

Electronic Supplementary Information (ESI)

Isatin *N*-phenylsemicarbazone: Effect of substituent and concentration on anion sensing selectivity and sensitivity

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EXPERIMENTAL SECTION – Supporting information

Synthesis

(*E*)-isatin-3-(4-phenyl)semicarbazone **Ia**:

Synthesis of the unsubstituted **Ia** *E*-isomer was published by Jakusová *et al.*, 2013.²⁷

(*E*)-isatin-3-[4-(4-fluorophenyl)semicarbazone] **IIIa**:

Obtained from 4-(4-fluorophenyl)semicarbazide (0.27g) in 83% yield (0.4g),

¹H NMR (300 MHz, DMSO-*d*₆) δ: 10.77 (s, 1H), 10.41 (s, 1H), 9.54 (s, 1H), 8.08 (d, *J* = 7.6 Hz, 1H), 7.66 – 7.55 (m, 2H), 7.38 (ddd, *J* = 7.7, 0.9 Hz, 1H), 7.25 – 7.13 (m, 2H), 7.09 (ddd, *J* = 7.7, 0.8 Hz, 1H), 6.92 (d, *J* = 7.7 Hz, 1H).

¹³C NMR (75 MHz, DMSO-*d*₆) δ: 164.72, 159.57, 156.39, 152.46, 143.11, 134.90, 131.98, 125.25, 121.61, 121.22, 121.12, 115.56, 115.26, 110.53.

Anal. Calcd. for C₁₅H₁₁FN₄O₂ (298.27) C, 60.40; H, 3.72; N, 18.78. Found C, 58.15; H, 4.19; N, 17.13.

(*E*)-isatin-3-[4-(4-bromophenyl)semicarbazone] **IVa**:

Obtained from 4-(4-bromophenyl)semicarbazide (0.16g) in 88% yield (0.22g),

¹H NMR (DMSO-*d*₆) δ: 6.92 (d, 1H, *J*=7.7Hz, ArH), 7.06 – 7.12 (dt, 1H, *J*=0.9Hz, *J*=7.7Hz, ArH), 7.36 – 7.41 (dt, 1H, *J*=0.9Hz, *J*=7.7Hz, ArH), 7.51 – 7.60 (m, 4H, ArH), 8.08 (d, 1H, *J*=7.6Hz, ArH), 9.64 (s, 1H, NH), 10.43 (s, 1H, NH), 10.77 (s, 1H, NH).

¹³C NMR (75 MHz, DMSO-*d*₆) δ: 164.55, 152.19, 143.02, 137.92, 134.89, 131.90, 131.52, 125.15, 121.48, 121.03, 115.45, 114.42, 110.41.

Anal. Calcd. for C₁₅H₁₁BrN₄O₂ (359.18) C, 50.16; H, 3.09; N, 15.60. Found C, 50.40; H, 3.05; N, 15.43.

(*E*)-isatin-3-[4-(4-trifluoromethylphenyl)semicarbazone] **Va**:

Obtained from 4-(4-trifluoromethylphenyl)semicarbazide (0.22g) in 100% yield (0.35g),

¹H NMR (DMSO-*d*₆) δ: 6.92 (d, 1H, *J*=7.7Hz, ArH), 7.07 - 7.10 (dt, 1H, *J*=0.8Hz, *J*=7.7Hz, ArH), 7.37 - 7.42 (dd, 1H, *J*=4.3Hz, *J*=11.2Hz, ArH), 7.70 – 7.83 (dd, 1H, *J*=8.7Hz, *J*=31.9Hz, ArH), 8.08 – 8.10 (d, 1H, *J*=7.6Hz, ArH), 9.90 (s, 1H, NH), 10.50 (s, 1H, NH), 10.79 (s, 1H, NH).

¹³C NMR (75 MHz, DMSO-*d*₆) δ: 164.73, 152.35, 143.35, 142.44, 135.71, 132.26, 126.28, 125.45, 123.19, 122.76, 121.72, 119.05, 115.59, 110.68.

Anal. Calcd. for C₁₆H₁₁F₃N₄O₂ (348.28) C, 55.18; H, 3.18; N, 16.09. Found C, 53.58; H, 3.27; N, 15.65.

(*E*)-isatin-3-[4-(4-cyanophenyl)semicarbazone] **VIa**:

Obtained from 4-(4-cyanophenyl)semicarbazide (0.19g) in 71% yield (0.24g),

¹H NMR (300 MHz, DMSO-*d*₆) δ: 10.80 (s, 1H), 10.54 (s, 1H), 10.00 (s, 1H), 8.08 (d, *J* = 7.6 Hz, 1H), 7.80 (s, 4H), 7.40 (td, *J* = 7.8, 0.9 Hz, 1H), 7.10 (td, *J* = 7.7, 0.9 Hz, 1H), 6.92 (d, *J* = 7.6 Hz, 1H).

¹³C NMR (75 MHz, DMSO-*d*₆) δ: 165.89, 153.50, 144.57, 144.38, 137.18, 134.62, 133.51, 126.68, 122.91, 120.38, 116.75, 111.88, 105.80.

Anal. Calcd. for C₁₆H₁₁N₅O₂ (305.29) C, 62.95; H, 3.63; N, 22.94. Found C, 63.01; H, 3.60; N, 22.87.

(E)-isatin-3-[4-(4-methylphenyl)semicarbazone] **VIIa**:

Obtained from 4-(4-methylphenyl)semicarbazide (0.28g) in 86% yield 0.43g),

¹H NMR (300 MHz, DMSO-*d*₆) δ: 10.76 (s, 1H), 10.37 (s, 1H), 9.39 (s, 1H), 8.09 (d, *J* = 7.6 Hz, 1H), 7.46 (d, *J* = 8.4 Hz, 2H), 7.38 (ddd, *J* = 7.7, 1.0 Hz, 1H), 7.15 (d, *J* = 8.2 Hz, 2H), 7.09 (ddd, *J* = 7.7, 0.9 Hz, 1H), 6.92 (d, *J* = 7.7 Hz, 1H), 2.27 (s, 3H).

¹³C NMR (75 MHz, DMSO-*d*₆) δ: 164.59, 151.98, 142.94, 135.81, 134.50, 131.80, 129.17, 125.03, 121.46, 119.09, 115.48, 110.39, 20.30.

Anal. Calcd. for C₁₆H₁₄N₄O₂ (294.31) C, 65.30; H, 4.79; N, 19.04. Found C, 64.97; H, 4.92; N, 18.60.

