Electronic Supplementary Material (ESI) for ChemComm.

ESI

for

Ratiometric determination of etomidate based on an albumin-based indicator displacement assay (IDA)

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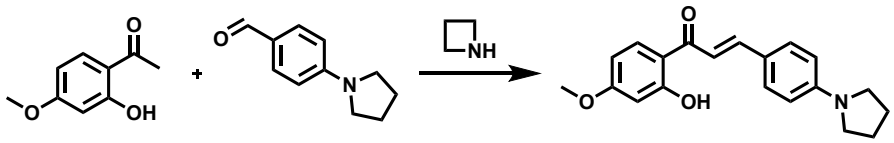
1. Materials and Instruments

Chemical reagents (AR grade) and solvents (HPLC grade) used in this study were purchased from J&K, Energy Chemical, Alfa Aesar, and Sigma-Aldrich, used directly without further purification. Albumin (ALB) and amino acids were purchased from Sigma-Aldrich. Phosphate buffered saline (PBS, 10×, pH ~7.4) was purchased from J&K scientific (Beijing, China). The fluorescent spectra were recorded using the Thermo-fisher Evolution 220 and Thermo Lumina spectrometer, respectively. Fluorescent images were captured by a smartphone in a dark box (Ultraviolet Analyzer). The RGB values of points in each color range were determined by the ImageJ software or phone application "color recognition".

2. Methods

2.1 Synthesis

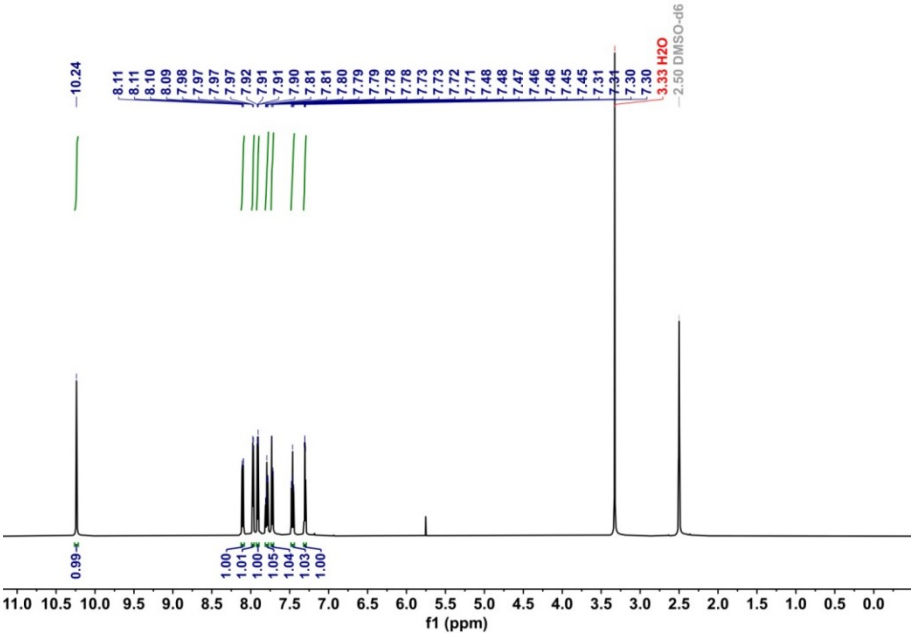


Dye 1

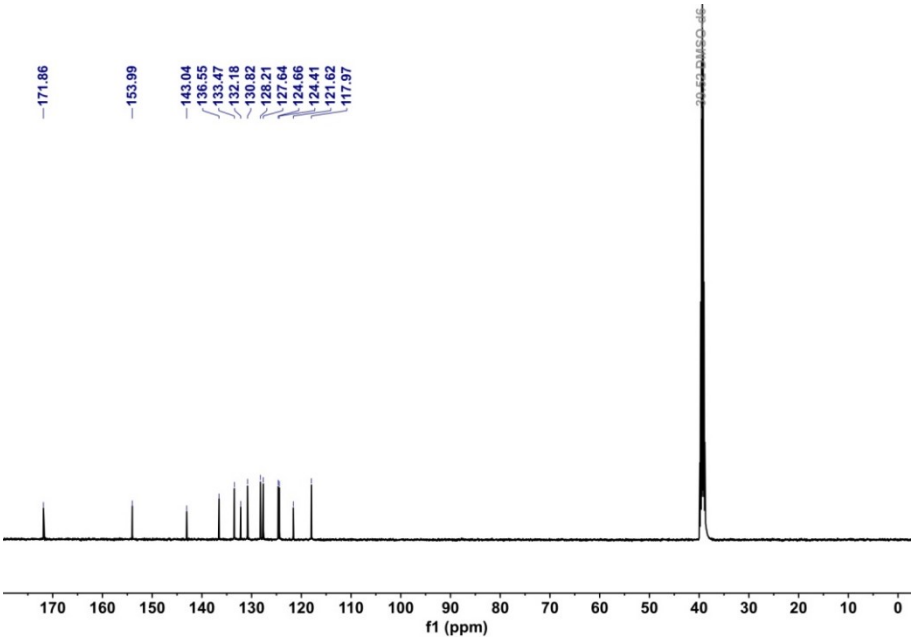
Synthesis of Dye 1: it was prepared according to the previous study[1].

