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種別(和文)	論文要旨
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（博士課程）
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論 文 要 旨
THESIS SUMMARY

系・コース： Department of, Graduate major in	Department of Life Science and Technology, Human Centered Science and Biomedical Engineering	系 コース	申請学位（専攻分野）： Academic Degree Requested	博士 Doctor of (Science)

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要旨（英文 800 語程度）
Thesis Summary (approx.800 English Words)

This thesis titled "Discovery of Inhibitors Targeting p300/Transcription Factors Protein-Protein Interactions" contains six chapters written in English. The contents of this thesis cover the construction of a fluorescence anisotropy (FA)-based screening assay for the discovery of HIF-1α/p300 protein-protein interaction (PPI) inhibitor and further investigation of the binding site and its effect in other p300/transcription factor PPIs.

Chapter 1 (Introduction) discusses the significance of HIF-1α/p300 PPI as a cancer drug target, along with drug development efforts targeting this interaction. It covers the HIF-1α/p300 PPI detection screening assay, the FA assay system, and the role of compound libraries in drug discovery. The chapter also discusses modifying the FA-based screening system and screening compound libraries to identify HIF-1α/p300 PPI inhibitors.

Chapter 2 (Construction of Fluorescence Anisotropy Screening Assay System for the Discovery of HIF-1α/p300 PPI Inhibitor) introduces the successful construction of the FA-based screening assay system. HIF-1α/p300 interaction happens between the CTAD domain of HIF-1α and the CH1 domain of p300. Thus, the dansyl-labeled and unlabeled CTAD peptides were synthesized, and CH1 p300 protein was produced by *E. coli*. The FA-screening assay system was validated by measuring the binding affinity and competition assay using unlabeled peptide and OHM1 as positive control. The statistical parameter for evaluate the quality of screening assay was also determined.

Chapter 3 (Screening of Compound Library using Constructed Assay System and Validation of Lead Compounds as HIF-1α/p300 PPI Inhibitors) demonstrates the screening and evaluation of potential HIF-1α/p300 interaction inhibitors. The NPDepo compound library is introduced as the source of candidate compounds. In this screening, several lead compound candidates that showed HIF-1α/p300 inhibition activity better than reported inhibitor OHM 1, e.g., cantharidin (**12**), flunarizine hydrochloride (**13**), perphenazine (**14**), and niclosamide (**15**) were discovered. From reporter gene assay, compounds **12-14** inhibited HIF transcriptional activity with IC_{50} values of 5.63 ± 1.88, 9.01 ± 0.63, and 14.27 ± 1.57 μM, respectively. Compound **15** showed direct inhibition in the luciferase assay. Thus, measurement of the HIF transcriptional activity of compound **15** using HRE-luciferase assay was not appropriate. The effect of candidate lead compounds on the protein expression levels of HIF-1α and its downstream targets in HeLa cells were investigated and it is shown that compound **15** suppressed the CAIX protein expression in a concentration-dependent manner without affecting HIF-1α protein levels. Finally, the effect of compound **15** on the gene expression of HIF-1α and its downstream targets is analyzed. It is shown that Compound **15** did not affect HIF-1α mRNA expression but did suppress the mRNA expression of CAIX, indicating that compound **15** can inhibit HIF-1α/p300 PPI.

Based on the findings of compound **15** as a promising inhibitor of the p300/HIF-1α interaction, in chapter 4 (Identification of the Binding Site of Niclosamide using Photoaffinity Labeling), its binding site in CH1 p300 protein were investigated. Niclosamide azide (**16**) was synthesized as a photoaffinity labeling (PAL) probe aiming for minimal structural changes to preserve its activity. The probe was evaluated for its effect on HIF-1α and CAIX protein expression in HeLa cells. Western blot results revealed that compound **16** suppresses CAIX protein levels without affecting HIF-1α. Compound **16** was also shown to inhibit luciferase enzyme directly, with comparable efficacy to

compound **15**. Thus, it can be used as a probe for PAL experiments. MS/MS analysis detected fragmented peptides with the modification probe attached to Trp^{181}. It is shown that the identified binding site is located in the Zn^{2+}-coordination site of p300/HIF-1α. The binding interaction between compound **16** and p300 CH1 protein was further investigated using Discovery Studio. Docking studies revealed that the azide moiety of compound **16** is located near Trp^{181} and forms key interactions, including a carbon-hydrogen bond and an attractive charge interaction with His^{180}. The same binding site was used to study the interaction between compound **15** and p300 CH1 protein. The molecular docking showed that the nitro group of compound **15** forms a carbon-hydrogen bond with Pro^{110}, while the benzene ring exhibits pi-alkyl interactions with several amino acids such as Pro^{190}, Val^{191}, and Ile^{116}. The chloro substituents also contribute to alkyl-binding interactions with Ile^{177}, Leu^{120}, and His^{180}. This result showed that the binding site of compound **15** was discovered.

Based on the finding of the identified binding site located in the Zn^{2+}-coordination site of p300/HIF-1α, in chapter 5 (Investigation of The Effect of Niclosamide in Other p300/Transcription Factor Protein-Protein Interaction), its effects on other p300-related transcription factors, such as tumor suppressor p53 and signal transducer and activator of transcription-3 (STAT3) were evaluated. To assess whether compound **15** affects p53, the mRNA expression levels of p53 and its downstream gene, BAX, were measured. It is shown that compound **15** significantly suppressed BAX gene expression without affecting the p53 gene levels. The mRNA expression levels of STAT3 and its downstream gene, c-MYC, were also measured. It is shown that compound **15** significantly suppresses c-MYC gene expression without affecting STAT3 gene levels. This result further supports the finding that compound **15** acts as an inhibitor of p300-transcription factor PPIs.

Chapter 6 (conclusion) summarizes the overall thesis about discovering inhibitor targeting p300/transcription factors PPIs. This thesis also proposes a new finding about drug discovery for cancer diseases, significantly contributing to science.