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Synthesis and Photophysical Characterization of a Photoaffinity Molecular Switch

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Abstract: In recent years, the development of some photoswitches based on azobenzene group provides a good tool for high spatio-temporal and precise regulation of ion channels and protein receports, but these small molecular photoswitches are very easily diffused in organisms. To overcome this limit, we have designed a photoswitches SY1 containing photocrosslinked group diazirine. The photoswitch was synthesized by the condensation reaction between azobenzene derivative and diazirine. The structure of the compound was characterized by ^{1}H NMR and HR-MS, and the photophysical properties of the photoswtich SY1 were studied by UV-vis spectroscopy in different solvent systems. The results show that SY1 has excellent photodependent isomerization.

Keywords: Photoswitch, Azobenzene, Diazirine, Photo-crosslinking

Introduction

The application of small molecular photoswitches in the photopharmacology has attracted increasing attention from researchers [1, 2]. Azobenzene is one of the most classic photoswitch ligand molecules [3-5], which can undergo cis-trans conformational tautomerism under irradiation of different wavelengths of light. This conformational change is sufficient to affect the activity of ligands. Therefore, the photoisomerization of ligands can be involved in regulating the structure and function of proteins. Although a series of novel azobenzene photoswitching molecules have been developed that can quickly and reversibly activate and deactivate ion channels under altering the different wavelengths light [6], most of their effects on ion channels lack targeting and specificity, and small molecule photoswitches are prone to diffusion in vivo. However, the covalent small molecule ligand strategy combines the rapid kinetics of photochemical reactions and the site specificity of protein ligand recognition, which can improve the targeted release of small molecules and effectively overcome the free diffusion of decaying small molecules [7].

Photochemical crosslinking is a commonly method for covalently capturing the interactions between small molecule and biomacromolecule, and widely used in the various fields such as pharmaceuticals, chemical biology, and materials science [8, 9]. The photo-crosslinking probe is usually composed of photo-crosslinking groups, active ligands and reporting groups. The diazirine group is one of the most commonly used photo-crosslinking chemical groups [10], which has excellent chemical properties [11-13]. Firstly, it is very stable under dark conditions, avoiding reacting with other biological macromolecules under physiological. On the other hand, diazirine has smaller steric hindrance, and can producing free radical under illumination, which can ensure capturing the target protein with spatial accuracy.

Based on photo-crosslinking technology, this study designed and synthesized a photoswitch with photo-crosslinking property by connecting azobenzene derivatives with diazirine groups containing -OH. The structure of novel photoswitch was confirmed by ^{1}H NMR and HR-MS, and the photophysical properties were characterized by UV-vis spectroscopy in different solvent systems.

Experimental

1. Materials and Instruments

All the reagents were purchased from the reagent company in a pure analytical grade and are used directly, unless otherwise stated. All experimental water was triple distilled water (Cascade Laboratory Pure Water System, PALL, USA). UV-vis spectrum was measured by UV spectrophotometer (UV-2600, Shimadzu, Japan). The pH value was measured by a digital pH meter (PHS-3C, Shanghai, China). The quartz colorimetric dish used in the experiment has a specification of 1 cm optical path and a capacity of 3.5 mL. Four fiber-coupled LED of 365 nm, 415 nm, 470 nm and 530 nm were driven by T-Cube™ LED to irradiate the quartz colorimetric dish added to the test solution, respectively.

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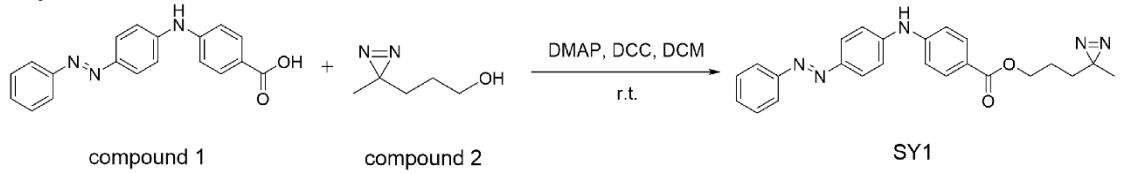
2. Synthesis of SY1



Scheme 1 Synthesis and response mechanism of SY1.

