

# Design of Photoactivated DNA Cleaving Agents and Sensitizers for Photodynamic Therapy

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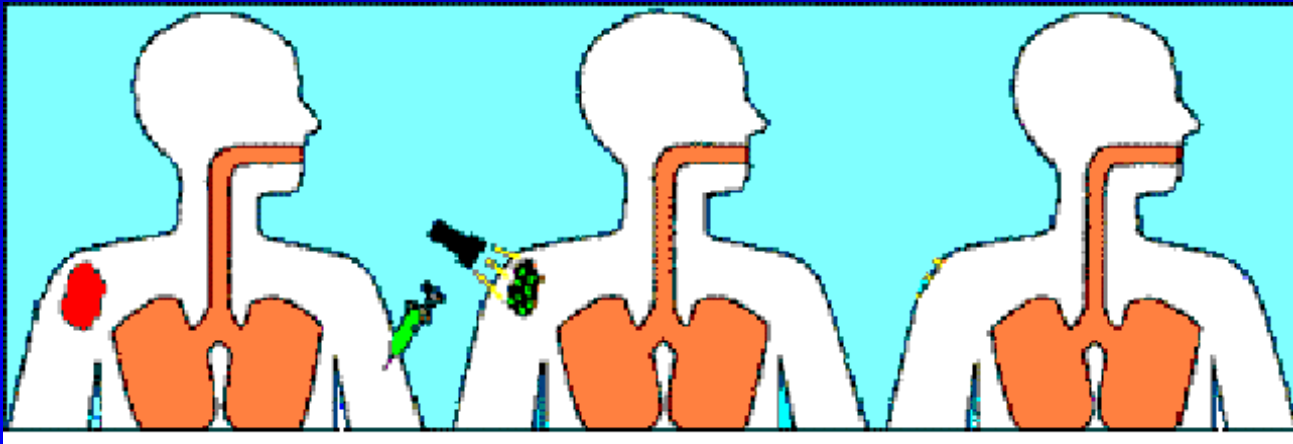
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# Squaraine Dye Based Sensitizers

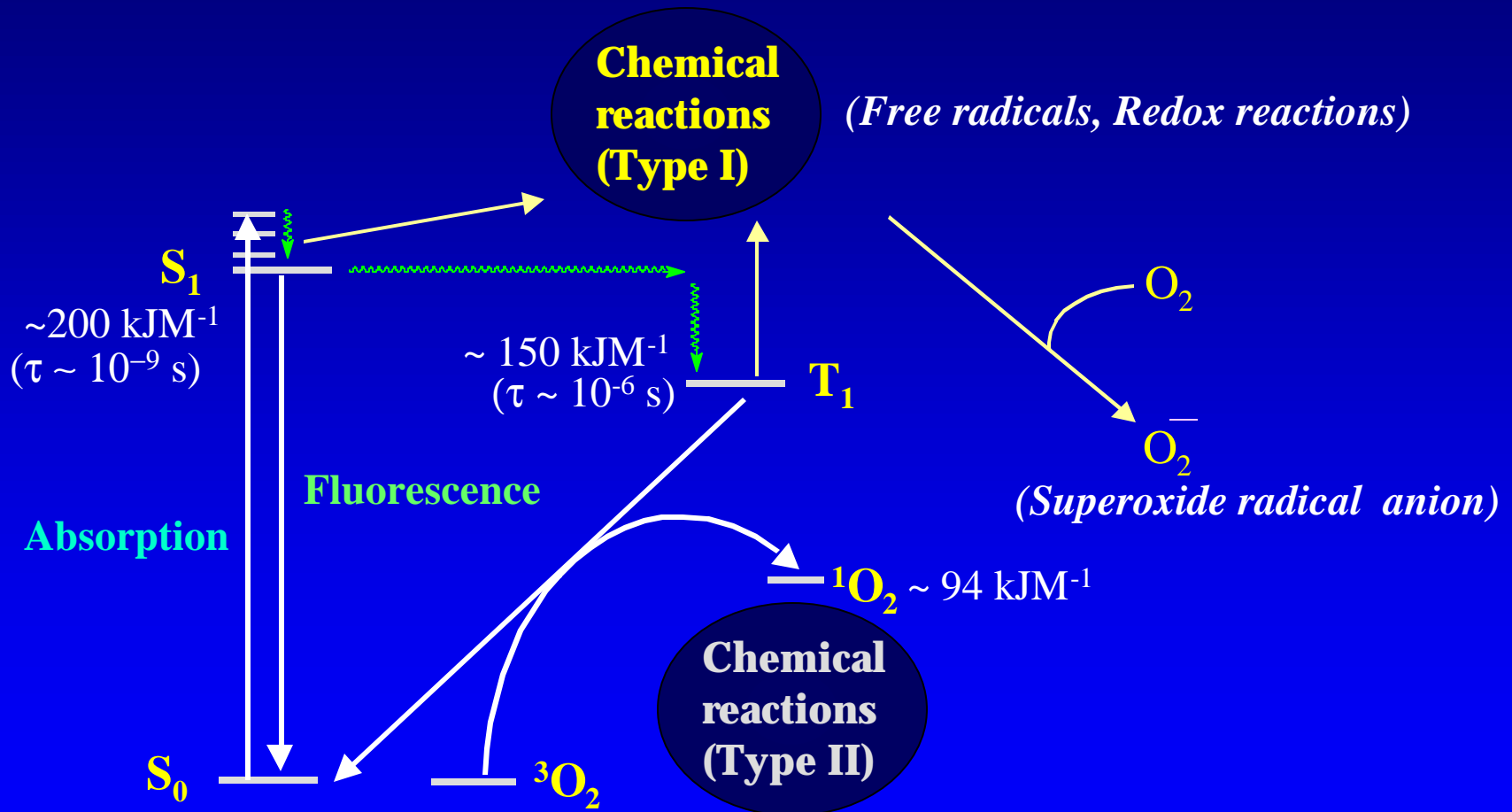
Photodynamic Therapy (PDT) is a fast developing treatment for both neoplastic and non-neoplastic diseases. It involves the inactivation of living cells by the combined action of light and a photosensitizer.

