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Modeling of peroxide activation in artemisinin derivatives by serial docking

Roy J. Little · Alexis A. Pestano · Zaida Parra

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Abstract Serial docking of artemisinin derivatives. A serial docking study was undertaken with the purpose to improve the understanding of the mechanism of production of biological activity in Artemisinin derivatives. The Heme molecule receptors were primarily chosen to represent the changing binding and oxidation states of this molecule, which are postulated to occur during the activation of the drug, in order to relate these results to the observed biological activity. The results of the docking runs were classified according to similarity to a standard orientation by a combination of automated and "by hand" procedures. One- and two-dimensional QSAR equations were used in an exploratory sense in order to study the influence of the type of receptor. Principal component and partial least squares regression techniques were used in the case of the multivariate (3D-QSAR) descriptors. The results obtained corroborate the postulated mechanism of production of biological activity as well as providing evidence that, at the moment of activation, the electronic structure of the Heme molecule approximates that of the oxygenated Heme, the drug molecule adopts a preferred orientation, and that, in addition to the important positive contribution of the O^1 - Fe interaction, there is as well a significant negative effect on the biological activity by carbons 4 – 6 of the artemisinin ring system.

Keywords Artemisinin · Modeling · Partial least squares · Serial docking

R. J. Little (✉) · A. A. Pestano · Z. Parra
Grupo de Fisicoquímica Orgánica, Departamento de Química,
Facultad de Ciencias, Universidad de los Andes,
Mérida 5101, Venezuela
e-mail: rlittle@ula.ve

Introduction

An improved understanding of the chemical basis of the mechanism of action of artemisinin is necessary in the rational development of more cost-effective modes of treatment of malaria. As a tropical disease, malaria is one of the most important causes of economic and social stagnation in third-world countries, and, although the scientific discussion has been marred by advocacy [1], it is incontestable that, because of anthropogenic global climate change, increased efforts to fight the disease will be necessary, in tropical as well as in temperate zones [2, 3].

In our concern to understand the molecular mechanisms involved in the production of biological activity in artemisinin and its derivatives, we have applied, reformulating the well-known scoring problem [4], the methods of modern QSAR studies to obtain greater understanding, in a chemical sense, of the molecular events involved in the production of biological activity. We have applied the multiple-receptor technique (serial docking) with the purpose of studying the variation in the QSAR relations as the receptor varies, according to the model of heme-activated initiation of the drug response. Thus, the study of deoxy- and oxy-hemoglobin, as well as hemin, is proposed as an approximation of the sequence of events of 1) initial binding, 2) orientation, and 3) reaction of the drug molecule with the receptor. Some of the more recent methods of the multivariate description of the dependence of biological activity upon chemical structure are based upon docking experiments [5] which model the interaction of the drug molecule with the postulated active site, often a protein-based receptor system, using molecular mechanics methods. Incorporating this type of data into a QSAR analysis is conceptually fundamentally different from the original Hansch approach in that the descriptors are not of intrinsic

properties of the drug, or ligand, molecule, but of the interaction itself between the two species.

The many docking routines available to the researcher are often used in scoring [6, 7] procedures, to rank the series of compounds on the basis of predicted biological activity. The scoring problem is currently of high priority in the field of QSAR research, but the result of such studies is often limited to the assignment of a single-valued (scalar) score, or rank, to a compound, reducing the amount of information that may be of help in the understanding of the mechanism of action of the series of compounds. Often, this scalar value is simply the binding energy, as estimated by the docking routine, or it can be derived from a multivariate analysis, reduced to a composite score, but still unidimensional.

The immediate result of a docking study is a population of geometries (also called orientations or poses) which need to be evaluated as to their representing the true state of affairs at the moment of the production of biological activity. In many studies, there is previous knowledge of this "true" state, which is usually the crystallographic structure of the bound ligand, and in such cases the goal is usually the testing of novel algorithms. In our case, the true state is the subject of investigation. An often incompletely documented problem with this is to choose exactly which one of these conformations represents the reality of the situation for the biologically significant event. Should one use the global minimum, or based on other knowledge, should the lowest energy geometry which is most similar to that "known" to be the preferred binding mode? Other work has not usually addressed this problem; indeed, it has been a contentious issue that "not all authors have cared to reveal the exact details of their functions," [5, p. 233] when referring to the problems of replicability in scoring. We test here the suitability of a particular pose based on some admittedly qualitative characteristics of preliminary QSAR-type studies. A study in which the correctness of the pose was determined by the understandability of its predictions, as determined in QSAR-type analysis, has been presented in the steroid field [8]. Thus, in order to test the significance of the interaction, which is postulated to be the determining event in the production of biological activity [9, 10], between the drug molecule and the heme receptor, we applied a docking method, using AutoDock [11], to model the drug-heme interaction. Although the conventional QSAR approach has seen increasing sophistication both in its techniques and areas of applicability, it remains subject to its original paradigm, which is that of an overarching, quasi-mathematical relationship between an observed, desired, property and a number of determining variables, which may be manipulated by the scientist, or entrepreneur, as the case may be. It is our opinion that methods adapted from the chemometrics field may be more advantageously applied as tools, individually, and in combination, and from a point of view that is more consonant with that of the experimental medicinal chemist. Thus, our focus here is radically different from the traditional QSAR approach, since our purpose is really more in line with that of a theoretical chemical study [e.g., 12] of the mechanism of electron transfer in the artemisinin - heme activated complex. With the explicit purpose, then, of gaining understanding of the elementary physico-chemical processes involved at the molecular level of the biological activity of artemisinin and derivatives, we present here some preliminary results of docking studies which have fundamental implications for the mechanism of action of this important series of medicinal compounds.

The mechanism of action of artemisinin and derivatives has recently become a subject of some controversy [13], and the original thinking that the interaction of the O1–O2 peroxide linkage of the Artemisinin molecule with the ferrous (or ferric) heme iron is the key event in the manifestation of biological activity [14] has come into question [15]. One of our purposes here then is to establish further evidence for the mechanism of heme activation of artemisinin and derivatives.

