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Synthesis of a dihydroquinoline based merocyanine as a 'naked eye' and 'fluorogenic' sensor for hydrazine hydrate in aqueous medium and hydrazine gas†

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1,2,2,4-Tetramethyldihydroquinoline (TMDQ) is formylated at the 6-position and the resulting quinoline aldehyde is condensed with 3-methyl-1-phenyl-1*H*-pyrazol-5(4*H*)-one to obtain a merocyanine 6-pyrazolinyl TMDQ. The merocyanine is a selective and sensitive sensor for hydrazine hydrate in aqueous ethanolic medium and also for hydrazine gas. The merocyanine detects hydrazine hydrate selectively in less than 5 s over other amines, anions, and metals. The detection can be done simply by the naked eye and quantification can be done by absorbance/fluorescence spectroscopy. Hydrazine hydrate as well as hydrazine gas can be detected by the TLC plate technique.

Introduction

Hydrazine is an important industrial chemical used widely in rockets and missiles as fuel,[1] as a reducing agent,[2] and as an antioxidant in nuclear and electrical power plants.[1] It has a vital function in the pharmaceutical and chemical industries as a catalyst, for dyes in textiles, and as an inhibitor.[3] Despite these many applications, hydrazine hydrate is extremely toxic. Hydrazine is most likely a carcinogen and its threshold limit value (TLV) is 10 ppb.[4] It can induce damage of the central nervous system (CNS), nose irritation, and temporary blindness.[5] Hence, designing a reliable and rapid detector that can provide on-site and real-time recognition of hydrazine is very desirable.

There are several reports on sensing of hydrazine.[6–13] But very few reports of 'naked eye' and 'fluorescent' probes for hydrazine hydrate can be found in the literature. Zhao reported a hydrazine sensor which contains expensive gold metal.[11] Further, the sensor does not show any fluorescence properties. Very recently, a hydrazine sensor which shows fluorescence was reported.[13] A similar molecule was reported by Cui almost at the same time.[14] However a probe which can be used either as a 'naked eye' detector, or in 'UV-vis absorption', as well as in 'fluorescence technique' is definitely a superior one.

Cyanines were initially used as developers in photography. Because of their conjugated structure and useful properties, people started using cyanines for diverse applications.[15] Merocyanines are a class of cyanines which have various applications.[16] Merocyanines can be used as probes and markers in chemical analysis, biology and medical applications, non-linear optics, and organic semiconductors.[16] Many cyanines contain the quinoline moiety.[17]

There are various detection methods for sensing toxic chemicals,[18] such as naked eye detection, spectrophotometry,[19] spectrofluorimetry,[20] electrochemistry,[21] GC,[22] and HPLC.[23] Each method has its own *pros* and *cons* with respect to portability, analysis time, sensitivity, and economy. However, a sensor which can be used in multiple detection methods can overcome these drawbacks and thus is advantageous. For example, 'naked eye' sensors particularly show potential because the alteration in colour can easily be observed without any equipment and hence are more economic. On the other hand, UV instrument can permit the quantification by the measurement of the absorption intensity. Further, if a sensor shows fluorescence, it is an added benefit. For example, the analyte may be determined with more accuracy in a spectrofluorimeter than in an UV instrument. In addition, fluorescence can be used in biological applications like cell imaging.

Merocyanines have a potential as sensors and many of them contain a quinoline nucleus. This prompted us to synthesize a merocyanine dye with dihydroquinoline joined to a heterocyclic ring through a conjugation extending linker. In this paper we would like to report the synthesis of (*E*)-3-methyl-1-phenyl-4-((1,2,2,4-tetramethyl-1,2-dihydroquinolin-6-yl)methylene)-1*H*-pyrazol-5(4*H*)-one which belongs to the class of merocyanines and is a conjugate of 1,2,2,4-tetramethyldihydroquinoline and pyrazolone. The merocyanine can effectively and competently sense hydrazine hydrate. It can act as a rapid and selective

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naked-eye, fluorescent, as well as absorbance sensor for hydrazine hydrate in an aqueous alcoholic medium. It is easy to synthesize, eco-friendly, sensitive, and selective. The sensing does not involve any complicated buffer-making procedure, and can be done simply in an ethanol–water system. To the best of our knowledge, this is the first report of a merocyanine acting as a hydrazine sensor in an aqueous alcoholic medium and also for hydrazine gas.