**Drug**  $\longrightarrow$  **Light + Photosensitizer**

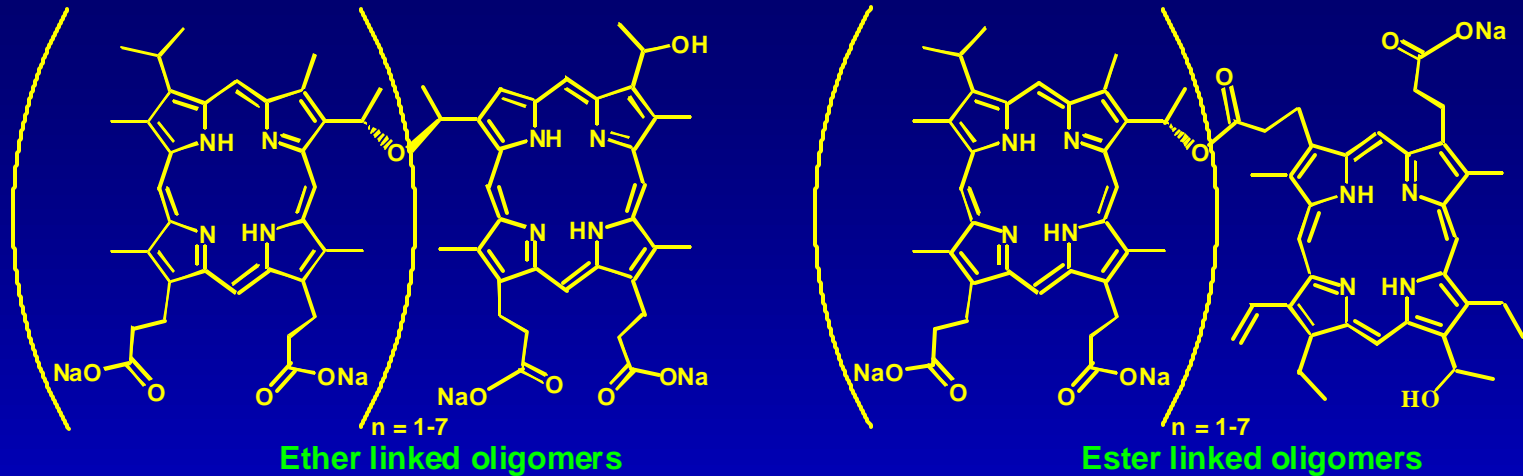


- ◆ **Injection of photosensitizer**
- ◆ **Selective accumulation**
- ◆ **Illumination**
- ◆ **Cell necrosis**

# Mechanism of photodynamic therapy



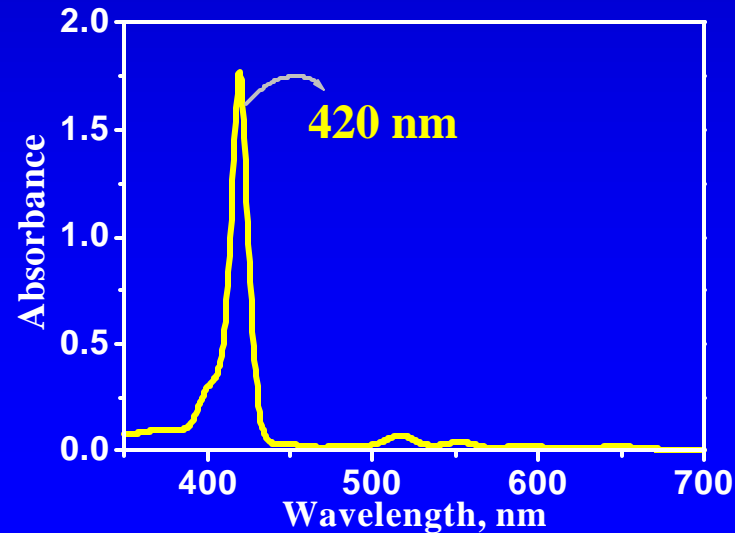
# First generation photosensitizer



**Photofrin**  
**Hematoporphyrin Derivative (HpD)**

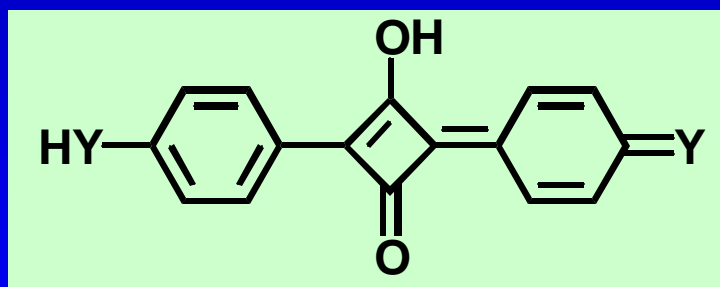
## Disadvantages of HpD

- *Mixture of at least nine components*
- *Preparation is highly sensitive to reaction conditions*
- *Causes cutaneous photosensitivity and immunosuppression*
- *Very low absorption in the red region of the spectrum*



# Objectives

- Design of sensitizers based on squaraine moiety that exhibit long wavelength absorption
- Quantification of triplet excited states and singlet oxygen
- Photophysical studies under physiological pH conditions
- Evaluation of *in vitro* photobiological properties (cytotoxicity, mutagenicity, cellular uptake and retention)
- Modification with cellular recognition elements



