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Thio-bisnaphthalimides as Heavy-Atom-Free Photosensitizers with Efficient Singlet Oxygen Generation and Large Stokes Shifts: Synthesis and Properties

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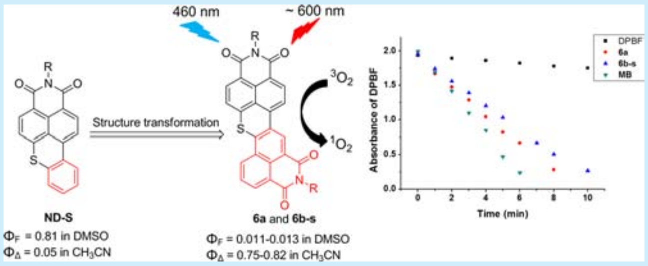
S Supporting Information

ABSTRACT: By structure transformation of benzo[*k*,*l*]-thioxanthene-naphthalimide derivatives (**ND-S**), a novel series of nonplanar thio-heterocyclic bisnaphthalimide derivatives are designed and synthesized. They display high molar absorptivity and large Stokes shifts. They are also heavy-atom-free photosensitizers with high singlet oxygen quantum yields of 0.75 and 0.82. Thus, these new structures based on the naphthalimide skeleton have great potential for singlet oxygen applications.



Singlet oxygen sensitizers are versatile molecules and have been widely applied in photodynamic therapy (PDT).[1] Upon photoexcitation, the photosensitizer enters a triplet excited state (T_1) via intersystem crossing (ISC) and reacts with triplet molecular oxygen to produce singlet oxygen ($^1O_2$).[2] One of the most common approaches to facilitate the ISC process is to introduce heavy atom effects, i.e. transition metal complexes with Ru(II), Ir(III), and Pt(II) or iodo-/bromo-containing compounds.[2b,3] However, such photosensitizers containing heavy atoms have often been reported to exhibit strong dark cytotoxicity.[4] Also, it is not always easy to modify a chromophore with heavy atoms.[5] Thus, intense efforts have been devoted to the development of efficient heavy-atom-free photosensitizers for singlet oxygen applications.

Developing heavy-atom-free photosensitizers is challenging.[6] Some effective examples are listed as follows: Akkaya[7] et al. reported the orthogonal boron-dipyrromethene (BODIPY) dimers as heavy-atom-free photosensitizers which displayed singlet oxygen quantum yields of 0.46–0.51. Fu[8] et al. designed several diperylene diimide (di-PDI) derivatives with high ISC rates for singlet oxygen generation, harnessing enhanced spin–orbital coupling of twisted di-PDI cores. Besides, the substituted perylene diimide (PDI) derivatives at core positions were also identified as efficient heavy-atom-free photosensitizers.[9] Nevertheless, these structures still show some limits in synthesis and structural derivation.

As is well-known, Naphthalimide is a facile chromophoric scaffold, exhibiting considerable molar absorptivity, low toxicity, and high photostability, and has been routinely employed in developing fluorescent probes and anticancer drugs.[10] In recent research, a large number of naphthalimide and heterocyclic fused naphthalimide derivatives have been reported as efficient DNA photocleavers,[11] while the mechanisms of photocleavage are the induced generation of a hydroxyl radical, superoxide anion, and singlet oxygen after DNA intercalating.[12] Moreover, naphthalimide-based metal complexes have been reported with a long-lived T_1 state because of the metal-to-ligand energy transfer (MLCT) at the excited state.[11d,13] However, further applications of naphthalimide derivatives as singlet oxygen sensitizers are seldom reported. Inspired by the previous studies of heavy-atom-free photosensitizers,[7,8] we consider developing some new singlet oxygen sensitizers based on the naphthalimide skeleton.