(E)-isatin-3-[4-(4-trimethoxyphenyl)semicarbazone] **VIIIa**:

Obtained from 4-(4-trimethoxyphenyl)semicarbazide (0.23g) in 51% yield (0.19g),

¹H NMR (DMSO-*d*₆) δ: 3.47 (s, 3H, OCH₃), 6.90 – 6.94 (m, 3H, ArH), 7.06 – 7.11 (dt, 1H, *J* = 0.9 Hz, *J* = 7.6 Hz, ArH), 7.35 – 7.40 (dt, 1H, *J* = 1.0 Hz, *J* = 7.8 Hz, ArH), 7.49 (m, 2H, ArH), 8.09 (d, 1H, *J* = 7.6 Hz, ArH), 9.35 (s, 1H, NH), 10.36 (s, 1H, NH), 10.84 (s, 1H, NH).

¹³C NMR (75 MHz, DMSO-*d*₆) δ: 164.02, 154.56, 141.25, 131.13, 124.67, 121.40, 120.83, 112.88, 107.96, 50.31.

Anal. Calcd. for C₁₆H₁₄N₄O₃ (310.31) C, 61.93; H, 4.55; N, 18.06. Found C, 58.04; H, 4.57; N, 17.91.

(E)-5-bromo-1-isatin-3-(4-phenyl)semicarbazone **IXa**:

Obtained from 4-phenylsemicarbazide (1g) in 43% yield (1.02g),

¹H NMR (600 MHz, DMSO-*d*₆) δ: 10.90 (s, 1H), 10.63 (s, 1H), 9.50 (d, *J* = 19.5 Hz, 1H), 8.36 (d, *J* = 1.8 Hz, 1H), 7.60 (dd, *J* = 8.6, 1.0 Hz, 2H), 7.55 (dd, *J* = 8.3, 1.9 Hz, 1H), 7.38 – 7.33 (m, 2H), 7.08 (tt, *J* = 7.5, 1.1 Hz, 1H), 6.88 (d, *J* = 8.3 Hz, 1H).

¹³C NMR (75 MHz, DMSO-*d*₆) δ: 164.41, 152.06, 142.15, 138.37, 134.07, 133.17, 128.90, 127.36, 123.13, 119.32, 117.25, 113.40, 112.32.

Anal. Calcd. for C₁₅H₁₁BrN₄O₂ (359.18) C, 50.16; H, 3.09; N, 15.60. Found C, 50.28; H, 3.11; N, 15.72.

(E)-5-trifluoromethoxyisatin-3-(4-phenyl)semicarbazone **Xa**:

Obtained from 4-phenylsemicarbazide (0.13g) in 100% yield 0.31g),

^1H NMR (300 MHz, DMSO- d_6) δ 10.97 (s), 10.73 (s), 9.47 (s), 8.24 (d, $J = 1.7$ Hz), 7.60 (d, $J = 7.6$ Hz), 7.46 – 7.30 (m), 7.08 (t, $J = 7.4$ Hz), 6.99 (d, $J = 8.6$ Hz).

^{13}C NMR (75 MHz, DMSO- d_6) δ 162.60, 151.01, 140.46, 138.22, 128.76, 128.66, 123.44, 123.21, 121.58, 119.69, 113.68, 111.87.

Anal. Calcd. for $\text{C}_{16}\text{H}_{11}\text{F}_3\text{N}_4\text{O}_3$ (364.28) C, 52.75; H, 3.04; N, 15.38. Found C, 51.96; H, 3.06; N, 15.2.

(*Z*)-5-trifluoromethoxyisatin-3-(4-phenyl)semicarbazone **Xb**:

Obtained from reaction mixture of **Xa** (0.08g) by column chromatography in 50% yield 0.04g),

^1H NMR (300 MHz, DMSO- d_6) δ 12.15 (s), 11.36 (s), 9.86 (s), 7.68 (d, $J = 1.5$ Hz), 7.63 (dd, $J = 8.6$, 1.1 Hz), 7.40 – 7.30 (m), 7.09 (s), 7.03 (d, $J = 8.6$ Hz).

^{13}C NMR (75 MHz, DMSO- d_6) δ 162.60, 151.01, 143.40, 140.46, 138.22, 131.30, 128.66, 123.45, 123.21, 121.57, 119.69, 113.69, 111.87.

Anal. Calcd. for $\text{C}_{16}\text{H}_{11}\text{F}_3\text{N}_4\text{O}_3$ (364.28) C, 52.75; H, 3.04; N, 15.38. Found C, 51.96; H, 3.06; N, 15.2.

(*E*)-5-methyl-1-isatin-3-(4-phenyl)semicarbazone **XIa**:

Obtained from 4-phenylsemicarbazide (0.47g) in 73% yield (0.67g),

^1H NMR (DMSO- d_6), δ : 2.34 (s, 3H, CH₃) 6.81 (d, 1H, $J=7.9$ Hz, ArH), 7.05 – 7.09 (m, 1H, ArH), 7.18 – 7.21 (dd, 1H, $J=0.7$ Hz, $J=7.9$ Hz, ArH), 7.32 – 7.38 (m, 2H, ArH), 7.58 – 7.61 (dd, 2H, $J=1.1$ Hz, $J=8.6$ Hz, ArH), 7.96 (s, 1H, ArH), 9.41 (s, 1H, NH), 10.40 (s, 1H, NH), 10.66 (s, 1H, NH).

^{13}C NMR (75 MHz, DMSO- d_6) δ : 164.64 (s), 152.21 (s), 140.71 (s), 138.35 (s), 134.78 (s), 132.13 (s), 130.45 (s), 128.75 (s), 125.58 (s), 122.90 (s), 119.17 (s), 115.55 (s), 110.09 (s), 20.52 (s).

Anal. Calcd. for $\text{C}_{16}\text{H}_{14}\text{N}_4\text{O}_2$ (294.31) C, 65.30; H, 4.79; N, 19.04. Found C, 65.32; H, 4.90; N, 18.58.

