Supporting Information for

6,7-Dimethyllumazine as a potential ligand for selective recognition of adenine opposite an abasic site in DNA duplexes

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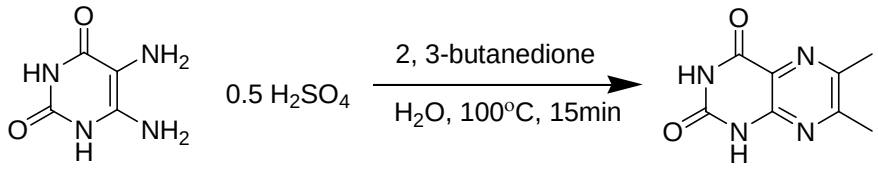
The following items have been included as supplementary material:



Scheme S1. Synthesis of 6,7-dimethyllumazine

Lumazine was obtained from Aldrich Chemical Co. (Milwaukee, WI) and used as received. 6,7-Dimethyl lumazine was synthesized according to the reported procedure[1-3] with slight modifications. A mixture of 5,6-diamino-2,4-dihydroxypyrimidine hemisulfate (0.93 g, 5 mmole) and 2,3-butanedione(0.90 g, 10 mmole) in 80 ml of water was boiled gently for 15 min and cooled at 2°C overnight. Purification by silica gel column chromatography with methanol-water (99:1 in v/v) as eluent gave the product as a white solid. The product was recrystallized from water (0.46g, 45%). ^{1}H NMR (270 MHz, $CDCl_3$+$C_2D_4O_2$): δ2.50 (3H, s), δ2.52 (3H, s). *m/z* (ESI positive): 192.0906; Anal. Calcd for $C_8H_8N_4O_2$ (192.0641). Elemental analysis: Calculated for $C_8H_8N_4O_2$/0.105H_2SO_4; C, 47.45; N, 27.67; H, 4.09. Found: C, 47.43; N, 27.62; H, 4.14.

references

1. R. Stewart, S. J. Gumbley, *Can. J. Chem.*, 1985, **63**, 3290.
2. R. Stewart, R. Srinivasan, S. J. Gumbley, *Can. J. Chem.*, 1981, **59**, 2755.
3. D. H. Bown, P. J. Keller, H. G. Floss, H. Sedlmaier, A. Bacher, *J. Org. Chem.*, 1986, **51**, 2461.

