Supporting Information

DyeLeS: A Web Platform for Predicting and Classifying Fluorescence Properties of Bioactive Molecules

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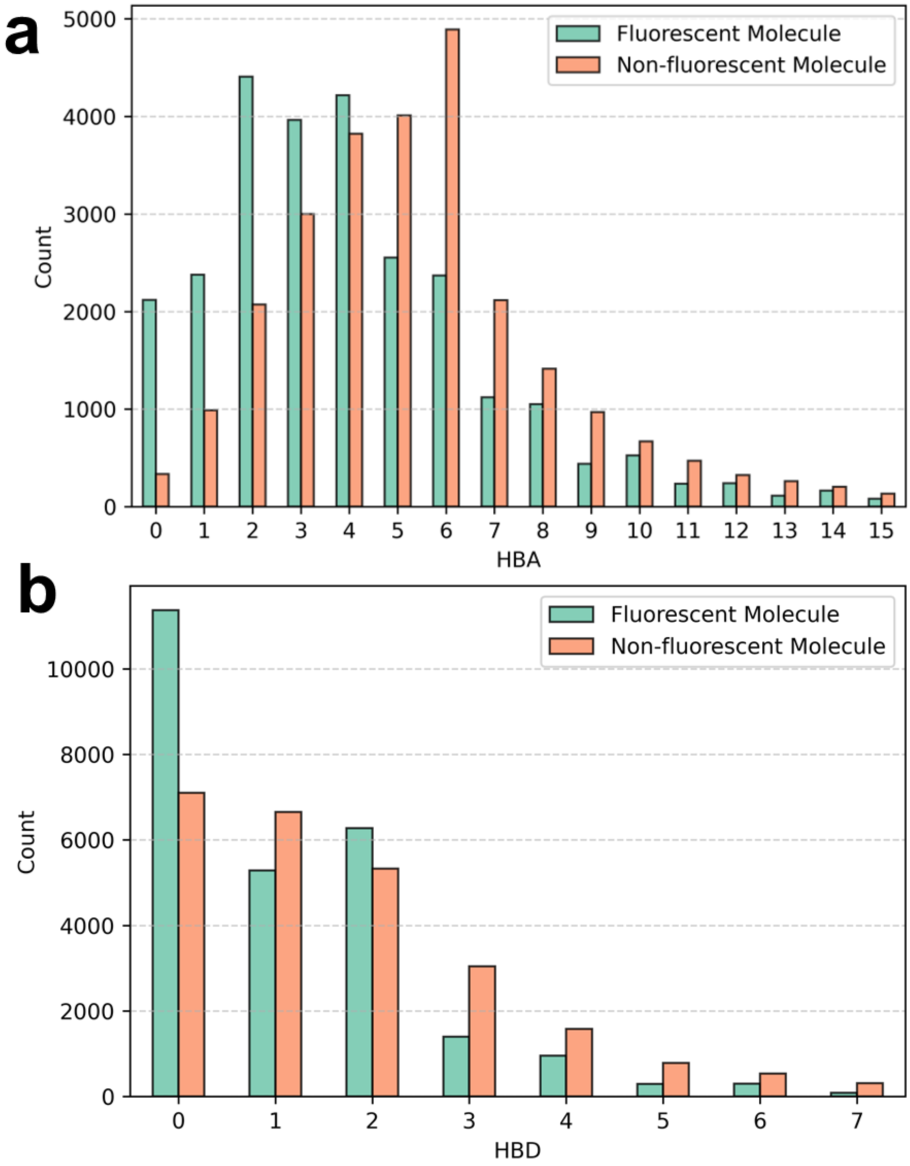


Figure S1. Statistical analysis of (**a**) hydrogen bond acceptors (HBA) and (**b**) hydrogen bond donors (HBD) in the fluorescent and non-fluorescent molecular databases. Despite differing in fluorescence properties, the two datasets show similar distributions of HBA and HBD counts, making them suitable for algorithms to capture structural features specific to fluorescent molecules.

