Lead Selection of a New Aminomethylphenol, JPC-3210, for Malaria Treatment and Prevention

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Structure-activity relationship studies of trifluoromethyl-substituted pyridine and pyrimidine analogues of 2-aminomethylphenols (JPC-2997, JPC-3186, and JPC-3210) were conducted for preclinical development for malaria treatment and/or prevention. Of these compounds, JPC-3210 [4-((tert-butyl)-2-((tert-butylamino)methyl)-6-(5-fluoro-6-(trifluoromethyl)pyridin-3-yl)phenol] was selected as the lead compound due to superior in vitro antimalarial activity against multidrug-resistant Plasmodium falciparum lines, lower in vitro cytotoxicity in mammalian cell lines, longer plasma elimination half-life, and greater in vivo efficacy against murine malaria.

Malaria continues to be a significant parasitic disease in tropical countries, with ~198 million cases and an estimated 584,000 deaths in 2013 (1). For the past decade, artemisinin combination therapies (ACTs) have been used for first-line treatment of uncomplicated Plasmodium falciparum malaria worldwide, with a marked impact on reducing the rate of malaria morbidity and mortality (2). However, reports of the development and spread of resistance to ACTs in Southeast Asia are alarming and highlight the urgent need to develop new antimalarial drugs (3–5).

Previously, we reported on a new 2-aminomethylphenol, JPC-2997, that showed high in vitro activity against P. falciparum lines, marked in vivo potency against Plasmodium berghei infections (6), and high efficacy in the Aotus monkey P. falciparum model (7) (Fig. 1). JPC-2997 synthesis was derived from the nonquinoline chlorophenylphenol WR 194,965, which was identified in the 1960s and 1970s and has good antimalarial activity against rodent malaria but limited potency in human challenge studies (8).

In addition to JPC-2997, structure-activity relationship studies revealed two other trifluoromethyl-substituted analogues, the pyrimidine JPC-3186 and the pyridine JPC-3210 (Fig. 1). Of these three compounds, we report on the selection of the lead 2-amino methylphenol JPC-3210 for future preclinical development as a new antimalarial compound based on superior in vitro activity against multidrug-resistant P. falciparum lines, lower in vitro cytotoxicity in mammalian cell lines, more favorable pharmacokinetic properties in mice, and greater in vivo efficacy against murine malaria.

The in vitro antimalarial activities of the three 2-aminomethylphenols were evaluated against the chloroquine-sensitive P. falciparum D6 line (Sierra Leone) (9) and seven multidrug-resistant lines, i.e., 7G8 (Brazil), TM90-C2B, TM91-C235 and TM93-1088 (Thailand), and MRA1239, MRA1240, and MRA1241 (Cambodia), by using the [3H]hypoxanthine growth inhibition assay (10).

These multidrug-resistant lines have been well characterized and have different levels of susceptibility to chloroquine, dihydroartemisinin, and mefloquine (11–14). TM90-C2B and TM93-1088 are also highly resistant to atovaquone (12).

The mean 50% inhibitory concentrations (IC50s) of JPC-2997, JPC-3186, JPC-3210, and WR 194,965 against the P. falciparum lines are shown in Table 1. Of the three trifluoromethylated analogues of WR 194,965, JPC-3210 was the most active, with mean IC50 ranging from 2.5 to 19 nM. Across the eight P. falciparum lines, the IC50 of JPC-3210 was on average 1.7-fold and 3.2-fold lower than those of JPC-2997 and WR 194,965, respectively. Although not statistically different (Mann-Whitney rank sum test, P > 0.05), the IC50 of JPC-3210 was 1.3-fold lower than that of JPC-3186. JPC-3210 was also far more active than either chloroquine.

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TABLE 1 In vitro antimalarial activities of JPC-2997, JPC-3186, and JPC-3210 against Plasmodium falciparum lines

