Supporting Information

Rapid detection of hydrazine in a naphthol-fused chromenyl loop and its effectiveness in human lung cancer cells: tuning remarkable selectivity *via* the reaction altered pathway supported by theoretical studies

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CONTENTS

❖ **Calculation of the detection limit:**

The detection limit (DL) of **NAC** in emission spectra for N_2H_4 was determined from the following equation[1]:

DL = K* Sb1/S

Where K = 2 or 3 (we take 3 in this case); Sb1 is the standard deviation of the blank solution; S is the slope of the calibration curve.

From the graph Fig.S_1, we get slope = 18.27, and Sb1 value is 27.776.

Thus using the formula we get the Detection Limit for N_2H_4 = 4.5 micromolar in emission.

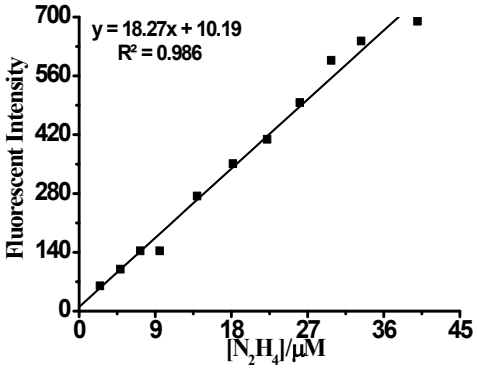

Figure S_1: (a) Changes of fluorescence of **NAC**(c = $2x10^{-5}$M) as a function of [N_2H_4] (c = $2x10^{-4}$M) at 420 nm.

❖ **Evaluation of cytotoxic activity on NCI-H460 cell lines:**

NCI-H460 cells were seeded in 96-well tissue culture plates. After 24 hrs fixed concentration of receptor **NAC** (c = 2x10^{-5}M) mixed with different concentrations of hydrazine (c = 1 x10^{-2}M, 2 x10^{-2} M, 4x10^{-2} M, 5 x10^{-2} M, 6 x10^{-2} M, 8x10^{-2} M, 1 x10^{-1} M, 2 x10^{-1}M) were added to the culture medium and incubated for 24 hrs at 37^{0}C. Non-treated cells were used as control. All the experiments were performed in triplicate. Some of the wells with cells were kept as DMSO and hydrazine control. Incubated cultured cells were then subjected to tetrazolium salt 3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide (MTT) colorimetric assay (1). The MTT is used to determine cell viability and cytotoxicity. MTT was added at a final concentration of 0.5mg/ml and the cells were incubated at 37^{0}C for 3.5 hrs. Then formazon dissolved with 100µL of DMSO in each well. The color changes were measured using a ELISA reader (Robonik, Readwell touch ELISA PLATE analyzer, India). The rate of survival was determined by using the following formulae: Cell viability (%) = (1-ODA$_1$/OD A$_o$)/100, where Ao=Absorbency of control cells and A$_1$=Absorbency of treated cells.

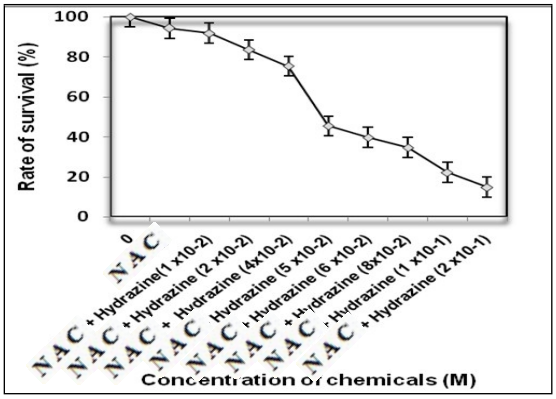

Figure S$_2$: The rate of survival of cells with different concentrations of N_2H_4 added to

NAC-incubated cell. The results are derived from three different experiments (SD<5).

After 5-6 hrs of treatment, fluorescence has been detected. But as the receptor when complexes with hydrazine, the cells started senescing within 3-4 hrs.

- **Evaluation of cytotoxic activity on NCI-H460 cell lines with NAC and N_2H_4 separately:**

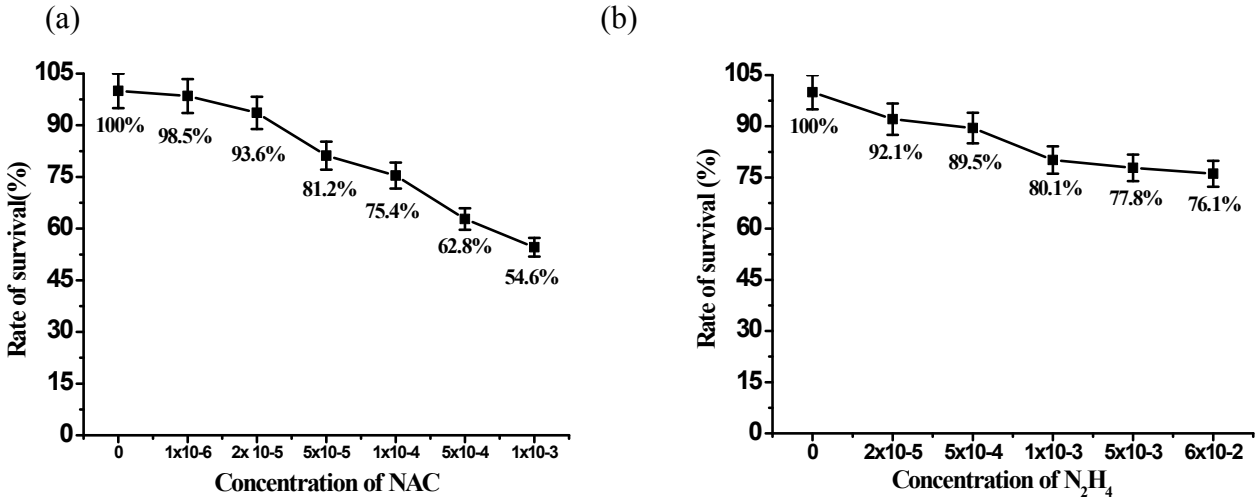

Figure S_3: (a) The rate of survival of cells with different concentrations of **NAC**. (b)The rate of survival of cells with different concentrations of N_2H_4 . The results are derived from three different experiments (SD<5).

It had been shown that when the concentration of **NAC** was $2x10^{-5}$ M and the concentration of Hydrazine was $6x10^{-2}$M, the survivability of cells was 39.77% . As per the new cytotoxicity data, the survivabilities of cells treated with NAC are 93.6% and cells treated with hydrazine are 76% respectively (Figure S_3). Therefore, it may be suggested that the survivability of cells decreased due to the additive effect of NAC & Hydrazine adduct (**NAP**)(Figure S_2).

