SUPPORTING INFORMATION

Introduction of a luminescent sensor for tracking trace level of hydrazine in insect pollinated cropland flowers

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Table S1. Performance comparison of existing methods and present method (fluorescence based chemosensor) for detection of N_2H_4

Analytes	Sensor type	Detection limit	Estimation	Application	Reference
N_2H_4	**Carbazole Naphthalidimide derivative**	**65 nM**	**Yes**	**In cropland flowers**	**Current method**
N_2H_4	Naphthalimide based	0.27 μM	No	Cell imaging	Sensors and Actuators B,2017, 244, 417–424
N_2H_4	Naphthalimide based	0.20 μM	No	Cell imaging	Sensors & Actuators: B. Chemical,2019, 285, 368–374
N_2H_4	Naphthalimide based	0.1 μM	No	Cell imaging	M. H. Lee, B. Yoon, J. S. Kim, J. L. Sessler, Chem. Sci., 2013, 4, 4121.
N_2H_4	Carbazole based	39 nM	No	Cell imaging	Journal of Photochemistry & Photobiology A: Chemistry,2020, 389, 112269
N_2H_4	Carbazole based	2.6 μM	No	Nil	W. D. Wang, Y. Hu, Q. Li, S. L. Hu, InorganicaChimicaActa, 2018, 477, 206-211
N_2H_4	Carbazole based	1.02 μM	Yes	In water	S. Goswami, S. Paul, A. Manna, RSC Adv., 2013, 3, 18872

1. NMR Studies:

^{1}H NMR of NCD in DMSO-d_6:

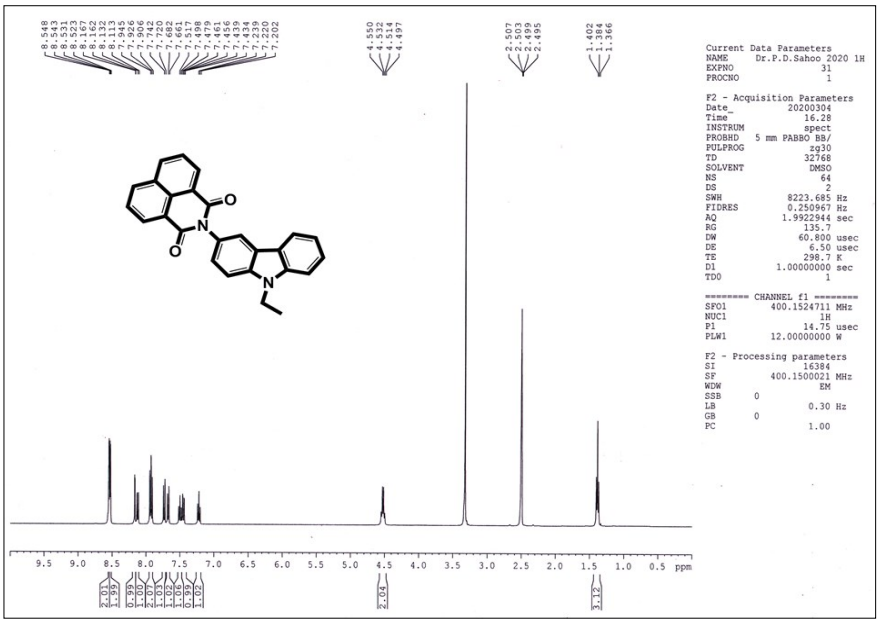

Fig.S1 ^{1}H NMR of **NCD** in DMSO-d_6 (400 MHz).

^{13}C NMR of NCD in DMSO-d_6:

