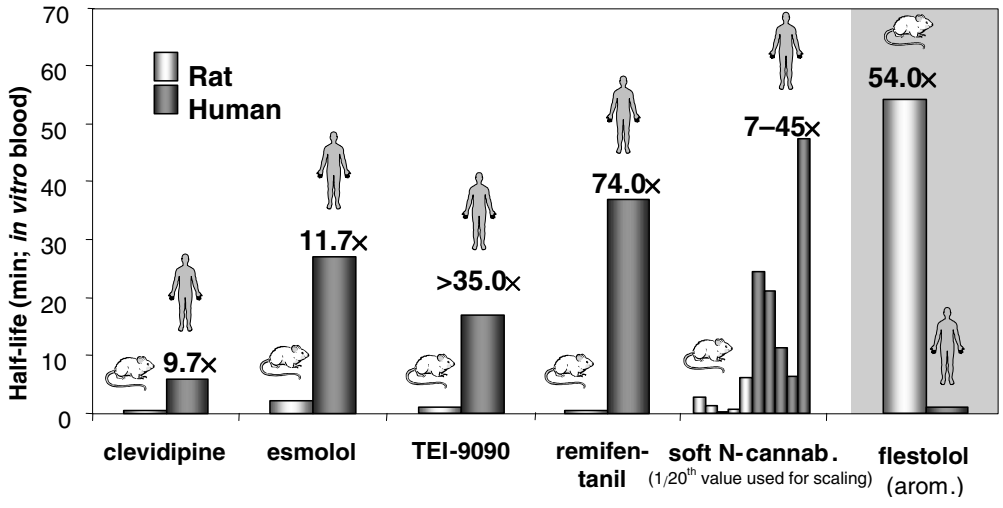

Fig. 1: The interspecies variability of hydrolytic half-lives illustrated by rat vs. human *in vitro* blood data for clevidipine [21], esmolol [17, 18], isocarbacyclin methyl ester (TEI-9090) [20], remifentanil [19], soft cannabinoid analogues [76], and the aromatic ester-containg flestolol [23]

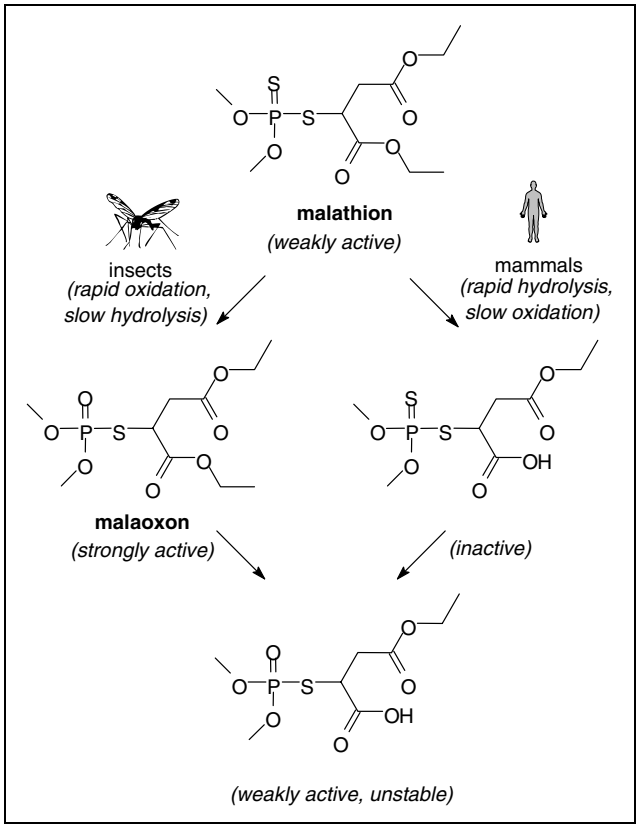

Fig. 2: Malathion, its oxidative activation to malaoxon, a much more active cholinesterase inhibitor, and its deactivation by carboxylesterases. Whereas oxidative activation is the dominant pathway in insect, hydrolytic deactivation dominates in mammals

ing the differences in the enzymatic constitution of vertebrates and insects (Fig. 2). It is detoxified through a variety of metabolic pathways, one of the most prominent one being the hydrolysis of one of its two ethyl carboxylester groups. The carboxylesterase that hydrolyzes and thereby detoxifies malathion is widely distributed in mammals, but only sporadically in insects, where in some rare cases is responsible for insecticide resistance (see [25, 26] and references therein). In the meantime, insects seem to posses a very active oxidative enzyme system that transforms malathion into malaoxon, a much more active cholinesterase inhibitor. Probably, all insects and all vertebrates possess both an esterase and an NADPH-dependent oxidase system, but the balance of action of these two systems varies from one organism to another and provides this selectivity of action. It is, therefore, quite likely that by incorporating the possibility of similar mechanisms into the structure of other insecticides from the parathion family, which tend to have unacceptably high mammalian toxicities and are activated by a similar oxidation mechanism, safer and more selective chemicals can be designed.

3. Interorgan and interindividual variability

Carboxylesterases are expressed in a variety of organs and tissues. For example, humans have been shown to express carboxylesterase in the liver, plasma, small intestine, brain, stomach, colon, macrophage, and monocytes [7]. Nevertheless, esterase activity is known to vary strongly between organs and tissues. In fact, for the activity of a nonspecific steroidal esterase, which was measured by two different assays, the observed interorgan variability was considerably larger than the observed interspecies variability [27]. Not unexpectedly, in all examined cases (human, rat, and mouse), liver gave the highest activity. If sufficiently tissue-specific esterase activity could be identified, retrometabolic or prodrug approaches can provide excellent means for tissue specific drug delivery.

