Characterizing Novel Psoralens by Photoadduct Formation

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INTRODUCTION

- Psoralens are flat, naturally occurring molecules used in phototherapies for various diseases (e.g., psoriasis, lymphomas, internal tumors).[1]

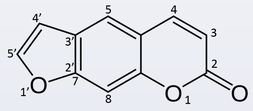


Fig. 1 – psoralen structure and numbering system

- These molecules slide in between DNA base pairs in a process called intercalation.[2]

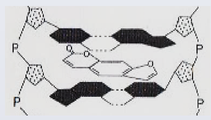


Fig. 2 – a psoralen molecule intercalated in the DNA double helix.[4]

- Upon excitation by UV photons, covalent bonds (photoadducts) can form with adjacent thymine bases.[3]

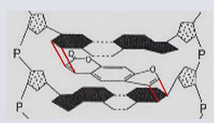


Fig. 3 – after UV irradiation, a diadduct (interstrand crosslink) can form. Adduct formation inhibits cell proliferation.[4]

- Adding functional groups changes the dynamics of this process, with the goal of creating a more potent drug.

MATERIALS & METHODS

- Nine psoralen derivatives were selected out of a library at Duke University based on high fractional kill of B16 melanoma cells and interesting structural features.

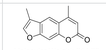
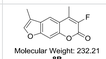
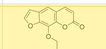
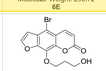
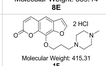
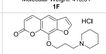
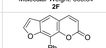
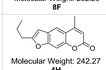
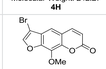
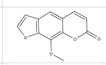
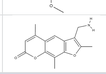
- Our model DNA, AT-10, is a 10 bp sequence alternating between adenine and thymine.
(Keck Biotechnology Resource Laboratory at Yale University, New Haven, CT)

Fig. 4 – synthetic AT-10 oligonucleotide forms a mini-helix below 20°C

- Samples irradiated using a UV source using three UV bulbs and a filter.
(Model UVA-1; Southern New England Ultraviolet Company, Branford, CT)

- Samples analyzed with MALDI-TOF MS.[5]
  - The relative percentage of AT-10 with increased mass is due to the addition of a psoralen molecule.

RESULTS

Compound Code	Structure	B16 Percent Cell Kill	Peak % mod. UVA (60 min.)	Peak % mod. UVB (60 min.)
1B	 Molecular Weight: 214.22 1B	98.5	18.1	10.1
8B	 Molecular Weight: 232.21 8B	96.9	6.6	5.4
6E	 Molecular Weight: 295.72 6E	**99.8**	**57.5**	**49.1**
8E	 Molecular Weight: 339.14 8E	99.6	0	0
1F	 Molecular Weight: 415.31 1F	99.7	18.6	21.5
2F	 Molecular Weight: 363.84 2F	99.6	48.6	43.2
8F	 Molecular Weight: 262.26 8F	99.4	3.6	5.0
4H	 Molecular Weight: 242.27 4H	99.4	0	0
5H	 Molecular Weight: 295.09 5H	82.1	3.7	0
Standards				
8-MOP		82.5	6.4	19.4
AMT		99.7	38.5	22.7

UV-Vis Absorbance Spectra

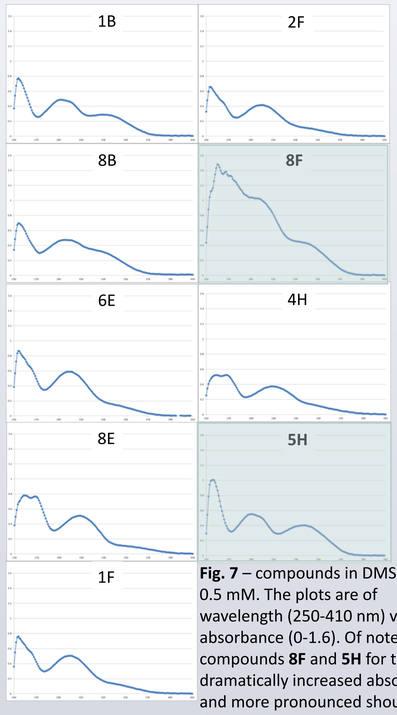


Fig. 7 – compounds in DMSO at 0.5 mM. The plots are of wavelength (250-410 nm) versus absorbance (0-1.6). Of note are compounds **8F** and **5H** for their dramatically increased absorbance and more pronounced shoulder in the UVA region, respectively.

Sample MALDI data

Before UVA

After UVA

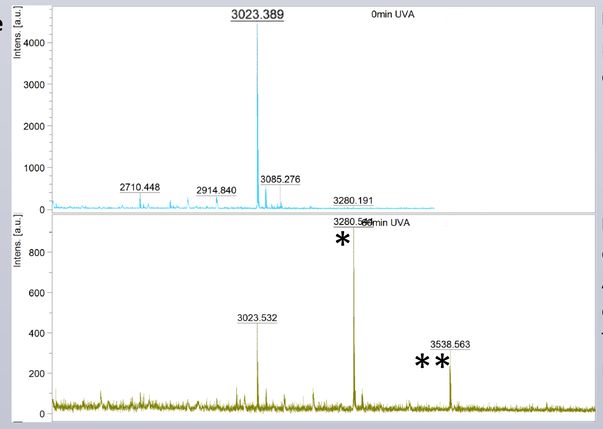


Fig. 5 – MALDI data for AMT before irradiation in the presence of AT-10. The peak at 3023 m/z is AT-10. The other peaks present are minor contaminants.

Fig. 6 – MALDI data for AMT after irradiation in the presence of AT-10. The peak at 3280 m/z (*) is the psoralen-modified AT-10. The difference between 3280 m/z and 3023 m/z is 257 corresponding to the molecular weight of one AMT molecule. The difference for 3538 m/z (**) is twice 257, so two AMT molecules have bonded with the same helix.

CONCLUSIONS

- No direct correlation can be made between absorption spectra and percent adduct formation for a given compound.
  - Other pathways for energy dissipation must play a major part in the photobiology for cell death.
- The positively charged aminomethyl group greatly increases adduct formation.
- Halogenated psoralens form almost no adducts due to photodegradation and/or electrostatic repulsion.
- In addition to steric interactions, the absorptivity of a substituent can majorly impact the quantum yield for this process.

FUTURE WORK

- Determine correlation between adduct formation and therapeutic impact.
- Run molecular dynamics simulations to approximate binding energies.
- Screen any derivatives with similar effective moieties.

REFERENCES

[1] - Scaffidi, J. P., et. al (2011) *ACS Nano*. **5**, 4679-4687

[2] - Mohammad, T. and H. Morrison (1992) *Photochem. Photobiol*. **55**, 631-638

[3] - Musajo, L., et. al (1965) *Experentia* **21**, 24-25

[4] - Lambert, M. E., et. al (1989) *Mol. Cell. Biol*. **9**, *847-850*

[5] - Buhimschi A., Gasparro F. (2013) *J. Photochem. Photobiol*. **90**, 241-246

ACKNOWLEDGEMENTS

We thank Hamden Hall Country Day School for funding; Dr. David Gooden and Immunolight, LLC at Duke University for supplying the compounds and cell kill data; and Yale University for use of equipment.

We also thank Dr. Irina Buhimschi at Ohio State University for graciously agreeing to run the samples in her lab as well as Alexandru Buhimschi for facilitating this arrangement and offering advice.