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Iridium(III) complex-based electrochemiluminescent probe for H_2S†

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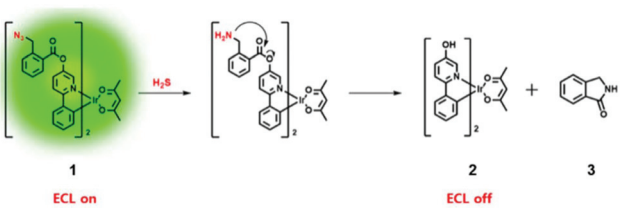
Since abnormal levels of hydrogen sulphide (H_2S) correlate with various diseases, simple methods for its rapid and sensitive detection are highly required. Herein, we introduce a new electrochemiluminescent probe **1** for H_2S based on a cyclometalated iridium(III) complex. *o*-(Azidomethyl)benzoate ester groups on the main ligands of probe **1** react selectively with H_2S, resulting in cascade reactions involving H_2S-mediated reduction and intramolecular cyclization/ester cleavage. With this structural change induced by H_2S, the intrinsic electrochemiluminescence (ECL) of **1** decreased greatly due to the unfavourable electron transfer of a tripropylamine (TPA) radical. Probe **1** showed a high ECL turn-off ratio and good selectivity for H_2S over various anions and biothiols. The sensing mechanism of H_2S was elucidated using ^{1}H NMR spectroscopy and MALDI-TOF mass spectrometry analyses.

Introduction

Hydrogen sulphide (H_2S) is a toxic gaseous molecule with a rotten egg smell. However, it is recognized as an important signalling molecule, along with nitric oxide (NO) and carbon monoxide (CO),[1] because it is involved in various physiological processes, including angiogenesis,[2] apoptosis,[3] vasodilation,[4] oxygen sensing,[5] inflammation,[6] and neuromodulation.[7] Endogenous H_2S is synthesized from L-cysteine, assisted by several enzymes like cystathionine β-synthase (CBS), cystathionine γ-lyase (CSE), and 3-mercaptopyruvate sulphur transferase (3-MST).[8,9] In blood plasma, normal concentrations of H_2S range between 10 and 100 μM.[2,10] Abnormal levels of H_2S are related with several diseases like Alzheimer's diseases,[11] Down's syndrome,[12] diabetes,[13,14] and liver cirrhosis.[15] Therefore, simple, rapid, and reliable detection methods for H_2S are required for the diagnosis of such diseases. To date, several H_2S detection methods have been reported, such as gas chromatography,[16] electrochemical analysis,[17] and metal nanoparticle sensors,[18] as well as fluorescence[19] and colorimetric assays.[20] In particular, fluorescent chemodosimeters for H_2S were developed based on nucleophilic,[21,22] reducing,[23,24] or transition metal-induced precipitation[25] properties of sulphide. Despite their good sensing properties, these probes require bulky light sources, and thus, cannot be used for point-of-care testing (POCT).

In this regard, electrochemiluminescence (ECL), coupled with chemodosimetry, provides many advantages, allowing simple analysis in a time- and cost-effective manner. ECL is a luminescent process involving electron transfer by electrochemically generated radical species at the surface of the working electrode.[26] Since ECL can be generated in the absence of light sources, it exhibits superior sensitivity without any background signal, compared to a conventional photoluminescence method. Moreover, it can be miniaturized for POCT applications.[26,27] Meanwhile, Ir(III) complexes have received increasing attention as ECL luminophores with good electrochemical stabilities, high phosphorescence efficiencies, and easy tunabilities of the emission color by modulating the ligand structure.[28,29]

Herein, we report a new ECL chemodosimetric probe (**1**) for H_2S (Scheme 1) based on a cyclometalated iridium(III) complex. Probe **1**, $(AzMB\text{-}ppy)_2Ir(acac)$, has 2-phenylpyridine-5-yl-2-(azidomethyl)benzoate (AzMB-ppy, AzMB = (azidomethyl) benzoate) as the main ligand and acetylacetonate (acac) as the ancillary ligand. Treatment of probe **1** with H_2S resulted in chemical reduction of the azide moiety to the corresponding



Scheme 1 ECL H_2S sensing mechanism of probe **1**.

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amine, which underwent simultaneous intramolecular cyclization and ester cleavage to afford compound **2**.[24,30] The structural change of **1** upon chemical reaction with H_2S significantly quenched the ECL of **1**. In order to compare the reaction trend according to the substitution position of AzMB on the main ligand, we also synthesized analogues (**16**, **21**) of **1** and compared their fluorescence and ECL properties with those of probe **1**. This is the first example of ECL probe for H_2S detection, which utilizes the reducing property of the sulphide ion.

Experimental

Materials and instruments

All the reagents were purchased from Sigma-Aldrich Corp., (MO, USA), Alfa Aesar (MA, USA), or Tokyo Chemical Industry (Tokyo, Japan) and used without further purification. Deuterated solvents were acquired from CIL (Cambridge Isotopic Laboratories, MA, USA). Merck silica gel 60 F254 on aluminum foil was used for analytical thin layer chromatography. SiliaFlash® P60 (230–400 mesh) from SILICYCLE was used as the stationary phase in chromatographic separation. ^{1}H NMR and ^{13}C NMR were recorded on Bruker DPX-300 (300 MHz for ^{1}H NMR), Agilent 400-MR (400 MHz for ^{1}H NMR) and Varian/Oxford As-500 (125 MHz for ^{13}C NMR) spectrometers. Chemical shifts (δ) were reported in ppm (chloroform = $CDCl_3$, dimethyl sulfoxide = DMSO-d_6). Matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectrometry data were obtained using a Bruker Microflex MALDI-TOF mass spectrometer. Gas chromatography high-resolution mass spectrometry (GC-HRMS, JEOL, JMS-700) data were received directly from the National Centre for Inter-University Research Facilities (NCIRF). Absorption spectra were recorded on a JASCO V-730 UV-visible spectrophotometer. Fluorescence emission spectra were recorded on a JASCO FP-6500 spectrofluorometer. Solutions of probes (**1**, **16** and **21**) for all the photophysical and electrochemical experiments were prepared from 2 mM stock solution in DMSO and stored in a refrigerator for use. NaHS was dissolved in 10 mM HEPES buffer (pH 7.4) as the source of sulphide ions.

