Pergamon

Tetrahedron 58 (2002) 4291–4298

TETRAHEDRON

Design of site specific DNA damaging agents for generation of multiply damaged sites

Alain Martelli, Jean-Francois Constant,* Martine Demeunynck,* Jean Lhomme and Pascal Dumy

LEDSS, CNRS/Université J. Fourier, BP53 38041 Grenoble Cedex 9, France

Received 21 January 2002; revised 11 March 2002; accepted 27 March 2002

Abstract—We describe the synthesis and DNA damaging activities of hybrid molecules in which a purine (adenine) is linked to an intercalating chromophore (acridine) by a polyamino linker. A DNA damaging agent, phenanthroline or *para*-nitrobenzamide, is tethered to the acridine moiety at various positions. Our goal is to induce upon activation other lesions in close proximity to the abasic site and therefore create cytotoxic multiply damaged sites.

A number of cytotoxic agents are known to produce DNA damage. Several classes of agents, mainly those involving radical processes (neocarzinostatin, bleomycin, ionizing radiations),[1–3] generate clustered lesions or multiply damaged sites (MDS) in tracks of a few base pairs. Such multiple damage have a high biological impact as they present a more challenging repair problem for the cell.[4,5] The strategy we developed to induce MDS is to target a DNA lesion (the abasic site) with a drug able to create other damage in close vicinity (Fig. 1). Abasic site[6] results from the loss of a base in DNA, either spontaneously or enzymatically as intermediate in the repair of modified bases.

These lesions are chemically unstable, and cleavage of the DNA strand at these sites may occur via a β-elimination reaction. We previously described the synthesis and the study of acridine-purine heterodimers such as **I** (see Fig. 2) designed to specifically interact and cleave DNA at abasic sites.[7] These molecules behave as 'artificial nucleases', and 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. NMR studies of the interaction of **I** with a duplex DNA undecamer containing a stable analogue of the abasic site have revealed that the drug fits perfectly the abasic site: the purine moiety being docked in the abasic pocket and the acridine moiety being intercalated at a two base pairs distance on the 5′ side of the lesion.[8]

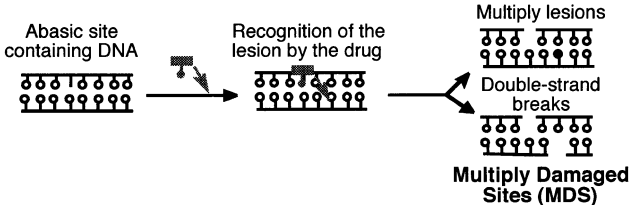


Figure 1. Targeting abasic sites for generation of multiply damage in DNA.

Starting from this optimized structure, we have modified the acridine nucleus to endow the heterodimer with a photodamaging or cleavage activity. Our goal was to induce upon activation other lesions in close proximity to the abasic site. In a first approach we have introduced various substituents (3-nitro, 3-amino, 3-amino-4-iodo) on the acridine

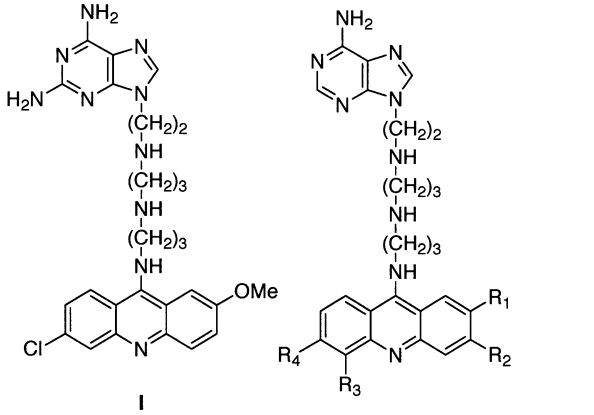
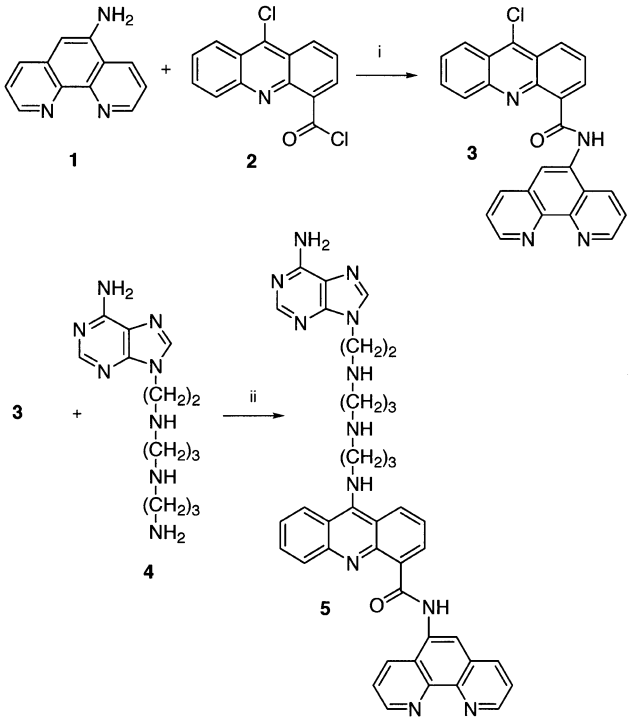


Figure 2.

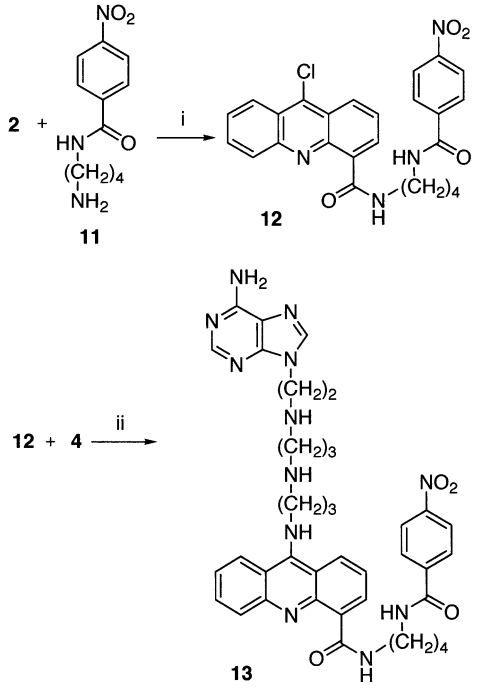
Keywords: DNA damaging agents; acridines; abasic site.

