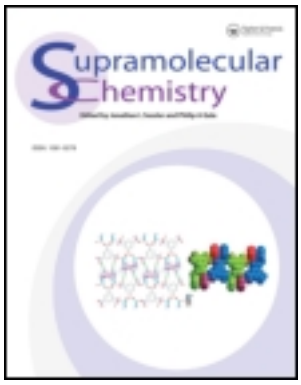

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Salicylaldehyde-indole-2-acylhydrazone: a simple, colorimetric and absorption ratiometric chemosensor for acetate ion

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A simple anion receptor (i.e. salicylaldehyde-indole-2-acylhydrazone) was synthesised and its recognition properties were investigated by naked-eye observation, UV–vis titration spectra, ^{1}H NMR spectroscopy and DFT calculations. The obtained results indicated that this receptor could realise the selective colorimetric sensing and absorption ratiometric response towards AcO^- in CH_3CN–DMSO medium, by virtue of threefold intermolecular hydrogen bonding interactions formed with phenolic OH, indole NH and amide NH.

Keywords: anion recognition; colorimetric sensing; absorption ratiometry; acetate ion

1. Introduction

Anions play a very important role in many chemical and biological processes, thereby development of anion chemosensors capable of selective recognition and sensing of the specific anions has been becoming a research focus in the field of supramolecular chemistry (*1–5*). Among common anions, fluoride and acetate ions are of particular interest to chemists because the amount of fluoride intake in the human body is closely related with some diseases (such as nephrolithiasis and kidney failure) (*6*, *7*), and carboxylate ions are pivotal components of many human metabolic processes (*8*). Compared with numerous selective sensors for F^-, the examples of selective sensing of AcO^- are relatively limited to date (*9–17*). Moreover, almost all the reported AcO^- chemosensors were completely relied on NH functionality; nevertheless, it had been validated that OH group, indole NH and amide NH in the *Salmonella typhimurium* sulphate-binding protein could work in concert to efficiently bind SO_4^{2-} in nature (*18*). Many anion receptors bearing indole NH and amide NH had been developed (*19–26*); to our surprise, there was little example of cooperative use of the above-mentioned three groups for construction of anion receptors (*27*).

Generally, a good anion chemosensor should produce easy-to-detect signal changes (e.g. colour, fluorescent and electrochemical changes) after complexation with specific anions. Relative to fluorescent and electrochemical sensors, colorimetric chemosensors have unique advantages as they can immediately provide qualitative information without resorting to any expensive equipment (*28*). In addition, compared with the usual detection method utilising an absorbance value at a single wavelength, the absorption ratiometric method provides more reliable quantitative information since it employs the ratio of absorption intensity at two different wavelengths as a function of analyte concentration, thereby a built-in correction for adverse environmental effects is obtained.

Bearing the above considerations in our mind, in this article we studied the recognition and sensing properties of salicylaldehyde-indole-2-acylhydrazone towards different anions. To our delight, a selective colorimetric sensing and absorption ratiometric response were seen upon addition of AcO^- to the solution of the receptor.

2. Results and discussion

2.1 Synthesis of salicylaldehyde-indole-2-acylhydrazone (the receptor)

Salicylaldehyde-indole-2-acylhydrazone was synthesised (see Scheme 1), and its structure was characterised by ^{1}H and ^{13}C NMR, MS, IR and elemental analysis. In order to evaluate the effect of phenolic OH on anion recognition properties, benzaldehyde-indole-2-acylhydrazone was also prepared as a reference compound.

2.2 UV–vis titration spectra and colorimetric sensing

Recognition properties of the receptor towards different anions were firstly studied in CH_3CN–DMSO (99.5/0.5, v/v) through UV–vis titration spectra. The receptor exhibited three characteristic absorption peaks at 308, 333 and 349 nm along with a very weak shoulder peak around 380 nm in the absence of anions. Upon addition of increasing amount of AcO^-, a moderate decrease in the

