

Membrane-Targeting Peptides Enhancing Uptake of Ruthenium(II) Polypyridyl Photosensitiser into Clinically Relevant Bacteria

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Introduction and Research Objectives

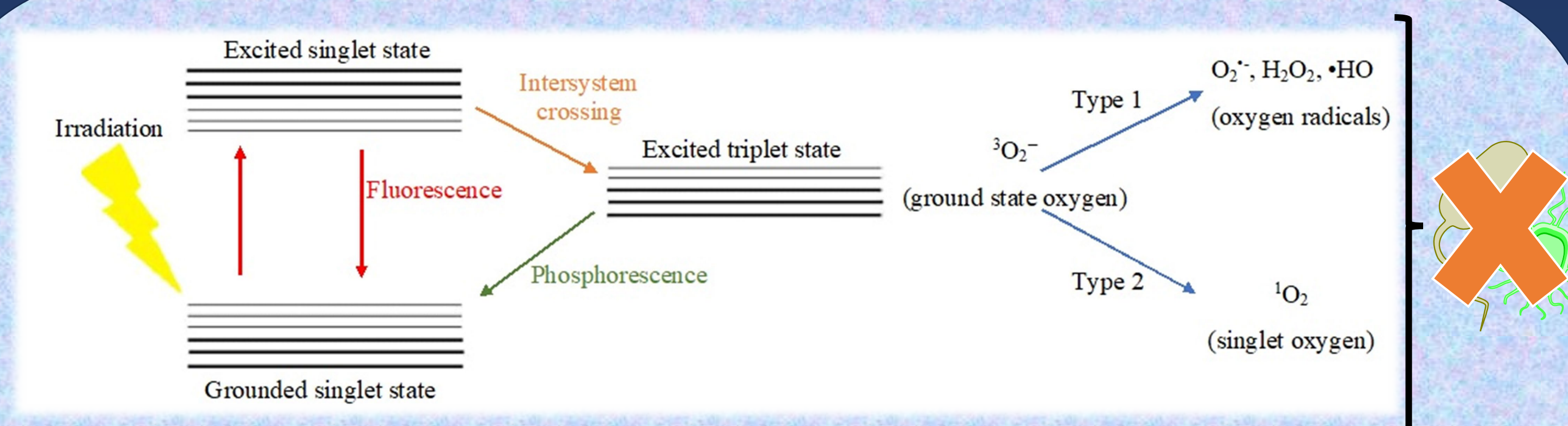
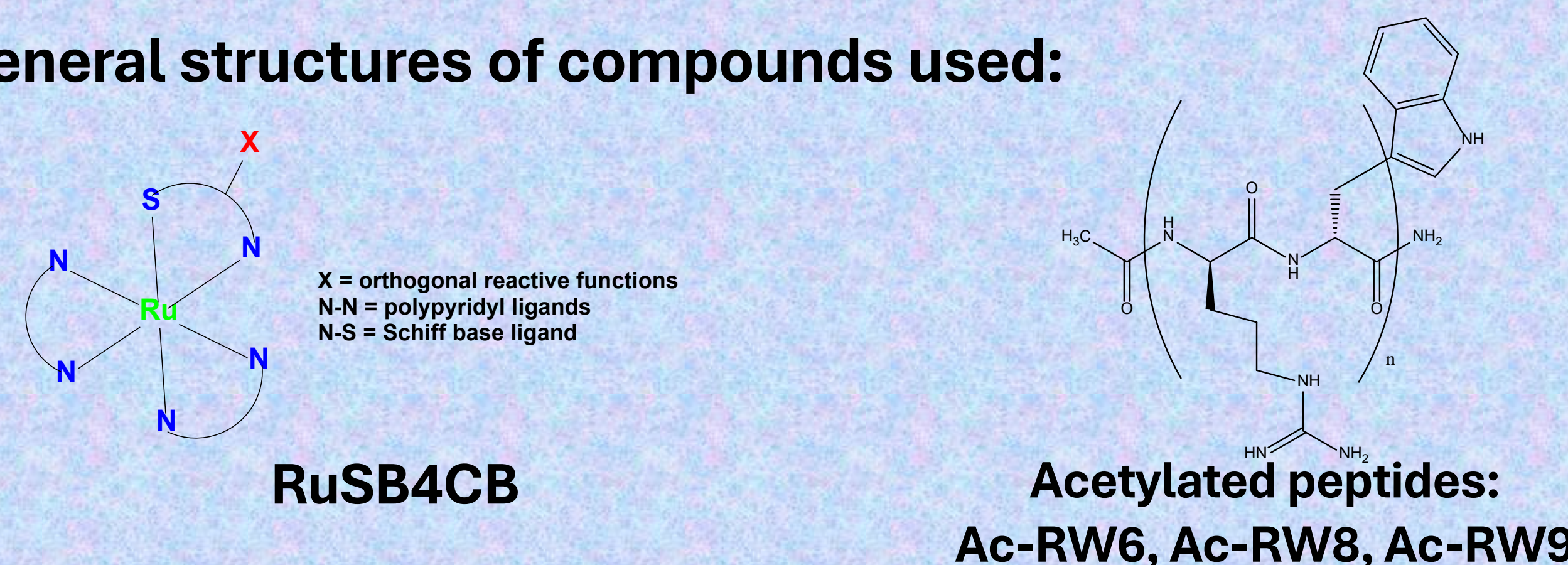


Fig. 1. Photochemical and photophysical mechanisms of photodynamic therapy involving Type 1 and Type 2 pathways.

