Selective Lighting Up of Epiberberine Alkaloid Fluorescence by Fluorophore-Switching Aptamer and Stoichiometric Targeting of Human Telomeric DNA G-Quadruplex Multimer

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This Material includes abbreviation list, experimental details for Fluorescence quantum yield and DNA melting (T_m) measurements, structures of investigated isoquinoline alkaloids (**Scheme S1**), Oligonucleotides used in this work (**Table S1**), HTG sequence-dependent fluorescence intensities of the isoquinoline alkaloids (**Figure S1**), Job's plot analysis of the stoichiometry of EPI binding to TA[Q] (**Figure S2**), fluorescence titration of isoquinoline alkaloids with different G-quadruplexes (**Figure S3**), absorption spectra (**Figure S4**), fluorescence lifetime (**Table S2**), T_m results (**Figure S5**), alkaloid concentration-dependent fluorescence (**Figure S6**), coexistence of BER with EPI (**Figure S7**), K^+-induced conformation experiments and comparison with RNA (**Figure S8**), HTG multimer experiments (**Figure S9, S10, S11**), and references.

Abbreviations used in this work:

[Q]: 5′-$G_3(TTAG_3)_3$-3′

EPI: epiberberine

BER: berberine

PAL: palmatine

JAT: jatrorrhizine

COP: coptisine

WOR: worenine

SAN: sanguinarine

CHE: chelerythrine

NIT: nitidine

HTG: human telomeric G-quadruplex

FSA: fluorophore-switching aptamer

NMR: nuclear magnetic resonance

SPR: Surface PlasmonResonance

ATP: adenosine triphosphate

GFP: green fluorescent protein

IA: isoquinoline alkaloid

PA: protoberberine alkaloid

BA: benzophenanthridine alkaloid

Fluorescence quantum yield measurements. The fluorescence quantum yields of EPI and EPI-TA[Q] were determined using that of BER in ethanol solution as the standard (φ_s= 0.028)[1,2] according to the following equation:

$$\varphi_x = (A_s F_x n_x^2 / A_x F_s\, n_s^2) \cdot \varphi_s$$

where A, F and n are the absorption value at the excitation wavelength, the integrated area for the fluorescence emission spectrum that is corrected after background subtraction, and the refractive index of the solvent, respectively. The fluorescence quantum yield of EPI alone in aqueous solution and EPI-TA[Q] are estimated to be 0.015 and 0.41, respectively. The fluorescence quantum yield of the DNA-bound EPI is obtained at excess of TA[Q]. The high quantum yield of EPI-TA[Q] can be also confirmed by the UV illuminated photographs shown in Figure 2 and 3.

DNA melting temperature (T_m) measurements. The melting temperatures (T_m) of the DNAs in the presence and absence of protoberberine alkaloids were determined using a UV2550 spectrophotometer (Shimadzu Corp., Kyoto, Japan), equipped with a TMSPC-8 T_m analysis system which can simultaneously control the chamber temperature and detect up to eight samples with a micro multi-cell. The absorbance of G-quadruplex at 295 nm as a function of solution temperature was collected in 0.5 °C increment, with a 30-second equilibration time applied after each temperature increment. It has been reported that benzophenanthridine alkaloids suffer from easy hydroxylation[3] (especially at the high solution temperature required for the T_m measurements) and bind to G-quadruplex with less structure specificity than protoberberine alkaloids.[4] Thus, the T_m measurements were carried out only for protoberberine alkaloids. A sigmoidal fitting method was used to estimate the T_m value.

