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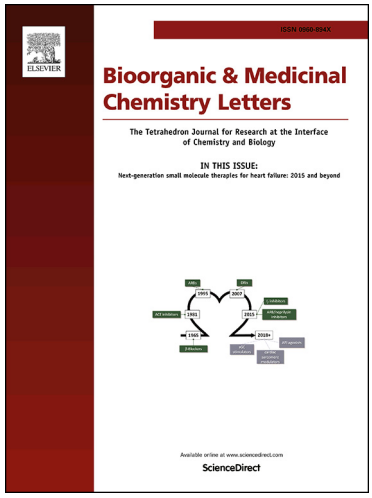

The design, synthesis, and evaluation of organic dithienopyrrole-based D-π-A dyes for use as sensitizers in photodynamic therapy

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The design, synthesis, and evaluation of organic dithienopyrrole-based D-π-A dyes for use as sensitizers in photodynamic therapy

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Abstract

Dithienopyrrole-based organic dyes that combine an electron-donating moiety (D), a π-conjugated bridge moiety (π), and an electron-accepting moiety (A) were designed and synthesized in short steps by previously developed one-pot Suzuki-Miyaura coupling approach. Absorption wavelengths of the dyes were readily tuned by altering the D and A moieties. The use of a strongly electron-withdrawing cyanopyridone acceptor enabled NIR absorption. A synthesized sensitizer, **2j**, exerted potent phototoxicity mainly via a Type I mechanism in cells. A nitrogen atom in the dithienopyrrole ring serves as a connecting point for the introduction of functional building blocks that can improve the properties of sensitizers, which makes this D-π-A sensitizer a valuable template for the further development of sensitizers.

Keywords: photodynamic therapy, Suzuki-Miyaura coupling, one-pot, dithienopyrrole

Photodynamic therapy (PDT) has garnered much attention as a non-invasive, selective, and cost-effective cancer therapy.[1] In PDT, a sensitizer is administered to cancer patients and damages cancer cells under photo irradiation via Type I (hydrogen/electron transfer) and/or Type II (singlet oxygen generation) pathways, which leads to cell death. Several sensitizers have been approved and clinically used for PDT.[2] Many porphyrin-based sensitizers have been developed in ongoing efforts to improve PDT.[3] Although tremendous effort has been extended to develop new sensitizers, the number of available sensitizers remains somewhat limited due to the many requirements: proper absorption wavelength (NIR absorption is desirable), high absorptivity, high stability under photo irradiation and physiological conditions, low levels of dark toxicity, high levels of tumor accumulation, and rapid excretion from the body following PDT.[3a] In addition, sensitizers that damage cancer cells involving Type I mechanisms are considered advantageous under hypoxic

conditions in cancers.[4] A sensitizer that could fulfill all requirements awaits development.

We have developed an efficient synthetic approach to thiophene-based, organic dyes that combines an electron-donating moiety (D), a π-conjugated bridge moiety (π), and an electron-accepting moiety (A).[5] We recently reported that the D-π-A sensitizer **1** exerted potent phototoxicity via both Type I and Type II mechanisms (Figure 1).[6] However, this did not demonstrate absorption in the NIR region. Herein, we report the design and synthesis of dithienopyrrole-based D-π-A sensitizer **2**. Use of strongly electron-withdrawing cyanopyridone acceptor enabled NIR absorption. Evaluation revealed that one of the synthesized sensitizers, **2j**, exerted potent phototoxicity mainly via a Type I mechanism.

