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The Abasic Site as a Target for Generation of Locally Multiply Damaged Sites

Alain Martelli, Nathalie Berthet, Jean-François Constant,* Martine Demeunynck* and Jean Lhomme

LEDSS, Chimie Bioorganique, UMR CNRS 5616, Université Joseph Fourier, BP 53, *38041 Grenoble cedex 9, France*

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Abstract—Abasic sites in DNA have been specifically targeted by synthetic compounds able to cleave DNA at abasic sites and to induce photodamages in the vicinity of the lesion. The synthesis and the photoactivity of the drugs on abasic sites containing DNA and oligonucleotides are reported.

Introduction

The cytotoxicity of many antitumor drugs is believed to result from massive production of DNA damages. Several classes of agents, mainly those involving radical processes (neocarzinostatin, bleomycin, ionizing radiations),[1,2] are known to produce clustered lesions or locally multiply damaged sites (LMDS) in tracks of a few base pairs. Such multiple damages are more toxic as they present a more challenging repair problem for the cell.[3,4]

A possible strategy to induce LMDS might be to target a DNA lesion in the cell with a drug able to generate another damage in close vicinity. Abasic sites (AP sites) which result from the loss of a base in DNA, either spontaneously[5] or enzymatically as intermediates in the repair of modified bases,[6,7] appear as good targets for this new strategy. Abasic sites are alkali-labile lesions subject to β-elimination of the 3′-phosphate strand occurring on the aldehydic form of the ring-opened deoxyribose unit.

In previous works, we designed heterodimeric drugs, such as compound **6**, that have been shown to recognize selectively the abasic site and incise the DNA strand at this site quite efficiently (cleavage occurred at nanomolar concentrations).[8–10] These molecules behave as ''artificial nucleases'', as cleavage is triggered in the pre-formed drug–DNA complex by a non-protonated secondary amine of the linking chain of the drug acting as a β-elimination catalyst. Spectroscopic studies (NMR, EPR) of the interaction of molecule **6** with a duplex DNA undecamer containing a stable analogue of the apurinic site have revealed that the drug fits perfectly the abasic site,[11,12] the purine moiety in **6** being docked in the abasic pocket opposite the thymine of the complementary strand and the acridine moiety being intercalated at a two base pair distance on the 5′ of the abasic site. The molecule was also shown to interfere with the AP site repair process.[13] Tested on L1210, this compound potentiated the cytotoxicity of *N,N′*-bis-(2-chloroethyl)nitrosourea (BCNU) in a concentration dependent fashion.[13] Based on these results, we have modified the heterodimer **6** to introduce a photochemical cleavage activity into the molecule while maintaining its selectivity for AP sites. The aim is to induce under irradiation a second lesion in the close proximity of the abasic site. In the present work, we describe the synthesis and the study of the new heterodimers **7**–**9** in which adenine is linked to different substituted acridines. Compound **7** contains a 6-nitroacridine moiety, the photocleavage activity of which is expected by analogy with the reactivity of other nitroaromatic compounds. Compound **8** is a derivative of ethacridine (6,9-diamino-2-ethoxyacridine), which was shown to induce photochemical cleavage of DNA.[14] Compound **9** contains a 5-iodoacridine susceptible to generate an aryl radical under UV irradiation as it was shown for 3-iodo-6-aminoacridine.[15]

Synthesis

The mode of preparation of molecules **7**–**9** is based on the synthesis of the parent compound **6**,[10] i.e., by

*Corresponding authors. Tel.: +33-4-76-51-4429; fax: +33-4-76-51-4382; e-mail: martine.demeunynck@ujf-grenoble.fr

