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On the Use of Diarylmaleimide Derivatives in Biological Contexts: An Investigation of the Photochromic Properties in Aqueous Solution

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Abstract

A series of photochromic diarylmaleimide derivatives has been synthesized and studied with respect to the photochromic properties in aqueous solution. The main rationale is to investigate if these compounds could be used as photoswitchable units in biological contexts, motivated by the fact that the diarylmaleimide structural motif is identified in many pharmacophores. Thus, photoswitchable variants of this class of compounds could be very useful in the quest for photoactivatable drugs. The photoinduced cyclization reaction (colorization) is suppressed in solvents of high polarity, whereas the ring-opening reaction (decolorization) still occurs with high efficiency. The photochromically active anti-parallel conformer of the open form is more abundant in the asymmetrically substituted derivatives, which in turn favors the formation of the closed isomeric form. The rates of the thermal isomerization reactions have also been assessed, together with the resistance toward thermal

degradation. Here it was observed that the maleimide derived compounds were not susceptible to the thermally driven reactions (hydrolysis and isomerization).

Keywords: photochromism, photoswitches, diarylmaleimides, photoisomerization

1. Introduction

The diarylmaleimide moiety features in a large number of biologically active natural products as well as known pharmaceuticals and bioactives.[1] Such examples include the natural product **rebeccamycin**, which exhibits potent anticancer properties, **Ro31-8220**, a known sirtuin inhibitor, and **MS-1**, a potent necrosis inhibitor (see Figure 1).[2]

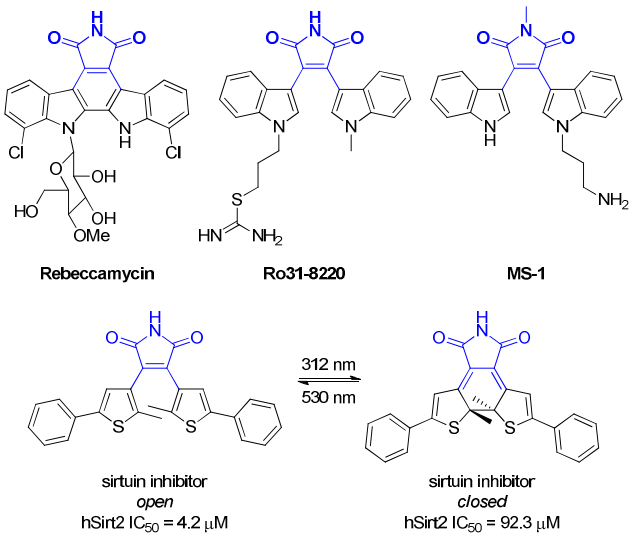


Figure 1. (*Top*) Maleimide-containing bioactive compounds. (*Bottom*) A photochromic diarylmaleimide inhibitor of the sirtuin kinase.[7]

Shown is also a photochromic sirtuin inhibitor built around the maleimide structural motif (*vide infra*). This compound serves as a good illustration of the emerging research field focusing on the design and synthesis of photoswitchable bioactives. The utilization of light to control the pharmacological activity of drugs in cells and diseased tissue has the potential to provide valuable insight into the fundamental molecular events of complex cellular processes.

It also allows for on-demand drug activation with unprecedented spatiotemporal control.[3] To date, a handful of photochromic core-structures have been documented in the literature including azobenzenes, diarylethenes, diarylmaleimides, fulgides and spiropyrans.[4] When compared to diarylethenes, photochromic diarylmaleimide derivatives present a number of favorable properties for biological applications such as red-shifted absorption spectra, increased water solubility, as well as increased stability toward hydrolysis.[5] Despite these advantages of the diarylmaleimides, water soluble diarylethene derivatives have already found much wider applications in biological settings.[3b, 3e–f, 6]

Due to the structural similarities between many bioactives and the photochromic core of the diarylmaleimide derivative, we and others are interested in transferring the photochromic properties to related pharmacophores to give rise to novel photoswitchable drugs. As indicated above, König and co-workers recently described the design and synthesis of a diarylmaleimide-based photochromic sirtuin inhibitor (see Figure 1, *bottom*).[7] Indeed, this inhibitor exhibits desirable photophysical properties and more importantly, the photoinduced isomerization between the open and closed forms results in significant changes in the affinity for the target enzyme. However, the photoisomerization had to be conducted in organic solvents rather than aqueous media, as the photocyclization reaction to the closed isomer of diarylmaleimide derivatives is strongly suppressed in aqueous media. This is due to the existence of a twisted intramolecular charge transfer (TICT) state, which is efficiently populated upon excitation in polar solvents, outcompeting the cyclization reaction.[8]

To surmount this obstacle, the UV-induced photocyclization must be performed in a less polar solvent prior to the administration/incubation of the closed isomer in aqueous milieu, where the photoswitch can be isomerized to the open isomeric form by visible light exposure. While this approach does not allow for reversible photoisomerization, it could still be useful if the desired effect can be achieved in a single activation step triggered by visible

light exposure. There are, however, additional requirements that have to be met. These include a high isomerization quantum yield of the ring-opening reaction, sufficiently high enrichment of the closed isomer in the UV-induced photostationary state (PSS), slow thermal isomerization of the closed isomer, and good stability toward thermal degradation. Employing the approach described above, we have investigated the photochromic properties of a small library of symmetrical and unsymmetrical diarylmaleimides and diarylmaleic anhydrides. The influence of the nature of the heterocycles as well as the photochromic diarylmaleimide core on the isomerization quantum yields, the PSS after UV-exposure, the thermal isomerization rate, and the stability toward degradation in aqueous media have been studied.

2. Results and Discussion

The primary objective of this work has been to investigate the various thermal and photochromic processes for compounds **1**–**8** shown in Figure 2. In order to study the structure-physical property relationship (SPPR) we opted for a series of symmetrical and unsymmetrical diarylmaleimides and diarylmaleic anhydrides that are structurally similar to biologically active compounds documented in the literature. Attached to the central pentacycle are indole- and benzothiophene derived heterocycles, decorated with various substituents, which may serve as examples of attachment points for structural motifs that could be tailor-made to improve water solubility as well as to optimize the interactions with the biomolecule of choice through, e.g. hydrogen bonding (c.f. the structures in Figure 1).

The sections below are organized as follows: After a brief description of the synthetic procedures, the general photochromic behavior of the compounds will be presented together with details on the UV-induced photocyclization (closing) reaction in THF. The thermal reactions (isomerization and degradation) of the closed isomeric form in aqueous solution will then be described. Finally, the visible-light induced opening reaction is investigated in both THF and aqueous solution. Note that the experimental results will be shown only for

compound **2**, whereas the corresponding data for the other derivatives is collected in the Supporting Information.

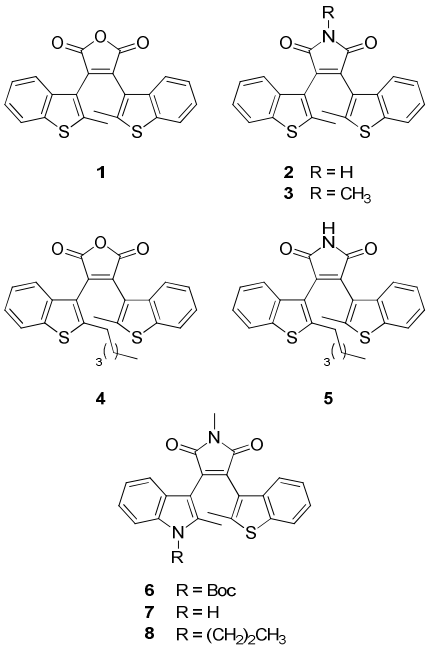
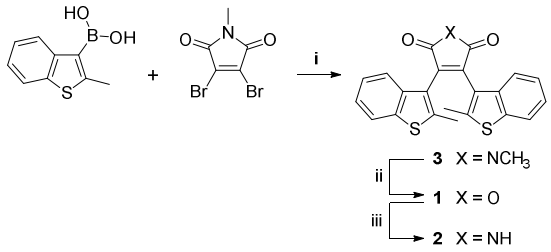


Figure 2. Structures of the open isomeric forms of the derivatives studied in this work.