We have studied separately two data sets from the literature. The first data set consists of 23 alkyl and acyl derivatives of artemisinin reduced at the 10-position to the hemiacetal stage studied by Cheng and co-workers [16], (cf. Fig. 1 of Cheng, et al.), the syntheses and biological activities of which had been reported in a previous publication [17]. Data set number two is from work published by Avery, et al. [18], also from 2002, from which we have taken only those derivatives of artemisinin which possess the full pyranobenzodioxepin structure, namely Table 1 (41 compounds), 3 (50 compounds), and 10 (14 compounds) of the Avery article (supplemental information), for a total of 105 compounds from this data set. When referring to the compounds of data set #2 in what follows, at times it is convenient to refer to a given substance by a single number, from 1 to 105, reflecting the collapse of the three tables to one. Both data sets have been previously studied with respect to the development of structure-activity relationships [16, 19, respectively]. The

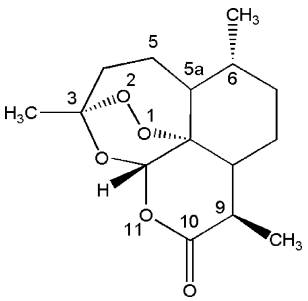


Fig. 1 Artemisinin. Only the positions discussed in the text are labeled

Table 1 Heme-type receptors used in study

Heme	Total charge	Mult.
1) 1CTJ	0	1
2) Hemin	1	2
3) 2DN1 - Fe++ α	0	1
ack4) 2DN1 - Fe++ β	0	1
5) 2DN1 - Fe+++ α	1	2
6) 2DN1 - Fe+++ β	1	2
7) 2DN2 - α	0	5
8) 2DN2 - β	0	5
9) 2DN3 - α	0	1
10) 2DN3 - β	0	1

Numbers (1–10) are used below to refer to hemes

heme-type molecules that were studied were representative of different oxidation states and different binding states. Also were included two heme-type receptors that have been used before in docking studies. The data sets were analyzed separately because of an observed incompatibility: the biological activities of the three compounds common to the two data sets are negatively correlated.

The computational techniques used in the present study are based nearly exclusively upon open-source programs and several utility scripts were developed to facilitate the interfacing of the main programs. This development was carried out in the spirit of the Unix philosophy [20] which emphasizes simplicity, modularity, and reliability. Both univariate (estimated free energy of binding, oxygen - Fe distances) and multivariate (individual atomic van der Waals potentials) regression techniques were applied in the development of the QSAR relations. Within the latter, we studied two methods of coefficient shrinkage (principal components and partial least squares). The multivariate analysis presented here can be thought of as a 3-D QSAR method, without being subject to the infamous alignment problem [21]. Additionally, descriptors derived from docking experiments could be thought to be more adequate than intrinsic descriptors in describing the dynamic, mutual interaction between drug and receptor and thus more important in determining the decisive moment of activation in the production of biological activity. In this sense, the use of QSAR methodology is subsumed under a more general goal of understanding or elucidating the chemical events underlying a biological phenomenon.

The various scripts used to process the docking data and perform the statistical analyses, together with an example data set, are available on the web site of the corresponding author [22]. Although the scripts are not being divulged under the authority of the Gnu public license [23], they are made available to enable other researchers to test the methods we introduce here.

Materials and methods

All calculations were performed on a Pentium-IV workstation using Debian Linux, version 4, and Linux kernel 2.6.16. Statistical analyses were carried out using scripts written using the statistical programming language R [24] and the add-on packages ‘mclust’ and ‘pls’. The open-source programs AutoDock 3.05 [11] and VMD 1.8.3 [25] were also used in the work. MOPAC 93 [26] was compiled for use on the workstation and was used in the preparation of models of the ligands. Further details of the experimental procedures have been published previously [8].

Automated docking

AutoDock uses a rigid receptor model and partially flexible ligands in that the side chains are allowed to rotate; any ring system is maintained in a fixed geometry. Of the several algorithms available in AutoDock, the hybrid genetic algorithm-local search (GALS) was used, a decision based on our previous experience with the program. The general procedures we employed in the use of AutoDock have been previously reported [8].

1. *Preparation of heme (receptor) models*

The heme molecular models used as receptors were prepared from crystallographic structures obtained from various sources. The high-resolution (1.25 Å) 2DN1.pdb, 2DN2.pdb, and 2DN3.pdb [27] structures were obtained from the Protein Data Bank, and from each of these we obtained two heme molecules, corresponding to the ? and ? chains of the protein, by extracting the HETATM records from the files. For the 2DN1(oxyhemoglobin)-derived structures both the Fe++ and Fe+++ oxidation states were modeled. For the other two hemes, deoxy (2DN2) and bound with CO (2DN3), only the Fe++ state was modeled. In order to compare our results with those of previous workers [16, 28–30], we also prepared receptor molecules based on hemin, which was prepared from the published data of Chlorohemin [31], and on the 1CTJ.pdb (Cytochrome - C6) structure, in which the covalently bound cysteinyl sulfurs were replaced with hydrogens. The total charge of the heme molecule in the receptors was assigned to reflect the oxidation state of the iron atom (a total charge of +1 reflecting a nominal charge on the iron atom of +3 and a neutral receptor molecule reflecting an iron with a nominal charge of +2).

The crystallographic structures were used without geometry optimization. The atomic charges were calculated from a single-point unrestricted Hartree-Fock calculation with the STO-3G basis set, using Gaussian 98 [32] for the calculations. The charges and multiplicities assigned are shown in Table 1. The high spin state chosen for deoxy

heme (2DN2), together with the low spin state chosen for oxy heme (2DN1 - Fe++) reflect the accepted spin state change on binding of O2 to hemoglobin [33].

After forming the.pdbq files with the Gaussian-calculated charges, using a Python script, the receptor molecules were put into final form (i.e., adding the solvation parameters) for use with AutoDock with AutoDock Tools, forming in the same step the potential energy maps. The grid for these maps was so chosen as to place the heme molecule at the bottom of the grid, with the O2 binding surface upward, and with dimensions of a regular cube of 38.3 Å per side. In this way, the docking of the artemisinin derivatives was limited to the oxygen binding face of the Heme molecule, in accordance with the postulated model that protein bound heme is the important species [9]. It is also important to note that the dimensions of the box are sufficiently large to accommodate all poses of the ligand molecules. This is a potential source of artefacts in docking studies. The spacing of the grid points was 0.3 Å. The ligand molecules contained more than the limit of seven different atom types and a modification of the AutoDock code, as has been treated in the AutoDock discussion list [34], was also necessary.