Interindividual variability and pharmacogenetics can also cause variability. For the above-mentioned nonspecific steroidal esterase, considerable (approx. 18-fold) interindividual variability was observed in human mammary tissues from 16 healthy female subjects. As an intriguing aspect, this esterase activity showed a statistically significant age-related increase [27].

Polymorphic rates of ester hydrolysis in New Zealand white rabbit blood and cornea were found for the metabolism of flestolol and other esters [23]. About 30% of the animals studied were found as "slow" metabolizing and about 70% were found as "fast" metabolizing. However, no bimodal distribution was found in blood from rats, dogs, and humans or in the aqueous humor and iris-ciliary body complex of rabbits [23]. For humans, genetic variants are known for butyrylcholinesterase and arylesterase. Atypical butyrylcholinesterase occurs in homozygous form in approximately 1 out of 3500 Caucasians. As a result of impaired ester hydrolysis, these patients exhibit prolonged paralysis after standard doses of neuromuscular blocking agents (e.g., succinylcholine, suxamethonium, and mivacurium) (see [28] and references therein).

4. Mechanism: catalytic triad and oxyanion hole

It has been suggested that hydrolysis by carboxylesterase involves a catalytic triad formed by a serine, a glutamate, and a histidine residue so that low-barrier hydrogen bonds facilitate a general base mechanism for the acylation of serine (Fig. 3) [7]. Sequences required for hydrolytic capability at the catalytic triad seem to be highly conserved in carboxylesterase, acetylcholinesterase, butyrylcholinesterase, and cholesterol esterase [7]. Two glycine residues also play important roles as parts of an oxyanion hole in which weak hydrogen bonds stabilize the tetrahedral adduct (Fig. 3). The important rate-influencing role of these glycine residues has been confirmed by site-directed mutagenesis studies in human acetylcholinesterase [29].

A stabilizing role of the hydrogen bonds agrees well with the observations of Page et al. [30]. For alkaline hydrolysis, they found that electron-withdrawing substituents increase the rate, and the corresponding Brønsted exponents indicate a transition state that resembles an anionic tetrahedral intermediate with a localized negative charge. By contrast, for enzymatic hydrolysis (by pig liver esterase), they found little dependence upon the electron-withdrawing power of substituents, which is consistent with a transition state that resembles a neutral tetrahedral intermediate [30].

In a recent quantitative structure-metabolism relationship (QSMR) study [31], we found the rate of enzymatic hydrolysis to strongly correlate with the steric hindrance of the carbonyl sp^2 oxygen. By contrast, chemical hydrolysis rates correlated more strongly with the steric hindrance of the reaction center, the carbonyl carbon. This also suggests an important, possibly even rate-determining, role for the hydrogen bonds formed in the oxyanion hole as they can both make the sp^2 carbon more susceptible toward a nucleophilic attack and stabilize the tedrahedral adduct by a partial proton transfer [31]. The important role of these hydrogen bonds could explain both a more neutral transition state and the importance of the steric

