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Access to Artemisinin–Triazole Antimalarials *via* Organo-Click Reaction: High *In Vitro*/*In Vivo* Activity against Multi-Drug-Resistant Malaria Parasites

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Cite This: *JACS Au* 2024, 4, 951–957
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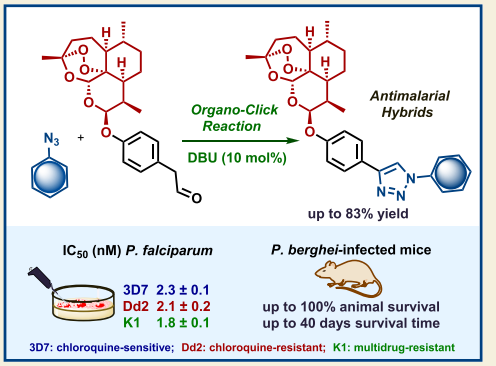
ABSTRACT: Malaria is one of the most widespread diseases worldwide. Besides a growing number of people potentially threatened by malaria, the consistent emergence of resistance against established antimalarial pharmaceuticals leads to an urge toward new antimalarial drugs. Hybridization of two chemically diverse compounds into a new bioactive product is a successful concept to improve the properties of a hybrid drug relative to the parent compounds and also to overcome multidrug resistance. 1,2,3-Triazoles are a significant pharmacophore system among nitrogen-containing heterocycles with various applications, such as antiviral, antimalarial, antibacterial, and anticancer agents. Several marketed drugs possess these versatile moieties, which are used in a wide range of medical indications. While the synthesis of hybrid compounds containing a 1,2,3-triazole unit was described using Cu- and Ru-catalyzed azide–alkyne cycloaddition, an alternative metal-free pathway has never been reported for the synthesis of antimalarial hybrids. However, a metal-free pathway is a green method that allows toxic and expensive metals to be replaced with an organocatalyst. Herein, we present the synthesis of new artemisinin–triazole antimalarial hybrids *via* a facile Ramachary-Bressy-Wang organocatalyzed azide-carbonyl [3 + 2] cycloaddition (organo-click) reaction. The prepared new hybrid compounds are highly potent *in vitro* against chloroquine (CQ)-resistant and multi-drug-resistant *Plasmodium falciparum* strains (IC_{50} (Dd2) down to 2.1 nM; IC_{50} (K1) down to 1.8 nM) compared to CQ (IC_{50} (Dd2) = 165.3 nM; IC_{50} (K1) = 302.8 nM). Moreover, the most potent hybrid drug was more efficacious in suppressing parasitemia and extending animal survival in *Plasmodium berghei*-infected mice (up to 100% animal survival and up to 40 days of survival time) than the reference drug artemisinin, illustrating the potential of the hybridization concept as an alternative and powerful drug-discovery approach.



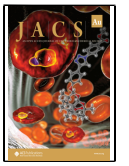
KEYWORDS: *multi-drug-resistant malaria, artemisinin–triazole hybrids, organo-click reaction, in vitro/in vivo studies*

INTRODUCTION

Malaria is still a major threat to the health of a big part of the world's population, afflicting 1 billion people in 84 countries, with nearly 247 million cases leading to 619,000 deaths in 2021.[1] The current frontline treatments utilized against malaria are based on artemisinin, a naturally occurring endoperoxide-bearing sesquiterpene lactone, and its semisynthetic derivatives like dihydroartemisinin (DHA). A growing number of people threatened by malaria due to the spread of parasite-carrying mosquitos facilitated by global warming can be observed, aggravating the need for new antimalarial drugs.[2] To slow the spread of drug resistance against known drug compounds, the WHO discourages the use of these drugs for monotherapy, and nowadays, mostly artemisinin-based combination therapy (ACT), combining an artemisinin derivative with a second drug, is used in clinical practice (Figure 1A).[1,3−6] A vastly proven concept to further exploit the advantages of using different drugs in parallel is known as hybridization.[7−9] A hybrid drug merges two biologically active pharmacophores by covalent bonding *via*, *e.g.*, copper(I)-catalyzed azide–alkyne cycloaddition (CuAAC) click reactions (Figure 1B)[10,11] with the potential to form highly active species able to overcome drug resistance. Although recently we reported access to new artemisinin-based antiviral compounds using an organo-click reaction,[12] to the best of our knowledge, an organo-click reaction has never been used before to synthesize antimalarial drug compounds.

