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Molecular recognition directed porphyrin chemosensor for selective detection of nicotine and cotinine

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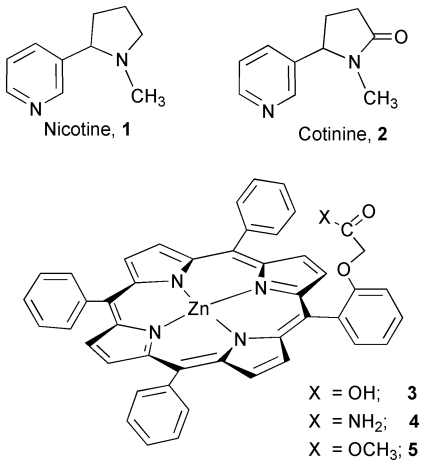
The first example of a metalloporphyrin based fluorescent chemosensor for selective detection of dinitrogen alkaloids such as nicotine and cotinine in solution by using a 'two-point' binding strategy and a modified fluorescence analysis procedure is reported.

Nicotine, a dinitrogen alkaloid is a major drug of abuse, acting as a potent agonist of the nicotinic acetylcholine receptor (*n*AChR). Recently, nicotinic agonists and antagonists have been considered as promising therapeutic agents for a variety of conditions including the treatment of pain, cognitive and attention deficits, Parkinson's disease, Tourette's syndrome, and anxiety.[1] Nicotine is also the most abundant and potent pharmacological agent in tobacco and tobacco smoke, hence, it is of considerable interest to medicine and society.[2] Several methods using HPLC, GC-MS or capillary electrophoresis are available for quantification of nicotine in tobacco, tobacco smoke, pharmaceutical agents, atmospheric air (an issue related to passive smoking), and in urine samples (involves cotinine, a metabolic product of nicotine).[3] Though these methods offer good detection sensitivity, they often suffer from low selectivity. Hence, a selective method for sensitive detection of nicotine and cotinine is highly desirable.

The development of sensors/biosensors for selective detection of analytes and improved transduction methods for higher detection sensitivity is considered to be a challenge in modern chemistry.[4] Among the different techniques utilized for developing chemical sensors, molecular fluorescence is an important one because of its high sensitivity of detection down to a single molecule, recognition and/or self-assembly directed selectivity, on–off switchability, sub-nanometer spatial resolution with sub-micron visualization, and sub-millisecond temporal resolution.[5] Among the different fluorophores that could be utilized to develop fluorescent sensors/chemosensors, porphyrins are attractive candidates because of their relatively high fluorescence quantum yields and the many different established synthetic procedures of functionalization.[6] In porphyrin based chemosensors, sensing is often achieved either by having suitable receptors at the ring periphery, by metal axial ligation, or a combination of both.[6] In the present study, we have successfully designed a porphyrin based fluorescence chemosensor for selective detection of nicotine and cotinine in solution. Our approach utilizes: (i) a 'two-point' binding mechanism which involves axial ligation of the nicotine pyridine entity through the zinc ion of the porphyrin cavity, and, hydrogen bonding of the pyrrolidine ring nitrogen to a carboxylic acid or amide group located on one of the phenyl rings of a tetraphenylporphyrinatozinc macrocycle and (ii) a modified porphyrin fluorescence data analysis procedure.

The binding of the dinitrogen alkaloid, **1** or **2**, to the newly synthesized, receptor porphyrin,† **3** or **4** was studied by ^{1}H NMR and UV-visible absorption spectral methods. Fig. 1 depicts ^{1}H NMR spectra of **1**, **3**, and, a mixture of **1** and **3** in $CDCl_3$. Upon binding to **3**, the *o*-pyridyl protons of **1** experience a shielding up to 5 ppm while the *m*- and *p*-pyridyl protons and the pyrrolidine ring protons of **1** experience less shielding (1–2 ppm). The protons of the substituted phenyl ring of **3** experience



a deshielding effect (up to 0.5 ppm) upon binding to **1**. Control experiments performed using *meso*-tetraphenylporphyrinatozinc, (TPP)Zn, (a compound without the side pendant arm) or compound **5**, bearing a methyl ester group at the substituted phenyl ring, reveal similar large deshielding of pyridyl protons of **1** indicating that the pyridyl group of **1** binds to the central zinc.[9]

The UV-visible absorption spectral studies reveal red shifted Soret and visible bands upon addition of **1** to a solution of the investigated receptor porphyrins confirming that the pyridyl entity of **1** binds to the central zinc.[10] The formation constant *K*

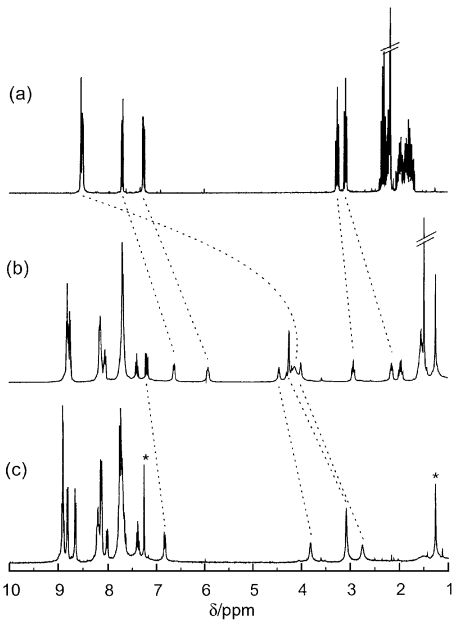


Fig. 1 ^{1}H NMR spectrum of (a) nicotine, **1** (31 mM), (b) **1** (31 mM) + porphyrin, **3** (27 mM), and (c), **3** (12 mM) in $CDCl_3$ at 298 K.

