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Squaraine rotaxanes for biomedical applications: a mini-review

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Squaraine dyes are important fluorescent probes and labels for biomedical applications because their molecular structure provides intense and narrow absorption and emission bands, high molar extinction coefficients, and high fluorescence quantum yields in the visible and near-infrared spectral regions. These favorable photophysical properties have led to the widespread use of squaraines in bioimaging, biosensing, photodynamic therapy, and other light-driven applications. However, their electron-deficient cyclobutenolate core makes them susceptible to nucleophilic attack, while, like other dyes, squaraine dyes absorbing in red and near-infrared regions, are prone to photobleaching, oxidation, and aggregation in aqueous media, thereby limiting their practical applications. To overcome these limitations, mechanically interlocked molecular architectures have been developed by encapsulating squaraine dyes within tetralactam macrocycles to form rotaxanes. The resulting squaraine rotaxanes demonstrate improved chemical and photochemical stability, reduced aggregation, and enhanced photophysical properties. This mini-review summarizes the methods used for the preparation of squaraine rotaxanes and discusses their applications in bioimaging, biomolecular sensing, and photodynamic therapy. The current limitations of squaraine rotaxanes and their future prospects for their biomedical applications are also considered.

Keywords: squaraine dye; rotaxane; supramolecular chemistry; bioimaging; biosensing; photodynamic therapy

Скваратн ротаксани для біомедичного застосування: міні огляд. *Р П. Свояков, П. М. Радіонов, О. С. Колосова, О. Г. Кулик*

Скваратнові барвники є одними з найважливіших флуоресцентних зондів та міток для біомедичних застосувань, оскільки їхня молекулярна структура забезпечує інтенсивні та вузькі смуги поглинання та випромінювання, високі молярні коефіцієнти екстинкції та високі квантові виходи флуоресценції у видимому та ближньому інфрачервоному діапазонах спектра. Ці сприятливі фотофізичні властивості зумовили широке використання скваратнів у біовізуалізації, біосенсориці, фотодинамічній терапії та інших застосуваннях і технологіях, що використовують світло.. Однак їхнє електронодефіцитне циклобутенолатне ядро робить їх чутливими до нуклеофільної атаки, тоді як, подібно до інших барвників червоного та ближнього інфрачервоного діапазону, скваратнові барвники також схильні до фотознебарвлення, окиснення та агрегації у водних середовищах, що обмежує можливості їх практичного застосування. Для подолання цих обмеженнь, були розроблені механічно зчеплені молекулярні архітектури шляхом інкапсуляції скваратнових барвників у тетралактамні макроцикли з утворенням ротаксанів. Отримані скваратнові ротаксани демонструють покращену хімічну та фотохімічну стабільність, знижену агрегацію та покращені фотофізичні властивості. У цьому міні-огляді узагальнено методи одержання скваратнових ротаксанів та обговорено їх застосування у біовізуалізації, біомолекулярній сенсориці та фотодинамічній терапії. Крім того, розглянуто сучасні обмеження скваратнових ротаксанів і перспективи їх подальшого розвитку для медико-біологічного застосування..

1. Introduction

Squaraine dyes, along with cyanine dyes, belong to an important class of polymethine dyes, but are distinguished by the presence in the structure of an electron-deficient central cyclobutenolate fragment, a square acid residue [1,2]. The electron density is delocalized and involved in conjugation with the entire π-system of the dye. In combination with electron-donating terminal substituents, a donor-acceptor-donor structure is formed, which contributes to the effective delocalization of charge within the chromophore system (Scheme 1) [3].

The molecular structure of squaraine dyes determines their unique spectral characteristics: the ability to absorb and emit in the long-wavelength spectral region, which corresponds to the window of increased optical transparency of biomaterials, high molar extinction coefficients and fluorescence quantum yields, narrow intense absorption and emission bands, as well as sensitivity to the polarity of the medium [4]. Due to these properties, squaraines are promising for use in bioimaging [5–7], diagnostics [8], theranostics [2], photodynamic [9–12] and photothermal therapy [13,14], as well as pH-sensitive systems [15], sensors for the detection of metal ions [16–18] and other functional materials.

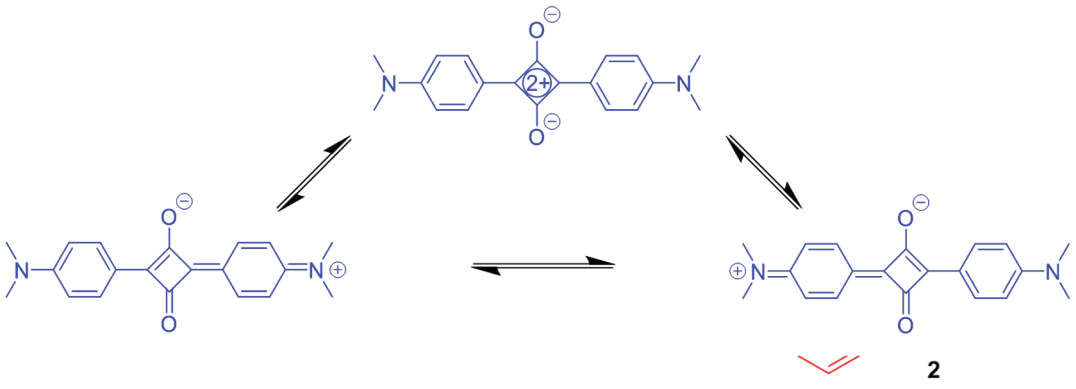
However, many fluorescence labels and probes, including squaraine dyes, suffer from limited photostability and susceptibility to oxidation and nucleophilic attack, which can lead to the degradation of the chromophore. Another limitation of squaraines is their tendency to aggregate in aqueous and/or biological media. Such aggregation often broadens the absorption and emission bands and decreases the optical response, thereby reducing their performance in biological and sensing applications [19].

