A Critical View on Antimalarial Endoperoxide QSAR Studies

R.R. Teixeira*,[1], J.W. de M. Carneiro[2], M.T. de Araújo[2] and A.G. Taranto[3]

[1]*Departamento de Química, Universidade Federal de Viçosa, Av. P. H. Rolfs, s/n, Campus Universitário, 36570-000 – Viçosa – MG, Brazil*

[2]*Departamento de Química Inorgânica e Departamento de Físico-Química, Instituto de Química, Universidade Federal Fluminense, Outeiro de São João Batista, s/n, 24020-141 Niterói - RJ, Brazil*

[3]*Laboratório de Bioinformática, Campus Centro-Oeste (CCO), Universidade Federal de São João del-Rei, Av. Sebastião Gonçalves Coelho 400, Bairro Chanadour, 35501-296, Divinópolis, Minas Gerais, Brazil*

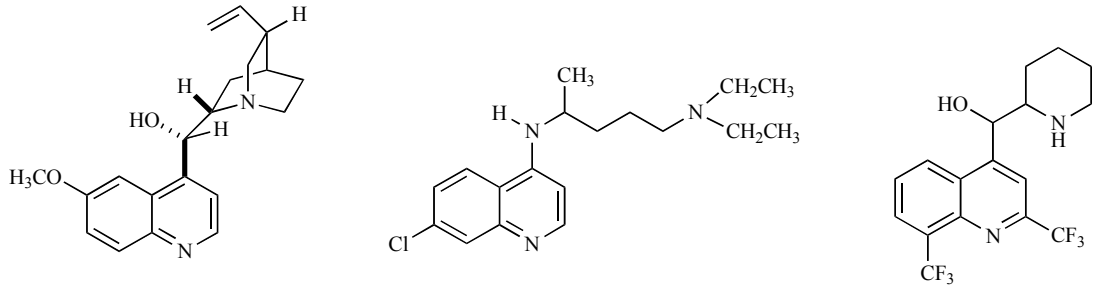
Abstract: Malaria is one of the most dangerous diseases in developing countries. The chemotherapy of malaria has been based on drugs developed more than half a century ago. These drugs are continuously losing their efficacy, mainly due to multi-drug resistance developed by the malaria-causing parasite. In the last three decades, artemisinin and artemisinin-like compounds have proven to be efficient alternatives to the chemotherapeutic control of malaria. These facts have led to an increasing interest in the development of Quantitative Structure Activity Relantioship (QSAR) models for these compounds. This work presents a critical view on some QSAR models, and shows that, due to lack of a rigorous selection of the descriptors entering the models, most of them are unable to accurately indicate the molecular cause of biological activity. Some reasons for the weakness of the published models are discussed.

Keywords: QSAR, malaria, artemisinin, endoperoxide, mechanism of action.

1. INTRODUCTION

Malaria continues to be a widespread endemic and devastating infectious disease in developing countries. It is estimated that 300-500 million malaria cases occur each year resulting in 750,000 – 2 million deaths [1,2]. The disease is caused by infections of protozoan parasites of the genus *Plasmodia,* with *Plasmodium falciparum* being the most significant [3,4]. According to a review by Guerra and co-workers [5], 2.37 billion people live in areas of risk of *P. Falciparum*. The traditional chemotherapeutic treatment of malaria is based on quinoline derivatives such as quinine, chloroquine and mefloquine (Fig. **1**). These drugs are continuously decreasing their efficacy, mainly due to multi-drug resistance developed by the parasite causing malaria [6,7]. In view of these facts, the search and development of new anti-malarial drugs have become a necessity.

One important discovery made by a group of Chinese researchers in the early 1970s initiated a new era in anti-malarial chemotherapy [8]. From the medicinal herb sweet wormwood (*Artemisia annua L.*), they isolated and elucidated the structure of artemisinin (**1**) [9,10]. This sesquiterpene lactone (Fig. **2**) and its more potent semi-synthetic analogues, such as artemether (**2**) and artesunate (**3**) [11,12], are effective not only against multi-resistant strains of *P. falciparum*, but also present a broad stage specificity against the *Plasmodium* life cycle, including activity through the asexual blood stages [13], as well as the



Quinine **Chloroquine** **Mefloquine**

Fig. (1). Structures of quinoline-based antimalarials.