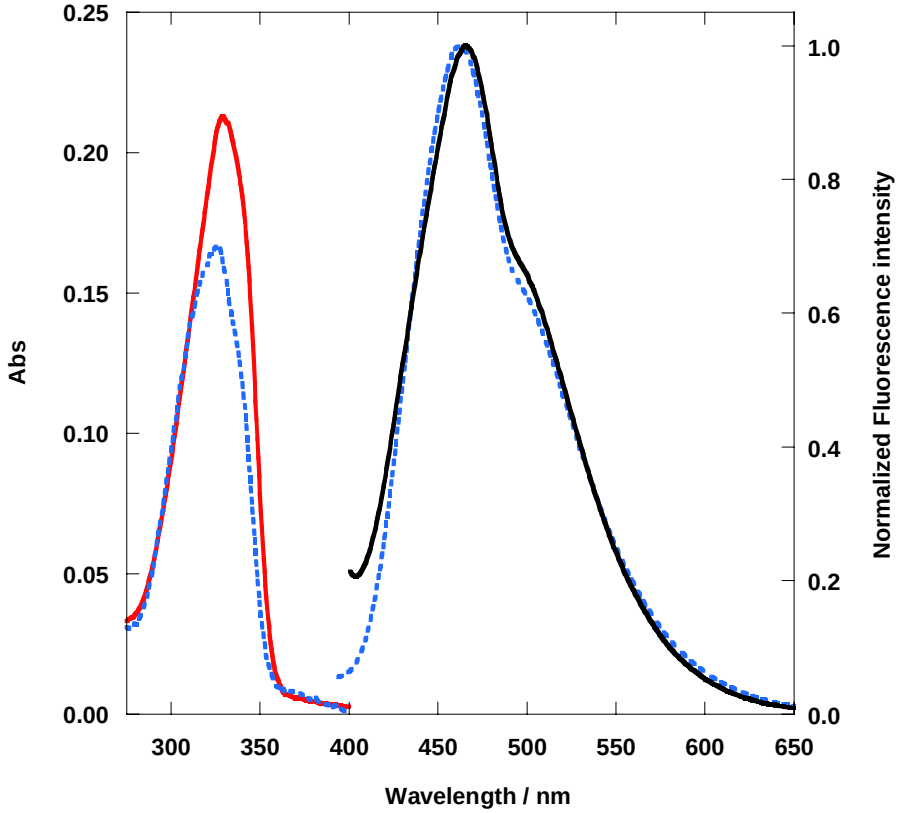


Figure S1 Absorbance and normalized fluorescence spectra of lumazine (dotted curve) and 6,7-dimethyllumazine (solid curve).

Table S1 Photophysical data of Lumazine and 6,7-dimethyllumazine.

sample	absorbance maxima (nm)	fluorescence maximum (nm)	molar extinction coefficients ($cm^{-1}.M^{-1}$)	logε
Lumazine	327	463	ε_{340nm}=5750[a]	3.76
6,7-dimethyllumazine	328	466	ε_{328nm} =10000	4.00

[a] From Michael D. Davis et al.[4]