2. *Preparation of ligand models*

The published crystallographic structure of artemisinin [35] was used as the starting point for the preparation of the models of the derivatives. We have observed that the optimization of the artemisinin molecule by either semi-empirical or ab initio quantum mechanical methods distorts the all-important O1 – O2 bond [27]. To avoid this distortion in the preparation of the models of the ligands, only modification to the ring system was made, and only when necessary, to atoms C9, C10 and O11, with all other ring system atoms held fixed. The MOPAC z-matrix thus was adjusted to optimize only the geometric parameters associated with these atoms in addition to those of the added side chains, using the PM3 basis set. The charges calculated by MOPAC were used to form the.pdbq files which were used as input to AutoDock Tools to put them into final form (i.e., assignment of rotatable bonds) for the docking runs, again using a Python script for the purpose. Other details with respect to the preparation of the ligand models have been previously discussed [8].

3. *Cluster analysis of docking runs*

In each docking run, 500 geometries (or poses), of the ligand were produced, each of which represents a local minimum on the potential energy surface. Although AutoDock provides a clustering algorithm, based on RMSD differences from the lowest energy geometry, we found it necessary for our purposes to develop our own method. Based on preliminary observation of the docking of artemisinin itself to heme, we found that a large proportion of the poses could be categorized into a principal binding mode (which we have designated the standard mode, (cf. Fig. 2), which was also the lowest energy pose for artemisinin. Significantly, the majority of the other compounds did *not* have the standard pose as their lowest energy pose. This mode, as well as a significant fraction of the others, have the peroxy oxygens oriented toward the heme iron. We then categorized the geometries from each docking run into one of four groups:

1) Standard;
2) Not standard (but α-side);
3) All α-side binding (the sum of standard and other rotated geometries); and
4) Not α-side; that is, with the peroxy oxygens orientated away from the heme iron (a pose that would not be expected to be correlated with the production of biological activity).

Groups 1 and 2 (and 3 and 4) are mutually exclusive; that is there are no poses in common between them. This grouping makes possible the test of whether an orientational preference is present (1 vs. 2) or if all that is necessary is that the drug molecule bind to the receptor with the peroxy group toward the heme (3 vs. 4). Due to the placement of the heme molecule in the grid as mentioned previously, we limited the docking to the α-side of the heme (the O_2 binding side). The averages of the values of binding energy, O^1 - Fe distance, and van der Waals potentials, as given by AutoDock, for each group, were used in the statistical analyses.

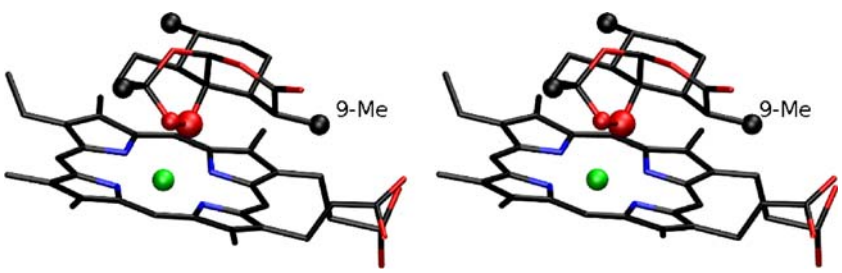


Fig. 2 The standard docking pose. The 9-Methyl group is used as a reference

In order to efficiently categorize the docking runs, a multivariate description of the binding geometries was necessary, and after some preliminary work, the variables (interatomic distances) in Table 2 were decided upon. The categorization of a given pose (Pose) is determined relative to two reference structures: 1) artemisinin, in its "standard" position (Ref.); and 2) the Heme molecule receptor (Heme). Other combinations of distances were tried, but this combination of four distances in particular seemed to aid most in the preliminary categorization of the groups. This idea of using a multivariate description of the poses to classify them has been applied in other instances e.g.,[36, 37].

Forming part of a utility script written in R, the R package 'mclust' [38], version 3, was used as a starting point to automatically cluster the multivariate description of the individual geometries. These preliminary groups were then transformed into trajectories, using a combination of procedures written in Python and TCL and using the facilities of VMD. This preliminary categorization was then refined "by hand" in a procedure in which the trajectories were visually classified (using VMD) and then assigned to one of the four groups mentioned above.

Statistical analysis - QSAR

The analysis we present here is more of an exploratory nature than is traditionally applied in studies of structure - activity relations, although the methodology indeed uses modern chemometrics techniques. In order to begin a study of the usefulness of AutoDock in QSAR, we used the binding energy, "estimated free energy of binding", given by AutoDock, as a unidimensional descriptor of the drug molecule. The average value of this variable for the group under consideration was used in the QSAR equations. Another variable that has been used, and that we report here also, to describe the artemisinin molecule and derivatives is the distance between O^1 of the drug molecule and the heme iron.

Next, in an approximation to 3-D QSAR, we used a multivariate descriptor of the drug molecule, namely, the van der Waals coefficients of each of the atoms of the artemisinin ring system, given as the column "vdW" in the AutoDock output. As a multivariate descriptor of structure, this variable also represents the spatial dependence of the van der Waals interaction energy of each atom of the drug molecule with the grid. This descriptor was then tested in three QSAR-type analyses, ordinary least squares, and two biased techniques, principal components regression (PCR) and partial least squares regression (PLS), in which the rotation of the descriptor matrix is conditioned on the prediction of biological activity. In the case of the biased regression methods, the regression coefficients of the original (vdW) descriptors, rather than the loadings of the transformed variables, were always used. There were 20 descriptors for data set #1, corresponding to the 20 common atoms: 16 of the ring system, the three methyl groups, at the 3−, 6−, and 9-positions, as well as the exocyclic oxygen at the reduced 10-position (not analyzed). For the case of data set #2, only the 16 ring atoms and the 6-methyl group were in common, for a total of 17 descriptors. Ordinary least squares could be applied because the number of cases exceeded (barely) the number of variables, but, of course, this analysis is statistically insignificant and is presented here only as a basis of comparison of the values of r2. The methods have often been used in QSAR studies (particularly, [19]), but their application in the present instance is quite different, and perhaps most similar to the original application of partial least squares analysis in CoMFA [39].

