ADVANCING PHOTODYNAMIC THERAPY: RUTHENIUM POLYPYRIDYL/SCHIFF BASE COMPLEXES FOR DUAL BIOMEDICAL APPLICATIONS

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01 INTRODUCTION

- Cancer - uncontrollable cell proliferation and metastasis, ineffective treatment and trigger side effects [1].
- Multidrug-resistant bacteria - limited potent antibiotics, and trigger sepsis [1,2].
- Photodynamic therapy (PDT) - targeted delivery of photosensitiser to eradicate diseases [1,2,3].
  - Trigger oxidative stress, vasculature damage and activate immune system.
  - Enhance treatment efficacy and patient compliance.
- Ruthenium (Ru) complexes - immense photoactivity upon photoirradiation at specific wavelengths,
  - Generate abundant reactive oxygen species (ROS) (Figure 1) [1,2,3].
  - Schiff bases - increases maximum absorption wavelength (λmax), photostability, and luminescence.

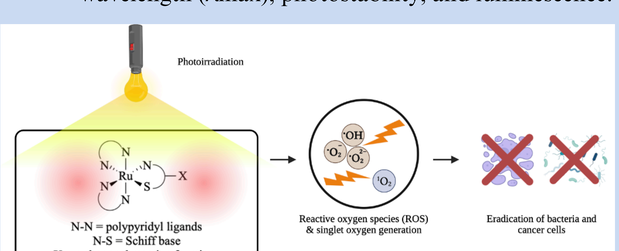


Figure 1: Application of Ru(II) Schiff base complexes for PDT to treat cancer and bacteria cells.

02 OBJECTIVES

- To synthesise and characterise Ru(II) Schiff base complexes.
- To determine the *in vitro* antibacterial and anticancer activity of Ru(II) Schiff base complexes.
- To evaluate the effectiveness of the combination therapy of Ru(II) Schiff base complexes with cisplatin.

$[Ru(Bpy)_2(SB4CB)]PF_6$	$[Ru(Bpy)_2(SBFH)]PF_6$
R_1 = 4-formylbenzoic acid	R_1 = 4-formyl-3-hydroxybenzoic acid
R_2 = benzyl	R_2 = benzyl

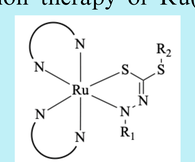


Figure 2: Chemical structure of Ru(II) Schiff base complexes.

03 METHODOLOGY

- **Synthesis and characterisation:** $Ru(bpy)Cl_2$ was refluxed with SB4CB and SBFH then precipitated with KPF_6, characterisations were confirmed with literature.
- **Minimum inhibitory concentration (MIC) test:** *Staphylococcus aureus (SA), Pseudomonas aeruginosa (PA), Escherichia coli (EC), Acinetobacter baumanii (AB)* were treated with Ru(II) Schiff base complexes. (Incubate 15 minutes, irradiate with 365 nm UV light, at 1.57 mW for 10 minutes.)
- **MTT assay:** MDA-MB-231, A549, HT29, MeL28, SW1990, MRC5 were treated with Ru(II) Schiff base complexes. (Incubate 4 hours, irradiate with 365 nm UV light at 1.72 mW for 2.5 minutes.)
- **Combination study:** Cancer cells were treated with Ru(II) Schiff base complexes and cisplatin, then analysed with Combenefit software (Cancer Research UK Cambridge Institute).

04 RESULTS AND DISCUSSION

A. MIC TEST

Table 1: MIC test results by the broth microdilution method in dark and light conditions.

Compounds	MIC (μM)							
	SA (S) ATCC 35923		*PA* (S) ATCC 27853		*EC* (S) ATCC 25922		*AB* (S) ATCC 19606	
	Dark	Light	Dark	Light	Dark	Light	Dark	Light
$[Ru(bpy)_2(SB4CB)]PF_6$	16	16	>128	>128	>128	>128	>128	>128
$[Ru(bpy)_2(SBFH)]PF_6$	8	16	>128	>128	>128	>128	>128	>128

- Both compounds exhibited activity against SA only, due to variance in the cellular membranes of Gram-ve & +ve bacteria.