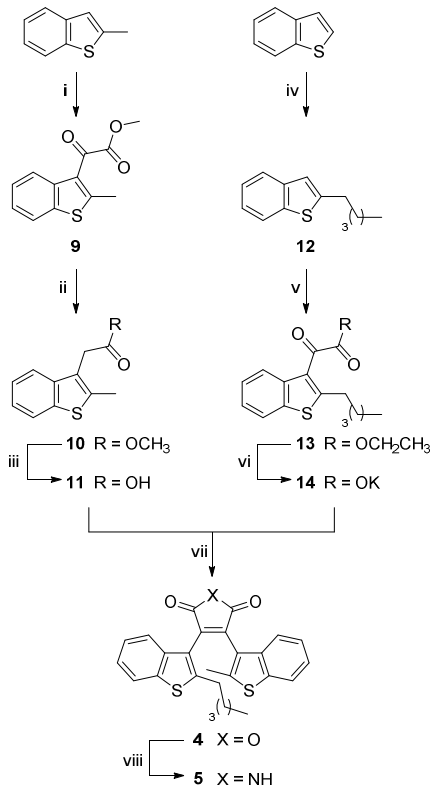
2.1. Synthesis

Employing the cross-coupling conditions described by Irie et al., symmetrical diarylmaleimide **3** was obtained in good yield (61%) by the Suzuki–Miyaura coupling of *N*-methyl-3,4-dibromomaleimide with a benzothiophene boronic acid (Scheme 1).[5c] Subsequent hydrolysis (using 40% KOH) and aminolysis with hexamethyldisilazane (HMDS) afforded **1** (84%) and **2** (74%), respectively.



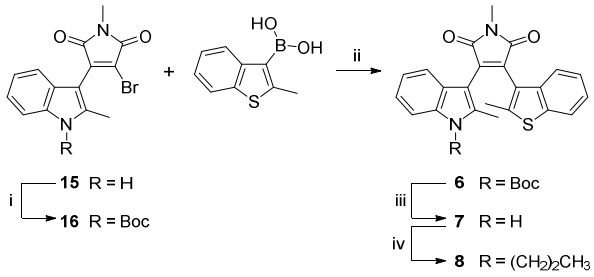
Scheme 1. Synthesis of compounds **1–3**. *Reagents and conditions:* (i) $Pd(PPh_3)_4$, CsF, 1,4-dioxane, 100 °C, 4 h (61%); (ii) EtOH, 40% KOH, reflux, 72 h (84%); (iii) HMDS, MeOH, DMF, room temperature, 12 h (74%).

Non-symmetrical diarylmaleimide derivatives can be readily accessed by employing a Perkin condensation between an appropriate carboxylic acid and α-keto acid precursor.[9] Utilizing this approach, the preparation of diarylmaleimide derivatives **4** and **5** are detailed in Scheme 2. Firstly, carboxylic acid **11** precursor was prepared from 2-oxoacetate **9**, which was synthesized according to Scandola et al.[9b] Using a modified literature procedure, reduction of 2-oxoacetate **9** with triethylsilane in trifluoroacetic acid at ambient temperatures, followed by hydrolysis of the methyl ester, afforded the corresponding carboxylic acid **11** in 61% yield over two steps.[10] The potassium salt **14** precursor was readily obtained in four steps beginning with lithiation at the 2-position of benzothiophene and subsequent addition of 1-bromopentane to give compound **12** in 98% yield. Friedel–Crafts acylation of benzothiophene **12** with ethyl chlorooxoacetate afforded compound **13** in high yield (80%). Hydrolysis of ethyl ester **13** to the corresponding potassium salt **14** was achieved in 63% yield. Next, condensation of potassium salt **14** with carboxylic acid **11** in acetic anhydride afforded the non-symmetrical diarylmaleimide **4** in modest yield (46%). Conversion of anhydride **4** to maleimide **5** was achieved in high yield (82%) by aminolysis with HMDS.



Scheme 2. Synthesis of compounds **4** and **5**. *Reagents and conditions*: (i) methyl chlorooxoacetate, $AlCl_3$, CH_2Cl_2, 0 °C to room temperature, 2 h (97%); (ii) Et_3SiH, TFA, room temperature, 16 h (64%); (iii) NaOH, 95% EtOH, room temperature, 4 h (96%); (iv) 1-bromo-pentane, *n*-BuLi, THF, −78 °C, 12 h (98%); (v) ethyl chlorooxoacetate, $AlCl_3$, CH_2Cl_2, 0 °C to rt, 2 h (80%); (vi) KOH, EtOH, room temperature, 4 h (63%); (vii) Ac_2O, 110 °C, 4 h (46%); (viii) HMDS, MeOH, anhydrous DMF, room temperature, 18 h (82%).

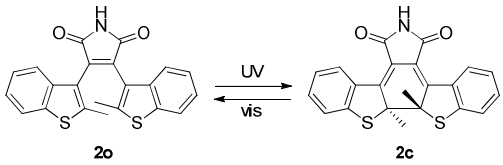
The non-symmetrical diarylmaleimide bearing an indole and benzothiophene ring as the aryl moieties (**6**) was accessed by the palladium-mediated Suzuki–Miyaura cross-coupling of benzothiophene boronic acid and *N*-methylmaleimide **16** (prepared from the Boc-protection of maleimide **15**, Scheme 3). Boc-deprotection, followed by *N*-alkylation of the indole heterocycle afforded non-symmetrical derivatives **7** (92%) and **8** (47%), respectively.



Scheme 3. Synthesis of compounds **6–8**. *Reagents and conditions*: (i) Boc_2O, DMAP, THF, room temperature, 1 h (80%); (ii) $Pd(PPh_3)_4$, CsF, 1,4-dioxane, 100 °C, 5 h (71%); (iii) TFA, CH_2Cl_2, room temperature, 2 h (92%); (iv) 1-iodopropane, NaH, DMF, 0 °C to room temperature, 12 h (47%).

2.2. General Photochromic Behavior

Scheme 4 shows the two forms of **2** and the corresponding isomerization processes. The open form **2o** is isomerized to the closed form **2c** in a photocyclization reaction induced by UV light (254 nm used here, 700 $\mu W/cm^2$), and the reverse reaction is triggered by visible light. The absorption spectrum of **2o** in THF is shown in Figure 3a, together with the spectrum recorded at the PSS after UV-exposure. The isomeric distributions at the PSS for **1–8** are shown in Table 1. The thermally stable isomer is the open form, and the rate of thermal isomerization to the open form varies substantially with the substitution pattern (*vide infra*).



Scheme 4. Isomerization scheme for compound **2**.

