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ARTICLE TYPE

A diarylethene as the SO_2 gas generator upon UV irradiation

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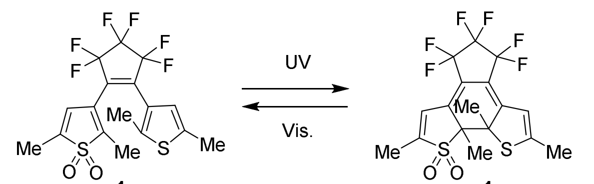
A closed-ring isomer of a diarylethene having sulfone group works as the reagent for SO_2 gas generator with thermal stability even at 70 °C, and it rapidly reverts to the open-ring isomer and generates the SO_2 gas to induce the cell death upon UV irradiation.

Since the development of the photochromic compounds in the late 1980s, diarylethenes have been attracted widespread attention as photocontrollable element in molecular devices and switches.[1, 2] This is due to the high fatigue resistance of the cyclization and cycloreversion reactions, which reversibly generate the open- and closed-ring isomers. However, the reports to the biochemical and biological applications were still very rare. Branda *et al.* demonstrated that the photoresponsive dithienylethene could be reversibly triggered in a living organism and that the photoswitch induced paralysis in *Caenorhabditis elegans* only when the closed-ring isomer was generated by exposure with UV light (365 nm).[3] Until today, the mechanism of the photoswitchable paralysis is not clear.

Some diarylethenes with sulfone groups are known as fatigue resistant photochromic compounds with dramatic fluorescence intensity changes upon alternate irradiation with UV and visible light.[4-6] We found that one of the diarylethene derivatives having sulfone group **1o** (Scheme 1), which was initially synthesized by S. I. Yang et al,[7] works as SO_2 gas generator to induce the cell death upon UV irradiation. Sulfur dioxide (SO_2) is an environmental pollutant and toxic at elevated concentrations. Although the mechanism of its cytotoxicity is yet unclear, SO_2 at elevated concentrations is known to induce oxidative damage to biomacromolecules such as proteins, lipids, and DNA.[8-11] Oxidation of SO_2 to sulphate is known to occur through radical intermediates (such as $\cdot SO_3$) in neutral pH conditions, which in turn can damage biomacromolecules.[8-11] In such background, reagents to generate SO_2 gas in situ have been developed.[8]

Diarylethene **1o** shows the reversible coloration and decoloration by alternate irradiation with UV and visible light. Open-ring isomer **1o** has the λ_{max} at 229 nm (ε: 1.67×10^4 $M^{-1}cm^{-1}$) and the band extends to 400 nm, while the closed-ring isomer **1c** has the λ_{max} at 477 nm (ε: 1.05×10^4 $M^{-1}cm^{-1}$) and the color is orange in hexane (Fig. 1), and quantum yields of the cyclization and cycloreversion reactions of **1** are 0.35 (366 nm) and 4.2×10^{-3} (533 nm), respectively.[12,13] The isomer **1c** is thermally stable and never generates the SO_2 gas by heating nor by visible light irradiation. By contrast, **1o** generate SO_2 gas not only by light irradiation but also heating. Therefore the fatigue resistance of the photochromism of **1** in hexane was poor (ESI†). The quantum yield of SO_2 gas generation of **1o** was estimated as 0.1 (ESI†). Herein we propose **1c** as the thermally stable unique regent for SO_2 gas storage and generator.