Synthesis of Dye 2: The 2′-hydroxyacetonphenone (5 mmol) and aryl aldehyde (5 mmol) were dissolved in 50 mL of ethanol, and then potassium hydroxide solution (1 g/mL, 3.5 mL) were dropwise added into the mixture. The reaction mixture was reflux for 24 h. Next, the mixture was placed in an ice-water bath and was slowly added with 10 mL of 30% H_2O_2. The mixture was stirred for 12 h and subsequently was neutralized with HCl (1 M). The precipitate was collected *via* filtration, washed with ethanol, and then dried in the vacuum oven. The pure products were obtained by using the column chromatography. ^{1}H NMR (500 MHz, DMSO-d_6): 10.24 (s, 1H), 8.10

(dd, J = 8.0, 1.7 Hz, 1H), 7.97 (dd, J = 3.8, 1.2 Hz, 1H), 7.91 (dd, J = 5.0, 1.2 Hz, 1H), 7.79 (ddd, J = 8.6, 6.9, 1.7 Hz, 1H), 7.72 (dd, J = 8.5, 1.1 Hz, 1H), 7.46 (ddd, J = 8.0, 6.9, 1.1 Hz, 1H), 7.30 (dd, J = 5.0, 3.8 Hz, 1H). ^{13}C NMR (125 MHz, DMSO-d_6): 171.86, 153.99, 143.04, 136.55, 133.47, 132.18, 130.82, 128.21, 127.64, 124.66, 124.41, 121.62, 117.97.



^{1}H NMR spectrum of Dye 2



^{13}C NMR spectrum of Dye 2

Synthesis of Dye 4: Dye 4 was synthesized and characterized based on our previous work (*Sens. Actuators B-Chem.,2021, 345, 130367*)

2.2 Construction of D1@ALB

D1@ALB solution (10 μM) was prepared by mixing 2 μL of Dye1 (10 mM in DMSO) and 40 μL of ALB (0.5 mM in water) into 2 mL of PBS buffer (1 mM, pH ~7.4). The mixtures were shaken for 30 s to complete the binding process and kept in steady for another 30 s before use.

2.3 Basic testing methods

Fluorescent spectra of Dye 1 (10 μM) and D1@ALB (10 μM) in phosphate-buffered saline (PBS, 1 mM, pH ~ 7.4) were measured under 400 nm excitation. Fluorescent spectra of Dye 2 (10 μM) and D2@ALB (10 μM) in phosphate-buffered saline (PBS, 1 mM, pH ~ 7.4) were measured under 380 nm excitation. Fluorescent spectra of Dye 3 (10 μM) and D3@ALB (10 μM) in phosphate-buffered saline (PBS, 1 mM, pH ~ 7.4) were measured under 420 nm excitation. Fluorescent spectra of Dye 4 (10 μM) and D4@ALB (10 μM) in phosphate-buffered saline (PBS, 1 mM, pH ~ 7.4) were measured under 420 nm excitation. Fluorescent titration experiments were measured by adding increasing concentrations of ET into 1 mL of D1@ALB solution (10 μM). Samples in anti-interference tests were prepared by directly dissolving accurate amounts of ions, amino acids and enzymes into testing solutions.