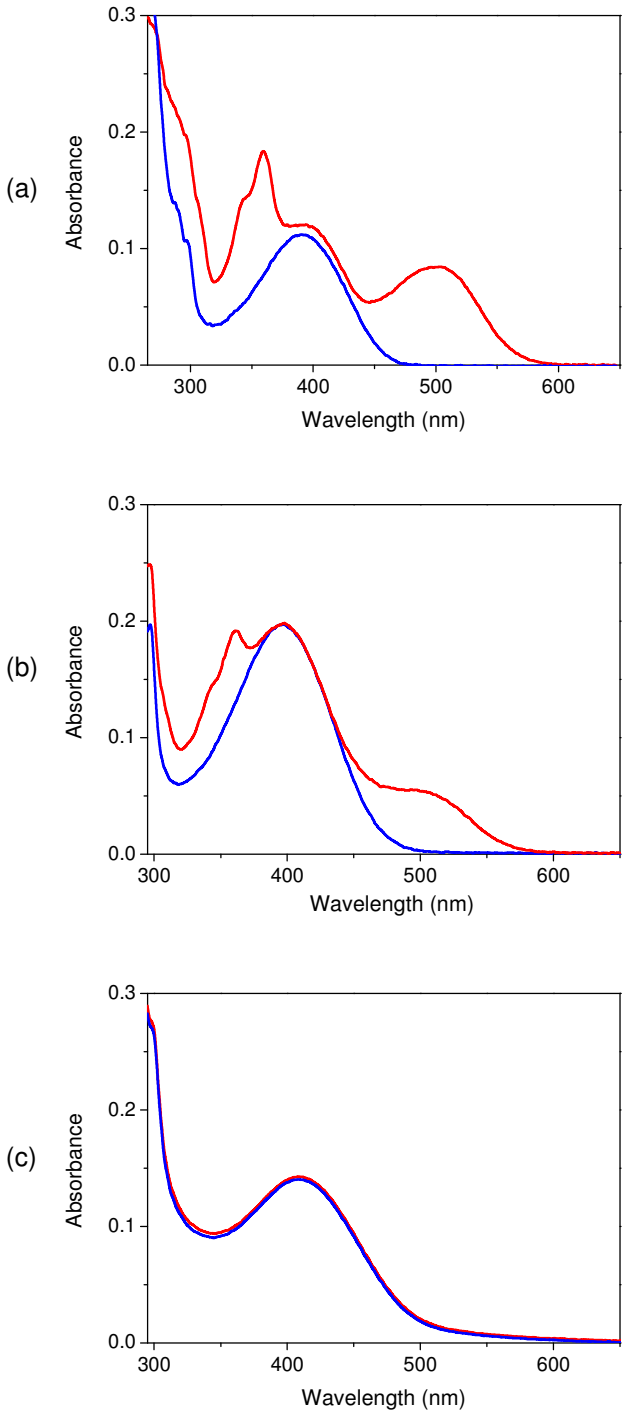


Figure 3. Absorption spectra of **2** in various solvents. (a) THF, (b) EtOH, (c) 10% DMSO/H_2O. Blue lines represent the spectra of **2o**, whereas the red lines represent the spectra recorded at the PSS after 254 nm UV-exposure.

As seen in Figure 3a, **2o** has a broad absorption band with λ_{max} at 390 nm in THF, and upon UV irradiation several new features appear in the spectrum. These include a band in the

visible region centered at around 500 nm and a sharp band at 360 nm, typical for these types of photoswitches. The isosbestic point observed at 272 nm indicates a clean conversion between the two forms. The PSS generated after 254 nm UV exposure yields 64:36 **2o**:**2c**. The reversed reaction is induced by visible light exposure, and converts the sample to 100% **2o**.

The same set of experiments was performed also in EtOH. As expected, in the more polar solvent the effect of the formation of the TICT state discussed above is much more pronounced. This results in a decreased efficiency of the photocyclization reaction open → closed and, hence, a lower concentration of the closed isomer at the PSS (Figure 3b). The formation of **7c** and **8c** was not observed (see Figures S9–S14 in the Supporting Information for spectra in EtOH). In aqueous solution (10% DMSO) the cyclization reaction was totally suppressed or barely significant (Figure 3c). In addition, the compounds did not dissolve readily in this solvent mixture, as judged by a scattering component in the absorption spectra, clearly suggesting the formation of aggregates (see Figures S15–S22 in the Supporting Information for absorption spectra). Dilution studies in aqueous solution show that for the symmetrical derivatives (**1**–**3**) and compound **7**, scattering significantly improved at concentrations below 1×10^{-5} M (see Figures S36–S38 and Figure S42 in the Supporting Information for dilution studies). The non-symmetrical derivatives displayed less scattering and no changes were observed upon dilution.

Table 1. Isomerization characteristics of compounds **1**–**8**.

Compound	λ_{max} open [nm][a]	λ_{max} closed [nm][a]	Conformation Ratio[c]		PSD in THF [% closed isomer]	Φ_{iso}(THF) [%][e]	E_{iso} (rel, aq)[f]
			parallel [%]	anti-parallel [%]			
1	403	371[b], 534	55	45	47	20	0.32
2	390	357[b], 503	55	45	36	22	0.54
3	397	358[b], 397, 503	60	40	31	28	0.43
4	403	374[b], 550	45	55	48	18	0.33
5	390	362[b], 395, 512	45	55	46	24	0.44
6	402	350[b], 402, 500	51	49	32[d]	33	0.67
7	447	356[b], 415, 555	61	39	22[d]	89	0.70
8	454	359[b], 565	60	40	18[d]	62	1.00

[a]In THF; [b]Shoulder; [c]The ratio of the two conformations of the open form in THF-d_8. For compounds **6**–**8**, the parallel/anti-parallel ratio was determined at −60 °C; [d]The relative intensities of the NCH_3 moiety was used to calculate the photostationary distribution (PSD); [e]Isomerization quantum yields for the photoinduced ring-opening reaction in THF; [f]Relative efficiencies of the photoinduced ring-opening reaction in aqueous solution. Please note that these numbers are not absolute, but instead normalized to the efficiency observed for compound **8**. See text for details. The ring-opening reactions were performed by exposure to λ = 503 nm or 545 nm.

2.3. Photocyclization in THF

The photostationary distributions (PSD) reported in Table 1 were determined by NMR analysis in THF-d_8. The ^{1}H NMR spectrum of **2o** displayed two non-equivalent CH_3 signals at δ_H 2.28 and 2.13 ppm, corresponding to the parallel and anti-parallel orientations of the heterocycles, respectively. The relative intensity of the signals corresponding to the methyl substituents at the 2-postion of the benzo[*b*]thiophenes shows that for **2o**, the ratio of anti-parallel and parallel conformations is 45:55 (at 25 °C). These observations are consistent with that previously reported for photochromic diarylmaleimide derivatives.[11]

Upon irradiation of **2o** with 254 nm UV light, the appearance of a new resonance (δ_H 1.96 ppm) assigned to the methyl moiety of the closed product was observed (see Figure 4). Additionally, the proton at the 4-position of the benzothiophene ring exhibited a significant shift downfield, from δ_H 7.49 ppm to δ_H 9.39 ppm; Δ 1.90 ppm. As highlighted in Figure 4,

this shift can be attributed to intramolecular hydrogen bonding between the proton at the 4-position of the heterocycle and the carbonyl group of the imide. As the photocyclization reaction can only originate from the anti-parallel conformer, the quantum yield for this process is dependent on the ratio anti-parallel/parallel. This ratio of the symmetrical compounds (**1**–**3**) is approximately 45:55, and the PSD after 254 nm UV-exposure is ranging from 31 to 47%. Interestingly, the introduction of the alkyl chain at the 2-position of one of the benzo[*b*]thiophenes (compounds **4** and **5**) results in an increase in the relative population of the anti-parallel conformation (from 45% for compound **2** to 55% for compound **5**). An increase in the PSD, from 36% for compound **2** to 46% for compound **5**, was also observed.

