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Selectivity advancement through chemical structure engineering: Long-term intracellular DNA recognition, chromosomal staining and micronuclei detection

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ABSTRACT

Simultaneous integration of the most desired properties such as high photostability, negligible cytotoxicity and phototoxicity, good brightness, large Stokes shift and high selectivity toward DNA over RNA into a single DNA stain has always been challenging. Despite several efforts, the practical development of such DNA stain has been rare. In this context, probes (**RD1**–**RD8**) were synthesized and the worth of crescent geometry toward DNA selectivity of **RD2** was established through careful structure–interaction relationship studies. Probe **RD2** displayed admirable selectivity toward dsDNA with noticeable fluorescence enhancement ($\phi = 0.23$). Molecular simulation was performed to comment on its stable conformation and optical changes observed due to electronic distribution in the presence of DNA. The excited state dynamics and binding mechanism were evaluated through fluorescence life-time and anisotropy measurements in the presence and absence of DNA. Further, the cell studies demonstrated its quick membrane permeability, extremely low cytotoxicity and potential toward specific nuclear staining with high contrast. Moreover, the negligible photoinduced cellular deterioration and high photostability under continuous laser scanning shed light on its long-term applicability for cellular investigations. Finally, the utility of **RD2** in fluorescence based detection of cell-proliferation and micronuclei reflected its encouraging potential as a bioprobe for cellular imaging.

1. Introduction

Being the fundamental unit of life, DNA plays the vital role in long-term storage of information in the form of genetic code to instruct the cellular development and genetic propagation. The temporal profile of structural and morphological architecture of DNA provides a deep insight into molecular pathology, medical diagnosis as well as bioanalytics [1–5]. Besides, the gross study revealed that DNA revolutionized the social aspects of life also through its influential impact on forensics [6], bioterrorism control [7] as well as agriculture and farming industry [8,9]. In this sense, because of its vitality for life, the selective detection and molecular imaging of DNA became a dire need and the subject of immense curiosity for biochemical research community. As a result of continuous efforts, fluorescence microscopy has grown as an ideal modality in the field of molecular imaging and cellular recognition [5]. In this context, commercialized dyes such as TOTO, YOYO [10] and cyanine dyes [11], well known for their DNA specificity and quantum efficiency, have been used successfully for the understanding of nuclear dynamics. Moreover, through decades of efforts stains such as thiazole orange [11], oxazole yellow [12], piperazine containing dyes [13] and indole-quinolinium cyanine [14] also came into existence. But the choice of a promising nuclear stain with enhanced discriminative expertise toward DNA as compared to RNA is still a critical issue. In addition, highly specific DNA dye with strong photostability and low phototoxicity is rare. Hence, the frequent inadequacies of the reported nuclear stains necessitated the rational design and adaptation of their molecular framework to meet their appreciable selectivity, biocompatibility, membrane permeability [15], low cytotoxicity [16] and less susceptibility toward photobleaching [17] for their wide applicability as