Photodynamic therapy has recently emerged as potential alternative in combating the growing threat of global antibiotic resistance. Free radicals will be generated in the presence of photosensitisers and oxygen under light irradiation of a specific wavelength and cause cytotoxic damage to bacteria. Ruthenium(II) photosensitisers with rich photochemical properties and potential antibacterial activities gained our focus of interest. However, they may experience low bacterial uptake when administered *in vivo*. Hence, the **objective** of our study is **to investigate the potential of bacterial membrane-targeting peptides - arginine-tryptophan (Arg-Trp or RW) peptides in enhancing bacterial uptake of the Ru(II) complex.**

Methodology

General structures of compounds used:



- Minimum inhibitory concentration (MIC) assays
- Combination assays and fractional inhibitory concentration indexes (FICI)
- Molecular docking studies
- ✓ EIGHT bacteria, both susceptible (S) and resistant (R) strains: *Staphylococcus aureus* (SA), *Pseudomonas aeruginosa* (PA), *Escherichia coli* (EC) & *Acinetobacter baumannii* (AB)

Results & Discussion

Minimum Inhibitory Concentration (MIC) assays

- RuSB4CB alone is **effective against SA only** with the MIC values of **16 μ M**, with or without irradiation.
- This may be due to membrane structural difference between Gram-positive and Gram-negative bacteria.
- Ac-RW9 alone is the **most effective** against **SA, EC, PA(S)** and **AB(R)** among three acetylated peptides with the lowest MIC values, followed by Ac-RW8 and Ac-RW6.
- All three acetylated peptides are not active against AB(S) and PA(R), regardless of irradiation.

Molecular Docking Studies of RuSB4CB with Multidrug Efflux Transporters in Bacteria

Table 2. Binding interaction and docking scores of RuSB4CB with residues in multidrug efflux transporters of various bacteria.

Bacteria	Multidrug efflux transporter	Residue	Ligand functional group	Binding interaction	Docking score (kcal/mol)
PA	MexB	LYS 292	Hydroxyl group	Hydrogen bonding	-6.757
		GLN 89	Carbonyl group	Hydrogen bonding	
		PHE 136, PHE 615	Aromatic ring	pi-pi stacking	
EC	AcrB	ARG 815	Hydroxyl group	Hydrogen bonding	-5.708
		PHE 617	Aromatic ring	pi-pi stacking	
SA	NorA	PRO 110	Aromatic ring	Hydrophobic	-5.403

*No result for AB as the molecule is rigid and the binding pocket is small.

- RuSB4CB has **lower docking score** in its interaction with **NorA** of **SA** compared to other bacteria, indicating **weaker binding interaction**.

Combination Assays and Fractional Inhibitory Concentration Indexes (FICI)

Table 1. MIC values of RW peptides when they are used alone and when they are used in combination with RuSB4CB.

Compound	Irradiated	MIC values (μ M)					
		SA (R)	SA (S)	PA (S)	EC (R)	EC (S)	AB (R)
Ac-RW6	Yes	-	-	-	64	-	-
	No	-	-	-	64	-	-
Ac-RW6 + RuSB4CB	Yes	8	8	-	64	128	-
	No	8	8	-	64	64	-
Ac-RW8	Yes	16	32	32	8	32	-
	No	8	32	32	8	32	-
Ac-RW8 + RuSB4CB	Yes	4	4	128	8	16	32
	No	4	4	128	4	8	32
Ac-RW9	Yes	8	16	16	4	16	128
	No	8	16	16	4	16	128
Ac-RW9 + RuSB4CB	Yes	4	4	64	4	8	16
	No	2	2	64	4	4	16

- Interestingly, drug combination between RuSB4CB with all three acetylated RW peptides shows **synergistic and/or additive** interaction when tested against **SA(S), SA(R), AB(R)** and **EC(S)**.
- ✓ “-”: Not effective, defined as MIC values >128 μ M; ORANGE indicates additivity; GREEN indicates synergism.
- ✓ FICI calculation as per formula:

$$FIC \text{ index} = FIC_x + FIC_y = \frac{(X)}{(MIC_x)} + \frac{(Y)}{(MIC_y)}$$

“FICI $\leq$ 0.5 indicates synergism; FICI > 0.5-1.0 indicates additivity.

Conclusion

- RuSB4CB alone is effective against SA only, supported by its lower docking score obtained in molecular docking studies compared to other bacteria.
- Combination of RuSB4CB with Ac-RW8 or Ac-RW9 shows additivity/synergism.

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