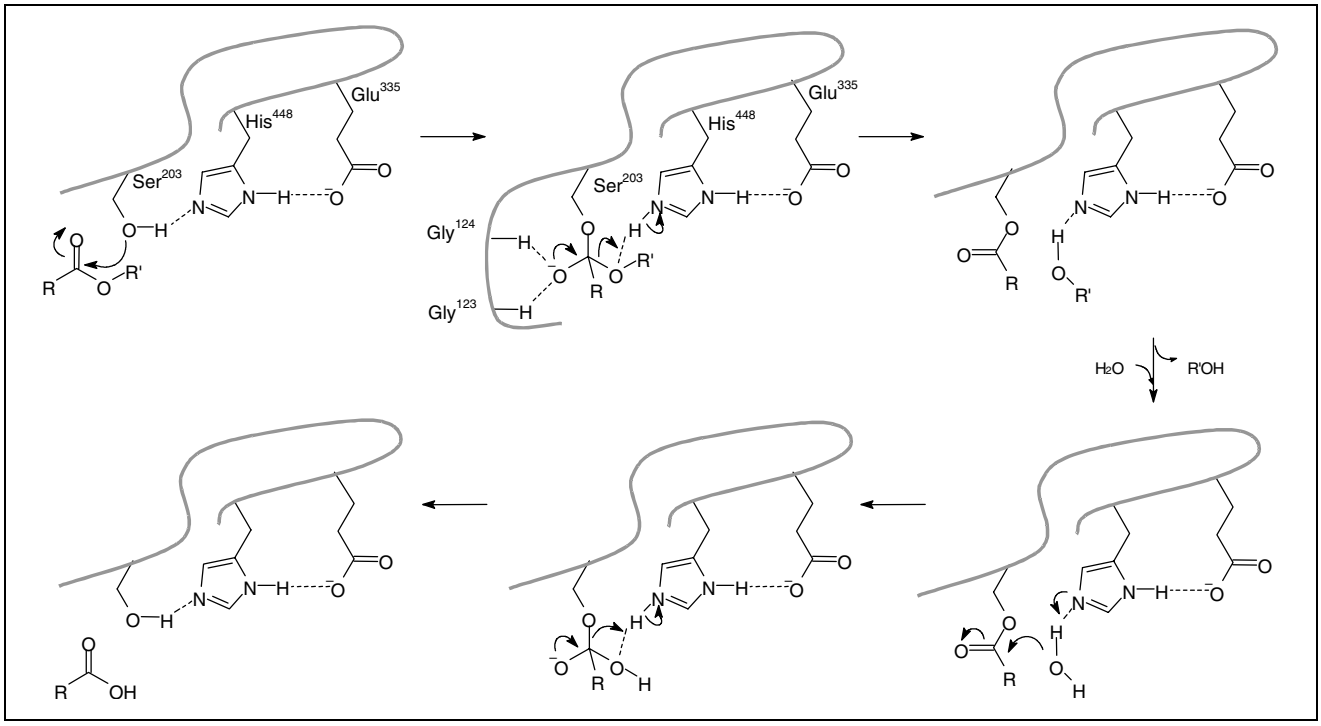

Fig. 3: Mechanism proposed for hydrolysis by carboxylesterases on the basis of analogy with other, similar mechanisms and a study of highly conserved motifs [7]. The mechanism involves serine, glutamate, and histidine residues as a catalytic triad and two glycine residues as part of an oxyanion hole

hindrance around the oxygen atom. It also can provide an explanation for the long-known observation (e.g., [32]) that chemical hydrolysis rates tend to afford just very low correlations with enzymatic hydrolysis rates.

5. Kinetics

A number of investigators found enzymatic hydrolysis reactions to obey Michaelis-Menten kinetics [24, 30, 33–36]. That is the rate of product formation, $v = dP/dt$, is connected to substrate concentration (S) through the well-known relationship characterized by a maximum velocity $V_{max} = k_{cat}E_0$, which is determined by k_{cat} and total enzyme concentration E_0, and a Michaelis constant K_m [37]:

$$v = \frac{V_{max}S}{K_m + S} . \qquad (1)$$

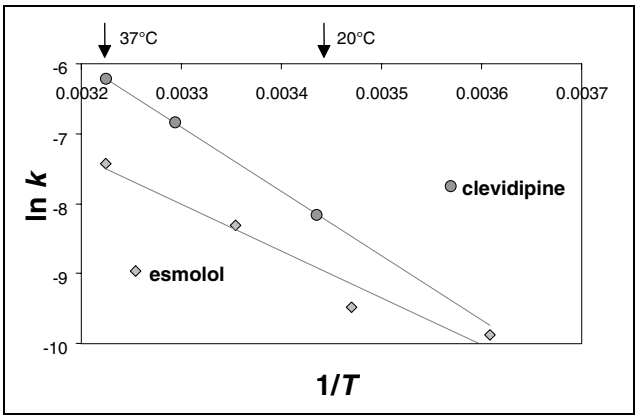

Fig. 4: The relationship between the logarithm of the rate (ln k) and the inverse of the absolute temperature (1/T) for esmolol and clevidipine hydrolysis is in good agreement with the linearity required by an Arrhenius-type equation, $\ln k = \ln A - E_a/R \times 1/T$. The corresponding slopes suggest apparent activation energies E_a of 55.5 kJ/mol ($n = 4$, $r^2 = 0.9586$) and 76.6 kJ/mol ($n = 3$, $r^2 = 0.9998$) for esmolol and clevidipine, respectively

However, under most pharmaceutically relevant conditions, the substrate concentration is sufficiently low ($S \ll K_m$), and the degradation shows good linearity (on a log scale). Hence, these reactions can be considered as pseudo-first-order and characterized by a constant rate, $v = kS = (V_{max}/K_m)\,S$, and a corresponding half-life, $t_{1/2} = 0.693/k$. The efficiency of these enzymatic reactions is well-illustrated by the enzyme rate-enhancement factor (EREF) concept introduced by Page et al. [30, 38]. This represents the ratio between the second-order rate constant for the enzyme-catalyzed reaction, k_{cat}/K_m, and that for the hydrolysis of the same substrate catalyzed by hydroxide ion, k_{OH}. For hydrolytic enzymes, EREFs are large values, usually within the 10^3–10^7 range [30, 38].

6. Stereoselectivity

Within the pharmaceutical field, only relatively limited data are available on the stereoselectivity of enzymatic hydrolysis despite this being an important aspect of enzyme-catalyzed reactions. Nevertheless, stereoselective hydrolysis has been documented in various media for a number of cases, such as ester prodrugs of oxazepam [39, 40], propranolol [41–44], or ibuprofen [45]. In the last case, *R*:*S* rate ratios as high as 50 were reported. Cocaine hydrolysis in baboon plasma provides an even more extreme example. The behaviorally inactive (+)-cocaine was found to hydrolyze at least 1,000 times faster than (−)-cocaine, the naturally occurring enantiomer [46]. Cocaine hydrolysis in rat hepatocytes also showed considerable stereoselectivity [47]. Elucidation of the rationale behind the enantioselectivity of certain hydrolytic enzymes may become possible with the accumulation of data on the mechanism of various enzymatic hydrolysis (see, e.g., [48] and references therein).

