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Full Paper

DNA Photocleavage and Binding Modes of Methylene Violet 3RAX and its Derivatives: Effect of Functional Groups

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With 4′-amino-*N*,*N*-diethylaniline and aniline as starting materials, methylene violet 3RAX **1** and its derivatives **2**–**5** were synthesised. The five compounds were characterised by IR, UV-vis, and ^{1}H NMR spectroscopy and mass spectrometry. The binding mode between the synthesised compounds and DNA were investigated. The results show that both compounds **1** and **5** bind to DNA by an intercalative mode, while compounds **2**–**4** interact with DNA through a mixed binding mode involving groove binding and electrostatic interactions. The photocleavage ability of the five compounds to DNA were calculated to be 38, 40, 30, 20, and 13 %, respectively, when their concentration was adjusted to 400 μM. The singlet oxygen production of compounds measured by the 1,3-diphenylisobenzofuran method was consistent with the trend of DNA photocleavage ability. The DNA studies suggest that the binding mode between methylene violet 3RAX and DNA, the ability of methylene violet 3RAX to generate singlet oxygen, and the DNA photocleavage activity could be adjusted through modification of the amino group on methylene violet 3RAX.

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Introduction

Singlet oxygen ($^1O_2$) is the main cytotoxic agent in the process of photodynamic therapy (PDT) to induce the apoptosis of cancer cells through a type II mechanism.[1,2] Singlet oxygen can effectively damage the tiny blood vessels in the tumour tissue through interaction with biological macromolecules,[3,4] such as DNA and proteins. Phenazine compounds and their derivatives with a conjugated structure have been widely developed and applied in dyes, biomedicine,[5–8] pesticides, photoelectric materials,[9] and analytical chemistry.[10] As one of the typical phenazine compounds, methylene violet 3RAX **1** displays a rigid planar phenazine structure. It has a potential application in the PDT of cancers as a photosensitiser, as it might change the molecular structure of DNA, undermine the spatial configuration of DNA, and even induce the generation of reactive singlet oxygen by its interaction with light to damage DNA and cause cell death through interaction with DNA in tumour cells.[11,12]

The functional groups of methylene violet 3RAX **1** allow easy further modification of its structure. In 2013, Saha and Kumar[13] prepared the methylene violet 3RAX derivative indoine blue by transforming the diethylamino group at one side of **1** into a (2-hydroxy-1-naphthalenyl) azo group and introducing a methyl group. Studies showed that the affinity of indoine blue to DNA was weaker than that of **1**. However, both of them still adopt an intercalation mode with DNA. To further investigate the effect of functional groups at the other side of **1** on its interaction with DNA, the methylene violet 3RAX derivatives **2**–**4** (Scheme 1) are synthesised here through transforming the amino group into hydroxy, 4-methyl-benzenesulfonimide, and benzene-1,2-dicarboxylic acid imide groups, respectively. In addition, the conjugation of **1** with indomethacin yields compound **5**. Indomethacin (INDO), as one of the first non-steroidal anti-inflammatory drugs (NSAID), has been chosen because it is widely used in the treatment of inflammatory diseases, such as rheumatoid arthritis, osteoarthritis, gut arthritis, burst, tendinitis, traumatic synovitis, and ankylosing spondylitis.[14] Preliminary experiments have indicated that indomethacin has antitumour activity.[15] The binding properties of compounds **1**–**5** to calf thymus (CT)-DNA are studied by means of various methods, such as UV-vis, fluorescence, and circular dichroism (CD) spectroscopy. The singlet oxygen generation ability of the compounds is determined using the 1,3-diphenylisobenzofuran (DPBF) method. Finally, we further evaluate the DNA photocleavage activities of the compounds through agarose gel electrophoresis.

Experimental

*Synthesis of Methylene Violet 3RAX **1** and its Derivatives **2**–**5***

*Synthesis of 3-Amino-7-(diethylamino)-5-phenylphenazinium (**1**)*

To a solution of 4′-amino-*N*,*N*-diethylaniline (5 g, 30.5 mmol), aniline (5.8 g, 61.0 mmol), and concentrated hydrochloric acid (8 mL) in acetic acid/sodium acetate buffer solution (200 mL)

