A NEW CLASS OF DNA INTERCALATOR AND PHOTOCLEAVER: BIS-NAPHTHALIMIDES WITH BROMO AND NITRO SUBSTITUENTS

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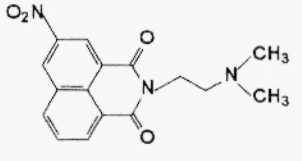
Abstract: Three bis-naphthalimides with bromo and nitro substitutents and different long linker arms have been synthesized and their excellent DNA intercalating and cleaving activities are reported. The difference of their intercalation activities is not quite big, but the cleavage activity of the bis-naphthalimide with 3-nitro and 4-bromo substituted groups and a longer aminoalkyl linker is obviously higher than that of the other two bis-naphthalimides: one with a 3-nitro and a 4-bromo substituted groups and a shorter alkyl linker, the other only with two 4-bromo substituted groups and a longer aminoalkyl linker.

Introduction

In recent years, the design and chemical synthesis of site-specific and nonselective DNA-cleaving agents that can be activated by UV or visible light have become a research topic. These synthetic chromophores have been used as structural probes in biological macromolecules, such as DNA and protein, as well as therapeutic agents.



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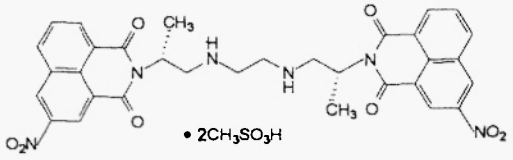

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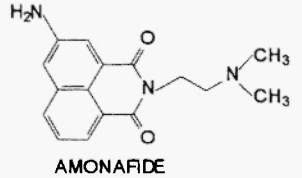

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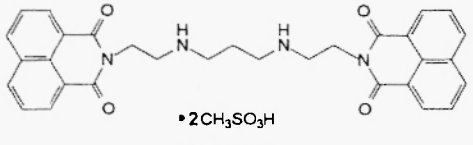
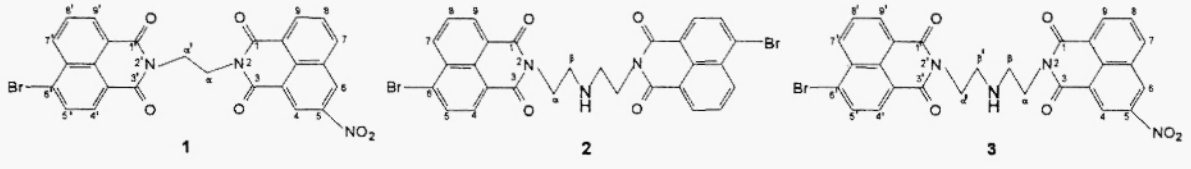


LU 79553

Of the chromophores, the studies on bio-activities, especially anti-cancer activity, of 1,8-naphthlimide derivatives have greatly attracted researchers' interest.[1-9] So far in the mononaphthalimide series, two of the most active compounds,

mitonafide and amonafide, have entered into clinical trials. Since bis-intercalating agents had demonstrated a higher binding affinity for DNA,[10] a new series of bis-intercalating naphthalimides (bis-naphthalimides) was designed and synthesized, of which (R,R)-2,2'-[1,2-ethanediylbis[imino-(1-methyl-2,1-ethanediyl)]]-bis[5-nitro-1H-benz[de]isoquinolone-1,3(2H)-dione]dimethanesulfonate (DMP-840) and 2,2'-[1,3-propanediylbis(imino-2,1-ethanediyl)]-bis[1H-benz[de] isoquinoline-1,3(2H)-dione]dimethanesulfonate (LU-79553) have also entered into clinical investigations.[7,11,12 13]

The novel bis-naphthalimides have bis-intercalating planar rigid 1,8-naphthalimide parent rings with more modified positions and different linker arms adjusted based on actual needs, so they are more potential intercalating agents[14] and have high antitumor activity in preclinical model systems.[15-17] It has been proved that linker arms containing at least one amino group and 1,8-naphthalimide moieties with at least one 3-nitro group are beneficial to enhance cytotoxic activity in the naphthalimide series.[2,5] They can recognize specific DNA sequences and conduct efficient photochemical strand cleavage at those sites.[18,19] Although the cytotoxic and antitumor activities for this family of bis-naphthalimides were extensively studied, there are few reports on DNA intercalating and cleaving activities for the bis-naphthalimides by a fluorescence approach and a photonicking method, respectively. Based on our previous studies,[20] we have designed and synthesized three bis-naphthalimides **1**, **2** and **3** (Scheme 1)[21] with different linker arms and substituents in naphthalene rings, in order to examine the influence of different substituents and the length and types of linker arms with bis-naphthalimides on their DNA intercalating and cleaving activities.



