

Electronic Supplementary Information (ESI)

Coumarin phenylsemicarbazones: Sensitive colorimetric and fluorescent “turn-on” chemosensors for low-level water content in aprotic organic solvents

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EXPERIMENTAL SECTION – Supporting information

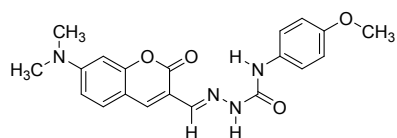
Synthesis

GENERAL

Elemental analyses were performed on a Carlo Erba Strumentazione 1106 apparatus. IR spectra were acquired on Nicolet FT-IR-ATR 6700 (Thermo Fisher Scientific, US) spectrometer and are given in cm^{-1} . The $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$ spectra were recorded at 600 MHz, 300 MHz and at 75 MHz, respectively, on a Varian Gemini 200 spectrometer in CDCl_3 or $\text{DMSO-}d_6$ with tetramethylsilane as internal standard. Chemical shift values are recorded in δ units (ppm) and coupling constants (J) are expressed in Hertz (Hz). The following abbreviations are used: s = singlet, d = doublet, dd = doublet of doublets, ddd = doublet of doublets of doublets, m = multiplet. Thin-layer chromatography was performed on Merck Millipore precoated aluminium TLC plates with silica gel 60 F254 (hexanes/EtOAc). Chemicals and solvents were purchased from the major chemical suppliers (Merck Millipore, Darmstadt, Germany; Acros Organics, Geel, Belgium; Sigma-Aldrich, St. Louis, MO, USA) as highest purity grade. All solvents were dried by standard methods and distilled prior to use. 7-(*N,N*-dimethylamino)-2-oxo-2H-chromene-3-carbaldehyde⁵⁷ and 4-(4-methoxyphenyl)semicarbazide were prepared according to literature procedures.⁵⁰

(*E*)-1-[(7-dimethylamino)-2-oxo-2H-chromen-3-yl]methylidene}-4-(4-methoxyphenyl)semicarbazone (4)

A solution of 4-(4-methoxyphenyl)semicarbazide (0.69 mmol, 0.125 g) in hot absolute ethanol (10 mL) was added to a solution of 7-(*N,N*-dimethylamino)-2-oxo-2H-chromene-3-carbaldehyde (0.69 mmol, 0.15 g) in hot absolute ethanol (10 mL). The reaction mixture was refluxed for 15 min. The precipitated product was filtered off, washed with cold ethanol, dried, recrystallized from ethanol, and obtained in 81% yield (0.22 g).



$^1\text{H NMR}$ (600 MHz, $\text{DMSO-}d_6$, 25°C) δ = 10.73 (s, 1H, -HN-Ph-OMe), 8.75 (s, 1H, C=N-NH-), 8.65 (s, 1H, -HC=N), 7.96 (s, 1H, 4-H), 7.50 (d, $^3J_{\text{H,H}} = 8.9$ Hz, 1 H, 5-H), 7.50 (d, $^3J_{\text{H,H}} = 8.9$ Hz, 2 H, 2'-H and 6'-H), 6.87 (d, $^3J_{\text{H,H}} = 8.9$ Hz, 2 H, 3'-H and 5'-H), 6.79 (dd, $^3J_{\text{H,H}} = 8.9$ Hz, 2.4 Hz, 1 H, 6-H), 6.59 (d, $^3J_{\text{H,H}} = 2.4$ Hz, 1H, 8-H), 3.73 (s, 3H, O-CH₃), 3.07 (s, 6H, N(CH₃)₂)

$^{13}\text{C NMR}$ (75 MHz, $\text{DMSO-}d_6$, 25°C) δ : 161.20 (C2), 156.21 (C8a), 155.44 (C4'), 153.66 (C7), 153.53 (N-C=O), 138.77 (HC=N), 135.07 (C4), 132.39 (C1'), 130.24 (C5), 122.36 (C2',C6'), 114.08 (C3',C5'), 114.01 (C3), 110.50 (C6), 108.95 (C4a), 97.61 (C8), 55.62 (O-CH₃), 40.28 (N(CH₃)₂).

IR (ATR) ν/cm^{-1} : 3365, 3082, 2938, 1720, 1668, 1598, 1510, 1356, 1270, 1152, 949, 823.

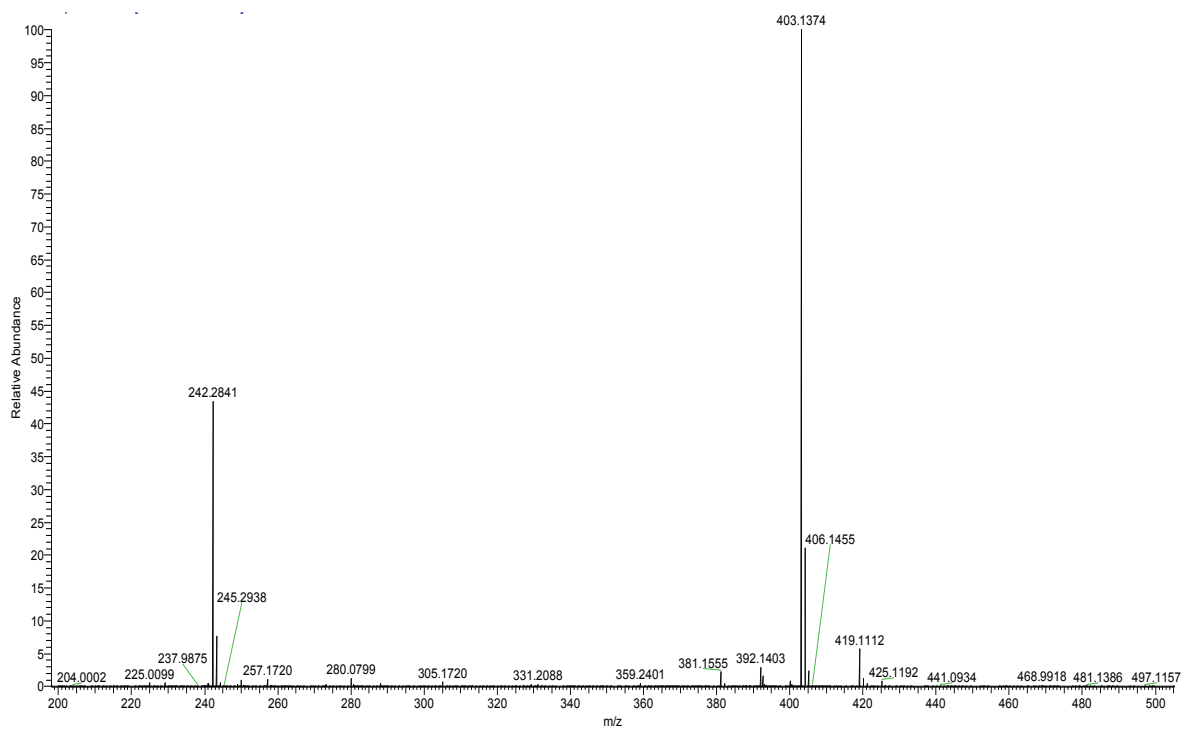
Anal. Calcd. for $\text{C}_{20}\text{H}_{20}\text{N}_4\text{O}_4$ (380.40) C, 63.15; H, 5.30; N, 14.73. Found C, 63.17; H, 5.29; N, 14.74.

HRMS (HESI): m/z [$\text{M}+\text{H}^+$] – calcd (for $\text{C}_{20}\text{H}_{20}\text{N}_4\text{O}_4 + \text{H}^+$): 381.1557, experimental: 381.1555