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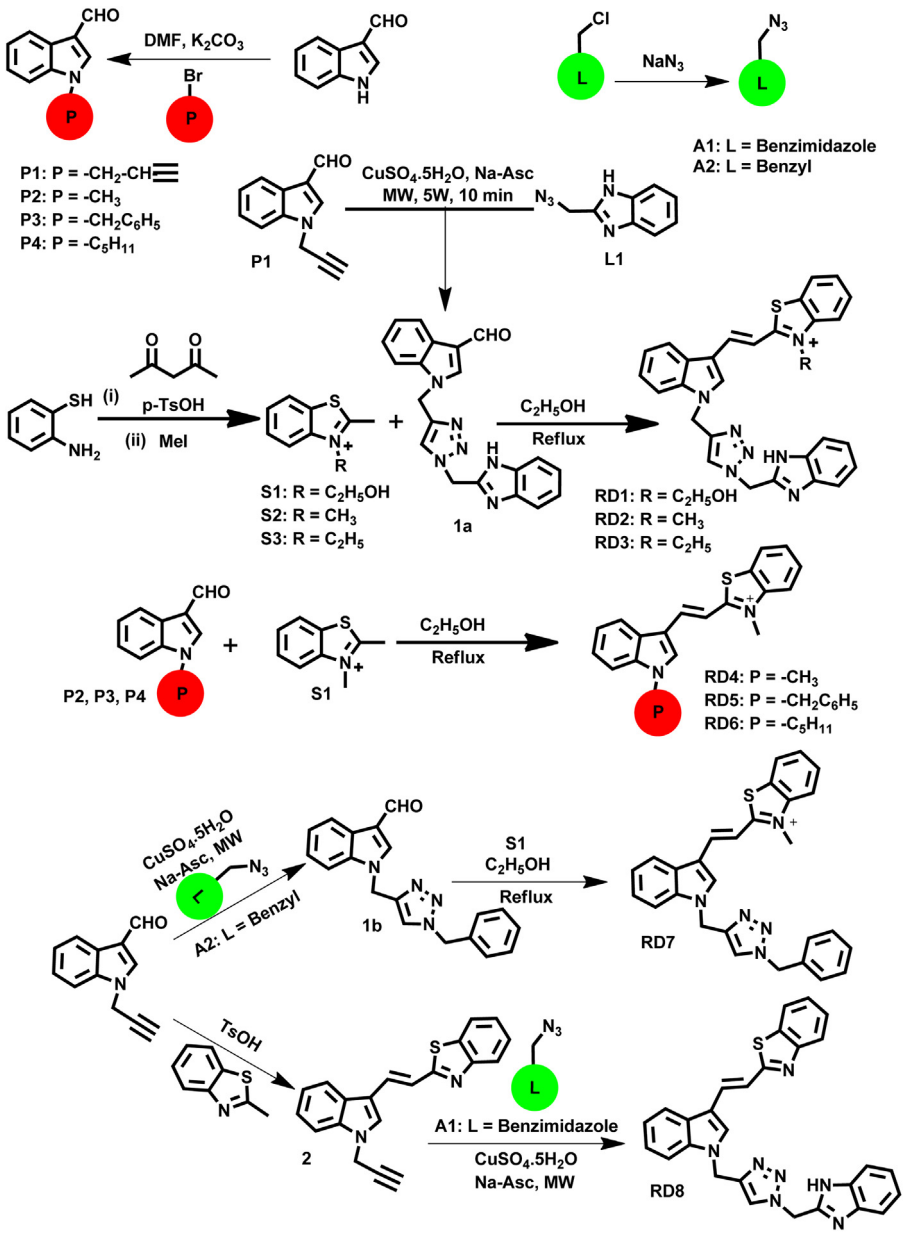

Scheme 1. Synthetic pathway of the probes **RD1**–**RD8**.

nucleus targeting stains in the fields of molecular imaging, clinical as well as biomedical research.

In this contribution, herein we report our newly devised molecular probes with a triazole linked benzimidazole-indole-thiazolium mixed heterocyclic framework as depicted in Scheme 1.

Their architecture was designed to achieve the advancement in selectivity and specificity toward DNA matrix over RNA through the incorporation of structural complexity into the molecular framework. The probes were synthesized by easy accessible synthetic protocols (see Scheme 1 and supplementary material). The optical behavior of the structurally characterized probes in the presence of DNA and other bioanalytes was examined by absorption and emission spectroscopy under physiological conditions. Moreover, a careful structure–interaction relationship study supported by the molecular simulation analysis revealed that the structurally engineered crescent geometry of the probes was the governing factor for their strong interaction with DNA matrix [18–22]. Furthermore, the set of cellular investigations established the potent standing of **RD2** as a probe of choice for long-term cellular investigations as well as cell-proliferation mimicking and micronuclei detection. Click reaction has become the most preferred smart chemical tool in recent times for functionalization of biomolecules [23]. However, we have successfully shown here that click reaction can be used to introduce crescent geometry through rational design into molecular architecture for enhancing biomolecular selectivity of optical probes.

2. Experimental

2.1. Materials

Solvents and chemicals were purchased from commercial resources and used without further purification. Spectroscopic grade dimethyl sulfoxide (DMSO) was used for photophysical studies. Doubly ionized water used in all experiments is from Milli-Q systems. PBS (pH 7.34, 0.1 M) was prepared using doubly ionized