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Novel Naphthalimide Hydroperoxide Photonucleases: The Role of Thiocyclic-Fused Area and the Difference in Spectra, Photochemistry and Photobiological Activity

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Abstract—Novel five- and six-membered thiocyclic-fused naphthalimide hydroperoxides (**7**, **8**) as photonucleases were designed, synthesized via unusual isomerization in Pschorr cyclization and photooxygenation. The five-membered **7** was able to induce single-strand nicks in duplex DNA pH independently (7.0–8.5) at 0.5 μM under 366 nm, while the six-membered **8** could photonick the duplex DNA pH dependently (7.0–8.5) at 5 μM under 450 nm and showed 'time-controlled' photo-bioactivity. Their thiocycles and the angular conjugated plane have contributions to their binding affinity with DNA and photocleaving efficiency.

Introduction

DNA cleavage by synthetic nucleases is of great interest in biology and bioorganic chemistry. During the past decades, many DNA cleavage reagents have been developed as potential antitumor agents or prothetic groups for antisenseoligonucleotides.[1–4] One of the most well-known DNA cleavage species is hydroxyl radical, and much effort has been devoted to the development of efficient methods for ·OH generation using organic precursors by low-energy irradiation, such as long-wavelength UV-light ($\lambda > 350$ nm) or more preferably by visible light irradiation.[5–8] Up till now, most of them showed photo-bioactivity around 350 nm, only few in the presence of metal cation under visible light,[9–11] and few examples on inorganic or organic metal compounds as well as aroyloxy-pyridinethiones with 'time-controlled' photocleaving activity[9–12] were known. However, no organic hydroperoxide releasing hydroxy radical with similar property was reported.

Among the exploited photochemical DNA cleavers, naphthalimide has been shown to be a class of chromophore capable of generating a multitude of reactive intermediates.[7,13–15] Coupled with the prospects of readily functionalizing (on the imide nitrogen) with suitable groups, the naphthalene-derived imides are ideal candidates for sequence-specific photooxidation and cleavage of oligonucleotide polymer.[15] The hydroperoxides of naphthalimides, such as **1** and **2**, could effectively generate hydroxyl radical under the irradiation of long-wavelength UV-light, and exhibited promising DNA photo-cleavage capabilities.[6,7,16] The high efficiency of these reagents may be attributed to their high DNA-intercalating capabilities due to their large conjugated planarities. Our previous studies[16–18] revealed that taking thio instead of oxo in naphthalimide (**3**, **4**) and using angular conjugated plane (**5**, **6**)[17] are useful strategies for constructing novel photocleaving agents and intercalators of DNA. Here we further explore a series of novel DNA cleaving reagents bearing a larger conjugated plane, thiocyclic fused naphthalene ring and a hydroxyl radical generating group, hydroperoxide. The former was used as the binding part, and the latter as the photonicking functionality. Upon photoirradiation the isomers 7 and **8** exhibited significantly higher cleaving abilities and interesting difference in biological activities to the supercoiled circular pBR322 DNA. In this paper, we report (a) the synthesis and spectra of two novel isomers of thiocyclic fused naphthalimide; (b) their photochemistry and binding affinities with DNA; (c) their highly efficient photodamage to DNA (Scheme 1).

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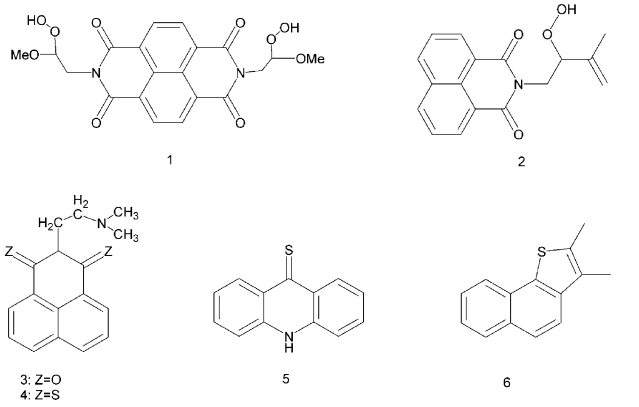


Scheme 1.

