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Electronic supplementary information

The "turn-on" small molecule fluorescent sensor for determination of Al³⁺ ion in real samples: theoretical calculations, photophysical and electrochemical properties

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Experimental

Material and Apparatus

Ethyl bromoacetate (99 %), tetrahydrofuran (anhydrous, ≥99.9%) trimethylamine (99%), absolute ethanol, hydrazine hydrate monohydrate (99%), 2-hydroxy-1-naphthaldehyde (99%), deuterated dimethyl sulfoxide (DMSOd₆), tri-, di- and monovalent metal ions (as their nitrate salts), anions (as their sodium salts), other solvents (analytical grade) were purchased from Sigma-Aldrich (USA). The steady-state fluorescence measurements, real samples analysis, and absorption measurements were carried out with Varian Cary Eclipse spectrofluorometer (USA) and Shimadzu 2101-UV spectrophotometer (Japan). Agilent 6530 Q-TOF LC/MS mass (USA) and Microflex spectrometers (Bruker Daltonics, USA) were performed to high resolution (HR-MS) and mass (MALDI-TOF) spectra of compound 3 and its complex. NMR (¹H, ¹³C) was recorded with INOVA 500 MHz spectrometer (Varian, USA). The spectroscopic quartz cuvette which has 10 mm pathlength was used for all molecular spectroscopic measurements where 5 nm was selected as slit width. The Horiba Jobin Yvon SPEX Fluorolog 3-2iHR (France) which has a charge-coupled device (CCD) and photomultiplier tube (R928) detectors were used for recording EEM analysis, 3D-fluorescence, and timeresolved fluorescence measurements. The signal acquisition was conducted by Single Photon Counting Controller (TCSPC, Fluoro Hub-B, France). The source of excitation for time-resolved fluorescence measurements was NanoLED (France) which excited samples at 390 nm. An electrochemical analyzer (USA, CH Instruments 842B) was used to perform CV and SWV voltammetries, which consisted of glassy carbon (GCE), counter electrode (Pt wire), and reference electrodes (Ag/AgCl). Tetrabutylammonium hexafluorophosphate (TBAPF₆, 0.10 mol dm⁻³) was used as the supporting electrolyte in voltammetric measurements which were performed in high purity of dimethylformamide (DMF) and nitrogen to deoxygenate the solution at least 10 minutes prior to each run at room temperature.

2.2. Photophysical calculations

The 3D-fluorescence and EEM analyses of **3** and **3** + AI^{3+} were measured in water:EtOH (1:4 v/v) at pH 6.0 via Horiba-Jobin-Yvon-SPEX with CCD detector. The time-resolved fluorescence measurements of **3** and **3** + AI^{3+} were recorded with TCSPC module (PMT detector) and the fluorescence lifetimes of samples were calculated directly via appropriate exponential calculations. The average lifetime of the charge carriers is estimated by using equation S1.¹

$$\tau_a = \frac{\sum a_i \tau_i^2}{\sum a_i \tau_i} \quad \text{(Eq. S1)}$$

where t_i demonstrates decay time constant and α_i is the pre-exponential factor corresponding to the i_{th} . The quinine sulfate dissolved in 0.1 M H₂SO₄ and used as a standard (ϕ_{Fstd} =0.54) for calculation of fluorescence quantum yields of **3** and **3** + Al³⁺ via equation S2.^{2, 3}

$$\phi F = \phi F_{Std} \frac{F.A_{Std}.n^2}{F_{Std}.A.n_{Std}^2}$$
(Eq. S2)

where F, F_{Std} , A, A_{Std} are fluorescence bands areas, absorbance values at the excitation wavelength of sample and standard, respectively while n represented refractive indices of solvents. The radiative rate constant (K_r) and non-radiative rate constants (K_{nr}) were determined by using equations S3 and S4.¹

$$K_r = \Phi_f / \tau$$
 (Eq. S3)
 $\tau^{-1} = K_r + K_{nr}$ (Eq. S4)

2.3. Theoretical calculations

DFT computations were carried with Gaussian 09 package to obtain the optimized structure of **3** and its AI^{3+} complex.⁴ Optimized geometries of **3** and its complex with AI^{3+} were obtained by Becke, 3-parameter, Lee-Yang-Parr (B3LYP)⁵ with a basis set of 6-31G (d, p) for describing the complex formation and support the sensing

mechanism. The energy values of LUMO and HOMO were calculated by the single point time-dependent DFT (TD-DFT) method at similar levels by performing the B3LYP/6-31G (d, p).