Table 2 Interatomic distances used to characterize docking poses

Ref.	Heme	Pose
O^2	-	O^2
-	Fe	C^{12}
O^2	-	C^4
O^2	-	C^{10}

Results

Docking studies

The use of several different receptors to study the dependence of biological activity upon the chemical structures of an analogous series of compounds has been termed inverse [5], cross [40], or serial [41] docking. The current work falls superficially into this category. However, it must be emphasized that the choice of the receptors to be studied was based on the supposed chemical reaction pathway of artemisinin activation and the focus of the work toward determining the key event of this pathway in the production of biological activity. The results of the docking studies are summarized in Fig. 3. It can be readily appreciated that the results with the heme molecules derived from the ?-chain of oxyhemoglobin reflect the highest percentage of "productive" docking, whether in the Fe++ or Fe+++ state, and we take these results as an indication that the docking to these two species is of higher "quality" than to the others. It is also apparent that the choice of the receptors based upon 1CTJ [29] and hemin [16, 18, 29] was perhaps not the most fortuitous. As stated before,? binding is that in which the ?-side of the artemisinin derivative is toward the heme molecule and standard binding is that portion of ? binding in which the artemisinin derivative adopts the orientation shown in

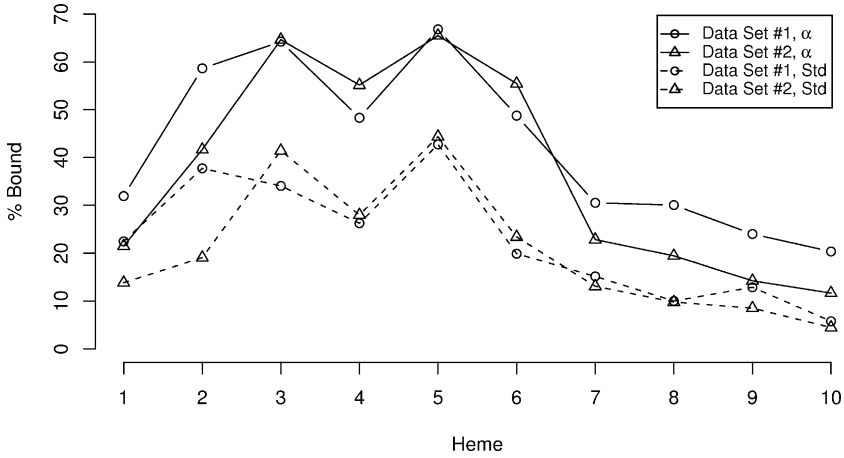


Fig. 3 Percent docked versus receptor type, according to type of docking. Heme numbers refer to

Fig. 2. In work not shown, there was no correlation observed between biological activity and either percent ? binding or standard binding, although it has been used as a criterion in other studies, e.g., [29, p 477]. Additionally, weighting the regression equation with percent of binding did not improve the correlation with any of the predictors tested.

1. *Conventional QSAR*

The measurement of biological activity in both data sets is an increasing function; that is, a higher number means higher activity. In data set #1 the experimental quantity is -log(inhibitory concentration$_{deriv}$) while in data set #2 it is log(conc$_{arte}$/conc$_{deriv}$). Thus a plot of biological activity versus binding energy would be expected to have a negative slope, if greater affinity for the receptor indeed translates to greater activity. The results with this predictor are presented in Fig. 4, segregated by the group (pose) being studied. In the graphs are plotted the Slopes obtained by linear regression analysis of the relationship:

$$\text{BiologicalActivity} = \text{Slope} * \text{EnergyofBinding} + \text{Intercept}.$$

The "error bars" in the figures are not confidence regions, but $1/(2*r^2)$ in the case of data set #1 and $1/(50*r^2)$ in the case of data set #2, indicating lower confidence in the data set #2 results. It is readily apparent that all cases have negative slopes. We look in these graphs for a similar aspect to that of Fig. 3, in which we introduced the subjective concept of "quality of docking", emphasizing the results obtained with hemes 3 and 5, which are derived from the oxyhemoglobin α-chain with nearly 70% α-side docking. In Fig. 4, we note that none of the graphs for data