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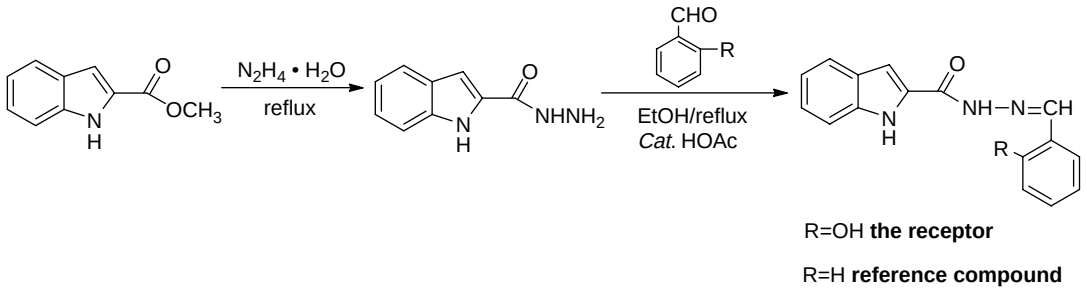


Scheme 1. Synthesis of the receptor and reference compound.

absorbance of the three characteristic peaks along with an obvious absorbance increase in the absorption band centred at 380 nm were simultaneously discovered (see Figure 1). In addition, a clear isosbestic point emerged at 350 nm, indicating the existence of only two absorbing species in the mixed solution (*29*). Noticeably, the colourless receptor solution was gradually changed into greenish-yellow upon addition of AcO^-. Interestingly, the above colour change was reversed after addition of several drops of water to the titrated solution, which fully proved that the above binding was reversible in essence.

The 1:1 stoichiometry for the receptor–AcO^- complexation was determined by Job's plot method (see Figure 2).

Gratifyingly, a good linear relationship was obtained between the A_{380}/A_{308} ratio and the concentration of AcO^- within the range of 0–32 μM (see Figure 3), thus enabling the receptor to work as an absorption ratiometric chemosensor for the quantitative detection of AcO^-.

Different with AcO^-, addition of F^- to the receptor solution promoted a modest enhancement in the absorbance at 333 and 349 nm (see Figure 4). Furthermore, the UV–vis spectral changes at 308 and 380 nm were similar but weaker than those in the presence of AcO^-. Noticeably, no perceptible colour change was detected under the range of fluoride concentration we used (0–6.4 equiv.).

Addition of 4.0 equiv. of $H_2PO_4^-$, Cl^-, Br^-, I^-, NO_3^- and HSO_4^- triggered a little or negligible changes in the UV–vis spectra of the receptor (see Figure 5), suggesting that very weak or no binding interactions occurred. The colorimetric responses of the receptor towards different anions were displayed in Figure 6, a visible colour change was found only in the case of AcO^-, so the receptor could act as a selective colorimetric chemosensor for detecting the AcO^-. Due to the subtle differences in the basicity and surface charge density among F^-, AcO^- and $H_2PO_4^-$,

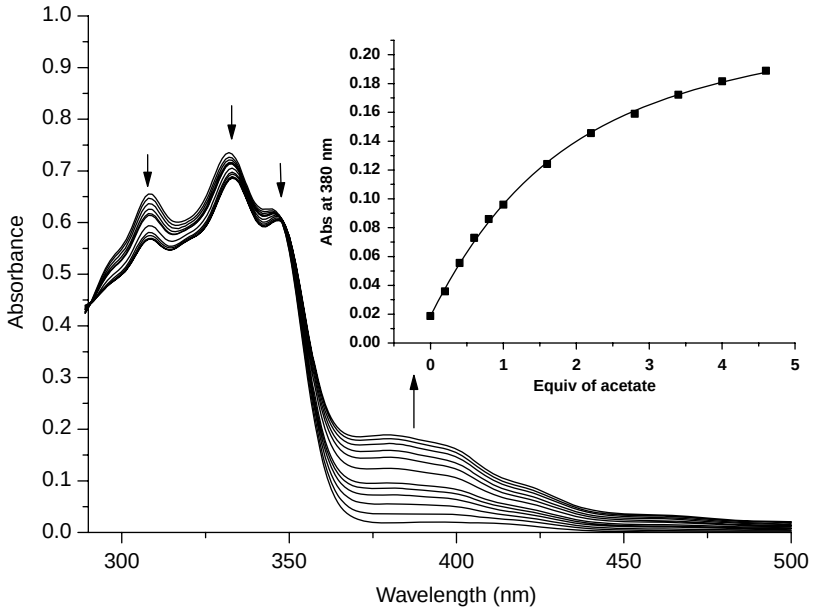


Figure 1. UV–vis absorption spectra of the receptor (20 μM) in CH_3CN–DMSO (99.5/0.5, v/v) upon titration with $Bu_4N^+AcO^-$ (0–4.6 equiv.). Inset: the nonlinear curve fitting of ΔA_{380} vs. equivalents of the added AcO^-.