Electrochemical and electrochemiluminescent measurements

The electrochemical study was conducted using a CH Instruments 650B Electrochemical Analyzer (CH Instruments, Inc., TX, USA). Cyclic voltammetry (CV) was applied to individual solutions in order to investigate electrochemical oxidative and reductive behavior. The ECL signals were obtained using a low-voltage photomultiplier tube (PMT) module (H-6780, Hamamatsu Photonics K. K., Tokyo, Japan) operated at 1.1 V. A 250 μL-sized ECL cell was directly mounted on the PMT module with home-made support mounts during the experiments. All the ECL data were collected *via* simultaneous cyclic voltammetry (CV). The ECL solutions commonly contained 10 mM TPA (tripropylamine, Sigma-Aldrich, MO, USA) as a co-reactant and 0.1 M tetrabutylammonium hexafluorophosphate ($TBAPF_6$, TCI) as a supporting electrolyte in acetonitrile (CH_3CN, spectroscopy grade, ACROS). The electrochemical measurements were referenced with respect to a Ag/Ag^+ reference electrode with ferrocene. A Pt working electrode was polished with 0.05 M alumina (Buehler, IL, USA) on a felt pad followed by sonication in a 1 : 1 mixed solution of deionized water and absolute ethanol for 5 min. The working electrode was dried using ultra-pure N_2 gas for 1 min. None of the solutions were reused. The reported ECL values were obtained by averaging the values of at least three repeated experiments in CH_3CN/DMSO (5 : 1 v/v).

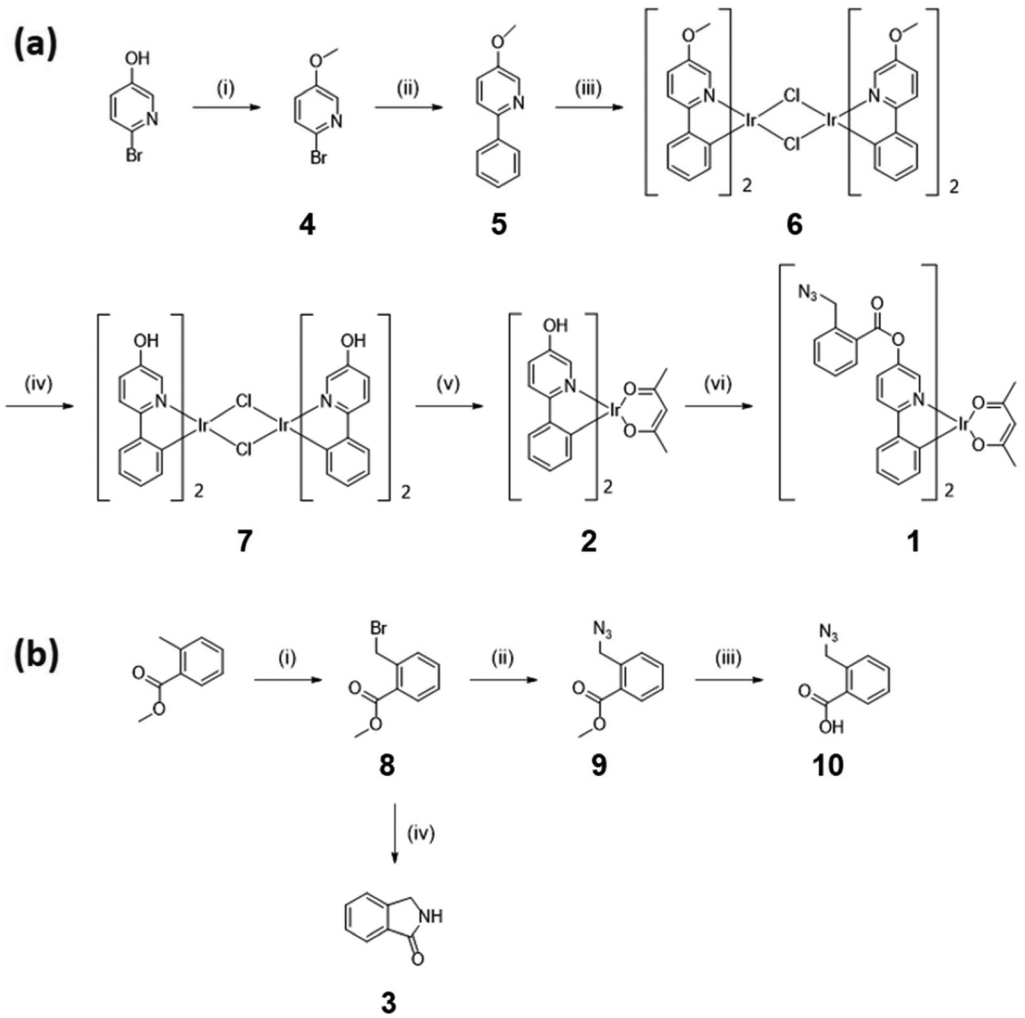
Synthetic procedures (Schemes 2 and 3)

Synthesis of 4. 2-Bromo-5-hydroxypyridine (1.74 g, 10 mmol) and K_2CO_3 (2.76 g, 20 mmol) were dissolved in DMF (80 mL). After stirring at room temperature for 20 min, iodomethane (0.94 mL, 15 mmol) was added. The mixture was stirred at room temperature for 6 h. The solvent was removed under reduced pressure and the reaction mixture was diluted with dichloromethane (DCM). The organic phase was washed with water and dried over anhydrous Na_2SO_4. The volatiles were removed under reduced pressure. The residue was purified by silica gel column chromatography using hexane/ethyl acetate (5 : 1 v/v) as the eluent to give a pale yellow liquid (1.51 g, 8.03 mmol, 80%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.08 (d, J = 3.0 Hz, 1H), 7.39 (d, J = 8.7 Hz, 1H), 7.12 (dd, J = 8.7, 3.1 Hz, 1H), 3.86 (s, 3H).

Synthesis of 5. A mixture of **4** (940 mg, 5 mmol), phenylboronic acid (671 mg, 5.5 mmol), K_2CO_3 (2.07 g, 15 mmol) and $Pd(PPh_3)_4$ (290 mg, 0.25 mmol) in THF (30 mL) and H_2O (30 mL) was refluxed for 7 h. After cooling to room temperature, the reaction mixture was extracted with DCM. The organic phase was washed with water and dried over anhydrous Na_2SO_4. The volatiles were evaporated under reduced pressure. The residue was purified by silica gel column chromatography with DCM/MeOH (100 : 1 v/v) as the eluent to give white solid (257 mg, 1.39 mmol, 60%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.42 (d, J = 2.8 Hz, 1H), 7.95 (d, J = 7.2 Hz, 2H), 7.69 (d, J = 8.7 Hz, 1H), 7.47 (t, J = 7.4 Hz, 2H), 7.41–7.36 (m, 1H), 7.31 (d, J = 2.9 Hz, 1H), 3.93 (s, 3H).

