Chemical Synthesis and Ex-Vivo Evaluation of Berberine Analogs as DNA-Binding Singlet Oxygen Photosensitizers and Anti-Bacterial Drugs

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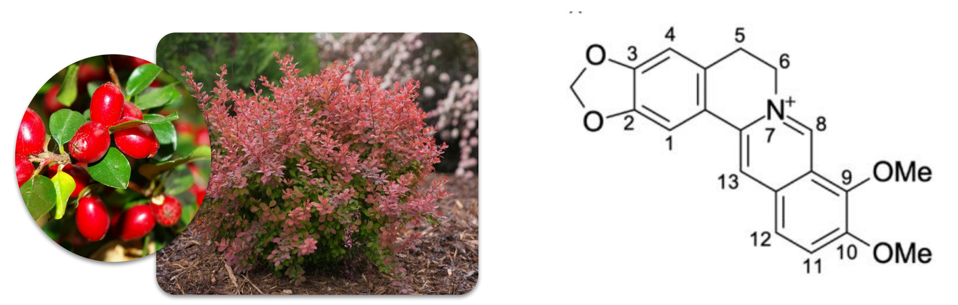
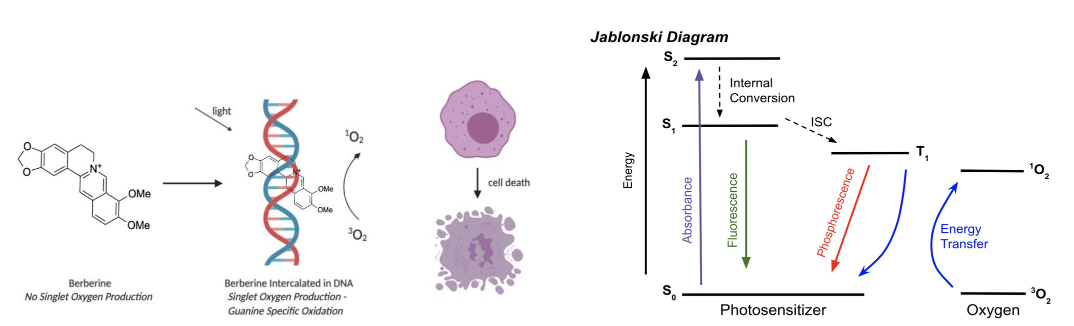
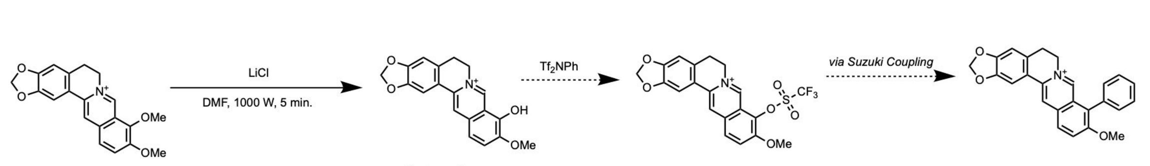
Introduction

Berberine, a natural product isoquinoline alkaloid, has been shown to exert its biological activity through in situ production of singlet oxygen, a highly reactive oxygen species, upon irradiation. Its putative mechanism of action as a DNA-binding singlet oxygen photosensitizer stems from its electronic structure, wherein upon irradiation, it sensitizes triplet oxygen to singlet oxygen to incur irreversible DNA damage, resulting in apoptosis. Through semisynthetic modifications of the berberine scaffold, we were able to modulate berberine's electronic structure bolstering its photosensitizing properties. Regioselective modifications, such as grignard additions to C8 and demethylation and cross couplings to C9, enabled the generation of a library of berberine analogues. Here, we present two ex-vivo experiments towards evaluating the DNA-binding singlet oxygen photosensitizing abilities of Berberine and related analogues. Through the use of a hetero Diels-Alder reaction between singlet oxygen and a terpene, we were able to quantitatively monitor singlet oxygen production with benchtop NMR. Moreover, we used HPLC in conjunction with in silico methods towards the construction of a structure activity relationship between berberine and various DNA structures.