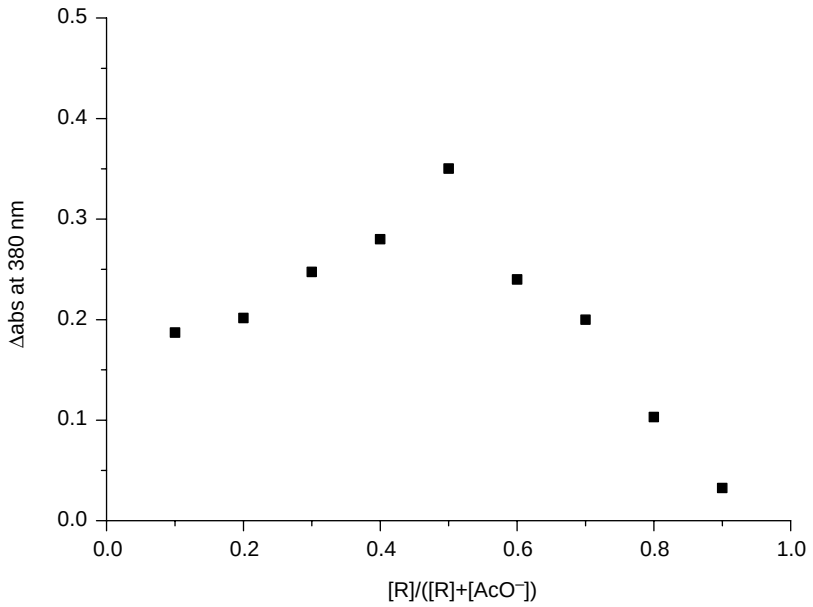


Figure 2. Job's plot of the receptor and AcO^- with a total concentration of 100 μM.

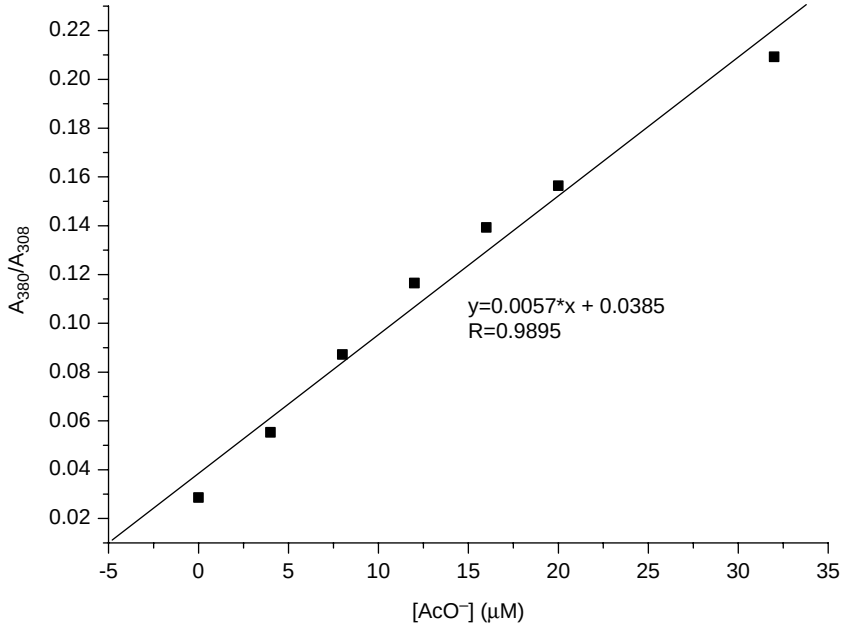


Figure 3. A linear relationship between the A_{380}/A_{308} ratio (for the receptor) and the concentration of AcO^- from 0 to 32 μM.

it was generally difficult to discriminate them from each other (*30*). However, the above aim could be realised to some extent on the basis of the different colour responses and absorption profiles of the receptor in the presence of these three anions.

