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Highly sensitive fluorescent probe for detection of alkaloids

Ivan Pietro Oliveri, Santo Di Bella *

Dipartimento di Scienze Chimiche, Università di Catania, I-95125 Catania, Italy

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ABSTRACT

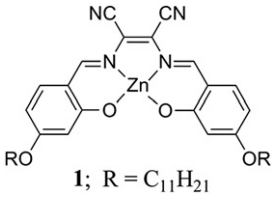
A new fluorescent probe, based on an amphiphilic Schiff-base zinc(II) complex, **1**, for the sensitive detection of some important classes of alkaloids is presented. It exhibits optical absorption changes and fluorescence enhancement upon formation of 1:1 **1**·alkaloid adducts. Four diverse classes of alkaloids, represented by their basic structures and related representative prototypes, are investigated, through the study of optical and binding properties of **1**·alkaloid adducts. It is found that the chromogenic and fluorogenic complex **1** is selective between these classes of alkaloids in the micromolar range, with a limit of quantification of 0.40 μM for nicotine and 0.43 μM for cinchonine.

1. Introduction

Alkaloids are natural organic compounds, generally secondary plant metabolites, containing basic nitrogen atoms. They are easily isolable, soluble both in polar and non-polar solvents, and characterized by a powerful physiological activity.[1–3] Actually, because of their pharmacological effects they are used as antibacterials[4,5] (berberine), vasodilators[6] (papaverine), anesthetics[7] (morphine, atropine), antimalarials[8] (quinine), and as stimulant[9,10] (nicotine, caffeine) or psychoactive[11] (cocaine) drugs, with a consequent considerable interest to medicine and society. More recently, alkaloids have attracted growing interest in synthesis and catalysis.[12–16]

Due to this broad range of applications, the molecular probing of alkaloids is certainly of relevance. In particular, the development of selective methods for sensitive detection of alkaloids is highly desirable. Among the different techniques utilized for developing chemical sensors, molecular fluorescence is one of most powerful because of the high sensitivity of detection.[17,18] However, literature on fluorescent chemosensors for alkaloids is rather limited. Except for a recent contribution on a cross-reactive array capable of classifying alkaloids,[19] literature data on fluorescent chemosensors are related to the detection of single alkaloids,[20–24] or a distinct class of them.[25–27]

We have recently demonstrated that some amphiphilic Zn^{II} Schiff base complexes of dichloromethane (DCM) solution exhibit substantial optical variations and a dramatic enhancement of the fluorescence emission upon addition of a coordinating species.[28,29] As this process occurs because of the axial coordination to the acidic Zn^{II} ion, it is expected to be selective and sensitive to the Lewis basicity of the coordinating species. Tetracoordinated Schiff-base Zn^{II} complexes have recently been investigated for their fluorescent features, related to the structure of the Schiff-base template[30–35] and the axial coordination.[36–40]



1; R = $C_{11}H_{21}$

The aim of this contribution is to explore the sensor properties of Schiff-base Zn^{II} complex **1** in DCM with respect to the most common classes of alkaloids, by investigating the optical changes and binding properties upon their basic structures (**2**–**5**) and related representative prototypes (**2a**–**6a**) (Scheme 1). It is found that the chromogenic and fluorogenic complex **1** is selective between these classes of alkaloids, with excellent detection sensitivity. Since isolation and/or purification of alkaloids generally involve extractions with non-polar solvents,[1–3] for example, as in the case of tobacco alkaloids,[41–43] the present approach represents a useful method for detection of alkaloids in non-polar solvents.

* Corresponding author. Tel.: +39 095 7385068; fax: +39 095 580138; e-mail address: sdibella@unict.it (S. Di Bella).

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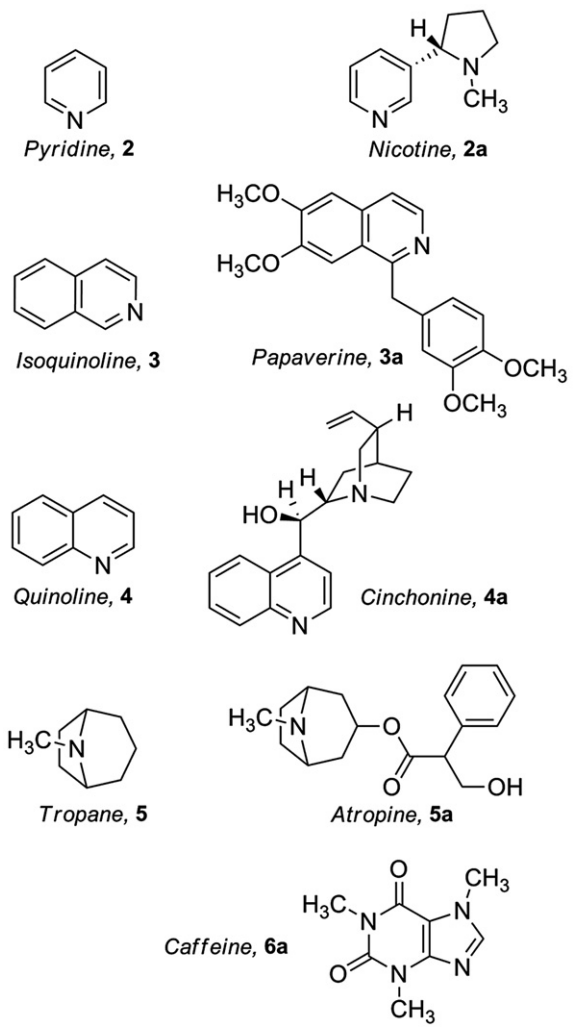