(a) (b)

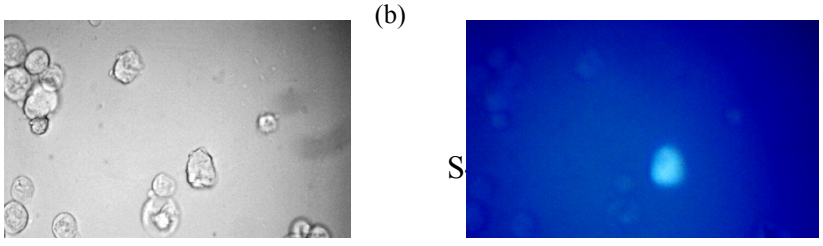

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Figure S_4 : Fluorescence images of NCI-H460 cells. (a) in presence of **NAC** (c=2x10^{-5} M**)** and Hydrazine (*c* = 1x10^{-4} M) (Bright field image)**.** (b) Corresponding dark field image.

- **The changes of emission curve of NAC(*c* =2x10^{-5}M) at different time interval by addition of N_2H_4 (*c* =2x10^{-4}) and calculation of first order rate constant:**

Fig S_5 (a) represents the changes of emission at different time interval by addition of N_2H_4.

From the time vs. emission intensity plot (Fig.S_5(b)) at fixed wavelength at 420 nm by using first order rate equation we get the rate constant K=slope x 2.303= 0.005 x 2.303=1.15 x 10^{-2} Sec^{-1}.

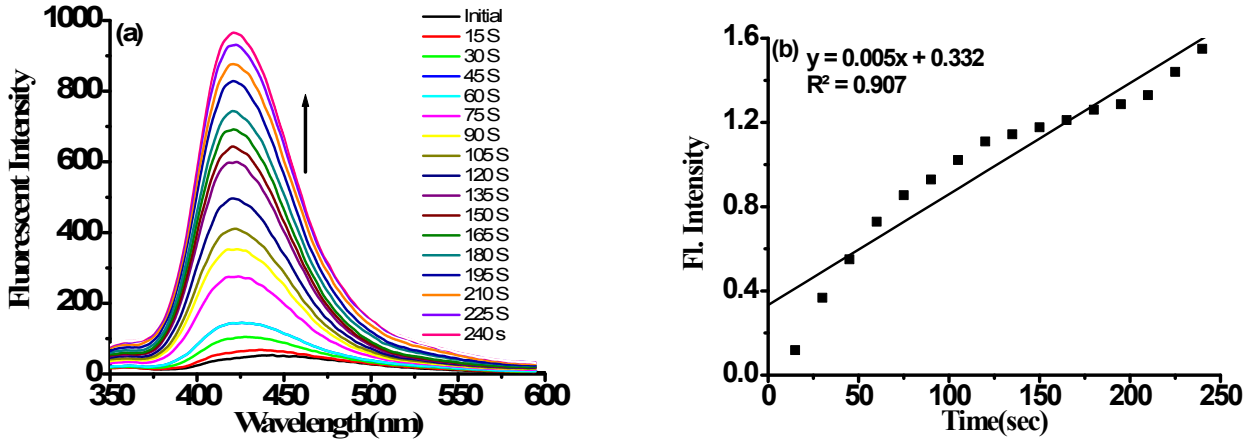


Figure S_5: (a) The changes of emission spectra at different time intervals of **NAC** in presence of N_2H_4 in CH_3CN: HEPES buffer solution (v:v, 50:50) at pH 7.4. **Inset:** Different time intervals are shown in the rectangle ('S' denotes Second). (b) The first order rate equation by using Time vs. fluorescent intensity plot at 420 nm.

❖ **UV–vis spectra of NAC with N_2H_4:**

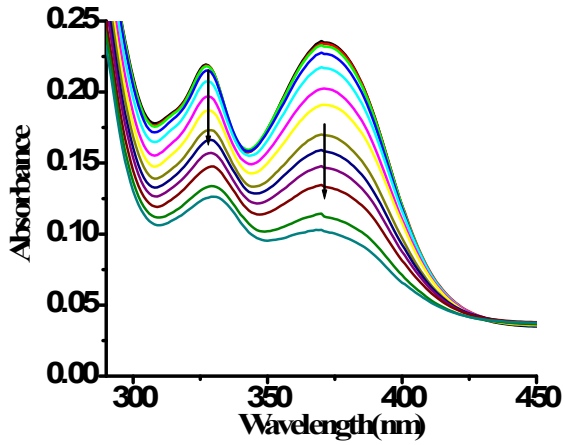


Figure S_6: UV–vis absorption spectra of **NAC** (c = 2.0 ×10^{-5} M) in CH_3CN:HEPES buffer solution (v:v, 50:50) at pH 7.4. solution upon titration with N_2H_4 (c = 2×10^{-4}M).

P^{H} titration:

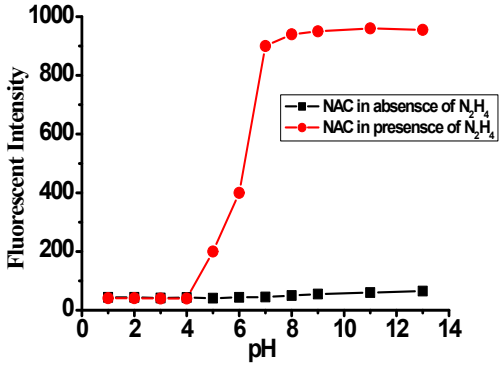


Figure S_7: The variation of fluorescence intensity at 420 nm of **NAC** in absence and in

presence of N_2H_4 as a function of different pH.