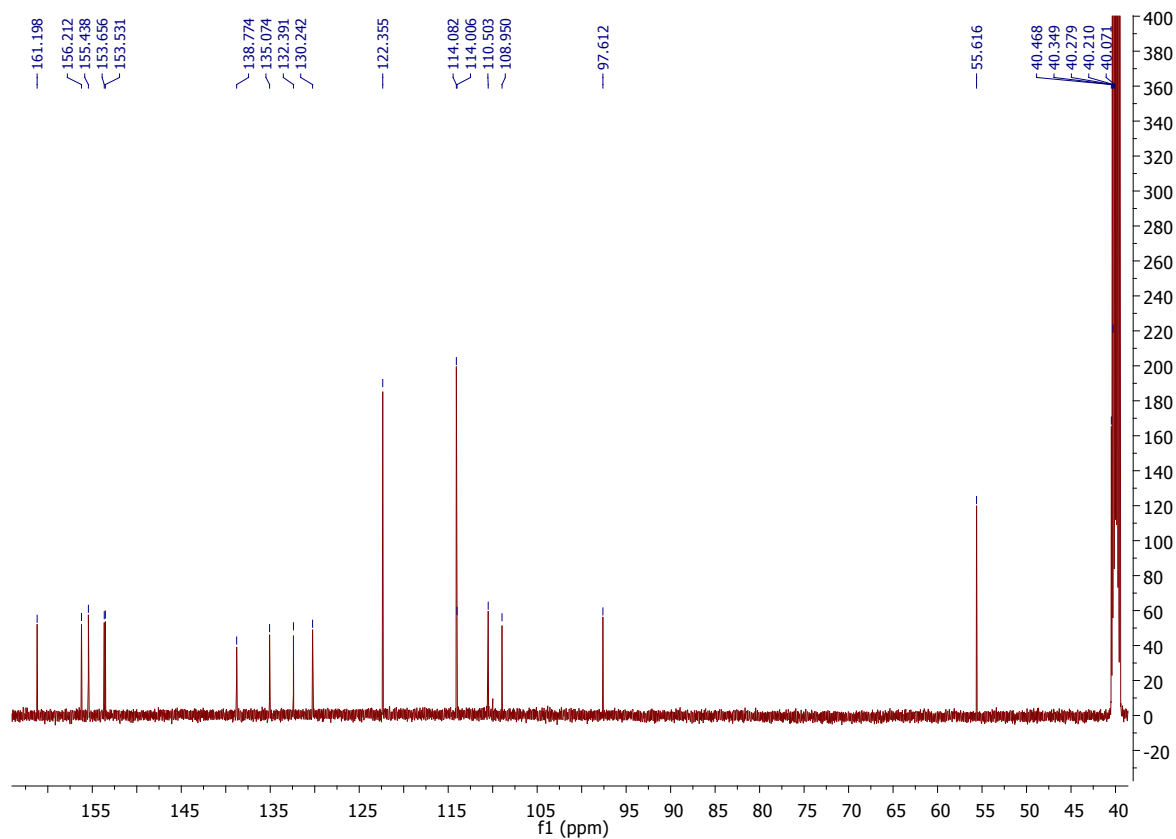
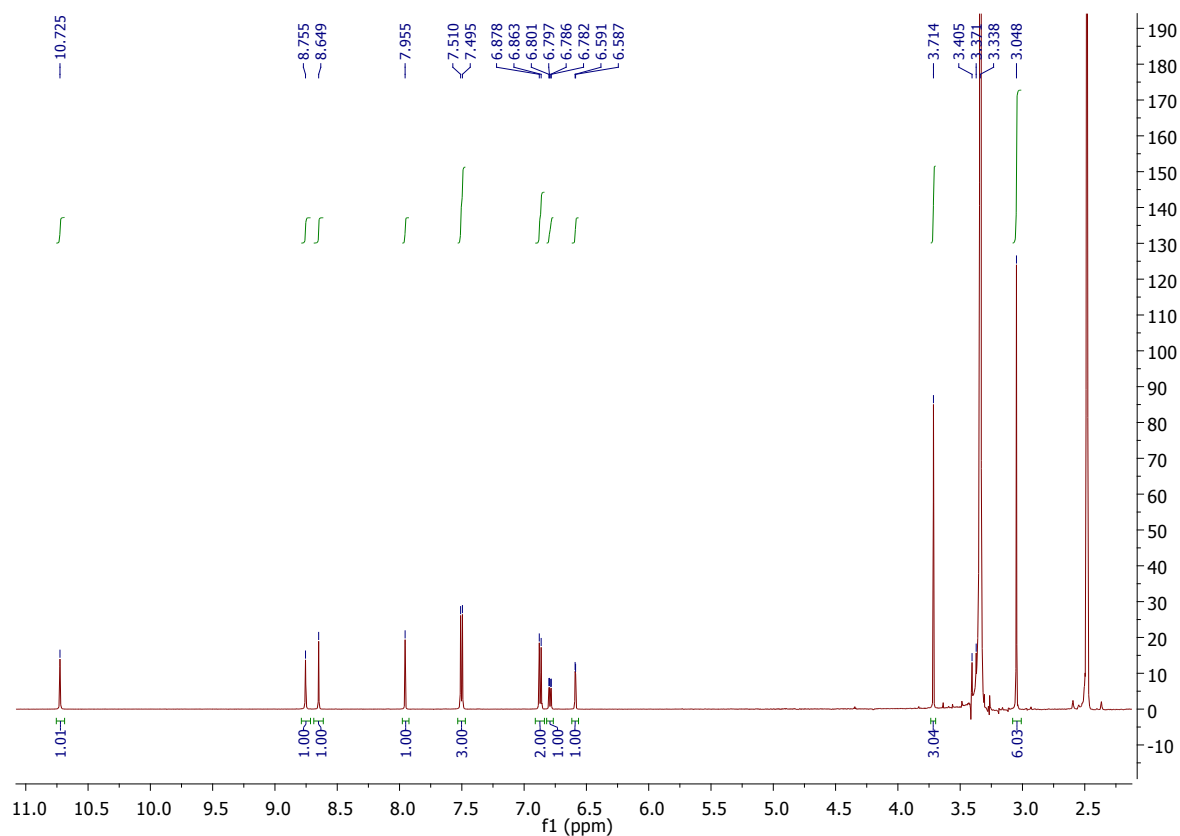
HRMS (HESI): m/z $[M+Na^+]$ – calcd (for $C_{20}H_{20}N_4O_4 + H^+$): 403.1377, experimental: 403.1374

m/z $[M+K^+]$ – calcd (for $C_{20}H_{20}N_4O_4 + H^+$): 419.1116, experimental: 419.1112

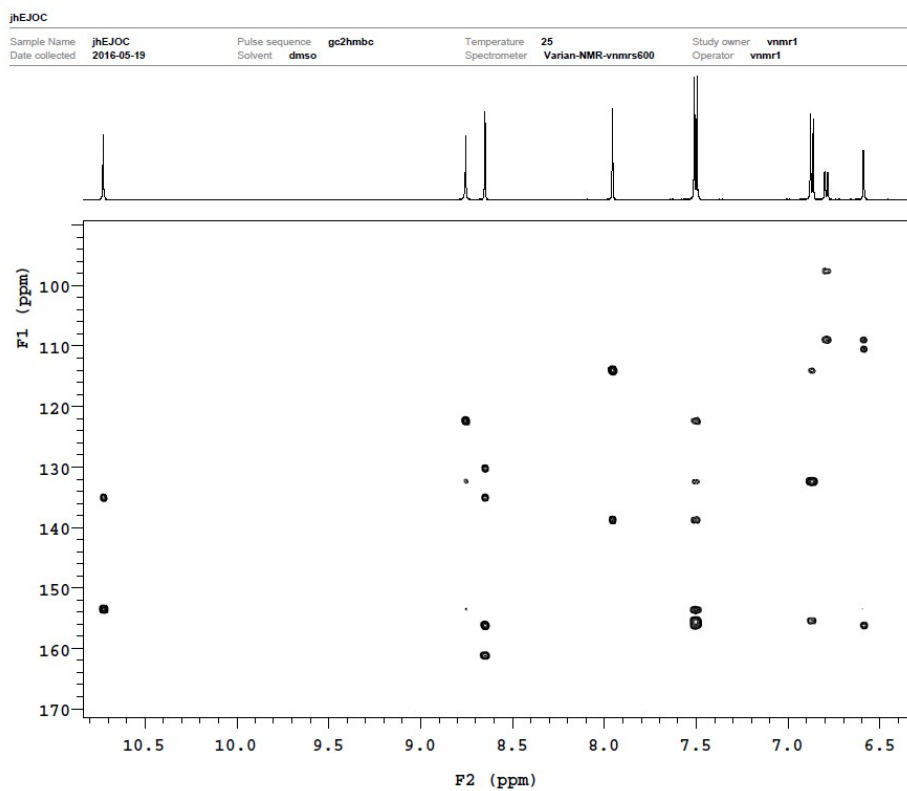
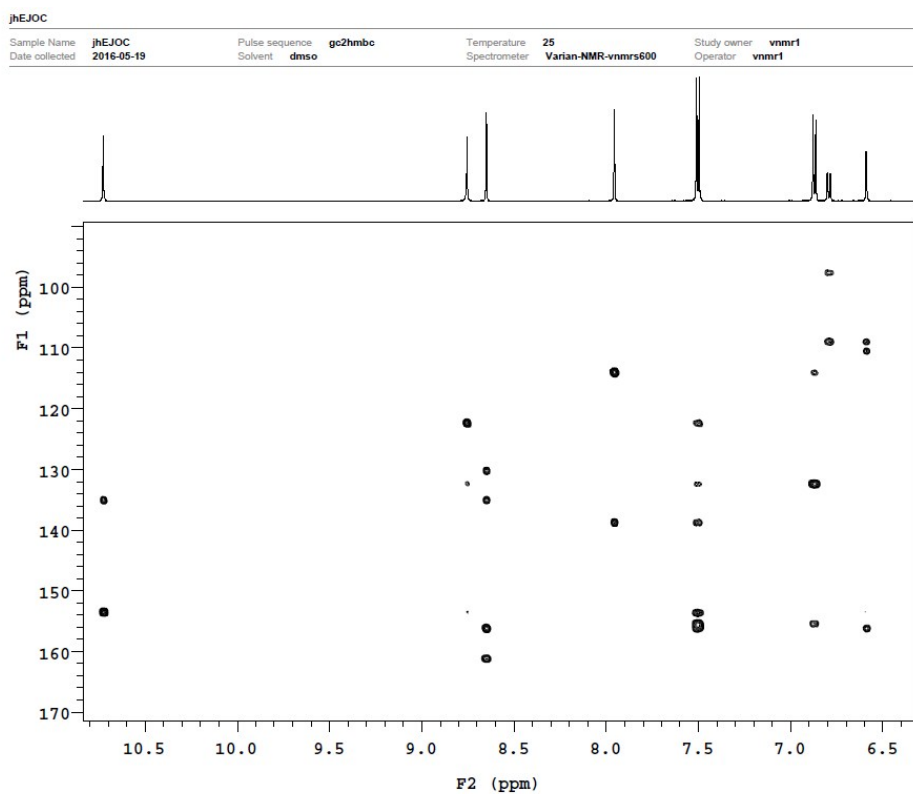
Coumarin phenylsemicarbazone **4**: HRMS spectrum (Orbitrap Fusion (Thermo Fisher Scientific, Inc.), positive mode, R 90000, MeCN:H₂O 80:20, 100 μ L/min, direct infusion to MS):



