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# Kinetics Study on Reaction between Dihydroartemisinic Acid and Singlet Oxygen: An Essential Step to Photochemical Synthesis of Artemisinin†

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Artemisinin is an excellent antimalarial drug widely used in clinical medicine. However, due to the limitation of natural source of artemisinin, the chemical synthesis of artemisinin has achieved substantial attention. Dihydroartemisinic acid is a key precursor for the synthesis of artemisinin. The reaction of dihydroartemisinic acid with singlet oxygen to form peroxide is a pivotal step in the photochemical preparation of artemisinin. Nevertheless, the reaction kinetics of dihydroartemisinic acid with singlet oxygen has not been investigated previously. Herein, we report the rate constants of the reaction between dihydroartemisinic acid and singlet oxygen. By directly detecting the luminescence decay kinetics of singlet oxygen at 1270 nm at room temperature, the reaction rate constants of singlet oxygen and dihydroartemisinic acid in different solvents are obtained to be $1.81\times10^5$ $(\mathrm{mol/L})^{-1}\mathrm{s}^{-1}$ in $CCl_4$, $5.69\times10^5$ $(\mathrm{mol/L})^{-1}\mathrm{s}^{-1}$ in $CH_3CN$, and $3.27\times10^6$ $(\mathrm{mol/L})^{-1}\mathrm{s}^{-1}$ in DMSO, respectively. It is found that the reaction rate constants of dihydroartemisinic acid with singlet oxygen increase as polarity of the solvent increases among the three solvents. These results provide fundamental knowledge to optimize experiment conditions of photochemical synthesis of artemisinin for improving the yields of artemisinin.

**Key words:** Dihydroartemisinic acid, Artemisinin, Singlet oxygen, Rate constant, Transient absorption spectroscopy

## I. INTRODUCTION

Artemisinin (ART), Qinghaosu in Chinese, is an endoperoxide lactone extracted from the medicinal herbal plant *Artemisia annua* L. Artemisinin is widely used as antimalarial drug in the clinic, which is first developed by Prof. Youyou Tu, the 2015 Nobel Prize in Physiology or Medicine for her discovery in 1972 of the drug artemisinin. Up to date, malaria still remains one of the most dangerous diseases worldwide, killing over one million people each year, which is caused by protozoan parasite *Plasmodium falciparum* [1–10]. The early antimalarial drug was quinolone-based drugs, which have large side effect. Additionally, the evolved *Plasmodium parasite* have also developed resistance to these quinolone-based drugs [2, 3]. Until 1972, artemisinin was extracted from the plant *Artemisia annua* L by Youyou Tu and was found to effectively kill malaria parasites [3, 4]. Therefore, artemisinin-based combination therapy has been recommended by the World Health Organization to be the most effective treatment against malarial infection currently [5, 6]. Although originally developed as an antimalarial drug, ART actually possesses many other therapeutic potentials on human diseases. In addition to malaria, artemisinin has also been found to have other pharmacological effects, including antitumor, antifungal, antifibrotic, choleretic, and treatment of systemic lupus erythematosus [2–6].

Presently, artemisinin is mainly obtained from the herbal plant *Artemisia annua*, and the drug artemisinin is often not sufficient due to its low product yields extracted from natural plant. Therefore, exploring chemical synthesis of artemisinin has attracted great attention in the past two decades. Although various attempts have been reported [11, 12], these synthesis methods are too complex to be considered a viable alternative for the large-scale production of artemisinin. Due to its complex structure, it is difficult to develop a process for the total synthesis of artemisinin. This problem was solved by synthesis of artemisinin from its closely related biosynthetic precursors, especially artemisnic acid [13–17]. However, it is still a formidable challenge for chemists to find a high-yielding, industrial, and low-cost process to convert artemisnic acid into artemisinin, es-

