Quantitative Chirality in Structure−Activity Correlations. Shape Recognition by Trypsin, by the D_2 Dopamine Receptor, and by Cholinesterases

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Abstract: We found that the quantitative degree of chirality of substrates correlates with their efficiency of reaction with active sites. The degree of chirality, a global shape descriptor, was determined by the use of the Continuous Chirality Measure (CCM) methodology developed previously (Zabrodsky et al. *J. Am. Chem. Soc.* **1995**, *117*, 462), which treats chirality as a continuous structural property and not as a binary quality (chiral/not chiral). The generality of this new type of shape−activity correlation is demonstrated for five receptor/substrate systems: trypsin/arylammonium inhibitors; the D_2-dopamine receptor/dopamine derivative agonists; trypsin/organophosphate inhibitors; acetylcholinesterase/organophosphates; and butyrylcholinesterase/organophosphates. The correlations were obtained both for active-site induced chiral conformers and for inherently chiral inhibitors. Interestingly, for some of these cases the correlation of activity with structure is hidden when classical parameters, such as chain length, are taken, but is revealed with this shape descriptor. For two cases we show that the CCM approach is capable of corroborating the assignment of the pharmacophore moiety. We define and make a distinction between the *quantitative enantioselectivity ratio*, which is the ratio of the slopes of the correlation lines for two enantiomeric series and which serves as a measure of enantioselectivity, and the *quantitative chirality−sensitivity ratio*, which compares the sensitivity to chirality changes of different enzymes toward the same set of inhibitors. The findings of this study are quite nontrivial because symmetry and chirality are *global* shape parameters and not specific descriptors of the intricate geometry of the drug or of the active site. We propose tentatively that these results may indicate two different recognition mechanisms: *shape recognition* and *chemical recognition*. The first is a low-resolution determination of the overall shape of the substrate and the second is the classical exact key-locking. We discuss possible implications of these results for predicting optimal shape from data of large libraries.

1. Introduction

The search for structure−activity correlations has been a major tool in contemporary biochemical and biomedical research and in rational drug design. A variety of molecular structural parameters and various similarity indices have served this purpose.[1] Having in mind that chirality is a common feature of practically all bioreceptors and of many of their natural and synthetic substrates, one would expect to find chirality as a common structural correlant with activity. This, however, is not the case. The main reasons for this lack have been the need to extend the conceptual notion of symmetry and chirality beyond the "either/or" picture and, instead, treat them as measurable on a quantitative scale and the need for a convenient and efficient methodology for the quantitative analysis of symmetry in general and of chirality as a special case.

Let us first emphasize the difference between two distinct ways to describe the structure of a molecule, in the context of a recognition action.[2] The first is the traditional, highly specific method of using bond lengths, angles, specific location of atoms, and so on; a picture as exact as instrumentation allows is thus obtained. The second method involves global, overall shape descriptors, asking questions such as: What is the symmetry of the molecule? Is it chiral? Is it planar? and so on. The interactions between molecules and recognition sites have been traditionally studied in terms of the first exact method of describing structure. This approach is fully justified since the specific details of an interaction are indeed dictated by the fine details of the structures of the involved species.

The question we have asked in this investigation is, can the second approach to structure, namely the global shape approach, be used to identify correlations between properties and structure, and what is the added information over the use of the first-type descriptors? The justification for this question lies in the experimental and computational observations that recognition is not an either-or property, but may vary in degree. Consider, for instance, active sites of enzymes which can operate at various degrees of efficiency on a variety of substrates, such as some esterases. Thus, recognition can be sharp, taking into account all the fine details of molecular structure, but it may also be fuzzy and vague, looking at the substrate molecule at low resolution where fine details become blurred, leaving as a relevant structure only the overall shape. Important contributions toward the implementation of fuzzy set theory for this purpose were recently made by Exner and Brickmann.[3] Despite the fact that shape descriptors have already been developed,[4] the vast majority of studies of molecular recognition have been in terms

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(1) Blaney, J. M.; Dixon, J. S. *Rev. Comput. Chem.* **1994**, *5*, 299.

(2) (a) Lipkowitz, K. B.; Pearl, G.; Coner, B.; Peterson, M. A. *J. Am. Chem. Soc.* **1997**, *119*, 600. (b) Lipkowitz, K. B. *J. Am. Chem. Soc.* **1992**, *114*, 1514.

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of the first type, using exact, atomic-level descriptors, and in contrast very few studies have been in terms of global shape.

The general need for symmetry and chirality metrics in chemistry has been addressed by a number of research groups[5] including our own.[6] Aimed at versatility, at convenience, and at conforming with chemical and physical intuition, we have developed the Continuous Symmetry Measure (CSM)[7−9] and the resulting Continuous Chirality Measure (CCM)[9] methodologies, which carry the following messages: First, structural chemistry is too rich to be described with the coarse binary language of having or not having the property of being symmetric or achiral. Second, it agrees with chemical, biochemical, and physical intuition to ask questions such as: Given a set of chiral molecules, by how much do they differ from each other in their achirality content? And third, the problem of how to quantify these structural properties is solvable: a concept of what is it exactly that is to be measured is provided (see below) and the practice of carrying it out is detailed and demonstrated. This approach already proved to be useful for a number of symmetry and chirality related issues, including the application of the symmetry measure as an order parameter in small clusters,[10a] the chirality properties of the cyclic trimer of water and of its enantiomerization pathways,[10b] the correlation between the degree of centrosymmetricity and hyperpolarizability,[10c] the chirality of large random objects,[10d] the energy/chirality correlations in the enantiomerization of chiral fullerenes,[10e] and the macroscopic chirality of Pasteur's tartrate crystals.[10f]