PA
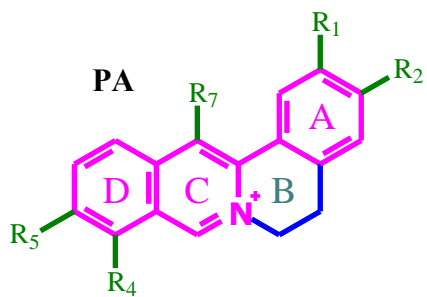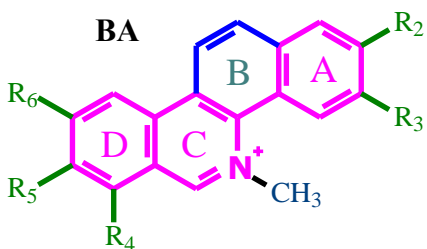
BER: $R_1, R_2 = $ -O-CH$_2$-O-; $R_4 = R_5 = $ -OCH$_3$; $R_7 = $ -H
PAL: $R_1 = R_2 = R_4 = R_5 = $ -OCH$_3$; $R_7 = $ -H
EPI: $R_1 = R_2 = $ -OCH$_3$; $R_4, R_5 = $ -O-CH$_2$-O-; $R_7 = $ -H
COP: $R_1, R_2 = $ -O-CH$_2$-O-; $R_4, R_5 = $ -O-CH$_2$-O-; $R_7 = $ -H
JAT: $R_1 = $ -OCH$_3$; $R_2 = $ -OH; $R_4 = R_5 = $ -OCH$_3$; $R_7 = $ -H
WOR: $R_1, R_2 = $ -O-CH$_2$-O-; $R_4, R_5 = $ -O-CH$_2$-O-; $R_7 = $ -CH$_3$

BA

SAN: $R_2, R_3 = R_4, R_5 = $ -O-CH$_2$-O-; $R_6 = $ -H
CHE: $R_2, R_3 = $ -O-CH$_2$-O-; $R_4 = R_5 = $ -OCH$_3$; $R_6 = $ -H
NIT: $R_2, R_3 = $ -O-CH$_2$-O-; $R_5 = R_6 = $ -OCH$_3$; $R_4 = $ -H

**Scheme S1.** Structures of investigated isoquinoline alkaloids and their substituent patterns.

Table S1. Oligonucleotides used in this work[a]

Entry	Name	Sequence	Entry	Name	Sequence
1	[Q]	5′-G$_3$(TTAG$_3$)$_3$-3′	13	TA[Q]TT	5′-TAG$_3$(TTAG$_3$)$_3$TT-3′
2	A[Q]	5′-AG$_3$(TTAG$_3$)$_3$-3′	14	[Q]T	5′-G$_3$(TTAG$_3$)$_3$T-3′
3	T[Q]	5′-TG$_3$(TTAG$_3$)$_3$-3′	15	[Q]TA	5′-G$_3$(TTAG$_3$)$_3$TA-3′
4	TA[Q]	5′-TAG$_3$(TTAG$_3$)$_3$-3′	16	mPu22	5′-TGAG$_3$TG$_4$AG$_3$TG$_4$AA-3′
5	AT[Q]	5′-ATG$_3$(TTAG$_3$)$_3$-3′	17	1XAV	5′-TGAG$_3$TG$_3$TAG$_3$TG$_3$TAA-3′
6	AA[Q]	5′-AAG$_3$(TTAG$_3$)$_3$-3′	18	PS2.M	5′-GTG$_3$TAG$_3$CG$_3$TTGG-3′
7	TT[Q]	5′-TTG$_3$(TTAG$_3$)$_3$-3′	19	T30695	5′-(G$_3$T)$_4$-3′
8	TA[Q]A	5′-TAG$_3$(TTAG$_3$)$_3$A-3′	20	2O3M	5′-(AG$_3$)$_2$CGCTG$_3$AGGAG$_3$-3′
9	TA[Q]T	5′-TAG$_3$(TTAG$_3$)$_3$T-3′	21	T$_3$TT	5′-G$_3$TTTG$_3$TG$_3$TG$_3$-3′
10	TTA[Q]	5′- TTAG$_3$(TTAG$_3$)$_3$-3′	22	T$_3$TT$_3$	5′-G$_3$TTTG$_3$TG$_3$TTTG$_3$-3′
11	TTA[Q]TT	5′-TTAG$_3$(TTAG$_3$)$_3$TT-3′	23	T$_3$T$_3$T$_3$	5′-G$_3$TTTG$_3$TTTG$_3$TTTG$_3$-3′
12	AAA[Q]AA	5′-A$_3$G$_3$(TTAG$_3$)$_3$AA-3′	24	rUA[Q]	r(5′-UAG$_3$(UUAG$_3$)$_3$-3′)

