Electronic Supplementary Material (ESI) for Analyst.

Electronic Supplementary Information

Estimation of bisulfate in edible plant foods, dog urine, and drug: Picomolar level detection and bio-imaging in living organisms

Ratish R. Nair, M. Raju, Surjit Bhai, Ishan H. Raval, Soumya Haldar,

Bishwajit Ganguly,* and Pabitra B. Chatterjee*

Analytical and Environmental Science Division and Centralized Instrument Facility,

CSIR-CSMCRI, G. B. Marg, Bhavnagar, India.

Academy of Scientific and Innovative Research (AcSIR),

CSIR-CSMCRI, G. B. Marg, Bhavnagar, India.

Corresponding author: pbchatterjee@csmcri.res.in

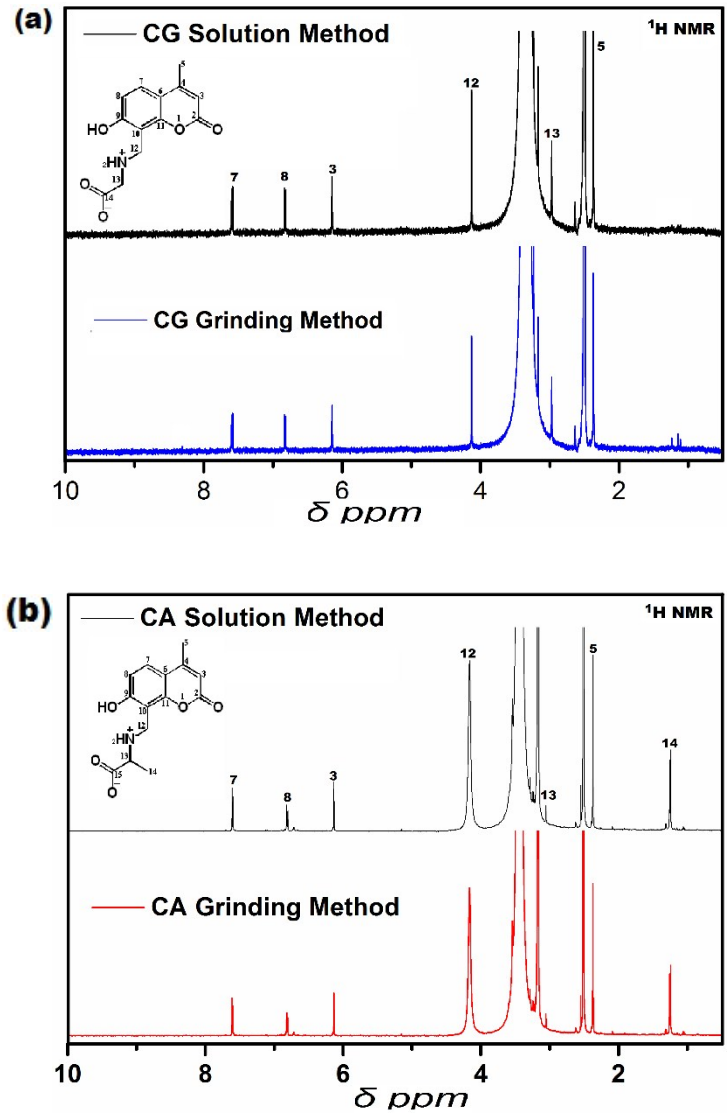

Fig. S1 ^{1}H NMR spectra of (a) CG and (b) CA, recorded in DMSO-d_6.

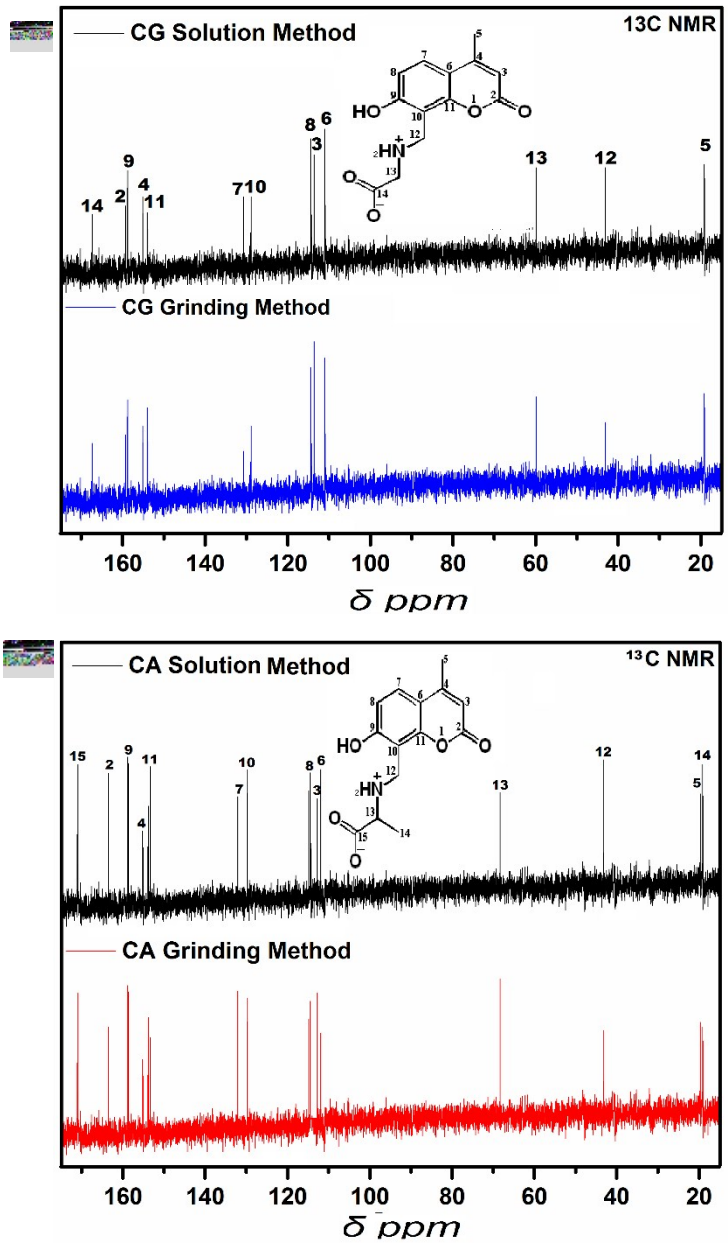

Fig. S2 ^{13}C NMR spectral stacks of (a) CG and (b) CA, recorded in H_2O.

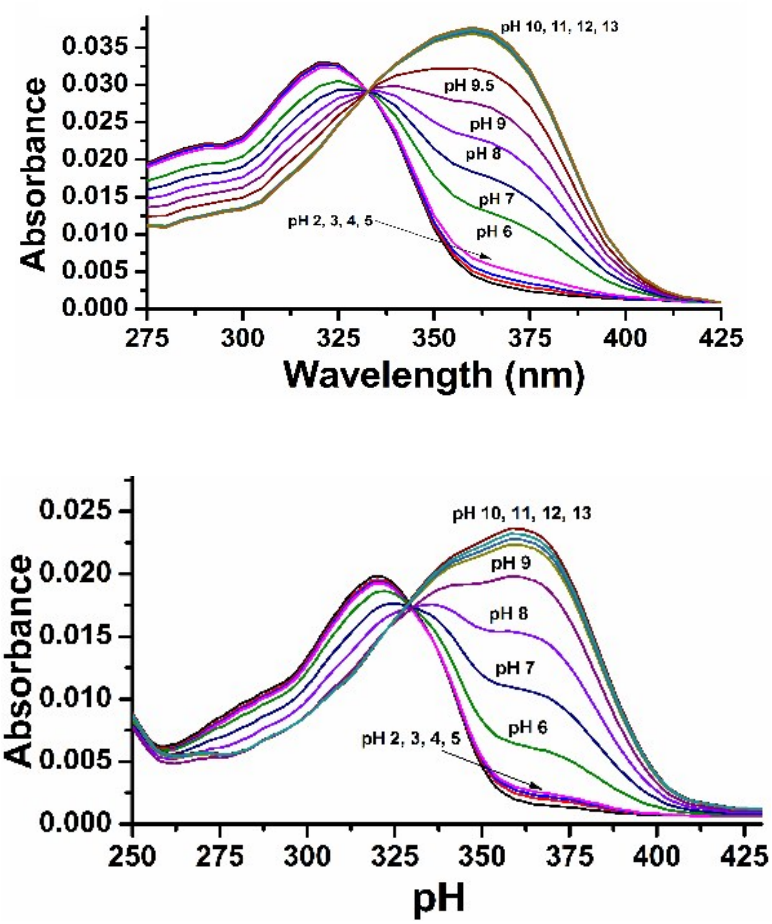

Fig. S3 Absorbance of CG (top) and CA (bottom) at different pHs. Solutions pH was varied between 2 and 13. Solutions of CG and CA were prepared in 100 % water.

