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Title	Heteroazoarenes as Smart Molecular Switches : From pH sensing to Light-Controlled Wnt Agonism [an abstract of dissertation and a summary of dissertation review]
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Abstract of Doctoral Dissertation

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Title of Doctoral Dissertation

Heteroazoarenes as Smart Molecular Switches: From pH sensing to Light-Controlled Wnt Agonism
(スマート分子スイッチとしてのヘテロアゾアレーン： pH センシングから光応答性 Wnt 受容体活性化作用まで)

Molecular switches undergo reversible transition between stable or metastable states in response to specific stimuli, affecting its physical, chemical, or electronic properties. Molecular switches show distinguishable changes in absorbance, fluorescence, color, conductivity, or conformation, making them useful for various material and biological applications. However, new designs of molecular switches are still very demanding in the context of unexplored applications. For instance, well-studied azobenzene molecular photoswitch that can respond to both photon and acid/base stimuli depending on the molecular design, however required harmful UV light and hence is not suitable for many applications as in the case of photopharmacology applications. Heteroazoarenes are novel category of molecular switches that has heteroarene segment either one or both sides of azo bond showing a red-shifted absorption compared with azobenzenes. My PhD research focused on the development of novel heteroazoarenes as smart molecular switches for near-neutral pH sensing application and light-controlled activation of Wnt signaling pathway.

Chapter 1: pH sensing is one of the applications of colorimetric molecular switches as it is essential in many fields, including chemical and biological processes, clinical diagnosis and environmental sciences. pH indicators are classified as fluorometry-based or colorimetry-based, with colorimetry offering the advantage of visual detection without instruments. Azobenzene dyes are well-known colorimetric indicators but are effective only in strong acidic or alkaline conditions (e.g., methyl yellow, pK_a 3.3, and alizarin yellow, pK_a 11). Near-neutral pH indicators are particularly useful for detecting pH changes in biological systems, such as tissue and cell cultures, where waste products can alter the pH. Chapter 1 research focused on the development of novel near-neutral pH indicators using heteroazoarenes with thiazole, benzothiazole, thiadiazole, pyrazole, and triazole groups (Figure 1). Our lab has previously studied heteroazoarenes with five-membered heteroarene as photoswitches. In the present study, I found that compounds **1–10** have very different spectral properties in acid and base aqueous solvents, showing red-shifts in base aqueous media. UV-vis titrations revealed dynamic spectral changes with pH variation. For example, compound **1** shifts from λ_{max} 479 nm (pH 10.2) to 364 nm (pH 4) with a color change from yellow (pH 6) to red (pH 7.8), and a pK_a of 7.4. Substituents, like NO₂, influence properties, as seen with absorption shifts from 435 nm (low pH) to 590 nm (high pH) and a detection range of pH 4.2–5.9. The hydroxyl group at para position of phenyl ring is critical, as OMe-substitution eliminates pH sensitivity. DFT calculations suggest pH sensing arises from hydroxyl anion formation and delocalization. Compound **1** demonstrated practical use in cell culture media, detecting pH changes (red to orange, pH 7.7 to 6.7) without cytotoxic effects. This highlights azoheteroarenes as promising pH indicators, with potential for distinguishing cancerous (pH 6.4–7.0) from normal tissues (pH 7.2–7.5).

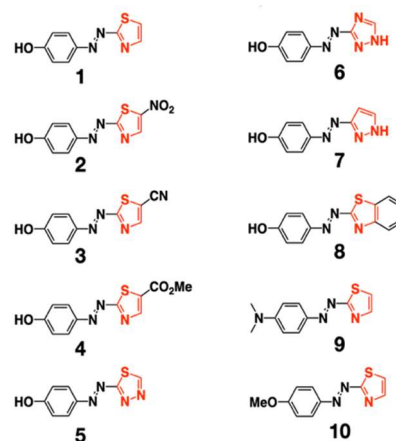


Figure 1. Novel heteroazoarenes pH indicators **1–10**.

Chapter 2: In chapter 2, I focused on photocontrolling of the Wnt signaling pathway which plays an important role in processes like gene regulation, embryonic development, cell adhesion, proliferation and differentiation. Heteroazoarenes as a photoswitchable agonist for Wnt signaling pathway based on the structure of the known Wnt agonist BML-284 were designed. Compound **1** and **2** to activate the Wnt signaling pathway only by the *cis* isomer because of the similar bent structure to BML-284 in the host protein T2R-TTL. Compound **1** and **2** were synthesized by Mills reaction between diamino methoxyphenyl pyrimidine and nitrosobenzene derivatives. These photoswitchable agonists undergo reversible *trans*-*cis* isomerization under visible light but activate the Wnt pathway only in the *cis* isomer as observed by a luminescence-based reporter assay in cultured cells (Figure 3). Compound **2** showed ~88 % agonist activity after visible light irradiation in comparison to the non-photoswitchable BML-284. I also achieved selective activation of the Wnt signaling pathway using **2** by light irradiation at specific region of interest in a model cell culture system highlighting the spatiotemporal regulation.

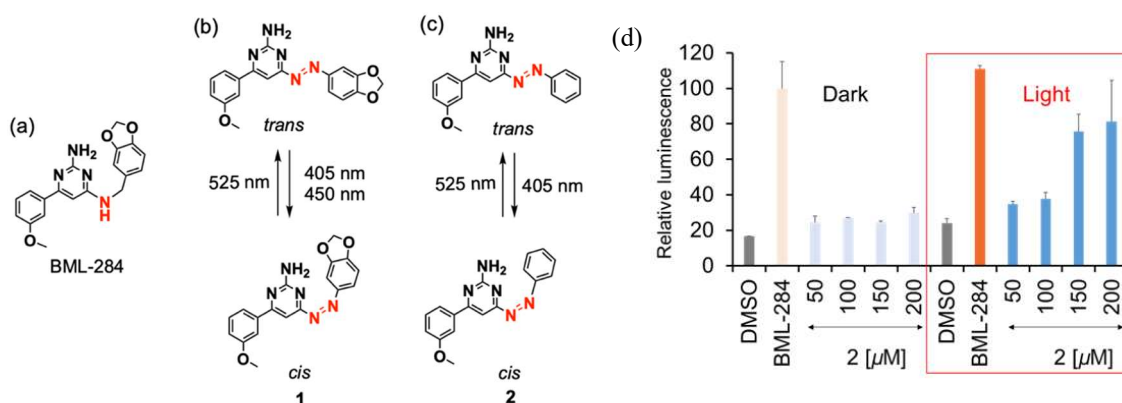


Figure 3. (a–c) Molecular structures of a non-photoswitchable Wnt agonist **BML-284** and photoswitchable Wnt agonists **1** and **2** along with their reversible *trans* – *cis* isomerization by visible lights (405 nm, 450 nm, or 525 nm). (d) Bar graph of relative luminescence measured after treatment with DMSO, BML-284 (5 μ M) and photoswitchable agonist **2** in dark or light.