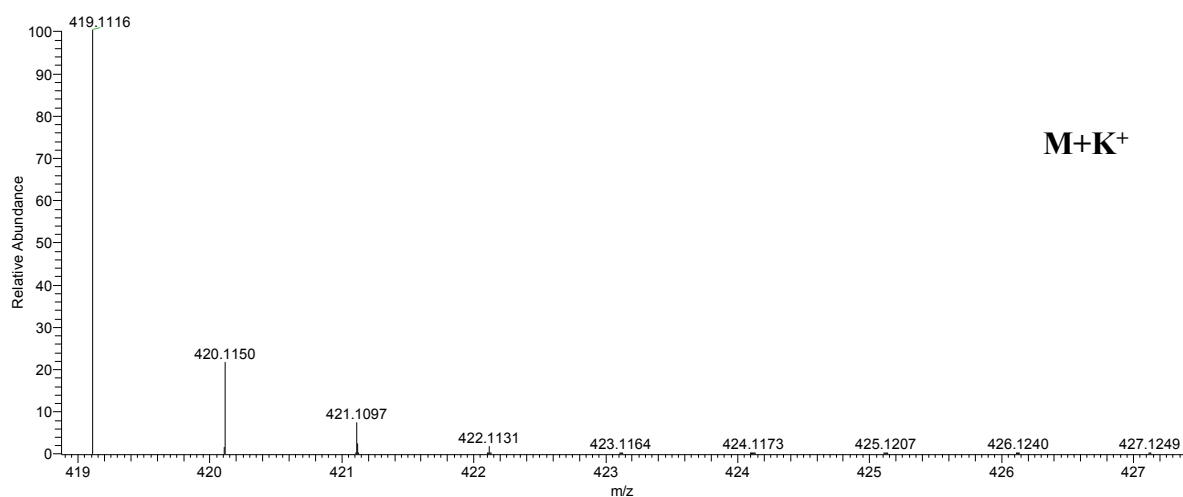
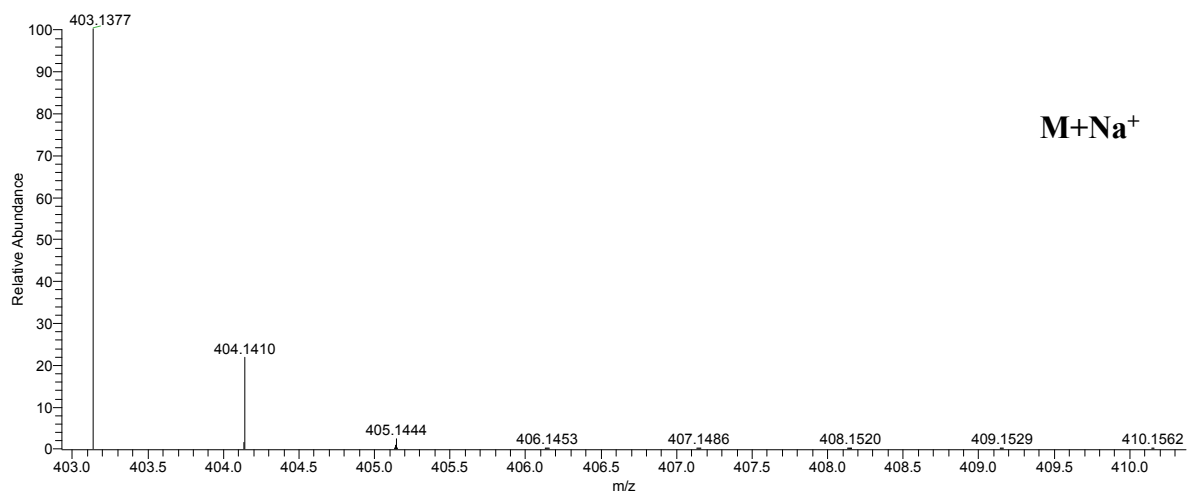
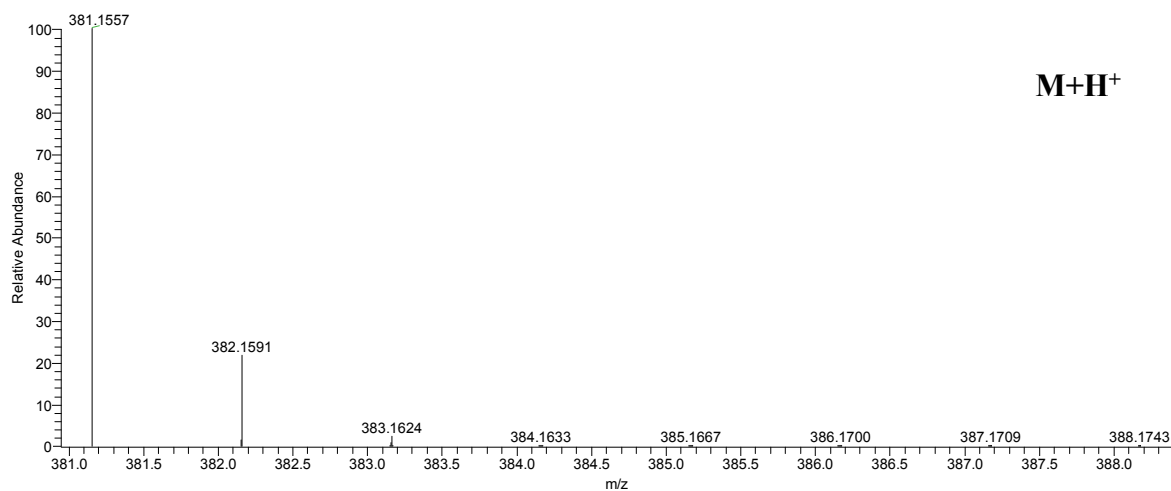
Coumarin phenylsemicarbazone **4**: ^1H NMR, ^{13}C NMR