Here we report the results of a study on the ability of the CCM approach to quantify structure−activity correlations in bioreceptors, i.e., of the ability to use chirality as a quantitative structural parameter. Five receptor/substrate systems were investigated: trypsin/arylammonium inhibitors; the D_2-dopamine receptor/dopamine derivative agonists; trypsin/organophosphate inhibitors; acetylcholinesterase/organophosphates; and butyrylcholinesterase/organophosphates. Quantitative correlations between the degree of chirality and activity were indeed identified. The correlations were obtained both for active-site induced chiral conformers and for inherently chiral substrates. These findings are quite nontrivial because symmetry and chirality are *global* shape parameters and not specific descriptors of the intricate geometry of the drug and of the active site. We discuss in Section 4 possible implications of this finding, as well as the potential value of the CCM approach for screening libraries and for predicting optimal active shape.

2. Quantifying Symmetry and Chirality

Our solution for quantifying symmetry in general, and chirality as a special case of asymmetry, has been described in detail elsewhere;[7−9] its main features, needed for this report, are outlined briefly here. In essence, CSM is a distance measure that quantifies the minimal translation that each vertex of a structure has to undergo to attain a desired symmetry. It is a special distance function[11] in that there is no ideal reference structure a-priori, but the nearest structure with the desired symmetry is searched and calculated.[12] In a formal way, given n vertexes of the original configuration, located at p_i, and given a symmetry point group G, the amount, $S(G)$, of this symmetry in this configuration is

$$S(G) = \frac{1}{nD^2}\sum_{i=1}^{n}(p_i - \hat{p}_i)^2 \qquad (1)$$

where $\hat{p}_i$ are the corresponding points in the nearest G-symmetric configuration. To avoid size effects, the original structure is normalized to the distance from the center of mass of the structure to the farthest vertex, D (other normalizations, such as root mean square, are possible). If a shape has the desired symmetry, $S(G) = 0$, $S(G)$ increases as the shape departs from G symmetry, up to a maximal value smaller than 1. For convenience we expand the scale by a factor of 100 (0 to 100). Equation 1 is general and allows one to evaluate the symmetry measure of any shape relative to any symmetry group or element. The main theoretical and computational task in applying eq 1 has been to find the set of $\hat{p}_i$, the solution to which is described in refs 7−9.

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(11) Rouvray, D. H. *Top. Current Chem.* **1995**, *173*, 1.

(12) The freedom from a reference structure is an advantage and a generalization of the measurement of chirality and symmetry according to the present methodology. For instance, in employing enantiomers overlap to estimate chirality, one enantiomer is used as a reference structure for the other.[5]

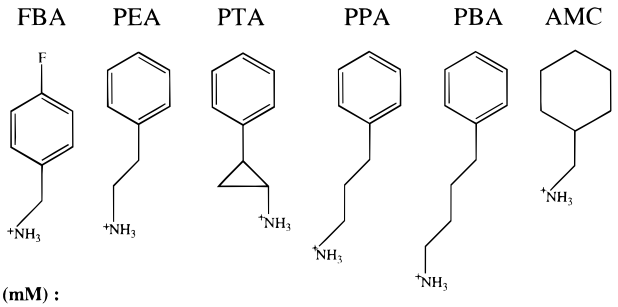

Figure 1. A series of phenylammonium and cyclohexylammonium inhibitors of trypsin and their K_i values.[14] FBA: 4-fluorophenylethylamine; PEA: phenylethylamine; PTA: tranylcypromine; PPA: phenylpropylamine; PBA: phenylbutylamine; AMC: aminomethylcyclohexane.

A particular family of symmetry point groups are the achiral ones, namely all groups containing improper elements such as reflection, inversion, and even-numbered improper rotations. In these cases $S(G)$ measures chirality. In most cases the G that leads to the minimal S value—the CCM—is the symmetry group composed of the identity element and of a symmetry plane. Thus, in its simplest form, the CCM is the minimal distance of the object from having a symmetry mirror plane, $S(\sigma)$. All cases below fall into this category.

3. Case Analyses

Searching for correlation between activity and structure of inhibitors, agonists, and antagonists has been based on three principally different methods of data acquisition,[13] which can be arranged hierarchically. The most relevant and direct method is the determination of the substrate structure within the active site, usually from X-ray or NMR analyses of the complexed substrate. In the absence of experimental measurement, the next method is based on the ever developing computational chemistry tools, tailored to identify an optimal conformation for the receptor-bound molecule. The third and still most common method has been to correlate activity with the structure of the unbound substrate obtained either from X-ray analysis of the crystalline substrate or from computations of the free, minimal-energy conformer. Each of these methods provides different insight on the substrate−active site interactions. Likewise, when analyzing CCM results based on each of these methods, one has to consider the special features of each. The following case analyses are arranged accordingly.

