Correlating the Molecular Electrostatic Potentials of Some Organic Peroxides with Their Antimalarial Activities†

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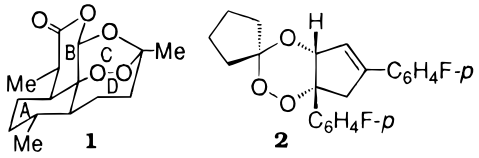
The molecular electrostatic potentials (MEPs) of artemisinin (also known as qinghaosu), yingzhaosu A, and some synthetic analogues have been calculated and studied as a means of distinguishing between high and low antimalarial activity. To facilitate comparison, the dimensionality of the MEP was reduced by Kohonen Neural Network transforms. The reduction revealed that peroxides exhibiting high antimalarial activity are characterized by a continuous strip of negative electric potential surrounding the molecule, whereas peroxides of lesser activity show a broken strip.

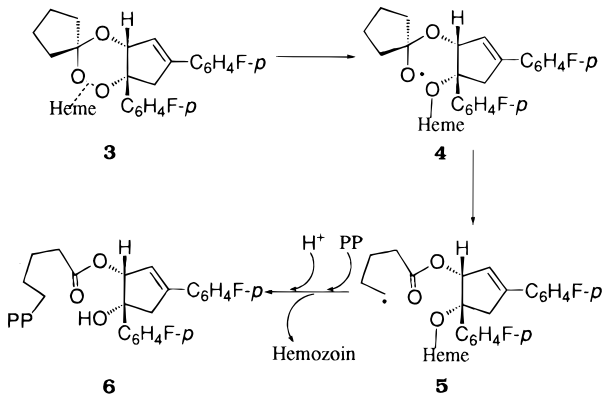
1. INTRODUCTION

It is now known that antimalarial peroxides, both of natural and synthetic origin, kill the Plasmodium parasite through a mode of action which is entirely different from that of the traditional quinoline-based drugs such as quinine and chloroquine.[1] For instance, artemisinin (also known as qinghaosu) (**1**) and the trioxane BO7 (**2**) effect their lethal action on the parasite by a process of chemical induction.[2,3]



During the trophozoïte stage of the intraerythrocytic cycle within the host, the parasites invade the red blood cells and digest the hemoglobin content as a nutritional source of amino acids. The prosthetic group, heme, released by proteolysis, because of its toxicity to the parasite, is immediately oxidized and polymerized to the insoluble malarial pigment, hemozoin. When the host is treated with **1** or **2**, the aforementioned detoxification process is interrupted by complexation with heme. For example, **2** by virtue of the flexibility conferred by the cis-fused rings, maneuvers its O−O bond over the iron atom and coordinates with it, forming a complex (**3**) (Scheme 1).[4] Next, within the complex, a single electron is transferred from the 3d orbital of iron to the σ antibonding orbital of the contiguous peroxide bond, causing it to break. The resulting oxygen radical **4** spontaneously rearranges to the highly reactive primary carbon-centered radical **5** which can then alkylate the protein

Scheme 1



(PP) of a nearby parasite causing its death. The alkylated protein **6** is then detached, releasing hemozoin.

The foregoing mechanism clearly depends on the optimal confluence of several molecular properties in order to produce the ultimate antimalarial pharmacophore. In other words, the parasiticidal action of potent peroxides such as **1** and **2** derives from the efficient operation of the above-mentioned sequence of chemical events, namely, docking with heme, electron transfer, formation of an oxy radical, scission to a primary C-centered radical, and finally, the coup de grâce, alkylation. In a 3D-QSAR study,[5] we showed that hydrophobic features and hydrogen bonding in certain cis-fused bicyclic trioxanes are responsible for binding with heme. Typically, the highest antimalarial activity correlates with those trioxanes which can adopt a conformation that ensures an intimate fit or close docking with heme. As a case in point, the *Catalyst* program predicted accurately that the activity of the cyclopentenotrioxane **2** exceeds that of the cyclohexeno analogue **7** because conformational accessibility to the critical fit is attained in **2**, but not in **7**.

While the foregoing 3D-QSAR study of active antimalarials confirmed the importance of docking, it provided no information on the electron-transfer process. As such a process is beyond the capability of the *Catalyst* software,

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† Dedicated to the memory of Dr. Colin Thomson.

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Table 1. Basic Steps in the Algorithm of Unsupervised Training of Kohonen Neural Networks

(1) At learning cycle (epoch) 0, the synaptic weights $\mathbf{w}_i$ of every neuron i are assigned randomly in the interval (0,1). The adaptation height parameter $\epsilon(t)$ and the adaptation width parameter $\Delta(t)$, controlling the changes in the synaptic weights, are set to 1.0 and to $N/2$, where N is the output square layer size. The values used for the parameters η_ϵ and η_Δ are set to 0.9997.

