Journal of Visualized Experiments

# Cercosporin-photocatalyzed [4+1]- and [4+2]-Annulations of Azoalkenes under Mild Conditions

--Manuscript Draft--

| | |
|---|---|
| **Article Type:** | Invited Methods Article - JoVE Produced Video |
| **Manuscript Number:** | JoVE60786R2 |
| **Full Title:** | Cercosporin-photocatalyzed [4+1]- and [4+2]-Annulations of Azoalkenes under Mild Conditions |
| **Section/Category:** | JoVE Chemistry |
| **Keywords:** | Perylenequinonoid, Cercosporin, Photocatalysis, 1,2,3-Thiadiazole, 1,4,5,6-Tetrahydropyridazine, Heterocycles |
| **Corresponding Author:** | Yijian Rao<br>Jiangnan University<br>Wuxi, Jiangsu CHINA |
| **Corresponding Author's Institution:** | Jiangnan University |
| **Corresponding Author E-Mail:** | raoyijian@jiangnan.edu.cn |
| **Order of Authors:** | Philippe ICYISHAKA |
| | Chenxing Li |
| | Liushen Lu |
| | Wenhao Bao |
| | Li Jia |
| | Yan Zhang |
| | Yijian Rao |
| **Additional Information:** | |
| **Question** | **Response** |
| Please indicate whether this article will be Standard Access or Open Access. | Standard Access (US$2,400) |
| Please indicate the **city, state/province, and country** where this article will be **filmed**. Please do not use abbreviations. | Lihu Avenue 1800，Wuxi, 214122, P.R.China |

## TITLE:

# Cercosporin-Photocatalyzed [4+1]- and [4+2]-Annulations of Azoalkenes under Mild Conditions

## AUTHORS AND AFFILIATIONS:

Philippe Icyishaka[1]*, Chenxing Li[1]*, Liushen Lu[1], Wenhao Bao[1], Jia Li[1], Yan Zhang[2], Yijian Rao[1]

*These authors contributed equally.

[1]Key Laboratory of Carbohydrate Chemistry and Biotechnology, Ministry of Education, School of Biotechnology, Jiangnan University, Wuxi, China
[2]School of Pharmaceutical Science, Jiangnan University, Wuxi, China

iphitunga@gmail.com
6170201015@stu.jiangnan.edu.cn
7170201045@stu.jiangnan.edu.cn
tzc19931029@163.com
lijiahb@jiangnan.edu.cn
zhangyan@jiangnan.edu.cn
raoyijian@jiangnan.edu.cn

Correspondence to:
Yijian Rao
raoyijian@jiangnan.edu.cn

## KEYWORDS:

Perylenequinonoid, Cercosporin, Photocatalysis, 1,2,3-Thiadiazole, 1,4,5,6-Tetrahydropyridazine, Heterocycles

## SUMMARY:

New routes for the synthesis of nitrogen-containing heterocycles utilizing cercosporin as a metal-free photocatalyst were developed.

## ABSTRACT:

The interest on nitrogen-containing heterocycles has expanded rapidly in the synthetic community since they are important motifs for new drugs. Traditionally, they were synthesized through thermal cycloaddition reactions, whereas today, photocatalysis is preferred due to the mild and efficient conditions. With this focus, a new photocatalytic method for the synthesis of nitrogen–containing heterocycles is highly desired. Here, we report a protocol for the biosynthesis of cercosporin, which could function as a metal-free photocatalyst. We then illustrate cercosporin-photocatalyzed protocols for the synthesis of nitrogen–containing heterocycles 1,2,3-thiadiazoles through annulation of azoalkenes with KSCN, and synthesis of 1,4,5,6-tetrahydropyridazines [4+2] through cyclodimerization of azoalkenes under mild conditions, respectively. As a result, there is a new bridge between the microbial fermentation method and organic synthesis in a mild, cost-effective, environmentally friendly and sustainable

manner.

## INTRODUCTION:

Nitrogen-containing heterocycles have drawn much attention since they are not only important skeletons for a wide range of natural products with bioactivities, but also the synthetic precursors for agrochemicals and drug molecules[1-2]. Among the various *N*-heterocycles, 1,2,3-thiadiazoles[3-4] and 1,4,5,6-tetrahydropyridazines[5-6] are the most important molecules, which are utilized as versatile intermediates in the synthetic chemistry (**Figure 1**). Since modification of their functional groups always induces distinctive pharmacological activities, extensive efforts have been devoted to developing effective strategies for the synthesis of nitrogen-containing heterocycles and they were mostly synthesized through thermal cycloaddition reactions[7-10]. Nowadays, to meet the requirements of sustainable development and green chemistry, photocatalysis has exerted great importance and advantages[11-14], which includes effectiveness[15-19] and avoidance of stoichiometric reagents for the activation[20-21]. The powerful and versatile four-unit intermediates, azoalkenes (1,2-diaza-1,3-dienes)[22-29], have been employed as precursors in metal-based Ru(bpy)$_3$Cl$_2$-photocatalyzed reactions with high efficiency for the annulation of halogeno hydrazine and ketocarbonyls[30]. Furthermore, it was also used in the metal-free Eosin Y photocatalyzed system, but affording the desired product in only 7% yield. Since metal-free photocatalysts show great advantage over transition metal-based photocatalysts, regarding to the environmental factor as well as the cheaper prices[18-19], it is highly important to develop new metal-free photocatalytic systems for the synthesis of *N*-heterocycles.

Cercosporin[31-35], hypocrellin[36-40], elsinochrome[41] and phleichrome[42-43] (**Figure 2**) belong to perylenequinonoid pigments (PQPs) in nature and are produced by endophytic fungi, which have been widely investigated regarding to their photophysical and photobiological properties, and applied in photodynamic therapy and photophysical diagnosis, due to their strong absorption in UV-vis region and unique properties of photosensitization[36,44-47]. Upon irradiation, those PQPs can be prompted to excited state and then generate active species through energy transfer (EnT) and electron transfer (ET)[35,38,44,48-54]. Thus, we envisioned that these natural PQPs may be utilized as “metal-free” photocatalysts to drive organic reactions, which have rarely been investigated[55-59].

Herein, we report the protocol for the biosynthesis of cercosporin from liquid fermentation and then apply it as a metal-free photocatalyst for the [4+1] annulation reaction of azoalkenes and KSCN, as well as the [4+2] cyclodimerization of azoalkenes, which supply 1,2,3-thiadiazoles and 1,4,5,6- tetrahydropyridazines with high efficiency under mild conditions, respectively (**Figure 3**).

## PROTOCOL:

NOTE: $\alpha$-Halo-*N*-acyl-hydrazones were prepared according to a published procedure[60]. All the solvents and other chemical reagents were obtained from commercial sources without further purification. We first described the synthesis of $\alpha$-Halo-*N*-acyl-hydrazones and the biosynthesis of cercosporin as a metal-free photocatalyst. Next, we illustrated the protocols of the cercosporin-photocatalyzed reactions for the synthesis of 1,2,3-thiadiazoles and 1,4,5,6-

tetrahydropyridazines.

CAUTION: All the manipulation should be conducted cautiously wearing gloves, lab-coat, and goggles. It is highly recommended to carefully read the MSDS for each chemical and solvent used in those reactions and purification process. Chemicals can be weighed out on a balance on the bench. All the organic reactions should be set up in the fume hood and the purified process should also be carried out in a fume hood.

## 1. Preparation of *α*-Halo-*N*-acyl-hydrazones

1.1. Weigh out 10 mmol of ketone and 10 mmol of benzoyl hydrazine into a flask.

1.2. Add 20 mL of $CH_3OH$ to the flask.

1.3. Equip the flask with a rubber stopper and a stirring bar.

1.4. Inject 0.25 mL of HCl slowly into the mixture.

1.5. Incubate the flask in the air at room temperature for 4 h.

1.6. Collect the precipitate after reaction by filtration and wash with acetone.

1.7. Dry the product by vacuum and identify by NMR.

## 2. Preparation of cercosporin

2.1. Charge a 3 L shake flask with 1 L of S-7 medium.

2.2. Inoculate the cercosporin-producing strain[56] into the shake flask.

2.3. Culture the mixture under light conditions at 135 R/min, 25 °C for 2 weeks.

2.4. Subject the fermentation broth to vacuum filtration using a vacuum pump to obtain the supernatant and pellet.

2.5. Collect the pellet and dry it in a freeze dryer.

2.6. Extract the pellet and the supernatant separately with 3 x 50 mL of dichloromethane.

2.7. Combine the organic phases and wash with water 2-3 times.

2.8. Concentrate the organic phase under vacuum.

2.9. Re-dissolve the residue with analytical methanol, and filter through a 0.18 µm organic microfiltration membrane.

2.10. Purify the cercosporin with a Sephadex LH-20 column and identify by HPLC.

## 3. Preparation of 1,2,3-thiadiazoles

3.1. Weigh out the $\alpha$-Halo-*N*-acyl-hydrazone (0.2 mmol, 1.0 eq), 1 mg of cercosporin (0.002 mmol, 0.01 equiv.), 27 mg of $^t$BuOK (1.2 equiv) and 39 mg of KSCN (2 equiv) into a 10 mL Schlenk tub equipped with a rubber stopper and a stirring bar.

3.2. Purge the Schlenk tube with $O_2$ three times.

3.3. Inject dry $CH_3CN$ (2 mL) to the Schlenk tube.

3.4. Subject the Schlenk tube to a 5 W blue LED from the bottom for 16 h.

3.5. Wash with 4 x 15 mL of saturated NaCl solution and combine the aqueous phase.

3.6. Re-extract the aqueous phase with 4 x 15 mL of ethyl acetate.

3.7. Combine organic phase and dry with anhydrous $Na_2SO_4$.

3.8. Remove the solvent with vacuum evaporator.

3.9. Purify the product **3** by silica gel column chromatography (eluent, petroleum: ethyl acetate=10:1) and identify by NMR.

## 4. Preparation of 1,4,5,6-tetrahydropyridazine

4.1. Weigh out the α-Halo-N-acyl-hydrazone (0.5 mmol), 2.7 mg of cercosporin (0.01 equiv), and 195 mg of $Cs_2CO_3$ (1.2 equiv) into a 10 mL Schlenk tub equipped with a rubber stopper and a stirring bar.

4.2. Purge the Schlenk tube with $N_2$ three times.

4.3. Inject $CH_3CN/H_2O$ (10:1, 2 mL) to the Schlenk tube.

4.4. Subject the Schlenk tube to a 5 W blue LED from the bottom for 16 h.

4.5. Wash with 4 x 15 mL of saturated NaCl solution and combine the aqueous phase.

4.6. Re-extract the aqueous phase with 4 x 15 mL of ethyl acetate.

4.7. Combine organic phase and dry with anhydrous $Na_2SO_4$.

4.8. Remove the solvent with vacuum evaporator.

4.9. Purify the product **4** by silica gel column chromatography (eluent, petroleum: ethyl acetate=10:1) and identify by NMR.

## REPRESENTATIVE RESULTS:

**Synthesis of *α*-Halo-*N*-acyl-hydrazones:** They are synthesized according to Protocol 1.

**Synthesis of cercosporin:** It was synthesized and purified according to Protocol 2. $^1$H NMR (400 MHz, $CDCl_3$): δ ppm 14.82 (s, 2H, ArH), 7.06 (s, 2H, ArH), 5.57 (s, 2H, $CH_2$), 4.20 (s, 6H, $2OCH_3$), 3.62-3.57 (m, 2H, $CH_2$), 3.42-3.37 (m, 2H, $CH_2$), 2.93-2.88 (m, 2H, $CH_2$), 0.63 (d, 6H, J = 8 Hz, $2CH_3$) (**Figure 4**). $^{13}$C NMR (101 MHz, $CDCl_3$): δ ppm 207.0, 181.8, 167.4, 163.4, 152.8, 135.4, 130.6, 127.9, 112.9, 109.3, 108.2, 92.6, 68.1, 61.2, 42.2, 19.3. HRMS (ESI-Q-TOF) exact mass calcd for $C_{29}H_{25}O_{10}$ $[M - H]^-$ 533.1448, found 533.1468.