A. Experimentally Derived Structures: Ammonium Inhibitors within the Active Site of Trypsin. Kurinov and Harrison[14] have studied a series of phenyl- and cyclohexylamines which act as nontransition state inhibitors of the N-terminal serine proteinase, trypsin. They determined the X-ray structures for six of the inhibitors (Figure 1) within the active site of the protein[14] and measured the inhibition, expressing it in terms of the enzyme−inhibitor dissociation constants, K_i (i.e., lower K_i values represent more efficient inhibitors). The pharmacophore of these inhibitors is the alkylammonium positively charged side chain that complexes to the negatively charged aspartate residues within the active site.[15] We therefore concentrate on it and compare the chirality properties of the various bound pharmacophores. Except for PTA, the pharmacophores of all other inhibitors are achiral in solution, but exhibit induced chirality in the bound form. Of the two enantiomers of PTA only one is active, and it too undergoes conformational changes within the active site. The induced chirality in the bound, solution-achiral pharmacophore is due to the arc conformation, as shown, for instance, for the butyl pharmacophore of PBA (Figure 2). Chiral arc induction is not possible in the smallest pharmacophores, namely those of FBA and AMC, but even here some residual chirality is observed, mainly due to the specific fixation of the hydrogens (the positions of which were calculated and given as well).[16]

For each of the pharmacophores, the chirality value, $S(\sigma)$, of the bound state was calculated from the X-ray coordinates, using eq 1. When the inhibition efficiency is plotted against chirality content of the pharmacophore, a clear correlation trend between the two parameters is revealed (Figure 3). The correlation expresses the ability of the active site to induce chirality within the bound pharmacophore, indicating that the farther the pharmacophore is from induced chirality, the stronger it inhibits the activity of the enzyme. The ability of the active site to induce chirality is indeed remarkable when one compares the closely related PTA and PEA: the former is chiral in solution whereas the latter is not; yet the degree of induced chirality in PEA exceeds the chirality of bound PTA. It seems therefore that the somewhat higher K_i value of PTA is a reflection of its inflexibility in forming the inhibition bonding, compared to PEA.

An important observation is that the correlation collapses if the alkyl chain length is taken as a structural parameter: The order of activity is ethyl, butyl, propyl, *which is detected by the chirality analysis*, and not the routinely expected order of ethyl, propyl, butyl. Thus, the correlation is between activity and a global shape parameter (chirality), and not between activity and a specific structural parameter (chain length).[17] Finally, the CCM analysis is also capable of corroborating the originally proposed hypothesis that the active chromophore is the alkylammonium chain: When activity is plotted as a function of the chirality value of the whole molecule, no correlation is apparent (Figure 4).

B. Computational 3D QSAR: Agonists of the D_2-Dopamine Receptor. Martin and Lin used computational 3D-quantitative structure activity relationships (QSAR) analysis in search of optimal agonists for the D_2-dopamine receptor.[18] Their methodology included molecular modeling, conformational searching, pharmacophore mapping, and Comparative Molecular Field Analysis (CoMFA)[19] for partial least squares (PLS) analysis.[20] The conformations obtained in that study were proposed to represent the best fit of the agonists within the active site. Biological activity was determined and expressed in terms of the inhibition constants, pK_i, of binding of [^{3}H]spiperone, a D_2 antagonist, to rat brain synaptosomes.[21] Twenty seven D_2 agonists were tested, out of which we concentrate here on the series that includes dopamine itself and its ethylamine derivatives (Figure 5).[22] Four molecules in this series are achiral in solution (**XX**−**XXIII**),[23] but their calculated optimal interacting

(13) Kubini, H. *Pharmazie* **1995**, *50*, 647.
(14) Kurinov, I. V.; Harrison, R. W. *Struct. Biol.* **1994**, *1*, 735.
(15) Persona, J. J. et al. *J. Mol. Biol.* **1993**, *230*, 934.
(16) Brookheaven PDB, entries: 1tnh, 1tni, 1tnj, 1tnk, 1tnl, 1tng.
(17) Note that the chirality of an arc must pass through a maximum when plotted as a function of the arc length; we are now studying this phenomenon in depth for the chiral helicenes series.
(18) Martin, Y. C.; Lin, C. T. In *The Practice of Medicinal Chemistry;* Wermuth, C. C., Ed.; Academic Press: New York, 1996; p 459.
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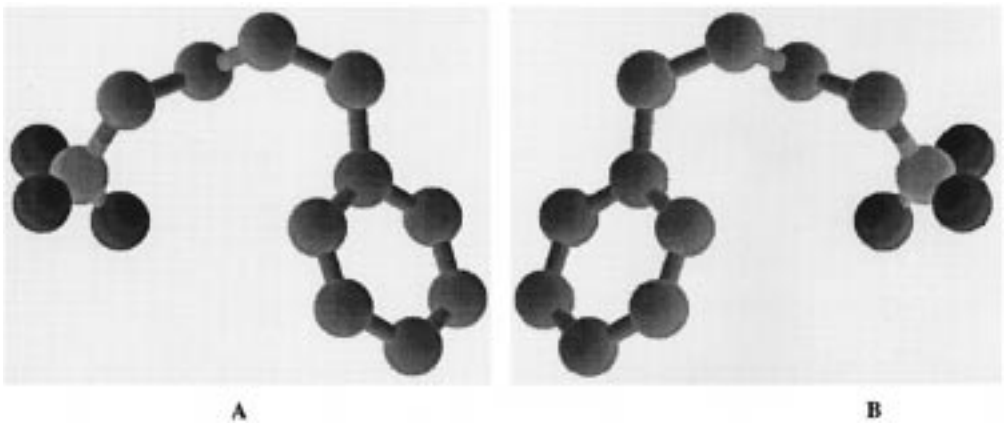


Figure 2. (A) The structure of PBA within the active site of trypsin. The chirality of the butylammonium pharmacophore is induced by the active site and is due to the arc shape. (B) PBA's enantiomer can only be reproduced artificially, by mirror, as in this case, or by creating the artificial enantiomer of trypsin.

