

Optical detection of scopolamine and ketamine with a BODIPY-Phen conjugate and Cu(II)

Jordi Hernández-Contreras^a, Paula Madrigal^a, Pau Arroyo^{a,b}, Malva Liu-González^c, Salvador Gil^{a,b,d}, Margarita Parra^{a,b,d}, José A. Sáez^{a,b,**}, Pablo Gaviña^{a,b,d,*}

^a Instituto Interuniversitario de Investigación de Reconocimiento Molecular y Desarrollo Tecnológico (IDM), Universitat de Valencia - Universitat Politècnica de València, C/ Doctor Moliner 50, Burjassot, 46100, Valencia, Spain

^b Departamento de Química Orgánica, Universitat de València, Doctor Moliner 50, Burjassot, 46100, Valencia, Spain

^c SCSIE-DRXM, Universitat de València, Doctor Moliner 50, Burjassot, 46100, Valencia, Spain

^d CIBER de Bioingeniería, Biomateriales y Nanomedicina (CIBER-BBN), Spain

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ABSTRACT

Chemical submission is becoming a global growing threat. Therefore, the development of fast and reliable methods for detecting the presence of chemical submission drugs in drinks is of great interest. For this purpose, a new BODIPY-Phen conjugate consisting of a 1,10-phenanthroline moiety connected through its 4-position to the *meso* position of a BODIPY dye has been synthesized and characterized by NMR, UV–vis spectroscopy and by single-crystal X-ray diffraction. BODIPY-Phen in combination with Cu(II) was used as a probe for the fluorescent and colorimetric detection of scopolamine and ketamine, two of the drugs used in cases of chemical submission. The determined limits of detection in water were 3 μM for scopolamine and 2.88 μM for ketamine. Selectivity of the probe in the presence of other drugs and some possible interferents that could be found in beverages has also been tested. The detection process seems to be due to a reduction of the Cu(II) ion to Cu(I) followed by coordination of Cu(I) to the BODIPY-Phen conjugate, which results in quenching of its fluorescence together with a visible color change. The probe is able to detect by the unaided eye the presence of scopolamine in real soft drinks and alcoholic beverages spiked with the drug.

1. Introduction

Chemical submission (CS) crimes are a growing threat due to the emergence of new abuse drugs on the market. CS is defined as the administration of a psychoactive substance to a person, without their knowledge or consent, for illicit or criminal purposes. The aim of the perpetrator is to manipulate or modify the victim's behaviour to commit the crime, being sexual assaults the most common [1]. Scopolamine and ketamine (see Scheme 1) belong to the group of substances most commonly used to perpetrate drug-facilitated sexual assaults (DFSA). The low dose required for these substances to achieve the victim unconsciousness make them attractive for criminal purposes. Scopolamine is one of the most studied tropane alkaloids naturally occurring in plants of the *Solanaceae* family [2]. On the other hand, ketamine is a synthetic

compound derived from phencyclidine, which belongs to the cathinone family of compounds and consists of a racemic mixture of (S)-ketamine and (R)-ketamine enantiomers.

Regarding their effects, scopolamine produces restlessness, excitation, hallucinations, euphoria, disorientation or even death, although it also shows some beneficial antidepressant effects for unipolar and bipolar depression patient [2]. Quite similar effects are produced by ketamine, although the latter has found medical applications as anaesthetic, analgesic and antidepressant [3–5].

While their use in medical applications is always controlled, their usage in leisure environments can lead to very dangerous situations. Due to this, different procedures have been developed to detect these drugs. Thus, several methods for the determination of scopolamine and ketamine have been reported in the literature such as gas chromatography-

* Corresponding author. Instituto Interuniversitario de Investigación de Reconocimiento Molecular y Desarrollo Tecnológico (IDM), Universitat de Valencia - Universitat Politècnica de València, C/ Doctor Moliner 50, Burjassot, 46100, Valencia, Spain.

** Corresponding author. Instituto Interuniversitario de Investigación de Reconocimiento Molecular y Desarrollo Tecnológico (IDM), Universitat de Valencia - Universitat Politècnica de València, C/ Doctor Moliner 50, Burjassot, 46100, Valencia, Spain.

E-mail addresses: jose.a.saez@uv.es (J.A. Sáez), pablo.gavina@uv.es (P. Gaviña).

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mass spectrometry (GC-MS) [6], high performance liquid chromatography (HPLC) with UV and indirect conductometric detection, liquid chromatography with tandem mass spectrometry (LC-MS/MS) [7], capillary electrophoresis [8], potentiometry and spectrophotometry [9, 10]. Nevertheless, many of these techniques are laborious, time-consuming and require specialized equipment and operators. Therefore, the development of preventive detection methods for scopolamine and ketamine that can be used both by citizens and law enforcement agencies are of crucial importance. In addition, these methods should overcome two additional problems: the low doses required by these substances to achieve the perpetrator's aim [11] and the specificity in an ever-growing market of illegal substances. The use of optical molecular sensors for the detection and quantification of different analytes has proven to be very useful due to the reliability, ease of use and low price of the systems being developed [12–15].

Although some optical sensors have been described for these and other drugs, they require quite complex methodologies (acetylcholine receptors, microfluidic paper-based ELISA systems or molecular imprinted polymers) [16–18]. As far as we know, simpler systems, such as those described here, have not been developed.