Long wavelength  
absorption

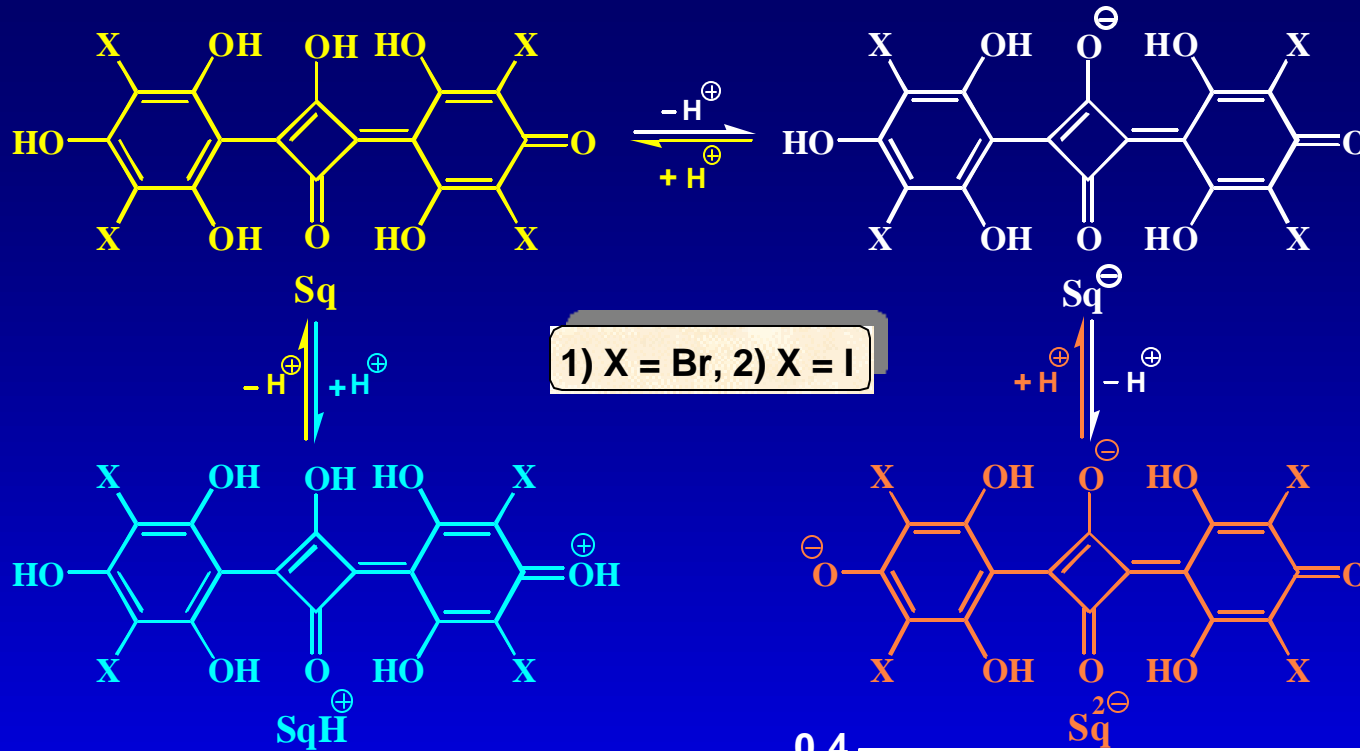


Industrial  
applications



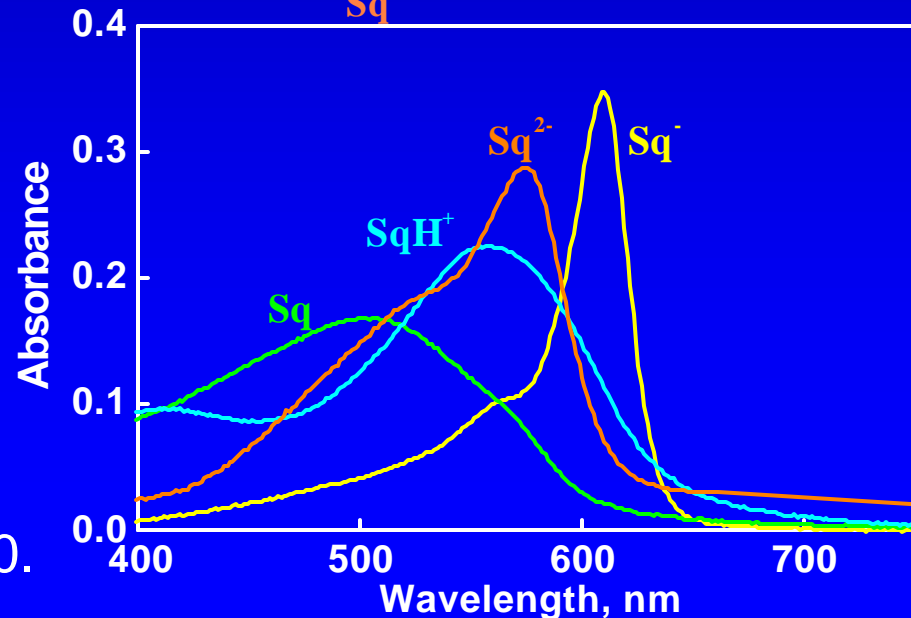
Negligible triplet  
quantum yields

# Absorption properties



★ Both 1 and 2 exist in protonation equilibria

★  $Sq^-$  exists as the predominant species under biological pH conditions

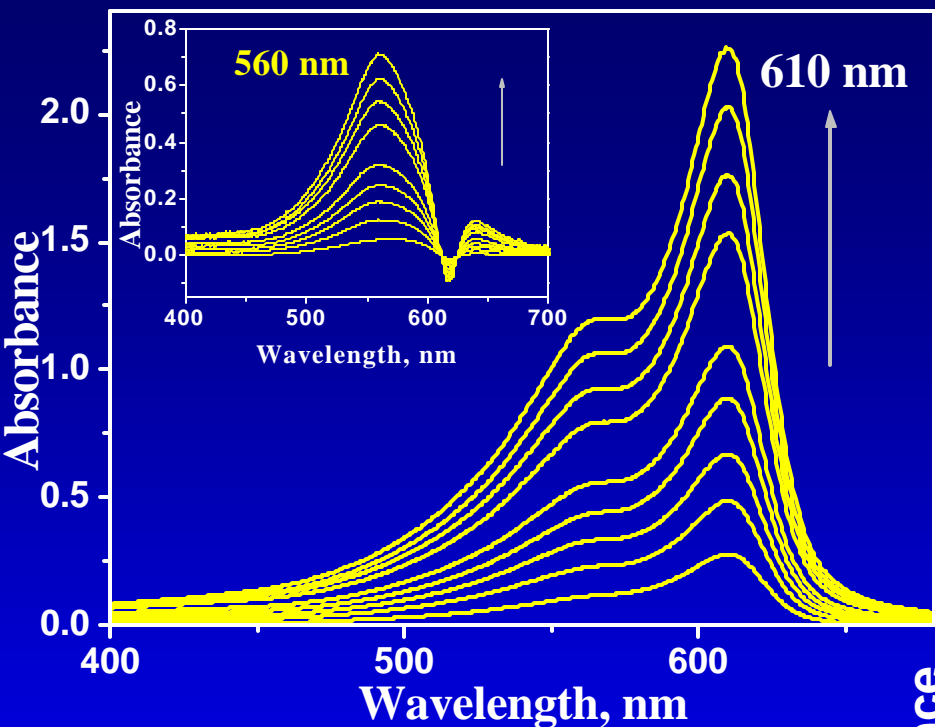


# Photophysical properties

Dye	$\lambda_{\text{max}}$ , nm	$\hat{\mathbf{I}}$ , $\text{M}^{-1}\text{cm}^{-1}$	$\mathbf{F}_T$	$\mathbf{t}$ , ns	$\mathbf{F}({}^1\text{O}_2)$
Bromo	610	47,000	0.22	132	0.13
Iodo	617	63,000	0.5	36	0.47
Hp	630	3,500	-	-	0.65
HpD	630	3,000	-	-	0.20

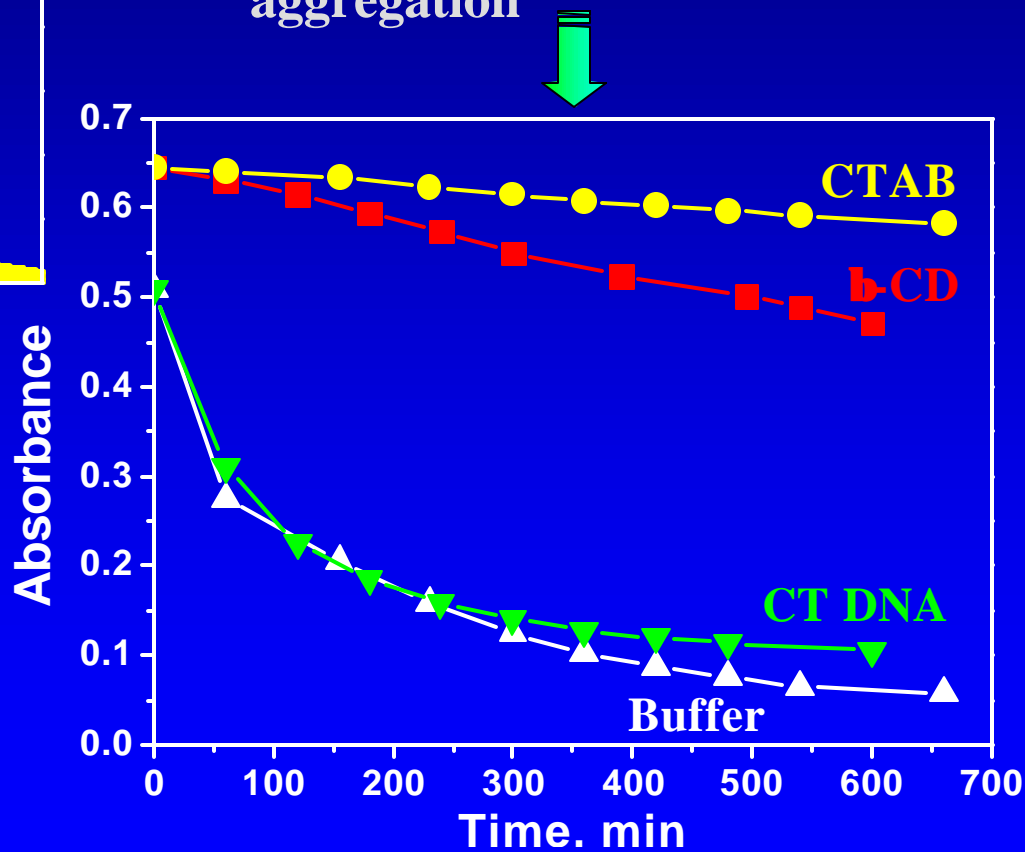
- *Efficiency of triplet formation and singlet oxygen generation is 50% and 47%, respectively for the iodo derivative*
- *Quantum yield of  ${}^1\text{O}_2$  increased in the presence of organized media*