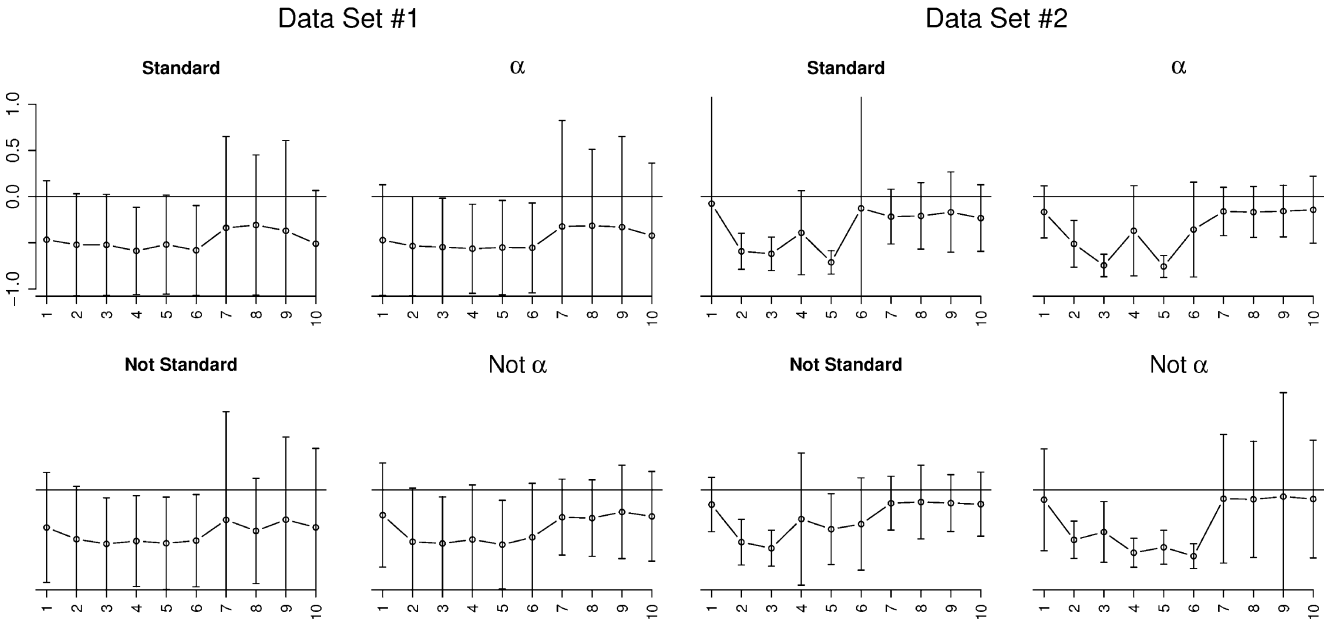


Fig. 4 Serial docking. Slopes of biological activity versus binding energy for the group are plotted. See text for discussion of "error bars"

set #1 have an aspect similar to that of Fig. 3, while, in particular, the α cluster for data set #2 does. This leads us to make an initial, again admittedly subjective, judgment that the results from data set #2 merit especial attention; this enthusiasm being tempered, of course, by the nonsensical results from the "Not α" plot. Thus, we must restate that, in our use of the QSAR-type analyses, they be taken as an indication, in a qualitative sense, of the relevance of the docking experiment to the biological activity. The preliminary observation in this case is that the dockings to the oxyhemoglobin molecules appear to be of "better quality" than the others, as was also observed in Fig. 3.

Another parameter that has been used to describe docking in artemisinin and derivatives is the O^1 - heme iron distance. In Fig. 5, we present the average of this value for the cluster versus the heme receptor. The values for the "Not O^1" cluster are not shown since these values are of course far removed from the other three. To compare, in the oxyhemoglobin molecule, 2DN1.pdb, the O^1 - Fe distance is 1.82 Å in the α-chain and 1.78 Å in the β-chain. In the other liganded Heme models, the corresponding distances are 2.22 Å in clorohemin and 1.74 Å(a) and 1.71 Å(b) in the CO - bound 2DN3.pdb. Thus, the docking of artemisinin derivatives is analogous to the binding of molecular O_2 to these molecules. It would be expected that the biological activity would increase with shorter distance between the two atoms in question, giving a value of <0 for the slope of the Biol. Act. Versus O^1 - Fe distance. We have also analyzed this parameter according to group and according to heme molecule, as above, with the results presented in Fig. 6. Again, as in the case of the free energy of binding study, the "error bars" are not standard errors, but in this case, for both data sets, are $1/(2*r^2)$. The relationship examined in this case is:

$$\text{Biological Activity} = \text{Slope} * O^1 - \text{Fe Distance} + \text{Intercept}.$$

In the case of Fig. 6, our subjective selection of the results from data set #2 receives support, this time clearly favoring the standard pose. Additionally, the nonsensical result observed for the "Not α" cluster is not found here, except in the case of data set #1. Indeed, the expected result: that biological activity should *not* correlate with "wrong-side" docking is indeed found for data set #2. Although the correlation between biological activity and O^1-Fe distance could perhaps be improved through the use of curvilinear or nonlinear regression techniques, we feel that the exploratory nature of this initial study would not justify an additional nonlinear analysis.

2. *Multivariate QSAR*

The presentation of multivariate data is often problematic and although the manner we have chosen may not be the best, we do believe that our conclusions are adequately supported by the particular manner in which we have chosen to represent the results. All dockings to all species were analyzed, but in light of the results from the univariate analyses, and for considerations of space, we limit the presentation here to those obtained with the 2DN1, Heme α, Fe^{++} (Heme 3) analysis.

We have not arbitrarily assigned a "test set" (a subset of the compounds under study), as is common in QSAR studies, for validation and have used cross-validation instead. There are several options in the 'pls' package for validation, and we used the validation='CV'instructive to randomly divide the data into ten segments, each of which is sequentially used as a test set, upon which the RMSEP statistic is then calculated. The statistic used for validation, as presented in the initial report on the CoMFA [39] method, is the prediction sum of squares, or PRESS. In the chemometrics literature, the square root of the mean of this value, called RMSEP (root mean square error of prediction), is used commonly and is the value provided by the PLSR and PCR routines in R and is used in the present work. The validation plots are presented in Fig. 7. There are two observations to be made concerning these plots. The first has to do with the number of components that are considered appropriate to effectively model the relationship between biological activity and chemical structure while avoiding over-fitting. In the present