In this context, we recently reported a chromo-fluorogenic probe, consisting of a mixture of a *meso*-aryl BODIPY and a Cu(II) salt, for the detection of synthetic cathinones [19]. The detection mechanism was based on the ability of these substances to reduce Cu(II) to Cu(I) [20], and the subsequent changes induced by the Cu(I) ions on the BODIPY core. We decided to extend these studies and explore the possibility of detecting other amine-containing drugs used in DFSA following this redox approach. Towards this end, we have incorporated into a BODIPY fluorophore, a 1,10-phenanthroline ligand (phen) [21–24]. Phen is known to form complexes both with copper(I) and copper(II) ions, with different coordination geometries and usually different coordination number [25]. Taking advantage of these differential coordination properties, we expected that coordination of the phen unit to the copper (I) ion, generated from copper(II) in the presence of these drugs, should influence the spectroscopic properties of the BODIPY dye giving rise to an optical response. Thus, we report herein the synthesis of a new BODIPY-Phen conjugate **1**, consisting of a BODIPY dye incorporating a 1,10-phenanthroline ligand attached to its *meso* position (Scheme 1), and its use, in combination with copper(II), as a chromo-fluorogenic probe for the detection of scopolamine and ketamine in aqueous solution and beverages.

2. Results and discussion

2.1. Synthesis, structural characterization and spectroscopic properties of **1**

Compound **1** was prepared in two steps following the procedure

depicted in Scheme 2. First, 4-methyl-1,10-phenantroline (**3**) was oxidized with SeO₂ in refluxing dioxane to obtain 1,10-phenantroline-4-carbaldehyde (**2**), as described previously in the literature [24]. In a second step, aldehyde **2** was reacted with 2,4-dimethyl pyrrole in dry DCM, in the presence of a catalytic amount of TFA under the usual conditions used for BODIPY synthesis. After a certain time, DDQ, NEt₃ and BF₃OEt₂ were added sequentially, to yield compound **1**, after solvent removal and chromatographic purification. BODIPY-phen **1** was completely characterized by ¹H NMR, ¹³C NMR and HRMS.

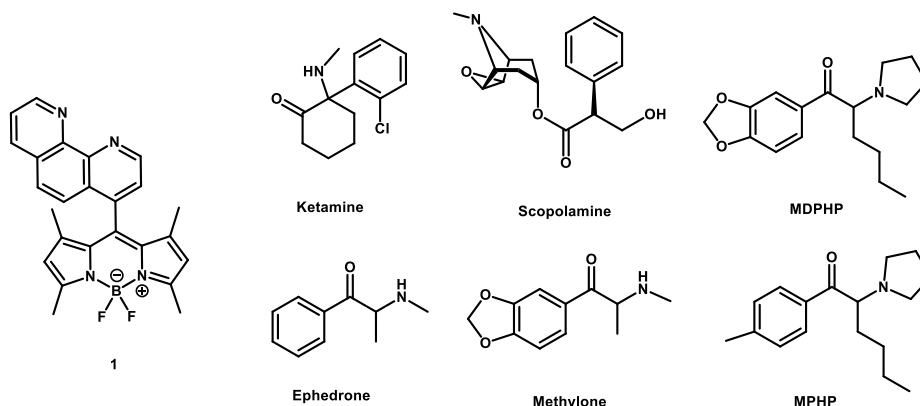
Single crystals of compound **1**, suitable for X-ray diffraction, were obtained by slow evaporation of dichloromethane solutions, as light red-orange prisms. The crystal structure of **1** reveals an orthorhombic system, (space group Pca2₁), with four molecules of **1** and four solvent molecules in the asymmetric unit (Fig. 1).

Bond lengths and angles within the BODIPY core are similar to other BODIPY derivatives previously reported [26,27]. For instance, the bond lengths found for the boron atom are B1–N2 1.543 (7) Å, B1–N1 1.546 (6) Å, B1–F1 1.393 (6) Å and B1–F2 1.395 (6) Å. The corresponding N1–B1–N2 and F1–B1–F2 angles are typical of a tetrahedral arrangement around the boron centre. The dihedral angle between the two pyrrole rings is 4.62° indicating a high level of planarity of the BODIPY core. Moreover, the plane of the phenanthroline moiety at the *meso* position of is almost perpendicular to the BODIPY plane with a dihedral angle of 98.30° (Fig. 1).

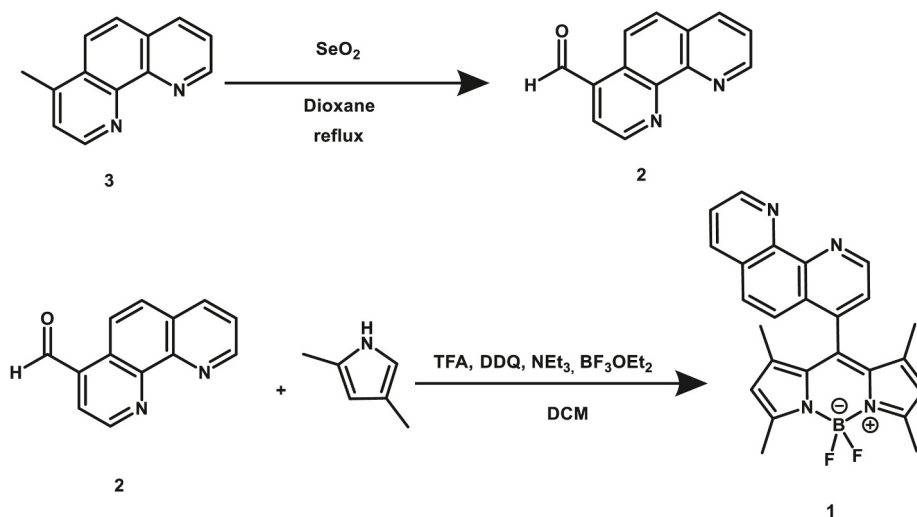
Fig. 2 depicts the absorption and normalized emission spectra of **1**. The UV–vis spectrum of chemosensor **1**, recorded in a mixture of DMSO/H₂O 95:5 as solvent (5 × 10^{−5} M), shows an intense absorption band at λ_{max} = 507 nm (ε = 69400 cm^{−1}M^{−1}) with a shoulder at 476 nm which can be attributed to S₀ → S₁ (π–π*), (0–0) and (0–1) transitions of the BODIPY chromophore [28]. Also, a strong absorption band (see Fig. S11) was observed at λ_{max} = 270 nm which can be attributed to the phen moiety [29]. The emission spectrum of **1** shows a strong band at λ_{em} = 520 nm (λ_{exc} = 470 nm). The relatively small Stokes' shift observed (13 nm) is characteristic of most BODIPY-based probes (around 5–15 nm) [30]. The fluorescence quantum yield and lifetime of **1** were measured in pure DMSO. **1** exhibits a high fluorescence quantum yield (φ_f = 0.87, using fluorescein free acid (φ_f = 0.91 in aq NaOH 0.1 M) as a standard [31]), with a lifetime value of 5.35 ± 0.01 ns (calculated from the slope of the corresponding decay curve [32]).