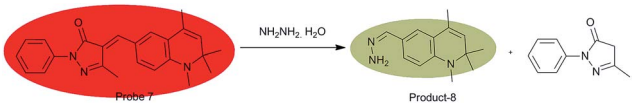
Results and discussion

1,2,2,4-Tetramethyl-1,2-dihydroquinoline (1) was prepared by the condensation of aniline with acetone.[24] 1 was *N*-methylated by methyl iodide in DMF in the presence of potassium carbonate to obtain 2. 2 was formylated using DMF/$POCl_3$ according to the reported procedure to 1,2,2,4-tetramethyl-1,2-dihydroquinoline-6-carbaldehyde (3).[24] Phenyl hydrazine was condensed with ethyl acetoacetate to obtain 3-methyl-1-phenyl-1*H*-pyrazol-5(4*H*)-ones (6) as per the reported procedure.[25] 3 and 6 were condensed in the presence of TEA in ethanol to obtain 7 in 82% yield (Scheme 1). The structure of 7 was confirmed by ^{1}H NMR and ^{13}C NMR as well as by ESI-MS. It was an intensely orange coloured powder.

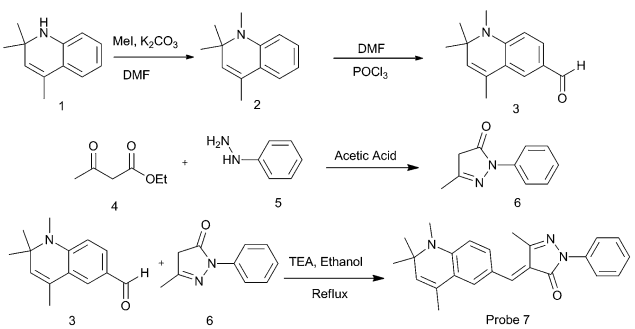
Water is the best solvent for any sensor. 7 was insoluble in water, but soluble completely in ethanol–water (8 : 2 v/v). Hence, all studies were done in ethanol–water (8 : 2 v/v). The UV-vis spectrum showed λ_{max} at 503 nm. When increasing concentration of hydrazine hydrate was put in to a fixed concentration of 7, the absorbance of the dye decreased. Hydrazine hydrate at different concentrations was added to 16.16 μM (concentration which obeyed Beer–Lambert's law) of probe-7, (Fig. 1). This showed that with increasing concentration of hydrazine hydrate the red color of dye decreased linearly and finally vanished. This visual response was instantaneous (<5 seconds). Thus, 7 is a 'naked-eye' colorimetric sensor. Moreover, the probe was not fluorescent initially, but after addition of hydrazine hydrate, it showed fluorescence at 432 nm.

The colour of the merocyanine is due to extended conjugation from quinoline ring to pyrazoline ring through the exocyclic double bond on the pyrazoline ring. Hydrazine hydrate may attack the linker double bond that is conjugated to the carbonyl group in pyrazolone and cleave the molecule to form hydrazone **8** and pyrazolone 6 (Scheme 2). This leads to a change in the absorbance of the probe. To confirm this, the probe-7 was mixed with 5 eq. of hydrazine hydrate in ethanol and stirred for 1 h at room temperature and the reaction was monitored by TLC which showed the formation of a new compound. This compound was matching with hydrazone of quinoline aldehyde 3. The reaction was worked up and purified by flash column chromatography to get an off white to light yellow coloured product. The structure was further confirmed by ^{1}H, ^{13}C NMR.

Fig. 1 Visual colour change in 7 (16.16 μM, in ethanol–water (8 : 2)) when hydrazine hydrate is added at different concentrations (0, 2, 3, and 4 mM, (a)–(d) respectively).



Scheme 2 Proposed reaction of hydrazine with probe-7.



Scheme 1 Synthesis of (*E*)-3-methyl-1-phenyl-4-((1,2,2,4-tetramethyl-1,2-dihydroquinolin-6-yl)methylene)-1*H*-pyrazol-5(4*H*)-one.

UV-Vis study

The probe sensed hydrazine hydrate very rapidly within few seconds and a visible response was seen. In order to follow the reaction between probe-7 and hydrazine hydrate, the absorbance of 7 at 503 nm was recorded with respect to time after mixing hydrazine hydrate. Using 160 × 10^{-5} M hydrazine hydrate (concentration where the absorbance is almost negligible) and 16.16 μM of 7, absorbance was measured with respect to time (Fig. 2). The initial response was very rapid. The reaction was complete after 90 min.

