Chapter-7

Summary, Conclusion and Future Perspectives

7. Summary, Conclusion and Future Prospectives

7.1. Summary and Conclusion of Work

In **Chapter 1**, we introduced the concept of fluorescence and explained the phenomenon using a Jablonski diagram. We also delved into a comprehensive discussion about fluorescence dyes, including their chemical classes, importance, and applications in bioimaging. Additionally, we explored the usage of fluorophores, auxochromes, and fluorescent detectors in various fluorescence-based applications. Furthermore, we elaborated on the advancement of amino-based auxochromes over the years, discussing their benefits and drawbacks and providing a brief insight into our aim and objective.

In **Chapter 2**, we delineated the challenges linked to existing auxochromes and the limitations of fluorescent probes designed over the years. Consequently, we identified a research gap and endeavoured to uncover viable solutions to address this issue, ultimately bridging this gap.

In **Chapter 3,** we examined the photophysical properties of azacoumarin fluorophores and identified dihydropyrrolo-4-methylcoumarin as the brightest and most stable. This compound also demonstrated excellent biocompatibility, pH stability, photostability as well as excellent cellular uptake, leading us to develop a probe (**PYCB)** from it for H_2O_2 detection. The probe exhibited strong selectivity and sensitivity within the 5-100 μM concentration range and remained stable in the pH range of 2-8. Substantial biocompatibility and successful fluorescence imaging of H_2O_2 in MCF-7 breast cancer cells confirmed the effectiveness of the **PYCB** probe, highlighting the potential of azacoumarin-based fluorescent probes.

Chapter 4 demonstrates the identification of cycloalkylamines as potential auxochromes for coumarin fluorophores. Herein, we have expanded the scope of luminogenic coumarins beyond the canonical azetidine analogue by synthesizing 7-cycloalkylaminocoumarins using Buchwald-Hartwig cross coupling reactions. The scope of the reaction extends to a variety of

substrates such as cycloalkylamines, alkylamines, and cyclic amines in good to excellent yields. The photophysical properties of synthesized analogues revealed that the cyclobuytylaminated- and the cyclopentylaminated-coumarin derivatives have the highest quantum yields, slightly elevated than standard azetidinylated coumarin analogue. Theoretical analysis suggests that the ring size of cycloalkylamine plays a drastic role in controlling the brightness of the coumarin scaffolds. Further evaluations show decent pH stability and photostability of these compounds. MTT assay in both cancer and non-cancer cell lines shows substantial biocompatibility of both these derivatives. Fluorescence imaging in MDA-MB-231 cell lines further demonstrates the future scope of the application of such new dyes in *in vivo* imaging. Cycloalkylamines, especially cyclobutylamine and cyclopentylamine, show all the perks of azetidine auxochrome and also provide a nitrogen centre for further functionalization into a biochemical-detecting probe.

In **Chapter 5**, we sought to explore the versatility and scope of cycloalkylamines as auxochromes beyond coumarins and thus intended to extend their applications to other fluorophores, such as naphthalimide and 4-nitrobenzoxadiazole. In this chapter, syntheses of cycloalkylamine-substituted naphthalimides and 4-nitrobenzoxadiazole were reported and these synthesized derivatives were compared with their azetidinylated counterparts for their light-emitting properties. The photophysical studies exhibited that the cyclobutylaminated analogues of both these fluorophores showed excellent quantum yield and brightness as compared to theor reference standards. The quantum chemical calculations also corresponded to the practically obtained photophysical results. Cytotoxicity assay of compounds in both cancer and non-cancer cells indicated excellent biocompatibility of these cyclobutylaminated analogues of naphthalimides and 4-nitrobenzoxadiazole. The molecules also demonstrated excellent cell penetration and better photostability as compared to their respective standards,

thus showcasing the plausibility of further application of such cyclobutylamine-substituted fluorophores in design and synthesis of fluorescent probes.

In **Chapter 6**, we delve into the design and development of a ratiometric probe known as **NAPB**. This probe utilizes a cyclobutylamino-functionalized naphthalimide as its fluorophoric unit and is tailored for the selective and sensitive detection of GSH. In presence of GSH, NAPB undergoes a ratiometric change in fluorescence emission from blue to greenish yellow due to the cleavage of the former to its parent fluorophore. The probe exhibits a linear relationship with GSH concentration within a range of 0 to 10 equivalents with a very low detection limit. It also demonstrates a swift response to GSH incubation and remains stable within the physiological pH range. MTT assay conducted in HepG2 cells confirms its acceptable biosafety up to a concentration of 5 μM. Fluorescence imaging in HepG2 cells underscores the potential of NAPB as a powerful tool for detecting GSH in living cells. Furthermore, the probe shows great promise in advancing research on GSH-related disease diagnosis and inspiring the development of other innovative fluorescent probes for GSH or other biothiol detection.

