New Active Drugs against Liver Stages of *Plasmodium* Predicted by Molecular Topology*††*

Nassira Mahmoudi, 1,2* Ramon Garcia-Domenech, 3 Jorge Galvez, 3 Khemais Farhati, 1,2 Jean-François Franetic, 1,2 Robert Sauerwein, 4 Laurent Hannoun, 5 Francis Derouin, 6 Martin Danis, 1,2,7 and Dominique Mazier 1,2,7*

Université Pierre et Marie Curie-Paris 6, UMR S511, Paris F-75013, France 1; INSERM, U511, Paris F-75013, France 2; Unidad de Investigación de Diseño de Fármacos y Conectividad Molecular, Dep. Química Física, Facultad de Farmacia, Universidad de València, Burjassot, Valencia, Spain 3; Department of Medical Microbiology, University of Nijmegen, Nijmegen, The Netherlands 4; AP-HP, Groupe Hospitalier Pitié-Salpêtrière, Service de Chirurgie Digestive Hépato-Bilio-Pancréatique et de Transplantation Hépatique, Paris F-75013, France 5; Laboratoire de Parasitologie-Mycologie, and Hôpital Saint-Louis, Assistance Publique-Hôpitaux de Paris 1, Avenue Claude Vellefaux, 75010 Paris, France 6; and AP-HP, Groupe Hospitalier Pitié-Salpêtrière, Service Parasitologie-Mycologie, Paris F-75013, France 7

Received 8 August 2007/Returned for modification 31 October 2007/Accepted 13 January 2008

We conducted a quantitative structure-activity relationship (QSAR) study based on a database of 127 compounds previously tested against the liver stage of *Plasmodium yoelii* in order to develop a model capable of predicting the in vitro antimalarial activities of new compounds. Topological indices were used as structural descriptors, and their relation to antimalarial activity was determined by using linear discriminant analysis. A topological model consisting of two discriminant functions was created. The first function discriminated between active and inactive compounds, and the second identified the most active among the active compounds. The model was then applied sequentially to a large database of compounds with unknown activity against liver stages of *Plasmodium*. Seventeen drugs that were predicted to be active or inactive were selected for testing against the hepatic stage of *P. yoelii* in vitro. Antiretroviral, antifungal, and cardiotonic drugs were found to be highly active (nanomolar 50% inhibitory concentration values), and two ionophores completely inhibited parasite development. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was performed on hepatocyte cultures for all compounds, and none of these compounds were toxic in vitro. For both ionophores, the same in vitro assay as those for *P. yoelii* has confirmed their in vitro activities on *Plasmodium falciparum*. A similar topological model was used to estimate the octanol/water partition of each compound. These results demonstrate the utility of the QSAR and molecular topology approaches for identifying new drugs that are active against the hepatic stage of malaria parasites. We also show the remarkable efficacy of some drugs that were not previously reported to have antiparasitic activity.

Each year, the malaria parasite *Plasmodium falciparum* infects 300 to 660 million persons worldwide and causes several million deaths (25). New antimalarial drugs are urgently needed, especially considering the increasing prevalence of drug-resistant *P. falciparum* strains and the lack of effective vaccines and vector control measures. The *Plasmodium* liver stage is an interesting drug target, as it precedes the emergence of blood stages that cause the symptoms and complications of malaria. Drugs that inhibit parasite maturation within hepatocytes could be used for short-term prophylaxis in areas of endemicity (refugees and travelers, etc.). In addition, such drugs would be unlikely to select resistant strains, as the number of mitoses that occur during hepatic schizogony is many orders of magnitude smaller than that during the erythrocyte stage, thereby limiting the risk of mutant selection.

* Corresponding author. Mailing address: Université Pierre et Marie Curie-Paris 6, UMR S511, Paris F-75013, France. Phone: 33140779736. Fax: 33145838858. E-mail for Nassira Mahmoudi: nasstira@netcourrier.com. E-mail for Dominique Mazier: mazier@chups.jussieu.fr.
† Supplemental material for this article may be found at http://aac.asm.org/.
* Published ahead of print on 22 January 2008.
The structure of each molecule is represented by specific subsets of topological indices (TIs) (4). These indices, when well chosen, provide a unique way of characterizing a molecular structure (14). Moreover, they correlate with many physical, chemical, and biological properties of structurally heterogeneous groups of compounds and can be used to find new drugs (8). These models have already been used to predict the activities of candidate antimicrobial and antimalarial agents (5, 10–12, 18, 19, 21).

