

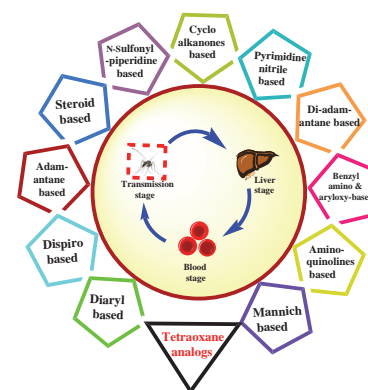
Endoperoxides as Antimalarials: Development, Structural Diversity, and Pharmacodynamic Aspects of 1,2,4,5-Tetraoxane-Based Structural Scaffolds

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Abstract Malaria poses a serious threat to human life and is prevalent in tropical and subtropical areas across the globe. Drugs such as quinine, chloroquine (a synthetic version of quinine), artemisinin, and its derivative compounds have been used to treat malaria. Developing highly effective chemical scaffolds with minimal toxicity is necessary because malarial parasites have become resistant to existing drugs. In this context, 1,2,4,5-tetraoxanes have emerged as a crucial framework with notable antimalarial properties. To improve the effectiveness and combat resistance to various antimalarial drugs, 1,2,4,5-tetraoxanes have been combined with a variety of alicyclic, aryl, heteroaryl, and spiro groups including steroid-based, aminoquinoline-based, dispiro-based, triazine-based, diaryl-based, and piperidine-based 1,2,4,5-tetraoxanes. We provide an overview of the synthesis and most important *in vitro* and *in vivo* investigations carried out on hybrids based on 1,2,4,5-tetraoxane as antimalarial drugs. The future development of malaria treatment may be influenced by the structural changes in different hybrids of 1,2,4,5-tetraoxane.

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Key words artemisinin, endoperoxide, cerebral malaria, sesquiterpene, central nervous system (CNS), 1,2,4,5-tetroxane, 1,2,4,5-tetraoxane

1 Introduction

Malaria poses a serious threat to human life and is prevalent in tropical and subtropical areas across the globe.¹ Drugs such as quinine,² chloroquine (a synthetic version of quinine), artemisinin, and its derivative compounds have been used to treat malaria. Developing highly effective chemical scaffolds with minimal toxicity is necessary because malarial parasites have become resistant to existing drugs. In this context, 1,2,4,5-tetraoxanes have emerged as a crucial framework with notable antimalarial properties.³ To improve effectiveness and combat resistance to various antimalarial drugs, 1,2,4,5-tetraoxanes have been combined with a variety of alicyclic, aryl, heteroaryl, and spiro groups including steroid-based, aminoquinoline-based, dispiro-based, triazine-based,⁴ diaryl-based, and piperidine-based 1,2,4,5-tetraoxanes. The objective of this review is to provide pharmacists and organic/medical chemists with a current comprehension of the science behind 1,2,4,5-tetraoxane compounds.

Malaria, a lethal infectious disease, has been around for thousands of years. In humans, this disease is transmitted by female *Anopheles* mosquitoes infected with the *Plasmodium* parasite. Rarely, it can also be passed through congenital transmission and contact with infected blood products (transfusion malaria).⁵ According to the World Malaria Report 2023 released by WHO, there were around 608,000 deaths and 249 million new cases of malaria documented globally in 2022.⁶ In humans, malaria is caused by five well-recognized species of *Plasmodium*: *Plasmodium falciparum*, *Plasmodium malariae*, *Plasmodium vivax*, *Plasmodium knowlesi*, and *Plasmodium ovale*. Of these, *P. falciparum* is the most prevalent and lethal species because it infects all types of red blood cells (RBCs) found at different stages of development (from immature young to old RBCs), causing severe types of malaria-like cerebral malaria. Pregnant

women and children under the age of five are the main victims of malaria fatalities, it causes the majority of global malaria deaths, resulting in a mortality rate of 20% to 50% when left untreated.^{6–13} *P. vivax* which is less fatal and found frequently in Central America, India, and some parts of the Eastern Mediterranean, while *P. falciparum* species are found in South and East Asia, South America, the Caribbean, the Middle East, and Africa.^{8,9,11} *P. ovale* and *P. malariae* are the two less prevalent and nonlethal species commonly found in Africa and Papua New Guinea.^{6–13} However, *P. knowlesi* has been proven to cause a type of malaria similar to monkey malaria in specific regions of Southeast Asia.¹⁴ Quinine (QN)^{15,16} and chloroquine (CQ)^{17,18} both quinoline α -acids from the cinchona plant, were first used to treat illnesses caused by *P. falciparum* infections.^{2,19–28} However, their use was stopped due to the development of resistance towards them.^{29–31} At the beginning of 1969, Youyou Tu was designated by the Chinese government as

the lead scientist for a drug development initiative called Project 523. In her role as the head scientist of Project 523, she traveled extensively throughout China, researching historical documents and 380 plant extracts that were being used to treat mice and monkeys. She discovered that *Artemisia annua* significantly decreased the *P. falciparum* parasite in these animals. Based on her findings, *Artemisia annua* successfully inhibited *P. falciparum* parasite in these animals. In 2015, the Nobel Prize in Physiology or Medicine was awarded for her discovery of artemisinin and dihydroartemisinin.^{32–34} Artemisinin (ART) is a compound with a sesquiterpene lactone and a 1,2,4-trioxane ring system where the peroxide group is placed within the ring structure of the molecule. It has been used to treat drug-resistant *P. falciparum* malaria. The study disclosed how artemisinin and its semi-synthetic analogues showed that their biological activity is due to the presence of the 1,2,4-trioxane ring.^{35–37} The rich heme content of malarial parasites

Biographical Sketches



Upendra Kumar Patel received his post-graduation (M.Sc.) from the University of Allahabad, (India). He is CSIR NET, CSIR-UGC NET, GATE qualified, and the recipient of a CSIR JRF, SRF fellowship. At present,

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Alka received her M.Sc. degree in organic chemistry from Mahatma Gandhi Kashi Vidyapith Varanasi. She qualified in the CSIR-UGC National Eligibility test and GATE and was awarded

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Prof. Alka Agarwal was awarded a Ph.D. in 1992 from Avadh University, Medicinal Chemistry, CDRI Lucknow, UP, India. She is a recipient of the INSA Visiting Scientist Fellowship award and visited Germany.

Prof. Agarwal has been working in diversified areas ranging from peptide chemistry to drug discovery. More specifically, she has been working on the synthesis of small molecules for antibacterial and antimalarial

studies, X-ray crystal structure analysis of small molecules, molecular docking studies of synthesized active molecules, nanomaterials, and their applications.

and its catalytic effect on breaking down the endoperoxide bridge may explain why antimalarial drugs like 1,2,4-trioxane are more harmful to the parasites compared to natural antimalarial terpenoids such as artemisinin (**1**) and its derivatives. Derivatives, like artemistene (**2**), dihydroartemisinin (**3**), artemether (**4**), arteether (**5**), artesunic acid (**6**), sodium artesunate (**7**), and artelinic acid (**8**), are specifically deadly to malaria-causing parasites^{38,39} (Figure 1).

