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N-Aroyloxylthioxo-naphthalimides as DNA photocleavers of aroyloxyl oxygen radicals: synthesis, evaluation, and substituents' effect

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Abstract—Novel *N*-Aroyloxylthioxo-naphthalimides as highly efficient 'time-resolved' DNA photocleavers of aroyloxyl radicals type were designed and synthesized. The substituents at the aroyloxyl moiety have an important and unusual influence on the DNA photocleavage, and DNA photodamages of the compounds were unusually not depended on the electronic effects of substituents on the corresponding oxygen-centered radicals. With AM1 semi-empirical quantum calculation, it was found that their photocleaving activities were correlated with the densities of electron clouds on the N–O bonds in the triplet state. *N*-(*m*-Dichloro-benzoyloxy)-thioxo-naphthalimide could photodamage DNA effectively at less than the concentration of 2 μM.

1. Introduction

Oxygen-centered radicals are very useful in chemistry, biology, and medicine.[1–3] In recent years, the design and study of DNA 'artificial photonucleases' of the oxygen-centered radical type with no need of a metal or external oxidant, have attracted much attention and have led to the development of both specific gene-targeted drugs and artificial restriction enzymes.[4,5] Recently, highly efficient 'time-resolved' photonuclease with releasing hydroxyl radical and aroyloxyl radicals were developed.[6] Previously we ever reported the remarkable promoting action to photocleaving activity of thio-moiety on heterocycles, which was attributed to the enhancement of the electronic transition from n to π^* for thio-counterparts by compared with oxo-counterparts.[6] There as yet have been few reports about substituent effects of oxygen-centered radicals on their DNA photocleaving activity, although ones knew that the electron-donating group was favorable to the formation and stability of oxygen-centered radicals in a chemical system. Here we report important and unusual substituent effects of oxygen-centered radicals derived from novel photonucleases of *N*-aroyloxylthioxo-naphthalimides.

It was known that *N*-hydroxypyridinethiones (**A**), *N*-aroyloxypyridine-2-thiones (**B**) were DNA photocleavers through release of hydroxyl or aroyloxyl radicals (Scheme 1).[7–9] The alkylacyl oxygen radicals derived from *N*-alkylacyloxypyridine-2-thiones almost had not photocleaving activity by comparison with highly efficient aroyl counterparts,[10–12] it was probably caused by that the oxygen-centered radical with aroyl moiety has better stability and was easy to form. In this paper, in order to study the role of substituents on oxygen-centered radicals in photonucleases, we synthesized novel *N*-aroyloxylthioxo-naphthalimides (**C**) through the introduction of monosubstituted, disubstituted benzoyl or heterocyclic aroyl groups to that oxygen atom of N–O bond for obtaining the corresponding oxygen-centered radicals and evaluated their DNA photocleaving activities (Scheme 1).

2. Results and discussion

2.1. Synthesis and spectra

We have tried three synthetic routes to the target *N*-aroyloxythionaphthalimides **C**. It was found that the

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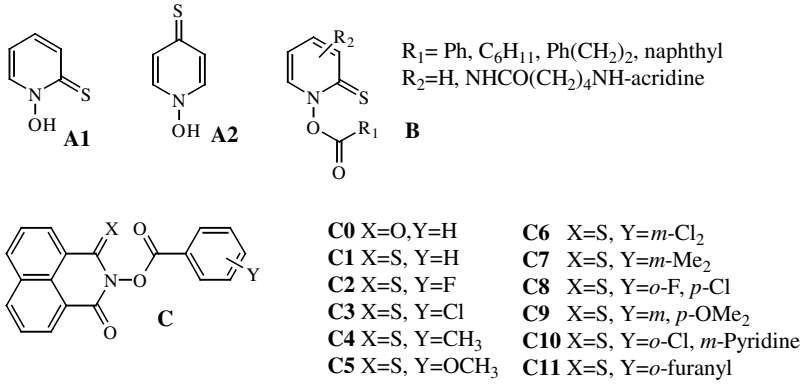


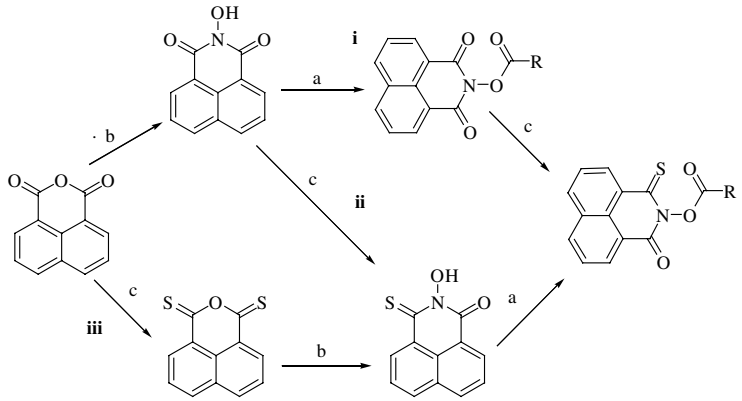
Scheme 1.