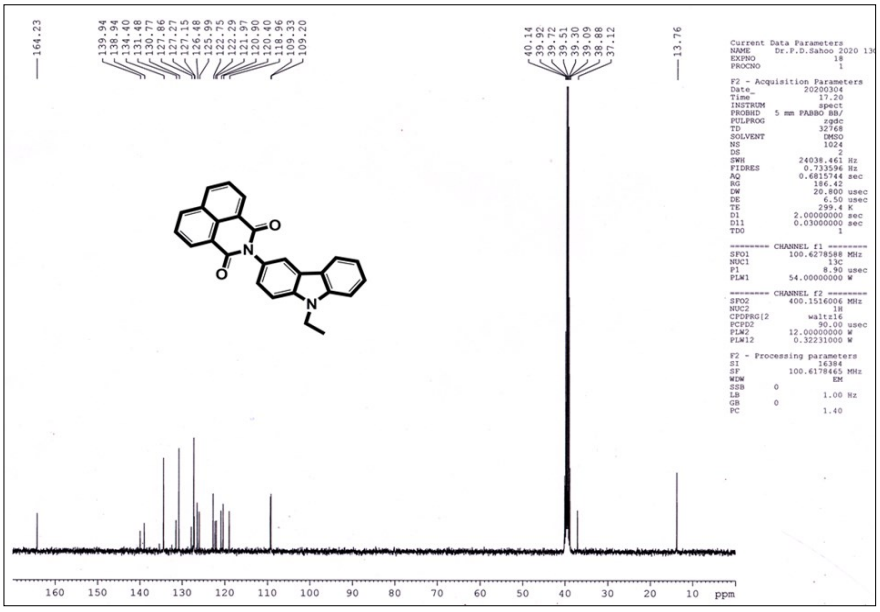

Fig.S2 ^{13}C NMR of **NCD** in DMSO-d_6 (400 MHz).

2.**Table S2**

Identification code	**NCD**
CCDC No.	**2070166**
Chemical formula	$C_{26}H_{18}N_2O_2$
Formula weight	390.1368g/mol
Temperature	296 K
Wavelength	0.71073 Å
Crystal system	monoclinic
Space group	P 21/n
Unit cell dimensions	a =10.321(7) Å α =90° b =16.443(11) Åβ =90.460(8)° c =23.688(16) Å γ =90°
Volume	4020(5) $Å^3$
Z	8
Density (calculated)	1.290 g/cm3
Absorption coefficient	0.082 mm-1
F(000)	1632.0
Reflections collected	6365
Refinement program	SHELXT 2014/4 (Sheldrick, 2014)

3. Materials and Instruments

1,8-naphthalic anhydride, 3-amino-9-ethyl carbazole, Methanol, Chloroform, Ethyl acetate and all other chemicals were purchased from Sigma-Aldrich Pvt. Ltd. Unless otherwise mentioned, materials were obtained from commercial suppliers and were used without further purification. Solvents were dried according to the standard procedures. Elix Millipore water was used throughout all the experiments. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker 400 MHz instrument. For NMR spectra, DMSO-d_6 and for NMR titration DMSO-d_6 and D_2O were used as solvent using TMS as an internal standard. Chemical shifts are expressed in δ ppm units and ^{1}H–^{1}H and ^{1}H–C coupling constants in Hz. The following abbreviations are used to describe spin multiplicities in ^{1}H NMR spectra: s = singlet; d = doublet; t = triplet; m = multiplet. The mass spectrum (HRMS) was carried out using a micromass Q-TOF Micro™ instrument by using DMSO as a solvent. Fluorescence spectra were recorded on a Perkin Elmer Model LS 55 spectrophotometer. UV spectra were recorded on a SHIMADZU UV-3101PC spectrophotometer.

Leica TCS SP8 laser scanning confocal microscope system was used for confocal microscopy. Images obtained through section scanning were analyzed by the LasX software with excitation at 340 nm monochromatic laser beam, and emission spectra were integrated over the range 446 nm (single channel) with 10X magnification.

4. UV-vis spectral studies. A stock solution of **NCD** (1 μM) was prepared in water-dimethylsulphoxide (1:1, v/v). **N_2H_4** solution of concentration 10 μM was prepared in Millipore water. All experiments were carried out in aqueous medium at neutral pH. During the titration, each time a 1 μM solution of **NCD** was filled in a quartz optical cell of 1 cm optical path length and **N_2H_4** stock solution was added into the quartz optical cell gradually by using a micropipette. Spectral data were recorded at 1 min after the addition of **N_2H_4.**