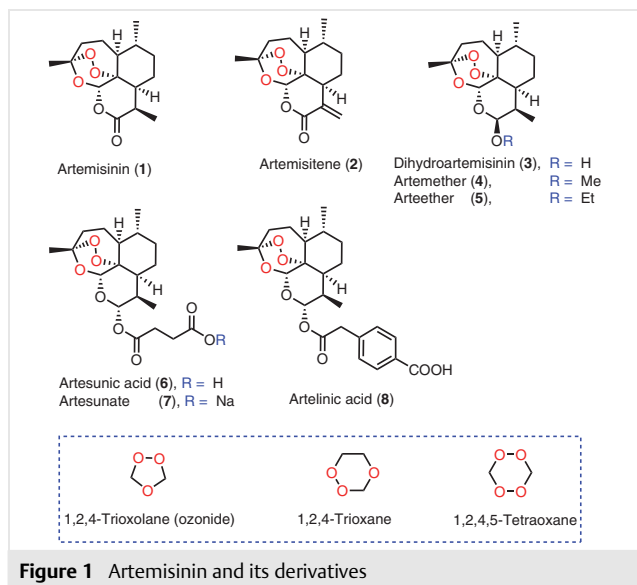


Figure 1 Artemisinin and its derivatives

In 2006, the World Health Organization (WHO) advised the utilization of a dual-drug combination in ART-based treatment to prevent the emergence of drug resistance in malaria parasites.^{40–43} However, the first case of antimalarial resistance to monotherapy with ART was documented in Cambodia in 2008 because the treatment reached its maximum effectiveness.^{44,45} This resistance is due to single nucleotide polymorphisms that can reduce the efficacy of artemisinin.⁴⁶ Artemisinin and its derivatives encounter obstacles such as resistance, as well as issues like low solubility in oil/water and sluggish parasite elimination in malaria patients. Dihydroartemisinin is a derivative of artemisinin which led the foundation for the development of related compounds such as artemether (**4**), arteether (**5**), sodium artesunate (**7**), and artelinic acid (**8**).⁴⁷ These substances are often known as the first-generation derivatives of ART and based upon solubility they can be classified into oil-soluble C(10) β -alkyl ethers (artemether and arteether) and water-soluble C(10) β -(substituted) esters (sodium artesunate and sodium artelinate). These drugs are more soluble in oil/water and more effective against malaria when compared to the ART drug. Yet, the early modifications of ART have downsides in aspects such as oil/water improvement and pharmacokinetics, observed in artemether and arteether, with decreased biological half-lives and potentially harmful effects on the blood system, heart, and cen-

tral nervous system in an animal model.^{48,49} Artesunate injection, given once daily for a week, shows a success rate of around 92% in treating malaria patients. However, to prevent resistance from spreading, artesunate is commonly administered in combination with other medications. Sodium artelinate has increased water stability and a longer biological half-life (1.5–3.0 h) than artemisinin, however, it showed decreased efficacy in both *in vivo* and *in vitro* experiments and induced nephrotoxicity in healthy rats.⁵⁰ Other significant issues related to ART-based antimalarials include higher treatment costs (compared to CQ or QN), inadequate physicochemical/pharmacokinetic properties (like inadequate lipid-/water-partitioning behavior, poor bioavailability, short plasma half-life, etc.), toxicities, and limited availability (scarcity in natural sources).^{51–58} Due to these obstacles, the treatment of malaria has become more complicated, prompting the search for new drugs to combat resistant strains of the disease. Recently, a variety of 1,2,4-trioxanes, 1,2,4-trioxolanes, and 1,2,4,5-tetraoxanes (Figure 1) have been developed and tested for their effectiveness in treating malaria.⁵⁹ 1,2,4,5-Tetraoxanes, IUPAC and CAS name these as 1,2,4,5-tetroxanes but both names are found in the literature, are entirely synthetic and can be synthesized from inexpensive, easily available materials. Various factors like substrate type, solvent, addition mode, temperature, concentration, and pH play a role in the synthesis of tetraoxanes, which can be accomplished using different techniques.⁶⁰ Tetraoxanes show enhanced efficacy against malaria and significantly greater stability.⁶¹ Unsymmetrical dispiro-tetraoxanes, as well as other synthetic tetraoxane compounds, were discovered to have comparable effectiveness to, or greater effectiveness than, artemisinin.⁶² Since the 1980s, researchers have developed different basic tetraoxanes and investigated their efficacy in combatting malaria. A series of research papers have assessed the efficacy of more than 250 tetraoxanes for combatting malaria. Many substances have exhibited promising *in vivo* antimalarial activity.^{63,64} The objective of this review is to provide pharmacists and organic/medical chemists with a current comprehension of the science behind 1,2,4,5-tetraoxane compounds.

2 Synthetic Methods for Tetraoxanes

Several synthetic approaches have been made to the synthesis of 1,2,4,5-tetraoxanes.^{56,65} Some commonly used methods are acid-catalyzed hydrogen peroxide cyclocondensation with ketones or aldehydes,^{65–68} ozonolysis of alkenes,⁶⁹ enol-ethers,⁷⁰ O-ether oxime,⁷¹ and cyclocondensation of bis(trimethylsilyl) peroxide with carbonyl compounds catalyzed by trimethylsilyl trifluoromethanesulfonate (TMSOTf).^{72,73} Asymmetric tetraoxanes can be synthesized by using different catalysts such as SSA ($\text{SiO}_2\text{-H}_2\text{SO}_4$),⁷⁴ aliphatic and alicyclic *gem*-hydroperoxides ($\text{MeReO}_3\text{-HBF}_4$

catalyst),⁶⁴ steroidal *gem*-bis-hydroperoxides (H_2SO_4 catalyst),⁷⁵ and *gem*-bis(trimethylsilylperoxy)alkanes (TMSOTf catalyst).⁷⁶ Various other catalysts such as $\text{Bi}(\text{OTf})_3$,⁷⁷ $\text{Cl-SO}_3\text{H}$,⁷⁸ I_2 ,⁷⁹ Re_2O_7 ,⁵⁹ PMA,⁸⁰ HPA/NaY,⁸¹ ADA-MNPs,⁸² and $\text{H}_{3+x}\text{PMo}_{12-x}^{+6}\text{Mox}^{+5}\text{O}_{40}$ ⁸³ are employed to improve the peroxy acetalization of aldehydes and ketones and promote the specific cyclocondensation of these substances (intermediates) with ketones/aldehydes to generate 1,2,4,5-tetraoxanes. Figure 2 depicts various techniques used in the synthesis of tetraoxanes.^{56,59,64–83} The yields of tetraoxanes obtained from these methods are influenced by a variety of factors, including the structure of the carbonyl compound, temperature, concentration, pH, addition mode, solvent, and the equilibrium between the ketone and precursors of cyclic peroxides.⁸⁴ The primary approach involves incorporating hydrogen peroxide into carbonyl compounds, aided by an acid catalyst, and subsequently initiating the cyclization of the hydroperoxide intermediate formed (Scheme 1).

