Diaza-18-crown-6 hydroxyquinoline derivatives as flexible tools for the assessment and imaging of total intracellular magnesium

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Supporting Information

Title

Diaza-18-crown-6 hydroxyquinoline derivatives as flexible tools for the assessment and imaging of total intracellular magnesium

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1. General Information and Instrumentation

General Spectroscopic Methods. All reagents were Ultrapure grade. The fluorescent probes were synthesized as reported in [1] and were dissolved in dimethyl sulfoxide (DMSO) to a final concentration of 1 mg/mL (DCHQ1 1.7 mM; DCHQ3 1.4 mM; DCHQ4 1.4 mM; DCHQ5 1.4 mM; DCHQ6 1.3 mM). Aliquots were kept at 4°C in the dark.

Photophysical measurements. Absorption spectra were recorded on a Perkin-Elmer Lambda 45 spectrophotometer.For the fluorescence spectroscopy measurements, uncorrected emission and corrected excitation spectra were obtained with a Perkin–Elmer LS 55 spectrofluorimeter.

Cell Culture. HL60 (Human Promyelocytic Leukemia) cells were grown at 37°C and 5% CO_2 in RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum (FCS), 2 mM L-glutamine, 1000 units/mL penicillin and 1 mg/mL streptomycin. Differentiation of HL60 cells was induced by treatment with 1.3 % DMSO for at least 3 days, as reported in [2].

Flow Cytometric Assay of DNA content in DMSO-treated HL60 cells. Control and DMSO-differentiated HL60 cells were collected following the Nusse protocol for the assessment of total DNA content.[3] Briefly, cell suspensions were washed and resuspended at a final concentration of $2x10^6$ cells/mL in Nusse-1 Solution (NaCl 0.584 g/L, trisodic citrate 1.139 g/L, DNA free RNAsi 10 mg/L, Nonidet P-40 0.03 % v/v).

After 30 minutes of incubation at 4°C, an equal volume of Nusse-2 Solution was added (sucrose 85.5 g/L, citric acid 15 g/L, propidium iodide 100 mg/L). Samples were analyzed on an Epics-XL cytometer (Beckman Coulter, USA) with excitation band centered on 488 nm and emission band on 605 nm in linear scale.

2. Photophysical properties of DCHQ1

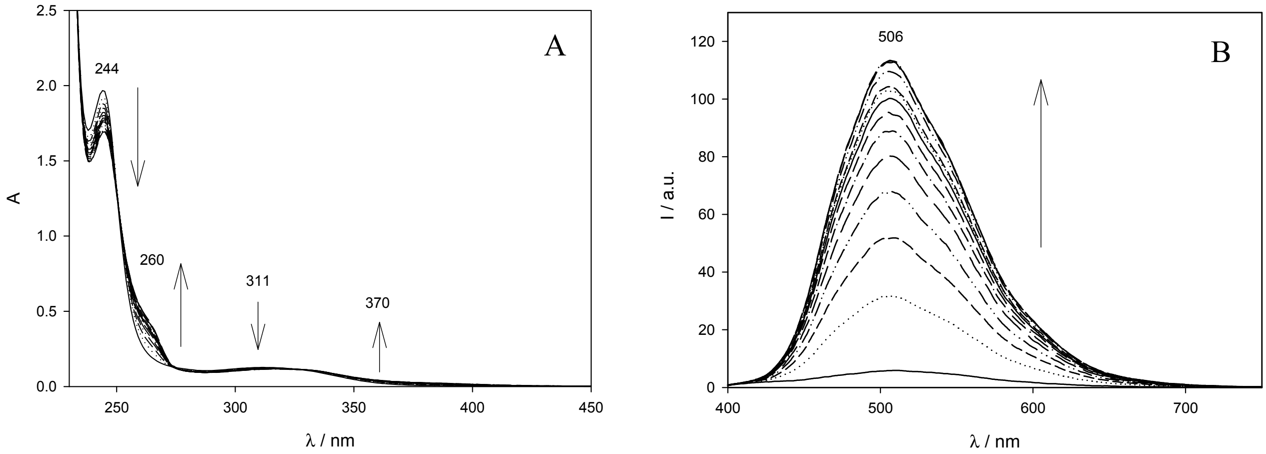

Figure 1 Titration with Mg^{2+}. Absorption (panel A) and emission (λ_{exc} = 324 nm, panel B) spectra of DCHQ1 (25 μM) in 1:1 CH_3OH:H_2O mixture buffered at pH=7.4 with MOPS at room temperature upon addition of increasing amounts (up to 2 equivalents) of $Mg(NO_3)_2 \cdot 6H_2O$solution (2.66 mM).