**Synthesis of 4-Phenyl-1,2,3-thiadiazole (3a)**: It was synthesized and purified using Protocol 3 with 88% yield. $^1$H NMR (400 MHz, $CDCl_3$): δ ppm 8.66 (s, 1H), 8.07-8.05 (m, 2H), 7.55-7.44 (m, 3H) (**Figure 5**). $^{13}$C NMR (100 MHz, $CDCl_3$): δ ppm 162.9, 130.8, 129.9, 129.4, 129.2, 127.4 (**Figure 6**). HRMS (ESI-Q-TOF) exact mass calcd for $C_8H_7N_2S$ $[M+H]^+$ 162.0330, found 163.0349.

**Synthesis of 4-(4-Fluorophenyl)-1,2,3-thiadiazole (3b):** It was synthesized and purified using Protocol 3 with 72% yield. $^1$H NMR (400 MHz, $CDCl_3$): δ ppm 8.60 (s, 1H), 8.09-8.02 (m, 2H), 7.19-7.19 (m, 2H). $^{13}$C NMR (100 MHz, $CDCl_3$): δ ppm 164.3-161.9 (d, $J_{C\text{-}F}$ = 240 Hz), 161.3, 133.6, 129.8 (d, $J_{C\text{-}F}$ = 9.0 Hz), 127.8 (d, $J_{C\text{-}F}$ = 3.0 Hz), 116.7 (d, $J_{C\text{-}F}$ = 22.0 Hz). HRMS (ESI-Q-TOF) exact mass calcd for $C_8H_6FN_2S$ $[M+H]^+$ 181.0196, found 181.0191.

**Synthesis of 4-(4-Chlorophenyl)-1,2,3-thiadiazole (3c):** It was synthesized and purified using Protocol 3 with 87% yield. $^1$H NMR (400 MHz, $CDCl_3$): δ ppm 8.65 (s, 1H), 8.00 (d, J = 8 Hz, 2H), 7.50 (d, *J* = 8 Hz, 2H). $^{13}$C NMR (100 MHz, $CDCl_3$): δ ppm 162.6, 135.5, 132.4, 129.4, 128.9, 128.7. HRMS (ESI-Q-TOF) exact mass calcd for $C_8H_6ClN_2S$ $[M+H]^+$ 196.9940, found 196.9940.

**Synthesis of 4-(4-Bromophenyl)-1,2,3-thiadiazole(3d):** It was synthesized and purified using Protocol 3 with 78% yield. $^1$H NMR (400 MHz, $CDCl_3$): δ ppm 8.66 (s, 1H), 7.94 (d, *J* = 8 Hz, 2H), 7.65 (d, *J* = 8 Hz, 2H). $^{13}$C NMR (100 MHz, $CDCl_3$): δ ppm 161.2, 134.3, 132.7, 130.4, 129.6, 119.1. HRMS (ESI-Q-TOF) exact mass calcd for $C_8H_6BrN_2S$ $[M+H]^+$ 240.9435, found 240.9429.

**Synthesis of (3,6-Diphenyl-5,6-dihydropyridazin-1(4H)-yl)(phenyl)methanone (4a):** It was synthesized and purified using Protocol 4 with 80% yield. $^1$H NMR (400 MHz, $CDCl_3$): δ ppm 7.84-7.82 (m, 2H), 7.60-7.58 (m, 2H), 7.49-7.44 (m, 3H), 7.33-7.30 (m, 5H), 7.26-7.24 (m, 1H), 7.18 (d, *J* = 8 Hz, 2H), 6.09 (s, 1H), 2.71-2.67 (m, 1H), 2.43-2.16 (m, 3H) (**Figure 7**).

**Synthesis of (3,6-Bis(4-fluorophenyl)-5,6-dihydropyridazin-1(4H)-yl) (phenyl)methanone (4b):** It was synthesized and purified using Protocol 4 with 72% yield. $^1$H NMR (400 MHz, $CDCl_3$): δ ppm

7.80-7.78 (m, 2H), 7.57-7.55 (m, 2H), 7.52-7.43 (m, 3H), 7.16-7.12 (m, 2H), 7.03-6.97 (m, 4H), 6.05 (s, 1H), 2.69-2.65 (m, 1H), 2.40-2.25 (m, 2H), 2.18-2.13 (m, 1H). $^{13}$C NMR (100 MHz, CDCl$_3$): δ ppm 170.2, 163.4 (d, $^1J_{C\text{-}F}$ = 248.1 Hz), 162.0 (d, $^1J_{C\text{-}F}$ = 244.1 Hz), 146.0, 135.5 (d, $^4J_{C\text{-}F}$ = 3.1 Hz), 135.1, 133.2 (d, $^4J_{C\text{-}F}$ = 3.2 Hz), 130.4, 129.9, 127.5, 127.2 (d, $^3J_{C\text{-}F}$ = 8.2 Hz), 127.1 (d, $^3J_{C\text{-}F}$ = 8.0 Hz), 115.7 (d, $^2J_{C\text{-}F}$ = 21.5 Hz), 115.4 (d, $^2J_{C\text{-}F}$ = 21.6 Hz), 50.9, 24.0, 18.7. $^{19}$F NMR (376 MHz, CDCl$_3$) (ppm) -111.7, -115.5. HRMS (ESI-Q-TOF) exact mass calcd for $C_{23}H_{19}F_2N_2O$ [M+H]$^+$ 377.1465, found 377.1482.

**Synthesis of (3,6-Bis(4-chlorophenyl)-5,6-dihydropyridazin-1(4H)-yl)(phenyl)methanone (4c):** It was synthesized and purified using Protocol 4 with 70% yield. $^1$H NMR (400 MHz, CDCl$_3$): δ ppm 7.78 (d, *J* = 4 Hz, 2H), 7.50-7.43 (m, 5H), 7.30-7.26 (m, 5H), 7.10 (d, *J* = 8 Hz, 2H), 6.03 (s, 1H), 2.68-2.63 (m, 1H), 2.39-2.26 (m, 2H), 2.20-2.11 (m, 1H). $^{13}$C NMR (100 MHz, CDCl$_3$): δ ppm 170.2, 145.8, 138.3, 135.4, 135.3, 134.9, 133.2, 130.5, 129.9, 129.0, 128.6, 127.5, 126.9, 126.6, 51.2, 29.7, 19.8, 18.6. HRMS (ESI-Q-TOF) exact mass calcd for $C_{23}H_{19}Cl_2N_2O$ [M+H]$^+$ 409.0874, found 409.0864.

**Synthesis of (3,6-Bis(4-bromophenyl)-5,6-dihydropyridazin-1(4H)-yl)(phenyl)methanone (4d):** It was synthesized and purified using Protocol 4 with 82% yield. $^1$H NMR (400 MHz, CDCl$_3$): δ ppm 7.78 (d, *J* = 8 Hz, 2H), 7.52-7.40 (m, 9H), 7.04 (d, *J* = 8 Hz, 2H), 6.01 (s, 1H), 2.67-2.62 (m, 1H), 2.39-2.25 (m, 2H), 2.20-2.11 (m, 1H). $^{13}$C NMR (100 MHz, CDCl$_3$): δ ppm 170.2, 145.9, 138.9, 135.8, 134.8, 132.0, 131.6, 130.5, 129.9, 127.5, 127.2, 126.9, 119.6, 121.2, 51.3, 29.7, 19.8, 18.5. HRMS (ESI-Q-TOF) exact mass calcd for $C_{23}H_{19}Br_2N_2O$ [M+H]$^+$ 498.9845, found 498.9799.

These representative results demonstrate how 4-aryl-1,2,3-thiadiazoles and 1,4,5,6-tetrahydropyridazines can be conveniently synthesized by cercosporin-catalyzed photocatalytic reactions from $\alpha$-Halo-*N*-acyl-hydrazone (**Figure 8**).

4-aryl-1,2,3-thiadiazoles were obtained with those conditions: **1** (0.2 mmol), KSCN (0.4 mmol), $^t$BuOK (0.24 mmol), $CH_3CN$ (2.0 mL), cercosporin (1 mol%), 5 W blue LEDs, 16 h, at room temperature under $O_2$ atmosphere (**Figure 3** and **Figure 8**). The procedure was suitable for substrates bearing both electron-donating and electron-accepting groups on the phenyl ring, providing the desired products with moderate to good yields.

1,4,5,6-tetrahydropyridazines were obtained with those conditions: **1** (0.5 mmol), $Cs_2CO_3$ (1.2 equiv) and cercosporin (1 mol%) in the mixture of MeCN and $H_2O$ (10:1) under $N_2$ atmosphere (**Figure 3** and **Figure 8**). The desired products were obtained in good to excellent yields.

**Figure 1. Bioactive molecules with N-heterocycles motifs.** Adapted with permission from Zhang Y., Cao Y., Lu L. S., Zhang S. W., Bao W. H., Huang S. P., Rao Y. J. Perylenequinonoid-Catalyzed [4+ 1]-and [4+ 2]-Annulations of Azoalkenes: Photocatalytic Access to 1, 2, 3-Thiadiazole/1, 4, 5, 6-Tetrahydropyridazine Derivatives, Journal of Organic. Chemistry. 84 (12), 7711-7721, (2019). Copyright (2019) American Chemical Society.

**Figure 2. Representative perylenequinonoid pigments in nature.** Adapted with permission from Zhang Y., Cao Y., Lu L. S., Zhang S. W., Bao W. H., Huang S. P., Rao Y. J. Perylenequinonoid-

Catalyzed [4+ 1]-and [4+ 2]-Annulations of Azoalkenes: Photocatalytic Access to 1, 2, 3-Thiadiazole/1, 4, 5, 6-Tetrahydropyridazine Derivatives, Journal of Organic. Chemistry. 84 (12), 7711-7721, (2019). Copyright (2019) American Chemical Society.

**Figure 3. Cercosporin-Catalyzed Synthesis of 1,2,3-Thiadiazoles and 1,4,5,6-Tetrahydropyridazines.** Adapted with permission from Zhang Y., Cao Y., Lu L. S., Zhang S. W., Bao W. H., Huang S. P., Rao Y. J. Perylenequinonoid-Catalyzed [4+ 1]-and [4+ 2]-Annulations of Azoalkenes: Photocatalytic Access to 1, 2, 3-Thiadiazole/1, 4, 5, 6-Tetrahydropyridazine Derivatives, Journal of Organic. Chemistry. 84 (12), 7711-7721, (2019). Copyright (2019) American Chemical Society.

**Figure 4. $^{1}$H-NMR spectrum of cercosporin (400 MHz, $CDCl_3$).** Reprinted with permission from Zhang Y., Cao Y., Lu L. S., Zhang S. W., Bao W. H., Huang S. P., Rao Y. J. Perylenequinonoid-Catalyzed [4+ 1]-and [4+ 2]-Annulations of Azoalkenes: Photocatalytic Access to 1, 2, 3-Thiadiazole/1, 4, 5, 6-Tetrahydropyridazine Derivatives, Journal of Organic. Chemistry. 84 (12), 7711-7721, (2019). Copyright (2019) American Chemical Society.

**Figure 5. Representative $^{1}$H-NMR spectrum of 3a (400 MHz, $CDCl_3$).** Reprinted with permission from Zhang Y., Cao Y., Lu L. S., Zhang S. W., Bao W. H., Huang S. P., Rao Y. J. Perylenequinonoid-Catalyzed [4+ 1]-and [4+ 2]-Annulations of Azoalkenes: Photocatalytic Access to 1, 2, 3-Thiadiazole/1, 4, 5, 6-Tetrahydropyridazine Derivatives, Journal of Organic. Chemistry. 84 (12), 7711-7721, (2019). Copyright (2019) American Chemical Society.

