Synthesis and Analysis of Benzothiazole Derivatives with π-π* Transition and Charge Transfer Characteristics

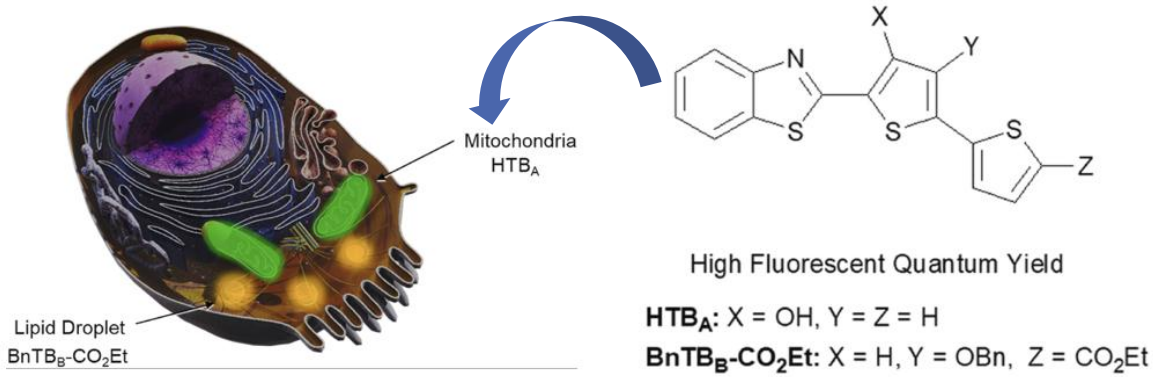
Fall 2025, Advanced Chemistry Major Experiment (CHE4107.01-00)

Biopolymer Lab (Prof. Woo-Dong Jang)

Jun Hyung Park

Experimental Objectives

- 1. Synthesize organic molecular probes exhibiting luminescence mechanisms such as ESIPT (Excited-State Intramolecular Proton Transfer) and ICT (Intramolecular Charge Transfer), and investigate how these mechanisms vary depending on molecular structure and environmental factors (solvent, pH).
- 2. Selectively stain intracellular mitochondria and lipid droplets (LDs) using the synthesized organic molecular probes.