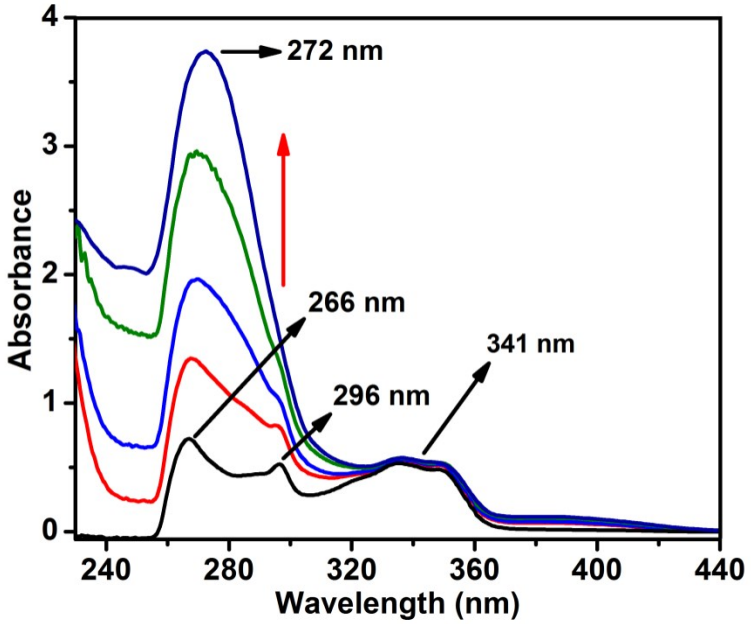

Fig.S3 UV−vis absorption spectra of **NCD** (1 μM) upon gradual addition of N_2H_4 up to 1.2 μM in H_2O:DMSO (1:1, v/v) at pH 7.0 (10 mM phosphate buffer).

5. Fluorescence spectral studies:

A stock solution of **NCD** (1 μM) was prepared in water-dimethylsulfoxide (1:1, v/v). **N_2H_4** solution of concentration 10 μM was prepared in Millipore water. All experiments were carried out in aqueous medium at neutral pH. During titration, each time a 1 μM solution of **NCD** was filled in a quartz optical cell of 1 cm optical path length and **N_2H_4** stock solution was added into the quartz optical cell gradually by using a micropipette. Spectral data were recorded at 1 min after the addition of **N_2H_4**. For all fluorescence measurements, excitations were provided at 340 nm, and emissions were collected from 390 to 580 nm.

6. Calculation of limit of detection (LOD) of NCD with N_2H_4:

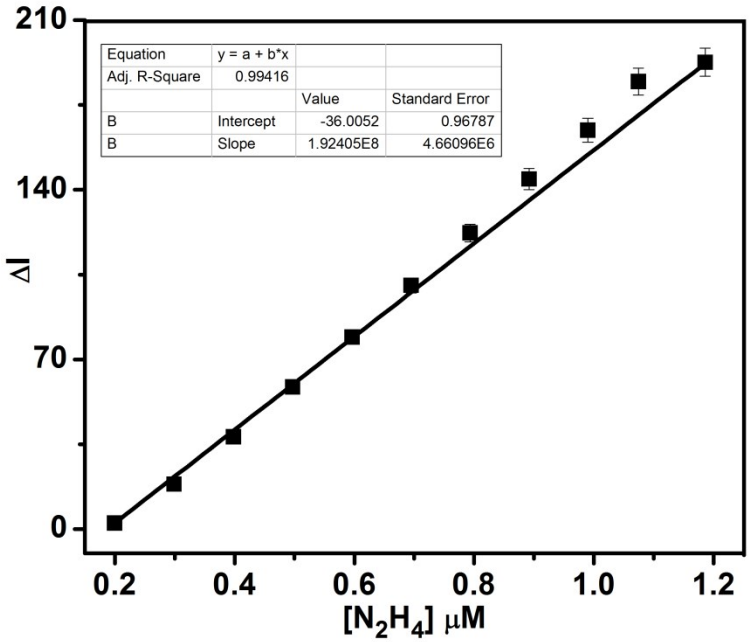

Fig. S4 Linear fit curve of **NCD** at 446 nm with respect to N_2H_4 concentration. Standard deviations are given by error bars where, n=3.

From the linear fit graph we get slope = 1.92405×10^8, and SD value is 4.17155

Thus using the above formula we get the Limit of Detection = 6.50×10^{-8} M, = 65 nM. Therefore **NCD** can detect $\mathbf{N_2H_4}$ up to this very lower concentration by fluorescence technique.