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC50 (nM)</th>
<th>D6</th>
<th>TM90-C2B</th>
<th>TM91-C235</th>
<th>TM93-C1088</th>
<th>7G8</th>
<th>MRA1239</th>
<th>MRA1240</th>
<th>MRA1241</th>
</tr>
</thead>
<tbody>
<tr>
<td>JPC-2997</td>
<td>13 ± 2</td>
<td>34 ± 1</td>
<td>25 ± 1</td>
<td>13 ± 1</td>
<td>4.6 ± 0.3</td>
<td>7.2 ± 0.2</td>
<td>19 ± 5</td>
<td>12 ± 1</td>
<td></td>
</tr>
<tr>
<td>JPC-3186</td>
<td>15 ± 5</td>
<td>23 ± 3</td>
<td>14 ± 0.3</td>
<td>9 ± 3</td>
<td>3.8 ± 0.5</td>
<td>6.1 ± 0.8</td>
<td>11 ± 4</td>
<td>11 ± 2</td>
<td></td>
</tr>
<tr>
<td>JPC-3210</td>
<td>9 ± 1</td>
<td>19 ± 2</td>
<td>17 ± 0.5</td>
<td>7 ± 2</td>
<td>2.5 ± 0.5</td>
<td>4.3 ± 0.7</td>
<td>12 ± 0</td>
<td>6.9 ± 0.4</td>
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<tr>
<td>WR-194,965</td>
<td>28 ± 9</td>
<td>53 ± 5</td>
<td>40 ± 2</td>
<td>18 ± 8</td>
<td>6 ± 1</td>
<td>14 ± 2</td>
<td>62 ± 40</td>
<td>26 ± 6</td>
<td></td>
</tr>
<tr>
<td>CQ</td>
<td>16 ± 5</td>
<td>144 ± 28</td>
<td>103 ± 25</td>
<td>599 ± 11</td>
<td>129 ± 6</td>
<td>76 ± 1</td>
<td>97 ± 21</td>
<td>220 ± 92</td>
<td></td>
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<tr>
<td>DHA</td>
<td>1.9 ± 0.1</td>
<td>1.7 ± 0.7</td>
<td>2.3 ± 0.7</td>
<td>1.4 ± 0.4</td>
<td>1.3 ± 0.2</td>
<td>0.3 ± 0.2</td>
<td>1.0 ± 0.7</td>
<td>0.7 ± 0.4</td>
<td></td>
</tr>
<tr>
<td>MQ</td>
<td>34 ± 6</td>
<td>130 ± 51</td>
<td>107 ± 41</td>
<td>16 ± 5</td>
<td>5.4 ± 1.7</td>
<td>26 ± 4</td>
<td>84 ± 8</td>
<td>51 ± 18</td>
<td></td>
</tr>
</tbody>
</table>

a CQ, chloroquine; DHA, dihydroartemisinin; MQ, mefloquine.

b Values are means ± SD, with ≥2 observations.

The cytotoxicity of the 2-aminomethylphenols was determined against two human cell lines (HEK293 [human embryonic kidney] and HepG2 [human hepatocellular carcinoma]) and a hamster cell line (BHK [baby hamster kidney]) by the alamarBlue fluorescent cell viability assay (Invitrogen Corporation, CA) (15). All compounds exhibited very low cytotoxicity in the mammalian cell lines, with mean IC50 of ≥36 µM, resulting in high selectivity index (SI) values of ≥2,769 when antimalarial activity was compared to mammalian cytotoxicity (see Table S1 in the supplemental material). Of the 2-aminomethylphenols, JPC-3210 was less cytotoxic than JPC-2997 and WR 194,965, with higher mean IC50 of 2.0-fold, 1.2-fold, and 2.6-fold, respectively, in the HEK293 cell line. Although the IC50 of JPC-3210 was 1.2-fold higher than that of JPC-3186 in the HEK293 cell line, the difference was not statistically significant (P > 0.05). The SI value of JPC-3210 was at least twice as high as those of chloroquine, with values of ≥9,000 for the three mammalian cell lines.

Previously, we reported on the pharmacokinetic properties of JPC-2997 in mice, revealing that the drug is widely distributed to tissues, with a low plasma clearance and a lengthy elimination half-life (6). In the present study, we determined the pharmacokinetics of JPC-2997, JPC-3186, and JPC-3210 in healthy ARC female mice (aged 6 to 7 weeks; mean ± standard deviation [SD] body weight, 28.0 ± 3.2 g; Animal Resources Centre, Perth, Australia). Groups of five mice were treated with a single 20-mg/kg oral dose of JPC-2997, JPC-3186, or JPC-3210. The mice were anesthetized with carbon dioxide and killed by cardiac puncture, and blood samples were collected at 0 (before dosing), 0.25, 0.5, 1, 2, 3, 6, 12, and 24 h and 2, 3, 4, 5, 7, 11, and 14 days after dosing. Plasma concentrations of the three 2-aminomethylphenols were measured by liquid chromatography-tandem mass spectrometry (LC-MS/MS) (for more information, see the supplemental material). Noncompartmental analysis was used to determine the area under the plasma concentration curve (AUC) from time zero to day 14 and the elimination half-life of the compounds (16).

The mean plasma concentration-versus-time profiles of JPC-2997, JPC-3186, and JPC-3210 in mice are shown in Fig. 2. The plasma maximum concentrations (Cmax) and the time the Cmax was reached for the 2-aminomethylphenols were similar (2,067 ng/ml at 1.0 h for JPC-2997, 2,359 ng/ml at 0.5 h for JPC-3186, and 2,086 ng/ml at 1.0 h for JPC-3210). The AUC0→14 values for JPC-2997, JPC-3186, and JPC-3210 were 190,140, 42,653, and 59,019 ng·h/ml, respectively. The corresponding elimination half-lives were 40.1, 43.5, and 94.3 h, revealing that JPC-3210 has an elimination half-life ~2-fold longer than that of JPC-2997 or JPC-3186.