(2) A four-dimensional input vector $\mathbf{v}(x,y,z,\text{MEP value})$ is chosen randomly on the Connolly surface and presented to the input layer.

(3) The neuron i_0, called the *winner* is chosen which has synaptic weights closest to the input vector $\mathbf{v}$:

$$||\mathbf{v} - \mathbf{w}_{i0}|| = \min_I ||\mathbf{v} - \mathbf{w}_i||$$

(4) The synaptic weights $\mathbf{w}_i$, the adaptation height parameter $\epsilon(t)$, and the adaptation width parameter $\Delta(t)$ are updated at every learning cycle (epoch), according to the rules

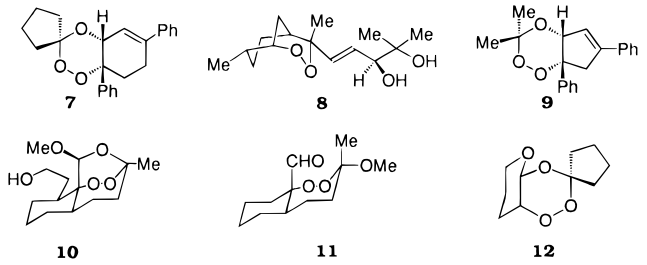
$$\mathrm{w}_i(t+1) = \mathrm{w}_i(t) + \epsilon(t)\, g(||i - i_0||)(\mathbf{v} - \mathbf{w}_i)$$

$$\epsilon(t+1) = \eta_\epsilon \epsilon(t) \qquad \eta_\epsilon > 0$$

$$\Delta(t+1) = \eta_\Delta \Delta(t) \qquad \eta_\Delta < 1$$

(5) If $||\mathbf{w}_i(t+1) - \mathbf{w}_i(t)|| < 10^{-6}$, for all neurons I in the output layer, exit the iterative procedure; otherwise go to step **1**.

we decided to see how the antimalarial data might be correlated with the ease of electron transfer in terms of the molecular electrostatic potential (MEP) of the peroxidic linkage. We previously showed[6] that active antimalarials such as artemisinin and some of its derivatives display a zone of negative potential of similar shape around the peroxide linkage, while in desoxyartemisinin, an inactive derivative, it is displaced. However, it emerged from this study that comparing MEPs of molecules of different shape proved difficult. We now describe how MEP values may be processed to enable a more meaningful comparison to be made. A representative set of bi-, tri-, and tetracyclic peroxides exhibiting different antimalarial activities was chosen, namely, artemisinin (**1**), yingzhaosu A (**8**), a *cis*-fused cyclopenteno-trioxane (**9**),[7] a structural mimic of artemisinin (**10**),[8] a bicyclic peroxide (**11**),[9] and a hypothetical trioxane (**12**).



2. METHODS, SOFTWARE, AND COMPUTATIONAL DETAILS

We have searched for descriptors that are directly obtainable as scalar numerical values from the MEPs. Since MEPs are four-dimensional objects, they are difficult to compare without reducing dimensionality. Clearly, dimensionality reduction requires a nonlinear projection method. In this study we decided to use the Kohonen neural network model[10] as ingeniously applied by Gasteiger and Zupan for comparing the properties, such as biological activities, of sets of nonplanar molecules.[11] The molecular and electronic structures of the candidate molecules were determined by recourse to the PM3 semiempirical method.[12] The MEPs were computed from the Merz−Kollman charges.[13] The numerical values of the MEPs were mapped to a color scale that ranges from −0.1 au (red) to +0.1 au (green).

The input neuron was fed with the Cartesian coordinates of 10 000 randomly selected points on the Connolly surface of the molecule in question along with the value of the MEP expressed in terms of a color code. The Kohonen Neural Network (KNN) used in all applications consisted of a grid of 60 by 60 neurons. For any point on the Connolly surface that enters the learning phase, the neuron with the three weights most similar to the input coordinates of this particular point is selected as the *winning neuron*, i.e., as the neuron whose weights, as well as the weights of some neurons in an appropriate neighborhood, will be corrected. When the KNN stabilizes, in the sense that no noticeable change in the weights can be detected upon further training, all neurons were checked to see which points they contain from the Connolly surface. The correctness of the procedure was confirmed by the finding that points lying very close on the Connolly surface (and therefore having nearly the same potential) map to the same neuron and that adjacent neurons are excited by points with similar electrostatic potentials.