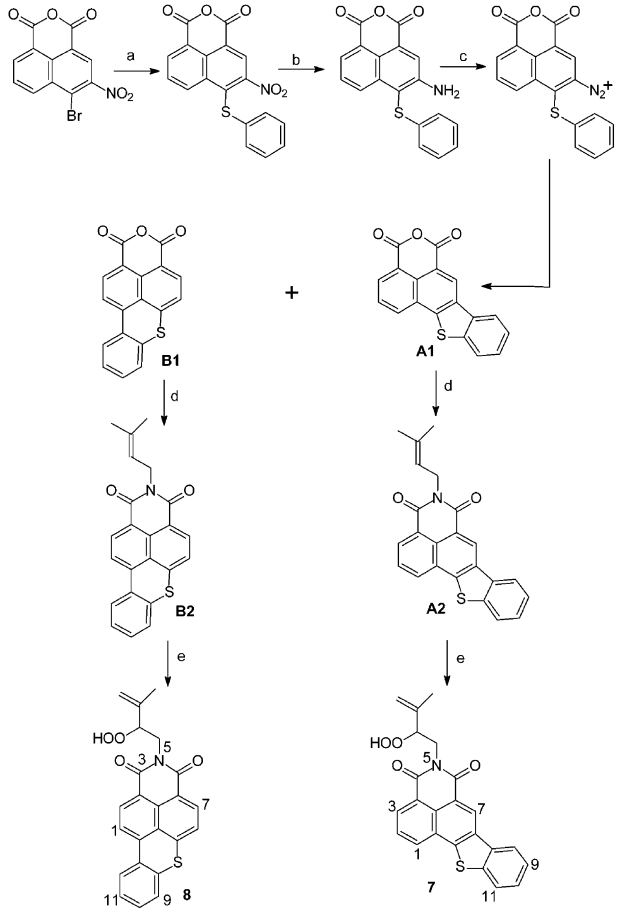
Results and Discussion

Synthesis and spectra

These reagents were synthesized from 4-bromo-3-nitro-1,8-naphthalic anhydride[20] as shown in Scheme 2. In the synthesis, that diazonium compound took an unusual Pschorr cyclization and gave two isomers **7** and **8** at the ratio of 6:1 via ^{1}H NMR.[21] So far, a similar isomerism was only observed in benzophenone derivatives,[22] while usually, Pschorr cyclization means two rings take the ring-closing reaction in the diazonium substituted positions to give multinuclear aromatic hydrocarbons, especially for derivatives of phenanthrene.

The photooxygenation of the corresponding *N*-isopentenyl-1,8-naphthalimides at −10 °C of **A2** and **B2** in methylene chloride gave products **7** and **8** as shelf-stable crystals, respectively. The hydroperoxides were identified via ^{1}H NMR, EI-MS, IR and elemental analysis, they displayed characteristic absorption peak of the O–O bond vibration around 800 cm^{-1} in FT-IR or FT-Raman spectra. The confirmation of similar structures was well established before.[16]

It can be seen in Table 1 that, the UV–vis absorption and fluorescence maximum of **7** and **8** are at longer wavelengths compared with those of **2**. **7** and **8** are strong electronic push–pull ICT (intramolecular charge transfer) chromophores, **7** has two electron-donating substituents on the same part of its naphthalene moiety (sulfur at the *para*, phenyl at the *meta* position to the same electron-withdrawing carbonyl group), while **8** has two electron-donating substituents on the different part of its naphthalene moiety (sulfur and phenyl at the *para* position to two electron-withdrawing carbonyl groups, respectively). Therefore, the maximal absorption and fluorescence of **7** are at shorter wavelengths, those of **8** at long wavelengths and **8** having stronger fluorescence. The fluorescence quantum yield of **8** is approximately three times higher than that of **7**. It implies a possibility that besides dissipated as heat in the system by internal conversion processes, the excitation energy of **7** might be more easily transferred from singlet excited state to cleave O–O bond compared with that of **8**.



Scheme 2. The synthetic route of heterocyclic-fused naphthalimides: (a) PhSH, EtOH, reflux, 4 h, 51 yield; (b) $SnCl_2$/HCl, 92.6% yield; (c) Pschorr cyclization; (1) $NaNO_2$, H_2O–HCl–HOAc, 0–5 °C, 2 h; (2) $CuSO_4$, HOAc, reflux, 2 h; 86% yield for B1 and A1 in the ratio of 6:1 mol/mol; (d) $H_2NCH_2CHC(CH_3)_2$, EtOH, 3 h, >90% yields; (e) hν, O_2, TPP/CH_2Cl_2, −10 to −15 °C, 3.5 h, 36% yield for **7**, 30% for **8**.

Photochemistry and DNA photocleavage

Under the irradiation of light matched with their maximal absorptions, both of **7** and **8** showed obvious hydroxyl radicals signal in ESR spectra with PBN (*N*-*tert*-butyl-α-phenylnitrone) trapping, it is important for the photocleavage profile whether hydroxyl radicals were involved.