**Figure 6. Representative $^{13}$C-NMR spectrum of 3a (400 MHz, $CDCl_3$).** Reprinted with permission from Zhang Y., Cao Y., Lu L. S., Zhang S. W., Bao W. H., Huang S. P., Rao Y. J. Perylenequinonoid-Catalyzed [4+ 1]-and [4+ 2]-Annulations of Azoalkenes: Photocatalytic Access to 1, 2, 3-Thiadiazole/1, 4, 5, 6-Tetrahydropyridazine Derivatives, Journal of Organic. Chemistry. 84 (12), 7711-7721, (2019). Copyright (2019) American Chemical Society.

**Figure 7. Representative $^{1}$H-NMR spectrum of 4a (400 MHz, $CDCl_3$).** Reprinted with permission from Zhang Y., Cao Y., Lu L. S., Zhang S. W., Bao W. H., Huang S. P., Rao Y. J. Perylenequinonoid-Catalyzed [4+ 1]-and [4+ 2]-Annulations of Azoalkenes: Photocatalytic Access to 1, 2, 3-Thiadiazole/1, 4, 5, 6-Tetrahydropyridazine Derivatives, Journal of Organic. Chemistry. 84 (12), 7711-7721, (2019). Copyright (2019) American Chemical Society.

**Figure 8. Cercosporin-Catalyzed Synthesis of 4-aryl-1,2,3-thiadiazoles and 1,4,5,6-tetrahydropyridazines.** Adapted with permission from Zhang Y., Cao Y., Lu L. S., Zhang S. W., Bao W. H., Huang S. P., Rao Y. J. Perylenequinonoid-Catalyzed [4+ 1]-and [4+ 2]-Annulations of Azoalkenes: Photocatalytic Access to 1, 2, 3-Thiadiazole/1, 4, 5, 6-Tetrahydropyridazine Derivatives, Journal of Organic. Chemistry. 84 (12), 7711-7721, (2019). Copyright (2019) American Chemical Society.

## DISCUSSION:

Nitrogen-containing heterocycles are important motifs for many new drugs and were traditionally synthesized through thermal cycloaddition reactions. Due to great interest, a new photocatalytic method for the synthesis of these compounds is highly desired. To take advantage of the excellent photosensitization properties of cercosporin, we applied cercosporin as a metal-free photocatalyst in two categories of annulation reactions to synthesize nitrogen–containing heterocycles.

First, we reported the protocol of cercosporin-photocatalyzed [4+1] annulation of azoalkenes with KSCN under standard conditions: α-halo-Nacyl-hydrazone **1** (0.2 mmol), $^t$BuOK (1.2 equiv), KSCN **2** (2 equiv), cercosporin (0.01 equiv), dry $CH_3CN$ (2 mL), and the resulting mixtures were subjected to 5 W blue LED for 16 h under an $O_2$ atmosphere. KSCN functionalized as an ambident nucleophilic unit here. Cercosporin, $^t$BuOK, blue light and $O_2$ were all prerequisites for this reaction. $CH_3CN$ supplied the best yield of product and 0.01 equiv. of cercosporin was the optimized ratio.

Second, we reported the protocol of cercosporin-photocatalyzed [4+2] annulation of azoalkenes under standard conditions: α-halo-*N*-acyl-hydrazone **1** (0.5 mmol), $Cs_2CO_3$ (1.2 equiv), cercosporin (0.01 equiv) ($CH_3CN/H_2O$ = 10:1) 2 mL, and the resulting mixtures were subjected to a 5 W blue LED for 16 h under a $N_2$ atmosphere. The control experiments have been done for the [4+2] reaction as it was for the [4+1] reaction. In this protocol, the addition of water and $Cs_2CO_3$ was critical for the self-condensation of α-halo-*N*-acyl-hydrazone. The ratios of water and $Cs_2CO_3$ were also critical to provide best yield for the product.

In summary, we have reported the biosynthesis protocol for cercosporin and then applied it as a metal-free photocatalyst for the synthesis of *N*-heterocycles 4-aryl-1,2,3-thiadiazoles and 1,4,5,6-tetrahydropyridazines under mild conditions, through [4+1] annulation of azoalkenes with KSCN and [4+2] annulation of azoalkenes, respectively. Those reactions made use of cost-effective 5 W LED and could be processed easily, which supplied a new application in synthesis. Most importantly, we built a bridge between biosynthesis and organic synthesis for the design of N-heterocycles in a mild, cost-effective, environmentally friendly and sustainable manner.

## ACKNOWLEDGMENTS:

We thank for the National Key R&D Program of China (2018YFA0901700), Natural Science Foundation of Jiangsu Province (Grants No. BK20160167), the Thousand Talents Plan (Young Professionals), the Fundamental Research Funds for the Central Universities (JUSRP51712B), the National First-class Discipline Program of Light Industry Technology and Engineering (LITE2018-14) and Postdoctoral Foundation in Jiangsu Province (2018K153C) for the funding support.

## DISCLOSURES:

The authors have nothing to disclose.

## REFERENCES:

(1) Majumdar, K. C., Chattopadhyay, S. K. *Heterocycles in Natural Product Synthesis*; 1st ed. Wiley-VCH (2011).

(2) Taylor, R. D., MacCoss, M., Lawson, A. D. Rings in drugs. *Journal of Medicinal Chemistry.* **57** (14), 5845-5859 (2014).

(3) Bakulev, V. A., Dehaen, W. *The Chemistry of 1,2,3-Thiadiazoles*. John Wiley & Sons (2004).

(4) Dong, W. L., Liu, Z. X., Liu, X. H., Li, Z. M., Zhao, W. G. Synthesis and antiviral activity of new acrylamide derivatives containing 1,2,3-thiadiazole as inhibitors of hepatitis B virus replication. *European Journal of Medicinal Chemistry.* **45** (5), 1919-1926 (2010).

(5) Combs, D. W., Reese, K., Phillips, A. Nonsteroidal Progesterone-Receptor Ligands .1. 3-Aryl-1-Benzoyl-1,4,5,6-Tetrahydropyridazines. *Journal of Medicinal Chemistry.* **38** (25), 4878-4879 (1995).

(6) Combs, D. W. et al. Nonsteroidal Progesterone-Receptor Ligands .2. High-Affinity Ligands with Selectivity for Bone Cell Progesterone Receptors. *Journal of Medicinal Chemistry.* **38** (25), 4880-4884 (1995).

(7) Xu, S. L., Chen, R. S., Qin, Z. F., Wu, G. P., He, Z. J. Divergent Amine-Catalyzed [4+2] Annulation of Morita-Baylis-Hillman Allylic Acetates with Electron-Deficient Alkenes. *Organic Letters.* **14** (4), 996-999 (2012).

(8) Ishikawa, T., Kimura, M., Kumoi, T., Iida, H. Coupled Flavin-Iodine Redox Organocatalysts: Aerobic Oxidative Transformation from N-Tosylhydrazones to 1,2,3-Thiadiazoles. *ACS Catalysis.* **7** (8), 4986-4989 (2017).

(9) Chen, J. F., Jiang, Y., Yu, J. T., Cheng, J. TBAI-Catalyzed Reaction between N-Tosylhydrazones and Sulfur: A Procedure toward 1,2,3-Thiadiazole. *Journal of Organic Chemistry.* **81** (1), 271-275 (2016).

(10) Liu, B. B., Bai, H. W., Liu, H., Wang, S. Y., Ji, S. J. Cascade Trisulfur Radical Anion (S3(*-)) Addition/Electron Detosylation Process for the Synthesis of 1,2,3-Thiadiazoles and Isothiazoles. *Journal of Organic Chemistry.* **83** (17), 10281-10288 (2018).

(11) Staveness, D., Bosque, I., Stephenson, C. R. J. Free Radical Chemistry Enabled by Visible Light-Induced Electron Transfer. *Accounts of Chemical Research.* **49** (10), 2295-2306 (2016).

(12) Corrigan, N., Shanmugam, S., Xu, J. T., Boyer, C. Photocatalysis in organic and polymer synthesis. *Chemical Society Reviews.* **45** (22), 6165-6212 (2016).

(13) Shaw, M. H., Twilton, J., MacMillan, D. W. C. Photoredox Catalysis in Organic Chemistry. *Journal of Organic Chemistry.* **81** (16), 6898-6926 (2016).

(14) Marzo, L., Pagire, S. K., Reiser, O., Konig, B. Visible-Light Photocatalysis: Does It Make a Difference in Organic Synthesis? *Angewandte Chemie-International Edition.* **57** (32), 10034-10072 (2018).

(15) Prier, C. K., Rankic, D. A., MacMillan, D. W. C. Visible Light Photoredox Catalysis with Transition Metal Complexes: Applications in Organic Synthesis. *Chemical Reviews.* **113** (7), 5322-5363 (2013).

(16) Reckenthaler, M., Griesbeck, A. G. Photoredox Catalysis for Organic Syntheses. *Advanced Synthesis & Catalysis.* **355** (14-15), 2727-2744 (2013).

(17) Nicewicz, D. A., Nguyen, T. M. Recent Applications of Organic Dyes as Photoredox Catalysts in Organic Synthesis. *ACS Catalysis.* **4** (1), 355-360 (2014).

(18) Pitre, S. P., McTiernan, C. D., Scaiano, J. C. Understanding the Kinetics and Spectroscopy of Photoredox Catalysis and Transition-Metal-Free Alternatives. *Accounts of Chemical Research.* **49** (6), 1320-1330 (2016).

(19) Romero, N. A., Nicewicz, D. A. Organic Photoredox Catalysis. *Chemical Reviews.* **116** (17), 10075-10166 (2016).

(20) Albini, A., Fagnoni, M. *Photochemically-Generated Intermediates in Synthesis*; John Wiley & Sons (2013).

(21) Chen, J. R., Hu, X. Q., Lu, L. Q., Xiao, W. J. Exploration of Visible-Light Photocatalysis in Heterocycle Synthesis and Functionalization: Reaction Design and Beyond. *Accounts of Chemical Research.* **49** (9), 1911-1923 (2016).

(22) Attanasi, O. A. et al. Cultivating the Passion to Build Heterocycles from 1,2-Diaza-1,3-dienes: the Force of Imagination. *European Journal of Organic Chemistry.* **19**, 3109-3127 (2009).

(23) Attanasi, O. A., Filippone, P. Working twenty years on conjugated azo-alkenes (and environs) to find new entries in organic synthesis. *Synlett.* **10**, 1128-1140 (1997).

(24) Deng, Y., Pei, C., Arman, H., Dong, K., Xu, X., Doyle, M. P. Syntheses of Tetrahydropyridazine and Tetrahydro-1,2-diazepine Scaffolds through Cycloaddition Reactions of Azoalkenes with Enol Diazoacetates. *Organic Letters.* **18** (22), 5884-5887 (2016).

(25) Guo, C., Sahoo, B., Daniliuc, C. G., Glorius, F. N-heterocyclic carbene catalyzed switchable reactions of enals with azoalkenes: formal [4 + 3] and [4 + 1] annulations for the synthesis of 1,2-diazepines and pyrazoles. *Journal of American Chemistry Society.* **136** (50), 17402-17405 (2014).

(26) Attanasi, O. A. et al. Interceptive [4 + 1] annulation of in situ generated 1,2-diaza-1,3-dienes with diazo esters: direct access to substituted mono-, bi-, and tricyclic 4,5-dihydropyrazoles. *Journal of Organic Chemistry.* **79** (17), 8331-8338 (2014).