As shown in Scheme 1, compound 1 (100 mg, 0.314 mmol, 1.0 eq) was dissolved in anhydrous DCM (30 mL) at 0 ℃, and 4-dimethylaminopyridine (DMAP, 57.54 mg, 0.471 mmol, 1.50 eq) was added while stirring. Compound 2 (143.28 mg, 1.256 mmol, 4.0 eq) was dissolved in 10mL anhydrous DCM and slowly added to the system. N, N'-dicyclohexylcarbodiimide (DCC, 97.18 mg, 0.471 mmol, 1.5 eq) was added three parts every 30 min. the solvent was evaporated, the residue was dissolved in ethyl acetate, and the reaction mixture was filtered to remove white precipitate. Extract three times with EtOAc/H_2O until the aqueous phase is clear. The organic phase was then washed with saturated sodium chloride aqueous solution and dried with anhydrous sodium sulfate. The solvent is concentrated and evaporated under reduced pressure. The crude product obtained was purified by silica gel column chromatography (EtOAc: PE = 1: 30, v/v) to obtain a yellow oily compound SY1. 3-(3-methyl-3H-diazirin-3-yl)propyl (E)-4-((4-(phenyldiazenyl)phenyl)amino)benzoate, ^{1}H NMR (600 MHz, DMSO-*d6*) δ 9.35 (s, 1H), 7.89 (d, *J* = 9.0 Hz, 4H), 7.84 (d, *J* = 7.1 Hz, 2H), 7.57 (t, *J* = 7.6 Hz, 2H), 7.51 (d, *J* = 7.3 Hz, 1H0, 7.34 (d, *J* = 8.9 Hz, 2H), 7.26 (d, *J* = 8.8 Hz, 2H), 4.20 (dt, *J* = 2279.0, 6.4 Hz, 2H), 1.64 - 1.53 (m, 2H), 1.54 - 1.44 (m, 2H), 1.04 (s, 3H). HRMS (ESI): m/z [M + H]$^+$ calculated for $C_{24}H_{23}N_5O_2$: 414.1930, found: 414.1930.

The structural identification spectrum is shown in Fig. 1 and Fig. 2.

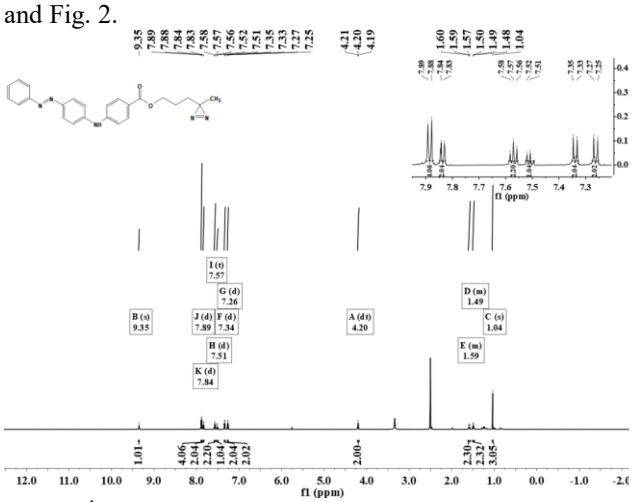


Fig. 1 ^{1}H NMR spectra of compound SY1 in DMSO-*d6*.

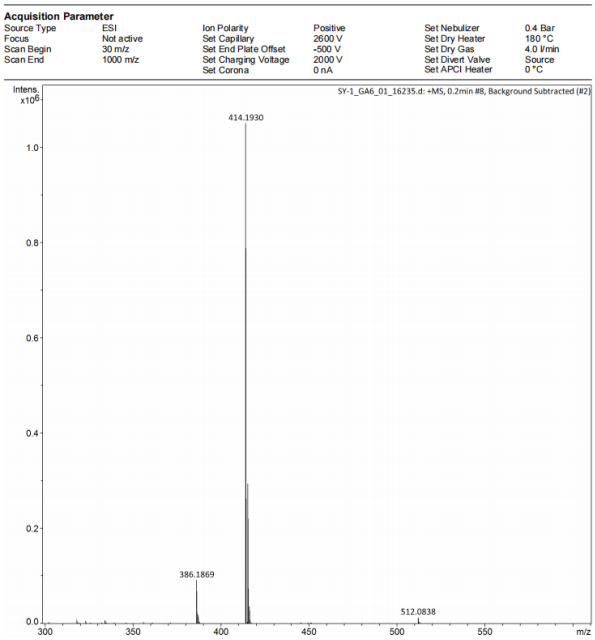


Fig. 2 Mass spectra of SY1.

