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ORIGINAL ARTICLE

First artificial acidic fluorescent receptors for caffeine and other xanthine alkaloids

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Abstract Recognition of three xanthine alkaloids caffeine, theobromine and theophylline with acidic fluorescent receptors has been achieved for the first time through the host–guest interaction. The designed receptors are based on the choice of different fluorophores as spacers which can accommodate the bicyclic xanthine guests in between the binding phenolic hydroxyl or carboxyl groups. Among all the xanthine alkaloids, caffeine being the strongest base, its binding constants were maximum though all the nitrogens of caffeine are methylated. Photochemically the *trans* isomers were converted to the *cis* isomers which generated cavities for better binding of the xanthine alkaloids. Receptor 4-{4-[4′-(3-Carboxypropoxy)phynylazo]phenoxy}butyric acid (**5**) has been shown to be most effective in enhancement of fluorescence intensity with caffeine complexation. The binding properties have been studied by a combination of experimental techniques such as UV-visible and fluorescence spectroscopy. Also solid state binding performance between receptor 4,4′-Diazenyldiphenol (**4**) and caffeine has been described.

Keywords Fluorescence sensors · Caffeine · Theobromine · Theophylline · UV–vis titration · Co-crystal

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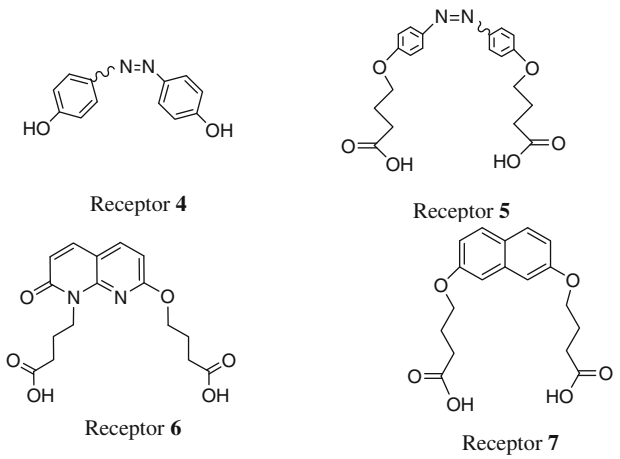
Introduction

Xanthine alkaloids are a class of alkylated oxopurines such as caffeine (**1**), theobromine (**2**) and theophylline (**3**). These are frequently consumed alkaloids [1]. Among these caffeine is one of the most consumed alkaloidal drug [2, 3]. These are natural constituent of tea, coffee, guarana paste, cola nuts and cocoa beans etc. These are also added to many popular soft drinks and these alkylated oxopurines are also widely used in the counter medications including analgesic, bronchodilator, diet aids, cold/flu remedies etc. Natural compounds like catechin [4], theaflavin [5], cyclodextrin [6, 7], potassium chlorogenate [8, 9] etc. are capable of binding caffeine or theobromine through hydrophobic interactions. Caffeine shows heterogeneous association with different dicarboxylic acids [10–13], hydroxy acid [14] and simple heterogeneous stacks with methyl gallate [15, 16], in their solid state. Recently we [17, 18] and several other research groups [19, 20], accomplished the synthesis of artificial alkylated xanthine receptors. It has been recently described a novel simple fluorescence receptor for caffeine by Waldvogel and coworkers [21]. They used scaffolds of triphenyleneketals based on amidic type H-bonding receptors for caffeine. It is also known that the interaction of caffeine has been studied with polyphenols which are present in the preparation of black tea, coffee [22, 23] and tea creaming. In aqueous solutions, the interaction of caffeine has also been studied with zinc based porphyrin peptide type receptor [24].

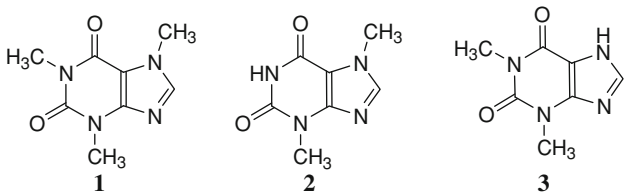
For the determination of caffeine, standard chromatographic methods are available such as (HP)TLC, HPLC-UV and CE^2. However all the above methods require a long period of time, so methods requiring shorter time for analysis is needed. A very few spectroscopic [25], approaches and the applications of molecular imprinted polymers (MIPS) occur in combination with quartz microbalance [26]. However these

types of sensors also required a long time and large concentration of analyte (caffeine). As such a promising approach is to use chemo-sensors, which represent a vivid field in supramolecular chemistry. This problem has been tackled by supramolecular chemists by devising several tailor-made chemo-receptors [27] which allow a design based interaction to occur in solution at very low concentration in the order of 10^{-5} (M). Various synthetic receptors have recently been discovered for caffeine [17–20], theobromine [18, 28, 29] and theophylline [30]. The most of the chemo-sensors are analytically useful in response to the binding event [31].The field of chemical sensor is therefore very attractive due to its easier applications and measurements by spectroscopical means. Fluorometric methods are extremely sensitive to appropriately designed systems. Most of the fluorescent chemo-sensors are based on hydrogen bonding phenomenon [21]. We have recently described a fluorescence sensing property of theobromine by simply using 2,6-diaminopyridine [18]. However, there is no such systematic study yet on detection of very minute quantity of caffeine, theobromine and theophylline in beverages and foods. It is, therefore, important and of major interest in sensor and/or separation technology to apply the concept of direct molecular interactions between alkylated xanthines and synthetic receptors.

We now report a series of novel azo, naphthyridine and naphthalene based receptors **4–7** (Scheme 1) which easily bind to caffeine, theobromine and theophylline (Scheme 2).



Scheme 1 Fluorescence receptors **4–7**



Scheme 2 Xanthine alkaloidal guests: caffeine **(1)**, theobromine **(2)** and theophylline **(3)**

Remarkably the binding sensitivity of these receptors towards caffeine is more than towards theobromine and theophylline using our previous work amide based receptors [17]. Our designed receptors for xanthine alkaloidal moiety combines the acidic part of the receptors which favours the H-bonding, with the ring oxygen and most basic imidazole 'N' of the guests. Due to the presence of azo sensing systems (**4** and **5**), receptors form complexes with xanthine derivatives through *cis–trans* isomerisation process.