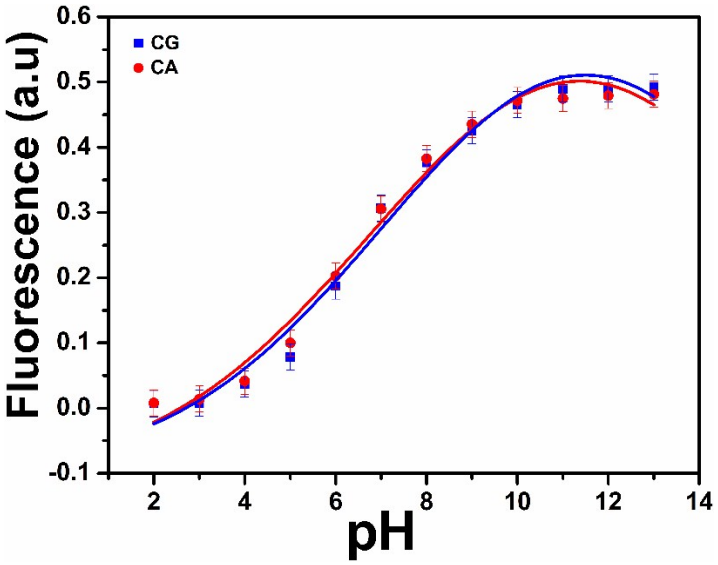

Fig. S4 Change of fluorescence peak intensities of CG and CA with pH of the aqueous solutions. λ_{exi}= 360 nm and λ_{emi}= 448 nm. The error bars indicate the standard deviations of three data points.

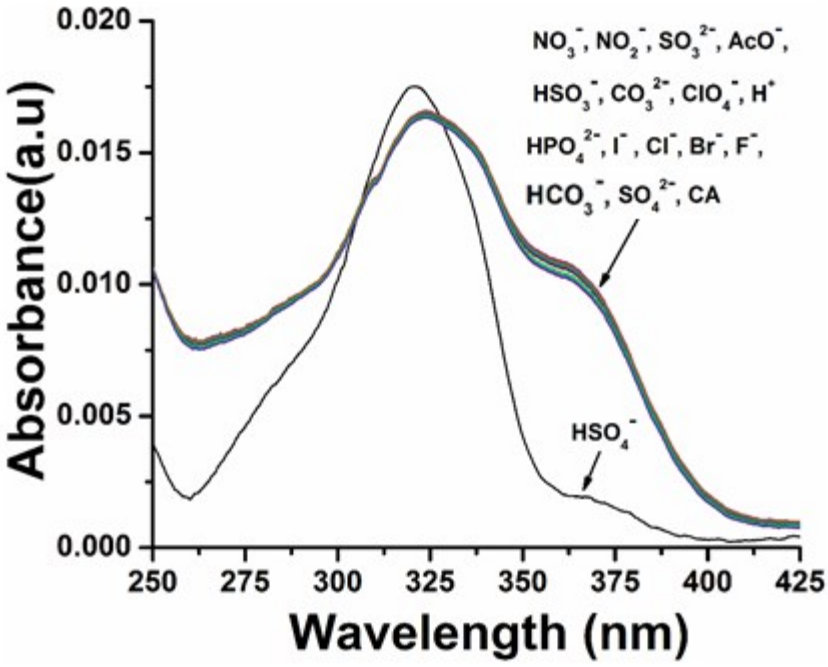

Fig. S5 Absorbance based selectivity plots of CA (10 μM) in 50 mM HEPES aqueous buffer at physiological pH (7.4). Resulting concentrations of various anion solutions is 2.5 mM, excepting bisulfate in which case, it is 25 nM.

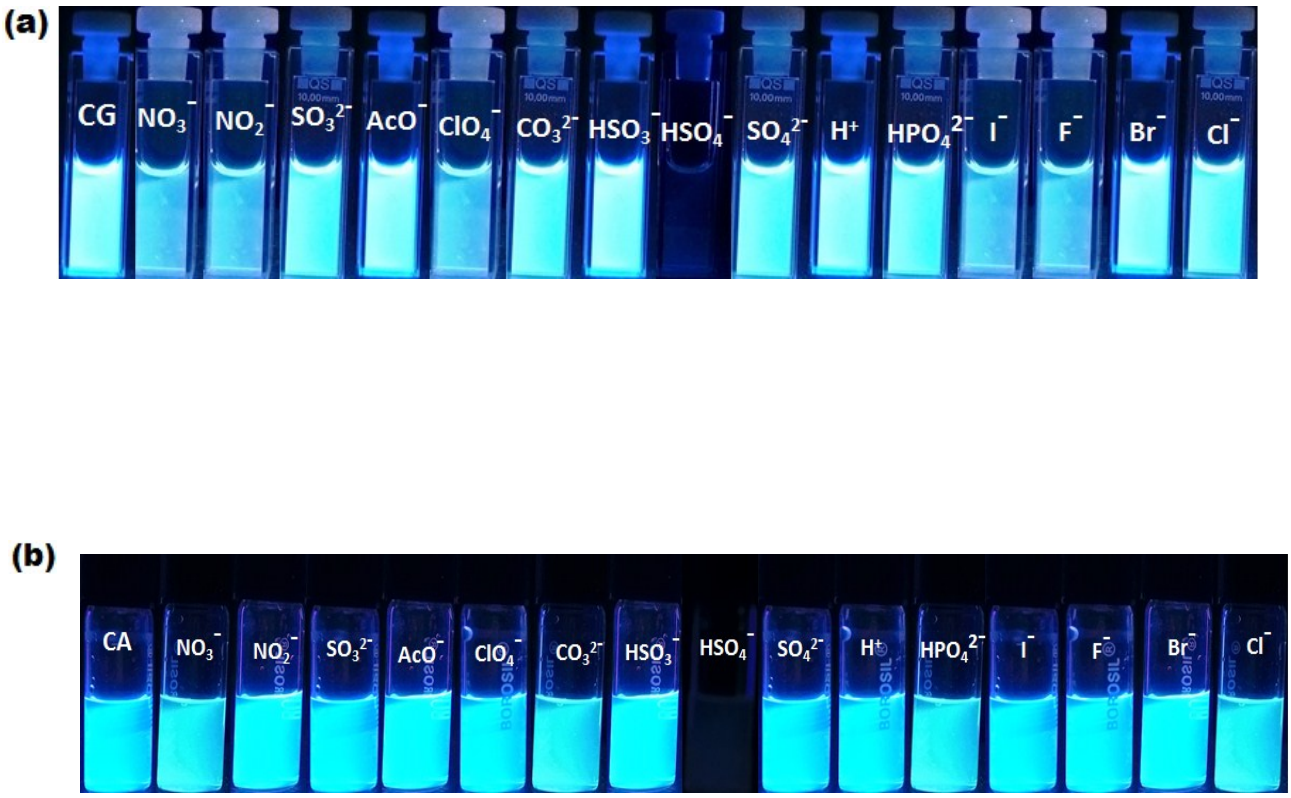

Fig. S6 Naked eye detection of HSO_4^- under UV lamp (365 nm) using aqueous HEPES buffer (pH=7.4) of (a) CG and (b) CA.