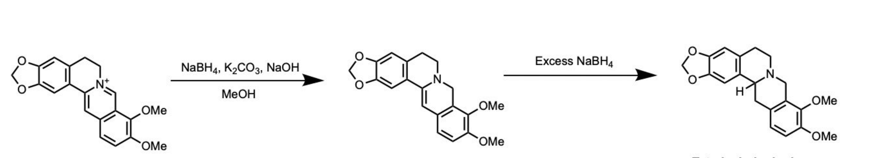




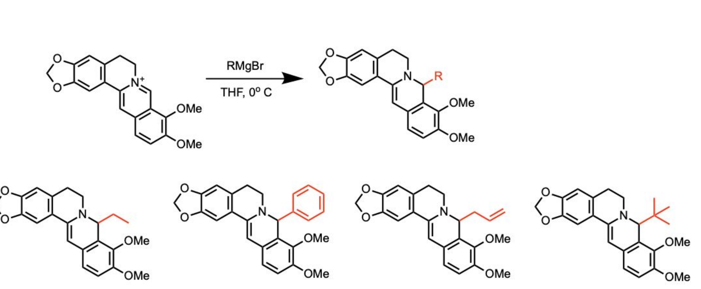
Synthesis of Berberrubine

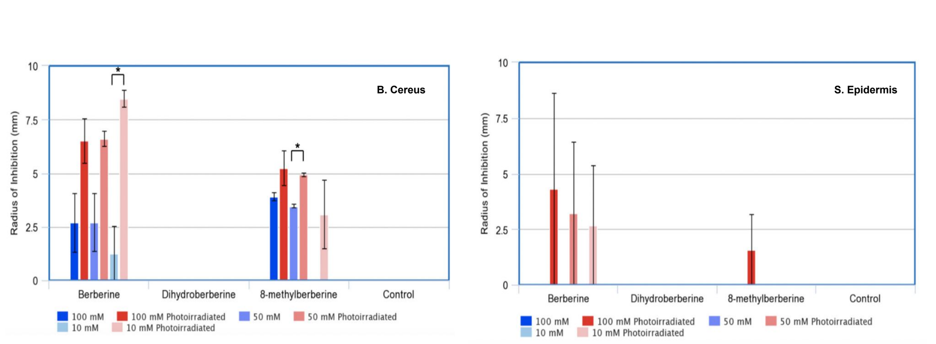


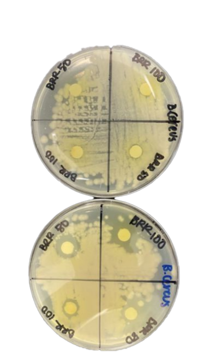
Synthesis of Reduced Analogs



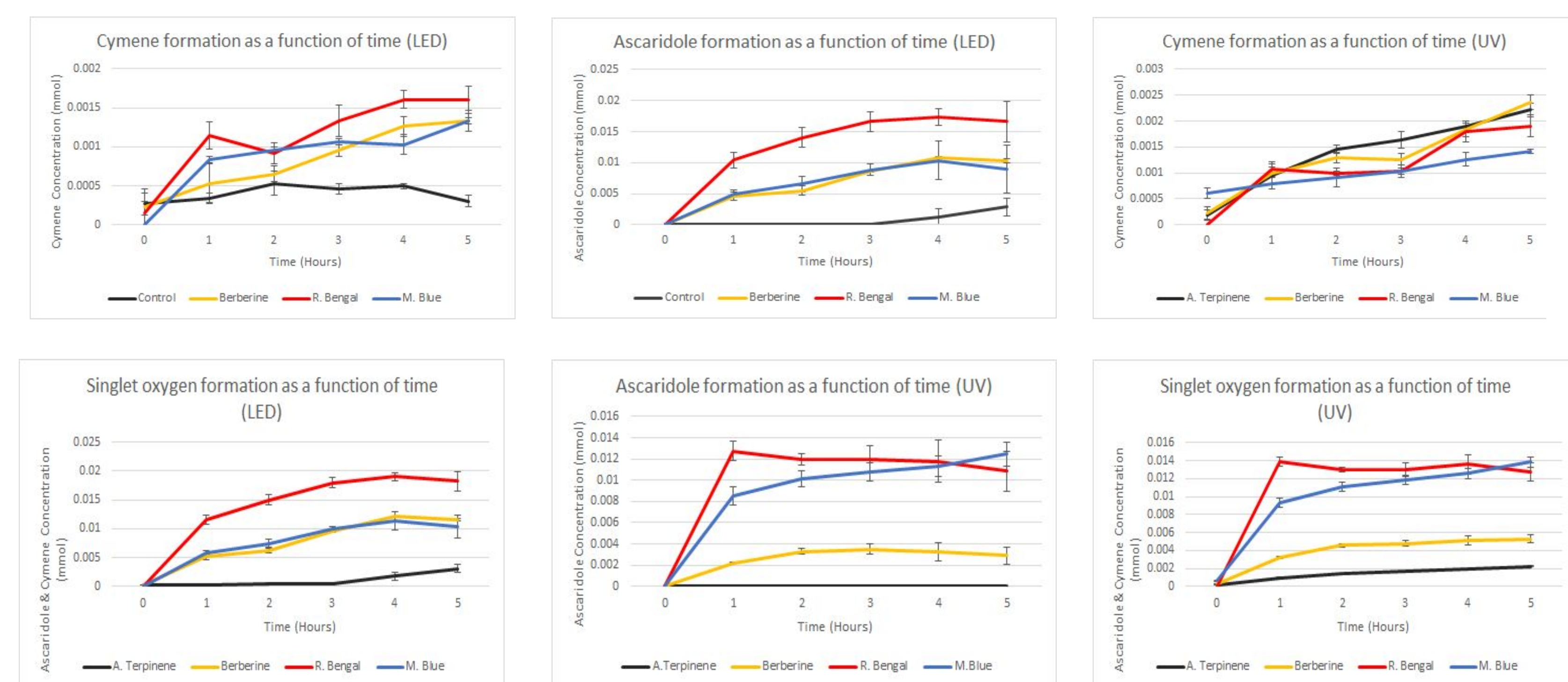
Photobiological Activity of C-8 Substitutions



