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New Anti-Malarial Compounds from Database Searching

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Abstract—In a serendipitous result, pharmacophores generated for the database searching for new non-nucleoside inhibitors of the HIV-1 reverse transcriptase enzyme unearthed 12 new lead compounds which were active against the *Plasmodium falciparum* strain of malaria.

Malaria is the number one infectious disease in the world and remains a serious endemic disease in more than 100 countries in Africa, Asia, Latin and South America.[1] The prolific mortality caused by malaria throughout the world sees of the order of 2.5 billion people being at risk and 250 million clinical cases arising each year.[2] The situation regarding control and treatment of malaria has progressively worsened with the spread of insecticide-resistant mosquito vectors[1,3] and drug-resistant parasites. Additionally, travellers from non-endemic areas are at risk of exposure and thousands of cases are reported in the US and Europe annually.[2] These combined factors emphasize the importance and significance of a continual supply of new and effective therapeutics for the long-term treatment of malaria.

Our studies originally centred on the discovery of new structural entities targeting the non-nucleoside inhibitor binding pocket of the HIV-1 reverse transcriptase (RT) enzyme. The approach was to generate a series of pharmacophores which were then used to mine the NCI database. Compounds that were generously supplied by the NCI were then screened for HIV-RT activity.[4] In a random (but clearly serendipitous) opportunity, the same compounds were further tested for anti-malarial activity. Of the 15 compounds supplied, nine showed activity in the micromolar range or better against the multidrug-resistant strain of *Plasmodium falciparum*. Such activity can be considered as being significant for lead compounds for further drug design and development. Table 1 illustrates these compounds and lists their activity against *P. falciparum*.

Pharmacophore Generation and Compound Selection

In summary, the automated pharmacophore development program Catalyst[5,6] was used to generate separate pharmacophore models for a series of four distinct non-nucleoside inhibitor (NNI) classes: nevirapine, HEPT, TIBO, and APA analogues.[7] Catalyst attempts to derive these models essentially by determining common descriptors (such as H-bond acceptors, hydrophobic regions, etc.) and correlating their location in space to the activity of a series (training set) of inhibitor compounds. The actual hypothesis scores are calculated according to the number of subunits required to completely describe a hypothesis. These hypotheses were then utilized in a unique filtering process, sequentially scanning the NCI database[8] with the final filter involving a manual selection from the remaining 500 compounds based upon excluding excessively bulky compounds (no size-exclusion constraints were used in pharmacophore generation), including compounds that both maintained a hydrophobic core and could be envisaged to adopt a butterfly shape (both characteristics of known NNIs).[9] Testing for anti-malarial activity utilized multidrug-resistant strains of *P. falciparum*.

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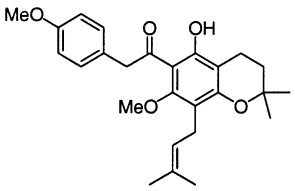
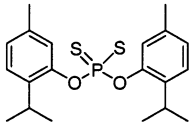
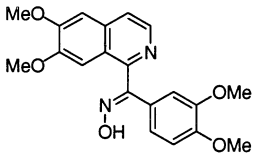
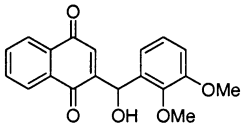
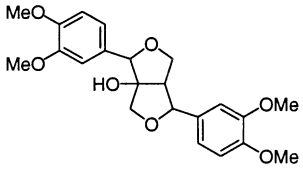
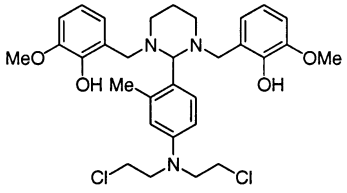
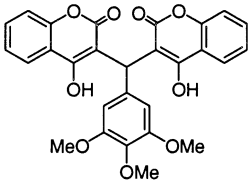
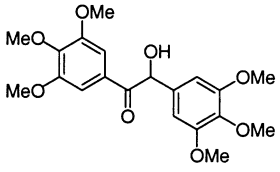
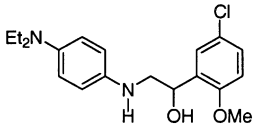
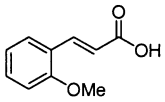
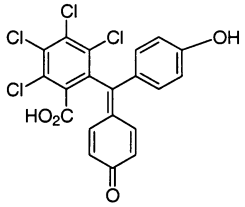
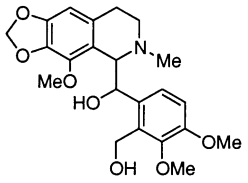
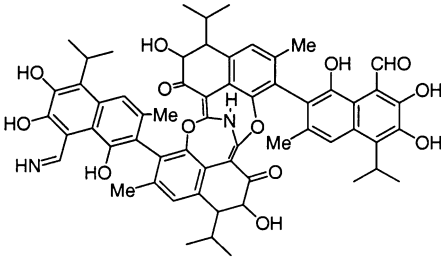
Table 1. The structure and anti-malarial activity (IC_{50}) against *P. falciparum* for selected compounds that emerged from database searching

Entry	Structure	Anti-malarial activity (M)
1		6.2×10^{-6}
2		Inactive
3		2.0×10^{-5}
4	 58342	4.6×10^{-6}
5		3.2×10^{-6}
6		1.2×10^{-6}
7		$>2.0\times10^{-6}$
8		2.0×10^{-5}
9		4.0×19^{-6}

(*continued*)

Table 1 (*continued*)

Entry	Structure	Anti-malarial activity (M)
10		3.0×10^{-6}
11		2.0×10^{-5}
12		8.0×10^{-6}
13		6.6×10^{-6}

Discussion

Table 1 lists the structures that emerged from our database searching, along with their corresponding anti-malarial activity against *P. falciparum*. To the best of our knowledge, none of these compounds have previously been reported as possessing anti-malarial activity.

