

Supporting Information

Development of Fluorescent Sensor for Illicit Date Rape Drug-GBL

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Materials and Methods

All the chemicals and solvents were purchased from Sigma Aldrich, Alfa Aesar, MERCK or Acros, and used without further purification. Normal phase purifications were carried out using Merck Silica Gel 60 (particle size: 0.040-0.063 mm, 230-400 mesh). Analytical characterization was performed on a HPLC-MS (Agilent-1200 series) with a DAD detector and a single quadrupole mass spectrometer (6130 series) with an ESI probe. Analytical method, unless indicated: eluents: A: H₂O (0.1% HCOOH), B: CH₃CN (0.1% HCOOH), gradient from 30 to 100% B in 5 min; C₁₈ (2) Luna column (4.6 x 50 mm², 3.5 μm particle size). ¹H-NMR and ¹³C-NMR spectra were recorded on Bruker Avance 300 NMR and 500 NMR spectrometers, and chemical shifts are expressed in parts per million (ppm) and coupling constants are reported as a *J* value in Hertz (Hz). High resolution mass spectrometry (HRMS) data was recorded on a Micromass VG 7035 (Mass Spectrometry Laboratory at National University of Singapore (NUS)). Spectroscopic and quantum yield data were measured on spectroscopic measurements, performed on a fluorometer and UV/VIS instrument, Synergy 4 of Biotek Company. The slit width was 1 nm for both excitation and emission, and the data analysis was performed using GraphPrism 5.0.

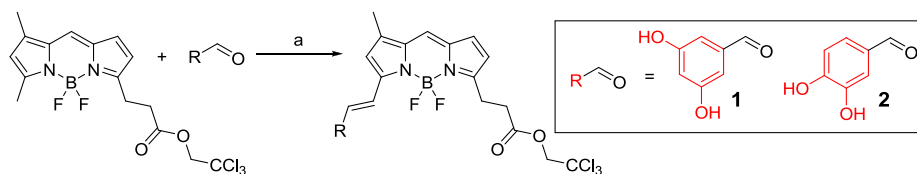
1. Quantum Yield Measurements

Quantum yields were calculated by measuring the integrated emission area of the fluorescent spectrum and comparing that value with the area measured for Rhodamine B in DMSO when excited at 500 nm ($\Phi_{\text{rho-B}} = 0.49$). Quantum yields were then calculated using equation (1), where Φ_{st} is the reported quantum yield of the standard, *I* is the integrated emission spectrum, *A* is the absorbance at the excitation wavelength, and η_x is the refractive index of the solvents used. The subscript x denotes unknown and st denotes standard. Emission was integrated between 560 to 700 nm.

$$\Phi_x = \Phi_{\text{st}}(I_x/I_{\text{st}})(A_{\text{st}}/A_x)(\eta_x^2/\eta_{\text{st}}^2) \quad (1)$$

2. General procedure for the synthesis of Green Date

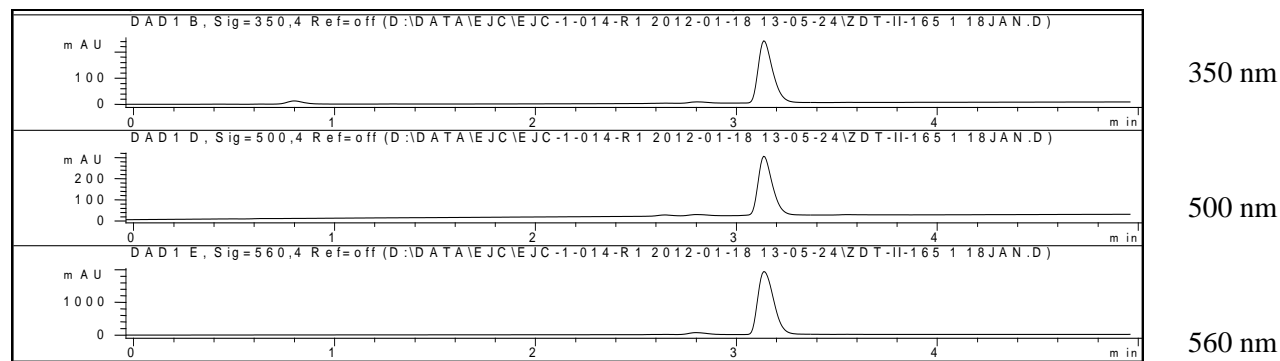
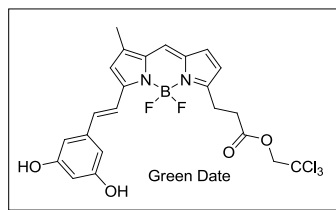
Scheme S1 Synthetic scheme of **Green Date**.



