Sveučilište u Zagrebu

FAKULTET KEMIJSKOG INŽENJERSTVA I TEHNOLOGIJE

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BIOKOMPATIBILNI FLUORIMETRIJSKI SENZORI ZA PREPOZNAVANJE METALNIH KATIONA, DNA i RNA

DOKTORSKI RAD

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Abstract

The development of bioactive and fluorescent small molecules for dual applications in bioimaging and therapy is an evolving field in modern chemical biology and medicinal chemistry. This doctoral thesis presents the synthesis, structural and spectroscopic characterization, and biological evaluation of three classes of novel fluorophores: (1) *N*-methylpyridinium-substituted pyrene derivatives, (2) diethynylarene-linked bis(triarylborane) tetracations, and (3) FRH-inspired peptidoid–coumarin conjugates.

The pyrene derivatives, 2- and 2,7-substituted *para*-*N*-methylpyridinium pyrene analogues (**P1** and **P2**) and their methylated cationic forms (**P1M** and **P2M**), show the ability to bind nucleic acids, exhibit pH-dependent fluorescence, and induce light-triggered cytotoxicity through singlet oxygen production. Notably, methylation and protonation cause significant bathochromic shifts in emission, while their absorption peaks stay mostly unchanged. **P1M** generally binds as a single molecule, either by intercalation with DNA or groove binding with RNA, localizes in mitochondria, and is photoactive at submicromolar levels. In contrast, **P2M** tends to form ligand aggregates on DNA and RNA, especially at higher concentrations, and displays a non-selective distribution across various cellular organelles.

Based on earlier findings, the bis(triarylborane) tetracations were studied for their dual optical sensing and therapeutic properties. Thiophene- and anthracene-bridged analogues showed efficient cellular uptake, low toxicity, and photoinduced cytotoxicity through the production of reactive oxygen species (ROS) under visible light. These compounds also displayed high-affinity, non-covalent binding to adenovirus type 5 (HAdV5) capsid proteins, with light activation strengthening antiviral effects. Their ability to generate singlet oxygen and their fluorescence/Raman–SERS sensitivity highlight their potential as light-triggered theranostic agents in cancer therapy and virology.

The third part of the study is focused on the design of four novel peptidoids derived from the FRH (Phe–Arg–His) peptide, wherein histidine was replaced with a triazole (*via* click chemistry) and arginine with lysine. Coumarin-functionalized FKA(triazole) and KA(triazole) conjugates exhibited strong nanomolar to submicromolar Cu^{2+} binding affinities, confirming the

triazole moiety as an effective metal-coordinating motif. Notably, KA(triazole)–coumarin emerged as the shortest known peptidoid capable of exhibiting both fluorescence and circular dichroism (CD) responses upon Cu^{2+} binding. Results that include DNA/RNA binding revealed that these peptidoid-Cu^{2+} complexes stabilized nucleic acid structures, likely *via* groove binding, and indicate potential for oxidative DNA cleavage due to the proximity of Cu^{2+} to the phosphate backbone. These findings highlight their utility as artificial nucleases and selective Cu^{2+} sensors.

Overall, the fluorophores developed in this thesis demonstrate considerable promise as theranostic agents. Their selective bioactivity, spectroscopic properties, and capabilities as photosensitizers or Cu^{2+} carriers support their applicability in molecular diagnostics, photodynamic therapy, and targeted biomolecular interactions. These results provide a foundation for future *in vivo* studies and the optimization of their pharmacological profiles.

Key words: Cu^{2+} binding, DNA/RNA noncovalent interactions, fluorescent sensors, peptide FRH, photodynamic therapy, theranostic agents

Sažetak

Razvoj bioaktivnih i fluorescentnih malih molekula za dvostruku primjenu u biooslikavanju i u terapijske svrhe predstavlja brzo rastuće područje unutar suvremene kemijske biologije i medicinske kemije. Ova doktorska disertacija obuhvaća sintezu, strukturnu i spektroskopsku karakterizaciju te biološka ispitivanja triju vrsta novih fluorofora: (1) *N*-metilpiridinijevim supstituiranih derivata pirena, (2) bis(triarilboran) tetrakationa povezanih dietinilarenskim mostovima te (3) peptidoidno–kumarinskih konjugata inspiriranih FRH peptidom.