*Address correspondence to this author at the Departamento de Química, Universidade Federal de Viçosa, Av. P. H. Rolfs, s/n, Campus Universitário, 36570-000 – Viçosa – MG, Brazil; Tel: (55) 313899-3209; Fax: (55)31 3899-2370; E-mail: robsonr.teixeira@ufv.br

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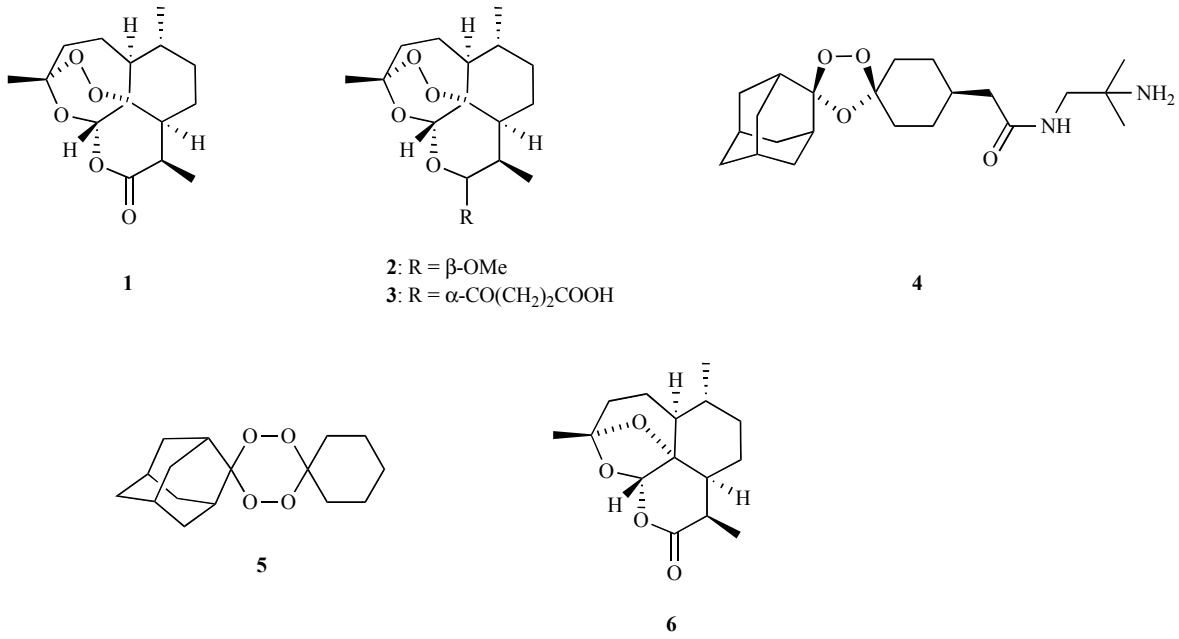


Fig. (2). Structures of compounds **1**-**6**.

sexual gametocyte stages, which may reduce the spread of the disease in low transmission areas [14].

It should be mentioned that artemisinin (**1**) has been evaluated against other parasites, including Leishmania [15], Schistosoma [16,17] and Toxoplasma [18,19]. Anti-viral [20] and anti-cancer [21,22] activities have also been reported for lactone **1**. In addition, a series of studies demonstrated that artemisinin and its synthetic analogues have a powerful inhibitory effect on plant growth [23-25]. This fact led to the investigation of phytotoxic effects of several synthetic heterocycles containing a peroxide structural moiety [26,27].

In view of its clinically useful anti-malarial activity, artemisinin (**1**) has been considered a leading or prototype structure toward the development of new and peroxide-based structures to fight malaria [28-32], resulting in the discovery of designed anti-malarial drugs *in vitro*, such as the compound encoded 0Z277 (**4**) [33-36] and RKA 182 [37] (**5**) (Fig. **2**). Over 2000 publications concerning artemisinin (**1**) and derivatives have been catalogued in the Science Citation Index in the last 20 years, with at least 30 being based on QSAR methodologies.

The structure of artemisinin (**1**) and other anti-malarial endoperoxides is considerably different from the structure of classical anti-malarial drugs, especially those based on quinoline analogues. The most relevant feature of artemisinin and artemisinin-like structures is the presence of a peroxide bridge within 1,2,4-trioxane heterocycle, probably the pharmacophoric group impairing anti-malarial activity to the drug [38,39]. It is a well-known fact that derivatives such as deoxyartemisinin (**6**), without the peroxide bridge (Fig. **2**), are inactive. This indicates that the mechanism of action of this class of compounds is probably distinct from that operating in traditional quinoline-based drugs. Therefore, there is an increasing interest in studying the mechanism of action of the anti-malarial endoperoxides, in particular the relationship between the molecular structure and the anti-malarial potency of each derivative. Due to the relevance of the subject, an assortment of QSAR models on artemisinin derivatives and similar structures has been published in recent years [40-58].

In this review, we discuss some of the QSAR models that have been published so far based on artemisinin (**1**) and artemisinin-like compounds. We demonstrate that, although highly relevant correlations have been found in some cases, it is clear that further investigations are still necessary to achieve a more detailed understanding of the relationship between the molecular structure and the anti-malarial potency of this class of compounds. It is worth mentioning that QSAR models with poor predictive quality are not uncommon [59].