4. M .D. Davis, J. S. Olson, and G. Palmerll, *J. biol. Chem.*, 1984, **259**, 3526.

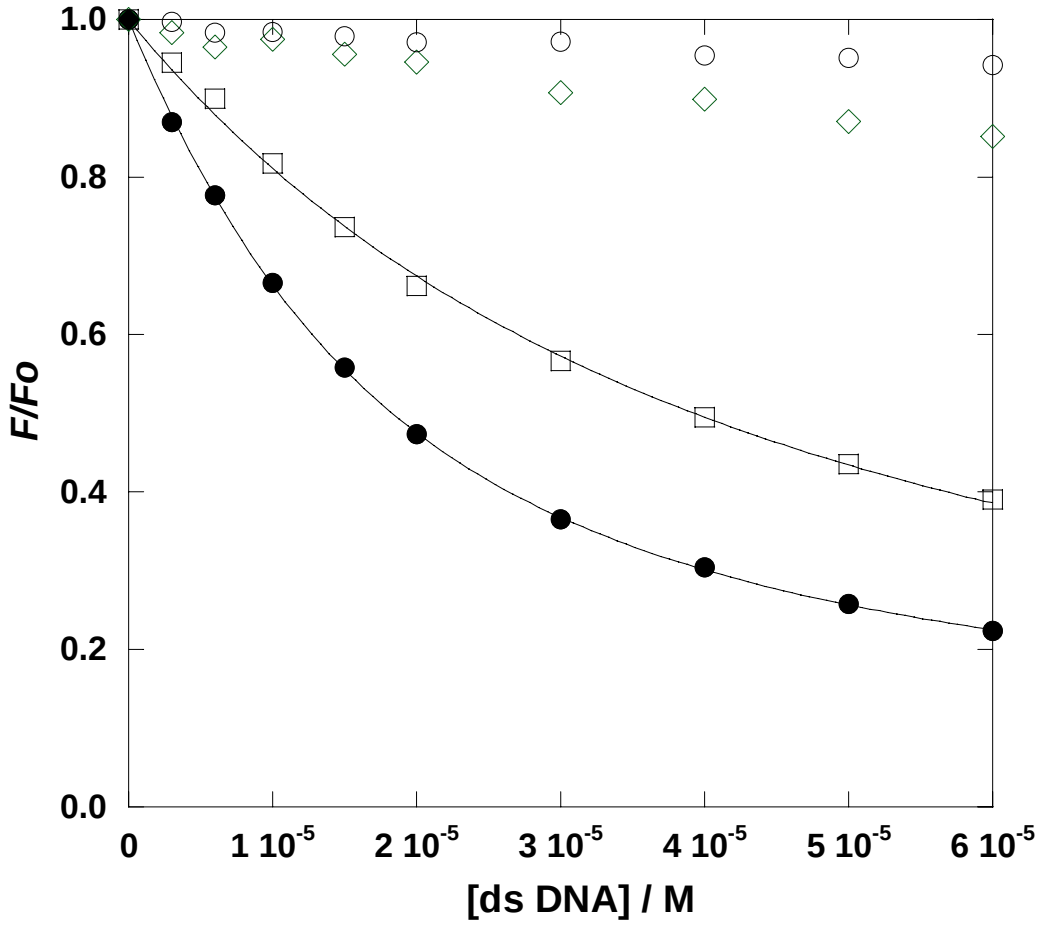


Figure S2 Fluorescence titration curves for the binding of lumazine to 11-mer AP site-containing DNA duplexes (5'-TCCAGXGCAAC-3'/3'-AGGTCNCGTTG-5', X = AP site; Spacer-C3, N = G (○), C (◊), A (●) and T (□)) obtained in solutions buffered to pH 7.0 (10 mM sodium cacodylate) containing 100 mM NaCl and 1.0 mM EDTA. The changes in the fluorescence intensity ratio at 463 nm were analyzed based on a 1:1 binding isotherm model. F and F_0 denote the fluorescence intensities of lumazine in the presence and absence of DNA duplexes, respectively. [lumazine] = 10.0 μM, λ_{ex} = 335 nm, λ_{em}= 463 nm, 5 °C.

Fitting equations for titration curves: The titration curves were analyzed by nonlinear least-squares regression based on a 1:1 binding isotherm

$$F / F_0 = \{1 + kK_{11}[\mathrm{D}]\}/\{ 1 + K_{11}[\mathrm{D}]\} \qquad (1)$$

where F and F_0 are the fluorescence intensities of ligand in the presence and absence of DNA duplexes, respectively, and k (= k_{11}/k_{L}) represents the ratio of proportionality constants connecting the fluorescence intensities and concentrations of the species (1:1 complex, k_{11}; free ligand, k_{L}). The free duplex concentration, [D], can be related to known total concentrations of duplex (D_0) and ligand (L_0), by the following equation:

$$D_0 = [\mathrm{D}] + \{ L_0 K_{11}[\mathrm{D}]\}/\{1 + K_{11}[\mathrm{D}]\} \qquad (2)$$

Together, eqs. (1) and (2) describe the system.