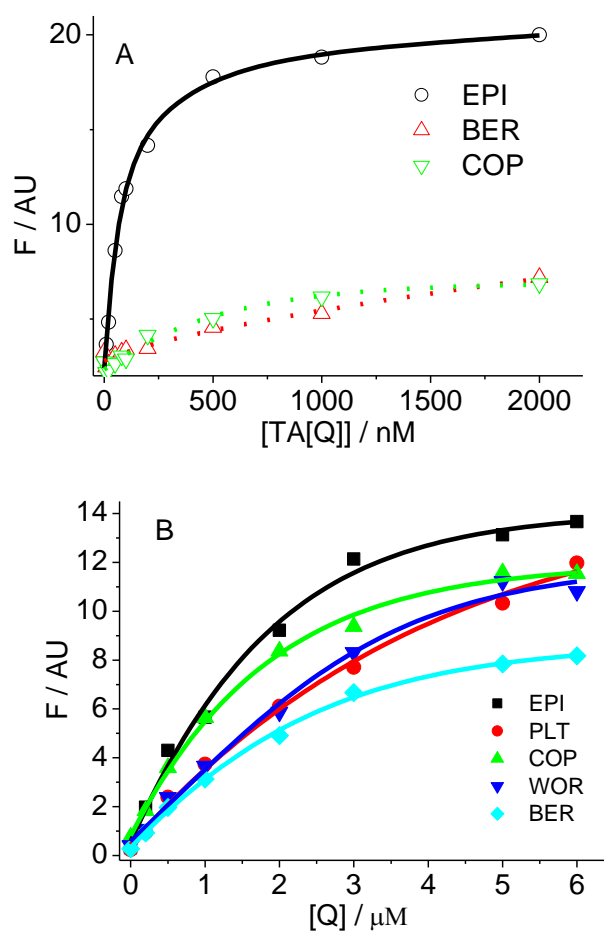[a] The sequences for the long telomeric DNA G-quadruplex multimeres are shown in the text.
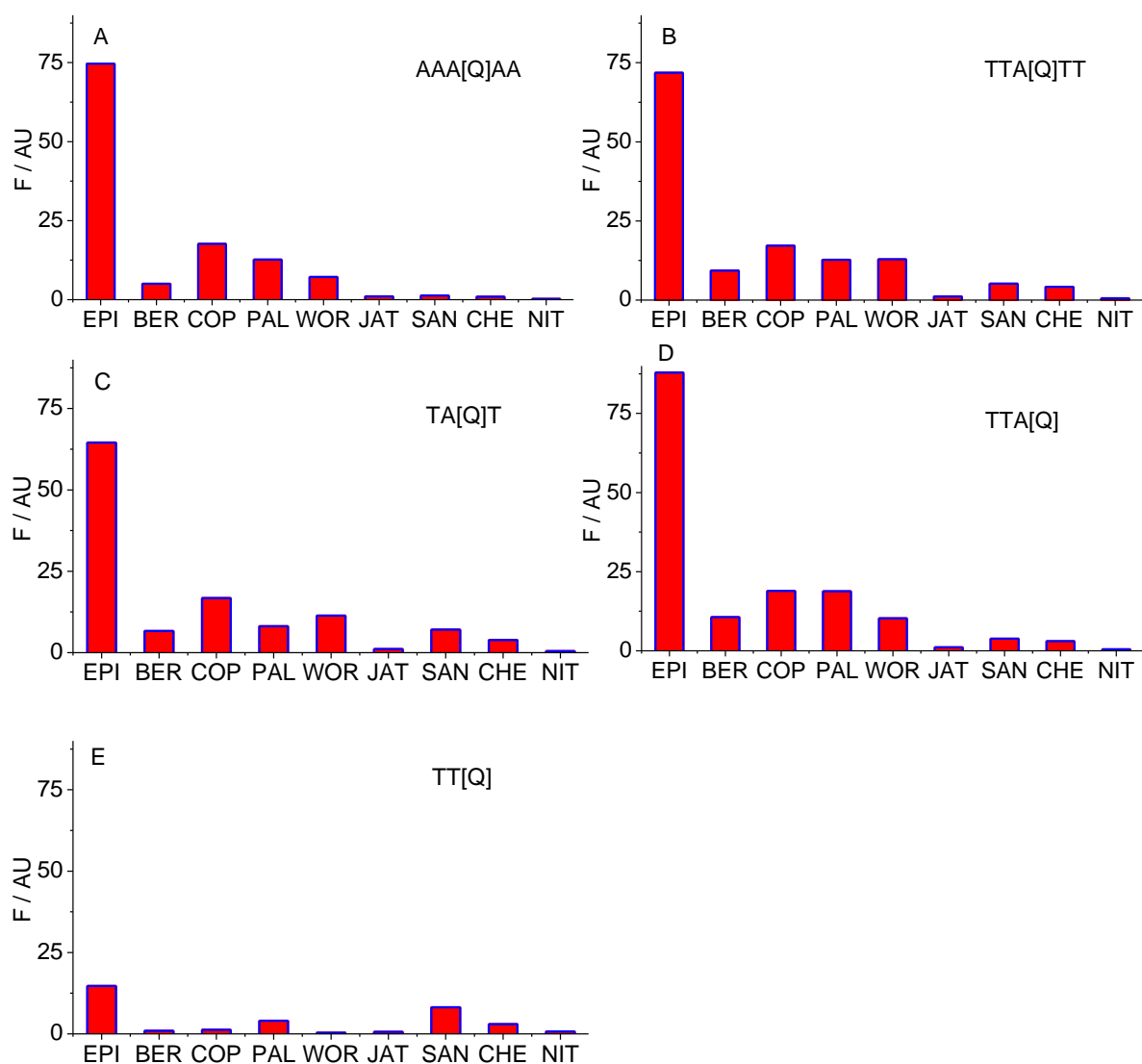
Figure S1. Fluorescence intensities of the investigated isoquinoline alkaloids (1 µM) in the presence of 1 µM AAA[Q]AA, TTA[Q]TT, TA[Q]T, TTA[Q], and TT[Q], respectively.
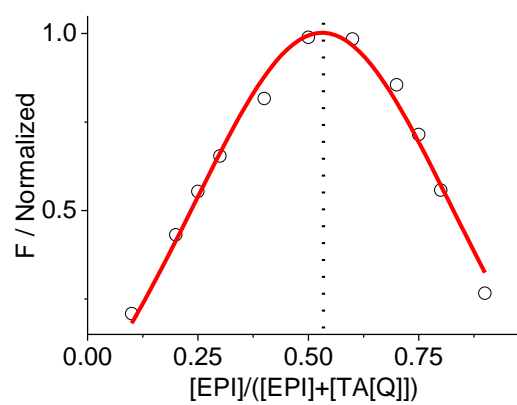
Figure S2. Job's plot analysis of the stoichiometry of EPI binding to TA[Q].