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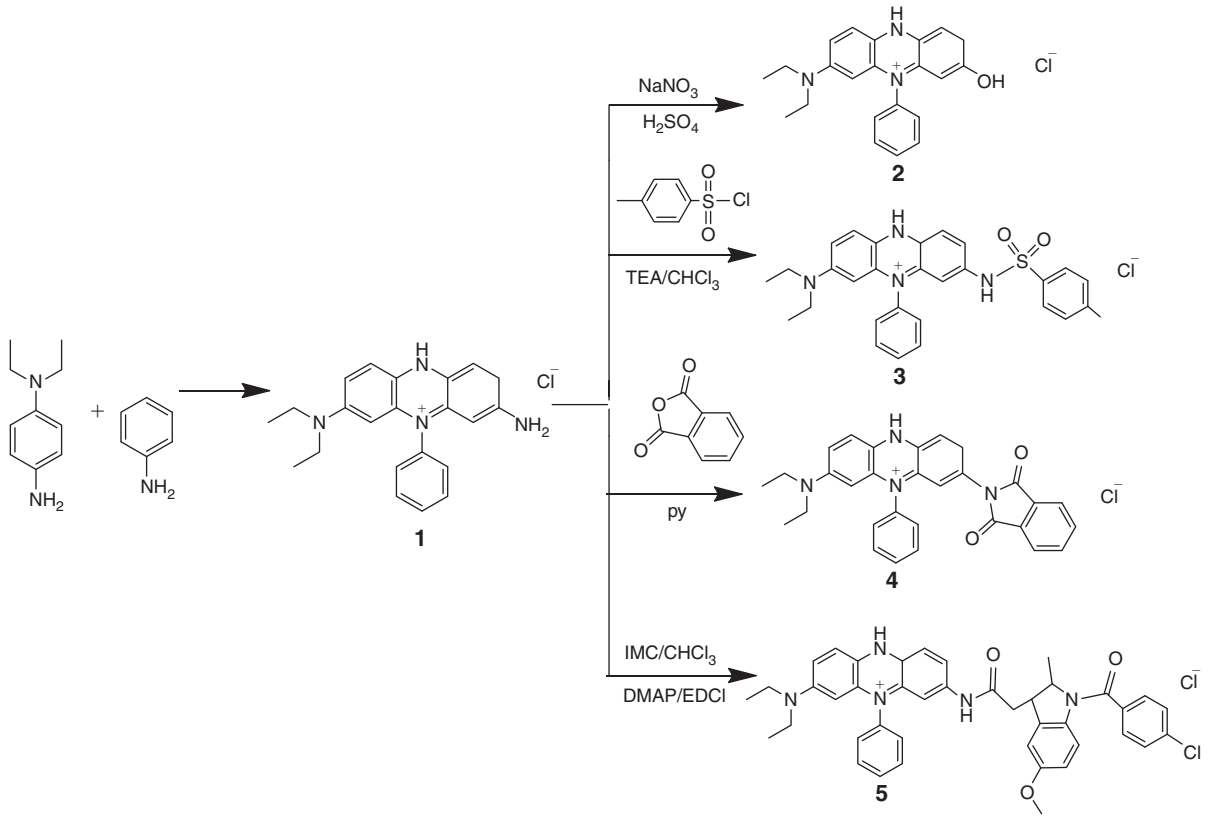

Scheme 1. Synthetic routes of compounds **1**–**5**.

was added to a large excess of potassium dichromate (19.7 g, 67.1 mmol). The reaction mixture was stirred for 3 h at 105°C, protected from light and atmospheric moisture. After cooling to room temperature, the mixture was filtered, affording 2.3 g of solid (yield 35 %). δ_H (400 MHz, $CDCl_3$) 1.09 (t, *J* 7.1, 6H), 3.33 (q, *J* 7.1, 4H), 5.63 (s, 1H), 5.99 (s, 1H), 7.13 (d, *J* 7.8, 1H), 7.32 (d, *J* 7.8, 2H), 7.69–7.80 (m, 4H), 7.85 (d, *J* 8.9, 1H). δ_C (400 MHz, $CDCl_3$) 149.3, 148.4, 143.8, 132.8, 130.2, 130.0, 129.5, 125.6, 124.4, 118.5, 115.3, 113.3, 111.9, 47.4, 12.8. Anal. Calc. for $C_{22}H_{23}N_4Cl$: C 69.74, H 6.12, N 14.79. Found: C 69.59, H 6.24, N 14.65 %. ν_{max} (KBr)/cm^{-1} 3058, 1649 (NH_2), 1339, 1198 (C–NH_2, N–H), 1601, 1524, 1484 (C=C, C–N), 1069, 1006, 991, 874, 849, 812, 776, 704 (C–H), 1392 (–CH_3). λ_{max} (DMSO)/nm 560. *m*/*z* (ESI) 343.58 $[M]^+$.