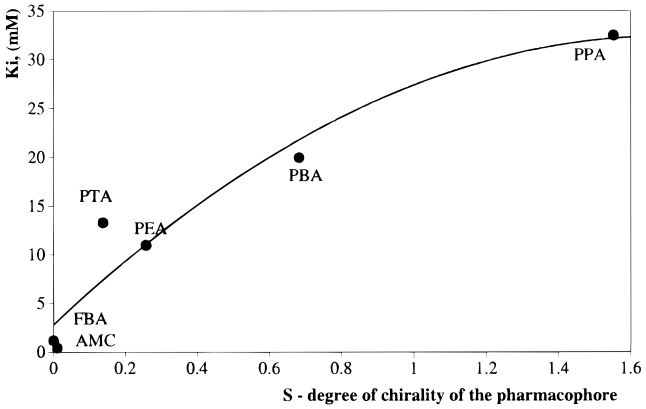


Figure 3. Plot of the inhibition activity as a function of the degree of chirality of the pharmacophores of Figure 1. (The fitted line here and in all figures serves to lead the eye, and does not imply the mechanisms leading to these correlations).

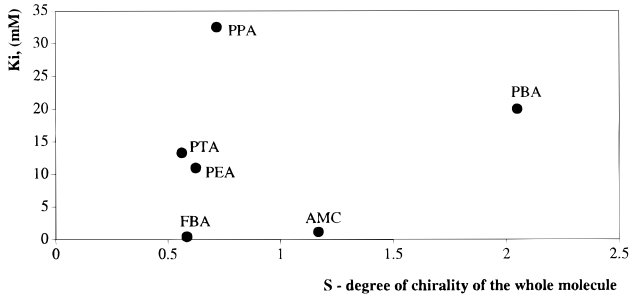


Figure 4. Plot of the tested and confirmed pharmacophore hypothesis: compared to Figure 3, no correlation is observed if the chirality of the whole inhibitor molecule is taken into account.

conformers are all chiral, i.e., chirality is induced by the active site, as in the previous case. The other two (**XXIV** and **XXV**) are a pair of enantiomers, but being in a chiral environment, their computed interacting conformers are quite different, Figure 6, and therefore of different chiralities as well. The chiral receptor recognizes these two as different molecules from each other and from the rest of the molecules in the series. In fact, when comparing two different conformers of an enantiomeric pair, the possibility of assigning opposite handedness to the two collapses. We return to this point in Section 4, Figure 13D.

(22) We shall devote a separate report to CCM screening of large libraries, such as the whole library of the 27 agonists.

(23) For clarity, we use the same notation as in the original report.[18]

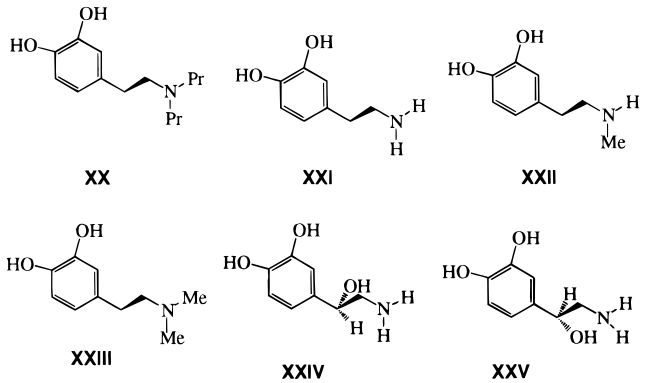


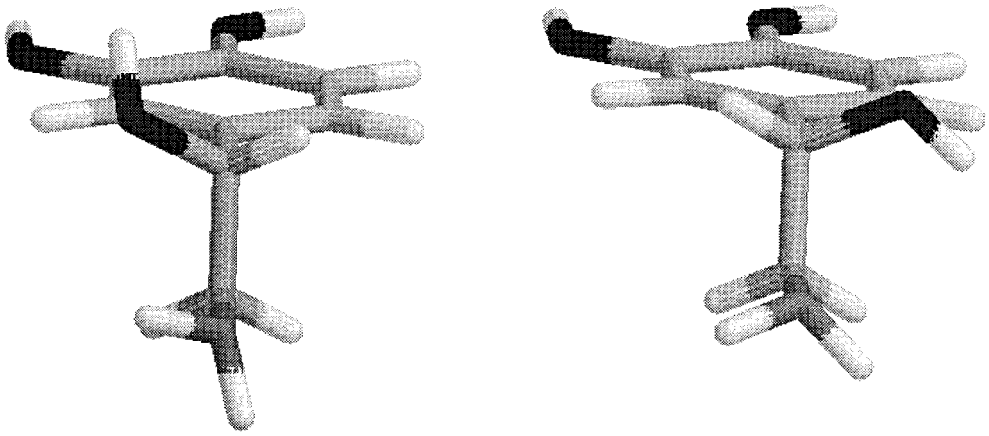
Figure 5. A series of six dopamine derivatives tested as agonists for the D_2-dopamine receptor (the numbering follows ref 18). Dopamine itself is XXI. The chirality of the optimal conformers of XX−XXIII is induced by the receptor. XXIV and XXV are an enantiomeric pair—see Figure 6.

A plot of activity vs degree of (induced) chirality reveals a second case of a clear correlation trend between these two properties, Figure 7. And, as we have seen in the trypsin analysis, whereas the size of the substituents as a parameter does not reveal any trend (the size order is **XXI** < **XXII** < **XXIII** < **XX**), the global shape parameter of the degree of chirality is capable of identifying how structure and activity are correlated. The authenticity of this correlation is further strengthened by testing the generally accepted hypothesis that the catechol moiety plays an important role in the binding to the D_2-dopamine receptor:[18] When the catechol moiety is removed, the activity−chirality correlation collapses (Figure 8). We recall that in the previous case we performed the same test but in an opposite direction.