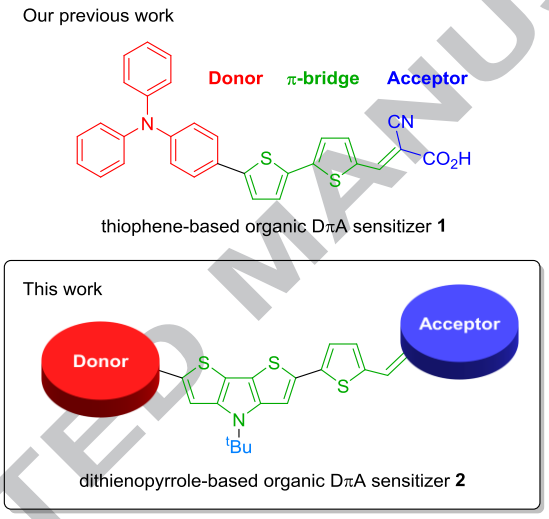
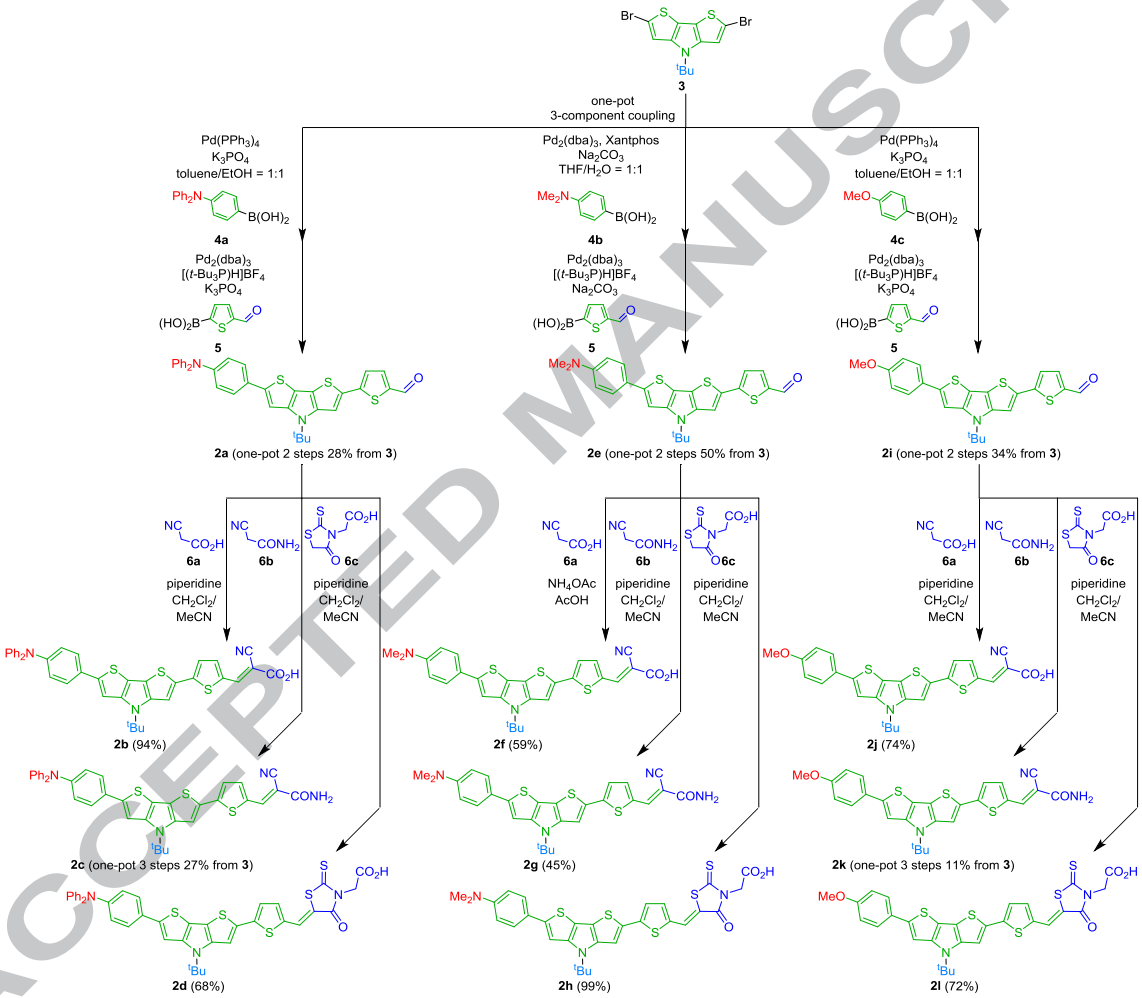


Fig. 1 Chemical structures of our previously reported thiophene-based organic D-π-A sensitizer **1**, and of the new dithienopyrrole-based organic D-π-A sensitizer **2**.

We designed the dithienopyrrole-based D-π-A sensitizer **2** for four reasons: 1) dithienopyrrole consists of a fused ring system with extended π-conjugation that enables a red shift of absorption; 2) dithienopyrrole consists of 5-membered heteroaromatic rings (pyrrole and thiophene), and when linked to a benzene ring, both dithienopyrrole and benzene rings reside on a common plane that allows an extension of the π-conjugated system, which results in a red-shift of absorption;[7] 3) a nitrogen atom in the dithienopyrrole ring serves as a connecting point for the introduction of a functional group; and, 4) dithienopyrrole is readily available.