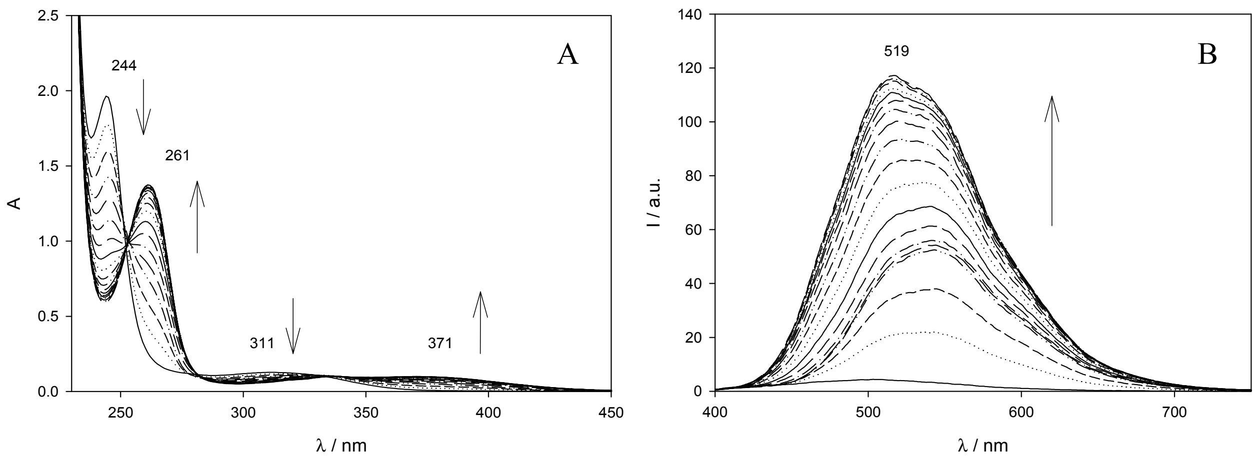

Figure 2 Titration with Zn^{2+}. Absorption (panel A) and emission (λ_{exc} = 324 nm, panel B) spectra of DCHQ1 (25 μM) in 1:1 CH_3OH:H_2O mixture buffered at pH=7.4 with MOPS at room temperature upon addition of increasing amounts (up to 3 equivalents) of $Zn(NO_3)_2 \cdot 6H_2O$ solution (1.89 mM).

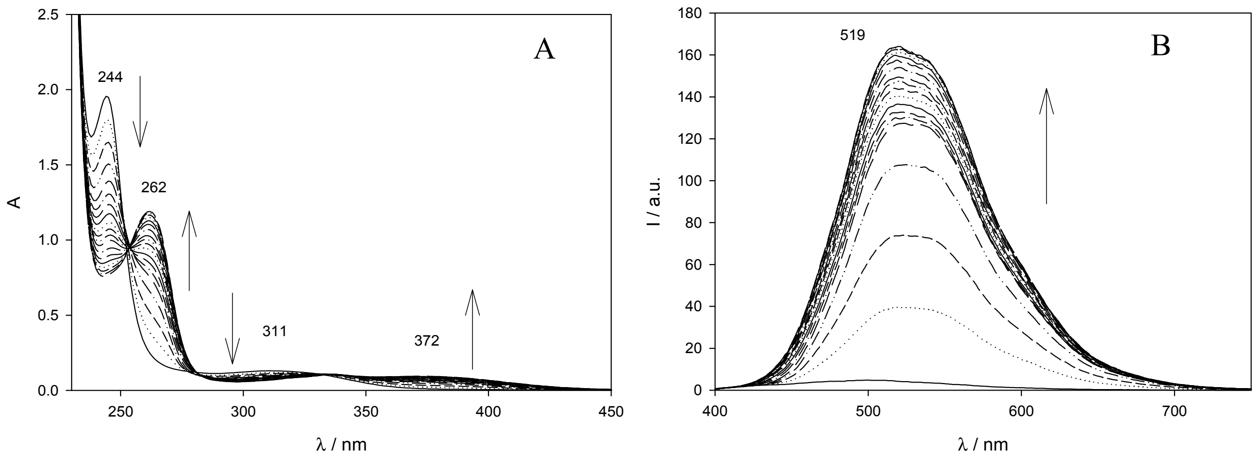

Figure 3 Titration with Cd^{2+}. Absorption (panel A) and emission (λ_{exc} = 330 nm, panel B) spectra oF DCHQ1 (25 μM) in 1:1 CH_3OH:H_2O mixture buffered at pH=7.4 with MOPS at room temperature upon addition of increasing amounts (up to 2.5 equivalents) of $Cd(NO_3)_2 \cdot 4H_2O$ solution (1.60 mM).

