Supplementary Information

A switch-on fluorophore by water molecules via hydrogen bonding and its application of bio-imaging of formaldehyde in living cells

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1. Fluorescence spectra of CHa1

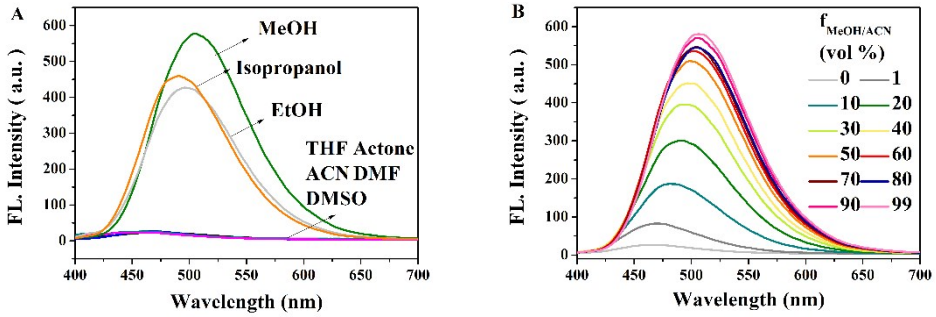

Fig S1. Fluorescence spectra of **CHa1** (10 μM) (A) in different solvents, (B) in MeOH/ACN mixtures with different MeOH fractions. (λ_{ex} = 363 nm)

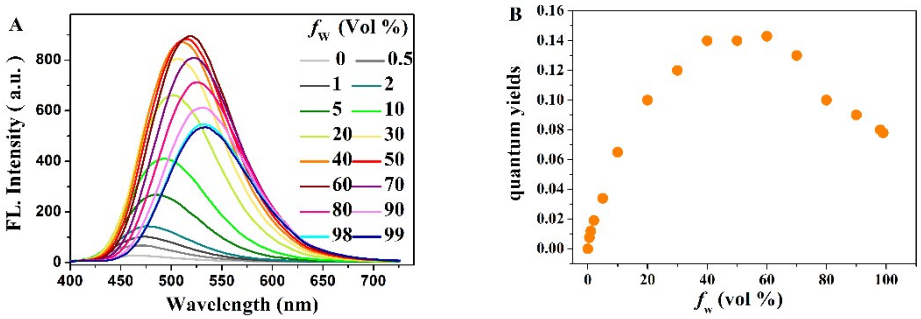

Fig S2. (A) Fluorescence spectra of **CHa1** (10 μM) in ACN/H_2O mixtures with different f_w. (B) Fluorescence quantum yields of **CHa1** (10 μM) in ACN/H_2O mixtures with different f_w. (λ_{ex} = 363 nm)

2. ^{1}H NMR spectra of CHa1

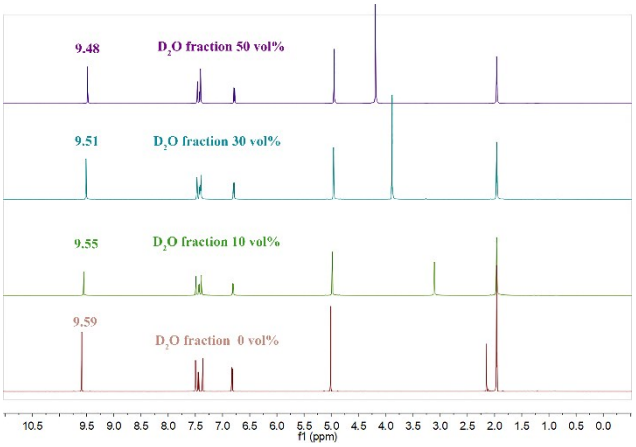

Fig S3. ^{1}H NMR spectra of **CHa1** (5 mg/mL) in Acetonitrile-d_3/D_2O mixture with different D_2O fractions (0, 10, 30, 50 vol%).

3. Temperature effect in fluorescence intensity of CHa1

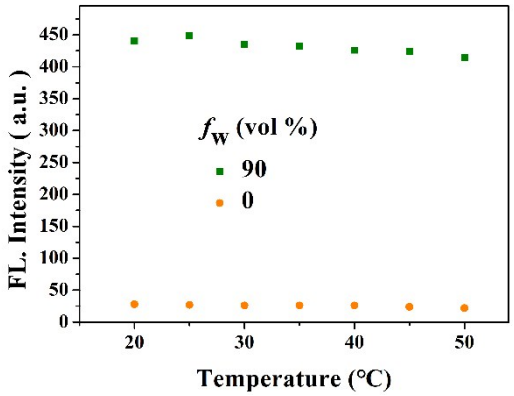

Fig S4. Fluorescence spectra of **CHa1** (10 μM) at different temperature in H_2O/ACN (90 : 10, v/v) system. (λ_{ex} = 363 nm).

4. pH effect in fluorescence intensity of CHa1

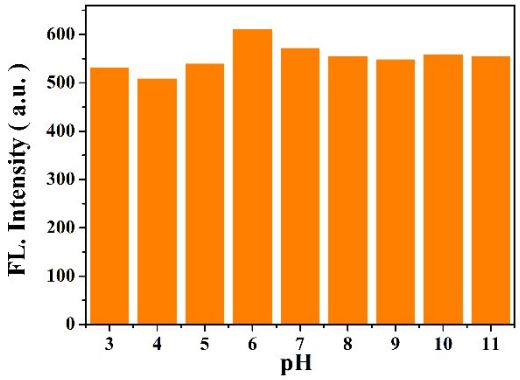

Fig S5. Fluorescence spectra of **CHa1** (10 μM) at different pH values in H_2O/ACN (90 : 10, v/v) system. (λ_{ex} = 363 nm).

5. Fluorescence intensities and quantum yields of chromene derivatives

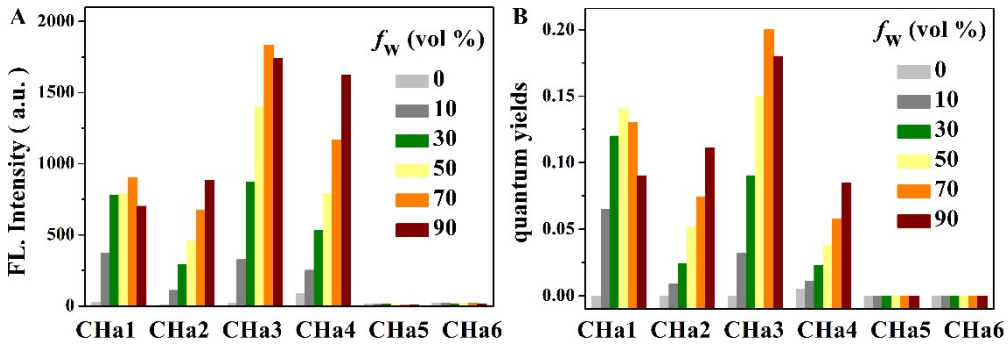

Fig S6. Fluorescence intensities and quantum yields of chromene derivatives (10 μM) in H_2O/ACN mixtures with different water fractions ($f_{w:}$: 0 vol%, 10 vol%, 30 vol%, 50 vol%, 70 vol%, 90 vol%). **CHa1** (λ_{ex} = 363 nm). **CHa2** (λ_{ex} = 358 nm). **CHa3** (λ_{ex} = 360 nm).**CHa4** (λ_{ex} = 365 nm). **CHa5** (λ_{ex} = 349 nm). **CHa6** (λ_{ex} = 363 nm).

6. Stability of CHa3

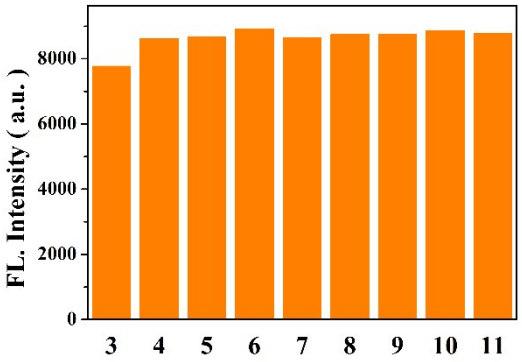

Fig S7. Fluorescence spectra of **CHa3** (100 μM) at different pH values in H_2O/ACN (90 : 10, v/v) system. (λ_{ex} = 360 nm).