* Corresponding authors. Tel.: +33-476-514429; fax: +33-476-514946; e-mail: jean-francois.constant@ujf-grenoble.fr; martine.demeunynck@ujf-grenoble.fr

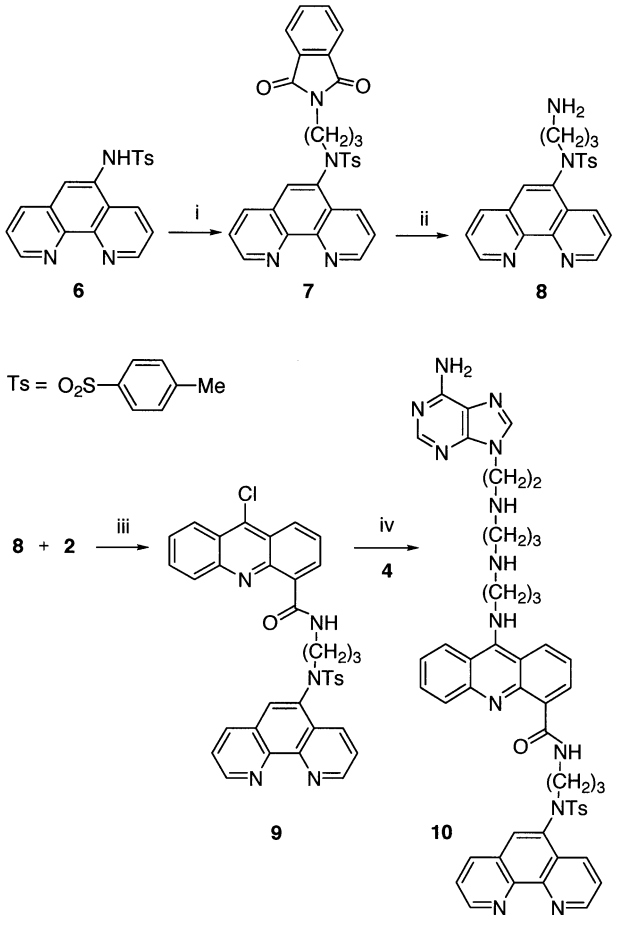
0040–4020/02/$ - see front matter
PII: S0040-4020(02)00345-9



Scheme 1. (i) DMF, NEt_3; (ii) PhOH, NEt_3, 70°C.



Scheme 3. (i) DMF, NEt_3, 0°C; (ii) PhOH, NEt_3, 70°C.

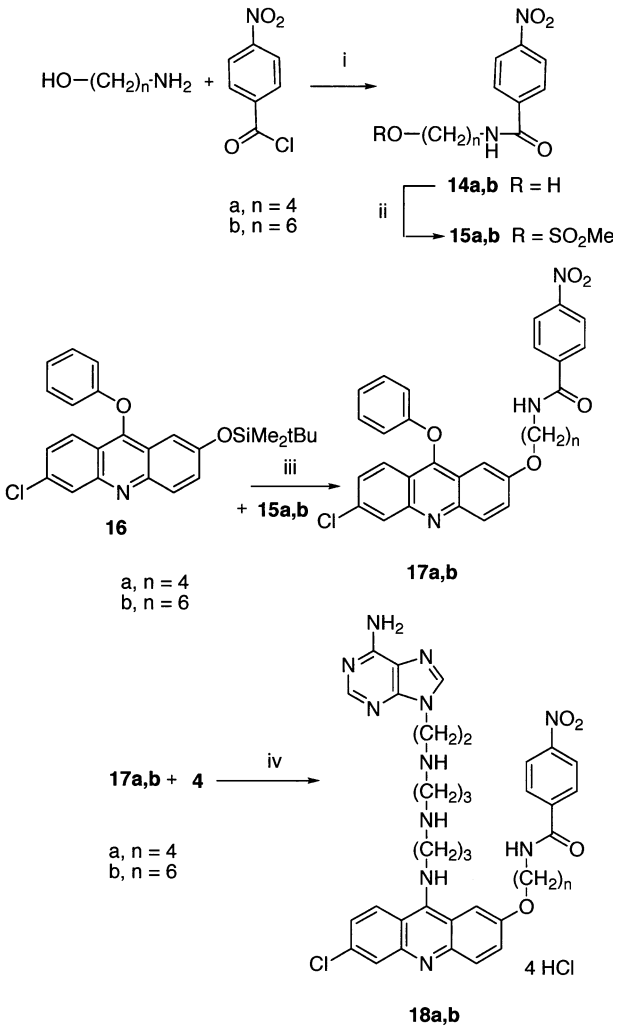


Scheme 2. (i) *N*-(bromopropyl) phthalimide; K_2CO_3, DMF, rt; (ii) $NH_2NH_2{-}H_2O$, EtOH, 60°C; (iii) DMF, NEt_3; (iv) PhOH, NEt_3, 70°C.

chromophore (Fig. 2), and studied the photodamaging activity of the corresponding heterodimers **II**–**V**.[9]

Two derivatives, **IV** and **V**, showed significant photochemical reactivity in close proximity to the abasic site. In order to increase both the selectivity and the efficiency of the cleavage, we designed new heterodimers in which the group responsible for DNA cleavage is tethered to the acridine chromophore. Denny and co-workers[10] have developed a large series of asymmetrically substituted acridines that binds by positioning a side chain in each groove of DNA. This mode of DNA binding, known as threading intercalation, gives to the molecules moderate DNA affinities but slow dissociation kinetics. In the design of 'triggered' DNA cleavage agents, this slow dissociation parameter should be advantageous as it confers to the molecules longer residence time in DNA that is likely to promote higher cleavage ratio. Introduction of the two chains at acridine 9 and 4 positions should place the two side chains in opposite grooves when the acridine ring intercalates with maximum overlap with the base pairs.

We therefore prepared new heterodimers (**5**, **10** and **13**) derived from molecule **I**, in which the damaging agents (1,10-phenanthroline that cleaves DNA in the presence of copper and a reducing agent, and the photochemically active *para*-nitrobenzamide group) were introduced as functional groups on the 4 position. The two acridine–phenanthroline conjugates differ in the absence (**5**) or presence (**10**) of a linker between the two chromophores. To modify the mode of binding we also synthesized two conjugates (**18a** and **b**) in which the *para*-nitrobenzamide group was tethered at the acridine 2 position. We describe here the synthesis of the conjugates and the preliminary studies of their DNA damaging activities.



Scheme 4. (i) CH_2Cl_2, 0.2N NaOH, 0°C; (ii) MsCl, pyridine, 0°C; (iii) 1 M TBAF in THF; (iv) PhOH, NEt_3, 80°C.