Titration experiments

Association constant determinations

Equation (1) was rewritten to the following form for nonlinear fit:

$$A = A_0 + c_1 * (P1 - A_0) * (c_0 + x + 1/P2 - \sqrt{(c_0 + x + 1/P2)^2 - 4 * c_0 * x}), \quad (2)$$

where: $c_0 = 1 \times 10^{-4}$ or 1×10^{-5} , $c_1 = 1/2c_0 = 5 \times 10^3$ or 5×10^4 , parameter $P1 = A_{lim}$, parameter $P2 = K_{ass}$ and $x = c_{A^-}$. The A_0 value was fixed to the absorbance value A for $x = 0$. The large difference in constants c_0 , c_1 and the x -data range led rescaling the nonlinear function (x and fitted parameter $1/P2$ by a factor of 10^4). Following this modification, the standard non-linear least-squares Nelder-Mead minimization method was employed to determine fitting parameters $P1$ and $P2$.²⁸ Four different wavelengths (425nm, 410 nm, 390 nm and 330 nm) were used to K_{ass} determination.

Where the titration curve and Job's plot indicated sensor:anion 2:1 stoichiometry, the K_{ass} value was determined by the hyperbolic equation:²⁵

$$\Delta A = \frac{\Delta A_{\infty} K_{ass} [S]^2}{1 + K_{ass} [S]^2}, \quad (3)$$

where ΔA is the change in sensor absorbance following addition of a constant amount of anion to the sensor solution compared to sensor solution absorbance without anion addition, ΔA_{∞} is the limit value for $\Delta A = f([S]^2)$ dependence and $[S]$ is the concentration of free sensor. Because the $A = f(c_{A^-})$ titration curves for 2:1 complexes were obtained at low anion equivalent, the overall sensor concentration was taken as free sensor concentration in solution. Standard non-linear least-squares fitting approach in Mathematica 9.0 was used to determine fitting parameters ΔA_{∞} and K_{ass} .²⁹

Detection and quantification limits

Detection limit ($3\sigma/S$) and quantification limit ($10\sigma/S$) were determined by the following equations:

$$3\sigma/S = 3 \sqrt{\frac{\sum (A - \bar{A})^2}{n-1}} / S; \quad (4)$$

$$10\sigma/S = 10 \sqrt{\frac{\sum (A - \bar{A})^2}{n-1}} / S, \quad (5)$$

where: σ is the standard deviation, A is the sensor (isatin *N*-phenylsemicarbazone) absorbance and S is the slope of the $A = f(c_{A^-})$ plot in the initial linear portion of the curve; in all cases $n = 4$.

Light initiated *E-Z* isomerization

Quantum yield determination

The *E-Z* isomerization quantum yield (Φ_{E-Z}) of isatinphenylsemicarbazone *E*-isomers **IIIa-XIa** in DMF solution was determined by equations (6) and (7):

$$\Phi_{E-Z} = \frac{\int_0^{c_1} dc}{\int_0^t I_a dt} = \frac{\Delta c}{\int_0^t I_a dt}; \quad (6) \quad \Delta c = \frac{\Delta A_\lambda}{\varepsilon_{\lambda Z} - \varepsilon_{\lambda E}}, \quad (7)$$

where: Δc is the concentration change in *E*- or *Z*-isomer, I_a is *E*-isomer absorbed photon flux at the irradiation wavelength λ using a monochromatic light source, ΔA_λ is the absorbance change at irradiation wavelength λ using a monochromatic light source, $\varepsilon_{\lambda E}$ is *E*-isomer molar extinction coefficient at irradiation wavelength λ using a monochromatic light source, $\varepsilon_{\lambda Z}$ is *Z*-isomer molar extinction coefficient of at irradiation wavelength λ using a monochromatic light source and t is irradiation time.

The *E*-isomer absorbed photon flux I_a at particular irradiation times during *E-Z* isomerization at irradiation wavelength $\lambda = 405$ nm and incident *E*-isomer concentration c_0 was calculated by Eq. (8):³⁰

$$I_{a,405 E} = I_0 \frac{\varepsilon_{E,405}(c_0 - \Delta c)}{\varepsilon_{E,405}(c_0 - \Delta c) + \varepsilon_{Z,405}\Delta c} \left[-10^{-[\varepsilon_{E,405}(c_0 - \Delta c) + \varepsilon_{Z,405}\Delta c]t} \right] \quad (8)$$

Neither photochemically nor thermally initiated back *Z-E* isomerization occurred during *Z*-isomer solution irradiation at 405 nm.¹⁵

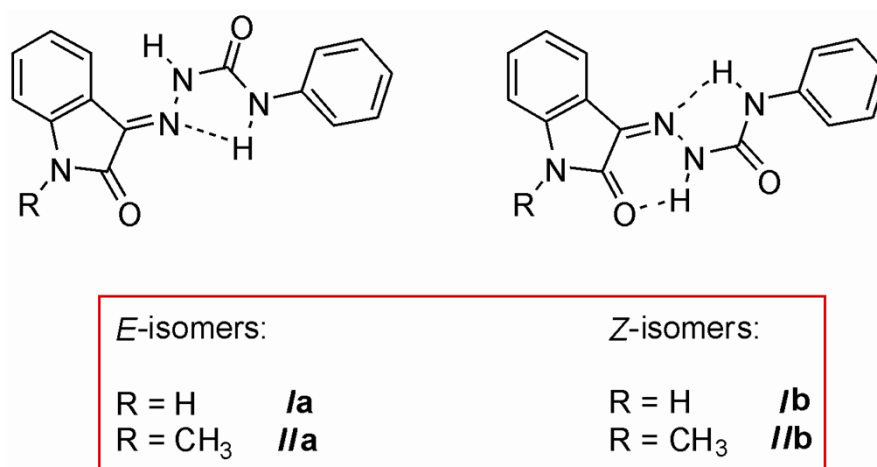
The incident photon flux I_0 was determined by 2-nitrobenzaldehyde (2-NB) as chemical actinometer, according to Eq. (9):³¹

$$I_0 = \frac{k}{2,303 \cdot \varepsilon_{o-NB,405} \cdot \Phi_{o-NB,405} \cdot l}, \quad (9)$$

where: $\varepsilon_{o-NB,405}$ is the molar extinction coefficient of 2-NB at irradiation wavelength 405 nm, $\Phi_{o-NB,405}$ is the quantum yield of 2-NB photodegradation at irradiation wavelength, l is the path length and k is the 2-NB first-order photodegradation slope plotted under low-light-absorbing conditions:³¹

$$\ln\left(\frac{[\rho - \text{NB}]_t}{[\rho - \text{NB}]_0}\right) = -kt. \quad (10)$$

SUPPORTING SCHEMES, TABLES AND FIGURES



Scheme S1. Molecular structure of the previously studied isatin *N*-phenylsemicarbazones.