7. Job's plot for determining the stoichiometry of binding by fluorescence method:

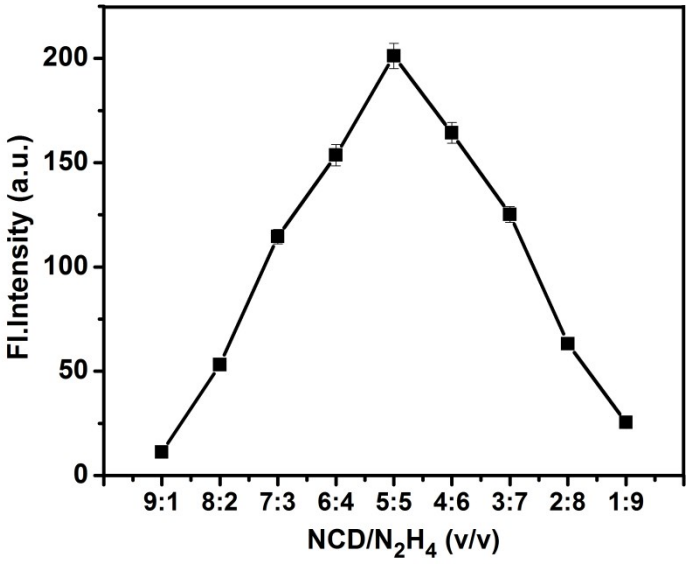

Fig. S5 Job's plot of **NCD** (1 μM) with N_2H_4 (1 μM) in water-DMSO (1:1, v/v) at neutral pH by fluorescence method, which indicate 1:1 stoichiometry for **NCD** with N_2H_4. Standard deviations are given by error bars where, n=3.

8. Dependence of the fluorescence intensity of NCD with N_2H_4 as a function of time:

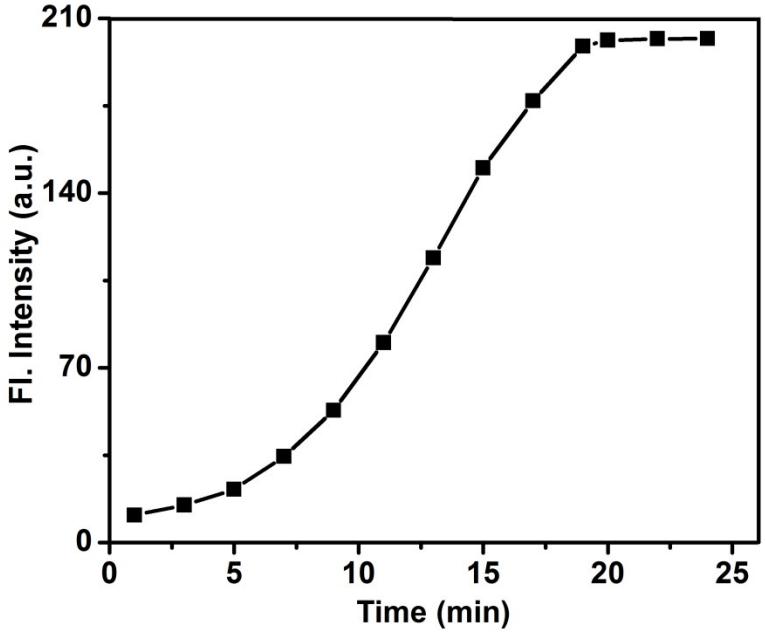

Fig. S6 Fluorescence intensity increase of **NCD** upon gradual addition of N_2H_4 with time in water-dimethylsulphoxide (1:1, v/v), buffered with 10 mM phosphate buffer (pH 7.0) (λ_{ex}= 340 nm).

9. Kinetic Study. The kinetic study of the reaction between **NCD** and hydrazine has been done by measuring the fluorescence spectra after mixing **NCD** and hydrazine in a cubic 4-sided quartz cell of 3 ml. The reaction was carried out at room temperature under the excess amount of hydrazine (initial concentration [**NCD**] << [N_2H_4]) and the reaction was expected to reach 100% conversion of **NCD** to the product carbazole amine fragment and cyclic diamide moiety. The excitation wavelength was 340 nm.