*Synthesis of 3-Hydroxy-7-(diethylamino)-5-phenyl-phenazinium (**2**)*

To compound **1** (1 g, 2.63 mmol), 5 mL of concentrated sulfuric acid and 10 mL of water was added. The solution was cooled to −10°C. An aqueous solution (10 mL) of sodium nitrite (0.27 g, 3.95 mmol) was then added and stirred for 1.5 h. After heating to room temperature, the reaction mixture was poured into a solution of 1 g of copper sulfate, 5 mL of concentrated sulfuric acid, and 5 mL of water. The mixture was stirred for another 3 h at 105°C and then cooled to room temperature. The pH of the solution was adjusted to 5 using sodium bicarbonate. The crude product was precipitated with sodium chloride (5 g), and then purified on a silica gel column with chloroform as eluent. The first fraction was collected and evaporated to yield 0.4 g of a solid product (yield 40 %). δ_H (400 MHz, $CDCl_3$) 1.03 (t, *J* 7.1, 6H), 3.24 (q, *J* 7.1, 4H), 5.55 (m, 2H), 6.81 (dd, *J* 2.3, 2.5, 1H), 6.91 (d, *J* 9.6, 1H), 7.25 (d, *J* 7.4, 2H), 7.53–7.57 (m, 2H), 7.60–7.64 (m, 2H), 7.74 (d, *J* 9.3, 1H). δ_C (100 MHz, $CDCl_3$) 158.5, 149.5, 143.8, 132.8, 131.5, 130.8, 129.4, 125.7, 121.1, 118.0, 113.2, 112.1, 47.2, 13.1. Anal. Calc. for $C_{22}H_{22}N_3ClO$: C 69.56, H 5.84, N 11.06. Found: C 69.45, H 5.77, N 11.12 %. ν_{max} (KBr)/cm^{-1} 3160 (OH), 1326, 1174 (C–O, OH), 1608, 1520, 1475 (C=C, C–N),1199 (C–N), 1060, 1010, 997, 870, 844, 818, 778, 702 (C–H). λ_{max} (DMSO)/nm 530, 562. *m*/*z* (ESI) 344.1781 $[M]^+$.

*Synthesis of 3-(4-Methyl-benzenesulfonimide)-7-(diethylamino)-5-phenyl-phenazinium (**3**)*

To compound **1** (1 g, 2.63 mmol) was added 4-toluene sulfonyl chloride (2.0 g, 10.52 mmol) and triethylamine (3 mL) in dry chloroform (15 mL). The solution was heated to 60°C for 2 h. After cooling to room temperature, the reaction mixture was poured into a saturated sodium chloride solution (10 mL) and extracted with chloroform (3 × 5 mL). The organic layers were combined, dried over anhydrous sodium sulfate, and then concentrated under vacuum. The residue was purified on a silica gel column eluted with chloroform/methanol (10 : 1) to yield the desired product (1.1 g, yield 78 %). δ_H (400 MHz, $CDCl_3$) 1.10 (t, *J* 7.1, 6H), 2.32 (s, 3H), 3.35 (q, *J* 7.1, 4H), 5.62 (s, 1H), 6.39 (s, 1H), 7.03 (d, *J* 8.9 , 1H), 7.11 (d, *J* 7.3, 2H), 7.31 (d, *J* 7.8, 2H), 7.46 (d, *J* 10.6, 1H), 7.57–7.62 (m, 3H), 7.80 (m, 4H) δ_C (100 M, $CDCl_3$) 149.7, 143.8, 137.6, 136.7, 133.0, 130.1, 129.4, 128.6, 125.4, 118.5, 116.5, 115.1, 113.8, 111.7, 47.3, 21.5, 13.1. Anal. Calc. for $C_{29}H_{29}N_4ClSO_2$: C 65.34, H 5.48, N 10.51. Found: C 65.41, H 5.40, N 10.58 %. ν_{max} (KBr)/cm^{-1} 2959, 2924, 2853 (C–H), 1593, 1514, 1493 (C=C, C–N), 1342, 1121 (–SO_2NH–), 1200 (C–N), 1072, 1017, 957, 895, 852, 803, 769, 695 (C–H). λ_{max} (DMSO)/nm 586. *m*/*z* (ESI) 498.29 $[M + H]^+$.

*Synthesis of 3-(Benzene-1,2-dicarboxylic acid imide)-7-(diethylamino)-5-phenyl-phenazinium (**4**)*

Phthalic anhydride (0.39 g, 2.65 mmol) and compound **1** (1 g, 2.63 mmol) were dissolved in pyridine (20 mL).The solution was heated to 120°C for 12 h under nitrogen atmosphere. After cooling to room temperature, the reaction mixture was poured into a saturated sodium chloride solution (100 mL). The precipitate was purified on a silica gel column with chloroform/methanol (100 : 1) as eluent. The first fraction was collected as the desired product (0.8 g, yield 60 %). δ_H (400 MHz, $CDCl_3$) 1.10 (t, *J* 7.1, 6H), 3.35 (q, *J* 7.1, 4H), 5.65 (s, 1H), 6.40 (s, 1H), 7.01 (d, *J* 8.9, 1H), 7.31 (d, *J* 7.8, 2H), 7.40 (d, *J* 10.6, 1H), 7.55–7.75 (m, 5H), 8.05 (m, 4H). δ_C (100 MHz, $CDCl_3$) 167.3, 150.4, 143.3, 133.6, 132.2, 130.1, 129.4, 128.4, 125.1, 123.0, 121.7, 120.5, 118.5, 113.8, 112.4, 47.3, 13.1. Anal. Calc. for $C_{30}H_{25}N_4ClO_2$: C 70.79, H 4.95, N 11.01. Found: C 70.82, H 4.90, N 11.08 %. ν_{max} (KBr)/cm^{-1}: 2935, 2856 (C–H), 1590, 1528, 1472 (C=C, C–N), 1678 (–CONH–), 1197 (C–N), 1073, 955, 877, 856, 801, 751, 682 (C–H). λ_{max} (DMSO)/nm 553, 588. *m*/*z* (ESI) 474.41 $[M + H]^+$.