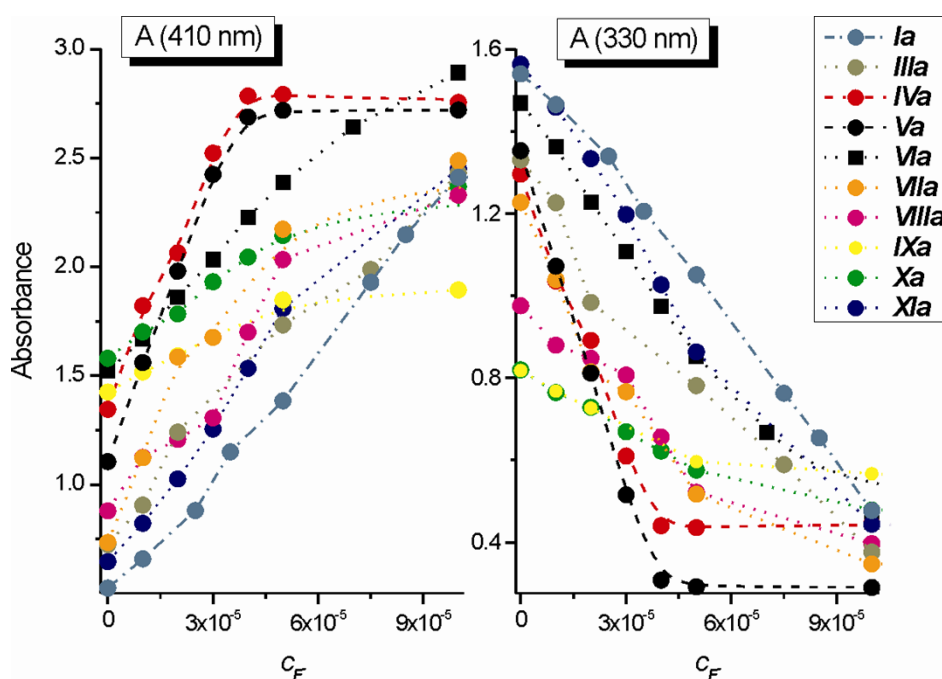


Fig. S1. Evolution of absorbance values at 330 nm and 410 nm for sensors *Ia* and *III-XIa* in DMF during *Ia-XIa* solution titration with TBA^+F^- ($c_{\text{sensor}} = 1 \times 10^{-4} \text{ mol dm}^{-3}$).

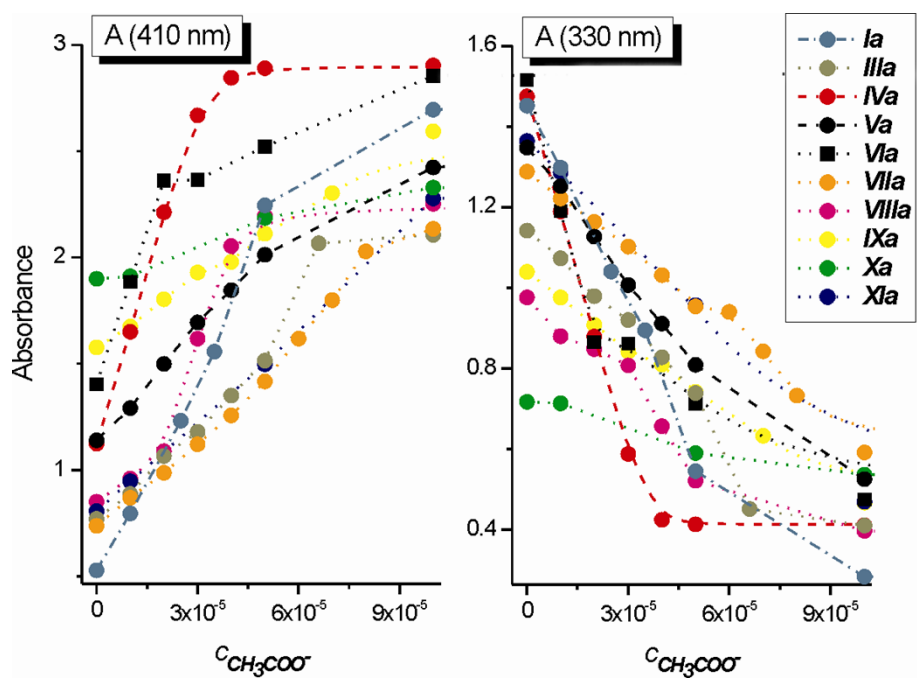


Fig. S2. Evolution of absorbance values at 330 nm and 410 nm for sensors *Ia* and *III-XIa* in DMF during *Ia-XIa* solution titration with $TBA^+ CH_3COO^-$ ($c_{\text{sensor}} = 1 \times 10^{-4}$ mol dm⁻³).