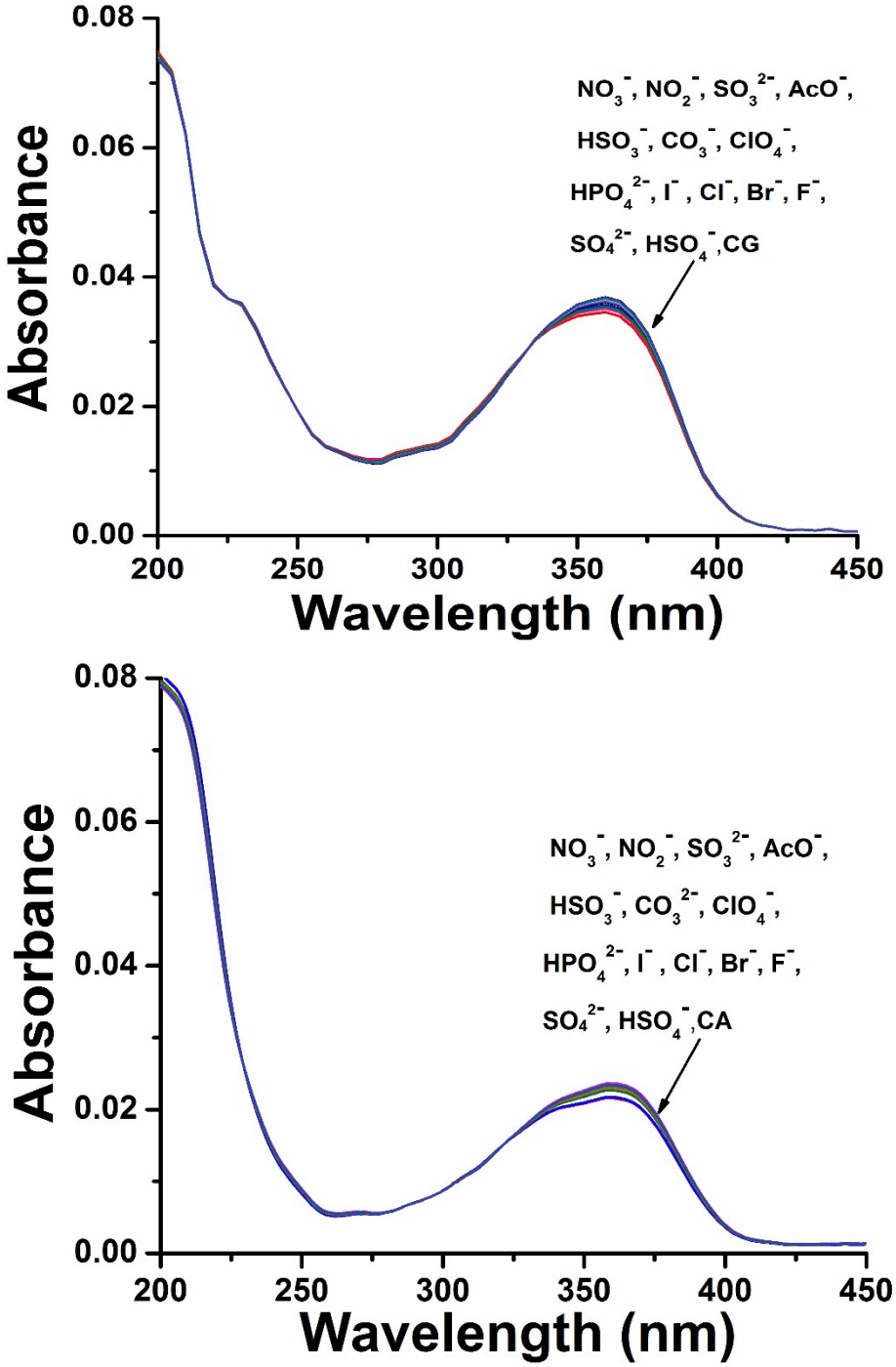

Fig. S7 Absorbance based selectivity plots of 20 μM CG/CA in aqueous CO_3^{2-}/HCO_3^- buffer (20 mM) at pH 10.5. Stock concentration of each anion is 2 mM.

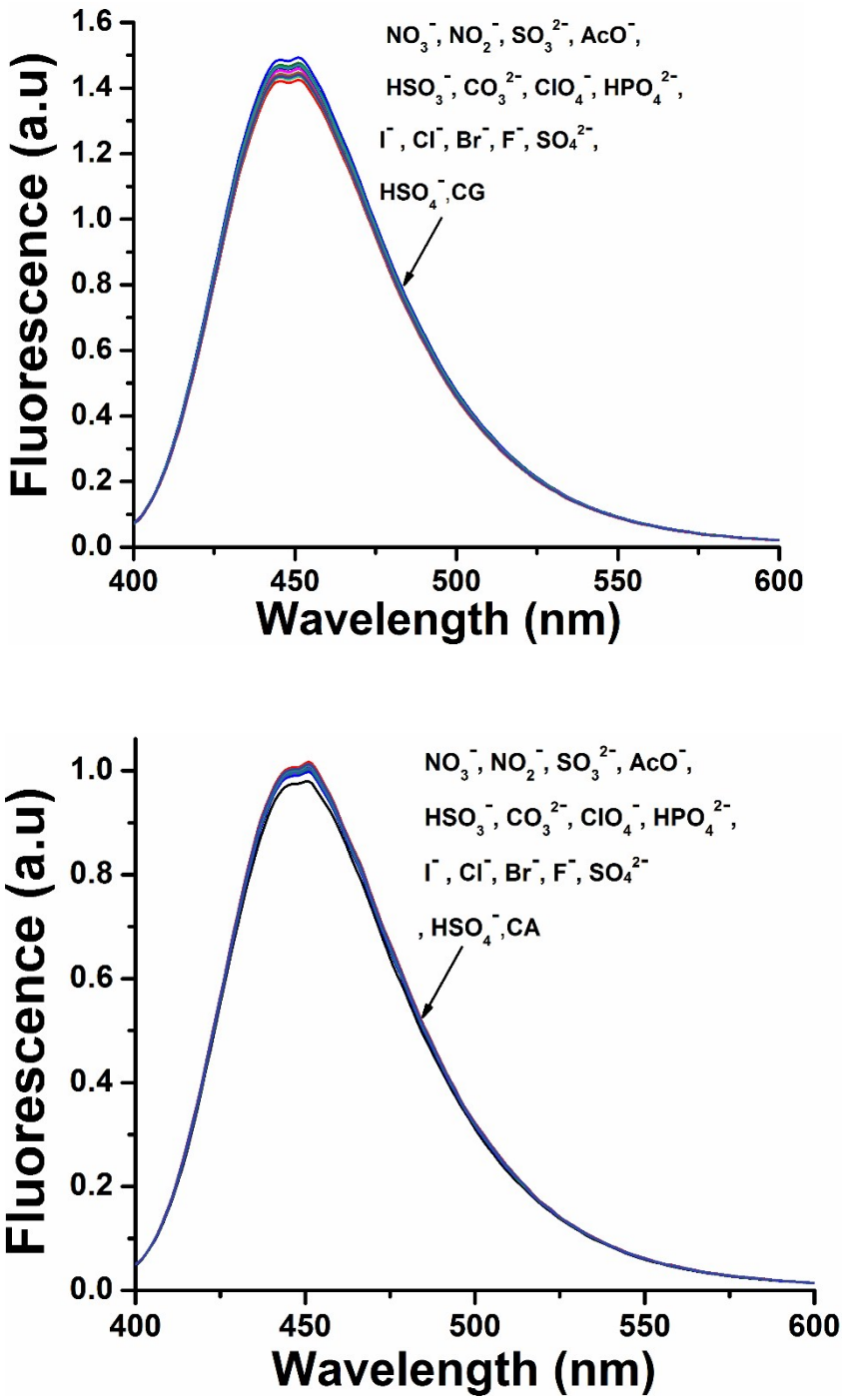

Fig. S8 Emission based selectivity profiles of 10 μM CG/CA in 20 mM aqueous CO_3^{2-}/HCO_3^- buffer at pH 10.5. Stock concentration of each anion is 2 mM.