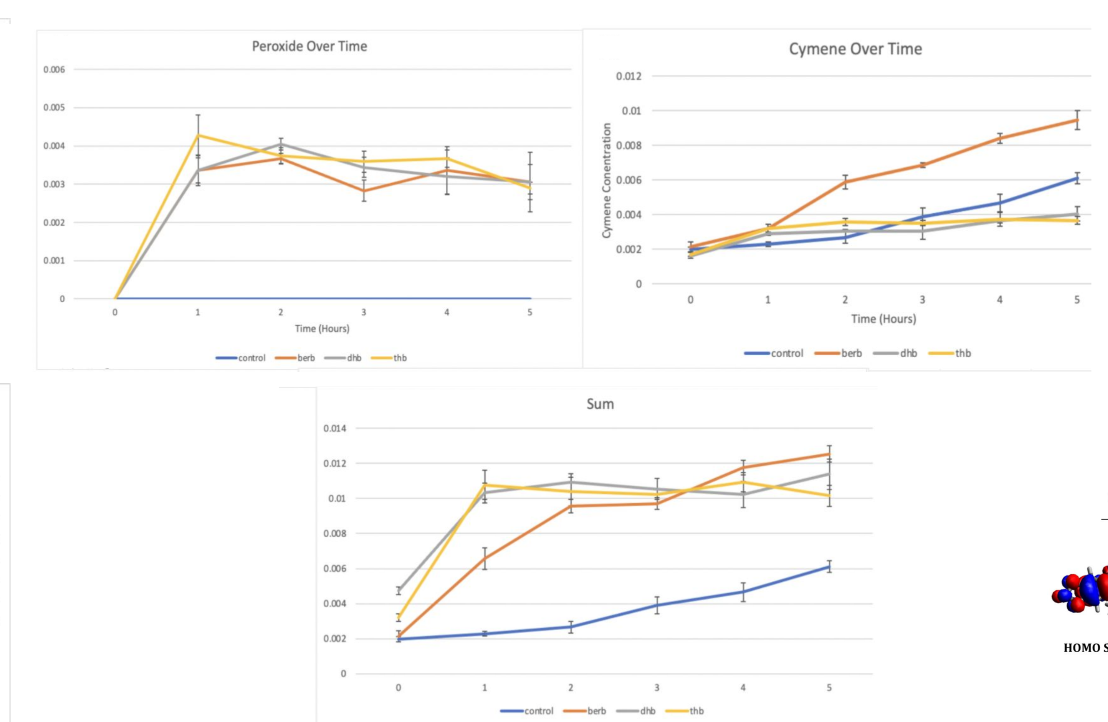




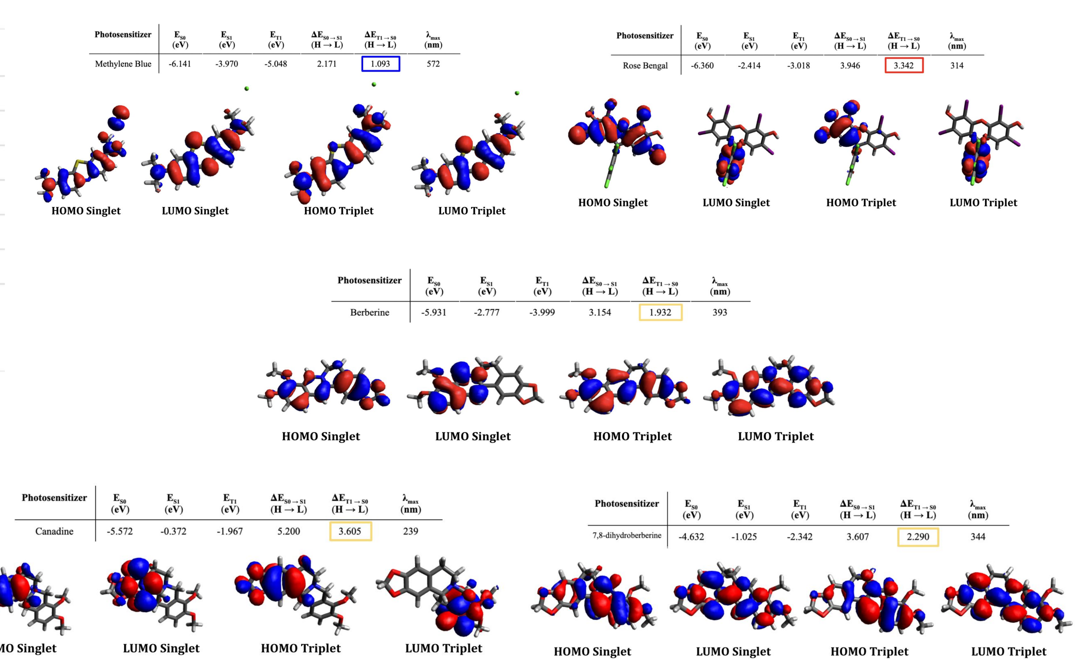
Benchtop NMR Enabled Monitoring of a Photochemical 4+2 Cycloaddition

