Supporting Information

Fluorogenic detection of hydrosulfide via reductive unmasking of o-azidomethylbenzoyl-coumarin conjugate

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Experimental procedure

2-(Azidomethyl)benzoic acid was obtained from Chemidate, Co. Nanjing, China. All other reagents were obtained from Alfa Aesar and used without further purification. Column chromatography was performed on silica gel (300-400 mesh). NMR spectra (^{1}H at 400 MHz and ^{13}C at 100 MHz) were recorded on a Bruker instrument using tetramethyl silane as the internal reference. HRMS was performed in Bruker En Apex ultra 7.0T FT-MS. Mass spectra were recorded by Bruker Dalton Esquire 3000 plus. The fluorescence spectra were performed on a spectrofluorimeter (Spectamax M5, Molecular Device). HeLa cells were obtained from American Type Culture Collection. Cells were analyzed using a fluorescence microscope (Ti-S; Nikon eclipse) equipped with a 100-W mercury lamp (C-SHG1, Nikon). Fluorescence microscopic images were obtained on LeicaSP2 using the following filters: λex@405 nm and *λem*@420-500 nm.

Synthesis of AzMB-coumarin

2-(Azidomethyl)benzoic acid (1.2 g) was added into thionyl chloride (20 ml). The solution was heated under reflux for 2 h and then evaporated *in vacuo*. The residue was dissolved in dry methylene dichloride (10 ml) containing 7-hydroxy-4-methylcoumarin. To the solution were added dropwisely diethylisopropylamine (2 ml). The reaction mixture were stirred at room temperature for 2 hours and then concentrated. The residue was purified by silica gel column chromatography using ethyl acetate/hexanes (1: 1，v/v) as the eluent to give 1.2 g of solid as the desired product in 70 % yield. ^{1}H-NMR (400 MHz, $CDCl_3$), δ:8.36 (d, 1H, J = 8.72 Hz), 8.23 (m, 1H), 7.71 (m, 1H), 7.55 (m, 1H), 7.29 (d, 1H, J = 2.56 Hz), 7.22 (dd, 1H ,J1 = 8.72 Hz, J2 = 2.32 Hz), 3.96 (s, 2H), 2.40 (s, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$): 168.81, 159.69, 155.39, 152.34, 149.66, 146.03, 139.04, 129.47, 127.56, 126.81, 125.45, 125.01, 124.05, 118.19, 115.72, 111.06, 36.18, 21.18 ppm; HRMS: ($C_{33}H_{41}N_3O_3$):calculated $(M+Na)^+$: 358.0798, found 358.0794