# Photophysical properties under physiological conditions



← Aggregation in methanol-buffer solutions at physiological pH

Effect of organized media on aggregation



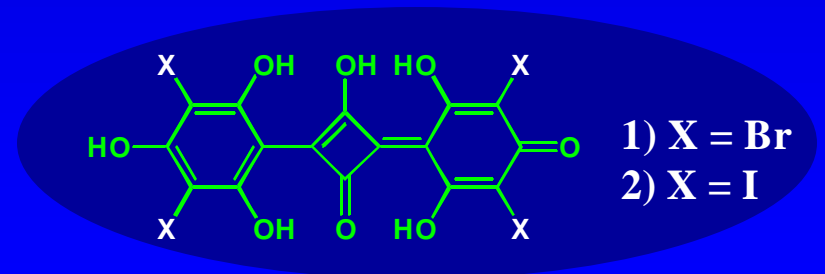
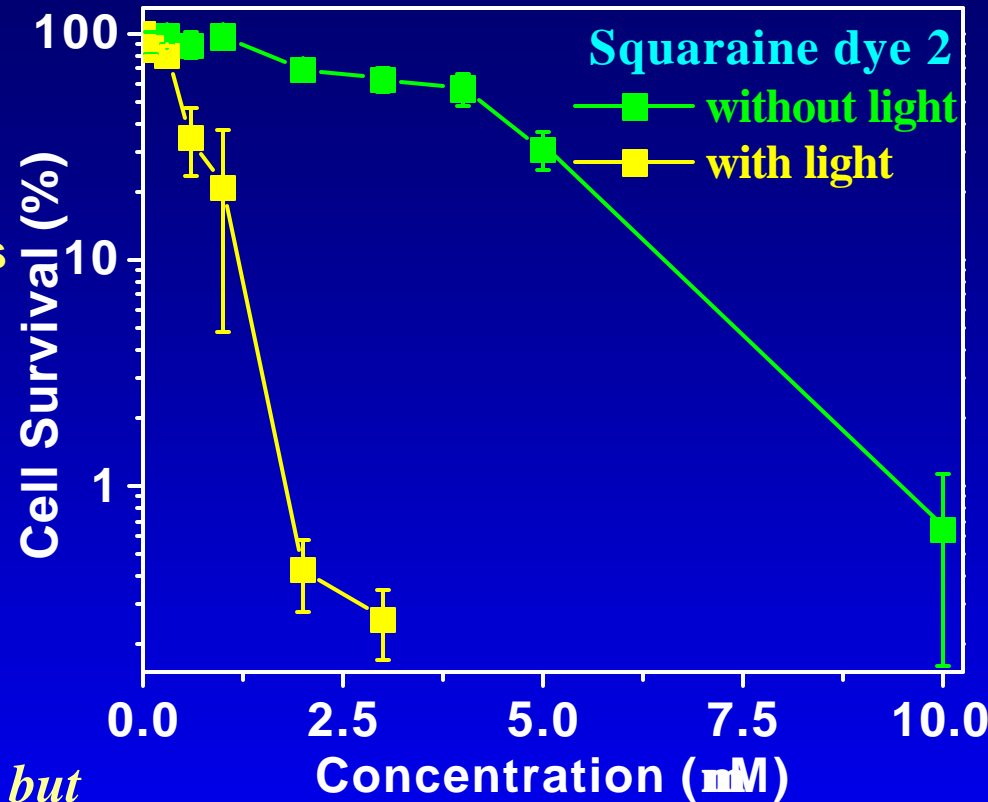
- Squaraines form H-type aggregates
- Aggregation enhanced in the presence of electrolytes
- Monomer stabilized in the presence drug carriers and membrane mimics

# Photobiological studies

## Cytotoxicity

- ★ Chinese hamster ovary AS52 cells
- ★ Mouse lymphoma L5178Y cells
- ★ Bacterial strains (TA100 and TA2638)

- *Squaraines are non-toxic in the dark but exhibited high photocytotoxicity*
- *The iodo derivative is a better candidate than the bromo derivative*



# Mechanism of biological activity

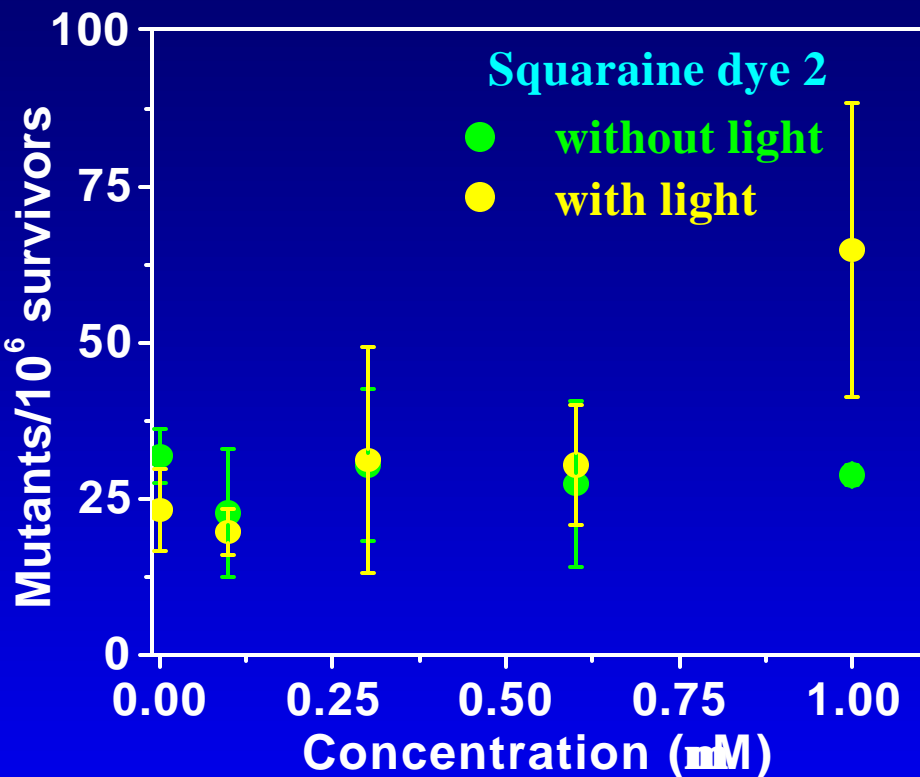
Dye	Relative number of SSB (%) in presence of				
	pH 7.0	pH 7.8	D <sub>2</sub> O buffer	SOD (20mg/mL)	Catalase (315 U/mL)
<b>1</b>	127±12	105±4	<b>526±28</b>	109±5	115±5
<b>2</b>	134±9	109±22	<b>614±97</b>	100±2	92±6
Hp	107±8	95±11	<b>648±36</b>	97±4	83±8
NDPO <sub>2</sub>	-	-	<b>960±190</b>	105±12	99±2

