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Evaluation of Mongolian compound library for potential antimalarial and anti-*Toxoplasma* agents

Orkhon Banzragchgarav[a, b, 1], Nanang R. Ariefta[a, 1], Toshihiro Murata[c], Punsantsogvoo Myagmarsuren[b], Badgar Battsetseg[b], Banzragch Battur[b, d], Javzan Batkhuu[e], Nishikawa Yoshifumi[a *]

[a]National Research Center for Protozoan Diseases, Obihiro University of Agriculture and Veterinary Medicine, Hokkaido 080-8555, Japan

[b]Institute of Veterinary Medicine, Mongolian University of Life Sciences, Ulaanbaatar 17024, Mongolia

[c]Division of Pharmacognosy, Tohoku Medical and Pharmaceutical University, Sendai 981-8558, Japan

[d]Graduate School, Mongolian University of Life Science, Ulaanbaatar 17024, Mongolia

[e]School of Engineering and Applied Sciences, National University of Mongolia, Ulaanbaatar 14201, Mongolia

[*] Corresponding author. Email: nisikawa@obihiro.ac.jp

[1] Both authors contributed equally to this study.

Abstract

179 compounds in a Mongolian compound library were investigated for their inhibitory effect on the *in vitro* growth of *Plasmodium falciparum* and *Toxoplasma gondii.* Among these compounds, brachangobinan A at a half-maximal inhibition concentration (IC_{50}) of 2.62 μM and a selectivity index (SI) of 27.91; 2-(2'-hydroxy-5'-*O*-methylphenyl)-5-(2",5"-dihydroxyphenyl)oxazole (IC_{50} 3.58 μM and SI 24.66); chrysosplenetin (IC_{50} 3.78 μM and SI 15.26); 4,11-di-*O*-galloylbergenin (IC_{50} 3.87 μM and SI 13.38); and 2-(2',5'-dihydroxyphenyl)-5-(2"-hydroxyphenyl)oxazole (IC_{50} 6.94 μM and SI 11.48) were identified as potential inhibitors of *P. falciparum* multiplication. Additionally, tricin (IC_{50} 12.94 μM and SI >23.40) was identified as a potential inhibitor of *T. gondii* multiplication. Our findings represent a good starting point for developing novel antimalarial and anti-*Toxoplasma* therapeutics from Mongolian compounds.

Keywords: Malaria, Toxoplasmosis, *Plasmodium falciparum, Toxoplasma gondii,* Mongolian compound library

1. Introduction

Malaria and toxoplasmosis are caused by *Plasmodium* spp and *Toxoplasma gondii*, respectively, of the phylum Apicomplexa. *Plasmodium* spp has a considerable effect on human morbidity and mortality, and they continue to cause significant global health problems [1]. *T. gondii* has an impact not only on human morbidity and mortality but also on animal morbidity, mortality, and productivity [2]. Currently, available drugs for treating these diseases have many limitations, such as drug resistance, adverse side effects, and low-to-medium drug efficacy [3]. Hence, there is an urgent need to develop new drugs for these protozoan diseases.

Malaria is considered one of the biggest public health issues because almost half of the world's population is at risk of contracting malaria. It causes 2% of the world's total deaths and millions of clinical infections [4]. According to the World Health Organization (WHO), 229 million new malaria cases and 409 thousand related deaths were reported globally in 2019; among these data, children under 5 years of age accounted for 274 thousand (67%) of all malaria deaths [5]. Several antimalarial drugs have been successfully developed over the last few years. WHO recommends combinational therapies, e.g., combinational artemisinin-based medicines including lumefantrine, mefloquine, amodiaquine, sulfadoxine/pyrimethamine, piperaquine, and chlorproguanil/dapsone. However, the current drugs have several limitations due to their toxicity and the prevalence of drug-resistant parasites. Moreover, drug options for liver-stage malaria parasites are limited [3,6]. Therefore, effective new drugs for malaria are still required.

T. gondii is the most commonly distributed parasite and can infect most warm-blooded animals, including humans. Approximately 30% of the human population is infected, and the infection rate may be as high as 70%–80% in some countries [7]. *T. gondii* infection can be life-threatening to immunodeficient individuals or congenitally infected children. Few therapeutics

against toxoplasmosis are available, such as a combinational therapy of pyrimethamine and sulfadiazine or clindamycin. However, these treatments are limiting owing to several toxic effects, such as bone marrow suppression and allergies in patients with AIDS, and ineffective to eliminate tissue cysts [2]. Thus, the development of new drugs is critical.

Natural products and their derived compounds have played a significant role and continue to increase drug discovery attention. Over one-third of the US Food and Drug Administration (FDA) approved medicine are derived from natural products [8]. In addition, their diversity in chemical structure and function makes them ideal starting points for drug development [9]. Previously, few studies have reported that Mongolian plants and their derived compounds have potential inhibitory properties against protozoan parasites such as *Babesia* and *Trypanosoma* [10–16]. Our recent finding showed that some Mongolian crude plant extracts exhibited promising inhibitor activities against *P. falciparum* and *T. gondii* [17]. In this study, we investigated the antimalarial and anti-*Toxoplasma* activities of the compounds in a Mongolian compound library for the first time.