1o 1c

Scheme 1. Molecular structures of the two isomers of diarylethene **1**

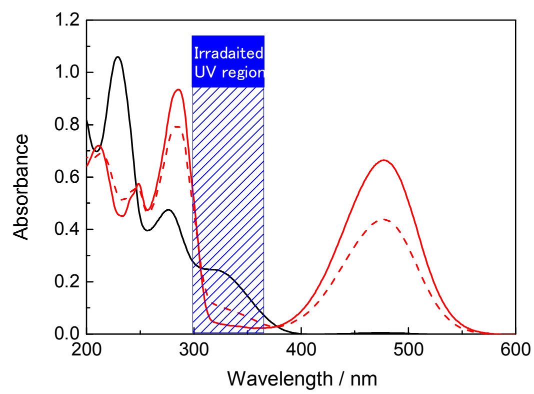


Fig. 1 Absorption spectral changes of **1o** in hexane solution (4.24×10^{-5} M). (a) Absorption spectrum of **1o** (black solid line), (b) absorption spectrum of **1c** (red solid line), and (c) photostationary state upon 365 nm light irradiation (**1o**: **1c**=34:66) (red dashed line).

Although diarylethenes are generally known as thermally irreversible photochromic compounds,[1] we confirmed the thermal stability of both isomers **1o** and **1c**. The hexane solution of the **1c** was heated at 70 °C for 7 days in the dark, SO_2 gas formation and thermal cycloreversion reaction from **1c** to **1o** were not observed. Upon visible light (λ> 480 nm) irradiation, only cycloreversion reaction to **1o** was observed without gas formation. In contrast, UV (300 nm <λ< 365 nm) irradiation to the hexane solution of **1o**, gas was detected accompanied with the cyclization reaction to **1c**. We identified the generated gas by the GC-Mass spectroscopy for the chloroform solution of **1o** in a closed-vessels upon irradiation with UV (300 nm<λ<365 nm). The gas consisted of SO, SO_2,

$(CH_3)_3$Si-OH, and the solvent (Fig. S1, ESI†). Then we used SO_2 gas test tube for the quantitative measurement (Fig. S2,S3 ESI†). By increasing the duration of UV irradiation, the amount of the generated SO_2 gas was increased (Fig. S4, ESI†). The SO_2 gas was also detected by heating the hexane solution of **1o** in a sealed tube at 70 °C for 1 day. These results indicate that the SO_2 generation ability was locked in the closed-ring isomer **1c** and unlocked by visible light irradiation (one way photoisomerization).

The protection was also unlocked by UV light irradiation (back and forth photoisomerization) and SO_2 gas was generated from **1o** after photo-converted from **1c**. The formation of SO_2 gas upon UV irradiation to the hexane solution of **1o** as well as **1c** are measured by a gas detector and summarized in Fig. 2 (Tables S1-S3, ESI†). Therefore, it is possible to use **1c** as the thermally stable regent for SO_2 gas storage. One of the useful applications of this finding for cell biological reserches is that, once UV light is irradiated, it works as photoinduced on-demand killing of adherent cells on culture substrates.

When we reduced the light intensity by using neutral density (ND) filters (Fig. S5, S6), the amount of SO_2 gas generation from **1c** was much suppressed than that from **1o**. This is attributed by the following reason. For the hexane solution of **1c**, the first photon absorbed by **1c** is used for the cycloreversion reaction to **1o**. The produced **1o** can generate SO_2 gas but the concentration of **1o** is low because of the reduced intensity of UV light at the initial stage of the experiment. Consequently, the ratio between the generation of SO_2 from **1o** initially and that from **1c** is larger for the reduced intensity of UV light (Fig. 2).

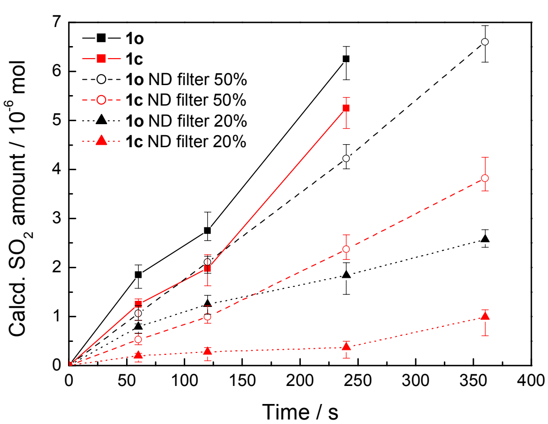


Fig. 2 SO_2 gas formations by the UV irradiation to the hexane solution of **1o** and **1c** with changing the UV intensity using ND filters.