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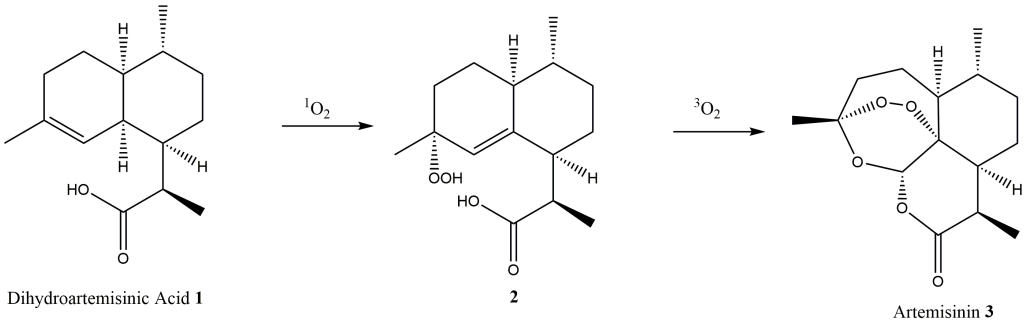


Scheme 1 Synthesis process of artemisinin from dihydroartemisinic acid by Roth and Acton.

pecially concerning the construction of peroxy bridge. In this synthesis process, Roth and Acton proposed the main reaction mechanisms of the conversion of dihydroartemisinic acid (DHAA) to artemisinin as shown in Scheme 1 [15]. DHAA was used as the intermediate. Through singlet oxygen oxidation and controlled two-step reactions, artemisinin was obtained in a total yield of 17%. Wu Yikang group used the singlet oxygen produced by disproportionation of hydrogen peroxide to construct the peroxy bond of artemisinin [17]. Seeberger research group firstly invented a continuous flow chemistry manufacturing process [18]. The raw material dihydroartemisinic acids flow continuously through the photoreactor to synthesize artemisinin. Although the chemical reactions took place in the solution without isolation and purification, the total yield reached 39%. Recently, Zhang Wanbin group used chemical methods to obtain singlet oxygen and converted artemisinin or dihydroartemisinic acid to artemisinin with a yield of up to 60% [19].

Among these synthesis processes, the essential step is the reaction between dihydroartemisinic acid and singlet oxygen, which triggers the construction of the endoperoxide group [20–24]. However, the reaction kinetics of dihydroartemisinic acid with singlet oxygen have not been studied previously. Knowing the reaction rate constants and the relationship with solvents is important to improve the photochemical synthesis process of artemisinin. Herein, we are motivated to measure the reaction rate constants between dihydroartemisinic acid and singlet oxygen in different solvents. Singlet oxygen is produced by irradiation of phenalenone with 355 nm laser, owing to its photochemical stability and poorly quenching effect on singlet oxygen [25, 26]. The 1270 nm near-infrared luminescence from $^1O_2$ is monitored, from which the quenching kinetics of singlet oxygen and the reaction rate constants of dihydroartemisinic acid with singlet oxygen are obtained. Our results reveal that the reaction rate constants of dihydroartemisinic acid with singlet oxygen increase with the solvent polarity. These results may provide fundamental knowledge to optimize the experimental conditions of photochemical synthesis of artemisinin.

## II. EXPERIMENTS

### A. Materials

Dihydroartemisinic acid (DHAA, Bide Pharmatech Ltd., 95%), phenalenone (PN, Sigma-Aldrich, 97%), acetonitrile ($CH_3CN$, Sigma-Aldrich, 99.9%), carbon tetrachloride ($CCl_4$, Sigma-Aldrich, 99%), and dimethyl sulfoxide (DMSO, Sigma-Aldrich, 99.7%) were used as received without further purification. 20 mmol/L solutions of dihydroartemisinic acid in three different solvents, *i.e.*, $CH_3CN$, $CCl_4$ and DMSO, were prepared as the stock solutions. Several concentration gradients of dihydroartemisinic acid in three different kinds of solvent are obtained from the dilution of their stock solutions. The concentration of PN used in the experiments were all adjusted to OD=0.3 at 355 nm unless stated otherwise. The sample solutions were bubbled with $O_2$ for 15 min prior to testing the luminescence kinetics and then maintained under the constant flowing oxygen condition to make sure the experimental conditions were not affected by the concentration of $O_2$. Note that each kinetic measurement was performed with a new sample.

### B. Steady-state spectral measurements

Ground state absorption spectra were recorded with a UV-Vis spectrometer (model U-3010, Hitachi) in 1 cm path length quartz cells.

