

Colorimetric Cyanide Chemosensor Based on 1',3,3',4-Tetrahydrospiro[chromene-2,2'-indole]

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A new class of chemosensors based on the 1',3,3',4-tetrahydrospiro[chromene-2,2'-indole] ring system, which detects cyanide with high specificity, is described. These chemosensors show a distinct color change when treated with cyanide in acetonitrile solution buffered with sodium phosphate, and this procedure is not affected by the presence of other common anions. The chemosensors exhibit high sensitivity to low concentrations of cyanide, meeting the European Union water quality control

criterion of sensitivity below 0.05 mg L⁻¹, and show a very fast response within tens of seconds. The mechanism for detection is rationalized by the nucleophilic substitution of the phenolic oxygen atom at the indoline C-2 atom by the cyanide anion to form a stable indolynitrile adduct and to generate the colored 4-nitrophenolate chromophore. These chemosensors can be synthesized by a simple procedure from commercially available starting materials.

Introduction

Cyanide (CN⁻) is a hazardous chemical that leads directly to death in humans, even at low concentrations.^[1,2] Chemical substances containing cyanide occur naturally^[3,4] or can be prepared artificially for use in various areas of industry, including mining,^[5] plastics manufacturing,^[6] and electroplating.^[7] Cassava (*Manihot esculenta*) plants, which are consumed by millions of people as a source of food,^[8,9] contain cyanogenic glucosides, such as linamarin.^[10] Linamarin can be digested by linamarase, a naturally occurring enzyme, or hydrolyzed chemically to liberate highly toxic hydrogen cyanide.^[11] Therefore, the presence of cyanide from raw carbohydrates containing cyanogenic glucosides in food products has to be tightly controlled.^[12] However, much more concerning are the cyanides used in industries, such as mining, where there is a high risk of contamination of drinking water sources, especially in the process of gold separation.^[13] It is well documented that over the last few decades, there have been many cases of transportation accidents and industrial dam failures, where large quantities of cyanide have entered river systems severely impacting aquatic life.^[5] In accordance with the the World Health Organi-

zation (WHO) recommendations, water containing more than 0.07 mg L⁻¹ (27 × 10⁻⁷ M) cyanide should not be used as a domestic supply,^[14] while the European Union (EU) Drinking Water Directive has set the maximum contaminant level for cyanide in drinking water at 0.05 mg L⁻¹ (19 × 10⁻⁷ M).^[15] These low limits require extremely sensitive methods for cyanide detection.

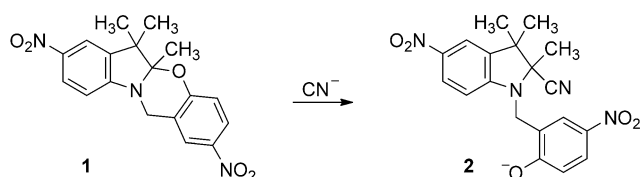
In order to control the presence of cyanide in food, feedstock, drinking water, and the environment, numerous studies have focused on the development of methods for its detection, including the use of chemosensors in which a change in color or fluorescence is monitored.^[16] Many previous designs for cyanide sensors relied on ditopic binding with crown ether conjugates, hydrogen-bonding interactions, or copper–cyanide affinity.^[17,18] In recent years, there has been increasing interest in the development of chemosensors based on the nucleophilic addition reactions of the cyanide anion to an electrophilic substrate to yield the covalent C–CN bond and the formation of a stable colored species. For example, the treatment of colorless [1,3]oxazine derivatives^[19,20] with cyanide anions yields adducts that absorb at approximately 430 nm, which is in the visual part of the spectrum.^[21,22] 2,8-Dinitroindolo[2,1-*b*] [1,3]benzoxazine **1** was commercialized by Sigma–Aldrich as a sensitive chemosensor for the detection of cyanide through the formation of a stable colored adduct (**2**), possessing the 4-nitrophenolate chromophore (Scheme 1).^[23]

It was recently shown that 6-nitro-1',3'-dihydrospiro[chromene-2,2'-indole] **3**, which is well known for its photochromic properties,^[24] also behaves as a selective and sensitive cyanide receptor in aqueous media.^[25] This compound is converted to the open-ring form upon UV irradiation, and when cyanide anions are present in a solution adduct **4** is produced, resulting in the formation of the colored 4-nitrophenolate chromophore causing a new absorption band to appear. However, adduct **4**

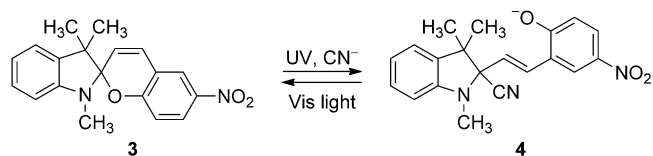
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Scheme 1. Mechanism for cyanide detection by the [1,3]oxazine-based chemosensor.



Scheme 2. Mechanism for cyanide detection by the 1',3'-dihydrospiro[chromene-2,2'-indole]-based chemosensor.