The cleavage activity of **7** was evaluated using supercoiled circular pBR322 (form I) DNA (50 μM/base pair) under photoirradiation with a transilluminator (366 nm, 2.3 mw/cm^2) at a distance of 20 cm at 0 °C for 0.5 h and analyzed on a 1% agarose gel. DNA photocleavage efficiency was defined as the degree of the relaxation of the supercoiled DNA.

As speculated above, **7** showed excellent cleavage activity which can effectively photonick form I DNA into form II DNA at a concentration as low as 0.5 μM (Fig. 1), while no obvious cleavage was observed for **2** even at as high as 50 μM concentration.

The significantly enhanced cleavage activity of **7** confirmed the rationality of the design of the molecular structures. On the one hand, UV spectra showed the maximum

Table 1. UV–vis and fluorescence spectra date for naphthalimide derivatives

Comp	UV–vis $\lambda_{max/nm}$ (logε)	FL $\lambda_{max/nm}$ (ϕ)	Stoke shift (nm)
7	379 (4.05)	457 (0.045)	78
8	455 (4.24)	524 (0.12)	69
2	332 (4.25)	372 (0.002)	40
A1	381(3.85)	430 (0.062)	49
A2	379 (3.52)	455 (0.057)	76
B1	456 (3.78)	521 (0.21)	65
B2	458 (4.40)	524 (0.11)	66

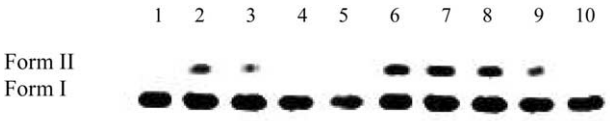


Figure 1. Cleavage of supercoiled circular pBR322 DNA by hydroperoxides **2** and **7**. Photoirradiation time 30 min. hν: 366 nm, DNA 50 μM/bp. Lane 1: DNA alone(no hν); Lanes 2∼5: DNA and **2** at concentrations of **2**: 100, 50, 10, and 1 μM, respectively; lanes 6–9: DNA and **7** at concentrations of **7**: 10, 5, 1, and 0.5 μM, respectively; lane 10: DNA alone.

absorption of **7**, was 379 nm (logε 4.05), close to the photoirradiation at 366 nm. On the other hand, the binding constants of **2** and **7** to calf thymus DNA measured using the fluorescence technique method[23] (Fig. 2) are 2.67×10^4 and 3.82×10^5 M^{-1}, respectively, the higher intercalating capability of **7** has contribution to its DNA cleaving capabilities.

When we firstly photoirradiated **8** and DNA with a transilluminator (366 nm, 2.3 mw/cm^2), no very obvious cleavage was observed at as high as 50 μM (data not shown). Obviously, because of the mismatch between the UV–vis absorption maximum of **8** and the photoirradiation wavelength, hydroxyl radicals can not be efficiently generated from **8**. When we changed photoirradition wavelength to 450 nm (2.3 mw/cm^2) through a light filter, **8** showed effective cleaving capability at the concentration of 5 μM (Fig. 3).

To illuminate the difference in photocleaving ability between isomers **7** and **8**, DNA binding capability of **8** was also evaluated by using fluorescence analysis. The apparent association constant K_a of hydroperoxides **8** was 4.26×10^3 M^{-1}. The association constant K_a of **7** was more than 100 times stronger than that of **8.**

On viewpoint of heterocyclic aromaticity and sulfur with two lone pairs, the sulfur atom at the six-membered ring of **8** is not conjugated with naphthalene ring, while that at the five-membered ring of **7** is, therefore, **7** is an extended planar conjugation system, while **8** is not. These were also supported by molecular mechanics calculation (Hyperchem 5.5). For example, the maximal dihedral angel of *peri*-hydrogen atoms on heterocyclic ring is about 30° for **8**, while that is 17° for **7**. The planarity and rigidity of **7** are helpful for its intercalation to DNA. It was known that, five-membered furan moiety in psoralens as famous DNA intercalators is more

