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Fluorescence sensing of theobromine by simple 2,6-diamino-pyridine and the novel cyclic chair-like hydrogen-bonded tetramer of its diacetyl derivative

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ABSTRACT

2,6-Diaminopyridine is found to be a simple fluorescent sensor for theobromine and its diacetyl derivative **2** also effectively binds theobromine. The receptor-binding sites are based on the co-operative hydrogen-bonding abilities of secondary amides. An unprecedented hydrogen-bonded self-organised cyclic tetrameric supramolecular network is shown for one such small molecule **2** containing one heterocyclic ring in contrast to the binuclear substrates like guanine or pterin which usually form cyclic tetrameric structures.

Theobromine **3** is an alkaloid belonging to methylxanthines. Among the methylxanthine derivatives, caffeine (**4**) is one of the most frequently consumed alkaloidal compound. Besides the traditional sources of caffeine, for example, coffee, black tea and green tea, nowadays caffeinated beverages with increased levels of the alkaloid are gathering larger shares among soft drinks and in various analgesics.[1] So, xanthine derivatives have several common pharmacological actions such as CNS-stimulation, diuretic, antibronchopastic and anti-cough medicine. The diverse pharmacological actions of the methylxanthines have found many therapeutic applications. So the recognition of xanthine alkaloids in both solution and solid state is important to understand their fundamental roles in biological systems.[2,5] Therefore, the design of artificial receptors and their synthesis for the recognition of xanthine alkaloids is reported by us and other research groups.[3] Supramolecular chemists have devised several tailor-made chemo-receptors that allow expected designed interaction in solution.[4] The application of molecular imprinting polymers (MIPs) also offers new possibilities.[5] Recently, several research groups accomplished the synthesis of artificial receptors for xanthine derivatives.[6]

Previously,[7] we developed a series of artificial receptors for xanthine alkaloids based on electron-rich aromatic moieties such as polyphenolic substrates and polyamides. Here, we report the photophysical sensing properties of xanthine alkaloids by simple 2,6-diaminopyridine (**1**) and its diacetyl derivatives (**2**) which can efficiently solubilise the notoriously insoluble xanthine substrate theobromine (**3**) in chloroform. We also report here the self-assembly in a solid state, crystalline sheet of *N*,*N*′-(pyridine-2,6-diyl)diacetamide(**2**) which makes a novel tetrameric cyclic structure like guanine or pterin.[4] To our knowledge, such a tetrameric hydrogen-bonded structure is rare in such mononuclear aromatic compounds. All these assemblies involve a hydrogen-bonding-induced-recognition process in self-organisation. The simple receptor **2** contains two donors and one acceptor hydrogen-bonding (**DAD**) site which has complementarity (**ADA**) with xanthine alkaloids, for example, especially theobromine guest **3** which has two lactam carbonyl groups and one imide N–H proton which makes complementary hydrogen bonding with receptor **2**. We therefore examined the photo-sensing property of simple 2,6-diaminopyridine **1** and its diacetyl derivative **2** which binds with the guest molecules of xanthine derivatives theobromine (**3**), caffeine (**4**) and theophylline (**5**) based on the triple hydrogen bonding complementarity as shown in Figure 1.

Theobromine makes possible hydrogen-bonding complexation of **1:3** and **2:3** (Fig. 2) of the **DAD**···**ADA** type arrangement between receptors **1** and **2** with the six membered ring containing the imide group of theobromine leaving the imidazole ring uncomplexed. Both UV–vis and fluorescence were studied with the xanthine derivatives (**3**, **4** and **5**) but only the diamine shows fluorescent properties, and not the diacetyl derivatives of compound **1** as expected. So, the xanthine derivatives theobromine (**3**), caffeine (**4**)

