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FEBRIFUGINE DERIVATIVE ANTIMALARIAL ACTIVITY: QUANTUM MECHANICAL PREDICTORS

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SUMMARY

Plasmodium falciparum resistant strain development has encouraged the search for new antimalarial drugs. Febrifugine is a natural substance with high activity against *P. falciparum* presenting strong emetic property and liver toxicity, which prevent it from being used as a clinical drug. The search for analogues that could have a better clinical performance is a current topic. We aim to investigate the theoretical electronic structure by means of febrifugine derivative family semi-empirical molecular orbital calculations, seeking the electronic indexes that could help the design of new efficient derivatives. The theoretical results show there is a clustering in well-defined ranges of several electronic indexes of the most selective molecules. The model proposed for achieving high selectivity was tested with success.

KEYWORDS: Malaria; Febrifugine; Molecular Modeling; Electronic Structure.

INTRODUCTION

Malaria is amongst the most important diseases caused by parasites in many tropical countries[7,9,14]. Quinine was the only drug used in the combat against this illness for a long time, but after the introduction of antimalarial synthetic drugs, such as chloroquine and mefloquine, its application diminished[9].

However, since strains of *Plasmodium falciparum* have acquired resistance to such drugs, the use of quinine has been retaken. What is becoming a worrying concern is that the quinine effectiveness is being progressively reduced in the treatment of malaria[9]. This asks for an urgent development of new substances with antimalarial properties.

Febrifugine and the isofebrifugine have been isolated from the *Dichroa febrifuga* - a Chinese plant used for centuries against malaria, without showing any reduction on their efficiency - as active principles against malaria. However, these substances present liver toxicity and emetic properties too high for routine clinical use[7,9,12,14]. This stimulates the search for new derivatives with equivalent efficiency, but with diminished toxicity.

The biological activity of a molecule with pharmaceutical properties is dominated by the electric interactions of its charge distribution with the target systems[8]. Structure and charge distribution are determinant since, for example, two isomers can have completely different activities. So, the quantitative investigation of the electronic structure may give us details of the biological activity of a compound. These computational physical-chemistry calculations have been intensively employed in the last decades to help the design of new substances with enhanced desired properties[1,3,15].

Recently, KIKUSHI *et al.* published a study of malarial activity of febrifugine, isofebrifugine, and 10 other derived molecules[9]. Our aim is to investigate the theoretical electronic structure of this group of molecules through semi-empirical molecular orbital theory looking for electronic indexes that could be correlated to their antimalarial properties.

METHODOLOGY

In this work we analyze febrifugine, isofebrifugine and 10 other derived molecules (see Fig. 1).

The biological properties of interest, which we have tried to correlate with the electronic structure data, are the cytotoxicity against mouse mammary FM3A cells, the antimalarial activity against *P. falciparum* FCR-3 strain, and the selectivity, which is defined as the ratio between the cytotoxicity and the antimalarial activity[9]. This apparently contradictory definition to the selectivity in fact leads to a proportional scale: the best selectivity is achieved with the highest pure number. The desired cytotoxicity (a low one) has the highest EC_{50} value and the opposite occurs to the antimalarial activity. So, the most selective compound will have the highest EC_{50} value to the cytotoxicity and the lowest EC_{50} value to the antimalarial activity. It is worthy noting that a high selectivity is commonly obtained with a high antimalarial activity, once its EC_{50} values are two or three orders of magnitude lower than those of the cytotoxicity.