2. Materials and methods

2.1. Test compounds

Overall, 179 compounds were isolated from 10 species of Mongolian plants [10–16,18–21] and were prepared as 10 mg/ml stock solutions in dimethyl sulfoxide (DMSO) (Table S1) with a guaranteed purity of ≥80-90% based on integration in their ^{1}H-nuclear magnetic resonance and stored at −20°C. The stocks were blindly screened (regardless of the structure) and further diluted to a single final screening concentration of 20 µg/ml. With the inhibition >70% on parasites growth, active compounds were further subjected to IC_{50} and CC_{50} determination (see SI). Six to eight

concentrations (μg/ml) were used to determine the IC_{50} of each parasite and CC_{50} of the HFF cells. The IC_{50} and CC_{50} values were converted and reported as μM after confirming the structure of the active compounds.

2.2. Anti-Plasmodium cultivation and growth inhibition assay

P. falciparum 3D7 strain was routinely cultivated in 2% washed human O-positive erythrocytes (Hokkaido Red Cross Blood Center, Hokkaido, Japan) in complete RPMI-1640 medium (Sigma-Aldrich, St. Louis, MO, USA) supplemented with (per L) 6 g HEPES (Sigma-Aldrich), 5 g AlbuMax II (Gibco, Carlsbad, CA, USA), 25 mg hypoxanthine, 2 g $NaHCO_3$, 250 μl of 50 mg/ml gentamicin. The cultures were maintained at 37°C with 5% each of CO_2 and O_2. The medium was changed daily. Subculturing was performed as required for maintenance, and parasitemia was monitored by Giemsa staining blood smear. This malarial parasite culture preparation in human erythrocytes underwent ethical review and received approval from Obihiro University of Agriculture and Veterinary Medicine (#2013-04-3).

The *in vitro* antimalarial activity was used to test compounds using the SYBR Green I-based fluorescence assay [22,23]. Briefly, the asynchronous parasites were synchronized in the ring stage using a 5% D-sorbitol solution. Next, the test compounds were diluted in a complete medium to eight concentrations (two-fold dilution) ranging from 5–0.039 μg/ml. Next, the synchronous parasites (ring-stage parasites ≥90%) at 0.5% parasitemia were seeded in the complete medium with 2% hematocrit. This medium and test compounds or the 1% DMSO control were added to 96-well plates at a final volume of 100 μl per well. The 96-well plates were incubated for 72 h at 37°C with 5% each of CO_2 and O_2. Next, 100 μL of lysis buffer containing SYBR Green I at the final concentration of 1× was added to each well, mixed, and incubated in the dark for 2 h.

Fluorescence intensity was then measured at an excitation wavelength of 485 nm and an emission wavelength of 518 nm using a Fluoroskan Ascent instrument (Thermo Scientific, Waltham, MA, USA). Chloroquine (Sigma-Aldrich) was used as a positive control, the 1% DMSO-treated cells served as a negative control, and a well containing only the medium was used to correct for any background signal. The assay was performed in quadruplicate for each compound concentration.

2.3. Red blood cell (RBC) hemolysis assay

RBC hemolysis assay was performed as previously described [24]. Briefly, 100 µL of each compound was prepared in PBS at 200 µM (final concentration 100 µM) on a 96-well plate, then 100 µL of 3% RBCs suspension in PBS was added. The plate was incubated at 37 °C for 3 h, then was centrifuged. Finally, 100 µL supernatant of each compound mixture was transferred to a new 96-well plate, and the absorbances were recorded at 540 nm using an MTP-500 microplate reader (Corona Electric, Ibaraki, Japan). Chloroquine (100 µM), 1% DMSO in PBS, and RBC lysis buffer (0.83% NH_4Cl; 0.01 M Tris-HCl, pH 7.2) were used as a reference drug, negative, and positive control, respectively. The hemolysis rate of RBC was calculated using the following formula: Hemolysis rate = $[(A_{sample} - A \text{ negative control}) / (A \text{ positive control} - A \text{ negative control}) \times 100]$; A, Absorbance. The experiments were conducted in triplicate and repeated three times independently.

2.4. Microscopic analysis of phenotype and parasitemia of P. falciparum

Compounds were diluted in complete medium to final concentrations equal to IC_{80}; 2-(2'-hydroxy-5'-*O*-methylphenyl)-5-(2",5"-dihydroxyphenyl)oxazole (IC_{80} 4.65 µM); 2-(2',5'-dihydroxyphenyl)-5-(2"-hydroxyphenyl)oxazole (IC_{80} 11.83 µM); 4,11-di-*O*-galloylbergenin

(IC$_{80}$ 5.81 µM); brachangobinan A (IC$_{80}$ 6.23 µM); chrysosplenetin (IC$_{80}$ 6.58 µM). A 50 µL of each compound or complete media (contained 1% DMSO, as negative control) was added to a 96-well plate. To these solutions, 50 µL of complete media with *P. falciparum* 3D7-infected RBC were added at 2% hematocrit and 0.5% parasitemia. The parasite was synchronized twice using the sorbitol method. The Giemsa-stained thin blood smears of each treatment were prepared at 1, 24, 48, 72 h incubation. After 72 h of incubation, we either continued the incubation with compound stress (unwashed group) or removed the active compounds (washed group)–replaced with fresh complete media– for up to 144 h. Subsequently, each treatment's Giemsa-stained thin blood smears were prepared at 96, 120, and 144 h. All prepared blood smears were observed using All-in-one Microscope BZ-9000 (Keyence BioRevo, Tokyo, Japan). The experiment was performed in triplicates for each compound and time point.