Figure S3. (A) Fluorescence intensity of isoquinoline alkaloids (20 nM) in 0.1 M K$^+$ is enhanced upon the addition of TA[Q]. The solid line for EPI is the fitted curve assuming a 1:1 binding of EPI to the aptamer. The dotted lines for BER and COP are only for clarity, without fitting due to the weak fluorescence especially at the low alkaloid concentrations. The fitting gives a binding constant of $(2.0\pm0.2)\times10^7$ M^{-1} for the EPI binding. (B) Fluorescence intensity of isoquinoline alkaloids (0.5 μM) in 0.1 M K$^+$ is also enhanced upon the addition of the core [Q] of G-quadruplex. The fitting gives the [Q] binding constants of $(7.1\pm1.3)\times10^5$, $(2.4\pm0.4)\times10^5$, $(8.8\pm1.3)\times10^5$, $(2.9\pm0.7)\times10^5$, and $(4.6\pm0.6)\times10^5$ M^{-1} for EPI, PLT, COP, WOR, and BER, respectively.
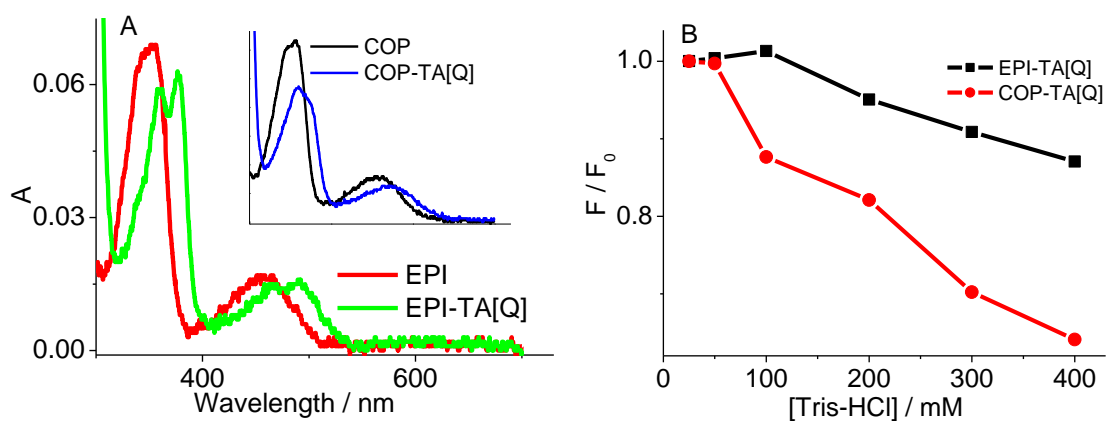
Figure S4. (A) Absorption spectra of EPI (5 µM) in 25 mM Tris-HCl containing 0.1 M KCl in the absence and presence of TA[Q] (10 µM). Inset: The absorption spectra for COP at the same experimental condition. (B) Effect of increasing Tris-HCl (pH 7.5) concentration on the fluorescence intensity of 1 µM EPI and COP in the presence of 1 µM TA[Q]. The solutions always contain 0.1 M KCl. F_0 and F are the fluorescence intensities at 25 mM and increased Tris-HCl concentrations, respectively.