1. Results

1.1. Synthesis

1.1.1. Phenanthroline–acridine conjugates. Two phenanthroline–acridine conjugates, **5** and **10**, have been synthesized. In both compounds the aminophenanthroline substituent was attached at the acridine 4 position. As described in Schemes 1 and 2, the strategy designed by Denny[11] was used to prepare both compounds, i.e. introduction of the first amino substituent at the 4 position of 9-choro-acridine-4-carbonyl chloride (**2**), followed by introduction of the aminoalkyl side chain at the 9 position. Reaction of 5-amino[1,10]phenanthroline (**1**) with the acridine **2**[11] gave the carboxamido derivative **3** in 62% yield. Introduction of the amino-substituent at the 9 position was achieved by reacting **3** with the adenine–polyamine synthon **4**[7] in phenol and in the presence of triethylamine. To prepare **10**, the 5-aminophenanthroline **1** was first functionalized with the amino chain in three steps (tosylation to give **6**,[12] alkylation and deprotection of the primary amino group of the linker). Synthesis of conjugate **10** was then achieved as described above by formation of the carboxamide **9** and subsequent substitution of chlorine at the 9 position of the acridine ring by synthon **4**.

1.1.2. Nitrobenzamide–acridine conjugates. In compound **13** the nitrobenzamido group was attached at position 4 of the acridine by formation of a carboxamide. In compounds **18a**, **b**, the reactive group was introduced at the acridine 2 position via an ether function.

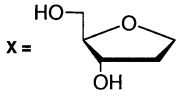
1.1.3. 4-Carboxamido acridine. As depicted in Scheme 3, we used the strategy described above to synthesize **13**. The nitrobenzamide synthon **11**[13] was reacted with acridine **2** to give **12** in good yield (80%). Substitution at position 9 was achieved by adding the adenine–polyamine synthon **4** to a solution of **12** in phenol. The final conjugate **13** was isolated in 42% yield.

1.1.4. 2-Substituted acridines. The synthesis of compounds **18a** and **b** is described in Scheme 4. We used the strategy we reported previously for selective alkylation of 2-hydroxy-9-phenoxyacridine.[14] Reaction of *O*-silyl protected 9-phenoxyacridine **16** with methanesulfonate activated synthons **15a**,**b** (prepared in two steps from ω-aminoalcohols and *para*-nitrobenzoylchloride) gave the intermediates **17a**, **b** in 51 and 48% yields, respectively. Substitution of the phenoxy group with adenine–polyamine synthon **4** afforded the desired conjugates **18a** and **b** in 52 and 74% yields, respectively.

1.2. DNA damaging activity

Due to the chemical instability of abasic sites, the damaging activities of the new conjugates were examined on a synthetic 23-mer duplex DNA containing the stable tetrahydrofurane analogue of the abasic site (X) in strand 1. This analogue was previously shown to be a good model of abasic site.[15] The oligonucleotide sequence and the structure of the analogue are shown in Fig. 3. Thymine T_{35} faces the abasic site. Each strand was successively ^{32}P 5′-end labeled using γ-^{32}P-ATP and T4 polynucleotide kinase.

1.2.1. Phenanthroline–acridine conjugates. The 23-mer duplex containing the abasic site was incubated with



Strand 1: $^{5'}C_1\ G_2\ C_3\ G_4\ T_5\ A_6\ C_7\ G_8\ C_9\ A_{10}C_{11}\mathbf{X_{12}}C_{13}A_{14}C_{15}G_{16}C_{17}A_{18}T_{19}G_{20}C_{21}G_{22}C_{23}\ ^{3'}$

Strand 2: $^{3'}G_{46}C_{45}G_{44}C_{43}A_{42}T_{41}G_{40}C_{39}G_{38}T_{37}G_{36}T_{35}G_{34}T_{33}G_{32}C_{31}G_{30}T_{29}A_{28}C_{27}G_{26}C_{25}G_{24}\ ^{5'}$

Figure 3.

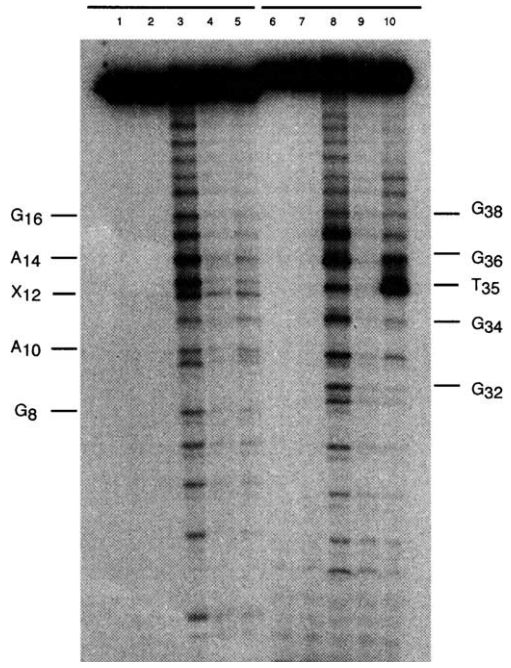


Figure 4. Autoradiogram of 20% denaturing polyacrylamide gel showing the DNA cleavage activity of the phenanthroline–acridine conjugates. Lane 1: strand 1 alone; lanes 2–5: strand 1 in the presence of added drug (lane 2: Cu; lane 3: phenanthroline–Cu; lane 4: **5**; lane 5: **10**); lane 6: strand 2 alone; lanes 7–10: strand 2 in the presence of added drug (lane 7: Cu; lane 8: phenanthroline–Cu; lane 9: **5**; 10: **10**).

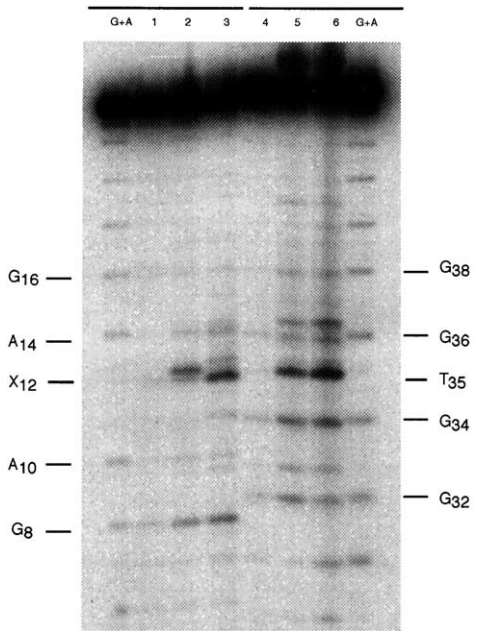


Figure 5. Autoradiogram of 20% denaturing polyacrylamide gel showing the photocleavage activity of the nitrobenzamide–acridine conjugates. Lanes G+A: Maxam–Gilbert sequencing; lane 1: strand 1 alone; lane 2: strand 1+**18a**; lane 3: strand 1+**18b**; lane 4: strand 2 alone; lane 5: strand 2+**18a**; lane 6: strand 2+**18b**.

phenanthroline or with compound **5** or **10**, in the presence of copper and mercaptopropionic acid (MPA) as a reducing agent. Incubation was carried out at 25°C for 40 min. The autoradiogram of the gel is shown in Fig. 4.