UV-Vis spectrum of 7 was recorded with increasing concentration of hydrazine hydrate (Fig. 3a and b). Addition of hydrazine to the solution of 7 led to a decrease in the absorbance, which vanished after addition of about 99 equiv. (160 × 10^{-5} M) of hydrazine hydrate. All the spectra were recorded 90 min after the addition of hydrazine hydrate. In the range of 2 × 10^{-5} to 180 × 10^{-5} M of hydrazine hydrate for 16.16 μM of 7, the change in absorption was inversely proportional to the concentration of hydrazine. Thus, 7 acted as an efficient 'turn-off absorbance' probe for the selective detection of hydrazine hydrate in an aqueous alcoholic medium. The experiments were repeated for three times to check the reproducibility of the results.

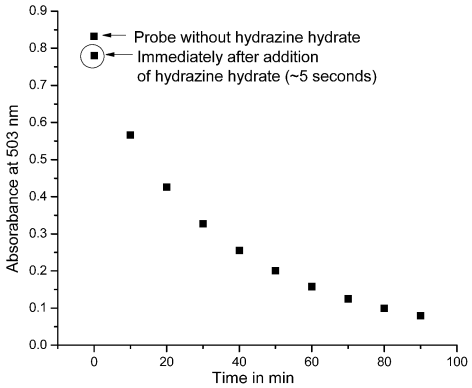


Fig. 2 Plot of absorbance at 503 nm with respect to time of a system containing 160 × 10^{-5} M hydrazine hydrate and 16.16 μM of **7**.

A graph of extinction ratio $(A^0_{503} - A_{503})/A^0_{503}$ *versus* hydrazine hydrate concentration was plotted (Fig. 3c and d). This showed good sensitivity of the probe upon addition of hydrazine hydrate. The extinction ratio showed a good linear relationship with concentration of hydrazine hydrate between 1.23 to 61.9 equivalents (*i.e.*, 2 to 100 × 10^{-5} M) (Co-efficient of determination (R^2) given in the graph). The detection limit[26] of probe-7 for hydrazine hydrate was estimated to be of 16 × 10^{-7} M (ESI†).

Selectivity of 7

Some metals may form complex with the probe and interfere with the sensing process. In fact anions and amines also may interfere. Hence the selectivity of the probe towards hydrazine hydrate was studied with respect to various common metals, anions, and neutral molecules. For 1 equivalent of probe-7, 50 equivalents of interfering anions/cations/neutral molecules were added separately and the absorption spectra was recorded (ESI†). All these spectra were recorded 90 minutes after the addition of anions/cations/neutral molecules. The graph showing the extinction ratio at 503 nm was plotted (Fig. 4a–c). Hydrazine hydrate (50 equivalents/80 × 10^{-5} M) only is found to affect the absorption of 7 to a significant extent. Thus probe-7 showed excellent selectivity towards hydrazine hydrate.

Fluorescence study

An interesting feature of probe-7 was that after addition of hydrazine hydrate is UV absorption was '*turned off*'. When the resulting solution was kept in an UV chamber at 254 nm, hydrazine hydrate '*turned on*' the emission feature of the probe. Hence the fluorescence study of the probe was carried out. The fluorescence spectra of probe-7 and 7 in the presence of increasing concentrations of hydrazine hydrate are given in Fig. 5a. The action of hydrazine with probe-7 turns-on the emission feature and shows peak at 432 nm. Fluorescence spectral changes were estimated after 90 minutes quantitatively

