Supplementary table 1. Raw source data for methanethiol (MeSH) quantification using the fluorometric DBD-F assay shown in Figure 2C.

	MeSH DBD-F fluorometric detection (AU)							
MeSH (µM)	**Replicate 1**	**Replicate 2**	**Replicate 3**	**Replicate 4**	**Replicate 5**	**Replicate 6**	**Replicate 7**	**Replicate 8**
0	1.5	1.6	1.4	1.8	1.4	1.4	1.4	1.6
0.0390625	1.7	1.8	1.7	2.0	1.8	1.9	2.2	1.9
0.078125	2.0	2.2	2.0	2.6	2.5	2.2	2.6	2.5
0.15625	3.1	3.4	3.4	3.6	4.0	4.1	4.2	4.5
0.3125	5.2	5.2	5.8	6.0	8.2	5.6	8.6	5.9
0.625	9.4	9.7	9.6	11.0	12.6	15.4	13.3	16.4
1.25	18.5	19.4	20.4	21.7	25.3	18.1	27.2	20.4
2.5	31.2	33.5	35.1	38.1	40.6	30.8	43.1	33.7
5	64.4	63.0	71.2	70.8	66.9	68.5	71.0	74.0
10	101.5	105.6	113.0	120.1	114.0	112.8	120.3	120.1

Supplementary table 2. Raw source data for methanethiol (MeSH) quantification using the gas chromatography with sulfur chemiluminescence detection (GC-SCD) shown in Figure 2C.

	MeSH GC-SCD detection (AUC)	
MeSH (µM)	**Replicate 1**	**Replicate 2**
0	0	0
0.0307	0	0
0.0614	14.8	15.8
0.1228	29.3	30.3
0.2456	47.5	46.5
0.4912	100.0	90.9

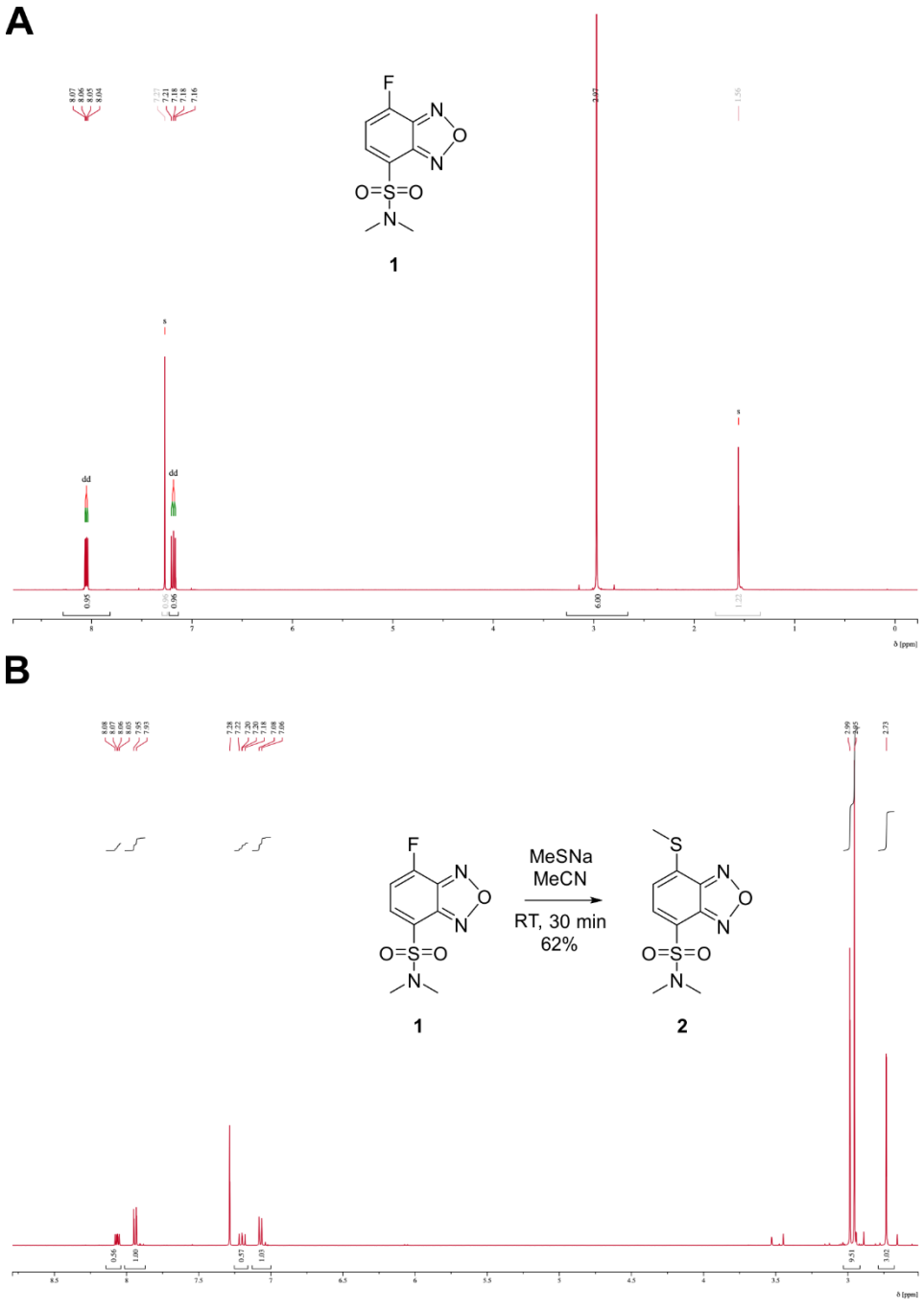
Supplementary table 3. Raw source data for MTO activity assessment using our real-time kinetic MTO assay shown in Figure 3I.