- **Application in real water samples:**

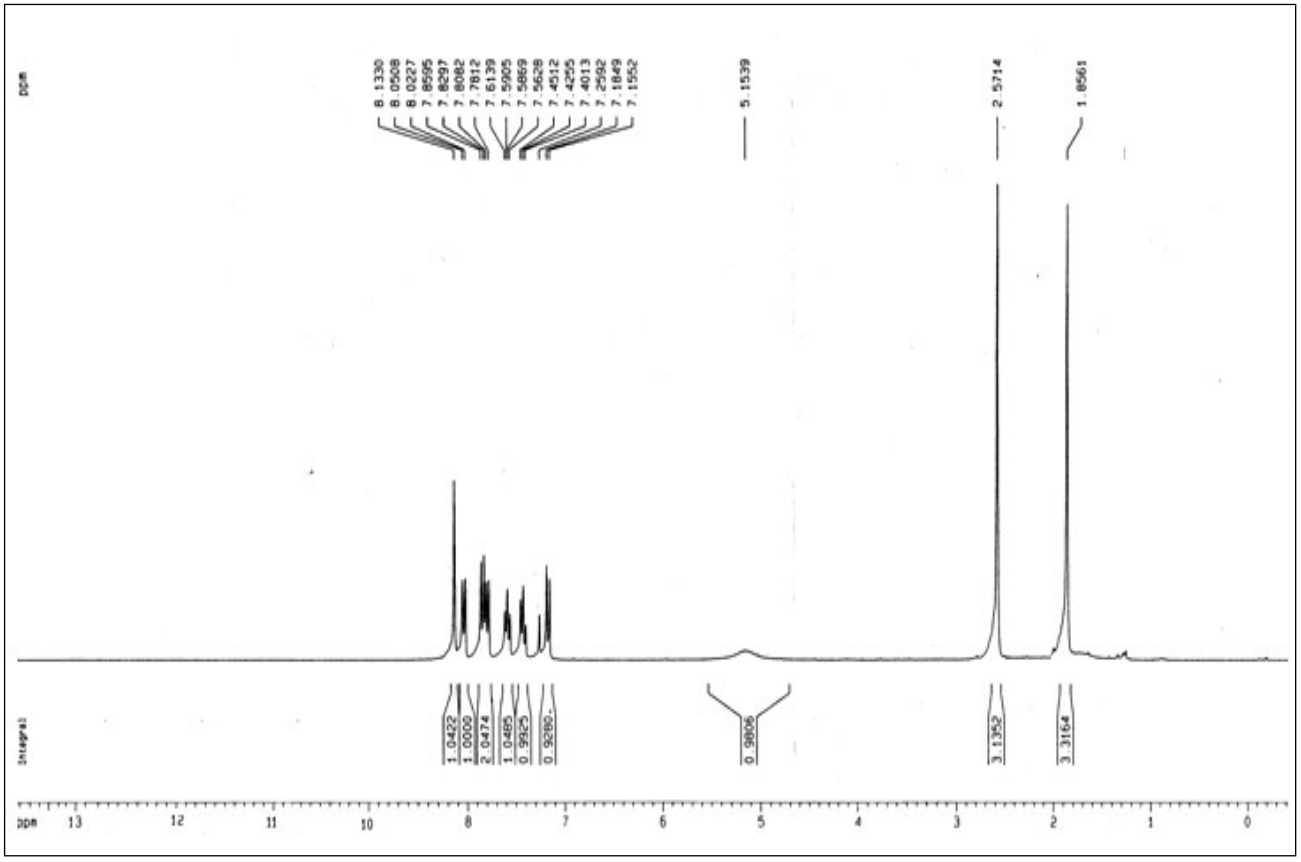
At first, **NAC** (20 μM) solution was prepared by using different water samples in CH_3CN: H_2O (v:v, 50:50) at pH 7.4 and the solution was treated with different concentration of hydrazine and left for sometime. The fluorescence intensity of each sample was recorded and the comparing curve Fig 5(a) was obtained at wavelength 420 nm.

Table S_1. Determination of hydrazine in water samples:

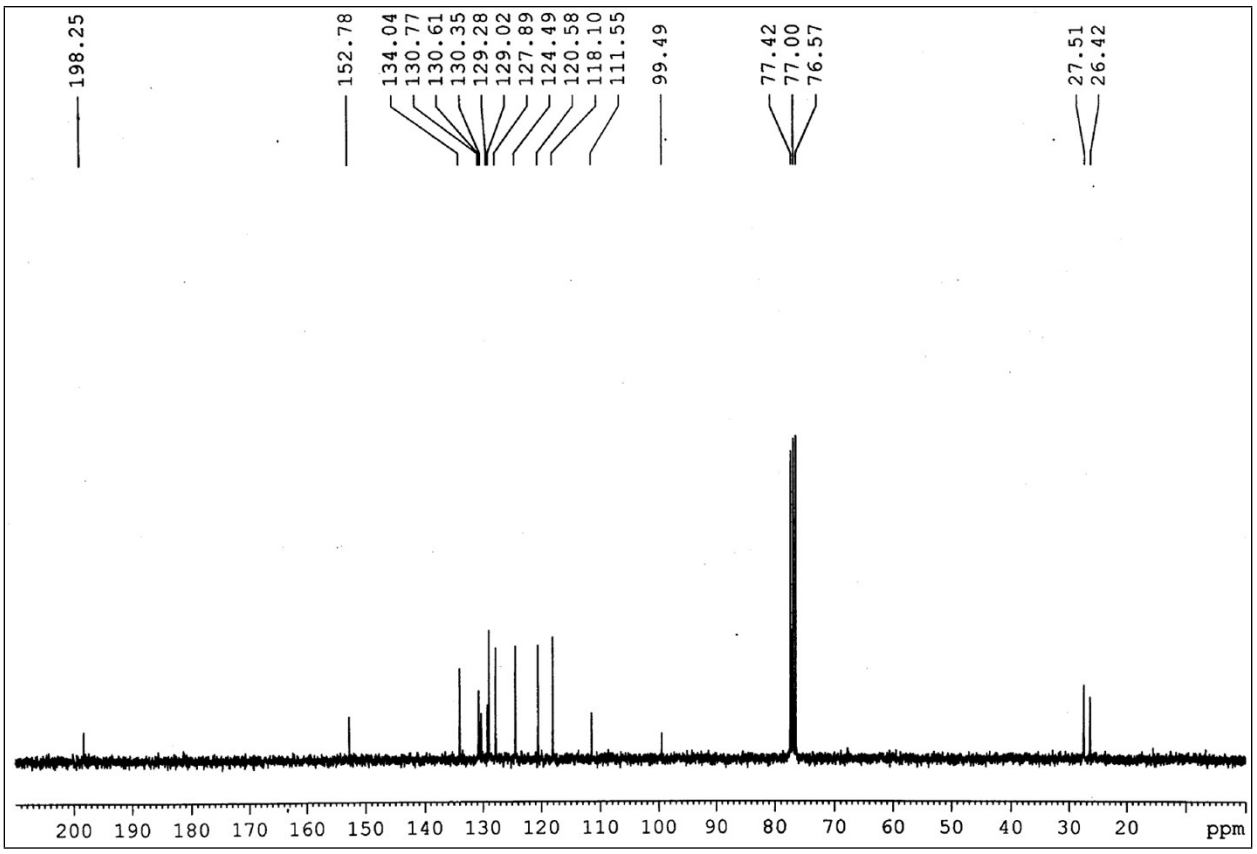
Water samples	**Hydrazine added(μM)**	**Found (μM)**	**Recovery (%)**
Drinking water	30	29.5	98.3
	50	48.3	96.6
	70	72	102.8
	30	28.9	96.3