C. Organophosphate Inhibitors of Trypsin and Acetyl- and Butyrylcholinesterase. The third category, as listed above, is the use of the structure of the unbound inhibitor in its minimum energy conformation. QSAR studies have traditionally used such data, assuming that there exists a secondary correlation between these structures and the unknown structures within the active site. This scenario has been a working hypothesis, widely used and with many demonstrated correlations.[13,24] We demonstrate here the use of the CCM approach to this type of data, and the information that can be deduced from it.

For this purpose we analyze data obtained for one of the most studied groups of hydrolytic enzyme inhibitors, namely the

(24) Bersuker, I. B. et al. *New J. Chem.* **1991**, *15*, 307.



XXIV XXV

Figure 6. The actual different conformers of the pair of enantiomers XXIV and XXV (Figure 5) within the active site.

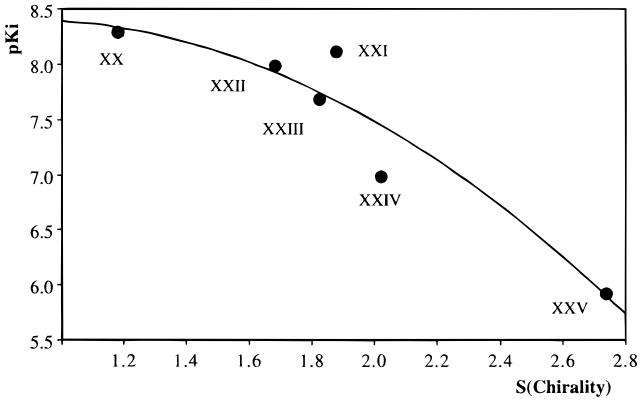


Figure 7. Plot of the activity of the D_2-dopamine receptor agonists (Figure 5) as a function of their degree of chirality (see the caption to Figure 3 for an explanation of the fitted line).

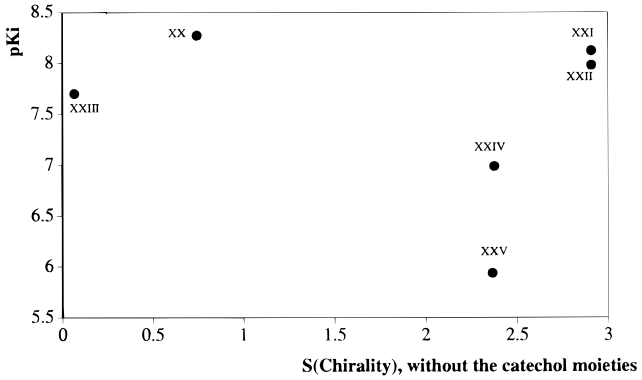
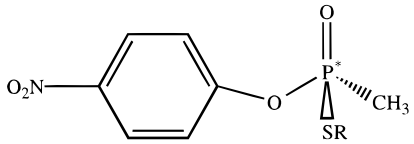


Figure 8. Plot of the tested and confirmed hypothesis of the importance of the catechol moiety for the inhibition activity of the compounds in Figure 5. The correlation of Figure 7 is lost upon removal of the catechol moiety.

organic phosphates. These phosphates are inherently chiral if the phosphorus atom carries four different substituents, as is the case for the *S*-alkyl-1-nitrophenylmethylphosphono-thiolates (Figure 9), which were the topic of the pioneering studies of Ooms and Boter.[25,26] These authors compared the inhibition activities of the racemates with those of the pure enantiomers for a number of enzymes including trypsin and acetyl- and butyrylcholinesterase (AcChE and BuChE). Inhibition activity was determined in these studies in terms of the rate constants of the irreversible disappearance of enzymatic activity (higher



R= CH_3, C_2H_6, n-C_3H_7, n-C_4H_9, n-C_5H_{11}

Figure 9. The L enantiomer of the series of five chiral organophosphate inhibitors of trypsin and of the cholinesterases studied by Ooms and Boter.[25,26]

rate constants indicate higher inhibition activity). In a later study,[27] the minimal energy conformers of these inhibitors were calculated (using Chem-X[28] as a molecular builder; structures were optimized with MOPAC[29] by using the AM1 semiempirical Hamiltonian[30]) and used for a structure−activity analysis.

Using the computed conformers of ref 27, we begin with the inhibition of trypsin, which was analyzed above (Case A). Here we shall test whether the CCM analysis can identify trypsin's chiral sensitivity for a different set of inhibitors, namely, whether the trend, identified above, that the more achiral inhibitors are better ones, is kept for another set of inhibitors. The degree of chirality of the organophosphate inhibitors was calculated according to eq 1, and a plot of the inhibition rate constants as a function of chirality for both the L and D series is shown in Figure 10: The two enantiomeric sets of inherently chiral inhibitors show a fairly good correlation between activity and the chirality of the computed minimal energy conformers. Interestingly, the trend is the same one as observed for the alkylammonium inhibitors: The more achiral inhibitors are the more active ones. (Recall that K_i and the inhibition rate constants indicate inhibition efficiency in an opposite way.) An important observation is that while the ammonium and phosphate inhibitors behave with opposite trends when it comes to the alkyl size (the smallest is the most active in the former, but the least active in the latter), they do show the same trend when chirality is taken as the structural parameter. Thus, the global shape parameter of chirality identifies a common trend for the two different families of inhibitors, not detected by looking at the obvious and standard parameter of the gradual change in size of the alkyl side chain.

