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Diaminopurine-acridine Heterodimers for Specific Recognition of Abasic Site Containing DNA. Influence on the Biological Activity of the Position of the Linker on the Purine Ring

Karine Alarcon,[a] Martine Demeunynck,[a,*] Jean Lhomme,[a] Danièle Carrez[b] and Alain Croisy[b]

[a]*LEDSS, Chimie Bioorganique, UMR CNRS 5616, Université Joseph Fourier, BP53, 38041 Grenoble cedex 9, France*
[b]*INSERM U350, Institut Curie Recherche, Laboratoire Raymond Latarjet, Centre Universitaire, 91405 Orsay cedex, France*

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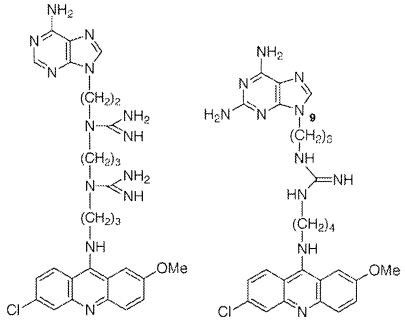
Abstract—Three acridine-diaminopurine heterodimers tethered by a linker containing an *N*,*N*′-substituted guanidine were prepared. The molecules differ by the site of introduction of the linker on the 2,6-diaminopurine. The interactions of the new heterodimers with abasic site containing oligonucleotide were compared, and their cytotoxicity was measured in the presence or absence of the antitumor alkylating agent BCNU.

The mode of action of many antitumor drugs involves the modification (mainly alkylation) of nucleic bases in DNA.[1] Drugs that interact specifically with such lesions, and inhibit the DNA repair system, may potentiate the action of antitumor drugs.[2] One major intermediate in the DNA repair cycle is the abasic site or AP-site (apurinic or apyrimidinic site) which results from the removal of modified or abnormal bases by hydrolysis of the *N*-glycosidic bond.[3] We have described in preceding papers the synthesis of a family of molecules that have been optimized for specific recognition of the abasic site in DNA.[4–7] They incorporate in their structure an intercalator with a high affinity for DNA, tethered to a nucleic base via a polyamine or guanidine containing linker. We recently reported on the synthesis and biological properties of **1** that contains two guanidine functions in the linker and that was shown to potentiate the action of the anticancer agent *N*,*N*′-bis(2-chloroethyl)-*N*-nitrosourea (BCNU) in vitro and in vivo.[5] High-field NMR measurements of the interaction of compound **1** with an oligonucleotide containing a stable analogue of the abasic site indicated that the purine ring of **1** is inserted inside the abasic pocket and forms hydrogen bonds with the unpaired base (thymine) located on the strand opposite to the abasic lesion.[5]



1 **2** **3** **4**

However, one limitation with compound **1** is a curare-like acute toxicity in vivo which led us to design new analogues containing basically the same structural units in an effort to modulate its biological activity and

*Corresponding author. Tel.: +33-476-514429; fax: +33-467-514382; e-mail: martine.demeunynck@ujf-grenoble.fr

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toxicity. We report herein on the synthesis of heterodimers **2**–**4** in which the linker contains one *N,N′*-guanidine function. The polymethylene units (butyl and propyl) that link the guanidine moiety to the heterocyclic units were chosen to adjust the length of the linker to 11 atoms, that is the same number of atoms as in **1**. The molecules differ by the position of attachment of the linker on the 2,6-diaminopurine ring. The linker was tethered at the N-9 position (**2**), in the same position as in compound **1**, but was also incorporated as substituent of the two exocyclic amines, at positions 2 and 6 (respectively, **3** and **4**). Varying the position of alkylation of the purine may modify the affinity and/or the specificity for abasic site containing DNA, as it may alter the formation of hydrogen bonds between the drug and the unpaired base located in the abasic site. The interaction with abasic site containing oligonucleotide and some pharmacological properties will be also presented in this paper.

Synthesis

The molecules were synthesized according to the retrosynthetic pathway shown in Figure 1.

