CHAPTER I

INTRODUCTION

1.1 Background and significance

Nitroreductases (NTRs) are FMN-dependent enzymes that can reduce a range of nitro-containing compounds in the presence of reduced nicotinamide adenine dinucleotide (NADH) or reduced nicotinamide adenine dinucleotide phosphate (NADPH) as the reducing agent (Parkinson et al., 2000; Peter F. Searle et al., 2004). In general, nitroreductase enzymes are found in various bacteria species, including ESKAPE pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter spp.*) that are common causes of life-threatening nosocomial infections amongst critically ill and immunocompromised individuals due to their potential drug resistance mechanisms (Santajit and Indrawattana, 2016).

In recent years, the development and utilization of fluorescent probes have emerged as powerful tools for studying nitroreductase activity with high sensitivity and selectivity (Jia et al., 2021; Wang et al., 2023). Previously, Wang and colleagues developed a two-photon fluorescent probe, TP-NO_2, with simple synthesis steps and high yield. Their probe has been successfully applied for NTR detection in cells under simulated hypoxia conditions (Wang et al., 2020). Wang and colleagues also developed NFP-NTR a benzoindole-based fluorescent probe with excellent selectivity and satisfactory sensitivity for detecting nitroreductases in live Hela cells under hypoxic conditions with low cytotoxicity (Wang et al., 2022). However, the development of NTR probes to enhance their efficacy is still attractive. In this study, a NTR-responsive fluorescent probe was tested to detect NTR in microorganisms (Figure 1.1). The reduction of the probe's nitro group was demonstrated by *E. coli* nitroreductase (*Ec*NfsB) catalyzed *in vitro* reaction to form an amino group, that generates the fluorescent signal. Therefore, we detected NTR activities in bacteria by using the probe, which could be a promising tool for the detection of ESKAPE pathogen infection.

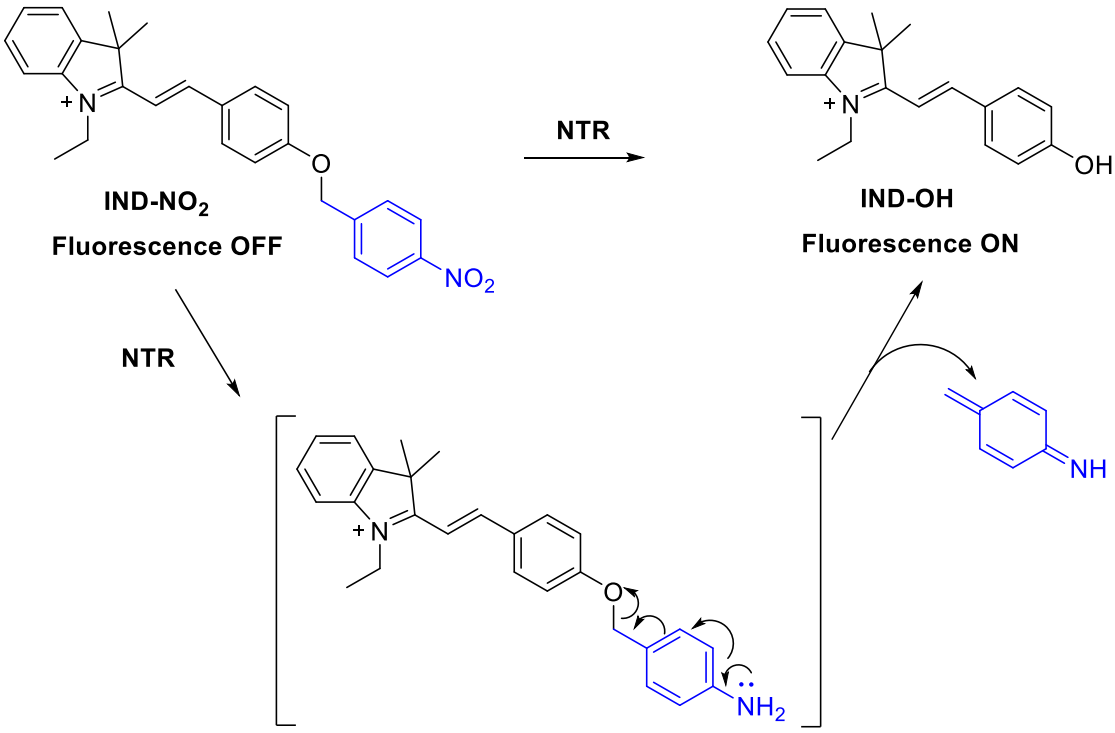

Figure 1.1 Proposed reaction mechanism of the probe IND-NO_2 reduced by NTR to generate the fluorescent IND-OH.

1.2 Research objective

1) To conduct *in vitro* assays using *E. coli* NTR as a model enzyme to reduce the fluorescence probe.
2) To test the probe in different microorganisms

1.3 Scope and limitations

In this work, *E. coli* NTR (*Ec*NfsB) was chosen to be a model enzyme to show that the probe's nitro group can be reduced by NTR to form an amino group, that generates the fluorescence signal. The reaction was confirmed by fluorescence spectroscopy and HPLC. The enzyme kinetics for the probe were determined. The reduction specificity of the probe was tested by conducting control experiments in the presence of various biological reductants. Lastly, the IND-NO_2 probe was tested in a few microorganisms,

including the Gram-positive bacterium *Staphylococcus aureus* and the Gram-negative bacteria *Escherichia coli* TISTR780 and *Pseudomonas aeruginosa* TISTR781.

1.4 References

Jia, C., Zhang, Y., Wang, Y., and Ji, M. (2021). A fast-responsive fluorescent turn-on probe for nitroreductase imaging in living cells. *RSC Advances. 11*(15), 8516-8520.

Parkinson, G. N., Skelly, J. V., and Neidle, S. (2000). Crystal Structure of FMN-Dependent Nitroreductase from Escherichia coli B: A Prodrug-Activating Enzyme. *Journal of Medicinal Chemistry. 43*(20), 3624-3631.

Santajit, S., and Indrawattana, N. (2016). Mechanisms of Antimicrobial Resistance in ESKAPE Pathogens. *BioMed Research International.* 2016, 2475067.

Searle, P. F., Chen, M.-J., Hu, L., Race, P. R., Lovering, A. L., Grove, J. I., Guise, C., Jaberipour, M., James, N. D., Mautner, V., Young, L. S., Kerr, D. J., Mountain, A., White, S. A., and Hyde, E. I. (2004). NITROREDUCTASE: A PRODRUG-ACTIVATING ENZYME FOR CANCER GENE THERAPY. *Clinical and Experimental Pharmacology and Physiology. 31*(11), 811-816.

Wang, H., Yang, M., Ji, M., and Wang, P. (2022). A benzoindole-based fluorescent probe for nitroreductase imaging in living cells under hypoxia conditions. *Journal of Photochemistry and Photobiology A: Chemistry. 427*, 113814.

Wang, S., Zhang, X.-F., Wang, H.-S., Liu, J., Shen, S.-L., and Cao, X.-Q. (2023). A highly sensitive NIR fluorescence probe for hypoxia imaging in cells and ulcerative colitis. *Talanta. 252*, 123834.

Wang, Y., Zhang, L., Huang, Y., Wang, X., Zhang, L., and Chen, L. (2020). Rational design of a nitroreductase-activatable two-photon fluorescent probe for hypoxia imaging in cell and in vivo. *Sensors and Actuators B: Chemical. 310*, 127755.