In order to judge the nature of absorption spectral changes of the receptor induced by AcO^-/F^- (complexation or deprotonation), the UV–vis titration of the receptor with $Bu_4N^+OH^-$ was also carried out (see Figure 7). After the careful observation of the obtained titration curves and colour change, three obvious features could be observed from the deprotonation event caused by OH^-: (i) a significant decrease in the absorbance at 308, 333 and 349 nm and a sharp increase in the peak intensity at 375 nm were found, which was obviously different with the observed spectral changes in Figures 1 and 4; (ii) a distinct new absorption peak at 375 nm evolved upon the addition of OH^-, different with the appearance of a broad and

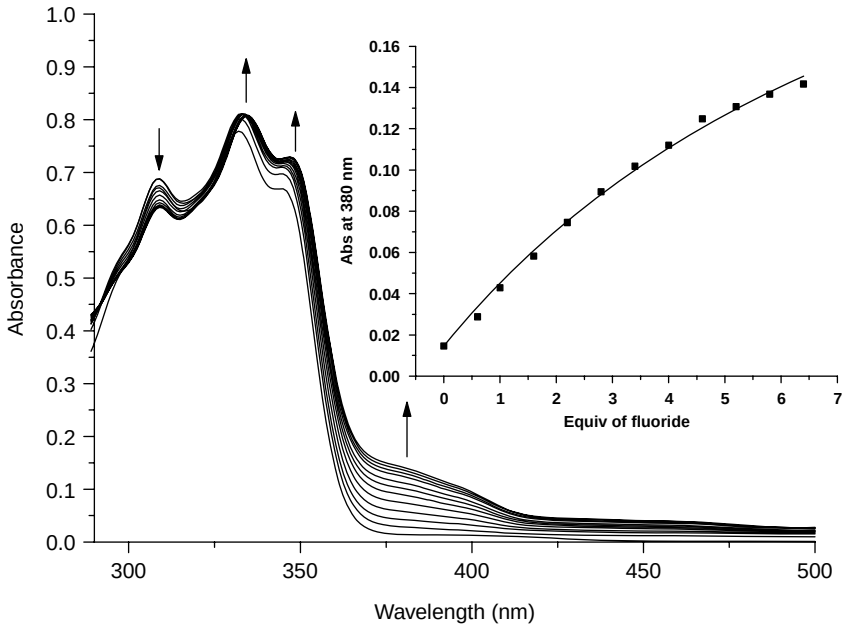


Figure 4. UV–vis absorption spectra of the receptor (20 μM) in CH_3CN–DMSO (99.5/0.5, v/v) upon titration with $Bu_4N^+F^-$ (0–6.4 equiv.). Inset: the nonlinear curve fitting of ΔA_{380} vs. equivalents of the added F^-.

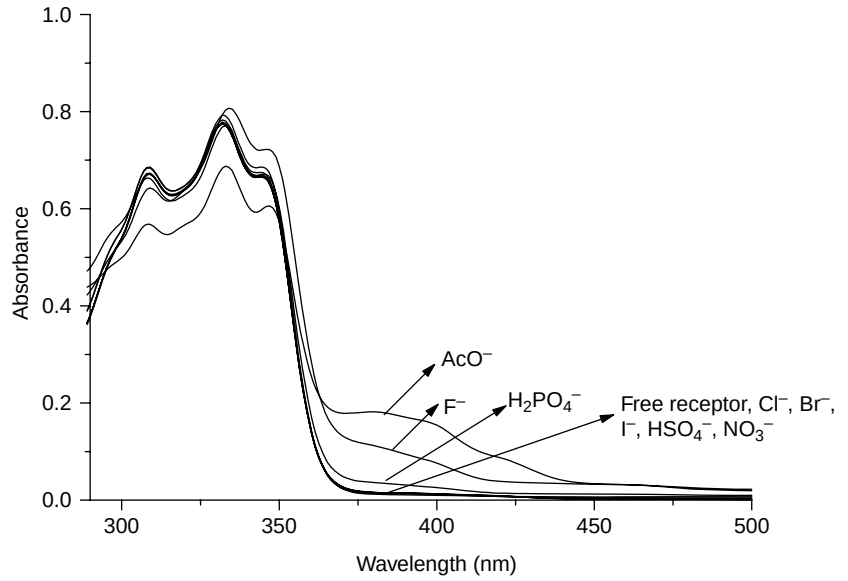


Figure 5. UV–vis absorption spectra of the receptor (20 μM) in CH_3CN–DMSO (99.5/0.5, v/v) upon titration with different anions (4.0 equiv.).