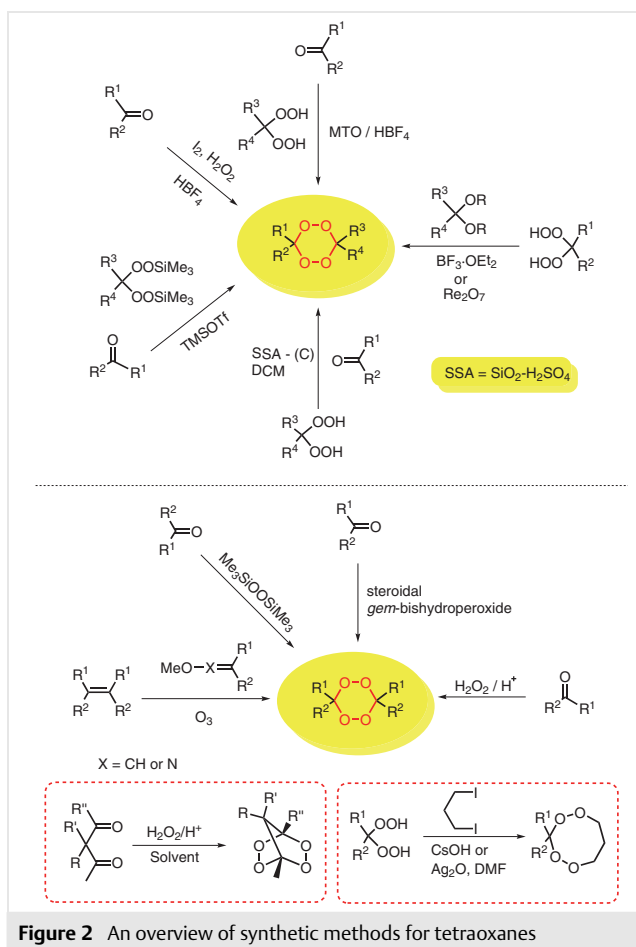
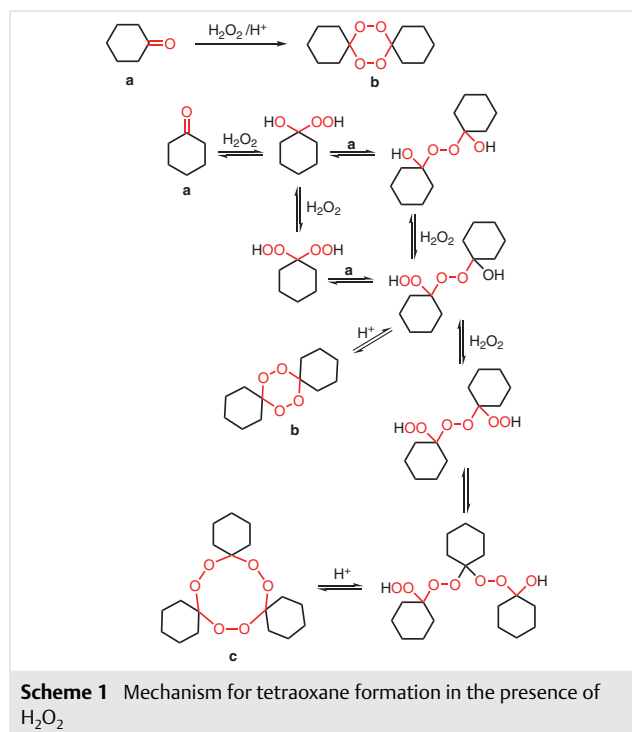


Figure 2 An overview of synthetic methods for tetraoxanes

It has been found that the yield of the tetraoxane can be affected by the reaction conditions, and it has been observed that hexaoxonanes might also result from as byproducts.⁸⁵ It is well known that tetraoxanes are thermodynamically

stable while hexaoxonanes are kinetically controlled products, but recent studies revealed that whether tetraoxanes or hexaoxonanes are kinetically preferred depends on the relative rates of several steps involved in their formation. A hypothesized mechanism for the generation of tetraoxane is illustrated in Scheme 1.⁸⁵



Scheme 1 Mechanism for tetraoxane formation in the presence of H_2O_2

Various reaction intermediates⁸⁵ depicted in Scheme 1 have been isolated under varying reaction conditions. It has always been difficult to purify tetraoxanes: hydroperoxide impurities can be eliminated by using potassium iodide or dimethyl sulfide and hexaoxonanes can be removed by recrystallization or washing the reaction mixture with cold methanol. If the conversion fails, hexaoxonanes can be transformed into tetraoxanes by heating the reaction mixture with perchloric acid in acetic acid.^{86,87} Spectroscopic techniques can validate the formation of hexaoxonanes.⁸⁸

2.1 Safety Concerns in the Synthesis of Cyclic Organic Peroxides

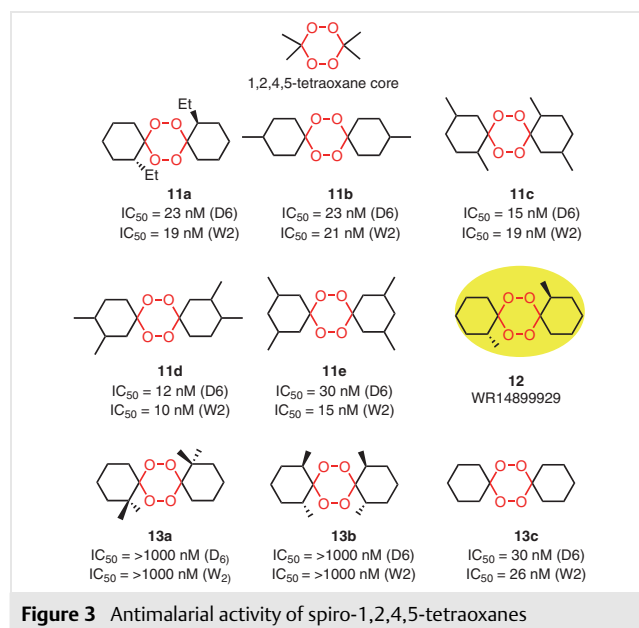
During the synthesis of cyclic organic peroxides, which are dangerous compounds when present in their solid form especially in quantities above 1 gram, explosions caused by peroxides may occur spontaneously and unpredictably when handled roughly or when the solid is subjected to impact, friction, static charge, or temperature changes. Solid peroxide samples should be handled very carefully and dissolved in a suitable solvent to minimize the risk of explosion. The synthesis of these substances may only be carried

out by highly qualified and experienced personnel, under the use of appropriate safety precautions, and only in small quantities (ca. 100 mg).⁸⁹

3 Antimalarial Activities of Tetraoxane Derivatives

3.1 Cycloalkanone-Based Tetraoxanes

In 2000, Vennerstrom and co-workers investigated a range of alkyl-substituted dispiro-1,2,4,5-tetraoxanes.⁹⁰ Of all the compounds investigated, five (**11a–e**) showed activity with IC_{50} values between 10 and 30 nM against the CQ-sensitive D6 and CQ-resistant W2 clones of *P. falciparum*, in contrast, the IC_{50} values for artemisinin (**1**) are 55 and 32 nM and for compound WR 1489929 (**12**) they are 8.4 and 7.3 nM (Figure 3).⁵⁸ Furthermore, certain compounds within this category have displayed moderate *in vivo* efficacy (achieving a 40–60% cure rate when given orally at 128 mg/kg/day) against mice infected with *P. berghei* on days 3, 4, and 5 after infection.



Vennerstrom and co-workers also studied the steric effect of the methyl group at C-1 and C-10 on dispiro-1,2,4,5-tetraoxanes. The study revealed that tetramethyl-substituted dispiro-1,2,4,5-tetraoxanes **13a,b** had significantly reduced antimalaria effectiveness ($IC_{50} = >1000$ nM) against

D6 and W2 strains when compared to WR 1489929 (**12**), whereas tetraoxane **13c**, without steric hindrance, was found to be active (Figure 3).⁹¹

3.2 Steroid-Based Tetraoxanes

In 1996, Šolaja and co-workers synthesized steroidal 1,2,4,5-tetraoxanes and tested their effectiveness against *P. falciparum* D6 and W2 strains for antimalarial activity.⁷⁵ They found that *cis*-isomer **14a** had no antimalarial effects on tested strains while *trans*-isomer **14b** exhibited some antimalarial activity ($IC_{50} = 155$ nM) against the D6 strain (Figure 4). The lack of effectiveness against malaria was identified as being due to a significant steric effect caused by obstructed ring structures and poor water solubility. In 2000, Šolaja and co-workers prepared tetraoxanes based on cholic acid with varying diester, acid, and diamide connections and they were tested against *P. falciparum* strains D6 and W2.⁷³ The *cis* compounds were more potent than the *trans* compounds. Two tetraoxanes, particularly **15a,b**, with an amide linker, showed strong effectiveness against the D6 strain with IC_{50} values between 9.29 and 20.08 nM and a significant SI value (Figure 4). The higher level of activity could be attributed to the existence of hydrophilic linker or a stereochemical structure distinct from **14a,b**.