Two controls were done: (1) the duplex in the absence of any added drug (lanes 1 and 6) and (2) the duplex in the presence of Cu and MPA (lanes 2 and 7) showed no cleavage. The duplex in the presence of 1,10-phenanthroline, Cu and MPA (lanes 3 and 8) showed cleavage at all bases on each strand. However, slightly preferential sites were observed on both strands on the 3′-side of the lesion. This might suggest that the distorted abasic site containing sequence is a preferential binding structure for $(Phen)_2Cu^+$. For instance, it has been reported that 1,10-phenanthroline copper complexes show preferential activity on particular nucleic acid structures.[16] Therefore, the preferential sites observed in this study might suggest the presence of distorted structures (such as kink) in the vicinity of the abasic site. In the presence of compound **5**, Cu and MPA (lanes 4 and 9), no significant cleavage was observed on either strand. Interestingly, in the presence of compound **10**, Cu and MPA (lanes 5 and 10) no cleavage was observed on strand 1, but a highly preferential site, corresponding to the unpaired base T_{35} facing the abasic site, appeared on strand 2. A secondary site of cleavage was also observed at the flanking G_{36} base.

1.2.2. Nitrobenzamide–acridine conjugates. The oligonucleotides were incubated with compounds **13** and **18a**,**b** and irradiated ($\lambda > 310$ nm) at 4°C for 3 h. After irradiation, the damaged sites were revealed by hot piperidine treatment (90°C, 10 min). Compound **13** in which the photodamaging agent is linked at the acridine 4 position, does not induce any alkali-labile site on either strand (data not shown). As shown in Fig. 5, compounds **18a** and **b** induce cleavage on both strands following irradiation and piperidine treatment. The cleavage is highly selective. The preferential sites are located at the abasic site X12 and at T35 on the opposite strand. Minor sites are also observed on G36, G34, G32 and G8.

2. Conclusion

We designed and prepared five new conjugates in which a reactive group capable of inducing DNA damage or cleavage was tethered to a vector targeting abasic lesions. From the three nitrobenzamide–acridine conjugates tested as photocleavage agents, two molecules, **18a** and **b**, have been shown to damage DNA upon illumination. As illustrated by molecular modeling calculations,[17] the two molecules possibly interact with DNA in a way very similar to that of the original artificial nucleases **I** and **II**. Both side chains of the intercalated acridine appear to be positioned in the minor groove. The acridine–phenanthroline conjugate **10** is very promising too as it cleaves efficiently and selectively the DNA strand at the base opposite the abasic site. This conjugate probably interacts with DNA by threading intercalation, i.e. by positioning a side chain in each groove of DNA.[10,18] The presence of the chain joining the phenanthroline to the acridine 4 position confers to the conjugate the degree of freedom required for adequate positioning of the DNA cleavage agent inside one groove. This compound seems a good candidate for the generation of double-stranded DNA cleavage (via its intrinsic β-elimination cleaving activity on the abasic strand and its radicalar cleavage activity on the opposite strand).

3. Experimental

3.1. General

Melting points were determined using a Reicher Thermovar apparatus and are uncorrected. NMR spectra were recorded on Bruker AC 200 and AM 300 spectrometers using solvent as the internal reference; the chemical shifts are reported in ppm, in δ units. The mass spectra were recorded on Varian Mat 311 and AET MS 30 instruments. High resolution mass spectra were obtained from 'Centre Regional de Mesures Physiques de l'Ouest', Université de Rennes. Absorption spectra were obtained on a Perkin–Elmer Lambda UV/Vis spectrometer. Microanalyses were performed by the 'Service Central de Microanalyses du CNRS', Lyon. Purifications were generally performed by chromatography on silica gel or alumina. The purity of the compounds was assessed by reversed-phase HPLC, performed on a μ-bondapak C18 analytical column (Waters Associates). The chromatographic system is equipped with two M-510 pumps and a photodiode array detector Waters 996 using Millenium 32 software. A linear gradient from 0 to 100% methanol in H_2O pH 2.5 (phosphoric acid), 2 mL min^{-1} flow rate, was used. Products were characterized by their retention time and UV/Vis absorption.

3.1.1. 9-Chloro-4-[*N*-([1,10]phenantrolin-5-yl)carboxamido]-acridine (3). 5-Amino[1,10]-phenanthroline (**1**) (0.146 g, 0.75 mmol) was added to a ice cooled solution of **2**[11] (0.2 g, 0.72 mmol) and triethylamine (0.2 mL, 1.44 mmol) in dry DMF (5 mL) kept under nitrogen. The mixture was kept at 4°C for 30 min, and then stirred at room temperature overnight. The solution was poured into cold 10% $NaHCO_3$. The solid was filtered off, and washed with cold water and cold methanol. Compound **3** was thus obtained in 62% yield (0.194 g) and was used without further purification: mp 185–190°C. ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm)=14.51 (1H, s, CO–N*H*); 9.29–7.60 (14H, m, Acr-H and Phen-H). MS (FAB, glycerol): *M*=434; *m/z*: 435 (100, $(M+1)^+$).

3.1.2. Acridine–phenanthroline conjugate (5). A solution of **3** (0.1 g, 0.23 mmol) in freshly distilled phenol (5 mL) was stirred at 60°C under nitrogen for 30 min. Compound **4**[7] (0.1 g, 0.23 mmol) and NEt_3 (0.046 mL, 0.33 mmol) were then added. The mixture was stirred at 70°C for 6 h. The mixture was cooled down to room temperature and poured into cold 1N NaOH. The precipitate was isolated by centrifugation, and washed several times with water. The solid was dissolved in methanol–methylene chloride mixture (1:1). 12N HCl was slowly added to allow formation and precipitation of **5** as the tetrahydrochloride (0.068 g, 35%): mp 245–250°C. ^{1}H NMR (300 MHz, DMSO-d_6: δ (ppm)=12.30 (0.5H, m, N*H*); 10.69 (0.5H, m, N*H*); 10.56 (0.5H, m, N*H*); 9.95 (1H, m, Acr-H or Phen-H); 9.65 (3H, m, Acr-H or Phen-H); 9.21 (1H, m, Acr-H or Phen-H); 9.15–8.70 (3H, m Acr-H or Phen-H); 8.61 (1H, s, Ade-H); 8.58 (1H, s, Ade-H); 8.52 (1H, m, Acr-H or Phen-H); 8.20–8.00 (2H, m, Acr-H or Phen-H); 7.70 (1H, m, Acr-H or Phen-H); 7.61 (2H, m, Acr-H or Phen-H); 7.39 (0.7H, m, N*H*); 4.67 (2H, m, CH_2–Ade); 4.27 (2H, m, CH_2–NHAcr); 3.47 (2H, m, CH_2–CH_2–Ade); 3.12 (6H, m, 3CH_2–NH); 2.70–2.30 (2H, m, CH_2); 2.12 (2H, m, CH_2). MS (FAB, NBA): *M*=690; *m/z*: 691 $(M+1)^+$. HRMS: $(M+H)^+$ calculated for $C_{39}H_{39}N_{12}O$: 691.3370; Found: 691.3365.