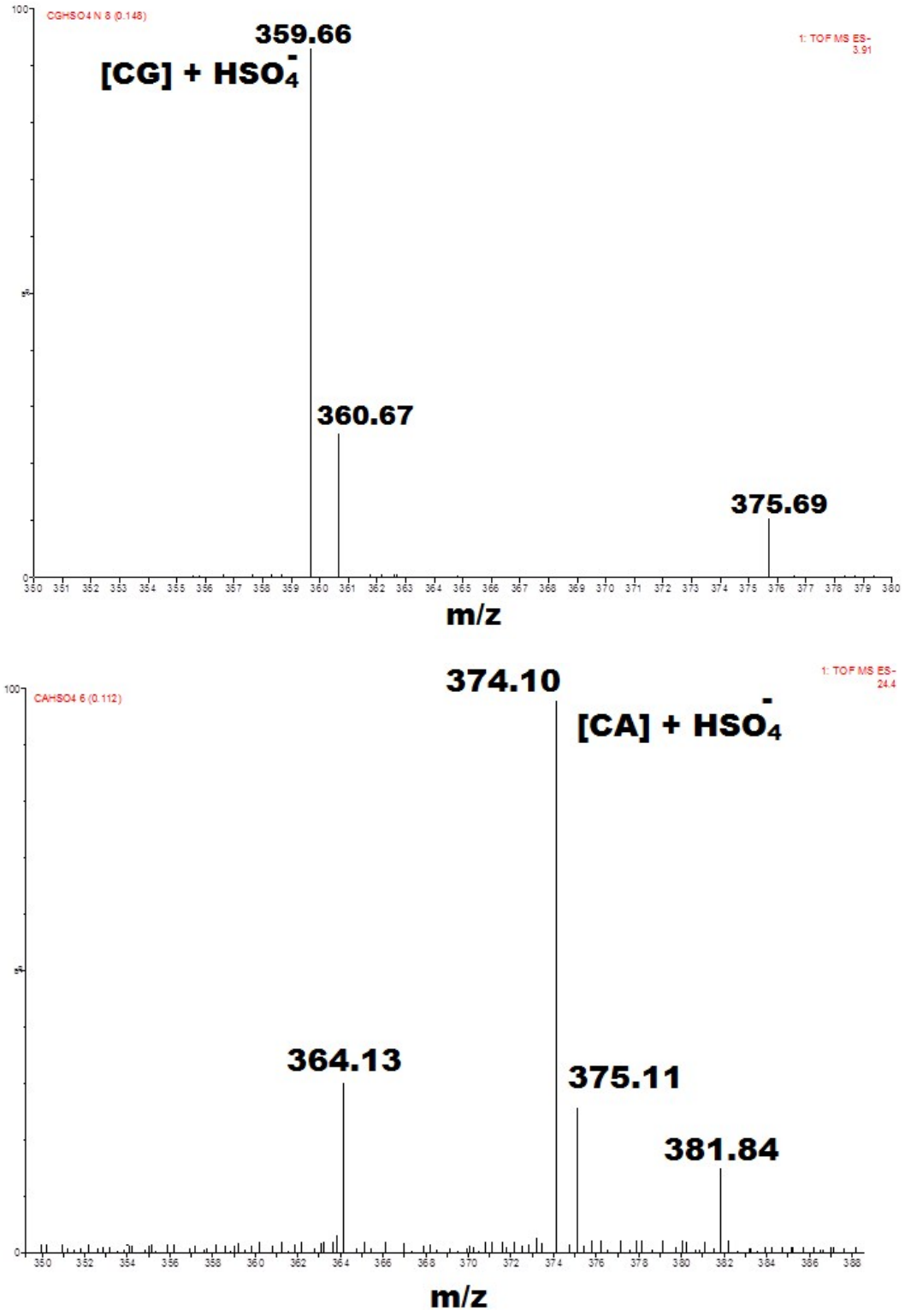

Fig. S9 ESI-MS spectra of (a) [CG] + [HSO_4^-] and (b) [CA] + [HSO_4^-] in the negative ion mode, recorded in water.

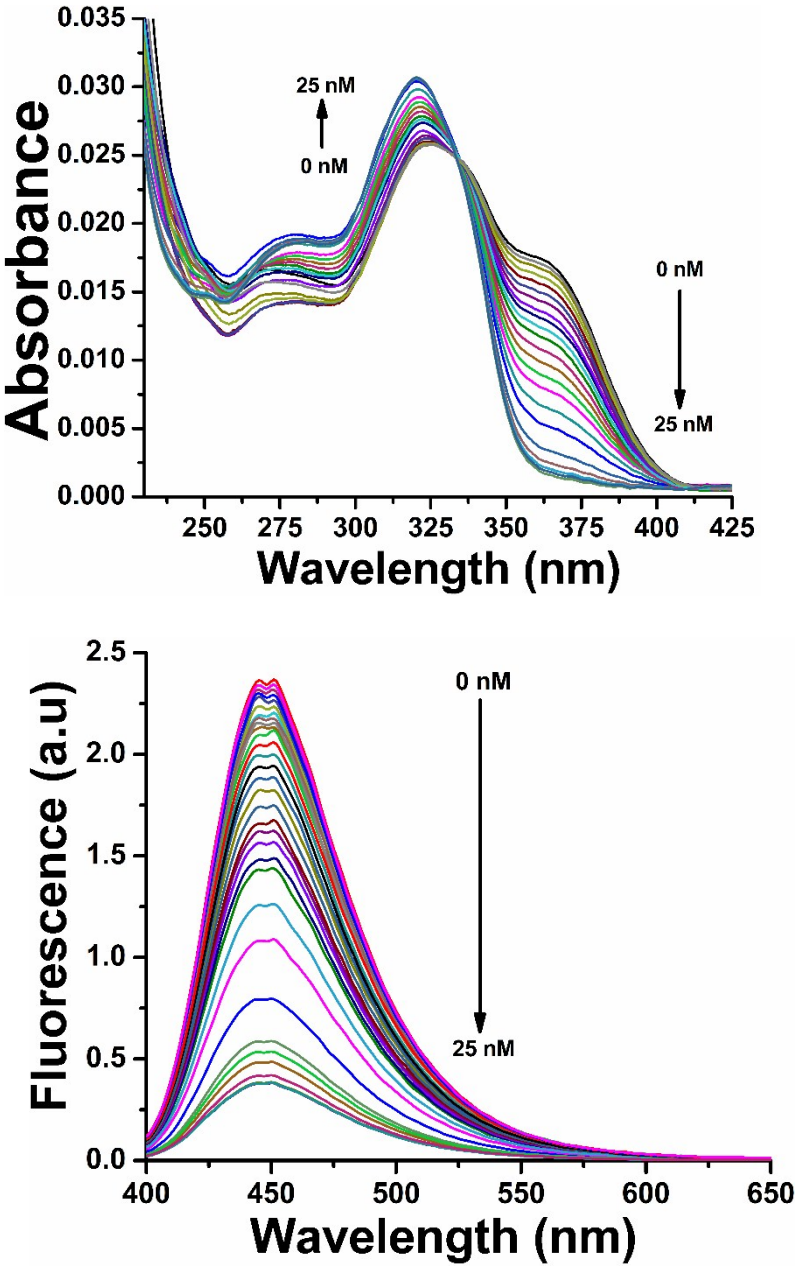

Fig. S10 Absorption (top) and fluorescence (bottom) titration profiles of CG upon addition of increasing concentrations of HSO_4^- (0-25 nM) in 50 mM aqueous HEPES buffer solution at pH 7.4. λ_{exi}= 360 nm.

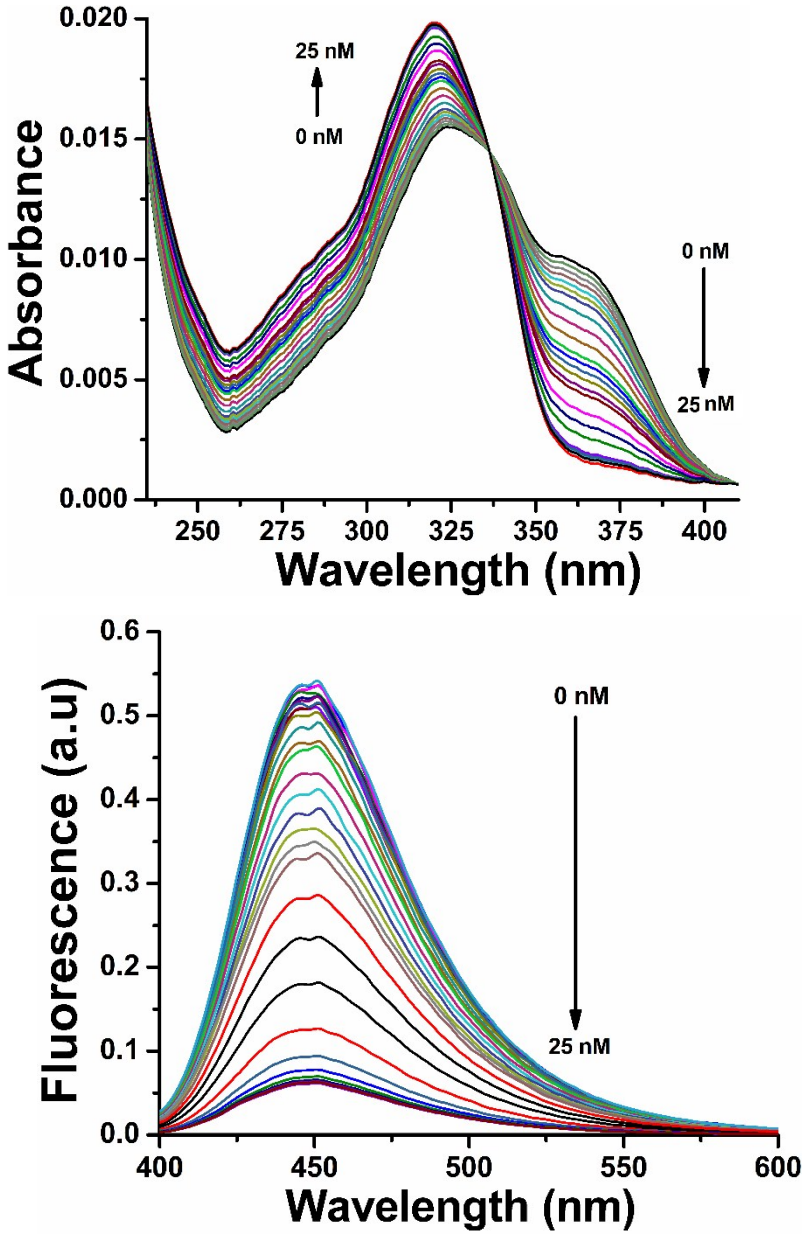

Fig. S11 Absorption (top) and fluorescence (bottom) titration profiles of CA upon addition of increasing concentrations of HSO_4^- (0-25 nM) in 50 mM HEPES aqueous buffer solution at pH 7.4. λ_{exi}= 360 nm.