Again in 2016, Šolaja and co-workers reported the *in vivo* antimalarial activity of steroidal tetraoxane **16** that inhibited liver-stage *P. berghei* infection ($IC_{50} = 0.33$ μ M) (Figure 4).⁹² Additionally, at a quantity of 100 mg/kg, it demonstrated a 91% drop in the parasite liver burden in mice. Moreover, the compound displayed a certain degree of efficacy with an IC_{50} of 1.16 μ M against IV-V *P. falciparum* gametocytes from the 3D7elo1-pfs16-CBG99 transgenic strain, compared to DHART with an IC_{50} of 0.44 μ M in the identical model.⁹³ In 2018, Kazakova and co-workers investigated the effectiveness of 1,2,4,5-tetraoxanes based on deoxycholic acids against *P. falciparum* isolates that are CQ-resistant K1 and CQ-sensitive (T96) (Figure 4).⁶³ In contrast to CQ ($IC_{50} = 29$ nM), compounds **17a,b** showed greater potency with IC_{50} values between 3.0–7.6 nM against the K1 strain. However, when it came to the T96 strain, **17a,b** exhibited moderate activity.

3.3 Adamantane-Based Tetraoxanes

In 2006, the O'Neill group developed unsymmetrical dispiro- and spiro-1,2,4,5-tetraoxanes and evaluated their efficacy against malaria under *in vitro* and *in vivo* studies.⁹⁴ Out of all the substances examined, compounds **18a,b** displayed notable *in vivo* efficacy (100% inhibition) when administered orally at a dose of 30 mg/kg (Figure 5).

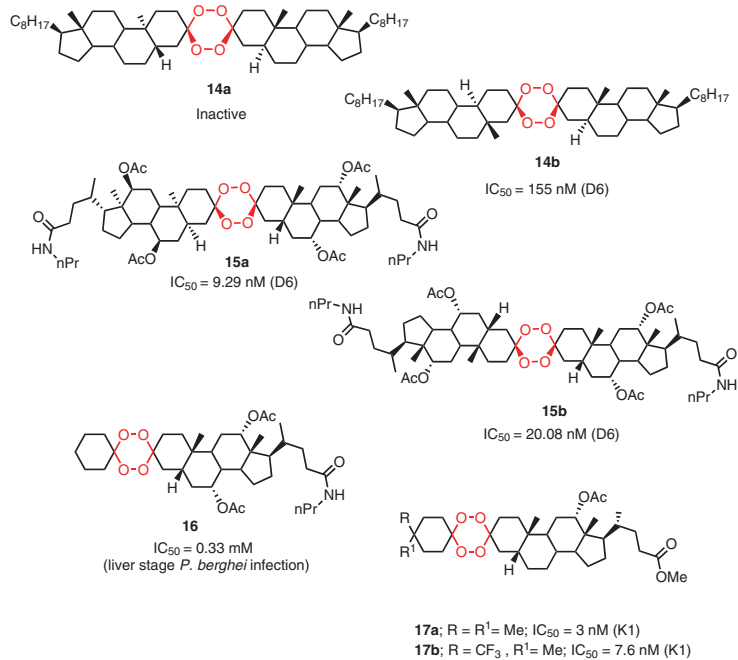


Figure 4 Antimalarial activity (*in vitro* and *in vivo*) of steroidal 1,2,4,5-tetraoxanes

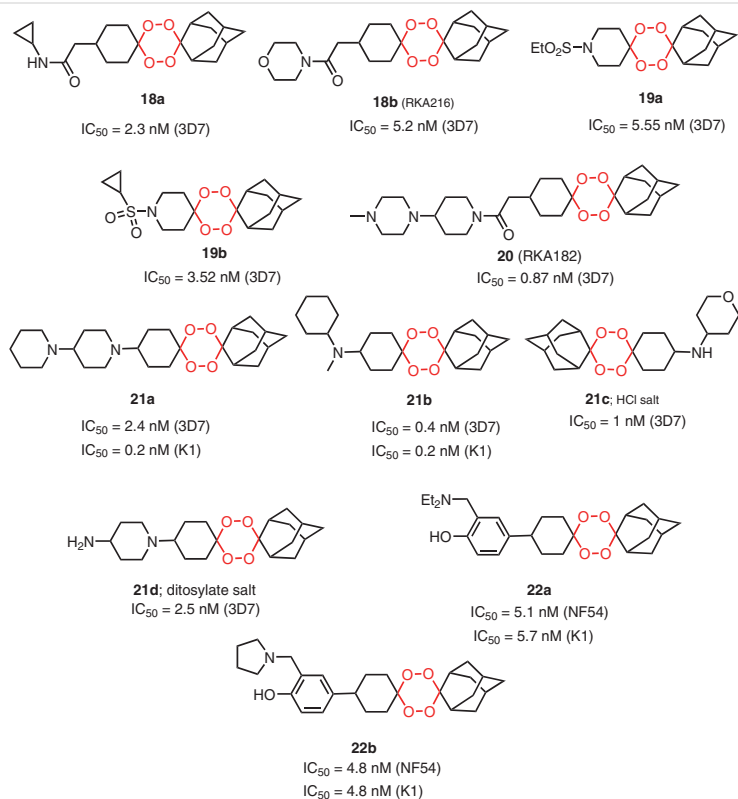


Figure 5 Antimalarial activity of novel adamantane-based 1,2,4,5-tetraoxanes

Compounds **18a,b** were tested in the 4-day Peter's test to determine their oral *in vivo* effectiveness against *P. berghei* (ANKA) in mice, comparing their ED₅₀ and ED₉₀ values to artemether (**4**) having ED₅₀ = 5.88 and ED₉₀ = 10.57 mg/kg. Compound RKA216 (**18b**) demonstrated exceptional efficacy when given orally with an ED₅₀ of 3.18 mg/kg and an ED₉₀ of 3.88 mg/kg.

In 2008, the O'Neill group investigated achiral dispiro-1,2,4,5-tetraoxanes and evaluated their efficacy in combating malaria.⁹⁵ Compound **19a,b** showed strong efficacy with IC₅₀ values of 5.55 and 3.52 nM, respectively, against the *P. falciparum* 3D7 strain (Figure 5). When compared to RKA216 (**18b**) with an ED₅₀ of 3.18 mg/kg, compounds **19a,b** showed considerable *in vivo* efficacy with ED₅₀ values of 6.61 and 7.93 mg/kg. It was also found that compounds **19a,b** showed no toxicity.