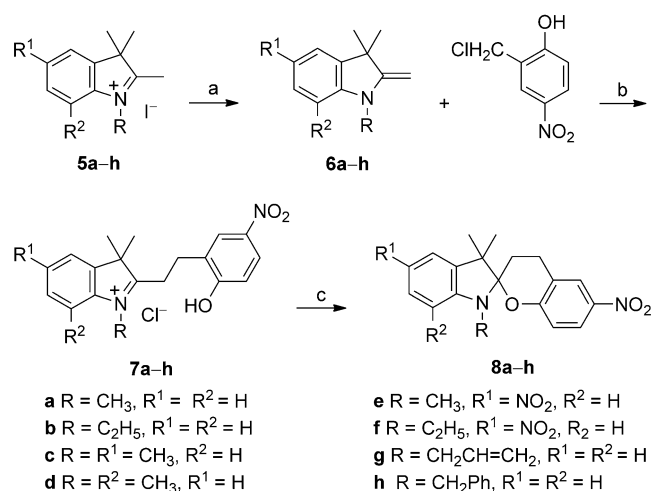
is not stable and reverts back to its spirocyclic ground state upon visible light irradiation (Scheme 2).

This study is focused on the design and investigation of a new class of highly sensitive, selective and stable chemosensors based on the 1',3,3',4-tetrahydrospiro[chromene-2,2'-indole] ring system.^[26] Recently, 1',3,3',4-tetrahydrospiro[chromene-2,2'-indoles], which are notable for the single C₃–C₄ bond in their pyran ring, have been described as ultrafast light-driven switches.^[27]

Results and Discussion

Synthesis

The synthesis strategy for 1',3,3',4-tetrahydrospiro[chromene-2,2'-indoles] **8a–h** outlined in Scheme 3 is based on the alkylation of Fisher's bases **6a–h** with 2-chloromethyl-4-nitrophenol



Scheme 3. Reagents and conditions: a) H₂O, Na₂CO₃, rt, 1 min; b) CH₃CN, rt, 6 h; c) H₂O, NH₄OH, rt, 1 min.

followed by work-up of the intermediate salts (**7a–h**) with base. The preparation of compounds **8a–c,f,g** and their characterization was previously described,^[27] while compounds **8d,e,h** are new 1',3,3',4-tetrahydrospiro[chromene-2,2'-indole] derivatives. The preparation of the starting material, 1,2,3,3-tetramethyl-5-nitroindolium iodide **5e**, was described elsewhere.^[28]

1',3,3',4-Tetrahydrospiro[chromene-2,2'-indoles] **8a–h** are chiral compounds with a single center of asymmetry. The single-crystal X-ray analysis of compound **8a** confirmed that the molecule consists of indole and benzopyran moieties that reside in near-perpendicular planes connected through the chiral spiro C-2' atom (Figure 1).^[29]

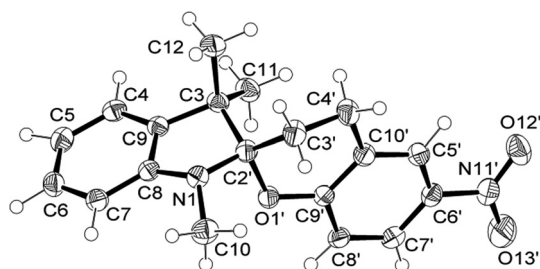


Figure 1. ORTEP image of the (S)-enantiomer of compound **8a**.

It was shown previously that 1',3,3',4-tetrahydrospiro[chromene-2,2'-indole] **8a** easily undergoes interconversion of the (R)- and (S)-enantiomers when dissolved in organic solvent due to the thermally induced C₍₂₎–O bond cleavage with formation of the intermediate planar 3H-indolium cation and subsequent ring-closure via the attack of the phenolate negatively charged oxygen atom on either face of the aforementioned planar intermediates. In sodium-phosphate-buffered acetonitrile solution, compound **8a** exists in an equilibrated mixture with the open form.^[27] The characteristic signal of compounds **8d,e,h** in the ¹H NMR spectra taken in deuterated chloroform at room temperature was a joint signal of 3,3-methyl groups in the area of 1.24–1.37 ppm, which reflects the coalescence of the separate singlets of the diastereotopic geminal methyl groups due to the inversion at the chiral spiro carbon atom similar to that of known compounds **8a–c,f,g**.

Chemosensing mechanism

Steady state absorbance spectra of compounds **8a–h**, measured for solutions in the acetonitrile/phosphate buffer, revealed absorption in the UV region of the electronic spectra (Table 1). However, when a sodium cyanide solution, buffered with sodium phosphate (pH 7.6), was added to the aforementioned solutions of compounds **8a–h** (0.1 mM of **8a–h**, 1 mM NaCN in the cell), a new absorption band was observed in the visible area at approximately 420 nm (Table 1, Figure 2).