Reagents and conditions: a) pyrrolidine, acetic acid, acetonitrile, 85 °C, 5 min.

BODIPY trichloroethyl ester (20 mg, 47 μmol) and aldehyde (94 μmol , 2 equiv) were dissolved in acetonitrile (3 mL), with 6 equiv. of pyrrolidine (23.5 μL , 282 μmol) and 6 equiv. of AcOH (16.1 μL , 282 μmol). The condensation reaction was performed by heating to 85 °C. After that, the reaction mixture was cooled down to room temperature and then monitored by TLC. The reactions were finished after 5 min. The resulting crude mixture was concentrated under vacuum, and purified by short silica column.

3. Characterization of Green Date

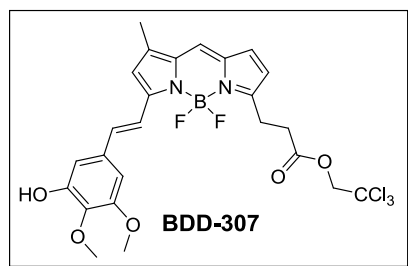


HPLC-MS characterization of **Green Date**. chromatograms (*descending order*) at 350 nm, 500 nm and 560 nm. HPLC conditions: A: H₂O-HCOOH: 99.9:0.1. B: CH₃CN-HCOOH: 99.9:0.1; gradient 30% B to 100% B (5 min.), isocratic 100% B (2.5 min). Reversephase Phenomenex C₁₈ Luna column (4.6 x 50 mm) 3.5 μm, flow rate: 1.2 mL/min.

HRMS *m/z* (C₂₃H₂₀BCl₃F₂N₂O₄) calculated: 542.0550 found: 541.0483 (M-H).

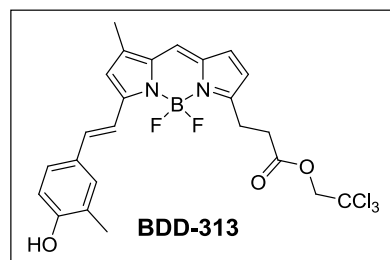
¹H NMR (300 MHz, CDCl₃+MeOD): 7.35 (d, *J*=16.2 Hz, 1H), 7.09 (d, *J*=16.2 Hz, 1H), 6.99 (s, 1H), 6.76 (d, *J*=3.9 Hz, 1H), 6.60 (s, 1H), 6.47 (d, *J*=2.1 Hz, 2H), 6.19 (t, *J*=2.1 Hz, 1H), 6.17 (d, *J*=3.9 Hz, 1H), 4.64 (s, 2H), 3.19 (t, *J*=7.5 Hz, 2H), 2.80 (t, *J*=7.5 Hz, 2H), 2.15 (s, 3H),.

¹³C NMR (75.5 MHz, CDCl₃+MeOD): 171.0, 158.5, 158.0, 155.2, 139.1, 137.7, 130.8, 127.2, 122.2, 118.1, 116.6, 116.3, 108.9, 107.9, 106.1, 104.2, 94.6, 73.6, 31.6, 23.4, 10.9.