3.1.3. 5-[*N*-(3-Phthalimidopropyl)-*N*-*p*-toluenesulfonyl]-amino[1,10]phenanthroline (7). A mixture of **6**[12] (0.2 g, 0.57 mmol) and potassium carbonate (0.117 g, 0.86 mmol) was stirred in dry DMF (5 mL) at room temperature for 30 min under nitrogen. *N*-(3-Bromopropyl)-phthalimide (0.23 g, 0.86 mmol) was then added and the mixture was stirred overnight at 60°C. After cooling down to room temperature, the mixture was poured into a large volume of water. Compound **7** precipitated, and was filtered off. Purification was achieved by crystallization from ethanol–water. Compound **7** was obtained in 57% yield (0.174 g). Mp 190–193°C. ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm)= 9.18 (2H, m, 2Phen-H); 8.60 (1H, dd, *J*=2 and 8.5 Hz, Phen-H); 8.04 (1H, dd, *J*=2 and 8.5 Hz, Phen-H); 7.77–7.52 (10H, m, 2Phen-H, Ph-H and Phth-H); 7.27 (1H, s, Phen-H); 3.96 (1H, m, CH); 3.72–3.56 (3H, m, CH_2 and CH); 2.43 (3H, s, Ph-CH_3); 1.82 (2H, m, CH_2–CH_2–CH_2). MS (FAB, NBA): *M*=536; *m/z*: 537 (100, $(M+1)^+$).

3.1.4. 5-[*N*-(3-Aminopropyl)-*N*-*p*-toluenesulfonyl]amino-[1,10]phenanthroline (8). Hydrazine hydrate (0.15 g, 3 mmol) was added dropwise to a solution of **3** (0.16 g, 0.3 mmol) in ethanol (10 mL). The mixture was stirred at 60°C for 2 h. The solvent was then cooled down to room temperature, and the precipitate was removed by filtration. The solvent was evaporated to dryness under vacuum. The solid residue was stirred again in ethanol (10 mL). The insoluble part was removed by filtration and the filtrate was evaporated to dryness. This procedure was repeated three times to eliminate most of the phthalhydrazide. The solid was finally dissolved in 10% aqueous $NaHCO_3$–CH_2Cl_2 mixture. Organic layer was separated, washed with water and dried over Na_2SO_4. After evaporation of the solvent, **8** was obtained in 41% yield (0.049 g): mp 162–165°C. ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm)=9.09 (2H, m, Phen-H); 8.53 (1H, m, Phen-H); 7.95 (1H, m, Phen-H); 7.58–7.15 (7H, m, Phen-H and Ph-H); 4.00 (1H, m, C*H*–NTsPhen); 3.65 (1H, m, C*H*–NTsPhen); 2.79 (2H, m, CH_2–NH_2); 2.41 (3H, s, CH_3–Ph); 1.60 (2H, m, CH_2–CH_2–NH_2). MS (FAB, NBA): *M*=406; *m/z*: 407 $(M+1)^+$.

3.1.5. 9-Chloro-4-[*N*-(3-(*N*-([1,10]phenanthrolin-5-yl)-*N*-*p*-toluenesulfonylamino)proyl)-carboxamido]-acridine (9). Compound **8** (0.063 g, 0.15 mmol) was added to a ice cooled solution of acridine **2**[11] (0.043 g, 0.15 mmol) and NEt_3 (0.021 mL, 0.15 mmol) in anhydrous DMF (3 mL) under nitrogen. The reaction was stirred for 10 min at 4°C and then the solution was poured into cold 10% aqueous $NaHCO_3$. The precipitate was filtered off, washed with water and dried to give compound **7** (0.023 g, 23%): mp 150–155°C. ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm)=11.68 (1H, m, CO–N*H*); 9.14 (2H, m); 8.89 (1H, dd, *J*=2 and 8.5 Hz); 8.65–8.57 (2H, m); 8.42 (1H, dd, *J*=2 and 8.5 Hz); 7.97 (1H, dd, *J*=1 and 7.5 Hz); 7.93 (1H, dd, *J*= 2 and 8.5 Hz); 7.77–7.47 (8H, m); 7.18 (1H, s); 7.14 (1H, s); 4.16 (1H, m, C*H*–NTsPhen); 3.79–3.71 (3H, m, CH_2–NHCOAcr and C*H*–NTsPhen); 2.38 (3H, s, Ph-CH_3); 2.02 (2H, m, CH_2–CH_2–CH_2). MS (FAB, NBA): *M*=645.5; *m/z*: 646 (100, $(M+1)^+$).

3.1.6. Acridine–phenanthroline conjugate (10). The procedure described above for **5** was used for the synthesis of **10**, starting from **9** (0.052 g, 0.085 mmol), **4**[7] (0.045 g, 0.102 mmol) and NEt_3 (0.035 mL, 0.25 mmol): mp 220–225°C. ^{1}H NMR (200 MHz, DMSO-d_6): δ (ppm)=10.55 (1H, m, N*H*); 9.62–8.67 (9H, N*H* and Acr-*H* and Phen-*H*); 8.58 (1H, s, Ade-H); 8.55 (1H, s, Ade-H); 8.42–7.81 (6H, Acr-H and Phen-H); 7.61–7.41 (5H, Ts-*H* and N*H*); 4.66 (2H, m, CH_2–Ade); 4.27 (2H, m, CH_2–NHCOAcr); 4.10–3.70 (2H, m, CH_2–NTsPhen); 3.42–3.35 (4H, m, 2CH_2); 3.10 (6H, m, 3CH_2); 2.40 (5H, m, CH_3–Ph and CH_2); 2.09 (2H, m, CH_2); 1.82 (2H, m, CH_2). MS (FAB, NBA): *M*=901; *m/z*: 902 (100, $(M+1)^+$). HRMS: $(M+H)^+$ $C_{49}H_{52}N_{13}O_3$: M calculated=902.4037; M found=902.4036.