A promising approach to overcome these limitations is the encapsulation of squaraine dyes into molecular hosts [20,21] or self-assembled nanocarriers, such as micelles [22] and liposomes [23]. These systems provide a protective environment for the squaraine core, shielding it from nucleophilic attack and other destabilizing factors. Encapsulation may also increase photostability, fluorescence quantum yield, brightness, and fluorescence lifetime. Accordingly, supramolecular host-guest systems based on macrocyclic hosts, including cyclodextrins [24,25],cucurbiturils [26,27], and triptycene-derived calixarenes [28,29] have been widely explored, although their inherent reversibility may limit long-term stability.

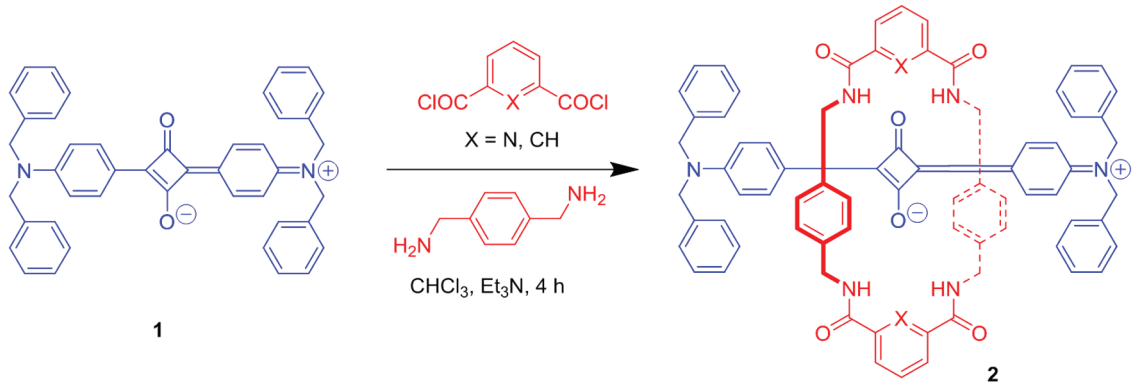
A more robust strategy was introduced by Smith and co-workers, who mechanically encapsulated *bis*-(*N,N*-dibenzylaniline) squaraine dye **1** as the thread component within a tetralactam macrocycle to form squaraine rotaxane **2** (Scheme 2) [19].

This mechanically interlocked architecture protects the chromophore from external chemical factors, restricts intramolecular motion, and suppresses aggregation, thereby improving the dye's stability and photophysical properties. Consequently, squaraine rotaxanes exhibit improved chemical stability [21], photostability [30], fluorescence quantum yield [31,32], brightness, and performance in complex biological environments [33].

These combined improvements have established squaraine rotaxanes as promising platforms for light-driven biomedical applications. Accordingly, this review provides a concise overview of squaraine rotaxanes developed for such purposes, with particular emphasis on the relationships among molecular design, photophysical properties, and biomedical per-



Scheme 1. Resonance structures of squaraine dye.



Scheme 2. Example of one of the first syntheses of squaraine rotaxane dyes.

formance. It represents their use as fluorescent probes and labels for bioimaging, biomolecular sensing, and photodynamic therapy. Finally, the principal limitations and future directions for the biomedical development of squaraine rotaxanes are discussed.

2. Molecular Design and Synthesis of Squaraine Rotaxanes

Among the wide variety of supramolecular systems, mechanically interlocked systems, particularly rotaxanes, have attracted considerable attention. Rotaxanes consist of a linear axle, which acts as the guest molecule, threaded through a macrocyclic host and trapped by bulky stopper groups at both ends (Figure 1) [34,35]. Pseudorotaxanes are unlocked analogues of rotaxanes that lack bulky stopper groups and can therefore undergo spontaneous threading and dethreading. Semirotaxanes are intermediate systems containing one bulky stopper group at only one end of the axle. They may serve as precursors in rotaxane synthesis or function as independent supramolecular systems [36,37].

The first encapsulation of a squaraine dye within a tetralactam macrocycle to form a squaraine rotaxane was reported by Smith and co-workers [19]. Squaraine rotaxanes of this type are commonly synthesized using the Leigh clipping method [38], in which the oxocyclobutenolate core of the squaraine dye acts as a template for the hydrogen-bond-directed cyclization of the tetralactam macrocycle around the dye. Bulky stopper groups located at both ends of the squaraine axle prevent dethreading of the macrocycle and ensure the mechanical stability of the resulted rotaxane [39].

Several general strategies have been developed for the synthesis of rotaxanes, with

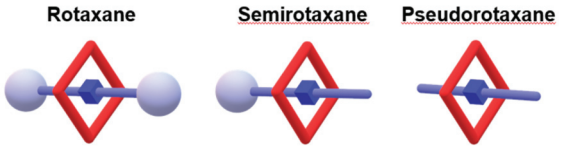


Figure 1. Schematic representation of rotaxane, semirotaxane, and pseudorotaxane supramolecular systems.