7. Activation energy/temperature dependence

Data on the temperature-dependence of the rate of hydrolysis in human blood are available for esmolol [49] and clevidipine [21]. With the decrease of media (blood) temperature, the rate (k) decreases and, hence, half-life increases. For both compounds, but especially for clevidipine, good linearity (Fig. 4) was observed between the logarithm of the rate (or the half-life) and the inverse of the absolute temperature (1/T) as required by an Arrhenius-type equation [50]:

$$k = Ae^{-\frac{E_a}{RT}}. \quad (2)$$

From the corresponding slopes, we obtained apparent activation energies E_a of 55.5 kJ/mol ($n = 4$, $r^2 = 0.9586$) and 76.6 kJ/mol ($n = 3$, $r^2 = 0.9998$) for esmolol and clevidipine, respectively. This appears to be in reasonable agreement, for example, with the activation energy of approximately 75 kJ/mol found for lactose hydrolysis by recombinant beta-glycosidases [51] or that of 43.5 kJ/mol found for anandamide hydrolysis by human brain fatty-acid amide hydrolase [52]. On the basis of data collected by Charton [53, 54], we also estimated activation energies for the chemical hydrolysis of a series of simple esters under acidic or basic catalyzes, and obtained values that also were somewhere in the 40–70 kJ/mol range. Values for the chemical hydrolysis of liposomal phosphatidylcholine under acidic conditions (pH 4.0) were also mostly in the 60–70 kJ/mol range [55].

Extrapolation of the above-mentioned *in vitro* enzymatic hydrolysis results for ester-containing drugs such as esmolol and clevidipine to *in vivo* situations has to be done carefully because a number of other effects are also temperature-dependent. Nevertheless, considering the E_a values obtained here, the data suggest that a reduction in body temperature from 37 °C to around 30 °C, which is routinely done during cardiac surgery, may approximately double the half-life of such drugs [21]. Indeed, pharmacokinetic studies in hypothermic and normothermic patients with remifentanil and clevidipine showed prolonged half-life and reduced clearance at lower temperatures [21, 56].

8. Structure-metabolism relationships

Many pharmaceuticals, usually members of some prodrug or soft drug series, have been investigated to establish the effect of structure on hydrolytic half-life (see [22, 57] and references therein). It was obvious from the beginning that increasing steric hindrance, such as that produced by branched substituents, increases half-life, but useful quantitative structure-metabolism relationships (QSMR) of general validity proved difficult to obtain. One has to emphasize that the ability to introduce rigorously measurable, quantitative aspects into structure-activity, structure-property, or similar type of relationships is one of the most important advancements in medicinal chemistry and drug design [58–63].

QSMR attempts for the hydrolysis of individual ester-containing series were made by Testa et al. [24, 33, 64, 65], by Charton [66], and by Altomare et al. [67]. However, such studies can only provide general guidelines and no quantitative predictions for other, noncongener series.

8.1. Solid-angle based QSMR model

Recently, we identified a more general relationship on the basis of human blood *in vitro* metabolism data of more than 80 compounds belonging to seven different classes [31] and including β-blocker series with ultrashort duration of action, ultra-short-acting ACE inhibitors, opioid analgetics, soft corticosteroids, short-acting antiarrhythmic agents, and buprenorphine prodrugs. *In vitro* human blood data was used because it represented the data of interest available in the largest number over the widest range of structures under comparable experimental conditions. Also, such data were of special interest for us as extrahepatic metabolism is expected to play a major role in the deactivation of soft drugs. The predictive power of the model has been tested on five separate ester-containing drugs with completely unrelated structures: vinyl acetate, isocarbacyclin methyl ester (TEI-9090), glycovir, clevidipine, and itrocinonide [22, 31].

In general agreement with previous results, we found steric effects as having the most important influence on the rate of enzymatic hydrolysis. Lipophilicity, as measured by the QLogP calculated log octanol-water partition coefficients 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. Half-lives were found to increase with increasing steric hindrance around the ester moiety as measured by the inaccessible solid angle Ω_h. An important novelty was the finding that the rate of metabolism as measured by log $t_{1/2}$ seems to be more strongly correlated with the steric hindrance of the carbonyl sp^2 oxygen ($\Omega_h^{O=}$: $r^2 = 0.58$, $n = 79$) than with that of the carbonyl sp^2 carbon as measured by ($\Omega_h^{C=}$: $r^2 = 0.29$). As mentioned, this seems to provide evidence for the important, possibly even rate-determining role played by hydrogen bonding at this oxygen atom in the mechanism of this reaction.

We settled on a final equation (eq. 3) to estimate log $t_{1/2}$ that in addition to $\Omega_h^{O=}$ includes the AM1-calculated charge on the carbonyl carbon ($q_{C=}$) and a calculated log octanol-water partition coefficient (QLogP) [68–70] as parameters:

$$\log t_{1/2} = -3.805 + 0.172\Omega_h^{O=} - 10.146q_{C=} + 0.112\mathrm{QLogP}$$

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

All the parameters in this equation are calculable from the molecular structure and are statistically relevant ($p < 0.01$). The present form was obtained after omission of a total of twelve outliers. However, eight out of these twelve compounds have very short half-lives that are difficult to determine and the corresponding experimental errors might be considerable, especially on a logarithmic scale. 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) half lives were not reported at all. For most of these compounds, our model fails to predict a half-life significantly larger than those of their structurally similar analogues. Some of their structural features might hinder their fit into the active site of the metabolizing enzyme(s), but no such features were obvious.