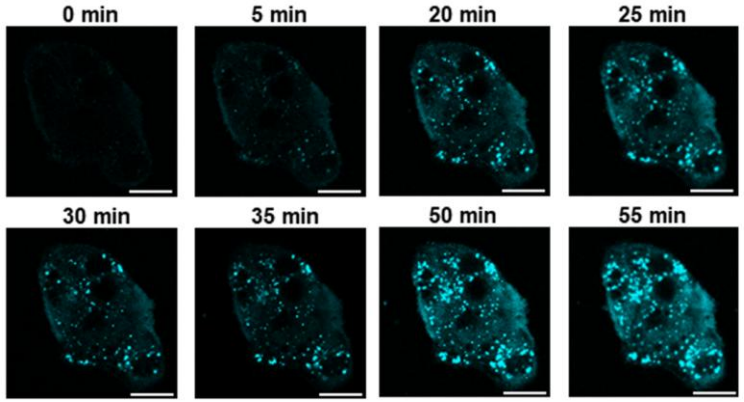


High Fluorescent Quantum Yield

HTB_A: X = OH, Y = Z = H

$BnTB_B$-CO_2Et: X = H, Y = OBn, Z = CO_2Et

Mitochondria with Fluorescent Probe

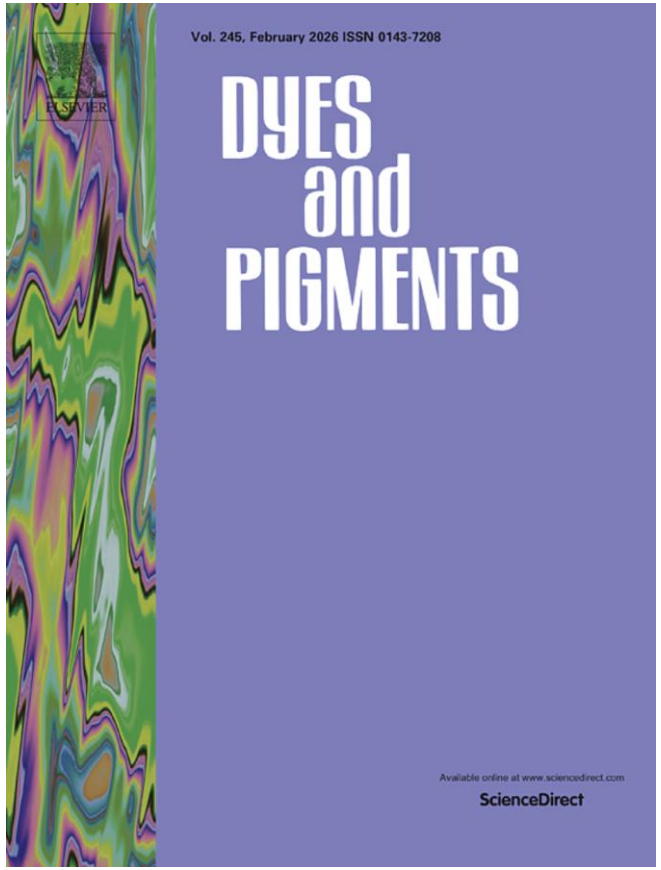


- Biomarker Monitoring
- Disease Diagnosis

Experimental Theory

- 1. π-π* transition:
  - An electronic transition that occurs when a molecule absorbs a photon, causing an electron to be excited from the π bonding orbital (HOMO) to the π* anti-bonding orbital (LUMO).
- 2. Charge transfer:
  - 2-1. ICT (Intramolecular Charge Transfer)
    - A process in the excited state where electron density moves from an electron donor to an electron acceptor through a π-conjugated bridge.
  - 2-2. ESIPT (Excited-State Intramolecular Proton Transfer)
    - A process in the excited state where a proton is transferred from a functional group (e.g., -OH) to an adjacent acceptor atom (e.g., the nitrogen atom within the benzothiazole ring).

Paper Referenced for Synthesis Procedure



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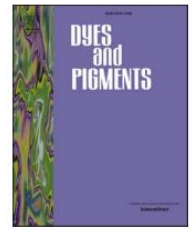

Check for updates

Thiophene-conjugated benzothiazole derivatives as versatile skeletons for staining subcellular organelles in living cells

