



Novel Inhibitors of Enzyme HGXPRT Targeting Malaria Parasite *Plasmodium falciparum* through Biochemical and Biophysical methods: A Step Toward Healthier Communities

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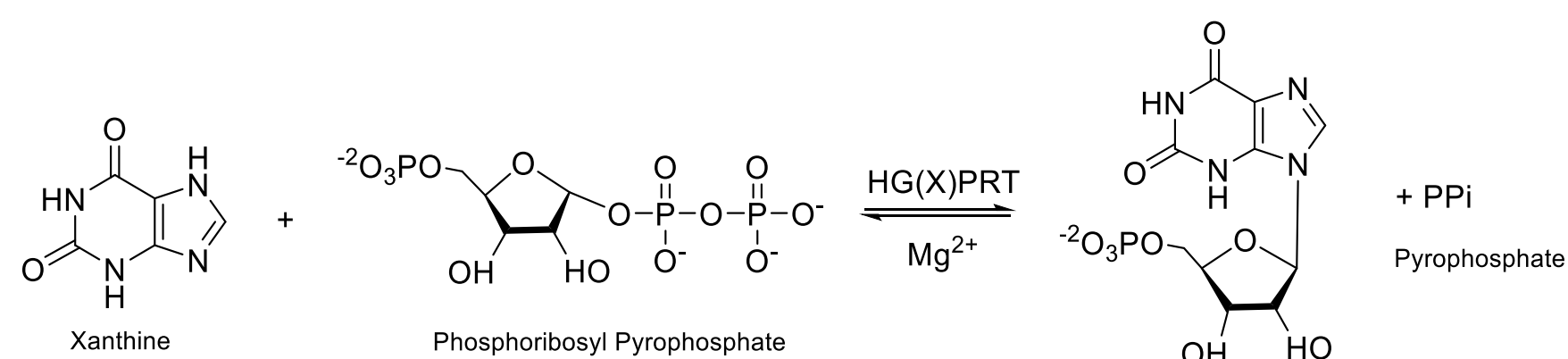
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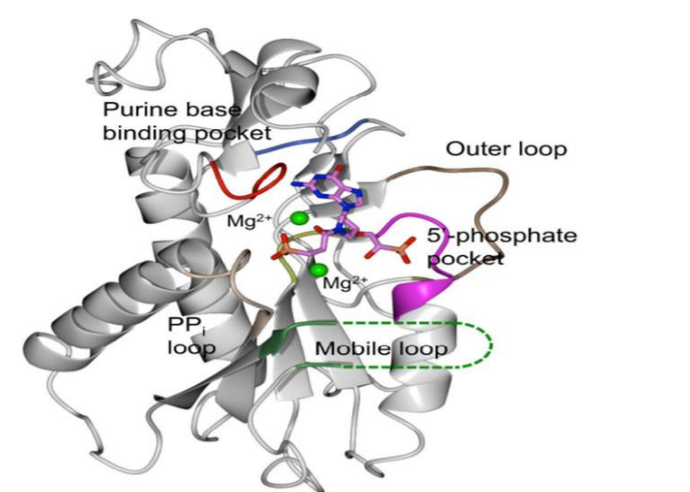


Introduction

- The mosquito-borne disease, Malaria, is widely spread through the bite of infected female Anopheles, and it is caused by the species of *Plasmodium falciparum*, *vivax*, and *ovale*.
- Clinical symptoms extend from minor chills, fever, and headache to severe fatigue, confusion, seizure, and difficulty in breathing [1].
- According to the WHO data reported in 2019, malaria accounts globally for approximately 230 million active cases, and 400,000 deaths each year [2].
- Plasmodium falciparum* and *Plasmodium vivax* are two of the most significant species of the malaria parasite affecting humans.
- In Pakistan, *Plasmodium falciparum* accounts for approximately 14.9% of malaria cases, while *Plasmodium vivax* is more prevalent, responsible for about 84% of cases [3].
- HGXPRT enzyme plays a crucial role in the purine salvage pathway, which allows the parasite to recycle purines from its environment and utilize them for nucleotide synthesis.
- This makes HGXPRT a potential target for drug development, as inhibiting its function could disrupt the parasite's ability to proliferate and survive within the host.
- It is a phosphoribosyl transferase, and a homo-tetrameric protein with each monomer (26kDa) and has binding sites.
- HG(X)PRT involves the conversion of purine bases (hypoxanthine, guanine, or xanthine) into their corresponding nucleotides (inosine monophosphate (IMP), guanosine monophosphate (GMP), or xanthosine monophosphate (XMP)) using 5-phosphoribosyl-1-pyrophosphate (PRPP) as a substrate.
- Chloroquine, Mefloquine, and Quinolines are drugs of choice for the treatment of malaria. To date, the RTS,S malaria vaccine is the most clinically advanced vaccine that intricately phase III clinical trials [4].