The high intrinsic in vitro antimalarial activities and long elimination half-lives of JPC-2997, JPC-3186, and JPC-3210 suggest that the compounds are suitable as prophylactic agents. To assess their potential prophylactic activity, CD-1 male or female mice (aged 7 to 9 weeks; mean ± SD body weight, 35.9 ± 2.8 g; Charles River Laboratories, Wilmington, MA), in groups of seven, were treated with various single oral doses of the 2-aminomethylphenols at 18 and 11 days before being infected with 5 × 105 parasitized erythrocytes of the lethal chloroquine-sensitive P. berghei KBG-173 strain, as previously described (17). The compounds were mixed in 0.5% hydroxyethylcellulose and 0.1% Tween 80 and administered via oral gavage. Mortality data were tabulated for 31 days postinfection because infected nontreated mice commonly survive for up to 10 days postinfection. Blood smears were also read up to day 31 postinfection.

A dose of 64 mg/kg of JPC-3210, administered to mice 18 days before parasite inoculation, was effective in suppressing the P.
The final assessment for the selection of the lead 2-amino methylphenol was the onset of drug action and recrudescence test against murine malaria, which measures the speed of parasite clearance by a candidate compound and the time when recrudescence occurs after a fixed single dose (20, 21). This was done with oral administration of the 2-aminomethylphenols (100 mg/kg) on day 4 after infection in groups of six ARC female mice (aged 6 to 7 weeks; mean ± SD body weight, 28.5 ± 2.6 g) intraperitoneally infected with 10^7 parasitized erythrocytes of the lethal chloroquine-sensitive *P. berghei* ANKA strain. Untreated control mice typically achieve a parasitemia of ∼30% by day 4 postinfection. Drugs were prepared in ethanol-Tween 80-water (10:10:80, vol/vol/vol). The reduction in parasitemia was monitored by readings of blood smears at 12-h intervals after treatment, and the time of recrudescence was assessed by daily blood smears for 17 days, followed by intermittent assessments for up to 31 days. The clearance rate of the high level of *P. berghei* infection (mean ± SD parasitemia, 30.1 ± 1.6% for the three treatment groups) was comparable for the 2-aminomethylphenols, taking 144 h for the animals to show negative blood smears (Fig. 3). Recrudescences were observed on days 17, 20, and 27 for mice treated with JPC-3186, JPC-2997, and JPC-3210, respectively. Of note, one of five mice treated with JPC-3186, two of six mice treated with JPC-2997, and two of five mice treated with JPC-3210 recrudesced with the rest of the animals having negative blood smears up to day 31 of follow-up. One mouse in the JPC-3186 treatment group and one in the JPC-3210 treatment group had to be euthanized at 84 and 120 h, respectively, as they had lost >20% of their body weight due to slower clearance of parasitemia. These findings suggest that the three 2-aminomethylphenols were similarly effective in reducing a high biomass of infection, with JPC-3210 exhibiting the longest recrudescence protection, most likely due to its greater potency and longer elimination half-life.

In summary, of the three 2-aminomethylphenols evaluated in the present study, JPC-3210 was the most active compound in vitro against multidrug-resistant *P. falciparum* lines, was the least

### TABLE 2 Prophylactic assessment of mefloquine, JPC-2997, JPC-3186, and JPC-3210 in the mouse *Plasmodium berghei* model

<table>
<thead>
<tr>
<th>Days before parasite inoculation</th>
<th>Single oral drug dose (mg/kg)</th>
<th>Mice alive with NPS on day 31 postinoculation with:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mefloquine</td>
</tr>
<tr>
<td>−18</td>
<td>64</td>
<td>0/7</td>
</tr>
<tr>
<td></td>
<td>32</td>
<td>0/7</td>
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<td></td>
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<td>−11</td>
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<td></td>
<td>32</td>
<td>0/7</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>0/7</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>ND(^b)</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>ND</td>
</tr>
<tr>
<td>Infected control</td>
<td></td>
<td>0/7</td>
</tr>
<tr>
<td>Noninfected control(^c)</td>
<td></td>
<td>7/7</td>
</tr>
</tbody>
</table>

\(^a\) No parasites seen (NPS) in 3 of 4 living mice.

\(^b\) ND, not done.

\(^c\) Not applicable for NPS.
cytotoxic in mammalian cell lines, and had the lowest elimination half-life. JPC-3210 also possessed the greatest prophylactic protection and duration of action against murine malaria in vivo. Because of the superior parasitological, pharmacokinetic, and pharmacodynamic properties of JPC-3210 compared with those of JPC-2997 and JPC-3186, it has been designated the lead compound for further preclinical studies, including those for safety, toxicology, mechanism of action, bioavailability, metabolism, and efficacy in the Aotus monkey P. falciparum/P. vivax model. Based on the favorable outcome of these investigations, the first human studies will be conducted with the intent of using JPC-3210 as a potential partner drug with a rapid-acting antimalarial for the treatment and/or monotherapy prevention of malaria. (The animal studies for the pharmacokinetics and the onset and duration assessments of antimalarial activity of the aminomethylphenols were approved by the Australian Army Malaria Institute Animal Ethics Committee [protocols 03-13 and 04-13]. Animal use in the prophylactic test was approved by the University of Miami Institutional Animal Care and Use Committee [protocol 12-217].)

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With the exception of G.D.H., G.A.S., L.R.J. and D.P.J. who work for the Jacobus Pharmaceutical Company, we have no conflicts of interest to declare.

REFERENCES