2.2. Sensing experiments

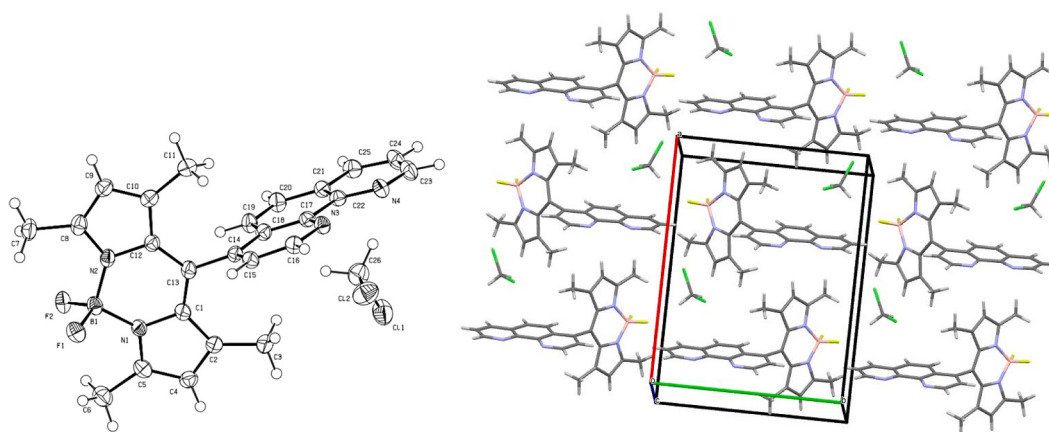
As we mentioned before, we decided to study the response of the probe, consisting of an equimolecular mixture of **1** and Cu(II) in DMSO/H₂O 95:5, towards scopolamine, ketamine and other amine containing drugs. In order to evaluate the response of the probe in the absence and in the presence of scopolamine, an excess of the drug dissolved in water was added to the solution of the probe, and after stirring the mixture for



Scheme 1. Structure of probe **1**, the drugs ketamine and scopolamine and other tested drugs.



Scheme 2. Synthesis of compound 1.

Fig. 1. (left) ORTEP drawing of compound 1. Thermal ellipsoids are drawn at the 50 % probability level. (right) Molecular packing of 1 along the *b* axis.

some time, the corresponding absorption and emission spectra were recorded. Three different reaction times (5, 10 and 20 min) and two different temperatures (room temperature and 60 °C) were assayed (see Fig. S12). The best response was observed after 10 min at 60 °C and thus, these conditions were selected for all the experiments.

As can be seen in Fig. 3, the addition of 1 equiv of a Cu(II) salt ($\text{Cu}(\text{NO}_3)_2$) did not induce any appreciable changes in the absorption or emission spectra of 1. Only a very slight decrease in the intensity of the emission maximum was observed. This could be due to a poor or negligible interaction between the Cu(II) ion and BODIPY-Phen 1 under the experimental conditions used. However, when excess of scopolamine was added to the mixture of 1 and Cu(II), a small decrease in the intensity together with bathochromic shift of the absorption band was observed in the UV–vis spectrum. More interestingly, the corresponding emission spectrum showed an important quenching of the emission band at 520 nm. These changes could be clearly observed by the unaided eye, either under visible light (from pale green to brownish pink color) or under irradiation with a common UV-lamp (see Fig. 3).

These changes were consistent with the proposed reduction of Cu(II) to Cu(I) promoted by scopolamine, followed by binding of the Cu(I) ion to the BODIPY-Phen probe. In fact, Cu(I) induced similar changes in the UV–vis and emission spectra of 1 than those observed in the presence of Cu(II) and scopolamine. As it can be observed in Fig. S8, the addition of 1 equiv of Cu(I) to 1 led to a small bathochromic shift of the corresponding absorption band and also a quenching in the maximum

emission band of 1. This fluorescence quenching is slightly smaller than that observed in the presence of Cu(II) and scopolamine. This can be explained by considering partial oxidation by air of Cu(I) to Cu(II), which would hindered in the presence of scopolamine. Also the same visual changes were observed for a mixture of 1 and Cu(I) and for a mixture of 1 + Cu(II) + scopolamine (Fig. S9).

In order to obtain more information about the binding of Cu(I) to 1, UV–vis titration experiments of 1 (60 μM in DMSO: H_2O 95:5 solution) in the presence of increasing amounts of CuBr were carried out. From these experiments, a 2:1 stoichiometry, corresponding to a bis(BODIPY-Phen)copper(I) complex was determined (Fig. S10).

Finally, to gain information about the redox process, we made an additional experiment by adding excess of scopolamine to a DMSO solution of a 1:1 mixture of neocuproine and Cu(II) [20]. After stirring the mixture at 60 °C for 10 min a clear color change from blue to red-orange characteristic of a tetrahedral copper(I) bis-phenanthroline complex was observed, and the corresponding UV–vis spectrum showed the typical MLCT absorption band at 450 nm (Fig. S15). Moreover, after letting stand the solution at room temperature for several weeks, we could observe the slow formation of red crystals of the bis-neocuproine Cu(I) complex (Fig. S16), thus confirming that under the studied conditions, scopolamine was able to reduce Cu(II) to Cu(I).