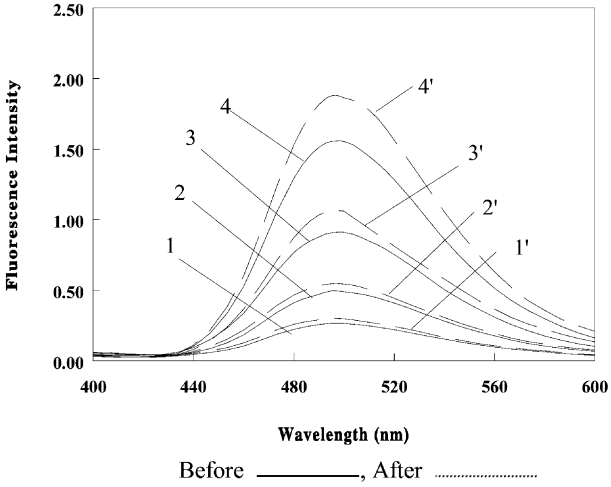
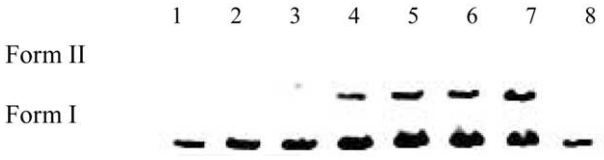


Figure 2. Fluorescence spectra before and after interaction of compound **7** and ctDNA. Curves 1–4 and 1′–4′ for compound **7** before and after mixed with DNA, respectively, at concentrations: 1, 5, 10, and 20 μM. DNA concentration: 50 μM (bp).



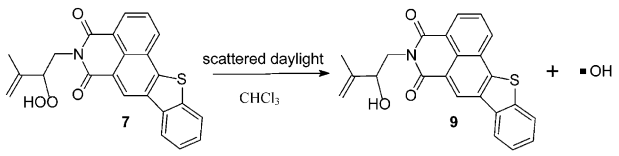
Photoirradiation time: 65 min. Lane 1:DNA alone (hν); Lane 2-7: DNA and 8 at concentration of 0.5 , 1, 5 ,10 , 20 and 50μM, respectively; Lane 8: DNA alone (no hν)

Figure 3. Effect of concentrations of compound **8** on the photocleavage of DNA at 450 nm.

important than pyrone moiety, which tends to maximize stacking interaction with the adjacent base pairs. It was also reported that anglicin which had the maximization of the angular furan ring stacking, was favorable intercalation geometry.[17,19] It implied that the five-membered thiocycle and angular conjugated plane of **7** might be the main reason for its high intercalating ability.

We also want to know the effect of photochemistry of **7** and **8** on their photocleaving activities. Under the irradiation of light, **7** and **8** should produce two parts: hydroxyl and allyloxyl radicals, as the reactivity and damage ability of hydroxyl radical to DNA should be same, their abilities to produce hydroxyl and the allyloxyl radicals will play important role in their photodamage behavior.

Interestingly, upon exposure of the chloroform solution of **7** to the scattered daylight at room temperature about 3 h, the corresponding hydroxyl derivative **9** was generated. The characteristic absorption peak of the O–O bond of **7** at 799 cm^{-1} gradually disappeared, accompanied by the emergence of the broad absorption peak of hydroxyl group at 3460 cm^{-1} by FT-Raman and IR



Scheme 3.

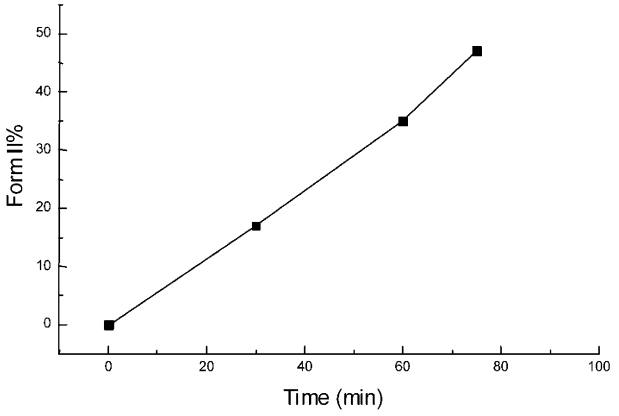


Figure 4. Effect of irradiation time on the photocleavage of DNA at 450 nm. **8**: 50 μM, hv: 450 nm. The conversion in percent of form I DNA to form II DNA was monitored by the computer imaging system.

monitoring, but the similar phenomena was not found for **8** under the same conditions.

Although it is known that most of organic hydroperoxides were converted into the corresponding ketones derivatives via an γ-H abstraction mechanism under irradiation by UV light with high energy,[6,24,25] **7** might decomposed in a different way under scattered daylight. Probably, because of O–O bond of hydroperoxyl group having weaker bond energy rather than γ-C–H bond, the released and transferred energy from photoexcited chromophore just meets the requirement of this photochemical reaction, that is, it can only selectively cleave O–O bond with the release of active species OH and substituted allyloxyl radical, which consequently was quenched in a hydrogen-donating solvent to give **9** (Scheme 3).