2. THE MECHANISM OF ACTION OF ANTI-MALARIAL ENDOPEROXIDES

Although the precise mechanism of action of artemisinin (**1**) and related endoperoxides is still under debate [60-63], it is known that they act in the intra-erythrocytic stage of the parasite development as a blood schizonticide, by interfering in the detoxification process, either sequestrating heme, thereby potentializing its toxicity, or reacting with it to produce a toxic species that can kill the parasite [8,9,64]. Free intra-parasitic heme released during hemoglobin digestion might play an essential role in the selective toxicity of artemisinin toward the parasites [8,9,64]. Heme catalyzes the reductive decomposition of artemisinin (**1**) and dihydroartemisinin. Incubation of artemisinin with a *Plasmodium sp* culture leads to the same products obtained in the reaction between artemisinin and heme [8,39]. These results indicate that artemisinin interacts with the heme units released in the degradation of hemoglobin, most likely *via* reduction of the peroxide bond. The leading role of the

peroxide bond in the mode of action of artemisinin has been thereafter confirmed in a large number of experimental investigations [8,39]. Based on these facts, most of the QSAR studies published until now have dealt with correlations that associate the anti-malarial activity with parameters intimately related to the peroxide bond. These parameters will be next discussed in this review.

3. PREMISES OF A QSAR STUDY

To develop a QSAR model that aside from being statistically acceptable can also help understanding the chemical and biological processes under investigation, some criteria must be met before the selection of the compounds forming the training set as well as before selecting the molecular descriptors appearing in the final model.

A careful selection of the compounds forming the training set for the development of a QSAR model with high predictive quality is of fundamental importance. Similarly, not all descriptors presenting a relevant statistical correlation can be associated with a causal dependence on activity [59,65]. Although the necessary conditions for development of a consistent QSAR model with satisfactory predictive power have been widely discussed in the literature [59,66-71], they were scarcely taken into account in some of the QSAR studies published on artemisinin and artemisinin-like compounds. To underline our further discussion, we will briefly summarize the minimal conditions for development of a sound QSAR model in terms of the biological activity data and the molecular descriptors that define it.

The first aspect that should be observed in a QSAR study is the quality of the activity (or potency) data of the compounds in the training set. Although the specific position where a drug actually exerts its function in the pathway from the point of entry into the body to the receptor-binding site is sometimes unknown, it has commonly been assumed that the activity (potency) of a drug is directly related to the interaction between the drug and its receptor [69]. Such an interaction results in an equilibrium process related to the free energy of the interaction (ΔG). Therefore, the effect of this interaction must be expressed in terms of the free energy changes that occur during the biological response. Changes in free energy may be calculated as a function of the logarithm of concentration expressed on a molar base. This is the reason why biological activities must be expressed in terms of molar concentrations necessary to give a specific biological response. To be used in QSAR studies, the potency of the drug should be given in the form of the logarithm of the concentration necessary to impart some standard effect.

Besides, to obtain an efficient QSAR model, the selected compounds for the training set should have values which span a large range of activities, preferentially *via* a normal distribution around the mean value. On a logarithmic scale, the lower limit for the range of activity values of the compounds in the training set should span at least two units [66-70]. Training sets with compounds having a small range of activity value data are not capable of reproducing the relevant features of the entire class of compounds.

On the one hand, compounds in the training set should pertain to the same chemical class, because different classes of compounds may have different mechanisms of action. On the other hand, they should have sufficiently dissimilar structures in order to produce a large range of activity values.

Finally, when the activity values form two or more clearly identifiable subgroups or clusters of compounds, they cannot lead to trustful correlations. In this case, a classification method should be used in a previous step to identify the different subgroups [66-70]. Compounds within a given subgroup do not show enough molecular diversity, and one of them should be chosen to represent that subgroup.

Apart from the aspects related to the biological activity, some rules must also be taken into consideration when selecting the molecular descriptors to be used in the final QSAR model. A large set of independent variables may be tested, particularly those representing steric, electronic and lipophylic properties of the molecules in the training set, as well as those obtained by quantum mechanical calculations [70]. In addition to being essentially independent, any variable selected in the final model must possess the highest possible variance to be able to causally correlate to the phenomena under investigation. One should also pay attention to possible errors associated with either the experimental measurements or the theoretical calculations of any variable in the model. It is important to mention that all independent variables should individually be validated by the appropriate statistical method.

In the following section, we will discuss the main aspects associated with the biological activity data, the quality of the descriptors and the final model using some of the QSAR studies published on artemisinin derivatives and other anti-malarial endoperoxides as a source of data for our discussion.

4. DESIRED FEATURES OF THE COMPOUNDS USED IN THE TRAINING SET

4.1. The Quality of the Training Set

Selection of a sound training set is the cornerstone in QSAR studies. QSAR models are usually developed for a chemically-related class of compounds. This is mainly because chemically-related compounds share a common mechanism of action. As a consequence, their potency may be directly related to the structural characteristics and, therefore, the chance of finding a mathematical correlation between structural parameters and potency increases significantly. However, using all compounds within a given chemical family may have a drawback: the activity usually spans a too small range of values. This is the case commonly found in QSAR studies on endoperoxides. One example that can be used to support this statement is the first reported QSAR study of artemisinin derivatives described by Rode and co-workers [40]. Multivariate adaptive regression splines (MARS), multiple linear regression (MLR), alternating conditional expectations (ACE) and project pursuit regression (PPR) based on net atomic charges as stereo-electronic structural descriptors to establish QSAR models for a congeneric set of dihydroartemisinin derivatives were reported [40]. The geometries of a training