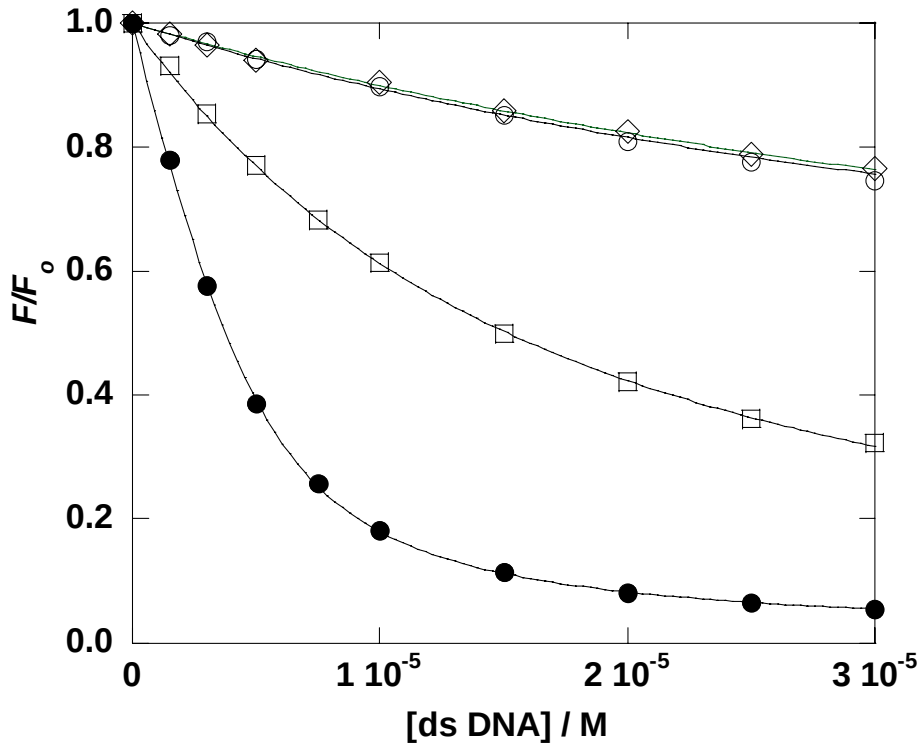


Figure S3 Fluorescence titration curves for the binding of 6,7-dimethyllumazine to 11-mer AP site-containing DNA duplexes (5'-TCCAGXGCAAC-3'/3'-AGGTCNCGTTG-5', X = AP site; Spacer-C3, N = G (○), C (◊), A (●) and T (□)) obtained in solutions buffered to pH 7.0 (10 mM sodium cacodylate) containing 100 mM NaCl and 1.0 mM EDTA. The changes in the fluorescence intensity ratio at 466 nm were analyzed based on a 1:1 binding isotherm model. F and F_0 denote the fluorescence intensities of 6,7-dimethyllumazine in the presence and absence of DNA duplexes, respectively. [6,7-dimethyllumazine] = 5.0 μM, λ_{ex} = 350.5 nm, λ_{em}= 466 nm, 5 ^{o}C.

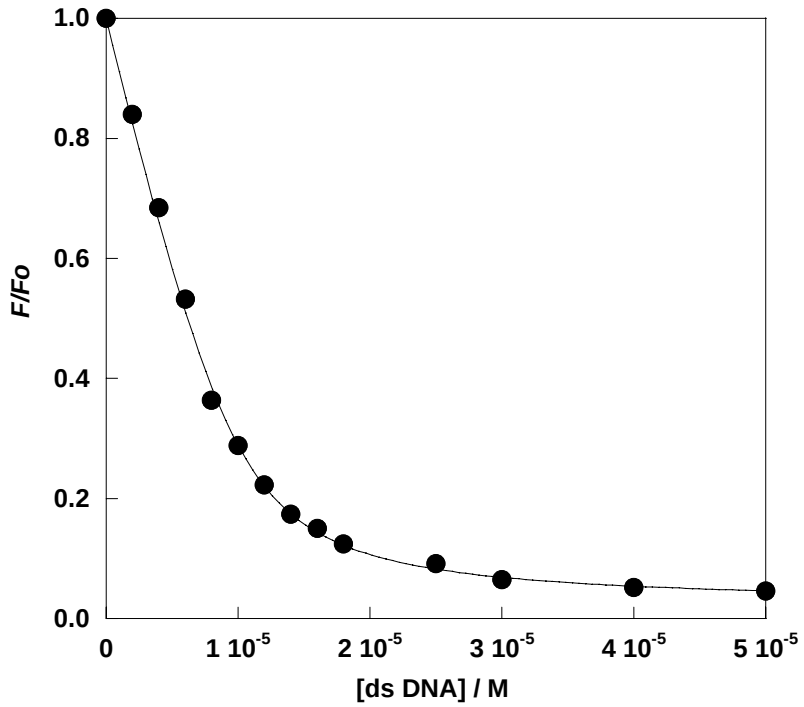


Figure S4 Fluorescence titration curves for the binding of 6,7-dimethyllumazine to 23-mer AP site-containing DNA duplexes (5'-TCTGCGTCCAGXGCAACGCACAC-3'/3'-AGACGCAGGTCACGTTGCGTGTG-5', X = AP site; Spacer-C3, A = Adenine, obtained in solutions buffered to pH 7.0 (10 mM sodium cacodylate containing 100 mM NaCl and 1.0 mM EDTA). The changes in the fluorescence intensity ratio at 466 nm were analyzed based on a 1:1 binding isotherm model. *F* and F_0 denote the fluorescence intensities of 6,7-dimethyllumazine in the presence and absence of DNA duplexes, respectively. [6,7-dimethyllumazine] = 10.0 μM, λ_{ex} = 350.5 nm, λ_{em}= 466 nm, 5 ^{o}C.