The appearance of this band in the visible region can be rationalized by spirochromene ring opening and formation of 4-nitrophenolate chromophores **9a–h** due to the nucleophilic substitution of the phenolic oxygen by a cyanide group. In this

Table 1. UV-vis spectral data for compounds 8a–h in CH ₃ CN/phosphate buffer.				
Compd	λ_{\max} of 8 [nm]	$\epsilon \times 10^3$ [dm ³ mol ⁻¹ cm ⁻¹]	λ_{\max} of 9 [nm]	$\epsilon \times 10^3$ [dm ³ mol ⁻¹ cm ⁻¹]
8a	205	51.4	250	17.2
	243	16.3	282	7.7
	326	14.6	422	29.0
8b	205	42.3	253	12.2
	246	14.4	286	9.1
	326	12.5	420	20.7
8c	206	46.3	255	41.9
	245	15.7	300	23.3
	313	13.6	422	24.8
8d	210	37.2	253	10.4
	246	10.7	293	4.2
	326	11.2	422	21.8
8e	229	11.8	254	13.8
	321	12.7	422	30.0
	375	19.0		
8f	230	14.1	257	14.5
	330	14.6	424	32.3
	380	22.1		
8g	205	46.1	254	42.3
	244	16.7	298	23.4
	313	12.4	419	23.4
8h	205	54.3	252	31.3
	242	17.9	298	17.2
	312	12.9	418	17.6

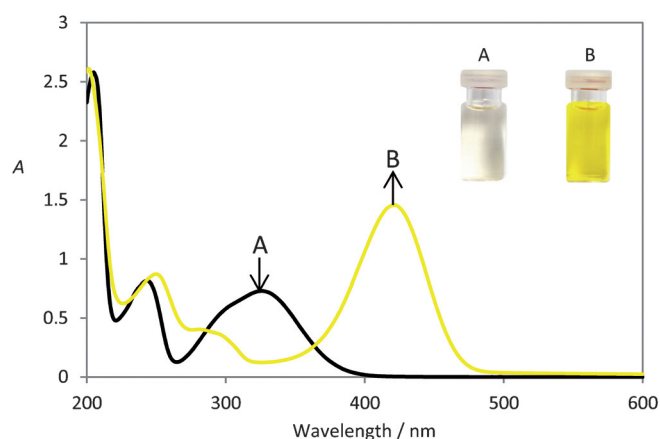
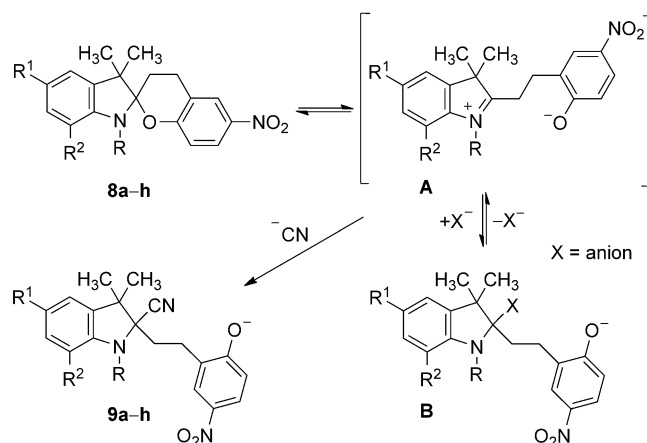
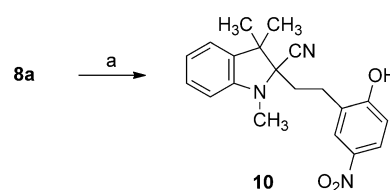


Figure 2. Absorption (A) spectra of **8a** (0.1 mm, 298 K) in a mixture of CH₃CN/phosphate buffer (Na₂HPO₄/NaH₂PO₄, 7.5 mM, pH 7.6) (19:1, v/v) without (spectrum A) and with (spectrum B) NaCN (10 equiv).

case, the limiting step of the reaction is the C_(spiro)–O covalent bond cleavage and the formation of intermediates **B**, which are in an equilibrated mixture with the starting 1',3,3',4-tetrahydrospiro[chromene-2,2'-indoles] (**8a–h**). However, intermediates **A** are quickly consumed when the nucleophilic addition of the cyanide anion to the C-2 carbon occurs to form stable



Scheme 4. Formation of 4-nitrophenolate chromophore **9**.



Scheme 5. Formation of 1H-indole-2-carbonitrile **10**. Reagents and conditions: a) THF, NaCN, H₂O, rt, 1 h.

nitriles **9a–h**. In such cases, the ring-opening reaction of **8a–h** becomes irreversible (Scheme 4).

When compound **8a** was treated with sodium cyanide in tetrahydrofuran (THF) containing a small amount of water, the reaction afforded indole-2-carbonitrile **10** (Scheme 5), the structure of which was confirmed by spectroscopic methods and elemental analysis. The IR spectrum of **10** contains an absorption band at 2222 cm⁻¹, characteristic for nitriles.^[30] The heteronuclear multiple bond coherence (HMBC) spectrum revealed three bonds range coupling between the methylene protons at 2.15 and 2.30 ppm and the nitrile carbon at 118.49 ppm.^[31,32] These protons also interact with the quaternary carbon at 76.75 ppm, separated by two bonds, and with the quaternary carbons at 46.76 and 128.16 ppm, separated by three bonds. The full assignments presented in Figure 3 were

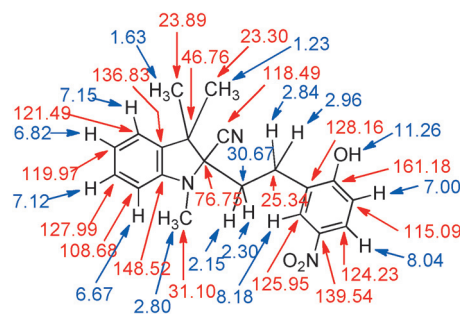


Figure 3. ¹H (blue) and ¹³C (red) NMR chemical shifts [ppm] for **10** in [D₆]DMSO.

based on the combined application of standard NMR techniques such as COSY, NOESY, ROESY, APT, DEPT, HSQC and HMBC spectra.