HGXPRT Catalyzed Reaction

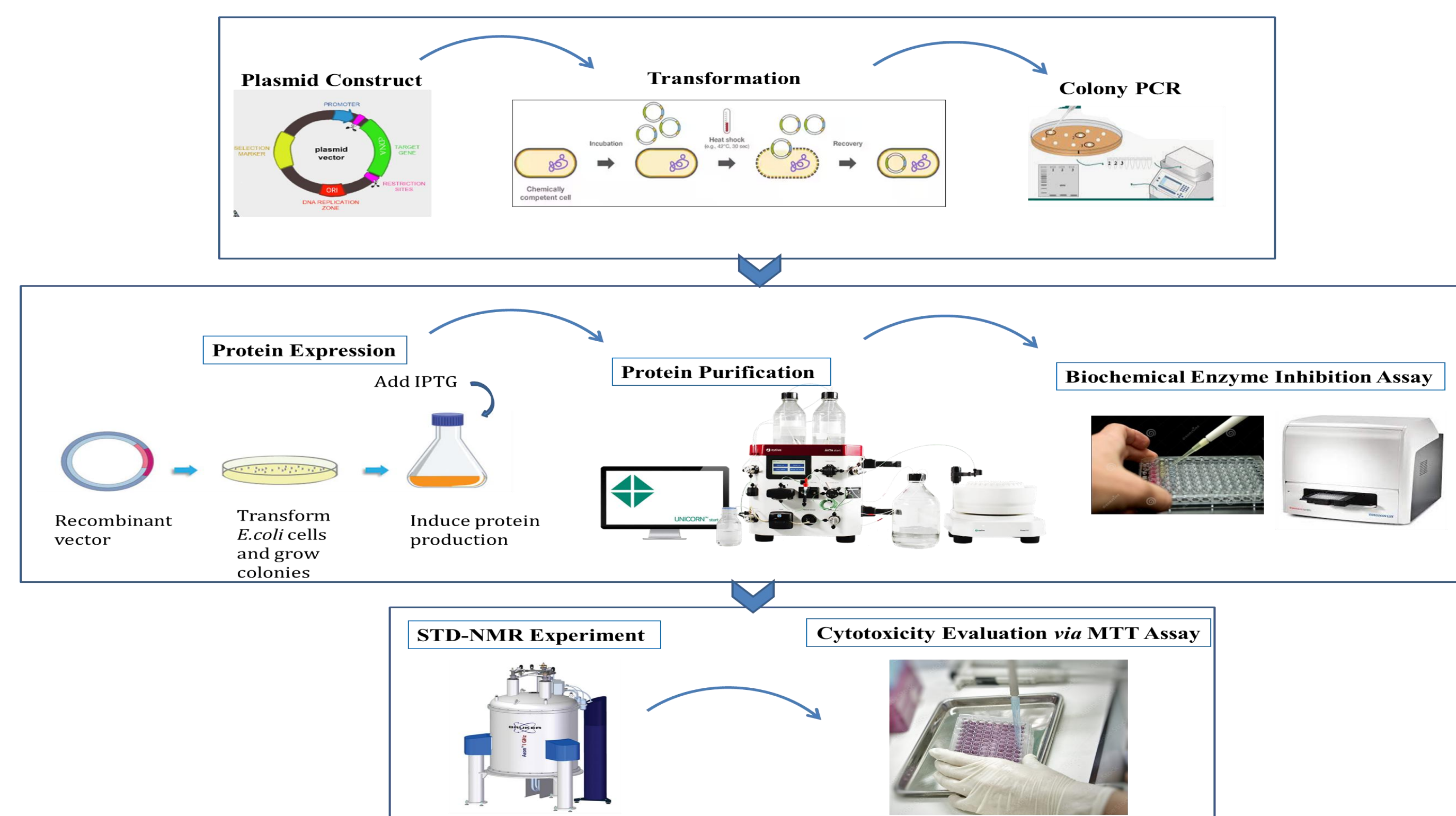


Structural Features of HGXPRT Enzyme

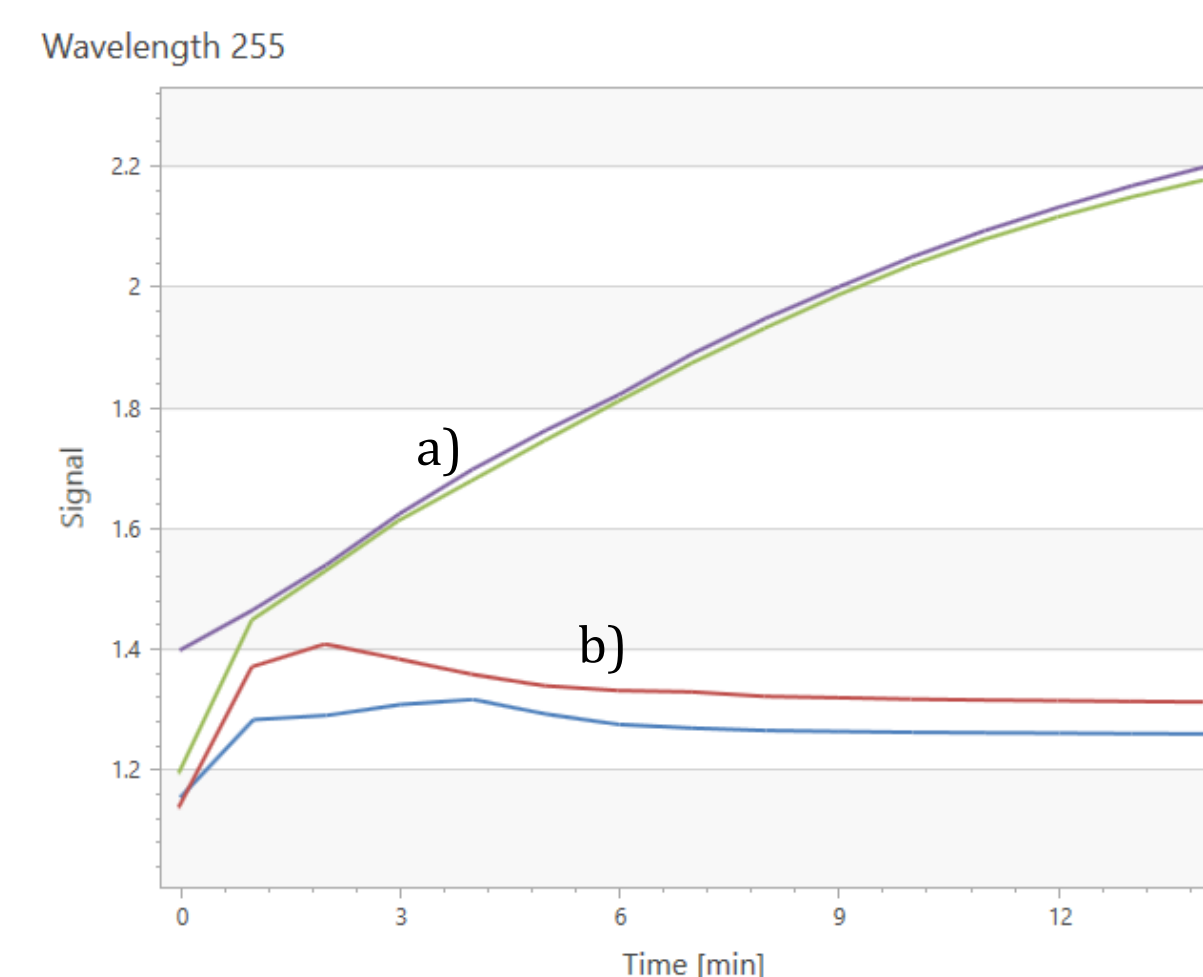
Objectives of the Study

- To transform, express, and purify protein HG(X)PRT in prokaryotic system
- To identify the inhibitors of the enzyme.