Preparation of target fluorescent sensor (3)

To access the target ligand, 1H-benzotriazol-I-acetic acid hydrazide (2) was synthesized by previously described methods.⁶ The fluorescent sensor **(3)** was also a known compound that was synthesized via conventional method before by Youhong and co-workers.⁷ However, in this presented study, it was synthesized using ultrasound sonication as a green method to both reduce the reaction time and increase the yield. For this purpose, the solution of compound 2 (10.00 mmol) and 2-hydroxy-1-naphthaldehyde (10.00 mmol) in ethanol (5 mL) was sonicated at 50 °C with the addition of 1-2 drops of acetic acid (glacial) at 40 kHz for 35 min (The reaction progress was followed by TLC). After evaporating the solvent under reduced pressure, a yellow solid was obtained, and it was recrystallized from methanol to afford the desired compound (Scheme 1). The results demonstrated that approximately 11-fold reduction in reaction time and a modest increase in reaction yield (Table S1). All synthesized compounds **(1-3)** were characterized by ¹H NMR and ¹³C NMR spectroscopic techniques. Further, the target compound **(3)** was also confirmed through the HR-MS technique. All spectroscopic results were found to be compatible with the literature (Fig. S1-S7).

The general spectrofluorimetric procedure for determination of Al³⁺ ions

The stock solution of **3** (25.00 mmol.L⁻¹), metal ions (from their nitrate salts, 1.00 mmol.L⁻¹), anions (from their sodium salts, 1.00 mmol.L-1), and single molecules (oleic, ascorbic, gallic, caffeic, citric acids, glucose, fructose, sucrose) were prepared with absolute ethanol or double deionized water. The relative fluorescence signal response of **3** at 454 nm was selected for the spectrofluorimetric determination of Al³⁺ ion content in real samples. The waters samples were collected from Istanbul and urine sample was collected from volunteer adult. sample was Briefly, 2.00 mL of 3, 0.8 mL real sample, and 0.2 mL buffer at pH 6.0 were added to 5.00 mL volumetric flask. After, the final volume of the flask was adjusted to 5.00 mL with absolute ethanol, and prior to determination processes obtained mixture was strongly stirred for 20 seconds. As can be seen from Scheme 1, although 3 has a naphthalene moiety, the faint fluorescence signal of 3 can be attributed to PET and ESIPT mechanism between imine (-C=N-) bond, naphthalene, and phenolic -OH. After treatment with real samples, obtained "turn-on" fluorescence response at 454 nm was used for spectrofluorimetric determination. The fluorescence "turn-on" response of 3 was proportionally increased between 1.00-20.00 µmol.L⁻¹ with a LOD of 0.34 µmol.L⁻¹ which pointed out that the trace level of Al³⁺ can be determined with "turn-on" response change of **3** in real samples. The accuracy of the novel determination method was carried out with the spike/recovery test and the analysis of certificated reference material (CRM-TMDW-500). Finally, paper-based analysis kits were developed and applied for the recognition of Al³⁺ ions as easily and rapid in-field applications.



Fig. S1. ¹H NMR spectrum of compound 1.



Fig. S2. ¹³C NMR spectrum of compound 1.



Fig. S3. ¹H NMR spectrum of compound 2.



Fig. S4. ¹³C NMR spectrum of compound 2.



Fig. S5. ¹H NMR spectrum of compound 3.



Fig. S6. ¹³C NMR spectrum of compound 3.

Sample Group		Info.	
Stream Name LC 1		Acquisition SW	6200 series TOF/6500 series O-TOE B 08 00 (B8058 0)
		V CI SIGII	Q 101 D.00.00 (D0000.0)

User Spectra



Fig. S7. HR-MS spectrum of compound 3.