¹H NMR (300 MHz, CDCl₃): 2.28 (s, 3H), 2.97 (t, *J*=7.5 Hz, 2H), 3.40 (t, *J*=7.5 Hz, 2H), 3.93 (s, 3H), 3.94 (s, 3H), 4.78 (s, 2H), 6.31 (d, *J*=3.9 Hz, 1H), 6.70 (s, 1H), 6.72 (d, *J*=2.1 Hz, 1H), 6.86 (d, *J*=2.1 Hz, 1H), 6.87 (d, *J*=3.9 Hz, 1H), 7.05 (s, 1H), 7.20 (d, *J*=16.2 Hz, 1H), 7.48 (d, *J*=16.2 Hz, 1H).

HRMS *m/z* (C₂₅H₂₄BCl₃F₂N₂O₅) calculated: 586.0812 found: 585.0745 (M-H).



¹H NMR (300 MHz, CDCl₃+MeOD): 2.16 (s, 3H), 2.18 (s, 3H), 2.87 (t, *J*=7.5 Hz, 2H), 3.28 (t, *J*=7.5 Hz, 2H), 4.69 (s, 2H), 6.19 (d, *J*=3.9 Hz, 1H), 6.62 (s, 1H), 6.69 (d, *J*=8.4 Hz, 1H), 6.74 (d, *J*=3.9 Hz, 1H), 6.94 (s, 1H), 7.20 (d, *J*=16.2 Hz, 1H), 7.23 (d, *J*=8.4 Hz, 1H), 7.29 (s, 1H), 7.34 (d, *J*=16.2 Hz, 1H).

HRMS *m/z* (C₂₄H₂₂BCl₃F₂N₂O₃) calculated: 540.0757 found: 539.0690 (M-H).

5. Details of Real Beverages Used in Experiment for Green Date

1. Absolute Vodka Pear; Anus Sweeden
 - 40% Alcohol/vol
 - Ingredients: Vodka, Pear Flavour
2. Arbor Mist Strawberry White Zinfandel; New York
 - 6% Alcohol/ Vol
 - Ingredients: White Zinfandel, water, high fructose corn syrup, natural flavours, carbon dioxide, citric acid, potassium sorbate, potassium benzoate, potassium metabisulfite
3. Red wine First Cavicchioli 1928 Lambrusco; Umberto Cavicchioli & Figli
 - 7.5% Alcohol/ Vol
 - Ingredients: Grapes from Emilia area
4. Guinness Foreign Extra; St James' gate Dublin
 - 6.8% Alcohol/ Vol
 - Ingredients: Water, malt, barley, hops.
5. F&N Fruit Tree Fresh Apple Juice ; Singapore
 - Ingredients: Apple juice Concentrate, Pear juice concentrate, Aloe Vera juice, flavouring, Malic acid, Vitamin C, Permitted colouring, Preservatives.
6. Cocktail Singapore Sling; Singapore
 - 6.8% Alcohol/ Vol
 - Ingredients: Pomegranate Fruit Juice, Pineapple Juice, Gin, Grenadine, Cherry Brandy, Cointreau, Benedictine, Angostura Bitters.
7. Johnnie Walker Whisky; Diageo
 - 40% Alcohol/ Vol
 - Ingredients: Scotch whisky