3. Photophysical properties of DCHQ3

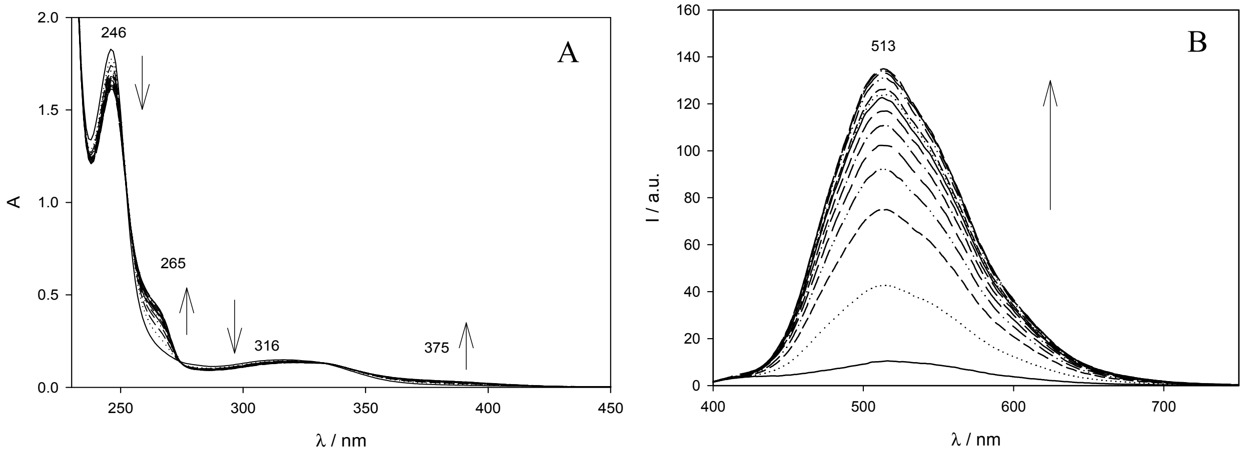

Figure 4 Titration with Mg^{2+}. Absorption (panel A) and emission (λ_{exc} = 336 nm, panel B) spectra of DCHQ4 (25 μM) in 1:1 CH_3OH:H_2O mixture buffered at pH=7.4 with MOPS at room temperature upon addition of increasing amounts (up to 1.5 equivalents) of $Mg(NO_3)_2 \cdot 6H_2O$ solution (2.50 mM).

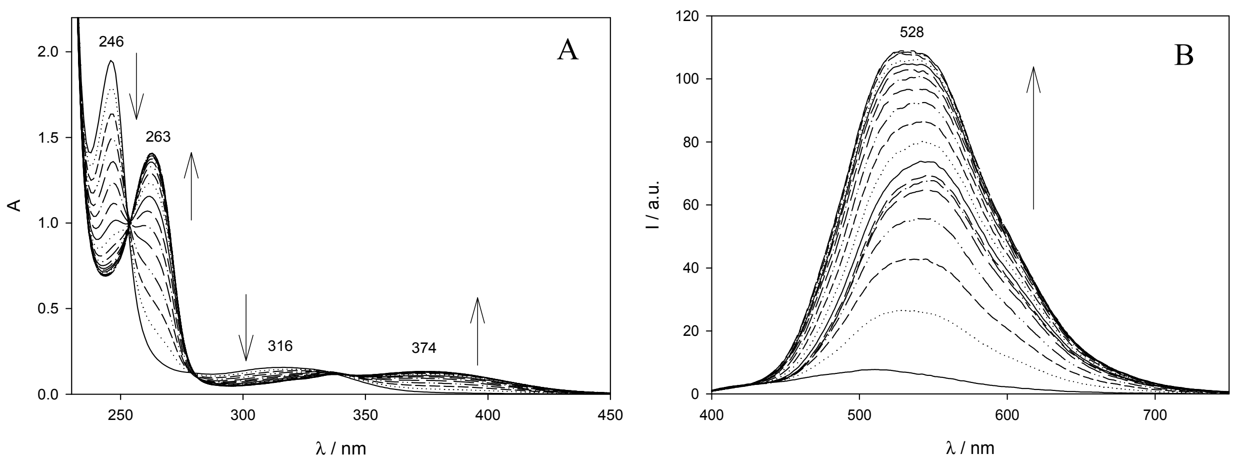

Figure 5 Titration with Zn^{2+}. Absorption (panel A) and emission (λ_{exc} = 336 nm, panel B) spectra oF DCHQ4 (25 μM) in 1:1 CH_3OH:H_2O mixture buffered at pH=7.4 with MOPS at room temperature upon addition of increasing amounts (up to 2.5 equivalents) of $Zn(NO_3)_2 \cdot 6H_2O$ solution (2.50 mM).