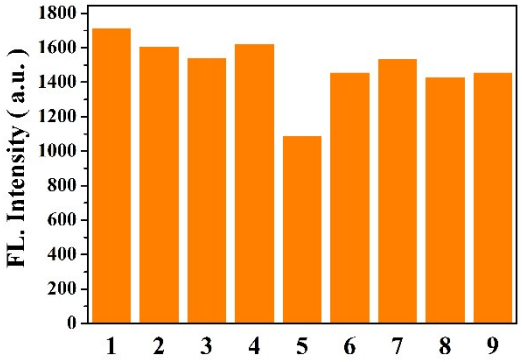

Fig S8. Fluorescence intensity of probe **CHa3** (10 μM) in the presence of 10 equiv. various analytes in PBS buffer (pH 7.4, containing 10% ACN) for 120 min at 37 °C. (1) blank; (2) cysteine; (3) Homocysteine; (4) glutathione; (5) $NaHSO_3$; (6) $NaHSO_4$ (7) Na_2S; (8) ascorbic acid; (9) H_2O_2. (λ_{ex} = 360 nm)

7. HPLC spectra and pH stability of the response

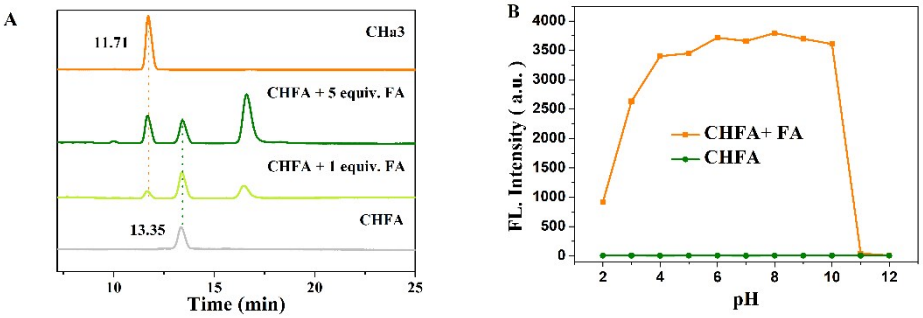

Fig S9. (A) Reverse-phase HPLC chromatogram analysis. The light gray: **CHFA** (20 μM), The light green line: **CHFA** (20 μM) + FA (1.0 equiv.), The olive line: **CHFA** (20 μM) + FA (5.0 equiv.), The orange line: **CHa3** (20 μM) Conditions: Incubation for 180 min in 10 mM PBS buffer at pH 7.4 containing 10% ACN at 37 °C. The eluent is MeOH/H_2O (v/v, 70: 30) mixed solvent. The flow rate is 0.5 mL/min^{-1}, the detection wavelength is 275 nm. (B) Fluorescence intensity of **CHFA** (20 μM) at 517 nm under different pH values in the absence and the presence of 100 equiv. of FA in PBS buffer (10 mM, containing 10% ACN). Spectra are recorded after incubation of FA for 180 min. (λ_{ex} = 360 nm)

8. Photostability and cytotoxicity assay of CHa3 and CHFA

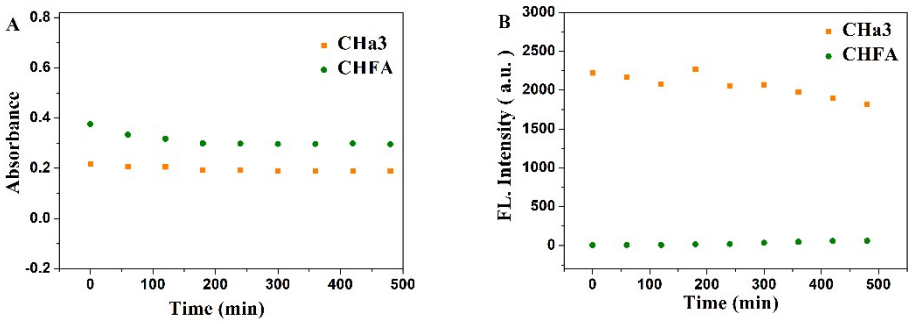

Fig. S10. Time-dependent of **CHa3** (20 μM) and **CHFA** (20 μM) in H_2O/ACN = 90:10 (v/v) solution. Under sustained illumination (254 nm). (A) UV absorption spectra. (B) Fluorescence spectra.

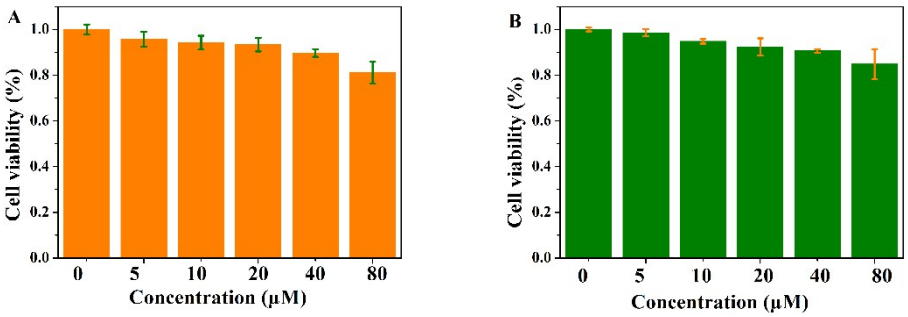

Fig. S11. Cell viabilities of HeLa cells after incubation with different concentrations of **CHa3** (A) and **CHFA** (B). The error bars are standard deviation.

9. Detection limits of CHFA responds to formaldehyde

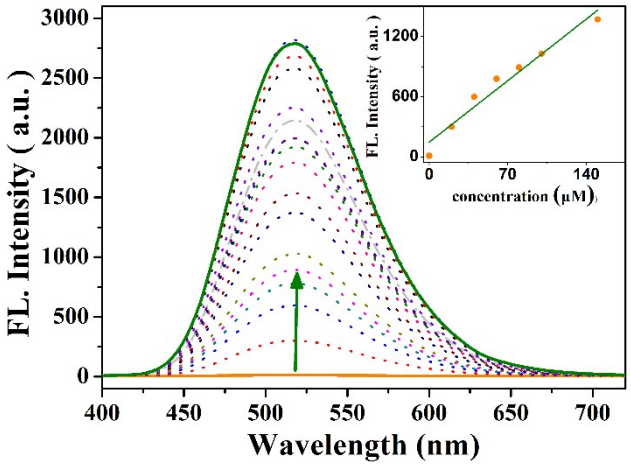

Fig. S12. Fluorescence spectra of 20 μM **CHFA** reaction with FA (0–3.0) mM for 180 min in pH 7.4 PBS buffer (10 mM containing 10% ACN). Excitation at 360 nm. Insert: The linear relationship between the fluorescence intensity and the concentration of FA.

10. Fluorescence lifetime of CHa1

Table 1 Fluorescence lifetime of **CHa1** in the ACN/H_2O mixture with different water fraction.

f_w (vol %)	A_1	T_1/ns	A_2	T_2/ns	T/ns
0	38600000	534.36536	680.63534	4938.55138	535.0833688
10	728.73216	4957.81339	28600000	552.22872	553.2360096
30	3140000	726.64389	510.63283	7882.58709	739.2510779
50	2430000	750.55805	548.16295	7505.85319	765.7796211
70	1890000	784.83447	531.83332	8593.56926	808.8822789

11. Characterization of compounds

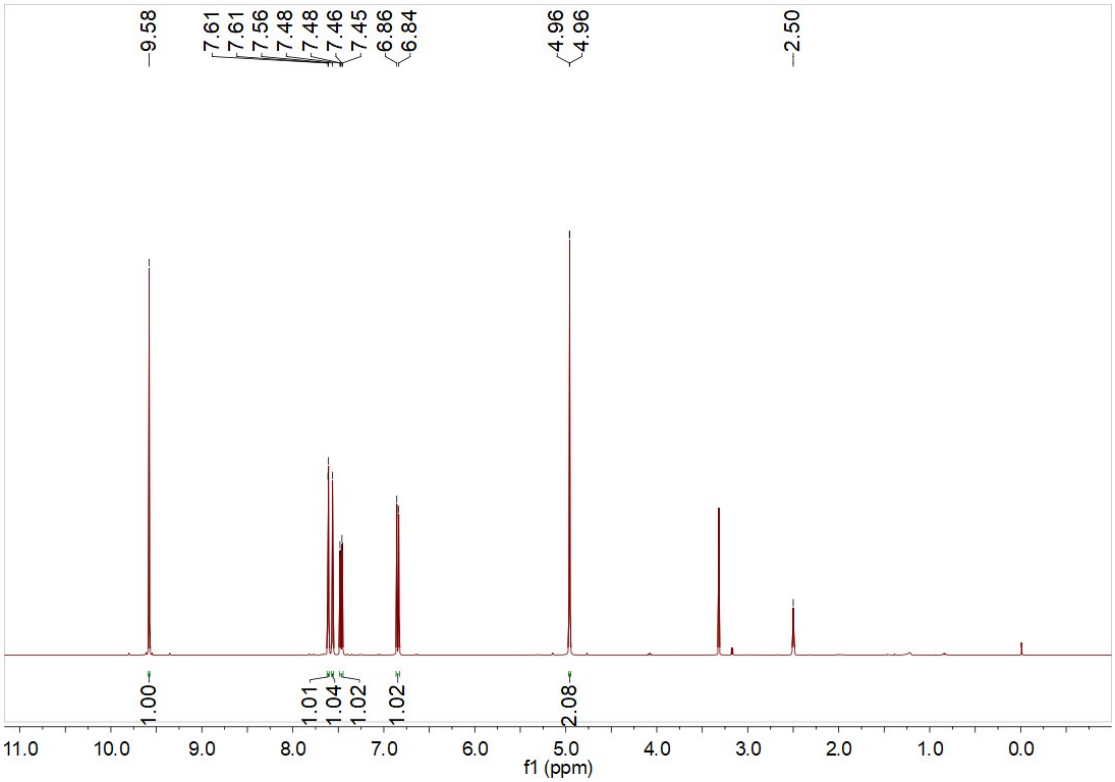

Fig S13a. ^{1}H NMR spectra of **CHa1** in DMSO-d_6.