6. Work flow of the high throughput screening for GBL fluorescent sensor development

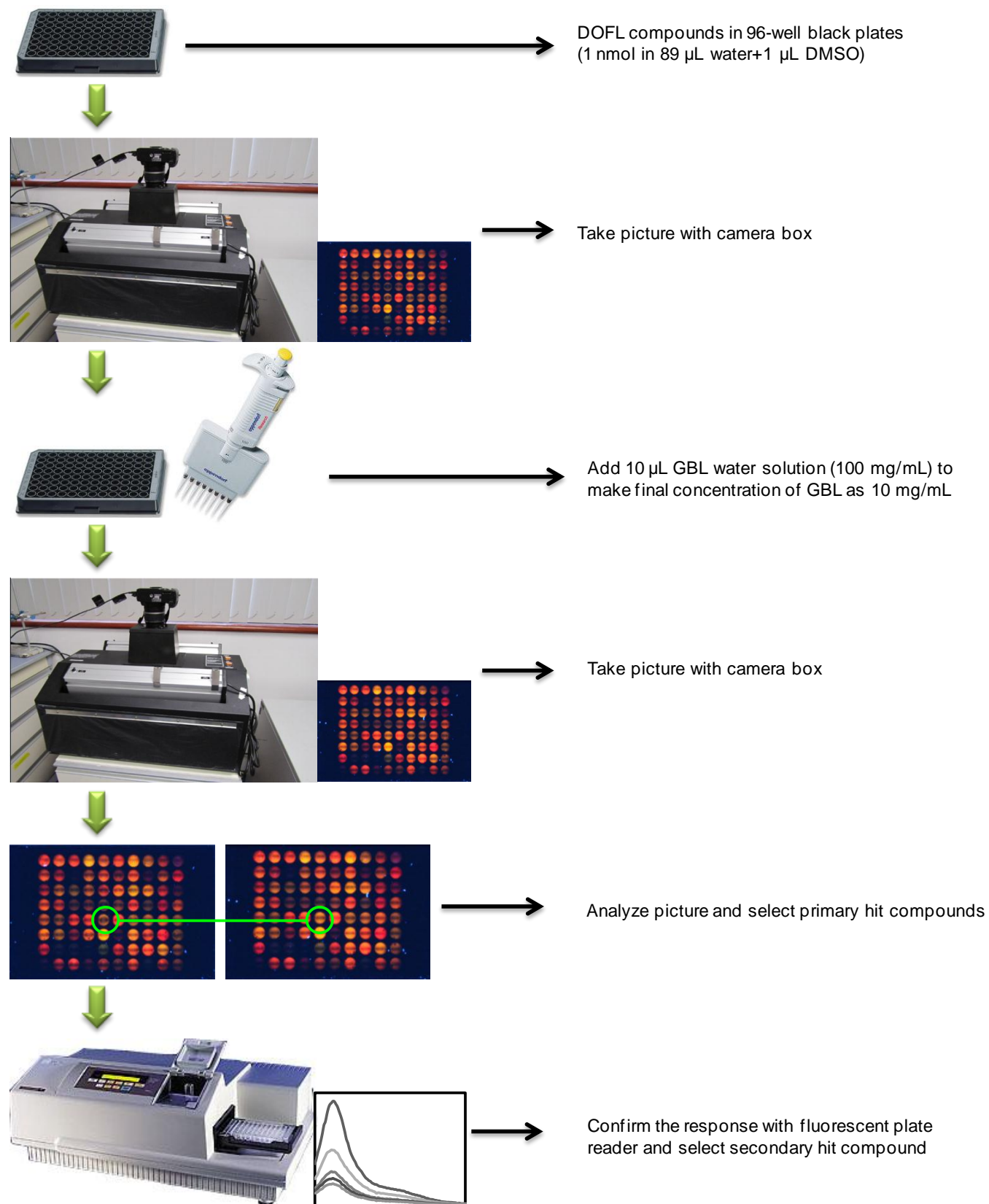


Figure S1

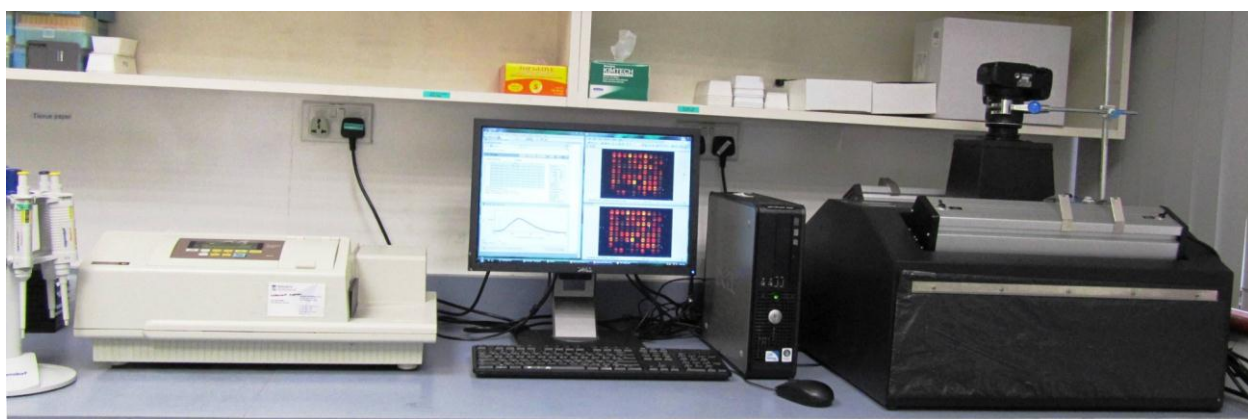


Figure S2 High throughput *in vitro* screening station .

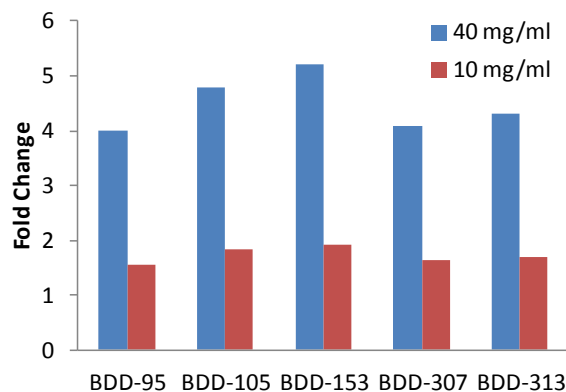


Figure S3 Fluorescence response of 5 hit compounds (10 μ M) to GBL in water.

