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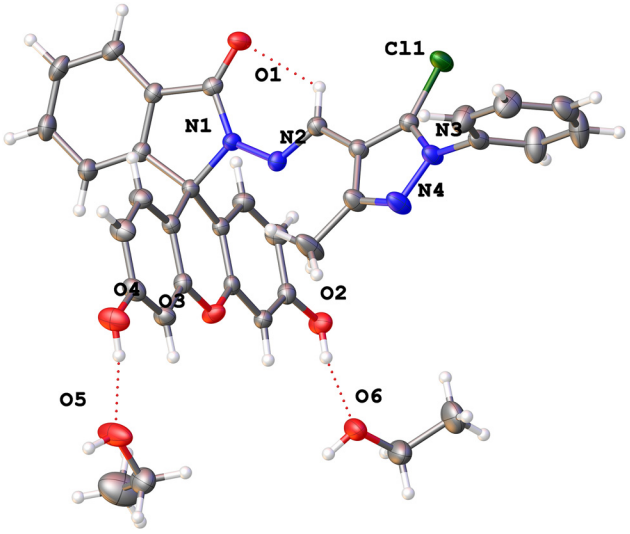
Crystal structure of (E)-2-(((5-chloro-3-methyl-1-phenyl-1H-pyrazol-4-yl)methylene)amino)-3′,6′-dihydroxyspiro[isoindoline-1,9′-xanthen]-3-one – ethanol (1/2), $C_{35}H_{33}ClN_4O_6$



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Abstract

$C_{35}H_{33}ClN_4O_6$, monoclinic, $P2_1/c$ (no. 14), a = 18.2808(8) Å, b = 9.0510(4) Å, c = 19.4323(7) Å, β = 93.406(10)°, V = 3209.6(2) Å^3, Z = 4, $R_{gt}(F)$ = 0.046, $wR_{ref}(F^2)$ = 0.129, T = 120.0 K.

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Table 1: Data collection and handling.

Crystal:	Light colourless block
Size:	0.28 × 0.15 × 0.10 mm
Wavelength:	Mo *Kα* radiation (0.71073 Å)
μ:	0.17 mm^{-1}
Diffractometer, scan mode:	Bruker APEX-II, φ and ω scans
θ_{max}, completeness:	27.5°, 99 %
$N(hkl)_{measured}$, $N(hkl)_{unique}$, R_{int}:	27117, 7343, 0.030
Criterion for I_{obs}, $N(hkl)_{gt}$:	$I_{obs} > 2\ \sigma(I_{obs})$, 6,152
$N(param)_{refined}$:	422
Programs:	Bruker,[1] Olex2,[2,3] SHELX[4,5]

The molecular structure is shown in the figure. Table 1 contains the crystallographic data and the list of the atoms including atomic coordinates and displacement parameters can be found in the cif-file attached to this article.

1 Source of material

The title compound is a natural product, which has been obtained by extraction and isolation from fungal fermentation products.[6] All chemicals were of analytical grade and used as received without further purification. The preparation of the title compound is similar to the previously reported literature procedure.[6] Fluorescein hydrazide (200 mg, 577.46 µmol) and 5-chloro-3-methyl-1-phenyl-1H-pyrazole-4-carbaldehyde (127.42 mg, 577.46 µmol) were mixed in ethanol (15 mL) with addition of a catalytic amount of acetic acid. The reaction mixture was refluxed at 78 °C for 2.5 h and the reaction was shown to be completed by thin layer chromatography (TLC). After cooling to room temperature, the solution was filtered, and the colorless crystals were obtained by slow evaporating in ethanol at room temperature.

2 Experimental details

The structure was treated with the Olex2 crystallographic software package,[2,3] solved with the SHELXT structure

solution program and refined with the SHELXL refinement package.[4,5] Carbon-bound hydrogen atoms were placed in calculated positions and refined with riding coordinates, with U_{iso}(H) fixed at 1.2 times of U_{eq}(C) (Tables 1 and 2).

3 Comment

Fluorescein serves as an ideal fluorescent reporter for chemosensors due to its superior photophysical properties.[7] Moreover, certain fluorescein hydrazones derived from substituted benzaldehydes have demonstrated significant biological activity, by functioning as non-intercalative topoisomerase catalytic inhibitors.[8] Notably, fluorescein hydrazone derivatives incorporating aldehyde moieties have been developed as effective fluorescent probes for the detection of Hg^{2+}, Zn^{2+} or Cu^{2+} ions.[9–11] Herein, we report the crystal structure of the title compound derived from fluorescein hydrazide and a substituted pyrazole-4-aldehyde, namely 5-chloro-3-methyl-1-phenyl-1*H*-pyrazole-4-carbaldehyde.