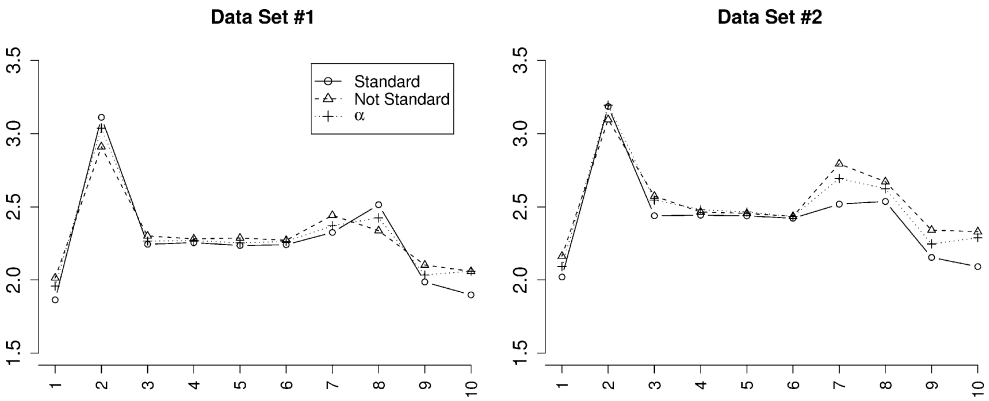


Fig. 5 Average O^1 - Fe distances (Å), in docked poses

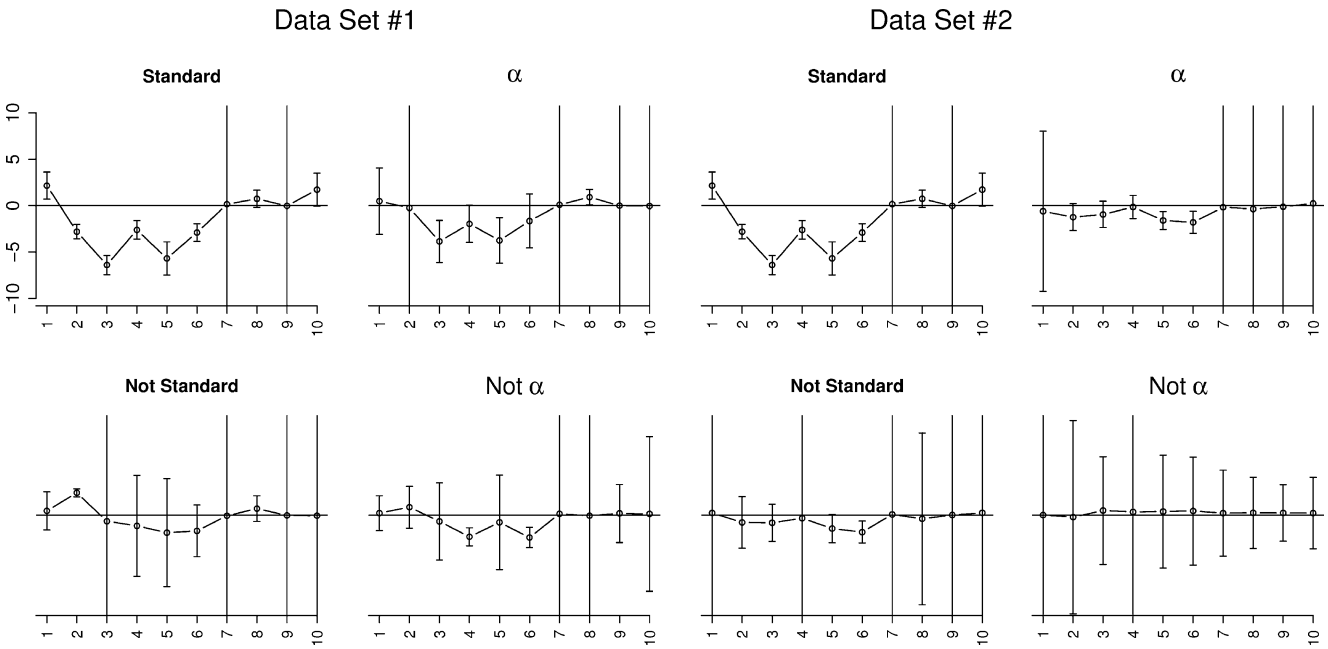
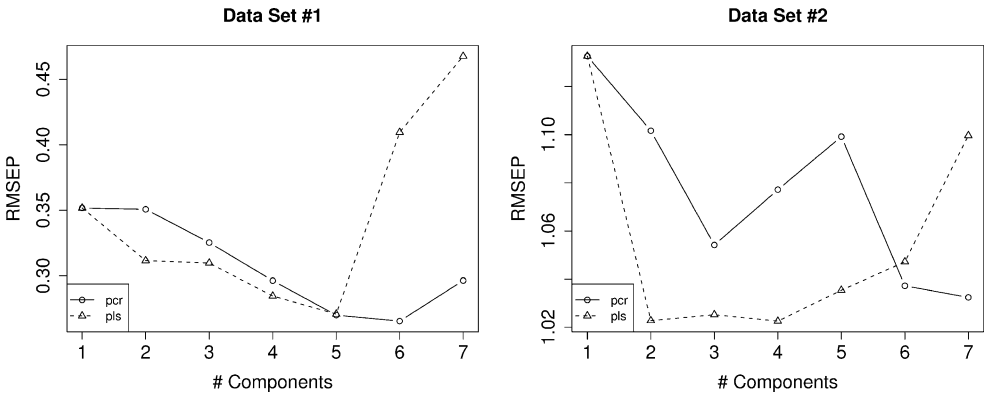


Fig. 6 Slopes of biological activity versus O^1 - Fe distance

case, although it has often been observed that the process of selection is not as objective as could be wished, the inflection point of the curve, or the first minimum found is often that which is chosen. It is to be noted that these curves present this situation at two components. The second observation has to do with the fact that, "in practice, there is hardly any difference between the use of PCR and PLSR..., but that PCR often needs more components to achieve the same prediction error" [42, p 4]. In the curves for PCR and PLSR analysis, it can be readily appreciated that the PCR analysis "follows" the tendency of the PLSR. In light of this observation, we continued with the results obtained from PLSR analysis exclusively.

The plots of predicted versus observed biological activity are presented in Fig. 8. The r^2 values are for the relationship between calculated and observed activity as is common in QSAR work. These values are to be differentiated from the value of r^2 calculated from the multivariate regression, which are much higher, due principally to the use of many descriptors. To compare, the values of this statistic for the OLS analyses are 0.9994 and 0.3704 for data sets 1 and 2, respectively. The effect of coefficient shrinkage is also evident from a consideration of the ranges of the values: The ranges for the coefficients are: OLS, −1377 to 1342; PCR (2 principal components), −0.018 to 0.023: and PLS (2 principal components), −0.062 to 0.062. Use was made of score plots, as recommended by Stanton [43], but we feel that the terms (products of regression coefficient and value of the variable) of the QSAR equations, as described below, have greater interpretability. Our interest here is of course a meaningful interpretation of the regression coefficients, and for this purpose, we chose two compounds from each data set to analyze further in this respect. From Data Set #1 we chose compounds #16 and #20 and from Data Set #2 compounds #100 and #33. The compounds are well fit by the regression treatment, and thus provide a test of the