Experimental section

General comments

All the chemicals were reagent grade and were used as supplied. The ^{1}H NMR spectra were recorded on Bruker-AM-200 and AM-500 spectrometers. The ^{1}H NMR chemical shift values are expressed in ppm (δ) relative to $CHCl_3$ ($\delta = 7.26$ ppm). UV–visible and fluorescence spectra measurements were performed on a JASCO V530 and a PerkinElmer LS-55 spectrofluorimeter respectively.

The receptors and guests were dissolved in dry UV grade acetonitrile and only for the guest theobromine stopper volumetric flask was sonicated for half an hour. The corresponding absorbance values during titration were noted and used for the determination of binding constant values. Binding constant were determined by the using expression $A_0/A - A_0 = [\varepsilon_M/(\varepsilon_M - \varepsilon_C)](Ka^{-1}\ Cg^{-1} + 1)$, where ε_M and ε_C are molar extinction co-efficients for receptor and the hydrogen bonding complex respectively at selected wavelengths, A_0 denotes the absorbance of the free receptors at the specific wavelength and Cg is the concentration of methyl xanthine guests. The measured absorbance $A_0/A - A_0$ as a function of the inverse of the methyl xanthine guest concentration fits a linear relationship, indicating a 1:1 complexation of the receptor and methyl xanthine. The ratio of the intercepts to the slope was used to determine the binding constant Ka.

Synthesis of receptors 4–7

Our designs are based on the structural features of xanthine alkaloids. Caffeine, theobromine and theophylline are difficult recognition targets as their nitrogen's are fully or partly methylated causing N-CH_3 moiety a poor recognition site for binding. Theobromine is also a difficult recognition substrate for its poor solubility in chloroform as well as in methanol.

The most important task to design artificial receptors for recognition of this type of bigger substrates is to place the hydrogen bonding groups (donors and acceptors) in an

appropriate big cavity where the particular binuclear guest can make maximum number of hydrogen bonds with the host leading to a stronger complexation.

The receptors **4**, **5**, **6** and **7** were synthesized according to the Schemes 3, 4, 5. Azo-diol **4** was prepared following the known literature procedures [32]. O-alkylation of **4** and **14** were synthesized from ethyl-4-bromobutanoate in dry acetone and K_2CO_3 mixture and subsequent hydrolysis of the resultant product afforded the corresponding acidic receptors **5** and **7** [33, 34]. Receptor **6** was prepared by the reaction between 2,7-dihydroxy naphthyridine [35, 36] and ethyl-4-bromobutyrate in the presence of K_2CO_3-dry acetone at refluxing condition and the resulting product was hydrolysed accordingly to give receptor **6**. All the synthesized compounds (**4**–**7**) were characterized structurally using ^{1}H NMR, mass, analysis, IR and UV-spectroscopic method (supporting information).

4,4′-Diazenyldiphenol **(4)**

A mixture of KOH (50 g, 760 mmol), *p*-nitrophenol (10 g, 72 mmol) and water (10 mL) was heated 120 °C for 1 h. Then temperature is allowed to rise to 195–200 °C for vigorous reaction. The large bubbles are produced. Then it is dissolved into water. A dark red solution is produced. It is acidified with pH 3 by conc. HCl and extracted with ether. Then it is dried by Na_2SO_4 and recrystallised by 50% EtOH (v/v) water. A brown crystalline product was obtained. A co-crystal of X-ray quality of the receptor **4** with caffeine was obtained by slow evaporation from a 1:1 saturated solution of the compounds in 2:1 chloroform:methanol mixture solvent.

4-{4-[4-(3-Carboethoxypropoxy)phynylazo] phenoxy}butyrate **(10)**

The azodiol (**4**) (2 g, 9.34 mmol), K_2CO_3 (3 g, 21 mmol) and ethyl 4-bromo butanoate (4.0 mL, 25 mmol) in acetone was refluxed overnight under an inert atmosphere. The reaction was evaporated to dryness, dissolved in water and extracted by chloroform. The organic layer was separated and dried over Na_2SO_4. The crude products were purified by doing column chromatography in chloroform solvent. The orange yellow powder was obtained (yield 80%); Mp. 102 °C; 1H NMR ($CDCl_3$, 200 MHz): δ (ppm): 7.87 (d, 4H, $J = 6$ Hz), 6.98 (d, 4H, $J = 6$ Hz), 4.16 (t, 4H, $J = 5$ Hz), 4.09 (qt, 4H, $J = 2.0$ Hz), 2.53 (t, 4H, $J = 5$ Hz), 2.14 (qt, 4H, $J = 4$ Hz), 1.26 (t, 6H, $J = 5$ Hz). $C_{24}O_6H_{30}N_2$ (442.504), Anal. Calcd. C 65.142, H 6.833, O 21.694, N 6.331; found C 65.132, H 6.833, O 21.689, N 6.337.

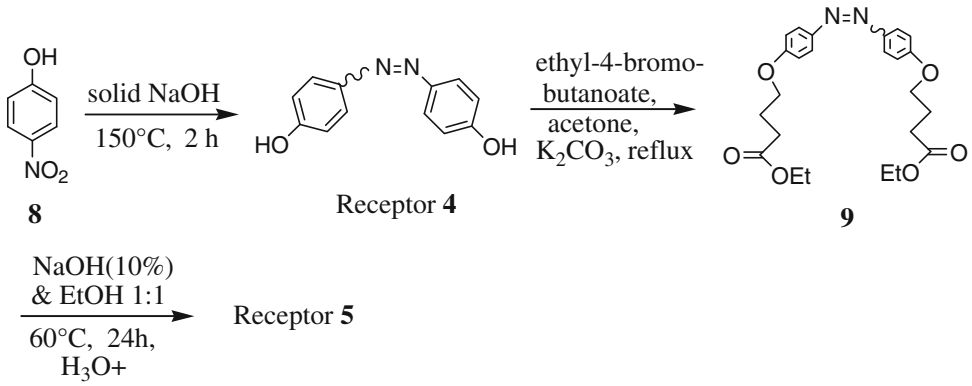
4-{4-[4-(3-Carboxypropoxy)phynylazo]phenoxy} butyric acid **(5)**