In 2010, the O'Neill group identified a powerful antimalarial medication named RKA182 (**20**) that was effective against the 3D7 and K1 strains of *P. falciparum*, with IC₅₀ values of 0.87 and 1.1 nM, respectively.⁹⁶ RKA182 (**20**) demonstrated noticeable *in vivo* efficacy in studies, with ED₅₀ and ED₉₀ doses of 1.33 and 4.18 mg/kg, outperforming the therapeutic medications artemether (**4**) and artesunate (**7**). Additionally, RKA182 (**20**) showed higher water solubility, reduced toxicity, and improved absorption, distribution, metabolism, and excretion (ADME) characteristics.⁹⁷ The O'Neill group developed a novel series of second-generation RKA182 (**20**) analogues by eliminating the amide bond to enhance metabolic stability.⁹⁸ They developed various polar dispiro-1,2,4,5-tetraoxanes and evaluated their *in vitro* efficacy against *P. falciparum* strains 3D7 and K1. Each compound exhibited strong antimalarial properties at very low nanomolar concentrations, outperforming both CQ and artesunate. Compounds **21a,b** displayed superior effectiveness against the CQ-resistant K1 strain, which is 25 times more potent than artesunate and 5 times stronger than RKA182 (**20**) (Figure 5). Compound **21c** (HCl salt) and **21d** (ditosylate salt) showed superior effectiveness compared to artesunate in treating *P. berghei* ANKA infected mice with ED₉₀ values of 11 and 10 mg/kg, respectively. These results show that this group of molecules has a high level of activity and better metabolic stability than RKA182 (**20**). Further investigation by the O'Neill group revealed that two mannoxane structure compounds **22a,b** containing a Mannich base displayed strong *in vivo* antimalarial properties (>99.7% decrease in parasitemia after 4 days in *P. berghei* infected mice) and *in vitro* tests (IC_{50s} = 4.8–5.7 nM against NF54 and K1 strains, respectively) (Figure 5).⁹⁹ Furthermore, mice that were given **22a,b** lived an average of 27 and 25 days, with **22a** curing 66% of them successfully. Compound **22a** exhibits significantly higher efficacy than atelolane (OZ277) (achieving a 60% cure rate in 25 days) and RKA182 (**20**) (requiring 22 days for cure). Unlike OZ277 and RKA182, compounds **22a,b** exhibit a level of *in vitro* inhibition of

hematin dimerization that is comparable to CQ and amodiaquine. This research also demonstrated the significance of the Mannich base pharmacophore and the adamantyl ring in the effectiveness against malaria.

3.4 Dispiro-Based Tetraoxanes

In 2010, Vennerstrom and co-workers synthesized unsaturated dispiro-1,2,4,5-tetraoxane (+)-dihydrocarvone and reported its antimalarial efficacy.¹⁰⁰ It was found that enhancement in the polarity of the compound leads to a decrease in the efficacy of antimalarial drugs. Compound **23a** showed the most effectiveness with an IC₅₀ of 2.1 nM against the K1 and NF54 strains (Figure 6).

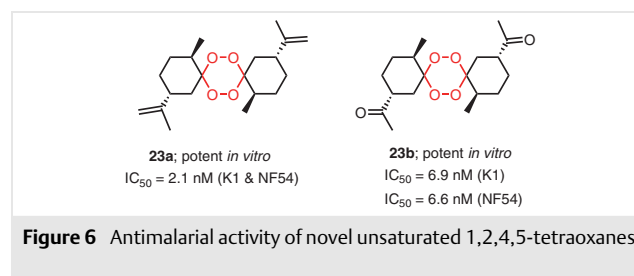


Figure 6 Antimalarial activity of novel unsaturated 1,2,4,5-tetraoxanes

Compound **23b** shows average *in vitro* antimalarial effectiveness with IC₅₀ of 6.9 and 6.6 nM against K1 and NF54 strains, respectively. In addition, it shows better *in vivo* effectiveness (99.9% cure) in mice infected with *P. berghei* when given orally, outperforming ART treatment (98% cure in the same study).¹⁰¹

3.5 Diaryl-Based Tetraoxanes

In 2009, Rawat and co-workers developed and assessed the *in vitro* efficacy of substituted 3,6-diaryl-1,2,4,5-tetraoxanes molecules against malaria.¹⁰²

The results indicate that symmetrical tetraoxanes **24a–c**, with ethyl, propyl, and isopropyl groups on the phenyl ring, have notable antimalarial activity against the D6 and W2 strains of *P. falciparum*. The values of IC₅₀ range from 0.61 to 0.99 μM and 0.76 to 1.03 μM, respectively (Figure 7). A few compounds **25a–f** in the asymmetric tetraoxanes displayed strong effectiveness against the D6 and W2 strains, showing IC₅₀ values ranging from 0.35 to 0.79 μM. Compounds **25a–f** are not as potent against the W2 strain as ART, but they have a similar effectiveness to CQ (0.41 μM). In 2011, Rawat and co-workers investigated the *in vitro* antimalarial efficacy of tetraoxane-imine/amine/amide conjugates against *P. falciparum* strains D6 and W2. When compared to therapeutic drugs like CQ and ART, four compounds **26a–d**, imine and amine derivatives, showed considerable effectiveness, with IC₅₀ values ranging from 0.38 to 2.64 μM (D6 strain) and 0.57–1.62 μM (W2 strain) (Figure 7).¹⁰³

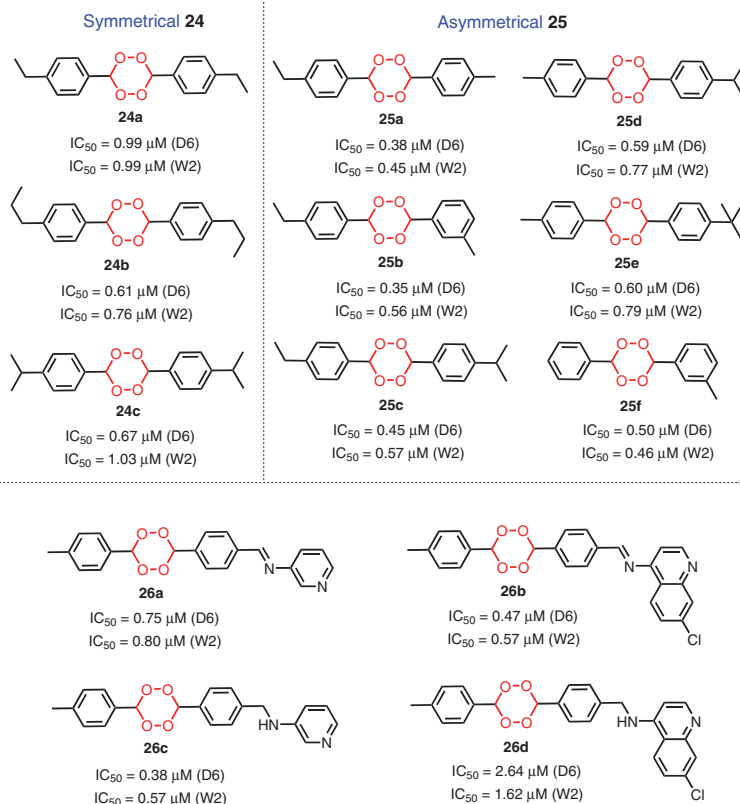


Figure 7 Antimalarial activity of 3,6-diaryl-1,2,4,5-tetraoxanes

3.6 Di-adamantane-Based Tetraoxanes

In 2013, the O'Neill group synthesized novel tetraoxane dimer compounds and evaluated their efficacy in treating malaria.¹⁰⁴ Among them compounds **27a–c** exhibited the highest *in vitro* antimalarial efficacy against the 3D7 strain with IC₅₀ values of 4.0, 3.5, and 4.7 nM, respectively (Figure 8).

Moreover, compounds **27a** and **27d** showed the best efficacy against the K1 strain with IC₅₀ values of 2.6 and 2.7 nM, respectively. Additionally, some compounds displayed moderate oral antimalarial effectiveness against *P. berghei* ANKA mice but were not as active as artesunate.

3.7 Benzylamino- and Aryloxy-Based Tetraoxanes

In 2016, the O'Neill group investigated an effective method for synthesizing aryloxy 1,2,4,5-tetraoxanes and evaluated their efficacy against malaria.¹⁰⁵ Each compound exhibited excellent antimalarial potency (less than 10 nM) against the *P. falciparum* 3D7 strain. Three compounds **28a–c** showed strong effectiveness against the strain being tested, with IC₅₀ values between 0.5–3.7 nM, similar to the IC₅₀ of 2.2 nM of artesunate (Figure 9).