Initially, we prepared dithienopyrrole-based 12 D-π-A sensitizers, **2a-2l** from a combination of 3 donors and 4 acceptors with different levels of electron-donating and -withdrawing abilities (Scheme 1). In order to improve the solubility of the sensitizers, a *tert*-butyl group was attached to the dithienopyrrole ring via the nitrogen atom. A one-pot Suzuki-Miyaura coupling[8] of dibromo dithienopyrrole **3**[9] with 3 donors, **4a-4c**, and thiophene boronic acid **5** was based on our previously

reported procedure,[5f] and afforded the desired compounds **2a**, **2e**, and **2i** in moderate yields. A subsequent Knoevenagel condensation of three aldehydes with 3 acceptors, **6a-6c**, afforded the desired D-π-A sensitizers **2b-2d**, **2f-2h**, and **2j-2l** in moderate to excellent yields. Knoevenagel condensations of **2e** with **6a** did not afford the desired product **2f** under basic conditions, and, therefore, acidic conditions were used for the synthesis of **6a**. In the cases of the syntheses of **2c** and **2k**, Knoevenagel condensation was performed in a one-pot fashion.[5f]



Scheme 1 Preparation of dithienopyrrole-based 12 D-π-A sensitizers, **2a-2l**.

The photo absorption spectra of **2a-2l** in DMSO were measured (Figure 2 and Table 1). The D-π-A sensitizers exerted good absorption intensity (ε = 20,000-53,000 L mol^{-1} cm^{-1}) as shown in Table 1. As expected, the introduction of a dithienopyrrole moiety to sensitizer **1** resulted in a *ca* 40 nm red shift (Table 1, entry 1 vs. 2). When a sensitizer had a more electron-donating donor (Me_2N- > Ph_2N- > MeO-), it tended to have longer wavelength absorption (**2e** with Me_2N-: λ_{max} = 488 nm, **2a** with Ph_2N-: λ_{max} = 477 nm, **2i** with MeO-: λ_{max} = 469 nm), as shown in entries 2, 7, and 11. On the

other hand, the electron-withdrawing ability of the acceptors (rhodanine > cyanoacrylic acid > cyanoacrylic amide > aldehyde) was not consistent with absorption wavelength. In detail, the sensitizer **2b** with more electron-withdrawing cyanoacrylic acid acceptor had similar or shorter wavelength absorption comparing with those of the sensitizers **2c** and **2a** with less electron-withdrawing cyanoacrylic amide and aldehyde acceptors, respectively (**2d** with rhodanine: λ_{max} = 557 nm, **2b** with cyanoacrylic acid: λ_{max} = 477 nm, **2c** with cyanoacrylic amide: λ_{max} = 526 nm, **2a** with aldehyde: λ_{max} = 477 nm), as shown in entries 2, 3, 5, and 6. We speculated that the deprotonation of cyanoacrylic acid generated less electron-withdrawing cyanoacrylate, which shortened the absorption wavelength. In order to confirm this speculation, a photo absorption spectrum of sensitizer **2b** in DMSO with 0.5% acetic acid was measured. As expected, longer wavelength absorption was observed (Table 1, entry 3 vs. 4).

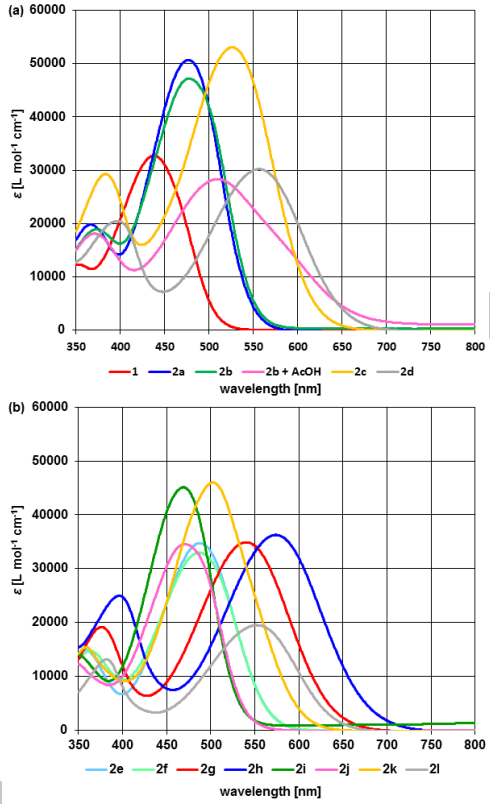


Fig. 2 Absorption spectra of D-π-A sensitizers (a) **1-2d** and (b) **2e-2l** in DMSO.