River water	50	49	98
	70	71.5	102.1

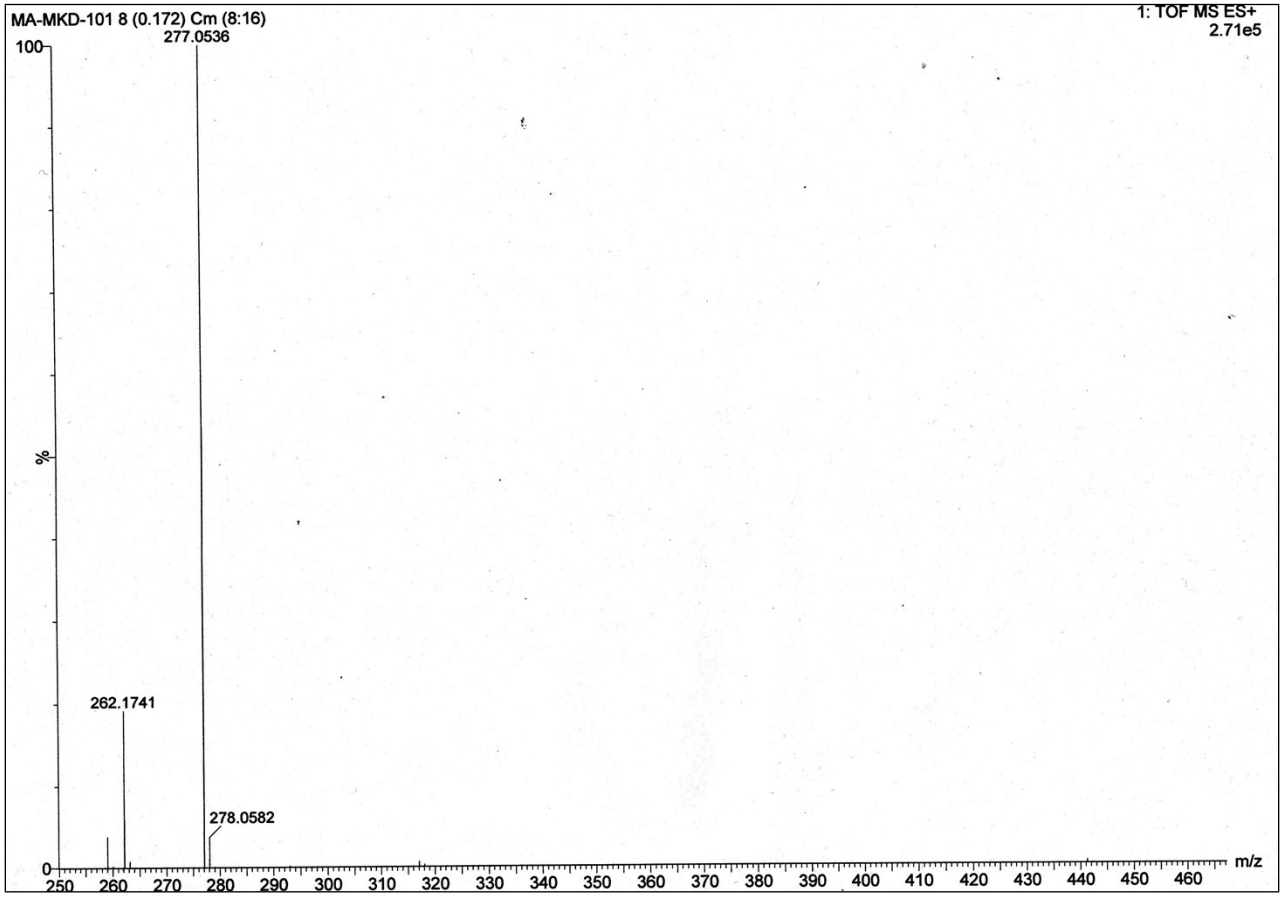
❖ **^{1}H NMR spectrum (S_8) of NAC:**



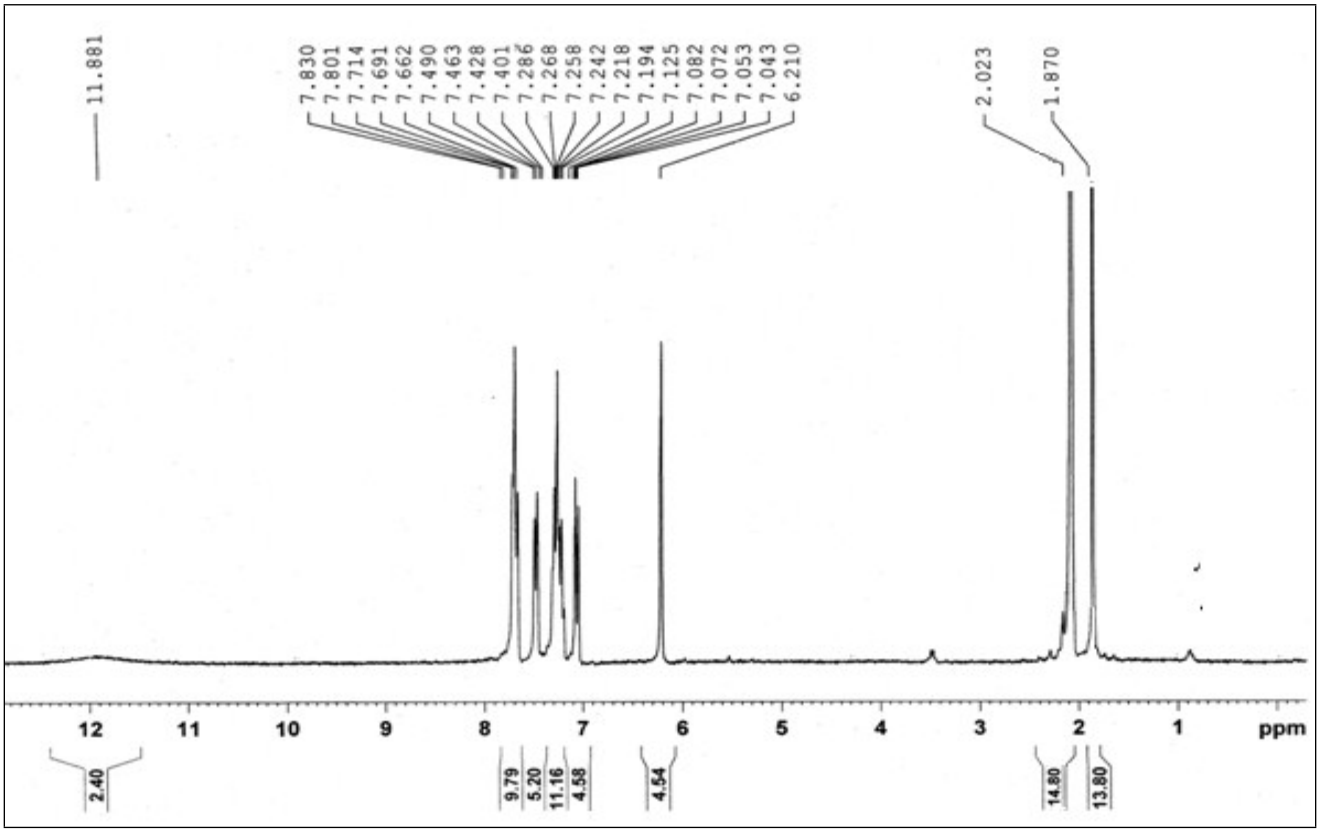
^{13}C NMR spectrum (S_9) of Compound NAC:



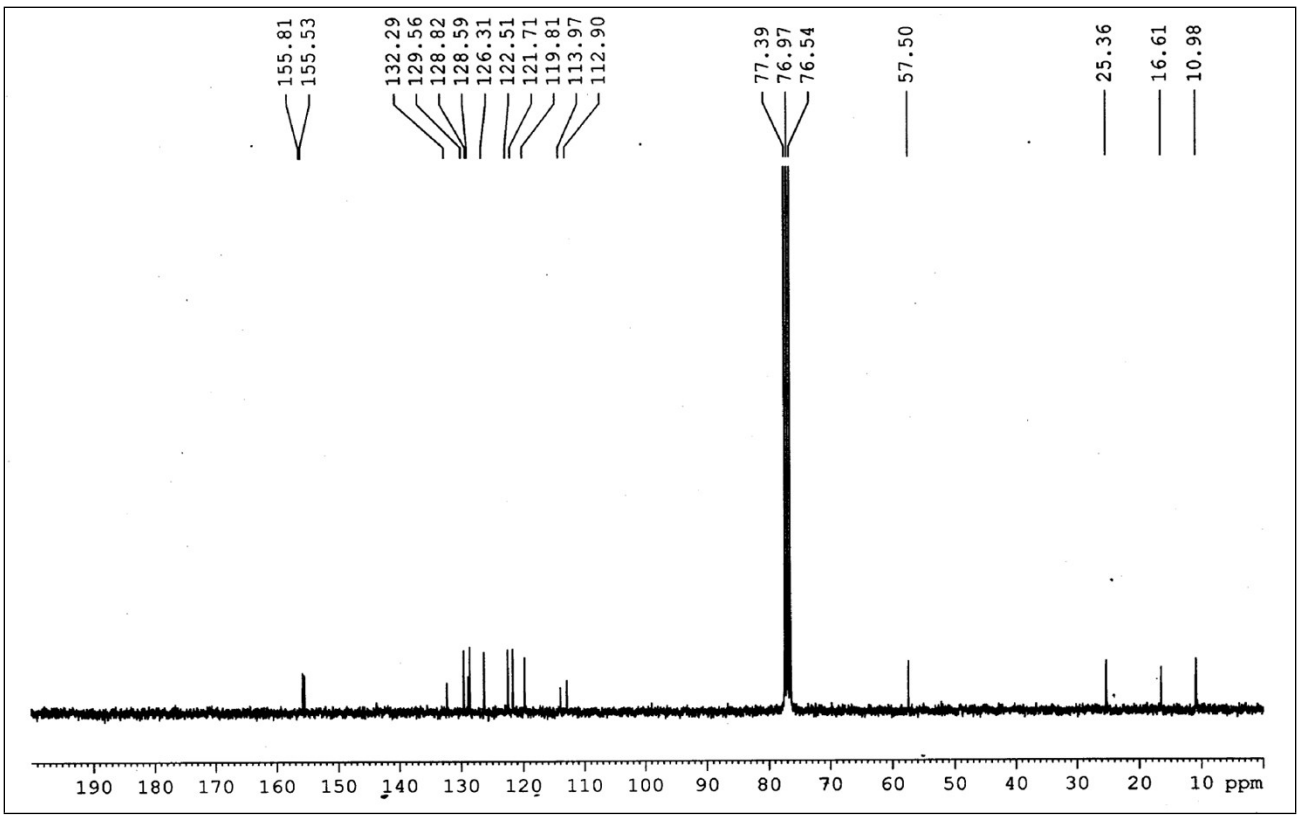
❖ **Mass spectrum (S_{10}) of Sensor NAC:**



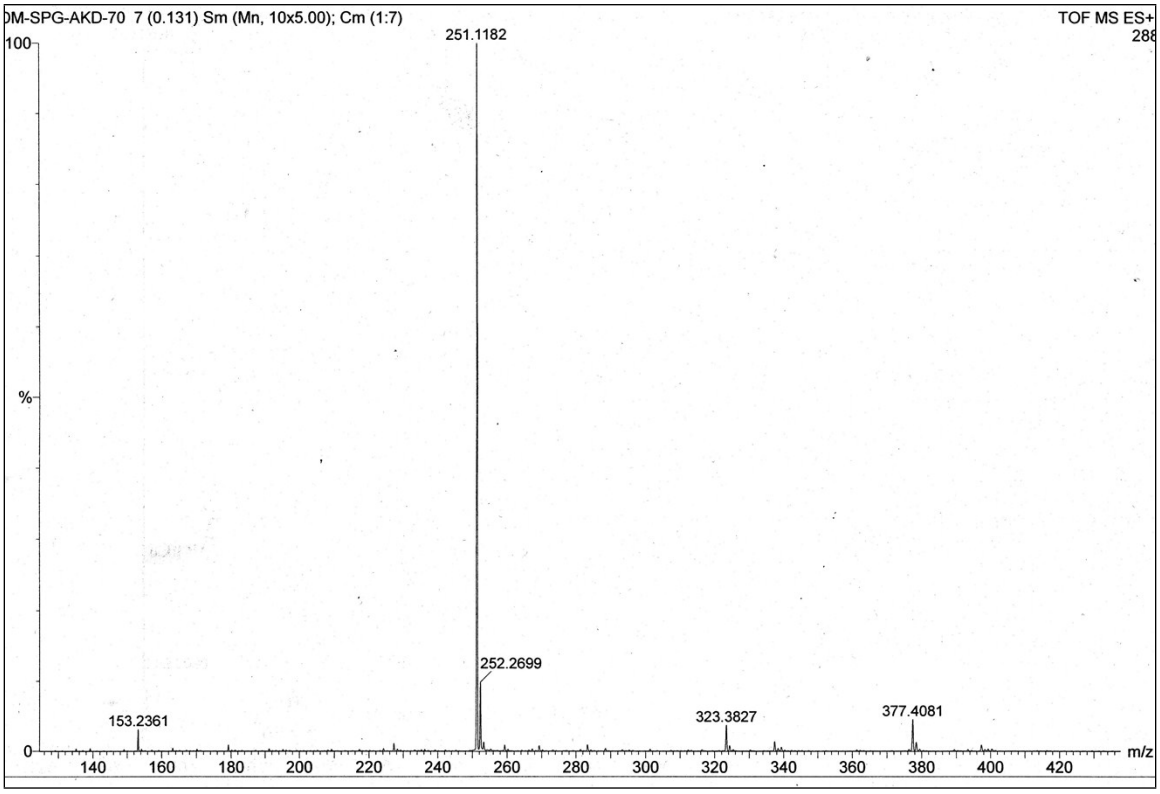
❖ **^{1}H NMR spectrum (S_{11}) of NAC+hydrazine adduct (NAP):**