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ARTICLE INFO

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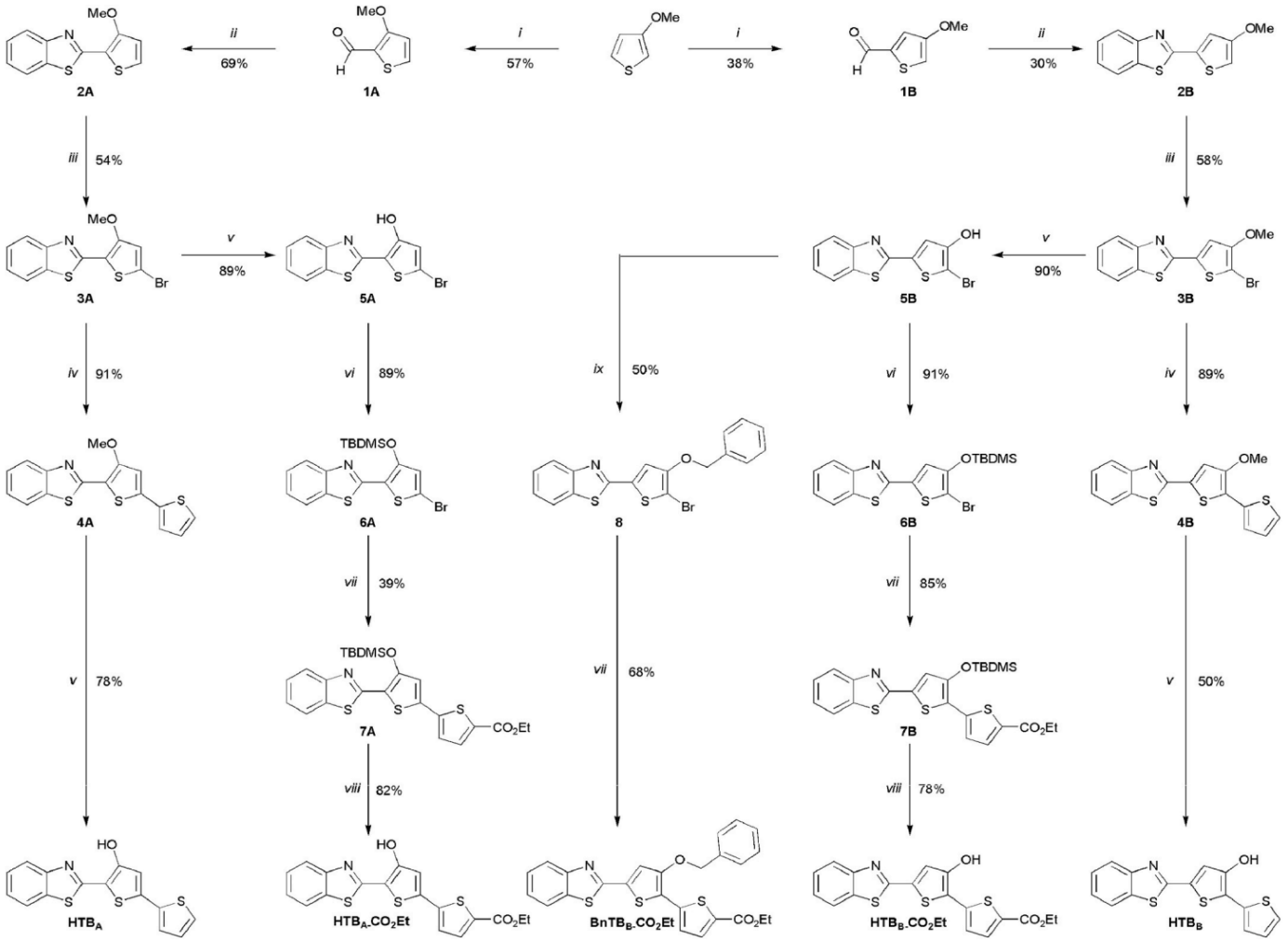
Lipid droplet
Intramolecular charge transfer
Thiophene

ABSTRACT

A series of hydroxy thiophene-conjugated benzothiazole derivatives (**HTBs**) was prepared to investigate their fluorescence emission behaviors and capability to selectively stain subcellular organelles for fluorescence imaging. The **HTB** derivatives exhibited excited-state intramolecular proton transfer (ESIPT) and intramolecular charge transfer (ICT) behaviors, according to their molecular structure. Subtle changes in the molecular structure of the **HTB** influenced its response to changes in the surrounding environment, such as the solvent polarity and/or pH. *In vitro* cellular imaging experiments revealed that **HTB_A** (5-(benzo[d]thiazol-2-yl)-[2,2′-bithiophen]-4-ol) could selectively stain mitochondria. In contrast, **HTB_B-CO_2Et** (ethyl 5'-(benzo[d]thiazol-2-yl)-3′-hydroxy-[2,2′-bithiophene]-5-carboxylate), which contains a regioisomeric hydroxy thiophene unit with an electron-withdrawing ethyl ester group, showed the capability to stain lipid droplets (LDs) in living cells. When the hydroxyl group in **HTB_B-CO_2Et** was protected with a benzyl group, effective staining of LDs in live cells was achieved. Thus, we successfully obtained an effective fluorescent probe. Collectively, the results indicated that small structural changes resulted in substantial changes in the intracellular localization and *in vitro* imaging ability of the prepared compounds.