unstructured absorption band from 350 to 450 nm upon addition of AcO^-/F^-; (iii) colourless receptor solution was changed into brightly yellow (not greenish-yellow induced by AcO^-). The above phenomena (especially the first one (*31*)) sufficiently attested that hydrogen-bonding complexation between the receptor and AcO^-/F^- was responsible for the spectral changes in Figures 1 and 4, instead of the deprotonation event. Last but not least, the observed consistent spectral changes throughout the entire titration process using AcO^-/F^- also stated that only one interaction process took place. Remarkably, the UV–vis spectral changes of the receptor upon addition of excessive F^- were nearly identical to those triggered by OH^- (see Figure 8), implying that the deprotonation event indeed happened in such a case. Interestingly, no deprotonation was seen upon titration with excessive AcO^- (up to 48

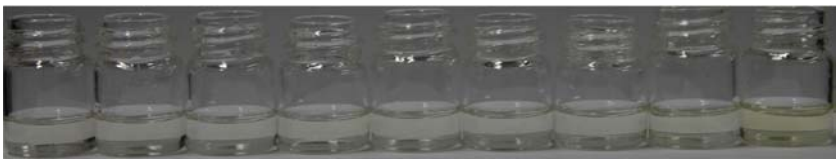


Figure 6. Colour change of the receptor (200 μM) in CH_3CN–DMSO (99.5/0.5, v/v) in the presence of 6.0 equiv. different anions (from left to right: free receptor, $+F^-$, $+Cl^-$, $+Br^-$, $+I^-$, $+NO_3^-$, $+HSO_4^-$, $+H_2PO_4^-$, $+AcO^-$).

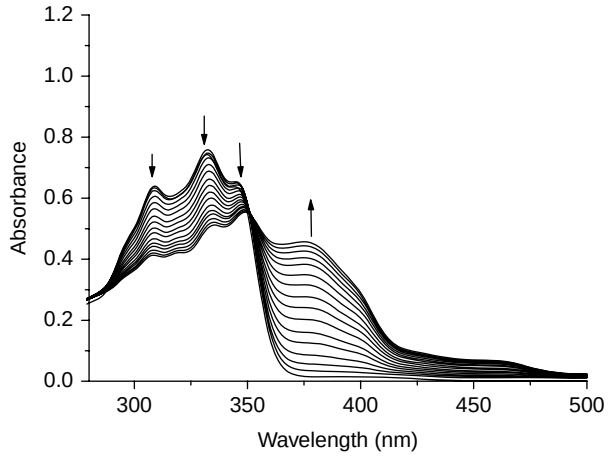


Figure 7. UV–vis absorption spectra of the receptor (20 μM) in CH_3CN–DMSO (99.5/0.5, v/v) upon titration with $Bu_4N^+OH^-$ (0–9.4 equiv.).

equiv.). The special stability of the species $[HF_2^-]$ in CH_3CN (*32*, *33*) was the main reason for the F^--induced deprotonation.

Unexpectedly, there was no detectable interaction between reference compound and all the tested anions (see Figure 9), suggesting that phenolic OH of the receptor was indispensable for realising effective anion binding.

2.3 *Determination of association constants (K_a)*

In a supramolecular system with the formation of a 1:1 binding complex, the association constant (K_a) could be determined through the method of nonlinear curve fitting using the following equation (*34*):

$$A = A_0 + [(A_{\lim} - A_0)/2C_0]\{(C_H + C_G + 1/K_a) - [(C_H + C_G + 1/K_a)^2 - 4C_H C_G]^{1/2}\}$$

where A is the absorbance of the whole system, A_0 is the absorbance of free receptor, C_H is the concentration of receptor compound, C_G is the concentration of guest anion and K_a is the association constant. The obtained K_a and R (correlation coefficient) from the above equation were listed in Table 1. Excellent correlation coefficients ($R > 0.99$) established the formation of a 1:1 complex between the receptor and AcO^-/F^- (*35–39*). The value of K_a for AcO^- ($4.10 \times 10^4\,M^{-1}$) was nearly sixfold larger than that for F^- ($5.92 \times 10^3\,M^{-1}$), which may be due to the stronger