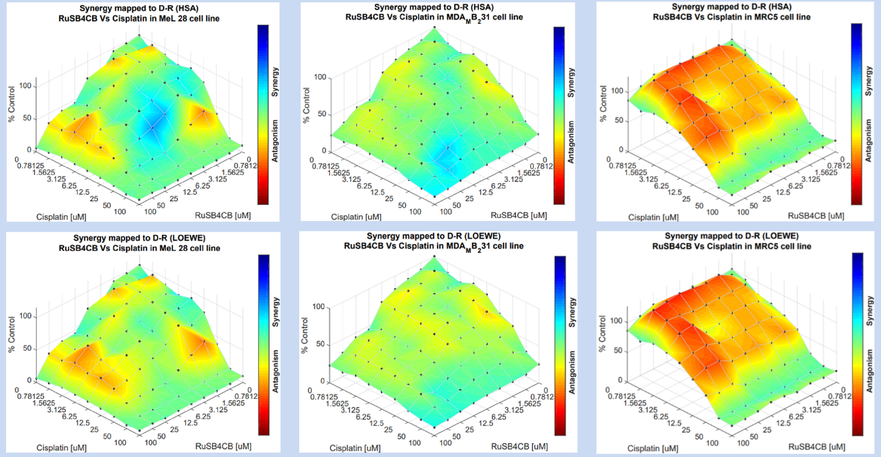
B. MTT ASSAY

Table 2: MTT test results of the compounds and cisplatin in both dark and light conditions.

Compounds	IC_{50}(μM)											
	Cancer cells										Healthy cells	
	MDA-MB-231 (Breast)		A549 (Lung)		HT29 (Colon)		MeL28 (Melanoma)		SW1990 (Pancreatic)		MRC5 (Lung fibroblast)	
	Dark	Light	Dark	Light	Dark	Light	Dark	Light	Dark	Light	Dark	Light
$[Ru(bpy)_2(SB4CB)]PF_6$	12.10 ±2.03	6.72± 2.45	>100	>100	>100	>100	20.88 ±1.34	9.39± 0.63	>100	>100	>100	>100
$[Ru(bpy)_2(SBFH)]PF_6$	15.40 ±1.67	11.31 ±2.62	>100	>100	>100	>100	55.74 ±0.69	13.09 ±3.14	>100	>100	>100	>100
Cisplatin	23.00		7.49		>100		4.00		>100		5.91	

- Both complexes exhibited selectivity against MRC5.
- $[Ru(bpy)_2(SB4CB)]PF_6$ was more cytotoxic than $[Ru(bpy)_2(SBFH)]PF_6$.
- Both successfully induced phototoxicity (MDA-MB-231 & MeL28).

C. COMBINATION STUDY



- Colour scale bar: level of synergism or antagonism (Dark blue SA: synergy; red SA: antagonism)
- Combination of $[Ru(bpy)_2(SB4CB)]PF_6$ with cisplatin in the MRC5 cell line - Red color (antagonistic effect)
- MDA-MB-231 & MeL28 cell lines, respectively, displayed an additive effect (light blue/green colour)

Figure 3: Combenefit mapped surface plot for the combinations involving $[Ru(bpy)_2(SB4CB)]PF_6$ and Cisplatin in the MRC5, MDA-MB-231, and MeL28 cell lines, respectively, using HSA, and Loewe synergy model (n = 3).

05 CONCLUSION

- $[Ru(bpy)_2(SB4CB)]PF_6$ & $[Ru(bpy)_2(SBFH)]PF_6$ was active against *SA(S)* (MIC = 16 μM; MIC = 16 μM and 8 μM).
- $[Ru(bpy)_2(SB4CB)]PF_6$ showed activity in MDA-MB-231 and MeL28 cell lines (IC_{50} = 6.72 μM and 9.39 μM).

06 REFERENCES

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