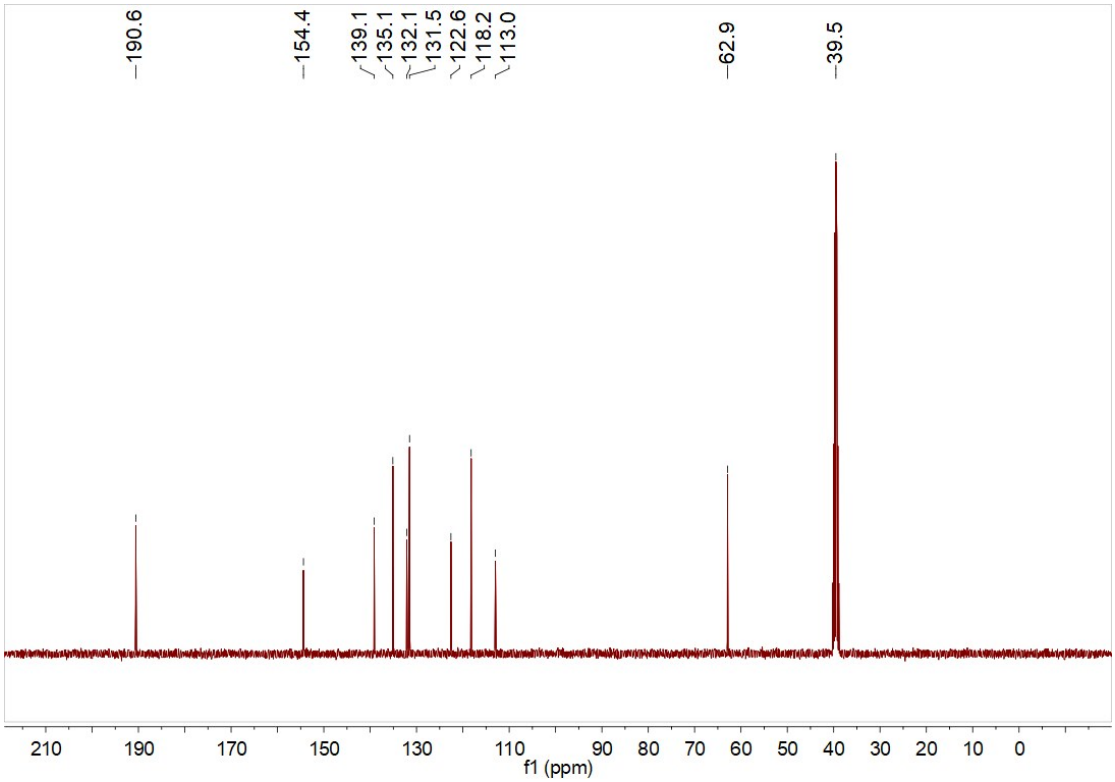

Fig S13b. ^{13}C NMR spectra of **CHa1** in DMSO-d_6.

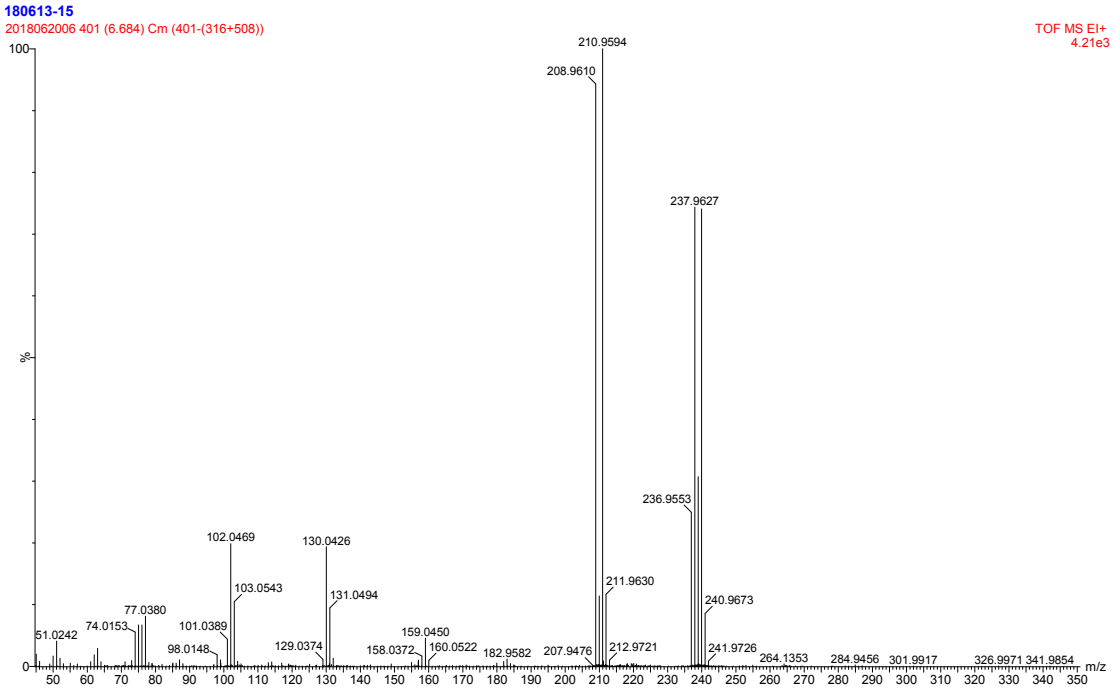

Fig S13c. Mass spectrum of **CHa1**.

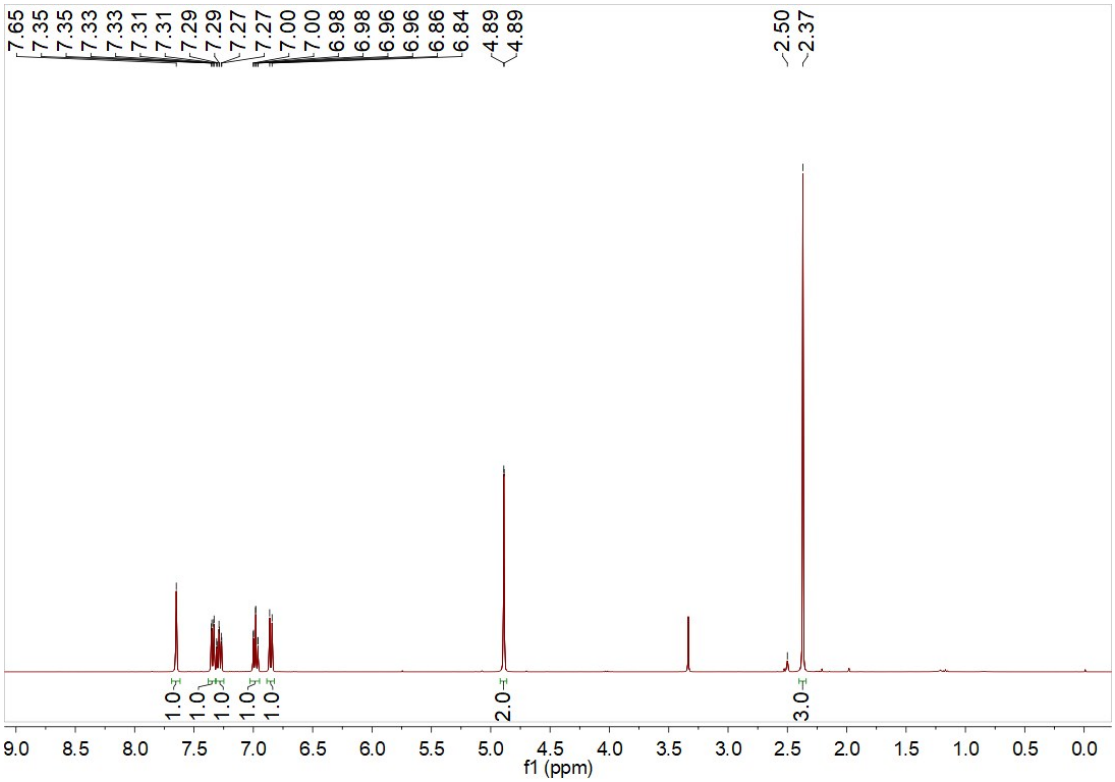

Fig S14a. ^{1}H NMR spectra of **CHa2** in DMSO-d_6.