- *Singlet oxygen is responsible for the biological activity*

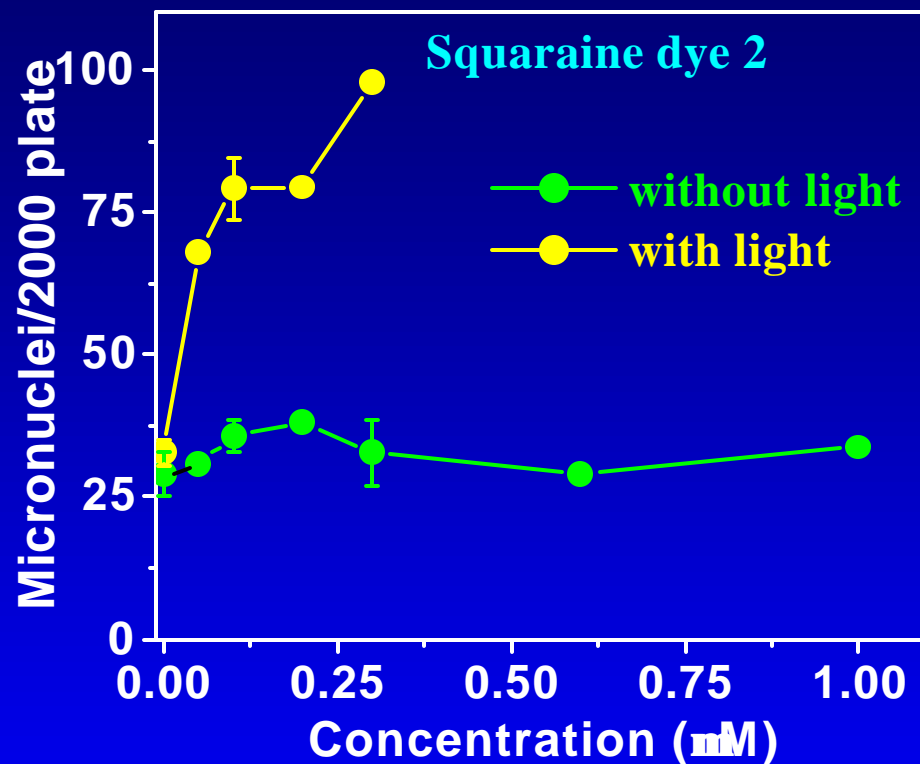
*Photochem. Photobiol.* **2002**, 76, 672-677.

US Patent application no.20020123532 dated 5.09.02

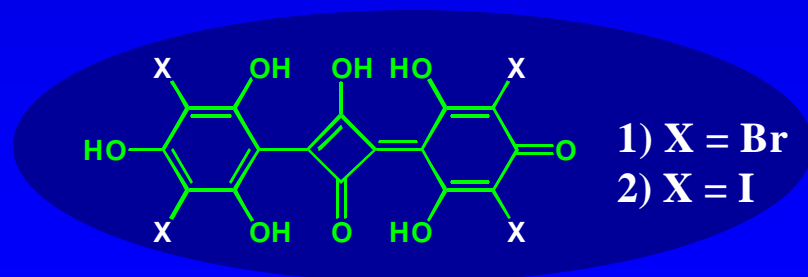
# Mutagenicity



# Micronuclei induction



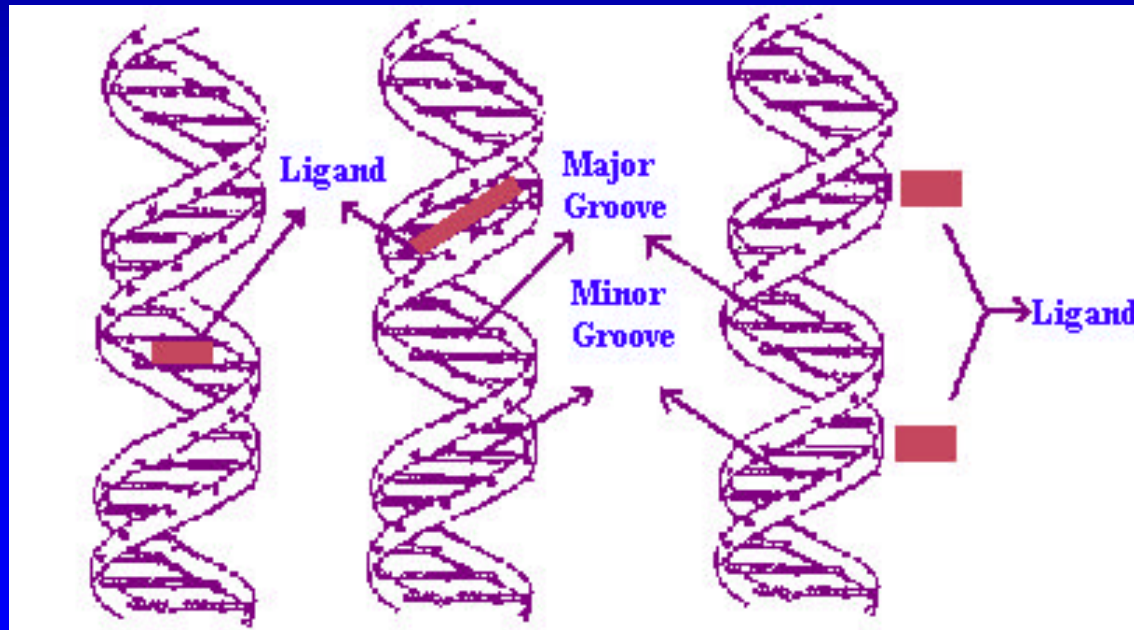
- *Squaraines are weakly photomutagenic*
- *Squaraines induced micronuclei only in the presence of light*