In the case of the non-symmetrical compounds containing an indole moiety (compounds **6**–**8**), at 25 °C only one set of singlets corresponding to the CH_3 moieties at the 2-position of the heterocycles was observed. This suggests a rapid interconversion between the two conformers. As such, the conformation of compounds **6**–**8** was determined using variable temperature NMR spectroscopy. At −60 °C, the rate of conversion slowed down for compounds **7** and **8** and the signals split into four resolved sets of singlets. From the relative intensity of these singlets, the population of the anti-parallel conformer was calculated to be 39% and 40% from compounds **7** and **8**, respectively (see Supporting Information, Figures S34–S35). In the case of compound **6**, the signals attributed to the methyl moieties at the 2-position were not well resolved. However, the singlet assigned to the *N*-Boc moiety (δ_H 1.62 ppm) split into two sufficiently resolved singlets, and was therefore used to determine the parallel/anti-parallel ratio (51:49, respectively, see Supporting Information, Figure S33).

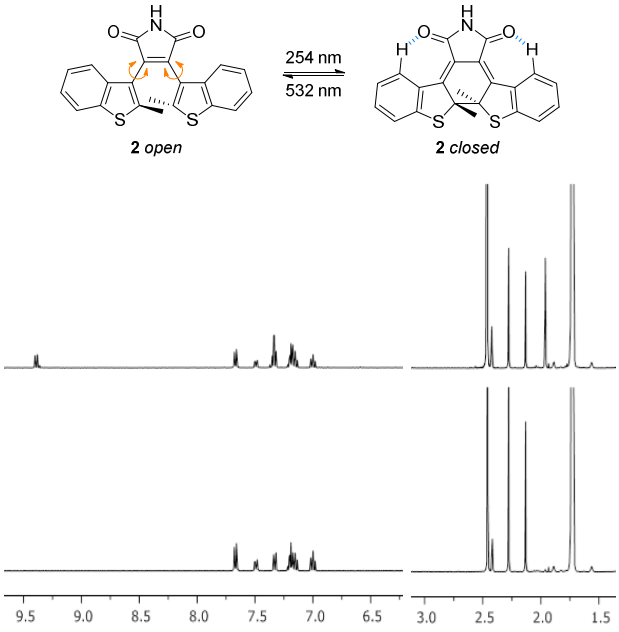


Figure 4. ^{1}H NMR spectra of **2** in THF-d_8, (*bottom*) open form; (*top*) after UV irradiation at 254 nm. For full spectra, see Supporting Information. Signals at δ_H 2.46 ppm and δ_H 1.73 ppm correspond to the water and residual solvent peak, respectively.

2.4. Thermal stability and photochromic properties of the closed isomers in aqueous solution

Following the approach mentioned above, we performed the UV-induced photocyclization reactions in THF until reaching the PSD. The samples were then purged with nitrogen to remove the THF, and re-dissolved in aqueous solution (10% DMSO as co-solvent). These samples were left in the dark for 48 hours, and UV/vis absorption spectra were recorded at regular intervals to monitor the thermal reactions (thermal isomerization to the open isomer and/or decomposition). No significant absorption changes were observed for compounds **2**–**8**, clearly showing that these derivatives are not isomerizing or decomposing during the time of the experiment. On the contrary, the spectral changes of maleic anhydride **1** clearly show the disappearance of the absorbance band corresponding to the closed isomer with a rate constant corresponding to a lifetime of 18 hours. Due to the fact that the overall spectral changes for **1** in this experiment also contain a contribution from hydrolysis of the

open isomeric form (complete hydrolysis of **1o** was observed within 90 mins as established from a separate experiment, data not shown), we cannot conclude whether such changes are due to isomerization, hydrolysis or a combination of the two. From these observations it is clear that compounds **2**–**8** display the most favorable thermal characteristics of the closed isomers.

2.5. Photoinduced opening reaction

The photoinduced ring-opening reactions, closed → open, induced by visible light (λ_{irr} = 503 nm or 545 nm) were studied in both THF and aqueous solution. The results are collected in Table 1. As mentioned above, the solubility of the compounds in DMSO/water was not sufficient to record absorption spectra free from scattering (see Figures S15–S22 in the Supporting Information). Thus, the molar absorption coefficients could not be properly determined in aqueous solution. This parameter is crucial in determining the isomerization quantum yields, as is the clean conversion between two non-aggregated isomeric forms. This is why we chose to only report the isomerization yields in THF. In aqueous solution, we instead report values that have been normalized to the compound displaying the most efficient isomerization (compound **8**). It should be noted, however, that the isomerization reactions in aqueous solution are of comparable efficiency to those in THF (isomerization quantum yields ranging between 18–89%), judging solely from the isomerization kinetics. Thus, the efficiencies of the photoinduced ring-opening reactions for **1**–**8** are all sufficiently high to promote isomerization to the open isomeric form without applying excessive exposure to visible light. Furthermore, the isomerization quantum yields obtained in THF are comparable or higher than those previously reported in the literature for structurally related photochromic diarylmaleimide derivatives.[8, 12]

3. Conclusion

As expected, none of the derivatives could be photocyclized by UV-exposure to the closed isomer in aqueous solution. In THF, the corresponding photostationary distribution contains between 18–48% of the closed isomer, the lower numbers belonging to *N*-methylated maleimide derivatives unsymmetrically substituted with indole- and benzothiophene units attached to the central pentacycle. As for the thermal stability of the closed isomeric forms in aqueous solution, derivatives **2**–**8** display very good characteristics as no dark reactions were observed over 48 hours. For the corresponding photoinduced isomerizations, we conclude that all derivatives isomerize readily to the open isomer by exposure to visible light in both aqueous and organic media. Although the poor solubility in aqueous solution (scattering observed when concentrations higher than 10 μM was used) refrained us from reporting absolute numbers on the isomerization quantum yields, we observe that the isomerization kinetics is comparable to what is observed in THF, in which the quantum yields vary between 18–89%. Given the amiable photochromic properties of the compounds studied, it can be concluded that diarylethene derivatives decorated with substituents optimized for biological targets are highly promising candidates for photopharmacological applications.

4. Experimental

4.1. General Experimental

All general reagents and solvents were purchased from commercial sources and used as supplied, unless stated otherwise. When anhydrous solvents were required, THF was distilled over Na/benzophenone, 1,4-dioxane over Na and CH_2Cl_2 was distilled over CaH_2. Argon was used as inert atmosphere. Flash column chromatography was performed using Merck silica gel 60. Melting points were determined using a Mettler FP82 hot-stage microscope (uncorrected). All NMR spectra (^1H, ^{13}C and 2D experiments) were collected on a Varian 400 MHz spectrometer at 25 °C. Samples were dissolved in either $CDCl_3$, DMSO-d_6 or THF-d_8.

The residual solvent peaks specific to that of the deuterated solvents were used as an internal reference; $CDCl_3$: 7.26 ppm (^{1}H NMR) and 77.20 ppm (^{13}C NMR), DMSO-d_6: 2.50 ppm (^{1}H NMR) and 39.50 ppm (^{13}C NMR), THF-d_8: 3.58 ppm (^{1}H NMR) and 25.31 ppm (^{13}C NMR). For compounds **1**–**6**, both anti-parallel and parallel conformations were observed in the NMR spectra ($CDCl_3$) at 25 °C and the methyl groups at the 2-position of the heterocycles have been assigned accordingly. Variable temperature NMR experiments were recorded on a Varian 500 MHz spectrometer at temperatures ranging from 25 to −60 °C, using THF-d_8 as the solvent. Chromatographic analysis was performed on an API SCIEX 150 EX Perkin Elmer ESI-MS (30 eV) connected to a Perkin Elmer gradient pump system using a C8 column (4.6 mm × 50 mm, 3.5 μM). High Resolution Mass Spectra (HRMS) analysis were conducted and recorded on an Agilent 1290 Infinity LC and an Agilent 6540 Ultra High Definition (UHD) Q-TOF LC/MS system. GC/MS analysis was performed on an Agilent 7890B GC and a JEOL AccuTOF GCX. High-performance liquid chromatography (HPLC) conditions were as follows; injection volume = 5 μL; solvent A = H_2O containing 0.1% formic acid; solvent B = MeCN containing 0.1% formic acid, compound was eluted with a gradient of 5–95% solvent B over 8–12 min; flow = 1 mL/min. UV-visible absorption spectra were collected using a Varian CaryBio 50 spectrophotometer. Samples were placed in a 1 cm quartz cuvette. Photoinduced ring-closing reactions were conducted using an UVP hand-held lamp, model UVGL-25 (λ = 254 nm, 700 μW/cm^2). Ring-opening reactions were performed using a light-emitting diode (λ = 523nm). The photostationary distribution was determined by ^{1}H NMR using THF-d_8 as the solvent. To calculate the quantum yields of the ring opening isomerization process, a 500 W Xe lamp equipped with an interference filter with a λ_{max} of transmission at either 545 nm or 503 nm was used.