### C. Transient spectroscopy measurements

Nanosecond time-resolved transient absorption spectra were measured using a nanosecond flash photolysis setup Edinburgh LP920 spectrometer (Edinburgh Instruments Ltd.), combined with a Nd:YAG laser (Surelite II, Continuum Inc.). All sample solutions in 1 cm×1 cm quartz cuvettes, were excited by a 355 nm laser pulse (1 Hz, 10 mJ/pulse, FWHM≈7 ns). The analyzing light was from a 450 W pulsed xenon lamp. A monochromator equipped with a photomultiplier for collecting the spectral range from 300 nm to 800 nm was used to ana-

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lyze transient absorption spectra and provided an overall resolution of approximately 10 ns. Data were analyzed by the online software of the LP920 spectrophotometer [27]. All the experiments were measured at room temperature.

The transient luminescence of $^1O_2$ at 1270 $cm^{-1}$ was recorded using a Edinburgh LP920 luminescence spectrometer equipped with a nitrogen cooled (−85 °C) photomultiplier (Hamamatsu R5509-73, $U$=1500 V), and combined with a Nd:YAG laser (Surelite II, Continuum Inc.), providing an overall resolution of approximately 10 ns. A cutoff optical filter with an 850 nm long-pass filter (Isuzu Optics, LP850) located between the sample and detector were used to cut off any stray light and scattered light with wavelengths shorter than 850 nm. All sample solutions in 1 cm×1 cm quartz cuvettes, were excited by a 355 nm laser pulse (10 Hz, 10 mJ/pulse, FWHM≈7 ns). All the experiments were measured at room temperature.

## III. RESULTS AND DISCUSSION

### A. Generation of singlet oxygen via photosensitization

To investigate the reaction kinetics of dihydroartemisinic acid with singlet oxygen, we firstly need to generate singlet oxygen through triplet-triplet energy transfer between triplet photosensitizer and ground molecular oxygen. Here, the photosensitizer phenalenone is excited by 355 nm laser, and the triplet PN leads to almost unity yields of singlet oxygen [25, 26]. The absorption spectra of PN and dihydroartemisinic acid (DHAA) were measured in $CH_3CN$, respectively. As shown in FIG. 1, PN presents two main absorption peaks at 357 nm and 250 nm, and DHAA only has absorption below 230 nm. Upon the addition of 1 mmol/L DHAA into the PN solution, there was no change in the main absorption peaks at 357 and 250 nm, indicating there were no reactions between ground state PN and DHAA. The 355 nm laser can selectively excite PN, but not DHAA.

Nanosecond transient UV-Vis absorption spectra were measured to monitor the excited state of PN. As shown in FIG. 2, with the excitation of 355 nm laser, a negative absorption peak at 400 nm due to the ground-state bleaching of PN were observed, whereas the other ground-state bleaching bands of PN was overlapped with the excited state absorption bands of PN and thus shielded. There are also two positive peaks at 340 and 380 nm, a positive absorption band at 430−600 nm, and some weak absorbances beyond 600 nm are observed, which are all ascribed to the absorption characterizations of the triplet state of PN. The assignment of the triplet state of PN can be confirmed by comparing the decay curves in $N_2$-saturated and air-saturated conditions (the inset in FIG. 2). A decay lifetime of 1.45 μs is obtained in $N_2$-saturated condition, which is decreased to 253 ns in air-saturated conditions. The decrease of

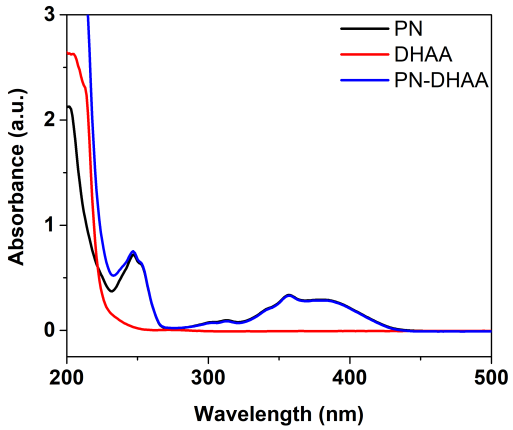


FIG. 1 Steady-state UV-Vis absorption spectra of PN (black), 1 mmol/L dihydroartemisinic acid (DHAA, red), and PN with 1 mmol/L dihydroartemisinic acid (PN-DHAA, blue) in $CH_3CN$.