Response time

For various types of chemosensors, the response time is very important for the practical detection of analytes. In our case, we observed that the response time depends on the structure of the compound. After the addition of sodium cyanide to the solutions of compounds **8a–d**, a strong new absorption band at 422 nm appeared within 1–3 min (Figure 4), while for compounds **8e,f**, this process took up to 1.5 h (Figure 5). We con-

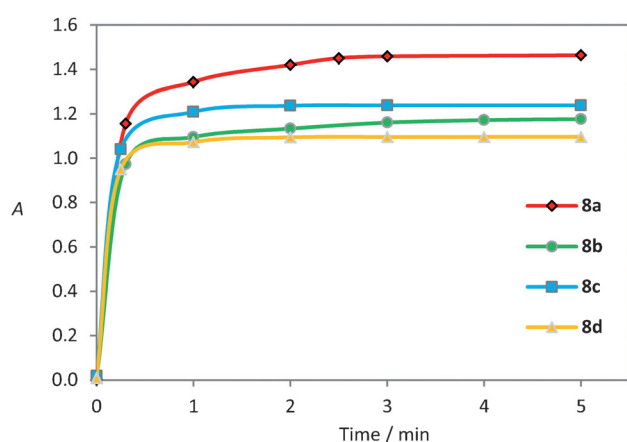


Figure 4. Absorbance changes at 422 nm for **8a–d** (0.1 mM, 298 K) in a mixture of CH₃CN/phosphate buffer (Na₂HPO₄/NaH₂PO₄, 7.5 mM, pH 7.6) (19:1 v/v) after addition of NaCN (10 equiv).

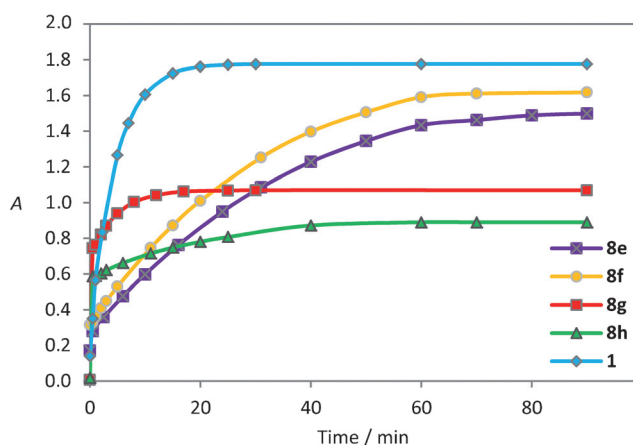


Figure 5. Absorbance changes at 422 nm for **8e–h** and at 411 nm for **1** (0.1 mM, 298 K) in a mixture of CH₃CN/phosphate buffer (Na₂HPO₄/NaH₂PO₄, 7.5 mM, pH 7.6) (19:1 v/v) after addition of NaCN (10 equiv).

cluded that the presence of a nitro group at C-5 of the indole nucleus stabilizes the closed form of the molecule and slows down the formation of the final adduct **9e,f**. Both electronic and steric effects can be considered for explaining the influence of the allyl and benzyl groups at the indole nitrogen

atom on the formation of adducts **9g,h** after the addition of sodium cyanide to a solution of compounds **8g,h**, respectively. The response time of the reference compound, 2,8-dinitroindolo[2,1-*b*][1,3]benzoxazine **1**, was evaluated in analogous conditions, and the formation of colored adduct **2** proceeds more slowly in comparison with compounds **8a–d**. The full process takes approximately 20 min (Figure 5), similar to that of compounds **8g,h**.

Selectivity and sensitivity

To test the selectivity of chemosensor **8a**, parallel investigations were carried out with a series of other anions (F⁻, Cl⁻, Br⁻, I⁻, CH₃COO⁻, C₂O₄²⁻, HCO₃⁻, HSO₃⁻, HSO₄⁻, NO₂⁻, NO₃⁻, SCN⁻, SO₃²⁻, SO₄²⁻, S₂O₃²⁻). The addition of excess amounts of these anions did not result in significant absorbance spectral changes during UV/vis titration (Figure 6), indicating that this

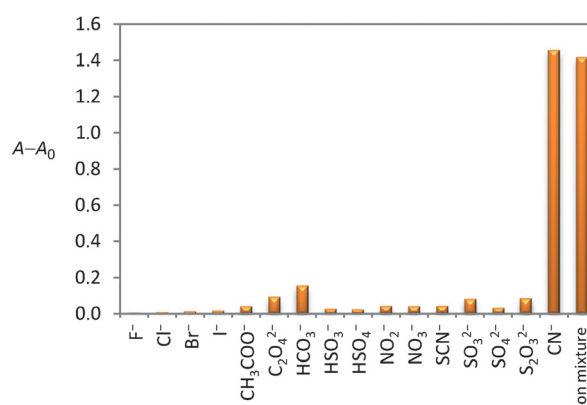


Figure 6. Absorbance at 422 nm of **8a** (0.1 mM, 298 K) in a mixture of CH₃CN/phosphate buffer (Na₂HPO₄/NaH₂PO₄, 7.5 mM, pH 7.6) (19:1 v/v) in the presence of CN⁻ or other common anions (10 equiv) or ion mixtures (10 equiv of CN⁻, HSO₄⁻, Cl⁻, Br⁻, and F⁻); A₀ is the absorbance of **8a** at 422 nm in the absence of CN⁻.