Derivati pirena, konkretno 2- i 2,7-supstituirani *para*-*N*-metilpiridinijevi derivati (**P1** i **P2**) te njihovi metilirani kationski analozi (**P1M** i **P2M**), pokazuju sposobnost vezanja za nukleinske kiseline, emisiju fluorescencije ovisnu o pH-vrijednosti te induciranu citotoksičnost pod utjecajem svjetla, putem stvaranja singletnog kisika. Metiliranje i protoniranje rezultiraju izraženim batokromnim pomacima u emisiji, dok apsorpcijski maksimumi ostaju uglavnom nepromijenjeni. **P1M** se, u strukturu nukleinskih kiselina, u pravilu veže kao monomer, interkalacijom u DNA ili vezanjem u utore RNA, lokalizira se u mitohondriju te pokazuje fotoaktivnost već pri submikromolarnim koncentracijama. Nasuprot tome, **P2M** ima tendenciju agregiranja na DNA i RNA pri višim koncentracijama te pokazuje neselektivnu unutarstaničnu distribuciju.

Nadovezujući se na prethodna istraživanja, bis(triarilboran) tetrakationi istraženi su u kontekstu njihove dvostruke funkcije, kao optičkih senzora te spojeva koji se koriste u terapijske svrhe. Analozi koji posjeduju tiofenski i antracenski most, učinkovitu ulaze u stanicu, te nisu intrinzično citotoksični, dok im je citotoksičnost izražena osvjetljavanjem pod vidljivim svjetlom što rezultira stvaranjem reaktivnih kisikovih vrsta (ROS). Ovi spojevi također pokazuju visok afinitet prema nekovalentnom vezivanju na kapsidne proteine adenovirusa tipa 5 (HAdV5), pri čemu aktiviranje svjetlom dodatno pojačava antivirusni učinak. Njihova sposobnost stvaranja singletnog kisika, zajedno s fluorescentnim i Raman–SERS svojstvima, potvrđuje njihov potencijal kao teranostičkih sredstava aktiviranih svjetlom u onkološkoj i virološkoj primjeni.

Treći dio istraživanja usmjeren je na dizajn četiri nova peptidoida izvedena iz FRH (fenilalanin–arginin–histidin) peptida, u kojima je histidin zamijenjen triazolnom skupinom (putem klik kemije), a arginin lizinom. Konjugati funkcionalizirani kumarinom, FKA(triazol) i KA(triazol), pokazali su visoke afinitete vezanja za Cu^{2+} ione (u nanomolarnom do submikromolarnom rasponu), čime je potvrđena učinkovitost triazola kao metal-koordinirajućeg motiva. KA(triazol)–kumarin posebno se ističe kao najkraći poznati peptidoid sposoban za istodobno iskazivanje fluorescentnog i odziva cirkularnog diktoizma na vezanje Cu^{2+}. Dodatna ispitivanja interakcija s DNA i RNA ukazuju na stabilizaciju nukleinskih kiselina putem vezanja u utore nukleinskih kiselina, uz mogućnost oksidativnog cijepanja DNA zbog blizine Cu^{2+} fosfatnoj okosnici. Ovi rezultati upućuju na njihovu primjenu kao endonukleaza i selektivnih senzora za Cu^{2+}.

Zaključno, fluorofori proučavani u sklopu ovog doktorskog rada pokazuju potencijal kao teranostička sredstva. Njihova selektivna bioaktivnost, povoljna spektroskopska svojstva te njihova sposobnost da djeluju kao fotosenzibilizatori ili nositelji Cu^{2+} iona ukazuju na široku primjenjivost u molekularnoj dijagnostici, fotodinamičkoj terapiji te ciljanom utjecaju na biomolekularne procese. Dobiveni rezultati pružaju čvrst temelj za daljnja *in vivo* istraživanja i optimizaciju farmakoloških svojstava ovih molekula.

Ključne riječi: vezanje Cu^{2+}, nekovalentne interakcije s DNA/RNA, fluorescentni senzori, peptid FRH, fotodinamička terapija, teranostički agensi