Scheme 1. The structures of the synthesized and examined compounds

Results and Discussion

As shown in Fig. 1, the intercalation of the three compounds **1**, **2** and **3** with calf thymus DNA in the dark was studied by a fluorescence quenching technique.[22] By assuming that the difference in intrinsic fluorescence intensity of the compounds studied between the free and bound states (DNA-compound complex), fluorescence quenching, is proportional to the amount of DNA-bound compound,[23] the apparent association constant (K_a) values for **1**-DNA, **2**-DNA and **3**-DNA complexes were calculated to be 3.48×10^4 M^{-1}, 5.53×10^4 M^{-1} and 4.98×10^4 M^{-1}, respectively, by Scachard analysis.[24] The difference between the apparent binding constants for compounds studied with DNA is indicative of different binding affinities towards DNA. The interaction mechanism of the bis-naphthalimides with DNA might be that the two naphthalimide rings bis-intercalate into DNA and are parallel to the base pairs; while their linkers lie in the major groove of the DNA molecule, resulting in the enhancement of the apparent length of linear DNA. And the amount of different compounds required to reach the maximum in relaxation is different.[25,26] So a possible reason leading to dissimilar intercalating activities is that the aminoalkyl chains for **2** and **3** allow their intercalative moieties sufficiently effective interaction with DNA. Also it is possible that a strong electron-withdrawing nitro group in **1** and **3** weakens their interaction with DNA. The cause of the smallest K_a for **1** among three compounds studied could be owing to the shorter linker arm enhancing steric hindrance of the intercalation of two bigger naphthalimide rings into DNA.

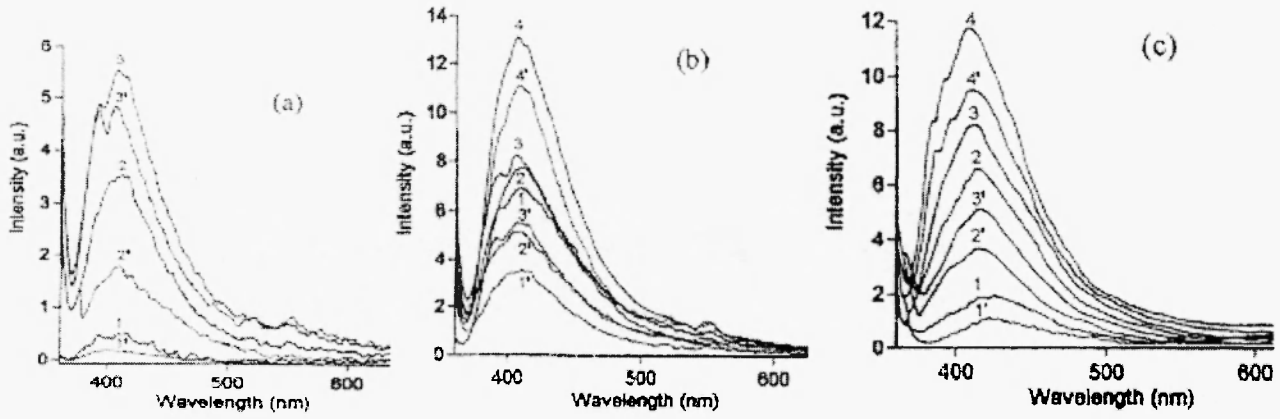


Figure 1. The fluorescence spectra before and after the interaction of compounds **1**, **2** and **3** with calf thymus DNA. Intrinsic fluorescence and dark binding of target compounds **1**, **2** and **3** to DNA were studied in a mixture of tris-HCl buffer : DMSO = 9:1 (v/v). The buffer used for all the measurements contains 10 mM Tris-HCl and 30 mM NaCl, adjusted to pH 7.4, without removal of soluble oxygen. All samples were swayed at 28 ℃ for three days in the dark in a incubator shaker and subsequently their UV and Fluorescence spectra using 348 nm as an excitation maximum were measured at r.t. (a) Curves 1-3: 0.1, 3, 6 μM of **1** respectively; curves 1'-3': DNA (5 μM/base pair) plus 0.1, 3, 6 μM of **1** respectively. (b) Curves 1-4: 1, 20, 40, 60 μM of **2** respectively; curves 1'-4': DNA (50 μM) plus 1, 20, 40, 60 μM of **2** respectively. (c) Curves 1-4: 1, 20, 40, 60 μM of **3** respectively; curves 1'-4': DNA (50 μM) plus 1, 20, 40, 60 μM of **3** respectively.