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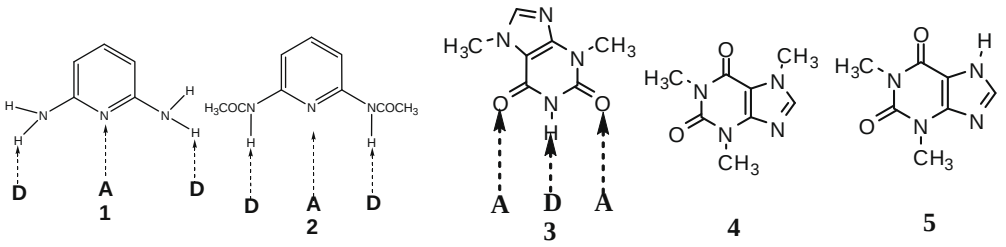

Figure 1. Receptors: **1** and **2**; guests: theobromine (**3**), caffeine (**4**) and theophylline (**5**).

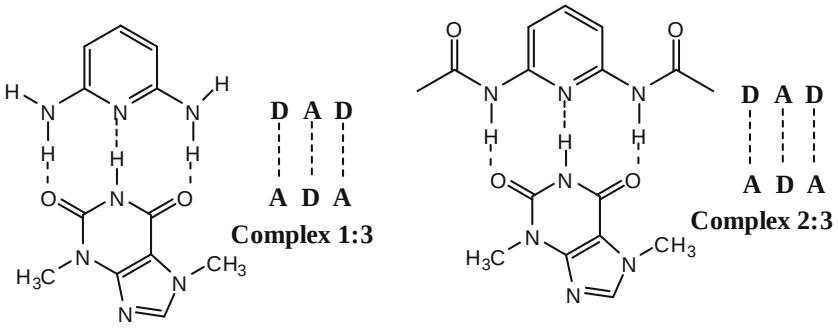

Figure 2. Possible hydrogen-bonding complexes of **3** with receptors **1** and **2**.

and theophylline (**5**) were studied with receptor **1** both by UV–vis and fluorescence methods, whereas receptor **2** was studied only by UV–vis.

Our previous observation[3] by proton NMR titration of theobromine (**3**) with a simple similar type diamide shows a 1:1 complex as evidenced by the appearance of the imide proton of theobromine along with one aromatic and six methyl protons for two $-CH_3$ groups. Here, receptor *N*,*N*′-(pyridine-2,6-diyl)diacetamide (**2**) also effectively solubilises theobromine in chloroform by forming a 1:1 complex by similar observation where the imide proton of theobromine appears at δ 11.37 ppm along with one aromatic and six methyl protons for two CH_3 groups (see Supplementary data).

Complexation studies by UV–vis and fluorescence methods and determination of association constants ($K_{ass.}$): The UV–vis absorption was studied[8] to see the interaction of receptors **1** and **2** with xanthine derivatives (**3, 4** and **5**) by the gradual addition of guest ($\sim 10^{-5}$ M concentration) to a solution of receptors **1** and **2** and the results are shown in Figures 3 and 4. Receptor **1** has absorbance maxima at λ_{max} = 306 nm and receptor **2** shows a peak at λ_{max} = 292 nm in acetonitrile solvent. In all the cases, a continuous decrease of absorbance is observed (except caffeine[2,7] because most of the nitrogens are methylated) on addition of the guest solution but the appearance of the isobestic point (λ = 290 nm) during titration of theobromine with receptor **1** revealed the formation of a 1:1 complex. To ascertain the selectivity and sensitivity of receptors **1** and **2** with xanthine alkaloids, we measured absorbance $[A_0/(A_0 - A)]$ at 306 nm for receptor **1** and at 292 nm for receptor **2** as a function of concentration of xanthine derivatives which fits with a linear relationship. The ratio of the intercept versus slope gave the association constant[9] $K_{ass.}$ (M^{-1}) as shown in Table 1. Among the xanthine derivatives **3**, **4** and **5**, the maximum association constant was observed for theobromine (**3**) ($K_{ass} = 1.8492 \times 10^4\ M^{-1}$ and $2.3894 \times 10^4\ M^{-1}$ with receptors **1** and **2**, respectively) (Fig. 5).