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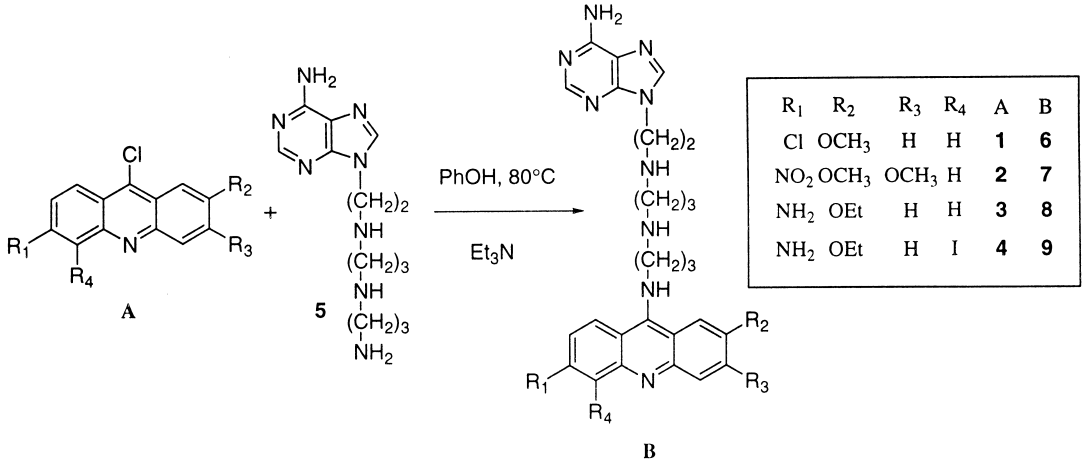
condensation of the polyamino functionalized adenine **5** with 9-chloro substituted acridines **(1–4)** (Fig. 1). 2,3-Dimethoxy-9-chloro-6-nitroacridine **2** is commercially available. 6-Amino-9-chloro-2-ethoxyacridine **3** was prepared in two steps from the 6,9-diamino-2-ethoxyacridine as already reported.[16] Iodination of 6-amino-9-chloro-2-ethoxyacridine **3** was performed by reaction of **3** with iodine in the presence of silver sulfate in ethanol.[17] 6-Amino-9-chloro-2-ethoxy-5-iodoacridine **4** was thus obtained in 50% yield.[18] Substitution of the 9-chloroacridines **1–4** by the amine **5** was performed in phenol at 80 °C in the presence of triethylamine. The desired compounds **6–9** were obtained in 50 to 55% yields. They were crystallized as their hydrochlorides.[19]

Cleavage Activity at Apurinic Sites

The abasic site cleavage activity of the new heterodimers **7–9** was tested using the depurinated plasmid assay already reported[20] and compared with the activity of **6**. Approximately 1.8 apurinic sites per plasmid DNA molecule were generated by thermal treatment in acidic conditions (pH 5, 70 °C, 20 min). Depurinated DNA was then incubated in the presence of varying concentrations of compounds **6–9** at 37 °C for 20 min. Conversion of supercoiled pBR322 plasmid (form I) into relaxed circular form (form II) was used to quantify the relative cleavage efficiencies. As shown in Figure 2, no significant difference in the cleavage efficiency was observed between compounds **6, 7, 8** and **9**, thus indicating that the substituents present on the acridine moiety have relatively low effect on the abasic site cleavage activity. This is consistent, as hypothesized, with a mode of interaction at the abasic site that is quite similar for the four drugs.

Photo-Induced Cleavage Activity on Abasic Site Analogue Containing Oligodeoxynucleotide

Due to the instability of abasic sites, the photocleavage activity of the molecules **6–9** was examined on a synthetic 23-mer duplex DNA containing the stable tetrahydrofurane analogue of the apurinic site (X). This analogue was previously shown to be a good model of abasic site.[21] The oligonucleotide sequence and the structure of the analogue are shown in Figure 3. Thymine T_{35} faces the apurinic site. Each strand was successively 5′-^{32}P labelled using ^{32}P-γ-ATP and T4 polynucleotide kinase. The oligonucleotides were incubated with the different compounds **6–9** and irradiated (λ >310 nm) at 4 °C for 3 h. After irradiation, the damaged sites were revealed by hot piperidine treatment (90 °C, 10 min). The results are presented in Figure 3. As expected, compound **6** is poorly photoactive. However cleavage is highly selective. It occurs exclusively on the abasic strand (lane 10 shows no cleavage on the opposite strand) at the site X_{12} of the lesion (see lane 5 compared to the control lane 1). Compound **7** also showed little activity suggesting that the 6-nitroacridine is less efficient in these conditions. Cleavage sites for **7** are observed on both strands, mainly located at the two cytosines C_{11} and C_{13} that flank the lesion on strand 1



R_1	R_2	R_3	R_4	A	B
Cl	OCH_3	H	H	**1**	**6**
NO_2	OCH_3	OCH_3	H	**2**	**7**
NH_2	OEt	H	H	**3**	**8**
NH_2	OEt	H	I	**4**	**9**

Figure 1.