potential chemosensor demonstrates excellent selectivity for cyanide over other common anions. The high selectivity of chemosensing can be explained by the fact that only the addition of a cyanide anion on the indole nucleus forms a strong enough covalent bond with the indole C-2 atom to afford stable nitrile **9**, while the addition of the other aforementioned anions is a reversible process.

To evaluate sensitivity, the calibration curve of cyanide concentration versus absorption at 422 nm for **8a** was plotted (Figure 7), showing that this chemosensor is sensitive to relatively low concentrations of CN⁻ and meets the European Union Drinking Water Directive criterion for water quality, with sensitivity below 0.05 mg L⁻¹ (19 × 10⁻⁷ M).

Conclusion

The derivatives of 1',3,3',4-tetrahydrospiro[chromene-2,2'-indole], synthesized by a simple procedure from commercially available starting materials, undergo transformations to the

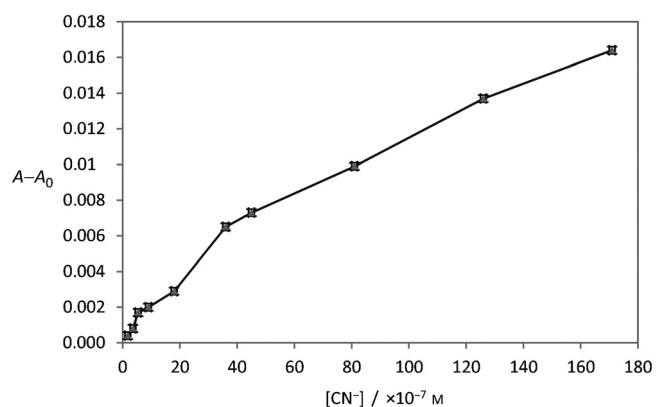


Figure 7. Absorbance at 422 nm of **8a** (0.1 mM, 298 K) in a mixture of CH₃CN/phosphate buffer (Na₂HPO₄/NaH₂PO₄, 7.5 mM, pH 7.6) (19:1 v/v) in the presence of different concentrations of CN⁻, where A₀ is the absorbance of **8a** at 422 nm in the absence of CN⁻ (SD = 0.0002).

colored ring-open form possessing the 4-nitrophenolate chromophore when treated with cyanide in acetonitrile solution buffered with sodium phosphate and show a distinct color change, which can be measured colorimetrically or detected by the naked eye. Furthermore, these compounds, in particular, exhibit a high selectivity and are not affected by the presence of halides or other anions, which are common interferents in the conventional sensing schemes for cyanide. This new chemosensor exhibits high sensitivity to low concentrations of cyanide, meeting the water quality control criterion of sensitivity below 0.05 mgL⁻¹ and also shows a very fast response up to tens of seconds.

Experimental Section

Synthesis procedures

General: Reactions were monitored by thin layer chromatography (TLC) on precoated silica gel plates (Kieselgel 60F₂₅₄, Merck). Compounds were visualized with UV light and charring after treatment with a 1% KMnO₄ solution. Column chromatography was performed using silica gel SI 60 (43–60 μm, Merck). Melting points (m.p.) were determined in open capillary tubes with a Büchi B-540 melting point apparatus and are uncorrected. Infrared (IR) spectra were recorded on a PerkinElmer Spectrum One spectrometer using KBr pellets. ¹H NMR spectra were recorded at 300 MHz on a Varian Unity Inova spectrometer and at 700 MHz on a Bruker Avance III spectrometer. ¹³C NMR spectra were recorded using the same instruments at 75 and 176 MHz, respectively. NMR experiments were performed at 25 °C. Chemical shifts (δ) are expressed in parts per million (ppm) downfield relative to tetramethylsilane (TMS), and coupling constants (*J*) referring to apparent peak multiplicity are reported in Hertz (Hz) using standard abbreviations. UV/vis spectra were determined on a PerkinElmer Lambda 35 spectrometer using quartz cells with a light path length of 0.5 cm. Mass spectra (MS) were measured using a Waters ZQ ion spray instrument and the Shimadzu LCMS-2020. High-resolution ESI-TOF mass spectra (HRMS) were measured on a Bruker maXis spectrometer. Elemental analyses were conducted using the Elemental Analyser CE-440 (Exeter Analytical Inc.) in the Microanalytical Laboratory, Department of Organic Chemistry, Kaunas University of Technology (Lithuania).

Reagents and solvents were purchased from Sigma–Aldrich and used without further purification. Water was doubly distilled. The anionic solutions for selectivity testing were prepared from sodium salts of various anions.