Herein, we report the straightforward synthesis of 13 artemisinin–triazole antimalarials **1**–**13** *via* an organo-click

Received: November 16, 2023
Revised: January 9, 2024
Accepted: January 23, 2024
Published: February 20, 2024



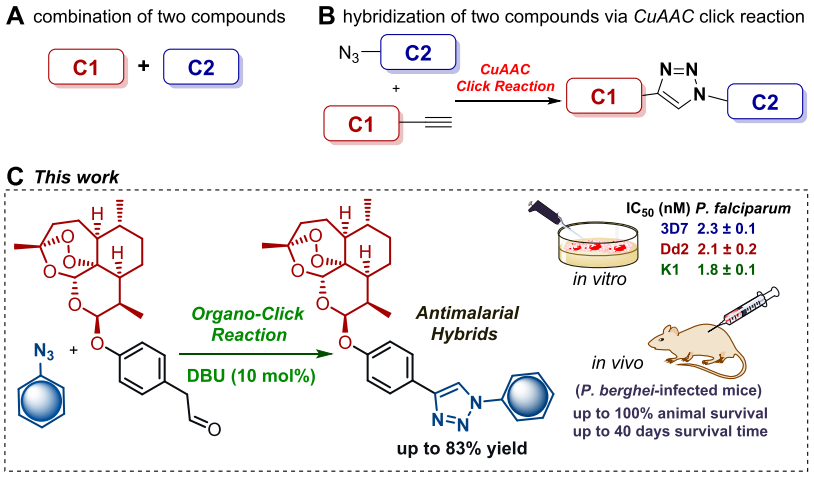


Figure 1. (A) Combination therapeutic approach for malaria treatment. (B) Schematic representation of antimalarial hybrid drugs accessible *via* a Cu(I)-catalyzed click reaction. C1 = compound 1; C2 = compound 2. (C) New efficient artemisinin−triazole antimalarial hybrids for *in vitro* and *in vivo* studies *via* organo-click reaction (this work).

reaction utilizing a Ramachary-Bressy-Wang organocatalyzed azide-carbonyl [3 + 2] cycloaddition from two readily available precursors (Figure 2A and Scheme 1). The library of compounds was complemented by our five recently reported antiviral artemisinin−triazole hybrids with different benzimidazoles as substituents on the triazole subunit **14**−**18** (Figure 2B).[12] In this work, we investigated the activity of compounds **1**−**18** against malaria *in vitro* and partly *in vivo* for the first time.

RESULTS AND DISCUSSION

Synthesis of New Artemisinin−Triazole Hybrids *via* Organo-Click Reaction

Starting from DHA, artemisinin-based aryl acetaldehyde **19** was synthesized *via* a Mitsunobu etherification reaction, utilizing PPh_3 and diisopropyl azodicarboxylate (DIAD)[13,14] as reagents, and was used as a substrate in the organo-click reaction. The second set of precursors includes readily available aromatic azides differently functionalized at the aryl ring, prepared *via* known procedures from commercially available aromatic amines, using $NaNO_2$ and NaN_3, or from aryl chlorides using NaN_3 (see the Supporting Information). Subsequently, the azide and aldehyde precursors were combined *via* a Ramachary-Bressy-Wang organocatalyzed azide-carbonyl [3 + 2] cycloaddition (organo-click) reaction to form a 1,2,3-triazole ring, which is a major pharmacophore system among nitrogen-containing heterocycles.[15−27] 1,2,3-Triazoles have been found to have a broad spectrum of applications, such as antiviral, antituberculosis, antibacterial, anticancer, antimalarial agents, and more.[28] In this work, we planned to vary the substituents on the 1,2,3-triazole pharmacophore to achieve the chemical diversity of artemisinin−triazole antimalarial hybrid compounds for structure−activity relationship studies (see compounds **1**−**18**, Figure 2). The reaction catalyzed by the base organocatalyst 1,8-diazabicyclo(5.4.0)undec-7-ene (DBU) (10 mol %) has been carried out in dimethyl sulfoxide (DMSO) at 50 °C for up to 24 h (Scheme 1). The reaction leads to the corresponding products in diverse yields ranging from 24 to 83%, significantly depending on the used aryl-azide substrate.