	H_2S (AzMC)				H_2O_2 (CBA)		
MTO (µg/ml)	**Replicate 1**	**Replicate 2**	**Replicate 3**	**Replicate 4**	**Replicate 1**	**Replicate 2**	**Replicate 3**
0	0	0	0	0	12.02	12.32	13.73
0.05					11.41	12.18	11.76
0.0976563					15.27	15.38	15.76
0.1953125	9.42	3.99	0.00	0.00	18.34	18.05	20.16
0.390625	8.87	10.99	9.05	6.50	30.58	30.32	28.99
0.78125	9.02	18.93	4.68	15.75	45.31	44.66	45.49
1.5625	30.72	30.66	26.83	23.96	68.84	68.93	81.77
3.125	40.85	46.05	44.50	42.53	99.45	96.65	103.90
6.25	65.77	70.01	69.02	69.50			
12.5	93.76	96.15	104.01	106.09			

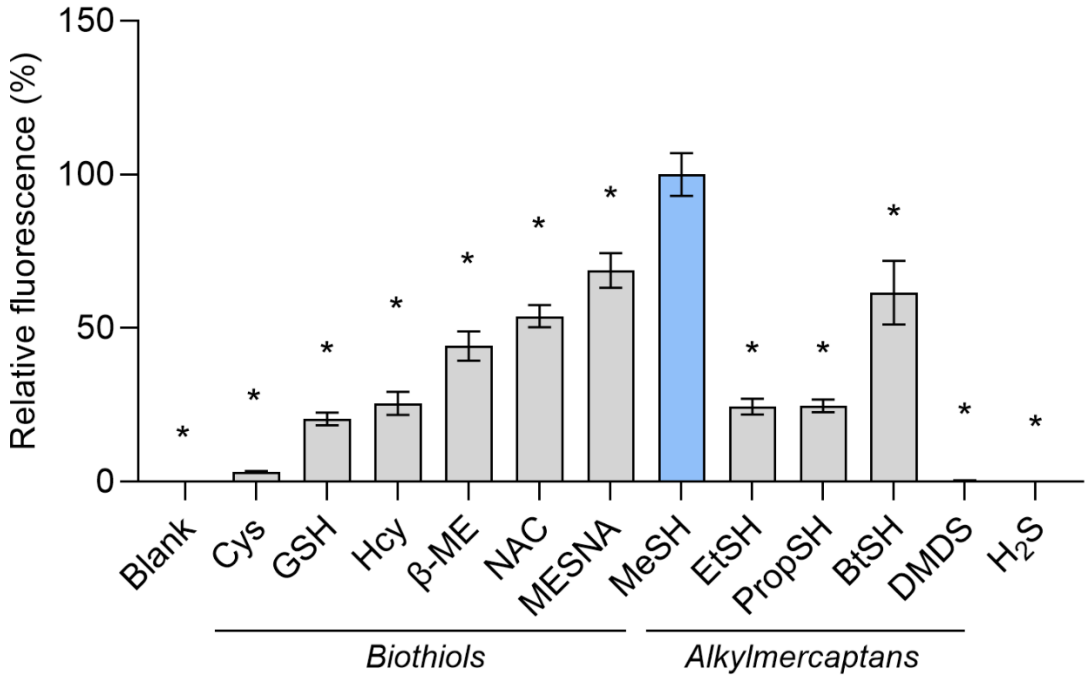
Supplementary table 4. Raw source data for MTO activity assessment using coupled MGL-MTO assay shown in Figure 3L.