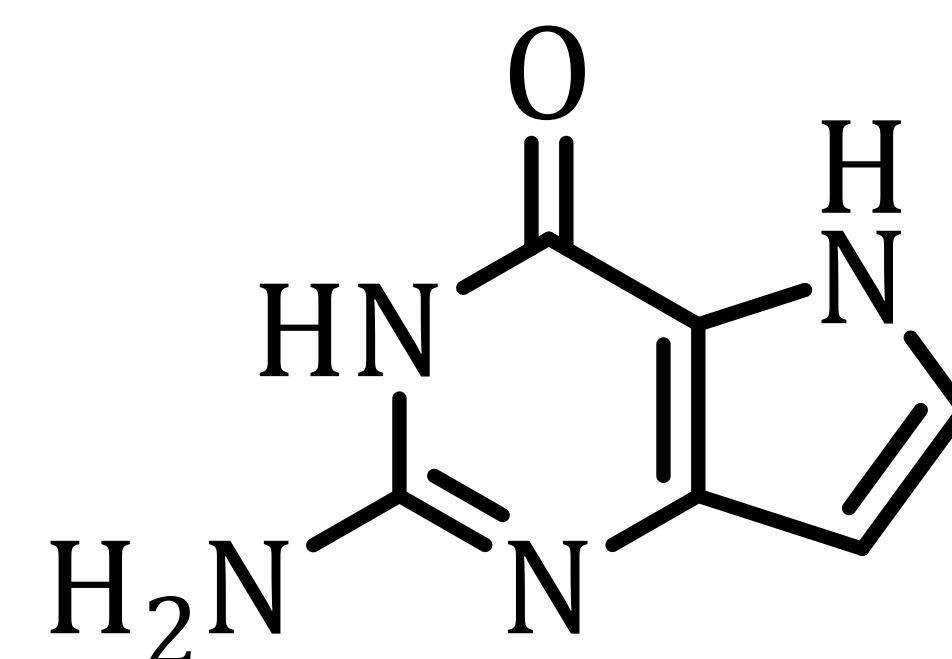
Methodology



Biochemical enzyme inhibition assay

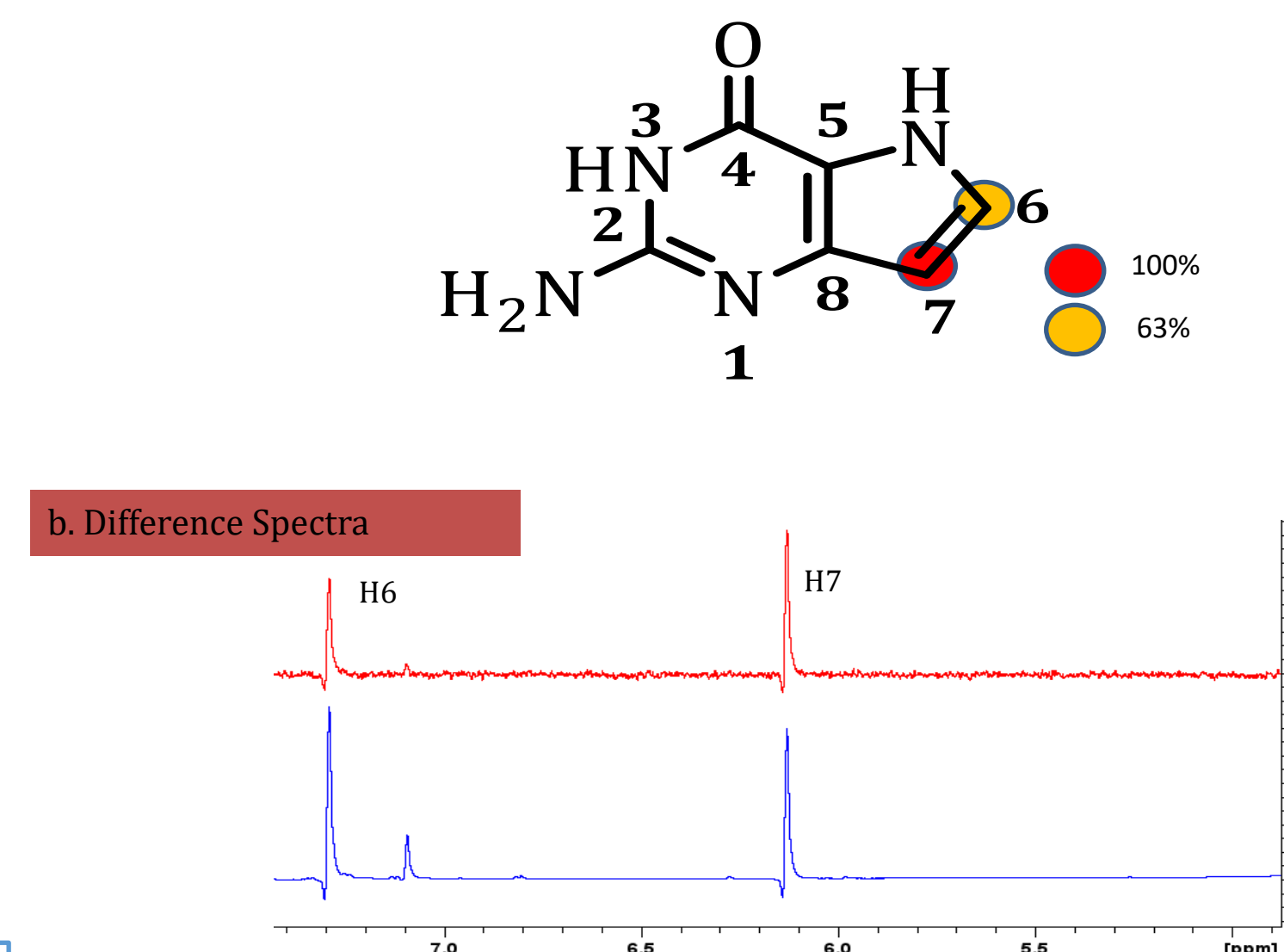


Enzyme reaction a) enzyme reaction without inhibitor (control reaction) b) reaction in the presence of inhibitor



