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Supplementary Information

Quantification of Synthetic Amino-Nitroquinoxaline Dyes: An Approach Using Image Analysis

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Procedure for quantification of compounds 2 and 3 through UV-Vis data

The first attempt to quantify the compounds **2** and **3** through UV-Vis data was using univariate calibration procedure. The results are summarized in Figures S1 and S2.

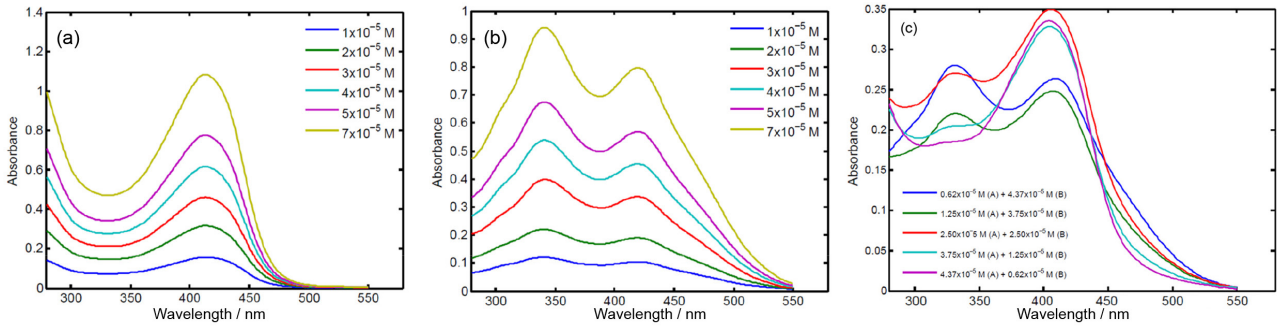

Figure S1. UV-Vis spectra of quinoxaline derivatives in DMSO: (a) **2**; (b) **3**; and (c) **2** + **3**.

Figure S2. Solutions of calibration set for solution studies of compounds **2** and **3**, with concentrations (in mmol L^{-1}) of (a) 5.0, (b) 2.5, (c) 1.25, (d) 0.62, (e) 0.31, (f) 0.16, (g) 0.078, (h) 0.039, (i) 0.019, (j) 0.0098, (k) 0.0049, and (l) 0.0024; and for a mixture of dyes **2**:**3** with proportions of (a) 5.0:0.0, (b) 4.5:0.5, (c) 4.0:1.0, (d) 3.5:1.5, (e) 3.0:2.0, (f) 2.5:2.5, (g) 2.0:3.0, (g) 1.5:3.5, (h) 1.0:4.0, (i) 0.5:4.5, and (j) 0.0:5.0.

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Quantification of compounds **2** and **3** through image analysis

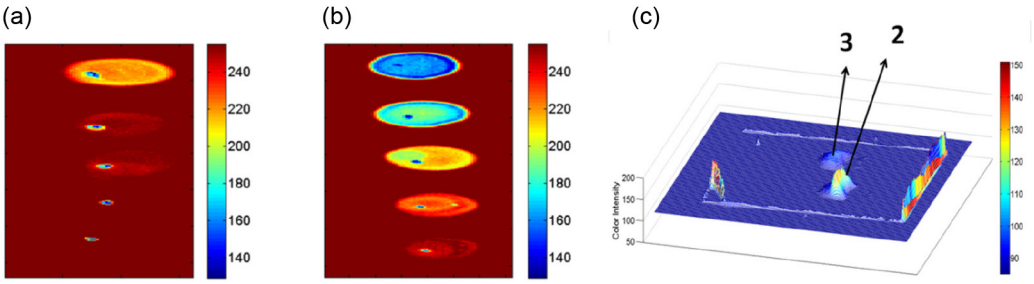

Figure S3. Scanned image of TLC plates containing the amino-nitro quinoxaline dyes (a) **2** and (b) **3**. (c) Contour plot obtained for **2** and **3**.

