SUMMARY

Synthesis of Hyptolide Derivative Compounds: *In Vitro* and *Insilico* Studies as Potential Anticancer Remedy

The changes in gene expression mechanism due to the failure of DNA replication processes mediated by Histone Deacetylases (HDAC) can lead to the growth of *Breast Cancer Stem Cells* (BCSCs). Therapeutic approaches targeting HDAC proteins have become an urgent problem, particularly by developing compounds derived from isolation with anticancer activity. Hyptolide compounds substituted diene with tertiary amine groups called *N,N-dimethylcyclohexylenamine-hyptolide* have been successfully synthesized using Diels-Alder method and tested with in vitro analysis for anticancer activity of BCSCs using the MTT assay and flow cytometry methods. The product is yellowish-white crystals with a melting point of 83-84ºC and 595 g/mol molecular weight. Identification using an IR spectrometer showed the presence of a new C=C cyclohexene bond at a wavelength of 1560 cm^{-1} and 1357 cm^{-1}, indicating the presence of a tertiary amine (C-N) group bound to the cyclohexene group. The spectrum from the 1H NMR spectrometer showed a doublet signal at a chemical shift of δ 5.73 ppm (J= 1.0 Hz; IH), a signal of the ethylene group in the cyclohexene structure. In vitro cytotoxicity test using MTT reagent against BCSCs cells gave an IC_{50} value of 47.42 µg/mL with cell cycle inhibition in the DNA synthesis phase (S phases) and cell division phase (G2/M phases). Substitution of the tertiary amine group in the compound *N,N-dimethylcyclohexylenamine-hyptolide* modifies the polarity of the hyptolide compound, thereby increasing its bioavailability in the body. The results of the In Silico study of HDAC proteins using the Density Functional Theory (DFT) principle and the B3LYP/6-31G** basis set with Hydroxamic Acid as a control, the inhibition constant value of the synthesized compound was 14.41 µM, higher than hyptolide 2.99 µM. The interaction energy of hyptolide against HDAC proteins gave the smallest value, namely -32.786 Å. The binding site of hyptolide and its derivatives to HDAC proteins through hydrogen bonds and hydrophobic interactions, at the amino acid residues HIS670, HIS669, HIS709, HIS760, and HIS843. The Gibbs free energy (ΔG) value of the HDAC…Hyptolide complex at pH 3 has the smallest value among other Hyptolide derivatives, which is -121.9 kJ/mol, indicating that the stability of the Hyptolide ligand towards HDAC proteins is better than its derivative ligands or Hydroxamic Acid. The presence of the *α,β*-Unsaturated δ-Lacton group as an electron-withdrawing group binds stably to the Histidine amino acid residue in the active site of the HDAC protein so that the activation of the deacetylation process by the HDAC protein will be inhibited. This stability makes Hyptolide and its derivatives potentially used as an HDAC inhibitor.

Keywords: Hyptolide and its derivatives, HDAC protein, Breast Cancer Stem Cell (BCSCs), Molecular docking