

## Supporting Information

A novel "on-off" peptide fluorescent probe for the detection of copper and sulfur ions in living cells

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**Tab. S1.** Comparison of probes for Cu<sup>2+</sup> assays reported in recent literature.

Probe	Detection condition	Detection limit	Detection method	cell imaging	Refs.
2',7'-dichlorofluorescein	DMSO/HEPES = 1/99 (v/v), and PH 7.4	5 nM	Turn on	Yes	1
Nitrogen and Fluorine Co-Doped Carbon Dots	deionized water	347 nM	Turn off	No	2
Nitrogen and sulfur doped carbon dots	aqueous solutions	23.4 nM	Turn off	No	3
The hybrid of phenanthraquinone and imidazole dye	DMSO, PBS buffer (PH 7.4)	—	Turn off	Yes	4
Chromone-3-aldehyde	THF	4.93 × 10 <sup>-6</sup> M	Turn off	No	5
Naphthaldehyde	EtOH: H <sub>2</sub> O (8:2 v/v)	9.342 × 10 <sup>-7</sup> M	UV-Vis	No	6
Diarylethene	Acetonitrile	1.76 × 10 <sup>-9</sup> M	Turn on	No	7
FITC-Ahx-Ser-Ser-His-Thr-Glu-Phe-NH <sub>2</sub>	PBS solution (10.0 mM, PH 7.4)	38.2 nM	Turn off	Yes	This work

**Tab. S2.** Comparison of probes for S<sup>2-</sup> assays reported in recent literature.

Probe	Detection condition	Detection limit	Detection method	cell imaging	Refs.
Naphthylamine derivatives	30% DMF and 0.1 M PBS buffer	$3.1 \times 10^{-7}$ M	Turn on	Yes	8
Salamo-based	EtOH/H <sub>2</sub> O (10 : 1)	$3.27 \times 10^{-8}$ M	Turn on	NO	9
Phenyl 2-(benzoylthio) benzoate-based	50 mM PBS buffer (pH 7.4) with 100 $\mu$ M cetrimonium bromide	100 nM	Turn on	Yes	10
(Dansyl-Glu-Glu)2-Lys-NH <sub>2</sub>	10 mM HEPES buffer solution at PH 7.4	87 nM	Turn on	Yes	11
ZnO nanoparticles	Methanol/water (1: 99, v/v)	6.4 nM	Turn on	No	12
Phen-anthro[9,10-d]imidazole platform	DMF/H <sub>2</sub> O solution	12.3 nM	Turn on	Yes	13
Cu (II)-dependent DNAzyme	Tris-HCl buffer	0.2 $\mu$ M	Turn on	No	14
FITC-Ahx-Ser-Ser-His-Thr-Glu-Phe-NH <sub>2</sub>	PBS solution (10.0 mM, pH 7.4)	37.9 nM	Turn on	Yes	This work

