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RECEIVED 19 December 2025

REVISED 21 February 2026

ACCEPTED 03 March 2026

PUBLISHED 13 May 2026

CITATION

Talgatov A, Kaur G, Shi G, Roque III JA,
Barrett PC, Sunday D, Sainuddin T, Kim S,
Deep G, Francés-Monerris A, Alberto ME,
Cameron CG and McFarland SA (2026)
Programmable Ru(II) phototherapeutics:
master dials, mode switches, and
coligand-controlled reactivity.
Front. Chem. Biol. 5:1771939.
doi: 10.3389/fchbi.2026.1771939

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Programmable Ru(II) phototherapeutics: master dials, mode switches, and coligand-controlled reactivity

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The ability to program excited-state pathways in Ru(II) polypyridyl complexes offers a powerful strategy for controlling phototherapeutic activity, yet how reliably such design principles translate across biological contexts remains unclear. In this work, we examine how systematic modulation of ligand structure alters excited-state accessibility and biological response across multiple test systems. Specifically, we investigate a complete series of oligothiophene-extended Ru(II) complexes, Ru(6,6'-dmb)₂(IP-*n*T) series (*n* = 0–4), using spectroscopy, computation, photochemistry, and photobiology to define how oligothiophene length and coligand environment govern excited-state accessibility and biological response. Increasing oligothiophene chain length progressively suppresses photodissociative ³MC pathways in favor of stabilized *n*T-centered ³ILCT states. In mammalian cancer cells, only **Ru-3T** and **Ru-4T** exhibit light-dependent cytotoxicity, with **Ru-4T** emerging as the most effective compound under both normoxic and hypoxic conditions despite similar singlet-oxygen yields and triplet lifetimes. Antibacterial studies reveal additional biological constraints: while **Ru-4T** displays light-selective activity in *Staphylococcus aureus*, responses are attenuated in *Enterococcus faecalis* and absent in Gram-negative bacteria. Preliminary *in vivo* tolerability studies show that **Ru-4T** is well tolerated and exhibits reduced systemic toxicity relative to related analogues. Together, these results demonstrate that stabilized ILCT-based excited states, rather than ³MC-driven photochemistry, underpin productive phototherapeutic activity in this family of compounds, while biological context ultimately defines the limits of predictability for excited-state design.

KEYWORDS

excited states, hypoxia, intraligand charge transfer, oligothiophene, photocytotoxicity, photosubstitution, ruthenium

1 Introduction

Ruthenium (II) polypyridyl complexes bearing oligothiophene-extended imidazo[4,5-*f*][1,10]phenanthroline ligands (IP-*n*T) have emerged as a programmable platform for designing photoactive coordination compounds whose excited-state reactivity can be tuned with molecular precision. Early work with 6,6'-dimethyl-2,2'-bipyridine (6,6'-dmb) scaffolds demonstrated that extending the oligothiophene chain increases triplet lifetimes and singlet oxygen (¹O₂) yields, suggesting that chain length *n* functions as a master dial governing the transition from ³MLCT/³MC to ³IL/³ILCT behavior (Sainuddin et al., 2016). Subsequent studies with unstrained 1,10-phenanthroline (phen) complexes established that ILCT activation is the principal determinant of potent PDT activity under normoxia (Cole et al., 2023), while investigations of highly strained 2,9-dimethylphenanthroline (dmp) analogues revealed extreme ILCT stabilization, suppressed ³MC accessibility, and unprecedented hypoxia performance in the resulting “ubertoxin” series (Roque et al., 2022). A direct comparison of dmb and dmp complexes further showed that coligand identity acts as a mode switch, altering the energetic accessibility of competing MLCT, ILCT, and ³MC states and thereby modulating photochemical pathways including photosubstitution (Cole et al., 2022).

Together, these studies suggest a unified design principle in which oligothiophene length defines the topology of the excited-state landscape, while coligand structure dictates which decay pathways are energetically accessible—and therefore which biological outcomes are realized. These observations prompted us to examine where molecular design principles succeed, where they break down, and which biological features impose new constraints. The present study therefore seeks to define how excited-state programming interacts with biological context to yield—or fail to yield—predictable photocytotoxic outcomes.

Here we test this principle explicitly by examining the complete Ru(6,6'-dmb)₂(IP-*n*T) (*n* = 0–4) family (Figure 1), combining computation, spectroscopy, and photobiology to determine how a moderately strained bipyridyl environment modulates ³ILCT–³MC competition across the *n*-series. This work establishes how master dials and mode switches cooperate to control reactivity in Ru(II) phototherapeutics, completing the mechanistic picture framed by our prior phen, dmb, and dmp studies.

2 Materials and methods

2.1 Instrumentation

Microwave reactions were performed in a CEM Discover microwave reactor. NMR spectra were collected using JEOL ECS 400 and ECA 500 NMR Spectrometers (¹H, ¹H–¹H COSY) at UNCG's NMR facility. ESI⁺ mass spectra were obtained using a Thermo Fisher LTQ Orbitrap XL coupled to a Water's Acuity Ultra-high Performance Liquid Chromatography (UPLC) stack using a BEH C18 column at UNCG's Triad Mass Spectrometry facility. HPLC analyses were carried out on an Agilent/Hewlett Packard 1100 series instrument (ChemStation Rev. A. 10.02 software) using a Hypersil GOLD C18 column (Thermo 25005-254630, guard 25003-014001) with an A–B gradient

(40 min run; 98% → 2% A; A = 0.1% formic acid in H₂O, B = 0.1% formic acid in MeCN). Reported retention times are accurate to within ±0.1 min. Flash chromatography was carried out on a Teledyne Isco CombiFlash EZ Prep system with Silicycle SiliaSep silica flash cartridges (FLH-R10030B-ISO25).

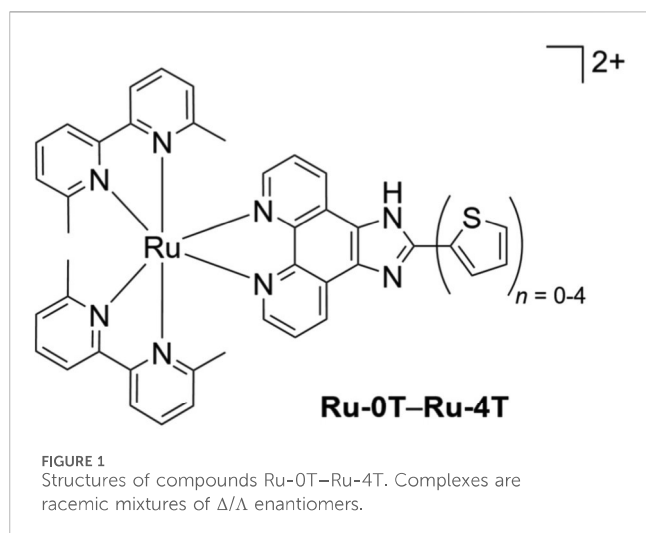
2.2 Synthesis and characterization

Ru(6,6'-dmb)₂Cl₂·2H₂O, compounds **Ru-1T–Ru-4T**, imidazo[4,5-*f*][1,10]phenanthroline (IP) ligands, IP-4T, and its precursor 4T-CHO were prepared following adapted literature procedures (Sainuddin et al., 2016; Cole et al., 2022; Wang, 2010; Ghosh et al., 2018; Zhang et al., 2014). Cl[−] salts of final complex products were obtained via anion metathesis on HCl-treated Amberlite IRA-410 resin (Alfa-Aesar, A1773436) with methanol as eluent and isolated *in vacuo*. Final complexes are a mixture of Δ/Λ isomers.

The full characterization of previously prepared **Ru-1T** to **Ru-4T** are reported in our prior publications (Sainuddin et al., 2016; Cole et al., 2022). These authenticated batches were used in the present study. Characterization of **Ru-0T** compound is described below. The reference compound [Ru(6,6'-dmb)₂(phen)]Cl₂ was synthesized but excluded from this work due to isolation difficulty from the coeluting tris homoleptic [Ru(6,6'-dmb)₃]Cl₂. All new products are characterized in Supplementary Material via ¹H NMR, ¹H–¹H COSY NMR, HPLC, and ESI⁺–MS.

Elemental analysis was attempted for **Ru-0T** and the related family members. However, reproducible values were not obtained despite extensive drying under high vacuum and elevated temperature. This may be due to reduced crystallinity and the tendency of extended, conformationally flexible dicationic chloride salts to retain variable amounts of lattice solvent or water, which significantly affects combustion-derived percentages. Detailed ¹H NMR spectroscopy with systematic 2D NMR-based resonance assignments confirmed ligand connectivity and coordination environment. High-resolution ESI⁺ supports the expected molecular formulation, and HPLC analysis confirms chemical purity (>95% by peak area). Taken together, these orthogonal methods establish the identity and purity of **Ru-0T** and the related complexes.

[Ru(6,6'-dmb)₂(IP-0T)]Cl₂ (**Ru-0T**). Ru(6,6'-dmb)₂Cl₂·2H₂O (92 mg, 0.16 mmol) and IP (35 mg, 0.16 mmol) were added to a microwave vessel containing argon-purged ethylene glycol (2.5 mL) and subjected to microwave irradiation at 180°C for 15 min. The resulting dark red mixture was transferred to a separatory funnel with deionized water (20 mL) and CH₂Cl₂ (30 mL). After gentle mixing, the CH₂Cl₂ was drained and the remaining aqueous layer was washed with CH₂Cl₂ (30 mL portions) until the CH₂Cl₂ was colorless. At that point, another 30 mL of CH₂Cl₂ was added and allowed to settle to the bottom of the separatory funnel. Then, saturated aqueous KPF₆ (5 mL) was added, and the mixture was shaken gently and allowed to settle over time to facilitate transfer of the product from the aqueous layer to the CH₂Cl₂ layer, which was concentrated under reduced pressure. The crude product was purified by silica gel flash column chromatography with a gradient of MeCN, 10% water in MeCN, followed by 7.5% water in MeCN with 0.5% KNO₃ to obtain the desired product as a dark red solid (37 mg, 24%). The PF₆[−] salt was converted in quantitative yield to its corresponding Cl[−] salt using Amberlite IRA-410 with



MeOH as the eluent. The solids were purified further using Sephadex LH-20 with MeOH as the eluent (22 mg, 77%). $R_f = 0.8$ (0.5% KNO_3 , 7.5% H_2O , 92% MeCN). ^1H NMR (500 MHz, $\text{MeOD}-d_3$, ppm): δ 8.91 (d, $J = 8.1$ Hz, 2H; c), 8.75 (d, $J = 8.2$ Hz, 2H; 3'), 8.59 (s, 1H; d), 8.54 (d, $J = 8.2$, 2H; 3), 8.31 (dd, $J = 5.5$, 1.2 Hz, 2H; a), 8.27 (t, 3H; 4'), 7.83 (d, $J = 8.3$, 5.5 Hz, 2H; b), 7.72 (t, 2H; 4), 7.65 (dd, $J = 7.7$, 0.6 Hz, 2H; 5'), 6.97 (dd, $J = 7.6$, 0.7 Hz, 2H; 5), 1.96 (s, 6H; 6'-Me), 1.59 (s, 6H; 6-Me). HRMS (ESI+) m/z for $\text{C}_{37}\text{H}_{32}\text{Cl}_2\text{N}_8\text{Ru}$ $[\text{M}-2\text{Cl}]^{2+}$ calc'd: 345.0891. Found: 345.0886 $[\text{M}-2\text{Cl}-\text{H}]^+$ calc'd: 689.1710. Found: 689.1696. HPLC retention time: 9.02 min (97% purity by peak area).

2.3 Solubility, counterion selection, and solvent choice

Solvent selection throughout this study was dictated primarily by counterion identity and experimental context rather than by intrinsic solubility limitations of the **Ru- n T** complexes. Complexes isolated as PF_6^- salts were readily soluble in MeCN, whereas Cl^- salts exhibited high solubility in MeOH and aqueous media. In all cases, optically transparent solutions were obtained at the concentrations used, with no visible particulates observed.

The Cl^- salt of **Ru-0T** was used for the characterization by NMR, HPLC, ESI $^+$ -MS. Solvent information and concentrations are listed in the Synthetic characterization section of Supplementary Material.

For determination of the log distribution coefficients ($\log D_{o/w}$) between saturated phosphate buffer (pH 7.4) and 1-octanol, the Cl^- salts were employed. **Ru-0T** to **Ru-2T** were prepared in phosphate buffer (pH 7.4) pre-saturated with 1-octanol, whereas for **Ru-3T** and **Ru-4T** were prepared in 1-octanol pre-saturated with phosphate buffer. Methods followed previously our reported procedure (Supplementary Figure S4, Supplementary Table S1) (Cole et al., 2024; Talgatov et al., 2026).

Absorption spectra were recorded in both MeCN (PF_6^- salts) and water (Cl^- salts) to highlight solvent-dependent behavior of the oligothiophene-centered transitions. In particular, Figure 3 compares spectra in polar aprotic and polar protic environments to emphasize solvatochromic responses of the n T-based bands, while maintaining comparable solution quality in both media.

Photophysical measurements were preferentially performed on PF_6^- salts in MeCN to enable direct comparison with the broader literature on Ru(II) polypyridyl complexes and to permit reliable singlet oxygen quantum yield determination, as protic solvents are known to quench singlet oxygen emission.