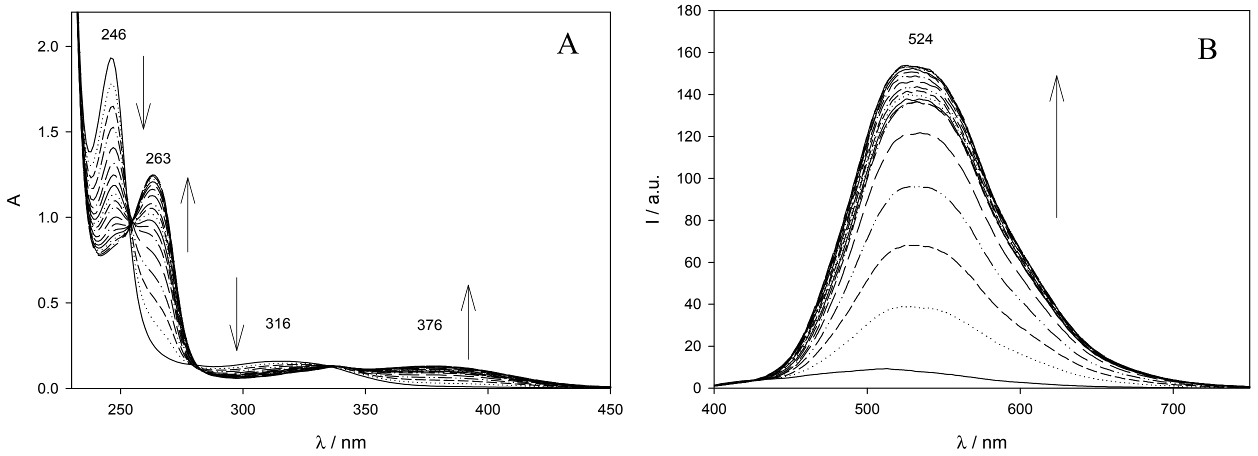

Figure 6 Titration with Cd^{2+}. Absorption (panel A) and emission (λ_{exc} = 336 nm, panel B) spectra of DCHQ4 (25 μM) in 1:1 CH_3OH:H_2O mixture buffered at pH=7.4 with MOPS at room temperature upon addition of increasing amounts (up to 3 equivalents) of $Cd(NO_3)_2{\cdot}4H_2O$ solution (1.60 mM).

Electronic Supplementary Material (ESI) for Chemical Science

4. Photophysical properties of DCHQ4

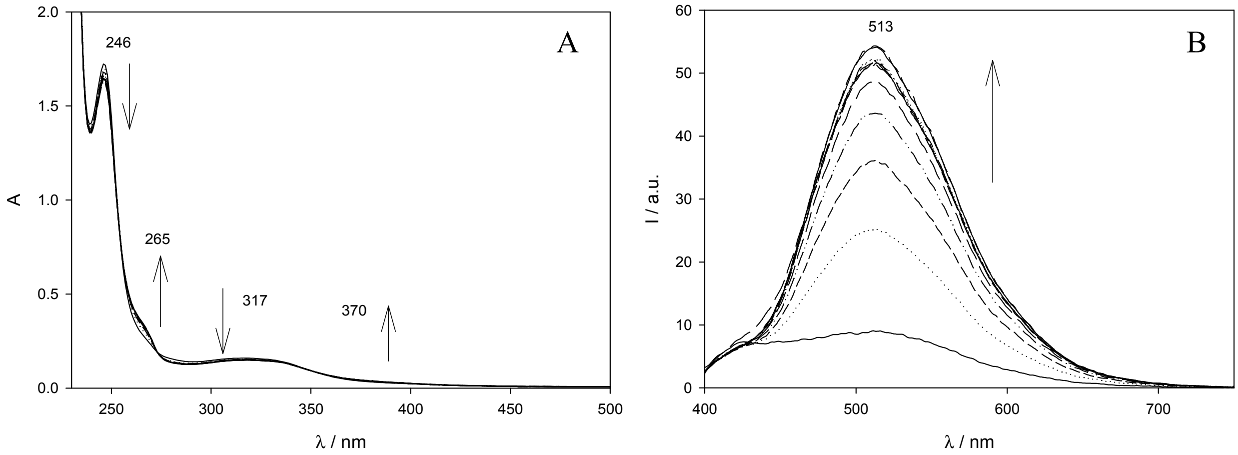

Figure 7 Titration with Mg^{2+}. Absorption (panel A) and emission (λ_{exc} = 330 nm, panel B) spectra of DCHQ4 (25 μM) in 1:1 CH_3OH:H_2O mixture buffered at pH=7.4 with MOPS at room temperature upon addition of increasing amounts (up to 1.5 equivalents) of $Mg(NO_3)_2 \cdot 6H_2O$ solution (2.50 mM).