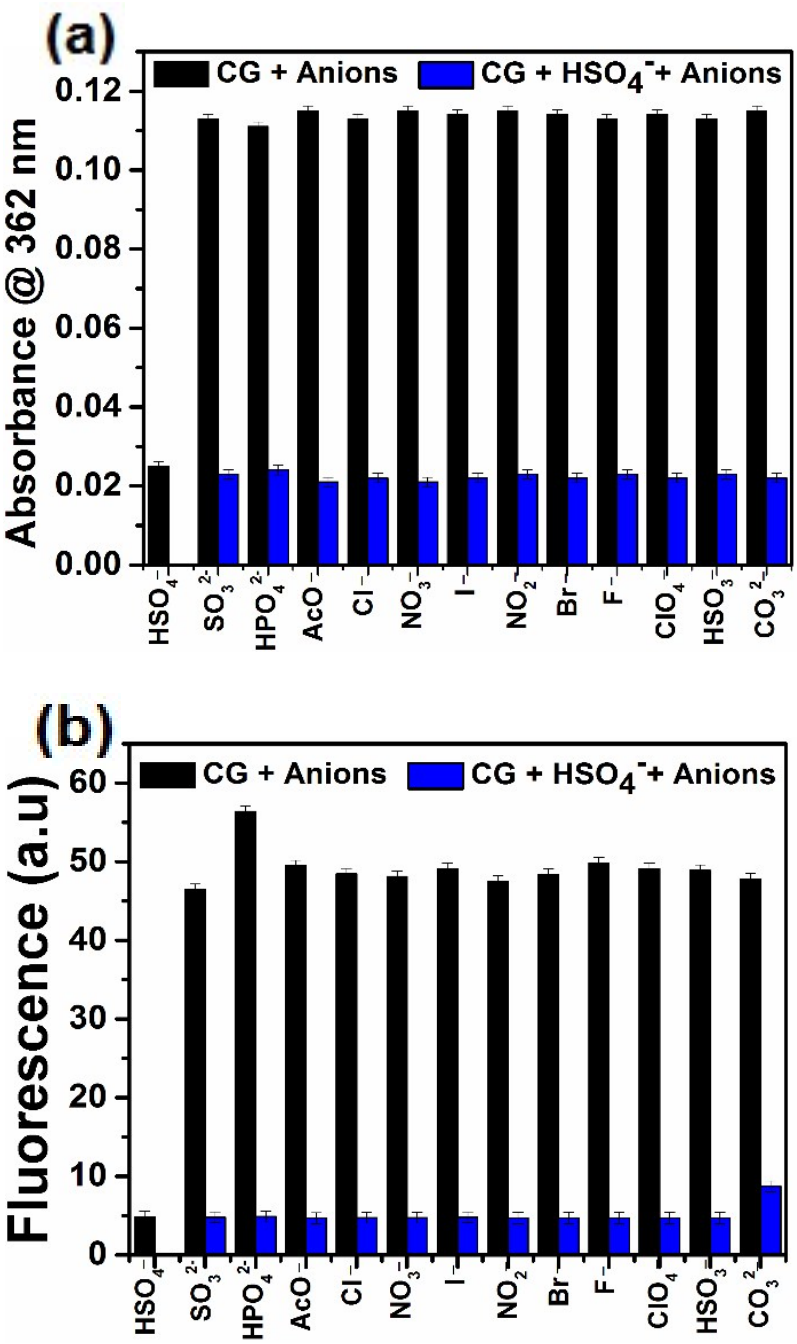

Fig. S12 Results from interference studies based on (a) UV-vis and (b) fluorescence of CG (10 μM). 50 mM aqueous HEPES buffer in presence of 25 nM bisulfate plus each of the oher anions (250 times of the probe) was used in this study. Error bars indicate standard deviations of three data sets.

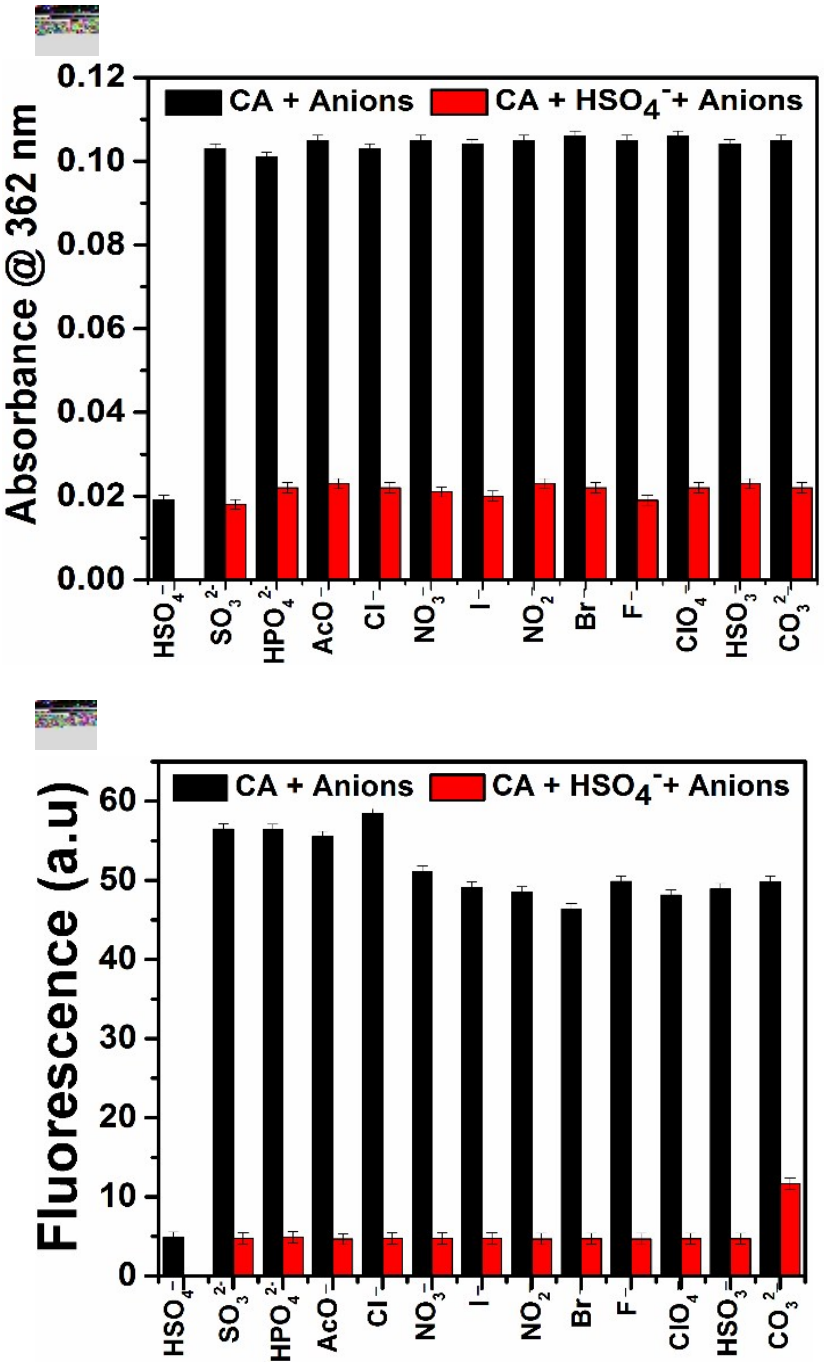

Fig. S13 Results from interference studies of 10 μM CA, which are based on (a) UV-vis and (b) fluorescence. 50 mM aqueous HEPES buffer in presence of bisulfate (25 nM) plus each of the other anions (250 times of the probe) was used in this study. Error bars indicate standard deviations of three data sets.