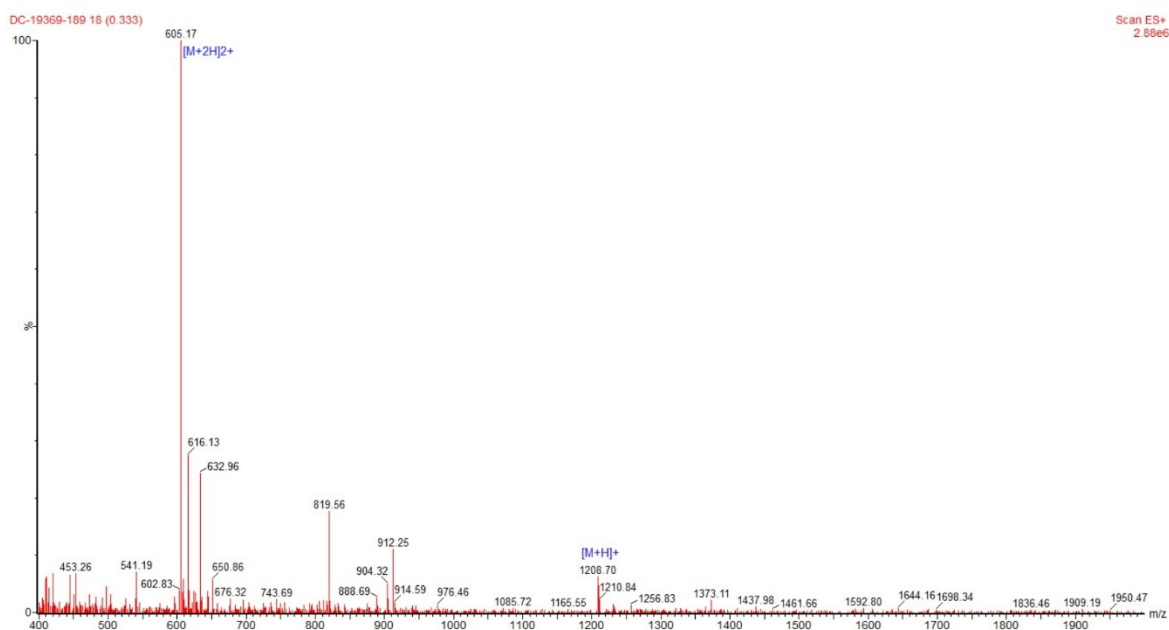
**Tab. S3.** Materials

	Reagent name	Abbreviated name
1	rink amide resin	AM
2	Fmoc-Phe -OH	/
3	Fmoc- Thr (Trt)-OH	/
4	Fmoc- His (Trt) - OH	/
5	Fmoc-Ser(tBu)-OH	/
6	6-Fmoc-Ahx	/
7	Fluorescein isothiocyanate isomer	FITC
8	1-hydroxybenzotriazole	HOBT
9	diisopropylethylamine	DIEA
10	trifluoroacetic acid	TFA
11	triisopropylsilane	TIS
12	Piperidine	/
13	dichloromethane	DCM

14	N, N-dimethylformamide	DMF
15	anisole	/
16	ethyl ether	Et <sub>2</sub> O

**Tab. S4.** Instruments

	Experimental project	Instrument
1	Absorption spectra	UV-2550 UV-Visible Spectrophotometer
2	Fluorescence spectra	F7000 fluorescence spectrometer
3	Electrospray ionization MS	Waters micromass ZQ2000
4	High performance liquid chromatography separation	Waters ailiance 2695 liquid chromatograph
5	Cytotoxicity	Infinite M200 PRO
6	cell images	Zeiss LSM 880 confocal microscope



**Fig. S1.** ESI-MS spectrum of FGP1

### HPLC chromatogram of FGP1

Sample ID: FGP1

Sequence: FITC-Ahx-SSHTEF-NH<sub>2</sub>

Column: 4.6\*150 mm, kromasil C18-5

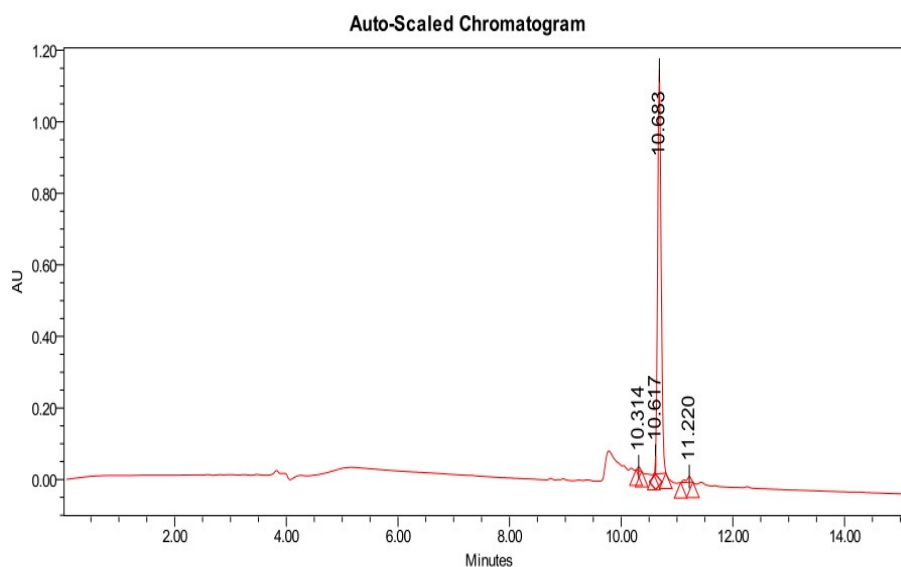
Solvent A: 0.1% Trifluoroacetic in 100% Acetonirile