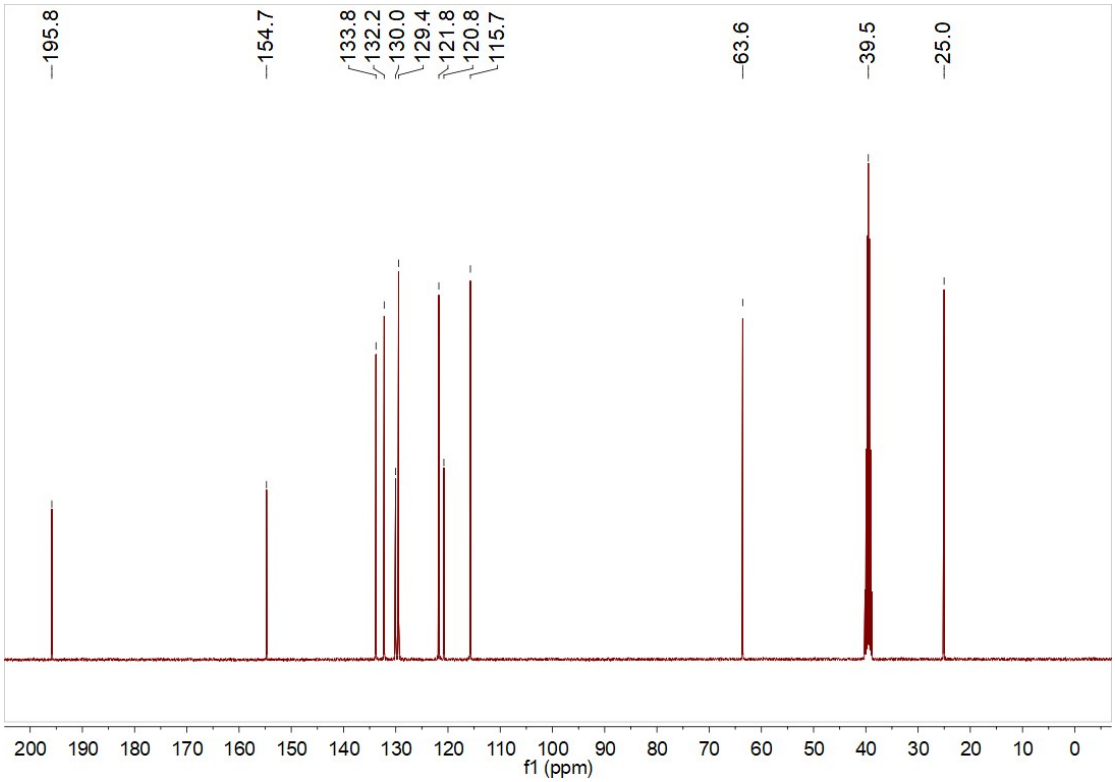

Fig S14b. ^{13}C NMR spectra of **CHa2** in DMSO-d_6..

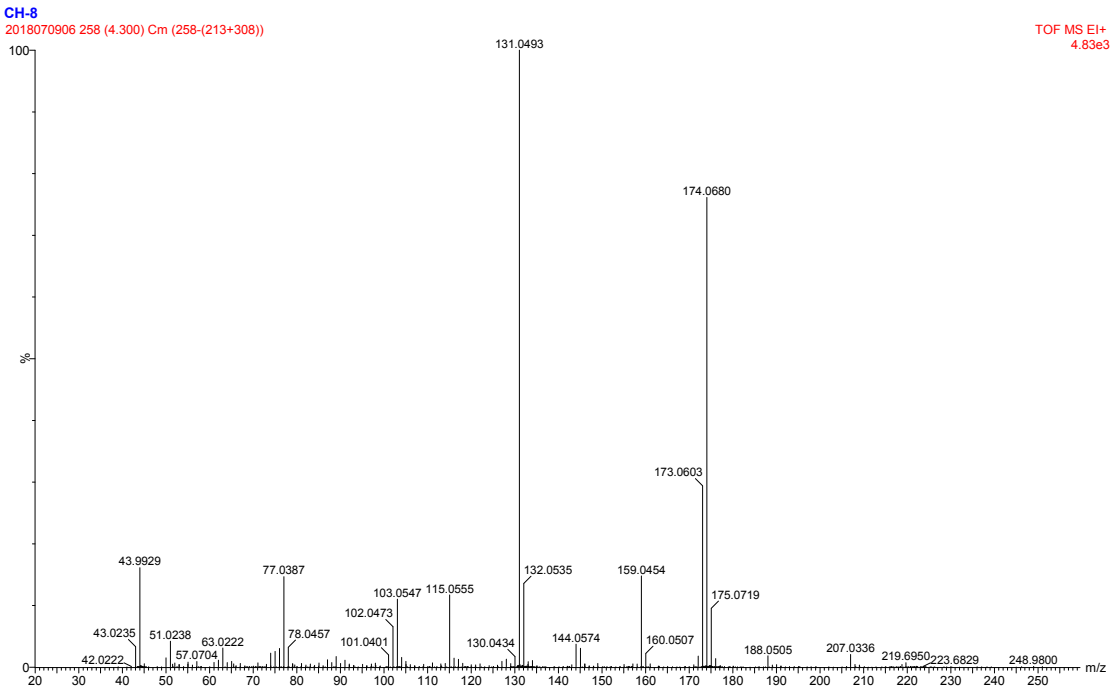

Fig S14c. Mass spectrum of **CHa2**.

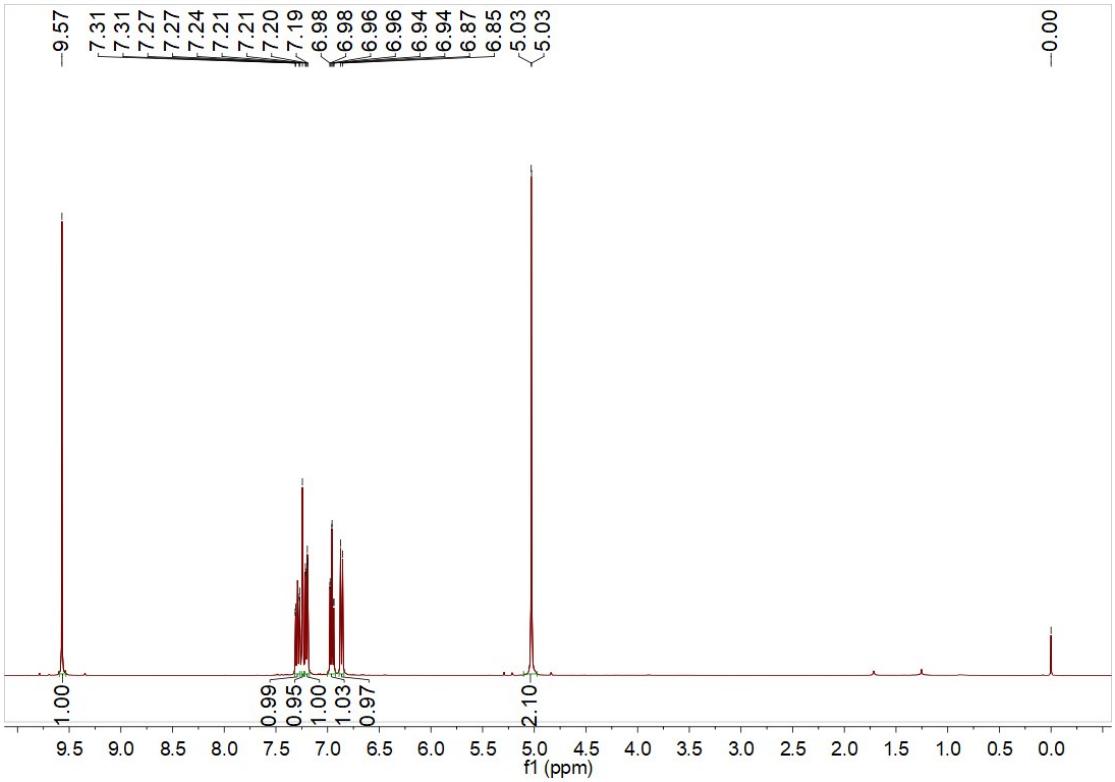

Fig S15a. ^{1}H NMR spectra of **CHa3** in $CDCl_3$

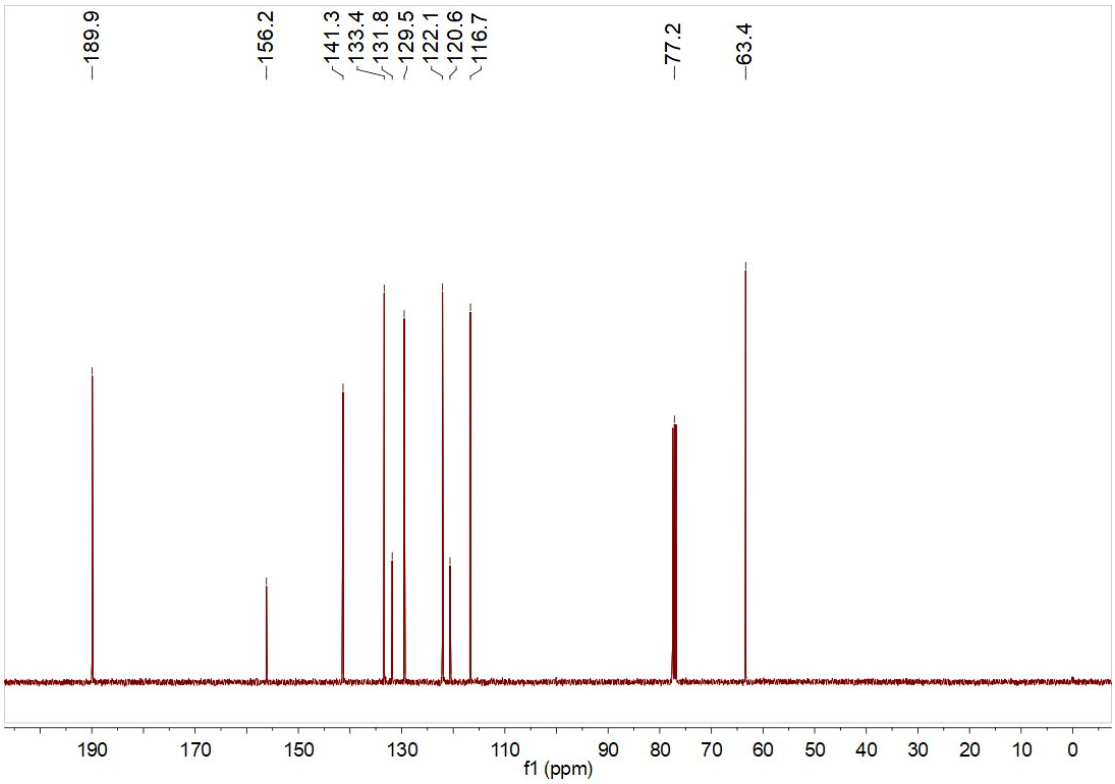

Fig S15b. ^{13}C NMR spectra of **CHa3** in $CDCl_3$.