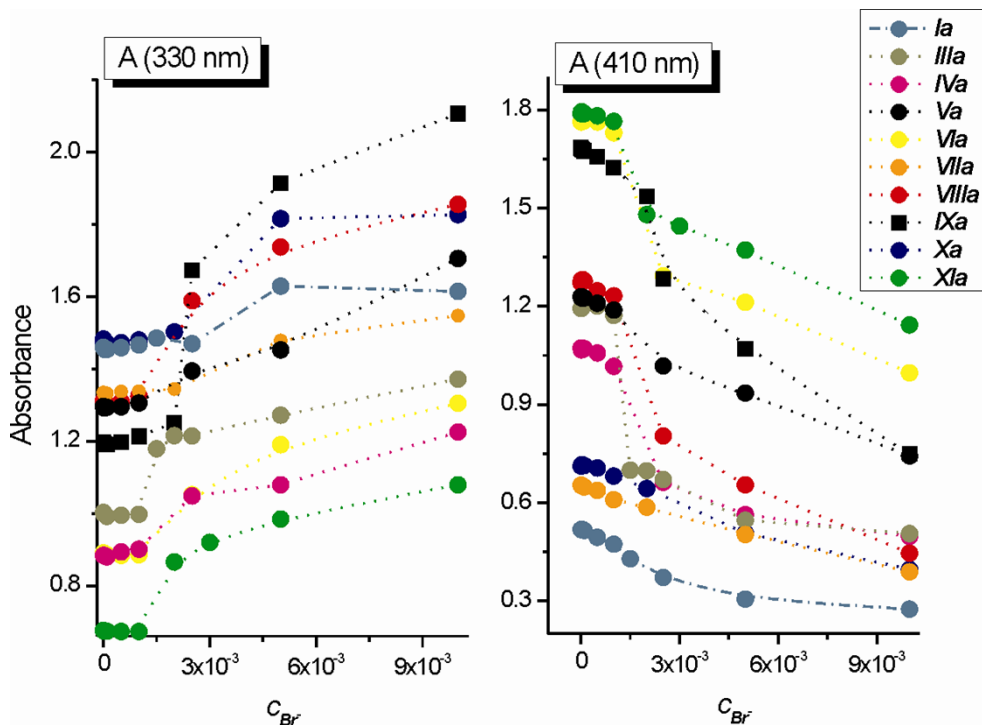


Fig. S3. Evolution of absorbance values at 330 nm and 410 nm for sensors *Ia* and *III-XIa* in DMF during *Ia-XIa* solution titration with $TBA^+ Br^-$ ($c_{\text{sensor}} = 1 \times 10^{-5}$ mol dm⁻³).

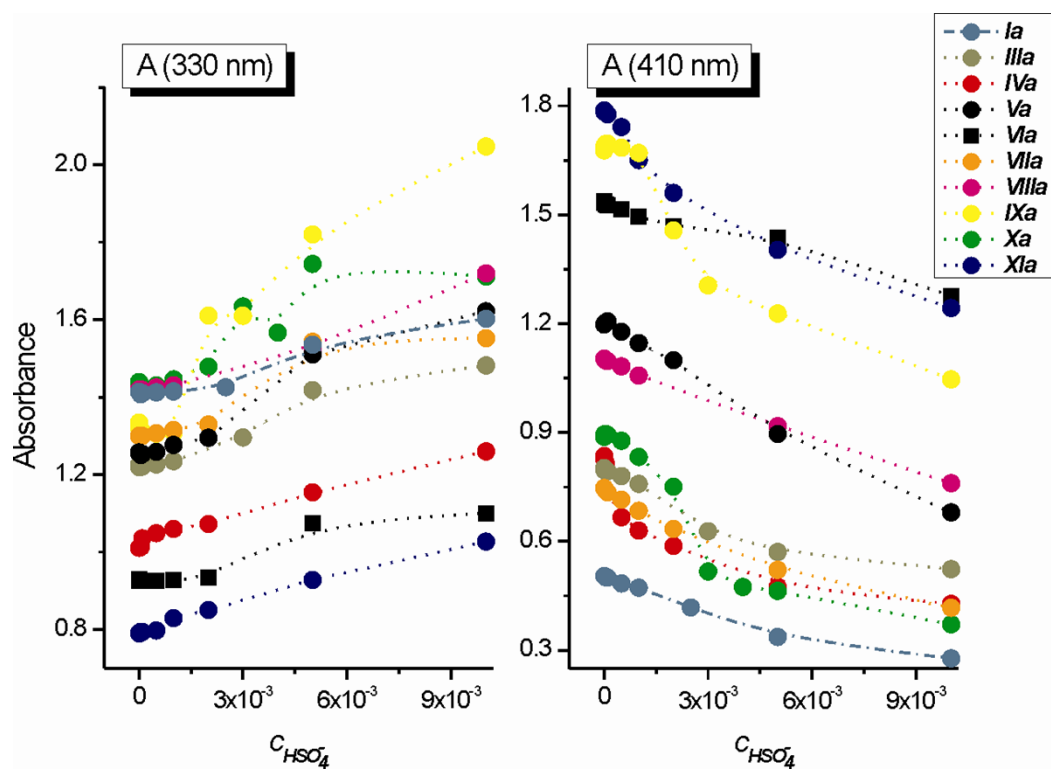


Fig. S4. Evolution of absorbance values at 330 nm and 410 nm for sensors *Ia* and *III-XIa* in DMF during *Ia-XIa* solution titration with $TBA^+HSO_4^-$ ($c_{\text{sensor}} = 1 \times 10^{-5} \text{ mol dm}^{-3}$).

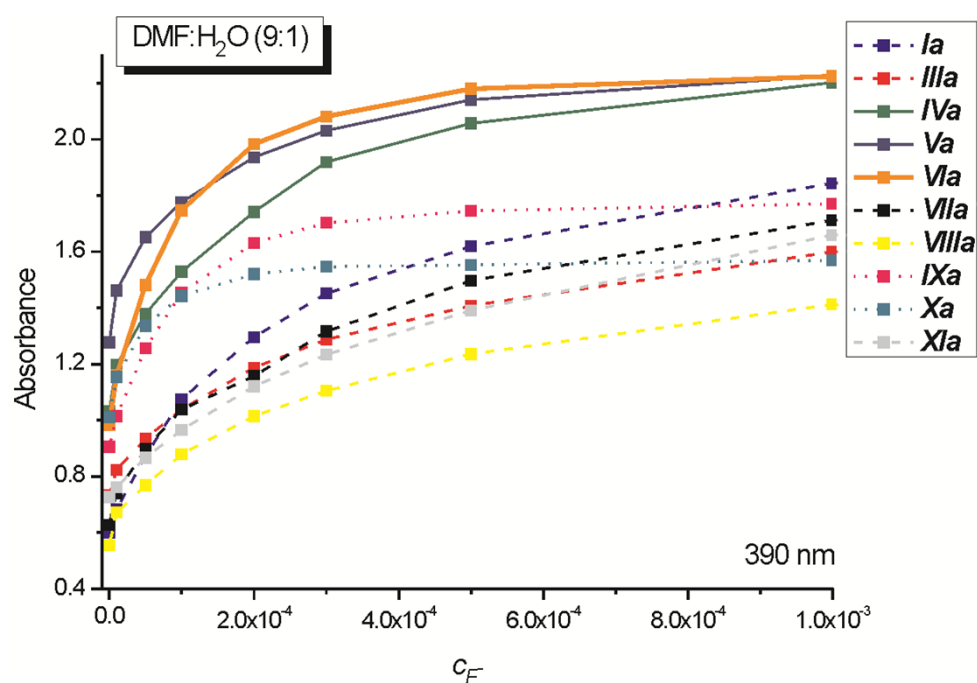


Fig. S5. Evolution of absorbance values at 330 nm and 410 nm for sensors *Ia* and *III-XIa* in DMF:H₂O (9:1; v/v) solvent mixture during *Ia-XIa* solution titration with TBA^+F^- ($c_{\text{sensor}} = 1 \times 10^{-4} \text{ mol dm}^{-3}$).