Synthesis Pathway for Probes 1 and 2

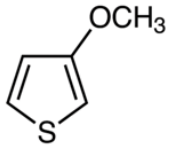
Starting Material



Product 1

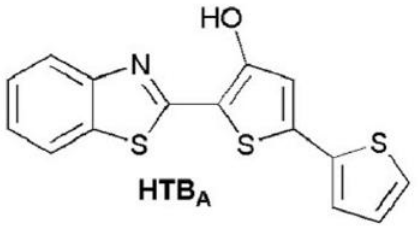
Product 2

Starting Material



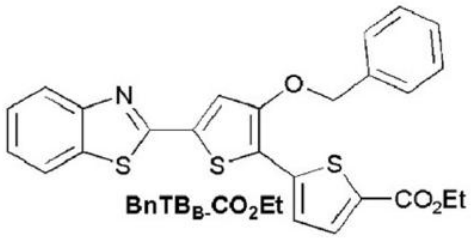
3-Methoxythiophene

Product 1



(5-(benzo[d]thiazol-2-yl)-[2,2′-bithiophen]-4-ol

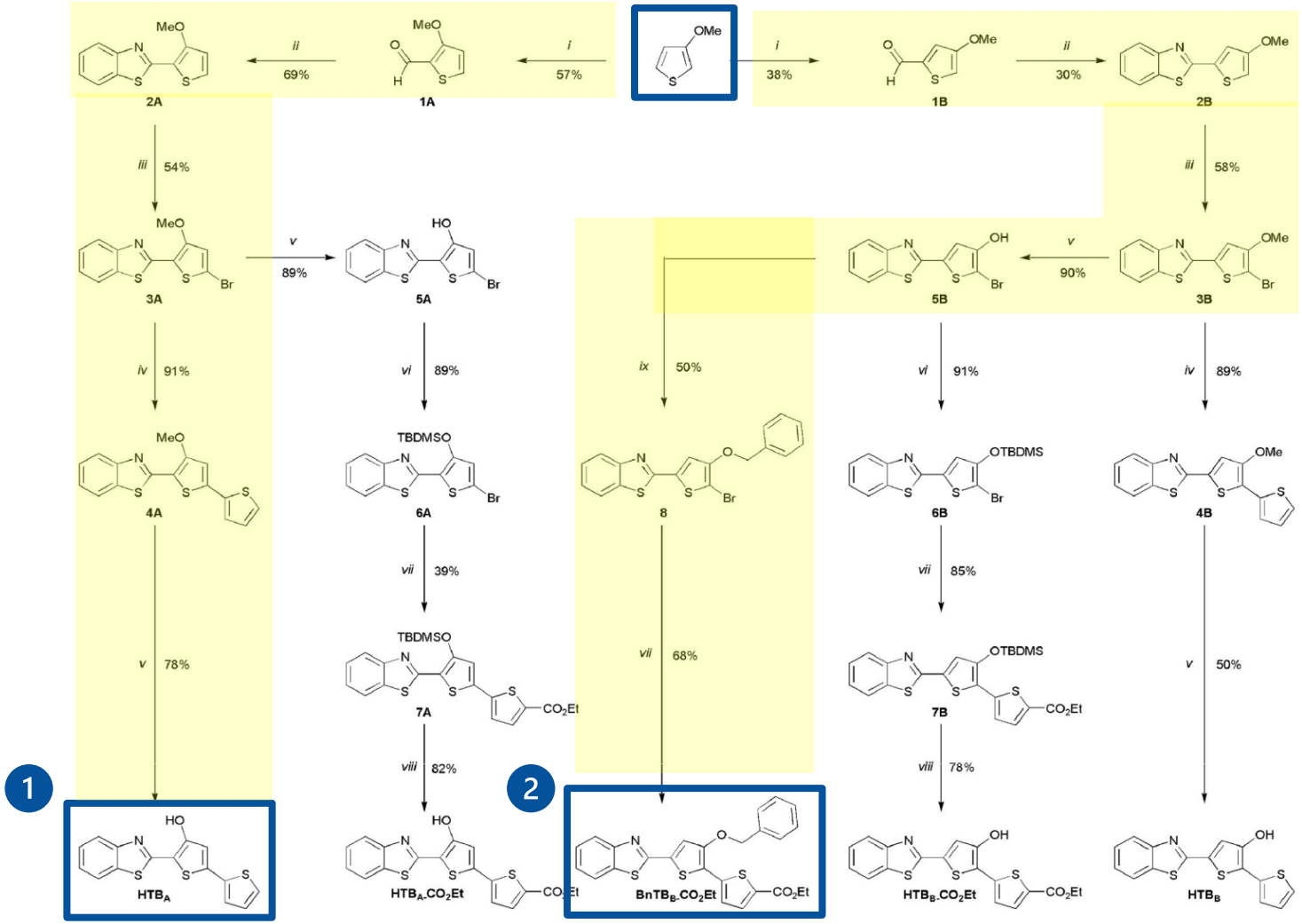
Product 2



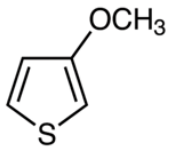
(ethyl 5′-(benzo[d]thiazol-2-yl)-3′-hydroxy-[2,2′- bithiophene]-5-carboxylate