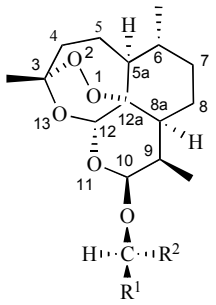
General Structure	R_1	R_2	RA[a]
	-H	*p*-PhCOOH	0.99
	-H	-H	1.07
	$-C_3H_7$	$-CH_2Ph$	0.99
	$-CH_2Ph$	$-C_3H_7$	1.05
	-Ph	$-COOC_2H_5$	1.15
	$-COOC_2H_5$	-Ph	1.10
	$-CH_3$	*p*-$PhCF_3$	1.02
	$-CH_2COOC_2H_5$	-Ph	1.11
	-Ph	$-CH_2COOC_2H_5$	1.13
	$-CH_2COOC_2H_5$	*p*-$PhNO_2$	1.11
	p-$PhNO_2$	$-CH_2COOC_2H_5$	1.19
	$-CH_3$	*p*-$PhCOOCH_3$	1.08
	p-$PhCOOCH_3$	$-CH_3$	1.08
	$-CH_3$	*p*-PhCOOH	0.98
	p-PhCOOH	$-CH_3$	1.02
	$-CH_2COOH$	*p*-$PhNO_2$	1.01

Fig. (3). Compounds investigated by Rode and co-workers [40]. [a] Relative activity taking artemisinin as reference.

set constituted of 16 dihydroartemisinin derivatives (Fig. **3**) were optimized using the semi empirical molecular orbital PM3 method. From these optimized geometries, charges on all skeletal carbon and oxygen atoms were determined. Their analysis revealed that the MARS methodology gives the best predictive ability. Based on this methodology, it was found that an improvement in activity against *P. falciparum* D-6 can be achieved if atomic charges at positions C_{5a} and C_8 (Fig. **3**) are maintained at moderately positive values while charges on O_2 and O_{11} are less negative [40]. The activity against *P. falciparum* W-2 is improved when electron density on O_{11} is decreased and the charges on O_1, C_{5a} and C_8 present moderate values [40]. This work has reinforced the importance of the peroxide oxygen atoms for anti-malarial activity, but has also included other atoms as significant for the biological activity.

The activity value data, for the set of compounds included in the training set are also presented in Fig. (**3**). The activity parameter has been usually expressed as relative value, taking artemisinin as reference. In the present example, it may be noticed that these activity values, after being expressed on a logarithmic scale, span a range of only 1.784 for *P. falciparum* W-2 strain and 1.438 log units for *P. falciparum* D-6, which are on the bottom limit for a qualified correlation.

Jiang and co-workers published two QSAR studies on artemisinin derivatives, one based on neural network methods and the other based on conventional partial least square (PLS) methods [41,42]. They optimized the geometries of 23 artemisinin ether and ester derivatives and calculated net atomic charges and bond orders with the AM1 and PM3 semi-empirical methods (Fig. **4**).

By means of a partial least squares (PLS) method, QSAR analysis involving these electronic parameters indicated that the activity of the compounds is directly related to i) the bond strength of the peroxide bridge, represented by bond orders; ii) the bond order of the O_{14}-C_{15} bond (C_{15} is the carbon bonded to O_{14}) and iii) the net atomic charge on carbon 10. From these results, the pharmacophore group of the artemisinin analogous, formed by a triangle including the peroxide group and the carbon 10, was deduced [41,42].

Fig. (**4**) data show that, similarly to the work published by Rode and co-workers [40], the range of activity values of the training set is again very small, spanning only 1.21 log units. As can be noticed, structural modifications carried on carbon 10 of artemisinin (Figs. **3** and **4**) usually impart only small changes in the activity.

As another example, we mention the study of Pinheiro and co-workers who used PCA and Hierarchical Cluster Analysis (HCA) to classify a set of 16 artemisinin derivates with anti-malarial activity against mefloquine resistant *P. falciparum* [51]. A total of 172 molecular descriptors, including parameters calculated by quantum chemical methods and topological indexes, were used to develop their models. The best ranking of compounds into high and low activity was obtained with a smaller set of 7 descriptors. Those results were then used to predict the activity of a test set of 12 unknown compounds, from which two were predicted as possessing high activity. The range of activity values of compounds in the training set [51] was very low,