route i was unsuccessful, in which all of the three carbonyl groups in the intermediate have the possibility to react with the Lawesson's reagent (LR, 2,4-bis(*p*-methoxyphenyl)-1,3-dithia-2,4-diphosphetane-2,4-disulfide), the reaction mixture always gave a tar-like oil from which no component could be isolated by silica gel or basic alumina preparative TLC. The route ii was also unattractive, as the hydroxyl group in the intermediate could react with LR reagent and resulted in the decomposition of LR, the total yield was very low. Only the route iii with one-pot procedure gave high yield, in which the target compounds were synthesized with 1,8-naphthalic anhydride as the starting material by the dithionation with LR reagent, the imidation with hydroxylamine hydrochloride at refluxing in acetonitrile and the condensation with substituted benzoyl chlorides. The structures of the target compounds were identified by using ^{1}H NMR, EI-MS, IR, and elemental analysis (Scheme 2).

All thioxo-compounds (**C1**–**C11**) had long-wavelength absorption and very weak fluorescence (Table 1). This implies that for the latter their excitation energy might be more easily transferred from the singlet excited state to cleave the N–O bond,[13] besides being dissipated as heat in the system by internal conversion processes. Meanwhile, the intercalation experiments of these compounds to the Calf thymus DNA with **C1** and **C6** as examples, were carried out by using an electronic absorption spectra technique instead of the fluorescence quenching method,[14] as **C1**–**C11** only have very weak fluorescence. During the addition of Calf thymus DNA the absorption intensities of **C1**, **C6** became weaker with the increase of the concentration of DNA (Fig. 1).

It was widely accepted that the DNA photocleaving activities of *N*-hydroxy-(**A**) and *N*-aroyloxypyridine-2-thione (**B**) are attributable to the formation of persistent hydroxyl or aroyloxyl radicals during the photolysis, and that they are able to give ESR signals through the spin trapping method, whilst the concomitant part, a thiyl radical signal was not detected under similar conditions.[7,13,15] In addition, the similar sulfur-centerd thiopyridyl radical is known not to contribute to the DNA damage.[10,12,13] In our case, PBN (*N*-*tert*-butyl-α-phenylnitrone) trapping ESR spectroscopy identified that **C1**–**C11** in benzene could generate a free aroyloxyl radicals signal under irradiation (Fig. 2), which should be responsible for the DNA damage.

The photocleaving ability for DNA double strand-scission was evaluated for all the novel *N*-aroyloxylthioxo-



Scheme 2. Reagents and conditions: (a) RCOCl, NEt_3, rt, 45 min; (b) $NH_2OH{\cdot}HCl$, NEt_3, CH_3CH, reflux, 2 h; (c) Lawesson's reagent, C_6H_5–Cl-*p*, reflux, 1 h.

Table 1. Photocleaving activities, spectra data,[a,b] and calculated parameters[c,d]

Compound	UV λ_{max}/nm (log ϵ)	FL λ_{max}/nm (ϕ)	Photocleaving activity (100 μM, hν1 h)		Electronic density of oxygen radical[c]	Polar surface area (Å^2)[d]
C1	392 (4.29)	491 (<0.001)	50.4	49.6	−0.069	66.958
C2	400 (4.37)	454 (<0.001)	100	0	−0.067	66.930
C3	390 (4.34)	466 (<0.001)	86.2	13.8	−0.066	67.005
C4	390 (4.23)	466 (<0.001)	63.4	36.7	−0.071	65.515
C5	390 (4.21)	454 (<0.001)	49.5	51.5	−0.072	76.744
C6	397.5 (4.19)	435.6 (0.0014)	29.7	70.3	−0.060	65.628
C7	397.0 (4.17)	435.6 (0.0016)	39.1	60.9	−0.070	65.211
C8	398.5 (4.21)	435.4 (0.0014)	39.5	60.5	−0.066	66.526
C9	398.5 (4.18)	436.0 (0.0016)	60.2	39.8	−0.068	84.946
C10	398.0 (4.15)	466.0 (<0.001)	41.6	58.4	−0.068	79.875
C11	397.5 (4.16)	468.3 (<0.001)	42.2	57.8	−0.058	78.771

[a] In absolute ethanol.
[b] With quinine sulfate in sulfuric acid as quantum yield standard.
[c] By semi-empirical SCF AM1 method (hyperchem 5.5).
[d] The polar surface area of the aroyl acid corresponding to a radical calculated by molecular modeling MMX-E method (Pcmodel 6.0).