The diester was dissolved in a mixture of 10% NaOH solution and EtOH (1:1) under reflux for 24 h. Then it is acidified with conc. HCl in ice. A bright yellow ppt which was filtered and recrystallised from acetone to give the final product. (yield 60%); Mp. > 250 °C; ^{1}H NMR (DMSO-d_6, 500 MHz): δ (ppm): 11.07 (s, 2H), 7.69 (d, 4H, $J = 6$ Hz), 6.96 (d, 4H, $J = 10$ Hz), 4.04 (t, 4H, $J = 7.5$ Hz), 2.39 (qt, 4H, $J = 9.0$ Hz), 2.01 (t, 4H, $J = 8.5$ Hz). $C_{20}O_6H_{22}N_2$ (386.399), Anal. Calcd. C 62.167, H 5.738, O 24.844, N 7.249; found C 62.159, H 5.441, O 28.721, N 8.366. TOF MS: m/z: $[M\text{-}2CO_2]^+$: 301.2697.

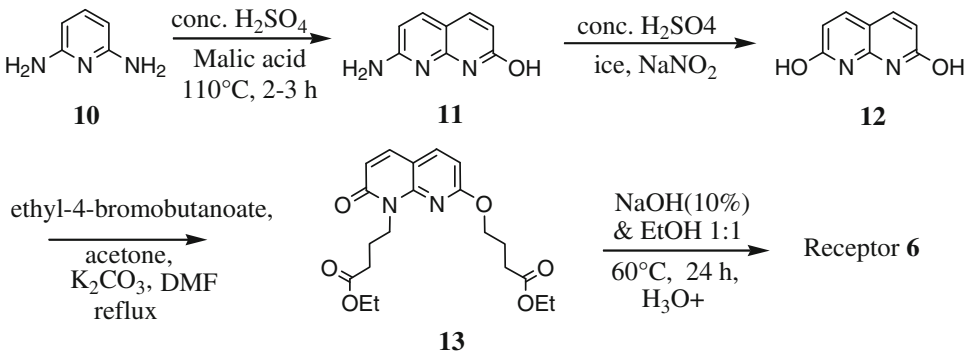
4-[8-(3-Carboethoxypropyl)-7-oxo-7,8-dihydro-[1, 8]naphthyridine-2-yloxy]butyrate **(14)**

Solid K_2CO_3 was ground smoothly. 2,7-dihydroxy-1,8-naphthyridine (2.0 g, 12.3 mmol), K_2CO_3 (3 g, 21 mmol) ethyl 4-bromo butanoate (4.0 mL, 25 mmol) in acetone (100 mL) was refluxed overnight under an inert atmosphere. The reaction was evaporated to dryness, dissolved in water and extracted with dichloromethane. The organic layer was separated and dried over Na_2SO_4. The diester was isolated. (yield 80%); Mp. 98 °C; 1H NMR ($CDCl_3$, 200 MHz): δ (ppm): 7.71 (d, 1H, $J = 8$ Hz), 7.55 (d, 1H, $J = 9$ Hz), 6.59 (d, 1H, $J = 8$ Hz), 6.55 (d, 1H, $J = 9$ Hz), 4.51 (q, 4H, $J = 5$ Hz), 4.48 (t, 2H, $J = 4$ Hz), 4.14 (t, 2H, $J = 3$ Hz),

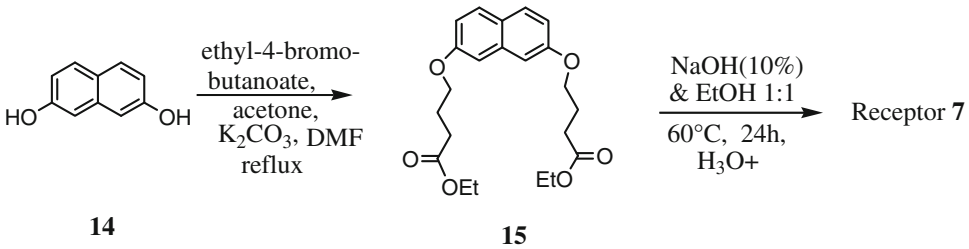
Scheme 3 Synthesis of receptors **4** and **5**



Scheme 4 Synthesis of receptor **6**



Scheme 5 Synthesis of receptor **7**



2.48 (t, 2H, $J = 3$ Hz), 2.42 (t, 2H, $J = 7$ Hz), 2.18 (qt, 4H, $J = 7$ Hz), 1.24 (t, 6H, $J = 4$ Hz). $C_{20}O_6H_{26}N_2$ (390.430), Anal. Calcd. C 61.525, H 6.712, O 24.587, N 7.175; found C 61.532, H 6.721, O 24.581, N 7.181.

4-[8-(3-Carboxypropyl)-7-oxo-7,8-dihydro-[1, 8]naphthyridine-2-yloxy]butyric acid **(6)**

The diester was dissolved in a mixture of 10% aqueous NaOH solution and EtOH under reflux for 24 h. The reaction mixture was evaporated and it was acidified with conc. HCl into ice. The light yellow ppt was collected. (yield 90%); Mp. > 250 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm): 12.08 (s, 2H), 8.04 (d, 1H, $J = 8$ Hz), 7.85 (d, 1H, $J = 9$ Hz), 6.70 (d, 1H, $J = 8$ Hz), 6.48 (d, 1H, $J = 9$ Hz), 4.39 (t, 2H, $J = 6$ Hz), 4.37 (t, 2H, $J = 7$ Hz), 2.39 (q, 2H, $J = 7$ Hz), 2.27 (q, 2H, $J = 7$ Hz), 1.99 (t, 2H, $J = 6.8$ Hz), 1.89 (t, 2H, $J = 7.1$ Hz). $C_{16}O_6H_{18}N_2$ (334.324), Anal. Calcd. C 57.481, H 5.427, O 28.714, N 8.379; found C 57.488, H 5.421, O 28.717, N 8.372. . TOF MS: m/z: $[M + Na]^+$: 358.1112.