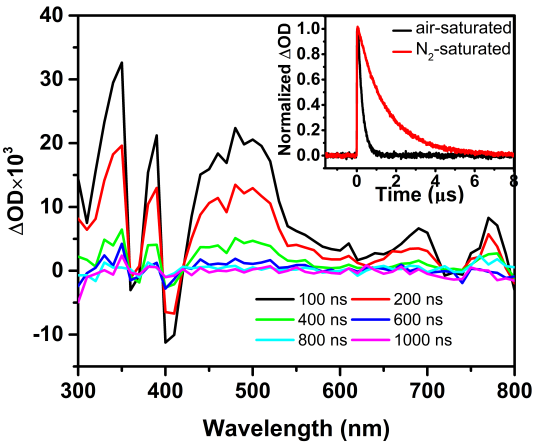


FIG. 2 Transient absorption spectra of PN ($\Delta OD_{355}$=0.3) in $CH_3CN$. The spectra were recorded in air-saturated condition at time delays for 100 ns to 1000 ns using an excitation wavelength of 355 nm. Inset: Decay kinetics of transient absorption at 700 nm under different conditions, air-saturated (black), $N_2$-saturated (red).

lifetime in air-saturated conditions is a typical indicator of triplet sensitizers for the molecular oxygen quenching effect on the triplet state.

Given that triplet state of PN can sensitize oxygen to produce singlet oxygen [25, 26], the time-resolved luminescence spectra were measured upon excitation of PN in $CH_3CN$ under $O_2$-saturated condition by 355 nm laser. As shown in FIG. 3, there is a broad peak at 1270 nm observed in the emission spectra, which is the characteristic luminescence of singlet oxygen, indicating the generation of singlet oxygen. Then, by monitoring the luminescence decay kinetics of singlet oxygen at 1270 nm, the reaction rates between singlet oxygen and DHAA can be investigated.

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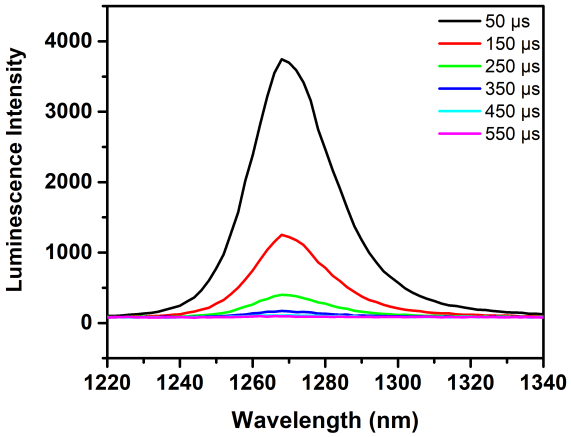


FIG. 3 Singlet oxygen luminescence spectra obtained from the 355 nm photoexcitation of PN in $CH_3CN$.

### B. Reaction rate constant measurements for DHAA with singlet oxygen in $CH_3CN$

The luminescence decay kinetics of singlet oxygen at 1270 nm were investigated upon addition of different concentrations of DHAA into the solution of PN. As is shown in FIG. 4(a), with the increase of DHAA concentration, the luminescence of singlet oxygen at 1270 nm decays faster. In the current experimental conditions, the concentration of DHAA should be much greater than that of the generated singlet oxygen. Therefore, the pseudo-first order reaction approximation can be applied for the reaction of DHAA with $^1O_2$. The decay kinetics of singlet oxygen at 1270 nm can be modeled as a unimolecular process given by a Stern-Volmer relationship, since the rate of singlet oxygen decay displays a linear relationship with concentration,

$$r = r_0 + k_q[\mathrm{DHAA}] \quad (1)$$

where $r$ is the apparent decay rate of singlet oxygen, $r_0$ is intrinsic decay rate of singlet oxygen in $CH_3CN$, and $k_q$ is the reaction rate constant of singlet oxygen with DHAA. Hence, $k_q$, can be determined from the slope of a plot of $r$ versus the concentration of DHAA in solution (FIG. 4(b)). And the extrapolation of the linear plot gives the intrinsic decay rate constant of singlet oxygen ($r_0$) in $CH_3CN$. The reciprocal of $r_0$ is the intrinsic lifetime of singlet oxygen in $CH_3CN$, which is in good agreement with the previously published value [26–29], demonstrating the reliability of our experimental method. The reaction rate constant for DHAA with singlet oxygen in $CH_3CN$ is determined to be $(5.69\pm0.17)\times10^5$ $(\mathrm{mol/L})^{-1}\mathrm{s}^{-1}$. This value indicates that the reaction rate of DHAA with singlet oxygen in $CH_3CN$ is not very fast. Generally, fast reaction rates are desirable to improve the yield of the drug artemisinin, thus the experimental conditions need to be optimized.