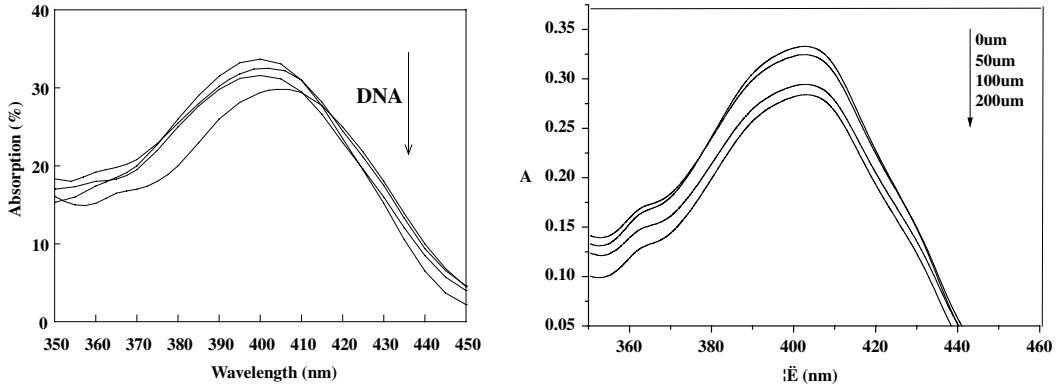


Figure 1. Interaction of **C1**, **C6**, and DNA. Absorption changes from 300 to 500 nm of **C1**, **C6** (20 μM) during the addition of Calf thymus DNA (0, 50, 100, 200 μM) in THF (10 mM), Tris–HCl (pH 7.4) solution (3:10, v/v).

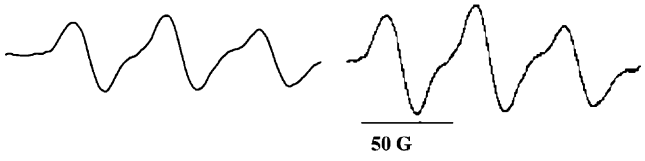


Figure 2. ESR spectroscopy of the radical released by **C1** and **C6**. ESR with PBN (*N*-*tert*-butyl-α-phenylnitrone) trapping in benzene under light. Microwave frequency 9.782 GHz, mid range 3430 G, scan range.

naphthalimides (Fig. 3). Obviously, the DNA damages were caused by the aroyloxyl radicals with different substituents,[16] which were formed from the corresponding *N*-aroyloxylthioxo-naphthalimides under photo-irradiation. It was found that the DNA photocleaving abilities of these compounds were in the order: **C6 > C7 ≈ C8 > C10 ≈ C11 > C5 > C1 > C9 > C4 > C3 > C2**. The photodamage abilities of these compounds should be correlated with the formation, stabilities, and reactivities of the corresponding oxygen-centered

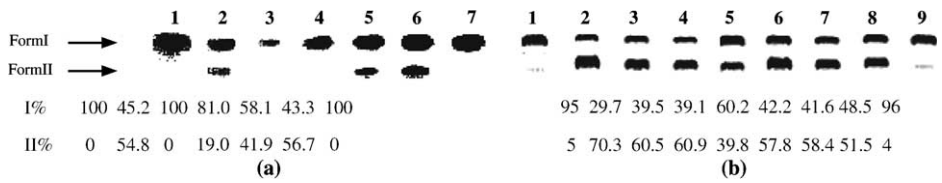


Figure 3. *Photocleavage on pBR 322 DNA.* The cleavage activities were evaluated using supercoiled circular pBR322 (form I) DNA (50 μM/bp) with the compound in the buffer Tris–HCl (pH 7.6) under photoirradiation (2300 μW/cm^2) with a transluminator (366 nm) at a distance of 20 cm at 0 °C and then analyzed on a 1% agarose gel. DNA photocleavage efficiency was defined as the degree of relaxation of the supercoiled DNA, relaxed circular DNA (single-stranded cleavage) as form II. (a) *Effects of different substituents on photocleavage* (100 μM; hν, 45 min). Lane 1: DNA alone (no hν); Lane 2: DNA and **C1**; Lane 3: DNA and **C2**; Lane 4: DNA and **C3**; Lane 5: DNA and **C4**; Lane 6: DNA and **C5**; Lane 7: DNA alone (hν). (b) *Effects of different substituents on photocleavage* (100 μM; hν, 60 min). Lane 1: DNA alone (hν); Lane 2–8: DNA and **C6**–**C11**, **C5**, respectively; Lane 7: DNA alone (no hν).

radicals. It was known that in radical chemistry, electron-donating substituents could improve the stabilities of oxygen-, and heteroatom-centered radicals, while electron-withdrawing substituents do not.[17] However, in our case the electron-donating effect was not so important here, for instance, the compound with the *m*-dichlorobenzoyl group (**C6**) showed the highest activity, while that with strong electron-donating *m*, *p*-dimethoxybenzoyl group (**C9**) was the worst, this observation implied that these radicals' stabilities were not only or important factor to influence on their photobioactivities.