Synthesis Pathway for Probes 1 and 2

Starting Material

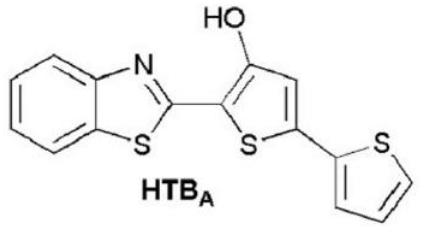


Starting Material



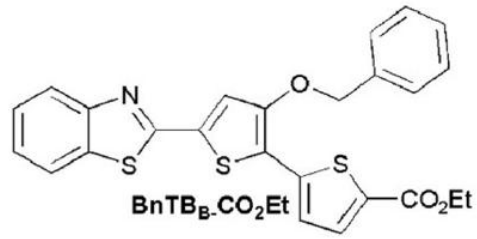
3-Methoxythiophene

Product 1



(5-(benzo[d]thiazol-2-yl)-[2,2′-bithiophen]-4-ol

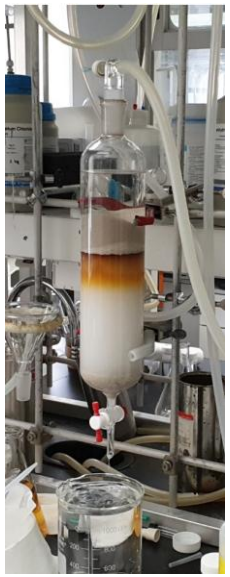
Product 2

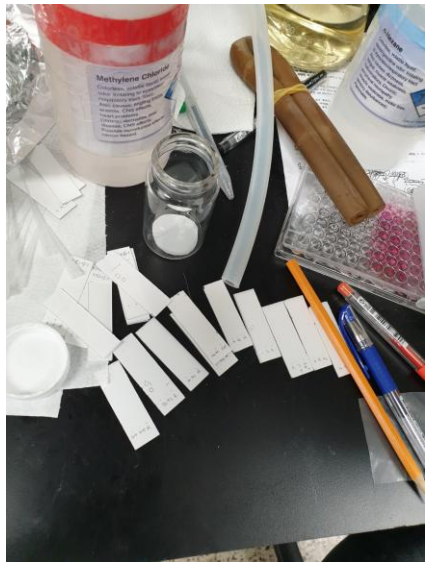


(ethyl 5′-(benzo[d]thiazol-2-yl)-3′-hydroxy-[2,2′- bithiophene]-5-carboxylate