An important feature is that the organo-click synthetic approach allows easy formation of triazole derivatives with different moieties, *e.g.*, oligoethylene glycol and morpholine (see, *e.g.*, compounds **11**, **12**, **16**, **17**), and even with an alkyne tag (see **18**), suitable for, *e.g.*, bioorthogonal click chemistry and/or proteomics experiments in live cells. In line with this, this synthetic approach was highly valuable for preparing proteolysis targeting chimeras (PROTAC) motifs.[12]

Aromatic azides with electron-withdrawing groups (EWGs) in *para* position lead, in accordance with the literature,[17] to elevated yields of up to 83% (see compounds **3** (83%), **5** (77%), **9** (78%), and **10** (77%)). Functionalization in the *para* position improves yields compared to *ortho* analogues (**3** compared to **4** or **5** compared to **6**). Since this effect was

Table 1. IC_{50} Values for Hybrids 1−18, Chloroquine (CQ), and Artemisinin (ART) Tested against *P. falciparum* Parasite Strains 3D7, Dd2, and K1

	3D7[a]	Dd2[a]	K1[a]
ART	26.8 ± 2.4[b]	11.3 ± 1.8[b]	5.4 ± 0.5[b]
CQ	12.7 ± 2.5[b]	165.3 ± 16[b]	302.8 ± 15.1[b]
1	17.4 ± 0.4	17.1 ± 0.8	14.2 ± 1.5
2	2.5 ± 0.2	2.4 ± 0.1	1.8 ± 0.6
3	3.4 ± 0.3	3.5 ± 0.2	4.2 ± 0.2
4	3.0 ± 0.1	2.1 ± 0.2	2.6 ± 0.3
5	11.1 ± 1.0	10.7 ± 1.1	10.7 ± 1.2
6	4.3 ± 0.2	6.0 ± 0.5	3.8 ± 0.4
7	2.3 ± 0.1	2.4 ± 0.7	3.0 ± 0.2
8	2.7 ± 0.2	2.9 ± 0.3	3.0 ± 0.4
9	4.1 ± 0.5	3.4 ± 0.3	3.0 ± 0.3
10	3.5 ± 0.4	3.8 ± 0.3	3.8 ± 0.3
11	3.0 ± 0.2	3.2 ± 0.2	3.5 ± 0.3
12	2.3 ± 0.2	2.3 ± 0.1	2.8 ± 0.3
13	4.3 ± 0.2	4.3 ± 0.1	4.3 ± 0.2
14	11.2 ± 0.2	8.5 ± 1	7.3 ± 0.1
15	7.3 ± 0.1	6.5 ± 0.5	5.1 ± 0.2
16	3.0 ± 0.2	3.0 ± 0.3	1.8 ± 0.1
17	14.4 ± 0.8	15.1 ± 0.4	10.7 ± 0.7
18	20.1 ± 3.1	25.5 ± 2.0	12.6 ± 1.0

[a]*In vitro* antiparasitic activity was determined in asexual blood stages of *P. falciparum* after 72 h of incubation. IC_{50} values are the mean ± SEM (in nM). [b]IC_{50} value has been previously reported.[10,33] **3D7**: chloroquine-sensitive strain; **Dd2**: chloroquine-resistant strain; **K1**: multi-drug-resistant strain.