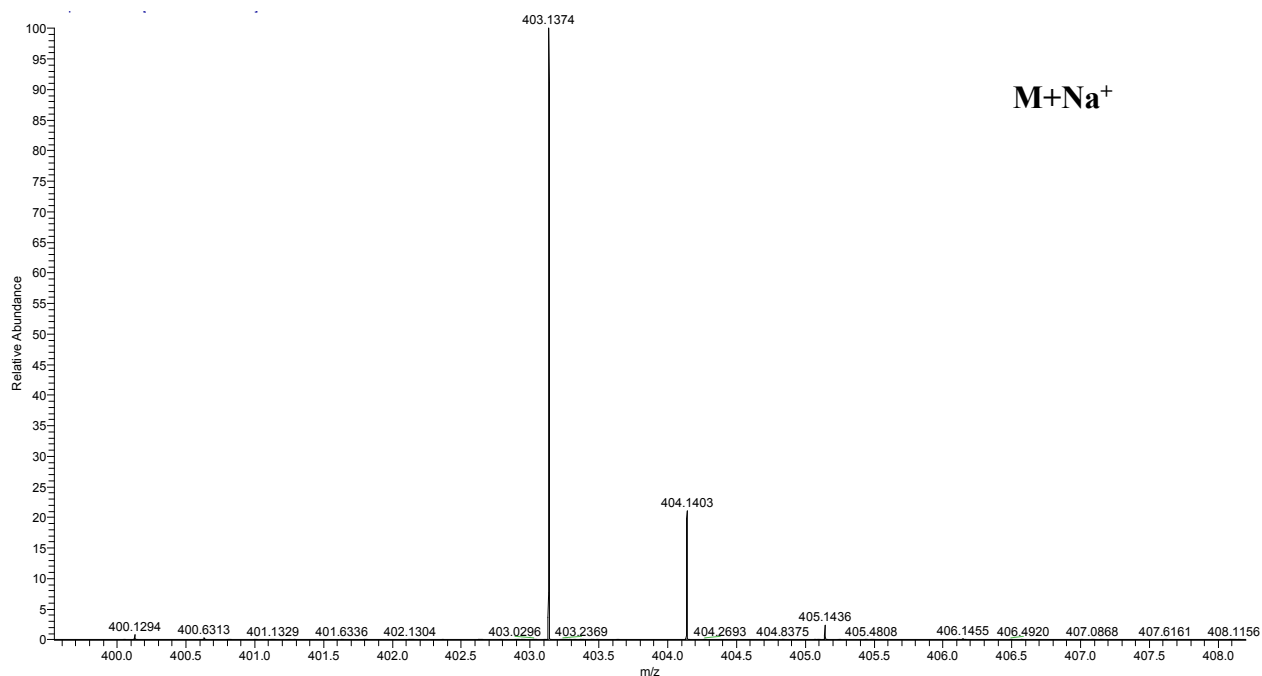
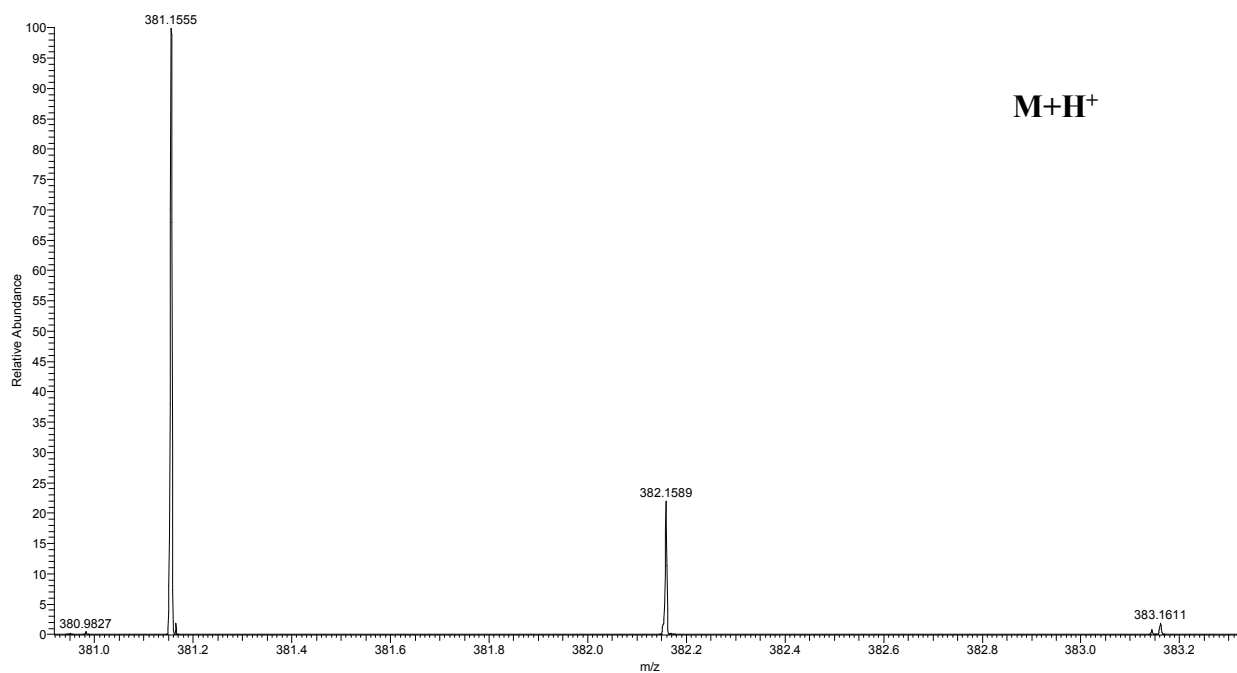
Coumarin phenylsemicarbazone **4**: 2D NMR: HMBC experiment, HSQC experiment

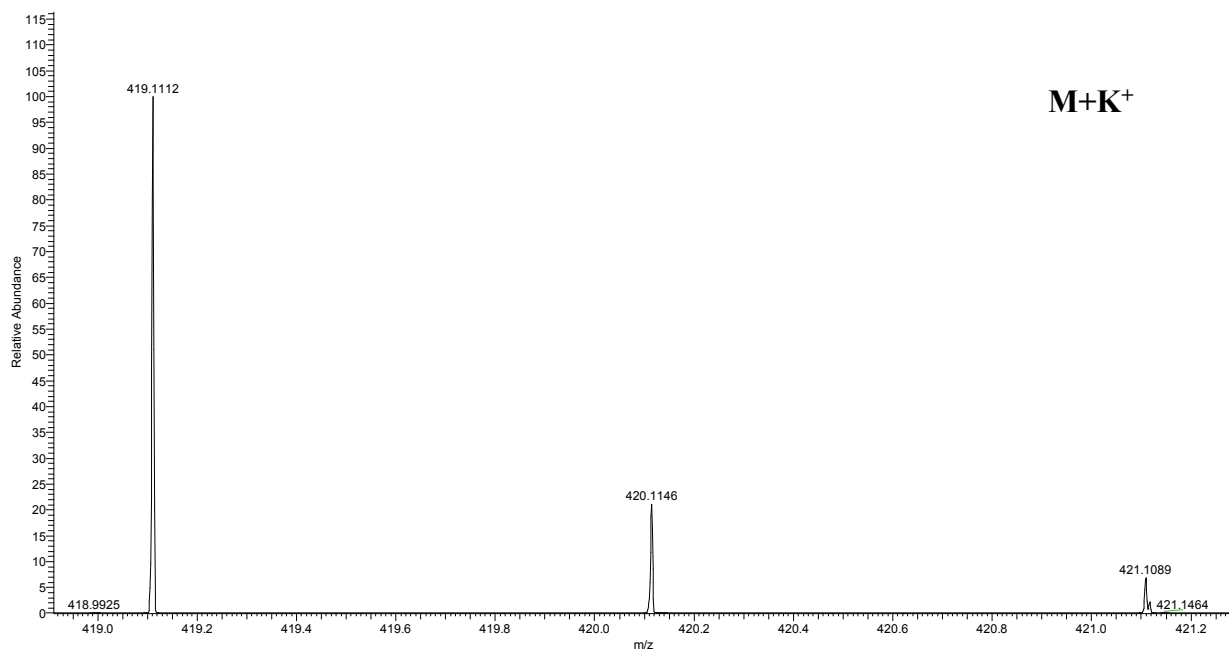


Coumarin phenylsemicarbazone **4**: Simulated MS spectra (software: Xcalibur 4.0)



Coumarin phenylsemicarbazone **4**: Details of molecular ion adducts (experimental data)





Titration experiments

Association constant determinations

Association constants K_{ass} for apparent coumarin phenylsemicarbazone: F^- 1:1 complex formation were determined by the acknowledged formula describing complex anion concentration:⁵⁸

$$F = F_0 + \frac{F_{\text{lim}} - F_0}{2c_0} \left[c_0 + c_{A^-} + 1/K_{\text{ass}} - \left[(c_0 + c_{A^-} + 1/K_{\text{ass}})^2 - 4c_0c_{A^-} \right]^{1/2} \right] \quad (5)$$

where: F_0 is the area under the fluorescence emission curve of free coumarin phenylsemicarbazone, F is the area under the fluorescence emission curve of coumarin phenylsemicarbazone measured after anion addition, F_{lim} is the area under the fluorescence emission curve of coumarin phenylsemicarbazone measured with the F^- anion excess, c_0 is the overall concentration of coumarin phenylsemicarbazone and c_{A^-} is the overall concentration of added anion F^- .

Equation (1) was rewritten to the following form for nonlinear fit in OriginPro 8.1 software:

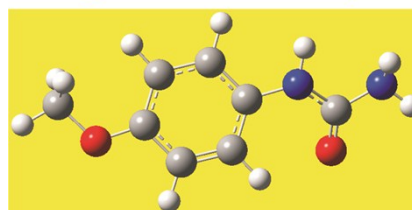
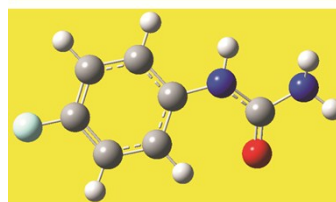
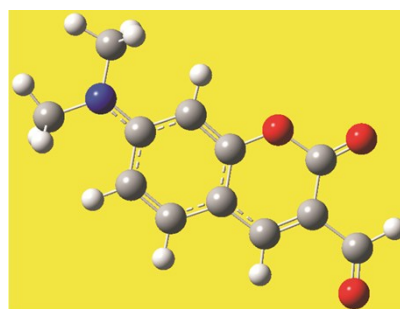
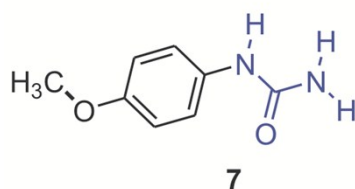
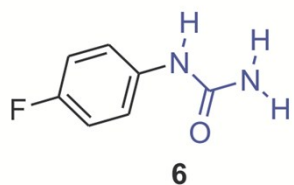
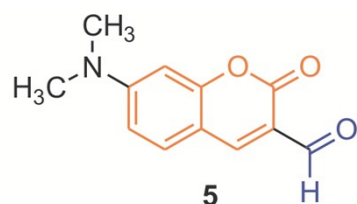
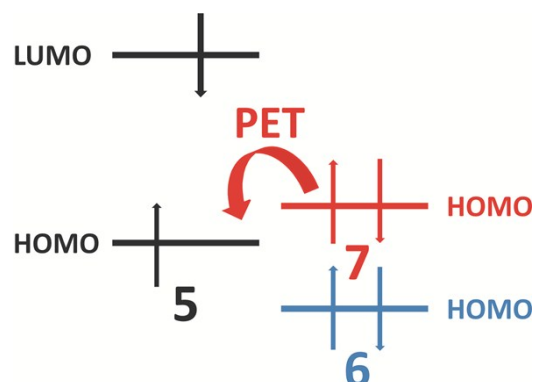
$$F = F_0 + c_1 * (P1 - F_0) * (c_0 + x + 1/P2 - \sqrt{(c_0 + x + 1/P2)^2 - 4 * c_0 * x}), \quad (6)$$

where: $c_0 = 3 \times 10^{-5}$, $c_1 = 1/2c_0 = 1.7 \times 10^4$, parameter $P1 = F_{\text{lim}}$, parameter $P2 = K_{\text{ass}}$ and $x = c_{F^-}$. The F_0 value was fixed to the fluorescence emission area value for $x = 0$.