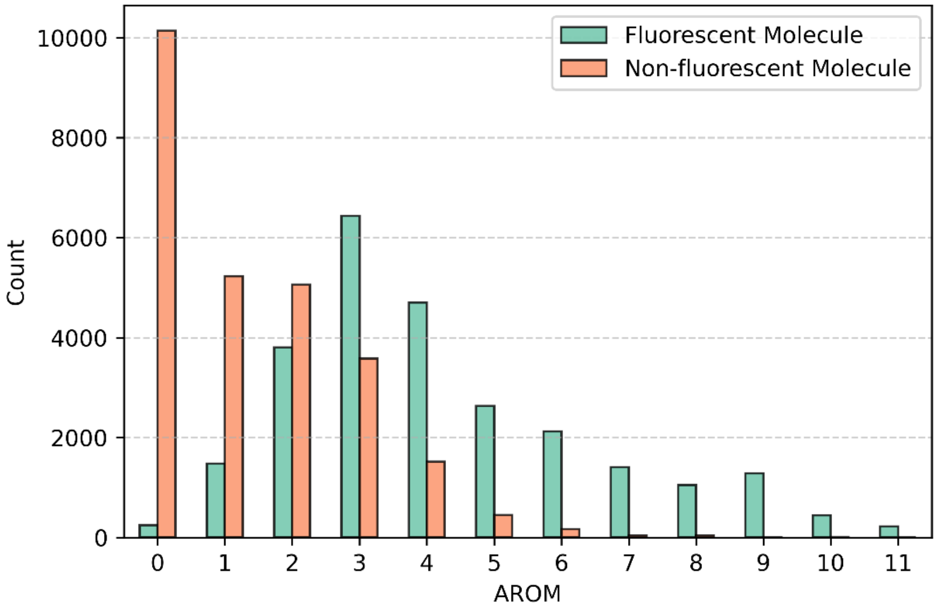


Figure S2. Distribution of aromatic ring counts in fluorescent and non-fluorescent molecular databases. Molecules in the fluorescent database contain significantly more aromatic rings than those in the non-fluorescent database. The delocalized π-electrons and structural rigidity of aromatic rings contribute fundamentally to the fluorescence properties of these molecules.

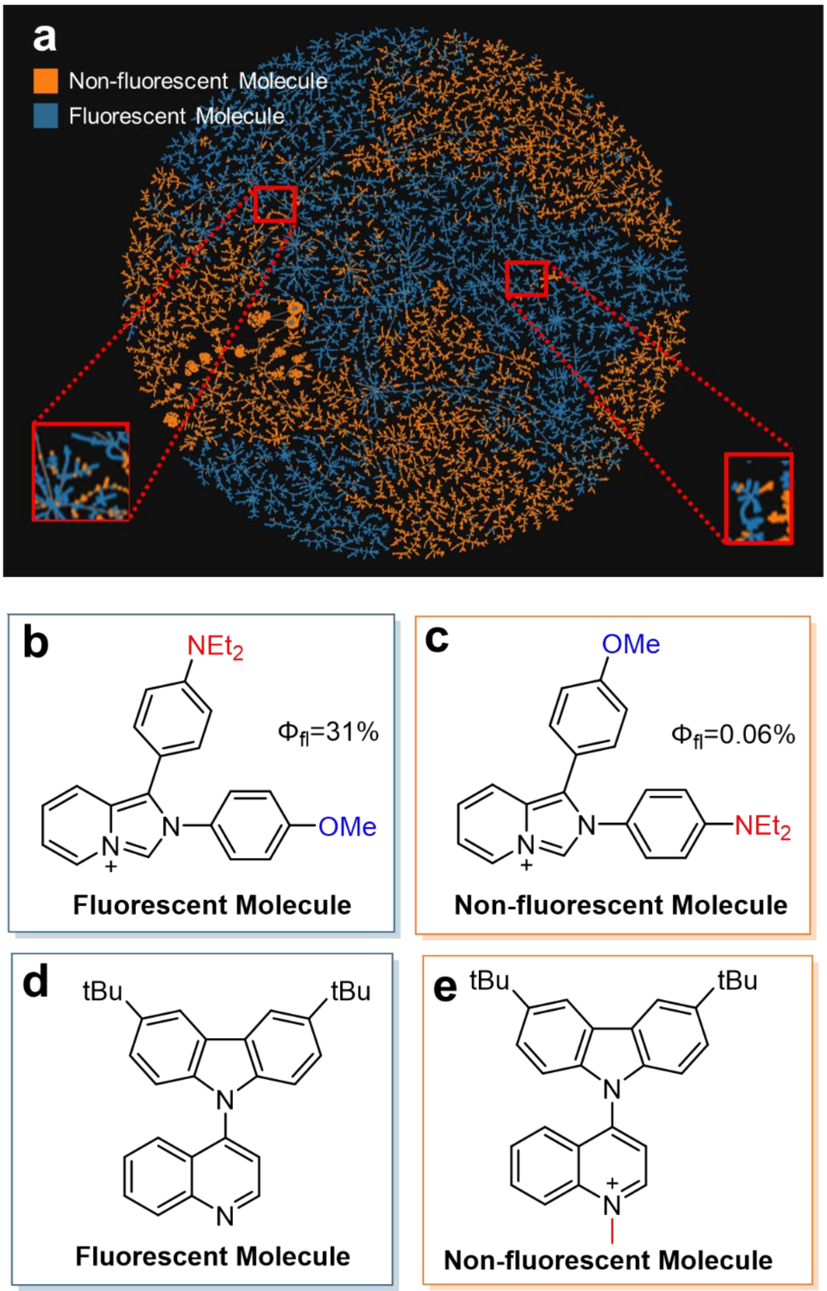
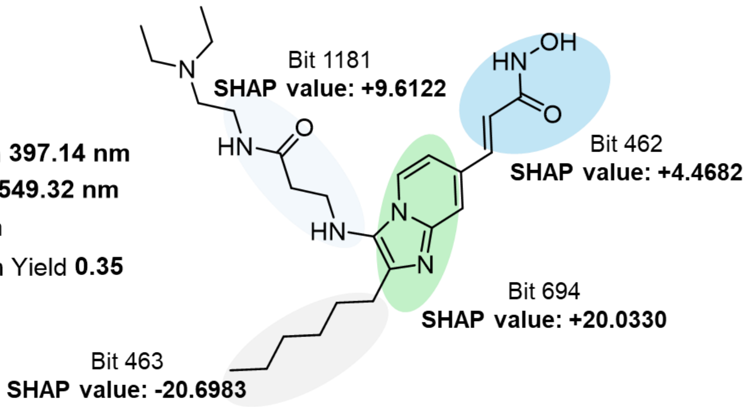