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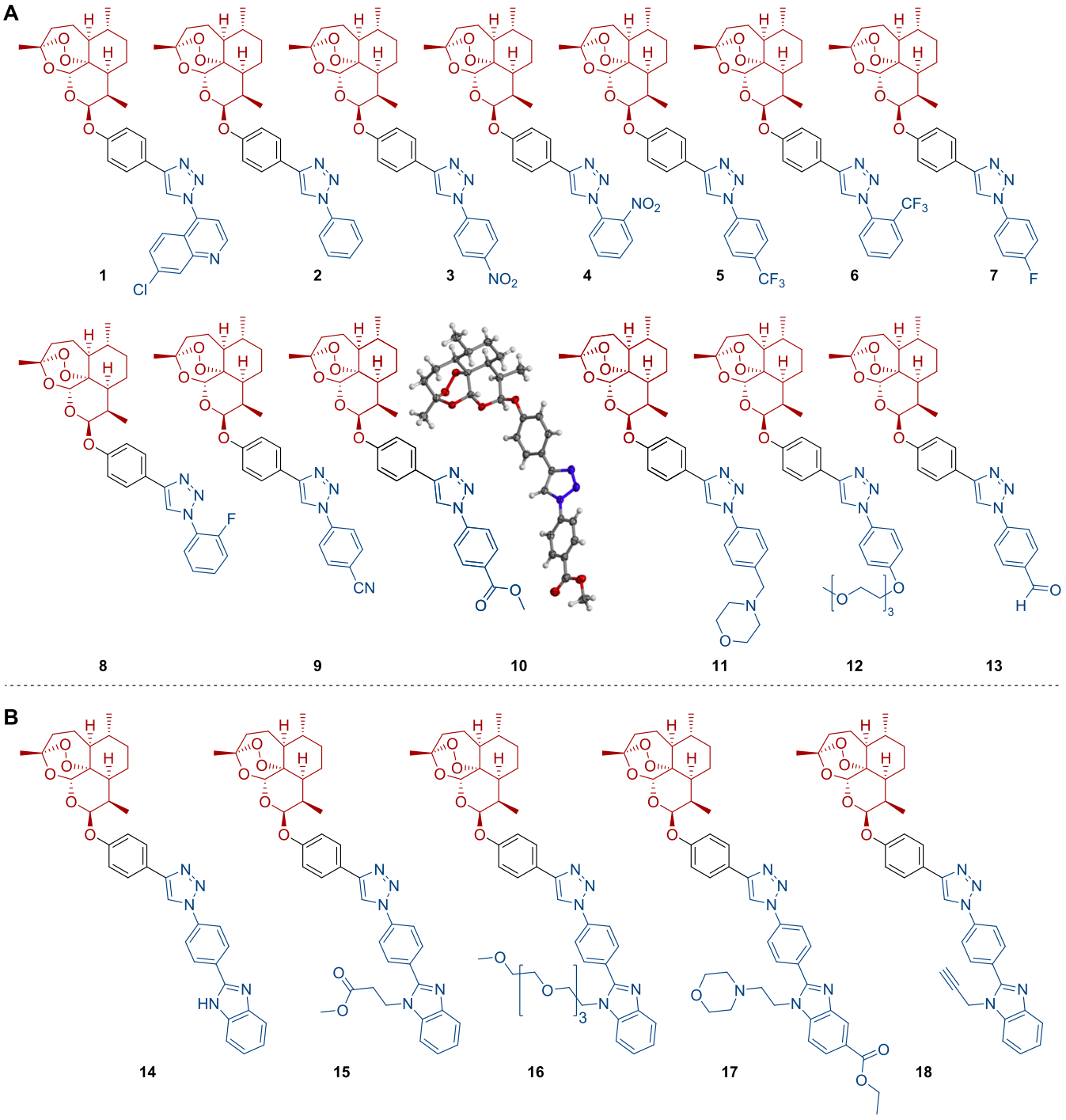

Figure 2. (A) Overview of new artemisinin−triazole hybrids **1**−**13** and X-ray structure of compound **10**. (B) Overview of five artemisinin−triazole hybrids **14**−**18**, previously studied against viruses.[12]

previously not observed in the literature for the organo-click reaction with simple phenylacetaldehyde, steric hindrance induced by the bulkiness of the artemisinin unit seems to play a considerable role in the reduced yields of *ortho* derivatives. Implementing an electron-donating group (EDG) in the *para* position of the aromatic azide decreases the yield (see, *e.g.*, product **12** (24% yield)), which is in accordance with the literature.[17] The C-10β configuration in all hybrid compounds with C-10β-DHA units was determined by ^{1}H NMR and verified vicariously for compound **10** by X-ray crystal structure analysis (Figure 2A).[29]