2,7-*Bis*[[4-(ethoxycarbonyl)propyl]oxo]naphthalene **(16)**

A solution of 2,7-naphthalenediol (2.0 g, 12.5 mmol), solid K_2CO_3 (3.6 g, 26 mmol), ethyl 4-bromo butanoate (4.0 mL, 25 mmol) in acetone (100 mL) was refluxed overnight under an inert atmosphere. The reaction was evaporated to dryness, dissolved in water and extracted with DCM. The organic layer was separated and dried over Na_2SO_4. The diester was isolated. (yield 80%); Mp. 80 °C; ^{1}H NMR ($CDCl_3$, 200 MHz): δ (ppm): 7.63 (d, 2H, $J = 8$ Hz), 7.02–6.89 (m, 4H), 4.52 (q, 4H, $J = 7$ Hz), 4.06 (t, 4H, $J = 5.5$ Hz), 2.39 (t, 4H, $J = 7$ Hz), 1.86–1.83 (m, 4H), 1.53 (t, 6H, $J = 7$ Hz), $C_{22}O_6H_{28}$ (388.45), Anal. Calcd. C 68.022, H 7.265, O 24.712; found C 68.013, H 7.254, O 24.742.

2,7-*Bis*[[4-(carboxyl)propyl]oxo]naphthalene **(7)**

The diester was dissolved in a mixture of 10% aqueous NaOH solution and EtOH under reflux for 24 h. The reaction mixture was evaporated and it was acidified with conc. HCl into ice. The light yellow ppt was collected. (yield 90%); Mp. 182 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm): 12.48 (s, 2H), 7.63 (d, 2H, $J = 8$ Hz), 7.02–6.89 (m, 4H), 4.06 (t, 4H, $J = 5.5$ Hz), 2.39 (t, 4H, $J = 7$ Hz), 1.86–1.83 (m, 4H), $C_{18}O_6H_{20}$ (332.347), Anal. Calcd. C 65.050, H 6.065, O 28.884; found C 65.048, H 6.071, O 28.877. TOF MS: m/z: $[M + Na]^+$: 355.1156.

Results and discussion

Interaction studies

The photophysical properties of **4**, **5**, **6** and **7** were evaluated in acetonitrile solution (for theobromine 0.8% DMSO containing acetonitrile was used). Here our designed receptors are all acidic in nature and the guest xanthine alkaloids are basic components due to the presence of N-CH_3 and imidazole nitrogen. Moreover it is reported that caffeine extraction from tea leaves can be done by using benzoic acid type acidic substrate. All three compounds (except **4**) reveal the same arrangement of hydrogen

bonding acceptors that is complementary to that of the binding site.

To investigate the sensitivity of photo-physical property of these receptors, we carried out both the UV and fluorescence titrations at very low concentration to effectively sense caffeine, theobromine and theophylline. All methyl xanthines do not absorb light at wavelength greater than 310 nm, whereas azo, naphthyridine and naphthalene based receptors show significant absorption in the range up to 347–400 nm respectively.

UV-visible spectroscopic studies

The colorimetric sensing ability of the receptors **4–7** with methyl xanthine alkaloids such as caffeine (**1**), theobromine (**2**) and theophylline (**3**) in acetonitrile was monitored by UV–vis absorption [37]. The solutions of guests (**1–3**) (4.639×10^{-4} M) were respectively added to the solutions of the receptors **4–7** (3×10^{-5} M). Figure 1a shows that UV–vis absorption spectra of a mixture of receptor **4** (*trans* form) with different concentrations of caffeine in acetonitrile. Addition of caffeine to a solution of receptor **4** results a new absorption band at 272 nm which is gradually enhanced, while the intensity of absorption at 211 nm slowly gets decreased by the formation of shoulder like peak which appear at 230 nm due to formation of new complex at higher concentration. Addition of caffeine to a solution of receptor **4** results in a bathochromic shift of the emission bands Fig. 1a by 6 nm and an increased signal intensity, from 205 to 211 nm. A clear isobestic point was observed at 297 nm.

This result demonstrates that a complex formation of **4** with caffeine is taking place via hydrogen bonding. The same phenomenon is observed in the case of *cis* isomer of receptor **4** by similar experiment where only difference is noted that no shoulder like peak occurs and sharp peak appear at 273 nm (Fig. 1c) with the formation of clear isobestic point at 300 nm. The formation of hydrogen bonded weak interactions with different fluoro-acidic groups, results stronger binding with basic xanthines. Similar type of experiments were done with other guests **2** and **3** by UV–vis method with receptors **4** and **5** and their results are shown in the supporting information.

Parallel investigations were carried out with a series same methyl xanthine alkaloids (**1–3**) with receptors **6** and **7**. A similar phenomenon of UV–vis absorptions was observed in Figs. 2a and 3a. In all the cases increasing concentration of guest, develops a new absorption band which gradually gets enhanced and exhibits sharp upward perturbation Fig. 4a. By contrast, most of the cases significant changes are observed in the presence of caffeine, so it is apparent that caffeine has more selectivity than other xanthine guests. The selectivity of caffeine can be rationalized on the basis of association constants (Table 1).