In the photocleavage profile the excitation was at 366 nm with high energy and that at 450 nm with low energy, so, the above experimental results at least implied that hydroxyl radical is more easily formed from **7** than **8**, which resulted in their differences in DNA photocleaving capability.

Therefore, the difference of **7** and **8** in photobiological property depends on that in molecular and electronic structure of their thiocyclic fused areas. These revealed that the high DNA intercalating capability and high radical-producing ability of **7** have contributions to its efficient DNA cleaving capability, by comparison with those of **8**. However, It should be stressed that for efficient photonick DNA at μM level concentration the photosensitizing wavelength of **7** was much shorter than that of **8**.

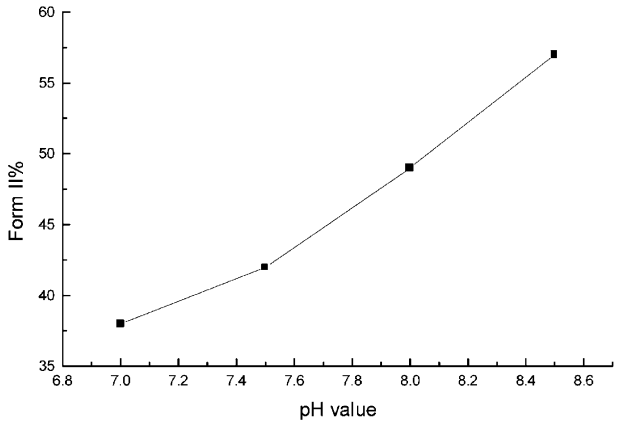


Figure 5. pH effect of the aqueous buffer on the photocleavage of **8** at 450 nm. Photoirradiation time: 75 min, **8**: 50 μM, hv:450nm, pH value = 7.0, 7.5, 8.0, 8.5. The conversion in percent of form I DNA to form II DNA was monitored by the computer imaging system.

The photocleavage activity of **8** was increased remarkably with the prolongation of photoirradiation time, which had not been seen in the experiment of **7** under the similar condition (at 366 or 450 nm, 2.3 mw/cm^2). **8** generated radicals at a relatively linear rate (from 30 to 75 min). It was time-controlled DNA photocleaving reagents (Fig. 4), which were different from the other hydroperoxide[17,18] and those involving metal complexes as well as classical Fenton reaction, where radicals are produced in a rapid burst.[24] Up to now, to our knowledge it was the first organic hydroperoxide as 'time-controlled' DNA photocleaver, which could release hydroxyl radicals without of metal oxidants under long-wavelength irradiation. This continuous generation of reactive hydroxy radical in the absence of metal oxidants provides a novel alternative to the famous Fenton-based chemistry, as one ever reported *N*-aroxyloxy-2-thiopyridones to produce aroyloxyl radical for 'time-controlled' DNA photocleavage.[12] **8** was much attractive for 'time-controlled' DNA cleavage studies and for in vivo biomedical application involving 'photodynamic therapy'.

In addition, when **8** and DNA were put in buffers with different pH values, **8** was sensitive to the outer environment and it was able to efficiently photocleave DNA during pH 7.5–8.5 and its photobioactivity was increased with the increasing pH value (Fig. 5). No pH-dependent change in the photonicking efficiency was found in the photocleavage of **7** to DNA under the same conditions.

So far ones wonder why some compounds show time-controlled or pH-independent DNA photocleaving activity and some others not. In our case, the isomers **7** and **8** might provide an opportunity to approach the reasons. Their differences in photochemical stability and availability of radicals possibly were major cause to result in 'time-controlled property'. **8** has high photochemical stability and its radical was not easy to form. However, **7** with lower photochemical stability will easily give radicals at a rapid burst under the irradiation of light. Similarly, the presence of the hydroxyl anion

was very important for **8** to produce radicals and high pH value was favorable, while the presence of hydroxyl anion did not give much help for the formation of radicals derived from **7**.

Conclusion

7, with high DNA binding ability, was very easily decomposed to unusually give hydroxyl derivative through losing hydroxyl radical in scattered daylight and it exhibits highly-effective photocleaving activity under the irradiation of light at 366 nm at 0.5 μM with pH-independent property (pH 7.0–8.5). **8** efficiently photo-cleaved DNA at a concentration of 5 μM, it was able to give promising results at pH 7.5–8.5 and its photobioactivity was increased with the increasing of pH value. Probably **8** was the first organic hydroperoxide which exhibits highly effective photocleavage with 'time-controlled' property, its radicals can be generated easily under the irradiation of visible light (450 nm). In general, it was demonstrated that sulfur-heterocyclic angular conjugated plane was an effective strategy for constructing novel photocleavers.