Our group has reported the synthesis of a series of benzo-heterocycle substituted naphthalimides and evaluated their abilities as effective DNA photocleavers.[14] According to their synthetic methods, we anticipated that using naphthalimide instead of a benzene ring might afford some novel nonplanar bisnaphthalimide derivatives (Scheme 1) to simulate the twisted cores of bay-substituted PDIs, which have been reported with efficient singlet oxygen productions.[9] Based on the above considerations, we designed and synthesized some novel thio-heterocyclic bisnaphthalime derivatives and evaluated their photophysical characteristics. Finally, we found that these thio-bisnaphthalimide derivatives demonstrated brilliant singlet oxygen generation along with heavy-atom-free properties.

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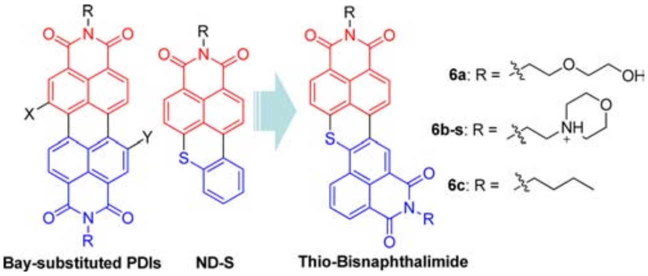
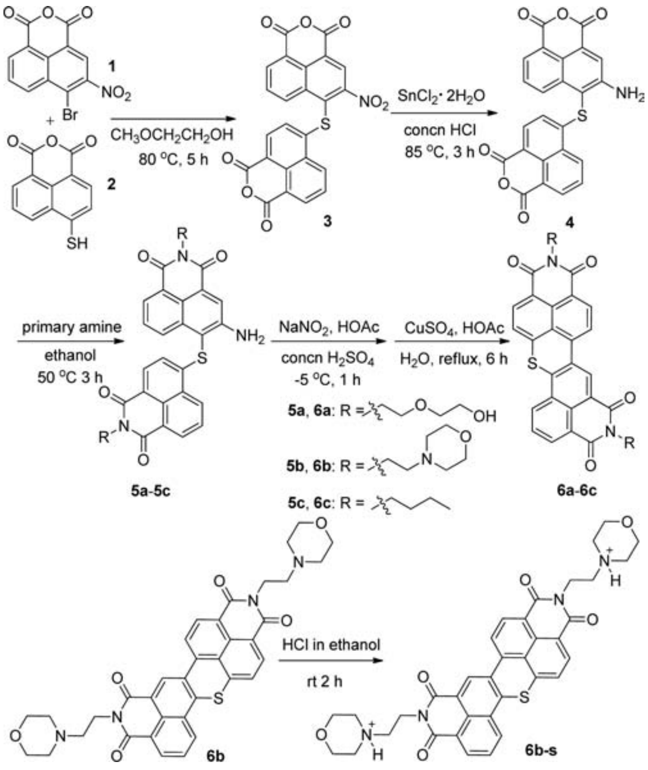
Scheme 1. Design Concept from ND-S and PDI to Thio-bisnaphthalimide



Scheme 2 summarizes the synthetic routes of thio-bisnaphthalimide compounds **6a**−**6c**. Naphthalic anhydride derivatives **1** and **2** were reacted in ethylene glycol monomethyl ether to produce additional product **3**, which was then reduced in HCl-$SnCl_2$ to generate **4**. Subsequently, substitution of **4** with different primary amines in ethanol obtained the key intermediates **5a**−**5c**. At last, the thio-bisnaphthalimide precursors **5a**−**5c** were diazotized in the presence of sodium nitrite and then cyclized by a copper-catalyzed reaction to afford **6a**−**6c** through a one-pot synthesis (yields: 40−52%).[14b,15] Moreover, to improve the solubility of the morpholine-substituted derivative, **6b** was treated with HCl in ethanol solution (1 mol/L) to prepare **6b**−**s**, which displayed characteristic peaks at the 2200−2700 cm^{-1} region in infrared spectroscopy (Figure. S1). Compared to the poor solubility of compound **6c**, **6a** and **6b**−**s** were soluble in multiple organic solvents including CH_2Cl_2, CH_3OH, DMSO, DMF, etc.