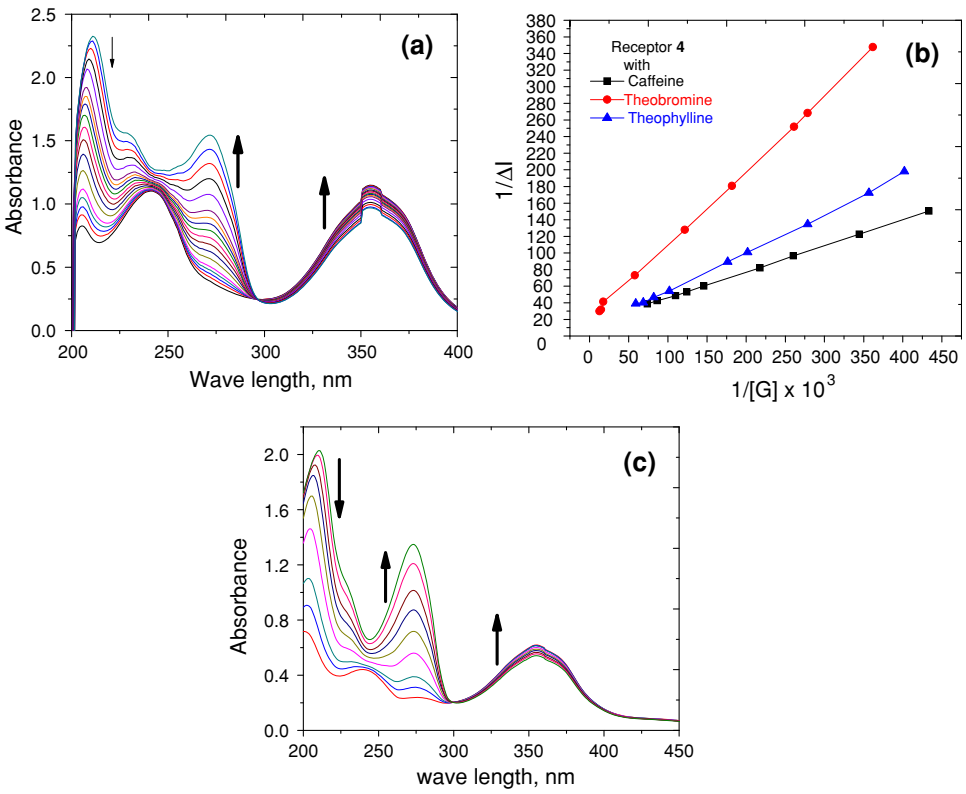


Fig. 1 **a** UV spectral change of *trans*-form of receptor **4** in CH_3CN ($c = 3.271 \times 10^{-5}$ M) upon gradual addition of caffeine ($c = 4.639 \times 10^{-4}$ M) dissolved in CH_3CN. **b** Binding constant calculation curves for receptor **4** versus guests. **c** UV spectral change of *cis*-form of receptor **4**

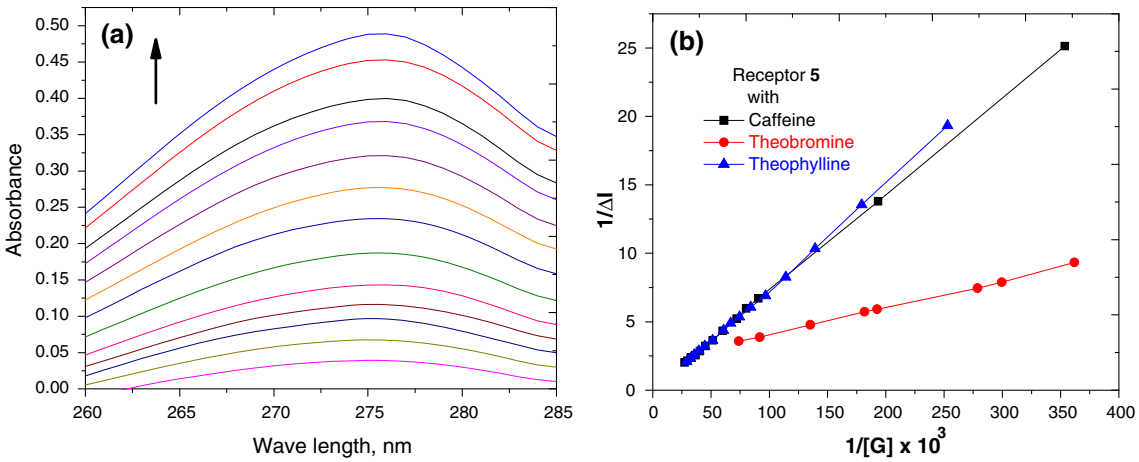


Fig. 2 **a** UV spectral change of receptor **5** in CH_3CN ($c = 3.6269 \times 10^{-5}$ M) upon gradual addition of caffeine ($c = 4.639 \times 10^{-4}$ M) dissolved in CH_3CN. **b** Binding constant calculation curves for receptor **5** versus guests

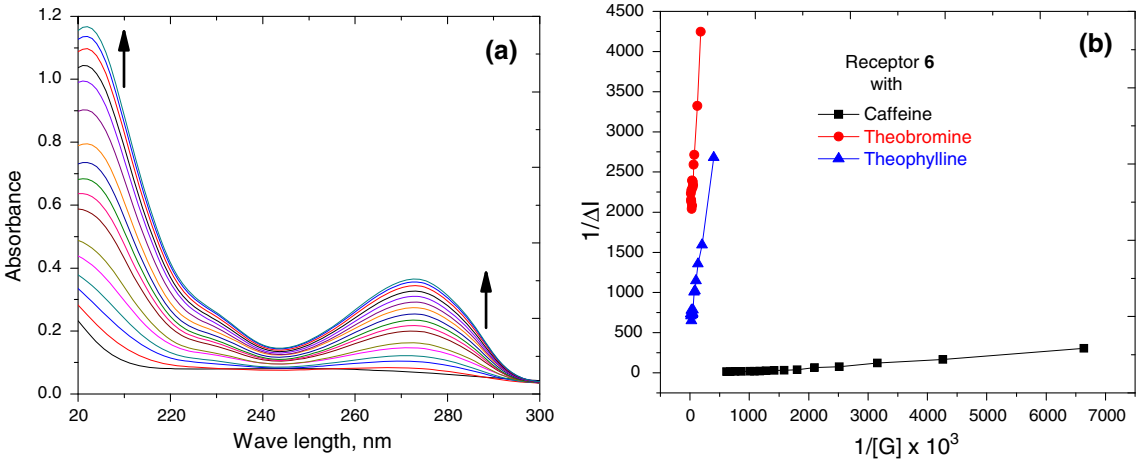


Fig. 3 **a** UV spectral change of receptor **6** in CH_3CN ($c = 3.5928 \times 10^{-5}$ M) upon gradual addition of caffeine ($c = 4.639 \times 10^{-4}$ M) dissolved in CH_3CN. **b** Binding constant calculation curves for receptor **6** versus guests