The aim of this study was to develop and assess QSAR models based on molecular topology in order to identify new compounds that are active on the liver stages of *Plasmodium* as well as predict physicochemical parameters influencing intestinal absorption.

**MATERIALS AND METHODS**

**Use of molecular topology to obtain structural descriptors.** A database of 127 compounds with known in vitro activities against the liver stage of *P. yoelii yoelii* was constructed from a literature search of several medical databases and from our own experimental data. The database comprises 24 families of congeners, naphthoquinones, amino-alcohols, amino-quinolines, acyclic and cyclic peroxides, quinolones and fluoroquinolones, cyclines, macrolides, lincosamides, penicillins, an herbal alkaloid, inhibitors of dihydrofuroate reductase, iron chelators, azidine type Mannich bases, vitamins, a sponge extract, anti-inflammatories, drugs, colchicine, antirhymnic, antiseptic, and antifungal compounds, and insecticides. The plane chemical structure of each drug was described with the aid of the Chemdaw software package (version 2002). Each compound was characterized by a set of 62 TIs. We used Kier and Hall’s connectivity indices in the aid of the Chemdraw software package (version 2002). Each compound was assessed with Kier and Hall’s connectivity indices through a computer-aided approach (5). The database used for this study was the BMDP New System 2 package, module 7M. The variables used to model the activities of each compound were included in the discriminant equation (or the variable that makes the largest contribution to the separation of each group). The quality of the database was assessed as the percentage of correct classifications in each set of compounds. The classification criterion was the Mahalanobis minimal distance (distance of each case to the mean of all the cases in a category). The quality of the discriminant function was evaluated by using the Wilks parameter, which was obtained by multivariate analysis of variance that tests the equality of group means for the variable in the discriminant model. The method used to select the descriptors was based on the Fisher-Snedecor parameter, which determines the relative importance of candidate variables. The software used for the LDA study was the BMDP New System 2 package, module 7M. The variables used to compute the linear classification function are chosen in a stepwise manner: at each step, the variable that makes the largest contribution to the separation of the groups is entered into the discriminant equation (or the variable that makes the smallest contribution is removed).

**PDAs.** The pharmacological distribution diagram (PDD) is a graphical representation that provides a straightforward way of visualizing the regions of minimum overlap as well as the regions in which the probability of finding active compounds is at maximum. A PDD is a frequency distribution diagram of dependent variables in which the ordinate represents the expectancy (probability of active) and the abscissa represents the values of DF in the interval (7). For an arbitrary interval of values of a given function, an “expectancy of activity” can be defined as $E_a = a/(i + 1)$, where $a$ is the number of active compounds in the interval divided by the total number of active compounds and $i$ is the number of inactive compounds in the interval divided by the total number of inactive compounds. The expectancy of inactivity is defined in a symmetrical way, as $E_{1-a} = (i+1)/i$. With these diagrams, we can visually determine the intervals in which there is a maximum probability of finding new active compounds and a minimum probability of finding inactive compounds.

**Topological virtual screening.** The topological model selected and constructed with the DF1 and DF2 functions was used to find new antimalarial agents from among a database of 479 compounds listed in the Merck index. This database was composed of drugs belonging to several therapeutic categories (antineoplastics, antivirals, antifungals, and antibacterials, etc.). A first step was performed by using the discriminant function DF1. The DF2 function was then used to sort the compounds selected as being active by DF1. PDDs were used to assign thresholds to discriminate active from inactive compounds with the highest probability of success. The compounds predicted to be active by the two equations (DF1 and DF2) were then evaluated and reclassified to be considered as potentially active. Among these, several commercially available compounds were assayed in vitro against the liver stage of *P. yoelii yoelii*.

