A turn-on fluorogenic probe for detection of MDMA from ecstasy tablets†

Daniel Moreno, Borja Diaz de Greñu, Begoña García, Saturnino Ibeas and Tomás Torroba*

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We report a fluorogenic probe that is able to discriminate a range of primary or secondary biogenic amines and their natural or synthetic mimics, in water or buffer, by means of the turn-on transient generation of green fluorescence, with high quantum yields and low detection limits, thus making the system suitable for the detection of abuse drugs, such as MDMA, from ecstasy tablets.

Some biogenic amines are neurotransmitters, their appropriate physiological interactions are crucial for a healthy condition and their synthetic mimics are some of the most widespread abuse drugs. Having in common only amine groups, discrimination between the structurally diverse biogenic amines and their synthetic mimics is only possible by lengthy chromatographic methods that do not allow in-field assays. Fluorimetric reagents for fast detection of amines are a good alternative but these methods suffer from lack of selectivity for biogenic amines. With this in mind, we have developed a new bis-diaryluerea receptor tagged with two units of a new highly solvatochromic fluorescent indicator. In this communication, we aim to report our findings on the selective fluorescent discrimination of biogenic amines and their natural or synthetic mimics such as ecstasy.

The synthesis of the fluorescent probe is depicted in Scheme 1. Compound 1 is a pale yellow ($\lambda_{\text{max}} \approx 398$ nm, $\varepsilon = 34500$ M$^{-1}$ cm$^{-1}$, in CH$_2$Cl$_2$), highly solvatochromic fluorescent indicator ($\lambda_{\text{max}} \approx 537$ nm, quantum yield $\Phi$ = 0.6 in CH$_2$Cl$_2$). Reaction of 1 with bis-isocyanate 2 in chloroform for 2 days at room temperature afforded bis-diaryluerea 3 in 77% yield, fully characterized by spectroscopic and analytical techniques (see ESI†). It is known that arylureas increase their colour in the presence of anions but, in our case, 3 diminished its yellow colour ($\lambda_{\text{max}} \approx 400$ nm in DMSO) in the presence of very few anions, showing very little increase of fluorescence. Although alkylurea-derivatives of 1 are indeed fluorescent, bis-diaryluerea 3 showed only a slight fluorescence because of the effective quenching of the fluorescence by the electron-rich p-substituent arylerather group. It is known that, in related systems, quenching of the luminescence is assigned to a photoinduced electron transfer (PET) from the electron-rich alkoxybenzene unit to the fluorescent unit. Therefore compounds that could get involved in intermolecular photoinduced electron transfer processes, such as the classic processes between acceptor dyes and amines, could restore the original fluorescence of the amidoindane group, giving rise to fluorescent probes for amines. Consequently, we tested solutions of 3 (10$^{-4}$ M in DMSO) with a large number of amines of different types, primary, secondary, tertiary, aliphatic, aromatic or heterocyclic, including diverse functionalities in their structures, and observed the influence of the structure of amine on the generation of fluorescence (see ESI†). Thus, primary or secondary aliphatic amines, including benzyl or allylic amines, were best suited for the generation of fluorescence in solutions of 3, but tertiary aliphatic amines, primary, secondary or tertiary anilines and aromatic amines such as pyridine, pyrrole, indole or carbazole did not show any effect. Diamines such as o/n/p-phenylenediamines, proton sponge, 9,10-diaminophenanthrene, or DMAP did not show any effect. The only exceptions were DBU, DBN and, more weakly, DABCO. Several amines of natural origin belonged to the class of amines for which the fluorogenic probe 3 was able to generate fluorescence, therefore we tested solutions of 3 with 11 common biogenic amines, selected from the most representative series of biological importance. Thus, upon adding one or more equivalents of commercial biogenic amines in water to solutions of 3 (10$^{-4}$ M in DMSO), the initial yellow colour of these solutions slightly lightened and an intense green fluorescence appeared under the UV lamp ($\lambda_{\text{exc}} = 366$ nm). We took quantitative measurements from representative examples, thus the quantum yield for 3 and cadaverine (1 equiv.) in DMSO was $\Phi = 0.57$, and for 3 and tryptamine (1 equiv.) QY was $\Phi = 0.43$. Some natural ephedrines or synthetic amphetamines and its analogues, 3,4-methylene-dioxymphetamine (MDA) and 3,4-methylenedioxyamphetamine (MDMA, ecstasy), are closely related to biogenic amines (Fig. 1). They are important therapeutic and recreational drugs, and constitute a large proportion of currently used drugs of abuse (ecstasy consumption is second only to cannabis in several countries). Therefore, we have studied the changes in fluorescence of 3 in

![Scheme 1 Synthesis of fluorescent probe 6.](image-url)
the presence of ephedrine, pseudoephedrine, amphetamine, MDA and MDMA, in order to detect and differentiate their presence. Thus, upon adding one or more equivalents of ephedrines or amphetamines in water to solutions of 3 (10⁻⁴ M in DMSO) the yellow colour of the solutions lightened very little but an intense green fluorescence appeared under the UV lamp (λₚₑₙ = 366 nm) (Fig. 1). MDA and MDMA, obtained as hydrochloride salts, were studied in neutralized aqueous solutions as well as in neutralized solutions in HEPES, pH = 8.2, showing comparable results.

