B-05

Effect of Desulphated Heparin Oligosaccharides in Inhibiting Iduronate-2-sulphatase Activity

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Introduction

- Mucopolysaccharidoses Type II (MPS II) is a rare inherited disease caused by mutation in *IDS* gene encoding iduronate-2-sulphatase (IDS), an important enzyme in the glycosaminoglycans degradation pathway of heparan sulphate and dermatan sulphate.
- Current treatments such as enzyme replacement therapy is not effective in reducing the central nervous system manifestation while finding the suitable donor quite challenging in bone marrow transplantation.
- The use of small molecules in assisting misfolding protein has been explored as an alternative approach for potential therapy in MPS II treatment.
- In this study, we performed the inhibition assay of desulphated heparin oligosaccharides (DSH) small molecules using recombinant human iduronate-2-sulphatase (rhIDS) to identify potential candidate as pharmacological chaperone for MPS II.

Material & Methods

- The small molecules of DSH (DSH004, DSH006, DSH008, DSH010 & DSH012) were used in this study.
- All of the DSH candidates were tested in the inhibition assay as described in product insert of recombinant human iduronate 2-sulphatase (rhIDS) (2) (Figure 1).
- All DSH candidates were also tested on ATCC cell for specificity testing.

SAMPLE DILUTION & INCUBATION

- DSH candidates were diluted into several concentrations and incubated with rhIDS

10 minutes at 0° C

INHIBITION ASSAY

- 50 µL of 2 mM ρ-nitrocatechol sulphate was added into 50 µL of each concentration of the respective DSH candidates in the microplate

24 hours at 37° C

END OF ASSAY & MEASUREMENT

- The reaction was terminated with 0.2 M sodium hydroxide and measured using microplate reader at 515 nm wavelength

Figure 1: Flow chart of inhibition assay

Results

- The results of inhibition assay for DSH candidates were shown in table 1.
- Figure 2 illustrated the inhibition profiling of each small molecules of DSH.
- There were no significant different in the inhibition of IDS activity in ATCC cells ($p>0.05$).

Table 1: Inhibition assay results

Small molecules of DSH	IC_{50} (µM)	K_i
DSH004	139	15.6
DSH006	19	11.2
DSH008	18	4.3
DSH010	13	6.2
DSH012	17	6.4

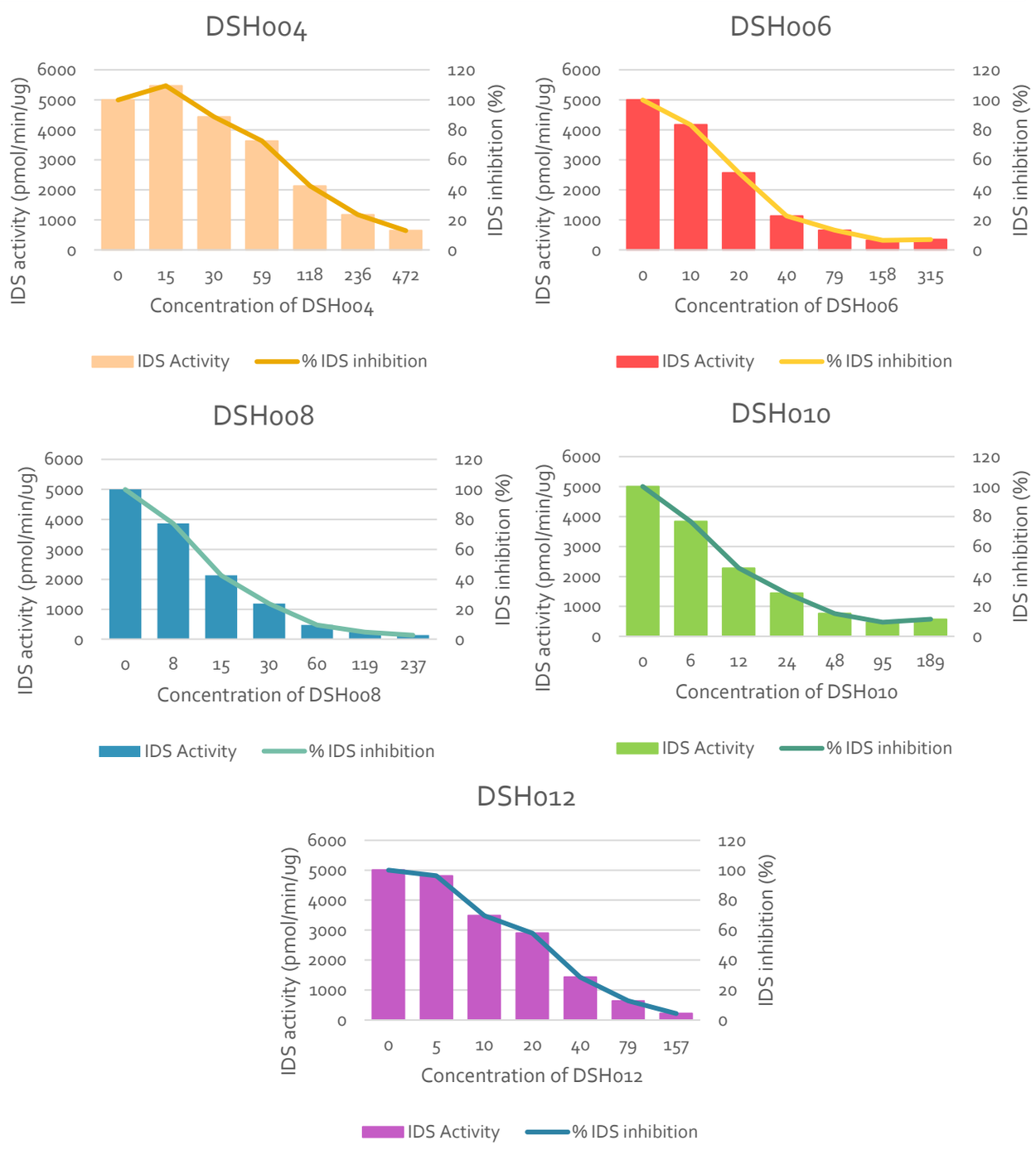

Figure 2: Inhibition profiling of respective DSHs

Discussion & Conclusion

- Inhibition concentration (IC_{50}) referring to concentration required to produce 50% inhibition of enzyme activity.
- A lower IC_{50} generally means a more potent inhibitor (3) while inhibition constant (K_i) indicates the binding affinity of the inhibitors.
- It was observed that DSH004 shows the highest value of IC_{50} and K_i compared to other DSH candidates.
- Although DSH004 demonstrate the highest K_i value, high IC_{50} value may cause toxicity to the cells.
- Overall, among all DSH candidates, DSH006 shows a promising outcome in inhibiting the IDS activity and therefore may have the potential to be a suitable candidate as the pharmacological chaperone for MPS II.

References

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