3. UV-Absorption Spectra

The compound SY1 to be tested was prepared with dimethyl sulfoxide (DMSO) to obtain a stock solution of 100 mM. The reserve solution was diluted to 50 μM with extracellular fluid free of Ca^{2+}, acetonitrile (chromatographically pure), cyclohexane and dimethyl sulfoxide to obtain the test solution. The test solution of 2.5mL was added to the quartz colorimetric dish, and the detection wavelength range of UV absorption spectrum was 250-600 nm. Two irradiation methods and measurement methods were used. On the one hand, four fiber-coupled LED of 365 nm, 415 nm, 470 nm and 530 nm are driven by T-Cube™ LED, and the colorimetric dish added to the test solution is irradiated for 3 min before measurement. The measurement was carried out after the light was turned off. On the other hand, different wavelengths of fiber coupled LEDs were used to directly irradiate the colorimetric dish, and real-time absorbance measurement was carried out during irradiation. The experiment was conducted in a dark environment at room temperature and repeated three times at 25 ± 2.0 ℃.

Results and discussion

1. The UV−vis spectra of photoswitch SY1 in Different Solvent Systems

After SY1 in calcium-free extracellular fluid was irradiated with different wavelengths of light for 3 min, and its UV-vis absorption spectrum was measured. As

shown in Fig. 3A, the maximum absorbance wavelength of SY1 in the extracellular fluid system is 473 nm (Table 1). Compared with the UV-vis absorption of SY1 under darkness, the corresponding absorption of SY1 change little after light irradiation at 530 nm, 470 nm, 415 nm or 365 nm. The reason for this may be the influence of solvent system on the stability and thermal relaxation of cis-isomer of SY1. So we changed irradiation mode or different solvents for further testing UV-vis absorption.

As shown in Fig. 3B, while vertically irradiating with 530 nm, 470 nm, 415 nm or 365 nm light, the UV-vis spectra changed obviously. Especially, under irradiation of 415 nm, UV absorbance changed the most. Next, we tested the UV-vis spectra in different solvent systems including acetonitrile, cyclohexane, and dimethyl sulfoxide. By changing the polarity of the solvent and the solubility of SY1, the optical properties of SY1 were observed and its photoisomerization properties were discussed. As shown in Fig. 3C, in acetonitrile system, the UV-vis absorption of SY1 has almost no change after 530 nm light irradiation. However, the UV-vis absorption of SY1 changed under the irradiation of 470 nm, 415 nm and 365 nm, and the absorbance decreased most obviously after 415 nm irradiation, indicating that the thermal relaxation property was slow. The maximum absorbance wavelength of SY1 in the acetonitrile system is 399 nm (Table 1).

Table 1 The maximum ultraviolet absorption wavelength of compound SY1 in different systems

Solvent system	Maximum absorption wavelength (λ)
extracellular fluid	473 nm
acetonitrile	399 nm
cyclohexane	395 nm
dimethyl sulfoxide	417 nm

As shown in Fig. 3D and 3E, the subsequent measurement results in the cyclohexane and dimethyl sulfoxide systems showed that the absorbance of SY1 solution changed obviously after 3 min of irradiation with different wavelengths of light. In both system, the UV-vis absorption changed the greatest after irradiation with 415 nm wavelength light, indicating that they both had slower thermal relaxation characteristics. The maximum absorbance wavelength of SY1 in the cyclohexane system is 395 nm, and the maximum absorbance wavelength of SY1 in the dimethyl sulfoxide system is 417 nm.