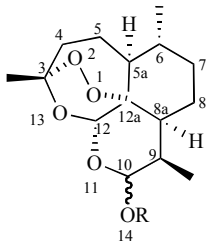
General Structure	R	RA[a]	R	RA[a]
	H(α)	1.08	$COOC_3H_7$(α)	1.26
	H(β)	1.05	$COOC_3H_7$(β)	1.17
	CH_3(α)	1.01	$CO(p\text{-}CH_3)C_6H_4$(α)	1.15
	CH_3(β)	1.17	$COCH{=}CHC_6H_5$(α)	1.23
	C_2H_5(β)	1.12	$COCH_2C_6H_5$(α)	1.20
	n-C_3H_7(β)	1.13	$CH_2CH_2COCH_3$(α)	1.11
	$CH(CH_3)_2$	1.10	COC_6H_5(β)	1.08
	$CH_2CH_2CH(CH_3)_2$(β)	1.03	$CH_2CH_2OCH_3$(β)	1.05
	$COCH_3$(α)	1.16	2-Oxacyclopentyl(α)	1.02
	COC_2H_5(α)	1.22	2-Oxacyclopentyl(β)	1.05
	COC_3H_7(α)	1.23	$C_2H_4OC_2H_4CH_3$(β)	1.07
	$COOC_2H_5$(α)	1.23		

Fig. (4). Structures of compounds calculated by Jiang and co-workers. [a]RA is relative activity taking artemisinin as reference.

only 0.5 log units, when expressed in terms of molar concentrations. Additionally, some molecular descriptors, such as total surface area and molecular volume, are very sensitive to molecular conformation.

4.2. The Quality of the Descriptors

Software development has led to the common use of automated procedures for the selection of relevant descriptors in the development of a QSAR model. This, however, must be done carefully. In addition to providing statistically significant correlation with the independent variable, appropriated descriptors may also be physical or chemically interpretable. Lastly, one should not only be looking for a mathematical correlation between parameters but also trying to understand the chemical or biological processes that are related to the biological activity. As it happens with the activity values, the descriptor values must also change sufficiently to discriminate among the several compounds in the training set. In the works cited above, where calculated atomic charges were used as descriptors, we have an illustrative example. There is no doubt about the relevance of the peroxide bond for the activity of the endoperoxides. However, chemical modifications on artemisinin (**1**) to afford derivatives are most easily made on the carbonyl group, which is considerably distant from the peroxide bond. As a consequence, these structural modifications have little or no influence on the electronic distribution in the vicinity of the peroxide bond. For example, charge densities on the oxygen atoms of the peroxide bond are only marginally affected by chemical modifications made on the carbonyl group of artemisinin (**1**). To illustrate this point, let us consider the values reported by Rode and co-workers [40], used to calculate semi-empirical approaches. First of all, the net atomic charges calculated by semi-empirical or *ab initio* methods are usually expressed by no more than four significant figures, but they are most commonly expressed with three significant figures [70]. The values of net atomic charges of large, flexible molecules are not reproducible with more than three or four significant figures. Small changes in conformation are sufficient to result in similarly small changes in net atomic charges that manifest from the fourth significant figure on. To obtain results that are perfectly reproducible up to the sixth significant figure, a very tight optimization criterion must be adopted. Rounding off the values reported in reference [40] to three significant figures leads to net atomic charges for most of the atoms that are essentially constant for all the compounds in the training set.

As a test case, we have re-optimized the geometry of the same set of compounds studied by Rode and co-workers (Fig. **3**), using the same PM3 semi-empirical method. In each case, the optimal geometry was determined by calculation of all rotamers obtained by a complete rotation around freely rotating single bonds. Net atomic charges were obtained for the most stable conformer. Table **1** gives the PM3 average net atomic charge of selected atoms calculated by us over the 16 compounds in the training set (Fig. **3**), the variance around the mean value and the higher individual differences between the value calculated by us as well as those calculated by Rode and co-workers. Analysis of Table **1** clearly shows the following points: the charges on carbon and oxygen atoms are essentially constant for all compounds in the set, and the variance around the mean value is in the order of $0.009e^-$ or less. For the atoms selected by the MARS methodology, only charge on O_{11} shows some significant variance; other atoms have charges that are constant along the set. The peroxide oxygen atoms, for example, show charges that change by only $0.001e,^-$ along the whole set. This aspect by itself precludes the use of these net atomic charges as molecular descriptors. An additional point to be noted in Table **1** is that, although net atomic charges calculated by us are on average similar to those reported by Rode *et al*., individual values show differences that are always higher than the variance around the mean value.

Table 1. Average values (taken over the 16 compounds studied by Rode and co-workers [40]) of net atomic charges for selected carbon and oxygen atoms; variance around the mean value (σ_{n-1}); and the higher individual difference (*MaxΔq*) between the values calculated in the present work (X) and those calculated by Rode and co-workers (Y)[a]