3.1.7. *N*-[4-(4-Nitrobenzamido)butyl]-9-chloro-acridine-4-carboxamide (12). The acridine derivative **2**[11] (0.212 g, 0.88 mmol) was added to a solution of amine **11**[13] (0.210 g, 0.85 mmol) and NEt_3 (0.319 mL, 2.29 mmol) in DMF (10 mL) kept at 0°C under nitrogen. The mixture was stirred at 0°C for 20 min, and then allowed to warm up to rt until complete solubilization of the reactants. The mixture was then poured into cold $NaHCO_3$. The solid was filtered off and washed with water. Compound **12** was thus obtained in 80% yield (0.325 g, 0.68 mmol). It was crystallized from ethanol: mp 124–128°C. ^{1}H NMR (200 MHz, $CDCl_3$): δ ppm=11.86 (1H, m, N*H*–CO–Acr); 8.98 (1H, dd, *J*=2 and 9 Hz, Acr-H); 8.64 (1H, dd, *J*=2 and 9 Hz, Acr-H); 8.46 (1H, d, *J*=9 Hz, Acr-H); 8.24 (2H, m, Ph-H); 8.15 (2H, d, *J*=9 Hz, Acr-H); 8.07 (2H, m, Ph-H); 7.90–7.65 (3H, m, 3Acr-H); 7.55 (1H, m, N*H*–CO–Ph); 3.74 (2H, q, *J*=6 Hz, CH_2–NH); 3.64 (2H, q, *J*=6 Hz, CH_2–NH); 1.90 (4H, m, 2CH_2). ^{13}C NMR (50 MHz, $CDCl_3$): δ (ppm)= 165.75; 149.56; 147.35; 146.37; 140.51; 135.84; 132.02; 129.30; 129.06; 128.65; 127.85; 126.67; 125.07; 124.71; 123.73; 108.74; 84.51; 40.45; 39.30; 28.20; 26.13. MS (CI, NH_3+isobutane): *M*=476.1; *m/z*=477 ($(M+1)^+$). Analysis: Found: C 62.32, H 4.44, N 10.71%; Calcd for $C_{25}H_{21}ClN_4O_4$ (0.5 EtOH): C 62.46, H 4.84, N 11.21%.

3.1.8. 6-Amino-9-[11-(*N*-(4-(4-nitrobenzamido)-butyl)-acridin-9-yl-4-carboxamido)-3,7,11-triazaundecyl]-9*H*-purine trihydrochloride (13). A solution of **12** (0.100 g, 0.21 mmol) in phenol was stirred under nitrogen at 60°C for 30 min. Compound **4**[7] (0.092 g, 0.21 mmol) and NEt_3 (0.030 mL, 0.21 mmol) were then added, and the mixture was stirred at 70°C for 7 h. The solution was slowly poured into cold 1N NaOH under vigorous stirring. The yellow solid was filtered off, washed several times with 1N NaOH and then water. The solid residue was dissolved in methanol and 12N HCl was added to allow isolation of **14** as the hydrochloride (78 mg, 42%): mp 192–195°C. ^{1}H NMR (200 MHz, D_2O): δ ppm=8.17 (1H, s, Ade-H); 8.11–8.07 (2H, m, Ade-H and Acr-H); 7.91 (1H, m, Acr-H); 7.63 (1H, m, Acr-H); 7.48–7.43 (2H, m, Ph-H); 7.28–7.18 (5H, m, 3Acr-H and Ph-H); 4.47 (2H, t, *J*=6 Hz, CH_2–NH–Acr); 3.90 (2H, t, *J*=7 Hz, CH_2–CO–Ph); 3.43 (2H, t, *J*=7 Hz, CH_2–Ade); 3.16 (4H, m, CH_2–CH_2–Ade and CH_2–NH–CO–Acr); 3.09–2.90 (6H, m, 3CH_2); 2.09 (2H, m, CH_2); 1.91 (2H, m, CH_2); 1.55 (4H, m, 2CH_2). ^{13}C NMR (75 MHz, D_2O): δ (ppm)=150.98; 150.56; 149.35; 148.71; 145.52; 144.85; 140.09; 136.46; 134.99; 128.17; 123.49; 119.35; 118.73; 46.91; 46.31; 45.39; 45.23; 44.94; 41.12; 40.15; 40.12; 26.22; 25.82; 25.35; 22.90. UV (H_2O): λ (ε): 423.1 (7900); 263.7 (47,000). MS (FAB, NBA): *m/z*=733 ($(M+1)^+$). HRMS: $(M+H)^+$ calculated for $C_{38}H_{45}N_{12}O_4$ 733.3687; Found: 733.3685.

3.1.9. 4-(4-Nitrobenzamido)butanol (14a). A solution of *p*-nitrobenzoyl chloride (0.500 g, 2.7 mmol) in CH_2Cl_2 (30 mL) was added to a cold solution of 4-amino-butanol (0.300 g, 3.36 mmol) in 0.2N NaOH solution (30 mL). The mixture was stirred at 0°C for 6 h. The organic layer was then separated, washed successively with 0.5N NaOH, water and brine, and finally dried over sodium sulfate. After evaporation of the solvent, compound **14a** was isolated as an oil in 32% yield (204 mg): ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm)=8.24–8.20 (2H, m, Ph-H); 7.94–7.89 (2H, m, Ph-H); 7.09 (1H, m, NH); 3.71 (2H, t, *J*=6 Hz, CH_2–OH); 3.47 (2H, q, *J*=6 Hz, CH_2–NH); 2.28 (1H, m, OH); 1.74–1.51 (4H, m, 2CH_2). ^{13}C NMR (200 MHz, $CDCl_3$): δ (ppm)=165.83; 149.59; 140.45; 130.87; 123.88; 62.46; 40.36; 29.92; 26.28.

3.1.10. 6-(4-Nitrobenzamido)hexanol (14b). The procedure described above for **14a** was used for the synthesis of **14b**, starting from 6-amino-hexanol. Compound **14b** was isolated as a white solid in 67% yield (1.09 g, 3.60 mmol): mp 72–73°C; ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm)=7.87–8.28 (4H, m, Ph-*H*); 6.27 (1H, s, N–*H*); 3.63 (2H, t, *J*=6 Hz, CH_2–OH); 3.43 (2H, q, *J*=6 Hz, NH–CH_2); 1.38–1.80 (9H, m, 4 CH_2 and O*H*).; ^{13}C NMR (50 MHz, $CDCl_3$): δ (ppm)= 165.57; 149.33; 140.22; 128.06; 123.68; 62.48; 40.15; 32.34; 29.32; 26.45; 25.21. MS (CI, NH_3+isobutane) *m/z*=267 (100, $(M+1)^+$); Analysis: Calcd for $C_{13}H_{18}N_2O_4$: C 58.63, H 6.81, N 10.52%; Found: C 58.76, H 6.85, N 10.56%.