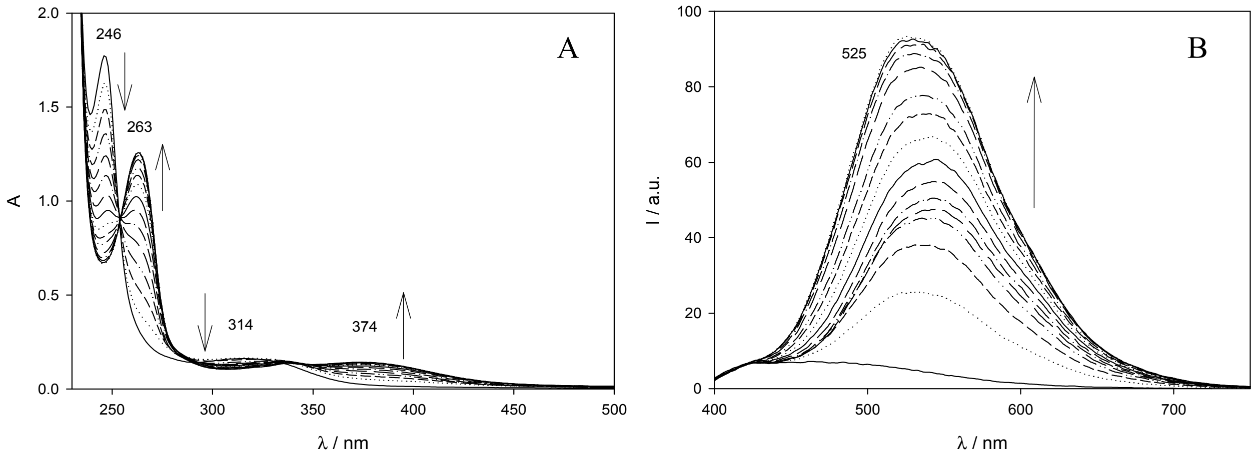

Figure 8 Titration with Zn^{2+}. Absorption (panel A) and emission (λ_{exc} = 330 nm, panel B) spectra of DCHQ4 (25 μM) in 1:1 CH_3OH:H_2O mixture buffered at pH=7.4 with MOPS at room temperature upon addition of increasing amounts (up to 2 equivalents) of $Zn(NO_3)_2 \cdot 6H_2O$ solution (2.50 mM).

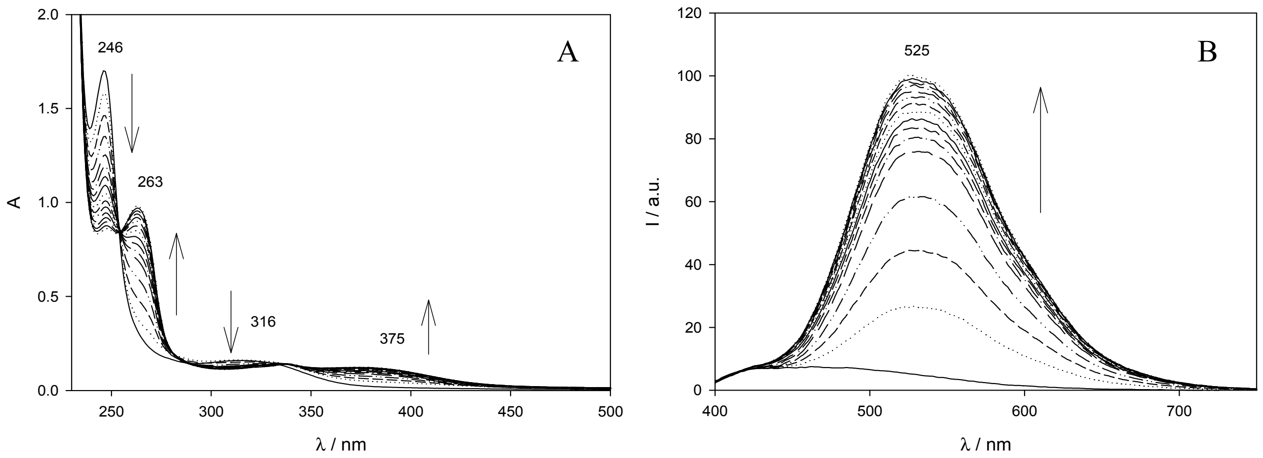

Figure 9 Titration with Cd^{2+}. Absorption (panel A) and emission ((λ_{exc} = 330 nm, panel B) spectra oF DCHQ4 (25 μM) in 1:1 CH_3OH:H_2O mixture buffered at pH=7.4 with MOPS at room temperature upon addition of increasing amounts (up to 2.5 equivalents) of $Cd(NO_3)_2 \cdot 4H_2O$ solution (1.60 mM).