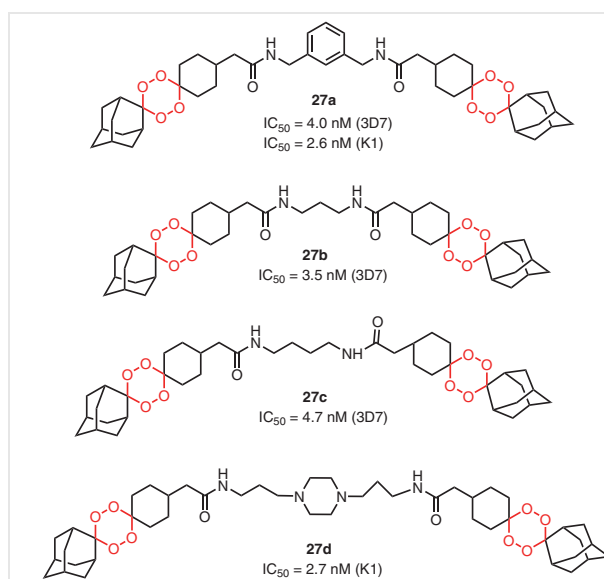
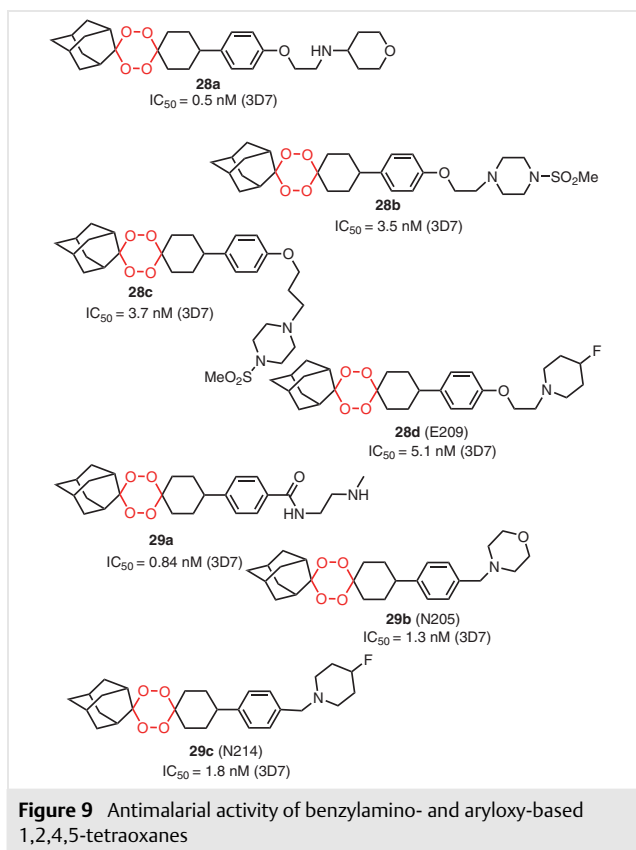


Figure 8 Antimalarial activity of dimer tetraoxanes



When given at a dosage of 30 mg/kg, the lead compound E209 (**28d**) exhibited the highest level of *in vivo* effectiveness (prevention rate of 99.65%) in mice from *P. berghei* infection. Furthermore, they described the most effective approach for synthesizing the lead compound E209.¹⁰⁵ After a thorough assessment, it was concluded that E209 (**28d**) fulfills all requirements specified in the Medicines for Malaria Venture (MMV) target candidate profile 1 (TCP1), having the pharmacokinetic attributes needed for a single treatment, alone or with other drugs, and a fast efficacy (PRR equal to or better than DHART).¹⁰² E209 (**28d**) exhibited strong *in vivo* inhibitory effects in the nanomolar range against various strains of *P. falciparum* and *P. vivax*. Additionally, its effectiveness against *P. falciparum* was validated in trials (rodent models). It demonstrates favorable PK-PD characteristics and reduced levels of parasites similar to DHART.¹⁰⁶

In 2018, the O'Neill group synthesized some new aryl-carboxamide and benzylamino-based dispiro-1,2,4,5-tetraoxanes.¹⁰⁷ Compounds **29a–c** exhibited the strongest *in vitro* effectiveness against *P. falciparum* strain 3D7 showing IC_{50} values ranging from 0.84–1.8 nM (Figure 9). It was also found that N205 (**29b**) showed greater efficacy against *P. falciparum* compared to artesunate (ED_{90} = 10 mg/kg) fol-

lowing four doses administered daily. According to the effectiveness of N205 (**29b**),¹⁰⁷ a single oral dose of N205 is equally potent as several doses of artesunate.

3.8 Aminoquinoline-Based Tetraoxanes

Several research groups have tried to synthesize hybrids of 1,2,4,5-tetraoxanes with other known pharmacophores to provide potential leads against malaria. In 2008, Šolaja and co-workers studied the development and antimalarial effects of hybrid compounds that merged tetraoxane with 7-chloro-4-aminoquinoline, which they called tetraox-quinines.¹⁰⁸ According to activity data compounds, **30a,b** exhibited *in vitro* activity against *P. falciparum* stains (D6 and W2) with IC_{50} values between 2.0–2.33 nM and 5.76–9.05 nM, respectively (Figure 10).

Additionally, compounds **30a,b**, with a minimal curative dose (MCD) of 80 mg/kg, a minimum active dose (MAD) of 20 mg/kg/day, and a maximum tolerated dose (MTD) of >960 mg/kg, successfully treated mice in a modified Thompson test for antimalarial blood stage activity.

In 2014, Lopes and co-workers investigated a few hybrids based on 1,2,4,5-tetraoxane and 8-aminoquinoline, assessing their effectiveness against malaria (Figure 10).¹⁰⁹ Intraperitoneal injection of the most effective molecule **31**, connected to the other two pharmacophores by an amide linker, effectively treated animals with blood-stage *P. berghei* infection.

Furthermore, Lopes and co-workers also conducted the development of another series of hybrids based on 1,2,4,5-tetraoxane-8-aminoquinoline, linking aryl/heteroaryl groups to the metabolically labile C-5 position of the 8-aminoquinoline.¹¹⁰ The majority of the compounds exhibited high effectiveness against *P. falciparum* W2 and *P. berghei* in laboratory tests, with EC_{50} values ranging from low nanomolar to micromolar concentrations. Compounds **32a–d** demonstrated the strongest potency and had comparable effects to ART on CaCo-2 cells, without causing toxicity (CC_{50} = >50 μ M) (Figure 10). Hybrid molecules with C-5 aryl modification on the 8-aminoquinoline were discovered to exhibit improved metabolic stability in microsomes compared to primaquine analogues (C-5 unsubstituted 8-aminoquinolines) while retaining their dual-stage antimalarial efficacy.