TABLE I Reaction rate constants of $^1O_2$ with dihydroartemisinic acid in different solvents.

| Solvents | Polarity/D | $k_q/((\mathrm{mol/L})^{-1}\mathrm{s}^{-1})$ |
|---|---|---|
| $CCl_4$ | 1.6 | $(1.81\pm0.11)\times10^5$ |
| $CH_3CN$ | 6.2 | $(5.69\pm0.17)\times10^5$ |
| DMSO | 7.2 | $(3.27\pm0.18)\times10^6$ |

### C. Solvent effect on the reaction rate constant

The solvent polarity may influence reaction rates. Therefore, we perform the reaction at different solvents with different polarity to explore the solvent effect on the reaction rates of DHAA with singlet oxygen. In the current study, $CCl_4$ and DMSO solvents were chosen to compare with $CH_3CN$. The polarity order is DMSO (7.2 D)>$CH_3CN$ (6.2 D)>$CCl_4$ (1.6 D). The experiments were performed under same conditions, including the DHAA concentration range and laser intensity, and the kinetics curves are presented in FIG. 4. In the absence of DHAA, the intrinsic lifetime of singlet oxygen is obtained to be ∼3636 μs in $CCl_4$, ∼123 μs in $CH_3CN$, and ∼6.7 μs in DMSO. The intrinsic lifetime of singlet oxygen in nonpolar solvent is much longer than that in polar solvents, since the polarity of solvents affects the singlet oxygen radiative deactivation rates [26]. Upon addition of DHAA, the decay rates of singlet oxygen are accelerated due to the chemical quenching. Single exponential fitting of the kinetics curves (FIG. 4 (a), (c), (e)) yield decay rates at various DHAA concentration, and the decay rates of singlet oxygen display a linear relationship with the concentration of DHAA (FIG. 4 (b), (d), (f)). As shown in Table I, in $CCl_4$, the reaction rate constant is obtained to be $(1.81\pm0.11)\times10^5$ $(\mathrm{mol/L})^{-1}\mathrm{s}^{-1}$, which is three times smaller than that in $CH_3CN$. While in the more polar solvent DMSO, the reaction rate constant is $(3.27\pm0.18)\times10^6$ $(\mathrm{mol/L})^{-1}\mathrm{s}^{-1}$, which is almost six times larger than that in $CH_3CN$. These results show that with the increase of solvent polarity, the reaction rate constant for DHAA with singlet oxygen becomes larger. Generally, if the reaction products are more polar than the reactants, a polar solvent may accelerate the reaction [24, 31]. As shown in Scheme 1, the reaction product **2** is more polar than DHAA due to the addition of singlet oxygen, which can explain the solvent polarity effect on the reaction of DHAA with singlet oxygen. Our results certify that with the increase of solvent polarity, the reaction rate of DHAA with singlet oxygen can be increased to $3.27\times10^6$ $(\mathrm{mol/L})^{-1}\mathrm{s}^{-1}$ in DMSO, more than an order of magnitude improvement than that in $CH_3CN$ ($1.81\times10^5$ $(\mathrm{mol/L})^{-1}\mathrm{s}^{-1}$). These results can provide useful guidance for the chemical synthesis of artemisinin. It indicates that if the photochemical synthetic routes are performed in more polar solvents, higher yield of the drug artemisinin can

