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Development of a fluorescent sensor for illicit date rape drug GHB†

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The first fluorescent sensor (GHB Orange) for date rape drug GHB was developed. It exhibits the fluorescence quenching property for GHB and allows its detection in various drinks. The interaction mechanism was elucidated as intramolecular charge transfer induced by a hydrogen bond. This discovery will help in solving the drug facilitated sexual assault problems.

Drug facilitated sexual assault (DFSA) has been defined as a sexual assault carried out after the victim has become incapacitated due to consumption of alcohol or drugs.[1] These drugs are referred to as date rape drugs, including gamma-hydroxybutyric acid (GHB), Rohypnol, Ketamine and Soma.[2] Among them, GHB is a central nervous system depressant, and was used as a general anaesthetic in the 1960s and 1970s.[3] Subsequently, GHB was employed for a variety of purposes including the treatment of sleep disorders and depression, and the promotion of fat reduction and muscle development, until being banned for sale as a supplement in US by FDA in 1990.[4] Over the past two decades, it has gained media notoriety as a drug allegedly used in instances of drink spiking which leads to DFSA. Small amount (<1 g) of GHB acts as a relaxant, causing loss of muscle tone and reduced inhibitions. At 1 to 2 g, it slows down the heart rate and respiration. At 2 to 4 g, it interferes with motor and speech control, and may induce a coma-like sleep.[4a,5] GHB takes effect in 15 to 30 minutes, and the effect lasts for 3 to 6 hours.[6] It is only detectable in urine for 6 to 12 hours after ingestion.[7] Due to the fact that GHB is odourless, colourless, and slightly salty, it is almost undetectable when mixed in a drink, thus making it desirable to sexual predators.[8] GHB is the most commonly used date rape drug in DFSA, likely because it is much more easily available, is cheaper and leaves the body more quickly than other drugs.[9] Therefore, development of a real time detection method for GBL would be a great contribution to prevent DFSA.

Several test kits for detection of GHB have been developed, such as "DrinkSafe™" cards, "DrinkSafe™" coasters,[10] "Drink Detective™",[11] and "drug detection straw".[12] However, there has been no fluorescent sensor reported for GHB detection so far. Fluorescent sensors are attractive tools for analytical sensing because of their high sensitivity, fast response time, and technical simplicity. The Diversity-Oriented Fluorescence Library Approach (DOFLA) has proved its ability in fast and unique fluorescence sensor development during the last decade.[13] Through this approach, our group has recently reported the development of the first fluorescent sensor for GBL (gamma-butyrolactone), the pro-drug of GHB.[14] As a systematic extension of our previous work, here we report the development of the first fluorescent sensor for the illicit date rape drug GHB.

A similar high throughput image based screening system was applied for novel GHB sensor discovery. In our previous report, a GBL sensor (Green Date) requires an extraction method to eliminate alcohol effects for GBL detection in real drinks, which is an obstacle for its practical usage.[14] Taking this fact into account, here we set the screening medium as 50% EtOH in water instead of pure water, to simulate the working conditions. The sedative dosage of GHB is between 2 and 4 g per ingestion.[4a] Therefore we set the screening concentration of GHB to 10 mg mL^{-1}, assuming the average volume of a drink to be between 150 and 200 mL. After screening 5500 dyes generated from different fluorescent scaffolds, 17 compounds (data not shown) were selected as primary hits due to their fluorescence intensity change from the pictures. Secondary screening was then carried out on a fluorescent microplate reader for a wide range of concentrations of GHB (*i.e.*, 10, 20, 40 mg mL^{-1}) to render one best hit compound, named **GHB Orange** (Fig. 1a).