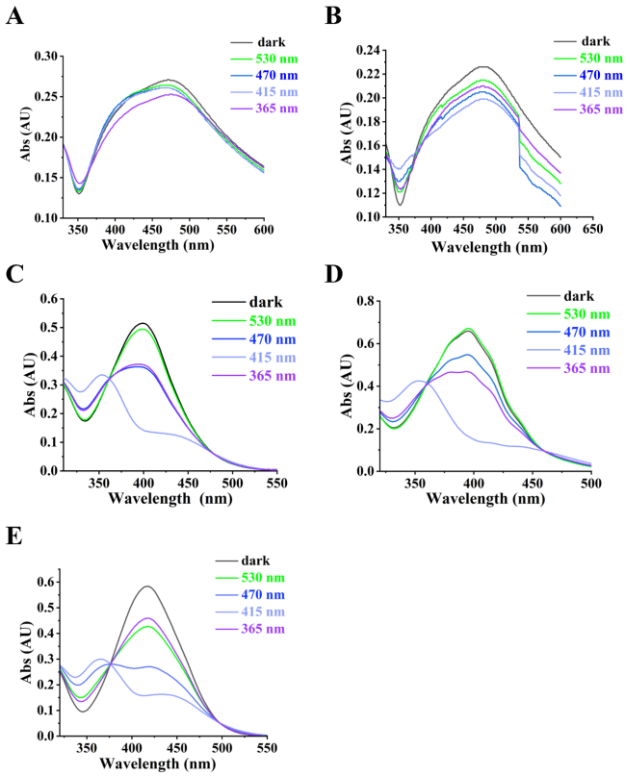


Fig. 3 Photoisomerization of SY1. (A) After 3 min of irradiation, the UV-vis spectra of SY1 in extracellular fluid. (B) Measurement of UV-vis spectra of SY1 when vertically irradiated in extracellular fluid. (C) After 3 min of irradiation, the UV-vis spectra of SY1 in acetonitrile system. (D) After 3 min of irradiation, the UV-vis spectra of SY1 in cyclohexane system. (E) After 3 min of irradiation, the UV-vis spectra of SY1 in dimethyl sulfoxide system.

2. Photo-circulation of isomerization in different system

In the photo-cycle of isomerization experiments, we measured the changes in maximum absorbance of SY1 by switching between different wavelengths of light in acetonitrile, cyclohexane and dimethyl sulfoxide systems. As shown in Fig. 4A and 4B, we measured the maximum absorbance change of SY1 in the acetonitrile or cyclohexane system under multiple switching of 415 nm and 530 nm wavelengths, respectively. And as shown in Fig. 4C, the maximum absorbance changes during multiple switching of 415 nm and 365 nm wavelengths in the dimethyl sulfoxide system. The results indicate that SY1 exhibits good photo reversibility and anti photo fatigue performance in different solvents.

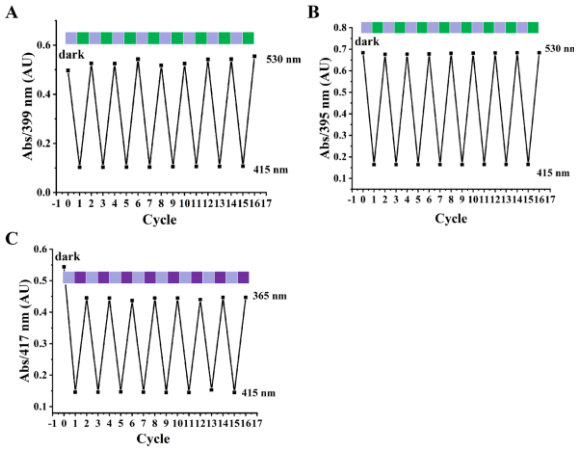


Fig. 4 Extracorporeal photo-cycling of compound SY1 under two wavelengths of light irradiation in different systems. (A) The UV-vis absorbance of SY1 was measured at 399 nm after repeated irradiation at 415 nm and 530 nm for 3 min in acetonitrile system. (B) The UV-vis absorbance of SY1 was measured at 395 nm after repeated irradiation at 415 nm and 530 nm for 3 min in cyclohexane system. (C) The UV-vis absorbance of SY1 was measured at 417 nm after repeated irradiation at 415 nm and 530 nm for 3 min in dimethyl sulfoxide system.

Based on the photochemical characterization of compound SY1, we speculate that compound SY1 exhibits photoisomerization characteristics similar to azobenzene. Different solvent systems can affect their solubility and photoisomerization results, and the thermal relaxation rate is slower. The isomerization of compound SY1 exhibits photo controllability, good photo stability and reversibility.

Conclusion

Photoswitch SY1 exhibits good photoisomerization characteristics in different solvents. Meanwhile, it is found that solvent can affect their photoisomerization properties. The isomerization of SY1 exhibits good photo controllability, photo stability and reversibility. This photoswitch provides a good molecular tool possessing photoaffinity property for photopharmacology.

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