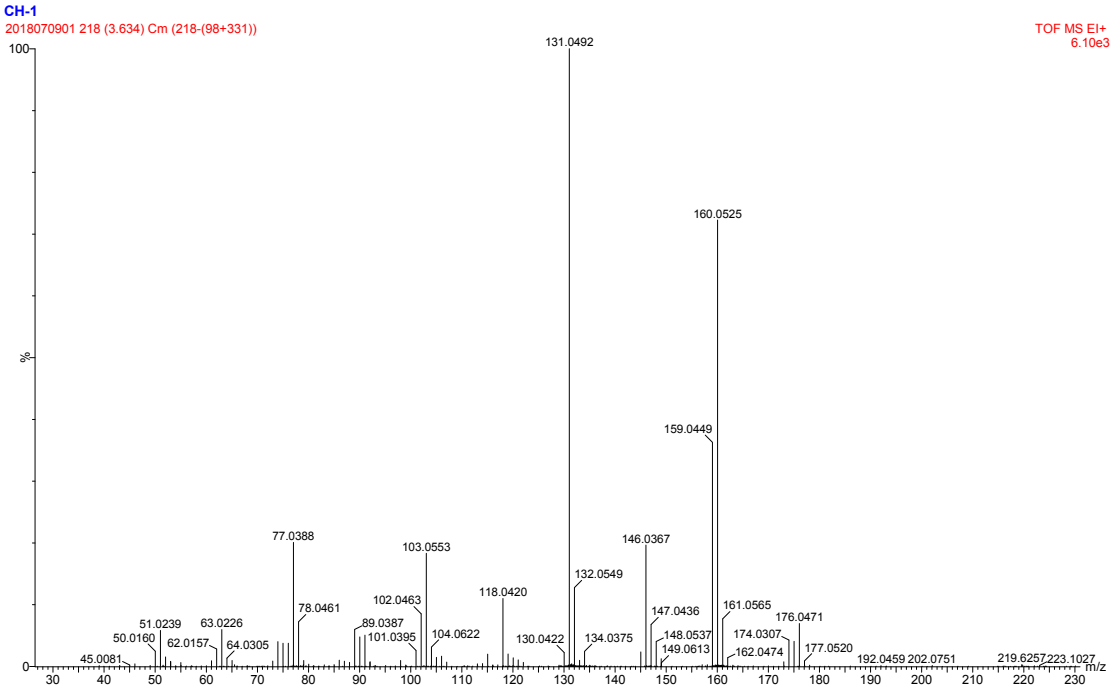

Fig S15c. Mass spectrum of **CHa3**.

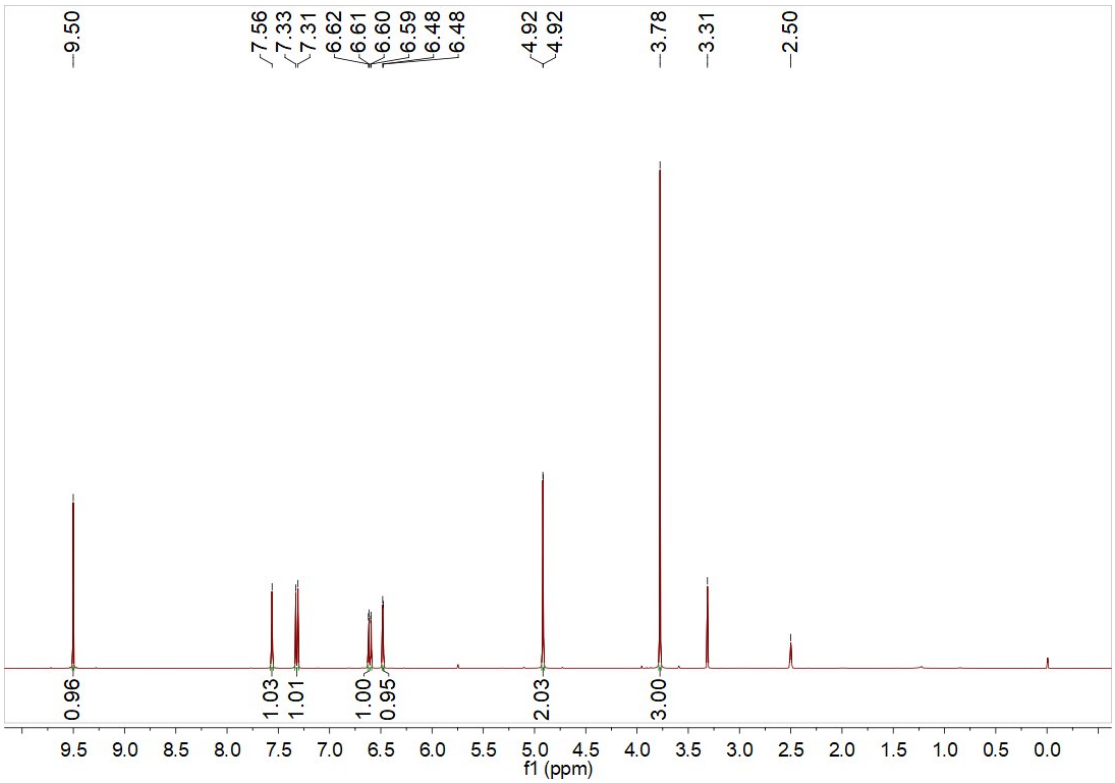

Fig S16a. ^{1}H NMR spectra of **CHa4** in DMSO-d_6

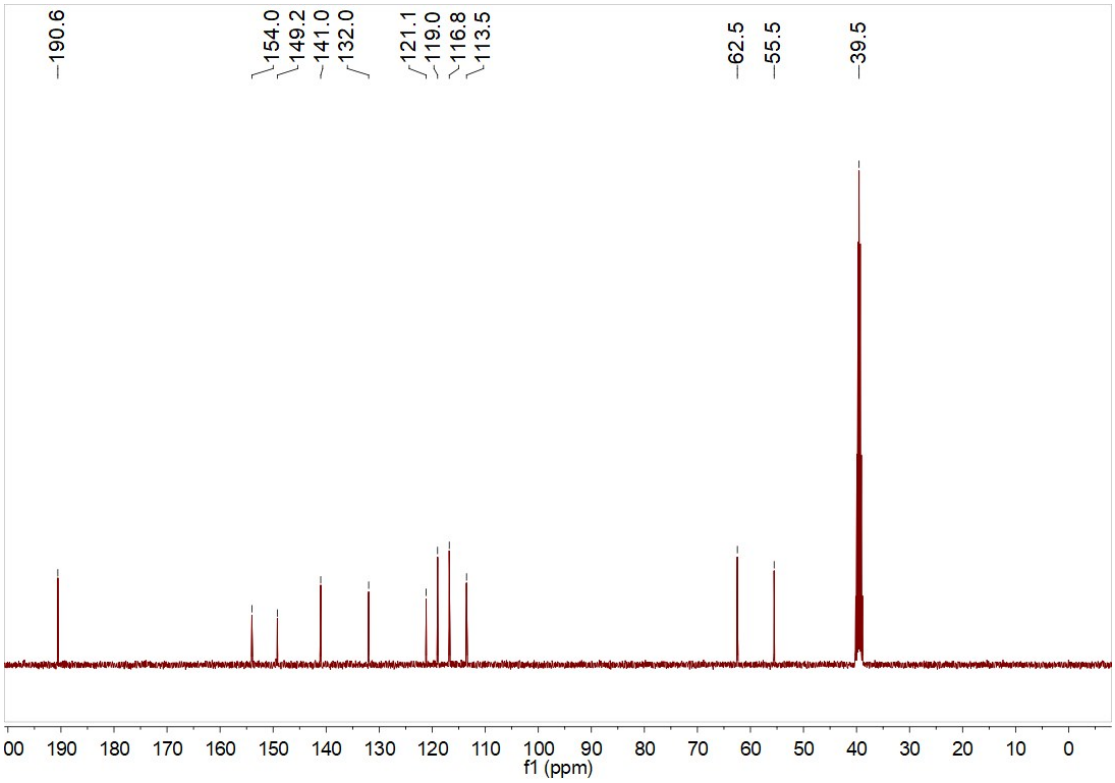

Fig S16b. ^{13}C NMR spectra of **CHa4** in DMSO-d_6.