3.1.11. (4-(4-Nitrobenzamido)butyl)methane-sulfonate (15a). Methanesulfonyl chloride (0.133 mL, 1.71 mmol) was added to a cold solution (4°C) of **14a** (0.204 g, 0.86 mmol) in pyridine (5 mL). After 4 h of stirring, the solution was diluted with water and extracted with CH_2Cl_2. The organic layer was separated, successively washed with 1N HCl, water and brine. After evaporation of the solvent, **15a** was isolated as an oil in 47% yield (0.126 g): ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm)=8.21–8.17 (2H, m, Ph-H); 7.92–7.87 (2H, m, Ph-H); 6.86 (1H, m, NH); 4.23 (2H, t, *J*=6 Hz, CH_2–OMs); 3.45 (2H, q, *J*=6 Hz, CH_2–NH); 2.98 (3H, s, SO_2–CH_3); 1.77 (4H, m, 2CH_2). MS (FAB, NBA) *m/z*=317 (100, $(M+1)^+$).

3.1.12. (6-(4-Nitrobenzamido)hexyl)methane-sulfonate (15b). Methanesulfonyl chloride (0.051 mL, 0.66 mmol) was added to a cold solution (4°C) of **14b** (0.100 g, 0.33 mmol) in pyridine (5 mL). After 6 h of stirring at 4°C, water was added to the solution. The solid was filtered off, washed with cold water and dried. **15b** was thus isolated in 80% yield (0.100 g, 0.26 mmol): mp 80°C; ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm)=7.89–8.29 (4H, m, Ph-*H*); 6.25 (1H, s, N*H*); 4.23 (2H, t, *J*=6 Hz, CH_2–OMs); 3.47 (2H, q, *J*=6 Hz, NH–CH_2); 1.40–1.85 (11H, m, 4CH_2 and –SO_2–CH_3); ^{13}C NMR (50 MHz, $CDCl_3$): δ (ppm)= 165.72; 149.71; 140.45; 128.32; 124.00; 70.01; 40.22;

37.61; 29.45; 29.09; 26.25; 25.13. MS (CI, NH_3+isobutane) *m/z*: 345 (100, $(M+1)^+$); Analysis: Calcd for $C_{14}H_{20}N_2O_6S$: C 48.83, H 5.85, N 8.13%. Found: C 49.11, H 5.89, N 8.24%.

3.1.13. 6-Chloro-2-[4-(4-nitrobenzamido)butyloxy]-9-phenoxy-acridine (17a). A solution of TBAF in THF (1 M, 0.370 mL, 0.37 mmol) was added to a mixture of 2-silyloxyacridine **16**[14] (0.157 g, 0.36 mmol) and **15a** (0.126 g, 0.40 mmol) in dry THF (5 mL). The resulting mixture was stirred for 10 days at room temperature. The solvent was then evaporated to dryness and the residue was triturated in diethylether. Compound **17a** was filtered off and was thus obtained in 51% yield (0.100 g, 0.18 mmol): mp 140–142°C; ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm)= 8.25–8.20 (3H, m, Ph-H and Acr-H); 8.09 (1H, d, *J*= 9 Hz, Acr-H); 7.93 (1H, d, *J*=9 Hz, Acr-H); 7.89–7.85 (2H, m, Ph-H); 7.45 (1H, dd, *J*=2 and 9 Hz, Acr-H); 7.34 (1H, dd, *J*=2 and 9 Hz, Acr-H); 7.26–7.22 (2H, m, Ph-H); 7.13 (1H, d, *J*=2 Hz, Acr-H); 7.11–7.03 (1H, m, Ph-H); 6.84–6.79 (2H, m, Ph-H); 6.28 (1H, m, NH); 3.99 (2H, t, *J*=6 Hz, Acr-O–CH_2); 3.55 (2H, q, *J*=6 Hz, CH_2–NH–CO–); 1.85 (4H, m, $2CH_2$). MS (FAB, glycerol) *m/z*=542 (100, $(M+1)^+$); Analysis: Calcd for $C_{30}H_{24}ClN_3O_5$: C 66.48, H 4.46, N 7.75%; Found: C 66.40, H 4.68, N 7.57%.

3.1.14. 6-Chloro-2-[6-(4-nitrobenzamido)hexyloxy]-9-phenoxy-acridine (17b). The procedure described above for **17a** was used for to the synthesis of **17b**, starting from **16** (0.100 g, 0.23 mmol) and **15b** (0.095 g, 0.28 mmol). **17b** was isolated as a yellow solid in 48% (0.063 mg, 0.11 mmol): ^{1}H NMR (200 MHz; $CDCl_3$): δ (ppm)= 8.27–8.22 (2H, m, Ph-H); 8.20 (1H, d, *J*=2 Hz, Acr-H); 8.09 (1H, d, *J*=9 Hz, Acr-H); 7.93 (1H, d, *J*=9 Hz, Acr-H); 7.90–7.86 (2H, m, Ph-H); 7.43 (1H, dd, *J*=2 and 9 Hz, Acr-H); 7.34 (1H, dd, *J*=2 and 9 Hz, Acr-H); 7.26–7.22 (2H, m, Ph-H); 7.12 (1H, d, *J*=2 Hz, Acr-H); 7.11–7.03 (1H, m, Ph-H); 6.84–6.79 (2H, m, Ph-H); 6.17 (1H, m, NH); 3.92 (2H, t, *J*=6 Hz, Acr-O–CH_2); 3.47 (2H, q, *J*=6 Hz, CH_2–NH–CO–); 1.90–1.40 (8H, m, $4CH_2$). MS (CI, NH_3+isobutane) *m/z*: 570 (100, $(M+1)^+$).

3.1.15. 6-Amino-9-[11-(6-chloro-2-(4-(4-nitrobenzamido)-butyloxy)acridin-9-yl)-3,7,11-triaza-undecyl]-9*H*-purine (18a). A mixture of **17a** (0.060 g, 0.11 mmol), **8** (0.053 g, 0.12 mmol) and triethylamine (0.017 mL, 0.12 mmol) in phenol (5 mL) was stirred at 80°C for 4 h. The solution was then added dropwise to ice-cold 1N NaOH. The solid thus formed was filtered off, washed with water. Compound **18a** was purified as the hydrochloride by crystallization in methanol acidified with 11N HCl. **18a** was isolated in 52% yield (42 mg): mp 190–195°C; ^{1}H NMR (300 MHz, D_2O): δ ppm=8.23 (1H, s, Ade-H); 8.17 (1H, s, Ade-H); 7.91 (1H, d, *J*=9.8 Hz, Acr-H); 7.52 (2H, m, Ph-H); 7.40–7.34 (3H, m, Acr-H); 7.24–7.14 (4H, m, Ph-H and Acr-H); 4.52 (2H, m, CH_2–NH–Acr); 4.09 (2H, m, Acr-O–CH_2); 3.90 (2H, m, CH_2–NH–CO); 3.49 (2H, t, *J*=5.6 Hz, CH_2–Ade); 3.21 (2H, m, CH_2–CH_2–Ade); 3.15–2.95 (6H, m, $3CH_2$); 2.15 (2H, m, CH_2); 1.97 (2H, m, CH_2); 1.68 (4H, m, $2CH_2$); UV (H_2O): λ (ε): 446.0 (6250); 427.0 (6340); 344.4 (4000); 269.0 (49,900). HRMS Calcd for $C_{37}H_{43}N_{11}O_4{}^{35}Cl$ 740.3188 $(M+H)^+$; Found: 740.3185.