1,2,3,3,7-Pentamethyl-3H-indolium iodide (5d): 2,3,3,7-Tetramethyl-3H-indole (0.865 g, 5 mmol) was mixed with iodomethane (1.42 g, 10 mmol), and the mixture was heated at reflux for 24 h. The resultant crystalline material was isolated by filtration and recrystallized from EtOH to afford **5d** as a brownish crystalline solid (1.12 g, 71%): m.p. 248–250 °C; ¹H NMR (300 MHz, TFA-*d*): δ = 1.65 (s, 6H, 2×3-CH₃), 2.86 (s, 6H, 2-CH₃, 7-CH₃), 4.34 (s, 3H, NCH₃), 7.41–7.59 ppm (m, 3H, Ar-H); ¹³C NMR (75 MHz, TFA-*d*): δ = 15.6, 20.8, 24.2 (2×CH₃), 40.8, 56.3, 123.3, 129.7, 132.8, 135.9, 142.4, 144.8, 198.1 ppm; IR (KBr): 3024, 2966, 1625 cm⁻¹ (C–N stretch); MS (ESI): *m/z* (%): 188 [M–I]⁺ (100); Anal. calcd for C₁₃H₁₈I_N: C 49.54, H 5.76, N 4.44; found: C 49.61, H 5.37, N 4.24.

[2-(2-Hydroxy-5-nitrophenyl)ethyl-1]-1,3,3,7-tetramethyl-3H-indolium chloride (7d): A stirred solution of iodide **5d** (1.575 g, 5 mmol) in distilled water (15 mL) was treated with Na₂CO₃ (1.06 g, 10 mmol) at rt. The mixture became turbid immediately and was then extracted with Et₂O (3×20 mL). The combined organic phase was dried over anhydrous Na₂SO₄, filtered, and concentrated in vacuo to afford crude 1,3,3,7-tetramethyl-2-methylidene-2,3-dihydro-1H-indole (**6d**) as an oily material.

Crude **6d** was dissolved in CH₃CN (3 mL), and 2-chloromethyl-4-nitrophenol (0.938 g, 5 mmol) was added to the solution. The mixture was stirred at rt for 6 h. The resultant crystalline material was isolated by filtration, washed with cold CH₃CN (1 mL), and dried in vacuo to afford **7d** as an off-white crystalline solid (0.90 g, 48%): m.p. 200–203 °C; ¹H NMR (300 MHz, [D₆]DMSO): δ = 1.58 (s, 6H, 2×3'-CH₃), 2.78 (s, 3H, 7-CH₃), 2.95–3.01 (m, 2H, CH₂), 3.32–3.38 (m, 2H, CH₂), 4.31 (s, 3H, NCH₃), 7.31 (d, *J* = 8.9 Hz, 1H, Ar-H), 7.39 (d, *J* = 7.5 Hz, 1H, Ar-H), 7.5 (t, *J* = 7.5 Hz, 1H, Ar-H), 7.67 (d, *J* = 7.5 Hz, 1H, Ar-H), 8.07 (dd, *J* = 8.9, 2.5 Hz, 1H, Ar-H), 8.35 (d, *J* = 2.5 Hz, 1H, Ar-H), 12.1 ppm (s, 1H, OH); ¹³C NMR (75 MHz, [D₆]DMSO): δ = 19.1, 21.5 (2×CH₃), 26.2, 38.8, 39.0, 53.5, 115.3, 121.1, 124.6, 126.3, 126.6, 127.2, 129.3, 132.5, 139.2, 140.3, 142.8, 162.3, 195.3 ppm; IR (KBr): 3624 (OH), 3050, 2923, 1520 (NO₂, asym), 1345 cm⁻¹ (NO₂, sym); MS (ESI): *m/z* (%): 339 [M]⁺ (100); Anal. calcd for C₂₀H₂₃ClN₂O₃: C 64.08, H 6.18, N 7.47; found: C 64.45, H 6.25, N 7.71.

1',3',3',7'-Tetramethyl-6-nitro-1',3',4'-tetrahydrospiro[chromene-2,2'-indole] (8d): A solution of **7d** (1.124 g, 3 mmol) in EtOH (5 mL) was diluted with water (15 mL). 10% aq NH₃ solution (~0.5 mL) was added dropwise to the solution while stirring until the mixture became turbid. The separated product was extracted with Et₂O (3×20 mL), and the combined extracts were dried over anhydrous Na₂SO₄, filtered, and concentrated in vacuo. The solid residue was recrystallized from CH₃CN to give **8d** as a yellowish crystalline solid (0.518 g, 51%): m.p. 206–209 °C (CH₃CN); ¹H NMR (300 MHz, CDCl₃): δ = 1.24 (s, 6H, 2×3'-CH₃), 2.31–2.36 (m, 2H, CH₂), 2.50 (s, 3H, 7'-CH₃), 3.05–3.10 (m, 5H, CH₂, NCH₃), 6.76–6.82 (m, 2H, 5'-H, 8-H), 6.91–6.96 (m, 2H, 4'-H, 6'-H), 7.99 (dd, *J* = 9.0, 2.7 Hz, 1H, 7-H), 8.04 ppm (d, *J* = 2.7 Hz, 1H, 5-H); ¹³C NMR (75 MHz, CDCl₃): δ = 20.1, 23.4, 24.0, 24.1, 25.6, 32.0, 48.8, 104.7, 116.7, 119.1, 119.2, 119.8, 121.5, 124.1, 125.1, 131.7, 137.6, 140.5, 146.5, 161.8 ppm; IR (KBr): 3084, 2975, 1513 (NO₂, asym), 1330 cm⁻¹ (NO₂, sym); HRMS (ESI): *m/z* [M+H]⁺ calcd for C₂₀H₂₃N₂O₃: 339.1703; found: 339.1706; Anal. calcd for C₂₀H₂₂N₂O₃: C 70.99, H 6.55, N 8.28; found: C 70.76, H 6.61, N 8.54.