**MLR.** Multilinear regression (MLR) analysis based on molecular topology was used to predict the octanol/water partition constant ($P$) of each molecule, as this parameter is closely related to the intestinal absorption of a molecule after oral administration. $P$ is the ratio of the concentration of the compound in octanol to the concentration of the compound in water and provides information on hydrophobicity (1, 16). For this prediction, the correlation between the calculated $P$ and the observed values of $P$ for 57 compounds was determined by MLR. The property of each group is entered into the discriminant equation (or the variable that makes the largest contribution to the separation of each group). The quality of the database was assessed as the percentage of correct classifications in each set of compounds. The classification criterion was the Mahalanobis minimal distance (distance of each case to the mean of all the cases in a category). The quality of the discriminant function was evaluated by using the Wilks parameter, which was obtained by multivariate analysis of variance that tests the equality of group means for the variable in the discriminant model. The method used to select the descriptors was based on the Fisher-Snedecor parameter, which determines the relative importance of candidate variables. The software used for the LDA study was the BMDP New System 2 package, module 7M. The variables used to compute the linear classification function are chosen in a stepwise manner: at each step, the variable that makes the largest contribution to the separation of the groups is entered into the discriminant equation (or the variable that makes the smallest contribution is removed).
The first equation, designated DF1, distinguished compounds that were predicted to have no significant in vitro activity from those likely to be active (IC\textsubscript{50} < 25 \textmu M) from those likely to be active (IC\textsubscript{50} < 25 \textmu M). The high cutoff of 25 \textmu M was chosen in order to avoid discarding drugs with potential activity in the first screening step.

This equation comprised 11 independent variables: DF1 = 3.17 + 1.00x\textsuperscript{1} + 1.28x\textsuperscript{2} - 14.04J\textsuperscript{x} + 22.94J\textsuperscript{y} + 96.23J\textsuperscript{z} + 65.98I\textsuperscript{x} + 1.88D\textsuperscript{4} - 23.53D\textsuperscript{5} + 0.29C\textsuperscript{x} - 0.51PR\textsuperscript{3} + 0.38I\textsubscript{5}.

Statistical parameters accounting for the significance of this equation were as follows: N = 76, F = 7.95, and \lambda = 0.42. The quality of this discriminant function was evaluated by using Wilks parameter, \lambda. With this function, a compound is considered to be either active or inactive depending on its DF1 value. DF1 values of <0 and >0 predict that a compound will be inactive or active, respectively, within a 95% confidence interval. In the training group (76 compounds), 35 out of 41 experimentally active compounds were correctly classified (85.4% accuracy), and 32 out of 35 experimentally inactive compounds were correctly classified (91.4% accuracy). Cross-validation (jackknifed matrix) of the training group showed that 30 (85.7%) out of the 35 inactive compounds and 33 (80.5%) out of the 41 active compounds were correctly classified. The PDD (Fig. 1) showed that drugs with DF1 values between 0.5 and 10 were classified as being active and those with DF1 values between -8 and -0.5 were classified as being inactive. The classification was uncertain for drugs with values between -0.5 and 0.5. Drugs with values above 10 or below -8 were considered to be “unclassified”.

The second LDA equation, DF2, was used to discriminate weakly to moderately active compounds (1 \textmu M < IC\textsubscript{50} < 10 \textmu M) from highly active compounds (IC\textsubscript{50} < 1 \textmu M). Once again, the 1 \textmu M cutoff was arbitrarily chosen.

Six independent variables composed DF2, as follows: DF2 = 81.90 - 3.45x\textsuperscript{5} + 7.41G\textsuperscript{3} - 70.21C - 1.44PR\textsuperscript{4} - 1.21V\textsuperscript{5} + 2.44V\textsuperscript{5}.

The statistical parameters were as follows: N = 28, F = 12.41, and \lambda = 0.21. By this function, a compound was considered to be active or highly active according the DF2 value. If DF2 was <0, the compound was predicted to be active, and if DF2 was >0, the compound was predicted to be highly active, with a 95% confidence interval. In the training set, consisting of 28 compounds with experimentally determined activities, 15 (93.8%) out of the 16 active compounds and 12 (100%) out of the 12 highly active compounds were correctly classified. Cross-validation of the training group showed that 14 (87.5%) out of the 16 active compounds and 12 (100%) out of the 12 highly active compounds were correctly classified. The corresponding PDD of this equation (Fig. 2) showed that compounds were classified as being highly active at DF2 values between 1 and 13 and as weakly or moderately active at values between -13 and 0. Drugs with values above 15 or below -13 were considered to be “unclassified”.