	H_2S (PbAc)			H_2O_2 (AmplexRed-HRP)		
MTO (µg/ml)	**Replicate 1**	**Replicate 2**	**Replicate 3**	**Replicate 1**	**Replicate 2**	**Replicate 3**
0	1.45	2.42	3.31	3.73	3.18	6.11
7.8125	2.23	3.32	2.02	15.95	14.23	20.84
15.625	1.07	2.96	2.04	35.81	35.32	36.24
31.25	4.52	6.79	6.76	49.37	61.58	62.72
62.5	11.83	25.35	15.46	79.41	129.06	91.54
125	22.96	50.92	37.70			
250	45.63	77.74	65.41			
500	89.68	105.87	104.45			

Supplementary figure 1. Identification of DBD-SCH$_3$ product. The ^{1}H-NMR spectrum (400 MHz, CDCl$_3$) of the derivatization probe DBD-F (**A**) and the reaction mixture of DBD-F with sodium methylthiolate (**B**). The spectrum shows a 1:2 mixture of unreacted DBD-F (1) and the product 4-methylthio-7-N,N-dimethylaminosulfonyl-2,1,3-benzoxadiazole (2). Key resonances for product (2): δ 2.72 (s, 3H, S–CH$_3$), 2.94 (s, 6H, N(CH$_3$)$_2$), 7.06 (d, J = 7.40 Hz, 1H, Ar–H), 7.93 (d, J = 7.40 Hz, 1H, Ar–H).



Supplementary figure 2. Direct reaction of biothiols and alkylmercaptans with DBD-F probe. Biothiols and alkylmercaptans (each at 10 μM) were directly reacted with DBD-F (20 μM) in solution for 10 minutes at room temperature (n=5). MeSH is highlighted in blue and used as a reference. Fluorescence was measured immediately thereafter at excitation/emission wavelengths of 390/526 nm. All data are presented as mean ± SEM and asterisk (*) indicates significance ($p<0.05$).



Supplementary figure 3. Determination of detection rate constants of the fluorescence probes. The respective substrates (100 µM MeSH for DBD-F (**A**), 100 µM H_2S for AzMC (**B**) and 100 µM H_2O_2 for CBA (**C**)) were directly reacted with each probe (10 µM DBD-F, 10 µM AzMC, or 10 µM CBA) in the respective assay buffers (DBD-F: 0.05 M NaOH, 0.5% ACN; AZMC/CBA: HBS). Fluorescence was monitored over time at the following excitation/emission wavelengths: 390/526 nm for DBD-F, 365/450 nm for AzMC, and 332/470 nm for CBA (n=6). All data are presented as mean ± SEM. The observed fluorescence increase was fitted to a pseudo-first-order reaction model to calculate the second-order rate constants (*k*) and midpoint of fluorescence change ($t_{1/2}$).