GHB Orange belongs to our previous reported BODIPY library BD.[15] It has an absorption and an emission maximum

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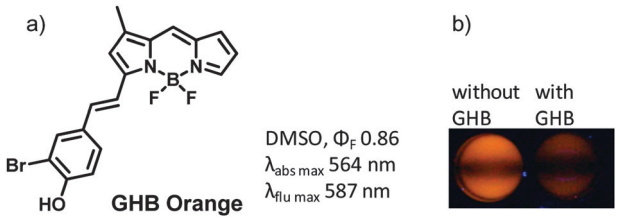

Fig. 1 (a) Structure of **GHB Orange**. (b) Picture of **GHB Orange** solution (20 μM in 50% EtOH) with and without GHB (10 mg mL^{-1}) taken from the screening camera box.

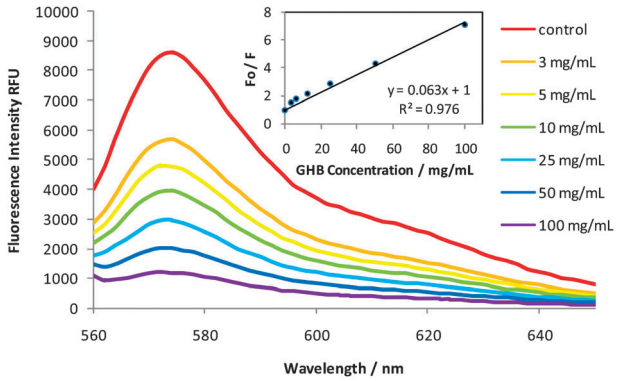

Fig. 2 Fluorescent spectra of **GHB Orange** (20 μM) after incubation with different concentrations of GHB. (inner) Linear correlation of fold change of fluorescence *versus* concentration of GHB.

at 557 and 574 nm in the testing medium (*i.e.*, 50% ethanol in water) respectively. It exhibited a 2.2-fold fluorescent decrease with 10 mg mL^{-1} GHB in 50% ethanol aqueous solution (Fig. 2), with an obvious fluorescence intensity decrease that can be observed by the naked eye (Fig. 1b). The fluorescence intensity fold change (F_0/F) of **GHB Orange** showed a linear decrease with respect to the concentration of GHB within the 0 to 100 mg mL^{-1} range (Fig. 2 inner).

In order to examine the efficiency of **GHB Orange** as a convenient GHB detection kit, we tested various real drink samples spiked with GHB. First, we tried to optimize the working conditions of **GHB Orange**. Several solvents which are miscible with water were tested, including DMSO, methanol, ethanol, acetone and acetonitrile. They were mixed with water in a 1 : 1 ratio (v/v) containing different concentrations of GHB, and their fluorescence responses to **GHB Orange** were measured (Fig. S2, ESI†). Among the different solvent systems, **GHB Orange** showed the best quenching response in 50% DMSO aqueous solution with a 5.5-fold fluorescence decrease at 10 mg mL^{-1} GHB. Hence, DMSO was selected as the working solvent for developing a GHB detection kit. Several beverages representing alcoholic, non-alcoholic, coloured and colourless drinks were selected for this test. **GHB Orange** was first dissolved in DMSO, and then mixed with beverage samples which were spiked with different concentrations of GHB (1 : 1 ratio by volume). The concentrations of GHB that caused a 1.3-fold fluorescence decrease (which were distinguishable by the naked eye) of **GHB Orange** in the beverages were calculated

Table 1 Concentrations of GHB to cause 1.3-fold of fluorescence decrease of **GHB Orange** in different beverages

Beverage (mg mL^{-1})	Water	Beer	Red wine	Vodka
	<1	4.7	2.3	<1
Beverage (mg mL^{-1})	Cola	Korean soju	Apple juice	Whiskey
	5.5	<1	6.1	10.1