Fig. S8. UV-Vis spectra of 3 in various solvent systems: water (a), DMF (b), DMSO (c), ethanol (d), ACN (e), dichloromethane (f), THF (g), hexane (h) and normalized absorption (i) spectra of 3 in various solvents.



Fig. S9. Fluorescence spectra of 3 in various solvent systems: water (a), DMF (b), DMSO (c), ethanol (d), ACN (e), dichloromethane (f), THF (g) and hexane (h).



Fig. S10. Optimized structure (a), HOMO (b) and LUMO (c) orbitals of 3



Fig. S11. Identification of optimal pH (a), initial sensor concentration (b), interaction time (c), and photostability (d) in water: EtOH (1:4 v/v, pH: 6.0, λ_{ex} : 365).



Fig. S12. UV-Vis spectra of compound **3** (**a**) and fluorescence signal change of $\mathbf{3} + Al^{3+}(\mathbf{b})$ at different water:EtOH ratios.



Fig. S13. Job's plot (a), Benesi-Hildebrand analysis (b), MALDI-TOF spectrum (c) of 3 after treatment with Al³⁺ ions in water:EtOH (1:4 v/v, pH:6.0, λ_{ex} = 365 nm) and ¹H NMR spectra (d) of 3 with Al³⁺ ion in DMSO-d₆.



Fig. S14. UV-Vis absorption spectra of 3 and its Al^{3+} complex were obtained by TD-DFT calculation.

Comp.	Method	Time	Yield	Temp.	m.p.	Ref
Name		(min)	(%)	(°C)	(°C)	
HNBTH	Conventional	300	92	Reflux	228-230	[36]
3	Ultrasonication	35	97	50	229-230	TW*

 Table S1. The comparison of known and novel methods.

*This Work

		e (L.mol ⁻¹ .cm ⁻¹) x10 ³									
λ_{abs}	λ_{ems}	*Wate	DMS	DM	EtOH	AC	DC	TH	Hxn	$\Phi_{\rm F}$	$\tau_a(ns)$
(nm)	(nm	r	0	F		Ν	Μ	F			
)										
371,	-	0.84	19.7	12.5	14.4	17.8	12.9	14.0	1.7	0.00	3.74 ± 0.0
359,										9	3
323,											
310											

Table S2: Photophysical properties of **3**.

* DMF; dimethylformamide, DMSO; dimethyl sulfoxide, EtOH, ACN, acetonitrile; DCM, dichloromethane; THF, Dxn, 1,4-dioxane; tetrahydrofuran; ethanol; Hxn, n-hexane;

Parameter	Value		
pH	6.0		
Sensor concentration (µmol.L ⁻¹)	50		
Measurement volume (mL)	5		
Working solvent	water:EtOH		
Interaction time (second)	20		
Linear range (µmol.L ⁻¹)	1.00-20.00		
Limit of detection (LOD) (µmol.L ⁻¹)	$0.34 (9.17 \ \mu g.L^{-1})$		
Limit of quantification (LOQ) (µmol.L ⁻¹)	1.02 (27.51 µg.L ⁻¹)		
Precision (RSD%)	2.98		
Correlation coefficient (R ²)	0.9945		
Excitation wavelength (nm)	365		
Emission wavelength (nm)	454		

Table S3. Optimum conditions and analytical parameters for determination of Al^{3+} with **3**.

Table S4: Application of the presented spectrofluorimetric method to the certified reference material (N = 3).

	CRM TMDW-500 Drinking Water						
Analyte	Certificated value (µg.L ⁻¹)	Found value (µg.L ⁻¹)	Recovery (%)				
Al ³⁺	120.0±0.6	117.8±3.2	98.17				

Table S5: Statistical Evaluation of accuracy by Student t-test.

	\$	X _R	\overline{X}	$t_{\text{exp.}} = \frac{\left X_R - \overline{X}\right }{s/\sqrt{N}}$	t _{ref.}	Results
A 13+	2.2	120.0	1170	1 10	4.2	0.19<4.3
Al	3.2 120.0	11/.8	1.19	4.3	(acceptable)	

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