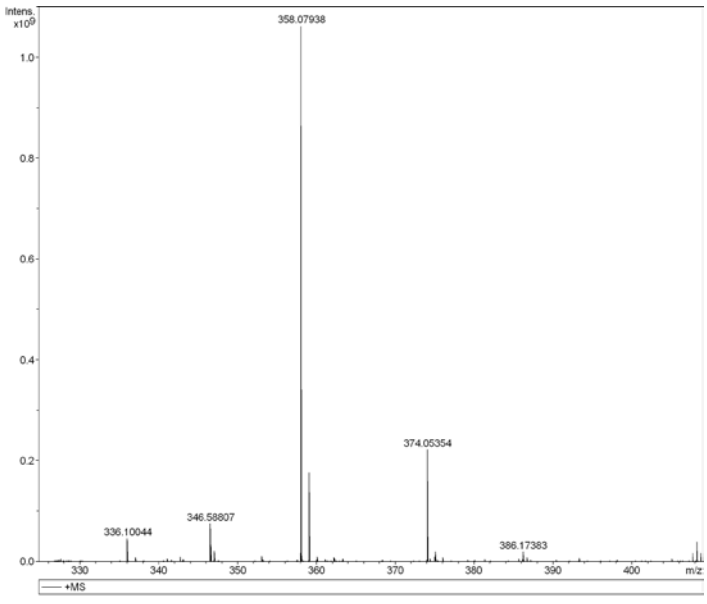


Fig. S1 Mass spectrometry spectrum of AzMB-coumarin

Synthesis of 7-acetoxy-4-methylcoumarin

7-hydroxy-4-methylcoumarin (4 g) and pyridine (6 ml) were added into acetic anhydride (20 ml). The solution was stirred at rt for 8 hrs and then evaporated *in vacuo*. The residue was dissolved in methylene dichloride (50 ml). The organic solution was washed with aqueous hydrogen chloride solution (1 M, 50 ml) and then washed with saturated sodium bicarbonate solution (30 ml). The organic layer was dried over sodium sulfate, and then concentrated. The residue was seperated by silica gel chromorography using ethyl acetate/hexanes (1:2, v/v) as the eluent to afford 3.9 g of pale yellow solid as the desired product in 90% yield. ^{1}H-NMR (400 MHz, $CDCl_3$), δ:7.62 (d, 1H, J = 8.56 Hz), 7.13 (d, 1H, J = 2.20 Hz), 7.09 (dd, 1H, J1 = 8.64 Hz, J2 = 2.28 Hz), 6.28 (d, 1H, J = 1.20 Hz), 2.45 (d, 3H, J = 1.24 Hz), 2.35 (s, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$):168.75, 160.51, 154.17, 153.06, 151.94, 125.39, 118.10, 117.85, 114.51, 110.46, 21.10, 18.71, ppm; HRMS: ($C_{33}H_{43}N_3O_2$):calculated $(MH)^+$: 241.0471, found 241.0475.

Characterization of AzMB-coumarin based assay with mass spectrometry

NaHS was added into acetnitrile solution containing AzMB-coumarin (20 μM) to a final concentration of 200 μM. The solution was incubated at room temperature for 60 minutes and then analyzed by mass spectrometry. The fluorescence emission of the as-preapred solution and 7-hydroxyl-4-methylcoumarin were respectively recorded using an excitation wavelength of 365 nm.

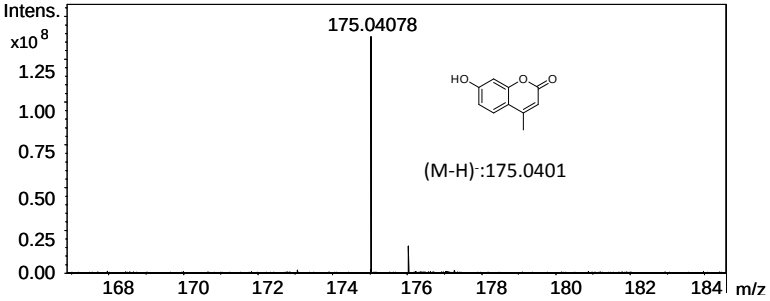
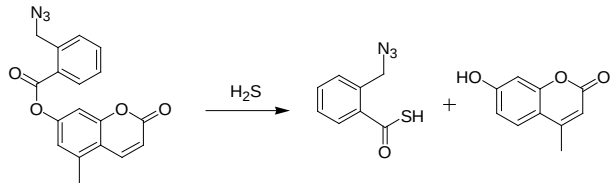


Fig. S2 HRMS analysis of hydrosulfide mediated reduction of AzMB-coumarin



Scheme S1. Potential cleave of 2-azidomethyl)benzoyl group by hydrosulfide.

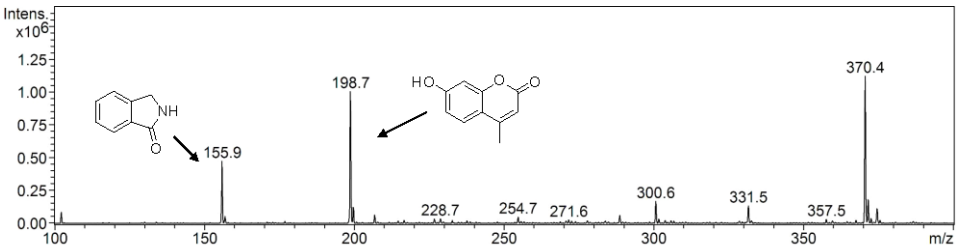


Fig. S3 Mass spectrometry analysis of hydrosulfide mediated reduction of AzMB-coumarin

Comparison of differential fluorescence responses of AzMB-coumarin and 7-acetoxycoumarin to hydrosulfide

NaHS sock solutions were respectively added to the following solbent conntaining AzMB-coumarin (20 μM) to a final concentration of 100 μM: acetonitrile , ethanol, DMF or sodium phosphate buffered acetonitrile (pH 7.4, 100 mM) (acetonitrile: 20%, v/v). The solutions were incubated at rt for 20 mins and then analyzed by fluorometry. The fluorescence emission of the solutions was recorded using an excitation

wavelength of 365 nm.