Scheme 1. Structure of investigated alkaloids.

2. Results and discussion

The binding interaction between **1** and the investigated alkaloids **2**–**5** and **2a**–**6a** always involves the formation of 1:1 adducts, as established by Job's plot analysis using the continuous variation method[44,45] (Figs. S1 and S2), and as demonstrated by ^{1}H NMR measurements in the case of formation of the **1**·pyridine adduct.[28,29] Moreover, it is characterized by appreciable optical variations and naked-eye observation of fluorescence intensity enhancement.

Spectrophotometric and spectrofluorimetric titrations of 10 μM DCM solutions of **1** were performed using DCM solutions of the alkaloids **2**–**5** and **2a**–**6a** as titrants. As representative example, the titration with nicotine, **2a**, is reported in Fig. 1.

Titrations of all investigated alkaloids are reported in the Supplementary data. They involve appreciable optical variations and an enhancement of the fluorescence emission, by almost an order of magnitude. In particular, on switching from **1** to **1**·alkaloid adducts, the optical absorption spectra show the appearance of a new, more intense band at ≈550 nm, indicative of the axial coordination of the alkaloid to the Zn^{II} metal center.[28,29,46] Moreover, the existence of multiple isosbestic points upon spectrophotometric titrations (Fig. 1 and Figs. S3–S10) further confirms the formation of defined 1:1 **1**·alkaloid adducts. The fluorescence emission wavelength (Table 1) is rather constant for all adducts (λ_{max}≈600 nm), except for cinchonine (λ_{max}=592 nm) and tropane (λ_{max}=606 nm) adducts, and is independent of the excitation wavelength.

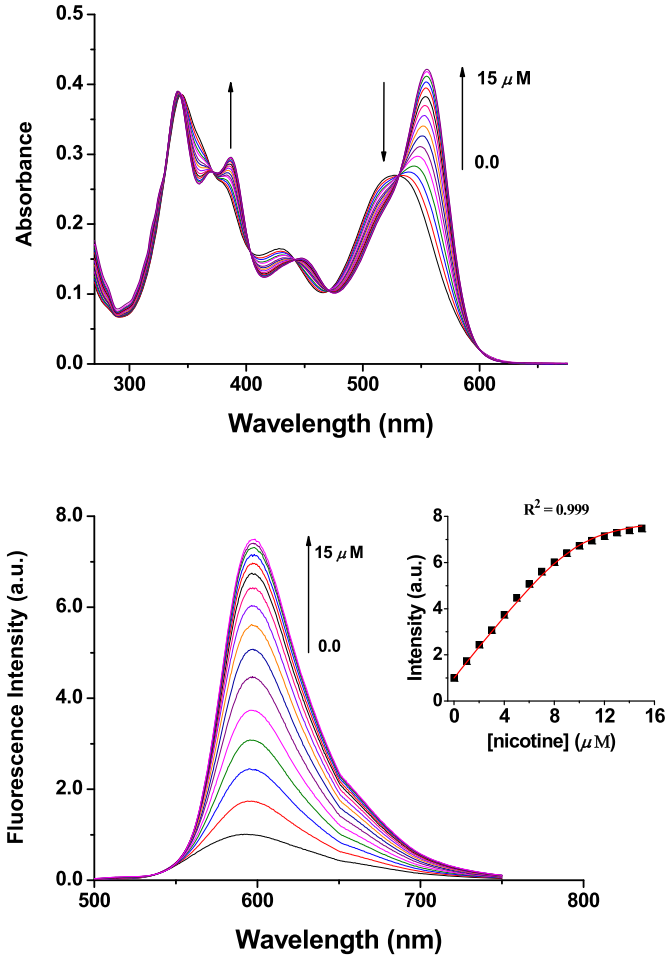

Fig. 1. UV/vis absorption (top) and fluorescence (bottom) (λ_{exc}=465 nm) titration curves of **1** (10 μM solution in DCM) with addition of nicotine. The concentration of nicotine added varied from 0 to 15.0 μM. Inset: variation of fluorescence intensity at 597 nm as a function of the concentration of nicotine added. The solid line represents the curve fitting analysis with [Eq. 1].