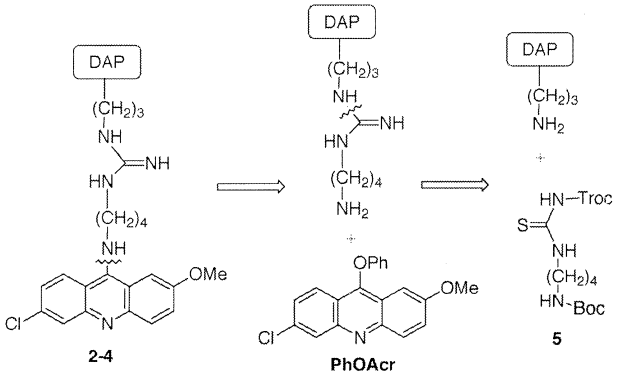
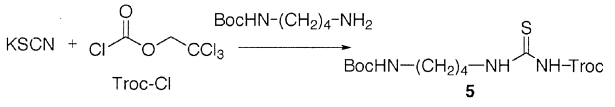


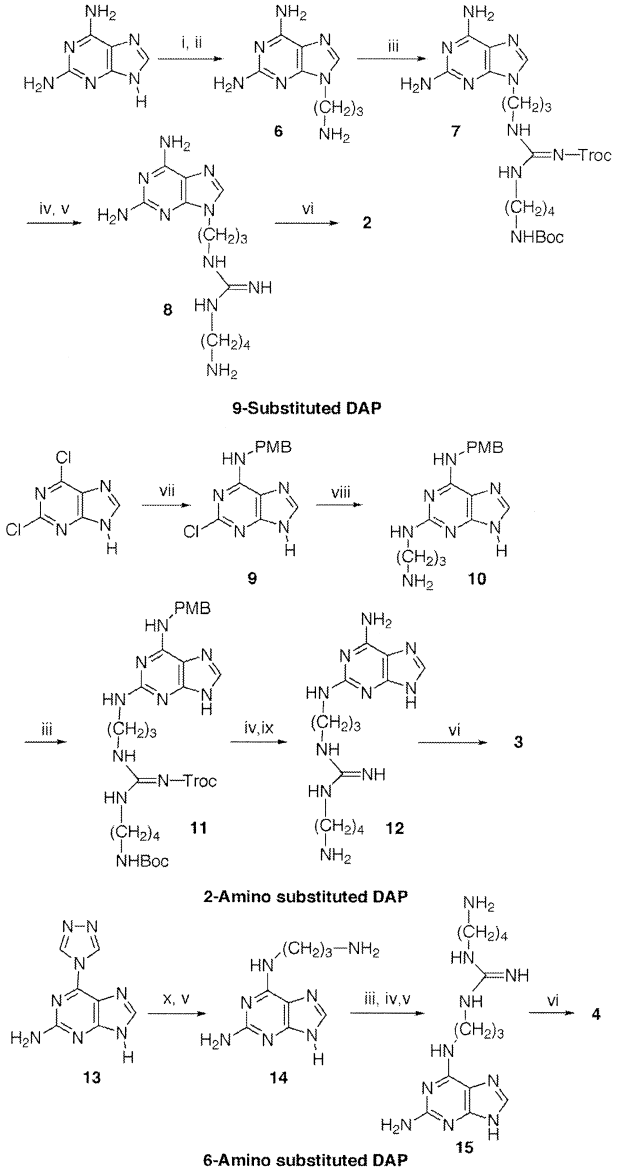
Figure 1.

In this strategy, the linker is built step-by-step by coupling in a key-step, a propylamino substituted 2,6-diaminopurine (DAP) with the protected amino thiourea **5** to form the *N,N′*-substituted guanidine.[8] The Troc protected thiourea **5** was prepared by the one-pot reaction published by Atkins.[9] Successive addition of 2,2,2-trichloroethylchloroformate (Troc-Cl) and mono-Boc protected diaminobutane to a solution of potassium thiocyanate gave **5** in 86% yield.



The syntheses of the molecules **2**–**4** are outlined in Scheme 1.