(27) Li, J., Huang, R., Xing, Y. K., Qiu, G., Tao, H. Y., Wang, C. J. Catalytic Asymmetric Cascade Vinylogous Mukaiyama 1,6-Michael/Michael Addition of 2-Silyloxyfurans with Azoalkenes: Direct Approach to Fused Butyrolactones. *Journal of the American Chemical Society.* **137** (32), 10124-10127 (2015).

(28) Huang, R., Chang, X., Li, J., Wang, C. J. Cu(I)-Catalyzed Asymmetric Multicomponent Cascade Inverse Electron-Demand Aza-Diels-Alder/Nucleophilic Addition/Ring-Opening Reaction Involving 2-Methoxyfurans as Efficient Dienophiles. *Journal of the American Chemical Society.* **138** (12), 3998-4001 (2016).

(29) Tong, M. C. et al. Catalytic asymmetric synthesis of [2,3]-fused indoline heterocycles through inverse-electron-demand aza-Diels-Alder reaction of indoles with azoalkenes. *Angew Chemistry International Edition English.* **53** (18), 4680-4684 (2014).

(30) Yu, J. M., Lu, G. P., Cai, C. Photocatalytic radical cyclization of alpha-halo hydrazones with beta-ketocarbonyls: facile access to substituted dihydropyrazoles. *Chemistry Communication (Camb).* **53** (38), 5342-5345 (2017).

(31) Kuyama, S., Tamura, T. Cercosporin. A pigment of Cercosporina kikuchii Matsumoto et Tomoyasu. I. Cultivation of fungus, isolation and purification of pigment. *Journal of the American Chemical Society.* **79** (21), 5725-5726 (1957).

(32) Kuyama, S., Tamura, T. Cercosporin. A pigment of Cercosporina kikuchii Matsumoto et Tomoyasu. II. Physical and chemical properties of cercosporin and its derivatives. *Journal of the American Chemical Society.* **79** (21), 5726-5729 (1957).

(33) Daub, M. E. Resistance of fungi to the photosensitizing toxin, cercosporin. *Phytopathology.* **77** (11), 1515-1520 (1987).

(34) Jalal, M. A. F., Hossain, M. B., Robeson, D. J., Vanderhelm, D. Cercospora-Beticola Phytotoxins - Cebetins That Are Photoactive, $Mg^{2+}$-Binding, Chlorinated Anthraquinone Xanthone Conjugates. *Journal of the American Chemical Society.* **114** (15), 5967-5971 (1992).

(35) Daub, M. E., Ehrenshaft, M. The photoactivated Cercospora toxin cercosporin: Contributions to plant disease and fundamental biology. *Annual Review of Phytopathology.* **38** (1), 461-490 (2000).

(36) Diwu, Z. J., Lown, J. W. Photosensitization with Anticancer Agents .14. Perylenequinonoid Pigments as New Potential Photodynamic Therapeutic Agents - Formation of Tautomeric Semiquinone Radicals. *Journal of Photochemistry and Photobiology A-Chemistry.* **69** (2), 191-199 (1992).

(37) Hu, Y. Z., An, J. Y., Jiang, L. J., Chen, D. W. Spectroscopic Study on the Photoreduction of Hypocrellin-a - Generation of Semiquinone Radical-Anion and Hydroquinone. *ournal of Photochemistry and Photobiology A-Chemistry.* **89** (1), 45-51 (1995).

(38) Hu, Y. Z., Jiang, L. J., Chiang, L. C. Characteristics of the reaction between semiquinone radical anion of hypocrellin A and oxygen in aprotic media. *Journal of Photochemistry and Photobiology A-Chemistry.* **94** (1), 37-41 (1996).

(39) Zhang, M. H. et al. Study of electron transfer interaction between hypocrellin and N,N-diethylaniline by UV-visible, fluorescence, electron spin resonance spectra and time-resolved transient absorption spectra. *Journal of Photochemistry and Photobiology A-Chemistry.* **96** (1-3), 57-63 (1996).

(40) He, Y. Y., An, J. Y., Jiang, L. J. pH Effect on the spectroscopic behavior and photoinduced generation of semiquinone anion radical of hypocrellin B. *Dyes and Pigments.* **41** (1-2), 79-87 (1999).

(41) Li, C. et al. Photophysical and photosensitive properties of Elsinochrome A. *Chinese Science Bulletin.* **51** (9), 1050-1054 (2006).

(42) So, K. K. et al. Improved production of phleichrome from the phytopathogenic fungus Cladosporium phlei using synthetic inducers and photodynamic ROS production by phleichrome. *Journal of Bioscience and Bioengineering.* **119** (3), 289-296 (2015).

(43) Hudson, J. B., Imperial, V., Haugland, R. P., Diwu, Z. Antiviral activities of photoactive perylenequinones. *Photochemistry and Photobiology.* **65** (2), 352-354 (1997).

(44) Diwu, Z. J., Lown, J. W. Photosensitization by Anticancer Agents .12. Perylene Quinonoid Pigments, a Novel Type of Singlet Oxygen Sensitizer. *Journal of Photochemistry and Photobiology A-Chemistry.* **64** (3), 273-287 (1992).

(45) Diwu, Z. J., Zimmermann, J., Meyer, T., Lown, J. W. Design, Synthesis and Investigation of Mechanisms of Action of Novel Protein-Kinase-C Inhibitors - Perylenequinonoid Pigments. *Biochemical Pharmacology.* **47** (2), 373-385 (1994).

(46)Guedes, R. C., Eriksson, L. A. Photophysics, photochemistry, and reactivity: Molecular aspects of perylenequinone reactions. *Photochemical & Photobiological Sciences.* **6** (10), 1089-1096 (2007).

(47) Mulrooney, C. A., O'Brien, E. M., Morgan, B. J., Kozlowski, M. C. Perylenequinones: Isolation, Synthesis, and Biological Activity. *European Journal of Organic Chemistry. (21), 3887-3904 (2012).*

(48) Daub, M. E., Hangarter, R. P. Light-induced production of singlet oxygen and superoxide by the fungal toxin, cercosporin. *Plant Physiololgy.* **73** (3), 855-857 (1983).

(49) Daub, M. E., Leisman, G. B., Clark, R. A., Bowden, E. F. Reductive Detoxification as a Mechanism of Fungal Resistance to Singlet Oxygen-Generating Photosensitizers. *Proceedings of the National Academy of Sciences of the United States of America.* ***89*** *(20), 9588-9592 (1992).*

(50) Leisman, G. B., Daub, M. E. Singlet Oxygen Yields, Optical-Properties, and Phototoxicity of Reduced Derivatives of the Photosensitizer Cercosporin. *Photochemistry Photobiology,* **55** (3), 373-379 (1992).

(51) Bilski, P., Li, M. Y., Ehrenshaft, M., Daub, M. E., Chignell, C. F. Vitamin B6 (pyridoxine) and its derivatives are efficient singlet oxygen quenchers and potential fungal antioxidants. *Photochemistry Photobiology.* **71** (2), 129-134 (2000).

(52) Xing, M. Z., Zhang, X. Z., Sun, Z. L., Zhang, H. Y. Perylenequinones act as broad-spectrum fungicides by generating reactive oxygen species both in the dark and in the light. *Journal of Agricultural and Food Chemistry.* **51** (26), 7722-7724 (2003).

(53) Weng, M., Zhang, M. H., Shen, T. Electron transfer interaction between hypocrellin A and biological substrates and quantitative analysis of superoxide anion radicals. *Journal of the Chemical Society-Perkin Transactions.* **2** (11), 2393-2397 (1997).

(54) Daub, M. E., Li, M., Bilski, P., Chignell, C. F. Dihydrocercosporin singlet oxygen production and subcellular localization: A possible defense against cercosporin phototoxicity in Cercospora. *Photochemistry and Photobiology.* **71** (2), 135-140 (2000).

(55) Zhang, S. W. et al. Perylenequinonoid-catalyzed photoredox activation for the direct arylation of (het)arenes with sunlight, *Organic & Biomolecular Chemistry*. **17** (17), 4364-4369 (2019).

(56) Zhang, Y. et al. Perylenequinonoid-Catalyzed [4+ 1]-and [4+ 2]-Annulations of Azoalkenes: Photocatalytic Access to 1, 2, 3-Thiadiazole/1, 4, 5, 6-Tetrahydropyridazine Derivatives, *Journal of Organic Chemistry,* **84** (12), 7711-7721 (2019).

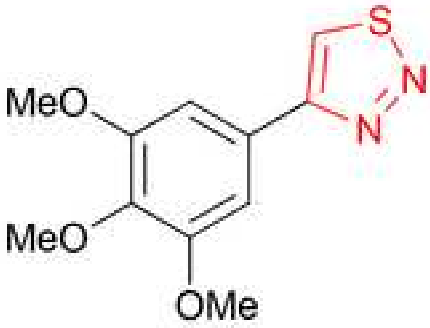
(57) Li, J. et al. Cercosporin-Bioinspired Selective Photooxidation Reactions under Mild Conditions, *Green Chemistry*. **21** (22), 6073-6081 (2019).

(58) Tang, Z. et al. Cercosporin-bioinspired photoreductive activation of aryl halides under mild conditions. *Journal of Catalysis.* **380**, 1-8 (2019).

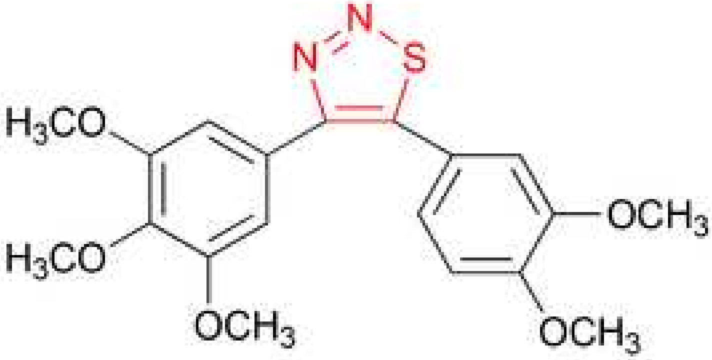
(59) Li, J., Bao, W., Zhang, Y., Rao, Y. Cercosporin-photocatalyzed $sp^3$ (C-H) Activation for the Synthesis of Pyrrolo[3,4-*c*]quinolones, *Organic & Biomolecular. Chemistry.* **17** (40), 8958–8962 (2019).

(60) Wang, F., Chen, C., Deng, G., Xi, C. J. Concise Approach to Benzisothiazol-3(2H)-one via Copper-Catalyzed Tandem Reaction of o-Bromobenzamide and Potassium Thiocyanate in Water. *Journal of Organic Chemistry.* **77** (8), 4148-4151 (2012).