# Photoactivated DNA Cleaving Agents

## ☆ Ligand - DNA interactions

- Covalent binding
- Non-covalent interactions

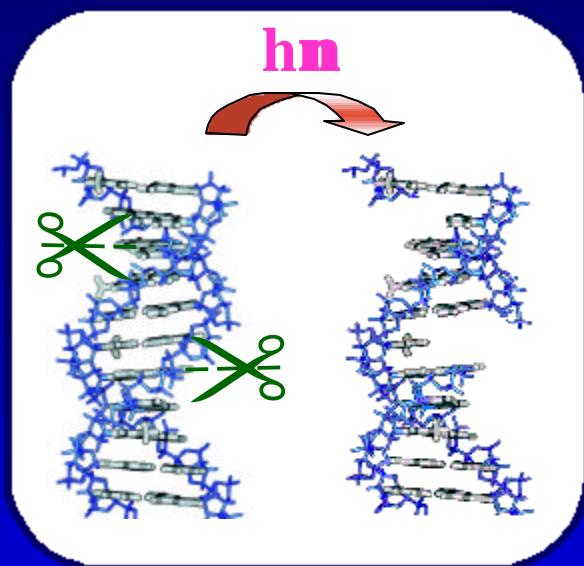


**Intercalative  
Binding**

**Groove  
Binding**

**Electrostatic  
Binding**

# Photoactivated DNA cleaving agents



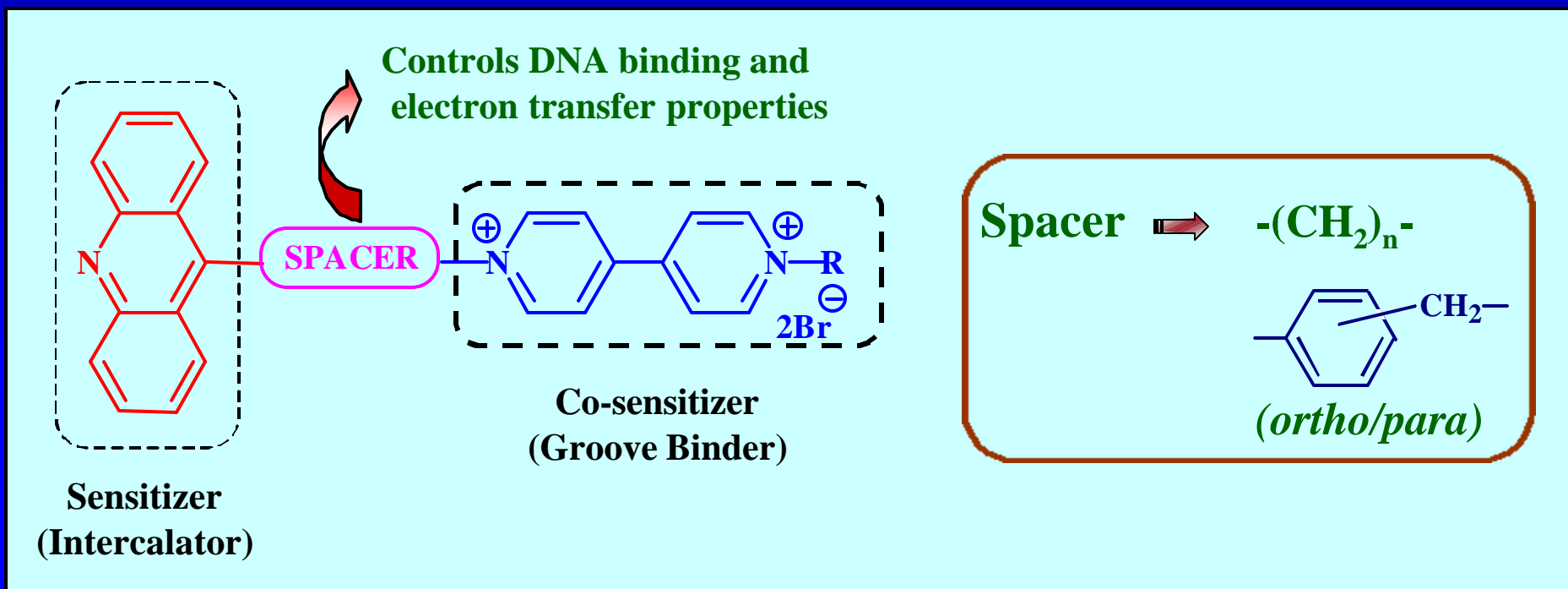
- *Generation of diffusible intermediates such as singlet oxygen, OH radical etc*
- *Photoinduced electron transfer reactions (oxidation of bases)*
- *Photoadducts*