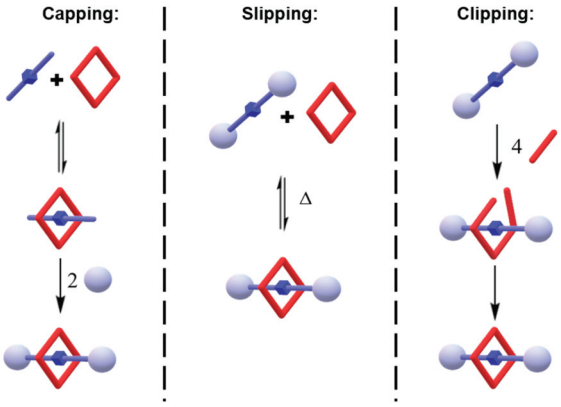
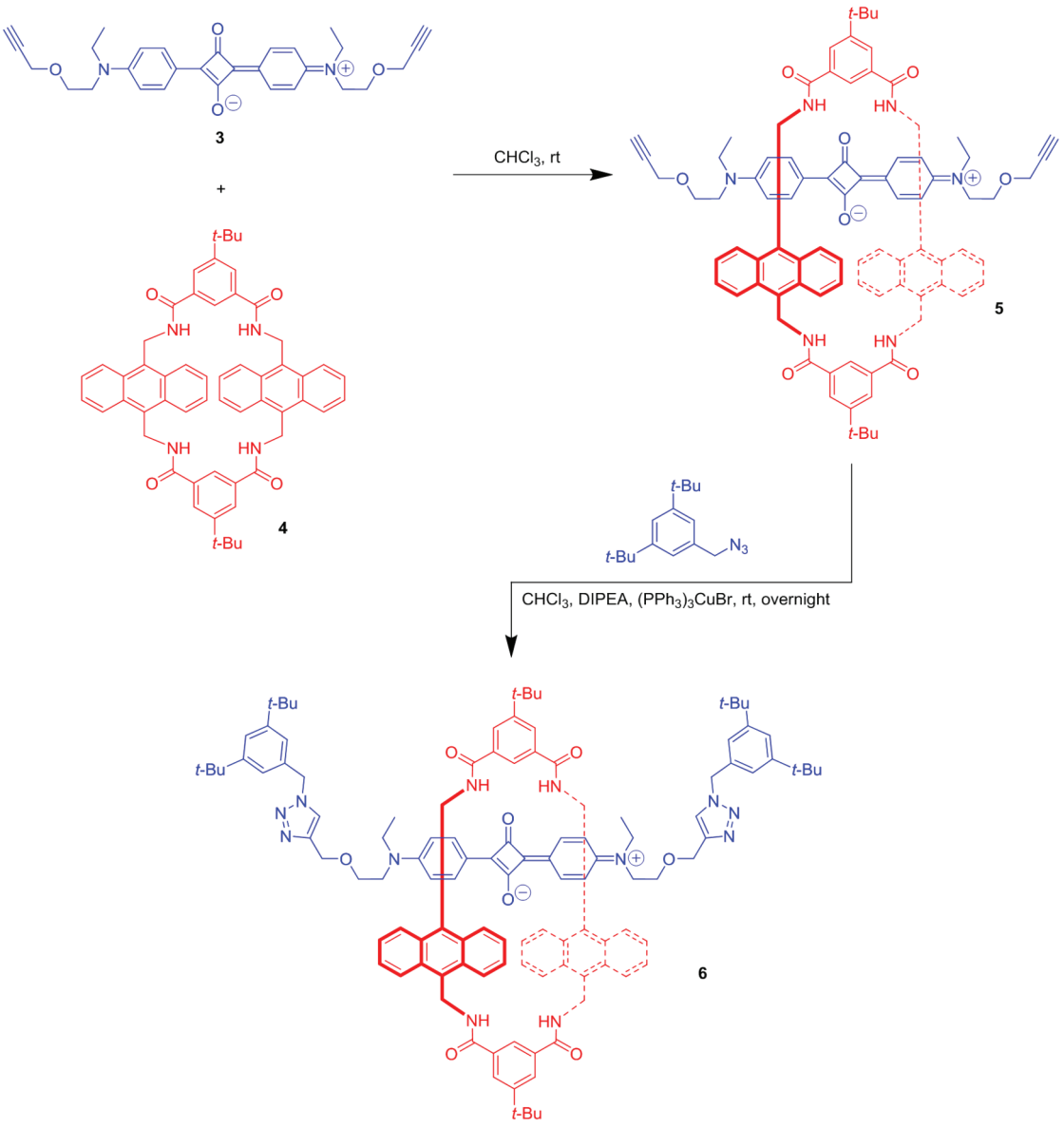


Figure 2. Methods for the synthesis of squaraine rotaxane systems.

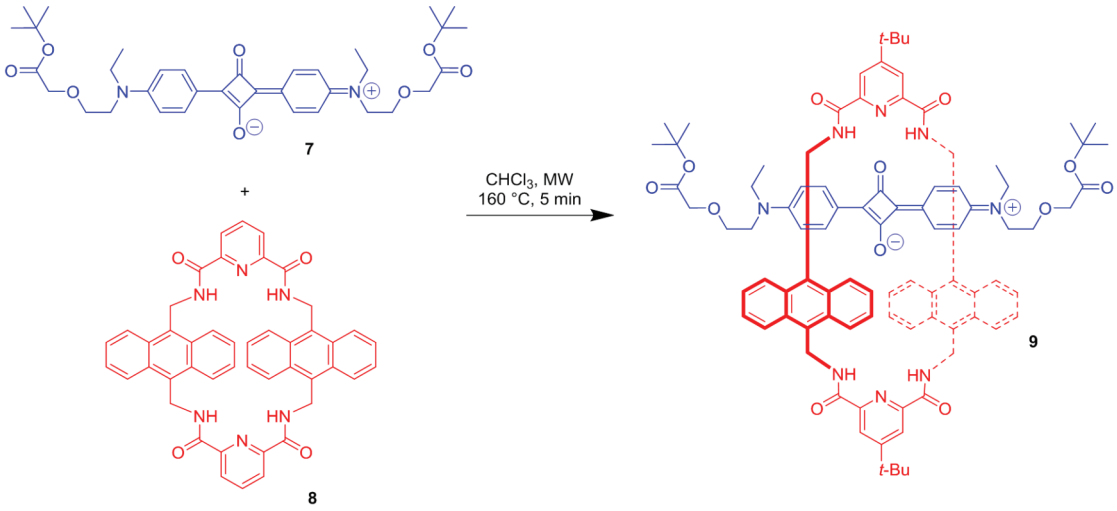
capping, slipping, and clipping being among the most widely used approaches (Figure 2) [40,41].