2.5. Anti-Toxoplasma cultivation and growth inhibition assay

T. gondii RH-GFP, a green fluorescent protein expressing-RH strain [25], was maintained in Vero (African green monkey kidney) cells [26] in minimum essential medium (EMEM, Sigma-Aldrich) with 8% (v/v) fetal bovine serum (FBS, Biowest, Nuaille, France) and 1% penicillin at 37°C and 5% CO_2. Tachyzoites were harvested from the infected host cells by passing through a 27-gauge syringe needle, filtering through a 5.0-µm filter (Millipore, Burlington, MA, USA), and centrifuging at 1,300 × *g* for 10 min. The parasite numbers were counted under a microscope using a hemocytometer.

The test compounds were evaluated for *T. gondii* growth inhibition via a fluorescence-based assay [27]. Briefly, human foreskin fibroblast (HFF) cells [28] were seeded in 96-well plates at 1 × 10^4 cells per well and incubated for 48 h at 37°C and 5% CO_2. The host cells were further infected

with the freshly harvested tachyzoites at 5×10^5 per well. After 4 h, the test compounds were added to the wells at six concentrations, 20, 15, 10, 5, 2.5, and 1 µg/ml. After 72 h of treatment, the fluorescence intensity of GFP produced by RH-GFP of *T. gondii* was measured using a microplate reader (GloMax-Multi Detection System, Promega, Madison, WI, USA). Sulfadiazine (Sigma-Aldrich) was used as a positive control, 1% DMSO-treated cells served as a negative control, and a well containing only the medium was used to correct any background signal. Each compound concentration was tested in triplicate.

2.6. Cytotoxicity assay on HFF cells

HFF cells were maintained in Dulbecco's modified eagle medium (Sigma-Aldrich) supplemented with 10% FBS and 1% penicillin at 37°C with 5% each of CO_2 and O_2. The cytotoxicity of the test compounds was assessed on HFF cells using a cell viability assay [29]. The HFF cells were seeded in 96-well plates at 1×10^4 cells per well and incubated at 37°C and 5% CO_2 to allow for cell attachment. After 48 h of incubation, the culture media was changed, and the cells were treated with eight concentrations (two-fold dilution) ranging from 100–0.78 µg/ml. After 72 h, the Cell Counting Kit-8 (Dojindo Molecular Technologies, Kumamoto, Japan) was used, and the absorbance was read at 450 nm using an MTP-500 microplate reader. Chloroquine and sulfadiazine were used as reference compounds. The experiments were conducted in triplicate and repeated three times independently. The selective index (SI) was calculated by dividing each compound's mean 50% HFF cells cytotoxic concentration (CC_{50}) value by the compound's IC_{50} of each parasite.

3. Results

3.1. Effects of the test compounds on P. falciparum

All 179 compounds were initially screened at a concentration of 20 μg/ml against *P. falciparum*. We identified 60 compounds that demonstrated >80% inhibition (Table S1). Secondary screening of these compounds at 2.5 μg/ml concentration yielded seven compounds with >50% inhibition. These compounds showed an IC_{50} of 2.62–6.94 μM (Table 1). Five compounds, 2-(2'-hydroxy-5'-*O*-methylphenyl)-5-(2",5"-dihydroxyphenyl) oxazole, 2-(2',5'-dihydroxyphenyl)-5-(2"-hydroxyphenyl) oxazole, 4,11-Di-*O*-galloylbergenin, brachangobinan A, and chrysosplenetin, met our selection criteria of SI ≥10 by showing SI values of 11.48–27.91. Brachangobinan A was found to be the most potent compound (IC_{50} of 2.62 μM and SI of 27.91), followed by 2-(2'-hydroxy-5'-*O*-methylphenyl)-5-(2",5"-dihydroxyphenyl) oxazole (IC_{50} of 3.58 μM and SI of 24.66). The chemical structures of these compounds are presented in Fig 1.

3.4 Effects of compounds on RBC hemolysis

The hemolysis rates of antimalaria active compounds were evaluated using healthy O-positive RBC. At 2% or less, the hemolysis rate is considered the standard of best value by the American Society for Clinical Pathology [30]. In Table 1, all active compounds showed hemolysis rates range from 0.69–4.60% at 100 μM, the concentration is more than tenfold of the IC_{50} values. In the effective inhibition concentration of each compound, the hemolysis rate was considered safe with values below 2%.