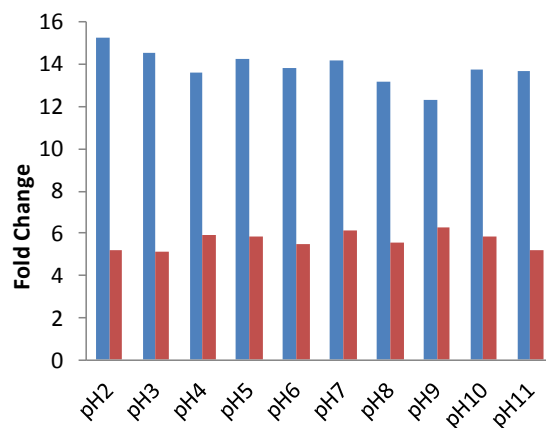


Figure S4 Fluorescent response of **Green Date** (10 μ M) to GBL (blue bar: 100 mg/mL; red bar: 50 mg/mL) under different pH conditions.

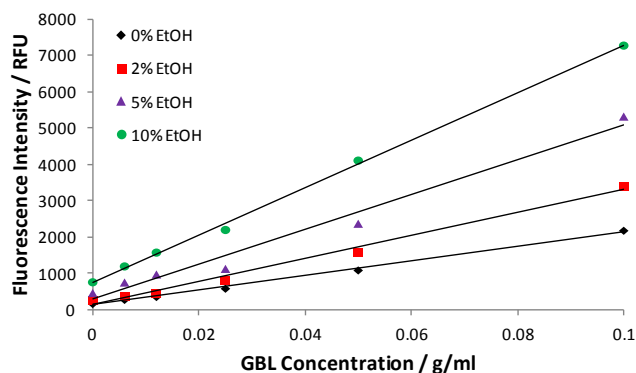


Figure S5 Linear correlation of fluorescence enhancement of **Green Date** (10 μ M) versus concentrations of GBL with different percentage of EtOH.