The rate of generation, intensity and duration of the fluorescence were different in every case, which can be seen by the naked eye, therefore we studied the kinetics of the interaction of probe 3 with 13 representative biogenic amines or their mimics, under the same experimental conditions, by stopped-flow measurements (Bio-Logic MOS-450/SFM-400) using both fluorescence and absorbance detection, because the absorption peak of the probe 3/amine complex blue shifts to UV ranges due to moderate disruption of conjugation of the chromophore. Conditions for pseudo-first-order kinetics were always maintained by using excess of amine in water over the concentration of 3 in DMSO. Ten-fold repeated runs were averaged and analyzed by non-linear functions. Two relaxation times (fast and slow) were observed by fluorescence detection (Fig. 2A) whereas only a single effect was observed by absorbance detection (Fig. 2B).

The absorbance effect is in fairly good agreement with the fast relaxation time deduced from fluorescence measurements; this finding reveals that the fast relaxation time, denoted as k₁, is related to fluorescent complex formation, whereas the slow rate constant, k₂, is related to a quenching process. The two observed rate constants, k₁ and k₂, differing by about one order of magnitude, are shown in the ESI. It is worth noting that MDMA had the highest k₁ and the lowest k₂ values, which will be important for its practical detection from ecstasy tablets. Fluorescent titrations were studied by preparing fresh individual solutions, corresponding to each titration point, and then measuring them independently at a fixed time. In this way, the quenching effect was circumvented and we obtained classic turn-on fluorescence titration curves and titration profiles that nicely fitted 1 : 1 complexes, from which the binding constants were calculated (see ESI†). As a typical example, quantitative fluorescent titration of a 10⁻⁴ M solution of 3 in DMSO and spermine in water (λₑₓᶜₑ = 288 nm) showed a 14-fold increase of the intensity of the green emission centred at 517 nm as spermine was added, the titration profile fitted a 1 : 1 binding model (Fig. 3). Epimers such as ephedrine and pseudoephedrine gave similar binding constants.

Titrations were also performed by dissolving the biogenic amines in buffer (HEPES, pH = 8.2), although in these cases the binding constants were different to the ones obtained by dissolving the amines in water. Job’s plot analysis of fluorescence titrations of 3 and tryptamine or spermine revealed a maximum at 50% mole fraction, according to the proposed 1 : 1 binding stoichiometry (Fig. 3, for spermine). It is noticeable that the formed fluorescent intermediate was always a 1 : 1 complex, independently of the number of amino groups in the analyte. We performed ¹H NMR titrations of 3 (10⁻¹ M in DMSO-d₆) with a simple secondary amine (pyrrolidine in D₂O or DMSO-d₆), which showed only coordination to the urea group but, after addition of one or more equivalents of amine, the NMR signals enlarged substantially, therefore we performed the EPR spectrum of the mixture, which showed signals consistent with the formation of a stable bi-radical complex. Therefore all experiments pointed to the formation of an initial intramolecular charge-transfer fluorescent complex between the amine donor and the bisurea acceptor that evolved by slow formation of stable bi-radical species (see ESI†). We calculated some detection limits of 10⁻⁴ M solutions of 3 in DMSO, calculated in fluorescence emission by the blank variability method, and selected amines. According to a turn-on fluorescent process, the experimental values were low. Thus, the detection limit for cadaverine in water was 6.13 × 10⁻⁸ M, for cadaverine in HEPES, pH = 8.2 was 9.51 × 10⁻⁷ M, for dopamine in HEPES,
obtained binding constants (log $K_{eq}$), rate constants ($k_i^p$, $k_i^h$) and the $pK_a$ values of the 13 amines, measured in 40% and 80% w/w DMSO/H$_2$O mixtures and calculated for 80% w/w DMSO/H$_2$O (see ESI†), were used as variable inputs in a principal components analysis (PCA). The purpose of the analysis was to obtain a small number of linear combinations of the four variables, which accounted for most of the variability in the data. In this case, two components were extracted, together they accounted for 72% of the variability in the original data. Separation between data points on the PCA plot described how unlike they are from one another. Thus, ephedrine and pseudoephedrine clustered closely, as well as diamines and polyamines, but amphetamine, MDA and MDMA were spatially separated between them, indicating the ability of the probe to discriminate the diverse analytes from one another (Fig. 4).

To test a practical use of the probe, we prepared a sample of ecstasy tablet$^{10}$ containing MDMA (45%) and common additives (sucrose, chalk dust, caffeine) and tested 20 µl of aqueous solution of the sample and 3, in comparison to every pure component, showing that the ecstasy tablet gave a fluorescent response as good as pure MDMA, thus proving that the system is suitable for a fast selective fluorescent detection of MDMA from ecstasy tablets (Fig. 5). The selectivity of 3 for primary or secondary amines could permit the detection of MDMA in the presence of other common tertiary amine additives such as methylenedioxy-pyrovalerone (MDPV, “bath salts”) and some synthetic opioids (such as meperidine) or cocaine analogs.

In summary, we have evaluated a fluorogenic probe that was able to detect primary or secondary amines selectively from anilines, tertiary and aromatic amines, and to discriminate a range of primary or secondary biogenic amines and their natural or synthetic mimics in water or buffer, by means of the turn-on transient generation of green fluorescence, with high quantum yields and low detection limits. Moreover, the sensitivity to the detected amines was preserved in a large extension by performing the experiments in the presence of other organic or inorganic compounds such as those found in commercial formulations. In this way, the system was suitable for the quantitative detection of abuse drugs, such as MDMA, from ecstasy tablets.

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**Notes and references**