For biological assays, compounds were initially dissolved in DMSO to ensure complete solvation prior to dilution into aqueous culture media. Final DMSO concentrations were maintained below 1% (v/v), and vehicle controls containing equivalent DMSO levels were included in all experiments. Although DMSO is not strictly required for this compound family, its use follows established laboratory protocols to ensure consistency across high-throughput biological screening campaigns.

2.4 Femtosecond transient absorption spectroscopy

Ultrafast pump-probe measurements were carried out using a HARPIA-LIGHT transient absorption spectrometer (Light Conversion). Samples were prepared at ≈ 50 μM solutions in MeCN and loaded into 1 mM quartz cuvettes. The UV-vis spectra of the samples were compared before and after data collection to ensure no significant photodegradation had occurred. Excitation pulses were produced at 343 nm with a temporal width of approximately 300 fs with magic angle polarization in transmission mode. Probe pulses consisted of a broadband white light continuum generated from the laser fundamental (1030 nm) in a sapphire crystal, producing a useable spectral range of around 450–850 nm. Spatial and temporal overlap of pump and probe beams at the sample position were optimized prior to acquisition. Transient spectra were recorded as a function of pump-probe delay using the HARPIA optical delay line, covering the time window of -10 ps to 7 ns. Raw data were corrected for probe chirp and baseline offsets using Light Conversion CarpetView software and processed for global fit lifetimes using the same package.

2.5 Statistical methods for biological replicates

Cancer cells. Following the assessment of cancer cell viability for five compounds under various light treatments and oxygen conditions, the acquired data underwent statistical analysis to facilitate multiple comparisons. A non-parametric statistical approach was implemented using GraphPad Prism (version 10.6.1) due to the unequal biological replicate numbers across treatment groups and limited sample sizes per group. The analysis commenced with a Kruskal-Wallis test to evaluate the presence of statistically significant differences among the mean ranks of the five compounds. Upon identification of significant differences ($p < 0.05$) via the Kruskal-Wallis test, Dunn's post hoc test was subsequently applied to conduct pairwise comparisons and determine which specific compound pairs exhibited statistically significant disparities. Dunn's test was adopted as the optimal post hoc procedure due to its reliance on the pooled rank data derived from the initial Kruskal-Wallis analysis, maintaining consistency in rank-based comparisons.

Bacteria. Statistical analyses were performed as described above, with inclusion of EC₉₀ evaluated and the following adaptations. For *Staphylococcus aureus* Newman D2C (three biological replicates per compound), data were analyzed using a one-way ANOVA assuming a normal Gaussian distribution, followed by Tukey's post hoc test to correct for multiple comparisons. Adjusted p-values <0.05 were considered statistically significant, with a family-wise significance level of 0.05 and 95% confidence intervals. For *Enterococcus faecalis* 29212, where replicate numbers were unbalanced across compounds, non-parametric analysis was performed using a Kruskal–Wallis test followed by Dunn's post hoc test with multiplicity-adjusted p-values; significance was assessed based on differences in mean ranks (adjusted p < 0.05). Statistical analysis was not performed for *Escherichia coli* W3110 or *Acinetobacter baumannii* 17978 due to the absence of measurable antibacterial activity and the availability of only a single biological replicate per condition.

2.6 Computational studies

The computational studies were performed with Gaussian 16 code (Frisch et al., 2016) using density functional theory (DFT) and its time-dependent formulation (TDDFT) (Casida and Seminario, 1996). The reliability of these methods for predicting photophysical and photochemical properties of photosensitizers for PDT has been demonstrated previously (Ponte et al., 2019; Alberto et al., 2016a; Abad-Montero et al., 2025; Romero-Castellón et al., 2026; Bertini et al., 2018; Ji et al., 2015). The protocol used herein is based on our previous benchmarking studies involving our related Ru(II) and Os(II) compounds (Roque et al., 2022; Alberto et al., 2016b; Roque III et al., 2020; Roque J. A. et al., 2020) as well as other metal complexes (Alberto and Adamo, 2017; Alberto et al., 2020). Detailed computational methods are available in Supplementary Material.

2.7 Cellular assays

General dose-response screens were performed on 96-well and 384-well plates for bacteria and cancer cells respectively. Regardless of well density, plates were only stacked up to 2-plates high in an incubator to facilitate rapid temperature equilibrium. Photocytotoxicity and cytotoxicity assays using various bacterial strains and SK-MEL-28 human melanoma cells were conducted following previously described procedures (Roque et al., 2022; Cole et al., 2022; Talgatov et al., 2026). For mammalian cells standard aseptic technique was employed in the absence of antibiotics. Controls included cells exposed to the light treatment in the absence of **Ru-*n*T**, and the light parameters employed were previously determined not to induce measurable temperature increases or thermal cytotoxicity under these conditions.

Compound stock solutions for cellular assays were prepared at 5 mM in H₂O containing 10% DMSO and stored at −20 °C. Stock solutions were serially diluted with autoclaved Milli-Q water (18.2 MΩ cm) for bacterial assays and 1x Dulbecco's Phosphate-Buffered Saline without Ca²⁺ and Mg²⁺ for cancer cell assays. The compounds were tested at final concentrations ranging from 1 × 10^{−3} to 300 μM, with the exception that **Ru-4T** was tested at additional lower concentrations (1 × 10^{−4} to 1 × 10^{−12} μM) in

cancer cells. The final DMSO concentration in assay wells was <1% for the highest test concentration tested (300 μM) in both bacteria and cancer cell assays.

2.7.1 Cell culture

Cancer cells. Photocytotoxicity and cytotoxicity assays against SK-MEL-28 human melanoma cells were performed in 384-well plates under both normoxia and hypoxia (1% O₂) and assessed using the resazurin-based metabolic assay according to previously reported protocols (Roque et al., 2022; Cole et al., 2022). For light treatment, plates were either maintained in the dark or exposed to irradiation using broadband visible light (400–700 nm), green light (523 nm), or red light (633 nm) at a radiant exposure of 100 J cm^{−2} and irradiance of 18–22 mW cm^{−2}. EC₅₀ values were determined by nonlinear regression of dose–response curves, and phototherapeutic indices (PI) were calculated as EC₅₀ (dark)/EC₅₀ (light).

Bacteria. Photocytotoxicity and cytotoxicity assays against *S. aureus* (strain Newman D2C), *E. faecalis* (strain ATCC 29212) (Gram-positive), *E. coli* (strain W3110, Genetic Stock Center (Yale)), and *A. baumannii* (strain ATCC 17978) (Gram-negative) were performed following earlier established protocols (Talgatov et al., 2026). For light treatment, plates were either maintained in dark or exposed to irradiation using broadband visible light (400–700 nm) at a radiant exposure of 100 J cm^{−2} and irradiance of 22–24 mW cm^{−2}. Detailed experimental conditions are provided in the Supplementary Material.

3 Results and discussion

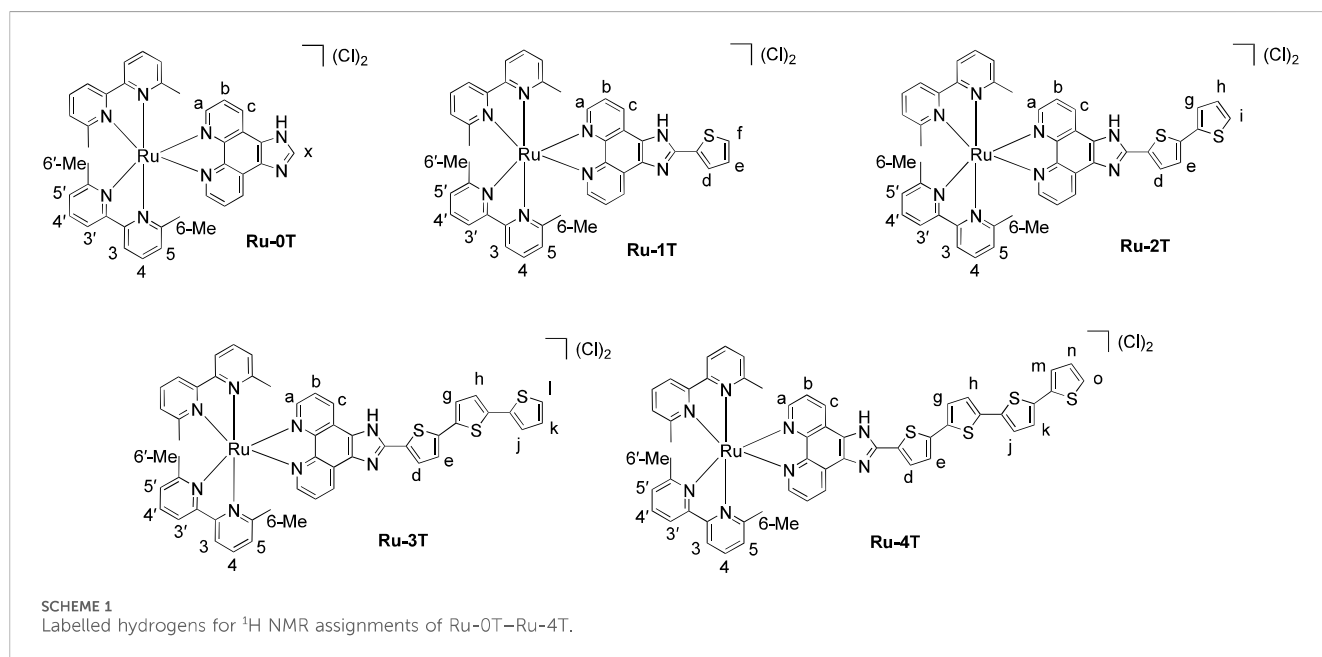
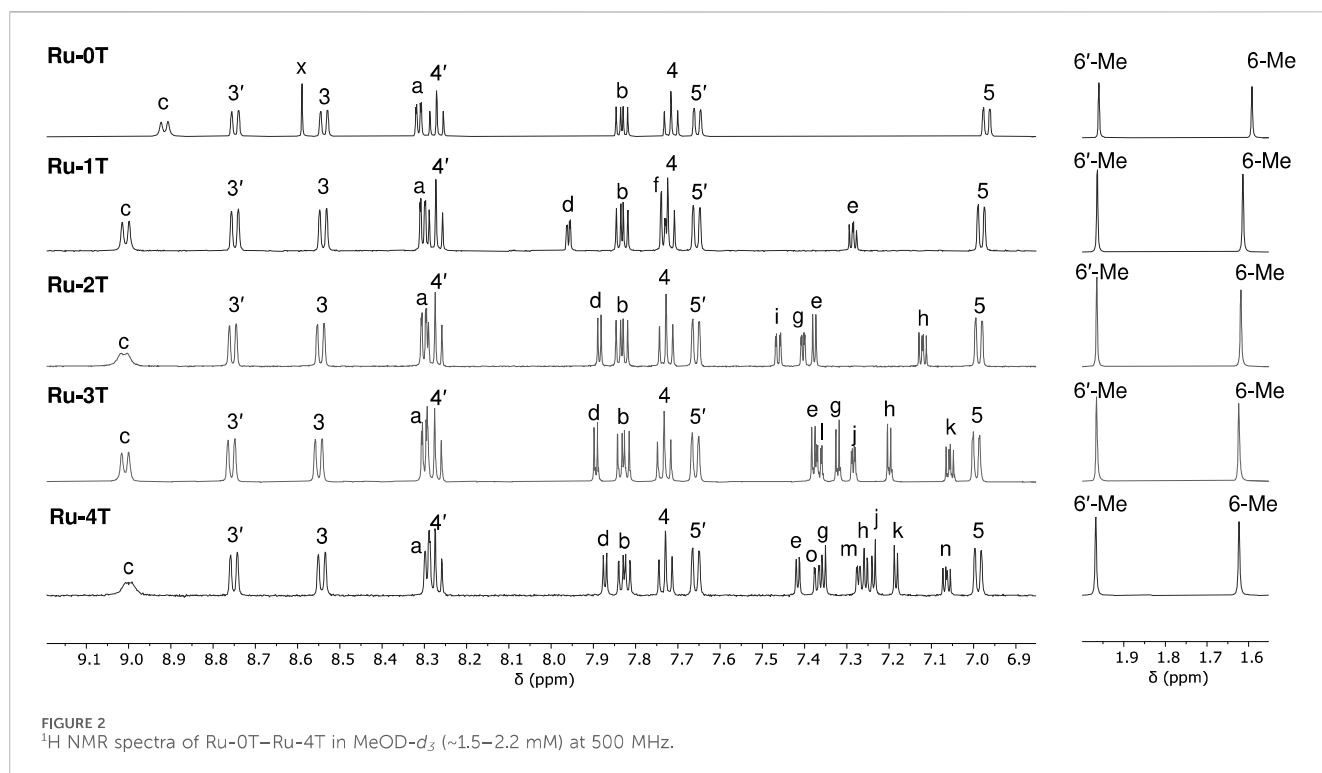
3.1 Synthesis and characterization

The synthesis of **Ru-0T** was carried out using the same general approach reported previously for the oligothiophene-extended complexes **Ru-1T** through **Ru-3T** (Sainuddin et al., 2016) and for **Ru-4T** (Cole et al., 2022). As the synthetic methodologies for these systems have been thoroughly described in those studies, they are not repeated here. In brief, coordination of the IP-*n*T ligand to the Ru(6,6'-dmb)₂Cl₂ precursor under microwave irradiation in ethylene glycol provides efficient access to the Ru(6,6'-dmb)₂(IP-*n*T) platform across the entire series.