*Synthesis of Methylene Violet 3RAX-INDO **5***

A mixture of compound **1** (1 g, 2.63 mmol), indomethacin (1.4 g, 3.95 mmol), dimethylaminopyridine (DMAP, 0.3 g, 2.63 mmol), and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI) (1 g, 5.26 mmol) in dry trichloromethane (10 mL) was heated to 60°C for 12 h. After cooling to room temperature, the reaction mixture was poured into a saturated sodium chloride solution (10 mL) and extracted with chloroform (3 × 5 mL). The organic layers were combined and dried over anhydrous sodium sulfate, and then concentrated under vacuum. The residue was purified on a silica gel column with chloroform/methanol (10 : 1) as eluent. The second fraction was collected as the desired product (1.5 g, yield 80 %). δ_H (400 M, $CDCl_3$) 1.15 (t, *J* 7.1, 6H), 2.31 (s, 3H), 3.49 (q, *J* 7.1, 4H), 3.80 (s, 3H), 4.10 (s, 2H), 5.76 (s, 1H), 5.56 (d, *J* 8.1, 1H), 6.86 (d, *J* 8.1, 2H), 7.43 (m, 5H), 7.59 (d, *J* 8.6, 2H), 7.79 (m, 3H), 8.01–8.10 (m, 3H), 8.58 (d, *J* 8.8, 2H). δ_C (100 MHz, $CDCl_3$) 167.2, 164.4, 154.4, 149.6, 143.6, 130.4, 135.7, 134.6, 132.2, 131.9, 129.3, 128.6, 127.6, 125.4, 124.1, 121.3, 120.4, 118.1, 116.3, 113.1, 111.7, 104.6, 55.9, 47.3, 12.8, 9.8. Anal. Calc. for $C_{40}H_{35}N_5Cl_2O_3$: C 68.18, H 5.01, N 9.94. Found: C 68.09, H 5.08, N 9.88 % ν_{max} (KBr)/cm^{-1} 2975 (C–H), 1662 (C=O), 1577, 1551, 1512, 1460 (C=C, C–N), 1259 (Ph–O–CH_3), 1199 (C–N), 1071, 1013, 925, 871, 829, 755, 699 (C–H). λ_{max} (DMSO)/nm 546, 578. *m*/*z* (ESI) 683.92 $[M + H]^+$.

*Determining the Mode of Interaction Between Compounds **1**–**5** and CT-DNA*

The spectroscopic measurements were performed at room temperature in buffer (pH 7.4, 0.05 M TRIS-HCl, 0.1 M NaCl). An extinction coefficient of 6600 M^{-1} cm^{-1} at 260 nm was used to determine the concentration of CT-DNA.[16] UV-Vis spectra were measured on a Shimadzu 1901 spectrometer. Fluorescence spectra were measured on a Perkin Elmer LS-55 spectrometer. Circular dichroism was run on a JASCO J-810 spectrometer. The UV-vis absorption spectra of compounds were recorded within a range of 450–740 nm. Fluorescence spectra of compounds were recorded at 557, 551, 574, 562, and 551 nm with excitation at 633, 621, 640, 674, and 675 nm, respectively. Induced CD spectra were recorded within a range of 500–700 nm.

Measurement of the Production Rate of Singlet Oxygen[17]

DPBF was used as a selective singlet oxygen ($^1O_2$) acceptor, which is bleached upon reaction with $^1O_2$.[18] Five sample solutions containing DPBF in DMSO (50 μM) were prepared in the dark. Each sample container was covered with aluminium foil with a yellow filter (with cut-off wavelength < 500 nm) on one side. The samples were then exposed to light (50 W) through the filter. After irradiation, the visible spectra of the sample was measured. The normalised absorbance of DPBF at 418 nm in the presence of **1**–**5** was reported as a function of the photo-irradiation time. From this plot, the production rates of $^1O_2$ were determined.

DNA Photocleavage Assay

The DNA photocleavage activities of compounds **1**–**5** were measured using the plasmid DNA relaxation assay. Briefly, plasmid DNA (0.5 μg), enriched with the covalently closed circular or supercoiled conformer (Form I), and the one-phor-all plus buffer (pH 7.5) was vortexed. Aliquots of the DNA were pipetted into different Eppendorf tubes. Various amounts of autoclaved water (control sample) or solutions of compounds **1**–**5** (test sample) were added into the Eppendorf tubes to give a final volume of 20 μL in each sample tube. The sample mixtures were then photo-irradiated at 400–500 nm for 60 min using a transilluminator (Vilber Lourmat) containing 4 × 15 W light tubes (Aqua Lux) with maximum emission at 435 nm. After photo-irradiation, 2 μL of the 6x sample dye solution was added to each Eppendorf tube and mixed well by centrifugation. The sample mixtures were loaded onto a 0.8 % (v/v) agarose gel (pH 8) used as supporting electrolyte, and electrophoresed at 1.3 V cm^{-1} for 3 h using a mini gel set. After electrophoresis, the gel was stained with 0.5 μg mL^{-1} ethidium bromide solution for 30 min and then washed with deionised water for 10 min. The resulting gel image was viewed under 365 nm and captured digitally using a gel documentation system (BioRad).[19]