The 9-substituted-DAP analogue **2** was prepared from the 2,6-diaminopurine. The 9-aminopropyl-2,6-diaminopurines **6** was obtained in two steps following the method reported by Leonard for the alkylation of adenine.[10] Introduction of the aminoalkyl chain was achieved by alkylation of the purine with 3-bromopropylphthalimide in the presence of sodium hydride. The reaction takes place regioselectively at position 9 (53% yield), no product alkylated at the N-7 position was observed. Deprotection of the amine was achieved in 72% yield by hydrolysis of the phthalimide group in acidic conditions ($HCl/AcOH/H_2O$). Reaction of the amine **6** with the protected thiourea **5** in the presence of coupling agent EDC (1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride) gave the Boc,Troc protected guanidine **7** (79% yield). Deprotections of the guanidino and amino protecting groups were achieved by one-pot successive treatments with Zn in acetic acid and HCl 1 N in acetic acid and gave **8** as a gum. The acridine moiety was introduced by reaction of **8** with 6-chloro-2-methoxy-9-phenoxyacridine (PhOAcr) as previously described.[7]



Scheme 1. (i) 3-Bromopropylphthalimide, DMF, NaH, rt, 6 days; (ii) 12 N $HCl/H_2O/AcOH$ 1:1:1 mixture, 100 °C, 24 h; (iii) **5**, EDC, DMF, rt, 4 h; (iv) Zn, $AcOH/H_2O$, 0 °C, 4 h; (v) 1 N HCl in AcOH, rt, 1 h; (vi) PhOAcr, NEt_3, PhOH, 80 °C, 5 h; (vii) *para*-methoxybenzylamine NEt_3, DMF, 80 °C, 2 h; (viii) 1,3-diaminopropane, 140 °C, 2 h; (ix) TFA, 70 °C, 5 h; (x) $BocNH–(CH_2)_3–NH_2$, 140 °C, 1.5 days.

2,6-Dichloropurine was chosen as starting material to prepare molecule **3**, in which the linker is attached on the 2-amino group of DAP. Regioselective substitution of the chlorine atom in position 6 with *para*-methoxybenzylamine gave the PMB protected 6-amino-2-chloropurine **9** in 76% yield. Higher temperature was required to introduce the second substituent, 1,3-diaminopropane. Compound **10** was thus obtained in 71% yield. Condensation with thiourea **5** in the presence of EDC gave the protected guanidine **11** (76% yield). The Troc group was first removed by treatment with Zn in acetic acid. Refluxing in trifluoroacetic acid achieved complete deprotection of the molecule, giving **12** as a gummy residue. Reaction of **12** with PhOAcr gave **3**. The third molecule, in which the linker is connected to the 6-amino group of DAP, was prepared using 2-amino-6-triazolylpurine **13**, which was prepared in one step from DAP.[11] Reaction of **13** with mono-Boc protected diaminopropane, followed by deprotection of the primary amine afforded **14** (40% yield from **13**). Condensation with thiourea **5**, one-pot deprotection of the two protecting groups followed by the introduction of the acridine moiety gave molecule **4**.[12]

Interaction with Synthetic Oligonucleotides Containing Abasic Sites

In preceding papers,[5,6] we used denaturation studies (T_m measurements) to assay binding of drugs to abasic DNA. In these experiments, the effect of adding increased concentrations of a drug on the thermal denaturation of a synthetic oligonucleotide (11-mer, TX duplex) containing a stable analogue of the abasic site [3-hydroxy-2-(hydroxymethyl)tetrahydrofuran, noted X] was measured (Fig. 2).

The same study was also performed on the corresponding natural duplex (TA duplex) in which the abasic site is replaced by adenine.

To compare the relative binding affinities of the drug, we used ΔT_m measurements ($\Delta T_m = T_m$(duplex in the presence of the drug)$-T_m$(duplex alone)). Results are given in Figure 3.

As reported previously for comparable heterodimers containing a DNA intercalator,[5,6] the three compounds significantly stabilize the TA duplex, as indicated by ΔT_m values that increase regularly when the drug to DNA ratio r is increased. Compound **2** appeared slightly more efficient than **3** and **4**. The stabilization is much more pronounced however on the TX duplex, with similar magnitudes for the three drugs. The shapes of the curves reflect the mode of binding. With the TX duplex, the slopes of the curves are steeper at low drug to DNA ratios ($r < 1$) than at ratios superior to 1. This effect suggests preferred formation of 1:1 complexes between the drugs and the abasic site containing oligonucleotides. Indeed a comparable curve was previously observed in the interaction of molecule **1** with the same TX duplex.[5] In this case, the high field NMR study had revealed formation of a very specific 1:1 complex. From these results, it appears that the site of tethering of the linker does not significantly modify the binding to abasic DNA although the geometry of the complex is probably different for the three drugs. The H-bonding capacities of the diaminopurine are preserved in all three analogues.