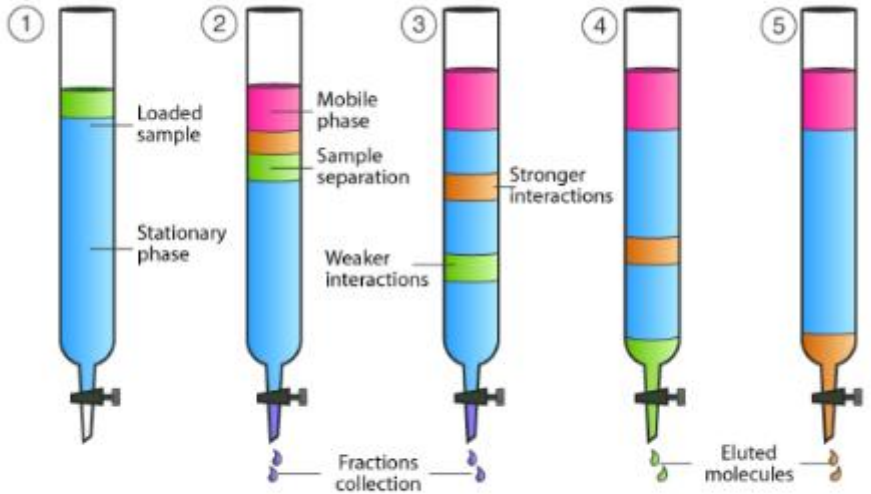
Synthesis

Column Chromatography + TLC



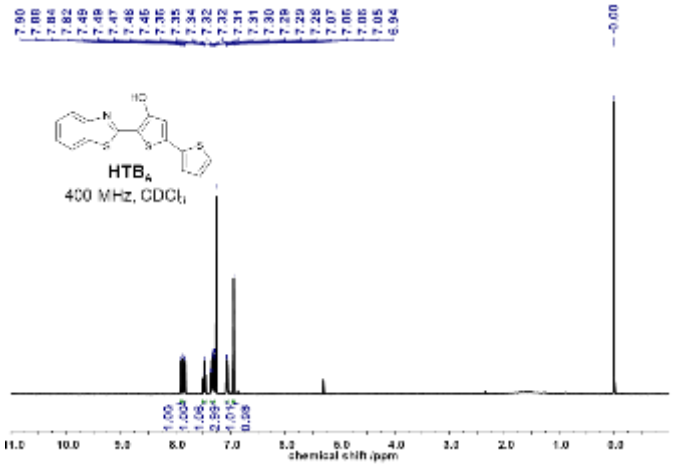


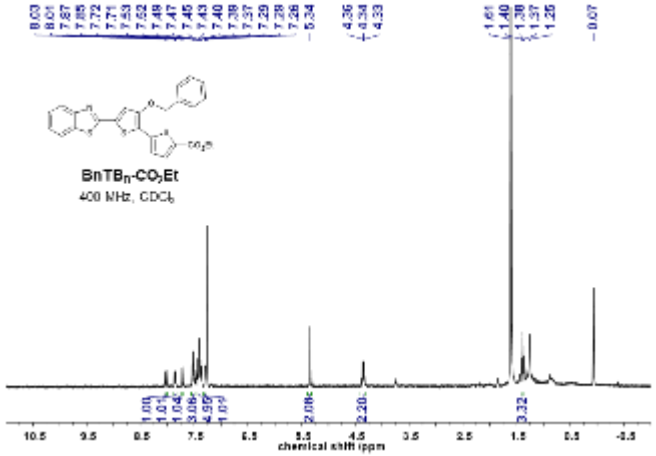
Column Chromatography



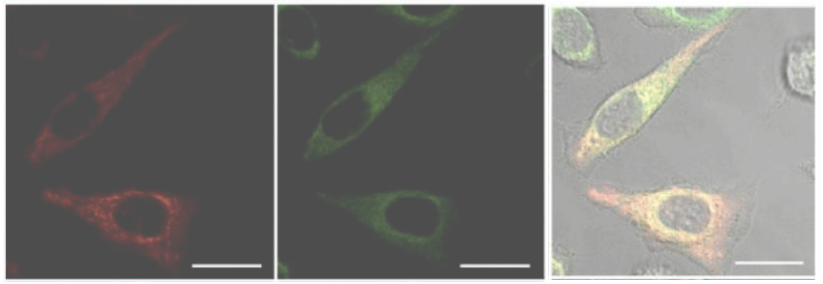
Column Chromatography Diagram

^{1}H NMR Spectrum

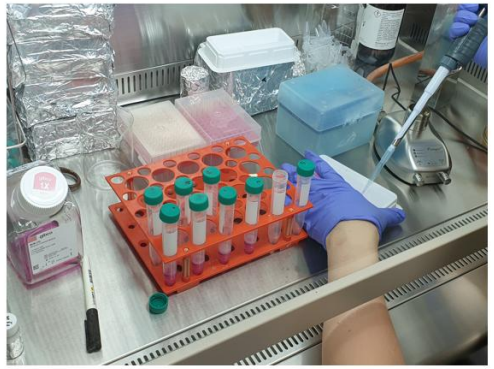




Fluorescence Imaging (HeLa Cell)