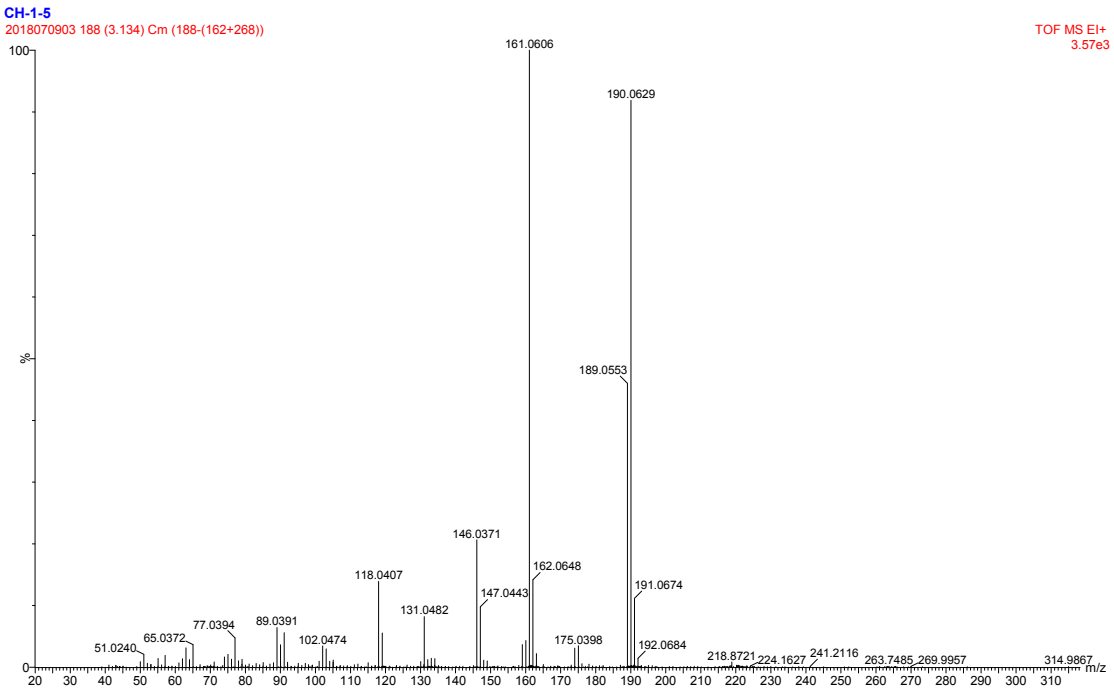

Fig S16c. Mass spectrum of **CHa4**.

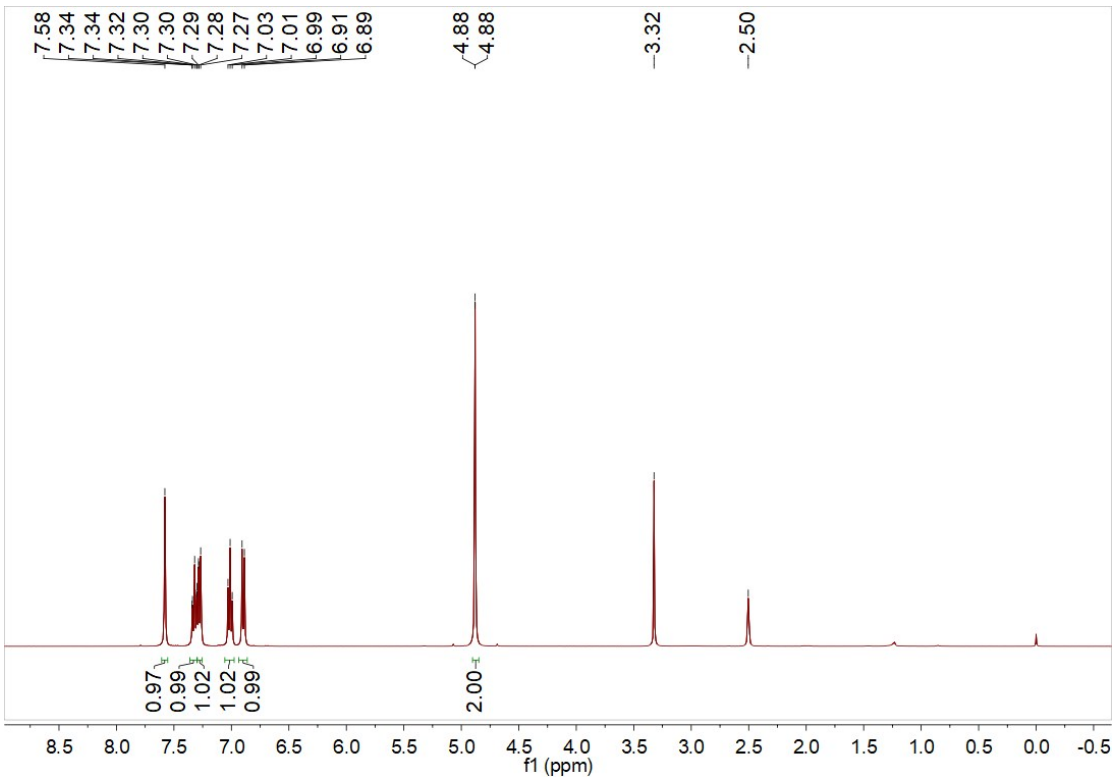

Fig S17a. ^{1}H NMR spectra of **CHa5** in DMSO-d_6.

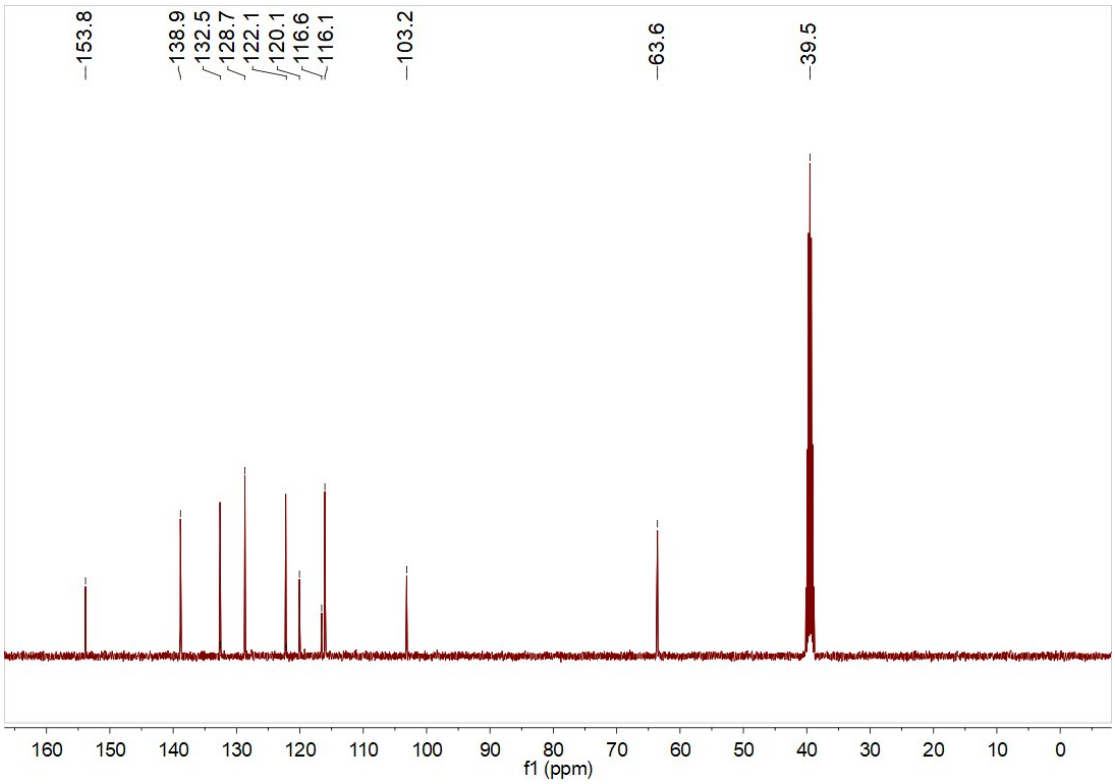

Fig S17b. ^{13}C NMR spectra of **CHa5** in DMSO-d_6.