3.2. Effects of compounds on parasitemia and phenotype of P. falciparum

To investigate the parasitemia level and morphological changes of active compounds, 2-(2'-hydroxy-5'-*O*-methylphenyl)-5-(2",5"-dihydroxyphenyl)oxazole, 2-(2',5'-dihydroxyphenyl)-

5-(2”-hydroxyphenyl)oxazole, 4,11-di-*O*-galloylbergenin, brachangobinan A, and chrysosplenetin, we performed a phenotyping analysis of the non-treated and treated culture of *P. falciparum* 3D7. Parasites were twice synchronized using sorbitol and incubated with the concentration equal to IC_{80} of each compound at the ring stage. Using this concentration allowed us to observe both inhibition and progression of the parasite [31]. The parasite’s life cycles were disturbed from 48 h of incubation under compound stress compared to the negative control, observed by the delayed formation of ring-stage at 48 h of incubation, except for 4,11-di-*O*-galloylbergenin (Fig. 3A). Sign of parasitic stress was also observed from 48 h incubation, with no significant increasing invasion into RBC in the treated groups, observed from the parasitemia level (Fig. 3C). At 72 h under compound stress, the parasitemia in negative control increased to more than seven folds from the beginning of incubation, and in the active compounds varies from 2–3 folds. From 72 h of incubation, the incubation was continued either under compound stress (unwashed group) or compounds were washed away and replaced by fresh complete media (washed group). At this time point, the parasite life cycle was highly unsynchronized (Fig. 3B). Along with continuous stress from the active compounds, the suppression of the parasites also continued (Fig. 3D). Parasitemia in the unwashed control group at 144 h was not countable due to many parasites outside RBC and integrity loss of RBC due to long-term culture without media replacement. On the other hands, up to 144 h in the washed group, the inhibitions of parasites were reverse, and parasite re-grow in the absence of 2-(2'-hydroxy-5'-*O*-methylphenyl)-5-(2”,5”-dihydroxyphenyl)oxazole, 2-(2',5'-dihydroxyphenyl)-5-(2”-hydroxyphenyl)oxazole, 4,11-di-*O*-galloylbergenin, and chrysosplenetin, but unreversible in the absence of brachangobinan A (Fig. 3E). These results demonstrate that the active compounds effectively inhibit the parasite’s growth in long-term treatment, and only brachangobinan A showed unreversible parasite growth after 72 h treatment.

3.3. Effects of compounds on T. gondii

Of the 179 compounds tested against *T. gondii*, 22 (12.3%) compounds showed >70% inhibition at 20 μg/ml (Table S1). Of these, seven compounds showed low cytotoxicity (< 40% inhibition) at 20 μg/ml, and these compounds were chosen to determine their IC_{50}. Tricin showed the most potent activity with an IC_{50} of 12.94 μM and an SI of >23.40 (Table 2). The chemical structures of these compounds are presented in Fig 1.

3.4. Effects of compounds on HFF cells

The compound's cytotoxicity effect was tested on HFF cells by a cell viability test to determine therapeutic indices. The CC_{50} of 16 compounds that were efficient against *P. falciparum* or *T. gondii* in the preliminary screenings were assessed. The CC_{50} values of compounds were ranged from 9.97 μM to >302.76 μM. Among them, brachangobinan B showed the lowest CC_{50} value, 9.97 μM, and tricin showed the highest CC_{50} value, > 302.76 μM (Tables 1, 2).

4. **Discussion**

We screened a Mongolian compound library against two closely related parasites, *P. falciparum* and *T. gondii*. As a result, we identified five compounds with antimalarial activity, brachangobinan A, 2-(2'-hydroxy-5'-*O*-methylphenyl)-5-(2",5"-dihydroxyphenyl)oxazole, chrysosplenetin, 4,11-di-*O*-galloylbergenin, and 2-(2',5'-dihydroxyphenyl)-5-(2"-hydroxyphenyl)oxazole, and one compound, tricin, with anti-*Toxoplasma* activity.

In our study, three derivatives of 2,5-diphenyloxazole, 2-(2'-hydroxy-5'-*O*-methylphenyl)-5-(2",5"-dihydroxyphenyl)oxazole, 2-(2',5'-dihydroxyphenyl)-5-(2"-hydroxyphenyl)oxazole, and

2-phenyl-5-(2",5"-dihydroxyphenyl)oxazole isolated from the roots of *Oxytropis lanata* (collected in July 2012; a common plant) as novel compounds [13], showed potent antimalarial activity at IC_{50} of 3.58–6.94 μM. These compounds were reported to be effective against *Trypanosoma congolense*, a causative agent of nagana, and the hydroxy groups of 2,5-diphenyloxazole were determined to contribute to its trypanocidal activity [13]. Likewise, the inhibitory activity of the 2,5-diphenyloxazole derivatives against *P. falciparum* was increased depending on the hydroxy groups on the main skeleton (Table 1). In the absence of a hydroxy group, 2,5-diphenyloxazole did not show significant antimalarial activity (Table S1). Moreover, 2-(2',5'-dihydroxyphenyl)-5-(2”-hydroxyphenyl)oxazole showed some activity against *T. gondii* at an IC_{50} of 54.26 μM and SI of 1.47 (Table 2). The 2,5-diphenyloxazole compounds are not abundant in natural products [13]. Synthetic oxazole derivatives have been reported to have various biological activities, such as antibacterial, anticancer, antidepressant, anti-obesity, and analgesic [32].