3.1.16. 6-Amino-9-[11-(6-chloro-2-(6-(4-nitrobenzamido)-hexyloxy)acridin-9-yl)-3,7,11-triaza-undecyl]-9*H*-purine (18b). The procedure described above for **18a** was used for the synthesis of **18b**, starting from **17b** (0.092 g, 0.16 mmol) and **4** (0.072 g, 0.16 mmol). **18b** was isolated as a yellow solid in 74% yield (0.120 g): mp 170–173°C; ^{1}H NMR (300 MHz, D_2O): δ (ppm)=8.39 (1H, s, Ade-H); 8.33 (1H, s, Ade-H); 8.10 (1H, d, *J*=9.4 Hz, Acr-H); 7.67–7.64 (2H, m, Ph-H); 7.48–7.29 (7H, m, Acr-H and Ph-H); 4.69 (2H, t, *J*=6.4 Hz, Ade-CH_2); 4.12 (4H, m, Acr-O–CH_2 and Acr-NH–CH_2); 3.66 (2H, t, *J*=6 Hz, CH_2–NH–CO); 3.38 (2H, m, Ade-CH_2–CH_2); 3.26–3.16 (6H, m, $3CH_2$); 2.33 (2H, m, CH_2); 2.14 (2H, m, CH_2); 1.88 (2H, m, CH_2); 1.66 (2H, m, CH_2); 1.57 (2H, m, CH_2); 1.45 (2H, m, CH_2); ^{13}C NMR (75 MHz, D_2O): δ (ppm)=168.19; 156.07; 155.68; 151.04; 149.41; 148.40; 146.14; 144.70; 141.23; 139.76; 139.55; 134.18; 128.21; 127.46; 124.83; 123.42; 120.49; 118.79; 117.62; 113.80; 109.76; 104.36; 68.63; 46.97; 45.93; 45.41; 45.23; 44.98; 41.12; 39.47; 27.97; 27.73; 26.50; 24.40; 24.30; 22.95. MS (FAB, NBA) *m/z*=768 (24, $(M+1)^+$); UV (H_2O): λ (ε): 446.0 (6250); 427.0 (6340); 344.4 (4000); 269.0 (49900). HRMS Calcd for $C_{39}H_{47}N_{11}O_4{}^{35}Cl$ 768.3501 $(M+H)^+$; Found: 768.3503.

3.2. DNA cleavage activities

The DNA damaging and cleavage activities of the drugs were tested on 5′-^{32}P end-labeled 23-mer oligonucleotide containing a tetrahydrofurane analogue of abasic site.

The 23-mer abasic duplex was incubated with compound **5** or **10** (2 μM) in buffered solution (Tris buffer, 50 mM, pH 8), in the presence of Cu (10^{-5} mol L^{-1}) and MPA (10^{-4} mol L^{-1}). Incubation was carried out at 25°C for 40 min. The DNA cleavage was quenched by adding neocuproine (3 mM solution) to the mixtures.

The abasic 23-mer (0.5 μM) was incubated with the nitrobenzamide–acridine conjugates **13**, **18a**,**b** (2 μM) in buffered solution (10 mM sodium phosphate buffer, pH 7, 20 mM NaCl, 1 mM EDTA. The solutions were then irradiated (with an ORIEL Xe/Hg 200 W 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.

After electrophoresis, the gels were exposed overnight at −30°C on Kodak BioMax MR-2 films. The films were scanned with an AGFA STUDIOscan IIsi scanner.

References

1. Norbury, C. J.; Hickson, I. D. *Annu. Rev. Pharmacol. Toxicol.* **2001**, *41*, 367–401.
2. Chu, G. *J. Biol. Chem.* **1997**, *272*, 24097–24100.
3. Dedon, P. C.; Goldberg, I. H. *Chem. Res. Toxicol.* **1992**, *5*, 311–332.
4. Chaudhry, M. A.; Weinfeld, M. *J. Biol. Chem.* **1997**, *272*, 15650–15655.
5. Harrison, L.; Hatahet, Z.; Purmal, A. A.; Wallace, S. S. *Nucl. Acids Res.* **1998**, *26*, 932–941.
6. Lhomme, J.; Constant, J. F.; Demeunynck, M. *Biopolym. Nucl. Acid Sci.* **1999**, *52*, 65–83.

7. Fkyerat, A.; Demeunynck, M.; Constant, J.-F.; Michon, P.; Lhomme, J. *J. Am. Chem. Soc.* **1993**, *115*, 9952–9959.
8. Coppel, Y.; Constant, J.-F.; Coulombeau, C.; Demeunynck, M.; Garcia, J.; Lhomme, J. *Biochemistry* **1997**, *36*, 4831–4843.
9. Martelli, A.; Berthet, N.; Constant, J. F.; Demeunynck, M.; Lhomme, J. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 763–766.
10. Wakelin, L. P. G.; Denny, W. A. *Molecular Basis of Specificity in Nucleic Acid–Drug Interactions*; Pullman, B., Jortner, J., Eds.; Kluwer Academic: Dordrecht, 1990; Vol. 23, pp 191–206.
11. Atwell, G. J.; Cain, B. F.; Baguley, B. C.; Finlay, G. J.; Denny, W. A. *J. Med. Chem.* **1984**, *27*, 1481–1485.
12. Del Guerzo, A.; Demeunynck, M.; Lhomme, J.; Kirsch-De Mesmaeker, A. *Inorg. Chem. Commun.* **1998**, *1*, 339–342.
13. Shinomiya, M.; Kuroda, R. *Tetrahedron Lett.* **1992**, *33*, 2697–2700.
14. Belmont, P.; Chapelle, C.; Demeunynck, M.; Michon, J.; Michon, P.; Lhomme, J. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 669–674.
15. Takeshita, M.; Chang, C. N.; Johnson, F.; Will, S.; Grollman, A. P. *J. Biol. Chem.* **1987**, *262*, 10171–10179.
16. Muth, G. W.; Hill, W. E. *Methods* **2001**, *23*, 218–232.
17. Ayadi, L.; Forget, D.; Martelli, A.; Constant, J. F.; Demeunynck, M.; Lhomme, J. *Theor. Chem. Acc.* **2000**, *104*, 284–289.
18. Hénichart, J.-P.; Waring, M. J.; Riou, J. F.; Denny, W. A.; Bailly, C. *Mol. Pharmacol.* **1997**, *51*, 448–461.