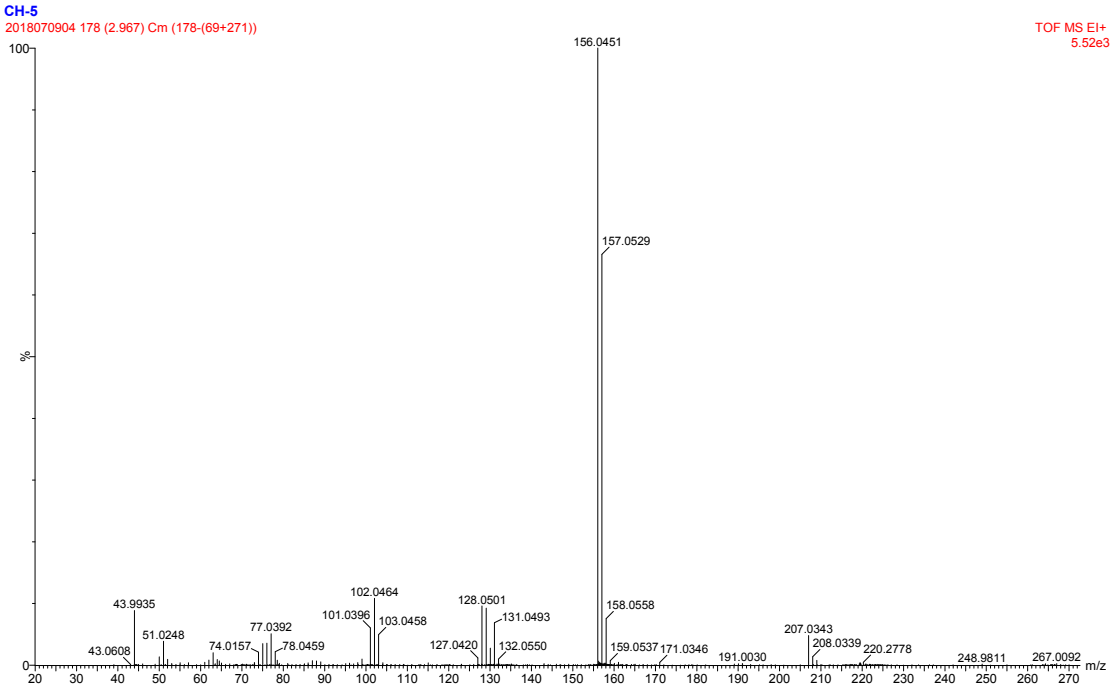

Fig S17c. Mass spectra of **CHa5**.

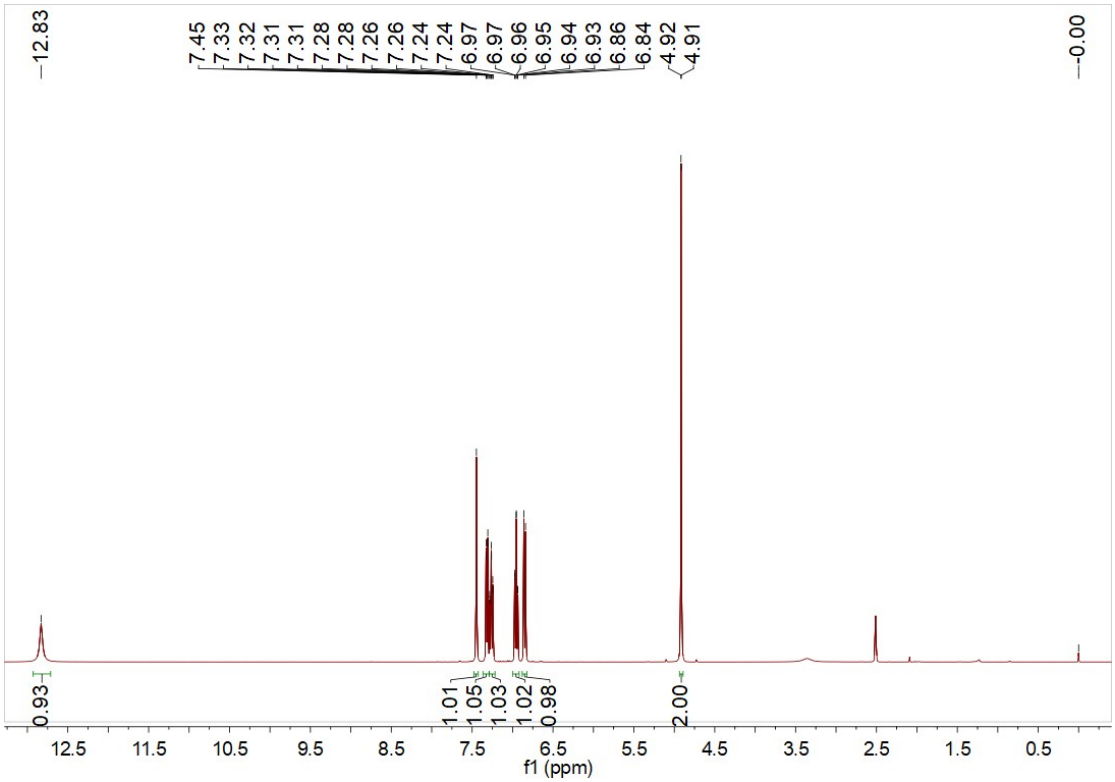

Fig S18a. ^{1}H NMR spectra of **CHa6** in DMSO-d_6.

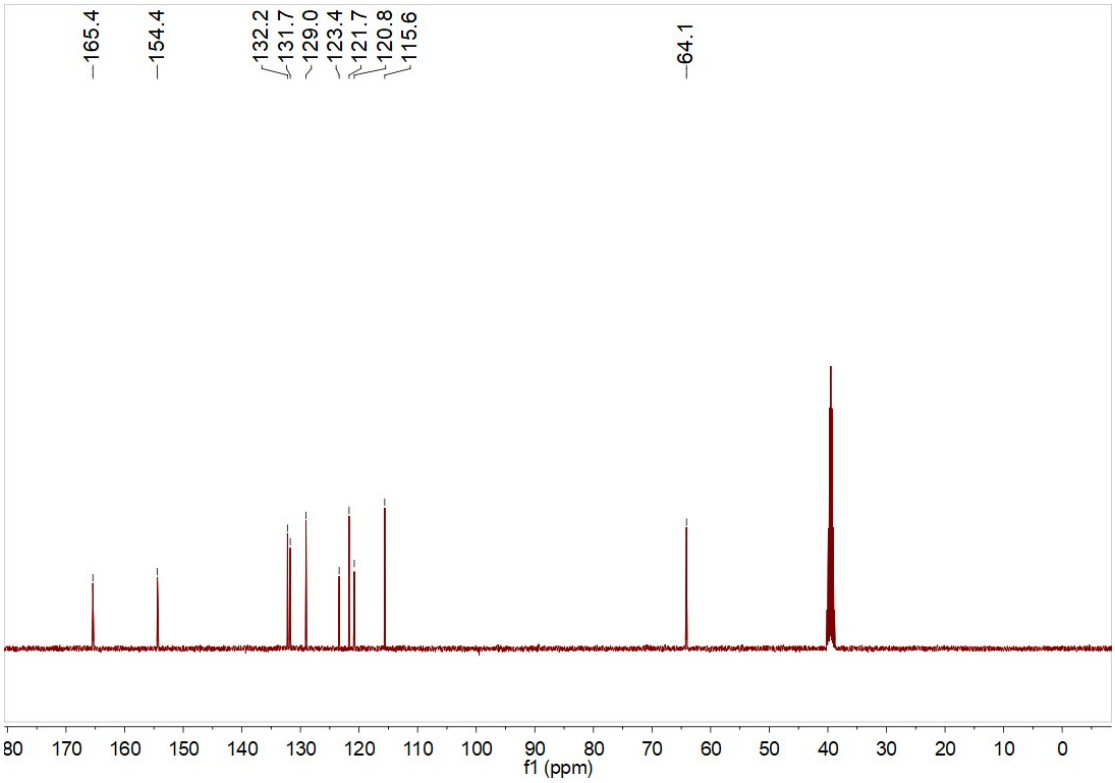

Fig S18b. ^{13}C NMR spectra of **CHa6** in DMSO-d_6.

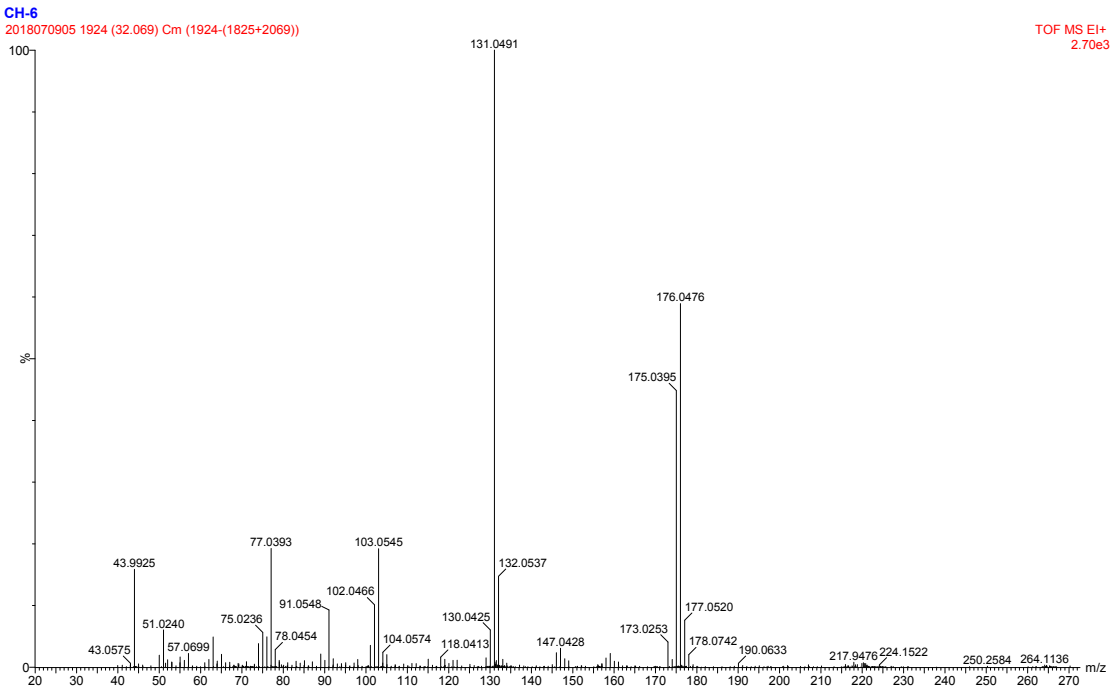

Fig S18c. Mass spectrum of **CHa6**.