Ru-0T, a reference complex which lacks an oligothiophene extension, was prepared analogously and isolated as its PF₆[−] salt before conversion to the corresponding Cl[−] salt to enable direct comparison with the previously reported complexes.

Because **Ru-1T** through **Ru-4T** have already been fully characterized, only data relevant to the structural trends within the series are discussed here.

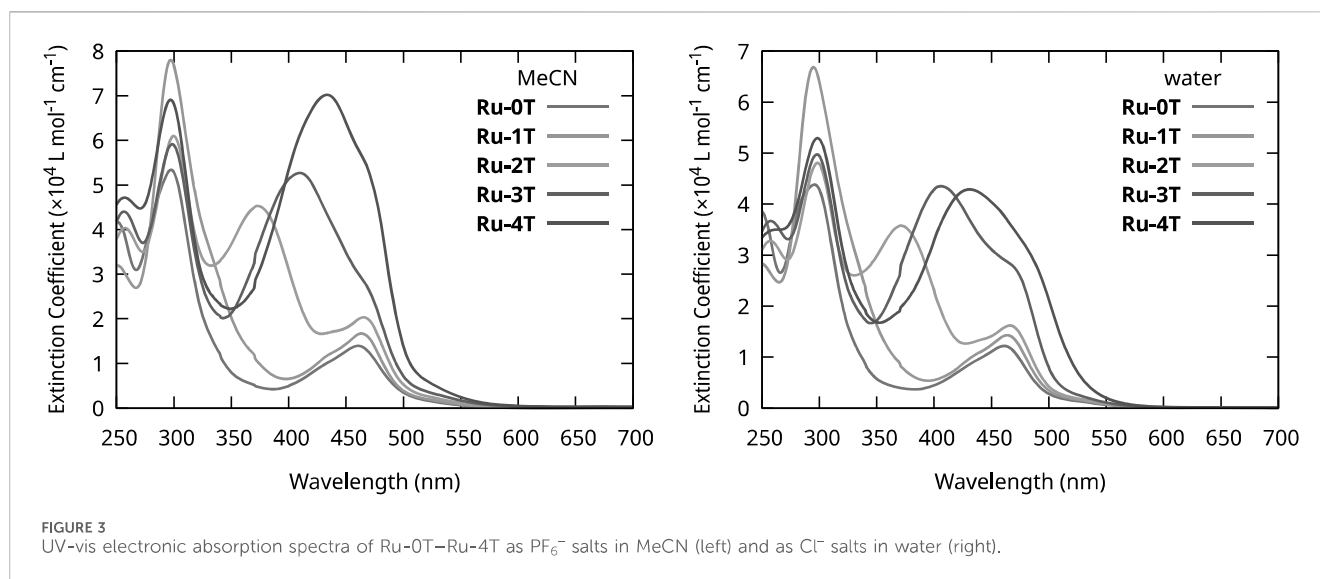
The ¹H NMR spectra of the series are shown in Figure 2 and the hydrogen labelling is depicted in Scheme 1. Consistent with the coordination-induced trends described by Pazderski for homoleptic Ru(II) polypyridyl complexes (Pazderski et al., 2010), each 6,6'-dmb ligand in **Ru-0T–Ru-4T** exhibits the characteristic intra-ring chemical shift ordering H3 (doublet) > H4 (triplet) > H5 (doublet), reflecting progressively weaker deshielding as electron-density perturbations diminish along the C3–C4–C5 bonding



positions after Ru–N coordination. However, the two 6,6'-dmb rings in these heteroleptic complexes are not magnetically equivalent. The resonance that shifts systematically with oligothiophene length is assigned to the 6-Me group on the 6,6'-dmb ring oriented toward the IP-*n*T ligand. This ring resides beneath the shielding influence of the extended IP–thiophene π -system, and its aromatic protons (3, 4, 5) correspondingly appear upfield relative to their counterparts on the other 6,6'-dmb ligand. The latter ring, identified by its more static 6'-Me resonance,

occupies the dmb–dmb cleft where anisotropic effects from the neighboring aryl ring generate greater deshielding, producing a consistently downfield pattern for the primed positions (3', 4', 5'). This assignment rationalizes both the relative positioning of methyl resonances across the series and the systematic chemical-shift differences observed between the two 6,6'-dmb ligands, while preserving the intrinsic H3 > H4 > H5 ordering within each ring.

With the coligand environments fully assigned, analysis of the IP-*n*T ligand was based on past assignments in analogous complexes



(Roque et al., 2022; Cole et al., 2022; Roque III. et al., 2020) and reveals a complementary but distinct set of chemical-shift trends arising from the imidazole core and the progressively extended thiophene chain. The *c* proton of the IP core is readily identified as the broadest and most downfield resonance, consistent with its proximity to the imidazole nitrogen. COSY correlations from *c* reveal the adjacent protons *a* and *b*, with *a* appearing farther downfield than *b* and exhibiting a simpler doublet-type pattern, while *b* appears as the characteristic doublet of doublets and is the most upfield of the three. This ordering (*c* > *a* > *b*) is fully consistent with that observed in our prior systems with other coligands, where proton *x* of the unsubstituted imidazo group appears as an isolated singlet in the case of **Ru-0T** (Supplementary Figure S1).

In the **Ru-*n*T** complexes, *d* remains the farthest downfield resonance within the extended *n*T-system, reflecting its proximity to the imidazole and provides a convenient anchor for assigning the rest of the protons. Its COSY cross-peak identifies *e*, which initiates the thiophene spin system. For all **Ru-*n*T** complexes, the most upfield proton in the *n*T spin system is the β-position of the terminal thienyl group which consistently appears as a doublet of doublets (*e* in **Ru-1T**; *h* in **Ru-2T**; *k* in **Ru-3T**; and *n* in **Ru-4T**). Each successive thienyl addition marks the disappearance of the terminal α-position proton and the appearance of three new resonances that typically appear below 7.5 ppm. These assignments are supported by past HSQC studies (Roque III. et al., 2020).

The identities and purities of **Ru-1T** through **Ru-4T** have been reported previously (Sainuddin et al., 2016; Cole et al., 2022). The estimated purity of **Ru-0T**, first disclosed in this study, was >97% based on HPLC analysis (Supplementary Figure S2). High-resolution ESI⁺ mass spectrometry (HRMS) showed two characteristic isotopic envelopes corresponding to the [M]²⁺ species, arising from loss of the counterions, and the [M-H]⁺ ion generated by deprotonation of the imidazole ring (Supplementary Figure S3).

3.2 Photophysical properties

Steady-state absorption and emission. The UV-visible absorption spectra **Ru-0T** through **Ru-4T** exhibit the characteristic features of

Ru(II) polypyridyl complexes, (Juris et al., 1988), sharing close similarity to related systems we have reported previously (Sainuddin et al., 2016; Ghosh et al., 2018; Roque III. et al., 2020; Roque J. A. et al., 2020; Roque et al., 2020c; Monro et al., 2019; McCain et al., 2019). As in other members of this structural class, three major types of electronic transitions are observed (Figure 3; Supplementary Figure S13, Table 1), and these features are largely invariant to solvent or counterion.

The intense bands at or below 300 nm correspond to $\pi \rightarrow \pi^*$ (¹IL) transitions localized to the 6,6'-dmb ligands and the proximal portion of the IP/IP-*n*T framework. The broader, lower-intensity bands with maxima near 465 nm are assigned to Ru²⁺(d π) → LL (π^*) (¹MLCT) transitions, consistent with well-established behavior of Ru(II) polypyridyl chromophores (Juris et al., 1988). Related complexes such as [Ru(6,6'-dmb)₃]²⁺ in MeOH (Fabian et al., 1980) and [Ru(phen)₃]²⁺ in MeCN (Juris et al., 1988; Stark et al., 2019) exhibit analogous bands near 445 nm, supporting this assignment. Although slightly red-shifted in the present series, the spectral shape and position of these ¹MLCT features remain essentially unchanged from **Ru-0T** through **Ru-4T**. For **Ru-3T** and **Ru-4T**, however, these MLCT absorptions become increasingly obscured by the *n*T-based transitions, which shift to longer wavelengths with increasing chain length and ultimately overlap with the ¹MLCT band. This overlap is further supported by TDDFT calculations and excited state configurations analysis (vide infra). The onset of solvatochromic behavior provides evidence of increasing charge-transfer character associated with the *n*T-centered IL/ILCT manifold, while the underlying MLCT transitions remain comparatively insensitive to thiophene chain extension and retain accepting orbitals localized primarily on the 6,6'-dmb ligands or the phen/IP portion of the coordinated scaffold. This trend is consistent with prior reports on *n*T-extended Ru(II) systems (Cole et al., 2023; Roque et al., 2022; Cole et al., 2024; Chettri et al., 2021a; Chettri et al., 2021b).

Examining this *n*T-centered IL/ILCT manifold in more detail, the thiophene-based $\pi \rightarrow \pi^*$ transitions evolve dramatically with chain length. A weak shoulder near 340 nm in **Ru-1T** grows into a well-defined and increasingly intense absorption, culminating in a

TABLE 1 UV–vis absorption peak maxima and molar extinction coefficients for Ru-0T–Ru-4T in MeCN and water. * = shoulder.

cmpd	$\lambda_{\text{max}}/\text{nm}$ ($\log \epsilon/M^{-1}\text{cm}^{-1}$)	
	MeCN	Water
Ru-0T	251 (4.62), 298 (4.73), 461 (4.14)	250 (4.59), 295 (4.64), 461 (4.09)
Ru-1T	250 (4.51), 297 (4.89), 463 (4.22)	250 (4.45), 295 (4.83), 464 (4.15)
Ru-2T	257 (4.61), 299 (4.78), 373 (4.66), 465 (4.31)	257 (4.52), 298 (4.68), 371 (4.55), 466 (4.21)
Ru-3T	256 (4.64), 298 (4.77), 410 (4.72), 466* (4.45)	258 (4.56), 298 (4.70), 406 (4.64), 470* (4.44)
Ru-4T	257 (4.67), 297 (4.84), 434 (4.85), 468* (4.74)	259 (4.54), 298 (4.72), 430 (4.63), 478* (4.51)

TABLE 2 Transient absorption lifetimes, singlet oxygen quantum yields, and photosubstitution quantum yields for Ru-0T–Ru-4T.^a

cmpd	$\tau_{\text{TA}}/\mu\text{s}^b$	Φ_{Δ} ($\lambda_{\text{ex}}/\text{nm}$) ^c	Φ_{PS} (5s) ^d
Ru-0T	—	0.03 (420)	0.0290
Ru-1T	—	0.03 (405, 460)	0.0266
Ru-2T	0.24, 1.2	0.09 (395), 0.34 (465)	0.0291
Ru-3T	4.7, 34	0.40 (415), 0.42 (466)	0.0162
Ru-4T	21	0.43 (460)	0.0047

^aExcitation wavelengths (nm) for $^1\text{O}_2$ emission are indicated in parentheses. Photosubstitution quantum yields were calculated from UV-vis absorption changes over the first 5 s of irradiation with broadband visible light (400–700 nm, 20 mW cm⁻²). A dash indicates a process too rapid to be detected on this instrument (<1 ns). ^bPF₆⁻ salts in MeCN, deaerated by five freeze-pump-thaw cycles. ^cPF₆⁻ salts in aerated MeCN. ^dCl⁻ salts in aerated water.

strong peak at ~434 nm for **Ru-4T**. These transitions become progressively more bathochromic and exhibit larger molar absorptivities with each added thiophene ring, consistent with enhanced conjugation along the oligothiophene chain. For **Ru-0T–Ru-2T**, these ^1IL transitions show little dependence on solvent or counterion; however, for $n > 2$ they display clear solvatochromic behavior, indicating increased charge-transfer character.

In the case of **Ru-3T** and especially **Ru-4T**, these low-energy $n\text{T}$ -based absorptions are best described as $^1\text{ILCT}$ transitions, in agreement with earlier observations for analogous thiophene-extended Ru(II) and Os(II) complexes with differing coligands (Roque et al., 2022; Roque III. et al., 2020; Roque J. A. et al., 2020). These assignments also align with the singlet–singlet electronic configurations determined computationally (*vide infra*), which reveal increasing participation of the oligothiophene π -system in the lowest-energy absorption features.

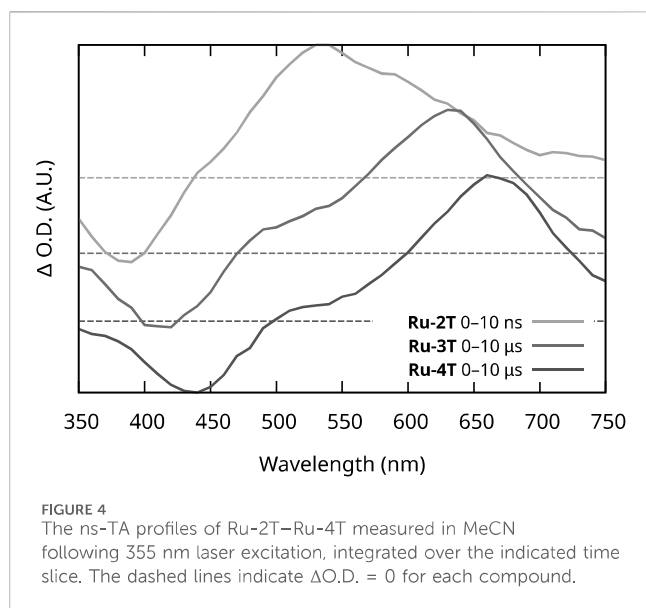
The complexes **Ru-0T** through **Ru-4T** were nonemissive at room temperature in both MeCN and water. This behavior is consistent with rapid nonradiative deactivation pathways that dominate in strained Ru(II) polypyridyl architectures, particularly when low-lying ^3MC states are energetically accessible (Sun et al., 2013). The absence of observable emission across the entire series therefore provides an early indication that the emissive $^3\text{MLCT}$, if formed, deactivates too rapidly to be observed under our experimental conditions, a conclusion reinforced by transient absorption studies discussed below.