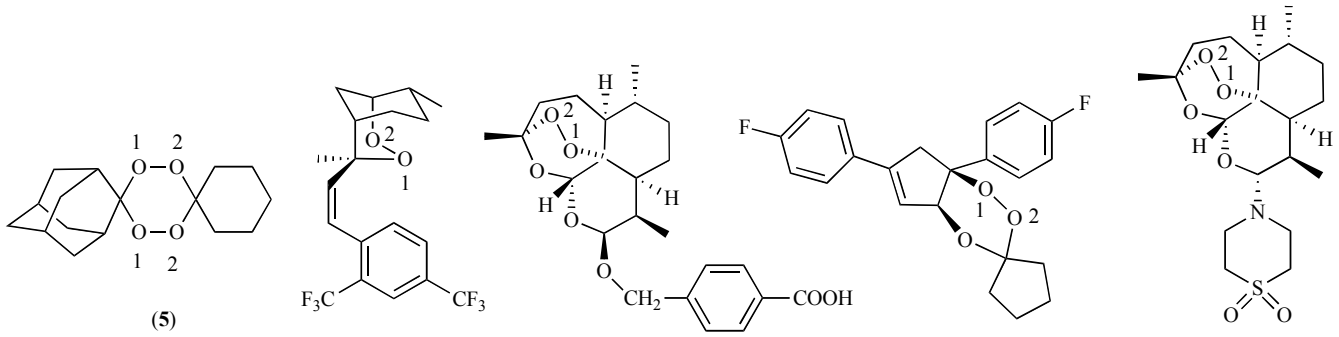
Atom	*X*	$\sigma_{n-1\,(X)}$	*Y*	$\sigma_{n-1\,(Y)}$	*MaxΔq*
O_1	-0.129	0.001	-0.128	0.001	0.003
O_2	-0.136	0.001	-0.136	0.001	0.002
C_{5a}	-0.168	0.001	-0.169	0.001	0.002
C_8	-0.258	0.005	-0.257	0.004	0.018
O_{11}	-0.262	0.009	-0.282	0.009	0.034

[a]X is the average value of net atomic charges taken over 16 compounds calculated by us. σ_{n-1} (X) is the variance around the mean value X. Y is the corresponding of X but calculated from the values published by Rode and co-workers. σ_{n-1} (Y) is the variance around the mean value Y. MaxΔq is the higher individual difference between charges calculated by us and those calculated by Rode and co-workers.

These differences probably result from differences in conformation. Atomic charges are strongly dependent on conformational changes. Therefore, different conformations naturally result in different atomic charges. Although this may be a positive feature to some QSAR studies, this is not the case in the present example. Conformational flexibility in the compounds of the training set arises as a consequence of rotation around single bonds. As shown in Table **1**, only for those atoms that are near the substituting group, charges of the different compounds show any variance.

We also calculated the charges on the peroxide oxygen atoms of other antimalarials RKA 182 (**5**), arteflene (**7**), artelinic acid (**8**), Fenozan B07 (**9**), and artemisone (**10**), shown in Fig. (**5**). The charge values do not significantly vary compared to the average values of net atomic charges calculated for the same atoms and presented in Table **1**. This fact corroborates the idea that structural modifications have little or no influence on the charge distribution in the vicinity of the peroxide bond.

Table **2** presents the average values of the bond orders and charges (the relevant descriptors) calculated by Jiang and co-workers [41,42] for the 23 artemisinin derivatives, expressed by three significant figures. Also presented in Table **2** is the variance around the mean value for each parameter and the corresponding data calculated by us. Table **2** analysis shows that the variance in bond orders for the bonds involved in the peroxide bridge, O_1-C_{12a}, O_2-C_3 and O_1-O_2 (Fig. **4**), which were identified by Jiang and co-



Compounds	Calculated Atomic Charges	
	O_1	O_2
5	-0.139	-0.147
7	-0.128	-0.130
8	-0.127	-0.132
9	-0.129	-0.134
10	-0.126	-0.132

Fig. (5). Structures of RKA 182 (**5**), arteflene (**7**), artelinic acid (**8**), fenozan B07 (**9**), and artemisone (**10**) and the calculated charge values on the oxygen peroxide atoms.

Table 2. Average Values (Taken Over the 23 Compounds Studied by Jiang and Co-Workers [41,42]) of Selected Bond Order and Net Atomic Charges and Variance Around the Mean Value[a]

	AM1			
	X	**σ_{n-1} (X)**	**Y**	**σ_{n-1} (Y)**
$P_{O(2)-C(3)}$	0.936	0.004	0.937	0.001
$P_{O(1)-C(12a)}$	0.941	0.002	0.942	0.003
$P_{O(1)-O(2)}$	1.003	0.001	1.003	0.000
P_{total}	2.881	0.005	2.882	0.003
$P_{O(14)-C(15)}$	0.947	0.025	0.978	0.027
$Q_{C(10)}$	0.164	0.060	0.157	0.188