The essential steps in the algorithm for the unsupervised training of the Kohonen networks are summarized (Table 1). As we can see from this table, a discrete time t is introduced in the algorithm, which goes through epochs, an epoch being the time needed to update sequentially all of the synaptic connections in the network. The unsupervised learning process is therefore iterative with a counter t. Another quantity of central importance is the function $g(||i - i_0||)$, inducing the so-called lateral inhibition among neurons. Here, $||i - i_0||$ designates the distance on the square output layer between any neuron i and the winner neuron i_0. The lateral inhibition function $g(||i - i_0||)$ takes maximal values on the winner neuron ($i = i_0$) and decays to 0 at large distances with a steepness determined by the adaptation width $\Delta(t)$. In practice the lateral inhibition function is chosen to be either a Gaussian or Heaviside step function. The adaptation height $\epsilon(t)$ controls the "strength" of the updating process through learning epochs. Both adaptation width and height are subject to gradual decrease as the unsupervised learning process goes on. The gradual decrease is obtained through the use of the multiplicative factors η_ϵ and η_Δ (cf. Table 1, step 4). The unsupervised training of a KNN is similar to a simulated annealing procedure, the choice of appropriate values for the parameters η_ϵ and η_Δ being crucial for a good convergence. To better distinguish the characteristic features of a Kohonen transform of a given MEP, it is important to surround the original transform by eight replicas, which eliminates discontinuities appearing at the edges of the original transform.

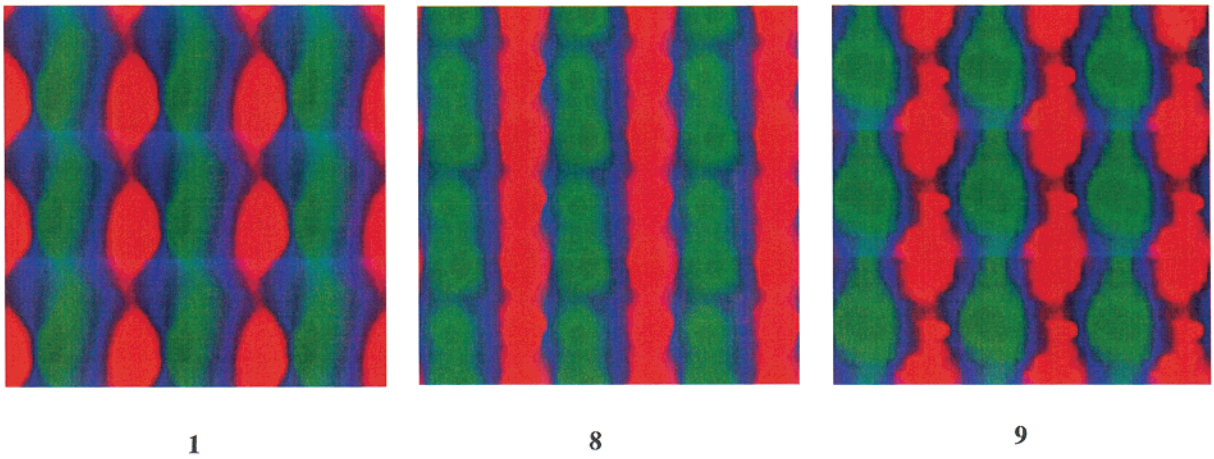


Figure 1. Tiled (3 × 3) Kohonen neural network transforms of the MEPs of the active peroxides **1**, **8**, and **9**.