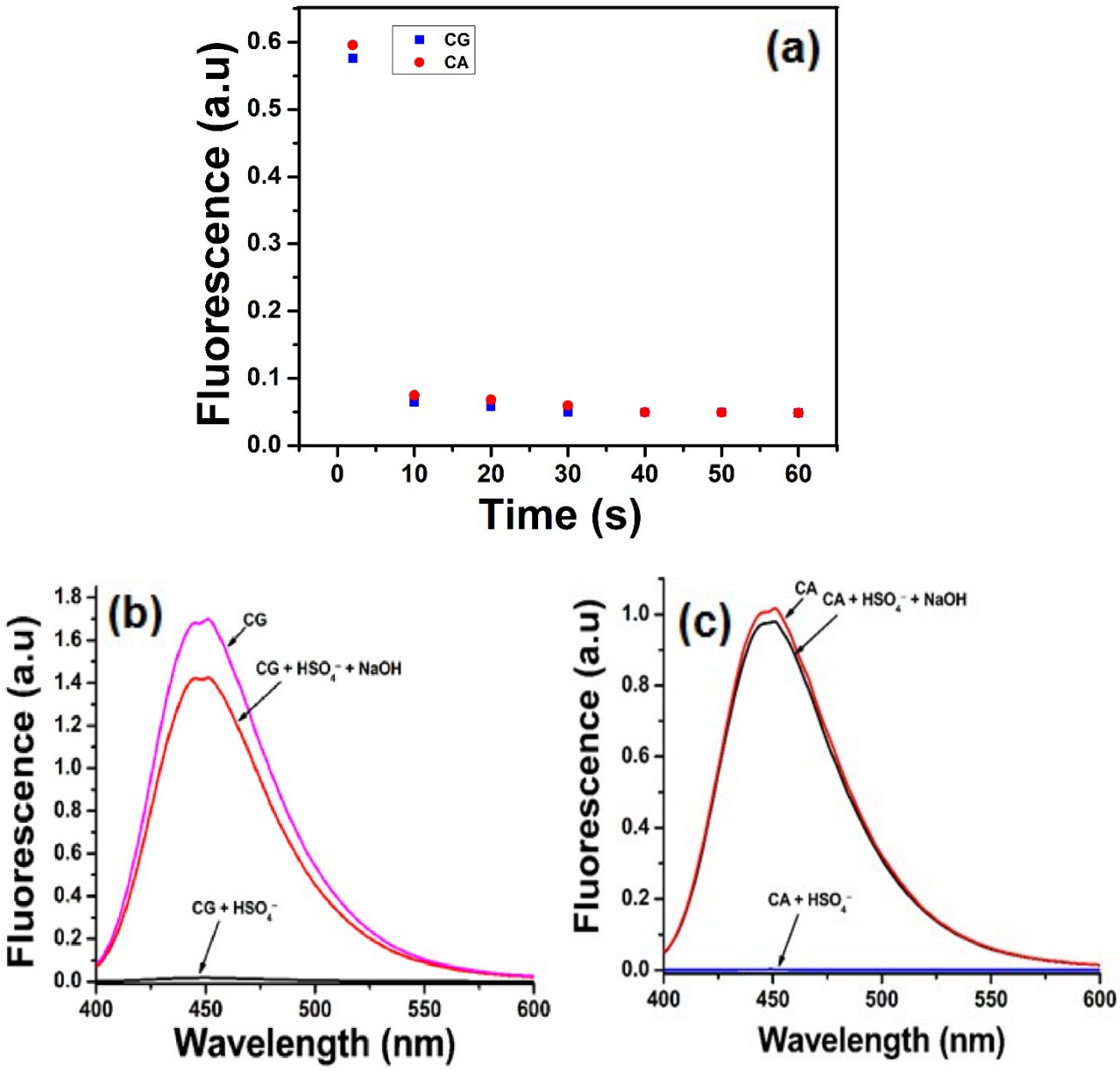

Fig. S14 (a) Time response plots of 10 µM CG and CA (50 mM HEPES buffer) in presence of 25 nM HSO_4^- at λ_{emi}=448 nm. Reversible behaviour of CG (b) and CA (c) in case of HSO_4^- detection.

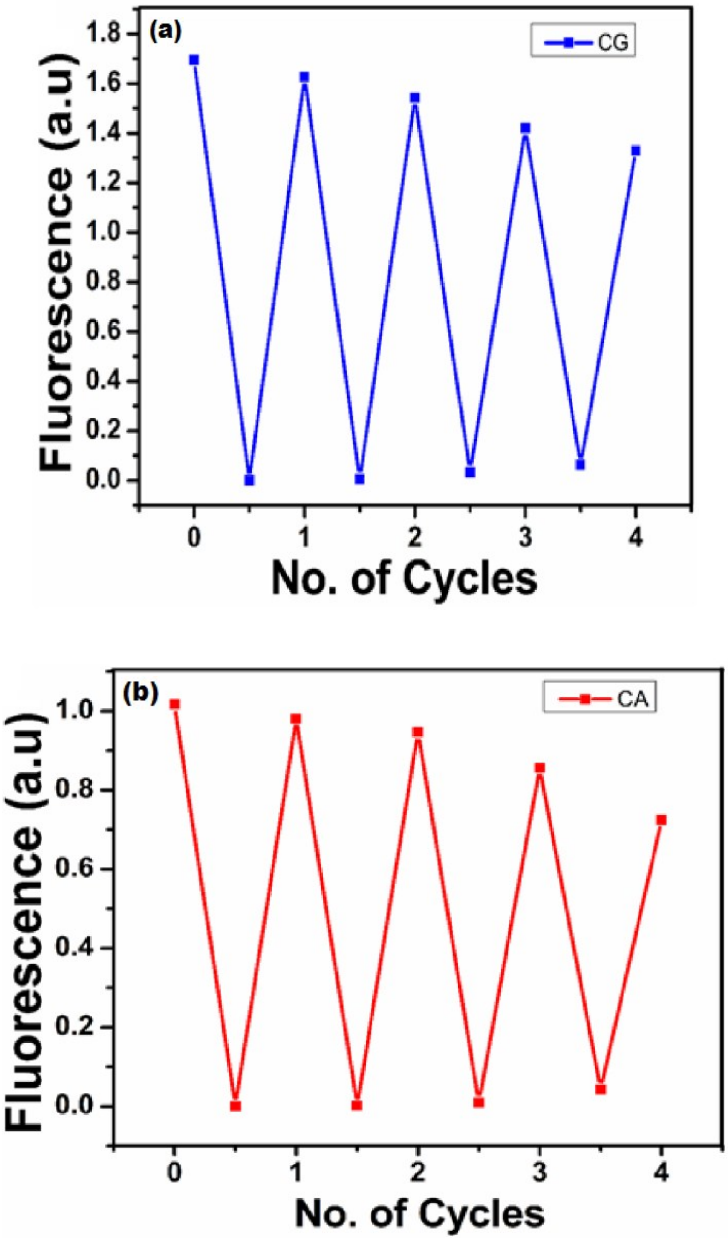

Fig. S15 Recyclability plots of (a) CG and (b) CA in the detection of HSO_4^- using OH^- and the probe, alternatively.

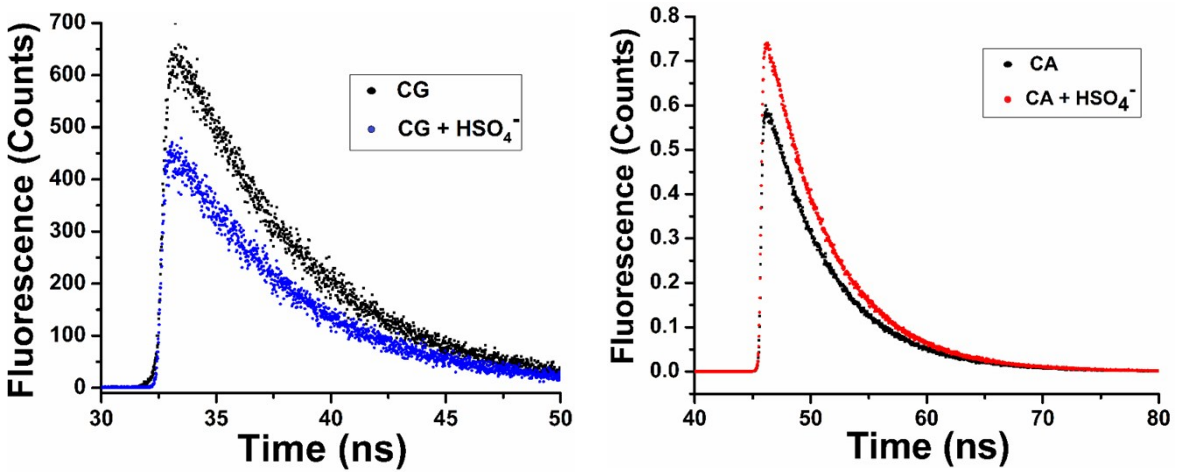

Fig. S16 Time correlated single photon counting (TCSPC) plots of 10 μM CG (left) and 10 μM CA (right) in aqueous HEPES buffer in absence and presence of 5 nM HSO_4^-.