From the Table 1 it could be found that the order of photocleaving activities of these radicals derived from **C**, is also not consistent with that of the electronic densities of aroyloxyl radicals at excited singlet state, which were calculated by semi-empirical SCF AM1 method (Hyperchem 5.5). In addition, no relationships were obtained between the photocleaving activities and the calculated steric parameters or others (Table 1). But, it seems that there is some correlation between the electron densities on the N–O bonds of the compounds at excited triplet state (not excited singlet state) and their photocleaving abilities (Fig. 4). For example, the density on the N–O bond of **C0** (oxo-compound) at excited triplet state is very high, it implies that the breakage of the N–O bond at excited triplet state is very difficult, so its photocleaving activity was very low (Form II 3.5%, 100 μM).[6] While for **C1**, with the same benzoyloxyl radical part, its density on the N–O bond at excited triplet state is low, so it's easy to break and its photocleaving activity is high (Form II 54.8%, 100 μM). A very similar conclusion could be obtained with other compounds, for example **C6**, its very low density is corresponding to very high photocleaving activity (Form II 70.3%, 100 μM). These suggested that the formation of these radicals was important factor to influence on their photobioactivities, as the radicals usually were very reactive.

Figure 5a indicates that the aroyloxyl radical was generated from **C6** at a relatively linear rate, this continuous generation of reactive oxygen-centered radicals provides an alternative to Fenton-based chemistry, where oxygen radicals are formed in a rapid burst. This kind of reagent is attractive for 'time-resolved' DNA cleavage studies.[18] Figure 5b also demonstrate that **C6** could damage DNA effectively at a concentration of 2 μM. It probably was the most potent compound in *N*-aroyloxypyridine-2-thione derivatives.[9] Figure 5c shows that pH had little influence on the photocleaving activity of **C6**, which means that these aroyloxyl radicals are quite stable in a wide range of pH values. In addition, the experiments showed that the solvents did not have influence on its photocleaving activity.