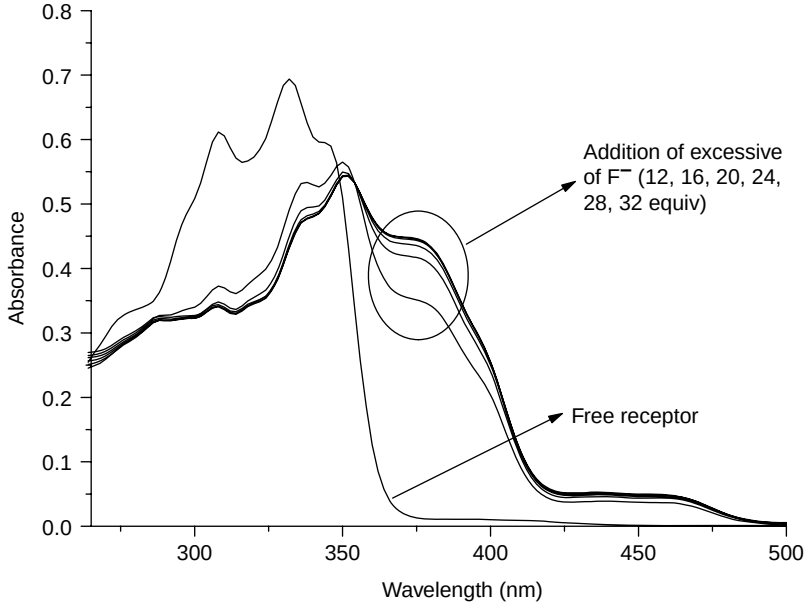


Figure 8. UV–vis absorption spectra of the receptor (20 μM) in CH_3CN–DMSO (99.5/0.5, v/v) upon titration with excessive F^-.

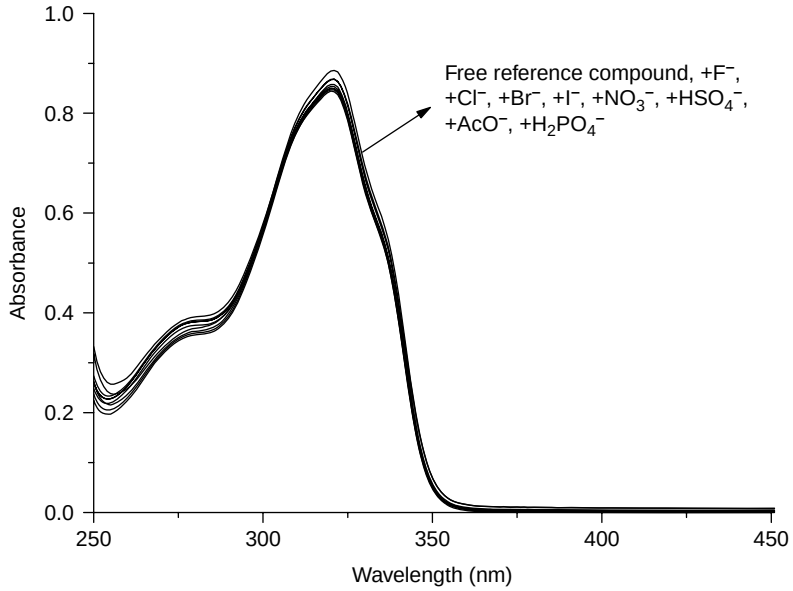


Figure 9. UV–vis absorption spectra of reference compound (20 μM) in CH_3CN–DMSO (99.5/0.5, v/v) upon addition of different anions (1.6 equiv.).

Table 1. Association constants (K_a/M^{-1}) and correlation coefficients (R) of the receptor with different anions in CH_3CN–DMSO (99.5/0.5, v/v).