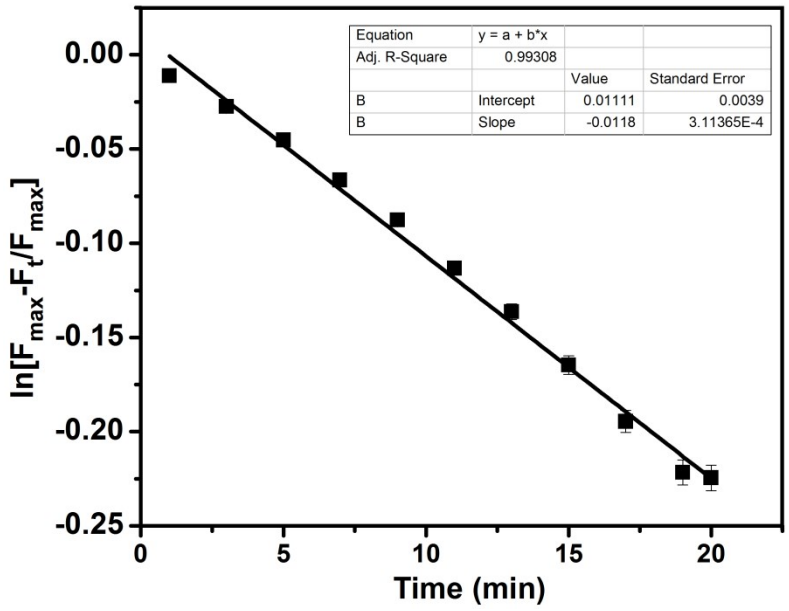

Fig. S7 Pseudo-first-order kinetics plot for the reaction of **NCD** and N_2H_4. Standard deviations are given by error bars where, n=3.

10. pH Titration

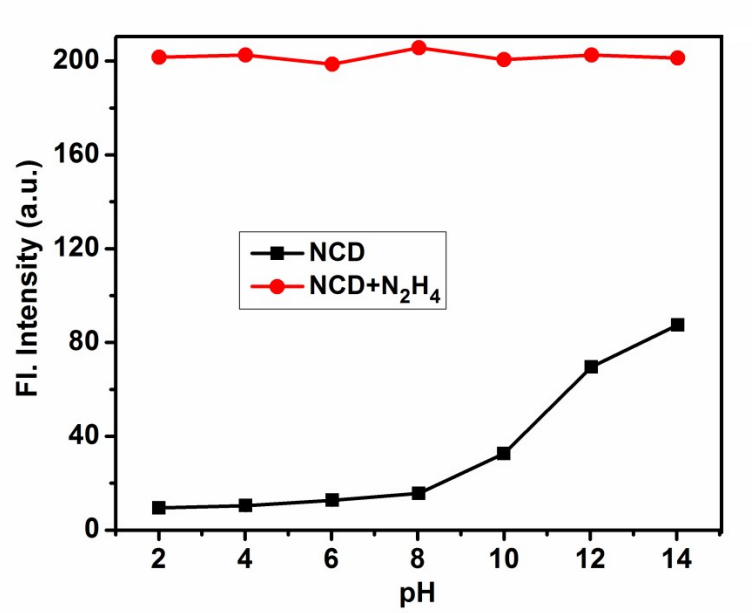

Fig. S8 Fluorescence responses of probe **NCD** (black) and **NCD+N_2H_4** (red) in different pH conditions in water-dimethylsulfoxide (1:1, v/v) (λ_{ex}= 340 nm)

11. Competitive selectivity in presence of other analytes

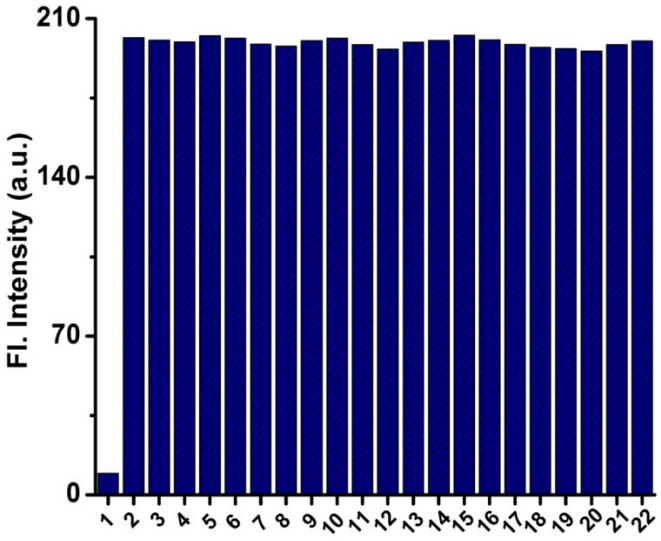

Fig. S9 Histogram representing competitive fluorescence spectra of **NCD+N_2H_4** in presence of different analytes at 446 nm (λ_{ex}= 340 nm) in H_2O-DMSO (1:1, v/v) at neutral pH. [1) Blank, 2)