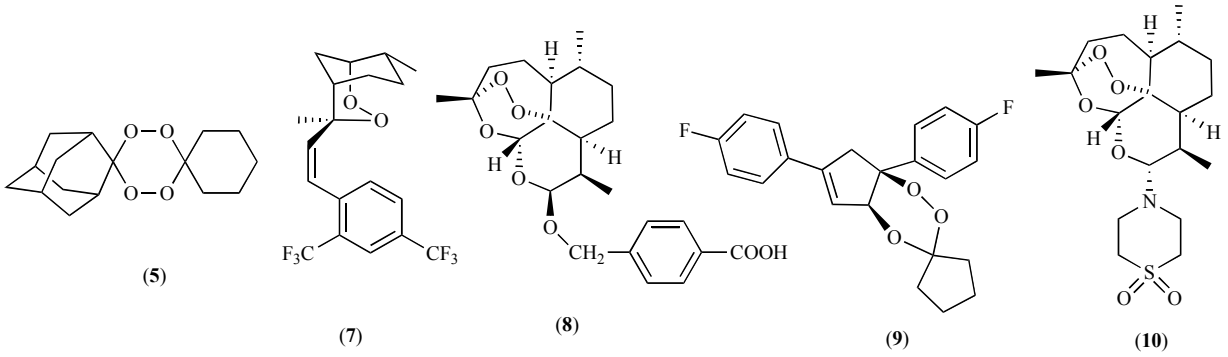
[a]X is the average value taken over 23 compounds calculated by us. σ_{n-1} (X) is the variance around the mean value X. Y is the corresponding of X but calculated from the values published by Jiang and co-worker. σ_{n-1} (Y) is the variance around the mean value of Y. P is for bond order and Q is for charge.

workers as the parameter determining the anti-malarial activity, is extremely low, $\sigma_{n-1} \leq 0.003$ unity. This clearly indicates that these parameters are not sensitive to structural modifications and cannot reflect the structural effects due to the different substituting group on the anti-malarial activity. Once again the parameters that show significant variance are charged on C_{15} ($\sigma_{n-1} = 0.188$) and on the O_{14}-C_{15} bond order ($\sigma_{n-1} = 0.027$), which are directly associated with the substitution position, albeit distant from the peroxide bond. Our AM1 calculations on the same set of compounds studied by Jiang and co-workers nicely reproduced their charge and bond order mean values (Table **2**).

Bond orders of the peroxide bond for the antimalarials **5, 7-10** were also calculated and are given in Fig. (**6**).

As can be seen, the calculated bond order values are essentially constant and do not vary from the values presented in Table **2**. Once again, the values shown in Fig. (**6**) support the idea that this parameter is not sensitive to structural modifications and cannot reflect the structural effects due to the different substituting group on the antimalarial activity.

The two cases discussed above illustrate the difficulties that arise when one tries to develop QSAR models for the family of artemisinin-like compounds mainly using derivatives with chemical modifications carried out on the carbonyl group. Structural modifications on this moiety of artemisin (**1**) barely alter any electronic or steric feature of the peroxide bond. As a consequence, the compounds in the training set usually have activities that do not vary significantly. When using a more diverse set of compounds, as in the case of the examples to be discussed shortly, a larger variance in the activity data is usually found.



Compounds	Calculated Bond Order
5	1.001
7	1.016
8	1.003
9	1.002
10	1.003

Fig. (6). Calculated bond order of the peroxide bond for compounds **5**, **7-10**.

4.3. Interpretability of the Final Model

A high quality QSAR model must provide clues for the interpretability of the biological process under investigation. This is, perhaps, the most challenging aspect in QSAR. 3D-QSAR studies using the CoMFA methodology have become widespread, and have also been applied to artemisinin derivatives [44, 45, 50, 53]. Automated software allows the use of a considerably larger data set of compounds in the training set. In the CoMFA study of Winger and co-workers, 40 compounds were screened [45], while in the CoMFA study of Avery and co-workers more than 150 compounds were included in the training set [44,53]. As the biological activity of this extended class of compounds could not be measured using exactly the same protocol, both these studies employed relative activities taking artemisinin as reference.

The HASL and CoMFA models proposed by Avery and co-workers [44] are based on a large set of compounds and show highly significant statistical results ($r^2 > 0.9$; cross-correlated $q^2 > 0.8$), and should, thus, be considered reliable models, with a high degree of prediction. These studies were further supplemented by CoMFA and hologram QSAR (HQSAR) [53]. The second CoMFA study [45] had as its main goal to compare the *ab* initio HF/3-21G versus the semi-empirical AM1 method to optimize the geometries needed to calculate the steric and electrostatic fields employed to develop the model. It was concluded that steric fields are better calculated with the HF/3-21G method, while electrostatic fields are strongly dependent on the model used to calculate atomic charges. A CoMFA study was also reported by Jung and Kim on 124 artemisinin derivatives [50]. This study shows that more reliable CoMFA models are obtained by elimination of descriptors that have small correlation with anti-malarial activity and perform the CoMFA within a restricted region containing a smaller number of probe atoms around the peroxide bond. Usually, CoMFA models show statistically significant correlations. However, their translation into useful information, with a physical meaning that may produce effective contribution to the understanding of the biological process, is normally not a simple task.