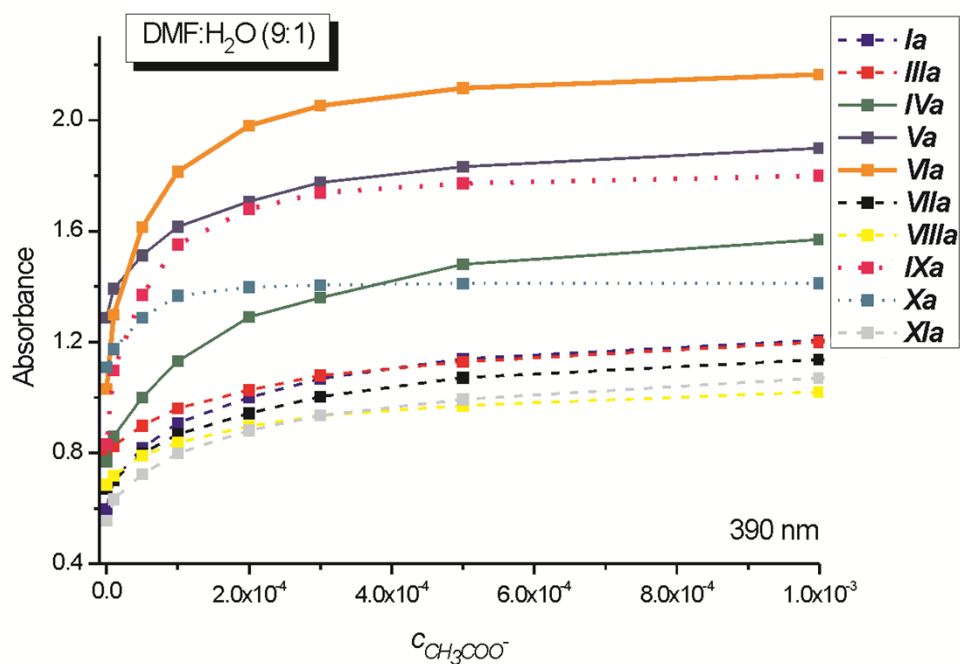


Fig. S6. Evolution of absorbance values at 330 nm and 410 nm for sensors *Ia* and *III-XIa* in DMF:H₂O (9:1; v/v) solvent mixture during *Ia-XIa* solution titration with $TBA^+ CH_3COO^-$ ($c_{\text{sensor}} = 1 \times 10^{-4}$ mol dm⁻³).

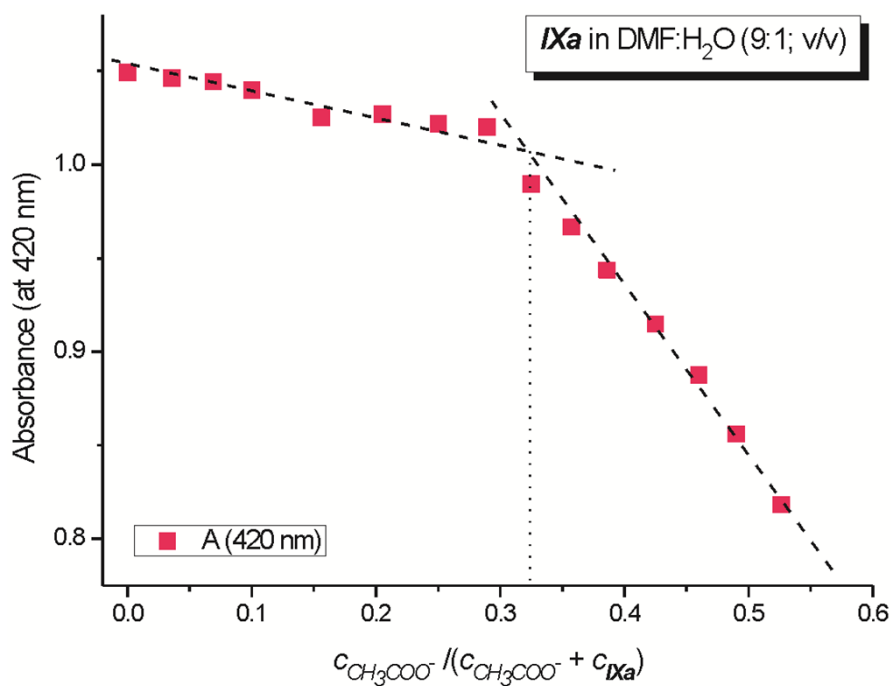


Fig. S7. Stoichiometric analysis of CH_3COO^- anion binding to sensor *IXa* – Job's plot.

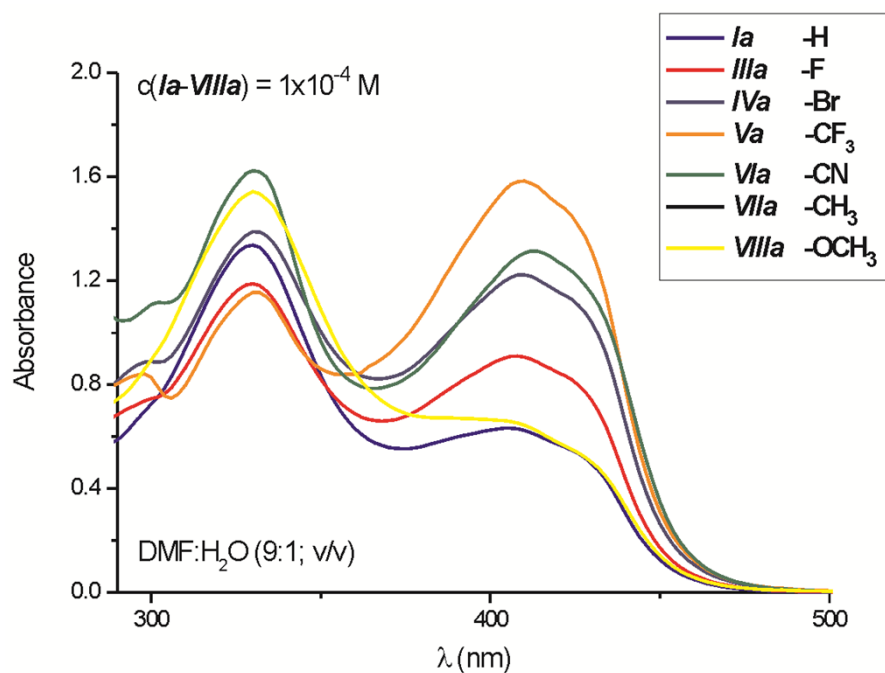


Fig. S8. UV-VIS spectra of isatin *N*-phenylsemicarbazone *E*-isomers *Ia*, *IIIa*, *IVa*, *Va* and *VIa* in DMF:H₂O (9:1; v/v) solvent mixture ($c_{\text{sensor}} = 1 \times 10^{-4} \text{ mol dm}^{-3}$; $T = 298.16 \text{ K}$).