Table 1 Absorption maxima (λ_{max}), molar absorption coefficients (ε), and optical edges of the sensitizers **1-2l**.

entry	sensitizer	λ_{max} [nm]	ε [$Lmol^{-1}cm^{-1}$]	optical edge*[1] [nm]
1	**1**	438	33,000	507

2	**2a**	477	51,000	548
3	**2b**	477	47,000	552
4*[2]	**2b**	510	28,000	669
5	**2c**	526	53,000	618
6	**2d**	557	30,000	656
7	**2e**	488	35,000	566
8	**2f**	487	33,000	567
9	**2g**	540	35,000	642
10	**2h**	574	36,000	684
11	**2i**	469	45,000	534
12	**2j**	471	35,000	540
13	**2k**	503	46,000	597
14	**2l**	553	20,000	642

*1 Optical absorption edge was defined by the wavelength for which the absorbance revealed 1/10 of the peak top.

*2 D-π-A sensitizer **2b** with 0.5% acetic acid.

The antitumor effects of sensitizers **2a-2l** and that of the positive controls, sensitizer **1**, merocyanine 540 (MC540),[10] and protoporphyrin IX (PPIX)[2b] on HeLa cells under photo irradiation are shown in Table 2.[11] Although our previously reported D-π-A sensitizer with a triphenyl amine donor exerted potent phototoxicity,[6] the triphenyl amine-containing D-π-A sensitizers **2a-2d** showed weak phototoxicity (entries 1-4). These results indicate the importance of the systematic preparation and evaluation of sensitizers by combining various building blocks for the development of highly potent sensitizers. Sensitizers containing dimethyl phenyl amine, **2e**, **2f-2h** (entries 5-8), or methoxy phenyl donor, **2i-2l** (entries 9-13), exerted a higher level of phototoxicity. With respect to an acceptor moiety, cyanoacrylic acid or cyanoacrylic amide acceptor contributed to a higher level of phototoxicity. In fact, highly phototoxic compounds, **2f**, **2j**, and **2k** (IC_{50} < 1 μM), are found in combinations of dimethyl phenyl amine or methoxy phenyl donor and cyanoacrylic acid or cyanoacrylic amide acceptor (entries 6, 10, and 12). These sensitizers exerted a level of phototoxicity that was higher than that of the positive controls MC540 and PPIX, and the same level of phototoxicity as that of sensitizer **1**. Since much stronger photo irradiation conditions are used in clinical PDT (laserphyrin: 150 mW/cm^2, 3-12 min), the antitumor activity of the most potent sensitizer, **2j**, was evaluated over an extended amount of photo irradiation time (60 min, entry 11), and exerted a significantly higher level of phototoxicity (IC_{50} of **2j**: 0.063 μM). In addition, **2j** was less toxic (IC_{50} = 43 μM) without irradiation by comparison with **2f**, **2k**, and **1** (IC_{50} = 15-22 μM).

Table 2 Antitumor activity of D-π-A sensitizers **2a-2l** against HeLa cells.

entry	Sensitizer	IC_{50} (mM)[*1]	
		dark	Irradiation (15 min)
1	**2a**	67.0 ± 13.8	45.0 ± 6.4
2	**2b**	> 100	> 100
3	**2c**	> 100	> 100
4	**2d**	60.2 ± 11.2	17.1 ± 1.8
5	**2e**	> 10	2.5 ± 0.1
6	**2f**	15.5 ± 2.7	0.6 ± 0.1
7	**2g**	54.0 ± 7.6	5.0 ± 0.4
8	**2h**	>100	12.1 ± 1.9
9	**2i**	68.6 ± 10.6	3.8 ± 0.2
10	**2j**	43.4 ± 3.3	0.19 ± 0.01
11	**2j**	43.4 ± 3.3	0.063 ± 0.005[*2]
12	**2k**	15.7 ± 1.7	0.56 ± 0.02
13	**2l**	63.9 ± 8.8	5.7 ± 0.3
14	**1**	21.9 ± 2.4	0.21 ± 0.03
15	MC540	>100	16 ± 3.0
16	PPIX	12.5 ± 4.3	0.75 ± 0.02

*1 The sensitizer concentration required to reduce cell viability by 50% (IC_{50}) was determined from semi-logarithmic, dose-response plots. *2 Irradiation time was 60 min.