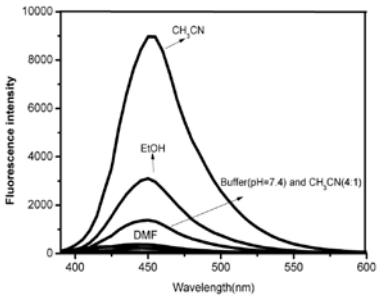


Fig. S4 Effects of the reaction media on the reduction of **AzMB-coumarin** (200 μM) with sodium hydrosulfide (200 μM) in acetonitrile, ethanol, buffered acetonitrile or dimethylformamide (DMF).

Reaction kinetics of AzMB-coumarin towards hydrosulfide

Reaction kinetics in acetonitrile: aliquots of AzMB-coumarin stock solution were added to acetonitrile supplemented with or without sodium hydrosulfide (100 μM) in acetonitrile to a final concentration of 20 μM. The rates of fluorescenece development in the reaction solutions were immediately recorded by fluorescence emission at 450 nm using an excitation wavelength of 365 nm on Spectamax-M5.

Reaction kinetics in aqueous acetonitrile: aliquots of AzMB-coumarin stock solution were added to sodlium phosphate buffer (pH 7.4, 6.4, 5.4, and 4.4) containing acetonitrile (20%, v/v) to a final concentration of 100 μM. The solution, supplemented with or without sodium hydrosulfide (1 mM), were analzied by fluorometry. The rates of fluorescenece development in the reaction solutions were immediately recorded by fluorescence emission at 450 nm using an excitation wavelength of 365 nm on Spectamax-M5.

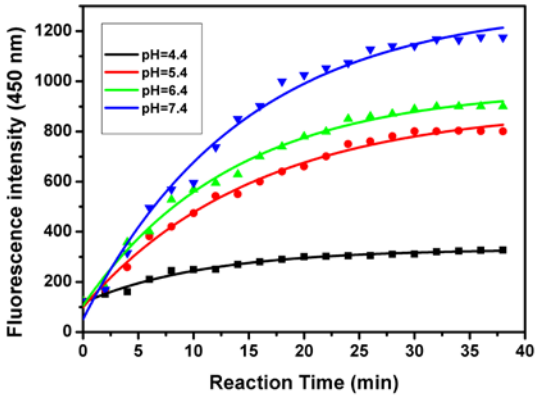


Fig. S5 pH dependent assay kinetics of AzMB-coumarin for hydrosulfide. The fluorescence emission intensity at 450 nm of AzMb-coumarin (100 μM) and NaHS (1 mM) in sodium phosphate buffer containing acetonitrile (20%, v/v) (pH 7.4, 6.4, 5.4, and 4.4) were recorded as a funtion of time (λ_{ex}@365 nm).

AzMB-coumarin based assay sensitivity for hydrosulfide

Assay limit in acetonitrile: various amounts of sodium hydrosulfide stock solution were added into a serial of acetonitrile solutions containing AzMB-coumarin (20 μM) to a final concentration of 0, 10, 20, 40, 60, or 100 μM. The reaction solutions were incubated at room temperature for 20 minutes and then directly analyzed for fluorescence emission spectra.

Assay limit in aqueous acetonitrile, various amounts of sodium hydrosulfide stock solution were added into a serial of soudium phosphate buffered acetonitrile (acetonitrile: 20%, v/v) (sodium phoaphate 100 mM, pH 7.4) and AzMB-coumarin (100 μM) to a final concentration of 0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8. 0.9, 1 mM. The reaction solutions were incubated at room temperature for 1 hr and then directly analyzed for fluorescence emission spectra.

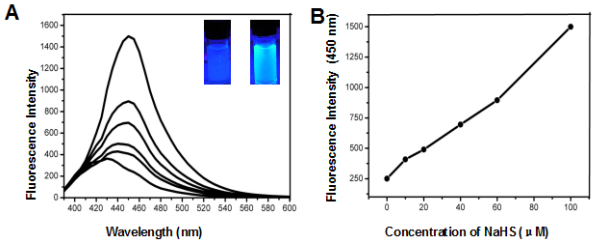


Fig. S6 Fluorescence emission spectra of AzMB-coumarin (20 μM) in the presence of NaHS (0, 10, 20, 40, 60, 100 μM, from bottom to top) (**A**). The insert showed the fluorescence image of AzMB-coumarin before (left) and after (right) addition of sodium hydrosulfide (100 μM). (**B**) The titration curve was plotted by fluorescence emission intensity at 450 nm *vs* analyte concentration. (λ_{ex}@365 nm)

Selectivity of AzMB-coumarin towards interfering species

Cobalt chloride(100 μM) and H_2O_2 (1 mM) were added in water at room temperature to generate hydroxyl radical (·OH); $\cdot O^{2-}$ was generated by dissolving of KO_2 in DMSO (100 μM); Nitric oxide was generated in-situ before use by dissolving sodium nitroferricyanide (III) dihydrate in deionizer water at room temperature; ROO· was generated from ammonium peroxydisulfate (100 μM).