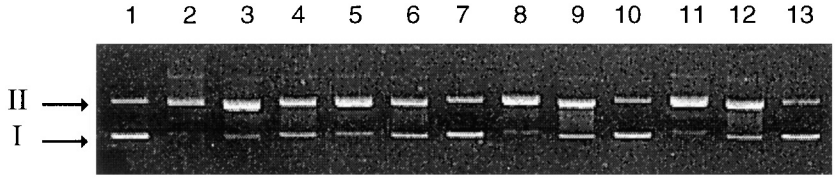


Figure 2. Abasic site cleavage activity on depurinated pBR322 plasmid. Plasmid DNA was incubated in 25 mM acetate buffer, pH 5, at 70 °C during 20 mm for depurination. The plasmid (15 ng/mL) was then incubated with different concentrations of synthetic molecules at pH 7 (1 mM phosphate buffer), 37 °C for 20 min. Lane 1: depurinated pBR322 (control); lane 2: DNA and 10 μM of **6**; lane 3: DNA and 1 μM of **6**; lane 4: DNA and 0.1 μM of **6**; lane 5: DNA and 10 μM of **7**; lane 6: DNA and 1 μM of **7**; lane 7: DNA and 0.1 μM of **7**; lane 8: DNA and 10 μM of **8**; lane 9: DNA and 1 μM of **8**; lane 10: DNA and 0.1 μM of **8**; lane 11: DNA and 10 μM of **9;** lane 12: DNA and 1 μM of **9;** lane 13: DNA and 0.1 μM of **9**.

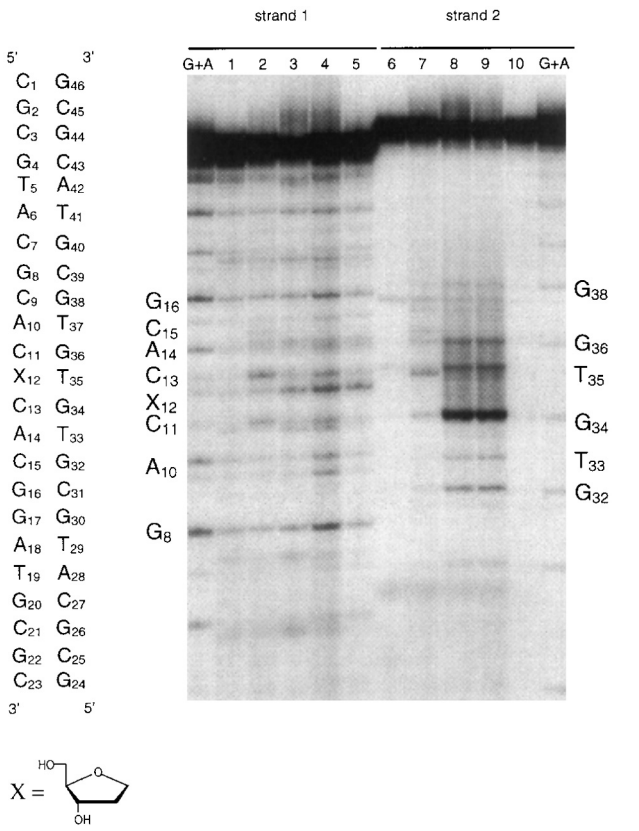


Figure 3. Autoradiogram of 20% denaturing polyacrylamide gel showing the photocleavage of 5′-^{32}P end-labeled 23-mer oligonucleotide containing an analogue of abasic site, induced by the compounds. Oligonucleotide (0.5 μM) was incubated with compound **6**, **7**, **8** or **9** (2 μM) in buffered solution (10 mM sodium phosphate buffer, pH 7, 20 mM NaC1, 1 mM EDTA) and then irradiated (with an ORIEL Xe/Hg 200W lamp) for 3 h at 4 °C. The resulting solution was treated with piperidine (1 M) at 90 °C for 10 min, followed by BuOH precipitation. Lanes 1–5: 5′-^{32}P end-labeled strand 1 duplex; lane 1: control; lane 2: oligonucleotide and **7**; lane 3: oligonucleotide and **8**; lane 4: oligonucleotide and **9**; lane 5: oligonucleotide and **6**; lanes 6–10: 5′-^{32}P end-labeled strand 2 duplex; lane 6: control; lane 7: oligonucleotide and **7**; lane 8: oligonucleotide and **8**; lane 9: oligonucleotide and **9**; lane 10: oligonucleotide and **6**.

(lane 2) and at T_{35} that faces the lesion on strand 2 (lane 7). However, the highest intensities for piperidine induced cleavage were observed for compounds **8** and **9** with cleavage occurring mainly on the strand opposite the abasic site (strand 2). On this strand, all strong cleavage sites are located three base pairs apart from the abasic site and the major band corresponded to G_{34}, which is adjacent to the unpaired base T_{35}. The similarity in the cleavage site distribution for compounds **8** and **9** suggests that the presence of the iodine at the C5 position is little or not involved in this photoreactivity.

Conclusion

It thus appears that modifying the artificial AP-endonuclease **6** by introducing a photoreactive acridine nucleus is a promising strategy to generate new lesions in the vicinity of the abasic site. The modification of the acridine does not alter the specificity of the drugs for the abasic site and the AP-site nuclease activity is maintained in compounds **7**–**9**. Quite interestingly all compounds show photochemical reactivity that leads to damages occurring quite selectively in close proximity to the lesion. The selectivity differs according to the drug considered, being probably dependant upon the mechanism of photocleavage and upon the fine structure of the drug/DNA complex.