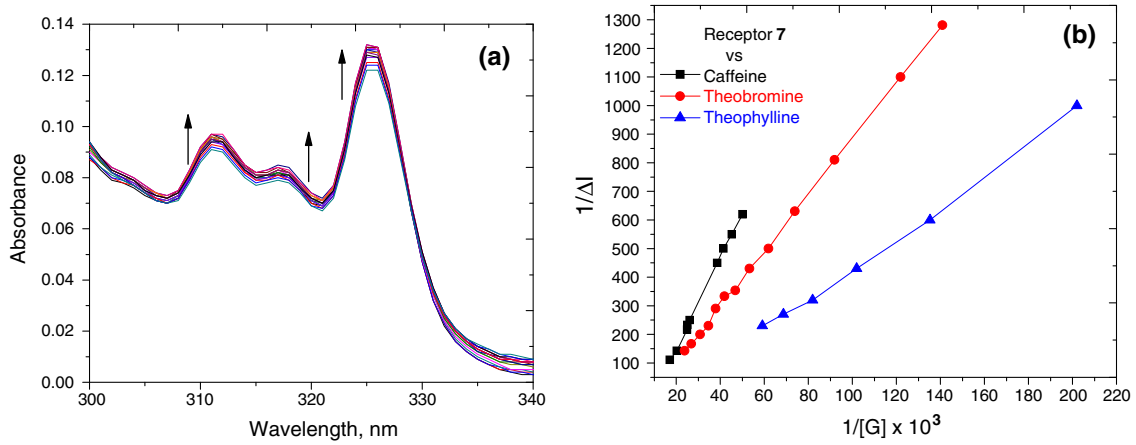


Fig. 4 **a** UV spectral change of receptor **7** in CH_3CN ($c = 3.4638 \times 10^{-5}$ M) upon gradual addition of caffeine ($c = 4.639 \times 10^{-4}$ M) dissolved in CH_3CN. **b** Binding constant calculation curves for receptor **7** versus guests

Table 1 Association constants of receptors **4**, **5**, **6** and **7** with xanthine alkaloids **1**, **2** and **3** in CH_3CN by UV–vis and fluorescence methods

Guests	UV–vis method				Fluorescence method			
	R4 (Ka M^{-1})	**R5** (Ka M^{-1})	**R6** (Ka M^{-1})	**R7** (Ka M^{-1})	**R4** (Ka M^{-1})	**R5** (Ka M^{-1})	**R6** (Ka M^{-1})	**R7** (Ka M^{-1})
1	1.877×10^4	2.076×10^4	6.586×10^5	1.184×10^4	1.451×10^5	1.024×10^7	3.236×10^7	4.192×10^5
2	1.084×10^3	1.330×10^4	1.419×10^5	6.97×10^3	2.513×10^4	1.004×10^6	1.471×10^6	6.250×10^4
3	1.133×10^3	3.268×10^3	1.120×10^5	1.818×10^3	1.026×10^4	5.008×10^6	1.056×10^6	5.340×10^4

All the errors are ±8%

For guests **1** and **3** dry CH_3CN were used for all experiments

For guest **2** dry CH_3CN containing 0.8% DMSO was used for experiments

Fluorescence study

Fluorescence spectroscopy studies were also carried out in order to evaluate the sensitivity or ascertain their excited state properties of receptors **4**–**7** to operate as fluorescent sensors for methylxanthines (**1**–**3**) (Figs. 5, 6, 7). The receptor **4** (3.271×10^{-5} M) when excited at 401 nm in acetonitrile produces large enhancements in the monomer emission bands at 398 nm on gradual addition of caffeine and much smaller changes occur in the corresponding excimer band at 417 nm (Fig. 5a).

The similar changes are observed in the fluorescence spectra of the receptor **5** (3.271×10^{-5} M) in acetonitrile on adding up caffeine which are depicted in Fig. 6a. The large increase in fluorescent intensity was observed. Spectra (a) in Fig. 6a was measured in the absence of caffeine where receptor **5** shows fluorescence spectrum with $\lambda_{max} = 543$ nm. As shown in spectra (o)–(q), **5** exhibits high perturbations upon addition of caffeine. Analogous investigations were carried out with the receptors **6** and **7** by adding caffeine in the titration experiments in the fluorescence spectroscopy (Fig. 7a, b).

For other guests **2** and **3**, parallel investigations were carried out with a series of receptor **4**–**7**. In all the cases slow increase of fluorescence intensity at different λ_{max} is observed according to their fluorophores (see supplementary data). The stronger fluorescence response was observed for caffeine for all the receptors, especially for the receptors **5** and **6**. In case of receptor **5**, large increase of fluorescent intensity was observed. On the basis of the change in fluorescent intensity, the association constants (Ka) were determined and are shown in Table 1**.** The values demonstrate that the association constants for caffeine are more among all of the studied guests. As caffeine is more basic than other two methyl xanthine alkaloids (**2** and **3**), it forms stronger complexes with the designed receptors (Fig. 8).

Crystallographic study of receptor 4 with caffeine

The crystal structure analysis reveals that an asymmetric unit of co-crystal **4** contains a minimum one molecule of caffeine and one molecule of azo-diol. The whole azo-diol molecule is disordered over two position with a site occupancy ratio of 0.638(5):0.362(5). The complex is neutral as determined from X-ray structure analysis.