## ☆ Advantages

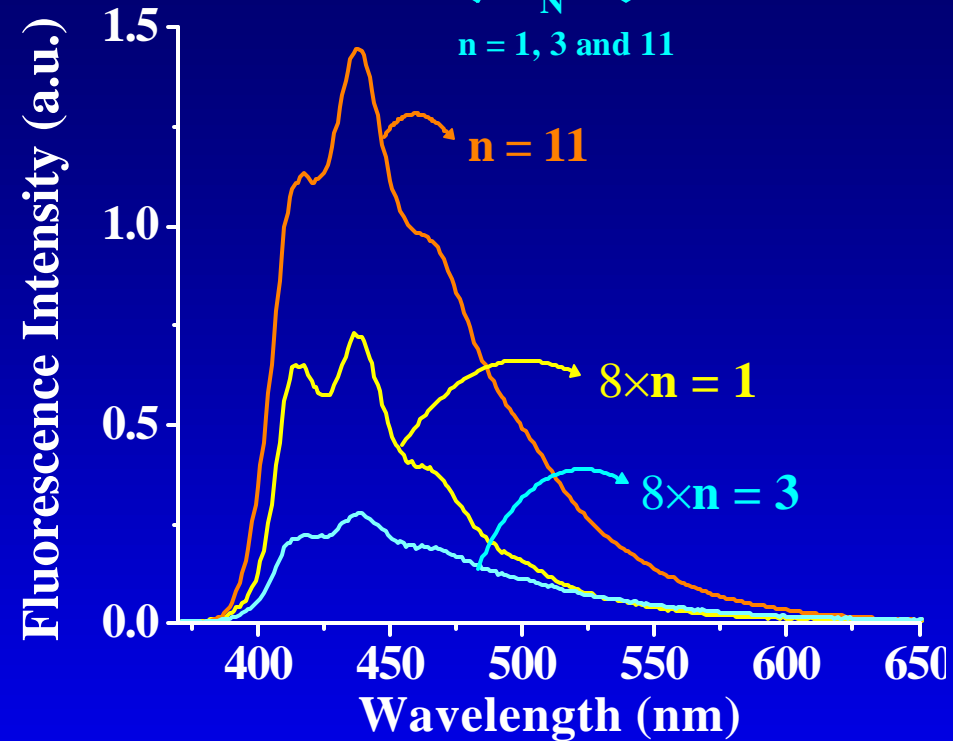
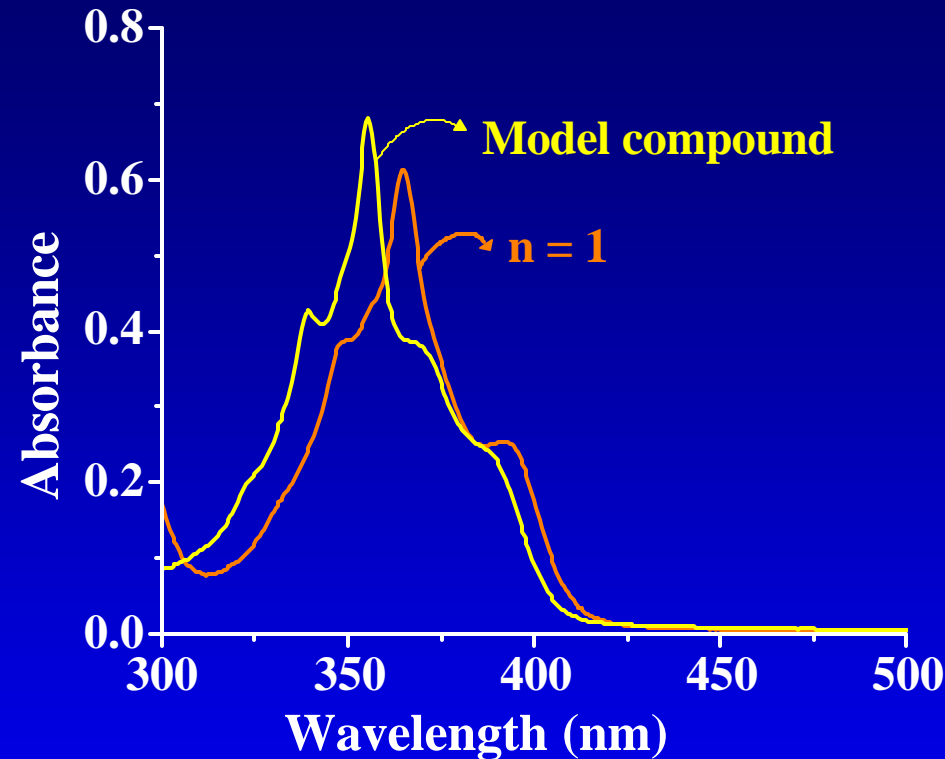
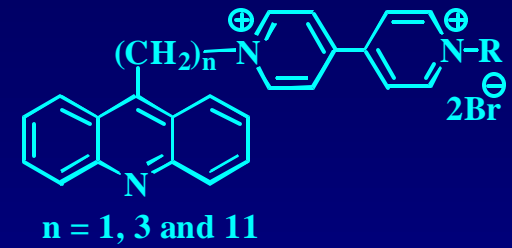
- *Better control of reaction trigger and reaction mode*
- *Selectivity of the reaction center by adjusting the wavelength of irradiating light*
- *Easy to elucidate the mechanism of cleavage*

# Objectives

- ☆ Design of photoactivated DNA cleaving agents which function through the mechanism of cosensitization
- ☆ Investigation of their inter- and intramolecular electron transfer reactions
- ☆ Study of their DNA binding and cleaving properties



# Photophysical properties

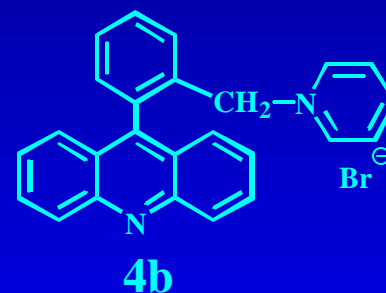
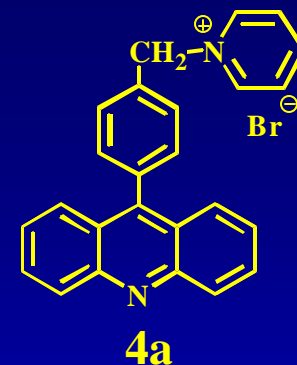
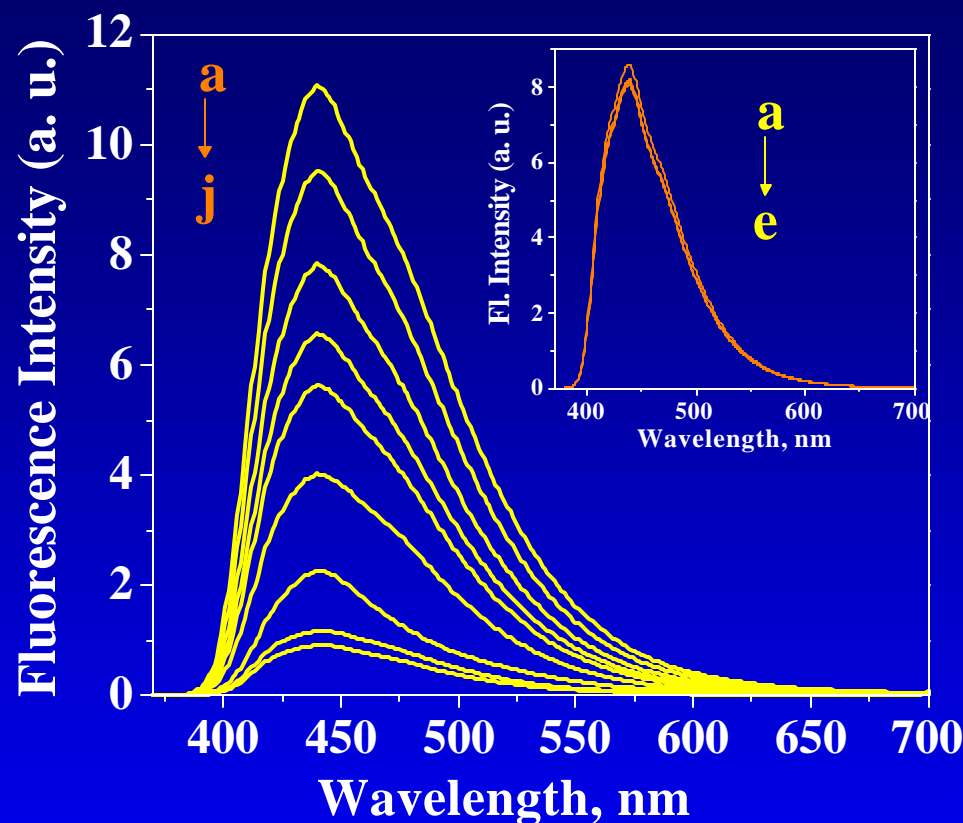


*Absorption and emission spectra of viologen linked acridines in water*

- *No ground state charge transfer complex formation*
- *Efficient fluorescence quenching in the case of the viologen linked acridines and bisacridines*
- *Rate of electron transfer depends on the spacer*