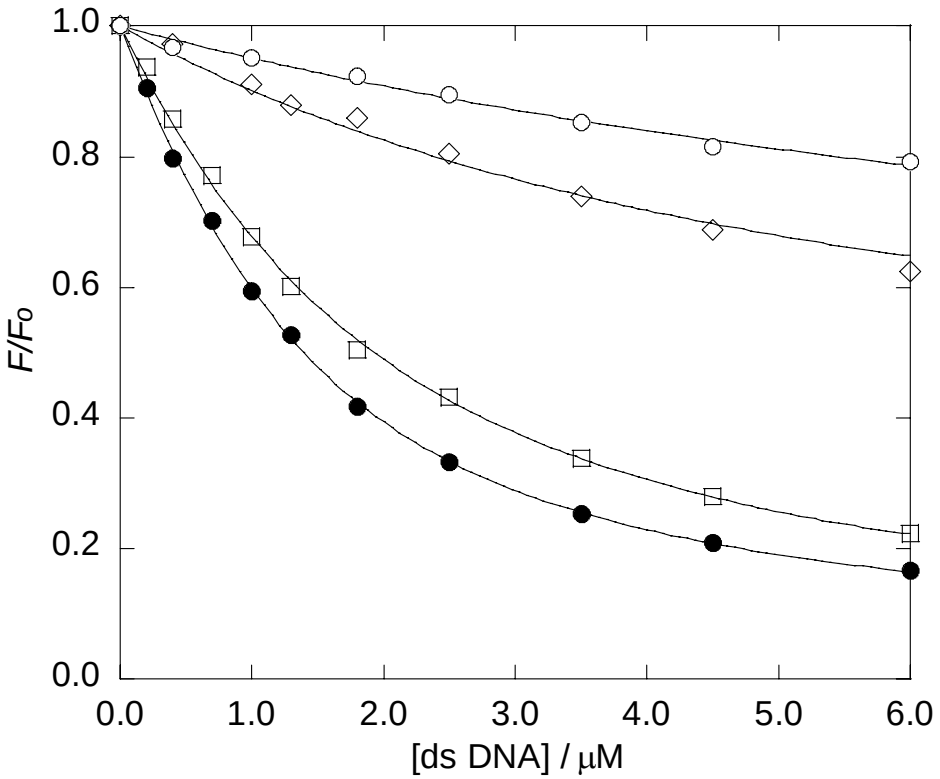


Figure S5 Fluorescence titration curves for the binding of alloxazine to 11-mer AP site-containing DNA duplexes (5'-TCCAGXGCAAC-3'/3'-AGGTCNCGTTG-5', X = AP site; Spacer-C3, N = G (○), C (◊), A (●) and T (□)) obtained in solutions buffered to pH 7.0 (10 mM sodium cacodylate containing 100 mM NaCl and 1.0 mM EDTA). The changes in the fluorescence intensity ratio at 453 nm were analyzed based on a 1:1 binding isotherm model. *F* and F_0 denote the fluorescence intensities of alloxazine in the presence and absence of DNA duplexes, respectively. [Alloxazine] = 1.0 μM, λ_{ex} = 385 nm, λ_{em}= 453 nm, 5°C.

Table S2 Binding constants (K_{11} / 10^6 M^{-1}) of lumazine and 6,7-dimethyllumazine to adenine base opposite an AP site in a 11-mer DNA duplex (5'-TCCAGXGCAAC-3'/3'-AGGTCACGTTG-5', X = AP site; Spacer-C3) under different salt concentrations.

$[Na^+]$ / mM(log$[Na^+]$)	Lumazine (logK)	6,7-dimethyl lumazine (logK)
110(-0.9586)	0.085 ± 0.002(4.9294)	0.83 ± 0.02(5.9191)
160(-0.7959)	0.081 ± 0.002(4.9085)	0.82 ± 0.01(5.9138)
210(-0.6778)	0.077 ± 0.003(4.8865)	0.80 ± 0.02(5.9031)
310(-0.5086)	0.074 ± 0.005(4.8692)	0.78 ± 0.02(5.8921)
410(-0.3872)	0.069 ± 0.002(4.8388)	0.77 ± 0.03(5.8865)

[a)] The binding constants were obtained by fluorescence titration experiments in solutions buffered to pH 7.0 with 10 mM sodium cacodylate containing 1.0 mM EDTA (cf. Figure 2). The concentration of NaCl was ranged from 110 mM to 410 mM. For the titration of lumazine: [lumazine]=10.0 μM, [DNA duplex] = 0-60 μM, λ_{ex} = 335 nm, λ_{em}= 463 nm, 5 ^oC. For the titration of 6,7-dimethyllumazine: [6,7-dimethyllumazine] = 5.0 μM, [DNA duplex] = 0-30 μM, λ_{ex} = 350.5 nm, λ_{em}= 466 nm, 5 ^oC.