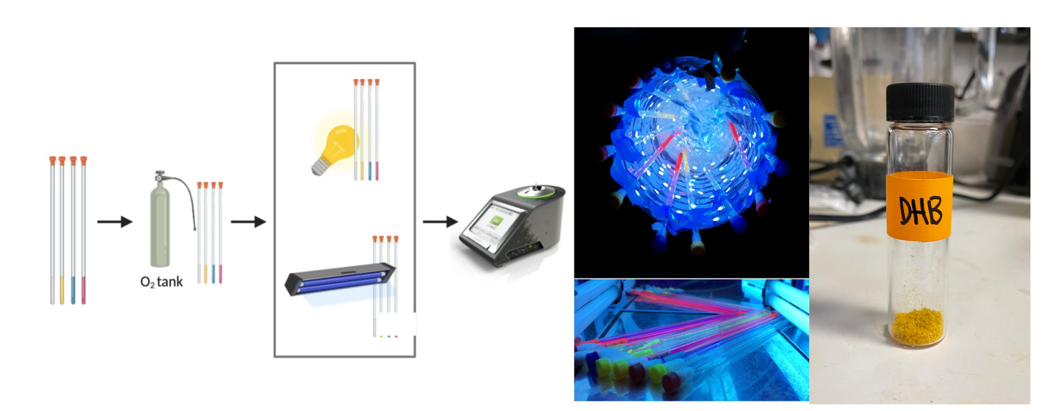


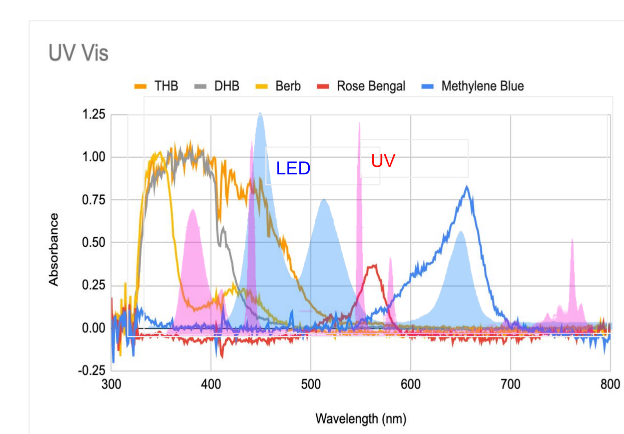
Singlet Oxygen Formation Over Time in Berberine Against Two Commercially Available Photosensitizers



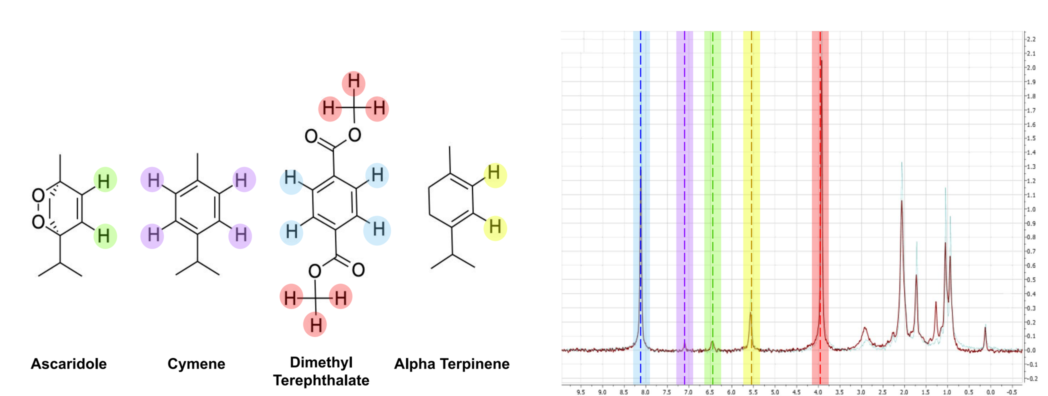
Singlet Oxygen Formation Over Time in Berberine Against Two Reduced Berberine Analogs