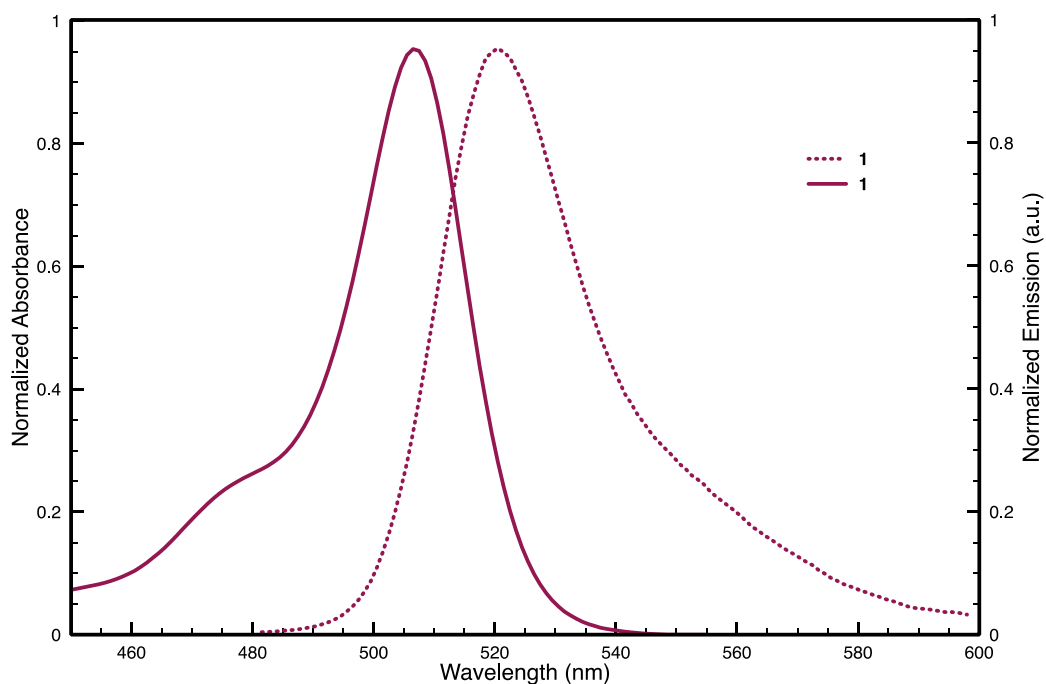


Fig. 2. Normalized UV-vis absorption (continuous line) and emission (dotted line) spectra of compound **1** ($5 \cdot 10^{-5}$ M in DMSO/H₂O 95:5). $\lambda_{\text{exc}} = 470$ nm.

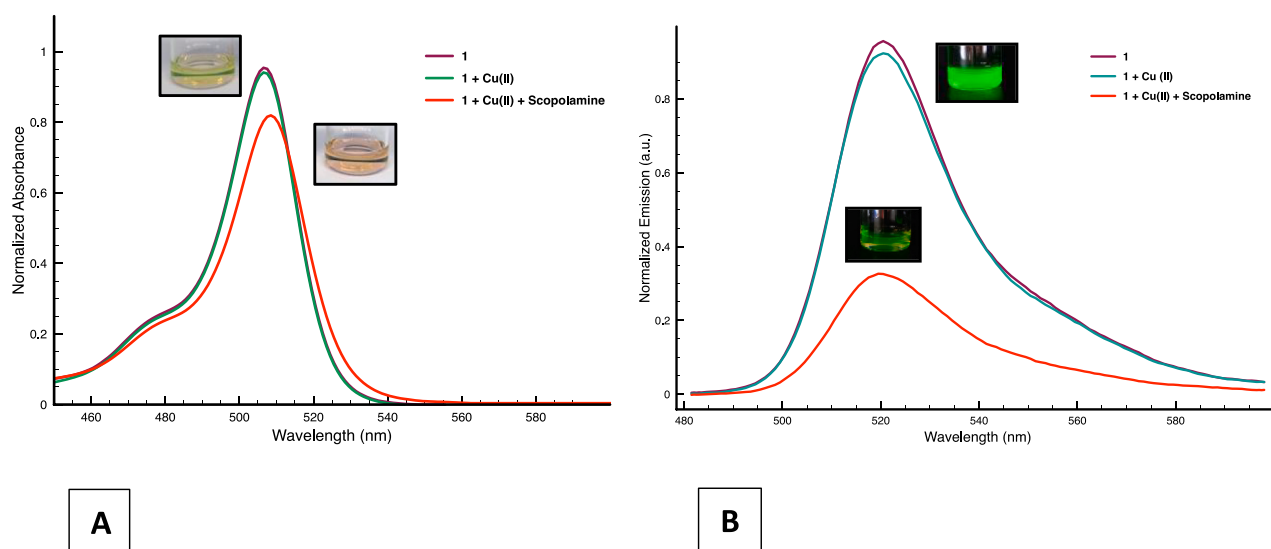


Fig. 3. UV-visible spectra (A) and emission spectra ($\lambda_{\text{exc}} = 470$ nm; $\lambda_{\text{em}} = 520$ nm) (B) of compound **1** ($50 \mu\text{M}$ in DMSO/H₂O 95:5), **1** + 1 equiv. of $\text{Cu}(\text{NO}_3)_2$ and **1** + 1 equiv. of $\text{Cu}(\text{NO}_3)_2$ + 3 equiv. of scopolamine.