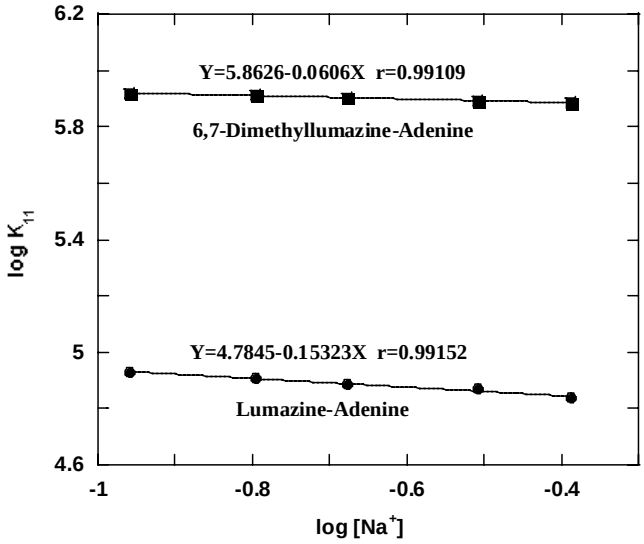


Figure S6. Salt-dependence of binding constants for the lumazine- and 6,7-dimethyllumazine - adenine interactions. The data are summarized in Table S1. The linear least squares fit to the data yielded a slope, and apparent charges of ligands were obtained from the value of the slope.

Salt dependence of the binding constants

The salt dependence of the binding constants is explained by the following relationship by Record *et al.* (13):

$$\delta \ln K_{11}/\delta \ln[Na^+] = -Z\psi = SK$$

where Z is the apparent charge on the ligand, and ψ (0.88 for B-type DNA) is the proportion of counterions associated with each DNA phosphate group. The slope (SK) is equivalent to the number of counterions released from DNA upon ligand binding.

13) Record, M.T., Anderson, C.F. and Lohman, T.M., Thermodynamic analysis of ion effects on binding and conformational equilibrium of proteins and nucleic-acids – Roles of ion association or release, screening and ion effects on water activity. *Q. Rev. Biophys.*, **11**, 103-178 (1978).