Despite their nonemissive nature, all members of the series produced measurable near-infrared phosphorescence from $^1\text{O}_2$ centered at 1276 nm following visible-light excitation. The

corresponding $^1\text{O}_2$ quantum yields (Φ_{Δ}) are compiled in Table 2. Complexes **Ru-0T** and **Ru-1T** generated $^1\text{O}_2$ inefficiently ($\Phi_{\Delta} \approx 0.03$), and **Ru-2T** provided only modest improvement ($\Phi_{\Delta} = 0.09$ when excited at 395 nm. Notably, **Ru-2T** is the only member of the series that exhibits wavelength-dependent sensitization: when excited at 465 nm, its Φ_{Δ} increases substantially to 0.34 (Sainuddin et al., 2016), an effect not observed for the other complexes.

Ru-3T and **Ru-4T** displayed the highest $^1\text{O}_2$ sensitization efficiencies ($\Phi_{\Delta} = 40$ –43%), although these values are still lower than those reported for oligothiophene-containing complexes of comparable chain length bearing coligands that are not strain-inducing (Roque III. et al., 2020; Roque J. A. et al., 2020; Monro et al., 2019; Chettri et al., 2021a). As we have observed previously across multiple metal complex families, higher $^1\text{O}_2$ quantum yields often occur alongside longer-lived excited states (*vide infra*); complexes in which the lowest-lying triplet is of $^3\text{ILCT}$ character are especially effective in this regard.

These trends are consistent with the structural evolution of the absorption spectra (*vide supra*), wherein oligothiophene extension lowers and intensifies IL/ILCT transitions and stabilizes the corresponding triplet states. In the present series, **Ru-3T** and **Ru-4T** best satisfy this criterion, correlating with both their enhanced Φ_{Δ} values and their long-lived transient absorption signals (*vide infra*). Conversely, the poor $^1\text{O}_2$ sensitization exhibited by **Ru-0T** and **Ru-1T** reflects rapid deactivation through energetically accessible ^3MC pathways—a conclusion supported by their inability to produce detectable TA signals on the nanosecond timescale.



Taken together, the steady-state photophysical data indicate that oligothiophene extension drives a transition from MLCT/MC-dominated deactivation (**Ru-0T–Ru-2T**) to IL/ILCT-dominated photophysics (**Ru-3T–Ru-4T**), providing the first experimental signature of the excited-state reorganization elucidated in the time-resolved and computational studies. To investigate these dynamics directly, we next performed nanosecond transient absorption (ns-TA) measurements on the series. The resulting kinetic and spectral features reveal a clear divide between short-lived MLCT/MC-dominated excited states and long-lived IL/ILCT triplets, thereby establishing the mechanistic basis for the photophysical and photochemical trends observed.

Nanosecond (ns) TA spectroscopy. The ns-TA measurements revealed pronounced differences in excited-state behavior across the series. No transient features were detected for **Ru-0T** or **Ru-1T** under our experimental conditions. The absence of characteristic $^3\text{MLCT}$ signatures indicates that any initially populated excited state decays too rapidly to be observed on the nanosecond timescale, and the lack of any ^3IL signals is consistent with complexes in which the lowest ligand-centered triplet states lie well above 3.5 eV and in which energetically accessible ^3MC pathways enable ultrafast nonradiative deactivation for both **Ru-0T** or **Ru-1T**.

In contrast, **Ru-2T** through **Ru-4T** exhibited strong and well-defined ns-TA signals (Figure 4; Supplementary Figure S14), with lifetimes summarized in Table 2. Their spectral profiles consist of a ground-state bleach at shorter wavelengths, arising from depletion of MLCT and IL/ILCT absorption bands (Figure 3), together with an intense excited-state absorption (ESA) at longer wavelengths. This ESA feature is characteristic of ligand-centered $^3\text{IL}/^3\text{ILCT}$ excited states in Ru and Os oligothiophene complexes (Roque et al., 2022; Roque III. et al., 2020; Roque J. A. et al., 2020; Lifshits et al., 2020). The ns-TA spectra of **Ru-2T–Ru-4T** closely resemble those of the corresponding uncomplexed IP-*n*T ligands (Roque III. et al., 2020), reinforcing the conclusion that the long-lived excited states probed by ns-TA are localized predominantly on the oligothiophene fragments.

Consistent with increasing π -conjugation, the ESA maxima red-shift systematically from **Ru-2T** to **Ru-4T**, reflecting progressive stabilization of the ligand-centered triplet manifold. Kinetic analysis revealed a monoexponential decay for **Ru-4T** ($\tau = 21 \mu\text{s}$), whereas **Ru-2T** and **Ru-3T** displayed biexponential kinetics ($\tau_1 = 0.24 \mu\text{s}$ and $\tau_2 = 1.2 \mu\text{s}$ for **Ru-2T**; $\tau_1 = 4.7 \mu\text{s}$ and $\tau_2 = 34 \mu\text{s}$ for **Ru-3T**). These differences reflect variations in the relative energies and population pathways of the triplet manifold.

Femtosecond (fs) TA spectroscopy. The earlier excited state dynamics of **Ru-0T–Ru-4T** were examined with fs-TA measurements, and the resulting spectra were compared to the ns-TA profiles (Figure 5). In the fs-TA datasets, the trace labeled “inf” is the transient from the ns-TA experiment, enabling direct comparison between ultrafast and microsecond timescales.

The fs-TA spectrum of **Ru-0T** exhibits a bleach near 450 nm corresponding to depletion of the $^1\text{MLCT}$ absorption, together with a broad, weak excited-state absorption extending from ~ 500 nm into the near-infrared. Analogous features in classical Ru(II) polypyridyl complexes such as $[\text{Ru}(\text{bpy})_3]^{2+}$ ($\text{bpy} = 2,2'$ -bipyridine) arise from ligand-localized transitions of the reduced $\text{bpy}^\cdot$ chromophore in the $^3\text{MLCT}$ state (McCusker, 2003; Damrauer et al., 1997). Ultrafast $^3\text{MLCT} \rightarrow ^3\text{MC}$ conversion has been characterized previously in strained Ru(II) bis- and tris-diimine complexes. In particular, Sun et al. used fs-TA to show that the homoleptic complex $[\text{Ru}(\text{m-bpy})_3]^{2+}$ ($\text{m-bpy} = 6\text{-methyl-}2,2'\text{-bipyridine}$), a close structural analog of $[\text{Ru}(6,6'\text{-dmb})_3]^{2+}$, undergoes rapid depopulation of the $^3\text{MLCT}$ state ($\tau = 1.6$ ps) followed by decay of the resulting ^3MC state with $\tau = 450$ ps (Sun et al., 2013). These features arise from ultrafast internal conversion to form a distorted ^3MC state. **Ru-0T** displays closely related behavior, whereby the $^3\text{MLCT}$ component decays with $\tau = 3.9$ ps, and the subsequently formed ^3MC state decays with $\tau = 83$ ps (Figure 5). Both processes occur entirely before the nanosecond timescale, consistent with the absence of any ns-TA signature, and mirror the behavior of **Ru-1T** and the reference compound $[\text{Ru}(6,6'\text{-dmb})_2(\text{bpy})]^{2+}$ that lacks an IP-based ligand.

In sharp contrast, the ultrafast dynamics of **Ru-2T–Ru-4T** show no evidence of involvement of a short-lived ^3MC state. Instead, all three complexes undergo extremely rapid evolution to form ligand-centered triplet states that persist into the microsecond regime. The growth of the long-wavelength ESA—characteristic of *n*T-based $^3\text{IL}/^3\text{ILCT}$ states—occurs on sub-picosecond timescales, with rise components of approximately 0.27 ps for **Ru-2T**, 0.42 ps for **Ru-3T**, and 0.53 ps for **Ru-4T**. By ~ 20 ps, the transient spectra of all three complexes are already almost indistinguishable from the ns-TA profiles (“inf”) (Figure 4). These observations demonstrate that, for $n \geq 2$, population of the ILCT-localized T_1 state occurs with high efficiency on the sub-picosecond timescale, and that branching into a ^3MC pathway is not evident under our experimental conditions.

Although the ILCT rise time increases modestly from **Ru-2T** to **Ru-4T**, all three complexes still form the ILCT-localized triplet on a sub-picosecond timescale. By analogy with the previously studied TLD1433 series (Chettri et al., 2021b), in which distinct picosecond components were assigned to vibrationally hot states and decay from a higher-lying, conformationally distorted $^3\text{ILCT}$ state, it is plausible that vibrational and conformational relaxation of the increasingly flexible oligothiophene substituent contributes to the early-time spectral evolution in **Ru-2T–Ru-4T**. However, our present data do not allow us to isolate a distinct planarization timescale, and any

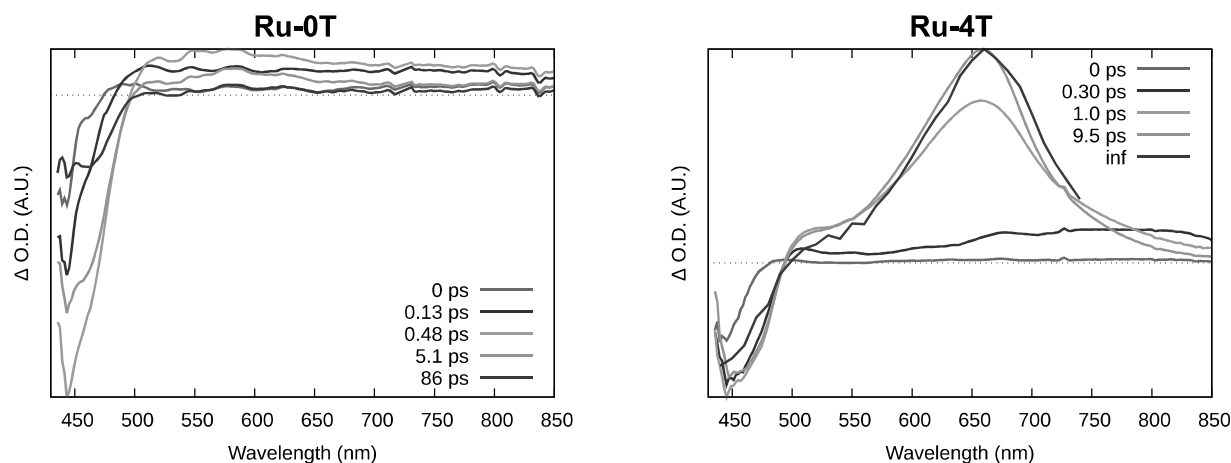


FIGURE 5

The fs-TA profiles of Ru-0T (left) versus Ru-4T (right) measured in MeCN following 343 nm laser excitation. The dashed lines indicate $\Delta O.D. = 0$ for each compound, and "inf" indicates the corresponding ns-TA spectrum.

such motion appears to be subsumed within the overall ultrafast relaxation into the ILCT-based triplet manifold.

Together, the fs-TA and ns-TA results establish a clear division between MLCT/MC-dominated dynamics at short oligothiophene chain lengths and ILCT-dominated excited-state behavior for $n \geq 2$. What remains unresolved from experiment alone is why these regimes emerge so sharply with increasing thiophene extension and how the underlying excited-state surfaces mediate the observed differences in lifetime, 1O_2 sensitization, and photochemical reactivity. To address these questions, we next turn to computational, which quantify how oligothiophene extension reorganizes the Frontier-orbital manifold, stabilizes ILCT configurations, and modulates access to dissociative 3MC pathways.

3.3 Computational studies

Ground and singlet states. Computational details are discussed below, with more details in Supplementary Tables S2-6 and Supplementary Figures S5-12 of the Supplementary Material. The optimized ground-state (S_0) structures of **Ru-0T** through **Ru-4T** (Figure 6) show that all complexes retain a pseudo-octahedral geometry in water, with the IP- nT ligand bound in a bidentate fashion and the (6,6'-dmb) coligands occupying the remaining coordination sites. The thienyl rings adopt a nearly coplanar trans arrangement at short chain lengths, but increased conformational flexibility emerges beginning at $n = 2$. Consistent with earlier Ru- and Os-based oligothiophene studies (Cole et al., 2023; Roque et al., 2022; Cole et al., 2024; Roque III. et al., 2020; Roque J. A. et al., 2020), the distal thiophene rings in **Ru-4T** exhibit an $\sim 18^\circ$ torsional twist relative to the IP core (Supplementary Table S2). Despite this increase in local flexibility, the Ru coordination sphere remains largely intact across the series, with only **Ru-0T** and **Ru-1T** showing slight axial Ru-N elongations that presage their tendency to access 3MC states in the excited-state manifold (*vide infra*).