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18. Physical data of **4**: mp = 187–190 °C. ^{1}H NMR (200 MHz; DMSO-d_6): δ ppm = 8.07 (1H, d, J = 9.0 Hz); 7.95 (1H, d, J = 9.6 Hz); 7.48 (1H, dd, J = 9.0 Hz and J = 2.0 Hz); 7.42 (1H, d, J = 2.0 Hz); 7.32 (1H, d, J = 9.6 Hz); 6.37 (2H, s); 4.22 (2H, q, J = 7.0 Hz); 1.42 (3H, t, J = 7.0 Hz). MS (electronic impact); M = 398.5, m/z: 398 (100, M^+ Cl^{35}), 400 (33.1, M^+ Cl^{37}), 369 (34.7, M^+ CH_2–CH_3), 341 (13.2, M^+ –CH_2–CH_2–CO), 271 (1.7, M^+–I); 242 (2.9, M^+–I–CH_2–CH_3). UV (ethanol): λ_{max} (ϵ): 439 (6900), 353 (6200), 278 (109,300), 240 (21,700) nm.
19. Physical data of final compounds: **7**: mp = 220–223 °C. ^{1}H NMR (200 MHz; $D_2$0): δ ppm = 8.18–8.40 (4H, m); 7.94 (1H, dd, J = 2.3 Hz and J = 9.6 Hz); 7.28 (1H, s); 6.75 (1H, s); 4.70 (2H, m); 4.05 (2H, m); 3.84 and 3.90 (3H, s); 3.51 (2H, m); 3.00–3.19 (6H, m); 2.17 and 1.96 (2H, m). ^{13}C NMR (200 MHz; D_2O): δ ppm = 156.9; 154.7; 149.1; 147.8; 145.4; 144.3;

137.8; 137.4; 127.4; 116.4; 114.7; 113.8; 107.8; 102.6; 97.8; 56.7; 56.4; 46.5; 45.8; 44.9; 44.8; 44.5; 40.7; 26.1; 22.5; 0.5. MS (FAB, NBA): M = 574; *m/z*: 575 (100, $(M+l)^+$). UV (H_2O): λ_{max} (ϵ) 446 (6200), 370 (7700), 308 (16,800), 260 (44,400) nm. HRMS: calcd 575.2843; found 575.2852. **8**: mp = 198 °C. ^1H NMR (300 MHz; D_2O): δ ppm = 8.52 (1H, s); 8.46 (1H, s); 7.82 (1H, d, *J* = 9.6 Hz); 7.31 (2H, m); 7.13 (lH, s); 6.85 (1H, d, *J* = 9.6 Hz); 6.35 (1H, s); 4.87–4.73 (2H, m); 4.12 (2H, q, *J* = 6.5 Hz); 3.98 (2H , m); 3.76 (2H, m); 3.22–3.35 (6H, m); 2.15–2.30 (4H, m); 1.52 (3H, t, *J* = 6.5 Hz). ^{13}C NMR (300 MHz; D_2O): δ ppm = 156.9; 154.7; 149.1; 147.8; 145.4; 144.3; 137.8; 137.4; 127.4; 116.4; 114.7; 113.8; 107.8; 102.6; 97.8; 56.7; 56.4; 46.5; 45.8; 44.9; 44.8; 44.5; 40.7; 26.1; 22.5; 0.5. MS (FAB, NBA): M = 528; *m/z*: 529 (51, $(M+1)^+$), 460 (100, $M^+ - Ade$). UV (H_2O): λ_{max} (ϵ): 419 (7010), 369 (13,800), 270 (50,500) nm. HRMS: calcd 529.3152; found 529.3170. **9**: mp = 200 °C. ^1H NMR (200 MHz; D_2O): δ ppm = 8.23 (1H, s); 8.18 (lH, s); 7.67 (1H, d, *J* = 9.0 Hz); 7.47 (1H, d, *J* = 9.0 Hz); 7.24 (1H, dd, *J* = 9.0 Hz and *J* = 2.0 Hz); 7.17 (1H, d, *J* = 2.0 Hz); 6.67 (1H, d, *J* = 9.0 Hz); 4.49–4.69 (2H, m); 4.02 (2H, q, *J* = 7.0 Hz); 3.86 (2H, m); 3.49 (2H, m); 2.90–3.15 (6H, m); 1.8–2.2 (4H, m); 1.32 (3H, t, *J* = 7.0 Hz). MS (FAB, glycerol): M = 654; *m/z*: 655 (97, $(M+1)^+$). UV (H_2O): λ_{max} (ϵ): 372 (11,500), 274 (39,200) nm. HRMS: calcd 655.2118; found 655.2130.

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