In Vitro Antimalarial Activity of Hybrids 1−18

The compounds **1**−**18** were tested *in vitro* against *Plasmodium falciparum*, and the IC_{50} values against parasite growth (SYBR green assay)[30] are given in Table 1. To gain a broader overview of antimalarial activity, three strains with different resistance profiles were tested: the chloroquine-sensitive 3D7 strain and the two multi-drug-resistant strains Dd2 and K1.[31,32] All new 13 artemisinin−triazole hybrids exhibit high antimalarial activity with IC_{50} values down to 2.3 nM (for **7**/**12**) against 3D7 as well as down to 2.1 nM (for **4**) and 1.8 nM (for **2**) for the multi-drug-resistant Dd2 and K1 strains, mostly out-

https://doi.org/10.1021/jacsau.3c00716
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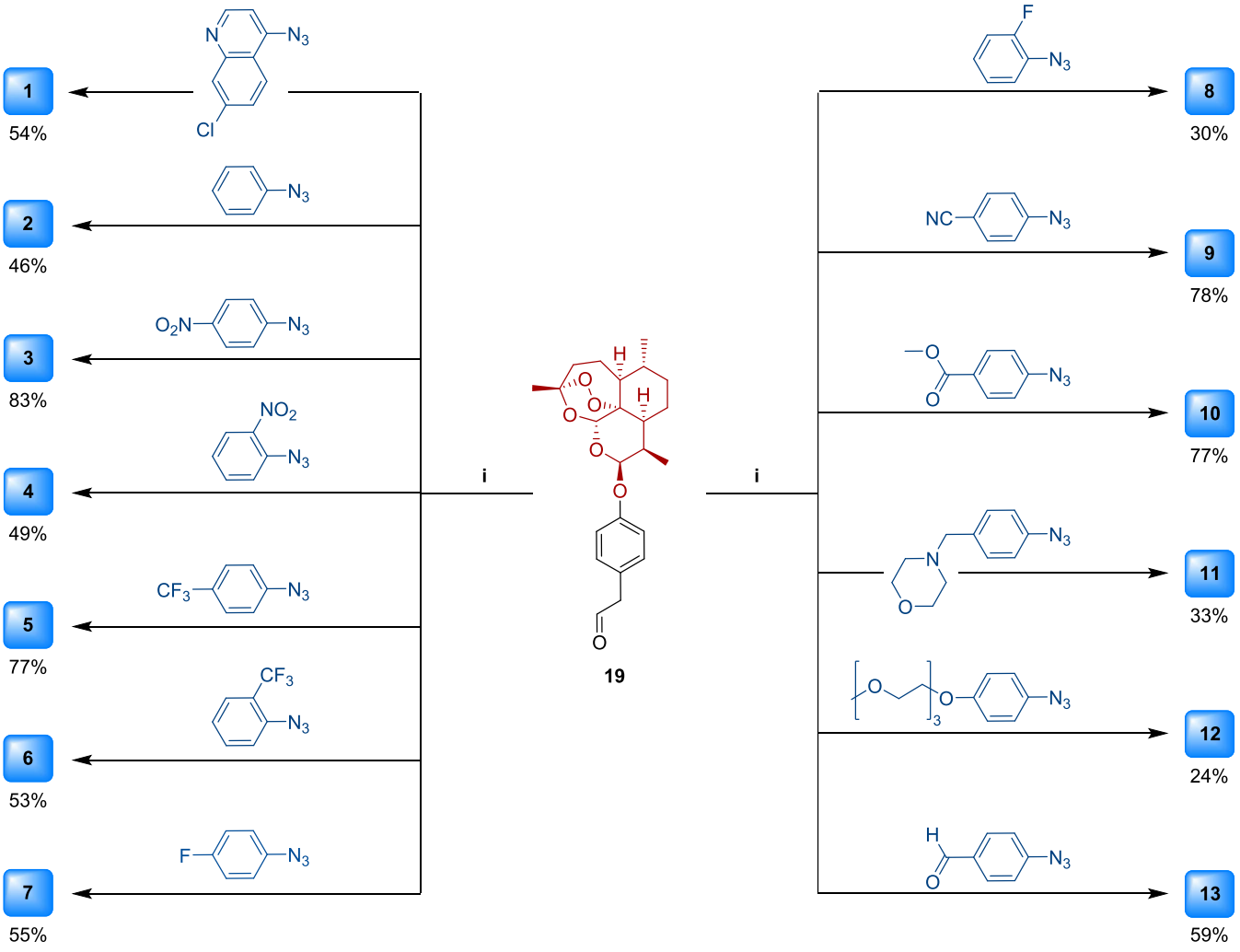
Scheme 1. Synthesis of the 13 Artemisinin−Triazole Hybrids 1−13; Reaction Conditions: (i) DBU (10 mol %), DMSO, 2−24 h, 50 °C (Reaction Times: Supporting Information)