A systematic restructuring of the frontier orbital manifold accompanies extension of the oligothiophene chain. The HOMO

becomes progressively destabilized and increasingly ligand-based with nT extension, evolving from metal-centered in **Ru-0T** and **Ru-1T** to predominantly oligothiophene-centered for **Ru-2T–Ru-4T**. Quantitatively, the thiophene contribution to the HOMO increases from 0% (**Ru-0T**) to 33% (**Ru-1T**), 61% (**Ru-2T**), and 71% (**Ru-4T**), while the Ru d -orbital contribution diminishes nearly to zero across the same progression (Figure 7, Supplementary Figure S5, Supplementary Table S3).

In contrast, the LUMO remains largely localized on the 6,6'-dmb ligands for all complexes, and its energy changes minimally along the series. As a result, the HOMO–LUMO gap decreases monotonically from 3.61 eV (**Ru-0T**) to 2.93 eV (**Ru-4T**) (Supplementary Figure S6, Supplementary Table S4), fully consistent with the systematic red shifts observed experimentally in the low-energy absorption bands. This frontier-orbital reorganization establishes the electronic foundation for the MLCT→ILCT evolution that emerges upon thiophene extension—a key mechanistic feature observed experimentally and theoretically throughout this work.

Time-dependent DFT simulations reproduce the major features of the experimental UV–vis spectra and capture the evolution from MLCT-dominated absorption at short chain lengths to ILCT/LLCT-dominated absorption for $n \geq 3$ (Figure 8, Supplementary Figures S7, 8, Supplementary Table S5). For **Ru-0T** and **Ru-1T**, the lowest-energy spin-allowed transitions possess clear 1MLCT character, as it is clearly revealed by the topology analysis reported in Figure 8 and also observed in the involved NTOs in Supplementary Figure S8. **Ru-2T** exhibits mixed $^1MLCT/{}^1LLCT$, reflecting the rising involvement of the thiophene π -system in the donor orbital, whereby the occupied NTOs retain substantial Ru and 6,6'-dmb contributions with only partial thiophene involvement, while the corresponding virtual NTOs reside predominantly on the 6,6'-dmb-based acceptor orbitals. A higher-lying transition begins to show emerging $^1LLCT/{}^1ILCT$ character, but the charge redistribution remains more localized within the IP-2T fragment and only modestly engages the 6,6'-dmb ligands.

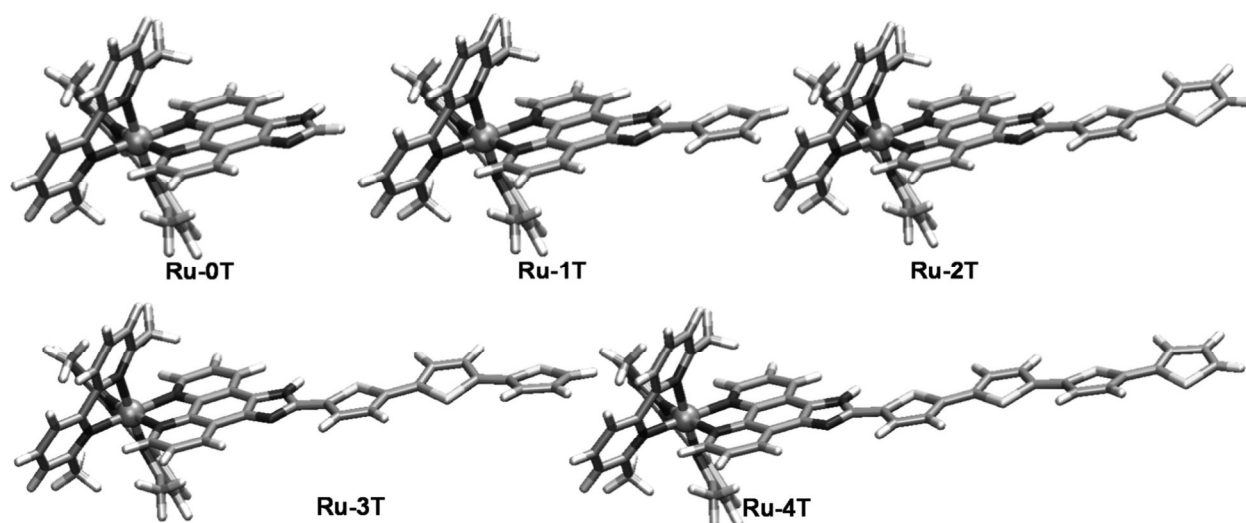


FIGURE 6
Optimized geometries of Ru- n T in a water environment at the PBE0/6-31+G (d,p)/SDD/level of theory.

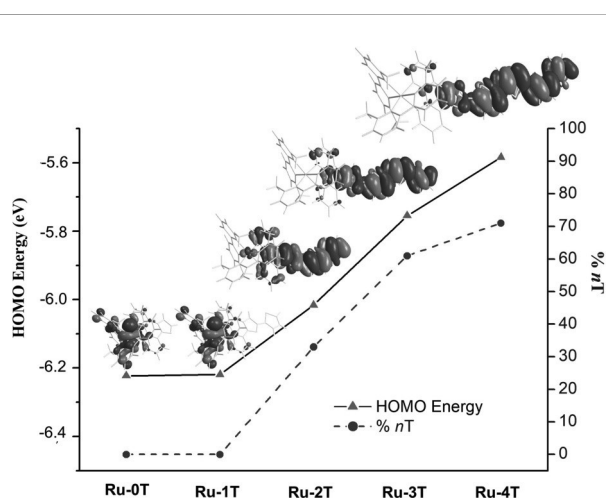


FIGURE 7
Calculated HOMO orbital energies (eV) (solid line) and percent contribution of the thienyl chain to the HOMO Frontier orbital (dashed line), for the compounds Ru- n T in the singlet ground state 1GS , at the M06/6-31+G (d,p)/SDD level of theory, in water. Plot of Ru(II)-based HOMO for Ru-0T, Ru-1T and thienyl-based HOMOs for n T with n = two to four, obtained at the same level of theory, are also reported in the picture. More details can be found in the SI section.

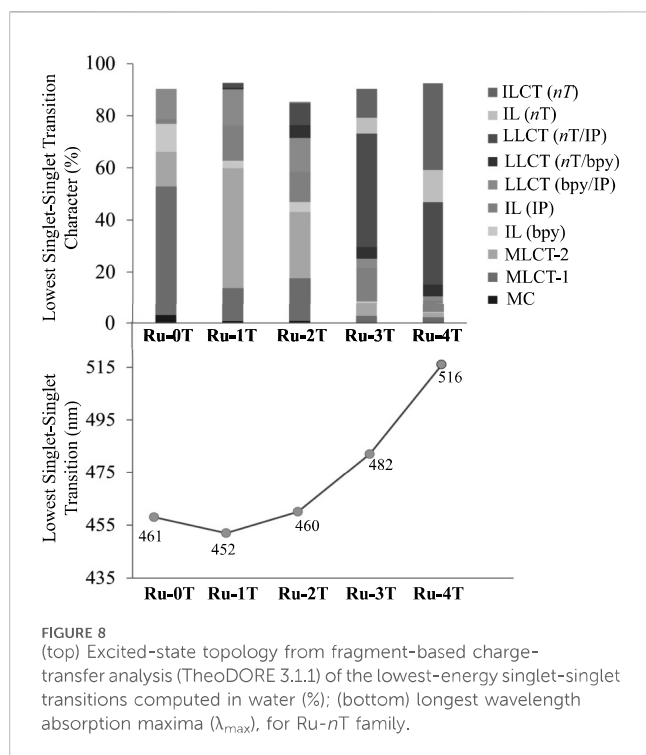
By contrast, in **Ru-3T** and **Ru-4T** the donor orbitals become strongly oligothiophene-centered, and the virtual NTOs are delocalized across the n T chain, the IP core, and even portions of the 6,6'-dmb ligands. This extended acceptor distribution, together with substantially larger oscillator strengths, defines the lowest-energy transitions as genuinely mixed $^1ILCT/^1LLCT$ in nature and marks the full emergence of ILCT character in the longer oligothiophene members of the series.

Together, these results refine the initial experimental UV-vis assignments. The lowest-energy bands for **Ru-0T** and **Ru-1T** remain unambiguously 1MLCT in origin, whereas **Ru-2T** is now understood to occupy a transitional regime: although its lowest-energy

absorptions were experimentally assigned as MLCT-derived, the topology (Figure 8) and NTO (Supplementary Figure S8) analyses reveal LLCT contributions with modest participation of the n T system, consistent with its modest bathochromic shift and intermediate intensity. For **Ru-3T** and **Ru-4T**, the computational analysis confirms that the intense, red-shifted absorptions arise from strongly allowed ILCT/LLCT transitions with donor density localized on the extended thiophene chain and acceptor density delocalized over IP and 6,6'-dmb in the case of the LLCT component (ILCT involves donor and acceptor within n T). Furthermore, the weak shoulder near 470–500 nm observed experimentally for **Ru-3T** and **Ru-4T** corresponds to a residual weak MLCT transition that persists across the series but becomes spectroscopically subordinate once ILCT/LLCT character is involved. Thus, the computational results both validate and sharpen the experimental interpretation, revealing a clear electronic crossover between $n = 2$ and $n = 3$.

Triplet states. Optimization of the lowest triplet states (T_1) for **Ru-0T** through **Ru-4T** reveals systematic structural and electronic differences that correlate with the experimentally observed photophysical regimes. In all complexes, the thienyl groups become more planar relative to the IP core upon triplet formation (Supplementary Figure S9), reflecting the well-known tendency of oligothiophenes to enhance π -conjugation in their lowest triplet manifolds (Lanzani et al., 1996; Yang et al., 2000). The Ru coordination environment remains largely intact for $n \geq 2$, whereas **Ru-0T** and **Ru-1T** exhibit pronounced lengthening of specific Ru–N bonds in T_1 (up to 2.48 Å), a signature of 3MC character and consistent with their ultrafast nonradiative decay observed experimentally.

The nature of the T_1 state evolves sharply across the series (Figure 9, Table 3, Supplementary Figure S11). For **Ru-0T** and **Ru-1T**, the occupied and virtual NTOs, together with the topology analysis, indicate dominant MLCT character. The corresponding Mulliken spin densities (MSDs) of ~ 1.5 on Ru further support significant metal involvement, consistent with a contribution from the 3MC configuration. This 3MC topology is predisposed



to rapid structural distortion and tends to undergo photodissociation, accounting for the lack of detectable $^3\text{MLCT}$ signatures in the ns-TA spectra.

In **Ru-2T**, the T_1 state transitions to a predominantly ligand-centered configuration with strong $^3\text{ILCT}/^3\text{IL}$ character. The metal

contribution vanishes ($\text{MSD} = 0$), and the excited-state spin density extends onto the *n*T and IP ligand framework. This change becomes even more pronounced in **Ru-3T** and **Ru-4T**, where the T_1 state is fully delocalized over the extended thiophene chain. These complexes exhibit the lowest T_1 energies in the series (1.86 and 1.70 eV, respectively), in excellent agreement with their long-lived μs TA signals and efficient $^1\text{O}_2$ generation.

A notable distinction emerges when the triplet manifold (Figure 9) is considered alongside the singlet-state analysis (Figure 8). For **Ru-3T** and **Ru-4T**, the lowest-energy singlet states exhibit substantial mixed $^1\text{ILCT}/^1\text{LLCT}$ character, reflecting extensive electronic communication between the Ru center, the IP core, and the extended oligothiophene framework. In contrast, the lowest triplet states are overwhelmingly $^3\text{ILCT}$ in nature, with comparatively diminished LLCT contributions. Thus, oligothiophene extension reshapes the singlet and triplet manifolds in a spin-dependent manner, with intersystem crossing selectively funneling population into a more ligand-centered triplet minimum rather than preserving the mixed charge-transfer character of the initially accessed singlet states.

All complexes possess T_1 energies sufficiently high to sensitize singlet oxygen, requiring $\approx 0.90\text{--}1.0$ eV (computed 0.90 eV, experimental 0.98 eV) (Alberto et al., 2015; Huber and Herzberg, 1979). For **Ru-0T** and **Ru-1T**, the $^3\text{MLCT}/^3\text{MC}$ character places the T_1 state at relatively high energy (2.27 eV), yet its extremely rapid deactivation through the ^3MC channel precludes effective $^1\text{O}_2$ sensitization—fully consistent with their low Φ_Δ values and lack of long-lived TA signals.