In the capping method, a pseudorotaxane is first formed through non-covalent association between the axle and the macrocycle (Figure 2). Bulky stopper groups are then introduced at the ends of the axle to prevent dethreading [42–44]. The efficiency of this approach is therefore largely governed by the thermodynamic stability of the intermediate pseudorotaxane under the reaction conditions. For example, Gassensmith et al. showed that encapsulation of squaraine **3** within macrocycles **4**, bearing anthracene moieties, yielded pseudorotaxane complex **5.** The pseudorotaxane was then covalently capped at the protruding alkyne termini to afford rotaxane **6** (Scheme 3) [21].



Scheme 3. Example of synthesis of squaraine rotaxane by the capping method.

In the slipping or pulling approach, a preformed macrocycle passes over a stopper group of an already capped axle to reach a favorable binding site on the thread (Figure 2). This process is generally promoted by heating, which enables the system to overcome the kinetic barrier associated with passage of the stopper through the macrocyclic cavity. Although formation of the threaded complex is accompanied by an unfavorable entropic contribution due to association of the two components, this can be compensated by favorable enthalpic interactions between the macrocycle and the axle. In tetralactam-based squaraine rotaxanes, stabilization is provided predominantly by hydrogen bonding between the amide NH groups of the macrocycle and the oxocyclobutenolate core of the squaraine dye. Thus, successful slipping requires an appropriate balance between the size and conformational flexibility of the macrocycle and stopper groups, the kinetic barrier to threading, and the thermodynamic stabilization of the resulting interlocked complex [45–47]. Once the macrocycle reaches the squaraine recognition site, these host–guest interactions stabilize the threaded structure, while the stopper groups provide a kinetic barrier against subsequent dethreading. For instance, Lee et al. synthesized squaraine rotaxane **9** by microwave-assisted slipping of tetralactam



Scheme 4. Example of synthesis of squaraine rotaxane by the slipping method.

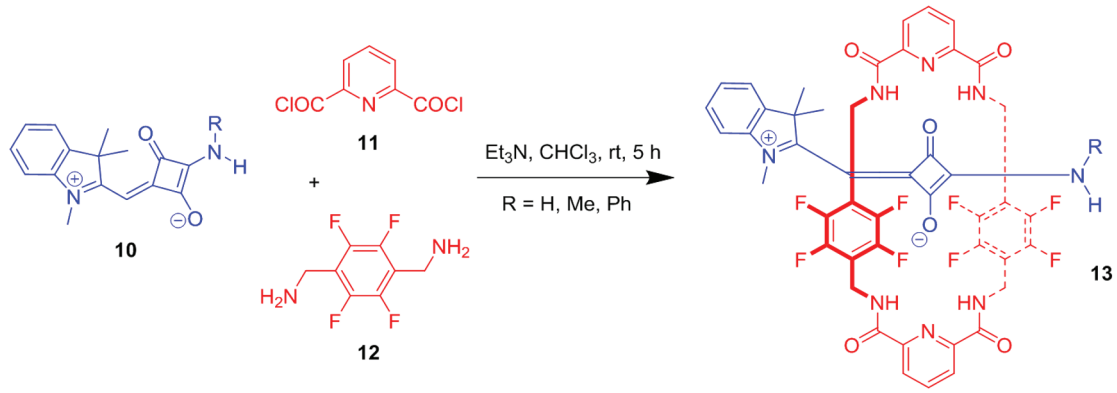
macrocycle **8** over the stopper group of preformed squaraine axle **7** in $CHCl_3$ at 160 °C for 5 min (Scheme 4) [48].

In the *clipping* method, the macrocycle is assembled directly around a template or axle through the formation of covalent bonds between its precursor fragments. Template-directed non-covalent interactions facilitate macrocyclization and can provide high selectivity for the desired mechanically interlocked product [19,20,49]. As an example, in our recent publication [50], we demonstrated the formation of rotaxanes **13** by a template-assisted macrocyclization of a tetralactam macrocycle around a hemisquaraine dye **10** (Scheme 5). Successful macrocyclization was observed for hemisquaraines bearing primary and secondary amino groups, whereas derivatives containing sterically hindered tertiary amino groups did not undergo encapsulation [50].

It is worth noting that, among these three strategies, the clipping approach is the most widely used, particularly for the preparation of template-directed squaraine rotaxanes.

Regardless of the synthetic strategy employed, encapsulation within the macrocycle not only protects the dye from external factors but also promotes structural rigidification of the fluorophore and restricts intramolecular motions. These effects suppress non-radiative relaxation pathways and can therefore enhance the fluorescence quantum yield and brightness [49].

Moreover, both the macrocyclic component of the rotaxane and the squaraine axle, including short- [39,50–52] and long-wavelength derivatives [44,49,53], can be functionalized with a wide range of substituents. These may include hydrophilic groups, targeting ligands [54,55], peptides [56], dendrimers [57,58], or other biomolecular moieties. In addition, various sidewall substituents can be introduced into the macrocycle [59]. The affinity of squaraine–macrocycle complexes can also be enhanced using the guest back-folding strategy [42], in which



Scheme 5. Example of synthesis of hemisquaraine rotaxanes by the clipping method/