The visual representation provides a picturesque illustration that succinctly encapsulates the fundamental aspects of our work (Figure 7.1). Our findings indicate that annulated amino auxochromes and cycloalkylamine auxochromes serve as superior alternatives to previously reported auxochromic moieties. They exhibit all the photophysical and physicochemical benefits of the desired auxochromes while also offering attachment sites for functionalization of response units. Notably, cyclobutylamine auxochrome enhances the brightness and various physicochemical properties of the entire fluorophore. Its impact has been demonstrated on three fluorophores, each from a different range of the spectrum: coumarin (blue), naphthalimides (green), and nitrobenzoxadiazole (yellow). These auxochromes have incredible potential for advancing research in the detection of various biomarkers, environmental toxicants, and other biochemicals.

7.2. Future Prospects

The present research underscores the distinctiveness of auxochrome units and their consequential contributions to the characteristics of fluorophores subsequent to their conjugation. However, the fluorophores investigated in this study exhibited emissions within the blue to yellow spectral range, raising concerns regarding potential *in vivo* toxicity upon prolonged application. Therefore, our impending course of action involves either substituting the existing fluorophores with those emitting red light or effecting modifications to the current fluorophores to engender emissions within the red region of the spectrum through enhanced conjugation.

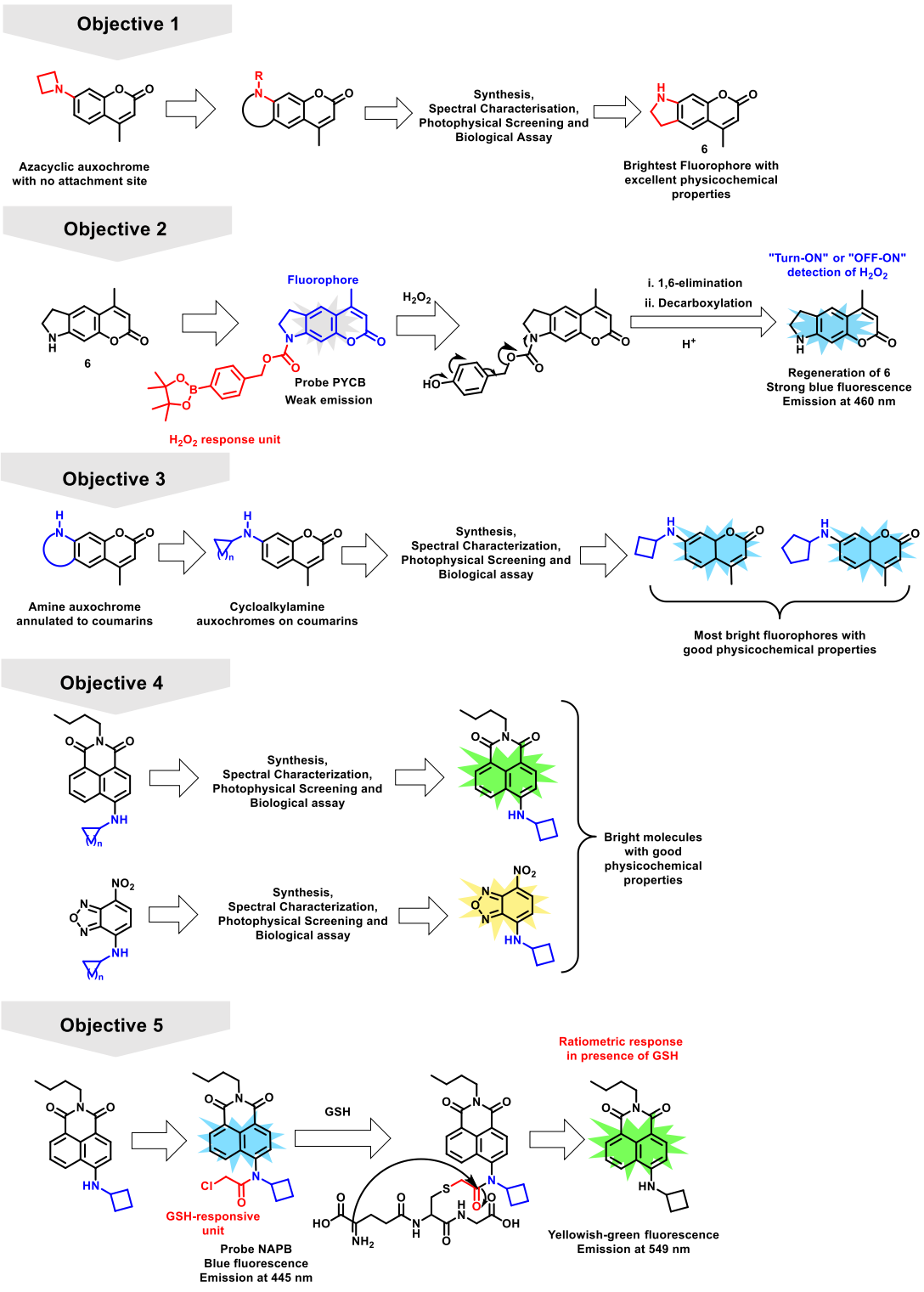

Figure 7.1. Illustration depicting the fundamental aspects of our work.