Synthesis of 6. A mixture of **5** (478 mg, 2.58 mmol) and iridium chloride hydrate (346 mg, 1.16 mmol) in 2-ethoxyethanol (30 mL) and H_2O (10 mL) was refluxed for 24 h. After cooling to room temperature, water (50 mL) was poured into the reaction mixture. The resulting green precipitate was filtered to give a crude cyclometalated Ir(III) chloro-bridged dimer (426 mg, 0.357 mmol, 62%), which was used in the next step without further purification. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.72 (d, J = 2.4 Hz, 2H), 9.24 (d, J = 2.6 Hz, 2H), 8.16 (d, J = 9.0 Hz, 2H), 8.05 (d, J = 8.9 Hz, 2H), 7.76 (dd, J = 9.0, 2.8 Hz, 2H), 7.69–7.62 (m, 4H), 7.58 (d, J = 7.9 Hz, 2H), 6.83 (t, J = 7.5 Hz, 2H), 6.77 (t, J = 7.3 Hz, 2H), 6.68 (t, J = 7.3 Hz, 2H), 6.62 (t, J = 7.4 Hz, 2H), 6.20 (d, J = 7.6 Hz, 2H), 5.60 (d, J = 7.4 Hz, 2H), 3.91 (d, J = 11.2 Hz, 12H).

Synthesis of 7. To a stirred solution of **6** (425 mg, 0.36 mmol) in DCM (5 mL) at 0 °C, BBr_3 (2.14 mL, 1.0 M in DCM) was added dropwise *via* syringe under N_2 atmosphere.

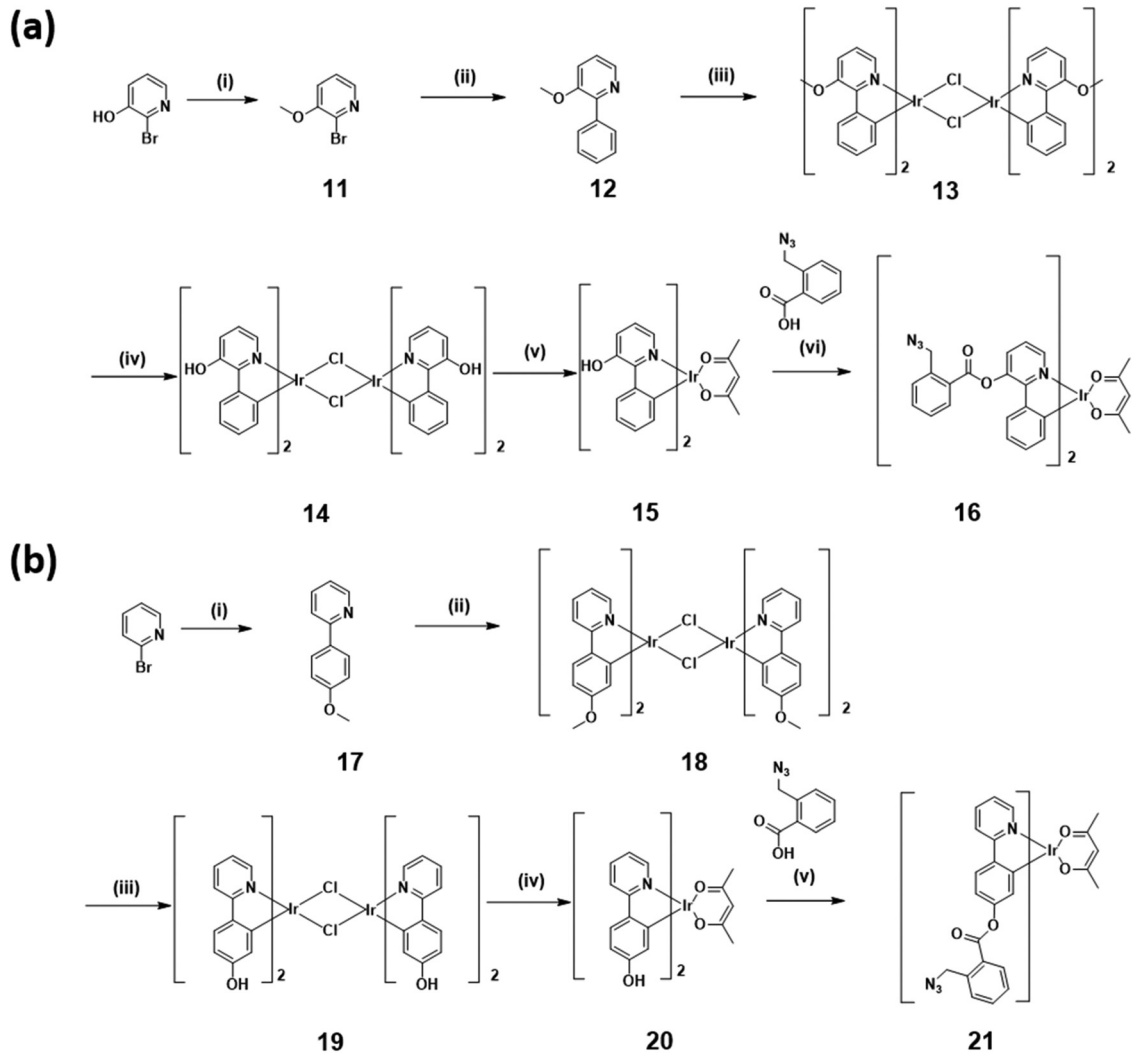


Scheme 2 (a) Synthesis of probe **1**, (i) K_2CO_3, CH_3I, DMF, r.t.; (ii) phenylboronic acid, $Pd(PPh_3)_4$, K_2CO_3, THF, H_2O, reflux; (iii) $IrCl_3{\cdot}xH_2O$, 2-ethoxyethanol, H_2O, reflux; (iv) BBr_3, DCM, 0 °C → r.t.; (v) acetylacetone, Na_2CO_3, 2-ethoxyethanol, 100 °C; (vi) $SOCl_2$, reflux; DCM, DIPEA, 0 °C → r.t. (b) Synthesis of 2-(azidomethyl)benzoic acid, (i) NBS, benzoyl peroxide, reflux; (ii) NaN_3, DMF, 70 °C; (iii) LiOH, THF, H_2O, r.t.; (iv) NH_3, MeOH, r.t.; (DMF = *N*,*N*-dimethylformamide, THF = tetrahydrofuran, DCM = dichloromethane, DIPEA = *N*,*N*-diisopropylethylamine, NBS = *N*-bromosuccinimide).