Experimental

Materials and methods

Melting points were taken on a digital melting point apparatus WRS-1 made in Shanghai and it was uncorrected. Infrared spectra were recorded on a Nicolet FT IR-20SX, mass spectra on a Hitachi M80, ^{1}H NMR on a Bruker AM-300 or AM-500 using TMS as an internal standard. Combustion analysis for elemental composition was done on Italy MOD.1106 analyzer. Absorption spectra were measured on Shimadzu UV-265, fluorescence spectra on a Hitachi 850.

Synthesis and photooxygenation

3-Nitro-4-phenylthio-1,8-naphthalic anhydride.[19] 4-Bromo-3-nitro-1,8-naphthalic anhydride (6.44 g) was stirred under reflux in ethanol (60 mL) with thiophenol (2.6 mL) for 5 h. The liquor was reduced in volume to 30 mL and filtered, washed with some ethanol, dried, giving 6.83 g (97.3%) of 3-nitro-4-phenylthio-1, 8-naphthalic anhydride. The recrystallization from glycol monomethyl ether gave long golden needles. Mp 177–178 °C (lit.[19] 178–179 °C).

3-Amino-4-phenylthio-1,8-naphthalic anhydride.[19] The above nitro compound (2.7 g) was stirred into a mixture of stannous chloride (8.81 g) and concentrated hydrochloric acid (12 mL). After warming to 40 °C, the temperature rose spontaneously to 85 °C and was maintained at 85 °C for 1 h (color change from orange to olive-green). The suspension was cooled and filtered to give 2.4 g crude product. Recrystallization from pyridine gave greenish-yellow needles, mp 224–225 °C[19] of 3-amino-4-phenylthio-1,8-naphthalic anhydride. ^{1}H NMR ($CDCl_3$, 300 Hz): δ 8.69 (d, J = 8.5 Hz, 1H, 7-H), 8.37 (d, J = 7.6 Hz, 1H, 5-H), 8.15 (s, 1H, 2-H), 8.15 (t, J = 7.6 Hz, J' = 8.5 Hz, 1H, 6-H), 7.22 (m,2H, 3′-H, 5′-H), 7.15 (m, 1H, 4′-H), 7.04 (m, 2H, 2′-H, 6′-H), 5.08 (s, 2H,–NH_2). EI-MS (m/z,%): 321 (M^+, 100), 277 ($M^+ - CO_2$, 6.16).

Benzothiophenonaphthalic anhydride (A_1) and benzothioxanthene-dicarboxylic anhydride (B_1). Sodium nitrite (0.7 g) and glacial acid (2 mL) were added dropwise to concd sulfuric acid (10 mL). The mixture was cooled to 5 °C and to it was added, portionwise over 1 h, 3-amino-4-phenylthio-1,8-naphthalic anhydride (3.2 g). After stirring for 1 h, the dark red viscous liquor was added over 90 min to a boiling solution of copper sulfate in water and glacial acetic acid. After the addition was complete, the liquor was refluxed for a further 30 min, cooled, filtered, dried and an orange solid (2.64 g, 86.3%) was collected. Recrystallisation from DMF gave deep red needles, but TLC indicated it contained compound $\mathbf{A_1}$ (R_f = 0.54) and $\mathbf{B_1}$ (R_f = 0.68), which were separated on preparative thin layer chromatography using dichloromethane as eluent (three times) and gave two pure compounds. The compound $\mathbf{A_1}$ was yellow-green solid, mp 284–286 °C. ^{1}H NMR (d-DMSO, 300 MHz): δ 9.4 (s, 1H, 7-H), 8.75 (m, 2H, 3-H, 1-H), 8.59 (dd, J_1 = 1.0 Hz, J_2 = 7.4 Hz, 1H, 11-H), 8.28 (m, 1H, 2-H), 8.04 (dd, J_1 = 8.2 Hz, J_2 = 7.4 Hz, 1H, 8-H), 7.68 (m, 2H, 9-H, 10-H); EIMS (m/z, %): 306 ($[M+2]^+$, 11.4), 304 (M^+, 100). The compound $\mathbf{B_1}$ was orange red solid, mp > 300 °C, ^{1}H NMR (-*d*DMSO, 300 MHz): δ 8.55(m, 3H, 2-H, 6-H, 7-H), 8.36 (d, J = 8.0 Hz, 1H, 2-H), 7.83 (d, J = 8.1 Hz, 9-H), 7.56∼7.70(m, 3H, 10-H, 11-H, 12-H); EIMS (m/z,%): 306 ($[M+2]^+$, 8.7), 304 (M^+, 100).