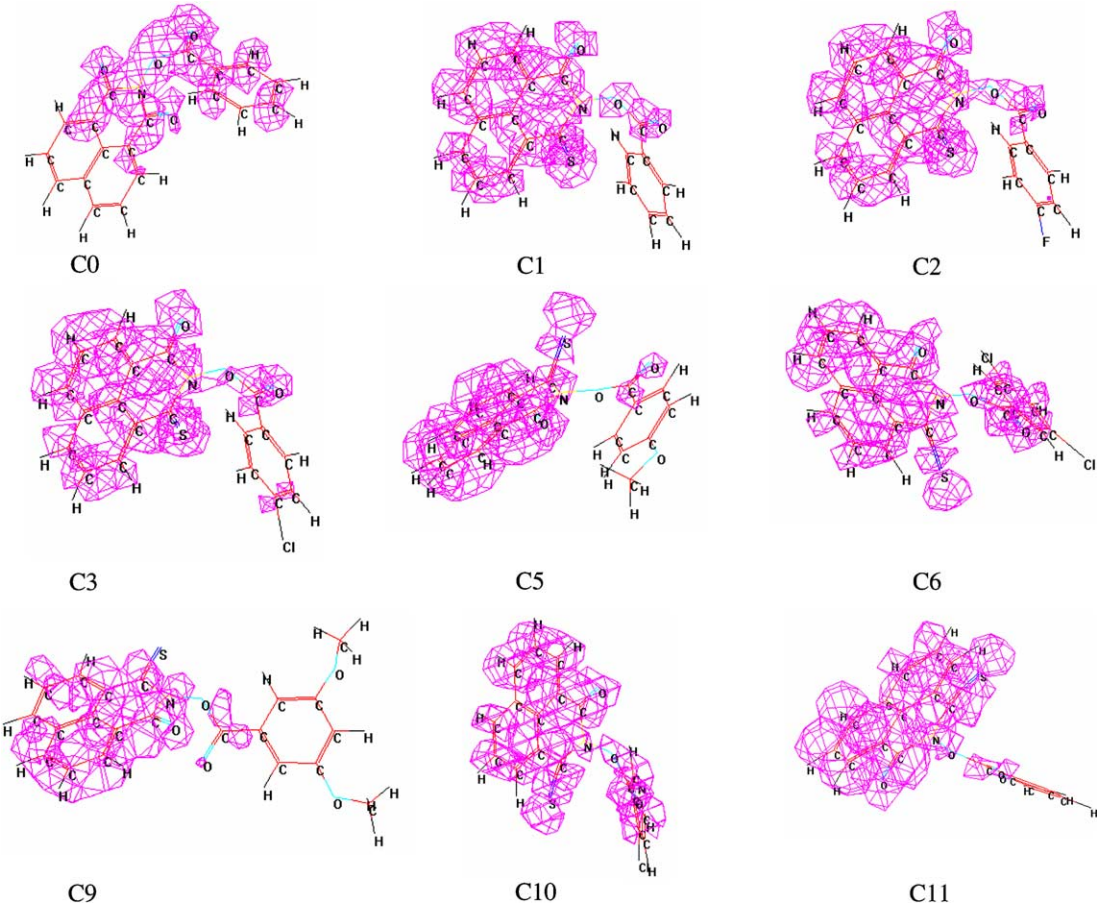


Figure 4. The distribution of electron clouds for the compounds.

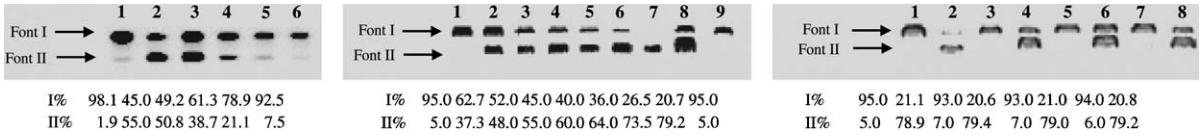


Figure 5. (a) *Time-dependant photocleavage of DNA using C6* (100 μM). Lane 1: DNA alone (no h*v*); Lane 2: **C6** and DNA (h*v*, 90 min); Lane 3: **C6** and DNA (h*v*, 75 min); Lane 4: **C6** and DNA (h*v*, 60 min); Lane 5:**C6** and DNA (h*v*, 30 min); Lane 6: DNA alone (h*v*, 1.5 h). (b) *Concentration-dependant photocleavage of DNA using C6* (h*v*, 1 h). Lane 1: DNA alone (no h*v*); Lane 2–8: DNA and **C6** at concentrations of 2, 5, 10, 20, 50, 100, and 200 μM, respectively. Lane 9: DNA alone (h*v*). (c) *pH effect on DNA photocleavage of C6* (100 μM; h*v*, 1 h). Lane 1, 3, 5, 7: DNA alone, pH = 8.5, 8.0, 7.5, 7.0, respectively; Lane 2, 4, 6, 8: DNA and **C6**, pH = 8.5, 8.0, 7.5, 7.0, respectively.

3. Conclusion

Novel *N*-aroyloxylthioxo-naphthalimides as highly efficient 'time-resolved' DNA photocleavers of aroyloxyl radicals type were designed and synthesized. It was found that substituents at the oxygen-centered radicals had important and unusual influence on the DNA photocleaving activity, the photocleaving activity of radical is correlated with the density of electron clouds on the N–O bond in the triplet state. It suggested that the formation of these radicals was important factor to influence on their photobioactivities. *N*-(*m*-Dichlorobenzoyloxy)-thioxo-naphthalimide could photodamage DNA effectively at less than the concentration of 2 μM, our study probably provides a novel 'time-resolved' and the most efficient DNA photocleaver in *N*-aroyloxypyridine-2-thione derivatives.

4. Experimental

4.1. 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.

5. Synthesis

5.1. Preparation of substituted benzoyl chloride

To a solution of substituted-benzoic acid was added thionyl chloride. The mixture was refluxed for 3 h until no hydrogen chloride gas produced and concentrated under reduced pressure to obtain the substituted benzoyl chloride.

5.2. Benzo[*de*]isochromene-1,3-dithione[19]

To a solution of 1,8-naphthlimide (5 g, 25 mmol) in chlorobenzene (90 mL), was added the Lawesson's Reagent under Ar protection, refluxed 24 h, then cooled, filtered, and dried to afford the crude product 4 g (17 mmol, 69%). The recrystallization in ethanol and chlorobenzene to give brown solid, mp: 211–212 °C (lit. 211–212 °C.). EI-MS: *m*/*z* 230 (M^+, 100).

5.3. 2-Hydroxy-benzo[*de*]isoquinoline-1,3-dithione

To a solution of hydroxylamine hydrochloride (0.24 g) in acetonitrile (25 mL), was added 1,8-thionaphthlimide (0.8 g), refluxed 2 h, cooled, filtered, the orange-red crude product was recrystallized in ethanol. EI-MS (*m*/*z*,%): 229 (M^+, 7.3), 213 ([M+H^+−$OH^·$], 56.5), 197 ([M−S]$^+$, 100).

