Electronic Supplementary Material (ESI) for Photochemical and Photobiological Science

Complexes of the antitumoral drugs Doxorubicin and Sabarubicin with telomeric G-quadruplex in basket conformation: ground and excited state properties

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Supplementary Information

S1) Absorption and Fluorescence spectra of the drugs 1 and 2

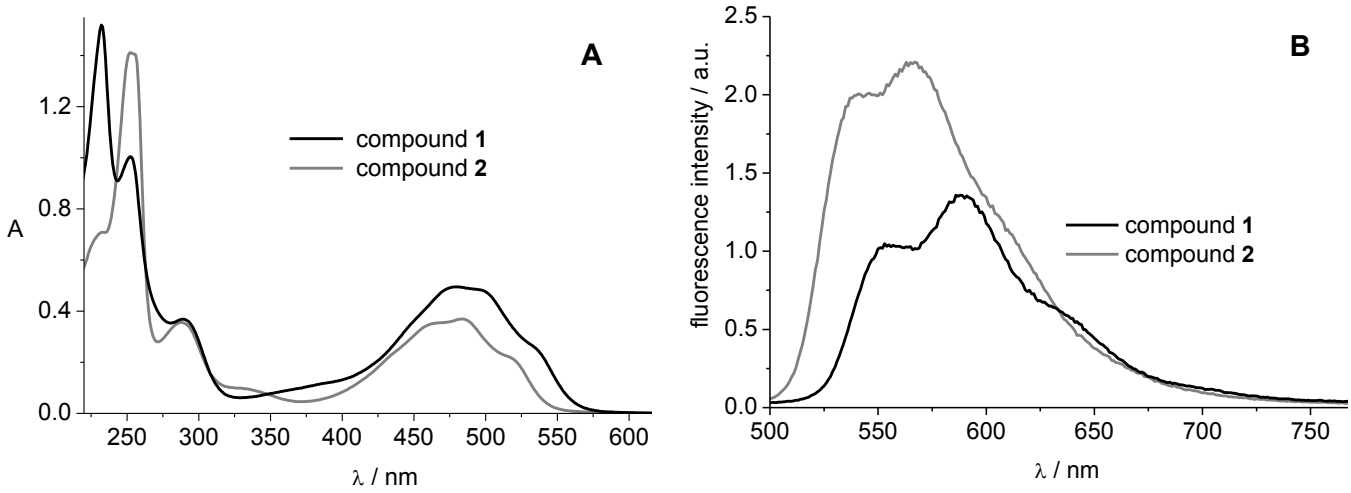

Fig. S1 (A) Absorption spectra of 4.9×10^{-5}M drug solution in TRIS/EDTA/NaCl buffer at pH 7.4, l =1.0 cm. (B) Fluorescence spectra normalized for absorbance at the excitation wavelength 485 nm of 1×10^{-5} M drug solution in TRIS/EDTA/NaCl buffer, pH 7.4.

S2) Global analysis of equilibrium spectroscopic data with Singular Value Decomposition and non linear regression modelling.

Modeling and fitting of the equilibrium titration spectroscopic data sets were performed using the commercial multivariate data analysis program SPECFIT/32^{TM}, based on the publications of A. Zuberbühler at the University of Basel, Switzerland (see refs. RS1 and RS2). Multiwavelength spectroscopic data are arranged in a matrix **Y** of dimension Nm × Nw , where Nw is the number of wavelengths, and Nm corresponds to the number of measurements (spectra), each for a couple of ligand and receptor concentrations. Thus each element Y_{ij} of the data matrix **Y** corresponds to an experimental quantity (absorbance, ellipticity, fluorescence intensity) at wavelength j for a given couple i of concentrations of ligand and receptor (typically in our experiments one of them is kept constant). The experimental 3D data matrix (**Y**) is first reduced to a much smaller basis set of concentration (**U**×**S**) and spectral (**V**) eigenvectors by Singular Value Decomposition (SVD), where the matrix product, **Y**' = **U**×**S**×**V**, is the least squares best estimator of the original data matrix. This **Y'** matrix of dimension Nm × Nw contains less noise than **Y** because the SVD procedure can factor