SUPPORTING SCHEMES, TABLES AND FIGURES

Table S1. Calculated HOMO and LUMO orbital energy of compounds **5-7** at the M062X 6-311++G(dp) level in vacuum (calculated in Gaussian 09).

Compd	5	6	7
LUMO (kJ mol ⁻¹)	- 161	- 53	- 40
HOMO (kJ mol ⁻¹)	- 695	- 732	- 678



Scheme S1. Molecular structure and calculated geometries of compounds **5-7** in vacuum at the M062X/6-311++G(d,p) level (performed by Gaussian 09 program package).

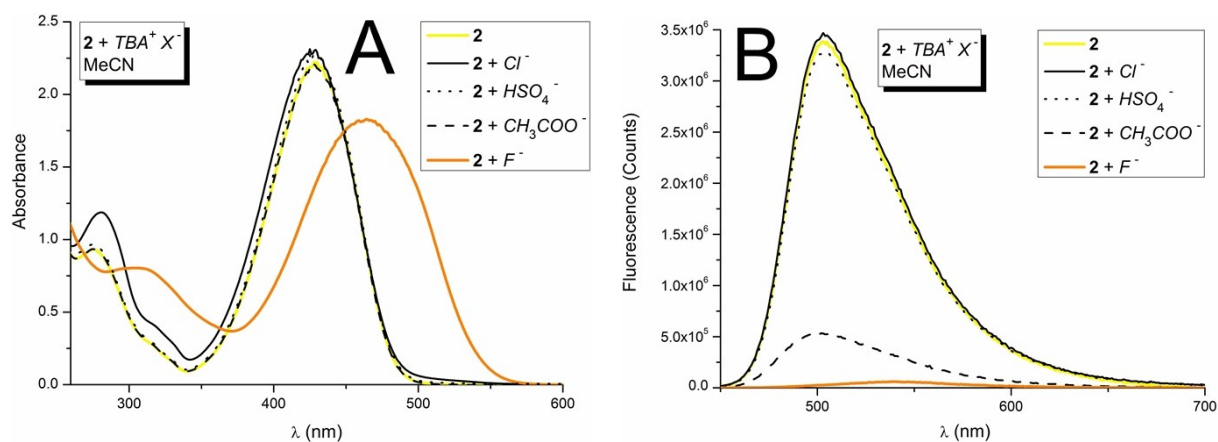


Fig. S1. UV-Vis (A) and fluorescence (B) spectrum of coumarin phenylsemicarbazone **2** in MeCN in the presence of various anions ($\sim 3 \times 10^{-5}$ mol L $^{-1}$ coumarin phenylsemicarbazone + 1×10^{-3} mol L $^{-1}$ TBA^+X^- ; $\lambda_{EX} = \lambda_{A(keto-form)}$; $T = 298.15$ K).

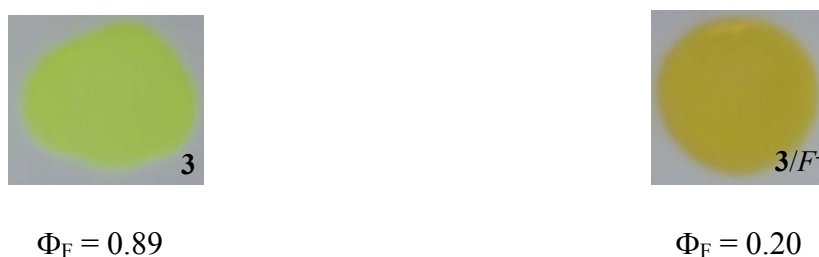


Fig. S2. Poly (propylene carbonate) thin polymer films of pure coumarin phenylsemicarbazone **3** and two-component coumarin phenylsemicarbazone **3/F $^-$** system (TBA^+F^- addition to chloroform solution of polycarbonate and coumarin **3**; $\lambda_{EX} = \lambda_{A(keto-form)}$; Φ_F were determined using integrating sphere; poly (propylene carbonate) was purchased from Sigma-Aldrich, St. Louis, MO, USA, $M_n \sim 50,000$ by GPC).

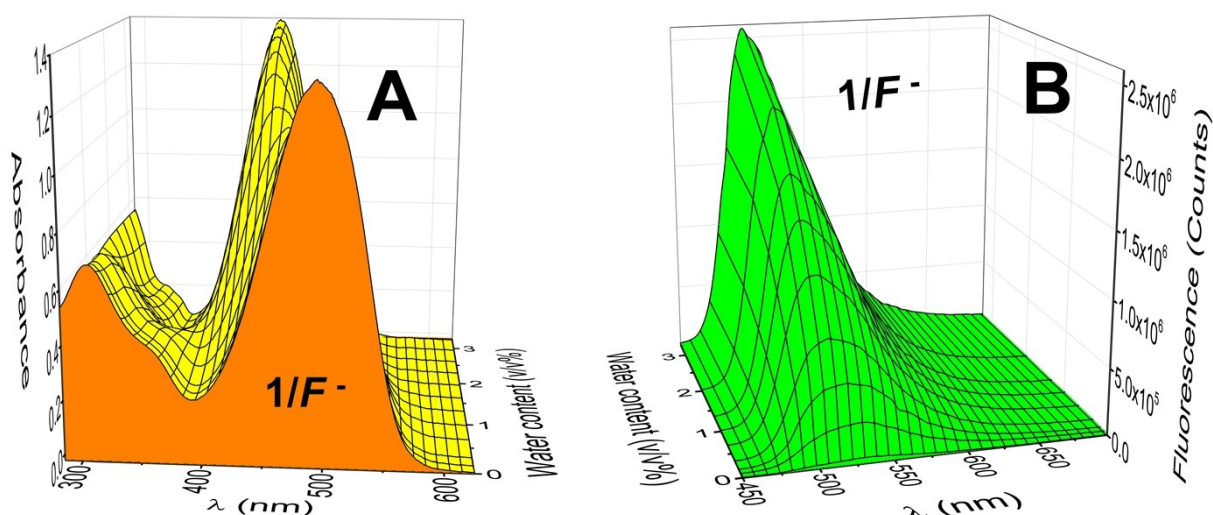


Fig. S3. Evolution of the UV-Vis (A) and fluorescence (B) spectrum of two-component coumarin phenylsemicarbazone **1/F $^-$** sensor in DMSO during its titration with water ($\sim 3 \times 10^{-5}$ mol L $^{-1}$ **1** + 1×10^{-3} mol L $^{-1}$ TBA^+F^- ; $\lambda_{EX} = \lambda_{A(keto-form)}$; $T = 298.15$ K).

Table S2. Detection limit (LOD) and quantification limit (LOQ) for water in MeCN using studied two-component coumarin phenylsemicarbazone/ F^- chemosensors (determined by absorption spectroscopy; $T = 298.16\text{ K}$).

	LOD ($3\sigma/S$)	
	wt%	v/v%
1/F^-	0.0044	0.0035
2/F^-	0.0046	0.0036
3/F^-	0.0056	0.0044
4/F^-	0.0055	0.0043
	LOQ ($10\sigma/S$)	
	wt%	v/v%
1/F^-	0.0148	0.0116
2/F^-	0.0153	0.0120
3/F^-	0.0185	0.0145
4/F^-	0.0182	0.0142

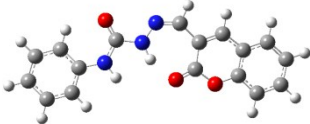
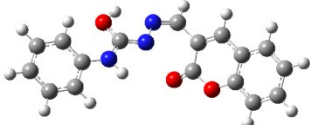
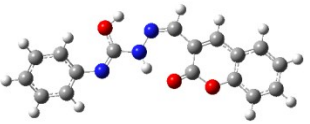
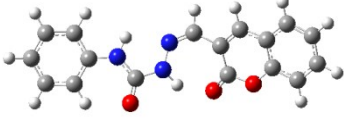
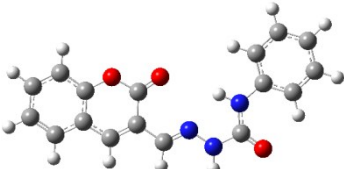
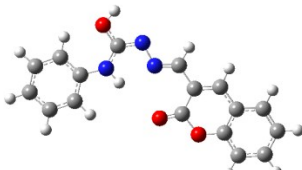
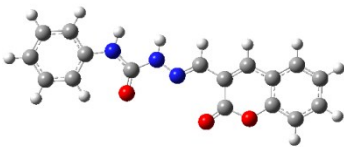
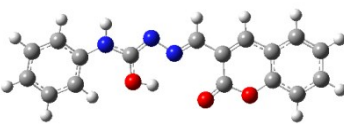
wt (%) – weight of water/weight of solution in %; v/v (%) – volume of water/volume of solution in %; Detection limit ($3\sigma/S$) and quantification limit ($10\sigma/S$) for water in MeCN were determined by the following equations:

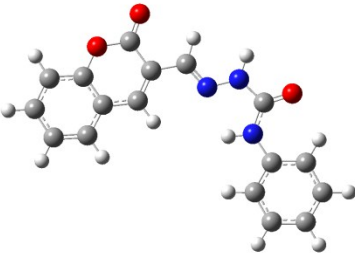
$$3\sigma/S = 3 \frac{\sqrt{\frac{\sum (A - \bar{A})^2}{n}}}{S}; \quad (7)$$

$$10\sigma/S = 10 \frac{\sqrt{\frac{\sum (A - \bar{A})^2}{n}}}{S}, \quad (8)$$

where: σ is the standard deviation, A is the absorbance of coumarin phenylsemicarbazone enolate form and S is the slope of the $A = f(\text{wt}\%)$ or $A = f(\text{v/v}\%)$ plot in the initial linear portion of the curve; in all cases $n = 10$.

Table S3. Gibbs free energy calculated for unsubstituted coumarin phenylsemicarbazone relative to most stable conformation.

Structure	ΔG (kJ mol ⁻¹) M06-2x/6-311++G(d,p)
 C1	39,01305
 C2	71,15716
 C3	83,58958
 C4	18,12382
 C5	4,189771
 C6	55,44739
 C7	51,89509
 C8	48,97527

Structure	ΔG (kJ mol ⁻¹)
	0
C	

The example of the 1-(2-oxo-2*H*-chromen-3-yl)methylidene]-4-phenylsemicarbazide in Table S3 can be generally applied to all studied compounds. Instead of a typical Y-shaped urea –NH-CO-NH- moiety conformation (*s-trans*, *s-trans*; conformers C1 and C7), quantum chemical calculations confirm *s-cis*, *s-trans* conformation of urea structural fragment with relatively strong intramolecular hydrogen bonding (conformer C). Intramolecular hydrogen bonding between –C=N- hydrazone nitrogen and the NH hydrogen at the phenyl semicarbazide moiety therefore contributes to the stabilization of coumarin phenylsemicarbazones.

Table S4. Apparent association constants K_{ass} of the studied coumarin phenylsemicarbazones **1-4** with strongly basic F^- anion in MeCN at 298.16 K (1:1 complex), determined by non-linear fitting from fluorescence data (please see ESI Association constant determinations†).

Compd	1	2	3	4
K_{ass} (mol ⁻¹ dm ³)	6946 ± 1733 (R ² =0.9621)	2655 ± 1168 (R ² =0.9305)	1628 ± 308 (R ² =0.9634)	975 ± 102 (R ² =0.9787)

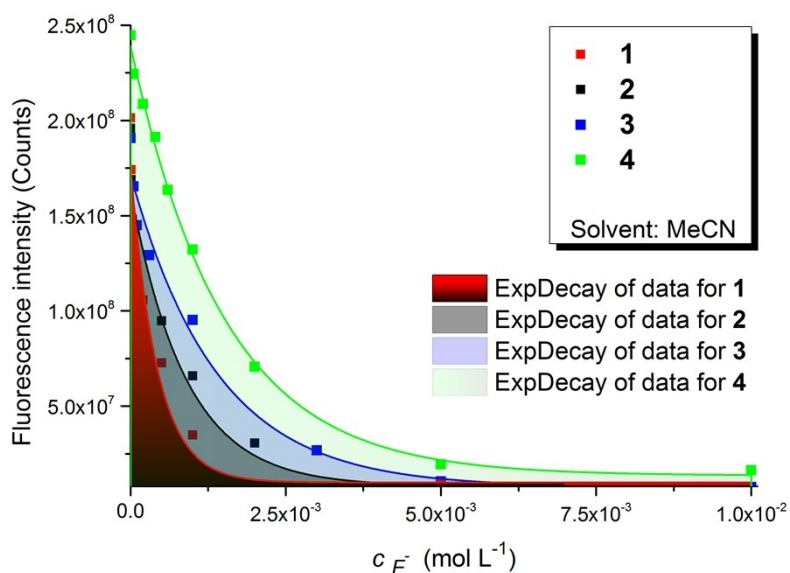


Fig. S4. Evolution of area under the emission curve of coumarin phenylsemicarbazones **1-4** in MeCN during **1-4** solution titration with TBA^+F^- ($c_{\text{coumarin}} = 3 \times 10^{-5}$ mol dm⁻³; $\lambda_{\text{EX}} = \lambda_{\text{A(coumarin keto-form)}}$; $T = 298.15$ K; fluorescence of derivative **4** was measured at different ExBW and EmbW due to significantly lower fluorescent quantum yield of this derivative).

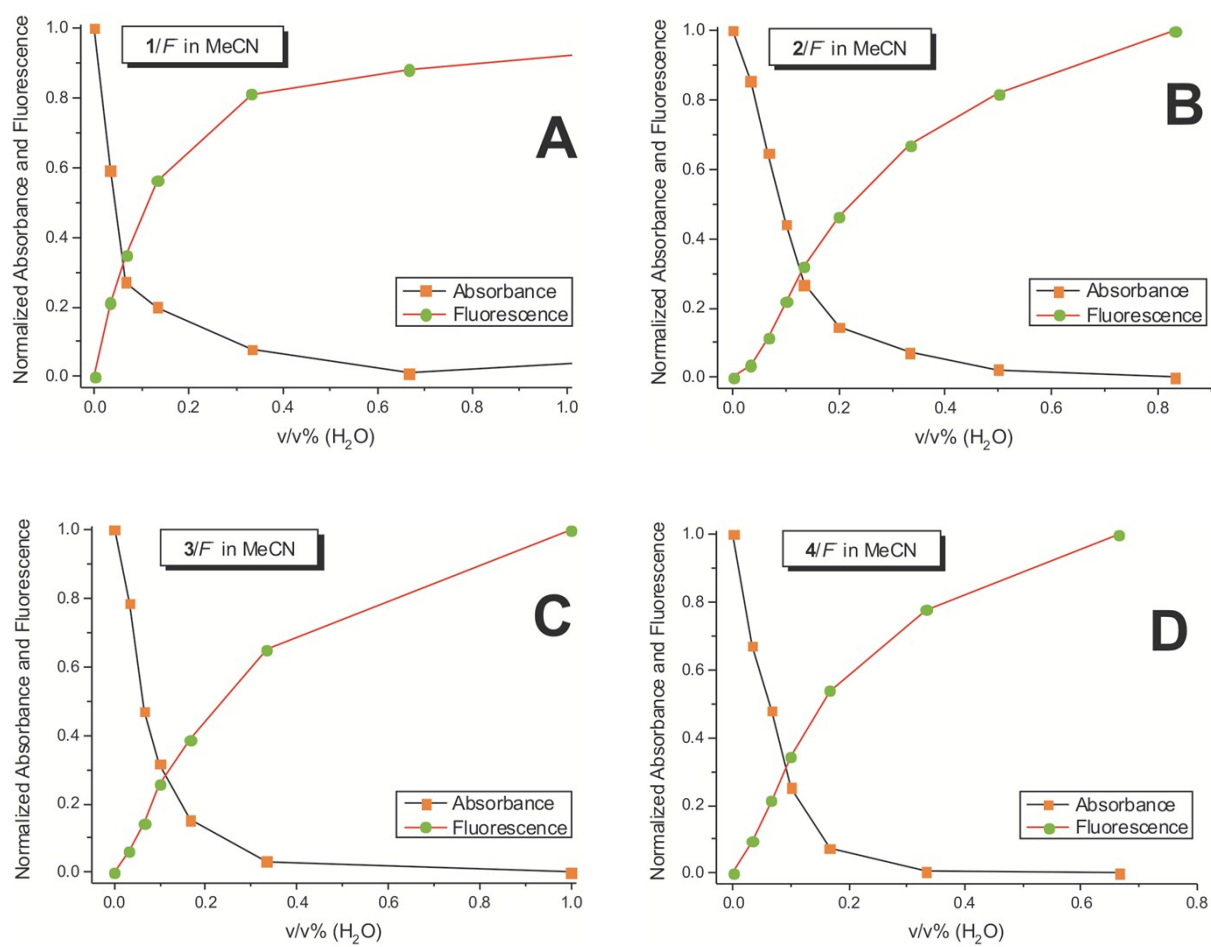


Fig. S5. Absorbance and fluorescence intensity behaviour of studied two-component coumarin phenylsemicarbazone sensors **1-4/F** in MeCN during titration with TBA^+F^- (absorbance was measured at $\lambda_{A(\text{coumarin enolate-form})}$ and fluorescence at $\lambda_{EX} = \lambda_{A(\text{coumarin keto-form})}$; $T = 298.15$ K).