8.2. QSMR model: predictive power

The predictive power of the model based on eq. 3 was tested until now on five compounds with completely unre-

lated structures: vinyl acetate, isocarbacyclin methyl ester, glycovir, clevidipine, and itrocinonide [22, 31]. It is unrealistic to expect accurate predictions of hydrolytic half-lives for arbitrary structures. Nevertheless, the present method should prove useful in distinguishing on the basis of chemical structure alone among compounds whose hydrolysis is fast, medium, or slow. Two important warnings have to be mentioned here. First, considering that eq. 3 is based on logarithmic half-lives and has a standard deviation of $\sigma = 0.36$, considerable errors in the actual predicted half-lives are possible. Second, because Ω_h is considerably conformation-sensitive, and because a small change in its value can cause large variations in the calculated $t_{1/2}$, careful conformational sampling is required to find the less hindered energetically favorable conformation before any estimates are made.

Obviously, eq. 3 in its present form cannot account for any specific effect. For example, insertion of a heteroatom substituent (in particular, sulfur, SO, or SO_2) in the β or γ position relative to the carbonyl was noted to dramatically increase the rate of enzymatic hydrolysis [71]. Similar observations were also made for ester prodrugs of benzoic acid [72], soft ACE inhibitors [73], or soft β-blockers [74]. For such sulfur atoms, a possible role in stabilizing the tetrahedral structure of the hydrate in the enzyme-inhibitor complex has been suggested based on an X-ray crystal structure of a hydrated trifluoromethyl ketone that showed intramolecular hydrogen bonding between the S-atom and the −OH group on the carbonyl of the hydrate [71]. Such trifluoromethyl ketones are putative transition state esterase inhibitors, thought to act by forming a tetrahedral covalent hydrate with the catalytically active serine of carboxylesterases.

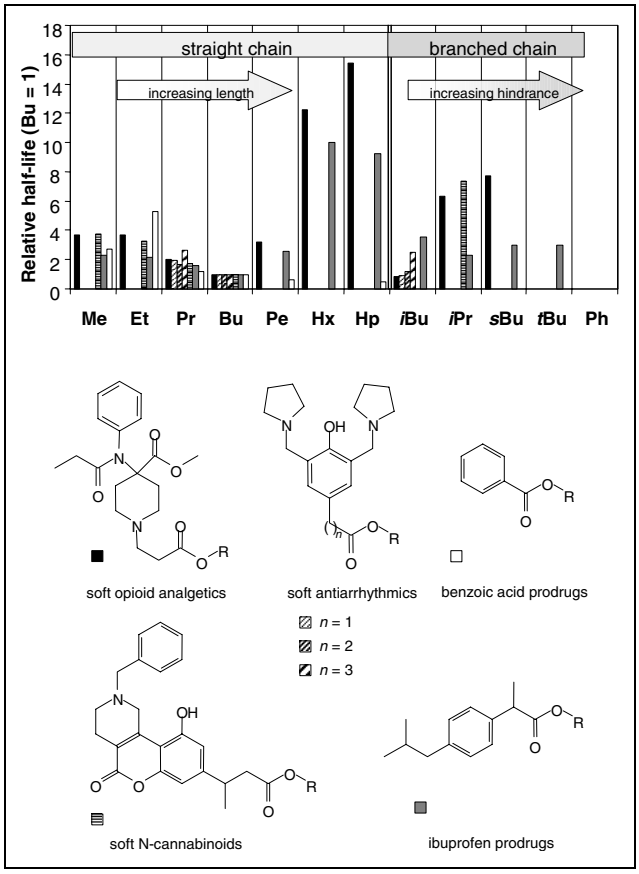

Fig. 5: Relative *in vitro* human blood or plasma hydrolysis half-lives for various alcohol substituents (R) in different congener series. Within each series, relative values were calculated compared to the butyl (Bu) substituent

9. Rate-influencing role of alcohol or acyl side chain

Equation 3 accounts for about 80% of the variance ($r^2 = 0.81$) in log $t_{1/2}$ (58% if all data are included). It is interesting to note that most of the unexplained variance remains within the series and not between the series. Hence, most unexplained variance is intraseries and not interseries. Therefore, we attempted to compare the rate-influencing role of the alcohol and acyl side-chains using data available for congener series with simple side-chains (e.g., Me, Et, Pr, Bu, Pe, Hx, iPr, sBu, tBu).

For alcohol side-chains, relatively consistent results were obtained (Fig. 5, Fig. 6) on the basis of *in vitro* human blood (or plasma) hydrolysis data for the following series: soft opioid analgetics [19], soft antiarrhythmics [75], soft nitrogen-containing cannabinoid analogues [76], ibuprofen prodrugs [77], and benzoic acid prodrugs (the only series containing aromatic esters) [72]. Among straight-chain substituents, butyl tends to result in the fastest degradation of aliphatic esters (Fig. 6). Both shorter and longer alcohol chains result in slower degradation. This might not be true for aromatic esters, but since comparable data from only one series (benzoic acid prodrugs) were available, it is difficult to make general assumptions. Obviously, increasing branching causes increasing steric hindrance and consequently increasing hydrolytic half-lives. In fact, data for *tert*-butyl (*t*Bu) substituents are missing from the graphics most likely because the corresponding compounds were to slow to hydrolyze to be of interest as possible soft drugs or prodrugs.