The oxidative damage to human serum albumin (HSA) caused by the photo irradiation of the sensitizers **2a-2l** and positive control (MC540) was monitored. HSA has only one tryptophan residue (Trp), and the intrinsic fluorescence of HSA originates almost entirely from it. This tryptophan residue is easily oxidized by photo-irradiated sensitizers. It results in a decrease in the characteristic fluorescence of tryptophan at around 350 nm, which makes HSA useful for evaluating the protein-damaging activity of photosensitizers.[4, 12] A sample solution containing 10 μM of sensitizer and 20 μM of HSA in a PBS buffer was irradiated via LED (ISL-150×150 H3WH4R, white light, 420-750 nm, 8.0 ± 0.5 mW/cm^2, CCS Inc.). MC 540 was used as a positive control. The observed protein-damaging activity of the sensitizers was almost consistent with their phototoxicity (Figure 3). The highly phototoxic sensitizers **2f**, **2j**, and **2k** obviously damaged HSA, whereas the other sensitizers showed no obvious damage to HSA. The most phototoxic sensitizer **2j** (Table 2, entry 10), showed the most significant oxidative damage to HSA (Figure 3).

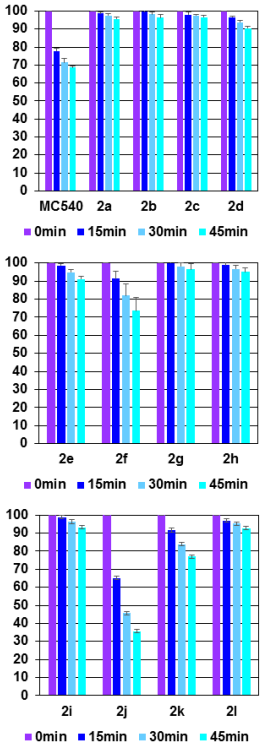
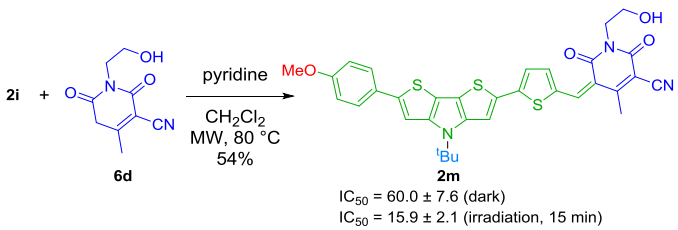
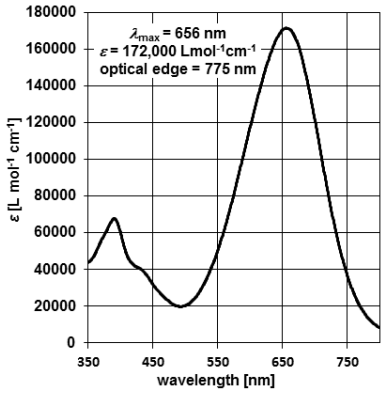


Fig. 3 Time-dependent damage of human serum albumin (HSA). A sample solution containing 10 μM of sensitizer and 20 μM of HSA in a PBS buffer was irradiated via LED.

In order to achieve NIR absorption, the acceptor moiety of the most potent sensitizer, **2j** was changed to strongly electron-withdrawing cyanopyridone. Knoevenagel condensation of aldehyde **2i** with the acceptor **6d**[13] afforded the desired sensitizer **2m** in a moderate yield. As expected, **2m** demonstrated excellent absorption in the NIR region (> 650 nm), as shown in Scheme 2. These results clearly demonstrated the ready tunability of the absorption wavelength of the D-π-A sensitizer. However, the antitumor activity of sensitizer **2m** on HeLa cells under photo irradiation was low. This result again indicated the importance of cyanoacrylic acid or cyanoacrylic amide acceptor for antitumor activity of sensitizers.





Scheme 2 Synthesis of a D-π-A sensitizer, **2m**, containing strongly electron-withdrawing cyanopyridone acceptor and its antitumor activity and photo absorption spectrum.