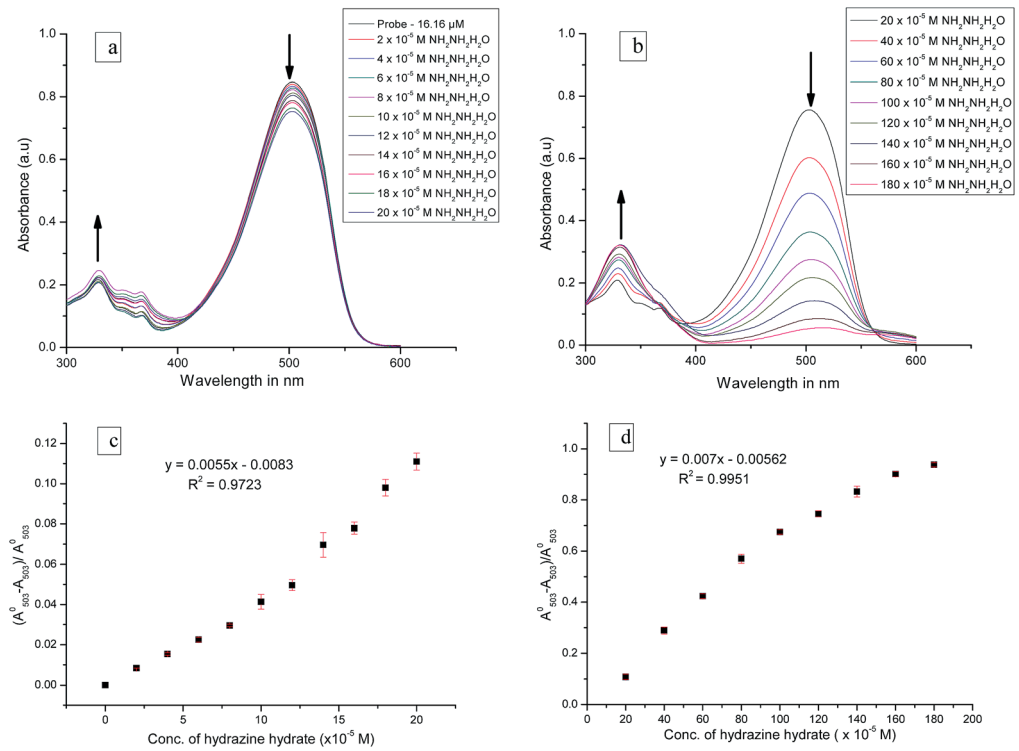


Fig. 3 (a) UV-Vis absorption spectra of the **7** upon the addition of hydrazine hydrate from 2 to 20 × 10^{-5} M (b) UV-Vis absorption spectra of the **7** upon the addition of hydrazine hydrate from 20 to 180 × 10^{-5} M. (c) Plot of $(A^0_{503} - A_{503})/A^0_{503}$ *vs.* hydrazine hydrate concentration from 0 to 20.0 × 10^{-5} M. (d) Plot of $(A^0_{503} - A_{503})/A^0_{503}$ *vs.* hydrazine hydrate concentration from 20 to 180 × 10^{-5} M; error bars for extinction ratio are shown using standard deviations from the mean.

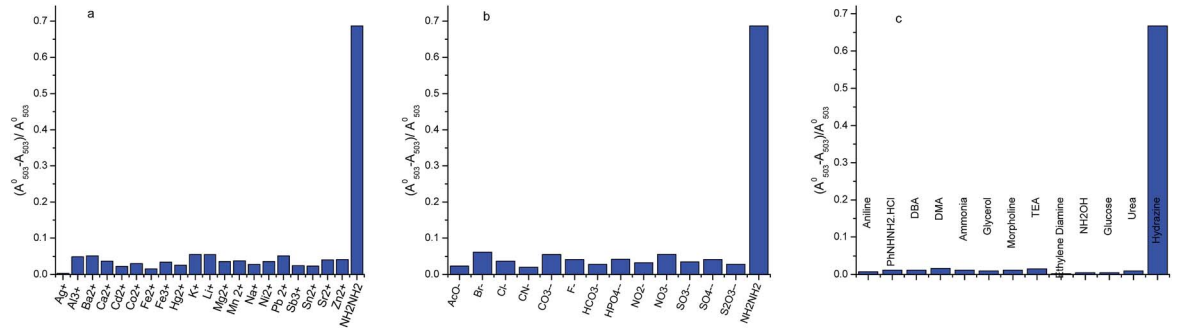


Fig. 4 (a) UV response of probe-7 (16.16 μM) to 50 equivalents of various metals and hydrazine. (b) UV response of probe-7 (16.16 μM) to 50 equivalents of various anions and hydrazine. (c) UV response of probe-7 (16.16 μM) to 50 equivalents of various neutral molecules.

with increasing concentrations of hydrazine (20–400 $\times$ 10^{-5} M) in ethanol–water system (8 : 2) (Fig. 5b). The fluorescence intensity reached a point of negligible deviation upon the addition of 99 equiv. (160.0 $\times$ 10^{-5} M) of hydrazine. The ratiometric changes under fluorescence were same as those observed in UV-vis studies. A linear relationship was found between the emission intensity at 432 nm and the hydrazine concentration over 20–140 $\times$ 10^{-5} M range. The fluorescence response of the probe with respect to other common interfering anions, cations, and related amino molecules also was checked. This study showed very high selectivity of the probe towards hydrazine hydrate among others (ESI†).