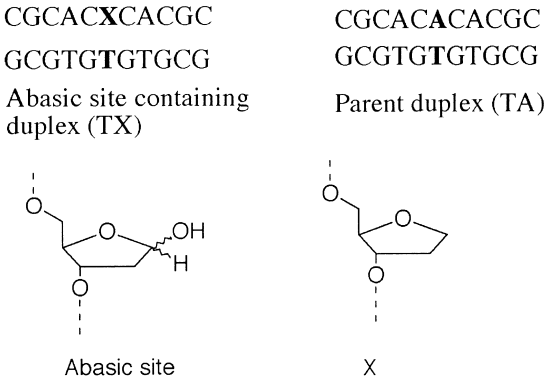


Figure 2. Sequences of the synthetic oligonucleotides.

Pharmacological Studies[5]

The three molecules were shown to be moderately cytotoxic on L1210 cells (IC_{50} of 36 μM for the most cytotoxic compound **3**) but displayed relatively high cytotoxicity on A549 cells (IC_{50} of 5–6.5 μM for the three compounds) (Table 1).

Tested in combination with BCNU, compound **2** led to a small but significant synergy (+16% increase). The efficiency of compound **2** to potentiate in vitro the activity of BCNU appeared much lower than the effect obtained previously with compound **1**. The observation is a new indication that the nature of the linker connecting the two heterocycles is determinant for the biological activities.

As a conclusion, the new heterodimers **2**–**4** interact specifically with abasic sites. The different sites of attachment

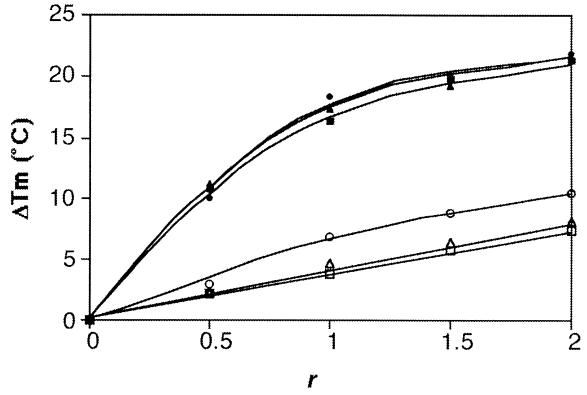


Figure 3. Influence of the drugs on the ΔT_m of the TA duplex (open symbols) and TX duplex (black symbols). Experiments were conducted at pH 7.0 in a buffer containing 10 mM sodium phosphate/1 mM EDTA/20 mM NaCl. The drugs tested were **2** (○/●), **3** (□/■) and **4** (△/▲). ΔT_m is defined as the difference between the T_m of the duplex in the presence of the drug and the T_m of the duplex alone. T_m were measured for various ratios r of drugs (r = [drug]/[duplex]).

Table 1. Cytotoxicity of compounds **1**–**4** alone and in association with BCNU

Compd	L1210 Cytotoxicity IC_{50} (μM)	A549 Cytotoxicity IC_{50} (μM)	Synergy with BCNU[a] (%)
1	33	10	+ 94%
2	60	6.5	+ 16%
3	36	5	—
4	> 100	5	—

[a]Simultaneous exposure of A549 cells to each tested compound (**1**–**4**) (10 μM) and BCNU (10 μM) for 24 h. Apparent synergy was measured when the cytotoxicity of the drug in association with BCNU was significantly higher than the addition of the toxic effect of both drugs alone.

of the linker tested in this study do not influence to a large extent the binding to abasic site containing oligonucleotides. One molecule, **2**, in which the linker is tethered to position 9, displayed a synergy effect with BCNU.

