Electronic Supplementary Material (ESI) for Chemical Communications


## Supporting Information

# Development of a Fluorescent Sensor for an Illicit Date Rape Drug-GHB

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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: $H_2O$ (0.1% HCOOH), B: $CH_3CN$ (0.1% HCOOH), gradient from 5 to 95% B in 5 min; $C_{18}$ (2) Luna column (4.6 x 50 $mm^2$, 3.5 µm particle size). $^1$H-NMR and $^{13}$C-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). Spectroscopic and quantum yield data were measured on spectroscopic measurements, performed on a fluorometer and UV/VIS instrument, Spectra Max M2 by Molecular Device. 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 ($\Phi_{rho\text{-}B} = 0.49$). Quantum yields were then calculated using equation (1), where $\Phi_{st}$ is the reported quantum yield of the standard, I is the integrated emission spectrum, *A* is the absorbance at the excitation wavelength, and $\eta_x$ 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.

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

## 2. Synthesis of GHB

Gamma-butyrolactone (GBL) was obtained from Sigma-Aldrich with purity of 99%. Solid anhydrous NaOH was obtained from TCI Chemicals. All chemicals were used without further purifications.

**Aqueous GHB**: Solid anhydrous NaOH crystals (6.00g, 0.150mol) were dissolved in 20ml of deionized water. 1 equivalent of GBL (10 ml, 0.131mol) was then slowly added to the NaOH solution under constant stirring for 1 hour. The resulting mixture was then brought to pH 7.4 with drop-wise addition of 1M HCl, under the monitoring of a pH metre.The final concentration of GHB is 3.81M. The product was confirmed using mass spectrometry.

**Sodium salt:**Solid anhydrous NaOH crystals (6.00g, 0.150mol) were dissolved in 100ml of ethanol under constant stirring for ½ hour. GBL was added dropwise through a dropping funnel. The solution was heated up to 70°C. The mixture was filtered and washed with excess cold ethanol. The solid was dried under high vacuum suction. $^{1}$H NMR (300MHz, $D_2O$): 1.79 (m, 2H); 2.23 (t, *J*=7.0 Hz, 2H); 3.59 (t, *J*=7.0 Hz, 2H).

## 3. High Throughput Screening

All fluorescent measurement data were recorded using a fluorescent plate reader, Spectra Max M2 by Molecular Device (Fig. S1a). Greiner Bio One 96-well U-Shaped black plate was used in conjunction with the plate reader for fluorescence measurement. All optical images were obtained in Spectroline Model CL-150 UV-fluorescence analysis cabinet equipped with two15W long wave tube of 365 nm (model: xx-15NF) – peak intensity of 550 µW/cm², taken with Canon EOS Digital Rebel XTi (Fig. S1b).

a) b)

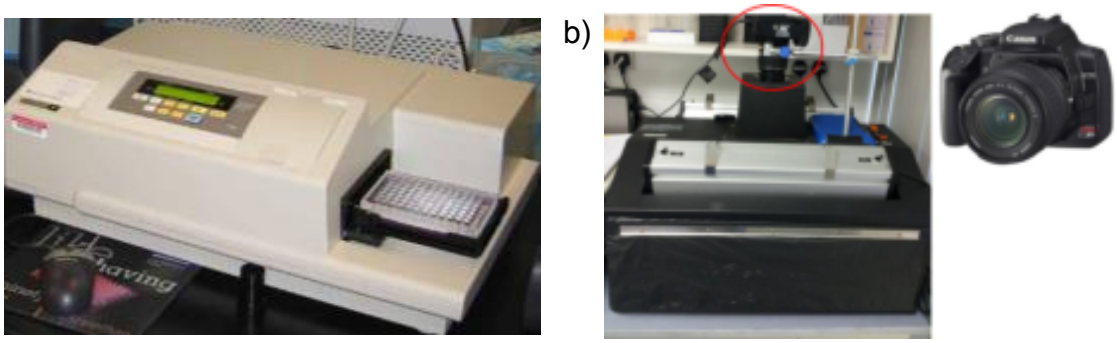
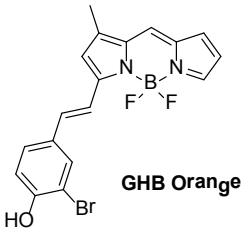


**Fig. S1**

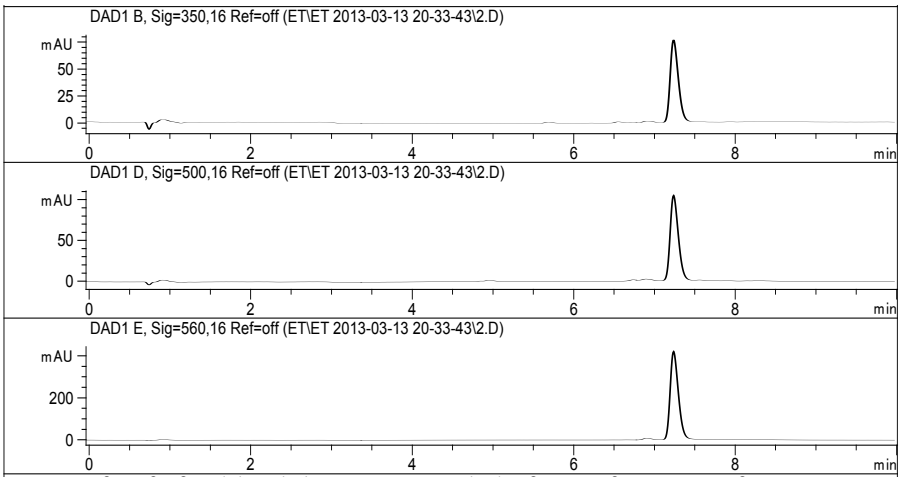
For the primary screening, 50% EtOH in $H_2O$ was used as the solvent to simulate the possible working environment of the beverages to be tested at a later stage. 90µL was added to each library of dyes arranged on the 96-well black plate.Readings were taken using the microplate reader and camera, before and after 10 µL of 100 mg/mL aq. GHB in 50% EtOH was spiked into each well.The final concentration of dye is 10 µM, while the final concentration of the analyte is 10mg/mL. The results were analysed and hit compounds were determined via their fold change, which is taking the fluorescence intensity after spiking it with GHB divided the by fluorescence intensity before spiking. All results were taken in triplicates for all the experiments, unless otherwise stated.