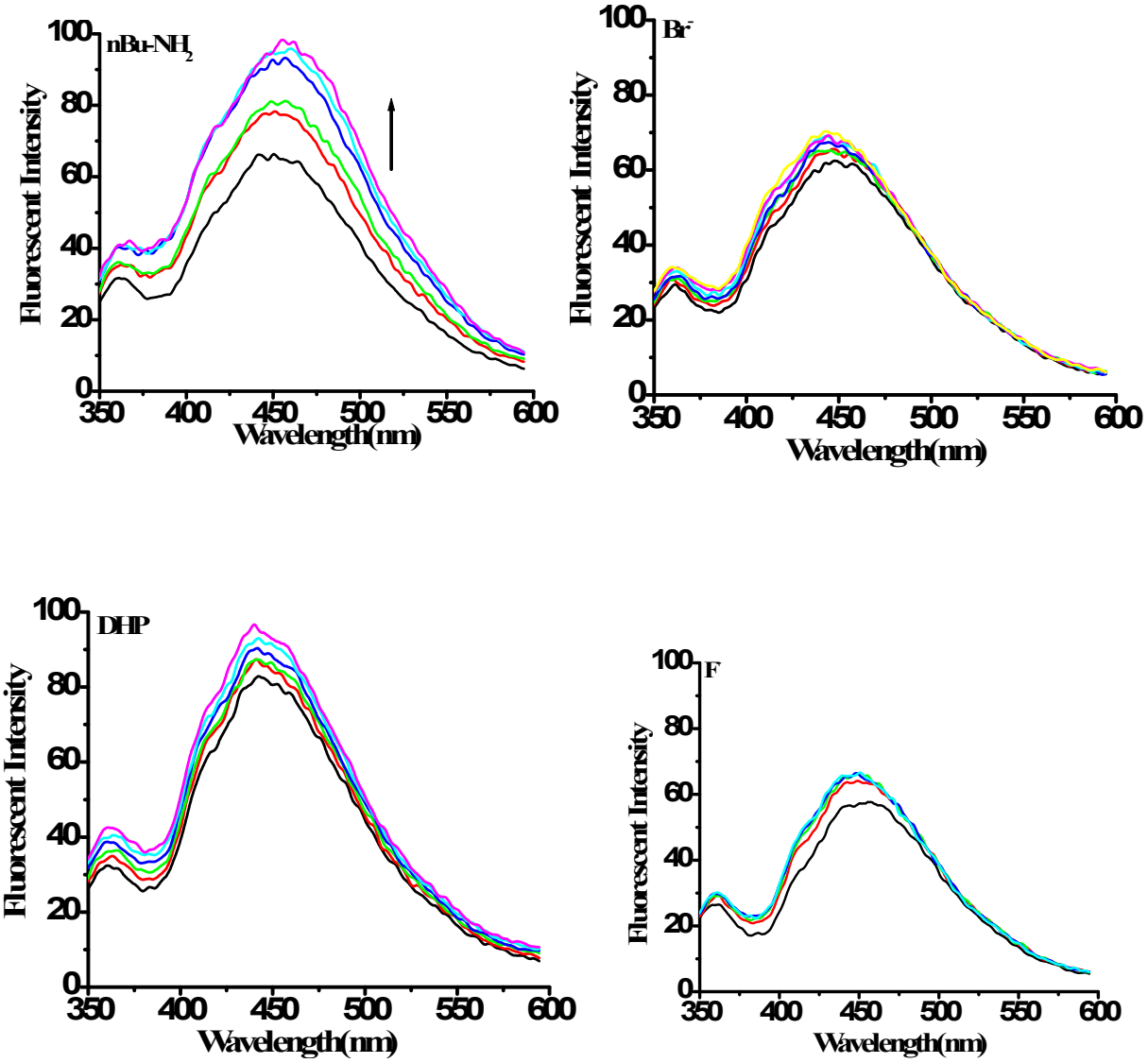
❖ **^{13}C NMR spectrum (S_{12}) of Compound NAP:**

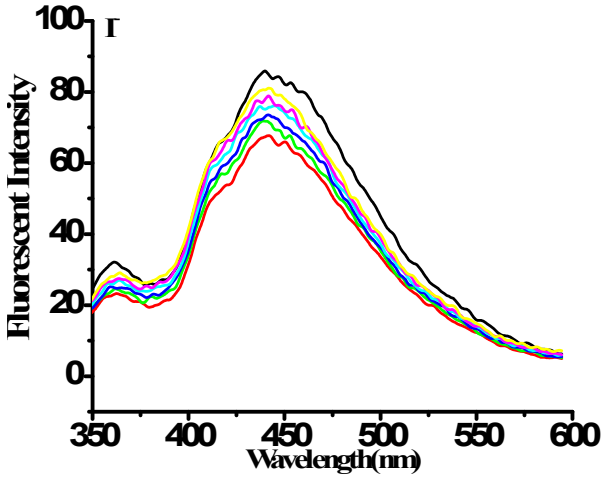


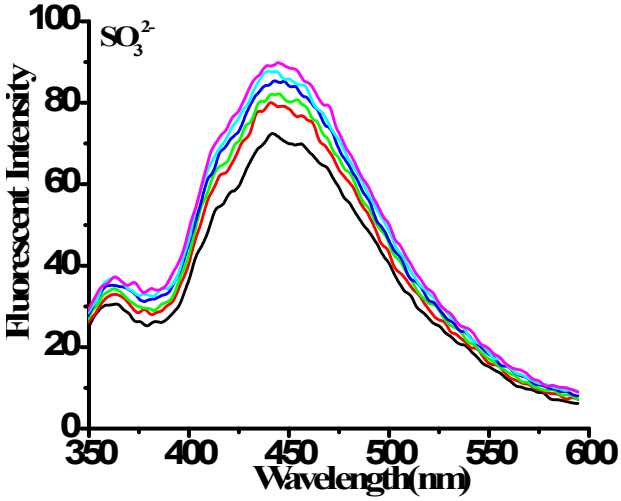
❖ **Mass spectrum (S_{13}) of Sensor NAP:**

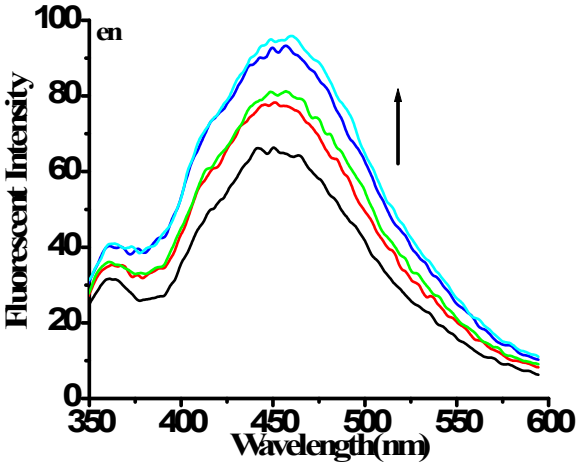


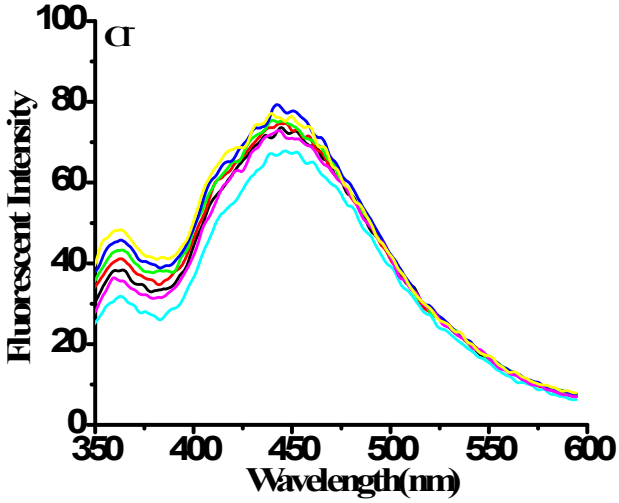
❖ **Fluorescence spectra of NAC (S_{14})(c = 2x10^{-5}M) with different analytes (c = 2x10^{-4}M) in CH_3CN-HEPES buffer (50/50, v/v, 25 ° C) at pH-7.4:**