Table S2 Fluorescence decay constants of EPI and COP (0.5 µM) in the presence of TA[Q] (2 µM). τ_0 is the average lifetime that is calculated from the bi-exponential lifetimes of τ_1 and τ_2.

|	τ_1 / ns	τ_2 / ns	τ_0 / ns	χ^2
EPI-TA[Q]	2.47 (16.9 %)	13.05 (83.1 %)	11.26	1.009
COP-TA[Q]	0.79 (18.5 %)	10.28 (81.5 %)	8.53	1.065
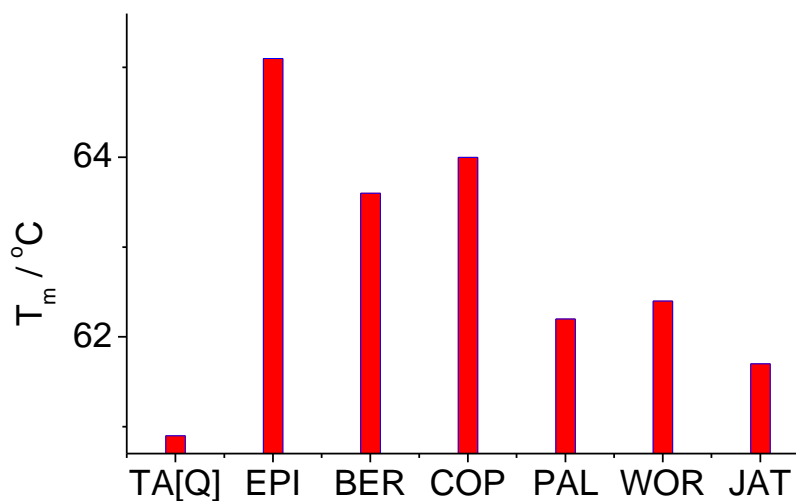
Figure S5. TA[Q] melting temperature (T_m) in the absence and presence of the protoberberine alkaloids. The melting temperatures were obtained in phosphate buffer (25 mM, pH 7.5) containing 0.1 M K^+, 2 μM TA[Q], and 4 μM alkaloids.
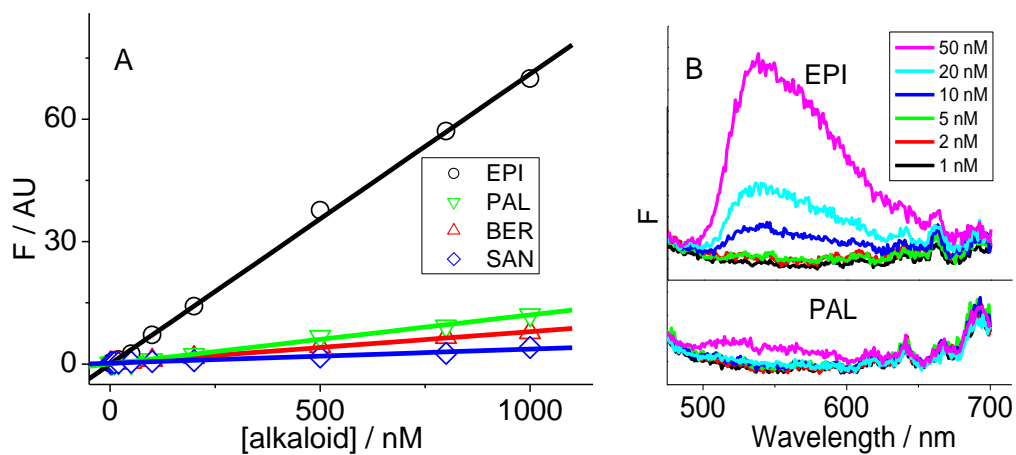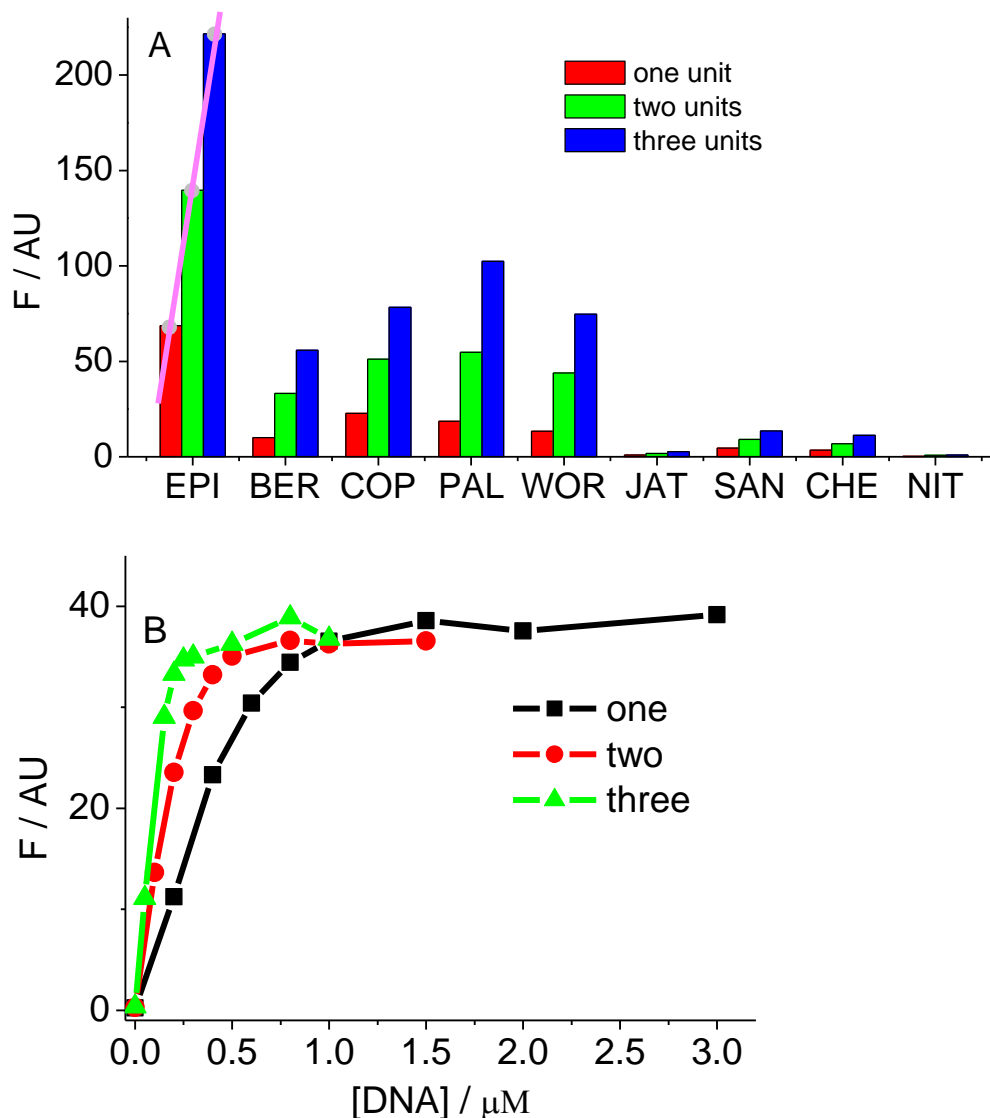
Figure S6. (A) Fluorescence intensity of alkaloids at 540 nm as a function of their concentrations between 0 and 1000 nM in the presence of 1 µM TA[Q]. (B) Fluorescence emission spectra of EPI and PAL at 1-50 nM. TA[Q]: 1 µM.
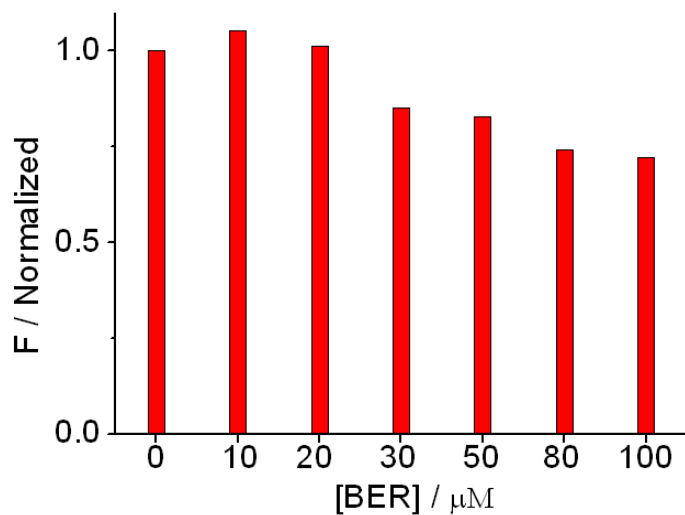
Figure S7. Fluorescence intensity of 1 µM EPI solution in the presence of 1 µM TA[Q] with the coexistence of 0, 10, 20, 30, 50, 80, 100 µM BER. Coexistence of BER with up to 20 times higher concentration produces little effect on the EPI fluorescence response. Additionally, EPI fluorescence decreases only 25% by increasing the BER concentration up to 100 times higher than that of EPI.