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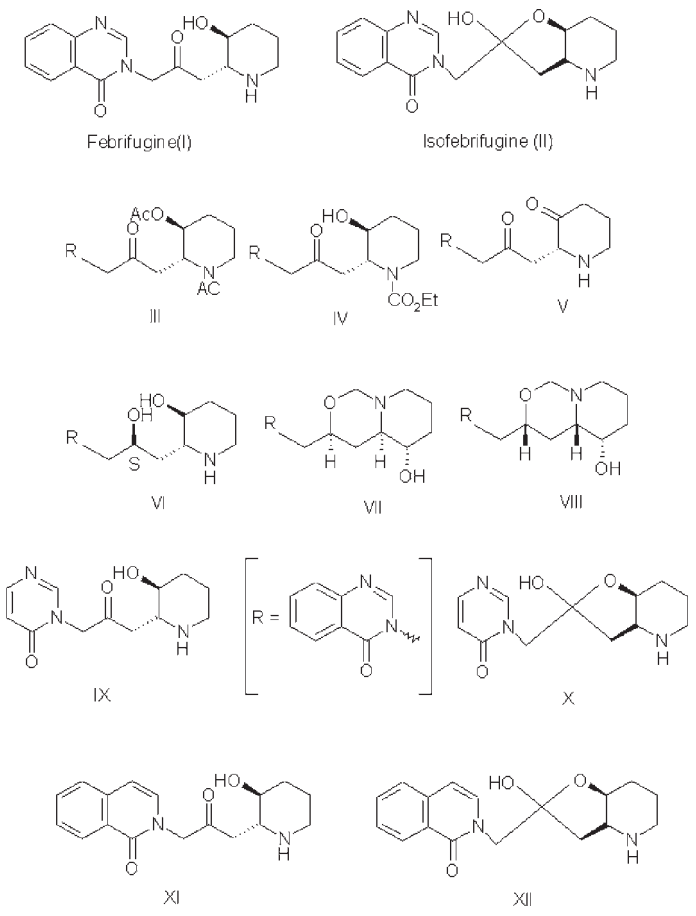

Fig. 1 - Febrifugine, isofebrifugine and 10 other derivatives.

All the calculation, including geometry optimization and electronic structure, were carried out employing the semiempirical method Austin Model 1 (AM1)[4], the package MOPAC version 6[11]. The optimization was considered completed when the gradient norm drops bellow 0.01. AM1 has proven to be highly efficient to predict heats of formation and to optimize the geometry of organic molecules[13]. The calculations for all molecules were done *in vacuo* employing Restricted Hartree-Fock.

A collection of electronic indexes, most of them related to the energy of the frontier molecular orbitals, were investigated as descriptors to these two sets of molecules: heat of formation, electric dipole moment, the energies of the highest occupied molecular orbital (the vertical ionization potential), E_{HOMO}, the level bellow, E_{HOMO-1}, the lowest unoccupied molecular orbital, E_{LUMO}, the level above, E_{LUMO+1}, the differences in energy (E_{LUMO}-E_{HOMO}), (E_{HOMO}-E_{HOMO-1}), (E_{LUMO+1}-E_{LUMO}), (E_{LUMO+1}-E_{HOMO-1}), and the chemical hardness, (E_{HOMO}+E_{LUMO})/2. To each one, we checked the existence of some kind of correlation with the available experimental results. So far, these electronic indexes have been employed successfully in several other groups of biologically active molecules for classifying carcinogenicity, antitumoral, antibiotic, hormonal and antioxidant activities[1,2,3,5,10,15].

The proposed model to design new molecules was corroborated through the use of the Linear Discriminating Analysis (LDA), a statistical method to determine the most correct way to distinguish between the two groups of highly selective and non-selective molecules[6].

RESULTS AND DISCUSSION

The experimental data and the theoretical results calculated which are relevant to febrifugine, isofebrifugine and derivatives are shown in Table 1. We made the option of taking the lowest limit to the experimental data in order to correlate it to the theoretical results. For example, the attributed selectivity to the compound V is 3.5.

From the calculated electronic structure, we could find the following indexes that can correlate the selectivity of the studied molecules (Fig. 2): (i) E_{LUMO}; (ii) E_{HOMO}; (iii) $\Delta E_{L,H}$, the energy difference E_{LUMO}-E_{HOMO}; (iv) $\Delta E_{L1,H1}$, the energy difference E_{LUMO+1}-E_{HOMO-1}; (v) E_{LUMO+1}. For all these indexes there is a common feature:

Table 1
Experimental data and theoretical results for febrifugine, isofebrifugine and derivatives