Results and Discussion

Synthesis of Compounds

The synthesis of compounds **1**–**5** was carried out as shown in Scheme 1. With 4′-amino-*N*,*N*-diethylaniline and aniline as starting materials, methylene violet 3RAX **1** was synthesised in 35 % yield following the procedure described in the literature.[20] The derivatives **2**–**5** were synthesised through a routine coupling reaction in moderate yield. The amino group in compound **1** was oxidised by an acid-catalysed reaction, affording 3-hydroxy-7-(diethylamino)-5-phenyl-phenazinium (**2**) in 40 % yield. In addition, the amino group in compound **1** was coupled with 4-toluene sulfonyl chloride, phthalic anhydride, or indomethacin in dry solvent, affording compounds **3**, **4**, and **5**, respectively, with moderate yields. The structures of **1**–**5** were confirmed by UV, NMR, and MS spectra.

UV-Vis Absorption Spectroscopy

The effect of the stoichiometric addition of CT-DNA on the UV-vis absorption spectra of compounds **1** to **5** is shown in Fig. 1. As shown in Table 1, with the increase of DNA concentration, moderate hypochromism for compounds **1**–**5** (H = 23–49 %) was found, together with a significant red or blue-shift for compound **1** and **5** ($\Delta\lambda = 12, -14$, respectively), indicating that compound **1** and **5** may intercalate DNA at high concentration.[15] Moreover, the bathochromic shift after

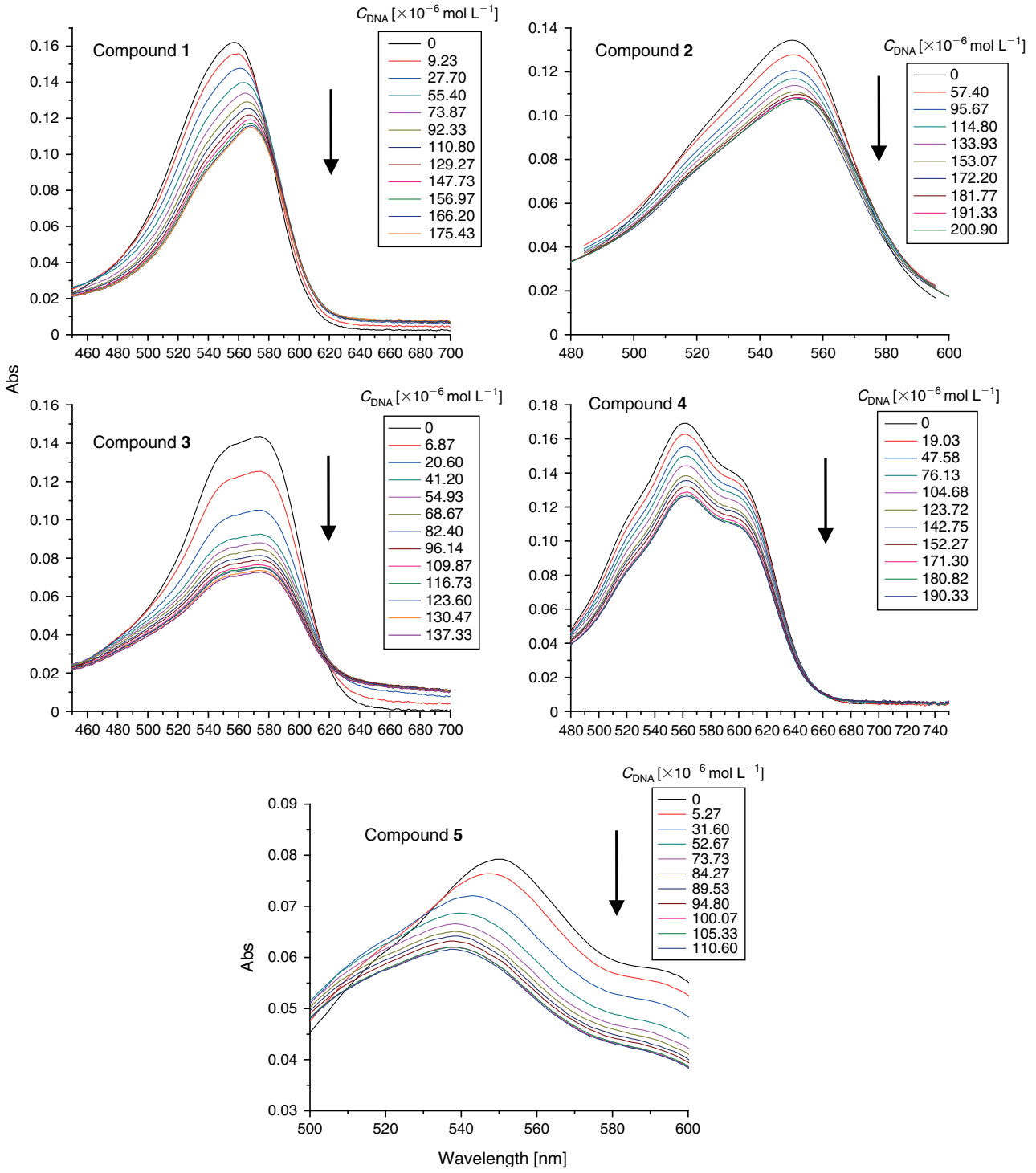

Fig. 1. UV-Vis absorption spectra of compounds **1**–**5** with the titration of CT-DNA, [compound **1**–**5**] = 4 μmol L^{-1}, buffer solution (pH 7.4, 0.05 mol L^{-1} TRIS-HCl + 0.1 mol L^{-1} NaCl).