(25) Ooms, A. J. J.; Boter, H. L. *Biochem. Pharmacol.* **1965**, *14*, 1839.
(26) Ooms, A. J. J.; Boter, H. L. *Biochem. Pharmacol.* **1967**, *16*, 1563.
(27) Seri-Levy, A.; Richards, W. G. *Tetrahedron: Asymmetry* **1993**, *4*, 1917.
(28) Chem-X, Chemical Design Ltd. Roundway House, Cromwell Business Park, Chipping North, Oxon, OX75SR, UK.
(29) Stewart, J. J. P. *MOPAC*; QCPE, No. 455.
(30) Dewar, M. J. J.; Zoebisch, E. G.; Healy, E. F.; Stewart, J. J. P. *J. Am. Chem. Soc.* **1985**, *107*, 3902.

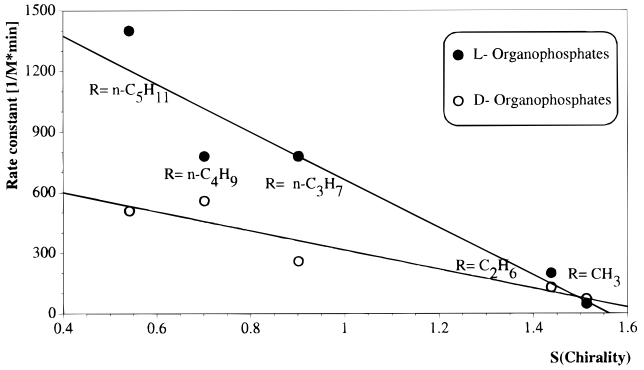


Figure 10. Plot of the inhibition rate constants of trypsin by the L- and D-organophosphates in Figure 9 as a function of their degree of chirality. (The correlation to a linear fit for the L series is $R^2 = 0.92$ and that for the D series is $R^2 = 0.89$.)

The clearly distinct different slopes for the D and L series indicate the different diastereomeric interactions of the two enantiomeric series. The ratio of the slopes of the correlation lines (Figure 10) can be used as an additional measure of enantioselectivity. The *quantitative enantioselectivity ratio* (QER)[31] for the present case is L/D = 2.4. This low QER values does not necessarily mean low sensitivity to chirality, since chirality and enantioselectivity are distinctly different issues—the first measures symmetry while the second relates to handedness; we explain this important point in the Discussion. Despite the low QER value and unlike Case B, it is not possible here to try and merge the data for the two enantiomer series, because information on the actual reactive conformers, which blur the assignment of handedness, is unavailable. For large QER values, as is our next example, one would not try to merge D and L anyway.

After we compared the chirality−activity correlations for the same enzyme, trypsin, with two different types of inhibitors, alkylamines and phosphates, let us make a different cross test, and use the same set of inhibitors, for different enzymes, to continue to explore the possible generality of this approach. This can be done with the same set of organophosphates because these compounds inhibit many other enzymes which employ serine as a catalytic moiety in the active site, such as AcChE and BuChE. Indeed, we continue with Ooms and Botter, who measured the irreversible inhibition activity of the compounds in Figure 9 on these two enzymes. They found that AcChE is much more enantioselective than BuChE and that "A rough correlation between the stereospecificity of AcChE and inhibitor activity exists". We are now in a position to study this statement by the quantitative CCM analysis.

Figure 11a shows the correlation between the reported inhibition rate constants of AcChE and the degree of chirality of the L and D organophosphates. Unlike the trypsin case, here the activities of the L and D series are very different, and therefore the graph for the D series is reproduced in Figure 11b on an enlarged scale. A good linear correlation is obtained for the D series for all inhibitors, and for the L series for all except the pentyl derivative. (It is in order to emphasize here that there is no implicit reason to expect that the correlation will be approximately linear on "all" scales. One would expect recognition and the degree of key/lock fitting to pass through an optimum.) The L/D-QER value, based on the methyl, ethyl, propyl, and butyl derivatives, is 60.4 (or 45.3, if all five are taken). This QER value is much higher than the trypsin value of 2.4 and accordingly AcChE is much more enantioselective. Finally, the known lower selectivity of BuChE (Figure 12) is reflected by the low QER of 1.9.

(31) Note that the QER compares slopes of sensitivity, while the eudismic ratio refers to specific L/D pairs.

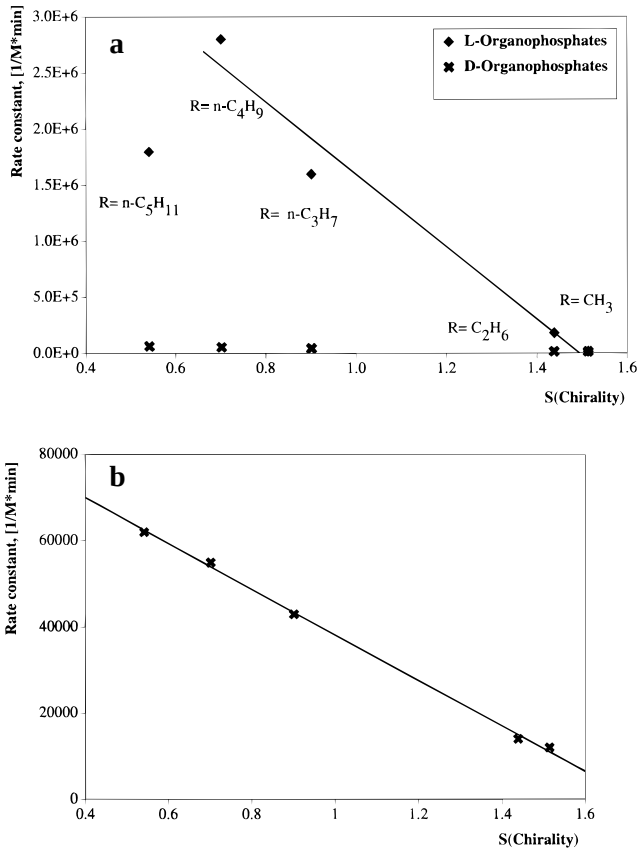


Figure 11. (a) Plot of the inhibition rate constants of acetylcholinesterase by the L- and D-organophosphates in Figure 9 as a function of their degree of chirality. (b) The D-series is shown on an enlarged scale. (The correlation to a linear fit for the L series (first four points—see text) is $R^2 = 0.968$, and that for the D series is $R^2 = 0.998$.)