2.3. Sensitivity studies

In order to evaluate the sensitivity of the probe towards the drug, fluorescence titration experiments with an equimolecular mixture of **1** and Cu(II) ($5 \mu\text{M}$ in DMSO: H₂O 95:5), in the presence of increasing amounts of scopolamine were undertaken. In a typical experiment, a $50 \mu\text{L}$ aliquot of an aqueous solution of scopolamine 5 mM was added to the solution of the probe ($5 \mu\text{M}$), always maintaining a 95:5 ratio of DMSO/H₂O as solvent. The mixture was stirred for 10 min at 60°C , and then the corresponding emission spectrum was recorded. As can be observed in Fig. 4 a gradual decrease in the maximum emission band was observed as the concentration of scopolamine was increased. The limit of detection (LOD) was determined from the plot of the % emission quenching at 520 nm ($\text{Quenching (\%)} = (1 - I_{\text{sample}}/I_{\text{blank}}) \times 100$) vs scopolamine

concentration, using equation $\text{LOD} = 3\sigma_b/m$ (where σ_b is the standard deviation of the blank and m is the slope of the titration curve). A LOD of $3 \mu\text{M}$ for scopolamine could be determined. This value is much lower than the amount of scopolamine necessary to induce severe effects in adults (1 mg/kg which in 150 mL of drink, represents a concentration of ca. 1.3 mM) [2]. These fluorescence changes could be observed by the unaided eye under a common UV-lamp (see Fig. 4C).

In view of the good response observed in the presence of scopolamine, we decided also to evaluate the use of the probe for the optical detection of ketamine, another drug used for chemical submission. Following the same previous procedure, fluorescence titrations with chemosensor **1** and Cu(II) in the presence of increasing amounts of ketamine were conducted. Similar to what was observed for scopolamine, a gradual quenching of the emission intensity **1** was observed (see

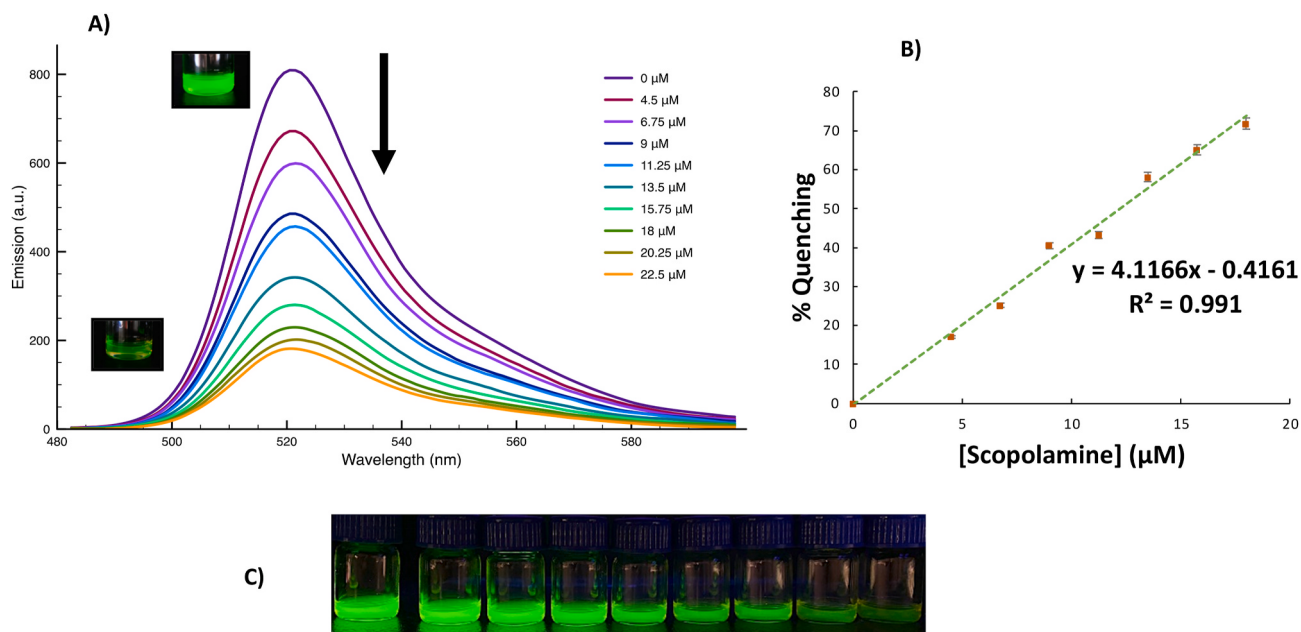


Fig. 4. (A) Emission spectra ($\lambda_{\text{exc}} = 470 \text{ nm}$; $\lambda_{\text{em}} = 520 \text{ nm}$) of compound 1 (5 μM in DMSO: H₂O 95:5 solution) in the presence of 1 equiv. of $\text{Cu}(\text{NO}_3)_2$ and increasing amounts of scopolamine after being heated to 60 °C for 10 min. (B) Calibration curve representing the % quenching of the emission intensity at $\lambda_{\text{em}} = 520 \text{ nm}$ ($\lambda_{\text{exc}} = 470 \text{ nm}$) vs the concentration of scopolamine. (C) Observed fluorescence changes under a common UV lamp.

Fig. 5). From the plot of the % fluorescence quenching versus ketamine concentration, a limit of detection of 2.88 μM could be determined, with a linear response in the 1–16 μM concentration range (Fig. 5B).

Finally, the recovery and accuracy of the method to detect scopolamine and ketamine in aqueous samples were also calculated and are shown in Table S2.

2.4. Selectivity studies

As our final goal is the detection of these chemical submission drugs in soft drinks and alcoholic beverages, we decided to test the selectivity of the probe with some of the potential interferents that can be found in these drinks, such as sodium ascorbate, sucrose or tartaric acid. As can be observed in Fig. 6, the quenching of fluorescence promoted by any of them is small when compared with that of scopolamine and thus, it does not interfere the detection of scopolamine in real samples under usual concentrations, as can be seen below (Fig. 8). Also, some other amine containing small molecules, such as dopamine or phenylglycine were tested, which also gave a much smaller response than scopolamine.

We also decided to test the selectivity of the probe in front of other common abuse drugs. For this, five equiv. of each drug (3,4-MDPH, ephedrone, diazepam, methylone, and MHPH) were added to a solution of the probe (50 μM of 1 with 1 equiv. of $\text{Cu}(\text{NO}_3)_2$ in DMSO/H₂O 95:5). The main conclusions (see Fig. 7) were the strong response of the probe towards scopolamine and ketamine (quite similar for both drugs), a medium response towards some synthetic cathinones and the lack of response of methylone and diazepam. However, if scopolamine is added to a sample containing methylone and diazepam, 50 % of quenching is still observed.