random noise from the principal components. **Y'** is utilized in the global fitting instead of the original data matrix **Y**. The global fitting procedure is designed to fit the concentration (**U**×**S**) eigenvector basis set with replacement of linear (amplitude) parameters by their linear regression best estimates. Complexation equilibria are solved assuming a complexation model (i.e. contemporary presence of complexes of given stoichiometries in equilibrium with free species in solution) and optimizing the numeric combination of the spectroscopic contributions of all "colored" species to best reproduce the **Y'** values. Optimization is performed by the least square method, using the Levenberg-Marquardt algorithm, for all the explored wavelengths and ligand-receptor concentration couples. The output from the global fitting procedure consists of the optimized association constants, the concentration profiles of the "colored" species and their individual spectra. The fits were evaluated on the basis of their Durbin-Watson (DW) factors. The DW test is very useful to check for the presence of auto-correlation in the residuals. This method is recommended for systematic misfit errors that can arise in titration experiments. It examines the tendency of successive residual errors to be correlated. The Durbin-Watson statistics ranges from 0.0 to 4.0, with an optimal mid-point value of 2.0 for uncorrelated residuals (i.e., no systematic misfit). In contrast to the χ^2 (Chi-squared) statistics, which requires the noise in the experimental data is random and normally distributed, the DW factor is meaningful even when the noise level in the data set is low. Since the factorized data usually have a significantly lower noise level than the original data, DW factor is ideal for the present type of data.

The SVD analysis of the data relevant to the fluorescence titration of **1** resulted to be the following:

[FACTOR ANALYSIS]
Tolerance = 1.000E-09
Max. Factors = 10
Num. Factors = 3
Significant = 1
Eigen Noise = 7.998E+02
Exp't Noise = 3.999E+02

#	Eigenvalue	Square Sum	Residual	Prediction
1	1.626E+13	2.310E+09	7.998E+02	Data Vector
2	9.370E+08	1.373E+09	6.167E+02	Probably Noise
3	2.888E+08	1.084E+09	5.481E+02	Probably Noise

The SVD analysis of the data relevant to the fluorescence titration of **2** resulted to be the following:

[FACTOR ANALYSIS]
Tolerance = 1.000E-09
Max.Factors = 10
Num.Factors = 3
Significant = 1
Eigen Noise = 7.705E+02
Exp't Noise = 3.852E+02

#	Eigenvalue	Square Sum	Residual	Prediction
1	5.828E+13	2.144E+09	7.705E+02	Data Vector
2	1.467E+09	6.763E+08	4.328E+02	Probably Noise
3	6.462E+08	3.012E+07	9.136E+01	Probably Noise

From the factor analysis for both compounds we retrieved only one emissive species. We tried to fit with exclusive presence of a 1:1 or a 2:1 complex or contemporary presence of 1:1 and 2:1 complexes admitting fluorescence from just one species, *i.e.* the free drug, but we could not obtain a satisfactory fit. The same binding models were tested including also fluorescence from 1:1 and/or 2:1 complexes. The best complexation model on the basis of the DW factor resulted to be that with 1:1 and 2:1 drug:21-mer complexes with binding constants $\log(K_{11}/M^{-1}) = 5.5\pm0.1$ and $\log(K_{21}/M^{-2}) = 10.8\pm0.2$ (DW = 1.8) for **1** and $\log(K_{11}/M^{-1}) = 5.6\pm0.1$ and $\log(K_{21}/M^{-2}) = 11.1\pm0.2$ (DW = 2.5) for **2**. In the case of drug **2** the fluorescence spectrum in the absence of 21-mer was fixed in the fitting procedure. This was not possible for drug **1** because of the presence of dimers in solution. The 2:1 complexes of **1** and **2** resulted emissive with a spectrum very similar in shape to that of the free drug, but less intense, and the 1:1 complexes were non emissive. On our opinion these analyses are reliable since the binding constants are in reasonable agreement with those determined by Isothermal Titration Calorimetry (ITC). Inclusion of the dimerization equilibrium in the case of **1** did not allow to obtain convergence.