One of the major outcomes of these drug design studies is that the pharmacophores generated, in combination with our novel filtering system,[4] have been shown to predict, in a surprisingly accurate manner, structures that display anti-malarial activity. Further, the compounds that emerged display a surprising degree of structural diversity, despite being derived from the same pharmacophore study. Additional evidence of the success of these pharmacophores is the concurrent unearthing of gossypol (**1**), which is already known to possess anti-malarial properties.[10] As an example, Figure 1 illustrates one of our pharmacophores with gossypol overlaid. This 'combined' hypothesis identifies two hydrophobic interactions, one possibly involved in π-stacking interactions, with the receptor. The additional feature is a hydrogen bond acceptor.

Clearly, the features revealed in the pharmacophores are directly related to the structures illustrated in Table 1. All have two hydrophobic regions, more often than not, corresponding to aromatic rings. The hydrophobicity of

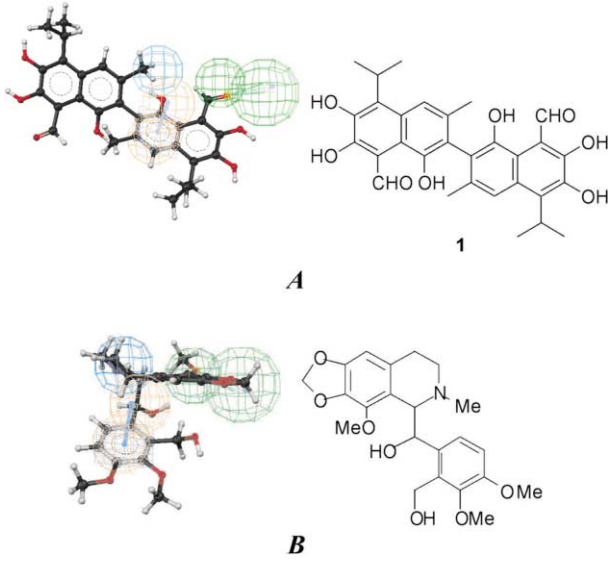


Figure 1. (A) An example of the generated pharmacophores with the known anti-malarial compound gossypol (**1**) overlaid. The mesh spheres illustrate the location constraints of each feature and are colour coded: hydrogen bond acceptor = green, with the proposed position of the H-bond illustrated with a projection; hydrophobic aliphatic region = blue; hydrophobic aromatic region = orange, with proposed π-stacking interaction shown by an arrow. Note, although only one example of the pharmacophores is illustrated here, aspects of commonality in these hypotheses were observed with a hydrophilic region (right) (hydrogen bond acceptor features) and a hydrophobic region (left) (common π-stacking regions). (B) Entry **12** (Table 1) overlaid on the same 'combined' hypothesis. Colour-coding is the same.

the NNI of HIV-1 RT, while possibly reflected by these (mostly) aromatic features, could play a smaller role in the anti-malarial activity—certainly, the general presence of a high number of electronegative functional groups would suggest a major role in determining the anti-malarial activity.

At this time, the exact site of action of these compounds is not known. Gossypol derivatives have been reported as lactate dehydrogenase inhibitors[11] and this would suggest that our novel compounds might target the same site.

Conclusions

We have generated a series of pharmacophores, and developed a simple filtering technique that predicts with outstanding success new structural entities as potential anti-malarial agents. While these compounds could not be considered therapeutics, they are most certainly significant lead compounds.

Biological Testing

Antiplasmodial assay

The parasite *P. falciparum* (K1, multidrug-resistant strain) was cultured continuously according to the method of Trager and Jensen.[12] Quantitative assessment of anti-plasmodial activity in vitro was undertaken by means of the microculture radioisotope technique based upon the method described by Desjardins et al.[13] Inhibition concentration (IC_{50}) represents the concentration which causes 50% reduction in parasite growth as indicated by the in vitro uptake of [^{3}H]-hypoxanthine by *P. falciparum*. An IC_{50} value of 0.16 mg/mL (3.1 mM) was observed for the standard sample, chloroquine diphosphate, in the same test system.

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7. The pharmacophores were generated using Catalyst® from MSI, using default parameters. 'Best conformation' model was used with a $\Delta E = 20$ kcal/mol, relatively large as a consequence of the poling algorithm used by the software. Of the 10 hypotheses that were always returned, the highest scoring example was used for each training set.
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