***N*-Iisopentenyl-benzothiophenonaphthalimide (A_2).** Compound A_1 (0.9 g) was refluxed in ethanol (40 mL) with isopentenylamine (0.4 g) for 3–5 h. After removal of the solvent, the residue was dissolved in CH_2Cl_2 and then purified on column chromatography with petroleum–dichloromethane (1:1) as eluent. The greenish-yellow main product was collected and recrystallized from petroleum–chloroform to give greenish-yellow needles (1.02 g), mp 207–208 °C, ^{1}H NMR ($CDCl_3$, 500 Hz): δ 1.79 (s, 3H, 3′-CH_3), 1.97 (s, 3H, 3′-CH_3), 4.66 (d, J = 6.7 Hz, 2H, –NCH_2–), 5.39 (t, $J_1 = J_2$ = 6.7 Hz, 1H, 2′-H), 7.32–7.37 (m, 2H, 9-H, 10-H), 7.49 (m, 1H, 2-H), 7.67 (d, J = 7.6 Hz, 1H, 8-H), 7.79 (d, J = 7.6 Hz, 11-H), 7.92 (d, 8.0 Hz, 1H, 1-H), 8.26 (d, J = 7.1 Hz, 1H, 3-H), 8.54 (s, 1H, 7-H); EI-MS (m/z, %): 373($[M+2]^+$, 5.4), 371(M^+, 53.5), 302 ($[M-CH_2CH{=}CMe_2]^+$, 100); ν $(KBr)_{max}/cm^{-1}$: 3060, 2980, 2850, 1690, 1660, 1580, 1440. Anal. calcd for $C_{23}H_{17}NO_2S$: C, 74.37, H, 4.62, N, 3.77. Found: C, 73.98, H, 4.62, N, 3.70.

***N*-Isopentenyl-benzothioxanthenediimide (B_2)**

In the similar manner to the synthesis of $\mathbf{A_2}$, $\mathbf{B_2}$ was obtained as orange red needles, mp 202–203 °C, ^{1}H NMR ($CDCl_3$, 500 Hz): δ 1.74 (s, 3H, 3′-CH_3), 1.77 s, 3H, 3′-CH_3), 4.73 (d, J = 7.5 Hz, 2H, –NCH_2–), 5.35 (t, $J_1 = J_2$ = 7.5 Hz, 1H, 2′-H), 7.39 (m, 3H, 10-12H), 7.49 (d, J = 8.0 Hz, 1H, 9-H), 8.20 (m, 2H, 1-H, 7-H), 8.42

(d, $J=8.0$ Hz, 6-H), 8.62 (d, $J=8.0$ Hz, 1H, 2-H); EI-MS (m/z, %): 373 ($[M+2]^+$, 5.1), 371 (M^+, 41.3), 302 ($[M+H–CH_2CH=CMe_2]^+$, 100); ν $(KBr)_{max}/cm^{-1}$: 3080, 2960, 2930, 1690, 1645, 1585, 1380. Anal. calcd for $C_{23}H_{17}NO_2S$: C, 74.37, H, 4.62, N, 3.77. Found: C, 74.08, H, 4.89, N, 3.79.

Benzothiophenonaphthalimide hydroperoxide (7). A solution of A_2 (200 mg) and tetraphenylporphyrin (TPP, 5 mg) in 40 mL of CH_2Cl_2 was irradiated externally by means of a sodium lamp (150 W) at −10 to −20 °C for 3–4 h while passing a continuous slow stream of dry oxygen gas through the solution. The solution was concentrated and purified by preparative thin layer chromatography on silica gel using petroleum ether–acetate (2:1) as eluent. The zone with $R_f=0.6$ was collected as product and gave greenish-yellow solid (36%). Mp 174–175 °C, ^{1}H NMR ($CDCl_3$, 500 Hz): δ 2.01 (s, 3H, 3′-CH_3), 4.70 (m, 3H, N-CH_2-CH-,), 5.12 (d, $J=1.4$ Hz, 2H, 3′-CH_2=), 7.56 (m, 2H, 9-H, 10-H), 7.75 (m, 1H, 2-H), 7.92 (d, $J=7.6$ Hz, 1H, 8-H), 8.19 (d, $J=7.6$ Hz, 1H, 11-H), 8.32 (d, $J=8.0$ Hz, 1H, 1-H), 8.53 (d, $J=7.1$ Hz, 1H, 3-H), 9.04 (s, 1H, 7-H), 10.17 (br, 1H, -OOH); EI–MS (m/z, %): 386 ($[M-OH]^+$, 5.7); ν $(KBr)_{max}/cm^{-1}$: 3258, 1688, 1640, 1587, 1335, 906, 799, 780; FT-Raman: 3065, 1689, 1587, 1400, 1377, 799 cm^{-1}. HRMS: calcd for $C_{23}H_{17}NO_4S$: 403.4878; Found: 403.0874.