An example of the quality of the agreement between the experimental data and the best fits is shown in Fig. S2 and S3.

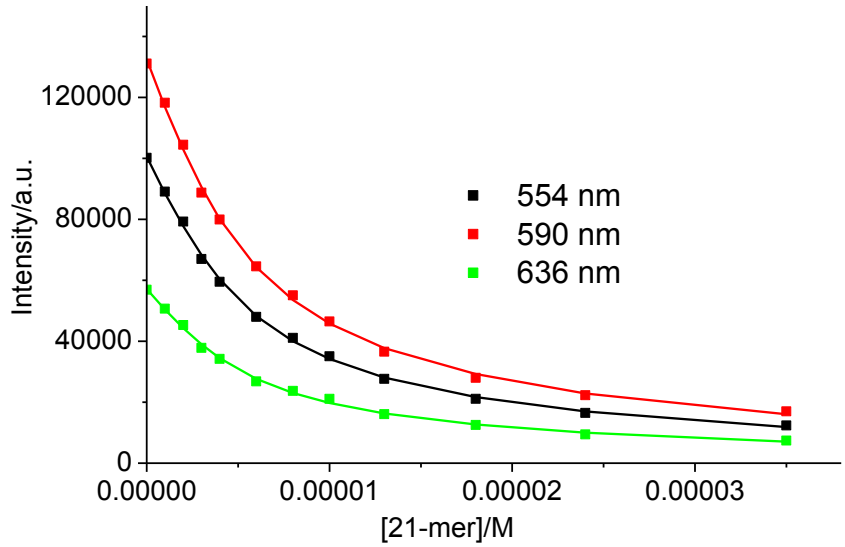

Fig. S2. Fluorescence intensity at key wavelengths for excitation at 485 nm of a 1×10^{-5} M **1** solution titrated with 21-mer in TRIS/EDTA/NaCl buffer, pH 7.4. Symbols, experimental values; lines, calculated values with $\log(K_{11}/M^{-1}) = 5.5$ and $\log(K_{21}/M^{-2}) = 10.8$.

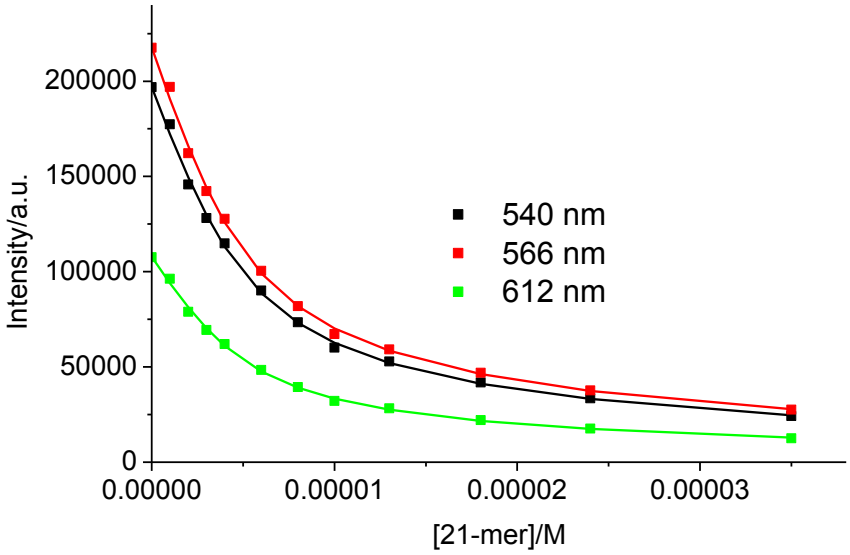

Fig. S3. Fluorescence intensity at key wavelengths for excitation at 485 nm of a 1×10^{-5} M **2** solution titrated with 21-mer in TRIS/EDTA/NaCl buffer, pH 7.4. Symbols, experimental values; lines, calculated values with $\log(K_{11}/M^{-1}) = 5.6$ and $\log(K_{21}/M^{-2}) = 11.1$.