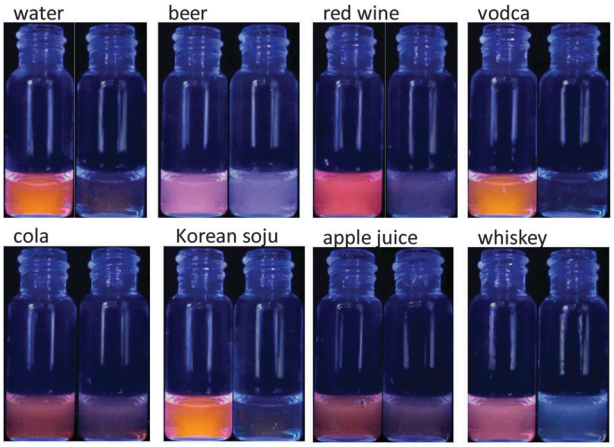

Fig. 3 Pictures of beverage samples with and without GHB containing **GHB Orange** under irradiation using a hand-held 365 nm lamp. (final concentrations: DMSO 50% by volume, **GHB Orange** 50 μM, GHB 5 mg mL^{-1}).

from this experiment (Table 1). On the other hand, the efficiency of **GHB Orange** as a visual detection kit was also explored. GHB-free and GHB spiked beverages (10 mg mL^{-1}) were mixed with **GHB Orange** DMSO solution (100 μM) in a 1 : 1 ratio, and the fluorescence intensity differences were directly observed under the irradiation of a hand-held 365 nm lamp. As shown in Fig. 3, the fluorescence intensity differences between GHB-free and GHB-spiked beverages are distinguishable by the naked eye. These results illustrated that **GHB Orange** can

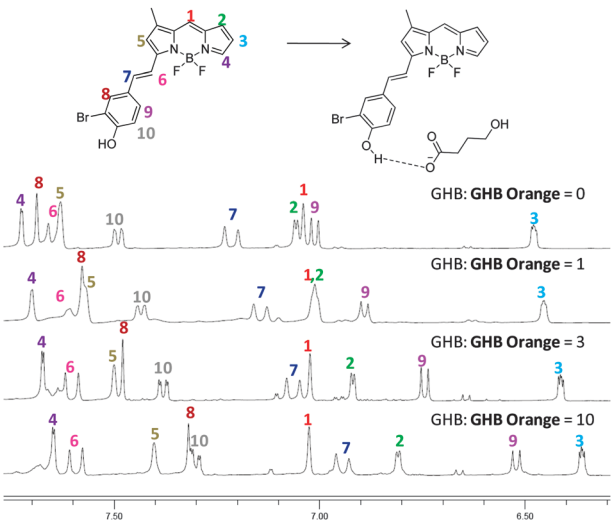

Fig. 4 Partial ^{1}H NMR spectra of **GHB Orange** (30 mM) upon addition of different equivalents of GHB in DMSO-d_6 and D_2O (9 : 1).

enable visual detection of GHB in various drinks. Remarkably, this detection can be done through a simple mix-and-see process, which takes less than 30 seconds.

The mechanism of the interaction between **GHB Orange** and GHB was further explored. The hydroxyl group in the structure of **GHB Orange** suggests that a hydrogen bond may form between **GHB Orange** and GHB. To confirm the assumption, NMR experiments were performed with different GHB:**GHB Orange** ratios (Fig. 4). Upon increasing equivalents of GHB, the proton in **GHB Orange** shifted up-field, especially for H8 and H9 (0.39 and 0.51 ppm respectively). This result illustrated the formation of a hydrogen bond between **GHB Orange** and GHB. Due to the formation of the hydrogen bond, the electron intensity of **GHB Orange** was increased, and photo-induced electron transfer (PET) effect was enhanced, which quenched the fluorescence of **GHB Orange**.

In summary, we have performed high-throughput screening using 5500 in-house compounds, and identified **GHB Orange** as a novel GHB fluorescent sensor. **GHB Orange** showed fluorescence quenching response to GHB. It was later proved to be working best in 50% DMSO aqueous solution. Through a simple mix-and-see process, **GHB Orange** is capable of detecting the presence of GHB in different kinds of beverages with explicit intensity change under the irradiation of a hand-held 365 nm lamp. NMR experiments confirmed the formation of a hydrogen bond between **GHB Orange** and GHB. This discovery will improve the protection against DFSA.

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