4,11-Di-*O*-galloylbergenin isolated from *Bergenia crassifolia* roots (collected in September 2015; a rare plant) showed potent antimalarial activity with SI of 13.38 (Table 1), which seem to be caused by galloyl groups on bergenins. Galloyl bearing compounds were found abundant in plants [33] and some synthetic strategies of galloyl bearing compounds were also developed [34,35]. The absence of the galloyl group in bergenins showed insignificant growth inhibition against *P. falciparum* (Table S1). This compound’s ability to inhibit *Babesia* species was tested and moderate activities were observed against *Babesia bovis* and *B. bigemina* with IC_{50}s of 6.04 and 8.60 μg/ml, respectively [10]. Moreover, another galloyl-bergenin derivative, 11-*O*-gallyolbergenin, was showed antimalarial activity against *P. falciparum* D10 with an IC_{50} of 7.85 μM [36]. Some studies also documented that galloyl groups of compounds are beneficial to the antiprotozoal activities [10,37–39] and this was confirmed in this work.

We also identified new lignans, brachangobinan A and B, as potential antimalarial compounds with IC_{50} of 2.62 and 3.69 μM, respectively (Table 1). They were previously purified from the aerial parts of *Brachanthemum gobicum* (collected in September 2012; a rare plant) and known to inhibit *Trypanosoma congolense* [12]. Here, brachangobinan A with a CC_{50} of 73.14 μM was less toxic than brachangobinan B with a CC_{50} of 9.97 μM. Consequently, brachangobinan A showed the highest SI value of 27.91 and unreversible inhibition of the parasite's growth after 72 h treatment in this study (Figure 3). Other neolignans isolated from plants have demonstrated robust antiprotozoal activities against *Leishmania, Trypanosoma,* and *Plasmodium* [40].

Chrysosplenetin, tricin, and epiyangambin were isolated from the aerial parts of *Artemisia sieversiana* (collected in August 2017; a common plant) [14]. In this study, chrysosplenetin was active against *P. falciparum* with a SI value of 15.26 (Table 1). Wei *et al*. investigated the *in vivo* antimalarial efficacy of chrysosplenetin. They identified that combined treatment with artemisinin and chrysosplenetin at a ratio of 1:2 was effective against *P. berghei*, whereas chrysosplenetin alone did not. They concluded that chrysosplenetin had a partially synergetic effect on artemisinin [41]. Furthermore, in our study, epiyangambin and tricin displayed potent anti-*Toxoplasma* activities at an IC_{50} of 18.16 and 12.94 μM and an SI of 6.32 and >23.40, respectively (Table 2). Another study revealed that tricin had an anti-*Leishmania* activity against *Leishmania infantum* with an IC_{50} of 56 μM and an SI of > 7; tricin was noted for being less toxic on NCTC clone 929 mammalian cells [42]. Additionally, chrysosplenetin, tricin, and epiyangambin exhibited moderate inhibition of *Trypanosoma congolense* with an IC_{50} of 2.90–90.2 μM [14]. These results suggest that *Artemisia sieversiana* and its compounds may be an excellent natural source of antiprotozoal drugs.

The hit compounds identified in this study may be potential compounds that can develop

into novel drugs against malaria and toxoplasmosis. It will be interesting to further examine theirs *in vitro* and *in vivo* mechanisms. The present data also suggest that Mongolian plants are potential natural sources of bioactive compounds to treat protozoan diseases.

5. Conclusions

This study identified five new potent antimalarial agents, brachangobinan A, 2-(2'-hydroxy-5'-*O*-methylphenyl)-5-(2",5"-dihydroxyphenyl)oxazole, chrysosplenetin, 4,11-di-*O*-galloylbergenin, and 2-(2',5'-dihydroxyphenyl)-5-(2"-hydroxyphenyl)oxazole, and an anti-*Toxoplasma* agent, tricin. Further study is needed to validate their suitability as candidates for antimalarial and anti-*Toxoplasma* drug development.

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Declaration of Competing Interest

The authors declare that they have no financial or competing interests concerning this study.

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Figure legends

Fig. 1. Chemical structure of antimalarial and anti-*Toxoplasma* active compounds with SI >10. (A) Antimalarial active compounds; (B) Anti-*Toxoplasma* active compound.

Fig. 2. Antimalarial and anti-*Toxoplasma* properties of the active compounds. (A) The IC_{50} and 95% CI values of the active antimalarial compounds. (B) The IC_{50} and 95% CI values of tricin for anti-*Toxoplasma*.

Fig. 3. Representative of parasite's morphologies and parasitemia level under no treatment (control) and treatments of compounds. (A) Parasite's morphologies at 1, 24, 48, and 72 h incubation; (B) Parasite's morphologies at 96, 120, and 144 h incubation, under compounds stress (unwashed) and no compound stress (washed); (C) Parasitemia levels at 1, 24, 48, and 72 h incubation; (D) Parasitemia levels at 96, 120, and 144 h incubation under compounds stress (unwashed); (E) Parasitemia levels at 96, 120, and 144 h incubation under no compounds stress (washed). Parasitemia levels were presented as the mean of triplicates experiments, and the error bar represents standard deviations. (a) 2-(2'-hydroxy-5'-*O*-methylphenyl)-5-(2",5"-dihydroxyphenyl)oxazole; (b) 2-(2',5'-dihydroxyphenyl)-5-(2"-hydroxyphenyl)oxazole; (c) 4,11-di-*O*-galloylbergenin; (d) brachangobinan A; (e) chrysosplenetin.