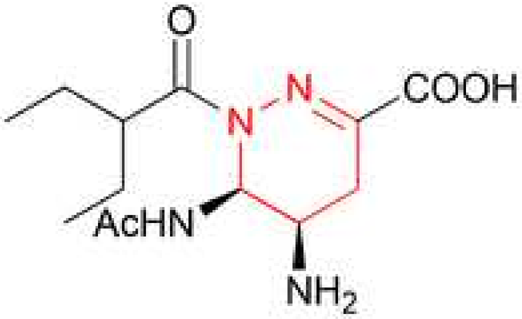
Figure 1



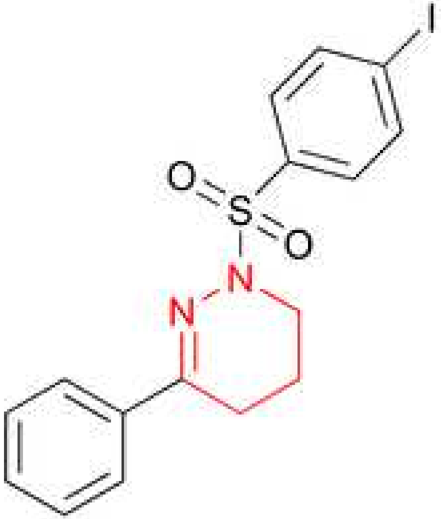
Neuroprotective regent



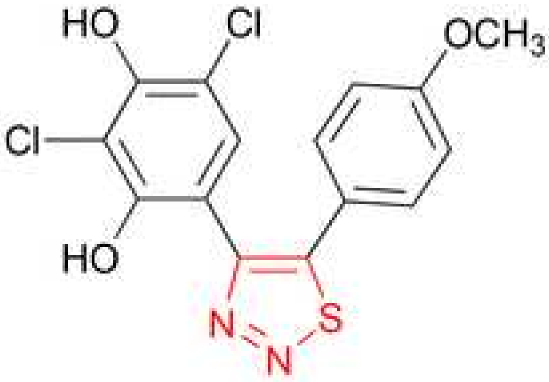
Antitumor agent



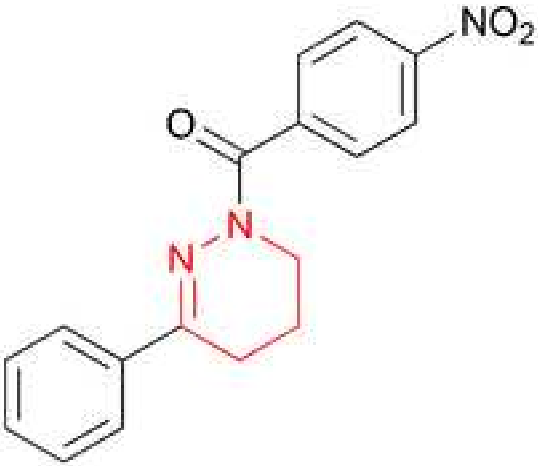
An influenza
neuraminidase inhibitor



Neuro-transmitter inhibitor

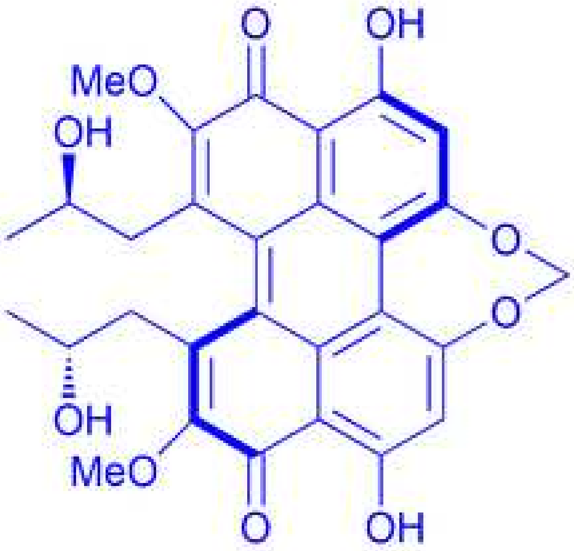


Inhibitor of Hsp90 chaperone

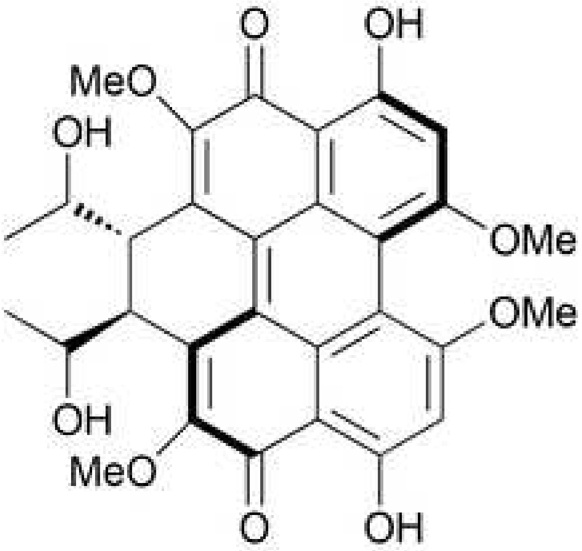


Nonsteroidal progesterone
receptor regulator

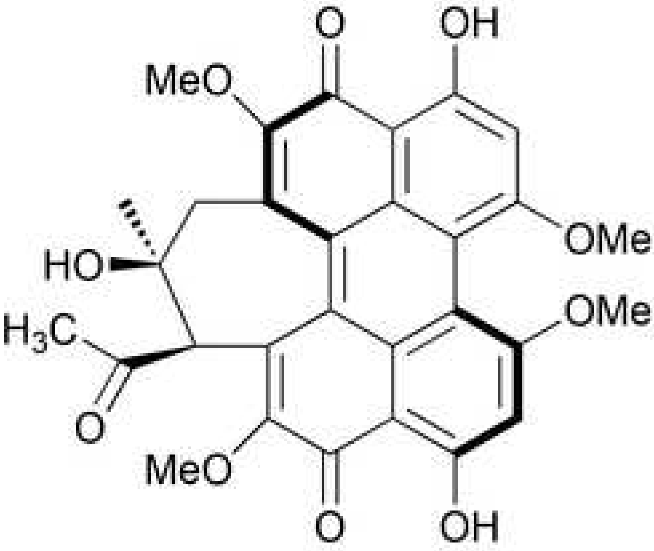
Figure 2



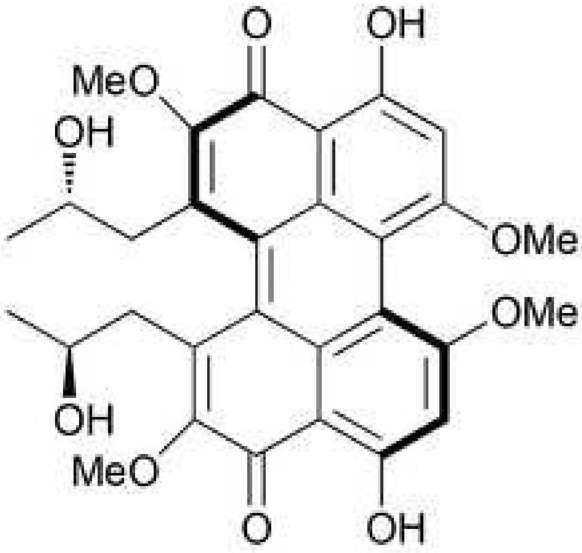
Cercosporin



Elsinochrome C



Hypocrellin A



Phleichrome

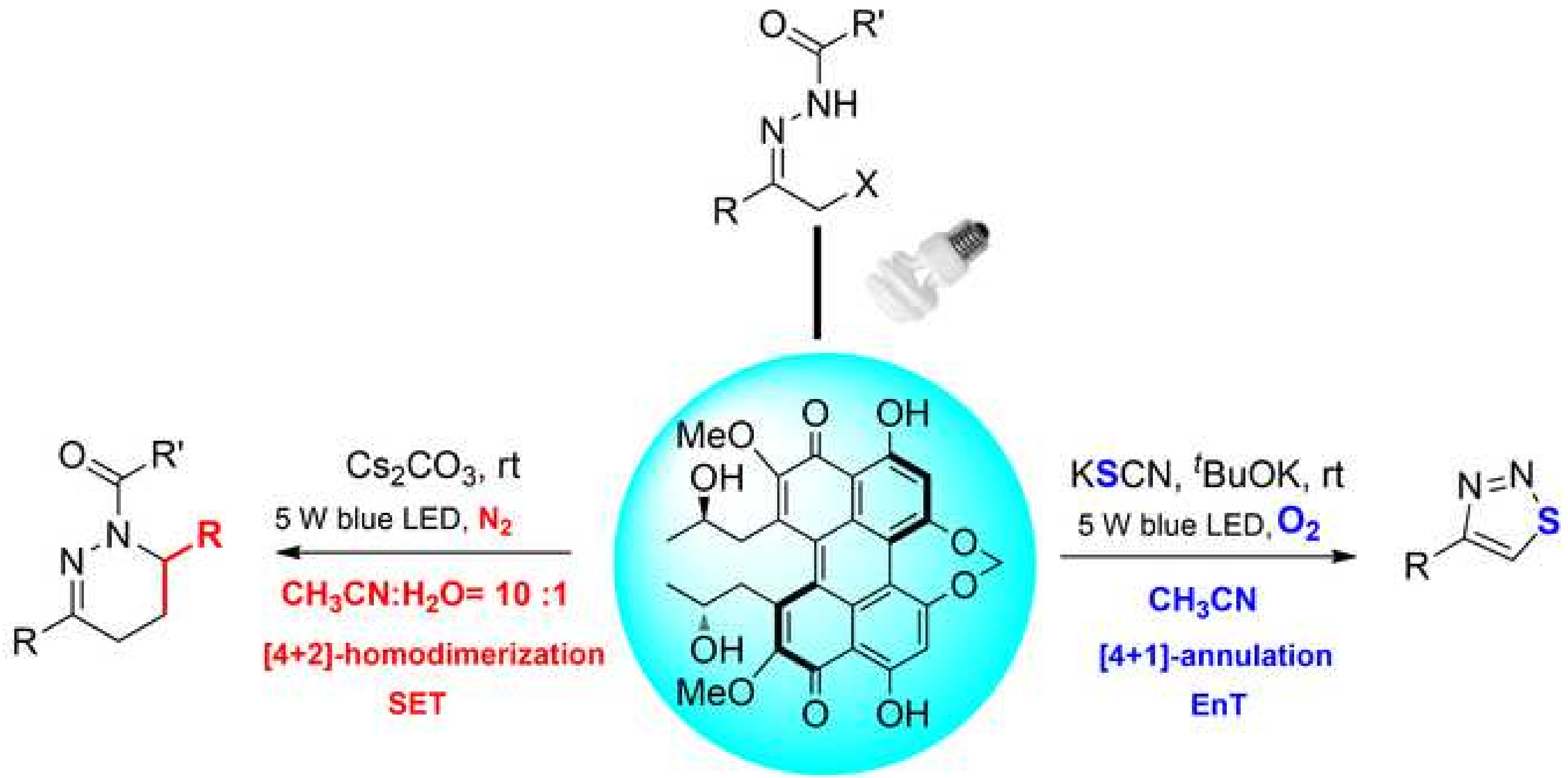

O
R'
NH
N
X
R
MeO
OH
O
OH
O
O
OH
MeO
O
OH
Cs$_2$CO$_3$, rt
5 W blue LED, N$_2$
CH$_3$CN:H$_2$O= 10 :1
[4+2]-homodimerization
SET
KSCN, $^t$BuOK, rt
5 W blue LED, O$_2$
CH$_3$CN
[4+1]-annulation
EnT
N
N
R
N=N
S