MASS SPECTROSCOPY ANALYSIS OF REACTION MECHANISM

The mass spectrum of derivate **4** dissolved in MeCN (yellow colour; $c = 0.25$ mg/ml - the solubility of derivate **4** in MeCN is limited); mobile phase MeCN, column C18

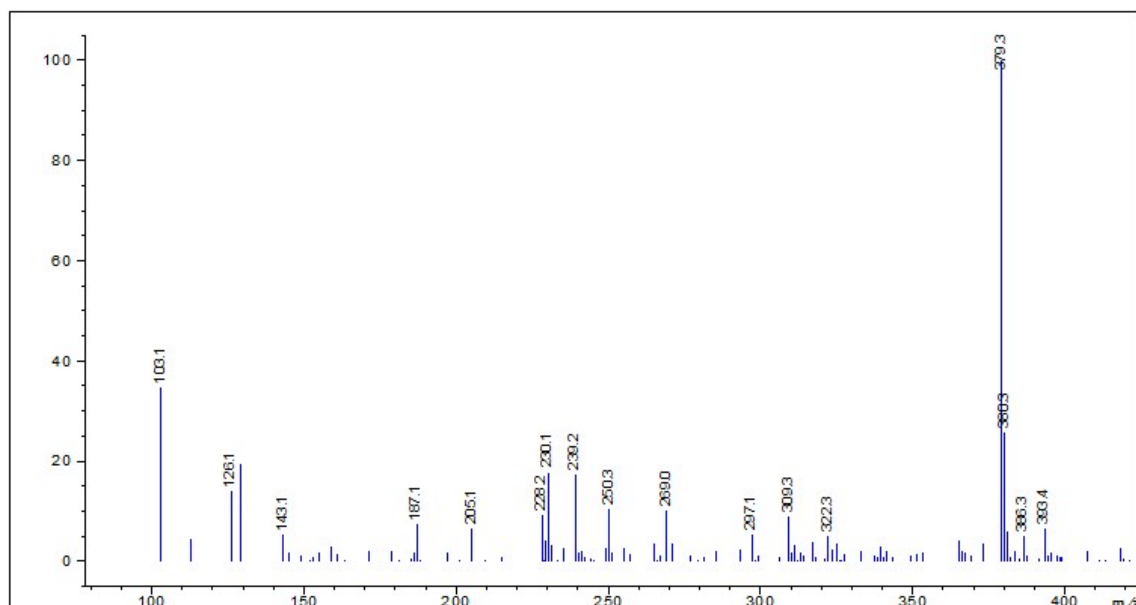


Fig. S6. Negative ionization mode; molecular peak m/z $[MH]^- = 379.3$ (LC MSD 6110, Agilent Technologies, MM (multimode ion source ESI/APCI)).

The mass spectrum of derivate **4** dissolved in MeCN ($c = 0.25$ mg/ml) after addition of 4 equiv. of TBA^+F^- (orange colour change; mobile phase MeCN, column C18

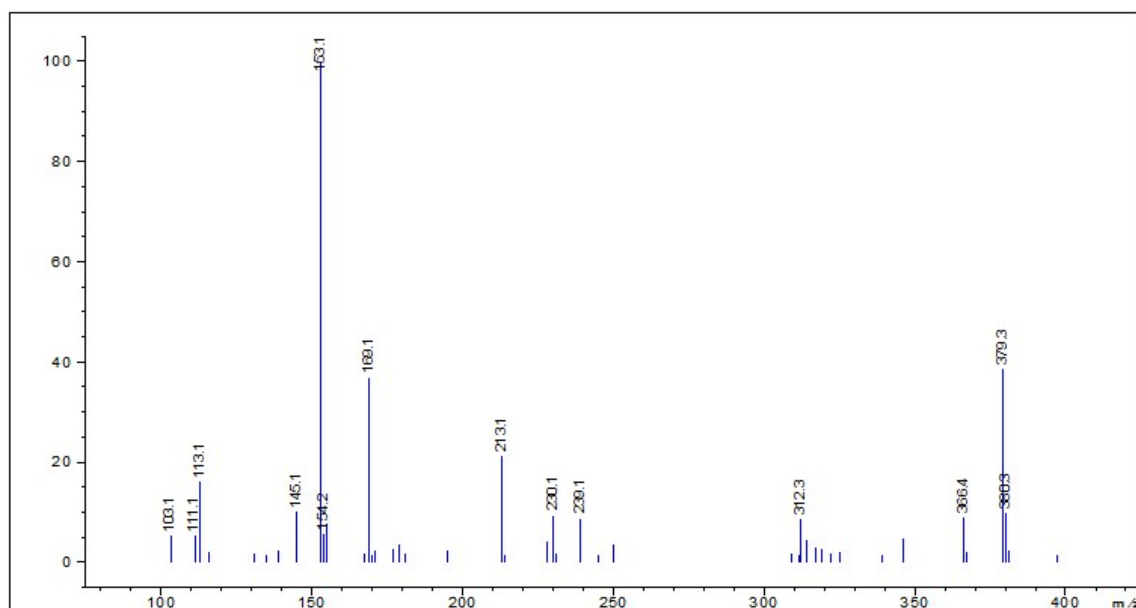


Fig. S7. Negative ionization mode; molecular peak m/z $[MH]^- = 379.3$ (LC MSD 6110, Agilent Technologies, MM (multimode ion source ESI/APCI)).

The mass spectrum of derivate **4** dissolved in MeCN ($c = 0.25$ mg/ml) + 4 equiv. of TBA⁺F⁻, followed by subsequent addition of 100 μ l DEMI H₂O (initial yellow colour appearance); mobile phase MeCN, column C18

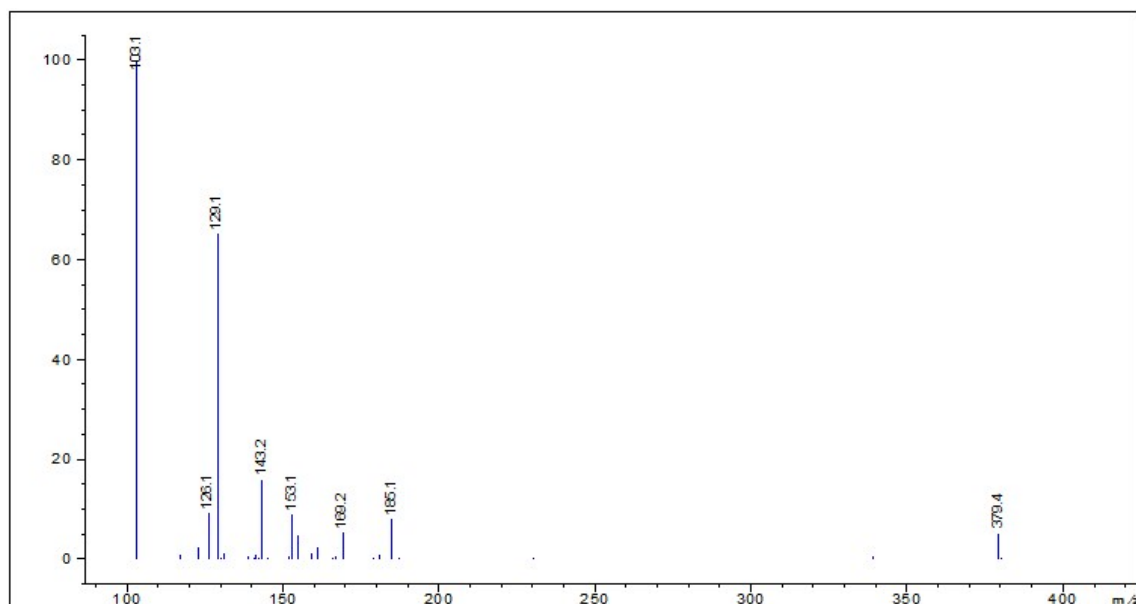


Fig. S8. Negative ionization mode; molecular peak m/z [MH]⁻ = 379.4 is poorly visible due to the detector saturation with a lot of ions (LC MSD 6110, Agilent Technologies, MM (multimode ion source ESI/APCI)).

Unfortunately, due to the limited solubility in MeCN, the signal of compound **4** in positive ionization mode was very low (on the detection limit level). Therefore, we performed experiments in positive mode after compound dissolution in DMSO.