2.5. Detection of scopolamine in real samples

Finally, we decided to test our probe under real conditions, by spiking some soft drinks and alcoholic beverages with a known amount of scopolamine. These experiments were carried out in organic/aqueous media (DMSO/water) at room temperature and the response could be observed almost immediately (within 1 min). For this, 30 μL of the adulterated sample was mixed with a known amount of the probe (1:1

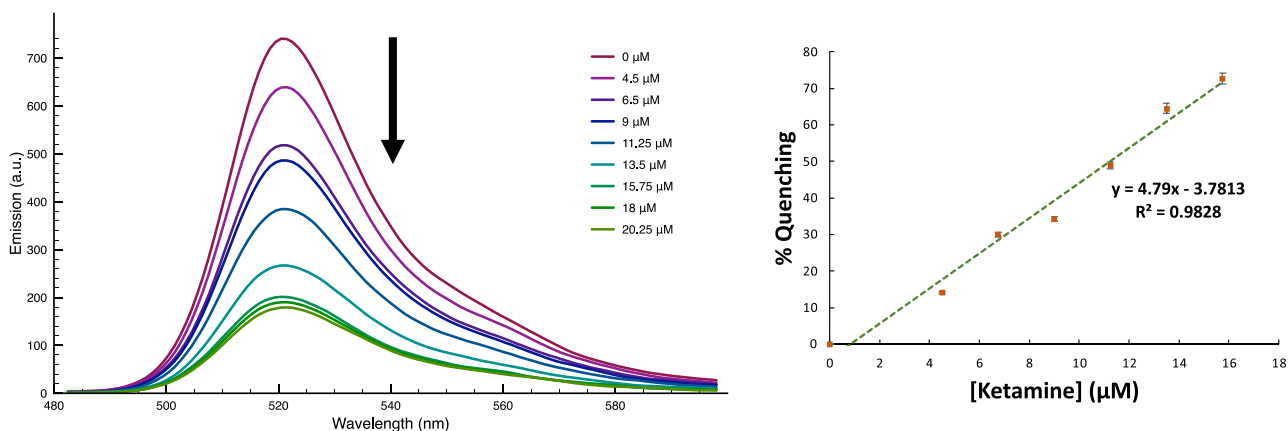


Fig. 5. (A) Emission spectra ($\lambda_{\text{exc}} = 470 \text{ nm}$; $\lambda_{\text{em}} = 520 \text{ nm}$) of compound 1 (5 μM in DMSO: H₂O 95:5 solution) in the presence of 1 equiv. of $\text{Cu}(\text{NO}_3)_2$ and increasing amounts of ketamine after being heated to 60 °C for 10 min. (B) Calibration curve.

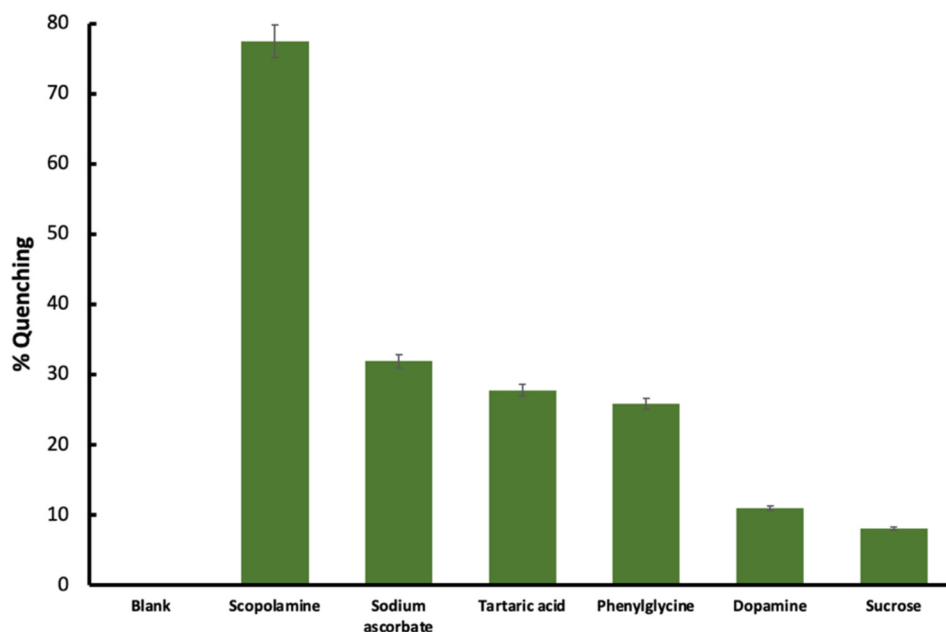


Fig. 6. Quenching of emission ($\lambda_{\text{exc}} = 470 \text{ nm}$; $\lambda_{\text{em}} = 520 \text{ nm}$) of compound **1** ($5 \mu\text{M}$ in DMSO/ H_2O 95:5) and 1 equiv. of $\text{Cu}(\text{NO}_3)_2$ (blank) and the same solution with 5 equiv. of scopolamine or the corresponding interferents. All spectra were registered after heating the mixture for 10 min at 60°C .