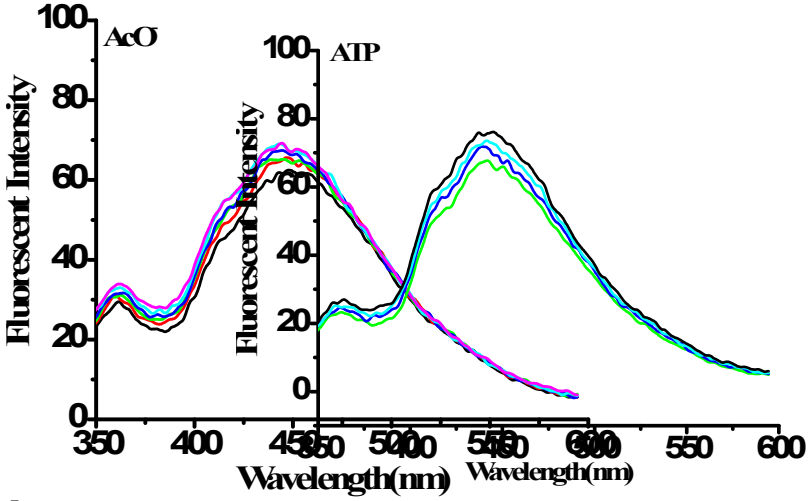


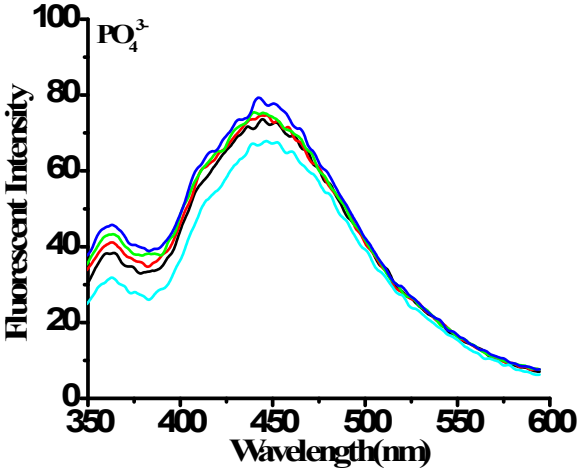

I⁻
Fluorescent Intensity
Wavelength(nm)

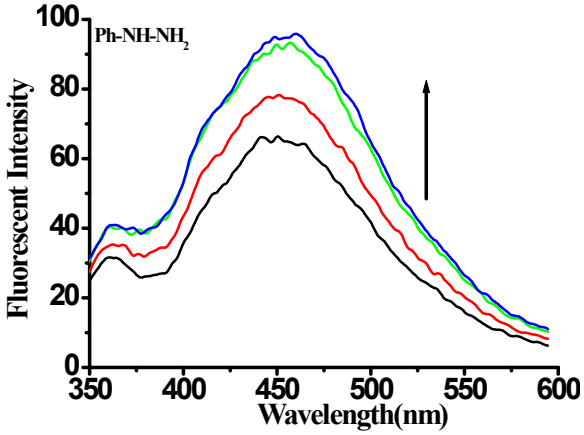

SO3 2-
Fluorescent Intensity
Wavelength(nm)


en
Fluorescent Intensity
Wavelength(nm)


Cl⁻
Fluorescent Intensity
Wavelength(nm)


AcO⁻
ATP
Fluorescent Intensity
Wavelength(nm)


PO4 3-
Fluorescent Intensity
Wavelength(nm)
0
20
40
60
80
100
350
400
450
500
550
600


Ph-NH-NH2
Fluorescent Intensity
Wavelength(nm)
0
20
40
60
80
100
350
400
450
500
550
600

Electronic transition	Composition	Excitation energy	Oscillator strength (*f*)	CI	Assign	λ_{exp} (nm)
$S_0 \rightarrow S_5$	HOMO-2 → LUMO	3.2748eV	0.0809	0.26601	ILCT	372
	HOMO → LUMO+1	(378 nm)		0.32052	ILCT	
	HOMO-3 → LUMO			0.25961	ILCT	
	HOMO-1 → LUMO			0.46771	ILCT	
$S_0 \rightarrow S_7$	HOMO-1 → LUMO	3.7220 eV	0.0940	0.66071	ILCT	327
	HOMO → LUMO+3	(333 nm)		0.18316	ILCT	
	HOMO-2 → LUMO			-0.15307	ILCT	

Theoretical calculation:

Table S_2 Selected Parameters for the vertical excitation (UV-vis absorptions) of **NAC**; electronic excitation energies (eV) and oscillator strengths (*f*), configurations of the low-lying excited states of **NAC**; based on optimized ground-state geometries (UV-vis absorption) by TDDFT/CPCM method (CH_3CN used as solvent).

Table S_3 Main calculated optical transition for the **NAP** with composition in terms of molecular orbital contribution of the transition, vertical excitation energies, and oscillator strength in acetonitrile solvent based on TDDFT/CPCM method.

Electronic transition	Composition	Excitation energy	Oscillator strength (*f*)	CI	Transition assigned	λ_{exp} (nm)
$S_0 \rightarrow S_{10}$	HOMO-3 → LUMO	3.3583 eV	0.0323	0.41639	ILCT	372
	HOMO-2 → LUMO	(369 nm)		0.56569	ILCT	
$S_0 \rightarrow S_{13}$	HOMO-3 → LUMO	3.7183eV	0.0441	0.10983	ILCT	327
	HOMO-3 → LUMO+1	(313 nm)		0.31383	ILCT	
	HOMO-2 → LUMO+1			0.46470	ILCT	
	HOMO → LUMO+1			0.22641	ILCT	
	HOMO → LUMO+2			0.18321	ILCT	