1',3',3'-Trimethyl-5',6-dinitro-1',3,3',4-tetrahydrospiro[chromene-2,2'-indole] (8e): A stirred solution of iodide **5e** (1.69 g, 5 mmol) in distilled water (15 mL) was treated with Na₂CO₃ (1.06 g, 10 mmol) at rt. The mixture became turbid immediately and was extracted with Et₂O (3 × 20 mL). The combined organic phase was dried over anhydrous Na₂SO₄, filtered, and concentrated in vacuo to afford crude intermediate enamine **6e** as a brownish oil.

Crude **6e** was dissolved in CH₃CN (3 mL), 2-chloromethyl-4-nitrophenol (0.938 g, 5 mmol) was added, and the mixture was stirred for 6 h at rt. Then, the reaction mixture was poured into water (50 mL), and 10% aq NH₃ solution (−0.75 mL) was added dropwise while stirring until the mixture became turbid. The separated product was extracted with Et₂O (3 × 20 mL), and the combined extracts were dried over anhydrous Na₂SO₄, filtered, and concentrated in vacuo. The residue was subjected to flash chromatography on silica gel (hexane/acetone, 4:1 v/v), and the obtained solid was recrystallized from acetonitrile to give **8e** as a yellowish crystalline solid (0.739 g, 40%); *R*_f = 0.17 (hexane/acetone, 4:1 v/v); m.p. 232–234 °C (CH₃CN); ¹H NMR (300 MHz, CDCl₃): δ = 1.28 (s, 6H, 2 × 3'-CH₃), 2.29–2.45 (m, 2H, CH₂), 2.96 (s, 3H, NCH₃), 3.03–3.17 (m, 2H, CH₂), 6.56 (d, *J* = 8.7 Hz, 1H, 7'-H), 6.79 (d, *J* = 9.0 Hz, 1H, 8-H), 7.91 (d, *J* = 2.4 Hz, 1H, 4'-H), 7.99 (dd, *J* = 9.0, 2.7 Hz, 1H, 7-H), 8.05 (d, *J* = 2.7 Hz, 1H, 5-H), 8.17 ppm (dd, *J* = 8.7, 2.4 Hz, 1H, 6'-H); ¹³C NMR (75 MHz, CDCl₃): δ = 21.9, 23.3, 24.1, 25.7, 28.6, 49.3, 104.1, 106.0, 117.0, 118.2, 121.3, 124.5, 125.4, 126.5, 137.9, 140.8, 141.3, 154.0, 161.0 ppm; IR (KBr): 3068, 2967, 1510 (NO₂, asymm), 1322 cm^{−1} (NO₂, symm); HRMS (ESI): *m/z* [M+H]⁺ calcd for C₁₉H₂₀N₃O₅: 370.1397; found: 370.1399; Anal. calcd for C₁₉H₁₉N₃O₅: C 61.78, H 5.18, N 11.38; found: C 61.63, H 5.19, N 11.60.

1'-Benzyl-3',3'-dimethyl-6-nitro-1',3,3',4-tetrahydrospiro[chromene-2,2'-indole] (8h): A solution of 2,3,3-trimethyl-3H-indole (1.59 g, 10 mmol) in CH₃CN (10 mL) was treated with benzyl iodide (2.39 g, 11 mmol), and the mixture was heated at reflux for 24 h. After cooling to rt, the solvent was removed in vacuo, and the reaction mixture was kept under high vacuum for 20 min to give crude **5h** as a brown amorphous solid, which was used for further reaction without purification.

A stirred solution of crude **5h** (1.955 g, 5 mmol) in CH₂Cl₂ (15 mL) was treated at rt with Et₃N (1.012 g, 1.39 mL, 10 mmol). The separated product was extracted with Et₂O (3 × 20 mL), and the combined extracts were washed with water (3 × 30 mL), dried over anhydrous Na₂SO₄, filtered, and concentrated in vacuo to afford crude intermediate enamine **6h** as a brownish oil.