The title compound $C_{35}H_{33}ClN_4O_6$ was crystallized in $P2_1/c$ with one $C_{31}H_{21}ClN_4O_4$ and two solvent molecules C_2H_6O in its asymmetric unit. The molecule structure comprises one xanthene ring and one 1-phenyl-1H-pyrazole moieties. The geometry of the title structure was characterized with the bond angles and lengths. In particular, the bond angles for C23···N4···N3, O1···C20···N1, and C22···C24···Cl1 are 106.14 (13)°, 125.74(14)° and 129.00(12)°, respectively. For another, the bond lengths for N2···C30, N2···N1, O1···C20, CI1···C24, N4···C23 and C22···C24, are 1.283(2) Å, 1.3661(17) Å, 1.2306(19) Å, 1.6973(16) Å, 1.324(2) Å and 1.377(2) Å. All bond angles and lengths of the title structure are within the expected range and comparable with those of reported fluorescein hydrazones[12–14] and a directly related spiro compound.[15] In the crystal, hydrazine molecules are arranged in layers by ethanol molecules via hydrogen bonds O–H···O and O–H···N, with max D–A distance 2.9 Å and minimum angle 120°.

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References

1. BRUKER. *SAINT, APEX2 and SADABS*; Bruker AXS Inc.: Madison, Wisconsin, USA, 2009.
2. Dolomanov, O. V.; Bourhis, L. J.; Gildea, R. J.; Howard, J. A. K.; Puschmann, H. OLEX2: a Complete Structure Solution, Refinement and Analysis Program. *J. Appl. Crystallogr.* **2009**, *42*, 339–341.
3. Bourhis, L. J.; Dolomanov, O. V.; Gildea, R. J.; Howard, J. A. K.; Puschmann, H. The Anatomy of a Comprehensive Constrained, Restrained Refinement Program for the Modern Computing Environment-Olex2 Dissected. *Acta Crystallogr.* **2015**, *71*, 59–75.
4. Sheldrick, G. M. A Short History of SHELX. *Acta Crystallogr.* **2008**, *64*, 112–122.
5. Sheldrick, G. M. Crystal Structure Refinement with SHELXL. *Acta Crystallogr.* **2015**, *71*, 3–8.
6. Sandhu, N.; Singh, A. P.; Saxena, A.; Pandey, S. K.; Yadav, R. K. X-ray Crystallographic, Electrochemical, Quantum Chemical and Antimicrobial Analysis of Fluorescein Based Schiff Base. *J. Mol. Struct*. **2020**, *1221*, 128762.
7. Chen, X. Q.; Pradhan, T.; Wang, F.; Kim, J. S.; Yoon, J. Fluorescent Chemosensors Based on spiroring-opening of Xanthenes and Related Derivatives. *Chem. Rev*. **2012**, *112*, 1910–1956.
8. Rahman, A. F. M. M.; Park, S.-E.; Kadi, A. A.; Kwon, Y. Fluorescein Hydrazones as Novel Nonintercalative Topoisomerase Catalytic Inhibitors with Low DNA Toxicity. *J. Med. Chem.* **2014**, *57*, 9139–9151.
9. Roy, S.; Mondal, T.; Dey, D.; Mane, M. V.; Panja, S. S. A New thiophene-Appended fluorescein-hydrazone-based chromo-fluorogenic Sensor for the Screening of Hg^2+ Ions in Real Water Samples. *Chemistryselect* **2021**, *6*, 10464–10479.
10. An, J.; Yan, M.; Yang, Z.; Li, T.; Zhou, Q.; An, J.; Yan, M.; Yang, Z.; Li, T.; Zhou, Q. A Turn-on Fluorescent Sensor for Zn(II) Based on fluorescein-coumarin Conjugate. *Dyes Pigments* **2013**, *99*, 1–5.
11. Chen, X.; Li, Z.; Xiang, Y.; Tong, A. Salicylaldehyde Fluorescein Hydrazone: a Colorimetric Logic Chemosensor for pH and Cu(II). *Tetrahedron Lett.* **2008**, *49*, 4697–4700.
12. Liu, B.; Xu, X.; Zhu, Z. Crystal Structure of (Z)-2-hydroxy-N′-(1-(o-tolyl) ethylidene)benzohydrazide, $C_{16}H_{16}N_2O_2$. *Z. Kristallogr. – N. Cryst. Struct.* **2025**, *240*, 423–424.
13. Zhang, L.; Wu, W.-N.; Wang, Y. Crystal Structure of (E)-2-(4-ethoxycarbonyl-3,5-dimethyl-2-(pyrrole-2-ylmethyleneamino)-3′,6′-dihydroxylspiro[isoindoline-1,9′-xanthen]-3-one-methanol (1/1), $C_{31}H_{29}N_3O_7$. *Z. Kristallogr. – N. Cryst. Struct.* **2021**, *236*, 239–241.
14. Polyakova, I. N.; Starikova, Z. A.; Parusnikov, B. V.; Krasavin, I. A.; Zhadanov, B. V. The Lactone Form of Fluorescein: Crystal Structure of the 1:1 Molecular Complex of Fluorescein with Methanol. *J. Struct. Chem*. **1984**, *25*, 752–757.
15. Song, Y.-F.; Wu, W.-N.; Wang, Y. Crystal Structure of (E)-2-(((1H-pyrrol-2-yl)methylene)amino)-3′,6′-dihydroxyspiro[isoindoline-1,9′-xanthen]-3-one-methanol (1/2), $C_{27}H_{25}N_3O_6$. *Z. Kristallogr. – N. Cryst. Struct.* **2021**, *236*, 257–258.