Solvent B: 0.1% Trifluoroacetic in 100% Water

Gradient:	A	B
0.01 min	5%	95%
25.0 min	95%	5%

30.0 min      90%      10%

Flow rate: 1.0 mL/min  
Wavelength: 214 nm  
Volume: 20  $\mu$ L



**Fig.S2** HPLC Chromatogram of FGP1

**Tab. S5.** HPLC chromatogram data of FGP1

Rank	RT	Area	Height	Area%
1	10.314	36807	10506	0.73
2	10.617	31020	49604	0.61
3	10.683	4895227	1104382	96.78
4	11.220	95253	15536	1.88

**MS Analysis Report**

Sample ID: FGP1  
Expected MS:1208.1  
Flow rate: 0.2 mL/min  
Run Time: 1 min  
Buffer A: 0.1% HCOOH in water  
Buffer B: 0.1% HCOOH in Acetonirile

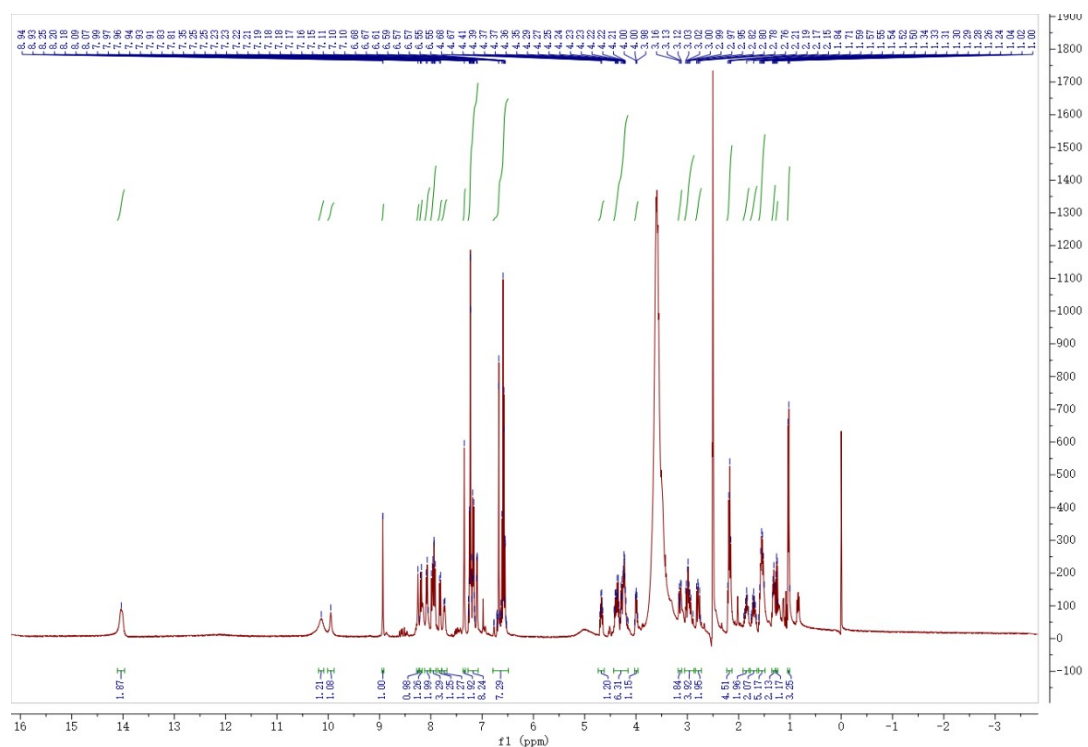


Fig. S3.  $^1\text{H}$  NMR spectrum of FGP1 in  $\text{DMSO}-d_6$ .

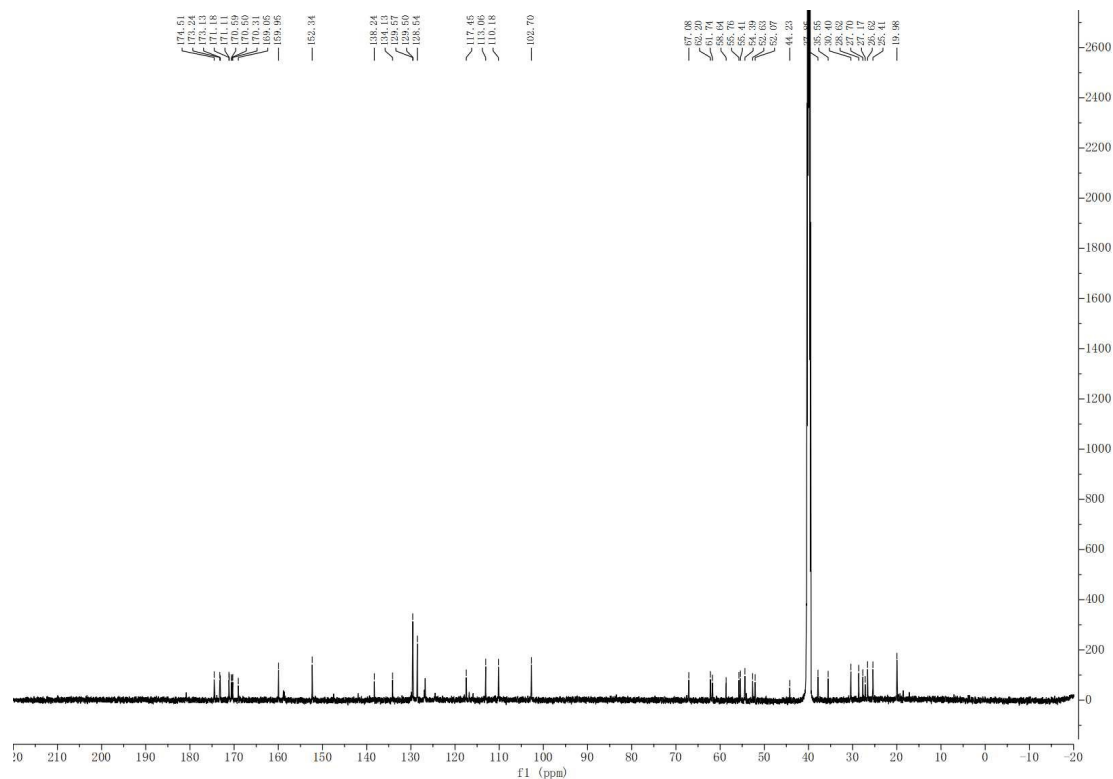
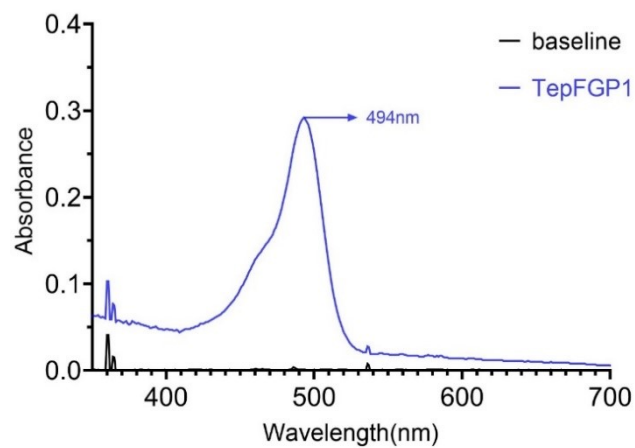
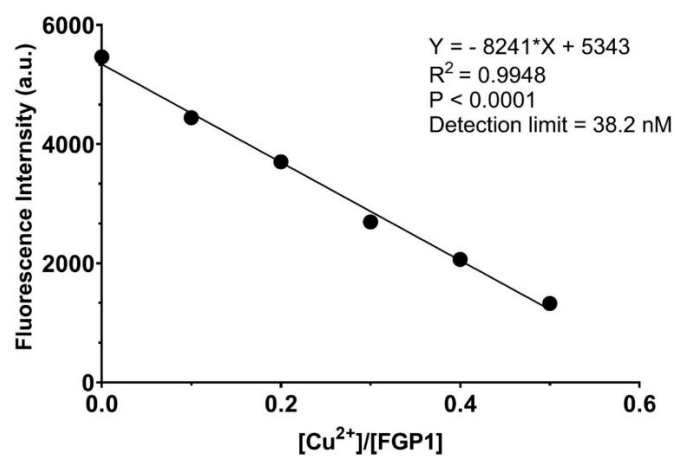