Comp.	Antimalarial Activity (EC_{50})*	Cytotoxicity (EC_{50})*	Selectivity*	Quantum Indexes (eV)					
				E_{HOMO-1}	E_{HOMO}	E_{LUMO}	E_{LUMO+1}	$\Delta E_{L,H}$	$\Delta E_{L1,H1}$
I	7.0×10^{-10}	1.7×10^{-7}	243	-9.842	-9.214	-0.375	0.227	8.839	10.069
II	3.4×10^{-9}	1.8×10^{-7}	53	-9.640	-9.273	-0.438	0.171	8.835	9.811
III	9.1×10^{-7}	$>2.9 \times 10^{-5}$	>32	-9.133	-8.950	-0.733	-0.308	8.217	8.825
IV	4.8×10^{-6}	$>1.7 \times 10^{-5}$	>3.5	-8.881	-8.287	-0.454	-0.046	7.833	8.835
V	2.0×10^{-8}	1.0×10^{-5}	500	-9.863	-8.996	-0.553	-0.134	8.443	9.729
VI	2.0×10^{-8}	1.5×10^{-5}	750	-9.445	-8.890	-0.473	-0.069	8.417	9.376
VII	3.7×10^{-9}	3.8×10^{-6}	1027	-9.397	-8.916	-0.497	-0.096	8.419	9.301
VIII	8.4×10^{-7}	$>2.5 \times 10^{-5}$	>30	-9.404	-8.980	-0.556	-0.155	8.424	9.249
IX	6.0×10^{-7}	$>1.9 \times 10^{-5}$	>32	-10.033	-9.221	-0.655	0.686	8.566	10.719
X	4.0×10^{-8}	7.0×10^{-6}	175	-10.001	-9.059	-0.471	1.150	8.588	11.151
XI	5.0×10^{-7}	$>1.6 \times 10^{-5}$	>32	-8.799	-8.786	-0.243	0.263	8.543	9.062
XII	2.1×10^{-6}	$>6.3 \times 10^{-6}$	>3	-9.797	-8.653	-0.104	0.374	8.549	10.171

* Reference 8.

the highly selective molecules (V, VI, and VII) are grouped in well-defined bands of energy.

To some indexes, there are other molecules not so selective in the high selectivity band (HSB): E_{LUMO}: molecules VIII and X; E_{HOMO}: molecules III and VIII; $\Delta E_{L,H}$: molecule VIII. The molecule VIII, which is an isomer of VII, a high selective molecule, is present in all HSB´s. The difference between both is subtle: two hydrogen atoms that change orientation relatively to the plane of the molecule. Despite our calculations showing differences between them, these two molecules have almost the same values for the indexes that have HSB´s. As they present a very different selectivity, it seems that the orientation of the hydrogen atoms plays an important role in the experimental events that are involved.

The indexes that show no other molecules in the HSB´s are $\Delta E_{L1,H1}$ and E_{LUMO+1}.

The observed effect is even more interesting since the molecules are structurally quite different. This fact seems to indicate that the clustering of electronic indexes in high selectivity bands can be employed as a scheme to predict the selectivity of new proposed compounds. The rule would be: to be a candidate for having a high selectivity, the new molecule should have its values of $\Delta E_{L1,H1}$ and E_{LUMO} within the HSB‘s of these indexes. Perhaps a combination of all the HSB‘s should be a better choice, since it improves the number of criteria.

LDA, a multivariate data analysis method, provides a discriminating function that can predict whether a new compound falls in the high-selectivity group or not. In order to get the best results before using LDA, we transform the values of the electronic indexes in such way that the HSB‘s are now located at the beginning of the horizontal axis (energy axis). The transformation equation is:

$$E^{(t)} = \left| E - \overline{E}_{sel} \right|$$

in which E is the value of the electronic indexes as shown in Table 1, $E^{(t)}$ is the transformed one, and $\overline{E}_{sel}$ is the average value of the electronic index being considered, for the high selectivity molecules (V, VI and VII).

The correlation matrix of the five electronic indexes with HSB tells us that E_{LUMO+1} and $\Delta E_{L1,H1}$, as well E_{HOMO} and ΔE_{LH}, are highly correlated (i.e., contain nearly the same information). Therefore, instead of five, after eliminating two correlated electronic indexes, we have calculated a discriminating function to the transformed indexes $E^{(t)}_{tLUMO+1}, \Delta E^{(t)}_{L,H}$ and $E^{(t)}_{LUMO}$:

$$D = 2.097E^{(t)}_{LUMO+1} + 6.566E^{(t)}_{LUMO} + 5.516E^{(t)}_{L,H} - 2.492$$

If D < 0, the molecule is predicted or classified as belonging to the high selectivity group. This discriminating function has a statistical significance higher than 97%, a canonical correlation of 0.810 (the squared canonical correlation is the percentage of variation of the selectivity discriminated by electronic indexes), and classifies correctly 91.7% of the original group of twelve molecules. The cross-validated