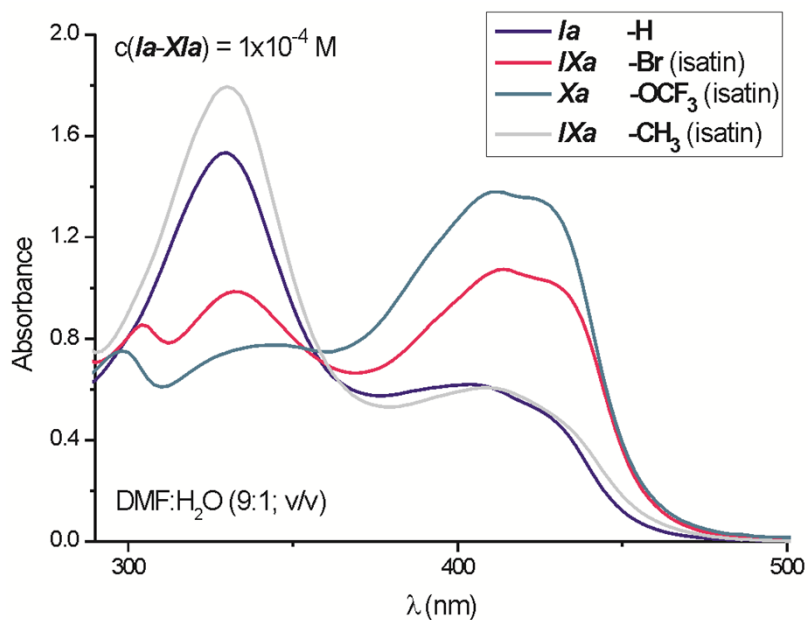


Fig. S9. UV-VIS spectra of isatin *N*-phenylsemicarbazone *E*-isomers *Ia*, *IXa*, *Xa* and *XIa* in DMF:H₂O (9:1; v/v) solvent mixture ($c_{\text{sensor}} = 1 \times 10^{-4} \text{ mol dm}^{-3}$; $T = 298.16 \text{ K}$).

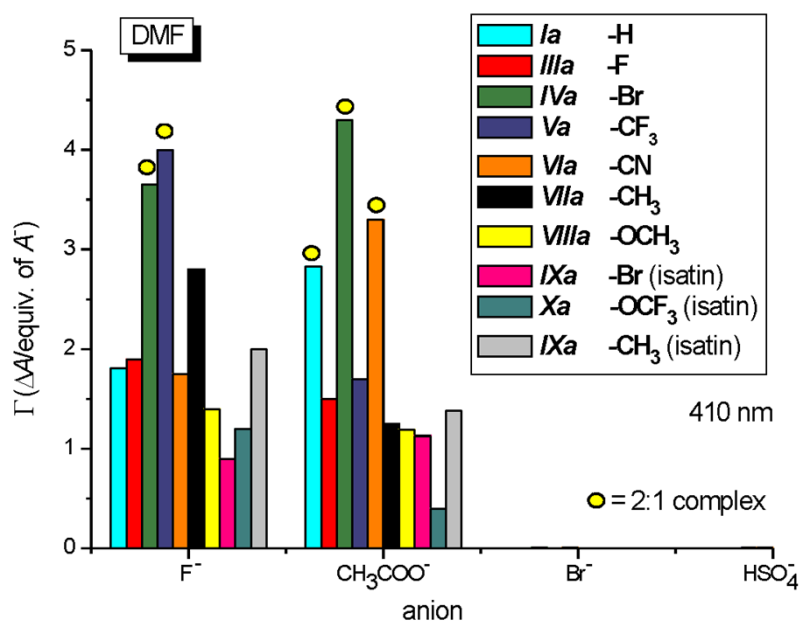


Fig. S10. The sensitivity Γ of the studied *E*-isomers of isatin *N*-phenylsemicarbazones **Ia** and **IIIa-XIa** at 410 nm for anions in DMF at 298.16 K ($c_{E\text{-isomer}} = 1 \times 10^{-4} \text{ mol}^{-1} \text{ dm}^3$ for F^- and CH_3COO^- sensing and $c_{E\text{-isomer}} = 1 \times 10^{-5} \text{ mol}^{-1} \text{ dm}^3$ for Br^- and HSO_4^- sensing). Parameter Γ defines $A = f(c_A^-)$ slope at low anion concentration where sensor absorbance increase/decrease is clearly linear.