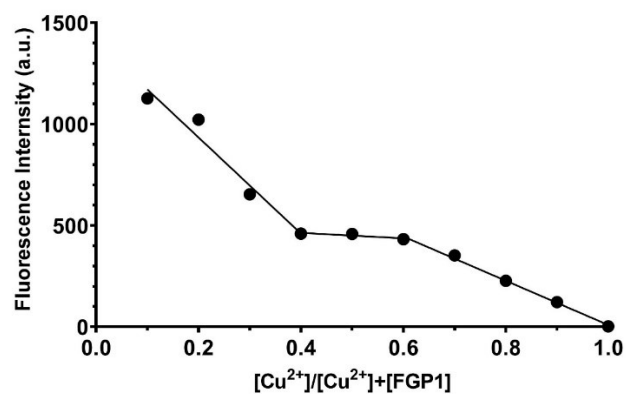
Fig. S4  $^{13}\text{C}$  NMR spectrum of FGP1 in  $\text{DMSO}-d_6$ .



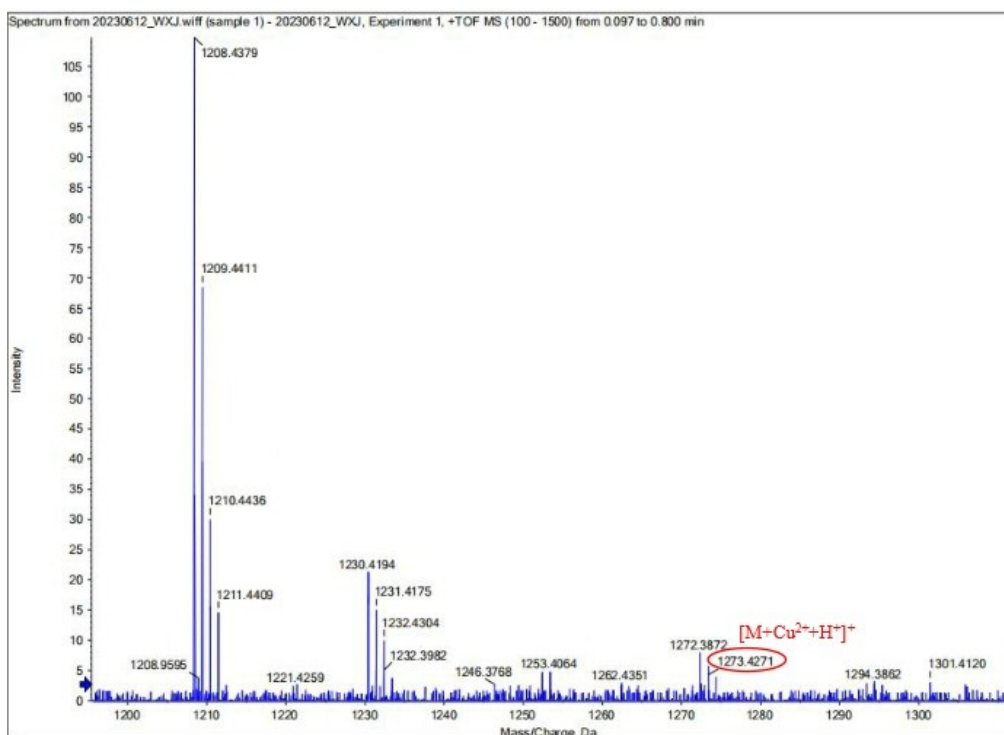
**Fig. S5.** UV absorption spectrum of **FGP1**