The reaction mixture was stirred at room temperature for 24 h, at which time ^{1}H NMR analysis indicates a complete disappearance of methoxy proton at δ 3.91. The reaction was quenched with water (50 mL) and the resulting green precipitate was filtered to give a crude cyclometalated Ir(III) chloro-bridged dimer (382 mg, 0.335 mmol, 94%), which was used in the next step without further purification. ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.80 (s, 2H), 10.53 (s, 2H), 9.49 (d, J = 2.1 Hz, 2H), 9.37 (d, J = 2.2 Hz, 2H), 8.06 (d, J = 8.9 Hz, 2H), 7.96 (d, J = 8.9 Hz, 2H), 7.59–7.43 (m, 8H), 6.86 (t, J = 7.4 Hz, 2H), 6.79–6.68 (m, 4H), 6.63 (t, J = 7.3 Hz, 2H), 6.16 (d, J = 7.5 Hz, 2H), 5.70 (d, J = 7.5 Hz, 2H).

Synthesis of 2. A mixture of 7 (421 mg, 0.37 mmol), acetylacetone (0.38 mL, 3.7 mmol) and Na_2CO_3 (392 mg, 3.7 mmol) in 2-ethoxyethanol (15 mL) was heated to 100 °C for 1.5 h. After cooling to room temperature, the reaction mixture was extracted with ethyl acetate. The organic phase was washed with water and dried over anhydrous Na_2SO_4. The volatiles were evaporated under reduced pressure. The residue was triturated with acetone and hexane, then the resulting green precipitate was filtered to give compound **2** (214 mg, 0.34 mmol, 46%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.44 (s, 2H), 8.04 (d, J = 2.3 Hz, 2H), 7.93 (d, J = 8.9 Hz, 2H), 7.48 (d, J = 7.5 Hz, 2H), 7.39 (dd, J = 8.8, 2.4 Hz, 2H), 6.70 (t, J = 7.3 Hz, 2H), 6.53 (t, J = 7.3 Hz, 2H), 6.02 (d, J = 7.4 Hz, 2H), 5.26 (s, 1H), 1.74 (s, 6H).

Synthesis of 1. To a stirred solution of **2** (80 mg, 0.13 mmol) in DCM (5 mL) at 0 °C, *N*,*N*-diisopropylethylamine (0.1 mL, 0.6 mmol) was added dropwise *via* syringe and stirred for 30 min. In a separate round-bottomed flask, compound **10** (180 mg, 1 mmol) was refluxed in thionyl chloride (0.37 mL, 5.1 mmol) for 2 h and then residual thionyl chloride was removed under reduced pressure to give 2-(azidomethyl) benzoyl chloride. The residue dissolved in DCM (5 mL) was added dropwise to a stirred solution of **2** prepared above. The



Scheme 3 (a) Synthesis of probe **16**, (i) K_2CO_3, CH_3I, DMF, r.t.; (ii) phenylboronic acid, $Pd(PPh_3)_4$, K_2CO_3, THF, H_2O, reflux; (iii) $IrCl_3{\cdot}xH_2O$, 2-ethoxyethanol, H_2O, reflux; (iv) BBr_3, DCM, 0 °C → r.t.; (v) acetylacetone, Na_2CO_3, 2-ethoxyethanol, 100 °C; (vi) $SOCl_2$, reflux; DCM, DIPEA, 0 °C → r.t. (b) Synthesis of probe **21** (i) (4-methoxyphenyl)boronic acid, $Pd(PPh_3)_4$, K_2CO_3, THF, H_2O, reflux; (ii) $IrCl_3{\cdot}xH_2O$, 2-ethoxyethanol, H_2O, reflux; (iii) BBr_3, DCM, 0 °C → r.t.; (iv) acetylacetone, Na_2CO_3, 2-ethoxyethanol, 100 °C; (v) $SOCl_2$, reflux; DCM, DIPEA, 0 °C → r.t.; (DMF = *N*,*N*-dimethylformamide, THF = tetrahydrofuran, DCM = dichloromethane, DIPEA = *N*,*N*-diisopropylethylamine).

reaction mixture was stirred at room temperature for 2 h and extracted with DCM. The organic phase was washed with water and dried over anhydrous Na_2SO_4. The volatiles were evaporated under reduced pressure. The residue was purified by silica gel column chromatography with hexane/ethyl acetate (5 : 1 v/v) as the eluent to give a yellow solid. (62 mg, 0.065 mmol, 52%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.55 (d, J = 2.1 Hz, 2H), 8.32 (d, J = 7.7 Hz, 2H), 7.92 (d, J = 8.9 Hz, 2H), 7.78–7.67 (m, 4H), 7.60 (dd, J = 20.7, 7.5 Hz, 6H), 6.87 (t, J = 7.3 Hz, 2H), 6.78 (t, J = 7.2 Hz, 2H), 6.38 (d, J = 7.4 Hz, 2H), 5.31 (s, 1H), 5.00–4.85 (m, 4H), 1.84 (s, 6H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 185.02, 166.60, 164.19, 146.99, 145.27, 143.85, 141.38, 138.62, 133.97, 132.99, 131.81, 130.72, 130.05, 129.33, 128.43, 126.89, 124.04, 120.98, 118.41, 100.80, 53.12, 28.82; HRMS (FAB^+, m-NBA): *m*/*z*: observed 950.2151 (calculated for $C_{43}H_{33}IrN_8O_6$ $[M]^+$ 950.2155).

Synthesis of 8.[31] Methyl 2-methylbenzoate (1.5 g, 10 mmol), NBS (1.96 g, 11 mmol) and benzoyl peroxide (48 mg, 0.2 mmol) were dissolved in $CHCl_3$ (50 mL) and refluxed for 5 h. The reaction mixture was cooled to room temperature and the organic phase was washed with saturated aqueous sodium thiosulfate solution and dried over anhydrous Na_2SO_4. The solvent was evaporated under reduced pressure. The residue was purified by silica gel column chromatography with hexane/acetone (5 : 1 v/v) as the eluent to give a pale yellow liquid (1.98 g, 8.7 mmol, 87%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.99 (d, J = 7.9 Hz, 1H), 7.56–7.47 (m, 2H), 7.43–7.36 (m, 1H), 4.98 (s, 2H), 3.97 (s, 3H).