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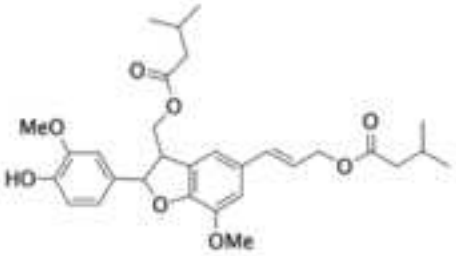
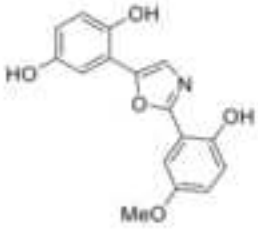
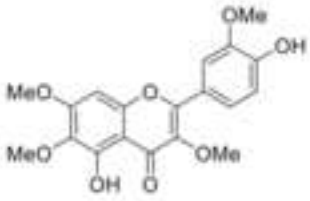
(A)



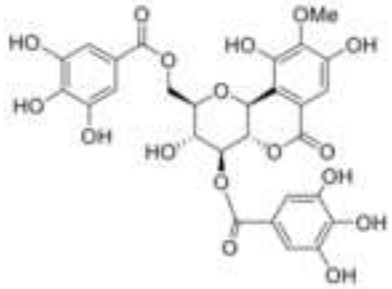
Brachangobinan A,
SI 27.91



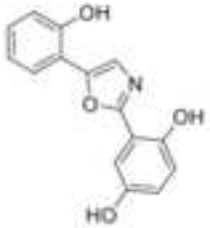
2-(2'-Hydroxy,5'-methoxyphenyl)-5-(2",5"-hydroxyphenyl)-oxazole,
SI 24.66



Chrysosplenetin,
SI 15.26

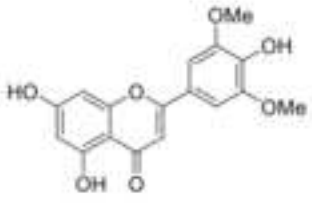


4,11-Di-O-galloylbergenin,
SI 13.38



2-(2',5'-Hydroxyphenyl)-5-(2"-hydroxyphenyl)-oxazole, SI 11.48

(B)



Tricin,
SI > 23.4

Figure 2

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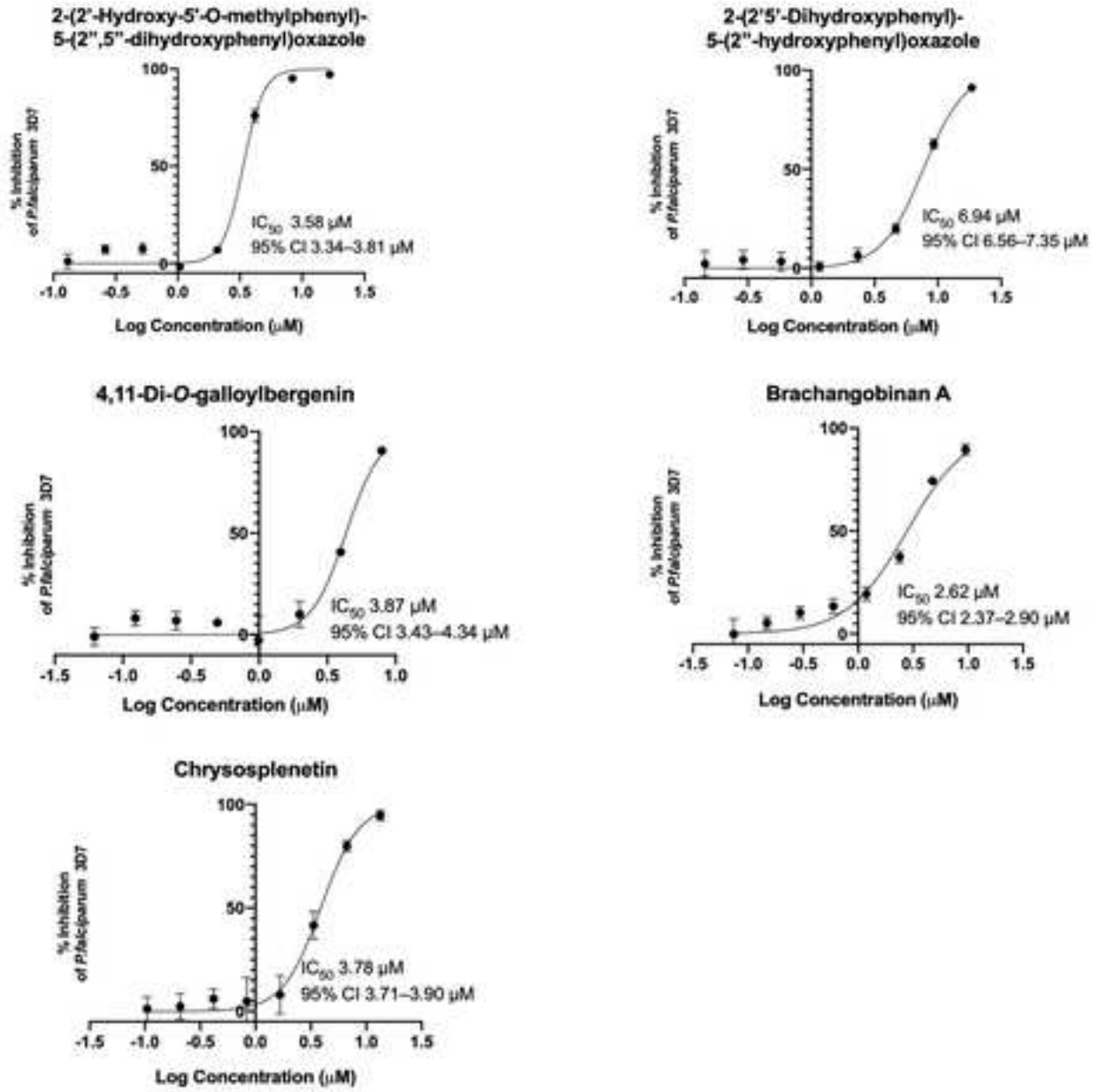
(A)