Figure S3. TMAP visualization of the fluorescent (blue) and non-fluorescent (red) molecule databases revealing cases of high structural similarity but contrasting fluorescence behavior. (**a**) A representative region of the TMAP highlights examples where structurally similar molecules exhibit opposite fluorescence properties. (**b**) and (**c**) share the same molecular scaffold, differing only in the positions of the methoxy and diethylamino groups. The molecule in (**b**) displays strong fluorescence ($\Phi_{fl} = 31\%$), whereas the molecule in (**c**) is non-fluorescent ($\Phi_{fl} = 0.06\%$).[1] (**d**) and (**e**) also share a common backbone, with the only difference being an additional methyl group on the nitrogen atom in (**e**).[2] This subtle change alters the nitrogen hybridization from sp^2 to sp^3, disrupting conjugation and resulting in loss of fluorescence in (**e**), which belongs to the non-fluorescent database.

Table S1. Machine learning feature contribution analysis using SHAP methods

Absorption Wavelength **397.14 nm**
Emission Wavelength **549.32 nm**
Stokes Shift **152.18 nm**
Fluorescence Quantum Yield **0.35**



O=C(NO)/C=C/C1=CC2=NC(CCCCCC)=C(NCCC(NCCN(CC)CC)=O)N2C=C1

Entry	Fragment (SMILES)	SHAP value	Role in Fluorescence
1 (Bit 463)	Long Alkyl Chain (CCCCCC)	-20.6983	Disrupts planarity but improves solubility.
2 (Bit 694)	Diazacyclic Core (N2=C1C=CC=N2)	+20.0330	Rigid π-conjugated scaffold; stabilizes excited state.
3 (Bit 294)	Bridge (CC)	+6.1891	minor flexibility penalty.
4 (Bit 922)	α,β-Unsaturated Nitroketone (O=C(NO)/C=C/)	+4.4682	Strong acceptor due to nitro (-NO_2) + conjugated ketone; likely dominates CT transition.
5 (Bit 1594)	Hydroxylamine (-NOH)	-4.3684	Weak donor/acceptor; may form H-bonds with C=O.
6 (Bit 1181)	CC(N)=O	+9.6122	The carbonyl group (C=O) acts as an electron acceptor, enabling intramolecular charge transfer (ICT) when paired with nearby donors (e.g., the tertiary amine NCCN(CC)CC), thereby promoting fluorescence emission.

Table S2. Photophysical property prediction performance of *DyeLeS-DyeP*

Entry		**R^2**	**RMSE**	**MSE**
1	Absorption wavelength (λ_{abs})	0.8839	38.1589	1456.0986
2	Amission wavelength (λ_{em})	0.8274	42.9814	1847.3987
3	Stokes shift	0.6629	29.6833	881.0986
4	Quantum yield (Φ_{fl})	0.4833	0.2107	0.0444

References

(1) Hutt, J. T.; Jo, J.; Olasz, A.; Chen, C.-H.; Lee, D.; Aron, Z. D. Fluorescence Switching of Imidazo[1,5-a]Pyridinium Ions: pH-Sensors with Dual Emission Pathways. *Org. Lett.* **2012**, *14* (12), 3162–3165. https://doi.org/10.1021/ol3012524.

(2) Klimash, A.; Prlj, A.; Yufit, D. S.; Mallick, A.; Curchod, B. F. E.; McGonigal, P. R.; Skabara, P. J.; Etherington, M. K. From Phosphorescence to Delayed Fluorescence in One Step: Tuning Photophysical Properties by Quaternisation of an Sp2-Hybridised Nitrogen Atom. *J. Mater. Chem. C* **2022**, *10* (25), 9484–9491. https://doi.org/10.1039/D2TC01737G.