Scheme 2. Synthesis of Thio-bisnaphthalimide Derivatives



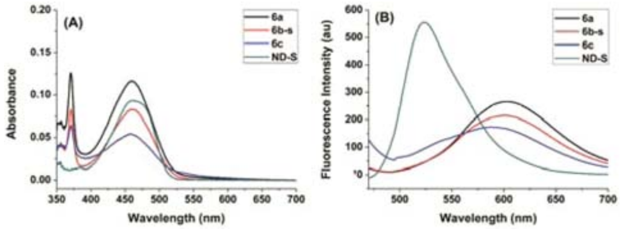


Figure 1. UV−vis absorption (A) spectra and fluorescent emission (B) spectra of **6a**, **6b**−**s**, **6c**, and **ND-S** in DMSO. Excitation wavelength: 460 nm, slit (**6a**, **6b**−**s**, and **6c**): (10, 10); slit (**ND-S**): (5, 2.5). The final concentrations of all compounds: 5 μM.

The UV−vis absorption and fluorescence emission spectra of **6a**, **6b**−**s**, and **6c** were acquired in DMSO and ethanol. Due to the low solubility of **6c** in ethanol, the data listed in Figure 1 and Table 1 were mainly obtained in DMSO (the data obtained in ethanol were shown in Figure S2 and Table S1). All of the thio-naphthalimide derivatives showed two separated absorption peaks at around 370 and 460 nm, while the benzo[*k*,*l*]-thioxanthene-naphthalimide derivative (**ND-S**) had only one peak at 460 nm (Table 1). The molar extinction coefficients of **6a** and **6b**−**s** in DMSO were 23 200 and 16 600 M^{-1} cm^{-1}, respectively. It suggested that they exhibited comparatively high absorptive abilities in the visible region, while **6c** displayed a weaker absorptive ability (ε = 10 800 M^{-1} cm^{-1}). Besides, the derivatives displayed broad emission spectra and the emission maxima of **6a** and **6b**−**s** were around 600 nm (red shift about 70 nm related to **ND-S**). Hence, the Stokes shifts were as large as 141 nm, which minimized self-quenching via homo-FRET.[16] In addition, the UV−vis absorption and fluorescent emission spectra of **6a** and **6b**−**s** upon variation of concentrations were evaluated in ethanol (Figure S3). Almost no shift was observed during the tests, which claimed that the molecular aggregation had a negligible effect on large Stokes shifts. For fluorescence, by comparison of **ND-S**, all of the thio-bisnaphthalimide derivatives were weakly emissive while the fluorescence quantum yields of **6a** and **6b**−**s** were determined to be around 0.01 in both ethanol and DMSO. We consequently supposed that efficient ISC processes might have happened in these compounds upon light irradiation.

Table 1. Optical Properties of 6a, 6b−s, 6c, and ND-S

compd	6a	6b−s	6c	ND-S
λ_{abs} (nm)[b]	371, 459	371, 460	371, 457	460
λ_{em} (nm)[b]	600	601	585	520
Stokes shift (nm)[a,b]	141	141	128	57
ε (M^{-1} cm^{-1})[b]	23200	16600	10800	18600
Φ_F[c]	0.012	0.011	0.013	0.81
Φ_Δ[d]	0.75	0.82	−	0.05

[a]Stokes shifts were listed in units of nanometers. [b]The spectroscopic properties were measured in DMSO. [c]Yields were calculated with *N*-butyl-4-butylamino-naphthalimide as standard (0.81 in ethanol), and the above data were obtained in DMSO. [d]Yields were calculated with Methylene blue as the standard (0.52 in acetonitrile).

