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SYNTHESIS, CRYSTAL STRUCTURE AND SPECTRAL PROPERTIES OF THIAZOLE ORANGE DERIVATIVE

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This work aims to study the crystal structure and spectral properties of the fluorescent dye, thiazole orange (TO) derivative. The single crystals with a size of 0.20×0.16×0.12 cm^3 have been obtained by the slow evaporation of a methanol solution at room temperature and characterized by NMR, TG/DTA thermograms, IR spectroscopy and single crystal X-ray diffraction. The compound crystallizes in the Triclinic system and belongs to P-1 space group. The fluorescence spectra of the crystal in solutions have been measured.

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1. Introduction

Fluorescent dyes probes have attracted much attention because of their specialities with fast detection speeds, good repeatability, low dosage, and non-radiation advantage. The cyanine dye based probe is used to detect the structures of RNA and DNA, study the remedy of damaged basic groups in DNA, identify the status of amino group and the active site of proteins, detect protein at the picomolar scale, distinguish nucleic acids with different conformations and the chemically reactive activities of related drugs [1-3].

It is remarkable that the embedded cyanine probe inserting into DNA structures by affinity has been used in live cellular imaging due to their significant fluorescence enhancements when bound to cellular nucleic acids or to a specific surface-expressed protein partner [4] in sharp contrast to their low quantum yield in solution.

Thiazole orange, a benzothiazole ring covalently linked to a quinoline ring through a monomethine bridge, has been widely used as the embedded cyanine dye for labeling nucleic acids, allowing the detection of DNA and RNA in gels, flow cytometry, or microscopy [5].

Embedded fluorescent dyes such as Thiazole Orange (TO) and Oxazole Yellow (YO) are of particular interest because they have lower quantum yield and weaker fluorescence in solution, while larger fluorescence enhancements are observed when bound to DNA or RNA partner [6].

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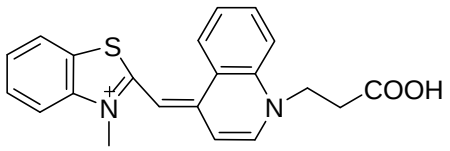
The obvious distinction of fluorescence between free dye and nucleic acid-bound dye provides an excellent way to image, label and detect cancer. TO and its derivatives can be synthesized in liquid phase by several different routes: (1) 2-mercaptobenzothiazole reacts with lepidine derivatives to yield TO [7-11]; (2) 2-aminobenzothiazole, instead of 2-mercaptobenzothiazole, reacts with lepidine derivatives to afford TO [12, 13]; (3) TO and its derivatives can also be prepared by reacting 2-methylbenzothiazole with lepidine derivatives [14].

Recently, we have designed and synthesized TO and its derivatives on the solid phase as a method for the traceless cleavage of Merrifield resin [15]. In recent years, researchers intensively investigated the modification of TO on the ring of benzothiazole or the side-chain binding with nitrogen on lepidine to improve their fluorescence intensities [16-25]. The fluorescent properties and the application of TO and its derivatives with carboxyl groups in biochemistry provoked us to study the crystal structure of TO derivative with carboxyl group.

In this paper, we present our results on the synthesis, crystal growth and spectral properties of TO derivative (we called TO-COOH)(Scheme 1). The crystal belongs to the P-1 space group in triclinic crystal system. The UV and fluorescence spectra of the crystal have been determined.



Scheme 1 The structure of the TO derivative.

2. Results and discussion

2.1. Infrared (IR) analysis

The IR spectrum of the compound (Fig.1) exhibits a wide absorption peak at 3387.2 cm^{-1} and a sharp intense absorption peak at 1609 cm^{-1}, which are corresponding to the stretch vibration of –OH and C=O group respectively. The bind of 1378 cm^{-1} and 1578 cm^{-1} can be assigned to the aromatic C–H stretching vibrations and 1506 cm^{-1} of C=C bending modes to α,β-unsaturated carbons. 1061 cm^{-1} is due to C=S vibrations. 746 cm^{-1} and 757 cm^{-1} are characteristic bands for o-substituted aromatic ring C-H out-of-plane wagging. The functional groups present in the TO-COOH molecule were identified and the assignments are summarized in Table 1.