9-deazaguaine (Lab Standard)

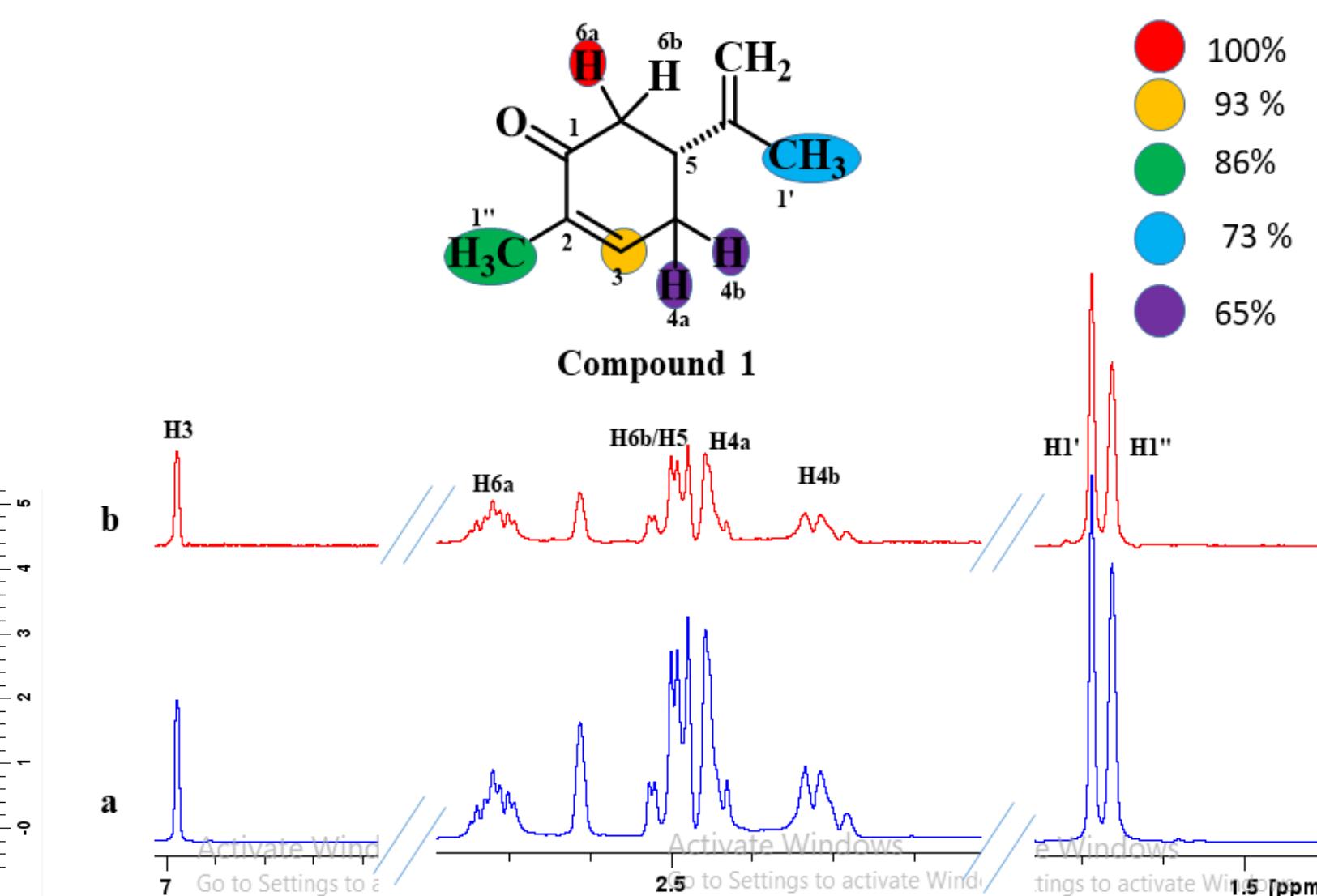
$$IC_{50} = 12 \pm 1.0 \mu M$$



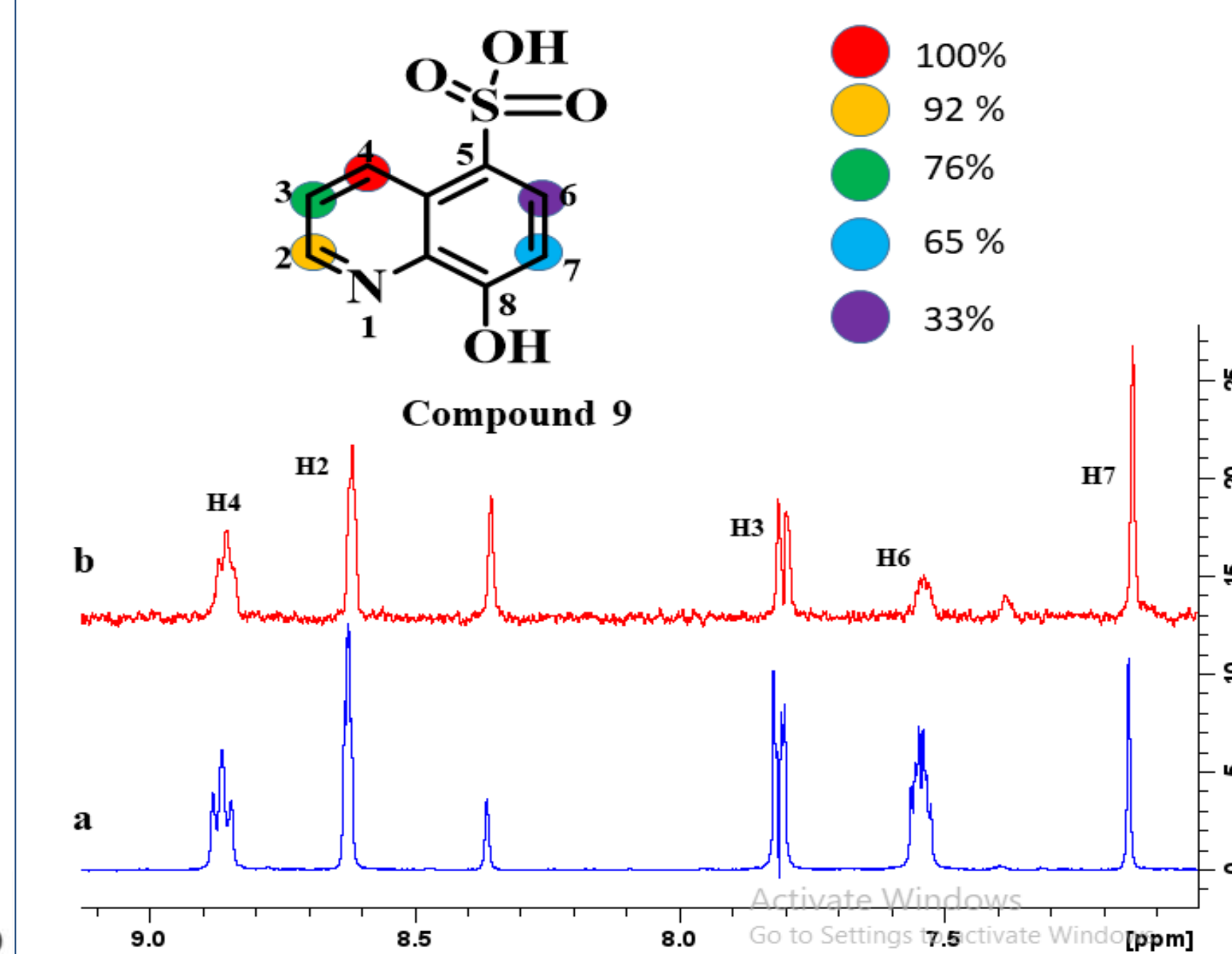
a. Off resonance Spectra

b. Difference Spectra

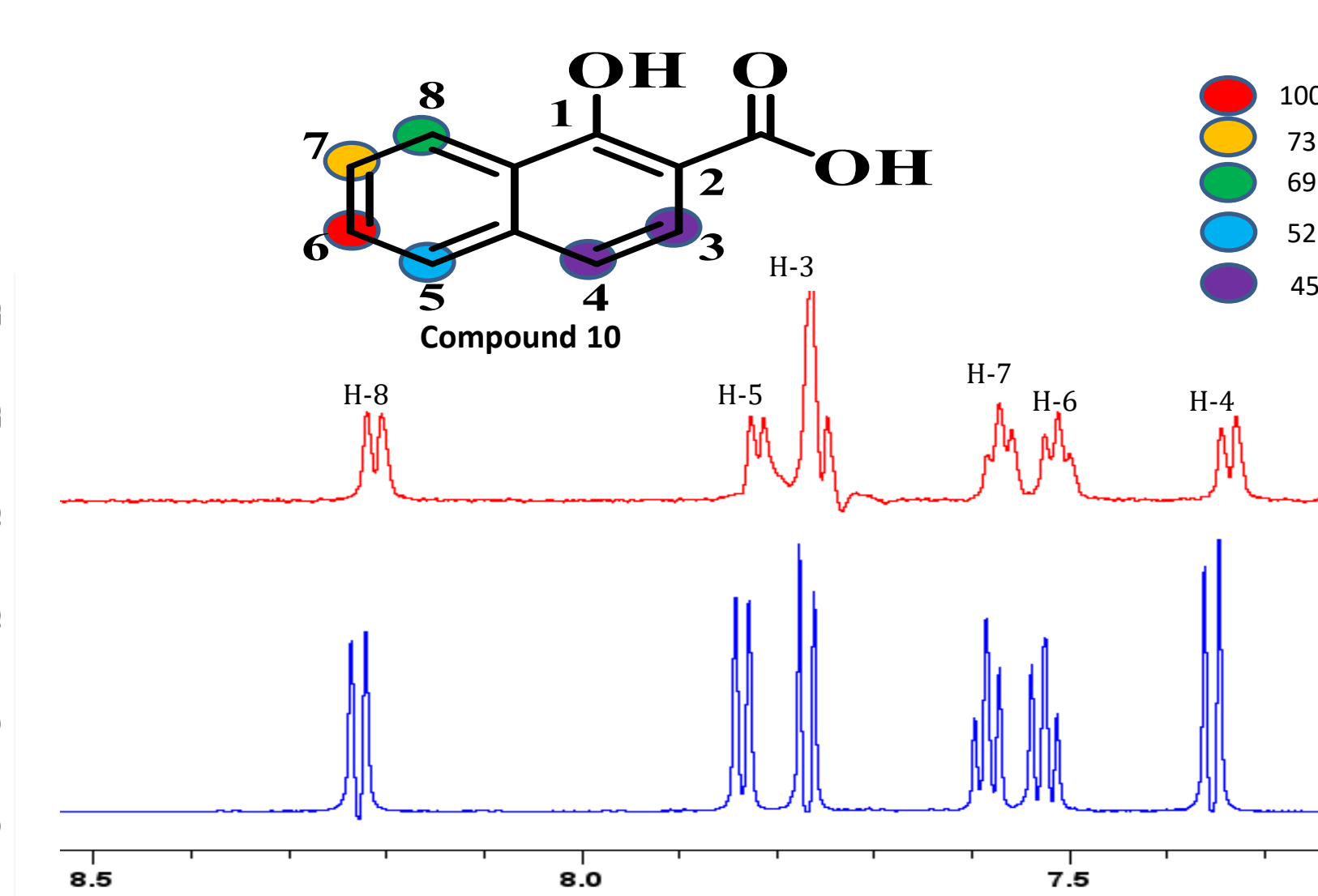
Results



$$IC_{50} = 15.7 \pm 0.94 \mu M$$

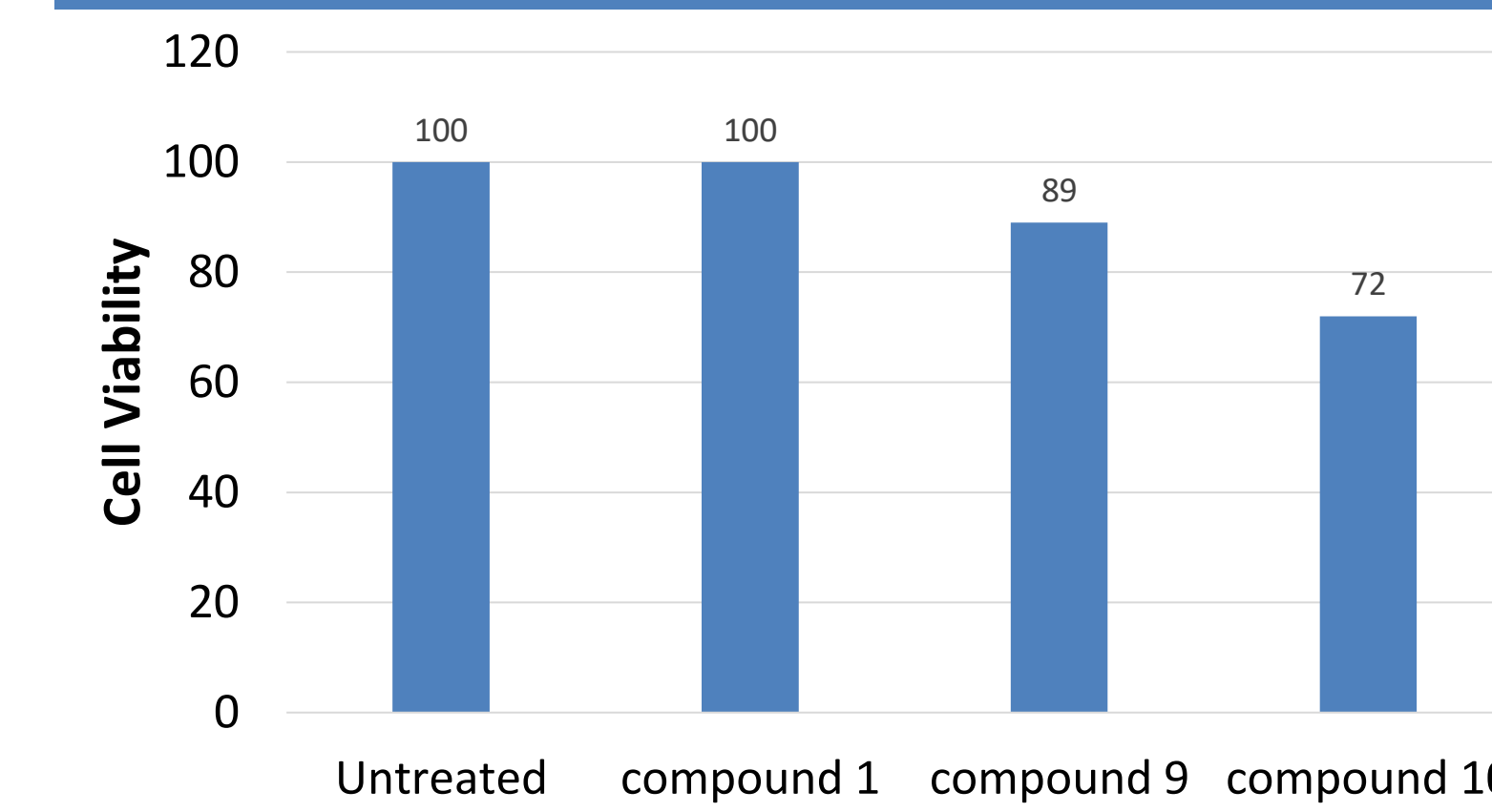


$$IC_{50} = 122.8 \pm 3.5 \mu M$$



$$IC_{50} = 128 \pm 3.349 \mu M$$

Cytotoxicity



Conclusion

- Transformation, expression, and purification of HGXPRT in *E. coli* was carried out successfully with 10mg /L yield.
- More than 200 molecules were screened and among them, fourteen compounds 1-14 showed a good to weak enzyme inhibitory potential.
- Biophysical study were also performed on these compounds to get structural insight into enzyme-ligand complex at atomic level. Compound 1, 9 and 10 exhibited significant interaction with the enzyme, reflected by the enhanced saturation transfer observed in the STD-NMR spectra.
- These compounds were found non-cytotoxic towards human fibroblast cells therefore, exhibit potential to serve as leads for further research towards anti-malarial drug design and development.