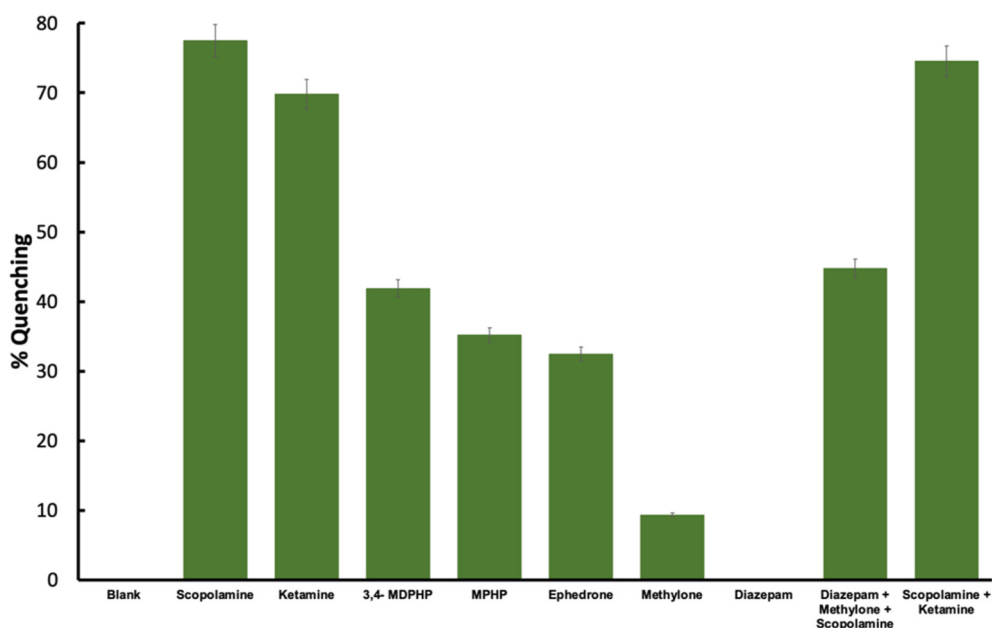


Fig. 7. Quenching of emission ($\lambda_{\text{exc}} = 470 \text{ nm}$; $\lambda_{\text{em}} = 520 \text{ nm}$) of compound **1** ($5 \mu\text{M}$ in DMSO: H_2O 95:5 solution) and 1 equiv. of $\text{Cu}(\text{NO}_3)_2$ (blank) in the presence of 5 equiv. of different abuse drugs.

stoichiometric mixture of **1** and $\text{Cu}(\text{NO}_3)_2$ ($50 \mu\text{M}$ in DMSO/ H_2O) to have the system ready. All adulterated samples presented a 1.3 mM scopolamine concentration, the required amount to induce chemical submission effects [2]. In all cases, the color change of the solution of the probe (from pale green to brownish pink color) was immediately appreciable by the unaided eye.

3. Conclusions

A new BODIPY-Phen conjugate **1**, consisting of a BODIPY dye incorporating a 1,10-phenanthroline moiety in its *meso* position, has been synthesized and characterized by NMR, UV vis absorption and emission spectroscopies and X-ray crystallography. In the presence of Cu

(II) this system acts as a selective optical probe for scopolamine and ketamine in water, via a fluorescent turn off mechanism, with determined LODs of around $3 \mu\text{M}$ for both drugs. These LODs are much lower than the usual amounts of drug in beverages required to induce chemical submission. The detection mechanism seems to be related to the ability of these drugs to reduce $\text{Cu}(\text{II})$ to $\text{Cu}(\text{I})$ under the studied conditions. The coordination of the resulting $\text{Cu}(\text{I})$ ions to the phenanthroline moiety of **1** result in change in color and a quenching of its fluorescence. The probe has been tested with real soft drinks and alcoholic beverages spiked with scopolamine, and the corresponding changes could be immediately observed by the unaided eye.

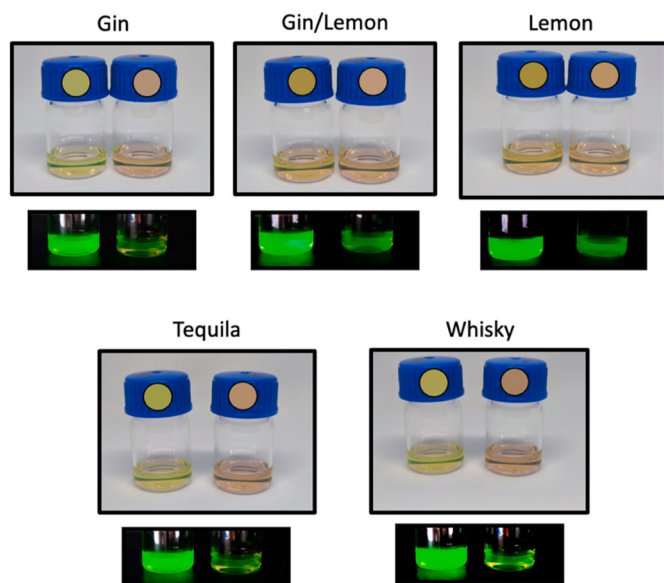


Fig. 8. Color and fluorescent changes of the probe with real samples after 1 min, under visible light/UV lamp. In each example, the sample on the left corresponds to addition of the pure beverage and on the right the same beverage spiked with scopolamine (1.3 mM).

4. Experimental section

4.1. Materials and instruments

All reagents employed in the synthesis were acquired from Sigma Aldrich and used without further purification. ^1H NMR and ^{13}C NMR spectra were registered with Bruker Avance 300 MHz spectrometers, all of them referenced to the corresponding residual solvent peak. All photophysical analysis were carried out in air-equilibrated DMSO at 298 K, unless otherwise specified. UV-vis absorption spectra were recorded with a PerkinElmer λ 40 or Shimadzu UV-2600 spectrophotometers using quartz cells with path length of 1.0 cm. The estimated experimental error was 2 nm on the band maximum. Mass spectrometry measurements were carried out with a Triple TOFTM 5600 LC/MS/MS System, with 2 gas sources (both to 35 psi), 450 °C and ion gas voltage of 5500 V. Plot2 was the program used to depict titrations. Single-crystal X-ray diffraction data were collected on a Bruker D8 Venture automated diffractometer, equipped with a micro-focus CuK α radiation source ($\lambda = 1.5418 \text{ \AA}$) and a Montel mirror, at 120 K. Intensities were collected for absorption effects using the multi scan technique SADABS. The structure was solved and refined by a full-matrix anisotropic least-squares method on F^2 using the SHELXTL software package. The extended packing plots and data from crystal packing were obtained using the software Mercury 2022.1.0. CCDC-2271350, contains the supplementary crystallographic data for **1**. These data can be obtained free of charge from the Cambridge Crystallographic Data Centre.