Figure 4

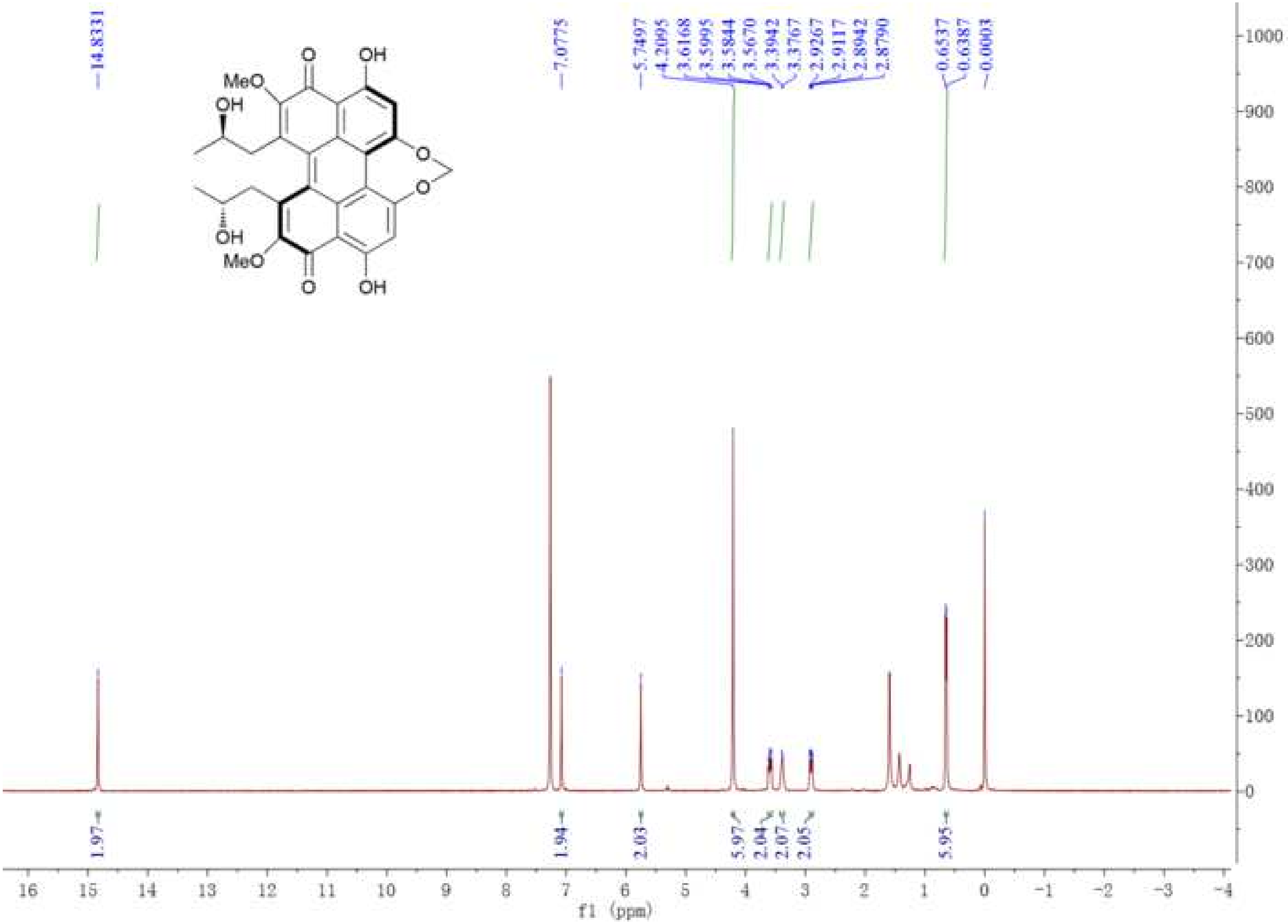

14.8331
7.0775
5.7497
4.2095
3.6168
3.5995
3.5844
3.5670
3.3942
3.3767
2.9267
2.9117
2.8942
2.8790
0.6537
0.6387
0.0003
MeO
OH
O
OH
O
O
OH
MeO
O
OH
1.97
1.94
2.03
5.97
2.04
2.07
2.05
5.95
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
-1
-2
-3
-4
f1 (ppm)
1000
900
800
700
600
500
400
300
200
100
0

Figure 5

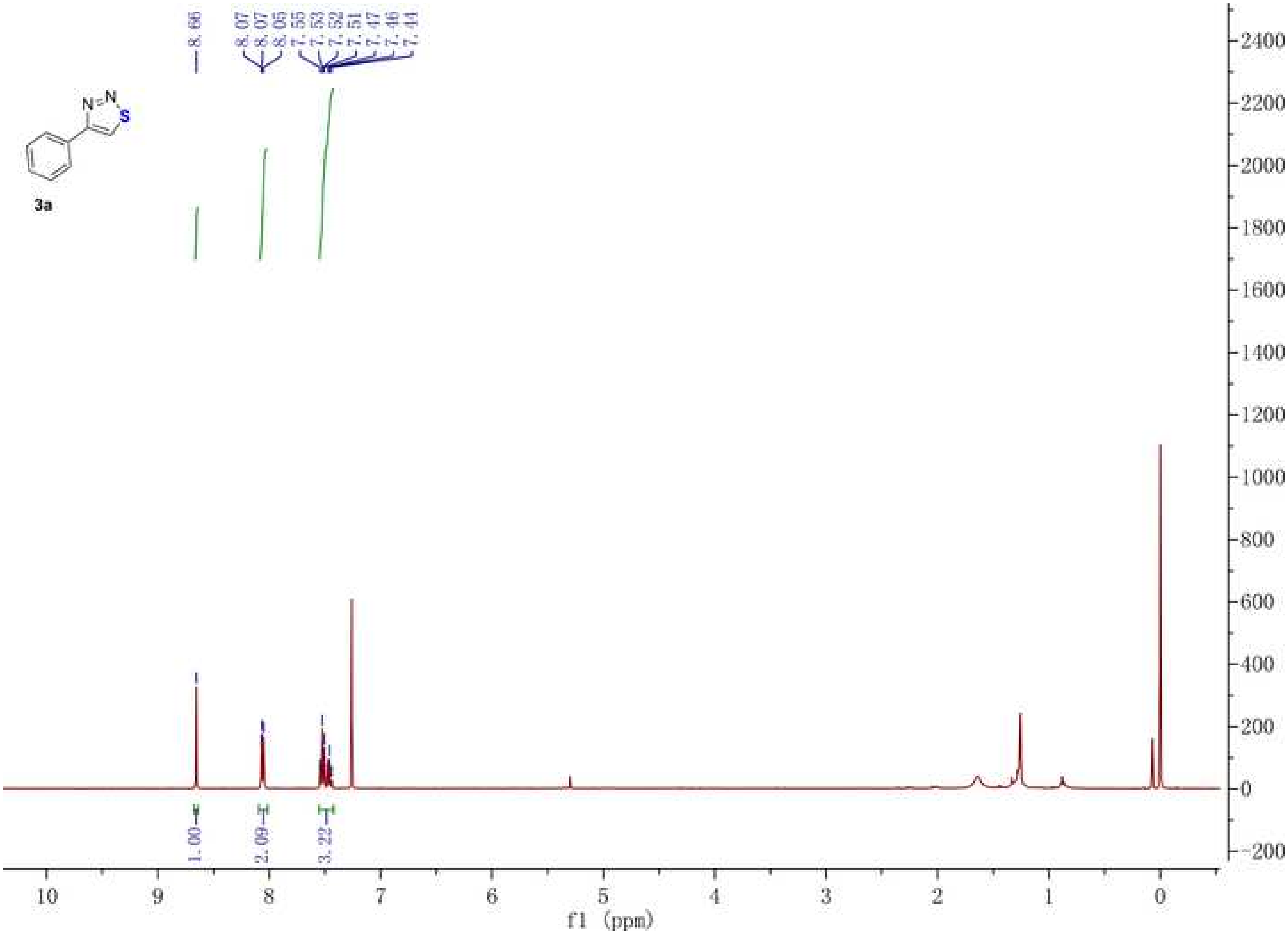
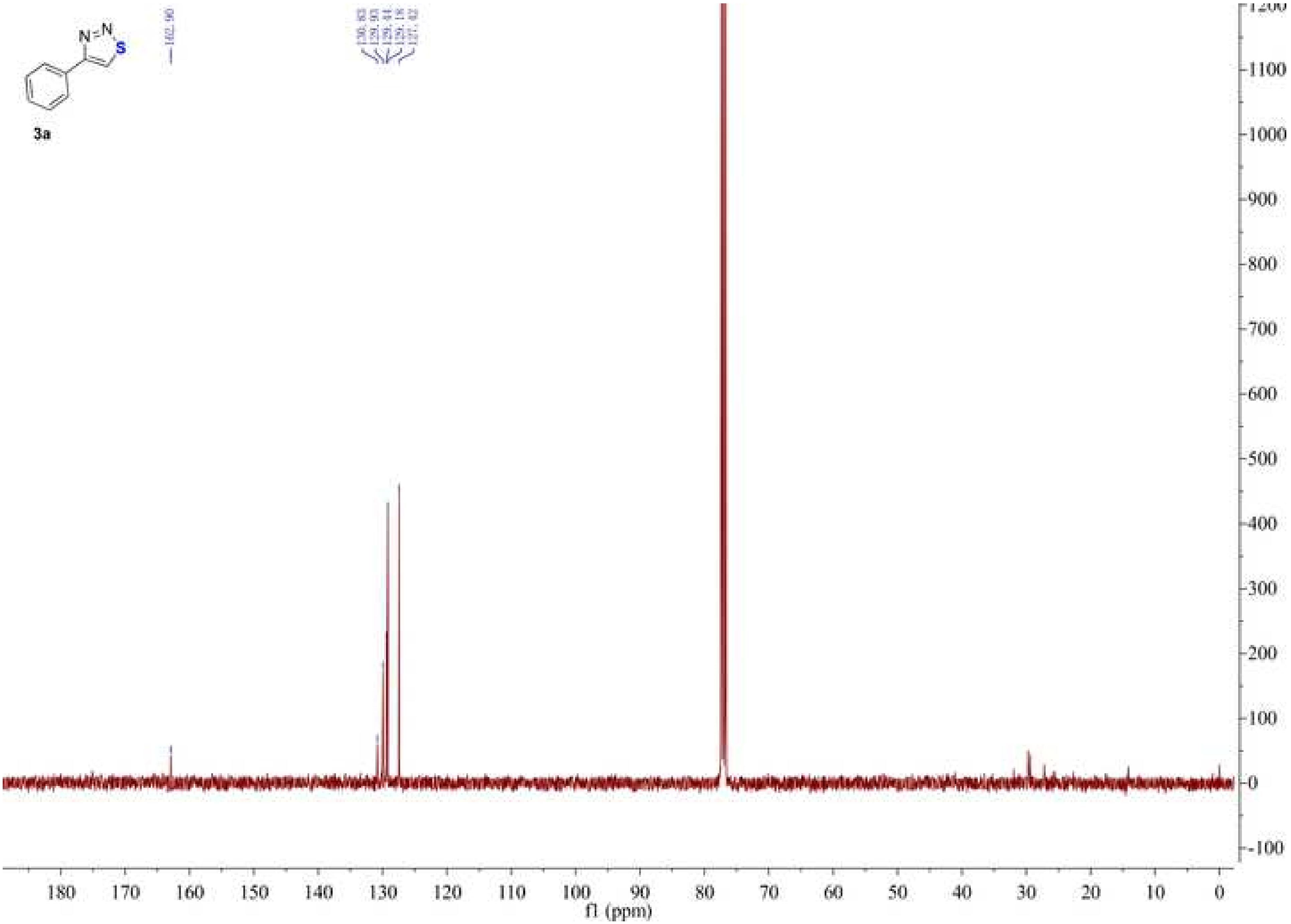

8.66
8.07
8.07
8.05
7.55
7.53
7.52
7.51
7.47
7.46
7.44
N
N
S
3a
1.00
2.09
3.22
2400
2200
2000
1800
1600
1400
1200
1000
800
600
400
200
0
-200
10
9
8
7
6
5
4
3
2
1
0
f1 (ppm)

Figure 6


N
N
S
3a
162.90
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
-100
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