Benzothiophenonaphthalimide hydroperoxide (8). In the similar manner to the synthesis of **7**, **8** was obtained as orange red solid. Mp 210–211 °C, ^{1}H NMR (DMSO-*d*, 500 Hz): δ 1.79 (s, 3H, 3′-CH_3), 4.07 (m, 1H, 1′-CH_2), 4.32 (m, 1H, 1′-CH_2), 4.64 (m, 1H, 2′-CH), 4.88 (2s, 2H, CH_2=), 7.53 (m, 2H, 10-H, 11-H), 7.62 (dd, $J_1=1.6$ Hz, $J_2=7.6$ Hz, 1H, 9-H), 7.78 (d, $J=8.0$ Hz, 1H, 12-H), 8.35 (d, $J=8.0$ Hz, 1H, 1-H), 8.48–8.55 (m, 3H, 2-H, 6-H, 7-H), 10.59 (br, 1H,-OOH); EI-MS (m/z, %): 386 ($[M-OH]^+$, 20.4); ν $(KBr)_{max}/cm^{-1}$: 3250, 2940, 1688, 1635, 1587, 1385, 800, 770 cm^{-1}. HRMS: calcd for $C_{23}H_{17}NO_4S$: 403.4878; Found: 403.0875.

***N*-hydroxyl-isopentenyl benzothiophenonaphthalimide (9).** Upon exposure of the chloroform solution of **7** to the scattered day light at room temperature for about 3 h, then collected sample at $R_f=0.4$ on preparative TLC with methylene chloride as eluent, after removal of solvent yellow solid was obtained. Mp 95–97 °C, ^{1}H NMR ($CDCl_3$, 500 Hz): δ 1.96 (s, 3H), 4.42 (d, $J=5.9$ Hz, 2H), 4.53 (t, $J=5.9$ Hz, 1H), 4.98 (s, 1H), 5.18 (s, 1H), 7.56 (m, 2H), 7.78 (m, 1H), 7.94 (m, 1H), 8.23 (m, 1H), 8.35 (d, $J=7.8$ Hz, 1H), 8.56 (d, $J=7.3$ Hz, 1H), 9.11 (s, 1H), 9.78 (br, 1H); EI-MS m/z 387 (M^+); IR (KBr): 3458, 1699, 1652, 1587, 1334, 913, 780 cm^{-1}; FT-Raman: 3061, 1700, 1588, 1401, 1377 cm^{-1}. HRMS: calcd for $C_{23}H_{17}NO_3S$, 387.0929; Found: 387.0923.

Spectroscopic measurements

The compounds were dissolved in absolute ethanol to give 10^{-5} M solutions which were read with Shimadzu UV-265 for absorption spectra and with Perkin-Elmer LS 50 using quinine sulphate in sulphuric acid as quantum yield for fluorescence spectra.

Intercalation studies of compounds 7 and 8 to DNA

0.1 mL of solution of a compound in DMSO (10^{-3}–10^{-4} M) mixed with 0.1 M Tris–HCl buffer (pH 7.4) to 10 mL. Then, two groups of samples were prepared in the concentration of chemical at 10^{-5}–10^{-6} M; one contained calf thymus DNA 50 μM, the other contained no DNA but had the same concentration of chemical as control. All the above solution was shaken for 3 days at 25 °C in the dark. Fluorescence wavelengths and intensity areas of samples were measured at following conditions: excitation: 365 or 450 nm, emission: 380–520 or 470–650 nm.

Photocleavage of supercoiled DNA using compounds 7 and 8

300 ng pBR 322DNA (form I), 1 μL of solution of chemical in DMSO and 10 mM Tris–HCl buffer (pH 7.6) were mixed to 10 μL and stood for 10 min at 0 °C, then irradiated for 30 min or more with light (2.3 mW/cm^2, 365 or 450 nm) using lamp placed at 20 cm from sample. Supercoiled DNA runs at position I, nicked DNA at position II, and linear DNA at position III. The samples were analyzed by gel electrophoresis in 1% agarose and gel was stained with ethidium bromide.

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