*4.2. Synthesis and Characterization of Compounds **1–8**.*

*4.2.1. N-Methyl-3,4-bis(2-methylbenzo[b]thiophen-3-yl)-1H-pyrrole-2,5-dione (**3**)*

A suspension of 3,4-dibromo-*N*-methylmaleimide (870 mg, 3.00 mmol), trimeric cyclic anhydride of (2-methylbenzo[*b*]thiophen-3-yl)boronic acid[5c] (1.15 g, 2.20 mmol), $Pd(PPh_3)_4$ (139 mg, 0.12 mmol) and CsF (2.28 g, 15.00 mmol) in anhydrous 1,4-dioxane (100 mL) was heated at 100 °C under an inert atmosphere with vigorous stirring for 4 h. The mixture was then poured into H_2O and extracted with $CHCl_3$. The combined organic phase was washed with H_2O, dried (Na_2SO_4), filtered and excess solvent removed *in vacuo*. The crude material was first purified by flash column chromatography (1:4, 1:2 EtOAc/hexane), then triturated with Et_2O to afford compound **3** (740 mg, 1.83 mmol, 61%) as light yellow solid; mp 256–257 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.69–7.64 (2H, m, Ar-H), 7.49–7.46 (1H, m, Ar-H), 7.32–7.26 (2H, m, Ar-H), 7.23–7.17 (2H, m, Ar-H), 7.06 (1H, ddd, *J* = 8.2, 7.2, 1.1 Hz, Ar-H), 3.26 (3H, s, NCH_3), 2.27 (3.3H, s, $CH_{3(parallel)}$), 2.02 (3.0H, s, $CH_{3(anti\text{-}parallel)}$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.17, 170.15, 143.5, 143.0, 138.4, 138.33, 138.32, 137.9, 136.5, 136.0, 124.7, 124.6, 124.4, 124.3, 122.5, 122.4, 122.2, 122.1, 122.03, 122.01, 24.8, 24.7, 15.64, 15.58; HRMS (ESI, *m/z*): Calculated for $C_{23}H_{17}NO_2S_2$ $[M + H]^+$ 404.0773; Found 404.0773.

*4.2.2. 3,4-Bis(2-methylbenzo[b]thiophen-3-yl)furan-2,5-dione (**1**)*

A suspension of *N*-methyl-3,4-bis(2-methylbenzo[*b*]thiophen-3-yl)-1*H*-pyrrole-2,5-dione **3** (403 mg, 1.00 mmol) in EtOH (20 mL) and 40% KOH (10 mL) was heated at 78 °C for 72 h. The reaction mixture was cooled to room temperature and the solvent was removed *in vacuo*. The crude residue was suspended in CH_2Cl_2, to which H_2O was added. The pH of the resulting suspension was adjusted to pH 1 using 6 M HCl. The organic layer was washed with H_2O, dried (Na_2SO_4), filtered and excess solvent removed *in vacuo*. Purification by flash column chromatography (2:10 EtOAc/hexane) afforded compound **1** (329 mg, 0.84 mmol, 84%) as yellow solid; mp 239–240 °C (lit.[11e] mp 238–240 °C); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.70 (2H, d, *J* = 9.6 Hz, Ar-H), 7.44–7.42 (1H, m, Ar-H), 7.33–7.28 (2H, m, Ar-H), 7.25–

7.22 (2H, m, Ar-H), 7.11 (1H, app. t, $J_{app.}$ = 7.6 Hz, Ar-H), 2.28 (3.6H, s, $CH_{3(parallel)}$), 2.11 (2.9H, s, $CH_{3(anti\text{-}parallel)}$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 164.14, 164.10, 145.6, 145.0, 138.3, 138.2, 137.8, 137.6, 137.3, 137.0, 125.1, 125.0, 124.9, 124.8, 122.2, 122.1, 121.0, 120.9, 15.7. The ^1H NMR data is in agreement with literature.[11e]

*4.2.3. 3,4-Bis(2-methylbenzo[b]thiophen-3-yl)-1H-pyrrole-2,5-dione (**2**)*

Compound **2** was synthesized according to the method previously described in the literature.[9b] Yield 74%, orange powder; mp 128–130 °C; ^1H NMR (400 MHz, $CDCl_3$): δ 7.70–7.65 (2H, m, Ar-H), 7.56 (1H, br s, NH), 7.49–7.47 (1H, m, Ar-H), 7.33–7.27 (2H, m, Ar-H), 7.25–7.18 (2H, m, Ar-H), 7.07 (1H, ddd, *J* = 8.2, 7.2, 1.1 Hz, Ar-H), 2.28 (3.1H, s, $CH_{3(parallel)}$), 2.04 (2.9H, s, $CH_{3(anti\text{-}parallel)}$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.95, 169.92, 143.8, 143.3, 138.28, 138.25, 137.8, 137.2, 136.8, 124.7, 124.6, 124.5, 124.4, 122.4, 122.3, 122.0, 121.9, 121.8, 15.6; LRMS (ESI, *m/z*): Calculated for $C_{22}H_{15}NO_2S_2$ $[M + H]^+$ 390.1; found 390.6. The ^1H NMR data is in agreement with the literature.[9b]

*4.2.4. Methyl 2-(2-methylbenzo[b]thiophen-3-yl)-2-oxoacetate (**9**)*

Compound **9** was synthesized according to the method previously described in the literature.[9b] Yield 97%, light brown solid; ^1H NMR (400 MHz, $CDCl_3$): δ 8.28–8.23 (1H, m, Ar-H), 7.77–7.73 (1H, m, Ar-H), 7.47–7.41 (1H, m, Ar-H), 7.40–7.34 (1H, m, Ar-H), 3.99 (3H, s, OCH_3), 2.74 (3H, s, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 182.1, 165.3, 156.0, 138.2, 137.0, 126.9, 126.1, 125.3, 123.7, 121.8, 53.1, 16.1. The ^1H NMR data is in agreement with the literature.[9b]

*4.2.5. Methyl 2-(2-methylbenzo[b]thiophen-3-yl)acetate (**10**)*

A solution of methyl 2-(2-methylbenzo[*b*]thiophen-3-yl)-2-oxoacetate **9** (2.81 g, 12.00 mmol) in TFA (48 mL) was cooled to 0 °C, to which triethylsilane (3.77 g, 32.40 mmol) was added dropwise. The reaction mixture was allowed to slowly warm to room temperature and

stirred for 16 h. The reaction was quenched with H_2O (48 mL) and extracted with CH_2Cl_2 (120 mL × 2). The combined organic layer was dried (Na_2SO_4), filtered and excess solvent removed *in vacuo*. Purification by flash column chromatography (1:4 EtOAc/hexane) afforded compound **10** (1.68 g, 7.68 mmol, 64%) as colorless oil; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.78 (1H, ddd, *J* = 8.0, 1.2, 0.8 Hz, Ar-H), 7.70 (1H, dt, *J* = 8.0, 1.0 Hz, Ar-H), 7.39 (1H, ddd, *J* = 8.2, 7.2, 1.2 Hz, Ar-H), 7.31 (1H, ddd, *J* = 8.2, 7.2, 1.2 Hz, Ar-H), 3.81 (2H, s, CH_2), 3.69 (3H, s, OCH_3), 2.58 (3H, s, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.0, 140.0, 138.1, 137.6, 124.2, 123.83, 123.78, 122.1, 121.2, 52.1, 32.1, 14.0. The ^{1}H NMR data is in agreement with published data.[9b]