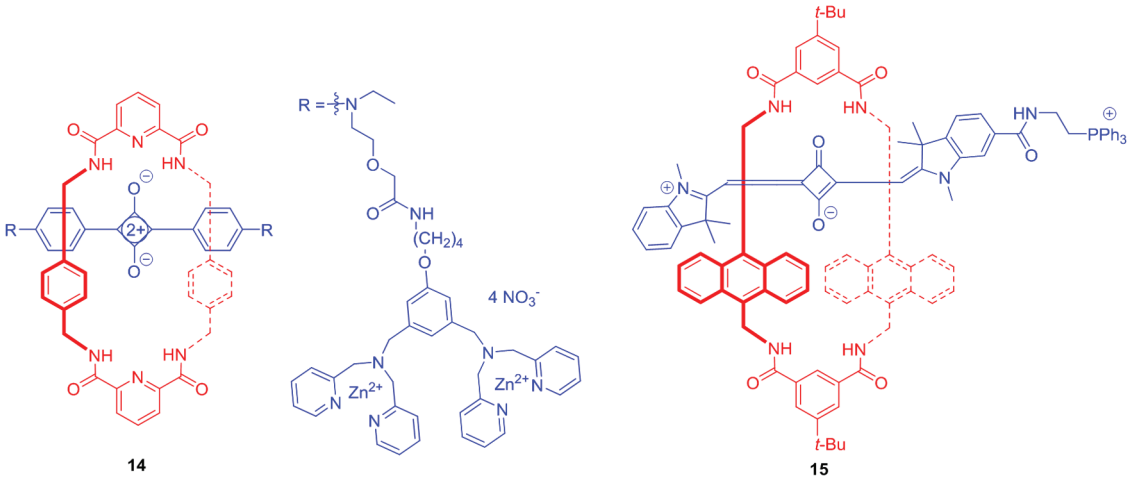


Figure 3. Examples of squaraine rotaxanes used for bioimaging.

flexible aromatic substituents of the guest fold toward the exterior of the macrocycle after threading, increasing the buried hydrophobic surface area and enabling additional favorable non-covalent contacts with the macrocycle. Such modifications can improve water solubility, cellular uptake, targeting selectivity, and preferential accumulation in specific tissues, cells, or subcellular organelles [60].

3. Biomedical Applications of Squaraine Rotaxanes

3.1 Bioimaging

Bioimaging is one of the most extensively investigated biomedical applications of squaraine rotaxanes [20,61,62]. Their intense absorption and fluorescence in the red or near-infrared spectral region, together with their enhanced resistance to chemical and photochemical degradation make them attractive fluorophores for visualizing biological structures and monitoring of biological processes [54,63]. Encapsulation of the squaraine dye within a macrocycle is particularly advantageous under physiological conditions, where unprotected squaraine dyes may undergo aggregation, nucleophilic attack, or oxidative degradation, resulting in fluorescence quenching and reduced imaging performance.

Squaraine rotaxane dyes have been successfully employed for optical imaging *in vitro* [60,64–66], in live [20,67,68] and fixed cells, and *in vivo* [54,55,69]. White et al. developed deep-red fluorescent squaraine rotaxane probe **14**, bearing bis[Zn(II)-dipicolylamine] targeting groups for the selective *in vivo* imaging of bacterial infections. The probe accumulated at sites infected with both Gram-positive *S. aureus* and Gram-negative *S. enterica*, while showing negligible uptake in sterile inflammatory lesions (Figure 3) [54].

Squaraine rotaxanes have also been incorporated into dendritic and multivalent architectures to improve their aqueous compatibility, targeting efficiency, and fluorescence performance in biological environments [57,58]. Xiao et al. developed a squaraine rotaxane bearing four alkyne groups that could be converted into a series of dendritic structures by copper-catalyzed azide-alkyne cycloaddition [57]. The resulting dendritic squaraine rotaxanes exhibited intense deep-red fluorescence and high chemical stability in biological media. In a subsequent study, the same authors developed multivalent squaraine rotaxane probes bearing multiple Zn(II)-dipicolylamine (ZnDPA) recognition units, that displayed bright fluorescence in water and broad-spectrum binding to both Gram-positive and Gram-negative bacteria [70]. Related multivalent Zn(II)-dipicolylamine squaraine rotaxanes were investigated by Smith et al. for imaging dead and dying cells, with tetravalent probes bearing four targeting ZnDPA units surpassing the corresponding divalent analogues bearing two ZnDPA units [71]. More recently, preassembled multivalent probes containing squaraine rotaxane fluorophores and multiple membrane-targeting units have been used to visualize anionic liposomes, bacterial membranes, and dead or dying mammalian cells [46].

Beyond bacterial and cell-death imaging, squaraine rotaxanes have also been designed for organelle-selective visualization. Squaraine rotaxane **15**, comprising an unsymmetrical squaraine dye encapsulated within a tetralac-

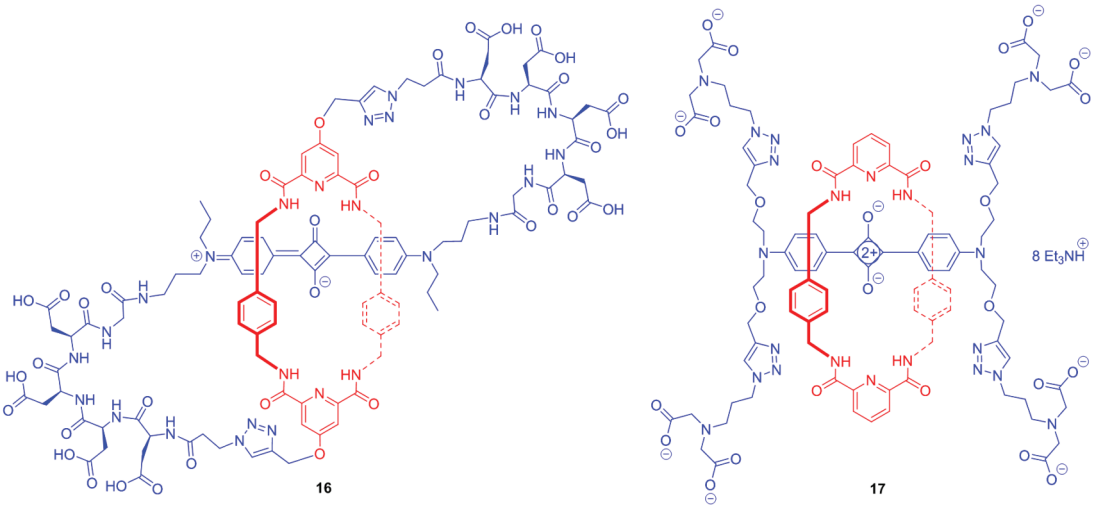


Figure 4. Examples of squaraine rotaxanes for selective targeting of different biological targets.

tam macrocycle bearing two anthracene sidewalls, was developed as a fluorescent probe for mitochondrial imaging (Figure 3) [20]. Singh et al. reported a red-emitting dual-targeting squaraine rotaxane that selectively entered cancer cells and localized in lysosomes, enabling real-time lysosomal tracking as well as three-dimensional and multicolour imaging in living cells [67].