References and Notes

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12. **2**: Mp 210–220 °C. ^{1}H NMR (200 MHz, CD_3OD) (free base): δ ppm 8.20 (1H, d, J = 9.2 Hz, Acr–H); 7.77 (1H, s, H-8); 7.72 (2H, m, Acr–H); 7.46 (1H, d, J = 1.7 Hz, Acr–H); 7.32 (1H, dd, J = 9.2, 2.4 Hz, Acr–H); 7.20 (1H, dd, J = 9.2, 2.1 Hz, Acr–H); 3.99 (2H, t, DAP-CH_2); 3.89 (3H, s, O–CH_3); 3.79 (2H, t, CH_2); 3.01 (4H, m, 2 CH_2); 1.90 (4H, m, 2 CH_2); 1.45 (2H, m, CH_2). ^{13}C NMR (50 MHz, D_2O): δ ppm 155.8; 155.6; 154.0; 151.4; 150.8; 148.7; 141.8; 140.6; 132.9; 127.5; 127.0; 124.5; 119.8; 116.8; 112.6; 110.0; 56.2 (O–CH_3); 47.8 (NH–CH_2); 40.5 (NH–CH_2); 40.4 (NH–CH_2); 38.0; 26.5; 25.6. MS (FAB(+), glycerol): M = 561, m/z: 562 ($(M+1)^+$). UV (H_2O): λ_{max} (ε): 423 (6000), 278 (36,900), 217 (32,900) nm. HR-MS (FAB(+), NBA): found: 562.2552; calcd for $C_{27}ClH_{32}N_{11}O$ + H: 562.2558.

3·4HCl: Mp > 350 °C. ^{1}H NMR (300 MHz, D_2O): δ ppm 7.85 (1H, d, J = 9.6 Hz, Acr–H); 7.50 (1H, s, Acr–H); 7.30–7.08 (5H, m, Acr–H and H-8); 3.83 (2H, t, DAP-CH_2); 3.75 (3H, s, O–CH_3); 3.23 (2H, t, CH_2); 3.07 (2H, t, CH_2); 2.87 (2H, t, CH_2); 1.87 (2H, m, CH_2–CH_2–CH_2); 1.69 (2H, m, CH_2–CH_2–CH_2–CH_2); 1.54 (2H, m, CH_2–CH_2–CH_2–CH_2). ^{13}C NMR (75 MHz, D_2O): δ ppm 155.5; 155.3; 154.1; 150.8; 140.5; 140.2; 126.5; 124.0; 119.5; 116.5; 55.8 (O–CH_3); 47.4 (NH–CH_2); 39.9 (NH–CH_2); 37.9 (NH–CH_2); 29.8 (CH_2–CH_2–CH_2); 27.1 (CH_2–CH_2–CH_2); 26.2 (CH_2–CH_2–CH_2); 25.2 (CH_2–CH_2–CH_2). MS (FAB(+), glycerol) m/z: 562 ($(M+1)^+$). UV (H_2O): λ_{max} (ε): 423 (6000), 279 (39,800), 217 (36,200) nm.

4·HCl: Mp 220 °C. ^{1}H NMR (300 MHz, D_2O): δ ppm 7.82 (1H, d, J = 9.2 Hz, Acr–H); 7.66 (1H, s, Acr–H); 7.24–7.06 (5H, m, Acr–H and H-8); 3.80 (2H, t, DAP-CH_2); 3.73 (3H, s, O–CH_3); 3.21 (2H, t, CH_2); 3.06 (4H, m, 2 CH_2); 1.82 (2H, m, CH_2–CH_2–CH_2); 1.65 (2H, m, CH_2–CH_2–CH_2–CH_2); 1.58 (2H, m, CH_2–CH_2–CH_2–CH_2). ^{13}C NMR (75 MHz, D_2O): δ ppm 156.2; 154.9; 153.3; 152.3; 145.5; 141.2; 141.0; 139.0; 127.3; 124.8; 120.2; 117.2; 113.4; 109.1; 106.0; 56.5 (O–CH_3); 48.1 (NH–CH_2); 40.7 (NH–CH_2); 38.8 (NH–CH_2); 38.3 (NH–CH_2); 27.8 (CH_2–CH_2–CH_2); 27.1 (CH_2–CH_2–CH_2); 25.9 (CH_2–CH_2–CH_2). MS (FAB(+), NBA) m/z: 562 ($(M+1)^+$). UV (H_2O): λ_{max} (ε): 423 (4900), 278 (32,100), 221 (27,900) nm. HR-MS (FAB(+), NBA): found: 562.2558; calcd for $C_{27}ClH_{32}N_{11}O$ + H: 562.2558.