For simple acyl side-chains, much less consistent results were obtained (Fig. 7). *In vitro* human blood or plasma hydrolysis data were included for the following series: etilefrine prodrugs [78], buprenorphine prodrugs [79], pilocarpine prodrugs [80], enol ester prodrugs [81], oxprenolol prodrugs [82], bispilocarpine diester prodrugs [83], and metronidazole prodrugs [84]. Even if the overall picture is much less consistent then for the alcohol side-chain, it can be concluded that a length of about three-four carbon atoms is also needed for fast hydrolysis and the often used acetyl derivatives may not provide the shortest half-lives. Hence, available *in vitro* human blood data suggest that shortest half-lives are achieved with sterically non-hindered alcohol and acyl chains that are neither too short nor too long and are of around four carbon-atoms long.

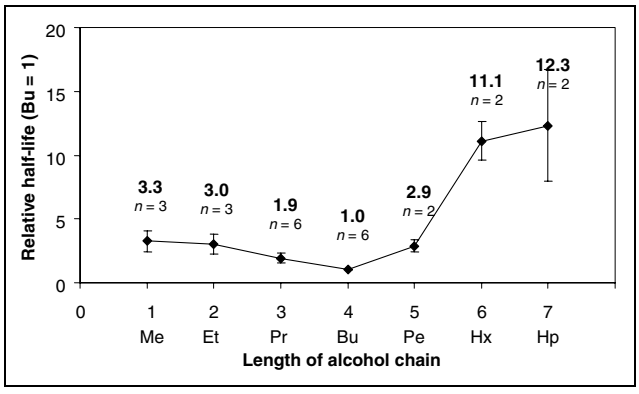

Fig. 6: Average relative *in vitro* human blood or plasma hydrolysis half-lives for various straight-chain alcohol substituents in different congener series of aliphatic esters. The graphic represents the average of the data of Fig. 5

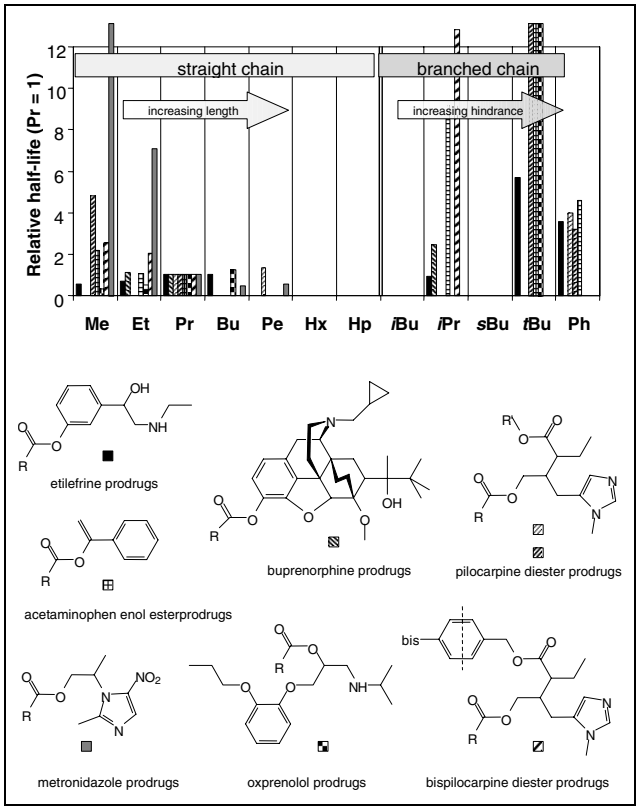

Fig. 7: Relative *in vitro* human blood or plasma hydrolysis half-lives for various acyl substituents (R) in different congener series. Within each series, relative values were calculated compared to the butanoic acid (R = Pr) substituent