Synthesis of 9.[31,32] A mixture of **8** (1.65 g, 7.25 mmol) and sodium azide (945 mg, 14.5 mmol) in DMF (50 mL) was heated to 70 °C with stirring for 24 h. The solvent was then removed under reduced pressure and the reaction mixture was

diluted with ethyl acetate. The organic phase was washed with water and dried over anhydrous Na_2SO_4. Ethyl acetate was evaporated under reduced pressure. The residue was purified by silica gel column chromatography with hexane/ethyl acetate (7 : 1 v/v) as the eluent to give a pale yellow liquid (1.27 g, 6.65 mmol, 92%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.04 (d, J = 7.7 Hz, 1H), 7.62–7.49 (m, 2H), 7.43 (t, J = 7.5 Hz, 1H), 4.84 (s, 2H), 3.95 (s, 3H).

Synthesis of 10.[31,32] A mixture of **9** (1.27 g, 6.65 mmol) and lithium hydroxide monohydrate (1.4 g, 33.3 mmol) in THF (40 mL) and H_2O (4 mL) was stirred at room temperature for 24 h. The reaction mixture was diluted with water, extracted with DCM, which was discarded. The aqueous phase was acidified with 2 N HCl, and extracted with DCM. The combined organic phases were washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated to give a white solid (1.15 g, 6.50 mmol, 98%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.19 (d, J = 7.7 Hz, 1H), 7.68–7.56 (m, 2H), 7.48 (t, J = 7.5 Hz, 1H), 4.91 (s, 2H).

Synthesis of 3.[33] To a solution of **8** (50 mg, 0.22 mmol) in methanol (5 ml), 2 M of ammonia solution in methanol (30 ml) was added and stirred at room temperature for 3 h. The reaction mixture was concentrated under reduced pressure. The residue was purified by silica gel column chromatography with hexane/ethyl acetate (1 : 1 v/v) as the eluent to give a white solid (25 mg, 0.19 mmol, 86%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.90 (d, J = 8.1 Hz, 1H), 7.63–7.57 (m, 1H), 7.51 (dd, J = 6.9, 4.6 Hz, 2H), 7.35 (s, 1H), 4.50 (s, 2H).

Synthesis of 11. 2-Bromo-3-hydroxypyridine (2.61 g, 15 mmol) and K_2CO_3 (4.05 g, 30 mmol) were dissolved in DMF (150 mL). After stirring at room temperature for 30 min, iodomethane (1.40 mL, 22.5 mmol) was added. The mixture was stirred at room temperature for 6 h. The solvent was removed under reduced pressure and the reaction mixture was diluted with DCM. The organic phase was washed with water and dried over anhydrous Na_2SO_4. All volatiles were removed under reduced pressure. The residue was purified by silica gel column chromatography using hexane/ethyl acetate (5 : 1 v/v) as the eluent to give a pale yellow liquid (2.04 g, 10.84 mmol, 72%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.97 (dd, J = 4.6, 1.4 Hz, 1H), 7.21 (dd, J = 8.1, 4.6 Hz, 1H), 7.13 (dd, J = 8.1, 1.4 Hz, 1H), 3.90 (s, 3H).

Synthesis of 12. A mixture of **11** (1.13 g, 6 mmol), phenylboronic acid (805 mg, 6.6 mmol), K_2CO_3 (2.48 g, 18 mmol) and $Pd(PPh_3)_4$ (348 mg, 0.18 mmol) in THF (50 mL) and H_2O (50 mL) was refluxed for 7 h. After cooling to room temperature, the reaction mixture was extracted with DCM. The organic phase was washed with water and dried over anhydrous Na_2SO_4. All volatiles were evaporated under reduced pressure. The residue was purified by silica gel column chromatography with DCM/MeOH (100 : 1 v/v) as the eluent to give white solid (800 mg, 4.32 mmol, 72%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.32–8.21 (m, 1H), 7.89 (d, J = 7.3 Hz, 2H), 7.39 (t, J = 7.5 Hz, 2H), 7.31 (t, J = 7.3 Hz, 1H), 7.11 (dt, J = 8.3, 6.0 Hz, 2H), 3.69 (s, 3H).

Synthesis of 13. A mixture of **12** (500 mg, 2.69 mmol) and iridium chloride hydrate (322 mg, 1.07 mmol) in 2-ethoxyethanol (30 mL) and H_2O (10 mL) was refluxed for 24 h. After cooling to room temperature, water (50 mL) was poured into the reaction mixture. The resulting yellow precipitate was filtered to give a crude cyclometalated Ir(III) chloro-bridged dimer (426 mg, 0.36 mmol, 67%), which was used in the next step without further purification.

Synthesis of 14. To a stirred solution of **13** (325 mg, 0.27 mmol) in DCM (5 mL) at 0 °C was added dropwise BBr_3 (1.63 mL, 1.0 M in DCM) *via* syringe under N_2 atmosphere. The reaction mixture was stirred at room temperature for 24 h. The reaction was quenched with water (50 mL) and the resulting green precipitate was filtered to give a crude cyclometalated Ir(III) chloro-bridged dimer (249 mg, 0.218 mmol, 80%), which was used in the next step without further purification.

Synthesis of 15. A mixture of **14** (400 mg, 0.35 mmol), acetylacetone (0.36 mL, 3.5 mmol) and Na_2CO_3 (371 mg, 3.5 mmol) in 2-ethoxyethanol (15 mL) was heated to 100 °C for 1.5 h. After cooling to room temperature, the reaction mixture was extracted with ethyl acetate. The organic phase was washed with water and dried over anhydrous Na_2SO_4. The volatiles were evaporated under reduced pressure. The residue was triturated with acetone and hexane, and then the resulting green precipitate was filtered to give compound **15** (150 mg, 0.23 mmol, 67%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.60 (d, J = 7.7 Hz, 2H), 7.80 (d, J = 4.9 Hz, 2H), 7.27 (d, J = 8.1 Hz, 2H), 7.00 (dd, J = 7.9, 5.6 Hz, 4H), 6.61 (t, J = 7.5 Hz, 2H), 6.43 (t, J = 7.3 Hz, 2H), 6.05 (d, J = 7.6 Hz, 2H), 5.14 (s, 1H), 1.64 (s, 6H).