The singlet oxygen generation of these thio-bisnaphthalimide derivatives were examined in air saturated acetonitrile with 1,3-diphenyl-isobenzofuran (DPBF) as the $^1O_2$ scavenger and methylene blue (**MB**) as the reference photosensitizer. Because the light irradiation at 460 nm could cause photobleaching of DPBF, we chose 470 nm as the experimental excitation wavelength for activating **6a** and **6b**−**s**. Figures 2 and S4 showed the absorbance values of DPBF at 410 nm decreased upon different irradiation times. There was no obvious change

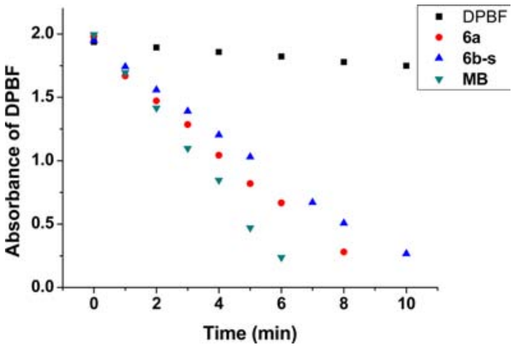


Figure 2. Absorbance values (at 410 nm) of DPBF (100 μM) in air saturated acetonitrile decreased upon time. The compounds' concentration: 3 μM.

in DPBF alone solution (no added compounds). When **6a** and **6b−s** were added to the DPBF solution, the absorbance decreased dramatically upon light irradiation (470 nm), which suggested that singlet oxygen had been produced in abundance. However, only a slight decrease was detected with the addition of **ND-S**. The relative singlet oxygen quantum yields were determined by comparison to methylene blue (whose yield was 0.52 in acetonitrile[2b]). Both two thio-bisnaphthalimide derivatives exhibited noteworthy yields of 0.75 (**6a**) and 0.82 (**6b−s**), respectively (much higher than **ND-S** of 0.05, showing in Table 1). These results were in accordance with the proposed efficient ISC processes from the singlet excited state to the triplet excited state. Besides, when fixing the wavelength and density of excitation light, the decreased absorbance of DPBF had a linear relationship with respect to the duration of irradiation. As a control, to verify the key role of oxygen in the decrease of DPBF's absorbance, **6a** and **6b−s** were further treated in deoxygenated solutions (Figures S5 and S6). The changes of absorbance value at 410 nm reduced significantly relative to the results in air saturated situations. It indicated that singlet oxygen generations were suppressed when removing the oxygen of solutions. The above results confirmed that these new thio-bisnaphthalimide compounds were highly efficient and stable singlet oxygen sensitizers.

Subsequently, the photocytotoxic effects of these thio-bisnaphthalimide derivatives were studied on human gastric carcinoma cells (MKN45) for PDT evaluation. After 20 min of treatment, both **6a** and **6b−s** displayed valid cytotoxic activities with IC_{50} values of 13.2 and 31.4 μM, respectively, while the control group without laser irradiation showed negligible cell death above 50 μM (Figure 3). These data proved that **6a** and **6b−s** might be utilized in potential applications as singlet oxygen sensitizers in PDT research. However, we also noted that photosensitive abilities were influenced by the relatively low water solubility of novel compounds. Further studies on improving these properties are under investigation.

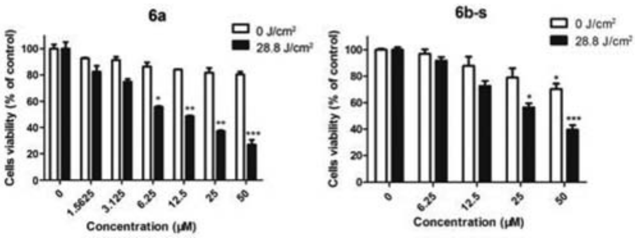


Figure 3. MTT assays of **6a** and **6b−s** exposed with laser irradiation of 0 and 28.8 J/cm^2 in MKN45 cells. All the data above are presented as mean ± SD ($n = 3$ per group). Significant differences are considered as $*p < 0.05$; $**p < 0.01$; $***p < 0.001$.