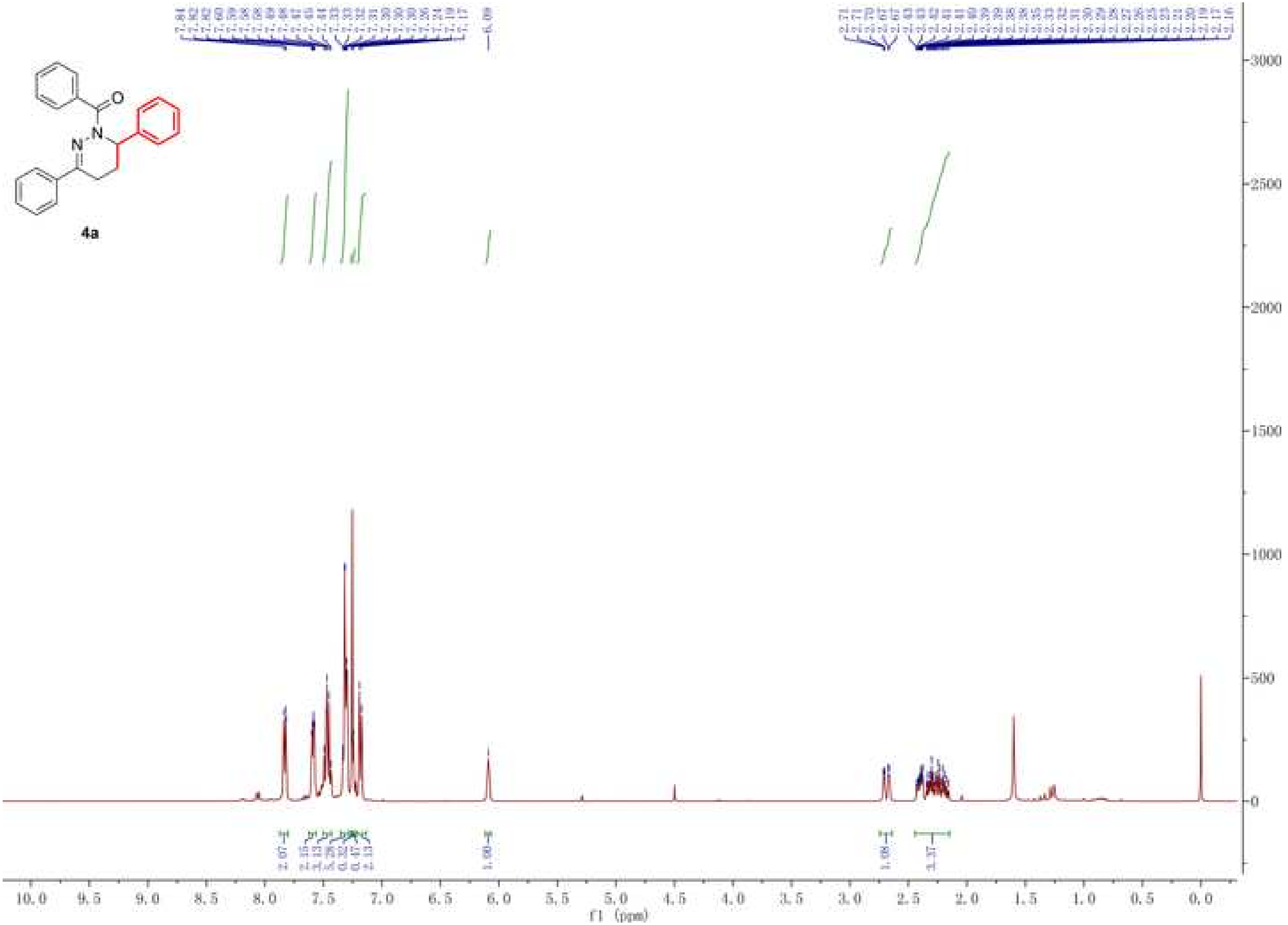
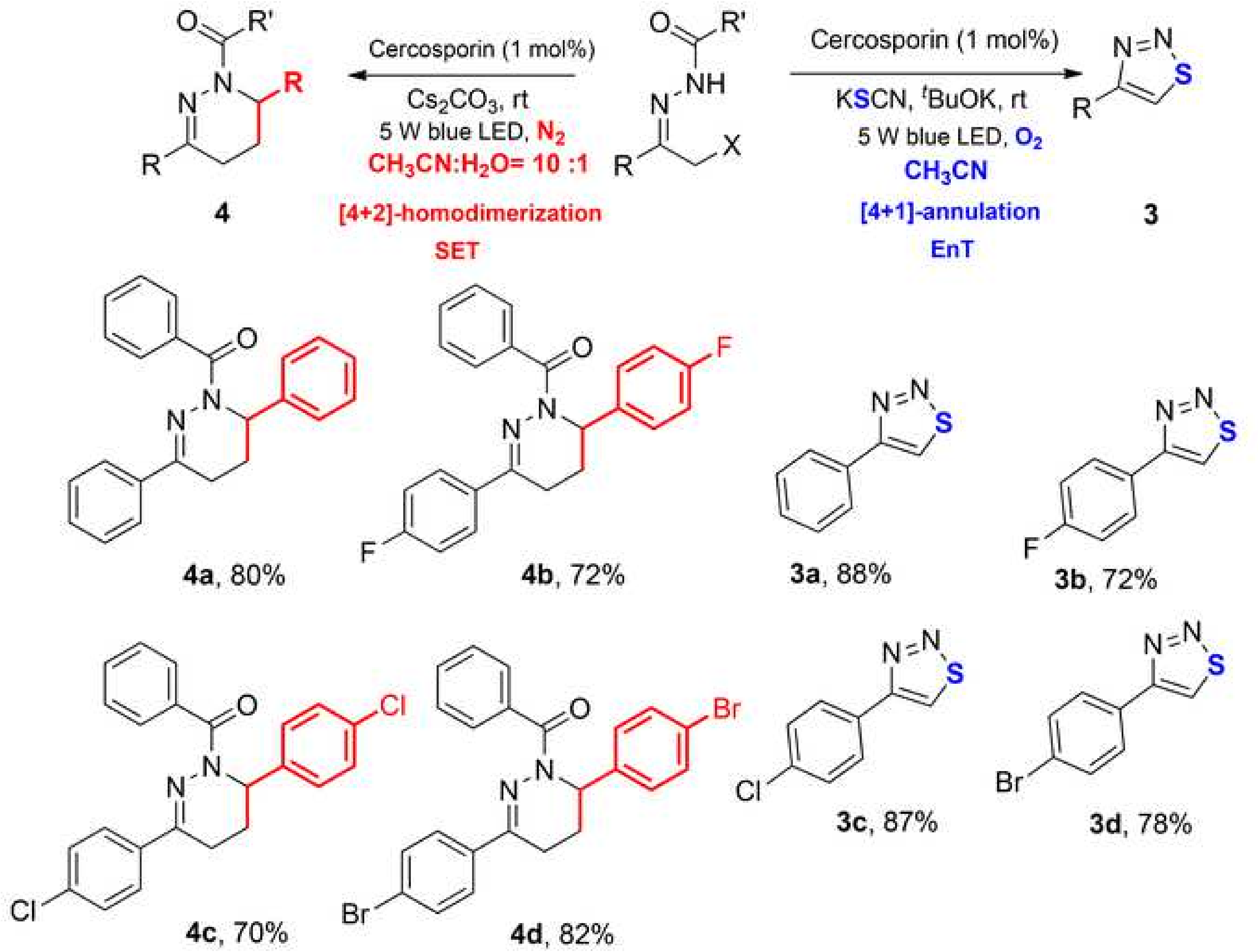

O
N
N
4a
1.00
1.00
3.37
3000
2500
2000
1500
1000
500
0
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

Figure 8


Cercosporin (1 mol%)
$Cs_2CO_3$, rt
5 W blue LED, $N_2$
$CH_3CN:H_2O$= 10 :1
[4+2]-homodimerization
SET
Cercosporin (1 mol%)
KSCN, $^tBuOK$, rt
5 W blue LED, $O_2$
$CH_3CN$
[4+1]-annulation
EnT
4
3
4a, 80%
4b, 72%
3a, 88%
3b, 72%
4c, 70%
4d, 82%
3c, 87%
3d, 78%

| Name of Material/Equipment | Company | Catalog Number |
|---|---|---|
| 2,4'-Dibromoacetophenone | ENERGY | D0500850050 |
| 2'-bromo-4-chloroacetophenone | ENERGY | A0500400050 |
| 2-Bromo-4'-fluoroacetophenone | ENERGY | A050037-5g |
| 2-Bromoacetophenone | ENERGY | A0500870050 |
| 4-Bromobenzhydrazide | ENERGY | B0103390010 |
| 4-Chlorobenzhydrazide | ENERGY | D0511130050 |
| 4-Fluorobenzhydrazide | ENERGY | B010461-5g |
| 5 W blue LED | PHILIPS | 29237328756 |
| Benzoyl hydrazine | ENERGY | D0500610250 |
| $CH_2Cl_2$ | SINOPHARM | 80047360 |
| $CH_3CN$ | SINOPHARM | S3485101 |
| $CH_3OH$ | SINOPHARM | 100141190 |
| $Cs_2CO_3$ | ENERGY | E060058-25g |
| Ethyl acetate | SINOPHARM | 40065986 |
| freeze dryer | LABCONCO | 7934074 |
| HPLC | Agilent | 1260 Infinity II |
| KSCN | ENERGY | E0104021000 |
| $Na_2SO_4$ | SINOPHARM | 51024461 |
| organic microfiltration membrane | SINOPHARM | 92412511 |
| S-7 medium | | |
| Schlenk tub | Synthware | F891910 |
| sephadex LH-20 column | GE | 17009001 |
| shaker | Lab Tools | BSH00847 |
| silica gel | ENERGY | E011242-1kg |
| $^t$BuOK | ENERGY | E0610551000 |
| vacuum bump | Greatwall | SHB-III |
| vacuum evaporator | | |

## Comments/Description

S-7 medium

Gluose 1g; Fructose 3g; Sucrose 6g; Sodium acetate 1g; Soytone 1g; Phenylalanine 5mg; Sodium benzoate 100mg; 1M $KH_2P0_4$ buffer ph6.8;Biotin 1mg; $Ca(NO_3)_2$ 6.5mg; Pyridoxal 1mg; Calcium pantothenate 1mg; Thiamine 1mg; $MnCl_2$ 5mg; $FeCl_3$ 2mg; $Cu(NO_3)_2$ 1mg; $MgSO_4$ 3.6mg; $ZnSO_4$ 2.5mg

**Editorial comments:**

The manuscript has been modified and the updated manuscript, **60786_R1.docx**, is attached and located in your Editorial Manager account.**Please use the updated version to make your revisions.**

1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues.

Response: We have revised the manuscript as suggested.

2. Please obtain explicit copyright permission to reuse any figures from a previous publication. Explicit permission can be expressed in the form of a letter from the editor or a link to the editorial policy that allows re-prints. Please upload this information as a .doc or .docx file to your Editorial Manager account. The Figure must be cited appropriately in the Figure Legend, i.e. "This figure has been modified from [citation]."

Response: We have obtained the copyright permission.

3. Please provide at least 6 keywords or phrases. There are only 5 in the manuscript.

Response: One keyword has added as suggested.

4. For in-text referencing, please insert the superscripted reference numbers before a comma or period.

Response: All reference numbers have been revised as suggested.

5. Please add a one-line space between each of your protocol steps.

Response: One-line space has been added between each of the protocol steps.

6. Please make scheme 1 as a figure and renumber all figures in the order of their appearance in the manuscript.

Response: Scheme 1 has been revised as Figure 3 and all other figures have been renumbered.

7. Please cite only one article per each reference number.

Response: Only one article is cited in each reference and the reference numbers in the manuscript have been revised accordingly.

8. Please revise the Discussion to explicitly cover the following in detail in 3-6 paragraphs with citations:

a) Critical steps within the protocol
b) Any modifications and troubleshooting of the technique
c) Any limitations of the technique
d) The significance with respect to existing methods
e) Any future applications of the technique

Response: The Discussion part has been revised as: Nitrogen-containing heterocycles are important motifs for lots of new drugs and were traditionally synthesized through thermal cycloaddition reactions. Due to their great importance, it is still highly desired to develop a new photocatalytic method for the synthesis of these compounds. To take advantage of excellent photosensitization properties of cercosporin, we applied cercosporin as a metal-free photocatalyst in two categories of annulation reactions to synthesize nitrogen–containing heterocycles.