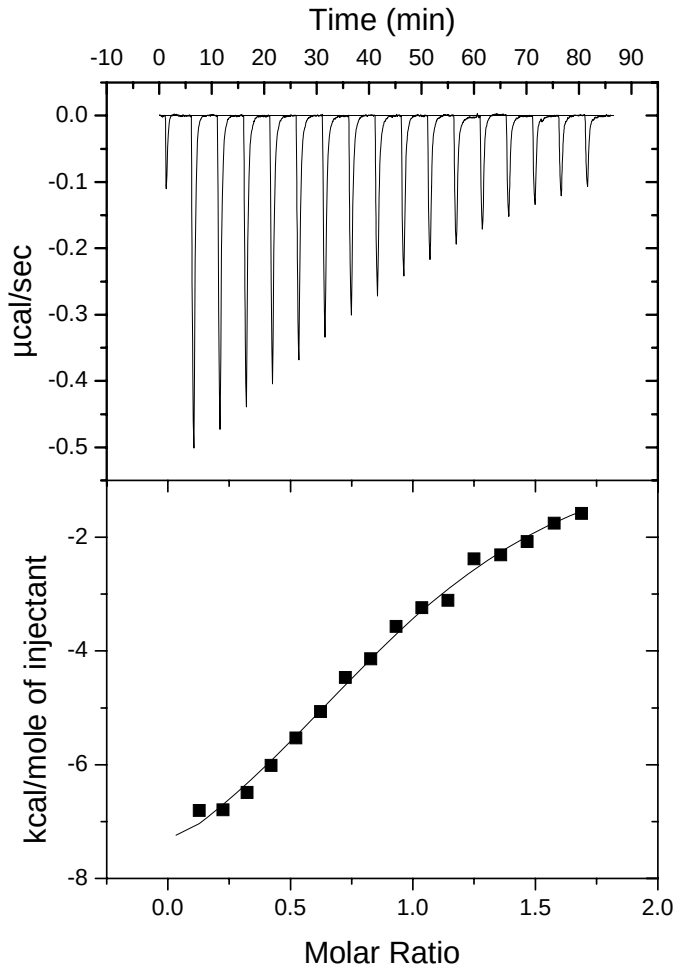


Figure S7. Isothermal titration calorimetry (ITC) analysis for the binding of lumazine to adenine base opposite the AP site in DNA duplex (5'-TCC AGX GCA AC-3'/3'-AGG TCA CGT TG-5', X = AP site) at 5°C for the addition of DNA aliquots (each 15 µl of 200 µM) into the solution containing lumazine (1.42 ml of 20 µM). Sample solutions were buffered to pH 7.0 (10 mM sodium cacodylate containing 100 mM NaCl, and 1 mM EDTA).

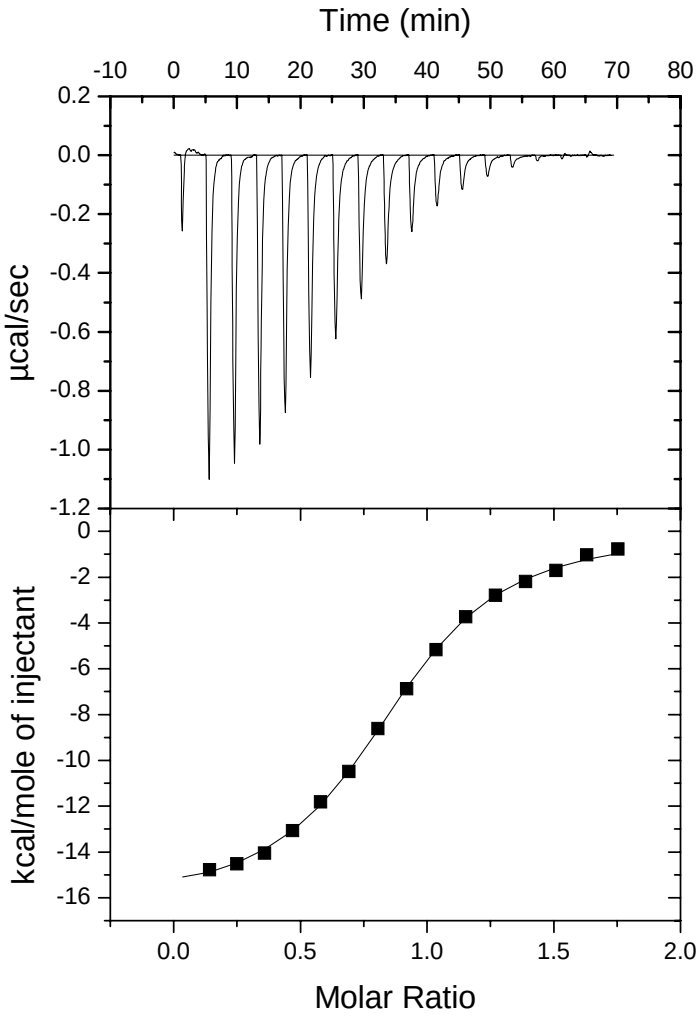


Figure S8. Isothermal titration calorimetry (ITC) analysis for the binding of 6,7-dimethyllumazine to adenine base opposite the AP site in DNA duplex (5'-TCC AGX GCA AC-3'/3'-AGG TCA CGT TG-5', X = AP site) at 5°C for the addition of DNA aliquots (each 15 µl of 200 µM) into the solution containing 6,7-dimethyllumazine (1.42 ml of 20 µM). Sample solutions were buffered to pH 7.0 (10 mM sodium cacodylate containing 100 mM NaC, and 1 mM EDTA).