5. Photophysical properties of DCHQ5

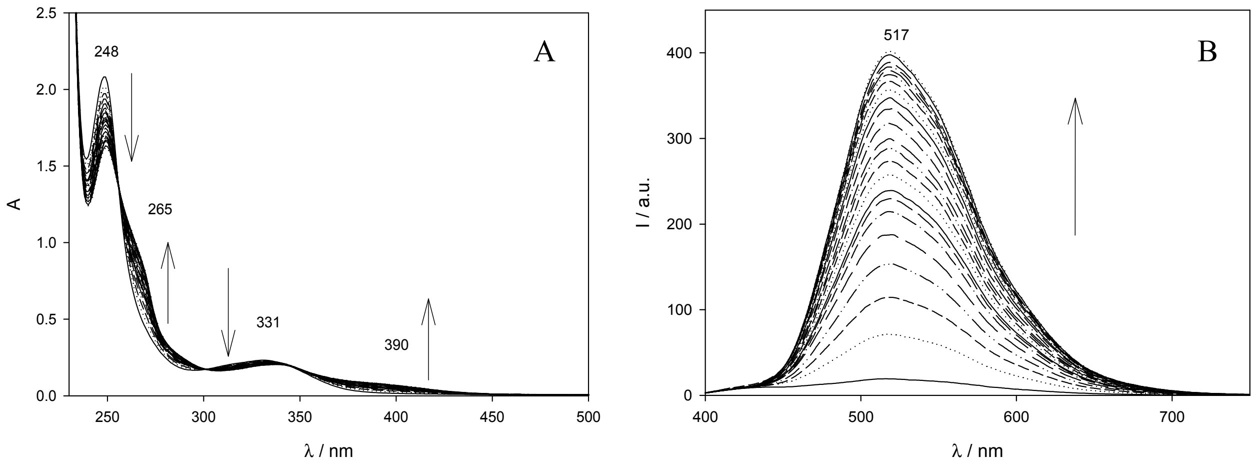

Figure 10 Titration with Mg^{2+}. Absorption (panel A) and emission (λ_{exc} = 344 nm, panel B) spectra of DCHQ5 (25 μM) in 1:1 CH_3OH:H_2O mixture buffered at pH=7.4 with MOPS at room temperature upon addition of increasing amounts (up to 4 equivalents) of $Mg(NO_3)_2 \cdot 6H_2O$ solution (2.50 mM).

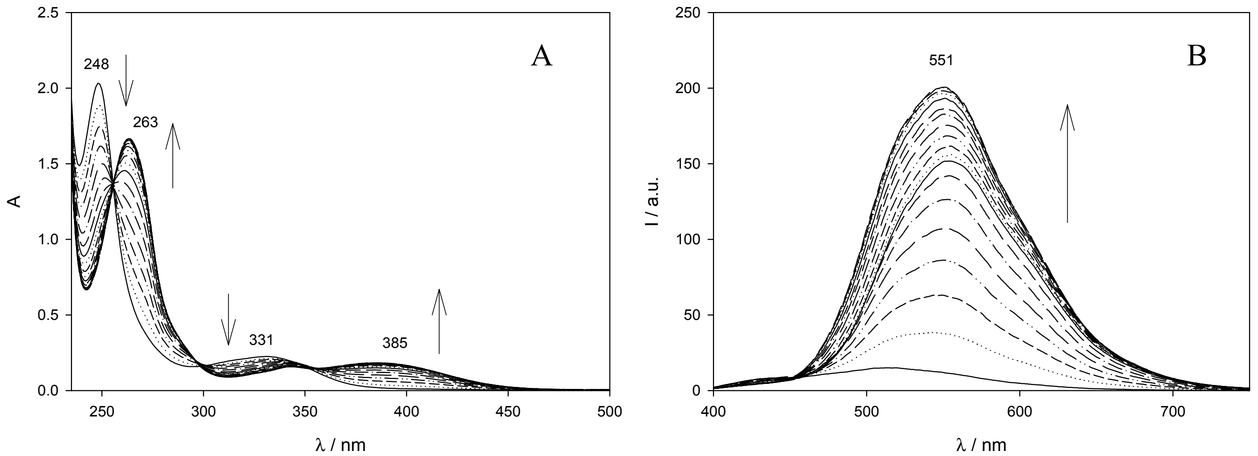

Figure 11 Titration with Zn^{2+}. Absorption (panel A) and emission (λ_{exc} = 344 nm, panel B) spectra of DCHQ5 (25 μM) in 1:1 CH_3OH:H_2O mixture buffered at pH=7.4 with MOPS at room temperature upon addition of increasing amounts (up to 2 equivalents) of $Zn(NO_3)_2 \cdot 6H_2O$ solution (2.50 mM).