Synthesis of 16. To a stirred solution of **15** (120 mg, 0.195 mmol) in DCM (10 mL) at 0 °C, *N*,*N*-diisopropylethylamine (0.15 mL, 0.9 mmol) was added dropwise *via* syringe and stirred for 30 min. In a separate round-bottomed flask, compound **10** (270 mg, 1.5 mmol) was refluxed in thionyl chloride (0.56 mL, 7.7 mmol) for 2 h and then residual thionyl chloride was removed under reduced pressure to give 2-(azidomethyl)benzoyl chloride. 2-(Azidomethyl)benzoyl chloride dissolved in DCM (10 mL) was added dropwise to a stirred solution of **15** prepared above. The reaction mixture was stirred at room temperature for 2 h and extracted with DCM. The organic phase was washed with water and dried over anhydrous Na_2SO_4. The volatiles were evaporated under reduced pressure. The residue was purified by silica gel column chromatography with hexane/ethyl acetate (5 : 1 v/v) as the eluent to give a yellow solid. (70 mg, 0.074 mmol, 39%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.56 (d, J = 5.5 Hz, 2H), 8.47 (d, J = 7.6 Hz, 2H), 7.87 (d, J = 8.0 Hz, 2H), 7.73 (d, J = 8.0 Hz, 2H), 7.68–7.64 (m, 4H), 7.59 (d, J = 7.0 Hz, 2H), 7.21 (d, J = 7.3 Hz, 2H), 6.65 (dd, J = 15.4, 7.7 Hz, 4H), 6.35 (d, J = 7.4 Hz, 2H), 5.25 (s, 1H), 4.90 (s, 4H), 1.83 (s, 6H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 184.94, 163.96, 161.37, 149.32, 146.25, 144.18, 143.75, 139.21, 134.16, 133.17, 132.47, 131.79, 130.03, 129.10, 128.57, 127.40, 121.15, 120.95, 100.48, 53.11, 30.86, 28.77; HRMS (FAB^+, m-NBA): *m*/*z*: observed 950.2147 (calculated for $C_{43}H_{33}IrN_8O_6$ $[M]^+$ 950.2155).

Synthesis of 17. A mixture of 2-bromopyridine (1.50 g, 9.49 mmol), (4-methoxyphenyl)boronic acid (1.59 g, 10.44 mmol), K_2CO_3 (4.33 g, 31.3 mmol) and $Pd(PPh_3)_4$

(329 mg, 0.28 mmol) in THF (50 mL) and H_2O (50 mL) was refluxed for 7 h. After cooling to room temperature, the reaction mixture was extracted with DCM. The organic phase was washed with water and dried over anhydrous Na_2SO_4. The volatiles were evaporated under reduced pressure. The residue was purified by silica gel column chromatography with DCM/MeOH (100 : 1 v/v) as the eluent to give white solid (1.1 g, 5.93 mmol, 63%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.67 (d, J = 4.7 Hz, 1H), 7.97 (d, J = 8.8 Hz, 2H), 7.84–7.63 (m, 2H), 7.20 (dd, J = 8.5, 3.2 Hz, 1H), 7.02 (d, J = 8.8 Hz, 2H), 3.89 (s, 3H).

Synthesis of 18. A mixture of **17** (200 mg, 1.08 mmol) and iridium chloride hydrate (128 mg, 0.43 mmol) in 2-ethoxyethanol (50 mL) and H_2O (5 mL) was refluxed for 24 h. After cooling to room temperature, water (30 mL) was poured into the reaction mixture. The resulting yellow precipitate was filtered to give a crude cyclometalated Ir(III) chloro-bridged dimer (153 mg, 0.13 mmol, 59%), which was used in the next step without further purification.

Synthesis of 19. To a stirred solution of **18** (100 mg, 0.08 mmol) in DCM (5 mL) at 0 °C, BBr_3 (0.5 mL, 1.0 M in DCM) was added dropwise *via* syringe under N_2 atmosphere. The reaction mixture was stirred at room temperature for 24 h. The reaction was quenched with water (30 mL) and the resulting green precipitate was filtered to give a crude cyclometalated Ir(III) chloro-bridged dimer (75 mg, 0.066 mmol, 78%), which was used in the next step without further purification.

Synthesis of 20. A mixture of **19** (100 mg, 0.09 mmol), acetylacetone (0.09 mL, 0.88 mmol) and Na_2CO_3 (92.9 mg, 0.88 mmol) in 2-ethoxyethanol (10 mL) was heated to 100 °C for 1.5 h. After cooling to room temperature, the reaction mixture was extracted with ethyl acetate. The organic phase was washed with water and dried over anhydrous Na_2SO_4. The volatiles were evaporated under reduced pressure. The residue was triturated with acetone and hexane, and then the resulting green precipitate was filtered to give compound **20** (30 mg, 0.05 mmol, 54%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.89 (s, 2H), 8.24 (d, J = 5.5 Hz, 2H), 7.87 (d, J = 8.3 Hz, 2H), 7.78 (t, J = 7.2 Hz, 2H), 7.46 (d, J = 8.4 Hz, 2H), 7.18 (t, J = 6.2 Hz, 2H), 6.17 (dd, J = 8.4, 2.3 Hz, 2H), 5.45 (d, J = 2.3 Hz, 2H), 5.18 (s, 1H), 1.66 (s, 6H).

Synthesis of 21. To a stirred solution of **20** (40 mg, 0.07 mmol) in DCM (5 mL) at 0 °C, *N*,*N*-diisopropylethylamine (0.05 mL, 0.3 mmol) was added dropwise *via* syringe and stirred for 30 min. In a separate round-bottomed flask, compound **10** (90 mg, 0.5 mmol) was refluxed in thionyl chloride (0.19 mL, 2.6 mmol) for 2 h and then residual thionyl chloride was removed under reduced pressure to give 2-(azidomethyl) benzoyl chloride. 2-(Azidomethyl)benzoyl chloride dissolved in DCM (5 mL) was added dropwise to a stirred solution of **20** prepared above. The reaction mixture was stirred at room temperature for 2 h and extracted with DCM. The organic phase was washed with water and dried over anhydrous Na_2SO_4. The volatiles were evaporated under reduced pressure. The residue was purified by silica gel column chromatography with hexane/ethyl acetate (5 : 1 v/v) as the eluent to give a yellow solid. (31 mg, 0.033 mmol, 47%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.38 (d, J = 5.4 Hz, 2H), 8.13 (d, J = 8.2 Hz, 2H), 7.93 (dd, J = 16.9, 7.3 Hz, 4H), 7.81 (d, J = 8.5 Hz, 2H), 7.64 (d, J = 6.3 Hz, 2H), 7.53 (d, J = 7.2 Hz, 2H), 7.47 (d, J = 7.4 Hz, 2H), 7.34 (d, J = 6.1 Hz, 2H), 6.74 (dd, J = 8.4, 2.3 Hz, 2H), 5.83 (d, J = 2.3 Hz, 2H), 5.25 (s, 1H), 4.70 (d, J = 2.9 Hz, 4H), 1.72 (s, 6H); HRMS (FAB$^+$, m-NBA): *m*/*z*: observed 950.2152 (calculated for $C_{43}H_{33}IrN_8O_6$ $[M]^+$ 950.2155).