Co-crystal of **4** with caffeine crystallizes in the monoclinic $P2_1/c$ space group. The caffeine and azo-diol

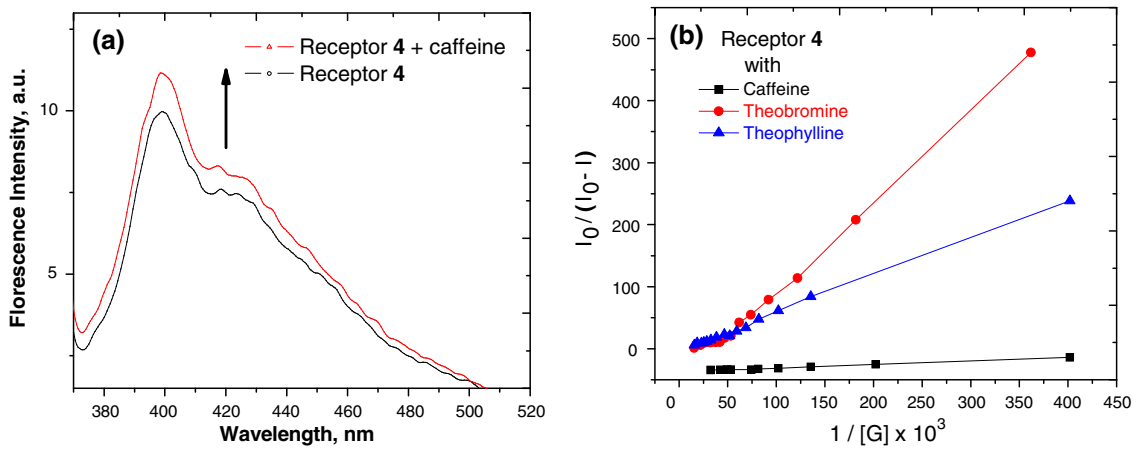


Fig. 5 **a** Fluorescence change of receptor **4** ($c = 3.271 \times 10^{-5}$ M) upon gradual addition of caffeine dissolved in CH_3CN ($\lambda_{max} = 398$ nm). **b** Binding constant calculation curves for receptor **4** versus guests

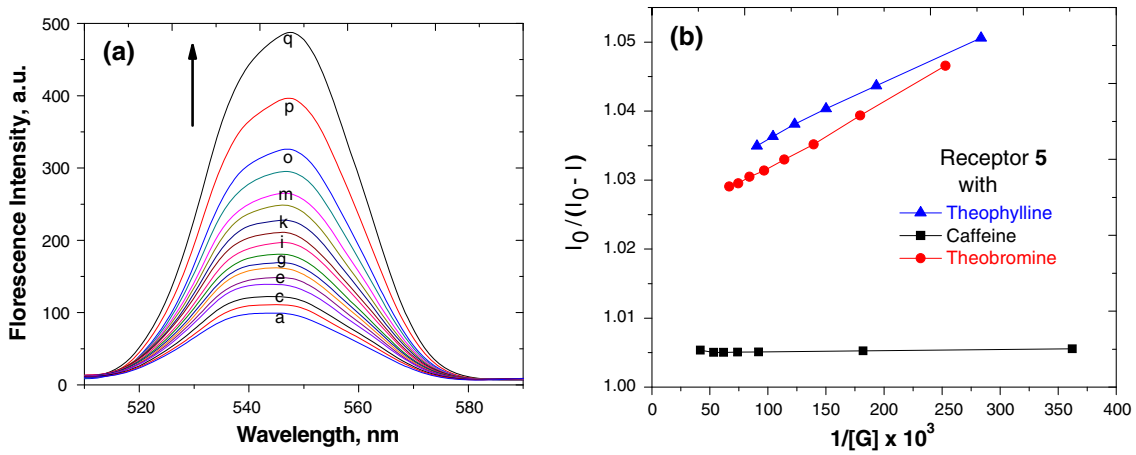


Fig. 6 **a** Fluorescence change of receptor **5** (c = 3.271 × 10^{-5} M) upon gradual addition of caffeine dissolved in CH_3CN (λ_{max} = 548 nm). **b** Binding constant calculation curves for receptor **5** versus guests

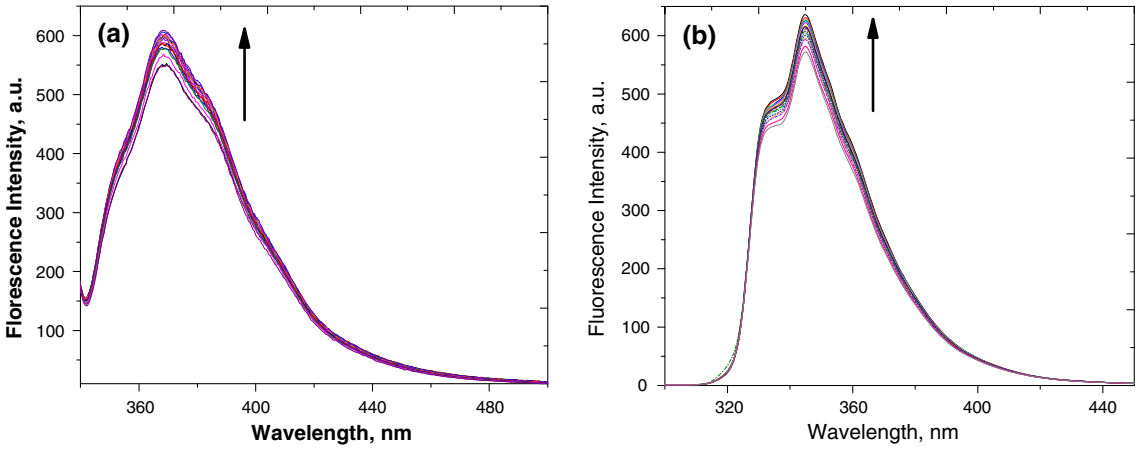


Fig. 7 **a** Fluorescence change of receptor **6** (c = 3.5928 × 10^{-5} M) and **b** receptor **7** (c = 3.4638 × 10^{-5} M) upon gradual addition of caffeine dissolved in CH_3CN (λ_{max} = 335 and 345 nm) respectively