*4.2.6. 2-(2-Methylbenzo[b]thiophen-3-yl)acetic acid (**11**)*

Compound **11** was synthesized according to the method previously described in the literature.[10] Yield 96%, white powder; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.38 (1H, br s, CO), 7.86 (1H, ddd, *J* = 8.0, 1.2, 0.8 Hz, Ar-H), 7.66 (1H, ddd, *J* = 8.0, 1.2, 0.8 Hz, Ar-H), 7.35 (1H, ddd, *J* = 8.2, 7.2, 1.2 Hz, Ar-H), 7.29 (1H, ddd, *J* = 8.2, 7.2, 1.2 Hz, Ar-H), 3.77 (2H, s, CH_2), 2.48 (3H, s, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6): δ 171.8, 140.0, 137.2, 136.6, 125.2, 124.1, 123.7, 122.0, 121.4, 31.6, 13.6. The ^{1}H NMR data is in agreement with published data.[9b]

*4.2.7 2-Pentylbenzo[b]thiophene (**12**)*

To a solution of benzo[*b*]thiophene (5.36 g, 40.00 mmol) in anhydrous THF (50 mL) was added *n*-BuLi (2.5 M in pentane, 21 mL, 52.00 mmol) at −78 °C and allowed to stir for 30 min. Next, 1-bromo-pentane (18.1 g, 120.00 mmol) was added dropwise and the resulting mixture was left to stir at room temperature for 12 h. The reaction was quenched with H_2O and extracted with Et_2O. The combined organic layer was dried (Na_2SO_4), filtered and excess solvent removed *in vacuo* to afford compound **12** (8.08 g, 39.20 mmol, 98%) as a colorless oil; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.80–7.76 (1H, m, Ar-H), 7.70–7.66 (1H, m, Ar-H), 7.34–

7.29 (1H, m, Ar-H), 7.28–7.24 (1H, m, Ar-H), 7.01 (1H, quint, J = 0.8 Hz, Ar-H), 2.96–2.87 (2H, m, CH_2), 1.83–1.73 (2H, m, CH_2), 1.44–1.34 (4H, m, $CH_2 \times 2$), 0.96–0.91 (3H, m, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 147.0, 140.4, 139.5, 124.1, 123.5, 122.8, 122.2, 120.5, 31.5, 31.0, 30.9, 22.6, 14.2; HRMS (EI, *m/z*): Calculated for $C_{13}H_{16}S$ $[M + \bullet]^+$ 204.0967; Found 204.0969.

*4.2.8. Ethyl 2-oxo-2-(2-pentylbenzo[b]thiophen-3-yl)acetate (**13**)*

To a cooled (0 °C) solution of anhydrous $AlCl_3$ (3.99 g, 30.00 mmol) and anhydrous CH_2Cl_2 (40 mL), was added ethyl chlorooxoacetate (1.38 mL, 15.00 mmol) and a solution of 2-pentyl-benzo[*b*]thiophene **12** (1.53 g, 7.50 mmol) in anhydrous CH_2Cl_2 (10 mL). After stirring at room temperature for 2 h, the reaction was quenched with ice H_2O (40 mL) and CH_2Cl_2 (80 mL) was added. The organic layer was separated, washed with saturated $NaHCO_3$, dried (Na_2SO_4), filtered and excess solvent removed *in vacuo*. Purification by flash column chromatography (1:4 EtOAc/hexane) afforded compound **13** (1.74 g, 6.00 mmol, 80%) as light yellow oil; ^1H NMR (400 MHz, $CDCl_3$): δ 8.16–8.14 (1H, m, Ar-H), 7.78–7.76 (1H, m, Ar-H), 7.45–7.40 (1H, m, Ar-H), 7.38–7.34 (1H, m, Ar-H), 4.44 (2H, q, J = 7.2 Hz, CH_2), 3.09 (2H, t, J = 7.8 Hz, CH_2), 1.79 (2H, quint, J = 7.8 Hz, CH_2), 1.46–1.30 (7H, m, CH_3, $CH_2 \times 2$), 0.92 (3H, t, J = 7.2 Hz, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 182.6, 164.8, 161.8, 137.9, 137.1, 126.6, 125.7, 125.0, 123.4, 121.9, 62.5, 31.6, 31.5, 30.3, 22.4, 14.1, 14.0; HRMS (ESI, *m/z*): Calculated for $C_{17}H_{20}O_3S$ $[M + H]^+$ 305.1206; Found 305.1208.

*4.2.9. Potassium 2-oxo-2-(2-pentylbenzo[b]thiophen-3-yl)acetate (**14**)*

A solution of ethyl 2-oxo-2-(2-pentylbenzo[*b*]thiophen-3-yl)acetate **13** (2.13 g, 7.00 mmol) in EtOH (21 mL) was treated with KOH (268 mg, 7.00 mmol) and left to stir at room temperature for 4 h. Excess solvent was then removed *in vacuo* and the resulting crude residue was suspended in Et_2O (100 mL). After stirring at room temperature for 12 h, the reaction mixture was filtered *via* vacuum filtration. The isolated material was further washed

with Et_2O and dried to afford compound **14** (1.40 g, 4.40 mmol, 63%) as a white solid; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.31 (1H, d, J = 8.0 Hz, Ar-H), 7.89 (1H, d, J = 7.6 Hz, Ar-H), 7.42–7.22 (2H, m, Ar-H), 3.18 (2H, t, J = 7.8 Hz, CH_2), 1.69 (2H, m, CH_2), 1.33 (4H, m, CH_2 × 2), 0.87 (3H, m, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6): δ 193.7, 170.1, 155.1, 138.5, 136.6, 129.0, 124.7, 124.1, 123.9, 121.8, 30.97, 30.96, 28.9, 21.9, 13.9; HRMS (ESI, *m/z*): Calculated for $C_{15}H_{16}O_3S$ $[M + H]^+$ 277.0893; Found 277.0893.