To further elucidate the mode of action for the most potent sensitizer, **2j**, damage to Trp was measured in the presence of L-ascorbic acid (ROS quencher), and NaN_3 (Type II singlet oxygen quencher) (Figure 4). Damage to the protein was obviously decreased in the presence of L-ascorbic acid and slightly decreased in the presence of NaN_3. These results indicate that sensitizer **2j** oxidizes HSA mainly through Type I mechanisms.

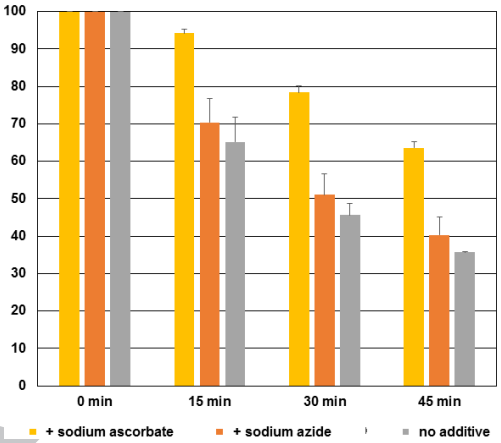


Fig. 4 Time-dependent damage of human serum albumin by sensitizer **2j**. A sample solution containing 10 μM of sensitizer and 20 μM of HSA in a PBS buffer was irradiated via LED.

In order to confirm the cellular uptake of sensitizer **2j**, HeLa cells that were treated with **2j** and Hoechst 33342 were observed via fluorescence confocal microscopy (Figure 5). The sensitizer **2j** obviously went into the HeLa cells and was distributed over the cytoplasm. In particular, **2j** seemed to localize near the nuclei.

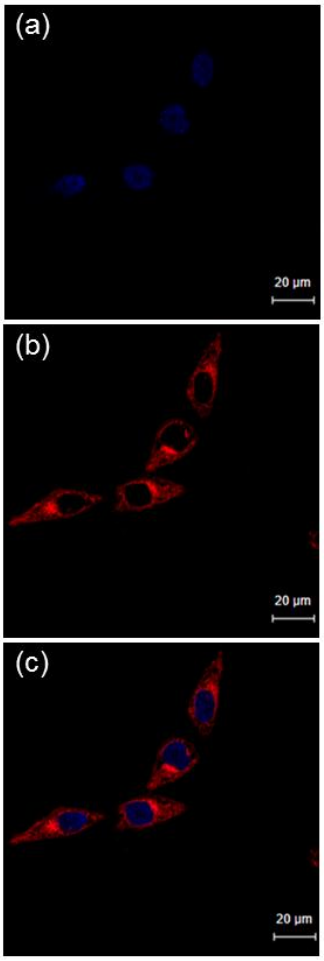


Fig. 5 Colocalization study (a) Hoechst 33342; (b) sensitizer **2j**; and, (c) a merging of two images, (a) and (b).

We designed dithienopyrrole-based D-π-A sensitizers and rapidly prepared them via our developed one-pot coupling approach. As expected, the D-π-A structure enabled a ready tuning of the absorption wavelength. The highly phototoxic sensitizer **2j** was developed, and it exerted phototoxicity mainly via a Type I mechanism following its absorption into cells. A nitrogen atom in a dithienopyrrole ring served as a connecting point to introduce functional building blocks that could improve sensitizer properties such as solubility and/or tumor targeting ability. Therefore, a D-π-A sensitizer with a dithienopyrrole structure could be a valuable template for further development of sensitizers that could fulfil the requirements for PDT.

A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.bmcl.xxxxx.

Acknowledgement

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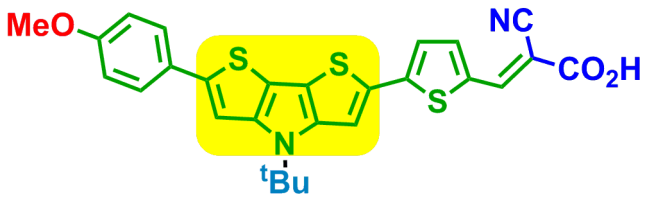
D-π-A sensitizer containing dithienopyrrole for PDT



antitumor activity
against HeLa cells

IC_{50} = 43.4 μM (irradiation)
IC_{50} = 0.063 μM (dark)

Highlights

- Dithienopyrrole-based organic dye exerted potent phototoxicity.
- The dye killed cancer cells mainly through Type I mechanism.
- D-π-A structure enabled a ready tuning of absorption wavelength.