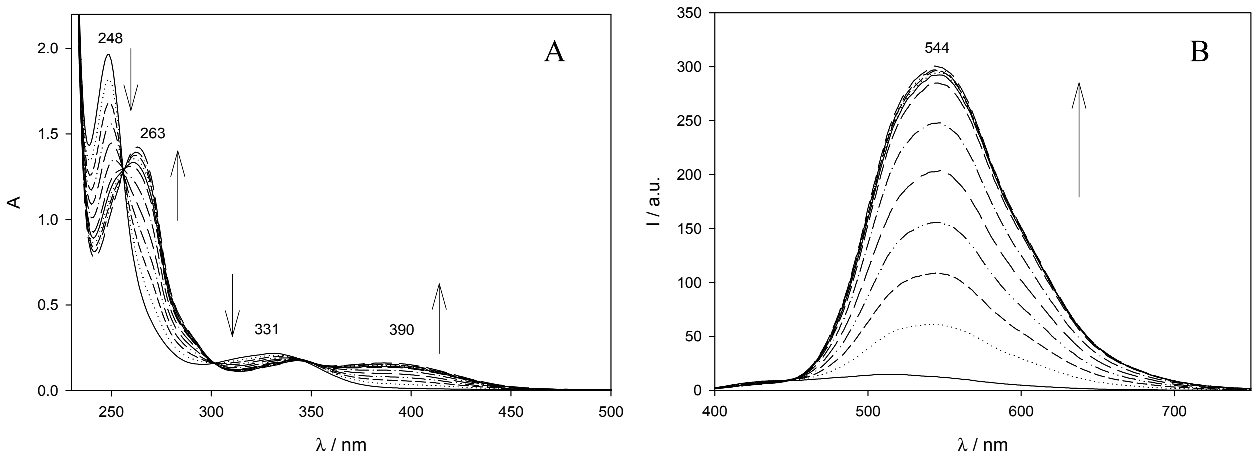

Figure 12 Titration with Cd^{2+}. Absorption (panel A) and emission (λ_{exc} = 344 nm, panel B) spectra of DCHQ5 (25 μM) in 1:1 CH_3OH:H_2O mixture buffered at pH=7.4 with MOPS at room temperature upon addition of increasing amounts (up to 1.5 equivalents) of $Cd(NO_3)_2 \cdot 4H_2O$ solution (1.60 mM).

6. Photophysical properties of DCHQ6

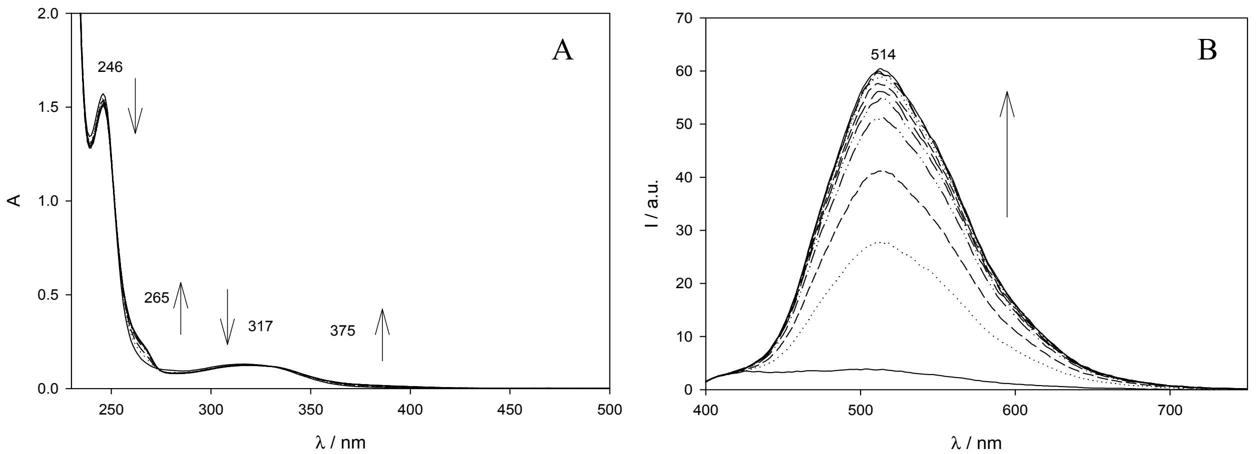

Figure 13 Titration with Mg^{2+}. Absorption (panel A) and emission (λ_{ex} = 337 nm, panel B) spectra oF DCHQ6 (18 μM) in 1:1 CH_3OH:H_2O mixture buffered at pH=7.4 with MOPS at room temperature upon addition of increasing amounts (up to 1 equivalent) of $Mg(NO_3)_2 \cdot 6H_2O$ solution (2.50 mM).