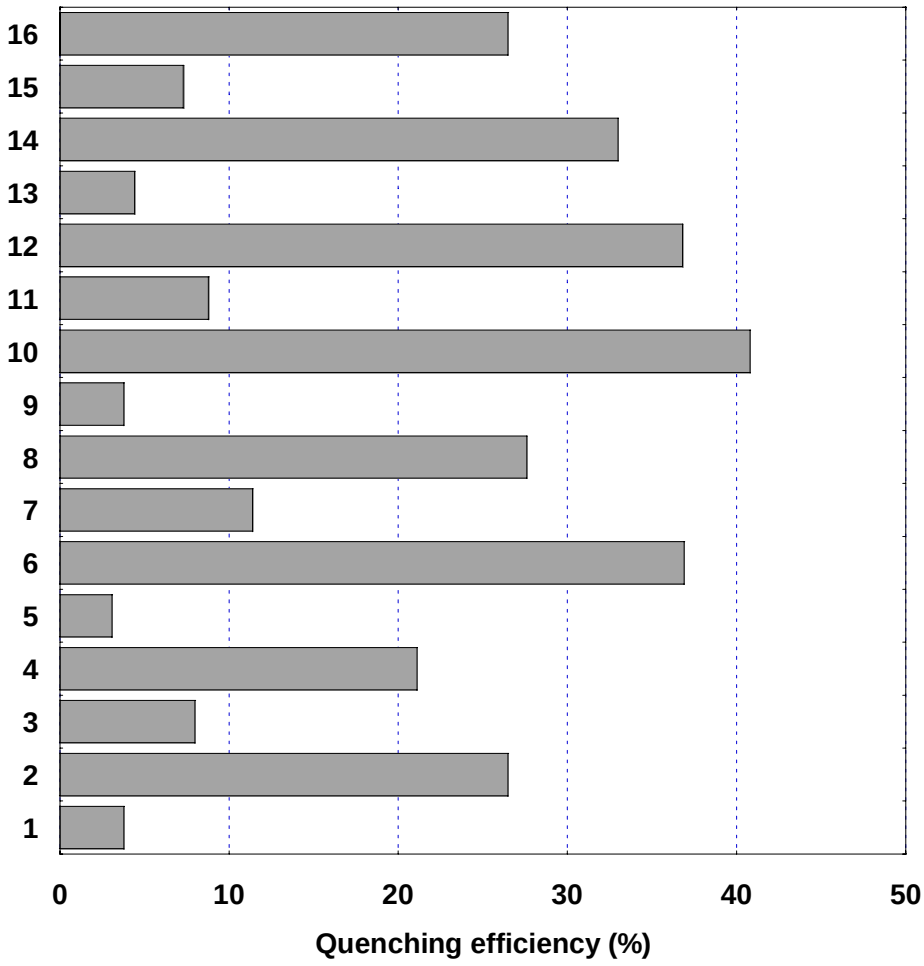


Figure S9. Effect of flanking nucleotides on the fluorescence quenching efficiency of 6,7-dimethyllumazine. DNA duplexes are 3'-AGA CGC AGG TN'G TTG CGT GTG-5′/5′-TCT GCG TCC AX'C AAC GCA CAC-3′, and N'/X' means 1) AAA/TXT 2) AAC/GXT 3) AAG/CXT 4) AAT/AXT 5) CAA/TXG 6) CAC/GXG 7) CAG/CXG 8) CAT/AXG 9) GAA/TXC 10) GAC/GXC 11) GAG/CXC 12) GAT/AXC 13) TAA/TXA 14) TAC/GXA 15) TAG/CXA 16) TAT/AXA. N' denotes adenine and its flanking bases. X' denotes the AP site and its flanking bases. Quenching efficiency (%) is given by $(F_o\text{-}F)/F_o\times100$, where F_o and F denote fluorescence intensities of 6,7-dimethyllumazine in the absence and presence of DNA duplexes, respectively. [DNA duplex] = 1.0 μM, [6,7-dimethyllumazine] = 1.0 μM in a solution buffered to pH 7.0 (10 mM sodium cacodylate containing 100 mM NaCl, and 1.0 mM EDTA). λ_{ex} = 350.5 nm, λ_{em}= 466 nm, 5°C.