performing the activity of established drugs artemisinin and chloroquine. All five previously reported antiviral benzimidazole-containing artemisinin−triazole hybrids **14**−**18**[12] were also active against *P. falciparum*. They reduced the parasite proliferation against the three parasite strains in a low nM range (down to 1.8 nM, Table 1).

Structure−activity relationship annotation for the series of artemisinin−triazole hybrids **1**−**13**, with different heteroaryl- and aryl-substituents on the triazole subunit, revealed a substituent effect regarding their antimalarial activity. While all *ortho*- and *para*-substituted aryl groups on triazole (see compounds **3**−**13**) are generally well tolerated, the best *in vitro* activity against CQ-resistant (Dd2) and multi-drug-resistant (K1) strains was obtained, interestingly, with phenyl-substituted triazole **2** (Table 1). Surprisingly, the 7-chloroquinoline moiety (which is a subunit of the antimalarial drug CQ) on the triazole ring reduced the antimalarial potency compared to the phenyl moiety (see compounds **1**, **2**, and Table 1).

As already mentioned above, benzimidazole-containing hybrids **14**−**18** demonstrated potent *in vitro* antimalarial activity and a selectivity index. Among the hybrid drugs, the tetraethylene glycol-functionalized hybrid **16** exhibited the highest activity against all three strains (IC_{50} (3D7) = 3.0 ± 0.2 nM; Dd2:3.0 ± 0.3 nM; K1:1.8 ± 0.1 nM), outperforming established drugs up to 4-fold against mammalian cells displayed.

In Vivo Antimalarial Efficacy of Selected Hybrid 16

While the *in vitro* activities of compound **2** (IC_{50} (K1) = 1.8 ± 0.6 nM) and compound **16** (IC_{50} (K1) = 1.8 ± 0.1 nM) were similar, we selected **16** for subsequent *in vivo* studies because it features improved drug-like properties such log *D*, log *P*, and solubility (data not shown). In addition, the intrinsic fluorescence of **16** is advantageous for a potential follow-up investigation of its mode of action, *e.g.*, *via* fluorescence imaging in living *P. falciparum*-infected red blood cells.[11,12]

Compound **16** and artemisinin displayed lower cytotoxicity for mammalian cells than the cytotoxic drug of the reference gentian violet. The selectivity index (S.I.) of compound **16** was higher than that of artemisinin (S.I. 55,555 for **16** vs S.I. 37,037 for artemisinin, see Table S1 in the Supporting Information). This shows a potency enhancement of the antiplasmodial activity for compound **16** in comparison to artemisinin, which is accomplished without an increase in mammalian toxicity (Table S1, Supporting Information). Furthermore, *in vivo* experiments using *Plasmodium berghei*-infected mice demonstrated that compound **16**, when given subcutaneously at a dose of 21.6 μmol/kg, resulted in complete