The photoinduced SO_2 gas generation from **1o** is much easier compared to other diarylethene derivatives.[4-6] This is most likely due to the shorter conjugation length in the molecular backbone. The thiophene 1,1-dioxide in which no lone pair electrons exist on the sulfur atom is not aromatic.[14,15] The SO_2 gas generated vigorously upon UV irradiation as well as on heating (ESI†).

Here, we further studied photoinduced cell damage on the substrates and found the cell death upon UV irradiation to the **1o** coated substrates with NIH/3T3 and MDCK cells. The phenomena were also observed for the cells on the **1c** coated substrates. At the photo-toxicity experiment, NIH/3T3 and MDCK cells were disseminated onto thin layers of **1o** and **1c**, respectively. The photoirradiation to the thin layers containing **1o** or **1c** can be controlled by selecting the wavelengths of irradiation light at 365 and 436 nm which are switchable on our PC-controlled microprojection system.[16] To both cells, 365 nm light was irradiated to the patterned area (four band shaped area) through the filter from bottom side of the culture substrate. Fig. 3a shows the NIH/3T3 cells on the thin layer of **1o**. The 365 nm light was irradiated on the patterned area (Fig. 3b, fluorescence from base polystyrene substrate was observed in the irradiated area), and the cells on the area were damaged and detached from the surface as shown in Fig. 3c. Also MDCK cells on the thin layer of **1o** were damaged after 365 nm irradiation (Fig. 3d, e), while no damage was observed with 436 nm light irradiation. Fig. 3d shows that the adhesion of the cells decreased in the irradiated area. Some of these cells were stained with Trypan which can stain the dead cells only. These results suggested that the cells were damaged significantly by 365 nm irradiation on the thin layer of **1o**. On the **1c** film, NIH /3T3 cells were also damaged upon 365 nm irradaition (Fig. 3f). The more magnified images (Fig. 3g, h) show cleary that the shape of the cells in the irradaited area were totally deformed indicating their critical damage.