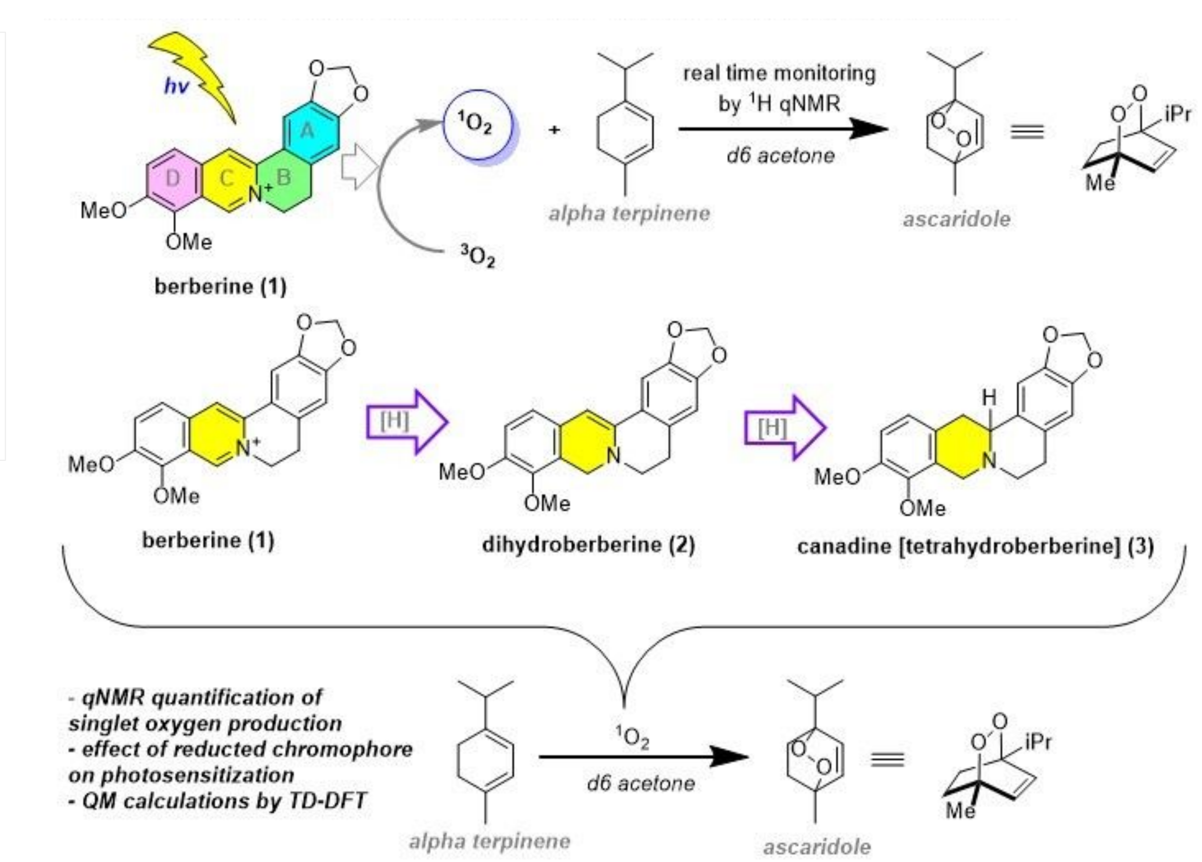


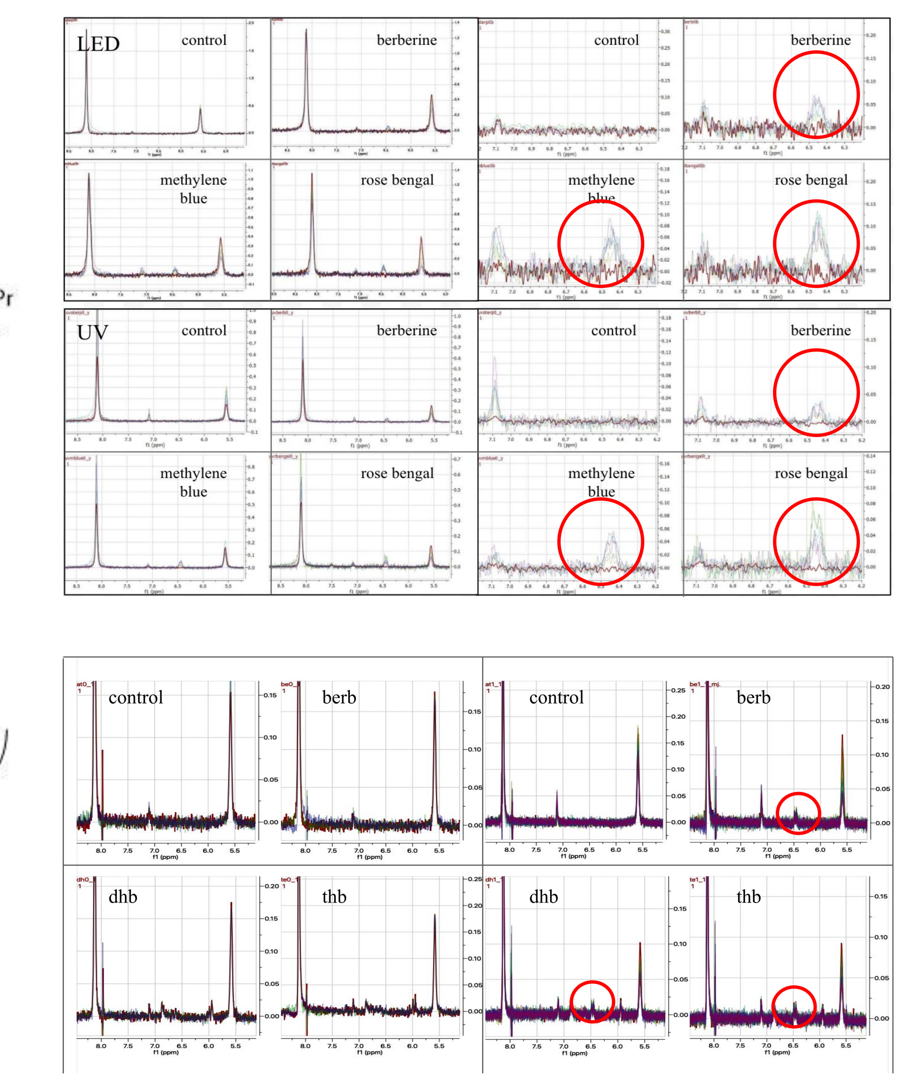




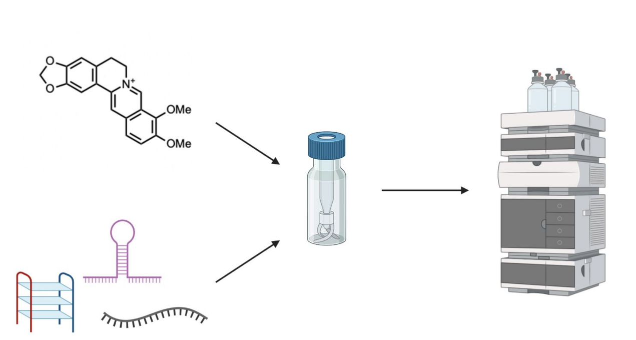
Comparison of Photosensitizer Absorption Spectra and Irradiation Sources