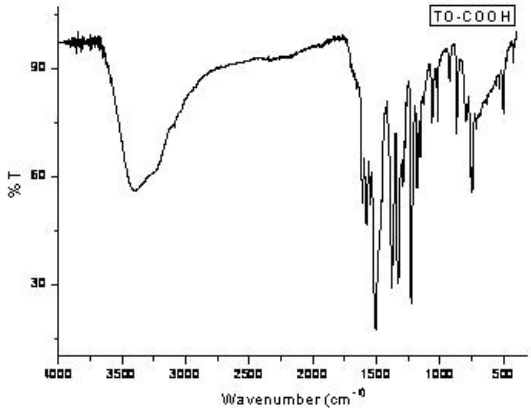


Fig. 1 The IR spectrum of the TO derivative.

Table 1 Assignment of vibrational frequencies of the TO derivative.

IR bands(cm^{-1})	Assignment
3387.2	-OH stretching
1609	C=O
1578	Aromatic ring vibrations
1506	C=C bengding modes of α,β-unsaturated carbons
1378	Aromatic ring vibrations
1185	C-O stretching
1061	C=S
1020	Aromatic ring stretching
925	O-H out-of-plane vibrations
746,757	o- substituted aromatic ring C-H out-of-plane wagging

2.2. Single Crystal XRD

The crystalline sample with a typical dimension of 0.20×0.16×0.12 cm^3 was used for the measurements. A summary of the refinement details obtained from single crystal XRD in this study are given in Table 2. From the XRD data, the compound crystallizes in the Triclinic P-1 space group with cell parameters a = 10.162(2) Å, b = 10.501(2) Å, c = 11.040(2) Å, α= 92.17(3) °, β= 117.10(3) °, γ= 92.28(3) ° and *Z*=2. The final refinement gave *R*=0.0625, *wR*= 0.1862 ($w = 1/[\sigma^2(F_o^2) + (0.0790P)^2 + 1.2740P]$, where $P = (F_o^2 + 2F_c^2)/3$), $(\Delta/\sigma)_{max} = 0.001$, $(\Delta\rho)_{max} = 0.752$, $(\Delta\rho)_{min} = -0.433$ e/Å^3 and $S = 1.086$.

The molecular structure of the compound and the crystal lattice are shown in Fig. 2 and Fig. 3 respectively. CCDC 739300 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the CCDC, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033; e-mail: deposit@ccdc.cam.ac.uk).

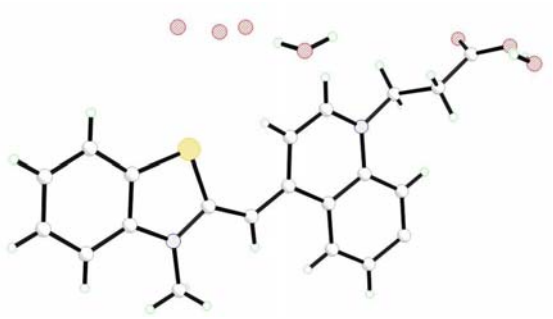


Fig. 2 The molecular structure of the TO derivative.

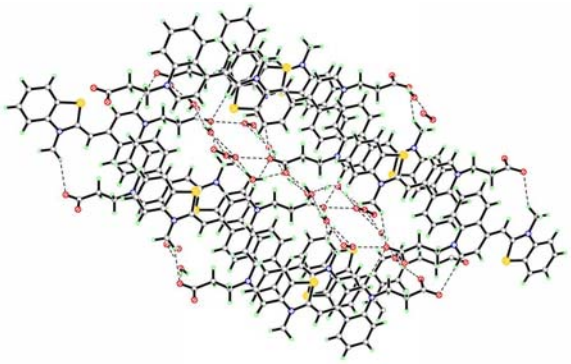


Fig. 3 The crystalline lattice of the TO derivative.

Table 2 Single-crystal XRD data of the TO derivative.

Formula	$C_{21}\ H_{26}\ N_2\ O_6\ S$
Formula weight	434.50
CCDC deposit no	CCDC-739300
Crystal system	Triclinic
Space group	p-1
a	10.162 (2) Å
b	10.501(2) Å
c	11.040(2) Å
α	92.17(3) ^{o}
β	117.10(3) ^{o}
γ	92.28(3) ^{o}
μ	0.2mm^{-1}
V	1045.9(4) $Å^3$
Z	2
D (calc)	1.380mg/m^{-3}
F(000)	460
Completeness to θ= 25.02	99.1%
Reflections collected / unique	8366 / 3658 [R(int) = 0.0358]
Goodness-of-fit on F^2	1.089
Final R indices [I>2sigma(I)]	R_1 = 0.0625, wR_2 = 0.1862
R indices (all data)	R_1 = 0.0787, wR_2 = 0.2079

2.3 Thermal studies

The sample was scanned in the 50–500 °C range at a rate of 5 °C/min under nitrogen atmosphere and the TG/DTA result is shown in Fig. 4. The endothermic peak observed at 88 °C is attributed to H_2O, the peak at 161°C and 190 °C are attributed to melting of the compound with crystal water and the compound, respectively.