Similarly, the binding properties of the xanthine derivatives were investigated with receptor **1** by observing the changes in fluorescence emission[10] at 352 nm (Fig. 5), where all the excitation spectra show λ_{max} at 352 nm. In the case of theobromine, association constant value was found to be greater than caffeine or theophylline. In this context, a negligible change occurs in fluorescence emission with caffeine due to its fully methylated hindered groups

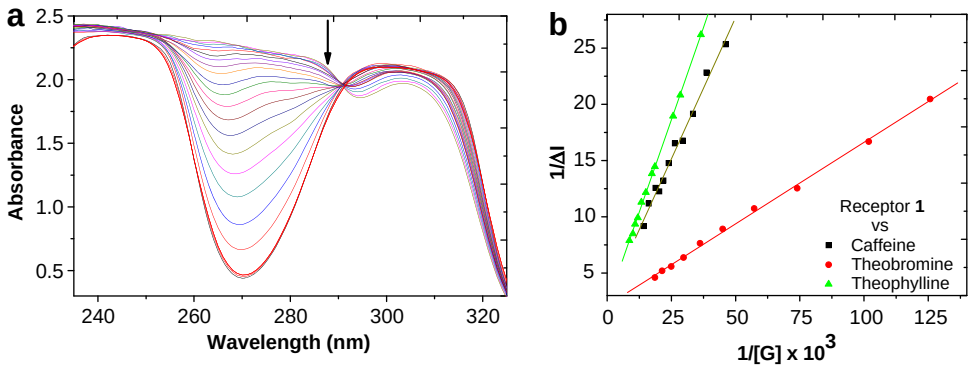

Figure 3. (a) Changes in UV–vis spectra for **1** ($c = 4.12 \times 10^{-5}$ M) in CH_3CN upon the addition of theobromine (**3**) guest. (b) Binding constant calculation curves.

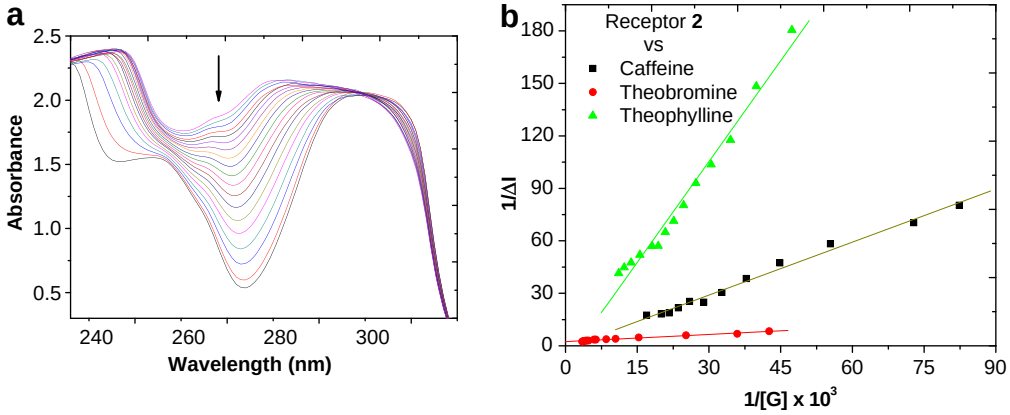

Figure 4. (a) Changes in UV–vis spectra for **2** ($c = 4.66 \times 10^{-5}$ M) in CH_3CN upon the addition of theobromine (**3**) guest. (b) Binding constant calculation curves.

Table 1
Association constants[a] of receptors **1** and **2** with xanthine alkaloids (**3**, **4** and **5**) in CH_3CN by UV–vis and fluorescence methods

Guest	UV–vis method		Fluorescence method
	Receptor **1** (Ka M^{-1})	Receptor **2** (Ka M^{-1})	Receptor **1** (Ka M^{-1})
Theobromine	1.8492×10^4	2.3894×10^4	3.9524×10^4
Caffeine	1.572×10^3	1.348×10^3	5.147×10^3
Theophylline	1.672×10^3	2.592×10^3	3.390×10^3

[a] All the errors are ±8%.

whereas in theophylline there is no methyl group in the imidazole part and hence better binding interaction is observed.