N_2H_4, 3) N_2H_4+Zn^{2+}, 4) N_2H_4+Cu^{2+}, 5) N_2H_4+Pb^{2+}, 6) N_2H_4+Mg^{2+}, 7) N_2H_4+Ca^{2+}, 8) N_2H_4+Na^{+}, 9) N_2H_4+K^{+}, 10) N_2H_4+F^{-}, 11) N_2H_4+Cl^{-}, 12) N_2H_4+Br^{-}, 13) N_2H_4+I^{-}, 14) N_2H_4+ClO_4^{-}, 15) N_2H_4+CN^{-}, 16) N_2H_4+NO_3^{-}, 17) N_2H_4+$H_2PO_4^{-}$, 18) N_2H_4+N_3^{-}, 19) N_2H_4+NH_4OH 20) N_2H_4+Pyridine, 21) N_2H_4+n-Butylamine and 22) N_2H_4+Ethylenediamine]

12. ^{13}C NMR titration

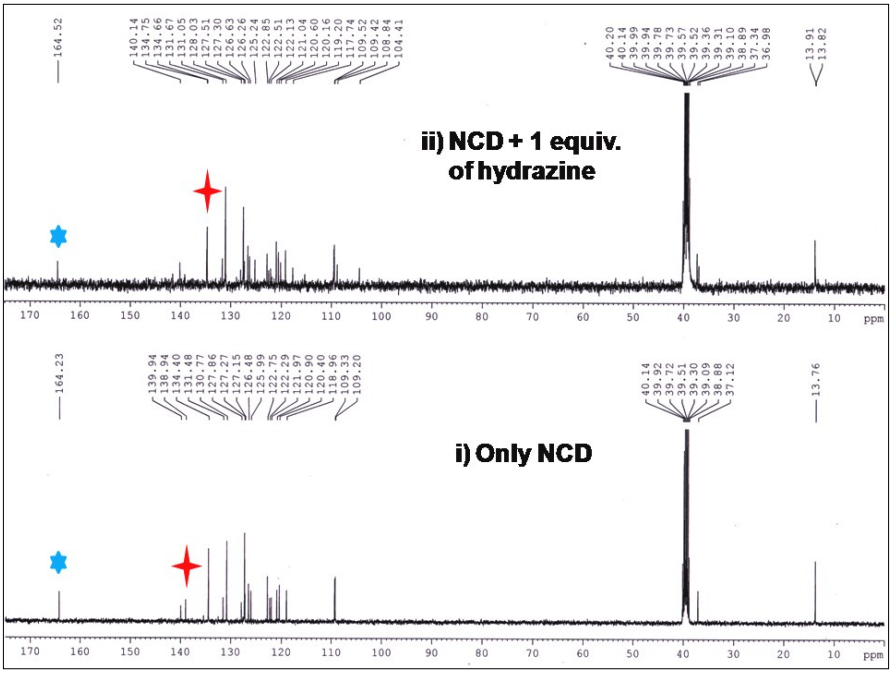

Fig. S10 ^{13}C NMR titration [400MHz] of **NCD** in DMSO-d_6 at 25^{0}C and the corresponding changes after the addition of 1 equiv. of hydrazine in D_2O from (i) only **NCD**, (ii) **NCD** + 1 equiv. of hydrazine.