We performed similar analyses of the absorption titration data in Fig. 3A and 4A in the manuscript. SVD yielded a number of coloured species consistent with the applied binding model involving three absorbing species (the free drug, the 1:1 and the 2:1 complexes). The best fit for **1** gave $\log(K_{11}/M^{-1}) = 5.6\pm0.3$ and $\log(K_{21}/M^{-2}) = 11.2\pm0.7$ (Durbin Watson factor = 2.5) in good agreement with the fluorescence results and ITC analysis, for **2** $\log(K_{11}/M^{-1}) = 5.7\pm0.4$ with

log(K_{21}/M^{-2}) fixed as 11.1 (DW 1.7) also in agreement with the fluorescence analysis. In the following Fig. S4 the quality of the agreement between experimental and calculated absorbances at key wavelengths is shown for compound **1**.

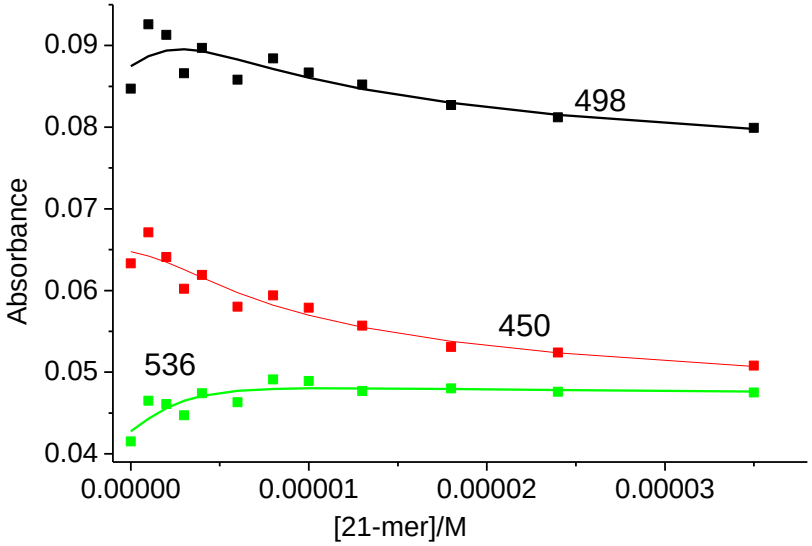

Fig. S4. Absorbance at key wavelengths of compound **1**, 1×10^{-5} M, titrated with 21-mer in TRIS/EDTA/NaCl buffer at pH 7.4, d=1.0 cm. Symbol, experimental values; line, calculated values with log(K_{11}/M^{-1}) = 5.64 and log(K_{21}/M^{-2}) = 11.16.

The UV-Vis circular dichroism variations for **1** and **2** titrated with 21-mer in Fig. 8A and 9A, respectively, were also analysed using the same approach. The number of colored species is 3 (the free drug, the 1:1 and the 2:1 complexes). The best fit binding constants were log(K_{21}/M^{-2}) = 11.8±0.6 (Durbin Watson factor = 1.8) with log(K_{11}/M^{-1}) = 5.5 (fixed) for **1** and log(K_{21}/M^{-2}) =10.1±0.7 with log (K_{11}/M^{-1}) = 5.6 fixed (Durbin Watson factor = 1.6) for **2**. The spectra of Fig. 8B and 9B were extracted. Examples of the quality of CD data reproduction for **1** and **2** are in Fig. S5 and S6.