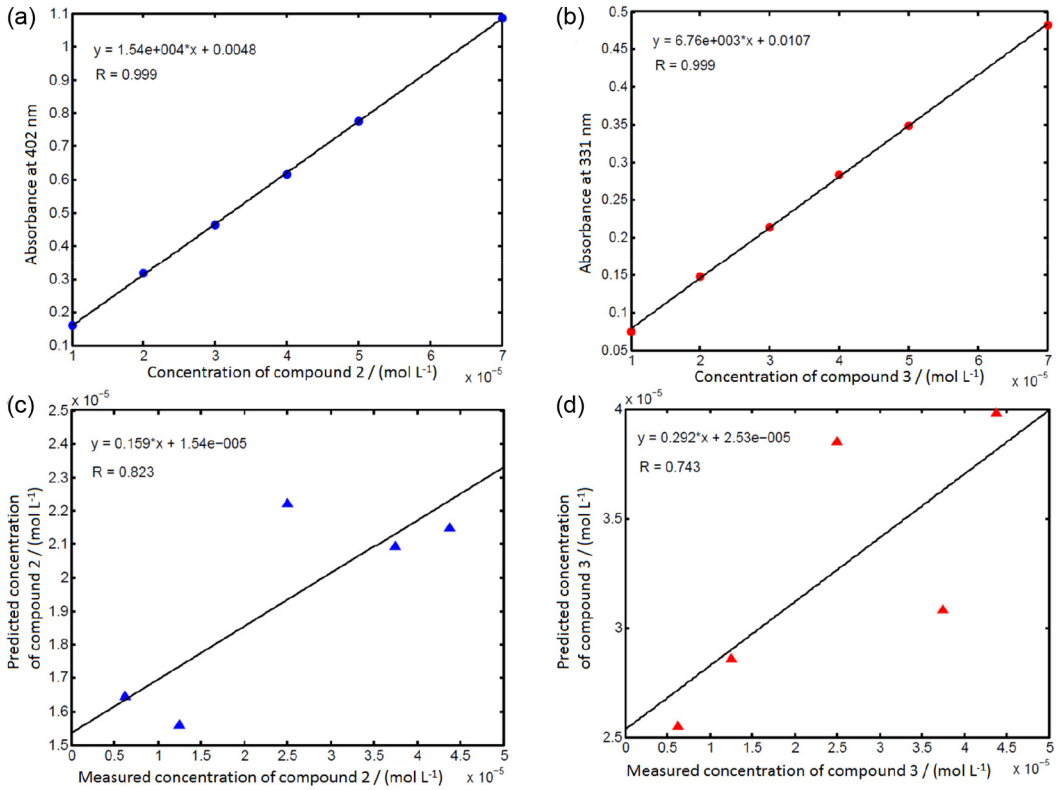

Figure S4. Best results found in the experiments involving quantification of compounds **2** and **3** through UV-Vis and univariate calibration. (a) Calibration set for compound **2** (correlation = 0.999) at 411 nm; (b) calibration set for compound **3** (correlation = 0.999) at 331 nm; (c) prediction of compound **2** in a mixture (correlation = 0.823); and (d) prediction of compound **3** in a mixture (correlation = 0.743).

Due to the unsatisfactory results, a second attempt was made using PLS regression of the UV-Vis data. In this case, the results are shown in Figure S5 and Table S1.

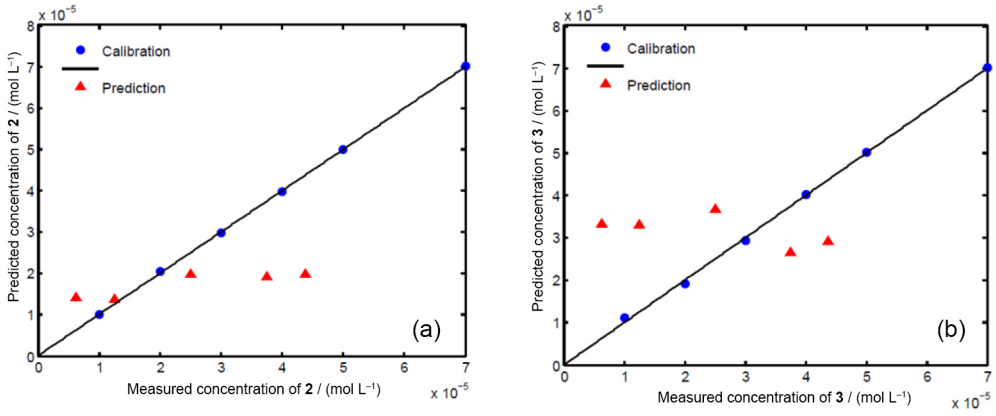

Figure S5. (a) PLS regression for compound **2**; (b) PLS regression for compound **3**. In both models, two latent variables (explained variance > 99%) was used.

Table S1. Figures of merit for PLS regression

Calibration	Compound	
	2	**3**
RMSEC	2.34×10^{-7} mol L^{-1}	6.27×10^{-7} mol L^{-1}
R^2 calibration	0.999	0.998
Cross-validation leave-one-out		
RMSECV	3.36×10^{-7} mol L^{-1}	1.22×10^{-6} mol L^{-1}
R^2 cross-validation	0.999	0.996
Prediction		
RMSEP	1.41×10^{-5} mol L^{-1}	1.80×10^{-5} mol L^{-1}
R^2 prediction	0.758	0.400

RMSEC: root mean square error of calibration; R^2: coefficient of determination; RMSECV: root mean square error of cross validation; RMSEP: root mean square error of prediction.

The same trend is observed using PLS regression, giving unsatisfactory results for the UV-Vis data.