We next examined DNA photocleavage activities of **1**, **2** and **3**. Herein, DNA photocleavage efficiency was defined as the amount of photonicking form I DNA into form II DNA. As seen from Fig. 2 and 3, the effects of photonicking DNA for **1**, **2** and **3** were obviously facilitated with elongation of photoirradiation time and the increases of the concentrations of **1**, **2** and **3** except for 70 μM for **2** almost not increasing the effect of photonicking DNA relative to 50 μM. Significantly, a result was observed that although the difference between apparent binding constants for compounds studied is not big, the difference of their cleavage activities is obviously observed in the order **3** > **1** > **2**. The reason is that there exists a nitro group, which can enhance DNA cleaving activity, at 3-posotion in a naphthalene ring for **1** and **3**; and, what is more, for **3** the longer linker arm with a amino group is more appropriate to the need of its interaction with DNA.[3,5] In addition, it is worth noting that the compounds **1** and **3** at a concentration of 30 μM have noticeably photonicking interaction on supercoiled circular pBR322 DNA, Fig. 3, however, shows the lowest concentration of obviously photonicking DNA for **2** is 50 μM. Though most of researchers consider that the photocleavage mechanism of halogenated organic compounds is similar to that of nitro-substituted compounds, that is, nitro-substituted naphthalimides are capable of cleaving DNA photochemically due to the nitro group abstracting hydrogen atoms from deoxyribose, leading to spontaneous cleavage, or from thymine methyl groups, resulting in T-selective cleavage after piperidine treatment, and halogen atoms (X) and carbon-bond radicals are produced by carbon-halogen bond homolysis upon irradiation, and then X mediates DNA strand scission via initial abstraction of H· from deoxyribose,[27,28] Fig. 2 and Fig. 3 both strongly suggest a major effect of a 3-nitro group, not a 4-bromo group, in a naphthalene ring on the cleavage activity for the bis-intercalating naphthalimides examined, then that of the linker

with a amino group, in spite of the biggest K_a for **2** only with bromo groups in three compounds. This is in accordance with the result of cytotoxic and antitumor assays for the type of compounds with 3-nitro groups.[3,5,6]

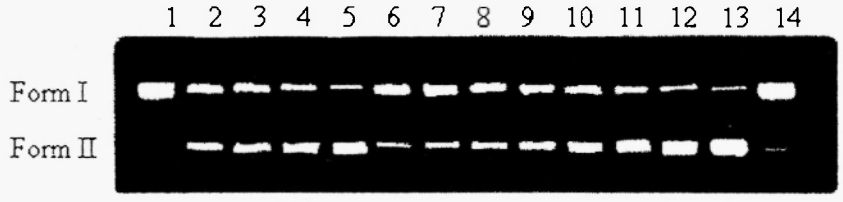


Figure 2. Effect of photoirradiation time on the photocleavage of supercoiled circular pBR322 DNA by **1**, **2** and **3**. The DNA-cleaving properties of **1**, **2** and **3** were examined in a mixture of tris-HCl buffer (pH = 8.0) : DMSO = 9:1 (v/v) using supercoiled circular pBR322 DNA (form I, 50 μM/base pair) under photoirradiation with a transilluminator (366 nm) at a distance of 20 cm at 0 ℃ in different photoirradiation times of substrates and then the reaction mixtures were analyzed on a 1% agarose gel (0.5 μg/ml ethidium bromide stain, gels were run at 120 V for 1.5h) with a DYY-III-8B electrophoresis instrument. Two DNA control samples were prepared without the bis-naphthalimides: one was irradiated and the other was covered and refrigerated at 4 ℃. In all cases, the final bis-naphthalimide concentrations are 50 μM in above mixture solvent and oxygen was removed from all samples and the controls before irradiation. Lane 1: DNA alone (no hv, 120 min); lanes 2–5: DNA plus **1** at different photoirradiation time: 30, 60, 90, and 120 min, respectively; lanes 6–9: DNA plus **2** at different photoirradiation time: 30, 60, 90, and 120 min, respectively; lanes 10–13: DNA plus **3** at different photoirradiation time: 30, 60, 90, and 120 min, respectively; lane 14: DNA alone (hv, 120 min).