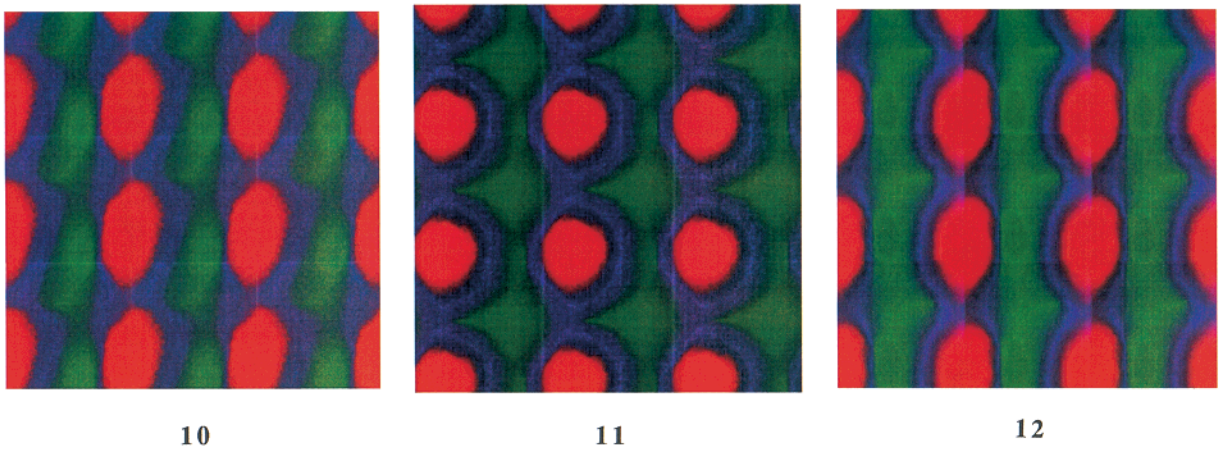


Figure 2. Tiled (3 × 3) Kohonen neural network transforms of the MEPs of the less active trioxane **10**, the inactive peroxide **11**, and the hypothetical peroxide **12** of unknown activity.

The KNN analysis of the MEPs, so generated, was performed with the Stuttgart Neural Network Simulator (SNNS) modified to handle the toroïdal topology of the network.[13]

3. RESULTS AND DISCUSSION

The peroxides selected (**1**, **8**−**12**) display a wide range of antimalarial activity. The high activity of the naturally occurring trioxane artemisinin (**1**), both in vitro and in vivo models, is the benchmark for the series. The IC_{50} value for **1** against the W-2 Indochina clone of *P. falciparum* in vitro is 1−2 ng/mL.[3] The activity of yingzhaosu A (**8**) is reputed to be equally high, although the evidence is purely anecdotal.[15] However, arteflene, a closely related synthetic analogue, displays an IC_{50} of 2.7 ng/mL against the K1 strain of *P. falciparum*, a value that can be taken as an index for **8**.[16] The synthetic cyclopentenotrioxane **9** has an IC_{50} of 8.4 ng/mL and can be regarded as being fairly active.[7] Interestingly, the trioxane alcohol **10** is considerably less potent than **1**, although several derivatives, particularly the benzyl ether, are several times more active than **1**.[8] We therefore assign poor activity, perhaps a notional value of 30−50 ng/mL, to **10**. The synthetic peroxide **11**, although retaining the AD rings of artemisinin, is completely inactive.[9] Last, the trans-fused bicyclic trioxane **12** embodies the BC rings of **1** and might be similarly active. However, it is a hypothetical entity yet to be synthesized. Consequently, **12** constitutes a test case.

The Kohonen transforms of the MEPs of (**1**, **8**−**12**) were obtained (Figures 1 and 2). Examination of the transforms reveals a net difference between active and inactive molecules. In the case of yingzhaosu A (**8**), which is a highly active, bridged bicyclic peroxide, the negative molecular electrostatic potential is completely delocalized appearing as a continuous strip on the Kohonen transform (Figure 1). Similarly, artemisinin (**1**), an equally highly active, rigid tetracyclic trioxane, displays a ribbon of contiguous ovoids of negative charge. The conformationally flexible, cis-fused bicyclic trioxane **9** is pretty active and is characterized by an array of extended fish-shaped zones of negative charge.

The inactive molecules reveal a patently different pattern (Figure 2). The inactive peroxide **11** which retains a portion of the artemisinin skeleton, unlike the active peroxide **8**, exhibits negative regions localized as circular islands on the transforms. Even the poorly active tricyclic trioxane **10**, also resembling artemisinin, shows discrete ovoid islands of negative charge (Figure 2). A similar ovoid pattern of negative charge is seen for the hypothetical trioxane **12**. Consequently, it is expected to be a poorly active antimalarial.

The foregoing results must be treated with caution, but they nevertheless seem to suggest that the Kohonen transform

may be a valid criterion of antimalarial potency. It seems likely, on comparing **1** with **10** by way of illustration, that both electronic and steric factors close to the O−O bond may be responsible for the observed differences in activity and the associated negative potential. Clearly, steric factors must also be taken into account, because, as we have mentioned elsewhere,[5] close docking with heme is a prerequisite for electron transfer. However, further calculations of the MEPs of other peroxidic antimalarials and reduction of their dimensionality need to be carried out by way of confirmation.

As a predictive tool for activity, the Kohonen transform procedure, in view of the time and cost involved, would be best used after initially sorting out the more from the less active peroxidic antimalarial candidates, preferably by using a 3D-QSAR model of the pharmacophore.

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