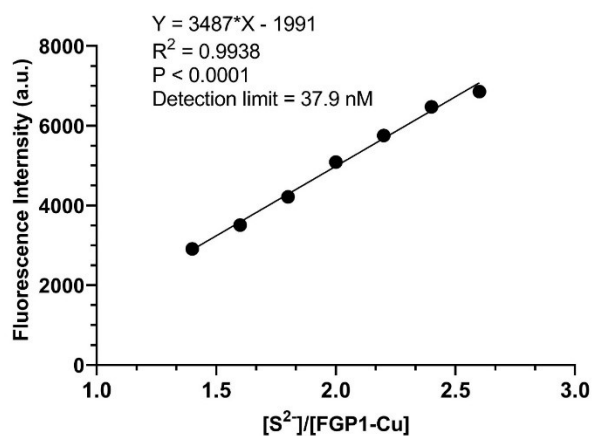
**Fig. S6.** Fluorescence intensity of **FGP1** (10.0 μM) with gradient concentration of Cu<sup>2+</sup> (0-15.0 μM) were added in PBS buffer solutions (10.0 mM, pH 7.4). The lowest detection limit of Cu<sup>2+</sup> was 38.2 nM.



**Fig. S7.** Job's plot for determining the stoichiometry of **FGP1** and Cu<sup>2+</sup> ions.

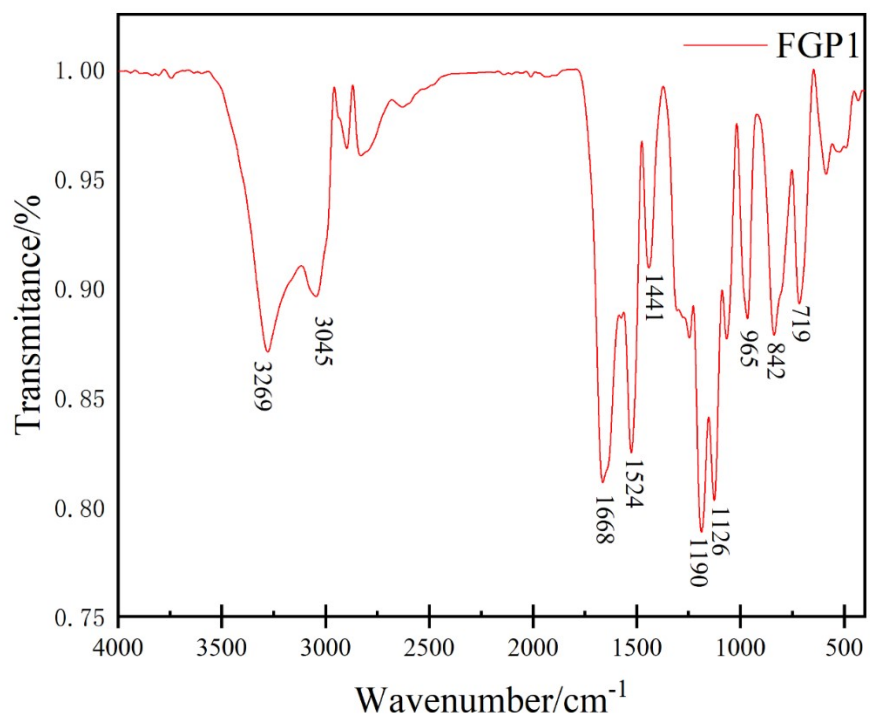


**Fig. S8.** TOF-MS of FGPI (100.0  $\mu\text{M}$ ) with  $\text{Cu}^{2+}$  ions (100.0  $\mu\text{M}$ ) in PBS buffer solutions (10.0 mM, pH 7.4).



**Fig. S9.** Fluorescence intensity of FGPI-Cu (10.0  $\mu\text{M}$ ) with gradient concentration of  $\text{S}^{2-}$  (0-40.0  $\mu\text{M}$ ) were added in PBS buffer solutions (10.0 mM, pH 7.4). The lowest detection limit of  $\text{S}^{2-}$  was 37.9 nM.

**Fig. S10.** The FTIR spectra of FGP1.





## References

1. M. J. Wei, Y. Y. Zhang, H. T. Li and S. Z. Yao, *Anal. Methods*, 2017, **9**, 3956-3961.
2. H. Wang, J. J. Cui, X. H. Fang, W. B. Zhang, J. J. Wang, S. Y. Chen and J. H. Qian, *Dyes Pigment.*, 2022, **197**, 10.
3. Y. T. Zeng, Z. B. Xu, J. Q. Guo, X. T. Yu, P. F. Zhao, J. Song, J. L. Qu, Y. Chen and H. Li, *Molecules*, 2022, **27**, 13.