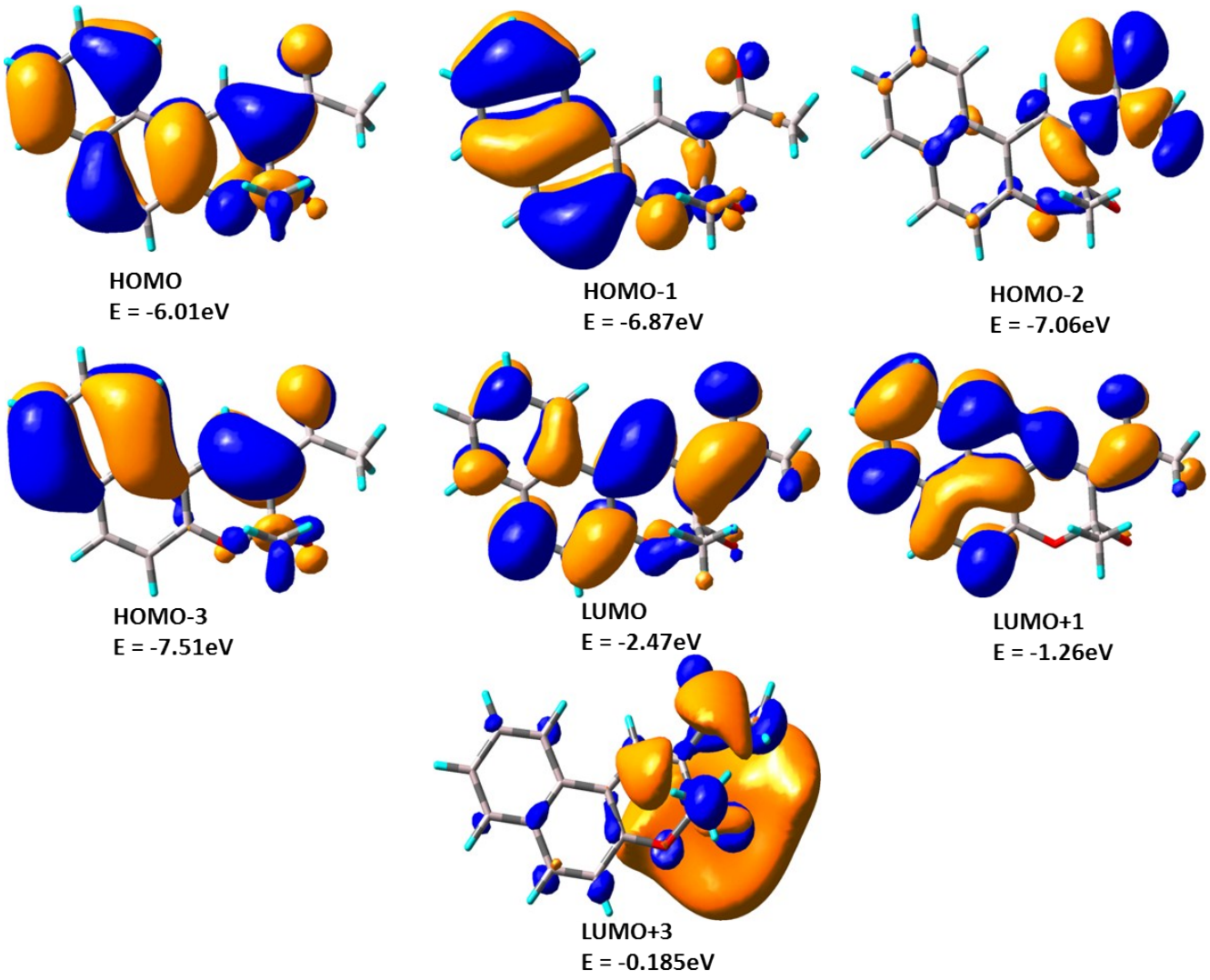


Figure S_{15}: Frontier molecular orbitals involved in the UV-vis absorption of **NAC**.

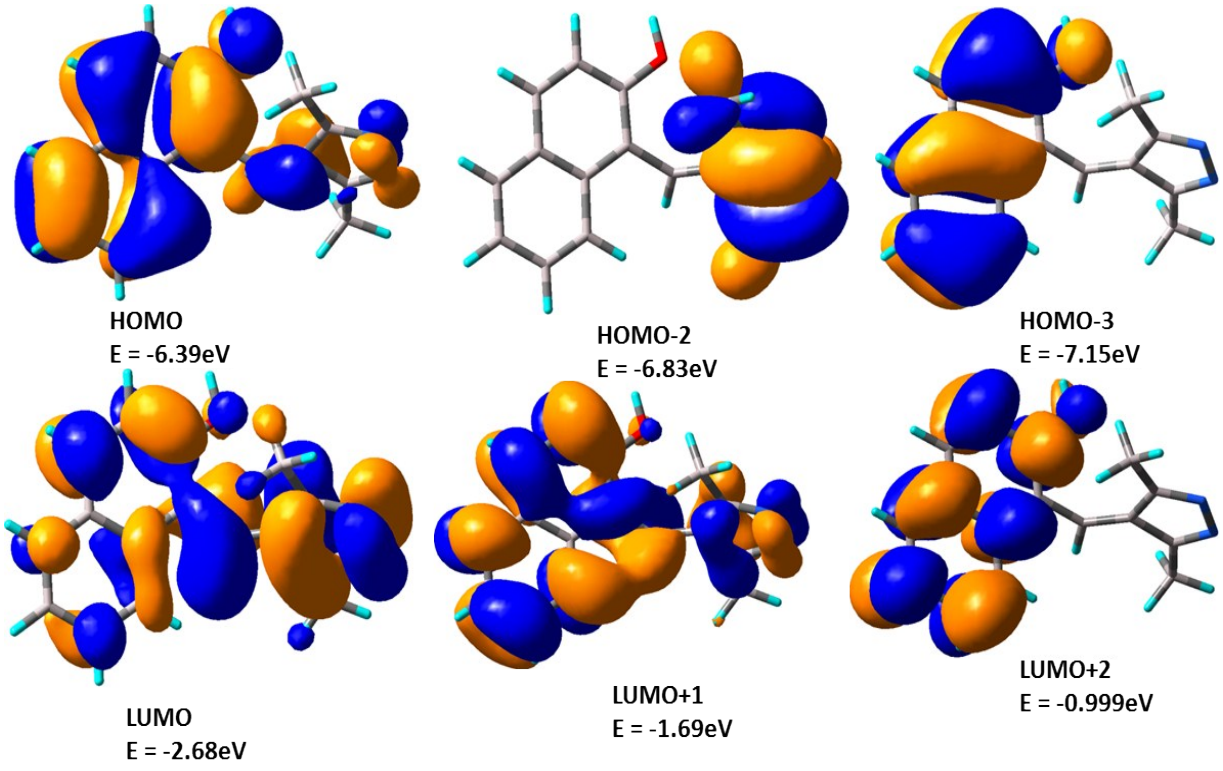


Figure S_{16}: Frontier molecular orbitals involved in the UV-vis absorption of **NAP**.

Ref:

1. M. Shortreed, R. Kopelman, M. Kuhn, B. Hoyland, *Anal. Chem*., 1996, **68**, 1414. (b) W. Lin,; Yuan, L.; Cao, Z.; Feng, Y.; Long, L. *Chem. Eur. J*. 2009, **15**, 5096.(c) Zhu, M.; Yuan, M.; Liu, X.; Xu, J.; Lv, J.; Huang, C.; Liu, H.; Li, Y.; Wang, S.; Zhu, D. *Org. Lett.* 2008, **10**, 1481-1484.