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References

1 Augustinsson, K.-B.: Ann. N.Y. Acad. Sci. **94**, 844 (1961)
2 Krisch, K.; in: Boyer, P. D. (Ed.): The Enzymes, Vol. 5, p. 43, Academic Press, New York 1971
3 Heymann, E.; in: Jakoby, W. B.; Bend, J. R.; Caldwell, J. (Eds.): Metabolic Basis of Detoxication, Biochemical Pharmacology and Toxicology, p. 229, Academic Press, New York 1982
4 Walker, C. H.; Mackness, M. I.: Biochem. Pharmacol. **32**, 3265 (1983)
5 Williams, F. W.: Clin. Pharmacokin. **10**, 392 (1985)
6 Leinweber, F.-J.: Drug Metab. Rev. **18**, 379 (1987)
7 Satoh, T.; Hosokawa, M.: Annu. Rev. Pharmacol. Toxicol. **38**, 257 (1998)
8 International Union of Biochemistry, Nomenclature Committee: Enzyme Nomenclature 1984. Recommendations of the Nomenclature Committee of the International Union of Biochemistry on the Nomenclature and Classification of Enzyme-Catalyzed Reactions, p. 646, Academic Press, Orlando 1984
9 Bodor, N.; Buchwald, P.: Pharmacol. Ther. **76**, 1 (1997)
10 Bundgaard, H., (Ed.): Design of Prodrugs, p. 360, Elsevier Science, Amsterdam 1985
11 Wermuth, C. G.; Gaignault, J.-C.; Marchandeau, C.; in: Wermuth, C. G. (Ed.): The Practice of Medicinal Chemistry, p. 671, Academic Press, London 1996
12 Stella, V. J., (Ed.): Adv. Drug Deliv. Rev. **19**, 111 (1996)
13 Bodor, N.; Buchwald, P.: Adv. Drug Deliv. Rev. **36**, 229 (1999)
14 Bodor, N.; Buchwald, P.: Med. Res. Rev. **20**, 58 (2000)
15 Morikawa, M.; Inoue, M.; Tsuboi, M.: Chem. Pharm. Bull. **24**, 1661 (1976)
16 Ammon, R.: Arzneim.-Forsch. (Drug Res.) **27**, 944 (1977)
17 Quon, C. Y.; Stampfli, H. F.: Drug Metab. Dispos. **13**, 420 (1985)
18 Quon, C. Y.; Mai, K.; Patil, G.; Stampfli, H. F.: Drug Metab. Dispos. **16**, 425 (1988)
19 Feldman, P. L.; James, M. K.; Brackeen, M. F.; Bilotta, J. M.; Schuster, S. V.; Lahey, A. P.; Lutz, M. W.; Johnson, M. R.; Leighton, H. J.: J. Med. Chem. **34**, 2202 (1991)
20 Minagawa, T.; Kohno, Y.; Suwa, T.; Tsuji, A.: Biochem. Pharmacol. **49**, 1361 (1995)
21 Ericsson, H.; Tholander, B.; Regårdh, C. G.: Eur. J. Pharm. Sci. **8**, 29 (1999)
22 Buchwald, P.: Min. Rev. Med. Chem. **1**, 101 (2001)
23 Stampfli, H. F.; Quon, C. Y.: Res. Commun. Mol. Pathol. Pharmacol. **88**, 87 (1995)
24 Durrer, A.; Wernly-Chung, G. N.; Boss, G.; Testa, B.: Xenobiotica **22**, 273 (1992)
25 Hassall, K. A.: The Biochemistry and Uses of Pesticides, 2nd Ed., p. 536, Macmillan, London 1990
26 Hodgson, E.; Kuhr, R. J., (Eds.): Safer Insecticides. Development and Use, p. 593, Marcel Dekker, New York 1990
27 Lund-Pero, M.; Jeppson, B.; Arneklo-Nobin, B.; Sjögren, H.-O.; Holmgren, K.; Pero, R. W.: Clin. Chim. Acta. **224**, 9 (1994)
28 Lin, J. H.; Lu, A. Y.: Pharmacol. Rev. **49**, 403 (1997)
29 Ordentlich, A.; Barak, D.; Kronman, C.; Ariel, N.; Segall, Y.; Velan, B.; Shafferman, A.: J. Biol. Chem. **273**, 19509 (1998)
30 Barton, P.; Laws, A. P.; Page, M. I.: J. Chem. Soc. Perkin Trans. 2, 2021 (1994)
31 Buchwald, P.; Bodor, N.: J. Med. Chem. **42**, 5160 (1999)
32 Wechter, W. J.; Johnson, M. A.; Hall, C. M.; Warner, D. T.; Berger, A. E.; Wenzel, A. H.; Gish, D. T.; Neil, G. L.: J. Med. Chem. **18**, 339 (1975)
33 Durrer, A.; Walther, B.; Racciatti, A.; Boss, G.; Testa, B.: Pharm. Res. **8**, 832 (1991)
34 Chelminska-Bertilsson, M.; Allenmark, S.; Edebo, L.: Biochim. Biophys. Acta **1202**, 56 (1993)
35 Rivory, L. P.; Bowles, M. R.; Robert, J.; Pond, S. M.: Biochem. Pharmacol. **52**, 1103 (1996)
36 McCarthy, T. J.; Witz, G.: Toxicology **116**, 153 (1997)
37 Tinoco, I.; Sauer, K.; Wang, J. C.: Physical Chemistry. Principles and Applications in Biological Sciences, 3rd Ed., p. 770, Prentice-Hall, Upper Saddle River, NJ 1995
38 Laws, A. P.; Page, M. I.: J. Chem. Soc. Perkin Trans. 2 , 1577 (1989)
39 Maksay, G.; Tegyey, Z.; Ötvös, L.: J. Pharm. Sci. **67**, 1208 (1978)
40 Yang, S. K.; Lu, X. L.: J. Pharm. Sci. **78**, 789 (1989)
41 Takahashi, K.; Haginaka, J.; Tamagawa, S.; Nishihata, T.; Yasuda, H.; Katagi, T.: J. Pharm. Pharmacol. **42**, 356 (1990)
42 Takahashi, K.; Tamagawa, S.; Sakano, H.; Katagi, T.; Mizuno, N.: Biol. Pharm. Bull. **18**, 1401 (1995)
43 Yoshigae, Y.; Imai, T.; Horita, A.; Matsukane, H.; Otagiri, M.: Pharm. Res. **15**, 626 (1998)
44 Udata, C.; Tirucherai, G.; Mitra, A. K.: J. Pharm. Sci. **88**, 544 (1999)
45 Mørk, N.; Bundgaard, H.: Pharm. Res. **9**, 492 (1992)
46 Gatley, S. J.; MacGregor, R. R.; Fowler, J. S.; Wolf, A. P.; Dewey, S. L.; Schlyer, D. J.: J. Neurochem. **54**, 720 (1990)
47 Melchert, R. B.; Goldlin, C.; Zweifel, U.; Welder, A. A.; Boelsterli, U. A.: Chem. Biol. Interact. **84**, 243 (1992)
48 Tafi, A.; van Almsick, A.; Corelli, F.; Crusco, M.; Laumen, K. E.; Schneider, M. P.; Botta, M.: J. Org. Chem. **65**, 3659 (2000)
49 Melendez, J. A.; Stone, J. G.; Delphin, E.; Quon, C. Y.: J. Cardiothorac. Anesth. **4**, 704 (1990)
50 Silbey, R. J.; Alberty, R. A.: Physical Chemistry, 3rd Ed., p. 969, Wiley, New York 2001
51 Petzelbauer, I.; Nidetzky, B.; Haltrich, D.; Kulbe, K. D.: Biotechnol. Bioeng. **64**, 322 (1999)
52 Maccarrone, M.; van der Stelt, M.; Rossi, A.; Veldink, G. A.; Vliegenthart, J. F.; Agrò, A. F.: J. Biol. Chem. **273**, 32332 (1998)
53 Charton, M.: J. Am. Chem. Soc. **97**, 1552 (1975)
54 Charton, M.: J. Am. Chem. Soc. **97**, 3691 (1975)
55 Zuidam, N. J.; Crommelin, D. J.: J. Pharm. Sci. **84**, 1113 (1995)
56 Russell, D.; Royston, D.; Rees, P. H.; Gupta, S. K.; Kenny, G. N.: Br. J. Anaesth. **79**, 456 (1997)
57 Buchwald, P.; Bodor, N.: Pharmazie **55**, 210 (2000)
58 Higuchi, T.; Davis, S. S.: J. Pharm. Sci. **59**, 1376 (1970)
59 Purcell, W. P.; Bass, G. E.; Clayton, J. M.: Strategy to Drug Design: A Guide to Biological Activity, p. 193, Wiley-Interscience, New York 1973
60 Martin, Y. C.: Quantitative Drug Design. A Critical Introduction, p. 425, Marcel Dekker, New York 1978
61 Hansch, C.; Leo, A. J.: Substituent Constants for Correlation Analysis in Chemistry and Biology, p. 339, Wiley-Interscience, New York 1979
62 Hansch, C.; Leo, A.; Hoekman, D.: Exploring QSAR. Hydrophobic, Electronic, and Steric Constants, p. 557, American Chemical Society, Washington, DC 1995
63 Kubinyi, H.; in: Wolff, M. E. (Ed.): Burger's Medicinal Chemistry and Drug Discovery, Vol. 1, p. 497, Wiley-Interscience, New York 1995
64 Pannatier, A.; Testa, B.; Etter, J.-C.: Int. J. Pharm. **8**, 167 (1981)
65 van de Waterbeemd, H.; Testa, B.; Marrel, C.; Cooper, D. R.; Jenner, P.; Marsden, C. D.: Drug Des. Deliv. **2**, 135 (1987)
66 Charton, M.: Method. Enzymol. **112**, 323 (1985)
67 Altomare, C.; Carotti, A.; Cellamare, S.; Ferappi, M.; Cagiano, R.; Renna, G.: Int. J. Pharm. **48**, 91 (1988)
68 Bodor, N.; Buchwald, P.: J. Phys. Chem. B **101**, 3404 (1997)
69 Buchwald, P.; Bodor, N.: Curr. Med. Chem. **5**, 353 (1998)