Alternative methodologies have also been employed to develop QSAR models for artemisinin derivatives [48, 49]. A kinetic energy density function method based on Molecular Quantum Similarity Measures (MQSM) was employed to develop QSAR models for two sets of artemisinin derivatives [48]. The quantitative relationship between the molecular descriptors and the activity was established by means of a multi-linear regression procedure using as independent variable some principal components (PC) obtained from Principal Component Analysis (PCA). The final equations in the model have each 4 descriptors for both training sets, which are formed by 18 and 15 compounds, respectively. The low compound/descriptor ratio increases the possibility of occurrence of correlation by chance [59] and hinders the interpretation of the model. Additionally, selection of PCs that are not among the first ones includes information with low degree of explanation of the whole variance. In PCA, the first few PCs retain most of the information present in the original data set. The remaining ones retain only a small fraction of the information present in the original data set. In the present case, the chosen PCs explain less than 40% of the whole variance [48].

A detailed analysis of the results published by Pinheiro and co-workers [51], including mainly the plot of the first two PC score vectors, reveals that two compounds (artemisinin and artemether) are mainly responsible for the sharp separation of the group of compounds having the highest activity along the PC_1 component. These two structures are the only ones which do not have bulky groups attached to the carbonyl position. Removal of these compounds from the PCA analysis would modify the pattern of distribution in the plot and, as a consequence, the identification of the highest activity region. A second study was recently published by the same group [56]. Using Molecular Electrostatic Potential maps, key molecular features relevant for activity could be identified. From their QSAR models, atomic orbital energies (HOMO), charge, molecular volume and hydrophobic index emerge as the descriptors determining activity. However, as it was the case with some of the works discussed previously, they also used a training set with low variance in activity and, additionally, relevant descriptors such as orbital energy and atomic charges were essentially constant for all compounds in the training set. It should also be observed that the final model has no independent term, an uncommon fact in QSAR models.

Parasuk and co-workers investigated the relation between the docking configuration and anti-malarial activity for 30 artemisinin derivatives [49]. The docking results were used to obtain a QSAR model using the binding energies of heme-artemisinin complexes as a descriptor. Quantum mechanical calculations at the HF/3-21G level were used to determine the geometries of all compounds and the combined force fields AMBER/MMFF were used for the docking calculations. A Monte Carlo simulation was employed to generate 100 docking calculations with different starting conformations, which were then classified into groups. In each group, the lowest energy conformation was selected as representative of that group. Relationships among biological activity and 5 parameters, i. e., O_1-Fe, O_2-Fe, O_{13}-Fe and O_{11}-Fe distances and the binding energy were investigated. Among these parameters, binding energies showed the best correlation, with r values of -0,93 and -0,81, respectively for the *P. falciparum* D-6 and *P. falciparum* W2 clones, indicating that compounds that more strongly bind to heme (lower binding energy) have a higher activity. The analysis of the O-Fe distances suggests that compounds with shorter O-Fe distances have higher activity.

The QSAR study of Katritzky and co-workers used two sets of compounds including not only artemisinin-like structures but also cyclic and acyclic peroxides [55], which were tested against the *P. falciparum* D-6 and the NF54 strains of malaria parasite. As they tried a large set of descriptors (their software allowed analyzing more than 800 descriptors), the final model includes descriptors with some degree of complexity, which are, however, associated in some way to shape, branching and charge-related interactions.

CONCLUSIONS

In the present review, we have briefly discussed the main results published for artemisinin and related endoperoxides using QSAR methodologies. We have focused on the fact that, although highly relevant models have been obtained, much still remains to be done in this field before a reasonable, statistically confident, chemically and biologically meaningful model can be achieved. Both the activity data and the descriptors must be rigorously selected to produce final models that may help not only find a mathematical relationship between structural parameters and the activity but also understand the chemical or biological process under investigation. The main reasons for such difficulties in the case of the endoperoxides arise, on the one hand, from the narrow range of activities expressed by the compounds usually used in training sets, which are most commonly derivatives presenting chemical modifications at the carbonyl carbon of the lactone ring, far away from the peroxide bond. On the other hand, these structural modifications usually result in descriptors (associated to the peroxide bridge) that are also essentially constant for a large set of compounds. The alternative use of a large and diverse set of descriptors resulted in models that cannot easily be rationalized (or are rationalized only in an indirect way) in terms of chemical and biological information. The published models point to the following facts: i) an improvement in the activity against *P. falciparum* D-6 can be achieved if atomic charges at positions C_{5a} and C_8 are kept at moderately positive values, while charges on O_2 and O_{11} are less negative [40]. The activity against *P. falciparum* W-2 is improved with electron density on O_{11} being decreased keeping charges on O_1, C_{5a} and C_8 at moderate values; ii) the activity is directly related to the bond strength of the peroxide bridge, to the bond order of the O_{14}-C_{15} bond and to the net atomic charge on the carbonyl carbon [9,10]; iii) compounds that more strongly bind to heme [49,52], with shorter O-Fe distances [49] have higher activity; iv) peroxides exhibiting high anti-malarial activity are characterized by a continuous, negative electrical potential surrounding the molecule [47]; and v) higher values for the HOMO energy combined with lower negative charge on O_1 and higher positive charge on C_3, higher volume and lower hydrophobicity increase anti-malarial activity [56].

CONFLICT OF INTEREST

The authors declared that there is no conflict of interest regarding the publication of this paper.

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