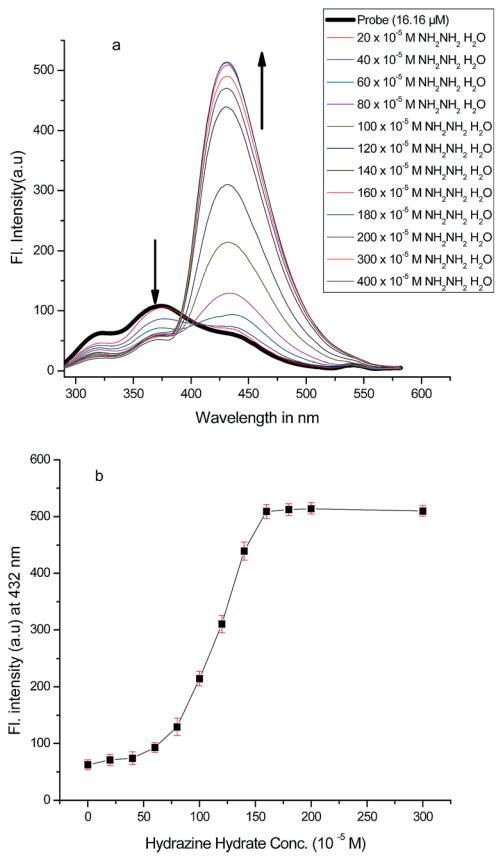


Fig. 5 (a) Fluorescence spectra of probe-7 (16.16 μM) with increasing concentrations of hydrazine hydrate. (b) Variation of Fl intensity of probe-7 (16.16 μM) at 432 nm with increasing concentrations of hydrazine hydrate; error bars for extinction ratio are shown using standard deviations from the mean.

NMR study

^{1}H NMR and ^{13}C NMR were used for characterizing the product. The ^{1}H NMR of the probe-7 and that of product-**8** on reaction of **7** with hydrazine hydrate were recorded. From the spectra it appears that the pyrazolone part was entirely removed in **8**. (Fig. 6). ^{1}H NMR of product-**8** shows loss of methyl (δ = 2.33) and phenyl groups of pyrazolone unit. The proton of probe-7 at 8.47 (=C*H*–C) is shifted to 8.58 (N=C*H*–C) in the product-**8**. Further when the ^{1}H NMR spectrum of **8** was compared with

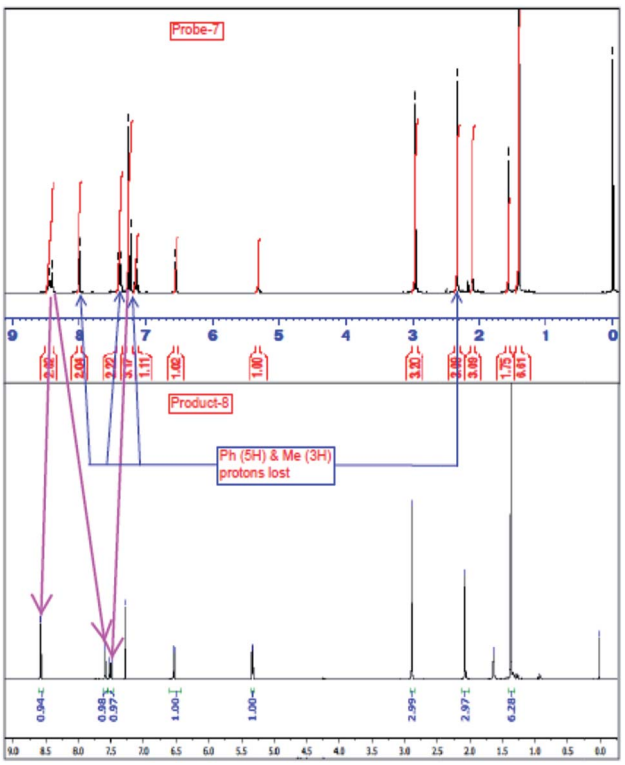


Fig. 6 ^{1}H NMR spectra in $CDCl_3$ of probe-7 and product-**8**.

that of 3, the only difference was found on the peak position of aldehydic H of 3 ($\delta = 9.8$) and the proton of imine ($\delta = 8.58$).

TLC plate detection techniques

Detection of hydrazine hydrate by TLC plates. Probe-7 was dissolved in ethyl acetate to get a 5 mM solution. Thin layer chromatography (TLC) plates were dipped into it and kept for 30 min at room temperature for drying. These plates were immersed into solutions of different concentrations of hydrazine hydrate in ethanol–water (8 : 2) medium and then exposed to air to get rid of the solvent. A minimum of 1 mM of hydrazine hydrate was easily recognizable by naked eye in this TLC plate technique. This is similar as observed in naked eye detection in aqueous ethanolic medium in Fig. 1. Hence the detection limit of hydrazine hydrate by TLC plate technique is 1 mM. In Fig. 7, the complete colour change from orange to colourless is shown.