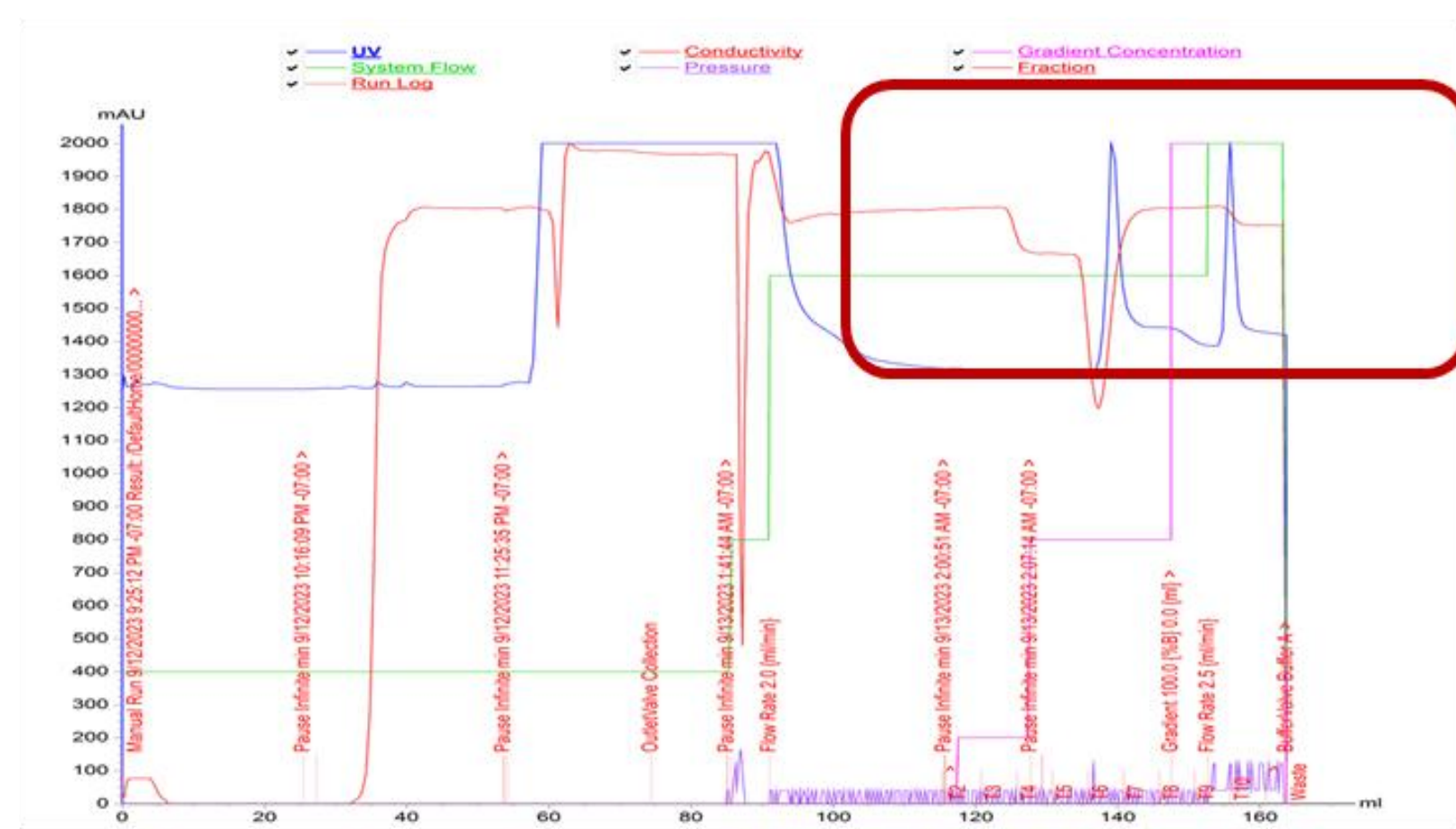
Future Perspective

- To evaluate the specificity of identified leads in *Plasmodium* strain
- To carry out the kinetics studies for the lead compounds in order to deduce their mechanism of action

References

- Malaria World Health Organization <https://www.who.int>
- Development of a continuous assay for high throughput screening to identify inhibitors of the purine salvage pathway in *Plasmodium falciparum*.
- Malaria – Pakistan World Health Organization <https://www.who.int> > Disease Outbreak News > Item17-Oct-2022
- Mills, B., Isaac, R. E., & Foster, R. (2021). Metalloaminopeptidases of the protozoan parasite *Plasmodium falciparum* as targets for the discovery of novel antimalarial drugs. *Journal of Medicinal Chemistry*, 64(4), 1763-1785.
- Gazanion, E., and Vergnes, B. (2018). Protozoan parasite auxotrophies and metabolic dependencies. In *Metabolic Interaction in Infection*, Springer Cham, 351-375.+