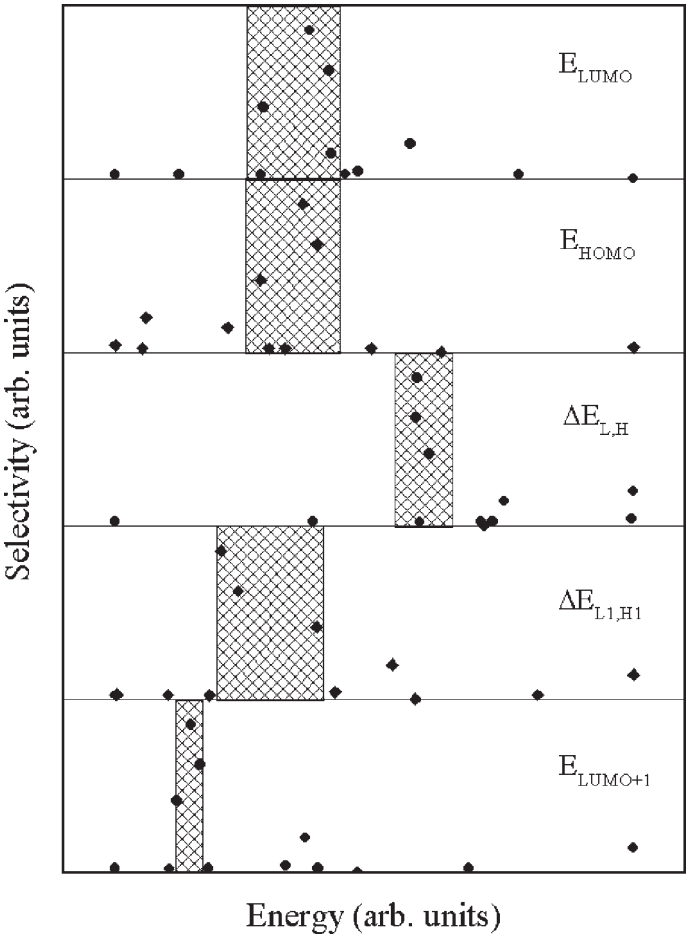

Fig. 2 - Quantum indexes vs selectivity (in arbitrary units) for febrifugine and isofebrifugine derivatives. The filled areas define the high selectivity bands.

classification results also show 91.7% of the molecules correctly classified. In cross-validation, each molecule is classified by the function calculated from all molecules other than that molecule. As expected, the observed error is due to molecule VIII, which has a low selectivity, but is classified as having high selectivity.

This model was tested with success on at least one molecule other than the 12 of the training set. KIKUSHI *et al.* studied another group of metabolites of febrifugine and its synthetic analogue[7]. Molecule 22 of this work, a stereoisomer of a metabolite of febrifugine, presents a very high selectivity. Thus, we followed the same procedure applied to the training set to calculate the electronic structure of the molecule 22. Applying the electronic indexes obtained for this molecule to the discriminating function, the result is D = -0.727, which implies that 22 is classified as having a high selectivity. Also, the obtained values to four of the five selected electronic indexes fall within the high selectivity bands, which we consider to be a very good result.

CONCLUSION

We propose a scheme for designing new derivatives of febrifugine and isofebrifugine with high antimalarial selectivity employing several

electronic indexes, which were tested with success.

This work is based uniquely on the correlation of electronic indexes against biological properties and presents a clear and fast method for qualitatively classifying the selectivity of new derivatives of febrifugine. The electronic indexes are simple descriptors to employ in order to model new compounds. Furthermore, it has been demonstrated that they are key descriptors to classify compounds with different biological activities[1,2,3,5,10,15].

RESUMO

Descritores da atividade antimalarial de derivados de febrifugina obtidos via mecânica qüântica

O desenvolvimento de linhagens resistentes de *Plasmodium falciparum* tem encorajado a busca por novas drogas antimalariais. A febrifugina é uma substância natural com alta atividade contra o *P. falciparum* que apresenta propriedade emética e toxicidade para o fígado tal que não permitem o seu uso clínico. A busca por análogos que possam ter uma performance clínica melhor é um tema de pesquisa atual. Nosso objetivo é investigar a estrutura eletrônica teórica de uma família de derivados da febrifugina empregando cálculos semi-empíricos de orbitais moleculares, procurando por índices eletrônicos que possam ajudar a modelar novos derivados mais eficientes. Os resultados teóricos mostram que para as moléculas mais seletivas existe um agrupamento dos valores de determinados índices em intervalos bem definidos. O modelo proposto para se obter alta seletividade foi testado com sucesso.

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