*4.2.10. 3-(2-Methylbenzo[b]thiophen-3-yl)-4-(2-pentylbenzo[b]thiophen-3-yl)furan-2,5-dione (**4**)*

A suspension of 2-(2-methylbenzo[*b*]thiophen-3-yl)acetic acid **11** (618 mg, 3.00 mmol) and potassium 2-oxo-2-(2-pentylbenzo[*b*]thiophen-3-yl)acetate **14** (943 mg, 3.00 mmol) in Ac_2O (18 mL) was stirred at 110 °C for 4.5 h. After cooling to room temperature, the reaction was quenched with H_2O (80 mL). The aqueous phase was extracted with Et_2O, dried (Na_2SO_4), filtered and excess solvent removed *in vacuo*. Purification by flash column chromatography (1:10 EtOAc/hexane) afforded compound **4** (617 mg, 1.38 mmol, 46%) as yellow solid; mp 133–134 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.75–7.66 (4H, m, Ar-H), 7.56–7.49 (2H, m, Ar-H), 7.39–7.27 (6H, m, Ar-H), 7.25–7.19 (1H, m, Ar-H), 7.19–7.14 (2H, m, Ar-H), 7.07–7.01 (1H, m, Ar-H), 2.74–2.64 (1H, m, CH_2), 2.54–2.45 (1H, m, CH_2), 2.43–2.38 (1H, m, CH_2), 2.36 (2.9H, s, $CH_{3(parallel)}$), 2.26–2.17 (1H, m, CH_2), 2.00 (3.3H, s, $CH_{3(anti-parallel)}$), 1.66–1.55 (1H, m, CH_2), 1.50–1.38 (1H, m, CH_2), 1.36–1.19 (5H, m, CH_2 × 3), 1.05–0.82 (7H, m, CH_3, CH_2 × 2), 0.70 (3H, t, J = 6.9 Hz, CH_3), 0.56–0.45 (1H, m, CH_2); ^{13}C NMR (100 MHz, $CDCl_3$): δ 164.3, 164.13, 164.10, 152.0, 151.2, 145.5, 144.8, 138.5, 138.31, 138.29, 138.23, 138.21, 138.15, 137.9, 137.6, 137.43, 137.36, 137.02, 136.98, 125.14, 125.12, 125.0, 124.94, 124.92, 124.8, 124.74, 124.73, 122.33, 122.25, 122.23, 122.19, 122.17, 122.15, 122.0, 121.0, 120.7, 120.0, 119.7, 31.9, 31.4, 31.0, 30.7, 30.43, 30.36, 22.5, 22.4, 15.8, 15.6,

14.1, 14.0; HRMS (ESI, *m/z*): Calculated for $C_{26}H_{22}O_3S_2$ [M + H]$^+$ 447.1083; Found 447.1083.

*4.2.11. 3-(2-Methylbenzo[b]thiophen-3-yl)-4-(2-pentylbenzo[b]thiophen-3-yl)-1H-pyrrole-2,5-dione (**5**)*

To a solution of 3,4-bis(2-methylbenzo[*b*]thiophen-3-yl)-4-(2-pentylbenzo[*b*]thiophen-3-yl)furan-2,5-dione **4** (320 mg, 0.81 mmol) in anhydrous DMF (4 mL), was added MeOH (0.12 mL, 3.00 mmol) and hexamethyldisilazane (1.26 mL, 6.00 mmol). After stirring at room temperature for 18 h, 0.1 M HCl (50 mL) was added and the reaction mixture was extracted with EtOAc. The combined organic layer was dried (Na_2SO_4), filtered and excess solvent removed *in vacuo*. Purification by flash column chromatography (1:5 EtOAc/hexane) afforded compound **5** (219 mg, 0.49 mmol, 82%) as an orange solid; mp 88–90 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.84–7.83 (2H, m, NH), 7.74–7.64 (4H, m, Ar-H), 7.58–7.53 (2H, m, Ar-H), 7.37–7.27 (6H, m, Ar-H), 7.25–7.19 (1H, m, Ar-H), 7.18–7.12 (2H, m, Ar-H), 7.02–6.97 (1H, m, Ar-H), 2.74–2.65 (1H, m, CH_2), 2.50–2.38 (2H, m, CH_2), 2.37 (2.6H, s, $CH_{3(parallel)}$), 2.22–2.13 (1H, m, CH_2), 1.94 (3.4H, s, $CH_{3(anti\text{-}parallel)}$), 1.64–1.54 (1H, m, CH_2), 1.43–1.20 (6H, m, $CH_2 \times 3$), 1.00–0.74 (7H, m, CH_3, $CH_2 \times 2$), 0.60 (3H, t, J = 7.1 Hz, CH_3), 0.49–0.39 (1H, m, CH_2); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.23, 170.16, 170.14, 170.07, 150.1, 149.6, 143.6, 143.2, 138.6, 138.3, 138.24, 138.18, 138.16, 138.14, 137.78, 137.75, 137.68, 137.3, 137.0, 136.7, 124.74, 124.71, 124.6, 124.5, 124.4, 124.3, 122.6, 122.5, 122.4, 122.2, 122.09, 122.08, 122.01, 121.9, 121.8, 121.7, 120.9, 120.7, 31.9, 31.4, 31.0, 30.7, 30.3, 30.2, 22.5, 22.4, 15.8, 15.3, 14.1, 13.9; HRMS (ESI, *m/z*): Calculated for $C_{26}H_{23}NO_2S_2$ [M + H]$^+$ 446.1243; Found 446.1243.

*4.2.12. 1-Methyl-3-bromo-4-(2-methyl-1H-3-indolyl)-2,5-dihydro-1H-2,5-pyrrole-dione (**15**)*

Compound **15** was synthesized according to the method previously published in the literature.[1d] Yield 81%, red powder; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.45 (1H, br s, NH),

8.50–8.44 (1H, m, Ar-H), 8.32–7.27 (1H, m, Ar-H), 7.22–7.13 (2H, m, Ar-H), 3.18 (3H, s, NCH_3), 2.46 (3H, s, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.3, 166.6, 139.5, 137.7, 135.7, 126.6, 122.6, 121.0, 120.7, 111.0, 110.2, 102.2, 25.1, 14.5. The NMR data is in agreement with published data.[1d]

*4.2.13. tert-Butyl 3-(4-bromo-1-methyl-2,5-dioxo-2,5-dihydro-1H-pyrrol-3-yl)-2-methyl-1H-indole-1-carboxylate (**16**)*

To a solution of 1-methyl-3-bromo-4-(2-methyl-1*H*-3-indolyl)-2,5-dihydro-1*H*-2,5-pyrrole-dione **15** (1.15 g, 3.60 mmol) and 4-dimethylaminopyridine (30 mg, 0.246 mmol) in anhydrous THF (50 mL), was added a solution of di-*tert*-butyl dicarbonate (1.02 g, 4.68 mmol) in anhydrous THF (10 mL) dropwise over a period of 10 min. After stirring at room temperature for 1 h, excess solvent was removed *in vacuo*. Purification by flash column chromatography (1:10 EtOAc/hexane) afforded compound **16** (1.20 g, 2.86 mmol, 80%) as yellow powder; ^1H NMR (400 MHz, $CDCl_3$): δ 8.17–8.12 (1H, m, Ar-H), 7.34–7.28 (2H, m, Ar-H), 7.26–7.22 (1H, m, Ar-H), 3.18 (3H, s, NCH_3), 2.58 (3H, s, CH_3), 1.71 (9H, s, *t*-Bu); ^{13}C NMR (100 MHz, $CDCl_3$): δ 168.6, 165.8, 150.2, 138.8, 138.5, 136.1, 126.9, 126.1, 124.4, 123.2, 119.9, 115.8, 108.1, 85.0, 28.4, 25.1, 16.9; HRMS (ESI, *m/z*): Calculated for $C_{19}H_{19}{}^{81}BrN_2O_4$ $[M + H]^+$ 421.0586; Found 421.0583.