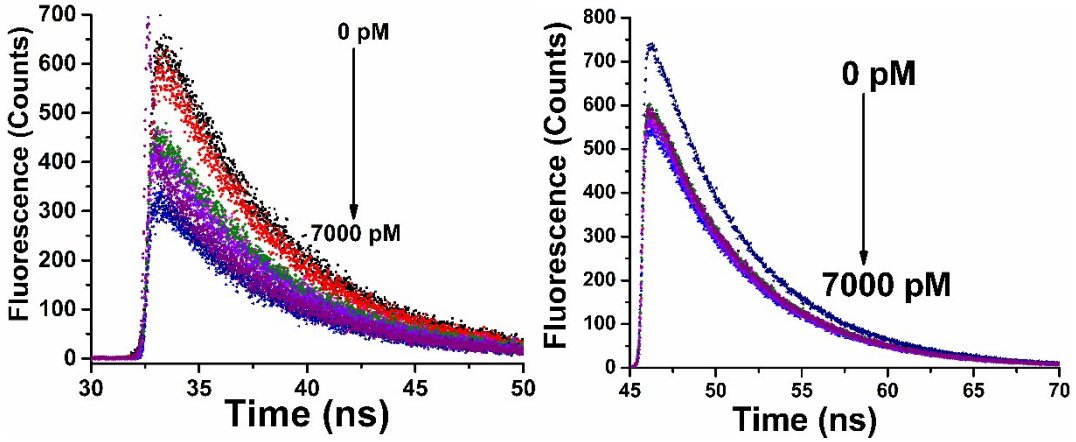

Fig. S17 Time correlated single photon counting (TCSPC) titration plots of 10 μM CG (left) and 10 μM CA (right) in aqueous HEPES buffer with increasing concentrations of HSO_4^- (0 to 7000 pM).

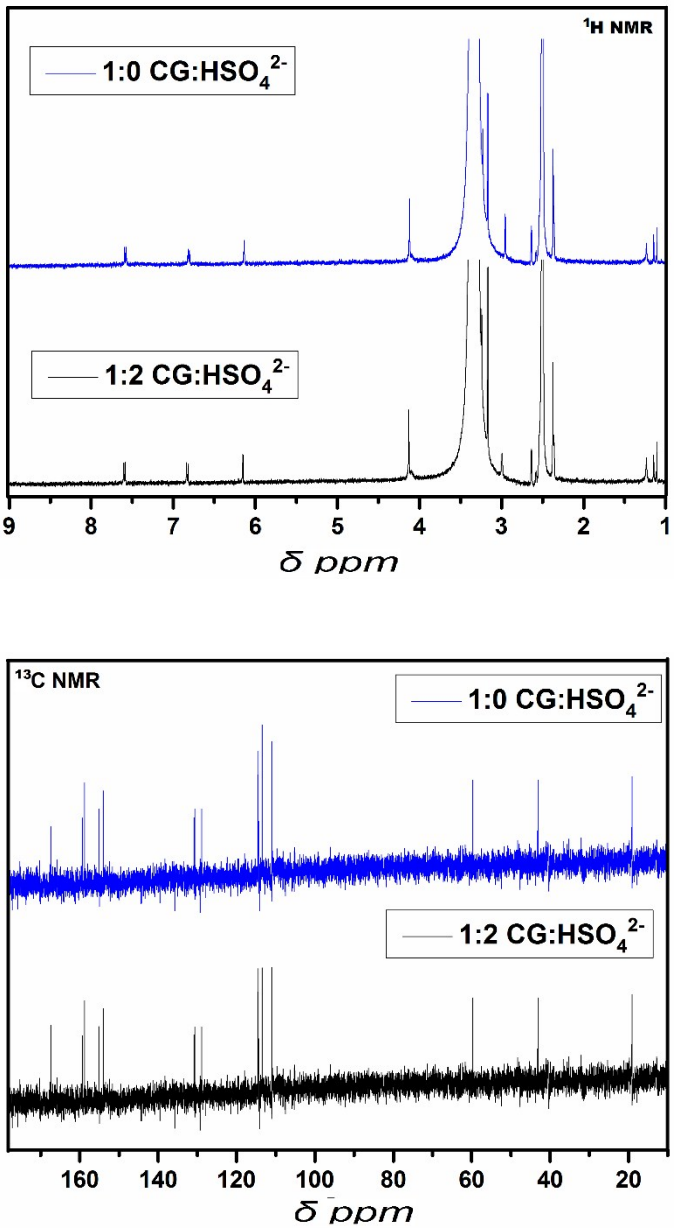

Fig. S18. NMR titration stack plots for (a) ^{1}H NMR of CG and (b) ^{13}C NMR of CG, recorded in DMSO-d_6 and water, respectively.

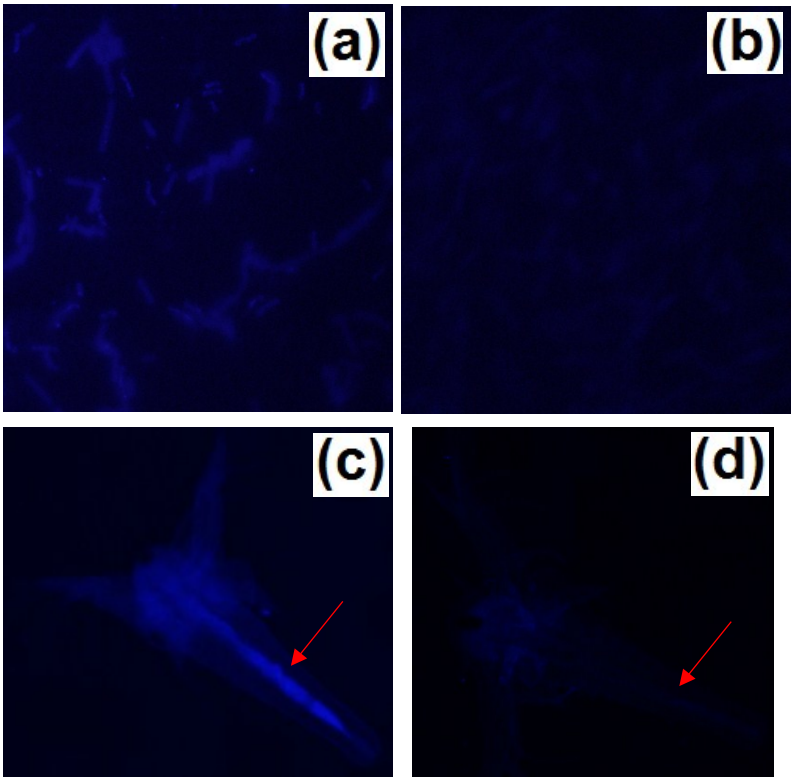

Fig. S19 Fluorescence microscopy images of *Bacillus subtilis* exposed to (a) 10 μM CG and (b) 10 μM CG plus 20 nM bisulfate. Fluorescence microscopy images of live whole *Artemia* (one day aged) after exposing to (c) 20 μM CG solution and (d) 20 μM aqueous solution of CG followed by the addition of 20 nM bisulfate solution. Arrows indicate the positions of the GI-tracts of the shrimps.