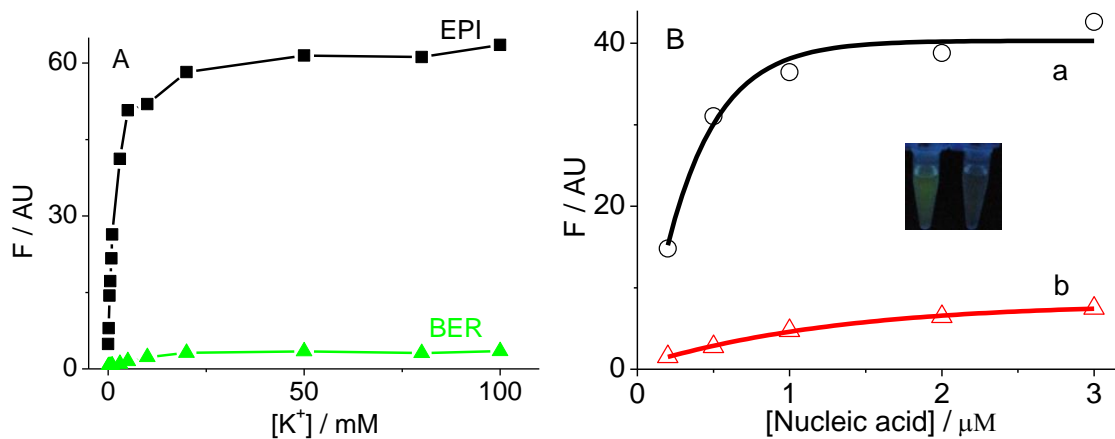
Figure S8. (A) Fluorescence intensity of 1 μM alkaloid (EPI, BER) and 1 μM TA[Q] in 25 mM Tris-HCl buffer (pH 7.5) containing 0.1 M Na^+ upon increasing K^+ concentration. Excitation wavelength: 377 nm. (B) Dependence of fluorescence intensity of EPI (0.5 μM) at 540 nm with the addition of TA[Q] and rUA[Q] at increasing concentrations. The solid lines are the fitted curves assuming a 1:1 binding of EPI to the G-quadruplexes. Inset: Photographs of the solutions of TA[Q] (left) and rUA[Q] (right) under UV illumination.
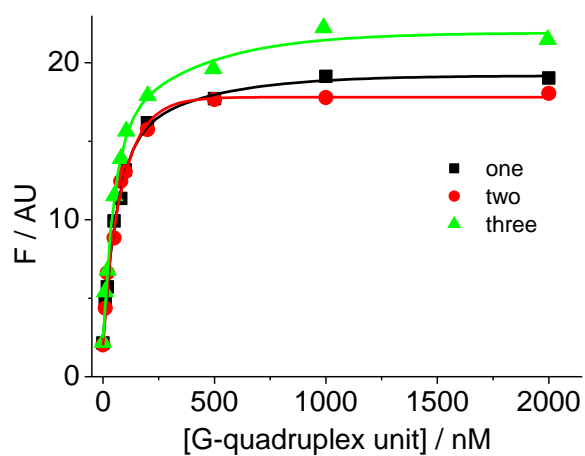
Figure S9. (A) Fluorescence intensity of the investigated isoquinoline alkaloids in the presence of DNAs (1 μM) containing one, two, and three G-quadruplex units (5′-(TTA[Q])$_n$TTA-3′ with n = 1, 2, 3), respectively. The concentrations of isoquinoline alkaloids are 1, 2, and 3 μM for the DNAs containing one, two, and three G-quadruplex units, respectively. The pink line shows the linear increase of EPI fluorescence upon increasing the number of G-quadruplex unit. EPI is the best alkaloid for targeting the long human telomeric G-quadruplexes. (B) Fluorescence