(B)

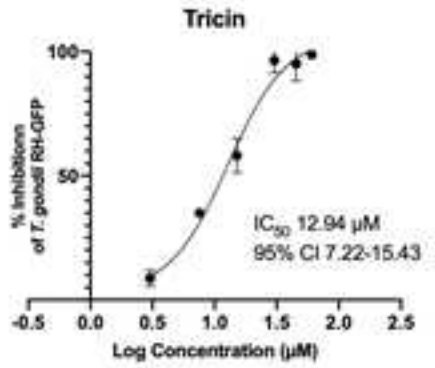


Figure 3

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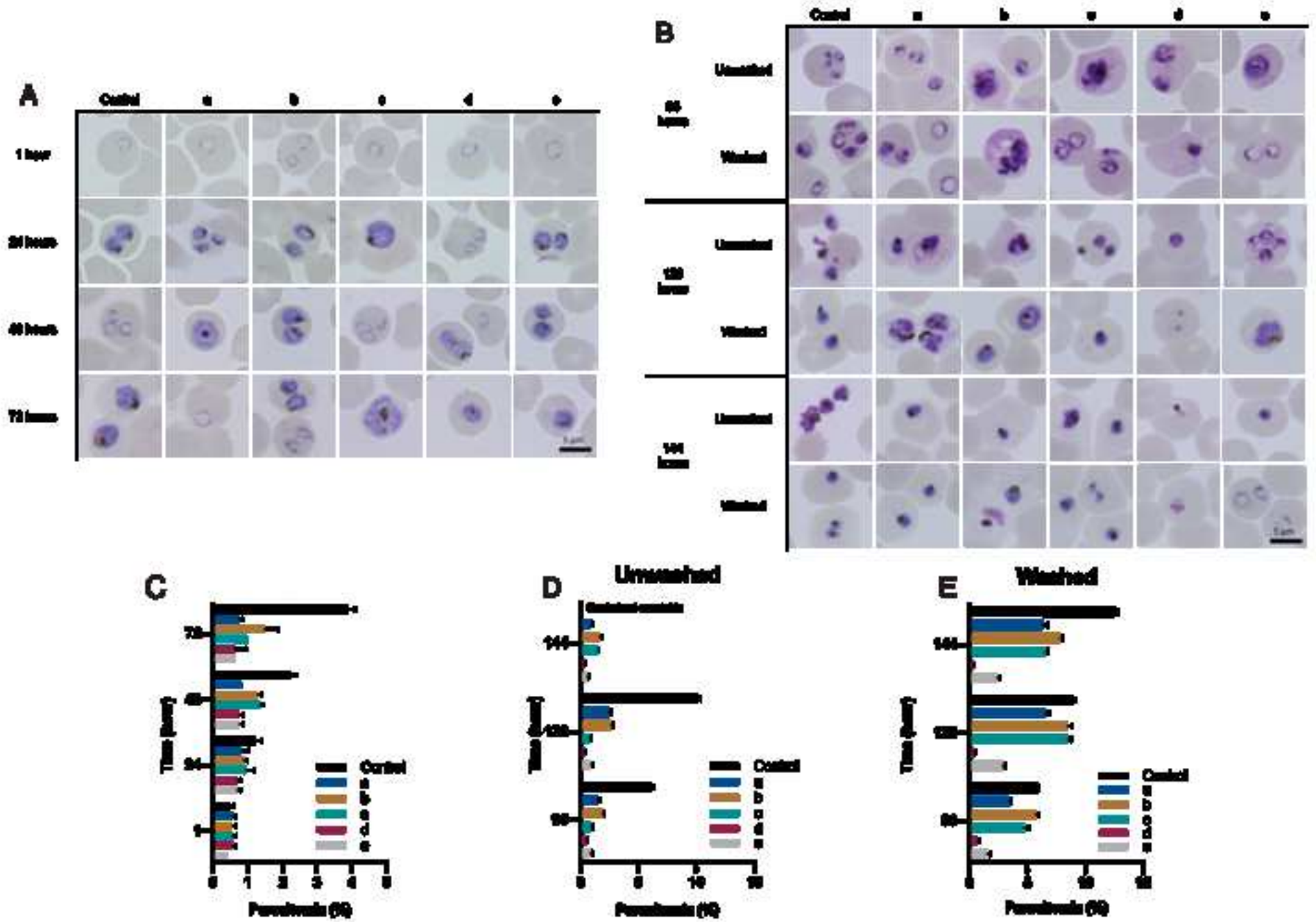