Anions[a]	AcO^-	F^-	$H_2PO_4^-$	Other anions
K_a[b]	$(4.10 \pm 0.19) \times 10^4$	$(5.92 \pm 0.89) \times 10^3$	ND[c]	NSC[d]
R	0.9997	0.9981	–	–

[a] Anions were used as their tetra-*n*-butylammonium salts.
[b] Values of K_a were determined by UV–vis titration spectra.
[c] The spectral changes were too small to calculate K_a accurately.
[d] No spectral changes were observed.

hydrogen-bonding accepting ability of AcO^- and the better shape complementarity for the receptor and AcO^-.

2.4 ^{1}H NMR titration spectra

In order to disclose the concrete binding sites within the receptor, the ^{1}H NMR titration of the receptor with AcO^- was conducted in DMSO-d_6 as an example (see Figure 10). The receptor displayed three sharp peaks at 12.16, 11.85 and 11.19 ppm, attributed to indole NH, amide NH and phenolic OH, respectively. Upon addition of 1.0 equiv. of AcO^-, these three peaks completely disappeared and no detectable chemical shift changes appeared for other protons. Obviously, the added AcO^- was complexed by the receptor via threefold hydrogen-bonding interactions, contributing from indole NH, amide NH and phenolic OH.

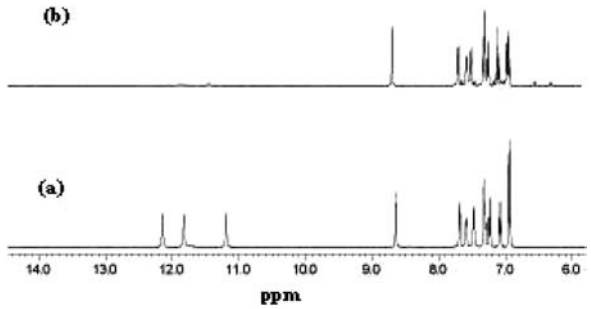


Figure 10. Partial ^{1}H NMR spectra of the receptor (6.4 mM) in DMSO-d_6 upon addition of AcO^-. (a) +0 equiv.; (b) +1.0 equiv.

2.5 DFT calculations

According to the DFT calculations (see Figure 11), indole NH, amide NH and phenolic OH formed H-bonds with AcO^- ion (1.69, 1.67 and 2.44 Å, respectively). In addition, it was worth mentioning that an intramolecular H-bond presumably existed between the imine nitrogen and phenolic hydroxyl proton.

Based on the above studies, a possible binding model between salicylaldehyde-indole-2-acylhydrazone and AcO^- was proposed in Scheme 2.

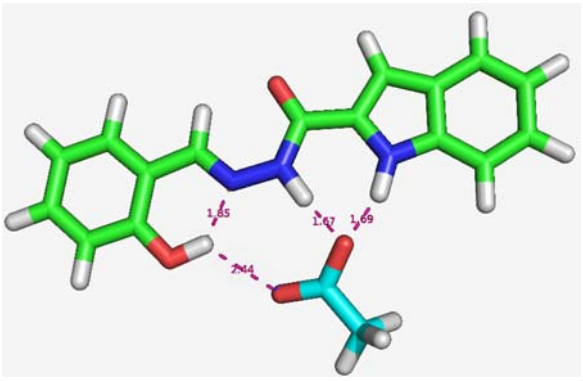


Figure 11. Calculated structure (B3LYP/6-31(d)) of the receptor–AcO^- complex.

3. Conclusions

In summary, salicylaldehyde-indole-2-acylhydrazone was found to provide the selective colorimetric sensing and absorption ratiometric response towards biologically importantly AcO^- in CH_3CN–DMSO (99.5/0.5, v/v) medium, which were driven by threefold intermolecular hydrogen-bonding interactions. The ease of synthesis of indole-2-acylhydrazone compounds provides the possibility to develop more elaborate and preorganised neutral anion receptors with a better affinity and selectivity.

4. Experimental

4.1 General

Acetonitrile was refluxed over CaH_2 and then distilled for use in the titration experiments. All the anions were utilised as their tetra-*n*-butylammonium salts. Other chemicals were purchased as analytical grade reagents and used directly. Melting points were determined on a XT-4 binocular microscope (Beijing Tech Instrument Co., Beijing, China). IR spectra were recorded on a Shimadzu IRPrestige-21 spectrometer in KBr disc. ^1H and ^{13}C NMR spectra were measured on a JEOL-ECX 500 NMR spectrometer at room temperature using TMS as an internal standard. Mass spectra were recorded on Agilent LC/MSD. Elemental analysis was performed on an Elementar Vario-III CHN analyzer. UV–vis spectra were recorded on TU-1900 spectrophotometer (Beijing Purkinje General Instrument Co., Beijing, China).