5.4. Benzoic acid 1-oxo-3-thioxo-1H,3H-benzo[*de*]isoquinolin-2-yl ester (C1)

To the solution of 2-hydroxy-benzo[*de*] isoquinoline-1,3-dithione, ice/salt bath cooled, was added drops of triethylamine and benzoyl chloride (0.49 g), the mixture reacted 1 h, filtered, concentrated, and silca gel purification ($R_f = 0.54$, petroleum ether: dichloromethene = 1:4, v/v) to give orange-red product **C1**. Mp: 189–190 °C EI-MS (*m*/*e*, %): 333 (M^+, 1.9), 213 ([M+H^+−PhCOO·], 2.8), 105 ($PhCO^+$, 100); IR (KBr): 3030, 1780, 1715, 1610, 1580, 1270, 780, 740 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 9.01 (dd, $J_1 = 7.6$ Hz, $J_2 = 0.9$ Hz, 1H, 7-H), 8.67 (dd, $J_1 = 7.2$ Hz, $J_2 = 1.1$ Hz, 1H, 2-H), 8.27 (m, 4H, 3–6H), 7.72 (m, 3H, 2′-, 4′-, 6′-H), 7.57 (m, 2H, 3′-, 5′-H); $C_{19}H_{11}NO_3S$ required: C, 68.46; H 3.32; N, 4.20. Found: C, 68.51, H, 3.49, N, 4.22.

5.5. 4-Fluoro-benzoic acid 1-oxo-3-thioxo-1H,3H-benzo[*de*]isoquinolin-2-yl ester (C2)

Preparation and purification of this compound (and the other compounds as follows) was accomplished following the procedure described for **C1**,4-fluorobenzoyl chloride (0.55 g) was used here. Orange product ($R_f = 0.56$). Mp: 186–187 °C, EI-MS (*m*/*z*, %): 351 (M^+, 3.6), 213 (4.6), 123 (*p*-$FPhCO^+$, 100); IR (KBr): 3040, 1780, 1710, 1600, 1580, 1500, 1270, 840 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 8.92 (d, $J = 7.5$ Hz, 1H, 2-H), 8.64 (m, 3H, 7-, 4-, 5-H), 8.32 (m, 2H, 3-, 6-H), 7.97 (m, 2H, -H, 3′-, 5′-H), 7.56 (m, 2H, 2′,6′-H); $C_{19}H_{10}FNO_3S$ required: C, 64.95; H, 2.87; N, 3.99. Found: C, 64.59; H, 2.87; N, 4.02.

5.6. 4-Chloro-benzoic acid 1-oxo-3-thioxo-1H,3H-benzo[*de*]isoquinolin-2-yl ester (C3)

The similar preparation and purification procedure as the above, 4-chlorobenzoyl chloride (0.61 g) was used here. Orange-red product ($R_f = 0.62$). Mp: 182–183 °C. EI-MS (*m*/*e*, %): 367 (M^+, 1.7), 213 (2.5), 139 (*p*-$ClPhCO^+$, 100); IR (KBr): 3035, 1780, 1710, 1650, 1580, 1270, 840 cm^{-1}. ^1H NMR (DMSO-d_6): δ 8.91 (d, $J = 7.5$ Hz, 1H, 2-H), 8.64 (m, 3H, 7-, 4-, 5-H), 8.23 (m, 2H, 3-, 6-H), 7.96 (m, 2H, 3′-, 5′-H), 7.79 (m, 2H, 2′,6′-H); $C_{19}H_{10}ClNO_3S$ required: C, 62.04; H, 2.74; N, 3.81. Found: C, 62.38; H, 2.90; N, 3.55.

5.7. 4-Methyl-benzoic acid 1-oxo-3-thioxo-1H,3H-benzo[*de*]isoquinolin-2-yl ester (C4)

4-Methylbenzoyl chloride (0.54 g) was used here. Orange product ($R_f = 0.55$). Mp: 204–205 °C. EI-MS (*m*/*z*, %): 347 (M^+, 2.3), 213 (2.8), 119 (*p*-$MePhCO^+$, 100); IR (KBr): 3040, 1770, 1715, 1610, 1585, 1510, 1270, 840 cm^{-1}; ^1H NMR ($CDCl_3$): δ 9.01 (dd, $J_1 = 7.6$ Hz, $J_2 = 0.9$ Hz, 1H, 2-H), 8.70 (dd, $J_1 = 7.3$ Hz, $J_2 = 1.1$ Hz, 1H, 7-H), 8.22 (m, 2H, 4-, 5-H), 8.18 (m, 2H, 3-, 6-H), 7.80 (m, 2H, 2′-, 6′-H), 7.40 (m, 2H, 3′-5-H), 2.50 (s, 3H, $-CH_3$); $C_{20}H_{13}NO_3S$ required: C, 69.15; H, 3.77; N, 4.03. Found: C, 68.95; H, 3.86; N, 3.99.