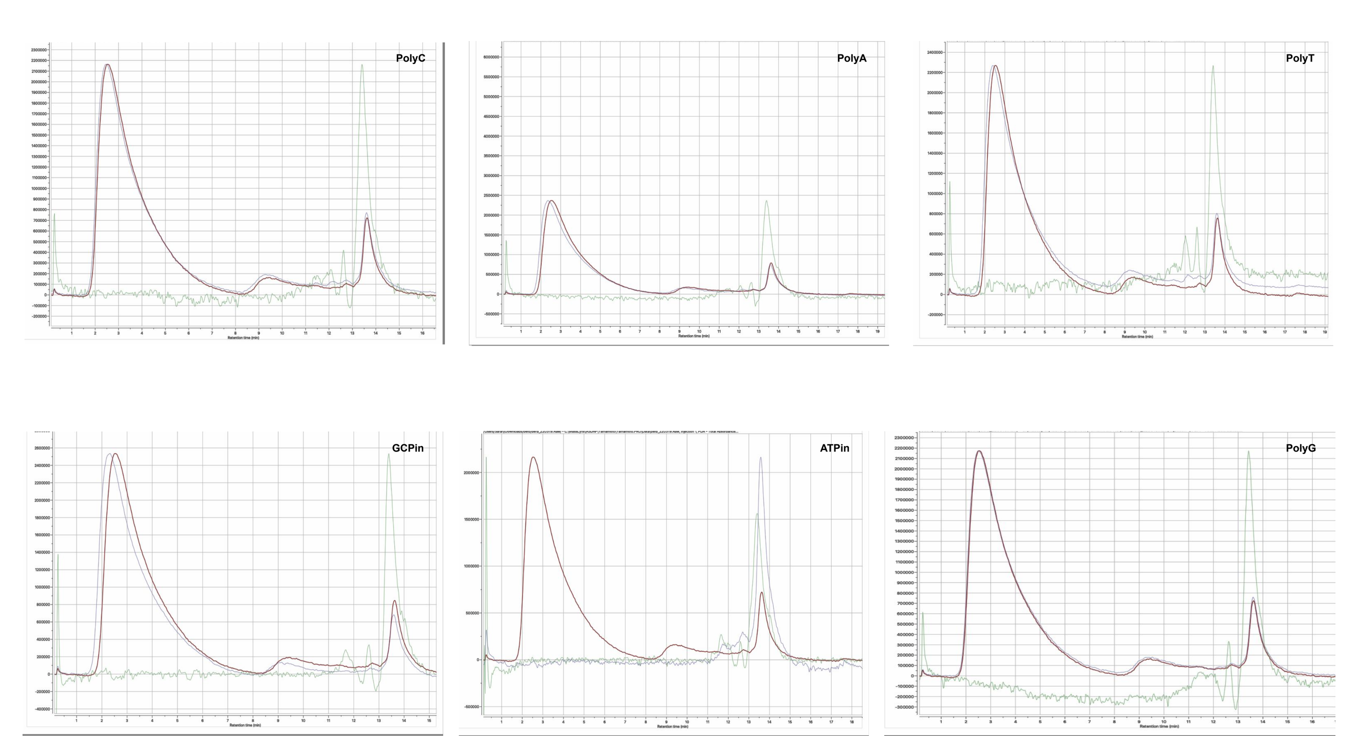




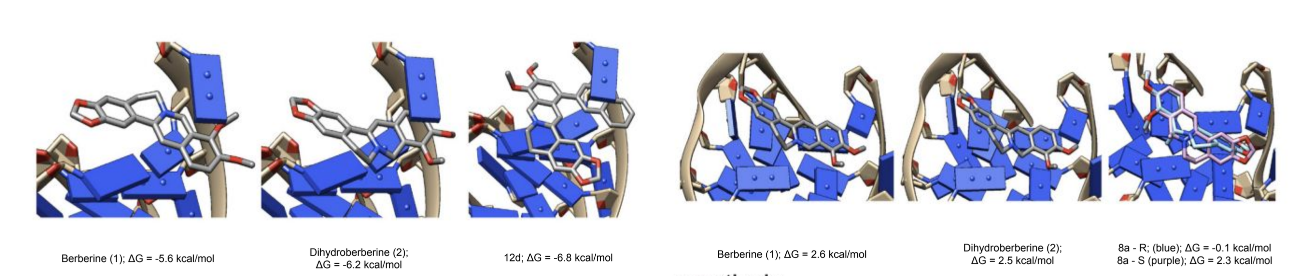


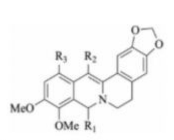
HPLC Analysis of DNA Interactions





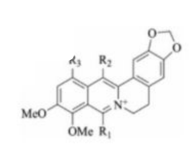
In Silico Evaluation of Analogs





Compound 2, 8a-h

Compound	R_1	R_2	R_3	ΔG - dsDNA	ΔG - G4DNA
2	H	H	H	-6.2	2.5
8a	CH_3	H	H	R = -4.0, S = -4.1	R = -0.1, S = 2.3
8b	C_2H_5	H	H	R = -4.2, S = -3.8	R = 0.8, S = 1.4
8c	C_4H_9	H	H	R = -4.5, S = -4.3	R = 3.3, S = 5.0
8d	C_6H_{13}	H	H	R = -3.8, S = -3.6	R = 3.0, S = 2.5
8e	C_8H_{17}	H	H	R = -3.7, S = -3.6	R = 6.3, S = 1.5
8f	$C_{10}H_{21}$	H	H	R = -3.0, S = -3.2	R = 9.1, S = 3.4
8g		H	H	R = -4.4, S = -3.4	R = 6.1, S = 9.8
8h		H	H	R = -2.1, S = -3.3	R = 9.3, S = 9.2
8i		H	H	R = -1.0, S = -2.2	R = 65.9, S = N/A



Compound 1, 12a-d, 13a-g

Compound	R_1	R_2	R_3	ΔG - dsDNA	ΔG - G4DNA
1	H	H	H	-5.6	2.6
12a	H	H	Br	-5.8	4.0
12b	H	H		-5.9	25.7
12c	H	H		-6.0	35.5
12d	H	H		-6.8	N/A
13a	H	C_4H_9	H	-4.8	3.2
13b	H	C_6H_{11}	H	-4.8	7.4
13c	H	C_8H_{17}	H	-4.6	4.1
13d	H	$C_{10}H_{21}$	H	-4.3	8.2
13e	H		H	-4.4	10.4
13f	H		H	-4.9	4.9
13g	H		H	-4.2	11.1