To rationalize the abnormal ISC processes and spectral behaviors of **6a** and **6b−s**, theoretical computational studies of ground states and excited states were performed and optimized by time-dependent density functional theory (TD-DFT) calculations at the B3LYP/6-31G(d) level (Figures S7 and S8). The optimized ground state geometries demonstrated that **6a** and **6b−s** displayed slightly twisted planars with the addition of a sulfur atom, which might decrease the self-aggregation of these thio-bisnaphthalimide derivatives.[8b] Table 2 listed the TD-B3LYP/6-31G(d) vertical excitation energies for the singlet and triplet excited states of **6a** and **6b−s** based on their optimized geometries of ground states. The calculations predicted notable transitions from S_0 to S_3 (for **6a**) and from S_0 to S_2 (for **6b−s**) according to the oscillator strengths. The transition energies in Table 2 were consistent with the determined data from absorption spectra (2.71 eV for **6a** and 2.70 eV for **6b−s**).[17] However, the calculated transition energies between S_1 and S_0 were much lower than the excitation energies. These results might lead to the large Stokes shifts of thio-bisnaphthalimide derivatives. More importantly, ISC processes of **6a** and **6b−s** were characterized by the low energy gaps between singlet excited states and triplet excited states (Table 2), which indicated that the $S_n{-}T_m$ ISC channels were energetically accessible for **6a** (i.e., $S_1 \rightarrow T_1$, $S_1 \rightarrow T_2$, and $S_3 \rightarrow T_3$) and **6b−s** (i.e., $S_1 \rightarrow T_1$, $S_1 \rightarrow T_2$, and $S_2 \rightarrow T_3$), in accordance with the previous experimental observations of high singlet oxygen quantum yields.[18]

Table 2. Lowest Vertical Singlet and Triplet Electronic Energies and Oscillator Strengths (f) of 6a and 6b−s at the TD-B3LYP/6-31G(d) Level of Theory

compd	state	E (eV)	f	ΔE ($S_n{-}T_m$, eV)
6a	S_1	2.14	0.029	–
	S_3	2.70	0.482	–
	T_1	1.42	–	0.72 ($S_1 \rightarrow T_1$)
	T_2	1.94	–	0.20 ($S_1 \rightarrow T_2$)
	T_3	2.63	–	0.07 ($S_3 \rightarrow T_3$)
6b−s	S_1	2.04	0.055	–
	S_2	2.68	0.530	–
	T_1	1.51	–	0.53 ($S_1 \rightarrow T_1$)
	T_2	1.87	–	0.17 ($S_1 \rightarrow T_2$)
	T_3	2.66	–	0.02 ($S_2 \rightarrow T_3$)

In summary, we have designed and synthesized a novel series of nonplanar thio-heterocyclic thio-bisnaphthalimide derivatives from single naphthalimide structures by a facile synthetic route. These outstanding structures (**6a** and **6b−s**) displayed high singlet oxygen quantum yields of 0.75 and 0.82 without heavy atoms, demonstrating that they were efficient singlet oxygen sensitizers. MTT assays also proved their possible application in PDT research. Furthermore, the photophysical properties of **6a** and **6b−s** demonstrated that they both had brilliant light absorptive abilities and large Stokes shifts. Additionally, theoretical calculations illustrated the slightly twisted planars of the thio-bisnaphthalimide cores for decreasing the self-aggregations and demonstrated that the efficient ISC processes were due to the low singlet−triplet energy gaps of **6a** and **6b−s**. We believe that these thio-bisnaphthalimide structures would present promising roles in not only singlet oxygen generation (as heavy atom free photosensitizers) but also many other applications.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.6b02902.

Experimental procedures and characterization data (PDF)

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Notes

The authors declare no competing financial interest.

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