5.8. 4-Methoxy-benzoic acid 1-oxo-3-thioxo-1H,3H-benzo[*de*]isoquinolin-2-yl ester (C5)

4-Methoxylbenzoyl chloride (0.60 g) was used here. Orange product ($R_f = 0.36$). Mp: 200–201 °C. EI-MS (*m*/*z*, %): 363 (M^+, 1.6), 213 (1.5), 135 (*p*-$MeOPhCO^+$, 100); IR (KBr): 3040, 1780. 1710, 1600, 1580, 1510, 1270, 845 cm^{-1}; ^1H NMR (DMSO-d_6): 8.92 (dd, $J_1 = 7.6$ Hz, $J_2 = 1.0$ Hz, 1H, 2-H), 8.63 (m, 3H, 7-, 4-, 5-H), 8.16 (m, 2H, 3-, 6-H), 7.96 (m, 2H, 2′-, 6′-H), 7.22 (m, 2H, 3′-, 5′)-H), 3.92 (s, 3H, $-OCH_3$); $C_{20}H_{13}NO_4S$ required: C, 66.10; H, 3.61; N, 3.85. Found: C, 66.57; H, 3.78; N, 3.65.

5.9. 3,5-Dichloro-benzoic acid 1-oxo-3-thioxo-1H,3H-benzo[*de*]isoquinolin-2-yl ester (C6)

3,5-Dichlorobenzoyl chloride (0.73 g) was used here. Orange-red product ($R_f = 0.54$). Mp: 212–214 °C; ^1H NMR δ (DMSO-d_6): 8.93 (dd, $J_{32} = 7.7$ Hz, 1H, 2-H), 8.62 (m, 3H, 7-, 4-, 5-H), 8.22 (m, $J_{2'4'} = J_{6'4'} = 1.9$ Hz, 4′-H), 8.19 (d, $J_{4'2'} = J_{4'6'} = 1.9$ Hz, 2H, 2′-, 6′-H), 7.97 (m, 2H, 3-, 6-H); EI-MS (*m*/*e*, %): 401 (M^+, 16.78), 213 ([M+H^+−3,5-2ClC_6H_3COO·], 56.69), 173 (3,5-2$ClC_6H_3CO^+$, 100); IR (KBr): 3040, 1780, 1715, 1610, 1580, 1270, 770, 740 cm^{-1}; $C_{19}H_9Cl_2NO_3S$ required: C, 56.73; H, 2.26; N, 3.48. Found: C, 56.53; H, 2.30; N, 3.67.

5.10. 2-Fluoro-4-chloro-benzoic acid 1-oxo-3-thioxo-1H,3H-benzo[*de*]isoquinolin-2-yl ester (C7)

2-Fluoro-4-chlorobenzoyl chloride (0.67 g) was used here. Orange product. Mp: 208–210 °C; ^1H HNMR δ (DMSO-d_6): 8.92 (d, $J_{32} = 7.2$ Hz, 1H, 2-H), 8.64 (m, 3H, 4-, 5-, 7-H), 8.24 (m, $J_{F-H(m)} = 8.0$ Hz, $J_{5'6'} = 8.3$ Hz, 1H, 3′-H), 7.96 (m, 2H, 3-, 6-H), 7.88 (dd, $J_{F-H(o)} = 10.8$ Hz, $J_{5'3'} = 1.8$ Hz, 1H, 3′-H), 7.65 (dd, $J_{F-H(p)} = 1.8$ Hz, $J_{6'5'} = 8.3$ Hz, 1H, 5′-H); EI-MS (*m*/*z*, %): 385 (M^+, 16.93), 213 ([M+H^+−2-F-4-ClC_6H_3COO·], 80.43), 157 (2-F-4-$ClC_6H_3CO^+$, 100); IR (KBr): 3040, 1780, 1710, 1610, 1580, 1500, 1280, 840 cm^{-1}; $C_{19}H_9ClFNO_3S$ required: C, 59.15; H, 2.35; N, 3.63. Found: C, 59.34; H, 2.13; N, 3.52.

5.11. 3,5-Dimethyl-benzoic acid 1-oxo-3-thioxo-1H,3H-benzo[*de*]isoquinolin-2-yl ester (C8)

3,5-Dimethylbenzoyl chloride (0.59 g) was used here. Brown product. Mp: 216–218 °C; ^1H NMR δ (DMSO-d_6): 8.92 (d, $J = 7.7$ Hz, 1H, 2-H), 8.63 (m, 3H, 4-, 5-, 7-H), 7.95 (m, 2H, 3-, 6-H), 7.81 (s, 2H, 2′-, 6′-H), 7.50 (s, 1H, 4′-H), 2.42 (s, 6H, $2CH_3$); ESI-MS (*m*/*z*, %): 384 (M^++Na), 745 ($2M^+$+Na), 1106 ($3M^+$+Na); IR (KBr): 3040, 1780, 1710, 1610, 1580, 1510, 1280, 840 cm^{-1}; $C_{21}H_{15}NO_3S$ required: C, 69.79; H, 4.18; N, 3.88. Found: C, 69.83; H, 4.30; N, 3.70.