2.4 Molecular docking

The 3D geometry of ligand was energy minimized in Chem3D by working with mm2. The ligand-free crystal structure of albumin (PDB ID: 4K2C) was taken from the Brookhaven Protein Data Bank (http://www.rcsb.org/pdb). Flexible ligand docking was

performed by AutoDock 4.2 molecular docking program using the implemented empirical free energy function and the Lamarckian Genetic Algorithm. The Autogrid was used to calculate Grids. The grid spacing was 0.375 Å as default. 20 docking runs with 25,000,00 energy evaluations were performed. Binding energy of each complex was recorded directly from AutoDock. The output from AutoDock was rendered with PyMol.

2.5 Limit of detection

The limit of detection (LOD) for ET was calculated by using $3\sigma/\kappa$ rule based on ET titration experiments. Where σ is the standard deviation of blank measurement for ten times and κ is the slope of the fluorescent intensity plotted against the ET concentration.

2.6 Analysis of real samples

Beverages samples (Sprite, Cola, Beer) were acquired from a local supermarket. Before the test, the samples (1 mL) were diluted tenfold with 10 mL PBS buffer (1 mM, pH ~7.4). After adding with different concentration of ET, the samples (2 mL) were measured by adding 20 μL of D1@ALB (1 mM).

Commercial vape juice samples were purchased from a local supermarket. Their autofluorescence were measured directly by the fluorometer without any pretreatment. In the case of the treated vape juice samples, 3 mL of the sample underwent slow filtration through a polyacrylamide (PMA) filter column and silicone filter column to eliminate the autofluorescence in vape juice. Then, the vape juice samples (50 μL) were then diluted tenfold with 10 mL PBS buffer (1 mM, pH ~7.4). Finally, the samples (2 mL) were tested by adding 20 μL of D1@ALB (1 mM).

3. Additional Figures

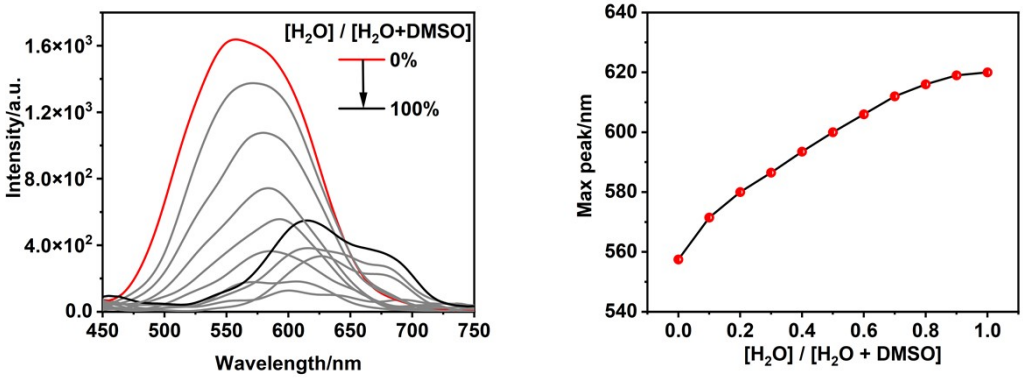


Fig. S1 Fluorescence spectra (left) and intensity peak (right) of the dye 1 in the water/DMSO mixture with different ratios.

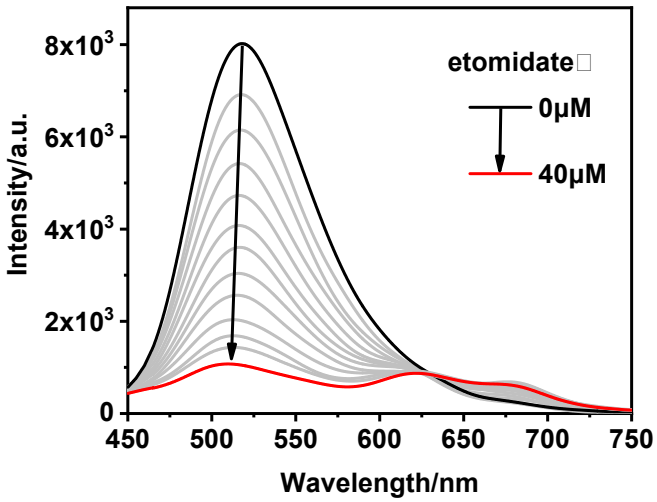


Fig. S2 Fluorescence spectra of D1@ALB (1 μM) upon addition of the different concentrations of etomidate (0-40 μM). Excitation wavelength: 400 nm.