4.2 Synthesis

4.2.1 Synthesis of indole-2-acylhydrazine

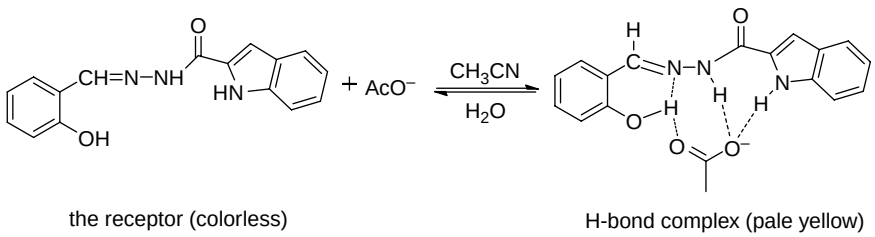
Indole-2-acylhydrazine was prepared according to the previous literature (*40*).

4.2.2 Synthesis of the receptor (salicylaldehyde-indole-2-acylhydrazone)

Indole-2-acylhydrazine (88 mg, 0.5 mmol), salicylaldehyde (53 μl, 0.55 mmol) and two drops of acetic acid were dissolved in 25 ml ethanol. The above mixed solution was heated to reflux for 5 h and then cooled to room temperature. The formed precipitate was filtered off and washed with ethanol to afford the receptor. Yield: 110 mg (79%), mp: $> 250^oC$. ^1H NMR (500 MHz, DMSO-d_6): δ 12.16 (s, 1H), 11.85 (s, 1H), 11.19 (s, 1H), 8.66 (s, 1H), 7.70 (d, $J = 8.0$ Hz, 1H), 7.61 (d, $J = 7.5$ Hz, 1H), 7.49 (d, $J = 8.5$ Hz, 1H), 7.33 (s, 1H), 7.31(d, $J = 8.0$ Hz, 1H), 7.24 (t, $J = 7.5$ Hz, 1H), 7.08 (t, $J = 7.5$ Hz, 1H), 6.96 − 6.93 (m, 2H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 158.0, 157.8, 147.8, 137.5, 131.9, 130.1, 129.8, 127.5, 124.6, 122.4, 120.6, 119.9, 119.4, 116.9, 113.0, 104.4. FT-IR (KBr): 3294, 1653, 1616, 1539, 1267, 752 cm^{-1}. MS (ESI): 302.1 ($M + Na^+$), 318.3 ($M + K^+$). Anal. Calcd for $C_{16}H_{13}N_3O_2$ C 68.81, H 4.69, N 15.05. Found: C 68.65, H 4.83, N 15.22.

4.2.3 Synthesis of reference compound (benzaldehyde-indole-2-acylhydrazone)

A similar synthetic method was utilised to prepare the reference compound by the reaction of indole-2-acylhydrazine with benzaldehyde. mp: 195 − 198°C. ^1H NMR (500 MHz, DMSO-d_6): δ 11.94 (s, 1H), 11.82 (s, 1H), 8.47 (s, 1H), 7.78 − 7.68 (m, 3H), 7.49 − 7.47 (m, 4H), 7.33 (s, 1H), 7.24 (t, $J = 7.5$ Hz, 1H), 7.08 (t, $J = 7.5$ Hz, 1H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 158.3, 147.7, 137.4, 134.8, 130.6, 130.5, 129.4, 127.6, 127.5, 124.5, 122.3,



Scheme 2. A possible binding model between the receptor and AcO^-.

120.6, 112.9, 104.2. FT-IR (KBr): 3308, 1636, 1603, 1545, 1246, 745, 689 cm^{-1}. MS (ESI): 286.1 ($M + Na^+$). Anal. Calcd for $C_{16}H_{13}N_3O$ C 72.99, H 4.98, N 15.96. Found: C 72.75, H 5.15, N 16.23.

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