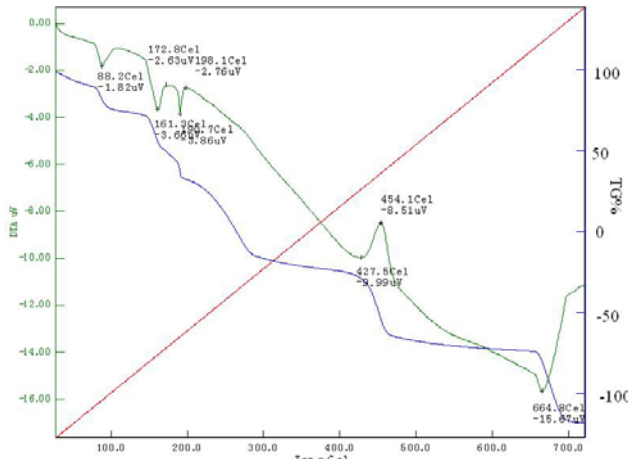


Fig. 4 The TG/DTA curve of the TO derivative.

2.4 Fluorescence studies

The fluorescence intensities in different solvents containing 0.05 mg/mL of the compound in CH_3OH, DMSO and DMF, respectively, were excited at 460 nm to give fluorescence spectra (Fig. 5), which clearly shows the fluorescent intensities are much higher in the DMSO and DMF than that in CH_3OH with no fluorescence emission wavelength shift.

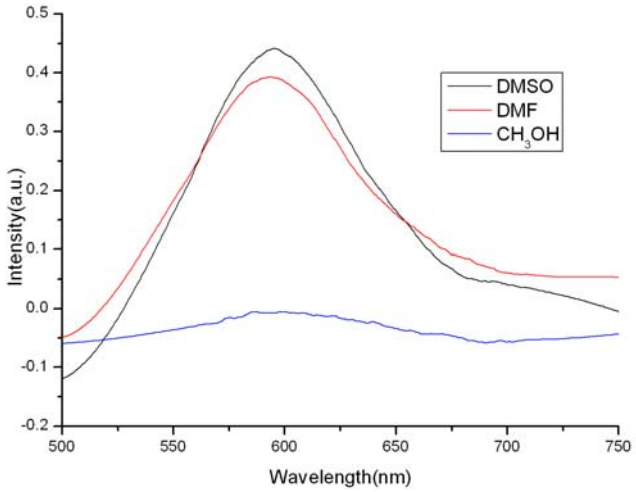


Fig. 5 The fluorescence of the TO derivative in different solvents.

The fluorescence intensities under different pH conditions are also measured. DMSO

(35mL) was used as the standard solvent in which 5mg of the compound was dissolved. The pH values of four solutions with the same concentration of 0.0142 mg/mL were adjusted to 2.8, 5.2, 8.5, 9.5, respectively, by addition of NaOH or HCl. The results show that the fluorescence emission wavelengths appear no shift when the pH was changed. However, the lower the pH value, the lower fluorescence intensity (shown in Fig. 6). We also found that there was a high increasing rate of the fluorescence intensity at lower pH values, whereas only small changes were observed in the pH range of 5-10.

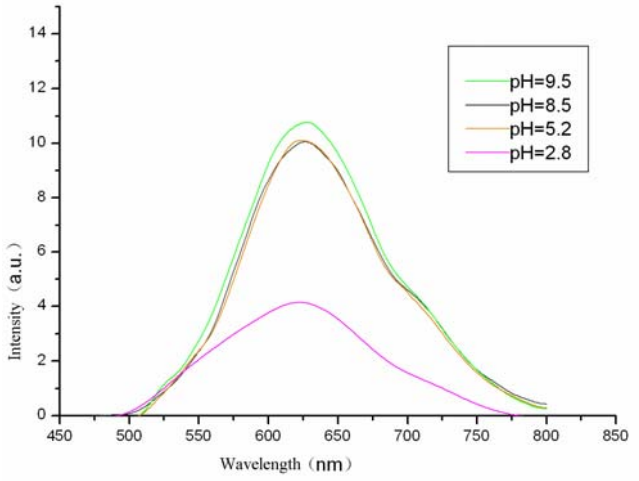


Fig. 6 The fluorescence of the TO derivative in DMSO at different pH values.