interaction with DNA suggests compound **1** adopts an end-to-end interaction with DNA (J aggregate). On the contrary, compound **5**, with hypsochromic shift, tends to exhibit a parallel stacking arrangement (H aggregate) probably due to the high steric hindrance, as reported previously.[21] Generally speaking, for a given DNA, intercalation will result in a large change of its absorption spectrum, while small changes occur upon the groove binding.[18] At a high concentration of CT-DNA, the absorption bands of compounds **2**–**4** exhibited substantial hypochromicity (> 20 %) without a significant spectroscopic shift. These weak spectroscopic variations suggest that compounds **2**–**4** may interact with DNA through a groove binding mode.

The binding affinities between compounds **1**–**5** and DNA were expressed by the binding constant (K), which was calculated using Eqn 1:[22,23]

$$D/|\varepsilon_A - \varepsilon_F| = D/|\varepsilon_B - \varepsilon_F| + 1/(|\varepsilon_B - \varepsilon_F|K) \qquad (1)$$

Table 1. Spectroscopic characterisation of compounds 1–5 in the presence of CT-DNA

Compd	UV-vis hypochromicity (H)/ bathochromic shift ($\Delta\lambda$) [%]/[nm]	Fluorescence emission hypochromicity (H)/ wavelength shift ($\Delta\lambda$) [%]/[nm]	CD $r = 0.01$	
			Positive band [nm]	Negative band [nm]
1	30.1/12	25.9/−14	—	598
2	22.8/2	−14.3/−5	604	584
3	49.3/1	−26.5/−2	608	573
4	25.2/1	13.9/−8	597	606
5	22.2/−14	58.3/−12	—	591