Mahmud and co-workers, in 2020, investigated the molecular docking and quantitative structure-activity relationship (QSAR) of various hybrids that linked 1,2,4,5-tetraoxane with 8-aminoquinoline.¹¹¹ Lopez and co-workers have already investigated the efficacy of these drugs against the W2 strain of *P. falciparum* within red blood cells.¹¹⁰ It was discovered through molecular docking analysis that 1,2,4,5-tetraoxane-8-aminoquinoline hybrids have a stronger binding affinity with *P. falciparum* lactate dehydrogenase

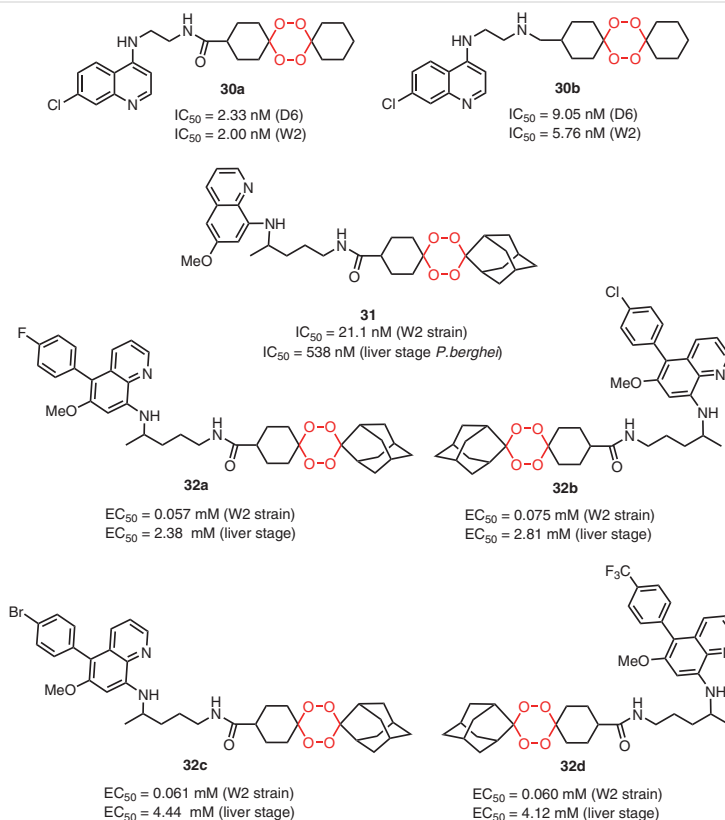


Figure 10 Antimalarial activity of aminoquinoline-based 1,2,4,5-tetraoxane hybrids

(pFLDH) than chloroquine (CQ). This study indicates that these substances may serve as more effective inhibitors of pFLDH than CQ.

3.9 2-Cyanopyrimidine-Based Tetraoxanes

In 2014, Moreira, O'Neill, and co-workers synthesized hybrids of 2-cyanopyrimidine-based 1,2,4,5-tetraoxanes and examined their efficacy against atovaquone-resistant (FCR3), 3D7, and W2 strains of *P. falciparum* for their antimalarial effects. Moreover, their ability to block falcipain-2 (FP-2) was also evaluated.¹¹²

Out of the tested compounds, three compounds **33a–c** exhibited the most favorable balance of activity and safety in the evaluation against HEK-293 cells. Compounds **33a** and **33c** effectively blocked the W2 and 3D7 strains with IC_{50} values between 9.8 and 13.1 nM (Figure 11). The hybrid compound **33b** was selected for its *in vivo* potent FP-2 inhibitory activity in mice infected *P. berghei*. In comparison to the control, the *in vivo* findings indicated a reduction in parasitemia levels and enhancements in the survival rate of mice.

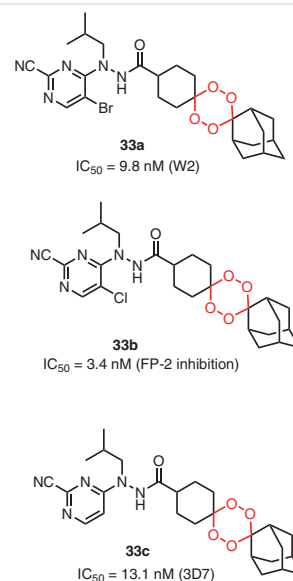
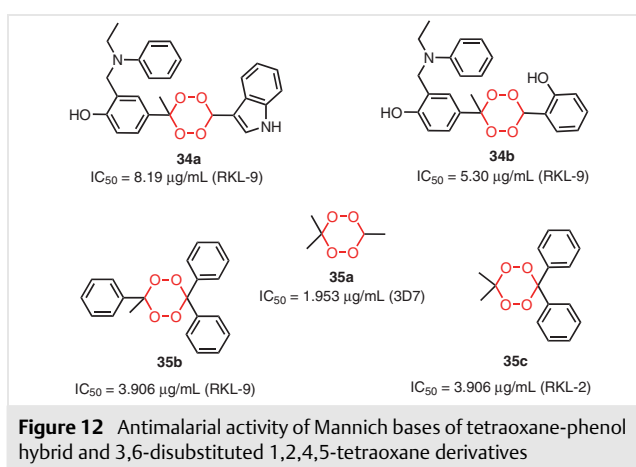


Figure 11 Antimalarial activity of 2-cyanopyrimidine-based 1,2,4,5-tetraoxane hybrids

4 Mannich Base Based Tetraoxanes

In 2016, Rudrapal and co-workers investigated a novel category of unique Mannich bases made from tetraoxane-phenol conjugate and evaluated their *in vivo* efficacy against *P. falciparum* strains, which included CQ-sensitive (RKL-2) and CQ-resistant (RKL-9) strains (Figure 12).¹¹³ Compared to other compounds, two compounds **34a,b** with indole and phenol rings, showed higher efficacy against RKL-9 (with IC_{50} of 8.19 and 5.30 $\mu\text{g/mL}$, respectively). However, they were not as effective as the standard drug CQ (IC_{50} = 0.04 $\mu\text{g/mL}$) when tested against the same strain.



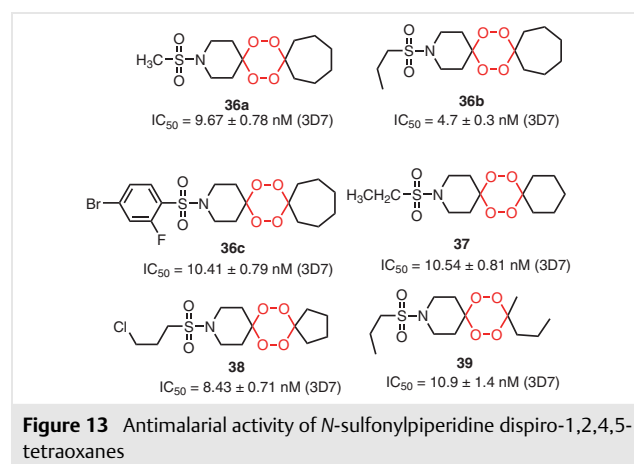
In 2019, Kumawat and co-workers synthesized modified forms of 3,6-disubstituted 1,2,4,5-tetraoxane derivatives and evaluated their efficacy in combating malaria¹¹⁴ (Figure 12). All the prepared compounds were examined for activity against *P. falciparum* strains 3D7, RKL-2, and RKL-9. Compounds with changes at the 3,6-positions of the 1,2,4,5-tetraoxane structure, like trimethyl **35a** (IC_{50} = 1.953 $\mu\text{g/mL}$ against 3D7), methyl triphenyl **35b** (IC_{50} = 3.906 $\mu\text{g/mL}$ against RKL-9), and dimethyl/diphenyl **35c** (IC_{50} = 3.906 $\mu\text{g/mL}$ against RKL-2), showed enhanced effectiveness.

The structure-activity relationship analysis showed that the antimalarial activity of the tetraoxane moiety was promoted by trimethyl substitution at positions 3 and 6 on the tetraoxane core. It could be because the peroxide bond has the proper steric barrier. The authors postulated that the enhanced effectiveness against malaria was observed with phenyl ring additions at positions 3 and 6 of tetraoxane which receive electrons from tetraoxane.