*4.2.14. tert-Butyl 2-methyl-3-(1-methyl-4-(2-methylbenzo[b]thiophen-3-yl)-2,5-dioxo-2,5-dihydro-1H-pyrrol-3-yl)-1H-indole-1-carboxylate (**6**)*

A suspension of *tert*-butyl 3-(4-bromo-1-methyl-2,5-dioxo-2,5-dihydro-1*H*-pyrrol-3-yl)-2-methyl-1*H*-indole-1-carboxylate **16** (890 mg, 2.13 mmol), trimeric cyclic anhydride of (2-methylbenzo[*b*]thiophen-3-yl)boronic acid[5c] (0.445 mg, 0.85 mmol), $Pd(PPh_3)_4$ (99 mg, 0.085 mmol) and CsF (1.62 g, 10.6 mmol) in anhydrous 1,4-dioxane (80 mL) was heated at 100 °C for 5 h with vigorous stirring under an inert atmosphere. The mixture was then poured into H_2O and extracted with $CHCl_3$. The combined organic phase was washed with H_2O,

dried (Na_2SO_4), filtered and excess solvent removed *in vacuo*. Purification by flash column chromatography (1:5 EtOAc/hexane) afforded compound **6** (740 mg, 1.51 mmol, 71%) as yellow solid; mp 181–182 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.04 (1H, dd, *J* = 8.4, 1.2 Hz, Ar-H), 7.68 (1H, d, *J* = 8.8 Hz, Ar-H), 7.49–7.33 (1H, m, Ar-H), 7.31–7.12 (4H, m, Ar-H), 6.98–6.88 (1H, m, Ar-H), 3.25 (3H, s, NCH_3), 2.39 (1.6H, s, $CH_{3(parallel)}$), 2.25 (1.7H, s, $CH_{3(parallel)}$), 2.18 (2.6H, s, $CH_{3(anti\text{-}parallel)}$), 2.16 (2.6H, s, $CH_{3(anti\text{-}parallel)}$), 1.64 (9H, s, *t*-Bu); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.4, 170.3, 150.1, 143.1, 142.4, 139.0, 138.5, 138.3, 138.1, 136.0, 135.6, 135.1, 134.9, 127.4, 126.9, 124.5, 124.21, 124.16, 123.2, 122.6, 122.4, 122.0, 119.3, 119.1, 115.4, 109.9, 109.7, 84.7, 28.3, 24.6, 16.1, 15.8, 15.7; HRMS (ESI, *m/z*): Calculated for $C_{28}H_{26}N_2O_4S$ $[M + H]^+$ 487.1686; Found 487.1686.

*4.2.15. 1-Methyl-3-(2-methyl-1H-indol-3-yl)-4-(2-methylbenzo[b]thiophen-3-yl)-1H-pyrrole-2,5-dione (**7**)*

A solution of *tert*-butyl 2-methyl-3-(1-methyl-4-(2-methylbenzo[*b*]thiophen-3-yl)-2,5-dioxo-2,5-dihydro-1*H*-pyrrol-3-yl)-1*H*-indole-1-carboxylate **6** (528 mg, 1.07 mmol) in CH_2Cl_2 (105 mL) was treated with trifluoroacetic acid (15 mL) for 2 h. Excess solvent was removed *in vacuo* and the resulting residue was dissolved in 10% 2-propanol in $CHCl_3$ (150 mL). The organic phase was washed with saturated $NaHCO_3$ and brine, dried (Na_2SO_4), filtered and excess solvent removed *in vacuo*. Purification by flash column chromatography (1:4 EtOAc/hexane) afforded compound **7** (382 mg, 0.99 mmol, 92%) as a red powder; mp 126–128 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.24 (1H, br s, NH), 7.70–7.65 (1H, m, Ar-H), 7.39–7.31 (1H, m, Ar-H), 7.28–7.01 (5H, m, Ar-H), 6.97–6.90 (1H, m, Ar-H), 3.23 (3H, s, NCH_3), 2 17 (3H, s, CH_3), 1.87 (3H, s, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.2, 171.0, 142.1, 138.43, 138.36, 137.8, 137.2, 135.6, 130.2, 126.8, 124.4, 124.1, 123.2, 122.8, 122.3, 122.0, 120.9, 120.0, 110.6, 104.0, 24.5, 15.7, 13.5; HRMS (ESI, *m/z*): Calculated for $C_{23}H_{18}N_2O_2S$ $[M + H]^+$ 387.1162; Found 387.1162.

*4.2.16. 1-Methyl-3-(2-methyl-1-propyl-1H-indol-3-yl)-4-(2-methylbenzo[b]thiophen-3-yl)-1H-pyrrole-2,5-dione (**8**)*

A solution of 1-methyl-3-(2-methyl-1*H*-indol-3-yl)-4-(2-methylbenzo[*b*]thiophen-3-yl)-1*H*-pyrrole-2,5-dione **7** (250 mg, 0.65 mmol) in anhydrous DMF (3 mL) was added slowly *via* syringe to a suspension of 60% NaH (31 mg, 0.78 mmol) in anhydrous DMF (5 mL) cooled to 0 °C. After stirring at 0 °C for an additional hour, 1-iodopropane (145 mg, 0.85 mmol) was added dropwise. After stirring at room temperature for 12 h, the reaction mixture was quenched with saturated NH_4Cl (10 mL). The aqueous layer was extracted with *tert*-butyl methyl ether (30 mL × 3). The combined organic layer was washed with brine, dried (Na_2SO_4), filtered and excess solvent removed *in vacuo*. Purification by flash column chromatography (1:4 EtOAc/hexane) afforded compound **8** (135 mg, 0.31 mmol, 47%) as red powder; mp 177–179 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.67 (1H, d, *J* = 8.0 Hz, Ar-H), 7.37–7.27 (2H, m, Ar-H), 7.23–7.16 (2H, m, Ar-H), 7.15–7.06 (2H, m, Ar-H), 6.99 (1H, t, *J* = 7.6 Hz, Ar-H), 4.05 (2H, m, CH_2), 3.24 (3H, s, NCH_3), 2.19 (3H, br s, CH_3), 1.91 (3H, s, CH_3), 1.60 (2H, q, *J* = 7.6 Hz, CH_2), 0.74 (3H, t, *J* = 6.8 Hz, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.3, 171.0, 141.8, 138.8, 138.3, 137.5, 136.7, 129.9, 126.1, 124.3, 124.0, 123.4, 122.8, 121.9, 120.8, 120.2, 109.4, 103.8, 45.0, 24.5, 23.0, 15.7, 12.2, 11.3; HRMS (ESI, *m/z*): Calculated for $C_{26}H_{24}N_2O_2S$ $[M + H]^+$ 429.1631; Found 429.1631.

4.3. Photophysical Characterization

4.3.1. Photoisomerization

The reversible photoisomerization of compounds **1**–**8** was investigated in THF and EtOH. Solutions were irradiated at 254 nm to obtain the closed photoisomer. The reactions were reversed using a LED (523 nm). In EtOH, the ring closing process was not observed for compounds **7** and **8**.

The ring opening photoisomerization of compounds **1**–**8** was investigated in 10% DMSO/H_2O using the approach described in the manuscript. Solutions were irradiated with

visible light using either a LED (λ = 523 nm) or a 500 W Xe lamp equipped with an interference filter (either, λ = 503 nm or λ = 545 nm).

4.3.2. Photostationary Distribution

The photostationary distributions were determined by ^{1}H NMR spectroscopy. NOESY experiments were used to assign the methyl substituents to either the parallel or anti-parallel conformations.

4.3.3. Determination of Isomerization Quantum Yields

The quantum yields for the ring opening isomerization reactions in 10% DMSO/H_2O were calculated following a procedure previously described in the literature.[13] The well-studied furylfulgide 2-[1-(2,5-dimethyl-3-furyl)ethylidene]-3-isopropylidenesuccinic anhydride was used as a reference for the decolorisation reaction.[14] The reference was measured in toluene, whereas compounds **1**–**8** were firstly prepared in THF to perform the closing isomerization process. After photoisomerization, THF was removed by bubbling N_2 through the solution and then re-dissolved in 10% DMSO/H_2O. Before measuring the ring-opening isomerization, the absorbance at the irradiation wavelength of the closed form in THF was adjusted to ~0.1. The decolorisation process was carried out at room temperature with continuous stirring. The absorption of the closed form was monitored until isomerization was complete and then fitted to a first order exponential decay function. The quantum yield of each sample can be calculated by comparison of the rate constant of the sample and the reference according to Equation 1 stated in the Supporting Information.

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