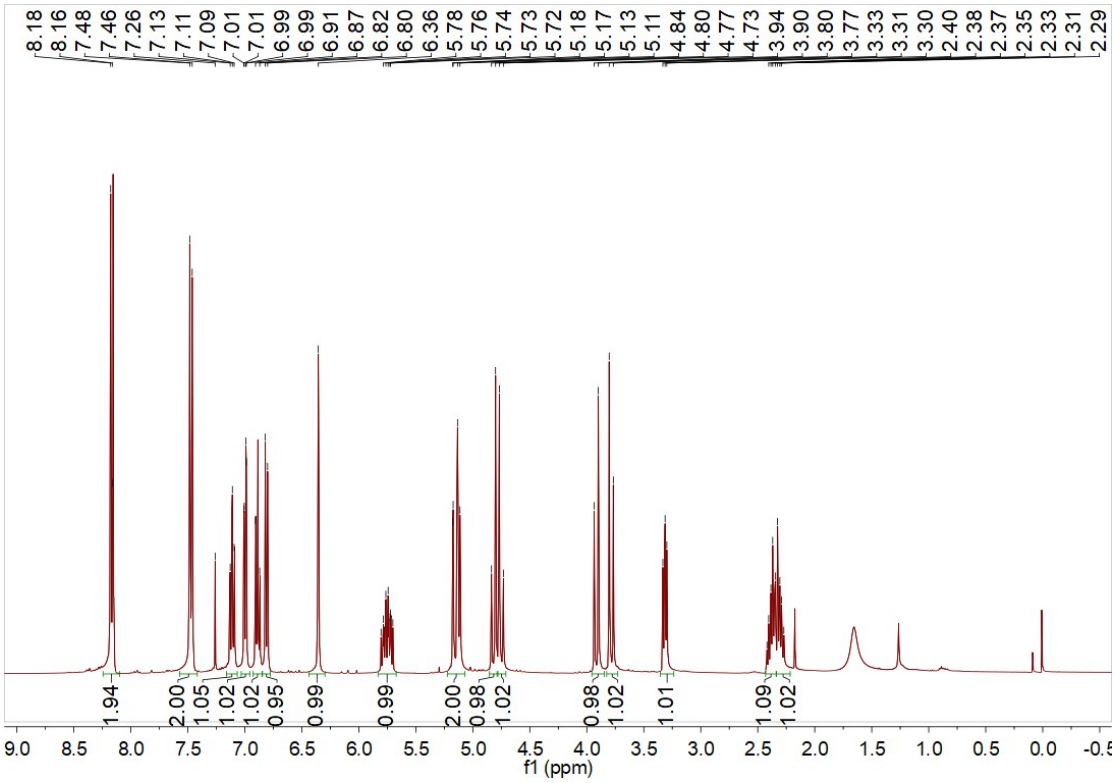

Fig S19a. ^{1}H NMR spectra of **CHFA** in $CDCl_3$

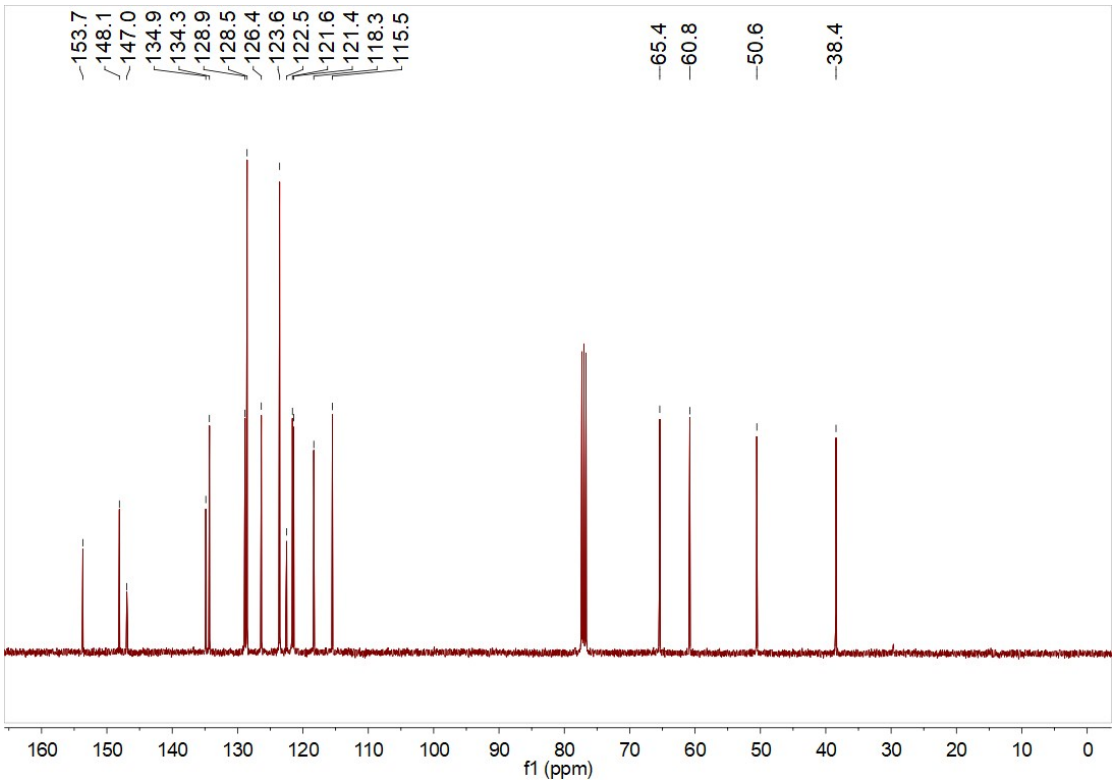

Fig S19b. ^{13}C NMR spectra of **CHFA** in $CDCl_3$.

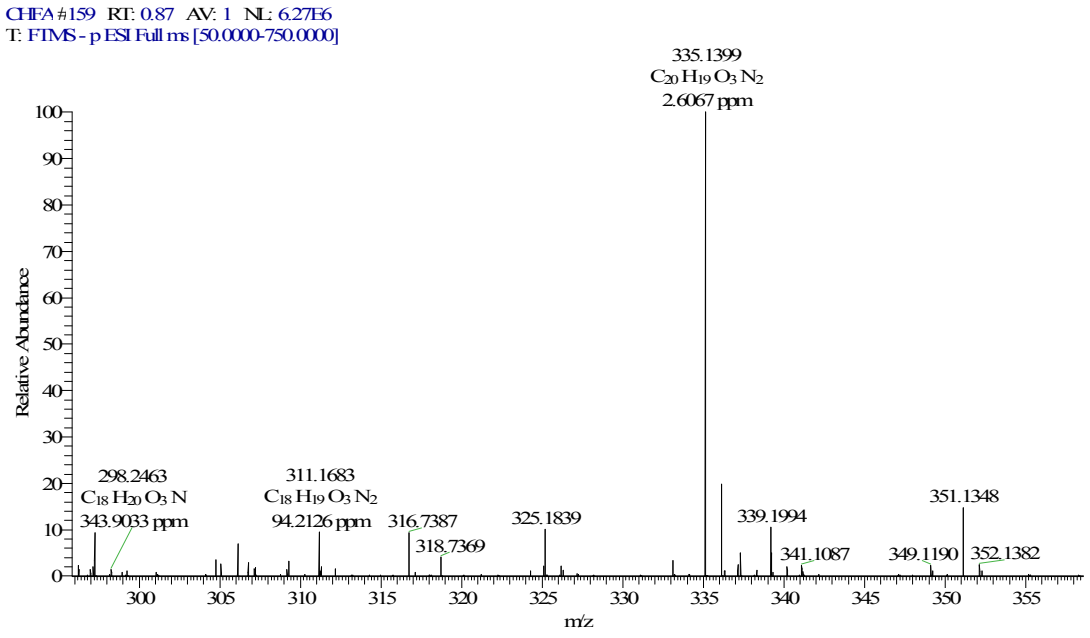

Fig S19c. Mass spectrum of **CHFA**.