9. Representative results: Please describe all data in complete sentences instead of just listing the data.
Response: All data has been revised as complete sentences.

**Reviewers' comments:**
Reviewer #1:

Manuscript Summary:
In this work, Rao and coworkers developed a method for the synthesis of 1,2,3-thiadiazole/ 1,4,5,6-tetrahydropyridazine derivatives mediated by cercosporin under visible light conditions. It's worth noting that this photoredox process exhibited good regioselectivity and broad functional-group with moderate yields for the construction of nitrogen-containing heterocycles. However, there are some doubts in the text that need to be further resolved.

Minor Concerns:

1. For the synthesis of 1,2,3-thiadiazole process, what's the byproduct?
Response: During the synthesis of 1,2,3-thiadiazole, 5% yield of 1,4,5,6- tetrahydropyridazine 4a derived from [4+2] cyclodimerization of azoalkene was detected with LC-MS during the optimization process as the byproduct.

2. The English need to be improved.
Response: We have revised the entire manuscript.

Reviewer #2:

Manuscript Summary:
The manuscript describe the synthesis of interesting heterocycles compounds through a photocatalyctic approach. The work starts with the biosynthesis of the photocatalyst which is good for the approach looking at the complexity of the Cercosporin catalyst used. However, the protocol for synthesis of this photocatalyts could not be reproduced with the information provided. Starting from α-Halo-N-acyl-hydrazone, 1,2,3-thiadiazoles are prepared by [4+1] annulation reaction with KSCN as source of sulphur in the presence of O2 and blue light irradiation. On the other hand, 1,4,5,6-tetrahydropyridazines are obtained by [4+2] reactions from the same substrates and light but in the absence of N2. In addition both protocols required the use of a base. Although the mechanisms was not discuss or analysed, the authors indicate an energy transfer vs an electron transfer based probably based in the reported photophysical and photochemical behaviour of the cercosporin.

Major Concerns:

Page 1: There is not reference in the work to cercosporin-producing strain and the access to it.
Response: Reference for the cercosporin-producing strain has been added as Ref. 56.

Page 3: There is a big problem with identity of compounds 4. All HRMS exact mass calcd are wrong and not correspond to the compounds synthesized. Besides the numbers of C in 13C NMR does not corresponde to the symetry and/or rotational behaviour of all compounds.
Response: Thanks the reviewer for pointing this out. We have checked the HRMS and CNMR for compounds 4 and they are correct. Actually, the formula for each compounds 4 are incorrect and have been revised.

Minor Concerns:

I agree with the author in respect to the advantages of the method but I should mention the discussion is a little biased in respect to green chemistry because nothing is mention about toxic -CN formed or atom economy with respect to other methodologies cited.
Response: The green description of the method is mainly derived from photocatalysis. We did not consider the environmental impact of the introduction of CN. According to the opinion of the reviewer, we deleted the description about green.

PROTOCOL
As a general comment to this section, I believe the synthesis of α-Halo-N-acyl-hydrazone should be included since they are not commercial available and its precursors are listed in the "Table of Material".
Response: A protocol for the synthesis of α-Halo-N-acyl-hydrazone has been added as suggested.

Page 1
There is not reference in the work to cercosporin-producing strain and the access to it.
Response: A reference regarding the cercosporin-producing strain and the access to it has been added as suggested.

Page 2

Protocol 2
2.4 Setup of irradiation should be specified, Is it one LED from the bottom used? Or Are many LED in another array used? From the comment on page 7 "household light" I believe the source of irradiation should be clearly specified since "5 W LED" usually refer to high power LED but "household light" could correspond to many different sources.
Response: This protocol has been revised as "Subject the Schlenk tube under 5 W blue LED from the bottom for 16 h."

2.5 which is the organic solvent for the extraction?
Response: This protocol has been revised as "Wash with 4x 15 mL of saturated NaCl solution and combine the aqueous phase."

2.6 how many times and with which volume?

Response: This protocol has been revised as "Re-extract the aqueous phase with 4x 15 ml of ethyl acetate."

2.9 eluent?

Response: The eluent was: petroleum: ethyl acetate=10:1

Protocol 3

3.2 according to scheme 1 and discussion this should "O2" should be changed for "N2"

Response: "O2" has been revised as "N2".

3.4 idem 2.4

Response: This protocol has been revised as "Subject the Schlenk tube under 5 W blue LED from the bottom for 16 h."

3.5 idem 2.5

Response: This protocol has been revised as "Wash with 4x 15 mL of saturated NaCl solution and combine the aqueous phase."

3.6 idem 2.6

Response: This protocol has been revised as "Re-extract the aqueous phase with 4x 15 ml of ethyl acetate."

3.9 eluent?

Response: The eluent was: petroleum: ethyl acetate=10:1.

Page 3

13C NMR for compound 3c should be corrected: typical JC-F are J1 ~ 245 Hz, J2 ~ 21, J3 ~ 8, J4 ~ 3 and 7 carbons are reported which is strange according to the compounds 3a, 3b, 3d and 3e and the expected rotational behaviour of this compounds.

Response: We have checked the CNMR for compound 3c. Since there is no F atom in this compound, JC-F should not be included here. Since 3b is the only compound which contains F atom, we have checked and revised its CNMR data.

There is a big problem with identity of compounds 4. All HRMS exact mass calcd are wrong and not correspond to the compounds synthesized. Besides the numbers of C in 13C NMR does not corresponde to the symetry and/or rotational behaviour of all compounds.

Response: Thanks the reviewer for pointing this out. We have checked the HRMS and CNMR for compounds 4 and they are correct. Actually, the formula for each compounds 4 are incorrect and have been revised.

Page 4

Discussion

In the second paragraph it should be mention that control experiment have been done for the formation 4+2 as it was for the 4+1 reactions.

Response: As suggested, we have added "The control experiments have been done for the [4+2] reaction as it was for the [4+1] reaction" in the Discussion part of the manuscript.

Scheme 1

Solvent must be specified in the "blue" approach

Response: Solvent has been added in the "blue" approach as suggested.

From the PROTOCOL in page 4, O2 is not a difference in the two approach in which case should not be missing in the "red" approach and should not in bold in the "blue" approach.

Response: $N_2$ has been added in the "red" approach.

Figure 3, 4 and 5

Please identify the impurities present in the spectra.

Response: These peaks are coming from solvents and water.

Table of Materials

"4-Chloro-2'-bromoacetophenone" should be change by "2'-bromo-4-chloroacetophenone"

Response: "4-Chloro-2'-bromoacetophenone" has been revised as "2'-bromo-4-chloroacetophenone"

Comments/Description

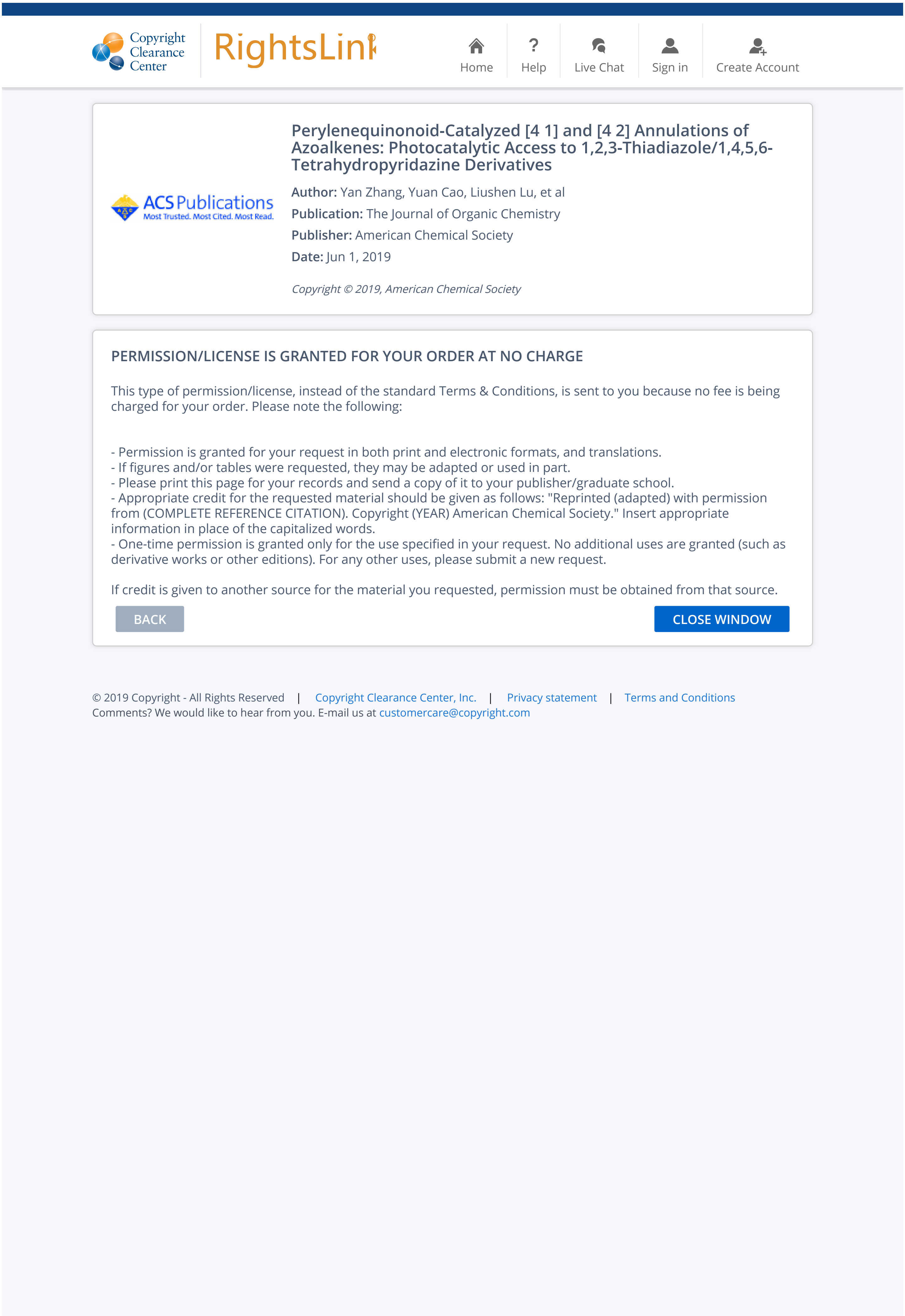
Is this "S-7 medium"? Please add a title

Response: "S-7 medium" has been added as a title in the Comments/Description.

Citation

Citation 4c should be corrected.

Response: Citation 4c has been corrected.



## Perylenequinonoid-Catalyzed [4 1] and [4 2] Annulations of Azoalkenes: Photocatalytic Access to 1,2,3-Thiadiazole/1,4,5,6-Tetrahydropyridazine Derivatives

**Author:** Yan Zhang, Yuan Cao, Liushen Lu, et al

**Publication:** The Journal of Organic Chemistry

**Publisher:** American Chemical Society

**Date:** Jun 1, 2019

### PERMISSION/LICENSE IS GRANTED FOR YOUR ORDER AT NO CHARGE

This type of permission/license, instead of the standard Terms & Conditions, is sent to you because no fee is being charged for your order. Please note the following:

- Permission is granted for your request in both print and electronic formats, and translations.
- If figures and/or tables were requested, they may be adapted or used in part.
- Please print this page for your records and send a copy of it to your publisher/graduate school.
- Appropriate credit for the requested material should be given as follows: "Reprinted (adapted) with permission from (COMPLETE REFERENCE CITATION). Copyright (YEAR) American Chemical Society." Insert appropriate information in place of the capitalized words.
- One-time permission is granted only for the use specified in your request. No additional uses are granted (such as derivative works or other editions). For any other uses, please submit a new request.

If credit is given to another source for the material you requested, permission must be obtained from that source.