https://doi.org/10.1021/jacsau.3c00716
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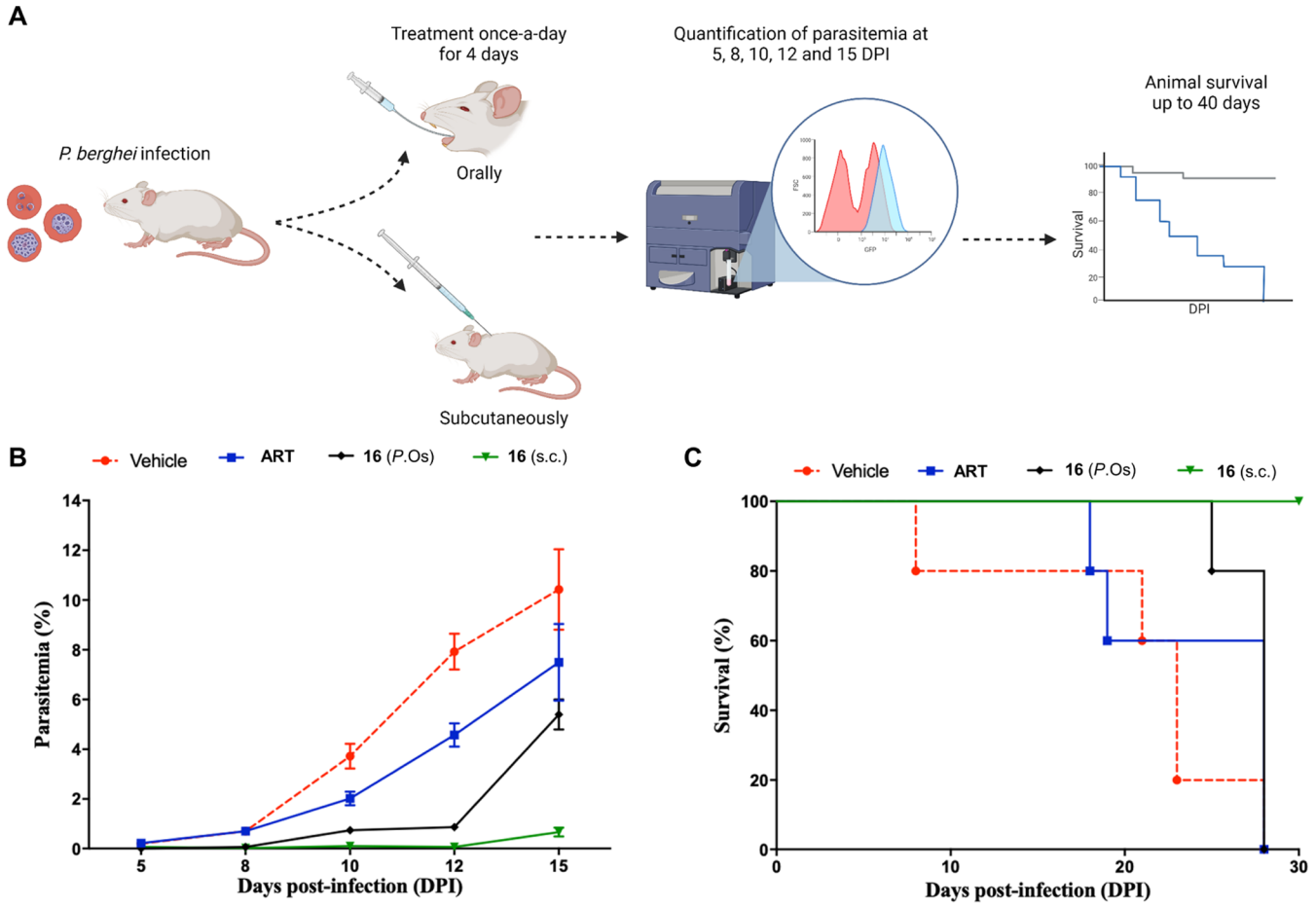

Figure 3. Efficacy of benzimidazole-containing artemisinin−triazole hybrid **16** and artemisinin (ART) in the experimental model of malaria. (A) Display of the experimental design. (B) Parasitemia in *P. berghei*-infected mice (n = 5 per group) (values are the mean and the standard deviation). (C) Animal survival in *P. berghei*-infected mice (n = 5 per group). ART was administered subcutaneously (S.C.). Drug dosages are shown in Table S2 (Supporting Information). Indicated route of administration: subcutaneously (S.C.) or orally by gavage (P.Os.). DPI = days postinfection.

parasite clearance in the Peters' standard 4-day suppressive test. Its efficacy to cure was twice as high as with artemisinin at the same dosage and route of administration (Figure 3 and Table S2, Supporting Information). Next, we evaluated the drug treatment given orally, and results showed that compound **16** could reduce parasitemia and prolong the median of animal survival, while artemisinin reduced parasitemia but not enough to increase the median of animal survival. The efficacy of compound **16**, which has surpassed artemisinin's efficacy, represents an improvement in terms of efficacy in comparison to previously related artemisinin-based hybrid drugs.[10,11,33] Moreover, it is anticipated that compound **16** may have an appropriate drug metabolism and pharmacokinetic profile, and further investigations of this are the subject of an ongoing study.