3. Experimental procedure

3. 1 General

^{1}HNMR spectra were recorded on a Bruker AC-P300 (300 MHz) spectrometer. Chemical shifts are reported in parts per million (ppm) downfield from TMS (tetramethylsilane), using DMSO-d_6 as a solvent. Mass spectral analyses were obtained using an electrospray ionization (ESI) mass spectrometer. Infrared spectra were recorded on a Nicolet380 FT-IR Spectrometer from ThermoNicolet Company, American with the following acquisition parameter: sample scan 6; frequency range 400-4000cm^{-1}. The thermal behavior was investigated by an EXSTAR simultaneous thermo-gravimetric/differential thermal analyzer (TG/DTA6300). The fluorescence intensities were measured on a Cary Eclipse, American Fluorescence spectrophotometer from Varian, Inc.

3.2 Synthesis

The dye was synthesized by a slightly modified procedure described previously [26]. The benzothiazolium and 4-methyl quinoline salts of the dye were prepared via nitrogen quaternization by reacting 2-benzylmercaptobenzothiazole with methyl *p*-methyltoluenesulfonate at 110°C for 24 h and 4-methylquinoline with 3-bromopropionic acid in refluxing acetone for 8 h, respectively. The methyl salt and quinoline salt were obtained in 81% and 39% yields, respectively. The dye

was obtained in 97% yield by condensation of the corresponding benzothiazolium with 1.35 equivalents of 4-methyl quinoline salts and 2.5 equivalents of triethylamine (Et_3N) in ethanol at room temperature for 1h.

To grow crystal of TO-COOH, the solubility of the TO-COOH was evaluated in numerous solvents such as acetone, methanol, DMF and DMSO. Its solubility in DMSO and DMF is higher compared to that in methanol and acetone. We used methanol as the solvent to grow crystal due to its moderate solubility. A saturated solution of TO-COOH in methanol at 25°C was filtered thrice to remove suspended particles. The solution was then transferred into a beaker and allowed for slow evaporation at ambient temperature. The resulting crystals are stable and non-hygroscopic at room temperature. IR (KBr) v (cm^{-1}): 3387.2, 1609, 1578, 1506, 1378, 1185, 1061, 1020,925. ^1H NMR (300MHz, DMSO-d6): δ2.41(t, J=6.15Hz, 2H), 4.00(s, 3H), 4.71(t, J=6.30Hz, 2H), 6.90(s, H), 7.34-7.42(m, 2H), 7.60(t, J=,7.80Hz, 1H), 7.75(t, J=7.65Hz, 2H), 7.96-8.04(m, 2H), 8.14(d, J=9.00Hz, 1H), 8.74-8.81(dd, J_1=7.20Hz, J_2=8.70Hz, 2H). ESI-MS: m/e 363.25 (M^+), 364.25 (M^++1).

3.4 X-ray crystallography

A red crystal of the title compound having approximate dimensions of 0.20×0.16×0.12 cm^3 was mounted on the top of a glass fiber. X-ray diffraction data were collected on a Bruker Smart-1000 CCD diffractometer equipped with a graphite-monochromatized MoK_α radiation (0.7170 Å) by using an ω/φ scan mode in the range of 1.94≤θ≤25.02° (–-12<=h<=12, -12<=k<=12, -13<=l<=13) at 113(2) K. A total of 8366 reflections were collected with 3658 unique ones (R_{int} = 0.0359), of which 2901 with $I > 2\sigma(I)$ were observed. The structure was solved by direct methods and refined on F^2 by full-matrix least-squares procedure with SHELXS-97[27] and SHELXL-97[28].

4. Conclusions

The single crystals of the TO derivative compound have been grown by the slow evaporation of a methanol solution at room temperature and characterized by NMR, TG/DTA thermograms, IR spectroscopy and single crystal X-ray diffraction. The fluorescent intensity varies in different solvents and at different pH values.

Acknowledgements

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