*The mass spectrum of derivate **4** dissolved in DMSO ($c = 1\text{mg/ml}$; orange colour); mobile phase MeCN, column C18*

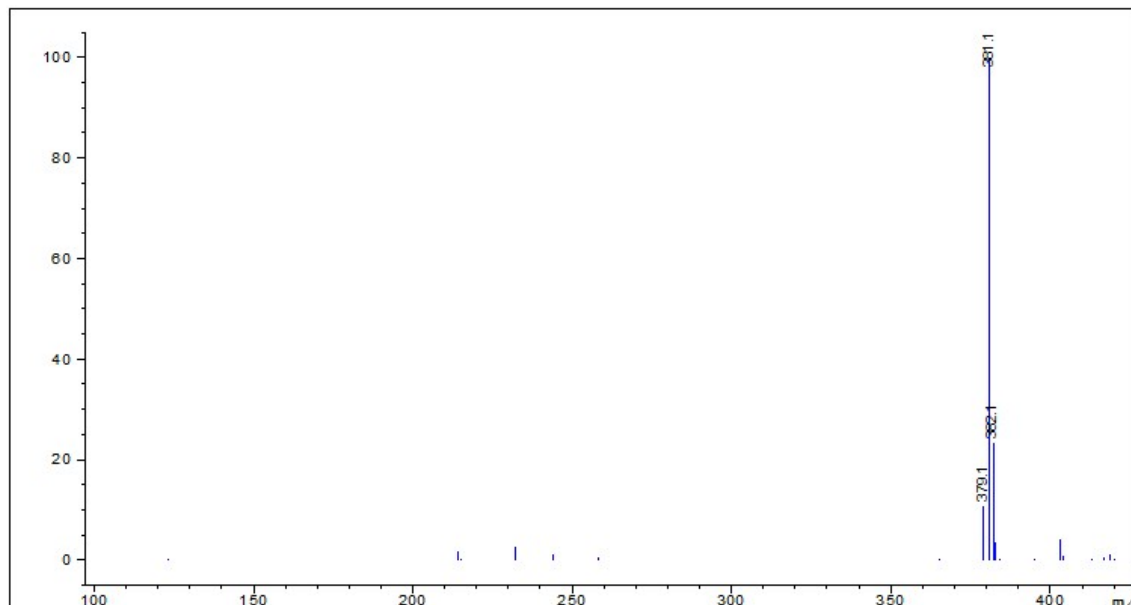


Fig. S9. Positive ionization mode; molecular peak m/z $[\text{MH}]^+ = 381.1$ (LC MSD 6110, Agilent Technologies, MM (multimode ion source ESI/APCI)).

*The mass spectrum of derivate **4** dissolved in DMSO ($c = 1\text{mg/ml}$) after addition of 2 equiv. of TBA^+F^- (brick red colour change); mobile phase MeCN, column C18*

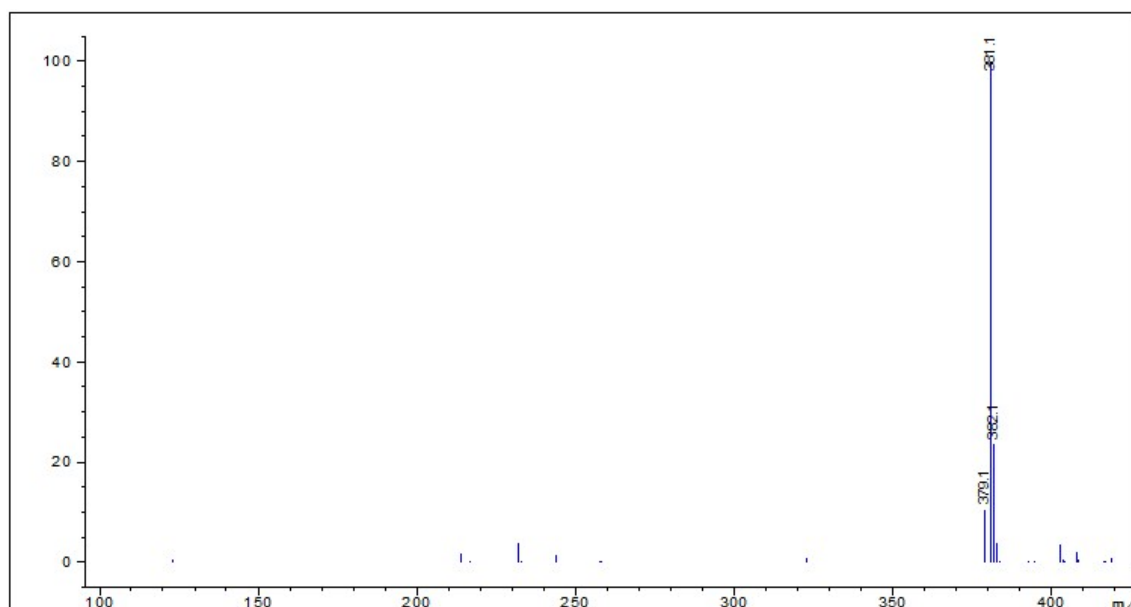


Fig. S10. Positive ionization mode; molecular peak m/z $[\text{MH}]^+ = 381.1$ (LC MSD 6110, Agilent Technologies, MM (multimode ion source ESI/APCI)).

The mass spectrum of derivate **4** dissolved in DMSO ($c = 1\text{ mg/ml}$) after addition of other 30 equiv. of TBA^+F^- (carmine red colour change); mobile phase MeCN, column C18

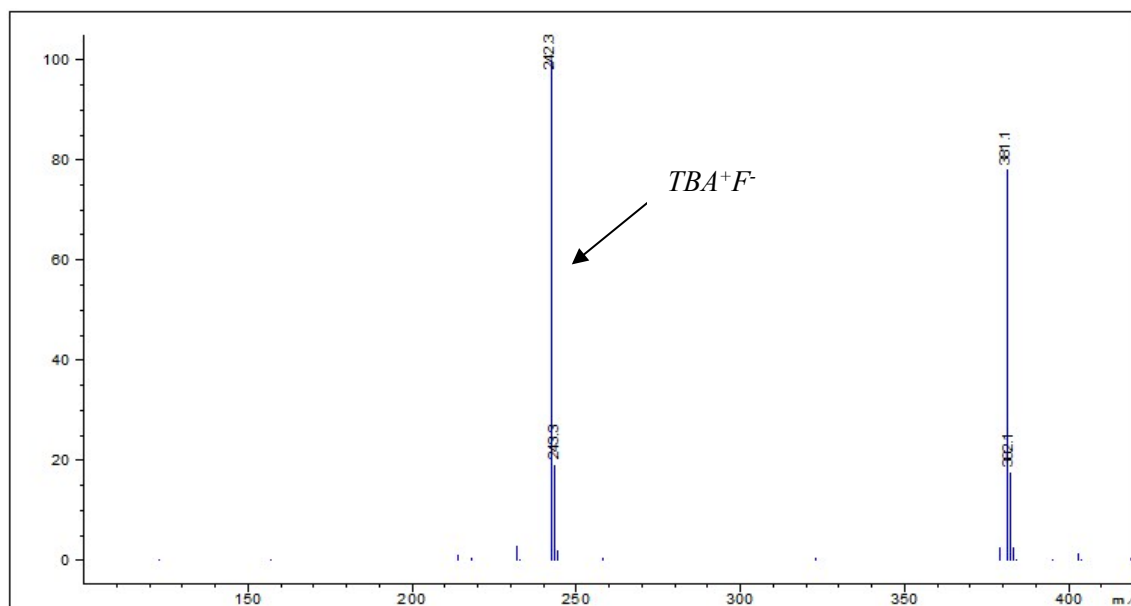
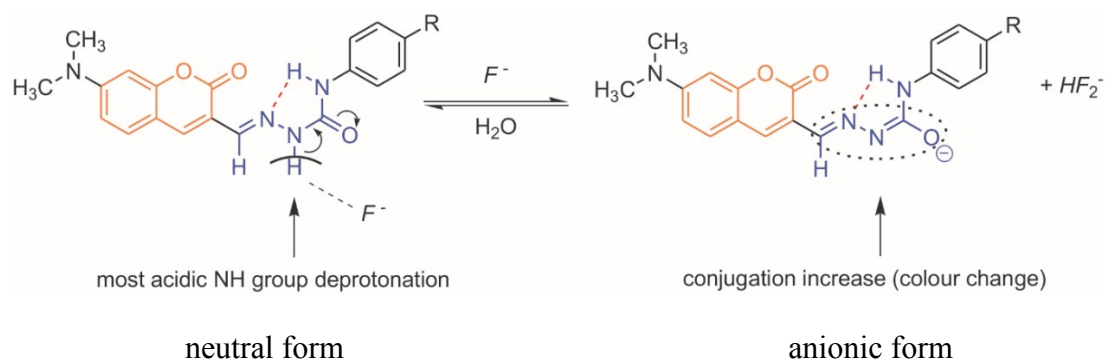


Fig. S11. Positive ionization mode; molecular peak: m/z $[MH]^+ = 381.1$, peak of TBA^+ cation: m/z $[M]^+ = 242.3$ (LC MSD 6110, Agilent Technologies, MM (multimode ion source ESI/APCI)).

As clearly indicate Figs. S8-S10, mass spectrum of coumarin anionic form (after F^- anion addition to initial coumarin solution) is almost identical to mass spectrum of initial neutral coumarin form; with only intense TBA^+ peak appearance (and its fragments) at high TBA^+F^- concentration as exception.

Mass Spectroscopy – Conclusion

The LC/MS method is not suitable for the confirmation of acid-base reaction mechanism of studied compounds during the low-level water sensing in aprotic solvents, because we demonstrated that the anionic form of studied compound **4** preserves the ion mass of initial neutral molecule in both positive and negative ionization mode (although reversible naked eye solution colour change in both cases clearly indicates running acid-base reaction).



ADDITIONAL REFERENCES

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