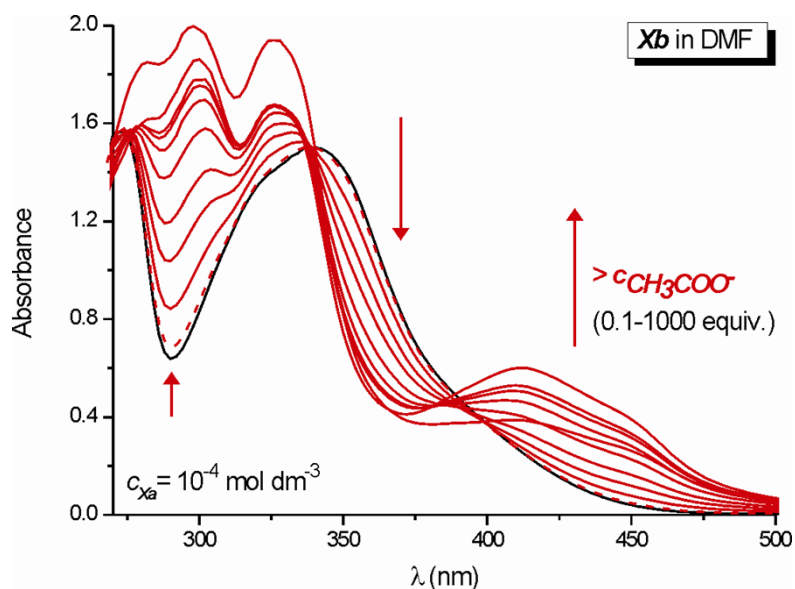
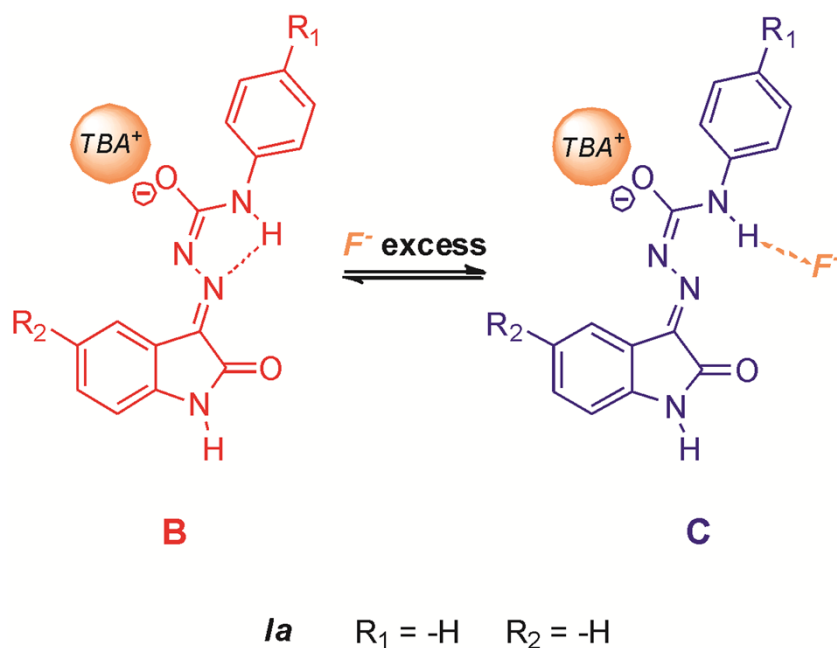


Fig. S11. Evolution of the UV-VIS spectrum of isatin *N*-phenylsemicarbazone *Z*-isomer **Xb** in DMF during **Xb** solution titration with $TBA^+CH_3COO^-$ ($c_{Ib} = 1 \times 10^{-4} \text{ mol dm}^{-3}$; $c_{CH_3COO^-} = 0 \text{ mol dm}^{-3}$, $1 \times 10^{-5} \text{ mol dm}^{-3}$, $3 \times 10^{-5} \text{ mol dm}^{-3}$, $5 \times 10^{-5} \text{ mol dm}^{-3}$, $1 \times 10^{-4} \text{ mol dm}^{-3}$, $2 \times 10^{-4} \text{ mol dm}^{-3}$, $3 \times 10^{-4} \text{ mol dm}^{-3}$, $4 \times 10^{-4} \text{ mol dm}^{-3}$, $5 \times 10^{-4} \text{ mol dm}^{-3}$, $1 \times 10^{-3} \text{ mol dm}^{-3}$ and $1 \times 10^{-2} \text{ mol dm}^{-3}$).

Table S1. The *E-Z* isomerization quantum yield for isatin *N*-phenylsemicarbazones **Ia-IXa** and **XIa** in DMF at 298.16 K ($c_{E\text{-isomer}} = 1 \times 10^{-4} \text{ mol}^{-1} \text{ dm}^3$).

Compd	$\Phi_{E-Z} \times 10^{-3}$
Ia	6.96 ± 0.37
IIIa	2.56 ± 0.16
IVa	2.20 ± 0.12
Va	2.24 ± 0.13
VIa	2.07 ± 0.15
VIIa	3.08 ± 0.14
VIIIa	2.96 ± 0.12
IXa	2.52 ± 0.13
XIa	1.68 ± 0.11

Φ_{E-Z} – the *E-Z* isomerization quantum yield



Scheme S2. The presumed non-covalent intermolecular interaction of derivative **Ia** with F^- anions during **Ia** solution titration with TBA^+F^- (self-associate hydrazone **A**/hydrazone **B**/hydrazone **C** equilibrium).

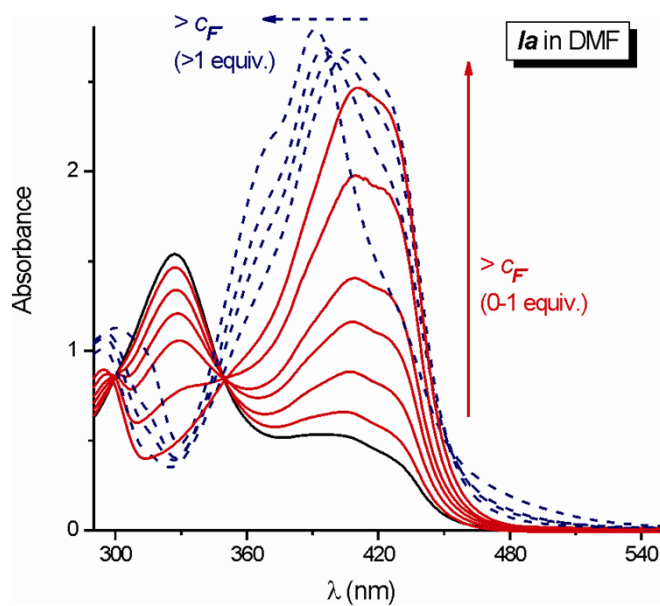


Fig. S12. Evolution of the UV-VIS spectrum of isatin *N*-phenylsemicarbazone *E*-isomer **Ia** in DMF during **Ia** solution titration with TBA^+F^- ($c_{Ia} = 1 \times 10^{-4} \text{ mol dm}^{-3}$; $c_{F^-} = 0 \text{ mol dm}^{-3}$, $1 \times 10^{-5} \text{ mol dm}^{-3}$, $2.5 \times 10^{-5} \text{ mol dm}^{-3}$, $3.5 \times 10^{-5} \text{ mol dm}^{-3}$, $5 \times 10^{-5} \text{ mol dm}^{-3}$, $7.5 \times 10^{-5} \text{ mol dm}^{-3}$, $1 \times 10^{-4} \text{ mol dm}^{-3}$, $1 \times 10^{-3} \text{ mol dm}^{-3}$, 5×10^{-3} , $7.5 \times 10^{-3} \text{ mol dm}^{-3}$ and $1 \times 10^{-2} \text{ mol dm}^{-3}$).

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