Selectivity in acetonitrile: a serial of solutions of AzMB-coumarin (20 μM) in acetonitrile were prepared to contain one of the following cations (1) NaF (1 mM), NaCl (1 mM), NaBr (1 mM), $NaHCO_3$ (1 mM), $Na_2S_2O_3$ (1 mM), Na_2HPO_4 (1 mM), Na_2SO_4 (1 mM), Na_2CO_3 (1mM), NaN_3 (1 mM), $NaNO_2$, sodium citrate (1 mM), CH_3COONa (1 mM), NaCN (1 mM), ROO· (100 μM), $\cdot O_2$ (100 μM), ·OH (100 μM), H_2O_2 (100 μM), NO· (100 μM), NaClO (100uM), glutathione (100 μM), cysteine (100 μM), homocysteine (100 μM), β-mercaptoethanol (128 μM). Fluorescence emission spectra of the solutions were recorded using excitation wavelength at 365 nm.

Selectivity in aqueous acetonitrile solution: a serial of solutions of AzMB-coumarin (100 μM) in sodium phoaphate buffered acetonitrile (acetonitrile: 20%, v/v) (sodium phosphate, 100 mM, pH 7.4) were prepared to contain one of the following cations glutathione (1 mM), cysteine (1 mM), homocysteine (1 mM), β-mercaptoethanol (1 mM) or hydrosulfide (1 mM). Fluorescence emission spectra of the solutions were recorded using excitation wavelength at 365 nm.

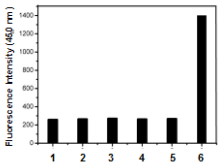


Fig. S7 Selectivity of AzMB-coumarin for hydrosulfide over endogenous biological thiols. Fluorescence responses of AzMB-coumarin (20 μM) in acetonitrile with no addition (1), or with addition of glutathione (100 μM) (2), cysteine (100 μM) (3), homocysteine (100 μM) (4), β-mercaptoethanol (128 μM) (5), or sodium hydrosulfide (100 μM) (6).

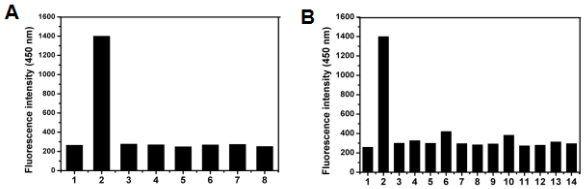


Fig. S8 Fluorescence responses of AzMB-coumarin towards hydrosulfide and potential interferences. (**A**) The fluorescence emission of AzMB-coumarin (20 μM) in acetonitrile spiked with selected reactive oxide species and reactive nitrogen species (100 μM): 1) no addition, 2) NaHS, 3) ROO·, 4) $\cdot O_2^-$, 5) ·OH, 6) H_2O_2, 7) NO·, 8) $NaClO^-$; (**B**) fluorescence responses of AzMB-coumarin (20 μM) in acetonitrile supplemented with each of the following species (100 μM): 1) no addition, 2) NaHS, 3) F^-, 4) Cl^-), 5) Br^-, 6) 6) HCO_3^-, 7) $S_2O_3^-$, 8) HPO_4^{2-}, 9) SO_4^{2-}, 10) N_3^-, 11) NO_2^-, 12) citrate, 13) CH_3COO^-, or 14) CN^- The fluorescence emission intensity at 450 nm was recorded (*ex* @ 365 nm).

Imaging of hydrogen sulfide in live cells

HeLa cells were grown at 37 °C under 5% CO_2 in Dulbecco's Modified Eagle Medium (DMEM, Gibco; Invitrogen) supplemented with 10% fetal bovine serum. Cells were seeded on 35 mm glass-bottom dishes (NEST) and incubated for 24 hours, followed by addition of AzMB-coumarin (100 μM). The cells were further incubated for 10 minutes. The medium was removed and replaced with PBS buffer supplemented with or without sodlium hydrosulfide (1 mM). Cells were further cultured for 60 min and then analyzed with a fluorescence microscope.