4.1 N-Sulfonylpiperidine-Based Tetraoxanes

In 2022, Awasthi and co-workers synthesized a range of symmetrical and non-symmetrical *N*-sulfonylpiperidine dispiro-1,2,4,5-tetraoxanes and assessed their effectiveness

against malaria (*in vitro* and *in vivo*).¹¹⁵ They initially tested the *in vitro* antiplasmodial effectiveness of both symmetrical and non-symmetrical *N*-sulfonylpiperidine dispiro-1,2,4,5-tetraoxanes using the HRP-2 assay on the erythrocytic phases of *P. falciparum* strain 3D7, found that the non-symmetrical tetraoxane showed higher antiplasmodial activity compared to the symmetrical tetraoxane (Figure 13).



Three compounds **36a–c** containing a cycloheptyl ring displayed superior efficacy compared to other analogues, particularly compound **36b** (IC_{50} = 4.7 \pm 0.3 nM against 3D7) which demonstrated nearly equivalent antimalarial activity to artemisinin (IC_{50} = 4.74 \pm 0.78 nM against 3D7). Compounds **37**, **38**, and **39** showed strong antimalarial effects with IC_{50} values of 10.54 \pm 0.81, 8.43 \pm 0.71, and 10.9 \pm 1.4 nM, respectively, against the 3D7 strain. Compounds **37** and **38** showed the best *in vivo* antimalarial effectiveness with only 0.25% parasitemia after 72 hours. Also, the SAR study of the synthesized tetraoxane revealed that ring size influenced the antimalarial activity.

4.2 N-Benzoylpiperidine-Based Tetraoxanes

Again in 2024, Awasthi and co-workers synthesized a certain number of mixed *N*-benzoylpiperidine-based 1,2,4,5-tetraoxane analogues and reported their antimalarial property against chloroquine-sensitive *P. falciparum* strain 3D7.¹¹⁶ Some of them showed very good antimalarial activity in the nanomolar range comparable to the artemisinin (IC_{50} = 5.97 \pm 0.61 nM). Compounds **40** and **41** both have the same structural units (*N*-benzoyl-substituted tetraoxane) except compound **40** with the formyl group present in the *para*-position showed higher antimalarial activity (IC_{50} = 6.35 \pm 0.55 nM) than **41** (IC_{50} = 7.28 \pm 0.57 nM); compounds **42** and **43** were found to be less active with than **40** and **41**.

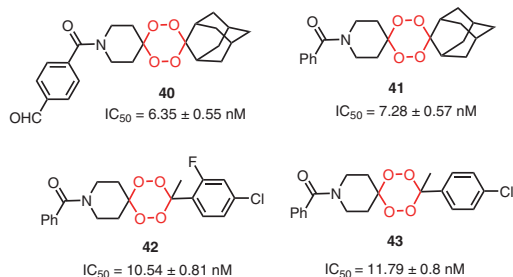
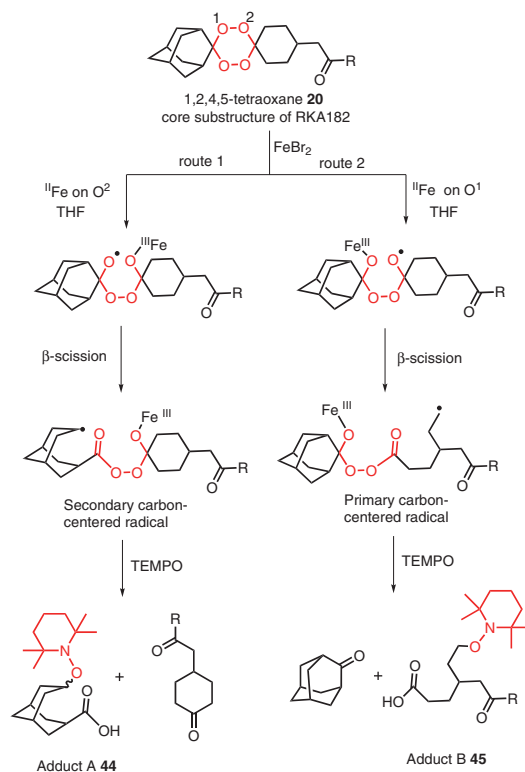


Figure 14 Antimalarial activity of *N*-benzoylpiperidine-based dispiro-1,2,4,5-tetraoxanes

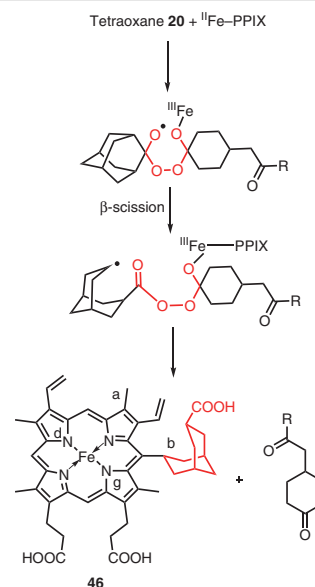
In all above reported compounds as shown in Figure 14, it is observed that substituents and their nature have a deciding role in antimalarial activity; formyl-, fluoro-, and chloro-substituted tetraoxanes possessed greater activity. They also performed molecular docking studies by taking falcipain-2 enzyme (receptor macromolecule) on synthesized tetraoxanes. Molecular docking studies revealed that tetraoxanes **40** and **43** bind the active site of the falcipain-2 enzyme through H-hydrogen bonding and hydrophobic interactions which are the foremost interpretation for their activity.

5 Mechanism of Action of Dispiro-1,2,4,5-tetraoxanes

The specific mechanism of action for these tetraoxanes remains to be identified. The O'Neill group have demonstrated the possible agents responsible for the antimalarial effects of RKA182 (**20**) in 1,2,4,5-tetraoxane activity,⁹⁶ by conducting mechanistic examinations of dispiro-1,2,4,5-tetraoxane **20** in THF with iron(II) bromide and a radical trapping agent TEMPO. The authors hypothesized that regioisomeric alkoxy radicals are formed by the cleavage of the O^1-O^2 bond. These regioisomeric radicals undergo β -scission, resulting in the formation of primary and secondary carbon-centered radicals. TEMPO captured these radicals to produce the respective aminoxy adducts **44** and **45** (Scheme 2). The O'Neil group¹¹⁷ conducted modeling research with heme and RKA182 (**20**), and found that heme favored binding with 1,2,4,5-tetraoxane through a less hindered oxygen atom. They also conducted docking experiments and discovered that the closest distance was between heme Fe(II) and the oxygen of RKA182, with the lowest energy conformations measuring between 2.4 and 2.8 Å (Scheme 3). Docking simulations showed that the heme Fe(II) tends to interact more with the less hindered oxygen atom of 1,2,4,5-tetraoxane.



Scheme 2 Proposed mechanisms for the iron-mediated degradation of RKA182 and its TEMPO adducts



Scheme 3 Heme alkylation by RKA182

6 Conclusion

Tetraoxane derivatives/hybrids have been identified as a new category of antimalarial endoperoxides, serving as an important treatment option for malaria with potent antimalarial properties over the last three decades. There is scope for further exploration in the synthesis of new 1,2,4,5-tetraoxane derivatives that have the potential to exhibit druglike effects against different types of *Plasmodium* species. 1,2,4,5-Tetraoxane compounds linked with a steroid, triazine, amine, aminoquinoline, dispiro, piperidine, or diaryl derivatives were designed and synthesized for their effectiveness against malaria. Some of these compounds exhibit impressive *in vitro* antimalarial activity. In this current review article, we have outlined the synthesis and effectiveness against malaria of 1,2,4,5-tetraoxane derivatives/hybrids and the impact of structural changes on their antimalarial properties. Eventually, these 1,2,4,5-tetraoxane derivatives coupled with different aryl/heteroaryl/alicyclic/spiro groups will assist medicinal chemists in concentrating on creating powerful new compounds against diverse strains of *Plasmodium* species.

Conflict of Interest

The authors declare no conflict of interest.

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