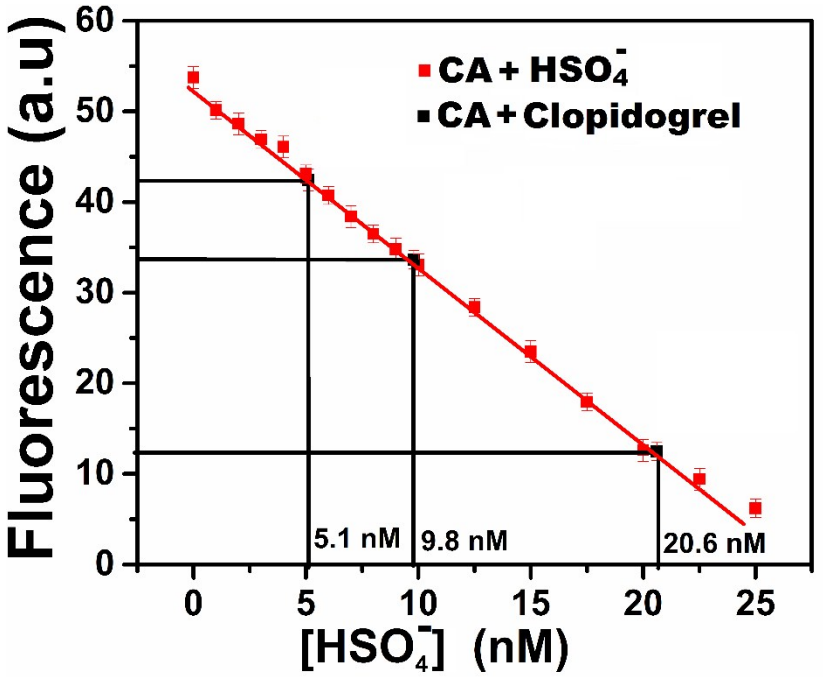

Fig. S20 Estimations of bisulfate in clopidogrel drug using standard plot of CA. Samples were titrated against 10 μM CA (in HEPES aqueous buffer at pH 7.4). Calculated concentrations of bisulfate produced from the drug clopidogrel are 5, 10, and 20 nM, respectively. Error bars indicate standard deviations of three data sets.

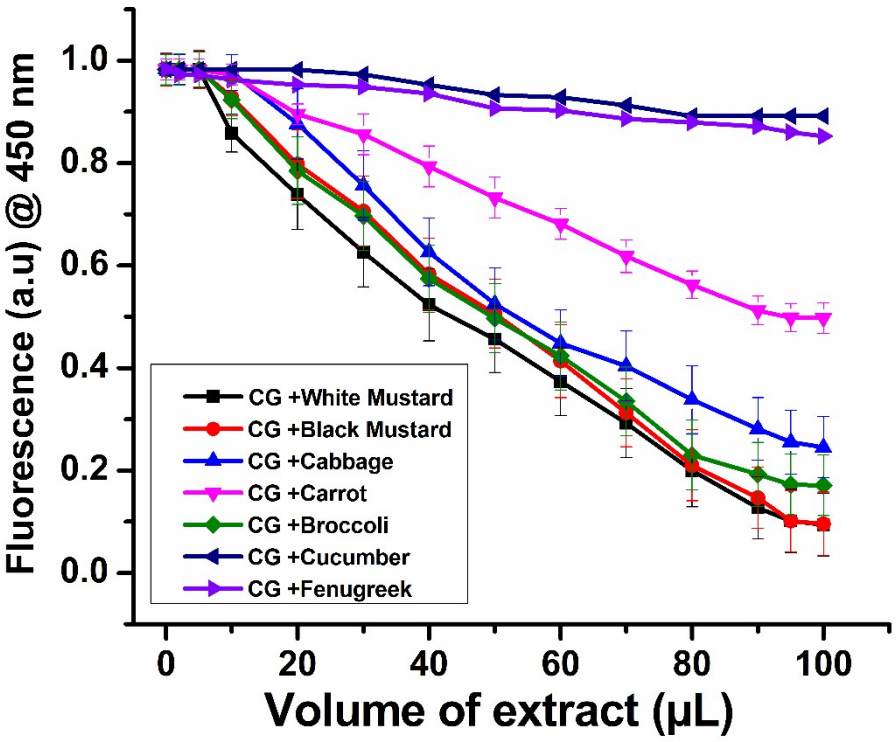

Fig. S21 Measurement of concentrations of bisulfate in different vegetables and seeds using the standard plot of CG. Samples were titrated against 10 µM CG (in HEPES buffer at pH 7.4). Error bars indicate standard deviations of three data sets.

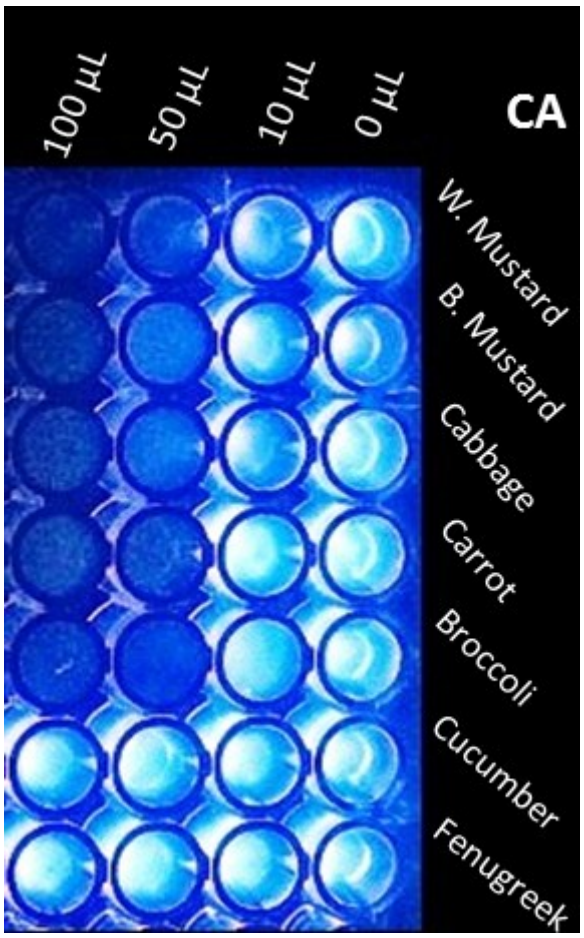

Fig. S22 Partial view of 96 well-plate showing the fluorescence changes of CA with the addition of varying volumes of aqueous extracts of different seeds and cruciferous vegetables. Samples were studied against 10 µM CA (dissolved in HEPES buffer at pH 7.4).

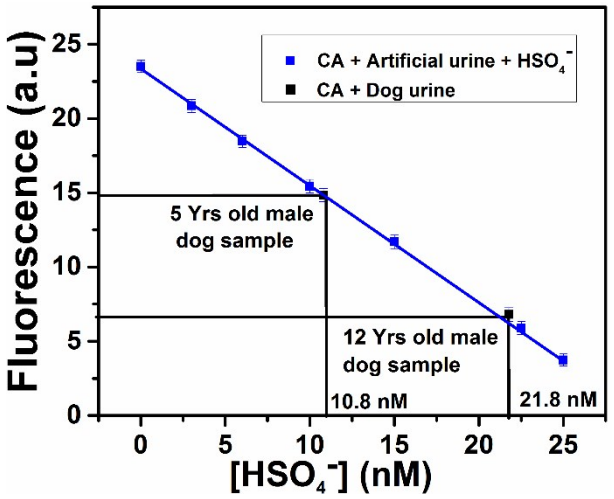

Fig. S23 Estimation of concentrations of bisulfate in dog urine samples using standard plot of CG produced from artificial urine samples. Samples were titrated against 10 μM CG (in HEPES buffer at pH 7.4). Error bars indicate standard deviations of three data sets.

Table S1. Results of toxicity studies of CG/CA with living brine shrimp *Artemia* based on % mortality counts.

Experiment	**Percentage of mortality**			
	Set 1	**Set 2**	**Set 3**	**Mean**
Control (in absence of any probe)	0	0	0	0
CG (10 μM)	1	0	0	0.22
CG (20 μM)	2	2	1	1.66
CA (10 μM)	1	1	0	0.66
CA (20 μM)	2	3	2	2.33

Table S2. Toxicity results of CG/CA against *Basillus subtilis* (CFU/mL).

Experiment	**Counts of *Basillus subtilis***
Control (in absence of any probe)	$8.3x10^9$
CG (20 μM)	$7.4x10^9$
CA (20 μM)	$5.8x10^9$

Table S3. Estimation of bisulfate present in the drug clopidogrel bisulfate by CG and CA.

Receptor	**Calculated concentrations (nM)**	**Found concentrations (nM)**	**Recovery (%)**
CG (10 μM)	5	4.9±0.2	98
	10	10.1±0.2	101
	20	20.4±0.2	102
CA (10 μM)	5	5.1±0.1	102
	10	9.8±0.1	98
	20	20.6±0.1	103

Table S4. Estimation of bisulfate present in dog urine samples.

Probe	Fluorescence intensity of 5 years old male dog's urine sample	Concentration of bisulfate found in 5 years old dog's urine sample	Fluorescence intensity of urine sample of 12 years old male dog	Concentration of bisulfate found in the urine sample of 12 years old dog
CG (10 μM)	88085±4000	10.8±0.3 nM	168460±4100	21.8±0.3 nM
CA (10 μM)	35207±860	10.5±0.2 nM	14302±940	20.1±0.2 nM