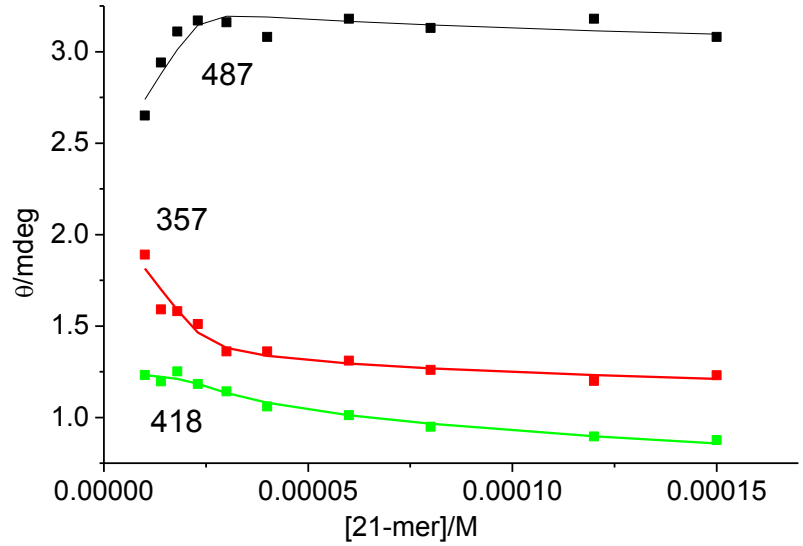

Fig. S5. Ellipticity at key wavelength of compound **1**, 5×10^{-5} M titrated with 21-mer in TRIS/EDTA/NaCl buffer at pH 7.4, d=1.0 cm. Symbol, experimental values; line, calculated values with $\log(K_{11}/M^{-1}) = 5.46$ and $\log(K_{21}/M^{-2}) = 11.80$.

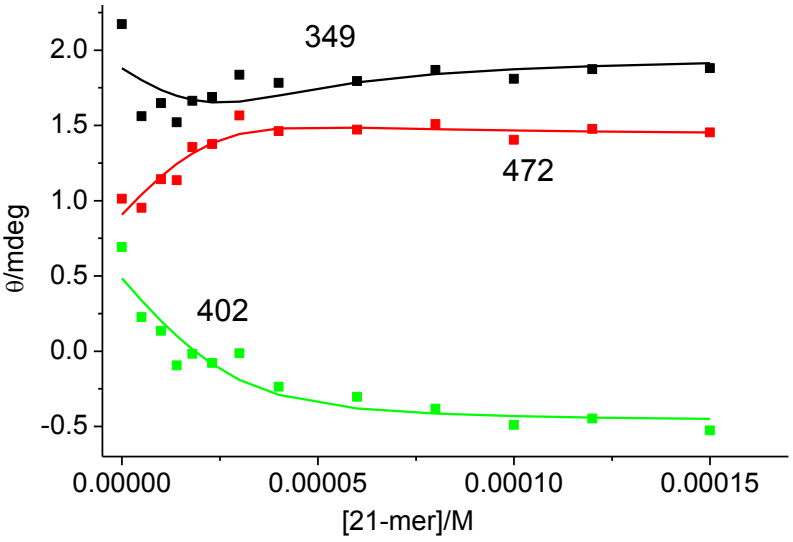

Fig. S6. Ellipticity at key wavelength of compound **2**, 5×10^{-5} M titrated with 21-mer in TRIS/EDTA/NaCl buffer at pH 7.4, d=1.0 cm. Symbol, experimental values; line, calculated values with $\log(K_{11}/M^{-1}) = 5.56$ and $\log(K_{21}/M^{-2}) = 10.13$.

References

RS1. Harald Gampp, Marcel Maeder, Charles J. Meyer, and Andreas D. Zuberbühler "Calculation Of Equilibrium Constants From Multiwavelength Spectroscopic Data I. Mathematical Considerations", Talanta, 1985, 32, 95-101.

RS2. Harald Gampp, Marcel Maeder, Charles J. Meyer, and Andreas D. Zuberbühler "Calculation Of Equilibrium Constants From Multiwavelength Spectroscopic Data II. SPECFIT: Two User Friendly Programs In BASIC And Standard FORTRAN 77", Talanta, 1985, 32, 257-264.