4. Y. M. Wang, M. Feng, B. He, X. Y. Chen, J. L. Zeng and J. Sun, *Appl. Surf. Sci.*, 2022, **599**, 10.
5. P. Bhalla, N. Tomer, A. Goel, Monika, A. Ansari and R. Malhotra, *Journal of Molecular Structure*, 2022, 1264.
6. P. Yadav, S. Gond, A. Singh and V. P. Singh, *Mater. Lett.*, 2021, **295**, 4.
7. X. T. Liu, H. L. Liu, X. G. Tang, G. Liu and S. Z. Pu, *Tetrahedron*, 2021, **78**, 8.
8. M. G. Ren, Q. Y. Xu, Y. Y. Bai, S. J. Wang and F. G. Kong, *Spectroc. Acta Pt. A-Molec. Biomolec. Spectr.*, 2021, **249**, 10.
9. Z. L. Wei, L. Wang, S. Z. Guo, Y. Zhang and W. K. Dong, *RSC Adv.*, 2019, **9**, 41298-41304.
10. W. Chen, A. Pacheco, Y. Takano, J. J. Day, K. Hanaoka and M. Xian, *Angewandte Chemie-International Edition*, 2016, **55**, 9993-9996.
11. Y. X. Li, Z. J. Ren, Y. S. Ge, C. X. Di, J. Zhou, J. Wu and L. Jia, *Microchem J.*, 2023, **184**, 7.
12. K. Kaur, S. Chaudhary, S. Singh and S. K. Mehta, *New Journal of Chemistry*, 2015.
13. Y. Q. He, B. Zhao, W. Kan, L. M. Ding, Z. C. Yu, M. Y. Wang, B. Song and L. Y. Wang, *Analyst*, 2020, **145**, 213-222.
14. G. Y. Yue, D. Huang, F. Luo, L. H. Guo and B. Qiu, *Journal of Luminescence*, 2019.