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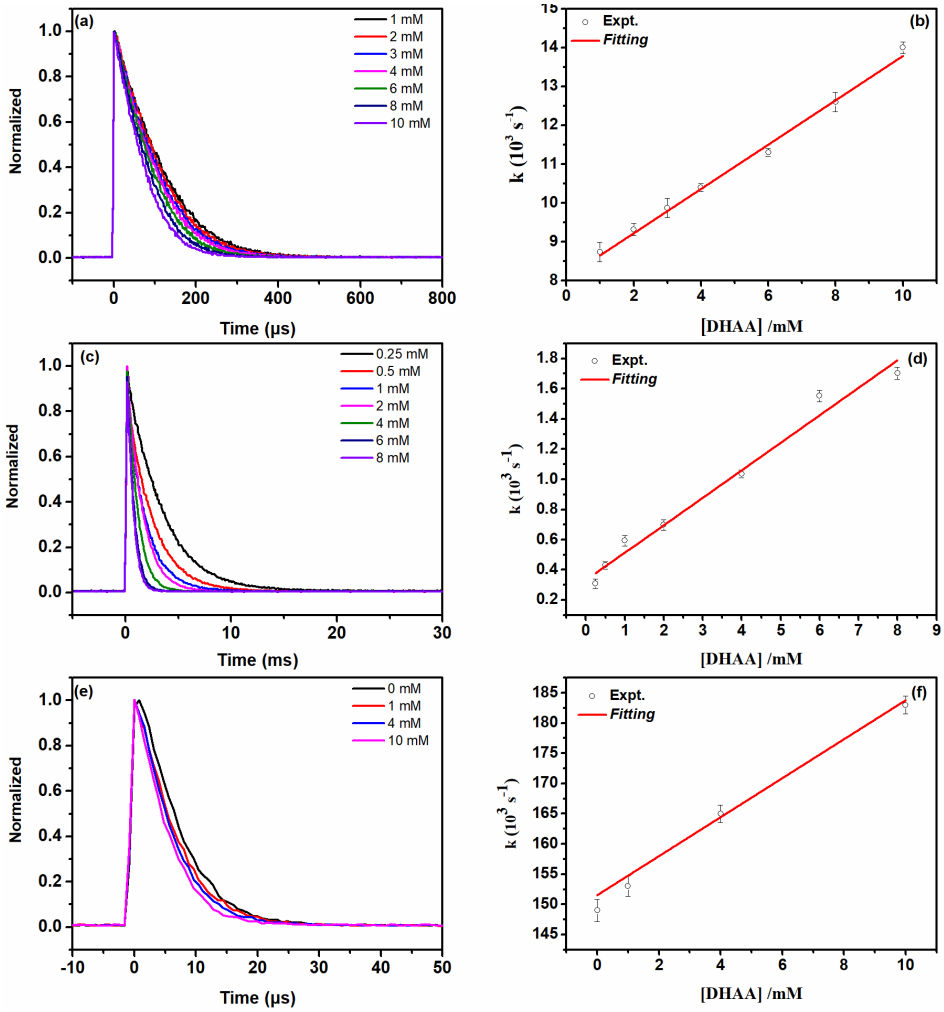


FIG. 4 Singlet oxygen luminescence decay kinetics monitored at 1270 nm obtained from the pulsed photoexcitation (355 nm, 7 ns pulse length, 10 mJ/pulse) of PN with different concentrations of DHAA in (a) $CH_3CN$, (c) $CCl_4$ and (e) DMSO under $O_2$-saturated condition, and the relationship between the decay rate of singlet oxygen and the concentration of DHAA in respective solvents ((b) $CH_3CN$, (d) $CCl_4$, (f) DMSO). The decay rate of singlet oxygen displays a linear increase with the increase concentration of DHAA.

be obtained.

## IV. CONCLUSION

The reaction rate constants of the essential step to the photochemical synthesis of artemisinin, DHAA with singlet oxygen, in different solvents were investigated by directly detecting the luminescence decay kinetics of singlet oxygen at 1270 nm. The reaction rate constant for DHAA with singlet oxygen is $1.81\times10^5$ $(mol/L)^{-1}s^{-1}$ in $CCl_4$, $5.69\times10^5$ $(mol/L)^{-1}s^{-1}$ in $CH_3CN$, and $3.27\times10^6$ $(mol/L)^{-1}s^{-1}$ in DMSO, respectively. It is found that the reaction rate constants of DHAA with singlet oxygen increase with the solvent polarity. According to the experimental results, the reaction rate constants for DHAA with singlet oxygen are not high, which rationalizes the relative low yield of artemisinin obtained from the reported photochemical synthetic routes. In addition, the polarity of the solvent can greatly affect the rate constant of the key reaction. These results provide fundamental knowledge for optimizing experimental conditions of photochemical synthesis of artemisinin. Considering that the photochemical synthesis of artemisinin belongs to a typical singlet oxygen addition reaction, the observation of the solvent effect contributes to further understanding of the reactivity of singlet oxygen in general as an important synthetic strategy.

## V. ACKNOWLEDGMENTS

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