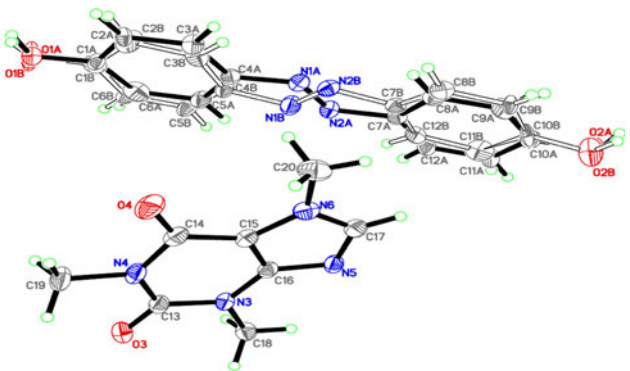


Fig. 8 The molecular structure (ORTEP diagram) of receptor **4** and caffeine

component form a two component assembly involving an intermolecular O–H···O and O–H···N hydrogen bonds. In azo-diol, one hydroxyl group binds with the 5-membered imidazole nitrogen (most basic) of one caffeine molecule and another hydroxyl group is linked with the oxygen of another molecule of caffeine. It is normal that the basic N of imidazole part of caffeine makes hydrogen bonds with acidic phenolic hydrogen. Selected hydrogen bond parameters are shown in Table 2. The acid–base pairs interact in parallel via weak van der Waals forces to form stacks. Co-crystal of receptor **4** with caffeine forms a wave-like polymeric chain when viewed along c axis. The another presentation is shown in Fig. 9c, which resembles an ivy climber plant curling up a pole.

Crystal data are as follows (**CCDC No. 726709**): $C_{20}H_{20}N_6O_4$, M_r = 408.42, 100 K, Monoclinic, $P2_1/c$, a = 10.1958(2) Å, b = 7.8938(2) Å, c = 25.4275(5) Å, α = 90.00°, β = 111.0550(10)°, γ = 90.00°, V = 1909.86(7) $Å^3$, Z = 4, μ(Mo Kα) = 0.103 cm^{-1}, Dc = 1.420 g cm^{-3}, F(000) = 856, range of indices = −14,14; −9,11; −36,27, no. of reflections collected = 24211, Unique reflections = 5892, Rint = 0.033, Reflections with I > 2σ(I) = 4,013, No. of parameters = 423, R(F^2), F > 2σ(F^2) = 0.0461,

Table 2 Selected hydrogen bond parameters of co-crystal of caffeine with receptor 4

D-H···A	*d*(D-H)/Å	*d*(H···A)/Å	*d*(D···A)/Å	*θ*(D-H···A)/deg
O1A-H1AA···N5[a]	0.8200	1.9800	2.772(5)	162.00
O2A-H2AA···O3[b]	0.8200	1.8900	2.654(12)	155.00
C12A-H12A···O3[c]	0.9300	2.4700	3.289(4)	147.00
C17-H17A···O2A[d]	0.9300	2.4900	2.958(11)	111.00
C18-H18B···O1A[e]	0.9600	2.4900	3.427(5)	166.00
C18-H18B···N5	0.9600	2.5500	2.9772(15)	107.00
C18-H18C···N5[d]	0.9600	2.6200	3.5400(15)	160.00
C19-H19C···O4	0.9600	2.3300	2.747(17)	106.00

Symmetry codes: [a] $x - 1, y, z$; [b] $x, -y + 3/2, z - 1/2$; [c] $-x + 1, -y + 1, -z + 2$; [d] $-x + 1, y + 1/2, -z + 3/2$; [e] $x + 1, y, z$

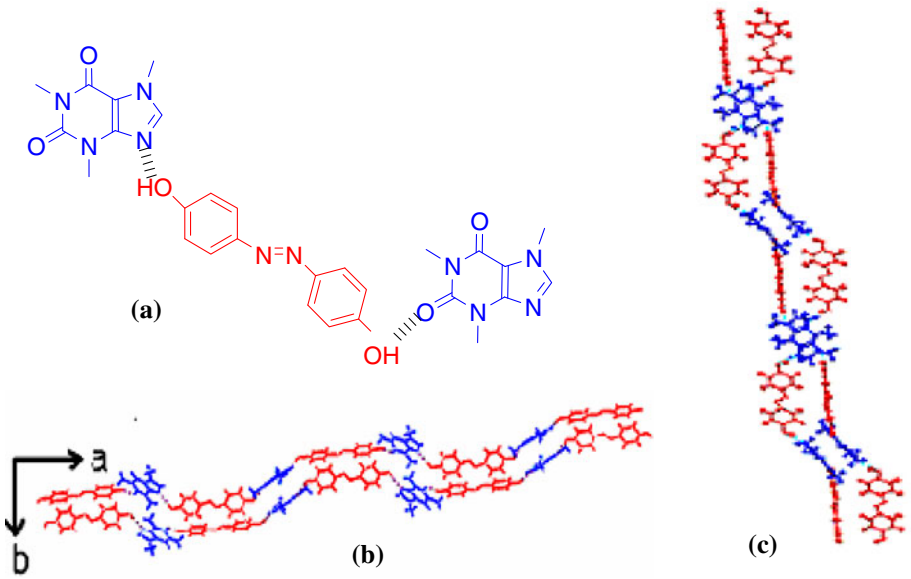


Fig. 9 Illustration of the co-crystal structure of receptor **4** (*red*) with caffeine (*blue*) **a** proposed H-bonding structure **b** 1D polymeric wave-like chain viewed down the crystallographic c axis. **c** supramolecular network which resembles an ivy climber plant curling up a pole

wR(F^2), $F > 2\sigma(F^2) = 0.1104$, R($F^2$), all data = 0.0771, wR(F^2), all data = 0.1232.

Conclusion

In summary, we have prepared the acidic receptors **4**, **5**, **6** and **7** based on different fluorescence probes. The simple hydrogen bonding acidic fluoro-receptors formed stronger complexes with methylxanthines. During the course of the investigations, it was found that the binding of caffeine with acidic receptors is stronger than that for theobromine and theophylline. The more basic nature of the caffeine among the other xanthines studied suggest stronger association constants with the acidic receptors. Here in fact, caffeine guest leads to a signal increase by a factor up to 10 and produces a reliable and fast identification method of the caffeine content in beverages. However, this system represents a powerful chemosensor method for the detection of medicinally and technically highly relevant analytes. Together with the results from the X-ray crystallography, a consistent picture of binding of the receptors with caffeine is presented.

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