13. Plausible Mechanism

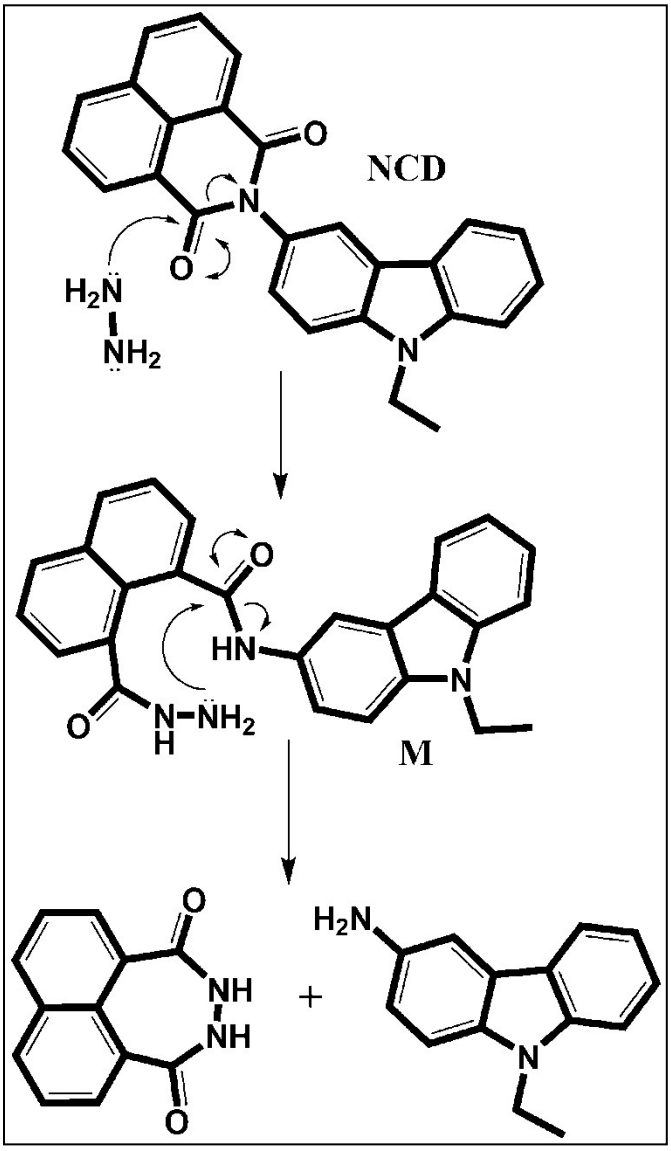

Fig. S11 A plausible mechanism of interaction of **NCD** with hydrazine

14. Mass spectrometry of products after reaction of NCD with hydrazine

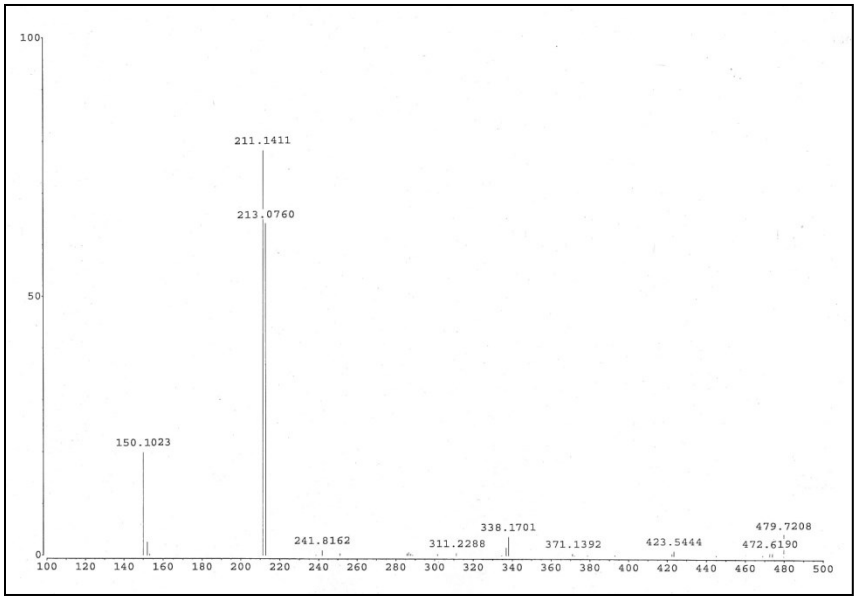

Fig. S12 HRMS of **NCD+hydrazine**

15. Table S3. Selected electronic excitation energies (eV), oscillator strengths (f), main configurations of the low-lying excited states of all the molecules and complexes. The data were calculated by TDDFT//B3LYP/6-311G(d,p)based on the optimized ground state geometries.

Molecules	Electronic Transition	Excitation Energya[a]	f[b]	Composition[c](%)
NCD	$S_0 \rightarrow S_3$	3.6793eV336.98 nm	0.3401	H-2 → L (69%)
	$S_0 \rightarrow S_{17}$	4.9949eV248.22nm	0.7592	H → L+4 (50%)
Carbazole amine fragment	$S_0 \rightarrow S_3$	4.6487eV266.70 nm	0.5484	H → L+1 (57%)
Cyclic diamide fragment	$S_0 \rightarrow S_2$	3.8583eV321.34 nm	0.1701	H → L (53%)

[a]Only selected excited states were considered. The numbers in parentheses are the excitation energy in wavelength.[b]Oscillator strength. [c]H stands for HOMO and L stands for LUMO.