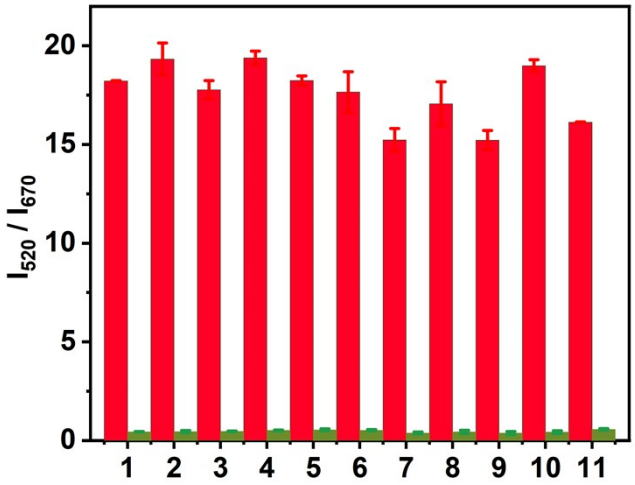


Fig. S3 Intensity ratios of D1@ALB in the presence (green bars) and absence (red bars) of etomidate (200 μM) co-existing with different components. Components No. 1-11: control, glucose, lactose, *D*-glycan, sucrose, epigallocatechin, epicatechin, epigallocatechin gallate, epicatechin gallate, BHT, and β-chlorophyll. Excitation wavelength: 400 nm. [D1@ALB] = 10 μM, Error bars: ±SD for three measurements.

Fig. S4 (a) The equipment component of device and the photographs of real device. (b) Illustration of analytical procedure of portable miniature device.

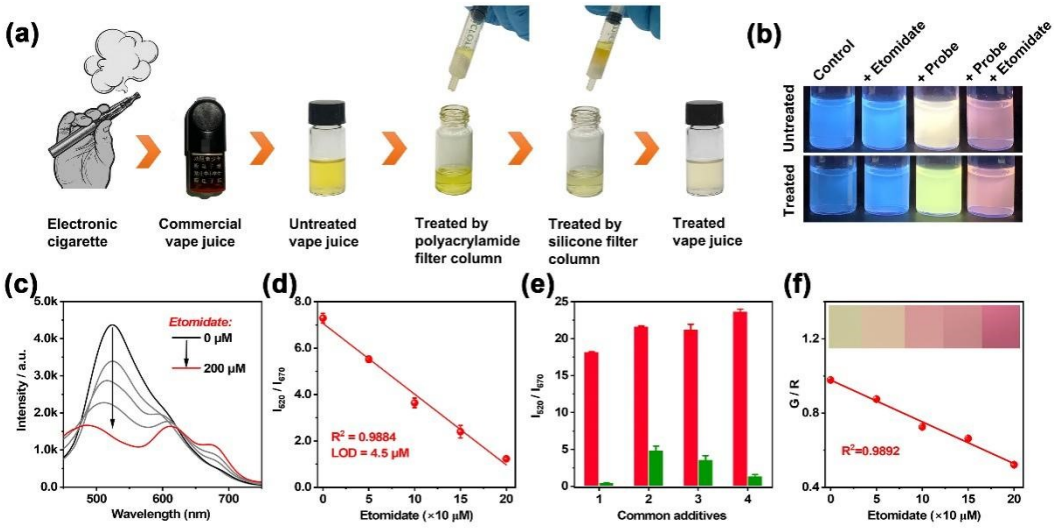


Fig. S5 (a) Illustration the treatment procedure of commercial vape juice. (b) fluorescence photographs of testing vape juice with and without treatment. (c) fluorescence spectra of D1@ALB with different etomidate in treated vape juice. (d) Relationship between the intensity ratio (I_{520} / I_{670}) and the concentration of etomidate in treated vape juice. (e) Intensity ratio (I_{520} / I_{670}) of D1@ALB (10 μM) in the presence (green bars) and absence (red bars) of etomidate (200 μM) co-existing with different common additives (200 μM). Additives No. 1-4: control, propylene glycol, nicotine, glycerol. (f) The ratio G/R value of vape juice with different concentrations of etomidate (0, 50, 100, 200 μM) by using the portable miniature device. Inset: photographs of the readout result from miniature device.

References

[1] C. Song, C. Zeng, T. Qin, T. Lv, Z. Xu, Z. Xun, L. Wang, X. Chen, B. Liu, X. Peng, Chemical Engineering Journal 468 (2023).