References

1. Grycová, Lenka, et al. "Quaternary Protoberberine Alkaloids." Phytochemistry, vol. 68, no. 2, 2007, pp. 150–175, doi:10.1016/j.phytochem.2006.10.004.
2. Wang, Ye, et al. "The Anti-Cancer Mechanisms of Berberine: A Review." Cancer Manag Res., vol. 12, 2020, pp 695–702, doi: 10.2147/CMAR.S242329
3. Hirakawa, Kazutaka, Shosuke Kawanishi, and Toru Hirano. "The mechanism of guanine specific photooxidation in the presence of berberine and palmatine: activation of photosensitized singlet oxygen generation through DNA-binding interaction." Chemical research in toxicology, vol. 18, no. 10, 2005, pp. 1545-1552, doi: 10.1021/tx0501740
4. Krey, Anne K., and Fred E. Hahn. "Berberine: complex with DNA." Science, vol. 166, no. 3906, 1969, pp. 755-757, doi: 10.1126/science.166.3906.755
5. Hirakawa, Kazutaka, Toru Hirano, Yoshinobu Nishimura, Tatsuo Arai, and Yoshio Nosaka. "Dynamics of singlet oxygen generation by DNA-binding photosensitizers." The Journal of Physical Chemistry B, vol.116, no. 9, 2012, pp. 3037-3044, doi: 10.1021/jp300142e
6. JC Atenco-Cuautle, MG Delgado-López, R. Ramos-García, JC Ramírez-San-Juan, J. Ramirez-Ramirez, and T. Spezzia-Mazzocco "Rose bengal as a photosensitizer in the photodynamic therapy of breast cancer cell lines", Proc. SPIE 11070, 17th International Photodynamic Association World Congress, 2019, 11070A1, doi:10.1117/12.2525456
7. Kofler, Barbara, Angela Romani, Christian Pritz, Teresa Bernadette Steinbichler, Volker Hans Schartinger, Herbert Riechelmann, and Jozsef Dudas. "Photodynamic effect of methylene blue and low level laser radiation in head and neck squamous cell carcinoma cell lines." International journal of molecular sciences, vol. 19, no. 4, 2018, pp. 1107, doi: 10.3390/ijms19041107
8. Dolmans, Dennis EJGJ, Dai Fukumura, and Rakesh K. Jain. "Photodynamic therapy for cancer." Nature reviews cancer, vol. 3, no. 5, 2003, pp. 380-387, doi: 10.1038/nrc1071
9. Horn, Clemens R., and Sylvain Gremetz. "A method to determine the correct photocatalyst concentration for photooxidation reactions conducted in continuous flow reactors." Beilstein Journal of Organic Chemistry, vol. 16, no. 1, 2020, pp. 871-879, doi: 10.3762/bjoc.16.78
10. Neese, Frank. "The ORCA program system." Wiley Interdisciplinary Reviews: Computational Molecular Science, vol. 2, no. 1, 2012, pp. 73-78, doi: 10.1002/wcms.81
11. Hanwell, Marcus D., Donald E. Curtis, David C. Lonie, Tim Vandermeersch, Eva Zurek, and Geoffrey R. Hutchison. "Avogadro: an advanced semantic chemical editor, visualization, and analysis platform." Journal of cheminformatics, vol. 4, no. 1, 2012, pp. 17, doi: 10.1186/1758-2946-4-17
12. Monroe, Bruce M. "Rate constants for the reaction of singlet oxygen with conjugated dienes." Journal of the American Chemical Society, vol. 103, no. 24, 1981, pp. 7253-7256, doi: 10.1021/ja00414a035.
13. Sun, et al. "Strain-specific and photochemically-activated antimicrobial activity of berberine and two analogs." Journal of Emerging Investigators, vol. 3, 2020, pp. 1-7.
14. Willcott, Mark Robert. "MestRe Nova." J. Am. Chem. Soc. vol. 131, no. 36, 2009, pp. 13180-13180, doi: 10.1021/ja906709t.
15. Bregnhøj, Mikkel, Michael Westberg, Frank Jensen, and Peter R. Ogilby. "Solvent-dependent singlet oxygen lifetimes: temperature effects implicate tunneling and charge-transfer interactions." Physical Chemistry Chemical Physics, vol. 18, no. 33, 2016, pp. 22946-22961, doi: 10.1039/C6CP01635A.
16. Hanwell, Marcus D., Donald E. Curtis, David C. Lonie, Tim Vandermeersch, Eva Zurek, and Geoffrey R. Hutchison. "Avogadro: an advanced semantic chemical editor, visualization, and analysis platform." Journal of cheminformatics, vol. 4, no. 1, 2012, pp. 17, doi: 10.1186/1758-2946-4-17
17. Sun, et al. "Computational Structure-Activity Relationship (SAR) of Berberine Analogs in Double-Stranded and G-Quadruplex DNA Binding Reveals Both Position and Target Dependence." Journal of Emerging Investigators, vol. 2, 2020, pp. 1-9.
18. Pettersen, Eric F., et al. "UCSF Chimera - A Visualization System for Exploratory Research and Analysis." Journal of Computational Chemistry, vol. 25, no. 13, 2004, pp. 1605–1612., doi:10.1002/jcc.20084.
19. Morris, Garrett M., et al. "AutoDock4 And AutoDockTools4: Automated Docking with Selective Receptor Flexibility." Journal of Computational Chemistry, vol. 30, no. 16, 2009, pp. 2785–2791., doi:10.1002/jcc.21256.
20. Trott, Oleg, and Arthur J. Olson. "AutoDock Vina: Improving the Speed and Accuracy of Docking with a New Scoring Function, Efficient Optimization, and Multithreading." Journal of Computational Chemistry, 2009, doi:10.1002/jcc.21334.
21. Su, et al. "Comparative singlet oxygen photosensitizer efficiency of berberine, rose bengal, and methylene blue by time course nuclear magnetic resonance (NMR) monitoring of a photochemical 4+2 cycloaddition endoperoxide formation." Journal of Emerging Investigators, vol. 4, 2021, pp. 1-9.

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