**Topological virtual screening.** Based on the mathematical model, virtual topological screening was applied to a database of 479 heterogeneous drug molecules (antiviral, antineoplastic, antifungal, and cardiotoxic, etc.). The model predicted that 62 (13%) of these compounds should be active against the liver stage of *Plasmodium*. Seventeen commercially available compounds (13 predicted to be active and 4 predicted to be inactive) were tested in vitro (see below) in order to assess the predictive capability of the model. The list of the 49 compounds predicted to be active and not tested in vitro is provided in Table S3 in the supplemental material. Table 1 illus-
TABLE 1. Predicted drug activity on liver stage of *P. yoelii yoelii*

<table>
<thead>
<tr>
<th>Drug (therapeutic category)</th>
<th>DFI* (class)</th>
<th>DF2* (class)</th>
<th>IC₅₀ exp (nM)</th>
<th>TC₅₀ exp (nM)</th>
<th>SI</th>
<th>Log P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Active drugs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Monensin (antibacterial/ionophore)</td>
<td>4.22 (A)</td>
<td>-21.62 (NC)</td>
<td>&lt;10⁻³</td>
<td>3.20 x 10⁴</td>
<td>&gt;3.20 x 10⁷</td>
<td>2.77</td>
</tr>
<tr>
<td>Nigericin (ionophore)</td>
<td>3.36 (A)</td>
<td>-24.22 (NC)</td>
<td>&lt;10⁻³</td>
<td>1.32 x 10⁴</td>
<td>&gt;1.32 x 10⁷</td>
<td>3.78</td>
</tr>
<tr>
<td>Delavirdine (antiviral)</td>
<td>-0.21 (NC)</td>
<td>6.75 (HA)</td>
<td>0.846</td>
<td>&gt;1 x 10⁵</td>
<td>&gt;1.18 x 10⁵</td>
<td>3.65</td>
</tr>
<tr>
<td>Mibefradil (antihypertensive)</td>
<td>7.59 (A)</td>
<td>6.66 (HA)</td>
<td>0.873</td>
<td>6.79 x 10⁴</td>
<td>7.7 x 10⁴</td>
<td>3.47</td>
</tr>
<tr>
<td>Licochalcone A (estrogenic flavonoid)</td>
<td>8.26 (A)</td>
<td>7.99 (HA)</td>
<td>0.927</td>
<td>6.34 x 10⁴</td>
<td>6.83 x 10⁴</td>
<td>2.57</td>
</tr>
<tr>
<td>Miconazole (antifungal)</td>
<td>1.46 (A)</td>
<td>1.77 (HA)</td>
<td>2.03</td>
<td>7.04 x 10⁴</td>
<td>3.47 x 10⁴</td>
<td>5.94</td>
</tr>
<tr>
<td>Dobutamine (cardiotonic)</td>
<td>5.74 (A)</td>
<td>1.52 (HA)</td>
<td>3.7</td>
<td>&gt;1 x 10⁵</td>
<td>&gt;2.70 x 10⁴</td>
<td>1.57</td>
</tr>
<tr>
<td>Ritonavir (antiviral)</td>
<td>8.80 (A)</td>
<td>-5.38 (A)</td>
<td>34.2</td>
<td>6.73 x 10⁴</td>
<td>1.96 x 10³</td>
<td>6.71</td>
</tr>
<tr>
<td>Saquinavir (antiviral)</td>
<td>9.14 (A)</td>
<td>-6.76 (A)</td>
<td>35.2</td>
<td>6.37 x 10⁴</td>
<td>1.80 x 10³</td>
<td>4.96</td>
</tr>
<tr>
<td>Epoximicin (antineoplastic)</td>
<td>8.17 (A)</td>
<td>7.92 (HA)</td>
<td>3.95</td>
<td>4.73 x 10⁴</td>
<td>4.73 x 10³</td>
<td>2.38</td>
</tr>
<tr>
<td>Indinavir (antiviral)</td>
<td>8.89 (A)</td>
<td>-10.35 (A)</td>
<td>5 x 10⁴</td>
<td>8.77 x 10³</td>
<td>1.75 x 10³</td>
<td>5.94</td>
</tr>
<tr>
<td>Vinblastine (antineoplastic)</td>
<td>1.21 (A)</td>
<td>38.71 (NC)</td>
<td>7.95 x 10⁴</td>
<td>5.45 x 10⁴</td>
<td>6.85</td>
<td>3.55</td>
</tr>
<tr>
<td>Nordihydroguaiaretic acid (antineoplastic)</td>
<td>9.03 (A)</td>
<td>2.88 (HA)</td>
<td>3 x 10⁴</td>
<td>&gt;1 x 10⁵</td>
<td>&gt;3.33</td>
<td>1.93</td>
</tr>
<tr>
<td>Inactive drugs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fenbendazole (anthelmintic)</td>
<td>-3.33 (I)</td>
<td>3 x 10⁴</td>
<td>&gt;1 x 10⁵</td>
<td>&gt;3.33</td>
<td>3.63</td>
<td></td>
</tr>
<tr>
<td>Quinacrine (anthelmintic/antimalarial)</td>
<td>-0.93 (I)</td>
<td>3 x 10⁴</td>
<td>&gt;1.16 x 10⁴</td>
<td>&gt;0.38</td>
<td>3.74</td>
<td></td>
</tr>
<tr>
<td>Rimantadine (antiviral)</td>
<td>-2.69 (I)</td>
<td>35.6</td>
<td>&gt;1 x 10⁵</td>
<td>&gt;3.33</td>
<td>3.85</td>
<td></td>
</tr>
<tr>
<td>Thiophanate (anthelmintic)</td>
<td>-4.04 (I)</td>
<td>3 x 10⁴</td>
<td>&gt;1 x 10⁵</td>
<td>&gt;3.33</td>
<td>1.93</td>
<td></td>
</tr>
<tr>
<td>Reference drugs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Atovaquone</td>
<td>9.37 (A)</td>
<td>3.63 (HA)</td>
<td>57</td>
<td>16.5 x 10³</td>
<td>289</td>
<td>3.67</td>
</tr>
<tr>
<td>Primaquine</td>
<td>0.91 (A)</td>
<td>1.79 (HA)</td>
<td>75.7</td>
<td>12.5 x 10³</td>
<td>165</td>
<td>1.73</td>
</tr>
</tbody>
</table>