Table 1 shows that theobromine, amongst the xanthine alkaloids, has strong affinity and good selectivity with both the receptors. This is due to the presence of the lactam moiety in the less hindered six membered ring, whereas in caffeine and theophylline, six membered ring nitrogens are fully methylated. So it is expected that theobromine makes a better fit with receptors **1** and **2** by DAD: ADA type triple point hydrogen-bonding interaction.

X-ray study: To investigate hydrogen-bonding selectivity between receptor **2** and theobromine (**3**) in the solid state, we tried to grow the co-crystal of *N*,*N*′-(pyridine-2,6-diyl)diacetamide (**2**) with theobromine (**3**). But we were unable to get single crystal of the complex. However, we were able to get single crystals of the *N*,*N*′-(pyridine-2,6-diyl)diacetamide (**2**) (Fig. 6). Receptor **2** was

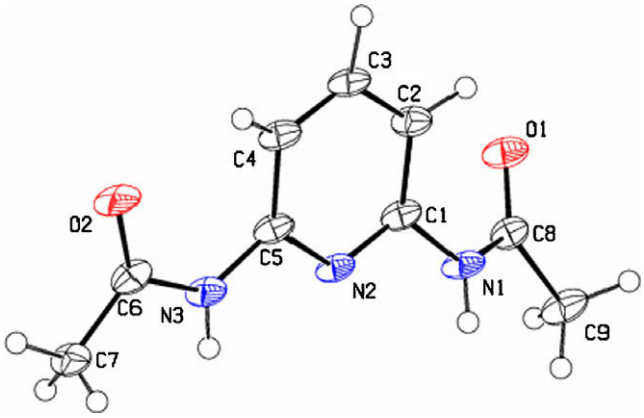

Figure 6. An ORTEP diagram of compound **2** (showing 50% probability displacement).

crystallised from the chloroform–methanol system in the monoclinic space group $P2_1/c$ and showed self-assembling. The hydrogen-bonding interactions in **2** according to X-ray structural studies are shown in Figure 7. The analysis of the crystal structure[11] shows a beautiful self-assembled cyclic tetramer where the self-association of receptor **2** forms an almost chair-like motif containing four molecules where two molecules are in one plane and the other two are present in the perpendicular planes but they are extended in the opposite direction.

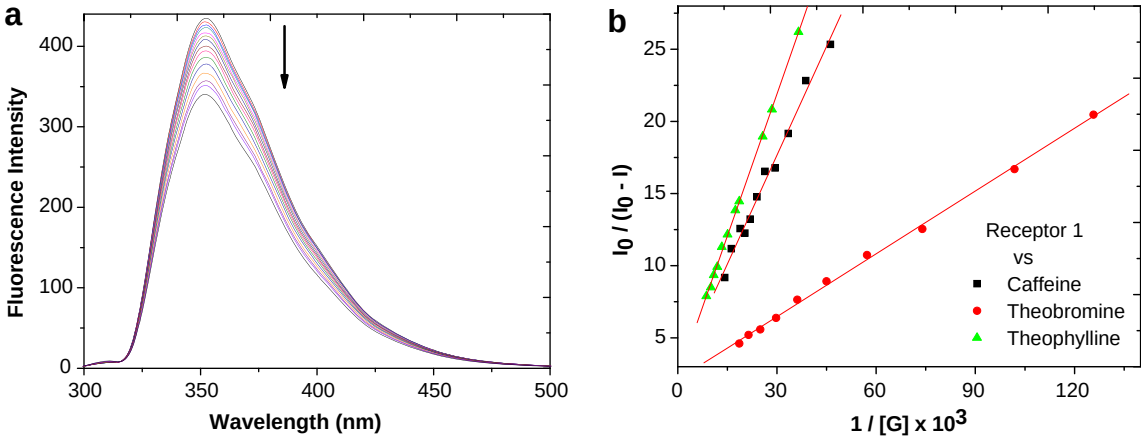

Figure 5. (a) Changes in fluorescence spectra for **1** ($c = 4.12 \times 10^{-5}$ M) in CH_3CN upon the addition of theobromine (**3**) guest. (b) Binding constant calculation curves.