titration of EPI (0.5 μM) in 0.1 M K$^+$ by the addition of DNAs of 5′-(TTA[Q])$_n$TTA-3′ (n = 1, 2, 3) containing one, two, and three G-quadruplex units, respectively. Note that the values of X-axis are the DNA strand concentration. The results with the X-axis values based on the G-quadruplex unit concentration are given in the main text.
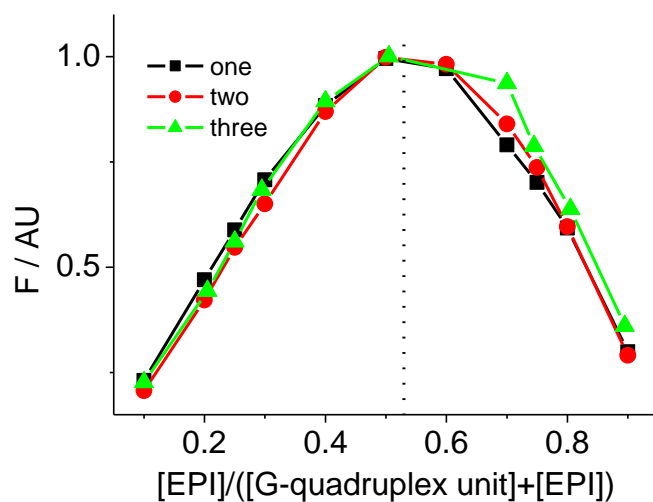
Figure S10. Job's plot analysis of the stoichiometric binding of EPI with DNAs of 5′-(TTA[Q])$_n$TTA-3′ (n = 1, 2, 3) containing one, two, and three G-quadruplex units, respectively. Note that the values of X-axis are the G-quadruplex unit concentration.

Figure S11. Fluorescence intensity of EPI (20 nM) in 0.1 M K$^+$ is enhanced upon the addition of DNAs of 5′-(TTA[Q])$_n$TTA-3′ (n = 1, 2, 3) containing one, two, and three G-quadruplex units, respectively. The solid lines are the fitted curves assuming a 1:1 binding of EPI to the G-quadruplex unit. The binding constants obtained are $(2.6\pm0.5)\times10^7$, $(3.1\pm0.6)\times10^7$, and $(2.9\pm0.5)\times10^7$ M^{-1}, respectively. Note that the values of X-axis are the G-quadruplex unit concentration, but not the strand concentration.

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