Results and discussion

Design of ECL probe 1

Density functional theory (DFT) calculations of $(ppy)_2Ir(acac)$ revealed that the highest occupied molecular orbital (HOMO) is mainly localized on the Ir(III) centre and the phenyl ring of ppy, while the lowest unoccupied molecular orbital (LUMO) is localized on the pyridine of ppy.[34] A recent study showed that chemical modifications of the substituent on the metal-coordinating pyridyl moiety affect the LUMO levels and may generate significant changes in the emission profiles.[35] These factor inspired us to develop a new chemodosimetric ECL sulphide probe based on an Ir(III) complex by modulating the electron density of the pyridyl moiety.

In this regard, we used $(hy\text{-}ppy)_2Ir(acac)$ (hy-ppy = 3-hydroxy-6-phenylpyridine) (**2**) as a model compound to apply the on–off system in ECL for H_2S. We predicted that functionalization of the 5′-position of the pyridyl moiety to the electron-donating hydroxyl group would destabilize the LUMO, which would stop the electron transfer from the HOMO of the TPA radical to the elevated LUMO level of **2**. On the other hand, the electron-withdrawing ester group of probe **1** would stabilize the LUMO and thus facilitate the electron transfer of TPA radical to the LUMO level of **1**. Therefore, we expected that the ECL intensity of **1** would be much larger than that of **2**.

Mechanism study

^{1}H NMR experiments in DMSO-d_6 were conducted to elucidate the sensing mechanism. ^{1}H NMR spectra (Fig. 1) revealed that

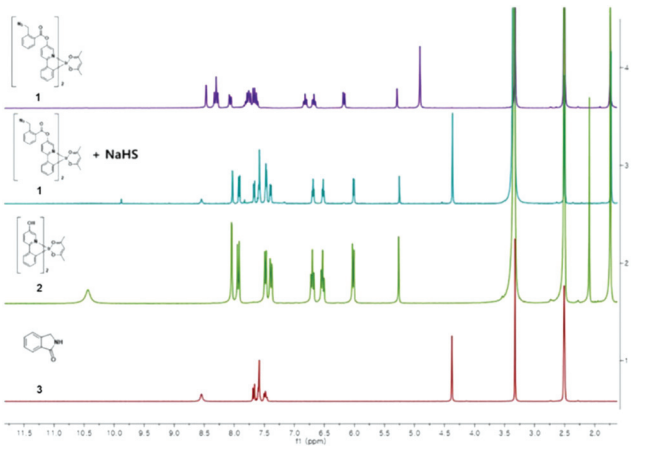


Fig. 1 Comparison of ^{1}H NMR spectra of **1**, **1** + NaHS (20 equiv.), **2** and **3** (2 mM in DMSO-d_6).

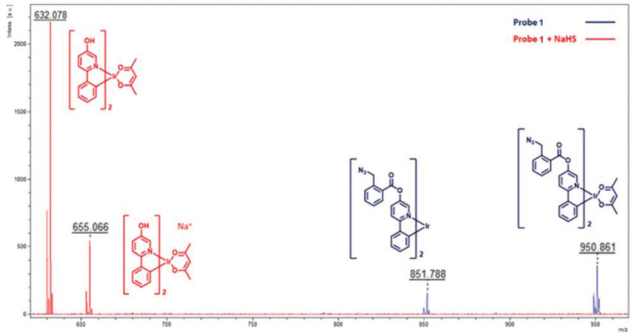


Fig. 2 MALDI-TOF mass spectra of **1** and **1** + NaHS (20 equiv.).

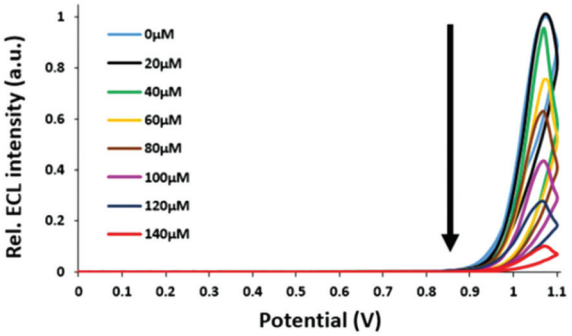


Fig. 3 ECL intensity of probe **1** (10 μM) in the presence of various concentrations of sulphide in CH_3CN/DMSO (5 : 1 v/v, 10 mM TPA, and 0.1 M $TBAPF_6$ as supporting electrolyte) while the potential is swept at a Pt disk electrode (diameter: 2 mm) in the range of 0–1.1 V (scan rate: 0.1 V s^{-1}).

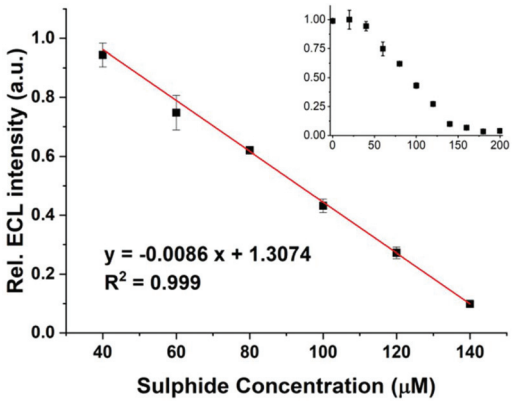


Fig. 4 ECL intensity of probe **1** (10 μM) upon the addition of various concentrations of sulphide in CH_3CN/DMSO (5 : 1 v/v, 10 mM TPA, and 0.1 M $TBAPF_6$ as supporting electrolyte).

1 was reacted with NaHS (20 eq.) to provide **2** and **3**. The ^{1}H NMR spectrum of **1** + NaHS is the same as a sum of ^{1}H NMR spectra of **2** and **3**. MALDI-TOF mass analysis of probe **1** was also performed following the addition of NaHS in CH_3CN (Fig. 2). Before NaHS addition, a peak was observed at 950.9 (*m*/*z*) corresponding to **1**, and after NaHS addition (20 eq.), a peak was observed at 632.1 (*m*/*z*) corresponding to **2**. These results support the proposed sensing mechanism of **1** for H_2S (Scheme 1).