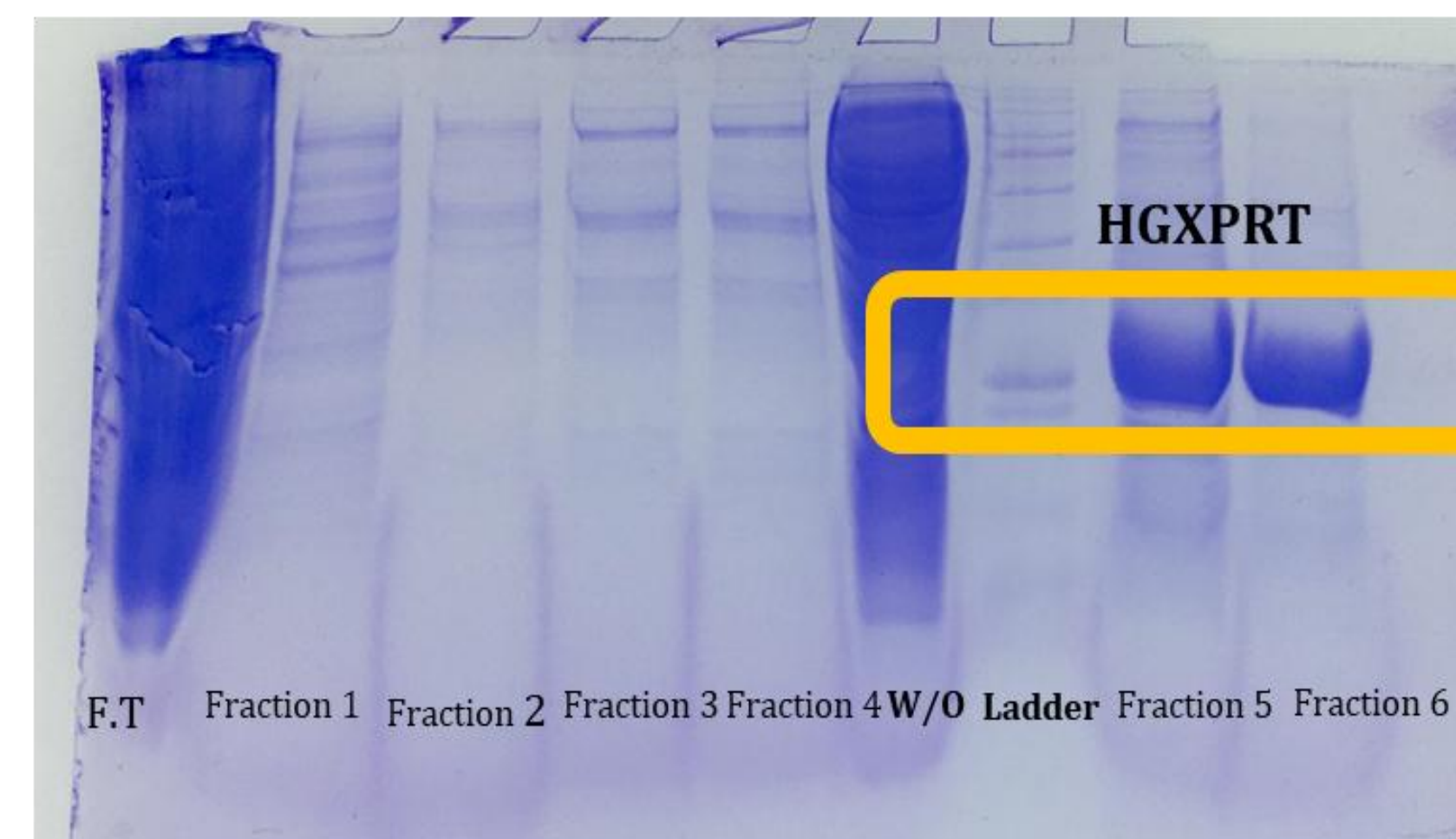
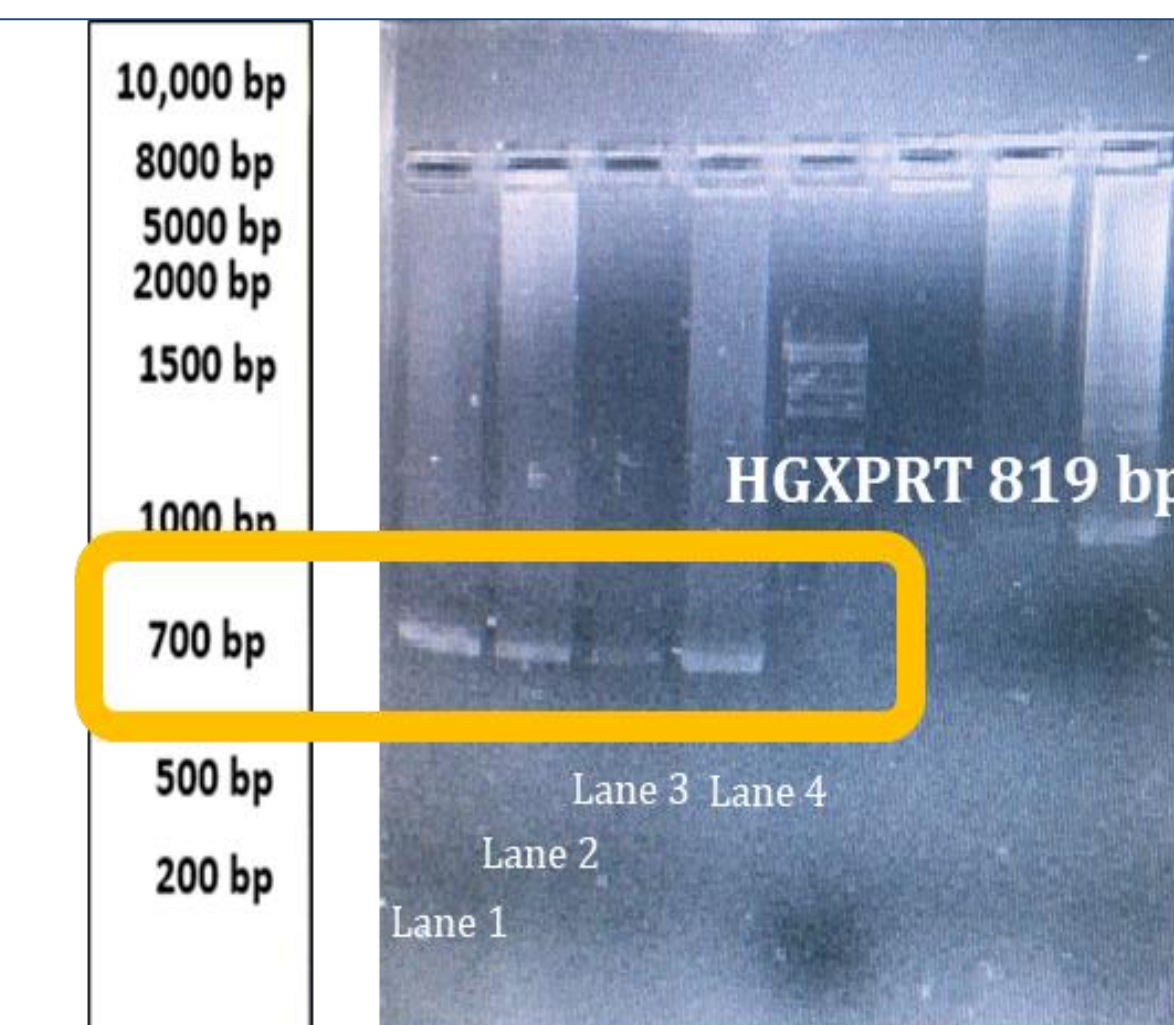
FPLC chromatogram



Transformation of the plasmid



1% agarose gel Lane 1-4 indicate HG(X)PRT gene



SDS-PAGE Gel After FPLC, L represents the protein ladder, FT is flow throw, W/o washout, and F1-F6 are FPLC fractions