* From discriminant function DFI.

b From discriminant function DF2. IC₅₀ exp, experimental IC₅₀; TC₅₀ exp, experimental TC₅₀; log P calc, calculated log P.

c Evaluation of antimalarial activity in vitro against the liver stage of *P. yoelii yoelii* as described previously by Mahmoudi et al. (17, 18).

From discriminant function DF1. IC₅₀ exp, experimental IC₅₀; TC₅₀ exp, experimental TC₅₀.

e Value of log P calculated with the MLR equation.

The statistical parameters were as follows: r² = 0.85, r² cv = 0.79, standard error = 0.92, F-stat = 59.0, and P (significance) < 0.00001. Figure 3 shows the good correlation obtained between experimental and calculated log P values. Detailed results for experimental and predicted log P values of these 57 compounds are provided in Table S2 in the supplemental material. Using this equation, we estimated log P values for selected antimalarial compounds. Values ranged between 1.57 and 6.71, reflecting a wide range of hydrophobicity among effective compounds.

In vitro antimalarial activity against the liver stage of *Plasmodium*. Table 1 shows the in vitro activities of the 17 compounds selected for in vitro testing. Twelve out of the 13 compounds predicted to be active showed an inhibitory effect on parasite growth, with IC₅₀ values ranging between 1 picomolar and 7.95 μM. The IC₅₀ values of primaquine and atovaquone, used as references, were 75.7 and 57 nM, respectively. Monensin and nigericin showed complete parasite inhibition, with picomolar IC₅₀ values. Seven compounds had IC₅₀ values below 50 nM (delavirdine, midefradil, dobutamine, licochalcone A, miconazole, saquinavir, and ritonavir), and three compounds had IC₅₀ values below 8 μM (indinavir, vinblastine, and epoximicin). Only one compound (nordihydroguaiaretic acid) that was predicted to be active was found to be inactive on parasite growth.