Fig. 7 Validation plots



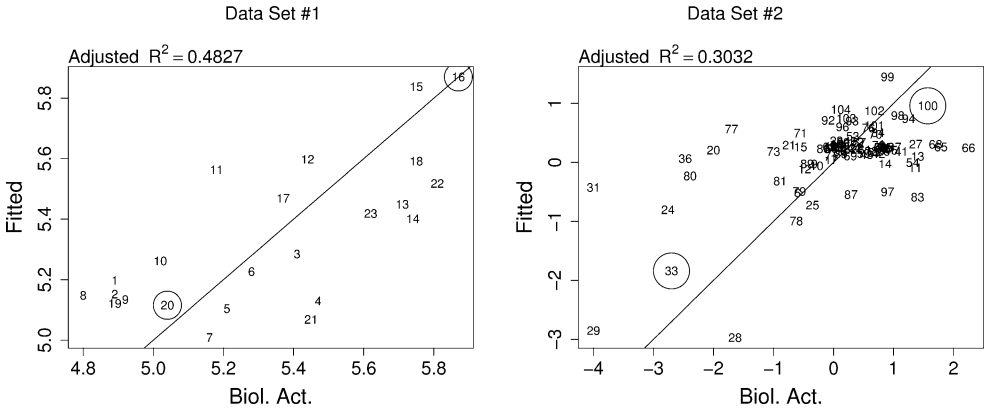


Fig. 8 Predicted versus observed biological activity. Compounds circled were chosen for more detailed analysis

model. The structures of the compounds are presented in Fig. 9.

In Fig. 10, we have plotted the terms of the regression equation for the compounds against atom name. It can be seen that in both plots the positive contribution to the activity of the O^1 atom is obvious, and is of greater magnitude in data set #2. As well, the negative influence of atoms C^4 through C^6 is more apparent in data set #2. These influences can perhaps be better appreciated in a plot in which the values of the terms for data set #2 are superimposed upon a three-dimensional representation of the interaction between drug and receptor, in Fig. 11.

As mentioned above, instead of plotting the coefficients themselves in this more detailed analysis, we considered

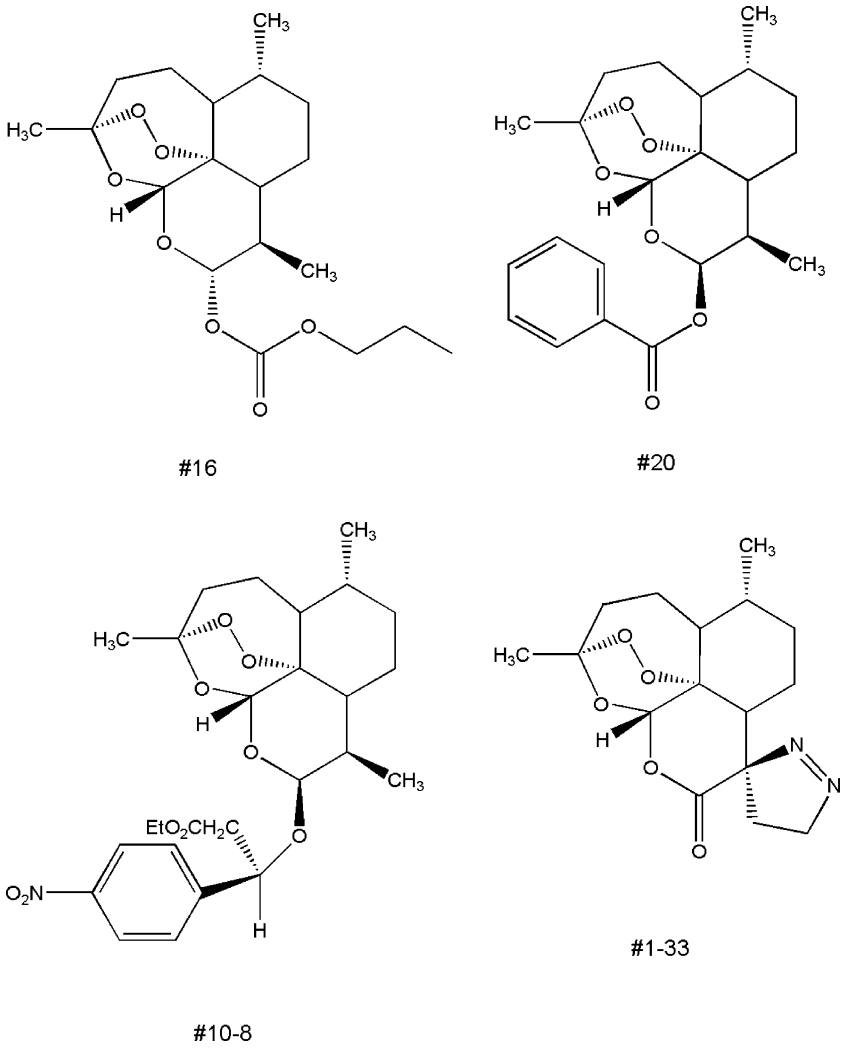


Fig. 9 Structures of compounds selected for further study

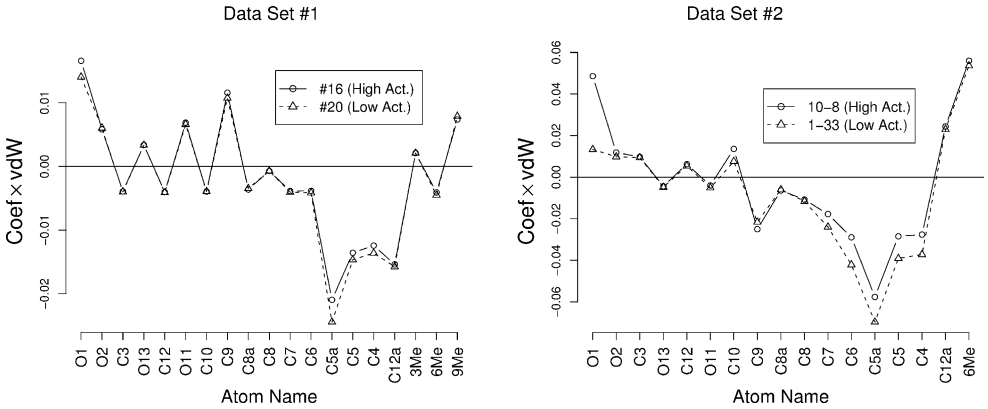


Fig. 10 Terms of QSAR equations for two components and standard pose

that it would be more instructive to plot the values of the terms of the QSAR equation for each compound, with respect to the atom under question. Thus, each point in the plot is the product of the compound's atomic vdW interaction potential (average value, in the standard pose) with the respective coefficient (PLSR, 2PC). In this way, the individual atomic contributions to the activity are more clearly appreciated. The immediate observation is that an active compound has a greater positive contribution from the O^1 position than an inactive compound and that, conversely, a compound of lower activity has a greater negative contribution from the series of atoms from C^4 to C^6, not including the 6-Methyl group, which makes a positive contribution to the biological activity.