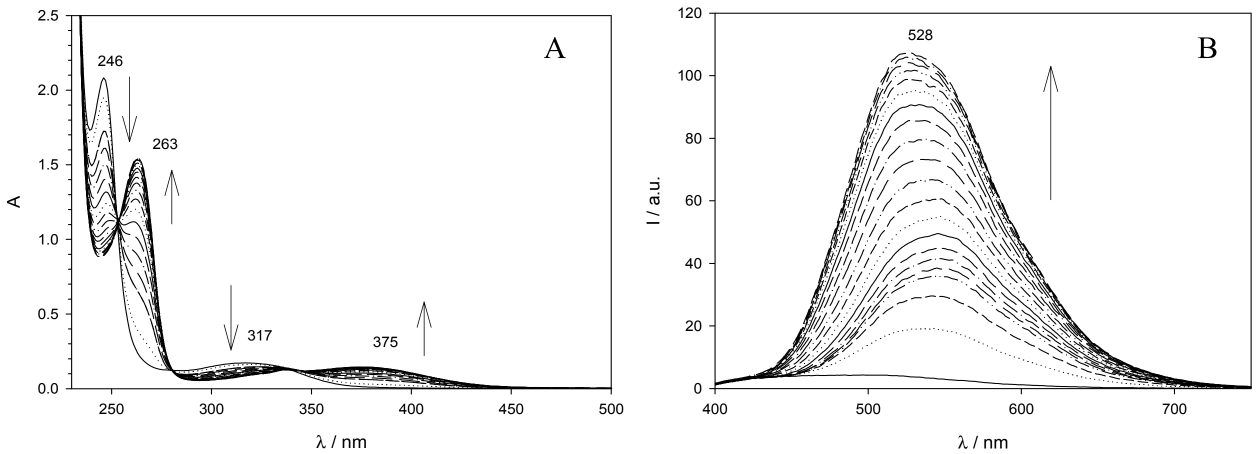

Figure 14 Titration with Zn^{2+}. Absorption (panel A) and emission (λ_{exc} = 337 nm, panel B) spectra oF DCHQ6 (25 μM) in 1:1 CH_3OH:H_2O mixture buffered at pH=7.4 with MOPS at room temperature upon addition of increasing amounts (up to 2 equivalents) of $Zn(NO_3)_2 \cdot 6H_2O$ solution (2.50 mM).

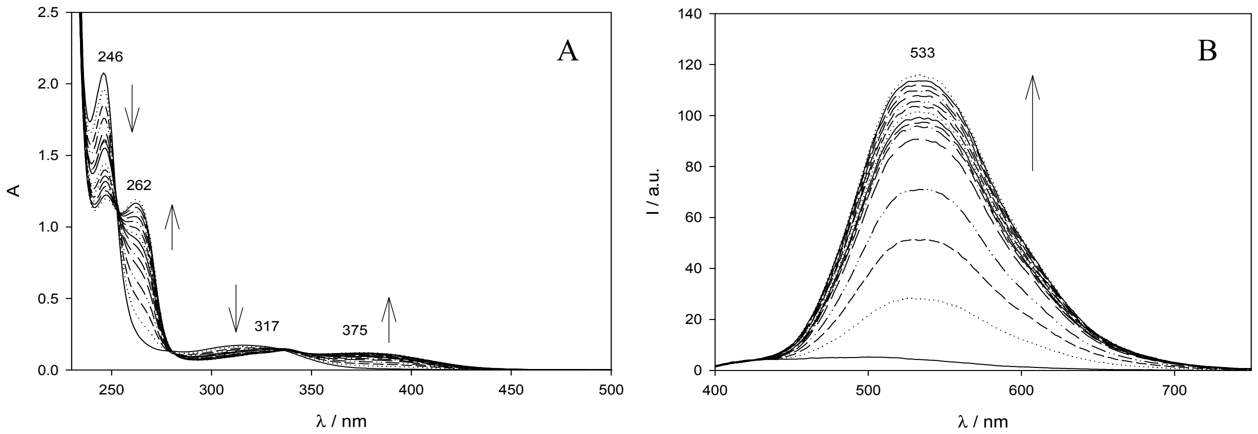

Figure 15 Titration with Cd^{2+}. Absorption (panel A) and emission (λ_{exc} = 335 nm, panel B) spectra oF DCHQ6 (25 μM) in 1:1 CH_3OH:H_2O mixture buffered at pH=7.4 with MOPS at room temperature upon addition of increasing amounts (up to 1.5 equivalents) of $Cd(NO_3)_2 \cdot 4H_2O$ solution (1.60 mM).

7. Flow Cytometric Assay of DNA content of HL60 treated with DMSO

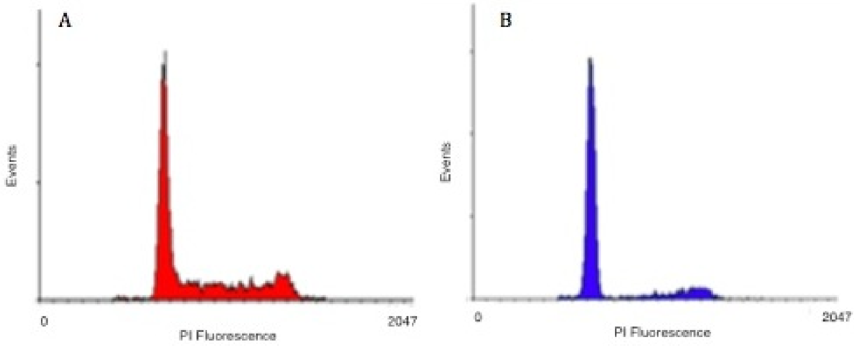

Figure 16 Assessment of the total DNA content of control (panel A) and DMSO-differentiated (panel B) HL60 cells. Hystograms show the PI fluorescence (expressed in fluorescence channels), that is directly proportional to the DNA content, with respect to the number of events acquired. Results from a typical experiment.

8. References

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[2] F. I. Wolf, V. Covacci, N. Bruzzese, A. Di Francesco, A. Sacchetti, D. Corda and A. Cittadini. *J. Cell. Biochem.*, 1998, 71:441–448.

[3] M. Nusse and J. Kramer. *Cytometry*, 1984, 5:20-5.