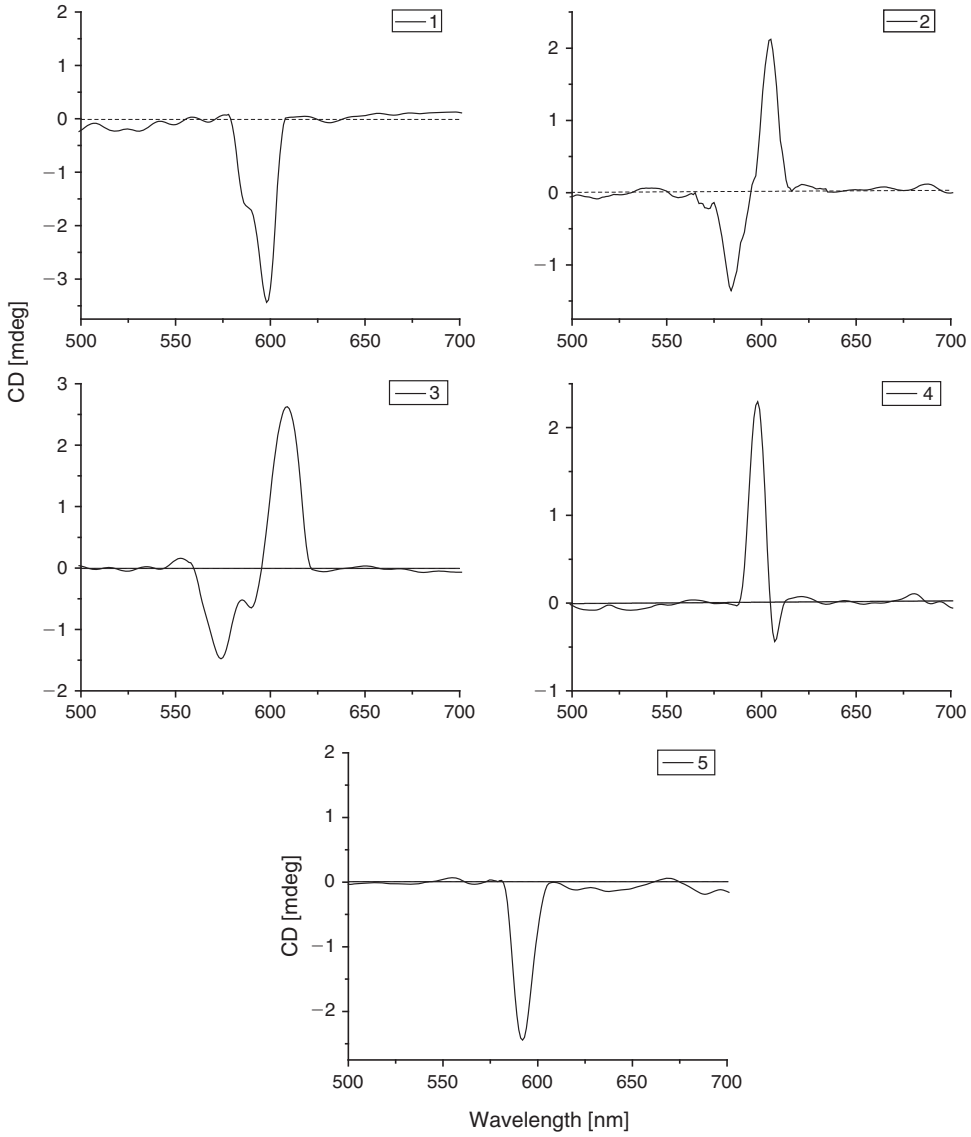

Fig. 2. Induced CD spectra of compounds **1**–**5**.

D is the concentration of DNA during the process of titration. ε_A is the ratio of A_{max}/[**1**–**5**] during the process of titration. ε_B is the ratio of A_{max}/[**1–5**] after saturating with DNA, and ε_F is the ratio of A_{max}/[**1–5**] without DNA. The values of K obtained for compounds **1–5** are 7.48×10^3, 2.72×10^3, 3.88×10^4, 2.82×10^3, and 1.56×104 mol L^{-1}, respectively.

Fluorescence Spectroscopy

A fluorescence titration was performed to further probe the binding mode between compounds **1**–**5** and CT-DNA. With the titration of CT-DNA, an increase in intensity of fluorescence emission of compounds **1**, **4**, and **5** is observed (Fig. S1, Supplementary Material). This might be ascribed to the

conjugated plane structures of compounds **1** and **5**, which could insert between the base pairs of the DNA double-helix and are protected by the strong hydrophobic core of the DNA so will avoid the quenching effect of water molecules.[24] As for compound **4**, it could insert into the grooves of the DNA double-helix. The hydrogen bonding of base pairs and the layer stacking of the bases provides a completely hydrophobic environment. However, compound **2** and **3** exhibited a decrease in fluorescence emission intensity upon titration with DNA, due to the insertion into the structure of DNA, which will change the electronic excited state.[22] At the same time, there were electrostatic interactions between the positive charges of compounds **1–5** and the negative charges of the phosphate groups of DNA. The electrostatic interaction will strengthen the heterozygosity.[25]

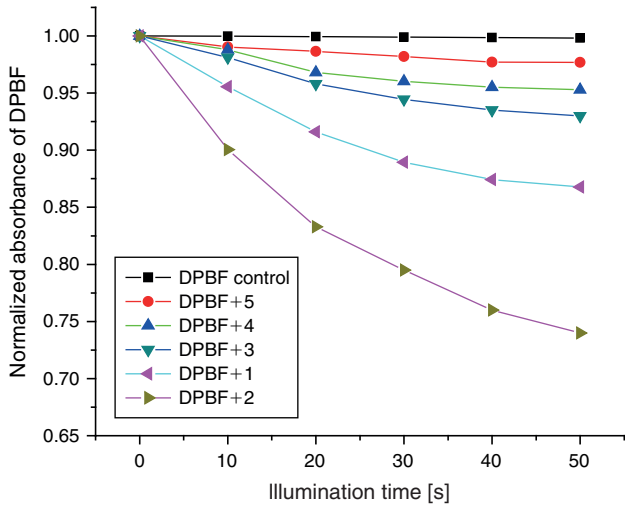

Fig. 3. Relation between irradiation time and DPBF at 418 nm ultraviolet absorption ratio (A/A_0).

Circular Dichroism Spectroscopy

To further clarify the binding mode between the compounds and DNA, the induced CD spectra of compounds **1–5** were recorded in the presence of CT-DNA (Fig. 2). In the case of duplex DNA, a positive induced CD band indicates groove binding, and a negative induced CD band indicates intercalation.[23] In solutions of DNA and compounds **1–5** (100 : 1, v/v), compounds **1** and **5** show a strong negative peak (centred at 598 and 591 nm, respectively), which further indicates they interact with DNA through intercalation. For compounds **2**, **3**, and **4**, a strong positive peak (centred at 604, 608, and 597 nm, respectively) and a weak negative peak (centred at 584, 573, and 606 nm, respectively) was obtained, suggesting they adopt a combination of groove binding and intercalation with DNA. The results suggest that the modification of the amino group could change the binding properties of these compounds to DNA.

Photogeneration of $^1O_2$

DPBF was able to capture the $^1O_2$ photogenerated by compounds **1–5**, which reduces its own light activity.[26] The A/A_0 value acquired from DPBF *v.* illumination time could indirectly reflect the $^1O_2$ yield of compounds **1–5**. As shown in Fig. 3, the absorbance of DPBF at 418 nm decreased gradually with the increase of illumination time in the presence of compounds **1–5**. From the slope of the line, the relative production rates of $^1O_2$ for compounds **1–5** could be obtained. The order of $^1O_2$ yield was **2** > **1** > **3** > **4** > **5**. The yield of $^1O_2$ by compound **2** was more than the other compounds owing to the hydroxy group on the methylene violet 3RAX, which could increase the electronic effect and intermolecular hydrogen bond. In addition, compound **1** generated $^1O_2$ much faster than compounds **3**, **4**, and **5**, as the acyl group could reduce the electron density of the compounds. Moreover, different yields of $^1O_2$ photogeneration indicated that modification of the amino group could affect the ability of the compounds to generate singlet oxygen.