In contrast, **Ru-3T** and **Ru-4T** populate strongly allowed $^3\text{ILCT}/^3\text{IL}$ states that do not efficiently couple to ^3MC surfaces, enabling long excited-state lifetimes (up to 34 μs for **Ru-3T** and

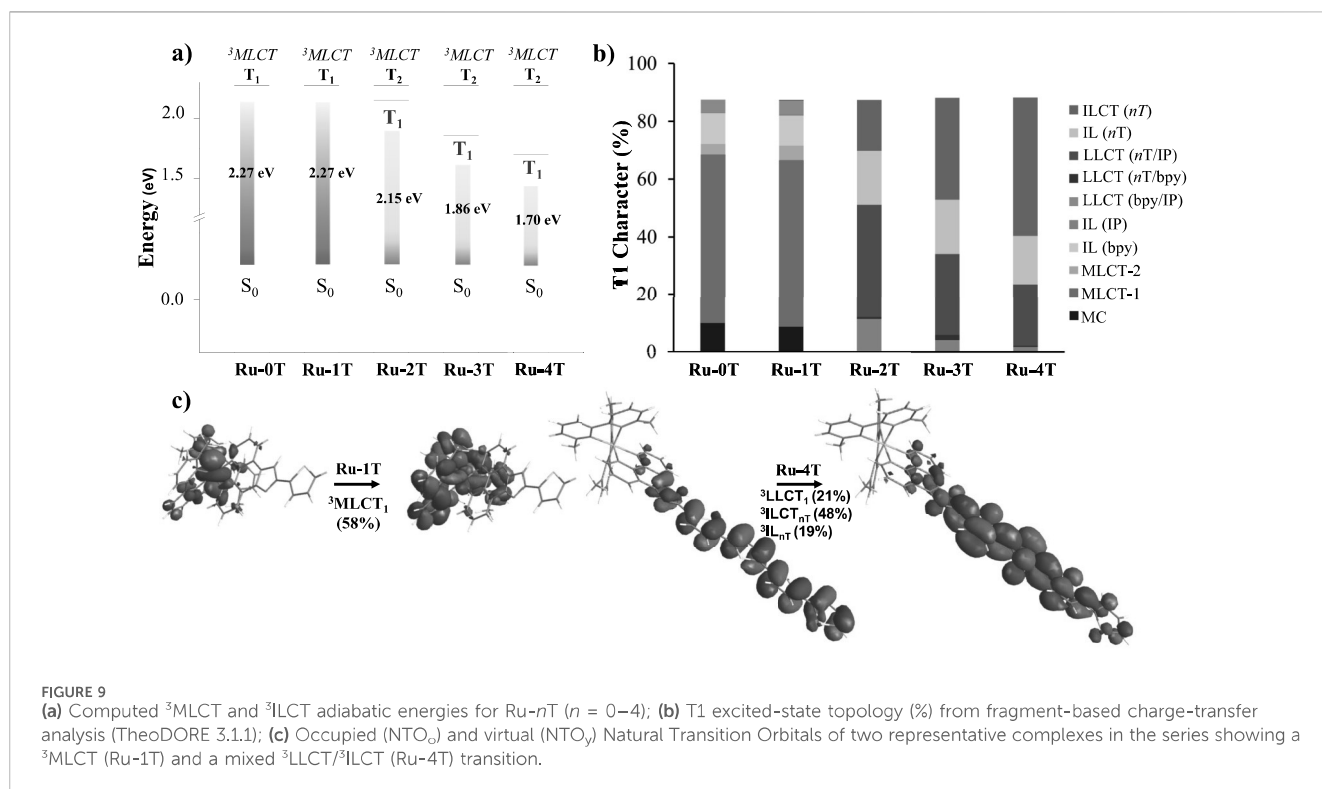
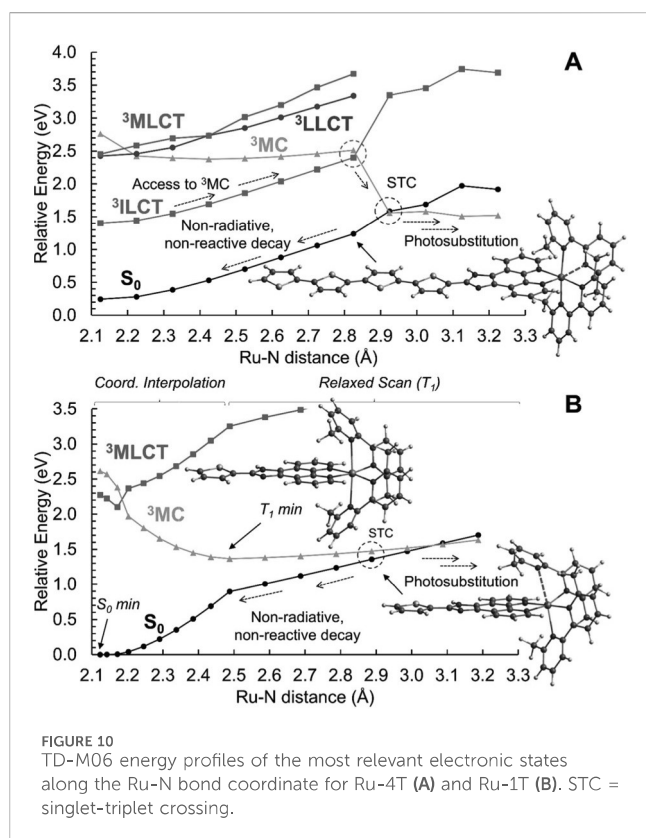


TABLE 3 Calculated T_1 parameters for Ru- n T ($n = 0-4$).

	T_1 energy (eV)	Configuration	MSD ^a	μ (D) ^b
Ru-0T	2.27	$^3\text{MLCT}$	1.53	4.83
Ru-1T	2.27	$^3\text{MLCT}$	1.53	9.01
Ru-2T	2.15	$^3\text{ILCT}/^3\text{IL}$	0	19.64
Ru-3T	1.86	$^3\text{ILCT}/^3\text{IL}$	0	30.13
Ru-4T	1.70	$^3\text{ILCT}/^3\text{IL}$	0	42.50

^aMSD, Mulliken spin density on the Ru metal center (no metal involvement was found for $n \geq 2$).

^bDipole moment (μ) of the optimized T_1 state.



21 μs for **Ru-4T**) and high Φ_{Δ} values (40–43%). The computed T_1 energies and spin densities thus provide a unifying mechanistic origin for the photophysical bifurcation across the series: thiophene extension stabilizes IL-dominated excited states that outcompete MC-mediated relaxation, enabling productive photosensitization pathways.

Notably, the calculated dipole moments (μ) of the T_1 state increase monotonically with oligothiophene chain length, rising from 4.83 D for **Ru-0T** to 42.50 D for **Ru-4T** (Table 3). While these values are not used to assign excited state character, the pronounced increase in polarization provides an independent indicator of the growing electronic asymmetry and charge separation accompanying thiophene extension, consistent with an increasing $^3\text{ILCT}$ contribution to T_1 for longer oligothiophenes.

Triplet energetic surfaces and mechanistic implications for photochemical reactivity. Triplet potential energy surfaces (PESs)

were mapped along a Ru–N stretching coordinate and are shown for **Ru-1T** and **Ru-4T** to demonstrate how chain length influences access to reactive ^3MC states (Figure 10; Supplementary Table S7). These two complexes represent limiting cases of MC-dominated (**Ru-1T**) and ILCT-dominated (**Ru-4T**) photophysics within the complexes bearing at thienyl group(s).

For **Ru-1T**, unconstrained T_1 optimization yields substantial elongation of two Ru–N bonds (>2.48 Å), placing the T_1 minimum squarely on the ^3MC surface. The PES features essentially barrierless relaxation from the Franck–Condon region to this ^3MC minimum, consistent with ultrafast quenching observed by fs- and ns-TA. Further elongation destabilizes S_0 and activates efficient STC crossings, enabling rapid (and largely nonproductive) return to the ground-state surface. The potential for photodissociative Ru–N cleavage exists but competes poorly with nonreactive decay.

By contrast, the PES for **Ru-4T** reveals that stretching an equatorial Ru–N bond destabilizes both the $^3\text{ILCT}$ and S_0 states while stabilizing the ^3MC state, leading to a $^3\text{ILCT} \rightarrow ^3\text{MC}$ crossing near 2.8 Å. However, this pathway carries an energy penalty of ~ 1 eV, making population of ^3MC significantly less competitive than internal ILCT-localized decay. This energetic barrier explains the long μs lifetimes of **Ru-3T/Ru-4T**, their efficient $^1\text{O}_2$ generation, and their reduced photosubstitution quantum yields (e.g., Φ_{PS} for **Ru-4T** = 0.47%).

A second mechanistic feature unique to **Ru-4T** is the intramolecular π -stacking that forms between pyridyl rings upon Ru–N elongation (>2.8 Å), which stabilizes the ^3MC pathway but only at geometries far removed from the Franck–Condon region. This structural motif is absent in the more rigid phenanthroline-based analogues (Roque et al., 2022), underscoring the coligand's influence on photoreactivity.

The combined triplet state energetics and PES analyses reveal how oligothiophene extension modulates access to photodissociative ^3MC pathways. For **Ru-0T** through **Ru-2T**, the T_1 surfaces lie close to the ^3MC manifold and the PES allows relatively facile population of dissociative coordinates. In **Ru-3T**, the ILCT-stabilized T_1 state still retains a thermally accessible ^3MC channel, whereas in **Ru-4T**, ^3MC formation becomes energetically penalized due to strong ILCT stabilization and the geometric constraints required to reach the ^3MC region.

These PES features also suggest the possibility of multiple population pathways in the longer oligothiophene complexes. In **Ru-4T**, for example, both an $^1\text{MLCT} \rightarrow ^3\text{MLCT} \rightarrow ^3\text{MC}$ route and an $^1\text{ILCT} \rightarrow ^3\text{ILCT}$ pathway that eventually intersects the ^3MC surface may contribute, with differing efficiencies depending on which excited state is initially accessed.

Overall, the computational results predict a shift from MLCT/MC-dominated to ILCT-dominated excited state deactivation as n increases. This reorganization of the excited-state landscape, driven by oligothiophene extension, is expected to have direct consequences for both photochemical behavior and biological response, as explored in the subsequent section.

3.4 Photochemical properties

The computational analyses predicted that access to the photodissociative ^3MC surface should vary systematically across the **Ru-0T–Ru-4T** series, with short-chain members favoring ^3MC population and longer oligothiophenes increasingly stabilizing ILCT-localized triplet states. Such differences in the excited-state landscape are expected to manifest directly in photochemical reactivity, particularly in the efficiency of photosubstitution pathways that require productive ^3MC formation. Guided by these predictions, we evaluated the photochemical behavior of **Ru-0T–Ru-4T** under continuous visible-light irradiation to quantify their photosubstitution quantum yields (Φ_{PS}) and identify any correlations with the experimentally and computationally determined excited state properties.

Complexes **Ru-0T–Ru-4T** were evaluated for their ability to undergo photosubstitution upon irradiation with visible light (400–700 nm, 20.5 mW cm^{-2}) in aqueous solution (Supplementary Table S8). Continuous illumination produced spectral changes for all members of the series that were consistent with ligand loss (Figure 11, Supplementary Figure S16, 17). HPLC analysis confirmed that the major photoproducts were the free 6,6'-dmb ligand and the corresponding aquated Ru complex, matching the photoproducts previously identified for **Ru-1T–Ru-3T** (Sainuddin et al., 2016).

Photosubstitution quantum yields (Φ_{PS}), estimated from the initial 5 s of irradiation, ranged from 2.6 to 3% for **Ru-0T–Ru-2T** and were approximately 1.6% for **Ru-3T**. These values fall within the typical range reported for distorted Ru(II) complexes (Roque et al., 2020c; Havrylyuk et al., 2018; Havrylyuk et al., 2020; Li et al., 2017; Loftus et al., 2018; Meijer and Bonnet, 2019). The trend is consistent with the computational prediction that the shorter-chain members possess ready access to photodissociative ^3MC states, whereas increasing thiophene extension progressively stabilizes ILCT triplet configurations that compete with or suppress ^3MC formation.

The behavior of **Ru-4T** was notably distinct. Its photosubstitution quantum yield was only 0.47%, substantially lower than the rest of the series. This dramatic decrease is aligned with the photophysical model established from TA spectroscopy and computation: the lowest-lying $^3\text{IL}/^3\text{ILCT}$ state in **Ru-4T** does not efficiently couple to the higher-lying $^3\text{MLCT}$ state that feeds into the ^3MC surface, and access to the ^3MC pathway becomes energetically disfavored. As a result, photodissociation is strongly suppressed.

Despite this reduced reactivity, the continuous-irradiation spectra of **Ru-4T** displayed clean isosbestic behavior, and HPLC endpoint analysis confirmed formation of a single set of photoproducts, indicating that the low Φ_{PS} value does not arise from competing photochemical channels. Instead, the biexponential photodissociation kinetics observed for **Ru-4T** suggest the involvement of two distinct excited-state population pathways—each ultimately producing the same photoproducts but with different efficiencies.

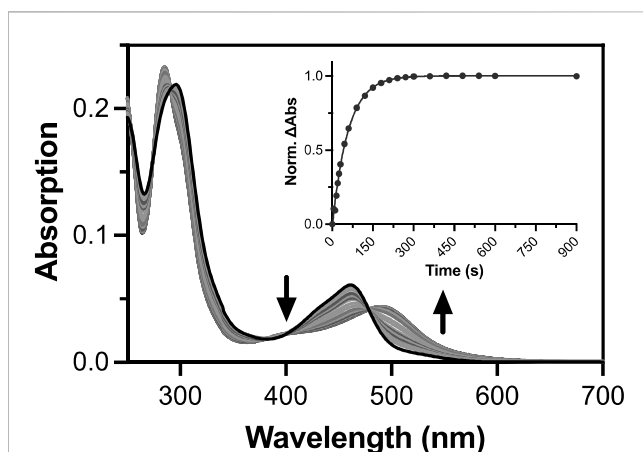


FIGURE 11
Representative changes in the absorption spectra of the complexes with light treatment shown for **Ru-0T** (10 μM in water) irradiated with visible light (400–700 nm, 20.5 mW cm^{-2}) using a 5-mm pathlength. The inset shows the change in absorbance around the isosbestic point as a function of time, where only the first 5 s was used for further analysis.