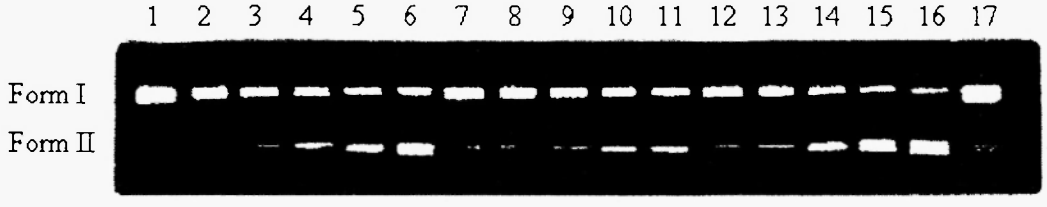


Figure 3. Effect of the concentration of **1**, **2** and **3** on the photocleavage of supercoiled circular pBR322 DNA under similar experimental conditions to Fig. **2**. Each reaction mixture of **1**, **2** and **3** with DNA (50 μM/base pair) was photoirradiated at 0 ℃ for 60 min. Lane 1: DNA alone (no hv); lanes 2–6: DNA plus 1, 10, 30, 50, and 70 μM of **1** respectively; lanes 7–11: DNA plus 1, 10, 30, 50, and 70 μM of **2** respectively: lanes 12–16: DNA plus 1, 10, 30, 50, and 70 μM of **3** respectively; lane 17: DNA alone (hv, 60 min). All samples were deoxygenated.

In short, the difference between their intercalation activities is not big for three bis-naphthalimides examined, but the cleavage activities of **1** and **3** with a 3-nitro substituent in a naphthalene ring are stronger than that of **2** and a marked enhancement of the cleavage activity for **3** with a longer aminoalkyl linker was also found. So the impact of the 3-nitro substitutent on the cleaving activity is bigger than that of the 4-bromo substitutent, furthermore the longer aminoalkyl linker is superior to the shorter alkyl linker in increasing their cleavage activities for the bis-naphthalimides with the same substitution pattern.