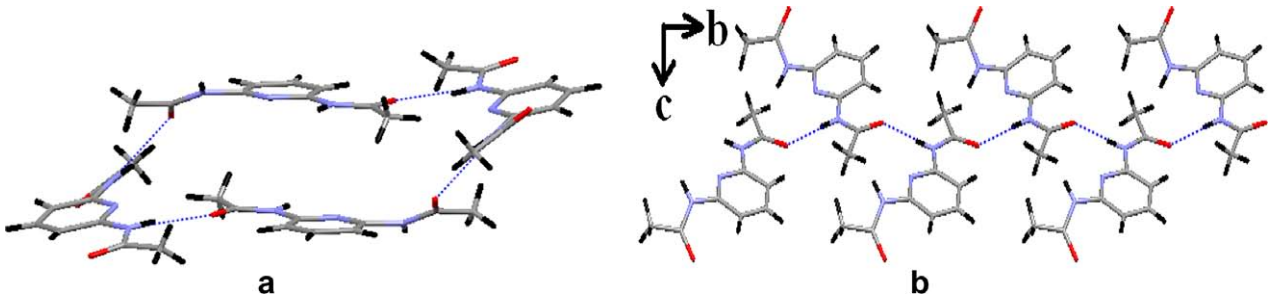

Figure 7. Illustration of the crystal structure of receptor **2** (a) almost chair-like supramolecular synthon; (b) 1D layer viewed down the crystallographic *a* axis.

In conclusion, we have developed a very simple new fluorescent chemosensor through complementary hydrogen-bonding-mediated complexation with theobromine which shows strong binding and good selectivity from other xanthine alkaloids, that is, caffeine and theophylline. This selectivity may be due to the presence of an unhindered lactam moiety present in the six membered part of theobromine. Further studies for the improvement of fluorescent sensors for these xanthines and co-crystallisation with different receptors are in progress.

Acknowledgements

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2008.10.095.

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11. *Crystal data of* **2:** $C_9H_{11}N_3O_2$, $M = 193.21$ monoclinic, $P2_1/c$, $a = 11.1279(12)$, $b = 9.3741(10)$, $c = 9.1878(9)$ Å, $\alpha = 90°$, $\beta = 98.218°(8)$, $\gamma = 90°$, $V = 948.57(17)$ Å^3, $T = 100.0(1)$ K, $Z = 4$, $D_c = 1.353$ g cm^{-3}, $\mu = 0.099$ mm^{-1}, 7619 total reflections were measured, no. of unique reflections = 1668 ($R_{int} = 0.0886$), no. of parameters = 172, $R_1 = 0.0785$ and $wR_2 = 1922$ with $I > 2\sigma(I)$. The diffraction data were collected on a Bruker SMART APEX2 CCD area detector diffractometer with MoK$_\alpha$ radiation ($\lambda = 0.71073$ Å) equipped with an Oxford Cryosystem Cobra low-temperature attachment. Cell refinement: APEX2; data reduction: SAINT;[12] programs used to solve structures: SHELXTL;[13] molecular graphics: SHELXTL. The crystallographic data (excluding structure factors) have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication number CCDC No. 698629. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax: +44(0)-1223-336033 or e-mail: deposit@ccdc.cam.ac.uk]. Compound data of **2**: mp 181 °C, IR V_{max} (KBr): 3320, 2950, 1669, 1531, 1455, 1304 cm^{-1}, ^{1}H NMR (200 MHz; $CDCl_3$); δ (ppm) 8.52 (2H, br s), 7.92 (2H, d, $J = 8$ Hz), 7.63 (1H, t, $J = 8$ Hz), 2.23 (6H, s). Single crystals were grown by slow evaporation of $CHCl_3$/MeOH (1:1 v/v) mixture solution of the compound.
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