Crude **6h** was dissolved in CH₃CN (3 mL), 2-chloromethyl-4-nitrophenol (0.938 g, 5 mmol) was added, and the mixture was stirred for 6 h at rt. Then, the reaction mixture was poured into water (50 mL), and 10% aq NH₃ solution (−0.75 mL) was added dropwise while stirring until the mixture became turbid. The separated product was extracted with Et₂O (3 × 20 mL), the combined extracts were dried over anhydrous Na₂SO₄, filtered, and concentrated in vacuo. The residue was subjected to flash chromatography on silica gel (hexane/acetone, 6:1 v/v). The powder-like solid was dried under high vacuum to give **8h** as a yellowish solid (0.581 g, 29%); *R*_f = 0.15 (hexane/acetone, 6:1 v/v); m.p. 62–64 °C; ¹H NMR (300 MHz, CDCl₃): δ = 1.37 (s, 6H, 2 × 3'-CH₃), 2.35–2.42 (m, 2H, CH₂), 3.02–3.06 (m, 2H, CH₂), 4.55 (s, 2H, NCH₂), 6.51–6.53 (m, 1H, 7'-H), 6.83–6.93 (m, 2H, 5'-H, 8-H), 7.28–7.32 (m, 2H, 6'-H, 4'-H), 7.34–7.39 (m, 5H, Ar-H), 7.98–8.02 ppm (m, 2H, 7-H, 5-H); ¹³C NMR (75 MHz, CDCl₃): δ = 23.6, 23.7, 24.5, 25.2, 47.2, 50.1, 107.9, 116.7, 118.0, 120.1, 121.4, 122.5, 124.2, 125.1, 126.1, 126.5, 127.1, 127.5, 128.0, 128.7, 137.0, 138.7, 140.5, 147.6, 161.7 ppm; IR (KBr): 3061,

2966; 1516 (NO₂, asymm), 1337 cm^{−1} (NO₂, symm); HRMS (ESI): *m/z* [M+H]⁺ calcd for C₂₅H₂₅N₂O₃: 401.1860; found: 401.1863; Anal. calcd for C₂₅H₂₄N₂O₃: C 74.98, H 6.04, N 7.0; found: C 74.84, H 5.59, N 6.65.

2-[2-(2-Hydroxy-5-nitrophenyl)ethyl-1]-1,3,3-trimethyl-2,3-dihydro-1H-indole-2-carbonitrile (10): A stirred solution of **8a** (0.15 g, 0.462 mmol) in THF (5 mL) was treated with NaCN (0.045 g, 0.924 mmol) and a drop of distilled water was added. The mixture was stirred for 1 h at rt. The solvent was evaporated in vacuo, and the residue was purified by flash chromatography on silica gel (hexane/acetone, 6:1 v/v) to afford **10** as yellow solid (0.079 g, 49%); *R*_f = 0.12 (hexane/acetone, 6:1 v/v); m.p. 176–178 °C; ¹H NMR (700 MHz, [D₆]DMSO): δ = 1.23 (s, 3H), 1.63 (s, 3H), 2.15 (td, *J* = 13.3, 4.8 Hz, 1H), 2.30 (td, *J* = 13.5, 4.1 Hz, 1H), 2.80 (s, 3H), 2.83 (td, *J* = 13.0, 4.1 Hz, 1H), 2.96 (td, *J* = 13.1, 4.9 Hz, 1H), 6.67 (d, *J* = 7.8 Hz, 1H), 6.82 (t, *J* = 7.4 Hz, 1H), 7.00 (d, *J* = 8.9 Hz, 1H), 7.12 (d, *J* = 7.6 Hz, 1H), 7.15 (d, *J* = 7.3 Hz, 1H), 8.04 (dd, *J* = 8.9, 2.8 Hz, 1H), 8.18 (d, *J* = 2.8 Hz, 1H), 11.26 ppm (s, 1H); ¹³C NMR (176 MHz, [D₆]DMSO): δ = 23.3, 23.9, 25.3, 30.7, 31.1, 46.8, 76.8, 108.7, 115.1, 118.5, 120.0, 121.5, 124.2, 126.0, 128.0, 128.2, 136.8, 139.5, 148.5, 161.2 ppm; IR (KBr): 3381 (OH), 3081, 2970, 2222 (C≡N), 1525 (NO₂, asymm), 1340 cm^{−1} (NO₂, symm); MS (ESI): *m/z* (%): 352 [M+H]⁺ (100); Anal. calcd for C₂₀H₂₁N₃O₃: C 68.36, H 6.02, N 11.96; found: C 68.56, H 6.07, N 11.61.

Analytical procedures

For the measurement of UV/vis absorption, compounds **8a–h** were dissolved in a mixture of CH₃CN/phosphate buffer (Na₂HPO₄/NaH₂PO₄, 7.5 mM, pH 7.6) (19:1, v/v, 298 K). Each solution (0.1 mM) was transferred to a spectrophotometer quartz cell (0.5 cm light path length) and 0.025 mL 72 mM aq NaCN solution was added. This volume was negligible compared with the initial volume of the solution in the cell (1.8 mL). The mixtures were shaken, and the absorption was measured from 200 to 600 nm against a blank of CH₃CN/phosphate buffer (19:1, v/v, 298 K).

To construct a calibration curve of cyanide concentration versus the most sensitive absorption at 422 nm for **8a**, different cyanide solutions (25, 50, 75, 125, 250, 500 μL of 0.72 mM and 25, 125, 250, 375 μL of 3.6 mM) were added to 100 mL of 0.1 mM **8a** solution in CH₃CN/phosphate buffer. A 72 mM NaCN stock solution was prepared from NaCN and diluted to 36 mM, 3.6 mM and 0.72 mM. All of the added volumes of cyanide were negligible, with 0.875 mL as the highest volume, compared with the initial volume of the **8a** solution.

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