Detection of hydrazine gas. The probe could rapidly and effectively sense hydrazine gas. This experiment was carried out using a TLC plate coated with probe-7. A 5 mM solution of the probe was prepared in ethyl acetate. A TLC plate was dipped into it and dried at room temperature for 30 min. The TLC plate was kept in a vial. Excess of hydrazine gas was passed into the vial for 5 s. The colour of the TLC plate changed from orange to colourless within 5 s (Fig. 8).

DFT study

In order to throw more light on the actual chromo/fluorophore, DFT studies were done on 7 and the possible reduced product-**8**. DFT study was done using Gaussian 03 program.[27] We performed B3LYP exchange correlation functional under 6-311G+, p, d basis set and calculated HOMO and LUMO levels. The optimized geometries and calculated electron distributions in the FMOs of probe-7 and product-**8** are shown in Fig. 9. The HOMO–LUMO orbital distribution is spread from the quinoline moiety to the pyrazolone moiety. There is a planarity over the major chromophore extending from quinoline to pyrazolone unit. The HOMO–LUMO energy gap in the probe-7 is less than that of the product-**8** because of extended conjugation. This observation is matching with the fact that the probe is coloured and the product is not.

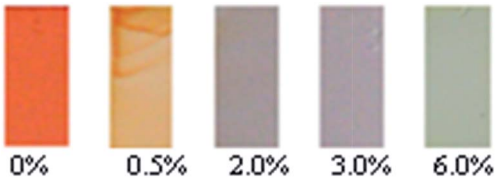


Fig. 7 Visible colour changes of probe-7 (5.0 mM)-coated on TLC plates after exposure to different concentrations (0%, 0.5%, 2.0%, 3.0%, and 6.0%) of hydrazine hydrate.

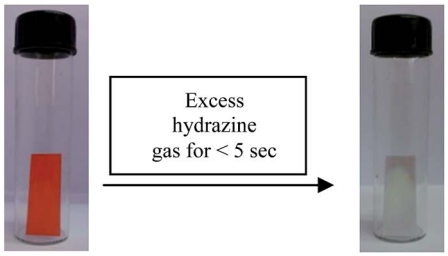


Fig. 8 TLC plate before hydrazine gas passing, and after excess hydrazine gas passing for 5 seconds.

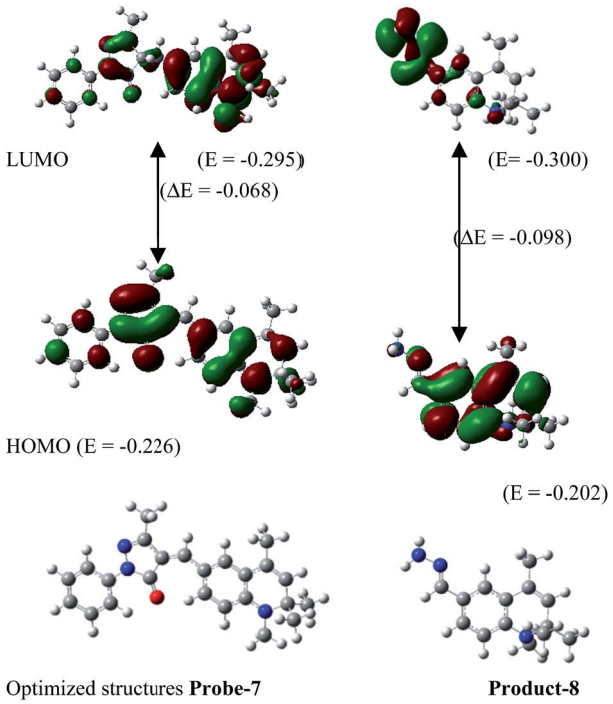


Fig. 9 HOMO–LUMO levels and optimized structures of probe-7 and its reduced product from Gaussian 03.

Conclusion

A merocyanine probe is described that provides very good sensitivity for detecting hydrazine without needing any advanced, complex readout equipment or complex buffer making procedure. The synthesis of the probe is very easy. The detection limit of the probe for hydrazine hydrate is 1.6 μM. The gaseous hydrazine is detected by the probe within 5 seconds. The TLC plate technique provides a more practical and sensitive detection method.

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