Table 1
Fluorescence emission, binding constants, and limits of quantification (LoQ) for the 1:1 **1**·alkaloid adducts

Adduct	λ_{max} (nm)	log *K*	LoQ (μM)
Pyridine, **2**	598	5.4±0.1	0.49 (39)
Nicotine, **2a**	597	5.6±0.1	0.40 (64)
Isoquinoline, **3**	596	6.1±0.2	0.46 (60)
Papaverine, **3a**	603	5.1±0.1	1.3 (4.3×10^2)
Quinoline, **4**	600	4.5±0.3	3.6 (4.7×10^2)
Cinchonine, **4a**	592	6.4±0.2	0.43 (1.3×10^2)
Tropane, **5**	606	4.6±0.1	4.0 (3.4×10^2)
Atropine, **5a**	599	3.8±0.3	15 (4.3×10^3)
Caffeine, **6a**	597	2.6±0.2	1.8×10^2 (3.6×10^4)

Values in parentheses refer to LoQs in μg L^{-1}.

The **1**·alkaloid binding constants, *K*, estimated from fluorescence titration data by the nonlinear curve fitting analysis of fluorescence intensity versus alkaloid concentration,[47] are rather large, log *K*>4, except for **5a** and **6a** (Table 1), indicating strong binding interactions. The order is isoquinoline>pyridine>>tropane≈quinoline for the basic structures **2**–**5**, and cinchonine>nicotine>papaverine>>atropine>caffeine for the representative alkaloids **2a**–**6a**, in spite of the stronger Lewis basicity[48,49] of the tertiary **5** and **5a** amines with respect to the pyridine-like **2**–**4** and **2a**–**4a** species, thus indicating that the binding interaction is strongly influenced by steric hindrance.[50] Within the **2a**–**6a** series, the largest **1**·cinchonine binding constant suggests that of the two

nitrogen atoms of cinchonine, the stronger Lewis basic and less encumbered quinuclidine nitrogen, rather than the quinoline one, is presumably involved in the formation of the 1:1 adduct (Fig. S2). Actually, fluorimetric titrations of DCM solutions of **1** using quinuclidine as titrant give a **1**·quinuclidine log K=6.9±0.3, comparable to that calculated for cinchonine (Table 1). This explains the larger binding constant value, by almost two orders of magnitude, on switching from quinoline to cinchonine, as previously found in the formation of diiodine·base adducts.[51,52] On the other hand, the stronger Lewis basicity of quinuclidine, in comparison to tropane, can be related to the less sterically encumbered nature of the former.[50]

The plots of relative fluorescence changes versus concentration of alkaloids in the micromolar range clearly indicate the selectivity of **1** within the investigated classes of alkaloids (Figs. 2 and 3). Thus, among the basic structures **2**–**5**, pyridines and isoquinolines can be selectively detected over the quinoline and tropane series. This can be easily verified by competitive experiments. For example, fluorimetric titrations of **1** with isoquinoline performed with and without the presence of an equimolar concentration of quinoline, indicate negligible variations of fluorescence intensity (Fig. S13). To observe a fluorescence response analogous to that for isoquinoline a concentration of quinoline one order of magnitude larger is required (Fig. S14). Within the **2a**–**6a** alkaloids, nicotine, cinchonine, and papaverine can selectively be detected, in the micromolar range, which respect atropine and caffeine, as can be also easily verified by competitive titration experiments. Again, to observe an analogous fluorescence response to nicotine or cinchonine, a concentration of atropine almost two orders of magnitude larger is required; while in the case of papaverine a concentration of atropine 15 times larger is required. Overall, the present results indicate high selectivity of **1** for pyridine-, isoquinoline-based, and cinchona alkaloids, as cinchonine possesses the quinuclidine group common to the cinchona family.[1–3]

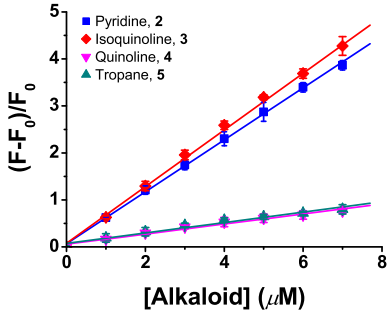

Fig. 2. Plots of relative fluorescence changes versus concentration of four alkaloids in DCM (basic structures **2**–**5**) added to **1** (10 μM solution in DCM), monitored at 597 nm.