Subsequently, the primary hit compounds proceeded to the next round of selection, known as secondary screening. In the secondary screening, the 1mM concentration stock of dyes dissolved in DMSO was used.It was diluted by 100 times before using it in secondary screening. A simple concentration dependency test was performed using 100 mg/mL, 200 mg/mL and 400 mg/mL of GHB. Therefore, the final concentration of GHB in the test solution 10 mg/mL, 20 mg/mL and 40 mg/mL, and the final dye concentration was 10 µM.

## 4. Characterization of GHB Orange




**GHB Orange**




HPLC-MS characterization of **GHB Orange**. chromatograms (*descending order*) at 350 nm, 500 nm and 560 nm. HPLC conditions: A: $H_2O$-HCOOH: 99.9:0.1. B: $CH_3CN$-HCOOH: 99.9:0.1; gradient 5% B to 100% B (10 min.), isocratic 100% B (5 min). Reversephase Phenomenex $C_{18}$ Luna column (4.6 x 50 mm) 3.5 µm, flow rate: 1.2 mL/min.

HRMS *m/z* ($C_{18}H_{14}BBrF_2N_2O$) calculated: 402.0 found: 401.0 (M-H).

$^1$H NMR (500 MHz, DMSO-$d_6$): 2.31 (s, 3H), 6.48 (dd, *J*=1.5, 3.5 Hz, 1H), 7.03, (d, *J*=8.5 Hz, 1H), 7.06 (s, 1H), 7.08 (d, *J*=3.5 Hz,1H), 7.23 (d, *J*=16.0 Hz,1H), 7.51 (d, *J*=8.5 Hz, 1H), 7.65 (s, 1H), 7.67 (d, *J*=8.5 Hz, 1H), 7.71 (s, 1H), 7.74 (d, *J*=1.5 Hz, 1H)

$^{13}$C NMR (125.5 MHz, DMSO-$d_6$): 11.6, 104.2, 106.1, 108.0, 113.6, 117.0, 120.0, 122.2, 125.3, 127.0, 130.6, 133.3, 137.2, 139.2, 141.2, 155.2, 158.3, 158.5.

5. **Detailes of Real Beverages Used in Experiment for GHB Orange**

1. Absolute Vodka Pear; Anus Sweeden
   - 40% Alcohol/vol
   - Ingredients: Vodka, Pear Flavour
2. Red wine First Cavicchioli 1928 Lambrusco; Umberto Cavicchioli & Figli
   - 7.5% Alcohol/ Vol
   - Ingredients: Grapes from Emilia area
3. Guinness Foreign Extra; St James' gate Dublin
   - 6.8% Alcohol/ Vol
   - Ingredients: Water, malt, barley, hops.
4. 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.
5. Johnnie Walker Whisky; Diageo
   - 40% Alcohol/ Vol
   - Ingredients: Scotch whisky
6. Chamisul fresh soju; South Korea
   - 19.5% Alcohol/ Vol
7. Coca Cola; Diageo
   - Ingredients: Carbonated Water, Sugar, Caramel, Colour, Phosporic Acids, Flavourings and Caffeine

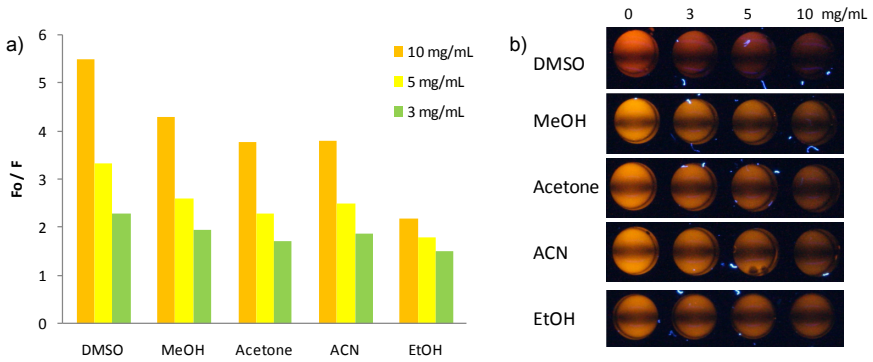



**Figure S2** (a) Fluorescence response of **GHB Orange** (20 μM) to GHB in different solvents mixed with water (1:1 by volume). (b) Picture of **GHB Orange** solution (20 μM) in 50% of different solvent with different concentrations of GHB take from the screening camera box.

COSY NMR (500 MHz, DMSO-$d_6$ : $D_2O$ = 9:1)