Other squaraine rotaxanes incorporate peptide loops or self-threaded architectures, providing additional opportunities for biological recognition and molecular targeting. The peptide sequence can be varied to tune aqueous compatibility, cellular uptake, and affinity for specific biomolecular targets: arginine-rich loops promoted plasma-membrane binding, whereas aspartate-rich loops selectively targeted the mineral matrix of bone [68,72]. In particular, figure-eight probe **16** combined bright deep-red fluorescence with high photochemical stability and resistance to proteolytic degradation, while solid-phase peptide synthesis enabled systematic variation of the peripheral loops to generate libraries of targeted probes (Figure 4) [56].

Squaraine rotaxanes have also enabled tissue and subcellular imaging *in vivo*. Harmatys et al. developed probe **17**, bearing four iminodiacetate groups, which showed strong affinity for bone tissue and provided bright, persistent fluorescence during *in vivo* bone imaging (Figure 4) [55]. Macrocyclic encapsulation enhanced its chemical stability and imaging performance relative to the commercial bone-imaging agent OsteoSense750. The red-emitting squaraine rotaxane SeTau-647 was used for long-term two-photon imaging of neurons in living tissue and, after conjugation to a targeting peptide, for visualization of the postsynaptic protein PSD-95 [61]. Rice et al. demonstrated that the lipophilic, uncharged squaraine rotaxane SRFluor680 selectively accumulated in interscapular brown adipose tissue, enabling deep-red fluorescence imaging in living mice [73].

Targeted tumour imaging represents another important application of squaraine rotaxanes. A squaraine rotaxane functionalized with the cyclic peptide cRGDfK was developed to recognize integrin receptors overexpressed on the surface of many tumour cells [74]. Schreiber et al. employed spectrally distinct targeted and untargeted squaraine probes for paired-agent imaging of tumours in living mice, allowing receptor-mediated probe accumulation to be distinguished from nonspecific uptake [69].

Taken together, these studies demonstrate the advantages of combining a protected fluorescent core with peripheral functional groups responsible for biological recognition and tissue distribution. The mechanically interlocked architecture preserves the fluorescence signal and enhances dye stability, whereas the peripheral molecular design governs interactions with specific biological targets.

3.2 Biosensing and Biomolecular Detection

Stimuli-responsive squaraine rotaxanes are an emerging class of fluorescent materials in which external triggers, including redox reactions and competitive guest binding, reversibly alter the co-conformation of the mechanically interlocked structure. These changes regulate

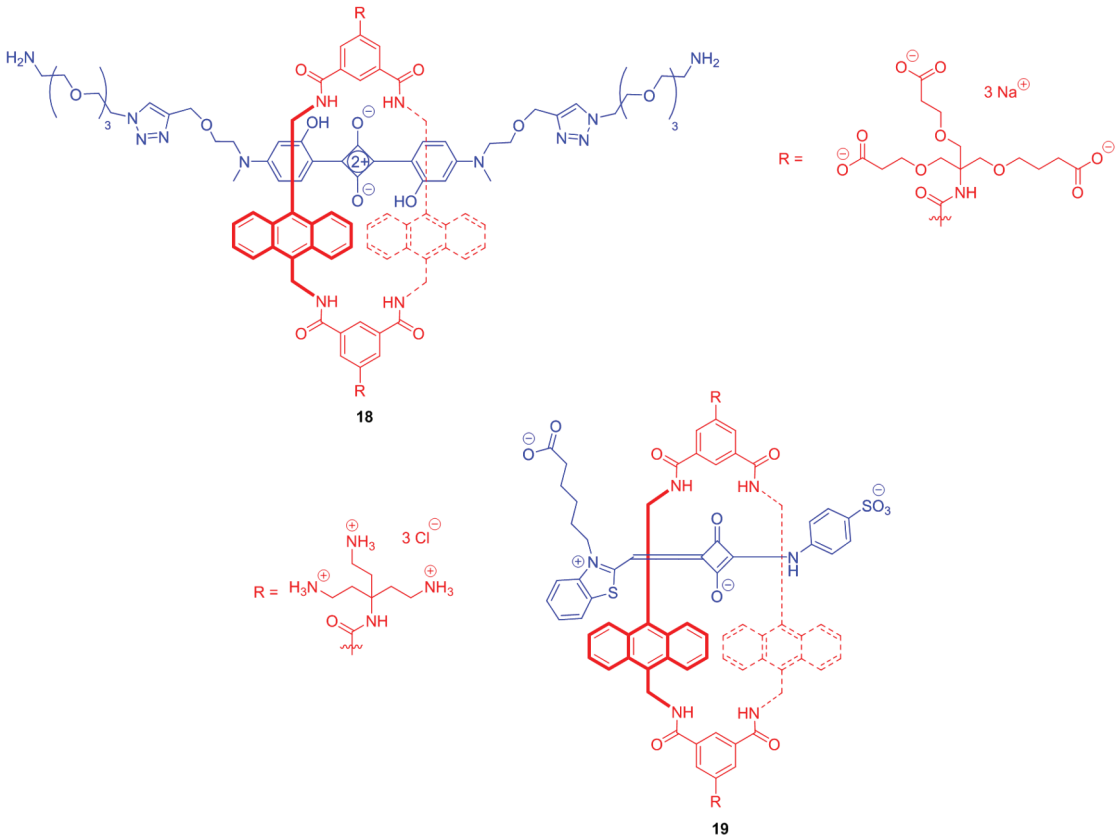


Figure 5. Examples of squaraine rotaxanes for biosensing and biomolecular detection.

the shielding of the squaraine chromophore, thereby modulating its fluorescence intensity, lifetime, emission wavelength, and photostability. Such responsive behaviour has enabled the development of mechanically interlocked squaraine systems for the detection of ions, small molecules, enzymes, and other biologically relevant analytes.