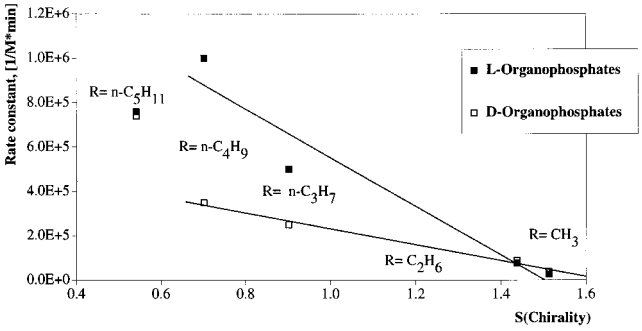


Figure 12. Plot of the inhibition rate constants of butyrylcholinesterase by the L- and D-organophosphates in Figure 9 as a function of their degree of chirality. (The correlation to a linear fit for the L series (first four points—see text) is $R^2 = 0.93$, and that for the D series is $R^2 = 0.99$.)

quantitative chirality−sensitivity ratio (QCSR) for the L inhibitors is trypsin: BuChE:AcChE = 1:770:2050; and for the D series, the QCSR

is 1:1215:110. Note the difference between the QER and the QSCR values: the former measures enantioselectivity of a series of chiral pairs; the latter compares chiralities for the homochiral series. The obtained QCSR values quantify the high sensitivity of the cholinesterases to chirality changes compared to the low sensitivity of trypsin, i.e., the relative nonspecificity of the latter. Interestingly, it also shows that while AcChE is more sensitive to changes of chirality within the L series, the higher sensitivity in BuChE is toward the D series.

4. Discussion

We have shown that the degree of chirality, a global shape descriptor, is capable of identifying a new type of structure−activity relationships. We have demonstrated it for several key bioreceptors, namely trypsin, two cholinesterases, and the D_2-dopamine receptor. We have shown that both induced and inherent chiralities obey these correlations; that the sensitivity to chirality of an enzyme is recognizable for different sets of inhibitors; that the same set of chiral inhibitors reveals this type of correlation for different enzymes; that when exact structural features, such as alkyl side chain length, fail to detect the correlation, the degree of chirality is capable of doing so; and that the CCM approach is capable of corroborating the assignment of the main chromophore in a series of bioactive substrates. It is evident quantitatively from all the cases we studied here (and as is actually well documented in many QSAR studies[13,24]) that it makes much more sense to relax the celebrated key/lock concept of Emil Fischer,[32] and speak on a *degree* of fitness.[33] For example, in the trypsin−amine inhibitors series, FBA (Figure 1) shows the best lock/key fit for this series, PBA has a lower degree of fitting, and PPA's degree of fitting is the lowest.

The CCM, as a structural parameter, shares with all other physical, chemical, and structural correlants used in QSAR studies the same inherent weakness: Finding a correlation with a single parameter and declaring this parameter as the dictating one, may be an oversimplification. However, the tradition in QSAR studies, and which we follow here, has been to assume that the main affecting feature within a homologous series is a gradually changing feature between one member of the series and the next, all other features being changed at a relatively slower pace. Thus, having identified the possible role of the degree of chirality, we illustrate now in Figure 13 several scenarios which may serve as a guideline as to what types of key-lock fits could lead to gradual changes in chirality and enantioselectivity. The illustrative model, which serves only as an educational tool without implying actual mechanisms, assumes that the active site is chiral (two-dimensional L- or T-shaped chirality) and distinguishes between four cases which were selected in view of the trends we found above (although not mimicking them, of course): a highly enantioselective situation in which the site recognizes only an L series but not a D series (Figure 13A; high enantioselectivity was observed in the AcChE case); induction of chirality in a set of a-priori achiral substrates (Figure 13B; cf., the trypsin-amines case); a chiral site that is sensitive to chirality, yet cannot distinguish between L and D (Figure 13C; cf., the BuChE case); and the role of induced chirality in a series comprised of both achiral substrates and an enantiomeric pair (Figure 13D; cf., the D_2-dopamine case). Note two important features: The order of chirality does not follow the order of arm length (Figure 13B), and the induction of chirality in a substrate that is already chiral may affect the handedness (Figure 13D).