Photophysical properties of 1

We first investigated the UV-vis absorption spectra of **1** (Fig. S20†). The absorption peak showed a slight change around 260 nm in the presence of NaHS (20 eq.). We also investigated the phosphorescence spectra of probe **1** (10 μM in CH_3CN/DMSO = 5 : 1, λ_{ex} = 380 nm). Probe **1** itself exhibited a strong phosphorescence emission peak at 538 nm. The addition of NaHS gradually reduced the emission intensity with a slight hypsochromic shift to 516 nm over 1 h (Fig. S21†). We found a good linear relationship between the phosphorescence intensity at 538 nm and the sulphide concentration ranging from 0 to 200 μM (Fig. S22 and S23†). The limit of detection (LOD) was estimated to be 102 nM (signal-to-noise (S/N) ratio = 3, *n* = 3). Moreover, the phosphorescence of probe **1** was selectively quenched by the sulphide (Fig. S21 and S22†). In contrast, no significant emission changes were observed in the presence of any other anions, such as F^-, Cl^-, Br^-, I^-, NO_3^-, NO_2^-, N_3^-, SO_4^{2-}, SO_3^{2-}, $S_2O_3^{2-}$, $S_2O_4^{2-}$, $S_2O_5^{2-}$, and SCN^- (Fig. S24 and S25†). It is difficult to distinguish biologically significant thiols like Cys, Hcy, and GSH from sulphide. However, these thiols did not induce significant changes in the phosphorescence of probe **1** (Fig. S24 and S25†).

ECL properties of 1

We performed ECL measurements in an CH_3CN/DMSO solution mixture (5 : 1 v/v) with 10 μM **1** and 10 mM TPA with 0.1 M $TBAPF_6$ as a supporting electrolyte. During cyclic voltammetry (CV), probe **1** itself showed high ECL intensity, which gradually decreased with addition of NaHS as expected (Fig. 3). A titration curve of **1** was obtained upon the addition of various concentrations of NaHS (Fig. 4), indicating that the ECL signal decreased linearly over a NaHS concentration range 40–140 μM (2–14 eq.). The estimated LOD was calculated to be 11 nM (S/N ratio = 3, *n* = 3), which is significantly lower than that obtained in the phosphorescence method. The selectivity of **1** was also tested in the presence of 200 μM of anions like F^-, Cl^-, Br^-, I^-, NO_3^-, NO_2^-, N_3^-, SO_4^{2-}, SO_3^{2-}, $S_2O_3^{2-}$, $S_2O_4^{2-}$, $S_2O_5^{2-}$, SCN^-, and biothiols like Cys, Hcy, and GSH (Fig. 5). This result showed that these anions and biothiols could not reduce the ECL intensity except for I^-. It is well documented that iodide adsorption onto the electrode can retard the oxidation processes necessary for ECL.[36]

CV analysis

To confirm our theoretical prediction of the on–off ECL mechanism, CV analysis was performed and energy levels were calculated based on the CV measurements (Fig. S26 and Table S1†).

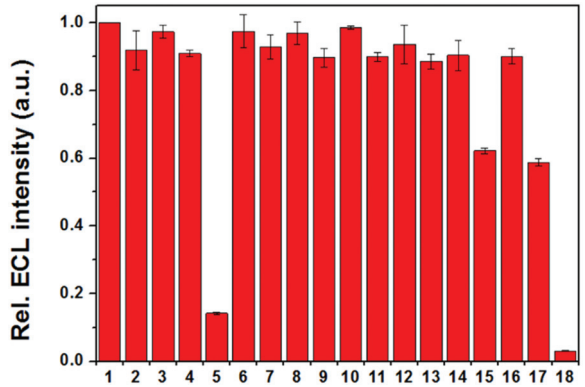


Fig. 5 ECL responses of probe **1** (10 μM) in the presence of various analytes (200 μM each, sodium salt) in CH_3CN/DMSO (5 : 1 v/v, 10 mM TPA, and 0.1 M $TBAPF_6$ as supporting electrolyte) (1) Probe **1** only (2) F^- (3) Cl^- (4) Br^- (5) I^- (6) NO_3^- (7) NO_2^- (8) N_3^- (9) SO_4^{2-} (10) SO_3^{2-} (11) $S_2O_3^{2-}$ (12) $S_2O_4^{2-}$ (13) $S_2O_5^{2-}$ (14) SCN^- (15) Cys (16) Hcy (17) GSH (18) HS^-.

The LUMO level of **1** is lower than that of HOMO of the TPA radical so that **1** can generate efficient ECL (Fig. S28†). On the other hand, the LUMO energy level of **2** is slightly higher than the HOMO of the TPA radical; thus, ECL could not be generated due to the unfavourable electron transfer from the TPA radical to the LUMO energy level of **2** (Fig. S28†). These results suggest that **1** could be a good on–off ECL probe for H_2S detection.

Comparison with analogues of probe 1

In order to compare the reaction trend of probes toward sulphide according the substitution position of the reaction site on the main ligand, we synthesized probes **16** and **21** (Scheme 3). First, we investigated the phosphorescence spectra of probes **16** and **21** (10 μM in CH_3CN/DMSO = 5 : 1, λ_{ex} = 380 nm) (Fig. S29†). The PL intensities of **16** and **21** are 0.5 and 0.4 times that of **1**, respectively. The phosphorescence spectra of probes **1** and **16** showed similar tendency upon reaction with sulphide. The emission intensity of **16** also gradually decreased over 1 h with the addition of sulphide (Fig. S30(a)†). However, **21** with green emission (emission maximum at 505 nm) exhibited no significant changes upon addition of sulphide (Fig. S30(b)†). **16** turned out to be selective for sulphide (Fig. S31†).

ECL measurements of **16** and **21** were performed in a mixture of CH_3CN and DMSO (v/v 5 : 1) in the presence of 10 μM probe (10 mM TPA with 0.1 M $TBAPF_6$ as the supporting electrolyte). The ECL intensities of **16** and **21** are 0.1 and 0.3 times that of **1**, respectively (Fig. S32†). The ECL intensity of **16** (10 μM) decreased with the addition of sulphide (200 μM) while other analytes showed relatively small changes. In contrast, **21** did not show any response to the addition of all the analytes (Fig. S33†). These results show that the substitution position of the reaction site on the main ligand of Ir(III) complexes is critical in designing ECL probes.

Conclusion

In summary, we have developed an ECL-based chemodosimetric probe **1** for sulphide. Treatment of **1** with sulphide produced **2** by cascade reactions involving hydrosulphide-mediated reduction and intramolecular cyclization/cleavage, which reduced ECL due to the unfavourable electron transfer from the TPA radical to the LUMO of **2**.

Conflicts of interest

There are no conflicts to declare.

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