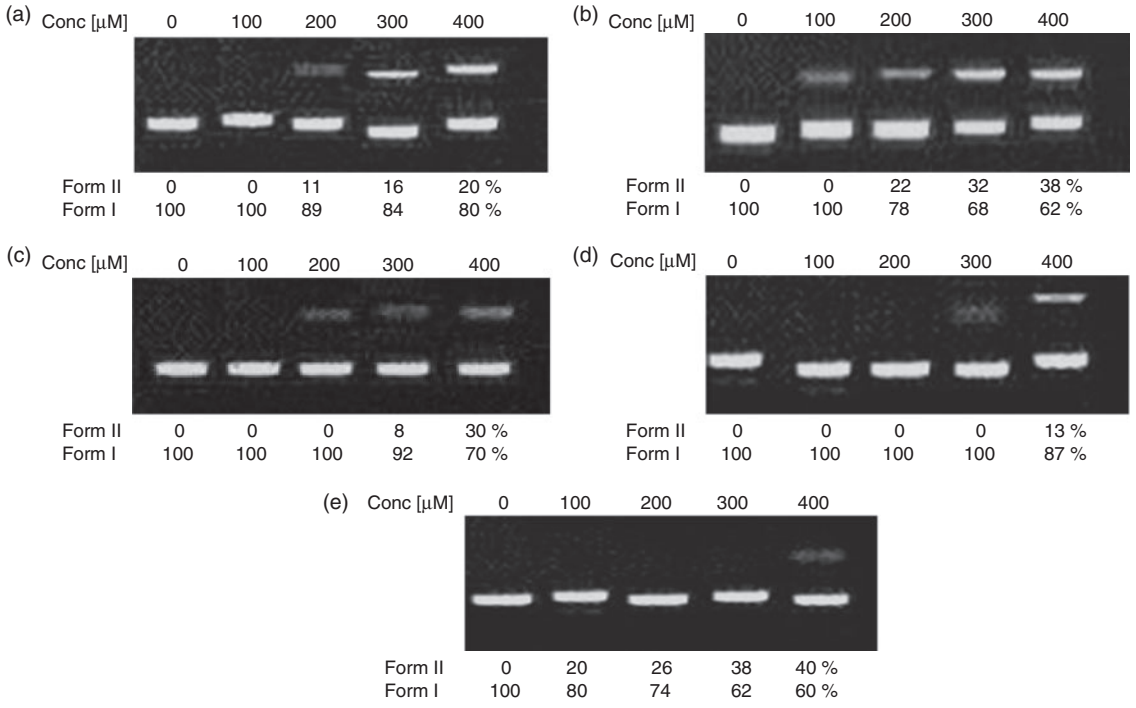

Fig. 4. Agarose gel electrophoresis images of DNA photocleavage assay of (a) **1**, (b) **2**, (c) **3**, (d) **4**, and (e) **5** at different concentrations.

DNA Photocleavage

The DNA photocleavage ability of compounds **1–5** were detected by monitoring the conversion of supercoiled form (form I) into the nicked circular form (form II).[27] As shown in Fig. 4, only compounds **1**, **2**, and **5** showed photocleavage activities towards DNA when their concentration was 200 μM. When the concentration of the compounds was elevated to 400 μM, the DNA photocleavage activities of compounds **1–5** were calculated to be 38, 40, 30, 20, and 13 %, respectively, with the order **2** > **1** > **3** > **4** > **5**, which was consistent with their ability to generate singlet oxygen. The DNA photocleavage activities of the five compounds were all less than 40 % due to their molecular structures and binding affinities with DNA.

Conclusions

We prepared methylene violet 3RAX **1** and four methylene violet 3RAX derivatives **2**–**5**. The five compounds were characterised by IR, UV-Vis, ^{1}H NMR, and MS analysis. The binding mode between the synthesised compounds and DNA and their DNA photocleavage ability were investigated. The results show that both **1** and **5** bind to DNA by intercalation, while compounds **2**–**4** interacted with DNA through a mixed binding mode involving groove binding and electrostatic interactions. The DNA photocleavage ability of compounds **1**–**5** were calculated to be 38, 40, 30, 20, and 13 %, respectively. The singlet oxygen production of compounds **1**–**5** measured by the DPBF method was consistent with the trend of DNA photocleavage ability, suggesting they have potential as PDT agents. These results suggest that the binding mode between methylene violet 3RAX and DNA, and its ability to generate singlet oxygen and DNA photocleavage activity could be adjusted through modification of the amino group on methylene violet 3RAX.

Supplementary Material

Fluorescence spectra of compounds **1**–**5** with the titration of CT DNA are available on the Journal's website.

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