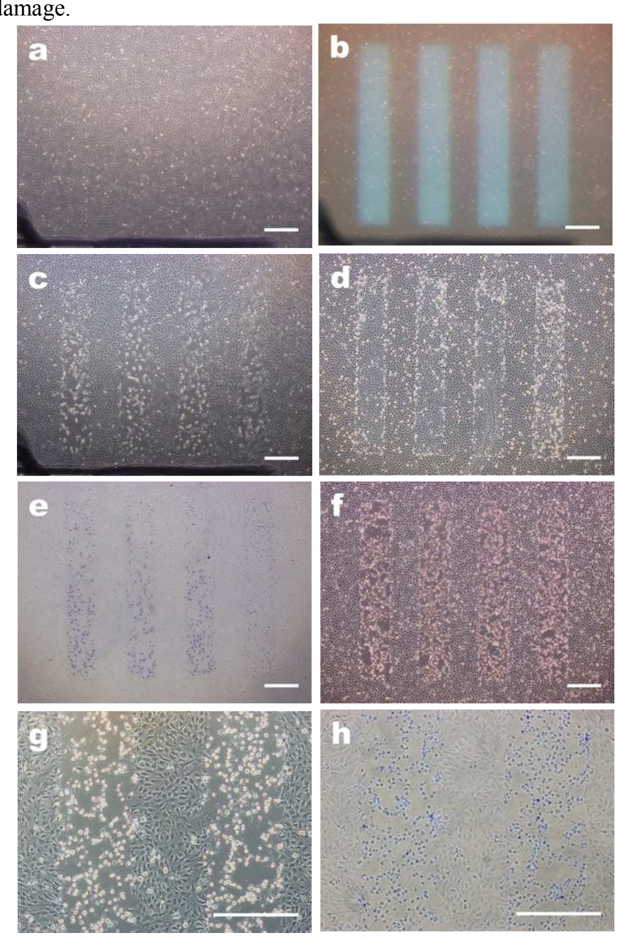


Fig. 3 Influence of patterned UV irradiation (wavelength: 365 nm, intensity: 90 mW/cm^2) on NIH/3H3 (a-c, f-h) and MDCK (d, e) cells on **1o** coated substrate (a-e), and **1c** coated substrate (f-h). Phase contract images of NIH/3T3 cells on **1o** coated substrate (0.44 μg/cm^2) before the irradiation (a), during the 365 nm light irradiation for 8 min (b), and 3 hours after the irradiation (c). Phase contrast image of MDCK cells on **1o**

coated substrate (0.44 μg/cm^2) 2 hours after 365 nm light irradiation for 12 min (d) and transmission image after subsequent Trypan blue dyeing (e). Phase contrast image of NIH/3T3 cells on **1c** coated substrate (0.30 μg/cm^2) 2 hours after 365 nm light irradiation for 12 min (f), phase contrast image after subsequent Trypan blue dyeing (g) and transmission image of the same view field (h). (Scale bars in all figures: 500 μm)

We then carried out the cell damage experiment on the PMMA films containing **1c**. The cells were attached before UV irradiation. UV light (365 nm, 95 mW/cm^2) was irradiated to the central part for 8 min (Figure 4).

Two hours later the irradiation, the cells in the irradiated area were damaged and departed from the surface. In the control experiment, such cell damage was not observed on the PMMA film without **1c** under the same UV irradiation conditions.

The by-products formed during the SO_2 gas generation were similar to those reported by Kobatake et al, and the by-products were maintained in PMMA matrix.[17]

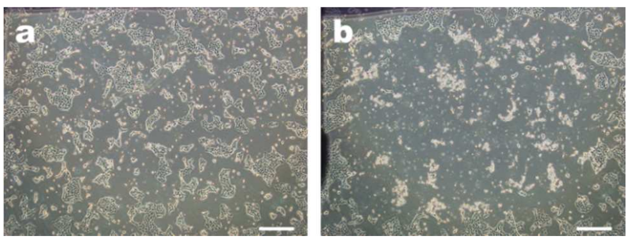


Fig. 4 Optical microscopic images of NIH/3T3 cells on a PMMA (64 μg/cm^2) film containing **1c** (7.2 μg/cm^2). (a) Before 365 nm light irradiation, (b) 2 h later after 365 nm (95 mW/cm^2) light irradiation for 8 min. (Scale bars: 500 μm)

The diarylethenes having longer conjugations showed high fatigue resistance even they possess the SO_2 groups.[5,6] The effective photoinduced elimination of sulfur dioxide will be limited within simple structured derivatives. Diarylethene **1o** is the special compound in that the SO_2 gas generation is moderate and the absorption wavelength is longer compared to that of thiophene 1,1-dioxide, and the degradation process is prohibited by forming the closed ring structure. Therefore, diarylethene **1c** has desirable characteristics as the photoinduced cell killing culture substrate for cell control.

Conclusions

Photoinduced elimination reaction of sulfur dioxide was found as biologically useful SO_2 gas generator accompanied with the photochromism of diarylethene **1o**. For the closed-ring isomer **1c**, the gas generation was prohibited without UV irradiation even at 70 °C, however once UV light (365 nm) was irradiated, it reverts to **1o** and sulfur dioxide gas was generated. Due to the generated gas, selective cell damage was observed. Such photoinduced on-demand killing of adherent cells on culture substrates will be applicable as one of the noncontact cell control technique.

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Notes and references

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† Electronic Supplementary Information (ESI) available: [Experimental Section, Absorption spectral changes of **1o** in hexane solution, Generated amount of SO_2 gas by gas detector without ND filters, GC-Mass spectroscopy of UV generated substance in gas phase, Detection of sulfur dioxide by a gas detector, and Discussion about decomposition mechanism]. See DOI: 10.1039/b000000x/

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