FIG. 3. Experimental versus calculated log P values obtained from MLR analysis. Shown is a comparison between experimental (y axis) and calculated (x axis) log P values for 57 compounds.
(IC$_{50}$ > 30 μM). Among the four compounds predicted to be inactive, three were completely inactive in vitro (IC$_{50}$ > 30 μM) (fenbendazole, quinacrine, and thiophanate), and one, rimantadine, was active (IC$_{50}$ of 35.6 nM). The most active compounds, monensin and nigericin, were examined for activity against _Plasmodium falciparum_. The same profile of activity was found with a complete inhibitory effect at picomolar concentrations (IC$_{50}$ < 10$^{-3}$ nM).

**Toxicity assay.** The drugs tested for in vitro activity on _P. yoelii yoelii_ were also examined for their toxicity on hepatocyte cells (MTT assay). TC$_{50}$ values are shown in Table 1. None of the 13 compounds with antimalarial activity was toxic for hepatocytes (TC$_{50}$ > 5 μM). An SI was then calculated as TC$_{50}$/IC$_{50}$ (Table 1). Ten of the 13 active compounds had an SI above 1,000. Three compounds had SI values between 1.77 and 12 (indinavir, vinblastine, and epoximicin). The primaquine and atovaquone SIs were 165 and 289, respectively.

**DISCUSSION**

The aim of this study was to build up a QSAR model suitable for screening a large molecular database for compounds that are active on _Plasmodium_ liver stages.

Based on molecular topology, a mathematical model was built up from a training set of 127 compounds whose activities against hepatic stages of _Plasmodium_ had previously been determined in vitro. This training set was comprised of heterogeneous molecular structures and a larger number of inactive than active molecules. The precise experimental IC$_{50}$ values of several compounds were not available from published sources. Because of this numerical imbalance between active and inactive compounds and the lack of firm IC$_{50}$ values, we had to build a stepwise model, as no single equation could reliably select active compounds.

The first equation (DF1) discriminated between inactive and active compounds by using a cutoff of 25 μM, while the second equation (DF2) selected highly active compounds among all active compounds (IC$_{50}$ < 1 μM). The discriminant function DF1 classified 83% of compounds in the training group correctly. There was little overlap between the active and inactive groups, but several compounds had DF1 values between −0.5 and 0.5, a range where no firm conclusions could be drawn. The second equation, DF2, classified about 97% of compounds in the training group correctly. Again, there was little overlap between the groups, confirming the quality of the discriminant function.

The significant role played by connectivity and topological charge indices in the different discriminant functions achieved must be emphasized.

The mathematical model was then applied to a database of 479 drugs with unknown activity against hepatic stages of _Plasmodium_. Despite a wide diversity of molecular structures and activities, the model predicted that 62 drugs (13%) would be active. Nine of the 13 compounds chosen for in vitro testing showed remarkable antimalarial activity. By comparison to the reference drugs primaquine and atovaquone, which had IC$_{50}$s of 75.7 nM and 57 nM, respectively, these nine compounds were 10- to 1,000-fold more potent in vitro. The most active compounds against the hepatic stages of _P. yoelii yoelii_ and _P. falciparum_ were monensin and nigericin, with IC$_{50}$s of <10$^{-3}$ nM. These results demonstrate the effectiveness of the topological model described herein for identifying new potential antimalarial drugs. However, the mathematical prediction was confirmed by the in vitro results only, and a confirmation of the in vivo activity will permit the validation of the mathematical model.

We have also examined the cellular toxicities of these drugs and calculated an SI based on the ratio between activity (IC$_{50}$) and toxicity (TC$_{50}$). Ten compounds had TC$_{50}$ values above those of primaquine and an SI above 10$^3$, compared to 165 for primaquine and 289 for atovaquone.