We have observed similar biexponential kinetics in related $[\text{Ru}(6,6'\text{-dmb})_2(\text{IP-R})](\text{Cl})_2$ scaffolds bearing aryl or oligothiophene substituents (Sainuddin et al., 2016; Roque et al., 2020d; Cole et al., 2021). In the present series, the most consistent mechanistic interpretation is that **Ru-4T** can reach the photoreactive ^3MC state through two distinct excited state pathways. The first is the classical dissociative channel, $^1\text{MLCT} \rightarrow ^3\text{MLCT} \rightarrow ^3\text{MC}$, which remains energetically viable for all chain lengths. The second, operative only when the ILCT manifold lies sufficiently low in energy, is an $^1\text{ILCT} \rightarrow ^3\text{ILCT} \rightarrow ^3\text{MC}$ route in which the ILCT-localized triplet crosses into the MC surface upon Ru–N elongation near $\sim 2.8 \text{ \AA}$. Computations show that this ILCT-initiated pathway carries a larger energetic penalty than the MLCT route and becomes increasingly disfavored as the oligothiophene chain is shortened, fully consistent with the higher Φ_{PS} values of **Ru-0T–Ru-3T** and the uniquely suppressed photoreactivity of **Ru-4T**.

3.5 Photobiological properties toward cancer cells

The photobiological evaluation of the **Ru- n T** series in mammalian cancer cells was designed to isolate how oligothiophene extension and coligand-induced strain reprogram light-triggered cytotoxicity under tightly controlled conditions. Accordingly, all compounds were evaluated in a single, well-characterized human cancer cell line using identical dosing, irradiation, and oxygenation protocols, allowing systematic comparisons across the series while minimizing biological variability. SK-MEL-28 melanoma cells were selected to enable direct comparison with related Ru(II) systems evaluated under matched photobiological conditions. The dark and light cytotoxicities of **Ru-0T–Ru-4T** were examined under normoxic ($\sim 18.5\text{--}21\% \text{ O}_2$) and hypoxic ($1\% \text{ O}_2$) conditions following our established photobiology protocols (Roque J. A. et al., 2020; Lifshits et al., 2020). All members of the series were highly soluble in DPBS

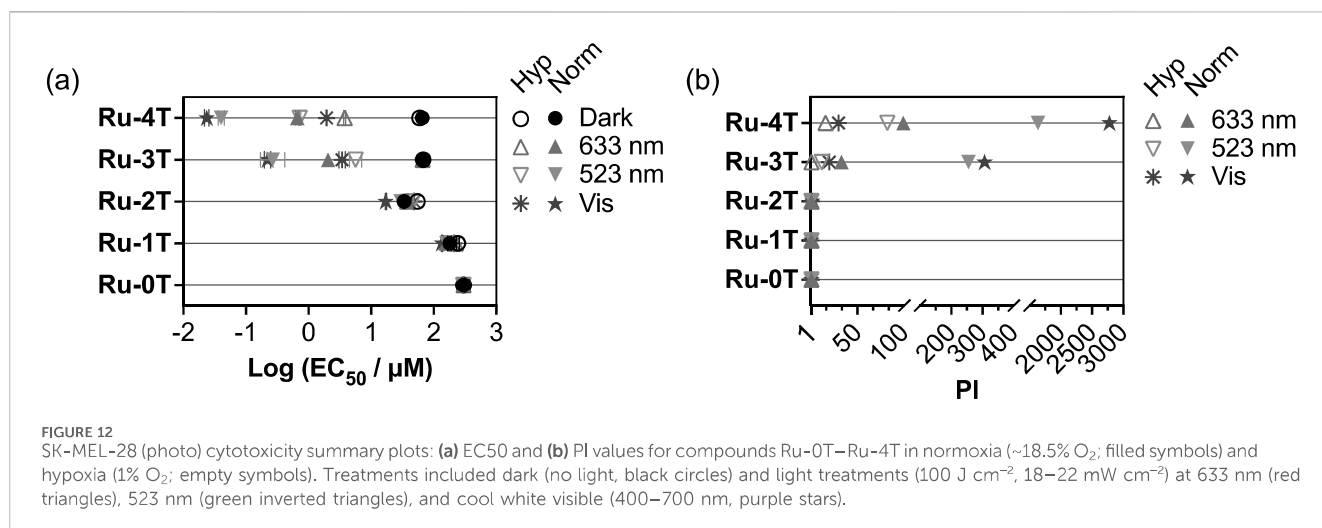


FIGURE 12

SK-MEL-28 (photo) cytotoxicity summary plots: (a) EC₅₀ and (b) PI values for compounds Ru-0T–Ru-4T in normoxia (~18.5% O₂; filled symbols) and hypoxia (1% O₂; empty symbols). Treatments included dark (no light, black circles) and light treatments (100 J cm⁻², 18–22 mW cm⁻²) at 633 nm (red triangles), 523 nm (green inverted triangles), and cool white visible (400–700 nm, purple stars).

and standard culture medium, enabling reliable determination of EC₅₀ values and phototherapeutic indices (PI = EC_{50,dark}/EC_{50,light}) across a wide concentration range (100 pM–300 μM). Hypoxia during irradiation was rigorously verified using [Ru(bpy)₂(dppn)]Cl₂, which produces a PI > 1 when trace oxygen is present. Each compound was tested in the dark and under three illumination conditions—broadband visible (400–700 nm), green (523 nm), and red (633 nm)—delivered at a standard fluence of 100 J cm⁻². Spectral output and absorbed photon flux values for each compound–light pairing are shown in Supplementary Figures S15, 18; the photobiological results are summarized in Figure 12, Supplementary Figures S19, 20 and Supplementary Tables S9–18. Because earlier work reported only normoxic photocytotoxicity for **Ru-1T–Ru-3T** and used different DLIs, light sources, and a different cell line, these compounds were re-evaluated here under matched normoxic and hypoxic conditions alongside **Ru-0T** and **Ru-4T** in SK-MEL-28 cells.

Dark cytotoxicity. Across the **Ru-0T–Ru-4T** family, dark cytotoxicity toward SK-MEL-28 cells remained low in both normoxia and hypoxia, establishing a consistent baseline from which light-driven effects could be evaluated. All complexes exhibited dark EC₅₀ values above 50 μM, with the expected exception of **Ru-2T**, which again showed modest intrinsic toxicity (EC_{50,norm} = 33.6 μM; EC_{50,hyp} = 54.9 μM). **Ru-3T** and **Ru-4T** displayed slightly lower dark EC₅₀ values (≈60–70 μM) than **Ru-0T** and **Ru-1T**, which were effectively nontoxic (EC₅₀ > 150 μM for **Ru-1T** and >300 μM for **Ru-0T**). These similarities across oxygen conditions indicate that oxygen-independent cellular interactions do not differentiate the series in the absence of light, an important point because it isolates the photocytotoxic behavior as arising from programmed excited-state differences rather than ground-state biology. This also aligns with the overarching design principle that coligand structure modulates photochemistry far more strongly than intrinsic dark activity, positioning excited-state topology—not ground-state reactivity—as the operative “mode switch” in this family.

Photocytotoxicity in normoxia. Under normoxic conditions, the photocytotoxicity profiles of **Ru-0T–Ru-4T** follow the excited state landscape shaped by oligothiophene extension. Photocytotoxicity is

observed when both the lowest-energy singlet and triplet excited states possess ILCT character, and when the resulting ³ILCT state is sufficiently long-lived. Consistent with this, only **Ru-3T** and **Ru-4T** exhibited appreciable light-amplified cytotoxicity, with EC₅₀ values typically in the submicromolar to nanomolar range (e.g., **Ru-4T**: EC_{50,vis} ≈ 20 nM; PI ≈ 3,800). Green light produced somewhat weaker normoxic responses, and red light was substantially less effective. **Ru-0T** and **Ru-1T**, which undergo rapid ³MLCT → ³MC deactivation, were inactive under any irradiation condition. Although the T₁ state for **Ru-2T** is also ILCT in nature, it lies higher in energy and is comparably short-lived, and therefore does not produce photocytotoxicity. This progression reflects the general photophysical trend that increasingly thiophene-centered S₁/T₁ and longer-lived ³ILCT states correlate with the ability to sustain phototoxic pathways under normoxia. The wavelength-dependent differences in PI cannot be explained solely by variations in absorbed photon flux, indicating that the character of the initially-formed excited states can also influence photocytotoxic outcomes. These observations reinforce the design principle emerging from this series: oligothiophene length functions as a master dial that reorganizes the excited state manifold, while the 6,6'-dmb coligands modulate which decay pathways remain accessible.

Photocytotoxicity in hypoxia. Under hypoxic conditions, the **Ru-0T–Ru-4T** series again separates into two groups, but with diminished overall responses. **Ru-0T**, **Ru-1T**, and **Ru-2T** remained inactive under all wavelengths tested, consistent with their inability to produce long-lived ³ILCT states. In contrast, **Ru-3T** and **Ru-4T** retained some photocytotoxicity, though highly attenuated relative to normoxia. **Ru-4T** displayed the strongest response, producing low-micromolar to submicromolar EC₅₀ values and PIs of ~100 under green light. A modest wavelength dependence was observed in hypoxia, with green light outperforming broadband visible despite similar absorbed photon flux. The origin of this behavior is unclear but may reflect subtle differences in the excitation pathways accessed at different wavelengths. Overall, these findings indicate that, within this family, only complexes capable of forming stabilized ³ILCT states exhibit photocytotoxicity under oxygen-limited conditions, although the efficiency of these pathways is diminished.

Overall, the photocytotoxicity trends were consistent across biological replicates, reinforcing the clear divide between inactive (**Ru-0T–Ru-2T**) and ILCT-active (**Ru-3T–Ru-4T**) complexes. Notably, **Ru-4T** consistently outperformed **Ru-3T** under every illumination and oxygen condition, even though the two complexes share similar $^1\text{O}_2$ quantum yields and **Ru-4T** possesses a slightly shorter $^3\text{ILCT}$ lifetime. Of note, its photosubstitution quantum yield is markedly lower. These observations indicate that the Φ_{Δ} , triplet lifetime, or Φ_{PS} do not, on their own, predict biological response in this family; rather, the decisive factor appears to be the ability to access and sustain ILCT-based excited states under otherwise identical cellular conditions in a way that promotes productive photobiological pathways. Within this programmed excited-state landscape, modest differences in ILCT stabilization or state accessibility can produce disproportionately large biological effects, as exemplified by the superior performance of **Ru-4T**. Together, these reproducible trends define a controlled benchmark for evaluating whether similar excited-state dependencies operate in bacterial photoinactivation, which we examine next.

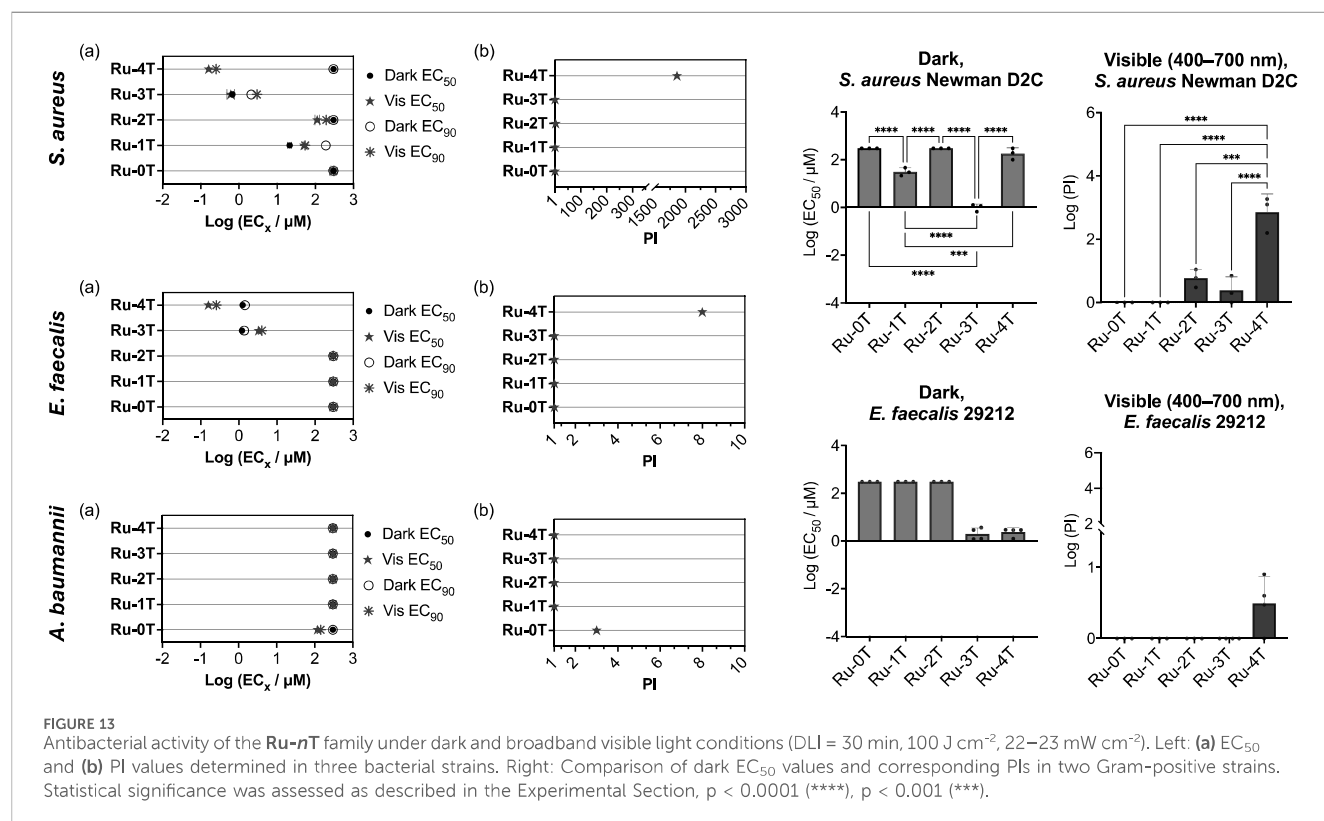
3.6 Photobiological properties toward bacteria