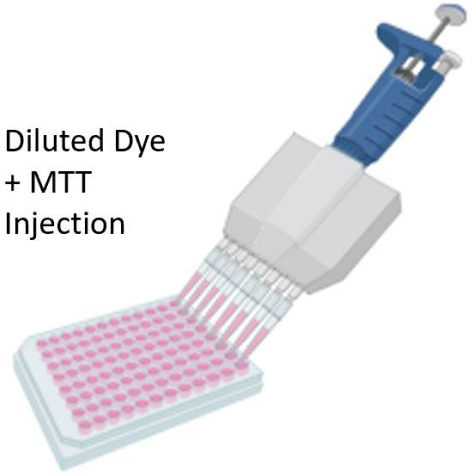


MTT Assay after Probe Synthesis

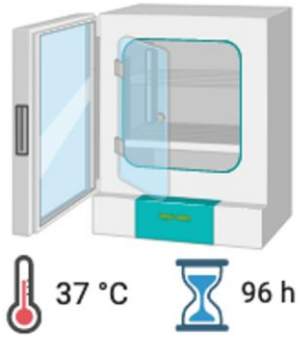


Dye Serial Dilution + DMSO

Diluted Dye
+ MTT
Injection



Incubation + UV-Vis Absorbance



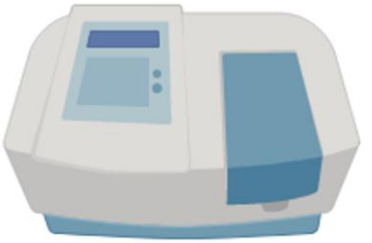
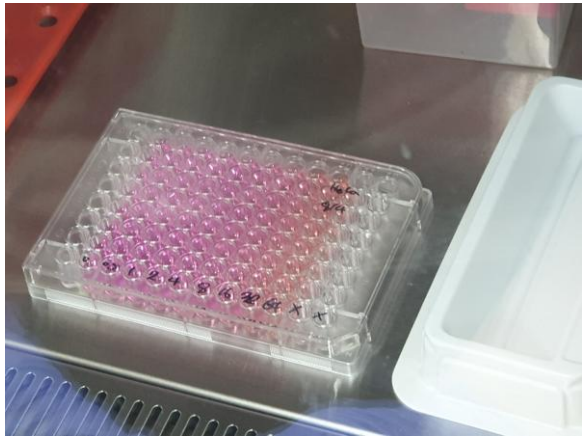
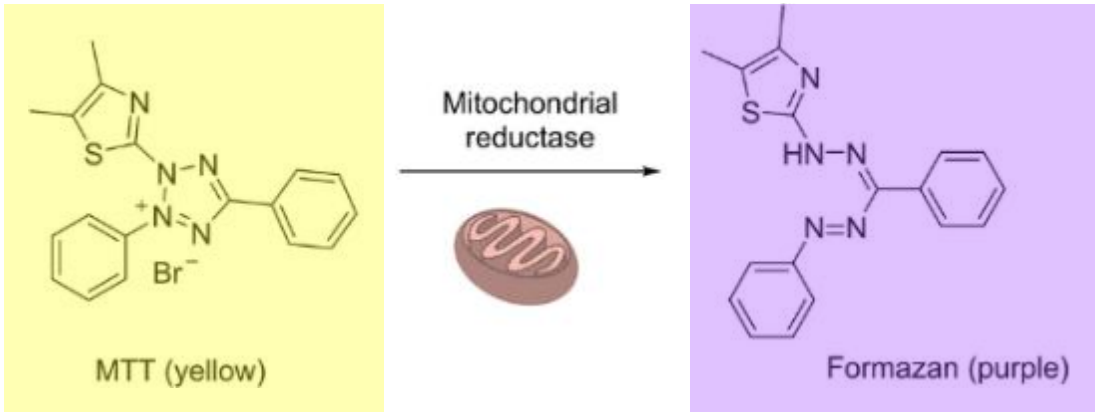


Plate read on microplate spectrophotometer at 540 nm



96-Well Plate

Assay Mechanism



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