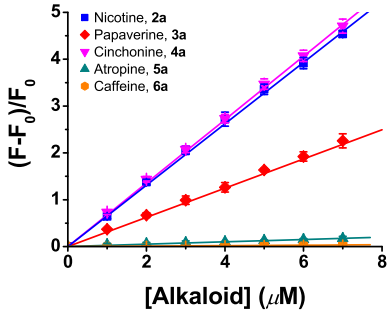

Fig. 3. Plots of relative fluorescence changes versus concentration of five alkaloids in DCM (representative prototypes **2a**–**6a**) added to **1** (10 μM solution in DCM), monitored at 597 nm.

The limit of quantification (LoQ) of a 10 μM DCM solution of **1**, calculated according to IUPAC recommendations,[53,54] indicates very low values for the series of investigated alkaloids, below the micromolar range for **2**, **3** and **2a**, **4a** (Table 1). When compared to literature fluorescent chemosensors of alkaloids,[19–27] the complex **1** is definitely more sensitive. Actually, even if in these investigations limits of detection are not reported, the investigated range of concentrations is generally higher[19–27] than that explored in this study. Moreover, the present LoQ values rival or exceed those reported in literature for detection of alkaloids using hyphenated analytical methods.[42,43,55–61] Thus, complex **1** is useful for fast detection of pyridine-, isoquinoline-based, and cinchona alkaloids in trace amounts (μg L^{-1}), with a linear dynamic range spanning almost two orders of magnitude (Figs. 2 and 3), up to 10 μM.

3. Conclusion

We have successfully developed a sensitive fluorescent probe for alkaloids in DCM, which exhibits fluorescence enhancement upon formation of 1:1 adducts. Its binding interaction can be related to Lewis basicity, strongly influenced by the steric characteristics of the coordinating alkaloid, leading to high selectivity, in the micromolar range, and sensitivity for pyridine-based and cinchona alkaloids. This approach represents a useful method for detection of alkaloids in non-polar solvents.

4. Experimental

4.1. Materials and general procedures

The Zn^{II} complex, **1**, was synthesized and fully characterized as previously reported.[28,29] Commercial alkaloids (Aldrich) **2**–**4** and **2a**, **4a**–**6a** were used without further purifications. Papaverine was obtained from papaverine hydrochloride (Aldrich) by extraction with a 3 M solution of NaOH saturated with Na_2CO_3 in diethyl ether. Dichloromethane stabilized with amylene was used to prepare solutions of **1**. Freshly prepared DCM solutions of **1**, obtained from stock solutions 1.0×10^{-3} M, were used for spectrophotometric and fluorimetric measurements.

4.2. Measurements

Optical absorption spectra were recorded at room temperature with a Varian Cary 500 UV/vis/NIR spectrophotometer. Fluorescence spectra were recorded at room temperature with a Fluorolog-3 (Jobin Yvon Horiba) spectrofluorimeter. Spectrophotometric and fluorimetric titrations were performed with a 1 cm path cell, using DCM solutions of **1**. Involved alkaloids in DCM solutions were added to the solution of the complex **1** via a 25-μL Hamilton syringe. At least three replicate titrations were performed for each alkaloid. In each fluorimetric titration (Fig. 1 and Figs. S3–S10) the wavelength of excitation is related to an isosbestic point.

4.3. Calculation of the binding constants

The **1**·alkaloid binding constants, K, were calculated from fluorescence titration data by the nonlinear curve fitting analysis of F versus c_p [Eq. 1]:[47]

$$F = F_0 + \frac{F_{lim} - F_0}{2c_0}\left[c_0 + c_p + 1/K - \left[(c_0 + c_p + 1/K)^2 - 4c_0c_p\right]^{1/2}\right] \quad (1)$$

where F_0 is the initial fluorescence of the solution having a concentration c_0, F is the fluorescence intensity after addition of a given

amount of alkaloid at a concentration c_p, and F_{lim} is the limiting fluorescence reached in the presence of an excess of alkaloid (see Supplementary data for further details).

4.4. Calculation of the limit of quantification

The limit of quantification was calculated from fluorescence data, according to IUPAC recommendations.[53,54] In particular, it was calculated using the following equation:

$$\mathrm{LoQ} = K \times S_b/S \quad (2)$$

where K=10, S_b is the standard deviation of the blank solution, S is the slope of the calibration curve. In our case, since the DCM solution of **1** gives a fluorescence signal without the presence of the alkaloid, therefore this signal is taken as the blank. Twelve blank replicates were considered. The calibration curve was obtained from plots of fluorescence intensity of **1** versus concentration of the alkaloid added. Each point is related to the mean value obtained from at least three replicate measurements (see Supplementary data for further details).

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tet.2011.09.100. These data include MOL files and InChIKeys of the most important compounds described in this article.

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