Discussion and conclusions

The present work is, in one respect, a reexamination of the scoring problem in docking applications in QSAR. One of the main uses of scoring functions is in high-throughput screening (HTS) [44], in which very large databases of drug candidates are screened. The scoring functions used in HTS are optimized for generality, to accommodate the tremendous variety of substances. In contrast, our scoring functions were adapted to the data sets in question, with the specific purpose to assign the poses to one of the four categories, all based upon the standard pose, and a more generalized approach would have been counterproductive. The preliminary observation of a preferred mode of docking by artemisinin itself led us to cluster the poses based on this "standard" pose, for the subsequent analysis of a possible dependence of predictive power on the pose chosen, the principal hypothesis of the work. Consideration of Figs. 4 and 6 (in particular, Fig. 6) gives a preliminary indication that the standard pose is indeed best able to explain the variation in biological activity over the series of compounds studied, as well as indicating that hemes 3 and 5 are the best models for the receptor in the moment of activation. This initial observation was then later substantiated in the multivariate analysis in which all receptors were studied. However, for reasons of space, we have only presented the results for all hemes for the univariate QSAR studies. The original objective of the study, to establish further evidence for the primacy of the O1 - Fe interaction, has been complemented with the discovery of an orientational preference for the production of biological activity, as well as the negative effect of carbons 4 – 6 on the activity. Our use of the techniques of scoring has been subsumed to an understanding of the physicochemical events surrounding the action of biologically active agents. The use of the coefficients from partial least squares analysis in QSAR dates from the original CoMFA report [39], and is similar in spirit to the approach we have taken here. Other workers have also used the technique in related, but distinctly different applications. In a study of the Avery [18] data, Guha and Jurs [19] used intrinsic descriptors and applied partial least squares analysis post facto to an equation produced from another procedure, in an application of Stanton's [43] recommendation regarding the use of scores

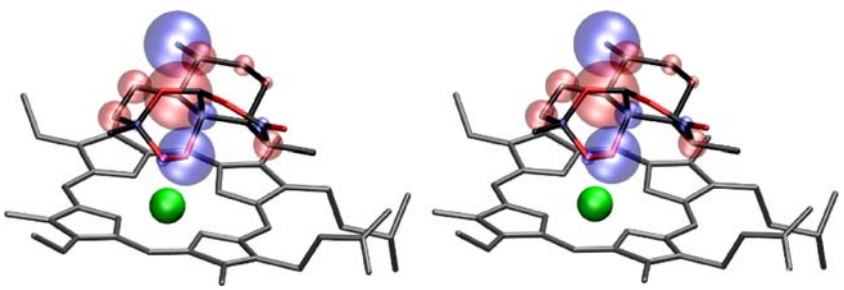


Fig. 11 Graphical representation of QSAR terms from data set #2

and loadings ("weights", according to Stanton) plots. As mentioned above, we feel that the coefficients of the original descriptors, rather than the loadings (which in the 'pls' package in R have assigned arbitrary signs) of the principal components should be used in interpreting the relationships found. We have gone further and have proposed the use of the terms of the QSAR equation when studying the contributions of individual atoms to the overall biological activity in the comparison of individual compounds.

A popular modern statistics book [45] begins with the somewhat apocryphal pronouncement (variously attributed on the Internet to Rutherford D. Roger (or Rogers) or even John Naisbitt) "We are drowning in information and starving for knowledge."[1] The application of statistical methods to the reduction of this volume of information is precisely what is involved in the scoring problem. The inadequacy of the abilities of the human mind to appreciate the subtle interrelationships in overly complex, multidimensional data has led to the use of computer-intensive methods and the development of overarching algorithms, often single-mindedly directed, for example, toward extracting the essence of "Drug-likeness" [46]. However, this is only one of the problems confronting the researcher interested in understanding the mysteries of Nature. The problem of interpretability is also of great current interest. It has been noted by several researchers that the "insights" provided by QSAR studies are all too often unintelligible [47] and even that a limit may have been reached in the field of drug design. The current introspective climate [48] is also evident in the field of HTS [49]. The fundamental assumption in QSAR, often implicit, is that the production of biological activity in a series of analogous compounds is due to a single mechanism. When combined with a docking study, in which this assumption is explicitly formulated, its defects become manifest, in the problem of which pose to use in the development of the QSAR equation. Thus, implicit in this unitary mechanism is a unitary binding mode of the analogous series which becomes quantifiable in terms of the multivariate description of the binding modes of the derivatives. In our work, this similarity of binding modes is a testable hypothesis. We have provided evidence that this unitary mode of production of biological activity is the standard pose. It is likely that this observation seldom applies due to the problem of multiple mechanisms of action.

With ever increasing sophistication, the field of QSAR research proposes ever more general goals, entailing a loss of understanding. The current work has been proposed and carried out with the much more specific purpose of understanding the mechanism of action of a single series of analogs, closely structurally related, through the application of the methods of QSAR as tools, small, efficient, and easily adapted to a given problem. The adaptability of the procedure presented here to new situations is one of its most recommendable aspects. The researcher has at his disposal a highly flexible set of procedures which have already been applied to sets of data as diverse as in vitro steroid-protein binding affinities [8] and, now, antimalarial biological activity.

[1] Rogers, Rutherford D., In Colin Campbell, "Torrent of Print Strains the Fabric of Libraries." New York Times, 25 Feb., 1985: A10.

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