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Table 1 Formation constant, *K*, and the thermodynamic parameters for zinc porphyrin–alkaloid complexes in toluene

Porphyrin	Alkaloid	K_a, M^{-1} [a]	ΔG, KJ mol^{-1} [a]	ΔH, KJ mol^{-1}	ΔS, J K^{-1} mol^{-1}
3	**1**	455.6×10^3	−32.28	−65.75	−112.31
	2	42.3×10^3	−26.39	−47.21	−69.87
4	**1**	63.1×10^3	−27.38	−49.12	−72.96
	2	17.5×10^3	−24.21	−40.25	−53.83
5	**1**	6.4×10^3	−21.69	−26.13	−14.89
	2	7.0×10^3	−21.92	−26.44	−15.17
(TPP)Zn	**1**	6.9×10^3	−21.91	−26.34	−14.87
	2	7.0×10^3	−21.93	−26.33	−14.77

[a] At 298 K.

calculated from the Scatchard method[11] of UV-visible absorption titration curves, is listed in Table 1. The binding constants for (TPP)Zn binding to alkaloids under these solution conditions is also given for comparison. The *K* values for **1** binding to **3** is found to be nearly two orders of magnitude higher than that observed for binding of either **1** to **5** or **1** to (TPP)Zn. The binding of alkaloids, **1** or **2** to porphyrins **3** or **4** are stronger as revealed by the *K* values and this effect could be attributed to the 'two-point' mode of binding. As expected, the *K* values for binding of **1** or **2** to **5** are comparable to that observed for alkaloid binding to (TPP)Zn indicating the absence of any hydrogen bonding between the methyl ester group of **5** with the pyrrolidine ring nitrogen of either **1** or **2**. The calculated thermodynamic parameters from the Van't Hoff plots of ln*K vs.* T^{-1} for **5** or (TPP)Zn binding to **1** or **2** also draw similar conclusions. Interestingly, both ΔH and ΔS decrease upon binding to **3** or **4** as compared to that observed for binding to **5** or (TPP)Zn. These results indicate that the enthalpy change is a main factor responsible for the observed higher stability of the 'two-point' bound porphyrin–alkaloid systems.

Fig. 2 shows the fluorescence emission spectra of porphyrin **3** in the presence of various amounts of **1** in toluene. The zinc porphyrin emission bands located at 605 and 650 nm decrease in intensity with the appearance of an isosbestic point at 670 nm indicating the presence of only one equilibrium in solution. It is observed that the decrease in intensity of the 650 nm band is much more than the 605 nm band.‡ Similar spectral features are observed for porphyrin **3** or **4** binding with either compound **1** or **2**.

In order to quantitate these results, we monitored the intensity ratio of these two bands as a function of alkaloid concentration.

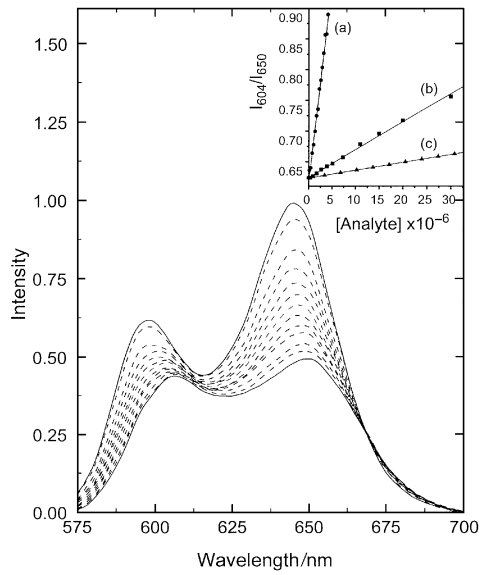

Fig. 2 Fluorescence emission spectrum of **3** (3.8 μM) in the presence of various amounts of **1** in toluene (λ_{ex} = 420 nm). The inset figure shows the relationship between the intensity ratio of the emission bands, I_{604}/I_{650} of **3** in the presence of (a) nicotine, (b) cotinine and, (c) pyridine substrates.

The inset in Fig. 2 shows such plots obtained for nicotine and cotinine binding. A linear relationship is obtained for the emission peak intensity ratio against the alkaloid concentration. This procedure is found to work well in non-coordinating solvents such as toluene, *o*-dichlorobenzene or acetonitrile. These plots offer the much-needed selectivity with respect to the presence of other axially coordinating nitrogenous bases. As shown in the inset of Fig. 2, the intensity ratio of the emission bands for a strongly coordinating ligand such as pyridine does not change significantly in the employed concentration range. These results along with the higher binding constants suggest that the present 'two-point' binding and fluorescence analysis procedure offers the much-needed selectivity for dinitrogen alkaloid detection. Further studies to expand this novel approach of employing a 'two-point' binding and modified fluorescence analysis procedure for developing porphyrin chemosensors for selective detection of compounds of biological and societal importance are in progress.

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Notes and references

† Free-base forms of porphyrins **3**–**5** were synthesized by reacting stoichiometric amounts of pyrrole, benzaldehyde and the appropriate *ortho* substituted benzaldehyde in propionic acid followed by column chromatography purification on either basic alumina or silica gel. The *ortho* substituted benzaldehydes, (2-formylphenoxy)acetic acid and (2-formylphenoxy)acetamide were synthesized according to the literature procedure given in ref. 7*a* and 7*b*, respectively. Zinc insertion was carried out according to the standard procedure (ref. 8). The molecular integrity of all the synthesized free-base and zinc porphyrins was established from FAB mass, elemental analysis and ^{1}H NMR studies (see Fig. 1 and text for ^{1}H NMR results).

‡ The details of the fluorescence quenching mechanism will be published elsewhere.

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