To sum up, we demonstrated that the chemical implantation of highly functionalized phenyl triazole groups, achieved by a straightforward and metal-free synthetic approach, helps to manipulate artemisinins' pharmacological activity.

This resulted in the discovery of compounds of enhanced *in vitro* antimalarial potency and *in vivo* efficacy compared to ART. These observations align with the fact that changes in the skeleton and implantation of chemical motifs surrounding the trioxane warhead can modulate the pharmacological activity of ARTs.[34−36] More specifically, these structural changes in the endoperoxide drugs can directly impact the speed of heme-mediated peroxide activation and, subsequently, in the protein alkylation profile.[37−39]

In line with previous findings,[12] our synthetic program and annotation of structure−activity relationships disclosed here are beneficial for boosting the ARTs chemotherapy not only in malaria but also in emerging applications, such as in oncology,[40] virology,[41] among others.[42]

CONCLUSIONS

We presented the synthesis of a library of 13 novel artemisinin−triazole hybrid compounds **1−13** *via* facile organo-click reaction in good yields (up to 83%) from readily available substrates. These novel compounds and five previously reported benzimidazole-containing artemisinin−triazole antiviral hybrids **14−18** were evaluated against *P. falciparum* strains for the first time. Remarkably, all hybrid compounds exhibited excellent antimalarial activity *in vitro* against CQ-resistant and multi-drug-resistant *P. falciparum* strains (IC_{50} (Dd2) down to 2.1 nM; IC_{50} (K1) down to 1.8 nM) compared to the reference drug CQ (IC_{50} (Dd2) 165.3 nM; IC_{50} (K1) 302.8 nM). The results demonstrated the high potential of artemisinin−triazole hybrid drugs to overcome drug resistance. Selected highly active hybrid **16** (IC_{50} (K1) = 1.8 ± 0.1 nM), whose selectivity index (S.I. 55,555) is higher than that of parent artemisinin (S.I. 37,037), was additionally proven to be efficacious *in vivo* in reducing parasitemia and

https://doi.org/10.1021/jacsau.3c00716
JACS Au 2024, 4, 951−957

prolonging animal survival to up to 100% with up to 40 days of survival time. Further study is necessary to profile the activity of artemisinin−triazole hybrids in multiple parasite stages of the *Plasmodium* life cycle.

■ ASSOCIATED CONTENT

Data Availability Statement

All data supporting the findings of this study are available within the article and its Supporting Information file.

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/jacsau.3c00716.

> Materials and methods; synthetic procedures; product characterizations; ^{1}H and ^{13}C NMR spectra for all new compounds; methods of antiplasmodial activity assessment *in vitro* and *in vivo*, pharmacological data; cytotoxicity in mammalian cells and selectivity index of compounds; X-ray crystallographic data, and references (PDF)

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Author Contributions

L.H. and B.W.G. carried out all synthetic work and conducted the click reactions. M.L. performed the *in vitro* studies under the supervision of O.F. and B.K.; H.C.Q. performed the *in vivo* studies under the supervision of D.R.M.M.; F.H. carried out X-ray structure analysis of compound **10**; and D.R.M.M., B.K., and S.B.T. designed and supervised the experimental outline of the corresponding studies. L.H., D.R.M.M., B.K., and S.B.T. wrote the manuscript with input from all authors. S.B.T. conceived and directed the research. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

S.B.T. thanks Deutsche Forschungsgemeinschaft (DFG) for funding (grant TS87/28-1). The authors also thank the Interdisciplinary Center for Molecular Materials (ICMM) and the Graduate School of Molecular Science (GSMS) for research support. H.C.Q. was supported through a CAPES doctoral scholarship (Finance code 001, Brazil). D.R.M.M. acknowledges CNPq (grant number. 305732/2019-6, Brazil), FAPESB (grant number APP0088/2016, Brazil), and Fiocruz/Proep (grant number IGM-002-FIO-20-2-25, Brazil).

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