Acknowledgements

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References and notes

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21 2-[2-(6'-bromo-1,3-dioxo-1H-benz[de]isoquinoline-2(3H)-yl)ethyl]-5-nitro-1H-benz[de]isoquinoline-1,3(2H)-dione **1** (yield: 52.3% from its precursor: 5-nitro-2-(2-aminoethyl)-1H-benz[de]isoquinoline-1,3(2H)-dione). a light brown powder, mp 268-270 ℃. IR (KBr) ν_{max}/cm^{-1}: 3100, 2950, 1680, 1600, 1550, 1350, 1100, 880, 800; ^{1}H-NMR (500 MHz, DMSO-d_6): δ 9.39 (d, $J_{4,6}$ 4.40, 1H, 4-H), 8.73 (d, $J_{6,4}$ 4.40, 1H, 6H), 8.68 (d, $J_{8,9}$ 8.34, 1H, 9-H), 8.47 (d, $J_{8',9'}$ 8.49, 1H, 9'-H), 8.43 (d, $J_{4',5'}$ 6.43, 1H, 4'-H), 8.31 (d, $J_{7,8}$ 6.84, 1H, 7-H), 8.07 (m, 2H, 7'-H, 8-H), 7.94 (m, 1H, 8'-H), 7.84 (m, 1H , 5'-H), 4.11 (m, 4H, α-H, α'-H); EI-MS (m/z, %): 545([M+2]$^+$, 84.92), 544 ([M+H]$^+$, 71.39), 543 ([M]$^+$, 84.92), 302 ([M-$C_{12}H_5O_2NBr$]$^+$, 71.39), 301 ([M-$C_{12}H_6O_2NBr$]$^+$, 100), 290 ([M$^+$+2-$C_{11}H_5O_2NBr$]$^+$, 50.14), 288 ([M-$C_{11}H_5O_2NBr$]$^+$, 52.50), 268 ([M-$C_{12}H_6O_4N_2$]$^+$, 75.11). UV (CH_3CN), λ^{ab}_{max}/nm (log ε): 234 (4.30), 269 (sh, 4.09), 357 (4.05), 372 (4.01); λ^{ex}_{max}/ nm: 355; FL (CH_3CN), λ^{em}_{max}/nm (Φ): 471 (0.038). Anal. Calcd. for $C_{26}H_{14}BrN_3O_6$: C, 57.41; H, 2.59; N, 7.72. Found: C, 57.64; H, 2.80; N, 7.67.
2,2'-[inino-bis(2,1-ethanediyl)]-bis[6-bromo-1H-benz[de]isoquinoline-1,3(2H)-dione] **2** (yield: 81.0 % from the starting compound: 4-bromo-1,8-naphthalic anhydride). a light yellow solid, mp 244-246℃. IR (KBr) ν_{max}/cm^{-1}: 3318, 3057, 2955, 2818, 1700, 1656, 1589, 1344, 1222, 778; ^{1}H-NMR (500 MHz, DMSO-d_6): δ 8.52 (d, $J_{4,5}$ 8.27, 1H, 4-H), 8.36 (d, $J_{9,8}$ 8.02, 2H, 9-H), 8.12 (m, 4H, 5-H, 7-H), 7.92 (dd, $J_{8,9}$ 8.02, $J_{7,8}$ 8.23, 2H, 8-H), 4.15 (m, 4H, α-H), 2.99 (m, 4H, β-H). ESI-MS (positive, %): 620.0 ([M+H]$^+$, 100); EI-MS (m/z, %): 619 (M$^+$, 2.2), 333 ([M+2-$C_{13}H_7BrNO_2$]$^+$, 47.0), 331 ([M-$C_{13}H_7BrNO_2$]$^+$, 49.1), 304 ([M+2-$C_{14}H_{10}Br$-N_2O_2]$^+$, 89.5), 302 ([M-$C_{14}H_{10}BrN_2O_2$]$^+$, 89.5), 262 ([M+2-$C_{16}H_{14}BrN_3O_2$]$^+$, 34.5), 260 ([M-$C_{16}H_{14}Br$ N_3O_2]$^+$, 33.4). UV (CH_3CN), λ^{ab}_{max}/nm (log ε): 223 (5.41), 237 (5.35), 339 (4.76), 354 (sh, 5.46); λ^{ex}_{max}/ nm: 355; FL (CH_3CN), λ^{em}_{max}/nm (Φ): 401 (0.095). Anal. Calcd. for $C_{28}H_{19}Br_2N_3O_4$: C, 54.13; H, 3.08; N, 6.76. Found: C, 54.03; H, 3.01; N, 6.81.
2-[2-[2-(6'-bromo-1,3-dioxo-1H-benz[de]isoquinoline-2(3H)-yl)ethylamino]ethyl]-5-nitro-1H-benz[de]isoquinoline-1,3(2H)-dione **3** (yield: 63.4% from the starting compound: 3-nitro-1,8-naphthalic anhydride). a brown powder, mp 208-210 ℃. IR (KBr) ν_{max}/cm^{-1}: 3319, 3090, 2954, 2841, 1701, 1660, 1595, 1545, 1346, 1250, 1240, 790; ^{1}H-NMR (500 MHz, DMSO-d_6): δ 9.37 (d, $J_{4,6}$ 4.40, 1H, 4-H), 8.71~8.59 (m, 2H, 6-H, 9-H), 8.49 (d, $J_{4',5'}$ 8.46, 1H, 4'-H), 8.40 (m, 1H, 9'-H), 8.28 (d, $J_{7,8}$ 6.90, 1H, 7-H), 8.12 (m, 2H, 7'-H, 8-H), 7.88 (m, 1H, 8'-H), 7.85 (m, 1H, 5'-H), 4.16 (m, 4H, α-H, α'-H), 2.90 (m, 4H, β-H, β'-H); ESI-MS (positive, %): 588.3 ([M+H]$^+$, 100); UV (CH_3CN), λ^{ab}_{max}/nm (log ε): 225 (4.68), 236 (sh, 4.51), 340 (4.19), 357 (sh, 3.97); λ^{ex}_{max}/ nm: 355; FL (CH_3CN), λ^{em}_{max}/nm (Φ): 476 (0.027). Anal. Calcd. for $C_{28}H_{19}BrN_4O_6$: C, 57.26; H, 3.26; N, 9.54. Found: C, 57.81; H, 3.63; N, 9.31.
The fluorescence quantum yields (Φ) for **1**, **2** and **3** were measured using quinine sulfate in 0.1 N sulfuric acid ($(C_{20}H_{24}N_2O_2)_2{\cdot}H_2SO_4{\cdot}2H_2O$, 1 μg / 1 ml) as a standard (Φ = 0.55)

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