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Table 1. Antimalarial activity, red blood cell (RBC) hemolysis rate, and selective index of the hit compounds

No	Compound name	Plant origin	Anti-*Plasmodium*		RBC hemolysis rate	Cytotoxicity	SI
			IC_{50}, µM	95% CI, µM	% at 100 µM	CC_{50}, µM	
1	2-(2'-Hydroxy-5'-*O*-methylphenyl)-5-(2",5"-dihydroxyphenyl)oxazole	*Oxytropis lanata*	3.58	3.34 - 3.81	0.69	88.17	24.66
2	2-(2',5'-Dihydroxyphenyl)-5-(2"-hydroxyphenyl)oxazole	*Oxytropis lanata*	6.94	6.56 - 7.35	0.94	79.71	11.48
3	2-Phenyl-5-(2",5"-dihydroxyphenyl)oxazole	*Oxytropis lanata*	6.77	6.42 - 7.13	0.94	59.70	8.82
4	4,11-Di-*O*-galloylbergenin	*Bergenia crassifolia*	3.87	3.43 - 4.34	1.24	51.82	13.38
5	Brachangobinan A	*Brachanthemum gobicum*	2.62	2.37 - 2.90	4.60	73.14	27.91
6	Brachangobinan B	*Brachanthemum gobicum*	3.69	3.42 - 3.99	0.88	9.97	2.70
7	Chrysosplenetin	*Artemisia sieversiana*	3.78	3.71 - 3.90	1.17	57.69	15.26
Reference	Chloroquine		0.0439	0.0417 – 0.0463	0.27	38.6*	879

*Data from Leesombun et al [22]. To determine the half-maximal inhibitory concentration (IC_{50}) values, eight concentrations (two-fold dilution) ranging from 5– 0.039 µg/ml were used. To determine the 50% cytotoxic concentration (CC_{50}) values, eight concentrations (two-fold dilution) ranging from 100–0.78 µg/ml were used. The IC_{50} and 95% confidence interval (95% CI) of each compound were calculated using nonlinear regression on GraphPad Prism 8 software (GraphPad Software, Inc. La Jolla, CA, USA). The selectivity index (SI) value of each compound was calculated by dividing the CC_{50} value by the IC_{50} value.

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Table 2. Anti-*Toxoplasma* activity and selective index of the hit compounds

No.	Compound name	Plant origin	Anti-*Toxoplasma*		Cytotoxicity	SI
			IC_{50}, µM	95% CI, µM	CC_{50}, µM	
1	2-(2',5'-Dihydroxyphenyl)-5-(2''-hydroxyphenyl)oxazole	*Oxytropis lanata*	54.26	44.7 – 60.39	79.71	1.47
2	3-Acetoxymethyl-5-[(*E*)-2-formylethen-1-yl]-2-(4-hydroxy-3-methoxyphenyl)-7-methoxy-2,3-dihydrobenzofuran	*Brachanthemum gobicum*	24.61	19.62 – 30.91	71.20	2.89
3	6-(Non-8-enyl) salicylic acid	*Comarum Salesovianum*	48.60	44.39 – 54.08	144.92	2.98
4	6-Nonyl salicylic acid	*Comarum Salesovianum*	37.89	33.39 – 42.62	169.19	4.47
5	Epiyangambin	*Artemisia sieversiana*	18.16	12.68 – 24.50	114.76	6.32
6	Kaempferol	*Pulsatilla flarescens*	24.61	18.98 – 31.20	177.57	7.21
7	Tricin	*Artemisia sieversiana*	12.94	7.22 - 15.43	>302.76	>23.40
Reference	Sulfadiazine		98.91	90.01 – 108.23	>1000*	>10.11

*Data from Leesombum et al [25]. To determine the half-maximal inhibitory concentration (IC_{50}) values, six different concentrations, 1, 2.5, 5, 10, 15, and 20 µg/ml, were used. To determine the 50% cytotoxic concentration (CC_{50}) values, a two-fold serially diluted compounds ranging from 3.13 to 100 µg/ml was used. The IC_{50} and CI_{95} of each compound were calculated by nonlinear regression on GraphPad Prism 8 software. The selectivity index (SI) value of each compound was calculated by dividing the CC_{50} value by the IC_{50} value.

Table S1

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Data in Brief
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