70 Buchwald, P.; Bodor, N.: J. Am. Chem. Soc. **122**, 10671 (2000)
71 Huang, T. L.; Shiotsuki, T.; Uematsu, T.; Borhan, B.; Li, Q. X.; Hammock, B. D.: Pharm. Res. **13**, 1495 (1996)
72 Nielsen, N. M.; Bundgaard, H.: Int. J. Pharm. **39**, 75 (1987)
73 Baxter, A. J. G.; Carr, R. D.; Eyley, S. C.; Fraser-Rae, L.; Hallam, C.; Harper, S. T.; Hurved, P. A.; King, S. J.; Meghani, P.: J. Med. Chem. **35**, 3718 (1992)
74 Yang, H.-S.; Wu, W.-M.; Bodor, N.: Pharm. Res. **12**, 329 (1995)
75 Stout, D. M.; Black, L. A.; Barcelon-Yang, C.; Matier, W. L.; Brown, B. S.; Quon, C. Y.; Stampfli, H. F.: J. Med. Chem. **32**, 1910 (1989)
76 Buchwald, A.: Ph.D. Thesis, University of Florida, Gainesville 2001
77 Bansal, A. K.; Khar, R. K.; Dubey, R.; Sharma, A. K.: Pharmazie **49**, 422 (1994)
78 Wagner, J.; Grill, H.; Henschler, D.: J. Pharm. Sci. **69**, 1423 (1980)
79 Stinchcomb, A. L.; Paliwal, A.; Dua, R.; Imoto, H.; Woodard, R. W.; Flynn, G. L.: Pharm. Res. **13**, 1519 (1996)
80 Bundgaard, H.; Falch, E.; Larsen, C.; Mosher, G. L.; Mikkelson, T. J.: J. Pharm. Sci. **75**, 775 (1986)
81 Patel, J. P.; Repta, A. J.: Int. J. Pharm. **9**, 29 (1981)
82 Jordan, C. G. M.: J. Pharm. Sci. **87**, 880 (1998)
83 Järvinen, T.; Poikolainen, M.; Suhonen, P.; Vepsäläinen, J.; Alaranta, S.; Urtti, A.: J. Pharm. Sci. **84**, 656 (1995)
84 Johansen, M.; Møllgaard, B.; Wotton, P. K.; Larsen, C.; Hoelgard, A.: Int. J. Pharm. **32**, 199 (1986)

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