4.2. Synthesis of 1,10-phenantroline-4-carbaldehyde (**2**)

4-Methyl-1,10-phenantroline (500 mg, 2.57 mmol) was dissolved in of dioxane (35 mL). Then, SeO_2 (714 mg, 6.44 mmol) was added, and the mixture was stirred at reflux for 5 h. After that, the crude was filtered and washed with ether. The organic phase was dried and concentrated under reduced pressure. Compound **2** was obtained as a yellow-brown solid (685 mg, 85 % yield). ^1H NMR (300 MHz, DMSO- d_6) δ (ppm) 10.69 (s, 1H), 9.43 (d, $J = 4.1\text{ Hz}$, 1H), 9.17 (dd, $J = 4.1, 2.0\text{ Hz}$, 1H), 8.98 (d, $J = 9.4\text{ Hz}$, 1H), 8.56 (dd, $J = 8.3, 2.0\text{ Hz}$, 1H), 8.25 (d, $J = 4.3\text{ Hz}$, 2H) 7.85 (dd, $J = 8.3, 4.2\text{ Hz}$, 1H).

4.3. Synthesis of chemosensor **1**

2,4-Dimethyl-1H-pyrrole (1.3 mL, 12.5 mmol) was dissolved in DCM (65 mL) dried under inert atmosphere. Then, 1,10-phenantroline-4-carbaldehyde (900 mg, 4.3 mmol) and TFA (20 μL , 0.26 mmol) was added and the mixture was stirred for 50 min at room temperature. Then, DDQ (1.04 g, 4.58 mmol) was added, and the reaction was stirred other 50 min. After that, NEt_3 (10.5 mL, 61.8 mmol) was added and, after stirring for 30 min, BF_3OEt_2 (10.5 mL, 83.9 mmol) was added dropwise in an ice-water bath and the mixture was kept stirring overnight. Finally, the solvent was removed and the crude was purified by chromatographic column, using DCM:MeOH 99:1. Compound **1** was obtained as a red solid (300 mg, 17 % yield). ^1H NMR (300 MHz, CDCl_3) δ (ppm) 9.31 (d, $J = 4.5\text{ Hz}$, 1H), 9.25 (dd, $J = 4.4, 1.4\text{ Hz}$, 1H), 8.23 (dd, $J = 8.0, 1.4\text{ Hz}$, 1H), 7.76 (d, $J = 8.9\text{ Hz}$, 1H), 7.72 (d, $J = 8.9\text{ Hz}$, 1H), 7.65 (dd, $J = 8.0, 4.4\text{ Hz}$, 1H), 7.56 (d, $J = 4.4\text{ Hz}$, 1H), 5.97 (s, 2H), 2.59 (s, 6H), 1.08 (s, 6H). ^{13}C RMN (75 MHz, CDCl_3) δ (ppm) 156.93, 150.82, 150.50, 146.42, 145.83, 142.82, 141.91, 136.58, 136.04, 131.06, 129.04, 128.19, 127.22, 123.86, 123.24, 123.00, 122.00, 14.82, 14.31. HRMS: m/z calculated for $\text{C}_{25}\text{H}_{22}\text{BF}_2\text{N}_4$ [$\text{M} + \text{H}$] $^+$: 427.1906; found 427.1907.

4.4. Fluorescence titration experiments with (–)-scopolamine in water

In a 3 mL quartz cell (1.0 cm of path length), DMSO/ H_2O 95:5 (2700 μL) was mixed with the reaction mixture (300 μL). The reaction mixture was prepared by mixing of DMSO (900 μL), **1** (50 μL , 1 mM in DMSO), $\text{Cu}(\text{NO}_3)_2$ (1 mM in H_2O , 50 μL) and increasing quantities of scopolamine hydrobromide (5 mM in water, 0–22.5 μM final concentration of scopolamine). In each case with a 10-min period of incubation at 60 °C.

4.5. Fluorescence titration with (R/S)-ketamine solutions in water

In a 3 mL quartz cell (1.0 cm of path length), DMSO/ H_2O 95:5 (2700 μL) was mixed with the reaction mixture (300 μL). The reaction mixture was prepared by mixing DMSO (900 μL), **1** (1 mM in DMSO, 50 μL), $\text{Cu}(\text{NO}_3)_2$ (1 mM in H_2O , 50 μL) and increasing quantities of (R/S)-ketamine 5 mM solution in water (0–22.5 μM) until arriving to saturation point. In each case with a 10-min period of incubation at 60 °C.

4.6. Interference studies

In a 3 mL quartz cell (1.0 cm of path length), DMSO/ H_2O 95:5 (2700 μL) was mixed with the reaction mixture (300 μL). The reaction mixture was prepared by mixing of DMSO (900 μL), **1** (1 mM in DMSO, 50 μL), $\text{Cu}(\text{NO}_3)_2$ (1 mM in H_2O , 50 μL) and 50 μL from 5 mM solution of the corresponding interferent, with a 10-min period of incubation at 60 °C.

CRediT authorship contribution statement

Jordi Hernández-Contreras: Investigation, Data curation, Methodology, Writing – original draft, preparation. **Paula Madrigal:** Investigation, Data curation. **Pau Arroyo:** Methodology, Data curation. **Malva Liu-González:** Investigation, Data curation. **Salvador Gil:** Methodology, Writing – original draft. **Margarita Parra:** Conceptualization, Funding acquisition, Supervision. **José A. Sáez:** Conceptualization, Supervision, Writing – review & editing. **Pablo Gaviña:** Conceptualization, Funding acquisition, Supervision, Writing – original draft.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.dyepig.2023.111806>.

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