In contrast to the mammalian photobiology studies, where activation wavelength and oxygen tension were key variables, antibacterial experiments were conducted exclusively under standard aerobic conditions using visible light. This design enabled direct comparison of light-dependent activity across biologically distinct bacterial systems without introducing additional experimental variables. The **Ru-*n*T** series was therefore

evaluated against representative Gram-positive and Gram-negative bacteria to examine how excited-state dependencies identified in mammalian cells engage organisms with fundamentally different cellular organizations. Gram-positive (*S. aureus* Newman D2C and *E. faecalis* 29212) and Gram-negative (*E. coli* W3110 and *A. baumannii* 17978) bacteria were selected to span distinct physiological profiles within each class. Antibacterial activity was assessed under established dark and visible-light treatment protocols using standard aerobic conditions, with $\text{EC}_{50/90}$ values and PIs determined as described (Talgatov et al., 2026). The results are shown in Figure 13, Supplementary Figures S21–25 and compiled in Supplementary Tables S19–38.

In gram-positive *S. aureus*, light-dependent antibacterial activity across the **Ru-*n*T** series exhibited a non-monotonic dependence on oligothiophene length. **Ru-0T** was inactive under both dark and light conditions in the tested concentration range, while **Ru-1T** showed modest dark activity ($\text{EC}_{50} \approx 21\text{--}46\text{ }\mu\text{M}$ for **Ru-1T**) but no enhancement upon illumination. **Ru-2T** was inactive in the dark but exhibited weak light-dependent antibacterial activity, yielding PIs in the single-digit range ($\approx 3\text{--}11$). Extension to **Ru-3T** produced a potent antimicrobial agent with single-digit micromolar dark activity, with negligible light amplification (Supplementary Figure S21). In marked contrast, **Ru-4T** exhibited a $\text{PI} > 10^3$ with minimal dark activity, resulting in the largest PI values observed in this series.

In *E. faecalis*, another gram-positive bacterial species but with higher stress-tolerance, **Ru-0T** through **Ru-2T** were inactive under both dark and light conditions across the tested concentration range. In contrast, **Ru-3T** and **Ru-4T** functioned as potent antibacterials in the absence of light, exhibiting low-micromolar dark EC_{50} values. Illumination produced no meaningful enhancement for **Ru-3T** (in



fact, the light response was somewhat lower), while **Ru-4T** showed only a marginal light-dependent response, with a mean PI ≈ 4 .

In Gram-negative bacteria, the **Ru-nT** series exhibited little to no antibacterial activity under either dark or light conditions across the tested concentration range. In *E. coli*, no measurable activity or light-dependent enhancement was detected for any complex. In *A. baumannii*, most compounds likewise remained inactive ($EC_{50,90}$ at the assay ceiling under both conditions); however, **Ru-0T** showed a weak light-dependent effect ($EC_{50} \approx 118 \mu\text{M}$; PI ≈ 3). Aside from this extremely small response in *A. baumannii*, oligothiophene extension did not translate to meaningful Gram-negative photocytotoxicity under the conditions employed.

Together, these results demonstrate that while oligothiophene length-dependent excited state programming can enable light-selective antibacterial activity in select Gram-positive contexts, this behavior is attenuated or absent in stress-tolerant and Gram-negative bacteria, indicating that photophysical design alone is insufficient to drive predictable antibacterial light-triggered responses across distinct biological classes.

3.7 Preliminary *in vivo* tolerability

The divergence in biological responses observed across bacterial systems underscores that photophysical design alone does not ensure predictable biological behavior, motivating additional evaluation of how selected compounds translate beyond cellular assays. In this context, preliminary *in vivo* tolerability was assessed exclusively for compound **Ru-4T**, which emerged as the most consistently photoresponsive member of the series across the limited set of mammalian and bacterial studies reported here. To minimize animal use, a limit-of-one dose-escalation design was employed, with a minimal number of dose levels selected based on range-finding MTD data from previously studied analogues.

Ru-4T was administered under dark conditions *via* intraperitoneal (IP) and intravenous (IV) injection over dose ranges of 25–150 mg kg⁻¹ (IP) and 12.5–75 mg kg⁻¹ (IV) following established protocols reported previously (Roque et al., 2022). Across the doses examined, **Ru-4T** was generally well tolerated, with no evidence of dose-limiting toxicity observed. Transient, mild clinical signs (including brief subdued behavior and transient hunching) were noted following IP administration and resolved within 2–3 h, and mild, reversible weight loss was observed at higher IP doses, resolving within several days. In contrast, no overt clinical signs were observed following IV administration across the tested dose range. No sustained adverse effects were detected by either route.

Within the tested dose range, no maximum tolerated dose was reached, establishing minimum MTD thresholds of $\geq 150 \text{ mg kg}^{-1}$ (IP) and $\geq 75 \text{ mg kg}^{-1}$ (IV) under dark conditions. Relative to the structurally related 2,9-dimethyl-1,10-phenanthroline (2,9-dmp) analogue reported previously (Roque et al., 2022), **Ru-4T** exhibits an expanded tolerability window by both IP and IV administration, with the improvement being particularly pronounced for IV dosing. Although **Ru-4T** is less photobiologically potent than its 2,9-dmp counterpart, its substantially reduced *in vivo* toxicity highlights the influential role of coligand identity in governing biological tolerability, demonstrating that coligand choice modulates not only excited-state accessibility but also whole-organism response.

4 Conclusion

This study set out to test whether excited-state design principles established for oligothiophene-extended Ru(II) phototherapeutics yield predictable biological outcomes when applied to a moderately strained 6,6'-dmb scaffold and evaluated across distinct biological contexts. By systematically examining the complete [Ru (6,6'-dmb)₂(IP-*n*T)]²⁺ series ($n = 0\text{--}4$) using spectroscopy, computation, photochemistry, and photobiological assays, we identify where excited-state programming succeeds, and where biological context imposes additional constraints.

Across this family, oligothiophene extension functions as a master dial that reorganizes the excited-state landscape from ³MLCT/³MC-dominated behavior (**Ru-0T–Ru-1T**) to stabilized ³ILCT states (**Ru-3T** and **Ru-4T**). This transition is directly supported by fs- and ns-TA spectroscopy, ¹O₂ measurements, photosubstitution quantum yields, and TD-DFT calculations. Importantly, access to photodissociative ³MC states—although efficient for **Ru-0T–Ru-1T** and associated with higher photosubstitution quantum yields—does not translate into photocytotoxicity under either normoxic or hypoxic conditions. These findings demonstrate that photosubstitution originating from ³MC is not, in this specific family of compounds, a productive pathway for photocytotoxic activity.

In mammalian cancer cells, only **Ru-3T** and **Ru-4T** exhibited light-dependent cytotoxicity, with **Ru-4T** consistently emerging as the most effective compound under all illumination and oxygen conditions. Although hypoxic activity was attenuated relative to normoxia, 6,6'-dmb retained measurable photocytotoxicity even at 1% O₂, a regime where most photosensitizers fail. The similar ¹O₂ quantum yields and comparable triplet lifetimes of **Ru-3T** and **Ru-4T** indicate that neither Φ_{Δ} nor excited-state lifetime alone predicts biological outcome. Instead, subtle differences in triplet configurations, energetic accessibility, or oxygen-independent reactivity—and potentially light-independent biological associations—appear sufficient to produce disproportionately large differences in photocytotoxic response.

The antibacterial results further underscore the limits of predictability based on photophysics alone. While **Ru-4T** displayed strong light-selective activity in *S. aureus*, responses in *E. faecalis* were dominated by intrinsic dark activity, and Gram-negative bacteria showed essentially no susceptibility across the series. These findings demonstrate that even when excited-state design successfully enables light-responsive behavior in mammalian cells, translation to bacterial systems depends strongly on organism-specific barriers and stress-tolerance mechanisms that are not encoded in photophysical parameters.

Consistent with these context-dependent biological responses, preliminary *in vivo* tolerability studies showed that **Ru-4T** is well tolerated over the tested dose range and exhibits reduced systemic toxicity compared to its more potent 2,9-dmp analogue reported previously. Although **Ru-4T** is not the most potent member of the broader IP-*n*T platform, its favorable tolerability profile highlights the importance of balancing excited-state reactivity with biological safety and supports its potential utility as a selectively

photoresponsive scaffold rather than a maximally aggressive phototoxin.

Taken together, this work provides a second, independent validation, beyond the previously reported phen and dmp systems, that stabilized ³ILCT states underpin productive photocytotoxic responses under both normoxic and hypoxic conditions. The progressive increase in excited state polarization across the series further underscores how thiophene extension alters the electronic landscape of the triplet manifold, complementing the trends observed in excited state lifetimes and photobiological activity. Importantly, thiophene extension reshapes the singlet and triplet manifolds in a spin-dependent manner. Although **Ru-3T** and **Ru-4T** display mixed ¹ILCT/¹LLCT character in their lowest singlet excitations, the corresponding triplet states are dominated by ³ILCT configurations.

At the same time, the divergence observed across bacterial systems and *in vivo* tolerability underscores that excited-state programming defines what is possible, not what is guaranteed, in complex biological environments. These results refine the master-dial/mode-switch framework by clarifying its scope and limitations, and they establish **Ru-4T** as a well-characterized reference point for future efforts aimed at disentangling photophysical control from biological contingency in metal-based phototherapeutics.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding authors.

Ethics statement

The animal study was approved by Wake Forest University Health Sciences Institutional Review Board (IRB). All animal experiments were conducted in accordance with institutional guidelines for the care and use of laboratory animals and were approved by the appropriate Institutional Animal Care and Use Committee (IACUC). All procedures complied with relevant national and international regulations, and every effort was made to minimize animal suffering and to reduce the number of animals used. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

AT: Writing – original draft, Writing – review and editing, Data curation. GK: Writing – original draft, Writing – review and editing, Data curation. GS: Writing – original draft, Writing – review and editing, Data curation. JR: Writing – original draft, Writing – review and editing, Data curation. PB: Writing – original draft, Writing – review and editing, Data curation. DS: Writing – original draft, Writing – review and editing, Data curation. TS: Writing – original draft, Writing – review and

editing, Data curation. SK: Writing – original draft, Writing – review and editing, Data curation. GD: Writing – original draft, Writing – review and editing, Data curation, Formal Analysis, Investigation, Methodology, Resources, Supervision, Validation, Visualization. AF-M: Writing – original draft, Writing – review and editing, Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Resources, Supervision, Validation. MA: Writing – original draft, Writing – review and editing, Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Resources, Supervision, Validation. CC: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Resources, Supervision, Validation, Writing – original draft, Writing – review and editing. SM: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing – original draft, Writing – review and editing.

Funding

The author(s) declared that financial support was received for this work and/or its publication. The authors would like to thank the National Science Foundation (NSF) (Awards CHE-2102459, CHE-2400127) for support. M. E. A. acknowledge financial support under the PRIN D.D. 104/2022 PNRR M4. C2.1.1.– Project Code: 2022AN47CACUP: H53D23003820001, funded by the European UnionNextGenerationEU–Project Title “La-G4-DACA”–Grant Assignment DD 862, 16/06/2023. She is also grateful for the CINECA award under the ISCRA initiatives, for the availability of high-performance computing resources and support (IsCc4_PAC-DACA, IsB30_MDPHOTO). AF-M. thanks the support from the Spanish Ministerio de Ciencia, Innovación e Universidades-Agencia Estatal de Investigación (MICIU/AEI/10.13039/501100011033) through the CNS 2024-154908 grant. He is also grateful for the support of the Generalitat Valenciana (GV) through the project CIAICO/2022/121.

Acknowledgements

The content in this work is solely the responsibility of the authors and does not necessarily represent the official views of the National Science Foundation. The authors also thank Light Conversion for the use of the HARPIA-LIGHT spectrometer. The authors acknowledge the Texas Advanced Computing Center (TACC) at The University of Texas at Austin for providing HPC, visualization, or storage resources that have contributed to the research results reported within this paper. URL: <http://www.tacc.utexas.edu>.

Conflict of interest

The author(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The reviewer ES declared a shared affiliation with the author MA at the time of review.

SM has a potential research conflict of interest due to a financial interest with Theralase Technologies, Inc. and PhotoDynamic, Inc. A management plan has been created to preserve objectivity in research in accordance with UTA policy.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fchbi.2026.1771939/full#supplementary-material>

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