We have also designed a mathematical model capable of predicting the octanol/water partition constant of these molecules, as this parameter is indicative of hydrophobicity. MLR analysis yielded an equation capable of predicting the log $P$ value. The predicted values were indicative of good intestinal absorption with most of the compounds tested (log $P$ between 1.5 and 3.5).

A search of the literature conducted after the experimental phase showed that none of these compounds had previously been shown to have antimalarial properties against the hepatic stage.

Molecular topology has some drawbacks; for example, it takes into account only the two-dimensional molecular structure, and it does not distinguish stereoisomers. However, based on our results and the known modes of action of several drugs that we found to inhibit _Plasmodium_, several targets can be highlighted, such as aspartic proteases (inhibited by human immunodeficiency virus [HIV] protease inhibitors); K$^+$, Na$^+$, and Ca$^{2+}$ channels (inhibited by nigericin, monensin, and mitofradil, respectively); microtubules (inhibited by vinblastine); the proteasome (inhibited by epoximicin); and fumarate reductase (inhibited by licochalcone A).

Some of these drugs were previously shown to be active on the erythrocytic stage of _Plasmodium_. Licochalcone A was tested in vitro against blood stages of _P. falciparum_ and in vivo against _P. yoelii_ (28). The latter experiment used synchronous cultures and strongly suggested that the main effect of licochalcone A is to inhibit erythrocytic invasion by merozoites and/or to inhibit the initial growth of internalized merozoites. Intravenous licochalcone A administration reduced parasitemia in mice infected by _P. yoelii_.

Adovelande and Schrevel (3) demonstrated that monensin and nigericin exhibited intrinsic antimalarial activities at picomolar levels in vitro and in vivo. Our results with monensin and nigericin suggest that these drugs could be used to block transmission, as we obtained 100% inhibition of the hepatic stage of _Plasmodium_ in vitro. Recently, Skinner-Adams et al. (24) reported that HIV protease inhibitors such as saquinavir, ritonavir, and indinavir directly inhibited the growth of erythrocytic stages of chloroquine-sensitive and chloroquine-resistant _P. falciparum_ strains in vitro at clinically relevant concentrations. Further studies are required to determine the activities of HIV protease inhibitors against malaria in vivo. It would be interesting to examine the possible impacts of these drugs on HIV-infected patients living in areas of endemicity.

In conclusion, a combination of structural description by using TIs and statistical treatment by LDA can reliably select new compounds that are effective against the liver stages of _Plasmodium_. The predictive model thus obtained can readily
be applied to large databases of drugs in order to identify active structures. These results confirm the utility of molecular topology as a powerful tool in the search for new antimalarial drugs.

The in vitro validation of the model was performed using cultures of *P. yoelii* *yoelii*, but we can reasonably assume that such results can be extrapolated to *P. falciparum* (most of the molecules known so far to prevent hepatic development are efficient in both rodent and human *Plasmodium* infections) (17, 26). Furthermore, we have tested two ionophores in vitro (monensin and nigericin) against the *P. falciparum* liver stage, and complete inhibition of parasite development was observed. However, there are exceptional examples of discrepancies between human and rodent species while inhibiting the development of *P. yoelii* in vitro and in vivo (20); for instance, doxycycline has never been shown to have any effect on the hepatic multiplication of *P. falciparum* in humans in areas of endemity (23).

ACKNOWLEDGMENTS
Nassira Mahmoudi was financially supported by the Ministère de l’Éducation Nationale, de la Recherche, et de la Technologie and by the Raoul Follereau Association. We acknowledge financial support from the following sources: Cooperation Franco-Espagnole en Recherche Médicale, accord INSERM-CSIC projet conjoint-2002-2005; Projet PAL and Ministère de la Recherche, Spanish Red de Investigación de Centros de Enfermedades Tropicales, RICET, (C03/04); and project SAF2005-P1052128 of the Fondo de Investigación Sanitaria, Ministerio de Sanidad, Spain, andImpact Malaria, Sanofi-Aventis. We thank Enrico Lazaro for providing the crambesicine analogue.

REFERENCES