5.12. 3,4-Dimethoxy-benzoic acid 1-oxo-3-thioxo-1H,3H-benzo[*de*]isoquinolin-2-yl ester (C9)

3,5-Dimethoxylbenzoyl chloride (0.70 g) was used here. Brown product. Mp: 221–223 °C, ^1H NMR δ (DMSO-d_6): 8.91(d, $J_{32} = 7.5$ Hz, 1H, 2-H), 8.61 (m, 3H, 4-, 5-, 7-H), 7.91 (m, 3H, 3-, 6-, 6′-H), 7.59 (d, $J_{6'2'} = 1.7$ Hz, 1H, 2′-H), 7.25 (dd, $J_{6'5'} = 8.6$ Hz, 1H, 5′-H), 3.93 (s, 3H, 3′-OCH_3), 3.87 (s, 3H, 4′-OCH_3); EI-MS (*m*/*z*, %): 393 (M^+, 79.55), 213 ([M+H^+−3,4-2$OCH_3C_6H_3COO$·], 94.21), 165 (3,4-2$OCH_3C_6H_3CO^+$, 100); IR (KBr): 3040, 1770, 1710, 1600, 1580, 1515, 1280, 840 cm^{-1}; $C_{21}H_{15}NO_5S$ required: C, 64.12; H, 3.84; N, 3.56. Found: C, 64.87; H, 4.03; N, 3.48.

5.13. 2-Chloro-nicotinic acid 1-oxo-3-thioxo-1H,3H-benzo[*de*]isoquinolin-2-yl ester (C10)

2-Chloronicotinic chloride (0.61 g) was used here. Brown product. Mp: 210–212 °C; ^1H NMR δ (DMSO-d_6): 8.94 (d, $J_{32} = 7.5$ Hz, 1H, 2-H), 8.68 (m, 3H, 4-, 5-, 7-H), 7.97 (m, 2H, 3-, 6-H), 8.83 (dd, $J_{3'4'} = 4.8$ Hz, $J_{2'4'} = 1.3$ Hz, 1H, 4′-H), 8.72 (dd, $J_{3'2'} = 7.7$ Hz, $J_{4'2'} = 1.3$ Hz, 1H, 2′-H), 7.80 (q, $J_{2'3'} = 7.7$ Hz, $J_{4'3'} = 4.8$ Hz, 1H, 3′-H); EI-MS (*m*/*z*, %): 368 (M^+, 11.52), 213 ([M+H^+−2-ClC_5H_3COO·], 28.33), 140 (2-$ClC_5H_3CO^+$, 100); IR (KBr): 3040, 1780, 1710, 1600, 1580, 1500, 1270, 840 cm^{-1}; HRMS: calcd for $C_{18}H_9ClN_2O_3S$: 368.0012. Found: 368.0022.

5.14. Furan-2-carboxylic acid 1-oxo-3-thioxo-1H,3H-benzo[*de*]isoquinolin-2-yl ester (C11)

Furan-2-carboxylic chloride (0.46 g) was used here. Red product. Mp: 209–211 °C, ^1H NMR δ (DMSO-d_6): 8.90

(d, $J_{32} = 7.7$ Hz, 1H, 2-H), 8.62 (m, 3H, 4-, 5-, 7-H), 7.94 (m, 2H, 3-, 6-H), 8.29 (d, $J_{4'2'} = 0.9$ Hz, 1H, 4′-H), 7.85 (d, $J_{3'2'} = 3.6$ Hz, 1H, 2′-H), 6.94 (q, $J_{2'3'} = 3.6$ Hz, $J_{4'3'} = 1.7$ Hz, 1H, 3′-H); EI-MS (m/z, %): 323 (M^+, 14.53), 213 ([M+H$^+$−C_4H_3OCOO·], 8.32), 95 ($C_4H_3OCO^+$, 100); IR (KBr): 3040, 1780, 1710, 1600, 1580, 1500, 1270, 840; HRMS: calcd for $C_{17}H_9NO_4S$: 323.0252. Found: 323.0260.

5.15. 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 sulfate in sulfuric acid as quantum yield for fluorescence spectra.

5.16. Intercalation studies of compounds C 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 wavelength and intensity area of samples were measured at following conditions: excitation: 365, emission: 380–520 nm.

5.17. Photocleavage of supercoiled DNA using compounds C

300 ng pBR322 DNA (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 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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