# Effect of spacer groups

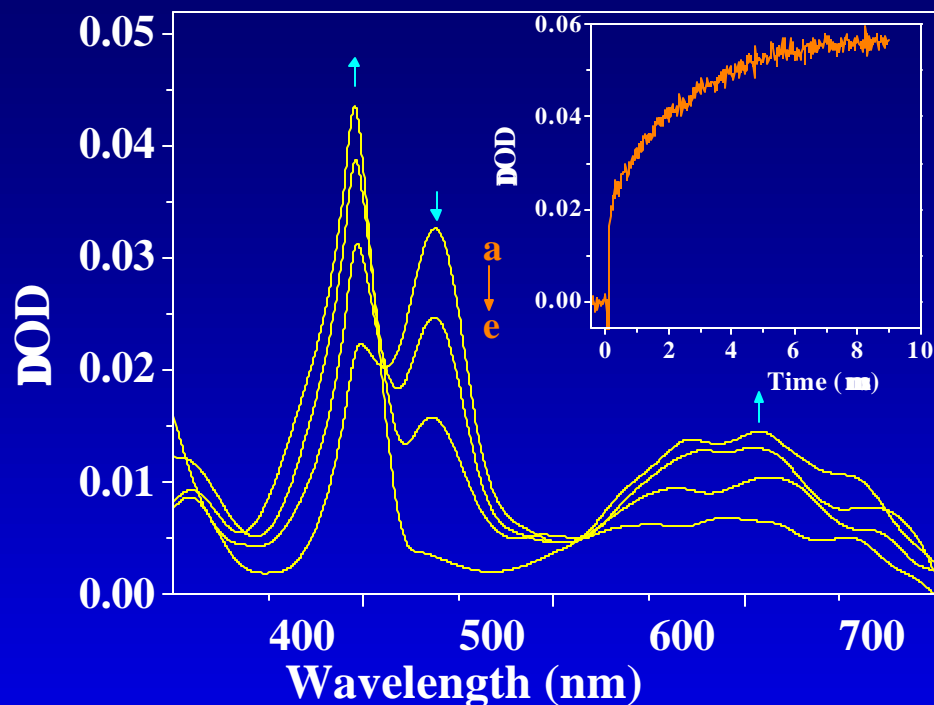


## *Absorption spectra of tolylacridine derivatives in presence of DNA*

- ☆ *Only the para-derivatives bind with DNA ( $K_{\text{DNA}} \sim 10^5 \text{ M}^{-1}$ )*
- ☆ *The ortho-derivatives fail to interact with DNA due to the conformational and steric effects*

*Chem. Lett. 2001, 438-439; J. Phys. Chem. B., 2003 (In press)*

# Laser flash photolysis studies



$n = 1, 3$  and  $11$

R = alkyl or  $-(\text{CH}_2)_n\text{-Acr}$

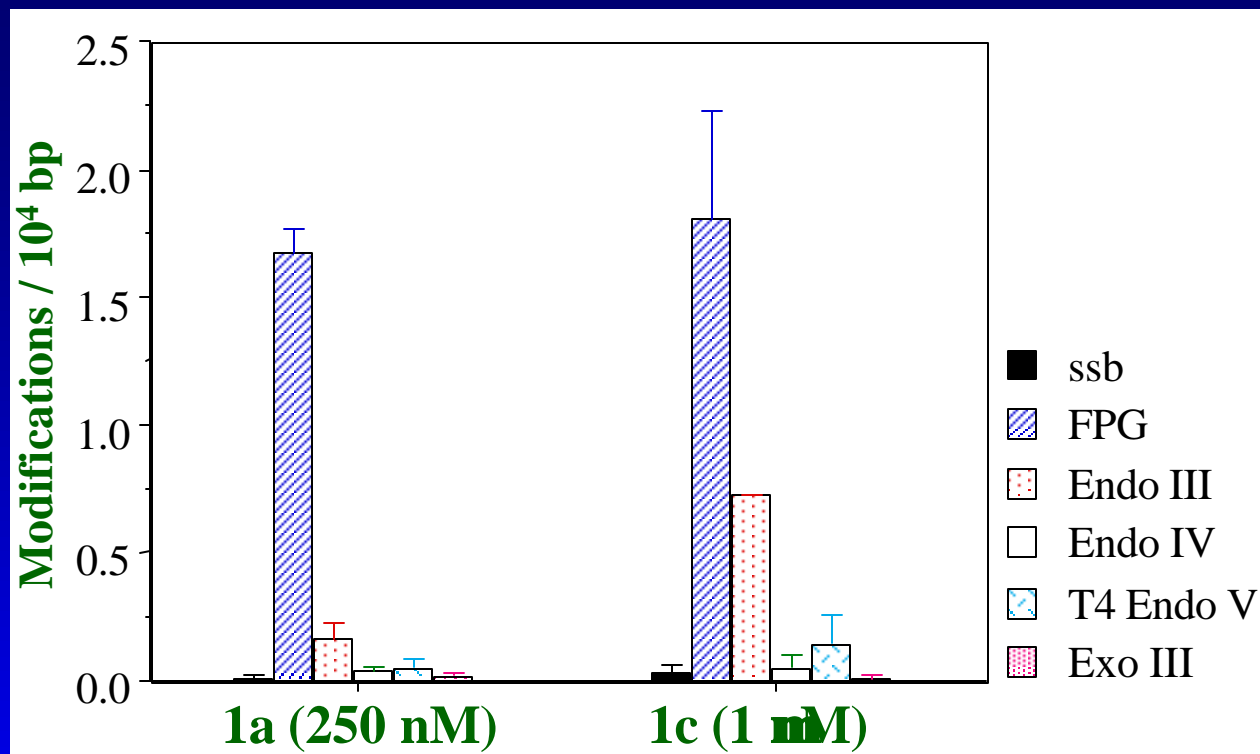
*Transient absorption spectra of **1c** ( $n = 11$ ) in presence of guanosine*

- ☆ *Both singlet and triplet excited states of acridine are capable of transferring an electron to the viologen moiety*
- ☆ *Reduced methyl viologen radical cation formed upon laser excitation of the viologen linked acridines in the presence of sacrificial donors (guanosine and DNA)*

# DNA Relaxation Assay



1a)  $n = 1$  and 1c)  $n=11$



*Single strand breaks and various endonuclease-sensitive modifications induced in PM2 DNA by photoexcited 1a (250 nm, 18 kJ/m<sup>2</sup>) and 1c (1 μM, 9 kJ/m<sup>2</sup>)*

- ☆ **Modifications induced were sensitive to FPG protein**
- ☆ **DNA cleavage enhanced with the increase in concentration of the ligand and time of irradiation**

# Summary

- ☆ Sensitizers based on intercalators and bisintercalators linked to electron acceptors were synthesized and examined their inter- and intramolecular electron transfer, DNA binding and cleavage properties.
- ☆ The results indicate that these molecules bind to DNA efficiently depending on the nature of the spacer and cleave DNA purely through the cosensitization mechanism.
- ☆ Squaraine dyes having amphiphilic substituents and cellular recognition elements were synthesized and their photophysical and *in vitro* photobiological properties were investigated.
- ☆ The results indicate that these dyes exhibit good absorption properties, significant triplet and singlet oxygen yields, *in vitro* photocytotoxicity and hence favorable for photodynamic therapeutical applications.

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