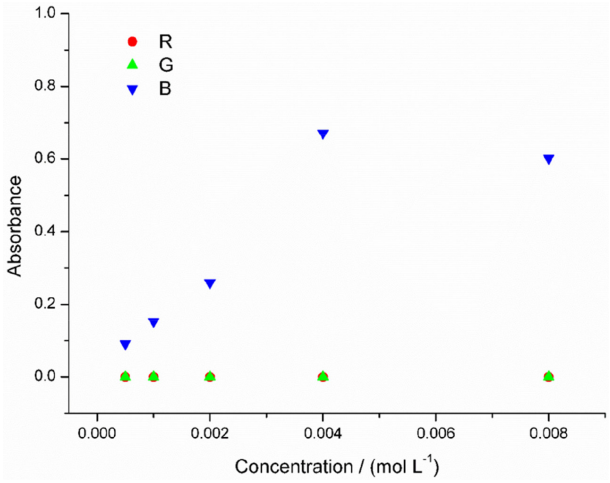

Figure S6. RGB absorbance signal for TLC plate of dye **3**. See the presence of only the B channel when the concentration is increasing, and the signal saturates at 0.004 mol L^{-1}.

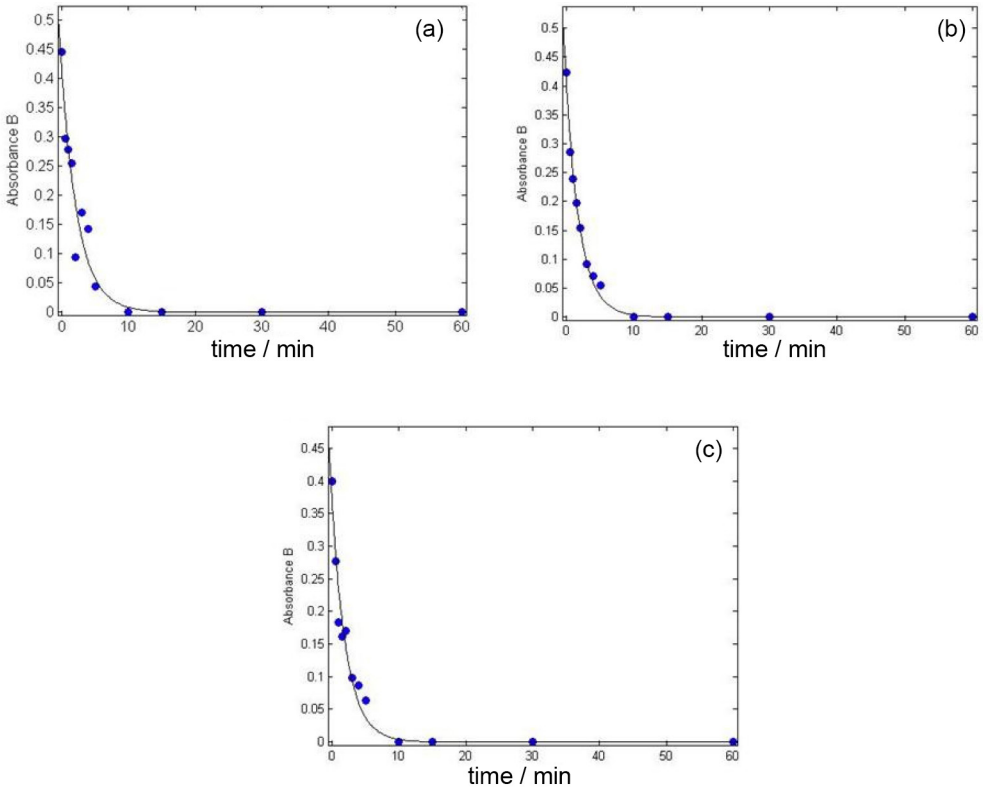

Figure S7. Kinetic profile (triplicate) obtained by TLC/IA for equimolar reaction of compound **2** and pyrrolidine, in $CHCl_3$.

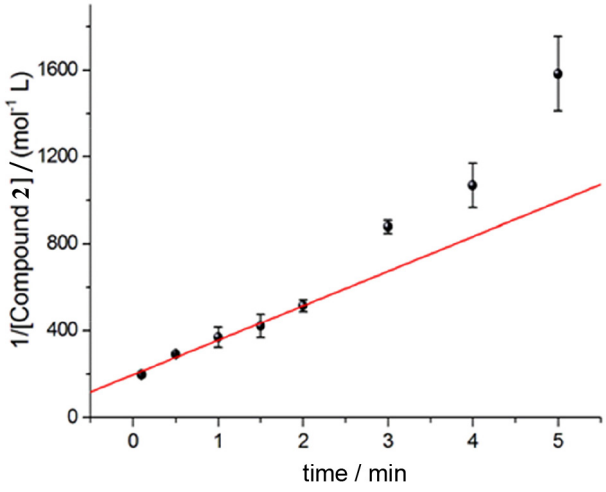

Figure S8. Nonlinear fit obtained for 1 / [**2**] *vs.* time for reaction of **2** with pyrrolidine in $CHCl_3$.

Figure S9. Color changes (naked eye colorimetric test) in DMSO solution of **4** (A) before and after addition of OH^- ((B) 10 eq; (C) 20 eq; (D) 40 eq; (E) 60 eq; (F) 80 eq; (G) 100 eq).

RGB-resolved absorbance

$$F = -\log\frac{I}{I_0} \quad (1)$$

where F is the RGB-resolved absorbance; I is the intensity of the R, G or B channels; and I_0 is the maximum value of intensity or blank: 255.