For example, Gassensmith et al. showed that formation of a pseudorotaxane complex between a squaraine dye and a tetralactam macrocycle resulted in an approximately 4-fold decrease in fluorescence intensity. Addition of strongly binding anions, such as chloride, acetate, or benzoate, displaced the squaraine dye from the macrocyclic cavity and nearly restored its fluorescence, enabling the system to function as a near-infrared fluorescent anion sensor [75]. Similarly, controlled displacement of a macrocycle along a squaraine axle in response to chloride binding enabled the development of a molecular sensing machine suitable for both instrumental detection and visual readout [76,77].

The same principles have been extended to biologically relevant sensing processes. Liu et al. developed a supramolecular fluorescent assay for neuraminidase activity based on enzyme-triggered threading of a squaraine dye into a tetralactam macrocycle to form rotaxane **18** (Figure 5). Neuraminidase cleavage of the bulky sialic acid groups enabled rapid threading, producing pronounced colorimetric and fluorescence changes and allowing enzyme activity and inhibitor efficacy to be evaluated [66]. Dharmarwardana et al. developed a supramolecular dye-capture system based on rotaxane **19**, in which encapsulation of an anionic amidosquaraine by a tetralactam macrocycle produced strong fluorescence enhancement, enabling a detection of phospholipase A_2-induced liposome leakage with an approximately 30-fold fluorescence increase [32].

Stimulus-induced changes in rotaxane co-conformation can also be exploited directly to control the optical properties of the squaraine chromophore. Wasternack et al. developed a redox-responsive co-conformational rotaxane in which the macrocycle reversibly shields or exposes a near-infrared squaraine dye, modulating its stability and fluorescence lifetime, while redox switching enables stepwise tuning of the emission wavelength from 570 to 683 nm

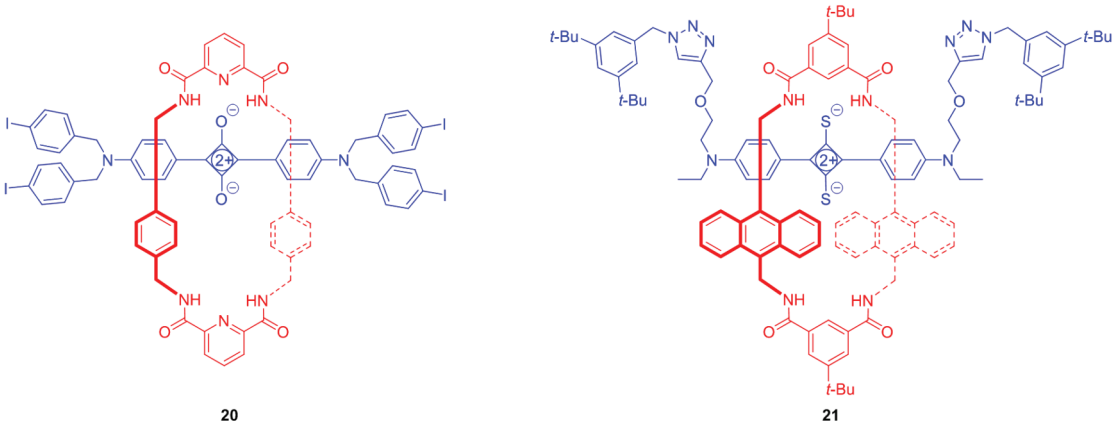


Figure 6. Examples of squaraine rotaxanes investigated as photosensitizers for PDT.

[43]. In another approach, a FRET-based fluorescent probe comprising a 1,8-naphthalimide donor and a squaraine rotaxane acceptor was developed for ratiometric detection of hydrogen peroxide in living cells, producing a deep-red fluorescence response with a large pseudo-Stokes shift [78].

Beyond direct analyte sensing, the high brightness, photostability, and versatile functionalization of squaraine rotaxanes provide opportunities for biomolecular recognition and the development of targeted biosensing platforms. Functionalized derivatives have demonstrated selective interactions with membrane proteins and receptors [79], biological membranes [64,65], and intracellular organelles such as lysosomes [67]. Further functionalization with selective recognition motifs could expand their application in biosensors targeting enzymes, proteins, nucleic acids, and disease-associated biomarkers [80]. These studies illustrate how incorporation of specific recognition motifs may further expand the application of squaraine rotaxanes toward the detection of biologically relevant analytes and disease-associated biomarkers.

3.3 Photodynamic Applications

The photodynamic activity of a photosensitizer depends on its ability to populate a long-lived triplet excited state, typically through intersystem crossing (ISC). The resulting triplet state can transfer energy to ground-state molecular oxygen to generate singlet oxygen through a Type II photochemical pathway or participate in electron-transfer processes leading to other reactive oxygen species through Type I pathways [81]. For many non-halogenated squaraine dyes, ISC is relatively inefficient; therefore, structural modifications that enhance spin-orbit coupling can substantially improve their photosensitizing properties [82,83].

In squaraine rotaxanes, these photophysical effects should be distinguished from the role of mechanical encapsulation. Rotaxane formation itself is not the primary origin of enhanced ISC or singlet-oxygen generation. Rather, encapsulation of the photosensitizing squaraine chromophore within a macrocycle can improve its chemical and photochemical stability and protect it from degradation by nucleophiles. The rotaxane structure improves molecular stability, while peripheral functional groups can be used to enhance water solubility, cellular uptake, and biological targeting. Incorporation of heavy atoms, such as iodine, can further increase singlet oxygen generation through the heavy-atom effect and thereby improve PDT efficiency.