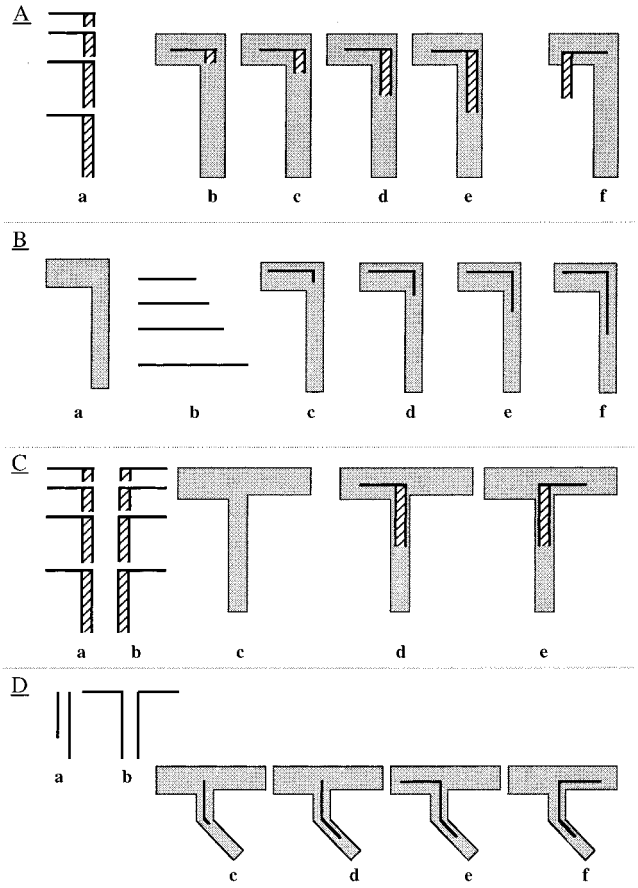


Figure 13. An illustration of various scenarios of active-site/substrate interactions, leading to changes of degree of chirality and of enantioselectivity: The active sites are the two-dimensional-chiral hollow L or T shapes; the substrates are the full lines. (A) We begin with the classical picture of high enantioselectivity—only one type of two dimensional handed substrates, **a**, can fit the active site, **b**−**e**, and a substrate of opposite handedness, **f**, cannot enter. The variation in the degree of chirality follows the inherent chiralities of the substrates. (B) Induced chirality: A set of achiral substrates, **b**, attains induced chirality upon interaction with the active site, **a**. Note that the degree of chirality does not follow the side chain length: it is highest for **d** and **f**, and lowest for **c** and **e**; **c** has just departed from being a straight line, and **e** is almost of equal-length arms. (C) Enantioselectivity and sensitivity to the degree of chirality may be different issues. This is a scenario for low enantioselectivity yet high sensitivity to chirality: Both sets of enantiomers, **a** and **b**, can interact with the active site **c**, as shown in **d** and **e**. While enantioselectivity is therefore low, the two sets follow a sensitivity to chirality, as in Case A. (D) When considering the case of a mixed set of achiral, **a**, and chiral, **b**, substrates, induced chirality may play a role in the interaction of both, as illustrated in **c**,**d** and **e**,**f**. It is important to note that in such cases, the original handedness assignment of an enantiomeric pair, **b**, may collapse within the active site: Whatever definition was used to assign specific handedness to each enantiomer of the **b**-pair, the different conformational changes in **e** and **f** do not necessarily preserve the same distinction—they now may be of the same handedness or switch under the same definition.

Beyond the identification of chirality as a structural correlant with activity, there is a principal finding that is quite nonobvious, and which must be addressed: Chirality is an overall descriptor—it does not go into specific fine details of the structure, which have been the focus of the search for a clear picture of chemical recognition—instead, chirality describes the whole shape. We

(32) Fischer, E. *Chem. Ber.* **1894**, *27*, 2985.

(33) See ref 10d for a related study on the concept of chirality of large random objects.

found that the recognition is not only the search for the best intricate fit, but also the identification of a suitable global shape. How can that be? One possible interpretation of this observation is that it indicates the operation of two different recognition steps involving two different recognition mechanisms: the *shape recognition* and the *chemical recognition*. The first step allows the receptor to probe at a low resolution the overall shape of the substrate and to make a preliminary assessment of whether that shape is potentially suitable for the second step of the exact key-locking. In other words, *shape recognition serves as a preliminary filter*. Since this tentative proposition agrees not only with the correlations we revealed, but also with the general accumulated know-how of the activity of bioreceptors, we believe it may be useful to put it on the discussion agenda. If corroborated, the finding that the active sites of enzymes respond to global shape may also shed some light on the understanding of evolutionary routes of these active proteins[34]—shape recognition may have preceded exact key/lock fit.

We conclude with two points: First, the two-step recognition concept also has potentially practical implications on the procedures employed for rational drug design, answering the growing need for fast screening of large (combinatorial) libraries: The globality of the CCM descriptor makes it a useful tool for a quick, preliminary search for trends in shape/activity (the first step) within large series of experimental drug molecules. Needless to say, the final details of the mode of action of a selected drug-molecule relate strongly to specific structural details (the second step), but a global shape descriptor may save a lot of computational and synthetic work, up to the stage where zooming-in on some promising candidates is required. Efforts in this direction are in progress in our laboratory.

Second, we recall that achirality is a special case of the general CSM methodology that is capable of measuring the degree of content of any symmetry group. QSAR studies can thus be extended beyond activity−chirality into activity−symmetry in general. Indeed, preliminary accounts of the CSM analyses of the degree of C_2 symmetry of the HIV-1 protease and its blockers[35] and of the analysis of the near-C_2 symmetry of the bacterial photosynthetic center[36] were made; full reports are in preparation.

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(34) For recent progress in evolutionary studies see: *J. Theor. Biol.* **1997**, *4* (August), *187*.

(35) Keinan, S.; Avnir, D. In *Book of Abstracts, 4th World Congress of Theoretically Oriented Chemists*; Jerusalem 7-12.7.96, and http://chem.ch.huji.ac.il/employee/avnir/watoc96/poster.html.

(36) Keinan, S.; Edelstein, J.; Plato, M.; Pinsky, M.; Avnir, D. In *Book of Abstracts, 63rd Meeting of the Israel Chemical Society*, Tel-Aviv, February 9−10, 1998.