In this context, Smith et al. investigated an iodinated squaraine and its mechanically interlocked rotaxane derivative **20** [84]. The iodine substituents promote ISC through the heavy-atom effect, thereby facilitating population of the squaraine triplet state and subsequent energy transfer to molecular oxygen. Indeed, laser flash photolysis confirmed formation of a long-lived triplet state for the iodinated squaraine rotaxane. Importantly, encapsulation within the tetralactam macrocycle substantially increased resistance to photobleaching, allowing the photosensitizer to generate singlet oxygen without rapid chromophore degradation. Thus, in this system, iodination is primarily responsible for enhanced triplet-state formation, whereas rotaxane encapsulation provides chemical and photochemical protection (Figure 6).

In another study, Peck et al. investigated a thiosquaraine rotaxane **21**, in which replacement of the squaraine carbonyl oxygen atoms by sulfur promotes ISC and enhances triplet-state formation (Figure 6) [85]. The thiosquaraine chromophore consequently acts as an efficient singlet-oxygen photosensitizer, while mechanical encapsulation within the tetralactam macrocycle protects the electrophilic core from nucleophilic attack. Upon red-light irradiation, the thiosquaraine rotaxane generated singlet oxygen with an efficiency comparable to that of methylene blue, demonstrating how triplet-state-promoting chromophore modification can be combined with mechanical protection to produce a more robust photosensitizer.

Although squaraine rotaxanes show considerable promise as photosensitizers, only a limited number of examples have so far been reported for PDT. Their performance depends on multiple factors, including singlet oxygen generation, photostability, aggregation, cellular localization, oxygen availability, dark toxicity, and phototoxicity. Further systematic biological evaluation will therefore be essential to assess their potential for translation into clinically relevant photodynamic therapeutic agents.

4. Challenges and Future Perspectives

Despite promising proof-of-concept studies, the biomedical potential of squaraine rotaxanes has yet to be fully realized. Future research should focus on improving their biocompatibility, aqueous solubility, synthetic accessibility, and functional versatility [86].

One major challenge is water solubility. Because squaraine rotaxanes typically contain extended aromatic squaraine chromophores and tetralactam macrocycles, additional hydrophilic functionalization, such as oligo(ethylene glycol), sulfonate, or carbohydrate substituents, is often required to ensure sufficient solubility and performance in physiological media [2].

Another limitation is the synthetic accessibility of squaraine rotaxanes. Current template-directed strategies generally require multistep procedures and may be highly sensitive to subtle structural variations in the axle and macrocycle. The development of simpler, more efficient, and scalable synthetic approaches would therefore greatly facilitate further research and biomedical applications [86]. In addition, the mechanisms underlying fluorescence enhancement upon macrocyclic encapsulation remain incompletely understood. Increased emission may result from a combination of chromophore rigidification, suppression of aggregation and other non-radiative decay pathways, changes in the local microenvironment, and electronic interactions between the squaraine dye and the macrocycle. A deeper understanding of these effects will support the rational design of brighter, more photostable, and functionally optimized fluorescent probes [87].

Improving selectivity toward specific biological targets represents another important research direction. Functionalization with targeting ligands, peptides, antibodies, aptamers, or other biomolecular recognition motifs could enable more precise interactions with specific cells, tissues, or disease-associated biomarkers. At the same time, the development of biodegradable and stimuli-responsive rotaxane systems could further expand their biomedical potential. Incorporating components that respond to biological or external stimuli, such as pH, redox conditions, enzymatic activity, or light, may enable spatiotemporal control over fluorescence, cargo release, or therapeutic activity [88]. Such advances could promote the transition of mechanically interlocked molecules from proof-of-concept systems to practical platforms for targeted imaging, biosensing, controlled drug delivery, and theranostic applications [89,90].

Comprehensive preclinical evaluation will also be essential for assessing the translational potential of squaraine rotaxanes. Future studies should address cytotoxicity, dark toxicity, phototoxicity, protein binding, cellular uptake, intracellular localization, metabolic stability, pharmacokinetics, biodistribution, and clearance in order to establish their safety and suitability for biomedical applications [2,91,92].

Another promising direction is the integration of squaraine rotaxanes into multifunctional delivery and nanomaterial platforms, including nanoparticles [93,94], polymeric materials [95,96], liposomes [97–99], DNA nanostructures [100,101], and peptide-based assemblies [102]. Such systems could combine the advantageous photophysical properties of squaraine rotaxanes with targeted delivery, molecular sensing, controlled cargo release, and therapeutic functions, thereby broadening their applications in imaging and theranostics. Although multifunctional platforms of this type remain relatively unexplored for squaraine rotaxanes, they represent a promising future direction inspired by advances in squaraine chemistry, supramolecular systems, and nanomedicine [103].

5. Conclusions

Squaraine rotaxanes represent a versatile class of mechanically interlocked fluorescent systems that combine the favorable optical properties of squaraine dyes with the protective effect of macrocyclic encapsulation. Encapsulation of the squaraine chromophore within a tetralactam macrocycle can partially suppress non-radiative relaxation, improve chemical and photochemical stability, and enhance fluorescence performance under biologically relevant conditions.

These advantages, together with the possibility of functionalizing both the squaraine axle and the macrocyclic component, have enabled the development of probes and labels for cellular and *in vivo* bioimaging, selective targeting of biological structures, biosensing, biomolecular detection, and photodynamic applications. Stimuli-responsive squaraine systems further demonstrate how threading, guest displacement, redox switching, and FRET can be exploited to generate analyte-dependent optical responses.

Despite these promising results, the biomedical application of squaraine rotaxanes remains at an early stage. Further progress will require improved water solubility and biocompatibility, simpler and more scalable synthetic strategies, and a deeper understanding of how the squaraine chromophore structure, macrocycle architecture, and peripheral functionalization influence photophysical properties, stability, and biological performance. The integration of squaraine rotaxanes with targeting motifs, stimuli-responsive elements, and multifunctional delivery or nanomaterial platforms may further expand their potential for advanced bioimaging, biosensing, photodynamic therapy, and theranostic applications.

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